COURSE: MSc Part -1

PAPER – 7

TOPIC- Cell biology (Various topics)

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Topic -1

NATURE OF GENETIC MATERIAL

Identification of Genetic Material

Chromosome was known to be the organ of heredity. It was proposed that it can pass on from one generation to another, to exhibit, continuity of life. While knowing about the chromosome it was hypothesized that chromosomes have linearly arranged units of character known as genes. Attempts were made to identify the physical and the chemical nature of genes, but it remained unsuccessful for quite some time until the classical experiment of Griffith (1928) on Mice.

Evidence that DNA is the genetic material

The famous mice experiment conducted by Frederick Griffith in 1928 Using Diplodocus pneumoniae (Pneumonia causing bacteria) provided preliminary evidence to support the contention that DNA is the genetic material.

He conducted the famous mice experiment (a test system used variously in genetic research). Two strains of Bacteria were used as test system:

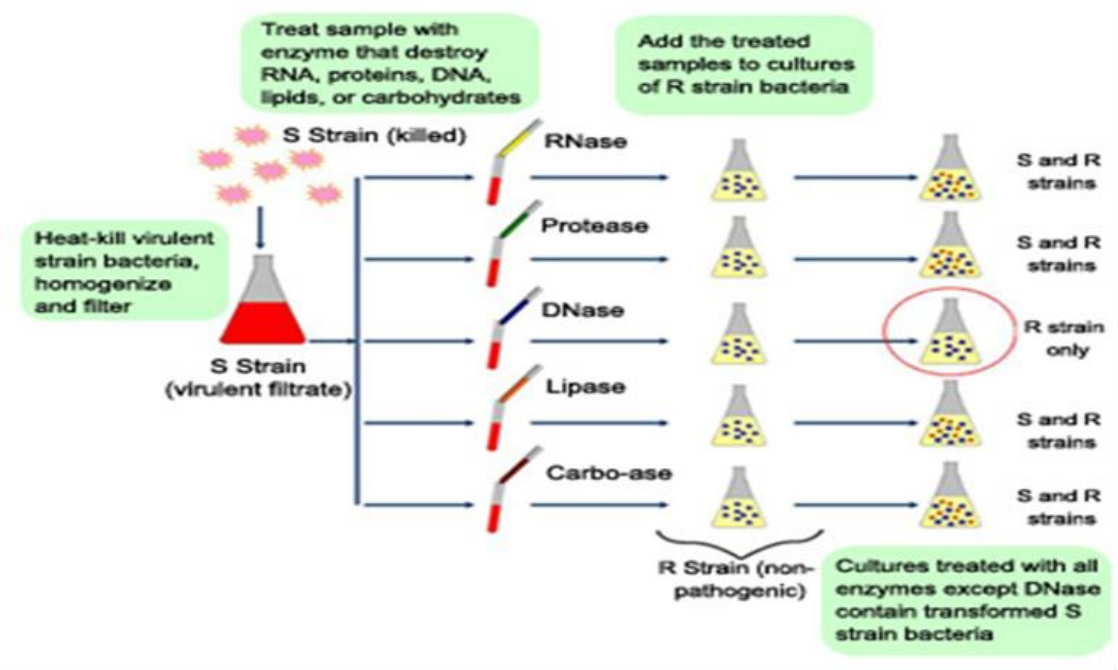
1. A virulent strain (pathogenic) forming smooth colonies on agar plate (S)
2. An a virulent strain (Non-pathogenic) forming rough colonies on agar plate (R)

When S type was injected into mice it caused death of mice, but no death occurred when R was injected. A mixture of heat killed S and live R could also kill the mice. But when heat killed S and live R were injected individually is caused no death to the mice: On examining the blood sample of mice injected with heat killed S and live R, the blood sample revealed the presence of virulent S form. Griffith could not explain the real cause for such happening and so,

concluded that something came out from the dead bacterium S (Virulent) during heating which changed R (Non virulent) to S type (Virulent). This something was known as transfer factor. Griffith could not explain the chemical nature of transfer factor, which was later on, identified by Oswald Avery, Collin Macleod and Maclyn Mcarty as DNA (1944).

Experiment of Avery, Macleod and Mcarty

Avery and his co-workers partially purified the transfer factor (Transforming principle) form the cell extract of heat killed S-type of bacteria. This transfer factor was mixed with live R-type. Enzyme treatment from Proteolytic enzyme, ribonuclease, and Lipase has no effect on transforming activity, means as a result of mixing of heat killed S-type and live R-type, no, damage to the transforming principle was observed. However, when the sample (mixture) was treated with DNA are enzyme, the transforming principle was in activated. Later on, such transforming principle was identified in several other bacteria. Chemical analysis of the transforming substance revealed that the ratio of nitrogen versus phosphorus was 1:67:1 which is not found in protein but is found in DNA. In January of 1944 studies of chemical nature of the substance inducing Transforming of Pneumococcal types was published, for which they got Nobel prize.



So, by conducting this experiment Avery and his colleague proved that DNA is the genetic material in bacteria and also there is a method of horizontal gene transfer in bacteria called as Transformation.

Universality that DNA is the genetic material in the entire living organism was not proved by this experiment. Alfred Hershey and Martha Chase in 1952 demonstrated that DNA is the genetic material in Bacteriophage.

Blender's Experiment (Experiment of Hershey and Chase)

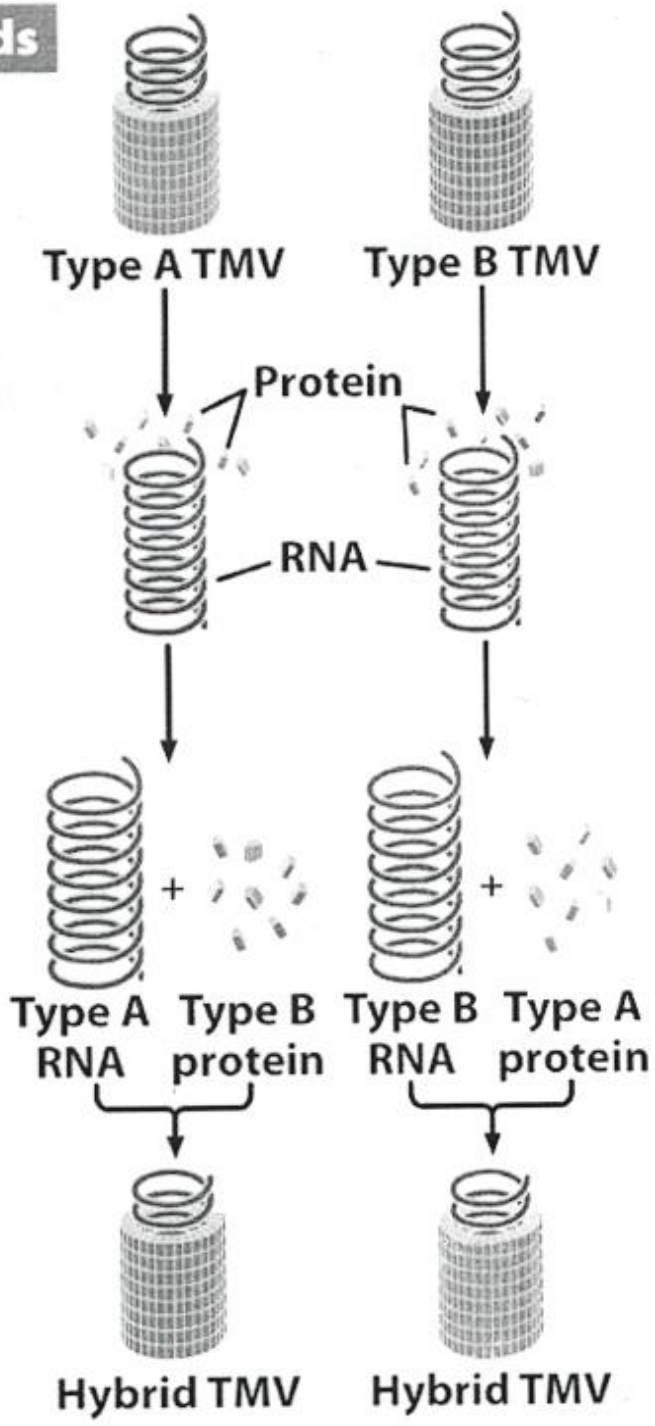
Phage T2 which spends its life in the host *E. coli* was used as the test material. The phage particle T2 has a protein coat which is DNA fibrils. Phosphorus is the chemical component of the DNA hence this forms the DNA of the bacteriophage. Similarly sulphur is the component of the viral protein. Because Sulphur is the element which makes, cysteine, homocysteine, and taurine and methionine, which contributes is mainly Viral protein. With this knowledge, Hershey and Chase cultivated *E.coli* in two sets, one containing radioactive Phosphorus (32p) and other containing radioactive sulfur (35s) with the sole intention to create *E.coli* with 32p and 35s. *E.coli* was grown in two separate flasks with these radioactive 32p and 35s. After certain stage of growth of *E.coli*, the flask was inoculated with T2 phage, allowed to grow for multiplication within the host. After certain period of growth the content of the flask was agitated in a Kitchen waring blender, hence the name, blender experiment was used. The sample collected after the agitation in the blender was collected in centrifuge tubes, for centrifugation at low rpm (to loosen the adhering particle phage). As a result of centrifugation both the samples exhibited different results.

In tube one the radioactivity was noticed in the pellet (broken bacterial cell containing 32p DNA). In another tube radioactivity was noticed in the supernatant (the detached protein coat containing 32s Protein). So, this experiment indicated that during infection it is the DNA that actually entered the bacteria.

Experimental proof to prove that RNA is the genetic material

H. Franenkel conrat and B. Singer in 1957 carried out experiment to prove that RNA is the genetic material is TMV. The workers selected two strains of TMV namely strain A and strain B. RNA molecule and the protein fraction of the TMV were carefully separated. These two separated samples were used to infect a healthy plan, it was noticed that only RNA fraction has the ability to transfer infection. In strengthen this contention they reunited the RNA of A with protein of strain B and Vice-versa. The two reconstituted viral particles caused infection on the healthy tobacco plant. So, this was proved that RNA alone has the capacity to act as genetic material.

Methods


Type A TMV
Type B TMV
Protein
RNA
+
+
Type A RNA
Type B protein
Type B RNA
Type A protein
Hybrid TMV
Hybrid TMV

Topic-2

Chemical Nature of DNA and RNA

The concept of Nucleic acid started during 1869 when Friedrick Miescher isolated a new molecule from the pus cells and called it as Nuclein. Miescher determined that chemically is made up of hydrogen, oxygen, nitrogen and phosporus. Miescher could also report an unique ratio of phosphorus of nitrogen in the Nuclein. In his further attempt Nuclein was also found in Salmon sperm. In 1889 Richard Altman discovered that Nuclein have acidic properties and hence the name was changed to Nucleic acid. In 1891 Albert Kossel discovered that Nuclein is made up of four bases and sugar molecule for which he got noble prize in medicine. In 1897 Eduard Zachans reported that Nuclein in an integral part of chromosome and his (1897) proposed the concept of chromatin with the chemical substance as Nuclein. Until 1940-1950, Nuclein as considered to be protein, until Avery et al (1944) and Hershey and chase (1952) proved that DNA is the genetic material in Escherichia coli (Bacteria) and T2 virus (A Bacteriophage of E.Coli).

Basic Structure of DNA and RNA

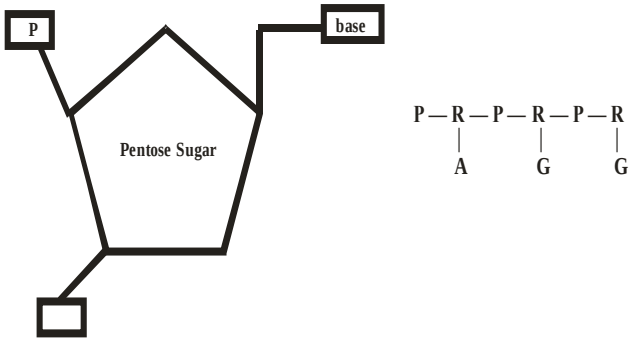
The basic structure of DNA and the RNA is the same and has the following structural configuration.

- Thread like in structure
- Made up of long chain of Polynucleotide.
- Each nucleotide consists of a nitrogen containing aromatic base attached to a pentose (five carbon) sugar, which in turn attached to a phosphate group.
- Each Nucleic acid contains four of five nitrogen base such as:

1. Adenine (A), Guanine (G), Cytosine (C) and Thymine (T) – in DNA.
2. Adenine (A), Guanine (G), Cytosine (C) and Uracil (U) – in RNA.

- A and G are categorized as purines and C, T and U are collectively categorized as Pyrimidines.
- A, T, C, G are common for DNA A, U, C, G makes the RNA.
- The Pentose sugar of DNA differs from pentose sugar (Ribose) by the absence of a hydroxyl group (–OH) from carbon position two and hence the name deoxyribose.

• The Phosphate group connects to the sugar group by 5` – hydroxyl group (known as 5` prime end) and the 3` – hydroxyl group (known as 3` prime end), by two ester bonds called as phospho-di-ester bond. This phosho-di-ester bond is common for both DNA and RNA. The arrangement plan has been shown in Fig……



Two successive bonds appear from the phosphate from 5` hydroxyl group of sugar and 3`– hydroxyl group of sugar. Henceforth named as 5` prime end and 3` prime end.

Topic-3

DNA and Chargaff's rule

A very and his associates proved that DNA is the genetic material. This opened up new scope of research in DNA biology. Several DNA samples were collected and char gaff and his associates proceeded to separate DNA. It was primarily hydrolyzed with acid, which would break the phosphodiester bonds to separate the components of DNA. The technique of paper chromatography and UV- spectrophotometer was used on Nitrogenous bases. During this it was observed that the prime bases (Adenine and Guanine) and pyrimidine bases (cytosine and Thynine) are not in equal amounts.

Chargaff further observed that GC equals to the concentration of AT. However, in RNA, Thymine is replaced with Uracil. Chargaff observed that the amount of certain bases were equal to each other. The base Guanine was equivalent to the amount of cytosine and the same held true for Adenine and Thymine. This means:

Total Amount of Purine = Total Amount of Pyridines

$A + G = T + G$

The ratio of $A : T = 1$

The ratio of $G : C = 1$

A is parried to thymine by 2 hydroxyl bonds and C to G by 3 hydrogen bonds.

Chargaff's conclusion of the significance of A, T, C and G, revolutionized the biological sciences. This helped James D. Watson and Francis crick in determination of structure of DNA. The idea that Adnine and Thymine always exist in the same proportion, they must remain bonded, and the same is true for cytosine and Guanine. So, a proposal that DNA is a double helical structure was established.

Topic- 4

Three-dimensional Structure of DNA

In 1953, Watson and Crick, on the basis of data collected from X-ray crystallography and chargaff's observation proposed a structure of DNA. Watson and Crick proposed that in DNA.

- In DNA two stands of polynucleotide coil around each other, forming a double helix.
- The two strands run in opposite directions due to their orientation of the 5` and 3` poshodiester bonds.
- The phosphate sugar association runs along the outside of the strands and the bases remain on the inside.
- The Nitrogenous bases remain bonded by hydrogen bonds, which forms a stable association.
- The normal DNA is called the B–DNA. This rotates along the axis in a right handed manner. The helix which is twined around each other takes a turn approximately at 10 base pairs.
- B-DNA has two principal grooves, a wide groove called major groove and a narrow groove called as minor groove.

A configurational change occurs in the structure of DNA, the data which could be collected from X-ray diffraction of the crystalline forms of DNA. The isolated DNA was converted into crystalline form for X-ray diffraction. Before conducting crystallography, the sample was hydrated and diffraction pattern was observed. The yielded two forms of pattern named B-form and elongated microcrystals (in more drier samples) as A form. Continued analysis of fibre pattern of B and A form could reveal the following facts:

- That is B-form, the second carbon molecule (C2) of ribose sugar was out of plane. This could in influence the rotation of the helix around the main axis of DNA. This also creates a distance of 7A0 between two phosphate group located on 3` and 5` end.
- That in A DNA the third carbon molecule (C3) was found to be out of plane. This resulted into shortening of the sugar-phosphate back-bone. This also resulted into displacement of base pair and hence a wider helix was found. This distance between the two adjacent phosphate group is also reduced to 5.8 – 6A0.

X-ray diffraction technique for analyzing the structure of DNA remained as the only means to determine the structure of form of B-DNA and A-DNA. The chemical methods for synthesizing obgonucteolides became successful. Ris and Boom undertook a collaborative study to synthesize oligonucleodite using G – C base pairs (being more stable). A CGCGCG sequence

of oligonucleotide was created a duplex DNA was created using this sequence which an unusual form was found which revealed.

- Left hander form of the double-helix.
- Two ant parallel chains held together A–T and G–C base pairs; but different from B–DNA.
- It is not stable and difficult to study.
- It was elongated and thinner molecule, that had only one groove.
- This DNA was name as Z–DNA or Zigzag DNA.
- In B–DNA and A–DNA the associated substituent (atoms or group of atoms) are located in opposite directions. Whereas, in Z–DNA the substituents are located in the same direction resulting into thinning of the helix.
- It has sugar in C3 conformation (like A DNA) and Guanine base in same conformation close to each other) and hence different from A and B form.
- Duplex in Z–DNA has to accommodate the distortion of nucleotide G in the same conformation.
- The cytosine in the adjacent nucleotide of Z DNA is in the C-2 endo anti conformation.

Genome Organisation in Living Systems

Naturally occurring DNA may be circular or linear in forms. It organization in different living system differs. Such as described below:

S N	Biological system	Genome organization
1	a. Bacteria and Archaebacteria (Prokaryotes) b. Mitochondria (Eukaryotes) c. Chloroplast (Eukaryotes) d. Plasmid (Bacteria small circular DNA (Extra-chromosomal DNA) Circular DNA (also called as Ring-Chromosome)	Circular DNA (also called as Ring-Chromosome
2	Eukaryotes	Liner Chromosome

Organization in Eukaryote

In Eukaryotic cell, the DNA, lies bounded by a protein coat called chromatin. The chromatin contains proteins, which determines expression of genes and also characteristic of the chromosome. The DNA which is negatively charged has a natural attraction for a positively charged protein, which helps the DNA to create a genetically more stable and compact structure called as chromosome. The nitrogenous bases of DNA, nearly 140 and 200 base pairs long winds around a discrete set of eight positively charged proteins called a histone forming a spherical structure called the Nucleosome. Additional histones and length base pairs (140 to 200) helps in creating a series of Nucleosomes like a beads on a string. This structure is now called as Solenoid. Various Solenoids get packed into to form a rod shaped chromosome. During replication and also during transcription and translation the eukaryotic genome has to be partially disassembled for the process.

Biochemical Properties of DNA

Besides being the genetic material DNA has certain other roles to place, such as

Denaturation: During adoption of various biotechnological principles, denaturation or melting of DNA is required. The principle of DNA denaturation is adopted during hybridization of DNA with complementary strands of DNA, a process called as renaturation. This practice is often used during manipulation of DNA. In denaturation, the DNA is heated in a solution to break the hydrogen bond, and cooled again for renaturation. The thermal denaturation profile of DNA is often used for establishing a genetic homology between two genetically unrelated species. It also helps in determining A, T rich and G, C rich biological systems, because G, C rich system is often considered more advanced.

Methylation

DN A molecules are prone to be affected during oxidation, ionizing radiation and also by carcinogens. Methylation can occur at two of the nucleotides, cytosine and adenine, when carbon molecule is replaced with CH3 molecule. This change leads to epigenetic change (not of heritable nature but acquired due to influence of environment). This change leads to perform various biological activity was hitherto, has not been carried by heitable genes. The methylation leads to modify the function of DNA. When present it acts to repress native gene of heritable change. DNA methyl transferase is the enzyme which performs this function. Methylation is the process which is concerned with reprogramming of gene regulation. Two of the DNA's four nucleotides, cytosine and adenine can be methylated. This rate of change differs in different plant and animal species. DNA methylation also forms the basis of chromatin structure which enables to single cell to grow into multiple organs or perform multiple functions. DNA methylation also plays important role in cancer development, cardio-vascular disease (antherosclerosis) and in aging. Methylation and demethylation occurs frequently in living system, which leads to abnormal activity of the cells.

Mutation

Chemical modification of DNA can lead to mutations in the genetic material. Cytosine can change to uracil which changes the genetic message (confined to Genetic Code). Many chemical and physical mutagens are known which can change the chemistry of genes and thus bringing about heritable changes.

Chemistry of Coiling and Super Coiling of DNA

The human DNA can stretch to three metres if it is unfolded from the 23 pairs of chromosome. It has to be packed into a nucleus which is 10 micro-meters in diameter. The enzyme gyrases and topo isomerases catalyze the winding and relaxation of DNA. Holding the long stretch of DNA, both in Prokaryotes and in Eukaryotes, is the function of these two enzymes present closely associated to DNA molecules.

Topic- 5

Replication of DNA

Continuity of life is based upon the principle of transfer of genetic material laden with genetic information to the next generation. So, a parental cell before entering into next generation must pass on its genetic information to a newly synthesized genetic material. This is an important event in the life of a cell, which is represented by S phase (synthesis phase) in the life of a cell. A cell can produce two daughter cells before undergoing cell division. During S phase the duplication of DNA takes place when a diploid cell (2N) becomes tetraploid (4N). This tetraploid cell, soon, get divided into two 2N (Diploid cells). The first question raised was that the genetic information to the newly synthesized DNA can be either semi conservative (one strand from the parent) or conservative (both the strands from the parent). Three models were also proposed to explain the replication of DNA as:

1. Semi conservative replication: One strand is new, while the other is parental.

2. Conservative replication: Both the strands are of parental origin.

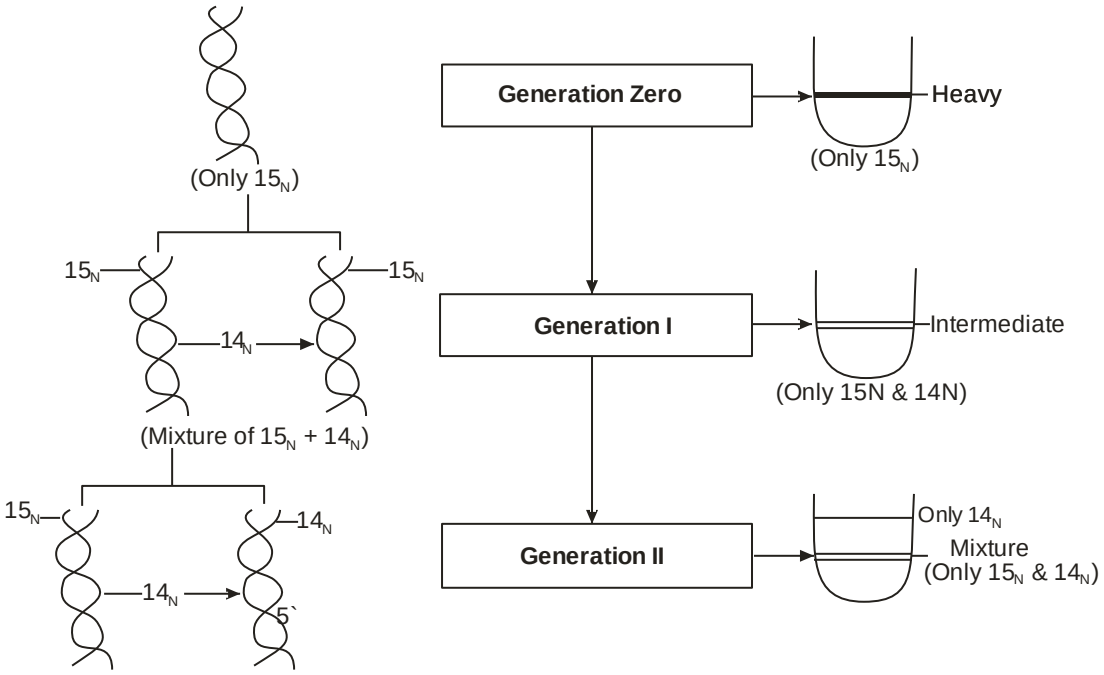
3. Dispersive: Both the strands of DNA acquires fragments of parental DNA as well as newly synthesized DNA.

Evidence to support conservative mode of replication and dispersive mode of replication could not be obtained, but evidence to support semi-conservative mode of replication could be obtained by the classical experiment of Meselson and Statil.

The Experiment

M. Meselon and F.W. Stathl (1958) differentiated parental and newly formed DNA, by incorporating normal isotope of Nitrogen (14N) or heavy isotope (15N). During metabolism nitrogen gets accumulated in the DNA. Culture media (for growth of bacteria) was supplemented with 14N and 15N for growth and development.

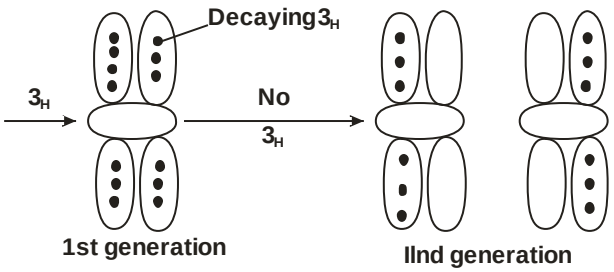
E. Coli was cultivated in culture media containing 15N as nitrogen source for several generations. DNA was extracted and centrifuged by density gradient centrifugation (separates molecules by its density) to obtain a heavy band at the bottom of the centrifuge tube (named as generation zero). Sample was withdrawn form the 15N growing culture and transferred to culture media which contained 14N source of nitrogen. DNA was again isolated and centrifuged to obtain a band in the centrifuge tube which also shifted its position (slightly raised) and hence called as Intermediate band (named as generation I). Sample withdrawn from 14N culture media was sub-cultured on 14N media a fresh. DNA isolated from *E. coli* cells demonstrated two tubes of band (named as generation II) as light band (upper most) and intermediate band. Interpretation of Meselson and Stahl report can be shown by Figure given below



The experiment conducted by Meselson and Stahl proved that Replication of DNA is semi conservative in *E.coli*. This raised doubt regarding the mode of replication of DNA in Eukaryotes.

Replication of Chromosomes in Eukaryotes

J.H. Taylor (1958) demonstrated the semi-conservative mode of replication in DNA and hence in the chromosomes. Root tip of Vicia faba. Member of family Fabaceae was used as experimental material. The technique of Antoradiography was used where radioactive material is used in biological system. Root tips were first fed with radioactive thymidrine 3H (half life few months and then it decays). This treatment was marked as first generation. After this generation root tip (another set) was transferred to colchicine (to induce cell division) marked as IInd generation. Slide was prepared after every generation, and left as such at low temperature to all 3H to decay. On the basis of observation made in the slide semi-conservative mode of DNA (chromosome) was established as shown in Figure given below.

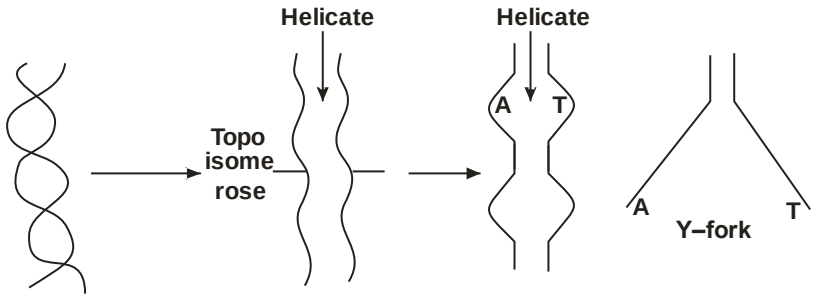


Both the chromatids in 1st generation have black dots (leaving dots of 3H during decay) but in generation II, one chromatid returned to parental type, while others have black dots. This proved that during replication of chromosome, one chromosome is old while other is fresh (new). This justifies the semi-conservative mode.

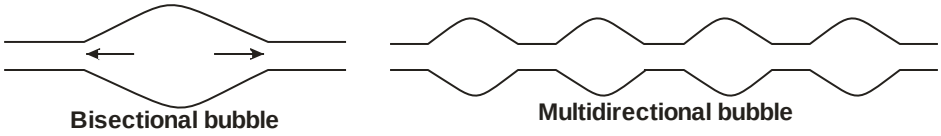
Mechanism of Replication of DNA

Replication occurs at specific times during the cell cycle. This requires soft of protein, enzymes and energy in the form of ATP. The Replication begins with initiation and ends in elongation. The initiation begins with separation of weak hydrogen bonds between the Nitrogenous bases. These two strands of DNA have nitrogenous bases complementary to each other (A to T and C to G). The separation of two strands is difficult due to their ant parallel nature and torsion generated by complementary strands. The point from where unwinding of DNA begins is called as initiation point. In viruses and also in bacteria the replicating unit in only one hence origin of replication initiates at only one point. IN Eukaryotes with large DNA molecule, there may be more than one initiation point, and each initiation point finally merge with one another.

Unwinding of the double helical ant parallel DNA is the first condition of replication. The unwinding of the DNA takes place by break down of hydrogen bonds with the help of enzyme helicases. To minimize the torsion effect of DNA helix, the enzyme Topoisomerases cut and rejoins the DNA strand to relax to torsion effect, leading to separation of DNA strand. Due to this upzipping of DNA strands replication bubbles are formed which subsequently extends as Y–shaped replication fork.



The formation of replication bubble and Y-fork in bacteria and phages is bidirectional but in eukaryote it multidirectional (Fig…….)

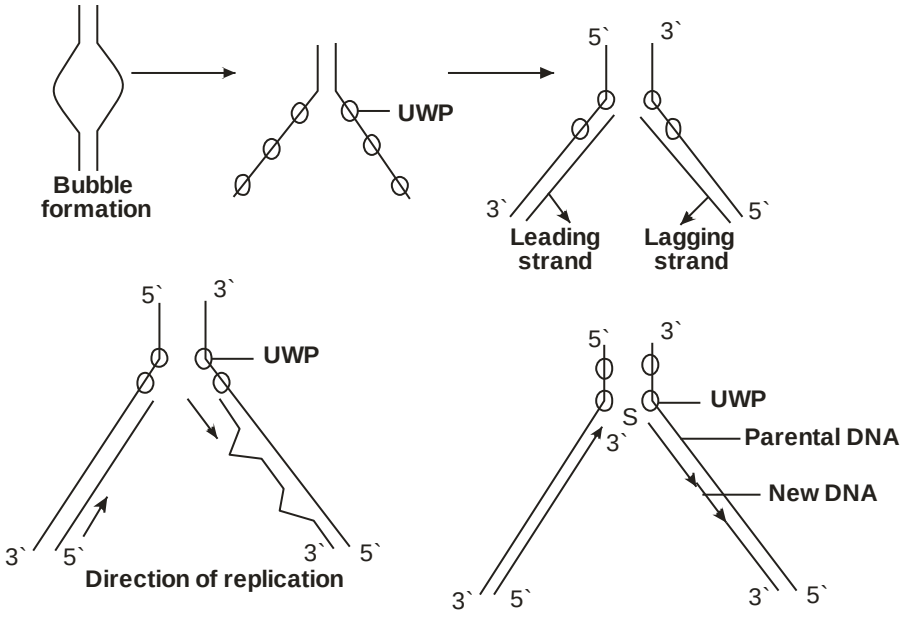


After the formation of replication fork, the unwinding protein called single strand binding protein (SSB protein) keeps the separated strand apart.

DNA directed RNA polymerase forms the RNA primer. This RNA primer forms hetero duplex structure (DNA–RNA) for sometimes. The RNA is ultimately removed enzymatically and the gap is filled up with newly synthesized nucleotides. The enzyme DNA polymerase polymerises the nucleotides only 5`–3` direction. Because the two strands of DNA are in antiparallel direction, the two strands have to be synthesized by growing in opposite direction.

The enzyme synthesizes a new strand in a continuous pieces 5`–3` direction and is called leading strand on the other strand of DNA, the enzyme forms DNA fragments in small pieces again 5`–3` direction called as Okazaki fragments.

The Okazaki fragment soon joins together with the help of enzyme DNA ligase to be called as lagging strand. The enzyme DNA primase helps in addition of new strand to the DNA template whereas enzyme exonuclease (a type of DNA polymerase) helps in removal of RNA primer. The process of DNA replication has been summarized in Figure given below



During replication of DNA, wrong base may get incorporated, which must be removed by another set of enzymes called as DNA polymerases. This enzyme removes the wrong base and performs editing. The enzyme nuclease (a type of DNA polymerase) cuts off the wrong base and replaces it with the correct one.

Topic- 6

Chemical Nature and Types of Ribonucleic Acid (RNA)

The nucleic acid in the cell has two important functions to perform. DNA is responsible for inheritance and its transfer to next generation; RNA is concerned with carrying out metabolic function. DNA can remain present in the cell, since birth. RNA has to be synthesized by DNA to carry out metabolic function. A sustainable life, appears to be interaction of DNA and RNA.

It is often postulated that life began as RNA molecule, concept becomes evident as some of the viruses (Influenza virus, foot and mouth virus, Rous sarcoma virus, Reovirus and Bacteriophage, Tobacco mosaic virus) contain RNA as genetic material.

Thus the RNA can be

1. Genetic
2. Non-Genetic

1. The Genetic RNA

The basic structure of RNA

Every RNA has two aspect of structure and function. The structural aspect is concerned with its unique assemblage of molecules to construct it, while the functional aspect deals with the modification of structure to perform an assigned job.

The RNA is a single stranded nucleic acid made up of four nucleotides; A, C, G and U joined together with a back alternating sequence of Phosphate and ribose sugar. It shows resemblance to DNA molecule so far as union of the three molecules is concerned. The Pentose sugar of RNA is Ribose sugar and the nitrogenous base Thymine of DNA is replaced by Uracil. It appears that functional DNA acquires the structure of RNA when functional aspect has to deal in the cell. By doing so the DNA (heritable material of cell) conserves itself. RNA is concerned with performing various metabolic functions of the cells, and hence acquires various forms and shapes to encounter various types of enzymes (acting as catalytic agent).

Types of RNA

The Non-genetic role of RNA are many, and the role with RNA has to play originates form the DNA. So, RNA is made by DNA by a process called as Transcription. The three RNA formed are

a. Messenger RNA (m RNA)

b. Ribosomal RNA (r RNA)

c. Transfer RNA (t RNA)

In order to remain functional, the cell, with the interaction of DNA and RNA has to perform much metabolic function for growth and development. Various kinds of proteins has to be made to perform this function. The DNA transfers knowledge for synthesis of proteins and the three RNA molecules helps in effective performance of this activity. The three RNA molecules are assigned with three separate jobs, such as:

- The mRNA carries the information from the DNA.
- The tRNA is concerned with supply of amino acids and
- The rRNA serves as a factory for aligment of different amino acids for the synthesis of a specific protein.

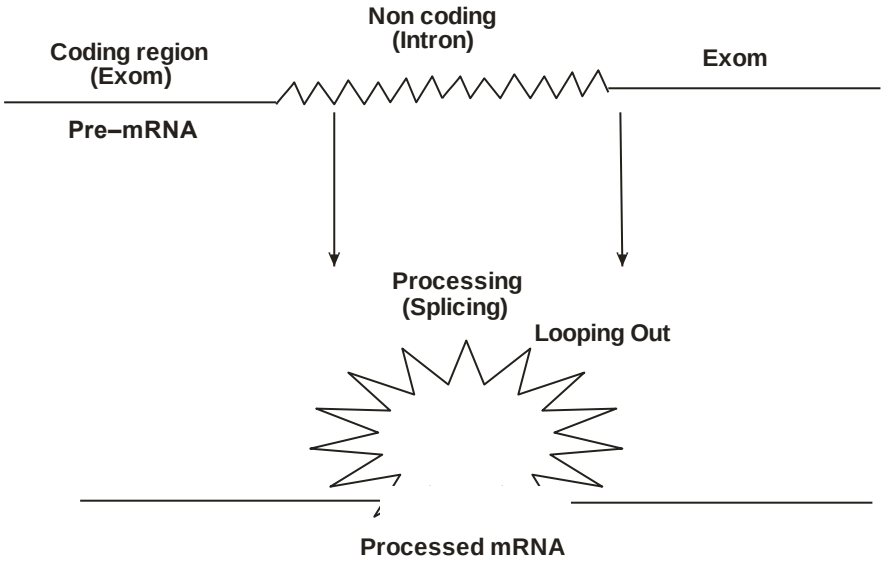
Messenger RNA (mRNA)

Messenger RNA is synthesized by DNA when metabolic activity of the cell begins. This RNA acquires information form DNA, in a coded language, regarding biochemical activity of the cell. This information can later on decode into a required protein. In Prokaryotes, m RNA's contain an exact transcribed copy of the DNA. This contains a terminal 5` end and a 3` end. In eukaryotes the terminal 5` end is further esterifies to form a cap. An additional segment of long adenosine residues (Poly A) are added enzymatic ally at the 3` end. This Poly A sequence is not encoded in the DNA. Due to cap and Poly A sequences, the eukaryotic RNA becomes more stable, the eukaryotic RNA becomes more stable, whereas, the prokaryotic mRNA is very short lived.

Processing of mRNA

Eukaryotic DNA is represented by functional DNA (required for the cell) called exon or coding sequence and intron (carried over DNA of the ancestors) called non-coding sequence.

The mRNA synthesized by this DNA, first forms a Pre-mRNA. This Pre-mRNA is processed during mRNA maturation, by a process called as splicing. The splicing is performed by group of enzymes called spliceosome. This splicing adopts the principle of cleavage (cutting of mRNA) and rejoining. This way the Pre-mRNA is processed into original mRNA, corresponding to the functional genes of the system..



rRNA

Mature rRNAs make up to 50-60 to of each ribosome. Some of the rRNA are purely structural, whereas others have catalytic activities to play. The eukaryotic ribosomes is composed of two sub units: a large sub-unit (60 S) and a small sub-unit (40S). The 60 S submit is composed of the 28 S rRNA, 5.8S rRNA, 5S rRNA and 50 proteins. The 40 S sub-units is composed of the 18S rRNA and 30 proteins. The bacterial (Prokaryotic) ribosome is composed of two similar sub units, with slightly different components. The bacterial large sub unit is called the 50 S sub-unit and is composed of the 23 S rRNA, 5 S rRNA and 31 proteins, while the bacterial small sub-unit is called 30S sub-unit and is composed of the 16S rRNA and 21 proteins.

The sub-units join to constitute the functional ribosome.

Processing of rRNA

In prokaryotes, which lack a nucleus, few rRNA genes helps synthesis of 50S, 30s sub-units as well as the protein sub-units. After synthesis of these two components of the ribosome, they unite to form the small and the large sub-units of functional nature. However, in Eukaryotes, the 28S, 5.8S, and 18S are synthesized in the Nucleolus (within the nucleus) while the protein (50) in synthesized in the cytoplasm. This protein synthesized in cyctoplasm is then transported to the nucleus for sub-assembly (large and small sub-units) in the nucleolus. After processing these sub-units are returned to the cytoplasm for final assembly.

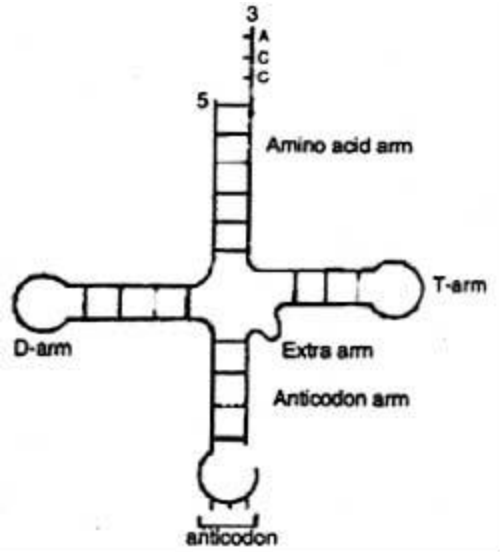
Role of 16S rRNA

A bacterial DNA sequence which is most amenable to change is that which enclodes for 16S rRNA. This 16S RNA gene is present is all bacteria, and also in eukaryotes. Analysis of the 16S rRNA sequences from many organisms has revealed that some portions of the molecule undergo rapid genetic changes, this creates a background for distinguishing different species within the genus, other positions of gene change very slowly, and this facilitates various levels of taxonomic significance, and hence used in modern classification.

Structure of tRNA

tRNA is a small type of tRNA with a size around 4S, consisting of less than 70-80 nucleotides. There are 40 to 50 known types, which represents about 5% of overall molecules. Transfer RNA carries individual amino acids into the ribosome for assembly of amino acids to make protein. Specialized tRNA exists for each of the 20 amino acids needed for synthesis of protein. Sometimes more than one tRNA for each amino acid is present for example a total of 40 different t RNAS are used to translate 61codons provided by the t RNA. Hence, t RNA has unique property to change its nucleotide sequence to accommodate all the 61 codons. The union of amino acid with tRNA is mediated by specialized enzymes called aminoacyl tRNA synthetases, usually one synthetase for each amino acid.

Each tRNA binds to a specific amino acid and transfers it to the ribosome. Mature to RNA made up of 70-80 nucleotides acquires a three dimensional form in a such a way that the position of the amino acid binding site is at one end and the anticodon an unpaired loop of nucleotides at the other. The anticodon is a three nucleotide sequence, unique to each different tRNA, which interacts with a mRNA by forming a complementary base pair.



Processing of tRNA

tRNA molecules are synthesized in the cell (synthesized in nucleus and transported to cytoplasm in Eukaryotes but in the cytoplasm in Prokaryotes) are Pre-tRNA molecule. This Pre-tRNA requires multiple processing steps before the mature t-RNA is formed for effective translation. This processing is less frequent in Prokaryote as Pre-tRNA is transcribed as a single RNA moiety. The first step involves digestion of the RNA to release individual Pre tRNAS. The process by which Pre-tRNA get converted to mature tRNA involves following steps.

1. The 5` end of Pre-tRNA, called the 5` leader sequence, is cleaved off.

2. The 3` end of the Pre-tRNA is cleaved off .

3. In Eukaryotes and many of the bacterial system, a sequence of CCA nucleotide is added to the 3` end. This CCA sequence at the mature tRNA is the site at which the amino acids are added.

4. Many nucleotides in the Pre-tRNA are chemically modified by altering their nitrogen bases. About 12 nucleotides are modified, as a result of which adenine (A) is modified to Pseudouridine adenine is also modified to inosine (1). In the same way Uridine may modified to dihydrouridine.

5. In eukaryotes the pre-tRNAS have introns (non-coding regions) which get spliced out during processing.

The mature of tRNA with the help of enzyme, aminoacyl tRNA gets attached to the specific amino acid, henceforth called as charging of tRNA. All tRNAs acquire the same structural configuration because they have to interact with ribosome on the same site.

Some Other Forms of RNA

Ribozyme

In 1989, Thomas cech and Sidney Altman shared the Nobel prize, for discovery of certain RNA which salely acts as enzyme, called as Ribozymes. The enzymes acitivity of Ribosome was notices in Protozoans, when to make the rRNA functional, some part of the rRNA (non-coding sequences or intron) could be removed itself from the rRNA molecule. This class of enzyme was named as Ribozyme due to its catalytic function which other-wise is the property of enzyme (proteins).

Antisense RNAS

In eukaryotes, the DNA synthesizes RNA having condons for protein synthesis. These condons are ultimately translated into respective proteins and hence named as sense condons. Sometimes the modified derivatives of RNA or DNA forms a sequence which is complementary to mRNA which blocks the synthesis of translated protein, and hence called as Antisense RNA (Opposite to RNA). The antisense RNA can block the synthesis of translation of protein by forming a double-stranded structure. Antisense RNA can be synthesized artificially and can be introduced in the cell to block the functioning of disease prone mRNA sequence. This RNA has thus great therapeutic value.

Topic- 7

Cell cycle

The cell cycle is an ordered series of events involving cell growth and cell division.The cell cycle has two major phases

1 The interphase and

2. the Mitotic phase.

During interphase the cell prepares itself by accumulating nutrient and raw material for mitotic phase, During this stage which is divided into G_1, S and G_2

G_1 Phase

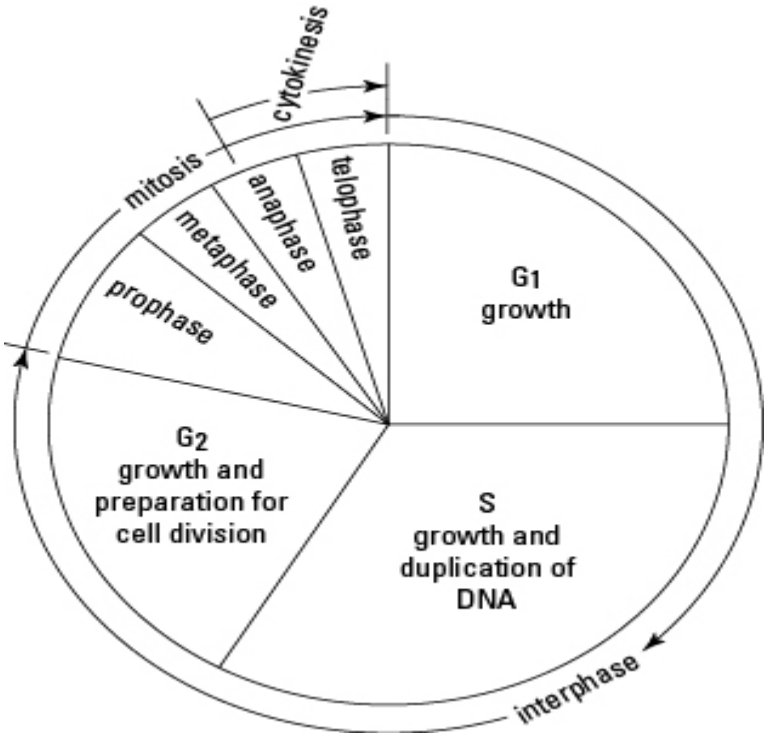
During this phase little change is visible although the cell is biochemically very active. The cell during this stage remains busy accumulating building blocks for new DNA molecule besides conserving the existence of native parental DNA. Associated proteins for such action are also synthesized. Enough energy sources is required for DNA replication for the cell conserves during this phase.

S phase

S phase is the period of wholesale DNA synthesis during which the cell replicates its genetic content; a normal diploid somatic cell with a 2N complement of DNA at the beginning of S phase acquires a 4N complement of DNA at its end. (Recall that N = 1 copy of each chromosome per cell [haploid]; 2N = 2 copies [diploid].) The duration of S phase may vary from only a few minutes in rapidly dividing, early embryo cells to a few hours in most somatic cells. Early embryo cells generally "live off" the accumulated stores of maternal RNA and proteins present in the egg and are transcriptionally silent, whereas cells in later development and mature organisms must actively transcribe subsets of their genes to survive and maintain specialized functions. The longer time required for the latter to complete S phase probably allows these cells to coordinate DNA replication with transcription and to preserve higher-order gene and chromatin structural information that influences gene expression for transmission to progeny cells.

G_2 phase

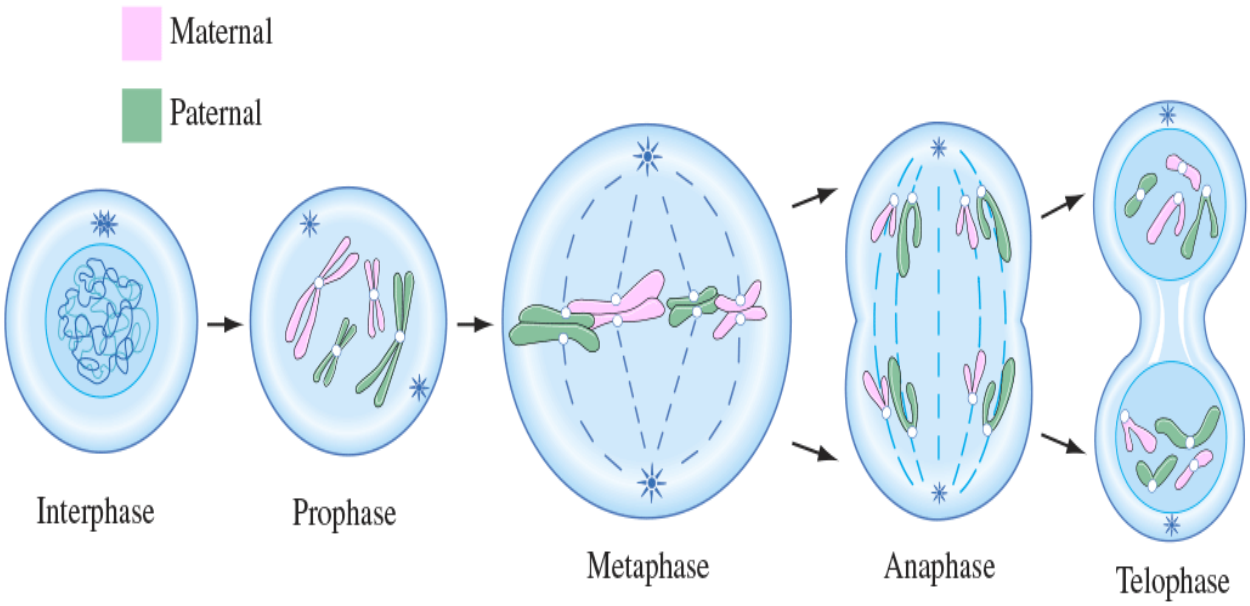
During this stage the cell stores energy and synthesizes proteins required for manipulation of Chromosomes. G2 phase and the beginning of mitosis are denoted by a 4-N DNA content. Following DNA replication and prior to cell division (cytokinesis), cells must maintain the integrity and proximity of the recently duplicated chromosomes (sister chromatids).



Different stages of Cell Cycle

Topic- 8

Mitosis



Detail can be studied from the provided lesson material

Topic- 9

Meiosis

Sexual reproduction in organisms takes place through the fusion of male and female gametes, the sperm and the egg respectively. Gametes are haploid in nature, i.e., they contain only half the number of chromosomes. This genetic content makes them different from other body cells. Meiosis leads to the formation of haploid cells.

Meiosis 1

Mitotic cell division is equational in nature while meiosis is a reduction division. The salient features of meiotic division that make it different from mitosis are as follows:-

It occurs in two stages of the nuclear and cellular division as Meiosis I and Meiosis II. DNA replication occurs, however, only once. It involves the pairing of homologous chromosomes and recombination between them. Four haploid daughter cells are produced at the end, unlike two diploid daughter cells in mitosis.Meiosis 1 separates the pair of homologous chromosomes and reduces the diploid cell to haploid. It is divided into several stages that include, prophase, metaphase, anaphase and telophase.

Meiosis

Meiosis 1 Stages

The different stages of meiosis 1 can be explained by the following phases :

Prophase 1

Metaphase 1

Anaphase 1

Telophase

Phases of Meiosis 1

Meiosis 1 **Prophase 1**

Prophase I is longer than the mitotic prophase and is further subdivided into 5 substages,

leptotene

zygotene

pachytene

diplotene

diakinesis

The chromosomes begin to condense and attain a compact structure during leptotene.

In zygotene, the pairing of homologous chromosomes starts a process known as chromosomal synapsis, accompanied by the formation of a complex structure called synaptonemal complex. A pair of synapsed homologous chromosome forms a complex known as bivalent or tetrad.

At pachytene stage, crossing over of non-sister chromatids of homologous chromosomes occurs at the recombination nodules. The chromosomes remain linked at the sites of crossing over.

Diplotene marks the dissolution of the synaptonemal complex and separation of the homologous chromosomes of the bivalents except at the sites of cross-over. The X-shaped structures formed during separation are known as chiasmata.

Diakinesis is marked by the termination of chiasmata and assembly of the meiotic spindle to separate the homologous chromosomes. The nucleolus disappears and the nuclear envelope breaks down.

Meiosis 1 **Metaphase 1**

The bivalents align at the equatorial plate and microtubules from the opposite poles attach to the pairs of homologous chromosomes.

Meiosis 1 **Anaphase 1**

The two chromosomes of each bivalent separate and move to the opposite ends of the cells. The sister chromatids are attached to each other.

Meiosis 1 **Telophase 1**

The nuclear membrane reappears and is followed by cytokinesis. This gives rise to a dyad of cells.

Phases of Meiosis II

During prophase II, chromosomes condense and the nuclear envelope breaks down, if needed. The centrosomes move apart, the spindle forms between them, and the spindle microtubules begin to capture chromosomes. The two sister chromatids of each chromosome are captured by microtubules from opposite spindle poles.

In metaphase II, the chromosomes line up individually along the metaphase plate.

In anaphase II, the sister chromatids separate and are pulled towards opposite poles of the cell.

In telophase II, nuclear membranes form around each set of chromosomes, and the chromosomes decondense.

Cytokinesis splits the chromosome sets into new cells, forming the final products of meiosis: four haploid cells in which each chromosome has just one chromatid. In humans, the products of meiosis are sperm or egg cells.

Necessary diagrams can be obtained from the provided lesson

Topic- 10

Technique in Cell Biology

TEM

Ernst Ruska developed the first electron microscope, a TEM, with the assistance of Max Knolls in 1931. After significant improvements to the quality of magnification, Ruska joined the Sieman's Company in the late 1930s as an electrical engineer, where he assisted in the manufacturing of his TEM.

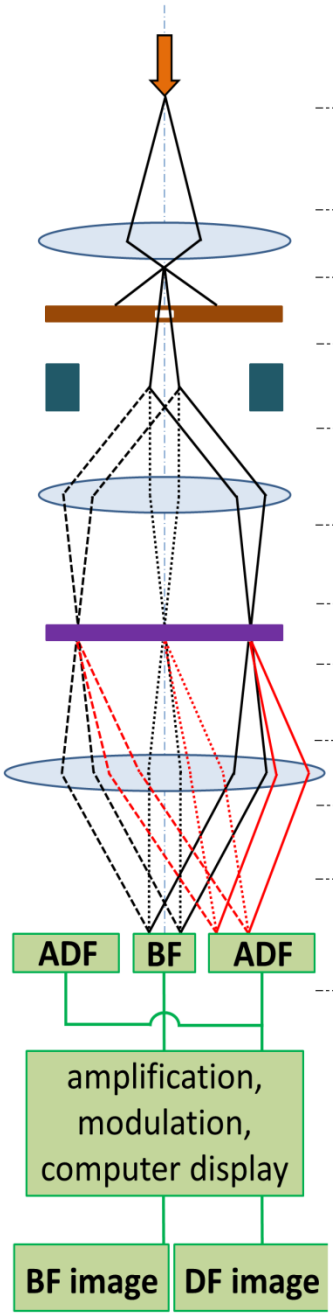
Transmission electron microscope (TEM), type of electron microscope that has three essential systems: (1) an electron gun, which produces the electron beam, and the condenser system, which focuses the beam onto the object, (2) the image-producing system, consisting of the objective lens, movable specimen stage, and intermediate and projector lenses, which focus the electrons passing through the specimen to form a real, highly magnified image, and (3) the image-recording system, which converts the electron image into some form perceptible to the human eye. The image-recording system usually consists of a fluorescent screen for viewing and focusing the image and a digital camera for permanent records. In addition, a vacuum system, consisting of pumps and their associated gauges and valves, and power supplies are required**.**

A transmission electron microscope fires a beam of electrons through a specimen to produce a magnified image of an object. A high-voltage electricity supply powers the cathode. The cathode is a heated filament, a bit like the electron gun in an old-fashioned cathode-ray tube (CRT) TV.

Transmission electron microscopes (TEM) are microscopes that use a particle beam of electrons to visualize specimens and generate a highly-magnified image. TEMs can magnify objects up to 2 million times.

The advantage of the transmission electron microscope is that it magnifies specimens to a much higher degree than an optical microscope. Magnification of 10,000 times or more is possible, which allows scientists to see extremely small structures.

STEM mode



Electron Gun

System of condenser lenses

Condenser aperture

Deflection scan coils

Upper-objective polepiece

Specimen

Lower-objective polepiece

STEM detector
(back focal plane of objective lens)

Scanning electron microscope

A typical SEM instrument, showing the electron column, sample chamber, EDS detector, electronics console, and visual display monitors.The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. In most applications, data are collected over a selected area of the surface of the sample, and a 2-dimensional image is generated that displays spatial variations in these properties. Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques (magnification ranging from 20X to approximately 30,000X, spatial resolution of 50 to 100 nm). The SEM is also capable of performing analyses of selected point locations on the sample; this approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions (using EDS), crystalline structure, and crystal orientations (using EBSD). The design and function of the SEM is very similar to the EPMA and considerable overlap in capabilities exists between the two instruments.

Fundamental Principles of Scanning Electron Microscopy (SEM)

Accelerated electrons in an SEM carry significant amounts of kinetic energy, and this energy is dissipated as a variety of signals produced by electron-sample interactions when the incident electrons are decelerated in the solid sample. These signals include secondary electrons (that produce SEM images), backscattered electrons (BSE), diffracted backscattered electrons (EBSD that are used to determine crystal structures and orientations of minerals), photons (characteristic X-rays that are used for elemental analysis and continuum X-rays), visible light (cathodoluminescence--CL), and heat. Secondary electrons and backscattered electrons are commonly used for imaging samples: secondary electrons are most valuable for showing morphology and topography on samples and backscattered electrons are most valuable for illustrating contrasts in composition in multiphase samples (i.e. for rapid phase discrimination). X-ray generation is produced by inelastic collisions of the incident electrons with electrons in discrete ortitals (shells) of atoms in the sample. As the excited electrons return to lower energy states, they yield X-rays that are of a fixed wavelength (that is related to the difference in energy levels of electrons in different shells for a given element). Thus, characteristic X-rays are produced for each element in a mineral that is "excited" by the electron beam. SEM analysis is

considered to be "non-destructive"; that is, x-rays generated by electron interactions do not lead to volume loss of the sample, so it is possible to analyze the same materials repeatedly.

Scanning Electron Microscopy (SEM) Instrumentation - How Does It Work?

Essential components of all SEMs include the following:

Electron Source ("Gun")

Electron Lenses

Sample Stage

Detectors for all signals of interest

Display / Data output devices

Infrastructure Requirements:

Power Supply

Vacuum System

Cooling system

Vibration-free floor

Room free of ambient magnetic and electric fields

SEMs always have at least one detector (usually a secondary electron detector), and most have additional detectors. The specific capabilities of a particular instrument are critically dependent on which detectors it accommodates.

Applications

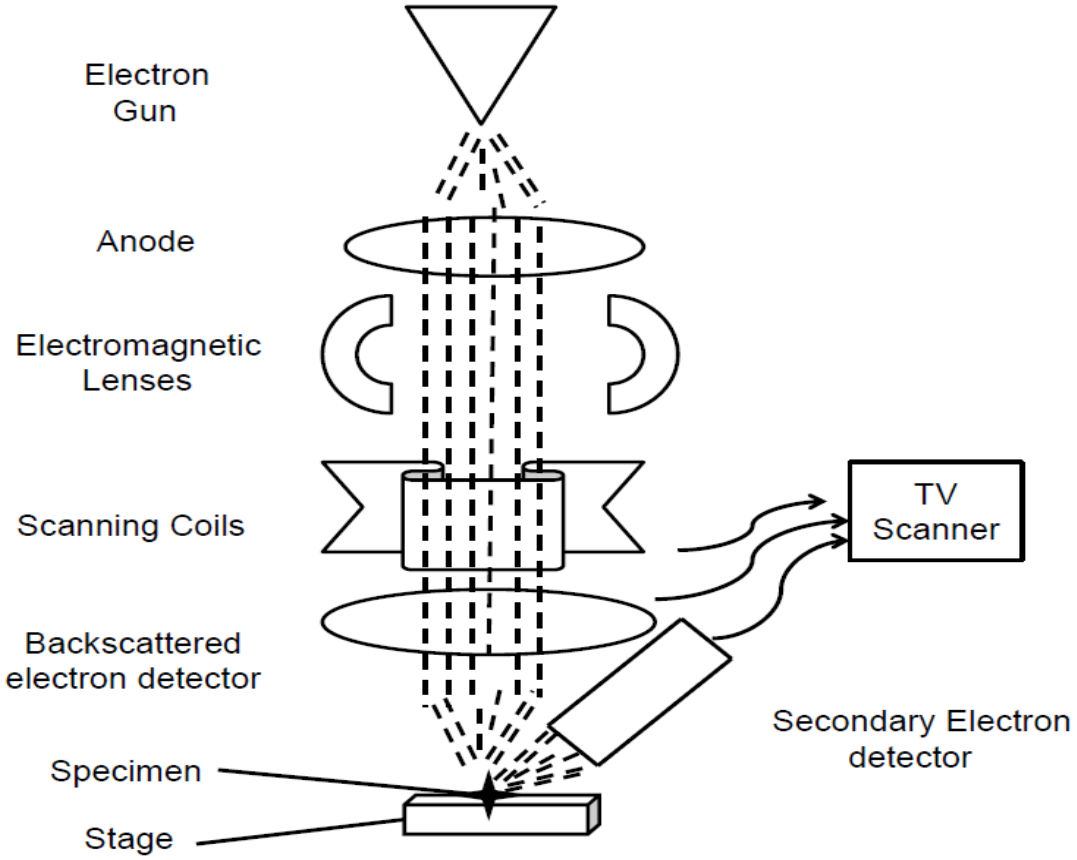
The SEM is routinely used to generate high-resolution images of shapes of objects (SEI) and to show spatial variations in chemical compositions: 1) acquiring elemental maps or spot chemical analyses using EDS, 2)discrimination of phases based on mean atomic number (commonly related to relative density) using BSE, and 3) compositional maps based on differences in trace element "activitors" (typically transition metal and Rare Earth elements) using CL. The SEM is also widely used to identify phases based on qualitative chemical analysis and/or crystalline structure. Precise measurement of very small features and objects down to 50 nm in size is also accomplished using the SEM. Backescattered electron images (BSE) can be used for rapid discrimination of phases in multiphase samples. SEMs equipped with diffracted backscattered

electron detectors (EBSD) can be used to examine microfabric and crystallographic orientation in many materials.

Strengths and Limitations of Scanning Electron Microscopy (SEM)?

Strengths

There is arguably no other instrument with the breadth of applications in the study of solid materials that compares with the SEM. The SEM is critical in all fields that require characterization of solid materials. While this contribution is most concerned with geological applications, it is important to note that these applications are a very small subset of the scientific and industrial applications that exist for this instrumentation. Most SEM's are comparatively easy to operate, with user-friendly "intuitive" interfaces. Many applications require minimal sample preparation. For many applications, data acquisition is rapid (less than 5 minutes/image for SEI, BSE, spot EDS analyses.) Modern SEMs generate data in digital formats, which are highly portable.



SCANNING ELECTRON MICROSCOPE

SPECTROSCOPY

Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelengt

Spectroscopy is the analysis of the interaction between matter and any portion of the electromagnetic spectrum. ... Spectroscopy can involve any interaction between light and matter, including absorption, emission, scattering, et

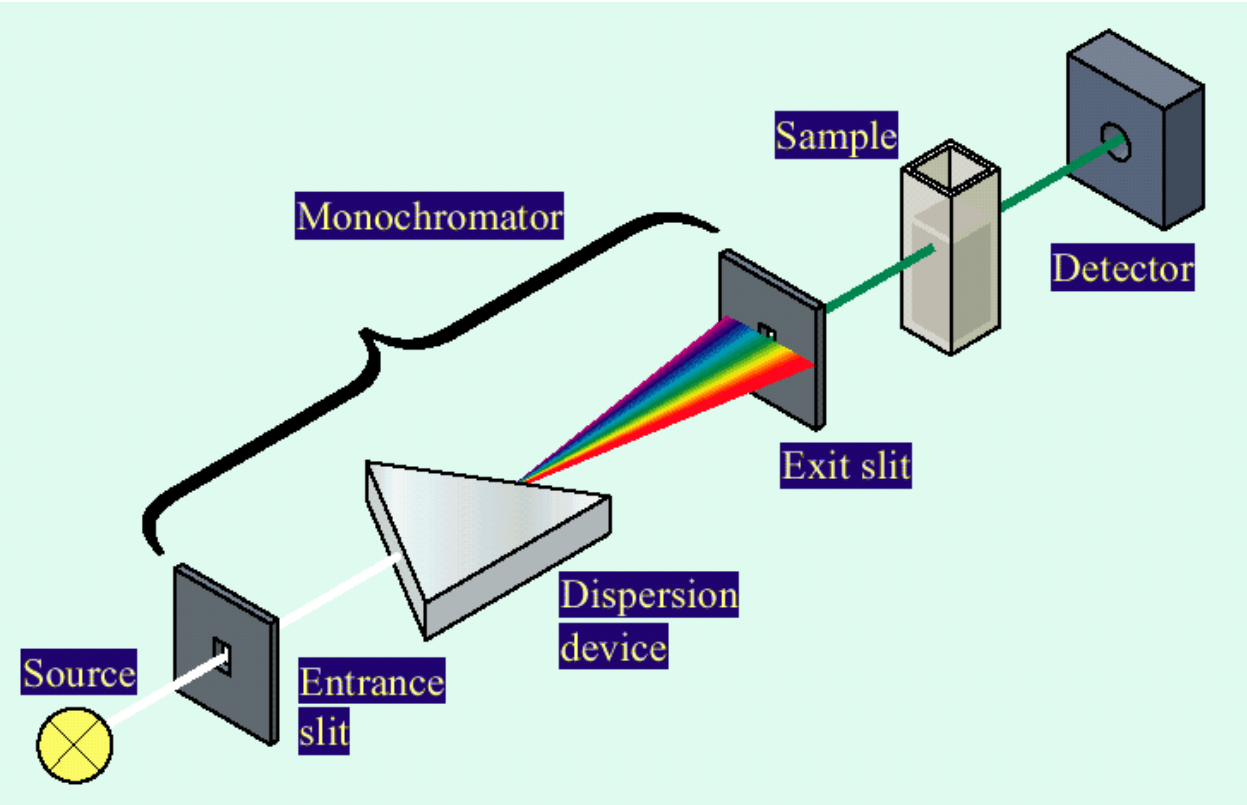
Spectroscopy is used as a tool for studying the structures of atoms and molecules. The large number of wavelengths emitted by these systems makes it possible to investigate their structures in detail, including the electron configurations of ground and various excited states

Spectroscopy pertains to the dispersion of an object's light into its component colors (i.e. energies). By performing this dissection and analysis of an object's light, astronomers can infer the physical properties of that object (such as temperature, mass, luminosity and composition).

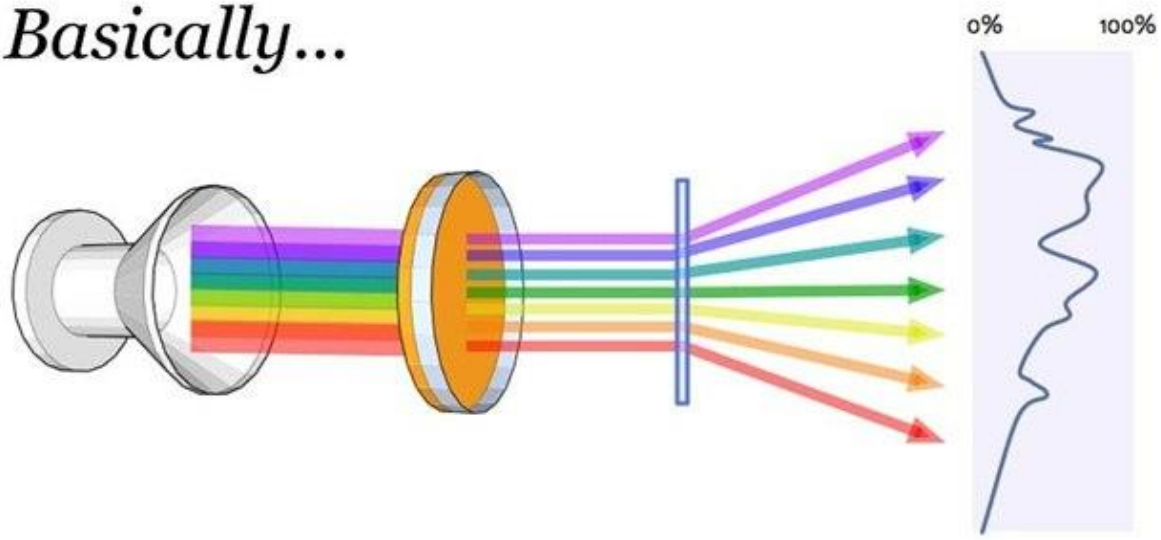
Spectroscopy is used in physical and analytical chemistry because atoms and molecules have unique spectra. As a result, these spectra can be used to detect, identify and quantify information about the atoms and molecules. Spectroscopy is also used in astronomy and remote sensing on Earth

Spectroscopy, study of the absorption and emission of light and other radiation by matter, as related to the dependence of these processes on the wavelength of the radiation.

Beer's Law is used in chemistry to measure the concentration of chemical solutions, to analyze oxidation, and to measure polymer degradation. The law also describes the attenuation of radiation through the Earth's atmosphere


Sample
Monochromator
Detector
Exit slit
Dispersion
device
Source
Entrance
slit

Basically...



1. A broad-spectrum light (halogen, incandescent) is shone through a sample

2. Some colors are absorbed more than others depending on its composition

3. Diffraction grating splits light into colors so they can be measured separately

4. A webcam measures each color and graphs their intensities. This is compared to known samples.

Chromatography

a technique for the separation of a mixture by passing it in solution or suspension through a medium in which the components move at different rates.

There are four main types of chromatography. These are

1.Liquid Chromatography,

2. Gas Chromatography,

3.Thin-Layer Chromatography and

4,Paper Chromatography.

Liquid Chromatography is used in the world to test water samples to look for pollution in lakes and rivers

Liquid Chromatography: Chromatography is used to separate proteins, nucleic acids, or small molecules in complex mixtures. Liquid chromatography (LC) separates molecules in a liquid mobile phase using a solid stationary phase. Liquid chromatography can be used for analytical or preparative applications. Sample carried by a moving gas stream of Helium or Nitrogen. High Performance Liquid Chromatography (HPLC) is a form of column chromatography that pumps a sample mixture or analyte in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase). HPLC works on the principle that some molecules take longer than others to pass through a chromatography column. This depends on the affinity of the molecule with the mobile phase (liquid or gas) and the stationary phase (solid or liquid). High-performance liquid chromatography (HPLC) is a chromatographic technique used to split a mixture of compounds in the fields of analytical chemistry, biochemistry and industrial. The main purposes for using HPLC are for identifying, quantifying and purifying the individual components of the mixture.

Gas Chromatography: Principle of gas chromatography: The sample solution injected into the instrument enters a gas stream which transports the sample into a separation tube known as the "column." (Helium or nitrogen is used as the so-called carrier gas.) The various components are separated inside the column. Gas chromatography is the process of separating, identifying and quantifying the various compositional elements of a compound. This is achieved by measuring how each of the different components reacts with being converted from a liquid or solid stationary phase into a mobile gas phase.

The equilibrium for gas chromatography is partitioning, and the components of the sample will partition (i.e. distribute) between the two phases: the stationary phase and the mobile phase.

Compounds that have a greater affinity for the stationary phase spend more time in the column and thus elute later and have a longer retention time (Rt) than samples that have a higher affinity for the mobile phase.

Affinity for the stationary phase is driven mainly by intermolecular interactions and the polarity of the stationary phase can be chosen to maximize interactions and thus the separation.

Ideal peaks are Gaussian distributions and symmetrical, because of the random nature of the analyte interactions with the column.

The separation is hence accomplished by partitioning the sample between the gas and a thin layer of a nonvolatile liquid held on a solid support.

A sample containing the solutes is injected into a heated block where it is immediately vaporized and swept as a plug of vapor by the carrier gas stream into the column inlet.

The solutes are adsorbed by the stationary phase and then desorbed by a fresh carrier gas.

The process is repeated in each plate as the sample is moved toward the outlet.

Each solute will travel at its own rate through the column.

Their bands will separate into distinct zones depending on the partition coefficients, and band spreading.

The solutes are eluted one after another in the increasing order of their kd, and enter into a detector attached to the exit end of the column.

Here they register a series of signals resulting from concentration changes and rates of elution on the recorder as a plot of time versus the composition of carrier gas stream.

The appearance time, height, width, and area of these peaks can be measured to yield quantitative data.

How Does Gas Chromatography Work?

Non-reactive gases such as helium (He) or hydrogen (H) are used as a carrier for vaporized molecules of interest. This gaseous mixture is flowed through the column of a gas chromatograph, which comprises a microscopic fluidic membrane and an inert, solid substrate. This column partitions vapors based on their mechanical properties and their affinity with the stationary fluid. The flow-through rates of the sample's constituent parts can be used for compound detection, identification, quantitation, and purification.

Elution in gas chromatography is electronically monitored using a detector at the outlet stream of the permeable column. This measures the retention time (tR) of compounds to qualitatively determine their adsorption characteristics with packing media of distinct chemical compositions.

Thin layer Chromatography:

What Is Thin Layer Chromatography?

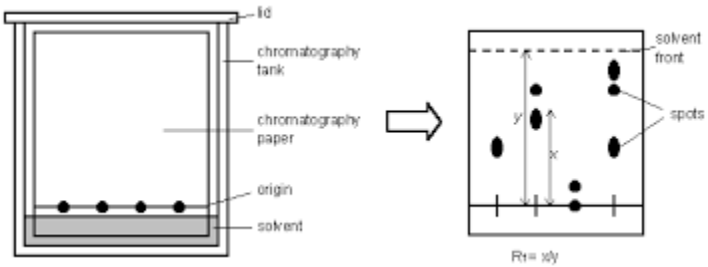
Thin Layer Chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminium foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminium oxide, cellulose, or silica gel.On completion of the separation, each component appears as spots separated vertically. Each spot has a retention factor (Rf) expressed as:

Rf = dist. travelled by sample / dist. travelled by solvent

The factors affecting retardation factor are the solvent system, amount of material spotted, absorbent and temperature. TLC is one of the fastest, least expensive, simplest and easiest chromatography technique.

Thin Layer Chromatography Principle

Like other chromatographic techniques, thin layer chromatography (TLC) depends on the separation principle. The separation relies on the relative affinity of compounds towards both the phases. The compounds in the mobile phase move over the surface of the stationary phase. The movement occurs in such a way that the compounds which have a higher affinity to the stationary phase move slowly while the other compounds travel fast. Therefore, the separation of the mixture is attained. On completion of the separation process, the individual components from the mixture appear as spots at respective levels on the plates. Their character and nature are identified by suitable detection techniques.



Thin Layer Chromatography Procedure

Before starting with the Thin Layer Chromatography Experiment let us understand the different components required to conduct the procedure along with the phases involved.

Thin Layer Chromatography Plates – ready-made plates are used which are chemically inert and stable. The stationary phase is applied on its surface in the form of a thin layer. The stationary phase on the plate has a fine particle size and also has a uniform thickness.

Thin Layer Chromatography Chamber – Chamber is used to develop plates. It is responsible to keep a steady environment inside which will help in developing spots. Also, it prevents the solvent evaporation and keeps the entire process dust-free.

Thin Layer Chromatography Mobile phase – Mobile phase is the one that moves and consists of a solvent mixture or a solvent. This phase should be particulate-free. The higher the quality of purity the development of spots is better.

Thin Layer Chromatography Filter Paper – It has to be placed inside the chamber. It is moistened in the mobile phase.

Thin Layer Chromatography Experiment

The stationary phase that is applied to the plate is made to dry and stabilize.

To apply sample spots, thin marks are made at the bottom of the plate with the help of a pencil.

Apply sample solutions to the marked spots.

Pour the mobile phase into the TLC chamber and to maintain equal humidity, place a moistened filter paper in the mobile phase.

Place the plate in the TLC chamber and close it with a lid. It is kept in such a way that the sample faces the mobile phase.

Immerse the plate for development. Remember to keep the sample spots well above the level of the mobile phase. Do not immerse it in the solvent.

Wait till the development of spots. Once the spots are developed, take out the plates and dry them. The sample spots can be observed under a UV light chamber.

Thin Layer Chromatography Applications

The qualitative testing of Various medicines such as sedatives, local anaesthetics, anticonvulsant tranquilisers, analgesics, antihistamines, steroids, hypnotics is done by TLC.

TLC is extremely useful in Biochemical analysis such as separation or isolation of biochemical metabolites from its blood plasma, urine, body fluids, serum, etc.

Thin layer chromatography can be used to identify natural products like essential oils or volatile oil, fixed oil, glycosides, waxes, alkaloids, etc

It is widely used in separating multicomponent pharmaceutical formulations.

It is used to purify of any sample and direct comparison is done between the sample and the authentic sample

It is used in the food industry, to separate and identify colours, sweetening agent, and preservatives

It is used in the cosmetic industry.

It is used to study if a reaction is complete.

Disadvantages Of Thin Layer Chromatography:

Thin Layer Chromatography plates do not have longer stationary phase.

When compared to other chromatographic techniques the length of separation is limited.

The results generated from TLC are difficult to reproduce.

Since TLC operates as an open system, some factors such as humidity and temperature can be consequences to the final outcome of the chromatogram.

The detection limit is high and therefore if you want a lower detection limit, you cannot use TLC.

Paper Chromatography

Paper chromatography is one of the types of chromatography procedures which runs on a piece of specialized paper. It is a planar chromatography system wherein a cellulose filter paper acts as a stationary phase on which the separation of compounds occurs.

Principle of paper chromatography: The principle involved is partition chromatography wherein the substances are distributed or partitioned between liquid phases. One phase is the water, which is held in the pores of the filter paper used; and other is the mobile phase which moves over the paper**.**

Uses and Applications of Paper Chromatography

Paper chromatography is specially used for the separation of a mixture having polar and non-polar compounds.

a) For separation of amino acids.

b)It is used to determine organic compounds, biochemicals in urine, etc.

c)In the pharma sector, it is used for the determination of hormones, drugs, etc.

d) Sometimes it is used for evaluation of inorganic compounds like salts and complexes.

Types or Modes of Paper Chromatography

Based on the way the development of chromatogram on paper is done in procedures, we have, broadly, five types of chromatography.

1. Ascending chromatography: As the name indicates, the chromatogram ascends. Here, the development of paper occurs due to the solvent movement or upward travel on the paper.

The solvent reservoir is at the bottom of the beaker. The paper tip with sample spots just dips into the solvent at the bottom so that spots remain well above the solvent.

2. Descending chromatography: Here, the development of paper occurs due to solvent travel downwards on the paper.

The solvent reservoir is at the top. The movement of the solvent is assisted by gravity besides the capillary action.

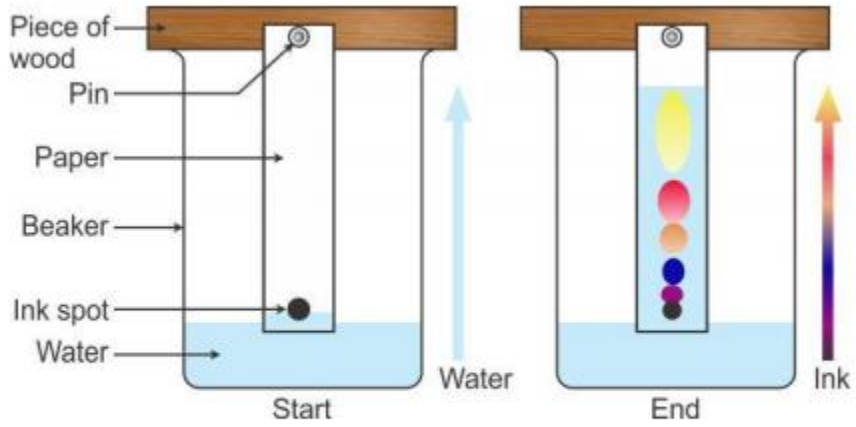
3. Ascending- descending mode: Here solvent first travels upwards and then downwards on the paper.

4. Radial mode: Here, the solvent moves from the center (mid-point) towards the periphery of circular chromatography paper. The entire system is kept in a covered Petri dish for the development of the chromatogram.

The wick at the center of paper dips into the mobile phase in a petri dish, by which the solvent drains on to the paper and moves the sample radially to form the sample spots of different compounds as concentric rings.

5. Two-dimensional chromatography: Here the chromatogram development occurs in two directions at right angles.

In this mode, the samples are spotted to one corner of rectangular paper and allowed for first development. Then the paper is again immersed in the mobile phase at a right angle to the previous development for the second chromatog



Paper Chromatography