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A CONCEPT BASED EXCLUSIVE MATERIAL

Molecular Genetics

B.Sc. Biotech Part-II

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Preface

I am glad to present this book, especially designed to serve the needs of the students. The book has been written keeping in mind the general weakness in understanding the fundamental concepts of the topics. The book is self-explanatory and adopts the "Teach Yourself" style. It is based on question-answer pattern. The language of book is quite easy and understandable based on scientific approach.

Any further improvement in the contents of the book by making corrections, omission and inclusion is keen to be achieved based on suggestions from the readers for which the author shall be obliged.

I acknowledge special thanks to Mr. Rajeev Biyani, *Chairman* & Dr. Sanjay Biyani, *Director* (*Acad.*) Biyani Group of Colleges, who are the backbones and main concept provider and also have been constant source of motivation throughout this Endeavour. They played an active role in coordinating the various stages of this Endeavour and spearheaded the publishing work.

I look forward to receiving valuable suggestions from professors of various educational institutions, other faculty members and students for improvement of the quality of the book. The reader may feel free to send in their comments and suggestions to the under mentioned address.

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Section A

Macromolecular Synthesis; Chromosome structure and replication & Gene Expression

---

Q.1 Describe the structure of DNA as suggested by Watson and crick?

Ans Watson and crick in 1953 deduce the double helical structure of B DNA.

DNA is a double stranded helical molecule where the two strands are complementary to each other. The structure of DNA consist of following.

(A) <u>Chemical composition:-</u>

The chemical analysis has indicated that DNA is confused of three different types of compounds:-

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(i) Sugar Molecule:- Represented by a pentose sugar the deoxyribose or 2' deoxyribose.

(ii) Phosphoric acid

(iii) Nitrogenous bases

These are nitrogen containing organic ring compounds. These are of the following four types:-

- Adenine (A)
- Thymine (T)
- Cytosine (C)
- Guanine (G)

These bases are separated into two categories:-

(a) Purines :- These are two-ringed nitrogen compounds Adenine and guanine are two purines found in DNA.

(b) Pyrimidines:- These are formed of one ring only and include cytosine and thymine.

(B) Molecular Structure:-

$[\vec{H}_3PO_3]$

(a) Nucleosides: - It is a nitrogenous base with a molecule of deoxyribose (without phosphate group). The nitrogenous base is attached to first carbon atom c_1 of deoxyribose through N-glycosidic bond. In all there are four nucleosides in a DNA molecule.

These are:-

→	Adenosine	-	Adenine	+	deoxyribose
	Guano sine	-	Guanine	+	deoxyribose
	Cytidine	-	Cytosine	+	deoxyribose
	Thymidine	-	Thymine	+	deoxyribose

(b) Nucleotides: - (The monomers of DNA):-

A nucleotide is formed of one molecule of deoxyribose. One molecule of phosphoric acid and one of the four nitrogenous bases, there are four types of nucleotides namely:

Deoxyadenylic acid - Adenine + Dcoxyribose + H_3PO_3

Deoxyadenylic acid – Guanine + Dcoxyribose + H_3PO_3

Deoxyadenylic acid - Cytosine + Dcoxyribose + H_3PO_3

Deoxyadenylic acid - Thymine + Dcoxyribose + H_3PO_3

(C) Polynucleotide Chain:- (Linking of Nucleotides in a DNA Molecule) Polynucleotide chain consists of many nucleotide as monomers. In nucleotide the phosphate molecule is attached of fifth atom (c-5) of deoxyribose through a phosphodiester linkage. The adjacent nucleotides are connected together forming the sugar PO_4 chain arranged in

alternate fashion. The PO_4 molecules of a nucleotide is joined to the third atom of the deoxyribose. These are directed at right angles to the long axis of the pohynucleotide chain and are stacked one above the other.

Fig:- linking of nucleotides in a polynucleotide chain has direction and are marked as 5'-3'. 3' or OH terminus and 5' or P terminus (triPo_4 group)

Watson and crick suggested that in a DNA molecule there are two such polynucleotide chains arranged ant parallel or in opposite directions i.c. one such polynucleotide chain runs in 5'-3' direction, the other in 3'-5' direction. It means the 3' end of one chain lies besides the 5' end of other. In such structure the PO_4 groups of nudeosides I each polynucleotide chain or strand lie on the outside of the deoxyribose and the nitrogenous bases are directed inward. The nitrogenous bases of the two chain are linked through H bonds formed between oxygen and nitrogen atoms of the adjacent bases. The unique feature of pairing b/w bases is:-

- Purines (adenine and guanine) pairs with pyrimidines. (Cytosine and thymine), and
- Adenine pairs with thymine and cytosine with guanine. DNA consists of two complementary chains twisted around each other forming a right handed helix. One turn of helix measures about $3.4A^{O}$. it contains 10 paired nucleotides placed at regular intervals of $34A^{0}$. The diameter of the helix is roughly $20A^{0}$. A narrow helical groove

and a wide helical groove run along the DNA helix. The narrow groove is the distance b/w the paired molecules while the wide groove is the space b/w successive turns when the pair is wound into a helix.

Fig:- Double Helical Structure of DNA

Q. 2 Describe the mechanism of DNA replication?

Ans. Watson and crick suggested a very simple mechanism of DNA replication on the basis of its double helical structure.

Watson & crick suggested semi-conservative mode of DNA replication. According to the mechanism suggested following steps are included in the entire replication process:-

1. Recognition of the Initiation or origin of replication
2. Unwinding of DNA
3. RNA priming

} Init

4.Formation of DNA on RNA primers. } Elongation
5.Excision of RNA primers

6. Joining of okazaki fragments. } Termination

(i) Recognition of initiation point:- The point at which DNA replication begins. c/d Initiation point or origin where replication fork begins.

This is a nucleotide sequence of 100 to 200 bp. Specific initiator proteins. Recognize the initiation point on DNA. The initiator proteins along with DNA directed RNA polymerase initiate the synthesis of RNA primer for the formation of DNA chain. In prokaryotic chromosomes there is only one new origin per chromosome but in eukaryotes each DNA molecule has many origins or replication fork. [~ 1000].

(ii) Unwinding of DNA:- The unwinding proteins bind to the nicked strand of the duplex end open up a 10bp or bubble, separating the two strands of DNA duplex.

(iii) RNA priming:- The DNA directed RNA pol now synthesizes the primer strands of RNA (RNA primer). The priming RNA strands are complementary to the two strands of DNA and are formed of 50 to 100 nucleotides.

(iv) Formation of DNA on RNA primers:- The new strands of DNA are formed in the 5^1-3^1 direction from the $3^{1-}5^1$ template DNA by the addition of deoxyribo nucleotides to the 3^1 end of primer DNA. The addition of deoxyeribo nucleotides to the 3^1 end of primer RNA. The addition is affected by DNA pol III (poly III- copal III) in the presence of ATP. Once the synthesis of DNA strand has been initiated, copol III detaches and copol III carries out replication of DNA strand. The unwinding separate the duplex strands ahead of the replication fork. The leading strand of DNA is synthesized in 5^1-3^1 direction one piece. The lagging strand of DNA is synthesized in its opposite direction in short segments consisting of 1000, 2000 nucleotides. These segments are c/d okazaki fragments.

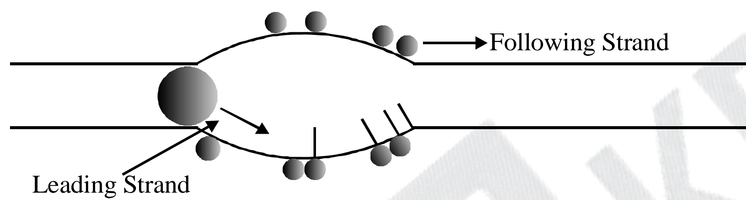
(v) Excision of RNA primers:- Once a small segment of an okazaki fragment has been formed, the nucleotides of RNA primer are removed from the 5^1 end one by one by the action of 5^1-3^1 exonuclease activity of DNA pol-I.

(vi) Joining of okazaki fragments:- The gaps left b/w okazaki fragments are filled with complimentary deoxyribo nucleotide residues by DNA pol I. Finally , the adjacent 5^1 and 3^1 ends are joined by DNA ligase.

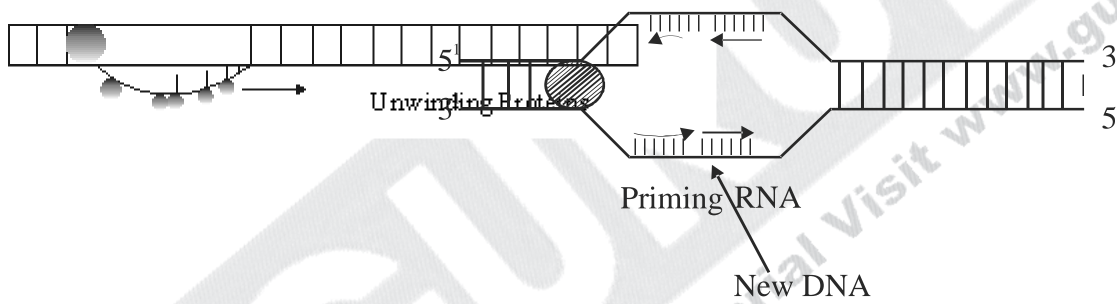
Fig. for DNA replication.

(a) Recognition of the origin by RNA polymerase

(b) Unwinding of DNA strands

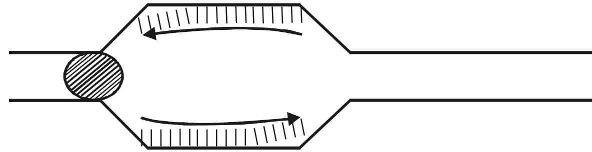


(C) Formation of DNA on RNA Primers by pol III*



(d) Formation of DNA on RNA primers by DNA pol III*

(e) excision of RNA primers by endonucleases to yield okazaki fragments



(f) Filling of gaps by DNA pol I and sealing by DNA ligase

Q.3 Describe the various enzymes required for the process of DNA replication?

Ans. About 20 or more different proteins and enzymes are required during DNA replication. These collectively form a system called DNA replicase system or replisome.

The enzymes fall into two categories:-

(i) DNA polymerase.

(ii) Poly-nucleotide ligase.

It has three sites for attachment one of them attached to the template DNA, the second to the triphosphate nucleotide and third one to the 3^1– OH end of the DNA primer. Thus DNA polymerase adds triphosphate nucleotides to primer DNA from 5^1 end and to the 3^1 end of the polynucleotide chain. The new strands are synthesized in fragments and these fragments are then added up by the enzymes, polynucleotide.

(i) DNA polymerase enzymes:-

They are of three types:-

(i) DNA pol – I (ii) DNA pol – II (iii) DNA pol – III

(a) <u>DNA pol – I</u>

This enzyme has been studied in E. Coli in detail. It is roughly spherical with a diameter of about 6.5 mm. it has a molecular weight of 1,90,000 and is formed of a single polynucleotide chain of about 1,000 amino acid residues. It consists of a sulphydryl group, single inter chain disulphide and one zinc molecule at the active site.

This DNA pol is formed of:-

DNA pol 3^1-5^1 exonuclease

· 5^1-3^1 exonuclease

There are five specific binding sites on the spherical molecule of pol-I:-

(i) Template site for binding the template DNA.

(ii) Primer site for binding primer strand of DNA.

(iii) Primer terminus site for 3^1-OH terminus of primer.

(iv) 5^1 triphosphate site – a locus for incoming deoxyribo nucleotide 5^1 triphosphate group.

(v) 3^1-5^1 exonuclease site, a locus for 3^1-5^1 exonuclease activity situated in the path of growing chain.

DNA pol-I was discovered by kornberg & his colleagues in 1955, the various fractions carried out by DNA pol-I

(i) It participates in the repair of DNA by catalyzing the addition of mononucleotide units (the deoxyribo nucleotide residues) to the free 3^1- on en dof DNA chain. A pure DNA pol-I can add about 1000 nucleotide residues per min per molecule of enzyme at 37^0C.

(ii) It catalyzes 3'-5' exonuclease activity and removes nucleotide residues of primer RNA at 3^1 end.

(iii) It catalyses 3'-5' exonuclease activity which is used in proofreading.

(b) DNA polymerase-II:-

The biological role of pol.II is not yet k/n. it is effective only on DNA duplex with gaps and cannot replicate leading strands.

(c) DNA pol-III:-

Discovered by T. Kornberg end M.L. Gefter(1972). It is the most active enzyme out of the three polymerases.

DNA pol III is mainly responsible for DNA chain elongation. It is a large end complex molecule with molecule weight about 5,50,000. the enzyme is formed of no. of subunits as shown in fig:-

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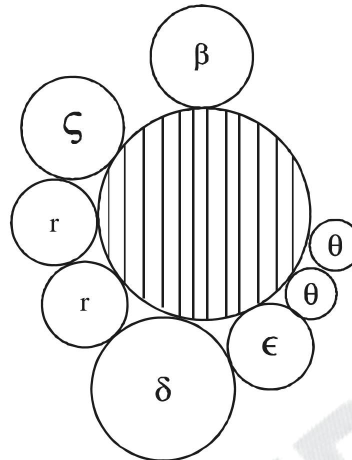

Fig:- DNA pol-III holoenzyme. It wnsists of subunits ....

The subunit β also c/d copolymerase-III recognizes and binds to the primer strand of the parental DNA. After pol-III binds to the correct initiation point the copolymerase-III is released. DNA pol-III now elongates DNA in 5^1-3^1 direction by adding new units to the 3^1 end of primer strand and thus cannot initiates replication. It can also act as 5^1-3^1 exonuclease and 3^1-5^1 exonuclease and can hydrolyze terminal nucleotides from either end of a DNA strand.

Polynucleotide ligase or DNA ligase: -

DNA- ligase can join the ends of two-fragments of DNA chain by catalyzing the synthesis of a phosphodiester bound b/w a 3^1-on group at the end of one chain and 5^1- PO_4 group at the end of other chain.

Functions of DNA-ligase:-

(i) To join the segments of DNA in the process of DNA replication.
(ii) To repair single-strand nicks in duplex DNA.
(iii) To link the ends of linear DNA duplexes to yield circles.
(iv) To join segments of DNA in the procen of recombination which occurs during meiosis, transduction or in genetic transformation.

Q.4 Describe the structure of chromosome with suitable diagram?

Ans. In resting no dividing eukaryotic cells the genome is nucleoprotein complex, called chromatin. It is amorphous and is randomly dispersed in the nuclear matrix as

interwoven network of line chromatin threads. When cell prepares to divide, the chromatin condenses into a species – specific number of well defined chromosomes.

Chromosomes structure

Chromosomes consist of different regions as part of their structure these include:-

(a) Primary constriction and Centro mere:-

A part of the chromosome is marked by a constriction which is comparatively narrow than the remaining chromosome. It is k/a primary constriction.

Its position is constant for a given chromosome and forms a feature of identification

The primary constriction divides the chromosome into two arms. It consist of DNA of repetitive type. This DNA is called centromeric hetero chromatin.

(b) **Secondary Constriction or Nucleolar Organizer:-**

Sometimes one or both the arms of a chromosome are marked by a constriction other then the primary constriction. During interphase the area is associated with the nucleolus and is funnel to participate in the formation of nucleolus. It is therefore, k/a nor / secondary constriction.

(c) **NOR / Nucleolar Organizer Region:-**

In certain chromosomes, the secondary constriction is intimately associated with the nucleolus during interphase. It contains genes coding for 18s and 28s or RNA and is responsible for the formation of nucleolus. The region k/a nucleolar organizer region (NOR).

(d) **Tertiary Constriction:-**

Are present in nearly all the chromosomes. Their significance is not k/n. these help to distinguish one chromosome from others.

(e) **Telomeres:-**

The tips of the chromosomes are rounded and sealed and are c/d Telomeres. These provide stability to the chromosomes and protect their individuality, because the telomeric ends do not form any permanent association with other parts of homologous or non-homologous chromosomes whereas the broken ends may join.

(f) **Chromatids :-**

A metaphase stage a chromosome consist of two chromatids joined at the common Centromere. In the beginning of anaphase. When Centro mere devices the two

chromatids acquire independent Centro mere and each one changes into a chromosome.

(g) **Centromere:-**

Under electron microscope the centromere appears as a plate-like or cup-like disc, plastered upon the primary constriction. It is about 1.20-0.25 in diameter and is formed of some non-chromatin material. The centromeres serves as a nucleation centre for the polymerization of tubulin, the protein used in the formation of microtubules. It may help in the formation of spindles fibres during prometaphase and metaphase.

Molecular organization of chromosomes:-

The main components of chromosomes that define its molecular identity includes the following:

(i) **Structural Protein / packaging proteins:-**

These are non-specifically binding proteins. These are bound to DNA along most of its length and help to package it without preventing the access of other DNA-binding protein.

Histones:-

Histones are main structural proteins found in eukaryotic cells. These are low molecular weight protein with high proportion of positively charged amino acids (basic amino acids- argeinine and lyoine). The positive charge helps histones to bind to DNA and play a crucial part in packing long DNA molecules. These are of 5 main types divided into two categories: -

(i) **Necleosomal hostones:-**

These are small proteins sesponsible for coiling DNA into nucleosome. These are H_2A_1 H_2B, H_3 and H_4. Each of them is formed of about 102-135 amino acids.

(ii) **H_1-histones:-**

These are large (about 200 amino acids) and are tissue specific. They are present once per 200 bp. These are loosely associated with DNA H1 histones are responsible for packing of nucleusomes into 30 nm fibre.

Nucleosomes: -

These are the fundamental packing units of chromatin and give chromatin the beads on a string appearance.

Each nucleosome is disc-shaped about 10nm in diameter. It consists of a core particle and a small spacer or linker DNA.

Core particles:-

They consist of the octamer of histones, having two copies of each H_2A_1 H_2B, H_3 and H_4. it is about 11 nm in diameter and 6nm in height. A strand of DNA having 146 base pairs is tightly wrapped around this core forming two cirdes.

Spacer DNA :-

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It is a small segment of DNA having just four base pairs. One unit of H_1 is associated with it.

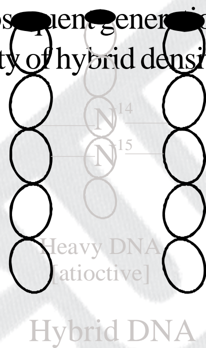
Q.5 Give evidences in support of semi conservative mode of DNA replication?

Ans: There are number of evidences in support of DNA replication given by different scientists:-

(i) **Meselson & stahl's experiment: -** In 1958 gave experimental evidence on semi conservative mode of DNA replication. They cultured species of bacteria (E. coli.) in a culture medium containing N^{15} isotopes of nitrogen. After these had replicated for a few generations in that medium both the strands of their DNA contained N^{15} as constituent of purines and pyrimidines. When these bacteria with N^{15}were transferred in cultural medium containing N^{14} it was found that DNA separated from fresh generation of bacteria possesses one strand heavier than the other. The heavier strand represents the parental strand heavier than the other. The heavier strand represents the parental strand and lighter one is the new one synthesized from the culture indicating semi conservative mode of DNA replication.

(ii) **Density determination experiments** By them. In this experiment the DNA of parental generation (labeled with N^{15}) was found to be heaviest. In first generation it was lighter than the first one. In second generation two bands were observed indicating the presence of DNA with two different densities, as is expected according to semi conservative method of DNA replication. These two bands were of equal intensity in the second generation. In the subsequent generation the intensity of light density bands gradually increased and the intensity of hybrid density band gradually decreased.

Hybrid DNA
(N^{15} -N^{14})



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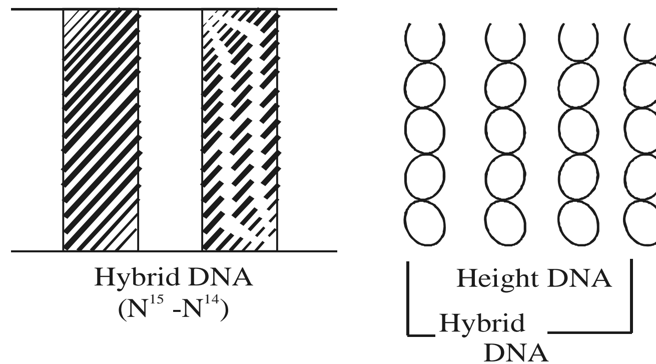

(iii) **Cairns Autoradiography Experiment:-**

I. cairns demonstrated the semi conservative mode of DNA replication in the chromosome of bacterium, E.Coli by autoradiography using radioactive thymine. Radioactive thymine or tritiated thymidine is obtained by using heavy isotope of hydrogen – H_3. By growing E. Coli in the culture medium containing tritiated thymidine, radioactive was incorporated in the daughter DNA molecules. Such radioactive DNA molecules are described as labeled DNA when mounted on a photographic emulsion for autoradiography; the labeled DNA exposes the film producing a diffused profile of the DNA molecule. The duplicating DNA molecule shows a replicating fork, the point at which two chains become four. After first replication, radioactivity is seen to be incorporated to only one of the strands of DNA and both strands are found to be labeled after second replication. This supports the semi conservative mode of DNA replication.

(iv) Taylor's experiments on Vicia Faba root tip:-

J.H. Taylor (1957) and his coworkers also demonstrated the semi conservative method of DNA duplication in the root tip cells of *vicia faba* by autoradiography. The roots were grown in a medium containing radioactive thymidine, so that radio-activity is in corporated in the DNA of these cells. The outline of this labeled chromosome on a photographic film appears in the form of scattered black dots of silver grains. When these root tips with labeled chromosomes were transferred. To the unlabelled medium containing colchicines and studied for radioactivity, the following observations were made:-

- In the chromosomes of first generation the radioactivity was found to be uniformly distributed in both the chromatids, because in them the original strand of DNA couble helix was labeled with radioactivity and the new one was non labeled.
- In the chromosomes of second generation only of the two chromatids in each chromosome was radioactive.

Q. 6 What do you mean by "Genetic Code"? Discuss in brief the special features of genetic code.

Ans. **Genetic Code:-**

A genetic code is the sequence of nitrogenous bases in mRNA molecule, which encloses information for the synthesis of protein molecules.

Features of genetic code:-

(a) Triplet:- A cod on of the modern genetic code comprises of three nitrogenous bases of mRNA in a specific sequence.

(b) Commaless:-There is no punctuation (Comma) b/w the adjacent codons i.e. each codon is immediately followed by the next codon with no intervening spaces of letters for comma.

(c) Non-Overlapping:- A triplet code with overlapping sequence was suggested. Under the overlapping triplet code the number of codons could be reduced to twenty. But evidences have suggested existence of non-overlapping code.

(d) Ambiguity:- The genetic code inside the cell medium(in vivo) is said to be non ambiguous, because a particular codon always codes for the same amino acid. The same amino acids may be coded by more than one codon (degeneracy), but one codon never codes for two different amino acids.

But it has been found ambiguous in some cases (some codons, coding for amino acids under different condition.)

For e.g.- In streptomycin sensitive strain of *E. Coli* UUU codon, normally codes for phenylalanine, but may also code for isoleucine, leucine or serine when ribosome's are treated with streptomycin. This ambiguity is enhanced at high Mg-ion concentration, 10w temperature and in presence of ethyl alcohol.

5. **Universality:-** The some genetic code is said to be present in all kinds of living organisms including viruses, bacteria unicellular and multicellular organisms.

6. Collinearity:- The codons is DNA and mRNA and the corresponding amino acid residues in the polypeptide chain have a linear arrangement is demonstrated by the studies of T4 Mutants. These produces incomplete head protein molecules. These

mutants can shown to map in linear sequence by recombination techniques. This suggests that the code is collinear.

Q.7 Write a note on degeneracy of genetic code?

Ans. Most of the amino acids can be directed to their specific places in the peptide chain by more than one codons. The multiple system of coding is k/a degenerate code and provides a protection to organisms against many harmful mutations, stabilizes phenotypes by lessening the effect of random mutations and minimizes the consequences of base pairing errors. The major degeneracy occurs at the third position. (3^1 end of the triplet codon). within the first two bases are specified the some amino acid, may be coded for whether the third base is C_1, C_1A or C_1. This base is described as 'wobbly base'.

For example, the genetic codes for the following amino acids.

(i) Serine – UCU, UCC, UCA, UCG and AGU, AGC.

(ii) Arginine – CGU,UGC, CGA, CGG and AGA, AGG.

(iii) Hevcine – CUU, CUU, CUA, CUG and UUA, UUG.

(iv) Valine – GUU, GUC, GUA, GUG.

<u>**Reasons for degeneracy :-**</u>

1. Crick proposed wobble hypothesis to describe this lack of specificity in the third base of codon. The base in the first position of antic don on tRNA is usually an abnormal base like inosine, pseudouridine, tyrosine etc.

   These abnormal bases are able to base pair with more than one base in the third position of the codon. Inosine (I) can pair with A, C and U.

II. Amino acyl tRNA- synthetase enzyme may commit some mistake in the attachment of proper amino acid.

Q.8 Describe the process of replication of bacterial chromosomes?

Ans. Bacterial chromosomes consist of circular DNA. The duplication of this chromosomes prior to cell division is carried out by a complex and efficient set of catalytically active proteins, each dedicated to different tasps needed to replicate a very large molecule.

The type of replication that occurs is called as bi – directional replication where two replication forks proceeding from an origin of replication (cori) to the terminus in opposite directions.

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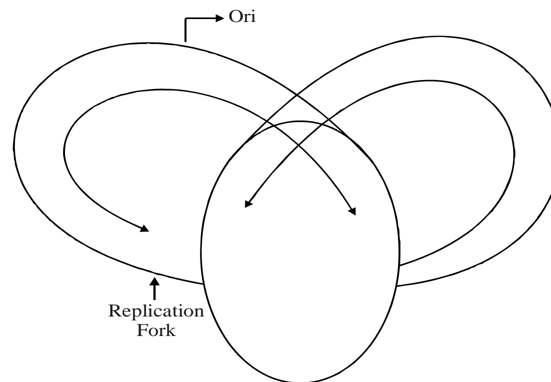

Half- chromosome replicating unit is called a replichore. The two replichores of a circular chromosome undergo very similar processes. The process of replication can be underlined under 3 major steps:-

(i) **Initiation:-**

Ori – The *E. Coli* bacterial replication origin c/d. Ori C consists of 245 be. Bearing DNA sequence that are highly conserved among bacterial replication origins. The chromosomal origin, function as a site where enzymes assemble to form the machinery that will generate the replication fork.

DNA sequence elements within ori that are important for its function include DnaA boxes, a repeat with a highly conserved consensus sequence 5'-TTATCCALA-3', that are recognized by the DnA protein. DnAa protein plays a crucial role in the initiation of chromosomal DNA replication. Bound to ATP, and with the assistance of bacterial histone like proteins (HU) DNA then unwinds an AT-rich region near the left boundary of oric, which carries three 13-mer motifs, and opens up the double-stranded DNA for entrance of other replication proteins. This region also contains four "GATC" sequences that are recognized by DNA adenine methyl's (DGM), an enzyme that modifies the adenine base when this sequence is un methylated or hemi methylated. The methylation of adenine is important as it alters the confirmation of DNA to promote strand separation, and it appears that this region of oric has a natural tendency to unwind. DnaA then recruits the replicative helicase, DnaB, from the DnaB-DnC complex to the unwound region to form the pre-priming complex. After DnaB Translocates to the apex of each replication fork, the helicase both unwinds

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the parental DNA and interacts momentarily with primase.

In order for DNA replication to continue, single stranded binding proteins are needed to prevent the single strands of DNA from forming secondary structures and to prevent them from re-annealing.

In addition, DNA by rose in needed to relieve the topological stress created by the action of DnaB helicase. DNA sequence motifs in oric of the E. coli. The gray bars represent GATC sequences recognized by DNA adenine methyl transfer ase. Small blue arouse are 13- mer sequences near the left border of oriC that become single-stranded when oric is bound by DnaA in association with ATP. The red boxes are DnaA box sequences recognized by DnaA protein. Smaller green boxes represent I sites bound by DnaA –ATP. The site within oric to which integration host factor(IHF) binds is shown b/w DnA boxes R1 &Rs (M). Dashed lines represent two regions bound by seqA protein.

(ii) **Elongation:-**

Bacterial chromosomal replication occurs in a bidirection manner. In specific experiments, E. Coli cells initiating chromosome replication after release from amino-acid starvation were inculcated in [3h] thymine plus [34] thymicline of very high specific activity. The grain troops produced in autoradiograph of chromosomes were denser on both ends than in the middle. The auto radiographic patterns are, therefore, evidence that replication of the chromosome in E. Coli is bidirectional.

The E.Coli replicase DNA pol III is a 900 KD complex possessing a dimeric structure. Each monomeric unit has a catalytic core a dimerization subunit and a processvityci component. Some of the core subunits of DNA pol are involved in synthesizing the leading strand continuously, while the other set of core subunits cycles form one okazaki fragments to the next on the looped lagging strand. Leading strand synthesis begins with the synthesis of a short RNA primer at the replication origin by the enzyme primase (DnaG protein).

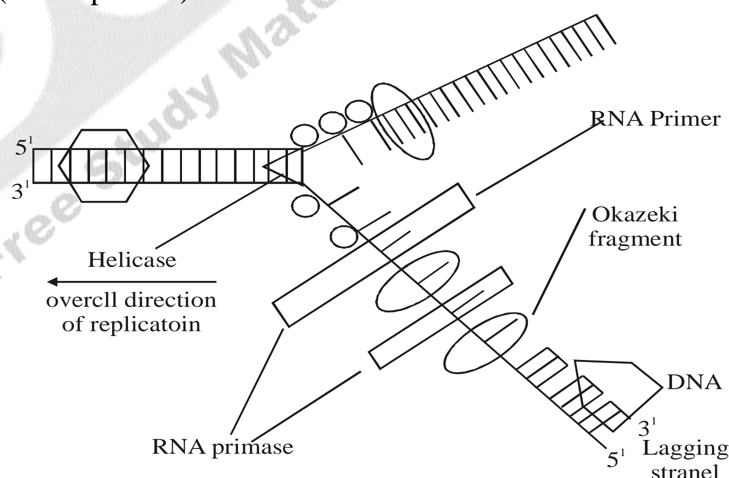

Fig :- Bacterial DNA replcation

Deoxynuleotides are then added to this primer by a single DNA Pol III dimer, in an integrated complex with DnB helicase. Leading strand synthesis proceeds continuocesly while the lagging strand synthesized accomplishes in short okazaki fragments. First an RNA primer is synthesis, DNA pol III binds to the RNA primerand acids deoxyribo nucleotides.

When the synthesis of an okazaki fragments has been completed, replication halts and the core subunits of DNA pol III dissociates from the B sliding clamp and the remaining nick is sealed by DNA ligase, which then ligates these fragments to form the lagging
strand. Since replication of a circular chromosome occurs bidi rectionally, when the replication fork moves around the circle, a structure shaped like Greek letter is formed that's why the model is named as theta replication model of circular bacterial chromosomes.

Termination:-Replication termination of prokaryote occurs at specific sequences called replication termini. These terminating sequences are designated as ter sequences located in a region opposite to the replication origin. The ter sites have polarity, that is, they arrest replication forks, when they are present in one orientation with respect to ori, but allow forks to pass through unrestricted in the opposite orientation. The arrangement of the Ter sites forms a replication trap that forces the two forks, initiated at ori C, to meet each other within a well-defined region of the chromosome.

- Ter sites specifically interact which is a polar contrahelicase, that is, it stops the DNA unwinding activity of DnaB in an orientation dependent manner.

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Section - B

RNA Structure a nd Function & Translation of protein Synthesis

Q.1 Write short notes on structure of with suitable diagrams?

(a) mRNA

(b) rRNA

(c) tRNA

Q.2. With suitable diagrams?

Ans. (a) <u>mRNA:-</u> Messenger RNA or nuclear RNA mRNA is synthesized inside the nucleus as a complementary strand to DNA and carries genetic information from chromosome DNA to the cytoplasm for the synthesis of proteins. For this reason, it is c/d as messenger RNA by Jacob and monod in 1961. It constitutes about 10% of the total RNA present in the cell.

<u>Structure of mRNA:-</u>

It is formed as a complementary strand to one of the two strands of a DNA. It carries same sequence of base arrangement as found in that part of DNA from C it is copied except that at the place of thymine RNA contains uracil. All mRNAs carry two non-coding regions. The region at 5^1 end is 50 bases where as at 3^1 end it is 90-100 bases. The 5^1 end non-coding region is c/d head while 3^1 end non-coding region is c/a tail.

mRNA acts as a template for protein synthesis and has a short life span and so has a high turn over. It consist of poly –A sequence at the 3^1 end and 7- methyl guano sine on the 5^1 end.

(b) **Ribosomal RNA(rRNA):-**Ribosome it is the major component of ribosome and is the most abundanof the cellular RNA(82%). It is made up of ribo nucleoproteins.

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Entire ribosome, rRNA makes 66% in prokaryotes and 60% in eukaryotes. E.Coli contains three types of rRNAs: 23, 16 and 55s rRNA Mammalian ribosome's contain 4 types of rRNAs: 28, 5.85, 85 (these 3 are cleaved from 45s precursors 55s (synthesized from an entirely different transcription unit).

Base composition of rRNA :-

rRNA differs in base content from tRNA and mRNA. It is relatively rich in guanine and cytosine. The base components in rRNA of E.Coli have a molar ratio of adenine 21: Uracil 19: guanine 36: cytosine 23.

Biogenesis of rRNA:-

The rRNA is present in ribosome's but form inside the nucleus. DNA associated with the nucleolus is responsible for coding rRNA. This part of DNA is known as nucleolar organizer. In bacteria about 10-210 cistrons are concerned with the rRNA synthesis. Whereas in higher organisms 210-2100 tightly clustered rDNA cistron are involved in rRNA synthesis.

The two types of rRNA 28s and 18s are transcribed form the nucleolar RNA as a single elongated unit of 45s. Inside the nucleolus the 45s RNA is methylated and complexed with protein, finally, via a number of steps it is cleaved into 32s and 18s segments. The 18s RNA gets associated with basic proteins to form the small subunit of ribosome. The 32s segment is further severed and finally changes to 28s RNA. This 28s RNA also gets associated with proteins and form the large subunit of ribosome. These units then come out into the cytoplasm. The 55s RNA has a extranucleolar origin, transcribed from genes located outside the nucleolar organizer. It gets associated with each large ribosomal subunit.

(c) **tRNA:-**

The transfer RNA is a family of about 60 small sized ribonucleic acids which can recognize the codons of mRNA and exhibit high affinity for 21 activated amino acids, combine with them and carry them to the site of protein synthesis. tRNA molecules are also named as soluble RNA, supernatant RNA or adaptor RNA. It is about 10-15% of the total weight of RNA of the cell.

Features & structural components of tRNA:-

1. tRNA molecules are smallest, containing 75-80 nucleotides.
2. Their sedimentation constant is 4s and molecular weight 25,000 Daltons.
3. Its polynucleotide chain is bent in the middle and folded, back on itself (clover leaf

model) and the two arms coiled over one another.

4. Some of the bases of the two arms coiled over one another.

5. The 3'end of the polynucleotide chain ends in CCA base sequence.This represents site for the attachment of activated amino acid. The end of the chain terminates with guanine base.

6. The bent in the chain of each tRNA molecule contains a definite sequence of three nitrogenous bases which constitute the anticodon. end of the chain terminates with guanine base.

7. Four different regions or special sites can be recognized in the molecule of tRNA. These are:-

(i) **Amino acid attachment site:-** It occurs at the 3'end of the tRNA chain and has OH group combines with specific amino acid in the presence of ATP forming amino-acyl tRNA. It is common to all the tRNA molecules.

(ii) **Recognition site:-** It contains a specific base sequence which dictate the attachment of correct amino acid to the tRNA molecule. It matches with the amino acid activating enzyme through which attachment of amino acid to tRNA takes place.

(iii) **Anticodon or codon recognition site:-** The site has three unpaired bases (triplet of base) whose sequence is complementary with a codon (triplet of base) in mRNA. Therefore, it determines the pairing of tRNA with the specific codon (triplet) of mRNA. It is therefore the most specific region of tRNA molecule.

(iv) **Ribosome recognition site:-** This helps in the attachment of tRNA to the ribosome. This site is common to all the molecules of tRNA.

8. In addition to the usual bases of RNA (adenine, uracil, cytosine, guanine) each tRNA molecule consists of several unusual bases. Some of them are pseudouridine inosinic acid, methyl guanine, methyl amino urine etc.

The presence of these rare nucleotides or unusual bases prevent intermolecular base pairing in the open tRNA loop or help in the recognition of ominously tRNA synthetase enzyme.

tRNA molecules occur in both active and inactive forms. The inactive molecules of tRNA lack the CCA sequence of nitrogenous bases at 3'end of the chain either in full or in part. By the addition of these nucleotide sequence an active tRNAs molecule can be activated. The process is governed by the enzyme cytidine triphosphate (CTP)

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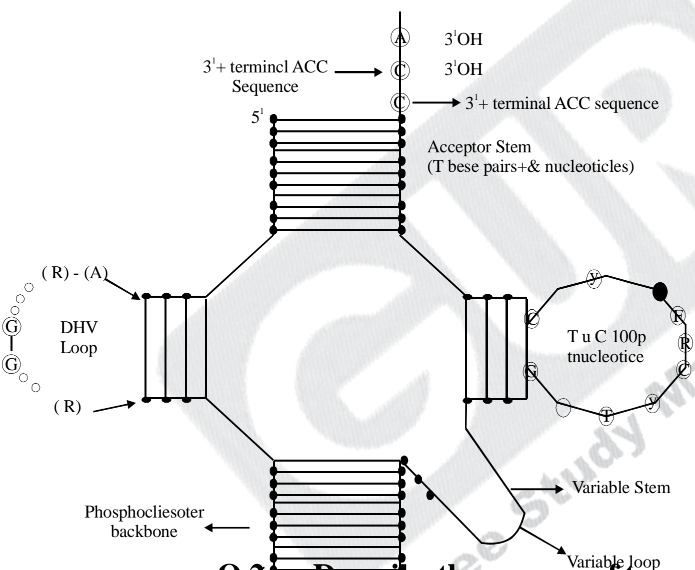
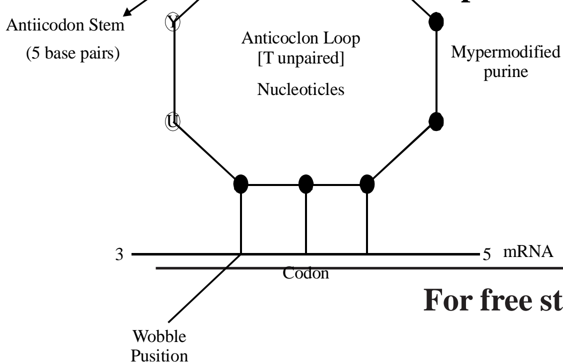
and the ATP.

Structure of tRNA- **Clover leaf structure.**



Q.2 Describe the process of transcription in prokaryotes?

Ans. DNA transcription: -



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DNA transcription is a process which involves the formation of mRNA on DNA tempelate. Transcription generates the mRNA that carry the information for protein synthesis, as well as the transfer, ribosomal and other RNA molecules that have structural or catalytic functions. All of these RNA molecules are synthesized by RNA pol enzymes, which makes an RNA copy of a DNA sequence. In eukaryotes three kinds of RNA pol molecules synthesize different types of RNA.

In prokaryotes RNA pol is a large multi subunit enzyme associated with several additional protein subunits that enter and leave the polymerase DNA complex at different stages of transcription.

RNA polymerase enzyme

In prokaryotes RNA pol, like DNA pol, is a complex enzyme made up of multiple polypeptide chains. The intact enzyme consists of 4 different subunits:-

and The subunit is relatively weakly bound and can be separated from the other subunits, resulting is a core polymerase consisting of two sub units.

The core polymerase is fully capable of catalyzing the polymerization of NTPs into RNA, indicating that is not required for the basic catalytic activity of the enzyme. The 6 subunit plays an important role in identifying the correct site for initiation.

Process of transcription: -

The process of transcription has been divided into 3 steps:-

(i) Initiation
(ii) Elongation
(iii) Termination

(i) Initiation: -

Promoter:- The DNA sequence to which RNA pol binds to initiate transcription. There are some DNA sequences involved in promoter function located ~ 10 & 35 base pairs upstream of the transcription initiation site. They are called the – 10 and -35 elements.

It the absence of , RNA pol binds nonspecifically to DNA with low affinity. The role of Is to direct the polymerase to promoters by binding specifically at both the -35 and -10 sequences, leading to the initiation of transcription at the beginning of a gene. The initial binding b/w the pol and a promoter is c/d as a closed-promoter complex because DNA is not unwound.

Elongation:-

After the formation of closed-promoter complex the polymerase unwinds approximately 15 bases of DNA around the initiation site to form an open promoter complex in which single stranded DNA is available as a tempelate for transcription. After addition of about the first 10 nucleotides, It is released from the polymerase, which then leaves the promoter and moves along the template DNA to continue elongation of the growing RNA chain. This marks the beginning of elongation. The pol enzyme unwinds the templeate DNA ahead of it and rewinds the DNA behind it, maintaining an unwound region of about 17 base pairs in the region of transcription.

RNA synthesis continues until the polymerase encounter a termination signal, at which point transcription steps, the RNA is released form the polymerase, and the enzyme dissociates from its DNA template.

Termination:-

As soon as RNA pol encounters a termination signal transcription stops. The transcription termination in both prok and euk. Cells, depends on the binding of proteins that terminate transcription to specific DNA sequences, rather than on the formation of a stem- loop structure in the RNA. The formation of stem-loop structure mination signals which is GC-rich inverted repeat resulting egment of RNA that can forma stable stem loop structure pairing.

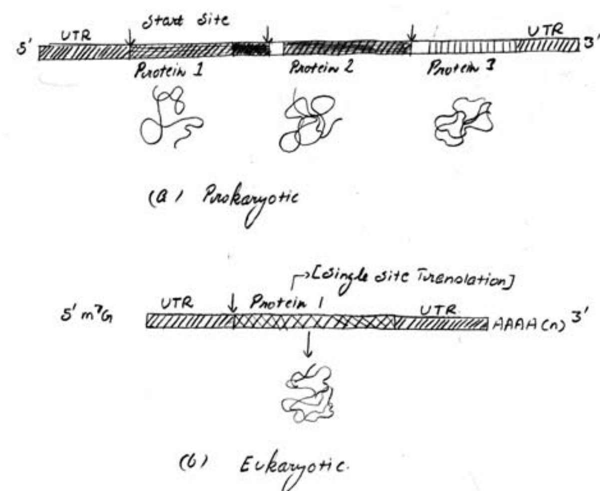

Fig.: Various steps in the process of transcription.

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Q. 3 Describe the mechanism of eukaryotic translation?

Ans Translation:-

The process of protein synthesis where mRNA formed as a result of DNA transcription which provide amino acid sequences via codons specific for each amino acid, later forming a polypeptide.

Components of translation:-

(a) Amino acid:-All type of amino acids essential as well as non-essential should be present at the site of protein synthesis or else the process is terminated.

(b) t-RNA:-

Type of t-RNA molecules carrying an anticodon for each codon are present in the cell. It they are attached at the 3'end with the specific amino acid, they are known as charged t-RNA. These are active and can participate in translation. The t-RNA molecules need to be aminoacylated is facilitated by the presence of 20 cifferent aminoacyl t-RNA systhetase enzymes one each for 20 individual amino acids used for protein synthesis.

(c) Ribosome:-These are ribonucleoprotein molecule containing two subunits. One large and one small. In prokaryotes they are of 50s and 30s, eukaryotes 60s and 40s types. Two types of binding site are present on the ribosome.

A-side- Amino acyl t-RNA

P-side – Peptide t-RNA

(d) mRNA:- Active mRNA coding for specific protein in form of codons is required properly attached with the ribosome.

(e) Amino acyl transferase:-

aa+tRNA-amino acyl tRNA (charged tRNA)

A transfer RNA molecule has to recognize several other molecules to perform its function. These molecule include both nucleic acid and proteins. Among nucleic acids, they recognize ribosomal RNA in the ribosome and codons in mRNA. Among proteins they recognize translation factors and aminoacyl synthesis enzymes.

(f) Energy:-GTP/ATP molecules required during the process.

Steps of protein synthesis:-

(a) Initiation:- When all the above components are present in the cell the process of translation starts. An assembly of the components containing m-RNA, small ribosomal

subunit is k/a initiating complex.

In prokaryotes there are more than one coding region on the m-RNA so more than polypeptides are formed such mRNA. In eukaryotes one m-RNA codes for one polypeptide k/a monocistronic mRNA

The initiator codon is recognized by the initiator t-RNA. In prokaryotes N- formylated methionine is attached to the initiator t-RNA. In eukaryotes non-formyated methionine is attached.

Energy is required for the attachment of larger subunits (50s/60s) of ribosome and the initiation of the process starts by acceptance of the t-RNA. This process is inhibited by streptomycin.

The initiation of polypeptide chain is always brought about by the amino acid methionine, which is regularly coded by the codon AUG, but rarely also by GUG by the initiation codon very rarely initiation may also be directed by UUG (~1%). In E. Coli there are two tRNAs for methionine, tRNA met non-formylable) and tRNA met (formylatable).

In eukaryotes, formylation of initiating methionine is not brought about due to the absence of tRNA met in plants due to the absence of tRNA met in plants and due to the absence of enzyme transformylase in animals. Initiation in higher organism will therefore, takes place without formylation..

Translation does not simply begin at 5^1 end of the mRNA, it starts at specific initiation sites the 5^1 terminal portions of both prokaryotes and eukaryotes mRNA are therefore non coding sequences, referred to as 5^1 untranslated regions.

In both prok. And euk. cells, translation always initiates with the amino acid methionine, usually encoded by AUG. alternative initiation codons such as GUG are used occasionally in bacteria, but when they occur at the beginning of a polypeptide chain, these codons direct the incorporation of methionine rather than of the amino acid they normally encode.

The process of initiation requires:-

- Ribosome subunits
- mRNA

- An energy source(GTP)
- Activated amino acid.
- The initiation factors.
- Ribosome have two sites
- Aminoacyl or A site
- Peptdyl or P site

The initiating codon AUG is positioned on the P site which is the only site to which f met- tRNA fmet (pro) or met tRNA can bind. All other incoming *aminoacyl tRNAs* bound to A-site. The initiation complex installs itself on mRNA. The anticodon of the tRNA pairs correctly with the initiation codon at the P site. This involves IF2, IF3 & GPT. The larger subunit combines with the smaller subunit later onto form the complete initiation complex. This step involves release of IF-2, IF-3 & GTP. The larger subunit combines with the smaller subunit later onto form the complete initiation complex. Thus step involves release of IF-2 and IF-3 simultaneously GTP is hydrolyzed to FDP.

Elongation of polypeptide chain:-

Elongation takes place due to the incoming of amino acid at A site and subsequently transfer of P site amino acids to A site.

(i) Elongation of polypeptide chain requires following 4 steps.

(ii) Initiation complex.

(iii) The next aminoacyl tRNA, specified by the next codon in the mRNA.

(iv) A set of 3 soluble cytosolic, proteins called elongation factors.

(vi) GTP.

The last 3 steps takes place in the addition of each amino acid residue and this cycle is repeated as many times as there are residues to be added.

Termination of polypeptide chain:-

Aminoacyl t-RNA does not normally bound to the a-site of a ribosome if the codon is UAA, UGA & UAG. Normal cell does not contain tRNAs with anticodons complementary to these stop signals. These stop signals are recognized by small protein molecules c/a release factors.

- Release factors bind to termination codons in the A site. Binding of release factors activates peptidye transferase so that it hydrolyses the bond b/w the peptide and that RNA in the P site.

- The polypeptide chain leaves the ribosome followed by t RNA & mRNA.
- Finally the ribosome dissociates and the subunit are ready for second round.

Translation Factors

Steps	**prokaryotes**	**eukaryotes**
Initiation	IF-1, IF-2, IF-3	elF-1, elF-1A, elF-2, elF-2B, elF-3, elF-4A, elF- 4B, elf-4E, elF-4G, elF-5
Elongation	EF-To,EF-Ts,EF-G	eEF-1x,eEF-1B, eEF-2
Termination	RF-1,RF-2,RF-3	eRF-1,eRF-3

Q. 4 Write note on post translational modification?

Ans. Following are the modifications that takes place after translation:

(a) Trimming: Removal of small portions of the polypeptide chain to make it active.
e.g. Trypsinogen (i) Trypsin, (ii) Peptide
Activation of trypsinogen, formation of active insulin from pro-insulin.
Insulin from pro insulin (51 aa)
Pre insulin [2 chain c- 30 and 20 aa]

(b) Phosphorylation : The enzyme dephosphophosphrylase which is an inactive protein is attached to phosphorus group to form phosphophosphorylase which is an active enzyme for metabolism of glycogen.

(c) Glycolsylation: For formation of active proteins of the cell membrane glyslation to form glycoprotein is required. It is the addition of carbohydrate molecule.

(d) Hydroxylation:- Hydroxylation of amino acid of the polypeptide chain forms hydroxyl amino acid.

Q.5 Describe the process of gene regulation in prokaryotes at transcriptional level?

Ans. Gene is expressed in the form of polypeptide so to control expression is required at the transcriptional and translational level.

The regulation of this expression takes place by two main processes:-

(I) Induction:-
The genes or DNA segments induces or activated to produce the required protein. The inducer is the substance which stimulates the synthesis of proteins.

(II) Suppression or Repression : -
In suppression the activity of the gene is suppressed and synthesis of the protein is stopped. Substances that suppress the action are c/d repressors.

At transcriptional level protein synthesis is regulated by operons in case of prokaryotes. The concept of operon was introduced by Jacob and Monod for the first

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time & explained that induction and repression are the two processes by which a gene expression is regulated at the transcriptional level.

Gene regulation can be best explained by the help of lactose operon:

In E.Coli there are three enzymes required for catabolism of lactose are expressed by 3 sets of genes.

The three enzymes are:-

B-galactosidase, galactose permease and thiogalactoside transacetylase.

The three genes coding for the 3 enzymes c/d cistrons and are represented as cistrons Z, Y and A. these are the structural genes.

Certain genes control the process of catabolism of regulator, promoter and operator gene and are k/a control genes.

An operon is consist of structural and control genes which regulates genetically controlled metabolic activity.

(a) Regulator Gene:-
Denoted by 'r'. it produces specific enzymes which acts as repressor. This repressor binds to operator gene and suppresses its activity.

(b) Promoter Gene:-
Denoted by 'p'. It is a DNA segment where RNA Pol binds. It starts the transcription of structural genes.

(c) Operator gene:- denoted by 'O'
It controls the process of transcription and if combines with the repressor or transcription stops.

(i) In the presence of inducer i.e. lactose the regulator gene produces a repressor protein which binds to the operator gene and prevents activation of promoter to synthesise mRNA so the catabolic enzymes are not formed.
(ii) When lactose is (t)nt in the blood it acts as an inducer and binds to the repressor protein and an inactivated complex is formed which cannot bind to the operator.
(iii) The operator gene is free and activates RNA Pol to synthesise MRNA and the corresponding enzymes.

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Section-c

Recombinant DNA techniques and cloning

Q.1 What is mutation? Describe various causes of mutations?

Ans. Mutation:-

Any hereditary change in the genetic make up of an individual other than that which may be caused by the simple recombination of genes.

These include changes in the gene structure or composition (the gene mutations or point mutations) and the changes in the chromosomes either in structure or in number (chromosomal mutations).

The term mutation was introduced by Hugo de vries. He used the term for large spontaneous inheritable changes which occurs suddenly in naturally reproducing populations and propounded 'mutation theory.'

Causes of Mutation:-

Mutations are result of mutagens, mutagens are those substances that cause mutation. They fall into two category.

1. Radiation:-

All forms of energy radiations that are capable of disrupting the chemical structure of chromosomes have been found to be mutagenic in almost all the organisms. These may be of two types:-

(i) Natural radiations:-

(ii) Man-Made radiations:-

(i) Natural radiations:-

Radiations that come from cosmic rays of the sun are the main source of spontaneous Mutation. These occur in small amount in our environment and background radiation.

Another source of natural radiation is the radioactive elements like thorium, radium and uranium, present in the earth crust.

The type of radiations that arise as a result of radioactive elements include ionizing radiations like x-ray, gamma rays, alpha rays and beta rays, neutrons protons and other fast moving particles. Alpha and beta rays do not penetrate beyond the human skin and therefore would not affect internal body cells. The gamma and x-rays collide with the molecules of the cells at high speed and eject electrons from the outer shells of atoms.

(ii) Man-Made radiations:-

These include x-rays, gamma rays and uv rays. J.J. Muller (1927) showed that x-rays cause an increase in the number of mutations in the offspring of irradiated **Drosophila**. He also demonstrated that there is a linear relationship b/w the dosage of radiation and the number of mutations.

2. Chemical Mutagens:-

Chemical mutagens include those chemicals that can cause mutations. No of chemicals mutagens that are considered to be most powerful mutagens are:-

(i) Mustard gas & its related compounds.
(ii) Ethyl urethane
(iii) Formaldehyde - Mutagenic in **Drosophila.**
(iv) Organic peroxides – Mutagenic for fungus etc.

Q.2 Discuss various types of Mutations?

Ans Mutations are of different types:-

(i) Based on- type of cells in which mutations occurs:

(a) Somatic Mutations:-

This type of mutations occurs in somatic cells. It produces a local phenotypic change

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in the organ. The descendants of that particular cell show the particular Mutation. Somatic Mutations are not heritable and die with the death of the organism.

(b) Germinal Mutations:-

This type of Mutations occur in the germ cells or gametes. These are heritable and are expressed in the next generation, and are established in the population.

· Some of the mutations which very limited segment of DNA, are called point mutations. They may be of the following types:-

GENE MUTATIONS

(i) Substitution Mutations
(ii) Frame-shift Mutations.

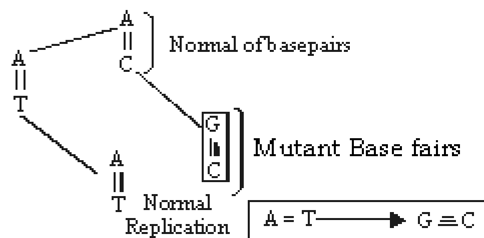
(A) Transition	(B) Transversion
Replacement of a	Replacement of purine
Purine pyrimidine	by a pyrimidine or vice
By another purine	versa is caused by
Pyrimidine in a	(a) alky lating agents
Polynucleotide chain,	(b) Depurination.
Caused by.	(a) Deletion Mutations
(b) Insertion	(a) Tautomerization, Mutations.
(b) Ionization	(c) Base analogs
(d) Deamination	

(i) **Substitution Mutations:-** In a substitution Mutation a nitrogenous base of a triplet codon of DNA is replaced by another nitrogen base or some derivative of the nitrogen base, changing the codon. The altered codon may code for a different amino acid and may result in the formation of a protein Molecule with a single amino acid substitution, whose effect may be seen in altered phenotype.

(a) **Transitions:-**Transition involve Mutations where a purine is replaced by another purine in same polynucleotide chain and replacement of one pyrimidine by another pyrimidine in the complementary chain. Transitional substitutions can be introduced by either of the following ways:-

(i) **Tautomerization:** In tautomerizaton four nitrogenous bases exist in their alternate form instead of their natural combinations (A=T and G=C). these tautomeric shifts or alternate forms are formed by the rearrangement in the distributions of hydrogen atoms-tautomeric shifts. Due to tautomerization the amino ($-NH_2$) group of cytosine and

adenine is converted into imino(-NH) group and likewise keto group (c=o) or thymine and guanine is converted to enol group(-OH).



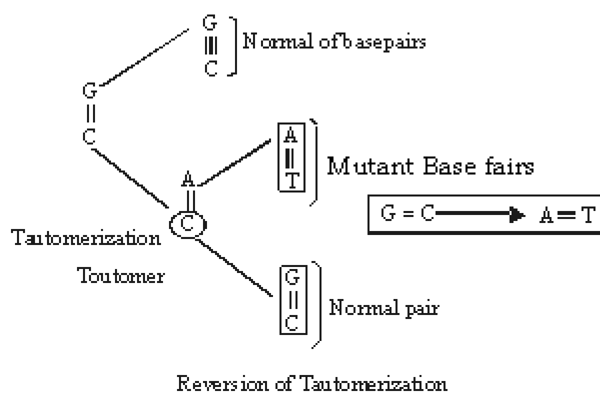


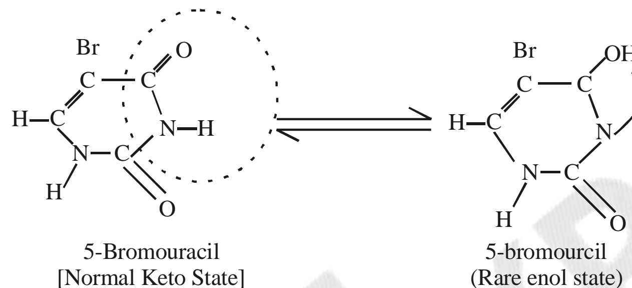
Fig :- Mechanism of substitution of A=T pair by G=C and G=C pair by A=T by tautomerization.

(b) **Ionization:**Transitions may also be introduced by ionization of a base at the time of DNA replication. Ionization involes the loss of the hydrogen from number-1 nitrogen of a nitrogenous base. e.g.- In its ionized. State, thymine pairs with normal guanine and ionized guanine links with normal thymine.

(c) **Base analogs:-** Certain chemical compounds have molecular structure similar to the nitrogenous base present in DNA nucleotides. These are called base analogs. These are usually derivatives of nitrogenous bases of DNA and occur as natural as well

as artificial base analogs. Some of the natural base analogs are 5-methyl cytosine (found in wheat and grasses), 5- hydroxymethyl cytosine and 5- glucosyl hydroxymethyl cytosine (in E.coil) etc.

Artificial base analogs:-5- bromouracil (5-BU), 5-iodo, URacil(5-IU), 2-bromo and 5-methyl cytosine. The first two examples are base analogs of thymine and later those of cytosine.e.g



(d) **Deamination:-**Certain chemical substances like nitrous acid. $CNNO_2$, hydroxylamine, diethyl sulphate(DES), ethyl methane sulphona(EMS) etc. change the base sequence in DNA by a series of chemical steps. Some of them like nitrous acid and hydroxylamine cause deamination of nitrogenous bases by replacing amino group (-NH_2) by hydroxyl group (-OH).

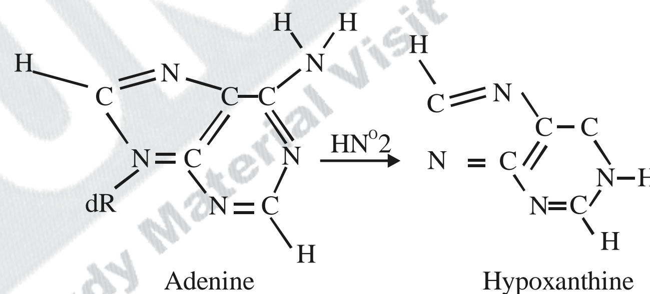
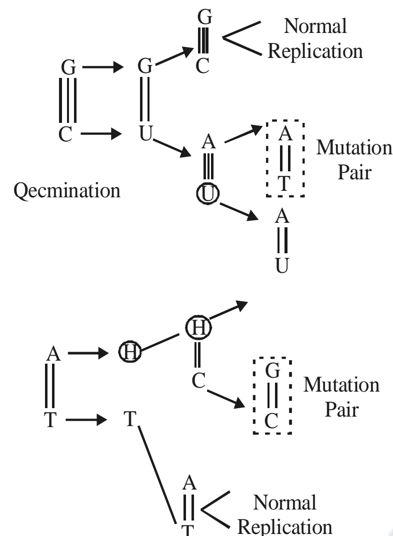


Fig – Deamination caused by nitrous acid.

(a) **Transition caused by hydroxylamine:-**

The hydroxylamine complexes with cytosine. This cytosine fairs with adenine rather than with guanine. This at the time of DNA replication introduces T=A at this level.



f.g – Mechaniom of mutation caused by nitrous acid.

(b) Transversions:- Certain alkylating agents, like ethyl methane sulphate (EMs) and methyl methane sulphate (MMS) induce substitutions by two ways:

(i) by substituting a purine for purine or pyrimidine for a pyrimidine.

(ii) by substituting a purine for a pyrimidine or a pyrimidine for a purine ctransversion), *i.e.* changes A=T to C=G.

· For transversion these chemicals affect the purine nitrogenous bases in the nitrogen at the seventh position in the guanine and adenine and finally lead to its separation from the DNA strand. This is k/a depurinations.

(ii) **Frame-shift Mutations:-**

The Mutations caused by the addition or deletion of nitrogenous base in the DNA or mRNA are k/a frame shift mutations, because In these shift the reading frame of codons from the site of change onward.e.g. of one and three base addition and deletions:-

Normal Sequence	CGT	CGT	CGT	CGT	CG
Sequence after deletion of one base	CGT	CTC	GTC	GTC	G
Sequence after deletion of three bases	CGT	CGC	TCG	TCG	GC
Sequence after deletion of three bases	CGT	- - -	CGT	CGT	CG
Sequence after cleletion of three bases	CGT	CGC	CGT	CGT	CG

fig.:- Frame shift Mutation and the changes in the reading of genetic code.

Types of Frame shift Mutation:-

They are of two types:-

(i) Deletion Mutations : These Mutations are caused due to the loss or deletion of one or more nucleotides.

(b) Insertion Mutations : These Mutations are caused by the addition of one or more extra nucleotides In DNA Molecule at one or more places.

Q.3 What are Plasmids? Describe its various features.

Ans. **Plasmid:-**A Plasmid is a replicon (unit of genetic material capable of independent replication) that is stably inherited (maintained without specific selection) in an extra chromosomal state. Most of the plasmids are not required for the survival of in which they reside.

Features of plasmids:-

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(i) **Replication:-**

Plasmids undergo independent replication due to the presence of certain sequences acting as the origin of replication. The smaller plasmids use the DNA replicative enzymes of the host cells.

Larger plasmids carry genes that code for. Special enzymes necessary for their replication. Under certain conditions, some plasmids may integrate into the bacterial chromosome. They are called episomes or integrative plasmids. At this stage they replicate along with the bacterial chromosome.

(ii) **Size of Plasmids:-**

The size of the plasmid varies from less than 1.0kb to more than 200kb. Smaller plasmids are much desirable for gene cloning experiments. Larger plasmids are less in number where as smaller ones are more in number.

(iii) **Copy number:-**

The number of molecules of a plasmid found in a single bacterial cell is termed as copy number. It ranges from one to more than50 per cell but this number is specific for a given plasmid residing in bacterial cell.

(iv) **Presence of special genes:-**

Plasmids contain some genes advantageous to the bacterial host, for e.g., resistance to antibiotics, productions of antibiotics, degradation of complex organic compounds and production of colicins, and enterotoxins.

Q.4 Describe the role of plasmid as a cloning vector?

Ans. Plasmids are double-stranded, closed circular DNA Molecules, which exist in the cell as extra chromosomal units. They have been exploited widely in the cloning process due to their various features making them best among other vectors.

Role as a cloning Vector:- A vector is the one that act as a carrier of specific genes that has to be used for transformation or cloning. Plasmids have proved to be the best among all other vectors due to their various features compatible with cloning process. They combine ease of purification with desirable properties s/a high transformation efficiency, convenient selectable markers for transform ants and recombinants and the ability to clone reasonably large (upto about 5kb) pieces of DNA. Most 'routine' gene cloning experiments make use of one or other of these plasmid vectors.

The most popular vector is PBR 322, PBR 322 has many such features that has make it the most useful out of other plasmids.

The first useful feature of PBR 322 is its size. A cloning vector ought to be less than l0kb in size, to avoid problems s/a DNA break down during purification. PBR322 is 4363 bp in size, which means that not only can the vector itself be purified with ease, but so can recombinant DNA Molecules constructed with it. Even with 6kb of additional DNA , a recombinant PBR 322 Molecule will still be a manageable size.

The second feature of pBR322 is that it carries two sets of antibiotic resistance genes. Either ampicillin or tetracycline resistance can be used as a selectable marker for cells containing the plasmid and each marker gene includes unique restriction sites that can be used in cloning experiments. Insertion of new DNA into pBR322 that has been restricted with Pst1, Puvl or Scal will inactivate the am p gene and insertion using any one of the eight restriction end nucleases (BamH1 & Hindi III) inactivates tetracycline resistance. This great variety of restriction sites that can be used for insert ional inactivation means that PBR 322 can be used to clone DNA fragments with any of several kinds of sticky ends.

A third advantage of PBR 322 is that it has a reasonably high copy number. Generally there are about 15 molecules present in a transformed E.Coli, but this no. can be increased upto 1000-3000, by plasmid amplification in the presence of a protein synthesis inhibitor such as chloramphenicol. An E.Coli culture will there fore provide a good yield of recombinant pBR322 molecules.

The other such plasmid vectors used in cloning include pBR325, pAT 153 and pBR 327 etc.

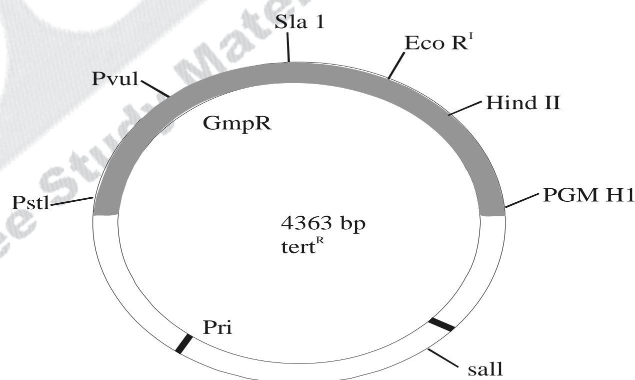

Fig:- A map of pBR 322 showing the positions of the ampicillin resistance (ampr, and tetracycline resistance(tetr, genes, the origh of replication orl) and a selection of the most important restriction sites.

Q.5 What is PCR? Describe the technique of PCR?

Ans. PCR- Polymerase chain reaction:-

· PCR technique was developed by Karry millis, who along with five other researchers demonstrated that oligonucloticle primers could be used specifically to amplify specific

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segments of genomic DNA or cDNA.

· PCR is an extremely powerful new procedure that allows one to make million fold copies of a selected. DNA sequence in a genome. PCR can be used to clone a given DNA sequence in vitro without using living cells during the cloning process. However, the procedure can only be applied when the nucleotide sequence of one short DNA segment on each side of the 'region of interest' is known.

· The PCR procedure makes use of synthetic oligonucleotides complementary to the k/n sequences spanning the region of interest to fatch enzymatic amplification of this segment of DNA.

Technique: -Technique of PCR involves three steps each repeated many times to produce cycles of amplification.

I. Denaturation:- denaturation of a template DNA duplex by heating at 94^0C

II. Annealing:- Oligonucleotide primers are annealed to the target sequences of separated DNA strands at 55-56^0C.

III. DNA synthesis:-
from the 3'-OH end of each primer by DNA Pol at 72^0C.

· Primer extension products of the previous cycle serve as new templates for the next cycle and number of target DNA copies has the potential to double each cycle.

Components in a polymerase chain reaction:-

1. Oligomer primers:-

   The amplification product of a PCR reaction is determined by the sequence of PCR primers.

   These primers are used at a concentration of 1 um, which sufficient for at least 30 cycles of amplification. These primers are designed considering some important features like length. [gen 16-24 nucleotides], duplex stability optimal distance b/w primers etc.

2. Amplification buffer:- The standard buffer for PCR reaction contains KCl, CL, and 15 mm $MgCl_2$.

3. Deoxyriboncleoside triphosphates:- dNTPs are used at a saturating concentration of 200 um for each dNTP.

4. Target sequence:- Template DNA containing the target sequences can be added in a single or double stranded form. Linear target sequences are preferred over closed circular DNAs as their amplification is better compared to the later.

5. Jag DNA pol:- Two forms of taq DNA Pol are available:-

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(a) The native enzyme purified from thermous aquaticus and

(b) a genetically engineered form synthesized in E.Coli (Ampli Taq-Tm). Taq DNA pol is the most frequently used and preferred enzyme.

The original PCR protocol used the klenow fragment of E.Coli DNA pol I to primer excision reaction.

- Optimal activity level of this enzyme is 37^{0}C, which greatly limits the specificity of the reaction due to degenerate primer annealing at this low temperature.
- Both these problems were solved by the use of Taq DNA pol. This enzyme retains activity even after repeated exposure to a temperature of 95^{0}C and is also fully active at ~ 75^{0}C, which essentially eliminates degenerate hetero-duplex formation.

Procedure:-

Polymerase chain reaction is used to amplify a segment of DNA that lies between the two regions of known sequence where two oligonucleotides (deoxy) act as primers can bind the opposite strands of DNA due to the complementary nature of base sequences. Taq DNA pol catalyzes the amplification reaction. The template DNA is first denatured by heating at 94^{0}C. the reaction mixture is then cooled to a temperature that allows the primers to anneal to their target sequences. These annealed primers are then extended (i.e. synthesis of DNA) with taq DNA pol. The cycle of denaturation, annealing and DNA synthesis is repeated many times, because the product of one round of amplification serves as template for the next, each successive cycle essentially doubles the amount of the desired DNA product. The major product of this exponential reaction is a segment of double stranded DNA whose ends are defined by the 5'-termini of the primers and whose length is defined by the distance between the primers. The products of first round of amplification are heterogeneously sized DNA molecules, whose lengths may exceed the distance between the binding sites of the two primers. In cycle 21 the original strands, and the new strands from cycle are separated, yielding a total of four primer sites with which primers anneal. The primers that are hybridized to the new strands from cycle are extended by polymerases as far as the ends of the tempelate, leading to a precise copy of the target region. In cycle 3_1 as DNA molecules are produced that are precisely identical to the target region. Further cycles lead to exponential doubling of the target region. The original DNA strands and the variable extended DNA strands will continuously be produced from the original template but at a linear rate and become negligible after the exponential increase of target fragments. The reaction can be continued for 25-45 cycles. Under normal conditions, the amount of taq DNA pol becomes limiting after 30cycles of amplification. It further amplification is required then a sample of the amplified DNA can be diluted and used as a tempelate for further rounds of synthesis in a fresh PCR reaction.

M.Sc./B.Sc. (Part II) Examination, 2011

(FACULTY OF SCIENCE)

(Common to Three and Five Year Integrated Course)

BIOTECHNOLOGY

Paper BT-401

(MOLECULAR GENETICS)

Year-2011

Time.: 3 Hours ***Max. Marks :50***

Question No. 1 is compulsory. Attempt FIVE questions in all, selecting ONE question from each section.

1. Answer the following questions as directed :

A. Answer briefly

(i) Write names of two bacteria which produce colicins.

(ii) Write one difference between transformation and conjugation.

(iii) Write names of termination codons.

(iv) What is genomic library?

(v) What is reverse transcription?

B. ***Fill in the blanks***

(vi) Taq polymerase is isolated from………..

(vii) …………discovered PCR.

(viii) When ribosomes are in clusters they are known as………….

(ix) In a……………method of DNA replication the old molecule disintegrates and two new molecules are synthesized.

(x) By…………model we can explain two dimensional structure of tRNA.

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(xi) Mg $^{++}$ ion concentration regulates the association and dissociation of two subunits of a ribososme. (True/False)

(xii) BY PCR technique we can obtain limited supply of identical copies of a gene sequence or DNA segment. (True/False)

(xiii) Bacteriophages provide us sources of cloning vectors. (True/False)

(xiv) mRNA functions as protein factory of a cell. (True/False)

Section-A

2. Describe in brief the structure DNA and compare it with that of RNA.
3. What is genetic code? Give an account of the properties of the genetic code.

Section-B

4. What is RNA processing? Give an account of processing of mRNA, rRNA and tRNA.
5. Describe regulation of protein synthesis with the help of 'Lac' operon model.

Section-C

6. What is mutation ? Give a brief account of different types of mutations.
7. Write brief notes on:

   (a) Plasmid cloning vectors

   (b) Non-homologons recombination

Section-D

8. What is recombinant DNA technology? How is it used in genetic engineering?
9. Write notes on:

   (i) Restriction endonucleases

   (ii) Bacterial gene cloning

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