COURSE: MSc Part -II

PAPER – XI

TOPIC- Molecular Biology

PREAPARED BY: Ajai Kishore Sharan

Topic -4

Packaging of DNA- Nucleosome

The haploid human genome contains approximately 3 billion base pairs of DNAs 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 × 10-9) × (6 × 109)]. 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?

The nucleosomes are structural building blocks of the packing of DNA within a chromosome. The packing problem of how to fit a very, very long stretch of DNA, which is about a yard of DNA, inside a very small cell, which is about a hundredth of a millimeter in diameter, has fascinated scientists for a long time. And it turns out how the cell does this--now--remember that each cell in the body has this problem--is that it coils and super coils the DNA in a multitude of complex ways. The fundamental building block of that coiling are nucleosomes, which are blocks of essentially little spheres of histone proteins around which the DNA is wrapped, and they look literally like beads on a string, except the beads have the DNA wrapped around them instead of having the DNA go through them, as in the case of a bead on a string.

Electron micrographs first confirmed that the eukaryotic genome is organized into repeating disk-shaped nucleosomal units composed of histones and their associated DNA. Those images made clear the function of the nucleosome in packaging and condensing the genome. Today, nucleosomes are recognized as highly dynamic units through which the eukaryotic genome can be regulated with epigenetically heritable consequences.

The packaging of DNA into chromatin represents one of the most fundamental layers of the biology of the cell. It provides the required structural compaction of DNA to fit in the nucleus and plays crucial roles in controlling cell fate and protecting genome integrity. The fundamental unit of chromatin is the nucleosome in which 147 base pairs (bp) of DNA are wrapped around an octameric protein complex composed of two copies of histone proteins H2A, H2B, H3 and H4 [1, 2, 3]. Nucleosomes are arranged as beads-on-a-string forming 10 nanometer (nm) wide fiber that subsequently condense into higher order structures [4]. Nucleosomes as the basis of chromatin are responsible for its dynamics. Chromatin state and changes in DNA accessibility are determined at the nucleosome level. These changes are mediated through interactions of histone proteins and nucleosomal DNA alike with a wide range of protein complexes that control the structure of chromatin. They interpret, write and erase post-translational modifications or act as ATP-dependent nucleosome remodelers. This allows changes in the functional state of chromatin and regulation of DNA-templated processes. While promoting a large variety of effects on chromatin structure, nucleosome-interacting proteins share the molecular basis of recognizing and binding the nucleosome.

Understanding the basis of chromatin dynamics therefore demands understanding the molecular basis of nucleosome-protein interactions.

DNA, Histones, and Chromatin

Histones are a family of basic proteins that associate with DNA in the nucleus and help condense it into chromatin. Nuclear DNA does not appear in free linear strands; it is highly condensed and wrapped around histones to fit inside of the nucleus and take part in the formation of chromosomes. Histones are basic proteins, and their positive charges allow them to associate with DNA, which is negatively charged. Some histones function as spools for the thread-like DNA to wrap around.

Under the microscope in its extended form, chromatin looks like beads on a string. The beads are called nucleosomes. Each nucleosome is made of DNA wrapped around eight histone proteins that function like a spool and are called a histone octamer. Each histone octamer is composed of two copies each of the histone proteins H2A, H2B, H3, and H4. The chain of nucleosomes is then wrapped into a 30 nm spiral called a solenoid, where additional H1 histone proteins are associated with each nucleosome to maintain the chromosome structure. Histones are a family of small, positively charged proteins termed H1, H2A, H2B, H3, and H4 . DNA is negatively charged, due to the phosphate groups in its phosphate-sugar backbone, so histones bind with DNA very tightly.

Stepwise description of Nucleosome model

Nucleosome model is a scientific model which explains the organization of DNA and associated proteins in the chromosome.

It also further explains the exact mechanism of the folding of the DNA in the nucleus.

The model was proposed by Roger Kornberg in 1974 and is the most accepted model of chromatin organization.

It was confirmed and christened by P. Oudet et al., (1975).

Features of the Nucleosome Model of Chromosomes

In eukaryotes, DNA is tightly bound to an equal mass of histones, which serve to form a repeating array of DNA-protein particles, called nucleosomes.

If it was stretched out, the DNA double-helix in each human chromosome would span the cell nucleus thousands of time.

Histones play a crucial role in packing this very long DNA molecule in an orderly way (i.e., nucleosome) into nucleus only a few micrometers in diameter.

Thus, nucleosomes are the fundamental packing unit particles of the chromatin and give chromatin a “beads-on-a-string” appearance in electron micrographs taken after treatments that unfold higher-order packing.

Each nucleosome is a disc-shaped particle with a diameter of about 11 nm and 5.7 nm in height containing 2 copies of each 4 nucleosome histones–H2A, H2B, H3, and H4.

This histone octamer forms a protein core [(i.e., a core of histone tetramer (H3, H4)2 and the apolar regions of 2(H2A and H2B)] around which the double-stranded DNA helix is wound 1¾ time containing 146 base pairs. In chromatin, the DNA extends as a continuous thread from nucleosome to nucleosome. Each nucleosome bead is separated from the next by a region of linker DNA which is generally 54 base pair long and contains single H1 histone protein molecule.

Generally, DNA makes two complete turns around the histone octamers and these two turns (200 bp long) are sealed off by H1 molecules.

On average, nucleosomes repeat at intervals of about 200 nucleotides or base pairs. For example, a eukaryotic gene of 10,000 nucleotide pairs will be associated with 50 nucleosomes and each human cell with 6 x 109 DNA nucleotide pairs contains 3 x 107

The Folding of the DNA

The first step is the assembly of the DNA with a newly synthesized tetramer (H3-H4), are specifically modified (e.g. H4 is acetylated at Lys5 and Lysl2 (H3-H4)), to form a sub-nucleosomal particle, which is followed by the addition of two H2A-H2B dimers.

This produces a nucleosomal core particle consisting of 146 base pairs of DNA bind around the histone octamer. This core particle and the linker DNA together form the nucleosome.

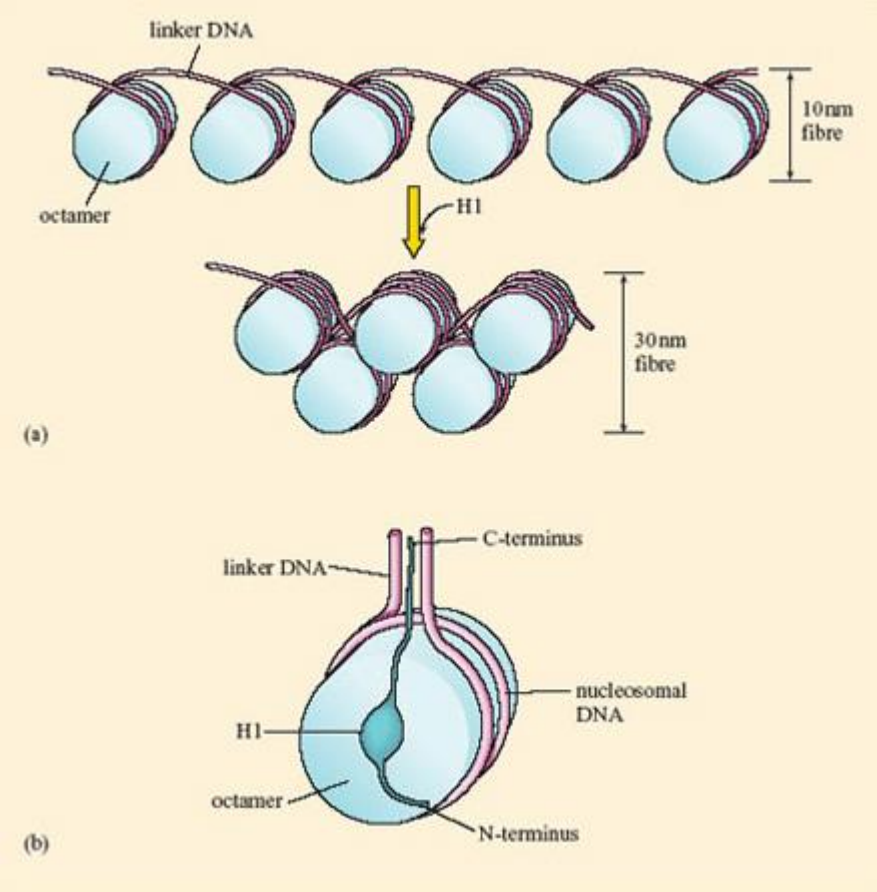
The next step is the maturation step that requires ATP to establish regular spacing of the nucleosome cores to form the nucleo-filament.

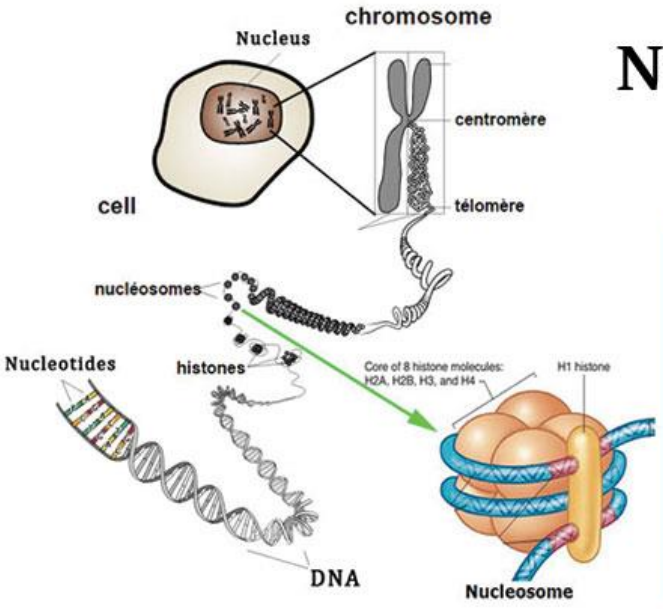
During this step the newly incorporated his-tones are de-acetylated.

Next, the incorporation of linker histones is accompanied by folding of the nucleo-filament into the 30 nm fiber, the structure of which remains to be elucidated.

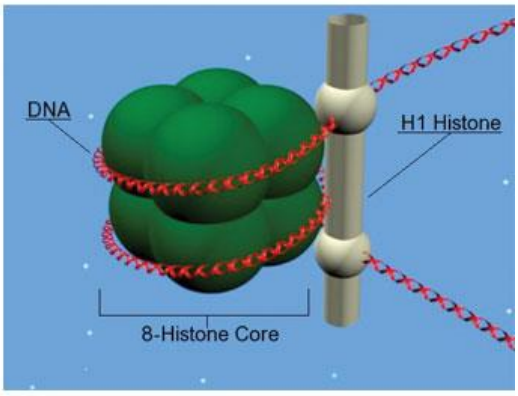
Two principal models exist- the solenoid model and the zig-zag.

Finally, further successive folding events lead to a high level of organization and specific domains in the nucleus.





Nucleosome Model of Chromosome



Topic-5

Types of RNA and their function

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 aspects 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 many metabolic functions for growth and development. Various kinds of proteins have 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 alignment of different amino acids for the synthesis of a specific protein.

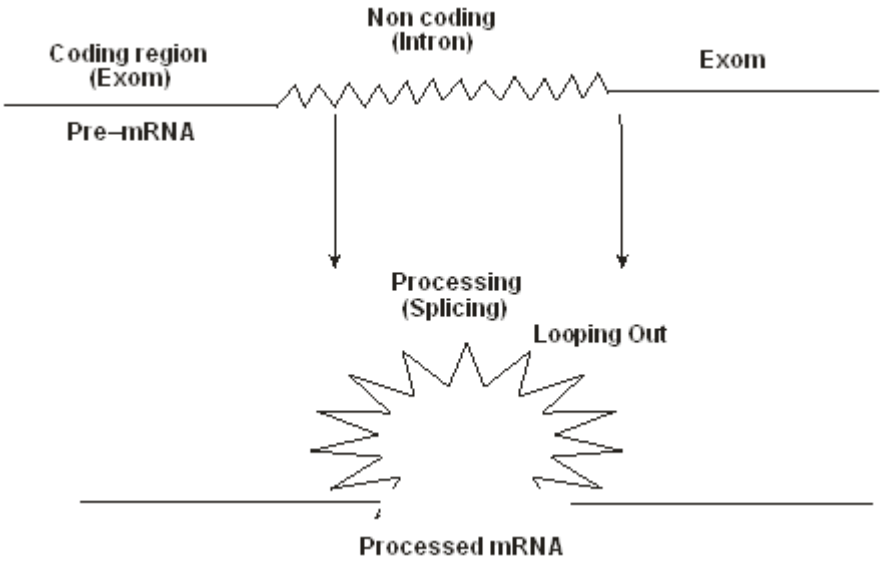
Messenger RNA (mRNA)

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 splicesome. 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.



Removal of Non coding sequence (intron) during processing of mRNA

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.

Both prokaryotic and eukaryotic ribosomes are made of a larger and smaller subunit and these two units come together during mRNA translation. The smaller subunit in prokaryotes is made of an RNA molecule about 1500 nucleotides in length with a Svedberg coefficient of 16S. Together with ribosomal proteins, the smaller subunit has a sedimentation rate of 30S. This is paired with the larger subunit, having two RNA molecules – one that is nearly 3000 nucleotides (23S) in length and the other is a short sequence of 120 nucleotides (5S). These RNA molecules are accompanied by proteins that give rise to the larger 50S subunit.

The eukaryotic ribosome is made of a 60S and a 40S subunit. There are two short rRNA molecules less than two hundred nucleotides in length (5S and 5.8S), and two RNA molecules that are much longer – one that has over five kilobases (28S), and another nearly two kilobases (18S). In all, the eukaryotic ribosome has a Svedberg coefficient of 80S. In addition, eukaryotic cells also have rRNA in mitochondria and chloroplasts. Ribosomes can be associated with the endoplasmic reticulum or be present as free floating complexes in the cytoplasm.

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) is synthesized in the cytoplasm. This protein synthesized in cytoplasm 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.

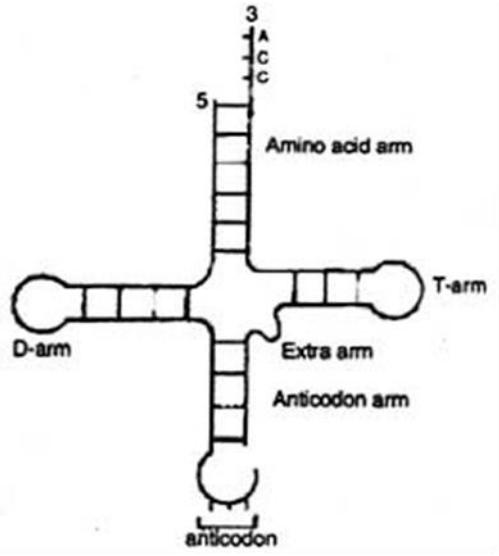
Role of 16S rRNA

A bacterial DNA sequence which is most amenable to change is that which encodes 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.



Sructure of tRNA

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) as 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 tRNA. The process by which Pre-tRNA get converted to mature tRNA involves following steps.

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

2. The 3` ends 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 (I). In the same way Uridine may modified to dihydrouridine.

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

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

Some Other Forms of RNA

.

Beyond the primary role of RNA in protein synthesis, several varieties of RNA exist that are involved in post-transcriptional modification, DNA replication, and gene regulation. Some forms of RNA are only found in particular forms of life, such as in eukaryotes or bacteria.

Small Nuclear RNA (snRNA)

snRNA is involved in the processing of pre-messenger RNA (pre-mRNA) into mature mRNA. They are very short, with an average length of only 150 nucleotides.

Regulatory RNAs

A number of types of RNA are involved in regulation of gene expression, including micro RNA (miRNA), small interfering RNA (siRNA) and antisense RNA (aRNA).miRNA (21-22 nt) is found in eukaryotes, and acts through RNA interference (RNAi). miRNA can break down mRNA that it is complementary to, with the aid of enzymes. This can block the mRNA from being translated, or accelerate its degradation.siRNA (20-25 nt) are often produced by breakdown of viral RNA, though there are also endogenous sources of siRNAs. They act similarly to miRNA. An mRNA may contain regulatory elements itself, such as riboswitches, in the 5' untranslated region or 3' untranslated region; these cis-regulatory elements regulate the activity of that mRNA.

Transfer-messenger RNA (tmRNA)

Found in many bacteria and plastids. tmRNA tag the proteins encoded by mRNAs that lack stop codons for degradation, and prevents the ribosome from stalling due to the missing stop codon.

Ribozyme

In 1989, Thomas cech and Sidney Altman shared the Nobel Prize, for discovery of certain RNA which solely acts as enzyme, called as Ribozymes. The enzymes activity of Ribozyme was noticed 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 codons for protein synthesis. These codons are ultimately translated into respective proteins and hence named as sense codons. 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

Double-stranded RNA (dsRNA)

This type of RNA has two strands bound together, as with double stranded DNA. dsRNA forms the genetic material of some viruses.

Topic-6

Northern, Southern and Western blotting

Blotting techniques were developed to discriminate fragments of nucleic acids. These techniques involve several processes; electrophoresis is one of the processes and is used to separate fragments of DNA and RNA. Blotting is technique in which nucleic acids i.e., RNA and DNA or proteins are transferred onto a specific membrane. This membrane may be nitrocellulose PVDF or nylon membrane. This process can be done just after the gel electrophoresis, by transferring the molecules from the gel onto the surface of blotting membrane. But sometimes it can be done by directly transferring the molecules onto the membrane. And then we can visualize these transferring molecules by using staining. Examples: Ethidium bromide, Crystal violet, Safranine and Ossmium tetroxide etc.

Types of Blotting

There are basically 4 types of blotting:

1) Southern blotting

2) Western blotting

3) Northern blotting

4) Eastern blotting

1) Southern blotting

Southern blotting is named after Edward M. Southern. This method is used for analysis of DNA sequences. It involves the following steps:

• Firstly, large weighted DNA is cut into small fragments by using Restriction endonucleases

• Then, these fragments are electrophoresed on separating gel so that they can separate according to their size.

• If DNA fragments are much larger in size so firstly the gel should be treated with HCl, causes depurination of DNA fragments.

• After separating these fragments, placed a nitrocellulose sheet over the separating gel. Apply pressure over the membrane so that proper interaction can occur between these two.

• After that the membrane is exposed to ultraviolet radiation so that the fragments are permanently attached to the membrane.

• Then the membrane is exposed to hybridization probe. But the DNA probe is labeled so that it can easily detect, when the molecule is tagged with a chromogenic dye .

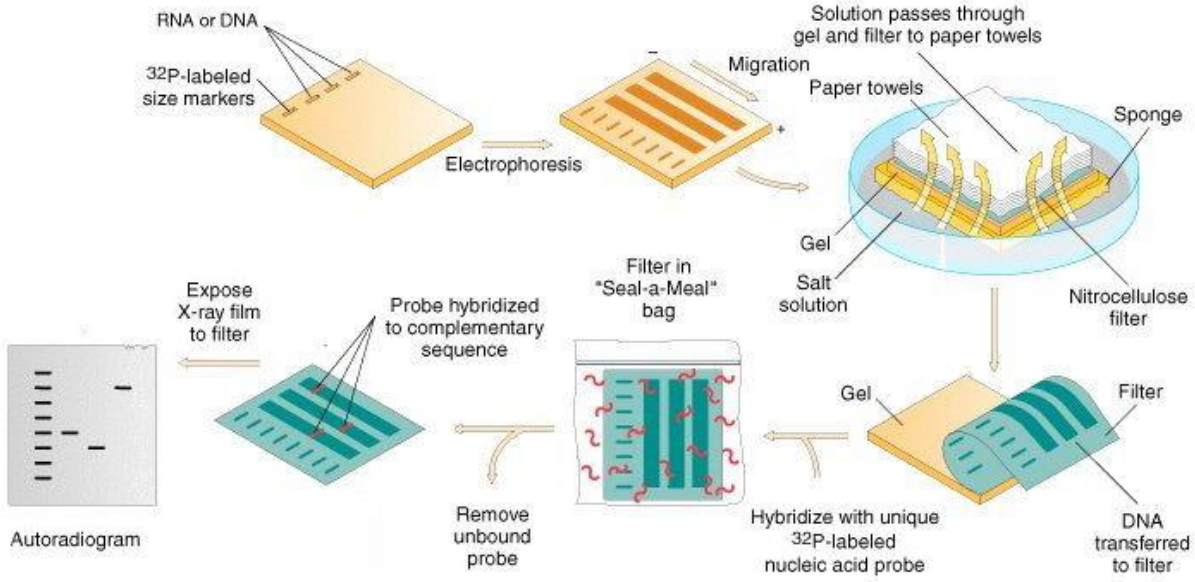
• After hybridization process, excess probe is washed away by using SSC buffer and it can be visualized on X-ray film with the help of autoradiography.

Applications

i) It is used in the technique called RFLP (Restriction fragment length polymorphism) mapping.

ii) Also used in phylogenetic analysis.

iii) To identify the gene rearrangements.



2) Western blotting

Western blotting is named after W. Neal Burnette. This method is used for detection and analysis of protein in each sample. It involves the following steps:

• Firstly, isolating the protein from particular sample.

After that beta- mercaptoethanol (BME) and Sodium dodecyl Sulfate (SDS) is added into the protein suspension.

• Then, protein- SDS complex is placed on top of the gel in the well. A molecular weight marker is also loaded in one of the well to determine the molecular weight of other proteins. After that the samples are added in the remaining wells.

• Once the samples and markers are loaded then current is passed across the gel. Protein is pulled down to the positive pole of the well because it is tightly bound to SDS which is negatively charged. Movement of protein is inversely proportional to its size.

• After this step, gel is placed against a membrane and current is passed across the gel so that all the proteins are transferred onto the membrane.

• Then Immunoblotting must be done. In this method, firstly block the membrane with non-specific protein to prevent antibody from binding to the membrane where the protein is not present.

• After that primary antibody is added to the solution. These antibodies are responsible for recognizing a specific amino-acid sequence. Then wash it to remove unbound primary antibody and add secondary antibody.

• Now these antibodies are conjugated with an enzyme and recognize the primary antibody. Lastly, another wash is done to remove unbound secondary antibody.

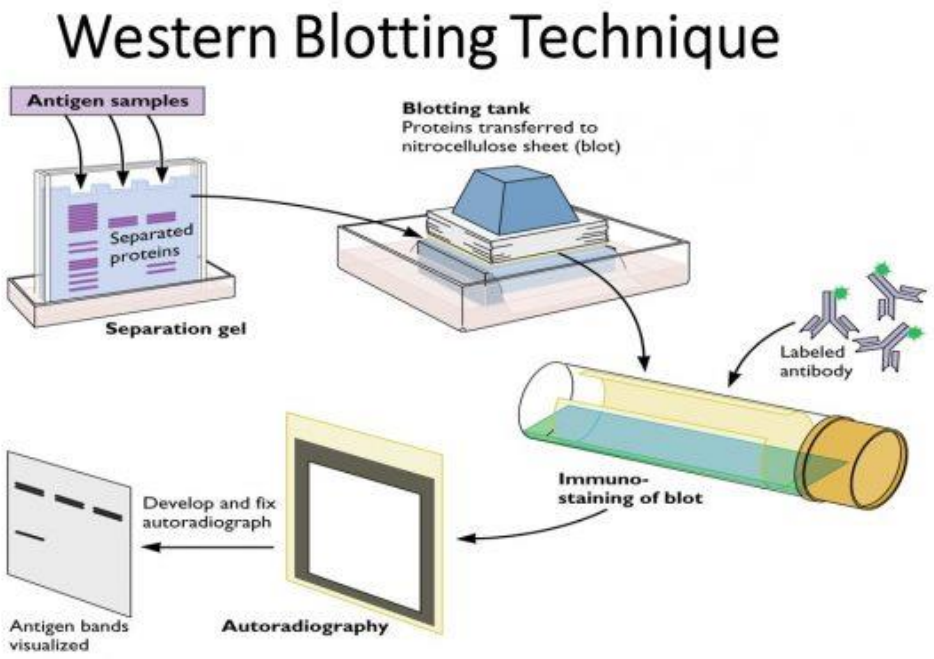
• Here, chemiluminescent substrate is used for detection. The light is being emitted once the substrate has been added and can be detected with film imager.

Applications:

i) Used in clinical purposes.

ii) Used to detect specific protein in low quantity.

iii) Used to quantifying a gene product.



3) Northern blotting

Northern blotting is given by Alwine. This method is used to analyse and detection of RNA in a sample.

• Firstly, extract and purify mRNA from the cells.

• Separate this RNA on agarose gels containing formaldehyde as a denaturing agent for the RNA.

• This gel is immersed in depurination buffer for 5-10 minutes and washed with water.

• Then transfer these RNA fragments onto the carrier membrane i.e aminobenzyloxymethyl filter paper.

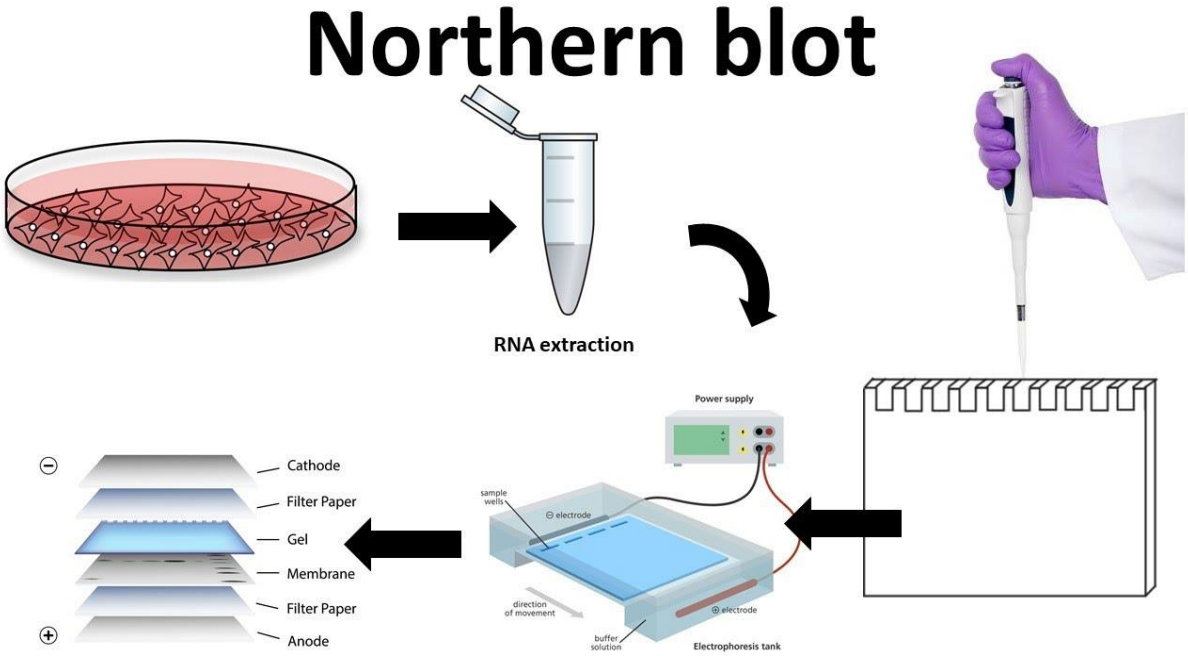
• After transferring the RNA,it is fixed to the membrane by using UV or heat.

• Add DNA labellled probe for hybridization.

Wash off the unbound probe and at the end mRNA-DNA hybrid are then detected by X-ray film.

Applications:

i) Used in screening.



Conclusion

Different blotting is used to detect different type of macromolecules such as southern blotting is used for DNA analysis, western blotting is for protein analysis, northern blotting is for RNA analysis and eastern for carbohydrate detection. The remaining of this article is focus on different techniques and applications used in particular blotting.