Uttar Pradesh Rajarshi Tandon
Open University

Bachelor of Science
DCEBY-108
Molecular Genetics & Biotechnology

Block -1 Molecular Genetic - I 3

Unit -1 Nucleic acid (DNA & RNA) and Genetic Materials 8

Unit -2 Structure of DNA, Replication and Types of RNA 29

Unit -3 Gene Expression, Transciption and Translation in Eukaryotes and Prokaryotes 54

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Course Design Committee

Prof. Ashutosh Gupta — **Chairman**
Director, School of Science
UPRTOU, Prayagraj.

Dr. Anil Tiwari — **Member**
Botany Department
Ewing Christen College.
Prayagraj.

Dr. Preeti Singh — **Member**
Botany Department
S.S.K.P.G. College
Prayagraj.

Dr. Sushma Chauhan — **Member/Secretary**
Asst. Prof. (Contractual) Botany
School of Science, UPRTOU, Prayagraj.

Course Preparation Committee

Dr. Rajivranjan — **Author**
Assistant Professor — **Block- 01 – 03** (Unit: 01- 09)
Department of Botany
MLK PG College, Balrampur (U.P)

Dr. Arun Kumar Pandey — **Editor**
Associate Professor, — **(All blocks and units)**
Department of Botany
PSM PG College, Anand Nagar, Maharajganj (U.P)

Dr. Sushma Chauhan — **Coordinator**
Asst. Prof. (Contractual) Botany
School of Science, UPRTOU, Prayagraj.

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COURSE INTRODUCTION

The objective of this course is to provide basic introduction to genetics in concerned to molecular biology. The aim is to provide brief introduction of DNA, RNA, gene expression and gene regulation etc. The course is organized into following three blocks:

Block 1 Molecular genetics- I

Block 2 Molecular genetics- II

Block 3 Biotechnology

The block 1st is organized into following three units as under:

Unit- 1 It covers nucleic acids (DNA & RNA) and genetic materials

Unit- 2 It covers structure of DNA, replication and types of RNA

Unit-3 It covers gene expression, transcription and translation in eukaryotes and prokaryotes.

The Block 2nd is organized into following three units as under:

Unit -4 Gene regulation
Unit-5 Genetic Engineering
Unit-6 Molecular antibodies, DNA probes and DNA finger printing.

The Block 3rd is organized into following three units as under:

Unit -7 Introduction and techniques of tissue culture
Unit-8 Culture of different tissues
Unit-9 Application of biotechnology

Block-I: Molecular Genetics-I

This is the first block on molecular genetics-I. It consists of following three units. These are as follows:

Unit 1:

This unit covers the different nucleic acids such as DNA and RNA as genetic material. Nucleic acid, naturally occurring chemical compound that is capable of being broken down to yield phosphoric acid, sugars, and a mixture of organic bases (purines and pyrimidines). Nucleic acids are biopolymers, or large biomolecules, essential to all known forms of life. They are composed of nucleotides, which are the monomers made of three components: a 5-carbon sugar, a phosphate group and a nitrogenous base. The two main classes of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). If the sugar is ribose, the polymer is RNA; if the sugar is the ribose derivative deoxyribose, the polymer is DNA.

Unit 2:

This unit covers the structure of nucleic acids, replication and types of RNA. This unit covers the chemistry of nucleic acids, describing the structures and properties that allow them to serve as the transmitters of genetic information. **Nucleic acids** are the most important macromolecules for the continuity of life. They carry the genetic blueprint of a cell and carry instructions for the functioning of the cell. The two main types of nucleic acids are **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA).** DNA is the genetic material found in all living organisms, ranging from single-celled bacteria to multicellular mammals. It is found in the nucleus of eukaryotes and in the organelles, chloroplasts, and mitochondria. In prokaryotes, the DNA is not enclosed in a membranous envelope.

Unit 3:

This unit covers gene expression, transcription and translation in prokaryotes and eukaryotes. When the information stored in our DNA is converted into instructions for making proteins or other molecules, it is called gene expression. Gene expression is a tightly regulated process that allows a cell to respond to its changing environment. It acts as both an on/off switch to control when proteins are made and also a volume control that increases or decreases

the amount of proteins made. There are two key steps involved in making a protein, transcription and translation. Transcription is when the DNA in a gene is copied to produce an RNA transcript called messenger RNA (mRNA). This is carried out by an enzyme called RNA polymerase which uses available bases from the nucleus of the cell to form the mRNA. RNA is a chemical similar in structure and properties to DNA, but it only has a single strand of bases and instead of the base thymine (T), RNA has a base called uracil (U).

Objectives:

After studying this unit you will be able:

- To know about the structure and function of nucleic acid.
- To know replication, types of RNA and genetic code.
- To know about structure of gene & their expression.
- To discuss transcription and translation in prokaryotes and eukaryotes.

Unit 1 Nucleic acids (DNA & RNA) and Genetic Materials

Structure

1.1 Introduction

Objectives

1.2 History

1.3 Occurrence and nomenclature

1.4 Molecular composition and size

1.5 Deoxyribonucleic acid (DNA)

1.6 Properties

1.7 Different uses in technology

1.7.1 Genetic engineering

1.7.2 DNA profiling

1.7.3 DNA enzymes or catalytic DNA

1.7.4 DNA nanotechnology

1.8 Ribonucleic acid (RNA)

1.9 Comparison with DNA

1.10 Structure of RNA

1.11 Synthesis

1.12 Types of RNA

1.13 Summary

1.14 Terminal questions

Further readings

1.1 Introduction

The history of molecular biology begins in the 1930s with the convergence of various, previously distinct biological and physical disciplines: biochemistry, genetics, microbiology, virology and physics. With the hope of understanding life at its most fundamental level, numerous physicists and chemists also took an interest in what would become molecular biology. In its modern sense, molecular biology attempts to explain the phenomena of life starting from the macromolecular properties that generate them. Two categories of macromolecules in particular are the focus of the molecular biologist:

- Nucleic acids, among which the most famous is deoxyribonucleic acid (or DNA), the constituent of genes.
- Proteins, which are the active agents of living organisms. One definition of the scope of molecular biology therefore is to characterize the structure, function and relationships between these two types of macromolecules.

This relatively limited definition will suffice to allow us to establish a date for the so-called molecular revolution, or at least to establish a chronology of its most fundamental developments. Nucleic acids are biopolymers, or large biomolecules, essential to all known forms of life. They are composed of nucleotides, which are the monomers made of three components: a 5-carbon sugar, a phosphate group and a nitrogenous base. The two main classes of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). If the sugar is ribose, the polymer is RNA; if the sugar is the ribose derivative deoxyribose, the polymer is DNA.

Nucleic acids are naturally occurring chemical compounds that serve as the primary information-carrying molecules in cells and make up the genetic material. Nucleic acids are found in abundance in all living things, where they create, encode, and then store information of every living cell of every life-form on Earth. In turn, they function to transmit and express that information inside and outside the cell nucleus to the interior operations of the cell and ultimately to the next generation of each living organism. The encoded

information is contained and conveyed via the nucleic acid sequence, which provides the 'ladder-step' ordering of nucleotides within the molecules of RNA and DNA. They play an especially important role in directing protein synthesis.

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Nucleic acids play an especially important role in directing protein synthesis. Nucleic acids are the main information-carrying molecules of the cell, and, by directing the process of protein synthesis, they determine the inherited characteristics of every living thing. The two main classes of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the master blueprint for life and constitutes the genetic material in all free-living organisms and most viruses. RNA is the genetic material of certain viruses, but it is also found in all living cells, where it plays an important role in certain processes such as the making of proteins.

The entire genetic content of a cell is known as its genome, and the study of genomes is genomics. In eukaryotic cells but not in prokaryotes, DNA forms a complex with histone proteins to form chromatin, the substance of eukaryotic chromosomes. A chromosome may contain tens of thousands of genes. Many genes contain the information to make protein products; other genes code for RNA products. DNA controls all of the cellular activities by turning the genes on or off. The other type of nucleic acid, RNA, is mostly involved in protein synthesis. The DNA molecules never leave the nucleus but instead use an intermediary to communicate with the rest of the cell. This intermediary is

the **messenger RNA (mRNA).** Other types of RNA-like rRNA, tRNA, and micro RNA-are involved in protein synthesis and its regulation.

Objectives:

This is the first block on molecular genetics I**.** It consists of following three units. Under first unit, we have following objectives. These are as under:

- To know the definition of nucleic acids
- To know about DNA and RNA
- To know the structure of DNA and types of RNA.
- To know about functioning of nucleic acids as a genetic material

1.2 History

Nucleins were discovered by Friedrich Miescher in 1869 at the University of Tübingen, Germany.

- In the early 1880s Albrecht Kossel further purified the substance and discovered its highly acidic properties. He later also identified the nucleobases.
- In **1889** Richard Altmann creates the term nucleic acid
- In **1938** Astbury and Bell published the first X-ray diffraction pattern of DNA.
- In **1944** the Avery–MacLeod–McCarty experiment showed that DNA is the carrier of genetic information.
- In **1953** Watson and Crick presented the structure of DNA.

Experimental studies of nucleic acids constitute a major part of modern biological and medical research, and form a foundation for genome and forensic science, and the biotechnology and pharmaceutical industries.

1.3 Occurrence and nomenclature

The term nucleic acid is the overall name for DNA and RNA, members of a family of biopolymers, and is synonymous with polynucleotide. Nucleic acids were named for their initial discovery within the nucleus, and for the presence of phosphate groups (related to phosphoric acid). Although first discovered within the nucleus of eukaryotic cells, nucleic acids are now known to be found

in all life forms including within bacteria, archaea, mitochondria, chloroplasts, and viruses. All living cells contain both DNA and RNA (except some cells such as mature red blood cells), while viruses contain either DNA or RNA, but usually not both. The basic component of biological nucleic acids is the nucleotide, each of which contains a pentose sugar (ribose or deoxyribose), a phosphate group, and a nucleobase. Nucleic acids are also generated within the laboratory, through the use of enzymes (DNA and RNA polymerases) and by solid-phase chemical synthesis. The chemical methods also enable the generation of altered nucleic acids that are not found in nature, for example peptide nucleic acids.

1.4 Molecular composition and size

Nucleic acids are generally very large molecules. Indeed, DNA molecules are probably the largest individual molecules known. Well-studied biological nucleic acid molecules range in size from 21 nucleotides (small interfering RNA) to large chromosomes (human chromosome 1 is a single molecule that contains 247 million base pairs. In most cases, naturally occurring DNA molecules are double-stranded and RNA molecules are single-stranded. There are numerous exceptions, however-some viruses have genomes made of double-stranded RNA and other viruses have single-stranded DNA genomes, and, in some circumstances, nucleic acid structures with three or four strands can form.

Nucleic acids are linear polymers (chains) of nucleotides. Each nucleotide consists of three components: a purine or pyrimidine nucleobase (sometimes termed nitrogenous base or simply *base*), a pentose sugar, and a phosphate group which makes the molecule acidic. The substructure consisting of a nucleobase plus sugar is termed a nucleoside. Nucleic acid types differ in the structure of the sugar in their nucleotides–DNA contains 2'-deoxyribose while RNA contains ribose (where the only difference is the presence of a hydroxyl group). Also, the nucleobases found in the two nucleic acid types are different: adenine, cytosine, and guanine are found in both RNA and DNA, while thymine occurs in DNA and uracil occurs in RNA.

The sugars and phosphates in nucleic acids are connected to each other in an alternating chain (sugar-phosphate backbone) through phosphodiester linkages. In conventional nomenclature, the carbons to which the phosphate groups attach are the 3'-end and the 5'-end carbons of the sugar. This gives nucleic acids directionality, and the ends of nucleic acid molecules are referred to as 5'-end and 3'-end. The nucleobases are joined to the sugars via an N-glycosidic linkage involving a nucleobase ring nitrogen (N-1 for pyrimidines and N-9 for purines) and the 1' carbon of the pentose sugar ring.

1.5 Deoxyribonucleic acid (DNA)

Deoxyribonucleic acid (DNA) is a molecule composed of two polynucleotide chains that coil around each other to form a double helix carrying genetic instructions for the development, functioning, growth and reproduction of all known organisms and many viruses. DNA and ribonucleic acid (RNA) are nucleic acids. Alongside proteins, lipids and complex carbohydrates (polysaccharides), nucleic acids are one of the four major types of macromolecules that are essential for all known forms of life.

The two DNA strands are known as polynucleotides as they are composed of simpler monomeric units called nucleotides. Each nucleotide is composed of one of four nitrogen-containing nucleobases (cytosine [C], guanine [G], adenine [A] or thymine [T]), a sugar called deoxyribose, and a phosphate group. The nucleotides are joined to one another in a chain by covalent bonds (known as the phospho-diester linkage) between the sugar of one nucleotide and the phosphate of the next, resulting in an alternating sugar-phosphate backbone. The nitrogenous bases of the two separate polynucleotide strands are bound together, according to base pairing rules (A with T and C with G), with hydrogen bonds to make double-stranded DNA. The complementary nitrogenous bases are divided into two groups, pyrimidines and purines. In DNA, the pyrimidines are thymine and cytosine; the purines are adenine and guanine.

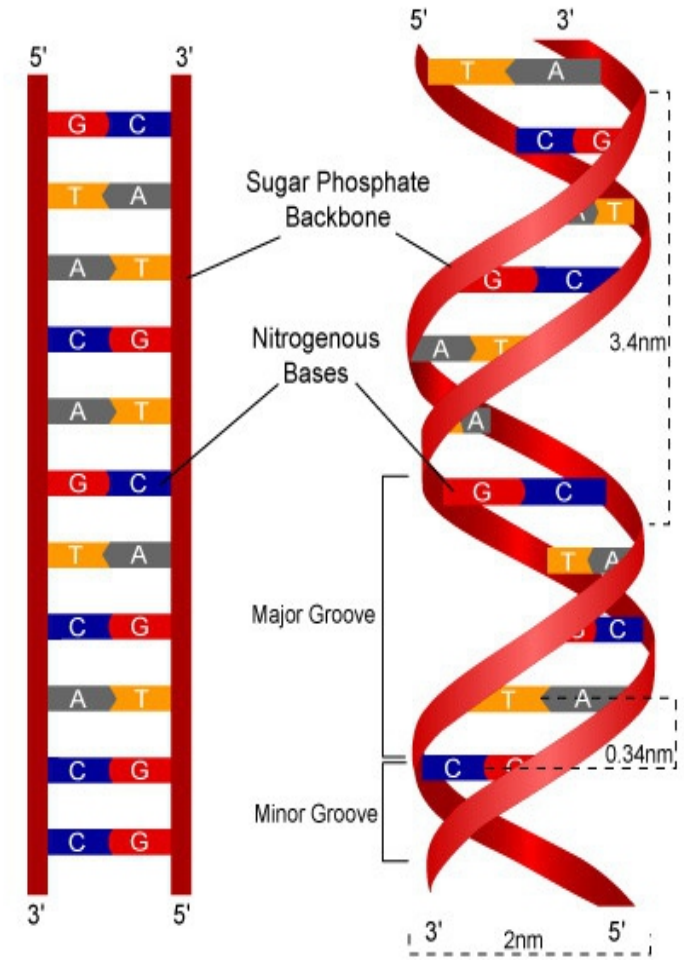

Fig. 1 Structure of DNA

Both strands of double-stranded DNA store the same biological information. This information is replicated as and when the two strands separate. A large part of DNA (more than 98% for humans) is non-coding, meaning that these sections do not serve as patterns for protein sequences. The two strands of DNA run in opposite directions to each other and are thus antiparallel. Attached to each sugar is one of four types of nucleobases (or bases). It is the sequence of these four nucleobases along the backbone that encodes genetic information. RNA strands are created using DNA strands as a template in a process called transcription, where DNA bases are exchanged for their corresponding bases except in the case of thymine (T), for which RNA substitutes uracil (U). Under the genetic code, these RNA strands specify the sequence of amino acids within proteins in a process called translation.

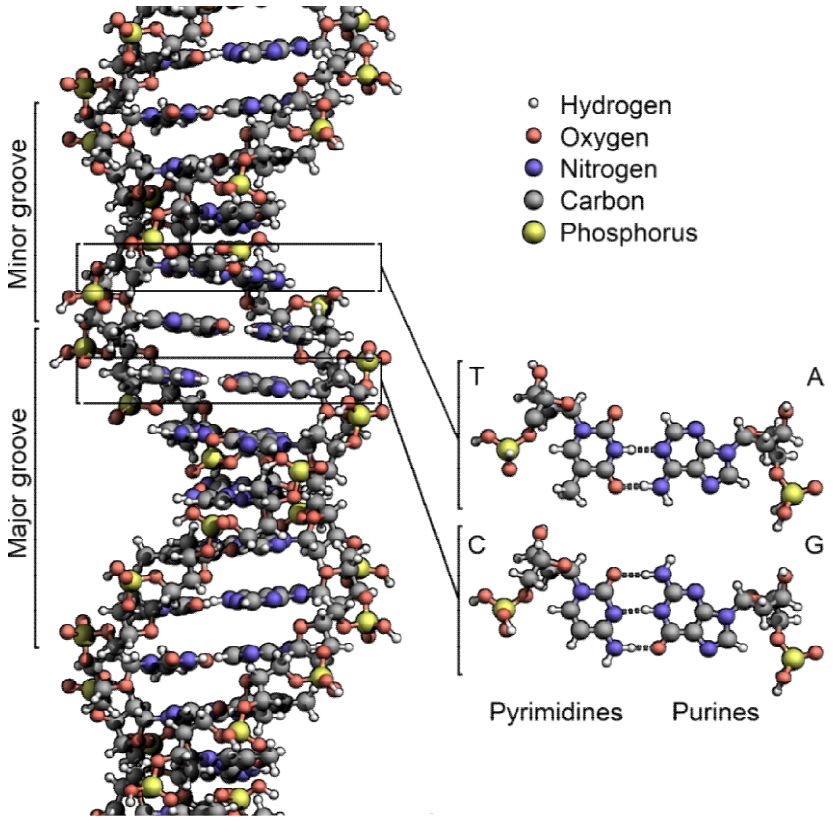

Fig. 2 The structure of the DNA double helix. The atoms in the structure are colour-coded by element and the detailed structures of two base pairs are shown in the bottom right.

Within eukaryotic cells, DNA is organized into long structures called chromosomes. Before typical cell division, these chromosomes are duplicated in the process of DNA replication, providing a complete set of chromosomes for each daughter cell. Eukaryotic organisms (animals, plants, fungi and protists) store most of their DNA inside the cell nucleus as nuclear DNA, and some in the mitochondria as mitochondrial DNA or in chloroplasts as chloroplast DNA. In contrast, prokaryotes (bacteria and archaea) store their DNA only in the cytoplasm, in circular chromosomes. Within eukaryotic chromosomes, chromatin proteins, such as histones, compact and organize DNA. These compacting structures guide the interactions between DNA and other proteins, helping control which parts of the DNA are transcribed.

1.6 Properties

DNA is a long polymer made from repeating units called nucleotides, each of which is usually symbolized by a single letter: either A, T, C, or G. The structure of DNA is dynamic along its length, being capable of coiling into

tight loops and other shapes. In all species it is composed of two helical chains, bound to each other by hydrogen bonds. Both chains are coiled around the same axis, and have the same pitch of 34 ångströms (3.4 nm). The pair of chains have a radius of 10 Å (1.0 nm). According to another study, when measured in a different solution, the DNA chain measured 22–26 Å (2.2–2.6 nm) wide, and one nucleotide unit measured 3.3 Å (0.33 nm) long. Although each individual nucleotide is very small, a DNA polymer can be very large and may contain hundreds of millions of nucleotides, such as in chromosome 1. Chromosome 1 is the largest human chromosome with approximately 220 million base pairs, and would be 85 mm long if straightened.

DNA does not usually exist as a single strand, but instead as a pair of strands that are held tightly together. These two long strands coil around each other, in the shape of a double helix. The nucleotide contains both a segment of the backbone of the molecule (which holds the chain together) and a nucleobase (which interacts with the other DNA strand in the helix). A nucleobase linked to a sugar is called a nucleoside, and a base linked to a sugar and to one or more phosphate groups is called a nucleotide. A biopolymer comprising multiple linked nucleotides (as in DNA) is called a polynucleotide.

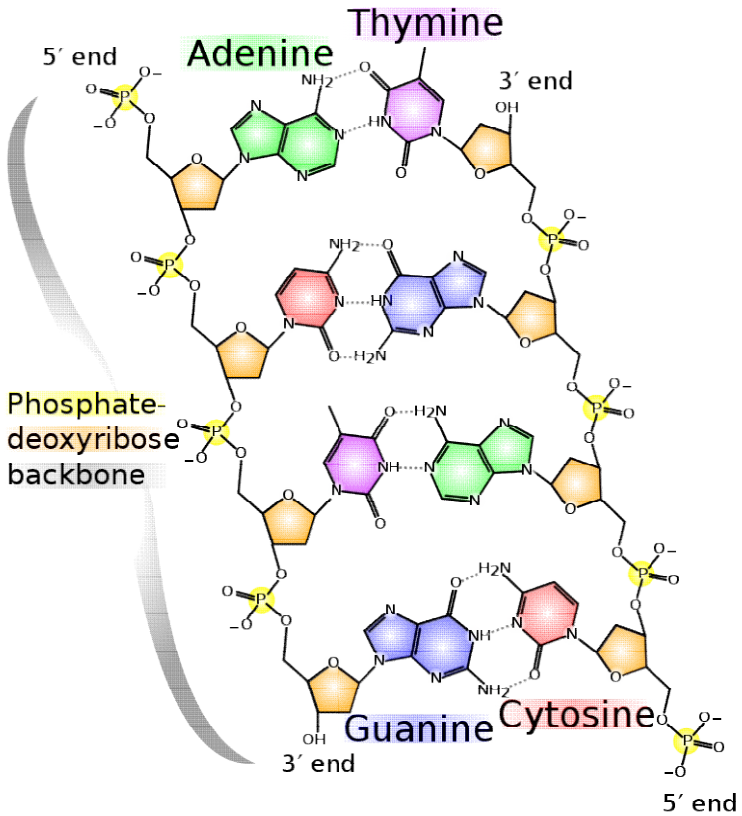

Fig. 3 Chemical structure of DNA; hydrogen bonds shown as dotted lines. Each end of the double helix
has an exposed 5' phosphate on one strand and an exposed 3' hydroxyl group (-OH) on the other.

The backbone of the DNA strand is made from alternating phosphate and sugar groups. The sugar in DNA is 2-deoxyribose, which is a pentose (five-carbon) sugar. The sugars are joined together by phosphate groups that form phosphodiester bonds between the third and fifth carbon atoms of adjacent sugar rings. These are known as the 3′-end (three prime end), and 5′-end (five prime end) carbons, the prime symbol being used to distinguish these carbon atoms from those of the base to which the deoxyribose forms a glycosidic bond. Therefore, any DNA strand normally has one end at which there is a phosphate group attached to the 5′ carbon of a ribose (the 5′ phosphoryl) and another end at which there is a free hydroxyl group attached to the 3′ carbon of a ribose (the 3′ hydroxyl). The orientation of the 3′ and 5′ carbons along the sugar-phosphate backbone confers directionality (sometimes called polarity) to each DNA strand. In a nucleic acid double helix, the direction of the nucleotides in one strand is

opposite to their direction in the other strand: the strands are antiparallel. The asymmetric ends of DNA strands are said to have a directionality of five prime end (5′), and three prime end (3′), with the 5′ end having a terminal phosphate group and the 3′ end a terminal hydroxyl group. One major difference between DNA and RNA is the sugar, with the 2-deoxyribose in DNA being replaced by the alternative pentose sugar ribose in RNA.

SAQ 1

Q. 1 Define nucleic acids.

Q. 2 Differentiate between DNA & RNA.

Q. 3 What do you mean by nitrogenous bases?

Q. 4 Define functions of nucleic acids.

1.7 Different uses in technology

1.7.1 Genetic engineering

Methods have been developed to purify DNA from organisms, such as phenol-chloroform extraction, and to manipulate it in the laboratory, such as restriction digests and the polymerase chain reaction.

Modern biology and biochemistry make intensive use of these techniques in recombinant DNA technology. Recombinant DNA is a man-made DNA sequence that has been assembled from other DNA sequences. They can be transformed into organisms in the form of plasmids or in the appropriate format, by using a viral vector. The genetically modified organisms produced can be used to produce products such as recombinant proteins, used in medical research, or be grown in agriculture.

1.7.2 DNA profiling

Forensic scientists can use DNA in blood, semen, skin, saliva or hair found at a crime scene to identify a matching DNA of an individual, such as a perpetrator. This process is formally termed DNA profiling, also called *DNA fingerprinting*. In DNA profiling, the lengths of variable sections of repetitive DNA, such as short tandem repeats and minisatellites, are compared between people. This method is usually an extremely reliable technique for identifying a matching DNA. However, identification can be complicated if the scene is contaminated with DNA from several people. DNA profiling was developed in

1984 by British geneticist Sir Alec Jeffreys, and first used in forensic science to convict Colin Pitchfork in the 1988 Enderby murders case.

The development of forensic science and the ability to now obtain genetic matching on minute samples of blood, skin, saliva, or hair has led to re-examining many cases. Evidence can now be uncovered that was scientifically impossible at the time of the original examination. Combined with the removal of the double jeopardy law in some places, this can allow cases to be reopened where prior trials have failed to produce sufficient evidence to convince a jury. People charged with serious crimes may be required to provide a sample of DNA for matching purposes. The most obvious defense to DNA matches obtained forensically is to claim that cross-contamination of evidence has occurred. This has resulted in meticulous strict handling procedures with new cases of serious crime.

DNA profiling is also used successfully to positively identify victims of mass casualty incidents, bodies or body parts in serious accidents, and individual victims in mass war graves, via matching to family members. DNA profiling is also used in DNA paternity testing to determine if someone is the biological parent or grandparent of a child with the probability of parentage is typically 99.99% when the alleged parent is biologically related to the child. Normal DNA sequencing methods happen after birth, but there are new methods to test paternity while a mother is still pregnant.

1.7.3 DNA enzymes or catalytic DNA

Deoxyribozymes, also called DNAzymes or catalytic DNA, were first discovered in 1994. They are mostly single stranded DNA sequences isolated from a large pool of random DNA sequences through a combinatorial approach called in vitro selection or systematic evolution of ligands by exponential enrichment (SELEX). DNAzymes catalyze variety of chemical reactions including RNA-DNA cleavage, RNA-DNA ligation, amino acids phosphorylation-dephosphorylation, carbon-carbon bond formation, etc. DNAzymes can enhance catalytic rate of chemical reactions up to

100,000,000,000-fold over the uncatalyzed reaction. The most extensively studied class of DNAzymes is RNA-cleaving types which have been used to detect different metal ions and designing therapeutic agents.

1.7.4 DNA nanotechnology

The DNA structure at left will self-assemble into the structure visualized by atomic force microscopy at right. DNA nanotechnology is the field that seeks to design nanoscale structures using the molecular recognition properties of DNA molecules. DNA nanotechnology uses the unique molecular recognition properties of DNA and other nucleic acids to create self-assembling branched DNA complexes with useful properties. DNA is thus used as a structural material rather than as a carrier of biological information. This has led to the creation of two-dimensional periodic lattices and three-dimensional structures in the shapes of polyhedra. Nanomechanical devices and algorithmic self-assembly have also been demonstrated, and these DNA structures have been used to template the arrangement of other molecules such as gold nanoparticles and streptavidin proteins.

1.8 Ribonucleic acid (RNA)

Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes. RNA and deoxyribonucleic acid (DNA) are nucleic acids. Along with lipids, proteins, and carbohydrates, nucleic acids constitute one of the four major macromolecules essential for all known forms of life. Like DNA, RNA is assembled as a chain of nucleotides, but unlike DNA, RNA is found in nature as a single strand folded onto itself, rather than a paired double strand. Cellular organisms use messenger RNA (mRNA) to convey genetic information (using the nitrogenous bases of guanine, uracil, adenine, and cytosine, denoted by the letters G, U, A, and C) that directs synthesis of specific proteins. Many viruses encode their genetic information using an RNA genome.

Some RNA molecules play an active role within cells by catalyzing biological reactions, controlling gene expression, or sensing and communicating responses to cellular signals. One of these active processes is protein synthesis, a universal function in which RNA molecules direct the synthesis of proteins

on ribosomes. This process uses transfer RNA (***tRNA***) molecules to deliver amino acids to the ribosome, where ribosomal RNA (***rRNA***) then links amino acids together to form coded proteins.

1.9 Comparison with DNA

The chemical structure of RNA is very similar to that of DNA, but differs in three primary ways:

- Unlike double-stranded DNA, RNA is a single-stranded molecule in many of its biological roles and consists of much shorter chains of nucleotides. However, a single RNA molecule can, by complementary base pairing, form intrastrand double helixes, as in tRNA.
- While the sugar-phosphate backbone of DNA contains *deoxyribose*, RNA contains *ribose* instead. Ribose has a hydroxyl group attached to the pentose ring in the 2' position, whereas deoxyribose does not. The hydroxyl groups in the ribose backbone make RNA more chemically labile than DNA by lowering the activation energy of hydrolysis.
- The complementary base to adenine in DNA is thymine, whereas in RNA, it is uracil, which is an unmethylated form of thymine.

Like DNA, most biologically active RNAs,
including mRNA, tRNA, rRNA, snRNAs, and other non-coding RNAs, contain self-complementary sequences that allow parts of the RNA to fold and pair with itself to form double helices. Analysis of these RNAs has revealed that they are highly structured. Unlike DNA, their structures do not consist of long double helices, but rather collections of short helices packed together into structures akin to proteins. In this fashion, RNAs can achieve
chemical catalysis (like enzymes). For instance, determination of the structure of the ribosome-an RNA-protein complex that catalyzes peptide bond formation-revealed that its active site is composed entirely of RNA.

1.10 Structure of RNA

Each nucleotide in RNA contains a ribose sugar, with carbons numbered 1' through 5'. A base is attached to the 1' position, in general, **adenine (A), cytosine (C), guanine (G), or uracil (U)**. Adenine and

guanine are purines, cytosine and uracil are pyrimidines. A phosphate group is attached to the 3' position of one ribose and the 5' position of the next. The phosphate groups have a negative charge each, making RNA a charged molecule (polyanion). The bases form hydrogen bonds between cytosine and guanine, between adenine and uracil and between guanine and uracil. However, other interactions are possible, such as a group of adenine bases binding to each other in a bulge, or the GNRA tetraloop that has a guanine–adenine base-pair.

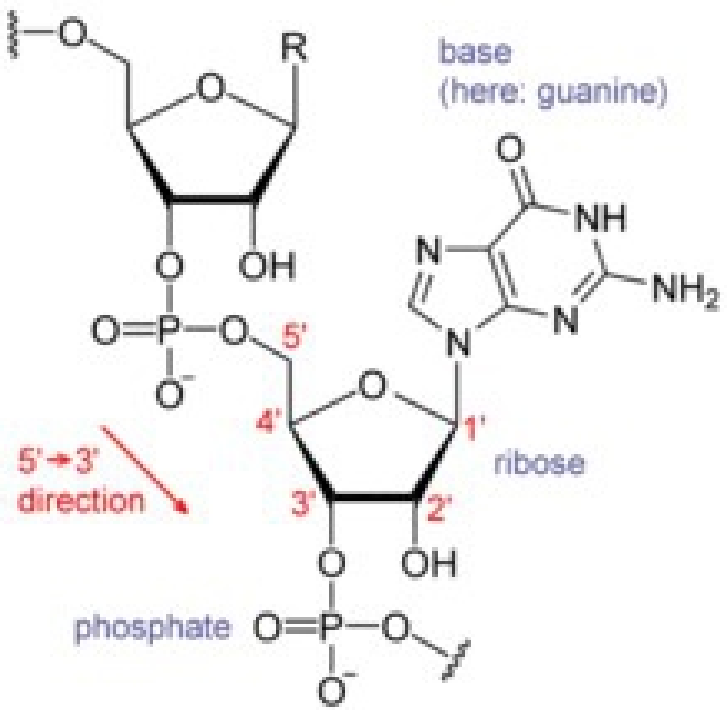

Fig. 4 Structure of a fragment of an **RNA**, showing a guanosyl subunit.

An important structural component of RNA that distinguishes it from DNA is the presence of a hydroxyl group at the 2' position of the ribose sugar. The presence of this functional group causes the helix to mostly take the A-form geometry, although in single strand dinucleotide contexts, RNA can rarely also adopt the B-form most commonly observed in DNA. The A-form geometry results in a very deep and narrow major groove and a shallow and wide minor groove. A second consequence of the presence of the 2'-hydroxyl group is that in conformationally flexible regions of an RNA molecule (that is, not involved

in formation of a double helix), it can chemically attack the adjacent phosphodiester bond to cleave the backbone.

RNA is transcribed with only four bases (adenine, cytosine, guanine and uracil), but these bases and attached sugars can be modified in numerous ways as the RNAs mature. Pseudouridine (Ψ), in which the linkage between uracil and ribose is changed from a C–N bond to a C–C bond, and ribothymidine (T) are found in various places (the most notable ones being in the TΨC loop of tRNA). Another notable modified base is hypoxanthine, a deaminated adenine base whose nucleoside is called inosine (I). Inosine plays a key role in the wobble hypothesis of the genetic code.

There are more than 100 other naturally occurring modified nucleosides. The greatest structural diversity of modifications can be found in tRNA, while pseudouridine and nucleosides with 2'-O-methylribose often present in rRNA are the most common. The specific roles of many of these modifications in RNA are not fully understood. However, it is notable that, in ribosomal RNA, many of the post-transcriptional modifications occur in highly functional regions, such as the peptidyl transferase center and the subunit interface, implying that they are important for normal function.

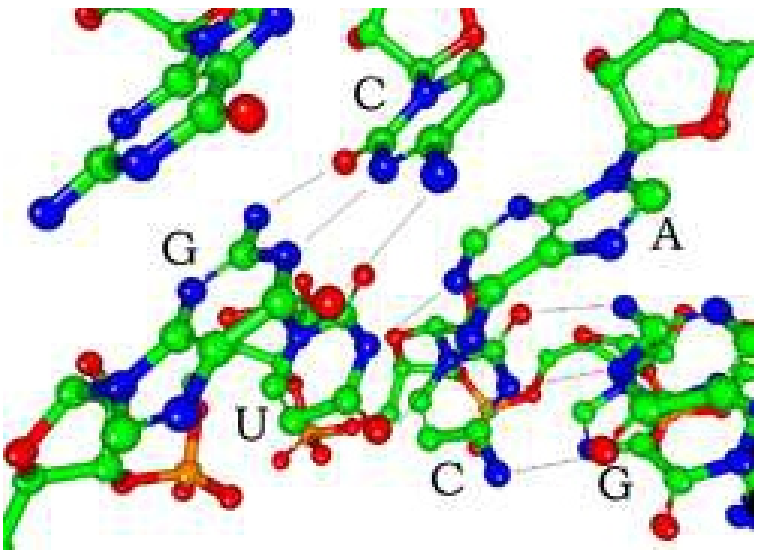

Fig. 5 Watson-Crick base pairs in a siRNA (hydrogen atoms are not shown)

The functional form of single-stranded RNA molecules, just like proteins, frequently requires a specific tertiary structure. The scaffold for this structure is

provided by secondary structural elements that are hydrogen bonds within the molecule. This leads to several recognizable domains of secondary structure like hairpin loops, bulges, and internal loops. In order create, i.e., design, a RNA for any given secondary structure, two or three bases would not be enough, but four bases are enough. This is likely why nature has chosen a four base alphabet: less than four does not allow creating all structures, while more than four bases are not necessary. Since RNA is charged, metal ions such as Mg^{2+} are needed to stabilize many secondary and tertiary structures.

SAQ 2

Q. 1 Define DNA profiling.

Q. 2 Define DNA nanotechnology.

Q. 3 Define genetic engineering.

Q. 4 Define types of RNA.

1.11 Synthesis

Synthesis of RNA is usually catalyzed by an enzyme-RNA polymerase-using DNA as a template, a process known as transcription. Initiation of transcription begins with the binding of the enzyme to a promoter sequence in the DNA (usually found upstream of a gene). The DNA double helix is unwound by the helicase activity of the enzyme. The enzyme then progresses along the template strand in the 3’ to 5’ direction, synthesizing a complementary RNA molecule with elongation occurring in the 5’ to 3’ direction. The DNA sequence also dictates where termination of RNA synthesis will occur.

Primary transcript RNAs are often modified by enzymes after transcription. For example, a poly (A) tail and a 5' cap are added to eukaryotic pre-mRNA and introns are removed by the spliceosome. There are also a number of RNA-dependent RNA polymerases that use RNA as their template for synthesis of a new strand of RNA. For instance, a number of RNA viruses (such as poliovirus) use this type of enzyme to replicate their genetic material. Also, RNA-dependent RNA polymerase is part of the RNA interference pathway in many organisms.

1.12 Types of RNA

Messenger RNA (mRNA) is the RNA that carries information from DNA to the ribosome, the sites of protein synthesis (translation) in the cell. The mRNA is a copy of DNA. The coding sequence of the mRNA determines the amino acid sequence in the protein that is produced. However, many RNAs do not code for protein (about 97% of the transcriptional output is non-protein-coding in eukaryotes. These so-called non-coding RNAs (ncRNA) can be encoded by their own genes (RNA genes), but can also derive from mRNA introns.

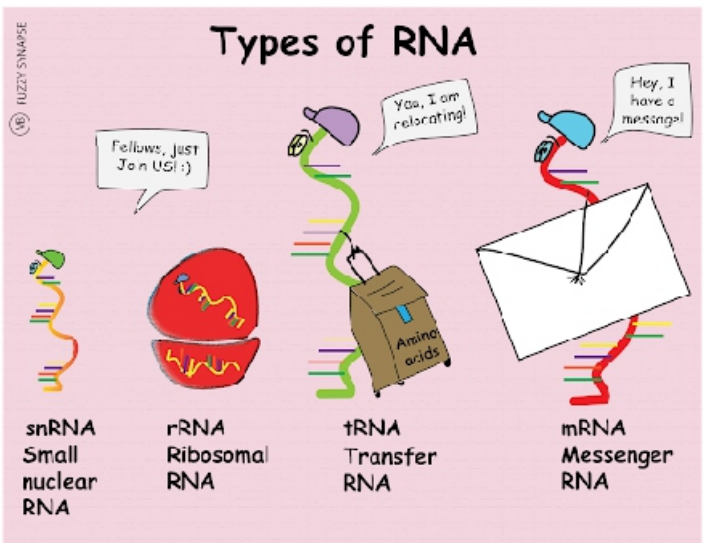

Fig. 6 Different types of RNA

The most prominent examples of non-coding RNAs are transfer RNA (tRNA) and ribosomal RNA (rRNA), both of which are involved in the process of translation. There are also non-coding RNAs involved in gene regulation, RNA processing and other roles. Certain RNAs are able to catalyse chemical reactions such as cutting and ligating other RNA molecules, and the catalysis of peptide bond formation in the ribosome; these are known as ribozymes.

1.13 Summary

The discovery of the double-helix structure of DNA 60 years ago led to a revolution in biological science, opening the floodgates for myriad subsequent discoveries and spawning new fields of research. Bio-Rad has been there from

the beginning, helping scientists, educators, and clinicians advance basic research and improve healthcare. As we celebrate Bio-Rad's diamond anniversary, we reflect on the major events in the evolution of life science research, from biochemistry to molecular biology and beyond, and the emergence of modern biotechnology. Although the origins of biotechnology arguably lie in prehistoric times with the advent of agriculture and fermentation, and the term "molecular biology" was originally coined in the late 1930s, the discoveries of the past six decades laid the foundations of modern molecular biology and biotechnology, giving rise to new technologies and industries that have changed the world in numerous ways, with applications in medicine, agriculture, and other fields.

Under this unit we have emphasized on RNA. The research on RNA has led to many important biological discoveries and numerous Nobel Prizes. Nucleic acids were discovered in 1868 by Friedrich Miescher, who called the material 'nuclein' since it was found in the nucleus. It was later discovered that prokaryotic cells, which do not have a nucleus, also contain nucleic acids. The role of RNA in protein synthesis was suspected already in 1939. Severo Ochoa won the 1959 Nobel Prize in Medicine (shared with Arthur Kornberg) after he discovered an enzyme that can synthesize RNA in the laboratory. However, the enzyme discovered by Ochoa (polynucleotide phosphorylase) was later shown to be responsible for RNA degradation, not RNA synthesis. In 1956 Alex Rich and David Davies hybridized two separate strands of RNA to form the first crystal of RNA whose structure could be determined by X-ray crystallography.

1.14 Terminal questions

Q.1. What do you mean by nucleic acids?

Answer:----------

Q.2. Distinguish between DNA and RNA.

Answer:----------

Q.3. Write a short note on DNA.

Answer:----------

Q.4. Describe double helical structure of DNA.

Answer:----------

Q.5. Describe RNA with its types.

Answer:----------

Q.6. Write a short note on RNA.

Answer:----------

Q.7. Explain DNA and RNA as a genetic material in brief.

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Text book of Botany – Singh -Pande-Jain.
3. The elements of Botany- James Hewetson Wilson
4. Textbook of Biotechnology –H. K. Das
5. Biochemistry and molecular biology- Wilson Walker.

Unit 2 Structure of DNA, Replication and Types of RNA

Structure

2.1 Introduction

Objectives:

2.2 Structure of DNA

2.3 Nucleobase classification

2.3.1 Non-canonical bases

2.3.2 Grooves

2.3.3 Base pairing

2.4 Biological functions

2.4.1 DNA replication

2.4.2 DNA Vs RNA

2.4.3 Types of replication

2.4.3.1 Semi-conservative replication

2.4.3.2 Conservative replication

2.4.3.3 Dispersive replication

2.5 Ribonucleic acid (RNA)

2.5.1 Types of RNA

2.5.1.1 Messenger RNA (mRNA)

2.5.1.2 Transfer RNA (tRNA)

2.5.1.3 Ribosomal RNA (rRNA)

2.5.1.4 Functions of RNA

2.5.1.5 Small Nuclear RNA

2.5.1.6 Micro RNA

2.5.1.7 Small Interfering RNA

2.5.1.8 Clinical Significance

2.6 Genetic Code

2.6.1 Properties of genetic codes

2.7 Summary

2.8 Terminal questions

Further readings

2.1 Introduction

DNA, or deoxyribonucleic acid, is the central information storage system of most animals and plants, and even some viruses. The name comes from its structure, which is a sugar and phosphate backbone which have bases sticking out from it--so-called bases. So that "deoxyribo" refers to the sugar and the nucleic acid refers to the phosphate and the bases. The bases go by the names of adenine, cytosine, thymine, and guanine, otherwise known as A, C, T, and G. DNA is a remarkably simple structure. It's a polymer of four bases--A, C, T, and G--but it allows enormous complexity to be encoded by the pattern of those bases, one after another.

DNA is the chemical name for the molecule that carries genetic instructions in all living things. The DNA molecule consists of two strands that wind around one another to form a shape known as a double helix. Each strand has a backbone made of alternating sugar (deoxyribose) and phosphate groups. Attached to each sugar is one of four bases--adenine (A), cytosine (C), guanine (G), and thymine (T). The two strands are held together by bonds between the bases; adenine bonds with thymine, and cytosine bonds with guanine. The sequence of the bases along the backbones serves as instructions for assembling protein and RNA molecules.

DNA is organized structurally into chromosomes and then wound around nucleosomes as part of those chromosomes. Functionally, it's organized into genes, of which are pieces of DNA, which lead to observable traits. And those traits come not from the DNA itself, but actually from the RNA that is made from the DNA, or most commonly of proteins that are made from the RNA which is made from the DNA. So the central dogma, so-called of molecular biology, is that genes, which are made of DNA, are made into messenger RNAs, which are then made into proteins. But for the most part, the observable traits of eye color or height or one thing or another of individuals come from individual proteins. Sometimes, we're learning in the last few years, actually, they come from RNAs themselves without being made into proteins--things like micro RNAs. But those still are relatively the exception for accounting for traits.

Objectives:

This is the second unit of first block on molecular genetics I**.** Under second unit, we have following objectives. These are as under:

- To know the structures of nucleic acids
- To discuss nitrogenous bases, grooves and base pairing
- To know about types of replication
- To know the types of RNA
- To discuss genetic code and its properties

2.2 Structure of DNA

DNA is a long polymer made from repeating units called nucleotides, each of which is usually symbolized by a single letter: either A, T, C, or G. The structure of DNA is dynamic along its length, being capable of coiling into tight loops and other shapes. In all species it is composed of two helical chains, bound to each other by hydrogen bonds. Both chains are coiled around the same axis, and have the same pitch of 34 ångströms (3.4 nm). The pair of chains have a radius of 10 Å (1.0 nm). According to another study, when measured in a different solution, the DNA chain measured 22–26 Å (2.2–2.6 nm) wide, and one nucleotide unit measured 3.3 Å (0.33 nm) long. Although each individual nucleotide is very small, a DNA polymer can be very large and may contain hundreds of millions of nucleotides, such as in chromosome 1. Chromosome 1 is the largest human chromosome with approximately 220 million base pairs, and would be 85 mm long if straightened.

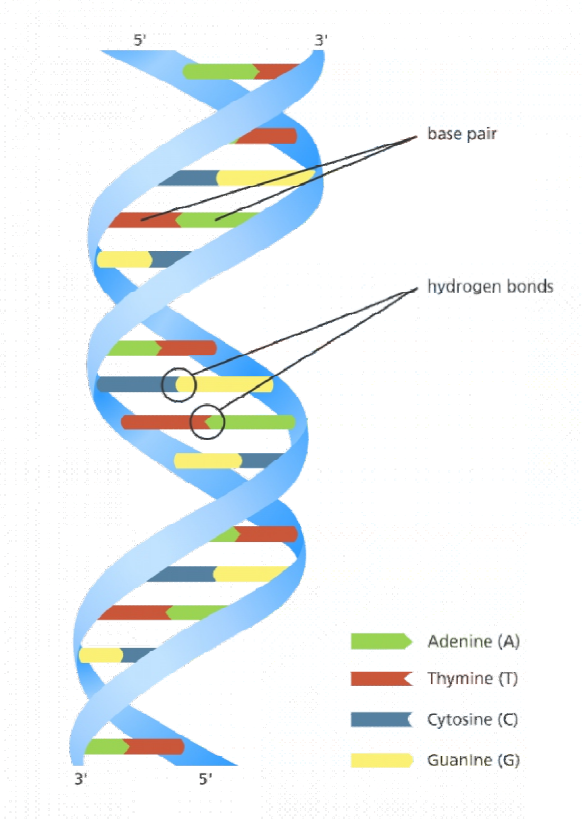

Fig. 1 DNA Structure

DNA does not usually exist as a single strand, but instead as a pair of strands that are held tightly together. These two long strands coil around each other, in the shape of a double helix. The nucleotide contains both a segment of the backbone of the molecule (which holds the chain together) and a nucleobase (which interacts with the other DNA strand in the helix). A nucleobase linked to a sugar is called a nucleoside, and a base linked to a sugar and to one or more phosphate groups is called a nucleotide. A biopolymer comprising multiple linked nucleotides (as in DNA) is called a polynucleotide.

The backbone of the DNA strand is made from alternating phosphate and sugar groups. The sugar in DNA is 2-deoxyribose, which is a pentose (five-carbon) sugar. The sugars are joined by phosphate groups that form phosphodiester bonds between the third and fifth carbon atoms of adjacent sugar rings. These are known as the 3′-end (three prime end), and 5′-end (five prime end) carbons, the prime symbol being used to distinguish these carbon atoms from those of the base to which the

deoxyribose forms a glycosidic bond. Therefore, any DNA strand normally has one end at which there is a phosphate group attached to the 5′ carbon of a ribose (the 5′ phosphoryl) and another end at which there is a free hydroxyl group attached to the 3′ carbon of a ribose (the 3′ hydroxyl).

The orientation of the 3′ and 5′ carbons along the sugar-phosphate backbone confers directionality (sometimes called polarity) to each DNA strand. In a nucleic acid double helix, the direction of the nucleotides in one strand is opposite to their direction in the other strand: the strands are antiparallel. The asymmetric ends of DNA strands are said to have a directionality of five prime end (5′), and three prime end (3′), with the 5′ end having a terminal phosphate group and the 3′ end a terminal hydroxyl group. One major difference between DNA and RNA is the sugar, with the 2-deoxyribose in DNA being replaced by the alternative pentose sugar ribose in RNA.

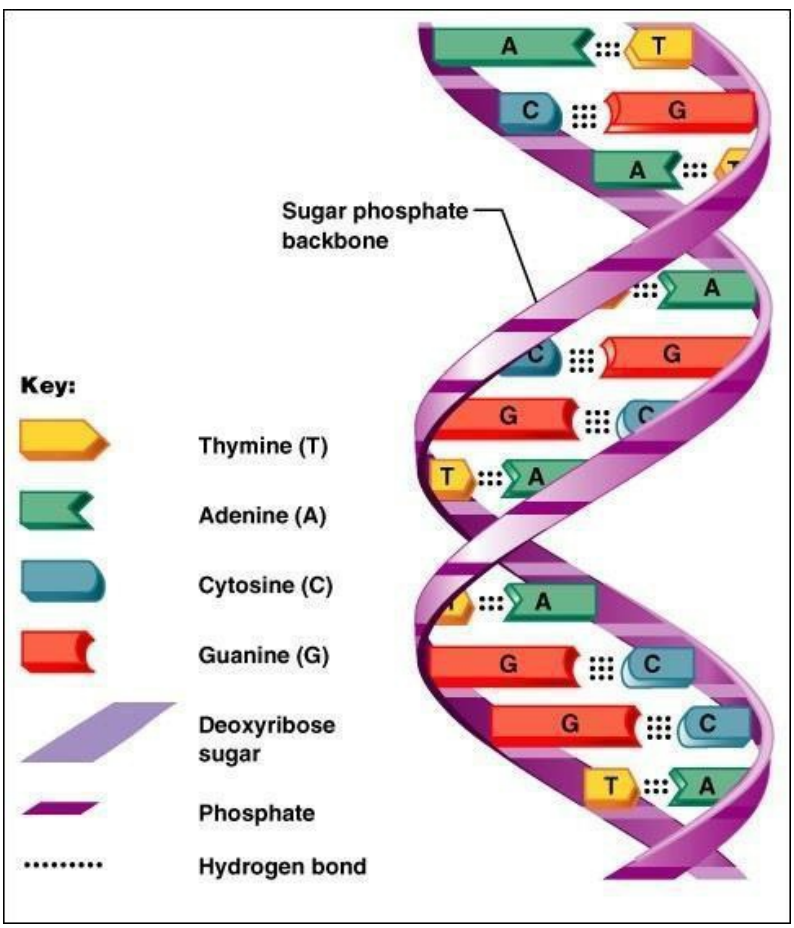

Fig. 2 Double helical structure of DNA

The DNA double helix is stabilized primarily by two forces: hydrogen bonds between nucleotides and base-stacking interactions among aromatic nucleobases. The four bases found in DNA are adenine (A), cytosine (C), guanine (G) and thymine (T). These four bases are attached to the sugar-phosphate to form the complete nucleotide, as shown for adenosine monophosphate. Adenine pairs with thymine and guanine pairs with cytosine, forming A-T and G-C base pairs.

2.3 Nucleobase classification

The nucleobases are classified into two types: the purines, A and G, which are fused five- and six-membered heterocyclic compounds, and the pyrimidines, the six-membered rings C and T. A fifth pyrimidine nucleobase, uracil (U), usually takes the place of thymine in RNA and differs from thymine by lacking a methyl group on its ring. In addition to RNA and DNA, many artificial nucleic acid analogues have been created to study the properties of nucleic acids, or for use in biotechnology.

2.3.1 Non-canonical bases

Modified bases occur in DNA. The first of these recognised was 5-methylcytosine, which was found in the genome of *Mycobacterium tuberculosis* in 1925. The reason for the presence of these noncanonical bases in bacterial viruses (bacteriophages) is to avoid the restriction enzymes present in bacteria. This enzyme system acts at least in part as a molecular immune system protecting bacteria from infection by viruses. Modifications of the bases cytosine and adenine, the more common and modified DNA bases, plays vital roles in the epigenetic control of gene expression in plants and animals.

2.3.2 Grooves

Twin helical strands form the DNA backbone. Another double helix may be found tracing the spaces, or grooves, between the strands. These voids are adjacent to the base pairs and may provide a binding site. As the strands are not symmetrically located with respect to each other, the grooves are unequally sized. One groove, the major groove, is 22 ångströms (2.2 nm) wide and the other, the minor groove, is 12 Å (1.2 nm) wide. The width of the major groove means that the edges of the bases are more accessible in the major groove than

in the minor groove. As a result, proteins such as transcription factors that can bind to specific sequences in double-stranded DNA usually make contact with the sides of the bases exposed in the major groove. This situation varies in unusual conformations of DNA within the cell, but the major and minor grooves are always named to reflect the differences in size that would be seen if the DNA is twisted back into the ordinary B form.

2.3.3 Base pairing

In a DNA double helix, each type of nucleobase on one strand bonds with just one type of nucleobase on the other strand. This is called complementary base pairing. Purines form hydrogen bonds to pyrimidines, with adenine bonding only to thymine in two hydrogen bonds, and cytosine bonding only to guanine in three hydrogen bonds. This arrangement of two nucleotides binding together across the double helix (from six-carbon ring to six-carbon ring) is called a Watson-Crick base pair. DNA with high GC-content is more stable than DNA with low GC-content. A Hoogsteen base pair (hydrogen bonding the 6-carbon ring to the 5-carbon ring) is a rare variation of base-pairing. As hydrogen bonds are not covalent, they can be broken and rejoined relatively easily. The two strands of DNA in a double helix can thus be pulled apart like a zipper, either by a mechanical force or high temperature. As a result of this base pair complementarity, all the information in the double-stranded sequence of a DNA helix is duplicated on each strand, which is vital in DNA replication. This reversible and specific interaction between complementary base pairs is critical for all the functions of DNA in organisms.

SAQ 1

Q. 1 Define DNA.

Q. 2 Define RNA.

Q. 3 What do you mean by base pairing?

Q. 4 Define semi-conservative replication.

2.4 Biological functions

DNA usually occurs as linear chromosomes in eukaryotes, and circular chromosomes in prokaryotes. The set of chromosomes in a cell makes up its genome; the human genome has approximately 3 billion base pairs of DNA

arranged into 46 chromosomes. The information carried by DNA is held in the sequence of pieces of DNA called genes. Transmission of genetic information in genes is achieved via complementary base pairing. For example, in transcription, when a cell uses the information in a gene, the DNA sequence is copied into a complementary RNA sequence through the attraction between the DNA and the correct RNA nucleotides. Usually, this RNA copy is then used to make a matching protein sequence in a process called translation, which depends on the same interaction between RNA nucleotides. In alternative fashion, a cell may simply copy its genetic information in a process called DNA replication.

2.4.1 DNA replication

In molecular biology, DNA replication is the biological process of producing two identical replicas of DNA from one original DNA molecule. DNA replication occurs in all living organisms acting as the most essential part for biological inheritance. This is essential for cell division during growth and repair of damaged tissues, while it also ensures that each of the new cells receives its own copy of the DNA. The cell possesses the distinctive property of division, which makes replication of DNA essential.

DNA is made up of a double helix of two complementary strands. The double helix describes the appearance of a double-stranded DNA which is thus composed of two linear strands that run opposite to each other and twist together to form. During replication, these strands are separated. Each strand of the original DNA molecule then serves as a template for the production of its counterpart, a process referred to as semiconservative replication. As a result of semi-conservative replication, the new helix will be composed of an original DNA strand as well as a newly synthesized strand. Cellular proofreading and error-checking mechanisms ensure near perfect fidelity for DNA replication.

In a cell, DNA replication begins at specific locations, or origins of replication, in the genome which contains the genetic material of an organism. Unwinding of DNA at the origin and synthesis of new strands, accommodated by an enzyme known as helicase, results in replication forks growing bi-directionally from the origin. A number of proteins are associated with the replication fork to help in the initiation and continuation of DNA synthesis.

Most prominently, DNA polymerase synthesizes the new strands by adding nucleotides that complement each (template) strand. DNA replication occurs during the S-stage of interphase.

DNA replication (DNA amplification) can also be performed *in vitro* (artificially, outside a cell). DNA polymerases isolated from cells and artificial DNA primers can be used to start DNA synthesis at known sequences in a template DNA molecule. Polymerase chain reaction (PCR), ligase chain reaction (LCR), and transcription-mediated amplification (TMA) are examples. In March 2021, researchers reported evidence suggesting that a preliminary form of transfer RNA, a necessary component of translation, the biological synthesis of new proteins in accordance with the genetic code, could have been a replicator molecule itself in the very early development of life, or abiogenesis.

DNA replication is the process by which DNA makes a copy of itself during cell division.

1. The first step in DNA replication is to unzip the double helix structure of the DNA molecule.
2. This is carried out by an enzyme called helicase which breaks the hydrogen bonds holding the complementary bases of DNA together (A with T, C with G).
3. The separation of the two single strands of DNA creates a 'Y' shape called a replication 'fork'. The two separated strands will act as templates for making the new strands of DNA.
4. One of the strands is oriented in the 3' to 5' direction (towards the replication fork), this is the leading strand. The other strand is oriented in the 5' to 3' direction (away from the replication fork), this is the lagging strand. As a result of their different orientations, the two strands are replicated differently:

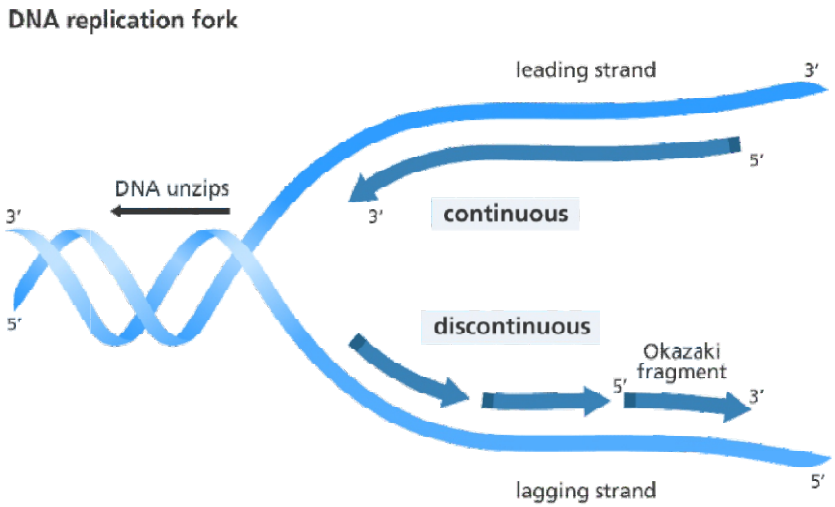

Fig. 3 An illustration to show replication of the leading and lagging strands of DNA

S. No.	Leading Strand	Lagging Strand
5.	A short piece of RNA called a primer (produced by an enzyme called primase) comes along and binds to the end of the leading strand. The primer acts as the starting point for DNA synthesis.	Numerous RNA primers are made by the primase enzyme and bind at various points along the lagging strand.
6.	DNA polymerase binds to the leading strand and then walks along it, adding new complementary nucleotide bases (A, C, G and T) to the strand of DNA in the 5' to 3' direction.	Chunks of DNA, called Okazaki fragments, are then added to the lagging strand also in the 5' to 3' direction.
7.	This sort of replication is called continuous.	This type of replication is called discontinuous as the Okazaki fragments will need to be joined up later.

2.4.2 DNA Vs RNA

DNA stands for deoxyribonucleic acid, while RNA is ribonucleic acid. Although DNA and RNA both carry genetic information, there are quite a few differences between them. This is a comparison of the differences between DNA versus RNA, including a quick summary and a detailed table of the differences.

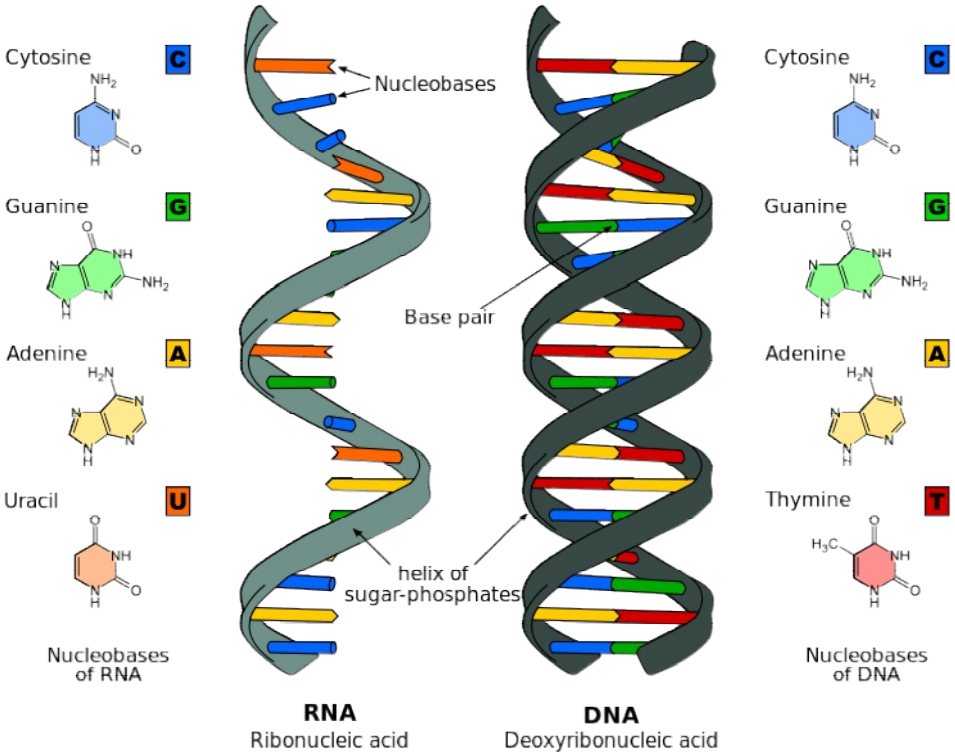

Fig. 4 Differences between DNA & RNA

Some important differences between DNA and RNA are as under:

1. DNA contains the sugar deoxyribose, while RNA contains the sugar ribose. The only difference between ribose and deoxyribose is that ribose has one more -OH group than deoxyribose, which has -H attached to the second (2') carbon in the ring.
2. DNA is a double-stranded molecule, while RNA is a single-stranded molecule.
3. DNA is stable under alkaline conditions, while RNA is not stable.
4. DNA and RNA perform different functions in humans. DNA is responsible for storing and transferring genetic information, while RNA directly codes for amino acids and acts as a messenger between DNA and ribosomes to make proteins.
5. DNA and RNA base pairing is slightly different since DNA uses the bases adenine, thymine, cytosine, and guanine; RNA uses adenine, uracil, cytosine, and guanine. Uracil differs from thymine in that it lacks a methyl group on its ring.

2.4.3 Types of replication

There were three basic models for DNA replication that had been proposed by the scientific community after the discovery of DNA's structure. These models are illustrated in the diagram below:

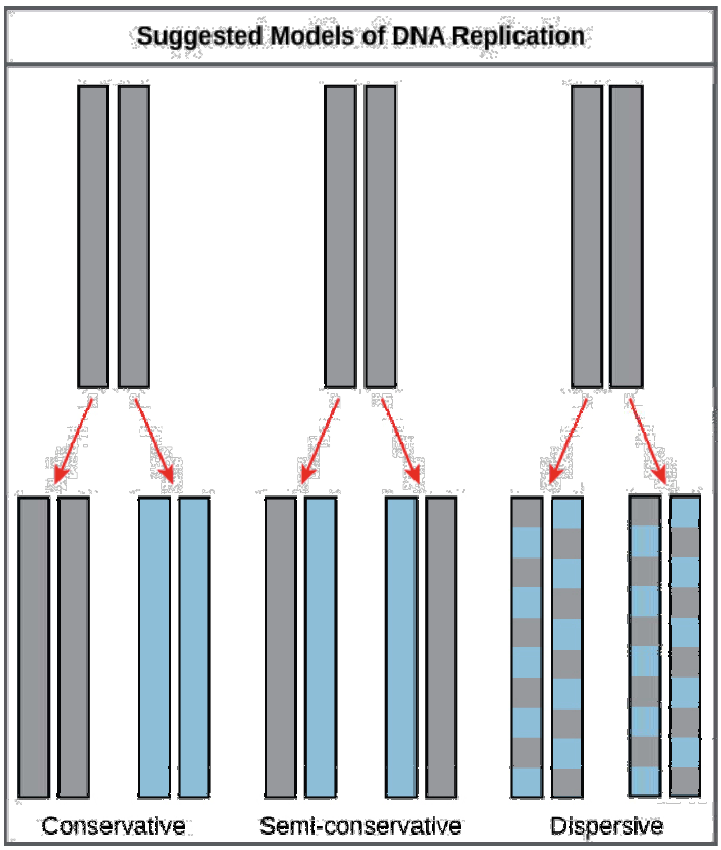

Fig. 5 Basics of DNA replication

2.4.3.1 Semi-conservative replication

In this model, the two strands of DNA unwind from each other, and each acts as a template for synthesis of a new, complementary strand. This results in two DNA molecules with one original strand and one new strand.

2.4.3.2 Conservative replication

In this model, DNA replication results in one molecule that consists of both original DNA strands (identical to the original DNA molecule) and another molecule that consists of two new strands (with exactly the same sequences as the original molecule).

2.4.3.3 Dispersive replication

In the dispersive model, DNA replication results in two DNA molecules that are mixtures, or "hybrids," of parental and daughter DNA. In this model, each individual strand is a patchwork of original and new DNA.

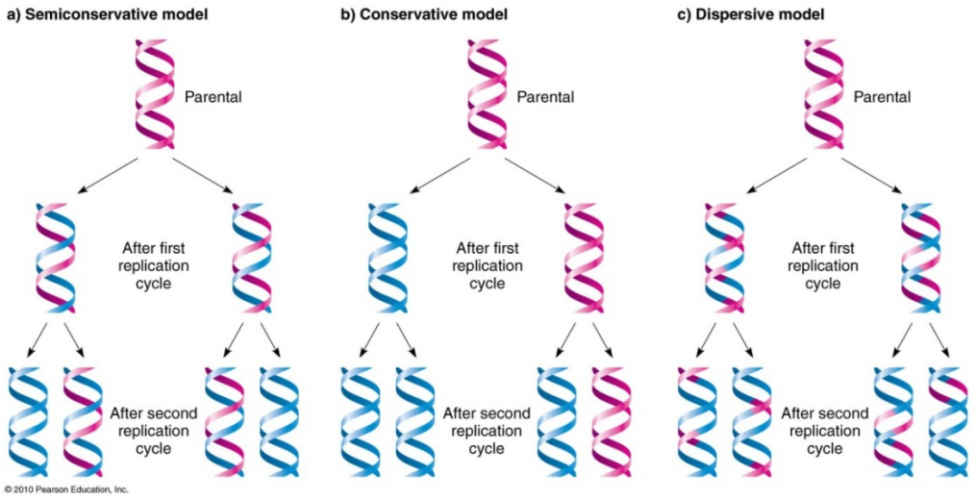

Fig. 6 Semi-Conservative, Conservative, & Dispersive models of DNA replication

Key Points

- There were three models suggested for DNA replication: conservative, semi-conservative, and dispersive.
- The conservative method of replication suggests that parental DNA remains together and newly-formed daughter strands are also together.
- The semi-conservative method of replication suggests that the two parental DNA strands serve as a template for new DNA and after replication, each double-stranded DNA contains one strand from the parental DNA and one new (daughter) strand.
- The dispersive method of replication suggests that, after replication, the two daughter DNAs have alternating segments of both parental and newly-synthesized DNA interspersed on both strands.
- Meselson and Stahl, using *E. coli* DNA made with two nitrogen istopes (^{14}N and ^{15}N) and density gradient centrifugation, determined that DNA replicated via the semi-conservative method of replication.

Key Terms

- **DNA replication**: a biological process occuring in all living organisms that is the basis for biological inheritance.
- **isotope**: any of two or more forms of an element where the atoms have the same number of protons, but a different number of neutrons within their nuclei

2.5 Ribonucleic acid (RNA)

Ribonucleic acid (RNA) is a molecule that is present in the majority of living organisms and viruses. It is made up of nucleotides, which are ribose sugars attached to nitrogenous bases and phosphate groups. The nitrogenous bases include adenine, guanine, uracil, and cytosine. RNA mostly exists in the single-stranded form, but there are special RNA viruses that are double-stranded. The RNA molecule can have a variety of lengths and structures. An RNA virus uses RNA instead of DNA as its genetic material and can cause many human diseases. Transcription is the process of RNA formation from DNA, and translation is the process of protein synthesis from RNA. The means of RNA synthesis and the way that it functions differs between eukaryotes and prokaryotes. Specific RNA molecules also regulate gene expression and have the potential to serve as therapeutic agents in human diseases.

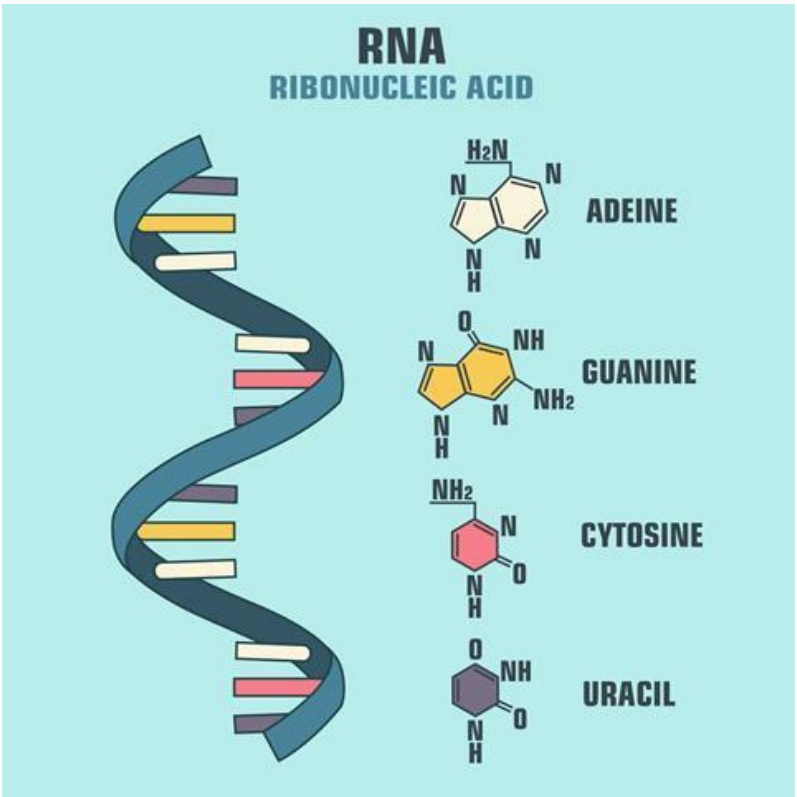

Fig. 7 Structure of RNA

2.5.1 Types of RNA

Three main types of RNA are involved in protein synthesis. They are messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA).

2.5.1.1 Messenger RNA (mRNA)

mRNA is transcribed from DNA and contains the genetic blueprint to make proteins. Prokaryotic mRNA does not need to be processed and can proceed to synthesize proteins immediately. In eukaryotes, a freshly transcribed RNA transcript is considered a pre-mRNA and needs to undergo maturation to form mRNA. A pre-mRNA contains non-coding and coding regions known as introns and exons, respectively. During pre-mRNA processing, the introns are spliced, and the exons are joined together. A 5' cap known as 7-methylguanosine is added to the 5' end of the RNA transcript and the 3' end is polyadenylated. Polyadenylation refers to the process where a poly(A) tail, which is a sequence of adenine nucleotides, is added to the transcript. The 5' cap protects the mRNA from degradation, and the 3' poly (A) tail contributes

to the stability of mRNA and aids it in transport. Researchers are also studying mRNA as an anti-cancer treatment due to its ability to modify cells.

2.5.1.2 Transfer RNA (tRNA)

tRNAs are RNA molecules that translate mRNA into proteins. They have a cloverleaf structure that consists of a 3' acceptor site, 5' terminal phosphate, D arm, T arm, and anticodon arm. The primary function of a tRNA is to carry amino acids on its 3' acceptor site to a ribosome complex with the help of aminoacyl-tRNA synthetase. Aminoacyl-tRNA synthetases are enzymes that load the appropriate amino acid onto a free tRNA to synthesize proteins. Once an amino acid is bound to tRNA, the tRNA is considered an aminoacyl-tRNA. The type of amino acid on a tRNA is dependent on the mRNA codon, which is a sequence of three nucleotides that codes for an amino acid. The anticodon arm of the tRNA is the site of the anticodon, which is complementary to an mRNA codon and dictates which amino acid to carry. tRNAs also regulate apoptosis through acting as a cytochrome c scavenger.

2.5.1.3 Ribosomal RNA (rRNA)

rRNA forms ribosomes, which are essential in protein synthesis. A ribosome contains a large and small ribosomal subunit. In prokaryotes, a small 30S and large 50S ribosomal subunit make up a 70S ribosome. In eukaryotes, the 40S and 60S subunit form an 80S ribosome. The ribosomes contain an exit (E), peptidyl (P), and acceptor (A) site to bind aminoacyl-tRNAs and link amino acids together to create polypeptides.

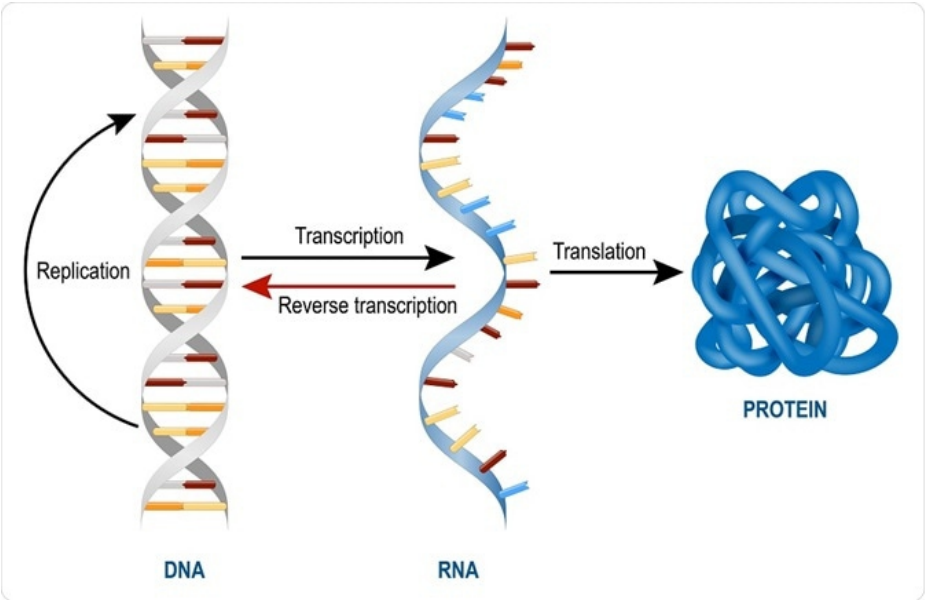

Fig. 8 Types of RNA: mRNA, rRNA and tRNA

2.5.1.4 Functions of RNA

The primary function of RNA is to create proteins via translation. RNA carries genetic information that is translated by ribosomes into various proteins necessary for cellular processes. mRNA, rRNA, and tRNA are the three main types of RNA involved in protein synthesis. RNA also serves as the primary genetic material for viruses. Other functions include RNA editing, gene regulation, and RNA interference. These processes are carried out by a group of small regulatory RNAs, which include small nuclear RNA, microRNA, and small interfering RNA.

2.5.1.5 Small Nuclear RNA

Small nuclear RNAs (snRNA) are non-coding RNAs that are responsible for splicing introns. The snRNAs join with proteins to form small nuclear ribonucleoproteins (snRNP), which most commonly contain U1, U2, U4, U5, and U6 snRNA molecules. Spliceosome assembly and activity begins once U1 of the snRNP binds a complementary sequence on the 5’ splice site of a pre-mRNA transcript. Introns are then removed from the pre-mRNA transcript by the spliceosome complex and mature mRNA forms.

2.5.1.6 Micro RNA

MicroRNAs (miRNA) are non-coding RNAs mainly involved in gene regulation. They are mostly processed from introns and are transcribed into primary miRNA from the host gene by RNA polymerase II. They are then modified by endonucleases, such as Drosha and Dicer into a mature miRNA. Studies have shown that miRNAs that bind to an untranslated region (3'UTR) on mRNAs suppress translation, while miRNA binding to promoter regions can upregulate transcription. miRNAs can also function similarly to hormones. They are released into the extracellular fluid and taken up by target cells for regulation of cellular activity. Additionally, researchers are studying these extracellular miRNAs as ideal biomarkers for various diseases. Research has already shown circulating miRNAs to be involved in cancer through its role in controlling oncogenes and tumor suppressors.

2.5.1.7 Small Interfering RNA

Small Interfering RNAs (siRNA) are double-stranded, non-coding RNAs that inhibit gene expression through RNA interference. They interfere with gene expression by degrading mRNA and preventing the translation of proteins. siRNAs form from long double-stranded RNAs with the assistance of Dicer. Once fully formed, siRNA binds to an RNA induced silencing complex (RISC) and cleaves mRNA through a catalytic RISC protein, Argonaute. Small interfering RNAs have the potential to be therapeutic agents for diseases due to their potency and ability to knock down genes. Unlike miRNAs, siRNAs can specifically target a gene of choice, and a single siRNA guide strand can function multiple times.

2.5.1.8 Clinical Significance

There are a wide variety of RNA viruses, such as coronavirus, rhinovirus, and retrovirus that can cause human diseases. These viruses can be classified as positive or negative sense depending on the polarity and have a naked or enveloped membrane. Coronavirus is most notable for causing severe acute respiratory syndrome. Its transmission is via droplets, and studies have found a higher viral load in the nasal mucosa compared to in the throat. It also has

associations with an increase in inflammatory cytokines, such as IL2, IL7, IL10, and TNFa, and has symptoms of fever, cough, sore throat, and pneumonia.

Another well-known RNA family that can cause a fatal human disease is a retrovirus, a positive sense, enveloped RNA virus consisting of HIV and HTLV. HIV destroys naïve and memory CD4+ T cells and is often symptomless until the last stage when it causes acquired immunodeficiency syndrome. With an impaired immune system, there is an increased risk of developing opportunistic infections and viral-induced cancers. Lastly, rhinovirus is the key viral agent in the common cold. They enter by endocytosis with the help of LDLR and ICAM-1 and produce symptoms of coughing, sore throat, and nasal congestion. Other notable human diseases caused by RNA viruses include hepatitis, West Nile fever, rabies, polio, and measles.

2.6 Genetic Code

Genetic code, the sequence of nucleotides in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) that determines the amino acid sequence of proteins. Though the linear sequence of nucleotides in DNA contains the information for protein sequences, proteins are not made directly from DNA. Instead, a messenger RNA (mRNA) molecule is synthesized from the DNA and directs the formation of the protein. RNA is composed of four nucleotides: adenine (A), guanine (G), cytosine (C),and uracil (U).

The **genetic code** is the set of rules used by living cells to translate information encoded within genetic material (DNA or mRNA sequences of nucleotide triplets, or **codons**) into proteins. Translation is accomplished by the ribosome, which links proteinogenic amino acids in an order specified by messenger RNA (mRNA), using transfer RNA (tRNA) molecules to carry amino acids and to read the mRNA three nucleotides at a time. The genetic code is highly similar among all organisms and can be expressed in a simple table with 64 entries.

The codons specify which amino acid will be added next during protein synthesis. With some exceptions, a three-nucleotide codon in a nucleic acid sequence specifies a single amino acid. The vast majority of genes are encoded with a single scheme. That scheme is often referred to as the canonical or standard genetic code, or simply *the* genetic code, though variant codes (such as in mitochondria) exist.

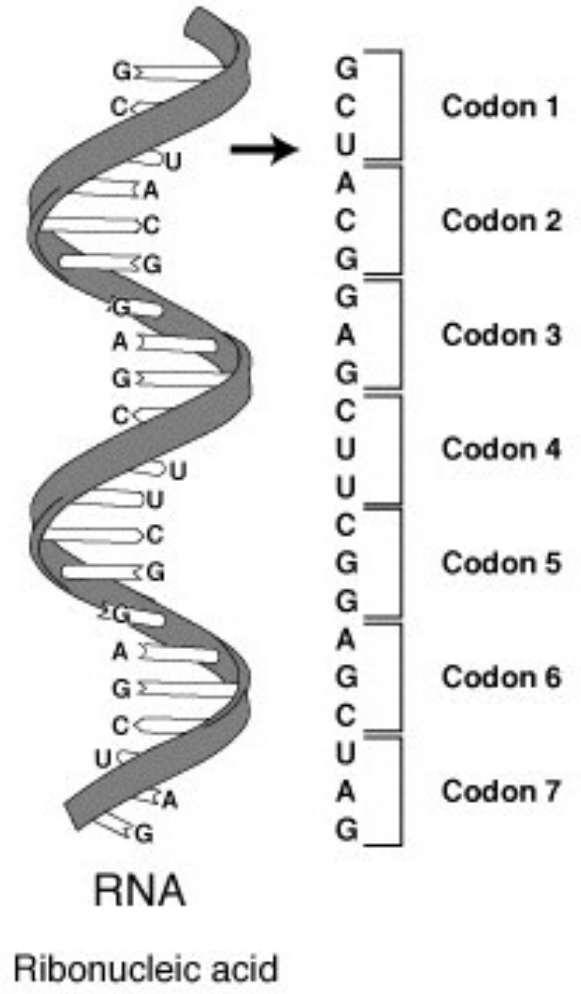

Fig. 9 A series of codons in part of a messenger RNA (mRNA) molecule. Each codon consists of three nucleotides, usually corresponding to a single amino acid.

Three adjacent nucleotides constitute a unit known as the codon, which codes for an amino acid. For example, the sequence AUG is a codon that specifies the amino acid methionine. There are 64 possible codons, three of which do not

code for amino acids but indicate the end of a protein. The remaining 61 codons specify the 20 amino acids that make up proteins. The AUG codon, in addition to coding for methionine, is found at the beginning of every mRNA and indicates the start of a protein. Methionine and tryptophan are the only two amino acids that are coded for by just a single codon (AUG and UGG, respectively). The other 18 amino acids are coded for by two to six codons. Because most of the 20 amino acids are coded for by more than one codon, the code is called degenerate.

The genetic code, once thought to be identical in all forms of life, has been found to diverge slightly in certain organisms and in the mitochondria of some eukaryotes. Nevertheless, these differences are rare, and the genetic code is identical in almost all species, with the same codons specifying the same amino acids. The deciphering of the genetic code was accomplished by American biochemists Marshall W. Nirenberg, Robert W. Holley, and Har Gobind Khorana in the early 1960s.

SAQ 2

Q. 1 Define r-RNA.

Q. 2 Define t-RNA.

Q. 3 What do you mean by genetic code?

Q. 4 Define conservative replication.

2.6.1 Properties of genetic codes

Some of the most important properties of genetic codes are as follows:

1. The code is a triplet codon:

The nucleotides of mRNA are arranged as a linear sequence of codons, each codon consisting of three successive nitrogenous bases, i.e., the code is a triplet codon. The concept of triplet codon has been supported by two types of point mutations: frame shift mutations and base substitutions.

(i) Frameshift mutations:

Evidently, the genetic message once initiated at a fixed point is read in a definite frame in a series of three letter words. The framework would be disturbed as soon as there is a deletion or addition of one or more bases. When such frame shift mutations were intercrossed, then in certain combinations they

produce wild type normal gene. It was concluded that one of them was deletion and the other an addition, so that the disturbed order of the frame due to mutation will be restored by the other

(ii) Base substitution:

If in a mRNA molecule at a particular point, one base pair is replaced by another without any deletion or addition, the meaning of one codon containing such an altered base will be changed. In consequence, in place of a particular amino acid at a particular position in a polypeptide, another amino acid will be incorporated. For example, due to substitution mutation, in the gene for tryptophan synthetase enzyme in E. coli, the GGA codon for glycine becomes a missence codon AGA which codes for arginine. Missence codon is a codon which undergoes an alteration to specify another amino acid.

2. The code is non-overlapping:

In translating mRNA molecules the codons do not overlap but are “read” sequentially. Thus, a non-overlapping code means that a base in a mRNA is not used for different codons. However, in actual practice six bases code for not more than two amino acids. For example, in case of an overlapping code, a single change (of substitution type) in the base sequence will be reflected in substitutions of more than one amino acid in corresponding protein. Many examples have accumulated since 1956 in which a single base substitution results into a single amino acid change in insulin, tryptophan synthelase, TMV coat protein, alkaline phosphatase, haemoglobin, etc.

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substitution results into a single amino acid change in insulin, tryptophan synthelase, TMV coat protein, alkaline phosphatase, haemoglobin, etc.

3. The code is commaless:

The genetic code is commaless, which means that no codon is reserved for punctuations. It means that after one amino acid is coded, the second amino acid will be automatically, coded by the next three letters and that no letters are wasted as the punctuation marks.

4. The code is non-ambiguous:

Non-ambiguous code means that a particular codon will always code for the same amino acid. In case of ambiguous code, the same codon could have different meanings or in other words, the same codon could code two or more than two different amino acids. Generally, as a rule, the same codon shall never code for two different amino acids. However, there are some reported exceptions to this rule: the codons AUG and GUG both may code for methionine as initiating or starting codon, although GUG is meant for valine. Likewise, GGA codon codes for two amino acids glycine and glutamic acid.

5. The code has polarity:

The code is always read in a fixed direction, i.e., in the 5'→3′ direction. In other words, the codon has a polarity. It is apparent that if the code is read in opposite directions, it would specify two different proteins, since the codon would have reversed base sequence:

2.7 Summary

The discovery of DNA stretches back to 1869, when Friedrich Miescher, a Swiss physician and biologist, began examining leucocytes, a type of white blood cell, he had sourced from pus collected on fresh surgical bandages. This he did while working in the laboratory of Felix Hoppe-Seyler in Tubingen, Germany as part of project to determine the chemical building blocks of cells. On looking through the microscope he observed that a substance separated from the solution of the cells whenever he added an acid and then dissolved again once alkali was added. The compound bore no resemblance to any

known protein. Believing the substance to originate from the nuceli of the cell, Miescher nicknamed it 'nuclein'. On investigating further he discovered nuclein to be present in many other tissues. While possessing only simple tools and methods, by 1874 Miescher had come close to working out the genetic role of nuclein. He lacked sufficient communication skills, however, to convey the importance of what he had found to the wider scientific world.

Under this unit we have discussed about the key role of DNA, RNA and genetic code. DNA is central to biotechnology and medicine by virtue of the fact that it not only provides the basic blueprint for all life, it is a fundamental determinant of how the body functions and the disease process. Understanding the structure and function of DNA has helped revolutionise the investigation of disease pathways, assess an individual's genetic susceptibility to specific diseases, diagnose genetic disorders, and formulate new drugs. It is also critical to the identification of pathogens. Aside from its medical uses, the fact that DNA is unique to each individual makes it a vital forensic tool identifying criminals, the remains of a missing person, and determining the biological parent of a child. Within agriculture DNA is also used to help improve animal livestock and plants.

2.8 Terminal questions

Q. 1 What do you mean by nucleic acids?

Answer:----------

Q.2 Distinguish between DNA and RNA.

Answer:----------

Q.3 Write a short note on DNA.

Answer:----------

Q.4 Describe double helical structure of DNA.

Answer:----------

Q.5 Describe RNA with its types.

Answer:----------

Q.6 Write a short note on RNA.

Answer:----------

Q.7 Explain DNA and RNA as a genetic material in brief.

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Text book of Botany – Singh -Pande-Jain.
3. The elements of Botany- James Hewetson Wilson
4. Textbook of Biotechnology –H. K. Das
5. Biochemistry and molecular biology- Wilson Walker

Unit 3 Gene Expression, Transcription and Translation in Eukaryotes and Prokaryotes

Structure

3.1 Introduction

3.2 Structure of gene

3.3 How do genes determine characteristics?

3.4 Gene expression

3.5 How is gene expression regulated?

3.6 Transcription

3.7 Transcription in Eukaryotes

3.8 Enzyme(s) Involved in Eukaryotic Transcription

3.9 Process of Eukaryotic Transcription

3.9.1 Elongation Phase

3.9.2 Termination Phase

3.9.3 Significance

3.10 Transcription in prokaryotes

3.11 RNA polymerase

3.12 Initiation

3.13 Elongation

3.14 Termination

3.15 Translation in Eukaryotes

3.16 Elongation

3.17 Termination

3.18 Translation in prokaryotes

3.19 Clinical significance

3.20 Summary

3.21Terminal questions

Further readings

3.1 Introduction

In biology, a gene meaning generation or birth or gender is a basic unit of heredity and a sequence of nucleotides in DNA that encodes the synthesis of a gene product, either RNA or protein. A gene is the basic physical and functional unit of heredity. Genes are made up of DNA. Some genes act as instructions to make molecules called proteins. However, many genes do not code for proteins. In humans, genes vary in size from a few hundred DNA bases to more than 2 million bases. An international research effort called the Human Genome Project, which worked to determine the sequence of the human genome and identify the genes that it contains, estimated that humans have between 20,000 and 25,000 genes.

Every person has two copies of each gene, one inherited from each parent. Most genes are the same in all people, but a small number of genes (less than 1 percent of the total) are slightly different between people. Alleles are forms of the same gene with small differences in their sequence of DNA bases. These small differences contribute to each person's unique physical features. Genes are made up of DNA. Each chromosome contains many genes.

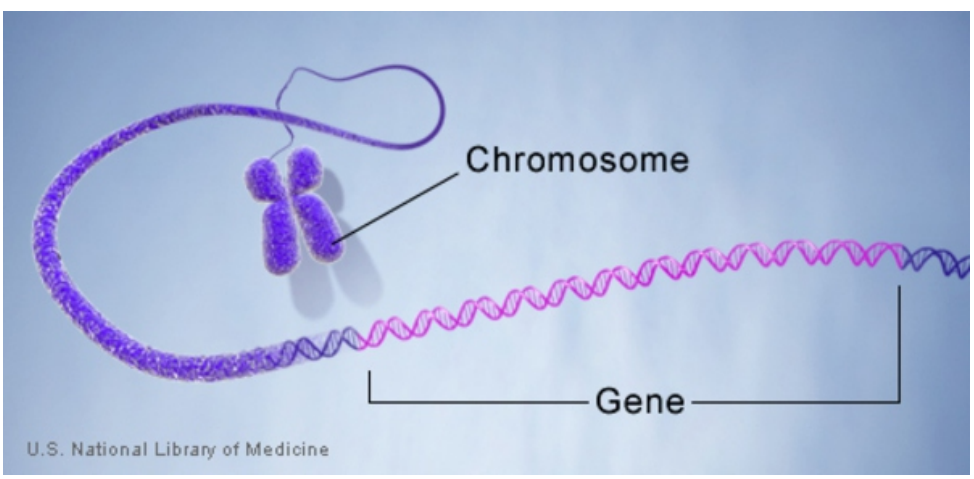

Fig. 1 U.S. National Library of Medicine

During gene expression, the DNA is first copied into RNA. The RNA can be directly functional or be the intermediate template for a protein that performs a function. The transmission of genes to an organism's offspring is the basis of the inheritance of phenotypic traits. These genes make up different

DNA sequences called genotypes. Genotypes along with environmental and developmental factors determine what the phenotypes will be. Most biological traits are under the influence of polygenes (many different genes) as well as gene–environment interactions. Some genetic traits are instantly visible, such as eye color or the number of limbs, and some are not, such as blood type, the risk for specific diseases, or the thousands of basic biochemical processes that constitute life.

Objectives:

This is the third unit of first block on molecular genetics I**.** Under third unit, we have following objectives. These are as under:

- To know the structures of gene
- To discuss gene expression
- To discuss about transcription in eukaryotes
- To discuss about transcription in prokaryotes
- To discuss about translation in prokaryotes.

3.2 Structure of gene

Genes can acquire mutations in their sequence, leading to different variants, known as alleles, in the population. These alleles encode slightly different versions of a protein, which cause different phenotypical traits. Usage of the term "having a gene" (e.g., "good genes," "hair color gene") typically refers to containing a different allele of the same, shared gene. Genes evolve due to natural selection / survival of the fittest and genetic drift of the alleles.

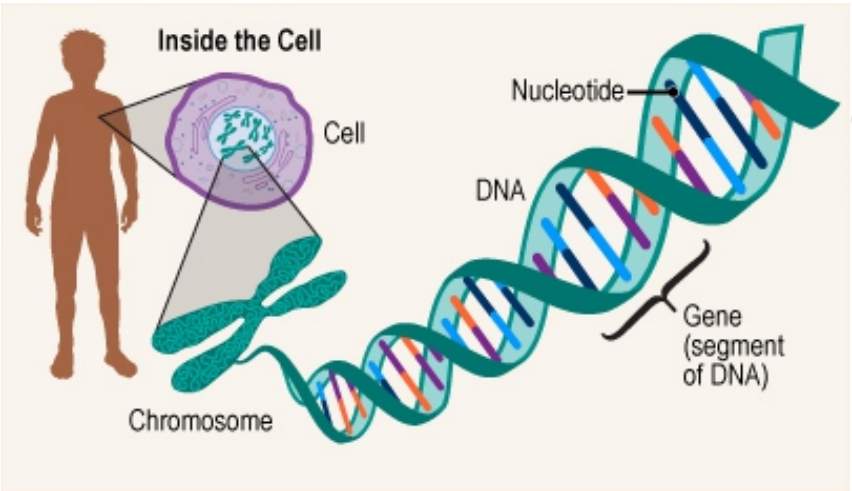

Fig. 2 Structure of gene

The concept of gene continues to be refined as new phenomena are discovered. For example, regulatory regions of a gene can be far removed from its coding regions, and coding regions can be split into several exons. Some viruses store their genome in RNA instead of DNA and some gene products are functional non-coding RNAs. Therefore, a broad, modern working definition of a gene is any discrete locus of heritable, genomic sequence which affect an organism's traits by being expressed as a functional product or by regulation of gene expression.

Advances in understanding genes and inheritance continued throughout the 20th century. Deoxyribonucleic acid (DNA) was shown to be the molecular repository of genetic information by experiments in the 1940s to 1950s. The structure of DNA was studied by Rosalind Franklin and Maurice Wilkins using X-ray crystallography, which led James D. Watson and Francis Crick to publish a model of the double-stranded DNA molecule whose paired nucleotide bases indicated a compelling hypothesis for the mechanism of genetic replication.

In the early 1950s the prevailing view was that the genes in a chromosome acted like discrete entities, indivisible by recombination and arranged like beads on a string. The experiments of Benzer using mutants defective in the rII region of bacteriophage T4 (1955–1959) showed that individual genes have a simple linear structure and are likely to be equivalent to a linear section of DNA. Collectively, this body of research established the central dogma of molecular biology, which states that proteins are translated from RNA, which is transcribed from DNA. This dogma has since been shown to have exceptions, such as reverse transcription in retroviruses. The modern study of genetics at the level of DNA is known as molecular genetics.

In 1972, Walter Fiers and his team were the first to determine the sequence of a gene: that of Bacteriophage MS2 coat protein. The subsequent development of chain-termination DNA sequencing in 1977 by Frederick Sanger improved the efficiency of sequencing and turned it into a routine laboratory tool. An automated version of the Sanger method was used in early phases of the Human Genome Project.

3.3 How do genes determine characteristics?

All individuals in a species have the same set of genes: in peas there is a gene for pod color, a gene for plant height, a gene for pea shape, and so on. What makes individuals different is that a gene can have several different forms, or alleles. Thus, in peas, the pod color gene has green and yellow alleles, the plant height gene has tall and dwarf alleles, and so on. Individuals have two copies of each gene, one inherited from each parent. How the two copies interact with each other determines an organism's characteristics. It doesn't matter whether a gene comes from the maternal or paternal side—a green-pod allele from a sperm cell is the same as a green-pod allele from an egg cell. What matters is how many of which type of allele an individual gets.

Mendel laid the foundation for our understanding of this business. He referred to the form of a trait that was visible in his first generation of hybrids (green pods, for example) as dominant. He called the other form of the trait (yellow pods, for example) recessive. Today, we know that the dominant and recessive forms of a trait are underlaid by dominant and recessive genes or, more precisely, alleles. In pea plants, for example, the green-pod allele is dominant, while the yellow-pod allele is recessive

3.4 Gene expression

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product that enables it to produce end products, protein or non-coding RNA, and ultimately affect a phenotype, as the final effect. These products are often proteins, but in non-protein-coding genes such as transfer RNA (tRNA) and small nuclear RNA (snRNA), the product is a functional non-coding RNA. Gene expression is summarized in the central dogma of molecular biology first formulated by Francis Crick in 1958, further developed in his 1970 article, and expanded by the subsequent discoveries of reverse transcription and RNA replication. The process of gene expression is used by all known life-eukaryotes (including multicellular organisms), prokaryotes (bacteria and archaea), and utilized by viruses-to generate the macromolecular machinery for life.

Genes encode proteins and proteins dictate cell function. Therefore, the thousands of genes expressed in a particular cell determine what that cell can do. Moreover, each step in the flow of information from DNA to RNA to protein provides the cell with a potential control point for self-regulating its functions by adjusting the amount and type of proteins it manufactures.

At any given time, the amount of a particular protein in a cell reflects the balance between that protein's synthetic and degradative biochemical pathways. On the synthetic side of this balance, recall that protein production starts at transcription (DNA to RNA) and continues with translation (RNA to protein). Thus, control of these processes plays a critical role in determining what proteins are present in a cell and in what amounts. In addition, the way in which a cell processes its RNA transcripts and newly made proteins also greatly influences protein levels.

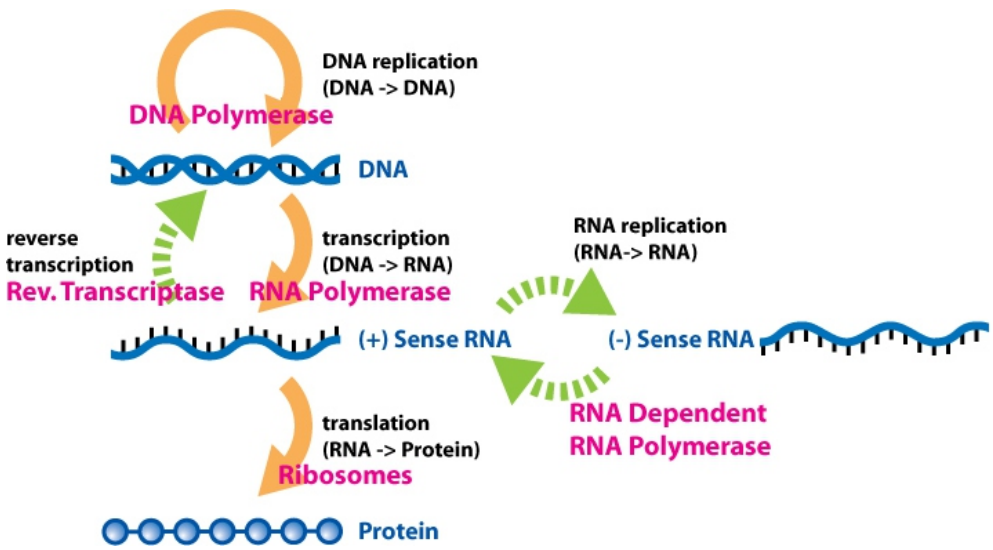

Fig. 3 The extended central dogma of molecular biology includes all the cellular processes involved in the flow of genetic information

In genetics, gene expression is the most fundamental level at which the genotype gives rise to the phenotype, *i.e.* observable trait. The genetic information stored in DNA represents the genotype, whereas the phenotype results from the "interpretation" of that information. Such phenotypes are often expressed by the synthesis of proteins that control the organism's structure and development, or that act as enzymes catalyzing specific metabolic pathways.

All steps in the gene expression process may be modulated (regulated), including the transcription, RNA splicing, translation, and post-translational

modification of a protein. Regulation of gene expression gives control over the timing, location, and amount of a given gene product (protein or ncRNA) present in a cell and can have a profound effect on the cellular structure and function. Regulation of gene expression is the basis for cellular differentiation, development, morphogenesis and the versatility and adaptability of any organism. Gene regulation may therefore serve as a substrate for evolutionary change.

3.5 How is gene expression regulated?

The amounts and types of mRNA molecules in a cell reflect the function of that cell. In fact, thousands of transcripts are produced every second in every cell. Given this statistic, it is not surprising that the primary control point for gene expression is usually at the very beginning of the protein production process- the initiation of transcription. RNA transcription makes an efficient control point because many proteins can be made from a single mRNA molecule. Transcript processing provides an additional level of regulation for eukaryotes, and the presence of a nucleus makes this possible. In prokaryotes, translation of a transcript begins before the transcript is complete, due to the proximity of ribosomes to the new mRNA molecules. In eukaryotes, however, transcripts are modified in the nucleus before they are exported to the cytoplasm for translation.

Eukaryotic transcripts are also more complex than prokaryotic transcripts. For instance, the primary transcripts synthesized by RNA polymerase contain sequences that will not be part of the mature RNA. These intervening sequences are called introns, and they are removed before the mature mRNA leaves the nucleus. The remaining regions of the transcript, which include the protein-coding regions, are called exons, and they are spliced together to produce the mature mRNA. Eukaryotic transcripts are also modified at their ends, which affects their stability and translation.

3.6 Transcription

Transcription is the process of copying a segment of DNA into RNA. The segments of DNA transcribed into RNA molecules that can encode proteins are

said to produce messenger RNA (mRNA). Other segments of DNA are copied into RNA molecules called non-coding RNAs (ncRNAs). Averaged over multiple cell types in a given tissue, the quantity of mRNA is more than 10 times the quantity of ncRNA (though in particular single cell types ncRNAs may exceed mRNAs).

The general preponderance of mRNA in cells is valid even though less than 2% of the human genome can be transcribed into mRNA (Human genome Coding vs. noncoding DNA), while at least 80% of mammalian genomic DNA can be actively transcribed (in one or more types of cells), with the majority of this 80% considered to be ncRNA. Both DNA and RNA are nucleic acids, which use base pairs of nucleotides as a complementary language. During transcription, a DNA sequence is read by an RNA polymerase, which produces a complementary, antiparallel RNA strand called a primary transcript.

- ✓ Transcription proceeds in the following general steps:
- ✓ RNA polymerase, together with one or more general transcription factors, binds to promoter DNA.
- ✓ RNA polymerase generates a transcription bubble, which separates the two strands of the DNA helix. This is done by breaking the hydrogen bonds between complementary DNA nucleotides.
- ✓ RNA polymerase adds RNA nucleotides (which are complementary to the nucleotides of one DNA strand).
- ✓ RNA sugar-phosphate backbone forms with assistance from RNA polymerase to form an RNA strand.
- ✓ Hydrogen bonds of the RNA–DNA helix break, freeing the newly synthesized RNA strand.
- ✓ If the cell has a nucleus, the RNA may be further processed. This may include polyadenylation, capping, and splicing.
- ✓ The RNA may remain in the nucleus or exit to the cytoplasm through the nuclear pore complex.

If the stretch of DNA is transcribed into an RNA molecule that encodes a protein, the RNA is termed messenger RNA (mRNA); the mRNA, in turn, serves as a template for the protein's synthesis through translation. Other

stretches of DNA may be transcribed into small non-coding RNAs such as micro RNA, transfer RNA (tRNA), small nucleolar RNA (snoRNA), small nuclear RNA (snRNA), or enzymatic RNA molecules called ribozymes as well as larger non-coding RNAs such as ribosomal RNA (rRNA), and long non-coding RNA (lncRNA). Overall, RNA helps synthesize, regulate, and process proteins; it therefore plays a fundamental role in performing functions within a cell.

In virology, the term transcription may also be used when referring to mRNA synthesis from an RNA molecule (i.e., equivalent to RNA replication). For instance, the genome of a negative-sense single-stranded RNA (ssRNA -) virus may be a template for a positive-sense single-stranded RNA (ssRNA +). This is because the positive-sense strand contains the sequence information needed to translate the viral proteins needed for viral replication. This process is catalyzed by a viral RNA replicase.

SAQ 1

Q. 1 Define gene.

Q. 2 Define transcription.

Q. 3 What do you mean by translation?

Q. 4 Define gene expression.

3.7 Transcription in Eukaryotes

Transcription occurs in eukaryotes in a way that is similar to prokaryotes with reference to the basic steps involved. However, some major differences between them include:

- Initiation is more complex.
- Termination does not involve stem-loop structures.
- Transcription is carried out by three enzymes (RNA polymerases I, II and III).
- The regulation of transcription is more extensive than prokaryotes.

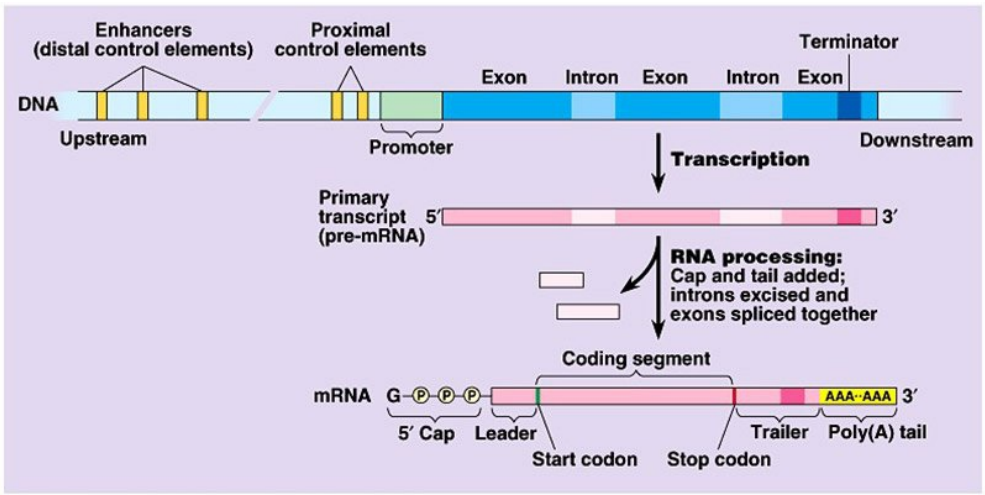

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Fig. 4 Transcription

3.8 Enzyme(s) Involved in Eukaryotic Transcription

Unlike prokaryotes where all RNA is synthesized by a single RNA polymerase, the nucleus of a eukaryotic cell has three RNA polymerases responsible for transcribing different types of RNA.

- RNA polymerase I (RNA Pol I) is located in the nucleolus and transcribes the 28S, 18S, and 5.8S rRNA genes.
- RNA polymerase II (RNA Pol II) is located in the nucleoplasm and transcribes protein-coding genes, to yield pre-mRNA, and also the genes encoding small nucleolar RNAs (snoRNAs) involved in rRNA processing and small nuclear RNAs (snRNAs) involved in mRNA processing, except for U6 snRNA.
- RNA polymerase III (RNA Pol III) is also located in the nucleoplasm. It transcribes the genes for tRNA, 5S rRNA, U6 snRNA, and the 7S RNA associated with the signal recognition particle (SRP) involved in the translocation of proteins across the endoplasmic reticulum membrane.
- Each of the three eukaryotic RNA polymerases contains 12 or more subunits and so these are large complex enzymes.
- The genes encoding some of the subunits of each eukaryotic enzyme show DNA sequence similarities to genes encoding subunits of the core enzyme of *E. coli* RNA polymerase.

- However, four to seven other subunits of each eukaryotic RNA polymerase are unique in that they show no similarity either with bacterial RNA polymerase subunits or with the subunits of other eukaryotic RNA polymerases.

3.9 Process of Eukaryotic Transcription

The basic mechanism of RNA synthesis by these eukaryotic RNA polymerases can be divided into the following phases:

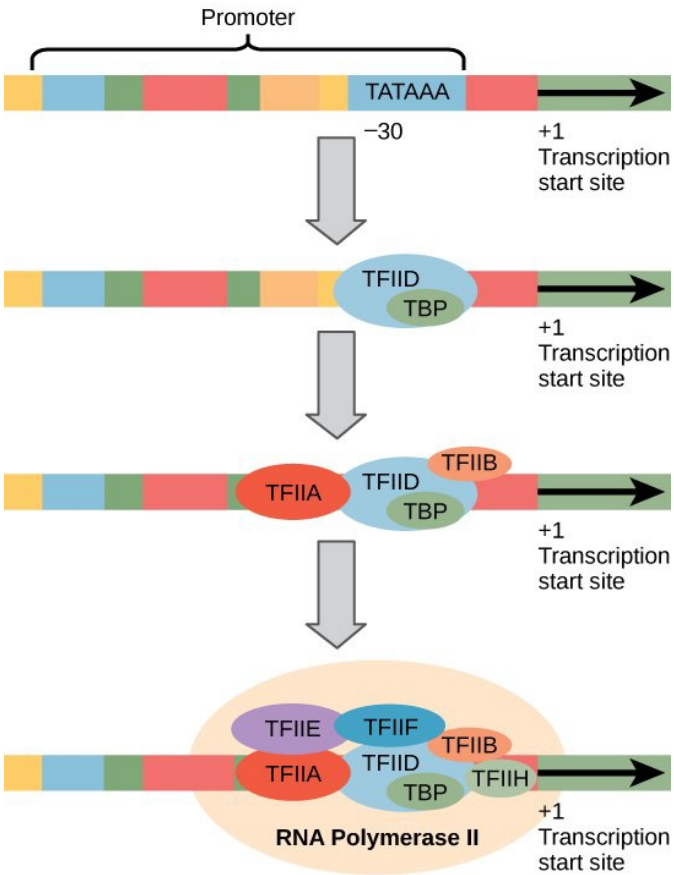

Fig. 5 Transcription

- ✓ During initiation, RNA polymerase recognizes a specific site on the DNA, upstream from the gene that will be transcribed, called a **promoter site** and then unwinds the DNA locally.
- ✓ Most promoter sites for RNA polymerase II include a highly conserved sequence located about 25–35 bp upstream (i.e. to the 5 side) of the start site which has the consensus TATA (A/T)A(A/T) and is called the TATA box.

- ✓ Since the start site is denoted as position +1, the TATA box position is said to be located at about position -25.
- ✓ The TATA box sequence resembles the -10 sequence in prokaryotes (TATAAT) except that it is located further upstream.
- ✓ Both elements have essentially the same function, namely recognition by the RNA polymerase in order to position the enzyme at the correct location to initiate transcription.
- ✓ The sequence around the TATA box is also important in that it influences the efficiency of initiation. Transcription is also regulated by upstream control elements that lie 5′ to the TATA box.
- ✓ Some eukaryotic protein-coding genes lack a TATA box and have an initiator element instead, centered around the transcriptional initiation site.
- ✓ In order to initiate transcription, RNA polymerase II requires the assistance of several other proteins or protein complexes, called general (or basal) transcription factors, which must assemble into a complex on the promoter in order for RNA polymerase to bind and start transcription.

3.9.1 Elongation Phase

It has two functions:

1. It is a helicase, which means that it can use ATP to unwind the DNA helix, allowing transcription to begin.
2. In addition, it phosphorylates RNA polymerase II which causes this enzyme to change its conformation and dissociate from other proteins in the initiation complex.

- The key phosphorylation occurs on a long C-terminal tail called the C-terminal domain (CTD) of the RNA polymerase II molecule.
- Interestingly, only RNA polymerase II that has a non-phosphorylated CTD can initiate transcription but only an RNA polymerase II with a phosphorylated CTD can elongate RNA.
- RNA polymerase II now starts moving along the DNA template, synthesizing RNA, that is, the process enters the elongation phase.

- RNA synthesis occurs in the 5' → 3' direction with the RNA polymerase catalyzing a nucleophilic attack by the 3-OH of the growing RNA chain on the alpha-phosphorus atom on an incoming ribonucleoside 5-triphosphate.
- The RNA molecule made from a protein-coding gene by RNA polymerase II is called a primary transcript.

3.9.2 **Termination Phase**

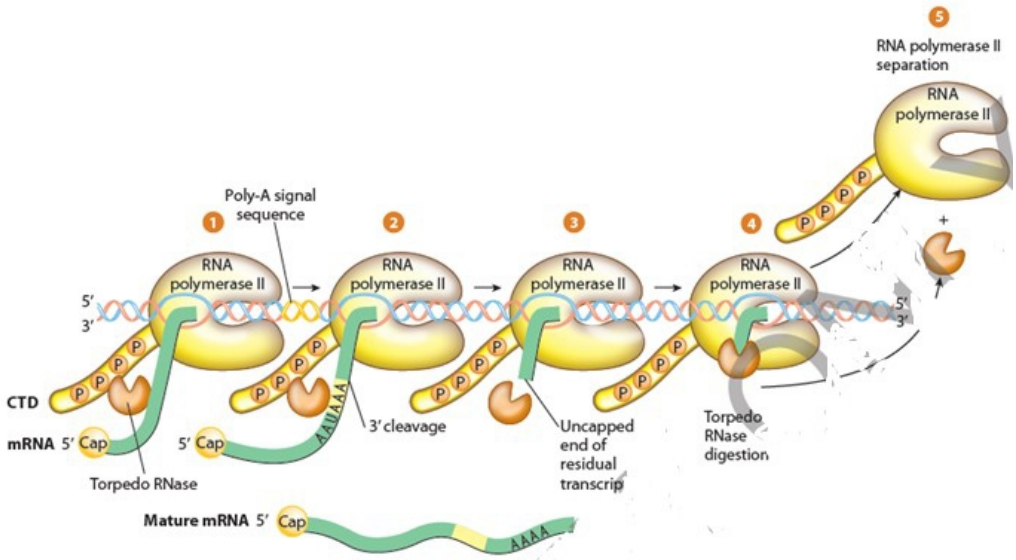

Fig. 6 Termination Phase

- Elongation of the RNA chain continues until termination occurs.
- Unlike RNA polymerase in prokaryotes, RNA polymerase II does not terminate transcription at a specific site but rather transcription can stop at varying distances downstream of the gene.
- RNA genes transcribed by RNA Polymerse II lack any specific signals or sequences that direct RNA Polymerase II to terminate at specific locations.
- RNA Polymerase II can continue to transcribe RNA anywhere from a few bp to thousands of bp past the actual end of the gene.
- The transcript is cleaved at an internal site before RNA Polymerase II finishes transcribing. This releases the upstream portion of the transcript, which will serve as the initial RNA prior to further processing (the pre-mRNA in the case of protein-encoding genes.)

- This cleavage site is considered the “end” of the gene. The remainder of the transcript is digested by a 5′-exonuclease (called Xrn2 in humans) while it is still being transcribed by the RNA Polymerase II.
- When the 5′-exonulease “catches up” to RNA Polymerase II by digesting away all the overhanging RNA, it helps disengage the polymerase from its DNA template strand, finally terminating that round of transcription.

Significance

- Transcription of DNA is the method for regulating gene expression.
- It occurs in preparation for and is necessary for protein translation.

3.10 Transcription in prokaryotes

Both prokaryotes and eukaryotes perform fundamentally the same process of transcription, with the important difference of the membrane-bound nucleus in eukaryotes. With the genes bound in the nucleus, transcription occurs in the nucleus of the cell and the mRNA transcript must be transported to the cytoplasm. In prokaryotes, which lack membrane-bound nuclei and other organelles, transcription occurs in the cytoplasm of the cell.

3.11 RNA polymerase

RNA polymerase is the enzyme that produces the mRNA molecule (just like DNA polymerase produced a new DNA molecule during DNA replication). Prokaryotes use the same RNA polymerase to transcribe all of their genes. In *E. coli*, the polymerase is composed of five polypeptide subunits. These subunits assemble every time a gene is transcribed, and they disassemble once transcription is complete. Each subunit has a unique role (which you do not need to memorize). The polymerase comprised of all five subunits is called the holoenzyme.

3.12 Initiation

Transcription in prokaryotes (and in eukaryotes) requires the DNA double helix to partially unwind in the region of mRNA synthesis. The region of unwinding is called a transcription bubble. The DNA sequence onto which the proteins and enzymes involved in transcription bind to initiate the process is called a promoter. In most cases, promoters exist upstream of the genes they regulate. The specific sequence of a promoter is very important because it determines whether the corresponding gene is transcribed all of the time, some

of the time, or hardly at all. The structure and function of a prokaryotic promoter is relatively simple as in given fig. One important sequence in the prokaryotic promoter is located 10 bases before the transcription start site (-10) and is commonly called the TATA box.

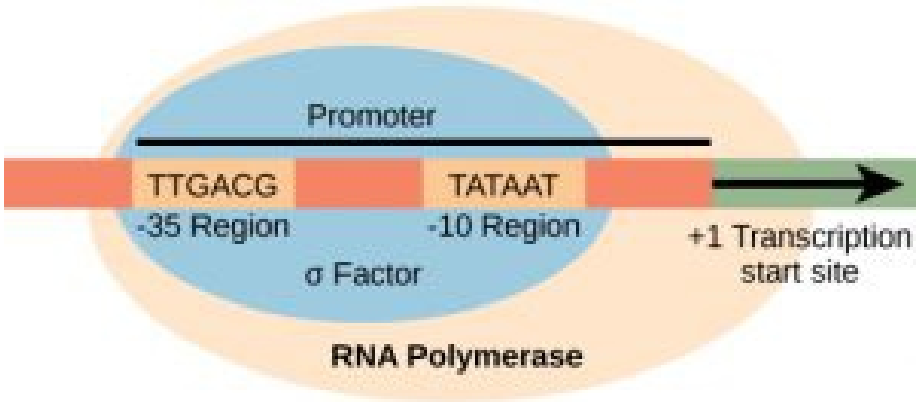

Fig. 7 The general structure of a prokaryotic promoter.

To begin transcription, the RNA polymerase holoenzyme assembles at the promoter. The dissociation of σ allows the core enzyme to proceed along the DNA template, synthesizing mRNA by adding RNA nucleotides according to the base pairing rules, similar to the way a new DNA molecule is produced during DNA replication. Only one of the two DNA strands is transcribed. The transcribed strand of DNA is called the template strand because it is the template for mRNA production. The mRNA product is complementary to the template strand and is almost identical to the other DNA strand, called the non-template strand, with the exception that RNA contains a uracil (U) in place of the thymine (T) found in DNA. Like DNA polymerase, RNA polymerase adds new nucleotides onto the 3′-OH group of the previous nucleotide. This means that the growing mRNA strand is being synthesized in the 5′ to 3′ direction. Because DNA is anti-parallel, this means that the RNA polymerase is moving in the 3′ to 5′ direction down the template strand in given fig. 8

3.13 Elongation

As elongation proceeds, the DNA is continuously unwound ahead of the core enzyme as the hydrogen bonds that connect the complementary base pairs in the DNA double helix are broken (Fig. 2). The DNA is rewound behind the core enzyme as the hydrogen bonds are reformed. The base pairing between

DNA and RNA is not stable enough to maintain the stability of the mRNA synthesis components. Instead, the RNA polymerase acts as a stable linker between the DNA template and the newly forming RNA strand to ensure that elongation is not interrupted prematurely.

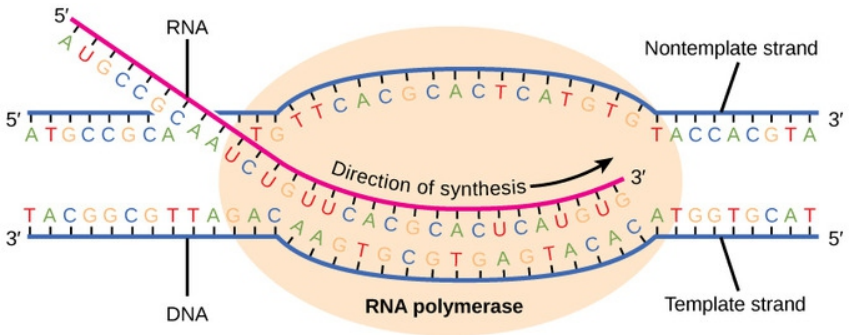

Fig. 8 During elongation, RNA polymerase tracks along the DNA template, synthesizes mRNA in the 5′ to 3′ direction, and unwinds then rewinds the DNA as it is read.

3.14 Termination

Termination once a gene is transcribed, the RNA polymerase needs to be instructed to dissociate from the DNA template and liberate the newly made mRNA. Depending on the gene being transcribed, there are two kinds of termination signals. One is protein-based and the other is RNA-based. Both termination signals rely on specific sequences of DNA near the end of the gene that cause the polymerase to release the mRNA.

In a prokaryotic cell, by the time transcription ends, the transcript would already have been used to begin making copies of the encoded protein because the processes of transcription and translation can occur at the same time since both occur in the cytoplasm (Figure 9). In contrast, transcription and translation cannot occur simultaneously in eukaryotic cells since transcription occurs inside the nucleus and translation occurs outside in the cytoplasm.

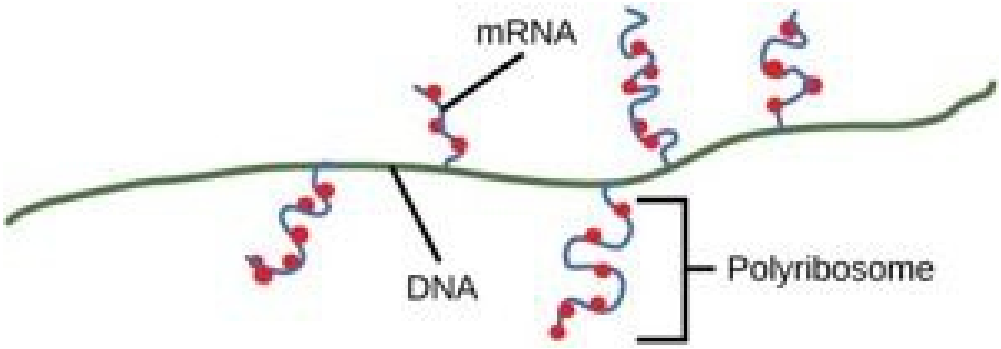

Fig. 9: Multiple polymerases can transcribe a single bacterial gene while numerous ribosomes concurrently translate the mRNA transcripts into polypeptides. In this way, a specific protein can rapidly reach a high concentration in the bacterial cell.

3.15 Translation in Eukaryotes

Translation

The process of protein synthesis from aminoacid sequence specified by the sequence of codons in messenger RNA is called translation.

Site

- ✓ Translation occurs in the cytoplasm where the ribosomes are located.
- ✓ Ribosomes are made of a small and large subunit which surrounds the mRNA.
- ✓ In eukaryotic translation 80S ribosomes with 40S and 60S subunits are used.
- ✓ The mRNA is synthesized from DNA only.
- ✓ In eukaryotes, there is single initiation and termination site..

Template

- ✓ This uses an mRNA sequence as a template to guide the synthesis of a chain of amino acids that form a protein.
- ✓ Many types of transcribed RNA, such as transfer RNA, ribosomal RNA, and small nuclear RNA are not necessarily translated into an amino acid sequence.

Enzymes Involved

- ✓ Three types of enzymes are used in translation. Aminoacyl tRNA synthetase (an enzyme) catalyzes the bonding between specific tRNAs and the amino acids.

- ✓ The enzyme peptidyl transferase connects A site and P site by forming a peptide bond [the nitrogen carbon bond] during elongation phase.
- ✓ Signal peptidase enzyme cleave the signal peptide sequence.

Starting amino acid

• In eukaryotes, starting amino acid methionine. Moreover, there is no overlapping of transcription and translation.

Mechanism of translation in eukaryotes

• Initiation,

• Elongation and

• Termination.

Initiation

• Formation of 43s preiniciation complex

• Attachment of mRNA

• Scanning for the start codon

• Assembly of large subunit

3.16 Elongation

Elongation depends on eukaryotic elongation factors. At the end of the initiation step, the mRNA is positioned so that the next codon can be translated during the elongation stage of protein synthesis. The initiator tRNA occupies the P site in the ribosome, and the A site is ready to receive an aminoacyl-tRNA. During chain elongation, each additional amino acid is added to the nascent polypeptide chain in a three-step microcycle. The steps in this microcycle are

(1) Positioning the correct aminoacyl-tRNA in the N site of the ribosome, which is brought into that site by eIF2,

(2) Forming the peptide bond, and

(3) Shifting the mRNA by one codon relative to the ribosome Unlike bacteria, in which translation initiation occurs as soon as the 5' end of an mRNA is synthesized, in eukaryotes, such tight coupling between transcription and translation is not possible because transcription and translation are carried out in separate compartments of the cell (the nucleus and cytoplasm). Eukaryotic mRNA precursors must be processed in the nucleus (e.g., capping, polyadenylation, splicing) in ribosomes before they are exported to

the cytoplasm for translation. Translation can also be affected by ribosomal pausing, which can trigger endonucleolytic attack of the tRNA, a process termed mRNA no-go decay. Ribosomal pausing also aids co-translational folding of the nascent polypeptide on the ribosome, and delays protein translation while it is encoding tRNA. This can trigger ribosomal frame shifting.

3.17 Termination

Termination of elongation depends on eukaryotic release factors. The process is similar to that of bacterial termination, but unlike bactrial termination, there is a universal release factor, eRF1, that recognizes all three stop codons. Upon termination, the ribosome is disassembled and the completed polypeptide is released. eRF3 is a ribosome-dependent GTPase that helps eRF1 release the completed polypeptide. The human genome encodes a few genes whose mRNA stop codon are surprisingly leaky: In these genes, termination of translation is inefficient due to special RNA bases in the vicinity of the stop codon. Leaky termination in these genes leads to translational read through of up to 10% of the stop codons of these genes. Some of these genes encode functional protein domains in their read through extension so that new protein isoforms can arise. This process has been termed functional translational read through.

SAQ 2

Define these terms in prokaryotes.

(a) Define initiation.

(b) Define elongation.

(c) What do you mean by termination?

3.18 Translation in prokaryotes

Translation involves translating the sequence of a messenger RNA (mRNA) molecule to a sequence of amino acids during protein synthesis. It is the process in which ribosomes in the cytoplasm or ER synthesize proteins after the process of transcription of DNA to RNA. In molecular biology and genetics, translation is the process in which ribosomes in

the cytoplasm or endoplasmic reticulum synthesize proteins after the process of transcription of DNA to RNA in the cell's nucleus. The entire process is called gene expression.

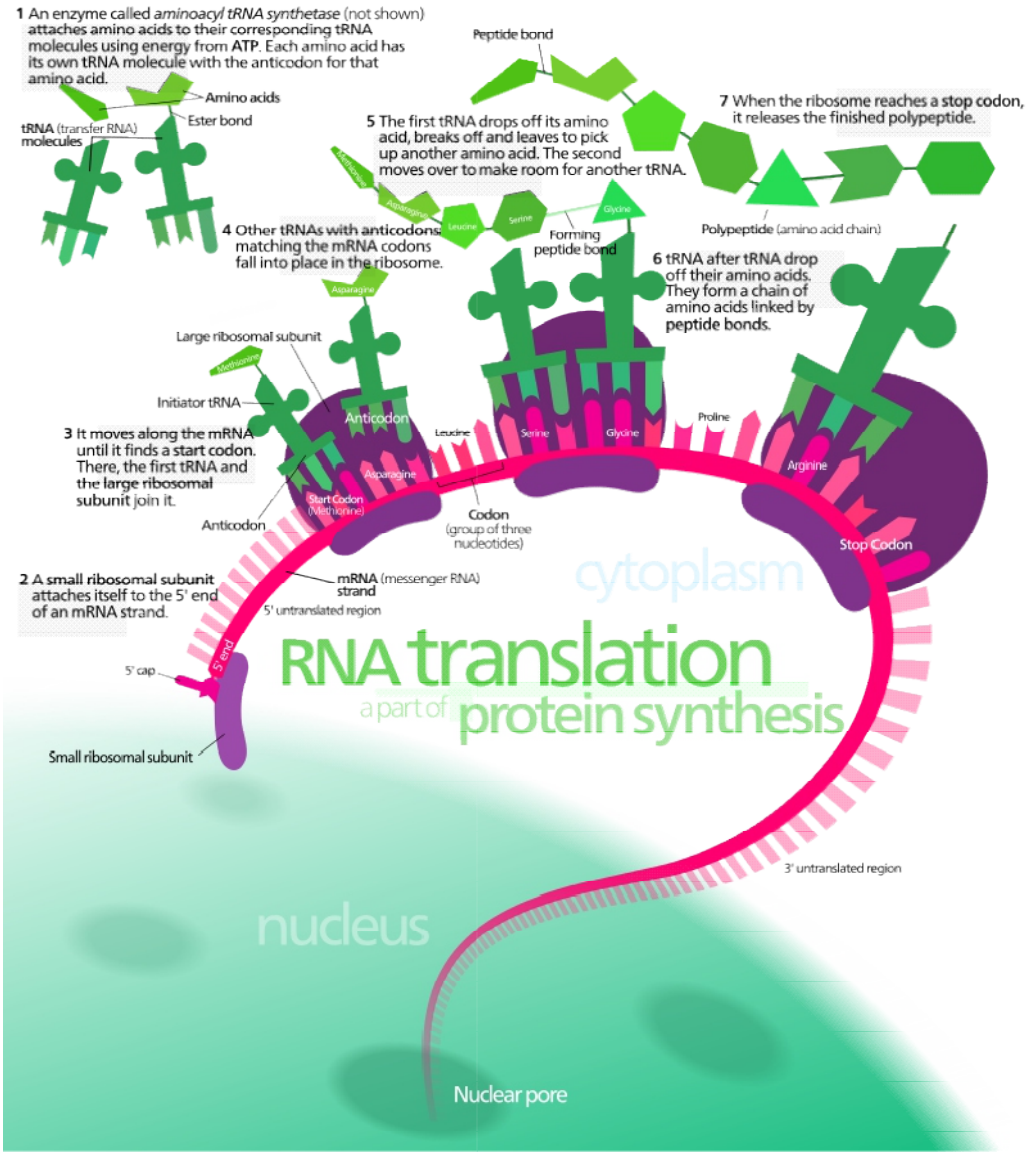

Fig. 10 Overview of the translation of eukaryotic messenger RNA

In translation, messenger RNA (mRNA) is decoded in a ribosome, outside the nucleus, to produce a specific amino acid chain, or polypeptide. The polypeptide later folds into an active protein and performs its functions in the cell. The ribosome facilitates decoding by inducing the binding of complementary tRNA anticodon sequences to mRNA codons. The tRNAs carry specific amino acids that are chained together into a polypeptide as the

mRNA passes through and is read by the ribosome. Translation proceeds in three phases as under:

Initiation

The ribosome assembles around the target mRNA. The first tRNA is attached at the start codon.

Elongation

The last tRNA validated by the small ribosomal subunit (accommodation) transfers the amino acid it carries to the large ribosomal subunit which binds it to the one of the precedingly admitted tRNA (transpeptidation). The ribosome then moves to the next mRNA codon to continue the process (translocation), creating an amino acid chain.

Termination

When a stop codon is reached, the ribosome releases the polypeptide. The ribosomal complex remains intact and moves on to the next mRNA to be translated.

In prokaryotes (bacteria and archaea), translation occurs in the cytosol, where the large and small subunits of the ribosome bind to the mRNA. In eukaryotes, translation occurs in the cytoplasm or across the membrane of the endoplasmic reticulum in a process called co-translational translocation. In co-translational translocation, the entire ribosome/mRNA complex binds to the outer membrane of the rough endoplasmic reticulum (ER) and the new protein is synthesized and released into the ER; the newly created polypeptide can be stored inside the ER for future vesicle transport and secretion outside the cell, or immediately secreted.

Many types of transcribed RNA, such as transfer RNA, ribosomal RNA, and small nuclear RNA, do not undergo translation into proteins. A number of antibiotics act by inhibiting translation.Theseinclude anisomycin, cycloheximide, chloramphenicol, tetracycline, streptomycin, erythromycin, and puromycin. Prokaryotic ribosomes have a different structure from that of eukaryotic ribosomes, and thus antibiotics can specifically target bacterial infections without any harm to a eukaryotic host's cells.

3.19 Clinical significance

Translational control is critical for the development and survival of cancer. Cancer cells must frequently regulate the translation phase of gene expression, though it is not fully understood why translation is targeted over steps like transcription. While cancer cells often have genetically altered translation factors, it is much more common for cancer cells to modify the levels of existing translation factors. Several major oncogenic signaling pathways, including the RAS–MAPK, PI3K/AKT/mTOR, MYC, and WNT–β-catenin pathways, ultimately reprogram the genome via translation. Cancer cells also control translation to adapt to cellular stress. During stress, the cell translates mRNAs that can mitigate the stress and promote survival. An example of this is the expression of AMPK in various cancers; its activation triggers a cascade that can ultimately allow the cancer to escape apoptosis (programmed cell death) triggered by nutrition deprivation. Future cancer therapies may involve disrupting the translation machinery of the cell to counter the downstream effects of cancer.

3.20 Summary

Under this unit we have focused on gene expression, transcription and translation. Gene expression is a fundamental life process providing a bridge between information encoded within a gene and a final functional gene product, such as a protein or non-coding RNA (ncRNA). For protein expression, it is a multi-stage process that includes transcription, mRNA splicing, translation, and post-translational protein modification. This process can be modulated at any stage, thus controlling the quantity and spatiotemporal parameters of the functional protein appearance. It is vital for maintaining normal cellular structure and function, and is also the basis for developmental changes, such as differentiation and morphogenesis. The ability to regulate gene expression allows cells to deliver a functional protein whenever it's needed for their normal functioning or survival. This mechanism underlies various physiological and pathological processes, including cellular adaptations to novel environments, maintenance of homeostasis, and recovery from damages.

Transcription and translation both are very important process in molecular biology. Transcription is the process in which a gene's DNA sequence is copied (transcribed) to make an RNA molecule. RNA polymerase is the main transcription enzyme. Transcription begins when RNA polymerase binds to a promoter sequence near the beginning of a gene (directly or through helper proteins). Translation has been discussed in eukaryotic and prokaryotic systems. It is the process that takes the information passed from DNA as messenger RNA and turns it into a series of amino acids bound together with peptide bonds. The ribosome moves along the mRNA, matching 3 base pairs at a time and adding the amino acids to the polypeptide chain.

3.2.1 Terminal questions

Q.1 Describe gene expression.

Answer:----------

Q.2 Distinguish between prokaryotes and eukaryotes.

Answer:----------

Q.3 Write a short note on transcription.

Answer:----------

Q.4 Describe transcription in eukaryotes and prokaryotes.

Answer:----------

Q.5 Describe translation in eukaryotes and prokaryotes.

Answer:----------

Q.6 Write a short note on translation.

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Text book of Botany – Singh -Pande-Jain.
3. The elements of Botany- James Hewetson Wilson
4. Textbook of Biotechnology –H. K. Das
5. Biochemistry and molecular biology- Wilson Walker

Uttar Pradesh Rajarshi Tandon
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Bachelor of Science
DCEBY-108
Molecular Genetics & Biotechnology

Block -2
Molecular Genetic - II

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Course Design Committee

Prof. Ashutosh Gupta — **Chairman**
Director, School of Science
UPRTOU, Prayagraj.

Dr. Anil Tiwari — **Member**
Botany Department
Ewing Christen College.
Prayagraj.

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Botany Department
S.S.K.P.G. College
Prayagraj.

Dr. Sushma Chauhan — **Member/Secretary**
Asst. Prof. (Contractual) Botany
School of Science, UPRTOU, Prayagraj.

Course Preparation Committee

Dr. Rajivranjan — **Author**
Assistant Professor — **Block- 01 – 03** (Unit: 01- 09)
Department of Botany
MLK PG College, Balrampur (U.P)

Dr. Arun Kumar Pandey — **Editor**
Associate Professor, — **(All blocks and units)**
Department of Botany
PSM PG College, Anand Nagar, Maharajganj (U.P)

Dr. Sushma Chauhan — **Coordinator**
Asst. Prof. (Contractual) Botany
School of Science, UPRTOU, Prayagraj.

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Block II: Molecular Genetics – II

In the previous block you have studied half part of molecular genetics. In this block you will study remaining part of molecular genetics which is divided into three units 4,5 and 6. The detail of units are given below:

Unit -4 Gene regulation contains operon concept, lactose operon model and transposons.

Unit-5 Genetic Engineering covers history of recombinant DNA technology, tools and process of DNA technology application of DNA technology in producing transgenic plants, improving crop yield and nutritional value

Unit-6 Contains molecular antibodies, DNA probes and DNA finger printing. After studying this unit you will be able to know production and application of monoclonal antibodies, types and use of DNA, methods and advantages of DNA finger printing.

Objectives:

After studying this unit you will be able to know:

- About gene regulation.
- Recombinant DNA technology and its application in crop improvement.
- Production and application of monoclonal antibodies.
- About DNA Probes and its advantages.
- Method of DNA finger printing and its application.

Unit 4 Gene Regulation, Operon Concept and Transposons

Structure

4.1 Introduction

Objectives:

4.2 Gene regulations

4.3 Prokaryotic and eukaryotic gene regulation

4.4 Operon concept

4.5 Regulation

4.6 The lactose operon

- 4.6.1 Structure
- 4.6.2 Genetic nomenclature
- 4.6.3 Regulation
- 4.6.4 Repressor structure
- 4.6.5 Mechanism of induction
- 4.6.6 Role of non-specific binding
- 4.6.7 Use in molecular biology

4.7 Transposons

- 4.7.1 Class II transposons
- 4.7.2 Miniature inverted –repeat transposable elements
- 4.7.3 Retrotransposons
- 4.7.4 Transposons and antibiotic resistance
- 4.7.5 Transposons and disease

4.8 Summary

4.9. Terminal questions

Further readings

4.1 Introduction

In the human genome, there are a little less than 20,000 genes. In some cells, many genes are active--say, 10,000--and the other 10,000 would be inactive. In other kinds of cells, maybe the other 10,000 would be active and the first 10,000 would be inactive. And so, gene regulation is the process by which the cell determines which genes will be active and which genes will not be active. And gene regulation is at the bottom of what makes a cell decide to become a red blood cell, or a neuron, or a hepatocyte in the liver, or a muscle cell. So different gene regulation will give you a different program of genes and different genes expressed. There are several different kinds of gene regulation. Some genes, called housekeeping genes, are expressed in almost every cell. And these require a regulatory network or machinery that keeps them on in almost every cell, so these are the enzymes that help make DNA, and perform glycolysis, and burn sugar, and things like that.

There are other genes that are called tissue-specific genes. These are genes that, say, would only be expressed in a red blood cell or a neuron. Very often, these genes have transcription factors, which are proteins that bind to DNA, near these genes. And those transcription factors actually help the RNA machinery get there and transcribe that gene in those cells, and those tissues, transcription factors, rather, are expressed specifically in those tissues. There are also factors expressed in those tissues that will be suppressors that can turn a gene off. And then there are genes that are regulated during development. Sometimes they're expressed in fetal life and then turned off in adults, and sometimes it's vice versa. So there are very complex different ways that genes are regulated. I kind of look at it as playing music: You have chords on a guitar, or you play with a right and a left hand on the piano. It depends what strings you push down and what strings you strum, or what keys are up and what keys are down, [that] determine what the profile of the gene expression will be or the sound that you hear.

Objectives:

This is the fourth unit of second block on molecular genetics II**.** Under fourth unit, we have following objectives. These are as under:

- To know about gene regulation
- To know about eukaryotic and prokaryotic gene regulation
- To know about operon concept and lactose operon
- To know about transposons and antibiotic resistance
- To discuss genetic nomenclature and retrotransposons

4.2 Gene regulations

Regulation of gene expression, or gene regulations includes a wide range of mechanisms that are used by cells to increase or decrease the production of specific gene products (protein or RNA). Sophisticated programs of gene expression are widely observed in biology, for example to trigger developmental pathways, respond to environmental stimuli, or adapt to new food sources. Virtually any step of gene expression can be modulated, from transcriptional initiation, to RNA processing, and to the post-translational modification of a protein. Often, one gene regulator controls another, and so on, in a gene regulatory network.

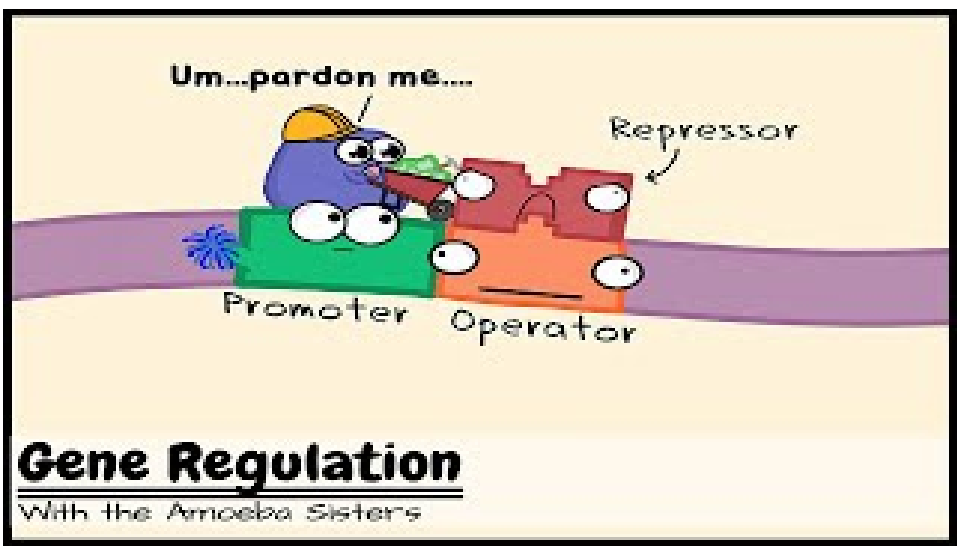

Fig. 1 Gene regulation and the order of the operon

Gene regulation is essential for viruses, prokaryotes and eukaryotes as it increases the versatility and adaptability of an organism by allowing the cell to express protein when needed. Although as early as 1951, Barbara McClintock showed interaction between two genetic loci, Activator (*Ac*) and Dissociator (*Ds*), in the color formation of maize seeds, the first discovery of a gene regulation system is widely considered to be the identification in 1961 of

the *lac* operon, discovered by François Jacob and Jacques Monod, in which some enzymes involved in lactose metabolism are expressed by *E. coli* only in the presence of lactose and absence of glucose.

In multicellular organisms, gene regulation drives cellular differentiation and morphogenesis in the embryo, leading to the creation of different cell types that possess different gene expression profiles from the same genome sequence. Although this does not explain how gene regulation originated, evolutionary biologists include it as a partial explanation of how evolution works at a molecular level, and it is central to the science of evolutionary.

4.3 Prokaryotic and eukaryotic gene regulation

To understand how gene expression is regulated, we must first understand how a gene codes for a functional protein in a cell. The process occurs in both prokaryotic and eukaryotic cells, just in slightly different manners. Prokaryotic organisms are single-celled organisms that lack a cell nucleus, and their DNA therefore floats freely in the cell cytoplasm. To synthesize a protein, the processes of transcription and translation occur almost simultaneously. When the resulting protein is no longer needed, transcription stops. As a result, the primary method to control what type of protein and how much of each protein is expressed in a prokaryotic cell is the regulation of DNA transcription.

All of the subsequent steps occur automatically. When more protein is required, more transcription occurs. Therefore, in prokaryotic cells, the control of gene expression is mostly at the transcriptional level. Eukaryotic cells, in contrast, have intracellular organelles that add to their complexity. In eukaryotic cells, the DNA is contained inside the cell's nucleus and there it is transcribed into RNA. The newly synthesized RNA is then transported out of the nucleus into the cytoplasm, where ribosomes translate the RNA into protein. The processes of transcription and translation are physically separated by the nuclear membrane; transcription occurs only within the nucleus, and translation occurs only outside the nucleus in the cytoplasm.

The regulation of gene expression can occur at all stages of the process as in fig. Regulation may occur when the DNA is uncoiled and loosened from

nucleosomes to bind transcription factors **(epigenetic** level), when the RNA is transcribed (transcriptional level), when the RNA is processed and exported to the cytoplasm after it is transcribed **(post-transcriptional** level), when the RNA is translated into protein (translational level), or after the protein has been made **(post-translational** level).

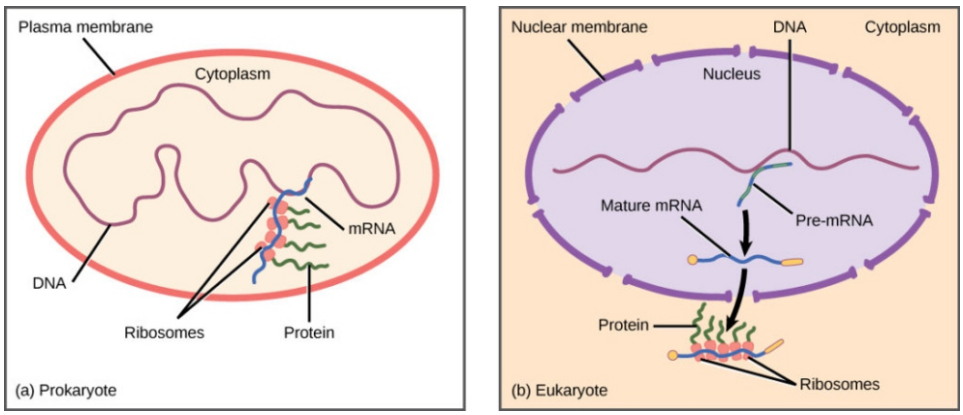

Fig. 2 Prokaryotic transcription and translation occur simultaneouslyin the cytoplasm, and regulation occurs at the transcriptional level.

- ✓ Gene regulation is the process of controlling which genes in a cell's DNA are expressed (used to make a functional product such as a protein).
- ✓ Different cells in a multicellular organism may express very different sets of genes, even though they contain the same DNA.
- ✓ The set of genes expressed in a cell determines the set of proteins and functional RNAs it contains, giving it its unique properties.
- ✓ In eukaryotes like humans, gene expression involves many steps, and gene regulation can occur at any of these steps. However, many genes are regulated primarily at the level of transcription.

The differences in the regulation of gene expression between prokaryotes and eukaryotes are summarized in the given table. The regulation of gene expression is discussed in detail in subsequent modules.

Table 1

Differences in the Regulation of Gene Expression of Prokaryotic and Eukaryotic Organisms	
Prokaryotic organisms	**Eukaryotic organisms**
Lack nucleus	Contain nucleus
DNA is found in the cytoplasm	DNA is confined to the nuclear compartment
RNA transcription and protein formation occur almost simultaneously	RNA transcription occurs prior to protein formation, and it takes place in the nucleus. Translation of RNA to protein occurs in the cytoplasm.
Gene expression is regulated primarily at the transcriptional level	Gene expression is regulated at many levels (epigenetic, transcriptional, nuclear shuttling, post-transcriptional, translational, and post-translational)

4.4 Operon concept

The term "operon" was first proposed in a short paper in the Proceedings of the French Academy of Science in 1960. From this paper, the so-called general theory of the operon was developed. This theory suggested that in all cases, genes within an operon are negatively controlled by a repressor acting at a single operator located before the first gene. Later, it was discovered that genes could be positively regulated and also regulated at steps that follow transcription initiation. Therefore, it is not possible to talk of a general regulatory mechanism, because different operons have different mechanisms. Today, the operon is simply defined as a cluster of genes transcribed into a single mRNA molecule. Nevertheless, the development of the concept is considered a landmark event in the history of molecular biology. The first operon to be described was the lac operon in *E. coli*. The 1965 Nobel Prize in Physiology and Medicine was awarded to François Jacob, André Michel Lwoff and Jacques Monod for their discoveries concerning the operon and virus synthesis.

In genetics, an operon is a functioning unit of DNA containing a cluster of genes under the control of a single promoter. The genes are transcribed together into an mRNA strand and either translated together in the cytoplasm, or undergo splicing to create monocistronic mRNAs that are translated separately, i.e. several strands of mRNA that each encode a single gene product. The result of this is that the genes contained in the operon are

either expressed together or not at all. Several genes must be *co-transcribed* to define an operon.

Originally, operons were thought to exist solely in prokaryotes (which includes organelles like plastids that are derived from bacteria), but since the discovery of the first operons in eukaryotes in the early 1990s, more evidence has arisen to suggest they are more common than previously assumed. In general, expression of prokaryotic operons leads to the generation of polycistronic mRNAs, while eukaryotic operons lead to monocistronic mRNAs.

Operons are also found in viruses such as bacteriophages. For example, T7 phages have two operons. The first operon codes for various products, including a special T7 RNA polymerase which can bind to and transcribe the second operon. The second operon includes a lysis gene meant to cause the host cell to burst.

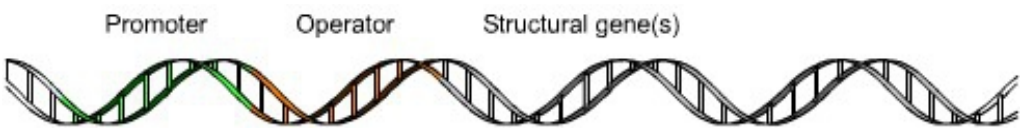

Fig. 3 A typical operon

Operon, genetic regulatory system found in bacteria and their viruses in which genes coding for functionally related proteins are clustered along the DNA. This feature allows protein synthesis to be controlled coordinately in response to the needs of the cell. By providing the means to produce proteins only when and where they are required, the operon allows the cell to conserve energy (which is an important part of an organism's life strategy). A typical operon consists of a group of structural genes that code for enzymes involved in a metabolic pathway, such as the biosynthesis of an amino acid. These genes are located contiguously on a stretch of DNA and are under the control of one promoter (a short segment of DNA to which the RNA polymerase binds to initiate transcription). A single unit of messenger RNA (mRNA) is transcribed from the operon and is subsequently translated into separate proteins.

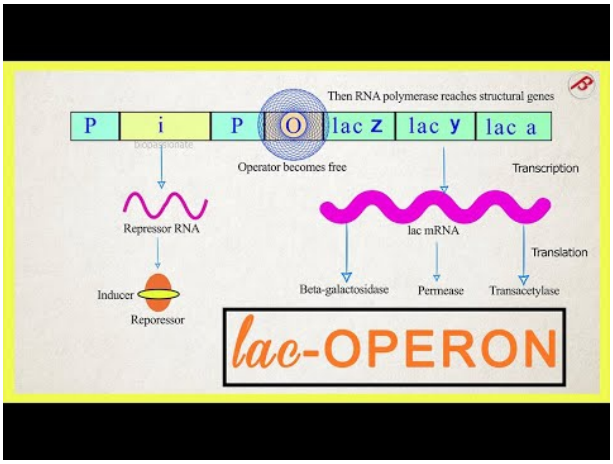

Fig. 4 Lac operon concept

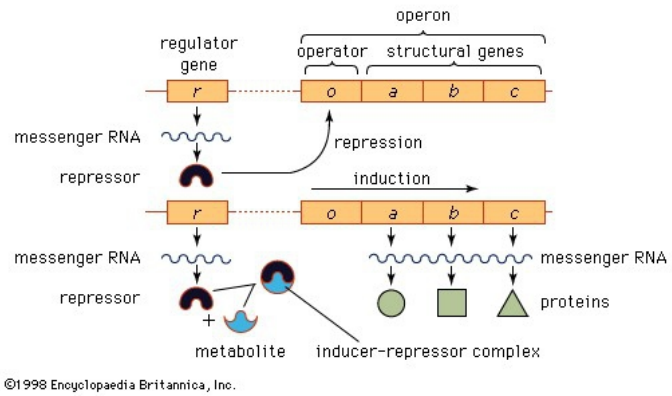

Fig. 5 Structure of operon

The promoter is controlled by various regulatory elements that respond to environmental cues. One common method of regulation is carried out by a regulator protein that binds to the operator region, which is another short segment of DNA found between the promoter and the structural genes. The regulator protein can either block transcription, in which case it is referred to as a repressor protein; or as an activator protein it can stimulate transcription. Further regulation occurs in some operons: a molecule called an inducer can bind to the repressor, inactivating it; or a repressor may not be able to bind to the operator unless it is bound to another molecule, the corepressor. Some operons are under attenuator control, in which transcription is initiated but is halted before the mRNA is transcribed. This introductory region of the mRNA is called the leader sequence; it includes the attenuator region, which can fold

back on itself, forming a stem-and-loop structure that blocks the RNA polymerase from advancing along the DNA.

The operon theory was first proposed by the French microbiologists François Jacob and Jacques Monod in the early 1960s. In their classic paper they described the regulatory mechanism of the *lac* operon of *Escherichia coli,* a system that allows the bacterium to repress the production of enzymes involved in lactose metabolism when lactose is not available.

4.5 Regulation

Control of an operon is a type of gene regulation that enables organisms to regulate the expression of various genes depending on environmental conditions. Operon regulation can be either negative or positive by induction or repression. Negative control involves the binding of a repressor to the operator to prevent transcription.

- ✓ In *negative inducible operons*, a regulatory repressor protein is normally bound to the operator, which prevents the transcription of the genes on the operon. If an inducer molecule is present, it binds to the repressor and changes its conformation so that it is unable to bind to the operator. This allows for expression of the operon. The *lac* operon is a negatively controlled inducible operon, where the inducer molecule is allolactose.
- ✓ In *negative repressible operons*, transcription of the operon normally takes place. Repressor proteins are produced by a regulator gene, but they are unable to bind to the operator in their normal conformation. However, certain molecules called corepressors are bound by the repressor protein, causing a conformational change to the active site. The activated repressor protein binds to the operator and prevents transcription. The *trp* operon, involved in the synthesis of tryptophan (which itself acts as the corepressor), is a negatively controlled repressible operon.

Operons can also be positively controlled. With positive control, an activator protein stimulates transcription by binding to DNA (usually at a site other than the operator).

- ✓ In *positive inducible operons*, activator proteins are normally unable to bind to the pertinent DNA. When an inducer is bound by the activator protein, it undergoes a change in conformation so that it can bind to the DNA and activate transcription.
- ✓ In *positive repressible operons*, the activator proteins are normally bound to the pertinent DNA segment. However, when an inhibitor is bound by the activator, it is prevented from binding the DNA. This stops activation and transcription of the system.

SAQ 1

Q. 1 Define gene regulation.

Q. 2 Define transposons.

Q. 3 What do you mean by lactose operon?

Q. 4 Define class II transposons.

4.6 The lactose operon

The *lactose* operon (*lac* operon) is an operon required for the transport and metabolism of lactose in *E. coli* and many other enteric bacteria. Although glucose is the preferred carbon source for most bacteria, the *lac* operon allows for the effective digestion of lactose when glucose is not available through the activity of beta-galactosidase. Gene regulation of the *lac* operon was the first genetic regulatory mechanism to be understood clearly, so it has become a foremost example of prokaryotic gene regulation. It is often discussed in introductory molecular and cellular biology classes for this reason. This lactose metabolism system was used by François Jacob and Jacques Monod to determine how a biological cell knows which enzyme to synthesize. Their work on the *lac* operon won them the Nobel Prize in Physiology in 1965.

Bacterial operons are polycistronic transcripts that are able to produce multiple proteins from one mRNA transcript. In this case, when lactose is required as a sugar source for the bacterium, the three genes of the *lac* operon can be

expressed and their subsequent proteins translated: *lacZ*, *lacY*, and *lacA*. The gene product of *lacZ* is β-galactosidase which cleaves lactose, a disaccharide, into glucose and galactose. *lacY* encodes Beta-galactoside permease, a membrane protein which becomes embedded in the cytoplasmic membrane to enable the cellular transport of lactose into the cell. Finally, *lacA* encodes Galactoside acetyltransferase.

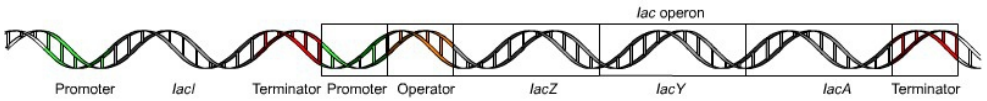

Fig. 6 The lactose operon

It would be wasteful to produce enzymes when no lactose is available or if a preferable energy source such as glucose were available. The *lac* operon uses a two-part control mechanism to ensure that the cell expends energy producing the enzymes encoded by the *lac* operon only when necessary. In the absence of lactose, the *lac* repressor, *lacI,* halts production of the enzymes encoded by the *lac* operon. The lac repressor is always expressed, unless a co-inducer binds to it. In other words, it is transcribed only in the presence of small co-inducer molecules. In the presence of glucose, the catabolite activator protein (CAP), required for production of the enzymes, remains inactive, and $EIIA^{Glc}$ shuts down lactose permease to prevent transport of lactose into the cell. This dual control mechanism causes the sequential utilization of glucose and lactose in two distinct growth phases, known as diauxie.

4.6.1 Structure

- ✓ The *lac* operon consists of 3 structural genes, and a promoter, a terminator, regulator, and an operator. The three structural genes are: *lacZ*, *lacY*, and *lacA*.
- ✓ *lacZ* encodes β-galactosidase (LacZ), an intracellular enzyme that cleaves the disaccharide lactose into glucose and galactose.
- ✓ *lacY* encodes Beta-galactoside permease (LacY), a transmembrane symporter that pumps β-galactosides including lactose into the cell using a proton gradient in the same direction. Permease increases the permeability of the cell to β-galactosides.
- ✓ *lacA* encodes β-galactoside transacetylase (LacA), an enzyme that transfers an acetyl group from acetyl-CoA to thiogalactoside.

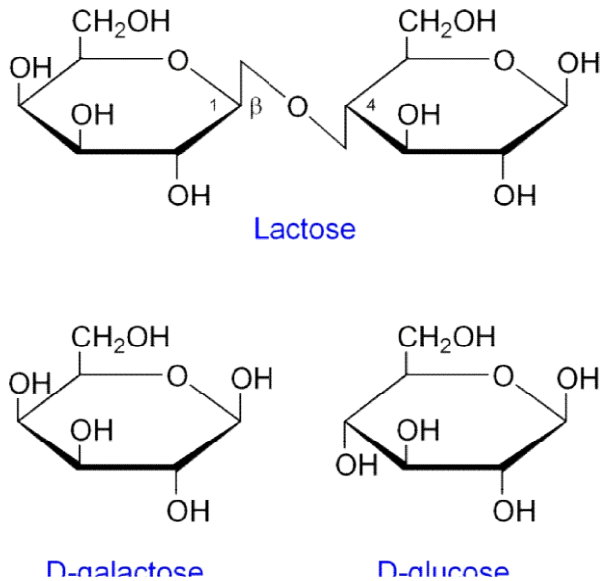

Fig. 7 Structure of lactose and the products of its cleavage.

Only *lacZ* and *lacY* appear to be necessary for lactose catabolic pathway.

4.6.2 Genetic nomenclature

Three-letter abbreviations are used to describe phenotypes in bacteria including *E. coli*.

Examples:

- ✓ Lac (the ability to use lactose),
- ✓ His (the ability to synthesize the amino acid histidine)
- ✓ Mot (swimming motility)
- ✓ Sm^R (resistance to the antibiotic streptomycin)

In the case of Lac, wild type cells are Lac^+ and are able to use lactose as a carbon and energy source, while Lac^- mutant derivatives cannot use lactose. The same three letters are typically used (lower-case, italicized) to label the genes involved in a particular phenotype, where each different gene is additionally distinguished by an extra letter. The *lac* genes encoding enzymes are *lacZ*, *lacY*, and *lacA*. The fourth *lac* gene is *lacI*, encoding the lactose repressor—"I" stands for *inducibility*.

One may distinguish between *structural* genes encoding enzymes, and regulatory genes encoding proteins that affect gene expression. Current usage expands the phenotypic nomenclature to apply to proteins: thus, LacZ is the protein product of the *lacZ* gene, β-galactosidase. Various short sequences that

are not genes also affect gene expression, including the *lac* promoter, *lac p*, and the *lac* operator, *lac o*. Although it is not strictly standard usage, mutations affecting *lac o* are referred to as *lac* o^c, for historical reasons.

4.6.3 Regulation

Specific control of the *lac* genes depends on the availability of the substrate lactose to the bacterium. The proteins are not produced by the bacterium when lactose is unavailable as a carbon source. The *lac* genes are organized into an operon; that is, they are oriented in the same direction immediately adjacent on the chromosome and are co-transcribed into a single polycistronic mRNA molecule. Transcription of all genes starts with the binding of the enzyme RNA polymerase (RNAP), a DNA-binding protein, which binds to a specific DNA binding site, the promoter, immediately upstream of the genes. Binding of RNA polymerase to the promoter is aided by the cAMP-bound catabolite activator protein (CAP, also known as the cAMP receptor protein). However, the *lacI* gene (regulatory gene for *lac* operon) produces a protein that blocks RNAP from binding to the operator of the operon. This protein can only be removed when allolactose binds to it, and inactivates it. The protein that is formed by the *lacI* gene is known as the lac repressor.

The type of regulation that the *lac* operon undergoes is referred to as negative inducible, meaning that the gene is turned off by the regulatory factor (*lac* repressor) unless some molecule (lactose) is added. Because of the presence of the *lac* repressor protein, genetic engineers who replace the *lacZ* gene with another gene will have to grow the experimental bacteria on agar with lactose available on it. If they do not, the gene they are trying to express will not be expressed as the repressor protein is still blocking RNAP from binding to the promoter and transcribing the gene. Once the repressor is removed, RNAP then proceeds to transcribe all three genes (*lacZYA*) into mRNA. Each of the three genes on the mRNA strand has its own Shine-Dalgarno sequence, so the genes are independently translated. The DNA sequence of the *E. coli lac* operon, the *lacZYA* mRNA, and the *lacI* genes are available from GenBank.

4.6.4 Repressor structure

The lac repressor is a four-part protein, a tetramer, with identical subunits. Each subunit contains a helix-turn-helix (HTH) motif capable of binding to DNA. The operator site where repressor binds is a DNA sequence with inverted repeat symmetry. The two DNA half-sites of the operator together bind to two of the subunits of the repressor. Although the other two subunits of repressor are not doing anything in this model, this property was not understood for many years.

Eventually it was discovered that two additional operators are involved in *lac* regulation. One (O_3) lies about −90 bp upstream of O_1 in the end of the *lacI* gene, and the other (O_2) is about +410 bp downstream of O_1 in the early part of *lacZ*. These two sites were not found in the early work because they have redundant functions and individual mutations do not affect repression very much. Single mutations to either O_2 or O_3 have only 2 to 3-fold effects. However, their importance is demonstrated by the fact that a double mutant defective in both O_2 and O_3 is dramatically de-repressed (by about 70-fold).

In the current model, *lac* repressor is bound simultaneously to both the main operator O_1 and to either O_2 or O_3. The intervening DNA loops out from the complex. The redundant nature of the two minor operators suggests that it is not a specific looped complex that is important. One idea is that the system works through tethering; if bound repressor releases from O_1 momentarily, binding to a minor operator keeps it in the vicinity, so that it may rebind quickly. This would increase the affinity of repressor for O_1.

4.6.5 Mechanism of induction

The repressor is an allosteric protein, i.e. it can assume either one of two slightly different shapes, which are in equilibrium with each other. In one form the repressor will bind to the operator DNA with high specificity, and in the other form it has lost its specificity. According to the classical model of induction, binding of the inducer, either allolactose or IPTG, to the repressor affects the distribution of repressor between the two shapes. Thus, repressor with inducer bound is stabilized in the non-DNA-binding conformation.

However, this simple model cannot be the whole story, because repressor is bound quite stably to DNA, yet it is released rapidly by addition of inducer. Therefore, it seems clear that an inducer can also bind to the repressor when the repressor is already bound to DNA. It is still not entirely known what the exact mechanism of binding is.

4.6.6 Role of non-specific binding

Non-specific binding of the repressor to DNA plays a crucial role in the repression and induction of the Lac-operon. The specific binding site for the Lac-repressor protein is the operator. The non-specific interaction is mediated mainly by charge-charge interactions while binding to the operator is reinforced by hydrophobic interactions. Additionally, there is an abundance of non-specific DNA sequences to which the repressor can bind. Essentially, any sequence that is not the operator, is considered non-specific. Studies have shown, that without the presence of non-specific binding, induction (or unrepression) of the Lac-operon could not occur even with saturated levels of inducer. It had been demonstrated that, without non-specific binding, the basal level of induction is ten thousand times smaller than observed normally. This is because the non-specific DNA acts as sort of a "sink" for the repressor proteins, distracting them from the operator. The non-specific sequences decrease the amount of available repressor in the cell. This in turn reduces the amount of inducer required to unrepress the system.

4.6.7 Use in molecular biology

The *lac* gene and its derivatives are amenable to use as a reporter gene in a number of bacterial-based selection techniques such as two hybrid analysis, in which the successful binding of a transcriptional activator to a specific promoter sequence must be determined. In LB plates containing X-gal, the colour change from white colonies to a shade of blue corresponds to about 20–100 β-galactosidase units, while tetrazolium lactose and MacConkey lactose media have a range of 100–1000 units, being most sensitive in the high and low parts of this range respectively. Since MacConkey lactose and tetrazolium lactose media both rely on the products of lactose breakdown, they require the presence of both *lacZ* and *lacY* genes. The many *lac* fusion techniques which

include only the *lacZ* gene are thus suited to X-gal plates or ONPG liquid broths.

4.7 Transposons

Transposon, class of genetic elements that can "jump" to different locations within a genome. Although these elements are frequently called "jumping genes," they are always maintained in an integrated site in the genome. In addition, most transposons eventually become inactive and no longer move. Transposons were first discovered in corn (maize) during the 1940s and '50s by American scientist Barbara McClintock, whose work won her the Nobel Prize for Physiology or Medicine in 1983. Since McClintock's discovery, three basic types of transposons have been identified. These include class II transposons, miniature inverted-repeat transposable elements (MITEs, or class III transposons), and retrotransposons (class I transposons).

4.7.1 Class II transposons

Class II elements are simply segments of DNA that move from one place to another via a "cut and paste" mechanism. Most, if not all, of these elements encode an enzyme called transposase, which acts to cleave the ends of the transposons, freeing it from its initial location in the genome. Transposase also cleaves target sites where the element is to be inserted. Once the transposon is ligated (bound) into its new position, gaps that are left in the DNA sequence are filled in through the synthesis of nucleotides. Class II transposons range in length from 1,000 to as many as 40,000 base pairs.

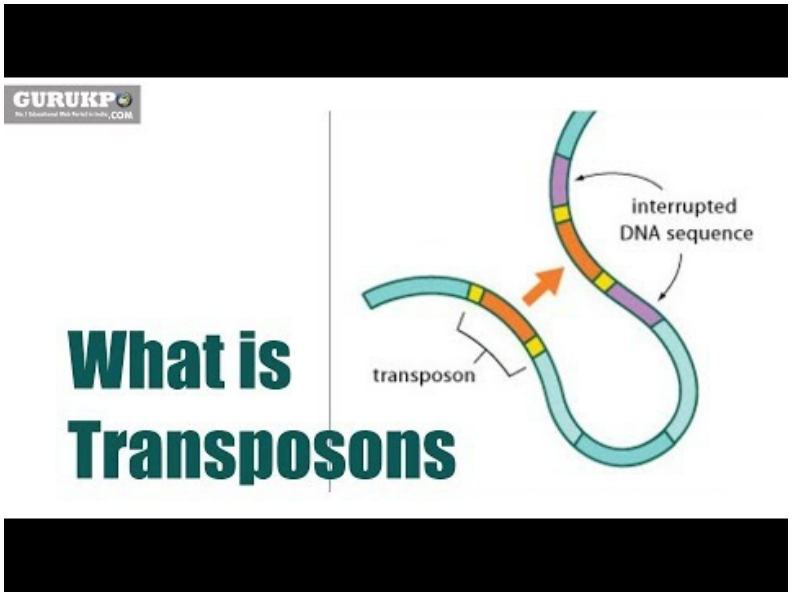

Fig. 8 What is transposons?

4.7.2 Miniature inverted-repeat transposable elements

MITEs are characterized by their short lengths, generally about 400 to 600 base pairs, and by a stretch of about 15 base pairs that occurs at each end of each element in an inverted fashion (as mirror sequences). The mechanism by which these elements move about genomes is not well understood. Thousands of MITEs have been identified in the genomes of *Oryza sativa* (cultivated rice), *Caenorhabditis elegans* (a type of nematode), and other organisms. Unlike some types of transposons, MITEs do not appear to encode proteins, and most insertions of these elements occur in euchromatin, the form of chromosomal material that contains the majority of active genes. As a result, a genetic regulatory function of MITEs has been proposed, and this has received support from evidence that some microRNAs (miRNAs), which play a role in RNA interference (a form of gene regulation), are derived from MITEs.

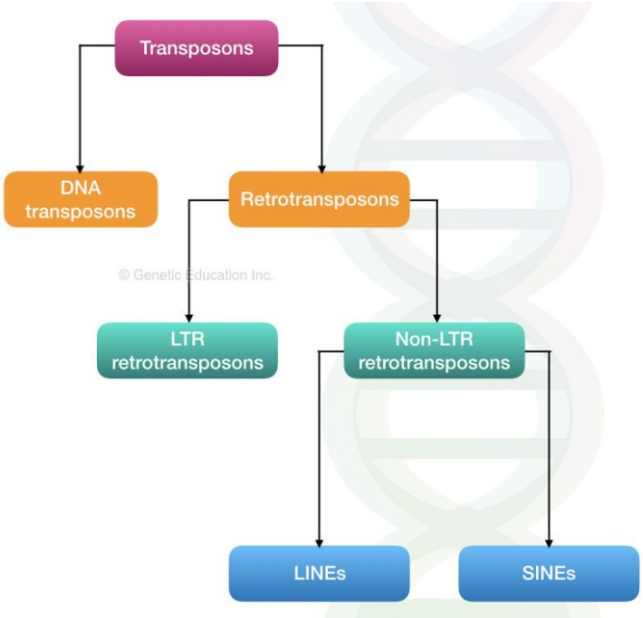

Fig. 9 Transposons and its types

4.7.3 Retrotransposons

Retrotransposons represent a highly unique group of transposable elements and form large portions of the genomes of many eukaryotes (organisms with cells containing a clearly defined nucleus). Retrotransposons function by a "copy and paste" mechanism. Thus, they leave behind the original copy and generate a second copy that is inserted elsewhere in the genome. This process results in the insertion of repetitive sequences of DNA throughout the genome and is the mechanism responsible for the vast spread of transposable elements in many higher organisms.

The first step in retrotransposition occurs when the transposable DNA is copied into RNA. The RNA segment then jumps to another location in the genome. However, in order to be inserted into the genome at the new site, the RNA must be copied back into DNA by an enzyme called reverse transcriptase. There are several different types of retrotransposons, including long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs). About 20 percent of the human genome is made up of LINEs.

SAQ 2

Q. 1 Define antibiotic resistance.

Q. 2 Define operon concept.

Q. 3 What do you mean by repressor structure?

4.7.4 Transposons and antibiotic resistance

The simplest kinds of transposons merely contain a copy of the transposase with no additional genes. They behave as parasitic elements and usually have no known associated function that is advantageous to the host. More often, transposable elements have additional genes associated with them—for example, antibiotic resistance factors. Antibiotic resistance typically occurs when an infecting bacterium acquires a plasmid that carries a gene encoding resistance to one or more antibiotics. Typically, these resistance genes are carried on transposable elements that have moved into plasmids and are easily transferred from one organism to another. Once a bacterium picks up such a gene, it enjoys a great selective advantage because it can grow in the presence of the antibiotic. Indiscriminate use of antibiotics actually promotes the buildup of these drug-resistant plasmids and strains.

4.7.5 Transposons and disease

The functions of transposons remain unclear. They have long been referred to as “junk” DNA because they appear to serve little or no purpose or as “selfish” DNA because they serve only to copy and amplify themselves within genomes. In rare cases, however, transposons are associated with genetic mutations or chromosomal rearrangements that cause disease in humans. Disease typically arises from the insertion of transposons into particular regions of genes that are involved in regulating gene activity. For example, insertions near promoter regions, which are short segments of DNA that are used to initiate gene transcription (the synthesis of RNA from DNA), can lead to overactivity of genes. In some cases this can give rise to cancer. In other cases the site where a class II element is cut out of the genome is not repaired correctly, resulting in mutations that interfere with gene regulation and thereby cause cell dysfunction. There are also several diseases, including hemophilia and Duchenne muscular dystrophy, that are associated with repetitive DNA arising from retrotransposons.

4.8 Summary

A gene is a part of DNA that codes for a particular protein. DNA is the information database of the cell and exists within the cell nucleus. It carries all

the important genetic instructions that produce proteins required by our cells. Each gene carries a particular set of instructions, which is usually in coded format, used for an accurate function or for a distinct protein. The said genes are first transcribed into mRNA and then get converted into a polypeptide chain. A polypeptide is then converted to a protein. All the hidden codes inside our genes emerged as our physical traits, are known as gene expression.

This is a process where the gene's genetic codes are used in managing the proteins synthesis that is required for our body to produces the cell structures. Genes that carry information required for the sequences of amino acids are termed as structural genes. This process has two main steps. First is transcription; In this step, with the help of RNA polymerase enzymes, the messenger RNA is produced resulting in the processing of mRNA molecule. Second step is Translation; the main function of mRNA is to direct the synthesis of a protein resulting in the succeeding post-translational processing of the protein molecules.

Transposons are small pieces of DNA (500–1500 bp long) capable of moving themselves from one place to another within a genome. These mobile genetic elements were first recognized in maize (corn), but are now known to be present in essentially all organisms. Transposons are mobile elements that can move about the plant (or animal) genome. There are two types of transposons, what may be termed true transposons such as the *Ac/Ds* and *MuDR/Mu* maize transposons. If transposons move into a gene or genes that control leaf or flower color during the development of that organ, they can cause flecks or streaks of different colors that resemble virus symptoms. This can be especially noticeable in flowers, where one can find apparent flower color break most likely caused by the movement of a transposon.

4.9 Terminal questions

Q.1. Explain gene regulations in detail.

Answer:----------

Q.2. Explain operon concept.

Answer:----------

Q.3. Write a short note on transposons.

Answer:----------

Q.4. Explain lactose operon.

Answer:----------

Q.5. Write a short note on genetic material.

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Text book of Botany – Singh -Pande-Jain.
3. The elements of Botany- James Hewetson Wilson
4. Textbook of Biotechnology –H. K. Das
5. Biochemistry and molecular biology- Wilson Walker

Unit 5 Recombinant DNA Technology and Transgenic Plant

Structure

5.1 Introduction

Objectives:

5.2 Recombinant DNA

5.3 History of recombinant DNA

- 5.3.1 Controversy of recombinant DNA

5.4 What is Recombinant DNA Technology?

5.5 Tools of recombinant DNA technology

- 5.5.1 Process of Recombinant DNA Technology
- 5.5.2 Applications of recombinant DNA technology
- 5.5.3 Recombinant chymosin
- 5.5.4 Recombinant human insulin
- 5.5.5 Recombinant human growth hormone (HGH, somatotropin)
- 5.5.6 Recombinant blood clotting factor VIII
- 5.5.7 Recombinant hepatitis B vaccine
- 5.5.8 Diagnosis of infection with HIV
- 5.5.9 Golden rice
- 5.10 Herbicide-resistant crops
- 5.11 Insect-resistant crops

5.6 Transgene & transgenic plants

- 5.6.1 Transgene
- 5.6.2 Transgenic plants

5.7 Gene transfer in plants

5.8 Agrobacterium-mediated gene transfer

5.9 Applications of transgenic plants

- 5.9.1 Resistance to biotic or a biotic stresses
- 5.9.2 Improving crop yield and nutritional value
- 5.9.3 Transgenic plants as bioreactors for recombinant proteins

5.10 Summary

5.11Terminal questions

Further readings

5.1 Introduction

DNA, or deoxyribonucleic acid, is the hereditary material in humans and almost all other organisms. Nearly every cell in a person's body has the same DNA. Most DNA is located in the cell nucleus (where it is called nuclear DNA), but a small amount of DNA can also be found in the mitochondria (where it is called mitochondrial DNA or mtDNA). Mitochondria are structures within cells that convert the energy from food into a form that cells can use. The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T). Human DNA consists of about 3 billion bases, and more than 99 percent of those bases are the same in all people. The order, or sequence, of these bases determines the information available for building and maintaining an organism, similar to the way in which letters of the alphabet appear in a certain order to form words and sentences.

DNA bases pair up with each other, A with T and C with G, to form units called base pairs. Each base is also attached to a sugar molecule and a phosphate molecule. Together, a base, sugar, and phosphate are called a nucleotide. Nucleotides are arranged in two long strands that form a spiral called a double helix. The structure of the double helix is somewhat like a ladder, with the base pairs forming the ladder's rungs and the sugar and phosphate molecules forming the vertical sidepieces of the ladder. An important property of DNA is that it can replicate, or make copies of itself. Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases. This is critical when cells divide because each new cell needs to have an exact copy of the DNA present in the old cell.

Objectives:

This is the fifth unit of second block on molecular genetics II**.** Under fifth unit, we have following objectives. These are as under:

- To know about recombinant DNA & RDT
- To know applications of RDT and human insulin
- To discuss transgene and transgenic plants

- To know about HGH, somatotropin and golden rice
- To discuss gene transfer in plants and agrobacterium-mediated gene transfer
- To discuss nutritional value and applications of transgenic plants

5.2 Recombinant DNA

Recombinant DNA (rDNA) molecules are DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) that bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome. Recombinant DNA is the general name for a piece of DNA that has been created by combining at least two fragments from two different sources. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure, and differ only in the nucleotide sequence within that identical overall structure. Recombinant DNA molecules are sometimes called chimeric DNA, because they can be made of material from two different species, like the mythical chimera. R-DNA technology uses palindromic sequences and leads to the production of sticky and blunt ends.

The DNA sequences used in the construction of recombinant DNA molecules can originate from any species. For example, plant DNA may be joined to bacterial DNA, or human DNA may be joined with fungal DNA. In addition, DNA sequences that do not occur anywhere in nature may be created by the chemical synthesis of DNA, and incorporated into recombinant molecules. Using recombinant DNA technology and synthetic DNA, literally any DNA sequence may be created and introduced into any of a very wide range of living organisms.

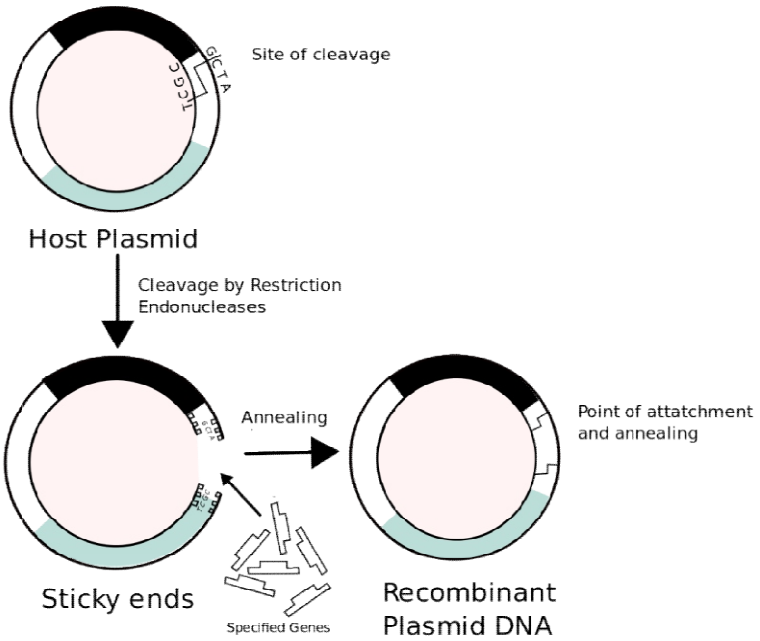

Fig. 1 Construction of recombinant DNA, in which a foreign DNA fragment is inserted into a plasmid vector.
In this example, the gene indicated by the white color is inactivated upon insertion of the foreign DNA fragment.

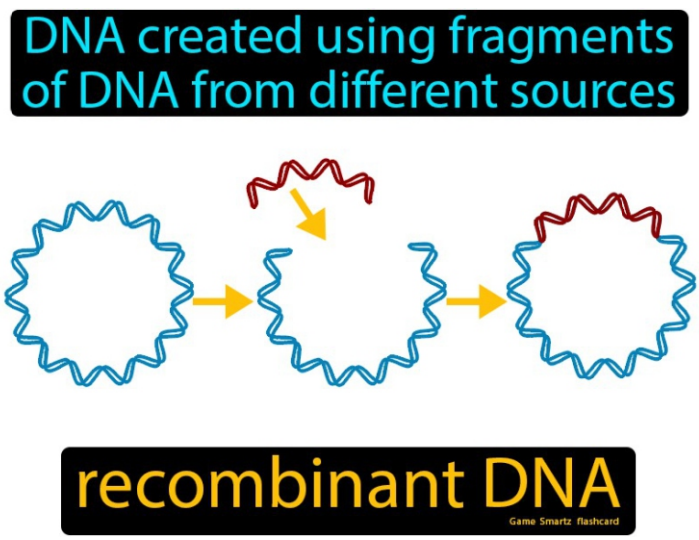

Fig.2 Recombinant DNA

Proteins that can result from the expression of recombinant DNA within living cells are termed *recombinant proteins*. When recombinant DNA encoding a protein is introduced into a host organism, the recombinant protein is not necessarily produced. Expression of foreign proteins requires the use of specialized expression vectors and often necessitates significant restructuring

by foreign coding sequences. Recombinant DNA differs from genetic recombination in that the former results from artificial methods in the test tube, while the latter is a normal biological process that results in the remixing of existing DNA sequences in essentially all organisms.

5.3 History of recombinant DNA

The idea of recombinant DNA was first proposed by Peter Lobban, a graduate student of Prof. Dale Kaiser in the Biochemistry Department at Stanford University Medical School. The first publications describing the successful production and intracellular replication of recombinant DNA appeared in 1972 and 1973, from Stanford and UCSF. In 1980 Paul Berg, a professor in the Biochemistry Department at Stanford and an author on one of the first papers was awarded the Nobel Prize in Chemistry for his work on nucleic acids "with particular regard to recombinant DNA". Werner Arber, Hamilton Smith, and Daniel Nathans shared the 1978 Nobel Prize in Physiology or Medicine for the discovery of restriction endonucleases which enhanced the techniques of rDNA technology.

Stanford University applied for a US patent on recombinant DNA in 1974, listing the inventors as Herbert W. Boyer (professor at the University of California, San Francisco) and Stanley N. Cohen (professor at Stanford University); this patent was awarded in 1980. The first licensed drug generated using recombinant DNA technology was human insulin, developed by Genentech and licensed by Eli Lilly and Company.

5.3.1 Controversy of recombinant DNA

Scientists associated with the initial development of recombinant DNA methods recognized that the potential existed for organisms containing recombinant DNA to have undesirable or dangerous properties. At the 1975 Asilomar Conference on Recombinant DNA, these concerns were discussed and a voluntary moratorium on recombinant DNA research was initiated for experiments that were considered particularly risky. This moratorium was widely observed until the National Institutes of Health (USA) developed and issued formal guidelines for rDNA work. Today, recombinant

DNA molecules and recombinant proteins are usually not regarded as dangerous.

However, concerns remain about some organisms that express recombinant DNA, particularly when they leave the laboratory and are introduced into the environment or food chain. These concerns are discussed in the articles on genetically modified organisms and genetically modified food controversies. Furthermore, there are concerns about the by-products in biopharmaceutical production, where recombinant DNA result in specific protein products. The major by-product, termed host cell protein, comes from the host expression system and poses a threat to the patient's health and the overall environment.

5.4 What is Recombinant DNA Technology?

A technique mainly used to change the phenotype of an organism (host) when a genetically altered vector is introduced and integrated into the genome of the organism. So, basically, this process involves the introduction of a foreign piece of DNA structure into the genome which contains our gene of interest. This gene which is introduced is the recombinant gene and the technique is called the recombinant DNA technology. There are multiple steps, tools and other specific procedure followed in the recombinant DNA technology, which is used for producing artificial DNA to generate the desired product.

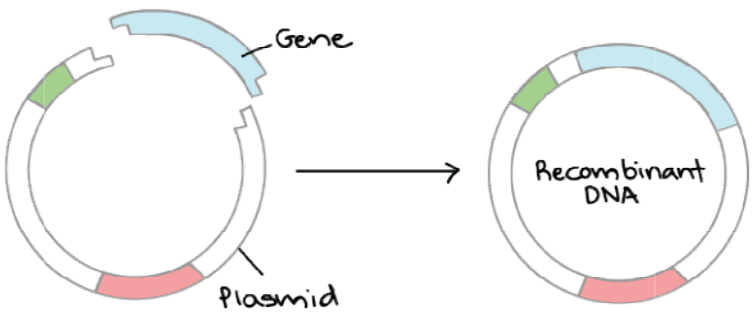

Fig. 3 DNA cloning

The technology used for producing artificial DNA through the combination of different genetic materials (DNA) from different sources is referred to as Recombinant DNA Technology. Recombinant DNA technology is popularly known as genetic engineering. The recombinant DNA technology emerged with the discovery of **restriction enzymes** in the year 1968 by Swiss

microbiologist Werner Arber, Inserting the desired gene into the genome of the host is not as easy as it sounds. It involves the selection of the desired gene for administration into the host followed by a selection of the perfect vector with which the gene has to be integrated and recombinant DNA formed. Thus the recombinant DNA has to be introduced into the host. And at last, it has to be maintained in the host and carried forward to the offspring.

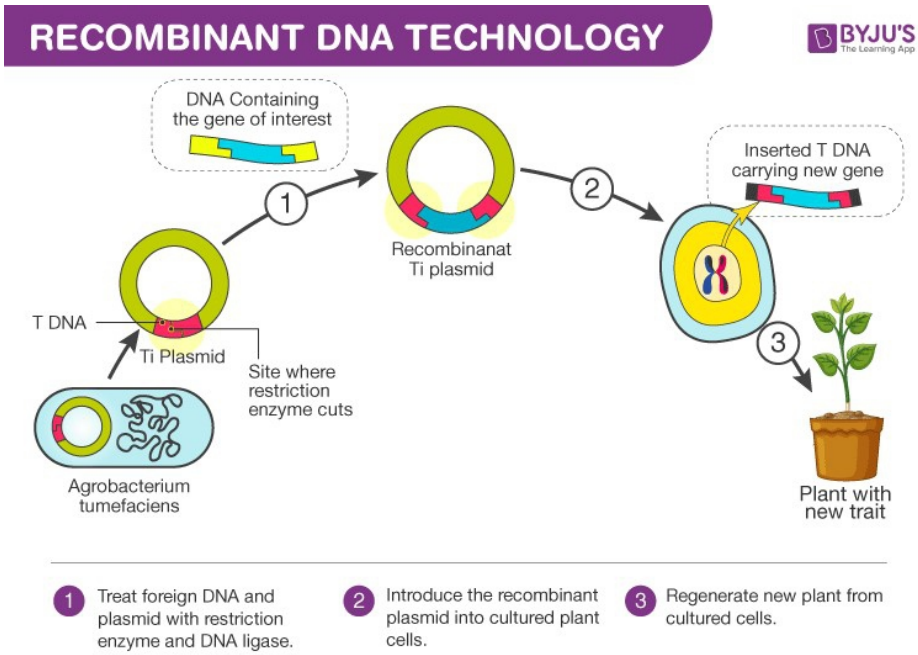

Fig. 4 Recombinant DNA technology

5.5 Tools of recombinant DNA technology

The enzymes which include the restriction enzymes help to cut, the polymerases- help to synthesize and the ligases- help to bind. The restriction enzymes used in recombinant DNA technology play a major role in determining the location at which the desired gene is inserted into the vector genome. They are two types, namely Endonucleases and Exonucleases. The Endonucleases cut within the DNA strand whereas the Exonucleases remove the nucleotides from the ends of the strands. The restriction endonucleases are sequence-specific which are usually palindrome sequences and cut the DNA at specific points. They scrutinize the length of DNA and make the cut at the specific site called the restriction site. This gives rise to sticky ends in the sequence. The desired genes and the vectors are cut by the same restriction enzymes to obtain the complementary sticky notes, thus making the work of the ligases easy to bind the desired gene to the vector.

The vectors – help in carrying and integrating the desired gene. These form a very important part of the tools of recombinant DNA technology as they are the ultimate vehicles that carry forward the desired gene into the host organism. Plasmids and bacteriophages are the most common vectors in recombinant DNA technology that are used as they have a very high copy number. The vectors are made up of an origin of replication- This is a sequence of nucleotide from where the replication starts, a selectable marker – constitute genes which show resistance to certain antibiotics like ampicillin; and cloning sites – the sites recognized by the restriction enzymes where desired DNAs are inserted.

Host organism – into which the recombinant DNA is introduced. The host is the ultimate tool of recombinant DNA technology which takes in the vector engineered with the desired DNA with the help of the enzymes. There are a number of ways in which these recombinant DNAs are inserted into the host, namely – microinjection, biolistics or gene gun, alternate cooling and heating, use of calcium ions, etc.

5.5.1 Process of Recombinant DNA Technology

The complete process of recombinant DNA technology includes multiple steps, maintained in a specific sequence to generate the desired product.

Step 1: Isolation of genetic material

The first and the initial step in Recombinant DNA technology is to isolate the desired DNA in its pure form i.e. free from other macromolecules.

Step 2: Cutting the gene at the recognition sites

The restriction enzymes play a major role in determining the location at which the desired gene is inserted into the vector genome. These reactions are called ‘restriction enzyme digestions’.

Step 3: Amplifying the gene copies through Polymerase chain reaction (PCR)

It is a process to amplify a single copy of DNA into thousands to millions of copies once the proper gene of interest has been cut using the restriction enzymes.

Step 4: Ligation of DNA molecules

In this step of Ligation, joining of the two pieces – a cut fragment of DNA and the vector together with the help of the enzyme DNA ligase.

Step 5: Insertion of recombinant DNA into host

In this step, the recombinant DNA is introduced into a recipient host cell. This process is termed as Transformation. Once after the insertion of the recombinant DNA into the host cell, it gets multiplied and is expressed in the form of the manufactured protein under optimal conditions. As mentioned in Tools of recombinant DNA technology, there are various ways in which this can be achieved. The effectively transformed cells/organisms carry forward the recombinant gene to the offspring.

5.5.2 Applications of recombinant DNA technology

Recombinant DNA is widely used in biotechnology, medicine and research. Today, recombinant proteins and other products that result from the use of DNA technology are found in essentially every western pharmacy, physician or veterinarian office, medical testing laboratory, and biological research laboratory. In addition, organisms that have been manipulated using recombinant DNA technology, as well as products derived from those organisms, have found their way into many farms, supermarkets, home medicine cabinets, and even pet shops, such as those that sell GloFish and other genetically modified animals.

The most common application of recombinant DNA is in basic research, in which the technology is important to most current work in the biological and biomedical sciences. Recombinant DNA is used to identify, map and sequence genes, and to determine their function. rDNA probes are employed in analyzing gene expression within individual cells, and throughout the tissues of whole organisms. Recombinant proteins are widely used as reagents in laboratory experiments and to generate antibody probes for examining protein synthesis within cells and organisms. Many additional practical applications of recombinant DNA are found in industry, food production, human and veterinary medicine, agriculture, and bioengineering. Some specific examples are as under:

5.5.3 Recombinant chymosin

Found in rennet, chymosin is an enzyme required to manufacture cheese. It was the first genetically engineered food additive used commercially. Traditionally, processors obtained chymosin from rennet, a preparation derived from the fourth stomach of milk-fed calves. Scientists engineered a non-pathogenic strain (K-12) of *E. coli* bacteria for large-scale laboratory production of the enzyme. This microbiologically produced recombinant enzyme, identical structurally to the calf derived enzyme, costs less and is produced in abundant quantities. Today about 60% of U.S. hard cheese is made with genetically engineered chymosin. In 1990, FDA granted chymosin "generally recognized as safe" (GRAS) status based on data showing that the enzyme was safe.

5.5.4 Recombinant human insulin

Almost completely replaced insulin obtained from animal sources (e.g. pigs and cattle) for the treatment of insulin-dependent diabetes. A variety of different recombinant insulin preparations are in widespread use. Recombinant insulin is synthesized by inserting the human insulin gene into *E. coli*, or yeast (Saccharomyces cerevisiae) which then produces insulin for human use.

5.5.5 Recombinant human growth hormone (HGH, somatotropin)

Administered to patients whose pituitary glands generate insufficient quantities to support normal growth and development. Before recombinant HGH became available, HGH for therapeutic use was obtained from pituitary glands of cadavers. This unsafe practice led to some patients developing Creutzfeldt–Jakob disease. Recombinant HGH eliminated this problem, and is now used therapeutically. It has also been misused as a performance-enhancing drug by athletes and others.

5.5.6 Recombinant blood clotting factor VIII

A blood-clotting protein that is administered to patients with forms of the bleeding disorder hemophilia, who are unable to produce factor VIII in quantities sufficient to support normal blood coagulation. Before the development of recombinant factor VIII, the protein was obtained by processing large quantities of human blood from multiple donors, which

carried a very high risk of transmission of blood borne infectious diseases, for example HIV and hepatitis B.

5.5.7 Recombinant hepatitis B vaccine

Hepatitis B infection is controlled through the use of a recombinant hepatitis B vaccine, which contains a form of the hepatitis B virus surface antigen that is produced in yeast cells. The development of the recombinant subunit vaccine was an important and necessary development because hepatitis B virus, unlike other common viruses such as polio virus, cannot be grown in vitro.

5.5.8 Diagnosis of infection with HIV

Each of the three widely used methods for diagnosing HIV infection has been developed using recombinant DNA. The antibody test (ELISA or western blot) uses a recombinant HIV protein to test for the presence of antibodies that the body has produced in response to an HIV infection. The DNA test looks for the presence of HIV genetic material using reverse transcription polymerase chain reaction (RT-PCR). Development of the RT-PCR test was made possible by the molecular cloning and sequence analysis of HIV genomes.

5.5.9 Golden rice

A recombinant variety of rice that has been engineered to express the enzymes responsible for β-carotene biosynthesis. This variety of rice holds substantial promise for reducing the incidence of vitamin A deficiency in the world's population. Golden rice is not currently in use, pending the resolution of regulatory and intellectual property issues.

5.10 Herbicide-resistant crops

Commercial varieties of important agricultural crops (including soy, maize/corn, sorghum, canola, alfalfa and cotton) have been developed that incorporate a recombinant gene that results in resistance to the herbicide glyphosate (trade name *Roundup*), and simplifies weed control by glyphosate application. These crops are in common commercial use in several countries.

5.11 Insect-resistant crops

Bacillus thuringeiensis is a bacterium that naturally produces a protein (Bt toxin) with insecticidal properties. The bacterium has been applied to crops as

an insect-control strategy for many years, and this practice has been widely adopted in agriculture and gardening. Recently, plants have been developed that express a recombinant form of the bacterial protein, which may effectively control some insect predators. Environmental issues associated with the use of these transgenic crops have not been fully resolved.

5.6 Transgene & transgenic plants

5.6.1 Transgene

A transgene is a gene that has been transferred naturally, or by any of a number of genetic engineering techniques from one organism to another. The introduction of a transgene, in a process known as transgenesis, has the potential to change the phenotype of an organism. *Transgene* describes a segment of DNA containing a gene sequence that has been isolated from one organism and is introduced into a different organism. This non-native segment of DNA may either retain the ability to produce RNA or protein in the transgenic organism or alter the normal function of the transgenic organism's genetic code. In general, the DNA is incorporated into the organism's germ line. For example, in higher vertebrates this can be accomplished by injecting the foreign DNA into the nucleus of a fertilized ovum. This technique is routinely used to introduce human disease genes or other genes of interest into strains of laboratory mice to study the function or pathology involved with that particular gene.

The construction of a transgene requires the assembly of a few main parts. The transgene must contain a promoter, which is a regulatory sequence that will determine where and when the transgene is active, an exon, a protein coding sequence (usually derived from the cDNA for the protein of interest), and a stop sequence. These are typically combined in a bacterial plasmid and the coding sequences are typically chosen from transgenes with previously known functions.

SAQ 1

Q. 1 Define recombinant DNA.

Q. 2 Define somatotropin.

Q. 3 What do you mean by RDT?

Q. 4 Define golden rice

5.6.2 Transgenic plants

Transgenic plants and associated bacteria constitute a new generation of genetically modified organisms for the treatment of polluted soil and water. This article focuses on the latest progresses in the development of transgenic organisms, including plants and associated bacteria, for the phytoremediation of major classes of organic environmental pollutants, such as chlorinated compounds, explosives, and pesticides. Phytoremediation is defined as the use of higher plants for the cost-effective, environmentally friendly rehabilitation of soil and groundwater contaminated by toxic metals and organic compounds. Phytoremediation involves a range of mechanisms, including uptake of pollutants from soil (phytoextraction), enzymatic transformation inside plant tissues (phytotransformation), volatilization into the atmosphere (phytovolatilization), and microbial degradation in the root zone (rhizoremediation). However, many persistent organic pollutants (POPs), such as explosives and polychlorinated biphenyls, are only slowly taken up and degraded by plants, resulting in accumulation and potential release of toxic metabolites into the environment. Similarly, bacteria naturally living in symbiosis with plants often do not possess the enzymatic machinery necessary for the efficient biodegradation of POPs. These limitations have led to the idea of genetic modification of plants and their associated bacteria by the introduction of key bacterial or mammalian genes known to be involved in the metabolism of xenobiotic pollutants, following a strategy similar to that used for the development of transgenic crops.

Transgenic or genetically modified organisms, be they bacteria, viruses or fungi, serve many research purposes. Transgenic plants, insects, fish and mammals (including humans) have been bred. Transgenic plants such as corn and soybean have replaced wild strains in agriculture in some countries (e.g. the United States). Transgene escape has been documented for GMO crops since 2001 with persistence and invasiveness. Transgenetic organisms pose ethical questions and may cause biosafety problems.

Transgenic plants have been created that are suitable for biodegradation of polymers, detoxification of heavy metals, for cleaning the environment from various types of pollution and to reduce the starvation and malnutrition. Plant resistance to insects was achieved by introducing the Bt genes from *Bacillus thuringiensis* into the plant genome, which encodes a protein, toxic to insects-Delta endotoxin, or CRY-protein - and is absolutely safe for mammals. Currently, so-called Bt-plants of cotton, potatoes and cornhave been producedand commercialized by the Environmental Protection Agency (EPA) in USA. The University of California (Toronto) bred a variety of tomatoes that are resistant to high levels of salt in the soil and retains salt in the leaf cells so that the fruit does not acquire a salty taste. The peculiarity and advantage of golden rice is the high content of provitamin A, iron, and folic acid.

Golden Rice was engineered to produce higher levels of beta-carotene, a precursor to vitamin A (major source of morbidity and mortality worldwide specially children) and later was found to express increased xanthophylls in substantial equivalence comparison high-pressure liquid chromatography (HPLC) analyses for carotenoids as an unintended effect of metabolites. This project was developed by international research team including European Union, India, the Philippines, Vietnam, and Bangladesh. Estimated number of 250,000 to 500,000 children (WHO) worldwide with deficiency of vitamin A cause blindness each year. Peoples living below poverty line and feeding on mainly on rice crop originated the concept of Golden Rice development where the rice genome was inserted with foreign genes to produce and accumulate the β-carotene in endosperm; rather than in green tissue naturally. Golden rice was an excellent example of positive effect of GM foods which ensured the recommended daily need of vitamin A—in form of β-carotene—in 100–200 g of rice, especially in rice-based feeding societies like India, Bangladesh, and Vietnam; that to without changing the cultural cuisine, significant reduction in clinical and subclinical vitamin A deficiency related diseases, and without creating new dependencies.

Fig. 5 Transgenic plant

Duckweed (*Lemna minor*) is a perennial plant whose habitat is the surface of stagnantfresh water pounds. Duckweed is a representative of monocotyledonous angiosperms, and to transfer foreign heterologous genes to its genome, agrobacterium mediated transformation system, and bioballistic methods can be used. Duckweed attracts the attention of researchers as a potential highly effective system for the expression of heterologous proteins due to its ability to rapidly accumulate biomass, which can double within 24–48 h. If appropriate signal sequences are present in the genetic structure, the recombinant protein can be secreted into the culture medium.

Green moss (*Physcomitrella patens*) is the only representative of mosses whose genome has been completely sequenced. A method for transforming *P. patens* was developed. The first successful example of using this type of moss as a recombinant protein expression system is the production of recombinant human erythropoietin. The attractiveness of this type of moss as a system of expression of recombinant proteins is that, in comparison with plants, fragments of exogenous DNA can be integrated into its genome by the mechanism of homologous recombination, which eliminates the possible inactivation of the expression of transferred genes (transgenes) in the future. An attractive aspect of *P. patens* is that it can be maintained as a suspension culture under controlled conditions.

Single-celled green algae (*Chlamydomonas reinhardtii, Phaeodactylum tricornutum, Tetraselmis suecica,* and *Odontella aurita*) are considered as

promising alternative systems for the expression of recombinant proteins. The most promising species is *C. reinhardtii*, whose recombinant protein production is based on chloroplast transformation. About 40% of the cell volume of this plant is occupied by one large chloroplast. The first GM algae based on *C. reinhardtii* were obtained in 1988. Cell populations of algae are homogeneous in cell size, with cell mass doubling every 4–8 h of cultivation. Large-volume bioreactors (up to 500,000 L) can be used for *C. reinhardtii* cultivation; moreover, the ability to secrete proteins into the culture medium can significantly reduce the cost of recombinant protein.

5.7 Gene transfer in plants

To add a desired trait to a crop, a foreign gene (transgene) encoding the trait must be inserted into plant cells, along with a "cassette" of additional genetic material. The cassette includes a DNA sequence called a "promoter," which determines where and when the foreign gene is expressed in the host, and a "marker gene" that allows breeders to determine which plants contain the inserted gene by screening or selection. For example, marker genes may render plants resistant to antibiotics that are not used medically (e.g., agromycin, canamycin) or tolerant to certain herbicides.

Two methods are used to transfer foreign genes into plants. The first method involves the use of a plant pathogen called Agrobacterium tumefaciens, which causes crown gall disease in many species. This bacterium has a plasmid, or loop of non-chromosomal DNA, that contains tumor-inducing genes (T-DNA), along with additional genes that help the T-DNA integrate into the host genome. For genetic engineering purposes, Agrobacterium must first be "disarmed" so that it does not make the plant sick. This is done by removing most of the T-DNA while leaving the left and right border sequences, which integrate a foreign gene into the genome of cultured plant cells. The second delivery method is a "gene gun," which fires gold particles carrying the foreign DNA into plant cells. Some of these particles pass through the plant cell wall and enter the cell nucleus, where the transgene integrates itself into the plant chromosome. Because both methods of gene transfer are fairly random, one must screen for the plant cells that contain the foreign gene.

5.8 Agrobacterium-mediated gene transfer

Introduction of additional DNA into plant cells or tissues is termed as plant transformation. A DNA of any source can be used for plant transformation. These transformation process has become an very important technique for both scientific and commercial purpose. These transgenic plants, which have a new set of DNA, aid in the understanding of plant gene regulation and also for identification and evaluation of useful traits in plants. Plant transformation is used for the introduction of these useful traits into other plants. Agrobacterium tumefaciens is the most efficient used vector to plant transformation. It has the advantage of evolved crown gall inducing mechanism of DNA transfer present in the common soil pathogen. Much has been learned about the mechanisms of this form of DNA movement and subsequent crown gall induction. A detailed protocol for Agrobacterium-mediated transformation of tobacco cells and their subsequent selection and regeneration into transgenic plants is as under.

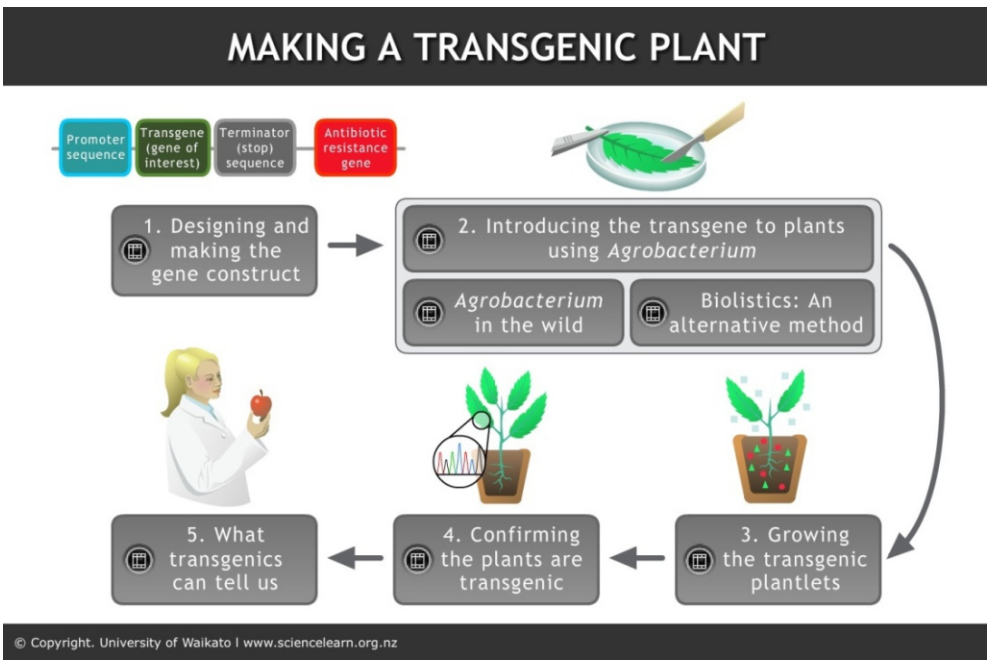

Fig. 6 Making a transgenic plant

The production of transgenic plants involves two major process. The first step is the introduction of new genetic material into plant cells, a process known as transformation. The second step uses tissue culture techniques for proliferation and to regenerate the transformed cells into a transgenic plants. Of various

methods used for plant transformation, transformation mediated by *Agrobacterium tumefaciens* is the most commonly used method.

Agrobacterium tumefaciens is a rod shaded, gram-negative bacteria found in soil and it is the natural causative agent for crown gall disease. The bacteria enters the plant through cuts or wounds present in its root or stem. The bacteria then inserts its DNA and stimulates the plant to grow swollen galls. *Agrobacterium tumefaciens* is capable of interkingdom DNA transfer, thus making it a potential vector in the production of transgenic plants. Transformation using this organism has its advantage in identification of transgenic expression. The presence of gall represents the manifestation of transfer and expression of additional DNA in plant cells. The application of this method is found both in scientific and commercial purpose.

Agrobacterium tumefaciens is attracted to the amino acids, sugars and organic acids released by the wounded plants. It binds the wounded tissue by polar attachment mechanism. During this attachment switches on the genetic operons critical to the start of gene transfer expression. The *vir* regulon is regulated and starts expression during the attachment stage. The wound phenolics and monosaccharides directly or indirectly cause the autophosphorylation of the *virA* transmembrane receptor kinase. This in turn activates the soluble cytoplasmic transcriptional factor *virG* through another phosphorylation event. Activated *Vir G* initiates the transcription of individual *vir* operon. These gene products produced in this process transfers the DNA fragment into the plant using the T-DNA present in the Tumour inducing (Ti) plasmid. T- strand is integrated with plant genome via non-homologous recombination by plant encode proteins. Most laboratory experiments use a binary system consisting of two plasmids. The oncogenes have been removed from the T-DNA sequence and replaced with DNA of interest. Different strains of *A. tumefaciens* display different virulence.

Once the plant cell has been introduced the new DNA in a stable manner, the next step is to regenerate a plant from the transformed cells. The isolation of transgenic plants is limited by lack of generation occurring in the transformed

cell population. Variability in the frequency and scope of regeneration among angiosperm species is high. Regeneration is done by two ways: organogenesis and somatic embryogenesis. Organogenesis is the formation of organized shoots and roots. Somatic embryogenesis is the formation of embryo like structure from somatic tissues.

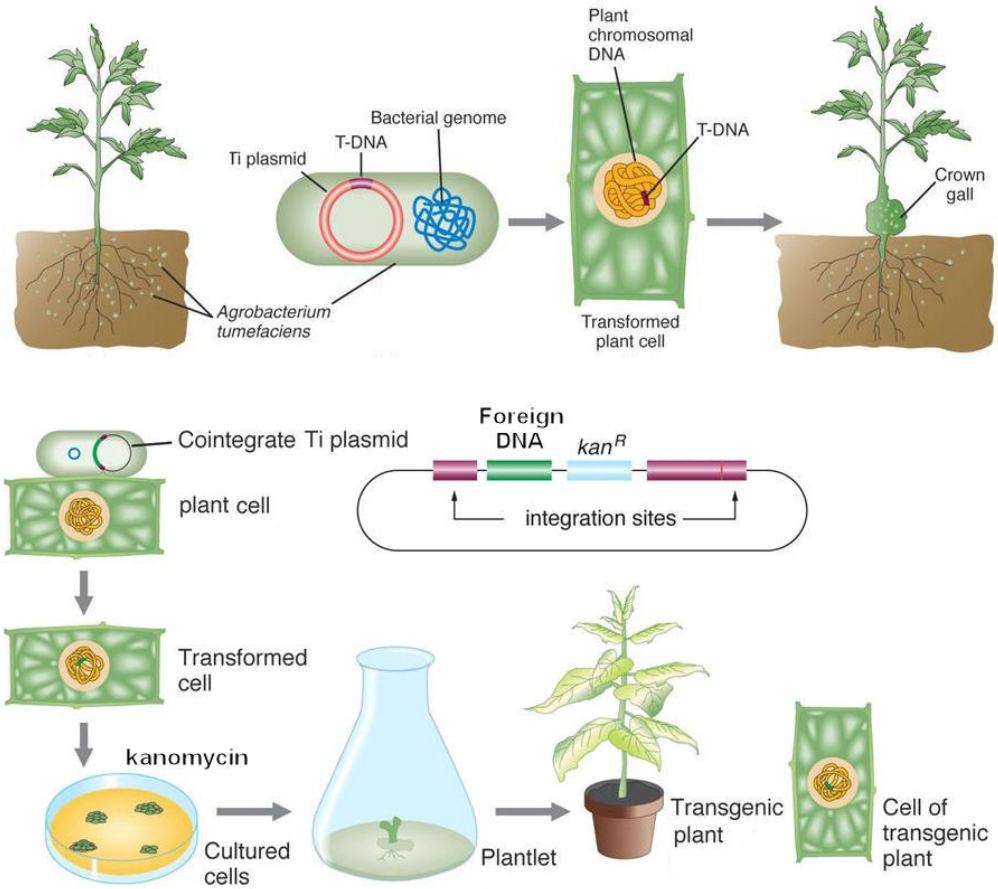

Fig. 7 Transgenic plant

5.9 Applications of transgenic plants

5.9.1 Resistance to biotic or a biotic stresses

Biotic stresses occur naturally as a result of stress exerted from other living organism within the same ecosystem. These include bacteria, viruses, herbivores, or native plants. Crop plants are incorporated with disease resistance gene to confer resistance toward these pathogenic diseases that are caused by pest, bacteria, and viruses; this includes tolerance to herbicides. The introduction of genetic modification technology could reduce the usage of expensive pesticides and herbicides in agriculture. The removal of natural pests

will lead to a greater yield and better quality of crops. As such, insecticidal toxin genes from a bacterium can be introduced into the plant of interest's genome, thus providing protection to the plant against insect pests. *Bacillus thuringiensis* (*Bt*) crops are an example of transgenic plant produced through this method. In addition, virus-resistant plants can be achieved through the introduction of viral coat proteins into plants.

Development of transgenic plants resistant to abiotic stresses is important in this "Global Warming's Terrifying Era". The world climate in the past few decades has changed tremendously culminating in changes to soil composition, humidity, water, sunlight availability, and many other agricultural problems that led to reduction in the crop yield. Hence, genetic engineering technology is needed as a tool to solve these problems by providing the plants with enhanced stress tolerant ability or protection. The manipulation of transcription factors (TFs), late embryogenesis abundant (LEA) proteins, and antioxidant proteins had successfully produced plants tolerant to drought and salinity. Overexpression of the proline biosynthesis enzyme (P5C), which allows the accumulation of osmoprotectant during drought season provides transgenic plants with osmotic stress resistance.

SAQ 2

Q. 1 Define transgene.

Q. 2 Define human insulin.

Q. 3 What do you mean by herbicide resistant plants?

Q. 4 Define recombinant hepatitis B vaccine

5.9.2 Improving crop yield and nutritional value

Malnutrition is a major health concern that is prevalent especially in the underdeveloped and developing countries due to limited access to nutritious food. Genetic engineering of staple crops has become one of the more effective solutions in addressing this problem. To date, a variety of crops had been successfully modified for better yield as well as for higher nutritional value. Biofortification is a technique used in agriculture to increase the nutritional value of crops. A well-known example would be the golden rice, a variety of *Oryza sativa*, produced to biosynthesize beta-carotene through genetic

modification. The golden rice was developed by adding two beta-carotene synthesis genes: phytoene synthase (*psy*) and lycopene β-cyclase (β-*lcy*) (originated from *Narcissus pseudonarcissus*). These genes were driven under the control of the endosperm-specific glutelin promoter together with a bacterial phytoene desaturase (*crt*I, from *Erwinia uredovora*).

5.9.3 Transgenic plants as bioreactors for recombinant proteins

Plants had been used as a biofactory in the production of the first recombinant human protein in 1989. Product yields from recombinant proteins using mammalian expression systems are low and expensive, while bacteria system is incapable of post-translational modification in complex protein formation. Due to this, the production methods had shifted to plant cell systems, which provide cheaper and better alternative sources for recombinant proteins production.

The recombinant proteins produced in transgenic plants include antibodies, metabolites or catabolites, proteins, and vaccines. Antibodies and vaccines against gastrointestinal tract diseases, cholera, and malaria are known to be produced in transgenic plants such as potato, banana, algae, and tobacco. An anticancer antibody that recognizes the cells of lung, breast, and colon cancer had also been successfully expressed in rice and wheat seed. However, despite a lot of successful plant-produced antibodies and vaccines, it is difficult to commercialize them and to date, the only plant-produced Newcastle disease vaccine had been approved by the United States department of agriculture for poultry farming with several vaccines in clinical trials.

5.10 Summary

In biology a clone is a group of individual cells or organisms descended from one progenitor. This means that the members of a clone are genetically identical, because cell replication produces identical daughter cells each time. The use of the word *clone* has been extended to recombinant DNA technology, which has provided scientists with the ability to produce many copies of a single fragment of DNA, such as a gene, creating identical copies that constitute a DNA clone. In practice the procedure is carried out by inserting a DNA fragment into a small DNA molecule and then allowing this molecule to replicate inside a simple living cell such as a bacterium. The small

replicating molecule is called a DNA vector (carrier). The most commonly used vectors are plasmids (circular DNA molecules that originated from bacteria), viruses, and yeast cells. Plasmids are not a part of the main cellular genome, but they can carry genes that provide the host cell with useful properties, such as drug resistance, mating ability, and toxin production. They are small enough to be conveniently manipulated experimentally, and, furthermore, they will carry extra DNA that is spliced into them.

It is essential that we improve food production and distribution in order to feed and free from hunger a growing world population, while reducing environmental impacts and providing productive employment in low-income areas. This will require a proper and responsible utilization of scientific discoveries and new technologies. The developers and overseers of GM technology applied to plants and micro-organisms should make sure that their efforts address such needs. Foods can be produced through the use of GM technology that are more nutritious, stable in storage, and in principle health promoting—bringing benefits to consumers in both industrialized and developing nations.

5.11Terminal questions

Q.1 What do you mean by recombinant DNA?

Answer:----------

Q.2 Describe recombinant DNA technology (RDT).

Answer:----------

Q.3 Write a short note on transgenic plant.

Answer:----------

Q.4 Write a short note on transgenic animal.

Answer:----------

Q.5 Write a short note on genetic engineering.

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Text book of Botany – Singh -Pande-Jain.
3. The elements of Botany- James Hewetson Wilson
4. Textbook of Biotechnology –H. K. Das
5. Biochemistry and molecular biology- Wilson Walker

Unit 6 Monoclonal Antibodies, DNA Probe and DNA Finger Printing

Structure

6.1 Introduction

Objectives:

6.2 Monoclonal antibodies

6.3 History

6.4 How do monoclonal antibody drugs work?

6.5 Producing monoclonal antibodies

6.6 Applications

- 6.6.1 Diagnostic tests
- 6.6.2 Analytic and chemical uses
- 6.6.3 Therapeutic uses
- 6.6.4 Cancer treatment
- 6.6.5 Autoimmune diseases

6.7 DNA Probes (labelling, types and uses)

6.7.1 Type of probe

- 6.7.1.1 DNA probe
- 6.7.1.2 Labelling of probe:
- 6.7.1.3 How probe hybridization occurs?
- 6.7.1.4 Applications: :

6.8 DNA fingerprinting

6.9 Methods of DNA Fingerprinting

6.10 Applications of DNA Fingerprinting

- 6.10.1 Forensic Science:
- 6.10.2 Paternity and Maternity Determination:
- 6.10.3 Personal Identification:
- 6.10.4 Diagnosis of Inherited Disorders:
- 6.10.5 Development of Cures for Inherited Disorders:
- 6.10.6 Detection of AIDS:
- 6.10.7 Breeding Program:

6.11 Summary

6.12 Terminal questions

Further readings

6.1 Introduction

The immune system is made up of a complex team of players that detect and destroy disease-causing agents, such as bacteria and viruses. Similarly, this system may eliminate damaged cells, such as cancer cells. One way the immune system finds and destroys invaders is with antibodies. An antibody attaches itself to a specific molecule (antigen) on the surface of the target cell, such as a cancer cell. When an antibody binds to the cell, it serves as a flag to attract disease-fighting molecules or as a trigger that promotes cell destruction by other immune system processes. Cancer cells are often able to avoid detection by the immune system. The cancer cells might mask themselves so they can hide or the cancer cells might release signals that block the immune system cells from working correctly.

A type of protein that is made in the laboratory and can bind to certain targets in the body, such as antigens on the surface of cancer cells. There are many kinds of monoclonal antibodies, and each monoclonal antibody is made so that it binds to only one antigen. Monoclonal antibodies are being used in the diagnosis and treatment of many diseases, including some types of cancer. They can be used alone or to carry drugs, toxins, or radioactive substances directly to cancer cells.

Objectives:

This is the sixth unit of second block on molecular genetics II**.** Under sixth unit, we have following objectives. These are as under:

- To know about monoclonal antibodies and applications
- To know about DNA probe, its type an applications
- To know about autoimmune disease, diagnostic tests and cancer treatment
- To discuss DNA fingerprinting and its applications
- To know about forensic science, breeding programme and detection of AIDS

6.2 Monoclonal antibodies

A monoclonal antibody (mAb or moAb) is an antibody made by cloning a unique white blood cell. All subsequent antibodies derived this way trace back

to a unique parent cell. Monoclonal antibodies can have monovalent affinity, binding only to the same epitope (the part of an antigen that is recognized by the antibody). In contrast, polyclonal antibodies bind to multiple epitopes and are usually made by several different antibody secreting plasma cell lineages. Bispecific monoclonal antibodies can also be engineered, by increasing the therapeutic targets of one monoclonal antibody to two epitopes. Monoclonal antibodies are laboratory-produced molecules engineered to serve as substitute antibodies that can restore, enhance, modify or mimic the immune system's attack on cells that aren't wanted, such as cancer cells.

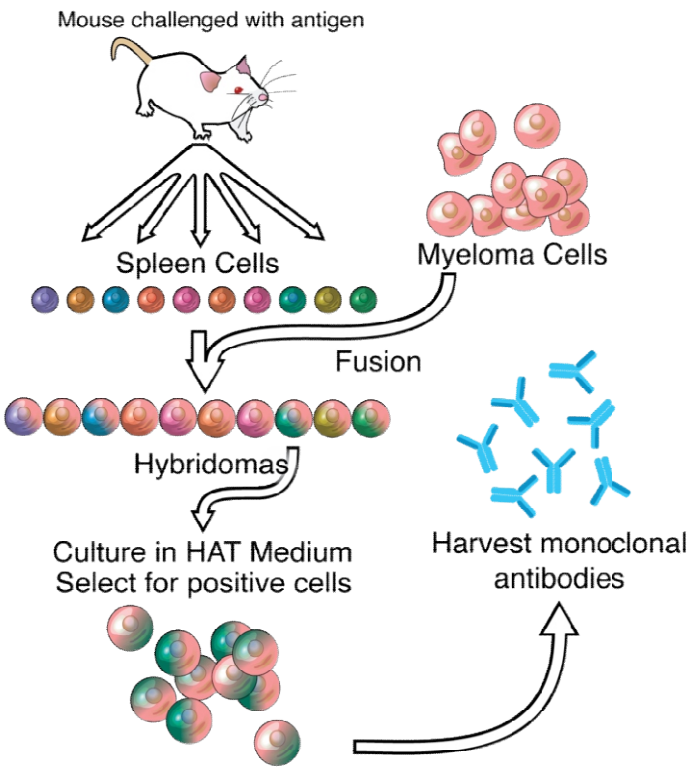

Fig. 1 A general representation of the method used to produce monoclonal antibodies

It is possible to produce monoclonal antibodies that specifically bind to virtually any suitable substance; they can then serve to detect or purify it. This capability has become an important tool in biochemistry, molecular biology, and medicine. Monoclonal antibodies are being used on a clinical level for both the diagnosis and therapy of several diseases.[3] The administration of monoclonal antibodies was authorized by several countries for the treatment of moderate COVID-19 symptoms.

6.3 History

In the early 1900s, immunologist Paul Ehrlich proposed the idea of a Zauberkugel – "magic bullet", conceived of as a compound which selectively targeted a disease-causing organism, and could deliver a toxin for that organism. This underpinned the concept of monoclonal antibodies and monoclonal drug conjugates. Ehrlich and Élie Metchnikoff received the 1908 Nobel Prize for Physiology or Medicine for providing the theoretical basis for immunology.

By the 1970s, lymphocytes producing a single antibody were known, in the form of multiple myeloma – a cancer affecting B-cells. These abnormal antibodies or paraproteins were used to study the structure of antibodies, but it was not yet possible to produce identical antibodies specific to a given antigen. In 1973, Jerrold Schwaber described the production of monoclonal antibodies using human–mouse hybrid cells. This work remains widely cited among those using human-derived hybridomas. In 1975, Georges Köhler and César Milstein succeeded in making fusions of myeloma cell lines with B cells to create hybridomas that could produce antibodies, specific to known antigens and that were immortalized. They and Niels Kaj Jerne shared the Nobel Prize in Physiology or Medicine in 1984 for the discovery.

In 1988, Greg Winter and his team pioneered the techniques to humanize monoclonal antibodies, eliminating the reactions that many monoclonal antibodies caused in some patients. By the 1990s research was making progress in using monoclonal antibodies therapeutically, and in 2018, James P. Allison and Tasuku Honjo received the Nobel Prize in Physiology or Medicine for their discovery of cancer therapy by inhibition of negative immune regulation, using monoclonal antibodies that prevent inhibitory linkages.

6.4 How do monoclonal antibody drugs work?

Monoclonal antibodies are designed to function in different ways. A particular drug may actually function by more than one means. The workings of monoclonal antibodies are as under:

- **Flagging cancer cells.** Some immune system cells depend on antibodies to locate the target of an attack. Cancer cells that are coated in monoclonal antibodies may be more easily detected and targeted for destruction.
- **Triggering cell-membrane destruction.** Some monoclonal antibodies can trigger an immune system response that can destroy the outer wall (membrane) of a cancer cell.
- **Blocking cell growth.** Some monoclonal antibodies block the connection between a cancer cell and proteins that promote cell growth — an activity that is necessary for cancer growth and survival.
- **Preventing blood vessel growth.** In order for a cancerous tumor to grow and survive, it needs a blood supply. Some monoclonal antibody drugs block protein-cell interactions necessary for the development of new blood vessels.
- **Blocking immune system inhibitors.** Your body keeps your immune system from being overactive by making proteins that control the activity of the immune system cells. Monoclonal antibodies can interfere with that process so that your immune system cells are allowed to work without controls against cancer cells.
- **Directly attacking cancer cells.** Certain monoclonal antibodies may attack the cell more directly. When some of these antibodies attach to a cell, a series of events inside the cell may cause it to self-destruct.
- **Delivering radiation treatment.** Because of a monoclonal antibody's ability to connect with a cancer cell, the antibody can be engineered as a delivery vehicle for other treatments. When a monoclonal antibody is combined with a small radioactive particle, it transports the radiation treatment directly to cancer cells and may minimize the effect of radiation on healthy cells.
- **Delivering chemotherapy.** Similarly, some monoclonal antibodies are combined with a chemotherapy drug in order to deliver the treatment directly to the cancer cells while avoiding healthy cells.
- **Binding cancer and immune cells.** Some drugs combine two monoclonal antibodies, one that attaches to a cancer cell and one that attaches to a specific immune system cell. This connection may promote immune system attacks on the cancer cells.

6.5 Producing monoclonal antibodies

Some types of assays require better antibody specificity and affinity than can be obtained using a polyclonal antiserum. To attain this high specificity, all of the antibodies must bind with high affinity to a single epitope. This high specificity can be provided by **monoclonal antibodies (mAbs)**. The given table compares some of the important characteristics of monoclonal and polyclonal antibodies.

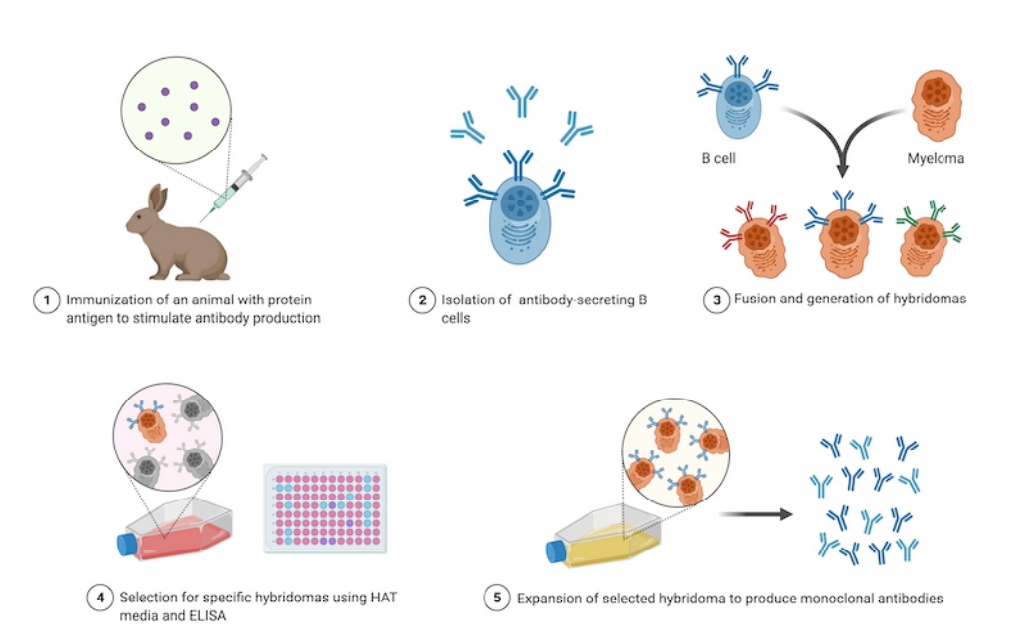

Fig. 2 Monoclonal antibodies (Mabs)

Unlike polyclonal antibodies, which are produced in live animals, monoclonal antibodies are produced *in vitro* using tissue-culture techniques. mAbs are produced by immunizing an animal, often a mouse, multiple times with a specific antigen. B cells from the spleen of the immunized animal are then removed. Since normal B cells are unable to proliferate forever, they are fused with immortal, cancerous B cells called myeloma cells, to yield **hybridoma** cells. All of the cells are then placed in a selective medium that allows only the hybridomas to grow; unfused myeloma cells cannot grow, and any unfused B cells die off. The hybridomas, which are capable of growing continuously in culture while producing antibodies, are then screened for the desired mAb. Those producing the desired mAb are grown in tissue culture; the culture medium is harvested periodically and mAbs are purified from the medium. This is a very expensive and time-consuming process. It may take

weeks of culturing and many liters of media to provide enough mAbs for an experiment or to treat a single patient. mAbs are expensive as under.

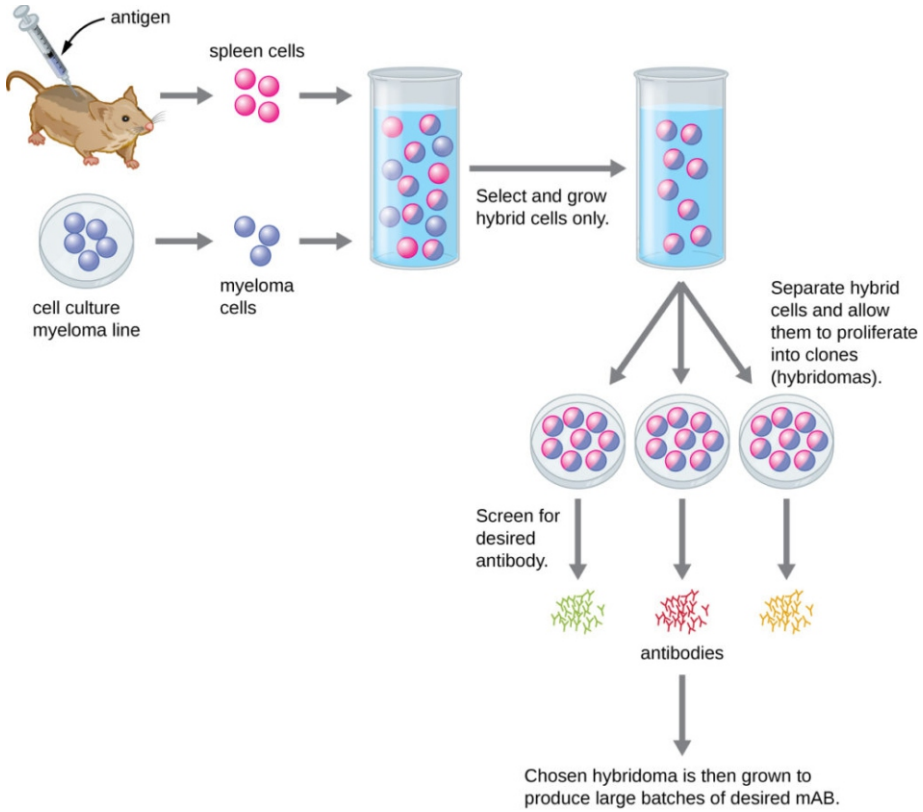

Fig. 3 Monoclonal antibodies (mAbs) are produced by introducing an antigen to a mouse and then fusing
polyclonal B cells from the mouse's spleen to myeloma cells. The resulting hybridoma cells are cultured and continue to produce antibodies to the antigen.

6.6 Applications

6.6.1 Diagnostic tests

Once monoclonal antibodies for a given substance have been produced, they can be used to detect the presence of this substance. Proteins can be detected using the Western blot and immuno dot blot tests. In immunohistochemistry, monoclonal antibodies can be used to detect antigens in fixed tissue sections, and similarly, immunofluorescence can be used to detect a substance in either frozen tissue section or live cells.

6.6.2 Analytic and chemical uses

Antibodies can also be used to purify their target compounds from mixtures, using the method of immunoprecipitation.

6.6.3 Therapeutic uses

Therapeutic monoclonal antibodies act through multiple mechanisms, such as blocking of targeted molecule functions, inducing apoptosis in cells which express the target, or by modulating signalling pathways.

6.6.4 Cancer treatment

One possible treatment for cancer involves monoclonal antibodies that bind only to cancer-cell-specific antigens and induce an immune response against the target cancer cell. Such mAbs can be modified for delivery of a toxin, radioisotope, cytokine or other active conjugate or to design bispecific antibodies that can bind with their Fab regions both to target antigen and to a conjugate or effector cell. Every intact antibody can bind to cell receptors or other proteins with its Fc region. MAbs approved by the FDA for cancer include:

- Alemtuzumab
- Bevacizumab
- Cetuximab
- Gemtuzumab ozogamicin
- Ipilimumab
- Nivolumab

6.6.5 Autoimmune diseases

Monoclonal antibodies used for autoimmune diseases include infliximab and adalimumab, which are effective in rheumatoid arthritis, Crohn's disease, ulcerative colitis and ankylosing spondylitis by their ability to bind to and inhibit TNF-α. Basiliximab and daclizumab inhibit IL-2 on activated T cells and thereby help prevent acute rejection of kidney transplants. Omalizumab inhibits human immunoglobulin E (IgE) and is useful in treating moderate-to-severe allergic asthma.

6.7 DNA Probes (labelling, types and uses)

DNA probes are the known short, single-stranded, labelled DNA sequences used to detect the presence or absence of nucleic acid in a sample." In situ hybridization allows the use of the DNA or RNA probes to employ in the detection of various nucleic acid present in any biological

sample. Therefore it is used in medical industries, food industries, microbial identification and environmental science. In general, the nucleic acid probes are 100 to 10000 basepair long, tagged with the coloured molecule either radioisotopes or fluorochromes. The aim of using the DNA probe is to identify the presence of a gene or DNA sequence of interest present on a chromosome or in a sample. A probe can be synthesised artificially or by the in vitro methods or amplification methods.

6.7.1 Type of probe

Based on our sample, the nucleic acid hybridization probe might be a DNA probe or RNA probe:

6.7.1.1 DNA probe

The DNA probe is used to detect the presence of the DNA or a gene present in a sample. 100 to 10000bp long complementary DNA sequence is used to detect the presence of complementary DNA sequence. For that, the single-stranded probe is first prepared by denaturing it and employed it for hybridization on the nitrocellulose paper. The DNA probe can also be synthesised chemically or prepared using the amplification method or using the cDNA library.

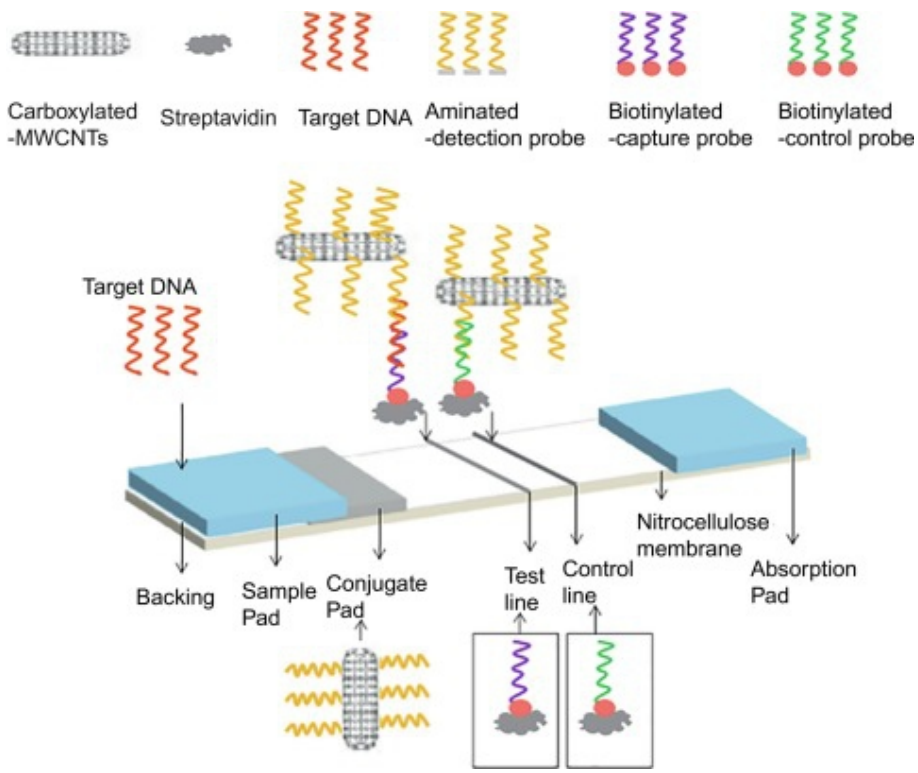

Fig 4 DNA probes- An overview

The DNA probes are adopted for detection of the microorganism present in a sample and DNA quantification. DNA probes are the first choice in the FISH-fluorescence in situ hybridization in which probes are allowed to hybridize directly on the chromosome. The computational software and high-end microscopes used to detect the results. TaqMan probe, scorpion probe and molecular beacons are commonly used DNA probes in the realtime PCR.

SAQ 1

Q. 1 Define monoclonal antibodies.

Q. 2 Define DNA fingerprinting.

Q. 3 What do you mean by autoimmune disease?

Examples of DNA probe

TaqMan probe

The TaqMan probe is one of the most popular types of probe chemistry available nowadays. A type of hydrolysis probe, commonly used in the real-time PCR assay, and developed by Kary Mullis, himself- the founder of PCR. The chemistry of the TaqMan probe is based on the exonuclease activity of the Taq DNA polymerase enzyme. The one end (5') of the probe contains a fluorophore while the other end (3') contains the quencher molecule which is in close proximity of the fluorophore.

Once the probe finds its complementary sequence, it binds to it, the DNA polymerase starts synthesising the strand, but removes the probe by the 3' to 5' exonuclease activity. The probe unquenched, the fluorophores detached from the quencher molecule and emits the fluorescent. The probe is hydrolysed and the fluorescent emitted by it is measured by the detector. This is the principle of the TaqMan probe.

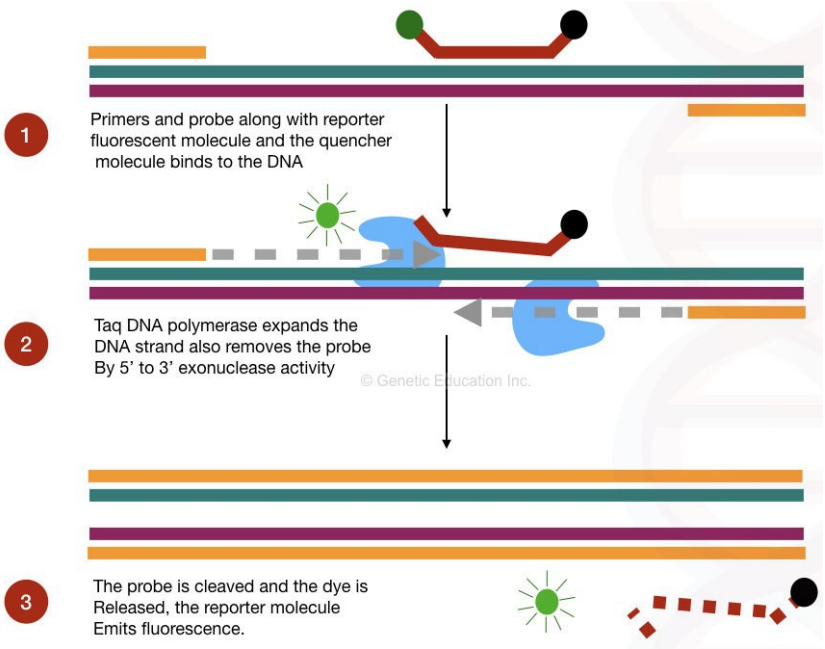

Fig. 5 The TaqMan probe hybridization method used in the realtime PCR.

Taqman probes are widely used in the DNA or gene quantification and gene expression studies. Some of the commercially available probes avail researchers for rapid detection and quantification of microorganism.

6.7.1.2 Labelling of probe:

One of the important characteristics of the probe is the label attached on its end which makes it different from the DNA primers. Either radioactive molecule or non- radioactive molecule are used to label the probe. Radio-active molecules such as 32P or 35S are used to do so. Autoradiography is used to detect the hybridization, however, the radiolabelled molecules are not safer to use. In order to achieve safety, non-radioactive compounds, such fluorophores are now used to label the probe which is safer than the radioactive compounds, however, the sensitivity is low. Other molecules such as biotin and digoxigenin are also used in the labelling of the probe as well.

6.7.1.3 How probe hybridization occurs?

Traditionally, the method is known as southern blot (for DNA hybridization) or northern blot (for RNA hybridization). In the very first step, the DNA sequence of our interest is digested with the restriction endonuclease and allow to immobilised on the nitrocellulose paper. A complementary probe is selected, labelled and denatured first. Then in the next step, the probe mixture allows

binding on the nitrocellulose paper, if it finds the exact complementary sequence, it will bind.

The unbound probes are removed by washing the nitrocellulose paper with the wash buffer. The final results are collected using the autoradiography method. In the modern version of this technique- called a microarray, the probes are hybridized instead of sample DNA. The sample is allowed to hybridize on the surface containing millions of probe, thus microarray enables to screen many mutations or alterations at once.

6.7.1.4 Applications:

Quantification of nucleic acid- one of the important application of the probe-based method is in the quantification of nucleic acid- DNA or RNA present in the sample. Besides this, it is used in the microbial identification/quantification, disease diagnosis and gene mapping. It is also used in forensic science and DNA profiling studies.

Limitation:

Non-specific binding is one of the key limitations of using DNA probes in molecular genetics. Sometimes the probe binds to the less complementary sequences and emits the fluorescence, also in some higher GC rich template the probe can not be bind properly. In both cases, positive results can not be obtained.

6.8 DNA fingerprinting

DNA fingerprinting, also called **DNA typing, DNA profiling, genetic fingerprinting, genotyping,** or **identity testing**, in genetics, method of isolating and identifying variable elements within the base-pair sequence of DNA (deoxyribonucleic acid). The technique was developed in 1984 by British geneticist Alec Jeffreys, after he noticed that certain sequences of highly variable DNA (known as minisatellites), which do not contribute to the functions of genes, are repeated within genes. Jeffreys recognized that each individual has a unique pattern of minisatellites (the only exceptions being multiple individuals from a single zygote, such as identical twins).

Fig. 6 DNA fingerprinting

The procedure for creating a DNA fingerprint consists of first obtaining a sample of cells, such as skin, hair, or blood cells, which contain DNA. The DNA is extracted from the cells and purified. In Jeffreys's original approach, which was based on restriction fragment length polymorphism (RFLP) technology, the DNA was then cut at specific points along the strand with proteins known as restriction enzymes. The enzymes produced fragments of varying lengths that were sorted by placing them on a gel and then subjecting the gel to an electric current (electrophoresis): the shorter the fragment, the more quickly it moved toward the positive pole (anode). The sorted double-stranded DNA fragments were then subjected to a blotting technique in which they were split into single strands and transferred to a nylon sheet. The fragments underwent autoradiography in which they were exposed to DNA probes—pieces of synthetic DNA that were made radioactive and that bound to the minisatellites. A piece of X-ray film was then exposed to the fragments, and a dark mark was produced at any point where a radioactive probe had become attached. The resultant pattern of marks could then be analyzed.

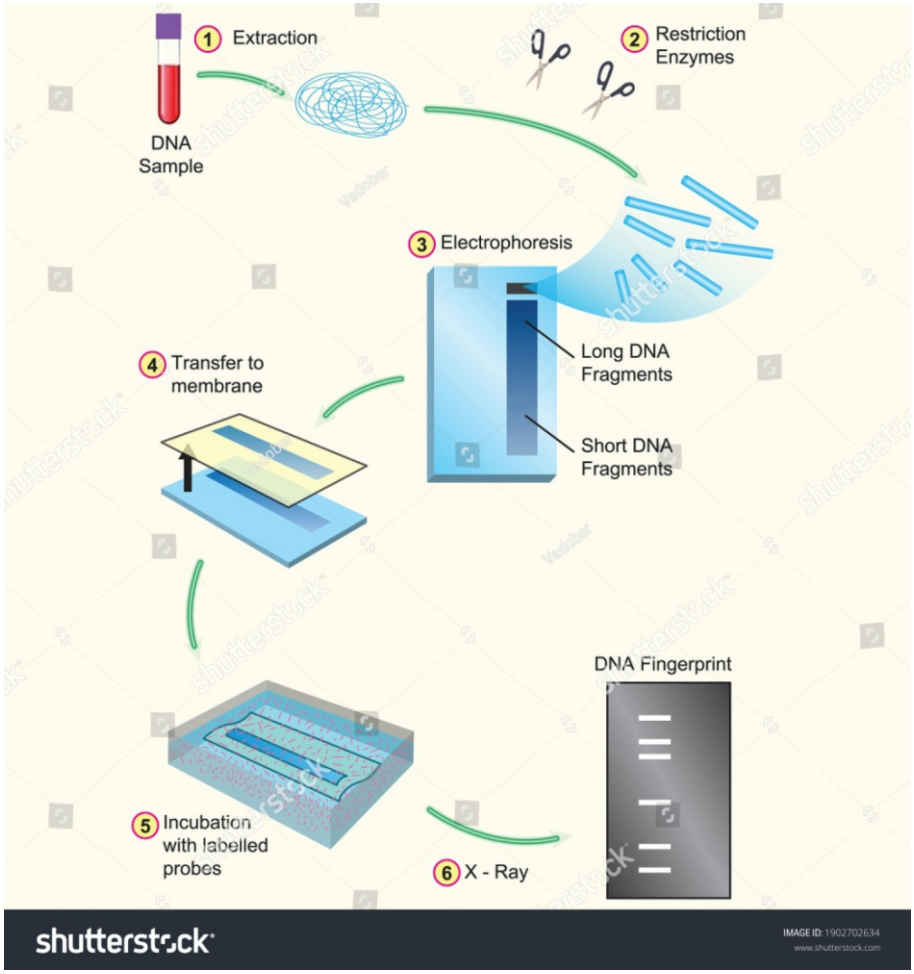

Fig. 7 Different steps in DNA fingerprinting

The assay developed by Jeffreys has been supplanted by approaches that are based on the use of the polymerase chain reaction (PCR) and so-called microsatellites (or short tandem repeats, STRs), which have shorter repeat units (typically 2 to 4 base pairs in length) than minisatellites (10 to more than 100 base pairs in length). PCR amplifies the desired fragment of DNA (e.g., a specific STR) many times over, creating thousands of copies of the fragment. It is an automated procedure that requires only small amounts of DNA as starting material and works even with partially degraded DNA. Once an adequate amount of DNA has been produced with PCR, the exact sequence of nucleotide pairs in a segment of DNA can be determined by using one of several biomolecular sequencing methods. Automated equipment has greatly increased the speed of DNA sequencing and has made available many new practical applications, including pinpointing segments of genes that cause genetic

diseases, mapping the human genome, engineering drought-resistant plants, and producing biological drugs from genetically altered bacteria.

An early use of DNA fingerprinting was in legal disputes, notably to help solve crimes and to determine paternity. Since its development, DNA fingerprinting has led to the conviction of numerous criminals and to the freeing from prison of many individuals who were wrongly convicted. However, making scientific identification coincide exactly with legal proof is often problematic. Even a single suggestion of the possibility of error is sometimes enough to persuade a jury not to convict a suspect. Sample contamination, faulty preparation procedures, and mistakes in interpretation of results are major sources of error. In addition, RFLP requires large amounts of high-quality DNA, which limits its application in forensics. Forensic DNA samples frequently are degraded or are collected postmortem, which means that they are lower-quality and subject to producing less-reliable results than samples that are obtained from a living individual. Some of the concerns with DNA fingerprinting, and specifically the use of RFLP, subsided with the development of PCR- and STR-based approaches.

6.9 Methods of DNA Fingerprinting

Restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) amplification of short tandem repeats (STRs) are two main DNA tests widely used for DNA fingerprinting.

A. Restriction fragment length polymorphism (RFLP)

1. The first step in this process is to **isolate the DNA** from the sample material to be tested. The sample size for RFLP test must be large enough to get the proper result.
2. Once the required size of the sample is available, the DNA is isolated from the sample and is **subjected to restriction digestion** using restriction enzymes.
3. The digested DNA sample is then **separated by agarose gel electrophoresis**, in which the DNA is separated based on the size.
4. The next step is **transfer of separated DNA from gel slab onto the nitrocellulose membrane** to hybridize with a labeled probe that is

specific for one VNTR region (radio activity labeled complimentary sequence for VNTR region nucleotide sequence).

5. This technique of transferring and hybridizing DNA onto nitrocellulose membrane is known as southern blotting, a most widely used DNA detection technique by molecular biologists.
6. After the hybridization with the radioactive probes, the **X- ray film** is developed form the southern blotting and only the areas where the radioactive probe binds will show up on the film.
7. Now these bands when **compared with the other known sample**s, will give the final result of the DNA fingerprinting.

Advantages

The RFLP is considered to be more accurate than the PCR, mainly because the size of the sample used more, use of a fresh DNA sample, and no amplification contamination.

Limitation

The RFLP, however, require longer time period to complete the analysis and is costly.

B. Polymerase Chain Reaction (PCR) amplification of short tandem repeats (STRs)

- ✓ Thousands of copies of a particular variable region are amplified by **PCR** which forms the basis of this detection.
- ✓ STR with a known repeat sequence is amplified and separated using gel-electrophoresis.
- ✓ For the amplification of STRs using PCR, a short synthetic DNA, called primers are specially designed to attach to a highly conserved common nonvariable region of DNA that flanks the variable region of the DNA.
- ✓ By comparing the STR sequence size amplified by PCR with the other known samples, will give the final result of the DNA fingerprinting.

Advantages

- ✓ Small amount of specimen is sufficient for the test.
- ✓ Takes a shorter time to complete.
- ✓ Less costly.

Limitation

- ✓ Less accurate than RFLP.
- ✓ Possibility of amplification contamination.

SAQ 2

Q. 1 Define applications of monoclonal antibodies.

Q. 2 Define applications of DNA fingerprinting.

Q. 3 What do you mean by forensic science?

6.10 Applications of DNA Fingerprinting

DNA Fingerprinting is used by scientists to distinguish between individuals of the same species using only samples of their DNA. It is a primary method for identifying an individual.

6.10.1 Forensic Science:

Biological materials used for DNA profiling are: Blood, Hair, Saliva, Semen, Body tissue cells etc. DNA isolated from the evidence sample can be compared through VNTR (Variable number of tandem repeats) prototype. It is useful in solving crimes like murder and rape.

6.10.2 Paternity and Maternity Determination:

A Person accedes to his or her VNTRs from his or her parents. Parent-child VNTR prototype analysis has been used to solve disputed cases. This information can also be used in inheritance cases, immigration cases.

6.10.3 Personal Identification:

It utilizes the concept of using DNA fingerprints as a sort of genetic bar code to pinpoint individuals.

6.10.4 Diagnosis of Inherited Disorders:

It is also useful in diagnosing inherited disorders in both prenatal and newborn babies. These disorders may include cystic fibrosis, hemophilia, Huntington's disease, familial Alzheimer's, sickle cell anemia, thalassemia, and many others.

6.10.5 Development of Cures for Inherited Disorders:

By studying the DNA fingerprints of relatives who have a history of some particular disorder, DNA prototypes associated with the disease can be ascertained.

6.10.6 Detection of AIDS:

By comparing the band of HIV "RNA" (converted to DNA using RTPCR) with the bands form by the man's blood, person suffering with AIDS can be identified.

6.10.7 Breeding Program:

Breeders conventionally use the phenotype to evaluate the genotype of a plant or an animal.As it is difficult to make out homozygous or heterozygous dominance from appearance, the DNA fingerprinting allows a fastidious and precise determination of genotype. It is basically useful in breeding race horses and hunting dogs.

6.11 Summary

Monoclonal antibodies (mAbs) are produced from the same, single B-lymphocyte clone which have a high specificity of recognition to the same epitope of an antigen. Methods to produce specific antibodies or immunoglobulins involving human–mouse hybrid cells were first introduced by Schwaber and Cohen. Subsequently, Kohler and Milstein introduced the hybridoma technique which enable a large production of mAbs. Their effort has earned them the prestigious Nobel Prize in Physiology or Medicine 1984. Unfortunately, the immunogenicity of the murine mAbs has limited their clinical applicability in human. To eliminate this major limitation, the technology has expanded and moved away from animal models to a humanization mAbs production.

A parallel increase in the knowledge of diseases pathology and a rapid progress in the antibody engineering have further accelerate the therapeutic use of mAbs. The specific selectivity of mAbs enables personalized medicine as the desired therapeutic outcomes can be achieved using a more targeted therapy, while minimizing the undesirable side effects of the non-specific, broad-spectrum conventional therapeutic agents. This chapter summarizes the history and development of mAbs, as well as their clinical use and the associated adverse events.

A DNA probe is a fragment of DNA that contains a nucleotide sequence specific for the gene or chromosomal region of interest. DNA probes employ

nucleic acid hybridization with specifically labeled sequences to rapidly detect complementary sequences in the test sample. A variety of methodologies for labeling DNA have been described. In short, these methods are used to generate end-labeled or continuously labeled probes. Most enzyme-mediated labeling techniques are very much dependent on polymerase activity, which is responsible for incorporation of the labeled nucleotides. Furthermore, the use of *Taq* or other thermostable DNA polymerases permits labeling reactions to be performed at higher temperatures via PCR, thereby reducing the incidence of enzyme-mediated point mutations during probe synthesis. PCR is an excellent method for probe synthesis, requiring very small quantities of template material. In the presence of appropriately labeled nucleotide primers, PCR products are labeled as they are being synthesized. Alternatively, the primers themselves may be labeled non-isotopically during their own synthesis, negating the requirement for the inclusion of labeled nucleotide precursors as part of the reaction mix.

6.12 Terminal questions

Q.1 What do you mean by monoclonal antibodies?

Answer:----------

Q.2 Describe DNA fingerprinting.

Answer:----------

Q.3 Write a short note on DNA probe.

Answer:----------

Q.4 Write a short note on antibodies.

Answer:----------

Q.5 Write a short note on functions of antibodies.

Answer:----------

Further readings

1 Biochemistry- Lehninger A.L.

2 Text book of Botany – Singh -Pande-Jain.

3 The elements of Botany- James Hewetson Wilson

4 Textbook of Biotechnology –H. K. Das

5 Biochemistry and molecular biology- Wilson Walker

Uttar Pradesh Rajarshi Tandon
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Bachelor of Science
DCEBY-108
Molecular Genetics & Biotechnology

Block -3
Biotechnology

Unit -7 Introduction and Techniques of Tissue Culture **150**

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Course Design Committee

Prof. Ashutosh Gupta — **Chairman**
Director, School of Science
UPRTOU, Prayagraj.

Dr. Anil Tiwari — **Member**
Botany Department
Ewing Christen College.
Prayagraj.

Dr. Preeti Singh — **Member**
Botany Department
S.S.K.P.G. College
Prayagraj.

Dr. Sushma Chauhan — **Member/Secretary**
Asst. Prof. (Contractual) Botany
School of Science, UPRTOU, Prayagraj.

Course Preparation Committee

Dr. Rajivranjan — **Author**
Assistant Professor — **Block- 01 – 03** (Unit: 01- 09)
Department of Botany
MLK PG College, Balrampur (U.P)

Dr. Arun Kumar Pandey — **Editor**
Associate Professor, — **(All blocks and units)**
Department of Botany
PSM PG College, Anand Nagar, Maharajganj (U.P)

Dr. Sushma Chauhan — **Coordinator**
Asst. Prof. (Contractual) Botany
School of Science, UPRTOU, Prayagraj.

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Block-III Biotechnology:

Block -3 is the last block of this SLM which covers biotechnology part in three units 7, 8 and 9. The detail of units are as under:

Unit -7 Covers introduction and techniques of tissue culture. in this unit you will study history and basis of plant tissues culture, germplasm conservation, embryo culture, production of haploid plant, micropropagation, somatic embryogenesis, organogenesis and application of tissue culture.

Unit-8 Culture of different tissues contains historical developments of tissue culture and culture environments. In this unit you will study about the process of meristem culture, anther culture, pollen culture and embryo culture.

Unit-9 Application of biotechnology covers history and definition of biotechnology, major goals and various branches of biotechnology. You will also study application of biotechnology in human welfare with special reference to agriculture.

Objectives:

After studying this unit you will be able to know about:

- The history and basis of tissue culture.
- The germplasm conservation and embryo culture.
- Meristem culture, anther culture, pollen culture and isolation of protoplast.
- The basic concept of biotechnology.
- Approaches of biotechnology in bioinformatics, biochemistry, microbiology, agriculture and medical.
- Applications of biotechnology in human welfare with special reference in agriculture.

Unit 7 Introduction and Techniques of Tissue Culture

Structure

7.1 Introduction

Objectives:

7.2 History of plant tissue culture

7.3 Basics of plant cell and tissue culture

7.4 Tissue culture in agriculture

7.5 Germplasm conservation

7.6 Embryo culture

7.7 Genetic transformation

7.8 Protoplast fusion

7.9 Haploid production

7.10 Current and future status of plant tissue culture

7.11 Techniques of plant tissue culture

- 7.11.1 Micropropagation
- 7.11.2 Stage 0: Preparation of donor plant
- Stage I: Initiation stage
- Stage II: Multiplication stage
- Stage III: Rooting stage
- Stage IV: Acclimatization Stage

7.12 Somatic embryogenesis

7.13 Organogenesis

7.14 Tissue culture in pharmaceuticals

7.15 Cell suspension culture

7.16 Hairy root cultures

7.17 Summary

7.18 Terminal questions

Further readings

7.1 Introduction

Tissue culture is the *in vitro* aseptic culture of cells, tissues, organs or whole plant under controlled nutritional and environmental conditions often to produce the clones of plants. The resultant clones are true-to type of the selected genotype. The controlled conditions provide the culture an environment conducive for their growth and multiplication. These conditions include proper supply of nutrients, pH medium, adequate temperature and proper gaseous and liquid environment.

Plant tissue culture technology is being widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites.Small pieces of tissue (named explants) can be used to produce hundreds and thousands of plants in a continuous process. A single explant can be multiplied into several thousand plants in relatively short time period and space under controlled conditions, irrespective of the season and weather on a year round basis. Endangered, threatened and rare species have successfully been grown and conserved by micropropagation because of high coefficient of multiplication and small demands on number of initial plants and space.

In addition, plant tissue culture is considered to be the most efficient technology for crop improvement by the production of somaclonal and gametoclonal variants. The micropropagation technology has a vast potential to produce plants of superior quality, isolation of useful variants in well-adapted high yielding genotypes with better disease resistance and stress tolerance capacities. Certain type of callus cultures give rise to clones that have inheritable characteristics different from those of parent plants due to the possibility of occurrence of somaclonal variability, which leads to the development of commercially important improved varieties.

Objectives:

This is the seventh unit of third block on Biotechnology**.** Under seventh unit, we have following objectives. These are as under:

- To discuss the history and basics of tissue culture
- To know about germplasm conservation and embryo culture
- To know about genetic transformation and protoplast fusion
- To know about somatic embryogenesis and organogenesis
- To discuss tissue culture in pharmaceuticals and cell suspension culture

Commercial production of plants through micropropagation techniques has several advantages over the traditional methods of propagation through seed, cutting, grafting and air-layering etc. It is rapid propagation processes that can lead to the production of plants virus free. *Coryodalisyanhusuo*, an important medicinal plant was propagated by somatic embryogenesis from tuber-derived callus to produce disease free tubers. Meristem tip culture of banana plants devoid from banana bunchy top virus (BBTV) and brome mosaic virus (BMV) were produced. Higher yields have been obtained by culturing pathogen free germplasm*in vitro*. Increase in yield up to 150% of virus-free potatoes was obtained in controlled conditions. The main objective of writing this chapter is to describe the tissue culture techniques, various developments, present and future trends and its application in various fields.

7.2 History of plant tissue culture

The science of plant tissue culture takes its roots from the discovery of cell followed by propounding of cell theory. In 1838, Schleiden and Schwann proposed that cell is the basic structural unit of all living organisms. They visualized that cell is capable of autonomy and therefore it should be possible for each cell if given an environment to regenerate into whole plant. Based on this premise, in 1902, a German physiologist, Gottlieb Haberlandt for the first time attempted to culture isolated single palisade cells from leaves in knop's salt solution enriched with sucrose.The cells remained alive for up to one month, increased in size, accumulated starch but failed to divide. Though he was unsuccessful but laid down the foundation of tissue culture technology for which he is regarded as the father of plant tissue culture. After that some of the landmark discoveries took place in tissue culture which are summarized as under:

- 1902 - Haberlandt proposed concept of *in vitro* cell culture.
- 1904 - Hannig cultured embryos from several cruciferous species.
- 1922 - Kolte and Robbins successfully cultured root and stem tips respectively.
- 1926 - Went discovered first plant growth hormone –Indole acetic acid.
- 1934 -White introduced vitamin B as growth supplement in tissue culture media for tomato root tip.
- 1939 -Gautheret, White and Nobecourt established endless proliferation of callus cultures.
- 1941 - Overbeek was first to add coconut milk for cell division in *Datura.*
- 1946 - Ball raised whole plants of *Lupinus* by shoot tip culture.
- 1954 - Muir was first to break callus tissues into single cells.
- 1955 - Skoog and Miller discovered kinetin as cell division hormone.
- 1957 - Skoog and Miller gave concept of hormonal control (auxin: cytokinin) of organ formation.
- 1959 - Reinert and Steward regenerated embryos from callus clumps and cell suspension of carrot (*Daucuscarota.*
- 1960 - Cocking was first to isolate protoplast by enzymatic degradation of cell wall.
- 1960 - Kanta and Maheshwari developed test tube fertilization technique.
- 1962 - Murashige and Skoog developed MS medium with higher salt concentration.
- 1964- Guha and Maheshwari produced first haploid plants from pollen grains of *Datura* (Anther culture).
- 1966 - Steward demonstrated totipotency by regenerating carrot plants from single cells of tomato.
- 1970 - Power *et al.* successfully achieved protoplast fusion.
- 1971 - Takebe *et al.*regenerated first plants from protoplasts.
- 1974 - Reinhardintroduced biotransformation in plant tissue cultures.
- 1977 - Chilton *et al*. successfully integrated Ti plasmid DNA from *Agrobacterium tumefaciens* in plants.
- 1981- Larkin and Scowcroft introduced the term somaclonal variation.

- 1984 - Horsh*et al*. developed transgenic tobacco by transformation with *Agrobacterium.*
- 1987 - Klien*et al*. developed biolistic gene transfer method for plant transformation.
- 2005 - Rice genome sequenced under International Rice Genome Sequencing Project.

7.3 Basics of plant cell and tissue culture

In plant cell culture, plant tissues and organs are grown *in vitro* on artificial media, under aseptic and controlled environment. The technique depends mainly on the concept of totipotentiality of plant cells which refers to the ability of a single cell to express the full genome by cell division. Along with the totipotent potential of plant cell, the capacity of cells to alter their metabolism, growth and development is also equally important and crucial to regenerate the entire plant. Plant tissue culture medium contains all the nutrients required for the normal growth and development of plants. It is mainly composed of macronutrients, micronutrients, vitamins, other organic components, plant growth regulators, carbon source and some gelling agents in case of solid medium.

Murashige and Skoog medium (MS medium) is most extensively used for the vegetative propagation of many plant species *in vitro*. The pH of the media is also important that affects both the growth of plants and activity of plant growth regulators. It is adjusted to the value between 5.4 - 5.8. Both the solid and liquid medium can be used for culturing. The composition of the medium, particularly the plant hormones and the nitrogen source has profound effects on the response of the initial explant.

Plant growth regulators (PGR's) play an essential role in determining the development pathway of plant cells and tissues in culture medium. The auxins, cytokinins and gibberellins are most commonly used plant growth regulators. The type and the concentration of hormones used depend mainly on the species of the plant, the tissue or organ cultured and the objective of the experiment. Auxins and cytokinins are most widely used plant growth regulators in plant

tissue culture and their amount determined the type of culture established or regenerated. The high concentration of auxins generally favors root formation, whereas the high concentration of cytokinins promotes shoot regeneration. A balance of both auxin and cytokinin leads to the development of mass of undifferentiated cells known as callus.

Maximum root induction and proliferation was found in *Stevia rebaudiana*, when the medium is supplemented with 0.5 mg/l NAA. Cytokinins generally promote cell division and induce shoot formation and axillary shoot proliferation. High cytokinin to auxin ratio promotes shoot proliferation while high auxin to cytokinins ratio results in root formation. Shoot initiation and proliferation was found maximum, when the callus of black pepper was shifted to medium supplemented with BA at the concentration of 0.5 mg/l. Gibberellins are used for enhanced growth and to promote cell elongation. Maximum shoot length was observed in *Phalaenopsis* orchids when cultured in medium containing 0.5 mg/l GA_3 (unpublished).

7.4 Tissue culture in agriculture

As an emerging technology, the plant tissue culture has a great impact on both agriculture and industry, through providing plants needed to meet the ever increasing world demand. It has made significant contributions to the advancement of agricultural sciences in recent times and today they constitute an indispensable tool in modern agriculture.

Biotechnology has been introduced into agricultural practice at a rate without precedent. Tissue culture allows the production and propagation of genetically homogeneous, disease-free plant material. Cell and tissue *in vitro* culture is a useful tool for the induction of somaclonal variation. Genetic variability induced by tissue culture could be used as a source of variability to obtain new stable genotypes. Interventions of biotechnological approaches for *in vitro* regeneration, mass micropropagation techniques and gene transfer studies in tree species have been encouraging. *In vitro* cultures of mature and/or immature zygotic embryos are applied to recover plants obtained from inter-generic crosses that do not produce fertile seeds. Genetic engineering can make

possible a number of improved crop varieties with high yield potential and resistance against pests. Genetic transformation technology relies on the technical aspects of plant tissue culture and molecular biology for:

- ✓ Production of improved crop varieties
- ✓ Production of disease-free plants (virus)
- ✓ Genetic transformation
- ✓ Production of secondary metabolites
- ✓ Production of varieties tolerant to salinity, drought and heat stresses

7.5 Germplasm conservation

In vitro cell and organ culture offers an alternative source for the conservation of endangered genotypes. Germplasm conservation worldwide is increasingly becoming an essential activity due to the high rate of disappearance of plant species and the increased need for safeguarding the floristic patrimony of the countries. Tissue culture protocols can be used for preservation of vegetative tissues when the targets for conservation are clones instead of seeds, to keep the genetic background of a crop and to avoid the loss of the conserved patrimony due to natural disasters, whether biotic or abiotic stress. The plant species which do not produce seeds (sterile plants) or which have 'recalcitrant' seeds that cannot be stored for long period of time can successfully be preserved via *in vitro* techniques for the maintenance of gene banks.

Cryopreservation plays a vital role in the long-term *in vitro* conservation of essential biological material and genetic resources. It involves the storage of *in vitro* cells or tissues in liquid nitrogen that results incryo-injury on the exposure of tissues tophysical andchemical stresses. Successful cryopreservation is often ascertained by cell and tissue survival and the ability to re-grow or regenerate into complete plants or form new colonies. It is desirable to assess the genetic integrity of recovered germplasm to determine whether it is 'true-to-type' following cryopreservation. The fidelity of recovered plants can be assessed at phenotypic, histological, cytological, biochemical and molecular levels, although, there are advantages and limitations of the various approaches used to assess genetic stability. Cryobionomics is a new approach to study genetic

stability in the cryopreserved plant materials. The embryonic tissues can be cryopreserved for future use or for germplasm conservation.

7.6 Embryo culture

Embryo culture is a type of plant tissue culture that is used to grow embryos from seeds and ovules in a nutrient medium. In embryo culture, the plant develops directly from the embryo or indirectly through the formation of callus and then subsequent formation of shoots and roots. The technique has been developed to break seed dormancy, test the vitality of seeds, production of rare species and haploid plants. It is an effective technique that is employed to shorten the breeding cycle of plants by growing excised embryos and results in the reduction of long dormancy period of seeds. Intra-varietal hybrids of an economically important energy plant "Jatropha" have been produced successfully with the specific objective of mass multiplication. Somatic embryogenesis and plant regeneration has been carried out in embryo cultures of Jucara Palm for rapid cloning and improvement of selected individuals.

In addition, conservation of endangered species can also be attained by practicing embryo culture technique. Recently a successful protocol has been developed for the *in vitro* propagation of *Khayagrandifoliola* by excising embryos from mature seeds. The plant has a high economic value for timber wood and for medicinal purposes as well. This technique has an important application in forestry by offering a mean of propagation of elite individuals where the selection and improvement of natural population is difficult.

7.7 Genetic transformation

Genetic transformation is the most recent aspect of plant cell and tissue culture that provides the mean of transfer of genes with desirable trait into host plants and recovery of transgenic plants. The technique has a great potential of genetic improvement of various crop plants by integrating in plant biotechnology and breeding programmes. It has a promising role for the introduction of agronomically important traits such as increased yield, better quality and enhanced resistance to pests and diseases.

Genetic transformation in plants can be achieved by either vector-mediated (indirect gene transfer) or vectorless (direct gene transfer) method. Among vector dependant gene transfer methods, *Agrobacterium*-mediated genetic transformation is most widely used for the expression of foreign genes in plant cells. Successful introduction of agronomic traits in plants was achieved by using root explants for the genetic transformation. Virus-based vectors offers an alternative way of stable and rapid transient protein expression in plant cells thus providing an efficient mean of recombinant protein production on large scale.

Recently successful transgenic plants of Jatropha were obtained by direct DNA delivery to mature seed-derived shoot apices via particle bombardment method. This technology has an important impact on the reduction of toxic substances in seeds, thus overcoming the obstacle of seed utilization in various industrial sector. Regeneration of disease or viral resistant plants is now achieved by employing genetic transformation technique. Researchers succeeded in developing transgenic plants of potato resistant to potato virus Y (PVY) which is a major threat to potato crop worldwide. In addition, marker free transgenic plants of *Petunia hybrida* were produced using multi-auto-transformation (MAT) vector system. The plants exhibited high level of resistance to *Botrytis cinerea,* causal agent of gray mold.

7.8 Protoplast fusion

Somatic hybridization is an important tool of plant breeding and crop improvement by the production of interspecific and intergeneric hybrids. The technique involves the fusion of protoplasts of two different genomes followed by the selection of desired somatic hybrid cells and regeneration of hybrid plants. Protoplast fusion provides an efficient mean of gene transfer with desired trait from one species to another and has an increasing impact on crop improvement. Somatic hybrids were produced by fusion of protoplasts from rice and ditch reed using electrofusion treatment for salt tolerance.

In vitro fusion of protoplast opens a way of developing unique hybrid plants by overcoming the barriers of sexual incompatibility. The technique has been applicable in horticultural industry to create new hybrids with increased fruit yield and better resistance to diseases. Successful viable hybrid plants were obtained when protoplasts from citrus were fused with other related citrinae species. The potential of somatic hybridization in important crop plants is best illustrated by the production of intergeneric hybrid plants among the members of Brassicaceae. To resolvethe problem of loss of chromosomes and decreased regeneration capacity, successful protocol has been established for the production of somatic hybrid plants by using two types of wheat protoplast as recipient and protoplast of *Haynaldiavillosa* as a fusion donor. It is also employed as an important gene source for wheat improvement.

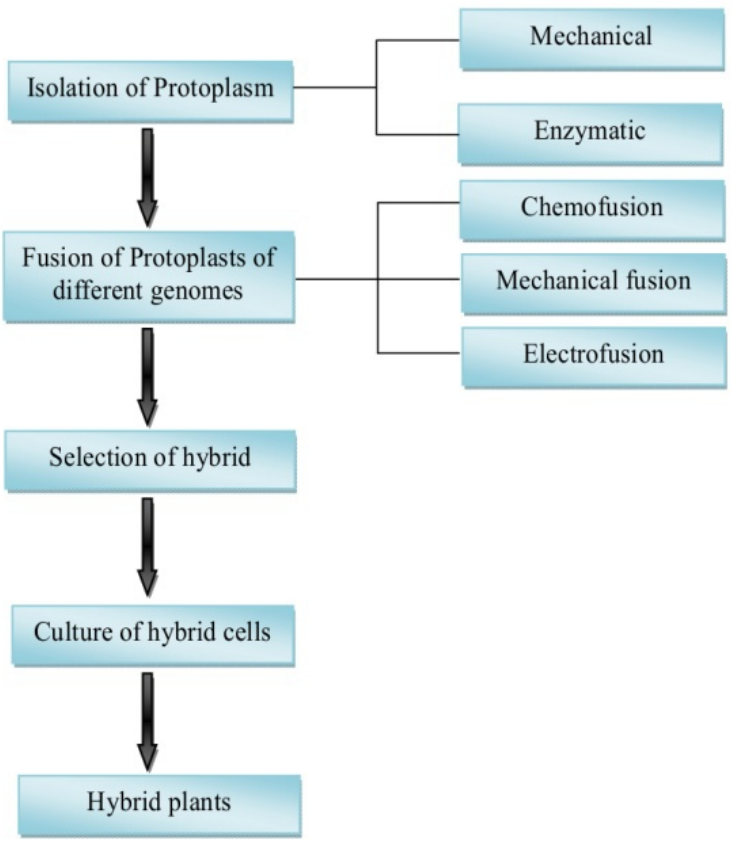

Fig. 1 Schematic representation of production of hybrid plant via protoplast fusion

7.9 Haploid production

The tissue culture techniques enable to produce homozygous plants in relatively short time period through the protoplast, anther and microspore

cultures instead of conventional breeding. Haploids are sterile plants having single set of chromosomes which are converted into homozygous diploids by spontaneous or induced chromosome doubling. The doubling of chromosomes restores the fertility of plants resulting in production of double haploids with potential to become pure breeding new cultivars. The term androgenesis refers to the production of haploid plants from young pollen cells without undergoing fertilization. Sudherson e*t al.* reported haploid plant production of sturt's desert pea by using pollen grains as primary explants.

The haploidy technology has now become an integral part of plant breeding programs by speeding up the production of inbred lines and overcoming the constraints of seed dormancy and embryo non-viability. The technique has a remarkable use in genetic transformation by the production of haploid plants with induced resistance to various biotic and abiotic stresses. Introduction of genes with desired trait at haploid state followed by chromosome doubling led to the production of double haploids inbred wheat and drought tolerant plantswere attained successfully.

7.10 Current and future status of plant tissue culture

The past decades of plant cell biotechnology has evolved as a new era in the field of biotechnology, focusing on the production of a large number of secondary plant products. During the second half of the last century the development of genetic engineering and molecular biology techniques allowed the appearance of improved and new agricultural products which have occupied an increasing demand in the productive systems of several countries worldwide. Nevertheless, these would have been impossible without the development of tissue culture techniques, which provided the tools for the introduction of genetic information into plant cells.

Nowadays, one of the most promising methods of producing proteins and other medicinal substances, such as antibodies and vaccines, is the use of transgenic plants. Transgenic plants represent an economical alternative to fermentation-based production systems. Plant-made vaccines or antibodies (plantibodies) are especially striking, as plants are free of human diseases, thus reducing

screening costs for viruses and bacterial toxins. The number of farmers who have incorporated transgenic plants into their production systems in 2008 was 13.3 million, in comparison to 11 million in 2007.

SAQ 1

Q. 1 Define tissue culture.

Q. 2 Define embryo culture.

Q. 3 What do you mean by micropropagation?

7.11 Techniques of plant tissue culture

7.11.1 Micropropagation

Micropropagation starts with the selection of plant tissues (explant) from a healthy, vigorous mother plant. Any part of the plant (leaf, apical meristem, bud and root) can be used as explant. The whole process can be summarized into the following stages as shown in fig.

Stage 0: Preparation of donor plant

Any plant tissue can be introduced *in vitro*. To enhance the probability of success, the mother plant should be *ex vitro* cultivated under optimal conditions to minimize contamination in the *in vitro* culture.

Stage I: Initiation stage

In this stage an explant is surface sterilized and transferred into nutrient medium. Generally, the combined application of bactericide and fungicide products is suggested. The selection of products depends on the type of explant to be introduced. The surface sterilization of explant in chemical solutions is an important step to remove contaminants with minimal damage to plant cells. The most commonly used disinfectants are sodium hypochlorite, calcium hypochlorite, ethanol and mercuric chloride ($HgCl_2$). The cultures are incubated in growth chamber either under light or dark conditions according to the method of propagation.

Stage II: Multiplication stage

The aim of this phase is to increase the number of propagules. The number of propagules is multiplied by repeated subcultures until the desired (or planned) number of plants is attained.

Stage III: Rooting stage

The rooting stage may occur simultaneously in the same culture media used for multiplication of the explants. However, in some cases it is necessary to change media, including nutritional modification and growth regulator composition to induce rooting and the development of strong root growth.

Stage IV: Acclimatization Stage

At this stage, the *in vitro* plants are weaned and hardened. Hardening is done gradually from high to low humidity and from low light intensity to high light intensity. The plants are then transferred to an appropriate substrate (sand, peat, compost etc.) and gradually hardened under greenhouse.

7.12 Somatic embryogenesis

Somatic embryogenesis: is an *in vitro* method of plant regeneration widely used as an important biotechnological tool for sustained clonal propagation. It is a process by which somatic cells or tissues develop into differentiated embryos. These somatic embryos can develop into whole plants without undergoing the process of sexual fertilization as done by zygotic embryos. The somatic embryogenesis can be initiated directly from the explants or indirectly by the establishment of mass of unorganized cells named callus. Plant regeneration via somatic embryogenesis occurs by the induction of embryogenic cultures from zygotic seed, leaf or stem segment and further multiplication of embryos.Mature embryos are then cultured for germination and plantlet development, and finally transferred to soil.

Somatic embryogenesis has been reported in many plants including trees and ornamental plants of different families. The phenomenon has been observed in some cactus species. There are various factors that affect the induction and development of somatic embryos in cultured cells. A highly efficient protocol has been reported for somatic embryogenesis on grapevine that showed higher plant regeneration sufficiently when the tissues were cultured in liquid medium. Plant growth regulators play an important role in the regeneration and proliferation of somatic embryos. Highest efficiency of embryonic callus was induced by culturing nodal stem segments of rose hybrids on medium supplemented with various PGR's alone or in combination.

This embryonic callus showed high germination rate of somatic embryos when grown on abscisic acid (ABA) alone. Somatic embryogenesis is not only a process of regenerating the plants for mass propagation but also regarded as a valuable tool for genetic manipulation. The process can also be used to develop the plants that are resistant to various kinds of stresses and to introduce the genes by genetic transformation. A successful protocol has been developed for regeneration of cotton cultivars with resistance to *Fusarium* and *Verticillium* wilts.

7.13 Organogenesis

It refers to the production of plant organs i.e. roots, shoots and leaves that may arise directly from the meristem or indirectly from the undifferentiated cell masses (callus). Plant regeneration via organogenesis involves the callus production and differentiation of adventitious meristems into organs by altering the concentration of plant growth hormones in nutrient medium. Skoog and Muller were the first who demonstrated that high ratio of cytokinin to auxin stimulated the formation of shoots in tobacco callus while high auxin to cytokinin ratio induced root regeneration.

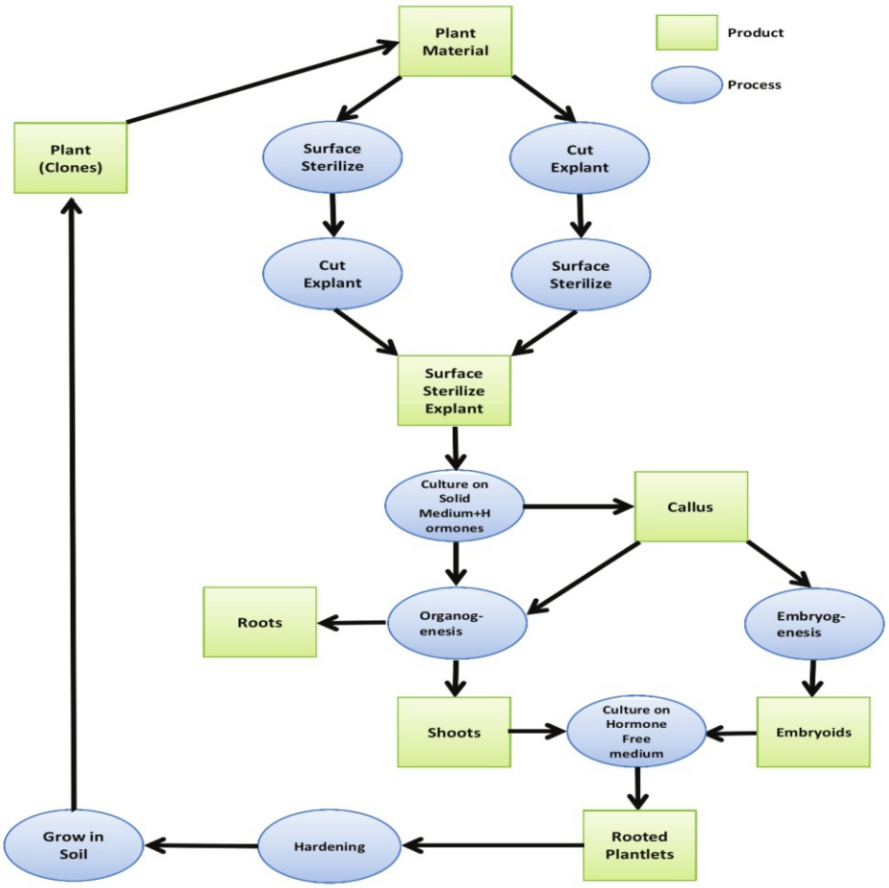

Fig. 2 Flow chart summarizing tissue culture experiments

7.14 Tissue culture in pharmaceuticals

Plant cell and tissue cultures hold great promise for controlled production of myriad of useful secondary metabolites. Plant cell cultures combine the merits of whole-plant systems with those of microbial and animal cell cultures for the production of valuable therapeutic secondary metabolites. In the search for alternatives to production of medicinal compounds from plants, biotechnological approaches, specifically plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites. Exploration of the biosynthetic capabilities of various cell cultures has been carried out by a group of plant scientists and microbiologists in several countries during the last decade.

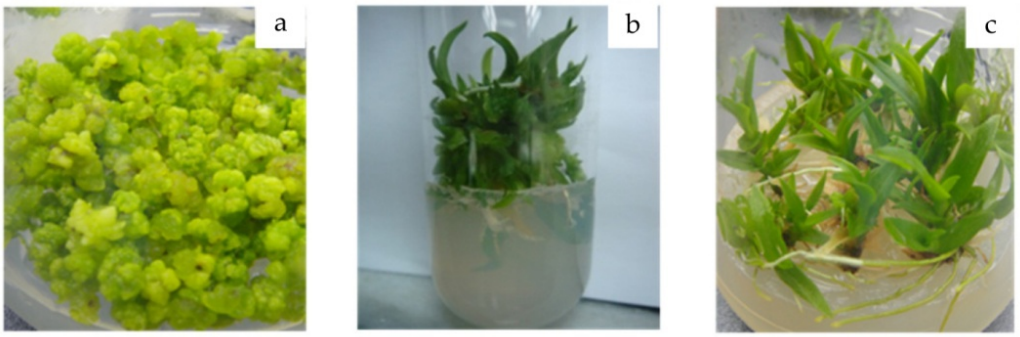

Fig. 3 Micropropagation of Orchids (a) callus culture (b) shoot regeneration (c) rooted plantlets

7.15 Cell suspension culture

Cell suspension culture systems are used now days for large scale culturing of plant cells from which secondary metabolites could be extracted. A suspension culture is developed by transferring the relatively friable portion of the callus into liquid medium and is maintained under suitable conditions of aeration, agitation, light, temperature and other physical parameters. Cell cultures cannot only yield defined standard phytochemicals in large volumes but also eliminate the presence of interfering compounds that occur in the field-grown plants. The advantage of this method is that it can ultimately provide a continuous, reliable source of natural products. The major advantage of the cell cultures include synthesis of bioactive secondary metabolites, running in controlled environment, independently from climate and soil conditions. A number of different types of bioreactors have been used for mass cultivation of plant cells. The first commercial application of large scale cultivation of plant cells was carried out in stirred tank reactors of 200 liter and 750 liter capacities to produce shikonin by cell culture of *Lithospermumerythrorhizon*. Cell of *Catharanthusroseus, Dioscoreadeltoidea, Digitalis lanata, Panaxnotoginseng, Taxuswallichiana* and *Podophyllumhexandrum* have been cultured in various bioreactors for the production of secondary plant products.

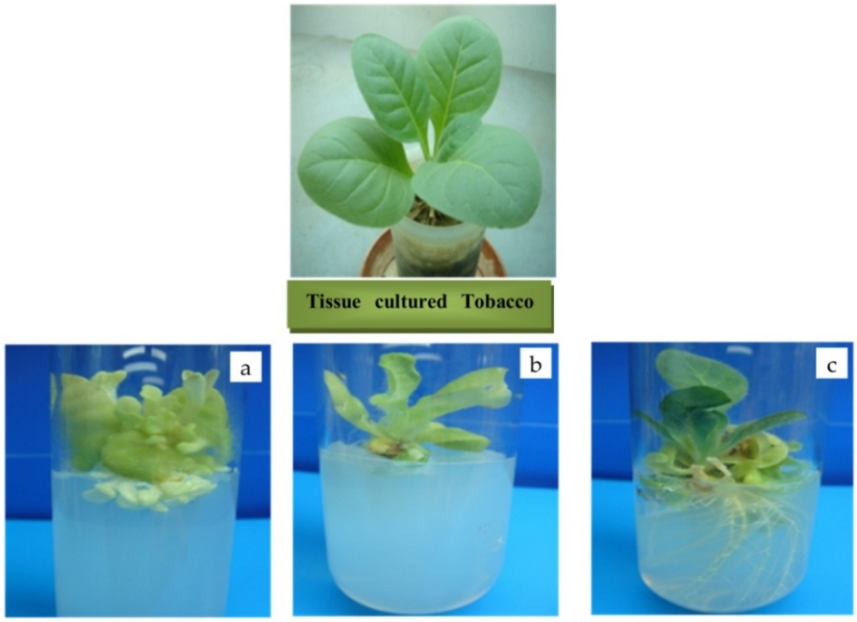

Fig. 4 Tissue culture of Nicotianatabacum (a) callus (b) shoot regeneration (c) root induction

A number of medicinally important alkaloids, anticancer drugs, recombinant proteins and food additives are produced in various cultures of plant cell and tissues. Advances in the area of cell cultures for the production of medicinal compounds has made possible the production of a wide variety of pharmaceuticals like alkaloids, terpenoids, steroids, saponins, phenolics, flavanoids and amino acids. Some of these are now available commercially in the market for example shikonin and paclitaxel (Taxol). Until now 20 different recombinant proteins have been produced in plant cell culture, including antibodies, enzymes, edible vaccines, growth factors and cytokines. Advances in scale-up approaches and immobilization techniques contribute to a considerable increase in the number of applications of plant cell cultures for the production of compounds with a high added value.

7.16 Hairy root cultures

The hairy root system based on inoculation with Agrobacterium rhizogenes has become popular in the last two decades as a method of producing secondary metabolites synthesized in plant roots. Organized cultures, and especially root cultures, can make a significant contribution in the production of secondary metabolites. Most of the research efforts that use differentiated cultures instead of cell suspension cultures have focused on transformed (hairy) roots.

Agrobacterium rhizogenes causes hairy root disease in plants. The neoplastic (cancerous) roots produced by A. rhizogenes infection are characterized by high growth rate, genetic stability and growth in hormone free media. High stability and productivity features allow the exploitation of hairy roots as valuable biotechnological tool for the production of plant secondary metabolites. These genetically transformed root cultures can produce levels of secondary metabolites comparable to that of intact plants. Hairy root technology has been strongly improved by increased knowledge of molecular mechanisms underlying their development. Optimizing the composition of nutrients for hairy root cultures is critical to gain a high production of secondary metabolites.

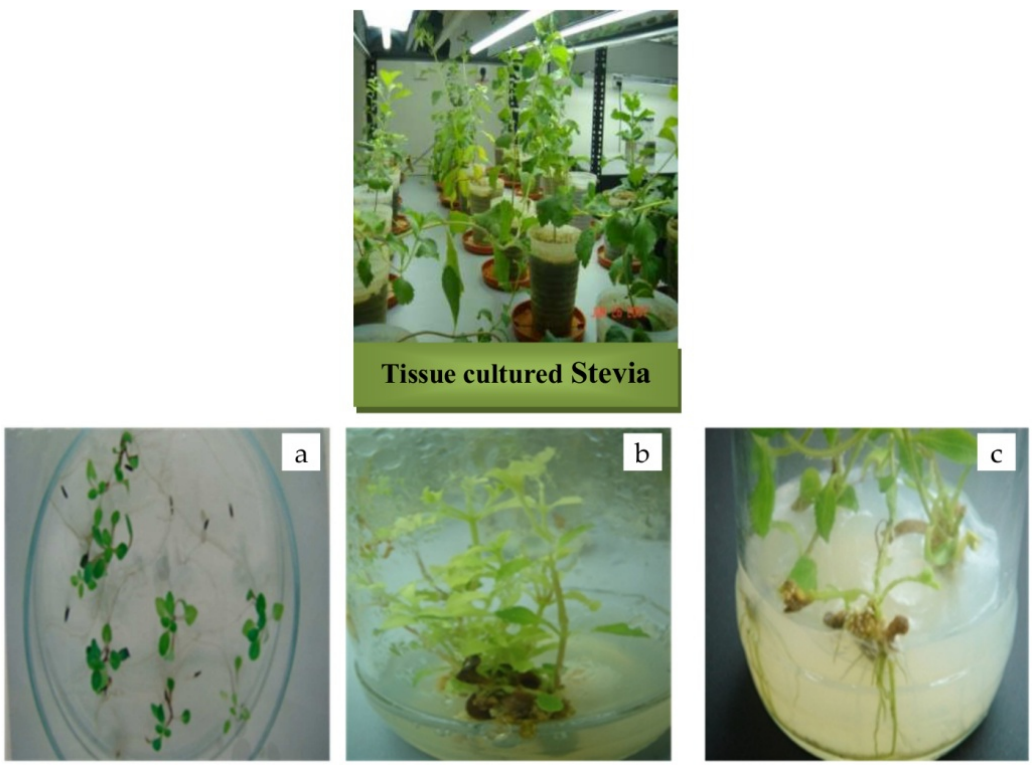

Fig. 5 In vitro propagation of S. rebaudiana (a) seed germination on MS medium (b) shoot multiplication (c) root development.

Quality control is also very essential to assure high quality plant production and to obtain confidence of the consumers. The selection of explants source, diseases free material, authenticity of variety and elimination of somaclonal variants are some of the most critical parameters for ensuring the quality of the plants.

SAQ 2

Q. 1 Define organogenesis.

Q. 2 Define suspension culture.

Q. 3 What do you mean by protoplast fusion?

The *in vitro* culture has a unique role in sustainable and competitive agriculture and forestry and has been successfully applied in plant breeding for rapid introduction of improved plants. Plant tissue culture has become an integral part of plant breeding. It can also be used for the production of plants as a source of edible vaccines. There are many useful plant-derived substances which can be produced in tissue cultures.

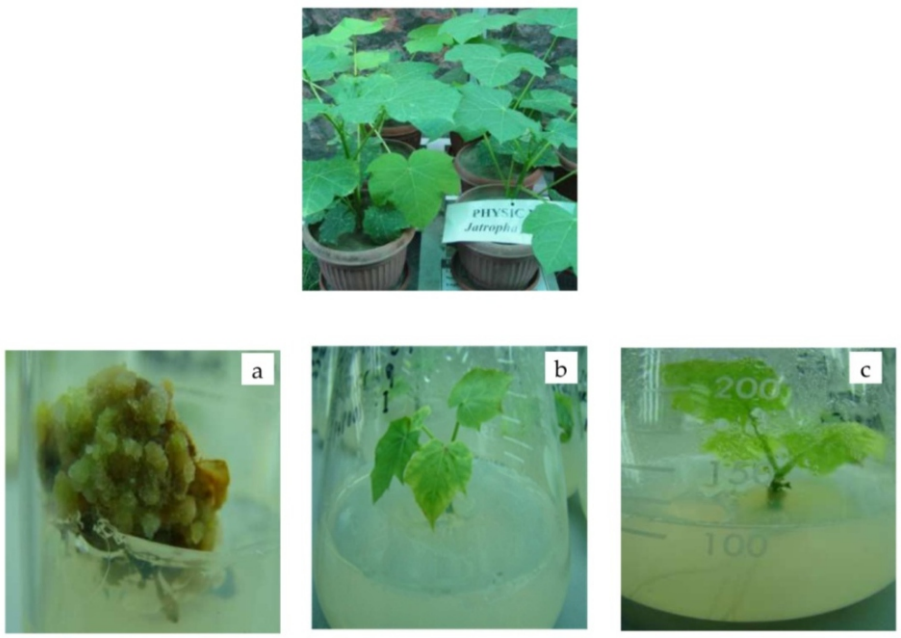

Fig. 6 Tissue culture of Jatrophacurcas (a) callus of Jatropha (b) shoot regeneration (c) root induction.

Since last two decades there have been considerable efforts made in the use of plant cell cultures in bioproduction, bioconversion or biotransformation and biosynthetic studies. The potential commercial production of pharmaceuticals by cell culture techniques depends upon detailed investigations into the biosynthetic sequence. There is great potential of cell culture to be use in the production of valuable secondary products. Plant tissue culture is a noble approach to obtain these substances in large scale.

Plant cell culture has made great advances. Perhaps the most significant role that plant cell culture has to play in the future will be in its association with transgenic plants. The ability to accelerate the conventional multiplication rate can be of great benefit to many countries where a disease or some climatic disaster wipes out crops. The loss of genetic resources is a common story when germplasm is held in field genebanks. Slow growth *in vitro* storage and cryopreservation are being proposed as solutions to the problems inherent in field genebanks. If possible, they can be used with field genebanks, thus providing a secure duplicate collection. They are the means by which future generations will be able to have access to genetic resources for simple conventional breeding programmes, or for the more complex genetic transformation work. As such, it has a great role to play in agricultural development and productivity.

7.17 Summary

Plant tissue culture represents the most promising areas of application at present time and giving an out look into the future. The areas ranges from micropropagation of ornamental and forest trees, production of pharmaceutically interesting compounds, and plant breeding for improved nutritional value of staple crop plants, including trees to cryopreservation of valuable germplasm. All biotechnological approaches like genetic engineering, haploid induction, or somaclonal variation to improve traits strongly depend on an efficient *in-vitro* plant regeneration system.

The rapid production of high quality, disease free and uniform planting stock is only possible through micropropagation. New opportunities has been created for producers, farmers and nursery owners for high quality planting materials of fruits, ornamentals, forest tree species and vegetables. Plant production can be carried out throughout the year irrespective of season and weather. However micropropagation technology is expensive as compared to conventional methods of propagation by means of seed, cuttings and grafting etc. Therefore it is essential to adopt measures to reduce cost of production. Low cost

production of plants requires cost effective practices and optimal use of equipment to reduce the unit cost of plant production.

It can be achieved by improving the process efficiency and better utilization of resources. Bioreactor based plant propagation can increase the speed of multiplication and growth of cultures and reduce space, energy and labor requirements when commencing commercial propagation. However, the use of bioreactors needs special care and handling to avoid contamination of culture which may lead to heavy economic losses. The cost of production may also be reduced by selecting several plants that provide the option for around the year production and allow cost flow and optimal use of equipment and resources. It is also essential to have sufficient mother culture and reduce the number of subculture to avoid variation and plan the production of plants according to the demand.

7.18 Terminal questions

Q.1. What do you mean by tissue culture?

Answer:----------

Q.2. Describe different techniques in tissue culture.

Answer:----------

Q.3. Write a short note on Biotechnology.

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Text book of Botany – Singh -Pande-Jain.
3. The elements of Botany- James Hewetson Wilson
4. Textbook of Biotechnology –H. K. Das
5. Biochemistry and molecular biology- Wilson Walker

Unit 8 Culture of Different Tissue, Meristem Culture and Anther Culture

Structure

8.1 Introduction

Objectives:

8.2 Historical developments

8.3 Culture environments

8.4 Primary cultures and established cell lines

8.5 Processing of cultured cells and tissues

8.6 Biological insights

8.7 Meristem culture

8.8 Meristem culture *in vitro*

8.9 Anther Culture

8.10 Pollen culture

8.11 Procedure for In-vitro Pollen culture:

8.12 Pollens are isolated either by squeezing or float culturing of anthers:

8.13 Protoplast isolation and culture

8.14 Embryo culture

8.15 Applications of embryo culture

8.16 Summary

8.17 Terminal questions

Further readings

8.1 Introduction

Tissue culture, a method of biological research in which fragments of tissue from an animal or plant are transferred to an artificial environment in which they can continue to survive and function. The cultured tissue may consist of a single cell, a population of cells, or a whole or part of an organ. Cells in culture may multiply; change size, form, or function; exhibit specialized activity (muscle cells, for example, may contract); or interact with other cells.

In modern usage, "tissue culture" generally refers to the growth of cells from a tissue from a multicellular organism *in vitro*. These cells may be cells isolated from a donor organism (primary cells) or an immortalised cell line. The cells are bathed in a culture medium, which contains essential nutrients and energy sources necessary for the cells' survival.[8] Thus, in its broader sense, "tissue culture" is often used interchangeably with "cell culture". On the other hand, the strict meaning of "tissue culture" refers to the culturing of tissue pieces, i.e. explant culture.

Tissue culture is an important tool for the study of the biology of cells from multicellular organisms. It provides an *in vitro* model of the tissue in a well defined environment which can be easily manipulated and analysed. In animal tissue culture, cells may be grown as two-dimensional monolayers (conventional culture) or within fibrous scaffolds or gels to attain more naturalistic three-dimensional tissue-like structures (3D culture). Eric Simon, in a 1988 NIH SBIR grant report, showed that electrospinning could be used to produced nano- and submicron-scale polymeric fibrous scaffolds specifically intended for use as *in vitro* cell and tissue substrates.

Fig. 1 What is tissue culture and its advantages in plants?

This early use of electrospun fibrous lattices for cell culture and tissue engineering showed that various cell types would adhere to and proliferate upon polycarbonate fibers. It was noted that as opposed to the flattened morphology typically seen in 2D culture, cells grown on the electrospun fibers exhibited a more rounded 3-dimensional morphology generally observed of tissues *in vivo*. Plant tissue culture in particular is concerned with the growing of entire plants from small pieces of plant tissue, cultured in medium.

Objectives:

This is the eighth unit of third block on Biotechnology**.** Under eighth unit, we have following objectives. These are as under:

- To know about historical developments and culture environments
- To discuss meristem culture, anther culture and pollen culture
- To discuss in-vitro pollen culture and in-vitro meristem culture
- To know about protoplast isolation, embryo culture and its application

8.2 Historical developments

An early attempt at tissue culture was made in 1885 by German zoologist Wilhelm Roux, who cultivated tissue from a chick embryo in a warm salt solution. The first real success came in 1907, however, when American zoologist Ross G. Harrison demonstrated the growth of frog nerve cell processes in a medium of clotted lymph. French surgeon Alexis Carrel and his assistant Montrose Burrows subsequently improved upon Harrison's technique, reporting their initial advances in a series of papers published in 1910–11. Carrel and Burrows coined the term ***tissue culture*** and defined the concept. Thereafter, a number of experimenters succeeded in cultivating animal cells, using as culture media a variety of biological fluids, such as lymph, blood serum, plasma, and tissue extracts. In the 1980s and '90s, methods were developed that enabled researchers to successfully grow mammalian embryonic stem cells under artificial conditions. Those breakthroughs ultimately enabled the establishment and maintenance of human embryonic stem cell lines, which advanced researchers' understanding of human biology and greatly facilitated progress in therapeutics and regenerative medicine.

8.3 Culture environments

Cells may be grown in a culture medium of biological origin such as blood serum or tissue extract, in a chemically defined synthetic medium, or in a mixture of the two. A medium must contain proper proportions of the necessary nutrients for the cells to be studied and must be appropriately acid or alkaline. Cultures are usually grown either as single layers of cells on a glass or plastic surface or as a suspension in a liquid or semisolid medium. To initiate a culture, a tiny sample of the tissue is dispersed on or in the medium, and the flask, tube, or plate containing the culture is then incubated, usually at a temperature close to that of the tissue's normal environment. Sterile conditions are maintained to prevent contamination with microorganisms. Cultures are sometimes started from single cells, resulting in the production of uniform biological populations called clones. Single cells typically give rise to colonies within 10 to 14 days of being placed under culture conditions.

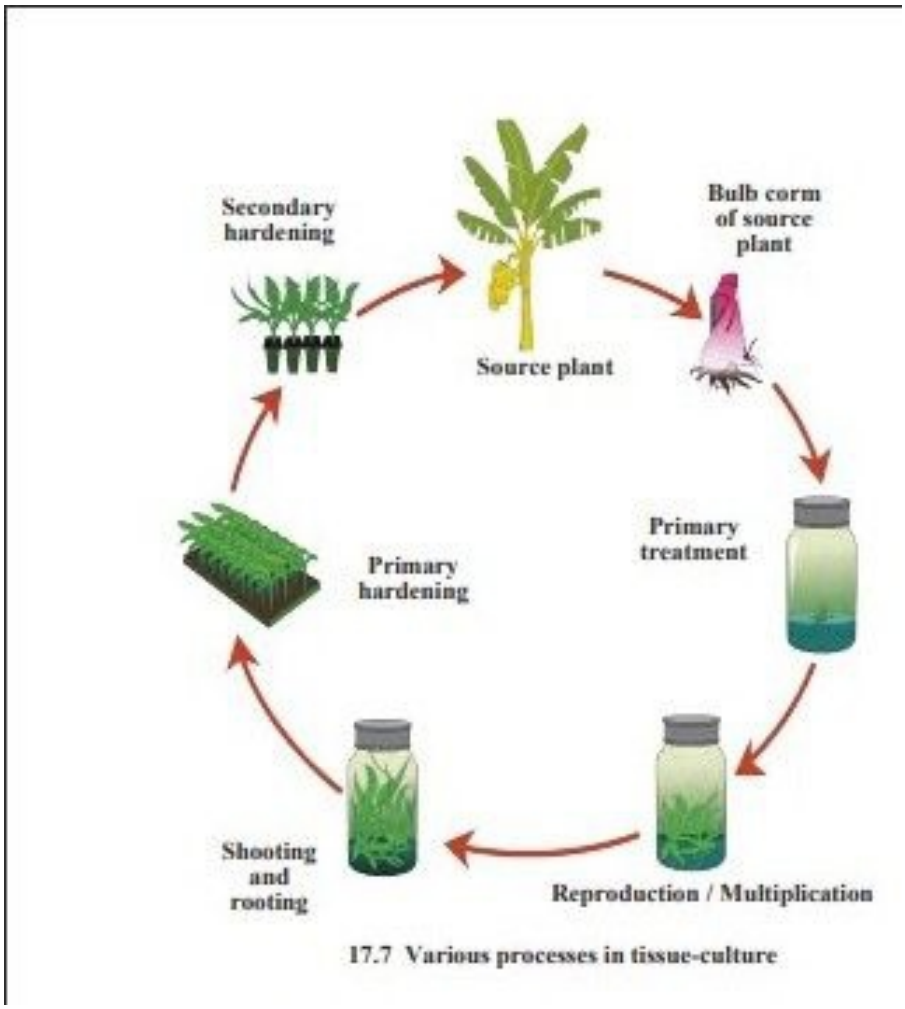

Fig. 2 Different processes in tissue culture

8.4 Primary cultures and established cell lines

There are two main types of cultures: primary (mortal) cultures and cultures of established (immortal) cell lines. Primary cultures consist of normal cells, tissues, or organs that are excised directly from tissue collected by biopsy from a living organism. Primary cultures are advantageous in that they essentially model the natural function of the cell, tissue, or organ under study. However, the longer the samples are maintained in culture, the more mutations they accumulate, which can lead to changes in chromosome structure and cell function. In addition, primary cultures generally are mortal. Cells undergo an aging process whereby they multiply for only 50 to 100 generations, after which the rate decreases markedly. The point at which cells in primary cultures stop growing, or undergo replicative senescence, marks the so-called Hayflick limit.

By contrast, established cell lines can be perpetuated indefinitely. Such cell lines generally are derived from tumour biopsies from patients, or they may be generated from primary cells that have undergone mutations that enabled them to overcome the Hayflick limit and continue replicating. Similar to cells in primary cultures, cells in established lines accumulate mutations over time that can change their character. Thus, in order for researchers from different laboratories to be able to compare results from experiments using the same cell lines, they must confirm the identity of the cells that they are working with. Cell identity is verified through a process known as authentication, in which the DNA profile of the cultured cells is compared against the known or standard profile for that cell line.

8.5 Processing of cultured cells and tissues

Live cultures may be examined directly with a microscope, or they may be observed by means of photographs and motion pictures taken through the microscope. Cells, tissues, and organs may also be killed, fixed (preserved), and stained for further examination. Following fixation, samples can also be embedded (e.g., in a resin) and cut into thin sections to disclose additional details under a light or electron microscope. Cells in tissue culture are subjected to a broad range of experimental treatment. For example, viruses, drugs, hormones, vitamins, disease-causing microorganisms, or suspected cancer-producing chemicals may be added to the culture. Scientists then observe the cells, looking for global changes in cell behaviour or function or for changes in specific molecules, such as alterations in the expression of a particular protein or gene.

8.6 Biological insights

Tissue culture has enabled numerous discoveries in the biological sciences. It has revealed, for example, basic information about cells regarding their composition and form; their biochemical, genetic, and reproductive activity; their nutrition, metabolism, specialized functions, and processes of aging and healing; the effects on cells of physical, chemical, and biological agents (drugs and viruses, for example); and the differences between normal

cells and abnormal cells, such as cancer cells. Work with tissue cultures has helped to identify infections, enzyme deficiencies, and chromosomal abnormalities, to classify tumours, and to formulate and test drugs and vaccines.

Since the discovery that certain viruses also grow in tissue culture, the technique has been used to produce vaccines against poliomyelitis, influenza, measles, mumps, and other infectious diseases. Cell cultures have also produced viral inhibitors, including interferon. Hormones are also produced from cultures of cells or organs. Cultured white blood cells from two individuals can be used to determine compatibility between potential donors and recipients of tissue transplants. By removing and culturing cells from a pregnant woman, it is possible to tell whether her fetus has certain chromosomal defects, such as those associated with Down syndrome and other trisomies.

The identification and diagnosis of chromosome abnormalities and inherited disorders has been greatly enhanced by the development of somatic cell genetics. Tissue culture techniques have been used to culture many kinds of hybrid cells that contain chromosomes from different species in the same cell, allowing the functions of individual chromosomes to be separately defined. Tissue culture studies have clarified the genetic causes of certain hereditary diseases, and methods have been developed for detecting environmental substances that may cause gene damage. The nature of certain cancers has been elucidated by the discovery of specific genes and chromosomal aberrations that are associated with the disease. The methods of somatic cell genetics have also been applied to plant cells; for example, such methods have been used in efforts to develop new strains of cereal crops with improved nutritional properties.

8.7 Meristem culture

Meristem culture is used for shoot apical meristem culture *in vitro*. Meristem culture was developed by Morel and Martin in 1952 for rivers eliminating from Dahlia. Orchid Cymbidium *was* micropropagated using meristem culture by Morel in 1965. An already existing shoot meristem grows in the meristem

culture and adventitious roots regenerate from these shoots. In the shoot tip beyond the youngest leaf lies the primordium meristem. It measures up to 250 mm in length and 100 mm in diameter. In addition to the apical meristem one or three leaf primordia would be present in a shoot tip of 100–500 nm. When virus elimination is the objective, to obtain disease-free plants, shoot tips of up to 10 mm are used. For rapid clonal propagation, a shoot-tip culture is followed in which (5–10 mm) explants are used. Hence, the majority of meristem culture are essentially shoot-tip cultures. Various sized nodal explants are also employed for rapid clonal propagation.

The size of the shoot tip used for culture is not important when the main objective is micropropagation. But when the objective is to obtain virus-free stock (or stock free from other pathogens) it is necessary for the excision of the apical meristem to be done with a minimum of the surrounding tissue. The shoot tip may be cut into fine pieces in order to obtain more than one plantlet from each shoot tip. Pieces of curd (the inflorescence) are used in some species such as cauliflower. Shoot tips or tissue pieces bearing buds of such stems may be used for those plants having an underground stem. Explants taken from actively growing plants early in the growing season are the most suitable.

Explants of meristem are then placed on Murashige and Skoog's (MS) medium, which is considered to be an effective medium for the majority of species. Lower salt concentration is suitable for some species. Fungicides (bavistin) or antibiotics (chloramphenicol/streptomycin) can be added to the medium during growth to remove the endophytic contamination. Similarly, meristem culture follows steps similar to micropropagation:

(i) Initiation of culture,

(ii) Shoot multiplication,

(iii) Rooting of the developed shoots, and

(iv) Transfer of the plantlets to the pots or soil.

8.8 Meristem culture *in vitro*

Meristem culture *in vitro* is used for the elimination of viruses and related pathogens from a large number of vegetatively propagated plants and it is the main method used in plant virus elimination programs. The shoot apical meristem is comprised of the leaf primordia, which turn into leaves, and the apical dome, where the stem elongates and it is usually free of pathogens. Limmaset and Cornuet (1949) observed that virus titers declined toward the apical meristem. As early as 1952, Morel and Martin produced VF dahlia plants by meristem culture.

The isolation of meristems and virus elimination involves the excision of a suitable explant from the infected parent plant, the aseptic culture of the explant in a nutrient medium, which in most cases is based on MS (Murashige & Skoog, 1962) and finally the establishment of the new plantlet in soil as in given figure. Other tissue culture techniques used for virus elimination are callus culture, protoplast culture, and the culture of reproductive tissues (George, 1993b).

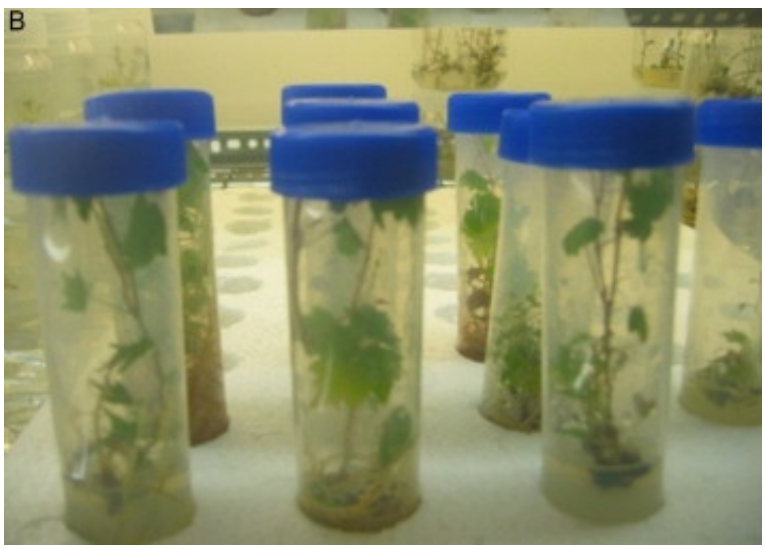

Fig. 3 Grapevine meristem culture *in vitro*. (A) Grapevine meristem and (B) rooted *in vitro* grapevine microplants in transparent polycarbonate tubes.

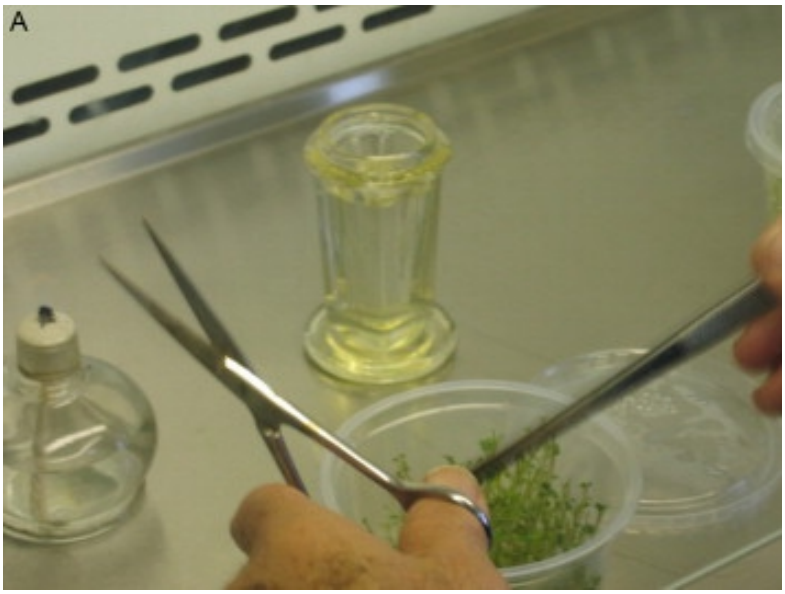

Fig. 4 Potato meristem culture *in vitro* (Aseptically prepared microcuttings for *in vitro* mass micropropagation)

Fig. 5 Potato meristem culture *in vitro* (culture with grown up and rooted Microcuttings ready to be transferred for acclimatization *ex vitro*)

8.9 Anther Culture

Anther culture means plant regeneration from the haploid microspore cells with the aim of haploid and dihaploid plant production. There are few reports on plant regeneration in the anther culture of buckwheat. Adachi et al. (1988) the first regenerated whole plants in anther culture of the diploid cultivar of common buckwheat Miyazaki-Zarai. The regenerants were all diploid but their

origin was unclear. The authors did not exclude the chromosome duplication in plant regenerants. In the experiment of Kong et al. (1992) regenerants were obtained in all six cultivars used. The adopted plants grew well but did not set the seeds. Unfortunately, the number of chromosomes was not counted in this case.

Bohanec et al. (1993) were the first who proved the presence of haploid plants among the regenerants of diploid cv. Daria. They noted the strong tendency toward endoduplication because they detected the presence of diploid, triploid, tetraploid, and aneuploid cells. Berbec and Doroszewska (1998) induced regeneration in anther culture of one tetraploid and two diploid cultivars. The most prevalent class of regenerants was tetraploids. No haploids were found among regenerants derived from the diploid cultivars; likewise no diploid regenerants were obtained from the tetraploid cultivar. The authors assumed that polyploid regenerants must have arisen through multiplication of the initial chromosome number in microspores or somatic tissues. Yui and Yoshida (2001) obtained a number of plantlets regenerated in anther culture of three diploid Japanese cultivars. Most of the plants showed the same amount of DNA as that of the control and were considered to be diploid.
Wang and Campbell (2006) tried to apply the anther culture in the buckwheat breeding process. Among the seven genotypes tested, three were the self-pollinated lines developed from interspecific hybridization between common buckwheat and wild *F. homotropicum*, and two genotypes were F_1 hybrids. Tetraploid and somatic diploid regenerants were predominant and no haploid plants were found. Based on the somatic origin of the regenerants the authors concluded that buckwheat is a difficult species in which to produce haploids or doubled haploids via anther culture.

Anther culture is used to develop instant homozygous inbred lines, avoiding the lengthy time needed using traditional selfing methods including bud pollination. Anther culture results in haploid plants, easily identified by their smaller sterile flowers. Chromosome numbers are doubled using colchicine to give doubled haploids. Spontaneous diploids can also

occur. Depending on incompatibility status, doubled haploids can be used as parents for hybrids or as 'cultivars' in their own right. The technique is successful in calabrese. Optimal conditions for anther culture include initial culturing at high temperatures (35 °C) for up to 48 hours, which stimulates embryogenesis; pretreatment of flower buds with high temperatures also stimulates embryogenesis.

Medium composition is important; increasing the auxin level tenfold increases embryo yields but added cytokinins were inhibitory or variable in effect with clearly different responses between cultivars. Cytokinins were not able to replace the inductive effect of high temperature shocks. In relation to pH of the medium, embryos were produced from responsive genotypes over the range 4.5 to 7.5, but the standard pH 5.5 to 5.8 was optimum with a decrease outside this range. The method of anther culture influences embryogenesis; anther culture with filaments attached lowered the production of embryos, and orientation of anthers with adaxial side uppermost was also found to be important. Fewer embryos were obtained using rotary shake culture compared with stationary culture. The proportion of responsive anthers shows a population effect, with fewer anthers being responsive at lower anther density. The number of embryos realized per responsive anther was, however, largely independent of density.

Responsiveness is dependent on genotype and genotype × developmental stage. For the best response plants are raised under controlled environmental conditions. However, anther culture from field-grown plants is possible in breeding programmes, as demonstrated in a survey of calabrese cultures where at least a small number of embryos were recovered in all but three plants (representing a 93% success rate).

Apart from calabrese, other broccolis including purple heading broccoli and sprouting broccoli grown under field conditions gave very poor responses; but one purple and one white sprouting broccoli gave good responses at 89 embryos per 100 cultured anthers and 76 embryos per 100 cultured anthers

respectively. Variation in response and the observation that populations of plants derived from microspores within cultured anthers do not necessarily represent a random genetic array imposes some limitation of its use in breeding programmes. It may be possible to select for improved responsiveness and reduce plant-to-plant variation by adjustment of the culture environment. Chilling the donor plants for several weeks significantly improved the response in the poorly responsive calabrese cultivar 'Green Belt'.

SAQ 1

Q. 1 Define anther culture.

Q. 2 Define pollen culture.

Q. 3 What do you mean by meristem culture?

8.10 Pollen culture

Pollen is a small grain that consists of a few cells. To the naked eye it appears as a yellowish (pale yellow) dust-like substance that is either dispersed by wind or insects.

Pollen is formed within the sacs (or microsporangia) in the anthers that are located in plant flowers. The development of plant anthers also involves the growth and differentiation of tissue that develop to produce the pollen sacs. This is then followed by a process of cell division (meiosis) that results in the formation of clusters referred to as quartets within the pollen sac chambers. The quartet then undergoes further divisions to produce pollen, which serves as the male gametophyte of plants (seed plants) for reproduction. Depending on the type of plant, pollen is either dispersed by wind or insect to the receptive stigma.

A plant that is pollinated through wind is referred to as **anemophilous** while those by insects are known as **entimophilous**. Once a pollen grain falls on the receptive stigma, it starts absorbing water and becomes activated. This causes the gametophyte in the grain to develop a tube reaching the ovule of the flower, which in turn allows male gamete cells to be transported to the ovule for fertilization. **Pollen culture** (microspore culture) is a technique in which haploid plants are obtained from isolated pollen grains while in anther culture

those are obtained from pollens, by placing anthers on a suitable, synthetic culture medium.

The technique was discovered by Guha and Maheshwari (1964). It is one of the various tissue culture techniques or methods used. Progeny developed by this technique contains a single set of chromosomes.

8.11 Procedure for In-vitro Pollen culture:

- **Collection of anthers** – Preferably should be taken from plants grown under controlled conditions or may be taken from plants grown in the field.
- **Transfer of anthers to the culture medium** – Flower buds of the appropriate developmental stage are collected and surface sterilized. Anthers from the buds are excised and placed horizontally on surface (or partially embed) on culture medium.
  It is important to note that injury to anthers may induce callus formation from anther walls. To avoid this, small anthers are cultured as such and sometimes entire inflorescence is cultured.

8.12 Pollens are isolated either by squeezing or float culturing of anthers:

Squeezing method

Anthers (about 50 to 60) are placed in 20 ml of medium. Squeezed gently with a glass rod to separate pollen from anther walls. The prepared solution is filtered through a nylon mesh of suitable pore size. Filtrate is centrifuged for about 5 min at 500-800 rpm. The resulting pollen dense pellet is collected, washed twice. Final concentration of pollen is adjusted to 103-104 pollen/ml.

Float culture

Anthers are excised. Those are placed in petridishes containing liquid medium. Anthers float in liquid medium. The anthers release their pollen grains into the medium in a few days.

Culture Medium for pollen culture:

Pollen can be cultured on a suitable medium containing sucrose (usually 2-3%), iron, vitamins, hormones etc. The hormonal component of the medium is important for initiation of growth. Usually to the culture medium auxin, cytokinin etc. are added either singly or in various combinations. Low concentration of auxin stimulates callus formation. In a medium supplemented with auxin, formation usually occurs at a faster rate. Medium used depends on various factors like:

- ✓ Species
- ✓ Genotype of donor plants and anthers

- ✓ Age of donor plants and anthers
- ✓ Growing conditions of donor plants
- ✓ Isolated pollen grains are cultured on a synthetic medium, the important ingredients of which are glutamine, L-serine and inositol. For anther cultures sucrose is essential ingredient.

8.13 Protoplast isolation and culture

Plant protoplasts are living cells that lack walls. They are produced by treating plant tissues with mixtures of enzymes that digest cell walls. As in other forms of *in vitro* culture, a variety of source tissues have been used to produce protoplasts, including nucellar tissue, pollen grains, roots, petioles, leaf laminae, cotyledons, shoots, flower petals, and xylem. Seedling cotyledon tissues are most often used in conifer protoplast research. Often callus or cell suspension cultures are developed from these tissues, and protoplasts are derived from these sources. In citrus, for example, cultured callus derived from nucellar tissues of the ovule is used to produce protoplasts. An unusual requirement for low ammonium concentrations in the media appears to be a feature of successful protoplast culture in some but not all cases.

The exact protoplast isolation procedure is quite genotype specific, but there are common features. Tissues often are finely dissected or shredded to enhance penetration of enzyme solutions. Plasmolysis also is induced by exposing the source tissue to solutions with low osmotic potential. The enzymes used to digest the cell wall matrix usually consist of various mixtures of cellulase, hemicellulase, and pectinase. They are obtained mostly from fungi or bacteria grown on cell wall substrates. Addition of a protein, bovine serum albumin (BSA), often improves protoplast yields, and it is thought that BSA serves as an alternate substrate for protease molecules that would otherwise attack proteins on the plasmalemma surface.

Liberated protoplasts have a distinctive spherical appearance in given figure, with chloroplasts, a thin layer of peripheral cytoplasm, and a large vacuole. In this state, numerous genetic manipulations can be accomplished with protoplasts. For example, protoplasts can be induced to take up DNA either spontaneously or through treatments such as electroporation and microinjection.

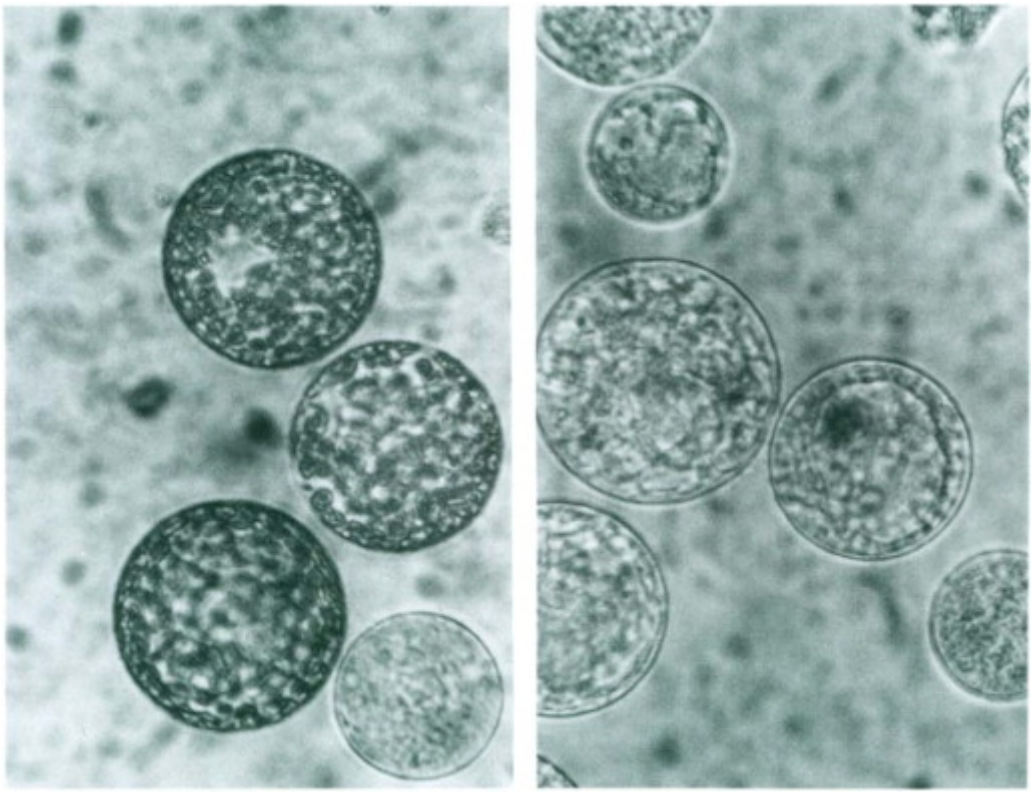

Fig. 6 Protoplasts of cluster pine (left) and giant sequoia (right)

Somaclonal selection can be conducted by exposing the millions of protoplasts in a small volume of culture medium to selective pressures such as high NaCl or heavy metal concentrations. Fusion may occur in culture when two protoplasts come into contact in the presence of polyethylene glycol. Application of an electric field also tends to promote protoplast fusion. Although the fusion product has a common cytoplasm, nuclei may or may not fuse. One or the other nucleus may be lost or destroyed by irradiation; in this case the product is known as a cytoplasmic hybrid or cybrid. If there is fusion of nuclei, a true somatic hybrid is created. The development of somatic hybrids is most advanced in the Rutaceae, and regenerated plants of true somatic hybrids between Citrus sinensis and Poncirus trifoliata cultivars have been produced. Intergeneric somatic hybrids between wild pear and Colt cherry also have been obtained.

Once protoplasts are liberated and removed from the enzyme solutions, they are purified to remove contaminating materials such as cell wall debris. Subsequently, protoplasts may be cultured in liquid or on semisolid media, again with genotype-specific requirements for media composition and protocols. Culture of protoplasts on a more solid matrix provides the physical isolation necessary to produce genetically identical colonies, and there is an optimal plating density above and below which cultures do not grow well. Low light levels are preferred for protoplast culture, perhaps because high light bleaches chloroplasts.

Protoplasts begin to synthesize a new cell wall within hours, and some regenerated cells may begin to divide within 1 to 2 weeks. Only a small proportion of cells may divide. For example, only 10 to 15% of cells regenerated from protoplasts of Douglas fir and lodgepole and cluster pines divided during the first week of culture. Applied electrical fields can stimulate protoplasts to divide, and higher osmotic potential in the culture medium, alteration of nitrogen nutrition, and other modifications are required for continued cell division and development of microcalli.

The ability of researchers to cycle woody plants through protoplast isolation to regeneration of plantlets remains limited, but the number of successful examples is increasing. For instance, whereas McCown and Russell reported only four such examples through Spring 1986, whole plants had been regenerated from protoplasts isolated from 45 woody plants by the time of a review by Russell. These systems were developed for a wide variety of plants, including gymnosperms (e.g., hybrid larch and white spruce), temperate deciduous angiosperms that are important timber trees (e.g., yellow poplar, black poplar, and aspen), and numerous fruit and ornamental trees (e.g., hybrid elm, citrus, apple, cherry, pear, and coffee).

8.14 Embryo culture

Embryo culture is the culture of isolated immature or mature embryos. Zygotic or seed embryos are often used advantageously as explants in plant tissue culture, for example, to initiate callus cultures. This embryo develops properly when nourishing tissue; endosperm was present in the seed during the development. But crossing between the two distant species resulted in the degeneration of endosperm tissue, which hinders embryo development and in such a case viable plant will not develop. To recover such a hybrid embryo, culturing of the embryo was followed. This culturing of embryo (hybrid) in *in vitro* conditions is known as embryo rescue and is widely used for crop improvement.

Hanning in 1904 first cultured isolated mature embryo of a cross between *Cochlearia* × Raphanus and successfully obtained the plants. In 1925 and 1929, Laibach isolated zygotic embryos from nonviable seeds of Linum *perenne* ×*L. austriacum*. These hybrids cannot be developed naturally. In 1941 for the first time Van Overbeek et al. introduced coconut milk for the development of embryo and callus formation in Datura.

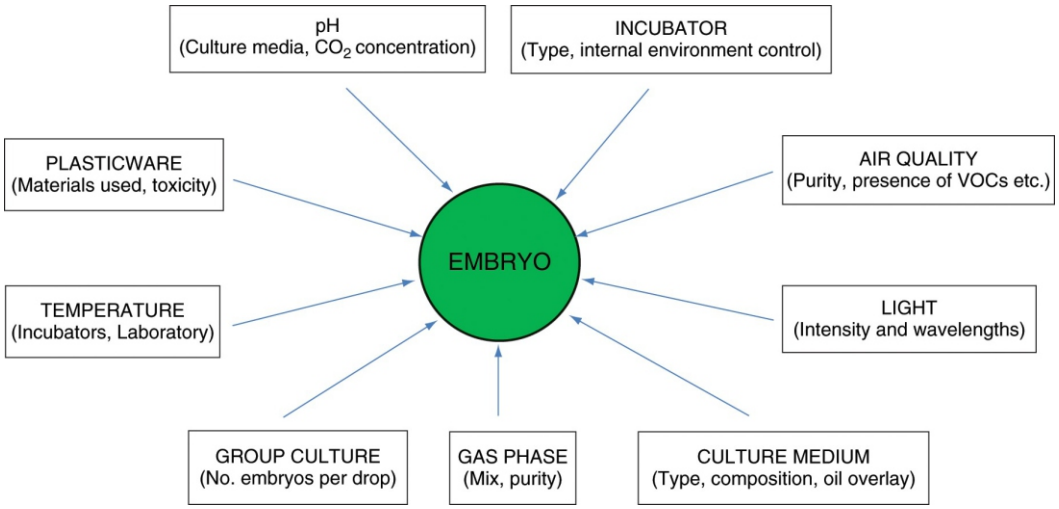

Fig. 7 Embryo culture

Other research has led to the development of a new area of hybrid embryos through embryo culture, which has given relief to those embryos that are usually aborted (due to various reasons) at early stages and thus cannot be raised naturally. This technique is used for culture where the embryo is incompletely developed (orchids), to overcome seed dormancy, for shortening the breeding cycle, determination of seed viability, microcloning of the source material, for rapid multiplication, and conservation of several medicinally important and endangered plants (e.g., recently embryo culture of medicinally important endangered forest tree species Strychnos *potatorum* with 100% germination rate was conserved by using this technique).

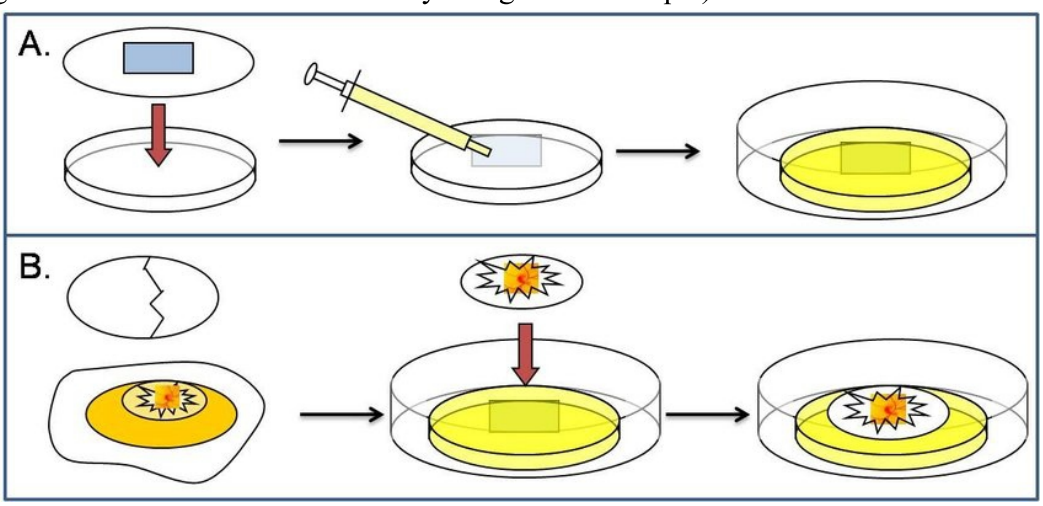

Fig. 8 Schematic representation of embryo culture method

In addition, embryo culture also acts as a rich source of therapeutic secondary metabolites, e.g., an alkaloid (neferine) having the potential for inhibiting human lung cancer has been recently explored from lotus seed embryo. Obtaining contamination-free seeds or embryos without damage or oversterilization is one of the most difficult obstacles in the successful establishment of *in vitro* cultures. Sugii demonstrated the various disinfestation protocols (bleach sterilization, gas sterilization, and ethanol dip and flame) that

are successful in establishing *in vitro* cultures of several endangered Hawaiian plant taxa.

8.15 Applications of embryo culture

Embryo production from sterile seeds (e.g., apricot), viable embryo production (interspecific and intergeneric crosses resulting in nonviable seeds), micropropagation of hard species (*Pinus palustris*), breaking of dormancy, haploid production (Hordeum *vulgare* and *Hordeum bulbosum*), and germination of seeds of obligatory parasites without the host can be achievable with embryo culture.

8.16 Summary

Under this unit we summarize that tissue culture plays a critical role in the genetic transformation of a plant species because the first requirement for most gene transfer systems is efficient regeneration of plantlets from cells carrying a foreign gene. Other than plant tissue culture, various methods of gene transfer are also necessary to obtain transgenic plants. Tissue culture is utilized to propagate large quantities of plants rapidly. High mutation rates and less desirable plant habits are difficulties encountered using tissue culture compared to propagation by terminal and leaf cuttings.

Plants propagated by tissue culture can originate from several different plant parts. Techniques have been developed to propagate hiemalis begonia from vegetative buds (Reuther, 1980), petiole segments, and flower stem segments. Propagation from vegetative buds is labor intensive and is primarily used to develop stock plants free of Xanthomonas *begoniae*. The basic principle of protoplast culture is the aseptic isolation of large number of intact living protoplasts removing their cell wall and cultures them on a suitable nutrient medium for their requisite growth and development. Protoplast can be isolated from varieties of plant tissues.

Embryo culture is the culture of isolated immature or mature embryos. Zygotic or seed embryos are often used advantageously as explants in plant tissue culture, for example, to initiate callus cultures. This embryo develops properly when nourishing tissue; endosperm was present in the seed during the development. But crossing between the two distant species resulted in the degeneration of endosperm tissue, which hinders embryo development and in such a case viable plant will not develop. Artificial embryo culture media basically contain **glucose, pyruvate, and energy**-providing components, but the addition of amino acids, nucleotides, vitamins, and cholesterol improve the performance of embryonic growth and development.

8.17 Terminal questions

Q.1. What do you mean by meristem culture?

Answer:----------

Q.2. Describe different types of culture methods.

Answer:----------

Q.3. Write a short note on pollen culture.

Answer:----------

Q.4. Write a short note on anther culture.

Answer:----------

Q.5. Describe embryo culture.

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Text book of Botany – Singh -Pande-Jain.
3. The elements of Botany- James Hewetson Wilson
4. Textbook of Biotechnology –H. K. Das
5. Biochemistry and molecular biology- Wilson Walker

Unit 9 Applications of Biotechnology

Structure

9.1 Introduction
Objectives:
9.2 Overview on Biotechnology
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9.5 Awareness in the fields of Bio-technology
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9.1 Introduction

Biotechnology, as the word suggests, is combination of biology and technology. Biotechnology is the use of technology to use, modify or upgrade the part or whole of biological system for industrial and human welfare. Biotechnology (commonly abbreviated as biotech) is the broad area of biology involving living systems and organisms to develop or make products, or "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use" (UN Convention on Biological Diversity).

Depending on the tools and applications, it often overlaps with the (related) fields of molecular biology, bio-engineering, biomedical engineering, biomanufacturing, molecular engineering, etc. For thousands of years, humankind has used biotechnology in agriculture, food production, and medicine. The term is largely believed to have been coined in 1919 by Hungarian engineer Károly Ereky. In the late 20th and early 21st centuries, biotechnology has expanded to include new and diverse sciences such as genomics, recombinant gene techniques, applied immunology, and development of pharmaceutical therapies and diagnostic tests.

Objectives:

This is the ninth unit of third block on Biotechnology**.** Under ninth unit, we have following objectives. These are as under:

- To know the basic concepts of biotechnology in reference of biochemistry.
- To recognize the approaches of biotechnology with its relevant braches viz. bioinformatics, biochemistry, microbiology, medical and agriculture biotechnology etc.
- To know the accessibility and approaches of biotechnology in different fields such as industry, medicine and agriculture etc.
- To discuss about applications of biotechnology in human welfare special reference with agriculture.

9.2 Overview on Biotechnology

Biotechnology is the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services. From its inception, biotechnology has maintained a close relationship with society. Although, now most often associated with the development of drugs, historically biotechnology has been principally associated with food and addressing such issues as malnutrition and famine etc.

9.3 History

The history of biotechnology begins with zymotechnology, which commenced with a focus on brewing techniques for beer. By World War I, however, zymotechnology expanded to tackle larger industrial issues, and the potential of industrial fermentation gave rise to biotechnology. However, both the single-cell protein and gasohol projects failed to progress due to varying issues including public resistance, a changing economic scene, and shifts in political power. Yet, the formation of a new field, genetic engineering, has brought biotechnology to the forefront of science in society, and the intimate relationship between the scientific community, the public, and the government would ensue. These debates gained exposure in 1975 at the Asilomar Conference, where Joshua Lederberg was the most outspoken supporter for this emerging field in biotechnology. By 1978, with the development of synthetic human insulin, Lederberg's claims proved valid and the biotechnology industry grew rapidly. Each new scientific advances became a media event designed to capture public support, and by the 1980s, biotechnology grew into a promising real industry. In 1988, only five proteins from genetically engineered cells had been approved as drugs by the United States Food and Drug Administration (FDA), but this number would skyrocket to over 125 by the end of the 1990s.

9.4 Definitions

Biotechnology can be defined by different ways as under:

- It is a technology which is used to manipulate living organisms and biological systems to produce products that advances healthcare, medicine, agriculture, food, pharmaceuticals and environmental control by modifying it according to human purposes.
- The field of science which deals with the use of biological processes, organisms or systems in various technological innovations in industrial, agricultural and medical fields, helping to build a better world.
- Biotechnology is the pinnacle of man & machine, its existence allows us to learn about the connection between the biological body and our technology, it will one day allow us to evolve far beyond our current evolutionary future; it's man-made evolution.
- It is science about methods and production technologies on the basis of natural biological objects.
- It is bio-production, genetic engineering, cloning rolled into one discipline.
- The science of using naturally occurring, living entities to develop new and unique applications and technologies.
- Biotechnology is the process whereby living systems, be it plants or organisms, or their derivatives are modified using techniques of biology, coupled at times with knowledge from chemical engineering,

bioinformatics or robotics, to develop products and services essentially aimed at bettering of human standard and way of life.

Biotechnology is the use of living things especially eukaryotic cells and bacteria for production of various products for benefiting human beings. It is a combination of various technologies, applied together to living cells, including not only biology, but also subjects like mathematics, physics, chemistry and engineering. Its application ranges from agriculture (Animal husbandry, cropping system, soil science and soil conservation, plant physiology, seed technology and crop management etc) to industry (food, pharmaceutical, chemical byproducts, textiles etc.), medicine, nutrition, environmental conservation, making it one of the fastest growing fields. Biotechnology is to modify genetic structure in animals and plants to improve them in desired way for getting beneficial products.

9.5 Awareness in the fields of Bio-technology

Biotechnology is distinguished and developing sciences in modern scientific world. It elucidates the newest pattern of scientific advances which focus at deriving valuable products for human race through merging the biological systems with the engineering practices. The phrase 'biotechnology' was coined by the Hungarian engineer Karl Ereky in 1919 but this term has been used by humans since ancient times for producing food. In biological literature, Biotechnology is knowledge based on biology, particularly when used in agriculture, food science, and medicine. According to the UN Convention on Biological Diversity, Biotechnology means any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.

Biotechnology is normally described as the branch of biological sciences which involves the practice of livings organisms that is associated with plants, animals, birds, bacteria, fungi, viruses or other defined groups of living organisms and the biological procedures being done within these living organisms that include all the anabolic, catabolic and reproductive systems and processes in particular ways, using the modern engineering tools and procedures to develop valuable commercial goods at small and large scale. Other group of theorists explained the term biotechnology as application of principles of engineering and biological science to produce new products from raw materials of biological source such as vaccines or food. It can also be elucidate as the use of living organisms or their products to alter or improve human health and human environment. Biotechnology is extensively used in numerous disciplines at global scale. Numerous important scientific research institutions have initiated huge programs and projects in biotechnology and address number of problems for human race on earth for example food

catastrophe through efficient utilization of biotechnological practices and approaches.

9.6 Development of Biotechnology

9.6.1 Ancient biotechnology

Early history as related to food and shelter; Includes domestication.

9.6.2 Classical biotechnology

Built on ancient biotechnology; Fermentation promoted food production, and medicine.

9.6.3 Modern biotechnology

Manipulates genetic information in organism; Genetic engineering

Conventionally, biotechnology was defined as ancient ways of using living organisms to produce new products or adapt existing ones. Broadly speaking, traditional biotechnology can be drawn back to human's evolution from hunter-gatherer to agriculturalist. As ranchers, humans collected uninhabited plants and cultivated them and the best yielding strains were selected for growing the following seasons. From time to time, farmers discovered many plant varieties and they progressively became proficient at upbringing specific plant varieties to obtain desired qualities such as disease resistance, better taste and higher yield.

In primordial time, humans unintentionally discovered the ways to use of natural processes that occur all the time within living cells. Though they did not have any scientific explanation for the processes, but they applied these techniques to get positive results such as food matures in a way that changes its taste and content, and makes it less consumable. When appraising modern biotechnology, it is denoted as several techniques that involve the planned manipulation of genes, cells and living tissue in an expectable and controlled manner to produce changes in the genetic make-up of an organism or produce new tissue. For example recombinant DNA techniques, tissue culture and mutagenesis. Modern biotechnology started with the 1953 discovery of the structure of deoxyribonucleic acid (DNA) and the way genetic information is transmitted from generation to generation.

This innovation was made possible by the previous discovery of genes (discrete, independent units that transmit traits from parents to offspring) by Gregor Mendel. These discoveries laid the foundation for the changeover from conventional to contemporary biotechnology. Plethora of studies have demonstrated that modern biotechnology is often related with the use of genetically altered microorganisms such as E coli or yeast for the production of materials like insulin or antibiotics. It can also be denoted as transgenic

animals or transgenic plants, such as Bt corn. Genetically modified mammalian cells, such as Chinese Hamster Ovary (CHO) cells which are also extensively used to manufacture pharmaceuticals. Other usage of new biotechnology is the development of plant-made pharmaceuticals.

9.6.4 Aspects of Biotechnology

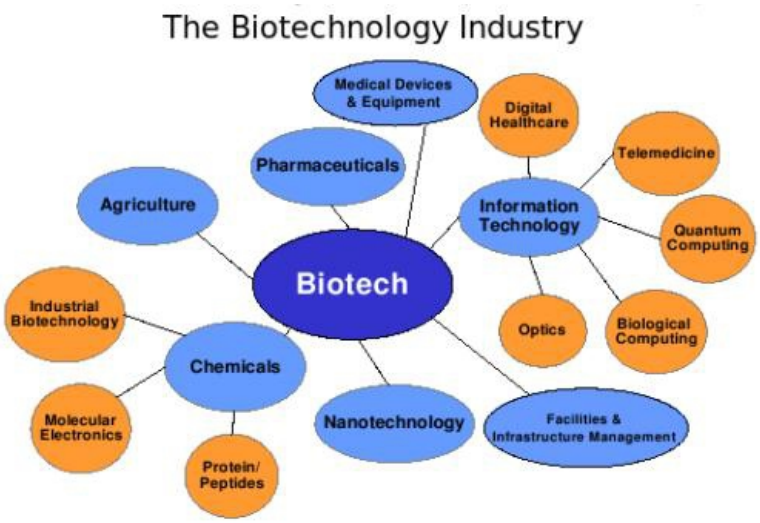

Fig. 1 Biotechnology and its scopes

9.6.5 Major goals of Biotechnology

- ✓ To understand more about the processes of inheritance and gene expression.
- ✓ To provide better understanding & treatment of various diseases, particularly genetic disorders.
- ✓ To generate economic benefits, including improved plants and animals for agriculture and efficient production of valuable biological molecules.

Major scientific disciplines where biotechnological processes frequently used are described below:

1. Genetic Engineering is perhaps the important arena among all sciences which biotechnological projects are used and deal with numerous problems. The use of technology to modify the genomes of viruses, bacteria, and other cells for medical or industrial purposes is called genetic engineering. Basically, genetic engineering encompasses the manipulation of genetic material of a living organism which may be plants, animals, birds, bacteria, fungi, viruses or other defined groups of living organisms in order to produce the anticipated changes in that living organism.

In fact, the genetic material of any living organism controls all the activities and products of that organism, therefore by bringing the required change in the genetic material, biotechnologist can make that living organism work exactly

in the desired way and produce exactly the desired product. It can be said that the use of genetic engineering becomes very important in biotechnology. Insertion of foreign genes into bacterium is as under:

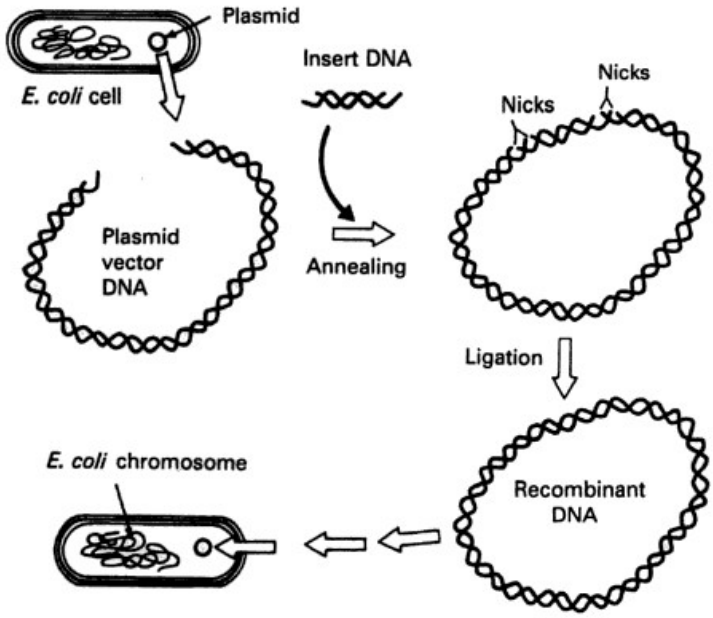

Fig. 2

2. Microorganism is also major source of biotechnology (Bryce, 2004). Biotechnologists are also interested in researching on microorganisms for their experiments and projects rather than dealing with macro organisms. Microorganisms include small microscopic organisms and macroscopic organisms involve large living organisms such as plants and animals. This is mostly because these microorganisms are easy to study and easy to work on as they have a relatively simple and understandable genetic makeup.

3. Embryology is also major branch of biology which involves biotechnological processes. Most of the biotechnological products depend upon the reproduction ability of the living organisms. As they reproduce, the scientists expect them to produce the desired product. The process of this reproduction is well understood by having good knowledge of embryology.

4. Other sciences like Physiology, Tissue culture, Histology, Molecular biology are also studied in biotechnology.

9.7 Branches of Biotechnology

There are several branches of biotechnology. They are classified as under:

9.7.1 Marine Biotechnology

This branch of biotechnology deals with the marine organisms and obtaining useful products from them. The use of this biotechnology is expanding now. This is also termed as “Blue Biotechnology” which is used to protect the

marine organisms from harmful diseases underwater. It relates with the exploring marine biodiversity and it's use in development novel bio products.

9.7.2 Bioinformatics

Bioinformatics is the combination of computer and biotechnology. It is used in developing databanks for DNA and protein sequences and chemicals and analysis of data for various purposes. It is also used for various purposes like development of drugs and medicines; it is also used to improve the fertility of crops and plants, and also for pest, drought and diseases resistance. Bioinformatics is known and referred by the term computational biology. It plays an important and vital role in areas like functional genomics, structural genomics and proteomics. These areas contribute a lot and become a key contributor to biotechnology and pharma sector.

9.7.3 Agriculture Biotechnology

Agriculture biotechnology is the term used for the agricultural sector. With the help of the process called micro-propagation (a practice of producing larger number of plants through the existing stocks of plants) it helps in selecting the right quality of plants and crops. Also with the help of transgenic plants (plants whose DNA is modified); this design of transgenic plants helps to grow in a specified environment with the help of certain chemicals.

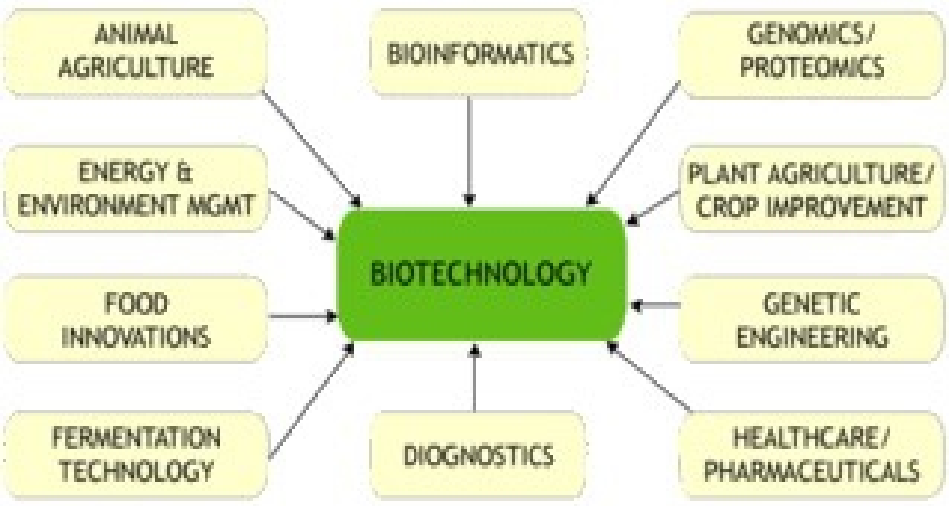

Fig. 3 Different branches of biotechnology

9.7.4 Medical Biotechnology

Medical biotechnology is referred to as "Red Biotechnology". It is used for the production of drugs and antibiotic medicines. It also helps to create or design organisms. Through the process of genetic manipulation, it helps to cure genetic issues in organisms. It also helps in analyzing diseases in organisms. It

also helps in developing new ways of diagnosis by performing tests. With the help of stem cell technology, it helps to develop new organs from the stem cells of patients for patients and also promises the cure for the damaged tissues in organisms.

9.7.5 Industrial Biotechnology

Industrial biotechnology is also called and known by the name "White Biotechnology". This area of biotechnology is used and applied in industries and its processes. Its various uses include; biopolymers (plastics) substitutes, new invention of vehicle parts and fuels for the vehicles, invention of fibers for the clothing industry, it is also involved in developing new chemicals and in the production process.

SAQ 1

Q.1 what do you mean by Biotechnology? Define it.
Q. 2 what are branches of Biotechnology?
Q. 3 Discuss future prospects of Biotechnology in brief.

9.8 Applications of Biotechnology

Biotechnology has offered modern medical devices for diagnostic and preventive purposes, which include diagnostic test kits, vaccines and radio-labeled biological therapeutics used for imaging and analysis. Human health is a major growing concern worldwide because of infectious diseases as well as life style diseases. Biotechnology plays dynamic role in facing the challenges related to human health as it has flexibility to reduce global health differences by the provision of promising technologies.

Malnutrition mainly arises due to the lack of essential nutrients and vitamins in food and ultimately results in death. Biotechnology is playing a major role in eliminating these problems by producing nutrients enriched food such as Golden Rice, Maize, potato and soybean etc. Biotechnology has also played an important role in controlling the environmental pollution through biodegradation of potential pollutants. This review sketches improvement of human health by the use of biotechnological advances in molecular diagnostics, medicine, vaccines, nutritionally enriched genetically modified crops and waste management.

Biotechnology contributes much towards the growing public and global health needs. It has revolutionized mankind since its existence. It provides effective diagnostics, prevention and treatment measures including production of novel drugs and recombinant vaccines. It gives effective drug delivery approaches, new methods for therapeutics, nutritionally enriched genetically modified crops and efficient methods for environmental cleanup. Health, life quality and expectancy of life have been increased worldwide through the services

provided by biotechnology. Parasitic and infectious diseases like *Acquired Immunodeficiency Syndrome* (AIDS) and *tuberculosis* (TB) are being diagnosed rapidly at relatively low cost. Molecular diagnostic tools including polymerase chain reaction (PCR), recombinant antigens and monoclonal antibodies are being used for this purpose.

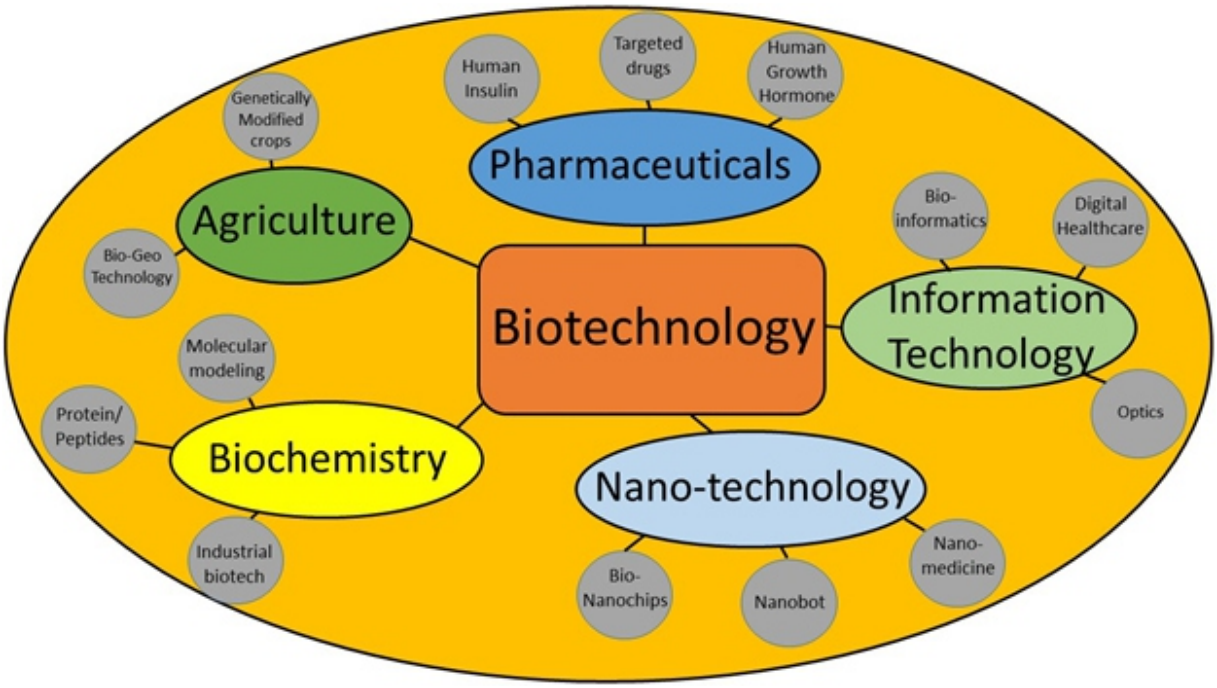

Fig. 4 Biotechnology and its brances

Biotechnology has offered modern diagnostic test kits, rickettsial, bacterial and viral vaccines along with radio-labelled biological therapeutics for imaging and analysis. Vaccines have eliminated small pox, polio and other deadly diseases for the last hundred years. Biotechnology has made advancements in vaccination by making recombinant vaccines that have the potential to eradicate non-communicable diseases like cancer. Naked DNA vaccines, viral vector vaccines and plant-derived vaccines are found to be most effective against a number of bacterial and viral disorders. Therapeutic proteins have a large influence on non-communicable diseases responsible for over 60% of deaths in developing countries. Transgenic bacteria, yeasts, plants and mammals are used as a factory of recombinant therapeutic proteins. New desired genes are introduced into these systems to produce therapeutic proteins of interest in large quantities and are then purified.

The most important recombinant therapeutic proteins include erythropoietin for anemia treatment. Interferon alpha against leukemia and viral diseases and insulin against type 1-diabetes mellitus have been produced. Other therapeutic agents include growth hormones, cytokines, recombinant blood products, monoclonal antibodies, gene therapy products, molecular pharming agents and

engineered tissue products. Xenografts, bone grafts, collagen and heart valves have been successfully engineered.

Biotechnology offers relatively cheaper drug and vaccine delivery tools. They eliminate blood-borne infections caused by re-use of needles. Drugs and vaccines are delivered efficiently in a controlled manner, thus avoiding the use of needles. Drugs can be propelled into the body speedily by gas jets, can be diffused into the body and can also be inhaled through nasal sprays. Drugs are efficiently delivered into the body using nanoparticles. Recent advances in biotechnology have reduced drug dosage required for a particular treatment.

Genetically modified crops are beneficial product of biotechnology which fulfills the requirement of essential nutrients and vitamins in the body. They are helpful in the physical and cognitive development of children. Novel and desired genes are introduced into these crops which become nutritionally enriched to cope with growing needs of health care among the people worldwide. Examples include iron rich rice, which fulfills the requirement of iron, thereby preventing anemia. Golden rice is rich in vitamin A, the deficiency of which causes blindness among a lot of children in developing countries. Potato has also been genetically engineered to have all the vitamins, thereby satisfying the nutritional needs of every individual. Genetically modified foods can become a part of staple food to increase the life quality.

Other enriched genetically modified foods include flax seeds, corn, maize, tomatoes, papaya, squash, soybeans, sugar beets, and canola and cotton seeds. Biological fungicides, herbicides and insecticides have also been produced to control pests. Biotechnology has tremendous applications in environmental cleanup for the sake of human health. Contaminated soil, water and air are being cleaned by using microorganisms or plants by the process of bioremediation.

Microorganisms and plants have the ability to detoxify organic waste pollution and heavy metal pollution caused by mercury, cadmium, and lead. Radioactive waste, acid mine drainage and oil spills are also treated effectively by using biotechnology. Plant biomass can be converted to ethanol, which can then be used as biofuel, offering much less air pollution. Biological enzymes can make the processing of paper and pulp industry easy, efficient and rapid.

9.9 Role of biotechnology in agriculture sector

The agricultural biotechnology sector (Ag Biotech) shares a common scientific foundation with the therapeutic biotechnology sector, including similar characteristics of a lengthy time to market for emerging products. But the challenges, goals, and opportunities for agricultural applications of

biotechnology provide a very different context for innovation and entrepreneurs. Now just 30 years old, the Ag Biotech sector is entering its third cycle of innovation, with the first wave being agricultural biotechnology trait creation followed by a second wave of Ag Biotech trait commercialization. The agricultural trait segment continues to focus on two product categories, herbicide tolerance and insect resistance, in spite of few entrepreneurial opportunities available. However, Ag Biotech applications to chemistries, biopesticides, microbials, and natural products offer new opportunities. These sectors offer new business opportunities that, like the first wave of Ag Biotech, enable entrepreneurs to think about creating disruptive businesses, not just disruptive technologies for today's business models.

Agricultural biotechnology is built with competencies and technical skills that describe the fundamental use of it in plant as medicine and food and defined as a set of tools that use living organisms or parts of it to make or modify a product, improve plants, trees, or develop microorganisms for specific uses pertaining to food production as a big picture to provide food security to the people. Agricultural biotechnology has products and process where in the crop improvement happens through rudimentary biotechnology following Mendelian genetics where two plant types of the same species are crossed to produce a better plant type. Crossing a plant that has a high tolerance to disease, drought tolerance, and pest resistance, with a plant that has a high fruit yield gives you a disease and drought tolerant and pest-resistant plant with a high fruit yield, which will be critical for helping farmers adapt to new growing conditions.

Professional plant breeders acquiring genetic material from ex situ collections benefit extensively from free access, information, and from materials that have been selected in various breeding programmers' for its desirable characteristics. Changing genetic traits of plants in a way to increase their resistance against pests or droughts thereby the commercial biotechnology steps from a successful transformational event into a plant breeding stage, where the benefits of investing in research are taken by partner organizations or countries through global technology transfer. Producing enough food for our growing global population comes with the added challenge of changing consumption patterns, as well as the need to cope with the dramatic effects of climate change and the increasing scarcity of water and land.

The current food system requires a global response to supply issues integrated in information, trade, and technology affected with rising population and more land utilization; thus, there is a need to increase food productivity in a sustainable manner, flexible to handle exigencies with better water and soil management. All these are possible with biotechnology revolution as an

integrative production system maintaining ecological diversity. Countries will have to pursue biotechnology applications that reduce waste from food and animal production, by altering animal metabolic processes to improve the environmental impact of animal waste management and disposal.

Agricultural Biotechnology is the use of new scientific techniques based on our understanding of DNA to improve crops and livestock that are not possible with conventional breeding alone. This can be achieved in part by modern molecular plant breeding techniques such as marker-assisted selection (MAS). MAS enables plant breeders to identify better traits in plants more rapidly than conventional breeding alone is capable of. Another aspect of agricultural biotechnology involves the use of recombinant DNA. Unlike molecular plant breeding, however, recombinant DNA technology results in new traits that cannot be achieved by conventional ways.

The genetic engineering of crops for improved agronomic and nutritional traits has been widely reviewed in the literature. Briefly, genetic engineering involves the introduction of a novel trait into a crop through the manipulation of its genetic material. Genetic material can be incorporated into the plant genome either via *Agrobacterium*-mediated transformation or by biolistic (gene gun) delivery, as illustrated in Figure 1. Transgenic, or genetically modified (GM) crops, have been commercially available in the United States since 1996. A well-known example of a transgenic plant is Golden Rice, which expresses β-carotene and was created philanthropically with the intent of alleviating vitamin A deficiency (VAD) in developing countries. Cisgenic plants, or plants that express genes from close wild relatives, are also being generated to obtain resistance genes which were lost over years of crop domestication. The Wheat Stem Rust Initiative, for example, is currently generating cisgenic versions of wheat which possess multiple resistance genes to the fungal pathogen Puccinia graminis f. sp. *tritici* Ugg99 from wild relatives. A third technology that falls under the umbrella of genetic engineering is RNA interference, or RNAi technology.

In this case, the plant is designed to produce an antisense RNA to a particular gene, whose expression is then blocked via gene silencing. Examples of the use of this technology are GM papaya which are resistant to Papaya ring spot virus. This technology is responsible for saving the Hawaiian papaya industry. More recently, a new technology known as ‘gene editing’ has come to the forefront. Gene editing does not require the introduction of new gene sequences; rather, it can direct only one or two nucleotide changes in a plant genome and thus is exempt from the regulations that govern the production of genetically modified organisms. While no examples of gene-edited crops are commercially available at present, much research is being undertaken in this field and many new

crop varieties will be realized in years to come using this biotechnological approach.

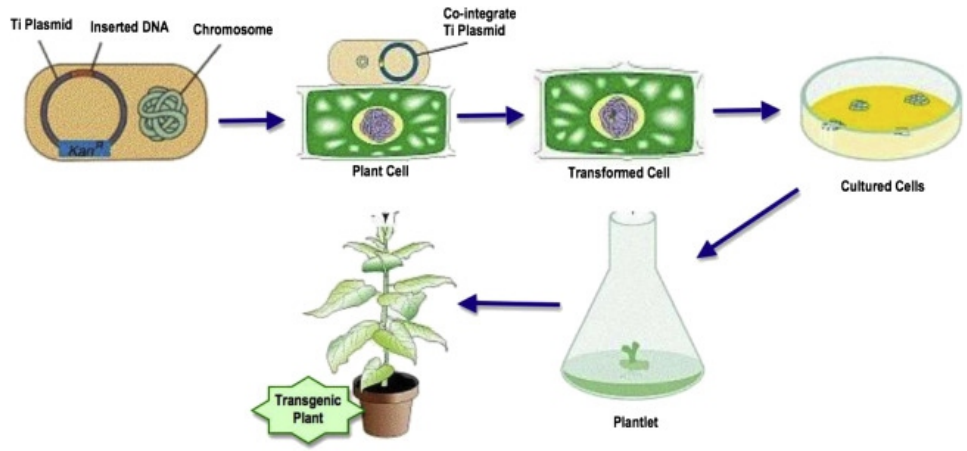

Fig. 5

It should also be noted that many varieties of crops available today have been generated using mutagenesis breeding. Not considered a form of genetic engineering, mutagenesis breeding involves the introduction of random mutations to plant cuttings using chemical or irradiation mutagenesis. Explants which express new traits are then propagated from these mutagenesis events. According to the Mutant Variety Database, over 17 000 varieties of crops have been developed using mutagenesis breeding. Ruby red grapefruit and single malt scotch are both derived from mutagenesis breeding.

Crops developed through biotechnology, specifically, transgenic crops, are being designed to address many of the world's significant agricultural challenges. For example, one of the most important products generated through agricultural biotechnology has been the introduction of transgenic crop plants which confer herbicide tolerance, in such a way that herbicides can be sprayed on transgenic crops without causing damage while detouring the growth of neighboring weeds. The utilization of herbicide-tolerant transgenic crops has therefore greatly reduced the levels of herbicide used on farms for weed management. Glyphosate [N-(phosphonomethyl)glycine] is the most widely utilized herbicide in the world.

SAQ 2

Q. 1 What do you mean by industrial biotechnology?

Q. 2 Define the applications of biotechnology in medicines?

Q. 3 Define the applications of Biotechnology in agriculture.

Almost 90% of all transgenic crops cultivated across the world possess the glyphosate-resistant trait. The second most commonly used trait generated in transgenic crops and used globally is Bt (a toxin from Bacillus thuringiensis). Insects which are susceptible for this toxin ingest the transgenic crop cultivar expressing the precursor Bt protein, which binds to the insect gut wall and

becomes activated in that alkaline environment. The insects soon die as a result of pores which are created in the gut cell membrane.

The first generation of biotechnology crops focused on agronomic input traits that helped farmers; however, their value was not clear to the general consumer who is disconnected to the demands of farming. Agronomic traits include improved crop yield and resistance against abiotic and biotic stresses. More recently, agricultural biotechnology has expanded to improve human health, including the design of biofortified and functional food crops. The next section provides examples of new traits for each.

9.10 Summary

Agricultural biotechnology has unfolded in India in form of a long drawn inconclusive battle. This political-scientific flux is aptly captured in words of Glenn Davis Stone as "the war over GM crops creates a demand for conclusive findings, parties on both sides seize on claims that confirm their antipathies and hold them up as conclusive even when they simply are not" (Stone, 2013). The greatest limitation of application of biotechnology in agriculture is the fact that it's exact course and implications are yet not known. Risks arising from GM crops cannot be ruled out altogether for all the times to come. At the same time, unnecessary politicization of public opposition tends to push the application of biotechnology in agriculture into doldrums. This may act to the detriment of large agrarian population in India. GM crops that have stood the test by independent and rigorous regulatory institutions will help in widening the horizon of agriculture in the country. To counter the monopoly of agribusiness companies, government should increase spending on the research projects in biotechnology in public sector agricultural research institutions as they are farmer-oriented.

Agricultural biotechnology, also known as agritech, is an area of agricultural science involving the use of scientific tools and techniques, including genetic engineering, molecular markers, molecular diagnostics, vaccines, and tissue culture, to modify living organisms: plants, animals, and microorganisms.[1] Crop biotechnology is one aspect of agricultural biotechnology which has been greatly developed upon in recent times. Desired traits are exported from a particular species of Crop to an entirely different species. These transgene crops possess desirable characteristics in terms of flavor, color of flowers, growth rate, size of harvested products and resistance to diseases and pests.

One can define agricultural biotechnology as a set of scientific techniques which can improve plants, micro-organisms, and animals based on DNA and its concepts.Arguably the use of biotechnology in agriculture is deemed to be more effective than that of agrochemicals. The latter is believed to be

responsible for causing environmental distress and is also somewhat unfeasible for farmers.

The role of biotechnology in agriculture is multifaceted. Some of the most prevalent benefits of biotechnology in agriculture include

Increase in Crop Production

With better disease control and increased tolerance to drought and flooding, biotechnology leads to a significant increase in crop production. This does not just match the ever-growing demand for food but also helps farmers to lower losses.

Better Crop Protection

The techniques of biotechnology serve as cost-effective solutions to problems about pests. Farmers have been able to transform crops like cotton, corn, and potato to synthesize a protein that tackles issues of pests effectively.

Increase in Nutrition Value

It has also enabled farmers to produce crops with a higher nutritional value and enhanced flavour and texture. For instance, the technology has made it possible to cultivate soybeans with high protein content, beans with more amino acids, and potatoes with starch.

Fresher Produce and Better Taste

It further helps to improve the taste and flavor of crops by enhancing the activity of enzymes present in plants. Also, it helps in keeping the yield fresh for longer.

Chemical Tolerance

Most farmers rely on herbicides to control the growth of weeds which often leads to soil erosion. However, genetically engineered food is resistant to a variety of chemicals, including herbicides; as a result, the scale of soil erosion is significantly low.

Disease Resistance

Viral infections spread by insects are often difficult to contain, and also the use of insecticides tends to pose a threat to both soil and the quality of produce. Nonetheless, genetically modified plants are less susceptible to viral infection and make it easier for farmers to contain crop damage.

9.11 Terminal questions

Q. 1 What do you mean by biotechnology? Give its definitions.

Answer:----------

Q.2 Describe biotechnological applications in agriculture field.

Answer:----------

Q.3 Write the name of any five biotechnology based company in India.

Answer:----------

Q.4 Write a short note on genetically modified foods (GMFs).

Answer:----------

Q.5 Write a short note on genetically modified organisms (GMOs).

Answer:----------

Q.6 What are the applications of biotechnology for the human welfare?

Answer:----------

Q.7 Discuss the role of biotechnology in industrial production of enzymes?

Answer:----------

Further readings

1. Biochemistry- Lehninger A.L.
2. Text book of Botany – Singh -Pande-Jain.
3. The elements of Botany- James Hewetson Wilson
4. Textbook of Biotechnology –H. K. Das
5. Biochemistry and molecular biology- Wilson Walker

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Open University

Bachelor of Science
DCEZY -108
Developmental Biology

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Uttar Pradesh Rajarshi Tandon
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Bachelor of Science
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Developmental Biology

Block -1
Developmental Biology - I

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Course Design Committee

Prof. Ashutosh Gupta — **Chairman**
Director, School of Science
UPRTOU, Prayagraj.

Dr. Shubhra Malviya — **Member**
Asst. Prof. Department of Zoology
S.S. Khanna Girls Degree College.
Prayagraj.

Dr. Sippy Singh — **Member**
Asst. Prof. Department of Zoology
S.S. Khanna Girls Degree College.
Prayagraj.

Dr. Deepa Chaubey — **Member/Secretary**
Asst. Prof. (Contractual) Zoology
School of Science, UPRTOU, Prayagraj.

Course Preparation Committee

Dr. Dharmendra Singh — **Author**
Assistant Professor, — Block – 1 (Unit – 01 to 04)
Department of Zoology — Block – 2 (Unit – 05)
SP PG College, Shohratgarh, Siddharth Nagar

Dr. Sadguru Prakash — **Author**
Assistant Professor, — Block – 2 (Unit – 06 to 08)
Department of Zoology
MLK PG College, Balrampur

Dr. Parmod Kumar Pandey — **Editor**
Associate Professor, — (All blocks and units)
Department of Zoology
PSM PG College, Anand Nagar, Maharajganj

Dr. Deepa Chaubey — **Coordinator**
Asst. Prof. (Contractual) Zoology
School of Science, UPRTOU, Prayagraj.

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COURSE INTRODUCTION

The objective of this course deals basic introduction to development biology in concerned to asexual reproduction, metamorphosis, regeneration, growth & ageing. The aim is to provide brief introduction of different concepts of sexual reproduction and practical aspects of parthenogenesis. The course is organized into following two blocks.

Block-I & Block II

Block I has four units, in which you are going to study developmental biology I (fission, budding, gemmule formation, gametogenesis, parthenogenesis, metamorphosis).

Block II has four units, in which you are going to study developmental biology II (Moulting, Regeneration Growth and aging)

Block I (Developmental Biology- I)

The block 1st is organized into following four units as under:

Unit-1

It covers the asexual reproduction (fission, budding, gemmule formation). The division of the cells into two daughter cells is known as binary fission. The formation of new bnd from the parent organism in known as budding. Gemmules are internal buds found in sponges and are involved in asexual reproduction.

Unit-2

It deals the basic concepts of sexual reproduction (spermetogenesis, oogenesis and vitellogenesis). Spermatogenesis and oogenesis are both forms of gametogenesis, the production of sperm (spermatogenesis) and eggs (oogenesis). Spermatogenesis is the process by which haploid spermatozoa develop from germ cells in the seminiferous tubules of the testis. Oogenesis is the process of producing the female gametes. It is the type of gametogenesis through which ova or the female gametes are formed. Vitellogenesis is the process of yolk formation

Unit-3

It describes the basic properties parthenogenesis. Parthenogenesis is the type of asexual reproduction involving the development of female gametes without any fertilization.

Unit-4

It covers the metamorphosis (morphogenetic processes and tissue reactivity). Metamorphosis occurs in animals such as amphibians insects and fish.

Objectives

This is the first unit on developmental biology. Under this unit, we have following objectives. These are as under:

- To know about developmental processes
- To discuss about sexual and asexual reproduction
- To know about morphogenetic processes
- To discuss about spermatogenesis and oogenesis
- To discuss about vitellogenesis and types of parthenogenesis, metamorphosis

Unit-1 Asexual reproduction (fission, budding, gemmule formation)

Structure

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1.2 Developmental processes

1.2.1 Cell differentiation

1.2.2 Regeneration

1.2.3 Asexual reproduction
1.3 Characteristics of Asexual Reproduction
1.3.1 Types of Asexual Reproduction
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1.5 Fission of prokaryotes

1.5.1 Process of FtsZ-dependent fission
1.6 Budding
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1.1 Introduction

Developmental biology is the study of the process by which animals and plants grow and develop. Developmental biology also encompasses the biology of regeneration, asexual reproduction, metamorphosis, and the growth and differentiation of stem cells in the adult organism. Developmental biology is a great field for scientists who want to integrate different levels of biology. We can take a problem and study it on the molecular and chemical levels (e.g., How are globin genes transcribed, and how do the factors activating their transcription interact with one another on the DNA), on the cellular and tissue levels (Which cells are able to make globin, and how does globin mRNA leave the nucleus), on the organ and organ system levels (How do the capillaries form in each tissue, and how are they instructed to branch and connect), and even at the ecological and evolutionary levels (How do differences in globin gene activation enable oxygen to flow from mother to fetus, and how do environmental factors trigger the differentiation of more red blood cells).

Developmental biology is one of the fastest growing and most exciting fields in biology, creating a framework that integrates molecular biology, physiology, cell biology, anatomy, cancer research, neurobiology, immunology, ecology, and evolutionary biology. The study of development has become essential for understanding any other area of biology.

Objectives

This is the first unit on developmental biology. Under this unit, we have following objectives. These are as under:

- To know about developmental processes
- To discuss about asexual reproduction
- To know about morphogenetic processes
- To discuss about gemmule formation
- To know about fission, budding, blastogenesis and embryogenesis

The main processes involved in the embryonic development of animals are: tissue patterning (via regional specification and patterned cell differentiation); tissue growth; and tissue morphogenesis.

- Regional specification refers to the processes that create spatial pattern in a ball or sheet of initially similar cells. This generally involves the action of cytoplasmic determinants, located within parts of the fertilized egg, and of inductive signals emitted from signaling centers in the embryo. The early stages of regional specification do not generate functional differentiated cells, but cell populations committed to develop to a specific

region or part of the organism. These are defined by the expression of specific combinations of transcription factors.

- Cell differentiation relates specifically to the formation of functional cell types such as nerve, muscle, secretory epithelia etc. Differentiated cells contain large amounts of specific proteins associated with the cell function.
- Morphogenesis relates to the formation of three-dimensional shape. It mainly involves the orchestrated movements of cell sheets and of individual cells. Morphogenesis is important for creating the three germ layers of the early embryo (ectoderm, mesoderm and endoderm) and for building up complex structures during organ development.
- Tissue growth involves both an overall increase in tissue size, and also the differential growth of parts (allometry) which contributes to morphogenesis. Growth mostly occurs through cell proliferation but also through changes of cell size or the deposition of extracellular materials.

The development of plants involves similar processes to that of animals. However plant cells are mostly immotile so morphogenesis is achieved by differential growth, without cell movements. Also, the inductive signals and the genes involved are different from those that control animal development.

1.2 Developmental processes

1.2.1 Cell differentiation

Cell differentiation is the process whereby different functional cell types arise in development. For example, neurons, muscle fibers and hepatocytes (liver cells) are well known types of differentiated cells. Differentiated cells usually produce large amounts of a few proteins that are required for their specific function and this gives them the characteristic appearance that enables them to be recognized under the light microscope. The genes encoding these proteins are highly active. Typically their chromatin structure is very open, allowing access for the transcription enzymes, and specific transcription factors bind to regulatory sequences in the DNA in order to activate gene expression. For example, NeuroD is a key transcription factor for neuronal differentiation, myogenin for muscle differentiation, and HNF4 for hepatocyte differentiation.

Cell differentiation is usually the final stage of development, preceded by several states of commitment which are not visibly differentiated. A single tissue, formed from a single type of progenitor cell or stem cell, often consists of several differentiated cell types. Control of their formation involves a process of lateral inhibition, based on the properties of the Notch signaling pathway. For example, in the neural plate of the embryo this system operates to generate a population of neuronal precursor cells in which NeuroD is highly expressed.

1.2.2 Regeneration

Regeneration indicates the ability to regrow a missing part. This is very prevalent amongst plants, which show continuous growth, and also among colonial animals such as hydroids and ascidians. But most interest by developmental biologists has been shown in the regeneration of parts in free living animals. In particular four models have been the subject of much investigation. Two of these have the ability to regenerate whole bodies: *Hydra*, which can regenerate any part of the polyp from a small fragment, and planarian worms, which can usually regenerate both heads and tails. Both of these examples have continuous cell turnover fed by stem cells and, at least in planaria, at least some of the stem cells have been shown to be pluripotent.

The other two models show only distal regeneration of appendages. These are the insect appendages, usually the legs of hemimetabolous insects such as the cricket, and the limbs of urodele amphibians. Considerable information is now available about amphibian limb regeneration and it is known that each cell type regenerates itself, except for connective tissues where there is considerable interconversion between cartilage, dermis and tendons. In terms of the pattern of structures, this is controlled by a re-activation of signals active in the embryo. There is still debate about the old question of whether regeneration is a "pristine" or an "adaptive" property. If the former is the case, with improved knowledge, we might expect to be able to improve regenerative ability in humans. If the latter, then each instance of regeneration is presumed to have arisen by natural selection in circumstances particular to the species, so no general rules would be expected.

1.2.3 Asexual reproduction

Asexual reproduction is a mode of reproduction in which a new offspring is produced by a single parent. The new individuals produced are genetically and physically identical to each other, i.e., they are the clones of their parent. Asexual reproduction is observed in both multicellular and unicellular organisms. This process does not involve any kind of gamete fusion and there won't be any change in the number of chromosomes either. It will inherit the same genes as the parent, except for some cases where there is a chance of rare mutation to occur.It can be defined as;

"Asexual reproduction is the mode of reproduction that is involved in the production of offsprings by a single parent."

1.3 Characteristics of Asexual Reproduction

Following are the important features of asexual reproduction:

- ✓ Single parent involved.
- ✓ No fertilization or gamete formation takes place.

- ✓ This process of reproduction occurs in a very short time.
- ✓ The organisms multiply and grow rapidly.
- ✓ The offspring is genetically similar.

1.3.1 Types of Asexual Reproduction

There are different types of asexual reproduction:

- Binary Fission
- Budding
- Fragmentation
- Vegetative Propagation
- Sporogenesis

1.3.2 Morphogenetic process

Morphogenesis (from the Greek *morphê* shape and *genesis* creation, literally "the generation of form") is the biological process that causes a cell, tissue or organism to develop its shape. It is one of three fundamental aspects of developmental biology along with the control of tissue growth and patterning of cellular differentiation. The process controls the organized spatial distribution of cells during the embryonic development of an organism. Morphogenesis can take place also in a mature organism, such as in the normal maintenance of tissue by stem cells or in regeneration of tissues after damage. Cancer is an example of highly abnormal and pathological tissue morphogenesis.

Morphogenesis also describes the development of unicellular life forms that do not have an embryonic stage in their life cycle. Morphogenesis is essential for the evolution of new forms. Morphogenesis is a mechanical process involving forces that generate mechanical stress, strain, and movement of cells, and can be induced by genetic programs according to the spatial patterning of cells within tissues.

Morphogenesis**,** the shaping of an organism by embryological processes of differentiation of cells, tissues, and organs and the development of organ systems according to the genetic “blueprint” of the potential organism and environmental conditions. Plant morphogenesis is brought about chiefly through differential growth. Permanent embryonic tissue results in a morphogenetic potential that varies greatly with the environment and continues to produce new organs throughout the life of the plant. Animal morphogenesis is accomplished by growth and by cell movement. A fixed pattern is established early; the organism is determined as to shape, size, and organ complement. Once organs are formed, no new ones (with few exceptions) are produced.

1.4 History

Some of the earliest ideas and mathematical descriptions on how physical processes and constraints affect biological growth, and hence natural

patterns such as the spirals of phyllotaxis, were written by D'Arcy Wentworth Thompson in his 1917 book On Growth and Form and Alan Turing in his The Chemical Basis of Morphogenesis (1952). Where Thompson explained animal body shapes as being created by varying rates of growth in different directions, for instance to create the spiral shell of a snail, Turing correctly predicted a mechanism of morphogenesis, the diffusion of two different chemical signals, one activating and one deactivating growth, to set up patterns of development, decades before the formation of such patterns was observed. The fuller understanding of the mechanisms involved in actual organisms required the discovery of the structure of DNA in 1953, and the development of molecular biology and biochemistry.

1.4.1 Blastema

Blastema, also called Regeneration Bud, in zoology, a mass of undifferentiated cells that has the capability to develop into an organ or an appendage. In lower vertebrates the blastema is particularly important in the regeneration of severed limbs. In the salamander, for example, tissues in the stump of a limb dedifferentiate—that is, they lose their individual characteristics—and revert to an embryonic appearance. Under the influence of regenerating nerve fibres, they will form a blastema, a mound of cells resembling the original limb bud, from which the replacement limb gradually emerges.

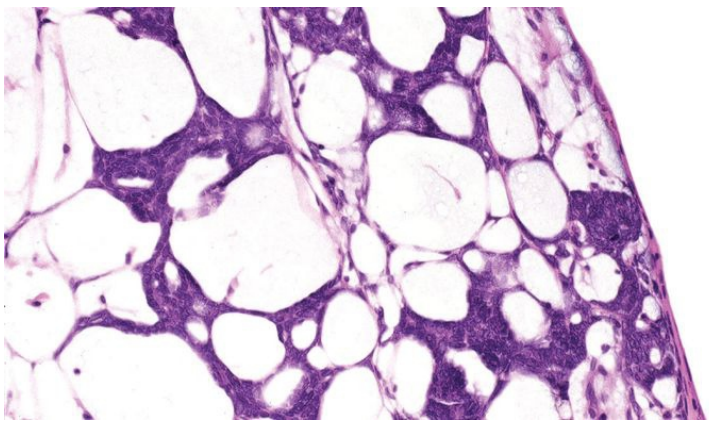


Fig. 1 Blastema cells surrounded by transparent cystic spaces

In some invertebrates, such as flatworms, reserve cells scattered throughout the body supply the cells of blastemas. In vertebrates, dedifferentiated skin and muscle cells at the site of a wound constitute the developing blastema. If for any reason the regenerating nerve fibres are damaged or destroyed, the blastema will fail to develop and scar tissue will form instead.

1.4.2 Blastogenesis

Normal human development begins with blastogenesis in the first 4 weeks after fertilization. This is followed by 4–5 weeks of organogenesis, which involves both morphogenesis and histogenesis. These embryonic organs continue to grow and differentiate during subsequent fetal development. These processes

are regulated by a finite number of developmental genes and pathways, the temporal and spatial expression of which are tightly regulated through control of gene expression at different developmental time points in different tissues or organs although there are some redundancies due to the presence of paralogous genes. Mutations in these developmental genes result in a variety of congenital malformations that may be explained through an understanding of the functions and the timing of the expression of these genes. Most organs that are malformed are histologically normal and hence not at risk of malignancies; however, abnormal histogenesis in a dysmorphogenetic organ might result in malignancy.

1.4.3 Blastozooids

It is a member of a colony of animals which are produced by asexual budding. Asexual reproduction occurs in prokaryotic microorganisms (bacteria) and in some eukaryotic single-celled and multi-celled organisms. Asexual reproduction produces offspring that are genetically identical to the parent because the offspring are all clones of the original parent. A single individual can produce offspring asexually and large numbers of offspring can be produced quickly.

In a stable or predictable environment, asexual reproduction is an effective means of reproduction because all the offspring will be adapted to that environment. In an unstable or unpredictable environment asexually-reproducing species may be at a disadvantage because all the offspring are genetically identical and may not have the genetic variation to survive in new or different conditions. On the other hand, the rapid rates of asexual reproduction may allow for a speedy response to environmental changes if individuals have mutations. An additional advantage of asexual reproduction is that colonization of new habitats may be easier when an individual does not need to find a mate to reproduce. There are a number of ways that animals reproduce asexually.

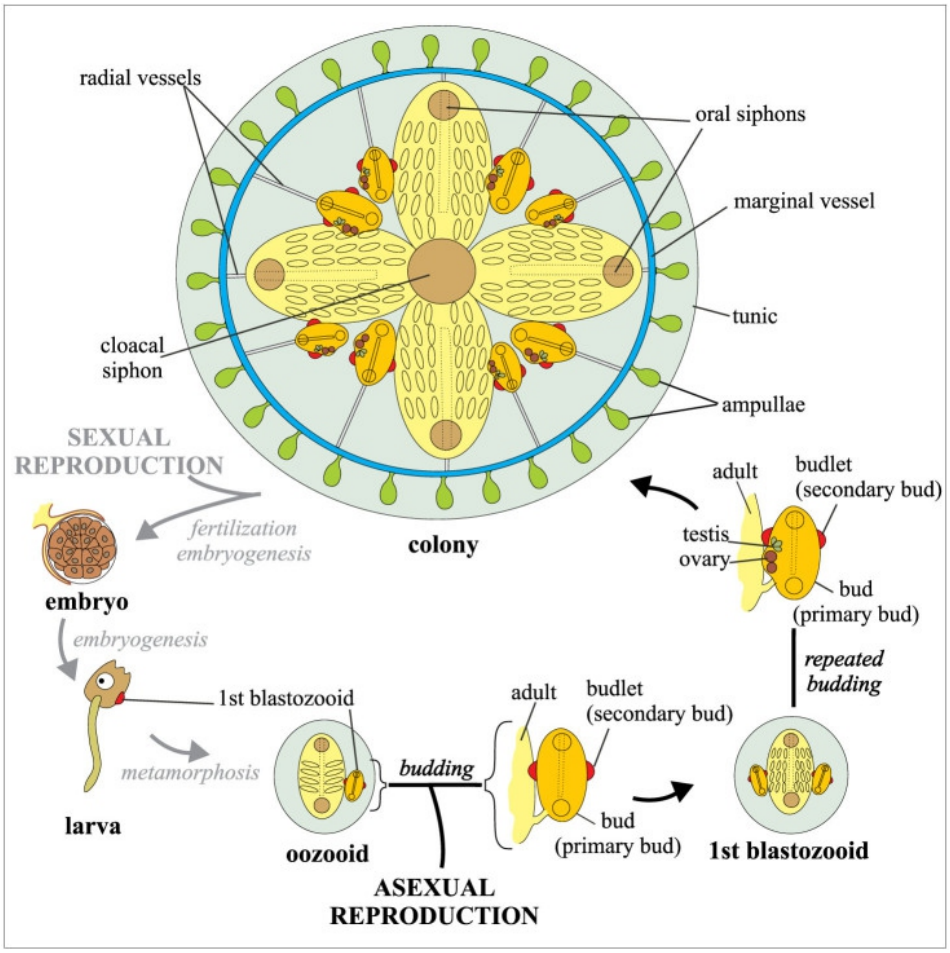


Fig. 2 Ozooid and blastozooids are shown in a dorsal view. A colony is represented as formed by a single
system of four adult blastozooids each bearing two buds, that in turn bear two budlets

1.4.4 Fission

The term "fission" means "to divide". During binary fission, the parent cell divides into two cells. The cell division patterns vary in different organisms, i.e., some are directional while others are non-directional. Amoeba and euglena exhibit binary fission. It is one of the simplest and uncomplicated methods of asexual reproduction. The parent cell divides into two, each daughter cell carrying a nucleus of its own that is genetically identical to the parent. The cytoplasm also divides leading to two equal-sized daughter cells. The process repeats itself and the daughter cells grow and further divide.

1.5 Fission of prokaryotes

The single DNA molecule first replicates, then attaches each copy to a different part of the cell membrane. When the cell begins to pull apart, the replicated and original chromosomes are separated. The consequence of this asexual method of reproduction is that all the cells are genetically identical, meaning that they have the same genetic material (barring random mutations). Unlike the

processes of mitosis and meiosis used by eukaryotic cells, binary fission takes place without the formation of a spindle apparatus on the cell. Like in mitosis (and unlike in meiosis), the parental identity is lost.

1.5.1 Process of FtsZ-dependent fission

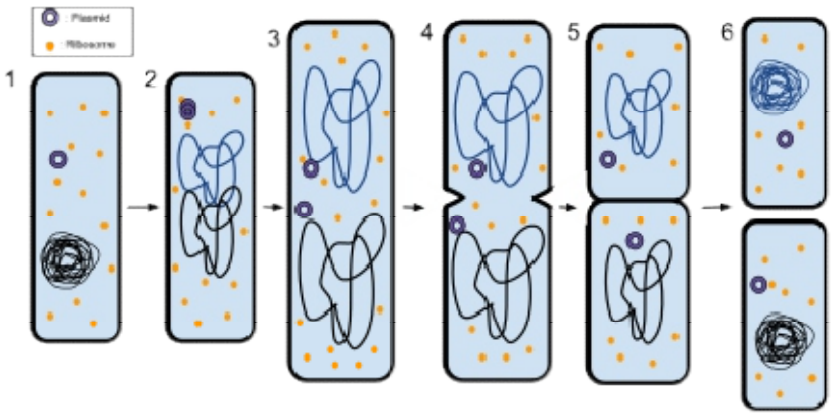


Fig. 3 Binary fission in a prokaryote

FtsZ is homologous to β-tubulin, the building block of the microtubule cytoskeleton used during mitosis in eukaryotes. FtsZ is thought to be the first protein to localize to the site of future division in bacteria, and it assembles into a Z ring, anchored by FtsZ-binding proteins and defines the division plane between the two daughter cells. MinC and MinD function together as division inhibitors, blocking formation of the FtsZ ring. MinE stops the MinCD activity midcell, allowing FtsZ to take over for binary fission.

More specifically, the following steps occur:

1. The bacterium before binary fission is when the DNA is tightly coiled.
2. The DNA of the bacterium has uncoiled and duplicated.
3. The DNA is pulled to the separate poles of the bacterium as it increases the size to prepare for splitting.
4. The growth of a new cell wall begins to separate the bacterium (triggered by FtsZ polymerization and "Z-ring" formation)[7]
5. The new cell wall (septum) fully develops, resulting in the complete split of the bacterium.
6. The new daughter cells have tightly coiled DNA rods, ribosomes, and plasmids; these are now brand-new organisms.

Studies of bacteria made to not produce a cell wall, called L-form bacteria, shows that FtsZ requires a cell wall to work. Little is known about how bacteria that naturally don't grow a cell wall divides, but it is thought to resemble the L-form's budding-like division process of extrusion and separation.

1.6 Budding

Budding is a type of asexual reproduction in which a new organism develops from an outgrowth or bud due to cell division at one particular site. For example, the small bulb-like projection coming out from the yeast cell is known as a bud. Since the reproduction is asexual, the newly created organism is a clone and excepting mutations is genetically identical to the parent organism. Organisms such as hydra use regenerative cells for reproduction in the process of budding.

In hydra, a bud develops as an outgrowth due to repeated cell division at one specific site. These buds develop into tiny individuals and, when fully mature, detach from the parent body and become new independent individuals. Internal budding or endodyogeny is a process of asexual reproduction, favored by parasites such as *Toxoplasma gondii*. It involves an unusual process in which two daughter cells are produced inside a mother cell, which is then consumed by the offspring prior to their separation.

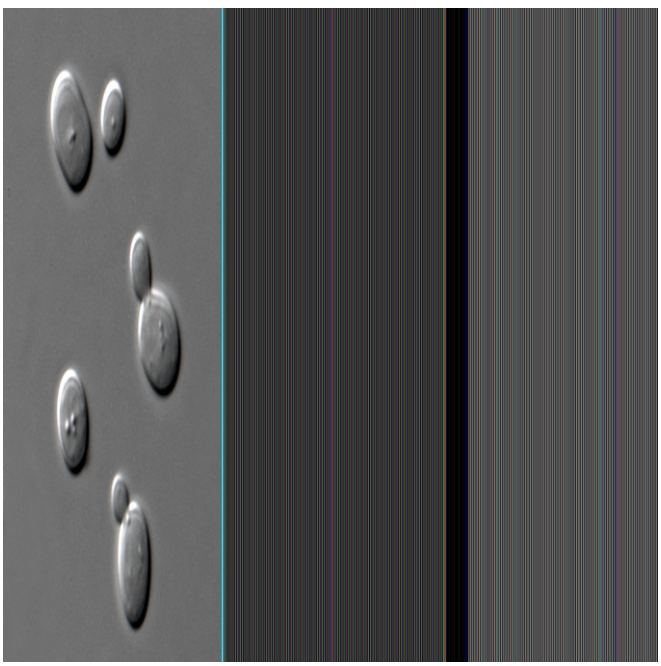


Fig. 4 Saccharomyces cerevisiae reproducing by budding

The new individual may separate to exist independently, or the buds may remain attached, forming aggregates or colonies. Budding is characteristic of a few unicellular organisms (e.g., certain bacteria, yeasts, and protozoans). However, a number of metazoan animals (e.g., certain cnidarian species) regularly reproduce by budding. In horticulture the term *budding* refers to a method of plant propagation in which a bud of the plant to be propagated is grafted onto the stem of another plant.

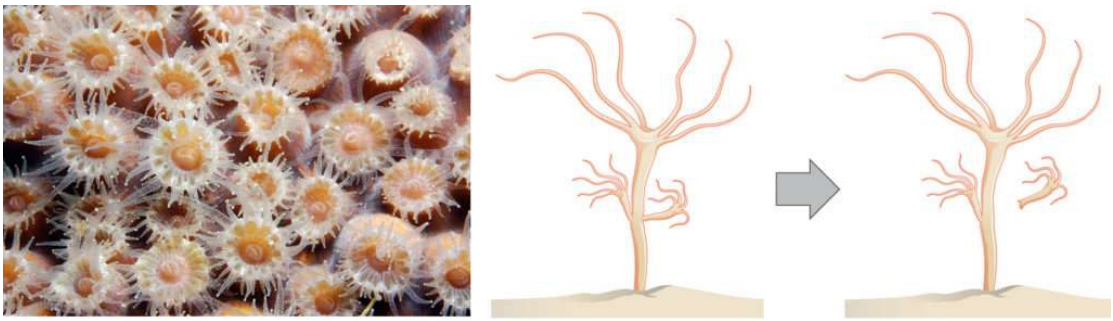


Fig. 5 Coral polyps reproduce asexually by fission. (b) Hydra reproduce asexually through budding

1.7 Gemmule

An asexually produced mass of cells, which are capable of developing into a new organism or into an adult freshwater sponge is termed as a Gemmule. They are small bud-like cells, which are formed by sponges to withstand unfavourable environmental conditions. A freshwater sponge reproduces both by sexually and asexually. Asexual reproduction is mainly carried out by budding and also by gemmulation. The internal buds, which are formed by the freshwater sponges are called gemmules. These gemmules are tough and coated with a dormant cluster of embryonic cells. Freshwater sponges are multicellular, marine living species of a Kingdom Phylum – Porifera. The species of this kingdom includes sponges, Ficulina ficus, sea sponges and much more. All the species related to the same kingdom and carry the same characteristic features.

Fig. 6 The Role of Gemmule in Sponge Reproduction

The internal buds, gemmules are resistant to dehydration, freezing and can survive even without an oxygen supply. The outer layer of the gemmule is enclosed and protected with the endospore that is surrounded by a layer of spicules, which helps the gemmule from all other unfavourable environmental conditions and also helps gemmules in growing into an adult freshwater sponge.

Fig. 7. Gemmules are usually found in a round or a ball-like structure, along with a central mass of amoeboid cells and a thick peripheral layer of a small siliceous spicule.

The structure of Gemmules includes Micropyle, Spicule, Inner layer, Archaeocytes and an Outer pneumatic layer. The micropyle is an outer coat of a cell with a minute opening and the peripheral layer is made up of a thick pneumatic layer and air chambers, which helps gemmules to float in water bodies. The Archaeocytes are reproductive cells and the spicule is the sharp-pointed structure, which is involved in protecting the gemmules from predators and also provide structural support to the gemmules.

1.8 How are gemmule produced?

Gemmule is produced by few genus of freshwater sponges such as Spongilla and some marine species including ficulina ficus, sea sponges, and other poriferans, which produces gemmules to survive in the unfavourable conditions and to germinate and produce new sponges. During gemmule formation, the archaeocytes- totipotent cells, which are loaded with food material in the form of glycoprotein or lipoprotein get combined into a mass. An amoebocyte – a cell with motility, move and surround the central mass of archaeocytes and starts to secrete thick solid chitin around archaeocytes and forms a layer around it. Amphidisc spicules are secreted by sclera oblasts in between the internal and external membranes.

A completely formed gemmule is normal in size, hardball like structure, having a mass of food loaded with reproductive cells, which are embedded in a double membrane layered thick envelope with amphidisc spicules in between. There is a minute opening called Micropyle through which the cells come out during development in favourable conditions. During autumn, freshwater sponges die by leaving behind numerous gemmules. These gemmules produced by the sponges remain active by withstanding unfavourable environmental conditions both in winter and summer season. Gemmules begin to develop into new sponges when there is an availability of an abundance of water.

1.9 Blastogenesis

Normal human development begins with blastogenesis in the first 4 weeks after fertilization. This is followed by 4–5 weeks of organogenesis, which involves both morphogenesis and histogenesis. These embryonic organs continue to grow and differentiate during subsequent fetal development. These processes are regulated by a finite number of developmental genes and pathways, the temporal and spatial expression of which are tightly regulated through control of gene expression at different developmental time points in different tissues or organs although there are some redundancies due to the presence of paralogous genes. Mutations in these developmental genes result in a variety of congenital malformations that may be explained through an understanding of the functions and the timing of the expression of these genes. Most organs that are malformed are histologically normal and hence not at risk of malignancies; however, abnormal histogenesis in a dysmorphogenetic organ might result in malignancy.

1.10 Embryogenesis

In developmental biology, animal embryonic development, also known as embryogenesis, is the developmental stage of an animal embryo. Embryonic development starts with the fertilization of an egg cell (ovum) by a sperm cell, (spermatozoon). Once fertilized, the ovum becomes a single diploid cell known as a zygote. The zygote undergoes mitotic divisions with no significant growth (a process known as cleavage) and cellular differentiation, leading to development of a multicellular embryo after passing through an organizational checkpoint during mid-embryogenesis.[3] In mammals, the term refers chiefly to the early stages of prenatal development, whereas the terms fetus and fetal development describe later stages.

The main stages of animal embryonic development are as follows:

- The zygote undergoes a series of cell divisions (called cleavage) to form a structure called a morula.
- The morula develops into a structure called a blastula through a process called blastulation.
- The blastula develops into a structure called a gastrula through a process called gastrulation.
- The gastrula then undergoes further development, including the formation of organs (organogenesis).

The embryo then transforms into the next stage of development, the nature of which varies between different animal species (examples of possible next stages include a fetus and a larva).

1.11 Differences between blastogenesis and embryogenesis

Blastogenesis is the formation of daughter individuals through budding, gemmation and other means of asexual reproduction. Embryogenesis is the

production of individuals through fertilization of ovum and development of embryo. Embryology deals with the study of changes that involve embryogenesis or formation of zygote and its development upto the birth of young one. The various steps of embryonic development are gametogenesis, gametes, fertilization, cleavage, blastulation, gastrulation, organogenesis, etc.

1.12 Summary

In this unit we summarize that the term morphogenesis generally refers to the processes by which order is created in the developing organism. This order is achieved as differentiated cells carefully organize into tissues, organs, organ systems, and ultimately the organism as a whole. Questions centered on morphogenesis have aimed to uncover the mechanisms responsible for this organization, and developmental biology textbooks have identified morphogenesis as one of the main challenges in the field. The concept of morphogenesis is intertwined with those of differentiation, growth, and reproduction. Each comprises the fundamental components of development that have commonly been used to categorize the problems that motivate developmental biology.

Fission is stated as the division of a single unit or cell into two or more parts. These parts regenerate to form a complete structure and resemble their parents. It is used mainly about the cell, but sometimes may also be used for organisms, species, etc. Binary fission is usually found in Bacteria but it is also present in a few eukaryotic organisms. It is of various types depending upon the direction of the fission. It may be irregular (fission takes place at any plane), longitudinal (fission occurs along the longitudinal axis), transverse (fission occurs along a transverse axis), and oblique (fission occurs obliquely).

New buds form externally on the body surface of the parent in budding, which is an asexual method of reproduction in yeasts, hydra and scypha. Gemmule formation, also known as internal budding, involves the formation of a mass of cells or gemmules inside the parent organism. Gemmule formation is a characteristic feature of sponges. The key difference between budding and gemmule formation is that buds develop externally while gemmules develop internally.

The basic developmental similarities between blastogenesis (development after asexual reproduction) and embryogenesis (development from the egg) are demonstrated. The development of different animals (Cnidaria, Bryozoa and Tunicata) was analyzed to show that such general embryological concepts as preformation, epigenesis, embryonic induction, recapitulation, phyloembryogenesis, palingenesis, coenogenesis, heterochrony, heterotropy, etc., apply to asexual reproduction as well.

1.13 Terminal questions

Q.1 What do you mean by asexual reproduction?

Answer:----------

Q.2 Describe blastema and blastogenesis stages.

Answer:----------

Q.3 Write a short note on fission and budding.

Answer:----------

Q.4 What do you mean by gemmule formation?

Answer:----------

Q.5 Compare between blastogenesis and embryogenesis?

Answer:----------

Q.6 Write a short note on blastozoides.

Answer:----------

Further readings

1. Vertebrate Endocrinology- David O. Norris
2. Invertebrate Zoology –Robert W. Hegner
3. Textbook of Biotechnology –B. D. Singh
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Unit 2 - Basic concepts of sexual reproduction (spermatogenesis, oogenesis and vitellogenesis)

Structure

2.1 Introduction

2.2 Evolution

2.3 Sexual selection

2.4 Animals

2.5 Gametogenesis

2.6 Spermatogenesis

2.7 Purpose

2.8 Location in humans

2.8.1 Duration

2.8.2 Stages

2.9 Oogenesis

2.9.1 Gamete

2.10 Evolution of gametes

2.11 Artificial gametes

2.12 Male & female gametes

2.13 Formation of the Female Gamete

2.14 Maturation of gametes

2.15 Difference between oogensis and spermatogenesis

2.16 Vitellogenesis

2.17 Summary

2.18 Terminal questions

Further readings

2.1 Introduction

Sexual reproduction is a type of reproduction that involves a complex life cycle in which a gamete (such as a sperm or egg cell) with a single set of chromosomes (haploid) combines with another to produce a zygote that develops into an organism composed of cells with two sets of chromosomes (diploid). Sexual reproduction is the most common life cycle in multicellular eukaryotes, such as animals, fungi and plants. Sexual reproduction does not occur in prokaryotes (organisms without cell nuclei), but they have processes with similar effects such as bacterial conjugation, transformation and transduction, which may have been precursors to sexual reproduction in early eukaryotes.

Objectives

This is the second unit on developmental biology. Under this unit, we have following objectives. These are as under:

- To know about sexual reproduction
- To discuss about spermatogenesis and oogenesis
- To know about maturation of gametes
- To discuss about vitellogenesis

In the production of sex cells in eukaryotes, diploid mother cells divide to produce haploid cells known as gametes in a process called meiosis that involves genetic recombination. The homologous chromosomes pair up so that their DNA sequences are aligned with each other, and this is followed by exchange of genetic information between them. Two rounds of cell division then produce four haploid gametes, each with half the number of chromosomes from each parent cell, but with the genetic information in the parental chromosomes recombined. Two haploid gametes combine into one diploid cell known as a zygote in a process called fertilisation. The zygote incorporates genetic material from both gametes. Multiple cell divisions, without change of the number of chromosomes, then form a multicellular diploid phase or generation.

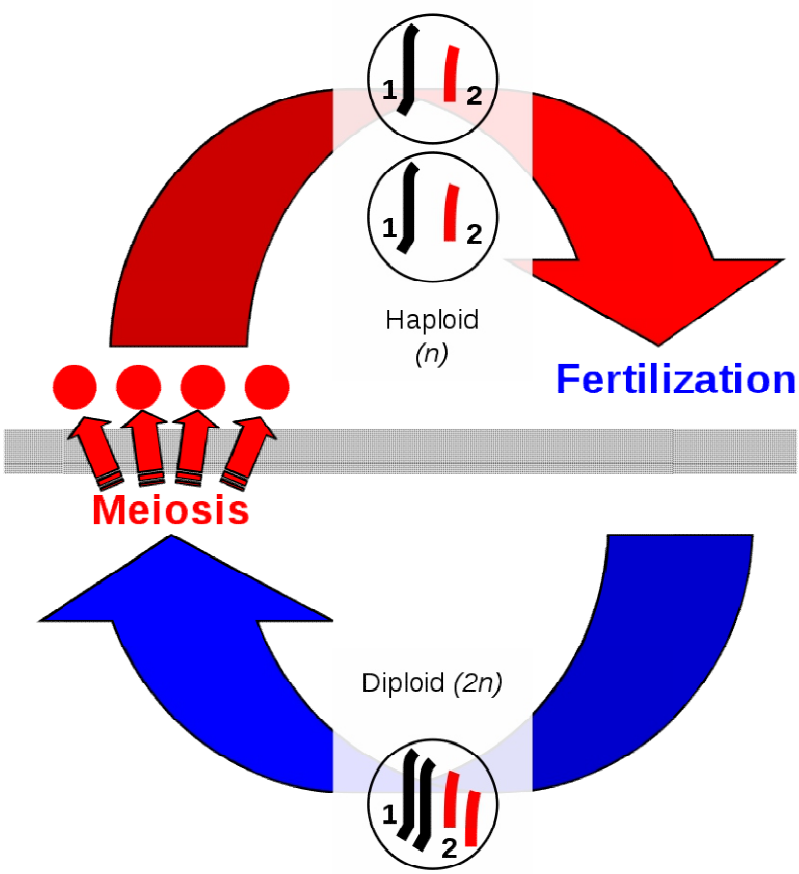


Fig. 1 In the first stage of sexual reproduction, "meiosis", the number of chromosomes is reduced from a diploid number (2n) to a haploid number (n).

In human reproduction, each cell contains 46 chromosomes in 23 pairs. Meiosis in the parents' gonads produces gametes that each contains only 23 chromosomes that are genetic recombinants of the DNA sequences contained in the parental chromosomes. When the nuclei of the gametes come together to form a fertilized egg or zygote, each cell of the resulting child will have 23 chromosomes from each parent, or 46 in total. In plants only, the diploid phase, known as the sporophyte, produces spores by meiosis that germinate and then divide by mitosis to form a haploid multicellular phase, the gametophyte, that produces gametes directly by mitosis. This type of life cycle, involving alternation between two multicellular phases, the sexual haploid gametophyte and asexual diploid sporophyte, is known as alternation of generations.

The evolution of sexual reproduction is considered paradoxical, because asexual reproduction should be able to outperform it as every young organism created can bear its own young. This implies that an asexual population has an intrinsic capacity to grow more rapidly with each generation. This 50% cost is a fitness disadvantage of sexual reproduction. The two-fold cost of sex includes this cost and the fact that any organism can only

pass on 50% of its own genes to its offspring. One definite advantage of sexual reproduction is that it impedes the accumulation of genetic mutations. Sexual selection is a mode of natural selection in which some individuals out-reproduce others of a population because they are better at securing mates for sexual reproduction. It has been described as "a powerful evolutionary force that does not exist in asexual populations."

2.2 Evolution

The first fossilized evidence of sexual reproduction in eukaryotes is from the Stenian period, about 1.05 billion years ago. Biologists studying evolution propose several explanations for the development of sexual reproduction and its maintenance. These reasons include reducing the likelihood of the accumulation of deleterious mutations, increasing rate of adaptation to changing environments, dealing with competition, DNA repair and masking deleterious mutations. All of these ideas about why sexual reproduction has been maintained are generally supported, but ultimately the size of the population determines if sexual reproduction is entirely beneficial. Larger populations appear to respond more quickly to some of the benefits obtained through sexual reproduction than do smaller population sizes.

Maintenance of sexual reproduction has been explained by theories that work at several levels of selection, though some of these models remain controversial. However, newer models presented in recent years suggest a basic advantage for sexual reproduction in slowly reproducing complex organisms. Sexual reproduction allows these species to exhibit characteristics that depend on the specific environment that they inhabit, and the particular survival strategies that they employ.

2.3 Sexual selection

In order to reproduce sexually, both males and females need to find a mate. Generally in animals mate choice is made by females while males compete to be chosen. This can lead organisms to extreme efforts in order to reproduce, such as combat and display, or produce extreme features caused by a positive feedback known as a Fisherian runaway. Thus sexual reproduction, as a form of natural selection, has an effect on evolution. Sexual dimorphism is where the basic phenotypic traits vary between males and females of the same species. Dimorphism is found in both sex organs and in secondary sex characteristics, body size, physical strength and morphology, biological ornamentation, behavior and other bodily traits. However, sexual selection is only implied over an extended period of time leading to sexual dimorphism.

2.4 Animals

Insect species make up more than two-thirds of all extant animal species. Most insect species reproduce sexually, though some species are

facultatively parthenogenetic. Many insects species have sexual dimorphism, while in others the sexes look nearly identical. Typically they have two sexes with males producing spermatozoa and females ova. The ova develop into eggs that have a covering called the chorion, which forms before internal fertilization. Insects have very diverse mating and reproductive strategies most often resulting in the male depositing spermatophore within the female, which she stores until she is ready for egg fertilization. After fertilization, and the formation of a zygote, and varying degrees of development, in many species the eggs are deposited outside the female; while in others, they develop further within the female and are born live.

2.5 Gametogenesis

Gametogenesis**,** in embryology, the process by which gametes, or germ cells, are produced in an organism. The formation of egg cells, or ova, is technically called oogenesis, and the formation of sperm cells, or spermatozoa, is called spermatogenesis. Gamete**,** sex, or reproductive, cell containing only one set of dissimilar chromosomes, or half the genetic material necessary to form a complete organism (i.e., haploid). Gametes are formed through meiosis (reduction division), in which a germ cell undergoes two fissions, resulting in the production of four gametes. During fertilization, male and female gametes fuse, producing a diploid (i.e., containing paired chromosomes) zygote.

Gametes may be identical in form (isogamy), as in certain species of algae, fungi, and protozoans, or there may be more than one morphological type (heterogamy, or anisogamy), as with many green algae of the genus *Chlamydomonas*. Gametes of animals, some algae and fungi, and all higher plants exhibit an advanced form of heterogamy called oogamy. In oogamy one of the gametes is small and motile (the sperm), and the other is large and nonmotile (the egg).

Gametogenesis is the production of gametes from haploid precursor cells. In animals and higher plants, two morphologically distinct types of gametes are produced (male and female) via distinct differentiation programs. Animals produce a tissue that is dedicated to forming gametes, called the germ line. Individual germline cells are called germ cells. During the process of gametogenesis, a germ cell undergoes meiosis to produce haploid cells that directly develop into gametes. Hence, in animals, meiosis is an integral part of gametogenesis. In plants, some fungi, and some algae, meiosis is temporally separated from gametogenesis. Diploid cells undergo meiosis to produce haploid spores, which give rise to a haploid generation called the ‘gametophyte’.

Cells in the latter eventually develop into gametes, sometimes in response to environmental or chemical stimuli. Many unicellular and simple multicellular eukaryotes produce gametes from haploid cells many generations after meiosis or, in some species, immediately following meiosis. Although of opposite mating types, gametes in fungi, multicellular algae, and some protists are usually not morphologically distinct and are designated (+) or (–) rather than as egg or sperm.

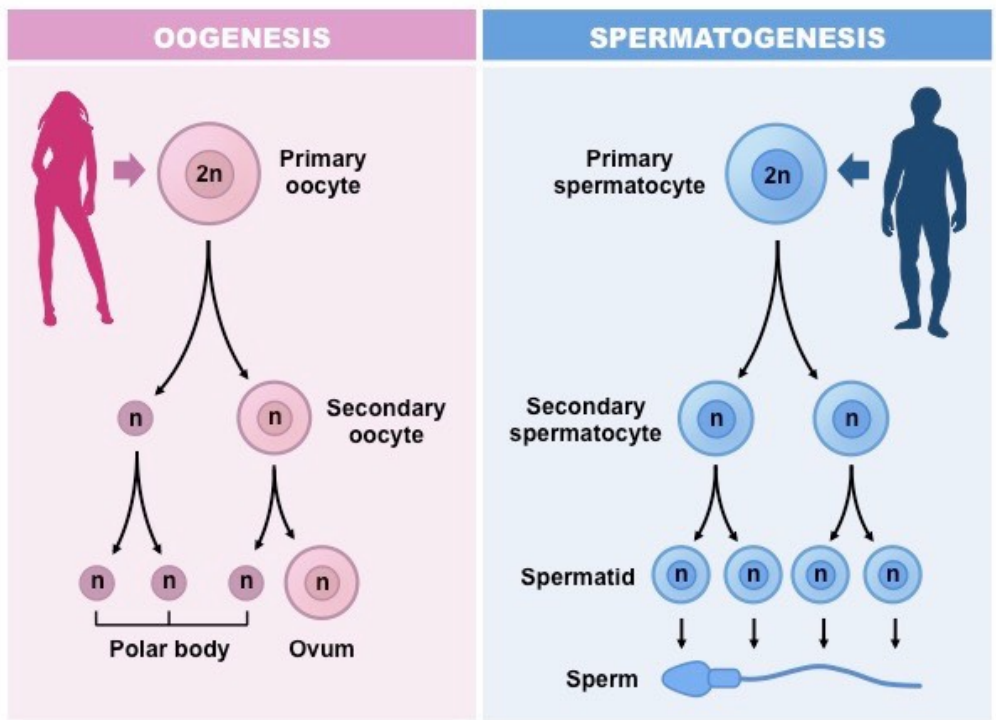


Fig. 2 Gametogenesis

Gametogenesis and intra-gonadal nutrient storage and utilization are linked processes in sea urchin reproduction. These processes involve the two cellular populations that make up the germinal epithelium of the sea urchin gonad. Uniquely, sea urchin gonads grow in size not only because gametogenesis increases the size and/or numbers of germinal cells present but also because somatic cells within the germinal epithelium, the nutritive phagocytes, store extensive nutrient reserves before gametogenesis begins. Knowledge of these phenomena has lead to successful manipulation of sea urchin reproduction. A thorough understanding of sea urchin reproduction will provide increased opportunities for aquaculture.

2.6 Spermatogenesis

Spermatogenesis is the process by which haploid spermatozoa develop from germ cells in the seminiferous tubules of the testis. This process starts with the mitotic division of the stem cells located close to the basement membrane of the tubules. These cells are called spermatogonial stem cells. The mitotic division of these produces two types of cells. Type A cells replenish the

stem cells, and type B cells differentiate into primary spermatocytes. The primary spermatocyte divides meiotically (Meiosis I) into two secondary spermatocytes; each secondary spermatocyte divides into two equal haploid spermatids by Meiosis II. The spermatids are transformed into spermatozoa (sperm) by the process of spermiogenesis. These develop into mature spermatozoa, also known as sperm cells. Thus, the primary spermatocyte gives rise to two cells, the secondary spermatocytes, and the two secondary spermatocytes by their subdivision produce four spermatozoa and four haploid cells.

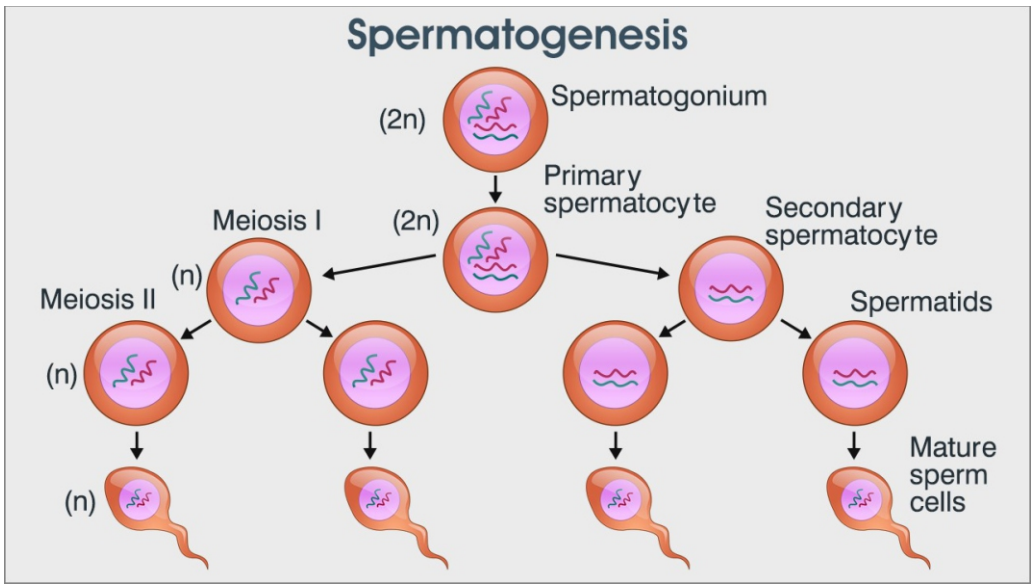


Fig. 3 The process of spermatogenesis

Spermatozoa are the mature male gametes in many sexually reproducing organisms. Thus, spermatogenesis is the male version of gametogenesis, of which the female equivalent is oogenesis. In mammals it occurs in the seminiferous tubules of the male testes in a stepwise fashion. Spermatogenesis is highly dependent upon optimal conditions for the process to occur correctly, and is essential for sexual reproduction. DNA methylation and histone modification have been implicated in the regulation of this process. It starts at puberty and usually continues uninterrupted until death, although a slight decrease can be discerned in the quantity of produced sperm with increase in age.

Spermatogenesis starts in the bottom part of seminiferous tubes and, progressively, cells go deeper into tubes and moving along it until mature spermatozoa reaches the lumen, where mature spermatozoa are deposited. The division happens asynchronically; if the tube is cut transversally one could observe different maturation states. A group of cells with different maturation states that are being generated at the same time is called a spermatogenic wave.

2.7 Purpose

Spermatogenesis produces mature male gametes, commonly called *sperm* but more specifically known as *spermatozoa*, which are able to fertilize the counterpart female gamete, the oocyte, during conception to produce a single-celled individual known as a zygote. This is the cornerstone of sexual reproduction and involves the two gametes both contributing half the normal set of chromosomes (haploid) to result in a chromosomally normal (diploid) zygote.

To preserve the number of chromosomes in the offspring – which differs between species – one of each gamete must have half the usual number of chromosomes present in other body cells. Otherwise, the offspring will have twice the normal number of chromosomes, and serious abnormalities may result. In humans, chromosomal abnormalities arising from incorrect spermatogenesis results in congenital defects and abnormal birth defects (Down syndrome, Klinefelter syndrome) and in most cases, spontaneous abortion of the developing foetus.

2.8 Location in humans

Spermatogenesis takes place within several structures of the male reproductive system. The initial stages occur within the testes and progress to the epididymis where the developing gametes mature and are stored until ejaculation. The seminiferous tubules of the testes are the starting point for the process, where spermatogonial stem cells adjacent to the inner tubule wall divide in a centripetal direction—beginning at the walls and proceeding into the innermost part, or *lumen*—to produce immature sperm. Maturation occurs in the epididymis. The location [Testes/Scrotum] is specifically important as the process of spermatogenesis requires a lower temperature to produce viable sperm, specifically 1°-8 °C lower than normal body temperature of 37 °C (98.6 °F). Clinically, small fluctuations in temperature such as from an athletic support strap, causes no impairment in sperm viability or count.

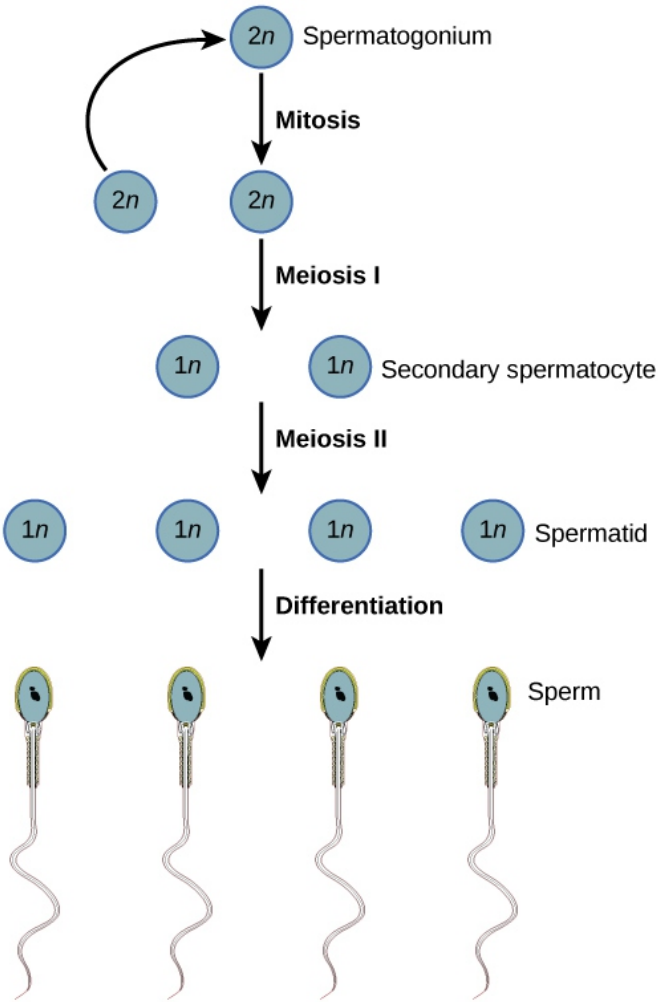


Fig. 4 During spermatogenesis, four sperm result from each primary spermatocyte, which divides into two haploid secondary spermatocytes

2.8.1 Duration

For humans, the entire process of spermatogenesis is variously estimated as taking 74 days (according to tritium-labelled biopsies) and approximately 120 days (according to DNA clock measurements). Including the transport on ductal system, it takes 3 months. Testes produce 200 to 300 million spermatozoa daily. However, only about half or 100 million of these become viable sperm.

2.8.2 Stages

The entire process of spermatogenesis can be broken up into several distinct stages, each corresponding to a particular type of cell in humans. In the following table, ploidy, copy number and chromosome/chromatid counts are for one cell, generally prior to DNA synthesis and division (in G1 if applicable). The primary spermatocyte is arrested after DNA synthesis and prior to division.

2.9 Oogenesis

Oogenesis, in the human female reproductive system, growth process in which the primary egg cell (or ovum) becomes a mature ovum. In any one human generation, the egg's development starts before the female that carries it is even born; 8 to 20 weeks after the fetus has started to grow, cells that are to become mature ova have been multiplying, and by the time that the female is born, all of the egg cells that the ovaries will release during the active reproductive years of the female are already present in the ovaries. These cells, known as the primary ova, number around 400,000. The primary ova remain dormant until just prior to ovulation, when an egg is released from the ovary. Some egg cells may not mature for 40 years; others degenerate and never mature.

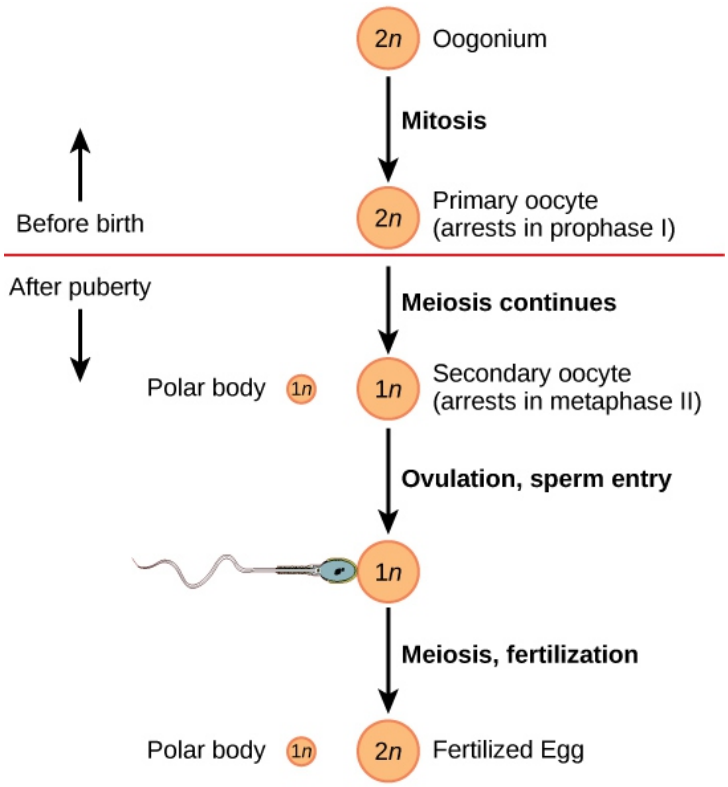


Fig. 5 The process of oogenesis occurs in the ovary's outermost layer

The egg cell remains as a primary ovum until the time for its release from the ovary arrives. The egg then undergoes a cell division. The nucleus splits so that half of its chromosomes go to one cell and half to another. One of these two new cells is usually larger than the other and is known as the secondary ovum; the smaller cell is known as a polar body. The secondary ovum grows in the ovary until it reaches maturation; it then breaks loose and is carried into the fallopian tubes. Once in the fallopian tubes, the secondary egg cell is suitable for fertilization by the male sperm cells.

2.9.1 Gamete

Oogenesis is the process of female gamete formation in animals. This process involves meiosis (including meiotic recombination) occurring in the diploid primary oocyte to produce the haploid ovum. Spermatogenesis is the process of male gamete formation in animals. This process also involves meiosis occurring in the diploid primary spermatocyte to produce the haploid spermatozoon. Gamete is a haploid cell that fuses with another haploid cell during fertilization in organisms that reproduce sexually.

Gametes are an organism's reproductive cells, also referred to as sex cells. In species that produce two morphologically distinct types of gametes, and in which each individual produces only one type, a female is any individual that produces the larger type of gamete-called an ovum; and a male produces the smaller type- called a sperm. Sperm cells or spermatozoa are small and motile due to the flagellum, a tail-shaped structure that allows the cell to propel and move. In contrast, each egg cell or ovum is relatively large and non-motile. In short a gamete is an egg cell (female gamete) or a sperm (male gamete).

In animals, ova mature in the ovaries of females and sperm develop in the testes of males. During fertilization, a spermatozoon and ovum unite to form a new diploid organism. Gametes carry half the genetic information of an individual, one ploidy of each type, and are created through meiosis, in which a germ cell undergoes two fissions, resulting in the production of four gametes. In biology, the type of gamete an organism produces determines the classification of its sex

This is an example of anisogamy or heterogamy, the condition in which females and males produce gametes of different sizes (this is the case in humans; the human ovum has approximately 100,000 times the volume of a single human sperm cell). In contrast, isogamy is the state of gametes from both sexes being the same size and shape, and given arbitrary designators for mating type. The name gamete was introduced by the German cytologist Eduard Strasburger. Male and female gametes set the basis for the sexual roles and sexual selection.

2.10 Evolution of gametes

It is generally accepted that isogamy is the ancestral state from which anisogamy evolved, although its evolution has left no fossil records. Oogamy also evolved from isogamy through anisogamy. There are almost invariably only two gamete types, all analyses showing that intermediate gamete sizes are eliminated due to selection. Intermediate sized gametes do not have same advantages as small or large ones; they do worse than small ones in mobility and numbers, and worse than large ones in supply.

2.11 Artificial gametes

Artificial gametes, also known as In vitro derived gametes (IVD), stem cell-derived gametes (SCDGs), and In vitro generated gametes (IVG), are gametes derived from stem cells. Research shows that artificial gametes may be a reproductive technique for same-sex male couples, although a surrogate mother would still be required for the gestation period. Women who have passed menopause may be able to produce eggs and bear genetically related children with artificial gametes. Robert Sparrow wrote, in the Journal of Medical Ethics, that embryos derived from artificial gametes could be used to derive new gametes and this process could be repeated to create multiple human generations in the laboratory. This technique could be used to create cell lines for medical applications and for studying the heredity of genetic disorders. Additionally, this technique could be used for human enhancement by selectively breeding for a desired genome or by using recombinant DNA technology to create enhancements that have not arisen in nature.

2.12 Male & female gametes

Gametes can be described as sex cells of plants. Like humans, plants have sperm and egg cells that need to fuse in order to produce a zygote, or fertilized egg. Unlike humans, however, plants produce both types of these cells.

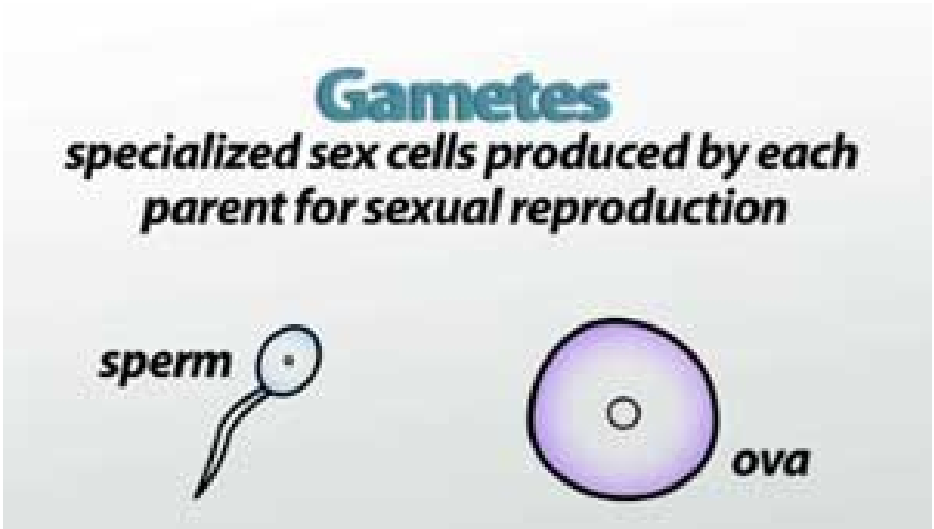


Fig. 6 Male and female gametes

The ovary of the plant produces the female ovule, or egg cell. The male sperm cell is generally encased by some sort of enclosure, like a pollen grain. This allows the egg and sperm cells to stay moist and safe from destruction.

The complexity of the different types of gametes will be seen shortly. However, they do have some commonalities; for example, each contains a sperm cell or, in some cases, multiple sperm cells. These sperm cells are all

formed by a specialized cell division and, in the case of vascular plants, are usually encased by some sort of protective shell that also contains nutrients for the cell.

Female gametes are also known as eggs or ova. They are haploid cells that, when fused during sexual reproduction with a male gamete (sperm), form a zygote. Let's break this down a little more. During sexual reproduction, two cells - one from a female organism and one from a male organism - fuse together to create a zygote, or a fertilized cell. This zygote will mature into a new individual of the same species as the mother and father. The female gamete is considered a haploid cell because it only has a half-set of chromosomes. When it fuses with the sperm of another haploid cell, their chromosomes come together to complete the set, resulting in a diploid zygote, which has a full set of chromosomes.

2.13 Formation of the Female Gamete

Gametes (both male and female) are the products of a special type of cellular reproduction called meiosis. Meiosis is special or different due to the number of chromosomes that results from it. During normal cell reproduction, **mitosis**, the resulting cells have the same number of chromosomes as the original cells. In **meiosis**, however, the resulting cell only has half of the original number of chromosomes. There are a lot of phases and processes that occur during meiosis, but there are two main stages: meiosis I and meiosis II. At the end of meiosis I, two cells result, each with a full set of chromosomes. These cells again go through a process of duplicating and splitting, but this time, the resulting cells only have half of the chromosomes. The resulting cells of meiosis are the gametes.

2.14 Maturation of gametes

Gametes (germ cells) are produced in the gonads. In females, this is called oogenesis and, in males, spermatogenesis. Most new mutations occur during gametogenesis, but there is a higher mutation rate in males, especially with increased paternal age. In females, meiosis I begins about 4 weeks before birth and then arrests in prophase, resulting in a primary oocyte. The primary oocyte persists in this stage until ovulation (after puberty). The arrest of meiosis I may contribute to the increased rate of chromosomal nondisjunction with advanced maternal age.

Gamete maturation occurs at the end of gametogenesis, with the formation of mature cells that are competent for fertilization, but in a state of meiotic and metabolic quiescence that can be reciprocally resumed by interaction with a partner. In fact, mutual activation of the gametes is an essential pre-requisite for fertilization, a process that involves numerous molecules, ions, cellular structures and metabolic pathways.

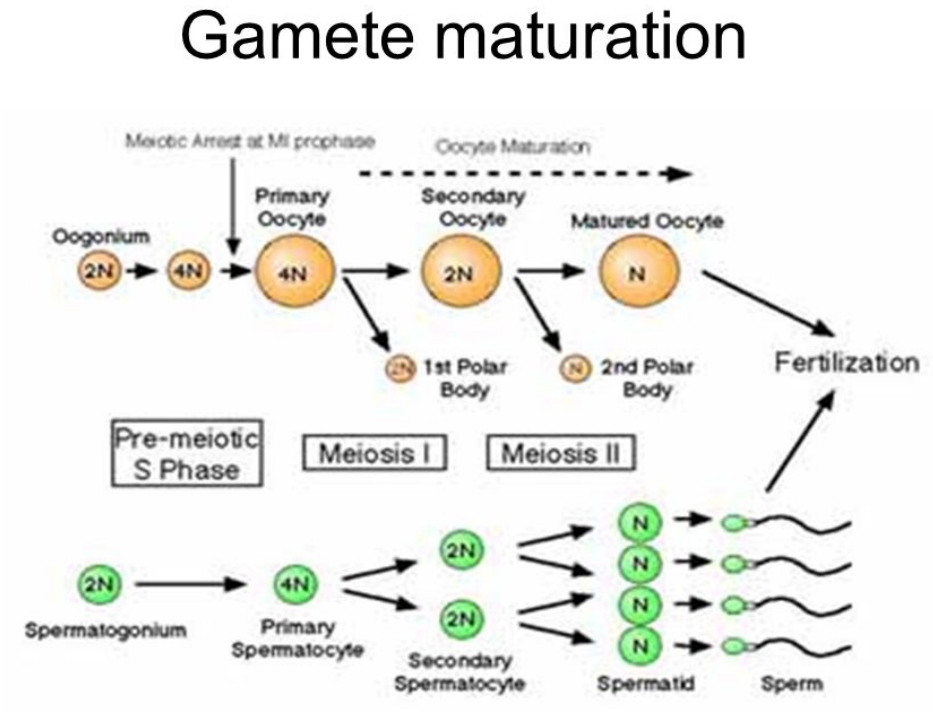


Fig. 7 Maturation of gametes

2.15 Difference between oogensis and spermatogenesis

Female oogenesis	Male spermatogenesis
• Meiosis initiated once in a finite population of cells	• Meiosis initiated continuously in a mitotically dividing stem cell population
• One gamete produced per meiosis	• Four gametes produced per meiosis
• Completion of meiosis delayed for months or years	• Meiosis completed in days or weeks
• Meiosis arrested at first meiotic prophase and reinitiated in a smaller population of cells	• Meiosis and differentiation proceed continuously without cell cycle arrest
• Differentiation of gamete occurs while diploid, in first meiotic prophase	• Differentiation of gamete occurs while haploid, after meiosis ends
• All chromosomes exhibit equivalent transcription and recombination during meiotic prophase	• Sex chromosomes excluded from recombination and transcription during first meiotic prophase

2.16 Vitellogenesis

Vitellogenesis (also known as yolk deposition) is the process of yolk formation via nutrients being deposited in the oocyte, or female germ cell involved in reproduction of lecithotrophic organisms. In insects, it starts when the fat

body stimulates the release of juvenile hormones and produces vitellogenin protein. It occurs in all animal groups other than the mammals. In cockroaches, for example, vitellogenesis can be stimulated by injection of juvenile hormone into immature females and mature males. Chemically yolk is lipoprotein composed of proteins, phospholipids and neutral fats along with a small amount of glycogen. The yolk is synthesised in the liver of the female parent in soluble form. Through circulation it is transported to the follicle cells that surround the maturing ovum, and is deposited in the form of yolk platelets and granules in the ooplasm. The mitochondria and Golgi complex are said to bring about the conversion of the soluble form of yolk into insoluble granules or platelets.

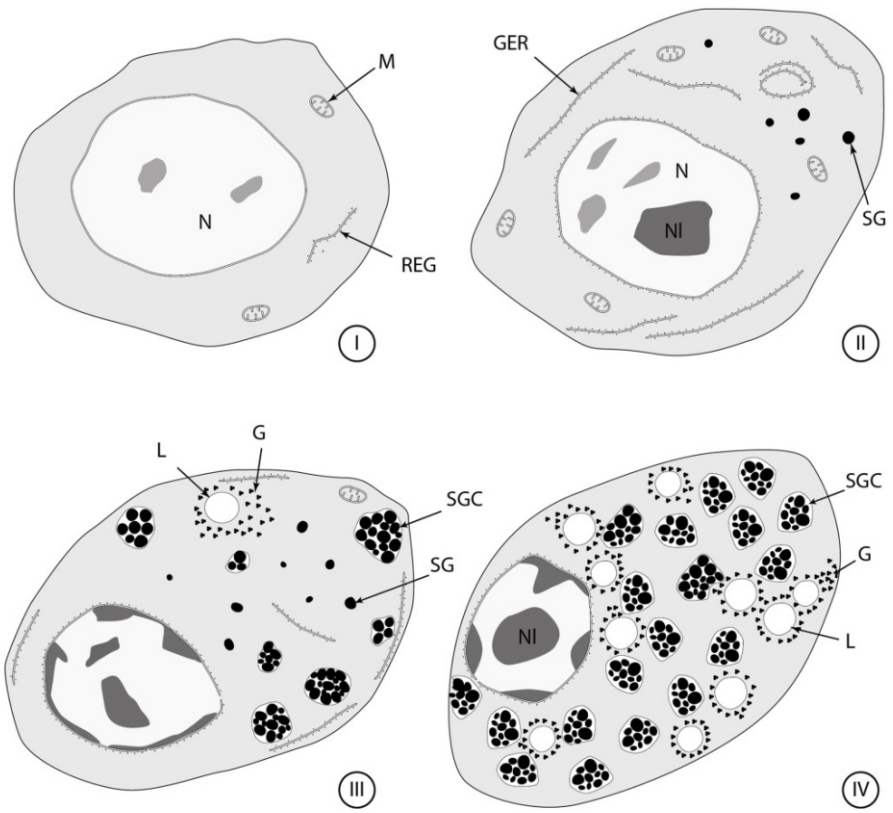


Fig. 8 Diagram of vitellogenesis in the digenean *Crepidostomum metoecus*

In mosquitoes infected with *Plasmodium*, vitellogenesis may be manipulated by the parasites to reduce fecundity. In mammalian vitellogenesis, vitellogenin is the major protein, produced by the Vit gene and regulated by oestrogen. The yolk consists of lipids (triglycerides, cholesterol etc.) and proteins, mainly vitellogenin. The term *vitellogenesis* comes from the Latin *vitellus* ("egg yolk").

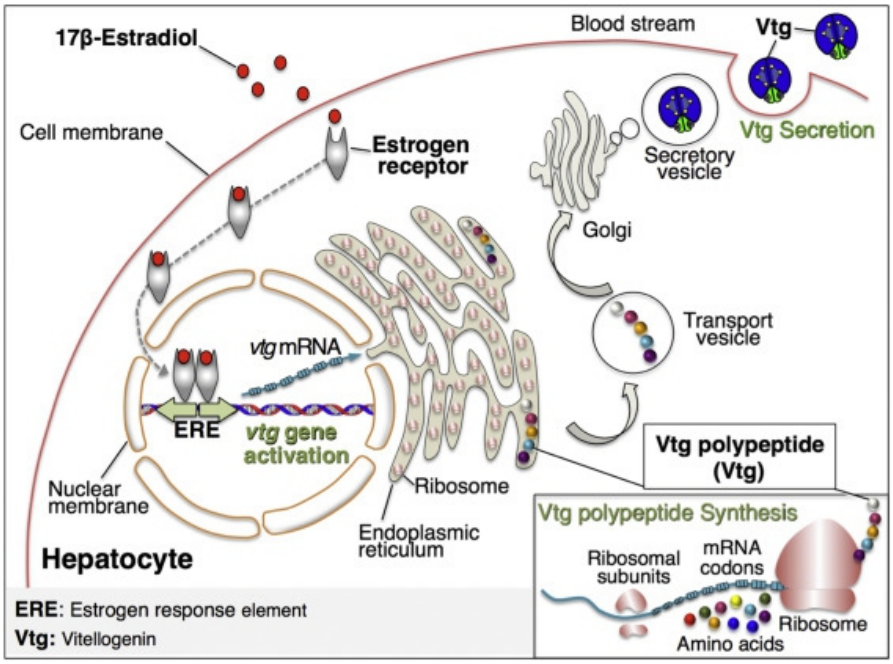


Fig. 9 Vitellogenesis - An overview

Yolk proteins provide embryos with nutrients essential for growth within the egg. Most are phosphoglycoproteins and provide amino acids, phosphate, lipids, and carbohydrates. The major yolk proteins are derived from vitellogenins, which are produced by the fat body and secreted for uptake by maturing oocytes. Yolk proteins in *Drosophila* consist of three polypeptides: YP1, YP2, and YP3. YP1 is expressed by the fat body and, after post-translational processing and glycosylation, the proteins are secreted into the hemolymph and delivered to the oocyte. YP2 is expressed in ovaries. The production and delivery of the three proteins is coordinately regulated and under the control of two hormones, 20-hydroxyecdysone and juvenile hormone. These two hormones regulate molting and metamorphosis during development, as well.

Production of yolk proteins begins during the first day of *Drosophila* adult life. The production rate is high, with yolk proteins representing about one-third of total proteins in hemolymph. YP1 and YP2 are closely linked genes on the X chromosome, while YP3 is sex-linked but more distant. YP1 and YP2 show much sequence homology and probably resulted from a fairly recent gene duplication event. Only one small intron is found in YP1 and YP2; there are two in YP3. Extensive yolk-protein synthesis in *Drosophila* is achieved because tissues are polytene and polyploid.

2.17 Summary

Under this unit, we have summarized that sexual reproduction is the production of new organisms by the combination of genetic information of two individuals of different sexes. In most species the genetic information is carried

on chromosomes in the nucleus of reproductive cells called gametes, which then fuse to form a diploid zygote. The zygote develops into a new individual. Sexual reproduction is the dominant form of reproduction in living beings. Gametes are an organism's reproductive cells. These reproductive cells are produced through a type of cell division called meiosis. During meiosis, a diploid parent cell, which has two copies of each chromosome, undergoes one round of DNA replication followed by two separate cycles of nuclear division to produce four haploid cells. These cells develop into sperm or ova.
The ova mature in the ovaries of females, and the sperm develop in the testes of males. Each sperm cell, or spermatozoon, is small and motile. The spermatozoon has a flagellum, which is a tail-shaped structure that allows the cell to propel and move. In contrast, each egg cell, or ovum, is relatively large and non-motile. During fertilization, a spermatozoon and ovum unite to form a new diploid organism. Gametogenesis, the production of sperm (spermatogenesis) and eggs (oogenesis), takes place through the process of meiosis.

In oogenesis, diploid oogonium go through mitosis until one develops into a primary oocyte, which will begin the first meiotic division, but then arrest; it will finish this division as it develops in the follicle, giving rise to a haploid secondary oocyte and a smaller polar body. The secondary oocyte begins the second meiotic division and then arrests again; it will not finish this division unless it is fertilized by a sperm; if this occurs, a mature ovum and another polar body is produced. In spermatogenesis, diploid spermatogonia go through mitosis until they begin to develop into gametes; eventually, one develops into a primary spermatocyte that will go through the first meiotic division to form two haploid secondary spermatocytes. The secondary spermatocytes will go through a second meiotic division to each produce two spermatids; these cells will eventually develop flagella and become mature sperm.

2.18 Terminal questions

Q.1. Describe gametogenesis with diagram?

Answer:----------

Q.2. What do you mean by spermatogenesis? Explain it.

Answer:----------

Q.3. What do you mean by oogenesis? Explain it.

Answer:----------

Q.4. Describe vitellogenesis with diagram.

Answer:----------

Q.5. Describe maturation of gametes.

Answer:----------

Q.6. Discuss between spermatogenesis and oogenesis.

Answer:----------

Further readings

1. Vertebrate Endocrinology- David O. Norris
2. Invertebrate Zoology –Robert W. Hegner
3. Textbook of Biotechnology –B. D. Singh
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Unit-3 Parthenogenesis

Structure

3.1 Introduction

Objectives

3.2 Parthenogenesis

3.3 Mechanisms

3.4 Parthenogenesis in order Hymenoptera

3.5 Variations

3.6 Types of parthenogenesis

3.6.1 Natural Parthenogenesis

3.6.2 Complete Parthenogenesis

3.6.3 Incomplete Parthenogenesis

3.7 Artificial Parthenogenesis

3.7.1 Physical Means

3.7.2 Chemical Means

3.8 Parthenogenesis in invertebrates

3.9 Parthenogenesis in humans

3.10 Parthenogenesis in birds

3.11 Parthenogenesis in reptiles

3.12 Examples of parthenogenesis

3.13 Significance of Parthenogenesis

3.14 Disadvantages of Parthenogenesis as a Form of Reproduction

3.15 Summary

3.16 Terminal questions

Further readings

3.1 Introduction

Parthenogenesis is a natural form of asexual reproduction in which growth and development of embryos occur without fertilization by sperm. In animals, parthenogenesis means development of an embryo from an unfertilized egg cell. In plants parthenogenesis is a component process of apomixis. Parthenogenesis occurs naturally in some plants, some invertebrate animal species (including nematodes, some tardigrades, water fleas, some scorpions, aphids, some mites, some bees, some Phasmatodea and parasitic wasps) and a few vertebrates (such as some fish, amphibians, reptiles and very rarely birds. This type of reproduction has been induced artificially in a few species including fish and amphibians.

Normal egg cells form in the process of meiosis and are haploid, with half as many chromosomes as their mother's body cells. Haploid individuals, however, are usually non-viable, and parthenogenetic offspring usually have the diploid chromosome number. Depending on the mechanism involved in restoring the diploid number of chromosomes, parthenogenetic offspring may have anywhere between all and half of the mother's alleles. The offspring having all of the mother's genetic material are called full clones and those having only half are called half clones. Full clones are usually formed without meiosis. If meiosis occurs, the offspring will get only a fraction of the mother's alleles since crossing over of DNA takes place during meiosis, creating variation.

Objectives

This is the third unit on developmental biology. Under this unit, we have following objectives. These are as under:

- To know about parthenogenesis.
- To discuss the mechanism of parthenogenesis.
- To know the types of parthenogenesis.
- To discuss the parthenogenesis in human, birds and reptiles
- To know the significance and disadvantages of parthenogenesis.

3.2 Parthenogenesis

Parthenogenetic offspring in species that use either the XY or the X0 sex-determination system have two X chromosomes and are female. In species that use the ZW sex-determination system, they have either two Z chromosomes (male) or two W chromosomes (mostly non-viable but rarely a female), or they could have one Z and one W chromosome (female).

Parthenogenesis is a form of reproduction in which an egg can develop into an embryo without being fertilized by a sperm. Parthenogenesis is derived from the Greek words for “virgin birth,” and several insect species including aphids, bees, and ants are known to reproduce by parthenogenesis. Recently,

parthenogenesis has received considerable attention as a tool for the production of stem cells. Human stem cells derived from embryos, fetal primordial germ cells, umbilical cord blood, and adult tissues provide potential cell based therapies for repair of degenerating or damaged tissues. Twenty years ago in the mouse model, the pluripotency of the cells and the efficacy of their derivatives were poorly explored. The possibility of deriving stem cells from parthenogenetic embryos could eliminate the requirement to produce and destroy viable embryos and may reduce the ethical concerns surrounding stem cell research. Because parthenogenetic stem cells contain only maternal genes, their use of may reduce the occurrence of immune-mediated rejection following graft transplantation.

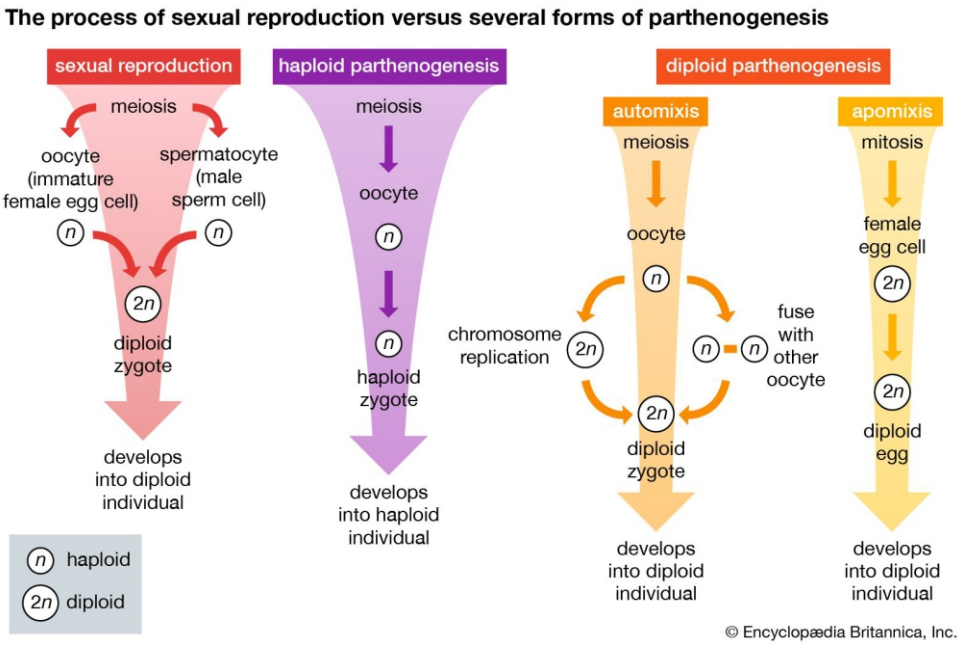


Fig. 1 Sexual reproduction and parthenogenesis compared

It is a reproductive strategy that involves development of a female (rarely a male) gamete (sex cell) without fertilization. It occurs commonly among lower plants and invertebrate animals (particularly rotifers, aphids, ants, wasps, and bees) and rarely among higher vertebrates. An egg produced parthenogenetically may be either haploid (i.e., with one set of dissimilar chromosomes) or diploid (i.e., with a paired set of chromosomes). Parthenogenic species may be obligate (that is, incapable of sexual reproduction) or facultative (that is, capable of switching between parthenogenesis and sexual reproduction depending upon environmental conditions). The term *parthenogenesis* is taken from the Greek words *parthenos*, meaning "virgin," and *genesis*, meaning "origin." More than 2,000 species are thought to reproduce parthenogenically.

Fig. 2 Parthenogenetic New Mexico whiptail, flanked on either side by whiptails that reproduce sexually

3.3 Mechanisms

Parthenogenesis is sometimes considered to be an asexual form of reproduction; however, it may be more accurately described as an "incomplete form of sexual reproduction," since offspring of parthenogenic species develop from gametes. Gametes are reproductive cells that result from meiosis (or reduction division)—in which a specialized cell with a (diploid) double set of chromosomes undergoes two fissions of its nucleus. Meiosis gives rise to four gametes, or sex cells, which are haploid—in that each possesses half the number of chromosomes of the original cell.

Parthenogenesis can operate on either a haploid or a diploid cell. In haploid parthenogenesis, a rare form of parthenogenesis that occurs in a few species of bees, nematodes, and plants, offspring develop from haploid eggs to produce haploid adults. On the other hand, the process of diploid parthenogenesis, a more common and varied form of the phenomenon, may proceed along two pathways. Automixis (automictic parthenogenesis) is a postmeiotic process in which a haploid cell may either duplicate its chromosomes or join with another haploid cell. In both cases, diploid zygotes develop and grow into diploid adults.

Such organisms are not true clones of the mother, however, because the meiotic process separates and recombines the genetic material. A second form of diploid parthenogenesis, apomixis (apomicitic parthenogenesis), forgoes complete meiosis altogether. Instead, two genetically identical diploid egg cells are produced from a parent cell through mitosis (the process of cell duplication), and one or more of these daughter cells, which are both diploid and clones (that is, genetically identical) of the original parent cell, develop into a diploid offspring. Diploid parthenogenesis occurs in insects such as aphids as well as in some rotifers and flowering plants (*see* animal reproductive system and plant reproductive system).

3.4 Parthenogenesis in order Hymenoptera

In the insect order Hymenoptera (which includes bees, wasps, and ants), parthenogenesis can take one of three forms: arrhenotoky, thelytoky, and deuterotoky. In arrhenotoky, haploid males are produced from unfertilized eggs laid by mated (impregnated) females or by so-called secondary, or supplementary, queens, which have not been impregnated. In thelytoky, which occurs in many species of the suborder Symphyta (a group that includes the sawflies, the horntails, and the wood wasps), unmated females produce males. In deuterotoky, unmated females of some Symphyta produce females as well as males. The occurrence of these forms is not always mutually exclusive. For example, in *Apis* (bees), about 1 percent of the eggs laid by secondary queens may be female.

Sometimes associated with arrhenotoky, thelytoky, and deuterotoky is pseudoarrhenotoky (or paternal genome elimination). Pseudoarrhenotoky is a nonparthenogenic form of reproduction that occurs in the hymenopteran superfamily Chalcidoidea (a group of small parasitic wasps) and in some mites, Like arrhenotoky, pseudoarrhenotoky results in the production of haploid males. In this process, development begins as diploid organisms within fertilized eggs; however, as development progresses, males become haploid after the paternal contribution to the genome has been lost, eliminated, or deactivated.

3.5 Variations

A number of parthenogenic variations have been observed. Some aphids and water fleas undergo a type of parthenogenesis called heterogony or cyclic parthenogenesis. In these species, generations of offspring produced from fertilized eggs may alternate with those produced from unfertilized ones. Such an alternation of generations in both groups of insects is thought to result partly from seasonal temperature changes, with eggs produced through sexual reproduction having a greater ability to withstand the winter cold. They lie dormant until temperatures rise.

Pseudogamy (gynogenesis, or sperm-dependent parthenogenesis) is another variation, which appears in the life cycle of a few insects, mites, and salamanders as well as the flatworm *Schmidtea polychroa*. *S. polychroa* is hermaphroditic and may be diploid (which can reproduce sexually) or polyploid (that is, with one or more additional sets of chromosomes). Whereas sexual reproduction requires sperm for fertilization, parthenogenic reproduction in this species involves sperm only to stimulate the initial development of the egg; the sperm's genetic material is not used.

3.6 Types of parthenogenesis

There are two types of parthenogenesis as under:

- Natural Parthenogenesis

- Artificial Parthenogenesis

3.6.1 Natural Parthenogenesis

In certain animals, parthenogenesis occurs naturally in their life cycles. This is known as natural parthenogenesis. Natural parthenogenesis can be further divided into:

3.6.2 Complete Parthenogenesis

A few insects have no males and no sexual phase. Such organisms depend upon self-reproduction. This is known as complete parthenogenesis.

3.6.3 Incomplete Parthenogenesis

The life cycle of a few insects involves two generations:

- Sexual generation
- Parthenogenesis generation

In this, the unfertilised eggs produce males and the diploid eggs produce females. This type of parthenogenesis is called partial or incomplete parthenogenesis.

3.7 Artificial Parthenogenesis

The fertilised eggs might sometimes develop parthenogenetically by various chemical and physical means. This is known as artificial parthenogenesis.

3.7.1 Physical Means

- ✓ Temperature induces parthenogenesis in eggs. For eg., parthenogenesis is induced if an egg is transferred from -30 to -10°C.
- ✓ Parthenogenesis is caused by ultraviolet light.
- ✓ Electrical shocks cause parthenogenesis.
- ✓ When an egg is pricked by a needle, the development occurs parthenogenetically.

3.7.2 Chemical Means

The chemicals that are responsible for the parthenogenesis of eggs are:

- ✓ Chloroform
- ✓ Urea and Sucrose
- ✓ Strychnine
- ✓ Fat solvents
- ✓ Acids
- ✓ Chlorides

3.8 Parthenogenesis in invertebrates

Parthenogenesis can be defined as the production of an embryo from a female gamete without any genetic contribution from a male gamete, with or without the eventual development into an adult. It is distinct from asexual reproduction since it involves the production of egg cells. Parthenogenesis is a normal method of reproduction in many lower organisms, but does not lead to viable mammalian offspring. Parthenogenetic development can proceed by various routes depending on whether meiosis has occurred or has been supressed, in which case the egg develops as as result of mitotic divisions. Whenever sex is determined by chromosome constitution, parthenogenetic offspring, in the absence of effective meiosis, all will be, mostly female. In birds, however (see below), the offspring are male as in this case females are the heterogametic sex.

In bees, males originate by haploid parthenogenesis while diploid females are produced by fertilization in the normal way. Other aphids, such as greenfly (Hemiptera) have generations which alternate between parthenogenesis and fertilization, so called cyclical parthenogenesis. The formation of female parthenogenetic offspring is widespread among many order of insects. For example in *Drosophila parthenogenetica*, a small proportion of eggs laid by virgin females develop to produce viable adults. Another example is the parthenogenetic grasshopper *Warramaba virgo*, a species which consists of females only. Parthenogenesis is also successful in some Crustaceae such as the brine shrimp, *Artemia salina*. Animals such as bees, wasps, ants have no sex chromosomes. These organisms reproduce by parthenogenesis. A few plants, reptiles and fish are also capable of reproducing in this manner. A few organisms such as crayfish, snakes, komodo dragons and sharks can reproduce sexually as well as by parthenogenesis. This is known as facultative parthenogenesis.

3.9 Parthenogenesis in humans

Parthenogenesis is common in some vertebrates including the whiptail lizard where the offspring develops from an oocyte without being fertilized by a sperm. So, no paternal inheritance occurs and the offspring genome is completely inherited from the mother. Parthenogenesis in humans, as well as androgenetic events, may spontaneously occur in humans. However, they can only produce tumors such as ovarian teratoma. The production of a parthenogenesis human depends on several factors including activation of the oocyte, meiosis, and genetic imprinting.

It is not naturally a common type of reproduction in mammals. However, artificial induction of parthenogenesis in mammals included stimulation of the

mammalian oocyte; The oocyte was activated and developed into an embryo by parthenogenesis. However, the resulting embryo will not develop further due to a lack of paternal genes, which are responsible for the establishment of the normal placenta. On the other hand, creating a fully fertile animal by parthenogenesis is possible by the technology of genetic modification, which manipulates the genome of the produced animal from an activated oocyte.

Asexual reproduction in humans is not common. Therefore, the parthenogenetic activation of a human oocyte is facing legal and ethical issues. However, this method of reproduction has medical, scientific, and economic reasons. For example, parthenogenesis may be used in different fields. Human stem cells have been studied for their regulatory mechanisms which are responsible for the development of an embryo or cloning experiments in different researches using the oocyte. Therefore, clinical human parthenogenesis in humans may reproduce cells such as embryonic stem cells that can be used in the favor of many patients. It can be used in organ transplantation with minimal risk of organ rejection.

Human oocyte activation is a complex process that requires several stimuli at different phases of the cell division cycle. At metaphase II, the unfertilized oocyte will not develop without a stimulus. The stimulus may occur due to the fertilization by a spermatozoon or using an artificial agent. The artificial agent permits the transition to anaphase, sister chromatids segregation, and expels the second polar body. The artificial agent must mimic the action of the sperm to trigger the development of an embryo. Oocyte parthenogenetic activation can be performed by different types of stimuli. These stimuli are divided into chemical and physical stimuli. Even though different methods provide oocyte stimulation, however, they have different degrees of embryonic development and activation rates which affect the degree of success.

The produced embryo is a *pseudodiploid*, which contains the two maternal sister chromatids chromosomes in the oocyte. Spontaneous diploidization occurs since the resulting embryo contains one copy of sister chromatids in order to develop into a homozygous embryo. Then, the activating agent induced the extrusion of the second polar body and transition from metaphase II without performing diploidization. Therefore, the resulting embryo from this stage is a haploid embryo. Activation of the human oocyte by parthenogenesis to treat infertility is now of great interest since it can be used to produce embryos in areas such as assisted reproduction technologies itself, somatic cell, and nuclear transfer experiments and for the derivation of clinical-grade pluripotent embryonic stem cells for regenerative medicine. Different methods have been employed to activate human and nonhuman female gametes with different degrees of embryonic development.

3.10 Parthenogenesis in birds

Parthenogenesis may occur naturally in some birds. However, surrounding conditions may affect the development of the unfertilized egg so it is usually abortive. Birds may reproduce by *facultative parthenogenesis*, producing diploid males only. The mechanism of parthenogenesis in birds is not clear yet, however, some researchers suggest that parthenogenesis may affect the normal fertilization as well as the natural development of an embryo. For example, *turkey* and *virgin quail* that reproduce by parthenogenesis have shown less reproductive performance after sexual mating. Environmental factors, as well as genetic selection such as exposure to virus vaccines, may initiate parthenogenesis in birds.

Parthenogenesis in avian species can be studied to get more information about parthenogenesis among vertebrates. This information is useful in understanding the pathogenesis of ovarian cancers that develop from mammalian oocytes by parthenogenesis. Avian species represent a great model for such studies due to their small size, the short interval of generations, and earlier sexual maturity in comparison with chickens and turkeys. On the other hand, parthenogenesis is abortive in most birds such as pigeons, quail, turkeys, and zebra finches. However, when they were exposed to genetic selection, researchers were able to hatch a small percentage of turkey's unfertilized eggs.

In parthenogenesis, the meiotic early stage is similar to fertilized egg where the number of chromosomes is reduced. Consequently, to restore the diploid number of chromosomes, the haploid egg nucleus and the haploid second polar body are fused or by endomitosis. As a result, the produced parthenogen has the same diploid chromosome number.

3.11 Parthenogenesis in reptiles

Many snake species reproduce sexually but there are other snake species that are capable of reproducing asexually. Apart from snakes, other reptiles that reproduce asexually are certain species of lizards. Both the snake and lizard asexual reproductions may be in the form of facultative or obligate parthenogenesis. In *facultative parthenogenesis,* a sexually reproducing reptile becomes parthenogenetic when males are lacking in their population. In this case, their population consists of members that may or may not be a clone of the mother's genome as they reproduce by mating as well when an opportunity presents. In *obligate parthenogenesis,* such as rock lizard and certain groups of geckos, they reproduce by parthenogenesis alone.

In these species, the female egg develops into a new offspring without the contribution of the male in fertilization. There are about 50 lizard species and only 1 snake species that reproduce by obligate parthenogenesis. Parthenogenesis in reptiles may produce either full clones that carry all the mother's genes or produce a half-clone that combines a haploid genome of the reptile egg. Full clones are produced due to a certain modification in the normal process of haploid egg production where the genome of a female's germ cell is doubled in a process known as *pre-meiotic genome doubling*.

In this process, two-division cycles of meiosis produce a diploid egg instead of a haploid egg where two similar sister chromosomes produced during premeiotic genome doubling are separated during *meiosis I*. Identical chromosomes pair instead of the two homologous chromosomes that pair in sexual reproduction. Then, two identical sister chromatids are separated during *meiosis II*. Full clones are found among obligate parthenotes, like *Lacerta*, as well as facultative parthenotes such as the Burmese python.

Other species produce half clones.

These usually reproduce by facultative parthenogenesis. Terminal fusion takes place between an egg cell and a haploid polar body. The polar body is a byproduct of meiosis. The fusion between the egg and polar body produces a diploid nucleus that develops into a diploid offspring. This method is similar to the fusion of an egg and sperm in sexual reproduction since it forms a diploid nucleus from the fusion of two different cells. The resulting offspring is a homozygote and gets about half of its mother's genetic variation.

This type of parthenogenesis could produce either a female or a male. Since the meiosis process takes place normally in individuals produced by this type of parthenogenesis, these individuals can reproduce either sexually or asexually such as some species of snakes and Komodo dragons. Several types of Lizards are parthenogenetic such as Caucasian rock lizard species of genus *Lacerta*. *Lacerta* is true parthenotes, therefore, their egg is not fertilized by a sperm. Another genus is the *Teiid* of whiptail lizards, which is the most known evolutionarily type of reptiles that reproduce by parthenogenesis.

SAQs

Choose the best answer from the given options.

1. Which of the following is parthenogenesis?

   (a) Reproduction by means of mating between male and female.
   (b) Reproducing offspring in the absence of a male gamete.

(c) Reproducing offspring in the absence of a female gamete.

2. Parthenogenesis is a form of…

(a) Asexual reproduction.
(b) Sexual reproduction.
(c) Either asexual or sexual reproduction.

3. Parthenogenesis that produces a full clone of the mother

(a) Apomixis parthenogenesis.
(b) Automixis parthenogenesis.
(c) Facultative parthenogenesis.

4. Which of the following is generally a disadvantage of parthenogenesis as a form of reproduction?

(a) Conserve time in finding a mate.
(b) Relatively a quicker way to reproduce offspring.
(c) Offspring are predisposed to the same diseases inflicting the present.

3.12 Examples of parthenogenesis

Parthenogenesis takes place spontaneously in rotifers, daphnia, nematodes, aphids, as well as other invertebrates and plants. Among vertebrates, birds, snakes, sharks, and lizards are the only species that can reproduce by strict parthenogenesis. While other amphibians, fish, and reptiles can perform different forms of hybridogenesis, which is a form of *incomplete parthenogenesis*. Other species such as the blacktip and hammerhead sharks as well as the Komodo dragon usually reproduce sexually; however, these species may sometimes reproduce asexually by parthenogenesis. Parthenogenesis may be artificially induced in mammals to produce animal clones, i.e., the offspring is genetically identical to the mother. In this process, the egg cell is artificially stimulated to undergo mitosis in order to produce a new organism.

3.13 Significance of Parthenogenesis

Parthenogenesis is important for the following reasons:

- Parthenogenesis helps in determining the sex of an individual in honey bees, wasps, etc.
- It supports the chromosomal theory of inheritance.
- Variations from populations are eliminated by parthenogenesis.
- It is the simplest, most stable and easy process of reproduction.
- Polyploidy in organisms is caused by parthenogenesis.
- It helps in the development of advantageous mutant characters.
- Non-adaptive combination of genes is controlled.
- There are no sterile races.

However, the organisms produced by parthenogenesis cannot survive for long due to no recombination of genetic material.

3.14 Disadvantages of Parthenogenesis as a Form of Reproduction

Parthenogenesis is a form of asexual reproduction. Thus, it is characterized by a lack of genetic diversity since the offspring gets all genetic material from one parent only. Lack of genetic diversity predisposes the offspring to the same diseases and same conditions as its parent. Moreover, negative mutations, as well as unfavorable traits, will persist in many generations. Furthermore, the offspring produced by parthenogenesis is a clone of the parent so it cannot adapt easily or survive when the condition changes and becomes unfavorable. That's why parthenogenesis may produce a large number of organisms that cannot survive through any slight change in their environment.

3.15 Summary

Parthenogenesis is a method of self-reproduction in which the egg cells develop into offspring in the absence of a mate. Thus, parthenogenetic animals and plants may still reproduce their own kind without the need to exert time and effort in looking for a suitable mate. The female can then use this time to search for food and shelter where these resources are available. For example, aphids reproduce asexually by parthenogenesis during summer since green leaves are available and the daytime is longer. Asexual parthenogenesis enables a population to grow as twice faster as a population that reproduces sexually. Parthenogenesis enables rapid reproduction and increases in population size without the need for fertilization. Therefore, parthenogenesis is faster and easier than sexual reproduction since it does not depend on the presence of a male. In fact, females that reproduce by parthenogenesis can produce the same number of offspring as sexually reproduced females but with only half the size of the population saving more resources.

In parthenogenetic species, one individual is capable of forming a colony without mating, producing female offspring that may grow and reproduce again to provide a large number of offspring in a short period of time. Moreover, some parthenogens can maintain the ability to incorporate new genes during the cycle of sexual reproduction. Therefore, they can maintain their evolutionary process while producing a great number of offspring.

Sexual reproduction does have greater genetic diversity than parthenogenesis. However, parthenogenesis may prove beneficial in a way that it produces clones having the same genes for favorable traits as inherited from their parents. So, if the mother lives in a habitat to which she has adapted, her offspring will carry the same genes to ensure their survival in such an environment. Parthenogenesis is now being tested on human eggs to stimulate the egg to develop without fertilization. This approach may help researchers to find a new method of producing human stem cells from an unfertilized egg or even to clone humans.

3.16 Terminal questions

Q.1 What do you mean by parthenogenesis?

Answer:----------

Q.2 Describe significance of parthenogenesis..

Answer:----------

Q.3 Write a short note on

Answer:----------

Q.4 What do you mean by parthenogenesis in birds?

Answer:----------

Q.5 What do you mean by parthenogenesis in reptiles?

Answer:----------

Q.6 Write a short note on parthenogenesis in invertebrates.

Answer:----------

Further readings

1. Vertebrate Endocrinology- David O. Norris
2. Invertebrate Zoology –Robert W. Hegner
3. Textbook of Biotechnology –B. D. Singh
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Unit-4 – Metamorphosis (morphogenetic processes and tissue reactivity)

Structure

4.1 Introduction
Objectives
4.2 Function of metamorphosis
4.3 Types of metamorphosis
4.3.1 Complete Metamorphosis
4.3.2 Incomplete Metamorphosis
4.3.3 Examples of Metamorphosis
4.3.3.1 Butterflies
4.3.3.2 Frogs
4.3.3.3 Fish
4.4 Morphogenesis
4.5 Genetic and molecular basis
4.6 Branching morphogenesis
4.6.1 Cancer morphogenesis
4.6.2 Virus morphogenesis
4.7 Amphibians
4.8 Thin-skinned
4.9 Leading a Double-Double Life
4.10 Just the Right Temperature
4.11 Amphibians in Danger: The Extinction Crisis
4.12Metamorphosis in amphibians
4.13 Metamorphosis in insects
4.14 Tissue reactivity
4.15 Foreign body reaction to biomaterial implantation
4.16 Protein adsorption
4.17 Immune recruitment
4.18 Macrophage fusion
4.19 Biological properties
4.20 Summary
4.21Terminal questions

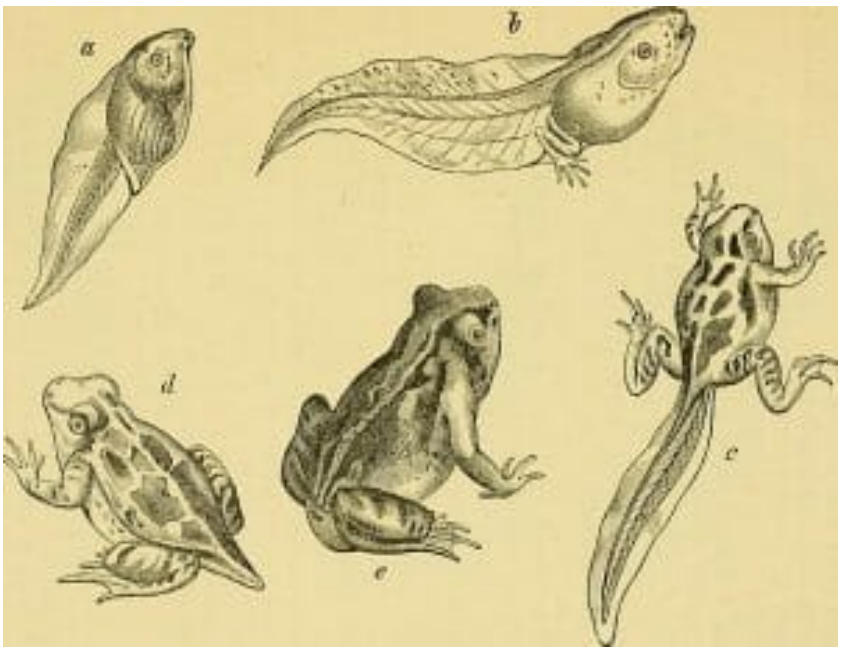
Metamorphosis is a process by which animals undergo extreme, rapid physical changes some time after birth. The result of metamorphosis may be change to the organism's entire body plan, such as a change in the animal's number of legs, its means of eating, or its means of breathing. In species that use metamorphosis, metamorphosis is also typically required for sexual maturity. Pre-metamorphic members of these species are typically unable to mate or reproduce. Commonly known examples of metamorphosis include the process undergone by most insects, and the transformation of tadpoles into frogs. The diagram below shows the stages of this change, wherein the small fish-like tadpoles transform into what seems a completely different animal.

Objectives

This is the first unit on developmental biology. Under this unit, we have following objectives. These are as under:

- To know about metamorphosis
- To discuss types of metamorphosis
- To discuss metamorphosis in butterflies, frogs and fish
- To know tissue reactivity and protein adsorption

Animals that you may not know undergo metamorphosis include fish, mollusks, and many other types of sea creatures which are related to insects, mollusks, or fish. Lobsters, for example, which are closely related to insects, do undergo metamorphosis as part of their life cycle. Metamorphosis is a remarkable process. The speed and extent of cell growth and differentiation is astonishing. In most species, such rapid growth and such sweeping changes to cell type only happen during embryonic development. Indeed, some scientists believe that the process of metamorphosis involves a sort of re-activating of genes that allow animal cells to change from one cell type to another.



The changes leading to metamorphosis are triggered by hormones, which the animal's body releases as the right conditions for metamorphosis approach. In some animals a hormone cascade follows, with the trigger hormone causing the release of several other hormones that act on different parts of the animal's body. The hormones cause drastic changes to the functioning of cells, and even behavioral changes such as the caterpillar spinning its cocoon. The effects of hormones on metamorphosis can be studied by artificially administering these hormones to pre-metamorphic animals. Tadpoles, for example, can be triggered to begin losing their tails and growing limbs early by the addition of thyroid hormones to their water supply. Unfortunately this has a detrimental effect on the animal's health.

4.2 Function of metamorphosis

Scientists remain uncertain why metamorphosis evolved. For the animals of today, its purpose is obvious: if metamorphosis did not occur, tadpoles could not become frogs and larvae could not become full-grown adults capable of reproduction. Without reproductively mature members, these species would quickly die off. But why would these species evolve to need this extra step in the first place? Why not just hatch full-grown butterflies or frogs from eggs?

At least some metamorphosing species did not start out that way: the earliest insects basically did hatch as full-grown adults. But a few hundred million years ago, some species stumbled upon the trick of metamorphosis. It was apparently wildly successful; it is thought that almost two-thirds of species alive today use metamorphosis to accomplish large changes between their adult and juvenile forms.

The benefit of metamorphosis may lie in its ability to reduce competition. Pre-metamorphic animals typically consume completely different resources from their adult forms. Tadpoles live in water, eating algae and plants. Frogs live on land, breathing air and eating insects. Caterpillars eat leaves; butterflies live off of nectar. Etc. This effectively prevents older members of the species from competing with younger members. This may lead more members of the species to successfully reach sexual maturity, without the risk of being out-competed by older members of their species.

4.3 Types of metamorphosis

4.3.1 Complete Metamorphosis

In complete metamorphosis, a larva completely changes its body plan to become an adult. The most famous example is that of the butterfly, which starts out as a worm-like, leaf-eating caterpillar and transforms into a flying, nectar-drinking creature with an exoskeleton. Organisms that undergo complete metamorphosis are called "holometabolous," from the Greek words "holo" for "complete" or "whole," "meta" for "change," and the noun "bole" for "to throw." "Holometabolous," then, means "completely changing," or "wholly changing."

This transformation is so swift and complete that the caterpillar must spin a cocoon and lie dormant for weeks while its body undergoes these radical changes. Other animals which transform from a worm-like larval stage into an animal that looks completely different include beetles, flies, moths, ants, and bees. Some scientists believe that the larval stage of complete metamorphosis may have evolved from insects which hatched from their eggs without developing properly. Some of these embryos may have survived long enough to find food in the outside world; and this may have ended up giving them an advantage, as they would be able to feed longer and gain more strength than their peers before metamorphosing into the adult stage.

4.3.2 Incomplete Metamorphosis

In incomplete metamorphosis, only some parts of the animal's body change during metamorphosis. Animals that only partially change their bodies as they mature are called "hemimetabolous," from the Greek words "hemi" for "half," "meta," for "change," and the verb "bole" for "to throw." "Hemimetabolous," then, is a word meaning "half-changing." Cockroaches, grasshoppers, and dragonflies, for example, hatch from eggs looking a lot like their adult selves. They do acquire wings and functioning reproductive organs as they grow, but

they do not completely remake their bodies like their completely metamorphosing cousins do.

4.3.3 Examples of Metamorphosis

4.3.3.1 Butterflies

Many of us may have witnessed the process of metamorphosis first hand, by raising caterpillars into butterflies in school. The idea of a worm-like caterpillar wrapping itself in a cocoon for weeks and then emerging as a beautiful butterfly is certainly strange. But the obvious changes of appearance, such as the growth of wings, don't do justice to just how strange this process is. In the cocoon, caterpillars don't simply gain legs, wings, and an exoskeleton. They also grow new eyes, lose their leaf-eating mouth parts and replace them with nectar-sucking proboscises, and gain mature reproductive organs. To accomplish this drastic change, a metamorphosing caterpillar basically digests itself.

A great deal of energy and raw materials are required to turn a caterpillar into a butterfly. So to make it possible, caterpillars release enzymes that dissolve most of their bodies! Indeed, the hard shell of the cocoon is required not just to protect the metamorphosing insect from attack: it is required to keep its liquefying body bound together, lest it ooze away! Not all of the caterpillar's cells are dissolved by these enzymes. Special tissues called imaginal discs survive – and they use the soup that used to be the rest of the caterpillar's body for nutrition. By consuming the proteins, vitamins, and minerals – everything you need to build a butterfly – these imaginal discs are able to grow incredibly quickly, developing into the butterfly's mature body parts. The new body has almost nothing in common with the old body. It has new legs, new sensory organs, a new exoskeleton, a new reproductive system. Even its digestive system does not work the same way, since it must now digest nectar instead of leaves. That's all in addition to the beautiful wings.

This radical change allows butterflies to complete their life cycle very efficiently, with no competition between adult butterflies and caterpillars for food. Many other insects pass through a similar process. They hatch as worm-like larva, eventually encase themselves in hard pupas, and emerge as adults with legs, exoskeletons, and other features that have little in common with the larva they once were. Bees, beetles, ants, and flies all use this strategy.

4.3.3.2 Frogs

The metamorphosis of a tadpole into a frog is a little less violent than that of a caterpillar into a butterfly, but the processes share some important common

features. Tadpoles do not dissolve their bodies into mush; but they do "digest" them in a less spectacular way. Using the process of apoptosis – or "programmed cell death" – the tadpoles "order" the cells they don't need anymore to shred their DNA and die. The dead cells are then cannibalized for energy and raw materials to make other cells. The cells of their tails are broken down and used to make their developing legs; a similar process happens with the gills, which disappear as the tadpole begins to develop air-breathing lungs. One interesting thing to note is that tadpole metamorphosis and insect metamorphosis likely developed separately; the common ancestor of insects and amphibians diverged long ago, and the ancestors of modern insects are not thought to have used metamorphosis. When the same phenomenon evolves twice in radically different organisms, that's a sure sign that it is a useful adaptation.

4.3.3.3 Fish

Some species of fish undergo metamorphoses similar to those of the tadpole. Though those changes are not so dramatic, they can result in changes in the fish's food source, its body plan, and where it's able to live. Just like the more drastic forms of evolution, this may function to prevent adults from competing with juveniles for food. The salmon, for example, is a freshwater fish in its juvenile form. After undergoing a partial metamorphosis, it becomes a saltwater fish. When thinking about this process it is important to keep in mind that all organisms must regulate their salt/water balance. This is why humans can't drink seawater without dying: the salt would overwhelm our cellular chemistry, and our cells would not function properly. In just the same way, freshwater fish typically cannot live in saltwater. To become saltwater fish, then, salmon must develop new organs and cellular mechanisms to cope with the salt water.
That's why salmon must perform their annual migration upstream; adult salmon live in the ocean, but their eggs must hatch in fresh water in order for the juveniles to survive. That means that adult salmon must leave their homes in the ocean for freshwater rivers, and swim as far upstream as possible before laying their eggs! Flounders, bizarrely, undergo a metamorphosis in which one of their eyes and nostrils move from one side of the head to the other. As juveniles, flounder look much like most fish: they swim vertical relative to the current, with one eye and one nostril on each side of their bladelike body. This body type allows them to swim fast like most other species of fish.

But in adulthood, flounder are flat fish which camouflage themselves by swimming on their bellies, pressed against the sea bed. To accomplish this lifestyle change, juvenile flounder essentially flip over on their sides and make one side of their body into their belly. Through cellular changes, the eye and

nostril from the belly side actually migrate to join the other eye and nostril on what is now the "top" side of the fish.

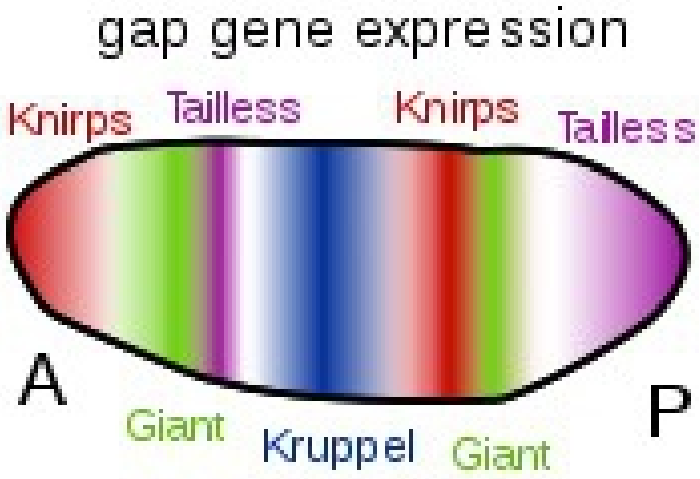
4.4 Morphogenesis

Morphogenesis is the biological process that causes a cell, tissue or organism to develop its shape. It is one of three fundamental aspects of developmental biology along with the control of tissue growth and patterning of cellular differentiation. The process controls the organized spatial distribution of cells during the embryonic development of an organism. Morphogenesis can take place also in a mature organism, such as in the normal maintenance of tissue by stem cells or in regeneration of tissues after damage. Cancer is an example of highly abnormal and pathological tissue morphogenesis. Morphogenesis also describes the development of unicellular life forms that do not have an embryonic stage in their life cycle. Morphogenesis is essential for the evolution of new forms.

Morphogenesis is a mechanical process involving forces that generate mechanical stress, strain, and movement of cells, and can be induced by genetic programs according to the spatial patterning of cells within tissues. Some of the earliest ideas and mathematical descriptions on how physical processes and constraints affect biological growth, and hence natural patterns such as the spirals of phyllotaxis, were written by D'Arcy Wentworth Thompson in his 1917 book *On Growth and Form* and Alan Turing in his *The Chemical Basis of Morphogenesis* (1952).

Where Thompson explained animal body shapes as being created by varying rates of growth in different directions, for instance to create the spiral shell of a snail, Turing correctly predicted a mechanism of morphogenesis, the diffusion of two different chemical signals, one activating and one deactivating growth, to set up patterns of development, decades before the formation of such patterns was observed. The fuller understanding of the mechanisms involved in actual organisms required the discovery of the structure of DNA in 1953, and the development of molecular biology and biochemistry.

4.5 Genetic and molecular basis



Morphogenesis is controlled by a "toolkit" of genes which switch development on and off at precise times and places.

Several types of molecules are important in morphogenesis. Morphogens are soluble molecules that can diffuse and carry signals that control cell differentiation via concentration gradients. Morphogens typically act through binding to specific protein receptors. An important class of molecules involved in morphogenesis are transcription factor proteins that determine the fate of cells by interacting with DNA. These can be coded for by master regulatory genes, and either activate or deactivate the transcription of other genes; in turn, these secondary gene products can regulate the expression of still other genes in a regulatory cascade of gene regulatory networks. At the end of this cascade are classes of molecules that control cellular behaviors such as cell migration, or, more generally, their properties, such as cell adhesion or cell contractility.

For example, during gastrulation, clumps of stem cells switch off their cell-to-cell adhesion, become migratory, and take up new positions within an embryo where they again activate specific cell adhesion proteins and form new tissues and organs. Developmental signaling pathways implicated in morphogenesis include Wnt, Hedgehog, and ephrins.

4.6 Branching morphogenesis

In the development of the lung a bronchus branches into bronchioles forming the respiratory tree. The branching is a result of the tip of each bronchiolar tube bifurcating, and the process of branching morphogenesis forms the bronchi, bronchioles, and ultimately the alveoli. Branching morphogenesis is also evident in the ductal formation of the mammary gland. Primitive duct formation begins in development, but the branching formation of the duct system begins later in response to estrogen during puberty and is further refined in line with mammary gland development.

4.6.1 Cancer morphogenesis

Cancer can result from disruption of normal morphogenesis, including both tumor formation and tumor metastasis. Mitochondrial dysfunction can result in increased cancer risk due to disturbed morphogen signaling.

4.6.2 Virus morphogenesis

During assembly of the bacteriophage (phage) T4 virion, the morphogenetic proteins encoded by the phage genes interact with each other in a characteristic sequence. Maintaining an appropriate balance in the amounts of each of these proteins produced during viral infection appears to be critical for normal phage T4 morphogenesis. Phage T4 encoded proteins that determine virion structures include major structural components, minor structural components and non-structural proteins that catalyze specific steps in the morphogenesis sequence. Phage T4 morphogenesis is divided into three independent pathways: the head, the tail and the long tail fibres as detailed by Yap and Rossman.

4.7 Amphibians

There are more than 6,000 species of amphibians living today. This animal class includes toads and frogs, salamanders and newts, and caecilians. Almost all amphibians have thin, moist skin that helps them breathe. No other group of animals has this special skin. Most amphibians undergo a unique change from larvae to adults, called metamorphosis. All amphibians are ectotherms (what used to be called "cold-blooded"), a trait they share with invertebrates, fish, and reptiles.

4.8 Thin-skinned

Most amphibians have thin skin that is very permeable (allowing liquids and gases to pass through it easily). This is important for two reasons. First, it means that their skin helps them breathe, since oxygen passes easily through it. Second, it means that amphibians lose a lot of water through their skin. This is why most amphibians are found in moist or humid environments, where they can re-load their water reserves.

4.9 Leading a Double-Double Life

The word amphibian comes from the Greek word *amphibios*, meaning "a being with a double life." Some say their name refers to the fact that amphibians live in two places -- on land and in water. While dual residence is the rule for most amphibians, some species are strictly aquatic (water-dwelling) and some are strictly terrestrial (land-dwelling).

More accurately, amphibians' "double life" refers to two distinct life stages -- a larval stage and an adult stage. Most amphibians lay eggs, which hatch into larvae and undergo an amazing transformation (or metamorphosis) as they move from larval to adult stages. For instance, tadpoles (the larval stage of

frogs) have gills and a tail -- features that enable them to live underwater. During metamorphosis, tadpoles lose their gills and develop lungs so they can breathe out of water. At the same time, they begin to grow limbs and lose their tails. The end result: adult frogs who spend much of their time on land.

4.10 Just the Right Temperature

Amphibians, like reptiles, are ectotherms. This means that they cannot produce sufficient internal heat to maintain a constant body temperature. Instead, amphibians' body temperature varies, depending on the surrounding temperature. So what does this mean for amphibians? It means that they're responsible for regulating their own body temperature. When it's cold outside and they need to warm up, amphibians often bask in the sun to raise their body temperature. When it's too cold to even bask, amphibians may brumate**.** This means they're in a hibernation-like state, but they may have periods of wakefulness and even drink when necessary. When it's hot outside, amphibians spend much of the time burrowing during the day, becoming active only at night.

4.11 Amphibians in Danger: The Extinction Crisis

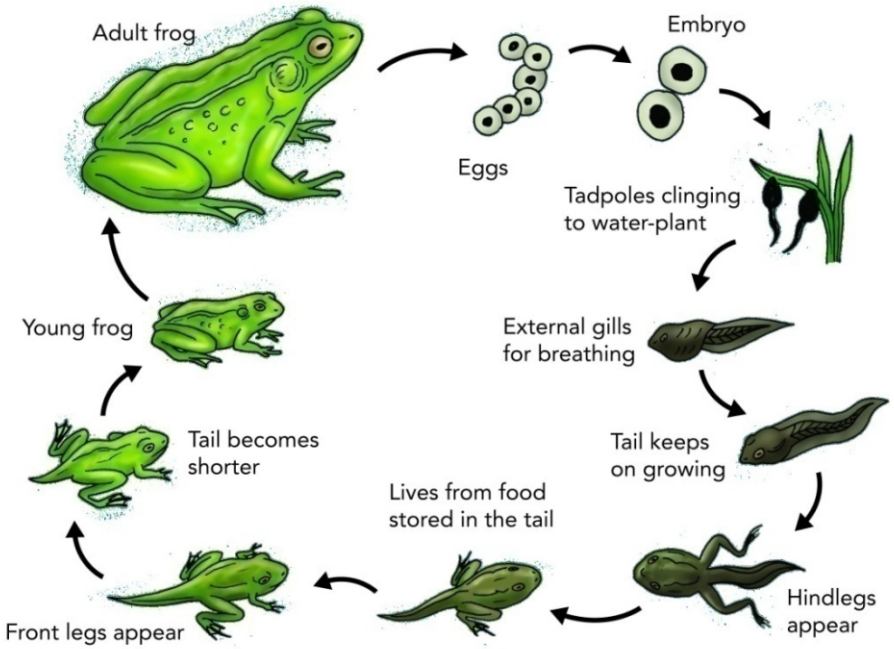
At this very moment, amphibians are facing an extinction crisis. More than 160 species of amphibians may already be extinct. More than 1,800 amphibian species are threatened with extinction – that's 32% of all known amphibian species. And at least 43% of all species have undergone population declines, while less than one percent show population increases. The greatest threat facing amphibians is habitat loss and degradation. Other significant threats include pollution, climate change, introduced species, and over-collection. Perhaps the most sinister threat is a newly-recognized fungal disease, which can cause rapid and severe amphibian declines. What is being done to help amphibians? As a first step, hundreds of experts have contributed to a Global Amphibian Assessment, an ongoing project that looks at the distribution and conservation status of all known species.

In addition, amphibian specialists around the world are working on understanding the causes of the declines, developing long-term conservation programs, and responding to immediate crises. Amphibians have been referred to as "canaries in the global coalmine" and "nature's indicators" because they are some of the first living things to be affected by environmental changes. When amphibians show a decline, it serves as a warning to other species, including humans. By helping amphibians, we are helping the world.

4.12Metamorphosis in amphibians

Metamorphosis in amphibians is the transformation of the larva to a miniature adult replicate, and usually from an aquatic to a terrestrial or semi-terrestrial lifestyle. Metamorphosis marks the beginning of the end of larval life. Once

begun, metamorphosis usually proceeds rapidly, which reduces the transforming amphibian's exposure to predation or other potential stresses when it is neither fully aquatic nor fully terrestrial.



Different stages of metamorphosis in frog

Metamorphosis is initiated internally and maintained by the hormone thyroxine (TH), and the process is obligatory. TH elicits extensive cellular, biochemical, and morphological changes to occur during metamorphosis. Events that occur during metamorphosis, including altered gene expression, morphogenesis, tissue restructuring, and extensive cell death, result from differential response of tissues to TH. The genetically determined developmental program is in place prior to the release of TH. The key element determining the response to the hormone is determined by the nuclear thyroid hormone receptor (TR). As in most vertebrates, two thyroid hormone receptors, TRα and TRβ, repress transcription in the absence of the TH, and whose concentration of TR in tissues is directly modulated by TH. Nevertheless, environmental factors can initiate early thyroxine release if a larva has completed certain morphogenic events.

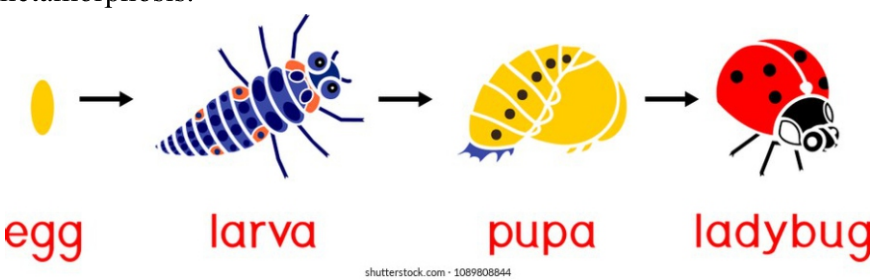
For example, crowding, reduced food or oxygen, drying of water bodies, or increased predation can result in TH release. Although TH and its derivatives promote metamorphosis, they do not operate alone. The thyroid is present early in larval life, but its secretory activity is apparently inhibited by corticoid hormones, such as corticosterone. Furthermore, prolactin is abundant in early larval stages and makes the body tissues insensitive to TH. When these inhibitions are removed, the thyroid secretes TH, effecting transformation.

Metamorphosis signals the completion of embryogenesis. Some developmental processes, such as maturation of gonads, continue through the juvenile stage, but the major structural and physiological features are in place at the conclusion of metamorphosis. Metamorphosis is nearly imperceptible in caecilians and salamanders but dramatic in frogs. Anuran larvae require major structural and physiological reorganization because of the striking differences between the larval and the juvenile–adult stages. Change does not occur all at once but gradually, each step leading to the next level of transformation. Unlike insect pupae, metamorphosing tadpoles remain active, capable of avoiding predators and environmental stresses.

4.13 Metamorphosis in insects

Amphibians and insects provide most people with their first contact with metamorphosis. The striking transformation of a tadpole into a frog is relatively rapid but one can track its progression from day to day as its limbs extend and its tail is resorbed. Metamorphosis in insects, though, seems more mysterious as their external exoskeleton hides the gradual changes occurring inside, and one only sees the results when the old outer cuticle is abruptly shed, such as when the butterfly emerges from its chrysalis. Metamorphosis is an ancient life history trait that extends deep to the roots of the amphibians and many groups of invertebrates.

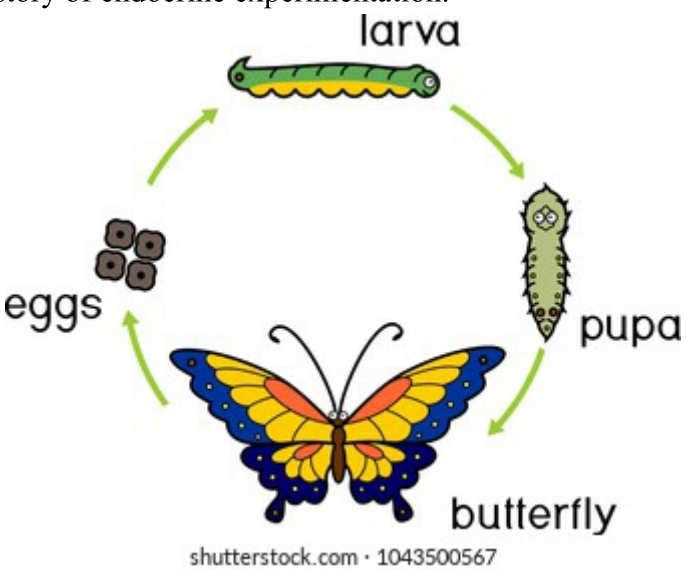
Insects are unusual, though, in that their metamorphosis is a derived trait that evolved well after they colonized the land. Insect orders approximating different steps in the evolution of metamorphosis exist today, so that comparative studies allow a piecing together of the molecular, developmental and endocrine changes that accompanied this transition. Insect metamorphosis is under hormonal control, which has been a subject of experimental study for almost a century. The importance of insects as competitors for food and fiber as well as their impact on human and animal health spurred extensive research into their physiology and development, and especially into how they control their metamorphosis.



The early stages of this research typically used large insects which facilitated surgical manipulations and the isolation of sufficient amounts of tissue for hormone extraction and chemical characterization. Ensuing genetic and molecular studies, though, gradually shifted to *Drosophila melanogaster*, which still serves as an unrivaled platform for gene discovery. However, many derived features of *Drosophila*, such as an invariant number of

larval instars and an almost complete replacement of larval cells by those of the adult at metamorphosis, have resulted in a de-emphasis or loss of some aspects of the hormonal control seen in less derived insects.

Consequently, despite the fact that work on *Drosophila* is responsible for the discovery of virtually all the genes involved in hormone reception, their downstream effects, and stage specificity of action, the broader functions of such genes often remained obscure for decades and were finally shown in insects with less derived life history patterns. For example, work on the flour beetle, *Tribolium castaneum*, provided the proof that the *Methoprene tolerant* gene (*Met*) does, indeed, encode the receptor for juvenile hormone (JH), a key hormone that suppresses metamorphosis. Also, studies on this beetle and the cockroach *Blattella germanica* showed that the helix-turn-helix transcription factor Eip93F (E93) is a master stage-specifying gene that stands on top of a gene cascade that produces the adult phenotype. Besides *Drosophila*, this discussion draws on other insect model systems such as *Bombyx mori*, *Manduca sexta*, *Tribolium castaneum*, *Oncopeltus fasciatus*, *Pyrrhocoris apterus* and *Blattella germanica*, for which genomic information and gene suppression techniques can be woven into a rich history of endocrine experimentation.



Although these few systems do not begin to cover the amazing range of variation that one sees in the insects, it is heartening that highly derived life history strategies, such as female neoteny in Strepsiptera and scale insects or the complete metamorphosis that evolved in parallel in thrips, utilize the same molecular switches that regulate more traditional life history patterns. This unit explores the developmental changes that accompanied the evolution of the insect larval and pupal stages. It then focuses on the two main hormonal systems that regulate insect molting and metamorphosis and considers how these systems evolved to support the increasing complexity of insect life histories. Finally, it examines our growing knowledge of the function of the

stage specification genes that regulate the phenotype of each major life stage and how these genes and their control have changed with the increasing complexity of insect life histories.

4.14 Tissue reactivity

A foreign body reaction (FBR) is a typical tissue response to a foreign body within biological tissue. It usually includes the formation of a foreign body granuloma. Tissue-encapsulation of an implant is an example, as is inflammation around a splinter. Foreign body granuloma formation consists of protein adsorption, macrophages, multinucleated foreign body giant cells (macrophage fusion), fibroblasts, and angiogenesis. It has also been proposed that the mechanical property of the interface between an implant and its surrounding tissues is critical for the host response.

In the long term, the foreign body reaction results in encapsulation of the foreign body within a calcified shell. For example, a *lithopedion* is a rare phenomenon which occurs most commonly when a fetus dies during an abdominal pregnancy, is too large to be reabsorbed by the body, and calcifies.

4.15 Foreign body reaction to biomaterial implantation

Following biomaterial implantation, blood and body fluids contact the implant surface. Host blood proteins adsorb onto the implant surface and a fibrin matrix forms. Acute and chronic inflammation follow the initial blood protein deposition and matrix formation. Macrophages at the implant site fuse to form foreign body giant cells. Following the inflammatory response, granulation tissue form. The end stage of the foreign body reaction is the fibrous capsule formation around the implanted biomaterial. The biocompatibility of the device affects the severity of the foreign body reaction. The foreign body reaction can lead to device failure.

4.16 Protein adsorption

During blood-biomaterial interaction, blood proteins spontaneously adsorb to the biomaterial surface. The biomaterial surface properties affect the types, concentrations, and conformation of proteins that adsorb to the surface. The Vroman effect can describe the time-dependent behavior of this protein adsorption. Surface-adsorbed proteins regulate inflammatory cell interaction and adhesion. The deposited proteins allow inflammatory cells to attach via integrins. The biomaterial surface can also recruit and activate complement proteins.

4.17 Immune recruitment

The composition and conformation of adsorbed proteins on the implant surface is critical to the foreign body reaction. For the first two days, neutrophils are

the primary cell type that deposit on the implant surface. Neutrophils release degradative enzymes and reactive oxygen intermediates that damage the implant. Platelets from the blood-biomaterial interaction release inflammatory cytokines that cause monocytes and macrophages to extravasate and migrate to the implant site. The degranulation and release of histamine from mast cells further recruits macrophages to the biomaterial. Macrophages adhere to the biomaterial surface based on the surface protein deposits and produce cytokines that further recruit macrophages. Foreign body granuloma forms as immune cells accumulate on the biomaterial surface in an attempt to eliminate the biomaterial.

4.18 Macrophage fusion

Adherent macrophages at the implant site can fuse into a multinucleated cell called foreign body giant cell. Foreign body giant cell formation depends on the biomaterial surface properties and on the presence of interleukin-4 and interleukin-13. Foreign body giant cells release reactive oxygen intermediates, degradative enzymes, and acid onto the biomaterial surface. Foreign body giant cells also attempt to engulf the biomaterial for degradation. Adherent macrophages and foreign body giant cells degrade biomaterials and can lead to device failure. Foreign body giant cells remain on the surface of the implanted device throughout the device's lifetime.

4.19 Biological properties

Biocompatibility of suture materials describes how sutures, which are foreign materials to the body, could affect surrounding tissues and how the surrounding tissues could affect the properties of sutures. Thus, biocompatibility is a two-way relationship. The extent of tissue reaction to sutures depends largely on the chemical nature of the suture and its degradation products, if it is absorbable. Sutures from natural sources like catgut and silk usually provoke more tissue reaction than synthetic sutures because of the availability of enzymes to react with natural biopolymers.

Besides chemical factors, physical form, the amount and stiffness of suture materials have also been reported to elicit different levels of tissue reactions. For example, a stiff suture would result in stiff projecting ends in a knot where cut. These stiff ends could irritate surrounding tissues mechanically, a problem associated with some monofilament sutures but generally not found in braided multifilament sutures. Because the extent of a reaction relates to the quantity of the buried structure, it is a well-known practice in surgery that one should use as little suture material as possible, such as a smaller knot and a smaller size, to close wounds. The use of a smaller size suture which provides adequate support to wounds and does not cut through tissue is preferred as a small

decrease in diameter would lead to a large decrease in buried suture volume. There are two basic ways to study biocompatibility of suture materials:

- Cellular response and
- Enzyme histo-chemistry

The former is the most frequently used and provides information about the type and density of inflammatory cells at a suture site. In this approach, sutures without tension are implanted in small animals, e.g. the gluteal muscle of rats. This implantation site has given a very consistent reproducible cellular response for comparisons. However, Walton (1989) raised the question of using this common test procedure, particularly for sutures used in orthopedic surgery due to the observed inflamed nature of the postoperative synovial tissue and the mechanically stressed nature of the suture.

Normal tissue reaction to sutures can be grouped into the following stages, according to the time taken for the appearance of inflammatory cells. They are:

- Initial infiltration of polymorphonuclear leukocytes;
- Lymphocytes and monocytes during the first 3–4 days (i.e., acute response);
- Appearance of macrophages and fibroblasts from day 4 to day 7; and
- Maturation of fibrous connective tissue formation with chronic inflammation after the 7th to the 10th day.

During the first 7 days post-implantation, there is virtually no difference in normal tissue reaction between synthetic absorbable and nonabsorbable sutures. However, a slightly higher inflammatory reaction to synthetic absorbable sutures could persist for an extended period until they are completely absorbed and metabolized, while synthetic nonabsorbable sutures, in general, produce a minimal chronic inflammatory reaction with a thin fibrous connective tissue capsule surrounding the suture usually by 28 days post-implantation. In addition to the tissue reactions that are normal to all sutures, there are several reactions that are suture and site specific. Some examples include urinary stone or calci formation, granuloma formation, thrombogenicity, propensity toward wound infection and recurrence of tumor after radiation treatment and allergy.

Monofilament sutures are considered to be a better choice than multifilament ones in closing contaminated wounds. Multifilament sutures elicit more tissue reactions which may lessen tissue ability to deal with wound infections. They also have a capillary effect which could transport microorganisms from one region of the wound to another. The reason that multifilament sutures generally elicit more tissue reactions than their monofilament counterparts is because inflammatory cells are able to penetrate into the interstitial space within a multifilament suture and invade each filament. Multifilament sutures also have

a larger surface area in contact with tissues which should be expected to elicit more tissue reaction.

4.20 Summary

Under this unit we have summarized metamorphosis and its types with examples. Metamorphosis is the change in the body form and habits during the development cycle of animals. Complete metamorphosis and incomplete metamorphosis are two growth types of insects where the body form of insects changes during their lifecycle. Both complete and incomplete metamorphosis extend from the egg stage to the adult stage. Complete metamorphosis consists of four stages: egg, larva, pupa, and adult. However, the incomplete metamorphosis consists of three stages: egg, nymph, and adult. The main difference between complete metamorphosis and incomplete metamorphosis is that complete metamorphosis consists of a very active, ravenously eating larva and an inactive pupa whereas incomplete metamorphosis consists of a nymph, which resembles a miniature adult. Complete metamorphosis occurs in wasps, ants, and fleas while incomplete metamorphosis occurs in termites, praying mantis, and cockroaches

Complete metamorphosis is the type of insect development that includes egg, larva, pupal, and adult stages, which differ greatly in morphology. The lifecycle of butterflies, ants, fleas, bees, beetles, moths, and wasps are examples of the complete metamorphosis. Complete and incomplete metamorphosis are two types of growth forms in insects. The complete metamorphosis occurs through four stages: egg, larva, pupa, and adult. The incomplete metamorphosis occurs through three stages: egg, nymph, and adult. The pupa stage is not developed during incomplete metamorphosis. Therefore, the main difference between complete and incomplete metamorphosis is the differential stages developed during each type of growth.

4.21 Terminal questions

Q.1. What do you mean by metamorphosis?

Answer:----------

Q.2. Describe metamorphosis in amphibians.

Answer:----------

Q.3. Write a short note on tissue reactivity.

Answer:----------

Q.4. What do you mean by metamorphosis in insects?

Answer:----------

Further readings

1. Vertebrate Endocrinology- David O. Norris
2. Invertebrate Zoology –Robert W. Hegner
3. Textbook of Biotechnology –B. D. Singh
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Uttar Pradesh Rajarshi Tandon
Open University

Bachelor of Science
DCEZY -108
Developmental Biology

Block -2
Developmental Biology - II

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Course Design Committee

Prof. Ashutosh Gupta — **Chairman**
Director, School of Science
UPRTOU, Prayagraj.

Dr. Shubhra Malviya — **Member**
Asst. Prof. Department of Zoology
S.S. Khanna Girls Degree College.
Prayagraj.

Dr. Sippy Singh — **Member**
Asst. Prof. Department of Zoology
S.S. Khanna Girls Degree College.
Prayagraj.

Dr. Deepa Chaubey — **Member/Secretary**
Asst. Prof. (Contractual) Zoology
School of Science, UPRTOU, Prayagraj.

Course Preparation Committee

Dr. Dharmendra Singh — **Author**
Assistant Professor, — Block – 1 (Unit – 01 to 04)
Department of Zoology — Block – 2 (Unit – 05)
SP PG College, Shohratgarh, Siddharth Nagar

Dr. Sadguru Prakash — **Author**
Assistant Professor, — Block – 2 (Unit – 06 to 08)
Department of Zoology
MLK PG College, Balrampur

Dr. Parmod Kumar Pandey — **Editor**
Associate Professor, — (All blocks and units)
Department of Zoology
PSM PG College, Anand Nagar, Maharajganj

Dr. Deepa Chaubey — **Coordinator**
Asst. Prof. (Contractual) Zoology
School of Science, UPRTOU, Prayagraj.

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Block-II

In the previous block you have studies half part of developmental biology. In this block you will study remaining part of developmental biology, which is divided into four units (units 5 to 8).

The block 2nd is organized into further following four units as under:

Unit-5

It deals the induction process (factors controlling moulting in insects). The process of moulting in insects beings with the separation of the cuticle from the underlying epidermal cells and formation of its replacement. Moulting which is regulated by harmons. Moulting known technically as ecdysis, is literally a period of growth for insects.

Unit-6

It describes regeneration (Ability of regeneration, amphibian limb regeneration). Regeneration is the natural process of replacing or restoring damaged or missing cells, tissues and organs or regeneration is one of the processes in which if an organism is cut into several pieces, each of its parts regrows to the original state.

Unit-7

It covers the growth and ageing (concepts of growth and mechanism of growth). Growth and aging appear to be opposites, growth is the progressive increase in the size of a child or parts of a child. Aging is the sequential or progressive , physiological change in an organism.

Unit-8

It covers the growth curve and its interpretation (types of cell growth, ageing). Aging changes occur in all of the body's cells, tissues and organs and these changes affect the functioning of all body system.

Objectives

This is the first unit on developmental biology. Under this unit, we have following objectives. These are as under:

- To know about genetic specificity
- To discuss moulting, regeneration
- To discuss about cellular molecular fundamentals (tissue, arthropods, planaria, humans and reptiles)
- To know about histology of regeneration process
- To know about concept of ageing and degrowth
- To know about mechanism of growth and cell growth
- To know about cancer cell growth and exponential growth
- To discuss about the factors that regulate growth

Unit-5 - Induction process (factors controlling moulting in insects)

Structure

5.1 Introduction

Objectives

5.2 Genetic specificity of induction

5.3 Moulting

5.4 When insects molt

5.5 Understanding the exoskeleton

5.6 The process of molting

5.7 Pros and cons of molting

5.8 Summary

5.9 Terminal questions

5.1 Introduction

Organs are complex structures composed of numerous types of tissues. In the vertebrate eye, for example, light is transmitted through the transparent corneal tissue and focused by the lens tissue (the diameter of which is controlled by muscle tissue), eventually impinging on the tissue of the neural retina. The precise arrangement of tissues in this organ cannot be disturbed without impairing its function. Such coordination in the construction of organs is accomplished by one group of cells changing the behavior of an adjacent set of cells, thereby causing them to change their shape, mitotic rate, or fate. This kind of interaction at close range between two or more cells or tissues of different history and properties is called proximate interaction, or induction. There are at least two components to every inductive interaction. The first component is the inducer: the tissue that produces a signal (or signals) that changes the cellular behavior of the other tissue. The second component, the tissue being induced, is the responder.

Objectives

This is the first unit on developmental biology. Under this unit, we have following objectives. These are as under:

- To know about induction
- To know about genetic specificity
- To discuss moulting
- To understand the process of moulting

Not all tissues can respond to the signal being produced by the inducer. For instance, if the optic vesicle (presumptive retina) of *Xenopus laevis* is placed in an ectopic location (i.e., in a different place from where it normally forms) underneath the head ectoderm, it will induce that ectoderm to form lens tissue. Only the optic vesicle appears to be able to do this; therefore, it is an inducer. However, if the optic vesicle is placed beneath ectoderm in the flank or abdomen of the same organism, that ectoderm will not be able to respond. Only the head ectoderm is competent to respond to the signals from the optic vesicle by producing a lens.

5.2 Genetic specificity of induction

The second property of epithelial-mesenchymal interactions is the genetic specificity of induction. Whereas the mesenchyme may instruct the epithelium as to what sets of genes to activate, the responding epithelium can comply with these instructions only so far as its genome permits. This property was discovered through experiments involving the transplantation of tissues from one species to another. In one of the most dramatic examples of interspecific induction, Hans Spemann and Oscar Schotté (1932) transplanted flank ectoderm from an early frog gastrula to the region of a newt gastrula destined

to become parts of the mouth. Similarly, they placed presumptive flank ectodermal tissue from a newt gastrula into the presumptive oral regions of frog embryos. The structures of the mouth region differ greatly between salamander and frog larvae.

The salamander larva has club-shaped balancers beneath its mouth, whereas the frog tadpole produces mucus-secreting glands and suckers. The frog tadpole also has a horny jaw without teeth, whereas the salamander has a set of calcareous teeth in its jaw. The larvae resulting from the transplants were chimeras. The salamander larvae had froglike mouths, and the frog tadpoles had salamander teeth and balancers. In other words, the mesodermal cells instructed the ectoderm to make a mouth, but the ectoderm responded by making the only kind of mouth it "knew" how to make, no matter how inappropriate.

5.3 Moulting

In biology, moulting (British English), or molting (American English), also known as sloughing, shedding, or in many invertebrates, ecdysis, is the manner in which an animal routinely casts off a part of its body (often, but not always, an outer layer or covering), either at specific times of the year, or at specific points in its life cycle. Moulting can involve shedding the epidermis (skin), pelage (hair, feathers, fur, wool), or other external layer. In some groups, other body parts may be shed, for example, the entire exoskeleton in arthropods, including the wings in some insects. It is also known as Ecdysis or Shedding. The periodic shedding of old cuticle and the subsequent formation of new one is called moulting or ecdysis moulting are accuring periodically till maturation of insects. The molting process is triggered by hormones released when an insect's growth reaches the physical limits of its exoskeleton. Each molt represents the end of one growth stage (instar) and the beginning of another. In some insect species the number of instars is constant (typically from 3 to 15), but in others it may vary in response to temperature, food availability, or other environmental factors.

An insect is known as an imago (adult) when it becomes sexually mature. At this point, molting stops and energy for growth is channeled into production of eggs or sperm. An insect cannot survive without the support and protection of its exoskeleton, so a new, larger replacement must be constructed inside the old one -- much like putting an overcoat under a sweater! The molting process begins when epidermal cells respond to hormonal changes by increasing their rate of protein synthesis. This quickly leads to apolysis -- physical separation of the epidermis from the old endocuticle. Epidermal cells fill the resulting gap with an inactive molting fluid and then secrete a special lipoprotein (the cuticulin layer) that insulates and protects them from the molting fluid's

digestive action. This cuticulin layer becomes part of the new exoskeleton's epicuticle.

After formation of the cuticulin layer, molting fluid becomes activated and chemically "digests" the endocuticle of the old exoskeleton. Break-down products (amino acids and chitin microfibrils) pass through the cuticulin layer where they are recycled by the epidermal cells and secreted under the cuticulin layer as new procuticle (soft and wrinkled). Pore canals within the procuticle allow movement of lipids and proteins toward the new epicuticle where wax and cement layers form. When the new exoskeleton is ready, muscular contractions and intake of air cause the insect's body to swell until the old exoskeleton splits open along lines of weakness (ecdysial sutures). The insect sheds its old exoskeleton (ecdysis) and continues to fully expand the new one. Over the next few hours, sclerites will harden and darken as quinone cross-linkages form within the exocuticle.

This process (called sclerotization or tanning) gives the exoskeleton its final texture and appearance. An insect that is actively constructing new exoskeleton is said to be in a pharate condition. During the days or weeks of this process there may be very little evidence of change. Ecdysis, however, occurs quickly (in minutes to hours). A newly molted insect is soft and largely unpigmented (white or ivory). It is said to be in a teneralcondition until the process of tanning is completed (usually a day or two).

Molting, known technically as ecdysis, is literally a period of growth for insects. In humans, an analogy can be drawn to molting as a period of personal transformation, such as the shedding of one's old self and the emergence of a new and improved person. Insects grow in increments. Each stage of growth ends with molting, the process of shedding and replacing the rigid exoskeleton. People often think molting is the simple act of an insect breaking out of its skin and leaving it behind. In truth, the process is complex and involves several parts.

5.4 When insects molt

After egg hatches, the immature insect feeds and grows. Its exoskeleton is like a shell. Eventually, the larva or nymph must shed its unyielding overcoat to continue its development.

The exoskeleton which serves as its external backbone is used for protection and support. Without an exoskeleton, the insect could not survive. An old exoskeleton is shed when a new one is ready underneath, a process that can take days or weeks.

5.5 Understanding the exoskeleton

To understand how molting occurs, it helps to know the three layers of the insect exoskeleton. The outermost layer is called the cuticle. The cuticle

protects the insect against physical injury and water loss, as well as provides rigidity for muscle. It is this outermost layer that sheds during a molt. Underneath the cuticle is the epidermis. It is responsible for secreting a new cuticle when it is time to shed the old one. Underneath the epidermis is the basement membrane. This membrane is what separates the insect's main body from its exoskeleton.

5.6 The process of molting

In molting, the epidermis separates from the outermost cuticle. Then, the epidermis forms a protective layer around itself and secretes chemicals that break down the insides of the old cuticle. That protective layer becomes part of the new cuticle. When the epidermis has formed the new cuticle, muscular contractions and air intake cause the insect's body to swell, thus splitting open the remains of the old cuticle. Finally, the new cuticle hardens. The bug squeezes out from the outgrown exoskeleton. The insect must continue to swell and expand the new cuticle, so it is large enough to allow room for more growth. The new overcoat is soft and much paler than the former one, but over a few hours, it becomes darker and begins to harden. Within a few days, the insect appears to be a slightly larger copy of its former self.

5.7 Pros and cons of molting

For some insects, a big benefit to having a system of molting for growth is that it allows damaged tissue and missing limbs to be regenerated or substantially reformed. Complete regeneration may require a series of molts, the stump becoming a little larger with each molt until it is a normal or nearly back to normal size. A major disadvantage to having to molt as a system of growth is that the animal in question is entirely incapacitated during the process. An insect is completely vulnerable to a predator attack while undergoing molting.

5.8 Summary

This unit summarizes molting that is the process of producing a new cuticle and the subsequent shedding of the old cuticle. The cuticle is the outer covering of the insect and is its exoskeleton to which the muscles are attached. The outermost layer is called the epicuticle; under this is the exocuticle followed by the endocuticle. In some systems, the exo- and endocuticle are classed together as the procuticle. In some insects, only the epi- and exocuticle are deposited before ecdysis, with the endocuticle following ecdysis, whereas in others, some endocuticle may be deposited before ecdysis. The epicuticle is composed of only protein, whereas the exo- and endocuticle contain both chitin and protein in varying proportions depending on the type of cuticle, that is, whether rigid or flexible. Chitin is a polymer of *N*-acetylglucosamine and can be cross-linked to the protein components of the cuticle in a process called sclerotization. After

sclerotization the insect is able to move, feed, fly, etc. The rigid parts of the cuticle are then set and cannot be expanded, whereas flexible cuticle may expand either by a simple unfolding of the new epicuticle or in response to hormonal signals. When the epicuticle has completely unfolded, further expansion is impossible and the larva must molt in order to grow further. Molting is also necessary at the end of larval life for metamorphosis.

5.9 Terminal questions

Q.1 What do you mean by induction process?

Answer:----------

Q.2 Describe moulting in insects.

Answer:----------

Q.3 Write a short note on moulting.

Answer:----------

Q.4 Write a short note on insects.

Answer:----------

Q.5 Describe controlling factors of moulting.

Answer:----------

Further readings

1. Vertebrate Endocrinology- David O. Norris
2. Invertebrate Zoology –Robert W. Hegner
3. Textbook of Biotechnology –B. D. Singh
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Unit-6 - Regeneration (Ability of regeneration, amphibian limb regeneration)

Structure

6.1 Introduction

Objectives

6.2 Regeneration

6.3 Ecosystems

6.4 Cellular molecular fundamentals

- 6.4.1 Tissues

6.5 In animals

- 6.5.1 Arthropods
- 6.5.2 Annelids
- 6.5.3 Echinoderms
- 6.5.4 Planaria (Platyhelminthes)
- 6.5.5 Aves (birds)
- 6.5.6 Mammals
- 6.5.7 Humans
- 6.5.8 Reptiles
- 6.5.9 Chondrichthyes
- 6.5.10 Amphibians limb regeneration
- 6.5.11 Hydra
- 6.5.12 Structure and tissue dynamics of Hydra

6.6 Characteristics of regeneration

- 6.6.1 Patterning process governing axial patterning in hydra
- 6.6.2 Histology of regeneration process (Metaplasia)
- 6.6.3 Tissue metaplasia
- 6.6.4 Polarity and gradient
- 6.6.5 Regulation of regeneration

6.7 Summary

6.8 Terminal questions

Further readings

Morphogenesis is the developmental process by which tissues and organs acquire the shape that is critical to their function. Here, we review recent advances in our understanding of the mechanisms that drive morphogenesis in the developing eye. These investigations have shown that regulation of the actin cytoskeleton is central to shaping the presumptive lens and retinal epithelia that are the major components of the eye. Regulation of the actin cytoskeleton is mediated by Rho family GTPases, by signaling pathways and indirectly, by transcription factors that govern the expression of critical genes. Changes in the actin cytoskeleton can shape cells through the generation of filopodia (that, in the eye, connect adjacent epithelia) or through apical constriction, a process that produces a wedge-shaped cell. We have also learned that one tissue can influence the shape of an adjacent one, probably by direct force transmission, in a process we term inductive morphogenesis. Though these mechanisms of morphogenesis have been identified using the eye as a model system, they are likely to apply broadly where epithelia influence the shape of organs during development.

Objectives

This is the first unit on developmental biology. Under this unit, we have following objectives. These are as under:

- To know about regeneration
- To discuss about cellular molecular fundamentals (tissue, arthropods, planaria, humans and reptiles)
- To know about amphibian limb generation
- To know about histology of regeneration process

Morphogenesis is the developmental cascade of pattern formation and body plan establishment, resulting in the final form of the organism. Morphogenesis is the basis of the fields of tissue engineering and regenerative medicine that is founded on the basis of morphogenetic signals, responding stem cells, and the extracellular matrix scaffolds. The goal of tissue engineering is the design and construction of spare parts for the human body to restore function to bone and articular cartilage and other tissues. Although bone has a high potential for regeneration, the adjacent articular cartilage is feeble in its capacity for repair and regeneration. Implantation of demineralized extracellular matrix from bone into subcutaneous sites induces local new bone induction. This experimental model permits the isolation and identification of bone morphogenetic proteins (BMPs), the morphogenetic signals for bone and cartilage morphogenesis. BMPs initiate and promote bone formation and a variety of tissues including articular cartilage, brain, eye, heart, and kidney, leading to the concept of BMPs as body morphogenetic proteins. This chapter

will discuss the role of BMPs in bone and articular cartilage regenerative medicine and the remaining challenges. The rules of tissue morphogenesis gleaned from BMPs and bone may be applied universally to morphogenesis and regeneration of other organs and tissues, ushering us into the brave new world of the creation of spare parts for the human body based on signals, stem cells, and scaffolds of extracellular matrix.

Many biological organisms can regenerate some of their tissues and organs. Some species, such as hydra or planarian, can regenerate the whole organism from its small parts. Other organisms, among them mammals, have more limited regeneration potential that includes wound healing but also regeneration of complex structures such as antlers, and fingertips.

Physiological mechanisms of regeneration are very complex and in many cases they are not completely known. However, the biggest knowledge gap concerns the overall dynamics of a large-scale anatomy which allows it to be maintained, remodelled, or regenerated toward a specific shape. Our goal is to understand what algorithm is used by cell networks to decide which activity brings the morphology closer to the correct state, and to know when that state has been reached so that growth can stop. We can group these mechanisms in two classes that can be related to large pattern formation and to cell (or tissue) memory. By pattern formation we understand here the tissue organization due to the molecular process of cell-cell communication, processes of cell growth, division and motion and other processes which use the information available at current time. Contrary to that, cell memory implies the existence of the information about some former states of the tissue, which orchestrates cellular activity towards restoring a particular anatomical configuration and allows growth and remodelling to stop when a particular goal-state has been reached.

Let us illustrate these mechanisms on the example of wound healing, in which some cytokines are produced at the injury site, they diffuse in the tissue and attract some special cells (e.g., fibroblasts) which divide and regenerate the tissue. Morphogenetic gradients, organizing centres and activator-inhibitor interaction can be involved in pattern formation and tissue regeneration. If, for example, tissue formation is controlled by an organizing centre, we can expect that the same centre will provide its regeneration assuming that the centre itself is preserved.

Experiments on planarian regeneration allow us to assume that its tissues can keep some information about their former states. Due to alteration of normal electrical connectivity among its cells, a planarian with two heads can be obtained from a normal one-headed animal. If two heads are amputated, then both of them will regenerate in subsequent rounds of regeneration. Hence, regenerations of one or two heads are possible. The choice between them is not determined at the genetic level because the genomic sequence is the same, and

because any “reprogrammed” (epigenetically) tissues are discarded at each cut. Therefore the information about the number of heads is kept in the remaining tissue. Moreover, this memory is preserved in any part of the tissue since the amputated part can vary. There are other examples of regeneration that suggest the existence of tissue memory.

A model of tissue regeneration based on cell memory is suggested in our previous work, where we consider a tissue as an ensemble of points on the plane, each point corresponding to a single cell. Each cell produces a signal that spreads in space and decays as a function of distance from the cell. At the same time, each cell receives the signals produced by all other cells, and consequently the total signal received by a cell depends on its location with respect to the other cells. Hence, the distribution of the total signal depends on the geometry of cell structure. We suppose that each cell keeps the value of the total signal that it receives from the other cells (memory). If a part of the cell structure is amputated, the signal distribution changes, and the difference between the old and the new signals stimulates cell proliferation. Under some additional conditions on the location of proliferation cells, the initial cell structure will be regenerated. This mechanism works for a local tissue regeneration with the characteristic size determined by the rate of signal decay in space.

In the current work we continue to model regeneration of cell structures. We suggest a model which allows a more distant regeneration. It contains two submodels – one global and one local. The main assumptions of the first submodel are similar to those in the previous work: cells exchange signals, keep the information about the values of these signals and react when these values are changed. In comparison with the previous work, we do not amputate a part of the cell structure but instead alter the positions of cells. The difference between the initial signal distribution and the new one after cell displacement initiates cell motion and returns them to the initial positions.

One of the important characteristics of multicellular regeneration is the minimal information necessary to restore the initial structure after its modification. In, all cells produce and receive the same signal. Hence, each cell is supposed to keep only one real number: the value of the total initial signal. It appears that this information is sufficient for the regeneration of cell structure when a part of it is amputated. The difference between the old and the new signals stimulate cell proliferation, and we can exactly restore the initial cell structure. In the current work we consider a more complex biological structure consisting of several different tissues, and we change its geometry. In this case, if all cells produce and receive the same signal, in general they will not return to their initial configuration. In order to characterise such structures, a more detailed pair wise cell communication is required, where each cell produces its own signal and distinguishes the signals from other cells.

Hence, we consider that only the cells of the first (global) submodel communicate by different signals. Those cells can be some special cells or groups of cells of the corresponding tissue (e.g. stem cells, instructor cells, or organizers. Then the number of such cells corresponds to the number of tissues or organs and it is much more limited than the number of cells in the whole structure. In this case we need to complete the first (global) submodel by the second (local) submodel, which describes tissue growth around the cells of the first submodel. While a more complete and biologically realistic models can be considered, here we choose a simple and schematic mechanism of circular tissue growth in order to illustrate the principle.

Therefore, the global submodel provides configuration of tissue centres, while the local submodel creates the tissues around the centres. If some parts of the tissues are amputated but their centres are preserved, then the tissues will be regenerated. If some centres are amputated, then they should be restored by some other mechanisms which we do not consider here. The same model is able to describe morphogenesis, assuming the cells of the global submodel emerge in the embryo. In this case the tissue centres move to their equilibrium positions and generate their corresponding tissues.

6.2 Regeneration

In biology, regeneration is the process of renewal, restoration, and tissue growth that makes genomes, cells, organisms, and ecosystems resilient to natural fluctuations or events that cause disturbance or damage. Every species is capable of regeneration, from bacteria to humans. Regeneration can either be complete where the new tissue is the same as the lost tissue, or incomplete where after the necrotic tissue comes fibrosis.

At its most elementary level, regeneration is mediated by the molecular processes of gene regulation and involves the cellular processes of cell proliferation, morphogenesis and cell differentiation. Regeneration in biology, however, mainly refers to the morphogenic processes that characterize the phenotypic plasticity of traits allowing multi-cellular organisms to repair and maintain the integrity of their physiological and morphological states. Above the genetic level, regeneration is fundamentally regulated by asexual cellular processes. Regeneration is different from reproduction. For example, hydra perform regeneration but reproduce by the method of budding.

The hydra and the planarian flatworm have long served as model organisms for their highly adaptive regenerative capabilities. Once wounded, their cells become activated and restore the organs back to their pre-existing state. The Caudata , an order of tailed amphibians, is possibly the most adept vertebrate group at regeneration, given their capability of regenerating limbs, tails, jaws, eyes and a variety of internal structures. The regeneration of organs is a common and widespread adaptive capability

among metazoan creatures. In a related context, some animals are able to reproduce asexually through fragmentation, budding, or fission. A planarian parent, for example, will constrict, split in the middle, and each half generates a new end to form two clones of the original.

Echinoderms (such as the sea star), crayfish, many reptiles, and amphibians exhibit remarkable examples of tissue regeneration. The case of autotomy, for example, serves as a defensive function as the animal detaches a limb or tail to avoid capture. After the limb or tail has been autotomized, cells move into action and the tissues will regenerate. In some cases a shed limb can itself regenerate a new individual. Limited regeneration of limbs occurs in most fishes and salamanders, and tail regeneration takes place in larval frogs and toads (but not adults). The whole limb of a salamander or a triton will grow again and again after amputation. In reptiles, chelonians, crocodilians and snakes are unable to regenerate lost parts, but many (not all) kinds of lizards, geckos and iguanas possess regeneration capacity in a high degree. Usually, it involves dropping a section of their tail and regenerating it as part of a defense mechanism. While escaping a predator, if the predator catches the tail, it will disconnect.

6.3 Ecosystems

Ecosystems can be regenerative. Following a disturbance, such as a fire or pest outbreak in a forest, pioneering species will occupy, compete for space, and establish themselves in the newly opened habitat. The new growth of seedlings and community assembly process is known as regeneration in ecology.

6.4 Cellular molecular fundamentals

Pattern formation in the morphogenesis of an animal is regulated by genetic induction factors that put cells to work after damage has occurred. Neural cells, for example, express growth-associated proteins, such as GAP-43, tubulin, actin, an array of novel neuropeptides, and cytokines that induce a cellular physiological response to regenerate from the damage. Many of the genes that are involved in the original development of tissues are reinitialized during the regenerative process. Cells in the primordia of zebrafish fins, for example, express four genes from the homeobox *msx* family during development and regeneration.

6.4.1 Tissues

Strategies include the rearrangement of pre-existing tissue, the use of adult somatic stem cells and the dedifferentiation and/or transdifferentiation of cells, and more than one mode can operate in different tissues of the same animal. All these strategies result in the re-establishment of appropriate tissue polarity, structure and form. During the developmental process, genes are activated that serve to modify the properties of cell as they differentiate into

different tissues. Development and regeneration involves the coordination and organization of populations cells into a blastema, which is "a mound of stem cells from which regeneration begins. Dedifferentiation of cells means that they lose their tissue-specific characteristics as tissues remodel during the regeneration process. This should not be confused with the transdifferentiation of cells which is when they lose their tissue-specific characteristics during the regeneration process, and then re-differentiate to a different kind of cell.

6.5 In animals

6.5.1 Arthropods

Arthropods are known to regenerate appendages following loss or autotomy. Regeneration among arthropods is restricted by molting such that hemimetabolous insects are capable of regeneration only until their final molt whereas most crustaceans can regenerate throughout their lifetimes. Molting cycles are hormonally regulated in arthropods, although premature molting can be induced by autotomy. Mechanisms underlying appendage regeneration in hemimetabolous insects and crustaceans are highly conserved. During limb regeneration species in both taxa form a blastema following autotomy with regeneration of the excised limb occurring during proecdysis. Limb regeneration is also present in insects that undergo metamorphosis, such as beetles, although the cost of said regeneration is a delayed pupal stage. Arachnids, including scorpions, are known to regenerate their venom, although the content of the regenerated venom is different from the original venom during its regeneration, as the venom volume is replaced before the active proteins are all replenished.

6.5.2 Annelids

Many annelids (segmented worms) are capable of regeneration. For example, Chaetopterus variopedatus and Branchiomma nigromaculata can regenerate both anterior and posterior body parts after latitudinal bisection. The relationship between somatic and germline stem cell regeneration has been studied at the molecular level in the annelid Capitella teleta. Leeches, however, appear incapable of segmental regeneration. Furthermore, their close relatives, the branchiobdellids, are also incapable of segmental regeneration. However, certain individuals, like the lumbriculids, can regenerate from only a few segments. Segmental regeneration in these animals is epimorphic and occurs through blastema formation. Segmental regeneration has been gained and lost during annelid evolution, as seen in oligochaetes, where head regeneration has been lost three separate times.

Along with epimorphosis, some polychaetes like Sabella pavonina experience morphallactic regeneration. Morphallaxis involves the de-differentiation, transformation, and re-differentation of cells to regenerate tissues. How

prominent morphallactic regeneration is in oligochaetes is currently not well understood. Although relatively under-reported, it is possible that morphallaxis is a common mode of inter-segment regeneration in annelids. Following regeneration in *L. variegatus*, past posterior segments sometimes become anterior in the new body orientation, consistent with morphallaxis.

Following amputation, most annelids are capable of sealing their body via rapid muscular contraction. Constriction of body muscle can lead to infection prevention. In certain species, such as Limnodrilus, autolysis can be seen within hours after amputation in the ectoderm and mesoderm. Amputation is also thought to cause a large migration of cells to the injury site, and these form a wound plug.

6.5.3 Echinoderms

Tissue regeneration is widespread among echinoderms and has been well documented in starfish *(Asteroidea)*, sea cucumbers *(Holothuroidea)*, and sea urchins *(Echinoidea)*. Appendage regeneration in echinoderms has been studied since at least the 19th century. In addition to appendages, some species can regenerate internal organs and parts of their central nervous system. In response to injury starfish can autotomize damaged appendages. Autotomy is the self-amputation of a body part, usually an appendage. Depending on severity, starfish will then go through a four-week process where the appendage will be regenerated. Some species must retain mouth cells to regenerate an appendage, due to the need for energy. The first organs to regenerate, in all species documented to date, are associated with the digestive tract. Thus, most knowledge about visceral regeneration in holothurians concerns this system.

6.5.4 Planaria (Platyhelminthes)

Regeneration research using Planarians began in the late 1800s and was popularized by T.H. Morgan at the beginning of the 20th century. Alejandro Sanchez-Alvarado and Philip Newmark transformed planarians into a model genetic organism in the beginning of the 20th century to study the molecular mechanisms underlying regeneration in these animals. Planarians exhibit an extraordinary ability to regenerate lost body parts. For example, a planarian split lengthwise or crosswise will regenerate into two separate individuals. In one experiment, T.H. Morgan found that a piece corresponding to 1/279th of a planarian or a fragment with as few as 10,000 cells can successfully regenerate into a new worm within one to two weeks. After amputation, stump cells form a blastema formed from neoblasts, pluripotent cells found throughout the planarian body. New tissue grows from neoblasts with neoblasts comprising between 20 and 30% of all planarian cells. Recent work has confirmed that

neoblasts are totipotent since one single neoblast can regenerate an entire irradiated animal that has been rendered incapable of regeneration. In order to prevent starvation a planarian will use their own cells for energy, this phenomenon is known as de-growth.

6.5.5 Aves (birds)

Owing to a limited literature on the subject, birds are believed to have very limited regenerative abilities as adults. Some studies on roosters have suggested that birds can adequately regenerate some parts of the limbs and depending on the conditions in which regeneration takes place, such as age of the animal, the inter-relationship of the injured tissue with other muscles, and the type of operation, can involve complete regeneration of some musculoskeletal structure. Werber and Goldschmidt (1909) found that the goose and duck were capable of regenerating their beaks after partial amputation and Sidorova (1962) observed liver regeneration via hypertrophy in roosters. Birds are also capable of regenerating the hair cells in their cochlea following noise damage or ototoxic drug damage. Despite this evidence, contemporary studies suggest reparative regeneration in avian species is limited to periods during embryonic development.

An array of molecular biology techniques have been successful in manipulating cellular pathways known to contribute to spontaneous regeneration in chick embryos. For instance, removing a portion of the elbow joint in a chick embryo via window excision or slice excision and comparing joint tissue specific markers and cartilage markers showed that window excision allowed 10 out of 20 limbs to regenerate and expressed joint genes similarly to a developing embryo. In contrast, slice excision did not allow the joint to regenerate due to the fusion of the skeletal elements seen by an expression of cartilage markers.

Similar to the physiological regeneration of hair in mammals, birds can regenerate their feathers in order to repair damaged feathers or to attract mates with their plumage. Typically, seasonal changes that are associated with breeding seasons will prompt a hormonal signal for birds to begin regenerating feathers. This has been experimentally induced using thyroid hormones in the Rhode Island Red Fowls.

6.5.6 Mammals

Fig. 1 Spiny mice (*Acomys cahirinus* pictured here) can regenerate skin, cartilage, nerves and muscle.

Mammals are capable of cellular and physiological regeneration, but have generally poor reparative regenerative ability across the group. Examples of physiological regeneration in mammals include epithelial renewal (e.g., skin and intestinal tract), red blood cell replacement, antler regeneration and hair cycling. Male deer lose their antlers annually during the months of January to April then through regeneration are able to regrow them as an example of physiological regeneration. A deer antler is the only appendage of a mammal that can be regrown every year. While reparative regeneration is a rare phenomenon in mammals, it does occur. A well-documented example is regeneration of the digit tip distal to the nail bed. Reparative regeneration has also been observed in rabbits, pikas and African spiny mice. In 2012, researchers discovered that two species of African Spiny Mice, *Acomys kempi* and *Acomys percivali*, were capable of completely regenerating the autotomically released or otherwise damaged tissue. These species can regrow hair follicles, skin, sweat glands, fur and cartilage. In addition to these two species, subsequent studies demonstrated that *Acomys cahirinus* could regenerate skin and excised tissue in the ear pinna.

Despite these examples, it is generally accepted that adult mammals have limited regenerative capacity compared to most vertebrate embryos/larvae, adult salamanders and fish. But the regeneration therapy approach of Robert O. Becker, using electrical stimulation, has shown promising results for rats and mammals in general.

Some researchers have also claimed that the MRL mouse strain exhibits enhanced regenerative abilities. Work comparing the differential gene expression of scarless healing MRL mice and a poorly-healing C57BL/6 mouse strain, identified 36 genes differentiating the healing process between MRL mice and other mice. Study of the regenerative process in these animals is aimed at discovering how to duplicate them in humans, such as deactivation of

the p21 gene. However, recent work has shown that MRL mice actually close small ear holes with scar tissue, rather than regeneration as originally claimed.

MRL mice are not protected against myocardial infarction; heart regeneration in adult mammals (neocardiogenesis) is limited, because heart muscle cells are nearly all terminally differentiated. MRL mice show the same amount of cardiac injury and scar formation as normal mice after a heart attack. However, recent studies provide evidence that this may not always be the case, and that MRL mice can regenerate after heart damage.

6.5.7 Humans

The regrowth of lost tissues or organs in the human body is being researched. Some tissues such as skin regrow quite readily; others have been thought to have little or no capacity for regeneration, but ongoing research suggests that there is some hope for a variety of tissues and organs. Human organs that have been regenerated include the bladder, vagina and the penis.

As are all metazoans, humans are capable of physiological regeneration (i.e. the replacement of cells during homeostatic maintenance that does not necessitate injury). For example, the regeneration of red blood cells via erythropoiesis occurs through the maturation of erythrocytes from hematopoietic stem cells in the bone marrow, their subsequent circulation for around 90 days in the blood stream, and their eventual cell-death in the spleen. Another example of physiological regeneration is the sloughing and rebuilding of a functional endometrium during each menstrual cycle in females in response to varying levels of circulating estrogen and progesterone.

However, humans are limited in their capacity for reparative regeneration, which occurs in response to injury. One of the most studied regenerative responses in humans is the hypertrophy of the liver following liver injury. For example, the original mass of the liver is re-established in direct proportion to the amount of liver removed following partial hepatectomy, which indicates that signals from the body regulate liver mass precisely, both positively and negatively, until the desired mass is reached. This response is considered cellular regeneration (a form of compensatory hypertrophy) where the function and mass of the liver is regenerated through the proliferation of existing mature hepatic cells (mainly hepatocytes), but the exact morphology of the liver is not regained. This process is driven by growth factor and cytokine regulated pathways. The normal sequence of inflammation and regeneration does not function accurately in cancer. Specifically, cytokine stimulation of cells leads to expression of genes that change cellular functions and suppress the immune response.

Adult neurogenesis is also a form of cellular regeneration. For example, hippocampal neuron renewal occurs in normal adult humans at an annual turnover rate of 1.75% of neurons. Cardiac myocyte renewal has been found to occur in normal adult humans, and at a higher rate in adults following acute

heart injury such as infarction. Even in adult myocardium following infarction, proliferation is only found in around 1% of myocytes around the area of injury, which is not enough to restore function of cardiac muscle. However, this may be an important target for regenerative medicine as it implies that regeneration of cardiomyocytes, and consequently of myocardium, can be induced.

Another example of reparative regeneration in humans is fingertip regeneration, which occurs after phalange amputation distal to the nail bed (especially in children) and rib regeneration, which occurs following osteotomy for scoliosis treatment (though usually regeneration is only partial and may take up to one year). Yet another example of regeneration in humans is vas deferens regeneration, which occurs after a vasectomy and which results in vasectomy failure.

6.5.8 Reptiles

The ability and degree of regeneration in reptiles differs among the various species, but the most notable and well-studied occurrence is tail-regeneration in lizards. In addition to lizards, regeneration has been observed in the tails and maxillary bone of crocodiles and adult neurogenesis has also been noted. Tail regeneration has never been observed in snakes. Lizards possess the highest regenerative capacity as a group. Following autotomous tail loss, epimorphic regeneration of a new tail proceeds through a blastema-mediated process that results in a functionally and morphologically similar structure.

6.5.9 Chondrichthyes

Studies have shown that some chondrichthyans can regenerate rhodopsin by cellular regeneration, micro RNA organ regeneration, teeth physiological teeth regeneration, and reparative skin regeneration. Rhodopsin regeneration has been studied in skates and rays. After complete photo-bleaching, rhodopsin can completely regenerate within 2 hours in the retina. White bamboo sharks can regenerate at least two-thirds of their liver and this has been linked to three micro RNAs, xtr-miR-125b, fru-miR-204, and has-miR-142-3p_R-. In one study, two-thirds of the liver was removed and within 24 hours more than half of the liver had undergone hypertrophy. Leopard sharks routinely replace their teeth every 9–12 days and this is an example of physiological regeneration. This can occur because shark teeth are not attached to a bone, but instead are developed within a bony cavity. It has been estimated that the average shark loses about 30,000 to 40,000 teeth in a lifetime. Some sharks can regenerate scales and even skin following damage. Within two weeks of skin wounding the mucus is secreted into the wound and this initiates the healing process. One study showed that the majority of the wounded area was regenerated within 4 months, but the regenerated area also showed a high degree of variability.

6.5.10 Amphibians limb regeneration

Limb regeneration in the axolotl and newt has been extensively studied and researched. The nineteenth century studies of this subject are reviewed in Holland (2021). Urodele amphibians, such as salamanders and newts, display the highest regenerative ability among tetrapods. As such, they can fully regenerate their limbs, tail, jaws, and retina via epimorphic regeneration leading to functional replacement with new tissue. Salamander limb regeneration occurs in two main steps. First, the local cells dedifferentiate at the wound site into progenitor to form a blastema. Second, the blastemal cells will undergo cell proliferation, patterning, cell differentiation and tissue growth using similar genetic mechanisms that deployed during embryonic development. Ultimately, blastemal cells will generate all the cells for the new structure.

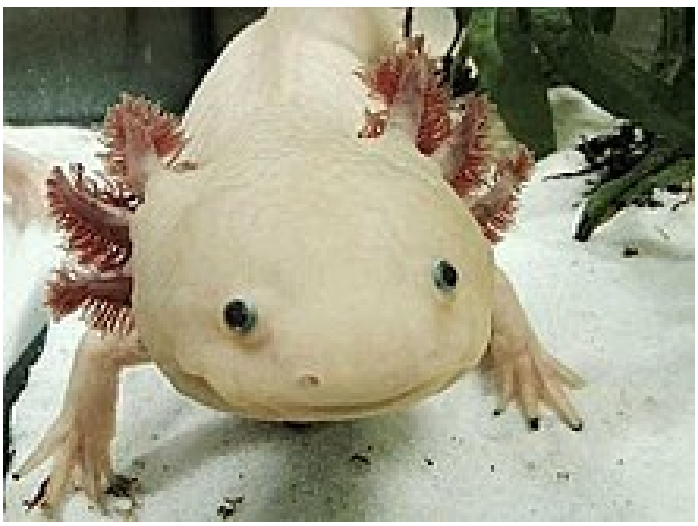

Fig. 2 Axolotls can regenerate a variety of structures, including their limbs

After amputation, the epidermis migrates to cover the stump in 1–2 hours, forming a structure called the wound epithelium (WE). Epidermal cells continue to migrate over the WE, resulting in a thickened, specialized signaling center called the apical epithelial cap (AEC). Over the next several days there are changes in the underlying stump tissues that result in the formation of a blastema (a mass of dedifferentiated proliferating cells). As the blastema forms, pattern formation genes – such as HoxA and HoxD – are activated as they were when the limb was formed in the embryo. The positional identity of the distal tip of the limb (i.e. the autopod, which is the hand or foot) is formed first in the blastema. Intermediate positional identities between the stump and the distal tip are then filled in through a process called intercalation. Motor neurons, muscle, and blood vessels grow with the regenerated limb, and reestablish the connections that were present prior to amputation. The time that this entire process takes varies according to the age of the animal, ranging from about a month to around three months in the adult and then the limb becomes fully functional.

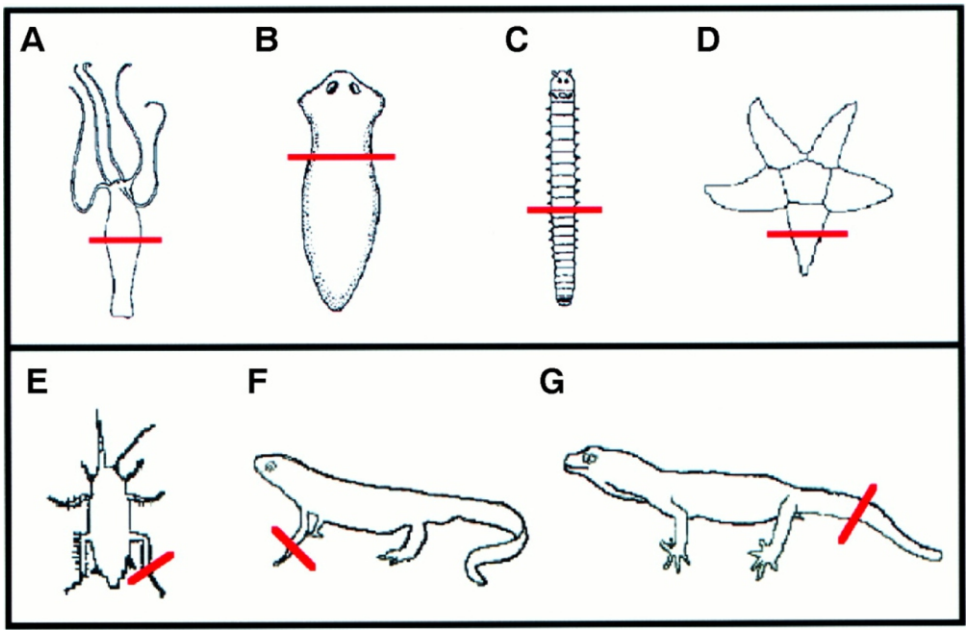

Fig. 3 Amphibian Limb Regeneration: Rebuilding a Complex Structure

In spite of the historically few researchers studying limb regeneration, remarkable progress has been made recently in establishing the neotenous amphibian the axolotl (*Ambystoma mexicanum*) as a model genetic organism. This progress has been facilitated by advances in genomics, bioinformatics, and somatic cell transgenesis in other fields, that have created the opportunity to investigate the mechanisms of important biological properties, such as limb regeneration, in the axolotl. The Ambystoma Genetic Stock Center (AGSC) is a self-sustaining, breeding colony of the axolotl supported by the National Science Foundation as a Living Stock Collection. Located at the University of Kentucky, the AGSC is dedicated to supplying genetically well-characterized axolotl embryos, larvae, and adults to laboratories throughout the United States and abroad. An NIH-funded NCRR grant has led to the establishment of the Ambystoma EST database, the Salamander Genome Project (SGP) that has led to the creation of the first amphibian gene map and several annotated molecular data bases, and the creation of the research community web portal.

Anurans can only regenerate their limbs during embryonic development. Once the limb skeleton has developed regeneration does not occur (Xenopus can grow a cartilaginous spike after amputation). Reactive oxygen species (ROS) appear to be required for a regeneration response in the anuran larvae. ROS production is essential to activate the Wnt signaling pathway, which has been associated with regeneration in other systems. Limb regeneration in salamanders occurs in two major steps. First, adult cells de-differentiate into progenitor cells which will replace the tissues they are derived from. Second, these progenitor cells then proliferate and differentiate until they have completely replaced the missing structure.

6.5.11 Hydra

Hydra, a primitive metazoan, has a simple structure consisting of a head, body column, and foot aligned along a single oral–aboral axis. The body column has a high capacity for regeneration of both the head and foot. Because of the tissue dynamics that take place in adult *Hydra*, the processes governing axial patterning are continuously active to maintain the form of the animal. Regeneration in hydra is morphallactic and closely related to these axial patterning processes. As might be expected, analysis at the molecular level indicates that the same set of genes are involved in head regeneration and the maintenance of the head in the context of the tissue dynamics of the adult. The genes analyzed so far play roles in axial patterning processes in bilaterians.

Hydra is a genus of freshwater polyp in the phylum Cnidaria with highly proliferative stem cells that gives them the ability to regenerate their entire body. Any fragment larger than a few hundred epithelial cells that is isolated from the body has the ability to regenerate into a smaller version of itself. The high proportion of stem cells in the hydra supports its efficient regenerative ability.

6.5.12 Structure and tissue dynamics of Hydra

The simple body plan of a *Hydra* is shown in given fig. The single axis consists of a cylindrical shell surrounding a gastric cavity that extends throughout the body column. The head consists of two parts. The apical part is the hypostome, or mouth region, whereas the basal part is the tentacle zone from which a ring of tentacles emerge. At the opposite end, the body column ends in the foot, or basal disk, with which the animal attaches itself to surfaces in its freshwater environment.

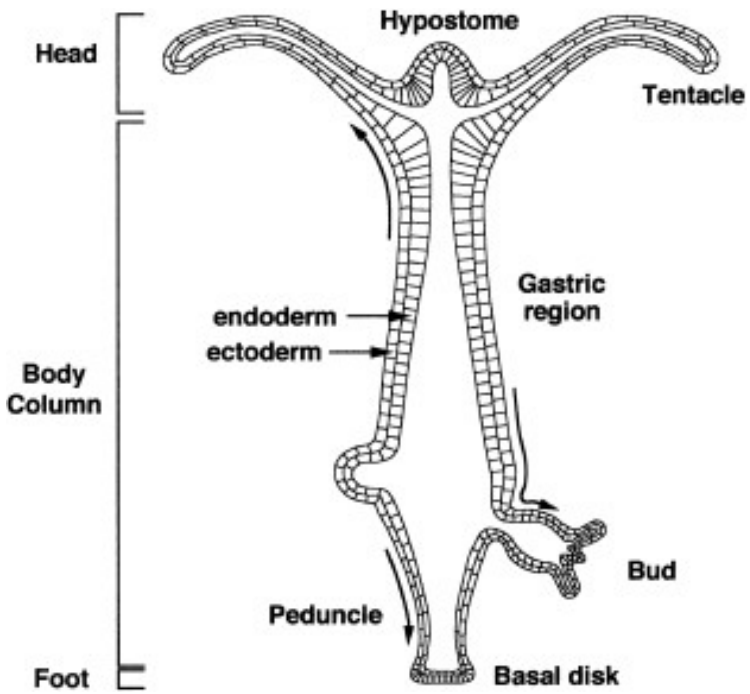

Fig. 4 Longitudinal cross-section of an adult *Hydra*.

Regeneration among hydra occurs as foot regeneration arising from the basal part of the body, and head regeneration, arising from the apical region. Regeneration tissues that are cut from the gastric region contain polarity, which allows them to distinguish between regenerating a head in the apical end and a foot in the basal end so that both regions are present in the newly regenerated organism. Head regeneration requires complex reconstruction of the area, while foot regeneration is much simpler, similar to tissue repair. In both foot and head regeneration, however, there are two distinct molecular cascades that occur once the tissue is wounded: early injury response and a subsequent, signal-driven pathway of the regenerating tissue that leads to cellular differentiation. This early-injury response includes epithelial cell stretching for wound closure, the migration of interstitial progenitors towards the wound, cell death, phagocytosis of cell debris, and reconstruction of the extracellular matrix.

Fig. 5 This tiny freshwater animal hydra can form two whole bodies after being cut in half

Regeneration in hydra has been defined as morphallaxis, the process where regeneration results from remodeling of existing material without cellular proliferation. If a hydra is cut into two pieces, the remaining severed sections form two fully functional and independent hydra, approximately the same size as the two smaller severed sections. This occurs through the exchange and rearrangement of soft tissues without the formation of new material.

6.6 Characteristics of regeneration

The head regeneration process is quite rapid. After bisection of the body column, head regeneration in the lower part consists of the following. The epithelia at the wounded upper end of the lower piece stretch to cover and close the wound, which takes place in 3 to 6 hr. Within 30 to 36 hr, tentacles begin to

emerge, and by 48 to 72 hr, a fully regenerated head has formed. Foot regeneration is a similar process in that the epithelia stretch to close the wound, and then a foot begins to form. As measured by the reappearance of a foot-specific peroxidase as well as the ability of the piece of tissue to stick to a surface, a foot regenerates in approximately 30 hr.

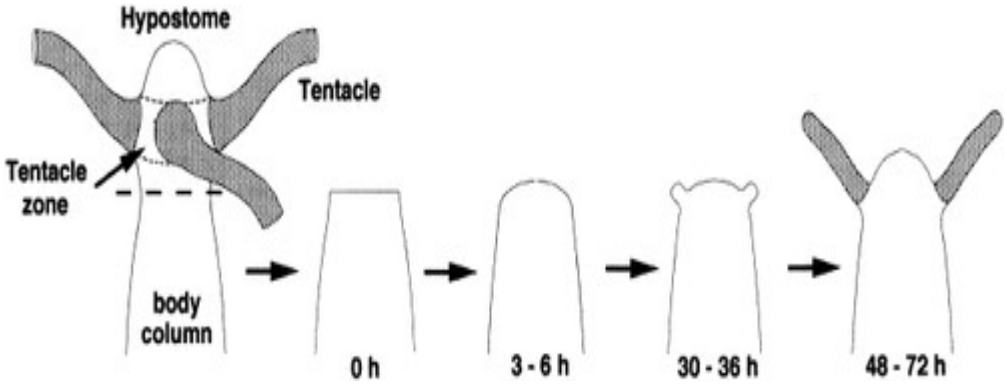

Fig. 6 Morphologic changes during head regeneration after decapitation.

Regeneration processes are of two kinds. In epimorphic regeneration, cell division takes place at and near the cut surface to provide tissue from which the removed structure regenerates. The regenerated structure, such as a limb, is the same size as the normal limb. In contrast, during morphallactic regeneration, cell division is unnecessary as the remaining tissue is remodeled to build the missing structures. In *Hydra*, regeneration is morphallactic, as illustrated by the regeneration of a complete, albeit much smaller, animal from a piece of the body column. That cell division is unnecessary for regeneration in *Hydra* was demonstrated by blocking cell division with γ-irradiation or hydroxyurea, bisecting the body column, and allowing regeneration to occur. The rates and extent of regeneration in pieces in which cell division was blocked were very similar in treated and untreated controls.

Another issue concerns which of the three cell lineages are responsible for head and foot regeneration, and what roles do they play. Obviously, the epithelial cells of both layers are involved. However, the interstitial cell lineage is not required. The interstitial cell lineage can be removed from hydra in several ways. Because of the shorter cell cycle time [1 day vs. 3 days], treatment with hydroxyurea or colchicine selectively reduces and eventually removes the interstitial cell population. Thereafter, the tissue displacement patterns coupled with sloughing at the extremities leads to the loss of all the interstitial cell differentiation products. Such animals, termed “epithelial animals” can be maintained indefinitely by hand-feeding, and will reproduce by budding as do normal animals. Epithelial animals maintained for over a year, when bisected, will regenerate a head and foot on the lower and upper parts, respectively, as do normal animals. Similar results were obtained with nf-1, a mutant of *Hydra magnipapillata* devoid of cells of the interstitial cell lineage. When bisected, animals of this strain regenerated a head at the same rate as a strain containing interstitial cells.

Although both epithelial lineages are required for head and foot regeneration simply for structural purposes, the question arises as to whether the control, or patterning, of the head during head regeneration is controlled by one or both layers. A similar question exists for foot regeneration. Smid and Tardent examined this issue by removing head and foot, separating the two epithelial layers, and generating chimeras by recombining the two layers either with the same head-foot polarity, or with an opposite polarity. Those chimeras with the same head-foot polarity all regenerated normally. However, the majority of those chimeras in which the ectoderm and endoderm had opposite polarities, formed a new axis that was perpendicular to the original axis, indicating that neither layer alone controlled where head and foot formed during regeneration. Instead, both layers play a role in determining the head/foot polarity.

Do the two layers provide the same or different information during head or foot regeneration? This question was examined by Zeretzke and Berking who made use of mh-1, a mutant of *Hydra magnipapillata* that forms ectopic heads along the body column. In addition, upon bisection, mh-1 animals regenerate a foot at a slower rate than does the 105 strain, which is the wild-type. By making ectoderm/endoderm chimeras [same head–foot polarity for both layers), they generated mh-1 ecto/105 endo and 105 ecto/mh-1 endo chimeras. They found that the mh-1 ecto/105 endo chimeras formed ectopic heads along the body column, whereas the 105 ecto/mh-1 endo chimeras regenerated feet at a reduced rate. These results suggest that, in this situation, the ectoderm influences head formation, whereas the endoderm affects foot formation. Whether this is generally true, will require a similar analysis using mutants with alterations in different characteristics of head and foot.

6.6.1 Patterning process governing axial patterning in hydra

Because an adult *Hydra* is in a steady state of production and loss of tissue coupled with continual displacement of tissue along the body axis, the processes governing axial patterning need to be constantly active to maintain the morphology of the animal. These same processes are also involved in head regeneration.

6.6.2 Histology of regeneration process (Metaplasia)

Metaplasia is the replacement of one differentiated cell type with another mature differentiated cell type that is not normally present in a specific tissue. It is important to distinguish metaplasia from transdifferentiation. Transdifferentiation is a process in which one differentiated cell type converts into a completely different cell type present in the tissue[2]. Although the change from one type of cell to another in metaplasia might be a part of the normal adult maturation processes, as will be discussed later, it is not known to occur during embryonic development. Metaplasia may be induced or accelerated by some sort of abnormal stimulus (for example, acid or base, and hence a change

in pH; hormones; cigarette smoke; and alcohol). In the context of an abnormal stimulus, the original cells adapt to the environmental stress by changing identity. If the stimulus that caused metaplasia is removed, it is not clear whether the tissues can return to their normal pattern of differentiation. However, if the condition promoting metaplasia persists, metaplasia can progress to dysplasia and occasionally malignancy, as will be discussed later (for example, in the oesophagus).

It is not realistically possible to determine the prevalence or incidence of tissue metaplasia of any type in the general population, as that information would require comprehensive and longitudinal monitoring, but one can glean some information about metaplasia and progression to dysplasia and cancer in the oesophagus, which has been studied more than metaplasia in other tissues. It is estimated that oesophageal intestinal metaplasia, termed Barrett oesophagus, will progress to cancer in 1 in 860 (0.12%) individuals with the condition.

The progression of metaplasia to dysplasia may be viewed as an ‘oncogenic’ phase, in contrast to the initial development of metaplasia, which is viewed as an ‘adaptive’ phase that occurs in response to environmental stress. While insights into the mechanisms leading to metaplasia are key to understanding tissue homeostasis as well as adaptation to stress, studying metaplasia progression to low-grade dysplasia and high-grade dysplasia can reveal major contributors to malignancy at early stages. The wide prevalence of tissue metaplasia and the limited progression to dysplasia and cancer, there is tremendous intersection of this topic with cancer prevention, early detection, risk stratification, prognosis and therapy. This unit focuses on the types of metaplasia, the potential cellular origins of metaplasia, the ways to model certain types of metaplasia and the potential opportunities for intervention to either reverse or arrest it.

6.6.3 Tissue metaplasia

Metaplasia tends to occur in tissues constantly exposed to environmental agents, which are often injurious in nature. For example, the pulmonary system (lungs and trachea) and the gastrointestinal tract are common sites of metaplasia owing to their contacts with air and food, respectively. As a result, the tissue epithelial structure adapts through metaplasia, with definitive morphological changes. The type of metaplasia depends upon the resident tissue. Metaplasia may be categorized broadly as squamous metaplasia, intestinal metaplasia or acinar–ductal metaplasia (ADM).

6.6.4 Polarity and gradient

Each living thing exhibits polarity, one example of which is the differentiation of an organism into a head, or forward part, and a tail, or hind part. Regenerating parts are no exception; they exhibit polarity by always growing

in a distal direction (away from the main part of the body). Among the lower invertebrates, however, the distinction between proximal (near, or toward the body) and distal is not always clear cut. It is not difficult, for example, to reverse the polarity of "stems" in colonial hydroids. Normally a piece of the stem will grow a head end, or hydranth, at its free, or distal, end; if that is tied off, however, it regenerates a hydranth at the end that was originally proximal. The polarity in this system is apparently determined by an activity gradient in such a way that a hydranth regenerates wherever the metabolic rate is highest. Once a hydranth has begun to develop, it inhibits the production of others proximal to it by the diffusion of an inhibitory substance downward along the stem.

When planarian flatworms are cut in half, each piece grows back the end that is missing. Cells in essentially identical regions of the body where the cut was made form blastemas, which, in one case gives rise to a head and in the other becomes a tail. What each blastema regenerates depends entirely on whether it is on a front piece or a hind piece of flatworm: the real difference between the two pieces may be established by metabolic differentials. If a transverse piece of a flatworm is cut very thin—too narrow for an effective metabolic gradient to be set up—it may regenerate two heads, one at either end. If the metabolic activity at the anterior end of a flatworm is artificially reduced by exposure to certain drugs, then the former posterior end of the worm may develop a head.

Appendage regeneration poses a different problem from that of whole organisms. The fin of a fish and the limb of a salamander have proximal and distal ends. By various manipulations, it is possible to make them regenerate in a proximal direction, however. If a square hole is cut in the fin of a fish, regeneration takes place as expected from the inner margin, but may also occur from the distal edge. In the latter case, the regenerating fin is actually a distal structure except that it happens to be growing in a proximal direction.

Amphibian limbs react in a similar manner. It is possible to graft the hand of a newt to the nearby body wall, and once a sufficient blood flow has been established, to sever the arm between the shoulder and elbow. This creates two stumps, a short one consisting of part of the upper arm, and a longer one made up of the rest of the arm protruding in the wrong direction from the side of the animal. Both stumps regenerate the same thing, namely, everything normally lying distal to the level of amputation, regardless of which way the stump was facing. The reversed arm therefore regenerates a mirror image of itself.

Clearly, when a structure regenerates it can only produce parts that normally lie distal to the level of amputation. The participating cells contain information needed to develop everything "downstream," but can never become more

proximal structures. Regeneration, like embryonic development, occurs in a definite sequence.

6.6.5 Regulation of regeneration

There are certain prerequisites without which regeneration cannot occur. First and foremost, there must be a wound, although the original appendage need not have been lost in the process. Second, there must be a source of blastema cells derived from remnants of the original structure or an associated one. Finally, regeneration must be stimulated by some external force. The stimuli often involve the nervous system. An adequate nerve supply is required for the regeneration of fish fins, taste barbels, and amphibian limbs. In the case of many tail regenerations, the spinal cord provides the necessary stimulus. Lens regeneration in salamander eyes depends upon the presence of a retina. Arthropod appendages regenerate in the presence of molting hormones. Protozoan regeneration requires the presence of a nucleus. In case after case, regeneration depends on more than a healed wound and a source of blastema cells. It is often triggered by some physiological stimulus originating elsewhere in the body, a stimulus invariably associated with the very function of the structure to be regenerated. The conclusion is inescapable that regeneration is primarily the recovery of deficient functions rather than simply the replacement of lost structures. The imperative of need is of further importance in suppressing excess regeneration. To be able to regenerate is to run the risk of regenerating too much or too often. If regeneration did not depend upon a physiological stimulus, such as those mediated by nerves or hormones, there would be no reason why simple wounds should not sprout whole new appendages.

It is not known why regeneration fails to occur in many cases, as in the legs of frogs or the limbs and tails of mammals. The nerve supply might be inadequate, for when the number of nerves is artificially increased, regeneration is sometimes induced. This cannot be the whole answer, however, because not all appendages depend on nerves for their regeneration; newt jaws, salamander gills, and deer antlers do not require nerves to regenerate. Possibly the failure to regenerate relates to the ways in which wounds heal. In higher vertebrates there is a tendency to form thick scar tissue in healing wounds, which may act as a barrier between the epidermis and the underlying tissues of the stump. In the absence of direct contact between these two tissues, the stump may not be able to give rise to the blastema cells required for regeneration.

6.7 Summary

In this unit we summarize that regeneration is the natural process of replacing or restoring damaged or missing cells, tissues, organs, and even entire body parts to full function in plants and animals. Scientists are studying regeneration for its potential uses in medicine, such as treating a variety of injuries and

diseases. Researchers also hope to learn more about the human aging process through studies of regeneration. This rapidly advancing field is called regenerative medicine.

All living organisms have some ability to regenerate as part of natural processes to maintain tissues and organs. Some animals have extensive regenerative abilities. For example, the tiny freshwater animal called Hydra can form two whole bodies after being cut in half. The axolotl, or Mexican salamander, is an animal with a backbone that can regenerate the form and function of almost any limb, organ, or other body part. More complex animals such as mammals have limited regenerative capacities. These include: Forming thick scars in tissues and skin to promote the healing of injured or amputated body parts, Regrowing hair and skin and Healing a bone fracture by using new tissue to knit the bone pieces together.

Organisms regenerate in different ways. Plants and some sea creatures, such as jellyfish, can replace missing parts by extensively remodeling their remaining tissues. Some animals such as lobsters, catfish, and lizards replace missing parts by first growing a blastema. The blastema cells rapidly divide to form the skin, scales, muscle, bone, or cartilage needed for creating the lost limb, fin, or tail. In other animals, including humans, organs such as the liver undergo what's called compensatory hypertrophy. When part of the liver is removed or destroyed, the remaining portion grows to the original size and allows the liver to function as it did before. Our kidneys, pancreas, thyroid, adrenal glands, and lungs compensate for organ loss in a similar, but more limited, way.

6.8 Terminal questions

Q.1 What do you mean by regeneration?

Answer:----------

Q. 2 Describe morphogenetic process of regeneration.

Answer:----------

Q. 3 Write a short note on ability of regeneration in different group of animal.

Answer:----------

Q.4 What do you mean by limb generation?

Answer:----------

Q.5 Describe limb generation in amphibians.

Answer:----------

Further readings

1. Vertebrate Endocrinology- David O. Norris
2. Invertebrate Zoology –Robert W. Hegner
3. Textbook of Biotechnology –B. D. Singh
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Unit-7 - Growth and Ageing

Structure

7.1 Introduction

7.2 Growth and development

Objectives

7.3 Ageing

7.4 Degrowth

7.5 Cell death

- 7.5.1 Programmed cell death
- 7.5.2 Apoptosis
- 7.5.3 Autophagy
- 7.5.4 Other variations of PCD
- 7.5.5 Necrotic cell death
- 7.5.6 Mechanism of growth

7.6 Mechanisms of cell growth

- 7.6.1 Cell growth regulation in animals
- 7.6.2 Cell populations
- 7.6.3 Cell size
- 7.6.4 Yeast cell size regulation
- 7.6.5 Linking Cdr2 to Wee1
- 7.6.6 Cell polarity factors
- 7.6.7 Other experimental systems for the study of cell size regulation
- 7.6.8 Cell division
- 7.6.9 Comparison of the three types of cell division

7.7 Sexual reproduction

7.8 Disorders

7.9 Measurement methods

7.10 Summary

7.11 Terminal questions

Further readings

7.1 Introduction

Growth, the increases in cell size and number that take place during the life history of an organism. It is seldom random. Rather, it occurs according to a plan that eventually determines the size and shape of the individual. Growth may be restricted to special regions of the organism, such as the layers of cells that divide and increase in size near the tip of the plant shoot. Or the cells engaged in growth may be widely distributed throughout the body of the organism, as in the human embryo. In the latter case, the rates of cell division and of the increase in cell size differ in different parts. That the pattern of growth is predetermined and regular in plants and animals can be seen in the forms of adults. In some organisms, however, notably the slime molds, no regular pattern of growth occurs, and a formless cytoplasmic mass is the result.

The rate of growth of various components of an organism may have important consequences in its ability to adapt to the environment and hence may play a role in evolution. For instance, an increase in the rate of growth of fleshy parts of the fish fin would provide an opportunity for the fish to adapt more easily to terrestrial locomotory life than could a fish without this modified fin. Without disproportionate growth of the fin-ultimately resulting from random changes in the genetic material (mutations); the evolution of limbs through natural selection might have been impossible.

7.2 Growth and development

Growth and development of the normal healthy breast is dependent on the sex steroid hormones, specifically estrogen and progesterone. This relationship is initially most evident in a woman's life during puberty, when undeveloped and quiescent breast tissue is exposed to increasing amounts of estrogen and progesterone, ultimately resulting in the development of the adult breast. Simply put, exposure to these hormones causes growth and development of the breast tissue. Throughout premenopausal life, during a normal menstrual cycling, physiological changes occur within the breast tissue in response to varying levels of the steroid hormones. Perhaps the most dramatic evidence for this change is during pregnancy, when the breast responds remarkably to the changing hormone milieu with breast enlargement, engorgement, lactation, and involution after breast-feeding has stopped. Finally, withdrawal of estrogen at menopause, either naturally or surgically, results in breast changes that are clinically measurable.

Biological development encompasses, therefore, all the processes concerned with implementing the instructions contained in the DNA. Those instructions can only be carried out by an appropriate executive machinery, the first phase of which is provided by the cell that carries the DNA into the next generation: in animals and plants by the fertilized egg cell; in viruses by the cell infected. In life histories that have more than a minimal degree of complexity, the

executive machinery itself becomes modified as the genetic instructions are gradually put into operation, and new mechanisms of protein synthesis are brought into functional condition. The fundamental problem of developmental biology is to understand the interplay between the genetic instructions and the mechanisms by which those instructions are carried out.

Objectives

This is the first unit on developmental biology. Under this unit, we have following objectives. These are as under:

- To know about concept of ageing and degrowth
- To discuss about programmed cell death, apoptosis and cell death
- To know about mechanism of growth and cell growth
- To know about sexual reproduction

Development and growth of bone are critically dependent on thyroid hormone, as evidenced by the typical short stature of adults when neonatal hypothyroidism remains untreated. The cell types that are involved in bone formation (osteoblasts) and bone resorption (osteoclasts) are both stimulated by T_3, with effects on several critical enzymes. In hyperthyroid patients, increased bone formation and resorption result in a net loss of bone thickness and increase in porosity, leading to greater risk of fractures. Little is known about the primary targets and mechanism of action of T_3 in bone cells, mainly because these cells are difficult to study in culture. Thyroid hormone is required for the effects of GH on bone growth and development, which involve the production of insulin-like growth factor 1. Some of the effects of T_3 may therefore result from this permissive action of thyroid hormone. However, studies using TR knockout mice indicate that many of the effects of T_3 on bone metabolism are nuclear mediated and independent of GH.

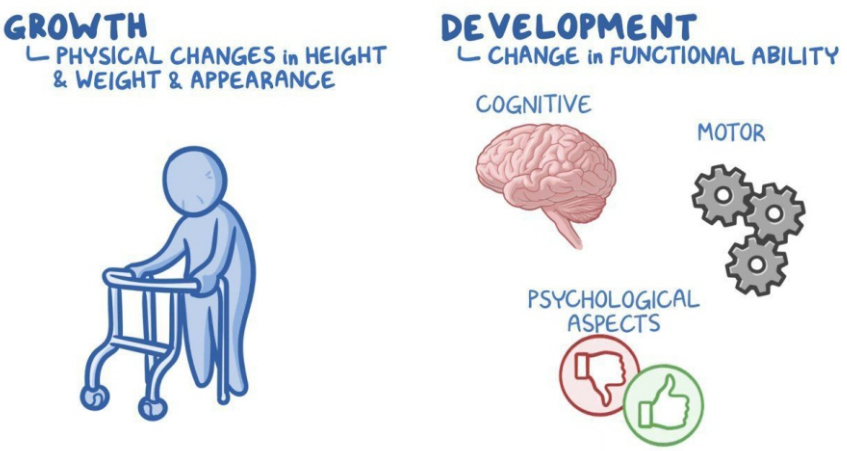

Fig. 1 Growth and development

The environment with all its potential toxins and pollutants may have a direct influence on growth and development, interfering with both physiologic and pathologic aspects. For the two past decades, we have known that programmed

cell death (or apoptosis) is part of normal development and that triggering factors result from both evolutionary pathways and external influences. This occurs at both anatomic and functional levels and opens the potential for many and varied investigations. Neurogenesis, particularly in specific parts of the brain such as the hippocampus, has long been thought to occur only during development, but recent evidence has shown that it continues throughout human life and both genetics and inflammation may hold the keys to neurodegenerative disorders. Implications of an interaction between memory and its influence on degenerative neurologic diseases are now firmly established. Anesthetic drugs have been shown to adversely affect the developing brain in rodents and primates, but with the discovery of neurogenesis in adults, we now know that similar changes occur in adults as well. However, recent evidence suggests it is quite unlikely that anesthetics affect the neurobehavioral changes in human infants and toddlers.

Evidence has confirmed that the functional aspect of brain development in the fetus is far more complex than previously thought and is not limited just to the last weeks before birth. A waking-like brain state exists that is present earlier in gestation and inducible with maternal behavioural changes and external stimuli (e.g., music). These factors may have long-term neuro developmental effects and the beginning of memorization likely occurs earlier than previously thought. A mother's voice, heartbeat, speech, and language all impact the fetal neural plasticity.

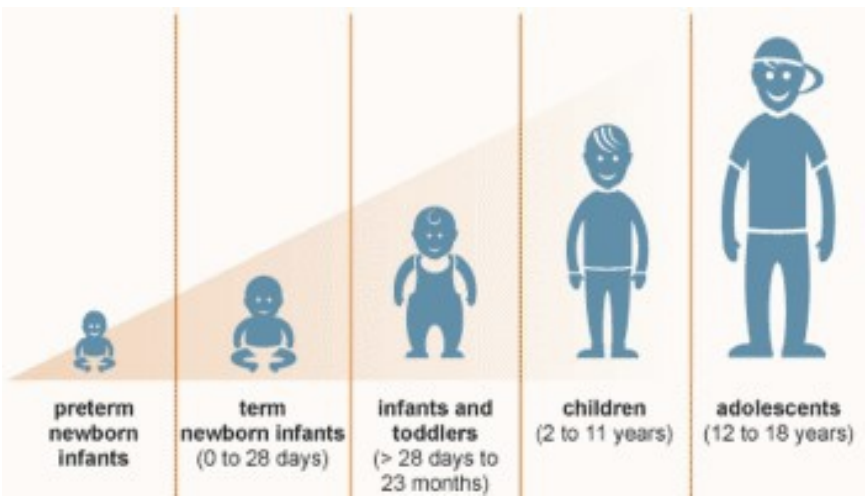

Fig. 2 Human Growth and Development

In addition, a large number of interactions between the neonate and the external world exist. The child has some direct influences on his or her external environment. The ethnologic concept of “baby schema” was proposed by Lorenz, who suggested that some type of positive features can result in a positive response in the human. Infantile physical features, such as a round face and big eyes, are perceived as cute and motivate care taking behavior in the

human, with the evolutionary function of enhancing offspring survival. This has been observed across animal species. A baby's smile, for example, can increase the speed of the caring response and narrow the intentional focus of the mother independently of face recognition. Comparisons between neurophysiologic and neuropsychological studies are difficult but may prove an important confounding factor in our search to understand the complexities of human growth and development.

As medicine has progressed over the past several decades, so has the development of hormonal medications. Tremendous advancements have been achieved using these medications in the areas of pregnancy prevention and relief of vasomotor symptoms related to estrogen withdrawal. Hormonal contraception and hormone replacement are the two most common medications prescribed today in the United States.

S.N	Growth	Development
1	Can be measured quantitatively e.g. change in height, weight	Multiple changes in behaviour, efficiency and capability
2	Limited to a definite age	Life long process
3	Part of Development	Includes physical, mental, social & emotional Growth
4	Linked to Food and Age	Linked to Physical activity, Education, social interaction
5	Related to one aspect of Personality	Related to all aspects of Personality

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FENIX RECORDER

Fig. 3 Difference between growth and development

Unfortunately, the most common cancer in U.S. women, affecting 1 in 8 women, is breast cancer. The incidence of breast cancer has risen over the past two decades, and there is mounting concern over the connection between exogenous hormone exposure and the development of breast cancer. This concern is not unfounded given that it stands to reason that the exposure of breast tissue to exogenous steroid hormones might affect breast physiology, including growth and development.

7.3 Ageing

Ageing or aging is the process of becoming older. The term refers mainly to humans, many other animals, and fungi, whereas for example, bacteria, perennial plants and some simple animals are potentially biologically immortal. Furthermore, ageing connotes a biological and social construct. It is usually associated with dynamic changes in the

biological, psychological, physiological, environmental, behavioral and social processes. In the broader sense, ageing can refer to single cells within an organism which have ceased dividing or to the population of a species.

In humans, ageing represents the accumulation of changes in a human being over time and can encompass physical, psychological, and social changes. Reaction time, for example, may slow with age, while memories and general knowledge typically increase. Ageing increases the risk of human diseases such as cancer, Alzheimer's disease, diabetes, Cardiovascular disease, stroke and many more. Of the roughly 150,000 people who die each day across the globe, about two-thirds die from age-related causes.

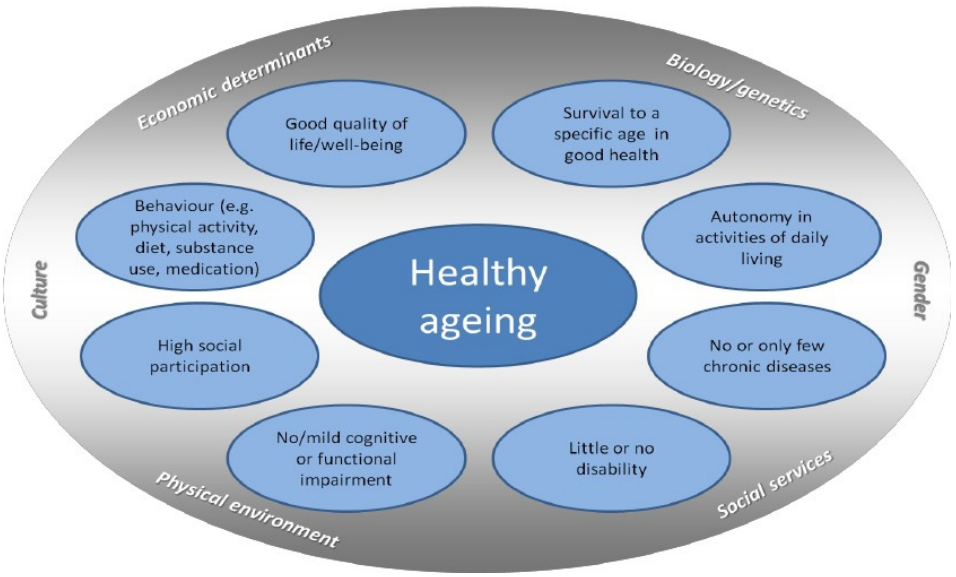

Fig. 4 Components of healthy ageing

Current ageing theories are assigned to the damage concept, whereby the accumulation of damage (such as DNA oxidation) may cause biological systems to fail, or to the programmed ageing concept, whereby problems with the internal processes (epigenetic maintenance such as DNA methylation) may cause ageing. Programmed ageing should not be confused with programmed cell death (apoptosis). Additionally, there can be other reasons, which can speed up the rate of ageing in organisms including human beings like obesity and compromised immune system.

Scientists have long known that dietary calorie restriction in non-primate animals slows ageing while maintaining good health and body functions. Rats and mice fed 30 to 50% fewer calories than they would freely eat beginning in early life shows various physiological health benefits, lower incidence of chronic diseases, and up to 50% increase in length of life. Though life-extending effects remain uncertain in primates (including humans), the diverse health benefits of limiting calorie intake are now well-established. They seem to result from a physiological response to food scarcity that evolved to enhance the body's capacity to survive adversity. Nevertheless, few people would be willing to maintain a substantially reduced diet for most of their lifespan. As a

result, scientists have begun to explore calorie-restriction mimetics-natural and synthetic drug compounds that might yield the same health effects as calorie restriction, without dieting. These investigations are still in their early stages.

Biologically, ageing results from the impact of the accumulation of a wide range of molecular and cellular damage over time. Thus, this leads to a gradual decline in physical and mental capacity, a growing risk of diseases, and ultimately, death. These changes are usually consistent, and they are associated with a person's age in years. While some people aged 70 years may be strong and enjoy good health, others who are 70 years may be weak and require others to help them.

7.4 Degrowth

Degrowth is a term used for both a political, economic, and social movement as well as a set of theories that critiques the paradigm of economic growth. It is based on ideas from a diverse range of lines of thought such as political ecology, ecological economics, feminist political ecology, and environmental justice, pointing out the social and ecological harm caused by the pursuit of infinite growth and Western "development" imperatives. Degrowth emphasizes the need to reduce global consumption and production (social metabolism) and advocates a socially just and ecologically sustainable society with social and environmental well-being replacing GDP as the indicator of prosperity. Hence, although GDP is likely to shrink in a "Degrowth society", i.e. a society in which the objectives of the degrowth movement are achieved, this is not the primary objective of degrowth.

Degrowth thought is in opposition to all forms of productivism (the belief that economic productivity and growth is the purpose of human organization). It is, thus, opposed to the current form of sustainable development. While the concern for sustainability does not contradict degrowth, sustainable development is rooted in mainstream development ideas that aim to increase capitalist growth and consumption. Degrowth therefore sees sustainable development as an oxymoron, as any development based on growth in a finite and environmentally stressed world is seen as inherently unsustainable. Critics of degrowth argue that a slowing of economic growth would result in increased unemployment, increased poverty, and decreased income per capita.

Many who understand the devastating environmental consequences of growth still advocate for economic growth in the South, even if not in the North. But, a slowing of economic growth would fail to deliver the benefits of degrowth-self-sufficiency, material responsibility—and would indeed lead to decreased employment. Rather, degrowth proponents advocate the complete abandonment of the current (growth) economic model, suggesting that

relocalizing and abandoning the global economy in the Global South would allow people of the South to become more self-sufficient and would end the overconsumption and exploitation of Southern resources by the North. Proponents of degrowth see it as a possible path to preserve ecosystems from human pressures. The environment is communally cared for, integrating humans and nature; degrowth implies the perception of ecosystems as inherently valuable, not just as a source for resources.

7.5 Cell death

Cell death is the event of a biological cell ceasing to carry out its functions. This may be the result of the natural process of old cells dying and being replaced by new ones, or may result from such factors as disease, localized injury, or the death of the organism of which the cells are part. Apoptosis or Type I cell-death, and autophagy or Type II cell-death are both forms of programmed cell death, while necrosis is a non-physiological process that occurs as a result of infection or injury.

7.5.1 Programmed cell death

Programmed cell death (or PCD) is cell death mediated by an intracellular program. PCD is carried out in a regulated process, which usually confers advantage during an organism's life-cycle. For example, the differentiation of fingers and toes in a developing human embryo occurs because cells between the fingers apoptose; the result is that the digits separate. PCD serves fundamental functions during both plant and metazoa (multicellular animals) tissue development.

7.5.2 Apoptosis

Apoptosis is the process of programmed cell death (PCD) that may occur in multicellular organisms. Biochemical events lead to characteristic cell changes (morphology) and death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. It is now thought that – in a developmental context – cells are induced to positively commit suicide whilst in a homeostatic context; the absence of certain survival factors may provide the impetus for suicide. There appears to be some variation in the morphology and indeed the biochemistry of these suicide pathways; some treading the path of "apoptosis", others following a more generalized pathway to deletion, but both usually being genetically and synthetically motivated. There is some evidence that certain symptoms of "apoptosis" such as endonuclease activation can be spuriously induced without engaging a genetic cascade, however, presumably true apoptosis and programmed cell death must be genetically mediated. It is also becoming clear that mitosis and apoptosis are toggled or

linked in some way and that the balance achieved depends on signals received from appropriate growth or survival factors.

7.5.3 Autophagy

Autophagy is cytoplasmic, characterized by the formation of large vacuoles that eat away organelles in a specific sequence prior to the destruction of the nucleus. Macroautophagy, often referred to as autophagy, is a catabolic process that results in the autophagosomic-lysosomal degradation of bulk cytoplasmic contents, abnormal protein aggregates, and excess or damaged organelles. Autophagy is generally activated by conditions of nutrient deprivation but has also been associated with physiological as well as pathological processes such as development, differentiation, neurodegenerative diseases, stress, infection and cancer.

7.5.4 Other variations of PCD

Other pathways of programmed cell death have been discovered. Called "non-apoptotic programmed cell-death (or "caspase-independent programmed cell-death"), these alternative routes to death are as efficient as apoptosis and can function as either backup mechanisms or the main type of PCD. Some such forms of programmed cell death are anoikis, almost identical to apoptosis except in its induction; cornification, a form of cell death exclusive to the eyes; excitotoxicity; ferroptosis, an iron-dependent form of cell death and Wallerian degeneration. Plant cells undergo particular processes of PCD similar to autophagic cell death. However, some common features of PCD are highly conserved in both plants and metazoa.

Activation-induced cell death (AICD) is a programmed cell death caused by the interaction of Fas receptor (Fas, CD95)and Fas ligand (FasL, CD95 ligand). It occurs as a result of repeated stimulation of specific T-cell receptors (TCR) and it helps to maintain the periphery immune tolerance. Therefore, an alteration of the process may lead to autoimmune diseases. In the other words AICD is the negative regulator of activated T-lymphocytes.

Ischemic cell death, or oncosis, is a form of accidental or passive cell death that is often considered a lethal injury. The process is characterized by mitochondrial swelling, cytoplasm vacuolization, and swelling of the nucleus and cytoplasm. Mitotic catastrophe is a mode of cell death that is due to premature or inappropriate entry of cells into mitosis. It is the most common mode of cell death in cancer cells exposed to ionizing radiation and many other anti-cancer treatments. Immunogenic cell death or immunogenic apoptosis is a form of cell death caused by some cytostatic agents such as anthracyclines, oxaliplatin and bortezomib,

or radiotherapy and photodynamic therapy (PDT). Pyroptosis is a highly inflammatory form of programmed cell death that occurs most frequently upon infection with intracellular pathogens and is likely to form part of the antimicrobial response in myeloid cells.

7.5.5 Necrotic cell death

Necrosis is cell death where a cell has been badly damaged through external forces such as trauma or infection and occurs in several different forms. In necrosis, a cell undergoes swelling, followed by uncontrolled rupture of the cell membrane with cell contents being expelled. These cell contents often then go on to cause inflammation in nearby cells. A form of programmed necrosis, called necroptosis, has been recognized as an alternative form of programmed cell death. It is hypothesized that necroptosis can serve as a cell-death backup to apoptosis when the apoptosis signaling is blocked by endogenous or exogenous factors such as viruses or mutations. Necroptotic pathways are associated with death receptors such as the tumor necrosis factor receptor.

7.5.6 Mechanism of growth

Mechanistic research has been extremely successful in identifying the parts, operations, and basic organization of a vast range of biological mechanisms. It has been less successful, though, in understanding the implications of various forms of organization — especially the temporal dynamics that orchestrate the functioning of biological mechanisms. Biochemists' basic strategy has been to put together linear sequences of reactions, moving to cyclic organization (e.g., the Krebs cycle) only as necessary. By focusing on near-equilibrium steady-state conditions and summary statistics (e.g., mean concentration of a metabolite), traditionally biologists have screened themselves off from oscillatory phenomena. We have suggested that the resulting mechanistic accounts are blind to crucial dynamics of the systems they target. The new mechanistic philosophy of science has tended to parallel biology in this respect, emphasizing the discovery of component parts and operations and simple organizational schemes, and providing little systematic attention to orchestration.

Although explanation in biology remains primarily mechanistic, small clusters of investigators have confronted the complex dynamics that serve to orchestrate the functioning of biological mechanisms. We have discussed a few of these endeavors, focusing especially on explorations of the oscillatory dynamics that can result from some common types of cyclic organization. All of the cases we discussed were grounded in accounts of parts, operations, and organization of a particular biological mechanism but added concepts and tools of mathematical modeling and dynamical systems. Hence, they well exemplify the project of dynamic mechanistic explanation that we have endorsed.

Dynamic mechanistic explanation stands in contrast not only to purely mechanistic explanation but also to theoretical inquiries that emphasize complex dynamics in living systems conceived abstractly — at best neglecting but in some cases explicitly rejecting the mechanistic project. Artificial life research, for example, is conducted on a plane removed from research on actual biological mechanisms. While accounts oriented purely to complexity or dynamics can make unique and valuable contributions, they provide no understanding of how the dynamic relations are actually realized in living systems if they do not get anchored to component parts and operations of actual mechanisms.

Some theoretical biologists have not only preferred to work on an abstract plane, but also aspired to achieve a unified, law-based theoretical framework. In the spirit of cybernetics and general systems theory, they direct themselves to the big picture that seems to be neglected in reductionistic inquiry. Again, this endeavor has produced some ingenious and valuable directions for further inquiry, but does not in itself achieve the integration we regard as crucial. The most promising contributions for near-term integration probably come not from comprehensive systems, but from particular proposed principles of organization: self-organization through positive feedback in non-equilibrium conditions, small-world organization, scale-free networks, and so forth.

A characteristic feature of modern biology is its particularity. Biochemical pathways, while showing common patterns across phyla, also reveal substantial differences that matter to the functioning of particular organisms. The same is true of circadian oscillators. The resulting extrapolation from studied models to other systems is very different from the generalization achieved by universal quantifiers in laws. Researchers do not know in advance which features change and which remain constant (or nearly so) when extrapolating, and must be prepared to modify specific parts and operations in mechanisms as they move to new instances. The same is likely to apply to the tools of complex systems analysis. That is, the general understanding of how small-worlds phenomena emerge from a few long-range connections in networks primarily constituted of short-range connections will need to be adapted given the particular long-range connections found in a given system. Complex systems analyses provide a rich toolkit for appreciating organization and orchestration of operations in biological mechanisms, and invoking these tools can illuminate how these mechanisms generate the rich phenomena biology seeks to explain.

Thus, we see an immediate future in which dynamic mechanistic researchers in biology will continue to offer piecemeal, context-specific accounts, even as they stretch them to incorporate dynamics. Systems biologists and philosophers of science can, and should, add insight and perspective while remaining grounded by examining a spectrum of these accounts. Such generalizations as we extract will be works in progress, with frequently modified contextual and

other limitations, and will not readily be systematized — including those regarding cycles, oscillations and autonomy.

7.6 Mechanisms of cell growth

Cells can grow by increasing the overall rate of cellular biosynthesis such that production of biomolecules exceeds the overall rate of cellular degradation of biomolecules via the proteasome, lysosome or autophagy. Biosynthesis of biomolecules is initiated by expression of genes which encode RNAs and/or proteins, including enzymes that catalyse synthesis of lipids and carbohydrates.

Individual genes are generally expressed via transcription into messenger RNA (mRNA) and translation into proteins, and the expression of each gene occurs to various different levels in a cell-type specific fashion (in response to gene regulatory networks).

- To drive cell growth, the global rate of gene expression can be increased by enhancing the overall rate of transcription by RNA polymerase II (for active genes) or the overall rate of mRNA translation into protein by increasing the abundance of ribosomes and tRNA, whose biogenesis depends on RNA polymerase I and RNA polymerase III. The Myc transcription factor is an example of a regulatory protein that can induce the overall activity of RNA polymerase I, RNA polymerase II and RNA polymerase III to drive global transcription and translation and thereby cell growth. In addition, the activity of individual ribosomes can be increased to boost the global efficiency of mRNA translation via regulation of translation initiation factors, including the 'translational elongation initiation factor 4E' (eIF4E) complex, which binds to and caps the 5' end of mRNAs. The protein TOR, part of the TORC1 complex, is an important upstream regulator of translation initiation as well as ribosome biogenesis. TOR is a serine/threonine kinase that can directly phosphorylate and inactivate a general inhibitor of eIF4E, named 4E-binding protein (4E-BP), to promote translation efficiency. TOR also directly phosphorylates and activates the ribosomal protein S6-kinase (S6K), which promotes ribosome biogenesis.
- To inhibit cell growth, the global rate of gene expression can be decreased or the global rate of biomolecular degradation can be increased by increasing the rate of autophagy. TOR normally directly inhibits the function of the autophagy inducing kinase Atg1/ULK1. Thus, reducing TOR activity both reduces the global rate of translation and increases the extent of autophagy to reduce cell growth.

7.6.1 Cell growth regulation in animals

Many of the signal molecules that control of cellular growth are called growth factors, many of which induce signal transduction via the PI3K/AKT/mTOR pathway, which includes upstream lipid kinase PI3K and the downstream serine/threonine protein kinase Akt, which is able to activate another protein kinase TOR, which promotes translation and inhibits autophagy to drive cell growth. Nutrient availability influences production of growth factors of the Insulin/IGF-1 family, which circulate as hormones in animals to activate the PI3K/AKT/mTOR pathway in cells to promote TOR activity so that when animals are well fed they will grow rapidly and when they are not able to receive sufficient nutrients they will reduce their growth rate. In addition, the availability of amino acids to individual cells also directly promotes TOR activity, although this mode of regulation is more important in single-celled organisms than in multicellular organisms such as animals that always maintain an abundance of amino acids in circulation.

A postulated model for mammalian size control situates mass as the driving force of the cell cycle. A cell is unable to grow to an abnormally large size because at a certain cell size or cell mass, the S phase is initiated. The S phase starts the sequence of events leading to mitosis and cytokinesis. A cell is unable to get too small because the later cell cycle events, such as S, G2, and M, are delayed until mass increases sufficiently to begin S phase.

7.6.2 Cell populations

Cell populations go through a particular type of exponential growth called doubling or cell proliferation. Thus, each generation of cells should be twice as numerous as the previous generation. However, the number of generations only gives a maximum figure as not all cells survive in each generation. Cells can reproduce in the stage of Mitosis, where they double and split into two genetically equal cells.

7.6.3 Cell size

Cell size is highly variable among organisms, with some algae such as Caulerpa taxifolia being a single cell several meters in length.[8] Plant cells are much larger than animal cells, and protists such as Paramecium can be 330 μm long, while a typical human cell might be 10 μm. How these cells "decide" how big they should be before dividing is an open question. Chemical gradients are known to be partly responsible, and it is hypothesized that mechanical stress detection by cytoskeletal structures is involved. Work on the topic generally requires an organism whose cell cycle is well-characterized.

7.6.4 Yeast cell size regulation

The relationship between cell size and cell division has been extensively studied in yeast. For some cells, there is a mechanism by which cell division is not initiated until a cell has reached a certain size. If the nutrient supply is restricted (after time t = 2 in the diagram, below), and the rate of increase in cell size is slowed, the time period between cell divisions is increased. Yeast cell-size mutants were isolated that begin cell division before reaching a normal/regular size.

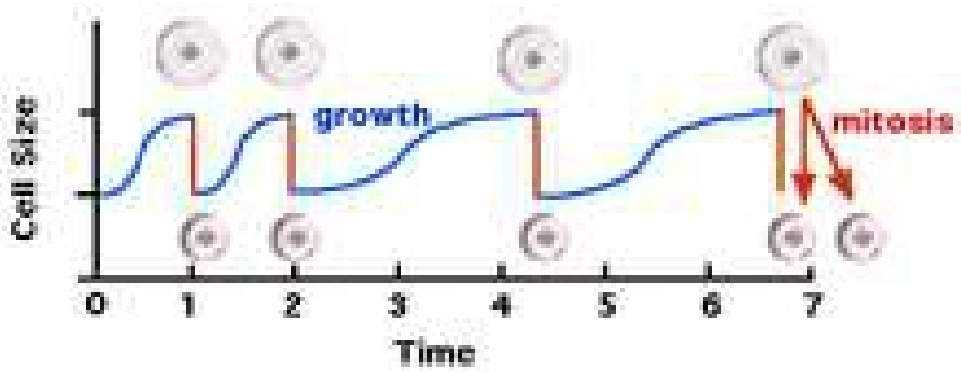

Fig. 5 Cell cycle and growth

Wee1 protein is a tyrosine kinase that normally phosphorylates the Cdc2 cell cycle regulatory protein (the homolog of CDK1 in humans), a cyclin-dependent kinase, on a tyrosine residue. Cdc2 drives entry into mitosis by phosphorylating a wide range of targets. This covalent modification of the molecular structure of Cdc2 inhibits the enzymatic activity of Cdc2 and prevents cell division. Wee1 acts to keep Cdc2 inactive during early G2 when cells are still small. When cells have reached sufficient size during G2, the phosphatase Cdc25 removes the inhibitory phosphorylation, and thus activates Cdc2 to allow mitotic entry. A balance of Wee1 and Cdc25 activity with changes in cell size is coordinated by the mitotic entry control system. It has been shown in Wee1 mutants, cells with weakened Wee1 activity, that Cdc2 becomes active when the cell is smaller. Thus, mitosis occurs before the yeast reach their normal size. This suggests that cell division may be regulated in part by dilution of Wee1 protein in cells as they grow larger.

7.6.5 Linking Cdr2 to Wee1

The protein kinase Cdr2 (which negatively regulates Wee1) and the Cdr2-related kinase Cdr1 (which directly phosphorylates and inhibits Wee1 *in vitro*) are localized to a band of cortical nodes in the middle of interphase cells. After entry into mitosis, cytokinesis factors such as myosin II are recruited to similar nodes; these nodes eventually condense to form the cytokinetic ring. A

previously uncharacterized protein, Blt1, was found to colocalize with Cdr2 in the medial interphase nodes. Blt1 knockout cells had increased length at division, which is consistent with a delay in mitotic entry. This finding connects a physical location, a band of cortical nodes, with factors that have been shown to directly regulate mitotic entry, namely Cdr1, Cdr2, and Blt1.

Further experimentation with GFP-tagged proteins and mutant proteins indicates that the medial cortical nodes are formed by the ordered, Cdr2-dependent assembly of multiple interacting proteins during interphase. Cdr2 is at the top of this hierarchy and works upstream of Cdr1 and Blt1. Mitosis is promoted by the negative regulation of Wee1 by Cdr2. It has also been shown that Cdr2 recruits Wee1 to the medial cortical node. The mechanism of this recruitment has yet to be discovered. A Cdr2 kinase mutant, which is able to localize properly despite a loss of function in phosphorylation, disrupts the recruitment of Wee1 to the medial cortex and delays entry into mitosis. Thus, Wee1 localizes with its inhibitory network, which demonstrates that mitosis is controlled through Cdr2-dependent negative regulation of Wee1 at the medial cortical nodes.

7.6.6 Cell polarity factors

Cell polarity factors positioned at the cell tips provide spatial cues to limit Cdr2 distribution to the cell middle. In fission yeast Schizosaccharomyces pombe (*S. Pombe*), cells divide at a defined, reproducible size during mitosis because of the regulated activity of Cdk1.The cell polarity protein kinase Pom1, a member of the dual-specificity tyrosine-phosphorylation regulated kinase (DYRK) family of kinases, localizes to cell ends. In Pom1 knockout cells, Cdr2 was no longer restricted to the cell middle, but was seen diffusely through half of the cell. From this data it becomes apparent that Pom1 provides inhibitory signals that confine Cdr2 to the middle of the cell. It has been further shown that Pom1-dependent signals lead to the phosphorylation of Cdr2. Pom1 knockout cells were also shown to divide at a smaller size than wild-type, which indicates a premature entry into mitosis.

Pom1 forms polar gradients that peak at cell ends, which shows a direct link between size control factors and a specific physical location in the cell. As a cell grows in size, a gradient in Pom1 grows. When cells are small, Pom1 is spread diffusely throughout the cell body. As the cell increases in size, Pom1 concentration decreases in the middle and becomes concentrated at cell ends. Small cells in early G2 which contain sufficient levels of Pom1 in the entirety of the cell have inactive Cdr2 and cannot enter mitosis. It is not until the cells grow into late G2, when Pom1 is confined to the cell ends that Cdr2 in the medial cortical nodes is activated and able to start the inhibition of Wee1. This finding shows how cell size plays a direct role in regulating the start of mitosis. In this model, Pom1 acts as a molecular link between cell growth and mitotic entry through a Cdr2-Cdr1-Wee1-Cdk1 pathway. The Pom1 polar gradient

successfully relays information about cell size and geometry to the Cdk1 regulatory system. Through this gradient, the cell ensures it has reached a defined, sufficient size to enter mitosis.

7.6.7 Other experimental systems for the study of cell size regulation

One common means to produce very large cells is by cell fusion to form syncytia. For example, very long (several inches) skeletal muscle cells are formed by fusion of thousands of myocytes. Genetic studies of the fruit fly Drosophila have revealed several genes that are required for the formation of multinucleated muscle cells by fusion of myoblasts. Some of the key proteins are important for cell adhesion between myocytes and some are involved in adhesion-dependent cell-to-cell signal transduction that allows for a cascade of cell fusion events.

Increases in the size of plant cells are complicated by the fact that almost all plant cells are inside of a solid cell wall. Under the influence of certain plant hormones the cell wall can be remodeled, allowing for increases in cell size that are important for the growth of some plant tissues. Most unicellular organisms are microscopic in size, but there are some giant bacteria and protozoa that are visible to the naked eye. (See Table of cell sizes—Dense populations of a giant sulfur bacterium in Namibian shelf sediments—Large protists of the genus Chaos, closely related to the genus Amoeba.)

In the rod-shaped bacteria *E. coli*, *Caulobacter crescentus* and *B. subtilis* cell size is controlled by a simple mechanisms in which cell division occurs after a constant volume has been added since the previous division. By always growing by the same amount, cells born smaller or larger than average naturally converge to an average size equivalent to the amount added during each generation.

7.6.8 Cell division

Cell reproduction is asexual. For most of the constituents of the cell, growth is a steady, continuous process, interrupted only briefly at M phase when the nucleus and then the cell divide in two. The process of cell division, called cell cycle, has four major parts called phases. The first part, called G_1 phase is marked by synthesis of various enzymes that are required for DNA replication. The second part of the cell cycle is the S phase, where DNA replication produces two identical sets of chromosomes. The third part is the G_2 phase in which a significant protein synthesis occurs, mainly involving the production of microtubules that are required during the process of division, called mitosis. The fourth phase, **M phase**, consists of nuclear division

(karyokinesis) and cytoplasmic division (cytokinesis), accompanied by the formation of a new cell membrane. This is the physical division of "mother" and "daughter" cells. The M phase has been broken down into several distinct phases, sequentially known as prophase, prometaphase, metaphase, anaphase and telophase leading to cytokinesis.

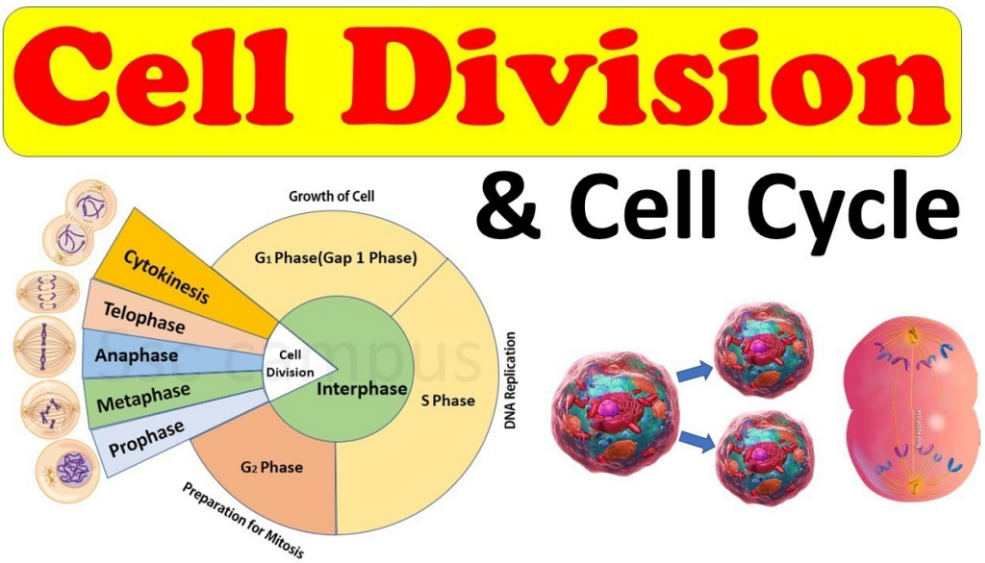

Fig. 6 Phases of the cell cycle

Cell division is more complex in eukaryotes than in other organisms. Prokaryotic cells such as bacterial cells reproduce by binary fission, a process that includes DNA replication, chromosome segregation, and cytokinesis. Eukaryotic cell division either involves mitosis or a more complex process called meiosis. Mitosis and meiosis are sometimes called the two "nuclear division" processes. Binary fission is similar to eukaryote cell reproduction that involves mitosis. Both lead to the production of two daughter cells with the same number of chromosomes as the parental cell. Meiosis is used for a special cell reproduction process of diploid organisms. It produces four special daughter cells (gametes) which have half the normal cellular amount of DNA. A male and a female gamete can then combine to produce a zygote, a cell which again has the normal amount of chromosomes.

7.6.9 Comparison of the three types of cell division

The DNA content of a cell is duplicated at the start of the cell reproduction process. Prior to DNA replication, the DNA content of a cell can be represented as the amount **Z** (the cell has Z chromosomes). After the DNA replication process, the amount of DNA in the cell is **2Z** (multiplication: 2 x Z = 2Z). During Binary fission and mitosis the duplicated DNA content of the reproducing parental cell is separated into two equal halves that are destined to end up in the two daughter cells. The final part of the cell reproduction process

is cell division, when daughter cells physically split apart from a parental cell. During meiosis, there are two cell division steps that together produce the four daughter cells.

After the completion of binary fission or cell reproduction involving mitosis, each daughter cell has the same amount of DNA (**Z**) as what the parental cell had before it replicated its DNA. These two types of cell reproduction produced two daughter cells that have the same number of chromosomes as the parental cell. Chromosomes duplicate prior to cell division when forming new skin cells for reproduction. After meiotic cell reproduction the four daughter cells have half the number of chromosomes that the parental cell originally had. This is the haploid amount of DNA, often symbolized as **N**. Meiosis is used by diploid organisms to produce haploid gametes. In a diploid organism such as the human organism, most cells of the body have the diploid amount of DNA, **2N**. Using this notation for counting chromosomes we say that human somatic cells have 46 chromosomes (2N = 46) while human sperm and eggs have 23 chromosomes (N = 23). Humans have 23 distinct types of chromosomes, the 22 autosomes and special category of sex chromosomes. There are two distinct sex chromosomes, the X chromosome and the Y chromosome. A diploid human cell has 23 chromosomes from that person's father and 23 from the mother. That is, your body has two copies of human chromosome number 2, one from each of your parents.

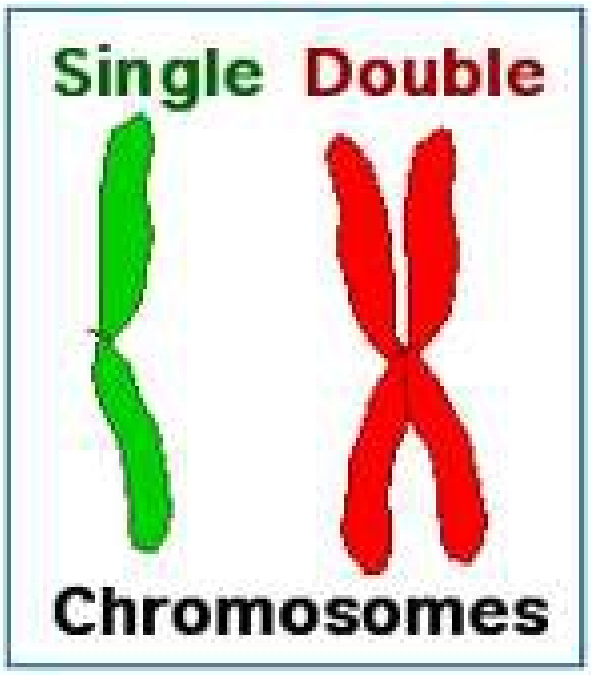

Fig. 7 Chromosomes

Immediately after DNA replication a human cell will have 46 "double chromosomes". In each double chromosome there are two copies of that chromosome's DNA molecule. During mitosis the double chromosomes are split to produce 92 "single chromosomes", half of which go into each daughter

split to produce 92 "single chromosomes", half of which go into each daughter cell. During meiosis, there are two chromosome separation steps which assure that each of the four daughter cells gets one copy of each of the 23 types of chromosome.

7.7 Sexual reproduction

Though cell reproduction that uses mitosis can reproduce eukaryotic cells, eukaryotes bother with the more complicated process of meiosis because sexual reproduction such as meiosis confers a selective advantage. Notice that when meiosis starts, the two copies of sister chromatids number 2 are adjacent to each other. During this time, there can be genetic recombination events. Information from the chromosome 2 DNA gained from one parent (red) will transfer over to the chromosome 2 DNA molecule that was received from the other parent (green). Notice that in mitosis the two copies of chromosome number 2 do not interact. Recombination of genetic information between homologous chromosomes during meiosis is a process for repairing DNA damages. This process can also produce new combinations of genes, some of which may be adaptively beneficial and influence the course of evolution. However, in organisms with more than one set of chromosomes at the main life cycle stage, sex may also provide an advantage because, under random mating, it produces homozygotes and heterozygotes according to the Hardy–Weinberg ratio.

7.8 Disorders

A series of growth disorders can occur at the cellular level and these consequently underpin much of the subsequent course in cancer, in which a group of cells display uncontrolled growth and division beyond the normal limits, *invasion* (intrusion on and destruction of adjacent tissues), and sometimes metastasis (spread to other locations in the body via lymph or blood). Several key determinants of cell growth, like ploidy and the regulation of cellular metabolism, are commonly disrupted in tumors. Therefore, heterogenous cell growth and pleomorphism is one of the earliest hallmarks of cancer progression. Despite the prevalence of pleomorphism in human pathology, its role in disease progression is unclear. In epithelial tissues, pleomorphism in cellular size can induce packing defects and disperse aberrant cells. But the consequence of atypical cell growth in other animal tissues is unknown.

7.9 Measurement methods

The cell growth can be detected by a variety of methods. The **cell size growth** can be visualized by microscopy, using suitable stains. But the **increase of cells number** is usually more significant. It can be measured by

manual counting of cells under microscopy observation, using the dye exclusion method (i.e. trypan blue) to count only viable cells. Less fastidious, scalable, methods include the use of cytometers, while flow cytometry allows combining cell counts ('events') with other specific parameters: fluorescent probes for membranes, cytoplasm or nuclei allow distinguishing dead/viable cells, cell types, cell differentiation, expression of a biomarker such as Ki67.

Beside the increasing number of cells, one can be assessed regarding the **metabolic activity growth**, that is, the CFDA and calcein-AM measure (fluorimetrically) not only the membrane functionality (dye retention), but also the functionality of cytoplasmic enzymes (esterases). The MTT assays (colorimetric) and the resazurin assay (fluorimetric) dose the mitochondrial redox potential. All these assays may correlate well, or not, depending on cell growth conditions and desired aspects (activity, proliferation). The task is even more complicated with populations of different cells, furthermore when combining cell growth interferences or toxicity.

7.10 Summary

Under this unit we summarize the concept of growth, development and ageing. Biological developmental, gradual changes in size, shape, and function during an organism's life that translate its genetic potentials (genotype) into functioning mature systems (phenotype). It includes growth but not repetitive chemical changes (metabolism) or changes over more than one lifetime (evolution). DNA directs the development of a fertilized egg so that cells become specialized structures that carry out specific functions. In humans, development progresses through the embryo and fetus stages before birth and continues during childhood. Other mammals follow a similar course. Amphibians and insects go through distinctive stages that are quite different. In plants, the basic pattern is determined by the arrangement of lateral buds around a central growing stem. Different rates of growth of the plant's component elements then determine its shape and that of various parts. In both animals and plants, growth is greatly influenced by hormones; factors within individual cells probably also play a role.

All organisms, including the very simplest, consist of two components, distinguished by a German biologist, August Weismann, at the end of the 19th century, as the "germ plasm" and the "soma." The germ plasm consists of the essential elements, or genes, passed on from one generation to the next, and the soma consists of the body that may be produced as the organism develops. In more modern terms, Weismann's germ plasm is identified with DNA (deoxyribonucleic acid), which carries, encoded in the complex structure of its molecule, the instructions necessary for the synthesis of the other compounds of the organism and their assembly into the appropriate structures. It is this whole collection of other compounds (proteins, fats,

carbohydrates, and others) and their arrangement as a metabolically functioning organism that constitutes the soma.

7.11Terminal questions

Q.1 What do you mean by concept of growth?

Answer:----------

Q. 2 Describe concept of cell death.

Answer:----------

Q. 3 Write a short note on degrowth.

Answer:----------

Q. 4 What do you mean by ageing?

Answer:----------

Q. 5 Describe mechanism of growth.

Answer:----------

Further readings

1. Vertebrate Endocrinology- David O. Norris
2. Invertebrate Zoology –Robert W. Hegner
3. Textbook of Biotechnology –B. D. Singh
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.

Unit-8 - Growth curve and its interpretation (types of cell growth, ageing)

Structure

8.1 Introduction

Objectives

8.2 Bacterial growth

8.3 Cancer cell growth

8.4 Exponential growth

8.4.1 Examples

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8.7.3 Cell reproduction that involves meiosis

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Further readings

8.1 Introduction

Growth is the irreversible increase of an organism's size over a given period. It may also be defined as one of the characteristics of a living thing. In *biology*, *"biological growth"* is associated with progressive development. An organism's growth may go on throughout its life, or end when that species is fully mature. An example of biological growth is a plant seed developing into a fully mature tree. At a cellular level, *cell growth* means an increase in size or an increase in number. In *medical science*, growth may be associated with pathology (disease). For example, *pathological growth* may indicate a tumor, which is an abnormal mass of cells or tissue. It can be defined as, An increase in the size of an organism or part of an organism, usually as a result of an increase in the number of cells. Growth of an organism may stop at maturity, as in the case of humans and other mammals, or it may continue throughout life, as in many plants. In humans, certain body parts, like hair and nails, continue to grow throughout life.

Living things tend to grow throughout their lifespan. However, it is a process that happens periodically and in stages. Growth usually commences with the presence of a singular cell. This cell may multiply and grow larger in specific regions like in plants or it may diversify and growth will be located in various parts of the organism, as seen in animals. Many factors including environmental and internal ones determine how quickly or how slowly an organism will grow. Centuries of change in these factors have influenced how living things develop and grow to this day.

Objectives

This is the first unit on developmental biology. Under this unit, we have following objectives. These are as under:

- To know about bacterial growth
- To know about cancer cell growth and exponential growth
- To know about types of cell growth
- To discuss about the factors that regulate growth

Growth curve, in biology, a curve in graph form that shows the change in the number of cells (or single-celled organisms) in an experimental culture at different times. Growth curves are also common tools in ecological studies; they are used to track the rise and fall of populations of plants, animals, and other multicellular organisms over time. The classic growth curve, as exemplified by a newly established bacterial colony, is divided into four phases, in order of their appearance:

(1) Lag phase;
(2) Log (logarithmic), or exponential, phase;
(3) Stationary phase; and

(4) Death, or decline, phase.

A growth curve is an empirical model of the evolution of a quantity over time. Growth curves are widely used in biology for quantities such as population size or biomass (in population ecology and demography, for population growth analysis), individual body height or biomass (in physiology, for growth analysis of individuals). Values for the measured property can be plotted on a graph as a function of time; as in given figure for an example.

8.2 Bacterial growth

In the given example, the number of bacteria present in a nutrient-containing broth was measured during the course of an 8-hour cell growth experiment. The observed pattern of bacterial growth is bi-phasic because two different sugars were present, glucose and lactose. The bacteria prefer to consume glucose (Phase I) and only use the lactose (Phase II) after the glucose has been depleted. Analysis of the molecular basis for this bi-phasic growth curve led to the discovery of the basic mechanisms that control gene expression.

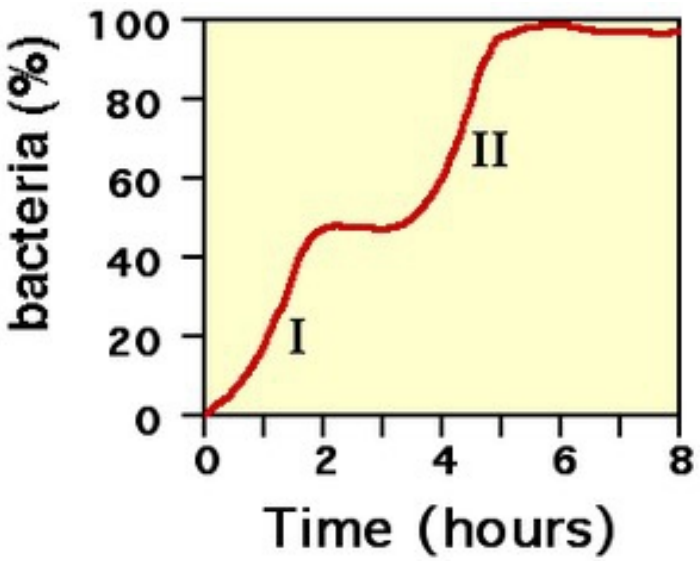

Fig. 1 A bi-phasic bacterial growth curve.

8.3 Cancer cell growth

Cancer research is an area of biology where growth curve analysis plays an important role. In many types of cancer, the rate at which tumors shrink following chemotherapy is related to the rate of tumor growth before treatment. Tumors that grow rapidly are generally more sensitive to the toxic effects that conventional anticancer drugs have on the cancer cells. Many conventional anticancer drugs (for example, 5-Fluorouracil) interfere with DNA replication and can cause the death of cells that attempt to replicate their DNA and divide. A rapidly growing tumor will have more actively dividing cells and more cell death upon exposure to such anticancer drugs.

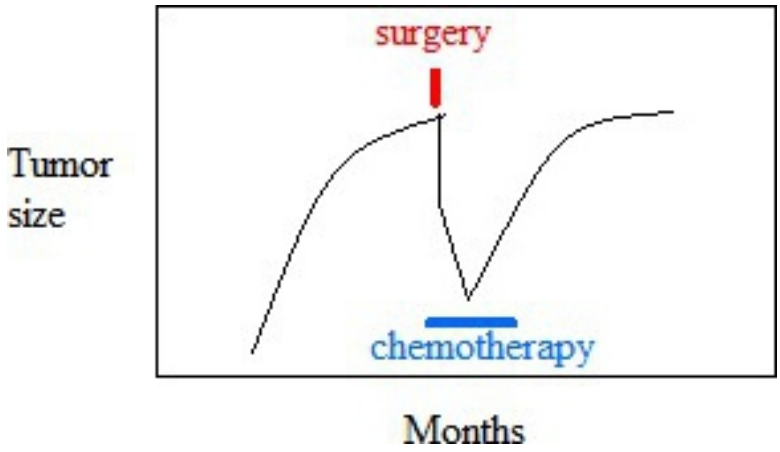

Fig. 2 Tumor growth curve

In the example shown in above Fig. a tumor is found after the cell growth rate has slowed. Most of the cancer cells are removed by surgery. The remaining cancer cells begin to proliferate rapidly and cancer chemotherapy is started. Many tumor cells are killed by the chemotherapy, but eventually some cancer cells that are resistant to the chemotherapy drug begin to grow rapidly. The chemotherapy is no longer useful and is discontinued.

8.4 Exponential growth

Exponential growth is a process that increases quantity over time. It occurs when the instantaneous rate of change (that is, the derivative) of a quantity with respect to time is proportional to the quantity itself. Described as a function, a quantity undergoing exponential growth is an exponential function of time, that is, the variable representing time is the exponent (in contrast to other types of growth, such as quadratic growth). If the constant of proportionality is negative, then the quantity decreases over time, and is said to be undergoing exponential decay instead. In the case of a discrete domain of definition with equal intervals, it is also called geometric growth or geometric decay since the function values form a geometric progression.

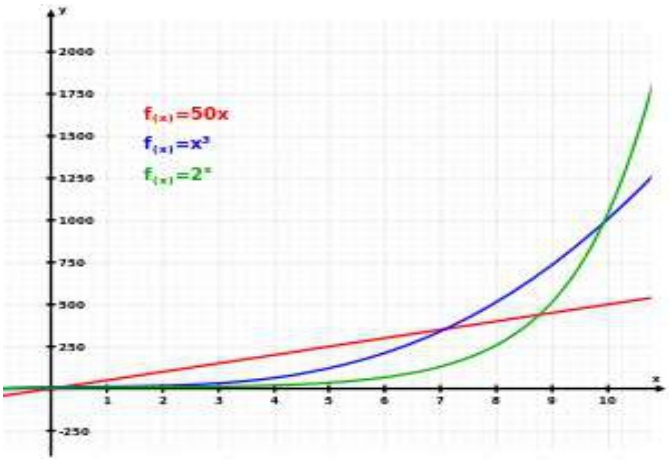

Fig. 3 The graph illustrates how exponential growth (green) surpasses both linear (red) and cubic (blue) growth.

The formula for exponential growth of a variable x at the growth rate r, as time t goes on in discrete intervals (that is, at integer times 0, 1, 2, 3,. ..), Where x_0 is the value of x at time 0. The growth of a bacterial colony is often used to illustrate it. One bacterium splits itself into two, each of which splits itself resulting in four, then eight, 16, 32, and so on. The rate of increase keeps increasing because it is proportional to the ever-increasing number of bacteria. Growth like this is observed in real-life activity or phenomena, such as the spread of virus infection, the growth of debt due to compound interest, and the spread of viral videos. In real cases, initial exponential growth often does not last forever, instead slowing down eventually due to upper limits caused by external factors and turning into logistic growth. Terms like "exponential growth" are sometimes incorrectly interpreted as "rapid growth". Indeed, something that grows exponentially can in fact be growing slowly at first.

8.4.1 Examples

8.4.1.1 In biology

- The number of microorganisms in a culture will increase exponentially until an essential nutrient is exhausted. Typically the first organism splits into two daughter organisms, who then each split to form four, who split to form eight, and so on. Because exponential growth indicates constant growth rate, it is frequently assumed that exponentially growing cells are at a steady-state. However, cells can grow exponentially at a constant rate while remodeling their metabolism and gene expression.
- A virus (for example COVID-19, or smallpox) typically will spread exponentially at first, if no artificial immunization is available. Each infected person can infect multiple new people.

8.4.1.2 In physics

- Avalanche breakdown within a dielectric material. A free electron becomes sufficiently accelerated by an externally applied electrical field that it frees up additional electrons as it collides with atoms or molecules of the dielectric media. These *secondary* electrons also are accelerated, creating larger numbers of free electrons. The resulting exponential growth of electrons and ions may rapidly lead to complete dielectric breakdown of the material.
- Nuclear chain reaction (the concept behind nuclear reactors and nuclear weapons). Each uranium nucleus that undergoes fission produces multiple neutrons, each of which can be absorbed by adjacent uranium atoms, causing them to fission in turn. If the probability of neutron absorption exceeds the probability of neutron escape (a function of the shape and mass of the uranium), the production rate of neutrons and

induced uranium fissions increases exponentially, in an uncontrolled reaction. "Due to the exponential rate of increase, at any point in the chain reaction 99% of the energy will have been released in the last 4.6 generations. It is a reasonable approximation to think of the first 53 generations as a latency period leading up to the actual explosion, which only takes 3–4 generations.

- Positive feedback within the linear range of electrical or electroacoustic amplification can result in the exponential growth of the amplified signal, although resonance effects may favor some component frequencies of the signal over others.

8.4.1.3 In economics

- Economic growth is expressed in percentage terms, implying exponential growth.

8.4.1.4 In finance

- Compound interest at a constant interest rate provides exponential growth of the capital.
- Pyramid schemes or Ponzi schemes also show this type of growth resulting in high profits for a few initial investors and losses among great numbers of investors.

8.4.1.5 In computer science

- Processing power of computers. See also Moore's law and technological singularity. (Under exponential growth, there are no singularities. The singularity here is a metaphor, meant to convey an unimaginable future. The link of this hypothetical concept with exponential growth is most vocally made by futurist Ray Kurzweil.
- In computational complexity theory, computer algorithms of exponential complexity require an exponentially increasing amount of resources (e.g. time, computer memory) for only a constant increase in problem size. So for an algorithm of time complexity 2^x, if a problem of size $x = 10$ requires 10 seconds to complete, and a problem of size $x = 11$ requires 20 seconds, then a problem of size $x = 12$ will require 40 seconds. This kind of algorithm typically becomes unusable at very small problem sizes, often between 30 and 100 items (most computer algorithms need to be able to solve much larger problems, up to tens of thousands or even millions of items in reasonable times, something that would be physically impossible with an exponential algorithm). Also, the effects of Moore's Law do not help the situation much because doubling processor speed merely allows you to increase the problem size by a constant. **e.g.** if a slow processor can

solve problems of size x in time t, then a processor twice as fast could only solve problems of size x + constant in the same time t. So exponentially complex algorithms are most often impractical, and the search for more efficient algorithms is one of the central goals of computer science today.

8.5 Cell growth

Cell growth refers to an increase in the total mass of a cell, including both cytoplasmic, nuclear and organelle volume. Cell growth occurs when the overall rate of cellular biosynthesis (production of biomolecules or anabolism) is greater than the overall rate of cellular degradation (the destruction of biomolecules via the proteasome, lysosome or autophagy, or catabolism). Cell growth is not to be confused with cell division or the cell cycle, which are distinct processes that can occur alongside cell growth during the process of cell proliferation, where a cell, known as the "mother cell", grows and divides to produce two daughter cells. Importantly, cell growth and cell division can also occur independently of one another. During early embryonic development (cleavage of the zygote to form a morula and blastoderm), cell divisions occur repeatedly without cell growth. Conversely, some cells can grow without cell division or without any progression of the cell cycle, such as growth of neurons during axonal path finding in nervous system development.

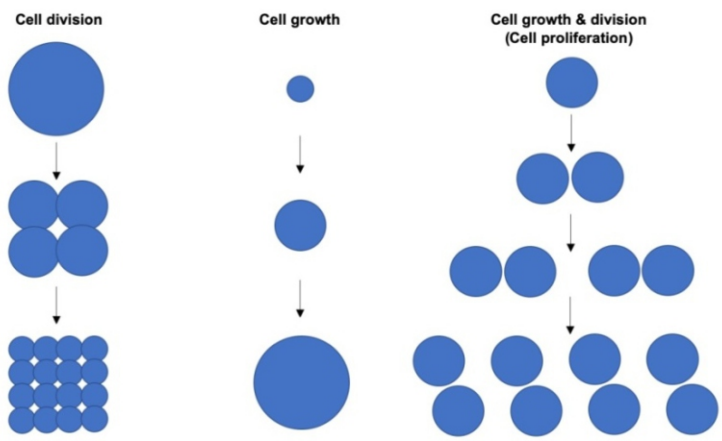

Fig. 4 Cell division, growth & proliferation

In multicellular organisms, tissue growth rarely occurs solely through cell growth without cell division, but most often occurs through cell proliferation. This is because a single cell with only one copy of the genome in the cell nucleus can perform biosynthesis and thus undergo cell growth at only half the rate of two cells. Hence, two cells grow (accumulate mass) at twice the rate of a single cell, and four cells grow at 4-times the rate of a single cell. This principle leads to an exponential increase of tissue growth rate (mass

accumulation) during cell proliferation, owing to the exponential increase in cell number.

Cell size depends on both cell growth and cell division, with a disproportionate increase in the rate of cell growth leading to production of larger cells and a disproportionate increase in the rate of cell division leading to production of many smaller cells. Cell proliferation typically involves balanced cell growth and cell division rates that maintain a roughly constant cell size in the exponentially proliferating population of cells.

Some special cells can grow to very large sizes via an unusual endoreplication cell cycle in which the genome is replicated during S-phase but there is no subsequent mitosis (M-phase) or cell division (cytokinesis). These large endoreplicating cells have many copies of the genome, so are highly polyploid.

Oocytes can be unusually large cells in species for which embryonic development takes place away from the mother's body within an egg that is laid externally. The large size of some eggs can be achieved either by pumping in cytosolic components from adjacent cells through cytoplasmic bridges named ring canals (Drosophila) or by internalisation of nutrient storage granules (yolk granules) by endocytosis (frogs).

8.6 Factors that regulate growth

Growth, like all the characteristics of living things, requires regulation to occur. Numerous different factors permit the process of growth to occur. For instance, if anything in the environment is not at its optimum this will either hinder or excessively speed up the growth of an organism. These physical factors can also give rise to different forms of certain structures. Plants that undergo extreme physical stress such as drought will eventually wither from lack of water. On the other hand, leaves grown in the air have a different physical shape from those grown in water for the buttercup plant. The ideal temperature is also necessary for all living things as most mammals would suffer from hypothermia in conditions that are too cold.

Within organisms, there are also internal factors that can promote or inhibit their growth. This is often what the organism possesses in its gene pool and it can result in either regular or abnormal growth in a living thing. Hormones – *those regulatory stimulants which activate the body into performing certain actions* – determine the speed, size, and physical trait the organism will develop in its system. These can even determine the likelihood of the organism to survive and its lifespan. For instance, the pituitary growth hormone has a massive effect on the speed at which mammals grow. A pig that is injected with the growth hormone will not only grow quicker than the ordinary pig but will also be leaner and require less food.

8.7 Types of cell growth

Three types of cell reproduction are compared: the relatively simple Binary fission and two more complicated types that either involve mitosis or meiosis.

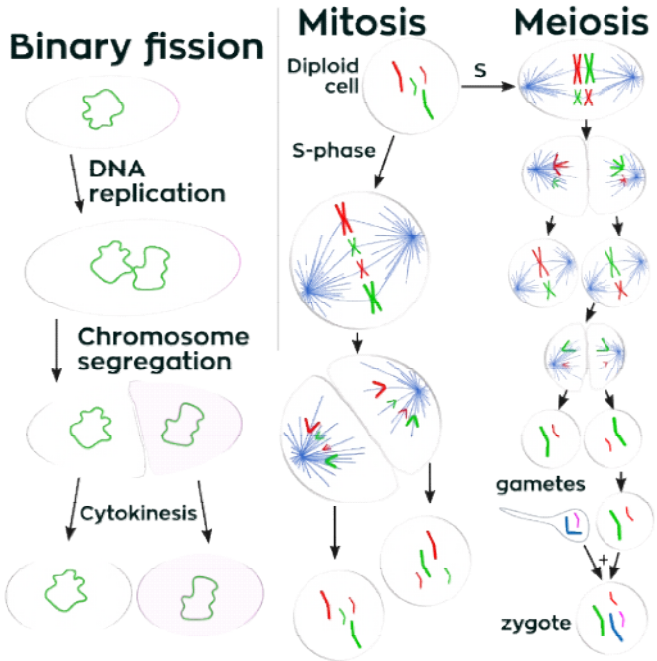

Fig. 5 Types of cell growth

8.7.1 Binary fission

Organisms such as bacteria typically have a single chromosome (green). At the start of the binary fission process, the DNA molecule of the cell's chromosome is replicated, producing two copies of the chromosome. A key aspect of bacterial cell reproduction is making sure that each daughter cell gets a copy of the chromosome. Cytokinesis is the actual physical separation of the two new daughter cells.

8.7.2 Cell reproduction that involves mitosis

Most eukaryotic organisms like humans have more than one chromosome. In order to make sure that a copy of each chromosome gets segregated into each daughter cell, the spindle apparatus is used (blue threads). The chromosomes are moved along the long thin microtubules like trains moving along train tracks. Humans are diploid; we have two copies of each type of chromosome, one from the father (red) and one from the mother (green).

8.7.3 Cell reproduction that involves meiosis

The human sex cells (gametes) are produced by meiosis. For sperm production there are two cytokinesis steps that produce a total of four cells, each with half

the normal number of chromosomes. The situation is different in the ovaries for egg production where one of the four sets of chromosomes that is segregated is placed in a large egg cell, ready to be combined with the DNA from a sperm cell

8.8 Ageing

Ageing or aging is the process of becoming older. The term refers mainly to humans, many other animals, and fungi, whereas for example, bacteria, perennial plants and some simple animals are potentially biologically immortal. Furthermore, ageing connotes a biological and social construct. It is usually associated with dynamic changes in the biological, psychological, physiological, environmental, behavioral and social processes. In the broader sense, ageing can refer to single cells within an organism which have ceased dividing or to the population of a species.

In humans, ageing represents the accumulation of changes in a human being over time and can encompass physical, psychological, and social changes. Reaction time, for example, may slow with age, while memories and general knowledge typically increase. Ageing increases the risk of human diseases such as cancer, Alzheimer's disease, diabetes, Cardiovascular disease, stroke and many more. Of the roughly 150,000 people who die each day across the globe, about two-thirds die from age-related causes.

Current ageing theories are assigned to the damage concept, whereby the accumulation of damage (such as DNA oxidation) may cause biological systems to fail, or to the programmed ageing concept, whereby problems with the internal processes (epigenetic maintenance such as DNA methylation) may cause ageing. Programmed ageing should not be confused with programmed cell death (apoptosis). Additionally, there can be other reasons, which can speed up the rate of ageing in organisms including human beings like obesity and compromised immune system.

Scientists have long known that dietary calorie restriction in non-primate animals slows ageing while maintaining good health and body functions. Rats and mice fed 30 to 50% fewer calories than they would freely eat beginning in early life shows various physiological health benefits, lower incidence of chronic diseases, and up to 50% increase in length of life . Though life-extending effects remain uncertain in primates (including humans), the diverse health benefits of limiting calorie intake are now well-established. They seem to result from a physiological response to food scarcity that evolved to enhance the body's capacity to survive adversity.

Nevertheless, few people would be willing to maintain a substantially reduced diet for most of their lifespan. As a result, scientists have begun to explore calorie-restriction mimetics-natural and synthetic drug compounds that might

yield the same health effects as calorie restriction, without dieting. These investigations are still in their early stages.

Biologically, ageing results from the impact of the accumulation of a wide range of molecular and cellular damage over time. Thus, this leads to a gradual decline in physical and mental capacity, a growing risk of diseases, and ultimately, death. These changes are usually consistent, and they are associated with a person's age in years. While some people aged 70 years may be strong and enjoy good health, others who are 70 years may be weak and require others to help them.

8.9 Summary

Under this unit we have summarized growth, growth curve and ageing. Growth in biology is the increase in size and mass of a living organism over time. Most importantly, it is an irreversible change that occurs in the body of the organism. Generally, growth is a result of two processes: cell division and cell enlargement. Here, the main form of cell division occurs during growth is mitosis, which is responsible for the production of cell offspring with the same genetic makeup. On the other hand, enlargement is the increase in the size of the divided cells by increasing the content inside the cell, mainly the water content in vacuoles. Furthermore, the growth of an organism can occur in two types: determinate growth and indeterminate growth. In determinate growth, the size of a part of the organism or the whole organism increases only up to a certain size. Thereafter, growth stops. In contrast, in indeterminate growth, the size of the organism continuously increases throughout its life. For example, plants show indeterminate growth while organs of the animal body undergo determinate growth. In addition, some may describe growth as the increase in the number of organisms in the population, mainly during ecological studies.

Development in biology is the increase in complexity of an organism. The three main processes that occur during development are growth, morphogenesis, and differentiation. As mentioned before, growth is the increase in size and number. However, morphogenesis is the acquisition of form and structure. It is responsible for developing the shape of the organism. Growth refers to the increases in cell size and number, which takes place during the life history of an organism while development refers to the progressive changes in size, shape, and function during the life of an organism by which its genetic potentials (genotype) are translated into functioning mature systems (phenotype). Thus, this explains the fundamental difference between growth and development in biology. That is; growth is the increase in size and mass over a period of time while development is the transformation of an organism into a more complex form in terms of function and organization. Hence, this is the main difference between growth and development in biology.

8.10 Terminal questions

Q.1 What do you mean by ageing?

Answer:----------

Q. 2Describe growth curve with its interpretation.

Answer:----------

Q.3 Write a short note on cell growth.

Answer:----------

Q.4 Describe type of cell growth.

Answer:----------

Q.5 Draw growth curve.

Answer:----------

Further readings

1. Vertebrate Endocrinology- David O. Norris
2. Invertebrate Zoology –Robert W. Hegner
3. Textbook of Biotechnology –B. D. Singh
4. Textbook of Biochemistry for Undergraduates - Rafi, M.D.
5. Biochemistry and molecular biology- Wilson Walker.