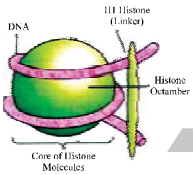


Chapter - 6

Molecular basis Of Inheritance

Anticodon : A sequence of three nitrogenous bases on tRNA which is complementary to the codon on mRNA.

Genome : Sum total of genes in haploid set of chromosomes.

DNA Polymorphism : The variations at genetic level, where an inheritable mutation is observed, in a population at high frequency.

Satellite DNA : The repetitive DNA sequences which form a large portion of genome and have high degree of polymorphism but do not code for any proteins.

Operon : A group of genes which control a metabolic pathway.

Exons : The regions of a gene which become part of mRNA and code for different regions of proteins.

Introns : The regions of a gene which are removed during the processing of mRNA.

Euchromatin : The region of chromatin which is loosely packed and transcriptionally active, it stains lighter.

Heterochromatin : The chromatin that is more densely packed, stains dark and is transcriptionally inactive.

Splicing : The process in eukaryotic genes in which introns are removed and the exons are joined together to form mRNA.

Bioinformatics : Science of use of techniques including statistics, storing as data bases, analysing, modelling and providing access to various aspects of biological information usually on the molecular level.

Central Dogma :

$$\text{replication} \quad \text{DNA} \xrightarrow{\text{Transcription}} \text{mRNA} \xrightarrow{\text{Translation}} \text{Protein}$$

Replication form : The Y shaped structure formed when double stranded DNA is unwound upto a point during its replication.

VNTR : Variable Number of Tandem Repeats

YAC : Yeast Artificial Chromosome

BAC : Bacterial Artificial Chromosome

SNPs : Single Nucleotide polymorphism

HGP : Human Genome Project

hnRNA : Heterogenous nuclear RNA. It is precursor of mRNA.

Friedrich Meischer	1869	First identified and isolated a acidic substance from pus cell and named it 'Nuclien'.
Altman	1889	Separated protein from nuclear substance and named it nucleic acid
Kossel	1893	Discover nitrogen bases (Adenine, guanine, cytocine, Thymine, uracil)
T.H. Morgan	1910	
Frederick Griffith	1928	Provide first clear-cut evidance that DNA in the hereditary material while working on *streptococus pneumoniae* Biochemical nature of genetic material was not defined
Avery, Macleod and McCarty	1944	Discover that transforming principle is DNA, not a protein or RNA. First identification that DNA is the hereditary material
Erwin Chargaff	1950	Purine and pyrimidine components occur in equal amount in a DNA molecule. $A + G = T + C$
Harshey and chase	1952	Performed experiment with *Escherichia Coli* and bacteriophage and show that it is the viral DNA and not protein that passed from virus to bacteria and therefore DNA serves as the genetic material.
Wilkins and Frankline	1952	Produce X-ray diffraction data of DNA.
Watson and Crick	1953	Double helical structure of DNA.
Messelson and Stahl	1958	Experimentaly proved the semiconservative nature of DNA replication.
Jacob and Monod	1961	Proposed operon model - genetic material has a number of functional unit called operon.
Alec Jaffery	1985	Discovered the technique of DNA finger printing.

Chemical Structure of Polynucleotide Chain (DNA/RNA) : A nucleotide has three components.

1. **Nitrogen base**
   (i) **Purines :** Adenine and Guanine
   (ii) **Pyrimidines :** Cytosine, Thymine and Uracil Thymine in DNA and Uracil in RNA.
2. **Pentose Sugar :** Ribose (in RNA) or Deoxyribose (in DNA).
3. **Phosphate Group**

- Nitrogen base is linked to pentose sugar through N-Glycosidic linkage.
- Nitrogen base + Sugar = Nucleoside
- Phosphate group is linked to 5′-OH of a nucleoside through phosphoester linkage.
- Nucleoside + Phosphate group = Nucleotide
- Two nucleotides are linked through 3'-5 phosphodiester linkage to form a dinucleotide
- A polynucleotide chain has free phosphate group at 5' end of ribose sugar and a free 3′-OH group at other end.

RNA is highly reactive than DNA : In RNA nucleotide has an additional OH group at 2′. positions in the ribose; RNA is also catalytic.

Double-helix Structure of DNA : Proposed by Watson and Crick in 1953.

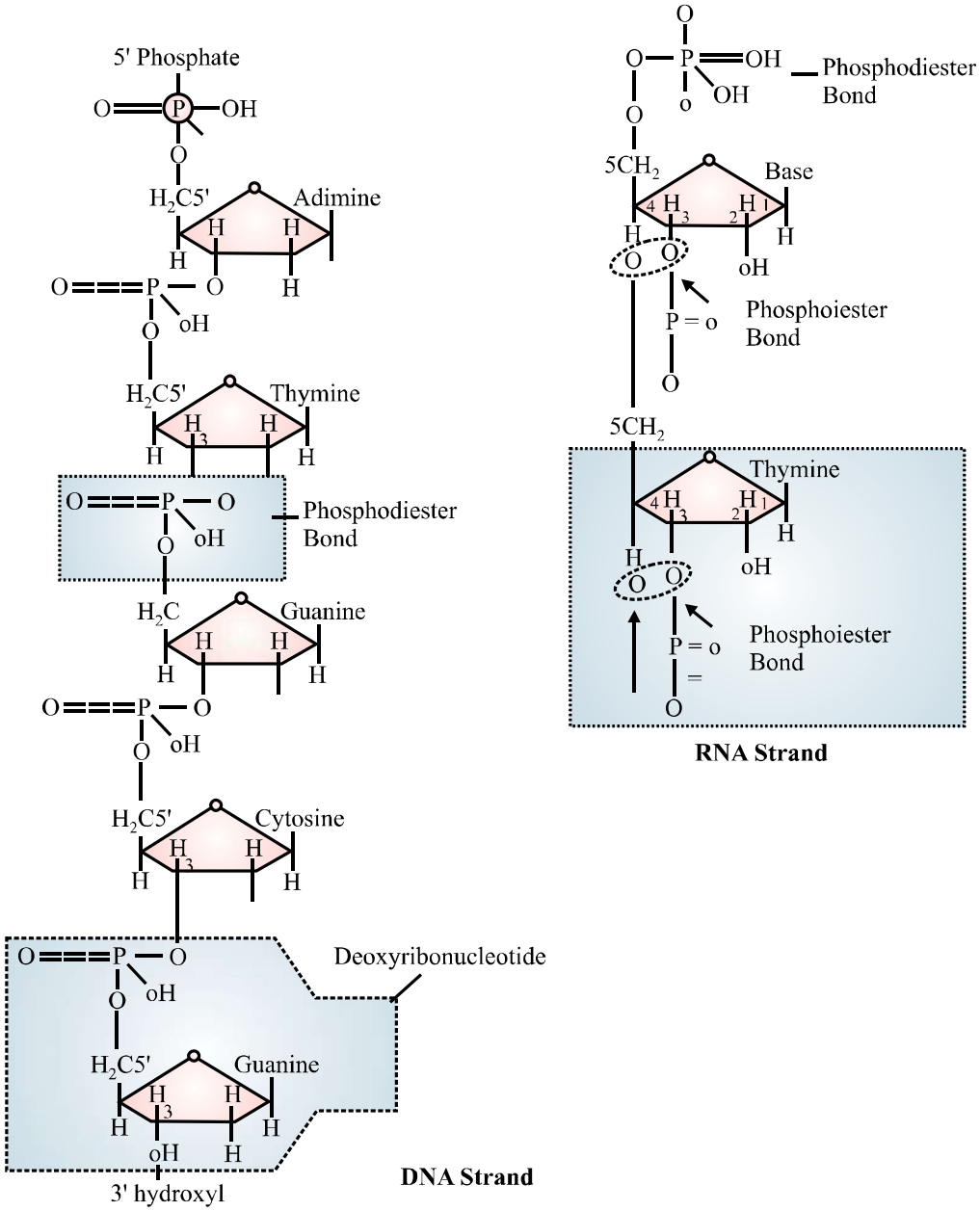
(i) DNA is made up of two polynucleotide chains.

(ii) The backbone is made up of sugar and phosphate and the bases project inside.

(iii) Both polynucleotide chains are antiparallel i.e. on chain has polarity 5′-3′ and other chain has 3′-5′.

(iv) These two strands of chains are held together by hydrogen bonds i.e. A = T, C ≡ G.

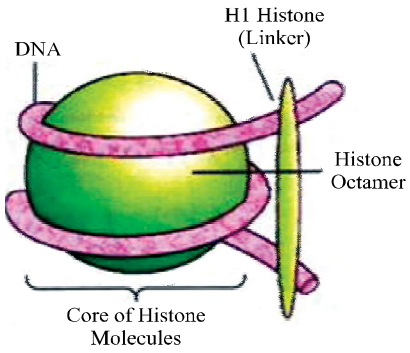
(v) Both chains are coiled in right handed fashion. The pitch of helix is 3.4 nm with 10 base pairs in each turn.



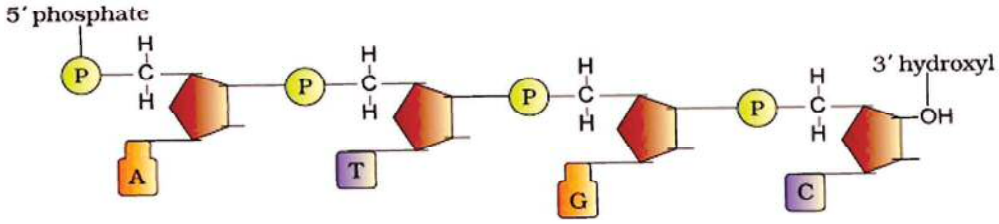
Packaging of DNA Helix

- The average distance between the two adjacent base pairs is 0.34 nm (0.34×10^{-9}m or 3.4°A)

- The number of base pairs in *Escherichia coli* is 4.6×10^6.
- **DNA Packaging in Prokaryotes :** DNA is not scattered throughout the cell. DNA (negatively Charged) is held by some proteins (has positive charges) in a region termed as nucleoid. The DNA in nucleoid is organised in large loops held by proteins.
- **DNA packaging in Eukaryotes :** There is a set of positively charged basic proteins called histones. Eight histone molecules combines together to form histone octamer.
- The negatively charged DNA is wrapped around positively charged histone octamer to form as structure called nucleosome.
- Histone H_1 is situated outside of nucleosomal DNA in linker region.
- Nucleosomes constitute the repeating unit of a structure in nucleus called chromatin.
- The beads-on-string structure in chromatin is packaged to form chromatin fibres that 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 protein that collectively are reffered to as Non-histone chromosomal (NHC) proteins. At places chromatin is density packed to form darkly staining heterochromatin. At other places chromatin is loosely packed to form euchromatin.
- Euchromatin is said to be transcriptionally active chromatin, whereas heterochromatin is inactive.



Structure of Nucleosome



A Polynucleotide Chain of DNA

Transforming Principle :

Frederick Griffith (1928) performed experiments with *Streptococcus phenumoniae* and mice. This bacterium has two strains.

1. S-strain (Virulent)-which possess a mucilage coat and has ability to cause pneumonia.
2. R-strain (Nonvirulent) which do not possess mucilage coat and is unable to cause pneumonia.

- Griffth injected R-strain bacteria into mice.

  → No disease noticed and mice remain live.

- On injecting S-strain bacteria into mice.

  → Mice died due to pneumonia.

- When heat-killed S-strain bacteria were injected into mice → No pneumonia symptoms noticed and nice remain live.
- He than injected a mixture of R-strain bacteria (Non virulent) and heat killed S-strain bacteria (virulent) into mice → mice died due to pnuemonia.
- Moreover Griffith recovered living S-strain (virulent) bacteria from the dead mice.

Conclusion : He concluded that presence of heat-killed S-strain bacteria caused transformation of some R-strain bacteria into virulent by a chemical substance, called 'transforming principle'. But biochemical nature of the genetic material was not defined by him.

Chemical Nature of Transforming Principle

In 1944, Avery MacLeod and McCarty worked to determine the chemical nature of 'transforming principle'.

The purified biochemicals from heat killed S-cells :

- Proteins $\xrightarrow{\text{Protcases}}$ Transformation takes place So, protein is not a 'transforming principle'.
- RNA $\xrightarrow{\text{RNases}}$ Transformation takes place So, RNA is not a 'transforming Principle'.
- DNA $\xrightarrow{\text{DNases}}$ Transformation inhibited. Therefore, DNA is the 'Transforming Principle'.

Hershey and Chase Experiment : In 1952, Hershey and Chase performed an experiment on bacteriophages (Virsues that infect bacteria) and proved that

DNA is the genetic material.

Bacteriophage Radioactive (S^{35}) Labelled protein coat	Bacteriophage Radioactive (P^{32}) labelled DNA
↓	↓
Infection : E. coli	E. coli
Blending : Viral coats removed from the bacteria.	
↓	↓
Centrifugation : Viral particles separated from the bacerial cell.	
↓	↓
No radioactive (S^{35}) Detected in bacterial cells but detected in supernatant	Radioactive (P^{35}) detected in bacterial cells but not in supernatant

Conclusion : DNA is the genetic material.

Meselson and Stahl's Experiment :

- Meselson and Stahl performed the experiment in 1958 on *E. coli* to prove that DNA replication is semiconservative.
- E. coli was grown in $^{15}NH_4CI$ for many generations.
- N^{15} was incorporated into newly synthesised DNA.
- This heavy DNA could be differentiated from normal DNA by centrifugation in cesium chloride (CsCl) density gradient.
- Then they transferred these *E.coli* into medium with normal $^{14}NH_4Cl$.
- After 20 minutes, it was found that all the DNA molecules of daughter cells were hybrid-**First generation.**
- After 40 minutes, it was found that 50% DNA molecules were hybrid and 50% were normal-**second generation.**

DNA replication

DNA strands start separating from ori (origin of replication). This unwinding is catalysed by many enzymes. Y-shaped structure is formed at ori called replication fork

↓

DNA polymerase attaches to the replication fork and add nucleotides complementary to the parental DNA strand. The direction of polymerisation is 5′-3′.

↓

DNA polymerase cannot initiate the polymerisation itself, so a small segment of RNA called primer is attached at replication start point

↓

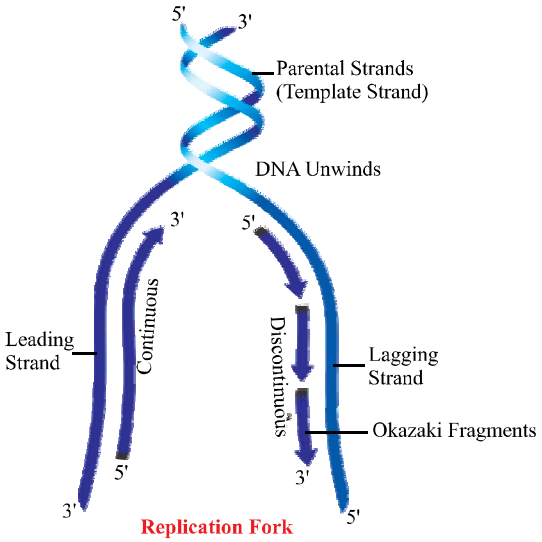
DNA polymerase adds nucleotides on one of the template strand called as leading strand (the template with polarity 3′-5′). In this strand nucleotides are added continuously therefore called as continuous replication

↓

On the other strand the replication is discontinuous, small fragment of DNA are formed called okazaki fragments which are later joined by DNA ligase. This strand is called as lagging strand.

↓

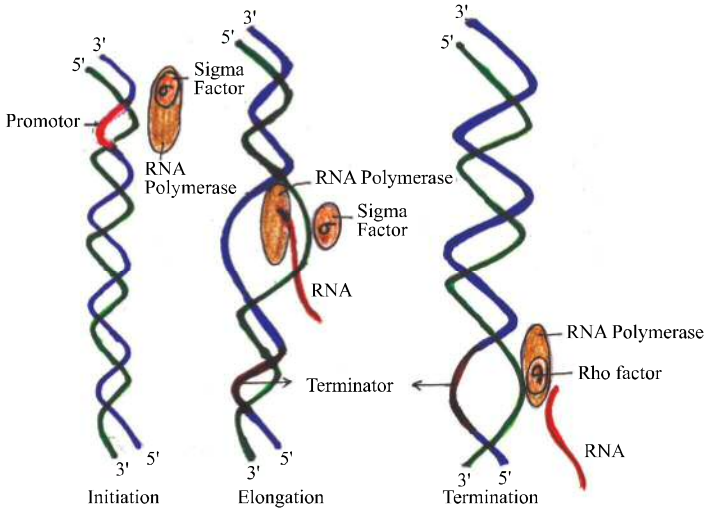
Accuracy of polymerisation is maintained by Proof reading and any wrong base added is removed by DNA polymerase



Replication Fork

Transcription in Prokaryotes : In prokaryotes the process of transcription is completed in three steps :

1. **Initiation :** RNA polymerase binds with initation factor (sigma factor) and then binds to promotor site.
2. **Elogation :** RNA polymerase separates from sigma factor and adds nucleoside triphosphate as substrate. RNA is formed during the process following the rule of complementary and remains bound to enzyme RNA polymerase.
3. **Termination :** On reaching terminator region RNA polymerase binds with who factor (terminator factor) As a result nascent RNA separates.



Transcription in Prokaryotes

Transcription in Eukaryotes :

- In eukaryotes three types of RNA polymerases found in the nucleus. (In addition to the RNA polymerase found in the organelles) are involved in transcription.

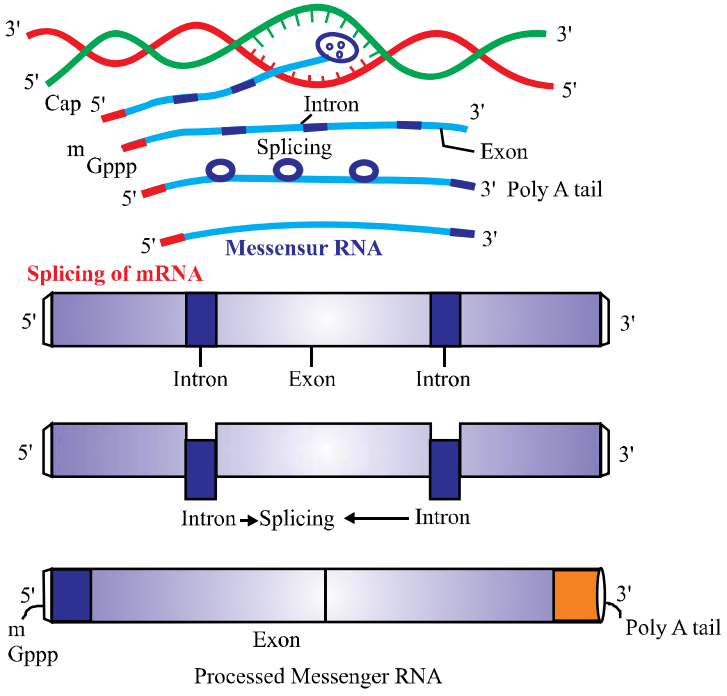
  RNA Polymerase I : Transribes rRNAs.

  RNA Polymerase II : Transcribes hnRNA (which is precursor of mRNA).

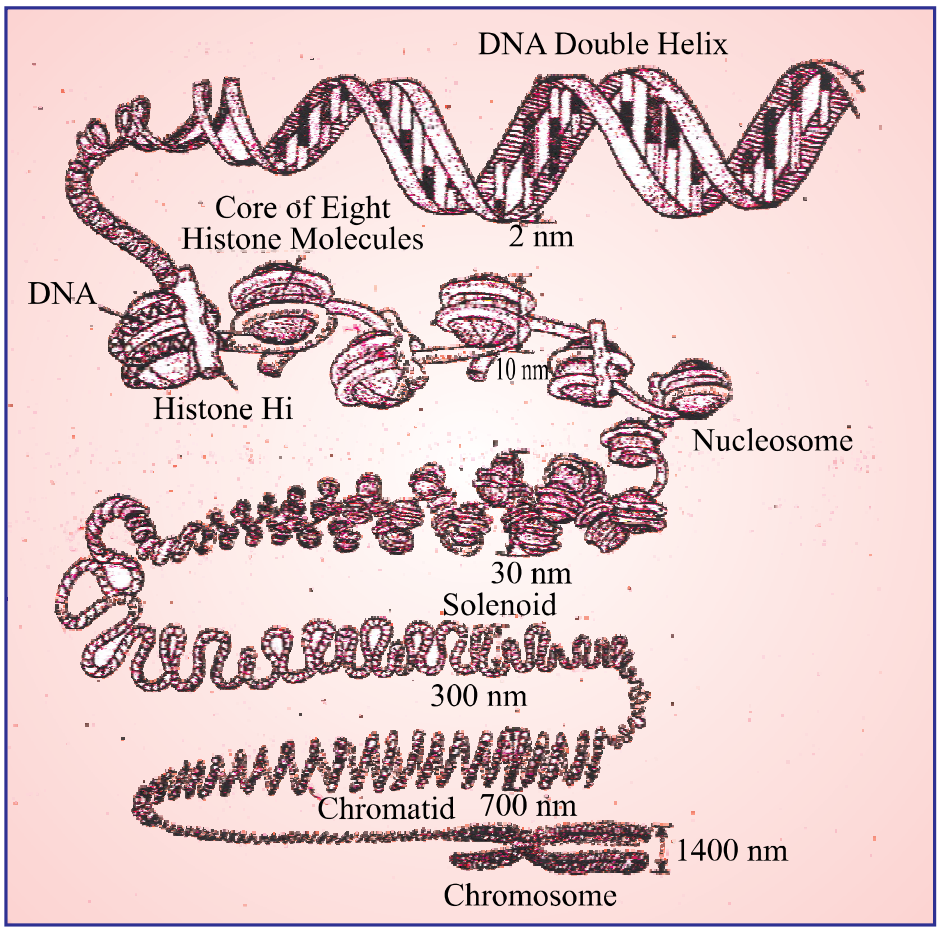
- **RNA Polymerase III :** Transcribes tRNA, 5 srRNA and sn RNA.
- The primary transcription has both exon and intron regions.
- Introns which are non-coding regions removed by a process called splicing.
- hnRNA undergoes who additional process :

  (a) **Capping :** An unusual nucleotide (methylguanosine triphosphate) is added to 5'–end of hnRNA.

  (b) **Tailling :** Adenylate residues (200-300) are added at 3′–end. It is fully processed hnRNA. Now called mRNA is transported out of the nucleus.



Transcription in Eukaryotes



Genetic Code

(i) The codon is triplet 61 codons code for amino acids and 3 codons function as stop codons (UAG, UGA, UAA)

(ii) One codon codes for only one amino acid, hence the codon is unambiguous and specific.

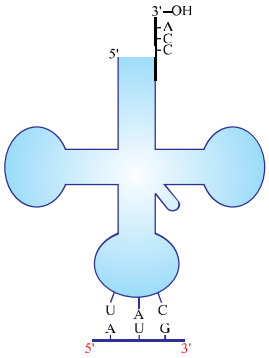
(iii) Some amino acids are coded by more than one codon, hence called as degenerate.

(iv) The codon is read in mRNA in a contiguous fashion. There are no punctuations.

(v) The code is nearly universal.

(vi) AUG has dual functions. It codes for Methionine (met) and it also acts as initiator codon.

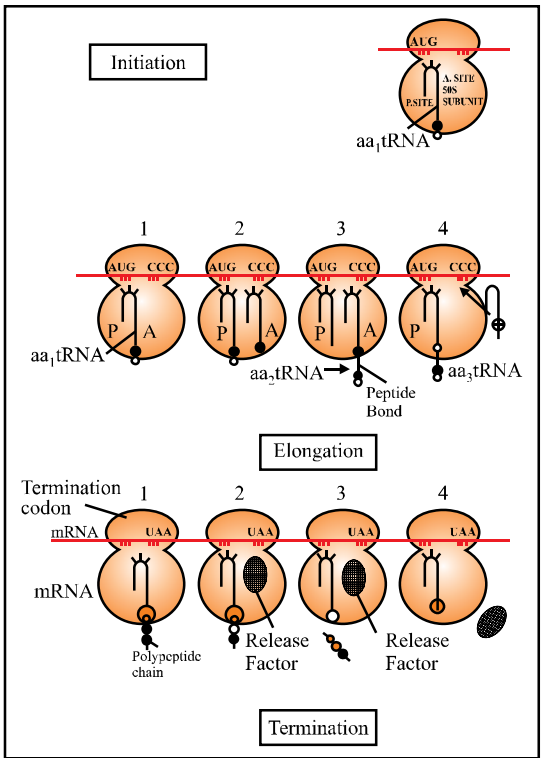
tRNA, the Adapter Molecule

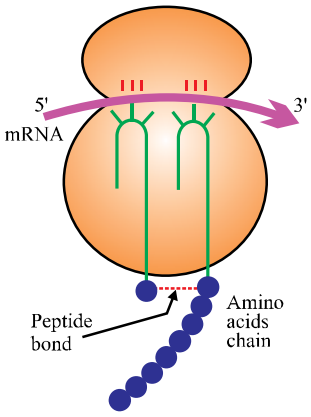


- tRNA has an anticoden loop that has bases complementary to the code, and also has an amino acid aceptor and through which it bind to amino acids.

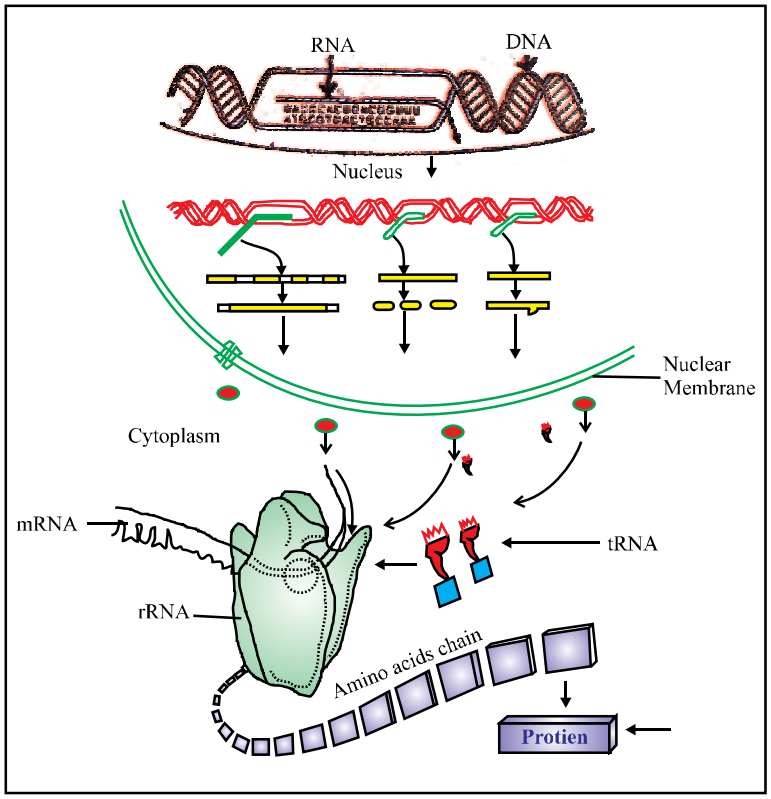
Translation

- 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. 20 amino acids participate in naturally occuring protein synthesis.





- First step is—charging of tRNA or aminoacylation of rRNA-here amino acids are activated in the presence of ATP and linked to specific tRNA.
- **Initiation :** Ribosome binds to mRNA at the start codon (AUG) that is recognized by the initiator tRNA.
- **Elongation phase :** Here complexes composed of an amino acid linked to tRNA. Sequentially bind to the appropriate codon in mRNA by forming complementary base pairs with rRNA codon. The ribosomes move from codon to codon along with mRNa. Amino acids are added one by one, translated into polypeptide sequences.
- **Termination :** Release facotors binds to the stop codon (UAA, UAG, UGA) translation and releasing the complete polypeptide from the ribosome.



Lac Operon

- The concept of operon was proposed by Jacob and Monod. Operon is a unit of prokaryotic gene expression.

- The lac operon consists of one regulatory gene (the i-gene) and three structural genes (z, y and a).
- The i-gene codes for repressor of lac operon.
- Promoter - It is the site where RNA-polymerase binds for transcription.
- Operator—acts as switch for operon.
- Lactose is an inducer.
- Operator : Act as switch for operon.
- Gene z—Codes for b-galactosidase

  Gene y—Codes for permease

  Gene a—Codes for transacetylase.

In the absence of Inducer (lactose)

Repressor (i-gene) binds with operator (o)

↓

Operator (O) turns off

↓

RNA polymerase stops the transcription

↓

structural genes (z, y and a) do not produce lac mRNA and enzymes

In the presence inducer (lactose)

Repressor binds to inducer (lactose)

↓

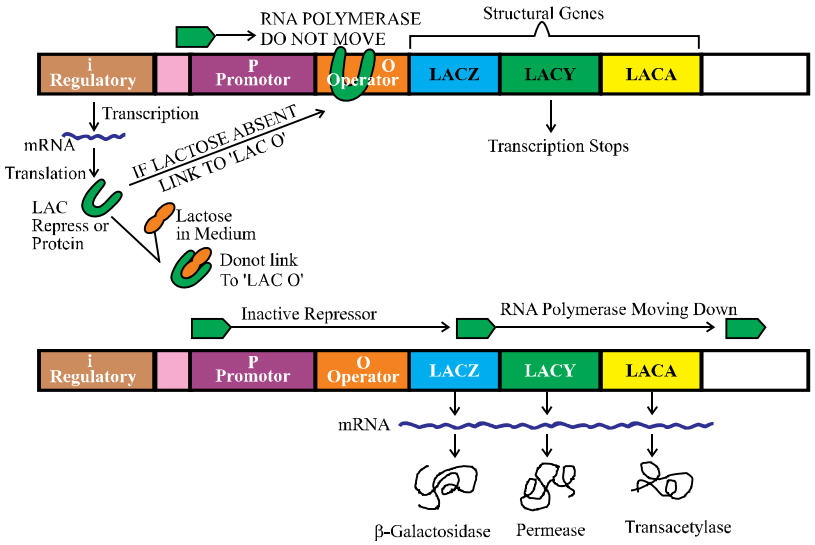
Operator (O) turns ON

↓

RNA polymerase starts the transcription

Structural genes (z, y and a) produce mRNA and enzymes

(β-galactosidase, permease and transacetylase respectively)



Human Genome Project was a 13 year project coordinated by the U.S. Department of energy and National institute of Health, it was completed in 2003.

Important goals of HGP

(i) Identify all the approzimately 20,000-25,000 genes in human DNA.

(ii) Determinate the sequence of the 3 millon chemical base pairs that make up human DNA.

(iii) Store this information in database.

(iv) Transfer the related technologies to other sectors, such as industries.

(v) Address the ethical, legal and social issues (ELSI) that may arise from the project.

Methodologies-2 major approaches

Expressed Sequences Tags (ESTs) (identifying all genes that are expressed as RNA	Sequence Annotation (Blind approach of simply sequencing the whole set of genome)

Steps for Sequencing :

- DNA isolated from cell and converted into fragments.
- DNA is cloned for amplification is suitable host using specialssed vectors.
- Commonly used hosts—Bacteria, Yeast
- Commonly used Vectors—BAC (Bacterial Artifical chromosomes) YAC (Yeast Artificial Chromosomes)

International Rice Genome Sequencing Project (IRGSP)

- Rice benefits from having the smallest genome of the major cereals, dense genetic maps.
- The IRGSP, formally established in 1998, pooled the resources of sequencing groups in 10 nations (Japan, Korea, UK, Taiwan, China, Thailand, India, United States, Canada and France)
- Estimated Cost— $ 200 million.
- India joined in June 2000 and chose to sequence a part of chromosome 11.
- Tools used in sequencing were :

  BAC (Bacterial Artificial chromosomes)

  PAC (P1-Phase derived artificial chromosomes)

- **How Sequenced**

  Shotgun sequencing involved—generation of short DNA fragments that are then sequenced and linearly arranged.

  It enables full coverage of the genome in a fraction of time required for the atternative BAC sequence approach.

- **Salient Features of Rice Genome**

  Rice is monocarpic annual plant, wind pollinated. It is with only 389 base pairs.

The world's first genome of a crop plant that was completely sequenced.

2,859 genes seem to be unique to rice & other cereals.

Repetitive DNA is estimated to constitute at least 505 of rice genome. The transposon content of rice genome is at least 35%.

- **Applications**

  To improve efficiency of Rice breeding.

  To improve nutritional value of rice, enhance crop yield by improving seed quality, resistance to pests and diseases and plant hardiness.

DNA Fingerprinting :

It is a technique of determine nucleotide sequence of certain areas of DNA which are unique to each individual.

Principle of DNA Fingerprinting : Short nucleotide repeats in the DNA are very specific in each individual and vary in number from person to person but are inherited. These are Variable Number Tandem Repeats. (VNTRs.) Each individual inherits these repeats from his/her parents which is used as genetic markers. One half of VNTR alleles of the child resembles that of mother and other half the father.

Steps/Procedure in DNA Fingerprinting

- Extraction of DNA—using high speed refrigerated centriguge.
- Amplification—many copies are made using PCR
- Restriction Digestion—using restriction enzymes DNA is cut into fragments.
- Separation of DNA fragments—using electrophoresis agarose polymer gel
- **Southern Blotting :** Separated DNA sequences are transferred on to nitrocellulose or nylon membranes.

- **Hybridization :** The nylon membranes exposed to radio active probes.
- **Autoradiography :** The dark bands develop at the probe site.

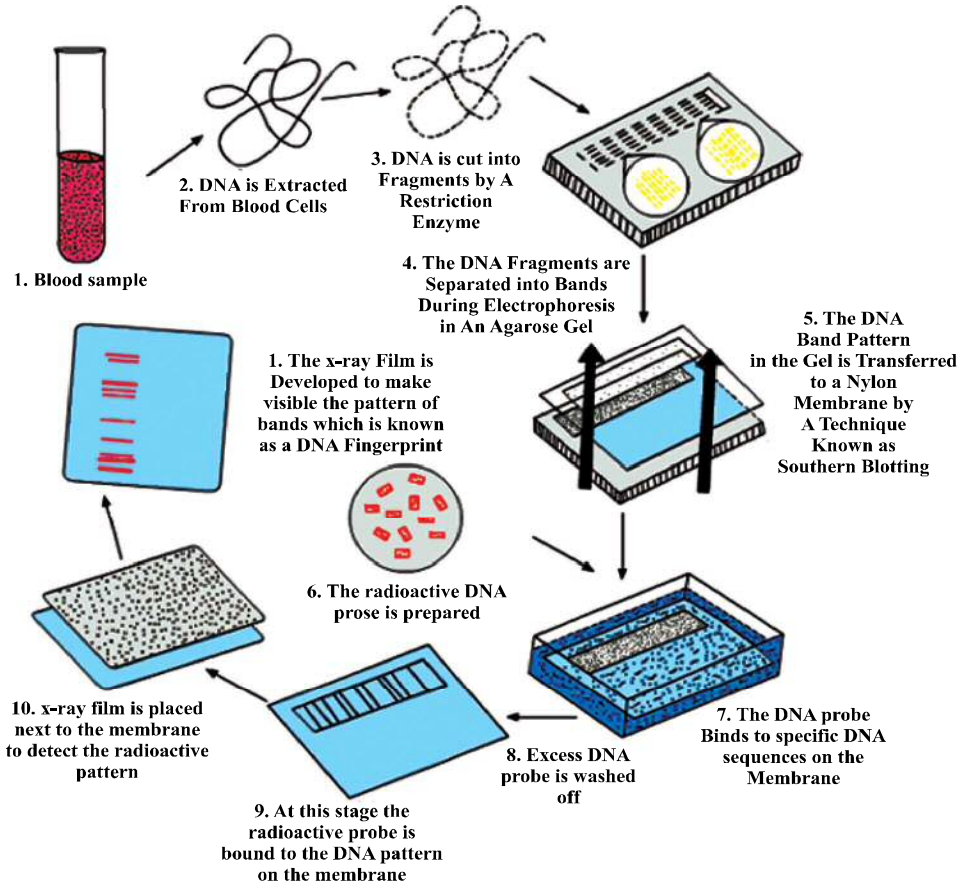
Applications of DNA Fingerprinting

(i) identify criminals if their DNA from blood, hair follicle, skin, bone, saliva, Sperm etc is available in forensic labs.

(ii) determine paternity

(iii) Verify whether a hopeful immigrant is really close relative of an already established resident.

(iv) identity racial groups to rewrite biological evolution.

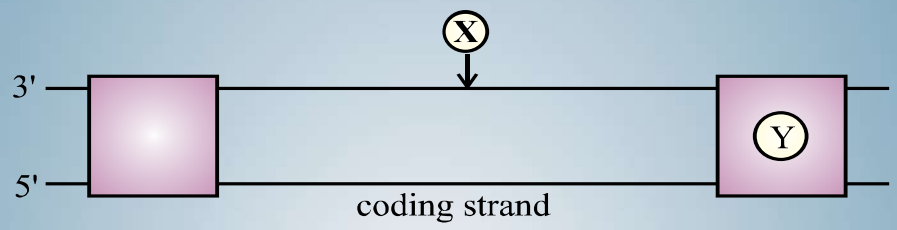


The DNA Fingerprinting Process

Questions

VSA (I Mark)

1. Name the factors for RNA polymerase enzymes which recognises the start and termination signals on DNA for transcription process in Bacteria.
2. RNA viruses mutate and evolve faster than other viruses. Why ?
3. Name the parts 'X' and 'Y' of the transcription unit given below.



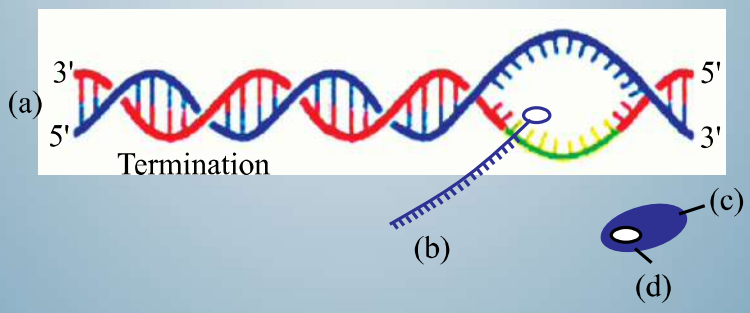
4. Name the two initiating codons
5. Write the segment of RNA transcribed from the given DNA

   3′ – A T G C A G T A C G T C G T A – 5′ – Template Strand

   5′ – T A C G T C A T G C A G C A T – 3′ – Coding Strand.

SA-I (2 Marks)

6. The process of termination during transcription in a prokaryotic cell is being represented here. Name the label a, b, c and d.



7. Give two reasons who both the standard of DNA are not copied during transcription.
8. State the 4 criteria which a molecule must fulfill to act as a genetic material.

SA-II **(3 Marks)**

9. Give six points of difference between DNA and RNA in their structure chemistry and function.
10. Explain how does the hnRNA becomes the mRNA.

OR

Explain the process of splicing, capping and tailing which occur during transcription in Eukaryotes.

11. Name the three major types of RNAs, specifying the function of each in the synthesis of Polypeptide.
12. A tRNA is charged with the aminoacids methionine.
    (i) Give the anti-codon of this tRNA.
    (ii) Write the Codon for methionine.
    (iii) Name the enzyme responsible for binding of aminoacid to tRNA.

LA **(5 Marks)**

13. State salient features of genetic code.
14. Describe the process of transcription of mRNA is an eukaryotic cell.
15. Describe the various steps involved in the technique of DNA fingerprinting.

Answers

VSA **(1 Mark)**

1. Sigma (σ) factor and Rho(ρ) factor
2. OH group is present on RNA, which is a reactive group so it is unstable and mutate faster.
3. X Template strand, Y – Terminator.
4. AUG and GUG
5. 5′ – U A C G U C A U G C A G C A U – 3′ (In RNA 'T' is replaced by 'U')

SA-1 **(2 Marks)**

6. (a) DNA molecule
   (b) mRNA transcript
   (c) RNA polymers
   (d) Rho factor

7. (a) If both the strands of DNA are copied, two different RNAs (complementary to each other) and hence two different polyeptides; if a segment of DNA produces two polypeptides, the genetic information machinery becomes complicated.

(b) The two complementary RNA molecules (produced simultaneously) would form a double-stranded RNA rather than getting translated into polypeptides.

(c) RNA polymerase carries out polymerisation in 5′ – 3′ direction and hence the DNA strand with 3′ – 5′ polarity acts as the template strand. (Any two)

8. (i) It should be able to generate its replica.

(ii) Should be chemically and structurally stable.

(iii) Should be able to express itself in the form of Mendelian characters.

(iv) Should provide the scope for slow changes (mutations) that are necessary for evolution.

SA-II **(3 Marks)**

9.	**DNA**	**RNA**
(i)	Double stranded molecules	Single stranded molecules
(ii)	Thymine as pyrimidine base	Uracil as pyrimidine base
(iii)	Pentose sugar is Deozyribose	Sugar is Ribose
(iv)	Quite stable and not very reactive	2′-OH makes it reactive
(v)	Dictates the synthesis of Polypeptides	Perform other function in protein synthesis.
(vi)	Found in the nucleus.	They are transported into the cytoplasm.

10. hnRNA is precursor of mRNA. It undergoes

(i) **Splicing :** Introns are removed and exons are joined together.

(ii) **Capping :** an unusual nucleotide (methyl guanosine triphosphate is added to the 5′ end of hnRNA.

(iii) Adenylate residues (200-300) are added at 3′ end of hnRNA.

Or

Refer fig. 6.11, page 110, NCERT book. Biology-XII

11. (i) mRNA-(Messenger RNA) : decides the sequence of amino acids.

(ii) tRNA-(Transfer RNA) : (a) Recognises the codon on mRNA (b) transport the aminoacid to the site of protein synthesis.

(iii) rRNA (Ribosomal RNA) : Plays the structural and catalytic role during translation.

12. (a) UAC (b) AUG

(c) Amino-acyl-tRNA synthetase.

LA **(5 Marks)**

13. Refer page 6.9.1., Page No. 120 NCERT Biology XII.

14. Refer notes 35 and figure 6.11, page 110, NCERT Biology XII.

15. Refer points to remember. Steps involved in DNA fingerprinting.

❑ ❑