Chapter-6

MOLECULAR BASIS OF INHERITANCE

I ONE MARKS QUESTIONS

1. Name the nitrogen base present in DNA, but absent RNA.
   ANS - Thymine
2. Define transcription.
   ANS- the process of synthesis of mRNA from DNA by enzymes
3. What are purines / pyrimidines?
   ANS - Double ring nitrogen base called purines. Ex- A& G
   Single ring nitrogen base called purines. EX- C, T & U.
4. Name the bond linked between nitrogenous base & sugar.
   ANS – N – glycosidic linkage
5. What is polynucleotide chain?
   ANS- more than 5 nucleotide are join end to end to form chain called polypeptide chain.
6. Who elucidate the structure of DNA?
   ANS- Watson & crick
7. Define Erwin Chargaff rule.
   ANS – amount of purines are equal to Pyrimidines.
8. Define central dogma.
   ANS - flow of genetic information from DNA to RNA to polypeptide or protein.
9. What is nucleosome?
   ANS- The negatively charged DNA is wrapped around the positively charged histones octomer to form a structure called nucleosome
10. What is replication?
    ANS – DNA produce an exact copy of itself called replication.
11. What is RNA splicing?
    ANS – The introns are removed & exons are joined in a definite order
12. How many sensible codons in genetic code dictionary?
    ANS - 61
13. UAA, UAG & UGA are called Nonsense codon, why?
    ANS – these codons do not code for any amino acids.
14. What is mutation?

ANS- the alteration of DNA sequences results in changes in the genotype & phenotype of an organism.

15. Define DNA finger printing.
    ANS- an analytical technique, sequence of DNA repeats to Identify of individual at DNA level is known as DNA finger printing
16. Define bioinformatics.
    ANS- the management & analysis of the biological information stored in the databases using computers.
17. In a double strand DNA the percentage of Adenine (A) is 15%. Calculate the % of Guanine (G).
    ANS – 35%

II **TWO MARKS QUESTIONS**

1. Nucleosome composed off?
   Histones octomer & DNA
2. Name the amino acids residue carry positive charge of histones.
   Lysines & arginines
3. Mention 4 properties of genetic material.
   i. Undergo replication
   ii. Chemically & structurally be stable
   iii. Slow changes (mutation) that are required for evolution.
   iv. Able to express itself in the form of ' Mendelian Characters'

4. Differentiate template strand & coding strand.
   One of the DNA strand act as template to produce mRNA called template strand.
   The DNA strand which does not code for anything is referred as coding strand
5. Write the function of DNA- dependent RNA polymerase & RNA- dependent DNA polymerase.
   DNA- dependent RNA polymerase - synthesis of mRNA, dependent on DNA strand
   RNA- dependent DNA polymerase - synthesis of DNA, dependent on genetic RNA strand.

6. What are exons & introns?
   The coding sequences or expressed sequence of mRNA called Exons.

The non coding sequence of mRNA called introns.

7. What is capping & tailing?
   Unusual nucleotide (methyl Guanosine triphosphate) is added to the 5' – end of hnRNA called capping
   Adenylate residue (200-300) are added at 3'– end called tailing
8. Mention the two essential role of ribosome during translation.
   i. Synthesizing polypeptide chain or protein
   ii. Act as catalyst for the formation of polypeptide bond.

9. Differentiate between Repetitive DNA & satellite DNA.
   Unusual base pairs sequence (10-15) repeated many times in DNA called Repetitive DNA. (It varies from person to person, but unique to a person)
   The small peaks are separated from bulk DNA during DNA finger printing called satellite DNA.
10. Mention the application of DNA finger printing.
    i. Solving disputed parentage
    ii. To identify criminals & rapists
    iii. Reuniting the lost children
    iv. Immigrant dispute
    v. To establish the identity of dead bodies.

III **THREE MARKS QUESTIONS**.

1. Name the three component of nucleotide.
   Nitrogen bases. Pentose sugar & phosphate groups.
2. Draw a schematic structure of a transcription unit.

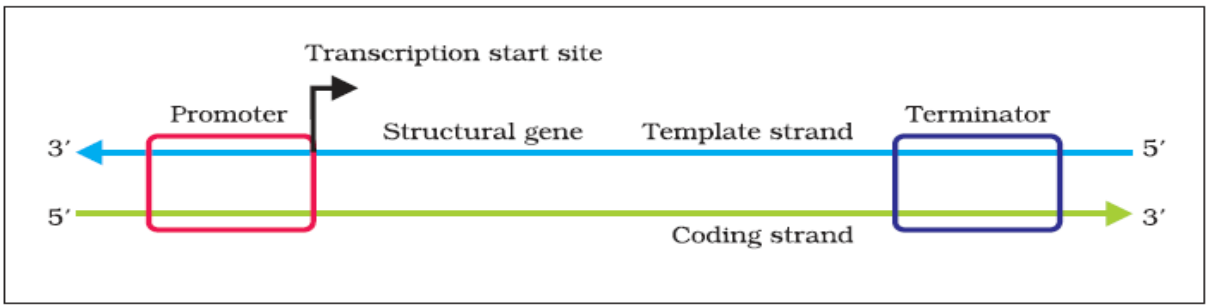


Figure 6.9 Schematic structure of a transcription unit

3. Where do you find code, codon & anticodon?

Code - DNA
Codon - mRNA
Anticodon - tRNA

4. Mention the function of RNA polymerase I, II & III.
   RNA polymerase I - Transcribe rRNA
   RNA polymerase II - Transcribe precursor of mRNA
   RNA polymerase III - Transcription of tRNA, 5srRNA & snRNAs

5. What are the goals of HGP?
   i. Mention a Identify genes in human DNA
   ii. Determine the sequence of human DNA
   iii. Store information in databases.
   iv. Improve data analysis

6. Mention any three level of regulation of gene expression.
   i. Transcriptional level
   ii. Splicing level
   iii. Translational level

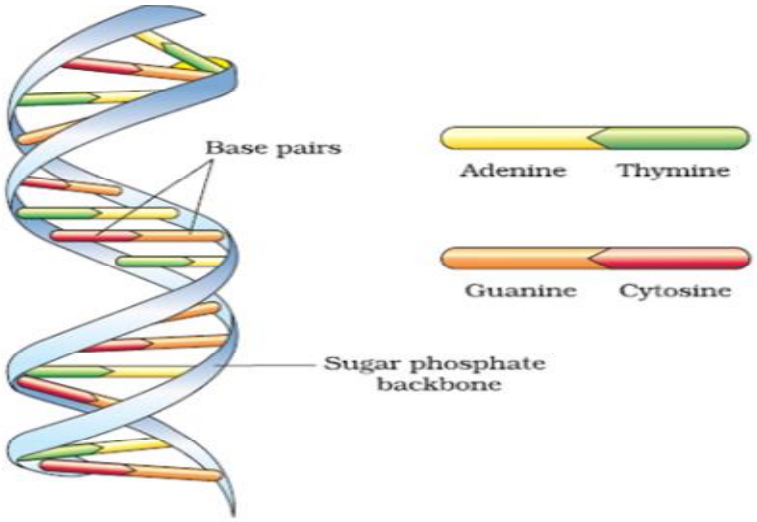
IV **FIVE MARKS QUESTIONS**

1. **Explain the structure of Watson & Crick model of structure of DNA**.

In 1953 **James Watson** and **Francis Crick**, based on the X-ray diffraction data produced by **Maurice Wilkins** and **Rosalind Franklin**, proposed a very simple but famous Double Helix model for the structure of DNA.

i. The backbone of DNA strand composed of repeated units of sugar and phosphate molecules.
ii. The pairing of nitrogen bases are always between a specific purins and specific pyrimidines that is between A and T, G and C and vice versa. This type of base pairing is called **complementary base pairing**.
iii. Due to complementary base pairing the amount of purines and pyrimidines in DNA are equal. The ratios between Adenine and Thymine, Guanine and Cytosine are

constant and equals to one (That is amount of A=T, and G=C) this is called **Chargaff's rule of base equivalence.**



iv. The bases in two strands are paired through hydrogen bond (H – bonds) forming base pairs (bp). There are two hydrogen bonds between A and T, three hydrogen bonds between G and C.
v. The two chains are coiled in a right –handed fashion.
vi. The pitch of the helix is 3.4 nm or 34 Å & there are roughly 10 bp in each turn.
vii. Double stranded DNA molecule has a diameter of 20Å and distance between two successive base pairs is 0.34nm (3.4 Å).
viii. The plane of one base pairs stacks over the other in double helix. This in addition to H – bonds, confers stability of the helical structures.

2. <u>With labeled diagram explain packaging of DNA helix.</u>

The human DNA in a cell contains 6.6 × 10^9 base pairs and its length is about 2.2 meters (6.6× 10^9x0.34x10^9m/bp). It is greater than the dimension of the nucleus (10^6). The long polymer DNA is present in highly folded or packed form in the nucleus.

In prokaryotes the negatively charged DNA held with some positively charged proteins in a region called **nucleoid**. Thus DNA forms large loops held by proteins in prokaryotes.

In eukaryotes

- A positively charged protein called **histones** held with DNA.
- Histones contain amino acids **lysine** and **arginines** residues that carry positive charges in their side chains.
- Eight histones molecules are organized to form a structure called **histone octomer**.

- The negatively charged DNA is wrapped around the positively charged histone octomer to form a complex called **nucleosome**.
- A typical nucleosome contains 200 bp of DNA helix.
- Nucleosomes constitute the repeating unit of a structure in nucleus called **chromatin**. The nucleosomes in chromatin are seen as ' beads –on –string'

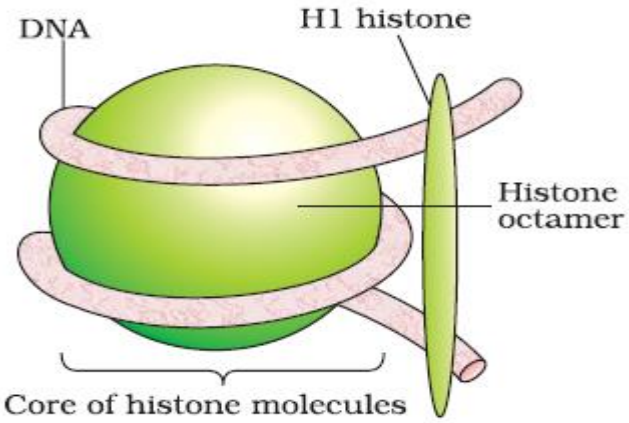


Figure 6.4a Nucleosome

The chromatin fibers are further coiled and condensed at metaphase stage of cell division to form chromosomes. The packaging of chromatin at higher level requires additional set of proteins called **Non-histone Chromosomal (NHC) proteins**. In a typical nucleus, some regions of chromatin are loosely packed and lightly stained called **euchromatin.** The highly coiled and darkly stained regions of chromatin are called **heterochromatin**. Euchromatin is said to be transcriptionally active chromatin, whereas heterochromatin is inactive.

3. Explain Griffith transforming principle to search for genetic material.

Frederick Griffith showed transformation in the bacterium ***Diplococcus pneumoniae*** which cause pneumonia disease in mammals.

This bacterium (***Diplococcus pneumonia)*** is found in two forms or strains as **Smooth Strain (S)** and **Rough strain (R).** The **S** strains are with mucous (polysaccharide) coat and pathogenic hence called **Virulent** Strains. The **R** Strains are without mucous coat and non-pathogenic called **avirulent** strains.

Griffith showed that injections of **S**-strains into mice produce Pneumonia disease. But an injection of **R**-strains does not produce the disease. The heat killed **S**-strains does not produce the disease. But the mixture of live **R**-strains and heat killed **S**-strains produce the disease in mice. Griffith recovered S-strains of bacteria from the dead mice.

S strain ——–> Inject into mice ——> Mice die

R strain ——–> Inject into mice ——–> Mice live

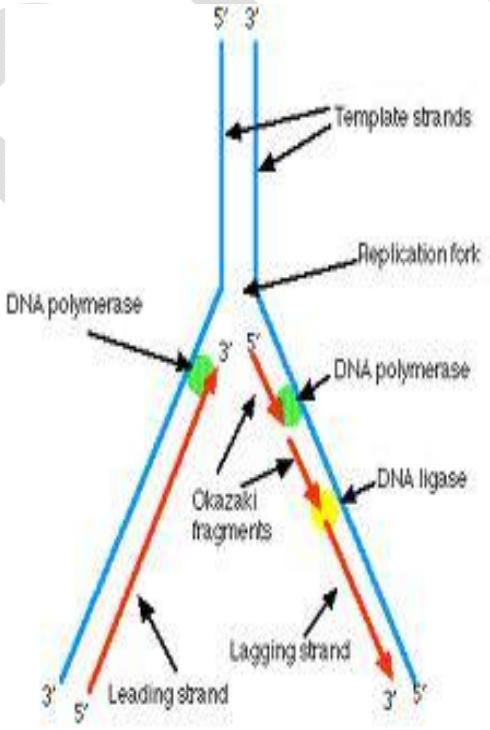
Heat killed-S strain ——–> Inject into mice ——> Mice live

S strain (heat killed) + R strain (live) ———> Inject into mice ——–> Mice die

Griffith concluded that some transforming principle transferred from the heat killed S-strains to produce mucous coat in R-strains and they become virulent. The transforming principle is genetic material, But he fail to define biochemical nature of it through his experiments.
(However, the biochemical nature of genetic material was not defined from his experiments).

4. **<u>Explain semi conservative replication of DNA</u>**.

The replication occurs during **S-phase** of Interphase during cell cycle. The process of replication is proved qualitatively by **J.Herbert Tayler** and quantitatively by **Meselson and Stahl**.



Requirements:

- Four types of nucleotides of DNA
- Energy source (ATP)
- RNA primers
- Inorganic ions:- Mg^{+2}
- Enzymes:

a) Topoisomerases: (DNA Gyrases)	- Breaking and resealing DNA strand.
b) Helicases:	- Unwinding DNA helix
C) DNA Polymerase I, II and III	- Catalyze replication
d) RNA Primase:	- Synthesize RNA primers
e) DNA Ligase:	- Join DNA fragments.

The main enzyme is referred to as **DNA –dependent DNA polymerase**. The average rate of polymerization has to be approximately 2000 bp per second.

Mechanism: The process of replication involves the following steps.

a. Activation of nucleotides:

The nucleotides of DNA such as d-AMP, d-TMP, d-GMP and d-CMP are activated and phosphorylated by ATP in to d-ATP, d-TTP, d-GTP and d-CTP respectively.

b. Unwinding of DNA helix:

The initiation of replication or uncoiling of the DNA helix starts at a specific point called origin of replication or Ori. There is a single Ori in prokaryotes but many Ori are present in eukaryotes.

The unwinding of DNA strands is catalyzed by Helicases. DNA Gyrases (Topoisomerases) remove the coils that accumulate in front of the replication fork. The separation of DNA strands during the initiation of replication forms a Y-shaped structure called **replication fork.** The separated DNA strands act as master strands or template strands for the formation of new strands.

c. Formation of RNA-primer:

The synthesis of new strand always proceeds in 5'→3' direction. During the initiation of replication a short segment of RNA is synthesized with the help of an enzyme RNA primase called RNA primer.

d. Initiation and elongation of DNA strand:

The DNA nucleotides are now added to exposed bases of parental DNA strand from the end of RNA primer. This process is catalyzed by DNA Polymerase III and Mg^{+2}. The addition of nucleotides of DNA proceeds only in 5'→3' direction. The two new strands of DNA produced in opposite or antiparallel direction called bidirectional replication.

In one strand the synthesis of new DNA strand goes on continuously in 5'→3' direction and this new strand is called leading strand. In the opposite strand (3'→5') the addition of nucleotides proceeds as short segments away from the replication fork called lagging strand. The short single stranded fragments of DNA of the lagging strand are called Okazaki fragments. The lagging strand has many RNA primers.

Later the RNA primers are removed and replaced by DNA nucleotides by an enzyme DNA polymerase I. The Okazaki fragments are joined by DNA Ligase enzyme.

e. Termination of replication:

The termination of replication is signaled by specific sequence of DNA nucleotides. After replication the DNA polymerase II takes an editing role to remove abnormal nitrogen bases and incorporate the normal bases **(proof reading**). This process is called **genetic repair mechanism**. **(**In E.coli the replication of DNA completes in 38 minutes. The average rate of polymerization is approximately **2000 base pairs per second**. It contains $4.6x10^6$ bp.)

5. **Explain the process of transcription**

The process of copying genetic information from one strand of the DNA into RNA is called transcription. (The biosynthesis of RNA from DNA is called **transcription**.)

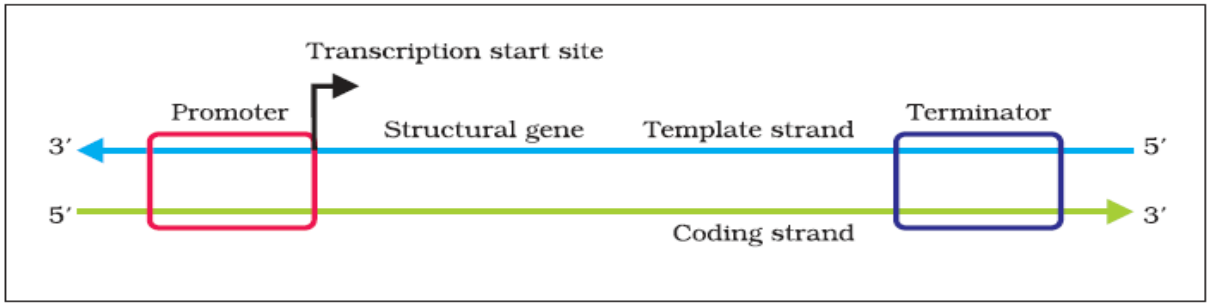
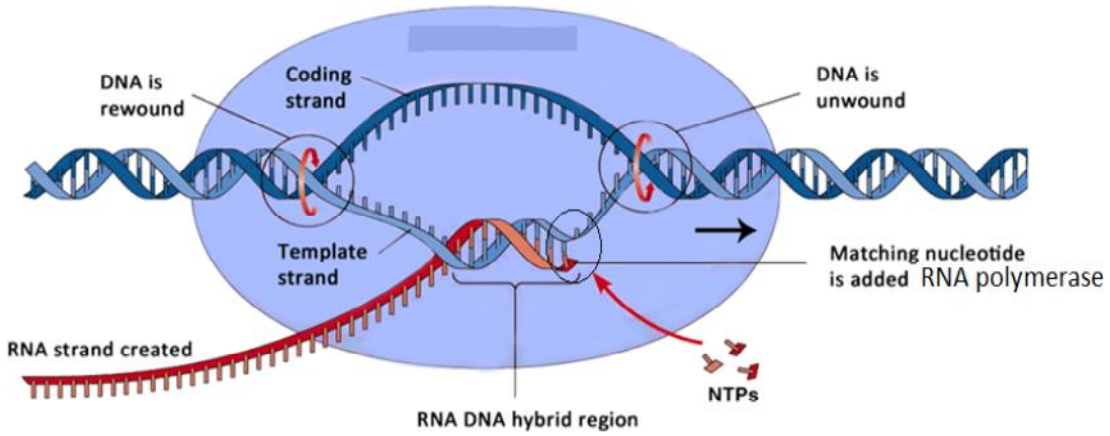


Figure 6.9 Schematic structure of a transcription unit

i. The transcription unit of DNA consists of three regions as a **promoter, structural gene** and **a terminator.**
ii. The transcription begins by the uncoiling of DNA strands due to the breakage of hydrogen bonds.
iii. After the unwinding **DNA dependent RNA polymerase** is only capable of catalyzing the process of elongation in association with **initiation – factor** (**σ**). It binds to promoter and initiate transcription.
iv. One of the strand of DNA (3'→5' strand) act as a template to produce RNA by complementary base arrangement is called **antisense strand**
v. The strand of DNA which bears the same sequence as the RNA and not used as template during transcription is called **sense strand** or **coding strand**.
vi. The nucleotides of RNA are attracted and assembled complementary to template in the presence of DNA dependent **RNA-polymerase and Mg+.** Only a short stretch of RNA remains bound to the enzyme.
vii. The termination of RNA chain is brought about by certain terminator sequences on DNA & **termination factor** (**ρ**).
viii. Finally the new RNA formed and RNA-polymerase gets detached from the DNA. Again the two strands of DNA rewind by the hydrogen bonds.



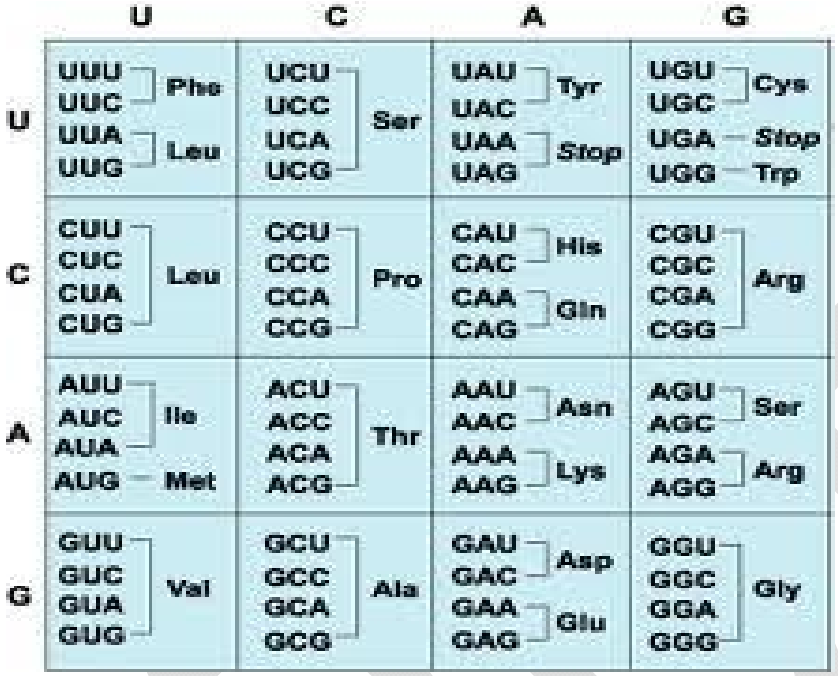
In eukaryotes the **introns** are removed from precursor m-RNA, the **exons** are joined in defined order to produce functional m-RNA. This process is called **splicing (**Capping and tailing processes occurs in hnRNA)

In eukaryotes there are at least three types of RNA polymerases performs different functions. (**RNA polymerase I** – transcribes t-RNA, **RNA polymerase II** – transcribes precursor m-RNA and heterogeneous nuclear RNA or hnRNA and **RNA polymerase III** – transcribes t-RNA, r-RNA and small nuclear RNA or snRNAs)

6. Explain any 5 salient feature of genetic code.

i. **Genetic code is triplet in nature:** The sequence of three nucleotides or nitrogen bases codes for one amino acid. Ex: AAA, UAC, AAU, etc.
ii. **Genetic code is universal**: A particular codon codes for the same amino acid in all organisms from bacteria to higher plants and animals. Ex: AUG codes for ***Methionine,*** UUU codes for ***phenylalanine***. (some exceptions in mitochondrial and protozoan codons)
iii. **Genetic code is non-overlapping**: The nitrogen bases are read continuously in groups of three without sharing or overlapping.
iv. **Genetic code is degenerate:** Most of the amino acids are coded by more than one codon, such codons are called degenerate or synonymous Codons and the phenomenon is called **degeneracy**. Ex: Alanine is coded by GCA, GCC, GCU and GCG.
v. **Genetic code is comma less**: The codons are read continuously from one end to other without any break or punctuation marks between the codons.
vi. **Genetic code is non-ambiguous or specific**: A particular codon always codes for the same amino acid without any mistake this characteristic is called **non-ambiguity.**
vii. **Genetic code has an initiator codon**: The protein synthesis starts or initiates by a particular codon called **initiator codon**. Ex: **AUG** present near the 5' end of the m-RNA act as initiator codon in most of the organisms which codes for **methionine**. Therefore methionine is the first amino acid in most of the proteins. Rarely **GUG** act as initiator codon in some bacteria which codes for **formyl-methionine**.
viii. **Genetic code has non-sense or terminator codons**: The codons which do not code for any amino acid and signal the termination of protein synthesis are called **non-sense codons**. Ex: **UAA, UAG** and **UGA**.
ix. **Principle of co linearity**: The linear order of the nitrogen bases in DNA determines the linear order of m-RNA codons. This in turn determines the linear order of amino acids in a polypeptide. This principle is called **co linearity**.

Total number of triplet codons	=64
Number of sense codons	=61
Number of non-sense codons	=03(UAA, UAG & UGA)



7. Explain the process of translation

Translation refers to the process of polymerization of amino acids to form a polypeptide. The order and sequence of amino acids are defined by the sequence of bases in the mRNA.

The amino acids are joined by a bond which is known as a peptide bond. Formation of a peptide bond requires energy.

- Therefore, in the first phase itself amino acids are activated in the presence of ATP and linked to their cognate tRNA–a process commonly called as charging of tRNA or aminoacylation of tRNA to be more specific. If two such charged tRNAs are brought close enough, the formation of peptide bond.
- The presence of a catalyst would enhance the rate of peptide bond formation. The cellular factory responsible for synthesizing proteins is the ribosome. The ribosome consists of structural RNAs and about 80 different proteins.
- Ribosome exists as two subunits; a large subunit and a small subunit.

- When the small subunit encounters an mRNA, the process of translation of the mRNA to protein begins. For initiation, the ribosome binds to the mRNA at the start codon (AUG) that is recognized only by the initiator tRNA.
- There are two sites in the large subunit, for subsequent amino acids to bind to and thus, be close enough to each other for the formation of a peptide bond. The ribosome also acts as a catalyst (23S rRNA in bacteria is the enzyme- ribozyme) for the formation of peptide bond. The ribosome moves from codon to codon along the mRNA. Amino acids are added one by one.
- At the end, a release factor binds to the stop codon, terminating translation and releasing the complete polypeptide from the ribosome.
- An mRNA also has some additional sequences that are not translated and are referred as untranslated regions (UTR). The UTRs are present at both 5′-end (before start codon) and at 3′-end (after stop codon). They are required for efficient translation process.

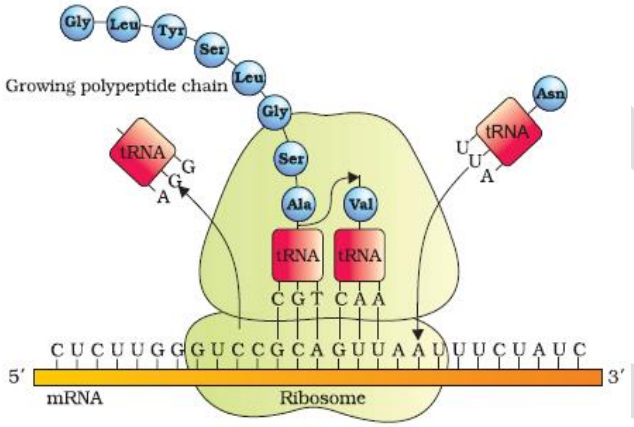


Figure 6.13 Translation

4. **With schematic representation, explain Lac Operon concept.**

Jacob and Monod proposed the Lac-Operon concept in 1961 to explain the gene action or regulation of protein synthesis. It is controlled by "switching on" and "switching off" of the different parts of the gene.

Jacob and **Monod** explained the activation and inactivation of genes that control lactose catabolism in ***Escherichia coli*** (**E.coli).** The group of closely related structural and control genes that regulate lactose catabolism in E.coli is called **Lac-operon**. According to lac-operon concept a gene consist of a structural gene and three control genes as follows.

The elucidation of the *lac* operon was also a result of a close association between a geneticist, Francois Jacob and a biochemist, Jacque Monod. They were the first to elucidate a transcriptionally regulated system.

In *lac* Operon (here *lac* refers to lactose), a Polycistronic structural gene is regulated by a common promoter and regulatory genes. Such arrangement is referred to as operon.

To name few such examples, *lac* operon, *trp* operon, *ara* operon, *his* operon, *Val* operon, etc.

The *lac* operon consists of

A.one regulatory gene.

i. The i *gene* codes for the repressor of the *lac* operon.

(Control gene –regulatory gene, operator gene & promoter gene)

b. **Three structural genes (*z*, *y*, and *a*)**.

ii. The *z* gene codes for beta-galactosidase (b-gal), which is primarily responsible for the hydrolysis of the disaccharide, lactose into its monomeric units, galactose and glucose.

iii. The *y* gene codes for permease, which increases permeability of the cell to b-galactosidase.

iv. The *a* gene encodes a transacetylase

Lactose is the substrate for the enzyme beta-galactosidase and it regulates switching on and off of the operon. Hence, it is termed as **inducer.**

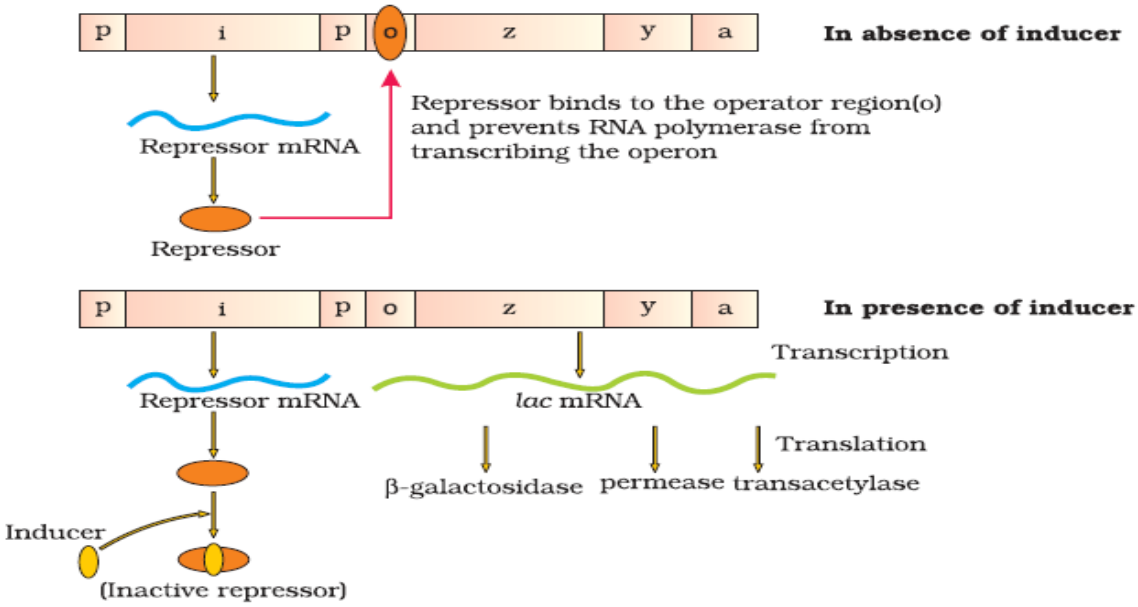
The lactose then induces the operon in the following manner.

The repressor of the operon is synthesized (all-the-time – constitutively) from the *i* gene.

In the absence of inducer-The repressor protein binds to the operator region of the operon and prevents RNA polymerase from transcribing the operon. The process is **switch off.**

In the presence of an inducer, such as lactose or allolactose, the repressor is inactivated by interaction with the inducer. This allows RNA polymerase access to the promoter and transcription proceeds. The process is **switched on**.

Regulation of *lac* operon by repressor is referred to as negative regulation.



6. **Mention the salient feature of HGP.**
   i. It contains **3164.7 million** nucleotide bases
   ii. The average gene consist of **3000** bases (largest human gene **dystrophin** contains 2.4 million bases)
   iii. Human genome consists of about **30**,**000** genes. (99.9% of nucleotide bases are same in all people)
   iv. The function of over **50**% discovered genes are unknown.
   v. Less than **2**% of the genome codes for proteins
   vi. Large portion of human genome contains **repetitive sequences**.
   vii. The repetitive DNA sequences are repeated hundred or thousand times not having any coding function. They shed light on chromosome structure, dynamics and evolution.
   viii. Chromosome -1 has most genes (**2968**) and Y has fewest genes (**231**)
   ix. Scientists have identified about 1.4 million locations where single base DNA differences (**SNP**-single nucleotide polymorphism) occur in humans. (This helps to find chromosomal locations for diseases and tracing human history).

Applications of HGP:

i. Helps in identifying genes associated with various diseases.
ii. Helpful for modern medical practice (production of antiviral proteins and pharmaceuticals)
iii. Knowledge can be used to study mechanism of drug action that leads to faster clinical trials.
iv. This knowledge can be used for the development of gene therapies.

8. What is DNA finger printing? Mention the steps of DNA finger printing.

The identification of an individual at genetic level through sample of DNA is called **DNA finger printing**. As we know 99.9% of human DNA is same among human beings. The DNA finger printing is based on identifying differences in some specific regions in DNA sequence that repeated many times called **repetitive DNA**. These repetitive DNA are separated from bulk genomic DNA by **density gradient centrifugation**. The small peaks of DNA separated from bulk DNA referred as **satellite** DNA. Depending upon the base composition, length of segment and number of repetitive units there are many types of satellite DNA as **micro satellites**, **mini satellites**, etc. these repetitive units show high degree of polymorphism and form the basis of DNA finger printing.

The junk DNA containing specific repeated sequence of nitrogen bases in different persons is called **variable number of tandem repeats** (**VNTR**). Therefore each individual has a unique type of VNTR's (except identical twins). Persons are genetically identified depending upon these specific VNTR's.

Steps of DNA finger printing technique:

- Collection of biological samples like blood, saliva, semen, root hair cells, skin cells, etc.
- Isolation of DNA from the sample and multiplying DNA by **polymerase chain reaction (PCR)** or **DNA amplification**. (If sample of DNA is very little).
- The DNA samples are cut into fragments using specific **REN**. The different length DNA fragments produced by REN are called **restriction fragment length polymorphs** (**RFLP**). They are the outcome of VNTR's hence specific to each person.
- The DNA fragments are separated according to their length and arranged on **electrophoretic gel slab** by a process called **gel electrophoresis**.
- The double stranded DNA is split into single stranded DNA by the action of alkaline chemicals.
- The separated DNA fragments are transferred from the gel slab into nylon or **nitro-cellulose sheet**. This technique is called **southern blotting**.
- Radioactive DNA probes are added to hybridize complementarily with VNTR sequence on nitrocellulose sheet. The un-hybridized radioactive probes of DNA are washed off with water.

- The entire nitrocellulose sheet is X-ray photographed; only the radioactive probes of DNA appear as dark bands in the **autoradiograph**.
- The position and number of such dark bands on the autoradiograph are unique to a person called **DNA finger prints**.
- By obtaining and comparing the DNA finger prints of sample and that of suspected person, it is possible to establish the identity of a person/criminal.