Conceptual Insights in Biology - II

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PREFACE

Biology plays an important role in the understanding of complex forms of life involving humans, animals and plants. Understanding the intricate details of life helps humans understand how to care for themselves, animals and plants in the proper manner. Biology helps individuals understand the interaction between humanity and the world.

Biology is an interesting subject as it includes study of living organisms and life processes and lays foundation of important disciplines like Zoology, Ecology, Bio informatics, Biotechnology, Biomedical Research, Molecular Biology, Genetics, Haematology, Oncology, Aerobiology, etc.

Often students find Biology as a boring subject; however, it is scoring subject if they adopt the systematic learning approach. The students who aim to make career in life sciences and medicine then they should seriously think about studying this subject right from senior secondary level.

Biology manuals in two volumes have been developed by SCERT for teachers teaching biology at the senior secondary level. The first volume consists of modules I, II and III and the second volume has modules IV, V and VI.

All the six modules have been carefully designed to cater to the pedagogic needs of lecturers teaching biology. Special focus is on the topics found difficult by the students of biology and on topics introduced in the recent years by C.B.S.E. Each module consists of topics from class XIth and XIIth. Objective of the modules is to facilitate the teachers in making the content easy and concise for the learners. At places content has been enriched for the teachers to satisfy their appetite to know more about a topic.

Each module is based on learner-centred objectives, brief introduction to the module, key words related to the module, content outline of the module, related activities, glossary of important words and references for further reading. Images are added wherever needed for reference. Glossary will be beneficial as ready-reckoner to find definitions of important words.

Module1 is on Human Life Processes and Diseases which deals with Digestion, Breathing, Excretion and Human Reproduction.

Module 2 is on Plant Diversity and Reproduction dealing with Plant Kingdom, Morphology and Anatomy of Flowering Plants, Plant Growth, Reproduction in Flowering Plants and Strategies for Enhancement of Food Production with special emphasis on Tissue Culture.

Module 3 is on Cell and Cell cycle, Heredity including Molecular basis of Inheritance and Variation and Evolution.

Module 4 is on Animal Diversity, Bio-Molecules and Bio-technology.

Module 5 is on Neural Control, Biotech Applications including Microbes in Human Welfare and Environmental Issues.

Module 6 is on Plant Physiology and Ecology including Photosynthesis and Respiration in Plants, Transport and Mineral Nutrition in Plants.

CONTENT

MODULE 4

MODULE - 5

MODULE 6

CHAPTER - 1

BIO-MOLECULES

Objectives : After going through the content learner will be able to :-

i) Differentiate between biomolecules, heteropolymer & homopolymer.

ii) Draw diagrammatic representation of peptide bond, phosphodiester bond and secondary structure of B-DNA.

iii) Understand the concept of activation energy in catalytic cycle of enzyme action.

iv) Classify types of proteins and enzyme.

Key words : Functional Group, Zwitterion, Activation Energy, Co-factor, Coenzyme, Prosthetic Group, Peptide bond, Phosphodiester bond, Heteropolymer.

Table of contents (CLASS -XI, LESSON-9)

INTRODUCTION

- There is a wide variety of living organisms in the biosphere but the chemical composition and metabolic reactions of all show striking-unity in diversity.
- In all living organisms, water is the most abundant chemical as well as there are thousands of small molecular weight biomolecules like amino acids, monosaccharides, disaccharides, fatty acids, glycerol, nucleotides, nucleosides and nitrogen bases.
- Proteins, Nucleic Acids and Polysaccharides are the three types of Biomacromolecules which are polymers of different building blocks.
- Biomacromolecules serve many functions viz energy storage, genetic material, enzymes, receptors, hormones etc in the living system.

1.1 Analysis of Chemical Composition of Living Tissue

Grinded Slurry of Living Tissue in Cl_3CCOOH (Trichloroacetic Acid)

- **FILTRATE** (Acid Soluble Pool of Cytoplasmic composition) consists of Biomolecules/ Micromolcules with molecular weight from 18-800 Dalton/Da
  - comprise of
    - **Amino Acids**

      $NH_2-\underset{R}{\overset{H}{C}}-COOH$
    - **Monosaccharides** (Can't be hydrolysed, with either free CHO or CO group)
      - Triose (3C) Glyceraldehyde
      - Tetrose (4C) Erythrose
      - Pentose (5C) Ribose, Deoxyribose
      - Hexose (6C) Glucose, Fructose, Galactose
      - Heptose (7C) Sedoheptulose
    - **Disaccharides** (with 2 monosaccharide) joined with glycosidic linkage
      - Maltose = Glu + Glu
      - Lactose = Glu + Galactose
      - Sucrose = Glu + Fructose
    - **Fatty Acids**
      - Saturated FA (butyric acid)
      - Unsaturated FA (Linoleic acid)
    - **Glycerol**
    - **Nucleoside**
    - **Nucleotides**
    - **Nitrogenous Bases**
- **FILTERED**
- **RETENTATE**

RETENTATE

(Acid insoluble fraction comprising of macromolecules of cytoplasm and cell organelles)

Comprise of

Polymeric Biomacromolecules with molecular weight of more than 1000 Da

Contains/Consists of

- Proteins (Polymers of amino acids) joined by peptide bond
- Nucleic Acids
  - DNA (Polymers of dexoyribonucleotides linked by phosphodiester bond)
  - RNA (Polymers of ribonucleotides linked by phosphodiester bond)
- Polysaccharides
  - Polymers of monosaccharides linked by glycosidic bond

Non Polymeric biomolecules with molecular weight of <800 Da

Consists of

Lipids (Esters of fatty acids with glycerol)

Types

- Simple
  - Oils
  - Fats
  - Waxes
- Compound
  - Phospholipids
  - Glycolipids
  - Sphingolipids

All carbon compounds in the living organism are called **biomolecules**. These organic compounds are also called as metabolites.

METABOLITES

Primary metabolites	Secondary metabolites
i) Proteins, carbohydrates & lipids	i) Alkaloids, phenols, sterols, essential oils, lignins etc
ii) Essential to growth of cell and are involved in primary metabolic processes like respiration & photosynthesis	ii) Not essential to sustain life of cells and produced during non-growth phase of cells.
iii) Identical in most organisms	iii) Numerous and wide spread

1.2 PROTEINS

i) Heteropolymers of a string/string of amino acids

ii) Successive Amino Acids are joined by peptide bond

1.2.1 AMINO ACIDS

i) Organic biomolecules containing an amino group and an acidic group as substituents on the same carbon hence called D-amino acids

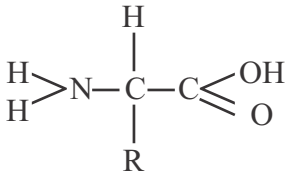
ii) Amino acids are substituted methanes with 4 substituent groups

a) Hydrogen

b) Carboxy

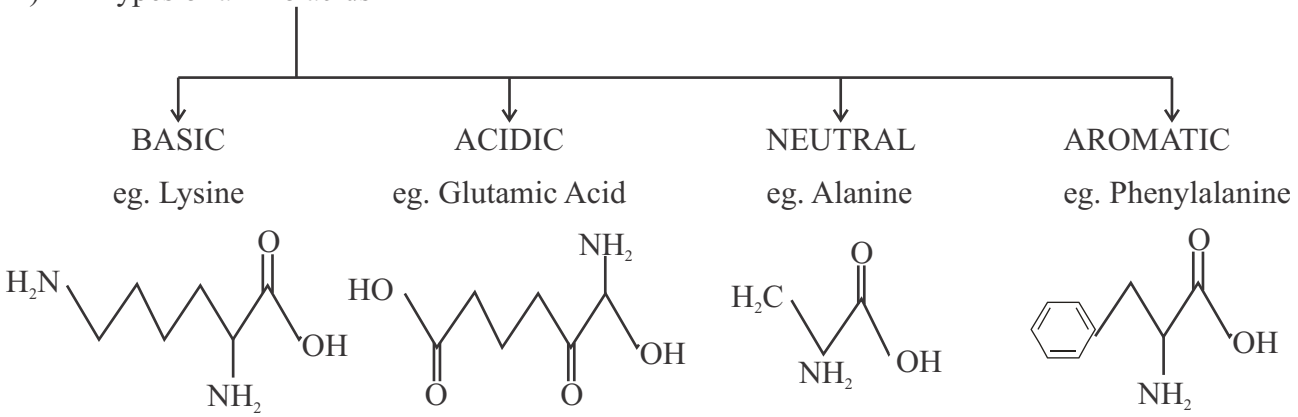
c) Amino

d) R (Variable)

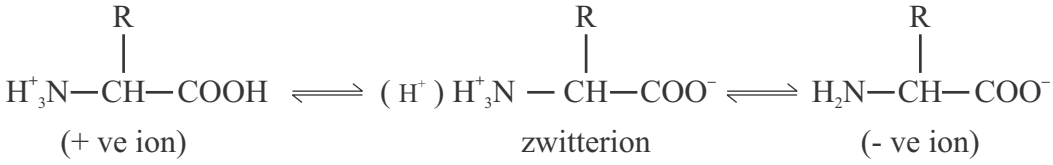


amino acid

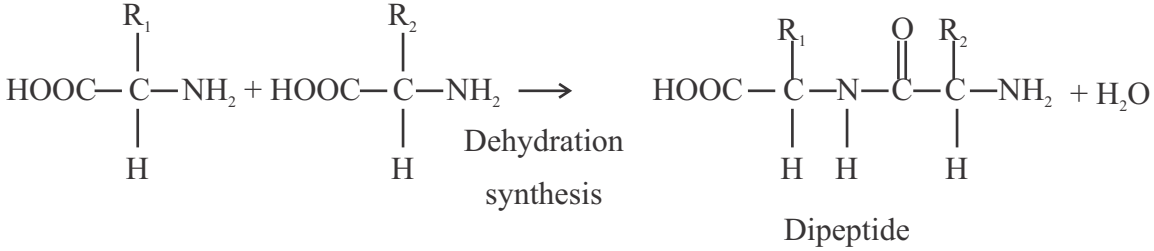
iii) Types of amino acids



iv) Zwitterion is a compound with no overall electrical charge, but which contains separate parts which are - vely & + vely charged



v) Peptide bond- Amide linkage between carboxylic group of one amino acid and amino group of another amino acid.



1.2.1 Level of protein structure.

There are 4 levels of protein structure:

a) **Primary**
- (i) Long chains of amino acid are arranged in a particular sequence
- (ii) Nonfunctional
- (iii) 1st amino acid on the left with free amino group is called N-terminal and the last with free carboxyl group is C-terminal.

b) **Secondary**
- (i) Interaction between every 4th amino acid by formation of hydrogen bond.
- (ii) Polypeptide form a right handed helix eg. keratin.
- (iii) If 3 or more chains are held together by intermolecular hydrogen bonds, the structure is called pleated sheet eg. silk fibres.

c) **Tertiary**
- (i) When a polypeptide chain becomes further stabilised by folding and coiling by the formation of ionic/hydrophobic/disulphide bonds/bridges.
- (ii) eg. amylase, pepsin etc.

d) **Quaternary**
- (i) When a protein has many subunits, each having primary, secondary and tertiary structure of its own.
- (ii) Haemoglobin, insulin.

Functions
- i) Body building eg. muscle protein actin, myosin
- ii) Support & protection eg. collagen & keratin
- iii) Biocatalysts eg. Enzymes
- iv) Transporters eg. haemoglobin, serum albumin
- v) Store & provide nutrients eg. milk casein,
- vi) Regulators of cellular/physiological activities eg. hormones like insulin, growth hormone.

Fig.1 Primary Structure

Fig. 2 Secondary Structure

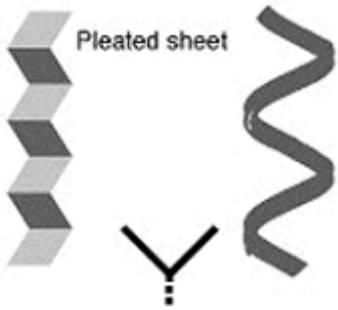


Fig.3 Tertiary Structure

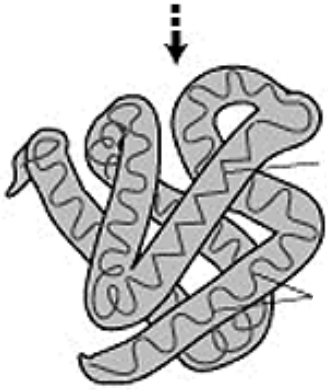
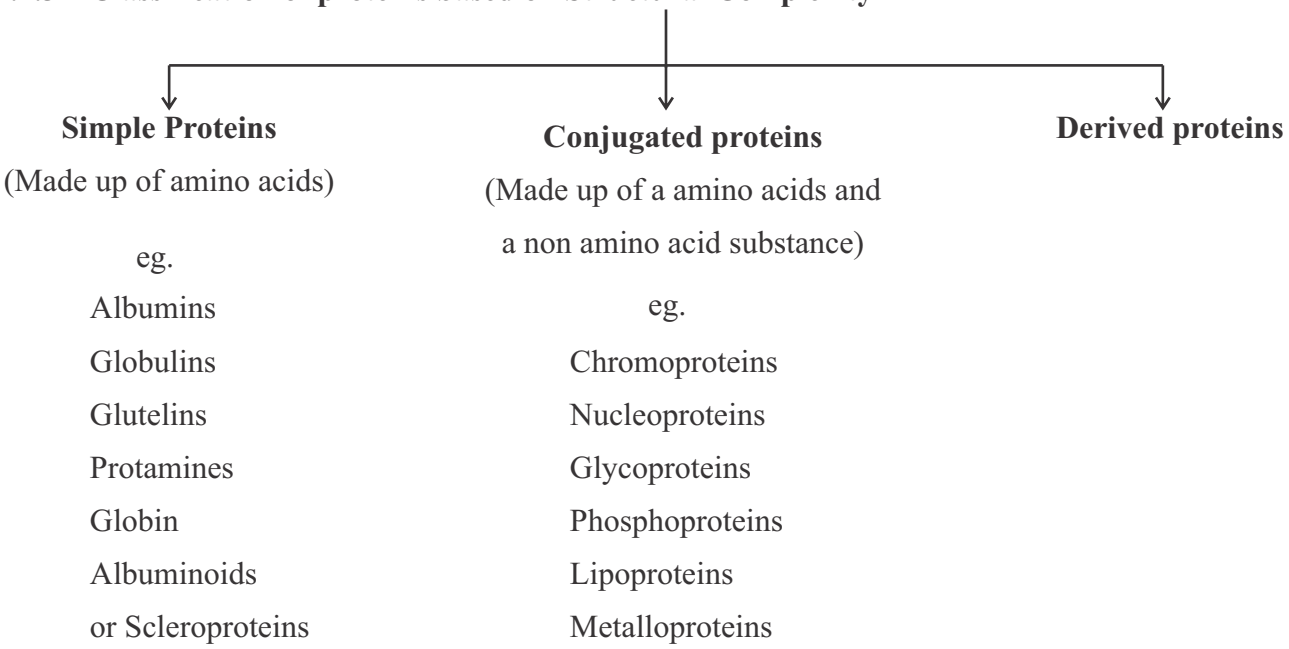


Fig. 4 Quaternary Structure

eg. hemoglobin consisting of 4 globin (Tertiary)

1.2.3 Classification of proteins based on Structural Complexity



Proteins occupy a prominent position in all biological systems both quantitatively & qualitatively. Irrespective of their structural or functional role, all proteins are built from the same fundamentals blocks, the amino acids. The physical and chemical properties of the constituent amino acids determine the structure & biological function of proteins.

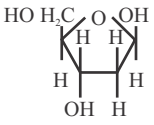
1.2.3 NUCLEIC ACIDS

DNA
(Deoxyribonucleic Acid)
Polymer of Deoxyribonucleotides
(Double stranded)

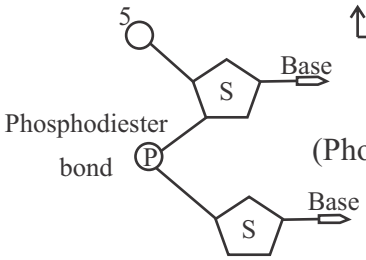
Dexoyribonucleoside + Phosphate

Nitrogenous Base
- Purine (6 member ring fused to 5 member ring)
  - Adenine + Sugar → Adenosine
  - Guanine + Sugar → Guanosine
- Pyrimidine (6 member ring)
  - Thymine + Sugar → Thymidine
  - Cytosine + Sugar → Cytidine

Sugar (5C) (pentose) → Deoxyribose



Nucleosides + Phosphate → Nucleotides (Phosphorylated nucleosides)



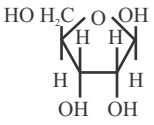
Phosphodiester bond
- Adenylic acid
- Guanylic acid
- Thymidylic acid
- Cytidylic acid

RNA
(Ribonucleic Acid)
Polymer of Ribonucleotides
(Single stranded)

Ribonucleoside + Phosphate

Nitrogenous Base
- Purine
  - Adenine
  - Guanine
- Pyrimidine
  - Thymine
  - Uracil

Suger (5C) (pentose) → Ribose



RNA Types
- m RNA
  - Carries information from DNA to ribosome
  - Decides sequence of amino acids in protein
- r RNA
  - Forms parts of ribosomes
- t RNA
  - Carries an amino acid from cytoplasm to ribosome

1.3.1 Structure of DNA((Blueprint of life)

1. Given by Watson Crick & Wilkins in the year 1953.
2. Watson & Crick had three sets of data :-
   a) Rosalind Franklins X-ray crystallography
   b) Chargaff's rules(No matter what ever the species A= T & G = C)
   c) Chemical structure of nucleotides
3. DNA is a double helical structure like a spiral stair case made of 2 strands of Polynucleotides running in the opposite direction (antiparallel) and are joined by hydrogen bonds.
4. The backbones of DNA molecules are made of alternating sugar and phosphates linked by phosphodiester bonds and rungs on the ladder are made of bases that are hydrogen bonded to each other.
5. Purine base always pairs up with pyrimidine base - complimentary base pairing.
   a) Adenine always pairs with thymine because they from 2 H bonds with each other.
   b) Cytosine always pairs with Guanine because they from 3 H bonds with each other.
6. Diameter and periodicity are consistent is 2.0nm/20 Aº, 10 base pairs/trun, 3.4nm/34 Aº is height of turn, width is consistent because of purine/pyrimidine pairing.
7. Double helical structure protects bases from attack by water soluble compounds & water itself as well as provides easy mechanism for replication.

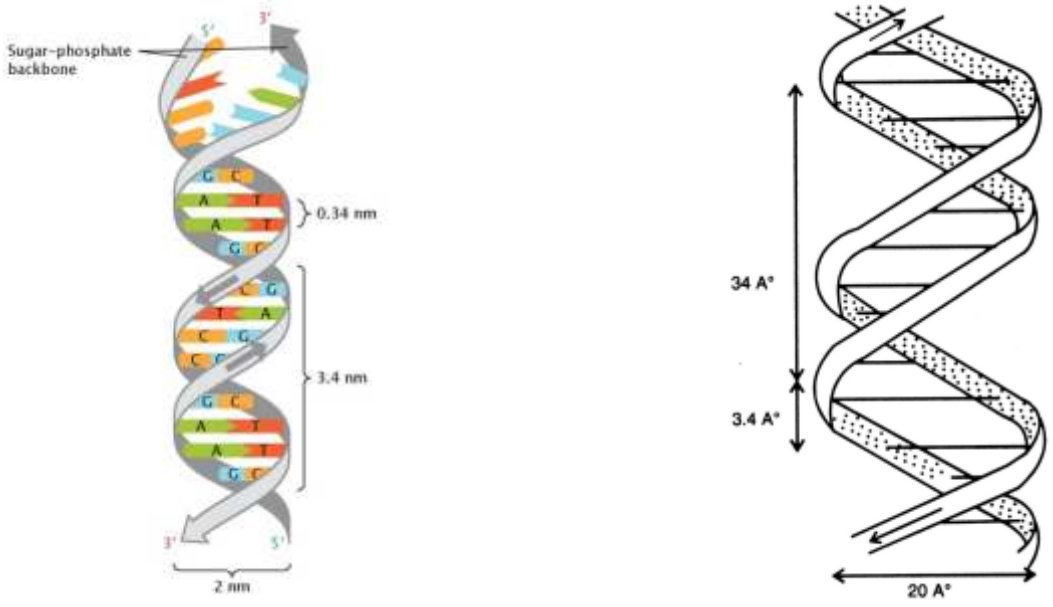
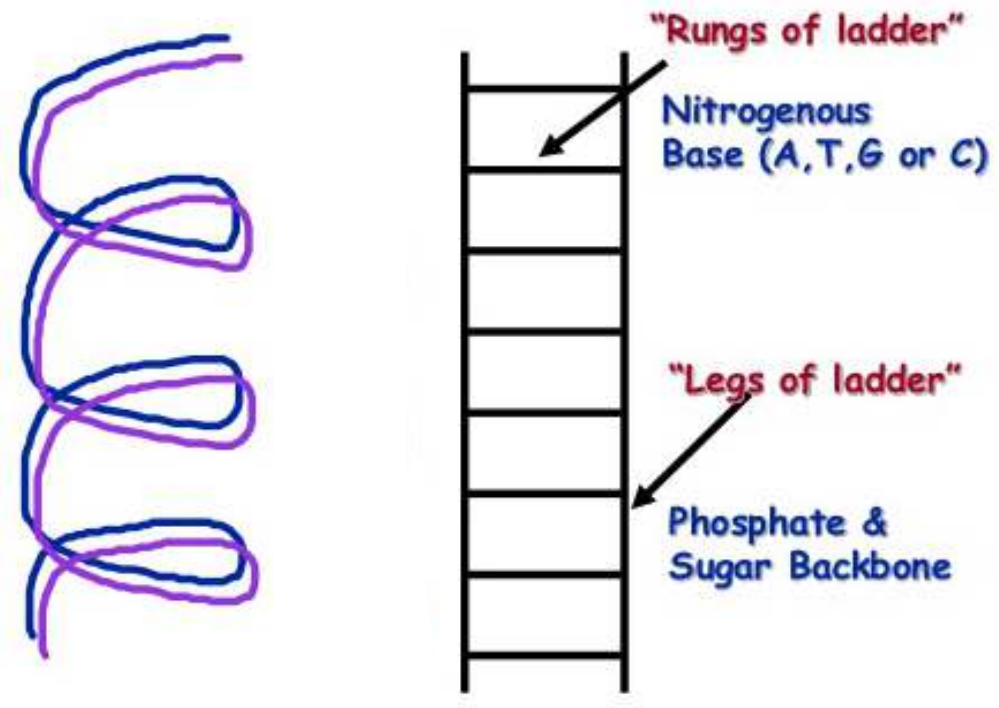
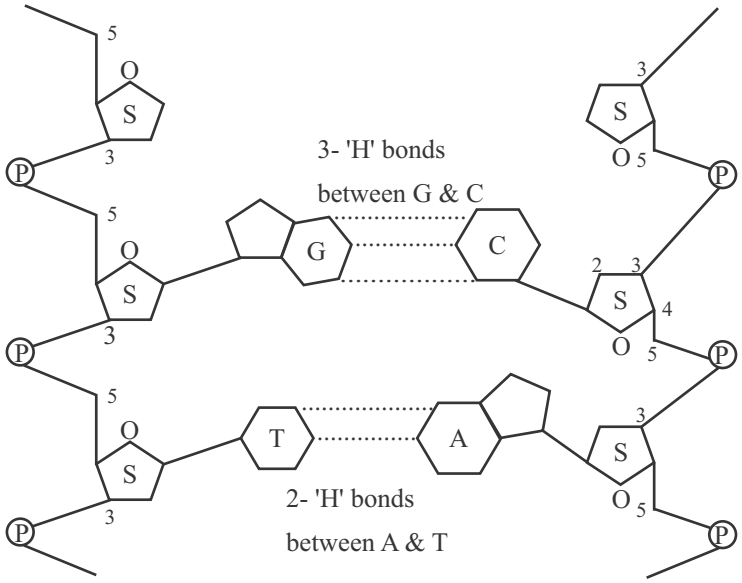


Fig. 5 Double helix DNA structure

Fig. 6 DNA Double Helix





1.3.1 Enzymes

1) Enzyme is a protein by an organism that enhance the rate at which bio-chemical reactions occur in the cell/body and act at specific pH & temperature without itself getting consumed at the end of the reaction.

2) Catalyst- A substance used in small amounts relative to the reactants, that modifies & increases the rate of a reactions without being consumed in the process

3) Similarities between Enzymes & Inorganic catalysts

   i) Remain unchanged at the end of the reaction & thus can be reused.

   ii) Required in very small quantities as compared to the substrate.

   iii) Do not initiate a reaction but change the rate of reaction by lowering activation energy.

   iv) Do not alter equilibrium but increase the rate of reaction by lowering activation energy.

   v) Forms short lived complexes with substrates

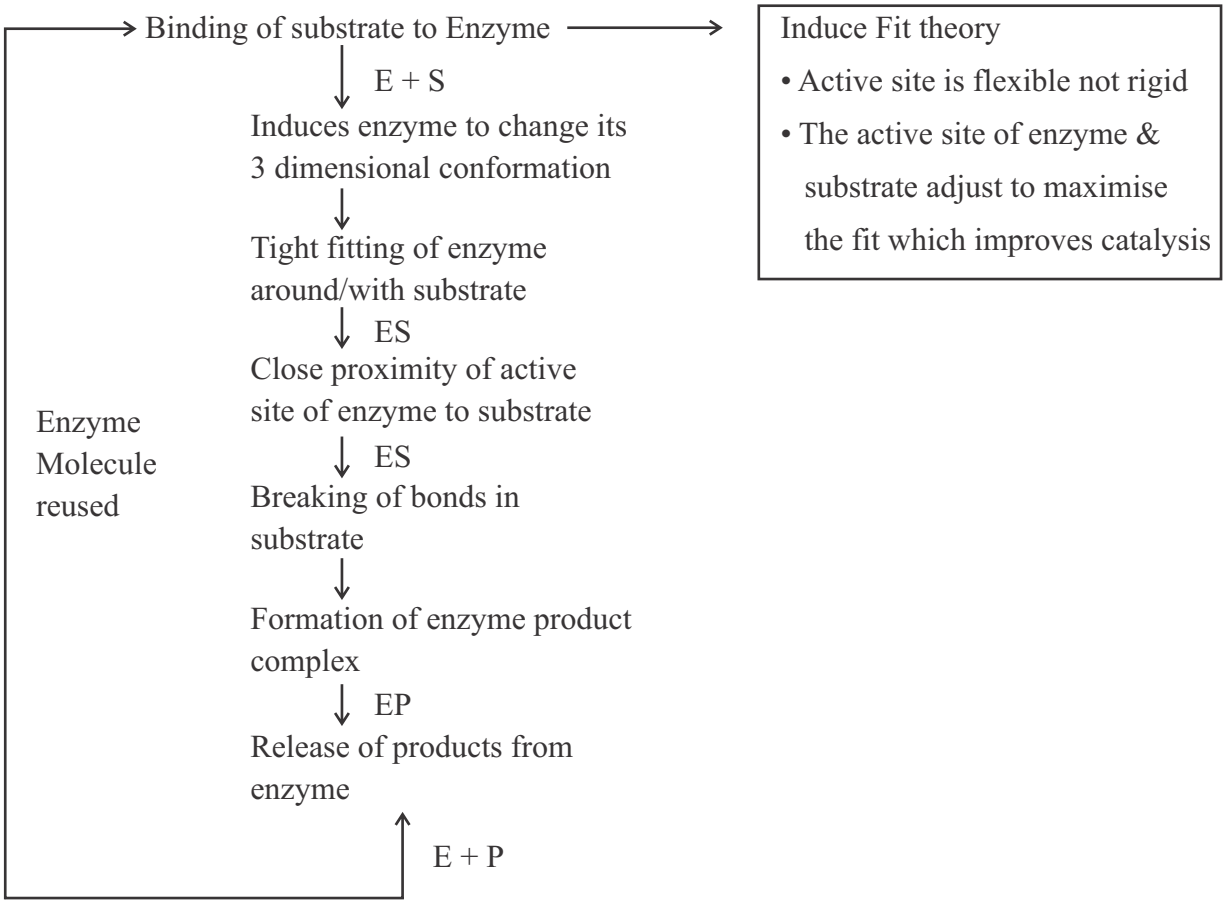
4. Difference between Enzymes & Inorganic catalysts

Enzymes	Inorganic catalysts
i) All enzymes are proteins with complex molecular organisation	i) Usually small & simple molecules
ii) An enzyme catalyses only a specific reaction	ii) A catalyst can catalyse more than one reaction
iii) Action of enzymes can be regulated by specific molecules	iii) Action cannot be regulated by any other molecule
iv) Sensitive to changes in pH, temperature or substrate concentration etc.	iv) Very less affected by such change.

1.4.1

Enzyme Action

A Catalytic Cycle



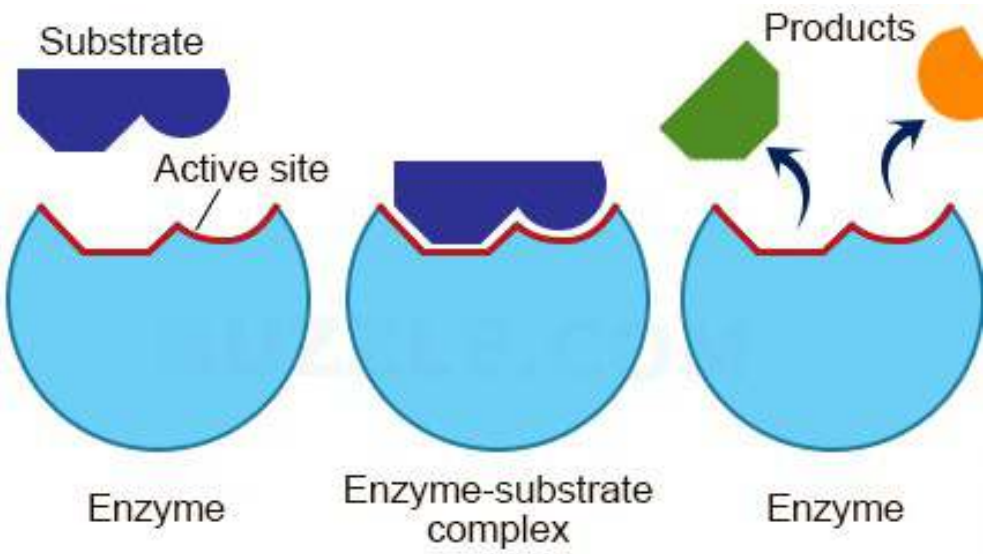


Fig 7 Enzyme - Product Complex

ENZYME COMPOSITION

Enzyme (Holoenzyme)

made up of

- Proteins
- Protein + Non Proteins

(Apoenzyme) + (Cofactor)

Coenzyme	Prosthetic group
• Large organic molecule	• Small organic molecules
• Loosely bound to apoenzyme by non covalent bond	• tightly bound by covalent bond
eg. vitamins	eg. heme

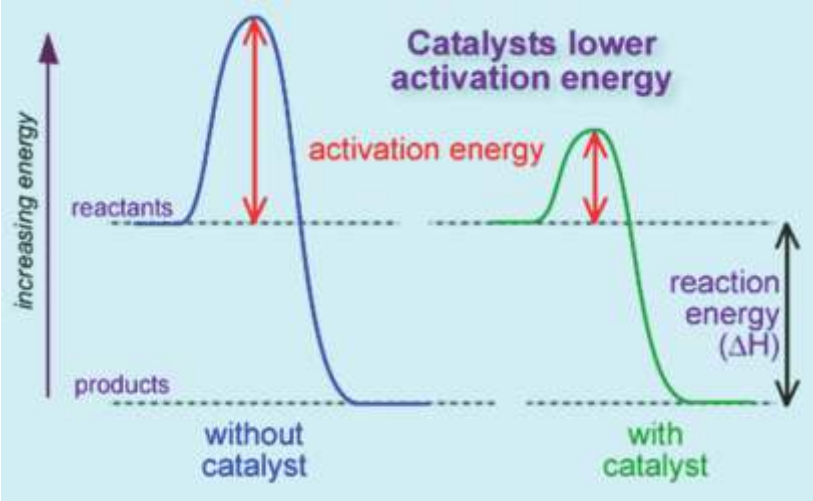
6) **ENZYME SPECIFICITY**

Absolute	Group	Linkage
Catalyse one type of reaction for a single substrate	Catalyse one type of reaction for a similar substance	Catalyse one type of reaction for a specific type of bond
eg urease catalyse hydrolysis of urea	eg. Hexokinase adds a phosphate group to hexoses	eg. Chymotrypsin catalyse the hydrolysis of peptide bonds

Activation Energy or Energy of Activation

- All chemical reactions require some amount of energy to get them started. This energy is called **activation energy**
- Enzymes being organic catalysts speed up the reaction rate by lowering activation energy

Without enzyme With enzyme



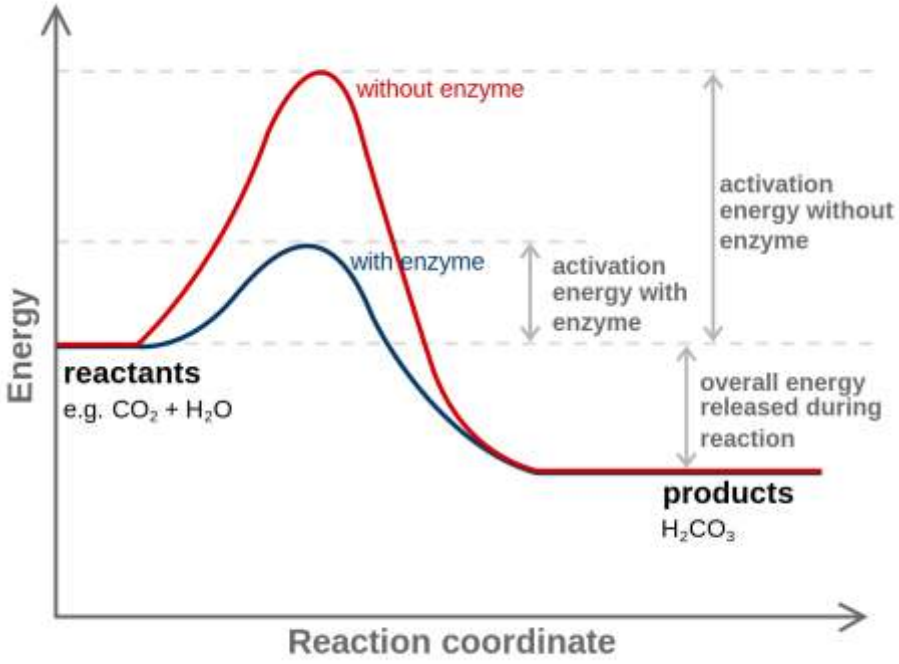


Fig. 8

1.4.2 Factors Affecting Enzyme Action

i) **Temperature -** Enzyme act best at optimum temperature. Below optimum temperature they are inactivated and above optimum enzymes are denatured.

ii) **pH -** Enzyme act best at optimum pH. Above or below optimum pH enzyme catalysed reaction is inhibited gastric enzymes act best at acidic pH while intestinal enzymes at basic/alkaline pH

iii) **Substrate Concentration -**

a) Rate of enzyme catalysed reactions increase with increase in substrate concentration.

b) At low substrate concentration there is a steep increase in the rate of reaction with increasing substrate concentration due to the availability of empty catalytic sites.

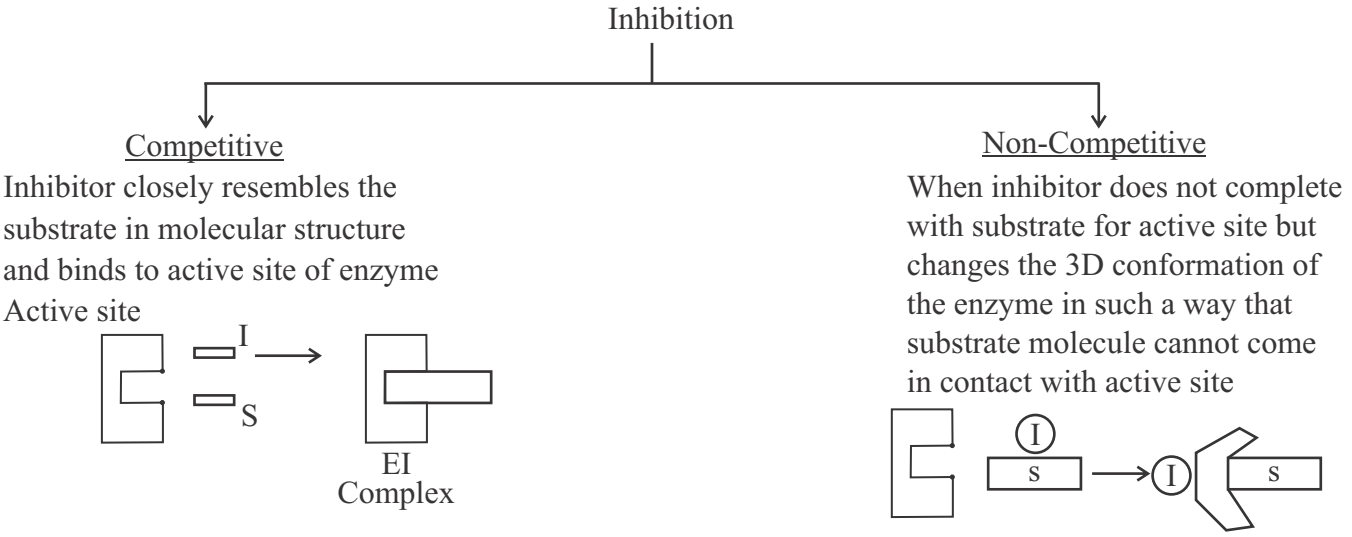
c) As the concentration of substrate increase the enzyme becomes saturated with substrate & the rate become maximum.

d) As soon as the catalytic site is empty, more substrate is available to the bind and undergo reaction.

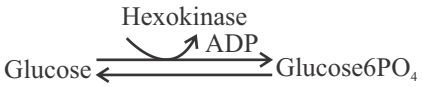
e) At this stage the rate of formation of product now depends on the activity of the enzyme itself & adding of substrate does not affect the rate of reaction to any significant effect

iv) **Effect of Chemicals/Inhibitors**

When binding of a certain chemical reduces/shuts off the enzyme activity, the chemical is called **inhibitor.**



v) **Feed back Inhibition :-** Inhibition of enzyme activity by product of same enzyme reaction.



Hexokinase enzyme which causes phosphorylation of glucose during respiration is inhibited by the product (glucose $6PO_4$) which it forms.

Use :- This type of inhibition is done as a regulatory mechanism to meet the metabolic needs of a cell or organism. It prevents overproduction of the product & also avoids energy depletion.

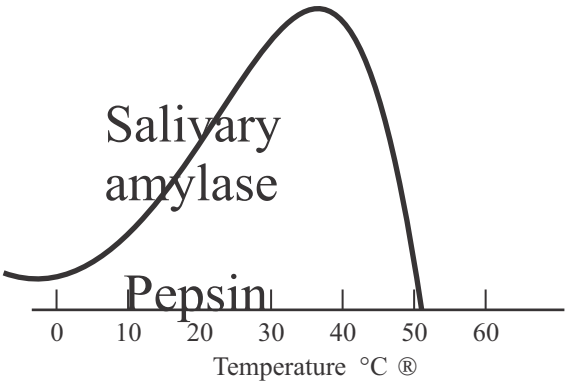


Fig 9.1
Effect of Temperature
on Enzyme Activity

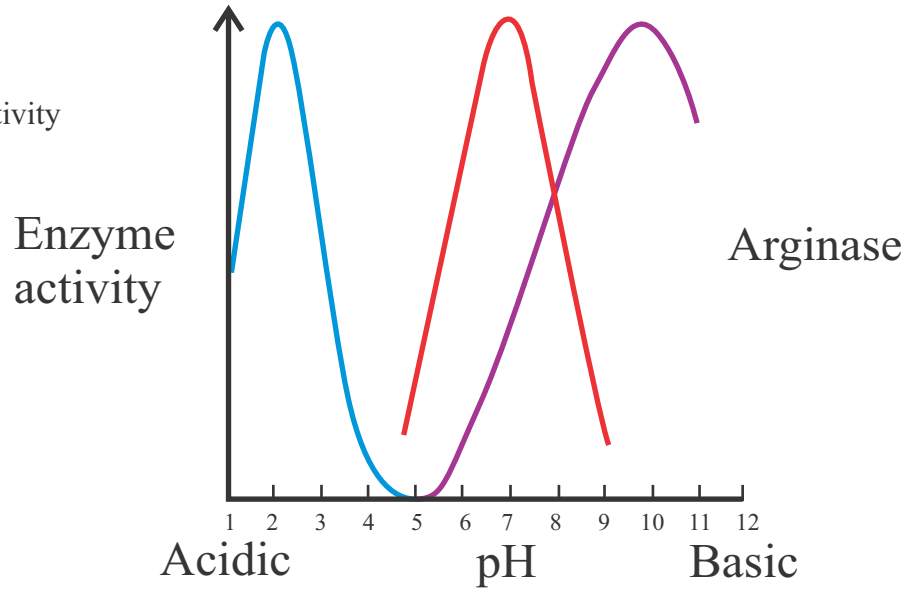


Fig 9.2
Effect of pH
on Enzyme Activity

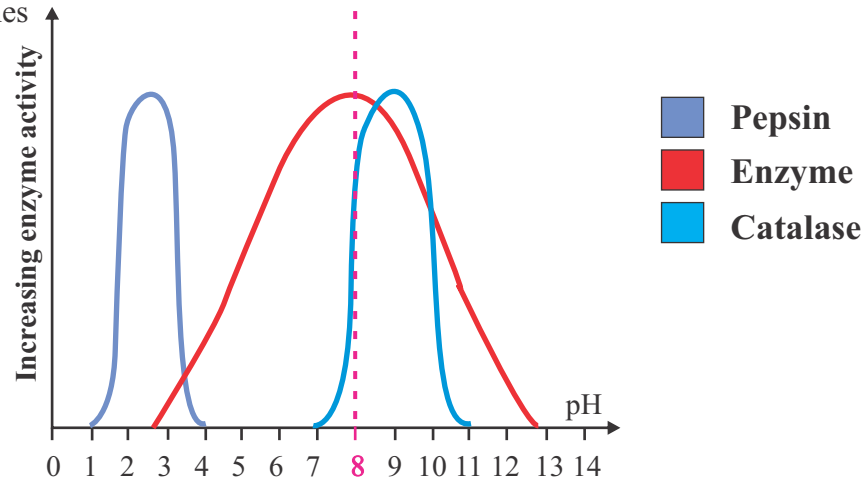


Fig 9.3
Effect of pH
on Activity of
different Enzymes

Fig 9.4
Effect of pH
on Reaction Rate of
different Enzymes

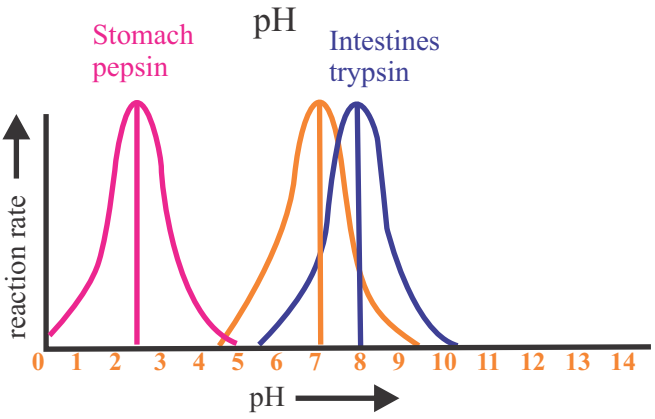
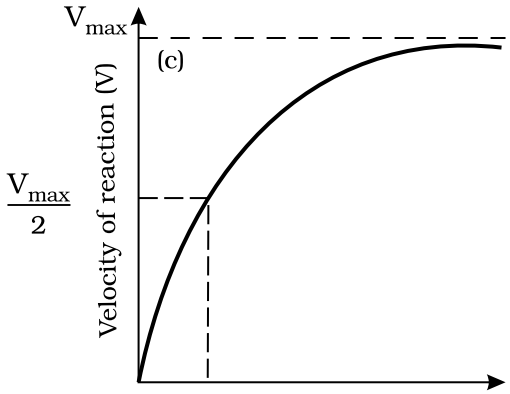


Fig 9.5
Effect of Substrate
Concentration on
Enzyme Action



1.4.3 Naming & Classification of Enzymes

A. Naming of Enzymes

- Use old names eg. pepsin, trypsin
- Adding suffix **-ase** eg. Sucrase catalysis hydrolysis of sucrose (Substrate)
- After the function eg. Oxidases catalyse oxidation reaction
- Describing both the substrate & function eg Alcohol dehydrogenase oxidises ethanol

B. Classification of Enzymes

Enzymes are grouped into 6 functional classes (EC number classification) by the International Union of Biochemists (IUB). On the basis of the types of reaction that they catalyse

EC 1. Oxidoreductases

EC 2. Transferases

EC 3. Hydrolases

EC 4. Lyases

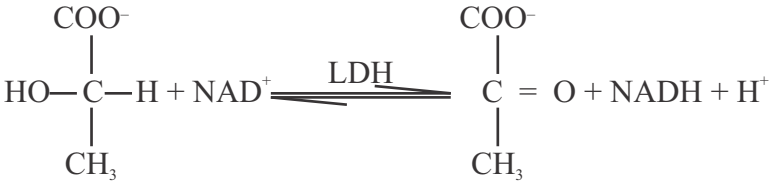
EC 5. Isomerases

EC 6. Ligases

Enzymes Classification

EC 1. OXIDOREDUCTASES

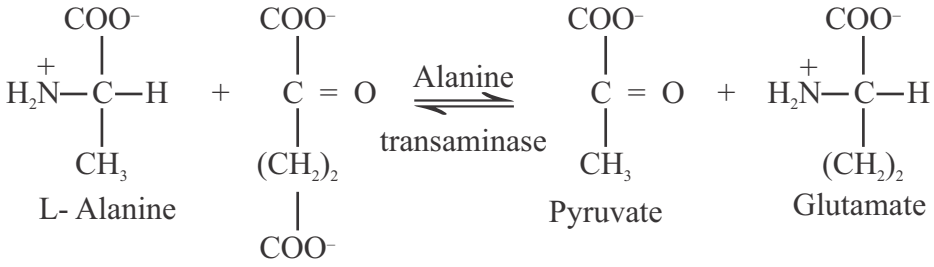
- Catalyse oxidation/reduction reaction
- Act on chemical groupings to add (reduction) or remove (oxidation) hydrogen atoms
- Peroxidase, catalase, glucose oxidase, Lactate dehydrogenase



Lactate Pyruvate

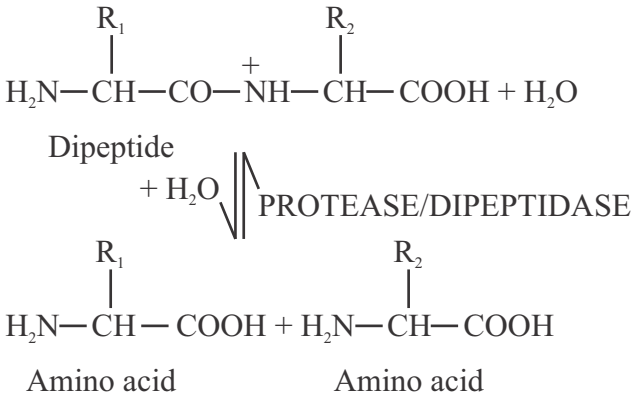
EC 2. TRANSFERASES

- Catalyse transfer of functional groups (eg methyl/phosphate) between donor & acceptor molecules
- Phosphotransferases (Kinases), transmethylases, transpeptidases, transminase



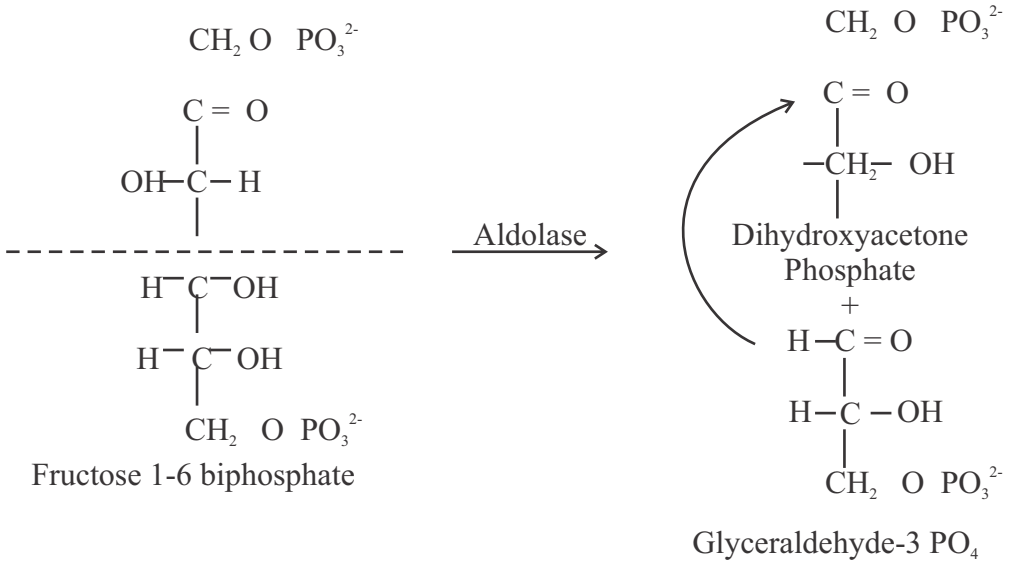
EC 3. HYDROLASES

- Catalyse hydrolysis of various bonds & add water across a bond.
- Proteases, carbohydrases, lipases, phosphatases etc.
- Catalyse hydrolysis reactions where water is the acceptor of transferred group.



EC 4. LYASES

- Break various bonds by means other than hydrolysis and oxidation
- Add water, ammonia or CO_2 across double bonds or remove these elements to produce double bonds.
- Fumarase, carbonic anhydrase, oxalate, decarboxylase, isocitrate, lyases, aldolase



Fructose 1-6 biphosphate

Glyceraldehyde-3 PO_4

EC 5. ISOMERASES

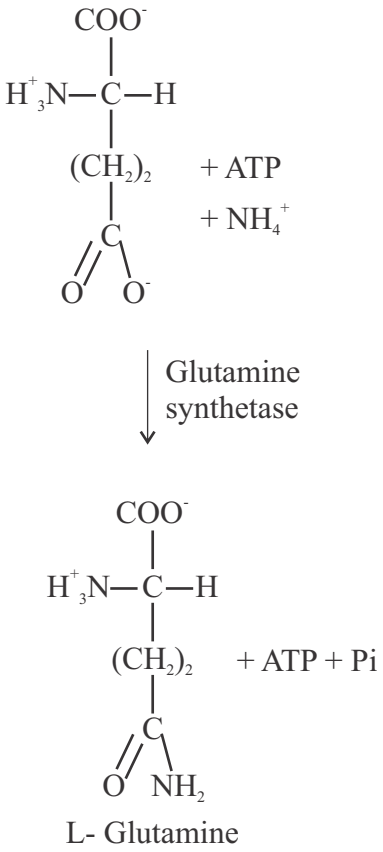
- Catalyse isomerisation changes within a single molecule
- Carry out L to D isomerisation mutase reactions
- Facilitate intermolecular rearrangements in which bonds are broken and formed or they can catalyse conformational changes.
- Mutase Isomerase

L- Alanine

D- Alanine

EC 6. LIGASES

- Join 2 molecules with covalent bonds.
- Catalyse reactions in which two chemical groups are joined or ligated with the use of ATP (energy)
- Acetyl- CoA carboxylase, Glutamine Synthetase, DNA ligase



L- Glutamine

1.5 RELATED ACTIVITIES

Catalase is an enzyme produced by living calls and it catalyse the break down of H_2O_2; a damaging substance produced by living tissue.

$H_2O_2 \xrightarrow{\text{catalase}} H_2O + O_2$

Bubbles of O_2 are produced in solution.

AIM. To identity the presence of catalase in living and non living substance

To compare catalase activity in different types of plant and animal tissues

PROCEDURE 1. Take 3filter paper discs.

2. Touch against freshly cut surface of non living (A), plant (B), and animal tissue (C)
3. Drop the 3 discs in test-tube containing H_2O_2

OBSERVATIONS 1. In which test tube the filter paper sinks?

2. In which test tube it floats?
3. In which test tube it takes less time to float and why?

1.6 GLOSSARY

1. **Activation energy:-** Energy required to start a reaction

2. **Biomolecules :-** Chemical compounds composed mainly of carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus, which are the building blocks of life and perform important functions in living organisms eg amino-acids, lipids, proteins, carbohydrates, nuclei acids etc.

3. **Co-Enzyme :-** Organic non-protein molecules that loosely bind with the protein molecule (Apoenzyme) to form active enzyme (Holo enzyme).

4. **Cofactor :-** Non-protein chemical compound that is required for a proteins biological activity. It can be a co-enzyme (loosely bound) or prosthetic group (tightly bound) to apoenzyme.

5. **Enzyme :-** Protein of RNA chain that catalyses a particular biochemical reaction involving specific substrate/reactant molecules. Also called biocatalyst.

6. **Filtrate :-** Acid soluble fraction obtained by filtration of grinded tissue/slurry through a filter (cheese cloth)

7. **Functional group :-** Specific group/moities of atoms bonds within molecules that are responsible for the characteristic chemical reaction of those molecules.

8. **Heteropolymers:-** Polymers formed from subunit that are not all the same eg proteins,DNA polynucleotide.

9. **Homopolymers :-** Polymers containing single type of repeat units eg glycogen.

10. **Prosthetic group :-** A cofactor that is tightly or even covalently bound to enzyme.

11. **Retentate :-** Acid insoluble material remaining on the filter after filtration of slurry.

12. **Zwitterion :-** An ion carrying both positive and negative charge in different parts of the molecule.

CHAPTER-2

ANIMAL KINGDOM

Objectives:

After going through the content learners will be able to

i) Classify kingdom Animalia upto phylum

ii) List the salient features of the various phylum with example

Introduction:

Kingdom Animalia includes a wide variety of animals but all of them do share some common features. They are multicellular eukaryotes having a heterotrophic mode of nutrition. All the animals of kingdom Animalia have the power of locomotion and respond to stimuli through a developed nervous system. Here, kingdom Animalia will be classified upto various phylum with salient features and examples.

Key Words: Coelom, metamerism, symmetry,

TABLE OF CONTENT: (CLASS XI, LESSON - 4)

4.1 Basis of classification:

Before we classify kingdom Animalia let us know the basis of classification. Well, the animals are classified on the basis of their organization, symmetry, body cavity, number of embryonic cell layers, metamerism and presence or absence of notochord.

Organization :

Although all animals are multicellular, they do not have the same level of organization. Animals may have a cellular level of organization or a tissue level of organization or organ level of organization. Animals such as sponges have a cellular level of organization. They have aggregates of cells. Cnidarians have group of cells performing specialized functions. They are at tissue level of organization. All other animals have organs (tissues are grouped together to form organs) and systems for performing body functions. Organs have associated to form functional systems, each system has a specific physiological function. This is known as organ system level of organization. Members of Phylum Platyhelminthes, phylum Nemathelminthes, phylum Annelida, phylum Arthropoda, phylum Mollusca, phylum Echinodermata, phylum Protochordata and phylum Chordata all belong to this organ system level of organization.

Symmetry : Symmetry means dividing the body into two equal and identical parts. Sponges are asymmetrical i.e. any plane that passes through the centre does not divide it into two equal halves. However, some of the sponges are **radially** symmetrical. Cnidarians are radially symmetrical. Echinoderm larvae are **bilaterally symmetrical**, however the adults are **radially symmetrical**. Radial symmetry means that any plane passing through the central axis of the body divides it into two identical halves. Radial symmetry occurs when a body is constructed around a central oral-aboral axis. All other animals are bilaterally symmetrical i.e. body can be divided into identical left and right halves in only one plane. There is a single median longitudinal plane through which the body can be divided into two similar halves.

Embryonic layers : Three layers of cells, ectoderm, mesoderm and endoderm (germinal layers) in the embryo give rise to various parts of the body of the animal. Sponges and Cnidarians have two germinal layers, an outer ectoderm and inner endoderm. An undifferentiated layer called the mesogloea is present between the ectoderm and endoderm. Mesoderm is not present in them. So, these animals are called diploblastic. All others have three germinal layers and are triploblastic.

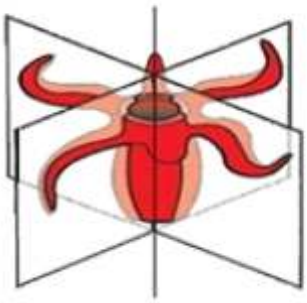


Fig.12 Radial symmetry

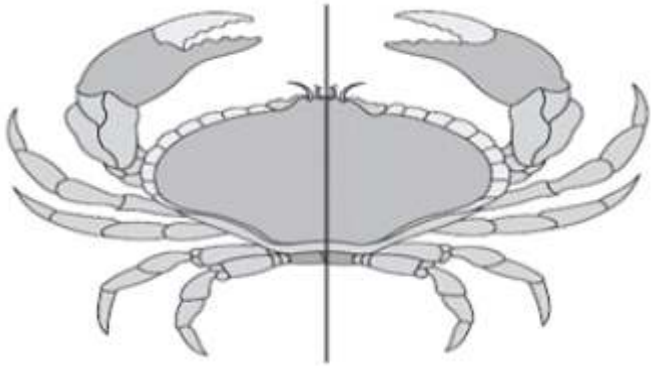


Fig.13 Bilateral symmetry

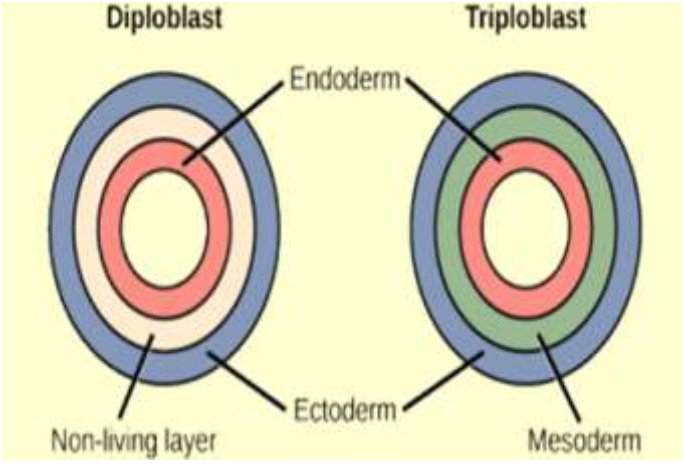


Fig.14 Embryonic Layers

Segmentation: In some animals, the body is externally and internally divided into segments along the anterior-posterior axis with a serial repetition of at least some organs. These animals are said to be metamerically segmented. Each segment is known as a metamere. For example, Nereis, Pheretima posthuma (earthworm), leech.

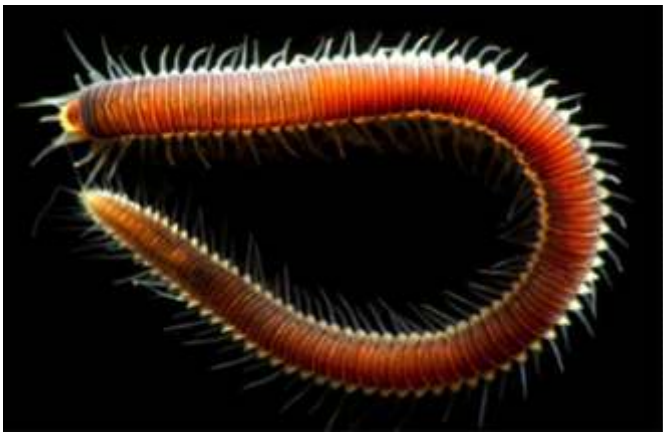


Fig.15 Nereis

Coelom or Body Cavity: Coelom is a cavity between body wall and gut wall and lined by mesoderm (peritoneum). Animals from phylum Annelida onwards are all eucoelomate (eu means true) animals. Animals belonging to phylum Platyhelminthes do not have a cavity between the body wall and gut wall. The space between the body wall and gut wall in them is filled up with parenchymal mesenchymal cells. They are known as Acoelomate animals. Animals belonging to group Aschelminthes (e.g. roundworms) have a pseudocoelom (pseudo = false). Pseudocoelom is not a true body cavity. A space is present between the body wall and the gut wall but it is not lined by the peritoneum.

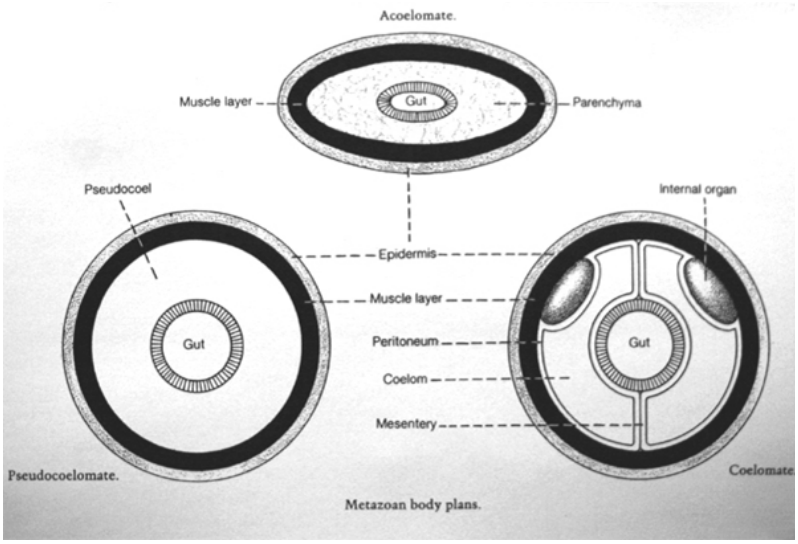
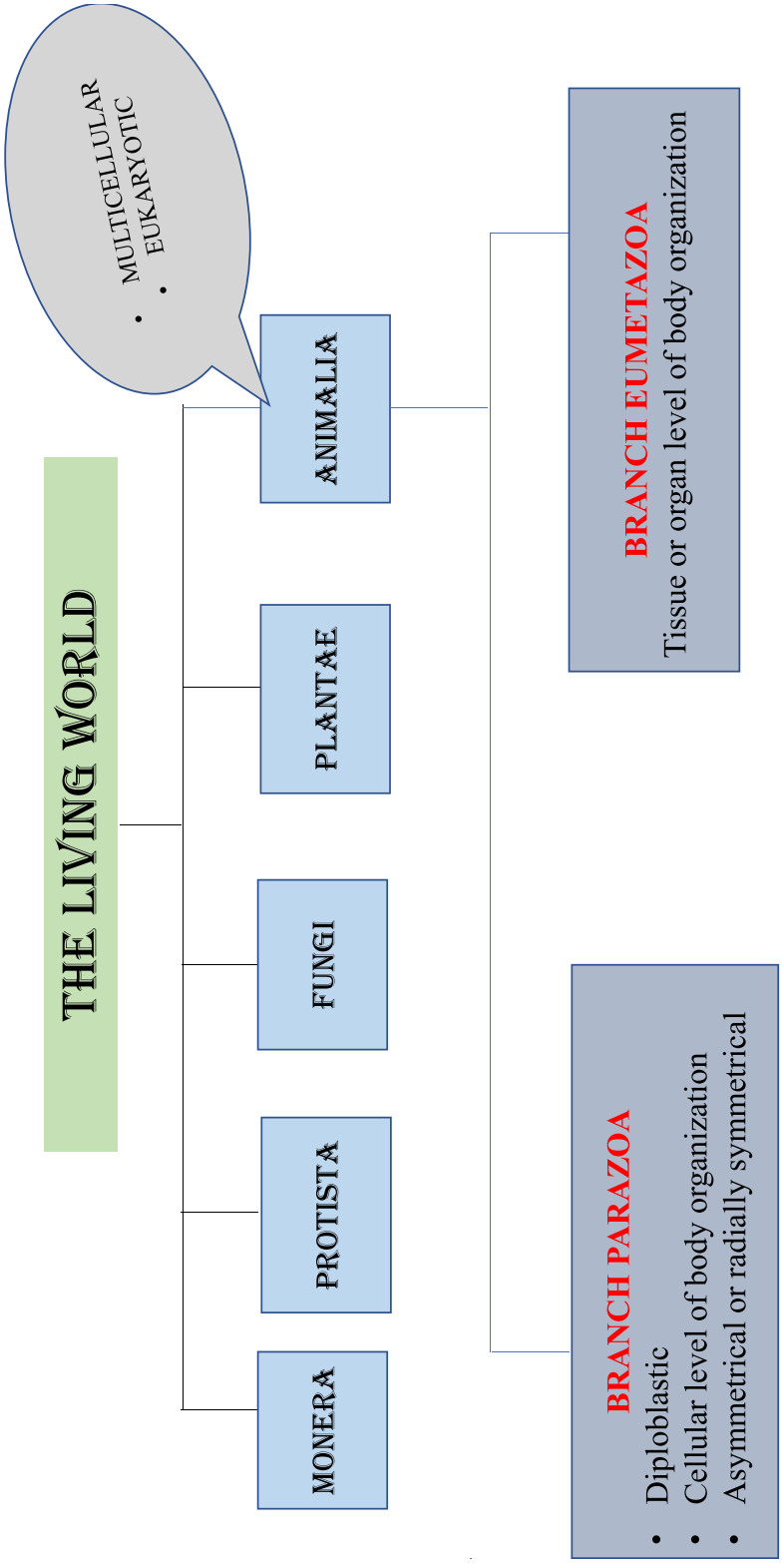


Fig.16

Notochord: is a solid rod like structure formed on the dorsal side during embryonic development in animals belonging to phylum Chordata. Notochord is absent in non-chordates.

4.2 Classification of Animalia



BRANCH PARAZOA

- Diploblastic
- Cellular level of body organization
- Asymmetrical or radially symmetrical

PHYLUM PORIFERA

Greek: Porus:pore; ferre: to bear

(THE SPONGES)

- Pore bearing (ostia, osculum) with a well developed canal system
- Internal skeleton made up of spicules and spongin fibres
- eg. *Sycon*, *Leucosolenia*

BRANCH EUMETAZOA

Tissue or organ level of organization

RADIATA

- Radially symmetrical
- Diploblastic
- Tissue level of body organization

BILATERIA

- Bilaterally symmetrical
- Triploblastic
- Organ level of body organization

RADIATA

PHYLUM CNIDARIA

- Exists either in **polyp** or **medusa** form or both.
- Possess a central gastrovascular cavity with a single opening, the **mouth** or **hypostome**.
- Presence of **cnidoblasts** for defense and offence.
- eg. *Hydra, Obelia, Aurelia, Metidium*

PHYLUM CTENOPHORA

- Commonly known as sea walnuts or comb jellies.
- Biradially symmetrical.
- Bears external rows of comb plates.
- Has special adhesive cells, the **colloblasts** for capturing prey.
- Exhibits bioluminescence.

eg. *Ctenoplana*

BILATERIA

ACOELOMATA
Coelom absent

PSEUDOCOELOMATA
False coelom

EUCOELOMATA
True coelom present

ENTEROCOELOMATA

SCHIZOCOELOMATA

GROUP ASCHELMINTHES

PHYLUM PLATYHELMINTHES

Greek: platys: broad or flat; helmin: worm

- Dorsoventrally flattened body.
- Free living or parasitic. Parasites either **ectoparasites** or **endoparasites**.
- Holdfast organs: **hooks** and **suckers**.
- Incomplete digestive tract with a **single opening**, the **mouth**, anus is absent.
- Hermaphrodite.
- eg. *Dugesia, Fasciola, Taenia,*

PHYLUM NEMATHELMINTHES
(Roundworms)

Greek: nema: thread; helmin: worm

- Pseudocoelomates.
- Syncytial epidermis; epidermis lack cilia.
- Complete digestive tract, both **mouth** and **anus** present.
- Sexes are separate.
- eg. *Ascaris, Wuchereria, Dracunculus*

SCHIZOCOELOMATA

Coelom develops by splitting up of the mesoderm

PHYLUM ANNELIDA

(Segmented animals)

Greek: annulus: ring; lidos: form

- Metamerically segmented.
- Eucoelomate.
- Hermaphrodite

eg. *Nereis, Pheretima, Hirudinaria*

PHYLUM ARTHROPODA

(animals with jointed appendages)

Greek: arthron: joint; podos: foot

- Thick exoskeleton of chitinous plates.
- Compound eyes present

eg. *Periplaneta, Julus, Palaemon*

PHYLUM MOLLUSCA

(Soft bodied animals)

Latin: Molluscus: soft

- Presence of shell, mantle and head-foot complex
- Presence of radula

eg. *Pila, Unio, Octopus, Sepia*

ENTEROCOELOMATA

Coelom develops from enteric pouches (outpocketing of the gut)

PHYLUM ECHINODERMATA

(spiny skinned animals)

Greek: echinos: spines; derma: skin

- Larva bilaterally symmetrical but adults are radially symmetrical.
- Presence of spines and pedicellariae.
- Water vascular system present.

eg. *Asterias, Holothuria, Antedon, Echinus*

PHYLUM HEMICHORDATA

Greek: hemi: half; chordata: notochord

- Worm like, cylindrical, bilaterally symmetrical and elongated animals.
- Body made up of anterior proboscis, a collar and trunk.

eg. *Balanoglossus*, *Saccoglossus*

PHYLUM CHORDATA

- Notochord present.
- A dorsal hollow nerve chord present.
- Presence of pharyngeal gill slits.

Fishes, amphibians, reptiles, birds and mammals are included in this category.

PHYLUM CHORDATA

SUBPHYLUM

UROCHORDATA

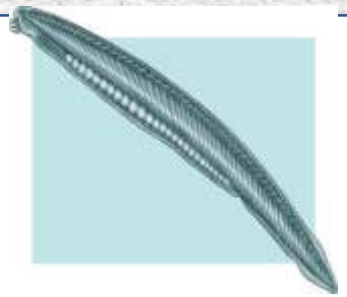
Gr.: uros: tail; chordata: notochord

- Notochord present only in the larval tail, disappears in adult.
- Dorsal tubular nerve chord found in larva, replaced by dorsal ganglia in adult.
- eg. *Herdmania, Salpa, Doliolum*

CEPHALOCHORDATA

Gr.: cephalos: head; chordata: notochord

- Notochord extends up to the anterior end of the body.
- Notochord persists throughtout life.
- Pharangeal gill slits are more numerous and better developed
- eg. *Branchiostoma (Amphioxus)*



VERTEBRATA

Vertebrata or craniata

- Cranium (brain box) present around the brain.
- Notochord present only in embryonic stage, replaced by vertebral column in the adults.
- High degree of cephalization.

SUBPHYLUM VERTEBRATA

DIVISION AGNATHA
Jaws absent

CLASS: CYCLOSTOMATA
(Circular mouthed fishes)
Gr.: cyclos: circular; stoma: mouth
- Eel like body with a compressed tail.
- Unpaired fins present.
- Soft skin without scales.
- eg. *Petromyzon, Myxine*

DIVISION GNATHOSTOMATA
Jaws present

SUPERCLASS: PISCES
- True fishes.
- Have paired appendages in the form of pectoral and pelvic fins.
- May possess an exoskeleton of dermal scales.
- Cold blooded.

SUPERCLASS: TETRAPODA
Gr. Tetra: four; podos: foot
- Four limbs present

SUPERCLASS PISCES

CLASS PLACODERMI

- Includes earliest fossil fishes that lived in fresh water.
- Body had an external protective armour of bony scales.
- Bony skeleton.

eg. *Climatius*

CLASS CHONDRICHTHYES

Gr. Chrondros: cartilage; ichthys: fish

- Cartilagenous fishes.
- Marine.

eg. *Scoliodon, Torpedo, Trygon, Sphyrna*

CLASS OSTEICHTHYES

Gr. Osteon: bone; ichthys: fish

- Bony fishes.
- Found in all types of water-fresh water, marine and brackish water.

eg. *Labeo, Catla, Hippocampus,*

SUPERCLASS TETRAPODA

CLASS AMPHIBIA

Greek: amphi: two or both; bios: life

- Amphibious, can live both in water and on land.
- Without scales.
- Paired fins absent, unpaired fins may be present.

eg. *Salamander, Bufo*

CLASS AVES

Latin: Avis: bird

- Bipedal, feathered and warm blooded animals.
- Forelimbs modified into wings.
- Hindlimbs adapted for perching, walking or swimming etc.
- Upper and lower jaws modified into beak which lacks teeth.

eg. Peacock, penguin, pigeon, crow

CLASS REPTILIA

Latin: reptare: to creep

- Creeping and burrowing cold blooded vertebrates bearing epidermal plates.
- Dry, rough skin without glands.

eg. *Calotes*, *Chameleon*, wall lizard, *Varanus*

CLASS MAMMALIA

Latin: mamma: breast

- Warm blooded, hairy with mammary glands.
- Oil glands (sebaceous glands) and sweat glands (sudoferous glands) present.
- Skull dicondylic (with two occipital condyles)

eg. Kangaroo, rodents, rabbit

2.3 GLOSSARY:

Coelom: A fluid filled perivisceral cavity lined by epithelial cells derived from the embryonic mesoderm

Metamerism: The serial segmentation of the body along an anterior- posterior axis
Symmetry: Division of the body into equal parts by lines or planes around a central axis

2.4 FURTHER READING:

1. Non- chordata by R.L.Kotpal, Rastogi publication, Meerut

2. Campbell, Biology by Jane B.Reece, Lisa A Urry, Michael L. Cain, Steven A. Wasserman, Peter V. Minorsky, Robert B. Jackson

CHAPTER - 3

MOLECULAR BASIS OF INHERITANCE

Objectives:

After going through this content, the learner will be able to understand the following processes

- DNA replication
- Transcription
- Translation
- Gene regulation through Lac Operon
- DNA fingerprinting

Introduction

A cell contains micromolecules and macromolecules which carry out specific cellular functions. When a cell divides, it doubles its components to pass to next generation. DNA being the basis of inheritance also replicates and expresses itself in the form of protein to carry out vital cellular functions. Slight variation in DNA has provided basis for criminal cases and paternity issues. This module will provide details on the process of DNA replication, gene expression and gene regulation, DNA fingerprinting.

Key words: replication, *ori,* helicase, DNA polymerase, okazaki fragments, promoter, genes, RNA polymerase, splicing ,capping, tailing, initiation complex, release factors, operon, RFLP, satellite DNA, VNTR.

TABLE OF CONTENT

MOLECULAR BASIS OF INHERITANCE (CLASS - XII, LESSON - 6)

3.1 DNA REPLICATION

Every cell makes copy of its DNA before cell division and passes the genetic information to daughter cells.

SEMI-CONSERVATIVE MODE OF DNA REPLICATION: DNA replication makes 2 new double helices of DNA, each with 1 old and 1 new strand. This mode of replication is described as semi-conservative: one-half of each new molecule of DNA is old; one-half new.

STEPS IN REPLICATION OF DNA

Identification of origin of replication

⇩

Enzyme topoisomerase relaxes the super coiled DNA.

⇩

DNA helicase and Initiator proteins binds to the DNA at the replication fork and untwist the DNA using energy derived from dNTP

⇩

DNA primase binds to helicase and produces a complex called a primosome

⇩

Primase synthesizes a short RNA primer of 10-12 nucleotides, to which DNA polymerase III adds nucleotides.

⇩

DNA Polymerase III adds nucleotides (dNTPs) 5' to 3' on both strands beginning at the RNA primer

⇩

.

The RNA primer is removed and replaced with DNA by polymerase I which also proof reads the newly synthesized strands and the gap is sealed with DNA ligase.

⇩

The single-stranded template DNA is further stabilized with the help of

Single-stranded DNA-binding proteins

⇩

Following replication the new DNA automatically winds up into a double helix

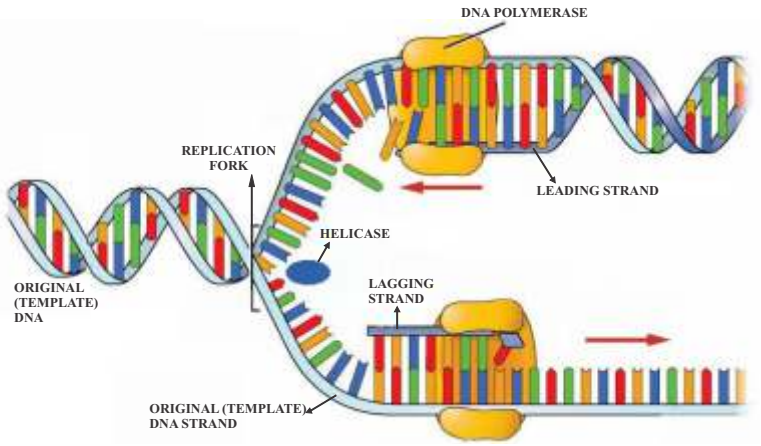


Fig.17 (a)

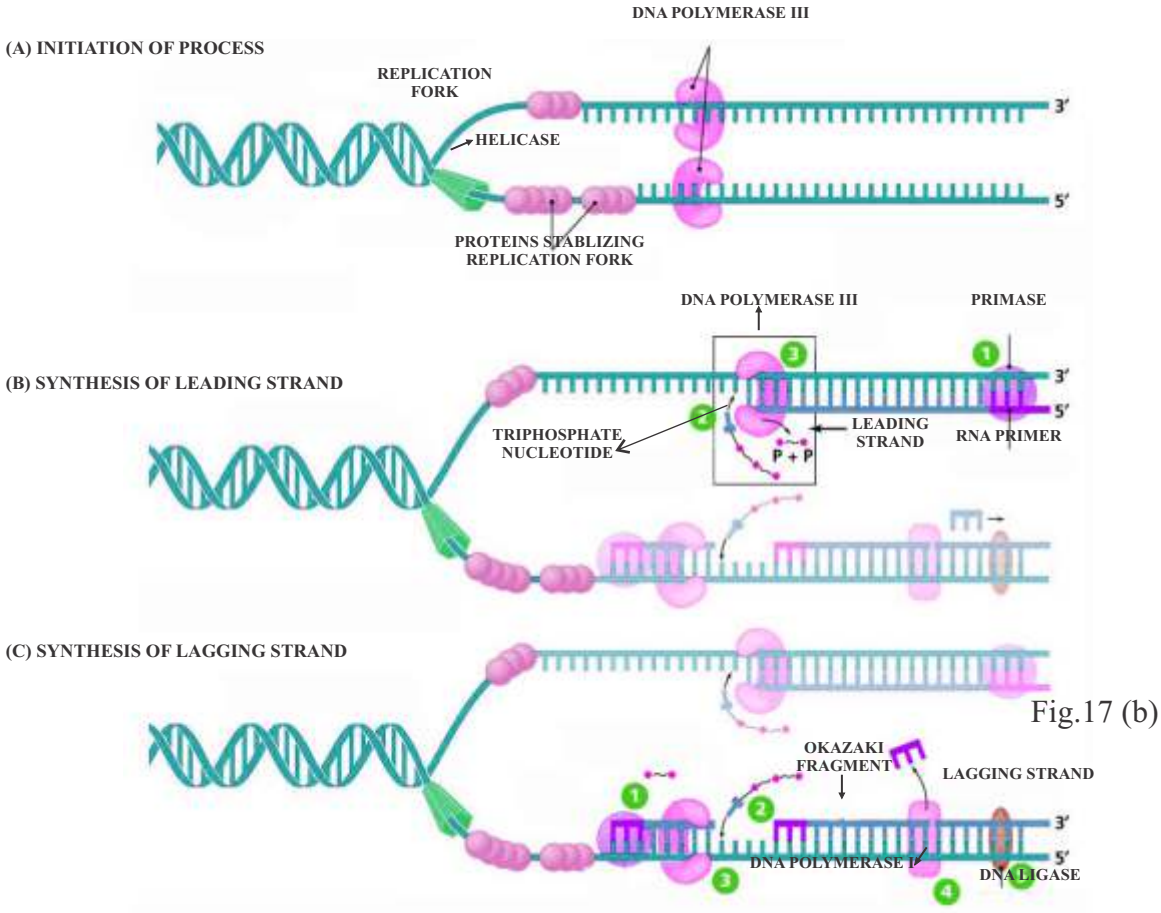


Fig.17 (b) PROCESS OF REPLICATION

CONTINUOUS AND DISCONTINUOS SYNTHESIS OF DNA

LEADING STRAND : It requires a single RNA primer to synthesize new strand in only 5' to 3' in the direction of the replication fork movement.

LAGGING STRAND : It requires many RNA primers to synthesize new strand in short fragments (Okazaki fragments) in 5' to 3', in the opposite direction of leading strand. It is semi discontinuous (i.e., not continuous)

6.2 TRANSCRIPTION

The first step of gene expression is transcription, in which a particular segment of DNA is copied into RNA by the enzyme DNA dependent RNA polymerase. RNA helps to synthesize, regulate, and process proteins; it therefore plays a very crucial role in performing functions within a cell.

TRANSCRIPTION UNIT The stretch of DNA transcribed into an RNA molecule is called a transcription unit and encodes at least one gene. DNA dependent RNA polymerase produces RNA from a transcription unit that extends from the promoter to the terminator.

UPSTREAM SEQUENCES are sequences present before the start site of gene

DOWNSTREAM SEQUENCES are sequences after the start site of gene.

Promoter are all the DNA sequences containing binding sites for DNA dependent RNA polymerase and the transcription factors necessary for normal transcription.

Terminator are those sequence of DNA where transcription stops.

Coding strand is the strand of DNA (5'to 3') which is complementary to template strand and do not get transcribed.

RNA Polymerases

a) Bacterial RNA polymerase has four subunits: alpha, beta, beta' and sigma; only the first three subunits are required for polymerase activity and are considered the core enzyme. The sigma factor is required for RNA polymerase to bind to the promoter; the enzyme has a loose affinity for DNA but when the sigma factor is present it will bind only at a promoter

b) **Eukaryotic RNA Polymerase** has two large subunits; <10 small subunits; largest subunit is homologous to beta', second largest subunit is homologous to the beta subunit. Many non-polymerase factors required for binding of the enzyme to DNA.

Type of Polymerase	Final product	Location
RNA Polymerase III	tRNA	nucleoplasm
RNA Polymerase II	hnRNA	nucleoplasm
RNA Polymerase I	rRNA	nucleolus

PROCESS OF TRANSCRIPTION

Binding of sigma factor protein to the DNA dependent
RNA polymerase (holoenzyme) ⇩

RNA polymerase binds to promoter DNA
⇩

RNA polymerase breaks the hydrogen bonds between complementary
DNA nucleotides and separates the two strands of the DNA helix
⇩

RNA polymerase adds matching RNA nucleotides to template strand
⇩

A sugar-phosphate backbone forms with assistance from
RNA polymerase to form an RNA strand.
⇩

Hydrogen bonds of the untwisted RNA-DNA helix break,
And newly synthesized RNA strand becomes free
⇩

In eukaryotes, the RNA may be further modified through
polyadenylation, capping and splicing
⇩

The RNA may remain in the nucleus or exit to the cytoplasm

Polycistronic mRNA encodes several polypeptides and is characteristic of many bacterial and chloroplast mRNAs. Polycistronic mRNAs consist of a leader sequence which precedes two or more genes interrupted by intercistronic region. A trailer sequence follows the last gene in the mRNA.

Monocistronic mRNA encodes only one polypeptide and all eukaryotic mRNAs are monocistronic.

POST-TRANSCRIPTIONAL MODIFICATIONS STEPS IN EUKARYOTES:

In Eukaryotes, primary transcript contain exons which are RNA sequences found in the mRNA and introns , RNA sequences between exons that are removed by splicing , and therefore, not coded .

Heterogeneous nuclear RNA (hnRNA) is the original product of transcription. hnRNA transcription is only a small portion of the transcription which occurs in the nucleus. Up to 90% of the transcription is for the production of rRNA. hnRNA actually exist in the nucleus in associatation with proteins and the complex designated as heteronuclear ribonuclearprotein (hnRNP) . All of the post-transcriptional modifications occur in the nucleus of the cell and the final product, the mRNA, is transported to the cytoplasm for translation.

The development of the mature monocistronic eukaryotic transcript involves several different processing steps. These steps are:

i. CAPPING

The first step in processing is the addition of a Cap (5' methyl guanosine) immediately after the start of transcription. Capping is a quick process. The linkage between the 5' methyl guanosine is a 5'-5' linkage. The reaction is catalyzed by the enzyme guanylyl transferase. In case cap 0,the guanosine that is added is always methylated at the 7 position of the guanine base (7mG). In addition a methyl group is added to 2'-OH of the original base in the mRNA. This is catalyzed by 2'-O-methyl-transferase, and this methyl group is referred to as cap 1.

ii. SPLICING

RNA splicing is the process by which introns are removed from the hnRNA and the remaining exons connected to re-form a single continuous molecule. Although most RNA splicing occurs after the complete synthesis and 5'end-capping of the hnRNA. Transcripts with many exons can be spliced co-transcriptionally. The splicing reaction is catalyzed by a large protein complex called the spliceosomes assembled from proteins and small nuclear RNA molecules.

iii. TAILING

The hnRNA processing at the 3' end of the RNA molecule involves cleavage of its 3' end and then the addition of about 250 adenine residues to form a poly(A) tail. As the poly (A) tail is synthesised, it binds multiple copies of poly (A) binding protein, which protects the 3'end from ribonuclease digestion.

DIFFERENCE BETWEEN PROKARYOTIC AND EUKARYOTIC TRANSCRIPTION

PROKARYOTES	EUKARYOTES
Occurs in cytoplasm	Occurs in nucleus
Transcription and translation are coupled process in cytoplasm	Transcription occurs in nucleus and translation in cytoplasm
A single RNA polymerase synthesizes all the three types of RNA (mRNA, tRNA, rRNA)	The RNA polymerases I, II and III synthesizes rRNA, mRNA and tRNA respectively.
No processing of mRNA takes place	hnRNAs are released and processes in the nusleus.
RNA polymerases are complexes of five polypeptides.	RNA polymerases are complexes of 10-15 polypeptides.
Polycistronic mRNA	Monocistronic mRNA
Conserved promoter sequences are TATAAT, TTGACA	Conserved promoter sequences are TATA (TATA box), CAAT (CAAT box)

6.3 TRANSLATION

Protein synthesis is vital to repair damages to the organelles and to add new organelles once the cell divides. Proteins are vital because they benefit the entire body through various ways as enzymes, hormones and component of cell membrane. A linear chain of amino acid residues is called a **polypeptide**. Proteins are assembled from amino acids using information encoded in genes.

The process of synthesizing a protein from an mRNA template is known as **translation**.

Translation involves three steps:

1. Initiation
2. Elongation
3. Termination

INITIATION	ELONGATION	TERMINATION
I. Small ribosomal subunit binds to a specific sequence on the mRNA chain with the help of initiation factor. **II.**A special tRNA molecule, fMet-tRNA, binds to the initiator codon (AUG). **III.**The large subunit binds to form the initiation complex. **IV.** The fMet-tRNA, occupies the P site of the ribosome and the A site is left empty.	**V.** Now, fMet-tRNA bearing Methionine is in the p site. **VI.** An aminoacyl tRNA with the complementary anticodon sequence reaches in the A site, and bind to the mRNA passing through the acceptor site. **VII.**The peptide bond occurs between the carboxyl group on the lowest link in the peptide chain located at the p site and the amine group on the amino acid in the A site catalyzed by Peptidyl transferase (ribozyme) with help of GTP. **VIII.**The peptide chain shifts over to the A site, with the original amino acid on the A site as the lowest link in the chain. **IX.**The tRNA in the A site becomes peptidyl RNA, and shifts over to the P site **X.** Ribosome moves ahead so that a new mRNA codon is accessible in the A site. **XI.** This process repeats, creating a polypeptide chain in the P site of the ribosome	**XII.** UAA, UAG, or UGA sequence enter the A site of the ribosome. **XIII.** No aminoacyl tRNA recognizes these sequences, then Release factors bind to the P site **XIV.** Release of the completed polypeptide chain and separation of the ribosome into its original small and large subunits.

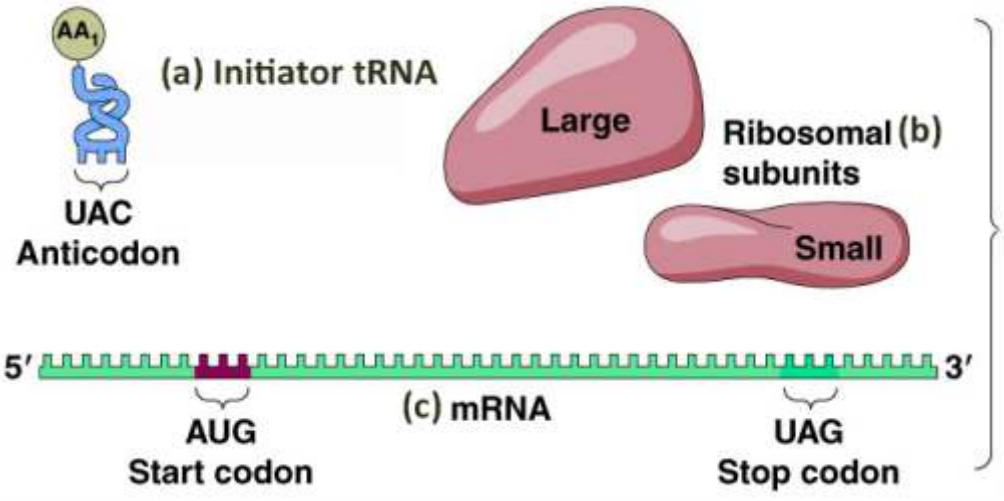


Fig. 18 COMPONENTS REQUIRED FOR TRNASLATION

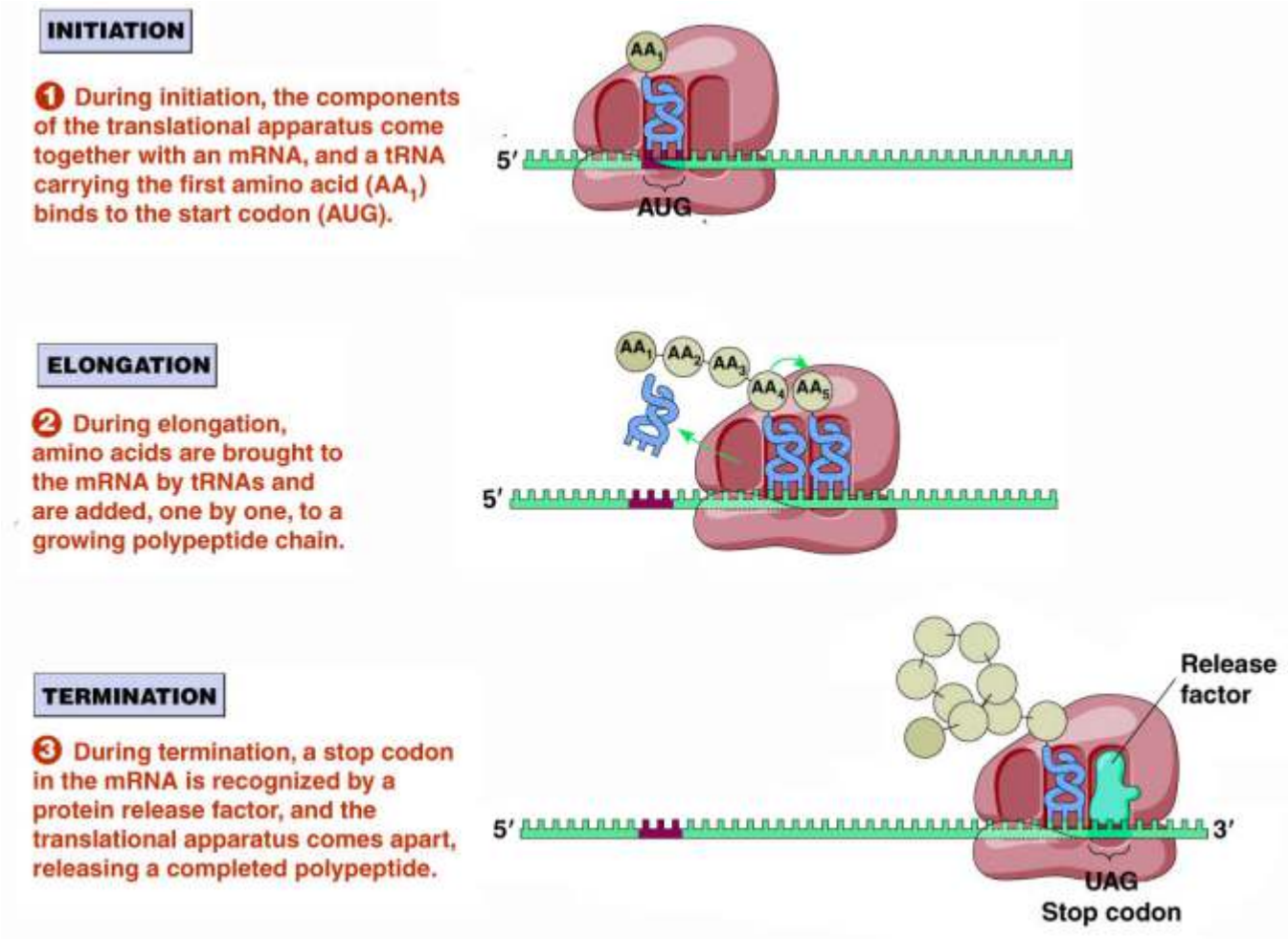
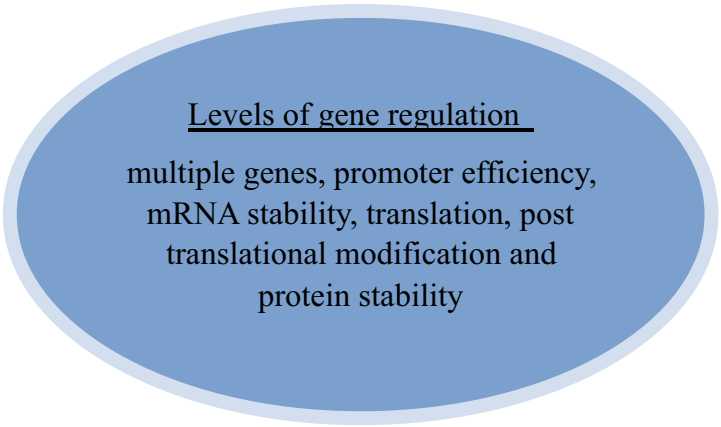


Fig. 19 STEPS OF DNA TRANSLATION

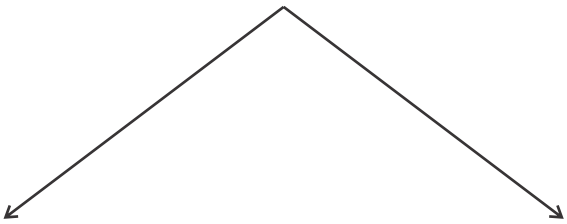
Table showing Differences in Prokaryotic and Eukaryotic Translation

PROKARYOTIC TRANSLATION	EUKARYOTIC TRANSLATION
Occurs in 70S ribosome	Occurs in 80S ribosome (free or ER membrane bound)
Translation and transcription are usually coupled processes in cytoplasm	Translation occurs in cytoplasm and transcription in nucleus
Faster process	Slower process
Require three initiation factors and three release factors	Require a set of nine initiation and single release factor
mRNA life is short (few second to 2 minutes)and mRNA is unstable	mRNA has life of few hours to few days and mRNA is quite stable
Starts with addition of f- methionine	Starts with addition of methionine

3.4 GENE REGULATION -- THE LAC OPERON



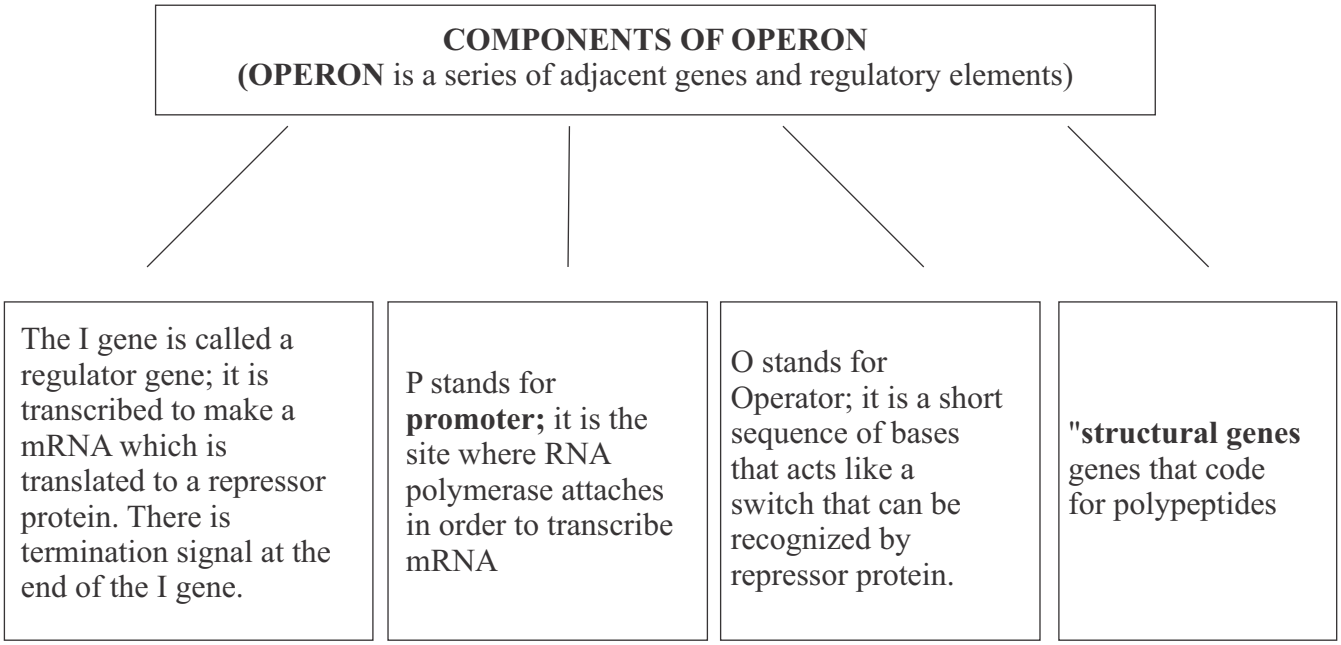
CATEGORIES OF GENES



HOUSEKEEPING GENES
Gene products are required by the cell under all growth conditions e.g. respiratory genes

REGULATORY GENES
Gene products are required under specific growth condition e.g. enzymes

Francois Jacob and Jacques Monod won a Nobel Prize for their work in describing how the lac operon functions.



Importance of Gene Regulation in Lac operon-

Regulation of transcription is especially effective because mRNA typically has a short half life (1.8 minutes in *E. coli*) so stopping mRNA synthesis leads to rapid changes in protein synthesis. It takes lots of energy to make mRNAs (and proteins) so making them when they are not needed is inefficient.

The Lac Operon has to do with the ability of *E. coli* to utilize the sugar lactose. Lactose is diassacharide of glucose and galactose. Glucose is a very efficient carbon source as it can enter directly into the metabolic paths that provide both energy and substrates for making more complex compounds. But if lactose is provided as the carbon source, it must be broken down into the two component sugars before it can be used. The enzyme for breaking down lactose in E. coli is called b-galactosidase. E. coli grown in glucose as the sole carbon source have about 3 copies of the enzyme b- galactosidase/cell. E. coli grown in lactose as the sole carbon source have about 3,000 copies of the enzyme b- galactosidase/cell. The system of regulation seen here is called "induction" since synthesis of the enzyme is "turned on" only when needed. Induction typically is used to regulate "breakdown" (catabolic) pathways as opposed to "synthetic" (anabolic) pathways.

z, 'y' and 'a' are all **"structural genes** (genes that code for polypeptides). 'z' codes for galactosidase; 'y' codes for lactose permease, a protein that functions to actively bring lactose from outside to cell to the inside, even against a concentration gradient and 'a' encodes for transacetylase, an enzyme that is also needed to breakdown many sugars related to lactose. One long mRNA is made for the 'z', 'y' and 'a' genes; this is the basis for the system being called an **operon**. All 3 genes that code for enzymes needed to use b-galactoside molecules as a source of carbon and energy are adjacent and are coordinately turned on or off by regulating transcription. Operons are only found in prokaryotes; in eukaryotes, each structural gene has its own promoter and regulatory elements

IN THE ABSENCE OF LACTOSE

Stepwise:

1. The Promoter for the 'i' gene is always "on".
2. The 'i' mRNA is translated into a polypeptide. (repressor protein)
3. In the absence of lactose, the repressor protein binds to the operator, preventing transcription from the second promoter.
4. Almost no 'z' 'y' 'a' mRNA is made.

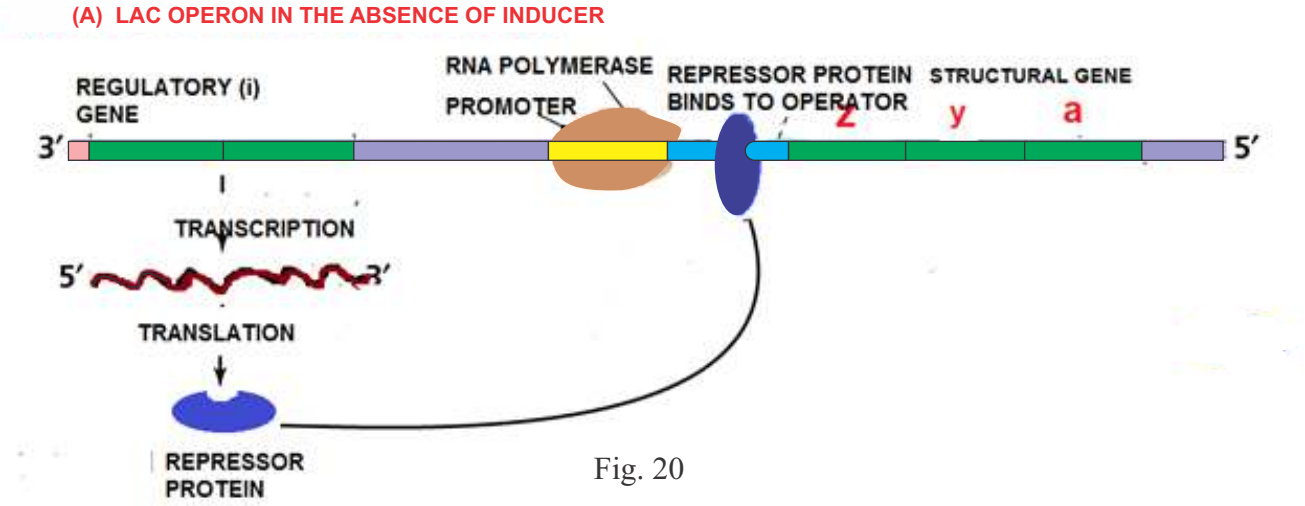


Fig. 20

IN THE PRESENCE OF LACTOSE

Stepwise: 1. The Promoter for the 'i' gene occasionally is bound by an RNA polymerase to initiate transcription and translation into repressor protein.

2. Lactose binds to the repressor very efficiently and converts the repressor into an inactive state, where it can't bind the Operator. The process is reversed when all the lactose is digested, so the system again will turn off.

3. When Promoter for making z-y-a mRNA is not blocked, many copies of the mRNA are made. The small amount of lactose that diffuses in is able to initiate induction of transcription of the z-y-a mRNA. Even as the message is being made, translation begins and the 3 proteins are made.

4. Translation begins at the 5' end of the mRNA and makes b-galactosidase from the z gene. There is a stop codon, followed immediately by another AUG start, so many, but not all, ribosomes read on through and make permease from the y gene. The same process allows some a gene product to also be made.

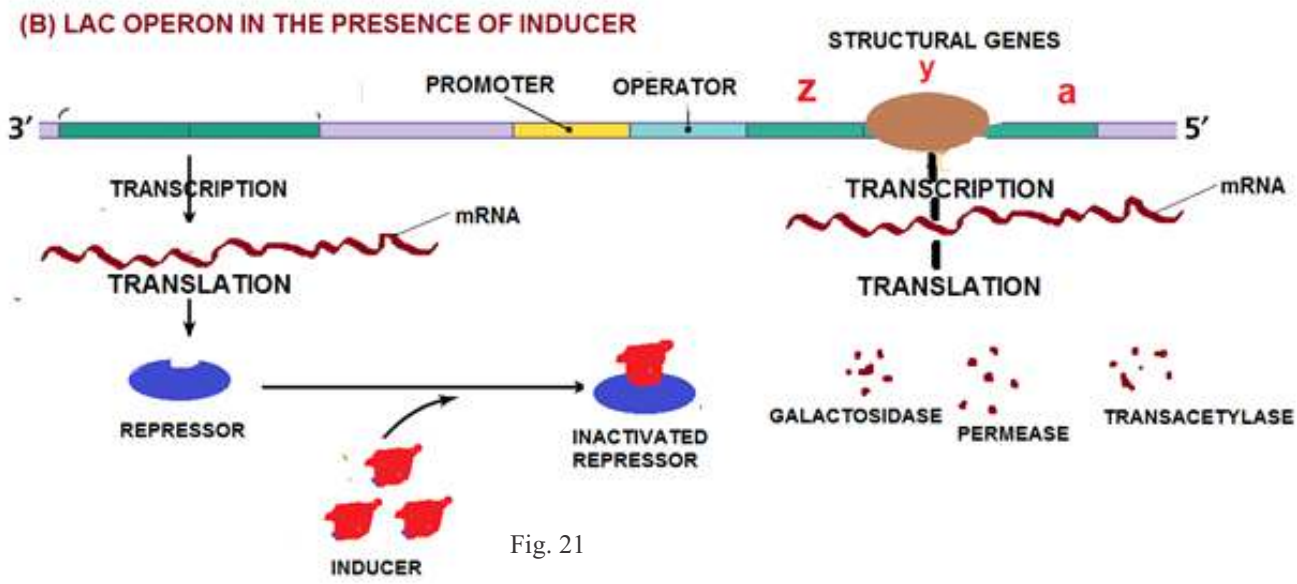


Fig. 21

6.5 DNA-FINGERPRINTING

Geneticist Alec J. Jeffreys from the University of Leicester discovered that there are patterns of DNA that are unique to almost every individual. The major uses for the information provided by DNA-fingerprinting analysis are for personal identification, the determination of paternity, criminal cases and family relationship tests.

DNA (deoxyribonucleic acid) represents the blueprint of the human genetic makeup and 99.9% of human genome is identical from one person to the next. It exists in virtually every cell of the human body and differs in its sequence of nucleotides. 0.1% variation, therefore, can be used to distinguish one individual from another.

DNA fingerprinting is based on DNA analyzed from regions in the genome that separate genes called introns or junk DNA which do not encode or any protein. They are spliced out during processing of the messenger RNA, which is an intermediate molecule that allows DNA to encode protein.
The original DNA fingerprinting procedure used Variable Number Tandem Repeats (VNTR), which are repetitive DNA sequences that are spread throughout the genome in noncoding regions. These targets are large, with repeat numbers that are variable from person to person and have a repeat size composed of hundreds of nucleotides which can be repeated a hundred times.

Satellite DNA consists of very large arrays of tandem repeats, non-coding DNA. Satellite DNA is the main component of functional centromeres and forms the main structural constituent of heterochromatin.
The name "satellite DNA" refers to how repetitions of a short DNA sequence tend to produce a different frequency of the nucleotides adenine, cytosine, guanine and thymine, and thus have a different density from genomic DNA - such that they form a second or 'satellite' band when genomic DNA is separated on a density gradient.

Tandem repeats occur in DNA when a pattern of one or more nucleotides is repeated and the repetitions are directly adjacent to each other

An example would be:
TTTCG TTTCG TTTCG
in which the sequence TTTCG is repeated three times.
Depending upon the length,GC ratio and no of nucleotide repeat they have been classified as follows :

a) A microsatellite consists of tandemly repeated (i.e. adjacent) DNA motifs that range in length from two to five nucleotides, and are typically repeated 5-50 times. For example, the sequence TATATATATA is a dinucleotide microsatellite, and GTCGTCGTCGTCGTC is a trinucleotide microsatellite

Microsatellites are distributed throughout the genome. Many are located in non-coding parts of the human genome and are therefore biologically silent. This allows them to accumulate mutations over the generations and gives rise to variability which can be used for DNA fingerprinting and identification purposes

b) A **minisatellite** is a tract of repetitive DNA in which certain DNA motifs (ranging in length from 10-60 base pairs) are typically repeated 5-50 times. Minisatellites occur at more than 1,000 locations in the human genome and they are notable for their high mutation rate and high diversity in the population. Minisatellites are prominent in the centromeres and telomeres of chromosomes, the latter protecting the chromosomes from damage.

Minisatellites are often referred to as VNTRs, and microsatellites are often referred to as short tandem repeats (STRs).

The commonly used DNA finger printing procedure are :
1) One of the most common DNA fingerprinting procedures is RFLP (Restriction Fragment Length Polymorphism) Selected Restriction Endonucleases are used to digest DNA. These segments are then separated using a technique called Electrophoresis, which separate them on the basis of size . Once they have been sorted in this way a visual representation of the results is created using a procedure known as Autoradiography.

The integrity of the sample as well as the quantity, therefore, can make reliable and definitive identity determination difficult in case of using VNTR because of its repeat size composed of hundreds of nucleotides and number of repetitions of the same and DNA extracted from samples in a crime scene is often broken up into tiny pieces due in most cases to natural DNA-degrading processes. Also, the smaller DNA sample degrades faster.

2) A more recent form of test is the STR Test (Short Tandem Repeat Test), which looks at DNA segments and counts the number of repeats at a number of different DNA sites - normally around thirteen. In this case only 50pg of DNA is required.

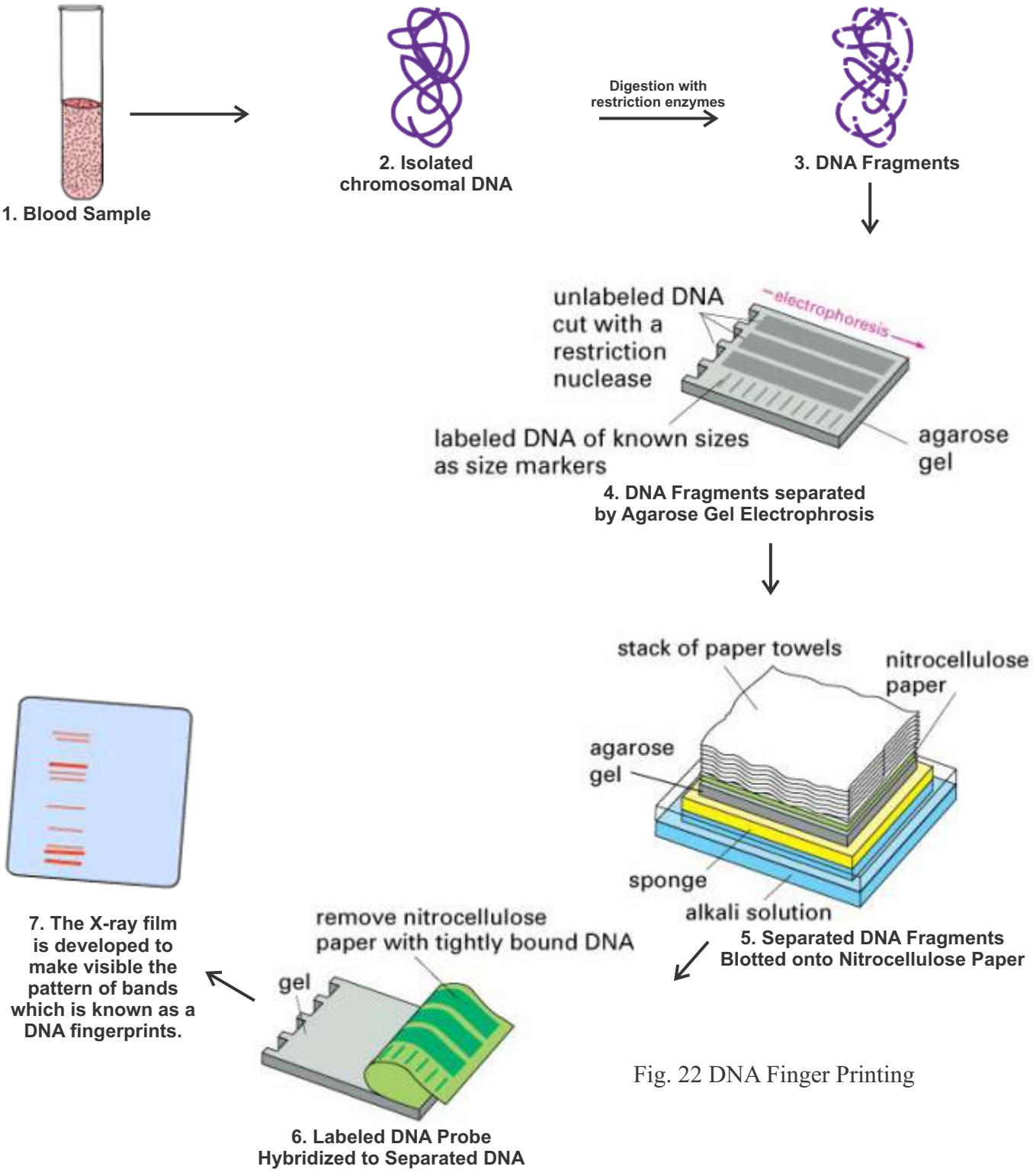


Fig. 22 DNA Finger Printing

3.6 Glossary

Capping: Addition of methylated Guanine at 5' end of mRNA.

Charging of tRNA: Addition of amino acid at 3' end of tRNA.

DNA polymorphism: Variation at genetic level which arise due to mutation.

DNA probe: Single stranded DNA having base sequences complementary to the possible VNTR.

Gel electrophoresis: A technique to separate DNA fragments on the basis of their sizes.

Gene- a segment of DNA that encodes for RNA

Housekeeping genes: Gene which constantly expresses themselves in cell as their product are needed for normal functioning of cells.

Inducer: The substrate which activates the operon.

Inducible operon: The operon which gets activated in the presence of substrate.

Okazaki fragments: Small segment of DNA formed at lagging strand.

Origin of replication : Definite region of DNA from where DNA replication originates.

Primer : A small stretch of RNA formed on template strand by RNA primer.

Regulatory genes: These genes get switched on or off as per cellular requirements.

Release factor: Translation terminates in the presence of release factor.

Replication fork: The Y shaped structure formed when double stranded DNA uncoiled during replication.

Repressor: The biomolecules whose binding the with operator switch off operon.

Rho factor : The factor required for termination of transcription.

Ribozyme: RNA molecule acting as enzyme.

Sigma factor: The factor which recognizes promoter sequences of DNA and bind with core enzyme.

Splicing : Removal of introns and joining of exons to form functional mRNA.

Tailing: Addition of Poly A segment at 3' end of mRNA.

3.7 Further reading:

1. Biology by Ravan, Johnson, Losos,Singev
2. Genetics by Strickberg
3. Textbook of Genetics by Cumin

CHAPTER - 4

BIOTECHNOLOGY : PRINCIPLES AND PROCESSES

Objectives: After going through the content the learner will be able to understand the following:

- Importance of genetic engineering
- Role of vectors
- Gel electrophoresis
- Methods of introduction of foreign DNA
- Screening methods

Table of content:

Genetic Engineering (CLASS - XII, LESSON - 11)

INTRODUCTION

Biotechnology has influenced every sphere of human life. Today, recombinant proteins and other products are in reach of common man. Organisms that have been manipulated as well as products derived from those organisms, have found their way into our lives. All this has been possible only when man learnt to alter the DNA and construct recombinant DNA. This process is called genetic engineering or recombinant DNA technology.

Let's understand what is recombinant DNA technology?

Recombinant DNA technology is the use of in vitro molecular techniques to isolate and manipulate the gene of interest either to get multiple copies (gene cloning) or desired effect.

Laboratory techniques for gene cloning were devised during early 1970s. Since then much technological advancement has been done in the field of biotechnology. Genetic engineering is based on the fact that nucleic acid is the genetic material of all the organisms without exception. Nucleic acid from one organism can be selectively isolated and introduced into target organism.

Genetic engineering has made it possible now to get cheap, tailor made products in substantial quantity.

Recombinant DNA technology involves the following steps:

1. Choice of host organism
2. Choice of cloning vector
3. Isolation and preparation of DNA to be cloned
4. Preparation of vector DNA
5. Creation of recombinant DNA
6. Introduction of recombinant DNA into host organism
7. Selection of transformants
8. Screening for recombinants and their biological products

(a) Selection of host organism : The selected host organism should be

- sophisticated,
- versatile
- easily and widely available
- simple growth needs
- high growth rate

In prokaryotes *E.Coli* is preferred choice.

In eukaryotic system, yeast and filamentous fungi are preferred as host for the production of recombinant proteins. The use of animal cell is difficult due to the fact that many need a solid support surface, unlike bacteria and have complex growth needs. But, some proteins are too large and complex to be produced so they need to be produced in eukaryotic cells.

(b) Selection of cloning vector: The choice of vector depends on the

- choice of host organism,
- size of DNA to be cloned and
- how foreign DNA is to be expressed.

Specific features of vectors

- Origin of Replication (ori) is necessary for the vector and its linked recombinant sequences to replicate inside the host organism. It also controls the copy number.
- One or more unique restriction endonuclease sites for the introduction of foreign DNA may be introduced in vector.
- Selectable marker gene will be used to enable the survival of the cells that have taken up vector sequences (transformants).
- A tag gene that can be used to screen the cells containing the foreign DNA (recombinants).

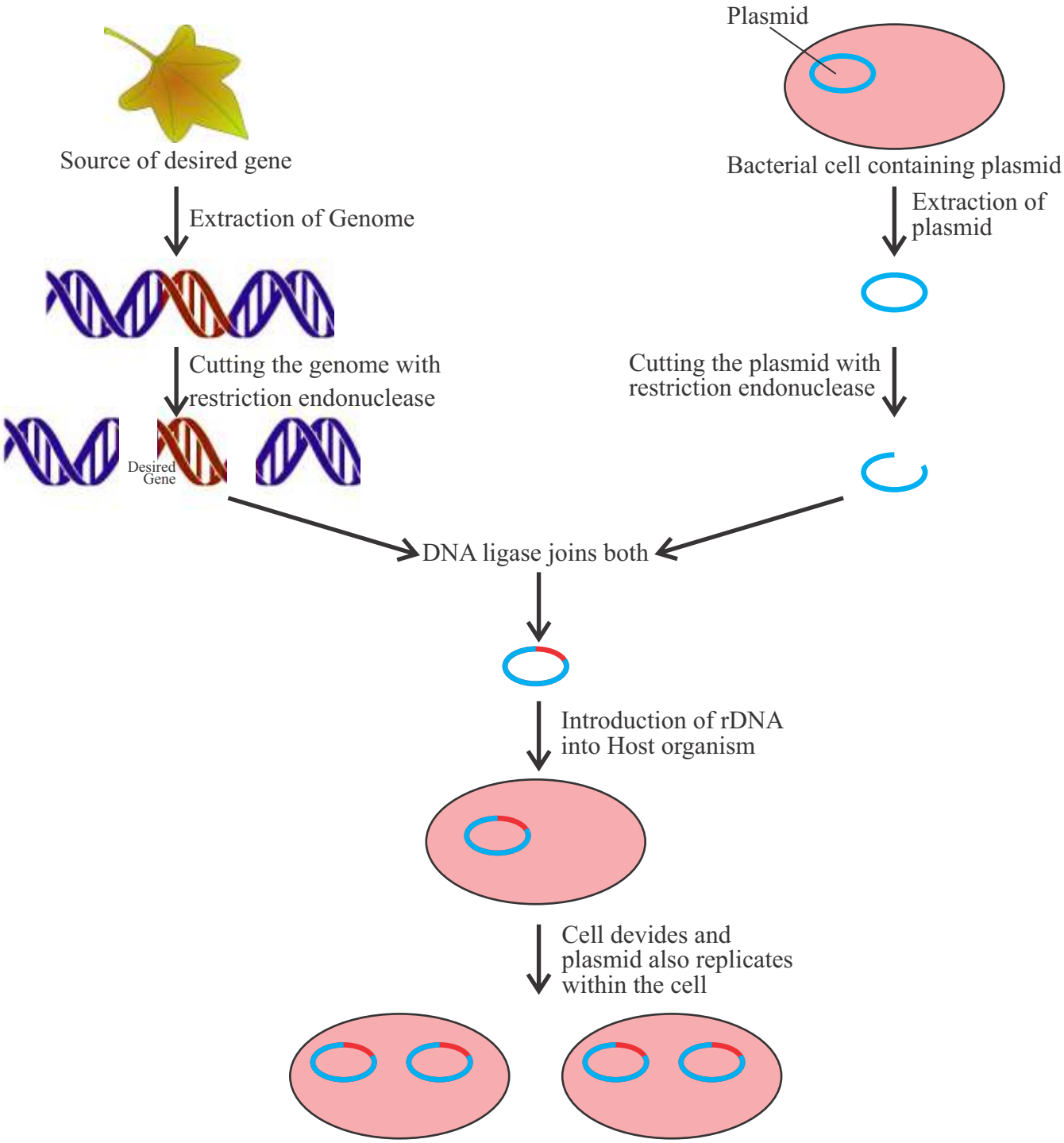


Fig 23 BASIC STEPS OF rDNA TECHNOLOGY

Vectors are generally derived from plasmids and viruses. Plasmids are self replicating, extra chromosomal, circular DNA found in bacterial cells. The vector selected must have their high copy number per host cells so that they have high copy no of their genome within the cell. If the DNA is to be cloned is exceptionally large, then the Bacterial artificial chromosome (BAC) or yeast artificial chromosome (YAC) is often used. Other vectors are cosmids, phasmids and bacteriophage etc.

c. **Isolation and preparation of DNA to be cloned :** Virtually any tissue source can be used to extract DNA to be cloned.DNA can be extracted from bacterial cells, plant cells ar animal cells with enzymes such as lysozymes (bacteria), cellulose (plant cells), chitinase (fungus).DNA is purified by removing contaminating proteins (proteases), RNA (ribonucleases) and small molecules (precipitation).

The purified DNA is then treated with restriction endonucleas resulting into many fragments of DNA which are separated by a technique called gel electrophoresis. The desired separated band is cut out from the agarose gel and extracted out of it (Elution).The cutting with restriction enonuclease generates sticky ends. In case of restriction endonuclease generating blunt ends, short double stranded segments of DNA (linkers) containing desired restriction sites may be added to create end stretches that are compatible with vector.

d. **Preparation of vector DNA:** Cloning vector is treated with a restriction endonuclease to cleave the DNA at the site where foreign DNA will be inserted. This site is located within the gene whose inactivation is useful in selection of recombinants at later step in process.

Restriction enzymes are made naturally by many species of bacteria in which they protect bacteria from viruses. Restriction endonuclease generally selected are those which cut the strand of DNA a little away from the centre of pallindromic site, but between the same two basis on the opposite strand resulting into sticky ends on each strand. Typically, vector DNA and foreign DNA is cleaved with the same restriction endonuclease, for example ECORI. If the resultant DNA fragments have the same kind of sticky ends and these can be joined together.

e. **Creation of recombinant DNA:** DNA prepared from vector and foreign source are simply mixed together along with DNA ligase that links the sticky ends together. The resulting DNA mixture contains randomly joined ends and is ready for introduction into the organisms.

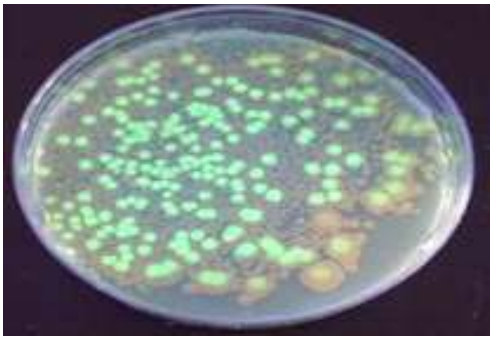
DNA ligase act on the end of linear DNA molecules with randomly joined ends (e.g. Foreign DNA linked to itself, vector DNA linked to itself) which is sorted out in subsequent steps.

f. **Introduction of recombinant DNA into host organism:** A DNA mixture, manipulated in vitro is introduced into host organism through variety of methods. These methods usually require preparation of the cells through chemical treatment processes to make them competent.

- Heat shock method (bacteria)
- Microinjection (animal cell)
- Biolostics or gene gun (plant cells)
- Disarmed pathogen
- Some latest methods like
- Electroporation
- Transduction

g. **Selection of transformants:** After you transform the cells, that is, induce the cells to take up the vector; you grow them on culture media containing the antibiotic cells that have the vector (the transformants) are resistant to the antibiotic and grow, cells that do not take the vector up are not resistant and do not grow.

h. **Screening for recombinants:** Foreign DNA is introduced into a sequence that encodes an essential part of beta- galactosidase ,an enzyme whose activity result in formation of blue colored colony on culture medium. Insertion of foreign DNA into the beta-galactosidase coding sequence disables the function of this enzyme, so colonies containing transformed DNA remain colorless (white) and ultimately help in selection of recombinants from non recombinants.



Bacterial colonies

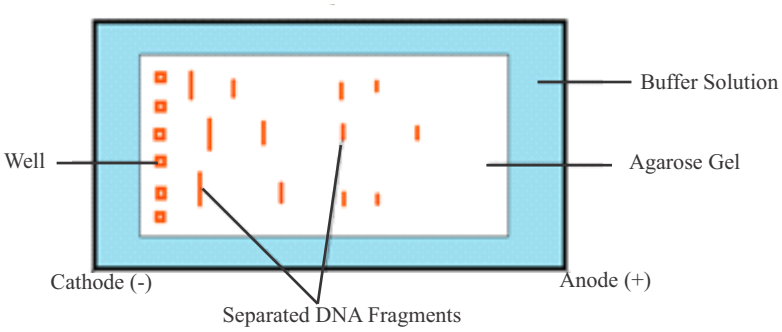


Fig. 25 Gel Electrophoresis

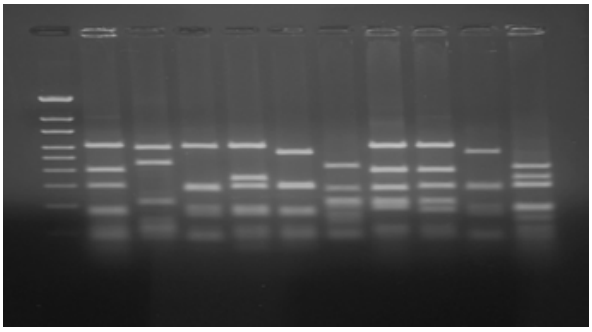


Figure: 26, 1.5% agarose gel electrophoresis showing the heterogeneity in a DNA sample upon restriction digestion.

Further studies on the transgenic bacterial colonies can be done.

After the gene cloning, optimization of growth condition is needed to induce its expression in heterologous host. The cells harboring cloned genes may be grown on the small scale in laboratory and on large scale in bioreactor.

After completion of biosynthetic phase the product is separated, purified and formulated with suitable preservatives.

ACTIVITY: Isolation of DNA from plant leaves.

Requirements: plant leaves, pestle and mortar, liquid detergent, salt, tap water and chilled ethanol.

Procedure:

1. Take a few tender plant leaves and tear them off to remove veins
2. Put leaves in mortar, add a pinch of salt and liquid detergent
3. Grind the content thoroughly with pestle to make a thick paste .Water can be added to facilitate the grinding
4. Now collect all the content in a test tube and allow it to stand for about half an hour
5. Separate out the supernatant in another test tube and then pour chilled ethanol
6 Mix both the contents gently and allow the mixture to stand for few minutes

Observation: Long and thin threads of DNA are visible

Glossary

Cloning vector: Any molecule of DNA to which desired DNA is ligated , multiple copies can be obtained by transferring the recombinant DNA into host cell.e.g. Plasmid

Elution: extraction of desired DNA fragment by cutting agarose gel containing the same.

Genetic engineering: Use of rDNA methods to confer new traits in organisms by introducing new genes.

Recombinants: The cells which has taken up recombinant DNA.

Recombinant DNA: A molecule of DNA formed by joining DNA segments from different resources.

Restriction Enzymes: Molecular scissors which recognize specific palindromic sequences of DNA and

cleave at specific site.

Transformant: cells containing vector.

Further reading :

1. Biotechnology and molecular biology by J.M.Walker

2 .Genes by B.Lewin

3. Biotechnology by Baltimore