KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act 1956)

Coimbatore – 641 021.

LECTURE PLAN

DEPARTMENT OF BIOTECHNOLOGY

STAFF NAME: Dr. BARATHKUMAR, S.
SUBJECT NAME: CELL BIOLOGY AND MOLECULAR GENETICS SUB.CODE:18BTP102
SEMESTER: I CLASS: I M.Sc.

SL.NO.	HOURS	TOPIC	SUPPORTING MATERIALS
		UNIT I	
1	1	Structure of prokaryotic and eukaryotic cells	T1- 10-18
2	1	Plasma membrane – Properties and functions, cell wall	T1- 117-125
3	1	Mitochondria	T1- 174-200
4	1	Chloroplast	T1- 208-209
5	1	Peroxisomes	
6	1	Golgi complex	
7	1	Endoplasmic reticulum	T1- 299-303
8	1	Lysosome	T1- 308
9	1	Cell division	T1- 560-599
10	1	Revision	
		UNIT II	
11	1	Replication –Types of replication	T1- 534-546
12	1	Transcription	T1- 425 - 440

13	1	Post Transcriptional Modification	
14	1	Translation	T1- 455 - 456
15	1	Post Translational modification	T1- 529
16	1	regulation of gene expression in Prokaryotes	T2- 1085 - 1097
17	1	regulation of gene expression in Eukaryotes	
18	1	Revision	
UNIT III			
19	1	Mendelian law	T3- 21-24
20	1	Non-Mendelian principles	
21	1	Genetic recombination	T4- 939-953
22	1	Genetic mapping	W1
23	1	Linkage	T4- 724 - 730
24	1	crossing over	T4- 730 - 734
25	1	Mutations and its types	W2
26	1	Genetic analysis of Mutations	
27	1	DNA repair Mechanisms	T4-851-864
28	1	Revision	
UNIT IV			
29	1	Transposons	T1- 402-415
30	1	Types of bacterial transposons	

31	1	Transposition	T1- 415-425
32	1	Detection of Transposition in Bacteria	
33	1	Excision of Transposons	
34	1	Types of Transposons in Eukaryotic cells	W1
35	1	Revision	
UNIT V			
36	1	Gene transfer in Bacteria	T1- 145-146
37	1	Transformation	T1- 147-156
38	1	Transduction	
39	1	Conjugation	
40	1	Bacteriophages - Structure & properties	T1-163-165
41	1	Lytic cycle	T1- 160-162
42	1	Lysogenic cycle	
43	1	Role of phages as vectors	T1-163-167
44	1	Revision	
45	1	Discussion of previous year ESE question paper	
46	1	Discussion of previous year ESE question paper	
47	1	Discussion of previous year ESE question paper	

References

T1 – Gernald and Karp, 2010, Cell and Molecular biology concepts and experiments 6th ed., Wiley International New York.

T2 – Lehninger and Nelson 2005, 4th ed., Principles of biochemistry, CBS publishers.

T3 – Gadner G 2001, Principles of Genetics, Wiley, New York.

T4 – Textbook of cell biology, 1st Ed. Aminul Islam, Books & Allied (P) Ltd., Kolkata.

W1 - www:biologydiscussion.com

W2- https://www.ncbi.nlm.nih.gov/books/NBK21475/.

Semester -I

18BTP102 CELL BIOLOGY AND MOLECULAR GENETICS 4H – 4C

Total hours/week: L:4 T:0 P:0 Marks: Internal:40 External:60 Total: 100

Course Objectives:

- **Students will understand the structures and purposes of basic components of cells, and how these cellular components are used to generate and utilize energy in cells**
- **To impart knowledge in genetics and genome organizations in organisms.**

Course Outcomes:

This paper will enable the students to learn the basics and lay strong foundation in understanding the composition of cells, how cells works is fundamental to living systems.
The structural and functional aspects of the cell provide the student with a strong foundation in the molecular mechanisms underlying cellular function.

UNIT-I

Cell Organization and regulation: Structure of prokaryotic and eukaryotic cells, Structural organization and function of intracellular organelles (Nucleus, Endoplasmic Reticulum, Golgi complex, Mitochondria, Chloroplast, Lysosomes, Peroxisomes and vacuoles, Cytoskeletons. Chromatin organization and packaging. Nucleic Acid - Replication, Types, Transcription, Post Transcriptional Modification, Translation and Post Translational modification, regulation of gene expression.

UNIT - II

Structure of model membrane, lipid bilayer and membrane protein diffusion, osmosis, ion channels, active transport, and ion pumps. Intracellular protein sorting- Mechanism and regulation of intracellular transport in mitochondria, chloroplast, endoplasmic reticulum and nucleus. Electrical properties of membranes. Cell cycle and its regulation, Molecular events Check points, Cyclins and protein kinases.

UNIT -III

Genetics: Mendelian and Non-Mendelian principles. Concept of gene : Allele, multiple alleles, pseudoallele, complementation tests. Genetic recombination, Genetic mapping, linkage and crossing over. Mutations- Types of Mutation, Genetic analysis of Mutations, DNA repair Mechanisms.

UNIT - IV

Methods of genetic transfers – transformation, conjugation, transduction. mapping genes by interrupted mating, Linkage maps, tetrad analysis, mapping with molecular markers,

mapping by using somatic cell hybrids. Introduction to Transposable elements – Discovery and types, Nomenclature - Insertion sequences - Mechanism – Transposons of E. coli, Bacteriophage and Yeast.

UNIT -V

Microbial and Human genetics - Gene transfer in Bacteria, Bacteriophages - properties, Structure, Role of phages as vectors.

Human genetics - Pedigree analysis, linkage testing, karyotypes, genetic disorders, Eugenics. Epigenetics & Genome Imprinting. Structural and numerical alterations of chromosomes, ploidy and their genetic implications, Quantitative genetics - Polygenetic inheritance, heritability and its measurements, QTL Mapping.

References

Gardner, E.J. (2001). *Principles of Genetics* (8th ed.). New York: John Wiley and Sons.

Karp, G. (2005). *Cell and Molecular Biology: Concepts and Experiments*. (7th ed.) London: John Wiley and Sons, Inc.

Maloy, S.R., Cronan, J.E., & Freifelder, D. (2006). *Microbial Genetics*. (5th ed) Sudbury: Massachusetts, Jones and Bartlett Publishers.

Cooper, G.M. & Hausman, R.E., (2004). *Cell : A Molecular Approach*. (5th ed.) Sunderland: Sinauer Associates, Inc.

Glick, B.R., & Pasternak, J.J. (2003). *Molecular Biotechnology* (3rd ed.). New Delhi: Panima Publishing Corporation,.

Frifielder, D. (2001). *Molecular Biology* (2nd ed.). New Delhi: Narosa Publishing House.

Lodish, B. (2004). *Molecular and cell biology* (5th ed.). New York: Freeman and company.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). *Molecular Biology of the Cell* (4th ed.). New York: Garland Publishing.

Unit I

SYLLABUS

Structure of prokaryotic and eukaryotic cells, Structural organization and function of intracellular organelles (Nucleus, Endoplasmic Reticulum, Golgi complex, Mitochondria, Chloroplast, Lysosomes, Peroxisomes and vacuoles, Cytoskeletons. Chromatin organization and packaging. Nucleic Acid - Replication, Types, Transcription, Post Transcriptional Modification, Translation and Post Translational modification, regulation of gene expression.

Cell

The cell is the basic unit of organization or structure of all living matter.

History*:*

- The cell was discovered by Robert Hooke in 1665.
- He examined very thin slices of cork and saw a multitude of tiny pores.
- He remarked that it looked like the walled compartments of a honeycomb, so he called them cells.
- However, Hooke did not know their real structure or function.
- His cell observations gave no indication of the nucleus and other organelles found in most living cells.

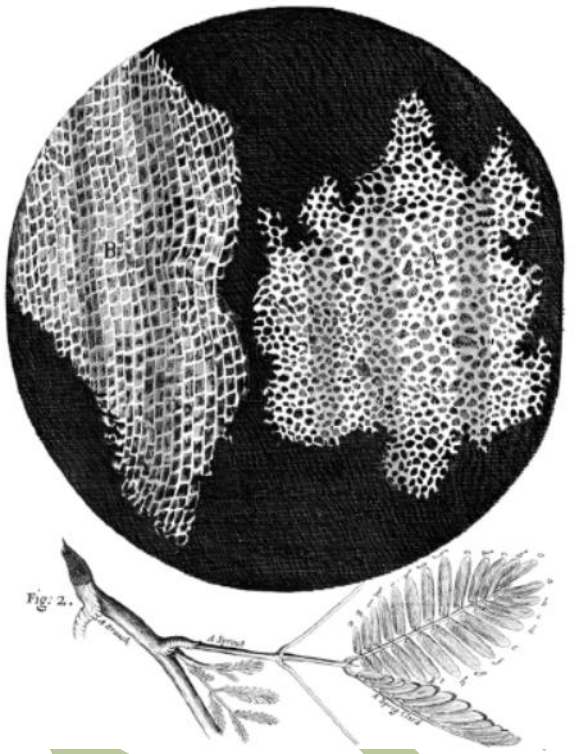

Fig: Drawing of the structure of cork by Robert Hooke that appeared in Micrographia.

"The cell is the fundamental element of organization"

The bservations of Hooke, Leeuwenhoek, Schleiden, Schwann, Virchow, and others led to the development of the cell theory.

The cell theory states:

- All living things or organisms are made of cells.
- New cells are created by old cells dividing into two.
- Cells are the basic building units of life.

Modern interpretation:

The generally accepted parts of modern cell theory include:

1. The cell is the fundamental unit of structure and function in living organisms.
2. All cells arise from pre-existing cells by division.
3. Energy flow (metabolism and biochemistry) occurs within cells.
4. Cells contain hereditary information (DNA) which is passed from cell to cell during cell division.
5. All cells are basically the same in chemical composition in organisms of similar species.
6. All known living things are made up of one or more cells.
7. Some organisms are made up of only one cell and are known as unicellular organisms.
8. Others are multicellular, composed of a number of cells.
9. The activity of an organism depends on the total activity of independent cells.

Exceptions

1. Viruses are considered alive by some, yet they are not made up of cells. Viruses have many features of life, but by definition of the cell theory, they are not alive.
2. The first cell did not originate from a pre-existing cell. There was no exact first cell since the definition of cell is
3. imprecise.
4. Mitochondria and chloroplasts have their own genetic material, and reproduce independently from the rest of the cell.

Classification of cell types

1. Prokaryotes :

- The prokaryotic *(Greek; pro = primitive or before; karyon = nucleus)* are small, simple and most primitive.
- Prokaryotes lack a nucleus *(though they do have circular DNA)* and other membrane-bound organelles *(though they do contain ribosomes)*.
- Bacteria and Archaea are two domains of prokaryotes.

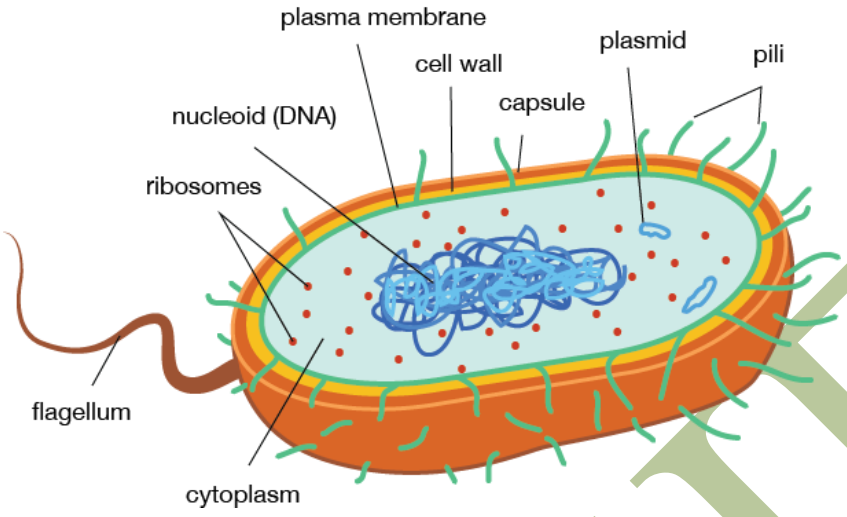

Fig: Schematic diagram of a prokaryotic cell.

Flagella:

- Long, whip-like protrusion found in most prokaryotes that aids in cellular locomotion.
- It also often functions as a sensory organelle, being sensitive to chemicals and temperatures outside the cell.

Capsule:

- It is found in some bacterial cells.
- This additional outer covering protects the cell when it is engulfed by phagocytes and by viruses.
- Assists in retaining moisture, and helps the cell stick to to surfaces and nutrients.
- The capsule is found most commonly among Gram-negative bacteria.
- Examples- *Escherichia coli (E.coli), Salmonella* etc.
- Examples of Gram positive bacteria -*Streptococcus pneumoniae, Streptococcus pyogenes* etc.

Cell wall:

- It is the outermost layer - protects the bacterial cell and gives it shape.
- One exception - Mycoplasma lacks cell wall.
- Bacterial cell walls are made of peptidoglycan which is made from polysaccharide chains cross-linked by unusual peptides containing D-amino acids.
- The antibiotic penicillin is able to kill bacteria by preventing the cross-linking of peptidoglycan and this causes the cell wall to weaken.
- There are two different types of cell wall in bacteria, called Gram-positive and Gram-negative. The names originate from the reaction of cells to the Gram stain, a test long-employed for the classification of bacterial species.

- Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids.
- Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins.

Cell membrane:

Cell membrane surrounds the cell's cytoplasm and regulates the flow of substances in and out of the cell.

Cytoplasm:

The cytoplasm of a cell is a fluid in nature that fills the cell and is composed mainly of 80% water that also contains enzymes, salts, cell organelles, and various organic molecules.

Cytosol:

(Gel like fluid other than nucleoid)

- The plasma membrane is followed by the colloidal organic fluid called ***matrix*** or***cytosol***.
- The cytosol is the aqueous portion of the ***cytoplasm***(the extra-nuclear protoplasm) and of the***nucleoplasm***(the nuclear protoplasm).
- It fills all the spaces of the cell and constitutes its true ***internal milieu***.
- Cytosol is particularly rich in differentiating cells and many fundamental properties of cell arebecause of this part of the cytoplasm.
- The cytosol serves to dissolve or suspend the great variety of small molecules concerned with cellular metabolism, *e.g.*, glucose, amino acids, nucleotides, vitamins, minerals, oxygen and ions.

Ribosomes:

Ribosomes are the organelles of the cell responsible for protein synthesis.

Nucleiod Region:

- The nucleoid region is possessed by a prokaryotic bacterial cell.
- It is the area of the cytoplasm that contains the bacterial DNA molecule.

Plasmids:

*(The term plasmid was first introduced by the American molecular biologist **Joshua Lederberg** in **1952**.)*

- Many species of bacteria also may carry extrachromosomal genetic elements in the form of small, circular and closed DNA molecules

- Plasmids usually occur naturally in bacteria, but are sometimes found in eukaryotic organisms. Their sizes vary from 1 to over 1,000 kbp.

2. Eukaryotes:

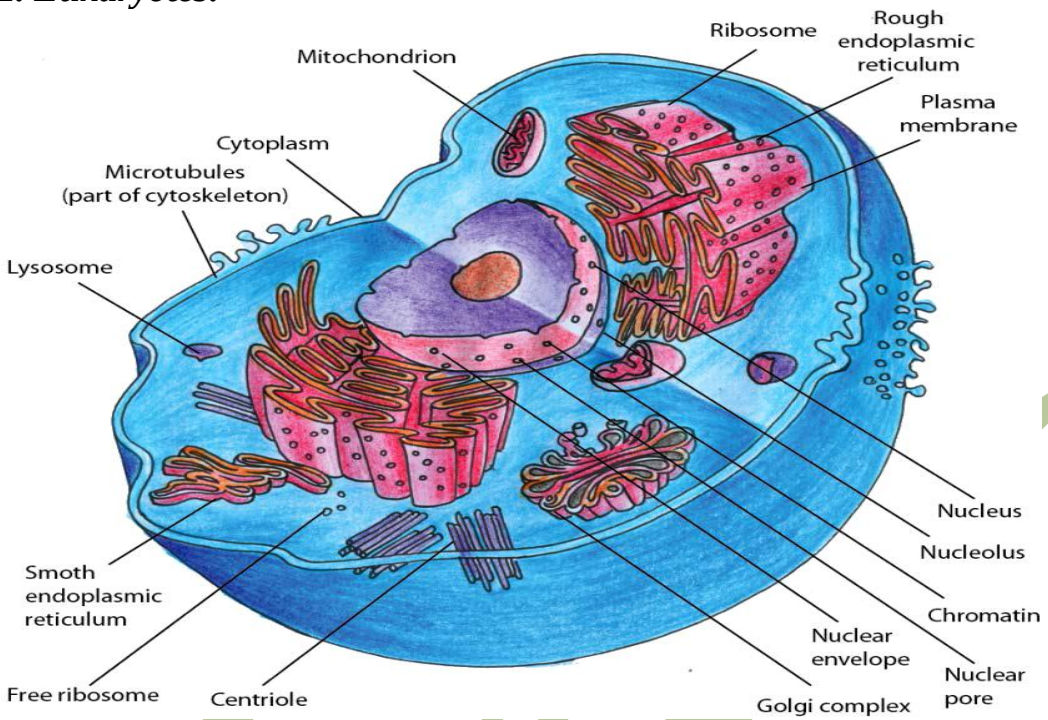

Fig: Eukaryotic cell.

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- The eukaryotic cells *(Greek; eu=good, karyotic=nucleated).*
- Eukaryotes, on the other hand, have distinct nuclei bound by a nuclear membrane and membrane-bound organelles *(mitochondria, chloroplasts, lysosomes, rough and smooth endoplasmic reticulum, vacuoles).*
- In addition, they possess organized chromosomes which store genetic material.

Difference between prokaryotes and eukaryotes:

Characteristic	Prokaryotes	Eukaryotes

Size of cell	Typically 0.2-2.0 m m in diameter	Typically 10-100 m m in diameter
Nucleus	No nuclear membrane or nucleoli (nucleoid)	True nucleus, consisting of nuclear membrane & nucleoli
Membrane-enclosed organelles	Absent	Present; examples include lysosomes, Golgi complex, endoplasmic reticulum, mitochondria & chloroplasts.
Flagella	Consist of two protein building blocks	Complex; consist of multiple microtubules
Glycocalyx	Present as a capsule or slime layer	Present in some cells that lack a cell wall
Cell wall	Usually present; chemically complex (typical bacterial cell wall includes peptidoglycan)	When present, chemically simple
Plasma membrane	No carbohydrates and generally lacks sterols	Sterols and carbohydrates that serve as receptors present
Cytoplasm	No cytosketeton or cytoplasmic streaming	Cytoskeleton; cytoplasmic streaming
Ribosomes	Smaller size (70S)	Larger size (80S); smaller size (70S) in organelles
Chromosome (DNA) arrangement	Single circular chromosome; lacks histones	Multiple linear chromosomes with histones

Cell division	Binary fission	Mitosis
Sexual reproduction	No meiosis; transfer of DNA fragments only (conjugation)	Involves Meiosis

Compartmentalisation of Eukaryotes :

- In Eukaryotes, cells are arranged into compartments *(as it is bound on all the sides by a cell membrane)*.
- It separates the protoplasm within the cell from the surrounding environment.
- Intracellular membrane systems, creats enclosed compartments that are separate from Cytosol.
- As a result, the cell is able to retain specific molecules and cartry out certain reactions in oderly manner.
- Prokaryotes evolved to form Eukaryotes, in the process *Cytosol compartmentalised* to form *Cytoplasm*.

(The cell cytoplasm contains *cytoplasm, cell organelles,* and *fluids - Cytosol*).

Plant cells

Plant cells are eukaryotic cells that differ in several key aspects from the cells of other eukaryotic organisms. Their distinctive features include the following organelles:

1. Vacuole:

- It is present at the centre and is water-filled volume enclosed by a membrane known as the tonoplast.
- The function is to maintain the cell's turgor, pressure by controlling movement of molecules between the cytosol and sap, stores useful material and digests waste proteins and organelles.

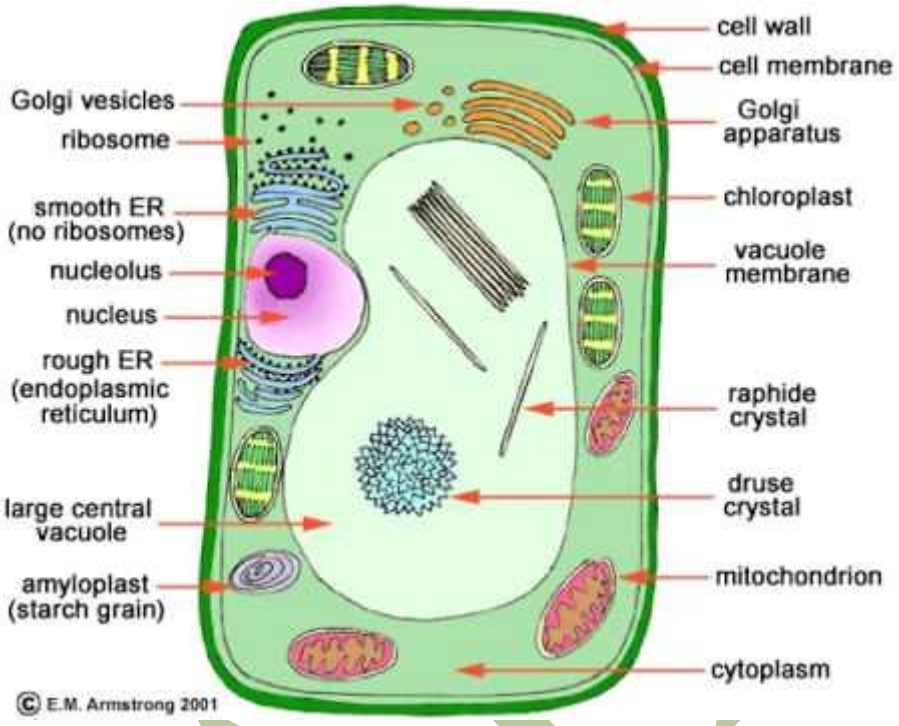


Fig: ***Anatomy of Plant Cell.***

2. Cell Wall:

- It is the extracellular structure surrounding plasma membrane.
- The cell wall is composed of cellulose, hemicellulose, pectin and in many cases lignin, is secreted by the protoplast on the outside of the cell membrane.
- This contrasts with the cell walls of fungi (which are made of chitin), and of bacteria, which are made of peptidoglycan.

3. Plasmodesmata:

Pores in the primary cell wall through which the plasmalemma and endoplasmic reticulum of adjacent cells are continuous.

4. Plastids:

- The plastids are chloroplasts, which contain chlorophyll and the biochemical systems for light harvesting and photosynthesis.
- A typical plant cell (e.g., in the palisade layer of a leaf) might contain as many as 50 chloroplasts.

Plant cell types :

Parenchyma cells:

These are living cells that have diverse functions ranging from storage and support to photosynthesis and phloem loading (transfer cells).

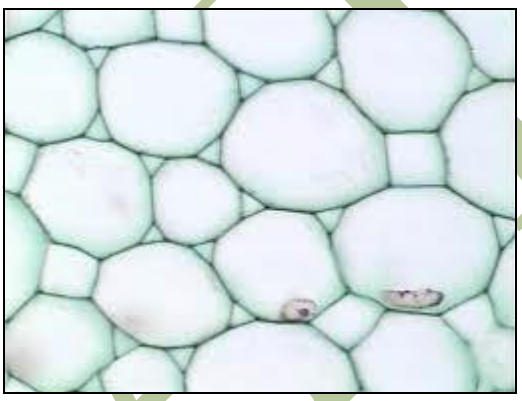


Fig: ***Parenchyma cells which have thin primary cell wall.***

Collenchyma cells:

- Collenchyma cells are alive at maturity and have only a primary wall.
- These cells mature from meristem derivatives that initially resemble parenchyma, but differences quickly become apparent.

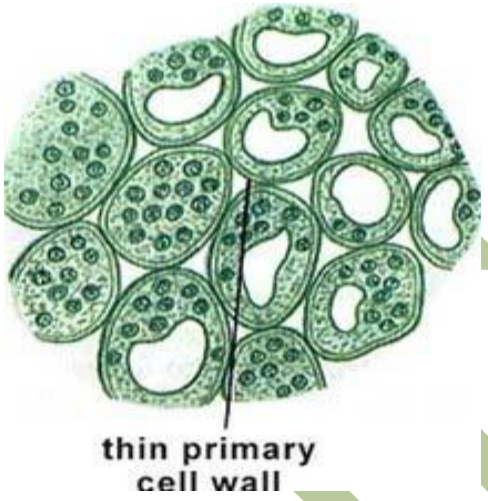


Fig:Typical collenchyma cell.

Sclerenchyma cells:

Sclerenchyma cells (from the Greek **skleros**, *hard*) are hard and tough cells with a function in mechanical support. They are of two broad types – sclereids or stone cells and fibres.

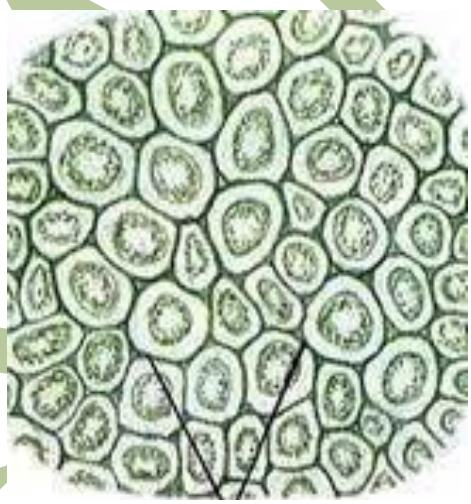


Fig:Sclerenchyma cells with irregularly thickened cell wall.

Animal cells:

- An animal cell is a form of eukaryotic cell that makes up many tissues in animals.

- The animal cell is different from plant cells, as they lack cell walls and chloroplasts, and they have smaller vacuoles.
- Due to the lack of a rigid cell wall, animal cells can adopt a variety of shapes, and a phagocytic cell can even engulf other structures.

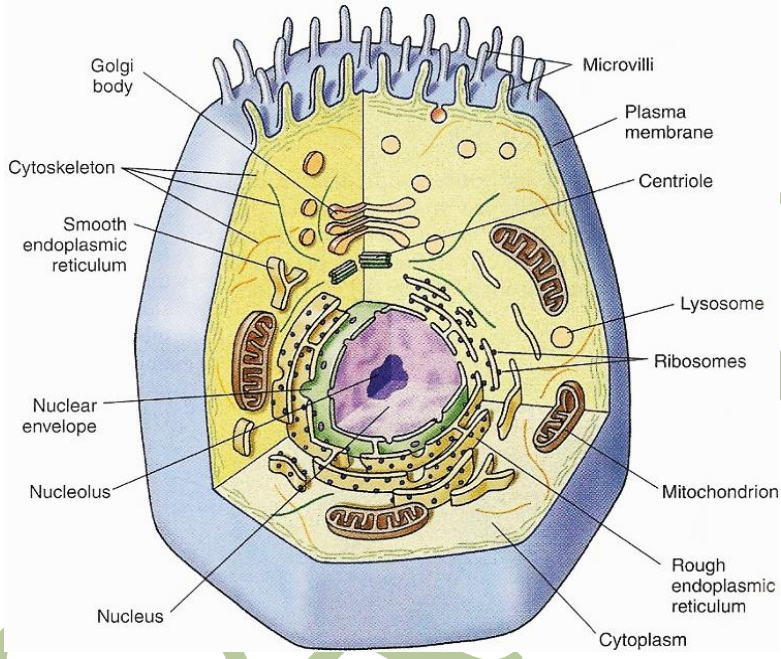


Fig:Schematic representation of a typical animal cell.

Cell organelles in animal cell:

1. Cell membrane:

- Plasma membrane is the thin layer of protein and fat that surrounds the cell (inside the cell wall – for plant cells).
- The cell membrane is semipermeable, allowing selective substances to pass into the cell and blocking others.

2. Nucleus:

- They are spherical body containing many organelles, including the nucleolus.
- The nucleus controls many of the functions of the cell (by controlling protein synthesis) and contains DNA (in chromosomes).
- The nucleus is surrounded by the nuclear membrane and possesses the nucleolus which is an organelle within the nucleus - it is where ribosomal RNA is produced.

3. Golgi apparatus:

It is a flattened, layered, sac-like organelle involved in packaging proteins and carbohydrates into membrane-bound vesicles for export from the cell.

4. Ribosome and Endoplasmic reticulum (ER) :

(Around the *Nucleus* there is ***densely granulated region*** – called ***Endoplasm***. ER is called so because it is present in the Endoplasm region **).**

Ribosomes are small organelles composed of RNA-rich cytoplasmic granules that are sites of protein synthesis and Endoplasmic reticulum are the sites of protein maturation and they can be divided into the following types:

a. Rough endoplasmic reticulum:

- These are a vast system of interconnected, membranous, infolded and convoluted sacks that are located in the cell's cytoplasm (the ER is continuous with the outer nuclear membrane).
- Rough ER is covered with ribosomes that give it a rough appearance.

b. Smooth endoplasmic reticulum:

- These are a vast system of interconnected, membranous, infolded and convoluted tubes that are located in the cell's cytoplasm (the ER is continuous with the outer nuclear membrane).
- The space within the ER is called the ER lumen. Smooth ER transport materials through the cell.

5. Mitochondria:

- These are spherical to rod-shaped organelles with a double membrane.
- The inner membrane is infolded many times, forming a series of projections (called cristae).

- The mitochondrion converts the energy stored in glucose into ATP (adenosine triphosphate) for the cell.

6. Lysosome:

- Lysosomes are cellular organelles that contain the hydrolase enzymes which breaks down waste materials and cellular debris.
- They can be described as the stomach of the cell.
- They are found in animal cells, while in yeast and plants the same roles are performed by lytic vacuoles.
- The membrane around a lysosome allows the digestive enzymes to work at the 4.5 pH they require.
- This means if enzymes escape from the lysosome they will be inactivated by the nuteral pH of the cell cytosol.
- Lysosomes digest excess or worn-out organelles, food particles, and engulf viruses or bacteria.

7. Centrosome:

- They are small body located near the nucleus and has a dense center and radiating tubules.
- The centrosomes are the destination where microtubules are made.
- During mitosis, the centrosome divides and the two parts move to opposite sides of the dividing cell. Unlike the centrosomes in animal cells, plant cell centrosomes do not have centrioles.

8.Peroxisome :

- Peroxisomes are organelles that contain oxidative enzymes, such as D-amino acid oxidase, ureate oxidase, and catalase.
- Peroxisomes function to rid the body of toxic substances like hydrogen peroxide, or other metabolites.
- They are a major site of oxygen utilization and are numerous in the liver where toxic byproducts accumulate.

9.Vacuoles and vesicles :

- Vacuoles are single-membrane organelles that are essentially part of the outside that is located within the cell.
- The single membrane is known in plant cells as a tonoplast.
- Many organisms will use vacuoles as storage areas.
- Vesicles are much smaller than vacuoles and function in transporting materials both within and to the outside of the cell.

Differences between Animal and Plant cell :

Sr. No.	Plant Cell	Animal Cell
01.	**Larger**	**Smaller** (Comparatively)
02.	**Cell wall is present** (made up of cellulose)**.**	**Cell wall is absent.**
03.	**Plastid is present.**	**Absent.**
04.	**Large Vacuole** (occupies 90% of cell space)**.**	**Vacuole is absent or *very small*.**
05.	**Absent**	**Lysosomes are present.**
06.	**Absent**	**Centriols are present.**
07.	**Plasmodesmata** (cytoplasmic strands connecting protoplast of adjacent cells through cell walls) **is present.**	**Absent** (tight junction between cells).
08.	**Absent**	**Desmosome is present.**
09.	**Ability to synthesis *amino acid, coenzymes, and vitamins* required by**	**Can not synthesis *amino acid, coenzymes, and vitamins* required by them.**

	them.	
10.	**Nucleus is near cell wall** (because vacuole occupies large space)**.**	**Nucleus lies in the center.**
11.	**Glyoscysomes present.**	**Absent**
12.	**Food stored in the form of *Starch*.**	**Food stored in the form of *Glycogen.*** *(It is animal starch- which is profusely branched)*
13.	**Spindel fibres are *Anastral.***	**Spindel fibres are *Astral.***
14.	**Cytokinesis occurs in *Plate method.***	**Cytokinesis occurs in *Burrowing method.***
15.	**Plant cells do not burst in *Hypotonic solution.*** (It is due to the presence of ***Cell wall***.)	**Animal cells do not burst in *Hypotonic solution.*** (It is due to the absence of ***Cell wall***.)

Theories on membrane structures

- In 1902 it was thought that the membranes had only lipids (Overton).
- In 1926 *Gorter and Grendell* proposed that lipids are capable of forming a double layer.
- In 1935 *Danielli* and *Davson* proposed the lipid bilayer model that includes proteins adhering to both lipid-aqueous interfaces
- Artificial model systems such as the liposomes supported the idea of *Danielli* and *Devson.*
- A droplet of lipid made soluble in an organic solvent can be spread over a small hole on a septum that divides two chambers containing water.
- This set up is useful to study biophysical properties of a bilayer such as permeability and electrical resistance.
- Channels for ions can be formed by adding certain proteins or polypeptides.
- Liposomes act as excellent carriers for different molecules such as chemotherapeutic compounds, insulin and antibodies.

Endoplasmic reticulum:

- Endoplasmic reticulum is a network of interconnected internal membranes generally, the largest membrane in a eukaryotic cell—an extensive network of closed, flattened membrane-bounded sacs called cisternae.

- The endoplasmic reticulum has a number of functions in the cell but is particularly important in the synthesis of lipids, membrane proteins, and secreted proteins.

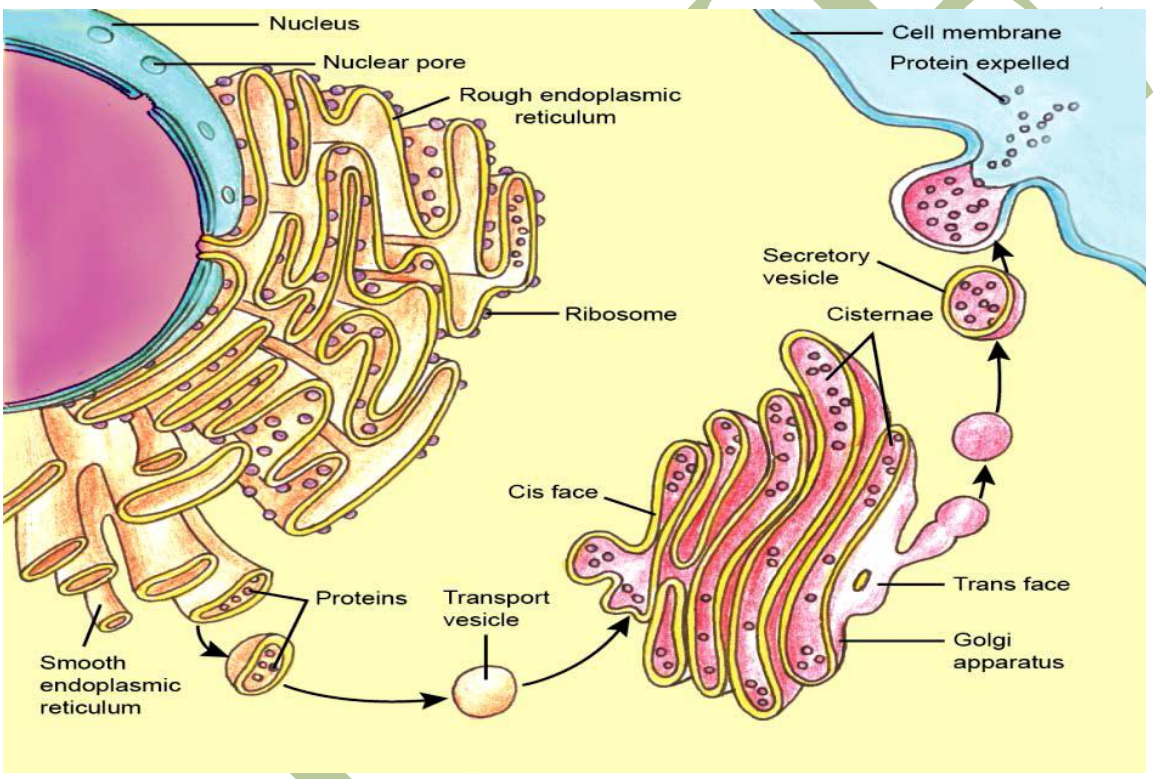


Fig :*The Endoplasmic reticulum.*

Occurrence:

- The occurrence of the endoplasmic reticulum is in eukaryotic cells with variation in its position from cell to cell.
- The erythrocytes (RBC), egg and embryonic cells lack in endoplasmic reticulum.

- ER is poorly developed in certain cells as the RBC which produces only proteins to be retained in the cytoplasmic matrix (haemoglobin), although the cell may contain many ribosomes).
- The spermatocytes also have poorly developed endoplasmic reticulum.

Morphology:

The endoplasmic reticulum occurs in three forms:

(i) Lamellar form or cisternae which is a closed, fluid-filled sac, vesicle or cavity is called ***cisternae***;
(ii) vesicular form or ***vesicle***and
(iii) tubular form or ***tubules***.

1. Cisternae:

- The cisternae are long, flattened, sac-like, unbranched tubules having diameter of 40 to 50 μm.
- They remain arranged parallely in bundles or stakes.
- RER mostly exists as cisternae which occur in those cells which have synthetic roles as the cells of pancreas, notochord and brain.

2. Vesicles:

- The vesicles are oval, membrane-bound vacuolar structures having diameter of 25 to 500 μm.
- They often remain isolated in the cytoplasm and occur in most cells but especially abundant in the SER.

3. Tubules:

- The tubules are branched structures forming the reticular system along with the cisternae and vesicles. They usually have the diameter from 50 to 190 μm and occur almost in all the cells.
- Tubular form of ER is often found in SER and is dynamic in nature, *i.e.*, it is associated with membrane movements, fission and fusion between membranes of cytocavity network.

Types of endoplasmic reticulum:

Smooth Endoplasmic Reticulum:

- ER with no embossed ribosomes makes it smooth in appearance.
- The adipose tissues, brown fat cells and adrenocortical cells, interstitial cells of testes and cells of corpus luteum of ovaries, sebaceous cells and retinal pigment cells contain only smooth endoplasmic reticulum (SER).
- The synthesis of fatty acids and phospholipids takes place in the smooth ER.

- It is abundant in hepatocytes. Enzymes in the smooth ER of the liver modify or detoxify hydrophobic chemicals such as pesticides and carcinogens by chemically converting them into more water-soluble, conjugated products that can be excreted from the body.
- High doses of such compounds result in a large proliferation of the smooth ER in liver cells.

Rough Endoplasmic Reticulum:

- Ribosomes bound to the endoplasmic reticulum make it appear rough.
- The rough ER synthesizes certain membrane and organelle proteins and virtually all proteins to be secreted from the cell.
- A ribosome that fabricates such a protein is bound to the rough ER by the nascent polypeptide chain of the protein.
- As the growing polypeptide emerges from the ribosome, it passes through the rough ER membrane, with the help of specific proteins in the membrane.
- Newly made membrane proteins remain associated with the rough ER membrane, and proteins to be secreted accumulate in the lumen of the organelle.
- All eukaryotic cells contain a discernible amount of rough ER because it is needed for the synthesis of plasma membrane proteins and proteins of the extracellular matrix.
- Rough ER is particularly abundant in specialized cells that produce an abundance of specific proteins to be secreted.
- The cells of those organs which are actively engaged in the synthesis of proteins such as acinar cells of pancreas, plasma cells, goblet cells and cells of some endocrine glands are found to contain rough endoplasmic reticulum (RER) which is highly developed.

Rough endoplasmic reticulum and protein secretion:

- The defined pathway taken by secreted protein is: Rough ER - Golgi - secretory vesicles- cell exterior.
- In mammalian cells most proteins are transferred into the ER while they are being translated on membrane bound ribosomes.
- Proteins that are destined for secretion are then targeted to the endoplasmic reticulum by a signal sequence (short stretch of hydrophobic amino acid residues) at the amino terminus of the growing polypeptide chain.
- The signal sequence is K/HDEL which is Lys/His-Asp-Glu-Leu.

- This signal peptide is recognized by a signal recognition particle consisting of six polypeptides and srpRNA. The SRP binds the ribosome as well as the signal sequence, inhibiting further translation and targeting the entire complex (the SRP, ribosome, and growing polypeptide chain) to the rough ER by binding to the SRP receptor on the ER membrane.
- Binding to the receptor releases the SRP from both the ribosome and the signal sequence of the growing polypeptide chain.

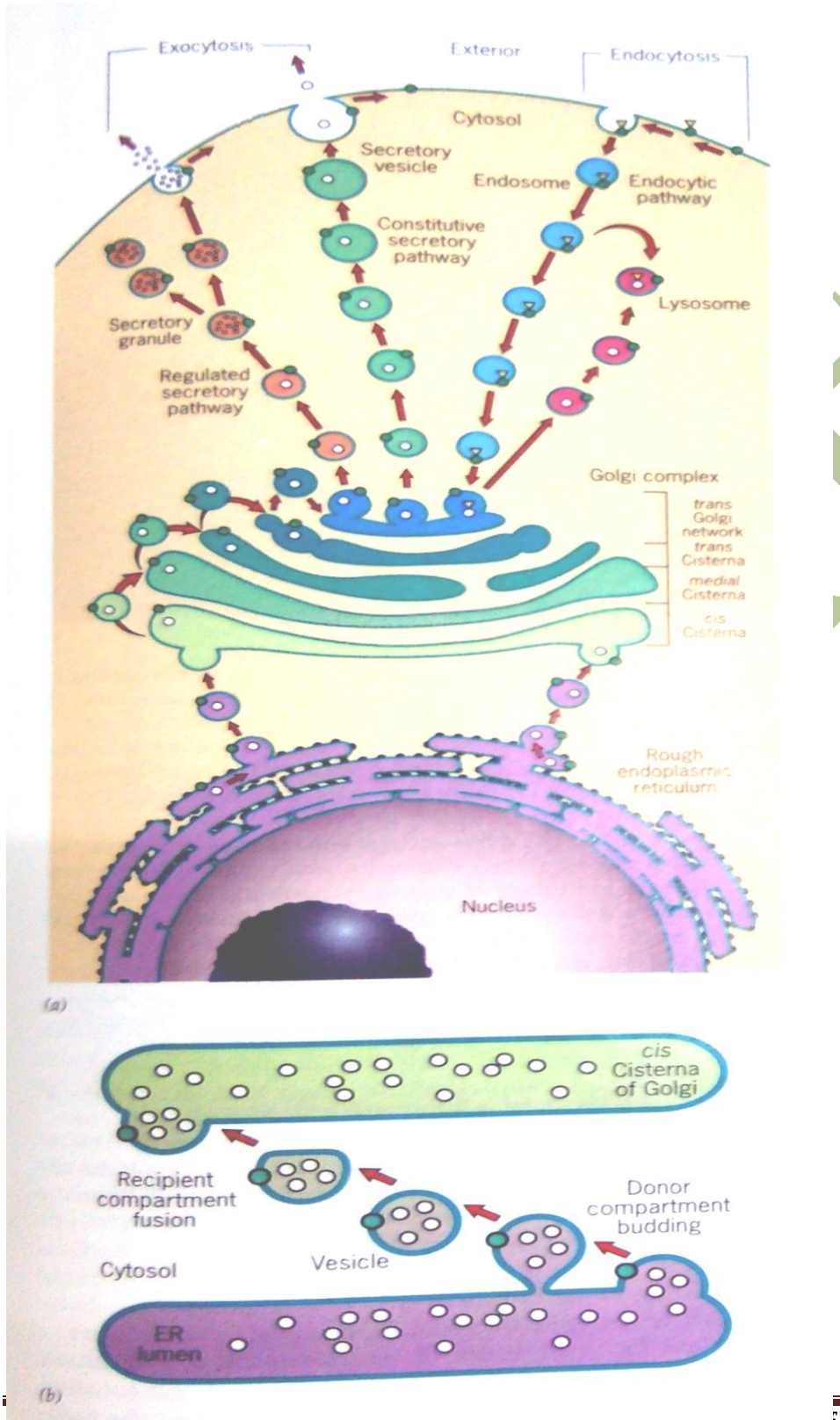


Fig : ***Biosynthetic and endocytic pathways unite the Endomembranes into a dynamic, interconnected framework.***

(a) The biosythetic pathways descibes the flow of materials (especially proteins) from the ER through the Golgi complex, and out to various locations including lysosomes, endosomes, secretory vesicles, vacuoles and plasma membrane.

(b) Illustrates the process of vesicle transport by wich materials are transported from a donar compartment to recipient compartment . vesicle form by membrane budding during which membrane proteins of the donor membrane can be incorporated into the vesicle membrane and soluble proteins in the donar compartment can be enclosed in the lumen of the vesicles. When the transport vesicle subsequently fuses, the proteins of the vesicle membrane become the part of the recipient membrane and the soluble proteins become sequestered within

- The ribosome then binds to a protein translocation complex in the ER membrane, and the signal sequence is inserted into a membrane channel or translocon with the aid of GTP.
- Transfer of the ribosome mRNA complex from the SRP to the translocon opens the gate on the translocon and allows translation to resume, and the growing polypeptide chain is transferred directly into the translocon channel and across the ER membrane as translation proceeds.
- As translocation proceeds, the signal sequence is cleaved by signal peptidase and the polypeptide is released into the lumen of the ER.

Smooth endoplasmic reticulum and lipid synthesis:

- Hydrophobic lipids are synthesized in the ER and then they are then transported from the ER to their ultimate destinations either in vesicles or by carrier proteins.
- Phospholipids are synthesized in the cytosolic side of the ER membrane from water-soluble cytosolic precursors.
- Other lipids that are synthesized in the ER are cholesterol and ceramide which is further converted to either glycolipids or sphingomyelin in the golgi apparatus.
- Smooth ER are also the site for the synthesis of the steroid hormones from cholesterol.
- Thus steroid producing cells in the testis and ovaries are abundant in smooth ER.

Common functions of SER and RER:

1. The endoplasmic reticulum provides an ultrastructural skeletal framework to the cell andgivesmechanical support to the colloidal cytoplasmic martix.
2. The exchange of molecules by the process of osmosis, diffusion and active transport occurs through the membranes of endoplasmic reticulum. The ER membrane has permeases and carriers.
3. The endoplasmic membranes contain many enzymes which perform various synthetic andmetabolic activities and provides increased surface for various enzymatic reactions.
4. The endoplasmic reticulum acts as an intracellular circulatory or transporting system. Various secretory products of granular endoplasmic reticulum are transported to various organelles as follows: Granular ER− agranular ER − Golgi membrane−lysosomes, transport vesicles or secretory granules. Membrane flow may also be an important mechanism for carrying particles, molecules and ions into and out of the cells. Export of RNA and nucleoproteins from nucleus to cytoplasm may also occur by this type of flow.
5. The ER membranes are found to conduct intra-cellular impulses. For example, the sarcoplasmic reticulum transmits impulses from the surface membrane into the deep region of the muscle fibres.

6. The sarcoplasmic reticulum plays a role in releasing calcium when the muscle is stimulated and actively transporting calcium back into the sarcoplasmic reticulum when the stimulation stops and the muscle must be relaxed.

The Golgi Complex:

Processes and Sorts Secreted and Membrane Proteins :

- The golgi complex was discovered by Camillo Golgi during an investigation of the nervous system and he named it the "internal reticular apparatus".
- Functionally it is also known as the post office of the cell.
- Certain important cellular functions such as biosynthesis of polysaccharides, packaging (compartmentalizing) of cellular synthetic products (proteins), production of exocytotic (secretory) vesicles and differentiation of cellular membranes, occurs in the Golgi complex or Golgi apparatus located in the cytoplasm of animal and plant cells.

Occurrence:

- The Golgi apparatus occurs in all eukaryotic cells.
- The exceptions are the prokaryotic cells (mycoplasmas, bacteria and blue green algae) and eukaryotic cells of certain fungi, sperm cells of bryophytes and pteridiophytes, cells of mature sieve tubes of plants and mature sperm and red blood cells of animals.
- Their number per plant cell can vary from several hundred as in tissues of corn root and algal rhizoids (*i.e*., more than 25,000 in algal rhizoids, Sievers,1965), to a single organelle in some algae.
- In higher plants, Golgi apparatuses are particularly common in secretory cells and in young rapidly growing cells. In animal cells, there usually occurs a single Golgi apparatus, however, its number may vary from animal to animal and from cell to cell.
- *Paramoeba* species has two golgi apparatuses and nerve cells, liver cells and chordate oocytes have multiple golgi apparatuses, there being about 50 of them in the liver cells.

Morphology :

- The Golgi apparatus is morphologically very similar in both plant and animal cells.
- However, it is extremely pleomorphic: in some cell types it appears compact and limited, in others spread out and reticular (net-like). Its shape and form may vary depending on cell type.
- It appears as a complex array of interconnecting tubules, vesicles and cisternae.
- There has been much debate concerning the terminology of the Golgi's parts.

- The simplest unit of the Golgi apparatus is the cisterna.
- This is a membrane bound space in which various materials and secretions may accumulate.
- Numerous cisternae are associated with each other and appear in a stack-like (lamellar) aggregation.
- A group of these cisternae is called the dictyosome, and a group of dictyosomes makes up the cell's Golgi apparatus.

All dictyosomes of a cell have a common function. The detailed structure of three basic components of the Golgi apparatus are as follows:

1. Flattened Sac or Cisternae :

- Cisternae of the golgi apparatus are about 1 μm in diameter, flattened, plate-like or saucer-like closed compartments which are held in parallel bundles or stacks one above the other.
- In each stack, cisternae are separated by a space of 20 to 30 nm which may contain rod-like elements or fibres.
- Each stack of cisternae forms a dictyosome which may contain 5 to 6 Golgi cisternae in animal cells or 20 or more cisternae in plant cells.
- Each cisterna is bounded by a smooth unit membrane (7.5 nm thick), having a lumen varying in width from about 500 to 1000 nm.
- The margins of each cisterna are gently curved so that the entire dictyosome of Golgi apparatus takes on a bow-like appearance.
- The cisternae at the convex end of the dictyosome comprise proximal, forming or cis-face and the cisternae at the concave end of the dictyosome comprise the distal, maturing or trans-face.
- The forming or cis face of Golgi is located next to either the nucleus or a specialized portion of rough ER that lacks bound ribosomes and is called "transitional" ER.
- Trans face of Golgi is located near the plasma membrane.
- This polarization is called cis-trans axis of the Golgi apparatus.

2. Tubules

A complex array of associated vesicles and tubules (30 to 50 nm diameter) surround the dictyosome and radiate from it. The peripheral area of dictyosome is fenestrated or lace-like in structure.

3. Vesicles

The vesicles are 60 nm in diameter and are of three types :

(i) Transitional vesicles are small membrane limited vesicles which are form as blebs from the transitional ER to migrate and converge to cis face of Golgi, where they coalasce to form new cisternae.

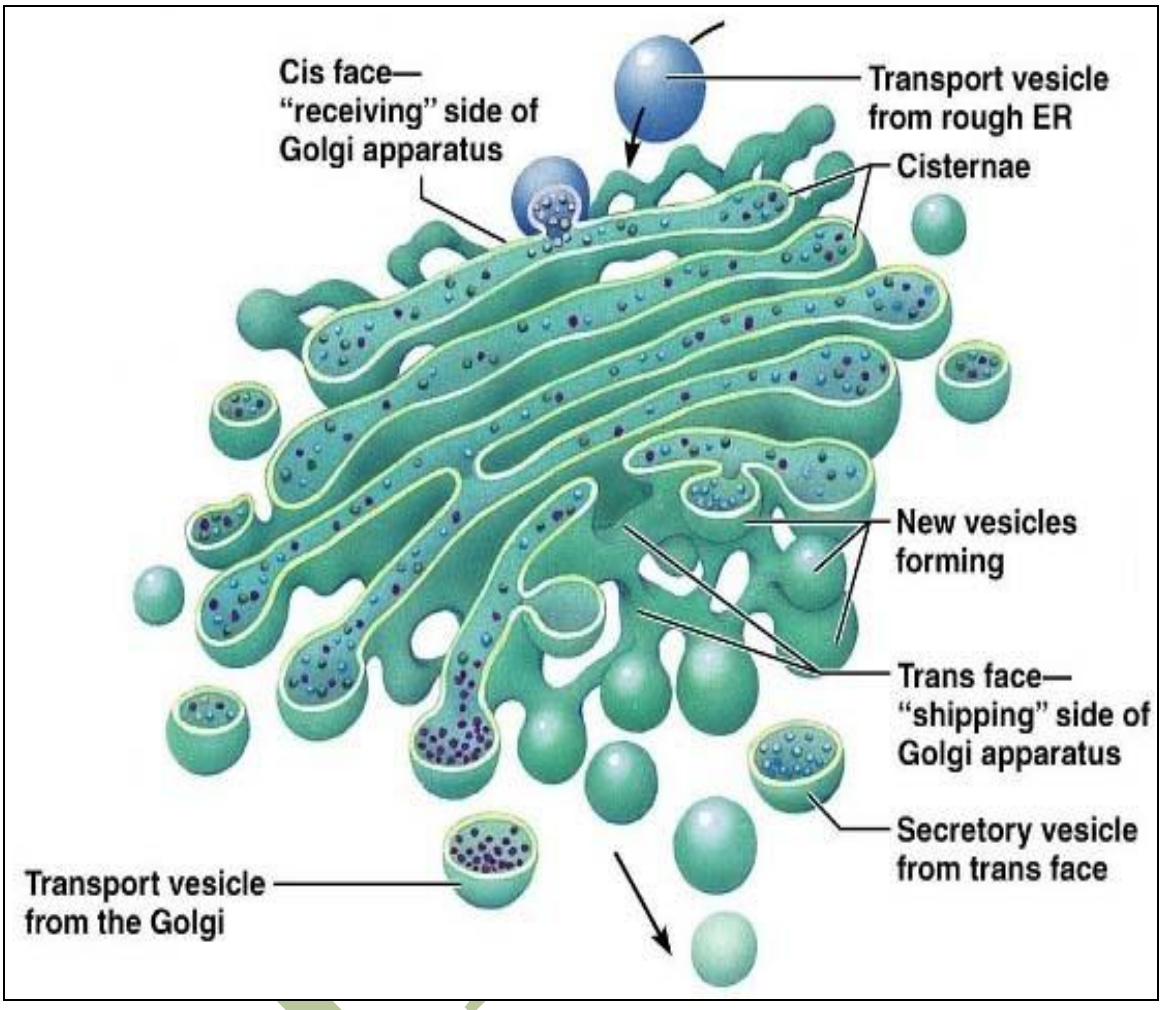


Fig :The Golgi complex.

(ii) Secretory vesicles are varied-sized membrane-limited vesicles which discharge from margins of cisternae of Golgi. They, often, occur between the maturing face of Golgi and the plasma membrane.

(iii) Clathrin-coated vesicles are spherical protuberances, about 50 μm in diameter and with a rough surface. They are found at the periphery of the organelle, usually at the ends of single tubules, and

are morphologically quite distinct from the secretory vesicles. Theclathrin-coated vesicles are known to play a role in intra-cellular traffic of membranes and of secretory products.

Origin or biogenesis :

- Origin of Golgi apparatus involves the formation of new cisternae and there is great variation in
- shape, number and size of cisternae in each stack (dictyosome).
- *The process of formation of newcisternae may be performed by any of the following methods:*

1. Individual stacks of cisternae may arisefrom the pre-existing stacks by division or fragmentation.
2. The alternative method of origin of Golgiis based on *de novo* formation.

- In fact, various cytological and biochemical evidences have establishedthat the membranes of the Golgi apparatus are originated from the membranes of the smooth ER whichin turn have originated from the rough ER.
- The proximal Golgi saccules are formed by fusion of ERderivedvesicles, while distal saccules "give their all" to vesicle formation and disappear.
- Thus, Golgisaccules are constantly and rapidly renewed.
- The cells of dormant seeds of higher plants generally lack Golgi apparatuses but they do displayzone of exclusion having aggregation of small transition vesicles.
- Photomicrographs of cells in earlystages of germination suggest progressive development of Golgi bodies in these zones of exclusion;and the development of Golgi apparatuses coincides with the disappearance of the aggregation of vesicles.

Functions:

1. Modifying, sorting, and packaging of macromolecules for cell secretion:

- The golgi complex is involved in the transport of lipids around the cell, and the creation of lysosomes.
- Proteins are modified by enzymes in cisternae by glycosylation and phosphorylation by identifying the signal sequence of the protein in question.
- For example, the Golgi apparatus adds a mannose-6-phosphate label to proteins destined for lysosomes.

- One molecule that is phosphorylated in the Golgi is Apolipoprotein, which forms a molecule known as VLDL that is a constituent of blood serum.
- The phosphorylation of these molecules is important to help aid in their sorting for secretion into the blood serum.

2. Proteoglycans and carbohydrate synthesis:

This includes the production of glycosaminoglycans (GAGs), long unbranched polysaccharides which the Golgi then attaches to a protein synthesised in the endoplasmic reticulum to form proteoglycans.

3. Golgi Functions in Animals:

- ***In animals, Golgi apparatus is involved in the packaging and exocytosis of the following:***

  1. Zymogen of exocrine pancreatic cells; Mucus (a glycoprotein) secretion by goblet cells of intestine;
  2. Lactoprotein (casein) secretion by mammary gland cells (Merocrine secretion);
  3. Secretion of compounds (thyroglobulins) of thyroxine hormone by thyroid cells;
  4. Secretion of tropocollagen and collagen;
  5. Formation of melanin granules and other pigments;
  6. and Formation of yolk and vitelline membrane of growing primary oocytes.

- It is also involved in the formation of certain cellular organelles such as plasma membrane, lysosomes, acrosome of spermatozoa and cortical granules of a variety of oocytes.

4. Golgi Functions in Plants:

- In plants, Golgi apparatus is mainly involved in the secretion of materials of primary and secondary cell walls (formation and export of glycoproteins, lipids, pectins and monomers for hemicellulose, cellulose, lignin).
- During cytokinesis of mitosis or meiosis, the vesicles originating from the periphery of Golgi apparatus, coalesce in the phragmoplast area to form a semisolid layer, called cell plate.
- The unit membrane of Golgi vesicles fuses during cell plate formation and becomes part of plasma membrane of daughter.

Lysosomes

- Lysosomes is an organelle which provides an excellent example of the ability of intracellular membranes to form closed compartments in which the composition of the lumen (the aqueous interior of the compartment) differs substantially from that of the surrounding cytosol.
- Found exclusively in animal cells, lysosomes are responsible for degrading certain components that have become obsolete for the cell or organism.
- Lysosomes are often budded from the membrane of the Golgi apparatus, but in some cases they develop gradually from late endosomes, which are vesicles that carry materials brought into the cell by a process known as endocytosis.
- The biogenesis of the lysosomes requires the synthesis of specialized lysosomal hydrolases and membrane proteins.
- Both classes of proteins are synthesized in the ER and transported through the Golgi apparatus, then transported from the trans Golgi network to an intermediate compartment (an endolysosome) by means of transport vesicles (which are coated by clathrin protein).

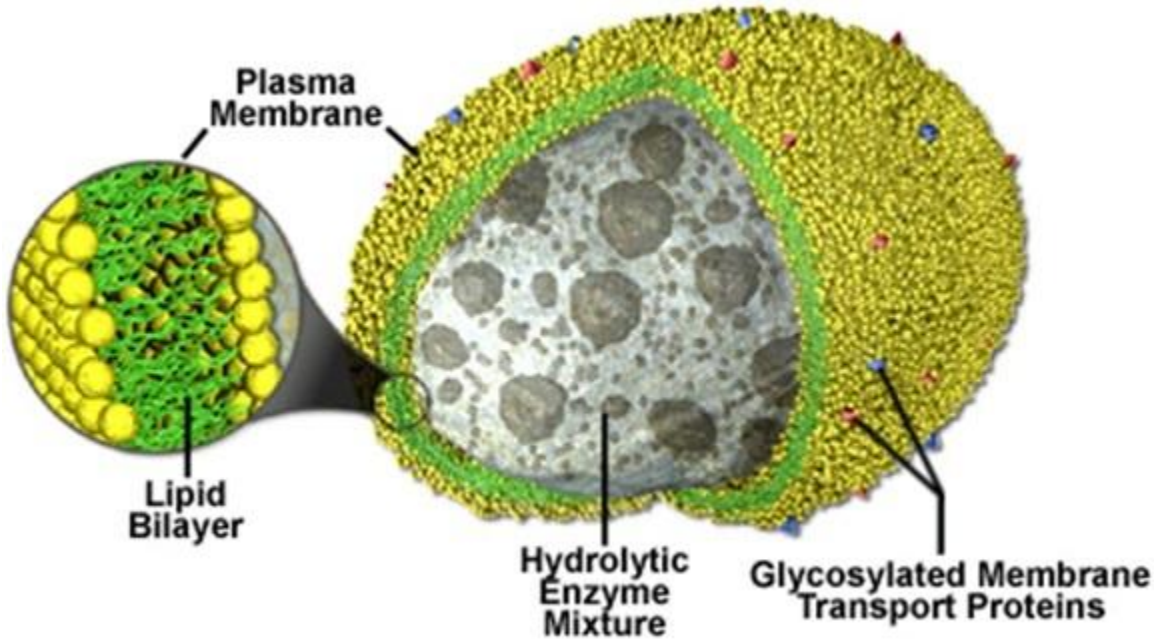


Fig : ***Anatomy of the Lysosome.***

Occurrence:

- The lysosomes occur in most animal and few plant cells.
- They are absent in bacteria and mature mammalian erythrocytes.
- Few lysosomes occur in muscle cells or in acinar cells of the pancreas.
- Leucocytes, especially granulocytes are a particularly rich source of lysosomes.

- Their lysosomes are so large-sized that they can be observed under the light microscope.
- They are also numerous in epithelial cells of absorptive, secretory and excretory organs (intestine, liver, and kidney).
- They occur in abundance in the epithelial cells of lungs and uterus.
- Phagocytic cells and cells of reticuloendothelial system (bone marrow, spleen and liver) are also rich in lysosomes.

Structure:

- The lysosomes are round vacuolar structures bounded by single unit membrane. Their shape and density vary greatly. Lysosomes are 0.2 to 0.5μm in size.
- Since, size and shape of lysosomes vary from cell to cell and time to time (they are polymorphic), their identification becomes difficult.

Isolation and chemical composition:

- Lysosomes are very delicate and fragile organelles.
- Lysosomal fractions have been isolated by sucrose-density centrifugation (Isopycnic centrifugation) after mild methods of homogenization.
- The location of the lysosomes in the cell can also be pinpointed by various histochemical or cytochemical methods. For example, lysosomes give a positive test for acid Schiff reaction.
- Certain lysosomal enzymes are good histochemical markers. For example, acid phosphatase is the principal enzyme which is used as a marker for the lysosomes by the use of Gomori'staining technique. Specific stains are also used for other lysosomal enzymes such as B- glucuronidase, aryl sulphatatase, N-acetyl-B-glucosaminidase and 5-bromo-4-chloroindolacetate esterase.

- A lysosome may contain up to 40 types of hydrolytic enzymes.
- They include proteases (cathepsin for protein digestion), nucleases, glycosidases (for digestion of polysaccharides and glycosides), lipases, phospholipases, phosphatases and sulphatases.
- All lysosomal enzymes are acid hydrolases, optimally active at the pH5. The membrane of the lysosome normally keeps the enzymes latent and out of the cytoplasmic matrix or cytosol (pH is ~7.2), but the acid dependency of lysosomal enzymes protects the contents of the cytosol (cytoplasmic matrix) against any damage even if leakage of lysosomal enzymes occur.
- The latency of the lysosomal enzymes is due to the presence of the membrane which is resistant to the enzymes that it encloses.
- Most probably this is due to the fact that most lysosomal hydrolases are membrane-bound, which may prevent the active centres of enzymes to gain access to
- susceptible groups in the membrane.

Lysosomal Membrane:

- The lysosomal membrane is slightly thicker than that of mitochondria.
- It contains substantial amounts of carbohydrate material, particularly sialic acid.
- In fact, most lysosomal membrane proteins are unusually highly glycosylated, which may help protect them from the lysosomal proteases in the lumen.
- The lysosomal membrane has another unique property of fusing with other membranes of the cell.
- This property of fusion has been attributed to the high proportion of membrane lipids present in the micellar configuration.

- Surface active agents such as liposoluble vitamins (A,K,D and E) and steroid sex hormones have a destabilizing influence, causing release of lysosomal enzymes due to rupture of lysosomal membranes. Drugs like cortisone, hydrocortisone and others tend to stabilize the lysosomal membrane and have an anti-inflammatory effect on the tissue.
- The entire process of digestion is carried out within the lysosome.
- Most lysosomal enzymes act in an acid medium.
- Acidification of lysosomal contents depends on an ATP-dependent proton pump which is present in the membrane of the lysosome and accumulates H+ inside the organelle.
- Lysosomal membrane also contains transport proteins that allow the final products of digestion of macromolecules to escape so that they can be either excreted or reutilized by the cell.

Functions:

1. Lysosomes serve as digestion compartments for cellular materials that have exceeded their lifetime or are otherwise no longer useful by autophagy. When a cell dies, the lysosome membrane ruptures and enzymes are liberated. These enzymes digest the dead cells. In the process of metamorphosis of amphibians and tunicates many embryonic tissues,
e.g., gills, fins, tail, etc., are digested by the lysosomes and utilized by the other cells.

2. Lysosomes break down cellular waste products, fats, carbohydrates, proteins, and other macromolecules into simple compounds, which are then transferred back into the cytoplasm as new cell-building materials. To accomplish the tasks associated with digestion, the lysosomes utilize about 40 different types of hydrolytic enzymes, all of which are manufactured in the endoplasmic reticulum and modified in the Golgi apparatus.

3. Digestion of large extracellular particles: The lysosomes digest the food contents of the phagosomes or pinosomes. The lysosomes of leucocytes enable the latter to devour the foreign proteins, bacteria and viruses.

4. Extracellular digestion: The lysosomes of certain cells such as sperms discharge their enzymes outside the cell during the process of fertilization. The lysosomal enzymes digest the limiting membranes of the ovum and form penetra path in ovum for the sperms. Acid hydrolases are released from osteoclasts and break down bone for the reabsorption; these cells also secrete lactic

acid which makes the local pH enough for optimal enzyme activity. Likewise, preceding ossification (bone formation), fibroblasts release cathepsin D enzyme to break down the connective tissue.

Mitochondria

Structure and Function:

- The mitochondria were first observed by Kolliker in 1850 as granular structures in the striated muscles.
- Mitochondria are called the 'powerhouse of the cell'.
- They are intracellular organelles found in almost all eukaryotic cells having bilayeredmembranes.
- Most eukaryotic cells contain many mitochondria, which occupy up to 25 percent of the volume of the cytoplasm.

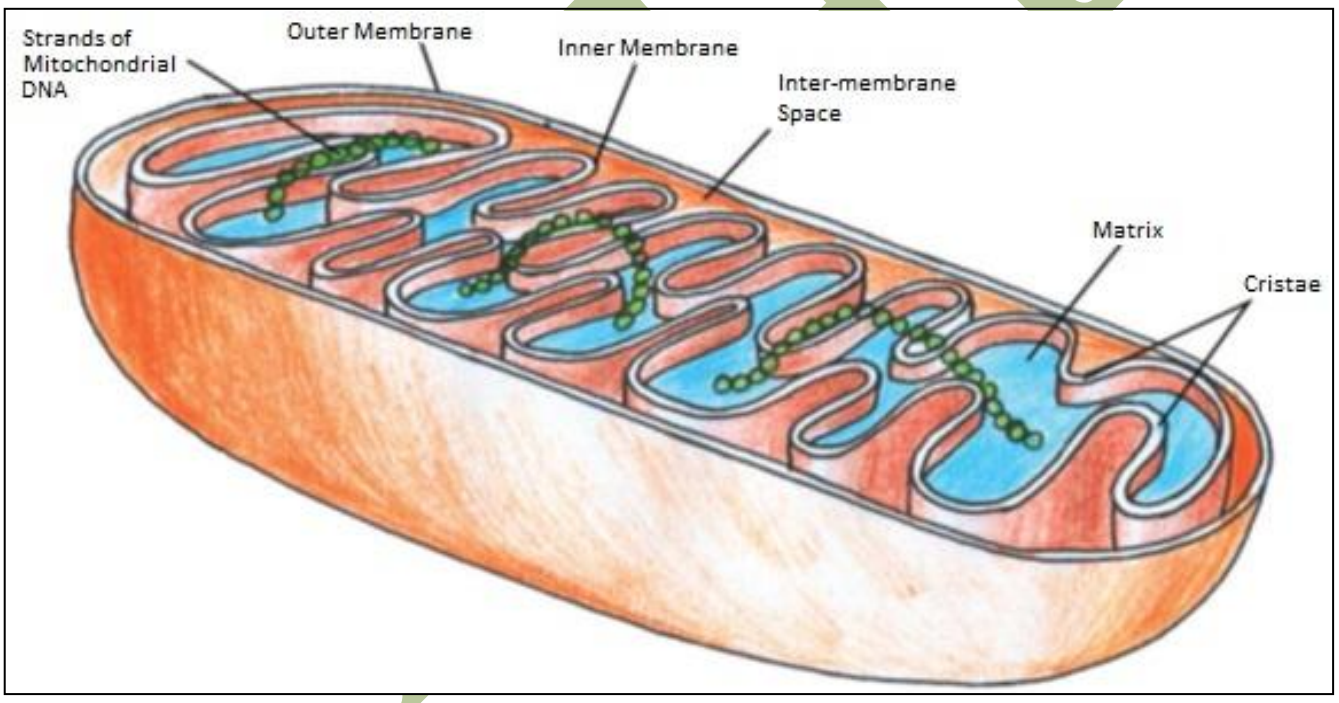


Fig : *Structure of Mitochondrion.*

- These crucial organelles, the main sites of ATP production during aerobic metabolism, are generally exceeded in size only by the nucleus, vacuoles, and chloroplasts.

- They are responsible for aerobic metabolism through oxidative phosphorylation, which leads to energy production in the form of adenosine triphosphate (ATP).
- Mitochondria contain a number of enzymes and proteins that help in processing carbohydrates and fats obtained from food we eat to release energy.
- Each human cell contains on average hundreds to thousands of mitochondria.
- The exception is mature red blood cells, which rely exclusively on anaerobic metabolism and contain no mitochondria.

Localisation:

- Mitochondria are present in all eukaryotic cells. They move autonomously in the cytoplasm, so they generally have uniform distribution in the cytoplasm, but in many cells their distribution is restricted.
- The distribution and number of mitochondria can be correlated with type of function the cell performs.
- Typically mitochondria with many cristae are associated with mechanical and osmotic work situations, where there are sustained demands for ATP *e.g*., between muscle fibres, in the basal infolding of kidney tubule cells, and in a portion of inner segment of rod and cone cells of retina.
- Myocardial muscle cells have numerous large mitochondria called sarcosomes that reflect the great amount of work done by these cells.
- Mitochondria are particularly numerous in regions where ATP-driven osmotic work occurs, *e.g.*, brush border of kidney proximal tubules, the infolding of the plasma membrane of dogfish salt glands and Malpighian tubules of insects, the contractile vacuoles of some protozoans as Paramecium.
- Non-myelinated axons contain many mitochondria that are poor ATP factories, since each has only single cristae.
- In this case, there is a great requirement for monoamine oxidase, an enzyme present in outer mitochondrial membrane that oxidatively deaminates monoamines including neurotransmitters (acetylcholine).

Orientation:

- The mitochondria have definite orientation. For example, in cylindrical cells the mitochondria
- usually remain orientated in basal apical direction and lie parallel to the main axis.
- In leucocytes, the mitochondria remain arranged radially with respect to the centrioles.
- As they move about in the mitochondria form long moving filaments or chains, while in others they remain fixed in one position where they provide ATP directly to a site of high ATP utilization,
- ***e.g.,*** they are packed between adjacent myofibrils in a cardiac muscle cell or wrapped tightly around the flagellum of sperm.

Structure:

• Each mitochondrion is bound by two highly specialized membranes that play a crucial role in its activities. Each of the mitochondrial membrane is 6 nm in thickness and fluidmosaic in ultrastructure.

• The membranes are made up of phospholipids and proteins. The space in between the two membranes is called the inter-membrane space which has the same composition as the cytoplasm of the cell.

• Inner and the outer membrane is separated by a 6–8 nm wide space.

Outer Membrane

• The two membranes that bound a mitochondrion differ in composition and function. The outer membrane, composed of about half lipid and half protein, contains porins that render the membrane permeable to molecules having molecular weights as high as 10,000 dalton.

• In this respect, the outer membrane of mitochondria is similar to the outer membrane of gram-negative bacteria.

• The outer membrane is smooth unlike the inner membrane and has almost the same amount of phospholipids as proteins.

• It has a large number of special proteins called porins that allow molecules of 5000 daltons or less in weight to pass through it.

• It is completely permeable to nutrient molecules, ions, ATP and ADP molecules.

Inner Membrane

• The inner membrane is much less permeable, than the outer membrane. It has about 20 percent lipid and 80 percent protein.

• The surface area of the inner membrane is greatly increased by a large number of infoldings, or finger like projections called cristae, that protrude into the matrix, or central space, increasing the surface area for the complexes.

• It contains the complexes of the electron transport chain and the ATP synthetase complex, they also serve to separate the matrix from the space that will contain the hydrogen ions, allowing the gradient needed to drive the pump.

• It is permeable only to oxygen, carbon dioxide and water and is made up of a large number of proteins that play an important role in producing ATP, and also helps in regulating transfer of metabolites across the membrane.

• In general, the cristae of plant mitochondria are tubular, while those of animal mitochondria are lamellar or plate-like. Some mitochondria, particularly those from heart, kidney and skeletal muscles have more extensive cristae arrangements than liver mitochondria.

• In comparison to these, other mitochondria (from fibroblasts, nerve axons and most plant tissues) have relatively few cristae.

• Attached to matrix face of inner mitochondrial membrane are repeated units of stalked particles, called elementary particles, inner membrane subunits or oxysomes.

Matrix

The matrix is a complex mixture of enzymes that are important for the synthesis of ATP molecules, special mitochondrial ribosomes, tRNAs and the mitochondrial DNA. Besides these, it has oxygen, carbon dioxide and other recyclable intermediates.

Function of mitochondria

1.The most important function of the mitochondria is to produce energy. The food that we eat is broken into simpler molecules like carbohydrates, fats, etc., in our bodies. These are sent to the mitochondrion where they are further processed to produce charged molecules that combine with oxygen and produce ATP molecules. This entire process is known as oxidative phosphorylation.

2.It is important to maintain proper concentration of calcium ions within the various compartments of the cell. Mitochondria help the cells to achieve this goal by serving as storage tanks of calcium ions.

3.Mitochondria help in the building of certain parts of the blood, and hormones like testosterone and estrogen.

4.Mitochondria in the liver cells have enzymes that detoxify ammonia.

Although most of the genetic material of a cell is contained within the nucleus, the mitochondria have their own DNA. They have their own machinery for protein synthesis and reproduce by the process of fission like bacteria do. Due to their independence from the nuclear DNA and similarities with bacteria, it is believed that mitochondria have originated from bacteria by endosymbiosis.

Chloroplasts

The chloroplast (*chlor*=green; *plast*=living) is most widely occurring chromoplast of the plants. It occurs mostly in the green algae and higher plants. The chloroplast contains the pigment chlorophyll 'a' and chlorophyll 'b' and DNA and RNA.

Chloroplasts were described as early as seventeenth century by Nehemiah Grew and Antonie van Leeuwenhoek.

Distribution:

The chloroplasts remain distributed homogeneously in the cytoplasm of plant cells. But in certaincells, the chloroplasts become concentrated around the nucleus or just beneath the plasma membrane.
The chloroplasts have a definite orientation in the cell cytoplasm. Chloroplasts are motile organelles, and show passive and active movements.

Morphology:

Shape:Higher plant chloroplasts are generally biconvex or plano-convex. However, in different plant cells, chloroplasts may have various shapes, *viz*., filamentous, saucer-shaped, spheroid, ovoid, discoid or club-shaped. They are vesicular and have a colourless centre.

Size:The size of the chloroplasts varies from species to species. They generally measure 2–3μm in thickness and 5–10μm in diameter (*Chlamydomonas*). The chloroplasts of polyploid plant cells are comparatively larger than those of the diploid counterparts. Generally, chloroplasts of plants grown in the shade are larger and contain more chlorophyll than those of plants grown in sunlight.

Isolation:

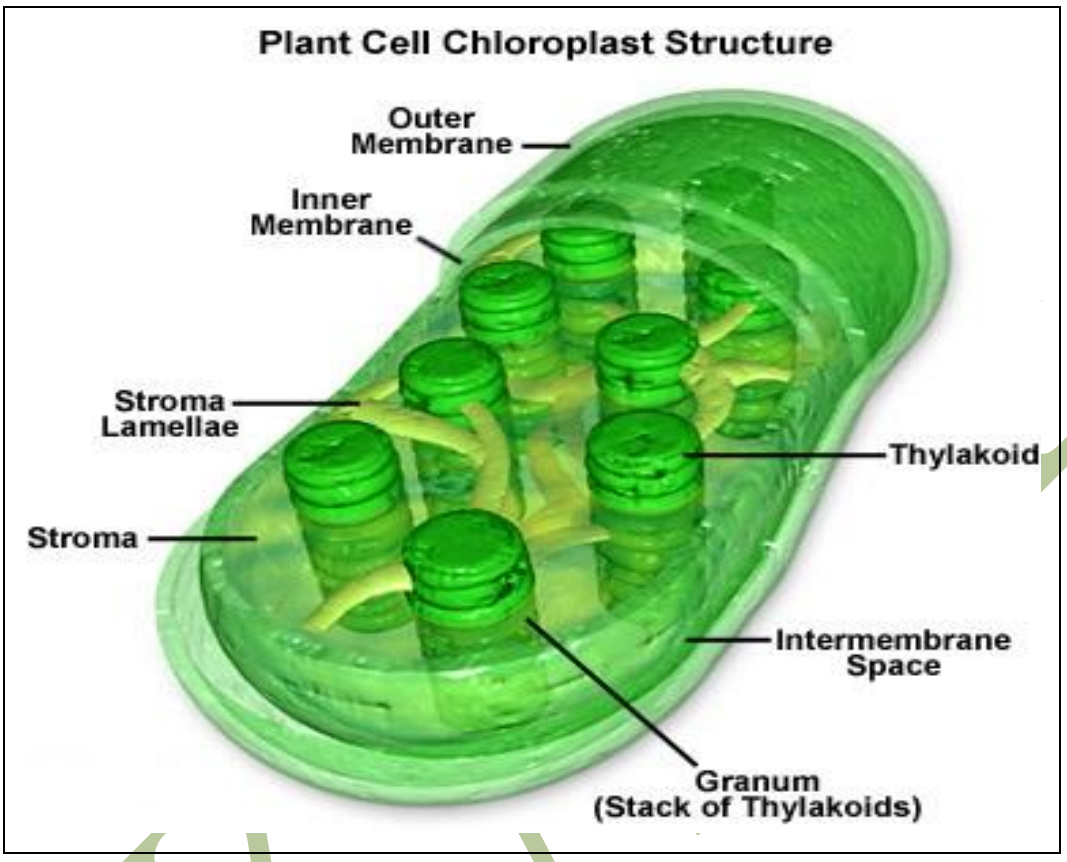
Chloroplasts are routinely isolated from plant tissues by differential centrifugation following the disruption of the cells.

Ultrastructure:

Chloroplast comprises of three main components:

1. Envelope :

- The entire chloroplast is bounded by a double unit membrane. Across this double membrane envelope occurs exchange of molecules between chloroplast and cytosol.



- Isolated membranes of envelope of chloroplast lack chlorophyll pigment and cytochromes but have a yellow colour due to the presence of small amounts of carotenoids. They contain only 1 to 2 per cent of the total protein of the chloroplast.

2. Stroma :

- The matrix or stroma fills most of the volume of the chloroplasts and is a kind of gel-fluid phasethat surrounds the thylakoids (grana).
- It contains about 50 per cent of the proteins of the chloroplast, most of which are soluble type.

- The stroma also contains ribosomes and DNA molecules both of which are involved in the synthesis of some of the structural proteins of the chloroplast.
- The stroma is the place where CO2 fixation occurs and where the synthesis of sugars, starch, fatty acids and some proteins takes place.

3. Thylakoids :

- The thylakoids (thylakoid = sac-like) consists of flattened and closed vesicles arranged as a membranous network.
- The outer surface of the thylakoid is in contact with the stroma, and its inner surface encloses an intrathylakoid space.
- Thylakoids get stacked forming grana. There may be 40 to 80 grana in the matrix of a chloroplast. The number of thylakoids per granum may vary from 1 to 50 or more.
- For example, there may be single thylakoid (red alga), paired thylakoids (Chrysophyta), triple thylakoids and multiple thylakoids (green algae and higher plants).

- Like the mitochondria, the chloroplasts have their own DNA, RNAs and protein synthetic machinery and are semiautonomous in nature.

- Chloroplasts are the largest and the most prominent organelles in the cells of plants and green algae.

- Chloroplasts and mitochondria have other features in common: both often migrate from place to place within cells, and they contain their own DNA, which encodes some of the key organellar proteins.

- Though most of the proteins in each organelle are encoded by nuclear DNA and are synthesized in the cytosol, the proteins encoded by mitochondrial or chloroplast DNA is synthesized on ribosomes within the organelles.

- Chloroplasts have a highly permeable outer membrane; a much less permeable inner membrane, in which membrane transport proteins are embedded; and a narrow intermembrane space in between.

- Together, these membranes form the chloroplast envelope. The inner membrane surrounds a large space called the stroma, and contains many metabolic enzymes.

- The electron-transport chains, photosynthetic light-capturing systems, and ATP synthase are all contained in the thylakoid membrane, a third distinct membrane that forms a set of flattened disclike sacs, the thylakoids**.**

• The lumen of each thylakoid is connected with the lumen of other thylakoids, defining a third internal compartment called the thylakoid space, which is separated by the thylakoid membrane from the stroma that surrounds it.

Photosynthesis

The many reactions that occur during photosynthesis in plants can be grouped into two broad categories:

1.Electron-transfer reactions or the light reactions: In the choloroplast, energy derived from sunlight energizes an electron of chlorophyll, enabling the electron to move along an electron-transport chain in the thylakoid membrane in much the same way that an electron moves along the respiratory chain in mitochondria.

The chlorophyll obtains its electrons from water (H_2O), producing O_2 as a by-product.

During the electron-transport process, H^+ is pumped across the thylakoid membrane, and the resulting electrochemical proton gradient drives the synthesis of ATP in the stroma.

As the final step in this series of reactions, high-energy electrons are loaded onto $NADP^+$, converting it to NADPH. All of these reactions are confined to the chloroplast.

2.Carbon-fixation reactions or the dark reactions wherein the ATP and the NADPH produced by the photosynthetic electron-transfer reactions serve as the source of energy and reducing power, respectively, to drive the conversion of CO_2 to carbohydrate.

The carbon-fixation reactions, which begin in the chloroplast stroma and continue in the cytosol, produce sucrose and many other organic molecules in the leaves of the plant.

The sucrose is exported to other tissues as a source of both organic molecules and energy for growth.

Thus, the formation of ATP, NADPH, and O_2 and the conversion of CO_2 to carbohydrate are separate processes, although elaborate feedback mechanisms interconnect the two. Several of the chloroplast enzymes required for carbon fixation, for example, are inactivated in the dark and reactivated by light-stimulated electron-transport processes.

Peroxisomes

Peroxisomes are membrane-bound organelles in most eukaryotic cells, primarily involved in lipid metabolism and the conversion of reactive oxygen species such as hydrogen peroxide into safer molecules like water and oxygen.

Fats are convenient energy storage molecules due to their high energy density. The number of ATP released from the oxidation of one gram of fat is much higher than that derived from carbohydrates or proteins. Lipids are also extremely useful molecules for the creation of membrane-bound subcompartments within cells or for delineating the cytoplasm from the extracellular space. Their lipophilic biochemistry, however, makes them difficult to metabolize within an aqueous cellular environment. Peroxisomes are structures where the metabolism of these hydrophobic molecules occurs.

Structure of Peroxisomes

Peroxisomes are organelles that can vary in shape, size and number depending on the energy needs of the cell. In yeast cells, a carbohydrate-rich growth medium shrinks peroxisomes. On the other hand, the presence of toxins or a lipid-rich diet can increase their number and size.

These organelles are made of a phospholipid bilayer with many membrane-bound proteins – especially those that act as protein transporters and translocators.

The enzymes involved in detoxification and lipid metabolism are synthesized on free ribosomes in the cytoplasm and selectively imported into peroxisomes, making them more similar to mitochondria and chloroplasts when compared to lysosomes that bud off from the endoplasmic reticulum (ER).

However, there is also some evidence linking ER-mediated protein synthesis to the enzymes present in peroxisomes.

DNA

- Deoxyribonucleic acids (DNAs) are polymeric molecules consisting of nucleotide building blocks. They are genetic material of almost all organisms except some RNA viruses.
- In prokaryotes, DNA is not separated from the rest of the cellular contents.In eukaryotes, however, DNA is located in the nucleus, where it is separated fromthe rest of the cell by the nuclear envelope.

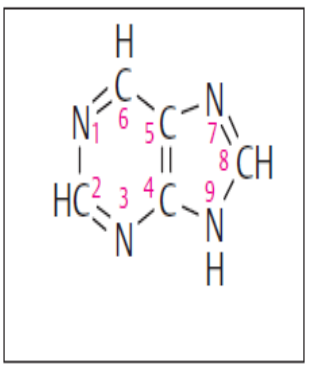
- Eukaryotic DNA is bound to proteins, forming a complex called chromatin. During interphase (when cells are not dividing), some of the chromatin is diffuse (euchromatin) and some isdense (heterochromatin), but no distinct structures can be observed.
- However,before mitosis (when cells divide), the DNA is replicated, resulting in two identical chromosomes called sister chromatids. During metaphase (a period in mitosis), these condense into discrete, visible chromosomes.
- Less than 0.1% of the total DNA in a cell is present in mitochondria. The genetic information in a mitochondrion is encoded in less than 20,000 base pairs of DNA.

Composition of DNA

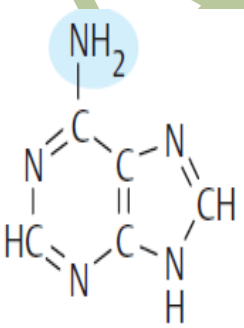
All nucleic acids are made up from nucleotide components, which in turn consist of a base, a sugar, and a phosphate residue. (phosphoric acid)

Bases

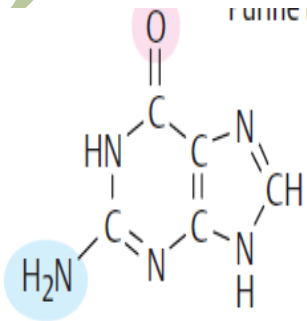
- They are aromatic heterocyclic compounds derived from either **pyrimidine** or **purine**.
- The purine bases **adenine** and **guanine** and the pyrimidine base **thymine** and **cytosine** are present in DNA.



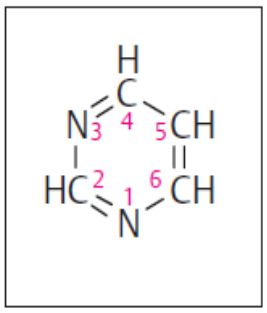
Purine



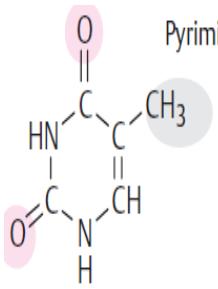
Adenine (Ade)



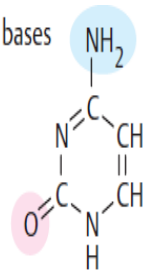
Guanine (Gua)



Pyrimidine



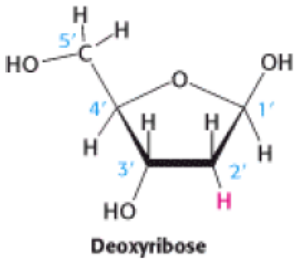
Thymine (Thy)



Cytosine (Cyt)

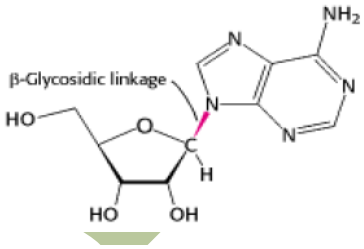
Sugars

- DNA contain 2'- deoxy-D-ribose, the pentose residues are present in the furanose form.
- The sugars and bases are linked by an *N*-glycosidic bond between the C-1 of the sugar and either the N-9 of the purine ring or N-1 of the pyrimidine ring. This bond always adopts the β-configuration.



Deoxyribose

Nucleosic



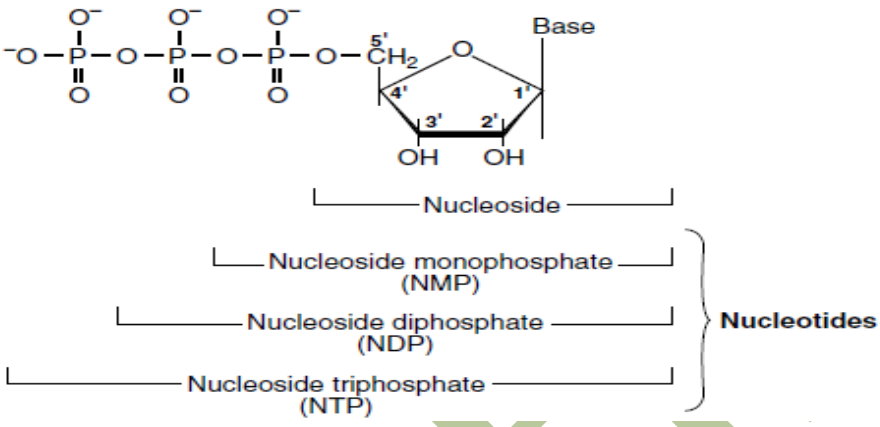
Glycosidic linkage in a nucleoside.

- In nucleosides, the nitrogenous base is linked by an *N*-glycosidic bond to the anomeric carbon of the sugar, i.e., deoxyribose.
- When a nucleic acid base is N-glycosidically linked to ribose or 2-deoxyribose, it yields a nucleoside. (Base + Sugar)

Nucleotides

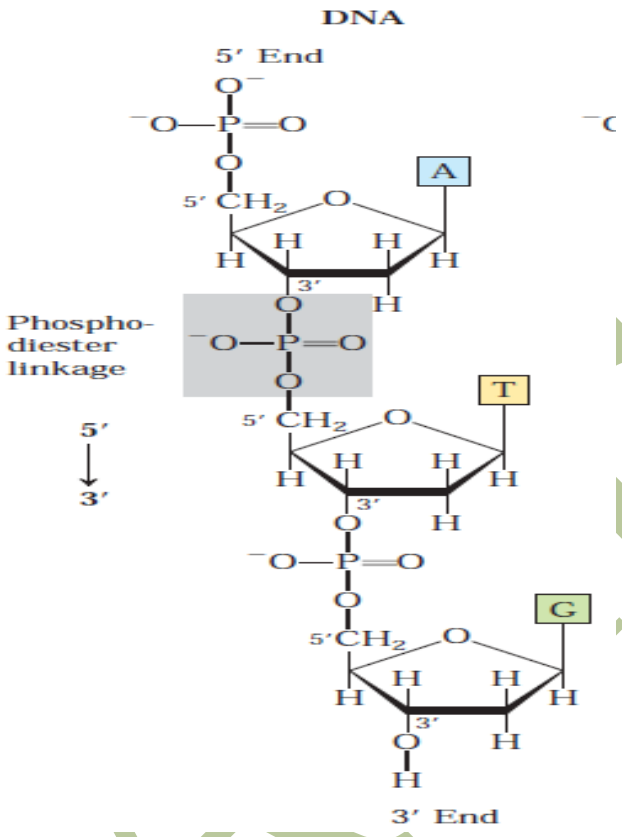
- A nucleotide is a nucleoside with an inorganic phosphate attached to a 5'-hydroxyl group of the sugar in ester linkage. The names and abbreviations of nucleotides specify the base, the sugar, and the number of phosphates attached (MP, **m**ono**p**hosphate; DP, **di**phosphate; TP, **tri**phosphate).

- In deoxynucleotides, the prefix "d" precedes the abbreviation. For example, GDP is guanosine diphosphate (the base guanine attached to a ribose that has two phosphate groups) and dATP is deoxyadenosine triphosphate (the base adenine attached to a deoxyribose with three phosphate groups).



Phosphodiester Bonds

- The successive nucleotides of DNA are covalently linked through phosphate-group "bridges," in which the 5_-phosphate group of one nucleotide unit is joined to the 3_-hydroxyl group of the next nucleotide, creating a **phosphodiester linkage**
- Thus the covalent backbones of nucleic acids consist of alternating phosphate and pentose residues, and the nitrogenous bases may be regarded as side groups joined to the backbone at regular intervals. The backbones of DNA are hydrophilic.
- All the phosphodiester linkages have the same orientation along the chain (Fig. 8–7), giving each linear nucleic acid strand a specific polarity and distinct 5' and 3' ends. By definition, the **5' end** lacks a nucleotide at the 5' position and the **3' end** lacks a nucleotide at the 3' position. Other groups (most often one or more phosphates) may be present on one or both ends.



Base paring

Concept of Base-Pairing was proposed by Chargaff in 1950. His proposal is called as Chargaff's rule of Base pairing.

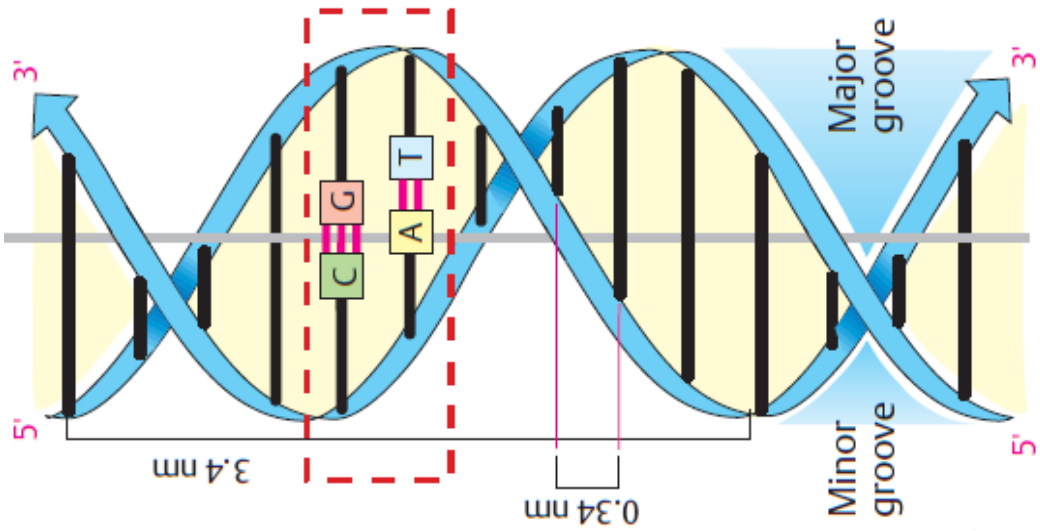
According to Chargaff's rule of Base pairing,

1) Total amount of purines equaled the total amount of pyrimidines. (A+G=T+C)
2) Adenine always pairs with thymine (A+T) and guanine always pairs with cytosine (G+C)
3) The amount of adinine equaled the amount of thymine (A=T), likewise, amount of guanine equaled the amount of cytosine (G=C).
4) Two hydrogen bonds are formed between adenine and thymine and three hydrogen bonds are formed between guanine and cytosine.

Watson and Crick model of DNA

Based on x-ray analysis, Watson and Crick proposed the structure of DNA, according to him,

- The two strands are complementary to each other.
- The two complementary strands of DNA run in opposite directions. On one strand, the 5'-carbon of the sugar is above the 3'-carbon. This strand is said to run in a 5_ to 3_ direction. On the other strand, the 3'-carbon is above the 5'-carbon. This strand is said to run in a 3' to 5' direction.
- Thus, the strands are antiparallel (that is, they run in opposite directions.)
- The two strands are wrapped helically around each other,with sugar-phosphate chain on the outside(forming ribbon like backbone of double helix) and purines and pyrimidines on the inside of the helix(projecting between two sugar phosphate backbones as transverse bars).
- Both polynucleotide strands remains separated by 20A° distance.
- The coiling of double helix is right handed and a complete turn occurs every 34A°. Since each nucleotide occupies 3.4A° distance along the length of a polynucleotide strand, ten mononucleotides occur per complete turn.(10 base pair per turn of the helix)
- The offset pairing of the two strands creates a **major groove** and **minor groove** on the surface of the duplex.



Polymorphism of DNA helix / Alternative forms of DNA double helix

For about 20 years after discovery of DNA double helix in 1953, some experiments shown that DNA is much more polymorphic. Thus DNA has following Types,

B-form/B DNA

- Biologically important form of DNA, naturally found in most living systems. Watson and Crick model DNA.

A-form/A DNA

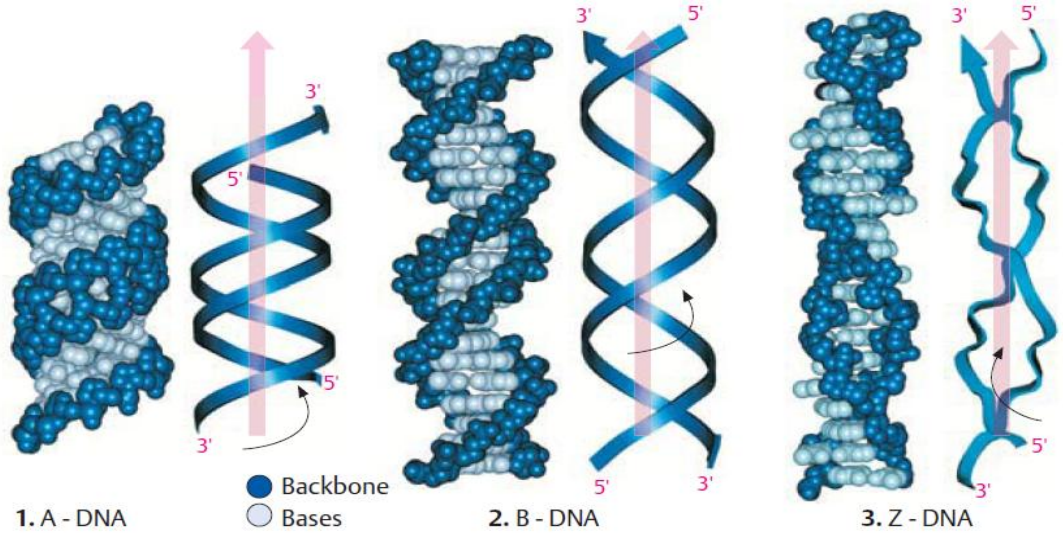
- It is right handled but less hydrated than B-form DNA.
- It is more compact with 11 base pair per turn of the helix.
- The double helix is 23A° in diameter.
- The bases are tilted more in relation to the axis of the helix than in the B-DNA.

Z-form/Z DNA

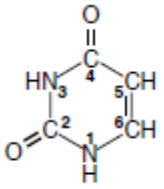
- It is observed by crystallographic studies. It reveals that synthetic nucleotides consists of alternating purines and pyrimidines such as GCGCGCGCGCGC.
- They are called as Z DNA because of its zigzag nature.
- They are left handed DNA, with 12 base pair per turn of helix.
- It is found in solutions of high-ionic strength, Example- 2M NaCl.
- The double helix is 18A° in diameter.

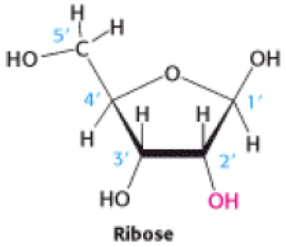
Other forms of DNA

- C form DNA found at 66 percent relative humidity with Li^+ ions.
- D form and E form DNA are found as rare extreme variants and has only 8 and 7.5 base pair per turn respectively. These DNAs lack guanine.



RNA

- RNA is the genetic material of some of viruses.
- RNA is similar to DNA. Like DNA, it is composed of nucleotides joined by 3'- to 5' phosphodiester bonds, the purine bases adenine and guanine, and the pyrimidine base cytosine. However, its other pyrimidine base is uracil rather than thymine.
- Uracil and thymine are identical bases except that thymine has a methyl group at position 5 of the ring. In RNA, the sugar is ribose, which contains a hydroxyl group on the 2´-carbon.
- 

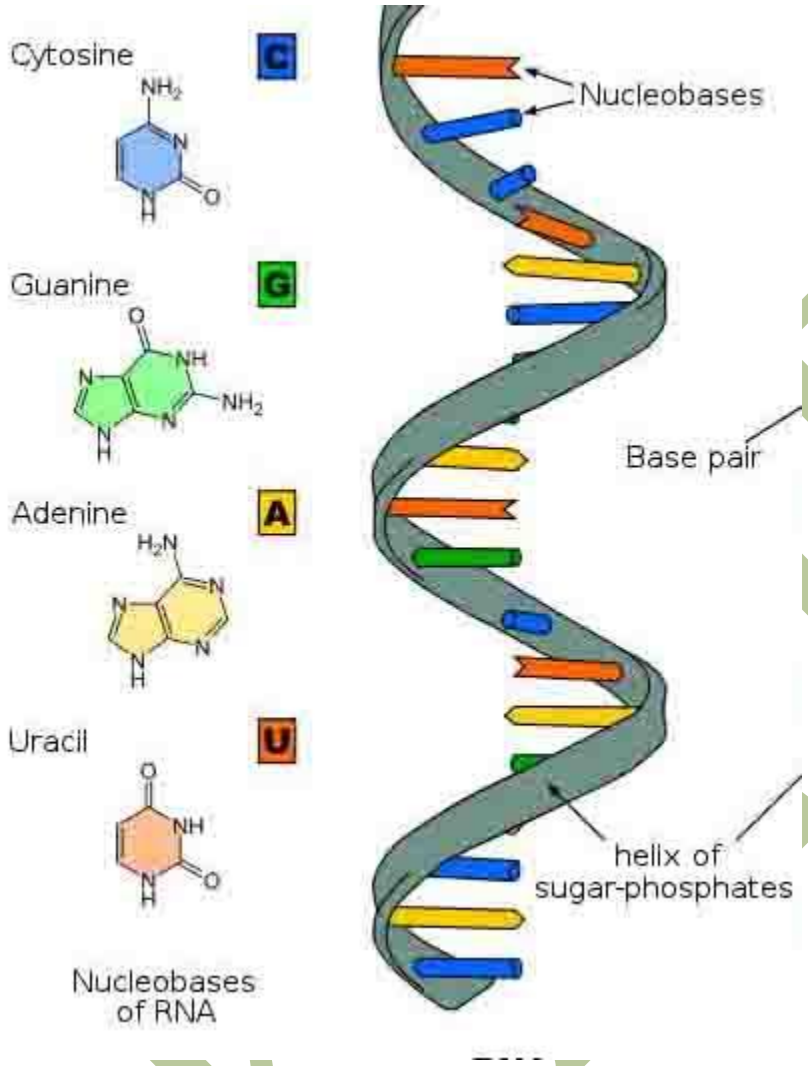


- RNA chains are usually single-stranded and lack the continuous helical structure of double stranded DNA.

- However, RNA still has considerable secondary and tertiary structure because base pairs can form in regions where the strand loops back on itself. As in DNA, pairing between the bases is complementary and antiparallel.
- But in RNA, adenine pairs with uracil rather than thymine. Basepairing in RNA can be extensive, and the irregular looped structures generated are important for the binding of molecules, such as enzymes, that interact with specific regions of the RNA.

Types of RNA

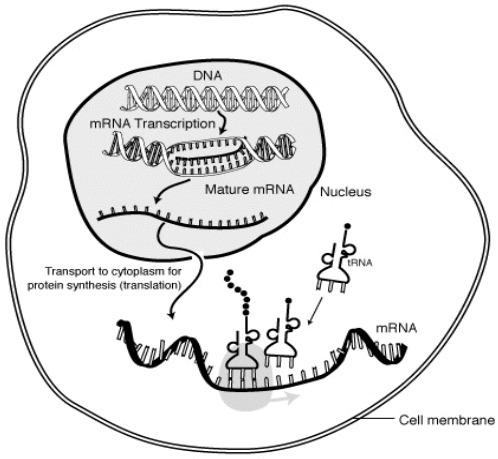
- The three major types of RNA (mRNA, rRNA, and tRNA) participate directly in the process of protein synthesis. Other less abundant RNAs are involved in replication or in the processing of RNA, that is, in the conversion of RNA precursors to their mature forms.



Structure of mRNA

- Each mRNA molecule contains a nucleotide sequence that is converted into the amino acid sequence of a polypeptide chain in the process of translation.
- In eukaryotes, messenger RNA (mRNA) is transcribed from protein-coding genes as a long primary transcript that is processed in the nucleus to form mRNA.
- The various processing intermediates, which are mRNA precursors, are called pre-mRNA or hnRNA (heterogenous nuclear RNA).

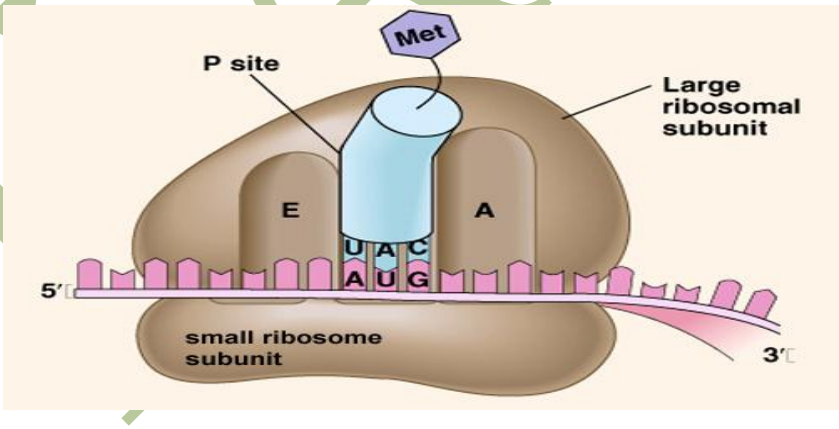
- mRNA travels through nuclear pores to the cytoplasm, where it binds to ribosomes and tRNAs and directs the sequential insertion of the appropriate amino acids into a polypeptide chain. Eukaryotic mRNA consists of a leader sequence at the 5´ end, a coding region, and a trailer sequence at the 3' end.
- The leader sequence begins with a guanosine cap structure at its 5' end. The coding region begins with a trinucleotide start codon that signals the beginning of translation, followed by the trinucleotide codons for amino acids, and ends at a termination signal.
- The trailer terminates at its 5' end with a poly (A) tail that may be up to 200 nucleotides long. Most of the leader sequence, all of the coding region, and most of the trailer are formed by transcription of the complementary nucleotide sequence in DNA.
- However, the terminal guanosine in the cap structure and the poly(A) tail do not have complementary sequences; they are added posttranscriptionally.



Structure of rRNA

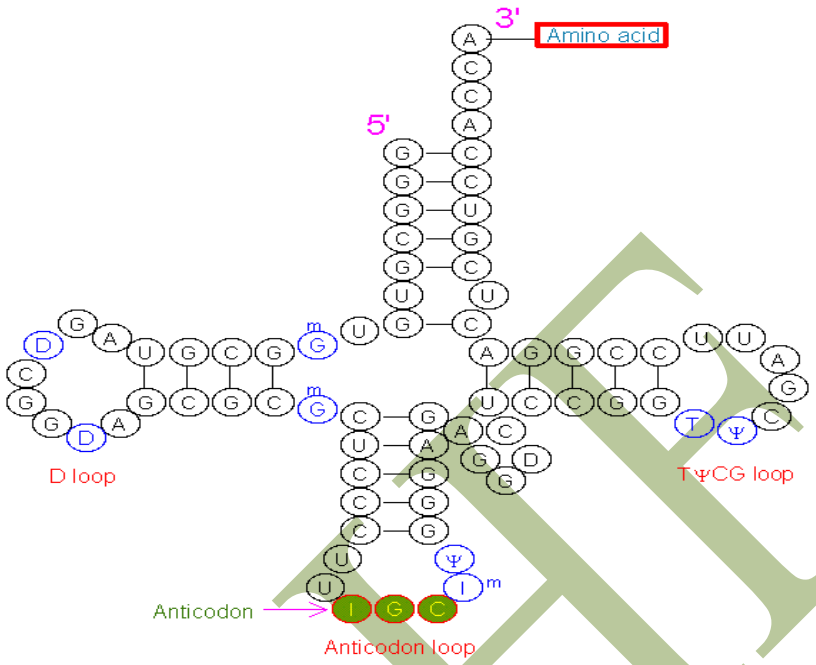
- Ribosomes are subcellular ribonucleoprotein complexes on which protein synthesis occurs. Different types of ribosomes are found in prokaryotes and in the cytoplasm
- and mitochondria of eukaryotic cells.
- Prokaryotic ribosomes contain three types of rRNA molecules with sedimentation coefficients of 16, 23, and 5S.

- The 30S ribosomal subunit contains the 16S rRNA complexed with proteins, and the 50S ribosomal subunit contains the 23S and 5S rRNAs complexed with proteins.
- The 30S and 50S ribosomal subunits join to form the 70S ribosome, which participates in protein synthesis.
- Cytoplasmic ribosomes in eukaryotes contain four types of rRNA molecules of 18, 28, 5, and 5.8S.
- The 40S ribosomal subunit contains the 18S rRNA complexed with proteins, and the 60S ribosomal subunit contains the 28, 5, and 5.8S rRNAs complexed with proteins. In the cytoplasm, the 40S and 60S ribosomal subunits combine to form the 80S ribosomes that participate in protein synthesis.
- Mitochondrial ribosomes, with a sedimentation coefficient of 55S, are smaller than cytoplasmic ribosomes. Their properties are similar to those of the 70S ribosomes of bacteria.
- rRNAs contain many loops and exhibit extensive base-pairing in the regions between the loops. The sequences of the rRNAs of the smaller ribosomal subunits exhibit secondary structures that are common to many different genera.



`Structure of tRNA

- During protein synthesis, tRNA molecules carry amino acids to ribosomes and ensure that they are incorporated into the appropriate positions in the growing polypeptide chain.
- This is done through base-pairing of three bases of the tRNA (the anticodon) with the three base codons within the coding region of the mRNA.
- Therefore, cells contain at least 20 different tRNA molecules that differ somewhat in nucleotide sequence, one for each of the amino acids found in proteins.
- Many amino acids have more than one tRNA. tRNA molecules contain not only the usual nucleotides, but also derivatives of these nucleotides that are produced by posttranscriptional modifications.
- In eukaryotic cells, 10 to 20% of the nucleotides of tRNA are modified. Most tRNA molecules contain ribothymidine (T), in which a methyl group is added to uridine to form ribothymidine. They also contain dihydrouridine (D), in which one of the double bonds of the base is reduced; and pseudouridine (Ψ), in which uracil is attached to ribose by a carbon–carbon bond rather than a nitrogen–carbon bond.
- The base at the 5'-end of the anticodon of tRNA is frequently modified. tRNA molecules are rather small compared with both mRNA and the large rRNA molecules. On average, tRNA molecules contain approximately 80 nucleotides and have a sedimentation coefficient of 4S.
- Because of their small size and high content of modified nucleotides, tRNAs were the first nucleic acids to be sequenced. Since 1965 when Robert Holley deduced the structure of the first tRNA, the nucleotide sequences of many different tRNAs have been determined. Although their primary sequences differ, all tRNA molecules can form a structure resembling a cloverleaf.



DNA replication

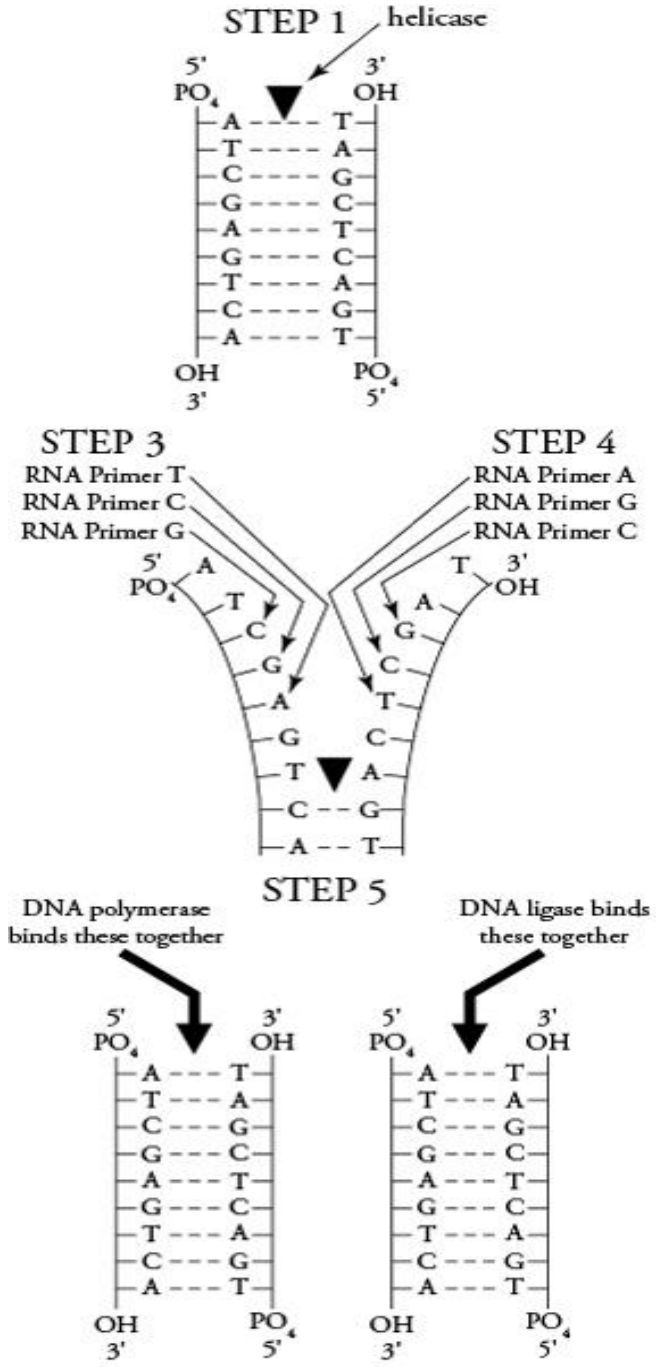
- During the S phase of interphase, a second chromatid is assembled. The second chromatid contains the exact same DNA found in the first chromatid.
- The copying process, called *DNA replication,* involves separating (“unzipping”) the DNA molecule into two strands, each of which serves as a template to assemble a new, complementary strand.
- The result is two identical double-stranded molecules of DNA that consist of a single strand of old DNA (the template strand) and a single strand of new, replicated DNA (the complementary strand).

Following are the steps involved in duplicating DNA. While studying the steps, refer to following Figure:

- Each strand of DNA is labeled as 3#x2032 and 5#x2032. The 3#x2032 area terminates with a hydroxyl group and the 5#x2032 area terminates with a phosphate group.
- The enzyme helicase “unzips” (unwinds) the DNA helix, producing a Y-shaped replication fork. Note: The DNA shown in Figure 4 is not depicted in a helical shape; it is drawn in a parallel form for ease of understanding.

- RNA primers "bring" in respective base pairings to each of the original strands. DNA polymerase is an enzyme that binds the base pairings together, but it can only work in the direction of 5#x2032 to 3#x2032.
- The other original strand also has to be "put together" 5#x2032 to 3#x2032 so it will be put together in a backward fashion.
- In order to bind those base pairings to the original strand, a different enzyme called *DNA ligase* is necessary. This is called the "lagging strand" since it basically takes longer to put together.

Figure. DNA Replication .


STEP 1
helicase
5' PO4
3' OH
OH 3'
PO4 5'
STEP 2
helicase
STEP 3
RNA Primer T
RNA Primer C
RNA Primer G
STEP 4
RNA Primer A
RNA Primer G
RNA Primer C
STEP 5
DNA polymerase binds these together
DNA ligase binds these together

Mutations

The replication process of DNA is extremely accurate; however, errors can occur when nucleotide bases between DNA strands are occasionally paired incorrectly. In addition, errors in DNA molecules may arise as a result of exposure to radiation (such as ultraviolet or X-ray) or various reactive chemicals. When errors occur, repair mechanisms are available to make corrections.

If a DNA error is not repaired, it becomes a mutation. A mutation is any sequence of nucleotides in a DNA molecule that does not exactly match the original DNA molecule from which it was copied. Mutations include an incorrect nucleotide (substitution), a missing nucleotide (deletion), or an additional nucleotide not present in the original DNA molecule (insertion). When an insertion mutation occurs, it causes all subsequent nucleotides to be displaced one position, producing a frameshift mutation. Radiation or chemicals that cause mutations are called mutagens. Mutagens that activate uncontrolled cell growth (cancer) are called carcinogens.

Transcription

Key points:

- Transcription is the first step in gene expression. It involves copying a gene's DNA sequence to make an RNA molecule.
- Transcription is performed by enzymes called RNA polymerases, which link nucleotides to form an RNA strand (using a DNA strand as a template).
- Transcription has three stages: initiation, elongation, and termination.
- In eukaryotes, RNA molecules must be processed after transcription: they are spliced and have a 5' cap and poly-A tail put on their ends.
- Transcription is controlled separately for each gene in your genome.

Introduction

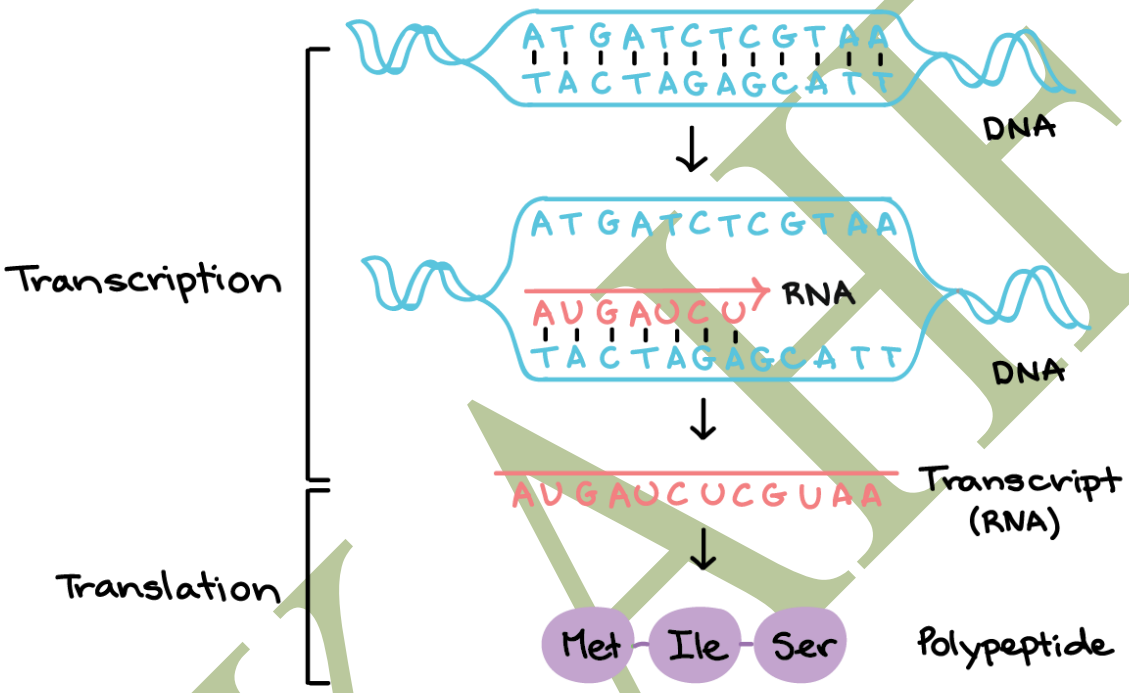
Have you ever had to transcribe something? Maybe someone left a message on your voicemail, and you had to write it down on paper. Or maybe you took notes in class, then rewrote them neatly to help you review.

As these examples show, *transcription* is a process in which information is rewritten. Transcription is something we do in our everyday lives, and it's also something our cells must do, in a more

specialized and narrowly defined way. In biology, transcription is the process of copying out the DNA sequence of a gene in the similar alphabet of RNA.

Overview of transcription

Transcription is the first step in gene expression, in which information from a gene is used to construct a functional product such as a protein. The goal of transcription is to make a RNA copy of a gene's DNA sequence. For a protein-coding gene, the RNA copy, or transcript, carries the



information needed to build a polypeptide (protein or protein subunit). Eukaryotic transcripts need to go through some processing steps before translation into proteins.

In transcription, a region of DNA opens up. One strand, the template strand, serves as a template for synthesis of a complementary RNA transcript. The other strand, the coding strand, is identical to the RNA transcript in sequence, except that it has uracil (U) bases in place of thymine (T) bases.

Example:

Coding strand: 5'-ATGATCTCGTAA-3' Template strand: 3'-TACTAGAGCATT-5' RNA transcript: 5'-AUGAUCUCGUAA-3'

For a protein-coding gene, the RNA transcript contains the information needed to synthesize a polypeptide (protein or protein subunit) with a particular amino acid sequence. In this case:

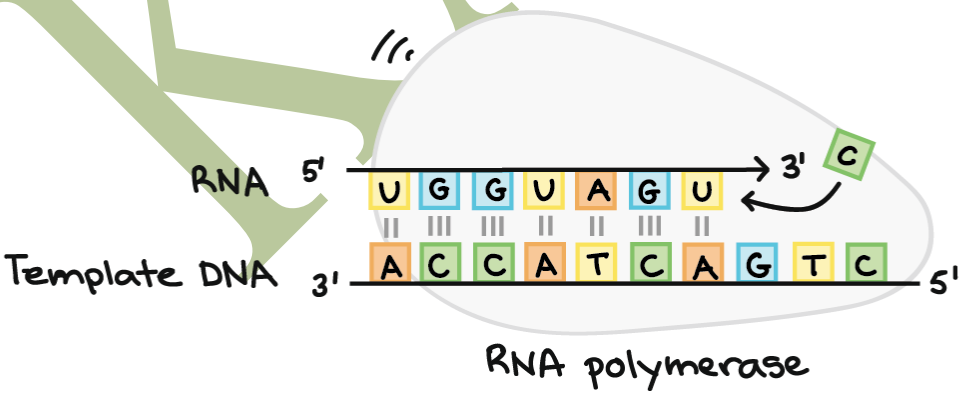
RNA transcript (acting as messenger RNA): 5'-AUGAUCUCGUAA-3' Polypeptide: Met-Ile-Ser-STOP

RNA polymerase

The main enzyme involved in transcription is RNA polymerase, which uses a single-stranded DNA template to synthesize a complementary strand of RNA. Specifically, RNA polymerase builds an RNA strand in the 5' to 3' direction, adding each new nucleotide to the 3' end of the strand.

RNA polymerase synthesizes an RNA strand complementary to a template DNA strand. It synthesizes the RNA strand in the 5' to 3' direction, while reading the template DNA strand in the 3' to 5' direction. The template DNA strand and RNA strand are antiparallel.

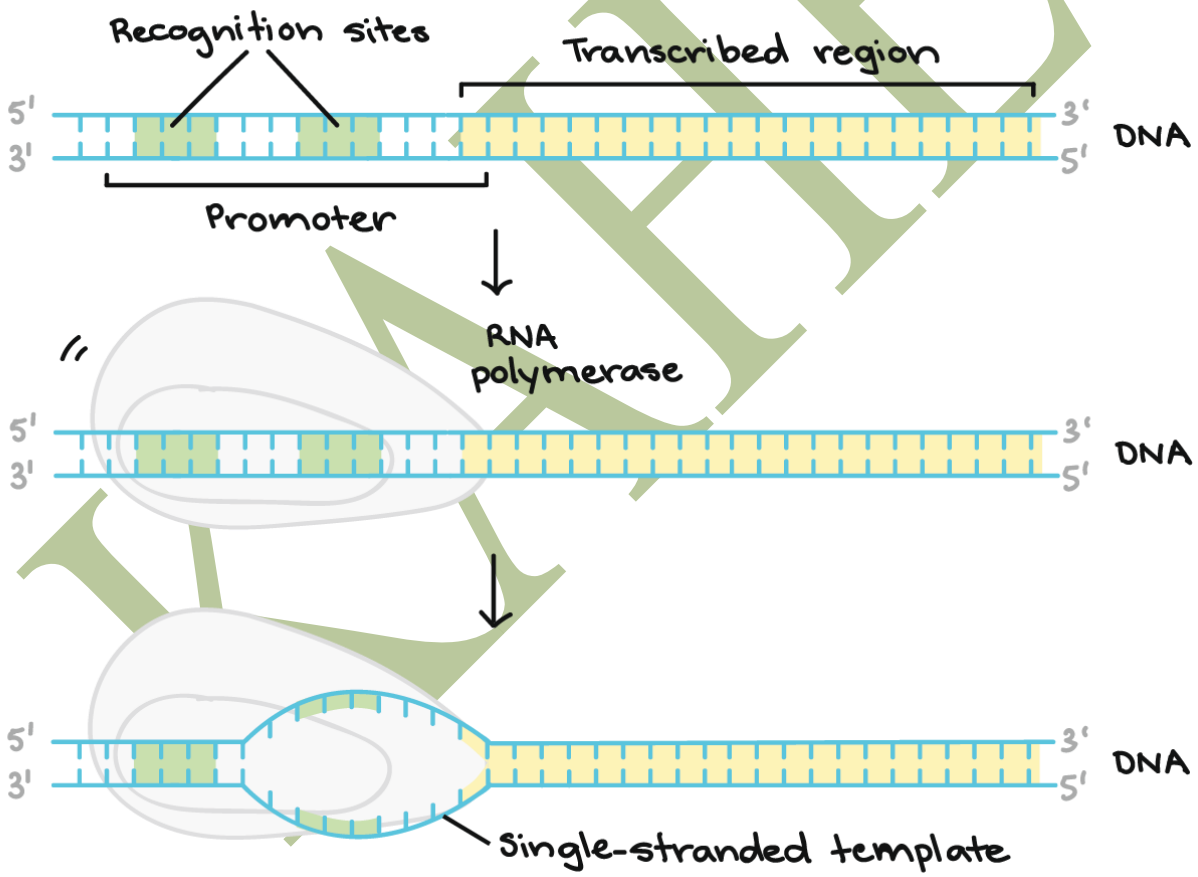
RNA transcript: 5'-UGGUAGU...-3' (dots indicate where nucleotides are still being added at 3' end) DNA template: 3'-ACCATCAGTC-5'



Stages of transcription

Transcription of a gene takes place in three stages: initiation, elongation, and termination. Here, we will briefly see how these steps happen in bacteria. You can learn more about the details of each stage (and about how eukaryotic transcription is different) in the stages of transcription article.

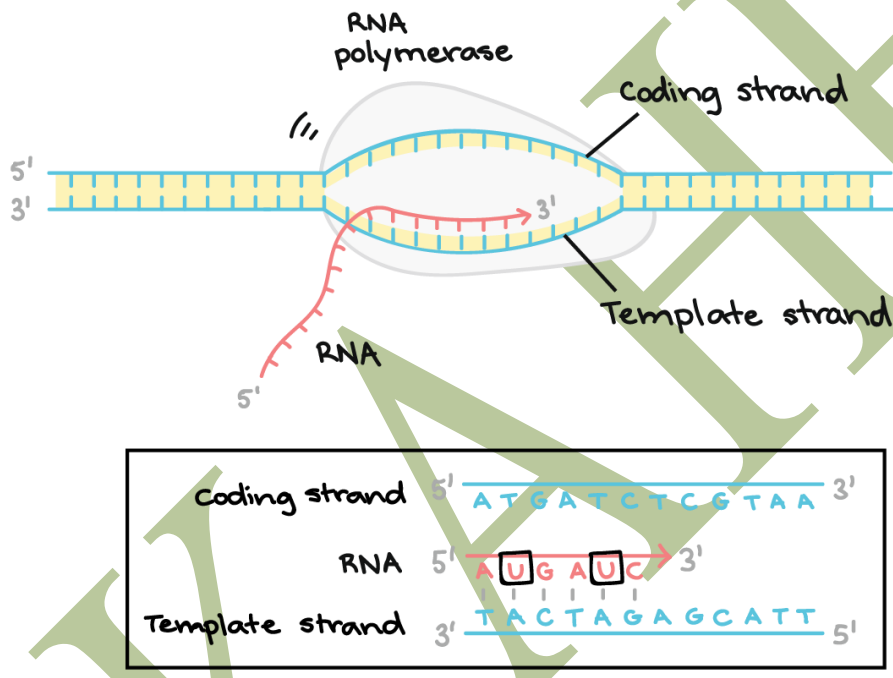
1. Initiation. RNA polymerase binds to a sequence of DNA called the promoter, found near the beginning of a gene. Each gene (or group of co-transcribed genes, in bacteria) has its own promoter. Once bound, RNA polymerase separates the DNA strands, providing the single-stranded template needed for transcription.



The promoter region comes before (and slightly overlaps with) the transcribed region whose transcription it specifies. It contains recognition sites for RNA polymerase or its helper proteins to bind to. The DNA opens up in the promoter region so that RNA polymerase can begin transcription.

2. Elongation. One strand of DNA, the template strand, acts as a template for RNA polymerase. As it "reads" this template one base at a time, the polymerase builds an RNA molecule out of complementary nucleotides, making a chain that grows from 5' to 3'. The RNA transcript carries the same information as the non-template (coding) strand of DNA, but it contains the base uracil (U) instead of thymine (T).

[What do 5' and 3' mean?]



RNA polymerase synthesizes an RNA transcript complementary to the DNA template strand in the 5' to 3' direction. It moves forward along the template strand in the 3' to 5' direction, opening the DNA double helix as it goes. The synthesized RNA only remains bound to the template strand for a short while, then exits the polymerase as a dangling string, allowing the DNA to close back up and form a double helix.

In this example, the sequences of the coding strand, template strand, and RNA transcript are:

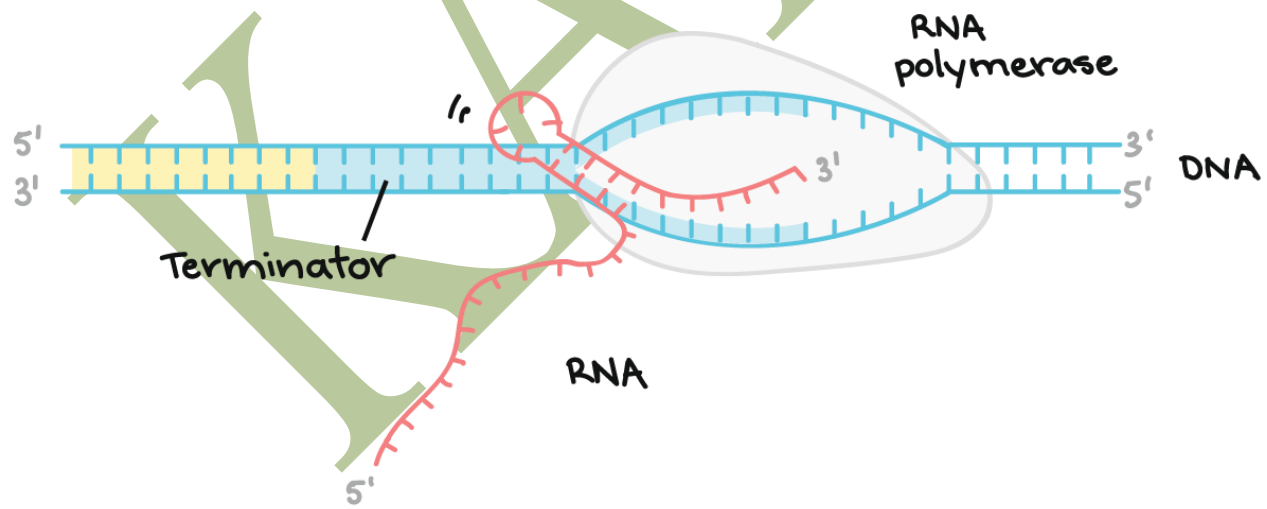
Coding strand: 5' - ATGATCTCGTAA-3'

Template strand: 3'-TACTAGAGCATT-5'

RNA: 5'-AUGAUC...-3' (the dots indicate where nucleotides are still being added to the RNA strand at its 3' end)

3. Termination. Sequences called terminators signal that the RNA transcript is complete. Once they are transcribed, they cause the transcript to be released from the RNA polymerase. An example of a termination mechanism involving formation of a hairpin in the RNA is shown below.

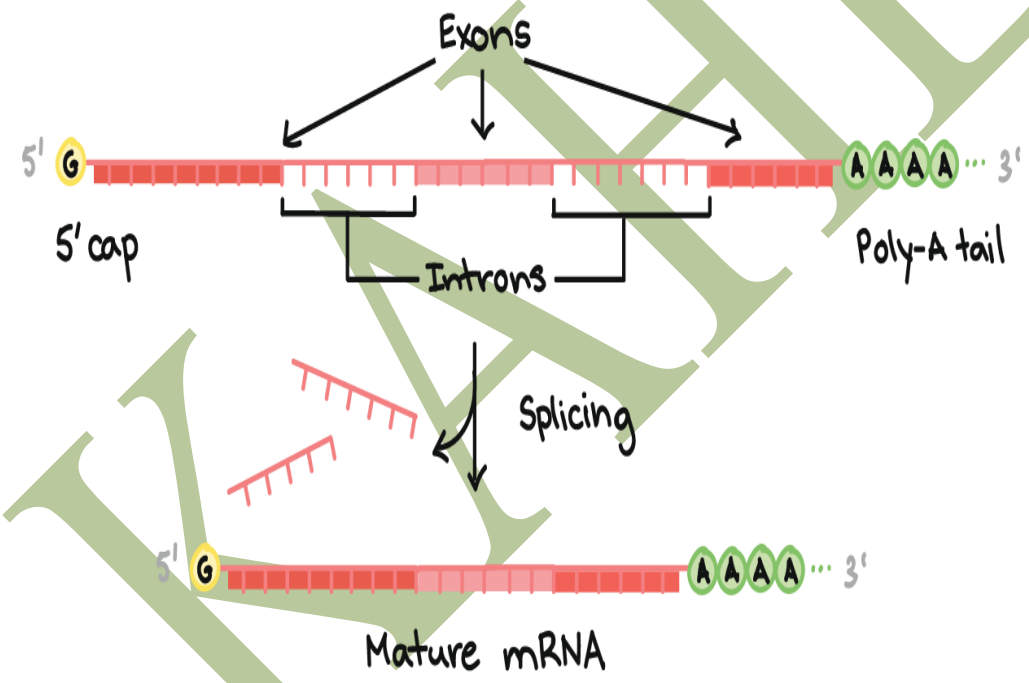
The terminator DNA encodes a region of RNA that forms a hairpin structure followed by a string of U nucleotides. The hairpin structure in the transcript causes the RNA polymerase to stall. The U nucleotides that come after the hairpin form weak bonds with the A nucleotides of the DNA template, allowing the transcript to separate from the template and ending transcription.



Post Transcriptional modification

In bacteria, RNA transcripts can act as messenger RNAs (mRNAs) right away. In eukaryotes, the transcript of a protein-coding gene is called a pre-mRNA and must go through extra processing before it can direct translation.

- Eukaryotic pre-mRNAs must have their ends modified, by addition of a 5' cap (at the beginning) and 3' poly-A tail (at the end).
- Many eukaryotic pre-mRNAs undergo splicing. In this process, parts of the pre-mRNA (called introns) are chopped out, and the remaining pieces (called exons) are stuck back together.



Top of image: Diagram of a pre-mRNA with a 5' cap and 3' poly-A tail. The 5' cap is on the 5' end of the pre-mRNA and is a modified G nucleotide. The poly-A tail is on the 3' end of the pre-mRNA and consists of a long string of A nucleotides (only a few of which are shown).

The pre-mRNA still contains both exons and introns. Along the length of the mRNA, there is an alternating pattern of exons and introns: Exon 1 - Intron 1 - Exon 2 - Intron 2 - Exon 3. Each consists of a stretch of RNA nucleotides.

During splicing, the introns are removed from the pre-mRNA, and the exons are stuck together to form a mature mRNA.

Bottom of image: Mature mRNA that does not contain the intron sequences (Exon 1 - Exon 2 - Exon 3 only).

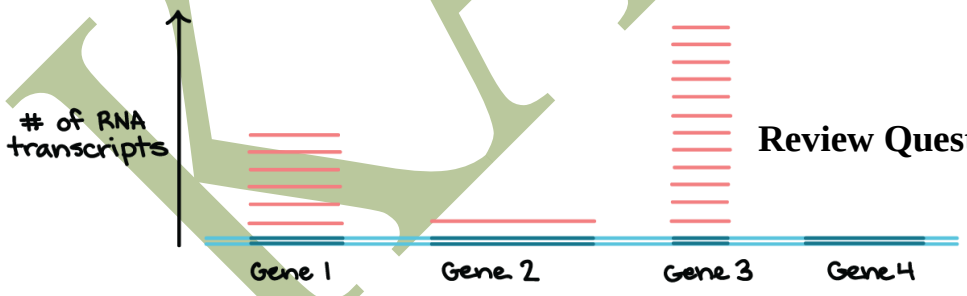
End modifications increase the stability of the mRNA, while splicing gives the mRNA its correct sequence. (If the introns are not removed, they'll be translated along with the exons, producing a "gibberish" polypeptide.)

To learn more about pre-mRNA modifications in eukaryotes, check out the article on pre-mRNA processing.

Transcription happens for individual genes

Not all genes are transcribed all the time. Instead, transcription is controlled individually for each gene (or, in bacteria, for small groups of genes that are transcribed together). Cells carefully regulate transcription, transcribing just the genes whose products are needed at a particular moment.

For example, the diagram below shows a "snapshot" of an imaginary cell's RNAs at a given moment in time. In this cell, genes 1, 2 and 3, are transcribed, while gene 4 is not. Also, genes 1, 2, and 3 are transcribed at different levels, meaning that different numbers of RNA molecules are made for each.



Review Questions

Protein synthesis (Translation)

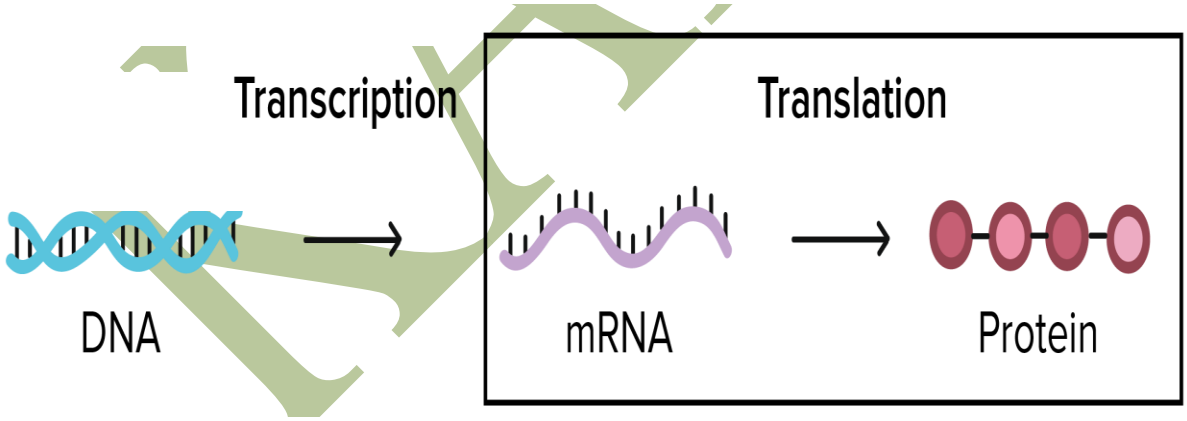
Introduction

Take a moment to look at your hands. The bone, skin, and muscle you see are made up of cells. And each of those cells contains many millions of proteins^11start superscript, 1, end superscript. As a matter of fact, proteins are key molecular "building blocks" for every organism on Earth!

How are these proteins made in a cell? For starters, the instructions for making proteins are "written" in a cell's DNA in the form of genes. If that idea is new to you, you may want to check out the section on DNA to RNA to protein (central dogma) before getting into the nitty-gritty of building proteins.

Basically, a gene is used to build a protein in a two-step process:

- Step 1: **transcription**! Here, the DNA sequence of a gene is "rewritten" in the form of RNA. In eukaryotes like you and me, the RNA is processed (and often has a few bits snipped out of it) to make the final product, called a messenger RNA or mRNA.
- Step 2: **translation**! In this stage, the mRNA is "decoded" to build a protein (or a chunk/subunit of a protein) that contains a specific series of amino acids.
[What exactly is an "amino acid"?]



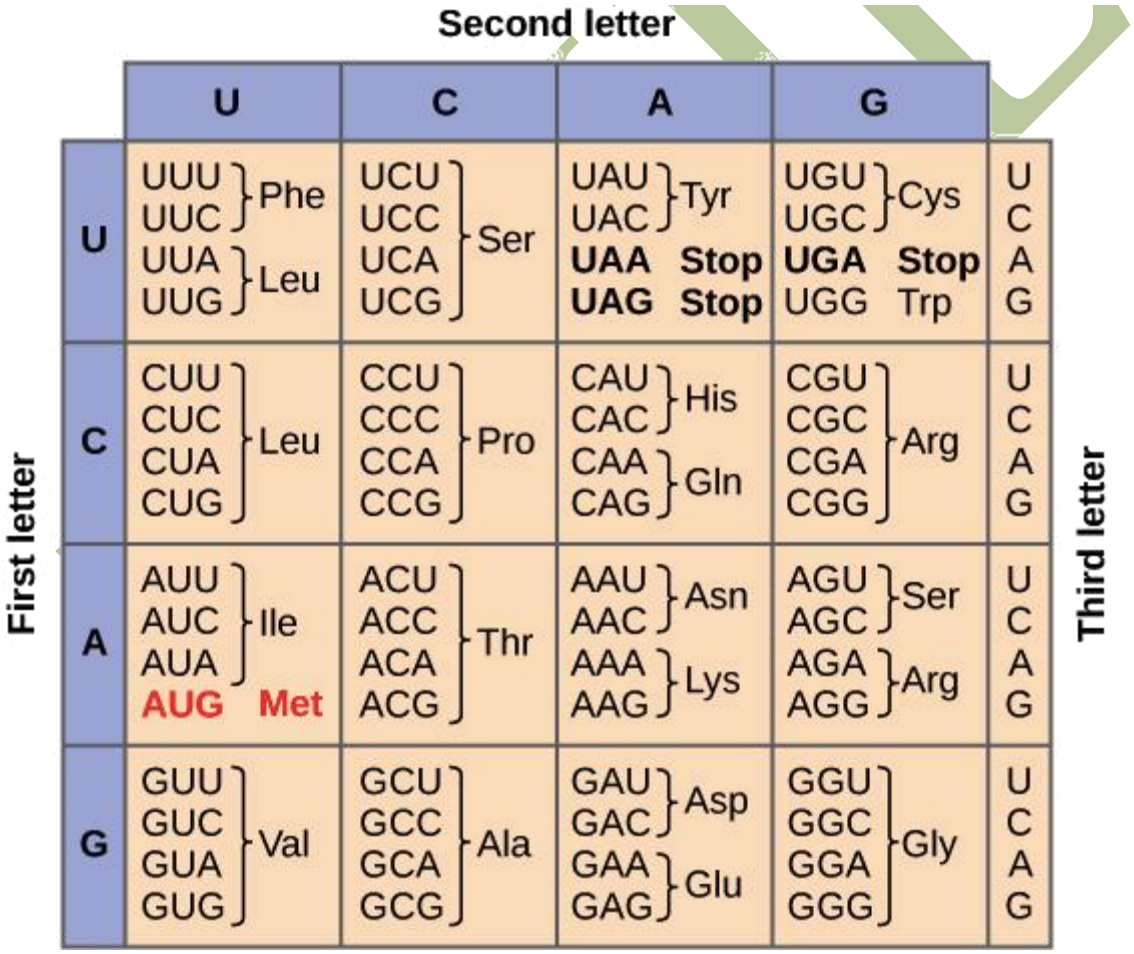
The central dogma of molecular biology states that information flows from DNA (genes) to mRNA through the process of transcription, and then to proteins through the process of translation.

Image modified from "Central dogma of molecular biochemistry with enzymes," by Daniel Horspool (CC BY-SA 3.0). The modified image is licensed under a CC BY-SA 3.0 license.

In this article, we'll zoom in on translation, getting an overview of the process and the molecules that carry it out.

The genetic code

During translation, a cell "reads" the information in a messenger RNA (mRNA) and uses it to build a protein. Actually, to be a little more techical, an mRNA doesn't always encode—provide instructions for—a whole protein. Instead, what we can confidently say is that it always encodes a **polypeptide**, or chain of amino acids.

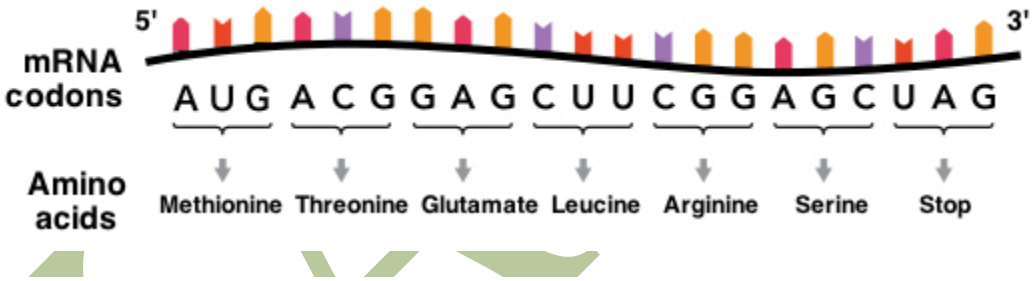


Each three-letter sequence of mRNA nucleotides corresponds to a specific amino acid, or to a stop codon. UGA, UAA, and UAG are stop codons. AUG is the codon for methionine, and is also the start codon.

In an mRNA, the instructions for building a polypeptide are RNA nucleotides (As, Us, Cs, and Gs) read in groups of three. These groups of three are called **codons**.

There are 616161 codons for amino acids, and each of them is "read" to specify a certain amino acid out of the 202020 commonly found in proteins. One codon, AUG, specifies the amino acid methionine and also acts as a **start codon** to signal the start of protein construction.

There are three more codons that do *not* specify amino acids. These **stop codons**, UAA, UAG, and UGA, tell the cell when a polypeptide is complete. All together, this collection of codon-amino acid relationships is called the **genetic code**, because it lets cells "decode" an mRNA into a chain of amino acids.



Each mRNA contains a series of codons (nucleotide triplets) that each specifies an amino acid. The correspondence between mRNA codons and amino acids is called the genetic code.

5' AUG - Methionine ACG - Threonine GAG - Glutamate CUU - Leucine CGG - Arginine AGC - Serine UAG - Stop 3'

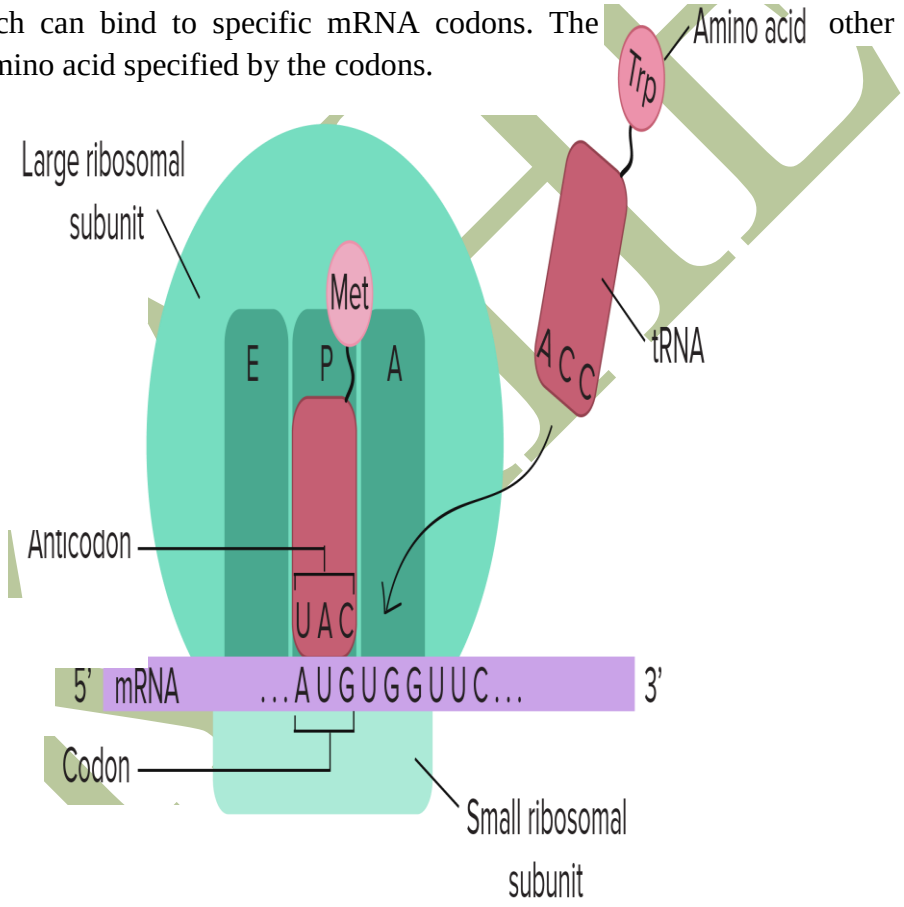
Image modified from "RNA-codons-aminoacids," by Thomas Splettstoesser (CC BY-SA 4.0). The modified image is licensed under a CC BY-SA 4.0 license.

Overview of translation

How is an mRNA "read" to make a polypeptide? Two types of molecules with key roles in translation are tRNAs and ribosomes.

Transfer RNAs (tRNAs)

Transfer RNAs, or **tRNAs**, are molecular "bridges" that connect mRNA codons to the amino amino acids they encode. One end of each tRNA has a sequence of three nucleotides called an **anticodon**, which can bind to specific mRNA codons. The other end of the tRNA carries the amino acid specified by the codons.



There are many different types of tRNAs. Each type reads one or a few codons and brings the right amino acid matching those codons.

Ribosomes are composed of a small and large subunit and have three sites where tRNAs can bind to an mRNA (the A, P, and E sites). Each tRNA vcarries a specific amino acid and binds to an mRNA codon that is complementary to its anticodon.

Ribosomes

Ribosomes are the structures where polypeptides (proteins) are built. They are made up of protein and RNA (**ribosomal RNA**, or **rRNA**). Each ribosome has two subunits, a large one and a small one, which come together around an mRNA—kind of like the two halves of a hamburger bun coming together around the patty.

The ribosome provides a set of handy slots where tRNAs can find their matching codons on the mRNA template and deliver their amino acids. These slots are called the A, P, and E sites. Not only that, but the ribosome also acts as an enzyme, catalyzing the chemical reaction that links amino acids together to make a chain.

Want to learn more about the structure and function of tRNAs and ribosomes? Check out the tRNA and ribosomes article!

Steps of translation

Your cells are making new proteins every second of the day. And each of those proteins must contain the right set of amino acids, linked together in just the right order. That may sound like a challenging task, but luckily, your cells (along with those of other animals, plants, and bacteria) are up to the job.

To see how cells make proteins, let's divide translation into three stages: initiation (starting off), elongation (adding on to the protein chain), and termination (finishing up).

Getting started: Initiation

In **initiation**, the ribosome assembles around the mRNA to be read and the first tRNA (carrying the amino acid methionine, which matches the start codon, AUG). This setup, called the initiation complex, is needed in order for translation to get started.

Extending the chain: Elongation

Elongation is the stage where the amino acid chain gets **long**er. In elongation, the mRNA is read one codon at a time, and the amino acid matching each codon is added to a growing protein chain.

Each time a new codon is exposed:

- A matching tRNA binds to the codon
- The existing amino acid chain (polypeptide) is linked onto the amino acid of the tRNA via a chemical reaction
- The mRNA is shifted one codon over in the ribosome, exposing a new codon for reading

Elongation has three stages:

1) The anticodon of an incoming tRNA pairs with the mRNA codon exposed in the A site.

2) A peptide bond is formed between the new amino acid (in the A site) and the previously-added amino acid (in the P site), transferring the polypeptide from the P site to the A site.

3) The ribosome moves one codon down on the mRNA. The tRNA in the A site (carrying the polypeptide) shifts to the P site. The tRNA in the P site shifts to the E site and exits the ribosome.

Image based on similar diagram in Reece et al.^22start superscript, 2, end superscript

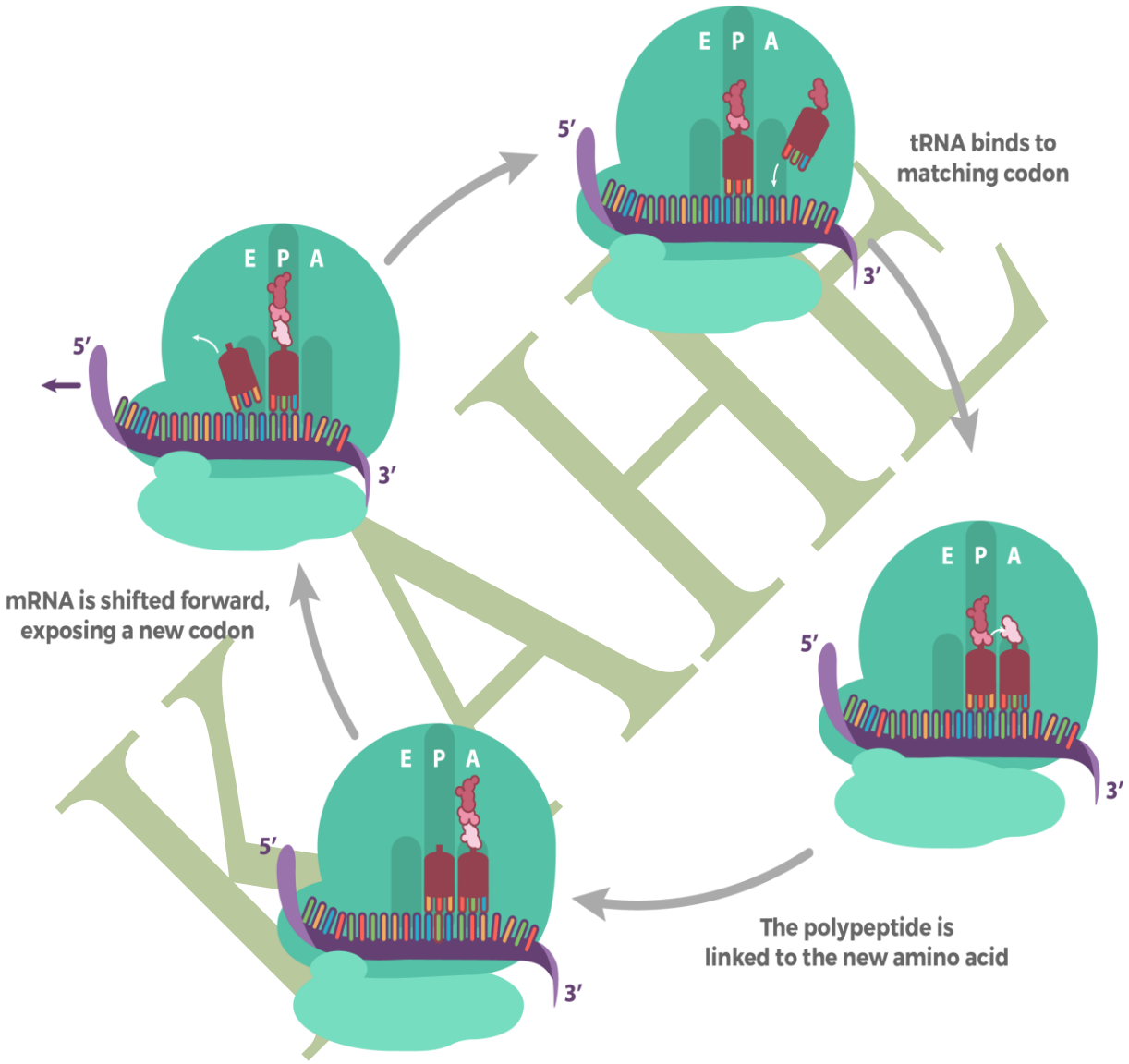
During elongation, tRNAs move through the A, P, and E sites of the ribosome, as shown above. This process repeats many times as new codons are read and new amino acids are added to the chain.

For more details on the steps of elongation, see the stages of translation article.

Finishing up: Termination

Termination is the stage in which the finished polypeptide chain is released. It begins when a stop codon (UAG, UAA, or UGA) enters the ribosome, triggering a series of events that separate the chain from its tRNA and allow it to drift out of the ribosome.

After termination, the polypeptide may still need to fold into the right 3D shape, undergo processing (such as the removal of amino acids), get shipped to the right place in the cell, or combine with other polypeptides before it can do its job as a functional protein.



Regulation of gene expression in prokaryotes

Introduction

We tend to think of bacteria as simple. But even the simplest bacterium has a complex task when it comes to gene regulation! The bacteria in your gut or between your teeth have genomes that contain thousands of different genes. Most of these genes encode proteins, each with its own role in a process such as fuel metabolism, maintenance of cell structure, and defense against viruses.

Some of these proteins are needed routinely, while others are needed only under certain circumstances. Thus, cells don't express all the genes in their genome all the time. You can think of the genome as being like a cookbook with many different recipes in it. The cell will only use the recipes (express the genes) that fit its current needs.

How is gene expression regulated?

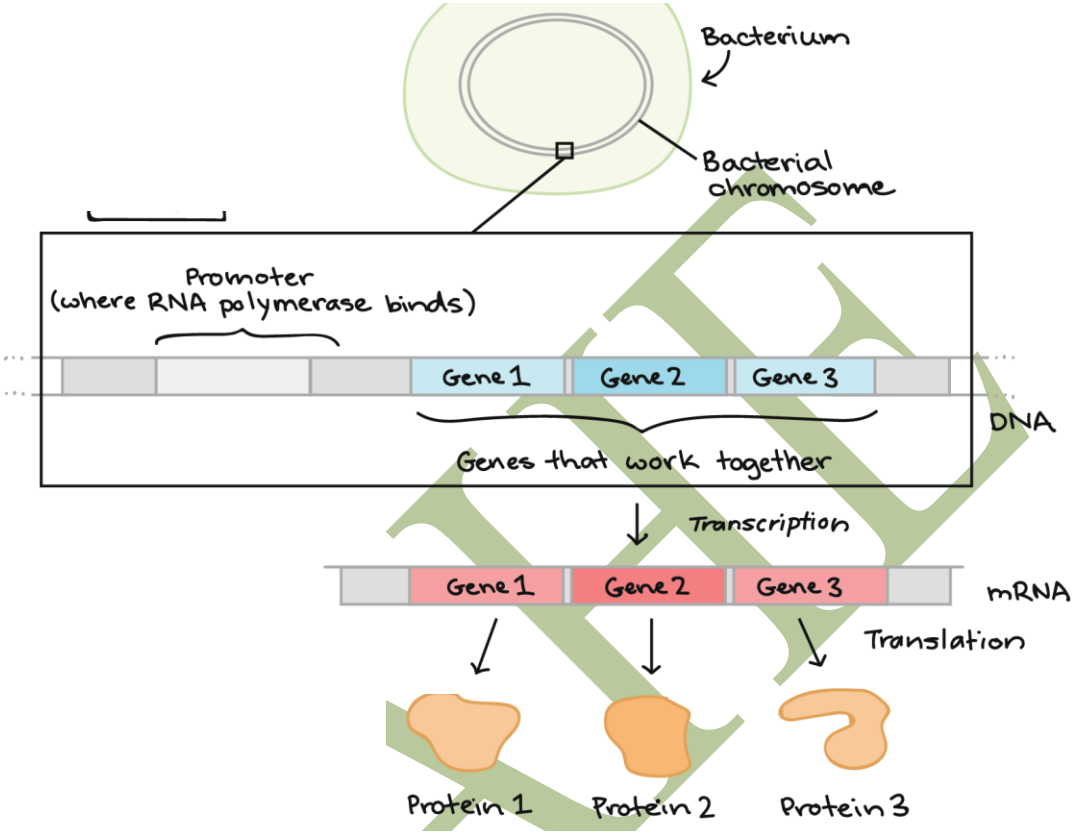
There are various forms of **gene regulation**, that is, mechanisms for controlling which genes get expressed and at what levels. However, a lot of gene regulation occurs at the level of transcription.

Bacteria have specific regulatory molecules that control whether a particular gene will be transcribed into mRNA. Often, these molecules act by binding to DNA near the gene and helping or blocking the transcription enzyme, RNA polymerase. Let's take a closer look at how genes are regulated in bacteria.

In bacteria, genes are often found in operons

In bacteria, related genes are often found in a cluster on the chromosome, where they are transcribed from one **promoter** (RNA polymerase binding site) as a single unit. Such a cluster of genes under control of a single promoter is known as an **operon**. Operons are common in bacteria, but they are rare in eukaryotes such as humans.

Diagram illustrating what an operon is. At the top of the diagram, we see a bacterial cell with a circular bacterial chromosome inside it. We zoom in on a small segment of the chromosome and see that it is an operon. The DNA of the operon contains three genes, Gene 1, Gene 2, and Gene 3, which are found in a row in the DNA. They are under control of a single promoter (site where RNA polymerase binds) and they are transcribed together to make a single mRNA that has contains



sequences coding for all three genes. When the mRNA is translated, the three different coding sequences of the mRNA are read separately, making three different proteins (Protein 1, Protein 2, and Protein 3).

Note: The operon does not consist of just the three genes. Instead, it also includes the promoter and other regulatory sequences that regulate expression of the genes.

In general, an operon will contain genes that function in the same process. For instance, a well-studied operon called the *lac* operon contains genes that encode proteins involved in uptake and metabolism of a particular sugar, lactose. Operons allow the cell to efficiently express sets of genes whose products are needed at the same time.

Anatomy of an operon

Operons aren't just made up of the coding sequences of genes. Instead, they also contain **regulatory DNA sequences** that control transcription of the operon. Typically, these sequences are binding sites for **regulatory proteins**, which control how much the operon is transcribed. The promoter, or site where RNA polymerase binds, is one example of a regulatory DNA sequence.

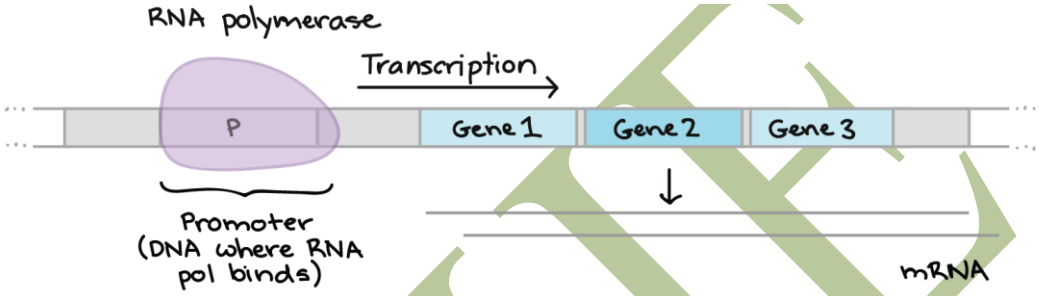


Diagram illustrating that the promoter is the site where RNA polymerase binds. The promoter is found in the DNA of the operon, upstream of (before) the genes. When the RNA polymerase binds to the promoter, it transcribes the operon and makes some mRNAs.

Most operons have other regulatory DNA sequences in addition to the promoter. These sequences are binding sites for regulatory proteins that turn expression of the operon "up" or "down."

- Some regulatory proteins are **repressors** that bind to pieces of DNA called **operators**. When bound to its operator, a repressor reduces transcription (e.g., by blocking RNA polymerase from moving forward on the DNA).

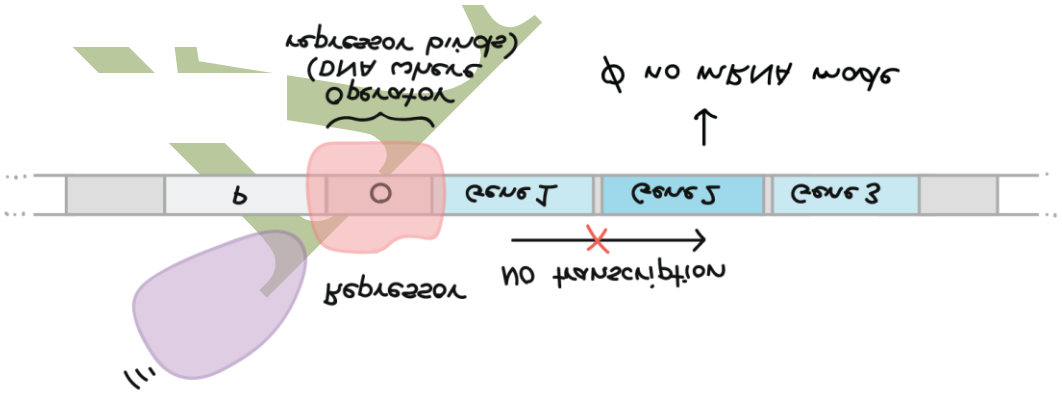


Diagram illustrating how a repressor works. A repressor protein binds to a site called on the operator. In this case (and many other cases), the operator is a region of DNA that overlaps with or

lies just downstream of the RNA polymerase binding site (promoter). That is, it is in between the promoter and the genes of the operon. When the repressor binds to the operator, it prevents RNA polymerase from binding to the promoter and/or transcribing the operon. When the repressor is bound to the operator, no transcription occurs and no mRNA is made.

- Some regulatory proteins are **activators**. When an activator is bound to its DNA binding site, it increases transcription of the operon (e.g., by helping RNA polymerase bind to the promoter).

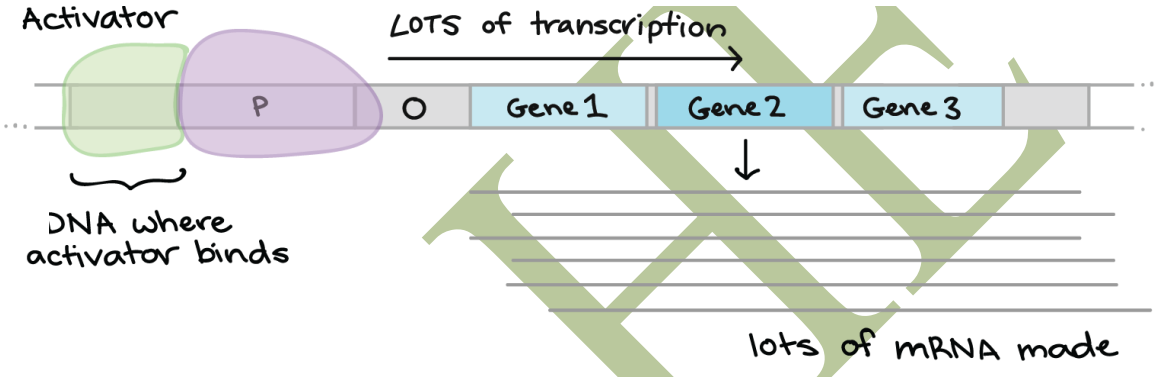


Diagram illustrating how an activator works. The activator protein binds to a specific sequence of DNA, in this case immediately upstream of (before) the promoter where RNA polymerase binds. When the activator binds, it helps the polymerase attach to the promoter (makes promoter binding more energetically favorable). This causes the RNA polymerase to bind firmly to the promoter and transcribe the genes of the operon much more frequently, leading to the production of many molecules of mRNA.

Where do the regulatory proteins come from? Like any other protein produced in an organism, they are encoded by genes in the bacterium's genome. The genes that encode regulatory proteins are sometimes called **regulatory genes**.

Many regulatory proteins can themselves be turned "on" or "off" by specific small molecules. The small molecule binds to the protein, changing its shape and altering its ability to bind DNA. For instance, an activator may only become active (able to bind DNA) when it's attached to a certain small molecule.

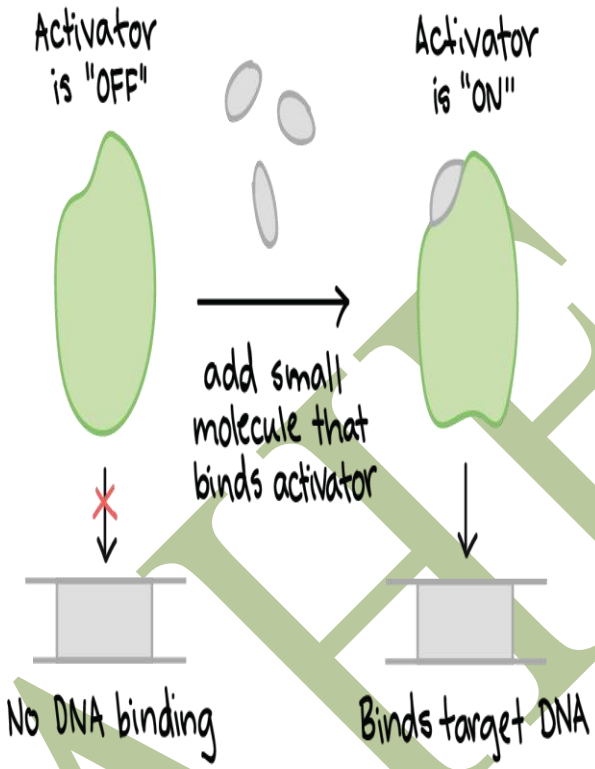


Diagram illustrating how a hypothetical activator's activity could be modulated by a small molecule. When the small molecule is absent, the activator is "off" - it takes on a shape that makes it unable to bind DNA. When the small molecule that activates the activator is added, it binds to the activator and changes its shape. This shape change makes the activator able to bind its target DNA sequence and activate transcription.

Operons may be inducible or repressible

Some operons are usually "off," but can be turned "on" by a small molecule. The molecule is called an **inducer**, and the operon is said to be **inducible**.

- For example, the *lac* operon is an inducible operon that encodes enzymes for metabolism of the sugar lactose. It turns on only when the sugar lactose is present (and other, preferred sugars are absent). The inducer in this case is allolactose, a modified form of lactose.

Other operons are usually "on," but can be turned "off" by a small molecule. The molecule is called a **corepressor**, and the operon is said to be **repressible**.

- For example, the *trp* operon is a repressible operon that encodes enzymes for synthesis of the amino acid tryptophan. This operon is expressed by default, but can be repressed when high levels of the amino acid tryptophan are present. The corepressor in this case is tryptophan.

These examples illustrate an important point: that gene regulation allows bacteria to respond to changes in their environment by altering gene expression (and thus, changing the set of proteins present in the cell).

Some genes and operons are expressed all the time

Many genes play specialized roles and are expressed only under certain conditions, as described above. However, there are also genes whose products are constantly needed by the cell to maintain essential functions. These **housekeeping genes** are constantly expressed under normal growth conditions ("constitutively active"). Housekeeping genes have promoters and other regulatory DNA sequences that ensure constant expression.

Gene regulation in eukaryotes

Introduction

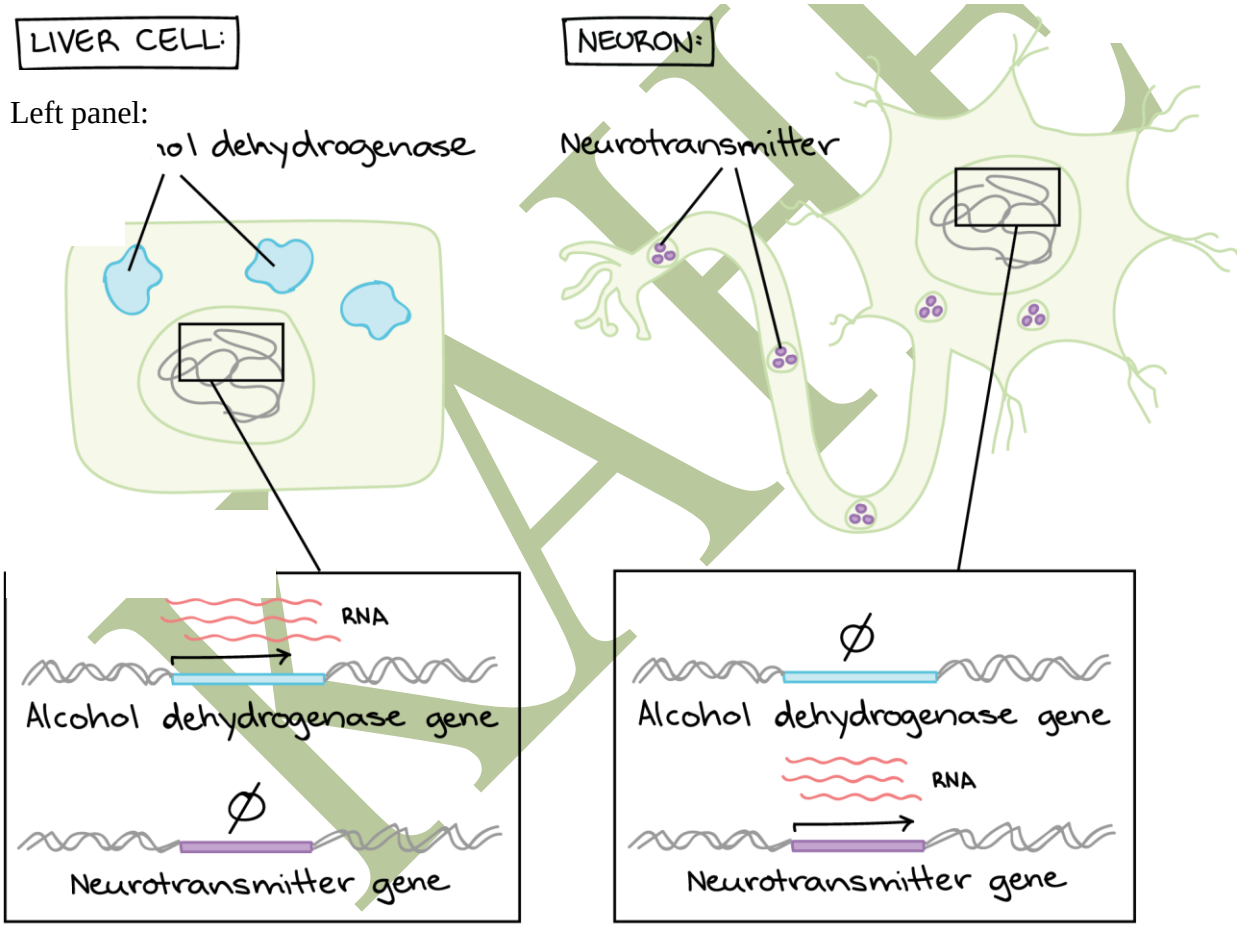
Your amazing body contains hundreds of different cell types, from immune cells to skin cells to neurons. Almost all of your cells contain the same set of DNA instructions – so why do they look so different, and do such different jobs? The answer: different gene regulation!

Gene regulation makes cells different

Gene regulation is how a cell controls which genes, out of the many genes in its genome, are "turned on" (expressed). Thanks to gene regulation, each cell type in your body has a different set of active genes – despite the fact that almost all the cells of your body contain the exact same DNA. These different patterns of gene expression cause your various cell types to have different sets of proteins, making each cell type uniquely specialized to do its job.

For example, one of the jobs of the liver is to remove toxic substances like alcohol from the bloodstream. To do this, liver cells express genes encoding subunits (pieces) of an enzyme called alcohol dehydrogenase. This enzyme breaks alcohol down into a non-toxic molecule. The neurons in a person's brain don't remove toxins from the body, so they keep these genes unexpressed, or "turned off." Similarly, the cells of the liver don't send signals using neurotransmitters, so they keep neurotransmitter genes turned off.

Left panel:



There are many other genes that are expressed differently between liver cells and neurons (or any two cell types in a multicellular organism like yourself).

How do cells "decide" which genes to turn on?

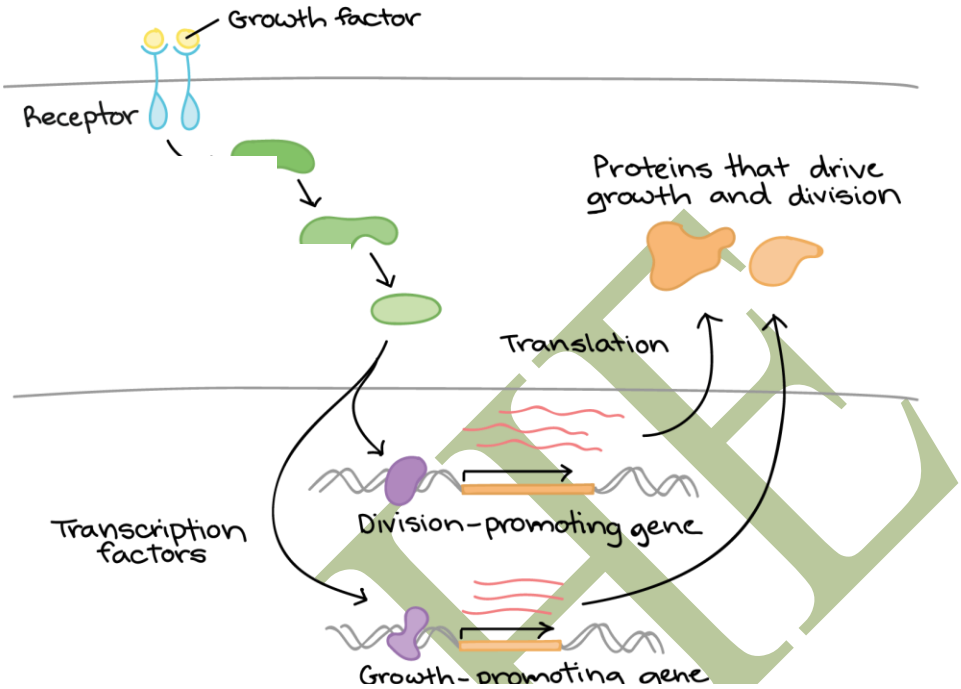
Now there's a tricky question! Many factors that can affect which genes a cell expresses. Different cell types express different sets of genes, as we saw above. However, two different cells of the same type may also have different gene expression patterns depending on their environment and internal state.

Broadly speaking, we can say that a cell's gene expression pattern is determined by information from both inside and outside the cell.

- Examples of information from inside the cell: the proteins it inherited from its mother cell, whether its DNA is damaged, and how much ATP it has.
- Examples of information from outside the cell: chemical signals from other cells, mechanical signals from the extracellular matrix, and nutrient levels.

How do these cues help a cell "decide" what genes to express? Cells don't make decisions in the sense that you or I would. Instead, they have molecular pathways that convert information – such as the binding of a chemical signal to its receptor – into a change in gene expression.

As an example, let's consider how cells respond to growth factors. A growth factor is a chemical signal from a neighboring cell that instructs a target cell to grow and divide. We could say that the cell "notices" the growth factor and "decides" to divide, but how do these processes actually occur?



Growth factors bind to their receptors on the cell surface and activate a signaling pathway in the cell. The signaling pathway activates transcription factors in the nucleus, which bind to DNA near division-promoting and growth-promoting genes and cause them to be transcribed into RNA. The RNA is processed and exported from the nucleus, then translated to make proteins that drive growth and division.

- The cell detects the growth factor through physical binding of the growth factor to a receptor protein on the cell surface.
- Binding of the growth factor causes the receptor to change shape, triggering a series of chemical events in the cell that activate proteins called transcription factors.
- The transcription factors bind to certain sequences of DNA in the nucleus and cause transcription of cell division-related genes.
- The products of these genes are various types of proteins that make the cell divide (drive cell growth and/or push the cell forward in the cell cycle).

This is just one example of how a cell can convert a source of information into a change in gene expression. There are many others, and understanding the logic of gene regulation is an area of ongoing research in biology today.

Growth factor signaling is complex and involves the activation of a variety of targets, including both transcription factors and non-transcription factor proteins. You can learn more about how growth factor signaling works in the article on intracellular signal transduction.

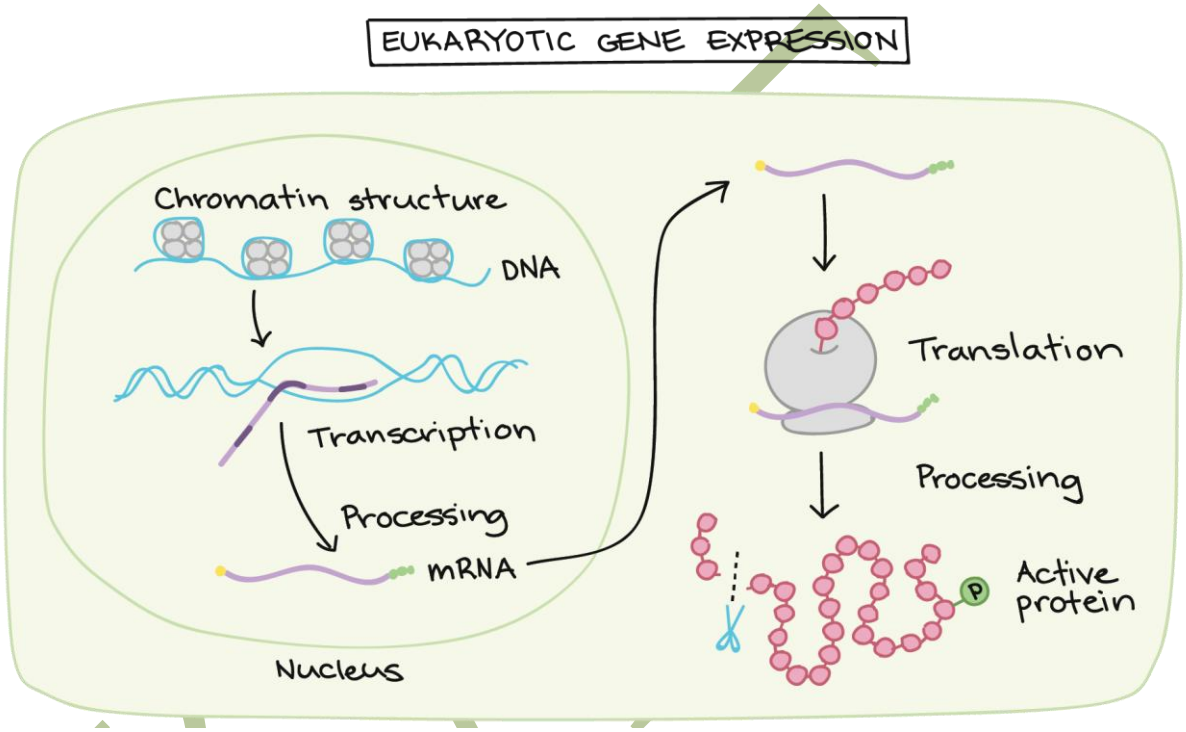
Eukaryotic gene expression can be regulated at many stages

In the articles that follow, we'll examine different forms of eukaryotic gene regulation. That is, we'll see how the expression of genes in eukaryotes (like us!) can be controlled at various stages, from the availability of DNA to the production of mRNAs to the translation and processing of proteins.

Eukaryotic gene expression involves many steps, and almost all of them can be regulated. Different genes are regulated at different points, and it's not uncommon for a gene (particularly an important or powerful one) to be regulated at multiple steps.

- Chromatin accessibility. The structure of chromatin (DNA and its organizing proteins) can be regulated. More open or "relaxed" chromatin makes a gene more available for transcription.
- Transcription. Transcription is a key regulatory point for many genes. Sets of transcription factor proteins bind to specific DNA sequences in or near a gene and promote or repress its transcription into an RNA.

- RNA processing. Splicing, capping, and addition of a poly-A tail to an RNA molecule can be regulated, and so can exit from the nucleus. Different mRNAs may be made from the same pre-mRNA by alternative splicing.



Stages of eukaryotic gene expression (any of which can be potentially regulated).

- Chromatin structure. Chromatin may be tightly compacted or loose and open.
- Transcription. An available gene (with sufficiently open chromatin) is transcribed to make a primary transcript.
- Processing and export. The primary transcript is processed (spliced, capped, given a poly-A tail) and shipped out of the nucleus.

- mRNA stability. In the cytosol, the mRNA may be stable for long periods of time or may be quickly degraded (broken down).
- Translation. The mRNA may be translated more or less readily/frequently by ribosomes to make a polypeptide.
- Protein processing. The polypeptide may undergo various types of processing, including proteolytic cleavage (snipping off of amino acids) and addition of chemical modifications, such as phosphate groups.
- All these steps (if applicable) need to be executed for a given gene for an active protein to be present in the cell.

RNA stability. The lifetime of an mRNA molecule in the cytosol affects how many proteins can be made from it. Small regulatory RNAs called miRNAscan bind to target mRNAs and cause them to be chopped up.

Translation. Translation of an mRNA may be increased or inhibited by regulators. For instance, miRNAs sometimes block translation of their target mRNAs (rather than causing them to be chopped up).

Protein activity. Proteins can undergo a variety of modifications, such as being chopped up or tagged with chemical groups. These modifications can be regulated and may affect the activity or behavior of the protein.

Although all stages of gene expression can be regulated, the main control point for many genes is transcription. Later stages of regulation often refine the gene expression patterns that are "roughed out" during transcription.

Review Questions

Short Answer Questions **(2 Marks)**

1. Define Nucleic acid.

2. Write key features of DNA replication.
3. Draw structure of RNA Polymerase enzyme.
4. Draw the structure of prokaryotic gene.
5. Explain briefly about translation.
6. List out the role of DNA polymerase..
7. Define transcription
8. Describe about mitotic cell division.
9. Describe in detail about functions of mitochondria.
10. Differentiate prokaryotic cells and eukaryotic cells.
11. Describe the structure of plasma membrane.
12. Explain about functions of chloroplast.

Essay Answer Questions **(6 & 8 Marks)**

1. Describe about DNA replecation.
2. Describe in detail about structure and functions of DNA polymerase.
3. Describe the structure of Eukaryotic gene.
4. Explain about post transcriptional modification.
5. Give a detailed note on protein synthesis.
6. Explain in detail about gene expression in prokaryotes.
7. Discuss in detail about eukaryotic gene expression.
8. Describe about mitotic cell division.
9. Describe in detail about functions of mitochondria.
10. Differentiate prokaryotic cells and eukaryotic cells.
11. Describe the structure of plasma membrane.
12. Explain about functions of chloroplast.
13. Give a detailed note on endoplasmic reticulum.
14. Explain in detail about golgi complex.

Unit II

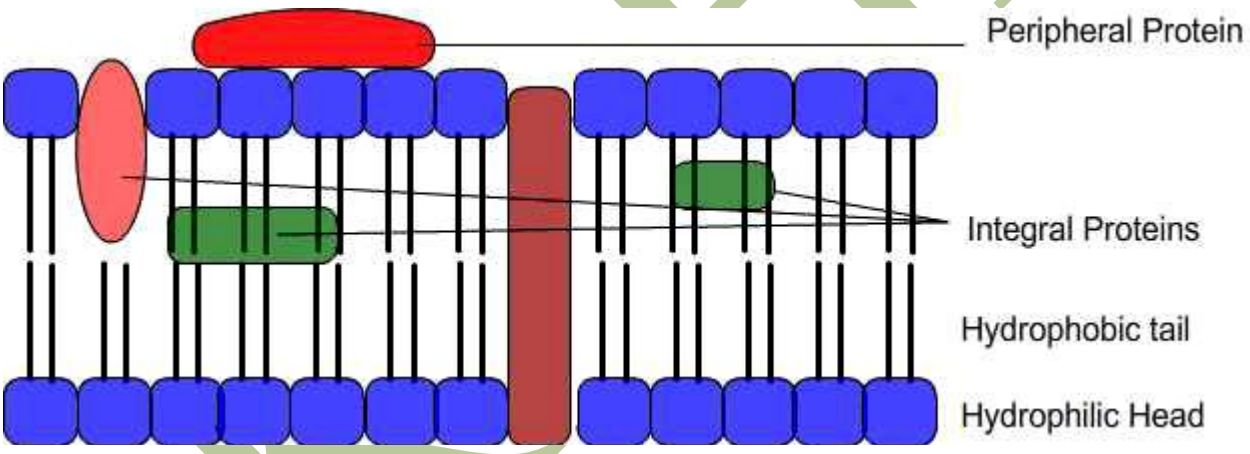
SYLLABUS

Structure of model membrane, lipid bilayer and membrane protein diffusion, osmosis, ion channels, active transport, and ion pumps. Intracellular protein sorting- Mechanism and regulation of intracellular transport in mitochondria, chloroplast, endoplasmic reticulum and nucleus. Electrical properties of membranes. Cell cycle and its regulation, Molecular events Check points, Cyclins and protein kinases.

Structure and model of biological membrane

Fluid mosaic model

- Fluid mosaic model proposed by S.J. Singer and G.L. Nicolson (1972) was finally acceptable to most biologists
- This model recognizes that lipids and proteins are in a mosaic arrangement.



- It also recognizes that there is translational movement of lipids and proteins within the lipid bilayer.
- Non covalent interactions ensure a fluid like state for the membranes.
- Integral proteins are intercalated into the continuous lipid bilayer.
- Polar/hydrophilic regions of proteins protrude from the surface while the nonpolar/hydrophobic regions are embedded inside.
- The concept of fluidity is attractive as it explains the considerable freedom of lateral movement for proteins and lipids observed within the bilayer.

Fluid mosaic model Permeability

- Permeability is an important property of the plasma membrane and other membranes in a living cell.
- This is important for maintaining the required intracellular conditions.
- Basically this determines as to what substances should enter or leave the cell and in turn this is essential to maintain life.
- The composition of important body fluids depends on the permeability.
- Osmotic pressure of intra and extra cellular fluids depends upon the permeability.

Electrical and ionic gradients

- Between the extracellular and intracellular compartments ionic and electrical gradients exist.
- Their interdependence is known since the distribution of ions on both sides of the membrane contributes to the electrical potential.
- Intracellular fluid contains more of K+ ions and organic anions
- Interstitial fluid contains more of Na+ and Cl- ions.
- The resting/steady potential is usually negative inside a cell and varies between -20 and -100 mV.
- The diffusion of ions depends both on the concentration and electrical gradients across the membrane.

Passive permeability :

- Membrane acts as a barrier to the passage of water soluble molecules.
- Lipid soluble substances more easily pass through the membrane.
- Size and solubility of molecules are important factors affecting their permeability.
- P=KD/t where P is permeability, K is partition coefficient, D is diffusion coefficient, and t is thickness of membrane.
- If two molecules have the same size, the one with higher solubility in lipids will penetrate the membrane faster.
- If two molecules have equal solubility in lipids, the smaller molecule will penetrate the membrane faster.

Types of passive transport:

1. Diffusion:

- The process of the net movement of solutes from a region of high concentration to a region of low concentration is known as diffusion.

- The differences of concentration between the two regions are termed as concentration gradient and the diffusion continues till the gradient has been vanished.
- Diffusion occurs down the concentration gradient.

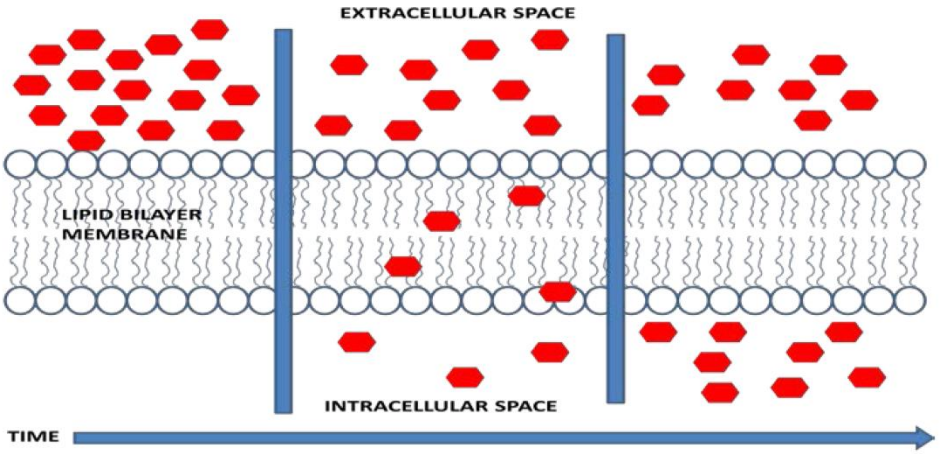


Figure 3: Diffusion.Extracellular space contains high concentration of solutes than intracellular space and hence the solutes move from extracellular space to intracellular space till there is no concentration gradient between the spaces.

2. Osmosis:

- Osmosis is the type of diffusion of water molecules across a semi- permeable membrane, from a solution of high water potential to a region of low water potential.
- A cell with a less negative water potential will draw in water but this depends on other factors as well such as solute potential (pressure in the cell e.g. solute molecules) and pressure potential (external pressure e.g. cell wall).

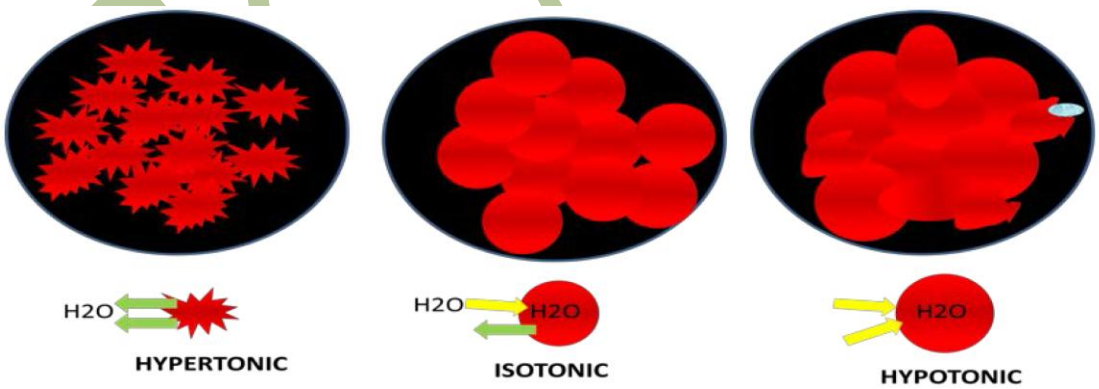


Fig:Osmosis.(A) In hypertonic solution, there are more solute molecules outside the cell, which causes the water to be sucked in that direction which leads to the shrinkage of cells. **(B)** In isotonic solution, there is equal concentration of solute on both sides, henceforth the water with move back in forth. **(C)** In hypotonic solution, there are less solute

molecules outside the cell, since salt sucks and water will move inside the cell. The cell will gain water and grow larger, and finally burst.

Active Transport :

- If only passive diffusion operates in cells any increase or decrease in membrane potential would result in asymmetric ionic distribution.
- Many experiments have shown that indeed there are active transport mechanisms in living cells that require energy.
- ATP provides energy for such active transport processes.
- Thus oxygen consumption is required when an ion is transported against the electrochemical gradient.
- Active transport is also needed to maintain the resting potential
- The cells are able to keep a constant osmotic pressure by regulating the ionic transport across the cell membrane.
- Potassium ions are present in higher concentrations inside the cells through a pumping mechanism that requires energy to work against the concentration gradient.

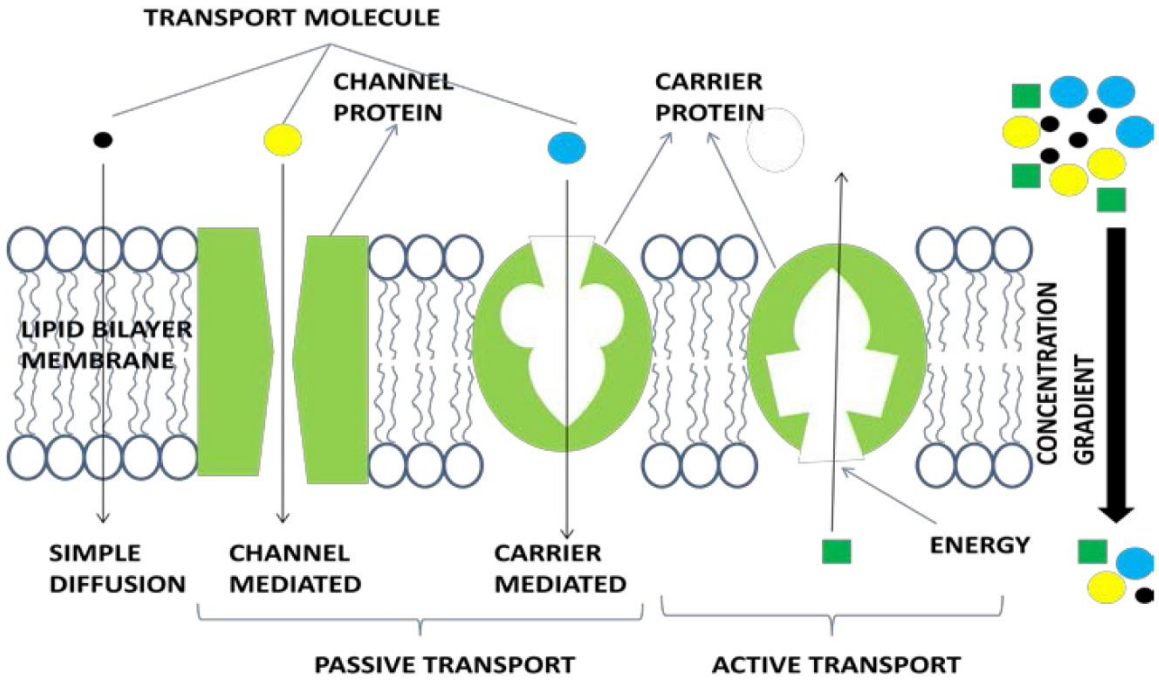


Figure 2: Mediated transport. (A) Passive transport and (B) Active transport

Sodium Pump

- Although Na+ does not have a higher mol. wt. in comparison to K+ and Cl- ions its ionic radius in the hydrated conditions is higher and thus it cannot enter the cell easily.
- Sodium pump throws out Na+ ions together water from inside to the outside of the cell by an active transport mechanism.
- Na+ K+ ATPase is an enzyme that couples the hydrolysis of ATP with the elimination of Na+ ions from the cytoplasm against electrochemical gradient.
- Hydrolysis of one ATP can give energy to transport two K+ ions towards inside and three Na+ ions towards outside.
- Thus both these ions can activate ATPase and ouabain can inhibit it by binding to it on the extracellular surface of the enzyme.
- Vanadate also inhibits this enzyme but this acts from the cytoplasmic side of the enzyme.
- The first step for this enzyme reaction is the formation of a covalent phosphoenzyme intermediate.
- This happens on the inner side of the membrane in the presence of Na+ ions but Ca++ ions inhibit this reaction
- In the second step the intermediate complex is hydrolyzed forming the free enzyme and phosphate ions and this requires K+ but is inhibited by ouabain.
- Other substances such as glucose and amino acids may use the sodium pump for their transport.

Sodium PumpTransport proteins

- Selective transport of molecules across the membranes is also achieved by means of carriers/permeases/transport proteins.
- High degree of specificity by this mechanism is related to the chemical structures of molecules being transported.
- For example the structures of glucose and galactose are very similar except for the position of OH group at carbon 4, but these two molecules cross the membranes by using different carriers.
- Permeases help in achieving this specificity related to the structure and in the process the permeases do not change and get recycled for another round.
- Some permeases work under a favorable concentration gradient in a mode of passive diffusion called facilitated diffusion.
- Some others work against the concentration gradient employing an active transport mechanism.

Mechanisms

- The **carrier mechanism** works by first binding of the molecule with the carrier protein at the outer surface of the cell.
- Then this complex translocates into the cytoplasm by a rotatory movement.
- However, this kind of a mechanism is not thermodynamically favorable as rotation and translocation across the bilayer may not be easy.
- **Fixed pore mechanism** suggests that the carriers are actually integral proteins and they undergo conformational change once a molecule to be transported gets attached with the carrier.

- In the above mechanism, the carrier proteins are suggested to be oligomers forming a channel or pore that has a hydrophilic lining in the middle.
- This mechanism can account for the sodium pump action and also for the transport of glucose and amino acids.

Protein Sorting and Transport

For a cell to function properly, each of its numerous proteins must be localized to the correct organelle like chloroplast, mitochondria, lysosome. Hormone receptor proteins must be delivered to the plasma membrane for the cell to recognize hormones, and specific ion-channel and transporter proteins are needed in the membrane, for the cell to import or export the corresponding ions and small molecules. Enzymes such as RNA and DNA polymerases must be targeted to the nucleus for gene expression and protein synthesis. Proteolytic enzymes or catalase, must go to lysosomes or peroxisomes, respectively for proper functioning. Hormones must be directed to the cell surface and secreted. The process of directing each newly made protein to its particular destination is critical to the organization and functioning of eukaryotic cells and this is referred to as protein targeting or protein sorting.

Protein Sorting

Except for a small number of proteins, coded in the genomes of mitochondria and chloroplasts, most of the proteins in a cell are encoded by nuclear DNA and are synthesized on ribosomes in the cytosol. For proper functioning, these proteins are to be distributed to their correct destinations in the cell. In

1999, Gunter Blobel was awarded Nobel Prize in Physiology or Medicine for the discovery that "proteins have intrinsic signals that govern their transport and localization in the cell." The sorting signals are present in the primary amino acid sequence levels mostly at its N terminal. For further sorting within the organelle, additional targeting information may be located in a secondary targeting sequence, either placed adjacent to the original targeting sequence or in other regions of the protein.

Proteins are translocated to their targeted location either cotranslationaly or posttranslationaly. In cotranslational translocation, the translocation starts while the protein is still being synthesized on the ribosome. Proteins targeted for ER, Golgi apparatus, plasma membrane, lysosome, vacuole and extracellular space uses the SRP-dependent pathway and are translocated cotranslationally. The N-terminal signal sequence of these proteins, is recognized by a signal recognition particle (SRP), while the proteins being translated in the free ribosome. The ribosome-protein complex is transferred to a SRP receptor on the ER and the synthesis pauses. There, the nascent protein is inserted into the the translocon that passes through the ER membrane. Transfer of the ribosome-mRNA complex from the SRP to the translocon opens the gate on the translocon and allows the translation to resume. The signal sequence is immediately cleaved from the polypeptide once it has been translocated into the ER by signal peptidase in secretory proteins. Within the ER, chaperone helps protein to fold correctly. From ER, proteins are transported in vescicles to the Golgi apparatus where they are further processed and sorted for transport to endosomes, lysosomes, plasma membrane or secretion from the cell. The proteins for ER will have various ER retention signals to keep them in the ER itself.

Most of the proteins targeted for mitochondria, chloroplast, nucleus and peroxisome are translocated posttranslationaly. In contrast to the cotranslationaly translocated proteins, these proteins are translated in the free ribosames in the cytosol. Once the translation is complete, they are released into the cytosol. These proteins which enter the non-secretory pathway are sorted to their destination site based on the presence of the targeting signal. Once the protein has reached its destination, the targeting signals are cleaved off. The targeting sequence for mitochondrial proteins, mitochondrial transfer peptide (mTP), will have 3 - 5 nonconsecutive Arg or Lys residues, often with Ser and Thr, at the N-terminal of the polypeptide chain. No Glu or Asp residues are generally found here. In the case of chloroplast, chloroplast transit peptide (cTP), no common sequence motifs are found but the N-terminal is generally rich in Ser, Thr, and small hydrophobic amino acid residues and the region is poor in Glu and Asp residues. For peroxisome proteins, the sorting signal is generally found at extreme C-terminal usually as Ser-Lys-Leu and these signals are not cleaved off after reaching the destination. Proteins destined for nucleus have a distributed sorting signal which is not cleaved off after sorting.

One cluster of 5 basic amino acids or two smaller clusters of basic residues, separated by around 10 amino acids are usually found as nuclear localization signal.

In the next section the major protein localization sites are discussed

Major Locations

Proteins are sorted to their locations with the help of an address signal present in the primary structure level. Each organelle has a mechanism to identify its own proteins. In this section, important protein localization sites like nucleus, mitochondrion, chloroplast, peroxisome, and secretory proteins are explained.

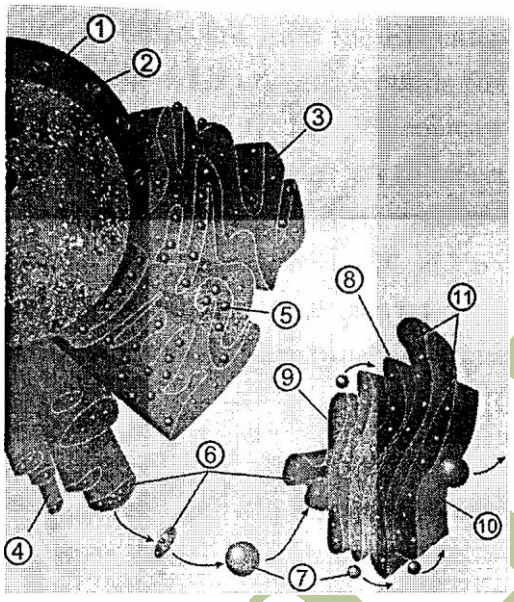
Endoplasmic reticulum

The Endoplasmic Reticulum is the first branching point in protein sorting. Figure shows nucleus, ER and Golgi Apparatus in eukaryote cell. Most of the proteins targeted for secretion, Golgi apparatus, plasma membrane, vac• uole, lysosome are translated on the ribosomes bounded to the Endoplasmic Reticulum and they enter into the ER cotranslationally. Only a few pro• teins enter the ER posttranslationally. The protein translation starts at the free ribosomes in the cytosol. The synthesis continues till the sorting signal which is present in the N-terminal emerges. This sorting signal is recognized by signal recognition particle. The SRP binds to the sorting signal and the translation pauses. The complex of SRP, ribosome, polypeptide chain and mRN A moves to the ER and the polypeptide chain enters the ER through translocon. The translocon is a protein complex containing various compo• nents used for protein translocation. The SRP receptor of the translocon binds with the SRP, the ribosome receptor binds with the ribosme and hold it in the correct position, the pore protein forms the channel through which the growing polypetide enter the ER lumen, the signal peptidase cut the sig• nal once it enters the ER. After the SRP and ribosomes are bound by SRP receptor and ribosme receptor respectively, GTP binds to the the complex of SRP and SRP receptor and the translation resumes. This causes the transfer of the signal sequence into the channel of pore protein. Then the GTP is hydrolysed and the SRP is

released. While the sorting signal remains bound at the the pore protein, the polypeptide grows into a loop and translocates into the ER lumen. When the polypetide synthesis is finished, the signal peptidase cleaves off the sorting signal, releasing the polypeptide into the ER lumen. After this, the ribosome detaches from the ER and dissociate into its subunits, and the mRNA is released. Inside the ER, the polypeptide chains are folded into their native forms usually with the help of molecular chaperones, which controls the quality of protein folding [23].

Integral membrane proteins of the plasma membrane or the membranes of the ER, Golgi apparatus, and lysosome are first inserted into the membrane of ER. These proteins do not enter the lumen cotranslationally but anchored to the ER membrane by membrane spanning *0:* helices that stop transfer of the growing polypeptide chain across the membrane.

Proteins travel along the secretory pathway in transport vesicle, which bud from the membrane of one organelle and then fuse with the membrane of another. The proteins are exported from the ER in vesicles that bud from the transitional ER and carry their cargo through the ER-Golgi intermediate compartment and then to Golgi apparatus. The proteins targeted for the ER has a retention signal in their C terminal that makes them come back to the ER even if they are exported from the ER. Two such retention signals are



1. Nucleus 2. Nuclear pore 3. Rough endoplasmic reticulum (RER) 4. Smooth endoplasmic reticulum 5. Ribosome on the rough ER 6. Proteins that are transported 7. Transport vesicle 8. Golgi apparatus 9. Cis face of the Golgi apparatus 10. Trans face of the Golgi apparatus 11. Cisternae of the Golgi apparatus. Source: Wikipedia

Figure : Nucleus, ER and Golgi Apparatus in eukaryote cell

KDEL (Lys-Asp-Glu-Leu) and KKXX (two lysine residues followed by any two amino acids) present in the C-terminal of the sequences. If the signal is removed from the ER proteins, they are transported to Golgi and then move out of the cell. The ER retention signals do not prevent the ER proteins from being packaged and exported from the ER. Instead these signals retrieve the ER proteins from Golgi apparatus or ER-Golgi intermediate compartments and put them back to ER using a recycling pathway. Specific recycling receptors bind to these retention signals and bring them back to ER. There are many retention signals other than EDEL and KKXX but they are not well characterized.

Nucleus

Nucleus is known as the control centre of the cell and is the largest organelle in animal cell. It is the storage place of the genetic material, DNA. A eukaryote nucleus and subnuclear locations are given in Figure.. Proteins are transported

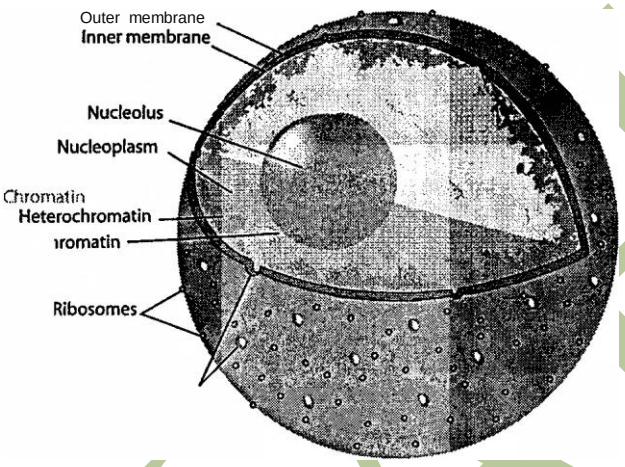


Figure:: The nucleus of eukaryotic cell

ported into the nucleus posttranslationally and in a folded state. Most of the nuclear proteins are imported to nucleus with the help of carrier proteins (eg importins). These carrier proteins form a complex with the proteins that are to be imported into the nucleus, and this complex is translocated through the nuclear pore. Inside the nucleus, the complex is dissociated and the importin is shuttled back to the cytoplasm and reused. The address signal for nucleus cleus is known as nuclear localization signal (NLS) and is a short stretch of amino acids. The deletion of the NLS from a nuclear protein disrupts nuclear

import and the addition of NLS to a non-nuclear protein facilitate nuclear import. These details have been widely used to experimentally unravel NLS motifs. The nuclear localization

signals can be present anywhere in the protein sequence. Since NLSs do not have any particular consensus sequence, it is difficult to differentiate an NLS from a non-NLS region. Usually NLS is rich with positively charged residues, since some of these positive residues bind to carrier proteins like importins. Mutating these positively charged amino acids will disrupt nuclear import. However, there are Glycine-rich NLS motifs with few positive charges like monopartite and

bipartite motifs . Monopartite consists of four basic and one helix• breaking residues, and the bipartite consists of two clusters of basic residues with a spacer of 9-12 amino acids in between . But these patterns also are not at all unique to nuclear proteins and may well be observed in many other proteins . Other observed NLS includes, the 38 amino acid long M9 sequence and the repeated G-R motif . However, these signals are in general significantly less frequent than the monopartite and bipartite NLS. There are also signals for nuclear protein export and retention.

Mitochondrion

Mitochondria is known as the power house of the cell as they generate most of the cell's supply of adenosine triphosphate (ATP) in the process of cellular respiration by breaking down carbohydrates and fatty acids. A typical mitochondrion is shown in Figure. Mitochondria consist of a smooth outer membrane and an inner membrane separated by an intermembrane space. The inner membrane forms numerous folds known as cristae. The space inside the inner membrane is called the mitochondrial matrix and contains the genetic material of mitochondria. The matrix and inner membrane represents the major working compartments of the mitochondria. As sugar is burned for fuel, a mitochondrion shunts various chemicals back and forth across the in• ner membrane. Even though mitochondrion has a genome of its own, it does not code for the proteins necessary for DNA replication, transcription and translation. All these proteins, the proteins required for oxidative phospho• rylation and the proteins to act as enzymes has to be generated from nuclear DNA and imported into the mitochondria. The double membrane structure of the mitochondrion makes the protein import a difficult task. The proteins for the matrix of mitochondria have to cross

two membranes. The proteins for other location have to be resorted with a secondary targeting signal, once they reach mitochondria. The sorting signal of mitochondrion is known as mitochondrial transfer peptide (mTP) and is on average 35 amino acids long. The mTP binds to the receptors on the surface of mitochondria. These re• ceptors are part of TOM (Translocase of the Outer Membrane) complex that

directs translocation across the outer membrane. The individual receptors

on the TOM complex are TOM20, TOM22 and TOM5. From these

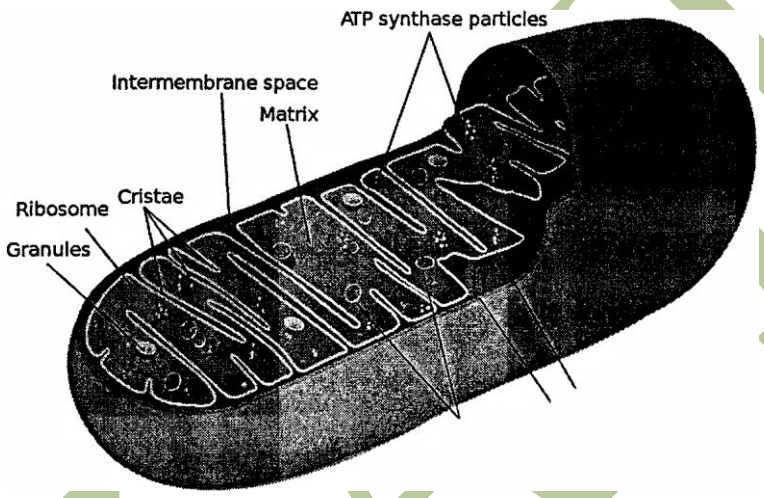
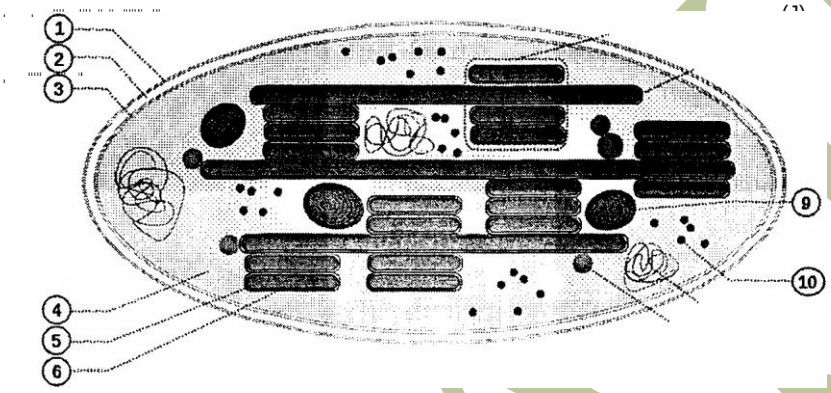


Figure : Typical mitochondrion

receptors, proteins are transferred to the TOM40 pore protein and translocated across the outer membrane. The protein is transported, via the GIP com• plex (general import pore), in an ATP-requiring process through the outer mitochondrial membrane. The proteins are then transferred to a secondprotein complex in the inner membrane, the TIM (Translocase of the Inner Membrane) complex for translocation into the matrix. The translocation is through a process that requires an electrochemical hydrogen ion gradient across the inner membrane. After entering mitochondrial matrix, the mTP is cleaved off by the mitochondrial processing peptidase, MPP (Matrix Processing Peptidase) by proteolytic cleavage. Some mitochondrial matrix proteins

are then cleaved again by the mitochondrial intermediate peptidase (MIP) which removes an additional eight or nine residues from the N-terminus. For some proteins, a second adjacent targeting signal that resembles the signal peptide for secretion is exposed after MPP cleav• age. These proteins are re-exported from the matrix to the intermembrane space (IMS), or inserted into the inner membrane, in a process very similar to bacterial protein secretion. Alternatively, the translocation over either of the membranes is halted by a stop-transfer signal, which is specifically recognized.



1. outer membrane 2. intermembrane space 3. inner membrane (1+2+3: envelope) 4. stroma 5. thylakoid lumen (inside of thylakoid) 6. thylakoid membrane 7. granum (stack of thylakoids) 8. thylakoid (lamella)

9. starch 10. ribosome 11. plastidial DNA 12. plastoglobule (drop of lipids). Source: Wikipedia

Figure : Typical chloroplast

by a TOM or TIM component [26, 49, 50], and the protein is subsequently inserted into the outer or inner membrane, respectively.

The inner membrane metabolite carrier proteins of mitochondria con•tain internal localization signals]. In mitochondrial targeting peptides (mTPs), Arg, Ala and Ser are over-represented while negatively charged amino acid residues (Asp and Glu) are rare . Other than this, there is no obvious features that distinguish the mTP from other N-

terminal se• quences. The degree of sequence conservation around the cleavage site is also poor. Many mTPs have an arginine in position -2 or -3 relative to the MPP cleavage site. It is reported that, the mTP forms an am• phipathic alpha-helix when bound to the receptor protein but adopts an extended structure, when processed by the MPP.

Chloroplast

The chloroplast is double membrane bound organelle present in photosynthetic plants and algae. Figure 2.5 shows a typical chloroplast. In addition to the inner and outer membranes of the envelope, chloroplasts have a third internal membrane system, called the thylakoid membrane. The thylakoid membrane forms a network of flattened discs called thylakoids, which are frequently arranged in stacks called grana. Because of this three – membrane structure, the internal organization of chloroplasts is more complex than that of mitochondria. In particular, the three membranes divide chloroplasts into three distinct internal compartments: the intermembrane space between the two membranes of the chloroplast envelope; the stroma, which lies inside the envelope but outside the thylakoid membrane; and the thylakoid lumen. Stroma is the site of the dark reactions, more properly called the Calvin cycle. Stacks of thylakoids are called granum. Even though it has a small genome of its own in stroma, the majority of chloroplast proteins are encoded in the nuclear genome and post-translationally imported into the organelle.

Protein import into chloroplasts generally resembles mitochondrial protein import. Proteins are targeted for import into chloroplasts by N-terminal sequences of 30 to 100 amino acids, called chloroplast transit peptides(cTP), which direct protein translocation across the two membranes of the chloro• plast envelope and are then removed by proteolytic cleavage. The transit peptides are recognized by the translocation complex of the chloroplast outer membrane (the Toe complex), and proteins are transported through this com• plex across the membrane. They are then transferred to the translocation complex of the inner membrane (the Tic complex) and transported across the inner membrane to the stroma. As in mitochondria, the translocation requires energy in the form of ATP. In contrast to the mTP, transit peptides are not positively charged and the translocation of polypeptide chains into chloroplasts does not require an electric potential across the membrane..

Inside the chloroplast, the cTP is cleaved off by the stromal processing peptidase (SPP). cTPs are rich in hydroxylated residues, especially serines, and have a low content of acidic residues [51]. The cTPs from different proteins varies from 20 to 120 residues in length. At the N-terminus of cTP, there is a conserved alanine next to the initial methionine. A semiconserved motif, V-R-A-(:)-A-A-V, around the SPP cleavage site (denoted by :) has also been recognized. The signal is not very strong and there are several proteins that are located to both mitochondria and chloroplasts using identical sorting signals.

Electrical potential of membranes

Unlike carrier proteins, channel proteins form hydrophilic pores across membranes. One class of channel proteins found in virtually all animals forms gap junctions between two adjacent cells; each plasma membrane contributes equally to the formation of the channel, which connects the cytoplasm of the two cells. Both gap junctions and porins, the channel-forming proteins of the outer membranes of bacteria, mitochondria, and chloroplasts have relatively large and permissive pores, which would be disastrous if they directly connected the inside of a cell to an extracellular space. Indeed, many bacterial toxins do exactly that to kill other.

In contrast, most channel proteins in the plasma membrane of animal and plant cells that connect the cytosol to the cell exterior necessarily have narrow, highly selective pores that can open and close. Because these proteins are concerned specifically with inorganic ion transport, they are referred to as ion channels. For transport efficiency, channels have an advantage over carriers in that up to 100 million ions can pass through one open channel each second—a rate 105 times greater than the fastest rate of transport mediated by any known carrier protein. However, channels cannot be coupled to an energy source to perform active transport, so the transport that they mediate is always passive ("downhill"). Thus, the function of ion channels is to allow specific inorganic ions—primarily Na^+, K^+, Ca^{2+}, or Cl^-—to diffuse rapidly down their electrochemical gradients across the lipid bilayer. As we shall see, the ability to control ion fluxes through these channels is essential for many cell functions. Nerve cells (neurons), in particular, have made a specialty of using ion channels, and we shall consider how they use a diversity of such channels for receiving, conducting, and transmitting signals.

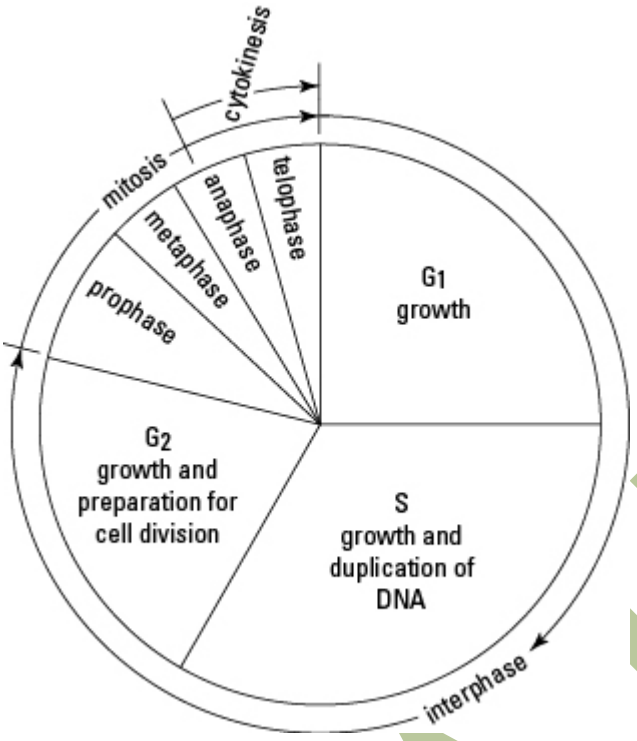
Cell Division

Cell division consists of two phases— *nuclear division* followed by *cytokinesis*.Nuclear division divides the genetic material in the nucleus, while cytokinesis divides the cytoplasm. There are two kinds of nuclear division—mitosis and meiosis. Mitosis divides the nucleus so that both daughter

cells are genetically identical. In contrast, meiosis is a reduction division, producing daughter cells that contain half the genetic information of the parent cell.

- The first step in either mitosis or meiosis begins with the condensation of the genetic material, chromatin, into tightly coiled bodies, the **chromosomes.** Each chromosome is made of two identical halves called sister **chromatids,** which are joined at the **centromere.** Each chromatid consists of a single, tightly coiled molecule of DNA. Somatic cells (all body cells except eggs and sperm) are diploid cells because each cell contains two copies of every chromosome.
- A pair of such chromosomes is called a homologous pair. In a **homologous pair of chromosomes,** one homologue originates from the maternal parent, the other from the paternal parent. In humans there are 46 chromosomes (23 homologous pairs). In males there are only 22 homologous pairs (autosomes) and one nonhomologous pair—the sex chromosomes of X and Y.
- When a cell is not dividing, the chromatin is enclosed within a clearly defined nuclear envelope, one or more nucleoli are visible within the nucleus, and two centrosomes (each containing two centrioles) lie adjacent to one another outside the nuclear envelope. These features are characteristic of *interphase,* the nondividing but metabolically active period of the cell cycle (Figure).
- When cell division begins, these features change, as described in the following sections.

Figure 1. Stages of the cell cycle .



Mitosis

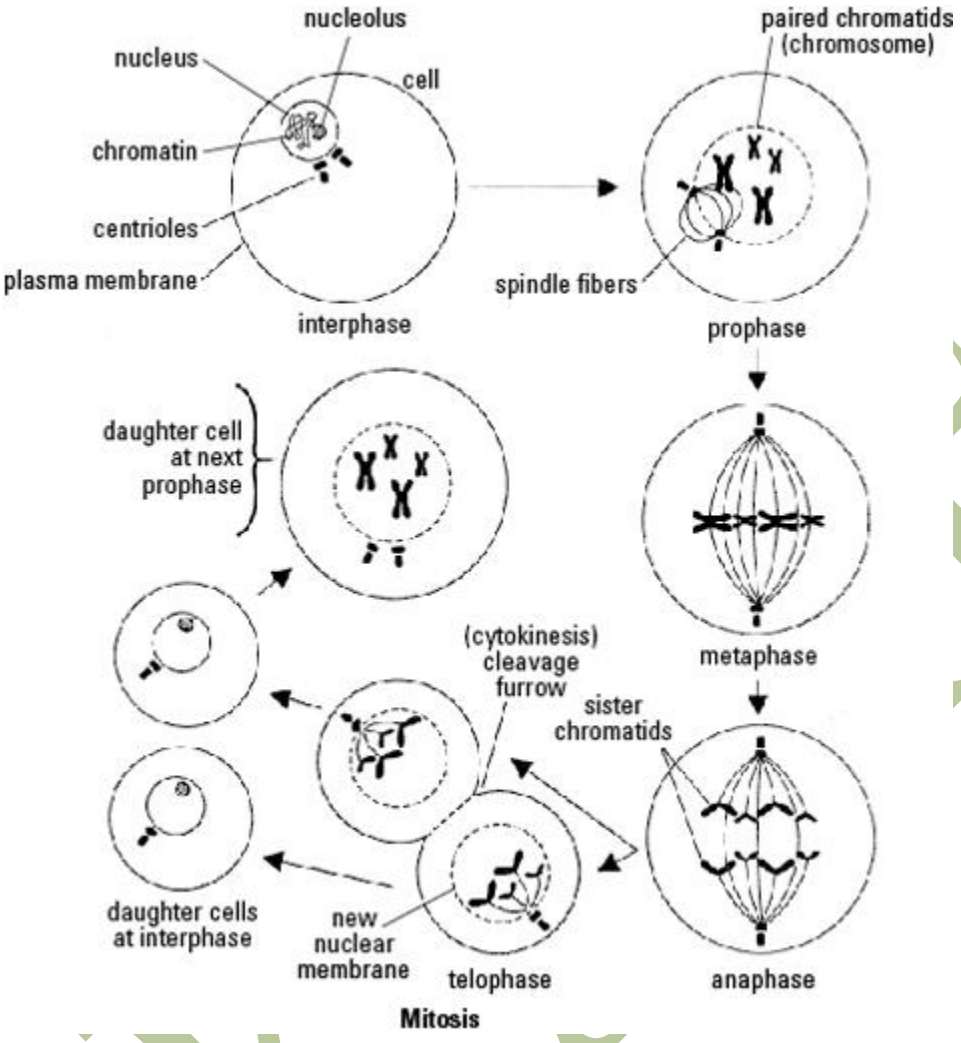
There are four phases in **mitosis** (adjective, mitotic): prophase, metaphase, anaphase, and telophase (Figure 2):

- During *prophase*, the nucleoli disappear, the chromatin condenses into chromosomes, the nuclear envelope breaks down, and the mitotic spindle is assembled. The development of the mitotic spindle begins as the centrosomes move apart to opposite ends (poles) of the nucleus. As they move apart, microtubules develop from each centrosome, increasing in length by the addition of tubulin units. Microtubules from each centrosome connect to specialized regions in the centromere called **kinetochores.** Microtubules tug on the kinetochores, moving the chromosomes back and forth toward one pole, then the other. Within the spindle, there are also microtubules that overlap at the center of the spindle and do not attach to the chromosomes.
- *Metaphase* begins when the chromosomes are distributed across the metaphase plate, a plane lying between the two poles of the spindle. Metaphase ends when the microtubules, still attached to the kinetochores, pull each chromosome apart into two chromatids. Each chromatid is complete with a centromere and kinetochores. Once separated from its sister

chromatid, each chromatid is called a chromosome. (To count the number of chromosomes at any one time, count the number of centromeres.)

- *Anaphase* begins after the chromosomes are separated into individual chromatids. During anaphase, the microtubules connected to the chromatids (now chromosomes) shorten, effectively pulling the chromosomes to opposite poles. Overlapping microtubules, originating from opposite centrosomes but not attached to chromosomes, interact to push the poles farther apart. At the end of anaphase, each pole has a complete set of chromosomes, the same number of chromosomes as the original cell. (Since it consists of only one chromatid, each chromosome contains only a single copy of the DNA molecule.)
- *Telophase* concludes the nuclear division. During this phase, a nuclear envelope develops around each pole, forming two nuclei. The chromosomes within each of these nuclei disperse into chromatin, and the nuclei reappear. Simultaneously, cytokinesis occurs, dividing the cytoplasm into two cells. Microfilaments form a ring inside the plasma membrane between the two newly forming nuclei. As the microfilaments shorten, they act like purse strings to pull the plasma membrane into the center, dividing the cell into two daughter cells. The groove that forms as the purse strings are tightened is called a *cleavage furrow*.

Figure 2. Cell reproduction and the four stages of mitosis .



Mitosis

Once mitosis is completed and interphase begins, the cell begins a period of growth. Growth begins during the first phase, called G_1 (gap), and continues through the S (synthesis) and G_2 phases. Also during the S phase the second DNA molecule for each chromosome is synthesized. As a result of this DNA replication, each chromosome gains a second chromatid. During the G_2 period of growth, materials for the next mitotic division are prepared. The time span from one cell division through G_1, S, and G_2 is called a *cell cycle* (Figure 1).

A cell that begins mitosis in the diploid state—that is, with two copies of every chromosome—will end mitosis with two copies of every chromosome. However, each of these chromosomes will consist of only one chromatid, or one DNA molecule. During interphase, the second DNA molecule is replicated from the first, so that when the next mitotic division begins, each chromosome will again consist of two chromatids.

Meiosis

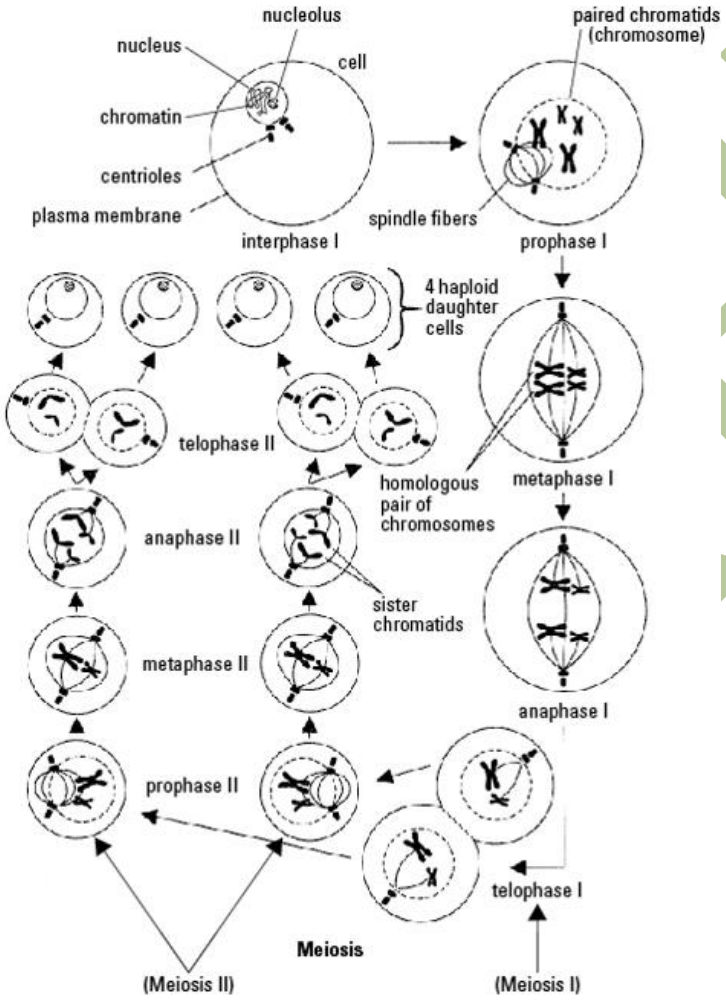
- Meiosis (adjective, meiotic) is very similar to mitosis. The major distinction is that meiosis consists of two groups of divisions, meiosis I and meiosis II (Figure 3). In meiosis I, homologous chromosomes pair at the metaphase plate and then migrate to opposite poles. In meiosis II, chromosomes spread across the metaphase plate, and sister chromatids separate and migrate to opposite poles. Thus, meiosis II is analogous to mitosis. A summary of each meiotic stage follows:

- Prophase I begins like prophase of mitosis. The nucleolus disappears, chromatin condenses into chromosomes, the nuclear envelope breaks down, and the spindle apparatus develops. Once the chromosomes are condensed, however, their behavior differs from mitosis. During prophase I, homologous chromosomes pair, a process called *synapsis.* These pairs of homologous chromosomes are called *tetrads* (a group of four chromatids) or bivalents. During synapsis, corresponding regions form close associations called **chiasmata** (singular, chiasma) along nonsister chromatids. Chiasmata are sites where genetic material is exchanged between nonsister homologous chromatids, a process called *crossing over*. The result contributes to a mixing of genetic material from both parents, a process called genetic recombination.
- At metaphase I, homologous pairs of chromosomes are spread across the metaphase plate. Microtubules extending from one pole are attached to kinetochores of one member of each homologous pair. Microtubules from the other pole are connected to the second member of each homologous pair.
- Anaphase I begins when homologues within tetrads uncouple as they are pulled to opposite poles.
- In telophase I, the chromosomes have reached their respective poles, and a nuclear membrane develops around them. Note that each pole will form a new nucleus that will have half the number of chromosomes, but each chromosome will contain two chromatids. Since daughter nuclei will have half the number of chromosomes, cells that they eventually form will be haploid.
- Cytokinesis occurs, forming two daughter cells. A brief interphase may follow, but no replication of chromosomes occurs. Instead, part II of meiosis begins in both daughter nuclei.
- In prophase II, the nuclear envelope disappears and the spindle develops. There are no chiasmata and no crossing over of genetic material as in prophase I.
- In metaphase II, the chromosomes align singly on the metaphase plate (not in tetrads as in metaphase I). Single alignment of chromosomes is exactly what happens in mitosis—except now there is only half the number of chromosomes.
- Anaphase II begins as each chromosome is pulled apart into two chromatids by the microtubules of the spindle apparatus. The chromatids (now chromosomes) migrate to their

respective poles. Again, this is exactly what happens in mitosis—except now there is only half the number of chromosomes.

- In telophase II, the nuclear envelope reappears at each pole and cytokinesis occurs. The end result of meiosis is four haploid cells. Each cell contains half the number of chromosomes and each chromosome consists of only one chromatid.

Figure 3. The stages of meiosis .



Meiosis ends with four haploid daughter cells, each with half the number of chromosomes (one chromosome from each homologous pair). These are **gametes**—that is, eggs and sperm. The fusing of an egg and sperm, fertilization (*syngamy*), gives rise to a diploid cell, the **zygote.** The single-celled zygote then divides by mitosis to produce a multicellular embryo fetus, and after nine

months, a newborn infant. Note that one copy of each chromosome pair in the zygote originates from one parent, and the second copy from the other parent. Thus, a pair of homologous chromosomes in the diploid zygote represents both maternal and paternal heritage.

Cyclin-Dependent Protein Kinase (Cdks)

A Cdks is an enzyme that adds negatively charged phosphate groups to other molecules in a process called phosphorylation. Through phosphorylation, Cdks signal the cell that it is ready to pass into the next stage of the cell cycle. As their name suggests, Cyclin-Dependent Protein Kinases are dependent on cyclins, another class of regulatory proteins. Cyclins bind to Cdks, activating the Cdks to phosphorylate other molecules.

Cyclins

Cyclins are named such because they undergo a constant cycle of synthesis and degradation during cell division. When cyclins are synthesized, they act as an activating protein and bind to Cdks forming a cyclin-Cdk complex. This complex then acts as a signal to the cell to pass to the next cell cycle phase. Eventually, the cyclin degrades, deactivating the Cdk, thus signaling exit from a particular phase. There are two classes of cyclins: mitotic cyclins and G1 cyclins.

peptide (LTP). There are two different pathways from the chloroplast stroma into the thylakoid lumen, the Sec-dependent pathway and the delta-pH or twin arginine translocation (TAT) pathway. The signals for the two pathways are very similar, the only significant difference being that the TAT pathway proteins contain a twin-arginine (RR) motif in the LTP (KR and RK may also be accepted). The -3, -1 motif found at the SP cleavage site in secreted proteins is present also in LTPs, and more strongly conserved.

Many proteins are needed in both mitochondria and chloroplasts. In

general the targeting peptide is of intermediate character to the two specific ones. The targeting peptides of these proteins have a high content of basic and hydrophobic amino acids, a low content of negatively charged amino acids. They have a lower content of alanine and a higher content of leucine and phenylalanine. The dual targeted proteins have a more hydrophobic targeting peptide than both mitochondrial and chloroplastic ones.

Review Questions

Short Answer Questions **(2 Marks)**

1. Define diffusion
2. List out the check points in cell cycle.
3. Define cell cycle.
4. Define active transport.
5. Explain briefly about cyclin.
6. List out the role of cell membranes.

Essay Answer Questions **(6 & 8 Marks)**

1. Describe about lipid bilayer structure of cell membrane..
2. Describe in detail active transport in cells.
3. Give a detailed account on cell cycle and its regulation.
4. Describe in detail about electrical properties of cell membrane.
5. Explain about role of cyclins and protein kinases.
6. Give a detailed note on ion pumps in cell membrane.

Unit III

SYLLABUS

Genetics: Mendelian and Non-Mendelian principles. Genetic recombination, Genetic mapping, linkage and crossing over. Mutations- Types of Mutation, Genetic analysis of Mutations, DNA repair Mechanisms.

MENDELIAN GENETICS

In the 1860's, an Austrian monk named Gregor Mendel introduced a new theory of inheritance based on his experimental work with pea plants. Prior to Mendel, most people believed inheritance was due to a blending of parental 'essences', much like how mixing blue and yellow paint will produce a green color. Mendel instead believed that heredity is the result of discrete units of inheritance, and every single unit (or gene) was independent in its actions in an individual's genome. According to this Mendelian concept, inheritance of a trait depends on the passing-on of these units. For any given trait, an individual inherits one gene from each parent so that the individual has a pairing of two genes. We now understand the alternate forms of these units as 'alleles'. If the two alleles that form the pair for a trait are identical, then the individual is said to be homozygous and if the two genes are different, then the individual is heterozygous for the trait.
Based on his pea plant studies, Mendel proposed that traits are always controlled by single genes. However, modern studies have revealed that most traits in humans are controlled by multiple genes as well as environmental influences and do not necessarily exhibit a simple Mendelian pattern of inheritance(see "Mendel's Experimental Results").

Mendel's Experimental Results
Mendel carried out breeding experiments in his monastery's garden to test inheritance patterns. He selectively cross-bred common pea plants (*Pisum sativum*) with selected traits over several generations.
After crossing two plants which differed in a single trait (tall stems vs. short stems, round peas vs. wrinkled peas, purple flowers vs. white flowers, etc), Mendel discovered that the next generation, the "F1" (first filial generation), was comprised entirely of individuals exhibiting only one of the traits. However, when this generation was interbred, its offspring, the "F2" (second filial generation), showed a 3:1 ratio- three individuals had the same trait as one parent and one individual had the other parent's trait.

Mendel then theorized that genes can be made up of three possible pairings of heredity units, which he called 'factors': AA, Aa, and aa. The big 'A' represents the dominant factor and the little 'a' represents the recessive factor. In Mendel's crosses, the starting plants were homozygous AA or aa, the F1 generation were Aa, and the F2 generation were AA, Aa, or aa. The interaction between these two determines the physical trait that is visible to us.

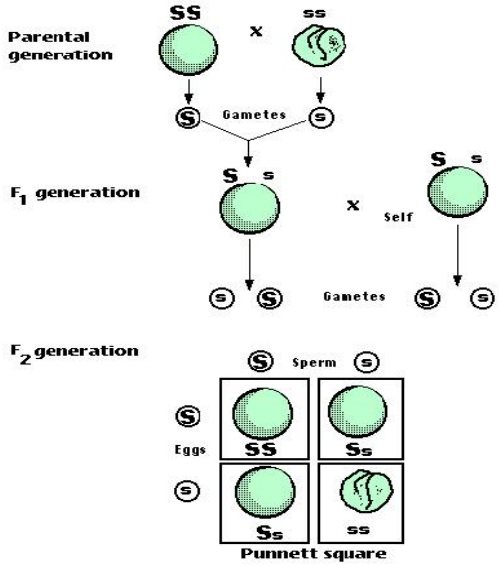
Mendel's Law of Dominance predicts this interaction; it states that when mating occurs between two organisms of different traits, each offspring exhibits the trait of one parent only. If the dominant factor is present in an individual, the dominant trait will result. The recessive trait will only result if both factors are recessive.

Mendel's Laws of Inheritance
Mendel's observations and conclusions are summarized in the following two principles, or laws.

Law of Segregation

The Law of Segregation states that for any trait, each parent's pairing of genes (alleles) split and one gene passes from each parent to an offspring. Which particular gene in a pair gets passed on is completely up to chance.
Law of Independent Assortment

The Law of Independent Assortment states that different pairs of alleles are passed onto the offspring independently of each other. Therefore, inheritance of genes at one location in a genome does not influence the inheritance of genes at another location.



NON-MENDELIAN GENETICS

Non-Mendelian inheritance is a general term that refers to any pattern of inheritance in which traits do not segregate in accordance with Mendel's laws. These laws describe the inheritance of traits linked to single genes on chromosomes in the nucleus. In Mendelian inheritance, each parent contributes one of two possible alleles for a trait. If the genotypes of both parents in a genetic cross are known, Mendel's laws can be used to determine the distribution of phenotypes expected for the population of offspring. There are several situations in which the proportions of phenotypes observed in the progeny do not match the predicted values.

Although inheritance of traits in fungi, viruses, and bacteria are all non-Mendelian, the phrase "non-Mendelian inheritance" is usually only used to describe the exceptions which occur in eukaryotic reproduction

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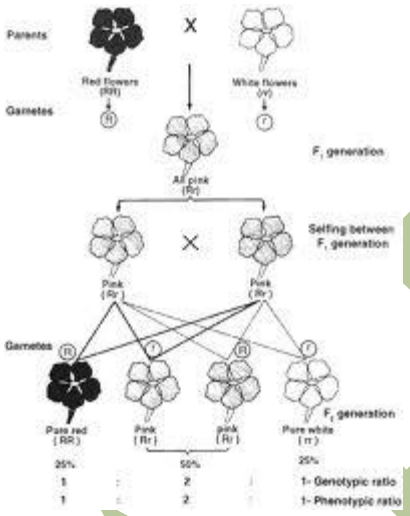
Incomplete Dominance:

Definition: Incomplete dominance is a form of intermediate inheritance in which one allele for a specific trait is not completely dominant over the other allele. This results in a combined phenotype.

Incomplete dominance:

Incomplete dominance occurs when the phenotype of the heterozygous genotype is an intermediate of the phenotypes of the homozygous genotypes. For example, the snapdragon flower color is either homozygous for red or white. When the red homozygous flower is paired with the white homozygous flower, the result yields a pink snapdragon flower. The pink snapdragon is the result of incomplete dominance. A similar type of incomplete dominance is found in the four o'clock plant

where in pink color is produced when true bred parents of white and red flowers are crossed. When plants of F_1 generation is self pollinated the phenotypic and genotypic ratio of the F_2 generation will be same and is 1:2:1



Over-dominance:

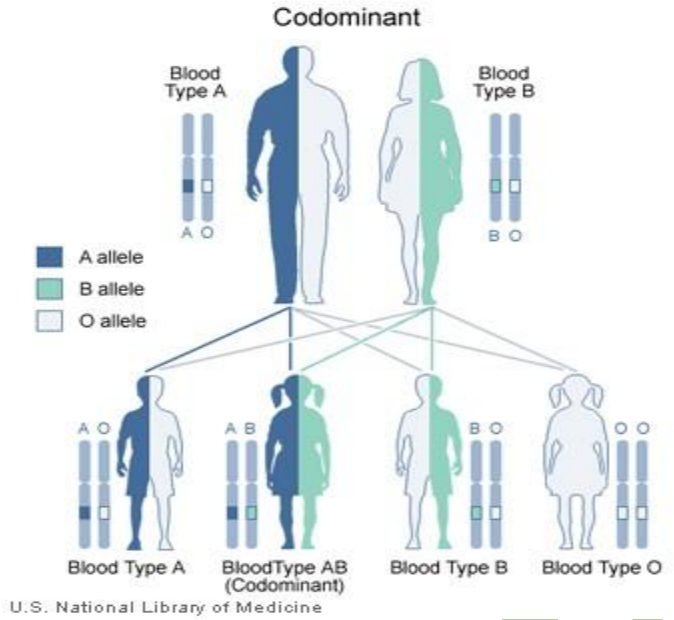
It is a condition in genetics where the phenotype of the heterozygote lies outside of the phenotypical range of both homozygote parents. Over dominance can also be described as heterozygote advantage, wherein heterozygous individuals have a higher fitness than homozygous individuals.

An example in humans is sickle cell anemia. This condition is determined by a single polymorphism. Possessors of the deleterious allele have lower life expectancy, with homozygotes rarely reaching 50 years of age. However, this allele also yields some resistance to malaria. A thus in region where malaria exerts or has exerted a strong selective pressure, sickle cell anemia has been selected for its conferred partial resistance to the disease. While homozygotes will have either no protection from malaria or a dramatic propensity to sickle cell anemia, heterozygotes enjoy a partial resistance to both

Codominance:

It is a situation in which two different alleles for a genetic trait are both expressed. Codominance is a relationship between two versions of a gene. Individuals receive one version of a gene, called an allele, from each parent. If the alleles are different, the dominant allele usually will be expressed, while the effect of the other allele, called recessive, is masked. In codominance, however, neither allele is recessive nor are the phenotypes of both alleles expressed.

A slightly more complicated multiple-allele system determines blood type in humans. The three alleles are: A, B, and O, corresponding to A, B, and O type blood respectively. The A and B alleles are dominant over the O allele, which is always recessive. However, there is an additional twist: the A and B alleles exhibit incomplete dominance and, when inherited together, give rise to AB blood type. Yet another catch is waiting, however: humans also have the Rh factor, a special type of protein found in most (but not all) human blood. Rh factor's presence is inherited in the ordinary Mendelian fashion, with Rh-positive dominant over Rh-negative. In total, this means humans have 8 simple blood types.



Epistasis: it is an interaction between genotypes at two *different* gene loci, which sometimes resembles a dominance interaction at a single locus. Epistasis modifies the characteristic 9:3:3:1 ratio expected for two non-epistatic genes. Most genetic systems involve complex epistatic interactions among multiple gene loci.

1. **Recessive epistasis**
2. **Dominant epistasis**
3. **Supplementary epistasis**

Multiple alleles

Although any individual has at most two different alleles, most genes exist in a large number of allelic forms in the population as a whole. In some cases, the alleles have different effects on the phenotype, and their dominance interactions with each other can be described as a series.

For example, the best known human blood groups, the ABO system, comprises three sets of alleles at the ***I*** locus, I^A, I^B, and I^O. The first two are dominant to the latter: that is, the **AA** and **AO** genotypes produce indistinguishable blood group phenotypes, called "*Type A*", as do **BB** and **BO**, which produce "*Type B*" blood. In another example, coat color in siamese cats and related breeds is determined by a series of alleles at the albino gene locus (***c***) that produce different levels of pigment and hence different levels of color dilution. Four of these are c^+, c^b, c^s, and c^a (standard, Birman, siamese, and albino, respectively), where the first allele is completely dominant to the last three, and the last is completely recessive to the first three.

CYTOPLASMIC INHERITANCE

Inheritance due to genes located in cytoplasm (plasmagenes) is called cytoplasmic inheritance. Since genes govering traits showing cytoplasmic inheritance are loc ated outside the nucleus and in the cytoplasm, they are refered to as plasmagenes. The sum total of genes present in the cytoplasm of a cell or an individual is known as plasmon. The plasmagenes are located in DNA present in mitochondria (mt DNA) and in chloroplasts (cp DNA). Together both the DNAs are called organelle DNA. Therefore, this type of inheritance is often referred to as organellar inheritance, plastid inheritance or mitochondrial inheritance. In this, generally, the character of only one of the two parents (usually female) is transmitted to the progeny. Hence such inheritance is usually referred to as extra - nuclear or extra-chromosomal or maternal or uniparental inheritance. The cytoplasmic inheritance is of two types: 1) Plastid inheritance and 2) mitochondrial inheritance.

Plastidial or Chloroplast Inheritance: Plastids self duplicated and have some amount of DNA and plays an important role in cytoplasmic inheritance. Plastids have green pigments called chloroplasts. Chloroplasts contain a unique circular DNA (cp DNA) in the stroma that is completely different from the nuclear genome. Some examples of plastid inheritance are given below.

a) Leaf variegation in *Mirabilis jalapa* : The conclusive evidence for cytoplasmic inheritance was first presented by C. Correns in *Mirabilis jalapa* (Four 'O' clock plant) in 1909. He studied inheritance of leaf variegation in *M. jalapa*. Variegation refers to the presence of white or yellow spots of variable size on the green background of leaves. In *M. jalapa*, leaves may be green, white or variegated. Some branches may have only green, only white or only variegated leaves. Correns made crosses in all possible combinations among the flowers produced on these three types of branches. When flowers from green branch were used as female parent, all the progeny were green irrespective of the phenotype (green, white or variegated) of male parent. Similarly, progeny from crosses involving flowers bloomed on white branches as female parent were all white irrespective of the phenotype of male parent. But in progeny from all crosses involving flowers born on variegated branches as female parent, all the three types i.e. green, white and variegated individuals were recovered in variable proportions.

Female Parent	x	Male Parent	
Green	x	Green	
	X	White	Green
	x	Variegated	
White	x	Green	
	X	White	pale Green
	x	Variegated	

Variegated	x	Green	
	X	White	Green, white and variegated
	x	Variegated	in variable ratio in each of the cases.

The green leaf branches have normal chloroplasts, white branches have mutant chloroplasts and variegated have a mixture of both normal and mutant chloroplasts. The above results indicated that the inheritance is governed by chloroplasts. Since the cytoplasm is contributed to the zygote mainly by female parent, the plastids are transmitted to the zygote from the female parent. Thus the plastids are responsible for variation in the crosses of green, white and variegated leaves.

b) Mitochondrial inheritance: The inheritance of some characters, such as cytoplasmic male sterility in plants, pokyness in *Neurospora* etc., is governed by mitochondrial DNA (mtDNA).

a) **Cytoplasmic Male Sterility (CMS) in maize :** In several crops, cytoplasmic control of male sterility is known. In maize, cytoplasmic male sterility (CMS) is governed by mitochondrial DNA. In such cases, if female parent is male sterile, F1 progeny also will be male sterile, because cytoplasm is mainly derived from female parent.

b) **Pokyness in *Neurospora*:** *Neurospora*, which is a breadmold has two strains i.e. wild and poky. The wild strain has normal growth. While the poky which is a mutant has very slow growth. A cross betw een a poky female and a wild male produce only poky progeny. In reciprocal cross (a cross between wild female and poky male) all the progeny would be wild. This suggests the presence of cytoplasmic inheritance because only difference between the reciprocal crosses is in the main contributor of cytoplasm.

Characteristic Features of Cytoplasmic Inheritance

a. Reciprocal difference: Reciprocal crosses show marked differences for characters governed by plasmagenes. In most cases, plasmagenes from only female parent are transmitted and hence this phenomenon is also called uniparental inheritance.

b. Lack of segregation: In general, F1, F2, F3 and subsequent generations do not show segregation for a cytoplasmically inherited trait, as F1 individuals receive plasmagenes from female parent only.

c. Somatic segregation: Plasmagenes generally show the features in somatic tissues such as leaf variegation features which is of rare occurrence in case of nuclear genes.

d. Association with organelle DNA: Several plasmagenes have been shown to be associated either with chloroplast or mitochondrial DNA. For example: Cytoplasmic Male Sterility (CMS) in sorghum and maize is associated with mitochondrial DNA.

e. Nuclear transplantation: Nuclear transplantation means nucleus of a cell is removed and replaced by nucleus of another genotype from a different cell. If nuclear transplantation reveals a trait to be governed by genotype of cytoplasm and not by that of nucleus, it clearly indicates that the trait or character is governed by cytoplasmic inheritance.

f. Mutagenesis: Some mutagens are highly specific mutagens which act only on the plasmagenes and do not affect nuclear genes Eg; ethidium bromide, Induction of mutations by such agents or chemicals in a gene clearly indicates that it is a plasmagene.

g. Lack of chromosomal location: In many organisms extensive linkage maps of nuclear genes are available. If a gene is shown to be located in one of these linkage groups, obviously it cannot be a plasmagene.

h. Transfer of nuclear genome through back crosses: Nucleus of a variety or species may be transferred into cytoplasm of another variety or species through repeated back crossing with former, which is used as recurrent male parent. Lines produced in this way are called alloplasmic lines, since they have cytoplasm and nucleus from different species.

i. Lack of association with a parasite or symbiont or virus: Only those cytoplasmically inherited traits which are not associated with parasites, symbionts or viruses can be regarded to be governed by plasmagenes.

Extranuclear Inheritance

It is the transmission of genes that occur outside the nucleus. It is found in most eukaryotes and is commonly known to occur in cytoplasmic organelles such as mitochondria and chloroplasts or from cellular parasites like viruses or bacteria.

Extranuclear Inheritance of Organelles

Mitochondria are organelles which function to produce energy as a result of cellular respiration. Chloroplasts are organelles which function to produce sugars via photosynthesis in plants and algae. The genes located in mitochondria and chloroplasts are very important for proper cellular function, yet the genomes replicate independently of the DNA located in the nucleus, which is typically arranged in chromosomes that only replicate one time preceding cellular division. The extranuclear genomes of mitochondria and chloroplasts however replicate independently of cell division. They replicate in response to a cells increasing energy needs which adjust during that cells lifespan. Since they replicate independently, genomic recombination of these genomes is rarely found in offspring contrary to nuclear genomes, in which recombination is common. Mitochondrial disease are received from the mother, sperm does not contribute for it.

Extranuclear Inheritance of Parasites

Extranuclear transmission of viral genomes and symbiotic bacteria is also possible. An example of viral genome transmission is perinatal transmission. This occurs from mother to fetus during the perinatal period, which begins before birth and ends about 1 month after birth. During this time viral material may be passed from mother to child in the bloodstream or breastmilk. This is of particular concern with mothers carrying HIV or Hepatitis C viruses. Examples of cytoplasmic symbiotic bacteria have also been found to be inherited in organisms such as insects and protists.

Types of Extranuclear Inheritance

Three general types of extranuclear inheritance exist. These are vegetative segregation, uniparental inheritance and biparental inheritance.

• **Vegetative segregation** results from random replication and partitioning of cytoplasmic organelles. It occurs with chloroplasts and mitochondria during mitotic cell divisions and results in daughter cells that contain a random sample of the parent cell's organelles. An example of vegetative segregation is with mitochondria of asexually replicating yeast cells.

Maternal inheritance

- The determination of the phenotype of offspring by the genotype of female parent is called *maternal inheritance* or *uniparental inheritance or maternal effect*.
- Example: Pattern of shell coiling in snail.
- oHere the shell coiling is determined by the genotype of the mother and not by the individual's own genotype.

Examples for cytoplasmic inheritance

Plastid inheritance in Mirabilis

Shell-coiling in snail

Kappa particles in Paramecium

Cytoplasmic male sterility in maize

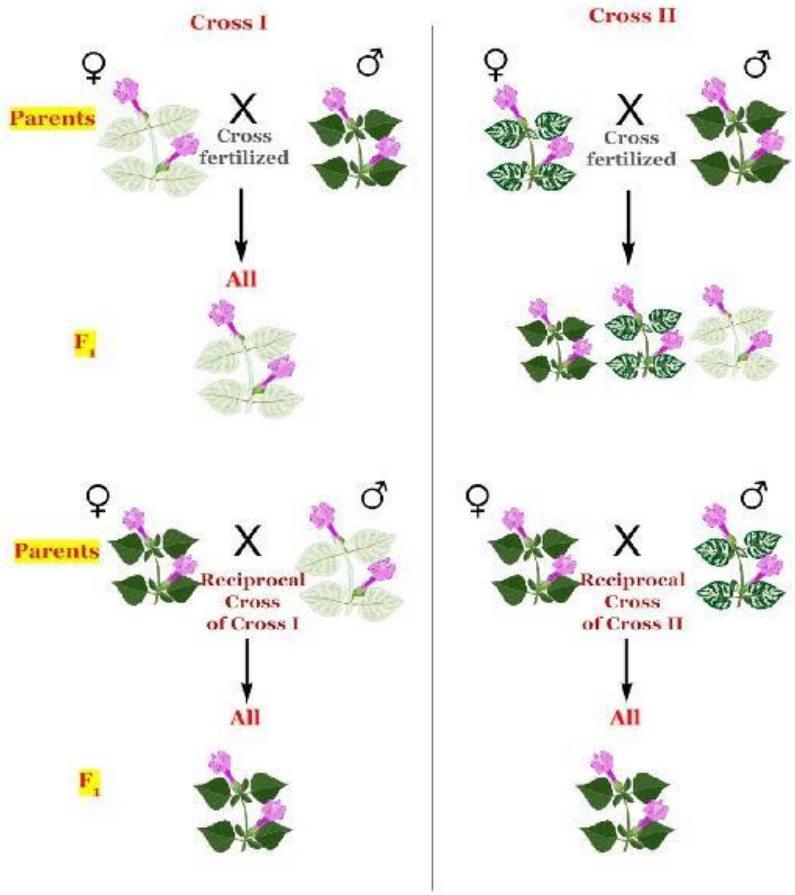
Sigma virus in *Drosophila melanogaster*

Milk factor in mice

LEAF VARIEGATION IN PLANTS

- The first example of cytoplasmic inheritance was reported by Correns (1909) in a variegated variety of the four-o'clock plant *Mirabilis jalapa.*
- Variegated plants have some branches which carry normal green leaves, some branches with variegated leaves (mosaic of green and white patches) and some branches which have all white leaves.
- Flowers on wholly green branches produce seeds that grow into normal plants.
- Flowers on variegated branches yield offspring of three kinds- green, white and variegated in variable proportions.
- Flowers from branches wholly white produce seeds that grow into white plants that is without chlorophyll.
- But in every case the source of pollen has no influence on the offspring.
- In other words, the phenotype of the progeny always resembled the female parent and the male made no contribution at all to the character. So cytoplasm of the egg influences the type of leaf in Mirabilis.
- The explanation for this unusual pattern of inheritance is that the genes concerned are located in the *plastids* within the cytoplasm, not in the nucleus and are therefore transmitted only through the female parent.
- Plastids are of two types, namely green *chloroplasts* and colourless *leucoplasts.*

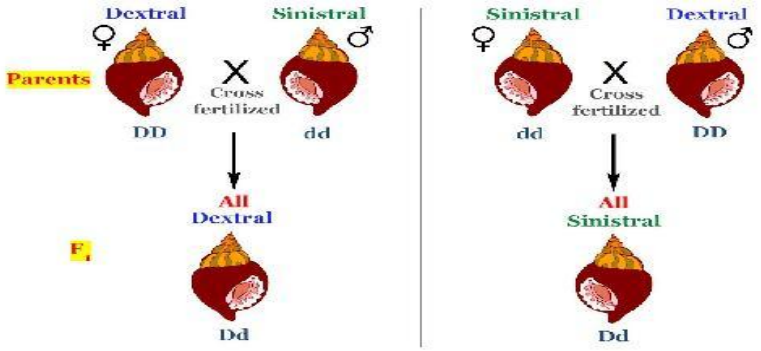
- *Green* branches contain Green plastids in their leaves, Variegated branches contain Green plastids and Colourless plastids and Colourless branches are due to the presence of Colourless plastids.



SNAIL SHELL COILING

- The classic phenotype which exhibits maternal effects is coiling direction of snail shells.
- Shell coiling in *Limnaea peregra*, a fresh water snail , is of two types, Dextral (clockwise) and Sinistral (anticlockwise).
- The dextral shell is dominant and is controlled by dominant gene D.
- The sinistral shell is recessive and is controlled by recessive gene d.

- The following crosses were made between pure line snails.
- When dextral female (DD) was crossed with sinistral male (dd), all the offsprings of F1 generation (Dd) have dextral coiling.
- If sinistral female (dd) is crossed with dextral male (DD), the offspring have Dd genotype but coiling is sinistral.
- In the above two crosses, the F1 snails have the same genotypes.
- The F1 phenotype is not the same for both crosses.

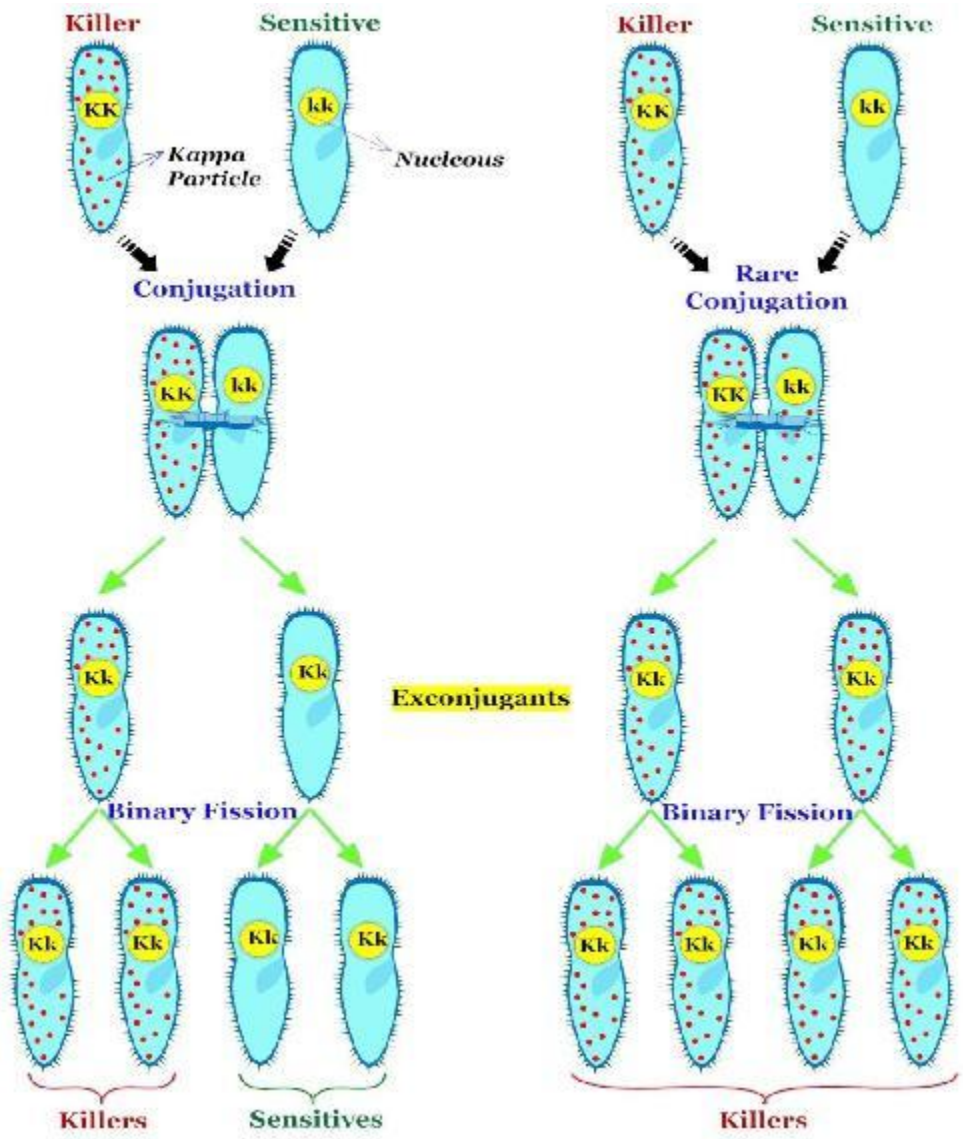


- The coiling phenotype that is seen in the offspring is controlled by the genotype of the mother.
- In the first cross, the offspring has dextral shell because the mother's genotype is DD.
- In the second cross, the offspring has sinistral shell because the mother's genotype is dd.
- Since, zygote receives whole of its cytoplasm from the egg, the direction of shell coiling in the offspring is governed by cytoplasm of the mother.

KAPPA PARTICLES IN PARAMECIUM

- T. M. Sonneborn described the inheritance of some cytoplasmic particles known as kappa and their relation to nuclear gene in the common cillate protozoan, *Paramecium aurelia.*

- There are two strains of Paramecium. They are killer and sensitive.
- Killer strain produces a toxic substance called paramecin that is lethal to other individuals called "sensitives" .
- The production of paramecin in killer type is controlled by certain cytoplasmic particles known as kappa particles. The sensitive strains lack these particles.
- The kappa particles are transmitted through the cytoplasm.
- The existence, production and maintenance of kappa particles
- are controlled by a dominant gene 'K' present in the nucleus. However, 'K' cannot initiate the production of kappa in the total absence of kappa in the cytoplasm.
- When a Paramecium of killer strain is having the genotype "KK" or (K+) conjugates with the Paramecium of non-killer strain having the genotype "kk", the exconjugants are all heterozygous for "Kk" genes.



- The development of a particular type depends upon the duration of cytoplasmic exchange
- oIf conjugation is normal, i.e., lasts only for a short time, and no exchange of cytoplasm takes place between the two, both killers and non-killers
- (sensitive) are produced.

- oHowever in rare or prolonged conjugation (i.e., lasting for long time) the cytoplasmic bridge between the two conjugants is larger. In such cases, in addition to the nuclear material, the cytoplasmic materials are also exchanged.
- oDuring this cytoplasmic exchange, the kappa particles present in the cytoplasm of the killer type enter the non-killer type and convert it into a killer type. So all the offspring produced by the exconjugants are killer type.
- This shows that a Paramecium becomes a killer when it receives kappa particles and it becomes a sensitive when it does not receive kappa particles.

• **Uniparental inheritance** occurs in extranuclear genes when only one parent contributes organellar DNA to the offspring. A classic example of uniparental gene transmission is the maternal inheritance of human mitochondria. The mother's mitochondria are transmitted to the offspring at fertilization via the egg. The father's mitochondrial genes are not transmitted to the offspring via the sperm. Very rare cases which require further investigation have been reported of paternal mitochondrial inheritance in humans, in which the father's mitochondrial genome is found in offspring Chloroplast genes can also inherit uniparentally during sexual reproduction. They are historically thought to inherit maternally, but paternal inheritance in many species is increasingly being identified. The mechanisms of uniparental inheritance from species to species differ greatly and are quite complicated. For instance, chloroplasts have been found to exhibit maternal, paternal and biparental modes even within the same species.

• **Biparental inheritance** occurs in extranuclear genes when both parents contribute organellar DNA to the offspring. It may be less common than uniparental extranuclear inheritance, and usually occurs in a permissible species only a fraction of the time. An example of biparental mitochondrial inheritance is in the yeast, *Saccharomyces cerevisiae*.

Non-Mendelian Inheritance

It is a general term that refers to any pattern of inheritance in which traits do not segregate in accordance with Mendel's laws. These laws describe the inheritance of traits linked to single genes on chromosomes in the nucleus. In Mendelian inheritance, each parent contributes one of two possible alleles for a trait. If the genotypes of both parents in a genetic cross are known, Mendel's laws can be used to determine the distribution of phenotypes expected for the population of offspring. There are several situations in which the proportions of phenotypes observed in the progeny do not match the predicted values.

Although inheritance of traits in fungi, viruses, and bacteria are all non-Mendelian, the phrase "non-Mendelian inheritance" is usually only used to describe the exceptions which occur in eukaryotic reproduction. Non-Mendelian inheritance plays a role in several disease processes.

VARIATION IN CHROMOSOME NUMBER & STRUCTURE

Changes in chromosome number can occur by the addition of all or part of a chromosome (**aneuploidy**), the loss of an entire set of chromosomes (**monoploidy**) or the gain of one or more complete sets of chromosomes (**euploidy**). Each of these conditions is a variation on the normal diploid number of chromosomes. As you would expect each of these can have drastic effects on phenotypic expression.

Aneuploidy - the abnormal condition were one or more chromosomes of a normal set of chromosomes are missing or present in more than their usual number of copies

Monoploidy - the loss of an entire set of chromosomes

Euploidy - an entire set of chromosomes is duplicated once or several times

Chromosome Number in Different Species

In "higher" organisms (**diploids**), members of same species typically have identical numbers of chromosomes in each somatic cell. **Diploid chromosome number** (2n). Nearly all chromosomes will exist in pairs (identical wrt length and centromere placement) except the sex chromosomes. Members of pair are **homologous chromosomes**. **Haploid number** (n) is the number of chromosome pairs.

		2n	n
Human		46	23
Horse	64	32	
Cat		38	19
Geometrid moth		224	112
Tomato		24	12
Pink bread mold		14	7

Autosomal monosomy and trisomy

Occasionally, one finds an organism that has an extra copy of a particular chromosome. This is known as **trisomy**--because there are now 3 copies of an autosome. Some trisomies are viable in animals, but the condition usually has severe effects. These effects are presumably related to the fact that there are 3 copies of every gene on the trisomic chromosome, but only 2 copies of all the

genes on the other chromosomes. We will see later, that organisms with three or more copies of **all** the chromosomes are often perfectly viable.

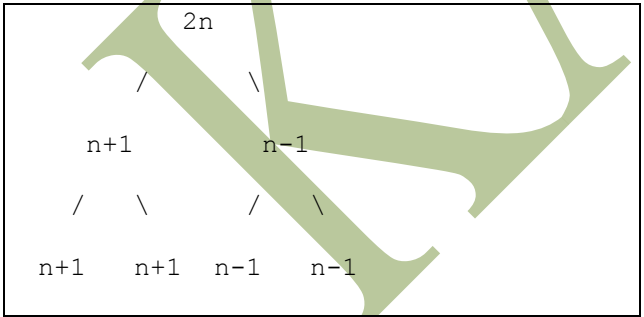
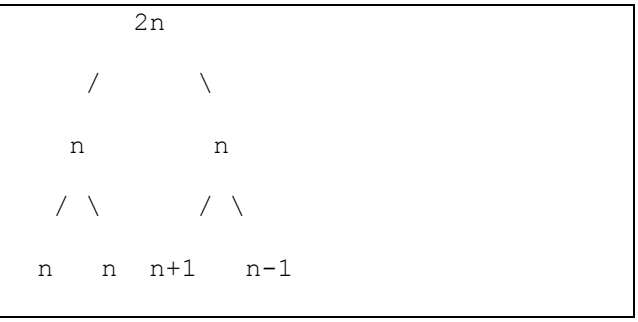
Trisomy of human chromosome 21 is the cause of the disorder known as **Down syndrome.**

(Remember, humans have 23 pairs of chromosomes--the pairs are numbered 1 through 22, plus the X and Y). It is characterized by multiple physical defects, including epicanthal fold, furrowed tongue, characteristic palm and finger print patterns, and lowered IQ. About 1 in 750 live births produces a child with this condition. It results from the **non-disjunction** of chromosome 21 during meiotic anaphase I or anaphase II, when the paired homologs (or paired chromatids) normally migrate to opposite poles of the cell.

Nondisjuction: Nondisjunction ("not coming apart") is the failure of chromosome pairs to separate properly during meiosis stage 1 or stage 2. This could arise from a failure of homologous chromosomes to separate in meiosis I, or the failure of sister chromatids to separate during meiosis II or mitosis. The result of this error is a cell with an imbalance of chromosomes. Such a cell is said to be aneuploid. Loss of a single chromosome (2n-1), in which the daughter cell(s) with the defect will have one chromosome missing from one of its pairs, is referred to as a monosomy. Gaining a single chromosome, in which the daughter cell(s) with the defect will have one chromosome in addition to its pairs is referred to as a trisomy.The members of a chromosome pair (homologs) line up at the metaphase plate during meiotic metaphase I, then separate to opposite poles of the cell during anaphase I--review this material in Klug and Cummings or any introductory Genetics text if you are not thoroughly familiar with it!). If the pair fails to separate, and both migrate to the same pole, half of the resulting gametes will have two copies of chromosome 21, rather than one. When this gamete unites with a normal gamete (bearing one copy of chromosome 21) during fertilization, the resulting gamete has 3 copies of chromosome 21, rather than the normal 2. Nondisjunction of chromosome 21 seems to occur more often in the production of eggs than sperm, and the frequency

increases with the age of the parent. Older individuals are often encouraged to test for trisomy 21 by amniocentesis at 15 to 16 weeks after conception. Nondisjunction can happen to other chromosomes in addition to chromosome 21. But human embryos that are trisomic for any other chromosome do not survive to birth. It should be obvious that the other half of the gametes resulting from a non-disjunction event at anaphase 1 will have **0 copies** of the chromosome. When a gamete with 0 copies of a chromosome unites with a normal gamete, the result is a zygote that has only one copy of that chromosome. This is **monosomy. Monosomy is not well tolerated in animals**—usually lethal. Some plants can survive (observed in maize, tomato, Oenothera, and Datura) but they have low viability and are usually sterile. Nondisjunction can also occur at anaphase II, when sister chromatids fail to separate and migrate to opposite poles. Nondisjuntion at anaphase one results in half the gametes being normal, and half being abnormal (see diagram in text).

If one surveys karyotypes of embryos that spontaneously abort, trisomies for all the autosomes are seen, and other forms of chromosomal abnormalities as well, but these conditions are apparently fatal early in development. Normal embryonic development requires a precise diploid complement of chromosomes.

	
Schematic of nondisjunction in meiosis I. Duplicated chromosomes in diploid cell (2n). All gametes are affected by nondisjunction in meiosis I. Two gametes have a single extra	Schematic of nondisjunction in meiosis II. Duplicated chromosomes in diploid cell (2n). Half of the gametes are affected by nondisjunction in meiosis II. One gamete has a

chromosome; two gametes are missing a single chromosome.	single extra chromosome; one gamete is missing a single chromosome.

CHROMOSOME STRUCTURE

1. During nuclear division, the DNA (as chromatin) in a Eukaryotic cell's nucleus is coiled into very tight compact structures called chromosomes. These are rod-shaped structures made of protein and DNA, which are visible (when stained) only during nuclear division.

2. The DNA in Eukaryotic cells is coiled tightly around proteins called histones, which help in the tight packing of DNA. During interphase, the DNA is not tightly coiled into chromosomes, but exists as chromatin.

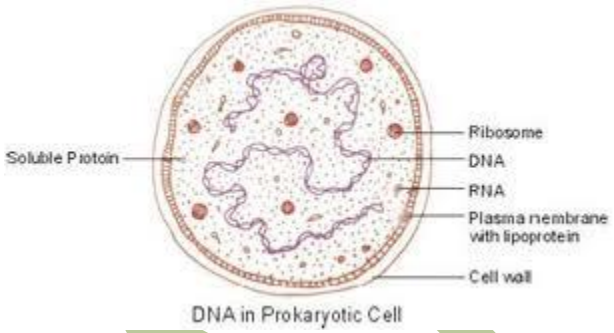
3. When preparing for nuclear division, during the S phase of interphase, the chromosomes copy themselves (i.e. DNA replication occurs). Each half of the chromosome is now called a chromatid. Note that there is still only one chromosome; it consists of two chromatids but has only one centromere. The function of this is to hold the two chromatids together until they separate during anaphase.

4. Chromosomes are simpler in Prokaryotes. Their DNA is in a single chromosome, and exists as a loop (ccc DNA).

Chromosome Structure of Prokaryotes: (Bacteria)

In contrast to the linear chromosomes found in eukaryotic cells, the strains of bacteria initially studied were found to have single, covalently closed, circular chromosomes. The circularity of the bacterial chromosome was elegantly demonstrated by electron microscopy in both Gram negative bacteria (such as *Escherichia coli*) and Gram positive bacteria (such as *Bacillus subtilis*). Bacterial

plasmids were also shown to be circular. In fact, the experiments were so beautiful and the evidence was so convincing that the idea that bacterial chromosomes are circular and eukaryotic chromosomes are linear was quickly accepted as a definitive distinction between prokaryotic and eukaryotic cells. However, like most other distinctions between prokaryotic and eukaryotic cells, it is now clear that this dichotomy is incorrect. Not all bacteria have a single circular chromosome: some bacteria have multiple circular chromosomes, and many bacteria have linear chromosomes and linear plasmids.



DNA in Prokaryotic Cell

Experimental evidence for multiple chromosomes and linear chromosomes initially came from studies using pulsed field gel electrophoresis (PFGE), an approach that uses alternating electric fields to separate large DNA molecules on an agarose gel. Subsequently genome sequencing projects have added to the list of bacteria with multiple or linear chromosomes.

Some examples of bacterial genome organization

Bacteria	Chromosome(s)	Plasmid(s)
Agrobacterium tumefaciens	one linear (2.1 Mb) + one circular (3.0 Mb)	two circular (450 + 200 Kb)
Bacillus subtilis	one circular (4.2 Mb)	
Bacillus thuringiensis	one circular (5.7 Mb)	six (each >50 Kb)
Borrella	one linear (0.91 Mb)	multiple circular + linear (5-200 Kb)
Bradyrhizobium japonicum	one circular (8.7 Mb)	
Brucella melitensis	two circular (2.1 + 1.2 Mb)	
Brucella suis biovars 1, 2, 4	two circular (1.0 + 2.0 Mb)	
Brucella suis biovar 3	one circular (3.1 Mb)	
Buchnera sp. strain APS	one circular (640 Kb)	two circular (< 7.8 Kb each)
Deinococcus radiodurans	two circular (2.6 + 0.4 Mb)	two circular (177 + 45 Kb)
Escherichia coli K-12	one circular (4.6 Mb)	
Leptospira interrogans	two circular (4.7 + 0.35 Mb)	
Paracoccus denitrificans	three circular (2.0 + 1.1 + 0.64 Mb)	
Pseudomonas aeruginosa	single circular (6.3 Mb)	
Rhizobacterium meliloti	two circular (3.4 + 1.7 Mb	one circular megaplasmid (1,400 Kb)
Rhodobacter sphaeroides	two circular (3.0 + 0.3 Mb)	
Ureaplasma urealyticum	one circular (0.75 Mb)	
Vibrio cholerae	two circular (2.9 + 1.1 Mb)	
Vibrio parahaemolyticus	two circular (3.2 + 1.9 Mb)	
Xylella fastidiosa	one circular (2.7 Mb)	two circular (51 + 1.3 Kb)

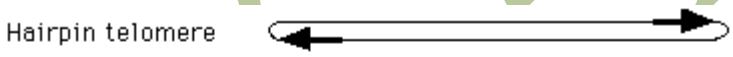
The first convincing evidence that some bacteria have multiple chromosomes came from studies on Rhodobacter sphaeroides. Both molecular and studies clearly demonstrated that R. sphaeroides has two large circular chromosomes. One of the chromosomes is 3.0 Mb and the other is 0.9 Mb. Genes encoding rRNAs and tRNAs required for translation, and metabolic enzymes are distributed between the two chromosomes. Multiple chromosomes have also been found in many other bacteria, including Agrobacterium tumefaciens, Rhizobium, Brucella, Paracoccus denitrificans, Ochrobactrum anthropi, Leptospira interrogans, Burkholderia, Vibrio cholerae, Deinococcus radiodurans, and many others from diverse groups of bacteria.

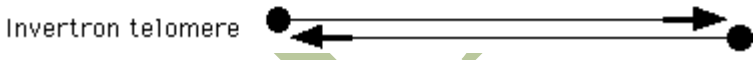
Furthermore, some bacteria have linear chromosomes. Borrelia have linear chromosomes and most strains contain both linear and circular plasmids; most of the bacteria in the genus Streptomyces have linear chromosomes and plasmids and some have circular plasmids as well. In addition, in

some cases there may be a dynamic equlibrium between linear and circular forms of a DNA molecule. There is some evidence that linearization may be due to integration of a linear phage genome into the circular DNA molecule.

Linear chromosomes and plasmids were not discovered in bacteria until relatively recently. The first published evidence for linear chromosomes was in 1979, but because the techniques used at that time were limited and because the dogma that all bacterial chromosomes are circular was so entrenched, few people believed that linear chromosomes and plasmids occured in bacteria until 1989. By that time pulsed field gel electrophoresis had been developed, and this new technique provided convincing evidence that the chromosome of Borrelia burgdoferi was linear.

The ends of linear DNA molecules (called telomeres) pose two problems that do not apply to circular DNA molecules. First, since free double-stranded DNA ends are very sensitive to degradation by intracellular nucleases, there must be a mechanism to protect the ends. Second, the ends of linear DNA molecules must have a special mechanism for DNA replication. These problems are solved by features of the telomeres. Two different types of telomeres have been observed in bacteria: hairpin telomeres and invertron telomeres.





There are examples of linear DNA molecules in bacteria that are protected by both types of telomeres: palindromic hairpin loops are protected by the lack of free double-stranded ends, and invertron telomeres are protected by proteins that bind to the 5'-ends. Both of these mechanisms are also used by some phage, eukaryotic viruses, and eukaryotic plasmids.

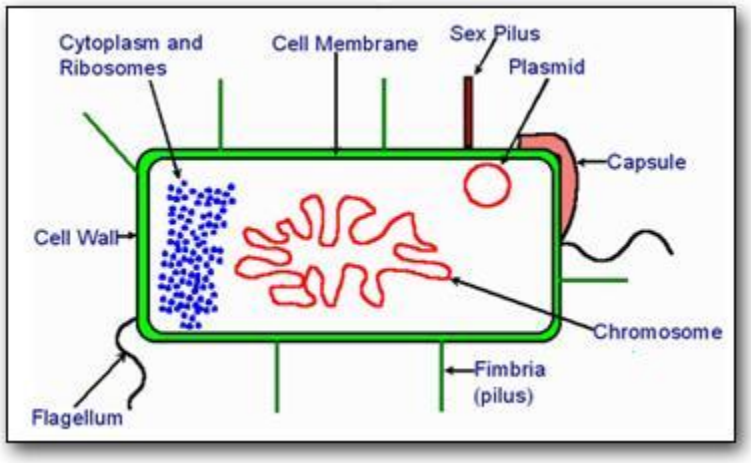
The two types of telomeres also solve the problem of DNA replication differently. Invertron telomeres have a protein covalently attached to the 5' ends of the DNA molecule (called the 5'-terminal protein or TP for short). DNA polymerase interacts with the TP at the telomere and catalyzes the formation of a covalent bond between the TP and a dNTP. The dNTP bound to the TP has a free 3'-OH group which acts as the primer for chain elongation. Replication of hairpin telomeres is less well understood. Apparently multiple hairpin sequences can pair to form concatemers that are replication intermediates.

The important take-home point is that we are just beginning to appreciate the similarity of many processes once thought to be completely different between bacteria and eukaryotes, partly because we now have better tools for studying these processes and partly because most of the earlier studies focused on relatively few types of bacteria. The more we study a wider diversity of bacteria, phages, and plasmids, the more obvious it becomes that E. coli is an excellent model for dissecting broad features of molecular and cell biology, but not all bacteria do everything the same way. Furthermore, we have only recently begun to attack the molecular genetics of the Archae, and what we have learned so far suggests that this diverse group of prokaryotes share even more common features with the eukaryotes.

1. The circular genomes of mitochondrial and chloroplast are a notable exception to the rule that eukaryotic chromosomes are linear. However, this nicely fit into the dichotomy that eukaryotic chromosomes are linear and bacterial chromosomes are circular because these organelles seem to have evolved from entrapped bacteria.
2. Other examples include the presence of introns, and poly-A tails on mRNA.
3. This genus includes B. burgdorferi, the causative agent of Lyme disease.
4. Streptomyces make a wide variety of useful antibiotics, including streptomycin.
5. For example, linear DNA was precipitated in the most commonly used procedures for purifying bacterial plasmids, and the procedures for purifying chromosomal DNA relied

upon the differential binding of ethidium bromide to "sheared DNA fragments" compared to circular DNA.

6. It is not intuitively obvious how the ends of a linear DNA molecule could be completely replicated. All known DNA polymerases require a pre-existing primer for initiation of DNA replication. The primer is usually a short RNA molecule with a free 3'-OH group that can be extended by DNA polymerase. If a linear DNA molecule was primed at one end, DNA synthesis could continue to the other end. However, once the primer is removed, the DNA corresponding to the primer could not be replicated.

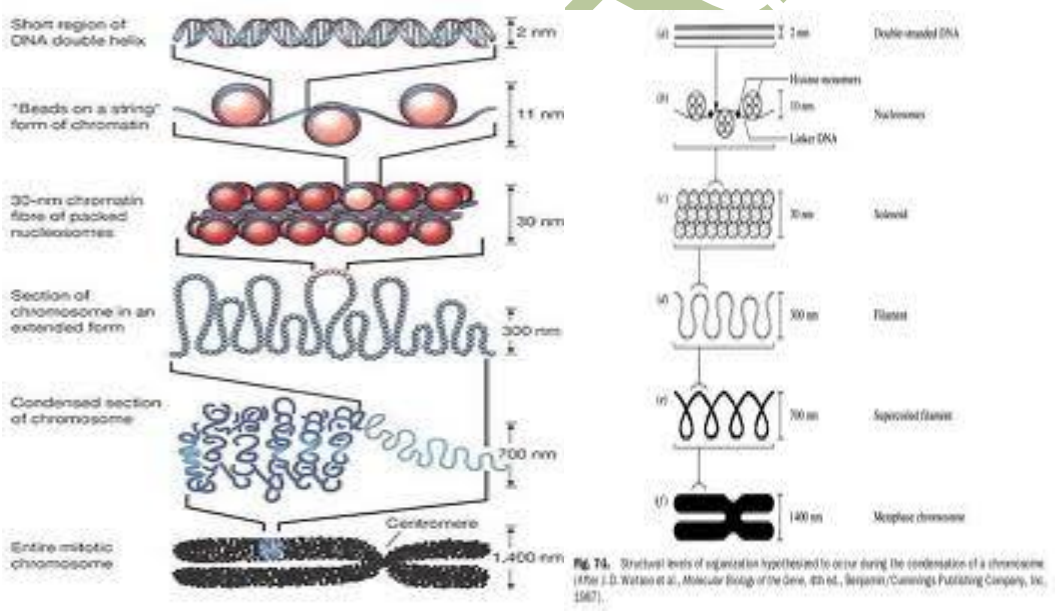


The telomers at the end of chromosomes of most eukaryotic cells are replicated by a different mechanism: most telomeres are short GC-rich repeats that are added in a 5' to 3' direction by the enzyme telomerase.

Eukaryotic Chromosome Structure (Plants and Animals)

In eukaryotes the chromosomes are found packaged within a nuclear membrane, unlike the case in prokaryotes. This membrane consists of a DNA double helix bound to an octamer of core histones (2 dimers of H2A and H2B, and an H3/H4 tetramer). Together, the DNA bound around this histone core forms what is known as the nucleosome. About 147 base pairs of DNA coil around 1 octamer, and ~20 base pairs are sequestered by the addition of the linker histone (H1), and various length of "linker" DNA (~0-100 bp) separate the nucleosomes.

Packaging of DNA is facilitated by the electrostatic charge distribution: phosphate groups cause DNA to have a negative charge, whilst the histones are positively charged. Most eukaryotic cells contain histones (with a few exceptions) as well as the kingdom Archaea, a protist group. Histones are positively charged molecules as they contain lysine and arginine in larger quantities and DNA is neatively charged. So they make a strong ionic bond in between them to form nucleosome.



Other variations in numbers of chromosomes—Polyploidy

Polyploidy is a term used to describe cells and organisms containing more than two paired (homologous) sets of chromosomes results from having **additional whole haploid sets** of chromosomes. If a normal diploid individual is said to have **2n** chromosomes, and a normal gamete is said to have **n** chromosomes, then polyploid individuals can be represented as: 3n, 4n, 6n, 8n, etc. Polyploidy is rare in many groups of animals, but we do observe it in fish, amphibians, and lizards.

Autotriploid. Many domestic plants are autopolyploids. **Usually sterile**. Problems at meiosis if there are odd numbers of chromosome sets (3,5, etc.). Unbalanced gametes. Some "seedless" varieties of fruit are polyploidy.

Allotetraploid. Non-homologous sets of chromosomes cannot synapse at meiosis. But if both sets are doubled (**mitotic failure**), all chromosomes now have a homolog to pair with, meiosis can take place normally.

Chromosomal Mosaics

It is when different cells within an individual, who has developed from a single fertilized egg, have a different chromosomal makeup. Most commonly there will be some cells with a typical number of chromosomes (46 chromosomes) and other cells with an altered number or structure of chromosomes. The most common kind of chromosomal mosaicism found at prenatal diagnosis involves trisomy, where the abnormal cells contain 47 chromosomes. Down syndrome mosaicism is an example of trisomy mosaicism. These individuals have some cells with the typical number of chromosomes (46) and some cells with an extra chromosome 21, for a total of 47 chromosomes. Mosaicism may exist for all kinds of chromosome abnormalities (monosomy, triploidy, structural changes, etc). Although more rare, there may even be mosaicism where both different cell types are abnormal in structure or number, and there are no normal cells involved.

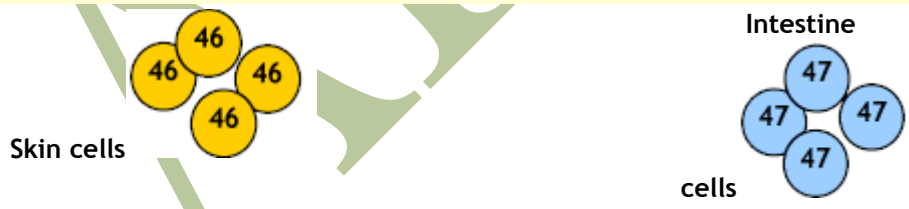
Chromosomal mosaicism describes a **group of disorders**. Any chromosomal change, in number or structure, can be present in a mosaic form. However, trisomy mosaicism is the most common kind ascertained during prenatal diagnosis.

All of the cells in our body come from a single cell, the fertilized egg or zygote. In order for the zygote to develop into a baby, this single cell must grow and divide. Before cells can divide each chromosome must make an identical copy of itself. At cell division each chromosome and its identical copy pull apart into two separate cells. Now the resulting cells, also called daughter cells, have the same chromosome make-up as the original cell. The two new cells will repeat this process. In each cell, the chromosomes will duplicate and divide into two new cells. The result, is 4 cells identical to the first original cell. This process of cell division is called mitosis. Sometimes a mistake can occur when the chromosomes are separating into the two daughter cells. An extra chromosome may travel into the wrong cell or a chromosome may get lost in the separation of the cells. The result would be two daughter cells with different chromosomal make-up.

In this illustration, the green cell represents a cell with an abnormal chromosome make-up. All cells that come from the green cell will share the same chromosome change. We say that all cells originating from that cell are in the same cell line. The baby that develops from this embryo will have some cells in his/her body which have the typical number of chromosomes and some that have the chromosome change.

a) Normal and abnormal cells are found in most tissues

b) Normal and abnormal cells are confined to specific tissues



Trisomy mosaicism can occur in one of two ways**:**

- In an abnormal fertilized egg with 47 chromosomes, one of the cells may lose the extra chromosome at cell division, leaving 46 chromosomes in that cell. All cells that are derived from that cell will have 46 chromosomes. The rest of the cells will have 47 chromosomes.

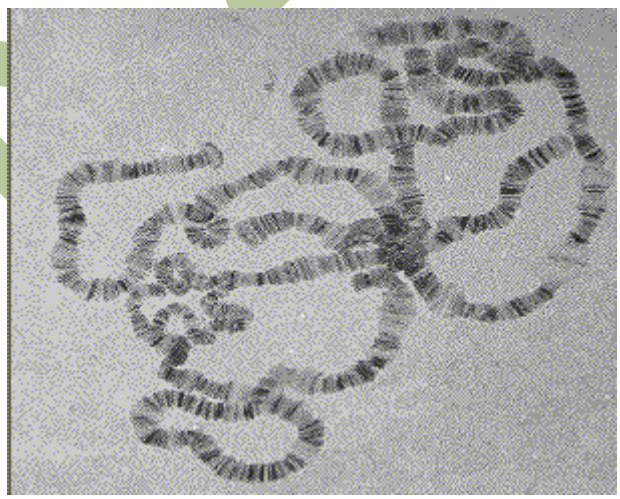
In a typical zygote with 46 chromosomes, at cell division one of the cells may retain a duplicated copy of one of the chromosomes. This produces a cell with 47 chromosomes. All cells that are derived from that cell also have 47 chromosomes. The rest of the cells will have 46 chromosomes.

Polytene Chromosome:

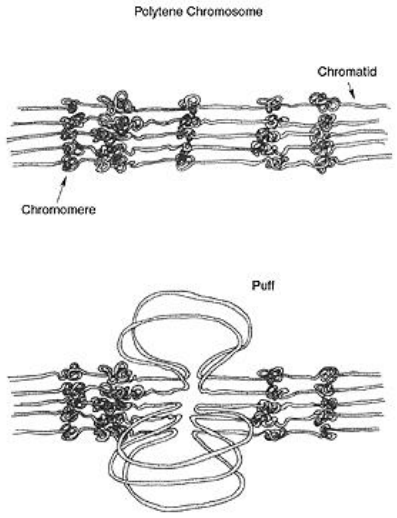
To increase cell volume, some specialized cells undergo repeated rounds of DNA replication without cell division (endomitosis), forming a giant **polytene chromosome**. Polytene chromosomes form when multiple rounds of replication produce many sister chromatids that remain synapsed together.

In addition to increasing the volume of the cells' nuclei and causing cell expansion, polytene cells may also have a metabolic advantage as multiple copies of genes permits a high level of gene expression. In *Drosophila melanogaster*, for example, the chromosomes of the larval salivary glands undergo many rounds of endoreplication, to produce large amounts of glue before pupation.

Polytene chromosomes have characteristic light and dark banding patterns that can be used to identify chromosomal rearrangements and deletions. Dark banding frequently corresponds to inactive chromatin, whereas light banding is usually found at areas with higher transcriptional activity. The banding patterns of the polytene chromosomes of *Drosophila melanogaster* were sketched in 1935 by Calvin B. Bridges, in such detail that his maps are still widely used today. The banding patterns of the chromosomes are especially helpful in research, as they provide an excellent visualization of transcriptionally active chromatin and general chromatin structure.



Chromosome puffs are diffused uncoiled regions of the polytene chromosome that are sites of RNA transcription. A **Balbiani ring** is a large chromosome puff.



Polytene chromosomes were originally observed in the larval salivary glands of *Chironomus* midges by Balbiani in 1881, but the hereditary nature of these structures was not confirmed until they were studied in *Drosophila melanogaster* in the early 1930s by Emil Heitz and Hans Bauer. They are known to occur in secretory tissues of other dipteran insects such as the Malpighian tubules of *Sciara* and also in protists, plants, mammals, or in cells from other insects. Some of the largest polytene chromosomes described thus far (see scale bar in figure below) occur in larval salivary gland cells of the Chironomid genus *Axarus*. Polytene chromosomes are about 200micron in length. The chromonema of these chromosomes divide but do not separate. Therefore, they remain together to become large in size. Another form of chromosomal enlargement that provides for increased transcription is the lampbrush chromosome.

Polytene chromosomes are also used to identify the species of Chironomid larvae that are notoriously difficult to identify. Each morphologically distinct group of larvae consists of a number of morphologically identical (sibling) species that can only be identified by rearing adult males or

by cytogenetic analysis of the polytene chromosomes of the larvae. Karyotypes are used to confirm the presence of specific species and to study genetic diversity in species with a wide range.

CHROMOSOME THEORY OF LINKAGE

The chromosome theory of linkage of Morgan and Castle states that:

1. The genes which show linkage, are situated in the same pair of chromosomes.
2. The linked genes remain arranged in a linear fashion on the chromosome. Each linked gene has a definite and constant order in its arrangement.
3. The distance between the linked genes determines the degree of strength of linkage. The closely located genes show strong linkage then the widely located genes which show weak linkage.
4. The linked genes remain in their original combination during the course of inheritance.

Kinds of Linkage:

Linkage is generally classified on the basis of three criteria viz., (i) Crossing over, (ii) Genes involved and (iii) Chromosomes involved.

Based on crossing over: Linkage may be classified into (a) complete and (b) incomplete / partial depending up on absence or presence of recombinant phenotypes in test cross progeny.

(a) Complete linkage: It is known in case of males of *Drosophila* and females of silkworms, where there is complete absence of recombinant types due to absence of crossing over.

(b) Incomplete / partial linkage: If some frequency of crossing over also occurs between the linked genes, it is known as incomplete / partial linkage. Recombinant types are also observed besides parental combinations in the test cross progeny. Incomplete linkage has been observed in maize, pea, *Drosophila* female and several other organisms.

Types of linkage

The types of linkage are two types.

1) Complete linkage
2) Incomplete linkage

Complete linkage

Linked genes inherit together for more generation in a continuous and regular fashion on the same chromosome. This phenomena of inheritance is called complete linkage.

Eg: linkage in *Drosophila melanogaster*.

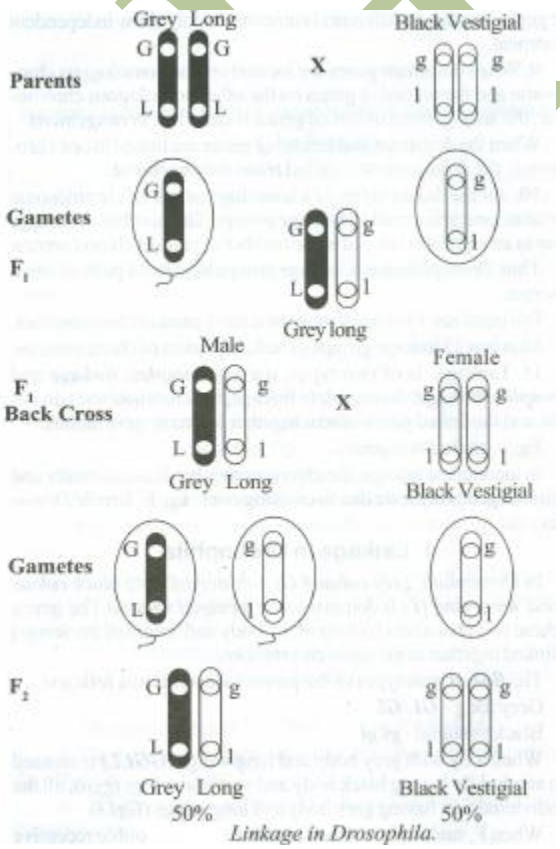
1. Complete Linkage

In complete linkage, linked genes inherit together for many generations. Here crossing over does not occur. In complete linkage the genes are closely situated.

Eg. 1. Body colour and shape of wings in male *Drosophila*.

2. Bent wings and shaven bristles in the 4^{th} chromosome of *Drosophila*.

Complete linkage is *the phenomenon in which two or moregenes or characters are inherited together Jor a number of generation.* In this, genes are closely associated and tend to inherit together. Complete linkage is due to the fact that there occurs no break in the chromosomes. As a result of complete linkage, the young ones inherit only the parental characters. New characters do not appear among the young ones. So complete linkage produces only parental combination; new combinations do not arise. This phenomenon is very rare. It is found only in male *Drosophila.* The F 1 male hybrid is back crossed with recessive female parent. The F 1 male hybrid produces only two types of gametes in which the linked genes (G and L or g and 1) are inherited together. So only two types of offspring are produced in the F2 generation in equal numbers.



Linkage in Drosophila.

Incomplete linkage

The linked genes which are widely located in chromosome and have changes (meiotic prophase) of separation by crossing over. This phenomena are incompletely linked genes and their inheritance is called incomplete linkage.

The separation of linked genes during inheritance is called *incomplete linkage.* The linked genes are separated due to crossing over, chromosomal breaks, etc.

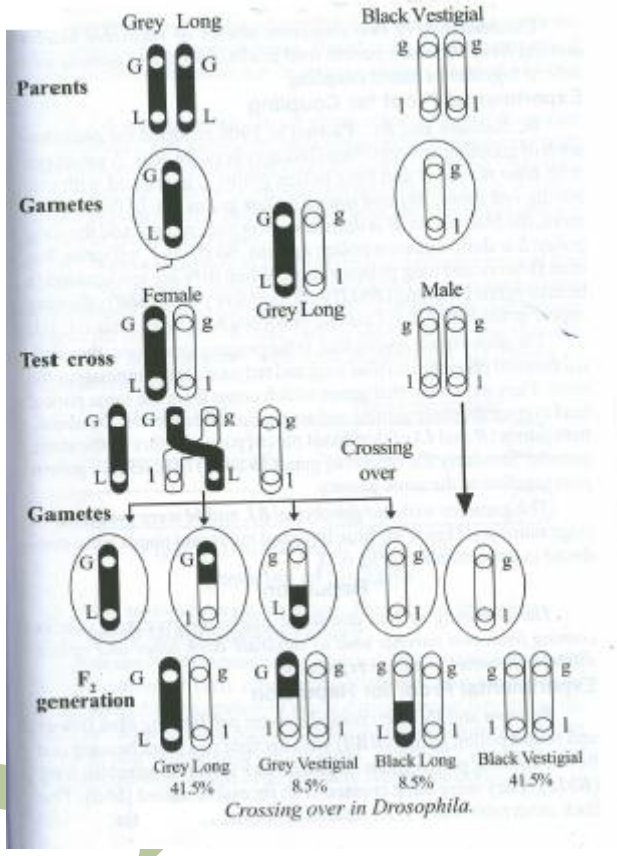
Eg. 1. *Body colour and wing shape in female Drosophila.*

2. Seed colour and seed shape in maize.

3. Flower colour and pollen grain shape in sweet pea.

- In incomplete linkage, the linked genes on certain occasions separate. This leads to the formation of new combinations among the young ones.
- Incomplete linkage is due to the breakage of chromosomes during gametogenesis. Incomplete linkage is found in *female Drosophila.*
- This breakage of chromosomes leads to the separation of linked genes and new combinations appear. Because of this new genetic combination the offspring produced in the F2 generation are different from their parent in their phenotype and genotype.
- So the incomplete linkage involves the accidental breakage of chromosomal segments or linked genes, resulting in new combination of genes.
- In the below experiment, the F 1 female hybrid produces four types of gametes. Among four types, two types of gametes carry new combinations due to the separation of linked genes. The gene G is separated from *L* and joins with *I.* In the same way the *L* joins with *g.*

- These combinations are different from the original combination. This type of inheritance is different from the *independent assortment.*
- If the genes are assorted independently the four types of offspring produced in the F2 generation of the above experiment should be in the 1:1:1:1 ratio.



Crossing over in Drosophila.

Importance of linkage

Morgan and *Castle* proposed the chromosomal theory of linkage. The main features of the chromosomal theory of linkage are the following:

1. The genes are arranged in a linear fashion on the chromosomes.

2. The genes on a chromosome are linked

3. Genes showing linkage are located on the same chromosome.

4. Linked genes will remain together during inheritance.

5. The distance between the genes will determine the strength of linkage. The closely located genes show strong linkage. Distantly located genes show weak linkage.

Factors effecting linkage

Linkage is affected by the following factors:

1. Distance: Closely located genes show strong linkage while genes widely located show weak linkage.

2. Age: With increasing age the strength of linkage decreases.

3. Temperature: Increasing temperature decreases the strength of linkage.

4. X-rays: X-ray treatment reduces the strength of linkage.

Based on genes involved: Depending on whether all dominant or some dominant and some recessive alleles are linked together, linkage can be categorized into (a) Coupling phase and (b) Repulsion phase.

(a) Coupling phase: All dominant alleles are present on the same chromosome or all recessive alleles are present on same chromosome.

(b) Repulsion phase: Dominant alleles of some genes are linked with recessive alleles of other genes on same chromosome.

Based on chromosomes involved: Based on the location of genes on the chromosomes, linkage can be categorized into (a) autosomal linkage and (b) X-chromosomal linkage / allosomal linkage / sex linkage.

(a) Autosomal linkage: It refers to linkage of those genes which are located in autosomes (other than sex chromosomes).

(b) X-chromosomal linkage / allosomal linkage / sex linkage: It refers to linkage of genes which are located in sex chromosomes i.e. either 'X' or 'Y' (generally 'X').

LINKAGE GROUPS:

Linkage group refers to a group of genes which are present in one chromosome. In other words, all those genes which are located in one chromosome constitute one linkage group. The number of linkage groups is limited in each individual. The maximum number of linka ge groups is equal to the haploid chromosome number of an organism. For example there are ten linkage groups in corn (2n = 20), seven in garden pea (2n = 14), seven in barley (2n = 14), four in *Drosophila melanogaster* (2n = 8) and 23 in man (2n = 46).

CROSSING OVER

The term crossing over was first used by Morgan and Cattell in 1912. The exchange of precisely homologous segments between non-sister chromatids of homologous chromosomes is called crossing over.

Types of crossing over:

Depending upon the number of chiasmata involved, crossing over is of three types.

Single crossing over: It refers to the formation of single chiasma between non-sister chromatids of homologous chromosomes. It involves two linked genes (Two point test cross).

Double crossing over: It refers to the formation of two chiasmata between non-sister chromatids of homologous chromosomes. It involves three linked genes (Three point test cross).

Multiple crossing over: Occurrence of more than two crossing overs between non-sister chromatids of homologous chromosomes is known as multiple crossing over. However, the frequency of such type of crossing over is extremely low.

Mechanism of Meiotic crossing over:

It is responsible for recombination between linked genes and takes place during pachytene stage of meiosis i.e. after the homologous chromosomes have undergone pairing and before they begin to separate. It occurs through the process of breakage and reunion of chromatids. During pachytene, each chromosome of a bivalent (chromosome pair) has two chromatids so that each bivalent has four chromatids or strands (four-strand stage). Generally one chromatid from each of the two homologues of a bivalent is involved in crossing over. In this process, a segment of one of the chromatids becomes attached in place of the homologous segment of the nonsister chromatid and vice-versa. It is assumed that breaks occur at precisely homologous points in the two nonsister chromatids involved in crossing over; this is followed by reunion of the acentric segments. This produces a cross (x) like figure at the point of exchange of the chromatid segments. This figure is called chiasma (which is seen in diplotene stage of meiosis) (plural-chiasmata).

Obviously, each event of crossing over produces two recombinant chromatids (involved in the crossing over) called cross over chromatids and two original chromatids (not involved in crossing over) referred to as noncrossover chromatids. The crossover chromatids will have new combinations of the linked genes, i.e. will be recombinant; gametes carrying them will produce the recombinant phenotypes in test-crosses, which are called crossover types. Similarly, the noncrossover chromatids will contain the parental gene combinations and the gametes carrying them will give rise to the parental phenotypes or noncrossover types. Therefore the frequency of crossing over between two genes can be estimated as the frequency of recombinant progeny from a test-cross for these genes. This frequency is usually expressed as percent. Thus, the frequency of crossing over (%) can be calculated using the formula;

$$\text{Frequency of crossing over(\%)} = \frac{\text{No. of recombinant progeny from a test cross}}{\text{}} \times 100$$

Total number of progeny

Kinds of crossing over

Single cross over

- When only one chiasma occurs at one point of the chromosome pair is called single cross over.
- It produces two known cross over chromatid and two cross over chromatid.

Double cross over

- When the crossing over occurs at two point between any points in the same chromosome pair is called double crossing over.
- It produces 4 cross overs

Triple cross over

- When the cross over occur at 3 point between any 3 points in the same chromosome pair is called triple cross over.
- It produces 6 cross over.

Multiple cross over

- When crossing over occur at more than 3 or 4 point between any two or more points in the pair of chromosomes is called multiple cross over.
- It produces multiple cross over.

Factors effecting crossing over

1) High temperature to increase the frequency of crossing over.

2) X – ray

3) Age

4) Some genetical mutations decrease the frequency of crossing over.

5) Inversion of chromosome segments suppress the crossing over.

Theories about the mechanism of Crossing over

Important Theories That Can Explain the Mechanism of Crossing Over are listed below:

1. Janssen's partial chiasma type theory.

2. Belling's copy choice theory.

3. Darlington's breakage and reunion theory.

1. Breakage and reunion theory:

This theory is based on the assumptions that:

1. Prior to crossing over each chromosome of each bivalent get duplicated to form tetrad.

2. Crossing over occurs only between non-sister chromatids.

3. Crossing over involves the mechanical breaks in non-sister chromatids due to twisting around each other and reunion or recombination of chromatids take place.

According to this theory first of all, chromatids break and then form chiasmata. Crossing over does not produce chiasmata but it is caused by chiasmata.

2. Copy choice theory:

This theory was proposed by Belling in 1933. According to this theory, the paired chromosomes duplicate their genes before the fibres join them to form strands.

When the chromosomes are twisted around each other, reciprocal exchange of the chromatids take place during pachytene or just before.

There may be some recombination during the period of DNA synthesis affecting short, unpaired segments of the chromosomes.

A small part of new DNA helix being synthesized may copy a non-sister helix rather than a sister helix to produce recombination in a very short period.

There are two main objections:

1. Only two chromatids out of four involved in crossing over thus newly formed chromatids would be altered by recombination.

2. Duplication should occur during late meiotic prophase but now it is clear that DNA duplication occurs even before syn-apsis.

Hence, these theories assume that crossing over occurs in the interphase rather than pachytene.

3. Partial chiasma type theory:

According to this theory breaks occur only in two chromatids out of the four at the pachytene stage. These four again rejoined and the chiasmata are formed i.e. chiasmata is the result of cross-ing over.

Cytological detection of crossing over.

- The genetical detection of crossing over in Drosophila melanogaster we will know using their (f1 off springs) phenotype characters. But we unable to see under microscopic examination whether the crossing over occur or not, because the chromosomes are in homologous strains or condition.

- This crossing over or blocks had changed places cannot observed until visible marker may be incorporated on the chromosome.

Stern's experiment for cytological detection of cross over

1) Crossing over is the interchange of chromosome parts between homologous chromosome, and this crossing over is proved by Stern, in 1931, on Drosophila melanogaster.

2) The female Drosophila carries XX chromosome and the male Drosophila carries one X chromosome and one Y chromosome.

3) In a type of female Drosophila the two X chromosomes are different from each other.

4) An X chromosome has a piece of Y chromosome attach to it, the other X chromosome has been broken into two unequal segments and it is shorter than the unbroken X chromosome. Thus the two X chromosome are structurally different from the normal X chromosome.

5) In Drosophila red eye (C) is dominant and carnation eye (c) is recessive. Similarly bar eye (B) is dominant and round eye (b) is recessive.

6) The broken X chromosome contains a recessive gene (c) for carnation eye colour and a dominant eye (B) for bar eye, while it is homologous contains C & b.

7) This female having red bar eyes is crossed with a double recessive male, having carnation round eyes.

8) In the absence of crossing over only two types of female gamates are produced, one type having broken X chromosome containing c & B genes, the other type X chromosome having with a piece of Y chromosome attached and contain C & b genes.

9) If crossing over occurs two more type of gamates are produced. One type having C & B on a broken X chromosome with a piece of Y chromosome. So these 4 types of gamates after fertilization will produce 4 types of off spring that are,

- Carnation colour & bar shape eyes
- Red colour & round shape eyes
- Carnation colour & round shape eyes
- Red colour & bar shape eyes

10) The X chromosome of above said 4 types we can identified under the microscopic examination. This experiment proves that inter change of chromosomal material takes place between the homologous chromosomes.

Creighton & Mc Clintock's experiment

1) Creighton & Mc Clintock proved experimentally the exchange of chromatids during crossing over in maize.

2) They used to strains of maize which showed difference in the 9th chromosome. Because the 9th chromosome has knob at one end and a cell maker at the other end.

3) The other strain has no knob and no cell maker.

4) In additional to genetical characters are selected they are, colour of Kernal and nature of endosperm. Coloured kernel is dominant (C) & the colourless kernel is recessive (c).

5) Starchy endosperm (WX) is dominant and waxy endosperm (wx) is recessive.

6) A maize with knobbed chromosome, coloured kernel and waxy endosperm is crossed with another maize having knobless chromosome colourless kernel and starchy endosperm.

7) Hybrid maize having heterozygous chromosomes and heterozygous genotype are produced in the F1 generation.

8) The F1 hybrid is test crossed with a double recessive knobless chromosome.

9) The outcome F2 generations have examine genetically and cytologically.

10) The outcome F2 off springs have the following characterestics features.

- Knobbed coloured waxy
- Knobbed coloured starchy
- Knobless coloured waxy
- Knobless coloured starchy

11) This result shows the knobbed chromosomes were transformed in meosis process through the crossing over.

Significance of crossing over

a. It produces new individuals having new combinations of traits.

b. Crossing over has helped in establishing the concept of linear arrangement of genes.

c. The frequency of crossing over helps in the mapping of chromosomes. i.e., determining the location of the genes in the chromosomes.

d. Selection of useful recombination by geneticists has brought about green revolution in our country.

Gene Mapping

Among the main goals of the Human Genome Project (HGP) was to develop new, better and cheaper tools to identify new genes and to understand their function.

One of these tools is genetic mapping. Genetic mapping - also called linkage mapping - can offer firm evidence that a disease transmitted from parent to child is linked to one or more genes. Mapping also provides clues about which chromosome contains the gene and precisely where the gene lies on that chromosome.

Genetic maps have been used successfully to find the gene responsible for relatively rare, single-gene inherited disorders such as cystic fibrosis and Duchenne muscular dystrophy. Genetic maps are also useful in guiding scientists to the many genes that are believed to play a role in the development of more common disorders such as asthma, heart disease, diabetes, cancer, and psychiatric conditions.

How do researchers create a genetic map?

To produce a genetic map, researchers collect blood or tissue samples from members of families in which a certain disease or trait is prevalent. Using various laboratory techniques, the scientists isolate DNA from these samples and examine it for unique patterns that are seen only in family members who have the disease or trait. These characteristic patterns in the chemical bases that make up DNA are referred to as markers.

DNA markers don't, by themselves, identify the gene responsible for the disease or trait; but they can tell researchers roughly where the gene is on the chromosome.

This is why: when eggs or sperm develop, the paired chromosomes that make up a person's genome exchange stretches of DNA. Think of it as a shuffling process, called recombination. The single

chromosome in a reproductive cell contains some stretches of DNA inherited from the person's mother and some from his or her father.

If a particular gene is close to a DNA marker, the gene and marker will likely stay together during the recombination process, and they will likely be passed on together from parent to child. If each family member with a particular disease or trait also inherits a particular DNA marker, it is very likely that the gene responsible for the disease lies near that marker.

The more DNA markers there are on a genetic map, the more likely it is that at least one marker will be located close to a disease gene-and the easier it will be for researchers to zero in on that gene. One of the first major achievements of the HGP was to develop dense maps of markers spaced evenly across the entire human genome.

What are genetic markers?

Markers themselves usually consist of DNA that does not contain a gene. But because markers can help a researcher locate a disease-causing gene, they are extremely valuable for tracking inheritance of traits through generations of a family.

The development of easy-to-use genetic maps, coupled with the HGP's successful sequencing of the entire human genome, has greatly advanced genetics research. The improved quality of genetic data has reduced the time required to identify a gene from a period of years to, in many cases, a matter of months or even weeks.

Genetic mapping data generated by the HGP's laboratories is freely accessible to scientists through databases maintained by the National Institutes of Health and the National Library of Medicine's National Center for Biotechnology Information (NCBI) [ncbi.nlm.nih.gov], as well as the Genome Browser of University of California, Santa Cruz.

Mutation

- Mutation is the change in sequence of nucleotide of DNA.
- Change in sequence of nucleotide brings sudden change in morphological characteristics of an organism. If such change are heritable, then it is called as mutation.
- So, mutation is defined as any heritable change in the sequence of nucleotide of DNA.

Organism with mutation is called mutant while the organism without mutation is wild type.

Types of Mutation

Based on change in genotype and phenotype, mutation are of two types

1. Point mutation
2. Frameshift mutation

1. Point mutation

- It occurs as a result of replacement of one nucleotide by other in specific nucleotide sequence of gene. Point mutation brings little phenotypic change as compared to frameshift mutation.

Point mutation are two types based on the base pair substitution

i) Translation:

- It is the point mutation occur by substitution of one purine by another purine or one pyrimidine by another pyrimidine.

ii) Transversion:

- It is the point mutation occur by substitution of purine by pyrimidine and vice versa.

Based on transcriptional property point mutation are of three types.

i) Silent mutation

ii) Missense mutation

iii) Non-sense mutation

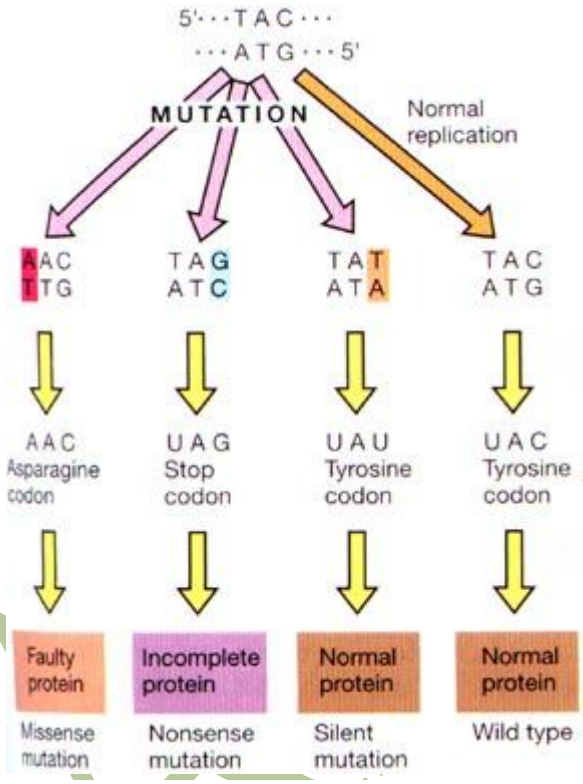


Figure: types of point mutation

i) Silent mutation:

- It is also known as neutral mutation.
- It is the mutation in which mutated codon codes same amino acids as the original codon. Since the aminoacid is same as original one, it does not effects the structure and composition of protein.
- Silent mutation causes phenotype of bacteria remain similar to that of wild type.

ii) Missense mutation:

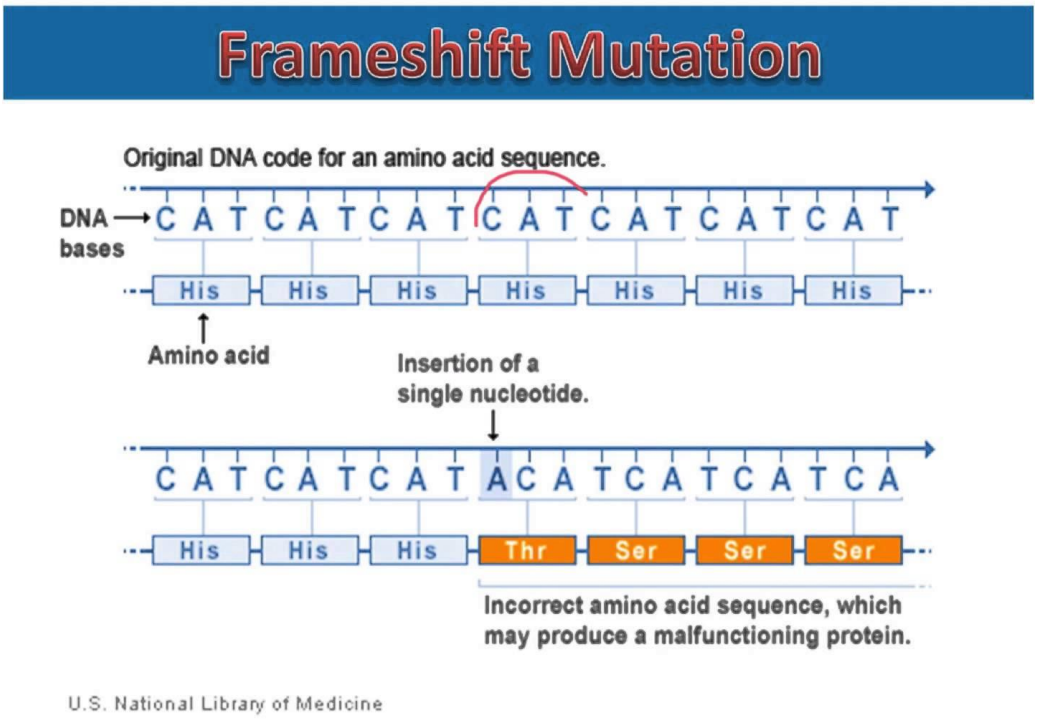
- In this mutation mutated codon codes different amino acid (other than original). Since new aminoacid coded by mutated codon is altered, the protein formed from it is also altered. Such protein can be less active or completely inactive.
- If altered aminoacids lie on active site of protein then such protein become completely non-functional.
- The missense mutation causes phenotypic change in organism.

iii) Non sense mutation:

- Mutation in which altered codon is stop codon or chain terminating codon, such mutation is called non-sense mutation.
- Non sense mutation causes incomplete synthesis. Such incomplete protein is always non-functional.
- Non-sense mutation bring greatest change in phenotype of an organism.

2. Frameshift mutation

- It occurs as a result of addition or deletion of nucleotide in the sequence of DNA. Addition or deletion of nucleotide causes shift of the reading frame of mRNA.
- In a mRNA each codon is represented by three bases without punctuation and insertion or deletion of a nucleotide changes the entire frame. So frame shift mutation bring greater phenotypic change than point mutation.
- Insertion or deletion of one or two base pair of nucleotide causes shift in frame. However, insertion or deletion of three base pair adds or remove a whole codon, this results in addition of removal of single amino acid from polypeptide chain.



DNA repair mechanism

There are three major DNA repairing mechanisms: base excision, nucleotide excision and mismatch repair.

Table -. Proteins involved in the DNA repairing of E. coli.

Repair System	Enzymes/proteins	Repair System	Enzymes/proteins
Base excision	DNA glycosylase	Mismatch	Dam methylase
	AP endonuclease		MutS, MutL, MutH
	DNA polymerase I		Exonuclease
	DNA ligase		DNA helicase II
Nucleotide excision	Uvr-A, Uvr-B, Uvr-C		SSB protein
	DNA polymerase I		DNA polymerase III
	DNA ligase		DNA ligase

Base excision

DNA's bases may be modified by deamination or alkylation. The position of the modified (damaged) base is called the "abasic site" or "AP site". In E.coli, the DNA glycosylase can recognize the AP site and remove its base. Then, the AP endonuclease removes the AP site and neighboring nucleotides. The gap is filled by DNA polymerase I and DNA ligase.

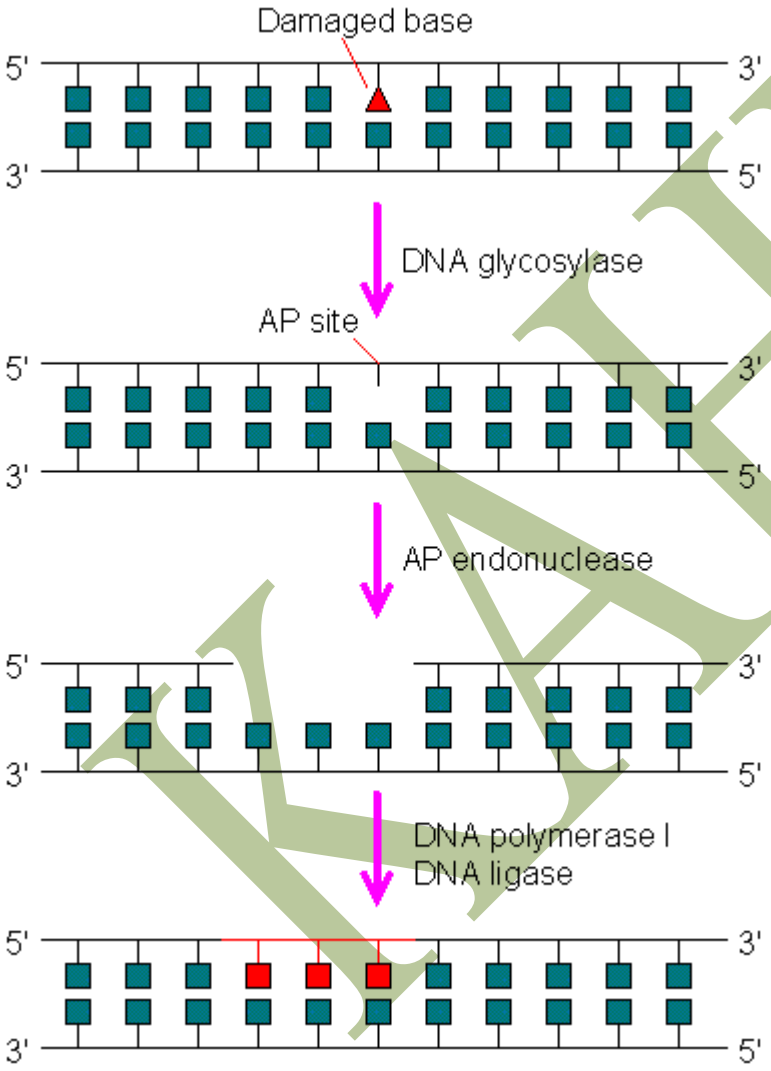


Figure -. DNA repair by base excision.

Nucleotide excision

In E. coli, proteins UvrA, UvrB, and UvrC are involved in removing the damaged nucleotides (e.g., the dimer induced by UV light). The gap is then filled by DNA polymerase I and DNA ligase. In yeast, the proteins similar to Uvr's are named RADxx ("RAD" stands for "radiation"), such as RAD3, RAD10. etc.

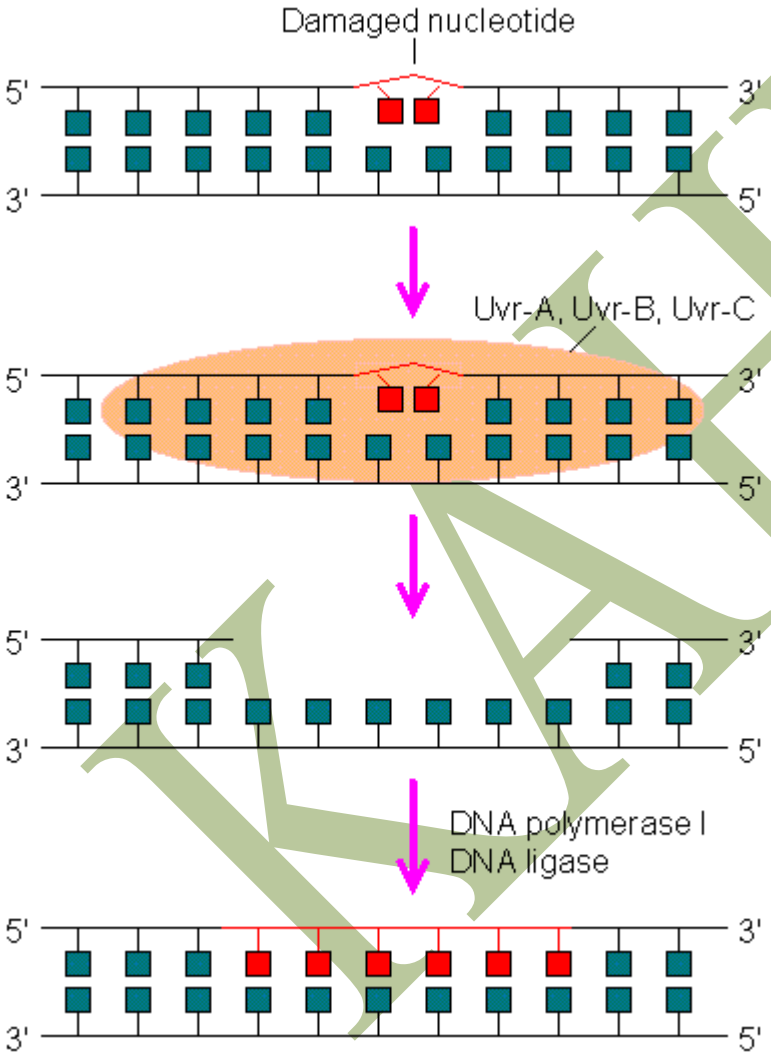


Figure -. DNA repair by nucleotide excision.

Mismatch repair

To repair mismatched bases, the system has to know which base is the correct one. In E. coli, this is achieved by a special methylase called the "Dam methylase", which can methylate all adenines that occur within (5')GATC sequences. Immediately after DNA replication, the template strand has been methylated, but the newly synthesized strand is not methylated yet. Thus, the template strand and the new strand can be distinguished.

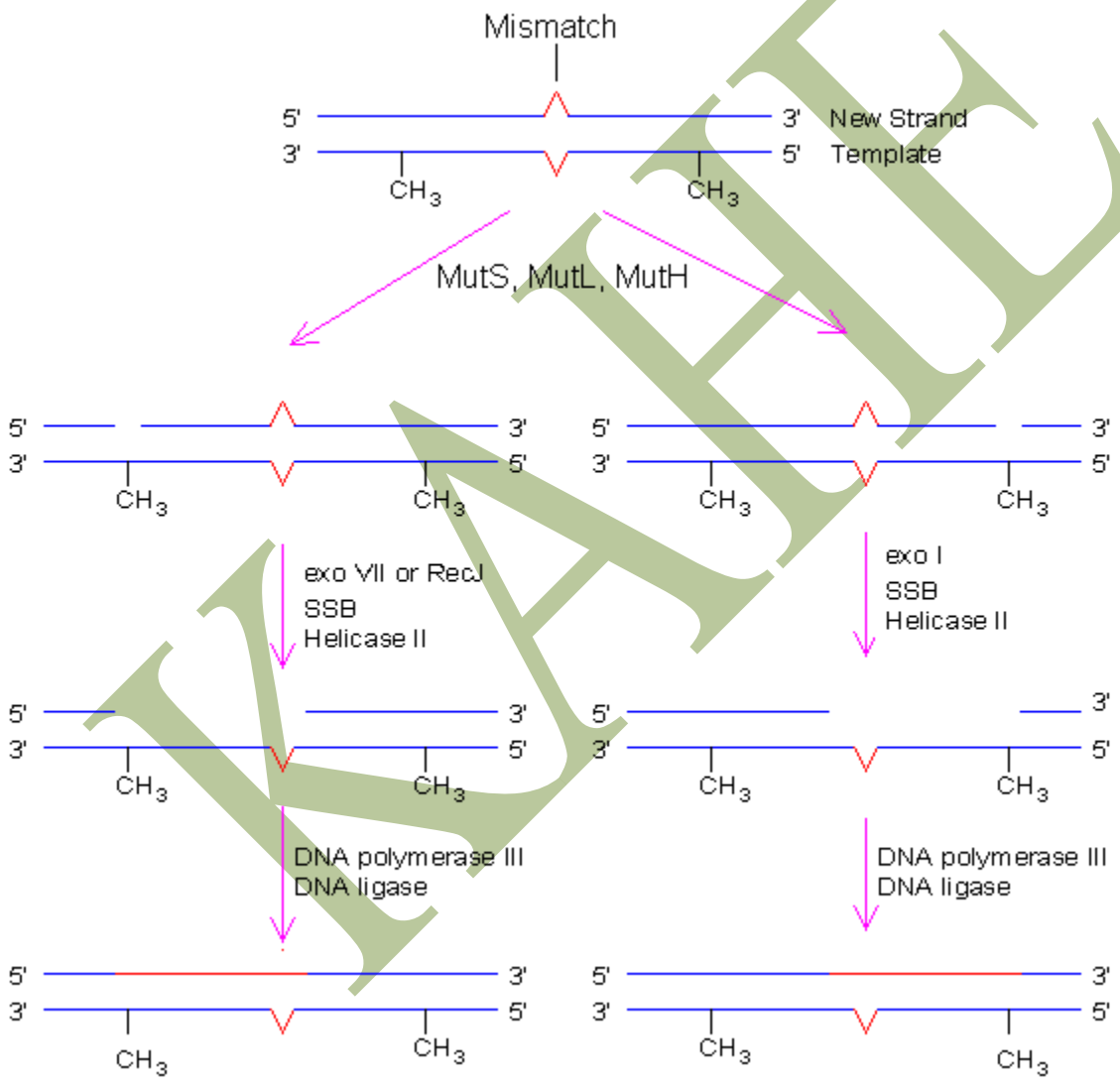


Figure -. Mismatch repair.

The repairing process begins with the protein MutS which binds to mismatched base pairs. Then, MutL is recruited to the complex and activates MutH which binds to GATC sequences. Activation of MutH cleaves the unmethylated strand at the GATC site. Subsequently, the segment from the cleavage site to the mismatch is removed by exonuclease (with assistance from helicase II and SSB proteins). If the cleavage occurs on the 3' side of the mismatch, this step is carried out by exonuclease I (which degrades a single strand only in the 3' to 5' direction). If the cleavage occurs on the 5' side of the mismatch, exonuclease VII or RecJ is used to degrade the single stranded DNA. The gap is filled by DNA polymerase III and DNA ligase.

The distance between the GATC site and the mismatch could be as long as 1,000 base pairs. Therefore, mismatch repair is very expensive and inefficient.

Mismatch repair in eukaryotes may be similar to that in E. coli. Homologs of MutS and MutL have been identified in yeast, mammals, and other eukaryotes. MSH1 to MSH5 are homologous to MutS; MLH1, PMS1 and PMS2 are homologous to MutL. Mutations of MSH2, PMS1 and PMS2 are related to colon cancer.

In eukaryotes, the mechanism to distinguish the template strand from the new strand is still unclear.

Review Questions

Short Answer Questions **(2 Marks)**

1. Define genetics
2. List out the characters selected by Mendel in pea plant.
3. Define allele..
4. Define co-dominance.
5. Explain briefly law of independent assortment.
6. List out the role of DNA repair mechanism..
7. Define gene linkage
8. What is crossing over.

Essay Answer Questions **(6 & 8 Marks)**

1. Describe about non mendelian principles.
2. Describe in detail crossing over.
3. Give a detailed account on gene linkage.
4. Describe in detail about gene mapping.
5. Explain about Mutation and types.
6. Give a detailed note on DNA repair mechanism.
7. Discuss in detail about gene recombination.

Unit IV

SYLLABUS

Methods of genetic transfers – transformation, conjugation, transduction. mapping genes by interrupted mating, Linkage maps, tetrad analysis, mapping with molecular markers, mapping by using somatic cell hybrids. Introduction to Transposable elements – Discovery and types, Nomenclature - Insertion sequences - Mechanism – Transposons of E. coli, Bacteriophage and Yeast.

Bacteria divide very rapidly. The doubling time is also called generation time and it may be as low as 20 minutes. Bacteria mainly reproduce by asexual reproduction but do not exhibit true sexual reproduction as they do not produce diploid phase. Thus, meiosis is lacking. However, bacteria exchange genetic material between two cells.

Modes of genetic transfer in bacteria:

Three modes of genetic transfer between bacterial cells are:

(a) Transformation

(b) Transduction

(c) Conjugation

Transformation

- Recipient cell uptake free DNA released into the environment.
- DNA is released it to the environment when another bacterial cell (i.e. donor) dies and undergoes lysis
- Not all bacteria are able to go for transformation, only some bacteria are able to take free DNA and are able to go transformation. These type of bacterial are called competent bacteria.

- Pathogenic bacteria showing competence
  - *Haemophilus* spp
  - *Streptococcus* spp
  - *Neisseria* spp

Transduction

Transduction is the transfer of genetic information from a donor to a recipient by way of a bacteriophage. The phage coat protects the DNA in the environment so that transduction, unlike transformation, is not affected by nucleases in the environment. Not all phages can mediate transduction. In most cases gene transfer is between members of the same bacterial species. However, if a particular phage has a wide host range then transfer between species can occur. The ability of a phage to mediated transduction is related to the life cycle of the phage.

Types of Transduction

1. Generalized Transduction – Generalized transduction is transduction in which potentially any bacterial gene from the donor can be transferred to the recipient. The mechanism of generalized transduction is illustrated in Figure.
2. Specialized transduction – Specialized transduction is transduction in which only certain donor genes can be transferred to the recipient. Different phages may transfer different genes but an individual phage can only transfer certain genes. Specialized transduction is mediated by lysogenic or temperate phage and the genes that get transferred will depend on where the prophage has inserted in the chromosome. The mechanism of specialized transduction is illustrated in Figure 4.Phages that mediate generalized transduction generally breakdown host DNA into smaller pieces and package their DNA into the phage particle by a "head-full" mechanism. Occasionally one of the pieces of host DNA is randomly packaged into a phage coat. Thus, any donor gene can be potentially transferred but only enough DNA as can fit into a phage head can be transferred. If a recipient cell is infected by a phage that contains donor

DNA, donor DNA enters the recipient. In the recipient a generalized recombination event can occur which substitutes the donor DNA and recipient DNA.

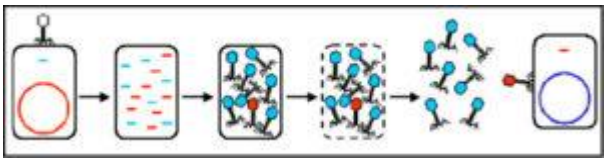


FIG. THE MECHANISM OF GENERALIZED TRANSDUCTION

During excision of the prophage, occasionally an error occurs where some of the host DNA is excised with the phage DNA. Only host DNA on either side of where the prophage has inserted can be transferred (*i.e.* specialized transduction). After replication and release of phage and infection of a recipient, lysogenization of recipient can occur resulting in the stable transfer of donor genes. The recipient will now have two copies of the gene(s) that were transferred. Legitimate recombination between the donor and recipient genes is also possible.

Significance

Lysogenic (phage) conversion occurs in nature and is the source of virulent strains of bacteria.

Conjugation:

The unidirectional transfer of DNA from one cell to another through a cytoplasmic bridge is called conjugation. The process is equivalent to sexual mating in eukaryotes. Two bacterial haploid cells of different strains come close to each other.

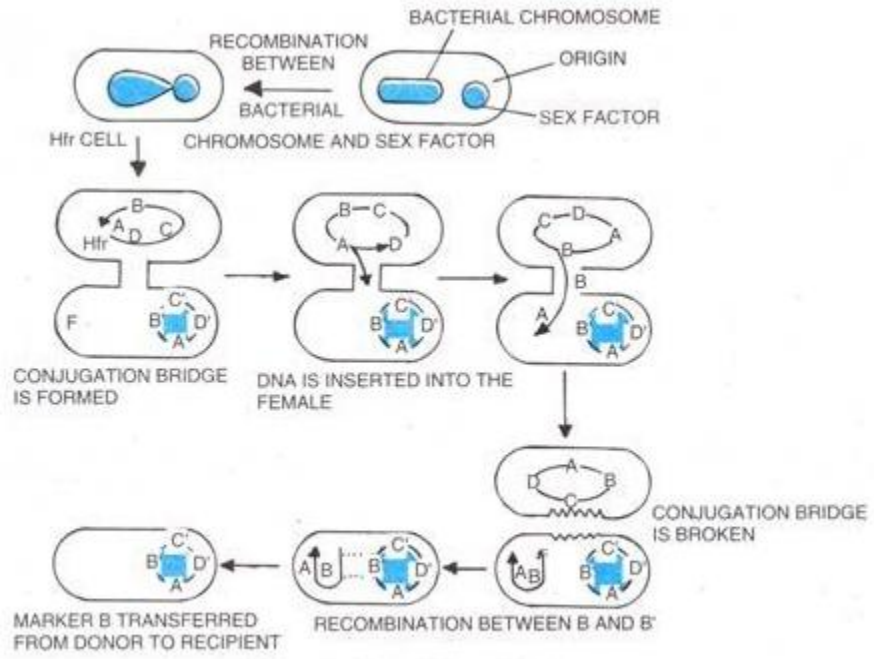
They recognise each other by complementary macromolecules borne on their surface. Donor or male cell passes part or whole of the chromosome into recipient or female cell. The ability of transferring the genetic material from male is controlled by sex or fertility factor (F gene) present in a plasmid.

Thus, genes can be transferred from donor to recipient cell on a molecule of DNA which acts as sex factor called F gene. This sex gene can reside in a bacterial chromosome or it may exist as an autonomous unit in cytoplasm.

Male bacterium with thorn-like protuberances called as sex pili come in contact with female bacterium which lacks pili and donate its DNA. F factor (a plasmid) carries genes for producing pili

and other functions required to transfer DNA. At times F factor integrates into bacterial chromosome.

Such bacteria can transfer their genetic material into female cell with high frequency (Hfr) in a particular sequence. They are called as Hfr -strains. Conjugation was first demonstrated by Lederberg and Tatum in E. coli. The frequency of recombination was very low in Lederberg's experiments.



Bacterial conjugation.

The Hfr cell acts as the male bacterium and when mixed with the female (F—) cell forms a conjugation bridge. The F factor containing DNA breaks at a particular point and starts inserting the DNA into the female and the sequence of chromosomal gene transfer is always in the same order (A, B, C and D genes).

The F factor is transferred last. The conjugation bridge usually breaks before the entire chromosome is transferred. Only the genes A and B have been transferred in the example given. These A and/or B genes can recombine with the corresponding genes in the F— chromosome.

Thus, if B' in the F— cell is a mutated form of B, theft the B' in the F— chromosome can become B as a result of recombination after conjugation. Thus, genetic markers can be transferred from a host to a suitable recipient lacking such markers.

The order in which such markers are transferred to the recipient would follow the order in which they are present in the donor. Thus, conjugation experiments are useful in constructing the gene maps (order of arrangement of genes in the chromosome) of organisms.

Hayes (1952) found a strain of E. coli in which the frequency of recombination was as high as 100 to 1000 times as reported by Lederberg. The strain was called high frequency recombinant (Hfr) strain.

Tetrad Analysis

The meiotic products of ascomycetes (occasionally some other organisms) stay together as the four products of single meiosis, as a *tetrad*. In some organisms, tetrad formation is followed by a post-meiotic mitosis within the ascus, resulting in spore *octads*. If the four spores are situated in the same linear order as produced by the two divisions of meiosis it is an *ordered tetrad*.

In the ordered tetrad, considering two genes *A* and *B*, three arrangements of the spores (parental ditype [PD], tetratype [TT], non-parental ditype [NPD]) can be distinguished as seen in the Figure . The parental ditype (PD) indicates no crossing over; tetratype (TT) reveals one recombination between the two genes and the second division segregation of the B/b alleles reveals recombination between the B/b gene and the centromere (see Fig.).

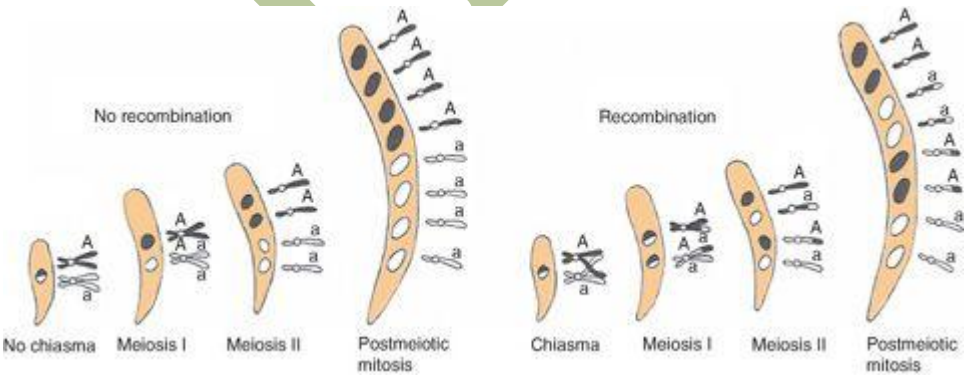


Figure .Spore tetrads and octads without and with recombination

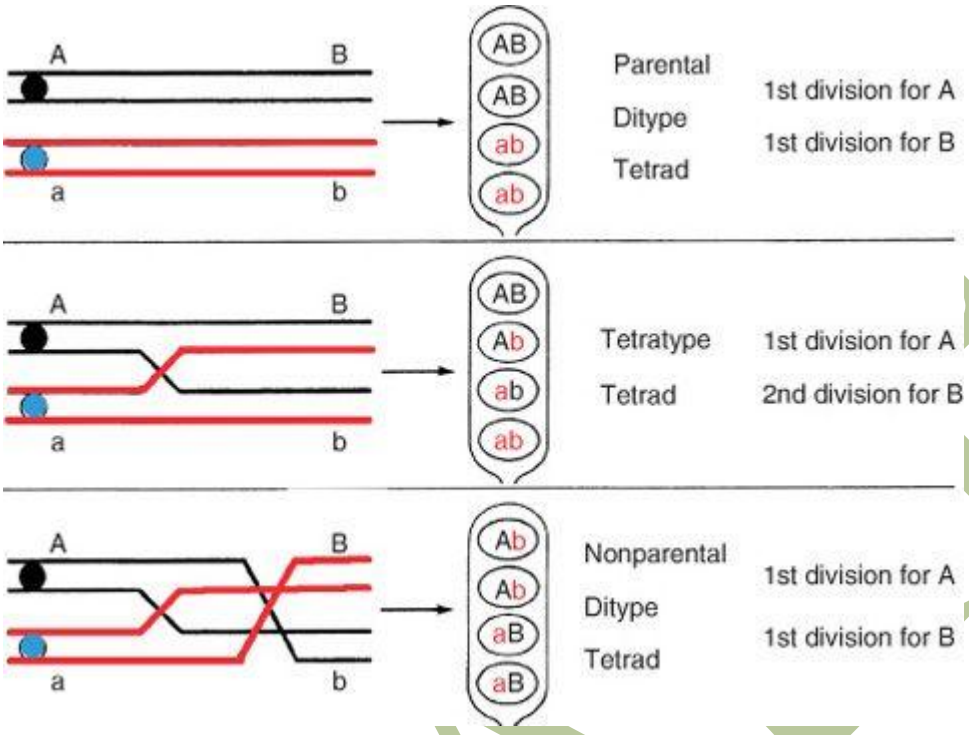


Figure Transactions and results examplified by two gene loci in an ordered tetrad. Gene *A* is so close to the centromere that practically no recombination occurs between them. (Diagram after Barratt RW et al 1954 Adv Genet 6:1)

The nonparental ditype (NPD) is an indication of double crossing over between the two gene loci. The PD, TT, and NPD may appear even if the genes are in separate chromosomes. An excess of PD over NPD is an indication of linkage. If the deviation from the 1:1 ratio between PD and NPD is small, a *chi square test* may be used to test the probability of linkage by the formula: $\chi^2 = (PD - NPD)^2/(PD + NPD)$.

By counting the number of tetrads of the above three types, *recombination frequency between the two loci* can be calculated as [1/2]TT+all NPDall tetrads,[1/2]TT+all NPDall tetrads, and recombination frequency between the B/b *gene and the centromere can be calculated* as TT[1/2]all tetrads.TT[1/2]all tetrads. (Dr. Fred Sherman recommended

to me to use for the gene-gene map distance the formula of Dr. David Perkins [Genetics 34:607], i.e. 100{[0.5(TT) + 6NPD]/[PD + TT + NPD]}.)

The recombination frequencies (if they are under 0.15) multiplied by 100 provide the map distances in centiMorgans. If the recombination frequencies are larger, mapping functions should be used. From the genetic constitution of the tetrads, a great deal of information can be revealed about recombination. When the four meiotic products are not in the order brought about by meiosis, the tetrad is *unordered*. For the estimation of gene–centromere distances from unordered tetrad data, one must rely on three markers, from which no more than two are linked, and algebraic solutions are required (e.g., Whitehouse 1950 Nature 165:893, see unordered tetrads). Tetrad analysis is most commonly used in ascomycetes (*Neurospora*, *Aspergillus*, *Ascobolus*, *Saccharomyces*, etc.) (see Fig.T37) yet it can be applied to higher plants where the four products of male meiosis stick together (*Elodea*, *Salpiglossis*, orchids, *Arabidopsis* mutants). Using transgene constructs encoding pollen-expressed fluorescent proteins of three different colors in the *qrt1* mutant, which retains pollen in the tetrad stage, segregation of the fluorescent alleles in 92,489 pollen tetrads could be observed (figure). Correlation between developmental position and crossover frequency, temperature dependence for crossingover frequency, meiotic gene conversion, as well as interference were detectable (Francis KE et al 2007 Proc Natl Acad Sci USA 104:3913). In *Drosophila* with attached X-chromosomes half-tetrad analysis is feasible. Since several genomes of higher eukaryotes have been sequenced, molecular markers are available for tetrad analysis for the cases when the products of individual meioses can be identified. unordered tetrads, half-tetrad analysis, meiosis, mapping, linkage, mapping function, four-point analysis of tetrads

Figure - *Neurospora* octads. (Courtesy of Dr. David Stadler)

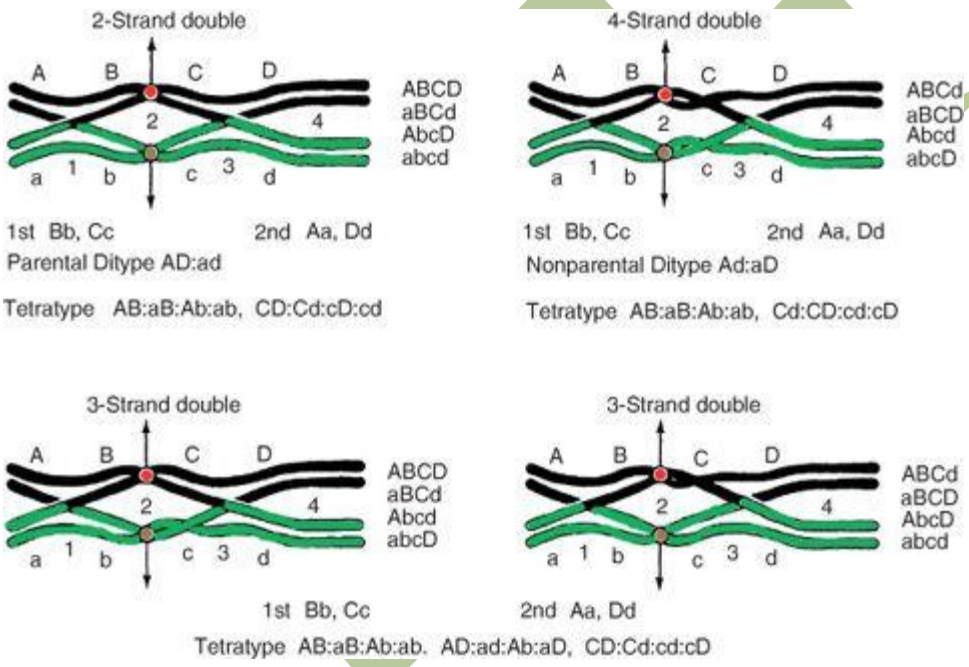


Figure - Four-point cross with genes in both arms of the chromosomes. It is a five-point cross if we consider the centromere as a genetic marker. From the spore order we can determine even if the chromatids rotated 180° after the exchange. (After Emerson S 1963 p 167.

Interrupted Mapping

The Interrupted Mating Experiment technique with bacterial cell was worked out by two French geneticists, François Jacob and Elie Wollman in the late 1950's. They were trying to demonstrate the mechanisms of gene transfer using *Escherichia Coli*.

This technique enable scientist to map, for the first time, any genome longer than that of a phage or a virus.

In their experiment, Jacob and Wollman mated an *Hfr* donor cell (which contains the *F* factor integrated into the main bacterial chromosome) with an F^- recipient cell, following the procedures of the conjugation experiment performed earlier by Hershey and Chase.

The genotypes of the two *E. coli* strains involved was:

Donor – HfrH: $thr^+\ leu^+\ azi^r\ ton^r\ lac^+\ gal^+\ str^s$

Recipient – F^-: $thr^-\ leu^-\ azi^s\ ton^s\ lac^-\ gal^-\ str^r$

(The superscripts *"s"* mean sensitive to, *"r"* resistant to, *"+"* able to synthesise or metabolise the compound, and *"-"* unable to synthesise or metabolise that compound).

The *Hfr* strain used was the *E. coli HfrH* (where H strands for Hayes, another scientist who had an important role in the discovery of the bacterial mating mechanism)

This strain was prototrophic (wild type strains that are able to synthesise all the essential nutriments) and sensitive to the streptomycin antibiotic. The F^- strain carries the gene for streptomycin resistance and a number of mutant genes, which cause it to be auxotrophic for threonine (thr^-) and leucine (leu^-), sensitive to sodium azid (azi^s) and to infection by bacteriophages T_1 (ton^s), and unable to ferment lactose (lac^-) and galactose (gal^-).

The two strains we mixed in nutrient medium and incubated at 37(C to allow conjugation to start.

In the beginning of conjugation, the integrated *F* factor is nicked at the origin and replication takes place by the rolling circle mechanism. The first genes to be transferred are those of the *F* factor. The bacterial genes close to the site of plasmid insertion, can also be sequentially transferred to the recipient cell if the conjugation process lasts long enough.

The donor and recipient cells alls are physically linked through a sex pili, which is synthesized by the donor cell. The pili is a very fragile structure and break easily. While the bacteria conjugate they jiggle around in a natural Brownian motion, which put the pili under physical stress and breaks it. This is why in nature only an average of 25-30% of a bacterial chromosome is transferred to the recipient cell.

The experimental design of Jacob and Wollman involved the use of a kitchen blender* to break the matting cell apart at various times after the beginning of conjugation. This stopped the transfer of DNA. The longer the genetic transfer was allowed to take place, the more genes were transferred. The genes that are passed to the recipient cell become incorporated into the main bacterial chromosome by two crossover events. The resulting recombinants are partially diploid. This means, that they are diploid for the genes that were transferred from the donor cell and haploid for all other genes.

Once the transfer was stopped the cells were removed from the mating mixture and then were plated on a selective medium, specially conceived to allow only the growth and division of the recombinant cells. The *HfrH* and F^- cell should not be able to grow. In this particular case the medium contained streptomycin that killed the *HfrH* cells and lacked threonine so the F^- cells could not grow. Other appropriate media were used to test the appearance of certain donor genes among the selected thr^+ leu^+ str^r transconjugants.

In this experiment the selected marques were thr^+ leu^+ str^r and the azi^r ton^r lac^+ and gal^+ genes were the unselective markers. The time of transfer of the first selected genes thr^+ and leu^+ was defined as time zero (measured in minutes).

The data collected from this experiment is shown in figure1.

From these results it is possible to determine the order of transfer of the unselected gene markers as a function of time. The first gene to be transferred was the one for azide resistance (azi^r), which is the result of a mutation in the gene *sec A* that is normally involved in protein secretion. This gene appeared at about 8 minutes.

The second gene to be transferred, the ton^r appeared at 10 minutes. The resistance to bacteriophages T_1 is determined by a mutation in the *fhuA* gene which codes for the outer membrane receptor for ferrichrome, *colicin M* and phages T_1, T_5 and *phi80*.

At about 17 minutes the lac^+ gene was transferred followed by the gal^+ at approximately 25 minutes. These two genes code for the lactose and galactose metabolisms respectively.

From the analysis of the appearance rates of each gene, which are indicated by the slope of the curves, and the maximum frequencies obtained for each recombinant type (the height of the plateau) it is possible to conclude that:

- As the conjugation time increases, the rate of appearance and the maximum frequencies of recombinant decrease.
- The rate of transfer from one mating couple to another is not constant because cells are not synchronised, that is they do not initiate DNA transfer all at the same time.

- The later the gene enters the recipient cell, the smaller is the maximum frequency of recombinants because the probability of the mating cells breaking apart as the result of the Brownian motions increases with time.

The time intervals between the appearance of each gene is used to determine the distance between them (the distances being measured in minutes).

From this information we can conclude that gene transfer occurs in a linear way, and that the genes that are far from the origin tend not to be transferred to recipient cell because of the higher probability that be mating pair will break apart before their transfer can take place. So being the *F*⁻ cell only very rarely receives the entire *F* factor (part of which is at the other end of the bacterial chromosome), thus becoming an *Hfr* cell.

Mapping with molecular markers

In the first 70 years of building genetic maps, the markers on the maps were genes with variant alleles producing detectably different phenotypes. As organisms became more and more researched, large numbers of such genes could be used as markers on the maps. However, even in those orga-nisms in which the maps appeared to be "full" of loci of known phenotypic effect, measurements showed that the chromosomal intervals between genes had to contain vast amounts of DNA. These gaps could not be mapped by linkage analysis, because there were no markers in those regions. What was needed were large numbers of additional genetic markers that could be used to fill in the gaps to provide a higher-resolution map. This need was met by the discovery of various kinds of molecular markers. Amolecular marker is a site of heterozygosity for some type of silent DNA variation not associated with any measurable phenotypic variation. Such a "DNA locus," when heterozygous, can be used in mapping analysis just as a conventional heterozygous allele pair can be used. Because molecular markers can be easily detected and are so numerous in a genome, when they are mapped by linkage analysis, they fill the voids between genes of known phenotype. Note that, in mapping, the biological significance of the DNA marker is not important in itself; the heterozygous site is merely a convenient reference point that will be useful in finding one's way around the chromosomes. In this way, markers are being used just as milestones were used by travelers in previous

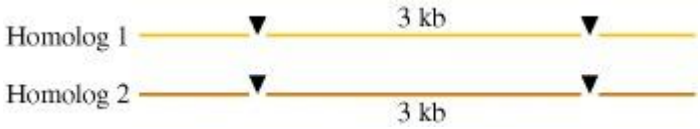
centuries. Travelers were not interested in the milestones (markers) themselves, but they would have been disoriented without them.

The two basic types of molecular markers are those based on restriction-site variation and on repetitive DNA.

Use of restriction fragment length polymorphisms in mapping

Bacterial restriction enzymes cut DNA at specific target sequences that exist by chance in the DNA of other orga-nisms. Generally, the target sites are found in the same position in the DNA of different individuals in a population; that is, in the DNA of homologous chromosomes. However, quite commonly, a specific site might be missing as a result of some silent mutation. The mutation might be within a gene or in a noncoding intergenic area. If an individual is heterozygous for presence and absence (+/−), that locus can be used in mapping. The +/− sites are found by Southern analyses using a probe derived from DNA of that region. A typical example follows:

On a Southern hybridization of such an individual, the probe would highlight three fragments, of size 3, 2, and 1 kb. Another individual might be homozygous for the long fragment and show only a 3-kb band in the Southern hybridization.



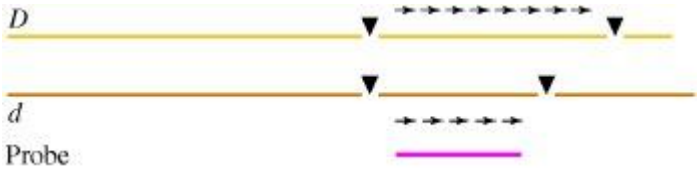
These multiple forms of this region constitute a restriction fragment length polymorphism (RFLP).

In a cross of the aforedescribed two individuals, half the progeny would show three fragments when probed and the other half only one fragment, following Mendel's law of equal segregation just as a gene would. Hence an RFLP can be mapped and treated just like any other chromosomal site. The following situation shows linkage of the heterozygous RFLP in our example to a heterozygous gene, with *D* in coupling conformation with the 1 plus 2 morph:

Crossovers between these sites would produce recombinant products that are detectable as *D*–3 and *d*–2–1. In this way, the RFLP locus can be mapped in relation to genes or to other molecular markers.

Use of VNTR

The number of repeated units in a tandem array is variable. The mechanisms for producing this variation need not concern us at present. The important fact is that individuals that are heterozygous for different numbers of tandem repeats can be detected, and the heterozygous site can be used as a marker in mapping. A probe that binds to the repetitive DNA is needed. The following example uses restriction enzyme target sites that are outside the repetitive array. The basic unit of the array is shown as an arrow.

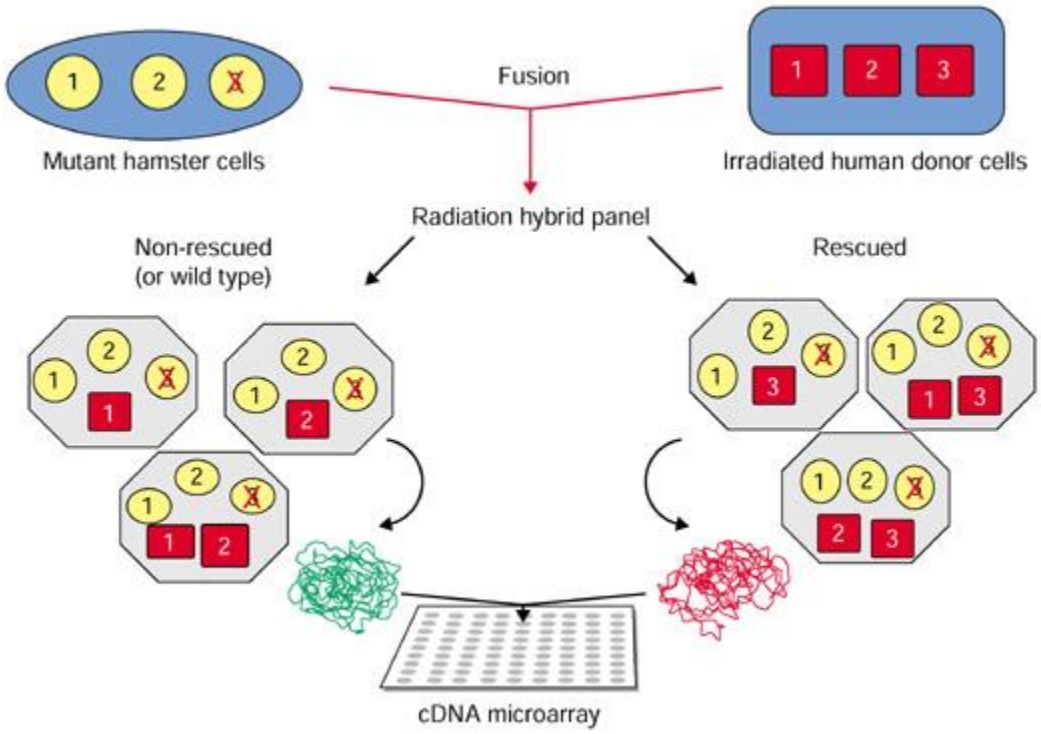


This VNTR locus will form two bands, one long and one short, on a Southern hybridization autoradiogram. Once again, this heterozygous site can be used in mapping just as the RFLP locus was.

Mapping genes with somatic cells

Mutant cell lines with a phenotype of interest are fused to irradiated wild-type donor cells by somatic cell fusion to generate a pool of radiation hybrids. Each radiation hybrid cell contains a full complement of the mutant genome and random fragments of the donor genome. The hybrids are selected for reconstitution (complementation) of their wild-type phenotype. Hybrid cells that survive the selection should all retain the chromosome fragment that bears the complementing gene(s), presumably the wild-type counterpart of the mutant gene, provided by the donor genome (Figure (Figure1).1). The genomic DNA from the rescued and the wild-type CHO populations are harvested and used as test and reference probes, respectively, in a comparative fluorescent hybridization to a DNA microarray. The DNA microarray contains human DNA sequences with known genomic positions. The preferential retention of the DNA fragments encompassing the gene of interest generates increased fluorescence ratios for the corresponding genes or expressed sequence tag (EST) elements on the array. The fluorescence ratios measured at each element are plotted along each chromosome on the basis of their positions in the genome. The position of the

wild-type gene is thus revealed by the clustering of increased fluorescence ratios in the region encompassing the complementing gene.



Figure

Schematic diagram of the radiation hybrid array-CGH methodology. Mutant rodent cells that have a recessive phenotype due to a mutation in a gene (gene 3, depicted as a red cross), are complemented by cell fusion with irradiated human donor cell lines. The resulting cells in the radiation-hybrid panel contain a full complement of the hamster genome as well as fragments of the human genome. Hybrids are complemented when they have retained the functional human chromosome fragment bearing the rescuing gene (in this case gene 3), resulting in restoration of the wild-type phenotype. Hybrids that were not complemented retain only random fragments of the donor genome (for example genes 1 and 2). The complemented and the non-complemented populations are separated by selection, resulting in enrichment of gene 3 in the complemented cells, which are used as the source of the test DNA probes (versus wild-type CHO DNA) on the array.

We first evaluated the feasibility of this methodology by analyzing a monochromosomal human-hamster hybrid cell line containing the human X chromosome. Test genomic DNA from the hybrid cell line and reference genomic DNA from CHO cells were labeled with Cy-5 (red) and Cy-3 (green) respectively, and the labeled DNAs were co-hybridized to a microarray comprising approximately 40,000 human cDNAs. Fluorescence ratios for the genes along the X chromosome were elevated (mean fluorescence ratio 1.9), reflecting DNA copy-number gain for X-chromosome-specific genes (Figure (Figure2).2). No significant elevations were observed along the other chromosomes (mean fluorescence ratio 0.93), indicating no significant gain of DNA copy number elsewhere in the genome. Thus, the method identified the presence of the human X chromosome in a monochromosomal human-hamster hybrid cell line.

Transposable Elements

Presence of transposable elements was first predicted by Barbara McClintock in maize (corn) in late 1940s. After several careful studies, she found that certain genetic elements were moving from one site to an entirely different site in the chromosome. She called this phenomenon of changing sites of genetic elements as transposition and those genetic elements were called by her as controlling elements.

These controlling elements were later on called as transposable elements by Alexander Brink. In late 1960s this phenomenon was also discovered in bacteria.

Consequently, the molecular biologists called them as Transposons. A transposon may be defined as: “a DNA sequence that is able to move or insert itself at a new location in the genome.” The phenomenon of movement of a transposon to a new site in the genome is referred to as transposition.

Transposons are found to encode a special protein named as transposase which catalyses the process of transposition. Transposons are particular to different groups of organisms. They constitute a fairly accountable fraction of genome of organisms like fungi, bacteria, plants, animals and humans. Transposons have had a major impact on changing or altering the genetic composition of organisms.

Transposons or transposable genetic elements are often referred to as 'mobile genetic elements' also. They can be categorized on different bases like their mode of transposition or on the basis of the organisms in which they are present.

Types of Transposons:

Different transposons may change their sites by following different transposition mechanisms.

(i) Cut-and-Paste Transposons:

They transpose by excision (cutting) of the transposable sequence from one position in the genome and its insertion (pasting) to another position within the genome (Fig. 1).

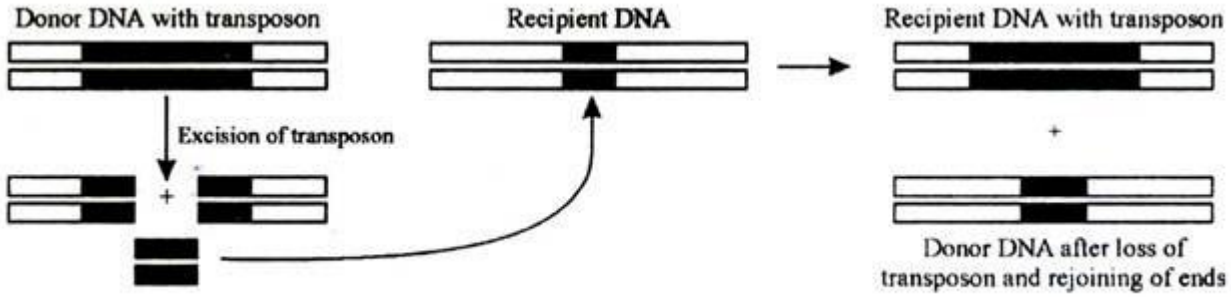


Fig. 1. Cut and Paste Transposons.

The cut-and-paste transposition involves two transposase subunits. Each transposase submit binds to the specific sequences at the two ends of transposon. These subunits of transposase protein then come together and lead to the excision of transposon.

This excised 'transposon-Transposase Complex' then gets integrated to the target recipient site. In this manner, the transposon is cut from one site and then pasted on other site by a mechanism mediated by transposase protein (Fig. 2). Role of Transposase protein in cut-and-paste transposition

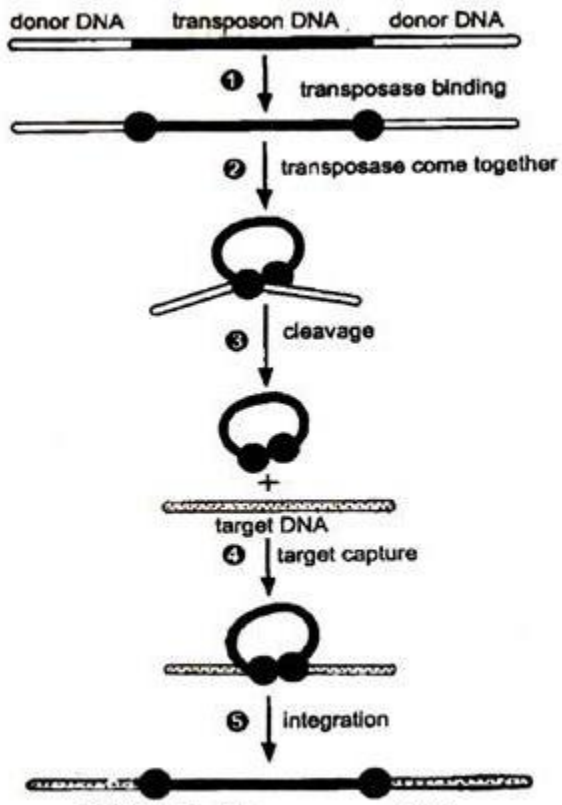


Fig. 2. Role of Transposase protein in cut-and-paste transposition.

Examples of cut-and-paste type of transposons are IS-elements, P-elements in maize, hobo-elements in Drosophila etc.

(ii) Replicative Transposons:

They transpose by a mechanism which involves replication of transposable sequence and this copy of DNA, so formed, is inserted into the target site while the donor site remains unchanged (Fig. 3). Thus, in this type of transposition, there is a gain of one copy of transposon and both-the donor and the recipient DNA molecule are having one-one transposable sequence each, after transposition.

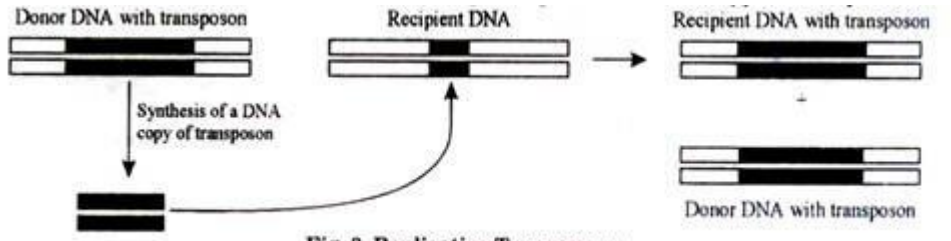


Fig. 3. Replicative Transposons.

Tn3-elements found in bacteria are good examples of such type of transposons.

(iii) Retro Elements:

Their transposition is accomplished through a process which involves the synthesis of DNA by reverse transcription (i.e. RNA DNA) by using elements RNA as the template (Fig. 4). This type of transposition involves an RNA intermediate, the transposable DNA is transcribed to produce an RNA molecule.

This RNA is then used as a template for producing a complementary DNA by the activity of enzyme reverse transcriptase. This single stranded DNA copy so formed, is then made double stranded and then inserted into the target DNA site. The transposable elements which require reverse transcriptase tor their movement are called retro transposons.

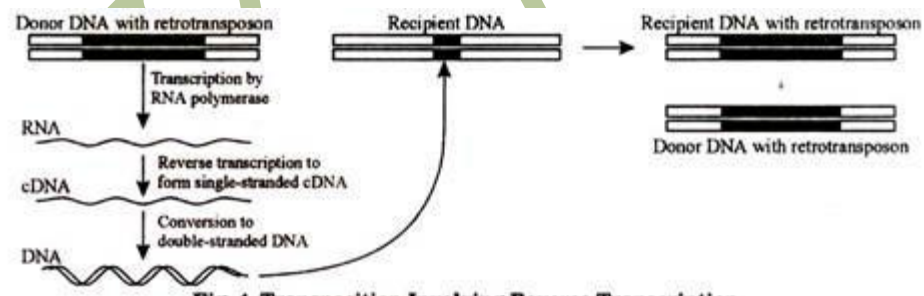


Fig. 4. Transposition Involving Reverse Transcription.

The Retro elements may be viral or non-viral. Out of these two, the non-viral retro elements are important and may further be classified as:

(a) Insertion Sequences or IS Elements:

They are the transposable sequences which can insert at different sites in the bacterial chromosomes.

IS-elements contain ITRs (Inverted Terminal Repeats), these were first observed in E.coli. IS elements are relatively short usually not exceeding 2500 bp. The ITRs present at the ends of IS-elements are an important feature which enables their mobility. The ITRs present in the IS-elements of E.coli usually range between 18-40 bp.

The term **'Inverted Terminal Repeat'** (ITR) implies that the sequence at 5 end of one strand is identical to the sequence at 5′ end of the other strand but they run in inverse opposite direction (Fig. 5). In Exoli chromosome, a number of copies of several IS-elements like IS1, IS2, IS3, IS4 and IS5 are present.

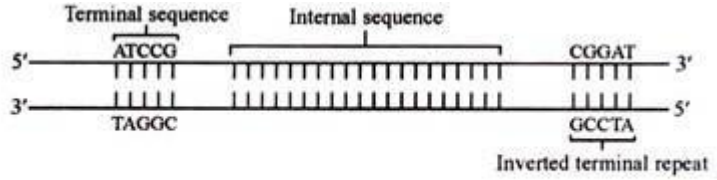


Fig. 5. An Insertion sequence (IS-element) with inverted terminal repeats (ITRs)

(b) Prokaryotic Transposon Element:

These are also called composite transposons and are shown by the symbol Tn. It is made up of two IS elements, one present at each end of a DNA sequence which contains genes whose functions are not related to the transposition process. These transposons have been found to have inverted repeats at the ends. The length of these inverted repeats ranges from a few nucleotides to about 1500 bp.

It can be said that these are the large transposons which are formed by capturing of an immobile DNA sequence within two insertion sequences thus enabling it to move. Examples of such transposons include the members of Tn series like Tn1, Tn5, Tn9, Tn10, etc.

Transposable Elements in Eukaryotes:

(a) Transposons in Maize:

Ac-Ds system:

This system of transposable elements in maize was analysed and given by Barbara Mc. Clintock. Here Ac stands for Activator and Ds for Dissociation. Barbara found that Ds and Ac genes were sometimes mobile and moved to different chromosomal locations thus resulting in different kernel phenotypes.

Ds element is activated by Ac and on activation it serves as the site provider for breakage in chromosome. Ac can move autonomously while Ds can move only in the presence of Ac (Fig. 6). The transposition involving this Ac-Ds system produces altered kernel phenotypes.

Other transposable elements of maize are:

i. spm (suppressor mutator) system,

ii. dt (dotted) system,

iii. Mu (Mutator) system, etc.

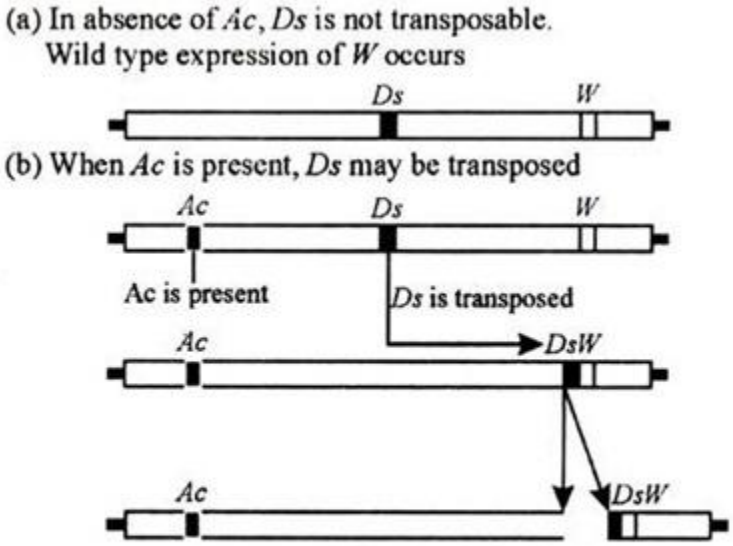


Fig. 6. Effects of Transposition involving *Ac-Ds* system in maize.

(b) Transposons in Drosophila:

A number of transposable elements are found in Drosophila which are of different types and account for a quite high fraction of Drosophila genome.

Some of these transposons are given below:

P-elements:

These were discovered during the study of 'hybrid-dysgenesis'which is a sterility causing condition. They are 2.9 kb long and contain 31 bp long inverted terminal repeats High rate of P-element transposition causes hybrid dysgenesis. P-elements encode transposase enzyme which helps in their transposition. These are also useful as vectors for introducing foreign genes into Drosophila.

Copia-elements:

Their transposition causes mutations for eye-colour in Drosophila. They are of size approximately 5-8 kb with direct terminal repeat (DTR) of about 276 bp at each end.

Within each of this direct repeats is present short inverted repeat (IR) of about 17 bp length. About 10-80 copia- elements are present in cell-genome (Fig. 7).

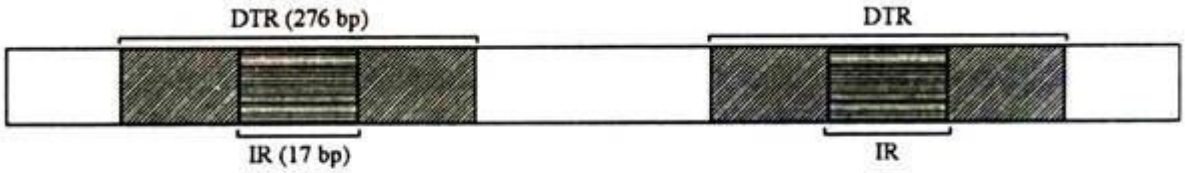


Fig. 7. Organization of a copia transposable element in *D. melanogaster*.

FB Elements:

These are the fold back elements present in Drosophila genome. These have ability to fold back to form a stem and loop structure due to the presence of long inverted terminal repeats. Their transposition results into a changed expression by causing mutation by insertion or by affecting the normal gene expression.

Other important types of transposable elements found in Drosophila are:

i. I elements,

ii. Mariner elements,

iii. Gypsy elements,

iv. Hobo elements, etc.

(c) Transposons in Humans:

Transposons in humans are in the form of repetitive DNA which consists of sequences that are interspersed within the entire human genome. These sequences are transposable and can move to different locations within the genome.

These are of following two types:

(1) SINEs (Short Interspersed Elements):

They are ~ 300 bp long and may be present about 5 lakh times in human genome. Alu sequences are the best characterized SINEs in humans.

These are termed as 'Alu' elements because they contain specific nucleotide sequences which are cleaved by the restriction enzyme named Alul. Alu elements contain Direct Terminal Repeats (DTR) of 7-20 bp length. These DTRs help them in the insertion process during transposition.

(2) LINEs (Long Interspersed Elements):

They are ~ 6400 bp long and are present about 1 lakh times in the human genome. Most prominent example is LI sequence. These transposable elements are some of the most abundant and common families of moderately repeated sequences in human DNA.

Significance of Transposable Elements:

1. Transposons may change the structural and functional characteristics of genome by changing their position in the genome.

2. Transposable elements cause mutation by insertion, deletion, etc.

3. Transposons make positive contribution in evolution as they have tremendous impact on the alteration of genetic organisation of organisms.

4. They are useful as cloning vectors also, in gene cloning. For example, P-elements are frequently used as vector for introducing transgenes into Drosophila.

5. Transposons may also be used as genetic markers while mapping the genomes.

6. Transposon-mediated gene tagging is done for searching and isolation of a particular gene.

Review Questions

Short Answer Questions **(2 Marks)**

1. Define transposition.
2. Define conjugation.
3. Explain linkage mapping.
4. What is tetrad analysis.
5. Explain about interrupted mapping.
6. List out the characters feature of transposase enzyme.
7. Define P- element.
8. Define Tn3 element.
9. Explain briefly about transposable element.
10. List out the types of transposons in eukaryotes.

Essay Answer Questions **(6 & 8 Marks)**

1. Describe in detail about transposable elements.
2. Explain in detail about physical mapping technique.
3. Give a detailed note on gene transfer methods
4. Give a detailed note on molecular markers.
5. Describe in detail about bacterial transposition.
6. Give a detailed note on types of eukaryotic transposable elements.
7. Describe in detail about prokaryotic transposable elements.
8. Explain about Excision of Transposons.
9. Give a detailed detection of transposable elements in prokaryotes.

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Unit IV

SYLLABUS

Microbial and Human genetics *- Gene transfer in Bacteria, Bacteriophages - properties, Structure, Role of phages as vectors.*

Human genetics - Pedigree analysis, linkage testing, karyotypes, genetic disorders, Eugenics. Epigenetics & Genome Imprinting. Structural and numerical alterations of chromosomes, ploidy and their genetic implications, Quantitative genetics - Polygenetic inheritance, heritability and its measurements, QTL Mapping.

Bacteria divide very rapidly. The doubling time is also called generation time and it may be as low as 20 minutes. Bacteria mainly reproduce by asexual reproduction but do not exhibit true sexual reproduction as they do not produce diploid phase. Thus, meiosis is lacking. However, bacteria exchange genetic material between two cells.

Modes of genetic transfer in bacteria:

Three modes of genetic transfer between bacterial cells are:

(a) Transformation

(b) Transduction

(c) Conjugation

Transformation

- Recipient cell uptake free DNA released into the environment.
- DNA is released it to the environment when another bacterial cell (i.e. donor) dies and undergoes lysis
- Not all bacteria are able to go for transformation, only some bacteria are able to take free DNA and are able to go transformation. These type of bacterial are called competent bacteria.

- Pathogenic bacteria showing competence
  - *Haemophilus* spp
  - *Streptococcus* spp
  - *Neisseria* spp

Transduction

Transduction is the transfer of genetic information from a donor to a recipient by way of a bacteriophage. The phage coat protects the DNA in the environment so that transduction, unlike transformation, is not affected by nucleases in the environment. Not all phages can mediate transduction. In most cases gene transfer is between members of the same bacterial species. However, if a particular phage has a wide host range then transfer between species can occur. The ability of a phage to mediated transduction is related to the life cycle of the phage.

Types of Transduction

1. Generalized Transduction – Generalized transduction is transduction in which potentially any bacterial gene from the donor can be transferred to the recipient. The mechanism of generalized transduction is illustrated in Figure.
2. Specialized transduction – Specialized transduction is transduction in which only certain donor genes can be transferred to the recipient. Different phages may transfer different genes but an individual phage can only transfer certain genes. Specialized transduction is mediated by lysogenic or temperate phage and the genes that get transferred will depend on where the prophage has inserted in the chromosome. The mechanism of specialized transduction is illustrated in Figure 4.Phages that mediate generalized transduction generally breakdown host DNA into smaller pieces and package their DNA into the phage particle by a "head-full" mechanism. Occasionally one of the pieces of host DNA is randomly packaged into a phage coat. Thus, any donor gene can be potentially transferred but only enough DNA as can fit into a phage head can be transferred. If a recipient cell is infected by a phage that contains donor

DNA, donor DNA enters the recipient. In the recipient a generalized recombination event can occur which substitutes the donor DNA and recipient DNA.

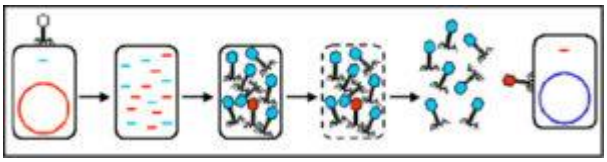


FIG. THE MECHANISM OF GENERALIZED TRANSDUCTION

During excision of the prophage, occasionally an error occurs where some of the host DNA is excised with the phage DNA. Only host DNA on either side of where the prophage has inserted can be transferred (*i.e.* specialized transduction). After replication and release of phage and infection of a recipient, lysogenization of recipient can occur resulting in the stable transfer of donor genes. The recipient will now have two copies of the gene(s) that were transferred. Legitimate recombination between the donor and recipient genes is also possible.

Significance

Lysogenic (phage) conversion occurs in nature and is the source of virulent strains of bacteria.

Conjugation:

The unidirectional transfer of DNA from one cell to another through a cytoplasmic bridge is called conjugation. The process is equivalent to sexual mating in eukaryotes. Two bacterial haploid cells of different strains come close to each other.

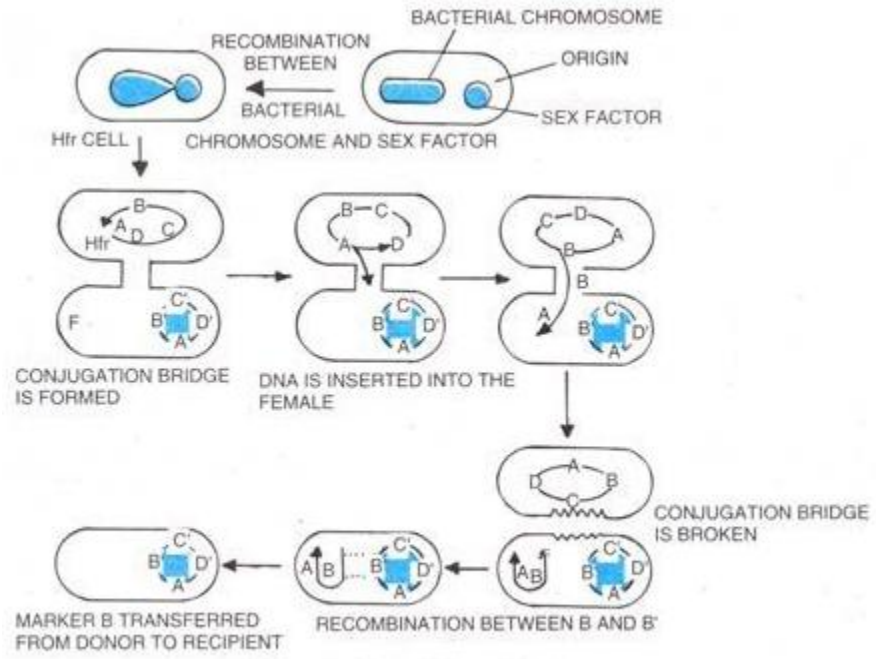
They recognise each other by complementary macromolecules borne on their surface. Donor or male cell passes part or whole of the chromosome into recipient or female cell. The ability of transferring the genetic material from male is controlled by sex or fertility factor (F gene) present in a plasmid.

Thus, genes can be transferred from donor to recipient cell on a molecule of DNA which acts as sex factor called F gene. This sex gene can reside in a bacterial chromosome or it may exist as an autonomous unit in cytoplasm.

Male bacterium with thorn-like protuberances called as sex pili come in contact with female bacterium which lacks pili and donate its DNA. F factor (a plasmid) carries genes for producing pili

and other functions required to transfer DNA. At times F factor integrates into bacterial chromosome.

Such bacteria can transfer their genetic material into female cell with high frequency (Hfr) in a particular sequence. They are called as Hfr -strains. Conjugation was first demonstrated by Lederberg and Tatum in E. coli. The frequency of recombination was very low in Lederberg's experiments.



Bacterial conjugation.

The Hfr cell acts as the male bacterium and when mixed with the female (F—) cell forms a conjugation bridge. The F factor containing DNA breaks at a particular point and starts inserting the DNA into the female and the sequence of chromosomal gene transfer is always in the same order (A, B, C and D genes).

The F factor is transferred last. The conjugation bridge usually breaks before the entire chromosome is transferred. Only the genes A and B have been transferred in the example given. These A and/or B genes can recombine with the corresponding genes in the F— chromosome.

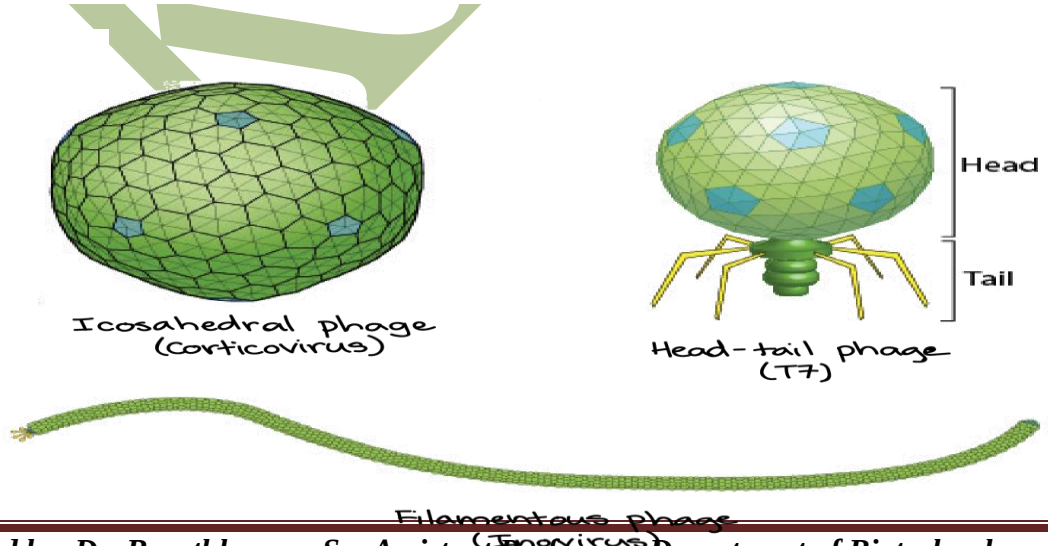
Thus, if B' in the F— cell is a mutated form of B, theft the B' in the F— chromosome can become B as a result of recombination after conjugation. Thus, genetic markers can be transferred from a host to a suitable recipient lacking such markers.

The order in which such markers are transferred to the recipient would follow the order in which they are present in the donor. Thus, conjugation experiments are useful in constructing the gene maps (order of arrangement of genes in the chromosome) of organisms.

Hayes (1952) found a strain of E. coli in which the frequency of recombination was as high as 100 to 1000 times as reported by Lederberg. The strain was called high frequency recombinant (Hfr) strain.

Bacteriophage

- A **bacteriophage**, or **phage** for short, is a virus that infects bacteria. Like other types of viruses, bacteriophages vary a lot in their shape and genetic material.
- Phage genomes can consist of either DNA or RNA, and can contain as few as four genes or as many as several hundred^{1,2,3}1,2,3start superscript, 1, comma, 2, comma, 3, end superscript.
- The capsid of a bacteriophage can be icosahedral, filamentous, or head-tail in shape. The head-tail structure seems to be unique to phages and their close relatives (and is not found in eukaryotic viruses)^{4,5}4,5start superscript, 4, comma, 5, end superscript.



Bacteriophages, just like other viruses, must infect a host cell in order to reproduce. The steps that make up the infection process are collectively called the lifecycle of the phage.

Some phages can only reproduce via a lytic lifecycle, in which they burst and kill their host cells. Other phages can alternate between a lytic lifecycle and a lysogenic lifecycle, in which they don't kill the host cell (and are instead copied along with the host DNA each time the cell divides).

Let's take closer look at these two cycles. As an example, we'll use a phage called lambda (\lambdaλlambda), which infects *E. coli* bacteria and can switch between the lytic and lysogenic cycles.

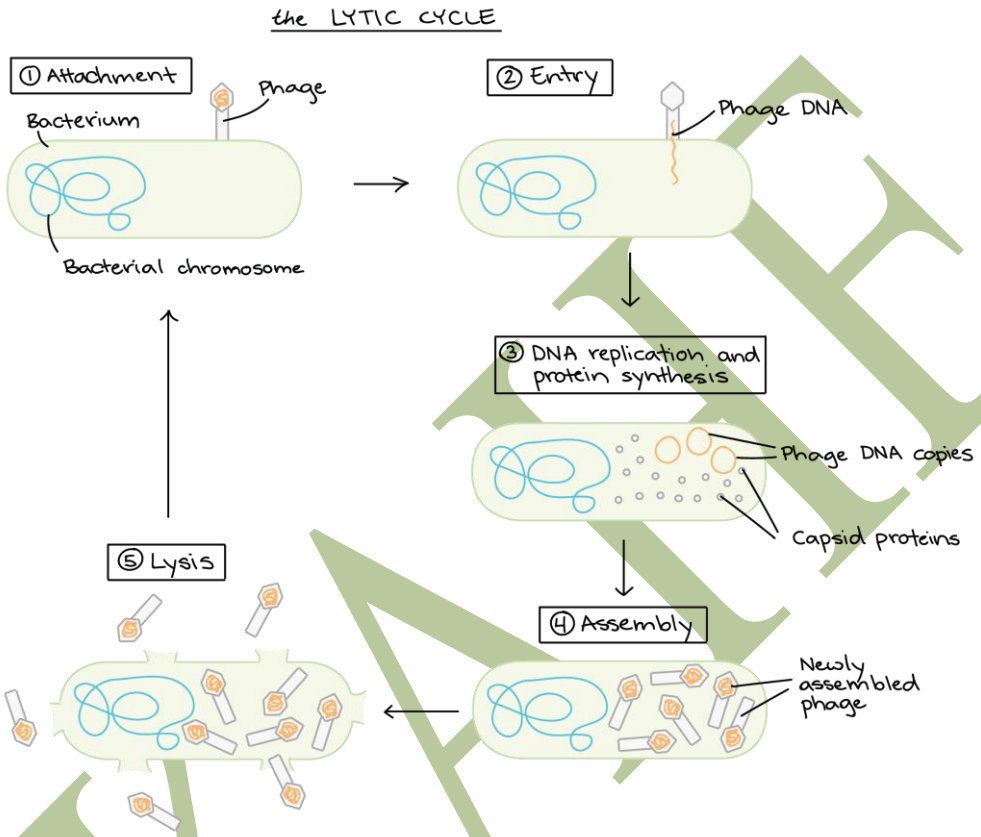
Lytic cycle

In the lytic cycle, a phage acts like a typical virus: it hijacks its host cell and uses the cell's resources to make lots of new phages, causing the cell to lyse (burst) and die in the process.

The stages of the lytic cycle are:

1. Attachment: Proteins in the "tail" of the phage bind to a specific receptor (in this case, a sugar transporter) on the surface of the bacterial cell.
2. Entry: The phage injects its double-stranded DNA genome into the cytoplasm of the bacterium.
3. DNA copying and protein synthesis: Phage DNA is copied, and phage genes are expressed to make proteins, such as capsid proteins.
4. Assembly of new phage: Capsids assemble from the capsid proteins and are stuffed with DNA to make lots of new phage particles.

5. Lysis: Late in the lytic cycle, the phage expresses genes for proteins that poke holes in the plasma membrane and cell wall. The holes let water flow in, making the cell expand and burst like an overfilled water balloon**.**

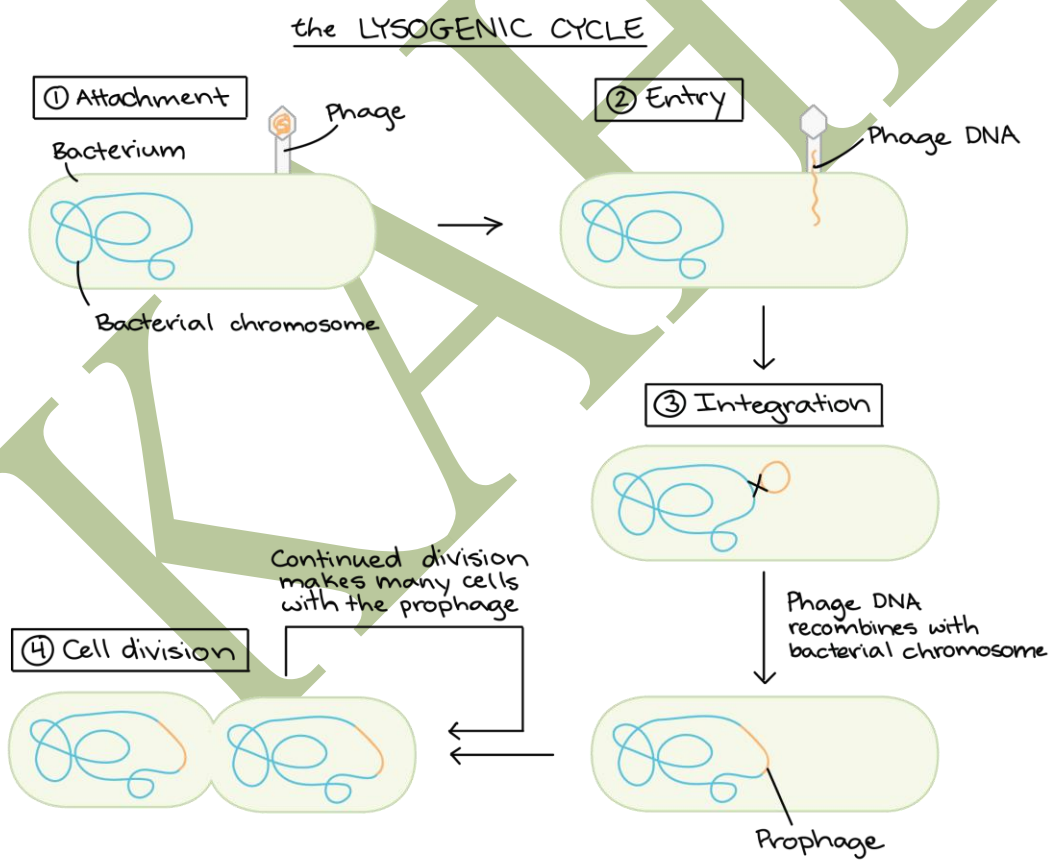


Cell bursting, or lysis, releases hundreds of new phages, which can find and infect other host cells nearby. In this way, a few cycles of lytic infection can let the phage spread like wildfire through a bacterial population.

Lysogenic cycle

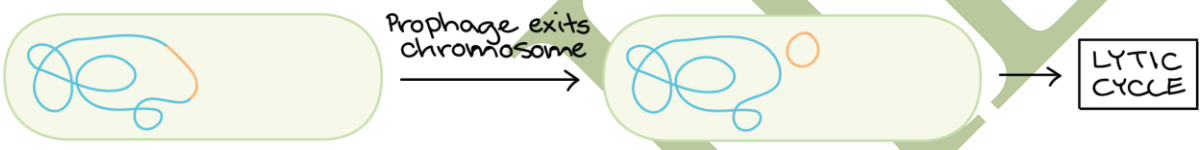
The lysogenic cycle allows a phage to reproduce without killing its host. Some phages can only use the lytic cycle, but the phage we are following, lambda (\lambdaλlambda), can switch between the two cycles.

In the lysogenic cycle, the first two steps (attachment and DNA injection) occur just as they do for the lytic cycle. However, once the phage DNA is inside the cell, it is not immediately copied or expressed to make proteins. Instead, it recombines with a particular region of the bacterial chromosome. This causes the phage DNA to be integrated into the chromosome.



The integrated phage DNA, called a prophage, is not active: its genes aren't expressed, and it doesn't drive production of new phages. However, each time a host cell divides, the prophage is copied along with the host DNA, getting a free ride. The lysogenic cycle is less flashy (and less gory) than the lytic cycle, but at the end of the day, it's just another way for the phage to reproduce.

Under the right conditions, the prophage can become active and come back out of the bacterial chromosome, triggering the remaining steps of the lytic cycle (DNA copying and protein synthesis, phage assembly, and lysis).



Bacteriaphage as vectors

1. Bacteriophage Vectors

- Bacteriophages are viruses that attack bacteria.
- Most phages lyse the bacterial cells they infect (lytic phages).
- But many others can choose to follow either a lytic or a lysogenic cycle; in the latter situation, the phage chromosome integrates into the bacterial chromosome and multiplies with the latter as prophage (temperate or lysogenic phages).
- The prophage may dissociate from the bacterial chromosome and follow the lytic cycle.

Several bacteriophages are used as cloning vectors,

- the most commonly used E. coli phages being λ (lambda) and M13 phages.

Plasmid vectors have to be introduced into bacterial cells, which are then cloned and selected for the recovery of recombinant DNA.

In contrast, the phage vectors are directly tested on an appropriate bacterial lawn (a continuous bacterial growth on an agar plate) where each phage particle forms a plaque (a clear bacteria-free zone in the bacterial lawn).

Phage vectors present two advantages over plasmid vectors.

(1) They are more efficient than plasmids for cloning of large DNA fragments; the largest cloned insert size in a λ vector is just over 24 kb, while that for plasmid vectors it is less than 15 kb. In addition,

(2) it is easier to screen a large number of phage plaques than bacterial colonies for the identification of recombinant plaques/clones.

Lambda (λ) Phage Vectors –

- The λ genome (total 48,502 bp) contains an
- origin of replication,
- genes for head and tail proteins and
- enzymes for DNA replication, lysis and lysogeny, and
- single-stranded protruding cohesive ends of 12 bases (5' GGGCGGCGACCT; the other end is complementary to it, i.e., CCCGCCGCTGGA 5').
- The λ genome remains linear in the phage head, but within E. coli cells the two cohesive ends anneal to form a circular molecule necessary for replication.
- The sealed cohesive ends are called cos sites, which are the sites of cleavage during and are necessary for packaging of the mature phage DNA into phage heads.

- The λ DNA must be larger than 38 kb and smaller than 52 kb to be packaged into phage particles.
- The genes for lysogeny are located in the segment between 20 and 38 kb; the whole or a part of this segment is deleted to create λ vectors to

(1) accommodate larger DNA inserts and
(2) to ensure that the recombinant phage is always lytic.

Several vectors were produced from wild type λ genome by mutation and recombination in vivo as well as by recombinant DNA techniques.

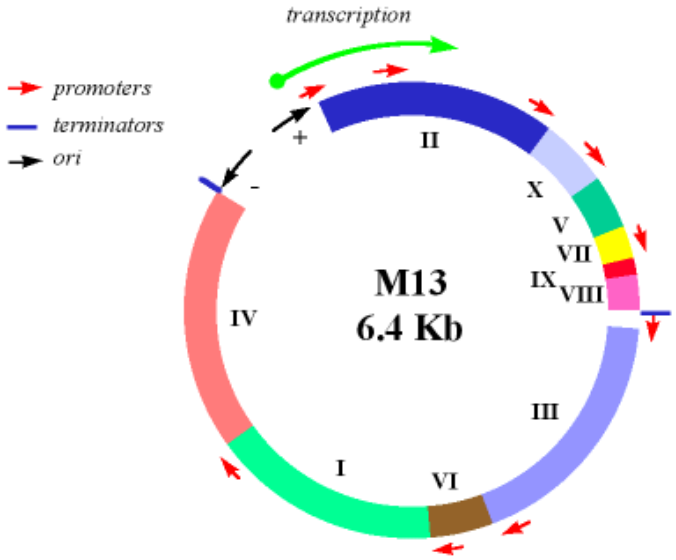
These vectors have the following two basic features.

(1) The vector itself can be propagated as phage in E. coli cells enabling preparation of vector DNA.
(2) They contain restriction sites, which allow the removal of the lysogenic segment and also provide insertion site for the DNA fragment.
(3) During annealing and ligation of the DNA insert with the λ vector, two or more recombinant DNAs may join end-to-end producing a concatemer, which is the proper precursor for packaging of λ genome into phage heads.

Phage M13 Vectors –

- These vectors are used for obtaining single-strand copies of cloned DNA, which are especially suited for DNA sequencing.
- They are derived from the 6.4 kb genome of the E. coli filamentous bacteriophage M13.
- This phage has a single-stranded linear DNA genome in phage particles, which converts into a double stranded circular replicative intermediate within the host cells.

- M13 infects only F+ cells; it does not kill the cells, but forms turbid plaques due to growth retardation of infected cells.
- Ordinarily, the double stranded form is used to produce recombinant molecules since single-stranded DNAs are not cleaved by type II restriction endonucleases; this form is readily isolated from M13-infected E. coli cells.



- However, the single-stranded form of M 13 is used to recover single-stranded copies of the DNA inserts; this form of vector is available from the phage particles abundant in the growth medium.

Properties of M13 Vectors –

M13 genome has been used to produce M13mp series of vectors, e.g., M13mp 8, M13mp 9, etc.

The desirable features of M13 vectors are as follows:

(1) very large inserts can be cloned since packaging does not depend on genome size (as is the case with λ vectors).

(2) Pure single-strand copies of double-strand DNA inserts are obtained in abundance.

(3) Since DNA inserts are accepted in either of the orientations (which is also the case for plasmid and λ vectors), some recombinant clones will produce single-strand copies of one strand of the DNA double-strand, while others would produce copies of the complementary strand of the DNA insert.

The phage particles in a single plaque, as a rule, will yield copies of the same single-strand. This property is very useful for a precise DNA sequencing (using both the strands of a DNA molecule) and for the synthesis of specific radio-labelled DNA probes.

(4) Bacterial cells infected by these vectors remain viable as in the case of plasmid vectors; this allows easy maintenance of the vector. Finally,

(5) they form plaques like λ phage vectors making selection of the recombinant DNAs rather easy, and

(6) the recombinant DNA is obtained within stable bacteriophage particles.

Two types of λ phage vectors

1. **Insertional vectors**
2. **Replacment vectors**

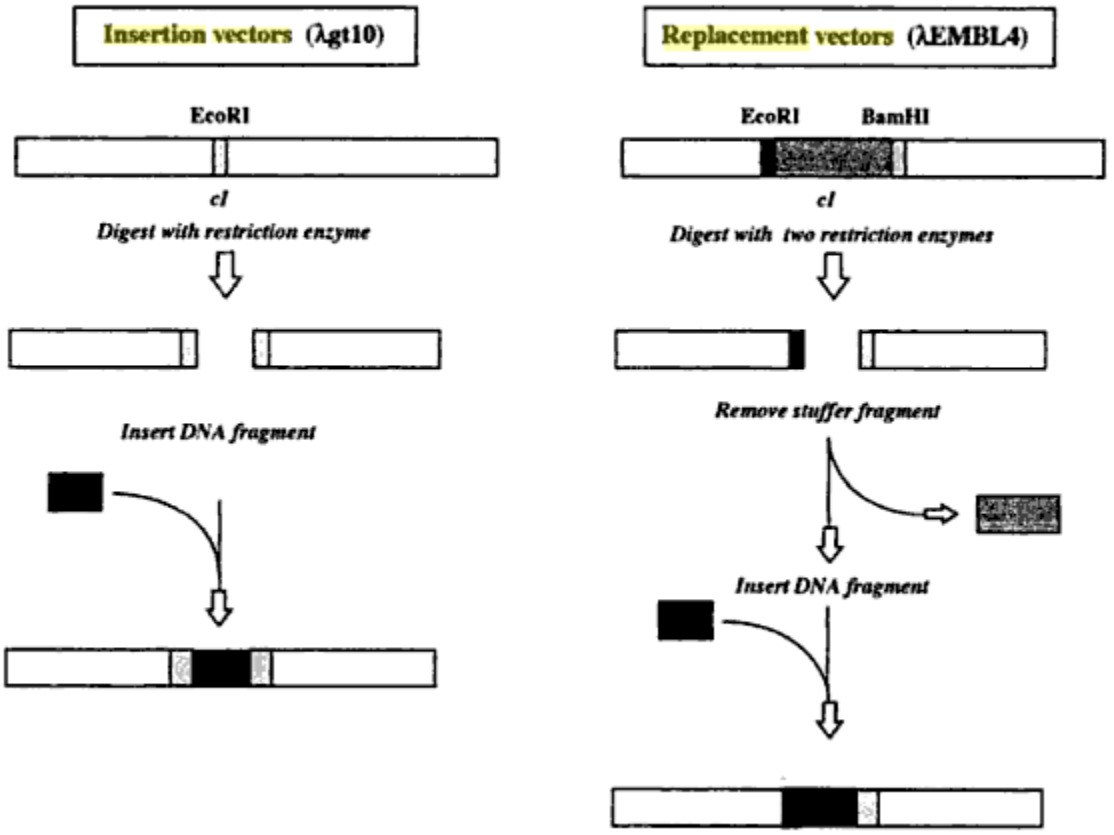
The λ insertional vectors – accept less DNA than the replacement type, the foreign DNA is simply inserted into a region of the phage genome with appropriate restriction sites.

Example - **λgt10, λcharon16A**

In λ replacement vectors – a central region of DNA not essential for lytic growth is removed. This creates two DNA fragments, called right and left arms. The central stuffer fragment is replaced by inserting foreign DNA between the arms to form a functional recombinant λ phage.

Example – λembl, λZAP

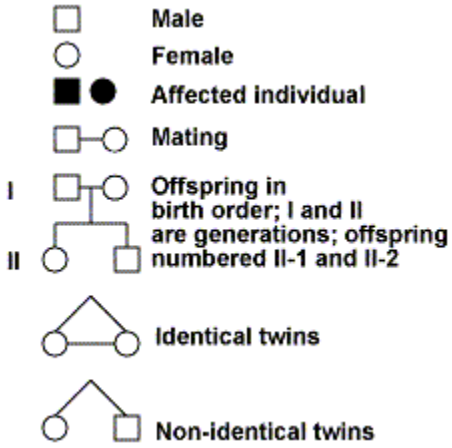
General schemes used for cloning in λ insertion and λ replacement vectors



Pedigree Analysis

All of the conclusions regarding gene action (dominant/recessive; codominant) we have discussed so far have been obtained from analyzing the results of controlled crosses. In some situations, we do not have the opportunity to perform controlled crosses. Rather we need to analysis an existing population. This is always the case when studying human genetics. Scientists have devised another approach, called **pedigree analysis**, to study the inheritance of genes in humans. Pedigree analysis is also useful when studying any population when progeny data from several generations is limited. Pedigree analysis is also useful when studying species with a long genration time.

A series of symbols are used to represent different aspects of a pedigree. Below are the principal symbols used when drawing a pedigree.

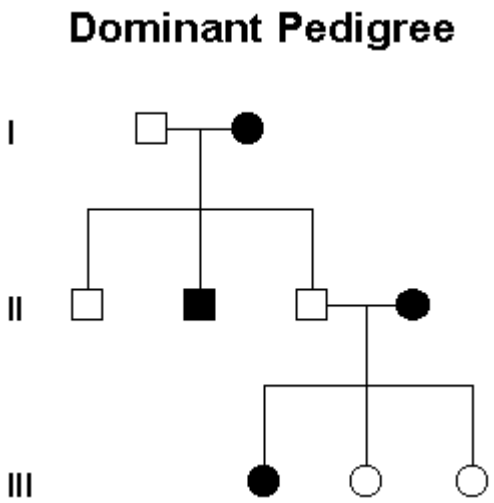


Once phenotypic data is collected from several generations and the pedigree is drawn, careful analysis will allow you to determine whether the trait is dominant or recessive. Here are some rules to follow.

For those traits exhibiting dominant gene action:

- affected individuals have at least one affected parent
- the phenotype generally appears every generation
- two unaffected parents only have unaffected offspring

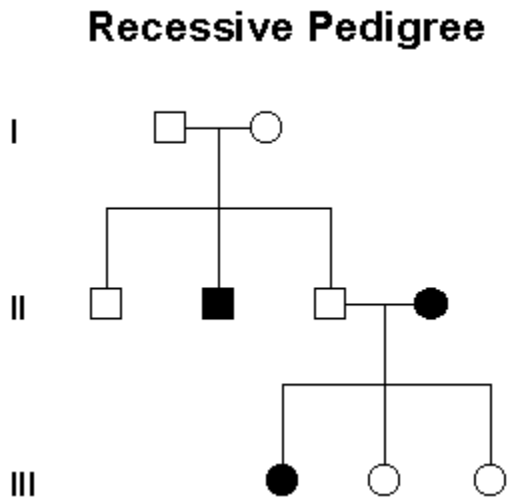
The following is the pedigree of a trait contolled by dominant gene action.



And for those traits exhibiting recessive gene action:

- unaffected parents can have affected offspring
- affected progeny are both male and female

The following is the pedigree of a trait contolled by recessive gene action.

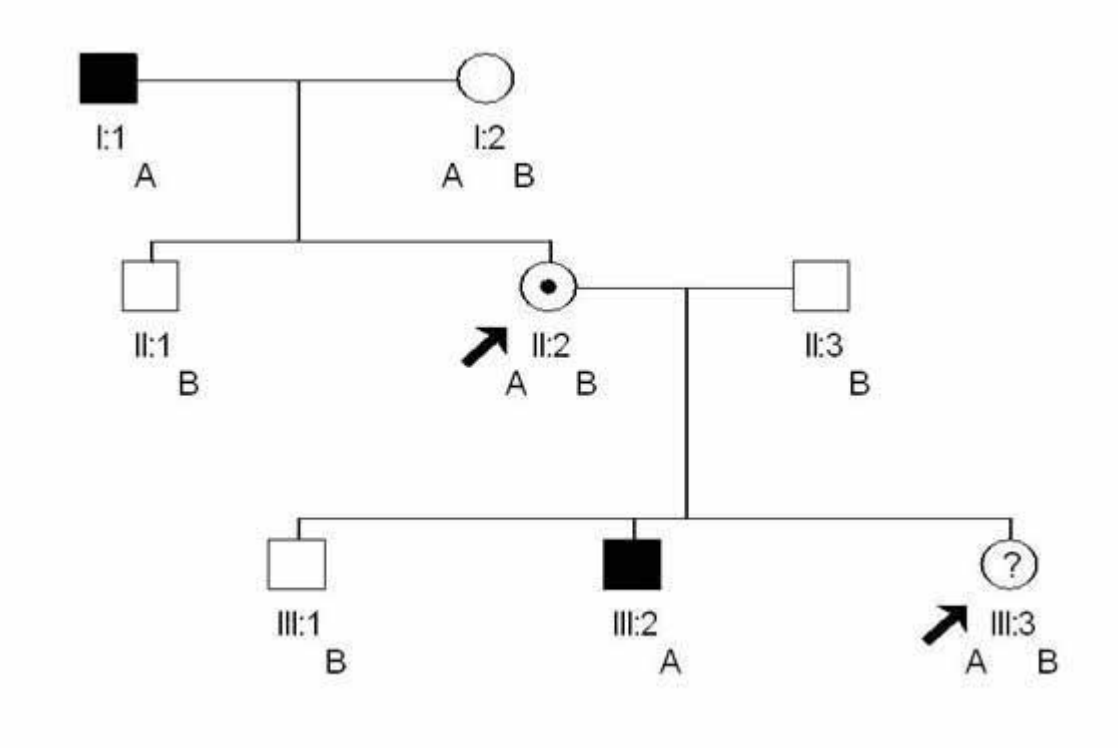


Linkage Testing

Linkage analysis is a method that is used in establishing the carrier status of female 'at-risk' carriers and for prenatal diagnosis. In many cases, linkage analysis has been replaced by mutational analysis but in a small number of families in whom the mutation cannot be identified, linkage analysis remains the only method for the genetic diagnosis of carriers.

Linkage: Two genetic loci are said to be in linkage if the alleles at these loci segregate together more often that would be expected by chance – that is the two loci are so close together on the same chromosome that the chances of them separating by a crossover event (recombination) during Meiosis is small. The probability that any two alleles at two randomly selected loci with be inherited together is 0.5. If two loci are closely linked then the chances of a crossover or recombination event occurring is <0.5. The chances of recombination taking places is linked to the distance between any two loci. The recombination fraction [θ] is a measure of the genetic distance between two loci. The distance between two loci is measured in centimorgans and 1 centimorgan is

defined as the genetic distance between two loci with a recombination frequency of 1%. Although the centimorgan is not a measure of physical distance, it typically equates to a physical distance of one million base pairs. So two loci close to the *F8* gene with a 5% probability of recombination would be 5 centimorgans apart i.e. approximately 5 million base pairs.
The aim of linkage analysis is to identify a marker that co-segregates with the gene of interest and so can be used to track the gene within a family without actually knowing the mutation. By definition this marker must co-segregate with the gene of interest and so be present in affected family members but absent in unaffected family members. In the era before rapid sequence analysis, linkage analysis was the principal method for establishing the carrier status of 'at-risk' females within a family and for pre-natal diagnosis.
Whilst we usually think of linkage analysis using DNA markers, other markers such as proteins can be also be used. Such a case is the gene for Glucose-6 Phosphate Dehydrogenase [G6PD] which maps to the long arm of the X-chromosome at Xq28 close to the gene for factor VIII [*F8*]. Close linkage between the loci for G6PD and *F8* has allowed prenatal diagnosis of haemophilia in the foetuses of women who are heterozygous for two electrophoretic variants (A and B) of G6PD.
The pedigree below illustrates the theoretical use of G6PD variants (A and B) for carrier detection in a family with severe haemophilia A.



In this pedigree, I:1 and III:2 have severe haemophilia A [VIII:C<1 IU/dL). II:2 must be an obligate carrier and III:3 wishes to know if she is a carrier or not. From the pedigree there is a 1/2 chance that she is or is not. Analysis shows that they both have the A variant of G6PD. In contrast, the unaffected males in this pedigree have the B variant. So in this family we can use the A variant of the G6PD protein to track the abnormal *F8* gene.

If we use the G6PD variants [remember the gene for G6PD is located on the X-chromosome at Xq28 close to the *F8* gene which also maps to Xq28] - then III:3 has inherited the B allele from her father and the A allele which tracks with the abnormal *F8* gene from her mother and she is, therefore, likely to be a carrier. Bayesian risk analysis would allow us to make more confident predictions as to her carrier status but to undertake this we would need to know the frequency of recombination occurring between the *F8* gene and the G6PD gene. Furthermore, measurement of the FVIII:C and VWF:Ag ratio would allow us to derive a VIII:C/VWF:Ag ratio and this may allow us to more accurately predict the carrier status for III:3.

There are, of course, serious limitations to this method of linkage analysis and in particular the risks of a recombination event occurring with each generation and as a result incorrectly assigning the carrier status of 'at-risk' females and in the case of pre-natal diagnosis. In addition, it relies upon the identification of women who are heterozygous for variants of G6PD. This is found in approximately 40% of black females in the USA but uncommon in other ethnic groups.

Karyotyping

It is the process of pairing and ordering all the chromosomes of an organism, thus providing a genome-wide snapshot of an individual's chromosomes. Karyotypes are prepared using standardized staining procedures that reveal characteristic structural features for each chromosome. Clinical cytogeneticists analyze human karyotypes to detect gross genetic changes—anomalies involving several megabases or more of DNA. Karyotypes can reveal changes in chromosome number associated with aneuploid conditions, such as trisomy 21 (Down syndrome). Careful analysis of karyotypes can also reveal more subtle structural changes, such as chromosomal deletions, duplications, translocations, or inversions. In fact, as medical genetics becomes increasingly integrated with clinical medicine, karyotypes are becoming a source of diagnostic information for specific birth defects, genetic disorders, and even cancers.

Preparing Karyotypes from Mitotic Cells

Karyotypes are prepared from mitotic cells that have been arrested in the metaphase or prometaphase portion of the cell cycle, when chromosomes assume their most condensed conformations. A variety of tissue types can be used as a source of these cells. For cancer diagnoses, typical specimens include tumor biopsies or bone marrow samples. For other diagnoses, karyotypes are often generated from peripheral blood specimens or a skin biopsy. For prenatal diagnosis, amniotic fluid or chorionic villus specimens are used as the source of cells.

The process of generating a karyotype begins with the short-term culture of cells derived from a specimen. After a period of cell growth and multiplication, dividing cells are arrested in metaphase

by addition of colchicine, which poisons the mitotic spindle. The cells are next treated with a hypotonic solution that causes their nuclei to swell and the cells to burst. The nuclei are then treated with a chemical fixative, dropped on a glass slide, and treated with various stains that reveal structural features of the chromosomes.

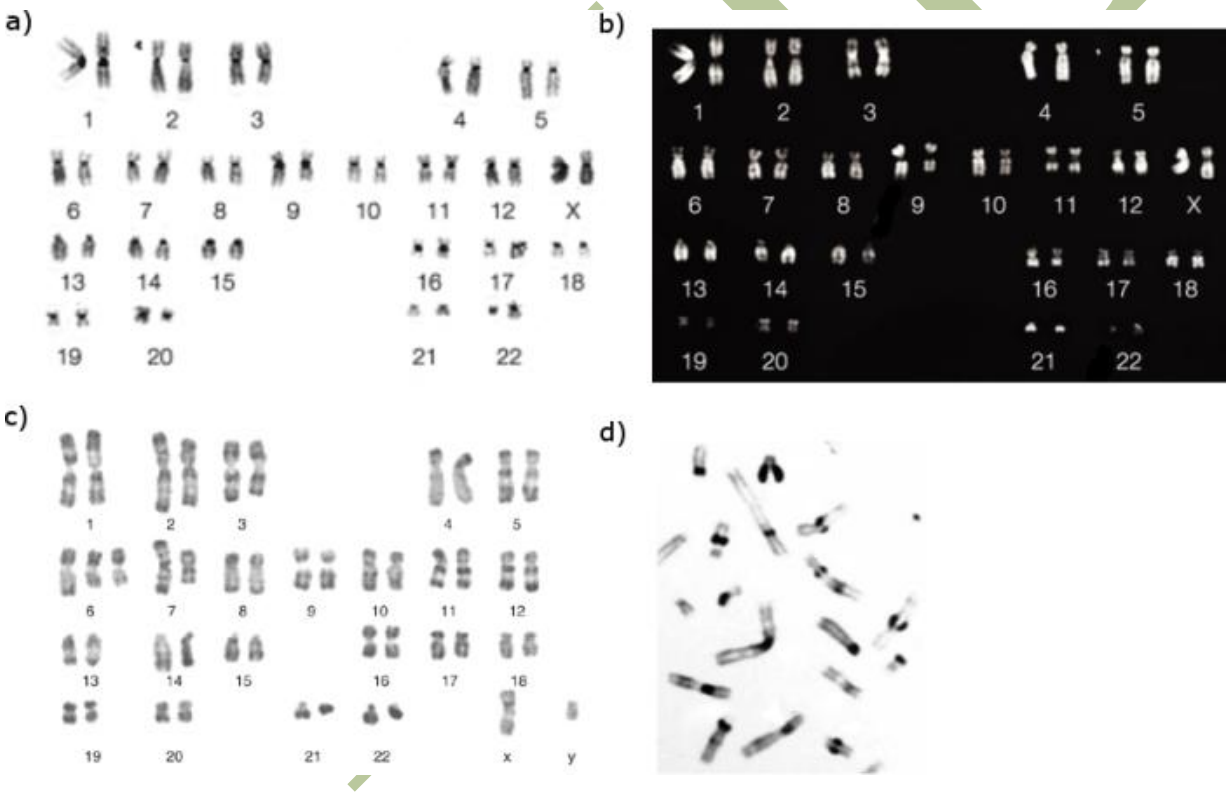
Banding Patterns Reveal the Structural Details of Chromosomes

Without any treatment, structural details of chromosomes are difficult to detect under a light microscope. Thus, to make analysis more effective and efficient, cytologists have developed stains that bind with DNA and generate characteristic banding patterns for different chromosomes. Prior to the development of these banding techniques, distinguishing chromosomes from one another proved very difficult, and chromosomes were simply grouped according to their size and the placement of their centromeres.

This changed in 1970, when Torbjorn Caspersson and his colleagues described the first banding technique, known as Q-banding. Q-banding involves use of the fluorescent dye quinacrine, which alkylates DNA and is subject to quenching over time. Caspersson *et al*. demonstrated that quinacrine produced characteristic and reproducible banding patterns for individual chromosomes. Since then, researchers have developed a variety of other chromosome banding techniques that have largely supplanted Q-banding in clinical cytogenetics. Today, most karyotypes are stained with Giemsa dye, which offers better resolution of individual bands, produces a more stable preparation, and can be analyzed with ordinary bright-field microscopy.

The molecular causes for staining differences along the length of a chromosome are complex and include the base composition of the DNA and local differences in chromatin structure. In G-banding, the variant of Giemsa staining most commonly used in North America, metaphase chromosomes are first treated briefly with trypsin, an enzyme that degrades proteins, before the chromosomes are stained with Giemsa. Trypsin partially digests some of the chromosomal proteins, thereby relaxing the chromatin structure and allowing the Giemsa dye access to the DNA.

In general, heterochromatic regions, which tend to be AT-rich DNA and relatively gene-poor, stain more darkly in G-banding. In contrast, less condensed chromatin—which tends to be GC-rich and more transcriptionally active—incorporates less Giemsa stain, and these regions appear as light bands in G-banding. Most importantly, G-banding produces reproducible patterns for each chromosome, and these patterns are shared between the individuals of a species. An example of Giemsa-stained human chromosomes, as they would appear under a microscope, is shown in Figure 1a. Typically, Giemsa staining produces between 400 and 800 bands distributed among the 23 pairs of human chromosomes. Measured in DNA terms, a G-band represents several million to 10 million base pairs of DNA, a stretch long enough to contain hundreds of genes.



Genetic Disorder

Genes are the building blocks of heredity. They are passed from parent to child. They hold DNA, the instructions for making proteins. Proteins do most of the work in cells. They move molecules from one place to another, build structures, break down toxins, and do many other maintenance jobs.

Sometimes there is a mutation, a change in a gene or genes. The mutation changes the gene's instructions for making a protein, so the protein does not work properly or is missing entirely. This can cause a medical condition called a genetic disorder.

You can inherit a gene mutation from one or both parents. A mutation can also happen during your lifetime.

There are three types of genetic disorders:

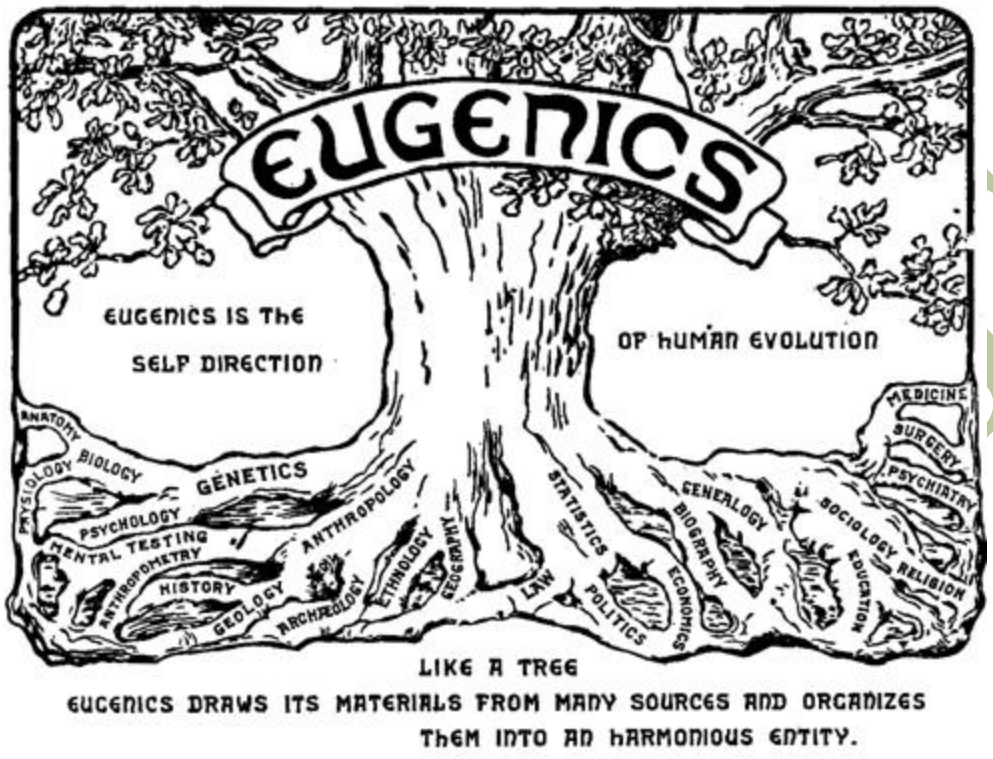
- Single-gene disorders, where a mutation affects one gene. Sickle cell anemia is an example.
- Chromosomal disorders, where chromosomes (or parts of chromosomes) are missing or changed. Chromosomes are the structures that hold our genes. Down syndrome is a chromosomal disorder.
- Complex disorders, where there are mutations in two or more genes. Often your lifestyle and environment also play a role. Colon cancer is an example.

Genetic tests on blood and other tissue can identify genetic disorders.

Introduction to Eugenics

Eugenics is a movement that is aimed at improving the genetic composition of the human race. Historically, eugenicists advocated selective breeding to achieve these goals. Today we have technologies that make it possible to more directly alter the genetic composition of an individual. However, people differ in their views on how to best (and ethically) use this technology.

History of Eugenics



In 1883, Sir Francis Galton, a respected British scholar and cousin of Charles Darwin, first used the term eugenics, meaning "well-born." Galton believed that the human race could help direct its future by selectively breeding individuals who have "desired" traits. This idea was based on Galton's study of upper class Britain. Following these studies, Galton concluded that an elite position in society was due to a good genetic makeup. While Galton's plans to improve the human race through selective breeding never came to fruition in Britain, they eventually took sinister turns in other countries.

The eugenics movement began in the U.S. in the late 19th century. However, unlike in Britain, eugenicists in the U.S. focused on efforts to stop the transmission of negative or "undesirable" traits from generation to generation. In response to these ideas, some US leaders, private citizens, and

corporations started funding eugenical studies. This lead to the 1911 establishment of The Eugenics Records Office (ERO) in Cold Spring Harbor, New York. The ERO spent time tracking family histories and concluded that people deemed to be unfit more often came from families that were poor, low in social standing, immigrant, and/or minority. Further, ERO researchers "demonstrated" that the undesirable traits in these families, such as pauperism, were due to genetics, and not lack of resources.

Committees were convened to offer solutions to the problem of the growing number of "undesirables" in the U.S. population. Stricter immigration rules were enacted, but the most ominous resolution was a plan to sterilize "unfit" individuals to prevent them from passing on their negative traits. During the 20th century, a total of 33 states had sterilization programs in place. While at first sterilization efforts targeted mentally ill people exclusively, later the traits deemed serious enough to warrant sterilization included alcoholism, criminality chronic poverty, blindness, deafness, feeble-mindedness, and promiscuity. It was also not uncommon for African American women to be sterilized during other medical procedures without consent. Most people subjected to these sterilizations had no choice, and because the program was run by the government, they had little chance of escaping the procedure. It is thought that around 65,000 Americans were sterilized during this time period.

The eugenics movement in the U.S. slowly lost favor over time and was waning by the start of World War II. When the horrors of Nazi Germany became apparent, as well as Hitler's use of eugenic principles to justify the atrocities, eugenics lost all credibility as a field of study or even an ideal that should be pursued.

Epigenetics

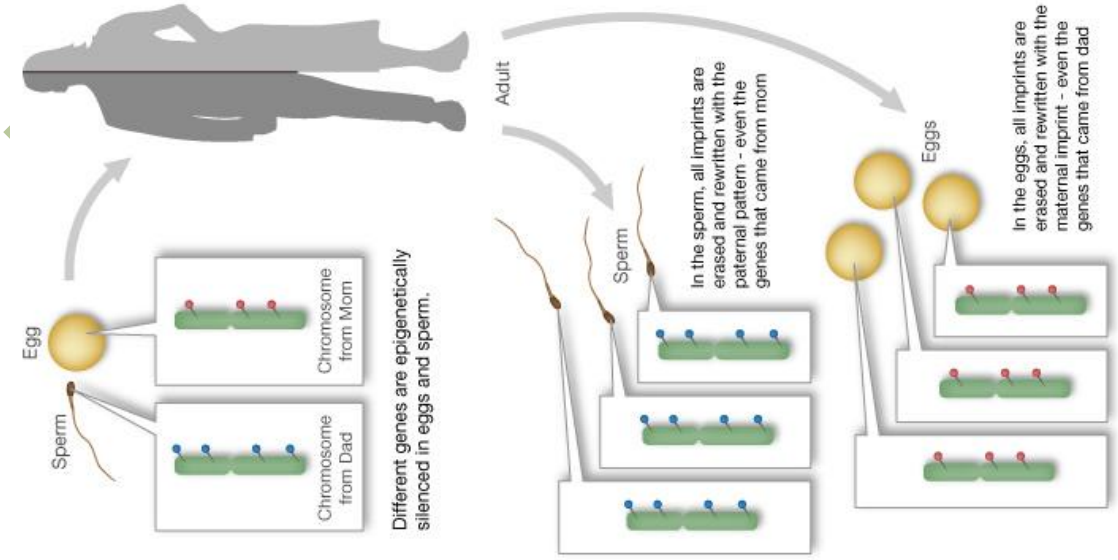
It is the study of heritable changes in gene expression (active versus inactive genes) that do not involve changes to the underlying DNA sequence — a change in phenotype without a change in genotype — which in turn affects how cells read the genes. Epigenetic change is a regular and natural occurrence but can also be influenced by several factors including age, the environment/lifestyle, and disease state. Epigenetic modifications can manifest as commonly as the manner in which cells terminally differentiate to end up as skin cells, liver cells, brain cells, etc. Or,

epigenetic change can have more damaging effects that can result in diseases like cancer. At least three systems including DNA methylation, histone modification and non-coding RNA (ncRNA)-associated gene silencing are currently considered to initiate and sustain epigenetic change.1 New and ongoing research is continuously uncovering the role of epigenetics in a variety of human disorders and fatal diseases.

Genome importing

For most genes, we inherit two working copies -- one from mom and one from dad. But with imprinted genes, we inherit only one working copy. Depending on the gene, either the copy from mom or the copy from dad is epigenetically silenced. Silencing usually happens through the addition of methyl groups during egg or sperm formation.

The epigenetic tags on imprinted genes usually stay put for the life of the organism. But they are reset during egg and sperm formation. Regardless of whether they came from mom or dad, certain genes are always silenced in the egg, and others are always silenced in the sperm.



Chromosomal aberrations

In an organism, any visible abnormality in chromosome number or structure from the diploid set is known as chromosomal aberration. The chromosomal aberrations based on the structure of the chromosome are of four types - deletion, duplication, inversion and transversion.

Structural chromosomal aberrations

Deletion

The loss of a segment of the genetic material in a chromosome is called deletion. It may be terminal or intercalary. When the deletion occurs near the end of the chromosome, then it is called terminal deletion. Eg. Drosophila and Maize. When the deletion occurs in the middle of the chromosome then, it is called intercalary deletion. Most of the deletions lead to death of an organism.

Duplication

When a segment of a chromosome is present more than once in a chromosome then, it is called duplication. For example, the order of genes in a chromosome is a, b, c, d, e, f, g and h. Due to aberration, the genes 'g' and 'h' are duplicated and the sequence of genes becomes a, b, c, d, e, f, g, h, g and h. In Drosophila, corn and peas a number of duplications are reported. Some duplications are useful in the evolution of the organism.

Inversion

It is another chromosomal abnormality in which, the order of genes in a chromosomal segment is reversed by an angle of 180'. For example, the order of genes in a chromosome is a, b, c, d, e, f, g and h. Due to aberration, the sequence of genes becomes, a, b, c, d, g, f, e and h. There are two types of inversion - pericentric and paracentric inversion.

In pericentric inversion, the inverted segment of the chromosome contains centromere. Sometimes, it is responsible for evolution of the organism. For example the 17th human chromosome is acrocentric, while in Chimpanzee the corresponding chromosome is metacentric. In paracentric inversion, the inverted segment of the chromosome has no centromere.

Translocation

It is a kind of a chromosomal abnormality in which the interchange of the chromosomal segments occurs. When translocation occurs between two non-homologous chromosomes, then it is called reciprocal translocation or illegitimate crossingover. It is of two kinds - heterozygous translocation and homozygous translocation.

In heterozygous translocation, one member of each pair of chromosomes is normal and the other member is with interchanged segment. But in homozygous translocation, both the members of paired chromosomes have translocated segments.

They play an important role in species differentiation. Translocations causes hereditary disorders.

Numerical chromosomal aberrations

Each species of an organism has a specific number of chromosomes in its somatic cells. These chromosomes are found in pairs. At the time of formation of gametes the chromosome number is reduced. Hence, the gemetes carry haploid set of chromosomes. Alterations in the number of chromosomes from the diploid set is called numerical chromosomal aberration. It is also known as ploidy. There are two types of ploidy x euploidy and aneuploidy.

Euploidy

Euploidy is the variation in the chromosome number that occurs due to increase or decrease of full set of chromosomes. Monoploidy, diploidy and polyploidy are the types in euploidy.

Diploidy

In most of the plants and animals, the somatic cells contain two sets of chromosome. Diploidy is formed by the union of two gametes during fertilization.

Polyploidy

Addition of one or more sets of chromosomes to the diploid set results in polyploidy. It is commonly noticed in plants and rare in animals. They are of two kinds - autopolyploidy and allopolyploidy.

Autopolyploidy

Addition of one or more haploid set of its own genome in an organism results in autopolyploidy. Watermelon, grapes and banana are autotriploids, whereas apple is an autotetraploid.

Allopolyploidy

Increase in one or more haploid set of chromosomes from two different species result in allopolyploidy. Triticale is the first man made cereal. It is obtained by crossing a wheat Triticum durum (2n = 4x = 28) and a rye

Secale cereale (2n = 2x = 14). The F1 hybrid (2n = 3x = 21) is sterile. Then the chromosome number is doubled using colchicine and it becomes an hexaploid.

Aneuploidy

Variation that involves one or two chromosomes within the diploid set of an organism results in aneuploidy. It is of two types - hypoploidy and hyperploidy.

Hypoploidy

Decrease in one or two chromosomes from the diploid set is described as hypoploidy. There are two types of hypoploidy - monosomy and nullisomy. Monosomy is due to loss of a chromosome from the diploid set i.e. 2n - 1. Nullisomy is the condition in which a pair of homologous chromosomes is lost from the diploid set i.e. 2n - 2.

Hyperploidy

Addition of one or two chromosomes to the diploid set of chromosome results in hyperploidy. There are two types of hyperploidy - trisomy and tetrasomy. Trisomy results due to the addition of one chromosome to diploid set of chromosomes. It is represented by 2n + 1. Trisomics are observed in Datura stramonium. Tetrasomy results due to the addition of two chromosomes to diploid set of chromosome. It is represented by 2n+2.

Significance of ploidy

1. Polyploidy plays an important role in plant breeding and horticulture.

2. Polyploidy has more vigorous effect than the diploids and results in the production of large sized flowers and fruits. Hence, it has economical significance.

3. It plays significant role in the evolution of new species.

4. Polyploidy results in the changes in the season of flowering and fruiting.

5. Polyploids are vigorous invaders of new habitats.

6. It leads to the formation of new varieties which show high resistance to disease and increase in yield.

7. Tetraploid cabbages and tomatoes contain more ascorbic acid whereas tetraploid corn contains more vitamin A.

8. Both euploidy and aneuploidy in man cause congenital diseases.

9. Polyploidy varieties like apple, pear, grape and watermelons are cultivated because of their large size.

Quantitative Genetics

All of the traits that we have studied to date fall into a few distinct classes. These classes can be used to predict the genotypes of the individuals. For example, if we cross a tall and short pea plant and look at F_2 plants, we know the genotype of s hort plants, and we can give a generalized genotype for the tall plant phenotype. Furthermore, if we know the genotype we could predict the phenotype of the plant. These type of phenotypes are called**discontinuous traits**.

Other traits do not fall into discrete classes. Rather, when a segregating population is analyzed, a continuous distribution of phenotypes is found. An example, is ear length in corn. Black Mexican Sweet corn has short ears, whereas Tom Thumb popcorn has long ears. When these two inbred lines are crossed, the length of the F_1 ears are intermediate to the two parents. Furthermore, when the F_1plants are intermated, the distribution of ear length in the F_2 ranges from the short ear Black Mexican Sweet size to the Tom Thumb popcorn size. The distribution resembles the bell-shaped curve for a normal distribution.

These types of traits are called **continuous traits** and cannot be analyzed in the same manner as discontinuous traits. Because continuous traits are often measured and given a quantitative value, they are often referred to as **quantitative traits**, and the area of genetics that studies their mode of inheritance is called **quantitative genetics**.

Many important agricultural traits such as crop yield, weight gain in animals, fat content of meat are quantitative traits, and much of the pioneering research into the modes of inheritance of these traits was performed by agricultural geneticists. Many human phenotypes such as IQ, learning ability and blood pressure also are quantitative traits. **These traits are controlled by multiple genes, each segregating according to Mendel's laws. These traits can also be affected by the environment to varying degrees**.

The following are examples of quantitative traits that we are concerned with in our daily life.

- Crop Yield
- Some Plant Disease Resistances
- Weight Gain in Animals
- Fat Content of Meat
- IQ
- Learning Ability
- Blood Pressure

Here are some images of quantitative traits in plants:

This image demonstrates the variation for flower diameter, number of flower parts and the color of the flower *Gaillaridia pilchella*. Each trait is controlled by a number of genes and is a quantitative trait.

Polygenic Inheritance

In a system which differs from *Mendelian Genetics*, where *monogenic* traits are determined by the different *alleles* of a single gene, polygenetic traits may display a range of possible phenotypes, determined by a number of different genes and the interactions between them.
The traits that are determined by polygenic inheritance are not simply an effect of dominance and recessivity, and do not exhibit *complete dominance* as in Mendelian Genetics, where one allele dominates or masks another. Instead, polygenic traits exhibit *incomplete dominance* so the phenotype displayed in offspring is a mixture of the phenotypes displayed in the parents. Each of the genes that contributes to a polygenic trait, has an equal influence and each of the alleles has an *additive effect* on the phenotype outcome.
Because of the inheritance mode patterns, the physical traits that are controlled by polygenic inheritance, such as hair color, height and skin color, as well as the non-visible traits such as blood pressure, intelligence, autism and longevity, occur on a continuous gradient, with many variations of quantifiable increments.

Polygenic inheritance should not be confused with the effects caused by *multiple alleles*. In the case of multiple alleles, a gene contains several different allele variants on the same locus of each *chromosome*, for example the three different alleles which control for blood type – A, B & O.

The probability of an offspring inheriting a certain characteristic from its parents can be determined using a *punnet square*, however, in reality there may be large numbers of different genes controlling for a single phenotype trait, so it becomes difficult to demonstrate. Fortunately, the distribution of phenotypes determined through polygenic inheritance usually fits into a *normal distribution* of probabilities, with most offspring displaying an intermediate phenotype of the two parents.

Using a simplified example of a polygenetic trait controlled by only three genes, this becomes easier to visualize.

Examples of Polygenic Inheritance

Skin Color

The pigment melanin is responsible for dark coloration in the skin and there are at least three genes, which control for human skin color. Using a hypothetical example where the production of melanin is controlled by *contributing alleles* (denoted here as A, B and C), resulting in dark skin color, and therefore light skin color is produced by *non contributing alleles* (denoted here as a, b and c), it is possible to see how the spectrum of different skin colors can result in the offspring.It is important to remember here that in polygenic inheritance, alleles do not display dominance over others, rather, each contributing allele gives an additive effect rather than a masking effect, and so the way that the alleles interact is different to those in Mendelian genetics. The additive effect means that each contributing allele produces one unit of color.

In an example using two parents, heterozygous for each of the melanin-producing genes (AaBbCc x AaBbCc), it is possible to see how the additive effects and combinations of alleles results in all the possible *genotypes*.

Heritability and its measurements

Heritability, amount of phenotypic (observable) variation in a population that is attributable to individual genetic differences. Heritability, in a general sense, is the ratio of variation due to differences between genotypes to the total phenotypic variation for a character or trait in a population. The concept typically is applied in behaviour genetics and quantitative genetics, where heritability estimates are calculated by using either correlation and regression methods or analysis of variance (ANOVA) methods.

MEASURING VARIATION

Before talking about the different flavors of heritability, it's useful to define what we mean by "variation" when we say things like the "the proportion of variation in a trait explained by" something.

Here, when we say "variation", we're referring to the mathematical concept of "variance". Variance is a common metric for measuring how much a trait differs between people in a group. Formally, it's the average squared difference between a randomly selected person and the "average" person. For example, across all men and women in the UK Biobank the variance of height in inches is 13.3 (86.0 for height in centimeters), corresponding to a standard deviation of 3.7 inches (9.3 cm). The standard deviation is simply the square root of the variance.

Statisticians like talking about variance (as opposed to more intuitive measures like the range or the mean absolute deviation from average) because it has nice mathematical properties. Most notably, if you have an outcome that is the sum of effects from independent sources (like, say, genes and environment) the variance of the effects from each source add up to the variance of the outcome. Being able to break up the total variance of a trait into different pieces that add up this way is very useful when we want to start talking about the "proportion of variance explained by genetics", as we will see below.

Lastly, talking about variance implicitly means we're talking about a group or population of individuals. You can't have an average difference between people with only one person. As we emphasize in Heritability 101, this means that whenever we talk about heritability we are talking about variation in some population of individuals, not about genetics determining some proportion of a trait in any given individual.

"EXPLAINING" VARIANCE

It's also worth clarifying the other half of the phrase "the proportion of variation in a trait explained by", namely what we mean by "explained". In this case, variance that is "explained" by genetics is variance that could be predicted based on genetic data if we had perfect information about the effects of all genetic variants (which, to be clear, we don't actually have).

If you've ever heard the phrase "correlation is not causation", that's the issue we're referring to here and why we aren't simply saying the proportion of variance caused by genetic effects.

We are closer to causation since it's fairly safe to assume that the heritable traits aren't causing the genetic variants, since our genetics is fixed at conception (with the exception of acquired mutations such as those seen in cancer). It is possible, however, for genetic variants to be correlated with environmental factors that have a direct causal impact on the trait. That doesn't mean the genetics aren't important and informative for that trait, but it does mean we have to be careful about describing effects as causal, even in genetics. So as a precaution against making any premature statements about causality we focus on "explained" variance instead.

BROAD-SENSE HERITABILITY

Our starting definition of heritability as "the proportion of variation in a trait explained by inherited genetic variants" refers to this most general version of heritability. Mathematically, we'd define the broad-sense heritability as: H2=σ2Gσ2PH2=σG2σP2 where σ2Gσ2G is the variance in the trait explained by genetics (G), and σ2Pσ2P is the total variance of the trait in the population.

We make three important observations about this definition. First, it's entirely flexible about how specific genetic effects contribute to σ2Gσ2G. The broad-sense H2H2 doesn't care whether σ2Gσ2G comes from a single Mendelian variant in just one gene, or the small additive effects from variants in 100 different genes, or complex interactions between every variant in the whole genome. We'll see below that this is an important distinction between broad-sense H2H2 and some of the other types of heritability.

Second, broad-sense H2H2 is entirely flexible about how σ2Gσ2G relates to σ2Pσ2P. We could choose to assume that the effects of genes and environment are independent and thus write:

H2=σ2Gσ2G+σ2EH2=σG2σG2+σE2 but that assumption isn't required. By simply writing the denominator as σ2Pσ2P we allow for the possibility that genetic and environmental factors are correlated or interact in some way. This is important since it highlights that the effect of environment on the trait isn't simply the "remainder" after accounting for all the genetic effects, instead they can overlap and interact in complex ways.

NARROW-SENSE HERITABILITY

In practice, the flexibility of broad-sense H2H2 makes it very hard to estimate without making strong assumptions. Allowing for effects of all possible interactions of all possible genetic variants means having a functionally infinite space of possible effects. One useful way to simplify this is to think of the total variance explained by genetics as a combination of

additive effects, dominant/recessive effects, and interaction effects between different variants.σ2G=σ2A+σ2D+σ2IσG2=σA2+σD2+σI2

For a number of reasons, we might expect the variance explained by additive genetic effects σ2AσA2 to be the largest and most immediately useful portion of the total σ2GσG2 [1]. Focusing on just this additive genetic component leads us to the definition of the narrow-sense heritability h2h2:h2=σ2Aσ2Ph2=σA2σP2

If there are no dominant/recessive or interaction effects (i.e. σ2D=σ2I=0σD2=σI2=0) then the narrow-sense and broad-sense heritability are the same (h2=H2)h2=H2). Otherwise the narrow-sense heritability will be smaller (h2<H2h2<H2) since it excludes these other types of genetic effects.

Historically, most scientific discussion of the heritability of different traits has focused on h2h2. One of the nice features of h2h2 is that it implies a simple relationship between between how genetically related two people are and how similar the trait will be for those two people. We can use this relationship to estimate h2h2 in twin and family studies.

In the simplest case, we can compare monozygotic twins (often called "identical" or MZ twins) to dizygotic ("fraternal" or DZ) twins. MZ twins shared all of their DNA [2], while DZ twins share half of their DNA on average. Twins also largely share the same environment regardless of whether they are MZ or DZ [3]. So to estimate h2h2 we can observe how correlated a trait is between pairs of MZ twins and how correlated the trait is between DZ twins and see if those correlations are different. If the MZ twins pairs, with their higher genetic similarity, are more strongly correlated than the DZ twin pairs, that suggests that genetics explains some of the variance in the trait [4].

There has been decades of scientific research on the heritability of human traits using this general approach. Helpfully, a recent effort by Danielle Posthuma and colleagues pooled together much of this work into a single webpage where you can browse twin-based estimates of h2h2 for a wide variety of traits.

SNP-HERITABILITY

The above flavors of heritability have referred to "genetic effects" conceptually without requiring any consideration of specific genetic variants and their association with the trait. Now that advances in genetics [5] have made it possible to actually collect data on these specific variants, there's the opportunity to evaluate how much each of these observed variants contribute to heritability.

In particular we can consider one type of genetic variant called a single nucleotide polymorphism (SNP), which is a change of a single base pair of DNA at a specific location in the genome. For example, some people may have an A at that location, while other people have a G. There are millions of these locations in the genome that commonly vary between different people, and much of the current research in human genetics is focused on understanding the effects of these variants [6].

So given a set "S" of SNPs that we've observed, how much of the variance in the trait can they explain? That leads us to define the SNP-heritability h2ghg2, the proportion of variance explained by additive effects of the observed SNPs, which we could write as:h2g=σ2SNPs∈Sσ2Phg2=σSNPs∈S2σP2

If we compare this to the above definitions, it's evident that h2g≦h2≦H2hg2≦h2≦H2 since h2ghg2 is limited to additive effects from only a subset of genetic variants.

This definition of h2ghg2 still hides the effects of individual SNPs though, so it's useful to introduce an alternate version. If we call our trait y, and say each SNP xjxj has an additive effect βjβj [7], then we can writey=∑SNPs∈Sxjβj+ϵy=∑SNPs∈Sxjβj+ϵ

where ϵϵ is a residual term for effects not explained by the sum of the SNP effects. We can then define h2ghg2 based on the variance of this sum of SNP effects compared to the total variance of the trait:h2g=var(∑SNPs∈Sxjβj)var(y)hg2=var(∑SNPs∈Sxjβj)var(y)

It's worth highlighting two key features of h2ghg2. First, you might notice that we've defined h2ghg2 based on some set of SNPs "S". In practice, this set of SNPs is going to depend on (a) the SNP data that has been observed and (b) the method used for estimating h2ghg2. This makes it tricky to compare values of h2ghg2 between different methods and different studies [8], though in most cases it's safe to at least assume it refers to commonly-occurring SNPs. Second, the variance explained by SNPs may or may not reflect the effects of those particular SNPs as opposed to the effects of other genetic variants the SNPs are correlated with. This is just an extension of our previous discussion above about the meaning of variance "explained", but worth reiterating since it would be easy to misinterpret SNP-heritability as fully excluding the causal effects of other types of genetic variation.

There are a couple of different methods that have been developed for estimating h2ghg2 from observed SNPs. In practice we don't know the true βjβj so we have to use other tricks. The first approach, known as GREML (Genomic relatedness matrix REstricted Maximum Likelihood; commonly implemented in GCTA), uses SNPs to estimate the genetic similarity between random individuals and compare that to their trait similarity. This is conceptually similar to the twin-based estimation described above, but uses the observed low-level genetic

similarity in SNP data from individuals who aren't directly related. You can read about the statistical details here with a more recent review here.

A second approach is called linkage disequilibrium (LD) score regression, implemented in ldsc. This is the method we are applying to the UK Biobank data set. LD score regression depends on the key observation that some SNPs are correlated with (i.e. in LD with) other genetic variants, so observing that SNP in turn "tags" information about the effects of other variants. The basic idea then is that if there are lots and lots of small genetic effects spread across the genome (i.e. the trait is "polygenic"), then the strength of the relationship between each individual SNP and the trait should be (on average) proportional to how much total genetic variation that SNP tags. Statistical details on the LD score regression method can be found here.

VARIANCE EXPLAINED BY KNOWN SNP EFFECTS

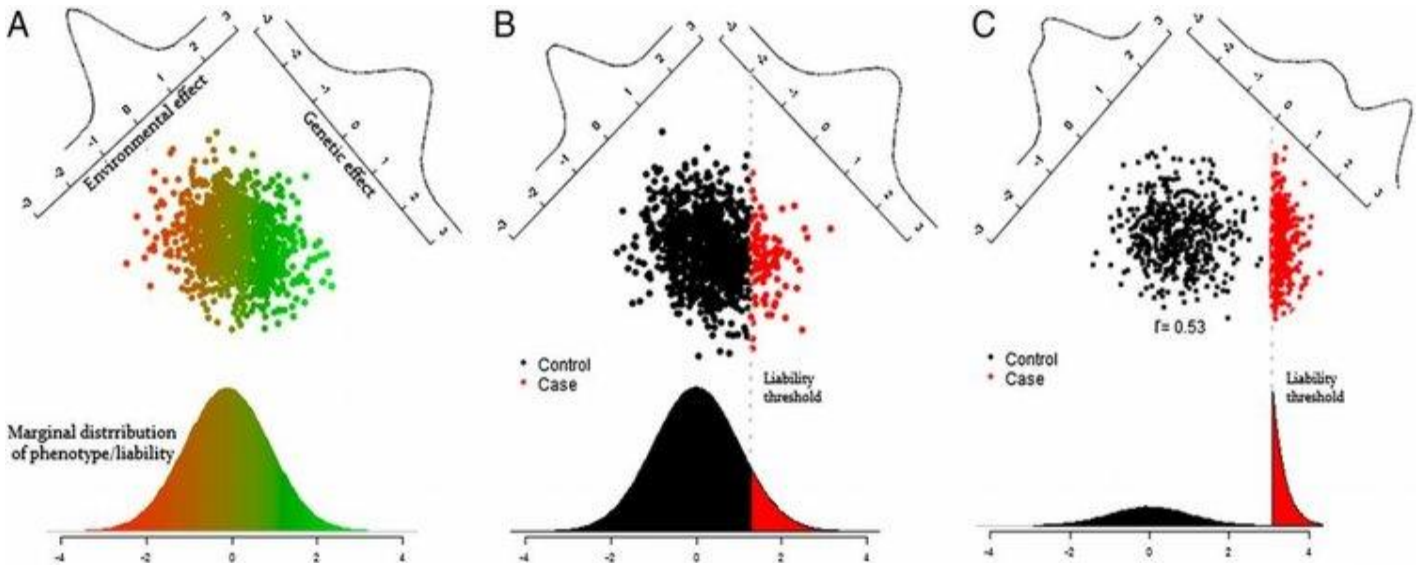
All of the above flavors of heritability are defined based on the "true" variance explained by genetic variants. Although we noted above that we don't know the true effects βjβj, we could estimate them from our observed SNP data and then use those estimated values to directly compute: h2PRS=var(∑SNPs∈Sxj^βj)var(y)hPRS2=var(∑SNPs∈Sxjβj^)var(y)

We refer to this version of heritability as h2PRShPRS2 since the sum with estimates βjβj is often known as a polygenic risk score (PRS). As with h2ghg2, it depends on the choice of a set S of SNPs. When this set is chosen to be only SNPs reaching genome-wide significance for evidence of association with the trait, this flavor of heritability is sometimes known as h2GWAShGWAS2.

Estimating h2PRShPRS2 is valuable because it indicates how well we can predict the trait from the observed SNPs with our current estimates of βjβj. In comparison, h2ghg2 indicates how well we could theoretically predict the trait from SNPs if we knew their true effect sizes. Inevitably h2PRS≦h2g(≦h2≦H2)hPRS2≦hg2(≦h2≦H2) since uncertainty in our estimates of βjβj reduces our prediction accuracy, but it's a useful way to contextualize h2ghg2 as the idealized maximum for h2PRShPRS2.

A NOTE ON LIABILITY VS. OBSERVED SCALE HERITABILITY

To some extent talking about components of the variance of a trait assumes the trait is continuous. For binary traits, such as whether or not someone is diagnosed with a disease, the use of variance as a convenient mathematical quantity becomes problematic.



The conventional solution is to treat the binary trait as if it has an underlying continuous liability, as depicted above, and then quantify the heritability of that continuous liability. In other words estimate the genetic contribution to the continuous liability as shown in the left plot, based on observing the binary outcome of that liability as shown in the center plot. In some cases we may intentionally select more individuals who have the binary outcome, as shown in the right plot, in which case we have to further adjust the heritability calculations for how that ascertainment has changed the distribution of liability in our sampled individuals.

The mathematical details of that adjustment to get heritability estimates on the liability scale [9] aren't critical, but it's important to be aware that we're having to make this additional adjustment for binary traits. This adjustment requires making assumptions about the prevalence of the trait in the population, which may or may not be safe in the UK Biobank data depending on the trait. As a result, the estimates of heritability for binary traits should be interpreted carefully, with an expectation that they are at a higher risk of statistical artifacts than than heritability estimates for continuous traits.

QTL mapping

A quantitative trait locus (QTL) is a region of DNA which is associated with a particular phenotypic trait, which varies in degree and which can be attributed to polygenic effects, i.e., the product of two or more genes, and their environment. These QTLs are often found on different chromosomes. The number of QTLs which explain variation in the phenotypic trait indicates the genetic architecture of a trait. It may indicate that plant height is controlled by many genes of small effect, or by a few genes of large effect.

Typically, QTLs underlie continuous traits (those traits which vary continuously, e.g. height) as opposed to discrete traits (traits that have two or several character values, e.g. red hair in humans, a recessive trait, or smooth vs. wrinkled peas used by Mendel in his experiments).

Moreover, a single phenotypic trait is usually determined by many genes. Consequently, many QTLs are associated with a single trait. Another use of QTLs is to identify candidate genesunderlying a trait. Once a region of DNA is identified as contributing to a phenotype, it can be sequenced. The DNA sequence of any genes in this region can then be compared to a database of DNA for genes whose function is already known.

Review Questions

Short Answer Questions **(2 Marks)**

1. Define reproduction.
2. Define conjugation.
3. Define transformation.
4. Define competence cell.
5. List out the steps in bacterial transformation.
6. Explain briefly about lytic cycle.
7. List out phage vectors.
8. Define pedigree analysis.
9. List some genetic disorders.
10. What is QTL mapping.
11. Define ploidy.

Essay Answer Questions **(6 & 8 Marks)**

1. Describe in detail about bacterial conjugation..
2. Describe in detail about bacterial transduction and its types.
3. Give a detailed note on bacterial transformation.
4. Describe in detail about bacteriophage.
5. Explain about M13 vectors.
6. Give a detailed note on phage vectors.
7. Structural and numerical alterations of chromosomes.

8. Describe in detail about Structural and numerical alterations of chromosomes.
9. Give a detailed notes on quantitative genetics.

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Question	Opt 1	Opt 2	Opt 3	Opt 4
Unit I				
Homologus chromosomes move towards the opposite poles of a dividing cell during	Mitosis	Meiosis I	Meiosis II	Fertilization
Bacterial cells are prokaryotic. Unlike a typcial eukaryotic cell they-----	Have no membrane-b	Lack a plasma membrane	have a smaller nucleus	have no ribosomes
Which of the following are similar between transcription in prokaryoes and eukaryotes?	RNA polymerase proc	b)RNA polymerase bind to	A polytail is added to th	Introns are present in genes which are spliced
Meiosis II is similar to mitosis in that	Sister chromatids sep	The daughter cells are dipl	Homologus chromosom	DNA replicates before the division
What is a chromatid?	A chromosome in G1	a replicated chromosome	a chromosome found o	a special region that holds two centromeres t
mt DNA is	simple double strand	simple,single stranded line	simple, single stranded	simple, double strand
The inner membrane of the mitochondria is usually higly convoluted forming a seris of infloding known as	Thylakoids	lamellae	cristae	grana
The beadlike unit of chromatic structure is the	chromatid	nucleosome	c)solenoid	kinetochore
The subunit of prokaryotic ribosomes are	60 S, 40S	70 S, 30S	60S, 30S	50 S, 30 S
Which of the following is NOT associated with animal cells?	plasmodesmata	an extracellualr matric	gap junctions	tight junctions
which of the following structure is found in eukaryotes but not in prokaryotes cells?	Mitochondria	Ribosomes	DNA	Plasma membrane
The sorting and packaging centre of the cell refers to what cellular component?	Golgi apparatus	Lysosomes	Nucleus	Mitochondria
Detoxification is a characteristic function of what type of organelle?	Mitochondria	Golgi apparatus	Peroxisomes	Nucleus
Which of the following cell structures exhibits selective permeability between a cell and its external environement?	The Plasma membrar	Mitochondria	Lysosomes	Chloroplast
The organelle involved in protein transport is	ER and Golgi	ER and mitochondria	lysosomes and golgi	Golgi and mitochondria
A component of the animal cell membranes that functions to stiffen the membrane and thus regualte its fluidity is	cholesterol	cellulose	pectin	lignin
Which of the following membrane has the large amount of proteins?	erythrocyte membrar	myelin sheath membrane	inner mitochondrial m	outer mitochondrial membrane
Nuclei were first discovered by?	Strasburger	Fonatana	Robert Brown	Robert Koch
Which of the following is the central commanding center of the cell?	Nucleus	Mitochondria	ER	d)Ribosomes
Nucleus is absent in	Red blood cells and b	Red blood cells, sieve cells	Red blood cells only	Bacterium only
Nucleus has	DNA only	DNA and Protein only	DNA, RNA and proteins	carbohydrates and lipids
Nuclear membrane is in continuous connection with-------	SER	RER	Golgi apparatus	lysosomes
The light stained and diffused region of chromatin is known as	Heterochromatin	Euchromatin	Chromatin	Chromosomes
The most important function of nuclear envelope is to	regulate nucelo cytop	protect genetic material	prevent the entrance of	synthesis rRNAs
The basic proteins of the nucleus are	nucleohistones	nucleoprotamines	nucleohistones, nucleo	protamines
Bacterial cell wall is made up of	N-acetyl glucosamine	N-acetyl muramic acid	N-acetyl muramic acid	N-acetyl glucosamine, N-acetyl muramic acid
Which of the following statements are not true regarding cell wall?	plant cell wall is made	Plant cell wall is a non-liv	Cell wall provides mech	Cell wall is semi permeable
Fine cytoplasmic connections between neighbouring cells through the cell wall for cell to cell communication is called	Plasmosome	plasmodesmata	mesosome	chromosomes
Which of the following organelle is involved in cell wall synthesis?	Mitochondria	Chloroplast	Golgi apparatus	Lysosomes
Plant cell wall is made up of	Cellulose only	Cellulose and chitin	Cellulose, hemicellulose	cellulose, hemicellulose and pectin
Cellulose is a polymer of D-glucose units joined by	α 1-4 linkage	β 1-4 linkage	α 1-6 linkage	β 1-6 linkage
Chitinous cell wall is present in	plants	Bacteria	protists	Fungi
Which statements best explains the evolutionary advantage if meiosis?	Meiosis is necessary f	Meiosis alternates with m	The same genetic syste	Genetic recombinations are possible from ge
Meiotic divisions occurs in	Vegetative cells	meristamatic cells	conductive cells	reproductive cells
Meiosis I is -----division	additive	reductional	subractive	oxidative
Meiosis II is equational division due to	crossing over	separation of chromatids	pairing of homologus cl	disjunction of homologus chromosomes
Meiosis II performs----	Separation of sex chrc	synthesis of DNA and cent	separation of homologu	separation of chromatids
Synapsis occurs between	mRNA and ribosomes	spindle fibres and centrom	a male and a female ga	Two homologus chromosomes
In Meiosis, the daughter cells differ from parent cell as well as amongst themselves due to	Segregation, indepen	Segregation and crossing c	Independent assortmer	segregation and independent assortment
When parental and maternal chromosomes change their materials with each other in cell division this vent called	synapsis	crossing over	Dyad forming	Bivalent forming
Continous variations are attirbuted to	polyploidy	mutation	crossing over	chromosomal aberrations
During which stage of prophase I of the crossing over takes place?	Leptotone	b)Pachytene	c)Zygotene	Diplotene
During mitosis, ER and nucleolus begin to disapper at	Late prophase	Early prophase	Late metaphase	early metaphase
Which among the following stage represents the best stage to view the shape, size and number of chromosomes	Metaphase	Anaphase	prophase	telophase
During metaphase mitosis chromosomes	undergo coiling	line up at the equator	breaks up	break up and disintegrate
What aspect of mitosis is affected by colchicine in inducing polyploidy?	DNA replciation	Spindle formation	Formation of cell plate	chromosome doubling
The site of light reaction is	Grana	stroma	thylakoid lumen	outer membrane
Which of these describes a holliday junction?	A Section of DNA whe	A strand of DNA containin	An interaction of two st	A three stranded DNA structure where single
Which of the following elements would be considered to be a complete transposable element?	An insertion sequenc	A composite element that	A replicative transposo	A retrotransposon containing long terminal r
A chromosome with a very short arm and a very logn arm is termed as	Telocentric	Acrocentric	Metacentric	d)sub-metacentric
Chromosme is thickest during	prophase	metaphase	anaphase	interphase
The lowest level of chromosome organization is	solenoid	nucelosome	30 nm fibre	super helix
The term chromosome was coined by	W. Flemming	W. Roux	Waldeyer	Sutton
The number of autosome in humans	44	21 pairs	46	45
The diagrammatic representation of karyotype (morphological representation of chromosomes) of a species is called	idiogram	cladogram	ecogram	chromogram
Chromatin has	DNA	DNA and proteins	DNA, RNA and proteins	Proteins and lipids
The fisrt man to observe live cell under microscope was	Robert hooke	Leeuwenhoek	Schleiden	Virchow
The nucleus was first described by	Robert Brown	Robert hooke	Weismann	Nageli
All the following statements are truw regarding the "cell theory" except	All living things or org	All cells arise spontaneous	cell is the basic structur	All cells arise from preexisting cells
The membrane around the vacuole is called	cytoplast	tonoplast	amyloplast	chromoplaast
Unit II				
Lipid bilayer is -----	hydrophilic	hydrophobic	hydrophilic and hydropl	depends on the surrounding medium
Which is the best definition of active transport?	Movement of molecu	movement of a substance	movement of molecules	Movement of molecules in and out of the pe
The current theroy of the structure of the plasma membrane is best described by the --------- model	sandwich	fluid mosaic model	electrochemical	unipermeable
In a phospholipid bilayer, the -----	phosphate groups are	fatty acid tails are ionized	fatty acid tails are hydr	phosphate heads are oriented toward the ex
Whether a molecule can cross the plasma membrane depends upon----	the charge of the mol	valency of the molecule	chemical bond of the m	atomic number of the moelcule
If a cell is placed in a hypertonic solution, which will occur?	Salts will move in to t	water will move out of th	Salts will move out of t	water will move out of the cell in to the surr
If a cell is placed in a hypotonic solution, which will occur?	Salts will move in to t	water will move in to the	Salts will move out of t	water will move out of the cell in to the surr
If a cell is placed in the isotonic solution which will occur?	Salts will move in to t	water will move in to the	Salts will move out of t	water will move in to and out of the cell in e
In plants --------is a shrinking of the cytoplasm due to osmosis	Plasmolysis	Endocytosis	Crenation	Diffusion
The sodium-potassium pump moves sodium and potassium ions across the plasma membrane by ------	Facilitated transport	active transport	cotransport	Endocytosis
Sugars and amino acids are carried in to the cell by means of ---	Facilitated transport	active transport	cotransport	Endocytosis
Cell products are secreted from the cell through	Facilitated transport	active transport	exocytosis	Endocytosis
Pinocytosis is an example of -----	Facilitated transport	active transport	endocytosis	exocytosis
Active transport ----------	requires an input of A	is involved in diffusion	occurs in osmosis and f	is needed for water to move through cell me
The major functions of the plasma membrane do NOT include	separation of the fluic	regulation of molecules ar	recognition and commu	production of proteins used in the constructio
The role of carbohydrate in cell membrane	cell adhesion	cell-cell recognition	assisting transport acro	cell storage reserve
The major biomolecule responsible for selective uptake of materials across plasma membrane	Carbohydrate	protein	lipids	phospholipids
At physiological pH increase in cholesterol level	Increases fluidity	decreases fluidity	no change in the fluidit	equal change in the fluidity
All the following substance pass through the cell membrane except	O2	b)H2O	CO2	d)H+
The major interactions responsible for stabilizing plasma membrane	hydrophilic interactio	covalent bonds	ionic bonds	hydrophobic interactions
Clathrin coated pits are associated with	phagocytosis	pinocytosis	receptor mediated endo	exocytosis
Na+ glucose transporter is an example of	facilitated diffusion	ATP driven active transpor	Symport	antiport
Which of the following transport induces conformational change in protein?	simple diffusion	active transport	facilitated diffusion	ion driven active transport
The erythrocyte glucose transporter is an example of	simple diffusion	active transport	facilitated diffusion	ion driven active transport
In cell membrane, carbohydrates in glycoproteins or glycolipids are oriented	towards outside	towards inside	towards outside and in	randomly distributed
The distribution of instrinsic proteins in the cell membrane is	symmetrical	assymetrical	random	uniform
High lipid content is a characteristic of	erythrocyte membrar	myelin sheath membrane	inner mitochondrial me	outer mitochondrial membrane
Which of the following membrane has the largest amount of proteins	erythrocyte membrar	myelin sheath membrane	inner mitochondrial me	outer mitochondrial membrane
The longer the fatty acid chain, fluidity of the lipid bi layer	increases	decreases	no change in fluidity	remains the same
Which transport mecahansim can bring whole cells into a cell?	pinocytosis	phagocytosis	facilitated diffusion	primary active transport
Water moves via osmosis	throughout the cytop	from an area with a high c	from an area with a hig	from an area with a low concentraion of wat
Cells may be said to be "sugar coated" due to the presence of	phospholipids	glycolipids	glycocalyx	sphingolipids
Which of the following molecular components of the lipid bilayer of the plasma membrane possesses a charged polar " head" and an uncharged nonpolar "tail"	glycoprotein	b)cholesterol	phospholipid	glycolipid
Vesicular transport is invloved in all of the following transport processes, except	exocyosis	Endocytosis	phagocytosis	solute pumps
All of the following are functions of membrane receptors, except	G protein-linking	contact signalling	chemical signalling	cell adhesion
Paracrine signalling is invloved in which of the following?	hormonal communiac	chemical signals that can	autostimualtion of a ce	receptor medaited signalling
Neurotransmitters are released in to the synapse at which of the following?	presynaptic membrar	postsynaptic membrane	golgi apparatus	axon hillock
Fluid mosaic model was given by	Robertson	Schwann	Dave Donson	Singer and Nicholson
Middle lamella is composed mainly of	protein	Hemicellulose	carbohydrate	calcium pectate
The lipids in the bilayer of the cell membrane have tails that are	hydrophilic	hydrophobic	zwitter ionic	polar
Unsaturated fatty acids in the lipid bilayer are bended giving rise to a	kink	rounded	straight	circular
Which of the following is not a form of active transport?	osmosis	bulk membrane transport	exocytosis	endocytosis
What is osmosis?	the diffusion of wate	the diffusion of water acrc	c)the active of transpor	the absorption of water from the environme
What is a ligand?	any molecule that bir	any molecule that can pas	the term for materials i	the term used for unprocessed lipids
Which of the following is not a typical function of membranes?	storage	site of recognition	selective transport	permeability barrier
The engulfing of bacteria by white blood cells is called as	Phagocytosis	Pinocytosis	Exocytosis	Endocytosis
The concentration gradient is said to exist when the	Concentration of ions	concentration of ions is le	hypertonic solution ent	water enters by osmosis
Which of these is part of the cell membrane?	aminoacids	phospholipids	ATP	glucose only
How do fat-soluble moelcules normally get into a cell?	they dissolve in the fl	they pass through protein	they are absorbed by pl	they never get in
Which of the statements best describes the "fluid mosaic model" of the structure of the cell membrane?	two layers of protein	b)two layers of lipid with	a double layer of lipid n	a single layer of protein on the outside and a
The movement of chloride iosn from an area where chloride is concentrated to an area where chloride is less concentrated is which of these?	diffusion	active transport	osmosis	exocytosis
If a cell solute concentration of 0.07% which od the solutions would be hypotonic to the cell?	0.01% solute	b)0.1% solute	1% solute	10% solute
which of the following is necessary in order for osmosis to occur?	a permeable membra	a semi-permeable membr	an isotonic solution	ATP
which of these are passive transport mechanisms?	osmosis	diffusion	phagocytosis	osmosis and diffusion
In an isotonic solution there would be----	no net movement of	net movement of water in	net movement of wate	d)bursting of the cell
The sodium-potassium pump carries sodium out of a cell and potassium in to a cell is an example of--------	active transport	Endocytosis	exocytosis	d)passive transport
when a cell bursts due to osmosis, it is in a solution that is-----	hypertonic	isotonic	hypotonic	normal
what is likely to happen to a plant cell that is placed in pure water?	it becomes turgid	it becomes flaccid	in undergoes plasmolys	it bursts
Simple nerve reflexes use signalling molecules called	neurotransmitters	nitric oxide	G proteins	proteases
In terms of cell communications, what do bacterial pathogens such as cholera and anthrax have in common?	They destroy the rece	They prevent the producti	They alter the chemical	They block the normal functioning of signal t
Unit III				
which of the following enzymes are used to join fragements of DNA?	DNA ligase	DNA polymerase	primase	endonuclease
Semiconservative replication of DNA was confirmed by the which scientist's experiments ?	Meselson and Stalh's	watson and crick	griffith's experiment	Mendels experiment
Unwinding of DNA is done by	helicase	ligase	hexonuclease	topoisomerase
During the replication of DNA, the sythesis of DNA on lagging strand takes place in segments, these segments are called	Satellite segments	Double helix segments	kornberg segments	Okazaki segments
Which of the following nitrogenous bases is found in DNA but is not found in RNA?	Adenine	Thymine	Guanine	Uracil
Nucleotides have a nitrogenous base attached to a sugar at the------	1' Carbon	2' Carbon	3' Carbon	5' carbon
The DNA of a certain organism has been found to have guanine as 30% of its bases. What percentage of its bases would be adenine?	30%	60%	20%	40%
The Process of DNA replication involves:	Multiple origins of re	Binding of ribosomes to or	Continous synthesis on	Conservative replciation with one original do
RNA is less stable than DNA because	RNAses are ubiquitou	RNAses are extremely he	denatured RNAses read	d)RNA is chemically stable
By convention, a DNA strand is written in an orientation that places the template strand	reading in a 5'→3' dir	reading in a 3'→5' directi	reading in a 2' → 5' dir	reading in a 1' →3' direction
The structural feature that allows DNA to replicate itself is the	sugar-phosphate bacl	complementary pairing of	phosphodiester bondin	twisting of the molecule to form an alpha he
If the base order in one strand of DNA is 5'-CATTAG-3', what is the order of bases in the complementary DNA strand?	3'-GTAATC-5'	b. 3'-GUAAUC-5 '	3'-CATTAG-5'	5'-GTAATC
In metabolically active cells the proportion of mRNAs is _____ and the proportion of rRNAs is _____	55-60%, 25-30%	25-30%; 55-60%	80-90%, 2.5-5%	2.5-5%; 80-90%
A nucleotide is made up of which of the following chemical components?	A nitrogenous base, a	A nitrogenous base, an ar	A nitrogenous base, an	A nitrogenous base, a phosphate group, and
Genetic code is	the sequence of nitro	is a triplet code	is non-overlapping	anticodon
A nucleoside is compsoed of	a base + a sugar	a base+ a sugar + phospha	a base+ a phosphate	lipids+ proteins
genetic mutation occurs in	protein	RNA	DNA	Nucleus
DNA is present in	viruses, prokaryotes a	prokaryotes and eukaryot	only in eukaryotes	in some viruses, prokaryotes and eukaryotes
The two strands in a DNA double helix is joined by	Co-valent bond	hydrogen bond	ionic bond	phosphodiester bond
The basic repeating units of a DNA molecule is	nuceloside	nucleotide	histones	amino acids

Adjacent nucleotides are joined by	covalent bond	phosphodiester bond	ionic bond	peptide bond
The length of one turn of DNA is	3.4 angstrom	34 angstrom	20 angstrom	3.04 angstrom
The type of sugar in DNA are	triose	tetrose	c)pentose	hexose
The width of DNA molecule is	15 angstrom	3.4 angstrom	20 angstrom	25 angstrom
The length of DNA having 23 base pairs is	78 angstrom	78.4 angstrom	78.2 angstrom	74.8 angstrom
Left handed DNA	A-DNA	B-DNA	c)Z-DNA	C-DNA
Z-DNA have a	double helical nature	Zig-zag appearance	uracil base	single stranded nature
Chargaff's rule states that in a double stranded DNA molecule	Concentration of deo	Concentration of Deoxyad	Concentration of Deoxy	Concentration of Deoxy uridine (U) nucelotid
Choose the incorrect statement out of the following	Double stranded DNA	The B-form is usually foun	single turn of B-DNA ab	d)The distance spanned by one turn of B-DN
When the DNA molecule is twisted in the direction opposite from the clockwise turns of the right-handed double helix found in B-DNA, such DNA is said to have acquired	Z form	A form	Positive supercoils	Negative supercoils
Choose the correct statement out of the following?	The common form od	The coding strand is copie	The two strands of the	The G-C bonds are much more resistant to de
RNA is a polymer of purine and pyrimidine ribonucleotides linked together by	Hydrogen bonds	hydrophobic interactions	vander wal's forces	3'-5' phosphodiester linkages
Which of the following types of RNA participate in RNA processing?	t-RNA	r-RNA	small nuclear RNA (snR	Small interfering RNAs (siRNAs)
The anticodon region is an imprtant structural component of	mRNA	DNA	rRNA	tRNA
The small nuclear RNAs are rich in	uracil	cytosine	thymine	adenine
The Z-DNA helix	has fewer base pairs	has alternating GC sequen	tends to be found at th	is inhibited by methylation of the bases
An endonuclease is an enzyme that hydrolyzes	A nucelotide from on	A nucelotide from either t	A phsophodiester bond	A bond only in the specific sequence of nucel
Which of the following has the highest percentage of modified bases?	hnRNA	b)t-RNA	sn-RNA	m-RNA
Choose the incorrect statement about an RNA?	has a single stranded	Does not obey chargaff's r	c)Does not exhibit wats	instead of uracil contains the ribonucleotides
Which of the following statements is incorrect about tRNA species?	they have the smalles	All tRNAs have between 1	tRNA molecules contair	They make up 15% of the tRNA molecule for
Semi conservative replication of DNA was first demonstrated in	E.Coli	S.penumonae	S.typhimuriam	D.melanogaster
Mode of DNA replication in E.Coli is	Conservative and unic	b)Semiconservative and u	Conservative and bidire	semiconservative and bidirectional
When DNA replication starts	The phosphodiester b	the bonds between the nit	the leading strand prod	the hydrogen bonds between the nucleotide
The elongation of the leading strand during DNA synthesis	Progresses away from	occur in 3'-5' direction	produces okazaki fragm	Depend on the action of DNA polymerase
Eukaryotes differ from prokaryote in mechanism of DNA replciation due to-----	Different enzyme for	use of DNA primer rather	unidirectional rather th	discontinous rather than semidiscontinous re
The accepted hypothesis for DNA replication is	conservative theory	b)dispersive theory	semi-conservative theo	evolutionary theory
When DNA polymerase is in contact with guanine in the parental strand, what dose it add to the growing daughter strand?	Phosphate	cytosine	uracil base	Guanine
Telomeres are usually rich in which nucleotide?	Adenine	Guanine	Thymine	Cytosine
Which is the largest among the following?	Nucleotide	Nitrogenous base	Phosphate	carbon
Each replication bubbles consists of	3 replication forks	2 replication forks	4 replication forks	1 replication fork
In a cesium chloride gradient, DNA labelled with N15 and centrifuged will form a band	above DNA containin	below DNA containing N1	with N14 containing DI	inbetween N14 and N15
The role of primase	dismantle RNA prime	celave and unwinds short	proofread base pairing	synthesize an RNA primer to begin the elong
DNA replciation in eukaryotes occurs only in	G1 phase	S phase	G2 phase	M phase
Who's X-ray work aided watson and crick in their discovery of the double helix?	W.H. bragg	R.Franklin	L.pauling	Leaderberg
Which polymerase is active in DNA proofreading during replication?	polymerase I	polymerase II	polymerase III	polymerase IV
The genetic code is	universal	not universal	species specific	kingdom specific
Which type of gene sequence occupies the most space in the bacterial chromosome?	Origins of replication	Structural gene sequence	intergenic regions	Equally origin of replication and structural ge
Which of the following is a description of the proof reading function of DNA polymerase?	Endonuclease cleavag	b)exonuclease cleavage	methylation	an induced fit phenomenon
What enzyme performs decatenation?	polymerase I	topoisomerase	c)telomerase	decatenase
What is a key difference between DNA polIII and DNA ligase?	OnlyDNA Pol III synth	only DNA ligase synthesize	DNA pol III can synthesi	DNA ligase can use energy from ATP ratehr t
Unit IV				
The normal function of the promoter is to	Bind the small subuni	Serve as an origin of replic	Serve as an acceptor fo	Serve as a binding site for RNA polymerase
Protein molecules are polymers of-----	DNA moelcule	Amino acid molecules	Fatty acid molecules	Sucrose moelcules
The peptide bond is -----	A covalent joing simp	A covalent bond joining n	A hydrogen bond joing	A covalent bond joining aminoacid together
In prokaryotes, the ribosomal binding site on mRNA is called	Hogness sequence	Shine-Dalgarno sequence	Pribnow Sequence	TATA box
During translation, the role of enzyme peptidyl transferase is	transfer of phosphate	amino acid activation	peptide bond formatio	binding of ribosome subunits to mRNA
Polysomes are	Aggregation of ribosc	aggregation of lysosomes	mRNA molecules to wh	a strech of tRNA assemblies
Translation is the	Synthesis of DNA fror	Synthesis of protein from	Synthesis of RNA from	Synthesis of RNA from a DNA template
During translation, proteins are synthesized	by ribosomes using tl	by lysosome using the infc	by ribosomes using the	by ribosomes using the information on rRNA
The enzyme involved in amino acid activation is	ATP synthetase	aminoacyl tRNA synthetas	aminoacyl mRNA synth	aminoacyl rRNA synthetase
Which of the following RNA molecules serves as an adaptor molecule during protein synthesis	rRNA	mRNA	tRNA	tRNA and mRNA
In eukaryotes, translation is inititated by binding of ribosomes to the	Pribnow box	Hogness box	5' CAP	poly A tail
Which is the energy rich molecule required for initiation of translation	ATP synthetase	GTP	CTP	AMP
Which of the following best explains the sequence of central dogma	DNA makes RNA mak	RNA makes DNAmakes Pr	DNA makes proteins m	Protein makes DNA makes RNA
Many primary transcripts of noncoding RNAs must be -------in order to be functional	translated	polyadenylated	processed	replicated
RNA polymerase used for the transcription of genes require a -------template	rRNA	DNA	RNA	mRNA
Which of the following statements is not true?	Transcription but not	Bacterial non coding RNAs	The regulation of the tr	Linking of exons must precede translation in
Regarding RNA polymerase in E.Coli which of the following describes the difference between the holo enzyme and the core enzyme?	The holoenzyme is us	The holoenzyme consists c	The core enzyme and n	The holoenzyme binds to DNA upstream of tl
RNA polymerase II transcribes all of the following except genes specifying	rRNAs	proteins	snRNAs	miRNAs
The recognition sequence to whiich RNA polymerase binds at the initiation of transcription is found-----	downstream of the pr	b)upstream of the gene to	withinthe first intron	downstream of the transcription bubble
Which of the following best describes a promoter?	An element that pron	A regulatory protein that	A specific target sequer	An extracellular environmental inducer that
Some eukaryotic promoters contain an element positioned around nucleotide +1 called----	the TATA box	the pribnow box	an initiator (Inr) sequer	an inverted repeat
Null genes are characterized by their-----	transcription by RNA	possession of neither TATA	Invariable start positior	inability to be transcribed
The conversion of a closed rpomoter complex to an open promoter complex in bacteria requires------	the activity of alterna	hydrogen bond breakage (	a G-C rich sequence aro	strong interaction between the core enzyme
An improtant difference between the initiation of prokaryotic and eukaryotic transcription of protein coding genes is that--------	only prokaryotic gene	only eukaryotic RNA polyr	eukaryotic promoters ir	d)only prokaryotes cells alter the rate of tran
Each time a ribonucleotide is added to the elongating RNA molecule during transcription a-----is released	deoxy ribonucleotide	sugar-base component	c)pyrophosphate	phosphate
The 5'end of mRNAs made by RNA polymerase II possess a distinct cap structure depicted in short-hand notation as-----	7-MeGppNpN	7-MeGpppNpN	pppN-7MeGpppNpN	pppNpN
The formation of hairpin loops at the 3' end of prokaryotic RNAs during transcription results in------	stabilization of DNA:F	activation of poly (polyme	a reduction in base pa	Rho-dependent activation of transcription
Which of the following statements regarding the poly (tail of eukaryotic RNA is incorrect?	The poly (tail is locat	The poly (tract is transcrit	poly (tails are found or	Tissue specific gene expression patterns soen
Translation occurs in the	nucleus	cytoplasm	nucleolus	lysosome
What is the main function of tRNA in relation to protein synthesis?	inhibits protein synth	proof reading	identifies amino acids a	promoter binding
Which site of tRNA molecule hydrogen bonds to a mRNA molecule?	codon	anticodon	5, ends of the tRNA mo	3' ends of the tRNA molecule
In the context of prokaryotic gene expression, which of the following is the msot appropriate definition of an operator?	A cluster of genes tha	A DNA-binding protein tha	A non-coding regulator	A non-coding regualtory DNA sequence that
In terms of lac operon regulation, what happens when E. Coli is grown in meidum containing both glucose and lactose?	Both CAP and the lac	CAP is bound to the DNA I	Lac repressor is bound t	d)Neither CAP nor the lac repressor are boun
Which of the following can be described as "a sequence that can be several thousand base pairs upstream or downstream of a eukaryotic promoter and which increases gene expression as much as 200-fold"?	CAAT box	TATA box	Insulator	TATA box
Which of the following statements, concerning regulation of trp operon expression by attenuation, is correct?	The leader peptide se	the leader peptide sequen	Rapid translation of the	Rapid translation of the leader peptide preve
Which of the following is true of the lac operon in E.Coli?	the operon is only sw	The lac operon messenger	The enzyme β-galactosi	The promoter is the binding site for the lac re
Transcription occurs along a ------template forming an mRNA in the ------direction	5' to 3'; 5' to 3'	5' to 3'; 3' to 5'	3' to 5'; 5' to 3'	3' to 5'; 3' to 5'
Which of the following statements below is false?	The genetic code is o	the genetic code is not un	Degenerate codons spe	The genetic code is triplet
The first mRNA codon to specify an amino acid is always	TAC	UAA	UAG	AUG
The aminoacid sequence of a polypeptide chain comprises the ----structure of the protein	primary	secondary	tertiary	Quaternary
Transfer RNA's bind during translation by the ----	codon	anticodon	template	mRNA
Which of the following statements is true regarding introns?	introns are the parts i	introns have no functions	In general human genes	Introns may be involved in exon shuffling
The effort to decipher the genetic code was led by-----who was awarded a nobel prize for his work	Nirenberg	Lederberg	Watson	Crick
What sequence on the template strand of DNA corresponds to the first amino acid inserted in to a protein?	TAC	UAA	UAG	AUG
Which of the following is an example of the degeneracy of the genetic code?	a given amino acid ha	each codon specifies more	the fiorst two bases spe	the genetic code is not degenerate
During translation, the-----site within the ribosome hold growing amino acid chain while the ----site holds the next amino acid to be added to the chain	A, P	P, A	A, B	B, A
The only methylated base in mammals is?	7-methyl guanine	thymine	methyl adenine	5-methyl cytosine
Repressor molecules bind to the	promoter	enhancer	c)operator	hormone response element
Which of the follwoiung is false about the E.Coli Lac operon?	It is polycistronic	It is an example of negativ	c)The presence of lacto	The repressor binds to the promoter
The part of the bacterial RNA polymerase responsible fopr recognizing the promoter is the ----	Alpha subunit	Rho protein	DNA pol III	Sigma subunit
In protein synthesis which out of the following is not a termination codon?	UAG	UGA	UUU	UAA
All are true for DNA polymerase except one---	Has exonuclease activ	Works only in the 5' to 3'	Edits as it synthesizes	Synthesizes RNA primer to initiate DNA synth
What is added to the 3' end of many eukaryotic tRNAs after transcription?	introns	b)Cap of modified G nucle	Poly A tail	Trinucleotide CCA
Which one of the following molecules is not a component of the 30S initiation complex?	GTP	mRNA	Initiation factor 2	ATP
Enhancer regions in eukaryotic DNA are---	DNA pol I binding site	Inhibit the binding of repr	Enhance the frequency	Specific for given set of genes
What are the coding segments of a strech of eukaryotic DNA called?	introns	exons	codons	replicons
A particular triplet of bases in the template strand of DNA is 5' AGT 3'. The corresponding codon for the mRNA transcribed is	3' UCA 5'	3' UGA 5'	5' TCA 3'	3' ACU 5'
RNA polymerase moves in which direction along the DNA?	3' to 5' along the tem	3' to 5' along the coding (s	5' to 3' along the templ	3' to 5' along the coding strand
What type of bonding is responsible for maintaining the shape of tRNA molecule?	covalent bonding bet	ionic binding between pho	c)hydrogen bonding bet	van der waals interactions between hydroger
To code phenylalanine aminoacid which codon on the mRNA strand coldes for this amino acid	UGG	GUG	GUA	UUC
Which of the following statements are true about protein synthesis in prokaryotes?	Extensive RNA proces	Translation can begin whil	Unlike eukaryotes, prok	d)Translation requires antibiotic activity
Unit V				
Which of the following statements is true regarding transposons?	They are sequences o	They exist in corn, but are	They are most abundan	they are coding sequences
Mutations	Are premanent chang	Produce genetic recombin	They are not harmful	They are permanent and found to be more h
Mutations that cause loss of a chromosome would be termed	Structural mutations	Chromosome mutations	Genome mutations	Single-gene mutations
A frame shift mutation could be caused by	A transition	A transversion	A deletion of 3 bp	Insertion or deletion of any number of base
Which of the following point mutations would be most likely to affect protein function?	TAA to TGA	CAA to TAA	AGG to AGA	CTT to CTC
The mutation which causes sickle cell anemia in humans	Is a base substitution	Is a deleterious mutation	Is a missense mutation	due to base substituion or deletion or misser
Which of the following would be a nexample of a germline mutation?	Exposure to excessive	In a very early human emt	A man with normal chr	An embryo missing one copy of the third chr
Mitochondrial DNA polyemrase does not have an error checking mechanism such as that of nuclear DNA polymerase. This would be expected to lead to a higher rate of which type of mutation in mtDNA?	Spontaneous mutatio	induced mutations	Deletions	Insertions
An alteration in a nucelotide in a nucelotide sequence that changes a triplet coding for an amino acid in to a termination codon	nonsense mutation	Mutation	Mutagenesis	Mutagen
The main differene between the directed mutation theory and the randome mutation theory is-----	The chemical nature (	The effect of the mutation	The cause of the mutati	The heritability of the trait
Examples of environmental mutagens include?	Alkylating agents	nucleotide base analogues	Ionizing radiation	Alkylating agnets, nucelotide base analogs ar
A bacterial cell experiences a mutation as a result of exposure to nitrogen mustard and then divides several times to produce a toal of eight cells. How many of the resulting cells would you expect to contain the mutation?	One	two	Four	Eight
In the Ames test:	mutagens cause letha	Mutagens cause mutation	Mutagens will cause an	only mutagens that cause transitions can be
Which of the following forms of DNA repair doest NOT require DNA polymerase?	Direct DNA repair	Base excision repair	Nucleotide excision rep	Mismatch repair
Why is alkyltransferase only able to be used once?	The protein is hydrol	The protein is chemically c	The protein becomes at	Inability to bind to another region
how does recombinational repair differ from nucelotide excision repair (NER)?	Unlike NER, recombir	NER replaces the thymine	NER requires DNA polyr	NER involves nick translation, which recombi
A point mutation that replaces a purine with another purine, or a pyrimisine with another pyrimidine	Transition	Transversion	c)Nonsense	Missense mutation
A point mutation that involves a purine being replaced by a pyrimidine or vice versa	Transition	Transversion	Nonsense	Silent mutation
A change in a DNA sequence that has no effect on the expression or functioning of any gene or gene product	Transition	Transversion	Nonsense	silent mutation
An alteration in a nucleotide sequence that converts a codon for one amino acid int a codn for a second amino acid	Transition	Transversion	missense	Silent mutation
An alteration to the normal chemical or physical structure of the DNA	Transition	Transversion	c)Lesion	Missense mutation
5-bromouracil is an analog of -----that can react with deoxyribonucleic acid to produce a polymer with increased susceptibility to mutation	Thymine	Guanine	cytosine	Uracil
All transposons encode a ----which catalyzes the insertion	DNA glycosylase	Excisionase	Transposase	d)integrase
Small DNA sequences that can move to virtually any position in a cell's genome	Exons	introns	LTRs	Transposons
In base excision repair, the lesion is removed by-----	DNA glycosylase	Transposase	Excisionsase	DNA polymerase
The exchange of non homologus regions of DNA at specific sites is independent of ----	IS elements	Illegitimate recombinatior	Retrotransposons	RecA
Cyclobultane pyrimidine dimers can be monomerized again by -----in the presence of visible light	Exonuclease	DNA photolyases	Transposases	DNA polymerase
The dispersed repetitive sequences found in higher eukaryotic DNA (eg LINES and SINES) probably spread through the genome by---	Transposition	homologus recombination	site specific recombinat	General recombination
The enzyme of E.Coli is a nuclease that initiates the repair of double stranded DNA breaks by homologus recombination	RNA polymerase	b)DNA polymerase	RecBCD	DNA ligase
Which of the following DNA mutation that result in the appearance of a stop codon in the resulting mRNA synthesis	Transition	Transversion	Nonsense	Missense mutation
Which type of mutation is most likely to revert?	deletion	translocation	inversion	transition
Which type of mutation is least likely to revert?	Deletion	translocation	inversion	transition
The hydrolysis of an -NH2 group from a base is called----, while intercalating agents such as proflavin function as mutagens by causing------	deamination; transve	deamination; deletions or	excision repair; deletior	excision repair; transversions
ultraviolet radiation is most likely to produce what form of mutation?	deaminations	b)depurination	double stranded breaks	thymine dimers
Duplication of multiple three- nucelotide repeats is responsible for	Sickel cell anemia	b)trisomy 21	alkaptonuria	xeroderma pigmentosum
Errors in DNA replication are most often corrected by	SOS systems	Base excision repair	Nucleotide excision rep	methyl-directed mismatch repair
A mutation in the UvrC protein of E.Coli would result in which of the following?	an increase in overall	a decrease in over allmuta	an increase in transposi	a decrease in transposition events
The---------- are heritable changes in base sequences that can affect phenotype	mutation	duplication	replication	d)transcription
A mutation that changes a wild type allele of a gene to a different allele is called a -----mutation	forward	reverse	inversion	deletion
A -------mutation causes a novel allele to be converted back to a wild type allele	forward	reverse	inversion	deletion
An----------is a type of mutation where a segment of a chromosome is rotated 180°	forward	reverse	inversion	d)deletion
Any physical or chemcial agent that increases the rate of mutation above the spontaneous rate is a	mutagens	b)plasmogens	antigens	antibodies

A mutation in which parts of two homologus chromosomes change places is called a	translocation	transition	transversion	deletion
The hydrolysis of a purine base from the deoxy-ribose phosphate backbone is called	depurination	deamination	replica plating	excision repair
UV light is a mutagen that can cause:	depurination	deamination	alkylation	thymine dimers
unequal crossing over results in	an exchange betweer	a loss of genetic material	repair of UV induced da	a creation of deletions and duplications
Base analogs differ from other classes of mutagen in that they	only alter bases	can only cause transversio	only work during DNA r	will not function in bacterial cells
Intercalating agents such as acridine orange function as mutagens to------	promote transitions	remove amine groups	fit between stacked bas	add ethyl or methyl groups
A complementation group is	a group of mutations	a group of mutations that	a group of mutations th	group of mutations in two different genes th
The condition sickle cell anemia is due to	the insertion of an an	the deletion of an amino a	substitution of an amin	failure to synthesize a hemoglobin molecule
Mutations that abolish the function encoded by the wild type allele are known as	null mutations	hypomorphic mutations	hypermorphic mutatior	conditional mutations
Choose the condition below that does not involve a defect in an enzymatic pathway	alkaptonuria	albinism	sickle cell anemia	phenylketonuria
The Ames test for mutagenicity is useful to identify potential carcinogens because	since bacteria do not	mutagens that affect bact	cbacteria thrive on sub	the same genes that cause cancer in humans
In the Ames test for mutagenicity:	auxotrophic bacteria	prototrophic bacteriaare c	cells are treated with m	cells are treated with excess amino acids killi
If a base analog such as 5- Bromouracil is used as a mutagen, how many generations will be required to mutate the codon for proline (CCin to the codon for alanine (GCC)?	one generation	two generations	three generations	it will not occur
The duplication of the triplet sequence CGG resulting in elongation or breakage of the X-chromosome is termed	Barr-eyed	huntington's disease	unequal crossing over	fragile X chromosome
The genetic condition xeroderma pigmentosum, which can lead to skin cancer, results from:	inability to correct UV	inability to process phenyl	inability to produce fun	breaks in the X chromosomes
Excision repair corrects DNA by:	removing a double str	detecting, removing and r	excising the incorrect b	correcting A=T to G=C transitions
The unit of gene mutation is---------	cistron	recon	muton	photon
Mutations that occurs under natural conditions are called------	point B	Induced C	base	Spontaneous
A----phenotype mutations result in the death of cells or organisms	Sub vital	Lethal	Super Vital	Induced

Answer

Meiosis I
Have no membrane-bounded organelles in their cytoplasm
RNA polymerase produces mRNAs which grow in the 5'-3' direction
Sister chromatids separate during anaphase
a replicated chromosome
simple double stranded linear DNA molecule
cristae
nucleosome
50 S, 30 S
plasmodesmata
Plasma membrane
Golgi apparatus
Peroxisomes
The plasma membrane
ER and Golgi
cholesterol
inner mitochondrial membrane
Robert Brown
Nucleus
Red blood cells, sieve cells and bacterium
DNA and Protein only
RER
Euchromatin
prevent the entrance of active ribosomes in to the nucleus
nucleohistones, nuceloprotamines
N-acetyl glucosamine, N-acetyl muramic acid and amino acids
Cell wall is semi permeable
Plasmodesmata
Gologi apparatus
cellulose, hemicellulose and pectin
β 1-4 linkage
Fungi
Genetic recombinations are possible from generation to generation
Reproductive cells
Reductional
separation of chromatids
separation of chromatids
Two homologus chromosomes
Segregation, independent assortment and crossing over
Crossing over
Crossing over
Pachytene

Metaphase
line up at the equator
Spindle formation
thylakoid lumen
An interaction of two strands of DNA from homologus chromosomes
An insertion sequence that include a transposase gene between the inverted repeats
Acrocentric
metaphase
nucleosome
W. Flemming
44
idiogram
DNA, RNA and proteins
Leeuwenhoek
Robert Brown
All cells arise spontaneously
tonoplast

hydrophilic and hydrophobic
movement of a substance against its concentraion through the release of energy from ATP
fluid mosaic model
phosphate heads are oriented toward the exterior of the cell or towards the cytoplasm
the charge of the molecule
water will move out of the cell in to the surrounding solution
water will move in to the cell from the surrounding solution
water will move in to and out of the cell in equal rates
Plasmolysis
active transport
Facilitated transport
exocytosis
endocytosis
requires an input of ATP
production of proteins used in the construction of cells
cell-cell recognition
proteins
decreases fluidity
H+
hydrophobic interactions
receptor mediated endocytosis
Symport
facilitated diffusion
facilitated diffusion
towards outside
assymetrical
myelin sheath membrane
inner mitochondrial membrane
increases
phagocytosis
from an area with a high concentration of water to one of lower concentration
glycolipids
phospholipid
solute pumps
cell adhesion
chemical signals that can only travel limited distances between cells
postsynaptic membrane
Singer and Nicholson
calcium pectate
hydrophobic
kink
bulk membrane transport
the diffusion of water across a permeable membrane
any molecule that binds specifically to another's receptor site
storage
Phagocytosis
concentration of ions is lesser inside the cell and more outside the cell
phospholipids
they dissolve in the fat layers of the membrane and enter the cell by diffusion
a double layer of lipid molecules with protein molecules suspended in the layer
diffusion
0.01%
permeable membrane
osmosis and diffusion
no net movement of water
active transport
hypotonic
it becomes turgid
neurotransmitters
They block the normal functioning of signal transduction mechansims

DNA ligase
Meselson and Stalh's experiment
helicase
Okazaki segments
Uracil
5' Carbon
20%
Multiple origins of replication per chromosme in eukaryotes
RNAses are ubiquitous

reading in a 5'-→3' direction
complementary pairing of the bases
3'-GTAATC-5'
80-90%, 2.5-5%
A nitrogenous base, a phosphate group, and a pentose sugar
is a triplet code
a base + a sugar
DNA
in some viruses, prokaryotes and eukaryotes
hydrogen bond
nucleotide

phosphodiester bond
34 angstrom
pentose
20 angstrom
74.8 angstrom
Z-DNA
Zig-zag apperarance
Concentration of deoxyadenosine (nucleotides equals that of thymidine (T) nucleotides
single turn of B-DNA about the axis of the molecule contains six base pairs
Negative supercoils
The G-C bonds are much more resistant to denaturation than A-T rich regions
3'-5' phosphodiester linkages
small nuclear RNA (snRN
tRNA
uracil
has alternating GC sequences
A phsophodiester bond located in the interior of a polynucelotide
t-RNA
instead of uracil contains the ribonucleotides of thymine
All tRNAs have between 10-20 nucleotides
E.Coli
semiconservative and bidirectional
the hydrogen bonds between the nucleotides of two strand break
Depend on the action of DNA polymerase
discontinous rather than semidiscontinous replication
semi-conservative theory
cytosine
Guanine
Nucleotide
2 replication forks
below DNA containing N14
d)synthesize an RNA primer to begin the elongation process
S Phase
R.Franklin
polymerase I
not universal
Equally origin of replication and structural gene sequences
exonuclease cleavage
decatenase
DNA ligase can use energy from ATP ratehr than nucleotides

Serve as a binding site for RNA polymerase
Amino acid molecules
A covalent bond joining aminoacid together to form a polypeptide
Shine-Dalgarno sequence
peptide bond formation between adjacent amino acids
mRNA molecules to which many ribosomes are attached simultaneously
Synthesis of protein from a mRNA template
by ribosomes using the information on mRNA
aminoacyl tRNA synthetase
tRNA
5' CAP
GTP
DNA makes RNA makes Proteins
processed
DNA
Transcription but not translation is regulated in bacteria
The holoenzyme consists of five subunits including σ, while the core enzyme lacks σ
rRNAs
upstream of the gene to be transcribed
A specific target sequence to which RNA polymeraase binds
an initiator Inr sequence
possession of neither TATA box nor an Inr sequence
hydrogen bond breakage of base pairs around the initiation site
eukaryotic promoters indirectly recognize core promoter sequences
pyrophosphate
7-MeGpppNpN
a reduction in base pairing between the template strand and the RNA transcript
The poly (tract is transcribed from the DNA template
cytopalsm
identifies amino acids and transport them to ribosomes
anticodon
A non-coding regulatory DNA sequence that is bound by a repressor protein
Neither CAP nor the lac repressor are bound to the DNA
Enhancer
Rapid translation of the leader peptide prevents completion of the mRNA transcript
The lac operon messenger RNA is the polycistronic mRNA
3' to 5'; 5' to 3'
The genetic code is overlapping
AUG
primary
anticodon
Introns may be involved in exon shuffling
Nirenberg
TAC
a given amino acid has more than one codon
P, A
thymine
operator
The repressor binds to the promoter
sigma subunit
UUU
Synthesizes RNA primer to initiate DNA synthesis
trinucleotide CCA
ATP
Enhance the frequency of transcription
Exons
3' ACU 5'
3' to 5' along the coding (sense) strand
hydrogen bonding between base pairs
UUC
Translation can begin while transcription is still in progress

They are most abundant type of repeat in the genome
They are permanent and found to be more harmful than beneficial
Chromosome mutations
Insertion or deletion of any number of base pairs that is not a multiple of 3
CAA to TAA
due to base substituion or deletion or missence mutation
A man with normal chromosomes exposed to radiations, his progeny will be with chromosomal deletion
Deletions
non sense mutations
The cause of the mutation
Alkylating agnets, nucelotide base analogs and ionizing radiations
Four
Mutagens will cause an increas in the number of revertants
Direct DNA repair
The protein is chemically changed by the addition of a methyl group
NER replaces the thymine dimer while recombinational repair leaves it in place
Transition
Transversion
silent mutation
missense
Lesion
Thymine
Transposase
Transposons
DNA glycosylase
RecA
DNA photolyases
Transposition
RecBCD
Nonsense
inversion
deletion
deamination; deletions or insertions
thymine dimers
alkaptonuria
Base excision repair
a decrease in transposition events
mutation
forward
reverse
inversion
mutagens

translocation
depurination
thymine dimers
a creation of deletions and duplications
only work during DNA replciation or repair
fit between stacked bases and disrupt replication
a group of mutations that are in the same gene and do not complement each other
substitution of an amino acid
null mutations
sickle cell anemia
mutagens that affect bacterial DNA are likley to casue human mutation
auxotrophic bacteria are converted to protrophs which survive
it will not occur
fragile X chromosome
Inability to correct UV induced dimers
detecting, removing and replacing a single stranded fragment of damaged DNA
muton
spontaneous
Lethal

Reg. No.....

[14BTP103]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2014 onwards)

M.Sc. DEGREE EXAMINATION, NOVEMBER 2014

First Semester

BIOTECHNOLOGY

CELL BIOLOGY AND MOLECULAR GENETICS

Time: 3 hours Maximum : 60 marks

PART – A (10 x 2 = 20 Marks)
Answer any TEN Questions

1. Define cell?
2. What are the characteristics of cell?
3. Define cell theory?
4. What are the nucleic acids present in organism?
5. Define chargaff's law?
6. What is nucleotide?
7. Define law of dominance?
8. What is mono-hybrid cross?
9. What are the dominance characters?
10. Define transposons.
11. What is the role of transposons?
12. Define class III transposons?
13. Define virulent phage.
14. What is lytic phage?
15. What is transduction?

PART B (5 X 8= 40 Marks)
Answer ALL the Questions

16. a. Give a detailed account on eukaryotic cell structural organization.

Or

b. Explain in detail about the process of mitotic cell division.

17. a. Explain in detail about Mendelian principles.

Or

b. Discuss in detail about mutation and its types.

18. a. Explain the types of transposons in prokaryotes.

Or

b. Discuss in detail about transposition in bacteria.

19. a. Explain the methods of gene transfer in bacteria.

Or

b. Explain in detail about transduction and conjugation.

20. **Compulsory : -**

Describe in detail about DNA replication.

Reg. No.....

[12BTP103]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2012 onwards)

M.Sc. DEGREE EXAMINATION, APRIL 2013

First Semester

BIOTECHNOLOGY

CELL BIOLOGY AND MOLECULAR GENETICS

Time: 3 hours

Maximum : 100 marks

PART – A (15 x 2 = 30 Marks)
Answer ALL the Questions

1. What is cell cycle?
2. Define eukaryotes
3. Who discovered and coined the word cell.
4. Define meiosis
5. What are histone protein and non –histone proteins.
6. Define homologous recombination
7. Define plasma membrane and any 2 of its functions.
8. Define active and passive transport
9. Define signal transduction and one example of it.
10. What is genetic code?
11. Define DNA replication
12. What is ribosomes?
13. Define translation
14. What are regulatory regions? Give some examples.
15. What mutagen and its types?

PART B (5 X 14= 70 Marks)
Answer ALL the Questions

16. a. Describe the structure, organization of eukaryotes and its organelles

Or

b Describe in detail the membrane transport and its types

17. a. Explain the process of translation and its post translational mechanisms

Or

b. What is an operon and explain the 'Lactose operon' switching on/off

18. a. What are mutations? Comment on types of mutations, mutagens and its effects.

Or

b. Explain the different types of DNA repair mechanisms.

19. a. What is a prokaryotic transposons and its mechanisms.

Or

b. Describe an eukaryotic transposons types and its mechanisms

20. a. What are bacteriophages? Comment on its general properties, structure

Or

b. Write an account on role of phage vectors

Reg. No.....

[12BTP103]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2012 onwards)

M.Sc. DEGREE EXAMINATION, NOVEMBER 2012

First Semester

BIOTECHNOLOGY

CELL BIOLOGY AND MOLECULAR GENETICS

Time: 3 hours Maximum : 100 marks

PART – A (15 x 2 = 30 Marks)
Answer ALL the Questions

1. What are postulates of cell theory?
2. Define prokaryotes.
3. What is cell and who discovered and coined the word cell?
4. Define mitosis and meiosis
5. What is replication?
6. Define genetic recombination and its models
7. Define plasma membrane and any 2 of its functions .
8. Define DNA repair
9. Define cell signalling and one example of it.
10. What is spilcing and its types
11. Define mendelian principles in brief
12. Draw the structure of t –RNA
13. Define transcription.
14. What is operon? Give some examples.
15. What transposon and its types.

PART B (5 X 14= 70 Marks)
Answer ALL the Questions

16. a. Explain the structure and properties and function of plasma membrane
(Or)
b. Describe the cell theory, properties of the cell and structure of prokaryotic cell.

17. a. Explain the models of DNA replication and its types
(Or)
b. Describe the process of transcription and its post transcriptional mechanisms.

18. a. Write an account on the principle of mendelian and non-mendelian inheritance.
(Or)
b. Explain the linkage and crossing over events.

19. a. What are mutations? Comment on types of mutations, mutagens and its effects.
(Or)
b. Comment on transposons and retro transposons.

20. a. What is gene transfer? Comment on types of gene transfer methods in bacteria
(Or)
b. Comment on Transformation and conjugation in bacteria.

Reg. No.....

[15MBP103]

KARPAGAM UNIVERSITY

Karpagam Academy of Higher Education
(Established Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2015 onwards)

M.Sc., DEGREE EXAMINATION, NOVEMBER 2015

First Semester

MICROBIOLOGY

MOLECULAR GENETICS

Time: 3 hours Maximum : 60 marks

PART – A (20 x 1 = 20 Marks) (30 Minutes)
(Question Nos. 1 to 20 Online Examinations)

(Part - B & C 2 ½ Hours)

PART B (5 x 6 = 30 Marks)
Answer ALL the Questions

21. a. Write down the difference between prokaryotic and eukaryotic DNA replication.

Or

b. Explain
i) Leading and Lagging strands ii) Single-strand DNA-binding protein.

22. a. What are mutagens? Write note on mutagenic agents.

Or

b. Give a brief account on SOS repair.

23. a. Explain post translational modification of proteins in eukaryotes.

Or

b. What are transcriptional factors?

24. a. Give an account on detection and isolation of phage T4 mutants.

Or

b. Describe the lytic cycle of a bacteriophage.

25. a. Write note on Yeast Artificial Chromosome.

Or

b. Explain about petite mutants.

PART C (1 x 10 = 10 Marks)
(Compulsory)

26. Illustrate regulation of gene expression using *Lac* operon model.
