Course- B.Sc. Part-II Botany Subsidiary

PAPER–II

Topic- Structure of DNA and RNA, Linkage and Crossing Over (GENETICS)

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TOPIC-1

STRUCTURE OF DNA AND RNA

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.

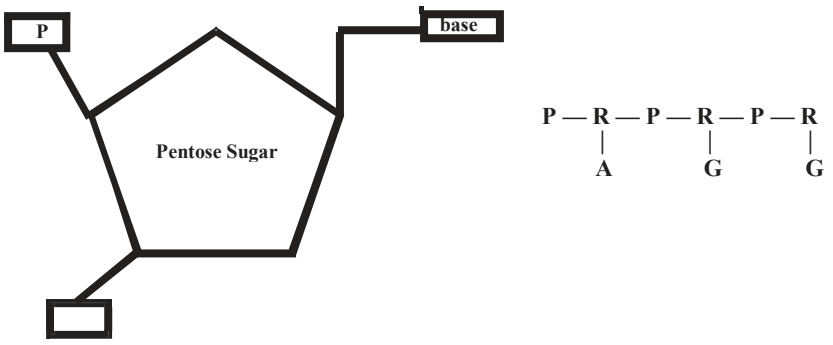
- Thread like in structure
- Made up of long chain of Polynucleotide.
- Each nucleotide consists of a nitrogen containing aromatic base attached to a pentose (five carbon) sugar, which in turn attached to a phosphate group.
- Each Nucleic acid contains four of five nitrogen base such as:

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 below



. Structural plan of Nucleoside of DNA/RNA

Chemical Components of Nucleotides in DNA and RNA

The Nucleotides of DNA and RNA are made up of Nitrogenous bases, Pentose sugar and Phospate group.

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

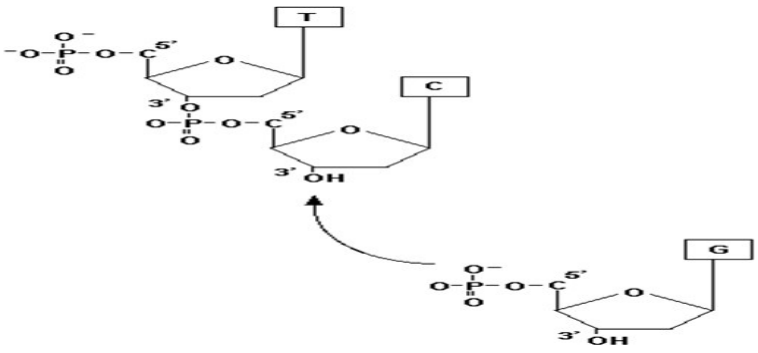
DN A molecules are prone to be affected during oxidation, ionizing radiation and also by carcinogens. Methylation can occur at two of the nucleotides, cytosine and adenine, when carbon molecule is replaced with CH3 molecule. This change leads to epigenetic change (not of heritable nature but acquired 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.



Phosphodiester bond connection between 5'prime and 3'prime of Pentose sugar

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` posho diester 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.

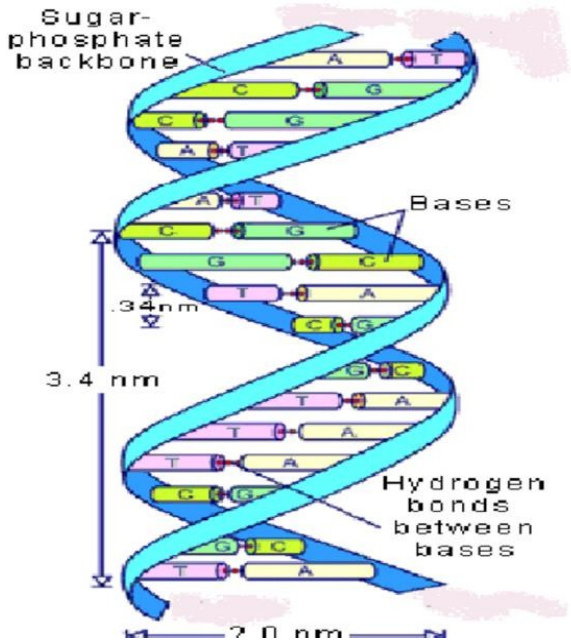
A configurational change occurs in the structure of DNA, the data which could be collected from X-ray diffraction of the crystalline forms of DNA. The isolated DNA was converted into crystalline form for X-ray diffraction. Before conducting crystallography, the sample was hydrated and diffraction pattern 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 oligonucteolides 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.



RNA

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.

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

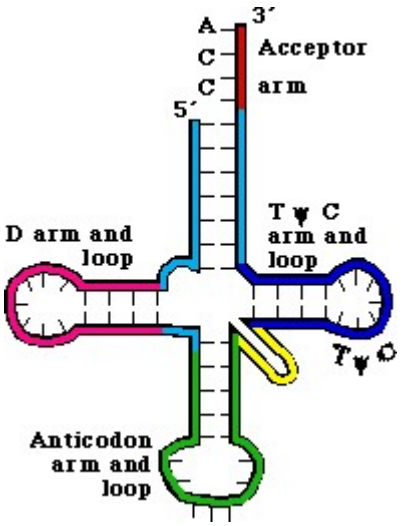
A bacterial DNA sequence which is most amenable to change is that which encodes for 16S

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` 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-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 have to interact with ribosome on the same site.

TOPIC-2

Linkage and Crossing over

Mendel considered inheritance of seven characters and all the characters showed random assortment. It was later demon•strated cytologically that peas have seven pairs of chromosomes. It was genetically shown that the genes for the characters studied by Mendel are each located in a different member of the seven pairs of chromosomes.

The independent assortment between two gene pairs, each with complete dominance and affecting different charac•ters, results in 9: 3: 3: 1 ratio .among the offspring of heterozygotes for both pairs (AaBb x AaBb).

But Bateson and Punnet in England in 1906 found exception to this law when they bred a di-hybrid strain of sweet pea. They crossed purple (P) flowered-long pollen grained (L) sweet peas with red flowered (p) round pollen grained (1) ones.The f1 gen•eration was purple flowered-long pollen grained (P-L-). But in f2 generation purple-long, purple- round, red-long and red-round appeared in the ratio of 11: 1: 1: 3 with too many parental types.Bateson and Punnet explained that the gametic coupling between P and L and p and 1 lead to such high ratio of parental combination. Again, in the next generation, Pl and pL characters tend to come together more often. This is called repulsion where the parental associations did not form but new combinations tend to persist.Both coupling and repulsion are possible when genes are arranged linearly along the length of chromosomes. When two genes remain in the same chromosome they tend to be inherited together to the offspring. This is called linkage and two genes are linked.

But, during reduction division, exchange of segments of a pair of chromosomes occurs, as a result linked genes are separated and recombination occurs. This is called crossing over . T. H. Mor•gan (1911) showed in his experiment with Drosophila, the linear arrangement of genes in a chromosome and gave explanation of both coupling and repulsion.

The phenomenon of tendency of linked genes to inherit together in the same combination for more than two generation is called linkage.

Morgan's View:

The degree of linkage between two genes depends on the distance between location of genes and they vary and form crossing over, if they are located at the distance. This phenomenon is explained by T.H. Morgan in 1911 in Drosophila melanogaster with grey body long wing and black body, vestigial wing. He stated that the pairs of genes of homozygous parents tried to enter the same gametes and to remain together, whereas same genes from heterozygous parents tend to enter different gametes and remain apart from each other.

Chromosomes Theory of linkage:

According to Morgan and Castle,

1. They concluded that chromosomes bear many genes.

2. The genes which show linkage are situated in the same chromosomes are bounded by the chromosomal material.

3. Gene are arranged in a linear fashion.

4. The strength of linkage depends upon the distance between the linked genes in the

chromosomes. 5.Linked gene remained in their original combination during the course of

inheritance.

Types of Linkages:

1.Complete linkage:If the parental combination of characters appear together for two or more generation in a continuous manner and regular manner. Such linked is called complete linkage.Example: Drosophila melanogaster

Here, gene are closely associated and tend to transmit together.

Parents: Grey, vestigial x Black long

(BBvv) (bbVV)

Gametes: (BV) (bV)

F1 generation : All grey, long

(BbVv)

Test cross: F1 male Grey, long x Female Black, vestigial

(BbVv) (bbvv)

Gametes: (BV) (bv) (bv)

(Due to complete linkage only two types of gametes are

formed). Test cross ratio: Grey, vestigial: Black long (1:1)

(Bvbv) (bVbv) The results show complete linkage.

Incomplete linkage : Incomplete linkage produces new combinations of the genes in the progeny due to the formation of chiasma or crossing over in between the linked genes present on homologous chromosomes. When in sweet peas a cross is made between blue flower and long pollen (BBLL) with red flower and round pollen (bbll) in F1 expected blue flower and long pollen (BbLl) heterozygous condition is got.

Significance of Linkage:

1. Linkage does not permit the breeders to bring the desirable characters in one variety.

2. Linked characters are maintained for generations because linkage prevents the incidence of recombination.

<u>Crossing over:</u>

Crossing over may be defined as an exchange of genetic material between non- sister chromatids of homologous chromosomes resulting in a new combination of genes.

The crossing over takes place during the early stage of prophase first of meiosis ce;; division.

Mechanism of crossing over:

The process of crossing over involves the following stages:

1. Synapsis : During zygotene substage of prophase I, the maternal and paternal homologous chromosomes come close to each other and start pairing along their length. The pairing of homologous chromosomes is called synapsis. They paired homologous chromosomes are called bivalents. It is mechanical basis of crossing over.

2.Duplication of chromosomes: The synapsis is followed by duplication of chromosomes. During pachytene substage of prophase I , the chromotids of each homologous chromosome splits lengthwise and forms two identical sister chromatids.Thus each bivalent contains four chromatids so it is known as tetrad.

3.Crossing over: The non-sister chromatids of homologous pair twist over each other at one or more points. The chromatid segments break at the corresponding points and the segment of one side fuses with the segment of the opposite side due to the action of enzyme. Thus the crossing over includes breaking of chromatid segments, their transposition and fusion.

4.Chiasmata Formation: Chiasmata are the points of attachment between two homologous chromosomes, where the crossing over occurs. The number of chiasmata depends on the length of the chromosomes; greater the length greater is the number.The crossing over may take place at one or several points in one tetrad and may result in the formation of one or more chiasma.

5.Terminalisation:After the process of crossing over, the non- sister chromatids start to repel each other due to lack of attraction force between them. The repulsion of chromatids starts from the centromere

towards the chiasma and the chiasma itself moves in a zipper fashion towards the end of the tetrad. The movement of chiasma is known as terminalization. Due to terminalisation the hlomologous are separated completely.