Semester – I

19BTP102 CELL BIOLOGY AND MOLECULAR GENETICS 4H – 4C

Instruction Hours / week: L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**
End Semester Exam: 3 Hours

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 (CO's):

1. 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.
2. 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.

SUGGESTED READINGS

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

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

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

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

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

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

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

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

KARPAGAM ACADEMY OF HIGHER EDUCATION

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Coimbatore – 641 021.

LECTURE PLAN

DEPARTMENT OF BIOTECHNOLOGY

STAFF NAME: Dr. T.SOUNDARA RAJAN
SUBJECT NAME: CELL BIOLOGY AND MOLECULAR GENETICS SUB.CODE:19BTP102
SEMESTER: I CLASS: I M.Sc.

SL.NO.	HOURS	TOPIC	SUPPORTING MATERIALS
		UNIT I	
1	1	Cell organization and regulation: Structure of prokaryotic and eukaryotic cells	T1: 10-18, W2
2	1	Structural organization and function of intracellular organelles - Nucleus	T2: 56-57
3	1	Endoplasmic reticulum, Golgi complex	T1: 299-303
4	1	Mitochondria, Chloroplast	T1: 174-209
5	1	Lysosomes, Peroxisomes and Vacuoles	T1: 308
6	1	Cytoskeleton	T2: 381- 397
7	1	Chromatin organization and packaging	T2: 71- 74
8	1	Nucleic acid – Replication, Types	T1: 386-390; 534-546
9	1	Transcription, Post Transcriptional modification	T1: 425-440
10	1	Translation, Post Translational modification	T1: 455-456, 529
11	1	Regulation of Gene Expression	T5: 1085-1097
		Total Hours for Unit I: 11	
		UNIT II	
1	1	Structure of model membrane, lipid bilayer and membrane protein	T1: 117-125
2	1	Diffusion, Osmosis, Ion Channels, Active transport and Ion pumps	T2: 51-53
3	1	Intracellular protein sorting – Mechanism and regulation of intracellular transport in Mitochondria	T2:213-215; 221

4	1	Chloroplast	W1
5	1	Endoplasmic reticulum and Nucleus	T2: 215-225
6	1	Electrical properties of membranes	T2: 51
7	1	Cell cycle and its regulation	T1: 560-599
8	1	Molecular events, Check points, Cyclins and Protein kinases	T2: 408
		Total Hours for Unit II: 8	
UNIT III			
1	1	Genetics: Mendelian and Non-Mendelian principles	T5: 21-24
2	1	Concept of gene: Allele, multiple alleles, Pseudoallele	T6: 462
3	1	Complementation tests and Genetic recombination	T4: 939-953
4	1	Genetic mapping	W2
5	1	Linkage and Crossing Over	T4: 724-734
6	1	Mutations – types of mutations	W3
7	1	Genetic analysis of Mutations	W3
8	1	DNA repair mechanisms	T4: 851-864
		Total Hours for Unit III: 8	
UNIT IV			
1	1	Methods of Genetic transfers	T1: 146-156
2	1	Transformation, Conjugation and Transduction	T1: 146-156
3	1	Mapping genes by interrupted mating	T6: 395
4	1	Linkage maps, Tetrad analysis	T6: 331, 441

5	1	Mapping with molecular markers	T6: 212, 397
6	1	Mapping by using somatic cell hybrids	T6: 215
7	1	Introduction to transposable elements – Discovery and types	W4
8	1	Nomenclature – Insertion sequences - Mechanism	
9	1	Transposans of E.Coli, Bacteriophage and Yeast	T1: 402-415
		Total Hours for Unit IV: 9	
UNIT V			
1	1	Microbial and Human Genetics	T3, T8
2	1	Gene transfer in Bacteria	T1: 145-146
3	1	Bacteriophages – properties, structure, role of phages as vectors	T1: 163-167
4	1	Human Genetics – Pedigree analysis, Linkage testing	T6: 102
5	1	Karyotypes, Genetic disorders	T3: 178, 478
6	1	Eugenics	T3: 756
7	1	Epigenetics and Genome imprinting	T3: 515
8	1	Structural and numerical alterations of chromosomes	T6: 423
9	1	Ploidy and their genetic implications	T3: 198
10	1	Quantitative genetics – polygenetic inheritance	T3: 668
11	1	Heritability and its measurements	T3: 228
12	1	QTL (Quantitative trait locus) Mapping	T6: 456
		Total Hours for Unit V: 12	

References

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

T2 – Bolsover et al., Cell Biology: A short course, 2nd ed. John-Wiley and Sons Publications, New Jersey.

T3 – Klug et al. Concepts of Genetics. 8th ed. 2006. Pearson. New York.

T4 – Aminul Islam. Textbook of Cell Biology, 1st ed., Books & Allied (P) Ltd., Kolkata.

T5 – Gardner G 2001, Principles of Genetics, Wiley, New York.

T6 – Strachan and Read, Human Molecular Genetics, 3rd ed. Garland Science, New York.

T7 – Maloy et al. Microbial Genetics. 2006. Jones and Bartlett Publishers. Massachusetts.

W1 - https://www.ncbi.nlm.nih.gov/books/NBK21652/

W2 - www:biologydiscussion.com

W3- https://www.ncbi.nlm.nih.gov/books/NBK21475/.

W4 - https://www.nature.com/scitable/topicpage/transposons-the-jumping-genes-518/

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). Cytoskeleton. 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.

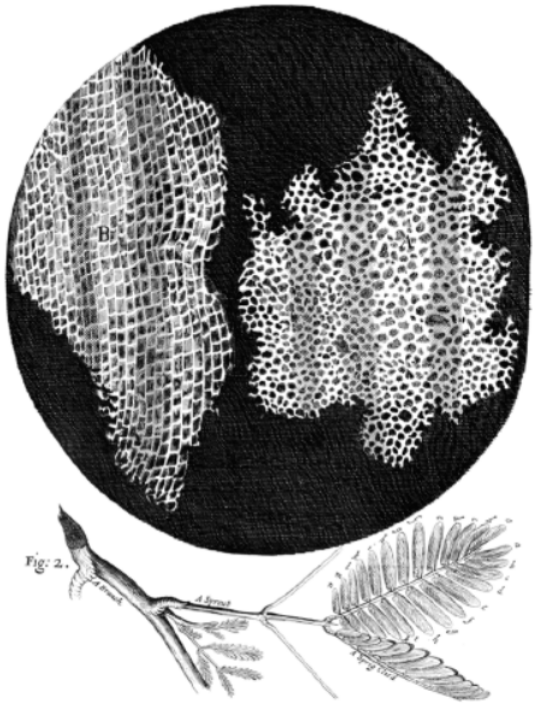

Fig: Drawing of the structure of cork by Robert Hooke that appeared in Micrographia.

"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.

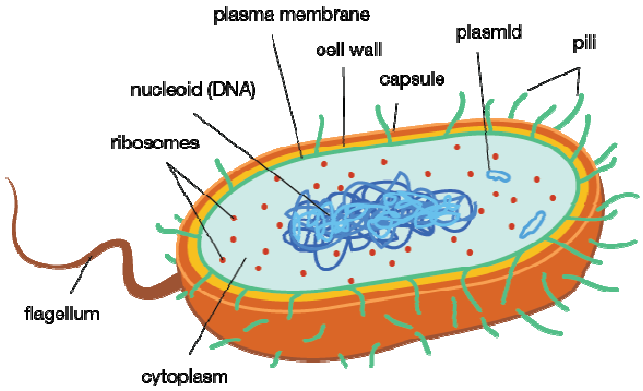

Fig: Schematic diagram of a prokaryotic cell.

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:

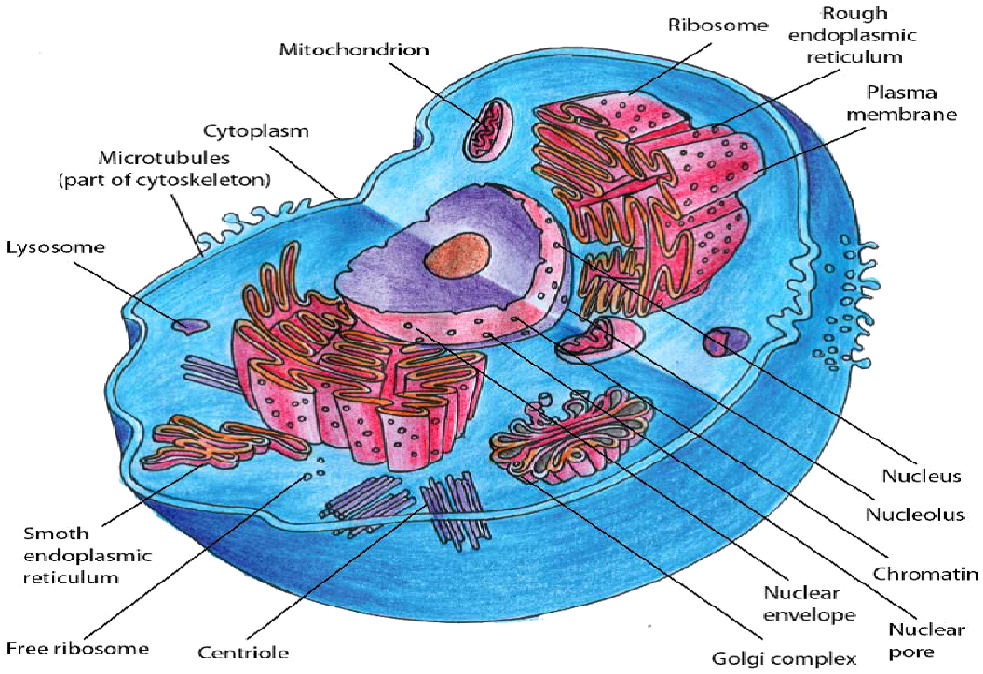

Fig: Eukaryotic cell.

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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.

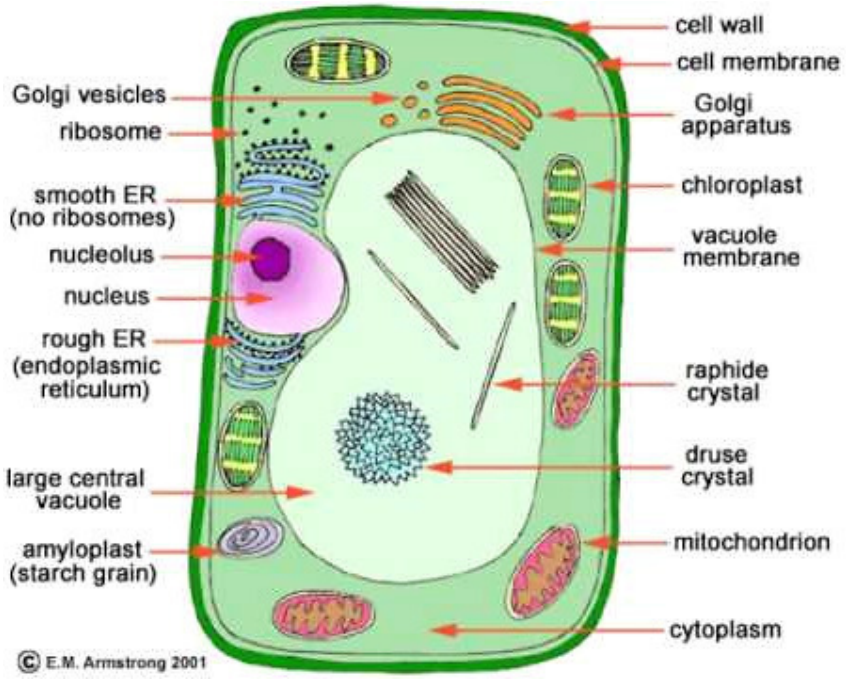


Fig: ***Anatomy of Plant Cell.***

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).

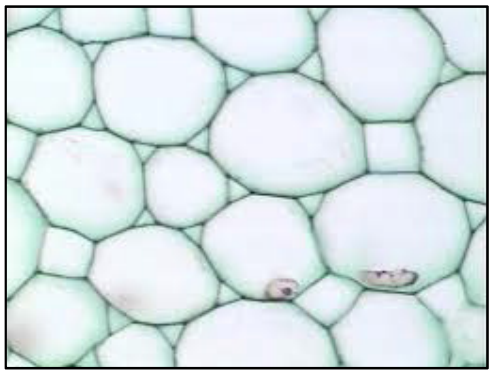


Fig: ***Parenchyma cells which have thin primary cell wall.***

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.

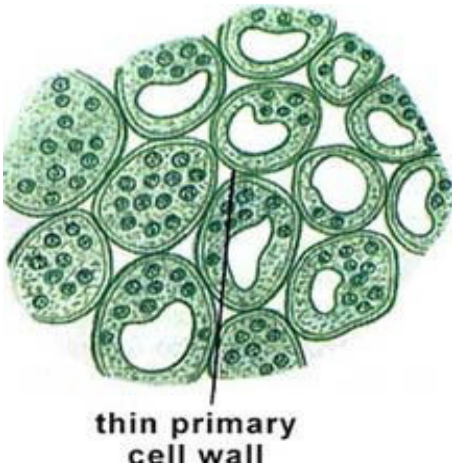


Fig: Typical collenchyma cell.

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.

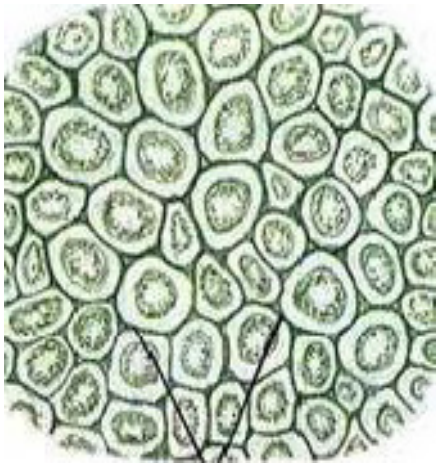


Fig:Sclerenchyma cells with irregularly thickened cell wall.

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.

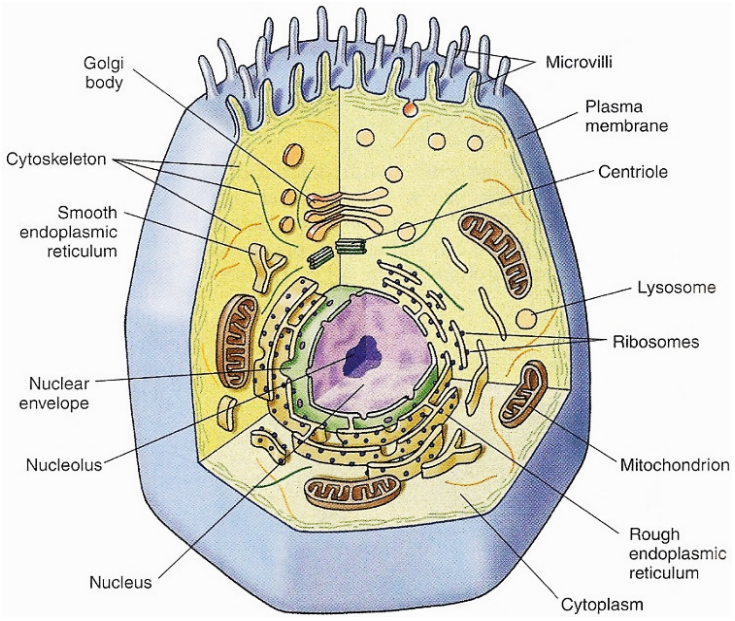


Fig:Schematic representation of a typical animal cell.

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 them.**	**Can not synthesis *amino acid, coenzymes, and vitamins* required by 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.***

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).

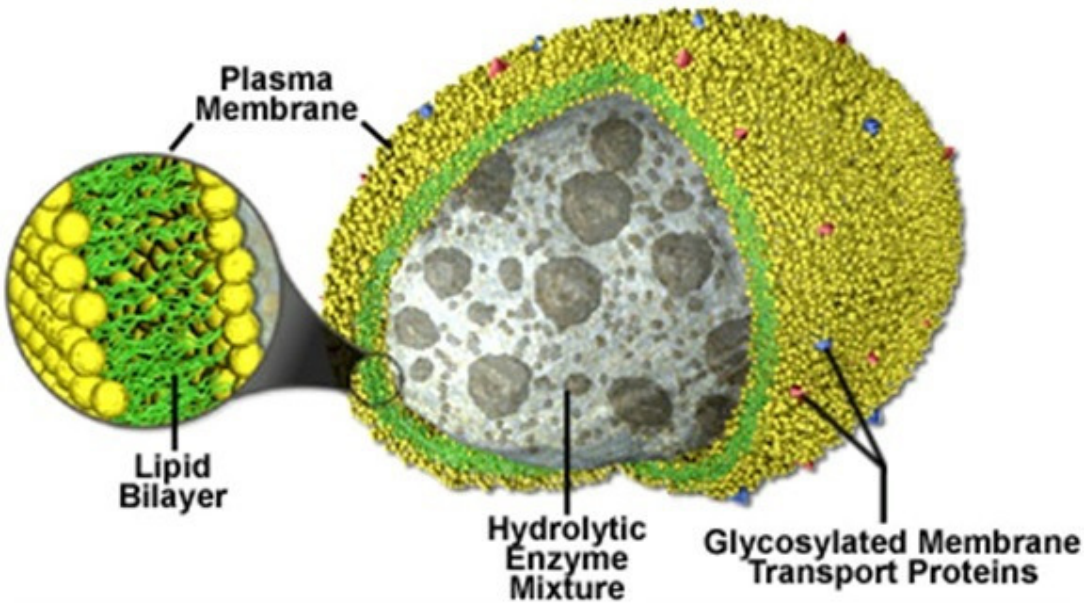


Fig : ***Anatomy of the Lysosome.***

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.

Ribosomes

- Ribosomes are the protein synthesis units of a cell described by G.E. Palade in 1952. They are complex of ribosomal RNA and various proteins.
- Ribosomes are small, dense, rounded and granular particles of the ribo-nucleoprotein.
- They occur either freely in the matrix of mitochondria, chloroplast and cytoplasm or remain attached with the membranes of the endoplasmic reticulum.
- They occur in most prokaryotic and eukaryotic cells and provide a scaffold for the ordered interaction of all the molecules involved in protein synthesis.
- They are the most abundant RNA-protein complex in the cell, which directs elongation of a polypeptide.

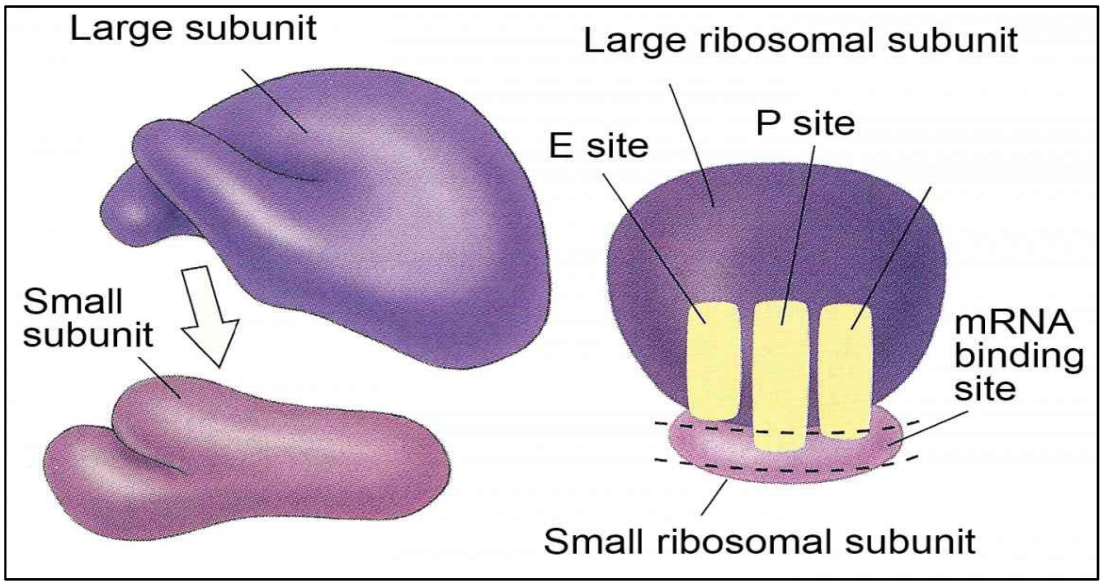


Fig :*Structure of Ribosome.*

Occurrence and distribution:

- The ribosomes occur in both prokaryotic and eukaryotic cells. In prokaryotic cells the ribosomes often occur freely in the cytoplasm or sometimes as polyribosome.
- In eukaryotic cells the ribosomes either occur freely in the cytoplasm or remain attached to the outer surface of the membrane of endoplasmic reticulum.
- The yeast cells, reticulocytes or lymphocytes, meristamatic plant tissues, embryonic nerve cells and cancerous cells contain large number of ribosomes which often occur freely in the cytoplasmic matrix.
- Cells like the erythroblasts, developing muscle cells, skin and hair which synthesize specific proteins for the intracellular utilization and storage also contain large number of free ribosomes.
- In cells with active protein synthesis, the ribosomes remain attached with the membranes of the endoplasmic reticulum.
- ***Examples:***are the pancreatic cells, plasma cells, hepatic parenchymal cells, Nissls bodies, osteoblasts, serous cells or the submaxillary gland, thyroid cells and mammary gland cells.

Types of ribosomes:

- Ribosomes are classified into two types based on their sedimentation coefficient, 70S and 80S.
- S stands for *Svedberg unit* and related to sedimentation rate (sedimentation depends on mass and size). Thus, the value before S indicates size of ribosome.

- ***70S Ribosomes :***Prokaryotes have 70S ribosomes. The 70S ribosomes are comparatively smaller in size and have sedimentation coefficient 70S with molecular weight 2.7×10^6daltons.

- ***80S Ribosomes:***Eukaryotes have 80S ribosomes. The 80S ribosomes have sedimentation coefficient of 80S and molecular weight 40×10^6daltons.

Number of ribosomes:

- An *E. coli* cell contains 10,000 ribosomes, forming 25 per cent of the total mass of the bacterialcell.
- Whereas, mammalian cultured cells contain 10 million ribosomes per cell.

Chemical composition:

- The ribosomes are chemically composed of RNA and proteins as their major constituents; both occurring approximately in equal proportions in smaller as well as larger subunit.
- The 70S ribosomes contain more RNA (60 to 40%) than the proteins (36 to 37%). The ribosomes of *E. coli* contain 63% rRNA and 37% protein.
- While the 80S ribosomes contain less RNA (40 to 44%) than the proteins (60 to 56%), yeast ribosomes have 40 to 44% RNA and 60 to 56% proteins; ribosomes of pea seedling contain 40% RNA and 60% proteins. There is no lipid content in ribosomes.

Ribosomal Proteins:

- A ribosome is composed of three (in bacteria) or four (in eukaryotes) different rRNA molecules and as many as 83 proteins, organized into a large subunit and a small subunit.
- When both 50S and 30S ribosomal subunits are dissociated by centrifuging both of them in a gradient of 5 M cesium chloride, then there are two inactive core particles (40S and 23S, respectively) which contain the RNA and some proteins called core proteins (CP) at the same time several other proteins—the so-called split proteins (SP) are released from each particle.
- There are SP50 and SP30 proteins which may reconstitute the functional ribosomal subunit when added to their corresponding core.
- Some of the split proteins are apparently specific for each ribosomal subunit.
- The split proteins have been further fractionated and divided into acidic (A) and basic (B) proteins.
- According to Nomura (1968, 1973) and Garett and Wittmann (1973) each 70S ribosome of *E. coli* is composed of about 55 ribosomal proteins.
- Out of these 55 proteins, about 21 different molecules have been isolated from the 30S ribosomal subunit, and some 32 to 34 proteins from the 50S ribosomal subunit.
- Similar organization of ribosomal proteins and RNA is found in 80S Ribosomes. Different rRNA molecules evidently play a central role in the catalytic activities of ribosomes in the process of protein synthesis.

Metallic Ions:

The most important low molecular weight components of ribosomes are the ***divalent metallic ions*** such as Mg++, Ca++ and Mn++.

Structure:

- The ribosomes are oblate spheroid structures of 150 to 250A^{o} in diameter.
- Each ribosome is porous, hydrated and composed of two subunits.
- One ribosomal subunit is large in size and has a domelike shape, while the other ribosomal subunit is smaller in size, occurring above the larger subunit and forming a cap-like structure.

- The small ribosomal subunit contains a single rRNA molecule, referred to as small rRNA. The large subunit contains a molecule of large rRNA and one molecule of 5S rRNA, plus an additional molecule of 5.8S rRNA in vertebrates.
- The lengths of the rRNA molecules, the quantity of proteins in each subunit, and consequently the sizes of the subunits differ in bacterial and eukaryotic cells.
- The assembled ribosome is 70S in bacteria and 80S in vertebrates.
- Both the subunits remain separated by a narrow cleft.
- The two ribosomal subunits remain united with each other due to high concentration of the Mg++ (.001M) ions.
- When the concentration of Mg++ ions reduces in the matrix, both ribosomal subunits get separated.
- Actually in bacterial cells the two subunits are found to occur freely in the cytoplasm and they unite only during the process of protein synthesis.
- At high concentration of Mg++ ions in the matrix, the two ribosomes (monosomes) become associated with each other and known as the dimer.
- Further, during protein synthesis many ribosomes are aggregated due to common messenger RNA and form the polyribosomes or polysomes.

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.

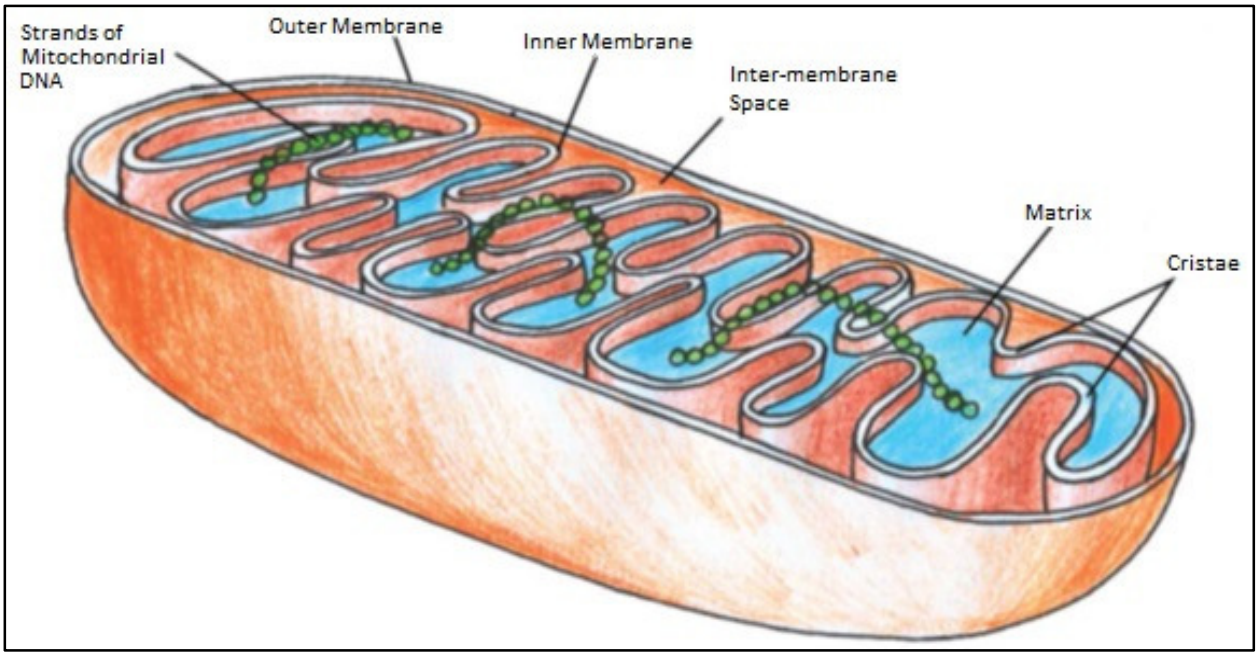


Fig : *Structure of Mitochondrion.*

- 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:

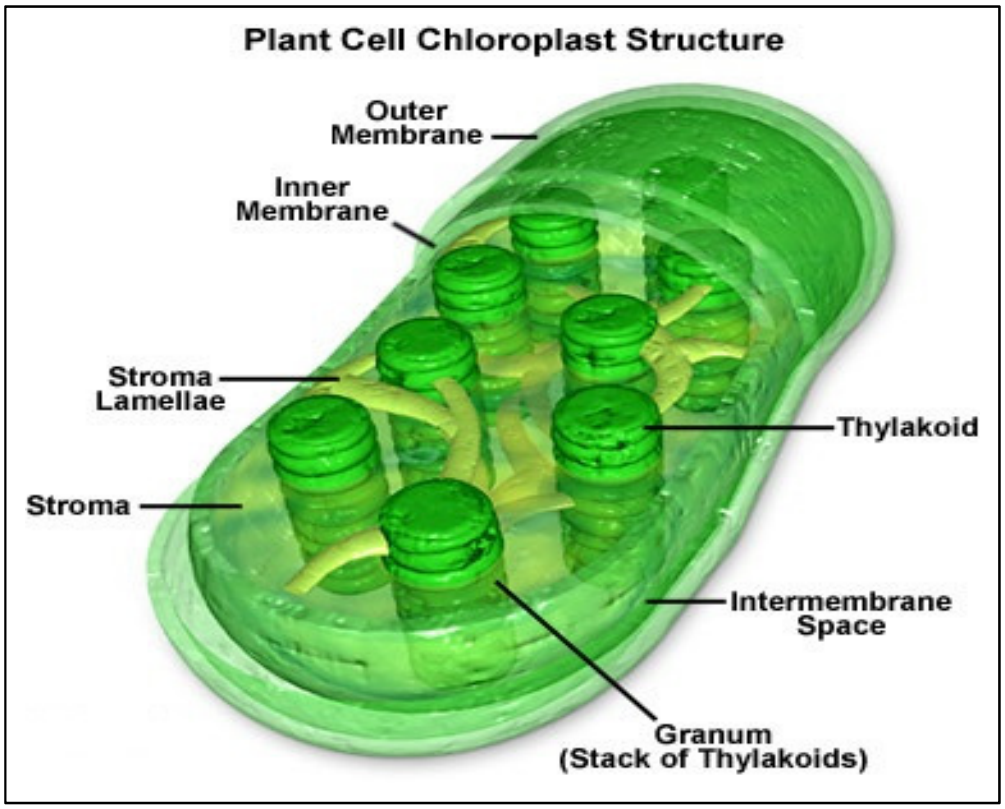
Chloroplasts are routinely isolated from plant tissues by differential centrifugation following the
disruption of the cells.

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 CO2 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 O2 and the conversion of CO2 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.

Nucleus

- Nucleus means kernel and was the first organelle to be discovered. It was discovered and named by Robert Brown in 1833 in the plant cells and is recognized as a constant feature of all animal and plant cells.
- Certain eukaryotic cells such as the mature sieve tubes of higher plants and mammalian erythrocytes contain no nucleus. It is the largest cellular organelle in eukaryotes. Prokaryotic cells lack nucleus and is complemented by nucleoid.

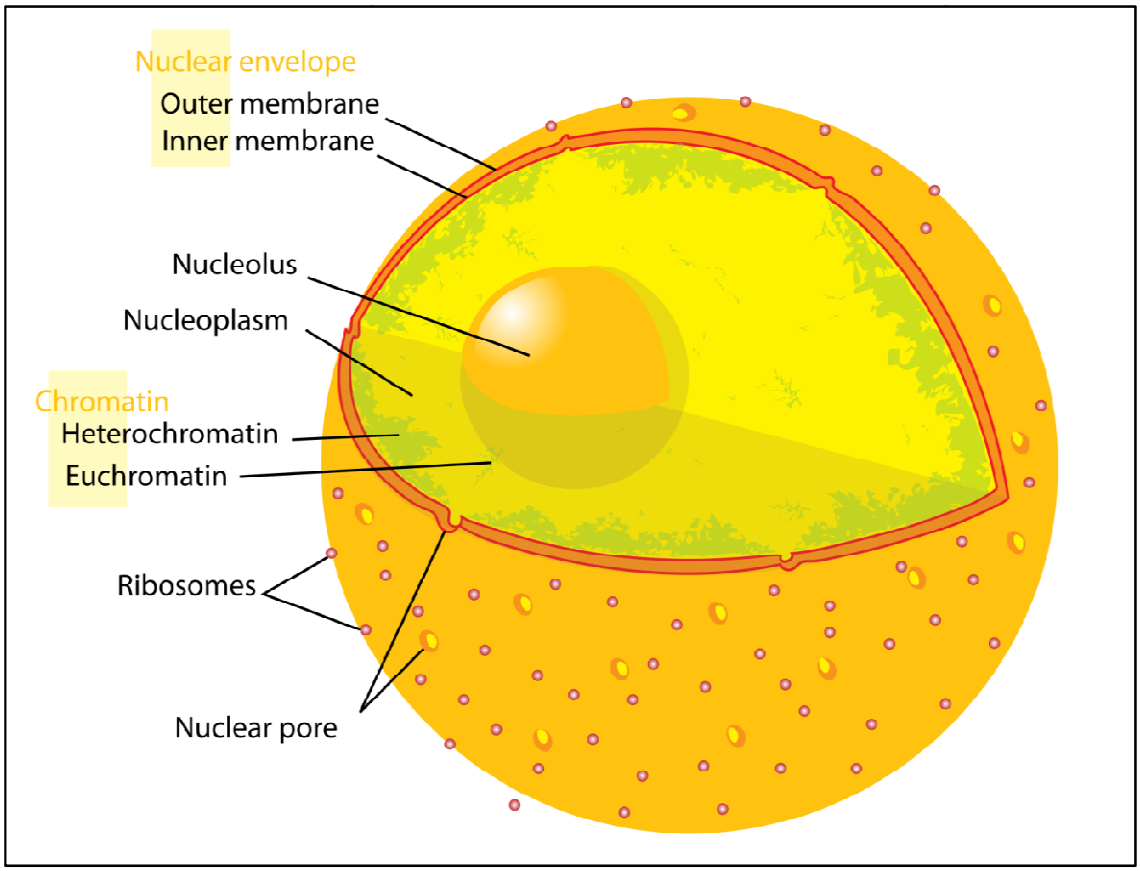


Fig :*The schematic representation of nucleus.*

- In mammalian cells, the average diameter of the nucleus is approximately 6 micrometers (μm), occupying about 10% of the total cell volume.
- The contents of the nucleus are DNA genome, RNA synthetic apparatus, and a fibrous matrix. It is surrounded by two membranes, each one a phospholipid bilayer containing many different types of proteins.
- The inner nuclear membrane defines the nucleus itself. In most cells, the outer nuclear membrane is continuous with the rough endoplasmic reticulum, and the space between the inner and outer nuclear membranes is continuous with the lumen of the rough endoplasmic reticulum.
- The two nuclear membranes appear to fuse at nuclear pores, the ringlike complexes composed of specific membrane proteins through which material moves between the nucleus and the cytosol. It contains cell's genetic material, organized as multiple long linear DNA molecules in complex with histones, to form chromosomes.
- The genes within these chromosomes are the cell's nuclear genome. The function is to maintain the integrity of the genes that controls the activities of the cell by regulating gene expression.
- In a growing or differentiating cell, the nucleus is metabolically active, replicating DNA and synthesizing rRNA, tRNA, and mRNA. Within the nucleus mRNA binds to specific proteins, forming ribonucleoprotein particles.
- Most of the cell's ribosomal RNA is synthesized in the nucleolus, a subcompartment of the nucleus that is not bounded by a phospholipid membrane. Some ribosomal proteins are added to ribosomal RNAs within the nucleolus as well.

- The finished or partly finished ribosomal subunits, as well as tRNAs and mRNA-containing particles, pass through a nuclear pore into the cytosol for use in protein synthesis.
- In a nucleus that is not dividing, the chromosomes are dispersed and not dense enough to be observed in the light microscope.
- Only during cell division are individual chromosomes visible by light microscopy. In the electron microscope, the nonnucleolar regions of the nucleus, called the nucleoplasm, can be seen to have dark and light staining areas.
- The dark areas, which are often closely associated with the nuclear membrane, contain condensed concentrated DNA, called heterochromatin.
- Fibrous proteins called lamins form a two-dimensional network along the inner surface of the inner membrane, giving it shape and apparently binding DNA to it. The breakdown of this network occurs early in cell division.

Cell Nucleus: Ultrastructure

The structure of a cell nucleus consists of a nuclear membrane (nuclear envelope), nucleoplasm, nucleolus, and chromosomes. Nucleoplasm, also known as karyoplasm, is the matrix present inside the nucleus.

Nuclear Membrane :

- It is a double-membrane structure each 5–10 nm thick . Numerous pores occur in the envelope, allowing RNA and other chemicals to pass, but not the DNA.
- Because the nuclear membrane is impermeable to most molecules, nuclear pores are required to allow movement of molecules across the envelope.
- These pores cross both of the membranes, providing a channel that allows free movement of small molecules and ions.
- The movement of larger molecules such as protein requires active transport regulated by carrier proteins.
- The nuclear envelope (or perinuclear cisterna) encloses the DNA and defines the nuclear compartment of interphase and prophase nuclei.
- The spherical inner nuclearmembrane contains specific proteins that act as binding sites for the supporting fibrous sheath ofintermediate filaments (IF), called nuclear lamina.
- Nuclear lamina has contact with the chromatin (or chromosomes) and nuclear RNAs.
- The inner nuclear membrane is surrounded by the outer nuclear membrane, which closely resembles the membrane of the endoplasmic reticulum, that is continuous with it.
- Like the membrane of the rough ER, the outer surface of outer nuclear membrane is generally studded with ribosomes engaged in protein synthesis.
- The proteins made on these ribosomes are transported into space between the inner and outer nuclear membrane, called perinuclear space.
- The perinuclear space is a 10 to 50 nm wide fluid-filled compartment which is continuous with the ER lumen and may contain fibres, crystalline deposits, lipid droplets or electrondense material.
- Nuclear pores and nucleocytoplasmic traffic. The nuclear envelope in all eukaryotic forms, from yeasts to humans, is perforated by nuclear pores.

Nucleo-cytoplasmic traffic:

- Quite evidently there is considerable trafficking across the nuclear envelope during interphase. Ions, nucleotides and structural, catalytic and regulatory proteins are

imported from the cytosol (cytoplasmic matrix); mRNA, tRNA are exported to the cytosol (cytoplasmic matrix).

- However, one of the main functions of the nuclear envelope is to prevent the entrance of active ribosomes into the nucleus.

Nucleoplasm:

- The space between the nuclear envelope and the nucleolus is filled by a transparent, semi-solid,
- granular and slightly acidophilic ground substance or the matrix known as the nuclear sap or
- nucleoplasm or karyolymph.
- The nuclear components such as the chromatin threads and thenucleolus remain suspended in the nucleoplasm which is composed mainly of nucleoproteinsbut it also contains otherinorganic and organic substances, namely nucleic acids, proteins, enzymes and minerals.
- The most common nucleic acids of the nucleoplasm are the DNA and RNA.
- The nucleoplasm contains many types of complex proteinscategorized into:

(i) Basic proteins. The proteins which take basic stain are known as the basic proteins. The most important basic proteins of the nucleus are nucleoprotamines and the nucleohistones.

(ii) Non-histone or Acidic proteins. The acidic proteins either occur in the nucleoplasm or in the chromatin. The most abundant acidic proteins of the euchromatin (a type of chromatin) are the phosphoproteins. The nucleoplasm contains many enzymes which are necessary for the synthesis of the DNA and RNA. Most of the nuclear enzymes are composed of non-histone (acidic) proteins.

- The most important nuclear enzymes are the DNA polymerase, RNA polymerase, NAD synthetase, nucleoside triphosphatase, adenosine diaminase, nucleoside phosphorylase, guanase, aldolase, enolase, 3-phosphoglyceraldehyde dehydrogenase and pyruvate kinase. The nucleoplasm also contains certain cofactors and coenzymes such as ATP and acetyl CoA. The nucleoplasm has small lipid content.

- The nucleoplasm also contains several inorganic compounds such as phosphorus, potassium, sodium, calcium and magnesium. The chromatin comparatively contains large amount of these minerals than the nucleoplasm.

- The nucleoplasm contains many thread-like, coiled and much elongated structures which take readily the basic stains such as the basic fuchsin. These thread-like structures are known as the chromatin (*chrome*=colour) substance or chromatin fibres. Chromosome will be discussed in detail in the next module.

Nucleolus:

- Most cells contain in their nuclei one or more prominent spherical colloidal acidophilic bodies, called nucleoli. However, cells of bacteria and yeast lack nucleolus.

- The nucleolus is mainly involved in the assembly of ribosomes.

- After being produced in the nucleolus, ribosomes are exported to the cytoplasm where they translate mRNA.

- Some of the eukaryotic organisms have nucleus that contains up to four nucleoli.

- The nucleolus plays an indirect role in protein synthesis by producing ribosomes.

- Nucleolus disappears when a cell undergoes division and is reformed after the completion of cell-division.

- The size of the nucleolus is found to be related with the synthetic activity of the cell.
- Therefore, the cells with little or no synthetic activities, sperm cells, blastomeres, muscle cell, etc., are found to contain smaller or no nucleoli, while the oocytes, neurons and secretory cells which synthesize the proteins or other substances contain comparatively large-sized nucleoli.

- The number of the nucleoli in the nucleus depends on the species and the number of the chromosomes. The number of the nucleoli in the cells may be one, two or four.

- A nucleolus is often associated with the nucleolar organizer (NO) which represents the secondary constriction of the nucleolar organizing chromosomes, and are 10 in number in human beings. Nucleolar organizer consists of the genes for 18S, 5.8S and 28S rRNAs. The genes for fourth type of r RNA, i.e., 5S rRNA occur outside the nucleolar organizer.

- Nucleolus is not bounded by any limiting membrane; calcium ions are supposed to maintain its intact organization. Nucleolus also contains some enzymes such as acid phosphatase, nucleoside phosphorylase and NAD+ synthesizing enzymes for the synthesis of some coenzymes, nucleotides and ribosomal RNA.

- RNA methylase enzyme which transfers methyl groups to the nitrogen bases occurs in the nucleolus of some cells. Functionally nucleolus is the site where biogenesis of ribosomal subunits (40S and 60S) takes place.

- In it three types of rRNAs, namely 18S, 5.8S and 28S rRNAs, are transcribed as parts of a much longer precursor molecule (45S transcript) which undergoes processing (RNA splicing) by the help of two types of proteins such as nucleolin and U3 sn RNP (U3 is a 250 nucleotide containing RNA, sn RNP represents small nuclear ribonucleoprotein).

- The 5S r RNA is transcribed on the chromosome existing outside the nucleolus and the 70S types of ribosomal proteins are synthesized in the cytoplasm.

- All of these components of the ribosomes migrate to the nucleolus, where they are assembled into two types of ribosomal subunits which are transported back to the cytoplasm.

- The smaller (40S) ribosomal subunits are formed and migrate to the cytoplasm much earlier than larger (60S) ribosomal subunits; therefore, nucleolus contains many more incomplete 60S ribosomal subunits than the 40S ribosomal subunits.

• Such a time lag in the migration of 60S and 40S ribosomal subunits, prevents functional ribosomes from gaining access to the incompletely processed heterogeneous RNA (hn RNA; the precursor of m RNA) molecule inside the nucleus.

Functions of the nucleus

• Speaking about the functions of a cell nucleus, it controls the hereditary characteristics of an organism.
• This organelle is also responsible for the protein synthesis, cell division, growth, and differentiation.
• Some important functions carried out by a cell nucleus are:

1.Storage of hereditary material, the genes in the form of long and thin DNA (deoxyribonucleic acid) strands, referred to as chromatins.

2.Storage of proteins and RNA (ribonucleic acid) in the nucleolus.

3.Nucleus is a site for transcription in which messenger RNA (mRNA) are produced for the protein synthesis.
4.Exchange of hereditary molecules (DNA and RNA) between the nucleus and rest of the cell.

5.During the cell division, chromatins are arranged into chromosomes in the nucleus.

6.Production of ribosomes (protein factories) in the nucleolus.

7.Selective transportation of regulatory factors and energy molecules through nuclear pores.

As the nucleus regulates the integrity of genes and gene expression, it is also referred to as the control center of a cell. Overall, the cell nucleus stores all the chromosomal DNA of an organism.

Peroxisomes

Peroxisomes are small, membrane-enclosed organelles that contain enzymes involved in a variety of metabolic reactions, including several aspects of energy metabolism. Although peroxisomes are morphologically similar to lysosomes, they are assembled, like mitochondria and chloroplasts, from proteins that are synthesized on free ribosomes and then imported into peroxisomes as completed polypeptide chains. Although peroxisomes do not contain their own genomes, they are similar to mitochondria and chloroplasts in that they replicate by division.

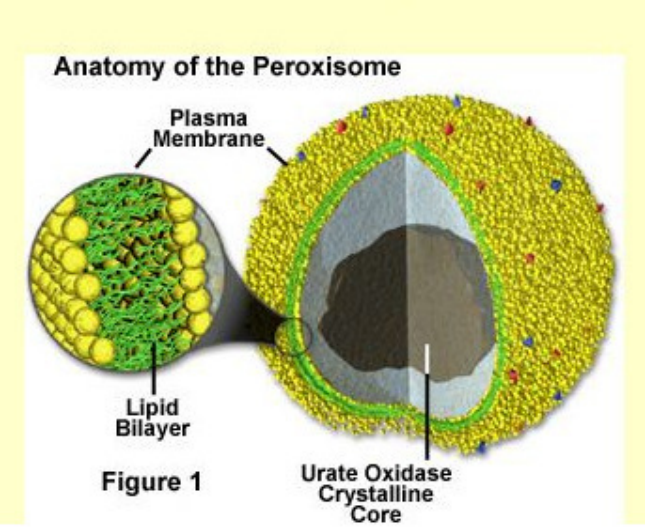


Figure 1

Functions of Peroxisomes

Peroxisomes contain at least 50 different enzymes, which are involved in a variety of biochemical pathways in different types of cells. Peroxisomes originally were defined as organelles that carry out oxidation reactions leading to the production of hydrogen peroxide. Because hydrogen peroxide is harmful to the cell, peroxisomes also contain the enzyme catalase, which decomposes hydrogen peroxide either by converting it to water or by using it to oxidize another organic compound. A variety of substrates are broken down by such oxidative reactions in peroxisomes, including uric acid, amino acids, and fatty acids. The oxidation of fatty acids is a particularly important example, since it provides a major source of metabolic energy. In animal cells, fatty acids are oxidized in both peroxisomes and mitochondria, but in yeasts and plants fatty acid oxidation is restricted to peroxisomes.

In addition to providing a compartment for oxidation reactions, peroxisomes are involved in lipid biosynthesis. In animal cells, cholesterol and dolichol are synthesized in peroxisomes as well as in the ER. In the liver, peroxisomes are also involved in the synthesis of bile acids, which are derived from cholesterol. In addition, peroxisomes contain enzymes required for the synthesis of plasmalogens (important membrane components in some tissues, particularly heart and brain, although they are absent in others).

Peroxisomes play two particularly important roles in plants. First, peroxisomes in seeds are responsible for the conversion of stored fatty acids to carbohydrates, which is critical to providing energy and raw materials for growth of the germinating plant. This occurs via a series of reactions termed the glyoxylate cycle, which is a variant of the citric acid cycle. The peroxisomes in which this takes place are sometimes called **glyoxysomes**. Second, peroxisomes in leaves are involved in **photorespiration**, which serves to metabolize a side product formed during photosynthesis.

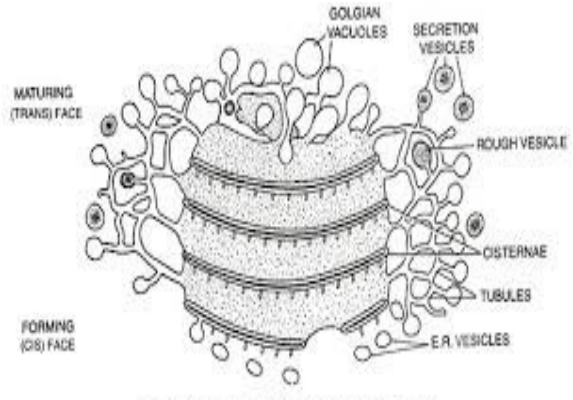
Peroxisome Assembly

As already noted, the assembly of peroxisomes is fundamentally similar to that of mitochondria and chloroplasts, rather than to that of the endoplasmic reticulum, Golgi apparatus, and lysosomes. Proteins destined for peroxisomes are translated on free cytosolic ribosomes and then transported into peroxisomes as completed polypeptide chains. Phospholipids are also imported to peroxisomes, via phospholipid transfer proteins, from their major site of synthesis in the ER. The import of proteins and phospholipids results in peroxisome growth, and new peroxisomes are then formed by division of old ones.

The Golgi Apparatus

The Golgi apparatus, or **Golgi complex**, functions as a factory in which proteins received from the ER are further processed and sorted for transport to their eventual destinations: lysosomes, the plasma membrane, or secretion. In addition, glycolipids and sphingomyelin are synthesized within the Golgi. In plant cells, the Golgi apparatus further serves as the site at which the complex polysaccharides of the cell wall are synthesized. The Golgi apparatus is thus involved in processing the broad range of cellular constituents that travel along the secretory pathway.

Organization of the Golgi



Morphologically the Golgi is composed of flattened membrane-enclosed sacs (cisternae) and associated vesicles. A striking feature of the Golgi apparatus is its distinct polarity in both structure and function. Proteins from the ER enter at its *cis* face (entry face), which is convex and usually oriented toward the nucleus. They are then transported through the Golgi and exit from its concave *trans* face (exit face). As they pass through the Golgi, proteins are modified and sorted for transport to their eventual destinations within the cell.

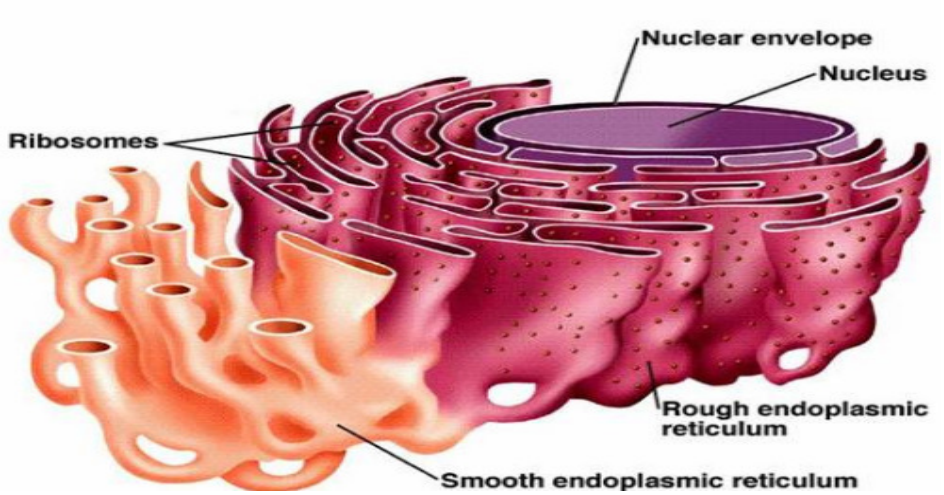
Distinct processing and sorting events appear to take place in an ordered sequence within different regions of the Golgi complex, so the Golgi is usually considered to consist of multiple discrete compartments. Although the number of such compartments has not been established, the Golgi is most commonly viewed as consisting of four functionally distinct regions: the ***cis*Golgi network**, the **Golgi stack** (which is divided into the *medial* and *trans* subcompartments), and the ***trans*Golgi network**. Proteins from the ER are transported to the ER-Golgi intermediate compartment and then enter the Golgi apparatus at the *cis* Golgi network. They then progress to the *medial* and *trans* compartments of the Golgi stack, within which most metabolic activities of the Golgi apparatus take place. The modified proteins, lipids, and polysaccharides then move to the *trans* Golgi network, which acts as a sorting and distribution center, directing molecular traffic to lysosomes, the plasma membrane, or the cell exterior.

Although the Golgi apparatus was first described over 100 years ago, the mechanism by which proteins move through the Golgi apparatus has still not been established and is an area of controversy among cell biologists. One possibility is that transport vesicles carry proteins between the cisternae of the Golgi compartments. However, there is considerable experimental support for an alternative model proposing that proteins are simply carried through compartments of the Golgi within the Golgi cisternae, which gradually mature and progressively move through the Golgi in the cis to trans direction.

The Endoplasmic Reticulum

The endoplasmic reticulum (**ER**) is a network of membrane-enclosed tubules and sacs (cisternae) that extends from the nuclear membrane throughout the cytoplasm. The entire endoplasmic reticulum is enclosed by a continuous membrane and is the largest organelle of most eukaryotic

cells. Its membrane may account for about half of all cell membranes, and the space enclosed by the ER (the lumen, or cisternal space) may represent about 10% of the total cell volume. As discussed below, there are two distinct types of ER that perform different functions within the cell. The **rough ER**, which is covered by ribosomes on its outer surface, functions in protein processing. The **smooth ER** is not associated with ribosomes and is involved in lipid, rather than protein, metabolism.



The Endoplasmic Reticulum and Protein Secretion

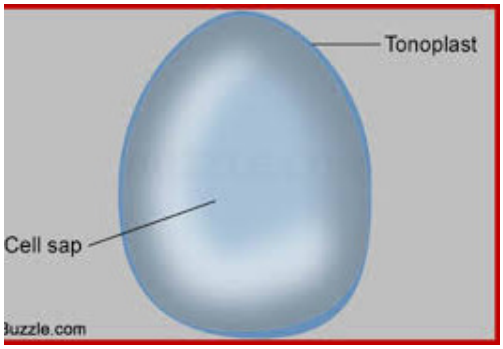
The role of the endoplasmic reticulum in protein processing and sorting was first demonstrated by George Palade and his colleagues in the 1960s. These investigators studied the fate of newly synthesized proteins in specialized cells of the pancreas (pancreatic acinar cells) that secrete digestive enzymes into the small intestine. Because most proteins synthesized by these cells are secreted, Palade and coworkers were able to study the pathway taken by secreted proteins simply by labeling newly synthesized proteins with radioactive amino acids. The location of the radiolabeled proteins within the cell was then determined by autoradiography, revealing the cellular sites involved in the events leading to protein secretion. After a brief exposure of pancreatic acinar cells to radioactive amino acids, newly synthesized proteins were detected in the rough ER, which was therefore identified as the site of synthesis of proteins destined for secretion. If the cells were then incubated for a short time in media containing nonradioactive amino acids (a process known as a chase), the radiolabeled proteins were detected in the Golgi apparatus. Following longer chase periods, the radiolabeled proteins traveled from the Golgi apparatus to the cell surface in secretory vesicles, which then fused with the plasma membrane to release their contents outside of the cell.

These experiments defined a pathway taken by secreted proteins, the **secretory pathway**: rough ER → Golgi → secretory vesicles → cell exterior. Further studies extended these results and demonstrated that this pathway is not restricted to proteins destined for secretion from the cell. Plasma membrane and lysosomal proteins also travel from the rough ER to the Golgi and then to their final destinations. Still other proteins travel through the initial steps of the secretory pathway but are then retained and function within either the ER or the Golgi apparatus.

The entrance of proteins into the ER thus represents a major branch point for the traffic of proteins within eukaryotic cells. Proteins destined for secretion or incorporation into the ER, Golgi apparatus, lysosomes, or plasma membrane are initially targeted to the ER. In mammalian cells, most proteins are transferred into the ER while they are being translated on membrane-bound ribosomes. In contrast, proteins destined to remain in the cytosol or to be incorporated into the nucleus, mitochondria, chloroplasts, or peroxisomes are synthesized on free ribosomes and released into the cytosol when their translation is complete.

Vacuoles

A vacuole is a membrane bound, multifunctional organelle found in the cells of plants (including algae and fungi) and some protists and bacteria. Vacuoles are acidic in nature and share some basic properties with lysosomes that are predominantly found in plant cells. Depending on the type of plant, there are different types of vacuoles with specific properties that are crucial to their functions. Unlike lysosomes in animals, there is only one of a few vacuoles in individual plant cells. Vacuoles take up 80-90 percent of the entire plant cell volume.



Lytic vacuoles: Lytic vacuoles share similar properties with lysosomes found in animals. As such, they contain different types of hydrolytic enzymes responsible for the degradation of such molecules as nucleic acids, proteins and polysaccharides.

Researchers have suggested that these particular organelles either originate from the trans-Golgi network or the dilation of a part of the smooth endoplasmic reticulum.

These types of vacuoles are also referred to as lytic compartments and are characterized by the optimum pH of 5. Research studies have found lytic vacuoles to contain the following types of hydrolytic and oxidizing enzymes:

- Hydrolases - Essentially, hydrolases are different types of hydrolytic enzymes that use water to break up chemical bonds. This allows them to divide larger molecules into smaller ones.
- Esterases - This includes hydrolase enzymes that specifically serve to break down esters (compounds made up of an acid and alkyl group) in to acids and alcohol group.
- Nucleases - Enzymes responsible for breaking down the bonds (phosphodiester bonds) to produce nucleotides.
- Peroxidases - Peroxidases include enzymes that typically break down hydrogen peroxide, removing it from chloroplast and cytosol among others in plants.

There are different processes through which cells eliminate unwanted/old material, unwanted cytoplasm or the entire cell.

In plant cells, this includes:

Autophagy

In plants, autophagy is an important process that helps in the elimination of unwanted material from the cells. Here, various materials in the cytoplasm that are no longer required by the cell are enclosed within a vesicle refered to as the autophagosome and transported to the vacuole where they are degraded.

The invagination of the double membrane of the autophagosome makes it possible for this vesicle to enclose and hold cytoplasmic material/components to be delivered to the vacuole. This process is also involved in recycling of material.

By breaking down various cell components, they are reduced to their basic components that can then be used by the cell. For instance, the breakdown of proteins produces peptides that can later be transported through the endoplasmic reticulum and Golgi apparatus to process proteins.

Autophagy in cells occurs in response to different conditions within the cells or in response to factors affecting the body in general. For instance, such stressful conditions as starvation result in the degradation of various components of the cell such a proteins and even lipids in order to produce energy.

While it was previously believed that autophagy non-selectively eliminates various components in the cell, recent studies have shown that this mechanism can and does selectively eliminate given components such as proteins in specific conditions or in response to given stressful conditions in yeast cells.

Cell Defence and Cell Death

Vacuoles play a crucial role in the defense and death of cells.

Although the process is yet to be fully understood, vacuoles play an important role in immunity of the cell by releasing various enzymes (hydrolytic enzymes) and antimicrobes that destroy the invading pathogen. However, the mechanism has also been associated with programmed cell death (PCD).

In reaction to invading organisms in the cell, an enzyme refered to as vacuolar processing enzyme triggers the disruption of the vacuolar membrane, causing the vacuole to collapse and release hydrolytic enzymes and other antimicrobes. This not only results in the destruction of the invaders, but also the cell itself.

On the other hand, the fusion of the central vacuole with the plasma membrane in the presence of proteasome can cause the vacuole to release antibacterial protease as well as other vacuole components that can cause the death of the cell.

Protein storage vacuoles (PSV)

Protein storage vacuoles can be found in the storage tissues where they accumulate proteins. Seeds are good examples of tissues where reserve proteins are stored. All proteins to be stored are first synthesized in the rough endoplasmic reticulum and then transported to the protein storage vacuole (PSV).

In some plants, this process involves the transport of proteins through autophagy and protein bodies (PBs). On the other hand, they may be released from the Golgi apparatus (having been synthesized in the ER) as prevacuoles before arriving at the vacuole for storage.

For proteins to be successfully transported from the Golgi apparatus to the vacuole, protein targeting is essential. Here, peptide targeting sequence target given receptors on the vacuole, which allows for proteins to be successfully transported and stored.

Depending on the type of plant, storage tissues (seeds, etc) will contain many, densely packed protein storage vacuoles. Moreover, depending on the plant, there may be one or different types of proteins (sub-domains) stored.

Gas vacuoles

Gas vacuoles are composed of hollow cylindrical gas vesicles. They are typically found in bacteria and have a permeable membrane that allows air to pass through. This membrane also serves to bind the vesicles. These vesicles can either inflate (fill with air) or deflate allowing the bacteria such as cyanobacteria to float or remain at a given desired depth in water.

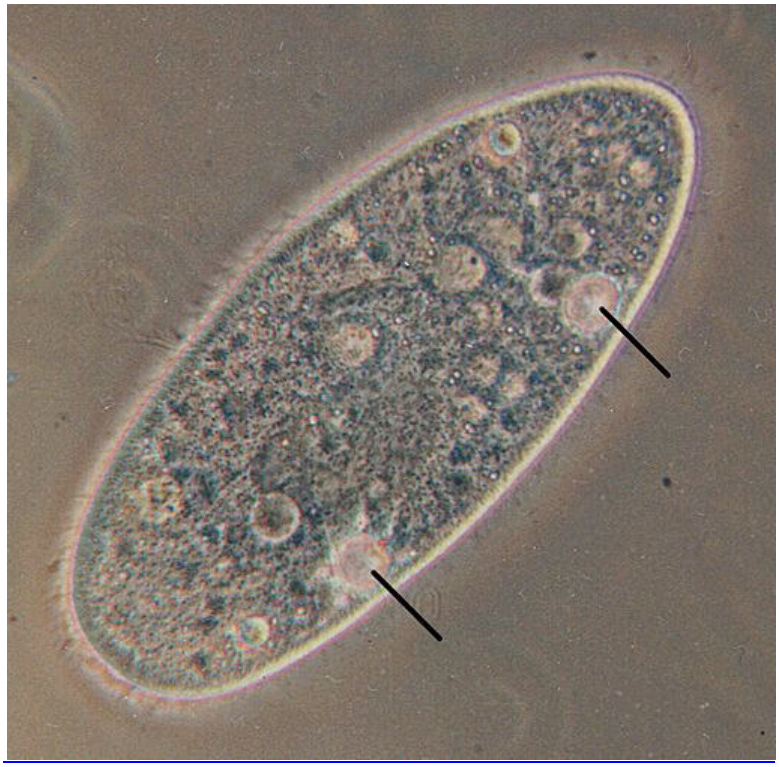
Contractile vacuoles

Contractile vacuoles are membrane bound organelles that are typically found among members of kingdom protista (algae, amoebas, and ciliates etc). In these cells, the contractile vacuole is particularly important given that it helps in osmoregulation (regulation of osmotic pressure).

Although the entre mechanism is yet to be understood, researchers suggest that the contractile vacuole system (contractive vacuole complex) functions through the activities of two compartments that are bound by two differentiated membranes.

The two membranes have different properties that make it possible for the vacuole to carry out osmoregulation. Here, the first membrane is divided into many vesicles and tubules and contains numerous proton-translocating V-ATPase enzymes. These components are responsible for producing an electrochemical gradient of protons and fuse with the membrane of the second compartment.

The second compartment expands into the reservoir for fluid storage and can fuse with the cell membrane of the cell. However, it lacks the V-ATPase holoenzymes. As such, it undergoes contraction periodically which enables the vacuole to expel fluids. Together with other solutes, this system works like a pump that pumps out excess water from time to time to prevent the cell from swelling and getting ruptured.



Paramecium contractile vacuoles

Sap vacuoles

The sap vacuole is also commonly refered to as the central vacuole of a cell. It is the large, central organelles that occupy most part of the cell volume. This organelle contains the fluid known as the cell sap, which consists of such contents as water, sugars, minerals and amino acids among others. As a plant and cells mature, provacules from the Golgi complex fuse to form the sap vacuole at the center of the cell.

Contents of the cell sap are transported to the vacuole from the cytoplasm in the cell.

Some of the other functions include:

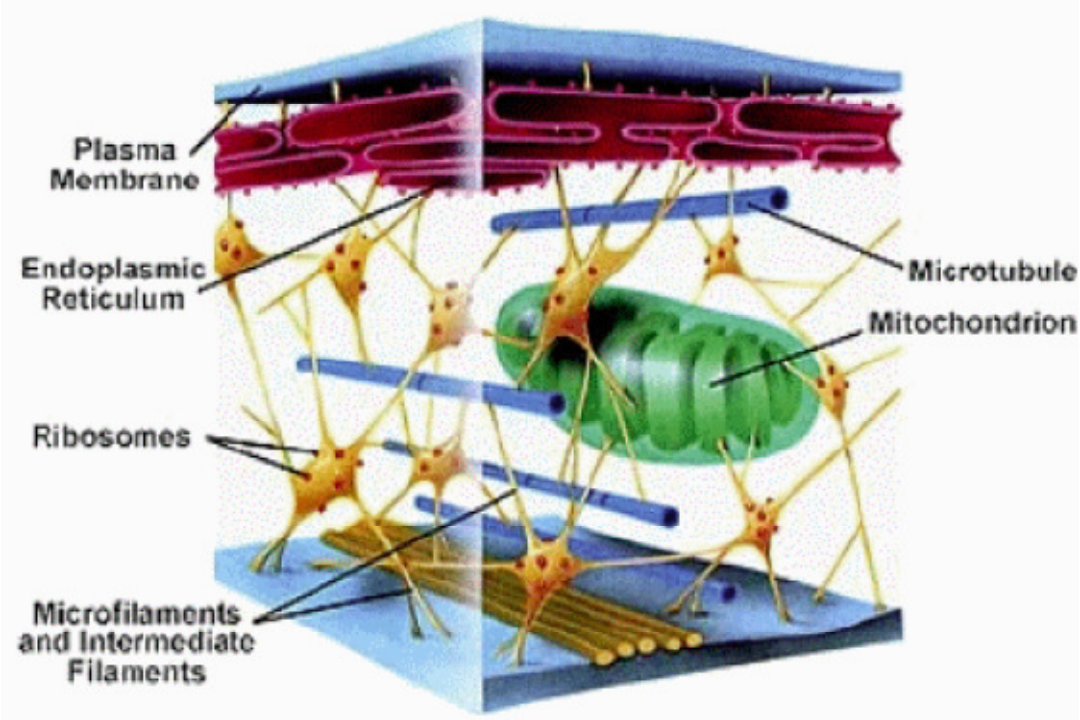
- Cell growth - Vacuoles are important in plant cells given that they help maintain turgidity of the cell. Enlargement of the vacuole consequently results in growth/increased size of the cell. This ultimately contributes to tissue rigidity.
- Storage - Apart from proteins, vacuoles are storage compartments for metabolites, organic acids and sugars among others

- Pigment deposition - Vacuoles are a site where pigments are deposited allowing for such vegetable colors as red, blue, scarlet etc.

Cytoskeleton

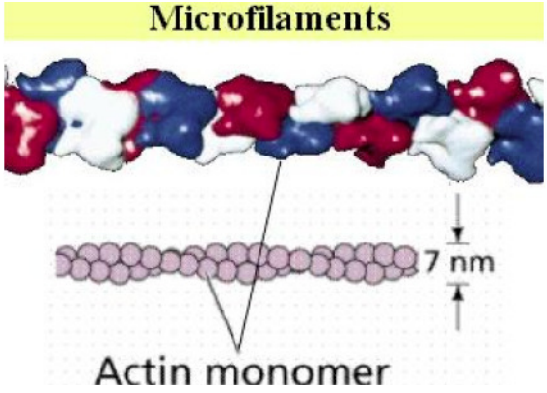
The cytoskeleton of a cell provides structure, strength, and motility. It provides a cellular scaffolding upon which the cellular organization is arranged. The figure shows a portion of a cell's cytoskeleton. Note that the cytoskeleton is very extensive. Also note that many ribosomes appear to be attached to the cytoskeleton. Polysome refers to two or more ribosomes. The ribosomes attached to the cytoskeleton are often referred to as "free" ribosomes to distinguish them from those ribosomes attached to the nuclear or ER membranes.

Cytoskeleteon is a network within the cytoplasm that maintains cell shape, moves substances within the cell - cellular trafficking, anchors cellular structures - organelles, proteins, flagella, cilia. (Various "motor" proteins are involved in moving cellular components).

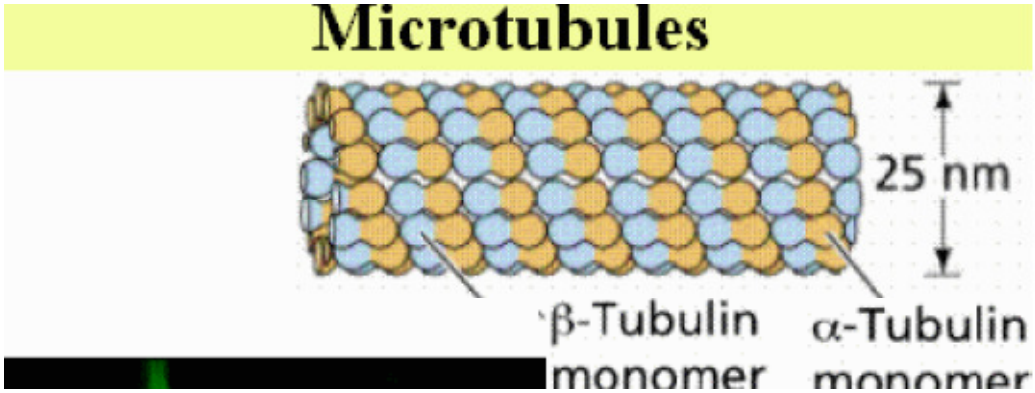


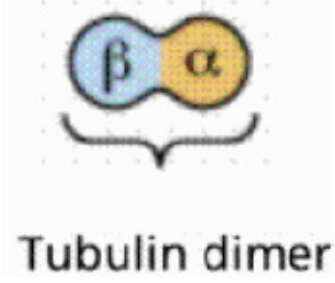
Three Types of Cytoskeleton Components:

1. Microfilaments: These are the thinnest cytoskeletal component and are composed of the globular protein Actin. Actin proteins associate in a head to tail fashion to form long chains called "microfilaments". When microfilaments associate they form a twisted double chain. When these chains associate in parallel they are referred to as Stress fibers.



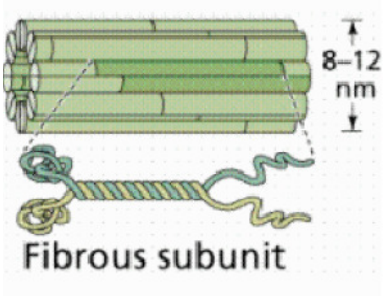
2. Microtubules: These are the thickest cytoskeletal component and are constructed of globular Tubulin proteins. Two tubulin proteins (alpha and beta tubulin) form a dimer. This tubulin dimer associates with other tubulin dimers which then arrange in a spiral to form a hollow tube - the microtubule. Microtubules radiate from center of cell in a "hub-spoke"fashion.

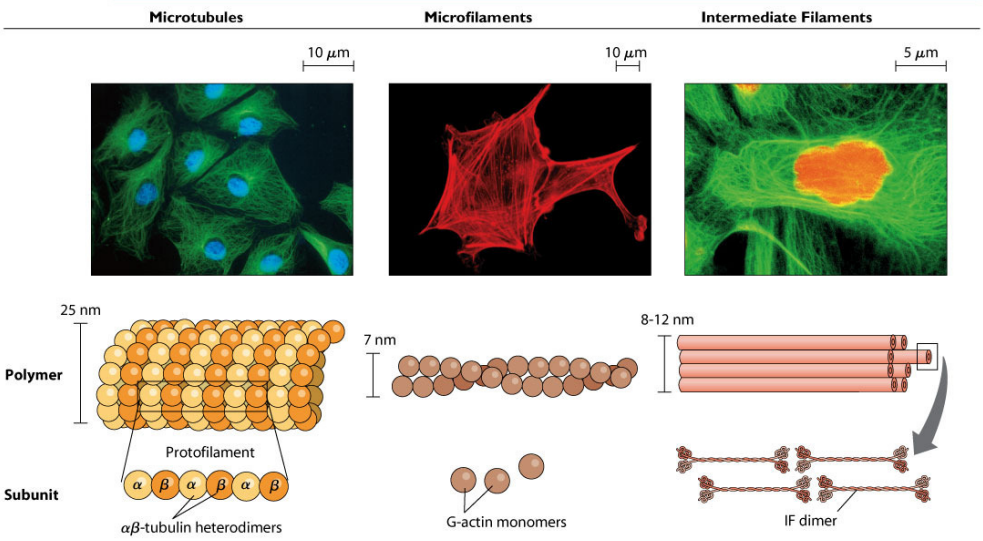




3. Intermediate filaments/ fibers (IF): Ifs are intermediate in thickness as compared to microtubules and microfilaments. Keratin is an example of an IF. Thought to be a triple twisted chain which extends throughout cytoplasm.

Intermediate fibers (IF)

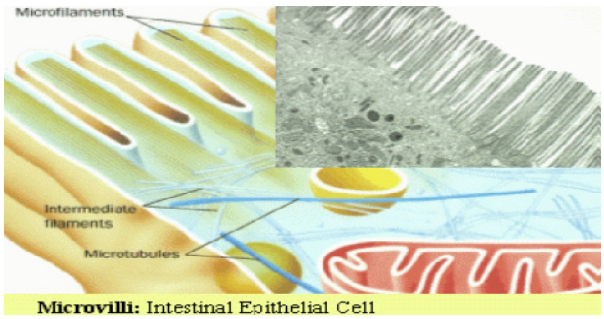


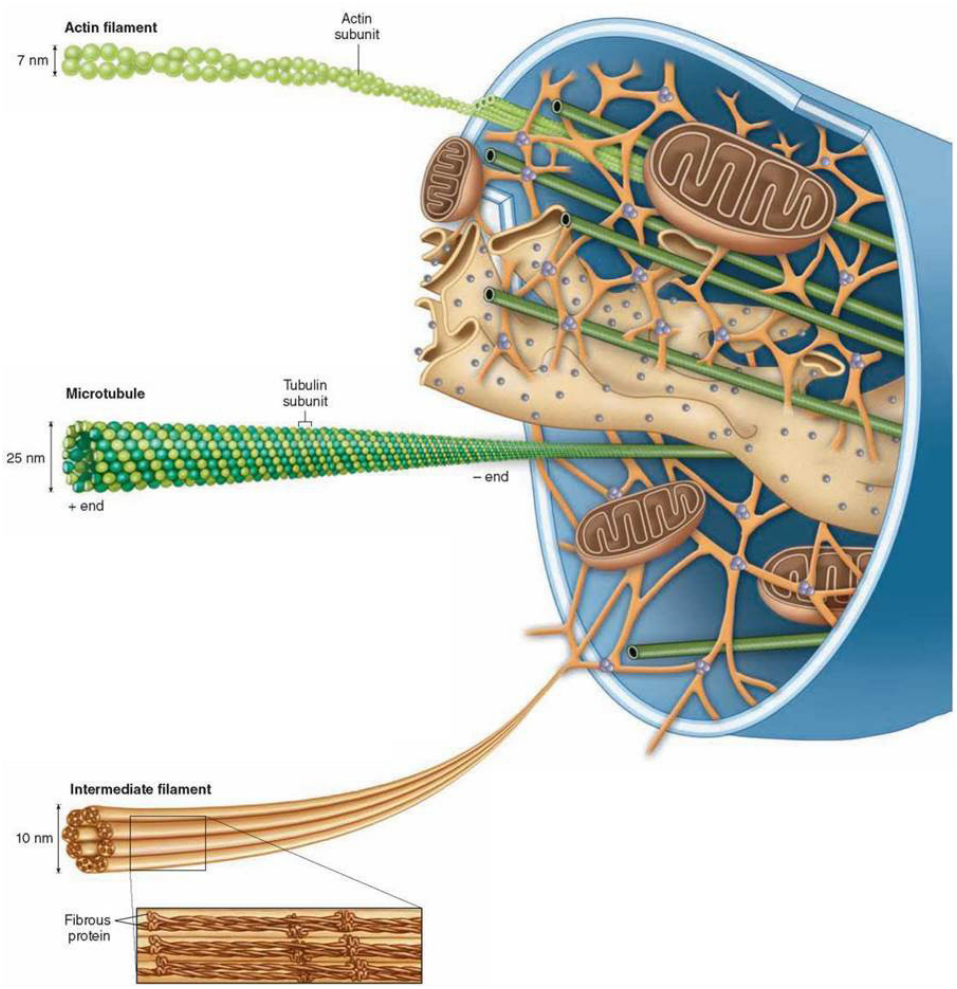


	Microtubules	Microfilaments	Intermediate Filaments
Structure	Hollow tube with a wall consisting of 13 protofilaments	Two intertwined chains of F-actin	Eight protofilaments joined end to end with staggered overlaps
Diameter	Outer: 25 nm Inner: 15 nm	7 nm	8–12 nm
Monomers	α-tubulin β-tubulin	G-actin	Several proteins; see Table 15-4
Polarity	(+), (−) ends	(+), (−) ends	No known polarity
Functions	Cytoplasmic: Organization and maintenance of animal cell shape and polarity Chromosome movements Intracellular transport/ trafficking, and movement of organelles Axonemal: Cell motility	Muscle contraction Cell locomotion Cytoplasmic streaming Cytokinesis Maintenance of animal cell shape Intracellular transport/trafficking	Structural support Maintenance of animal cell shape Formation of nuclear lamina and scaffolding Strengthening of nerve cell axons (neurofilament protein) Keeping muscle fibers in register (desmin)

Microvilli: The plasma membrane may be folded into finger-like projection scalled Microvilli - these increase the surface area of a cell to allow greater interaction with the environment; therefore greater absorptive capability. The microvilli finger-like protrusions of the cell membrane are possible due to the underlying cytoskeletal arrangement of actin (microfilaments).

An example of specialized cells containing microvilli are the small intestinal epithelial cells.



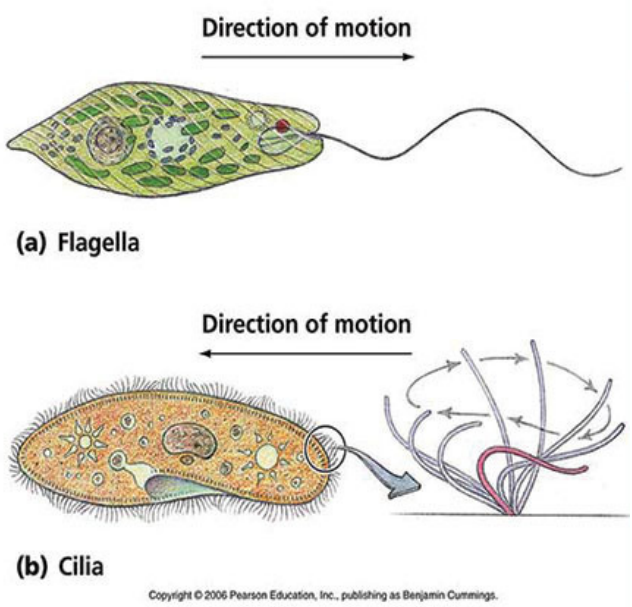


Cilia and Flagella: Structure and Movement

Cilia and *Flagella* are complex filamentous cytoplasmic structure protruding through cell wall. They are minute, specially differentiated appendices of the cell.

Flagella (singular = flagellum) are long, hair-like structures that extend from the plasma membrane and are used to move an entire cell.

Cilia (singular = cilium) are short, hair-like structures that are used to move entire cells (such as paramecia) or substances along the outer surface of the cell (for example, the cilia of cells lining the Fallopian tubes that move the ovum toward the uterus, or cilia lining the cells of the respiratory tract that trap particulate matter and move it toward the nostrils).



The Cilia and Flagella

- The terms cilium (meaning an eyelash) and flagellum (meaning a whip) are often used arbitrarily.
- Generally, cilia are shorter than flagella (<10 μm compared to >40μm).
- Cilia are present on the surface of the cell in much greater numbers (ciliated cells often have hundreds of cilia but flagellated cells usually have a single flagellum).
- The real difference, however, lies in the nature of their movement. Cilia row like oars. The movement is biphasic, consisting of an effective stroke in which the cilium is held rigid and bends only at its base and a recovery stroke in which the bend formed at the base passes out to the tip.
- Flagella wriggle like eels. They generate waves that pass along their length, usually from base to tip at constant amplitude.
- Thus the movement of water by a flagellum is parallel to its axis while a cilium moves water perpendicular to its axis and, hence, perpendicular to the surface of the cell.

Functions of Cilia

- Cilia are used for locomotion in isolated cells, such as certain protozoans (e.g., *Paramecium*).
- Motile cilia use their rhythmic undulation to sweep away substances, as in clearing dirt, dust, micro-organisms and mucus, to prevent disease.
- Cilia play roles in the cell cycle as well as animal development, such as in the heart.
- Cilia selectively allow certain proteins in to function properly.
- Cilia also play a role of cellular communication and molecular trafficking.
- Non-motile cilia serve as sensory apparatus for cells, detecting signals. They play crucial roles in sensory neurons. Non-motile cilia can be found in the kidneys to sense urine flow, as well as in the eyes on the photoreceptors of the retina.

- They also provide habitats or recruitment areas for symbiotic microbiomes in animals.
- Cilia have also been discovered to participate in vesicular secretion of ectosomes.

Functions of Flagella

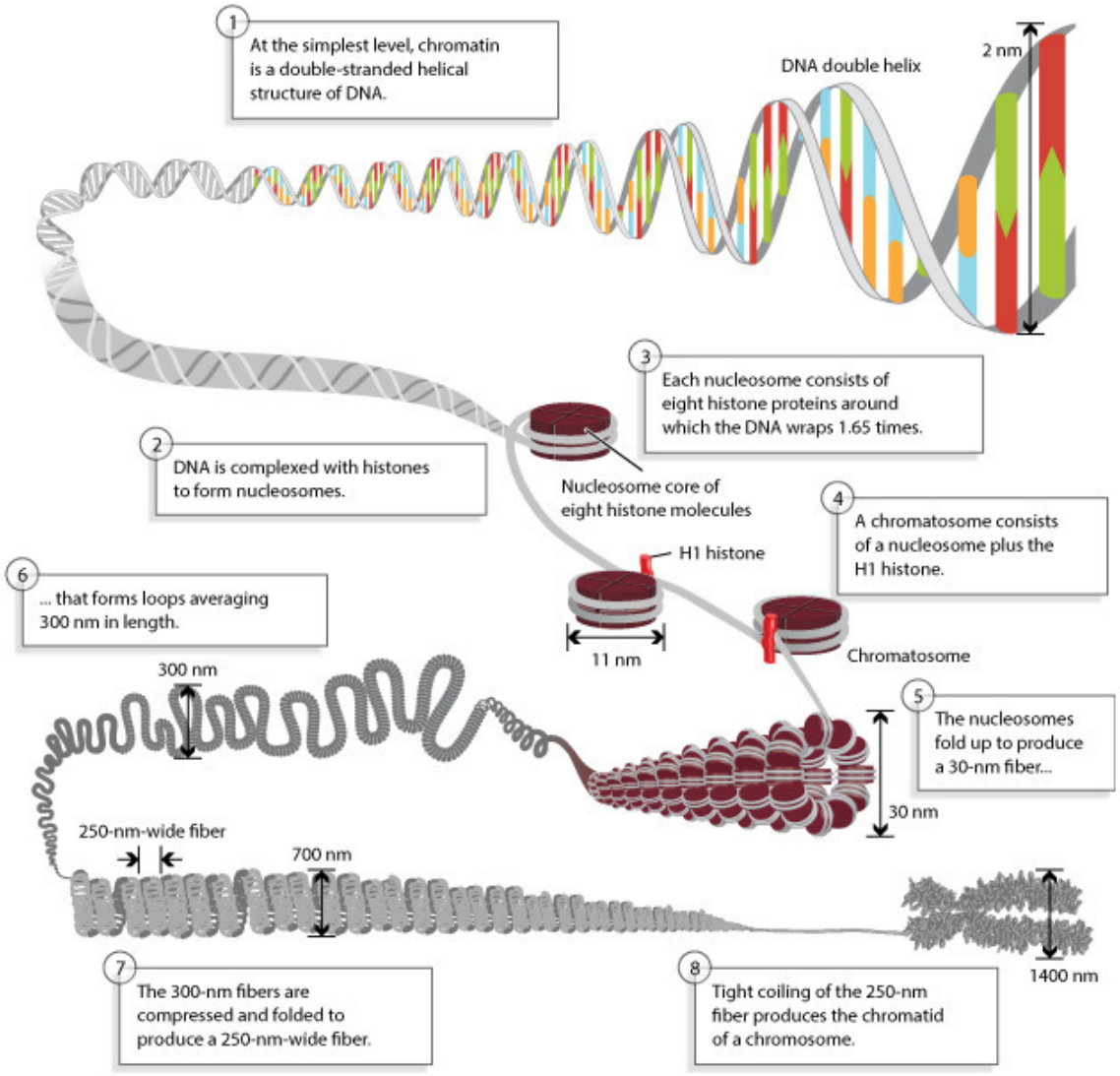
- Flagella are generally used for locomotion of cells, such as the spermatozoon and Euglena (protozoan).
- Flagella have active role in aiding cell feeding and eukaryotic reproduction.
- In prokaryotes such as bacteria, flagella serve as propulsion mechanisms; they're the chief way for bacteria to swim through fluids.
- It also provides a mechanism for pathogenic bacteria to aid in colonizing hosts and therefore transmitting diseases.
- Flagella also function as bridges or scaffolds for adhesion to host tissue.

Chromatin organization and packaging

The haploid human genome contains approximately 3 billion base pairs of DNA packaged into 23 chromosomes. Of course, most cells in the body (except for female ova and male sperm) are diploid, with 23 pairs of chromosomes. That makes a total of 6 billion base pairs of DNA per cell. Because each base pair is around 0.34 nanometers long (a nanometer is one-billionth of a meter), each diploid cell therefore contains about 2 meters of DNA $[(0.34 \times 10^{-9}) \times (6 \times 10^{9})]$. Moreover, it is estimated that the human body contains about 50 trillion cells—which works out to 100 trillion meters of DNA per human. Now, consider the fact that the Sun is 150 billion meters from Earth. This means that each of us has enough DNA to go from here to the Sun and back more than 300 times, or around Earth's equator 2.5 million times! How is this possible?

DNA, Histones, and Chromatin

The answer to this question lies in the fact that certain proteins compact chromosomal DNA into the microscopic space of the eukaryotic nucleus. These proteins are called histones, and the resulting DNA-protein complex is called chromatin. It may seem paradoxical that proteins are added to DNA to make it more compact. Within the nucleus, histones provide the energy (mainly in the form of electrostatic interactions) to fold DNA. As a result, chromatin can be packaged into a much smaller volume than DNA alone.



The Nucleosome: The Unit of Chromatin

The basic repeating structural (and functional) unit of chromatin is the nucleosome, which contains eight histone proteins and about 146 base pairs of DNA.

Nucleosomes are structured as follows: Two each of the histones H2A, H2B, H3, and H4 come together to form a histone octamer, which binds and wraps approximately 1.7 turns of DNA, or about 146 base pairs. The addition of one H1 protein wraps another 20 base pairs, resulting in two full turns around the octamer, and forming a structure called a chromatosome. Every chromosome contains hundreds of thousands of nucleosomes, and these nucleosomes are joined by the DNA that runs between them (an average of about 20 base pairs). This joining DNA is referred to as linker DNA. Each chromosome is thus a long chain of nucleosomes, which gives the appearance of a string of beads when viewed using an electron microscope.

Note that only eukaryotes (i.e., organisms with a nucleus and nuclear envelope) have nucleosomes. Prokaryotes, such as bacteria, do not.

Chromatin Is Coiled into Higher-Order Structures

The packaging of DNA into nucleosomes shortens the fiber length about sevenfold. In other words, a piece of DNA that is 1 meter long will become a "string-of-beads" chromatin fiber just 14 centimeters (about 6 inches) long. Despite this shortening, a half-foot of chromatin is still much too long to fit into the nucleus, which is typically only 10 to 20 microns in diameter. Therefore, chromatin is further coiled into an even shorter, thicker fiber, termed the "30-nanometer fiber," because it is approximately 30 nanometers in diameter.

Histone H1 is very important in stabilizing chromatin higher-order structures, and 30-nanometer fibers form most readily when H1 is present. Processes such as transcription and replication require the two strands of DNA to come apart temporarily, thus allowing polymerases access to the DNA template. However, the presence of nucleosomes and the folding of chromatin into 30-nanometer fibers pose barriers to the enzymes that unwind and copy DNA. It is therefore important for cells to have means of opening up chromatin fibers and/or removing histones transiently to permit transcription and replication to proceed.

Generally speaking, there are two major mechanisms by which chromatin is made more accessible:

- Histones can be enzymatically modified by the addition of acetyl, methyl, or phosphate groups.
- Histones can be displaced by chromatin remodeling complexes, thereby exposing underlying DNA sequences to polymerases and other enzymes.

It is important to remember that these processes are reversible, so modified or remodeled chromatin can be returned to its compact state after transcription and/or replication are complete.

Chromosomes Are Most Compacted During Metaphase

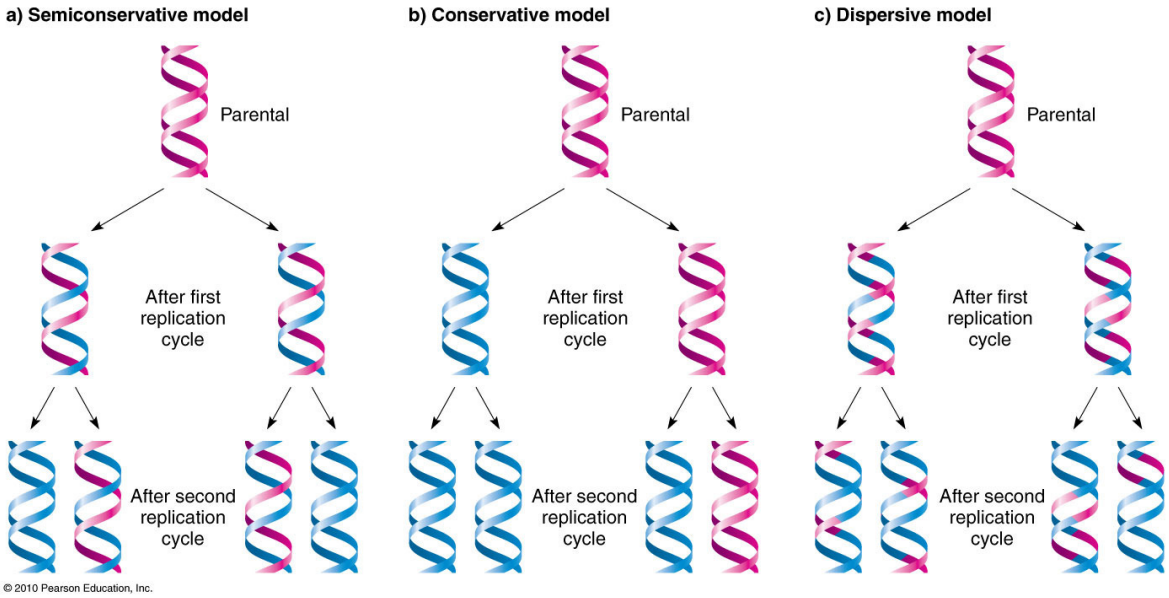
When eukaryotic cells divide, genomic DNA must be equally partitioned into both daughter cells. To accomplish this, the DNA becomes highly compacted into the classic metaphase chromosomes that can be seen with a light microscope. Once a cell has divided, its chromosomes uncoil again.

Comparing the length of metaphase chromosomes to that of naked DNA, the packing ratio of DNA in metaphase chromosomes is approximately 10,000:1 (depending on the chromosome). This level of compaction is achieved by repeatedly folding

chromatin fibers into a hierarchy of multiple loops and coils. Exactly how this is accomplished is unclear, but the phosphorylation of histone H1 may play a role.

DNA Replication types

After Watson and Crick proposed the double helix model of DNA, three classic models for DNA replication were proposed: ***semiconservative, conservative, and dispersive***.



a) **Semiconservative DNA replication:** Two strands of the original DNA are separated, and each is duplicated by the synthesis of the complementary strand, thus producing two exact replica of the parent DNA. Each of the two molecules thus contains one original strand and one newly synthesized strand. Thus, only one of the two strands of the original DNA is conserved. This mode of replication is known as semiconservative mode of replication.

b) **Conservative replication:** When complementary polynucleotide chains are synthesized, and a exact replica of parental DNA is formed. But the two newly created strands then come together and the parental strands reassociate. The original helix is conserved.

c) **Dispersive replication:** Here the parental strands are dispersed in to two new double helices following replication. Hence each strand consists of both old and new DNA. This mode will involve cleavage of the parental strands during replication. It is the most complex of the three possibilities and is, therefore, considered to be least likely to occur.

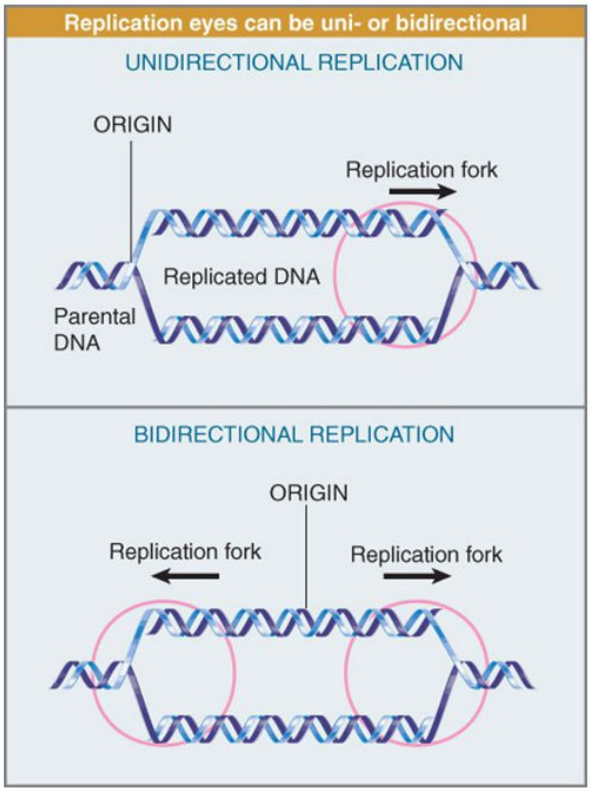
Unidirectional and bidirectional DNA replication

DNA replication can be unidirectional or bidirectional, depending upon whether the replication from the point of origin proceeds only in one direction or proceeds in both the directions. A replication eye may appear in both the situations, unless the replication starts from one of the two ends of a linear DNA molecule.

However, in unidirectional replication, one of the two ends of the replication eye will be stationary and the other end will move with replication.

On the other hand, in bidirectional replication, none of the two ends will be stationary and both will be moving.

A replicon is the length of the DNA that is replicated following one initiation event at a single origin.



Enzymes involved in DNA replication

- •DNA polymerase
- •DNAgyrase
- •DNAligase
- •Helicase
- •Primase

DNA Polymerase

The primary enzyme which carries out the condensation of the nucleotides to form a polynucleotide chain is the DNA polymerase. It is a single polypeptide with a molecular weight of 109 KD.

It catalyses the synthesis of the DNA from 5' to 3' direction, i.e. starts copying the template DNA from its 3' end. It cannot act in opposite direction.

DNA polymerase has three distinct properties.

i) One of the important functions of DNA polymerase is 5' →3' polymerase activity, which is the predominant function of the enzyme and is responsible for the nucleotides to form the DNA chain. A new nucleotide is added to an existing oligonucleotide at the 3'- OH group. For the formation of a new strand, a single-stranded DNA is required which is to be copied and is known as template. The enzyme thus depends on a template DNA and is referred to as the DNA dependent DNA polymerase.

ii) Another function of DNA polymerase is 5'→3' exonuclease activity, which helps in the removal of nucleotides from the DNA chain. RNA primer is removed from the newly synthesized chain by 5'→3' exonuclease activity.

iii) DNA polymerase shows another function of 3'→5' exonuclease activity, which catalyzes the removal of nucleotides from the 3' end of the DNA chain.

Arthur Kornberg was the first scientist who discovered the DNA polymerase.

DNA Polymerase I

DNA Polymerase I was the first enzyme suggested to be involved in DNA replication. The enzyme is now considered to be a DNA repair enzyme and has five active sites. The enzyme is mainly involved in removing RNA primers from okazaki fragments and fills up the gap due to its 5'→3' polymerizing activity.

The important function of this enzyme is proofreading which includes polymerizing activity and exonuclease activity (exonuclease activity means cleavage of nucleotides only at the end).

DNA polymerase I consists of two fragments: (1) a larger fragment, called Klenow fragment, which contains 3'–5' exonuclease activity with 5'–3' polymerizing activity and (2) a smaller fragment which contains 5'–3' exonuclease activity.

DNA Polymerase II

DNA Polymerase II resembles DNA polymerase I in its activity to bring about the growth in 5'–3' direction, using free 3'-OH groups, but mainly uses duplexes with short gaps

only. It cannot use nicked duplexes. Although it has 3'–5' exonuclease activity, it lacks 5'–3' exonuclease activity. DNA polymerase II is not the replication enzyme, and is involved in DNA repair.

DNA Polymerase III

DNA Polymerase III (Pol III) is a hetro-multemeric enzyme with a molecular mass of about 900 KD in that has catalytic activity consists of three its complex or holoenzyme form. The core polymerase subunits: α-subunit (coded by *dnaE* gene) has 5–3' synthetic activity; ε (coded by *dnaQ* gene) has 3'–5' exonuclease activity; and θ (coded by *holE* gene) is stimulator of the 3'–5' exonuclease activity. The core enzyme which has the ability to synthesize DNA, consists of subunits α, β and θ. The τ subunit (coded by *dnaX* gene) is responsible for dimerization of catalytic core and increased activity. The catalytic core synthesizes rather short DNA strands because of its tendency to fall off the DNA template. In order to synthesize the long DNA molecules present in chromosomes, this frequent dissociation of the polymerase from the template must be eliminated. For this purpose two homodimers of the β subunit (coded by *dnaN* gene), provides the ring structure that encircles the replicating DNA molecule and allows DNA polymerase III to slide along the DNA while remaining tethered to it. The DNA polymerase III holoenzyme, which is responsible for the synthesis of both nascent DNA strands at a replication fork, contains at least 20 polypeptides. The structural complexity of the DNA polymerase III holoenzyme shows 16 of the best characterized polypeptides encoded by seven different genes.

DNA Gyrase/Topoisomerases

Two strands of a ds DNA are wound with each other in a spiral manner. Further, the DNA molecule is supercoiled under normal cellular conditions. It is, therefore, necessary to unwind the DNA, so that the two strands can be opened and form ss regions during the DNA replication.

In order to get the DNA strands unwound, it will require to rotate at a speed of about 4500 rpm. The rotation will require a large amount of energy. The rotation of long strands of DNA at such a high speed can also cause mechanical shearing of the DNA. Thus the entire process will create an undesirable condition for the cell. This problem is overcome by the enzyme topoisomerase which opens the coiled turns by creating a small nick in one strand, letting the molecule uncoiled by one turn and closing the gap.

The topoisomerases are of two types: (1) DNA topoisomerase I enzyme produce temporary single stranded break or nick in DNA and (2) DNA topoisomerase II enzyme produce transient double stranded break in DNA.

Type I topoisomerase are remarkable in that they don't require ATP to work, they bind covalently to the cut DNA strand storing the chemical energy of the phosphodiester bridge, which they then use to reseal the strand after the tension has been removed from the DNA. An important result of this difference is that topoisomerase I activities remove supercoils from DNA one at a time, whereas, topoisomerase II enzyme remove and introduce supercoils two at a time. The best characterized type II topoisomerase is an enzyme named DNA gyrase in *E. coli*. Gyrases or topoisomerases II is a tetramer with 2 α-subunits encoded by the gene *gyrA* and 2 β-subunits encoded by gene *gyrB*.

DNA ligase

It can join two pieces of DNA by the formation of a phosphodiester bond between these molecules. For this, the two DNAs should have a free 3'-OH and a 5'-PO_4 groups respectively. It should be noted that the DNA ligase can only close a nick but cannot incorporate any new nucleotide to fill the gap. The enzyme is used to join the polypeptide fragments in the lagging strand during the DNA replication. DNA ligase catalyses the covalent closure of nicks in DNA molecules by using energy from NAD (Nicotiamide adenine dinucleotide) or ATP (Adenosine triphosphate).

Helicase

The enzyme also referred as unwinding protein. It is responsible for the melting of DNA and formation of ss DNA at the beginning of the replication fork. The major DNA replicative helicase in *E. coli* is the product of *dnaB* gene. DNA helicases unwind DNA molecules using energy derived from ATP. The active form of helicase is the homo-hexamer of a 330 KD protein.

Primase

It is a specific RNA polymerase which is responsible for the synthesis of a sequence specific RNA molecule which serves as the primer to initiate the DNA synthesis. This is a monomer of 66 KD and is the product of the gene dnaG. This enzyme is simpler and much smaller than the RNA polymerase involved in the transcription and is distinct from it.

PROCESS OF DNA REPLICATION

The synthesis of DNA starts at a defined locus in the genome which is known as the 'origin of replication'. There are no known conserved sequences to define the origin of replication. Prokaryotes have only one origin of replication instead of eukaryotes which have

got multiple origins of replication.

The origin of E. coli genome has been well characterized. It is referred as 'OriC' and it consists of 245 base pairs characterized by repeating sequences of 9 and 13 bases (called 9mers and 13mers). The sequences at the origin of replication are found A:T rich, which makes the melting of ds DNA easier. The origins of replication in yeast are referred as autonomous replicating sequences (ARS). However, the origins from higher organisms have not been well identified.

A unique sequence at which replication is initiated with the formation of a localized region of strand separation, called the replication bubble. The formation of this replication bubble is due to the interaction of prepriming proteins with *OriC*. A particular 82 KD protein called DnaA, encoded by gene *dnaA,* is responsible for the initial step of unwinding of helix. DnaA proteins bind cooperatively to form a core of 20-40 polypeptides with OriC DNA wound on the surface of the protein complex. Strand separation begins within the 3 tandem 13-bp repeats in OriC and spreads until the replication bubble is created. A complex of DnaB protein (the hexamer DNA helicase) and DnaC proteins (six molecules) joins the initiation complex, and contributes to the formation of two bidirectional replication forks. The DnaT protein also is present in the prepriming protein complex, but its function is unknown. Other proteins associated with the initiation complex at OriC are DnaJ protein, DnaK protein,

PriA protein, PriB protein, PriC protein, DNA binding proteins, DNA gyrase and single stranded DNA binding (SSB) proteins.

The single-stranded character of the replication fork is maintained by the binding of single strand specific DNA binding proteins, referred as SSB proteins. The association of these proteins prevent the reformation of ds DNA. Tthe activity of DnaB is facilitated by the binding of another protein DnaC to DnaB. DnaB is present in form of a hexamer. There are six molecules of DnaC for each DnaB molecule. One monomer of DnaC binds to each of the subunits of the DnaB hexamer. Once the primer has initiated the DNA synthesis, DNA polymerase takes over and adds more nucleotides, one at a time to the primer and continues to extend the DNA chain. Thus a number of factors get associated to DNA to initiate the replication. This entire complex of DNA primase and DNA helicase is referred to as primosome. Once a replication fork has formed the synthesis of new DNA strand is initiated by RNA primers synthesized by DNA primase. A single RNA primer is sufficient for the continuous replication of the leading strand, but the discontinuous replication of the lagging strand requires an RNA primer to start the synthesis of each okazaki fragment. During the entire process the helicase keeps binding ahead of the fork so that there is a forward movement of the fork.

Leading and Lagging strands

The mechanism of DNA replication explains the synthesis of DNA for one strand, namely 3'-5' of template which will be copied in the 5'-3' direction by the DNA polymerase. How does the synthesis of the other strand (5'-3' of template) which apparently seems to grow in 3'-5' direction, takes place? It was a big puzzle for a long time as there is no known DNA polymerase which can carry out such an extension.

The problem was solved in 1960s when Reiji Okazaki and his colleagues discovered the presence of a large number of small DNA fragments, each with an RNA primer attached to it, and was called as Okazaki fragments. It has been well established now that the synthesis of this strand also takes place only in the 5'-3' direction.

The primase initiates the synthesis of DNA at the 3'-end of the opened region of the fork and polymerase extends this primer towards the 5□-side (of template), synthesizing the new DNA in 5'-3' direction. When more bases of template DNA open up and the size of the fork is increased, a new primer is synthesied and gets annealed at the 5'-end of the fork, and the entire process is repeated until the polymerase has reached to the first primer.

The 5'-3' exonuclease activity of pol I, at this stage, digests the RNA primer and the polymerase fills the gap with DNA. Thus the synthesis of this strand takes place in pieces. These pieces are then joined by DNA ligase to form a continuous DNA strand. There are separate primases for leading and lagging strands. The synthesis of two strands thus takes place in slightly different manner. One strand which is known as the leading strand is synthesized in a continuous manner while the other strand is called as lagging strand and is synthesized in discontinuous manner.

The DNA replication is, therefore, semi-discontinuous in nature. The small fragments of DNA which are synthesized in the lagging strand are known as okazaki fragments. It should be noted that there is a single primosome formation for the leading strand, while multiple primosome are formed on the lagging strand. Each primosome results in the formation of a single Okazaki fragment.

The initiation of Okazaki fragments on the lagging strand is carried out by primosome. The primosome gets energy from ATP to move along the DNA molecule. The unwinding of double helix takes place with the help of enzyme DNA helicase and the DNA primase synthesizes the RNA primers for successive okazaki fragments.

The RNA primers are covalently extended with deoxiribonucleotides by DNA polymerase III. DNA topoisomerases provide transient breaks in DNA that serves as swivels for DNA unwinding and keep the DNA untangled. Single-stranded DNA binding protein coats the unwound prereplicative DNA and keeps it in an extended state for DNA polymerase III. The RNA primers are replaced with DNA by DNA Polymerase I and the single-stranded breaks left by polymerase are sealed by DNA ligase. The DNA is then condensed in to the nucleoid, or folded genome, of *E. coli*, in part through negative supercoiling introduced by DNA gyrase. All of these enzymes and DNA binding proteins function in concert at each replication fork.

The complete replication apparatus moving along the DNA molecule at a replication fork is called the replisome. The replisome contains the DNA pol III holoenzyme; one catalytic core replicates the leading strand, the second catalytic core replicates the lagging strand and the primosome unwinds the parental DNA molecule and synthesizes the RNA primers needed for the discontinuous synthesis of the lagging strand. In order for the two catalytic cores for the polymerase III holoenzyme to synthesize both the nascent leading and lagging strands, the lagging strand is thought to form a loop from the primosome to the second catalytic core of DNA pol III.

Termination of DNA replication

The *E. coli* chromosome carries a large termination zone, diametrically opposite from *oriC*, which blocks the progress of replication forks (of bidirectional replication), meeting at this region. *E.coli* chromosome and several plasmids carry specific sequences, called *ter* sites, where TBP (ter binding protein) or 'Tus protein' binds. In the termination zone of *E. coli*, there are 3 ter sites (*ter A*, *ter D* and *ter E*) for counter-clockwise fork and three *ter* sites (*ter B*, *ter C* and *ter F*) for clockwise fork. These six sites are arranged in overlapping manner, leaving no 'replication-free' gap on the chromosome. TBP-ter complexes formed at '*ter*' sites stalls the replication fork, by inhibiting the DNA helicase or DnaB. When this termination zone is deleted, replication stops simply by the meeting of opposite replication forks, suggesting that the termination zone is not essential.

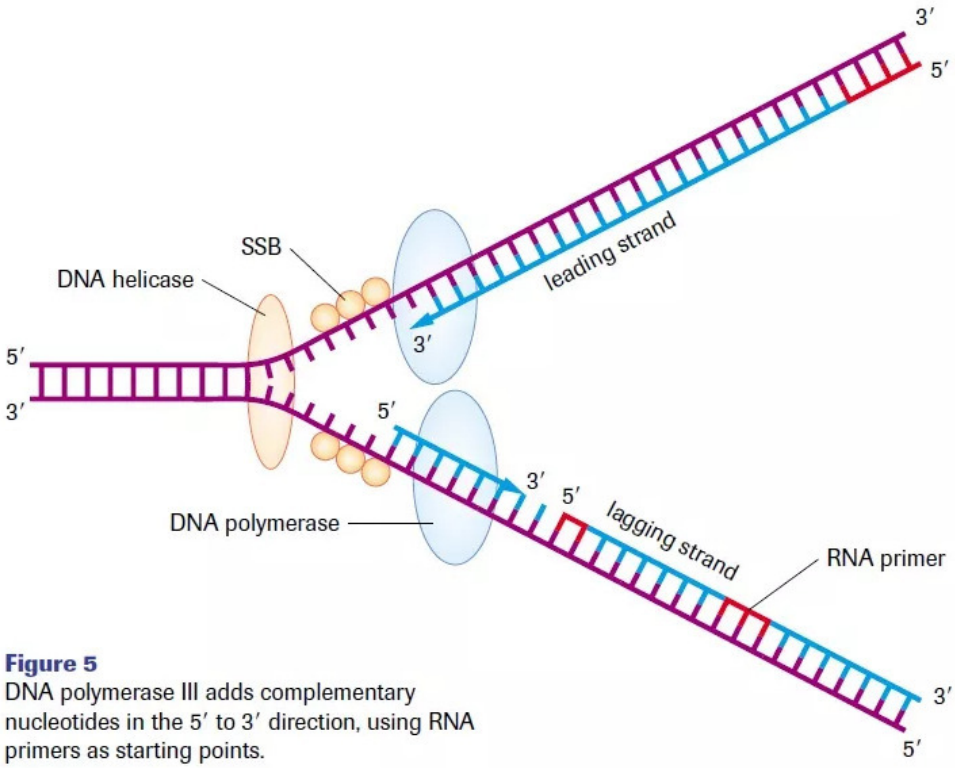
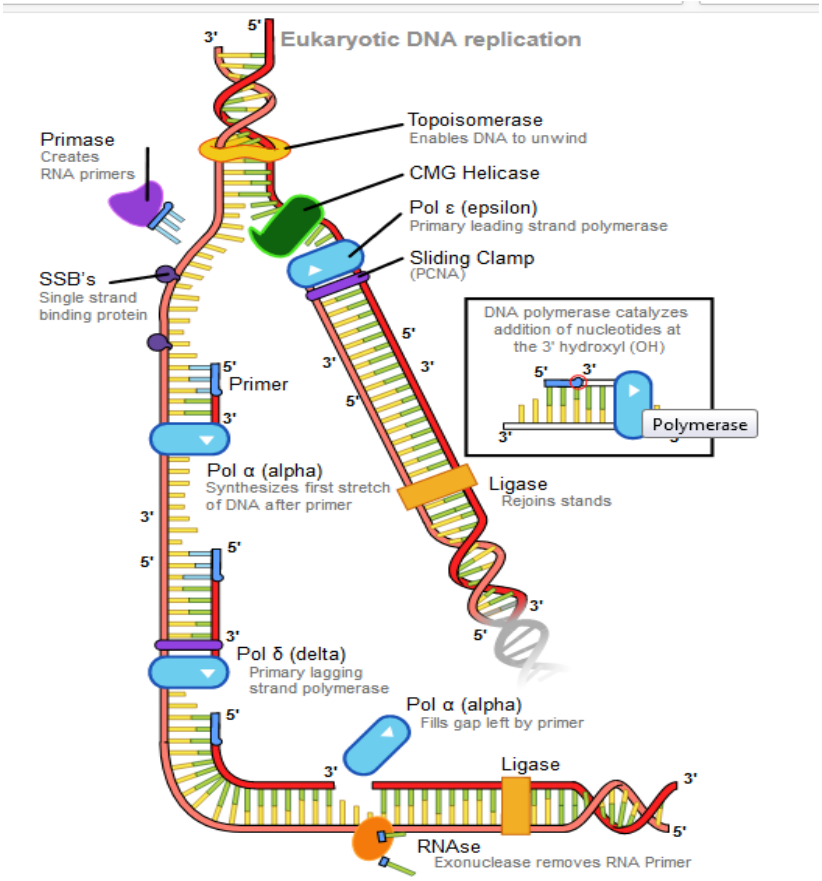


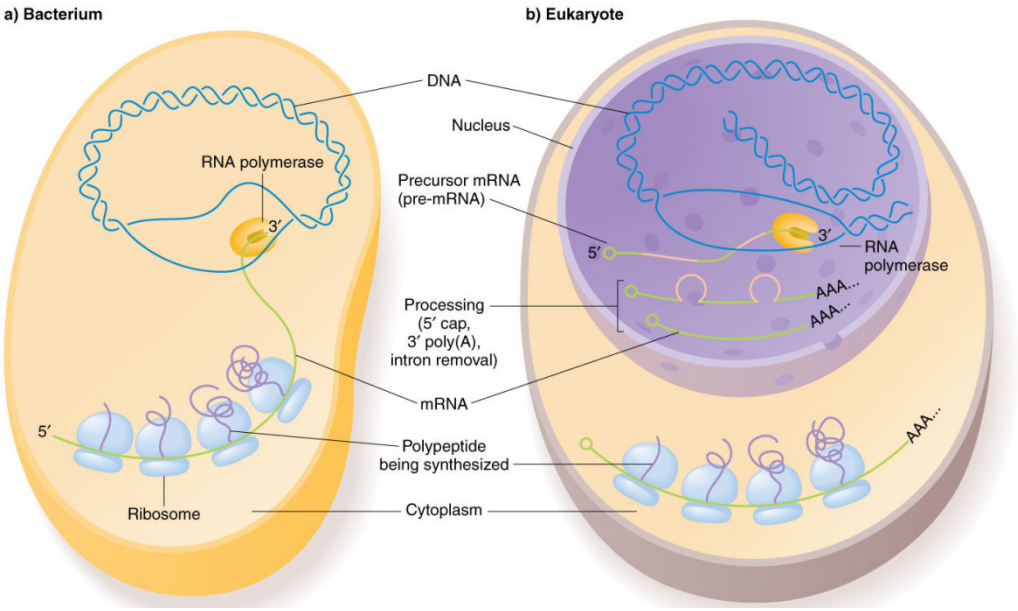
Figure 5
DNA polymerase III adds complementary nucleotides in the 5′ to 3′ direction, using RNA primers as starting points.

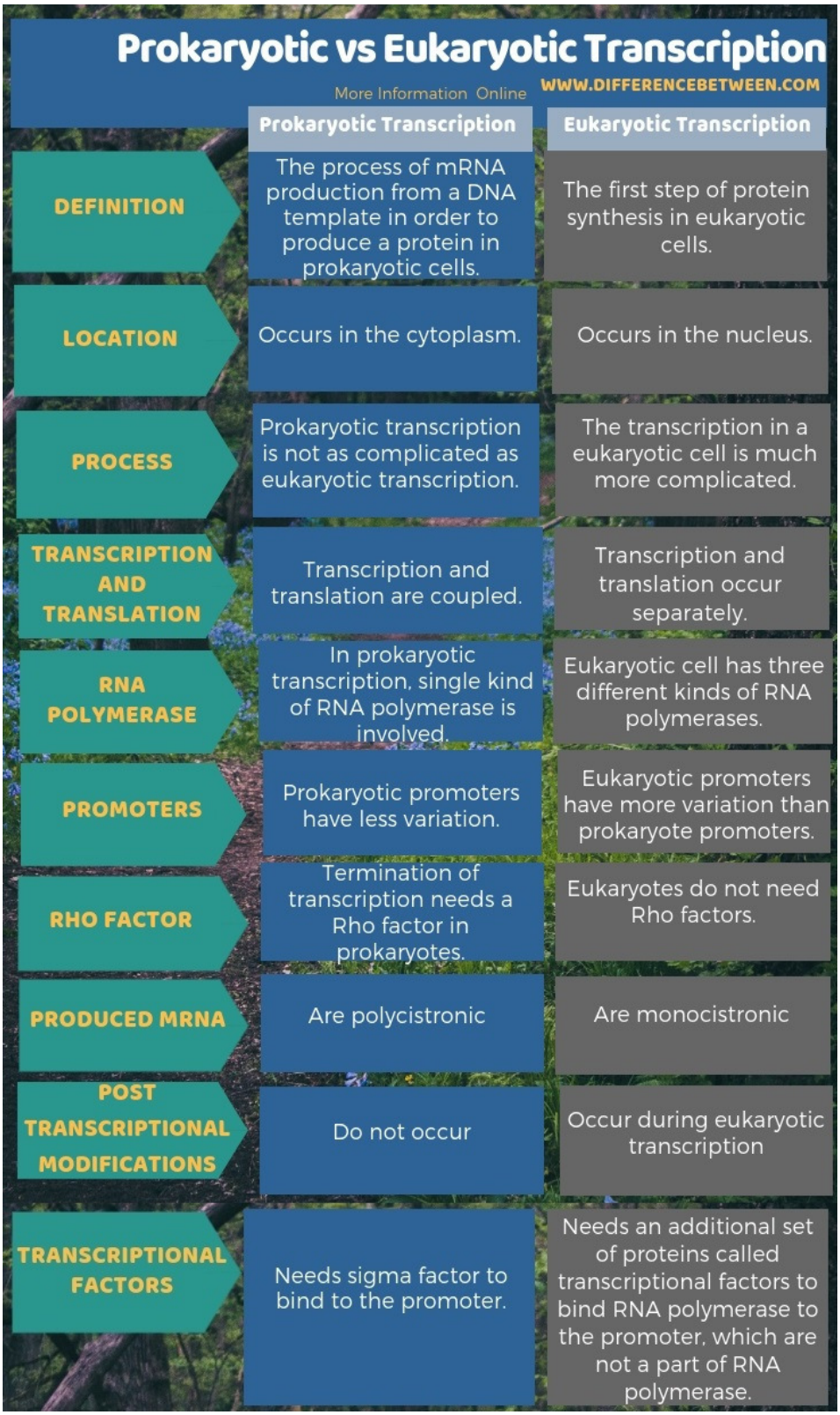


Comparison of Replication

Prokaryotes	Eukaryotes
1. It occurs inside the cytoplasm.	1. It occurs inside the nucleus.
2. There is single origin of replication.	2. Origin of replications are numerous.
3. DNA polymerase III carries out both initiation and elongation.	3. Initiation is carried out by DNA polymerase α while elongation by DNA polymerase δ and ε.
4. DNA repair and gap filling are done by DNA polymerase I.	4. The same are performed by DNA polymerase β.
5. RNA primer is removed by DNA polymerase I.	5. RNA primer is removed by DNA polymerase β.
6. Okazaki fragments are large, 1000-2000 nucleotides long.	6. Okazaki fragments are short, 100-200 nucleotides long.
7. Replication is very rapid, some 2000 base pairs per second.	7. Replication is slow, some 100 nucleotides per second.
8. DNA gyrase is needed.	8. DNA gyrase is not needed.

Transcription




Prokaryotic vs Eukaryotic Transcription
More Information Online
WWW.DIFFERENCEBETWEEN.COM
Prokaryotic Transcription
Eukaryotic Transcription
DEFINITION
The process of mRNA production from a DNA template in order to produce a protein in prokaryotic cells.
The first step of protein synthesis in eukaryotic cells.
LOCATION
Occurs in the cytoplasm.
Occurs in the nucleus.
PROCESS
Prokaryotic transcription is not as complicated as eukaryotic transcription.
The transcription in a eukaryotic cell is much more complicated.
TRANSCRIPTION AND TRANSLATION
Transcription and translation are coupled.
Transcription and translation occur separately.
RNA POLYMERASE
In prokaryotic transcription, single kind of RNA polymerase is involved.
Eukaryotic cell has three different kinds of RNA polymerases.
PROMOTERS
Prokaryotic promoters have less variation.
Eukaryotic promoters have more variation than prokaryote promoters.
RHO FACTOR
Termination of transcription needs a Rho factor in prokaryotes.
Eukaryotes do not need Rho factors.
PRODUCED MRNA
Are polycistronic
Are monocistronic
POST TRANSCRIPTIONAL MODIFICATIONS
Do not occur
Occur during eukaryotic transcription
TRANSCRIPTIONAL FACTORS
Needs sigma factor to bind to the promoter.
Needs an additional set of proteins called transcriptional factors to bind RNA polymerase to the promoter, which are not a part of RNA polymerase.

Transcription in Prokaryotes

In prokaryotic organisms transcription occurs in three phases known as initiation, elongation and termination.

RNA is synthesized by a single RNA polymerase enzyme which contains multiple polypeptide subunits. In E. coli, the RNA polymerase has five subunits: two α, one β, one β' and one σ subunit ($\alpha_2\beta\beta'\sigma$). This form is called the holoenzyme. The σ subunit may dissociate from the other subunits to leave a form known as the core enzyme.

These two forms of the RNA polymerase have different roles in transcription.

(i) Initiation:

Transcription cannot start randomly but must begin specifically at the start of a gene. Signals for the initiation of transcription occur in the promoter sequence which lies directly upstream of the transcribed sequence of the gene. The promoter contains specific DNA sequences that act as points of attachment for the RNA polymerase.

In E. coli, two sequence elements recognized by the RNA polymerase known as the -10 sequence and the -35 sequence arc present. The exact sequences can vary between promoters but all conform to an overall pattern known as the consensus sequence. The σ subunit of the RNA polymerase is responsible for recognizing and binding the promoter, probably at the -35 Box.

In the absence of the σ subunit the enzyme can still bind to DNA but binding is more random. When the enzyme binds to the promoter it initially forms a closed promoter complex in which the promoter DNA remains as a double helix. T he enzyme covers about 60 base pairs of the promoter including the -10 and -35 boxes. To allow transcription to begin, the double helix partially dissociates at the – 10 box, which is rich in weak A-1 bonds to give an open promoter complex.

The σ subunit then dissociates from the open promoter complex leaving the core enzyme. At the same time the first two ribonucleotides bind to the DNA, the first phosphodiester bond is formed and transcription is initiated (Fig. 7.7).

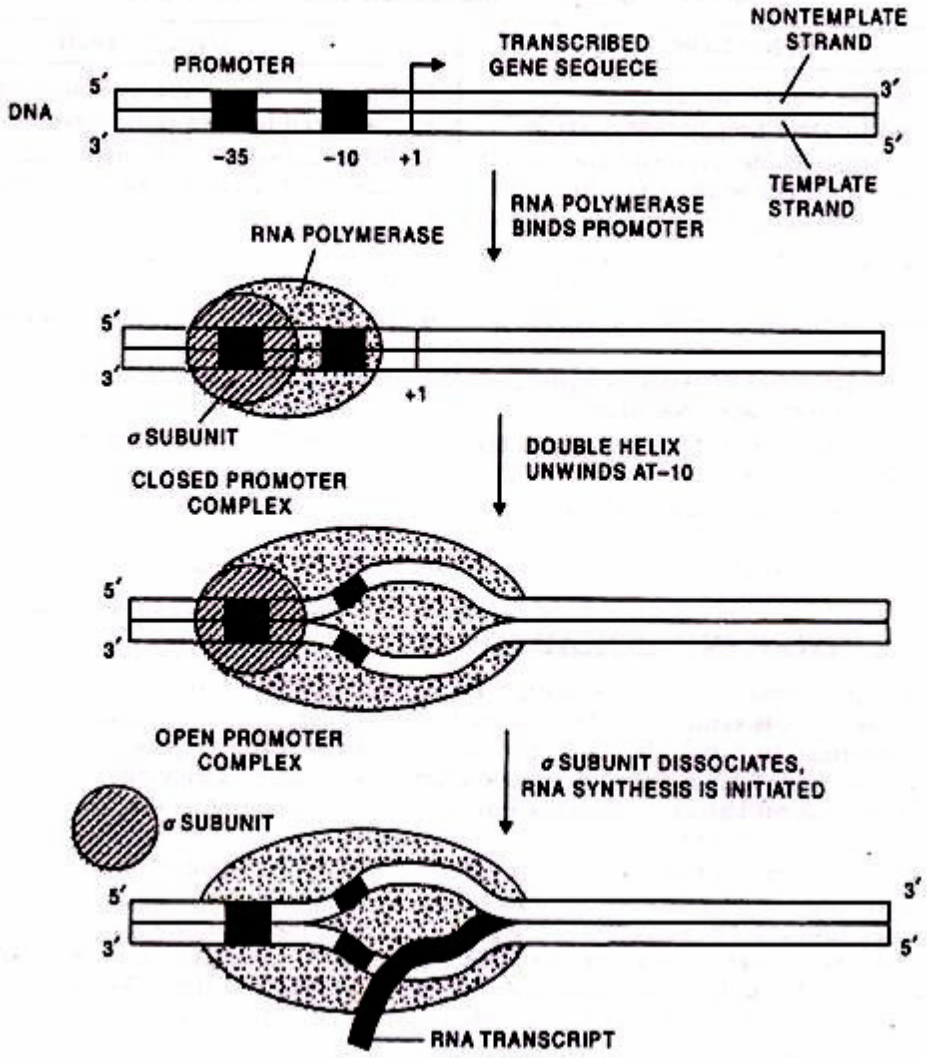


Fig. 7.7. Initiation of transcription in prokaryotes.

(ii) Elongation:

During elongation the RNA polymerase moves along the DNA molecule melting and unwinding the double helix as it progresses. The enzyme adds ribonucleotides to the 3′ end of the growing RNA molecule with the order of addition determined by the order of the bases on the template strand.

In most cases, a leader sequence of variable length is transcribed before the coding sequence of the gene is reached. Similarly, at the end of the coding sequence a noncoding trailer sequence is transcribed before transcription ends.

During transcription only a small portion of the double helix is unwound at any one time. The unwound area contains the newly synthesized RNA base-paired with the template DNA strand and extends over 12-17 bases.

The unwound area needs to remain small because unwinding in one region necessitates over-winding in adjacent regions and this imposes strain on the DNA molecule. To overcome this problem, the RNA is released from the template DNA as it is synthesized allowing the DNA double helix to reform (Fig. 7.8).

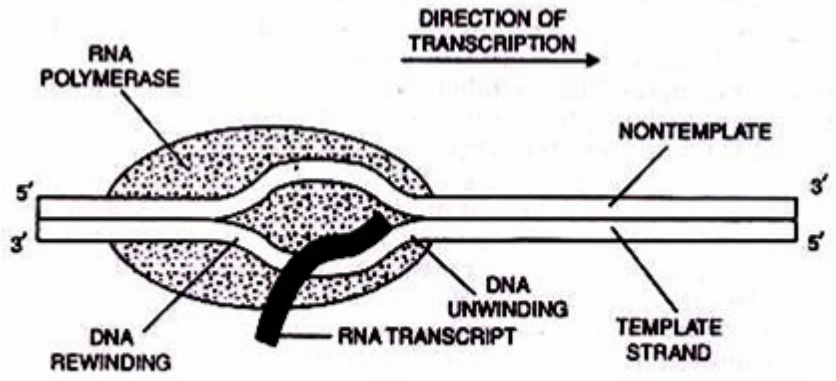


Fig. 7.8 Transcription elongation in prokaryotes.

(iii) Termination:

The termination of transcription occurs non-randomly and takes place at specific points after the end of the coding sequence. In E. coli, termination occurs at sequences known as palindromes. These are symmetrical about their middle such that the first half of the sequence is followed by its exact complement in the second half.

In single-stranded RNA molecules this feature allows the first half of the sequence to base pair with the second half to form what is known as a stem-loop structure (Fig. 7.9). These appear to

act as signals for termination. In some cases the stem-loop sequence is followed by a run of 5-10 As in the DNA which form weak A-U base pairs with the newly synthesized RNA.

It is thought that the RNA polymerase pauses just after the stem-loop and that the weak A-U base pairs break causing the transcript to detach from the template. In other cases the run of As is absent and a different mechanism occurs based on binding of a protein called Rho (ρ) which disrupts base-pairing between the template and the transcript when the polymerase pauses after the stem-loop. The termination of transcription involves the release of the transcript and the core enzyme which may then re-associate with the σ subunit and go on to another round of transcription (Fig. 7.9 & 7.10).

In many bacteria, genes of related functions are grouped together in operons. An operon acts as a single transcription unit and thus produces polycistronic mRNA. In eukaryotes, only monocistronic mRNAs are generally produced.

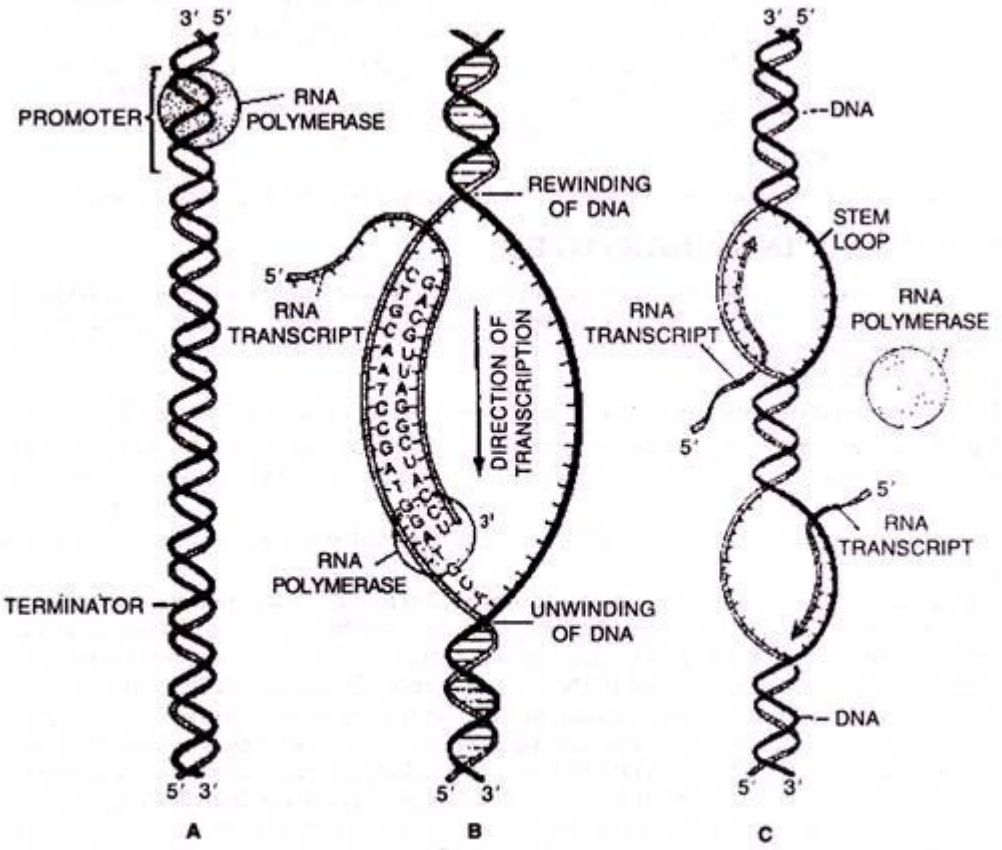
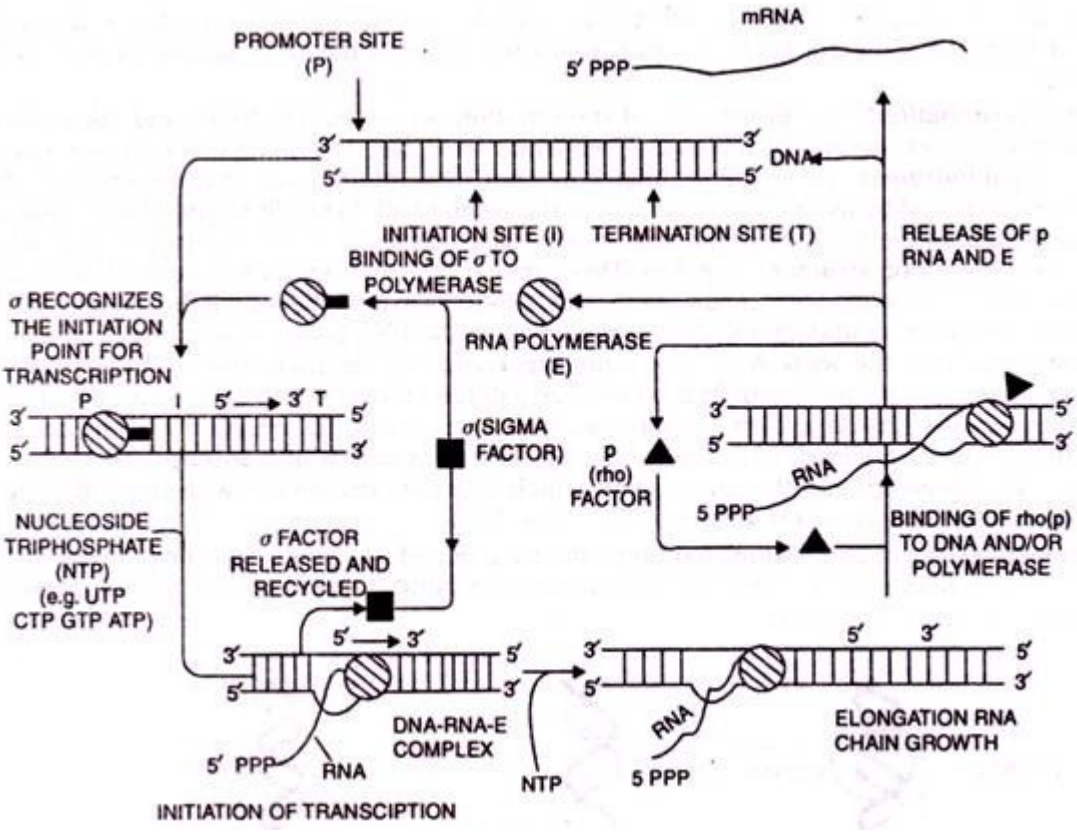


Fig. 7.9 A–C. A summarized diagram of general process of transcription in prokaryotes : **A.** Binding to promoter and RNA chain initiation : **B.** Elongation of RNA polypeptide chain, **C.** Termination of transcription.



Diagrammatic presentation of the synthesis of RNA by E.coli polymerase

Transcription in Eukaryotes

Transcription occurs in eukaryotes in a way similar to prokaryotes. However, initiation is more complex, termination does not involve stem-loop structures and transcription is carried out by three enzymes (RNA polymerases I, II and III) each of which transcribes a specific set of genes and functions in a slightly different way.

RNA polymerase I transcribes genes encoding three of the four ribosomal RNAs (18S, 28S and 5.8S). RNA polymerase II enzyme transcribes genes that encode proteins. Binding of RNA polymerase II to its promoter involves several different DNA sequence elements and a number of proteins called transcription factors. RNA polymerase III transcribes a set of short genes that encode transfer RNAs and the 5S ribosomal RNA.

Unlike the situation in prokaryotic genes, transcription in eukaryotes occurs within the nucleus and mRNA moves out of the nucleus into the cytoplasm for translation. The initiation and regulation of transcription is more extensive than prokaryotes. Another major difference between prokaryotes and eukaryotes lies in the fact that the mRNA in eukaryotes is processed from the primary RNA transcript, a process called maturation.

Initially at the 5′ end a cap (consisting of 7-methyl guanosine or 7 mG) and a tail of poly A at the 3′ end are added (Fig. 7.11) The cap is a chemically modified molecule of guanosine triphosphate (GTP). The primary eukaryotic mRNA transcript is much longer and localised into the nucleus, when it is also called heterogenous nuclear RNA (hnRNA) or pre- mRNA.

The eukaryotic primary mRNAs are made up of two types of segments; non-coding introns and the coding exons. The introns are removed by a process called RNA splicing. Of a pair of small nuclear ribonucleoprotein (SnRNPs pronounced “snurps”), one binds to 5′ splice site and the other to 3′ splice site.

A spliceosome forms because of interaction between SnRNPs and other proteins. This spliceosome uses energy of ATP to cut the RNA, releases the introns and joins two adjacent exons to produce mature mRNA. Besides, these two post-transcriptional modifications, RNA editing may also take place before translation begins.

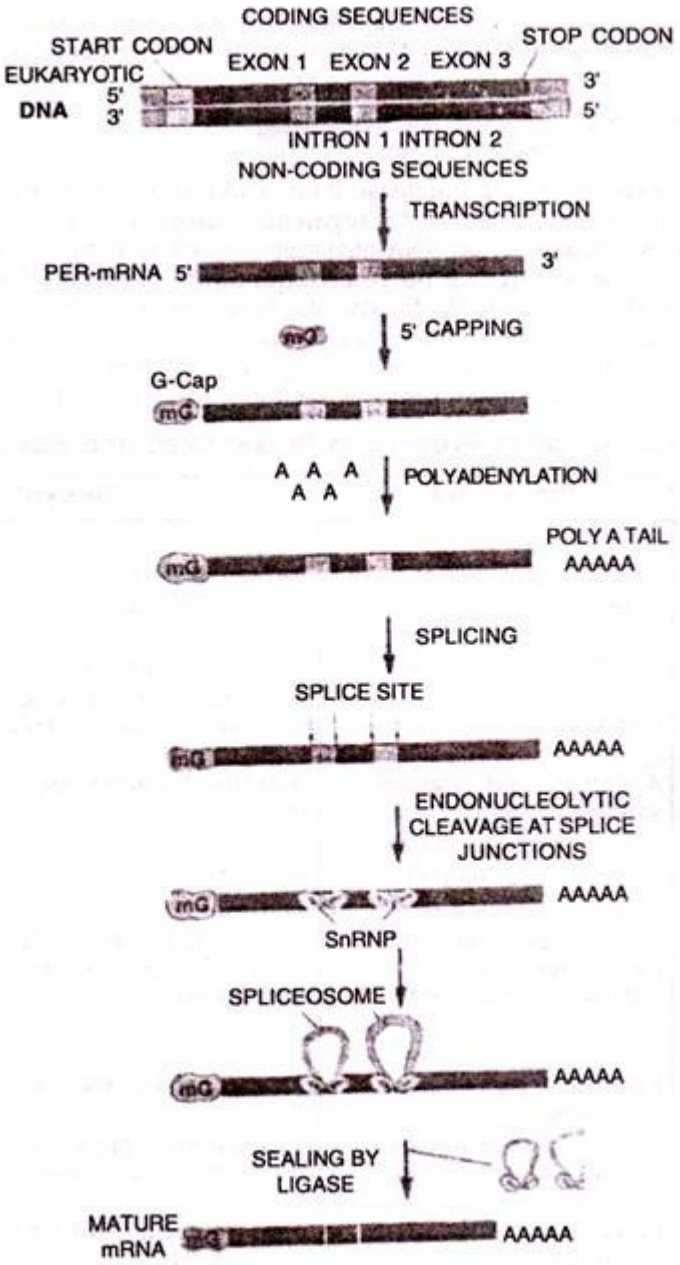


Fig. 7.11. Transcription in eukaryotes

Post transcriptional modification

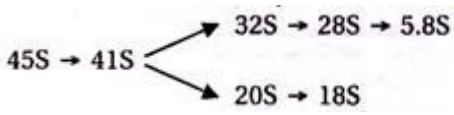
Post-transcription processing is required to convert primary transcript into functional RNAs.

It is of four types:

(i) Cleavage:

Larger RNA precursors are cleaved to form smaller RNAs. Primary transcript of rRNA is 45S in eukaryotes.

It is cleaved to form the following:



Primary transcript is cleaved by ribonuclease-P (an RNA enzyme) to form 5-7 tRNA precursors,

(ii) Splicing:

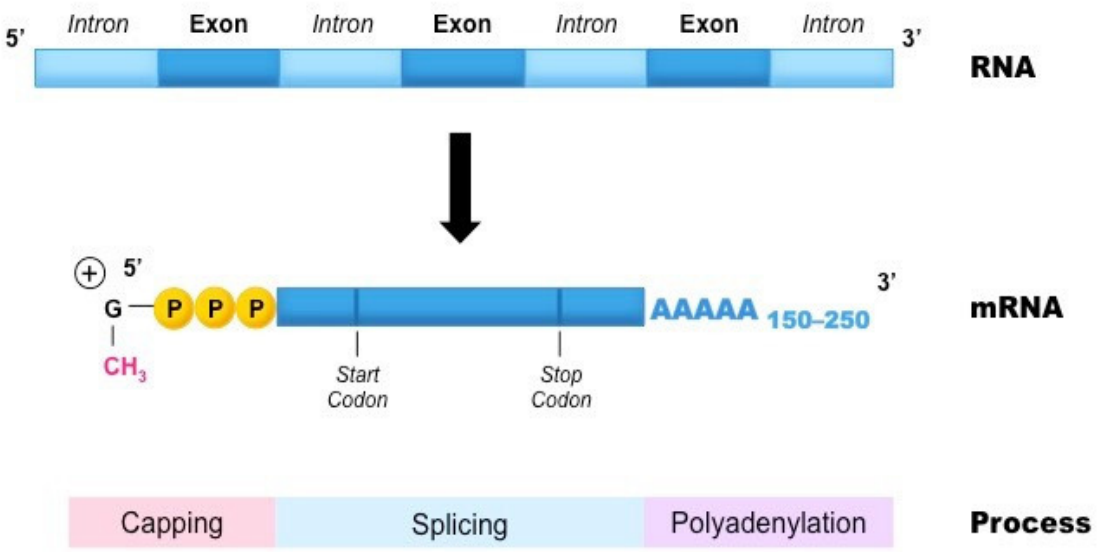
Eukaryotic transcripts possess extra segments (introns or intervening sequences). They are removed by nucleases. Ribozyme (an-RNA enzyme) is a self splicing intron involved in some of these reactions as well as catalysing polymerisation.

(iii) Terminal Additions:

Additional nucleotides are added to the ends of RNAs for specific functions, e.g., CCA segment in tRNA, cap nucleotides at 5′ end of mRNA or poly-A segments at 3′ end of mRNA.

(iv) Nucleotide Modifications:

They are most common in tRNA-methylation (e.g., methyl cytosine, methyl guanosine), deamination (e.g., inosine from adenine), dihydrouracil, pseudouracil, etc.



Translation

Prokaryotic and Eukaryotic Translation

Translation is similar in prokaryotes and eukaryotes. Here we will explore how translation occurs in *E. coli*, a representative prokaryote, and specify any differences between bacterial and eukaryotic translation.

Initiation

The **initiation of protein synthesis** begins with the formation of an initiation complex. In ***E. coli***, this complex involves the small 30S ribosome, the mRNA template, three initiation factors that help the ribosome assemble correctly, guanosine triphosphate (GTP) that acts as an energy source, and a special initiator tRNA carrying ***N*-formyl-methionine** (fMet-tRNAf^{Met}).

The initiator tRNA interacts with the start codon AUG of the mRNA and carries a formylated methionine (fMet). Because of its involvement in initiation, fMet is inserted at the beginning (N terminus) of every polypeptide chain synthesized by *E. coli*.

In *E. coli* mRNA, a leader sequence upstream of the first AUG codon, called the **Shine-Dalgarno sequence** (also known as the ribosomal binding site AGGAGG), interacts through complementary base pairing with the rRNA molecules that compose the ribosome. This interaction anchors the 30S ribosomal subunit at the correct location on the mRNA template. At this point, the 50S ribosomal subunit then binds to the initiation complex, forming an intact ribosome.

In eukaryotes, initiation complex formation is similar, with the following differences:

- The initiator tRNA is a different specialized tRNA carrying methionine, called Met-tRNAi
- Instead of binding to the mRNA at the Shine-Dalgarno sequence, the eukaryotic initiation complex recognizes the 5′ cap of the eukaryotic mRNA, then tracks along the mRNA in the 5′ to 3′ direction until the AUG start codon is recognized. At this point, the 60S subunit binds to the complex of Met-tRNAi, mRNA, and the 40S subunit.

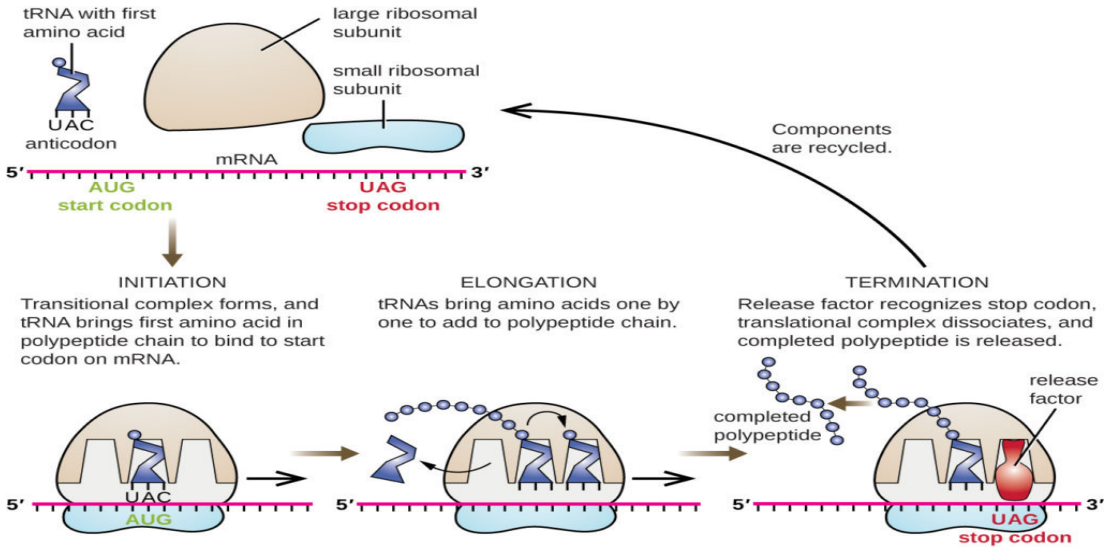


Figure. Translation in bacteria begins with the formation of the initiation complex, which includes the small ribosomal subunit, the mRNA, the initiator tRNA carrying N-formyl-methionine, and initiation factors. Then the 50S subunit binds, forming an intact ribosome.

Elongation

In prokaryotes and eukaryotes, the basics of **elongation of translation** are the same.

In ***E. coli***, the binding of the 50S ribosomal subunit to produce the intact ribosome forms three functionally important ribosomal sites: The **A (aminoacyl) site** binds incoming charged aminoacyl tRNAs. The **P (peptidyl) site** binds charged tRNAs carrying amino acids that have formed peptide bonds with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA.

The **E (exit) site** releases dissociated tRNAs so that they can be recharged with free amino acids. There is one notable exception to this assembly line of tRNAs: During initiation complex formation, bacterial fMet−$tRNA^{fMet}$ or eukaryotic Met-tRNAi enters the P site directly without first entering the A site, providing a free A site ready to accept the tRNA corresponding to the first codon after the AUG.

Elongation proceeds with single-codon movements of the ribosome each called a translocation event. During each translocation event, the charged tRNAs enter at the A site, then shift to the P site, and then finally to the E site for removal. Ribosomal movements, or steps, are induced by conformational changes that advance the ribosome by three bases in the 3′ direction.

Peptide bonds form between the amino group of the amino acid attached to the A-site tRNA and the carboxyl group of the amino acid attached to the P-site tRNA. The formation of each peptide bond is catalyzed by **peptidyl transferase**, an RNA-based ribozyme that is integrated into the 50S ribosomal subunit. The amino acid bound to the P-site tRNA is also linked to the growing polypeptide chain.

As the ribosome steps across the mRNA, the former P-site tRNA enters the E site, detaches from the amino acid, and is expelled. Several of the steps during elongation, including binding of a charged aminoacyl tRNA to the A site and translocation, require energy derived from GTP hydrolysis, which is catalyzed by specific elongation factors. Amazingly, the *E. coli* translation apparatus takes only 0.05 seconds to add each amino acid, meaning that a 200 amino-acid protein can be translated in just 10 seconds.

Termination

The **termination of translation** occurs when a **nonsense codon** (UAA, UAG, or UGA) is encountered for which there is no complementary tRNA. On aligning with the A site, these nonsense codons are recognized by release factors in prokaryotes and eukaryotes that result in the P-site amino acid

detaching from its tRNA, releasing the newly made polypeptide. The small and large ribosomal subunits dissociate from the mRNA and from each other; they are recruited almost immediately into another translation initiation complex.

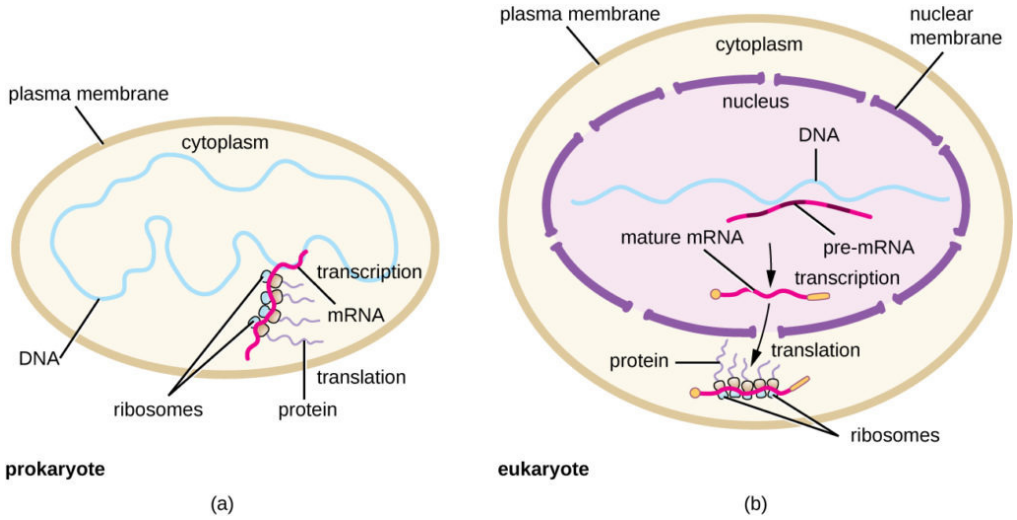


Figure. (a) In prokaryotes, the processes of transcription and translation occur simultaneously in the cytoplasm, allowing for a rapid cellular response to an environmental cue. (b) In eukaryotes, transcription is localized to the nucleus and translation is localized to the cytoplasm, separating these processes and necessitating RNA processing for stability.


PROKARYOTIC TRANSLATION
VERSUS
EUKARYOTIC TRANSLATION
Prokaryotic transcription and translation are simultaneous processes
Eukaryotic transcription and translation are discontinuous processes
30S and 50S = 70S ribosomes
40S and 60S = 80S ribosomes
Porkaryotic mRNAs occur in the cytoplasm
Eukaryotic mRNAs occur in the nucleus
mRNAs are unstable - live for few seconds to two minutes
mRNAs are quite stable - live for about few hours to days
Performed by 70S ribosomes in the cytoplasm
Performed by the 80S ribosomes attached with the ER
No definite phase for the occurrence
Occurs in G1 and G2 phases in the cell cycle
Cap-independent initiation
Cap-dependent & cap-independent initiation
Three initiation factors are involved: IF1, IF2 and IF3
Nine initiation factors are involved: eIF 1, 2, 3, 4A, 4B, 4C, 4D, 5 and 6
A faster process
A slower process
A single release factor is involved: eRF1
Two released factors are involved: RF1 & RF2
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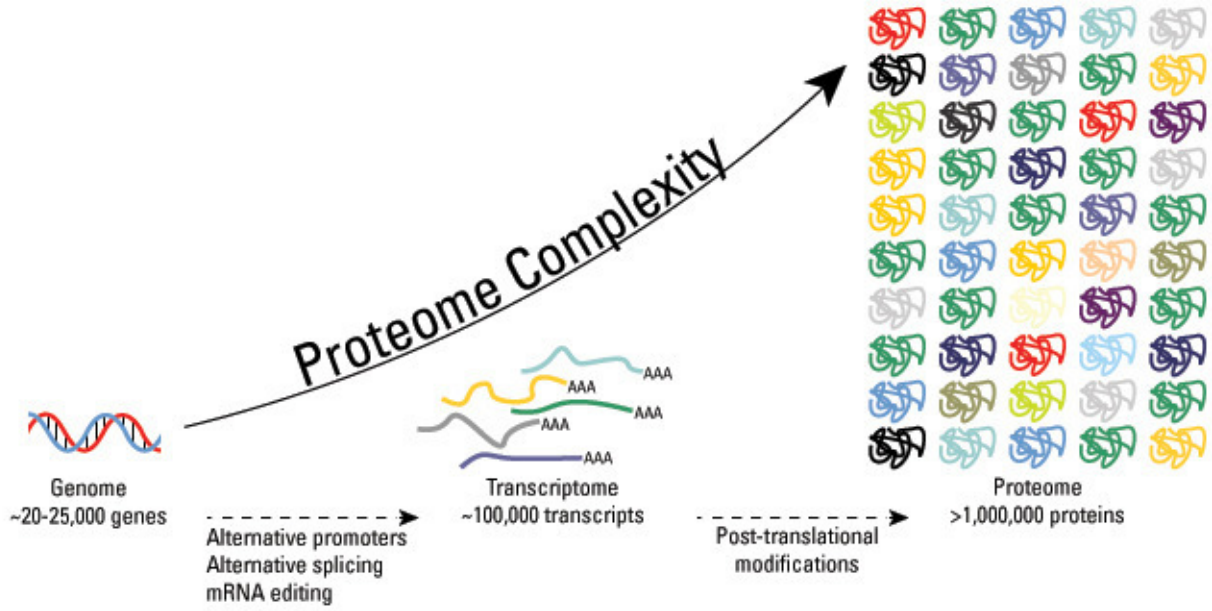
Post translational modifications

The increase in complexity from the level of the genome to the proteome is further facilitated by protein post-translational modifications (PTMs). PTMs are chemical modifications that play a key role in functional proteomic because they regulate activity, localization, and interaction with other cellular molecules such as proteins, nucleic acids, lipids and cofactors.



Post-translational modifications are key mechanisms to increase proteomic diversity.

While the genome comprises 20,000 to 25,000 genes, the proteome is estimated to encompass over 1 million proteins. Changes at the transcriptional and mRNA levels increase the size of the transcriptome relative to the genome, and the myriad of different post-translational modifications exponentially increases the complexity of the proteome relative to both the transcriptome and genome.

Additionally, the human proteome is dynamic and changes in response to a legion of stimuli, and post-translational modifications are commonly employed to regulate cellular activity. PTMs occur at distinct amino acid side chains or peptide linkages, and they are most often mediated by enzymatic activity.

Indeed, it is estimated that 5% of the proteome comprises enzymes that perform more than 200 types of post-translational modifications. These enzymes include kinases, phosphatases, transferases and ligases, which add or remove functional groups, proteins, lipids or sugars to or from amino acid side chains; and proteases, which cleave peptide bonds to remove specific sequences or regulatory subunits. Many proteins can also modify themselves using autocatalytic domains, such as autokinase and autoprotolytic domains.

Post-translational modification can occur at any step in the "life cycle" of a protein. For example, many proteins are modified shortly after translation is completed to mediate proper protein folding or stability or to direct the nascent protein to distinct cellular compartments (e.g., nucleus, membrane). Other modifications occur after folding and localization are completed to activate or inactivate catalytic activity or to otherwise influence the biological activity of the protein.

Proteins are also covalently linked to tags that target a protein for degradation. Besides single modifications, proteins are often modified through a combination of post-translational cleavage and the addition of functional groups through a step-wise mechanism of protein maturation or activation.

Protein PTMs can also be reversible depending on the nature of the modification. For example, kinases phosphorylate proteins at specific amino acid side chains, which is a common method of catalytic activation or inactivation. Conversely, phosphatases hydrolyze the phosphate group to remove it from the protein and reverse the biological activity. Proteolytic cleavage of peptide bonds is a thermodynamically favorable reaction and therefore permanently removes peptide sequences or regulatory domains.

Consequently, the analysis of proteins and their post-translational modifications is particularly important for the study of heart disease, cancer, neurodegenerative diseases and diabetes. The characterization of PTMs, although challenging, provides invaluable insight into the cellular functions underlying etiological processes.

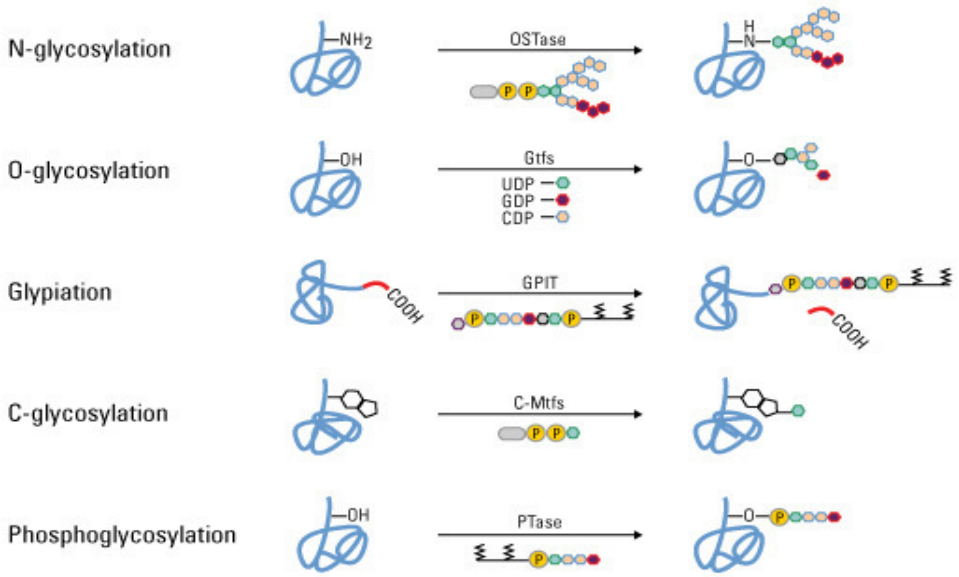
As noted above, the large number of different PTMs precludes a thorough review of all possible protein modifications. Therefore, this overview only touches on a small number of the most common types of PTMs studied in protein research today. Furthermore, greater focus is placed on phosphorylation, glycosylation and ubiquitination, and therefore these PTMs are described in greater detail on pages dedicated to the respective PTM.

Phosphorylation

Reversible protein phosphorylation, principally on serine, threonine or tyrosine residues, is one of the most important and well-studied post-translational modifications. Phosphorylation plays critical roles in the regulation of many cellular processes, including cell cycle, growth, apoptosis and signal transduction pathways.

Glycosylation

Protein glycosylation is acknowledged as one of the major post-translational modifications, with significant effects on protein folding, conformation, distribution, stability and activity. Glycosylation encompasses a diverse selection of sugar-moiety additions to proteins that ranges from simple monosaccharide modifications of nuclear transcription factors to highly complex branched polysaccharide changes of cell surface receptors. Carbohydrates in the form of aspargine-linked (N-linked) or serine/threonine-linked (O-linked) oligosaccharides are major structural components of many cell surface and secreted proteins.



Types of glycosylation: Glycopeptide bonds can be categorized into specific groups based on the nature of the sugar–peptide bond and the oligosaccharide attached, including N-, O- and C-linked glycosylation, glypiation and phosphoglycosylation.

Ubiquitination

Ubiquitin is an 8-kDa polypeptide consisting of 76 amino acids that is appended to the Îµ-NH2 of lysine in target proteins via the C-terminal glycine of ubiquitin. Following an initial monoubiquitination event, the formation of a ubiquitin polymer may occur, and polyubiquitinated proteins are then recognized by the 26S proteasome that catalyzes the degradation of the ubiquitinated protein and the recycling of ubiquitin. The following experiment provides an example of methods used to detect ubiquitinated proteins.

S-nitrosylation

Nitric oxide (NO) is produced by three isoforms of nitric oxide synthase (NOS), and it is a chemical messenger that reacts with free cysteine residues to form S-nitrothiols (SNOs). S-nitrosylation is a critical PTM used by cells to stabilize proteins, regulate gene expression and provide NO donors, and the generation, localization, activation and catabolism of SNOs are tightly regulated.

S-nitrosylation is a reversible reaction, and SNOs have a short half-life in the cytoplasm because of the host of reducing enzymes, including glutathione (GSH) and thioredoxin, that denitrosylate proteins. Therefore, SNOs are often stored in membranes, vesicles, the interstitial space and lipophilic protein folds to protect them from denitrosylation. For example, caspases, which mediate apoptosis, are stored in the mitochondrial intermembrane space as SNOs. In response to extra- or intracellular cues, the caspases are released into the cytoplasm, and the highly reducing environment rapidly denitrosylates the proteins, resulting in caspase activation and the induction of apoptosis.

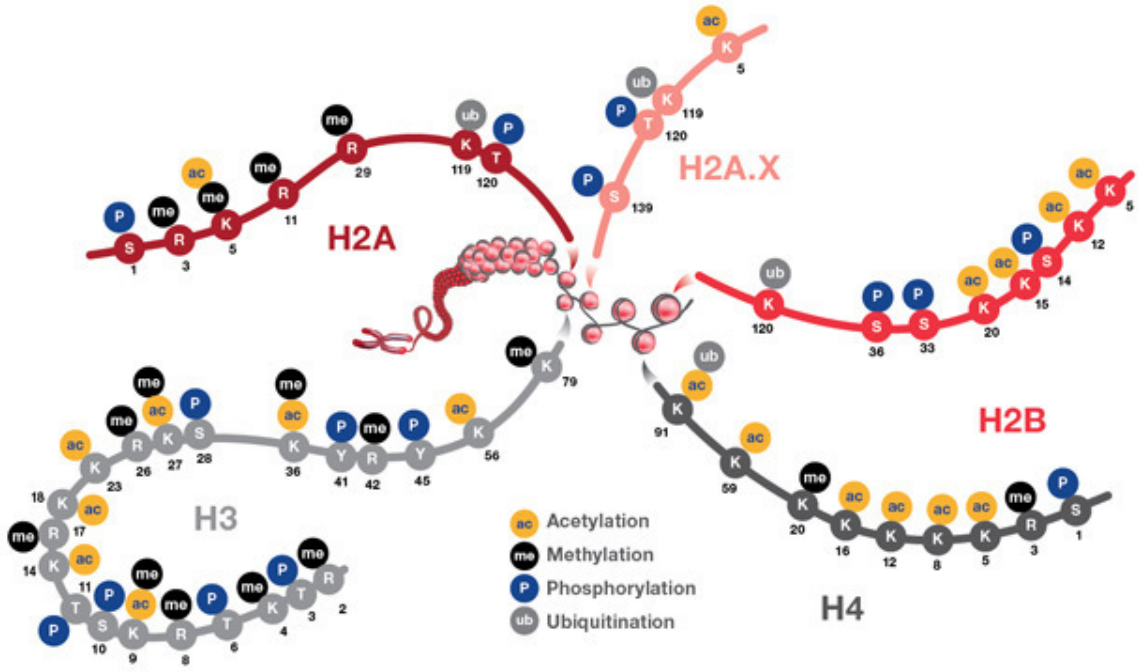
S-nitrosylation is not a random event, and only specific cysteine residues are S-nitrosylated. Because proteins may contain multiple cysteines and due to the labile nature of SNOs, S-nitrosylated cysteines can be difficult to detect and distinguish from non-S-nitrosylated amino acids.

Methylation

The transfer of one-carbon methyl groups to nitrogen or oxygen (N- and O-methylation, respectively) to amino acid side chains increases the hydrophobicity of the protein and can neutralize a negative amino acid charge when bound to carboxylic acids. Methylation is mediated by methyltransferases, and S-adenosyl methionine (SAM) is the primary methyl group donor.

Methylation occurs so often that SAM has been suggested to be the most used substrate in enzymatic reactions after ATP. Additionally, while N-methylation is irreversible, O-methylation is potentially reversible. Methylation is a well-known mechanism of epigenetic regulation, as histone methylation and demethylation influences the availability of DNA for transcription. Amino acid residues can be conjugated to a single methyl group or multiple methyl groups to increase the effects of modification.

The figure below provides an illustration of PMTs associated with nucleosome core particles.



Representation showing post-translational modifications associated with histone particles. Nucleosomes are represented by red spheres wrapped by DNA (shown in gray). Also depicted are the positions of PTMs located on the histone proteins H2A (and H2A.X), H2B, H3, and H4. These PTMs impact gene expression by altering chromatin structure and recruiting histone modifiers. PTM events mediate diverse biological functions such as transcriptional activation and inactivation, chromosome packaging, and DNA damage and repair processes.

N-acetylation

N-acetylation, or the transfer of an acetyl group to nitrogen, occurs in almost all eukaryotic proteins through both irreversible and reversible mechanisms. N-terminal acetylation requires the cleavage of the N-terminal methionine by methionine aminopeptidase (MAP) before replacing the amino acid with an acetyl group from acetyl-CoA by N-acetyltransferase (NAT) enzymes. This type of acetylation is co-translational, in that N-terminus is acetylated on growing polypeptide chains that are still attached to the ribosome. While 80 to 90% of eukaryotic proteins are acetylated in this manner, the exact biological significance is still unclear.

Acetylation at the ε-NH2 of lysine (termed lysine acetylation) on histone N-termini is a common method of regulating gene transcription. Histone acetylation is a reversible event that reduces chromosomal condensation to promote transcription, and the acetylation of these lysine residues is regulated by transcription factors that contain histone acetyletransferase (HAT) activity. While transcription factors with HAT activity act as transcription co-activators, histone deacetylase (HDAC) enzymes are co-repressors that reverse the effects of acetylation by reducing the level of lysine acetylation and increasing chromosomal condensation.

Sirtuins (silent information regulator) are a group of NAD-dependent deacetylases that target histones. As their name implies, they maintain gene silencing by hypoacetylating histones and have been reported to aid in maintaining genomic stability.

While acetylation was first detected in histones, cytoplasmic proteins have been reported to also be acetylated, and therefore acetylation seems to play a greater role in cell biology than simply transcriptional regulation. Furthermore, crosstalk between acetylation and other post-translational modifications, including phosphorylation, ubiquitination and methylation, can modify the biological function of the acetylated protein.

Protein acetylation can be detected by chromatin immunoprecipitation (ChIP) using acetyllysine-specific antibodies or by mass spectrometry, where an increase in histone by 42 mass units represents a single acetylation.

Lipidation

Lipidation is a method to target proteins to membranes in organelles (endoplasmic reticulum [ER], Golgi apparatus, mitochondria), vesicles (endosomes, lysosomes) and the plasma membrane. The four types of lipidation are:

- C-terminal glycosyl phosphatidylinositol (GPI) anchor
- N-terminal myristoylation
- S-myristoylation
- S-prenylation

Each type of modification gives proteins distinct membrane affinities, although all types of lipidation increase the hydrophobicity of a protein and thus its affinity for membranes. The different types of lipidation are also not mutually exclusive, in that two or more lipids can be attached to a given protein.

GPI anchors tether cell surface proteins to the plasma membrane. These hydrophobic moieties are prepared in the ER, where they are then added to the nascent protein en bloc. GPI-anchored proteins are often localized to cholesterol- and sphingolipid-rich lipid rafts, which act as signaling platforms on the plasma membrane. This type of modification is reversible, as the GPI anchor can be released from the protein by phosphoinositol-specific phospholipase C. Indeed, this lipase is used in the detection of GPI-anchored proteins to release GPI-anchored proteins from membranes for gel separation and analysis by mass spectrometry.

N-myristoylation is a method to give proteins a hydrophobic handle for membrane localization. The myristoyl group is a 14-carbon saturated fatty acid (C14), which gives the protein sufficient hydrophobicity and affinity for membranes, but not enough to permanently anchor the protein in the membrane. N-myristoylation can therefore act as a conformational localization switch in which protein conformational changes influence the availability of the handle for membrane attachment. Because of this conditional localization, signal proteins that selectively localize to membrane, such as Src-family kinases, are N-myristoylated.

N-myristoylation is facilitated specifically by N-myristoyltransferase (NMT) and uses myristoyl-CoA as the substrate to attach the myristoyl group to the N-terminal glycine. Because methionine is the N-terminal amino acid of all eukaryotic proteins, this PTM requires methionine cleavage by the above-mentioned MAP prior to addition of the myristoyl group; this represents one example of multiple PTMs on a single protein.

S-palmitoylation adds a C16 palmitoyl group from palmitoyl-CoA to the thiolate side chain of cysteine residues via palmitoyl acyltransferases (PATs). Because of the longer hydrophobic group, this anchor can permanently anchor the protein to the membrane. This localization can be reversed, though, by thioesterases that break the link between the protein and the anchor; thus, S-palmitoylation is used as an

on/off switch to regulate membrane localization. S-palmitoylation is often used to strengthen other types of lipidation, such as myristoylation or farnesylation (see below). S-palmitoylated proteins also selectively concentrate at lipid rafts.

S-prenylation covalently adds a farnesyl (C15) or geranylgeranyl (C20) group to specific cysteine residues within five amino acids from the C-terminus via farnesyl transferase (FT) or geranylgeranyl transferases (GGT I and II). Unlike S-palmitoylation, S-prenylation is hydrolytically stable. Approximately 2% of all proteins are prenylated, including all members of the Ras superfamily. This group of molecular switches is farnesylated, geranylgeranylated or a combination of both. Additionally, these proteins have specific 4-amino acid motifs at the C-terminus that determine the type of prenylation at single or dual cysteines. Prenylation occurs in the ER and is often part of a stepwise process of PTMs that is followed by proteolytic cleavage by Rce1 and methylation by isoprenyl cysteine methyltransferase (ICMT).

Proteolysis

Peptide bonds are indefinitely stable under physiological conditions, and therefore cells require some mechanism to break these bonds. Proteases comprise a family of enzymes that cleave the peptide bonds of proteins and are critical in antigen processing, apoptosis, surface protein shedding and cell signaling.

The family of over 11,000 proteases varies in substrate specificity, mechanism of peptide cleavage, location in the cell and the length of activity. While this variation suggests a wide array of functionalities, proteases can generally be separated into groups based on the type of proteolysis.

Degradative proteolysis is critical to remove unassembled protein subunits and misfolded proteins and to maintain protein concentrations at homeostatic concentrations by reducing a given protein to the level of small peptides and single amino acids. Proteases also play a biosynthetic role in cell biology that includes cleaving signal peptides from nascent proteins and activating zymogens, which are inactive enzyme precursors that require cleavage at specific sites for enzyme function. In this respect, proteases act as molecular switches to regulate enzyme activity.

Proteolysis is a thermodynamically favorable and irreversible reaction. Therefore, protease activity is tightly regulated to avoid uncontrolled proteolysis through temporal and/or spatial control

mechanisms including regulation by cleavage in cis or trans and compartmentalization (e.g., proteasomes, lysosomes).

The diverse family of proteases can be classified by the site of action, such as aminopeptidases and carboxypeptidase, which cleave at the amino or carboxy terminus of a protein, respectively. Another type of classification is based on the active site groups of a given protease that are involved in proteolysis. Based on this classification strategy, greater than 90% of known proteases fall into one of four categories as follows:

- Serine proteases
- Cysteine proteases
- Aspartic acid proteases
- Zinc metalloproteases

Regulation of gene expression in Prokaryotes and Eukaryotes

Gene is a part of DNA that specifies a protein/RNA. All the proteins/RNA are not required by the cell all the time. Some proteins are required at some time and yet other proteins are required at another time. Moreover these proteins are required in lesser quantities at one time, yet at other times they may be required in higher quantities. There are yet another class of proteins which are constantly (always) present in the cell, like the enzymes of the TCA cycle.

Therefore genes can be conveniently grouped under two classes:

1. Constitutive genes:

Those genes whose products are constantly present in the cell are called constitutive genes or housekeeping genes.

2. Inducible genes:

Those genes whose products vary with time and need, both in their presence and concentration are called inducible genes or those genes whose products (proteins/RNA) are induced by some inducer molecule. The activity of the constitutive genes is not regulated as their

products don't vary much with time, whereas the activity of inducible genes is always regulated. The regulation is primarily at the level of transcription. The gene or a set of related genes are switched on or off as per the need of the cell. These changes are brought about by some proteins or modulator.

If a particular protein/compound puts a gene into operation then that protein is called stimulatory protein/compound and the process is called positive regulation. If a protein/compound stops the operation of a gene then it is called the repressor protein/ compound and this process is referred to as negative regulation, ex. a steroid hormone acts as a positive modulator, wherein its presence enhances the rate of gene expression.

As soon as the hormone is destroyed the gene expression diminishes. The mechanism of regulation, though similar in the prokaryotes and eukaryotes, it differs in some aspects. Hence regulation of gene expression in prokaryotes and eukaryotes will be taken separately.

Regulation of Gene Expression in Prokaryotes:

Many prokaryotic genes are regulated in units called operons. Operon is unit of genetic expression consisting of one or more related genes and sequences (gene) controlling them, which includes the operator and promoter sequences that regulate their transcription.

The LAC operon:

It is the operon for utilization and metabolism of lactose in bacteria.

It consists of the following set of genes:

P_I = The promoter gene for regulatory genes

I = The gene for regulatory protein (repressor protein)

P = The promoter sequence for the related genes

O = Operator sequence for these genes

Z = The first gene for utilization of lactose, which forms the enzyme beta-galactosidase

Y = The second gene for the membrane protein galactoside permease

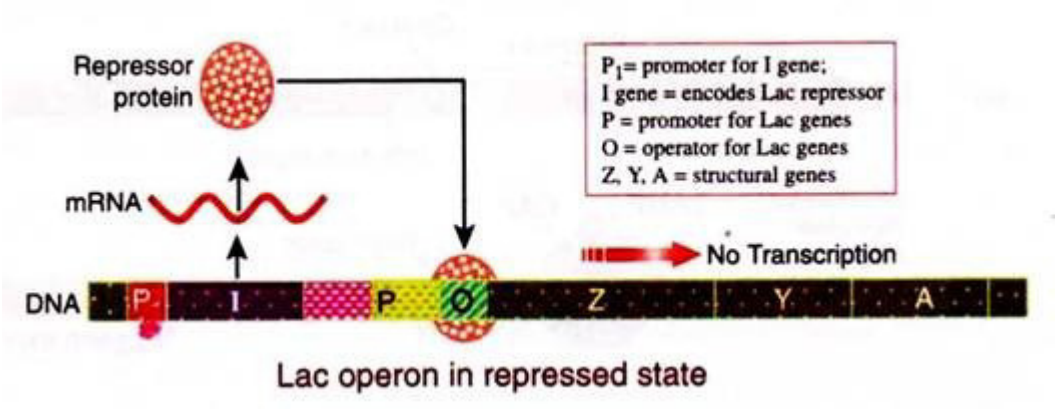
A = The third gene for the enzyme thiogalactoside trans-acetylase

This complete set of sequences (i.e. the operon) helps in switching on/off, the machinery for the utilization of the carbohydrate-lactose by the bacteria E. coli. When glucose is present in the media where the cell is growing, then the lac operon is switched off and when the medium is

devoid of glucose, and instead lactose is present as the sole source of carbon, then the Lac operon becomes operational.

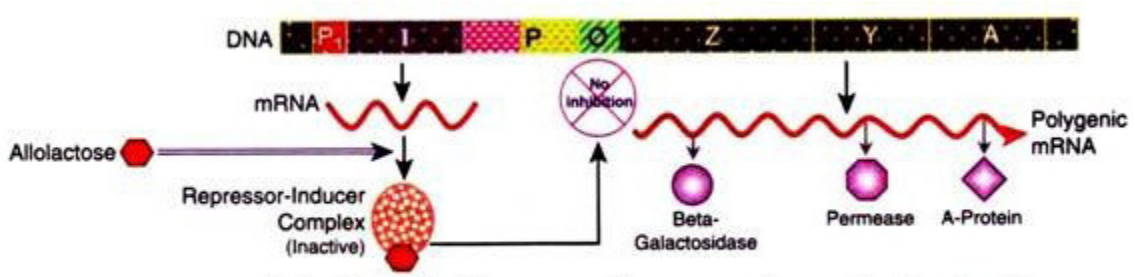
The transcription by RNA polymerase begins at the promoter site i.e. the enzyme binds to the promoter and moves along the DNA towards the structural genes of the operon to transcribe the mRNA for these genes and in this process it passes through the operator region of the operon.

Under all circumstances i.e. whether glucose or lactose is to be utilized by the cell, the I gene of the lac operon synthesizes a protein called repressor protein. This protein binds to the operator site in the DNA and thus prevents the movement of the RNA polymerase beyond this point (site), which results in the inhibition of the synthesis of the structural genes Z, Y and A.



Thus, when the cell is utilizing glucose as the only carbon source, the lac operon is switched off. Then, if the cell shifts over to the utilization of lactose as the carbon source then lactose is first converted to allolactose by the enzyme beta-galactosidase (which is always present in the cell in a few copies, irrespective of glucose or lactose is being utilized), and this allolactose acts as a positive modulator or inducer for the lac operon.

Here the allolactose binds to the repressor protein present at the operator site resulting in the release of the repressor protein from the operator site thereby permitting the enzyme RNA polymerase to pass freely through this operator site from the promoter site and thus transcribe all three structural genes Z, Y, & A.



Induction of the Lac operon in response to a molecular signal

The activity of the lac operon is not only dependent upon the binding and release of repressor molecule (with modulator) but it is also cAMP dependent. When glucose is low in the media/cell, then the cellular cAMP concentration increases. This increased amount of cAMP results in its binding at a particular site (sequences) on the promoter.

The promoter site can be divided into two parts:

(1) The site for the binding of RNA polymerase

(2) The site for a protein called catabolite gene activator protein (CAP).

The RNA polymerase can bind to the promoter site only if the CAP is bound to the promoter sequence and CAP can bind to the promoter only if cAMP is bound to it and cAMP binds to CAP only when its cellular concentration increases, which occurs when the cell is devoid of glucose and hence this facilitates the utilization of these sugars and the presence of lactose converts it to allolactose.

This acts as a positive modulator for switching on the lac operon genes by releasing the repressor protein from the operator site and producing the products of the three structural genes which produces:

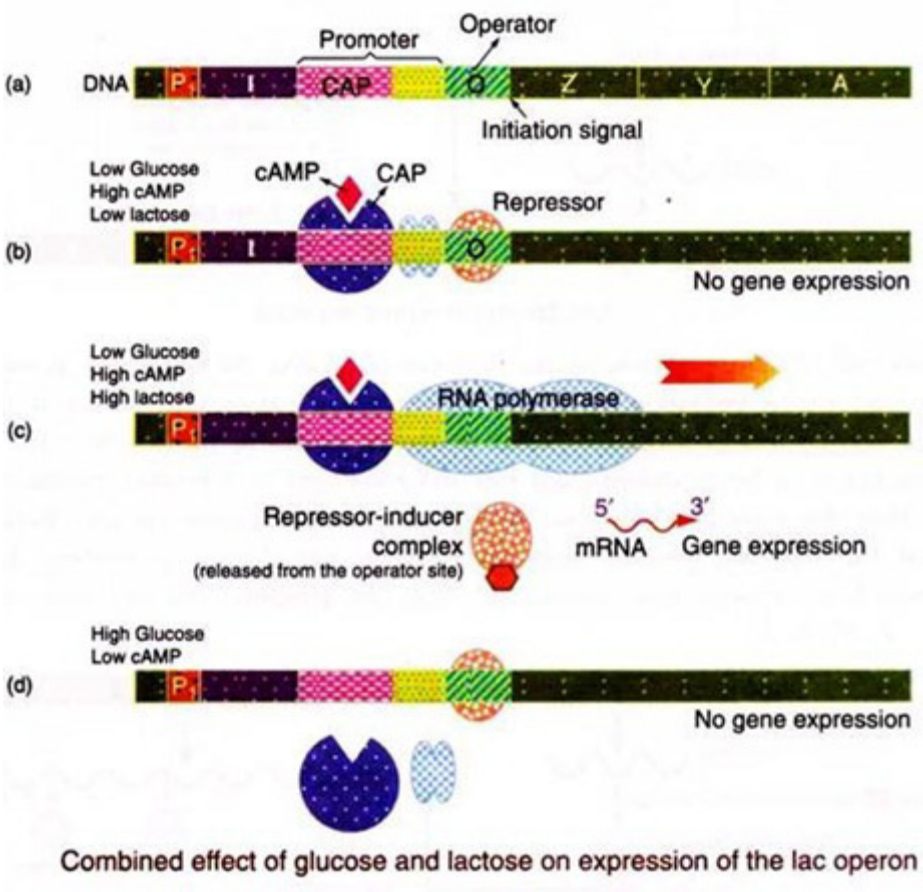
(1) The membrane protein β-galactoside permease, that enhances the uptake of lactose by the cells

(2) β-galactosidase which hydrolysis lactose to allolactose and then to glucose and galactose

(3) The enzyme thiogalacatosidase-transacetylase, whose function is unknown.

When glucose is again available to the cell the cAMP concentration decreases in the cytosol, resulting in its release from the CAP, this in turn results in the release of CAP from promoter site, which in turn results in release of the enzyme RNA polymerase from the promoter site and further prevents its binding to promoter.

This again results in the diminished synthesis of the structural genes, one of which is beta-galactosidase, that results in low production of allolactose (or no synthesis of allolactose), this is turn results in the repressor protein (formed from I gene) being devoid of the modulator and thus is free to bind at the operator site thereby prevent the movement of RNA polymerase and thus resulting in the inhibition of lac operon.



Combined effect of glucose and lactose on expression of the lac operon

Each and every metabolite has got its own operon, with different number of structural genes and whenever the genes for that metabolite are required it is switched on by a similar mechanism as that of the lac operon and switched off whenever not required.

The other operons and their details are as under:

Operon for	No. of structural genes	Function
His operon	9	Enzymes required in synthesis of histidine
Leu operon	4	Conversion of alpha-keto-isovalerate to leucine
Ara operon	4	Transport and utilization of the carbohydrate arabinose

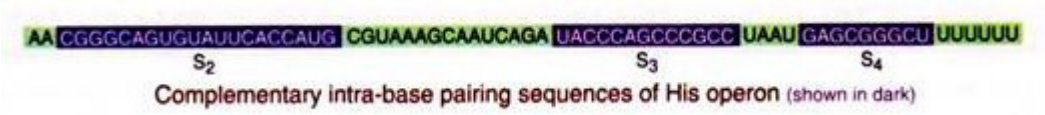
All of the operons found in the bacteria do not function only by completely switching on or off their genes. Some operons function at differential rates depending upon the need of the cell by a mechanism called the transcription attenuation i.e. slowing down of the rate of synthesis of enzymes, ex. those enzymes involved in the synthesis of amino acids (His).

Attenuation:

Transcription attenuation is a process in which transcription is initiated normally but is abruptly halted before the complete operon genes are transcribed. The frequency with which transcription is attenuated depends upon the cellular concentration of that particular amino acid for which the operon is meant for.

Attenuation of His operon:

In bacteria, transcription and translation are closely coupled. The rate at which RNA is transcribed and the rate at which that protein is translated is almost the same. Most of the transcribed RNAs for amino acid metabolism in the cell contain various complementary intra base pairing sequences. For example the following is the part of RNA that is being transcribed for His operon, which is also simultaneously being translated.



Complementary intra-base pairing sequences of His operon (shown in dark)

The sequence 2 and 3 are complementary and can base pair with each other. Likewise sequences 3 and 4 are also complementary and can also base pair with each other. If 2 and 3 bases pair, then transcription can proceed normally and if 3 and 4 bases pair the transcription is terminated, just like the termination of transcription due to appearance of a hair pin structure in

DNA. The base pairing between the sequences 2 & 3 or 3 & 4 is dependent upon the rate of translation of the mRNA, which in turn is dependent upon the concentration of His-tRNA^{His} that reflects the concentration of histidine in the cell.

If the concentration of His-tRNA^{His} is more and the rate of translation is very fast such that it passes the 2nd site before site 3 is transcribed, then this results in the site 3 base pairing with site 4 as soon as it is transcribed resulting in the termination of transcription.

On the other hand when the His-tRNA^{His} concentration is low, the rate of translation is very slow and thus the process of translation does not pass the 2nd site on mRNA by the time site (sequences) 3rd is transcribed then this result in the continuation of transcription because this will result in the 2 & 3 sites base pairing and so site 3 is not free for base pairing with site 4. Thus this results in a continuous operation of His operon.

Regulation of Gene Expression in Eukaryotes:

The genes in eukaryotes are also regulated in more or less the same manner as that of prokaryotes, but the regulation is mostly positive and very rarely negative regulation is seen. In higher eukaryotes the regulation of gene expression is solely by positive modulation and negative inhibition of the genes/operon is totally absent.

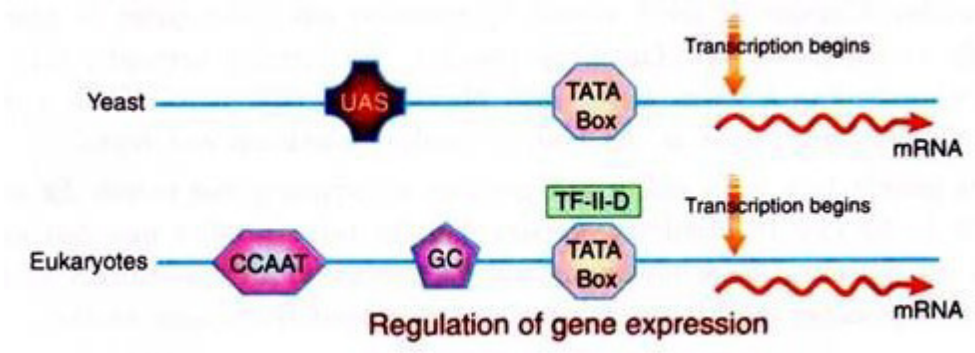
However in yeast some genes are regulated by negative modulation. Further, there is a physical separation between the process of transcription and translation is eukaryotes as transcription takes place in the nucleus and translation occurs in the cytosol.

Mechanism:

The gene regulation is only by positive regulation. Most of the genes are normally inactive in eukaryotes i.e. RNA polymerases cannot bind to the promoters. The cells synthesize only the selected group of activator proteins needed to activate transcription of the small subset of genes required in that cell.

There are at least five regulatory sites for RNA polymerase promoter sites in higher eukaryotes designated as (a) TATA box (b) GC box and (c) CAT box. In yeast there are two types of promoter sequences i.e. TATA box and UAS i.e. upstream activator sequence.

These sequences are the binding sites for the transcription factors called TF-II-D that is required for RNA polymerase binding. Each of these sequences are recognised and bound specifically by one or more regulatory proteins called transcription factors. These regulatory sequences are about 1000 bases away form the main gene, thus to activate the main gene a protein-protein interaction is required which can reach the main gene sequence.



Regulation of gene expression

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

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 and check points, Cyclins and protein kinases.

Cell membrane:

- Cell membrane or plasma membrane mainly regulates the cellular entry and exit of molecules and ions.
- This function of cell membrane is called cell permeability.
- Plant cells have a thick cell wall that covers plasma membrane and protects it.
- Animal cells have a cell coat or external laminae.
- Isolation of membranes from erythrocytes is relatively easy.
- Hypotonic solutions cause swelling of membranes forcing out hemoglobin from red blood cells forming a red cell ghost and hemolysis.

Components of cell membrane :

- The membrane is made up of a lipid bilayer embedded with proteins some of them protruding out from the membrane.
- Red blood cell membrane has 52% protein, 40% lipids and 8% carbohydrates.
- There is a wide variation in this composition among various membranes.
- Phospholipids, cholesterol and galactolipids generally constitute the major lipid portion of membranes but their composition varies among different membranes.
- Major phospholipids include the neutral phospholipids (no net charge at neutral pH) such as phosphatidyl choline, phophatidyl ethanolamine and sphingomyelin.
- Acidic phospholipids (5-20%) are negatively charged that include phosphatidyl inositol, phosphatidyl serine, phosphatidyl glycerol, cardiolipin and sulfolipids.

Membrane proteins

- Proteins are not only structural components but also serve as carriers or channels.
- They also serve as receptors for various signaling ligands in addition being enzymes and antigens.
- Membrane proteins are generally classified into extrinsic (peripheral) or intrinsic (integral) proteins.
- Peripheral proteins are soluble in water/aqueous solutions and do not contain much of lipids. Eg: Spectrin, cytochrome c.
- The majority of membrane proteins are integral proteins that are insoluble in water.
- They are in strong association with lipids and carbohydrates. Membrane bound enzymes, histocompatibility antigens and various drug and hormone receptors belong to this category.
- Hydrophilic or polar /hydrophilic amino acids are mainly present near the surface in the peripheral proteins whereas the nonpolar/hydrophobic amino acids are buried inside
- In the case of integral proteins the nonpolar amino acids are more exposed to the surface.
- Outer surface of some membranes contain acetyl choline esterase, nicotinamide dinunucleotide-adenine dinucleotidase and the ouabain binding site of Na+ K+ ATPase.
- Inner surface contains NADH-diaphorase, adenylatecyclase, protein kinase and Mg++ ATPase.

Table : ***Composition of different membrane lipids.***

Type	Composition	Example/ Remarks
Phosphoglycerides	esters of phosphoric acid and a trifunctional alcohol-glycerol	Phosphatidate four common substituents for phosphatidate; Serine, ethanolamine, choline and inositol.
Sphingolipids	Phosphoglycerides where glycerol is substituted with sphingosine.	Sphingomyelin, Glycosphingolipid Found in particularly nerve cells and brain tissues

Table : ***Cellular Architecture and function of Cell Membrane.***

Organism	Lipid composition	Membrane properties	Functionalities
Bacteria	Phosphatidylethanolamine and Phosphatidylglycerol	Robust Different shapes	Membrane protein incorporation
Yeast	Sphingolipids, Glycerophospholipids and Sterols	Robust Different shapes Complex organelle morphology	Membrane protein incorporation Membrane budding Vesicular trafficking
Higher Eukaryotes	Glycerophospholipids, sterols, and tissue-specific Sphingolipids	Robust Different shapes Complex organelle morphology Complex and specific cellular architecture	Membrane protein incorporation Membrane budding Vesicular trafficking Specific functions depending on the cell type

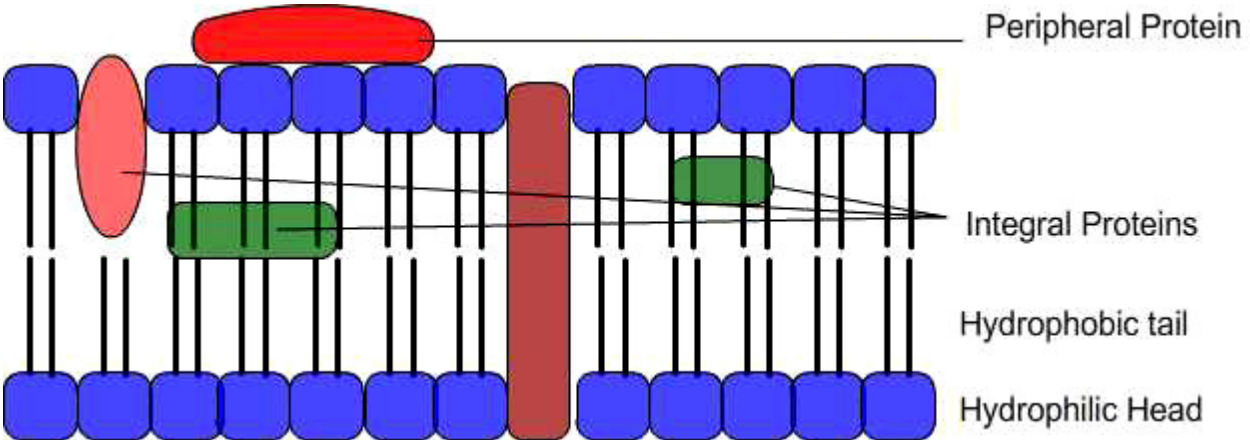
Theories on membrane structures

- 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.

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.

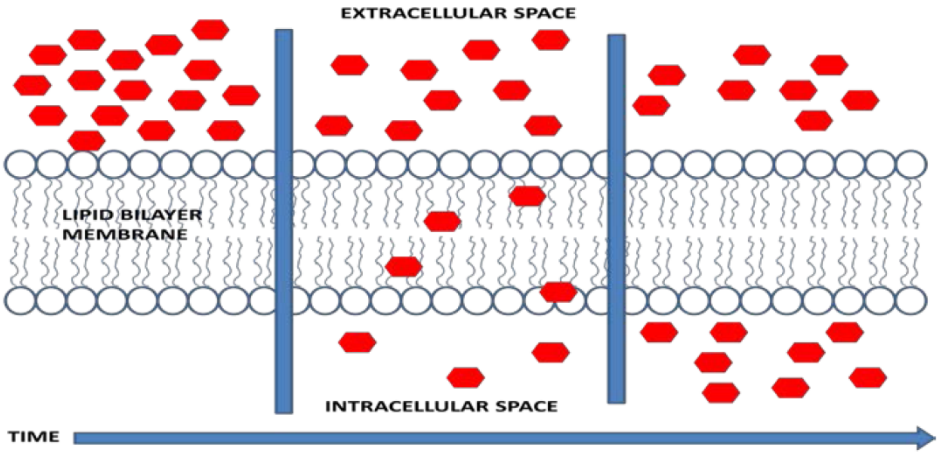


Figure 3: Diffusion.Extracellular space contains high concentration of solutes than intracellular space and hence the solutes move from extracellular space to intracellular space till there is no concentration gradient between the spaces.

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).

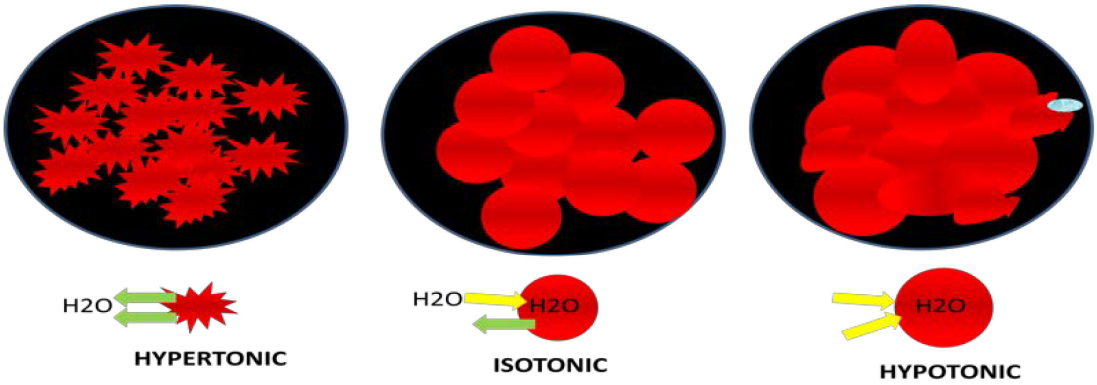


Fig:Osmosis.(A) In hypertonic solution, there are more solute molecules outside the cell, which causes the water to be sucked in that direction which leads to the shrinkage of cells. **(B)** In isotonic solution, there is equal concentration of solute on both sides, henceforth the water with move back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

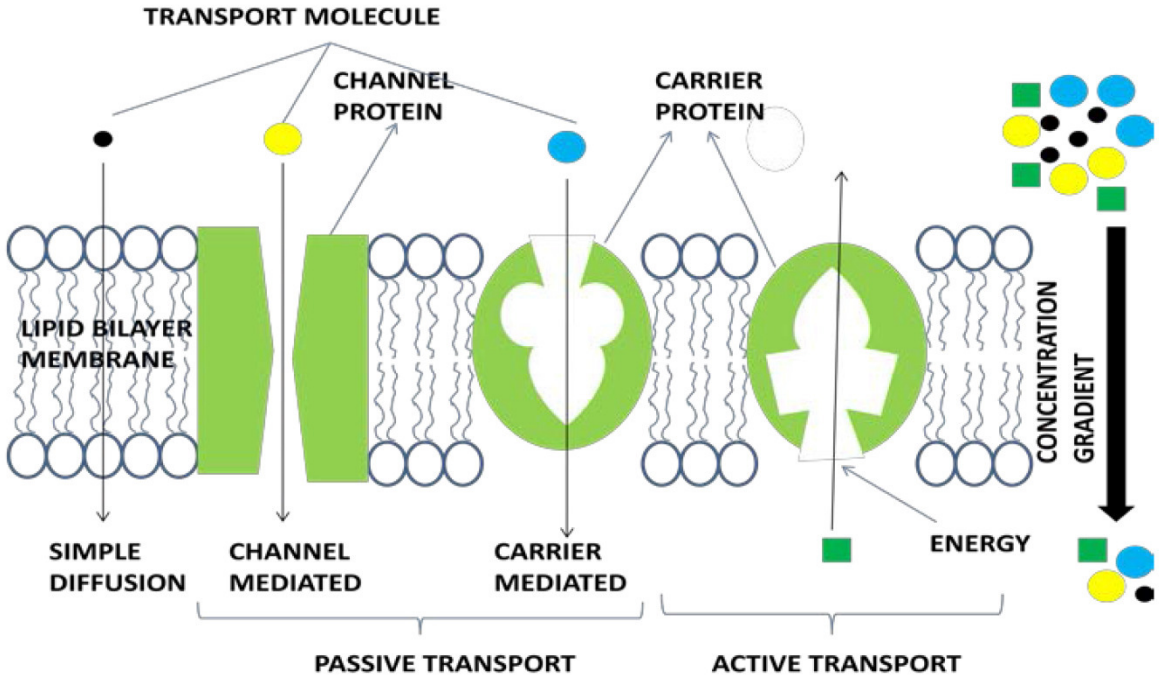


Figure 2: Mediated transport. (A) Passive transport and (B) Active transport

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

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. Prote• olytic 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.

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 cotranslation• aly 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 pro• cessed 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 cotrans lationaly translocated proteins, these proteins are translated in the free rib-

sames 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 desti• nation. 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

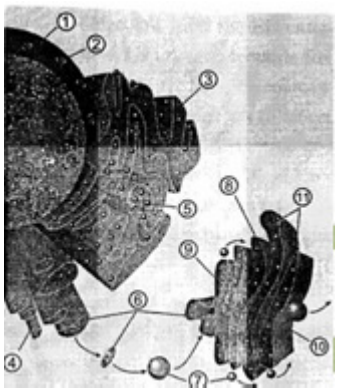
a few pro• teins enter the ER posttranslationally. The protein translation starts at the free ribosomes in the cytosol. The synthesis continues till the sorting signal which is present in the N-terminal emerges. This sorting signal is recognized by signal recognition particle. The SRP binds to the sorting 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 .

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

o: 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



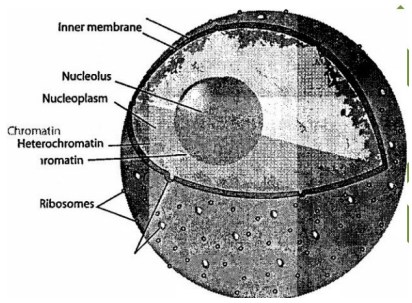
1. Nucleus 2. Nuclear pore 3. Rough endoplasmic reticulum (RER) 4. Smooth endoplasmic reticulum 5. Ribosome on the rough ER 6. Proteins that are transported 7. Transport vesicle 8. Golgi apparatus 9. Cis face of the Golgi apparatus 10. Trans face of the Golgi apparatus 11. Cisternae of the Golgi apparatus.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



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 thenucleus, 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 ashort stretch of amino acids. The deletion of the NLS from a nuclear protein disrupts nuclear

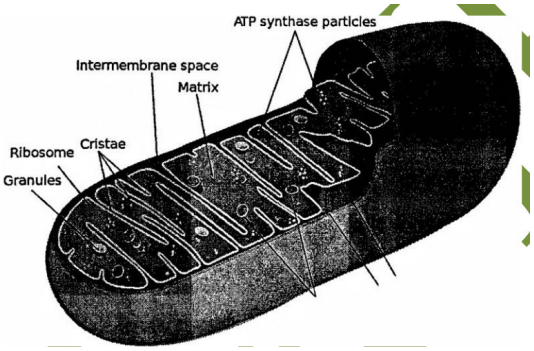
import and the addition of NLS to a non-nuclear protein facilitate nuclear import. These details have been widely used to experimentally unravel NLS motifs. The nuclear localization signals can be present anywhere in the protein sequence. Since NLSs do not have any particular consensus sequence, it is 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.

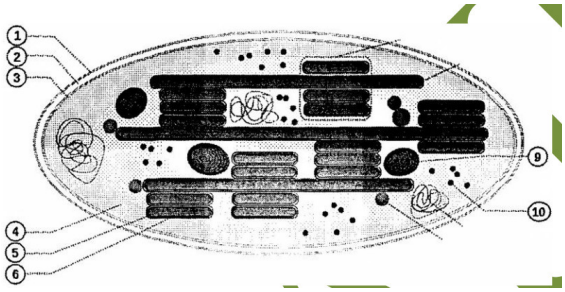
Mitochondrion

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 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 by a TOM or TIM component, and the protein is subsequently inserted into the outer or inner membrane, respectively.



9. 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

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 sequences. 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+, Ca2+, 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

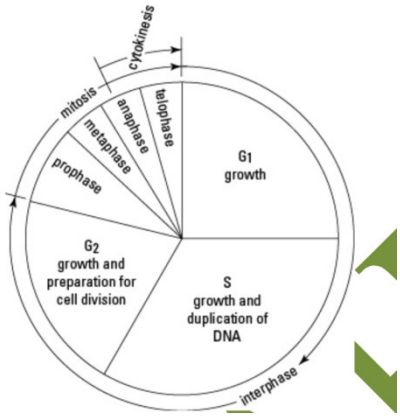
  made of two identical halves called sister **chromatids,** which are joined at the **centromere.** Each chromatid consists of a single, tightly coiled molecule of DNA. Somatic cells (all body cells except eggs and sperm) are diploid cells because each cell contains two copies of every chromosome.

- 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):

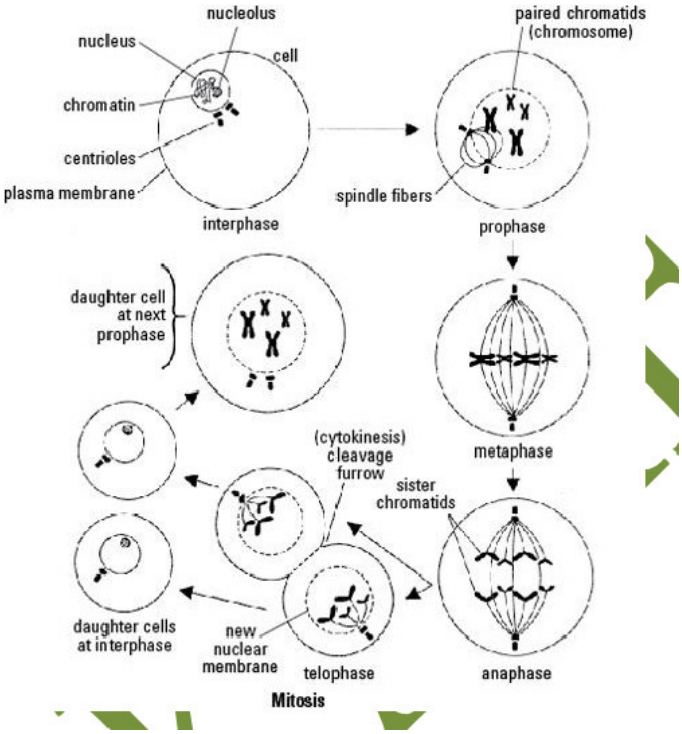
- During *prophase,* the nucleoli disappear, the chromatin condenses into chromosomes, the nuclear envelope breaks down, and the mitotic spindle is assembled. The development of the mitotic spindle begins as the centrosomes move apart to opposite ends (poles) of the nucleus. As they move apart, microtubules 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.



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.

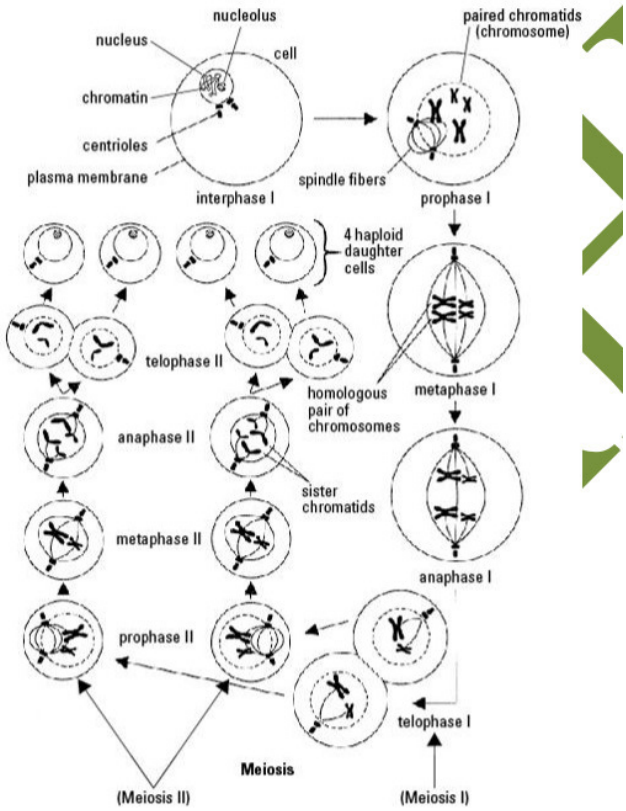
- In prophase II, the nuclear envelope disappears and the spindle develops. There are no chiasmata and no crossing over of genetic material as in prophase I.

- 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.

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

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, analysis of Mutations, DNA Genetic 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

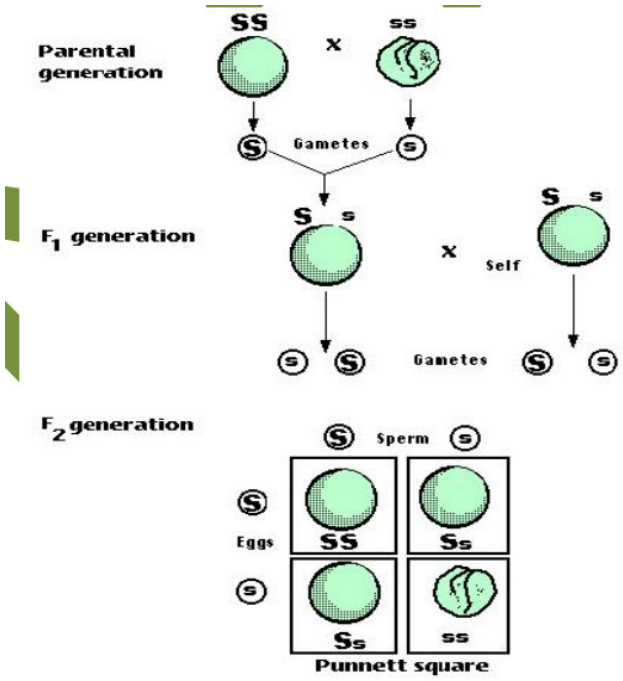
Mendel‘s observations and conclusions are summarized in the following two principles, or laws.

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.

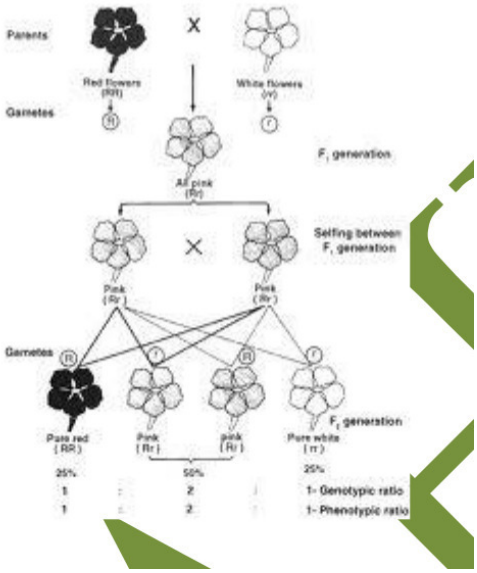
Although inheritance of traits in fungi, viruses, and bacteria are all non-Mendelian, the phrase "non-Mendelian inheritance" is usually only used to describe the exceptions which occur in eukaryotic reproduction.

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.

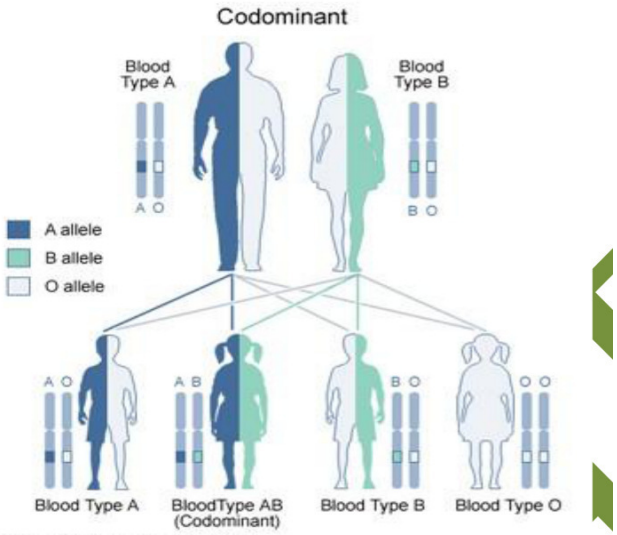
An example in humans is sickle cell anemia. This condition is determined by a single polymorphism. Possessors of the deleterious allele have lower life expectancy, with homozygotes rarely reaching 50 years of age. However, this allele also yields some resistance to malaria. A thus in region where malaria 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 transmittd 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

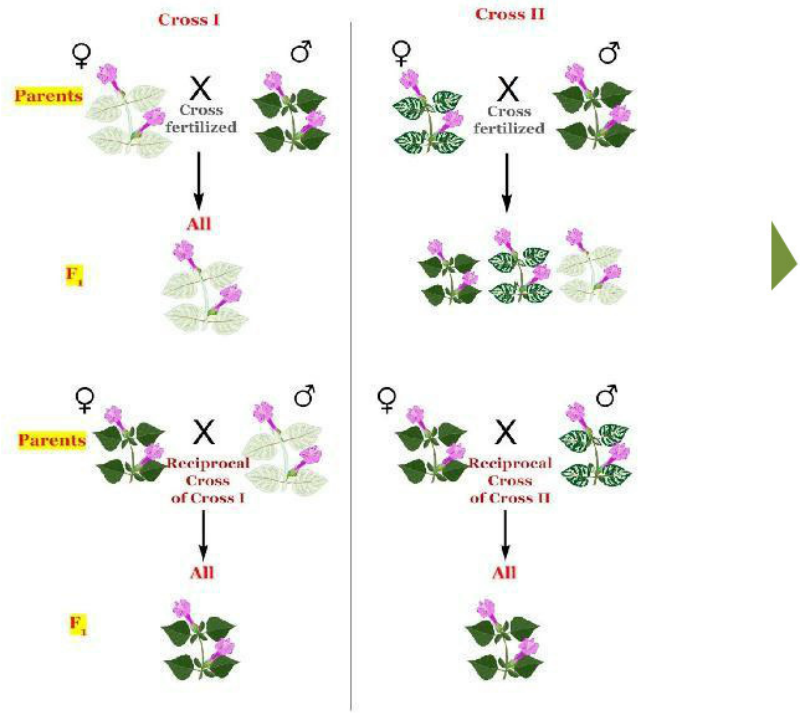
Sigma virus in *Drosophila melanogaster*

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.

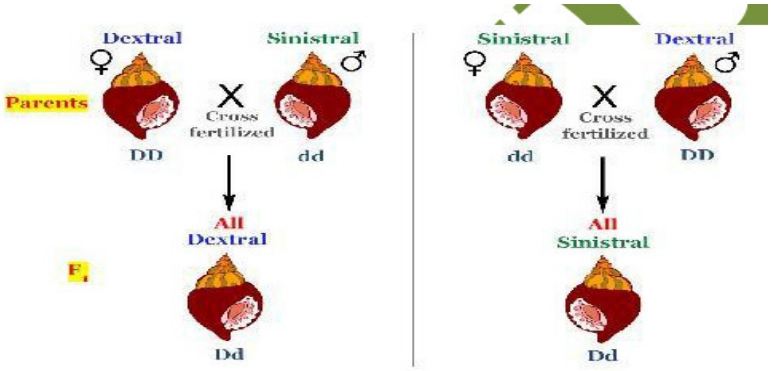
- Flowers on variegated branches yield offspring of three kinds- green, white and variegated in variable proportions.
- Flowers from branches wholly white produce seeds that grow into white plants that is without chlorophyll.
- But in every case the source of pollen has no influence on the offspring.
- 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.

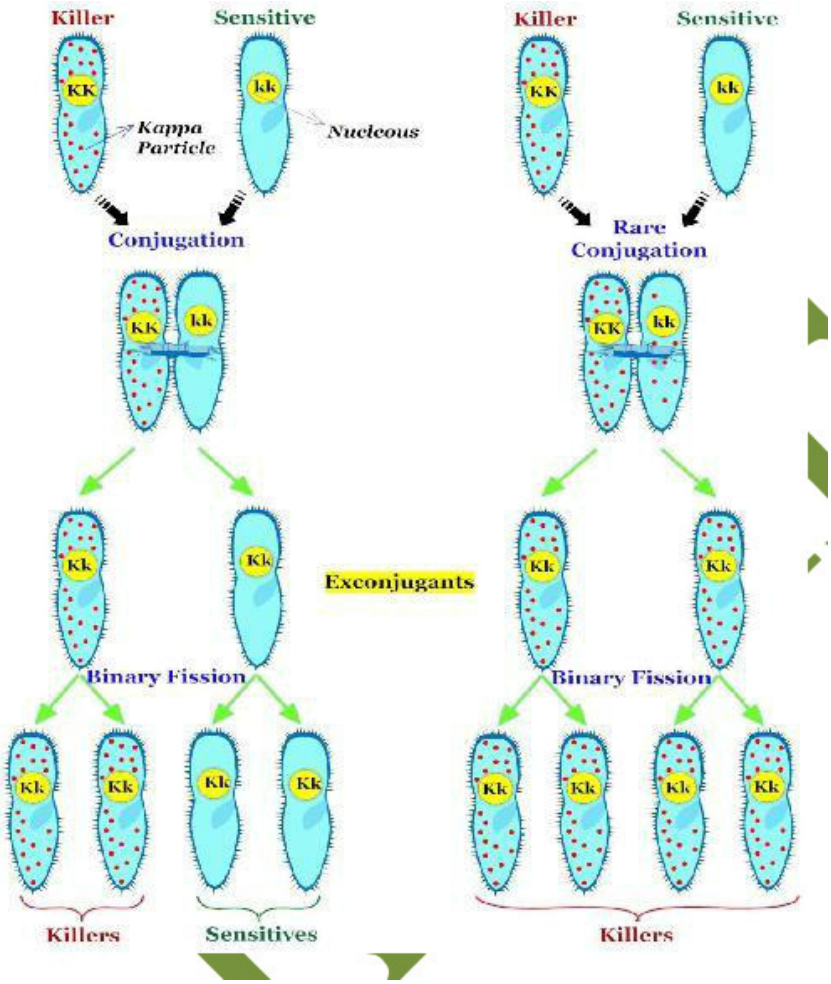


- The coiling phenotype that is seen in the offspring is controlled by the genotype of the mother.

- 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.
- If 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.
- However 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.

- During 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.

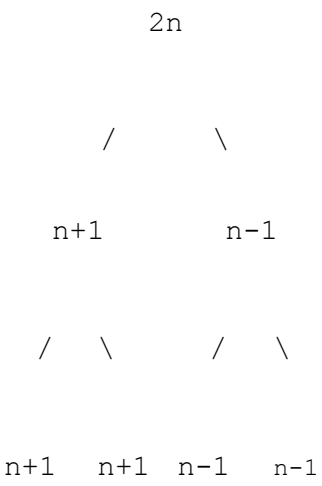
Nondisjuction: Nondisjunction ("not coming apart") is the failure of chromosome pairs to separate properly during meiosis stage 1 or stage 2. This could arise from a failure of homologous chromosomes to separate in meiosis I, or the failure of sister chromatids to separate during meiosis

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).

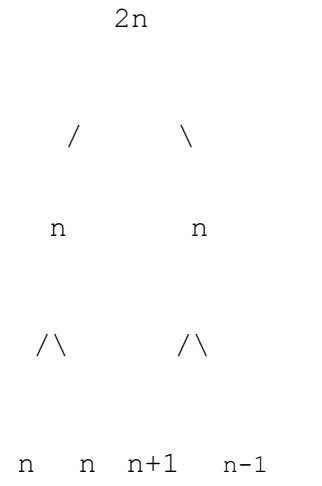
If one surveys karyotypes of embryos that spontaneously abort, trisomies for all the autosomes are seen, and other forms of chromosomal abnormalities as well, but these conditions are apparently fatal early in development. Normal embryonic development requires a precise diploid complement of chromosomes.



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 chromosome; two gametes are missing a single chromosome.



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 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.

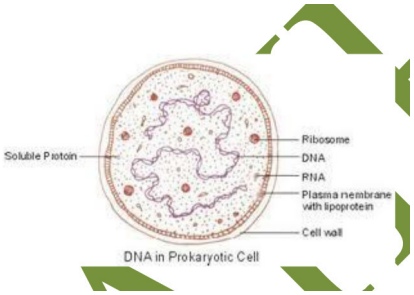
3. When preparing for nuclear division, during the S phase of interphase, the chromosomes copy themselves (i.e. DNA replication occurs). Each half of the chromosome is now called a chromatid. Note that there is still only one chromosome; it consists of two chromatids but has only one centromere. The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



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)
Borrelia	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

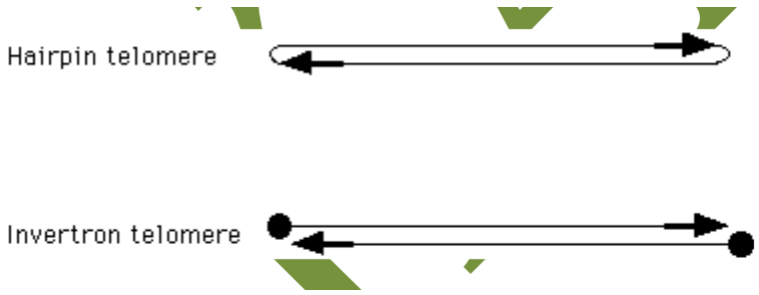
found in many other bacteria, including Agrobacterium tumefaciens, Rhizobium, Brucella, Paracoccus denitrificans, Ochrobactrum anthropi, Leptospira interrogans, Burkholderia, Vibrio cholerae, Deinococcus radiodurans, and many others from diverse groups of bacteria.

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.

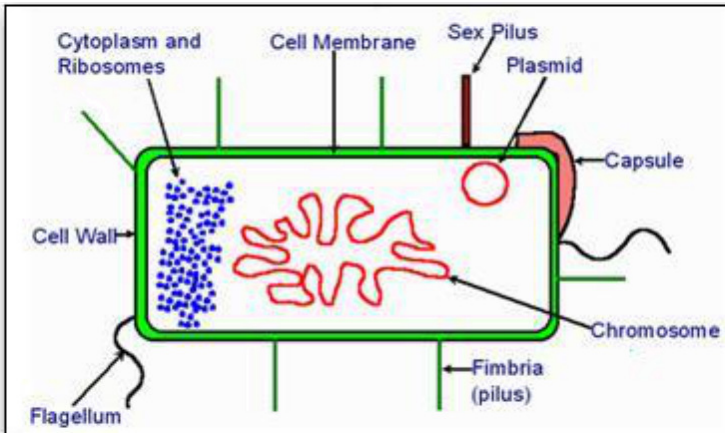
The important take-home point is that we are just beginning to appreciate the similarity of many processes once thought to be completely different between bacteria and eukaryotes, partly because we now have better tools for studying these processes and partly because most of the earlier studies focused 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.

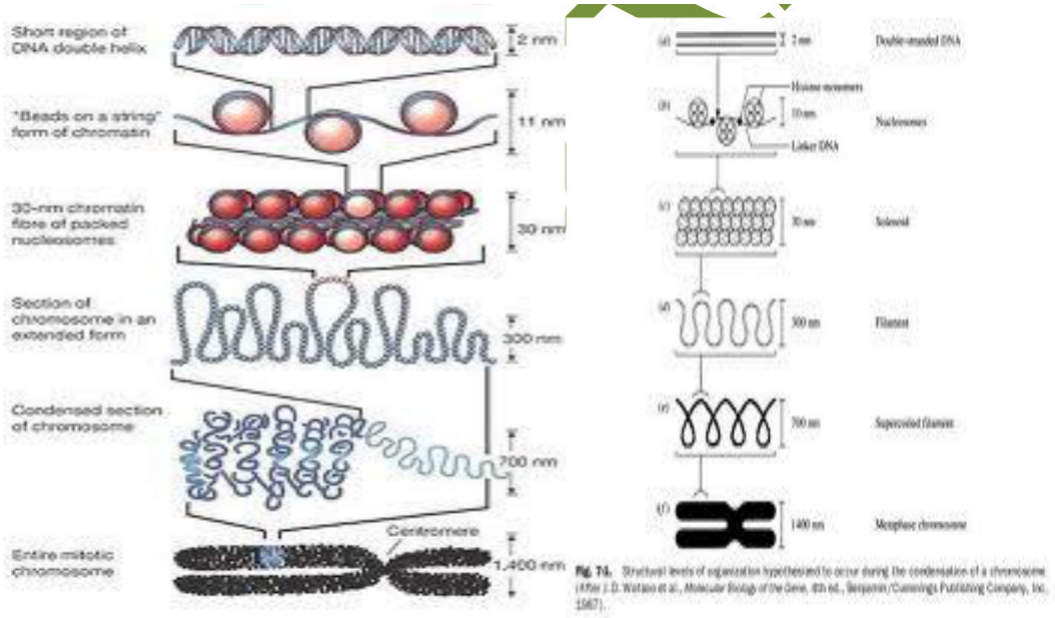


The telomers at the end of chromosomes of most eukaryotic cells are replicated by a different mechanism: most telomeres are short GC-rich repeats that are added in a 5' to 3' direction by the enzyme telomerase.

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.

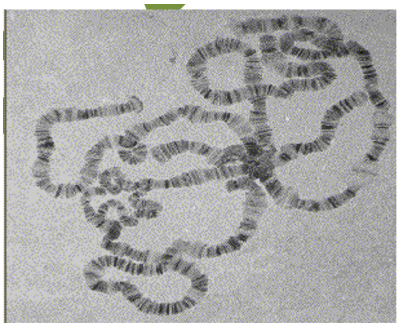
In a typical zygote with 46 chromosomes, at cell division one of the cells may retain a duplicated copy of one of the chromosomes. This produces a cell with 47 chromosomes. All cells that are derived from that cell also have 47 chromosomes. The rest of the cells will have 46 chromosomes.

Polytene Chromosome:

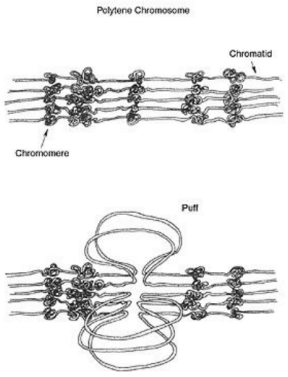
To increase cell volume, some specialized cells undergo repeated rounds of DNA replication without cell division (endomitosis), forming a giant **polytene chromosome**. Polytene chromosomes form when multiple rounds of replication produce many sister chromatids that remain synapsed together.

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

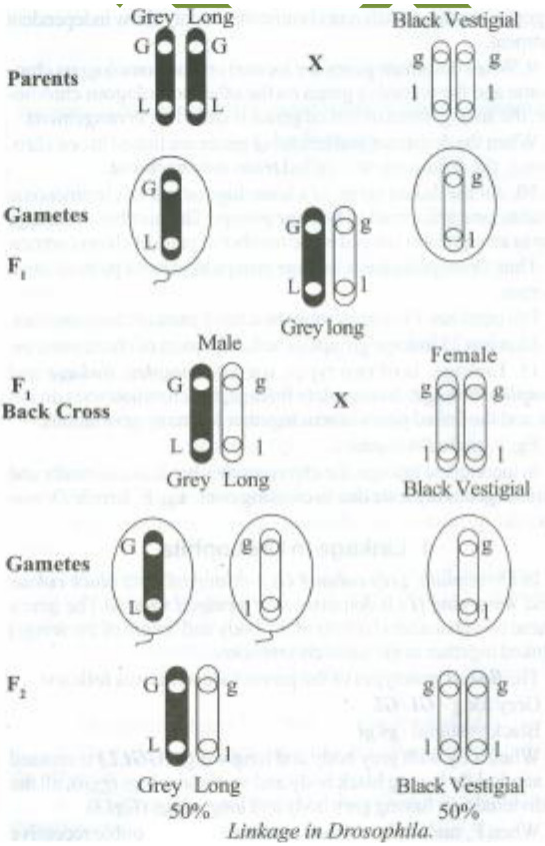
1. Complete Linkage

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 4th 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.

2) 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.

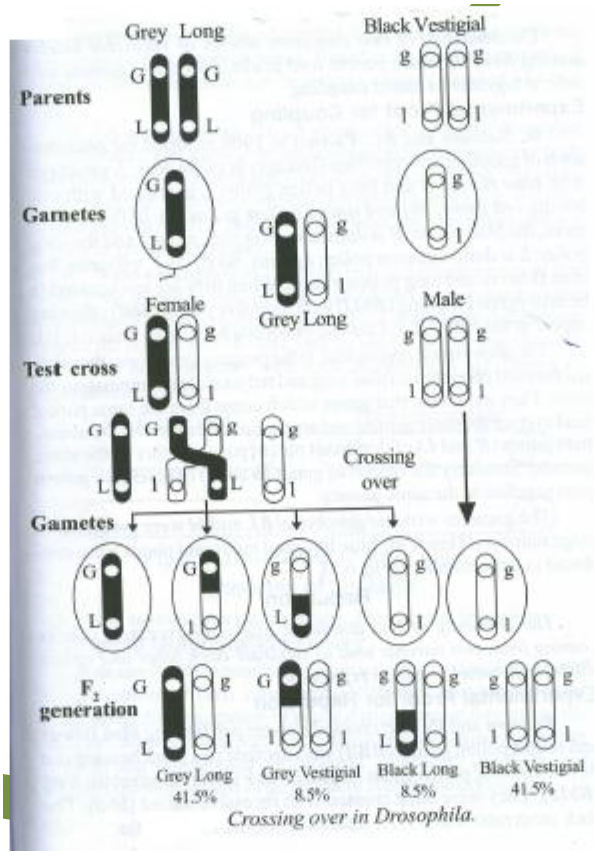
Eg. 1. *Body colour and wing shape in female Drosophila.*

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;

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

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 synapsis.

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 crossing 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

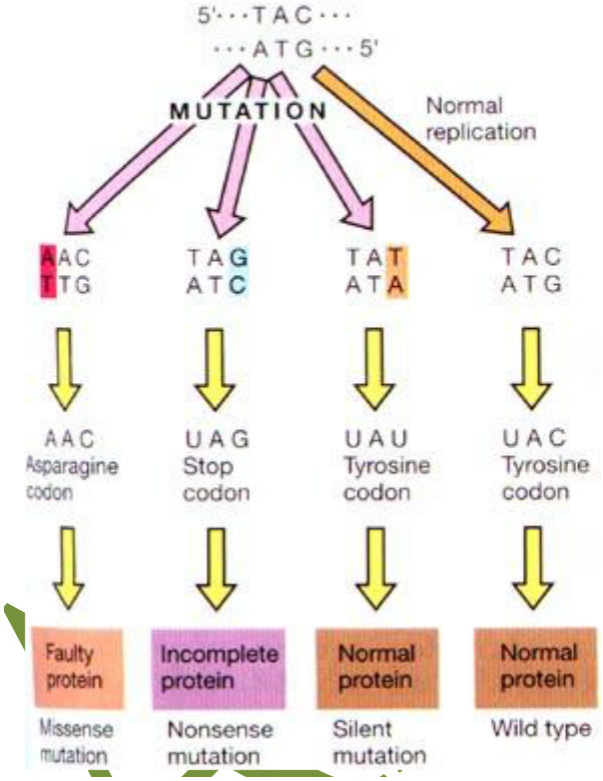


Figure: types of point mutation

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:

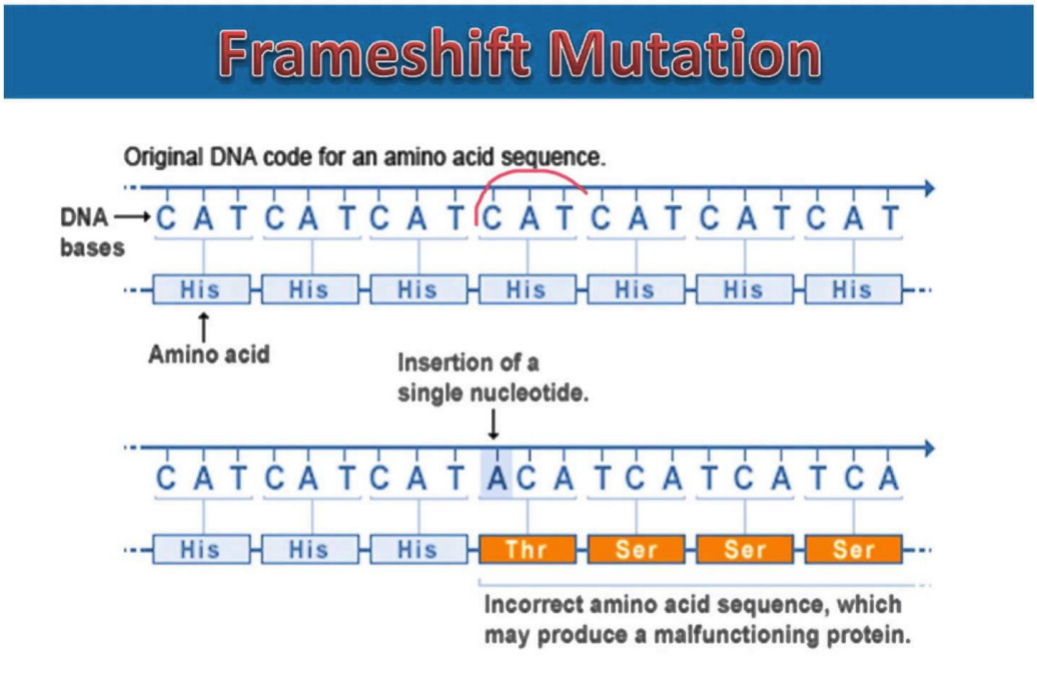
- In this mutation mutated codon codes different amino acid (other than original). Since new aminoacid coded by mutated codon is altered, the protein formed from it is also altered. Such protein can be less active or completely inactive.
- If altered aminoacids lie on active site of protein then such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

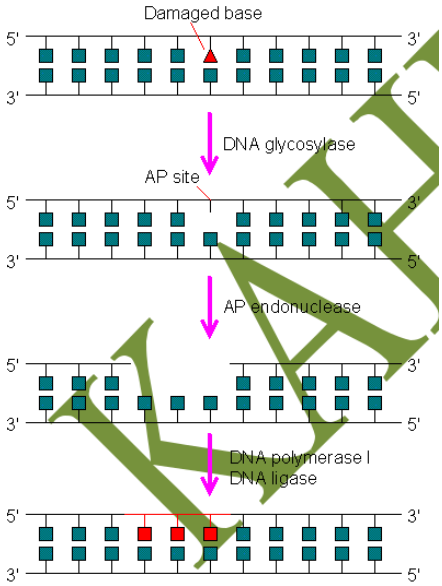
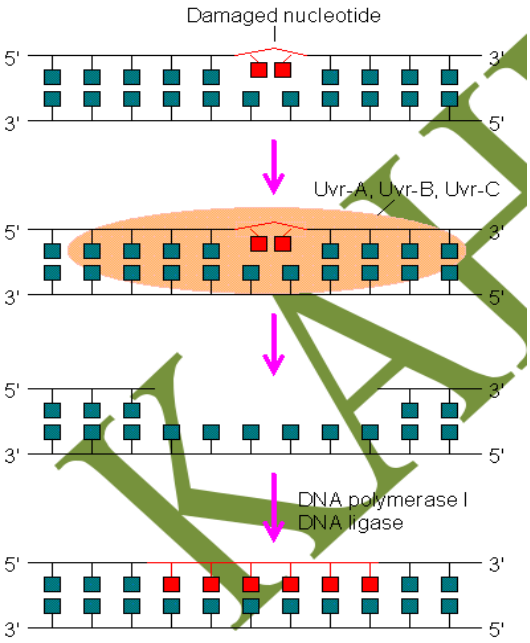


Figure -. DNA repair by base excision

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.



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.

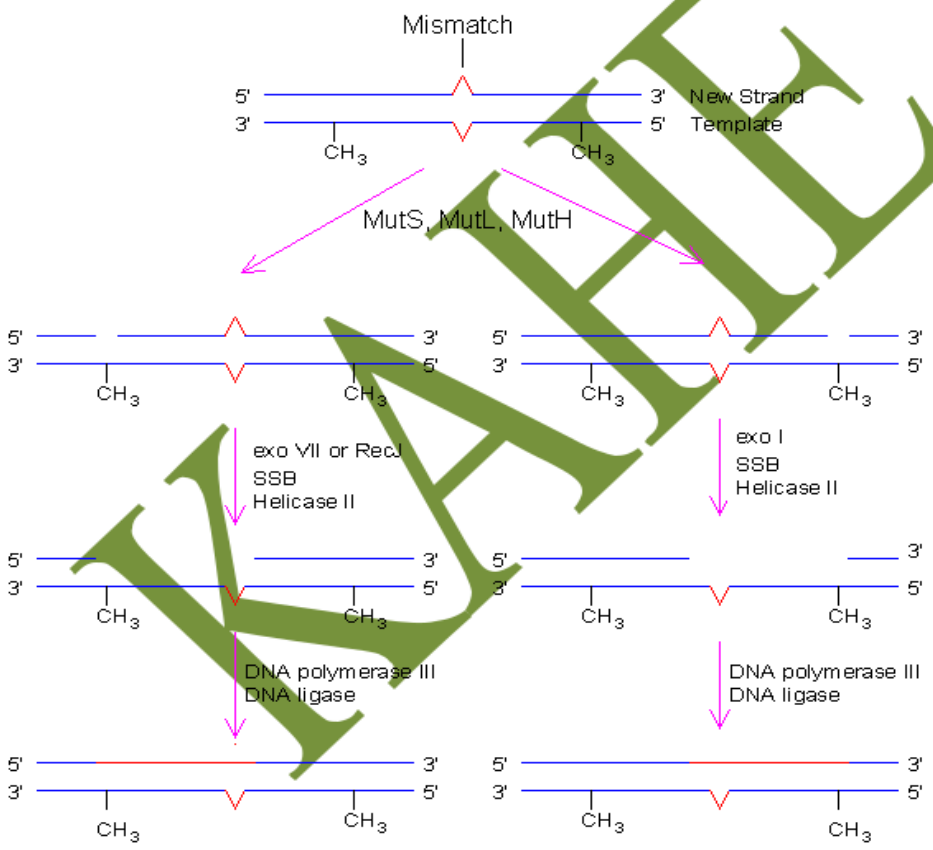


Figure -. Mismatch repair.

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.

I M.Sc Sem I
Cell Biology and Molecular Genetics
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.

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.

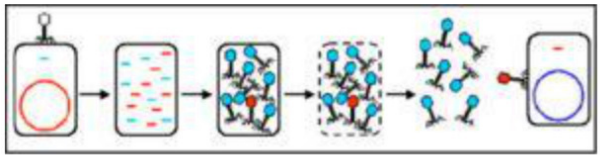


FIG. THE MECHANISM OF GENERALIZED TRANSDUCTION

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:

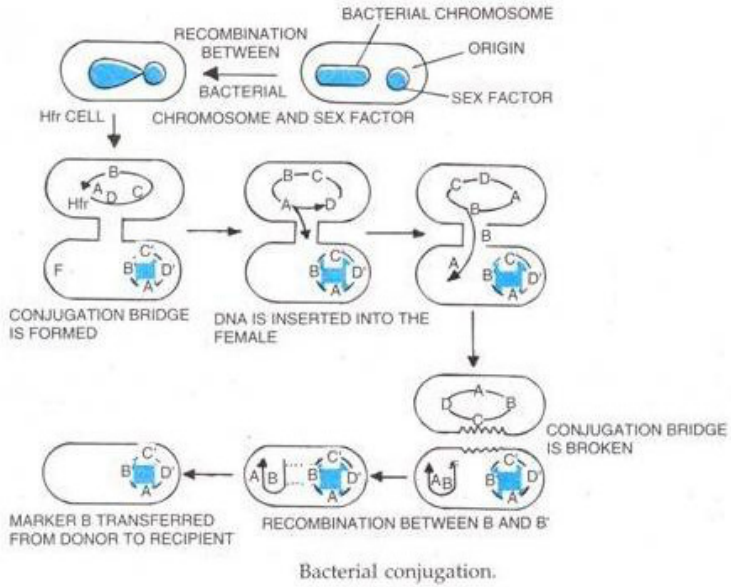
The unidirectional transfer of DNA from one cell to another through a cytoplasmic bridge is called conjugation. The process is equivalent to sexual mating in eukaryotes. Two bacterial haploid cells of different strains come close to each other.

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.).

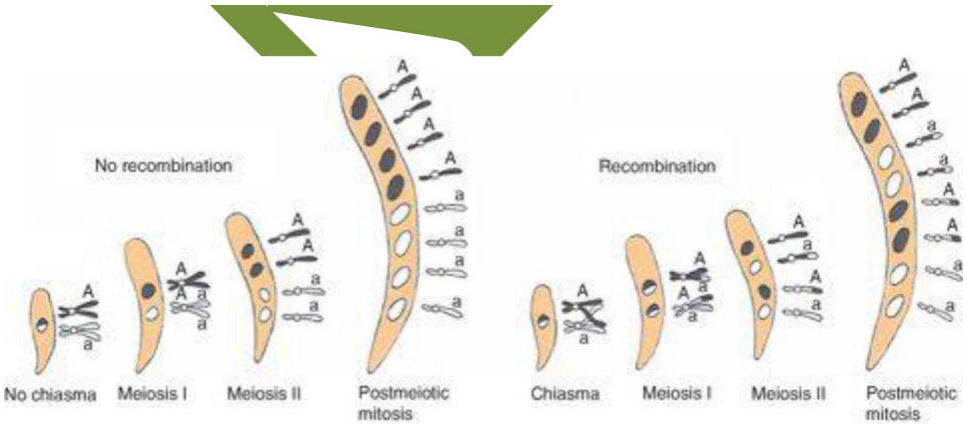


Figure .Spore tetrads and octads without and with recombination

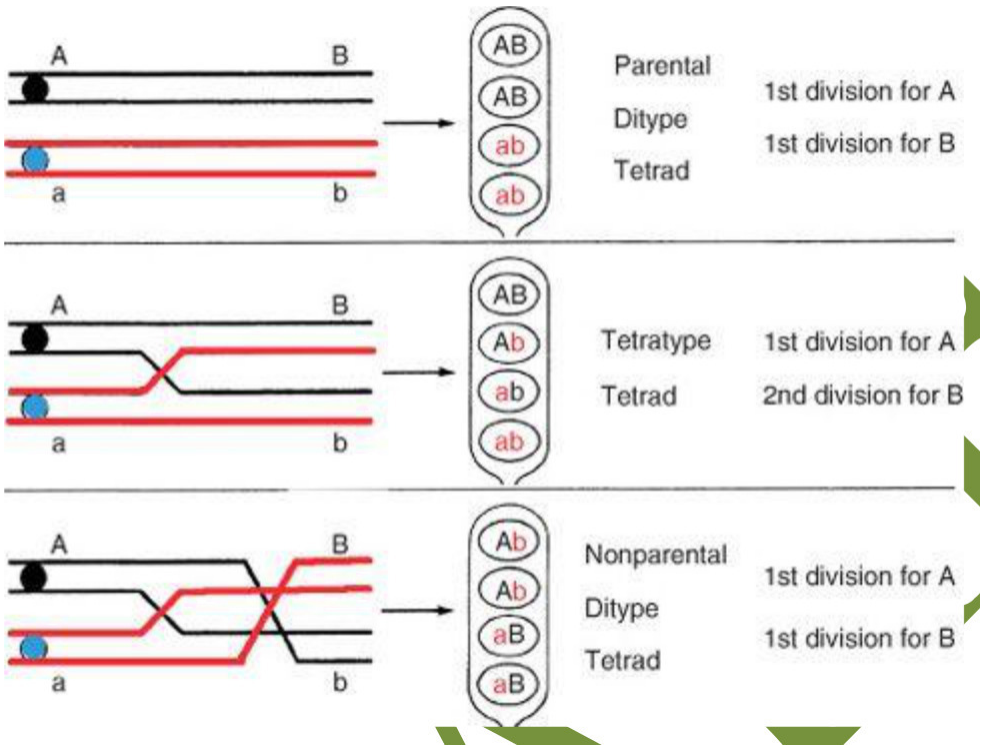


Figure: Transactions and results examplified by two gene loci in an ordered tetrad. Gene *A* is so close to the centromere that practically no recombination occurs between them. (Diagram after Barratt RW et al 1954 Adv Genet 6:1)

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 NPD tetrads and recombination frequency between the B/b *gene and the centromere can be calculated* as TT[1/2] all tetrads.

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)

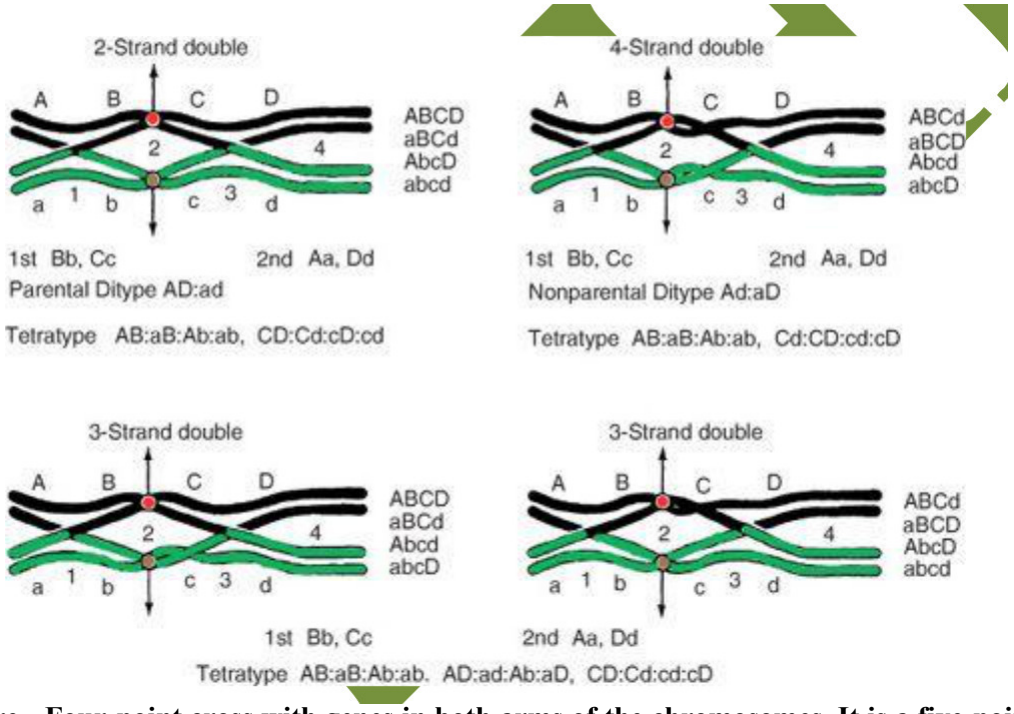


Figure - Four-point cross with genes in both arms of the chromosomes. It is a five-point cross if we consider the centromere as a genetic marker. From the spore order we can determine even if the chromatids rotated 180° after the exchange. (After Emerson S 1963 p 167.

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

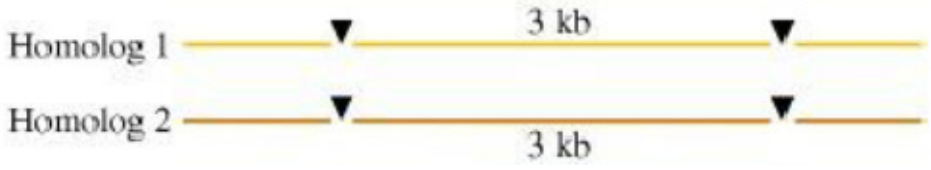
In the first 70 years of building genetic maps, the markers on the maps were genes with variant alleles producing detectably different phenotypes. As organisms became more and more researched, large numbers of such genes could be used as markers on the maps. However, even in those orga-nisms in which 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.



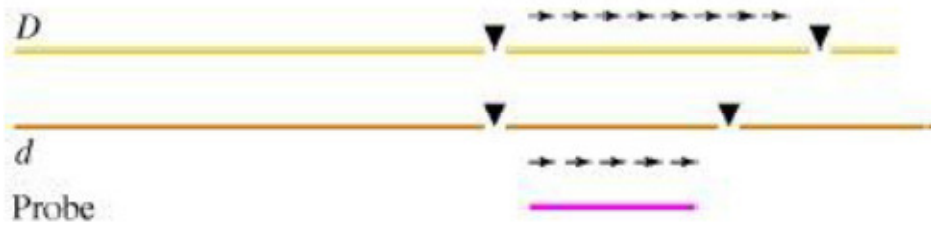
These multiple forms of this region constitute a restriction fragment length polymorphism (RFLP).

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.

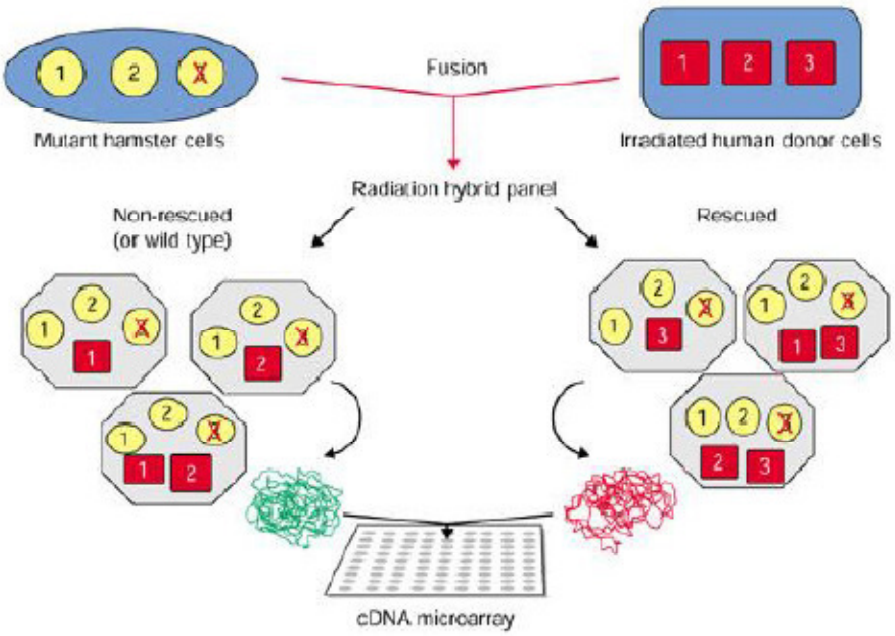


Figure: Schematic diagram of the radiation hybrid array-CGH methodology. Mutant rodent cells that have a recessive phenotype due to a mutation in a gene (gene 3, depicted as a red cross), are complemented by cell fusion with irradiated human donor cell lines. The resulting cells in the radiation-hybrid 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 (see Fig.1).

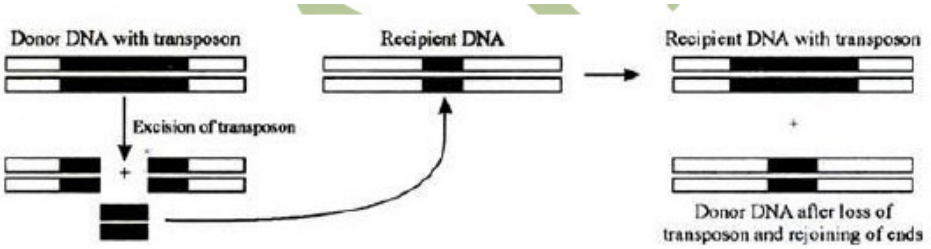


Fig. 1. Cut and Paste Transposons.

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

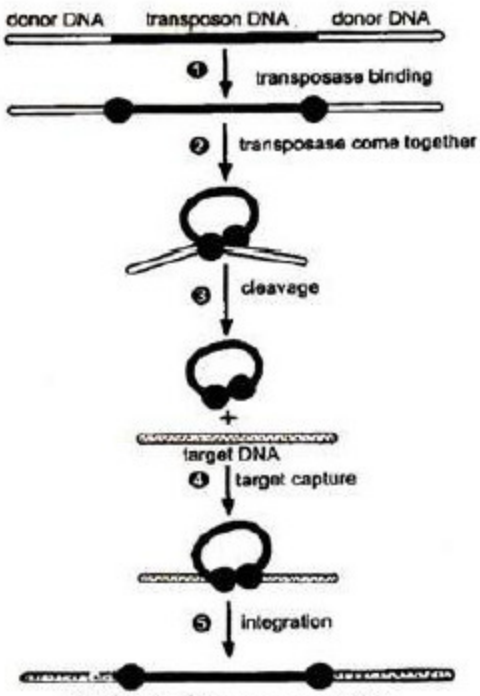


Fig. 2. Role of Transposase protein in cut-and-paste transposition.

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.

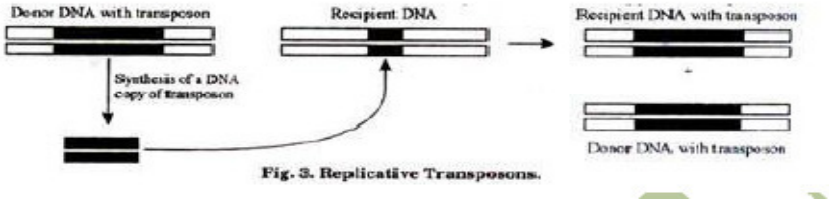


Fig. 3. Replicative Transposons.

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.

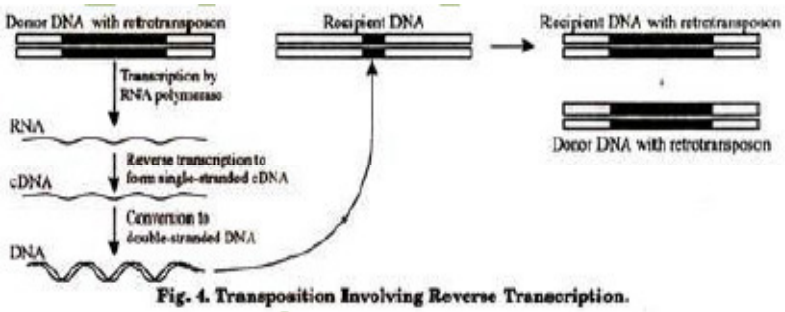


Fig. 4. Transposition Involving Reverse Transcription.

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.

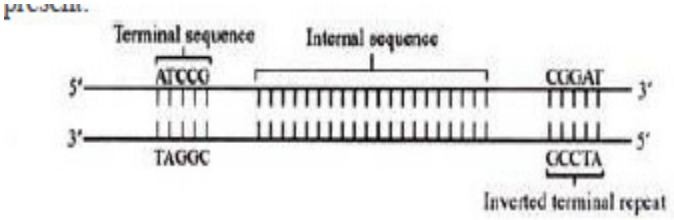


Fig. 5. An Insertion sequence (IS-element) with inverted terminal repeats (ITRs)

(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.

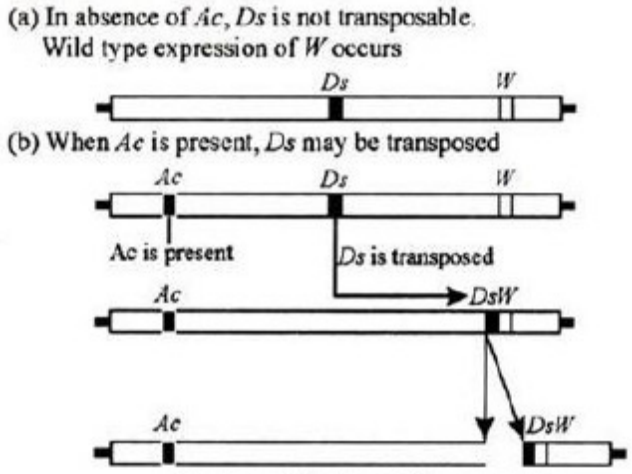


Fig. 6. Effects of Transposition involving *Ac-Ds* system in maize.

(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).

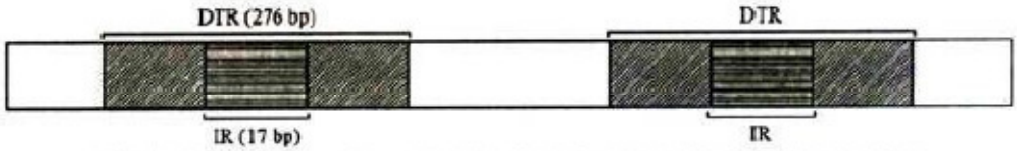


Fig. 7. Organization of a copia transposable element in *D. melanogaster*.

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 AluI. 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.

I M.Sc I SEM
Cell Biology and Molecular Genetics
UNIT - V

Methods of genetic transfers:

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.

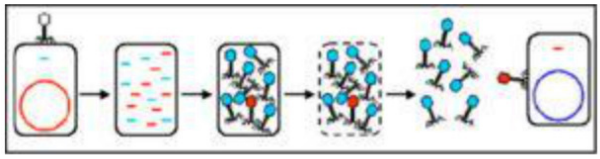


FIG. THE MECHANISM OF GENERALIZED TRANSDUCTION

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:

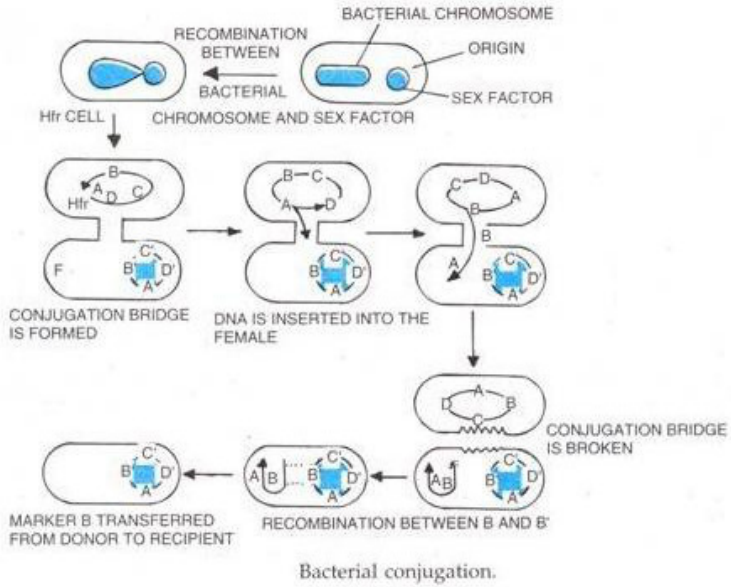
The unidirectional transfer of DNA from one cell to another through a cytoplasmic bridge is called conjugation. The process is equivalent to sexual mating in eukaryotes. Two bacterial haploid cells of different strains come close to each other.

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.

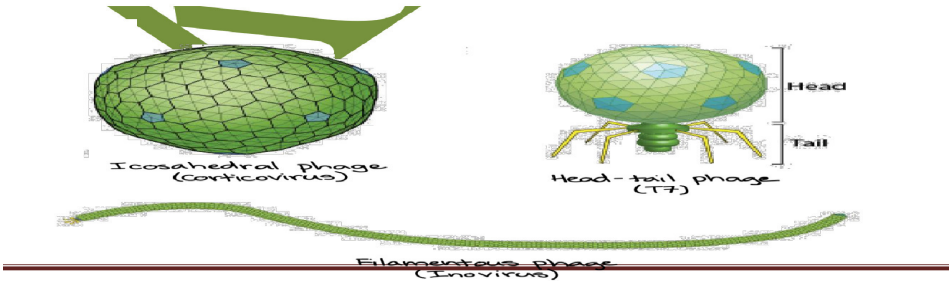
Hayes (1952) found a strain of E. coli in which the frequency of recombination was as high as 100 to 1000 times as reported by Lederberg. The strain was called high frequency recombinant (Hfr) strain.

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).

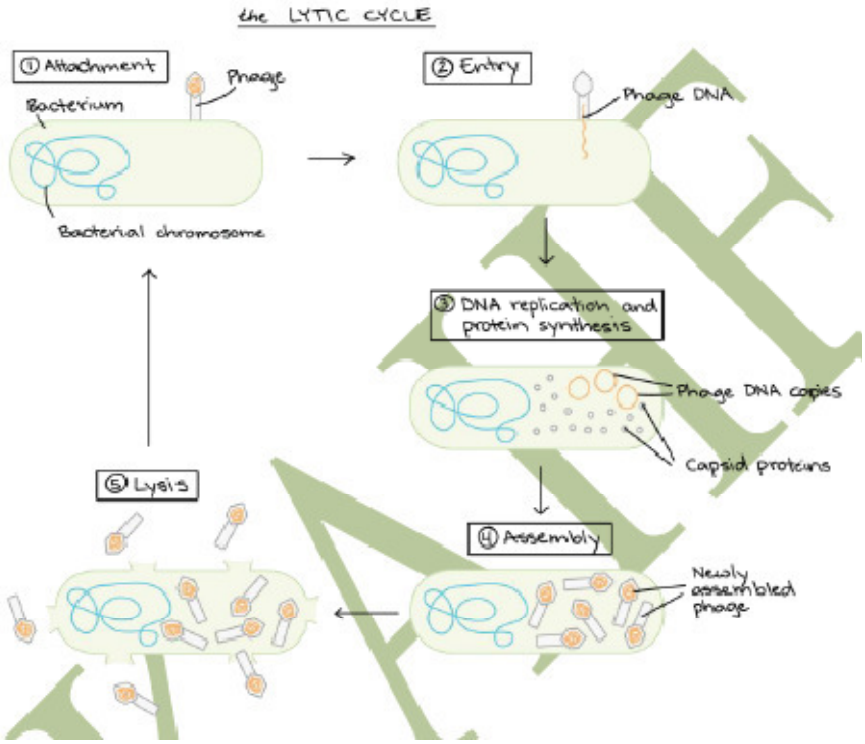
Let's take closer look at these two cycles. As an example, we'll use a phage called lambda (\lambdaλlambda), which infects *E. coli* bacteria and can switch between the lytic and lysogenic cycles.

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:

- 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.
- Entry: The phage injects its double-stranded DNA genome into the cytoplasm of the bacterium.
- DNA copying and protein synthesis: Phage DNA is copied, and phage genes are expressed to make proteins, such as capsid proteins.
- Assembly of new phage: Capsids assemble from the capsid proteins and are stuffed with DNA to make lots of new phage particles.
- 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**.**

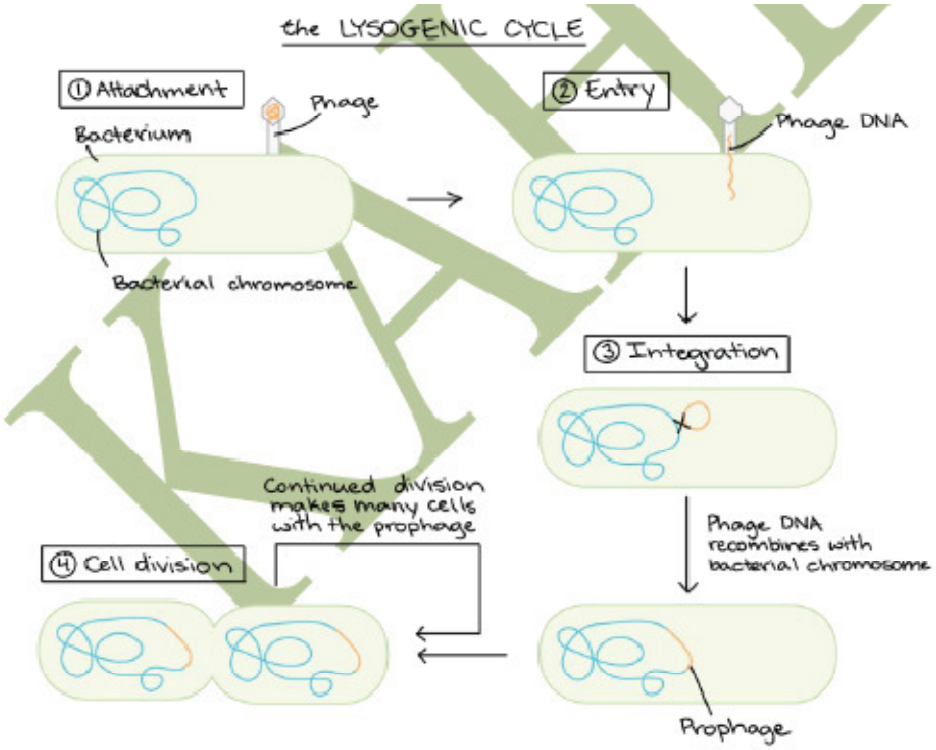


Cell bursting, or lysis, releases hundreds of new phages, which can find and infect other host cells nearby. In this way, a few cycles of lytic infection can let the phage spread like wildfire through a bacterial population.

Lysogenic cycle

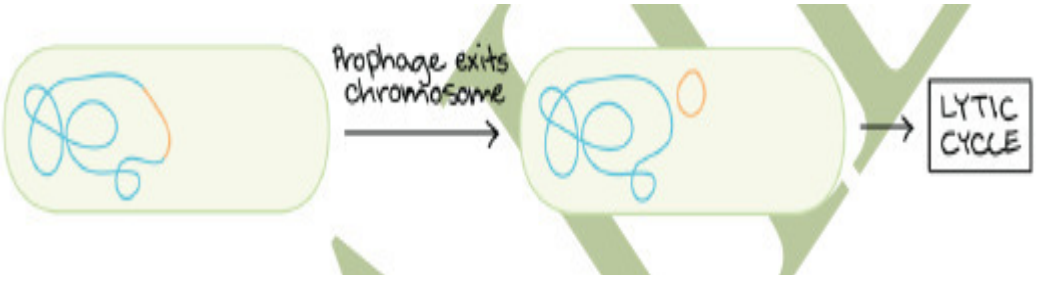
The lysogenic cycle allows a phage to reproduce without killing its host. Some phages can only use the lytic cycle, but the phage we are following, lambda (\lambdaλlambda), can switch between the two cycles.

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

1. The λ genome (total 48,502 bp) contains an

2. origin of replication,

3. genes for head and tail proteins and

4. enzymes for DNA replication, lysis and lysogeny, and

5. single-stranded protruding cohesive ends of 12 bases (5' GGGCGGCGACCT; the other end is complementary to it, i.e., CCCGCCGCTGGA 5').

6. 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.

7. 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.

8. The λ DNA must be larger than 38 kb and smaller than 52 kb to be packaged into phage particles.

9. 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

- accommodate larger DNA inserts and
- 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.

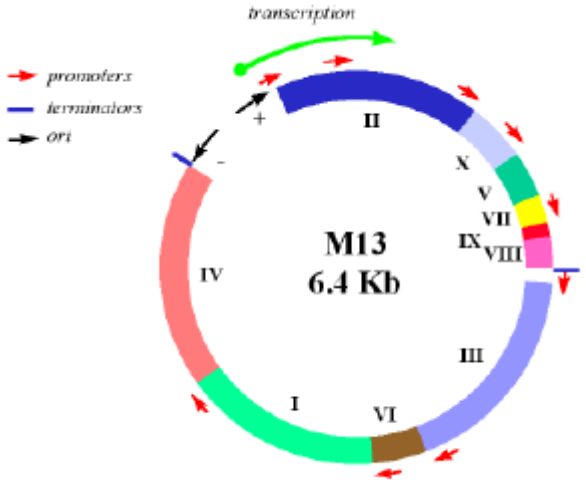
(2) They contain restriction sites, which allow the removal of the lysogenic segment and also provide insertion site for the DNA fragment.

(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

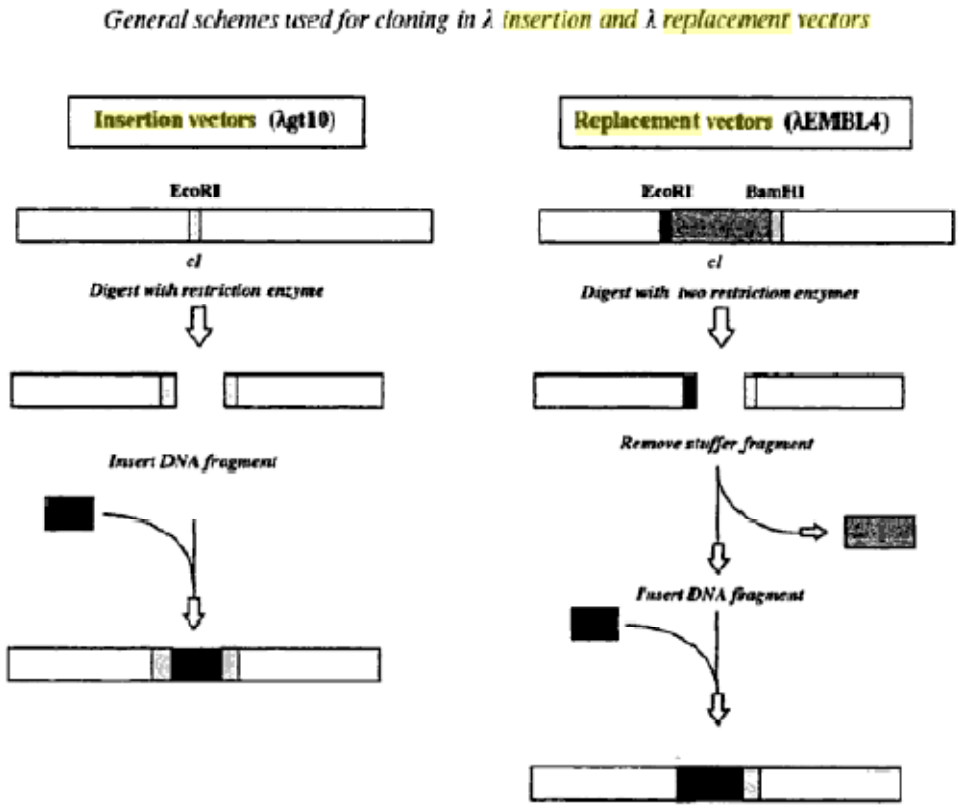
1. **Insertional vectors**
2. **Replacment vectors**

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.

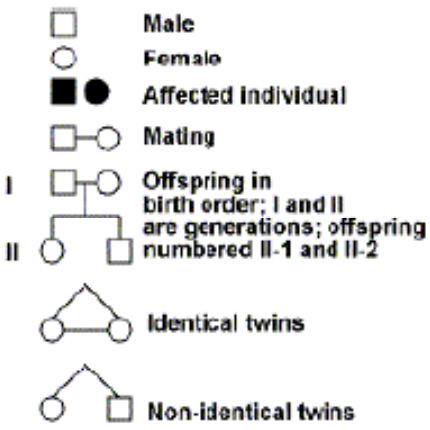
Example – **λembl, λZAP**



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.

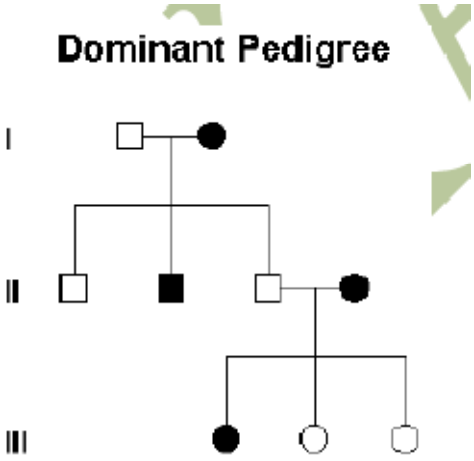


Once phenotypic data is collected from several generations and the pedigree is drawn, careful analysis will allow you to determine whether the trait is dominant or recessive. Here are some rules to follow.

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

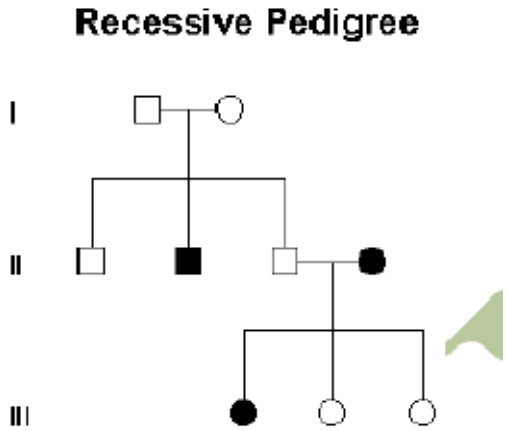
The following is the pedigree of a trait contolled by dominant gene action.



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.



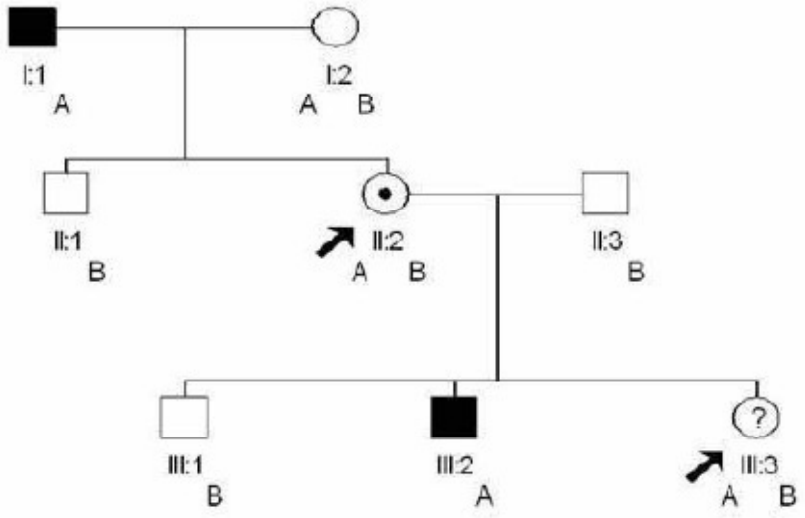
Linkage Testing

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.

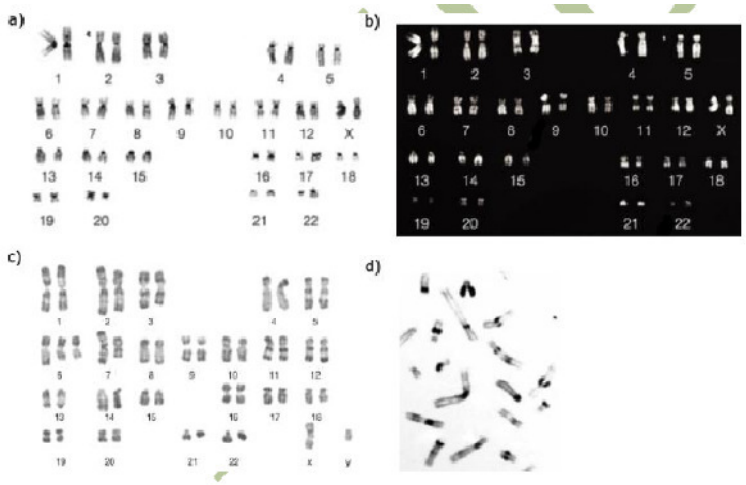
Banding Patterns Reveal the Structural Details of Chromosomes

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:

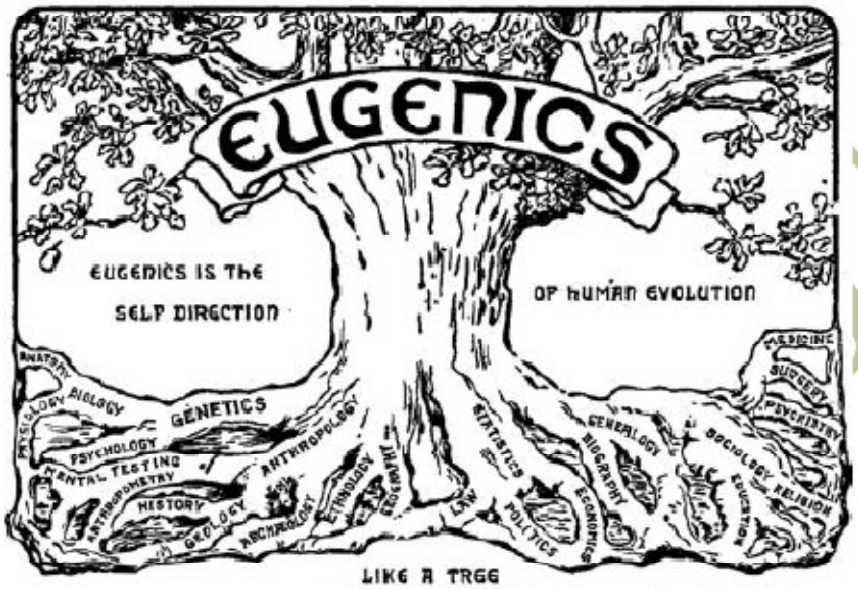
- Single-gene disorders, where a mutation affects one gene. Sickle cell anemia is an example.
- Chromosomal disorders, where chromosomes (or parts of chromosomes) are missing or changed. Chromosomes are the structures that hold our genes. Down syndrome is a chromosomal disorder.
- Complex disorders, 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

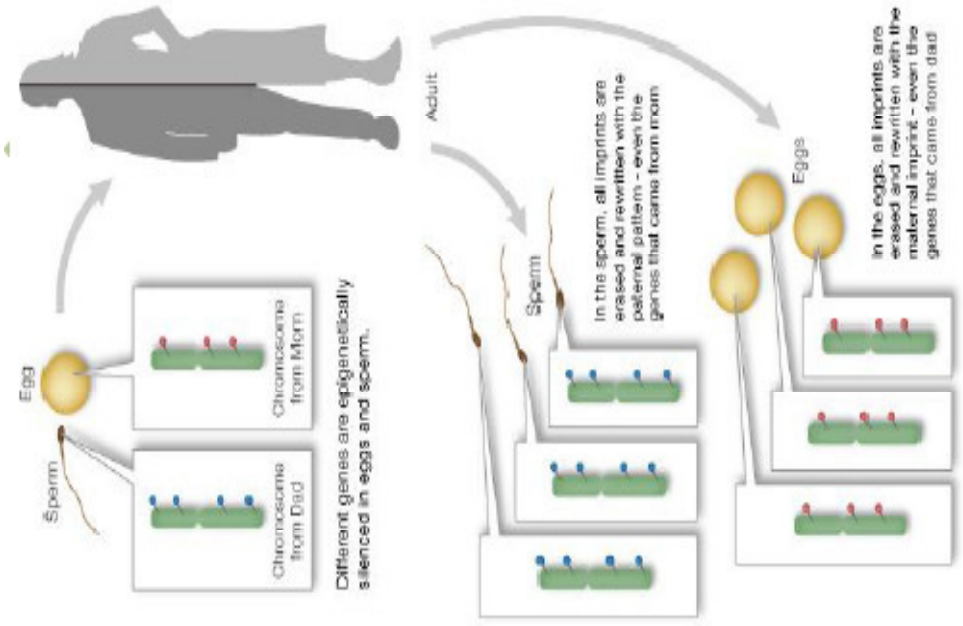
It is the study of heritable changes in gene expression (active versus inactive genes) that do not involve changes to the underlying DNA sequence — a change in phenotype without a change in genotype — which in turn affects how cells read the genes. Epigenetic change is a regular and natural occurrence 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. 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 do es 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: $H^2=\sigma^2_G/\sigma^2_P$ $H^2=\sigma_G^2/\sigma_P^2$ where σ^2_G G^2 is the variance in the trait explained by genetics (G), and σ^2_P P^2 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 σ2G G2. The broad-sense H2H2 doesn‘t care whether σ2G G2 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 eve ry 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 σ2G G2 relates to σ2P P2. 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 σ2P P2 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 σ2A A2 to be the largest and most immediately useful portion of the total σ2G G2 [1]. Focusing on just this additive genetic component leads us to the definition of the narrow-sense heritability h2h2:h2=σ2Aσ2Ph2=σA2σP2

there are no dominant/recessive or interaction effects (i.e. σ2D=σ2I=0 D2= I2=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, 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 . 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.

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 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.

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 βj j [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 βj j 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

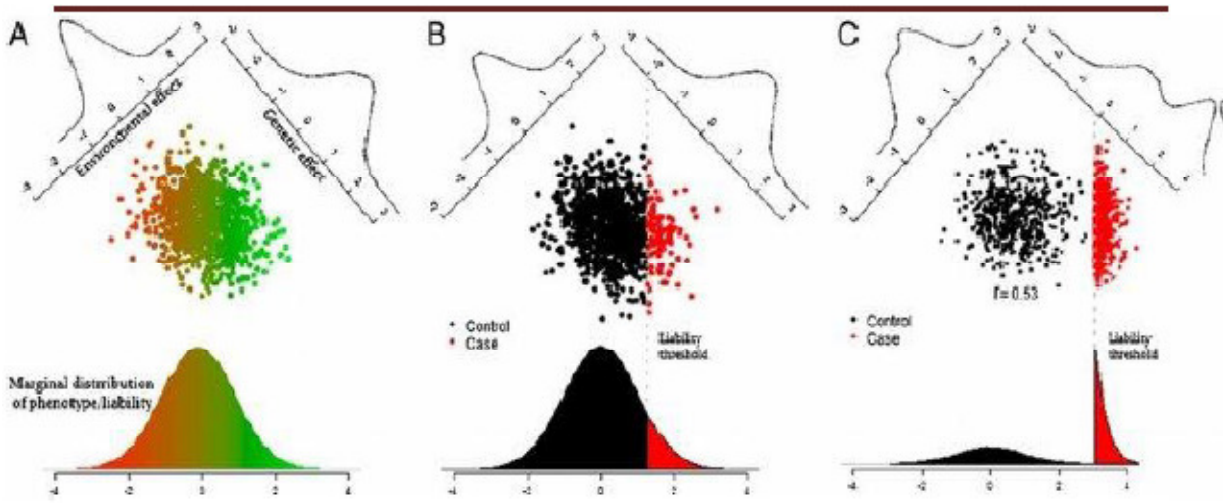
All of the above flavors of heritability are defined based on the ―true‖ variance explained by genetic variants. Although we noted above that we don't know the true effects βj j, we could estimate them from our observed SNP data and then use those estimated values to directly compute: h2PRS=var(∑SNPs∈Sxj^βj)var(y)hPRS2=var(∑SNPs∈Sxjβj^)var(y)

We refer to this version of heritability as h2PRShPRS2 since the sum with estimates βj j 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 βj j. 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 βj j 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 ce nter 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 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.

S.No.	Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
	Unit I					
1	Bacterial cells are prokaryotic. Unlike a typcial eukaryotic cell they-----	Have no membrane-bounded organelles in their cytoplasm	Lack a plasma membrane	have a smaller nucleus	have no ribosomes	Have no membrane-bounded organelles in their cytoplasm
2	Which of the following are similar between transcription in prokaryotes and eukaryotes?	RNA polymerase produces mRNAs which grow in the 5'-3' direction	RNA polymerase bind to ribosomes to allow transcription	A polytail is added to the 3' end of messenger RNAs	Introns are present in genes which are spliced out after transcription	RNA polymerase produces mRNAs which grow in the 5'-3' direction
3	mt DNA is	simple double stranded linear DNA molecule	simple,single stranded linear DNA molecule	simple, single stranded circular DNA molecule	simple, double stranded circular DNA molecule	simple double stranded linear DNA molecule
4	The inner membrane of the mitochondria is usually higly convoluted forming a seris of infloding known as	Thylakoids	lamellae	cristae	grana	cristae
5	The beadlike unit of chromatic structure is the	chromatid	nucleosome	c)solenoid	kinetochore	nucleosome
6	The subunit of prokaryotic ribosomes are	60 S, 40S	70 S, 30S	60S, 30S	50 S, 30 S	50 S, 30 S
7	Which of the following is NOT associated with animal cells?	plasmodesmata	an extracellualr matric	gap junctions	tight junctions	plasmodesmata
8	which of the following structure is found in eukaryotes but not in prokaryotes cells?	Mitochondria	Ribosomes	DNA	Plasma membrane	Plasma membrane
9	The sorting and packaging centre of the cell refers to what cellular component?	Golgi apparatus	Lysosomes	Nucleus	Mitochondria	Golgi apparatus
10	Detoxification is a characteristic function of what type of organelle?	Mitochondria	Golgi apparatus	Peroxisomes	Nucleus	Peroxisomes
11	Which of the following cell structures exhibits selective permeability between a cell and its external environement?	The Plasma membrane	Mitochondria	Lysosomes	Chloroplast	The plasma membrane

12	The organelle involved in protein transport is	ER and Golgi	ER and mitochondria	lysosomes and golgi	Golgi and mitochondria	ER and Golgi
13	A component of the animal cell membranes that functions to stiffen the membrane and thus regualte its fluidity is	cholesterol	cellulose	pectin	lignin	cholesterol
14	Which of the following membrane has the large amount of proteins?	erythrocyte membrane	myelin sheath membrane	inner mitochondrial membrane	outer mitochondrial membrane	inner mitochondrial membrane
15	Nuclei were first discovered by?	Strasburger	Fonatana	Robert Brown	Robert Koch	Robert Brown
16	Which of the following is the central commanding center of the cell?	Nucleus	Mitochondria	ER	d)Ribosomes	Nucleus
17	Nucleus is absent in	Red blood cells and bacterium	Red blood cells, sieve cells and bacterium	Red blood cells only	Bacterium only	Red blood cells, sieve cells and bacterium
18	Nucleus has	DNA only	DNA and Protein only	DNA, RNA and proteins	carbohydrates and lipids	DNA and Protein only
19	Nuclear membrane is in continuous connection with-------	SER	RER	Golgi apparatus	lysosomes	RER
20	The light stained and diffused region of chromatin is known as	Heterochromatin	Euchromatin	Chromatin	Chromosomes	Euchromatin
21	The most important function of nuclear envelope is to	regulate nucelo cytoplasmic traffic	protect genetic material	prevent the entrance of active ribosomes in to the nucleus	synthesis rRNAs	prevent the entrance of active ribosomes in to the nucleus
22	The basic proteins of the nucleus are	nucelohistones	nucleoprotamines	nucleohistones, nucleoprotamines	protamines	nucleohistones, nucleoprotamines
23	Bacterial cell wall is made up of	N-acetyl glucosamine	N-acetyl muramic acid	N-acetyl muramic acid branches	N-acetyl glucosamine, N-acetyl muramic acid and amino acids	N-acetyl glucosamine, N-acetyl muramic acid and amino acids
24	Which of the following statements are not true regarding cell wall?	plant cell wall is made up of cellulose	Plant cell wall is a non-living structure	Cell wall provides mechanical support to the cell	Cell wall is semi permeable	Cell wall is semi permeable
25	Fine cytoplasmic connections between neighbouring cells through the cell wall for cell to cell communication is called	Plasmosome	plasmodesmata	mesosome	chromosomes	Plasmodesmata
26	Which of the following organelle is involved in cell wall synthesis?	Mitochondria	Chloroplast	Golgi apparatus	Lysosomes	Gologi apparatus
27	Plant cell wall is made up of	Cellulose only	Cellulose and chitin	Cellulose, hemicellulose and chitin	cellulose, hemicellulose and	cellulose, hemicellulose and pectin

					pectin	
28	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	β 1-4 linkage
29	Chitinous cell wall is present in	plants	Bacteria	protists	Fungi	Fungi
30	The site of light reaction is	Grana	stroma	thylakoid lumen	outer membrane	thylakoid lumen
31	Which of these describes a holliday junction?	A Section of DNA where base pairing is not exact	A strand of DNA containing genetic material from two different chromosomes	An interaction of two strands of DNA from homologus chromosomes	A three stranded DNA structure where single stranded DNA has invaded a double helix	An interaction of two strands of DNA from homologus chromosomes
32	Which of the following elements would be considered to be a complete transposable element?	An insertion sequence that include a transposase gene between the inverted repeats	A composite element that carries an antibiotuc resistance gene	A replicative transposon that includes a reverse transcriptase gene	A retrotransposon containing long terminal repeats	An insertion sequence that include a transposase gene between the inverted repeats
33	A chromosome with a very short arm and a very logn arm is termed as	Telocentric	Acrocentric	Metacentric	d)sub-metacentric	Acrocentric
34	Chromosme is thickest during	prophase	metaphase	anaphase	interphase	metaphase
35	The lowest level of chromosome organization is	solenoid	nucelosome	30 nm fibre	super helix	nucleosome
36	The term chromosome was coined by	W. Flemming	W. Roux	Waldeyer	Sutton	W. Flemming
37	The number of autosome in humans	44	21 pairs	46	45	44
38	The diagrammatic representation of karyotype (morphological representation of chromosomes) of a species is called	idiogram	cladogram	ecogram	chromogram	idiogram
39	Chromatin has	DNA	DNA and proteins	DNA, RNA and proteins	Proteins and lipids	DNA, RNA and proteins
40	The fisrt man to observe live cell under microscope was	Robert hooke	Leeuwenhoek	Schleiden	Virchow	Leeuwenhoek
41	The nucleus was first described by	Robert Brown	Robert hooke	Weismann	Nageli	Robert Brown
42	All the following statements are truw regarding the "cell theory" except	All living things or organisms are made of cells	All cells arise spontaneously	cell is the basic structural and functional unit of life	All cells arise from preexisting cells	All cells arise spontaneously
43	The membrane around the vacuole is called	cytoplast	tonoplast	amyloplast	chromoplaast	tonoplast
44	which of the following enzymes are used to join fragements of DNA?	DNA ligase	DNA polymerase	primase	endonuclease	DNA ligase

45	Semiconservative replication of DNA was confirmed by the which scientist's experiments ?	Meselson and Stalh's experiment	watson and crick	griffith's experiment	Mendels experiment	Meselson and Stalh's experiment
46	Unwinding of DNA is done by	helicase	ligase	hexonuclease	toposiomerase	helicase
47	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	Okazaki segments
48	Which of the following nitrogenous bases is found in DNA but is not found in RNA?	Adenine	Thymine	Guanine	Uracil	Uracil
49	Nucleotides have a nitrogenous base attached to a sugar at the------	1' Carbon	2' Carbon	3' Carbon	5' carbon	5' Carbon
50	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%	20%
51	The Process of DNA replication involves:	Multiple origins of replication per chromosme in eukaryotes	Binding of ribosomes to origin of replication	Continous synthesis on both strands of the double helix	Conservative replciation with one original double helix and one totally new double helix as products	Multiple origins of replication per chromosme in eukaryotes
52	RNA is less stable than DNA because	RNAses are ubiquitous	RNAses are extremely heat stable	denatured RNAses readily renature	d)RNA is chemically stable	RNAses are ubiquitous
53	By convention, a DNA strand is written in an orientation that places the template strand	reading in a 5'→3' direction	reading in a 3'→5' direction	reading in a 2' → 5' direction	reading in a 1' →3' direction	reading in a 5'→3' direction
54	The structural feature that allows DNA to replicate itself is the	sugar-phosphate backbone	complementary pairing of the bases	phosphodiester bonding of the helices	twisting of the molecule to form an alpha helix	complementary pairing of the bases
55	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	3'-GTAATC-5'
56	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%	80-90%, 2.5-5%

57	A nucleotide is made up of which of the following chemical components?	A nitrogenous base, a fatty acid, and an amino acid	A nitrogenous base, an amino acid, and a phosphate group	A nitrogenous base, an amino acid, and a pentose sugar	A nitrogenous base, a phosphate group, and a pentose sugar	A nitrogenous base, a phosphate group, and a pentose sugar
58	Genetic code is	the sequence of nitrogenous bases in mRNA molecule that codes for a protein	is a triplet code	is non-overlapping	anticodon	is a triplet code
59	A nucleoside is compsoed of	a base + a sugar	a base+ a sugar + phosphate	a base+ a phosphate	lipids+ proteins	a base + a sugar
60	genetic mutation occurs in	protein	RNA	DNA	Nucleus	DNA
61	DNA is present in	viruses, prokaryotes and eukaryotes	prokaryotes and eukaryotes	only in eukaryotes	in some viruses, prokaryotes and eukaryotes	in some viruses, prokaryotes and eukaryotes
62	The two strands in a DNA double helix is joined by	Co-valent bond	hydrogen bond	ionic bond	phosphodiester bond	hydrogen bond
63	The basic repeating units of a DNA molecule is	nuceloside	nucleotide	histones	amino acids	nucleotide
64	Adjacent nucleotides are joined by	covalent bond	phosphodiester bond	ionic bond	peptide bond	phosphodiester bond
65	The length of one turn of DNA is	3.4 angstrom	34 angstrom	20 angstrom	3.04 angstrom	34 angstrom
66	The type of sugar in DNA are	triose	tetrose	c)pentose	hexose	pentose
67	The width of DNA molecule is	15 angstrom	3.4 angstrom	20 angstrom	25 angstrom	20 angstrom
68	The length of DNA having 23 base pairs is	78 angstrom	78.4 angstrom	78.2 angstrom	74.8 angstrom	74.8 angstrom
69	Left handed DNA	A-DNA	B-DNA	c)Z-DNA	C-DNA	Z-DNA
70	Z-DNA have a	double helical nature	Zig-zag apperarance	uracil base	single stranded nature	Zig-zag apperarance
71	Chargaff's rule states that in a double stranded DNA molecule	Concentration of deoxyadenosine (nucleotides equals that of thymidine (T) nucleotides	Concentration of Deoxyadenosine (nucelotides equals that of deoxy guanosine (G) nucleotides	Concentration of Deoxy cytidine © nucleotides equals that of thymidine (T) nucleotides	Concentration of Deoxy uridine (U) nucelotides equals that of deoxy guanosine (G) nucleotides	Concentration of deoxyadenosine (nucleotides equals that of thymidine (T) nucleotides
72	Choose the incorrect statement out of the following	Double stranded DNA exists in at least three forms (A, B and Z)	The B-form is usually found under physiologic conditions	single turn of B-DNA about the axis of the molecule contains six base pairs	d)The distance spanned by one turn of B-DNA is 3.4 nm	single turn of B-DNA about the axis of the molecule contains six base pairs

73	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	Negative supercoils
74	Choose the correct statement out of the following?	The common form od DNA is said to be left-handed	The coding strand is copied during RNA synthesis	The two strands of the double helical DNA molecule run parallel to each other	The G-C bonds are much more resistant to denaturation than A-T rich regions	The G-C bonds are much more resistant to denaturation than A-T rich regions
75	RNA is a polymer of purine and pyrimidine ribonucleotides linked together by	Hydrogen bonds	hydrophobic interactions	vander wal's forces	3'-5' phosphodiester linkages	3'-5' phosphodiester linkages
76	Which of the following types of RNA participate in RNA processing?	t-RNA	r-RNA	small nuclear RNA (snRN	Small interfering RNAs (siRNAs)	small nuclear RNA (snRN
77	The anticodon region is an imprtant structural component of	mRNA	DNA	rRNA	tRNA	tRNA
78	The small nuclear RNAs are rich in	uracil	cytosine	thymine	adenine	uracil
79	The Z-DNA helix	has fewer base pairs per turn than the B-DNA	has alternating GC sequences	tends to be found at the 3' end of genes	is inhibited by methylation of the bases	has alternating GC sequences
80	An endonuclease is an enzyme that hydrolyzes	A nucelotide from only the 3' end of an oligonucelotide	A nucelotide from either terminal of an oligonucelotide	A phsophodiester bond located in the interior of a polynucelotide	A bond only in the specific sequence of nucelotides	A phsophodiester bond located in the interior of a polynucelotide
81	Which of the following has the highest percentage of modified bases?	hnRNA	b)t-RNA	sn-RNA	m-RNA	t-RNA
82	Choose the incorrect statement about an RNA?	has a single stranded structure	Does not obey chargaff's rule	c)Does not exhibit watson and crick's base pairing	instead of uracil contains the ribonucleotides of thymine	instead of uracil contains the ribonucleotides of thymine
83	Which of the following statements is incorrect about tRNA species?	they have the smallest size of the three major species	All tRNAs have between 10-20 nucleotides	tRNA molecules contain unsual bases	They make up 15% of the tRNA molecule for each of the 20 amino acids	All tRNAs have between 10-20 nucleotides
84	Semi conservative replication of DNA was first demonstrated in	E.Coli	S.penumonae	S.typhimuriam	D.melanogaster	E.Coli
85	Mode of DNA replication in E.Coli is	Conservative and unidirectional	b)Semiconservative and unidirectional	Conservative and bidirectional	semiconservative and bidirectional	semiconservative and bidirectional
86	When DNA replication starts	The phosphodiester bonds between the	the bonds between the nitrogen base and	the leading strand produces okazaki	the hydrogen bonds between the	the hydrogen bonds between the nucleotides

		adjacent nucleotide break	deoxyribose sugar break	fragments	nucleotides of two strand break	of two strand break
87	The elongation of the leading strand during DNA synthesis	Progresses away from the replication fork	occur in 3'-5' direction	produces okazaki fragment	Depend on the action of DNA polymerase	Depend on the action of DNA polymerase
88	Eukaryotes differ from prokaryote in mechanism of DNA replciation due to----	Different enzyme for synthesis of lagging and leading strand	use of DNA primer rather than RNA primer	unidirectional rather than bidirectional replication	discontinous rather than semidiscontinous replication	discontinous rather than semidiscontinous replication
89	The accepted hypothesis for DNA replication is	conservative theory	b)dispersive theory	semi-conservative theory	evolutionary theory	semi-conservative theory
90	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	cytosine
91	Telomeres are usually rich in which nucleotide?	Adenine	Guanine	Thymine	Cytosine	Guanine
92	Which is the largest among the following?	Nucleotide	Nitrogenous base	Phosphate	carbon	Nucleotide
93	Each replication bubbles consists of	3 replication forks	2 replication forks	4 replication forks	1 replication fork	2 replication forks
94	In a cesium chloride gradient, DNA labelled with N15 and centrifuged will form a band	above DNA containing N14	below DNA containing N14	with N14 containing DNA	inbetween N14 and N15	below DNA containing N14
95	The role of primase	dismantle RNA primer	celave and unwinds short sections of DNA ahead of the replication fork	proofread base pairing	synthesize an RNA primer to begin the elongation process	d)synthesize an RNA primer to begin the elongation process
96	DNA replciation in eukaryotes occurs only in	G1 phase	S phase	G2 phase	M phase	S Phase
97	Who's X-ray work aided watson and crick in their discovery of the double helix?	W.H. bragg	R.Franklin	L.pauling	Leaderberg	R.Franklin
98	Which polymerase is active in DNA proofreading during replication?	polymerase I	polymerase II	polymerase III	polymerase IV	polymerase I
99	The genetic code is	universal	not universal	species specific	kingdom specific	not universal
100	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 gene sequences	Equally origin of replication and structural gene sequences

101	Which of the following is a description of the proof reading function of DNA polymerase?	Endonuclease cleavage	b)exonuclease cleavage	methylation	an induced fit phenomenon	exonuclease cleavage
102	What enzyme performs decatenation?	polymerase I	topoisomerase	c)telomerase	decatenase	decatenase
103	What is a key difference between DNA polIII and DNA ligase?	OnlyDNA Pol III synthesizes phosphodiester bonds	only DNA ligase syntehsizes phosphodiester bonds	DNA pol III can synthesize DNA from 3'-5'	DNA ligase can use energy from ATP ratehr than nucleotides	DNA ligase can use energy from ATP ratehr than nucleotides
104	The normal function of the promoter is to	Bind the small subunit of the ribosome	Serve as an origin of replication	Serve as an acceptor for transfer RNA	Serve as a binding site for RNA polymerase	Serve as a binding site for RNA polymerase
105	Protein molecules are polymers of-----	DNA moelcule	Amino acid molecules	Fatty acid molecules	Sucrose moelcules	Amino acid molecules
106	The peptide bond is -----	A covalent joing simple sugars together to form a polypeptide	A covalent bond joining nucleotides together to form a nucleic acid	A hydrogen bond joing nucleotides together to form polypoetide	A covalent bond joining aminoacid together to form a polypeptide	A covalent bond joining aminoacid together to form a polypeptide
107	In prokaryotes, the ribosomal binding site on mRNA is called	Hogness sequence	Shine-Dalgarno sequence	Pribnow Sequence	TATA box	Shine-Dalgarno sequence
108	During translation, the role of enzyme peptidyl transferase is	transfer of phosphate group	amino acid activation	peptide bond formation between adjacent amino acids	binding of ribosome subunits to mRNA	peptide bond formation between adjacent amino acids
109	Polysomes are	Aggregation of ribosomes	aggregation of lysosomes	mRNA molecules to which many ribosomes are attached simultaneously	a strech of tRNA assemblies	mRNA molecules to which many ribosomes are attached simultaneously
110	Translation is the	Synthesis of DNA from a mRNA template	Synthesis of protein from a mRNA template	Synthesis of RNA from a mRNA template	Synthesis of RNA from a DNA template	Synthesis of protein from a mRNA template
111	During translation, proteins are synthesized	by ribosomes using the information on DNA	by lysosome using the information on DNA	by ribosomes using the information on mRNA	by ribosomes using the information on rRNA	by ribosomes using the information on mRNA
112	The enzyme involved in amino acid activation is	ATP synthetase	aminoacyl tRNA synthetase	aminoacyl mRNA synthetase	aminoacyl rRNA synthetase	aminoacyl tRNA synthetase
113	Which of the following RNA molecules serves as an adaptor	rRNA	mRNA	tRNA	tRNA and mRNA	tRNA

	molecule during protein synthesis					
114	In eukaryotes, translation is inititated by binding of ribosomes to the	Pribnow box	Hogness box	5' CAP	poly A tail	5' CAP
115	Which is the energy rich molecule required for initiation of translation	ATP synthetase	GTP	CTP	AMP	GTP
116	Which of the following best explains the sequence of central dogma	DNA makes RNA makes Proteins	RNA makes DNAmakes Proteins	DNA makes proteins makes RNA	Protein makes DNA makes RNA	DNA makes RNA makes Proteins
117	Many primary transcripts of noncoding RNAs must be -------in order to be functional	translated	polyadenylated	processed	replicated	processed
118	RNA polymerase used for the transcription of genes require a ------- template	rRNA	DNA	RNA	mRNA	DNA
119	Which of the following statements is not true?	Transcription but not translation is regulated in bacteria	Bacterial non coding RNAs mus t be processed by cleavage to form functional molecules	The regulation of the transcription initiation complex is a key feature of gene expression control in eukaryotes	Linking of exons must precede translation in eukaryotes	Transcription but not translation is regulated in bacteria
120	Regarding RNA polymerase in E.Coli which of the following describes the difference between the holo enyzyme and the core enzyme?	The holoenzyme is used to synthesize mRNA, while the core enzyme synthesizes noncoding RNAs	The holoenzyme consists of five subunits including σ, while the core enzyme lacks σ	The core enzyme and not the holoenzyme is required for the initation of RNA synthesis	The holoenzyme binds to DNA upstream of the promoter while the core enzyme binds to dounwstream of the promoter	The holoenzyme consists of five subunits including σ, while the core enzyme lacks σ
121	RNA polymerase II transcribes all of the following except genes specifying	rRNAs	proteins	snRNAs	miRNAs	rRNAs

122	The recognition sequence to whiich RNA polymerase binds at the initiation of transcription is found-----	downstream of the promoter target sequence	upstream of the gene to be transcribed	within the first intron	downstream of the transcription bubble	upstream of the gene to be transcribed
123	Which of the following best describes a promoter?	An element that promotes termination of transcription	A regualatory protein that accelerates mRNA turnover	A specific target sequence to which RNA polymeraase binds	An extracellular environmental inducer that controls gene expression	A specific target sequence to which RNA polymeraase binds
124	Some eukaryotic promoters contain an element positioned around nucleotide +1 called----	the TATA box	the pribnow box	an initiator (Inr) sequence	an inverted repeat	an initiator Inr sequence
125	Null genes are characterized by their-----	transcription by RNA polymerase I and II	possession of neither TATA box nor an Inr sequence	Invariable start position of transcription	inability to be transcribed	possession of neither TATA box nor an Inr sequence
126	The conversion of a clsoed rpomoter complex to an open promoter complex in bacteria requires------	the activity of alternative promoters	hydrogen bond breakage of base pairs around the initiation site	a G-C rich sequence around to +1 site	strong interaction between the core enzyme and the -10 box	hydrogen bond breakage of base pairs around the initiation site
127	An improtant difference between the initiation of prokarytoic and eukaryotic transcription of protein coding genes is that--------	only prokaryotic genes use upstream sequence	only eukaryotic RNA polymerase possess a σ subunit	eukaryotic promoters indirectly recognize core promoter sequences	only prokaryotes cells alter the rate of transcription	eukaryotic promoters indirectly recognize core promoter sequences
128	Each time a ribonucleotide is added to the elongating RNA molecule during transcription a-----is released	deoxy ribonucleotide	sugar-base component	c)pyrophosphate	phosphate	pyrophosphate
129	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-7MeGppppNpN	pppNpN	7-MeGpppNpN

130	The formation of hairpin loops at the 3' end of prokaryotic RNAs during transcription results in------	stabilization of DNA:RNA pairing promoting further elongation of the transcript	activation of poly (polymerase	a reduction in base pairing between the template strand and the RNA transcript	Rho-dependent activation of transcription	a reduction in base pairing between the template strand and the RNA transcript
131	Which of the following statements regarding the poly (tail of eukaryotic RNA is incorrect?	The poly (tail is located at the 3, end	The poly (tract is transcribed from the DNA template	poly (tails are found on transcripts made by RNA polyemrase II	Tissue specific gene expression patterns soemtimes correlate with laternative polyadenylation	The poly (tract is transcribed from the DNA template
132	Translation occurs in the	nucleus	cytoplasm	nucleolus	lysosome	cytopalsm
133	What is the main function of tRNA in relation to protein synthesis?	inhibits protein synthesis	proof reading	identifies amino acids and transport them to ribosomes	promoter binding	identifies amino acids and transport them to ribosomes
134	Which site of tRNA molecule hydrogen bonds to a mRNA molecule?	codon	anticodon	5, ends of the tRNA molecule	3' ends of the tRNA molecule	anticodon
135	In the context of prokaryotic gene expression, which of the following is the msot appropriate definition of an operator?	A cluster of genes that are rqgulated by a single promoter	A DNA-binding protein that regulates gene expression	A non-coding regulatory DNA sequence that is bound by RNA polymerase	A non-coding regualtory DNA sequence that is bound by a repressor protein	A non-coding regulatory DNA sequence that is bound by a repressor protein
136	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 repressor are bound to the DNA	CAP is bound to the DNA but the lac repressor is not	Lac repressor is bound to the DNA but CAP is not	Neither CAP nor the lac repressor are bound to the DNA	Neither CAP nor the lac repressor are bound to the DNA
137	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	Enhancer	Insulator	TATA box	Enhancer

138	Which of the following statements, concerning regulation of trp operon expression by attenuation, is correct?	The leader peptide sequence encodes enzymes required for tryptophan synthesis	the leader peptide sequence contains no tryptophan residues	Rapid translation of the leader peptide allows completion of the mRNA transcript	Rapid translation of the leader peptide prevents completion of the mRNA transcript	Rapid translation of the leader peptide prevents completion of the mRNA transcript
139	Which of the following is true of the lac operon in E.Coli?	the operon is only switched on in the absence of lactose in the growth medium	The lac operon messenger RNA is the polycistronic mRNA	The enzyme β-galactosidase is only rpoduced in large quantitites when the lac repressor is bound to the operator	The promoter is the binding site for the lac repressor	The lac operon messenger RNA is the polycistronic mRNA
140	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'	3' to 5'; 5' to 3'
141	Which of the following statements below is false?	The genetic code is overlapping	the genetic code is not universal	Degenerate codons specify the same aminoacids	The genetic code is triplet	The genetic code is overlapping
142	The first mRNA codon to specify an amino acid is always	TAC	UAA	UAG	AUG	AUG
143	The aminoacid sequence of a polypeptide chain comprises the ---- structure of the protein	primary	secondary	tertiary	Quaternary	primary
144	Transfer RNA's bind during translation by the ----	codon	anticodon	template	mRNA	anticodon
145	Which of the following statements is true regarding introns?	introns are the parts if mRNA that are translated	introns have no functions	In general human genes have fewer introns than genes of other organisms	Introns may be involved in exon shuffling	Introns may be involved in exon shuffling
146	The effort to decipher the genetic code was led by-----who was awarded a nobel prize for his work	Nirenberg	Lederberg	Watson	Crick	Nirenberg
147	What sequence on the template strand of DNA corresponds to the first amino acid inserted in to a protein?	TAC	UAA	UAG	AUG	TAC

148	Which of the following is an example of the degeneracy of the genetic code?	a given amino acid has more than one codon	each codon specifies more than one amino acid	the fiorst two bases specify the amino acid	the genetic code is not degenerate	a given amino acid has more than one codon
149	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	P, A
150	The only methylated base in mammals is?	7-methyl guanine	thymine	methyl adenine	5-methyl cytosine	thymine
151	Repressor molecules bind to the	promoter	enhancer	operator	hormone response element	operator
152	Which of the follwoiung is false about the E.Coli Lac operon?	It is polycistronic	It is an example of negative control	The presence of lactose acts as an inducer	The repressor binds to the promoter	The repressor binds to the promoter
153	The part of the bacterial RNA polymerase responsible fopr recognizing the promoter is the ----	Alpha subunit	Rho protein	DNA pol III	Sigma subunit	sigma subunit
154	In protein synthesis which out of the following is not a termination codon?	UAG	UGA	UUU	UAA	UUU
155	All are true for DNA polymerase except one---	Has exonuclease activity	Works only in the 5' to 3' direction	Edits as it synthesizes	Synthesizes RNA primer to initiate DNA synthesis	Synthesizes RNA primer to initiate DNA synthesis
156	What is added to the 3' end of many eukaryotic tRNAs after transcription?	introns	b)Cap of modified G nucleotide	Poly A tail	Trinucleotide CCA	trinucleotide CCA
157	Which one of the following molecules is not a component of the 30S initiation complex?	GTP	mRNA	Initiation factor 2	ATP	ATP
158	Enhancer regions in eukaryotic DNA are---	DNA pol I binding sites	Inhibit the binding of repressor	Enhance the frequency of transcription	Specific for given set of genes	Enhance the frequency of transcription
159	What are the coding segments of a strech of eukaryotic DNA called?	introns	exons	codons	replicons	Exons
160	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'	3' ACU 5'
161	RNA polymerase moves in which direction along the DNA?	3' to 5' along the template strand	3' to 5' along the coding (sense) strand	5' to 3' along the template strand	3' to 5' along the coding strand	3' to 5' along the coding (sense) strand

162	What type of bonding is responsible for maintaining the shape of tRNA molecule?	covalent bonding between sulfer atoms	ionic binding between phosphates	c)hydrogen bonding between base pairs	van der waals interactions between hydrogen atoms	hydrogen bonding between base pairs
163	To code phenylalanine aminoacid which codon on the mRNA strand coldes for this amino acid	UGG	GUG	GUA	UUC	UUC
164	Which of the following statements are true about protein synthesis in prokaryotes?	Extensive RNA processing is required before prokaryotic transcripts can be translated	Translation can begin while transcription is still in progress	Unlike eukaryotes, prokaryotes require no initiation or elongation factors	Translation requires antibiotic activity	Translation can begin while transcription is still in progress
	Unit II					
165	Lipid bilayer is -----	hydrophilic	hydrophobic	hydrophilic and hydrophobic	depends on the surrounding medium	hydrophilic and hydrophobic
166	Which is the best definition of active transport?	Movement of molecules from an area from their higher concentration to an area of lower concentration	movement of a substance against its concentraion through the release of energy from ATP	movement of molecules across a permeable barrier utilizing energy	Movement of molecules in and out of the permeable barrier without utilizing energy	movement of a substance against its concentraion through the release of energy from ATP
167	The current theroy of the structure of the plasma membrane is best described by the --------- model	sandwich	fluid mosaic model	electrochemical	unipermeable	fluid mosaic model
168	In a phospholipid bilayer, the -----	phosphate groups are hydrophobic	fatty acid tails are ionized	fatty acid tails are hydrophilic	phosphate heads are oriented toward the exterior of the cell or towards the cytoplasm	phosphate heads are oriented toward the exterior of the cell or towards the cytoplasm
169	Whether a molecule can cross the plasma membrane depends upon----	the charge of the molecule	valency of the molecule	chemical bond of the molecule	atomic number of the moelcule	the charge of the molecule

170	If a cell is placed in a hypertonic solution, which will occur?	Salts will move in to the cell from surrounding solution	water will move out of the cell in to the surrounding solution	Salts will move out of the cell in to the surrounding medium	water will move out of the cell in to the surrounding medium	water will move out of the cell in to the surrounding solution
171	If a cell is placed in a hypotonic solution, which will occur?	Salts will move in to the cell from surrounding solution	water will move in to the cell from the surrounding solution	Salts will move out of the cell in to the surrounding medium	water will move out of the cell in to the surrounding medium	water will move in to the cell from the surrounding solution
172	If a cell is placed in the isotonic solution which will occur?	Salts will move in to the cell from surrounding solution	water will move in to the cell from the surrounding solution	Salts will move out of the cell in to the surrounding medium	water will move in to and out of the cell in equal rates	water will move in to and out of the cell in equal rates
173	In plants --------is a shrinking of the cytoplasm due to osmosis	Plasmolysis	Endocytosis	Crenation	Diffusion	Plasmolysis
174	The sodium-potassium pump moves sodium and potassium ions across the plasma membrane by ------	Facilitated transport	active transport	cotransport	Endocytosis	active transport
175	Sugars and amino acids are carried in to the cell by means of ---	Facilitated transport	active transport	cotransport	Endocytosis	Facilitated transport
176	Cell products are secreted from the cell through	Facilitated transport	active transport	exocytosis	Endocytosis	exocytosis
177	Pinocytosis is an example of -----	Facilitated transport	active transport	endocytosis	exocytosis	endocytosis
178	Active transport ----------	requires an input of ATP	is involved in diffusion	occurs in osmosis and faciliated transport	is needed for water to move through cell membranes	requires an input of ATP
179	The major functions of the plasma membrane do NOT include	separation of the fluid environments inside and outside the cell	regulation of molecules and ions that pass into and out of the cell	recognition and communication between different cells and tissues	production of proteins used in the construction of cells	production of proteins used in the construction of cells

180	The role of carbohydrate in cell membrane	cell adhesion	cell-cell recognition	assisting transport across cell membrane	cell storage reserve	cell-cell recognition
181	The major biomolecule responsible for selective uptake of materials across plasma membrane	Carbohydrate	protein	lipids	phospholipids	proteins
182	At physiological pH increase in cholesterol level	Increases fluidity	decreases fluidity	no change in the fluidity	equal change in the fluidity	decreases fluidity
183	All the following substance pass through the cell membrane except	O2	b)H2O	CO2	d)H+	H+
184	The major interactions responsible for stabilizing plasma membrane	hydrophilic interactions	covalent bonds	ionic bonds	hydrophobic interactions	hydrophobic interactions
185	Clathrin coated pits are associated with	phagocytosis	pinocytosis	receptor mediated endocytosis	exocytosis	receptor mediated endocytosis
186	Na+ glucose transporter is an example of	facilitated diffusion	ATP driven active transport	Symport	antiport	Symport
187	Which of the following transport induces conformational change in protein?	simple diffusion	active transport	facilitated diffusion	ion driven active transport	facilitated diffusion
188	The erythrocyte glucose transporter is an example of	simple diffusion	active transport	facilitated diffusion	ion driven active transport	facilitated diffusion
189	In cell membrane, carbohydrates in glycoproteins or glycolipids are oriented	towards outside	towards inside	towards outside and inside	randomly distributed	towards outside
190	The distribution of instrinsic proteins in the cell membrane is	symmetrical	assymetrical	random	uniform	assymetrical
191	High lipid content is a characteristic of	erythrocyte membrane	myelin sheath membrane	inner mitochondrial membrane	outer mitochondrial membrane	myelin sheath membrane
192	Which of the following membrane has the largest amount of proteins	erythrocyte membrane	myelin sheath membrane	inner mitochondrial membrane	outer mitochondrial membrane	inner mitochondrial membrane
193	The longer the fatty acid chain, fluidity of the lipid bi layer	increases	decreases	no change in fluidity	remains the same	increases
194	Which transport mecahansim can bring whole cells into a cell?	pinocytosis	phagocytosis	facilitated diffusion	primary active transport	phagocytosis

195	Water moves via osmosis	throughout the cytoplasm	from an area with a high concentration of outer solutes to a lower one	from an area with a high concentration of water to one of lower concentration	from an area with a low concentraion of water to one of higher concentration	from an area with a high concentration of water to one of lower concentration
196	Cells may be said to be "sugar coated" due to the presence of	phospholipids	glycolipids	glycocalyx	sphingolipids	glycolipids
197	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	phospholipid
198	Vesicular transport is invloved in all of the following transport processes, except	exocyosis	Endocytosis	phagocytosis	solute pumps	solute pumps
199	All of the following are functions of membrane receptors, except	G protein-linking	contact signalling	chemical signalling	cell adhesion	cell adhesion
200	Paracrine signalling is invloved in which of the following?	hormonal communiaction	chemical signals that can only travel limited distances between cells	autostimualtion of a cell	receptor medaited signalling	chemical signals that can only travel limited distances between cells
201	Neurotransmitters are released in to the synapse at which of the following?	presynaptic membrane	postsynaptic membrane	golgi apparatus	axon hillock	postsynaptic membrane
202	Fluid mosaic model was given by	Robertson	Schwann	Dave Donson	Singer and Nicholson	Singer and Nicholson
203	Middle lamella is composed mainly of	protein	Hemicellulose	carbohydrate	calcium pectate	calcium pectate
204	The lipids in the bilayer of the cell membrane have tails that are	hydrophilic	hydrophobic	zwitter ionic	polar	hydrophobic
205	Unsaturated fatty acids in the lipid bilayer are bended giving rise to a	kink	rounded	straight	circular	kink
206	Which of the following is not a form of active transport?	osmosis	bulk membrane transport	exocytosis	endocytosis	bulk membrane transport

207	What is osmosis?	the diffusion of water across a selectively permeable membrane	the diffusion of water across a permeable membrane	c)the active of transport of water between cells	the absorption of water from the environment	the diffusion of water across a permeable membrane
208	What is a ligand?	any molecule that binds specifically to another's receptor site	any molecule that can pass through membrane	the term for materials inside the vesicle	the term used for unprocessed lipids	any molecule that binds specifically to another's receptor site
209	Which of the following is not a typical function of membranes?	storage	site of recognition	selective transport	permeability barrier	storage
210	The engulfing of bacteria by white blood cells is called as	Phagocytosis	Pinocytosis	Exocytosis	Endocytosis	Phagocytosis
211	The concentration gradient is said to exist when the	Concentration of ions is more inside the cell and less outside the cell	concentration of ions is lesser inside the cell and more outside the cell	hypertonic solution enters the cells	water enters by osmosis	concentration of ions is lesser inside the cell and more outside the cell
212	Which of these is part of the cell membrane?	aminoacids	phospholipids	ATP	glucose only	phospholipids
233	How do fat-soluble moelcules normally get into a cell?	they dissolve in the fat layers of the membrane and enter the cell by diffusion	they pass through protein pores in the cell membrane	they are absorbed by phagocytosis	they never get in	they dissolve in the fat layers of the membrane and enter the cell by diffusion
234	Which of the statements best describes the "fluid mosaic model" of the structure of the cell membrane?	two layers of protein with lipid layers between the protein layers	b)two layers of lipid with proteins between the lipid layers	a double layer of lipid molecules with protein molecules suspended in the layer	a single layer of protein on the outside and a single layer of lipids on the inside	a double layer of lipid molecules with protein molecules suspended in the layer

235	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	diffusion
236	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	0.01%
237	which of the following is necessary in order for osmosis to occur?	a permeable membrane	a semi-permeable membrane	an isotonic solution	ATP	permeable membrane
238	which of these are passive transport mechanisms?	osmosis	diffusion	phagocytosis	osmosis and diffusion	osmosis and diffusion
239	In an isotonic solution there would be----	no net movement of water	net movement of water in to the cell	net movement of water out of the cell	d)bursting of the cell	no net movement of water
240	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	active transport
241	when a cell bursts due to osmosis, it is in a solution that is-----	hypertonic	isotonic	hypotonic	normal	hypotonic
242	what is likely to happen to a plant cell that is placed in pure water?	it becomes turgid	it becomes flaccid	in undergoes plasmolysis	it bursts	it becomes turgid
243	Homologus chromosomes move towards the opposite poles of a dividing cell during	Mitosis	Meiosis I	Meiosis II	Fertilization	Meiosis I
244	Meiosis II is similar to mitosis in that	Sister chromatids separate during anaphase	The daughter cells are diploid	Homologus chromosome synapse	DNA replicates before the division	Sister chromatids separate during anaphase
245	What is a chromatid?	A chromosome in G1 of the cell cycle	a replicated chromosome	a chromosome found outside the nucleus	a special region that holds two centromeres together	a replicated chromosome

246	Which statements best explains the evolutionary advantage if meiosis?	Meiosis is necessary for sexual reproduction	Meiosis alternates with mitosis from generation to generation	The same genetic system is passed on from generation to generation	Genetic recombinations are possible from generation to generation	Genetic recombinations are possible from generation to generation
247	Meiotic divisions occurs in	Vegetative cells	meristamatic cells	conductive cells	reproductive cells	Reproductive cells
248	Meiosis I is -----division	additive	reductional	subractive	oxidative	Reductional
249	Meiosis II is equational division due to	crossing over	separation of chromatids	pairing of homologus chromosomes	disjunction of homologus chromosomes	separation of chromatids
250	Meiosis II performs----	Separation of sex chromosomes	synthesis of DNA and centromere	separation of homologus chromosomes	separation of chromatids	separation of chromatids
251	Synapsis occurs between	mRNA and ribosomes	spindle fibres and centromere	a male and a female gamete	Two homologus chromosomes	Two homologus chromosomes
252	In Meiosis, the daughter cells differ from parent cell as well as amongst themselves due to	Segregation, independent assortment and crossing over	Segregation and crossing over	Independent assortment and crossing over	segregation and independent assortment	Segregation, independent assortment and crossing over
253	When parental and maternal chromosomes change their materials with each other in cell division this vent called	synapsis	crossing over	Dyad forming	Bivalent forming	Crossing over
254	Continous variations are attirbuted to	polyploidy	mutation	crossing over	chromosomal aberrations	Crossing over
255	During which stage of prophase I of the crossing over takes place?	Leptotone	b)Pachytene	c)Zygotene	Diplotene	Pachytene
256	During mitosis, ER and nucleolus begin to disapper at	Late prophase	Early prophase	Late metaphase	early metaphase	
257	Which among the following stage represents the best stage to view the shape, size and number of	Metaphase	Anaphase	prophase	telophase	Metaphase

	chromosomes					
258	During metaphase mitosis chromosomes	undergo coiling	line up at the equator	breaks up	break up and disintegrate	line up at the equator
259	What aspect of mitosis is affected by colchicine in inducing polyploidy?	DNA replciation	Spindle formation	Formation of cell plate	chromosome doubling	Spindle formation
260	Simple nerve reflexes use signalling molecules called	neurotransmitters	nitric oxide	G proteins	proteases	neurotransmitters
261	In terms of cell communications, what do bacterial pathogens such as cholera and anthrax have in common?	They destroy the receptors for keysignalling molecules	They prevent the production of key signaling molecules	They alter the chemical structure of key signaling molecules	They block the normal functioning of signal transduction mechansims	They block the normal functioning of signal transduction mechansims
	Unit III					
262	Mutations	are premanent changes in the DNA sequence or structure	produce genetic recombinations	are not harmful	are permanent and found to be more harmful than beneficial	are permanent and found to be more harmful than beneficial
263	Mutations that cause loss of a chromosme would be termed	Structural mutations	Chromosome mutations	Genome mutations	Single-gene mutations	Chromosome mutations
264	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 pairs that is not a multiple of 3	Insertion or deletion of any number of base pairs that is not a multiple of 3
265	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	CAA to TAA

266	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 missence mutation	due to base substituion or deletion or missence mutation
267	Which of the following would be a nexample of a germline mutation?	Exposure to excessive UV radiation causes changes in the DNA of a skin cell, leading to basal cell carcinoma	In a very early human embryo a mistake in mitosis causes loss of a Y chromosome in one daugther cell leading to Turner's syndrome	A man with normal chromosomes exposed to radiations, his progeny will be with chromosomal deletion	An embryo missing one copy of the third chromosome is miscarried very early in pregnancy	A man with normal chromosomes exposed to radiations, his progeny will be with chromosomal deletion
268	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 mutations	induced mutations	Deletions	Insertions	Deletions
269	An alteration in a nucelotide in a nucelotide squence that changes a triplet coding for an amino acid in to a termination codon	nonsense mutation	Mutation	Mutagenesis	Mutagen	non sense mutations
270	The main differene between the directed mutation theory and the randome mutation theory is-----	The chemical nature of the mutagens	The effect of the mutation on the phenotype	The cause of the mutation	The heritability of the trait	The cause of the mutation
271	Examples of environmental mutagens include?	Alkylating agents	nucleotide base analogues	Ionizing radiation	Alkylating agnets, nucelotide base analogs and ionizing radiations	Alkylating agnets, nucelotide base analogs and ionizing radiations

272	A bacterial cell experiences a mutation as a result of expsoure 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	Four
273	In the Ames test:	mutagens cause lethal mutations, reducing the numebr of colonies	Mutagens cause mutations that disrupt the ability of the cell to produce histidine	Mutagens will cause an increas in the number of revertants	only mutagens that cause transitions can be identified	Mutagens will cause an increas in the number of revertants
274	Which of the following forms of DNA repair doest NOT require DNA polymerase?	Direct DNA repair	Base excision repair	Nucleotide excision repair	Mismatch repair	Direct DNA repair
275	Why is alkyltransferase only able to be used once?	The protein is hydrolyzed in the process of repairing the DNA	The protein is chemically changed by the addition of a methyl group	The protein becomes attached to the DNA strand	Inability to bind to another region	The protein is chemically changed by the addition of a methyl group
276	how does recombinational repair differ from nucelotide excision repair (NER)?	Unlike NER, recombinational repair is unable to repair damage caused by thymine dimers	NER replaces the thymine dimer while recombinational repair leaves it in place	NER requires DNA polymerase and ligase function, while recombinationalrepair does not	NER involves nick translation, which recombinational repair does not	NER replaces the thymine dimer while recombinational repair leaves it in place
277	A point mutation that replaces a purine with another purine, or a pyrimisine with another pyrimidine	Transition	Transversion	Nonsense	Missense mutation	Transition
278	A point mutation that involves a purine being replaced by a pyrimidine or vice versa	Transition	Transversion	Nonsense	Silent mutation	Transversion
279	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	silent mutation

280	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	missense
281	An alteration to the normal chemical or physical structure of the DNA	Transition	Transversion	c)Lesion	Missense mutation	Lesion
282	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	Thymine
283	All transposons encode a ----which catalyzes the insertion	DNA glycosylase	Excisionase	Transposase	Integrase	Transposase
284	Small DNA sequences that can move to virtually any position in a cell's genome	Exons	introns	LTRs	Transposons	Transposons
285	In base excision repair, the lesion is removed by-----	DNA glycosylase	Transposase	Excisionsase	DNA polymerase	DNA glycosylase
286	The exchange of non homologus regions of DNA at specific sites is independent of ----	IS elements	Illegitimate recombination	Retrotransposons	RecA	RecA
287	Cyclobuitane pyrimidine dimers can be monomerized again by -----in the presence of visible light	Exonuclease	DNA photolyases	Transposases	DNA polymerase	DNA photolyases
288	The dispersed repetitive sequences found in higher eukaryotic DNA (eg LINES and SINES) probably spread through the genome by---	Transposition	homologus recombination	site specific recombination	General recombination	Transposition
289	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	RecBCD
290	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	Nonsense
291	Which type of mutation is most likely to revert?	deletion	translocation	inversion	transition	inversion
292	Which type of mutation is least likely to revert?	Deletion	translocation	inversion	transition	deletion

293	The hydrolysis of an -NH2 group from a base is called----, while intercalating agents such as proflavin function as mutagens by causing------	deamination; transversions	deamination; deletions or insertions	excision repair; deletion or insertions	excision repair; transversions	deamination; deletions or insertions
294	ultraviolet radiation is most likely to produce what form of mutation?	deaminations	depurination	double stranded breaks	thymine dimers	thymine dimers
295	Duplication of multiple three-nucelotide repeats is responsible for	Sickel cell anemia	trisomy 21	alkaptonuria	xeroderma pigmentosum	alkaptonuria
296	Errors in DNA replication are most often corrected by	SOS systems	Base excision repair	Nucleotide excision repair	methyl-directed mismatch repair	Base excision repair
297	A mutation in the UvrC protein of E.Coli would result in which of the following?	an increase in overall mutation rate	a decrease in over allmutation rate	an increase in transposition events	a decrease in transposition events	a decrease in transposition events
298	The---------- are heritable changes in base sequences that can affect phenotype	mutation	duplication	replication	transcription	mutation
299	A mutation that changes a wild type allele of a gene to a different allele is called a -----mutation	forward	reverse	inversion	deletion	forward
300	A -------mutation causes a novel allele to be converted back to a wild type allele	forward	reverse	inversion	deletion	reverse
301	An----------is a type of mutation where a segment of a chromosome is rotated 180°	forward	reverse	inversion	deletion	inversion
302	Any physical or chemcial agent that increases the rate of mutation above the spontaneous rate is a	mutagens	b)plasmogens	antigens	antibodies	mutagens
303	A mutation in which parts of two homologus chromosomes change places is called a	translocation	transition	transversion	deletion	translocation
304	The hydrolysis of a purine base from the deoxy-ribose phosphate backbone is called	depurination	deamination	replica plating	excision repair	depurination
305	UV light is a mutagen that can cause:	depurination	deamination	alkylation	thymine dimers	thymine dimers

306	Unequal crossing over results in	an exchange between nonhomologus chromosomes	a loss of genetic material	repair of UV induced damage	a creation of deletions and duplications	a creation of deletions and duplications
307	Base analogs differ from other classes of mutagen in that they	only alter bases	can only cause transversions	only work during DNA replciation or repair	will not function in bacterial cells	only work during DNA replciation or repair
308	Intercalating agents such as acridine orange function as mutagens to------	promote transitions	remove amine groups	fit between stacked bases and disrupt replication	add ethyl or methyl groups	fit between stacked bases and disrupt replication
309	A complementation group is	a group of mutations that produce the same phenotype	a group of mutations that are in the same gene and complement each other	a group of mutations that are in the same gene and do not complement each other	group of mutations in two different genes that do not complement each other	a group of mutations that are in the same gene and do not complement each other
310	The condition sickle cell anemia is due to	the insertion of an amino acid	the deletion of an amino acid	substitution of an amino acid	failure to synthesize a hemoglobin molecule	substitution of an amino acid
311	Mutations that abolish the function encoded by the wild type allele are known as	null mutations	hypomorphic mutations	hypermorphic mutations	conditional mutations	null mutations
312	Choose the condition below that does not involve a defect in an enzymatic pathway	alkaptonuria	albinism	sickle cell anemia	phenylketonuria	sickle cell anemia
313	The Ames test for mutagenicity is useful to identify potential carcinogens because	since bacteria do not get cancer they can survive lethal carcinogens	mutagens that affect bacterial DNA are likley to casue human mutation	bacteria thrive on substances that could cause cancer in humans	the same genes that cause cancer in humans can be mutated in bacteria	mutagens that affect bacterial DNA are likley to casue human mutation

314	In the Ames test for mutagenicity:	auxotrophic bacteria are converted to protrophs which survive	prototrophic bacteriaare converted to auxotrophs which survive	cells are treated with mutagen and only thos ewith no mutations survive	cells are treated with excess amino acids killing cells that carry mutations	auxotrophic bacteria are converted to protrophs which survive
315	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	it will not occur
316	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	fragile X chromosome
317	The genetic condition xeroderma pigmentosum, which can lead to skin cancer, results from:	inability to correct UV induced dimers	inability to process phenylamine	inability to produce functional hemoglobin	breaks in the X chromosomes	Inability to correct UV induced dimers
318	Excision repair corrects DNA by:	removing a double stranded fragment of damaged DNA	detecting, removing and replacing a single stranded fragment of damaged DNA	excising the incorrect base from a nucleotide	correcting A=T to G=C transitions	detecting, removing and replacing a single stranded fragment of damaged DNA
319	The unit of gene mutation is---------	cistron	recon	muton	photon	muton
320	Mutations that occurs under natural conditions are called-------	point B	Induced C	base	Spontaneous	spontaneous
321	A-----phenotype mutations result in the death of cells or organisms	Sub vital	Lethal	Super Vital	Induced	Lethal
322	Unit IV					
323	Direct DNA uptake by protoplasts can be stimulated by	polyethylene glycol	decanal	luciferin	GFP	polyethylene glycol
324	Which technique is used to introduce genes into dicots?	electroporation	particle acceleration	microinjection	Ti plasmid infection	Ti plasmid infection
325	The size of the virulent plasmid of	40-80 kb	80-120 kb	140-235 kb	>235 kb	140-235 kb

	Agrobacterium tumefaciens is					
326	Virulence trait of *Agrobacterium tumefaciens* is borne on	chromosomal DNA	tumour inducing plasmid DNA	both chromosomal and plasmid DNA	cryptic plasmid DNA	tumour inducing plasmid DNA
327	Introduction of DNA molecules into the recipient organism is termed as _____	transformation	translation	transduction	transcription	transformation
328	F plasmid is often used in conjugation. The correct statement is?	The F plasmid encodes the factor which is transferred from one cell to another	The factor encoded by the F plasmid is called as Filamentous (F) factor	It is transferred from one cell to another by filament	The bacteria must belong to same species to carry out the conjugation	The F plasmid encodes the factor which is transferred from one cell to another
329	Plasmids can be classified into how many types depending on the genes present for their transformation?	1	2	3	4	2
330	Electroporation is also used for taking up the DNA by the cells. It constitutes of _____	inserting the DNA into the cells via an electric shock	increased efficiency than both natural and chemical methods	causing the least amount of damage in comparison to other methods	decreased efficiency than both natural and chemical methods	inserting the DNA into the cells via an electric shock
331	Transformation carried out using a particle gun is known as biolistic transformation. It falls under which category of transformation?	Natural	Electroporation	Chemical	Physical	Physical
332	The injection of DNA into developing inflorescence using a hypodermic syringe is called	macroinjection	micromanipulator mediated DNA delivery	microfection	microinjection	macroinjection
333	Fibre mediated DNA delivery uses	silicon carbide fibres that will create pores in the membarne	aluminium carbide fibres that will create pores in the membarne	boron carbide fibres that will create pores in the membarne	lead carbide fibres that will create pores in the membarne	a) silicon carbide fibres that will create pores in the membarne
334	DNA solution injected directly into the cell using micromanipulators is called	macroinjection	b) micromanipulator mediated DNA delivery	microfection	microinjection	microinjection

335	The method widely used for transforming invitro animal cell cultures that uses lipid vescicles or liposomes	lipotransformation	liposome mediated transformation	lipofection	lipid mediated DNA transfer	lipofection
336	The virus mediated gene transfer using genetically modified bacteriophages is called	transfection	transduction	transformation	conjugation	transduction
337	The ability of cells to take up DNA fragments from surrounding is called	transfection	transduction	transformation	conjugation	transformation
338	Which of the following bacterium is considered as 'natural genetic engineer'	Agrobacterium tumefaciens	Agrobacterium radiobactor	Psueudomonas putida	Thermus aquaticus	Agrobacterium tumefaciens
339	Which of the following chemical enhances vir gene expression	cyanidin	glutennin	acetosyringone	dextran	acetosyringone
340	Ti plasmid vectors include	binary vectors and cointegrate vectors	cointegrate vectors and multiple vectors	multiple vectors and binary vectors	Ti plasmid based vector	binary vectors and cointegrate vectors
341	Agrobacterium tumefaciens is a	Gram negative soil bacterium causing crown gall disease in dicots	Gram negative soil bacterium causing crown gall disease in monocots	Gram positive soil bacterium causing crown gall disease in dicots	Gram positive soil bacterium causing crown gall disease in dicots	Gram negative soil bacterium causing crown gall disease in dicots
342	The removal or replacement of tumor causing genes from Ti plasmid is termed as	gene replacement	disarming	insertional inactivation	gene displacement	disarming
343	Which of the following statements is true regarding transposons?	They are sequences of mRNA that can move around in the genome	They exist in corn, but are not found in the human genome	They are most abundant type of repeat in the genome	they are coding sequences	They are most abundant type of repeat in the genome
	Unit V					
344	A cDNA library contains clones representing which of the following?	mRNA	Genomic DNA	Introns	Repeated DNA sequences	mRNA

345	A candidate gene for a disorder is tested for linkage. At $\theta = 0$, the lod score is -8. The implication of this finding is:	The candidate gene must be the gene for the disorder.	The candidate gene has not been excluded as being the cause of the disorder.	The candidate gene has been excluded as being the cause of the disorder.	The candidate gene is not linked to the gene for the disorder.	The candidate gene has been excluded as being the cause of the disorder.
346	The CAG repeat in the Huntington disease gene encodes:	A signal to methylate the promoter	A signal to alter patterns of splicing	A polyglutamine repeat	An RNA–protein binding segment	A polyglutamine repeat
347	The best estimate for the number of human genes is:	1 000 000	100 000	50 000	20 000	20 000
348	Genetic counseling includes all of the following EXCEPT:	Assessment of the occurrence or recurrence risk	Recommendation of specific reproductive options	Discussion of available therapies	Discussion of the impact of the disease on the patient and family	Recommendation of specific reproductive options
349	Why is □-thalassemia major usually evident only after birth?	The switch from □-gene to □-gene expression occurs around the time of birth.	The fetal Hb persists after birth when it should have been shut off.	The product of the □-globin pseudogene is highly expressed only after birth.	The mother's normal red blood cells provide oxygen to the fetus in utero.	The switch from □-gene to □-gene expression occurs around the time of birth.
350	To establish a successful and cost-effective screening program for detecting heterozygous carriers of an autosomal recessive disease, all of the following are essential, EXCEPT:	The screening test has a positive predictive value of 100%	A high-risk population can be identified	Genetic counseling is provided with the testing	The disease is severe enough to be clinically significant	The screening test has a positive predictive value of 100%
351	A QTL	is one of the genes that influences a trait.	is a chromosomal region containing genes that influence a quantitative trait.	will not contain any genes other than the ones influencing a trait.	is a measure of the phenotypic variation in a quantitative trait.	is a chromosomal region containing genes that influence a quantitative trait.
352	Genomic imprinting in autosomes is seen in _____ percent of the genes.	<1%	25%	50%	100%	<1%
353	Differential expression of the genetic material depending on its parentage of	Imprinting	Penetrance	Expressivity	Non-penetrance	Imprinting

	inheritance gives_____					
354	What will happen if an individual receives two copies of a gene from the same parent?	One gene will be automatically inactivated	Uniparental disomy	Both the genes will show their product	Nothing much will be seen	Uniparental disomy
355	Which of the following is not true for a bacteriophage?	A very simple structure	Consist either DNA or RNA	Bacteriophages are viruses	Complex structure that infects bacteria	Complex structure that infects bacteria
356	What is the capsid (protective coat) of the bacteriophage made up of?	DNA	Organic acids	RNA	Protein	Protein
357	Which of the following is an example of head-and-tail bacteriophage?	M16	Pbr322	Lambda phage	M13	Lambda phage
358	The replication of phage DNA molecule is associated with which step in the infection cycle of a bacteriophage?	Second step	First step	Third step	Preparation stage	Second step
359	The cycle which is completed quickly in the infection by a phage is _____	Lytic	Lysogenic	Replication	Capsid formation	Lytic
360	Which infection cycle is characterized by retention of the phage DNA molecule in the host bacterium for many thousands of cell division?	Lysogenic cycle	Lytic cycle	Integrative Phase	Protein synthesis	Lysogenic cycle
361	Approximate size of lambda phage is _____	49 kb	12 kb	23 kb	100 kb	49 kb
362	Euploidy is a chromosomal variation in _____	Size	Position of genes	Number	Structure	Number
363	Which of the following will be sterile?	Tetraploid	Triploid	Diploid	Monoploid	Triploid
364	Colchicine is used to cause_____	Meiotic disjunction	Mitotic disjunction	Meiotic non-disjunction	Mitotic non-disjunction	Mitotic non-disjunction
365	Choose the odd one out?	Leaf	Endosperm	Fertilized egg	Petals	Endosperm
366	Farmers often practice polyploidy as _____	It makes the plants more durable	They take longer time to undergo meiosis	It increases complexity and there is a hope of new species	It produces larger plant parts and products	It produces larger plant parts and products

367	In animals polypoidy is rarely practiced as _____	Giants are harder to train and take care of	Increase in size is not appreciable	It is often sterile	Animal cells are resistant to colchicine	Increase in size is not appreciable