MOLECULAR BASIS OF INHERITENCE

HSSLIVE.IN

Introduction

- There are 2 types of nucleic acids
  - **DNA (Dexoy ribonucleic acid)**
  - **RNA (Ribo nucleic acid)**
- DNA is the genetic material in most of the organism including human being
- But there are some viruses (**Retrovirus**) in which genetic material is RNA.
  Eg: **HIV, TMV, QB Bacteriophage**
- DNA is acidic substance present in the nucleus was first identified by **Friedrich Meischer (1869).** He called it as **Nuclein**.
- Due to **technical limitation** he could not isolate such a long polymer

The Search for the Genetic Material

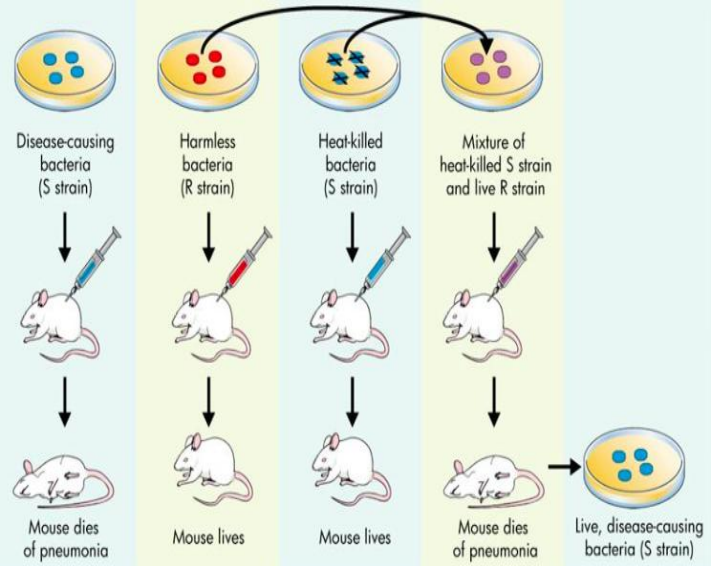
1-Transforming principle/ Griffith effect (1928)

- This experiment is conducted by Frederick Griffith in 1928.
- He conducted experiment on *Streptococcus pneumoniae (*Pneumococcus-It is a coccus bacteria that cause pneumonia)
- There are 2 strains of pneumococcus bacteria
  - **S-Strain**
  - **R-Strain**
- **S strain bacteria:** The bacteria produce smooth and shiny colony in culuture plate. This is ecause the S strain bacteria has mucous (Polysacharide)Coat. This strain of bacteria is virulent and cause pneumonia
- **R strain :** It produce rough colonies when grown on culture plate. The rough appearance is due to the lack of mucous coat. This type of bacteria is avirulent and do not cause pneumonia

Experiment

- S strain ⟶ Inject into mice ⟶ Mice die
- R strain ⟶ Inject into mice ⟶ Mice live
- S strain (heat-killed) ⟶ Inject into mice ⟶ Mice live
- He then injected a mixture of Heat killed S strain and Live R strain bacteria, the mice died due to pneumonia, moreover , he recovered living S bacteria from died mice

- S strain (heat-killed) + R strain (live) ⟶ Inject into mice ⟶ Mice die



- He concluded that the **R strain bacteria had somehow been transformed by the heat-killed S strain bacteria**. Some **'transforming principle'**, transferred from the heat-killed S strain, had enabled the R strain to synthesise a smooth polysaccharide coat and become virulent.
- This must be due to the transfer of the genetic material. However , the biochemical nature of genetic material was not defined from his experiments.

Biochemical Characterisation of Transforming Principle

- Prior to the work of **Oswald Avery, Colin MacLeod and Maclyn McCarty (1933-44**), the genetic material was thought to be a **protein.**
- They worked to determine the biochemical nature of 'transforming principle' in Griffith's experiment.
- They purified biochemicals (**proteins, DNA, RNA, etc.)** from the heat-killed S cells to see which ones could transform live R cells into S cells. They discovered that DNA alone from S bacteria caused R bacteria to become transformed.
- They also discovered that protein-digesting enzymes (**proteases)** and RNA-digesting enzymes **(RNases)** did not affect transformation, so the transforming substance was not a protein or RNA.
- **Digestion with DNase did inhibit transformation, suggesting that the DNA caused the transformation.**

- They concluded that DNA is the hereditary material, but not all biologists were convinced.

2-Hershey-Chase experiment (1952)

- The unequivocal proof that DNA is the genetic material came from the experiments of Alfred Hershey and Martha Chase (1952).
- They worked with viruses that infect bacteria called bacteriophages.

Life cycle of Bacteriophage

- The bacteriophage attaches to the bacteria
- Bacteriophage introduce its genetic material into the bacterial cell.
- The bacterial cell treats the viral genetic material as if it was its own and subsequently manufactures more virus particles.

Hershey and Chase worked to discover whether it was protein or DNA from the viruses that entered the bacteria

Experiment

- Hershey and chase grew some viruses in a medium containing radioactive phosphorus (^{32}P). Viruses grown in the presence of radioactive Phosphorus (^{32}P) contain radioactive DNA but not radioactive protein, because DNA contains phosphorus but protein does not
- They also grew some viruses (bacteriophages) on a medium containing radioactive Sulphur (^{35}S). Viruses grown in the presence of radioactive sulphur (^{35}S) contains radioactive protein but not radioactive DNA because protein contains sulphur but DNA does not.
- Radioactive phages were allowed to attach to E. coli bacteria. This step is called **Infection**
- Then, as the infection proceeded, the viral coats were removed from the bacteria by agitating them in a blender. This process is called **Blending**
- The virus particles were separated from the bacteria by spinning them in a centrifuge. This process is called **centrifugation**
- Bacteria which was infected with viruses that had radioactive DNA were radioactive, indicating that DNA was the material that passed from the virus to the bacteria.
- Bacteria that were infected with viruses that had radioactive proteins were not radioactive. This indicates that proteins did not enter the bacteria from the viruses.

- **DNA is therefore the genetic material that is passed from virus to bacteria**
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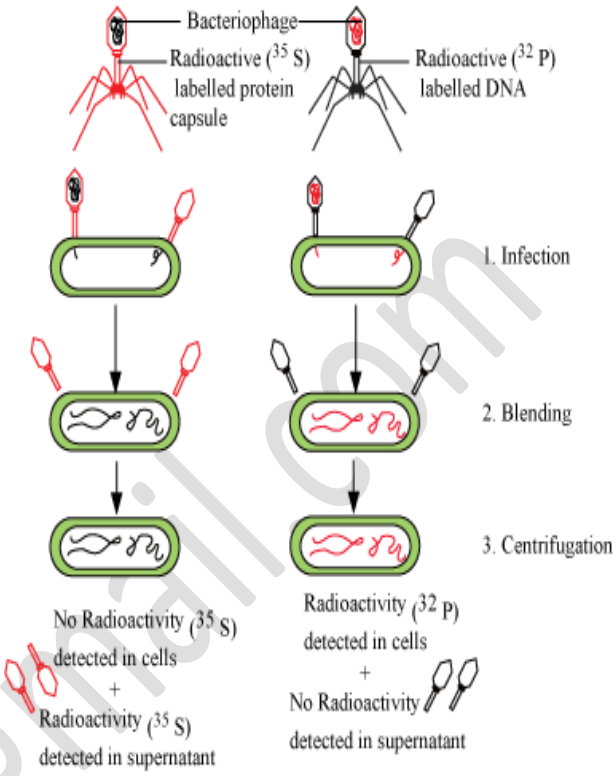


DNA (Deoxy Ribonucleic acid)

- It is an acidic substance present in the nucleus
- It was first identified by **Friedrich meischer** in 1869
- He named it as **Nuclein**. However due to technical limitation in isolating such a long polymer, the elucidation of structure of DNA remains elusive
- **In 1953, James Watson and Francis Crick** proposed a very simple but famous **Double Helical Structure of DNA**. They proposed this model based on X ray diffraction data proposed by Maurice Wilkins and Rosalind Frankiln
- The length of DNA is usually defined as the **number of nucleotides** (Or Base pairs: a pair of nucleotides)

Eg: Man = 6.6×10^9 BP
E.Coli = 4.6×10^6 BP
Lambda phage =48502 BP
Ø X 174 phage =5386 nucleotides (Single stranded DNA)

Structure of DNA

(Read your notebook)

The salient features of the Double-helix structure of DNA

01- It is made of **two polynucleotide chains**, where the backbone is constituted by sugar -phosphate, and the bases project inside.

02- The two chains have **anti-parallel polarity**. It means, if one chain has the polarity 5'→3', the other has 3'→5'.

03- The bases in two strands are **paired through hydrogen bond** (H-bonds) forming base pairs (bp). Adenine forms two hydrogen bonds with Thymine from opposite strand and vice-versa. Similarly, Guanine is bonded with Cytosine with three H-bonds. As a result, always a purine comes opposite to a pyrimidine. This generates approximately uniform distance between the two strands of the helix (20A^0)

04- The two chains are coiled in **a right -handed fashion**. The pitch of the helix is 3.4 nm (a nanometre is one billionth of a metre, that is 10^{-9} m) and there are roughly **10 bp in each turn**. Consequently, the distance between a bp in a helix is approximately equal to 0.34 nm.

05- The plane of one base pair stacks over the other in double helix. This, in addition to H-bonds, confer stability of the helical structure

Chargaff Rule

- ✓ Proposed y Erwin Chargaff
- ✓ For a double stranded DNA the ratios between Adenine and Thymine, and Guanine and Cytosine are constant and equal one.

Ie: A+G=T+C **(or)** A+G/T+C=1

Packaging of DNA Helix

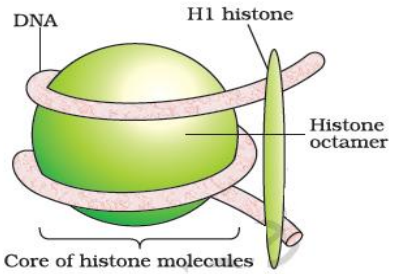
a)Packaging of DNA in Eukaryote

Length of DNA =Total number of Base pair × distance between adjacent base pair

= 6.6 × 10^9BP × 0.34 ×10^{-9}

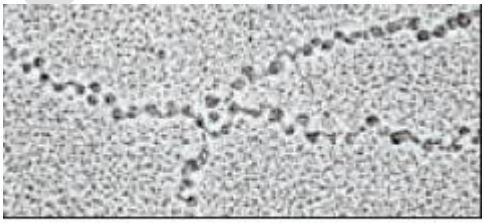
= 2.2m

- This length that is far greater than the dimension of a typical nucleus (approximately 10–6 m).
- The negatively charged DNA is wrapped around the positively charged histone octamer to form a structure called nucleosome.
- In Eukaryotes there is a set of +vely charge basic proteins called **Histones**
- Histones are rich in +vely charged basic amino acids (Amino acids are **Lysine and arginine**)
- The +ve charge is due to the presence of +ve charge on both the amino acid residues.
- Histones are organised to form a unit of eight molecules called as histone octamer.



-NUCLEOSOME-

- Nucleosomes constitute the repeating unit of a structure in nucleus called **chromatin**, thread-like stained (coloured) bodies seen in nucleus.
- The nucleosomes in chromatin are seen as '**beads-on-string'** structure when viewed under electron microscope (EM)



EM picture - 'Beads-on-String'

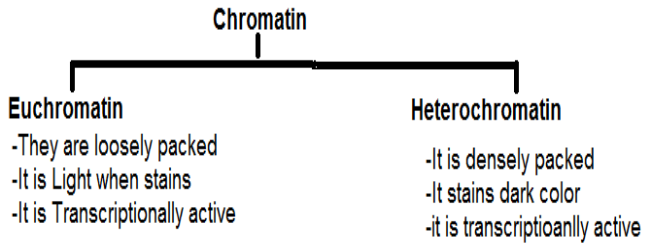
- A typical nucleosome contains **200 bp** of DNA helix.

There for total number of nucleosome in human :

$$\frac{6.6x10^9 bp}{200} = 3.3x10^7$$

- The beads-on-string structure in chromatin is packaged to form chromatin fibers 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 proteins that collectively are referred to as **Non-histone Chromosomal (NHC) proteins.**

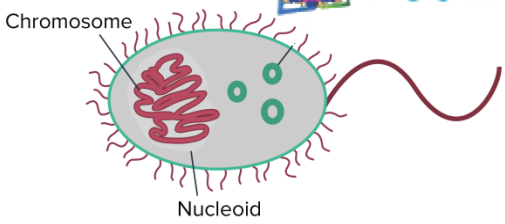
Chromatin is packaged → chromatin fibres → coiled and condensed at metaphase stage → chromosomes.



b)Packaging of DNA in Prokaryote

Eg : E.coli

- ✓ In prokaryotes, such as, E. coli, though they do not have a defined nucleus, the DNA is not scattered throughout the cell.
- ✓ -vely charged DNA is held with +vely charged proteins in a region termed as '**nucleoid'.**
- ✓ The DNA in **nucleoid** is organised in **large loops** held by proteins.



RNA (Ribo nucleic acid)

- ✓ It is formed of a single polynucleotide chain
- ✓ It act as the genetic material in some viruses. Such viruses are called Retroviruses
  Eg: HIV, TMV, QB Bacteriophage.
- ✓ RNA was the first genetic material.
- ✓ RNA used to act as a genetic material as well as a catalyst (there are some important biochemical reactions in living systems that are catalyzed by RNA catalysts (**RIBOZYME**) and not by protein enzymes).
- ✓ RNA being a catalyst was reactive and hence unstable. Therefore, DNA has evolved from RNA with chemical modifications that make it more stable.

RNA (Unstable) ----Chemical Modification----> DNA (Stable)

Properties of Genetic Material

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

- Both the nucleic acids (DNA and RNA) have the ability to direct their duplications/Replication
- But protein do not replicate

(ii) It should chemically and Structurally be stable.

- ✓ The genetic material should be stable enough not to change with different stages of life cycle, age or with change in physiology of the organism.
- ✓ Stability as one of the properties of genetic material was very evident in Griffith's 'transforming principle' itself that heat, which killed the bacteria, at least did not destroy some of the properties of genetic material.
- ✓ But in RNA, Present of 2' OH group make it catalytic and reactive hence RNA is unstable.

(iii) It should provide the scope for slow changes (mutation) that are required for evolution.

- ✓ RNA is unstable so RNA viruses mutates and evolve faster

(iv) It should be able to express itself in the form of 'Mendelian Characters'.

- ✓ For the storage of genetic information, DNA is better due to its stability.
- ✓ But for the transformation of genetic information RNA is better. Because RNA can directly code for the protein synthesis. Hence can easily express the character. But DNA depend on RNA for protein synthesis

Q- Among two nucleic acid DNA is better genetic material. Explain

Ans : DNA is stable and less reactive. For the storage of genetic information, DNA is better due to its stability.

Q- What is the reason for the stability of DNA?

Ans: -DNA is double stranded
-Presence of thymine
-Absence of 2' OH in sugar

Q- Why retrovirus mutates and Evolve faster?

Ans : Retroviruses are viruses in which genetic material is RNA. RNA is unstable, due to the presence of 2'OH in the sugar (Ribose). It make RNA unstable and catalytic. Hence retrovirus mutate faster rate.

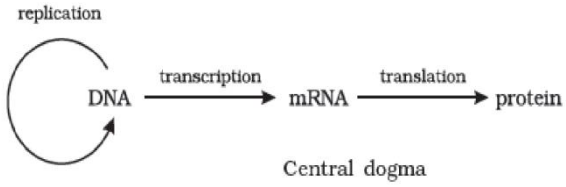
Q- Difference between DNA and RNA ?

DNA	RNA
-It is formed of 2 poly nucleotide chain	-It is formed of single poly nucleotide chain
-the sugar in DNA is dexoy ribose	-The Sugar in RNA is Ribose
-The nitrogen base in DNA is A,T,G,C	-The nitrogen base in RNA is A,U,G,C

-Stable	-Unstable and catalytic

CENTRAL DOGMA OF MOLECULAR BIOLOGY

- Proposed by Francis Crick
- It is the unidirectional flow of information form DNA-RNA-Protein



Central dogma

- In some viruses the flow of information is in reverse direction

RNA----------Reverse transcription----------> DNA

- It is an exception to central dogma of molecular biology.

DNA REPLICATION

- DNA replication is the copying for DNA from parent DNA
- Watson and crick proposed Semiconcervative method of DNA replication
- According to this 2 daughter DNA are produced from parent DNA. Each daughter DNA consist of 2 strands, one strand is newly synthesised and other strand belongs to parent.
  Ie: Parent's strand is conserved
- While proposing the double helical structure for DNA, Watson and Crick had immediately proposed a scheme for replication of DNA.
  To quote their original statement that is as follows:
  ''It has not escaped our notice that the specific Pairing we have postulated immediately suggests a possible copying mechanism for the genetic material'' (Watson and Crick, 1953).
- DNA replication takes place in the S phase of cell division cycle.
- Failure of cytokinesis after DNA replication results **Polyploidy** (Increase in the wholes set of chromosome). Polyploidy is common in **plant cells**

Experimental verification of semiconcervative method of DNA replication

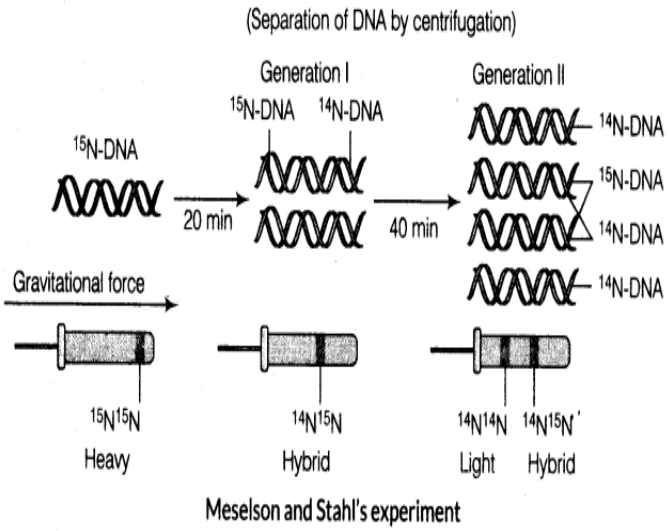
- It was first shown in E.coli and later in higher organisms such as plants and Human cell.

1) Meselson-Stahl experiment (1958)

- Proposed by Matthew Meselson and Franklin Stahl performed the following experiment in 1958
  - ❖ They grew *E. coli* in a medium containing $^{15}NH_4Cl$ (^{15}N is the heavy isotope of nitrogen).^{15}N was incorporated in to both strand of bacterial DNA and the DNA become heavier. This DNA is called Heavy DNA.
  - ❖ They also grew *E. coli* in a medium containing $^{14}NH_4Cl$ (^{14}N is the normal isotope of nitrogen).^{14}N was incorporated in to both strand of bacterial DNA and the DNA become lighter. This DNA is called light DNA.
  - ❖ They took E.coli from ^{15}N medium (Heavy DNA) and transferred into ^{14}N medium. After one generation (20 minutes), they isolated and centrifuged the DNA. Its density was intermediate (hybrid). This shows that , in the newly formed DNA, one strand is newly formed (^{14}N) and other strand belongs to parent (^{15}N).This confirms semi conservative method DNA replication.
  - ❖ After second generation (40 minutes), there was equal amount of hybrid DNA and light DNA.

Heavy DNA can be distinguished from light DNA y centrifugation in **CSCl (cesium chloride**) density gradient.

(See your note book for the picture)



Meselson and Stahl's experiment

2) Taylor's Experiment (1958)

- Conducted by Taylor and Colleague
- He used *Vicia faba* (Faba beans) as experimentall material .
- He used Radioactive thymidine to detect presence of newly synthesised DNA

Enzymes in DNA Replication

a) Helicase

- During replication, the 2 strands **unwind** and separated by breaking the H bond in the presence of Helicase enzyme
- DNA Replication is **energetically expensive** that is why during replication 2 strands of DNA cannot be separated in its entire length

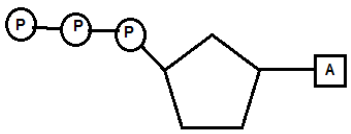
b) RNA Primase

- It synthesise **RNA Primer** (Small stretch of RNA)

c) DNA Dependent DNA polymerase

- It is the main enzyme in DNA replication
- It uses a DNA template to catalyse polymerisation of dexoy nucleotides
- In E.Coli, it polymerise the nucleotides in faster rate (2000BP/second).
- In E.coli DNA replication completes in 18 minutes
- This enzyme has high accuracy
- It polymerise the nucleotides in 5'→ 3 direction

Deoxy Ribonucleoside Triphsophate



- It has 2 functions,
  i)It act as substrate.
  ii)It provide energy for polymerisation

The 2 terminal phosphate in a dexoy ribo nucleoside triphosphates are high energy phosphate. It provides energy for polymerisation.

Replication Origin

DNA replication starts at a point called Replication Origin.

Replication Fork

Unwinding of DNA molecule at a point forms Y shaped structure called Replication Fork

Template

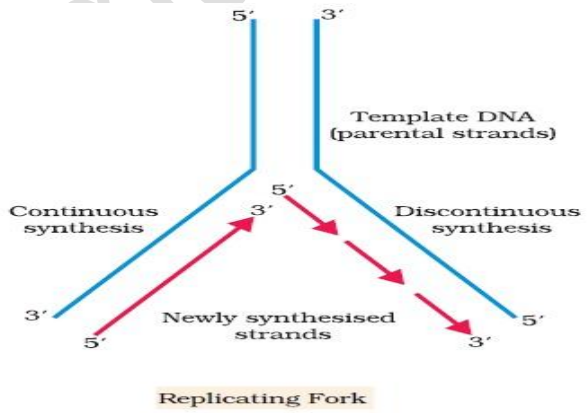
During DNA replication, the 2 strands separate and act as a template for the synthesis of new strand. New strands are synthesised based on template sequence.

Leading strand and Lagging strand
(Continous and discontinuous strand)

- ✓ In the presence of DNA dependent DNA polymerase, many nucleotides are joined to one another to form poly nucleotide (new strand). The DNA polymerase forms one new strand (leading strand) in continous stretch in 5'-3' direction (Continous synthesis)
- ✓ The other new strand is formed in small fragment. This fragment of DNA is called Okazaki fragment. Okazaki fragment is seen in discontinues strand.

d) DNA Ligase

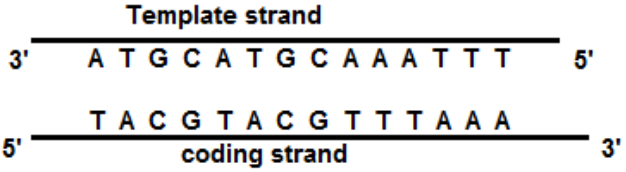
- ✓ Okazaki fragments are joined together to form new strand by DNA ligase.



Replicating Fork

DNA transcription

- ✓ The process of copying genetic information from one strand of DNA (Template) into RNA is called transcription.
- ✓ The enzyme involved in transcription is DNA dependent RNA polymerase.
- ✓ In DNA transcription only a segment (Gene) of DNA and only one of the strand (Template strand, 3'—5') is copied to RNA



- ✓ The strand with polarity 3'—5' act as a template (For mRNA synthesis), the other strand with

polarity 5'—3' is called coding strand (It do not code for anything).

Q-Why Both strands of DNA are not copied into RNA during transcription?

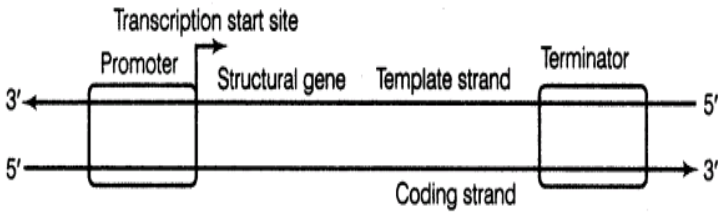
Ans : If both strand act as a template

i)Two RNA molecule will be produced with different sequences . It result in the formation of 2 different protein.

ii)Two RNA molecule will be produced, they are complementary to each other, hence it may for double stranded DNA. This RNA will not translate into protein.

Transcription Unit

- A transcription unit in DNA is defined byu 3 regions
  a) A promoter
  b) The structural gene
  c) A terminator



Schematic structure of a transcription unit

a) A promoter

- It is the site where DNA dependent RNA polymerase bind
- Transcription starts from promoter
- Promoter is located towards the 5' end (Upstream) of the structural gene (The reference is made with respect to the polarity of Coding strand)

b) **The structural gene**

- The promoter and Terminator flank the structural gene in transcription unit. RNA is produced from the structural gene

c) Terminator

- It is the site at which transcription stops.
- It is located towards the 3' end (Down stream)of coding strand

Steps in DNA Transcription

- It consist of 3 steps
  i)Initiation
  ii)Elongation
  iii)Termination

i)Initiation

- The sigma factor (σ factor/initiation factor) bind with RNA polymerase.
- This RNA polymerase bind to the promoter of Transcription unit and initiate transcription

ii) Elongation

- The DNA dependent RNA polymerase, polymerase nucleotides in a template dependent manner in 5'-3' direction.

iii) Termination

- Terminator is located towards the 3' end of coding strand, it usually defines the end process of transcription.
- The Rho factor (termination factor) terminates the process of transcription.
- The RNA Produces as a result of transcription in prokaryote is called mRNA (Messenger RNA).
- The RNA Produces as a result of transcription in Eukaryote is called hnRNA (Heterogeneous nuclear RNA).
- Both transcription and translation are couple in bacteria. Many times translation can begin much before the m RNA is fully transcribed

Transcription in Eukaryotes

- The process of transcription is same in both prokaryotes and eukaryotes.
- In Eukaryotes, there are 2 additional complexities
  i) There are at least 3 RNA polymerease in the nucleus
     - RNA Pol I : it transcribe r RNA (28S,18S,5.8S)
     - RNA Pol II : It transcribe hnRNA (Heterogenous nuclear RNA/ Precursor of mRNA)
     - RNA Pol III : It transcribe tRNA , 5srRNA, and snRNAs (small nuclear RNAs)
  ii) The hnRNA Produced as a result of transcription contains both Exons (Coding sequences) and Introns (non coding sequences),such RNA are non functional.

This hnRNA is subjected to processing (Splicing, capping, tailing) to become mRNA . Henc hnRNA is called precursor of mRNA .

hn RNA ----Splicing, capping,tailing----> mRNA

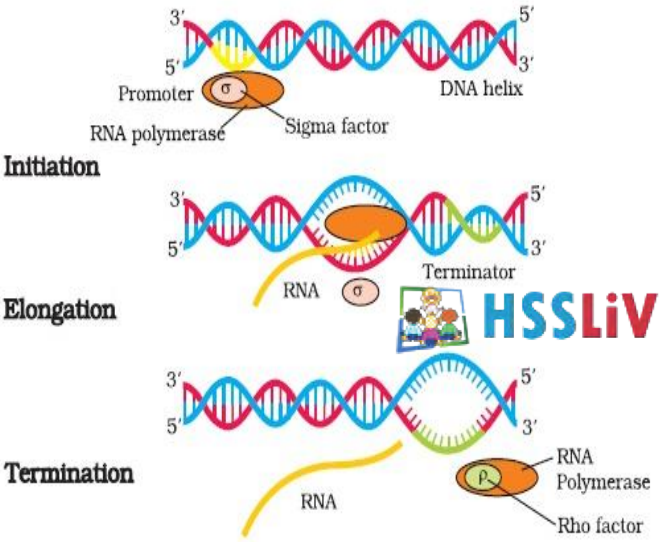
Splicing : It is the process by which Introns (Non coding sequences) are removed and Exons are join together in a defined order

Capping : Here an unusual nucleotides $^{m}G_{ppp}$ (methyl guanosine triphosphate) is added to the 5'-end of hnRNA.

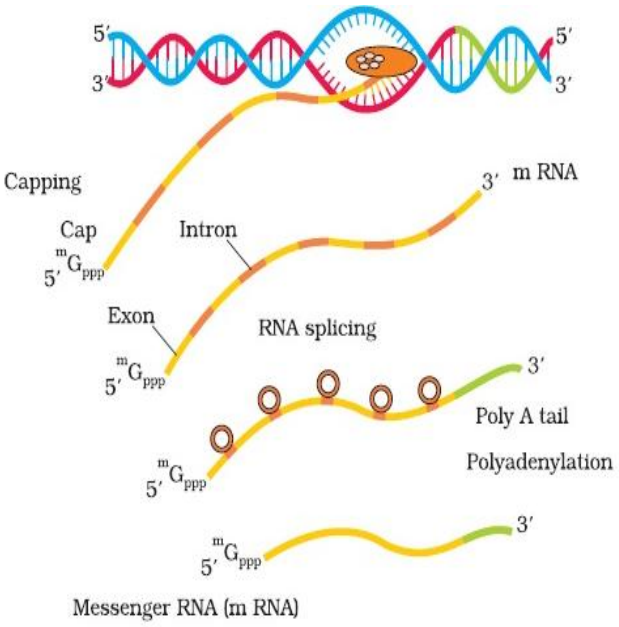
Tailing: In tailing, adenylate residues (200-300) are added at 3'-end in a template independent manner.

- The fully processed hnRNA, now called mRNA, It is then transported out of the nucleus for translation.

(Read pictures in the notebook-Transcription)



Process of Transcription in Bacteria



Process of Transcription in Eukaryotes

Difference between DNA replication and Transcription

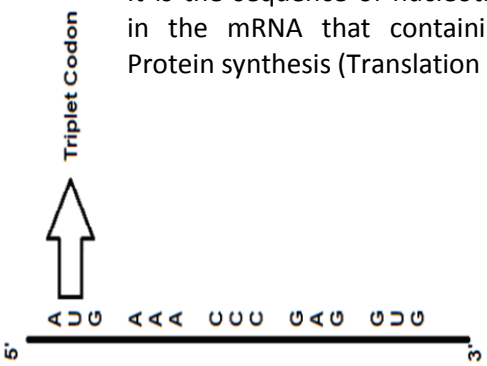
DNA REPLICATION	DNA TRANSCRIPTION
DNA→DNA	DNA→RNA
Enzyme in DNA replication is DNA dependent DNA polymerase	Enzyme in DNA transcription is DNA dependent RNA polymerase
The entire DNA is duplicated	Only one of the strand (Template) and a segment (Gene) DNA is copied into RNA

Difference between Prokaryotic and Eukaryotic transcription

Prokaryotic transcription	Eukaryotic transcription
Both transcription and translation are couple in prokaryotes	They are separate
The RNA contains only exons, this RNA is called mRNA	The RNA contains exons and Introns ,hence this RNA is called hnRNA
No processing required for mRNA	HnRNA Undergo processing (Splicing,capping, tailing) to become mRNA
A single DNA dependent RNA polymerase is involved in synthesizing all types of RNA	At least 3 types of RNA polymerase is involved

Genetic Code

- It is the sequence of nucleotides (Nitrogen base) in the mRNA that containing information for Protein synthesis (Translation)



Scientists involved in cracking the Genetic code

Several scientists belongs to several branches of science involved in cracking the genetic code such as it physicists, organic chemists, biochemists and geneticists. Some of the scientists are given below

1-George Gamow (Physicist) : he argued that since there are only 4 bases and if they have to code for 20 amino acids, the code should constitute a combination of bases. He suggested that in order to code for all the 20 amino acids, the code should be made up of three nucleotides (Triplet codon)

2-Har Gobind Khorana : The chemical method developed by Har Gobind Khorana was instrumental in synthesising RNA molecules with defined combinations of bases (homopolymers and copolymers).

3-Marshall Nirenberg : He developed cell free system for protein synthesis

4-Severo Ochoa : Severo Ochoa enzyme (polynucleotide phosphorylase) was also helpful in polymerising RNA with defined sequences in a template independent manner (enzymatic synthesis of RNA).

- Finally a checker-board for genetic code was prepared which is given below .

	U		C		A		G		
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys	C
	UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop	A
	UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg	C
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg	A
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg	G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser	C
	AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg	A
	AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg	G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly	C
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly	A
	GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly	G

The salient features of genetic code are as follows:

i) The codon is triplet. 61 codons code for aminoacids and 3 codons do not code for any amino acids, hence they function as stop codons.

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

iii) Some amino acids are coded by more than one codon, hence the code is degenerate.

Nondegenerate codon
AUG :Methionine
UGG: tryptophan

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

v) The code is nearly universal: for example, from bacteria to human UUU would code for Phenylalanine (phe). Some exceptions to this rule have been found in mitochondrial codons, and in some protozoans.

vi) AUG has dual functions.
-It codes for Methionine (met) ,
- it also act as initiator/start codon

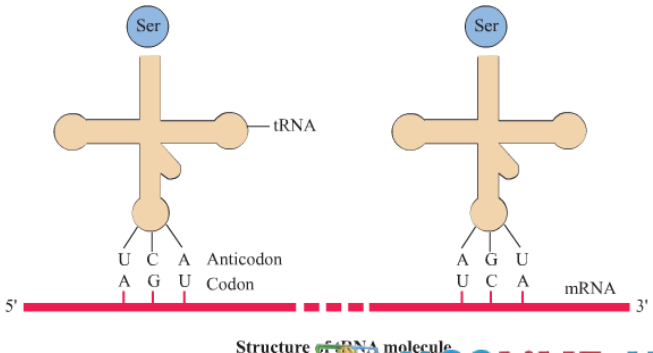
Genetic code	Amino acids
AAA	Lysine
CCC	Proline
GGG	Glycine
UUU	Phenyl alanine
GAG	Glutamic acid
GUG	Valine
AUG	Methionine
AGU	Serine
UAC	Tyrosine
UAA	STOP (do not code for any amino acid)
UGA	
UAG	

MUTATION

(Refer principles of inheritance notes-Mutation and Types)

tRNA (Transfer RNA)/Adapter molecule

- Proposed by Francis crick
- Presence of tRNA was known before the genetic code was postulated
- tRNA has
  - **Anticodon loop :** That has bases (Anticodon) complementary to the triplet codon on the mRNA
  - **Aminoacid acceptor end** to which it bind to specific amino acids .

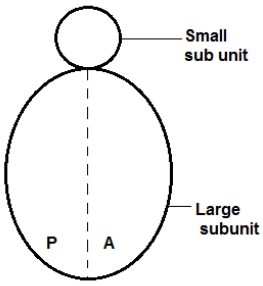


Structure of tRNA molecule

- tRNA has specific for each amino acids
- there are **no tRNA for STOP codon**
- For initiation of translation process, there involved initiator tRNA
- The secondary structure of tRNA looks **like Clover Leaf structure**
- 3D structure of tRNA looks like **Inverted 'L'**

Ribosome

- Ribosome is the cellular factory for protein synthesis.
- The ribosome consists of structural RNA and 80 different proteins.



- It exists as 2 sub units, a large sub unit and small unit.
- The large sub units has 2 sites (P and A site).
- The ribosome also acts as catalyst (23srRNA) in Bacteria is the enzyme **Ribozyme.** It helps in for the formation of peptide bond.

Q-Why tRNA is called adapter molecule?
Ans : A tRNA can read triplet codon on the mRNA with one hand and can carry a specific amino acids with other hand hence tRNA is called Adapter molecule.
Q-mRNA : Triplet codon
tRNA : Anticodon

DNA Translation

- The process of polymerisation of amino acids to form Polypetide chain (Protein) is called Translation.
- **The order and sequence of amino acids in a protein is defined by the sequence of triplet codon in the mRNA**
- In a Protein, amino acids are joined by **Peptide bond**
- The process of translation consists of 4 steps

1-Charging Of Trna/ Aminoacylation of tRNA

- ✓ Formation of peptide bond required energy. In the first steps amino acids are activated in the presence of ATP

Ie: Aminoacid+ATP= Activated amino acids

- ✓ Such charged Amino acids are linked to specific tRNA. This process is called charging of tRNA / Aminoacylation of tRNA

2-Inititation

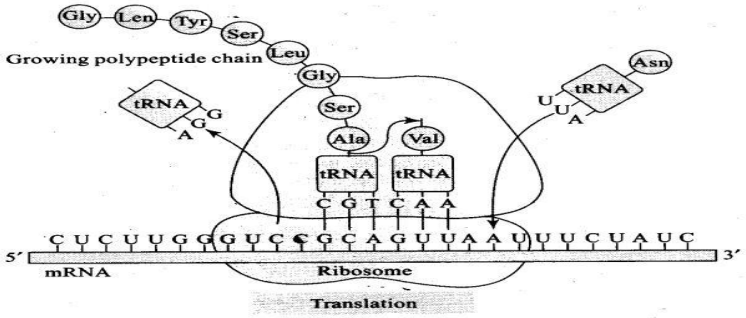
- Small sub unit of ribosome bind to the mRNA
- The tRNA with methionine (Inititator tRNA) enter to the P site , then another Amino acids with t RNA Enter to the A site
- If 2 such charged tRNA are brought close enough, the **formation of peptide bond** between them would be favoured **energetically.**

3-Elongation

- During this linkage 1st amino acid and 2nd amino acid, 1st amino acid's tRNA linkage is broken. This tRNA is removed from the P site. And the 2nd tRNA at the A site is pulled to the P site along with mRNA . this process is known as translocation
- It result the 3rd codon coming into the A site and appropriate tRNA with amino acid bind to the A site. This process is repeated result in the elongation of Protein chain

4-Termination

- When the 'A site' reaches on to the stop codon termination of translation occur because there is ni tRNA for stop codon (UAA,UGA,UAG)
- Release factor binds to Stop codon also helps in termination
- **(See the picture in your Note book)**



UTR (Untranslated region)

- 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.**

REGULATION OF GENE EXPRESSION

A. Regulation of gene expression in Eukaryotes

- In eukaryotes, the regulation could be exerted at
  - i) Transcriptional level (formation of primary transcript),
  - ii) Processing level (regulation of splicing),
  - iii) Transport of mRNA from nucleus to the cytoplasm,
  - iv) Translational level.

B. Regulation of gene expression in Prokaryotes

- The metabolic, physiological and environmental conditions regulate gene expression of genes in Prokaryotes
- Eg: In E coli the enzyme, beta galactosidase hydrolyses lactose into glucose and galactose. In the absence of lactose, the synthesis of beta galactosidase stops.
- The development and differentiation of embryo into adult organisms are also a result of the coordinated regulation of expression of several sets of genes.

OPERON

- Operons are cluster of genes responsible for controlling metabolic reaction within a living system.

OR

- All the genes regulating a metabolic reaction constitute an Operon.

  Eg: Lac operon
  Trp Operon
  Ara Operon
  His Operon
  Val Operon

LAC OPERON

- Proposed by a geneticist, Francois Jacob and a biochemist, Jacque Monod
- The operon controlling lactose metabolism is called Lac Operon. It consists of

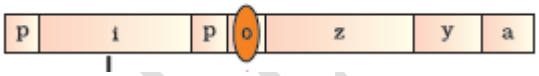
  1-A regulator gene (i gene/ inhibitor gene) : It code for repressor protein

  2-three structural gene

  a)Lac Z gene : It code for Beta galactosidase (It hydrolyze lactose to glucose and galactose)

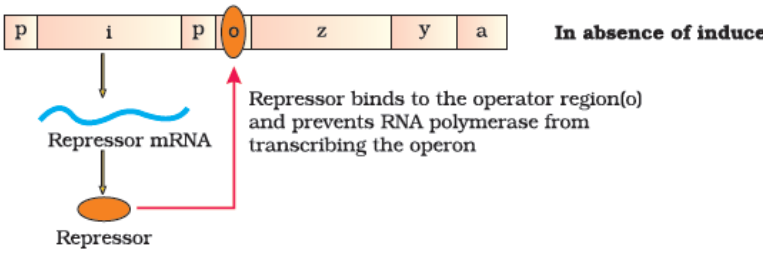
  b)Lac y gene : It code for Permease (Increase permeability of cell to Lactose)

  c)Lac a gene : it code for Transacetylase



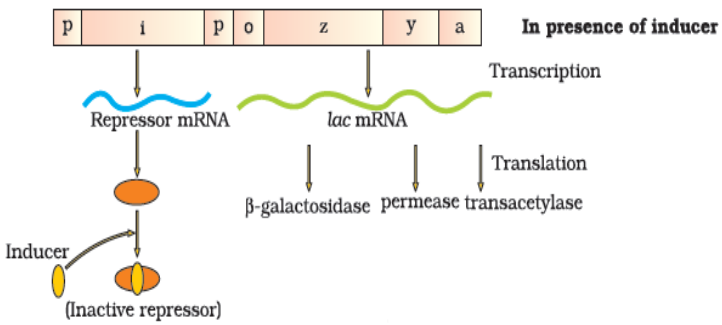
In the Absence of Lactose (Inducer)

- If there is no inducer (Lactose), lac operon remains switched off. The regulator gene synthesizes mRNA to produce the repressor protein. This protein binds to the operator gene and blocks RNA polymerase movement. So structural genes are not expressed.



In the presence of Lactose (Inducer)

- If lactose is provided in the growth medium, the lactose is transported into E.coli cells by the action of permease.
- Lactose (Inducer) binds with repressor protein. So Repressor protein cannot bind to operator gene. The operator gene become free and induces the RNA polymerase to bind with promoter gene. Then RNA polymerase transcribe the structural RNA result in lac mRNA formation. The lac Mrna translated to produce beta galactosidase, trans acetylase and permease.



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

Human Genome Project

- It is the Finding out the complete DNA sequence of human genome.
- It is launched in **1990**
- Completed in **2003**
- It was a **13 year project**
- **But** the sequence of **chromosome 1** was completed only in **May 2006** (this was the last of the human chromosomes – 22 Autosomes and X and Y – to be sequenced).
- The project was coordinated by
    - **the U.S. Department of Energy and**
    - **the National Institute of Health**
- During the early years of the HGP, the **Wellcome Trust (U.K.)** became a major partner;
- additional contributions came from **Japan, France, Germany, China and others.**

Why HGP is called a Mega project...?

- Human genome is said to have approximately **3 x 10^9 bp,** and if the cost of sequencing required is **US $ 3 per bp** (the estimated cost in the beginning), the total estimated cost of the project would be approximately **9 billion US dollars.**
- Further, if these sequences were to be stored in typed form in books, and if each page of the book contained **1000 letters** and each book contained **1000 pages**, then **3300 such books** would be required to store the information of DNA sequence from **a single human cell.**

Goals of HGP

(i) Identify all the approximately **20,000-25,000 genes in human DNA**;

(ii) Determine the sequences of the 3 **billion chemical base pairs** that make up human DNA;

(iiii) Store this information in databases;

(iv) Improve tools for data analysis;

(v) Transfer related technologies to other sectors, such as industries;

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

Methodologies in Human genome project

The methods involved two major approaches.

a)Expressed sequence tags(ESTs).

Here we focused on identifying all the genes that are **expressed as RNA**

b)Sequence Annotation

this is a blind approach of simply sequencing the whole set of genome that contained **all the coding and non-coding sequence**

Steps in HGP

For sequencing,

1. the total DNA from a cell is **isolated**
2. convert DNA **into random fragments** of relatively smaller sizes (because DNA is a very long polymer, and there are technical limitations in sequencing very long pieces of DNA)
3. **clone** each piece of DNA in suitable host using specialised **vectors**. The cloning resulted into amplification of each piece of DNA fragment so that it subsequently could be sequenced with ease. The commonly used hosts were bacteria and yeast, and the vectors were called as **BAC (bacterial artificial chromosomes), and YAC (yeast artificial chromosomes).**
4. The fragments were **sequenced** using **automated DNA sequencers** that worked on the principle of a method developed by Frederick **Sanger**. (Sanger is also credited for developing method for determination of amino acid sequences in proteins).
5. These sequences were then arranged based on some overlapping regions present in them. This required generation of overlapping fragments for sequencing.
6. Alignment of these sequences was humanly not possible. Therefore, specialized **computer based programs were developed (bioinformatics—It is the application of computer science and information technology to the field of biology and medicine)**.
7. These sequences were subsequently annotated and were assigned to each chromosome.

Salient Features of Human Genome

I. The human genome contains **3164.7 million nucleotide bases.**

II. The average gene consists of 3000 bases, but sizes vary greatly, with the largest known

human gene being **dystrophin at 2.4 million bases.**

III. The total number of genes is estimated at 30,000–much lower than previous estimates of 80,000 to 1,40,000 genes. Almost all (99.9 per cent) nucleotide bases are exactly the same in all people.

IV. The functions are unknown for over 50 per cent of the discovered genes.

V. Less than 2 per cent of the genome codes for proteins.

VI. Repeated sequences make up very large portion of the human genome.

VII. Repetitive sequences are stretches of DNA sequences that are repeated many times, sometimes hundred to thousand times. They are thought to have no direct coding functions, but they shed light on chromosome structure, dynamics and evolution.

VIII. **Chromosome 1 has most genes (2968), and the Y has the fewest (231).**

IX. Scientists have identified about 1.4 million locations where singlebase DNA differences **(SNPs – single nucleotide polymorphism, pronounced as 'snips')** occur in humans. This information promises to revolutionise the processes of finding chromosomal locations for disease-associated sequences and tracing human history.

Application

1-One of the greatest impacts of having the HG sequence may well be enabling a radically **new approach to biological research.**

2- **it can study all the genes in a genome**, for example, all the transcripts in a particular tissue or organ or tumor, or how tens of thousands of genes and proteins work together in interconnected networks to orchestrate the chemistry of life.

Genome sequencing in other organisms

Genome Sequencing also done in bacteria, yeast, *Caenorhabditis elegans* (a free living non-pathogenic nematode), *Drosophila* (the fruit fly), plants (rice and *Arabidopsis*),

Repetitive DNA

These are sequences, **a small stretch of DNA is repeated many times**. These repetitive

DNA can be separated from bulk genomic DNA as different peaks during **density gradient centrifugation**. The bulk DNA forms a major peak an the other small peaks are referred to as satellite DNA.

Depending on

- **base composition (A : T rich or G:C rich),**
- **length of segment, and**
- **number of repetitive units (Copy number)**

the satellite DNA is classified into many categories, such as

a)micro-satellites,
b)mini-satellites

- The **VNTR** belongs to a class of satellite DNA referred to as **mini-satellite**. A small DNA sequence is arranged tandemly in many copy numbers. The copy number varies from chromosome to chromosome in an individual(this number varies from person to person).
- The size of VNTR varies in size from **0.1 to 20 kb**

Functions of repetitive DNA

- It normally **do not code for any proteins,**
- These sequence **show high degree of polymorphism** (Variation at genetic levels).
- DNA Polymorphism means any difference in the nucleotide sequence observed in a population.
- It is inheritale and arises due to mutation) and form the **basis of DNA fingerprinting.**
- Since DNA from every tissue (such as blood, hair-follicle, skin, bone, saliva, sperm etc.), from an individual show the same degree of polymorphism, they become very useful identification tool **in forensic applications**

DNA Fingerprinting

- ✓ DNA fingerprinting was initially developed by **Alec Jeffreys.**
- ✓ DNA finger printing is a very quick way to compare the DNA sequences of any two individual.
- ✓ The DNA from **a single cell** is enough to perform DNA fingerprinting.
- ✓ DNA fingerprinting involves identifying differences in some specific regions in DNA called repetitive DNA, because in these sequences, a small stretch of DNA is repeated many times
- ✓ Alec Jeffrey used satellite DNA as the basis of DNA fingerprinting that shows very high degree of polymorphism**. It was called as Variable NumberTandem Repeats.(VNTR**)

• Different steps of DNA fingerprinting are:-

- ➢ Isolation of DNA.
- ➢ Digestion of DNA by restriction endonucleases.

- Separation of DNA fragments by gel electrophoresis.
- Transferring (blotting) of separated DNA fragment to synthetic membranes, such as nitrocellulose or nylon.
- Double stranded DNA made single stranded. Hybridization using labeled VNTR probe.
- Detection of hybridized DNA fragments by After hybridization with VNTR probe the autoradiogram gives many bands of different sizes
- These bands give a characteristic pattern for an individual DNA. It differs from individual to individual.

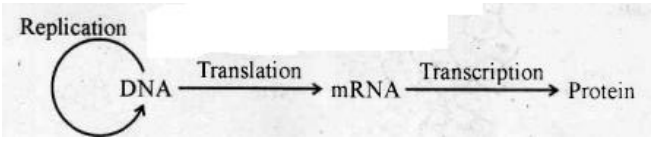
Applications:

• Test of paternity.
• Identify the criminals.
• Population diversity determination.
• Determination of genetic diversity.

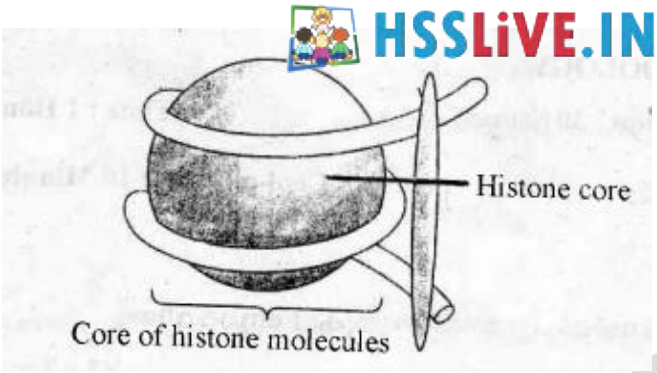
Previous Year Question Papers

HSE-March-2019

1. Diagrammatic representation of the central dogma given below is not correct. Make necessary corrections and redraw it (1)



2. Observe the figure given below : (2)

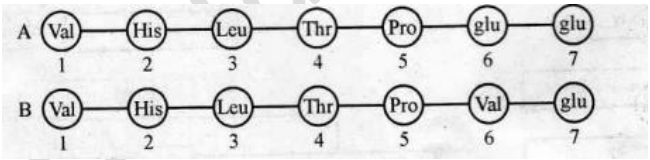


a) Identify the figure.

b) How many histone molecules are present in the Histone core ?

c)Distinguish Euchromatin and Heterochromatin.

3. The amino acid composition of the relevant portion of β chain of 2 Haemoglobin molecules (A & B) are shown below : (3)



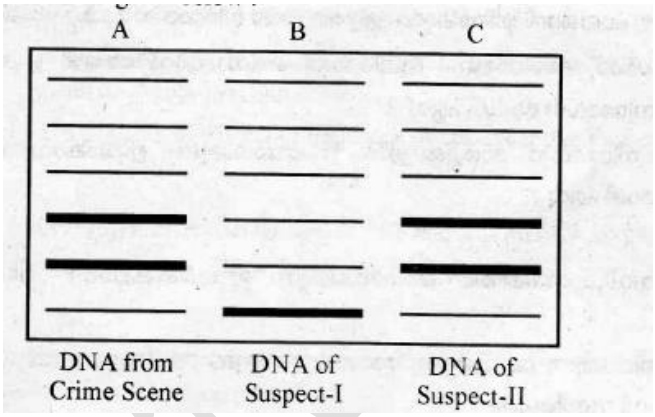
(a) Which one of the polypeptide chain is abnormal ?

(b) Name the disorder caused by it.

(c) What is the reason for this abnormality?

(d) What is the effect of this abnormality in such individuals ?

4. The diagrammatic representation of the DNA fingerprint from a crime scene and that of suspected persons are given below : (3)

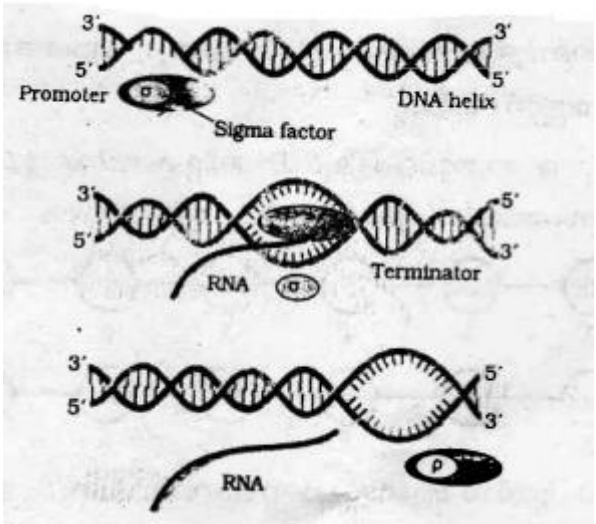


a)What is your conclusion about the suspects based on DNA Fingerprint given ?

(b) What is VNTR ?

(c) Who developed this technique first ?

5. The diagrammatic representation of a process in bacteria is given below : (3)



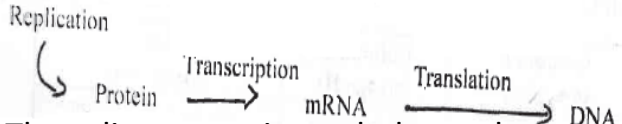
a)Identify the process.

b)Name the enzyme involved in this process.

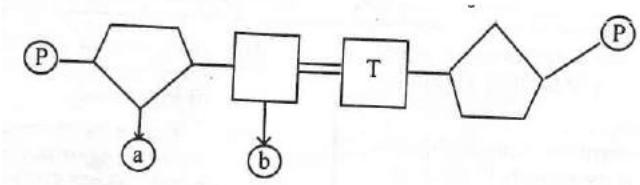
c)Explain the three major steps in this process.

HSE-Model-2019

6. Analyze the figure, find out the error and correct it. (2)

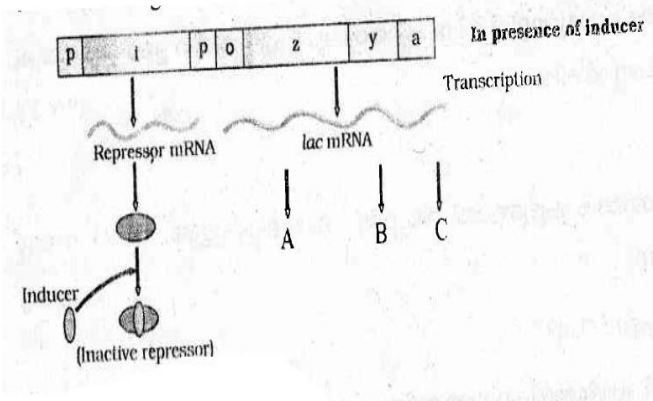


7. The diagram given below show a segment of DNA, Label the part (a) and (b) (3)



HSE JUNE 2018

8. "Human genome project is a mega project" give two reason to explain this? (2)
9. Observe the diagram and answer the following (2)

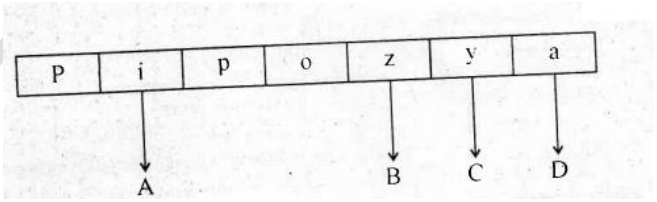


a)Identify the diagram ?
b)Name the enzymes A,B, and C

10. "Genetic code is universal in nature"
a)Substantiate this statement ?
b)mention any two other salient features of genetic code (2)
11. Expand the following (3)
a)SNP b)BAC c)YAC

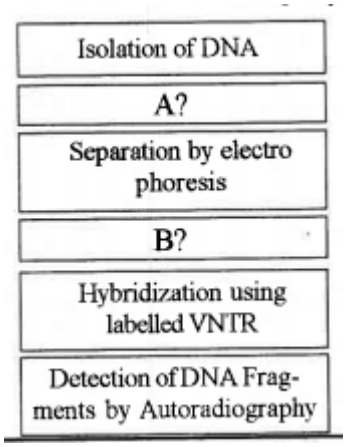
HSE MARCH 2018

12. Expresses sequence in the gene is called (1)
a)Introns b)Muton
c)Exons d)Cistron
13. DNA is tightly packed structure and is found as units called nucleosomes
(a) Explain the concept of nucleosomes
(b)Differentiate between euchromatin and hetero chromatin (2)
14. Identify the disadvantages of RNA over DNA as a genetic material and explain it ? (2)
15. a) In Lac-operon lactose act as inducer molecule. Evaluate the statement and explain it (3)
b) Observe the diagram of Lac –Operon and Identify Labelled part A,B,C and



HSE-Model-2018

16. Complete the flow chart of Southern blot hybridization (2)



c)Mention two uses of DNA fingerprinting.

17. Read the following statements and answer the following questions

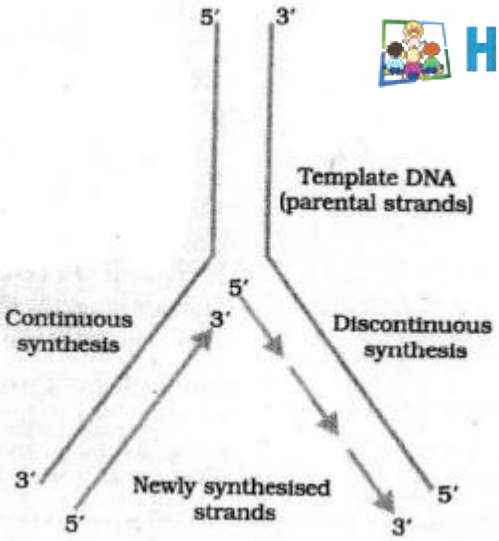
1-A genetic material should be able to generate its replica

2-A genetic material should not provide scope for mutation

3- A genetic material should be able to express itself in the form of mendelian characters.

a. Choose the correct statements from the above. b. Rewrite the wrong statement to correct one (2)

18. Observe the given diagram and answer the following questions. (2)



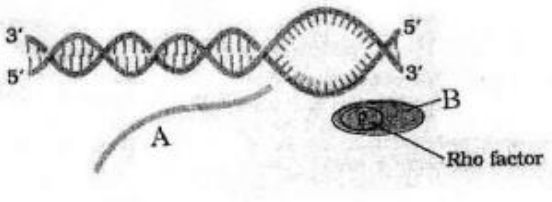
a)Identify the above process.

b) Name the enzyme required to polymerise the DNAstrand.

c) Name the enzyme required to join the discontinuous strands

d) In eukaryotes replication of DNA occurs at ……phase of cell cycle.

19.



a) Name 'A' and ' B' from the above diagram.

b. Describe the following terms

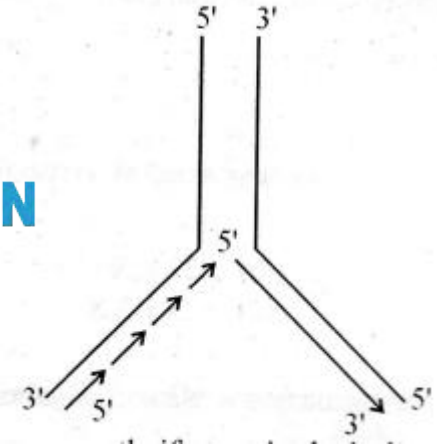
i) Capping ii)Tailing

HSE-JUNE-2017

20. Find the odd one and write the common feature of the other (1)

Cytidine,adenine,Thymine,guanine

21. Observe the diagram (2)

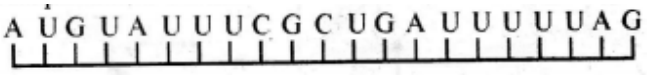


a)Redraw the diagram correctly if any mistake is there ?

b)what does the diagram indicate?

b)What is the function of DNAL ligase in this process ?

22. Read the codon sequence in the mRNA unit which is undergone translation (3)



a)What will happend if the nitrogen base 'U' in the 6th position is replaced by 'A' by point mutation

b)Name and define this type of mutation

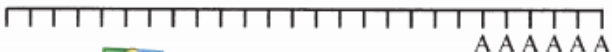
c)draw the base sequence in the coding DNA strand from which the above mRNA is transcribed ?

HSE-March 2017

23. Which of the following combinations do not apply to DNA ? (1)

(a)	Deoxyribose, Guanine	(1)	(a) and (b)
(b)	Ribose, Adenine	(2)	(b) and (c)
(c)	Deoxyribose, Uracil	(3)	(c) and (d)
(d)	Guanine, Thymine	(4)	(a) and (d)

24. Examine the diagram of Mrna given below . Mark the 5' end 3' end of Mrna by giving reason (2)



25. A small fragment of a skin of different person was extracted from nails of a murdered person. This fragment of skin led the crime investigators to the murder. Ased on this incident answer the following questions (3)

(1) What technique was used by the investigators

(2) What is the procedure involved in this technique

Or

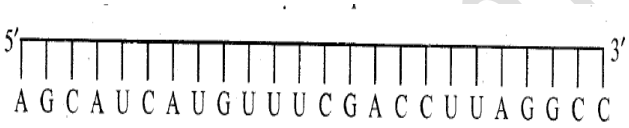
26. In an E.coli cultre lactose is used as food instead of glucose. If So, answer the following questions (3)

(1) How do the bacteria respond to the above situation at genetic level?

(2) If lactose is removed from the medium what will happen?

HSE-June 2016

27. Observe the figure of mRNA and answer the following question (3)



a)Find the start codon and stop codon?

b)How many amino acids will be present in the protein translated from this Mrna ?

c)The additional sequences that are not translated in the mRNA is called.....

28. a) The hints of lac Operon is given below (HSE-June-2016) (3)

Hints :

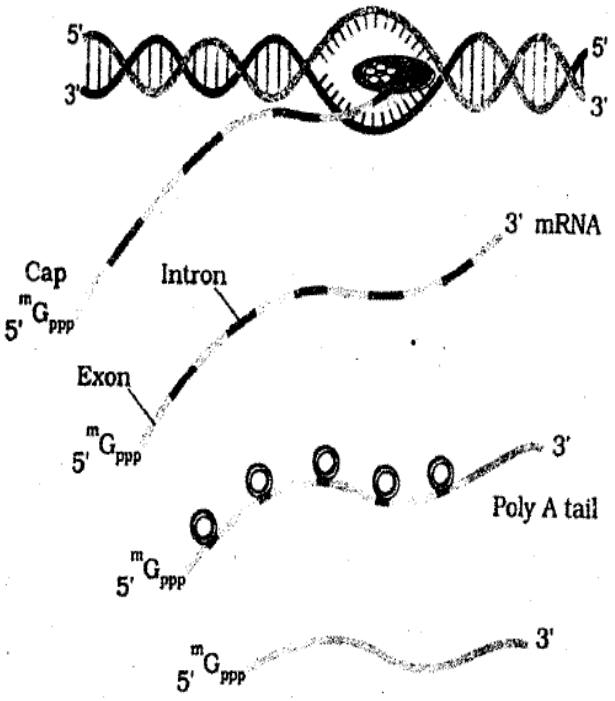
Inducer, Repressor,
Structural genes, operator
Regulatory gene

a)which substance is acting as inducer in this operon ?

b)explain the working of operon in the presence of inducer ?

OR

29. b)With the help of the figure given, explain the processing of hnRNA to mRNA in eukaryotes (3)

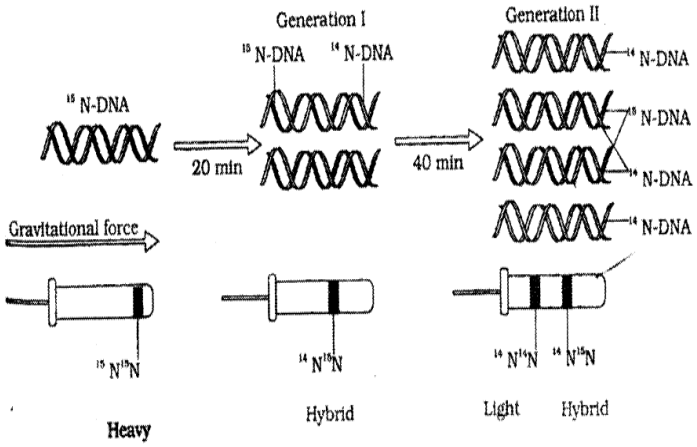


HSE-March 2016

30. Results of a famous experiment is given in the figure .Answer all (2)

a)Identify the experiment ?

b)which property of DNA is proved by experiment ?



(Separation of DNA by Centrifugation)

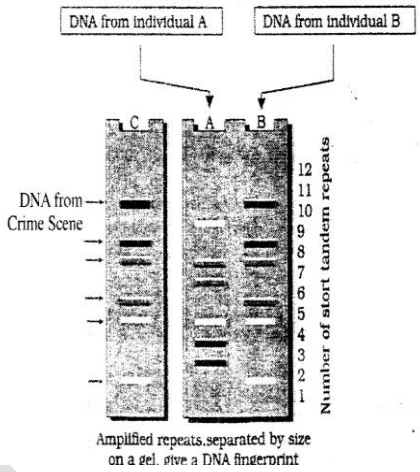
31. Read carefully the sequence of codon in the mRNA unit and answer the question (2)

a)what changes is needed in the first codon to start the translation process ?

b)if translation starts by that change, till which codon it can be continues ?

32. Schematic representation of DNA finger prints as shown below

[Hints : C is a sample taken from a crime scene, A and B from two suspected individuals]

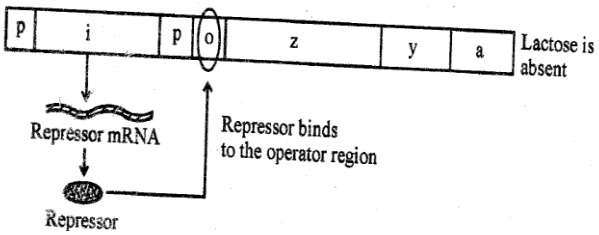


a)which one of the suspected individual may involve in the crime ?

b)write any other use of DNA figure print ? (2)

HSE-June -2015

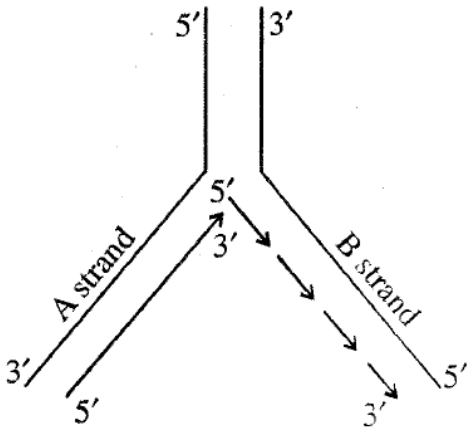
33. Observe the following diagram and answer the question? (3)



a) Diagrammatically represent changes takes place when lactose is added to medium?

b) What is the role of z,y, and a gene in this metabolic pathway ?

34. Observe the diagram and answer the question? (3)



a)what is the difference in the replication process ins strand A and strand B ?

b)what is the role of DNA ligase in the replication process in B strand ?

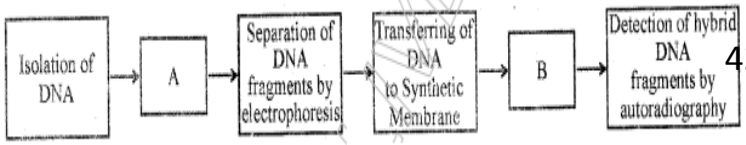
c)what is meant by replication fork ?

HSE-March-2015

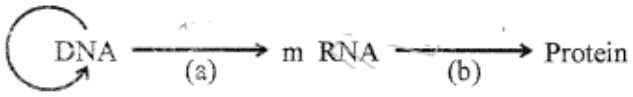
35. Explain Transcription. A transcription unit in a DNA is defined by 3 regions. Write the name of any 2 regions? (2)

36. a) The steps in DNA Finger printing are given below. Complete the flow chart (A and B)

b)Mention the application of DNA finger printing (3)

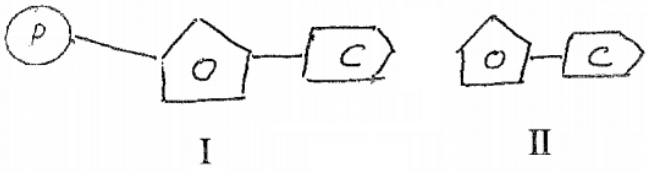


37. The flow of genetic information is shown below. Name the process of A and B (1)



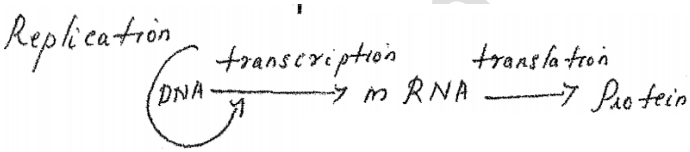
HSE-June-2014

38. Diagrams of components of DNA are given below: (1)



Identify and differentiate the two diagrams I and II

39. a)Identify the diagram and explain



b)In some cases DNA is produced from RNA. Name this process and give example ? (2)

40. a)Paternity and maternity can be determined by certain scientific methods. What is it? Define?

b)Briefly write the methodology involved in the technique ?

c)Comment on its other application ? (3)

41. a)Define mutation ?

b)What are the different types of mutation ? (2)

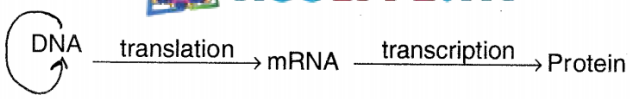
HSE-March-2014

42. "Prediction of the sequence of aminoacids from the nucleotide sequence in mRNA is very easy, but the exact prediction of nucleotide sequence in mRNA from the sequence of amino acids coded by mRNA is difficult"

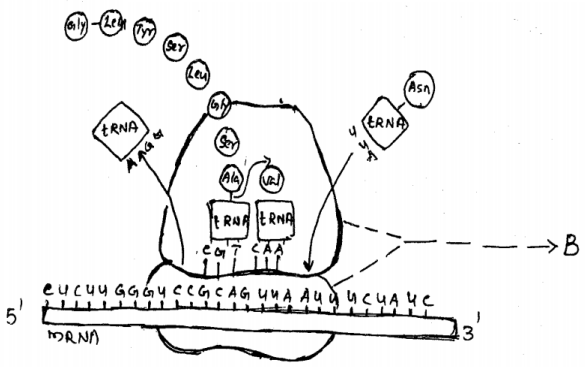
a)Which property of genetic code is the reason for the above condition ? Explain

b)Which are the stop codons in DNA transcription ? (3)

43. Diagrammatic representation of 'Central Dogma' is given below :
Observe the diagram carefully and redraw it making appropriate corrections (1)



44. Observe the diagram and answer the question (2)
a)Identify the process shown in the figure and define it ?
b)Identify the structure 'B', write any one function of it in the process shown in the diagram ?



HSE-Sept-2013

45. Presence of lactose enhances the production of beta galactosidase and other enzymes in bacteria . How will you explain this phenomenon ? (1)

46. A DNA sequence for coding a peptide is given below

"CAAGTAAATTGAGGACTC"
(Hint : Codons and Aminoacids)

UUA – Leu	ACU – Thr
CCU – Pro	GUU – Val
CAU – His	GAG – Glu

a)Write the complementary mRNA coding sequence for it ?

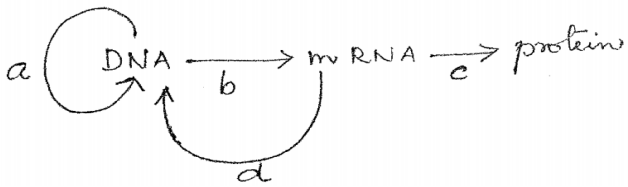
b)Find out the amino acids sequence of peptide chain using the codon given in the hints

c)if a mutation causes a change in the sixth codon CTC to CAC. It leads to a mendelian disorder. Identify the disease and write the specific characteristic of the disease ? (4)

47. Draw the flow chart showing the steps of southern blot hybridisation using radiolabelled VNTR ? (3)

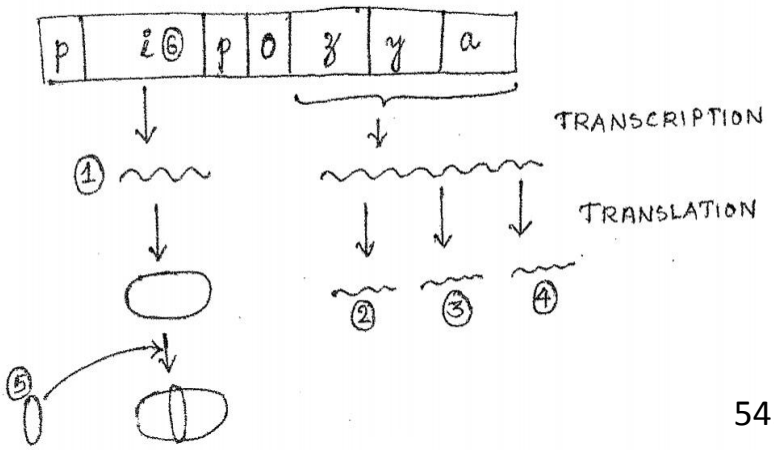
HSE-March 2013

48. The flow of genetic information is shown below (2)



a)Name the process a,b,c and d

49. Given below is the figure showing the functioning of lac operon in presence of lactose. Redraw the figure and label the parts numbered 1 to 6 (3)



50. RNA is not an ideal molecule as genetic material because (1)

a) 2′OH group of ribose is reactive and make it labile
b) It is catalytic and hence reactive
c) Both (a) and (b)
d) None of the above

HSE-June-2012

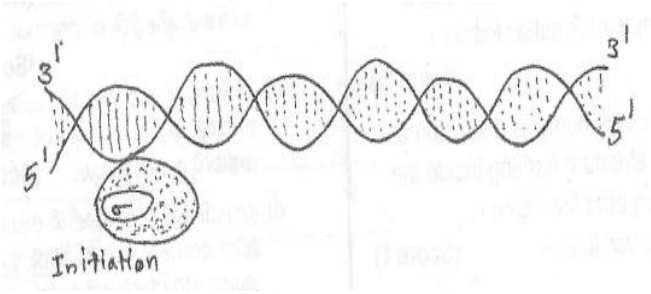
51. Following are the first two steps in Griffiths transformation experiment

1) S strain → Inject into mice → mice live
2) R strain → Inject into mice → mice die

a)If there is any mistake correct it
b)write the remaining steps ? (1.5)

52. DNA is the better genetic material than RNA, Do you agree with this statement? Substantiate (1.5)

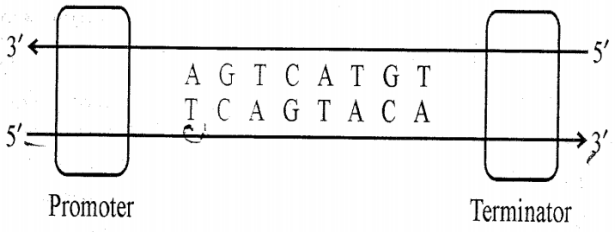
53. Given below is the diagrammatic representation of first stage of a process in a bacteria



a)Identify the process
b)Name the enzyme catalyses this process
c)What are the additional complexities in eukaryotes in this process ? (3)

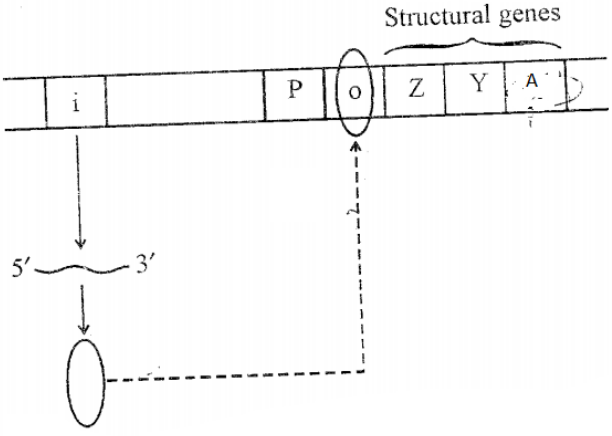
HSE-March-2012

54. A transcription unit is given below. Observe it and answer the question (3)



a)How can you identify the coding strand ?
b)Write the sequence of RNA formed from this unit ?
c)what would happened if both strand of DNA act as template for transcription ?

55. In E.coli Lactose catabolism is controlled by Lac Operon. Lac operon in the absence of inducer (Lactose) is given below. (3)



a)What is ‘P’?

b)Name the enzyme produced by the structural gene ‘Z’,’Y’, and ‘A’ ?

c)Re draw the diagram in the presence of an Inducer