Tuesday

Molecular Basis of Inheritance

The DNA

→ Full form Deoxyribo Nucleic Acid

→ DNA is an acidic substance present in nucleus was first identify by Friedrich Meischer in 1869. He named it as nuclein

→ It is the polynucleotide of Deoxyribonucleoside

→ Component of DNA

① Nitrogen bases

- Adenine
- Guanine
- Cytosine
- Thymine

② Deoxyribose Sugar / Pentose Sugar

③ Phosphorus

→ Human haploid Content of DNA = 3.6×10^9 bp

→ E. Coli (bacteria) = 4.6×10^6 bp.

→ Bacteriophage = 5386 bp.

→ Size of Human DNA = 2.2 meter.

In 1953

→ James watson and Francis crick, based on the X-ray difraction data produced by Maurus Wielkins and Rosalian Franklin produced a fomous double Helix model for the structure of DNA.

Genetic material

is that substance which controls the inheritance of traits from one generation to next.

Properties of Genetic Material (DNA / RNA)

i. It should be able to replicate itself. itself.

ii. It should be stable both chemically and structurally.

iii. It should provide the scope for slow changes, which is required for evolution.

iv. It should be able to express itself in the form of Mendelian characters.

The Search of Genetic Material

① Transforming Principle [Griffith Frederick Experiment]

→ In 1928 this principle was given by Frederick Griffith. He taken two types bacterial strain namely R-strain & S-strain

✱ Bacterial Strain [Streptococcus pneumoniae]

R-Strain	S-Strain
[Non-Virulent]	[Virulent Strain]
[NON-Poisnous]	[Poisnous] because wall Contain Sulphofolymucosaccha

Step - I

Mice ← inject R-Strain → MICE LIVE

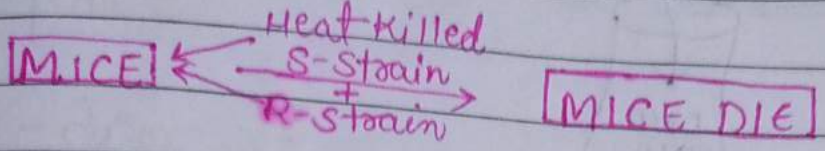
Step - II

MICE ← inject S-Strain → MICE DIE

Step - III

MICE ← Heat Killed (inject) at 60°C S-Strain → MICE LIVE

Step - IV



Why Called Transforming Principle

→ Something is there which transformed R-Strain bacteria into virulent strain bacteria, He named it 'transforming Principle' which transformed R-Strain to virulent.

→ He didn't identify the nature of material.

② Biochemical characterisation of transforming Principle

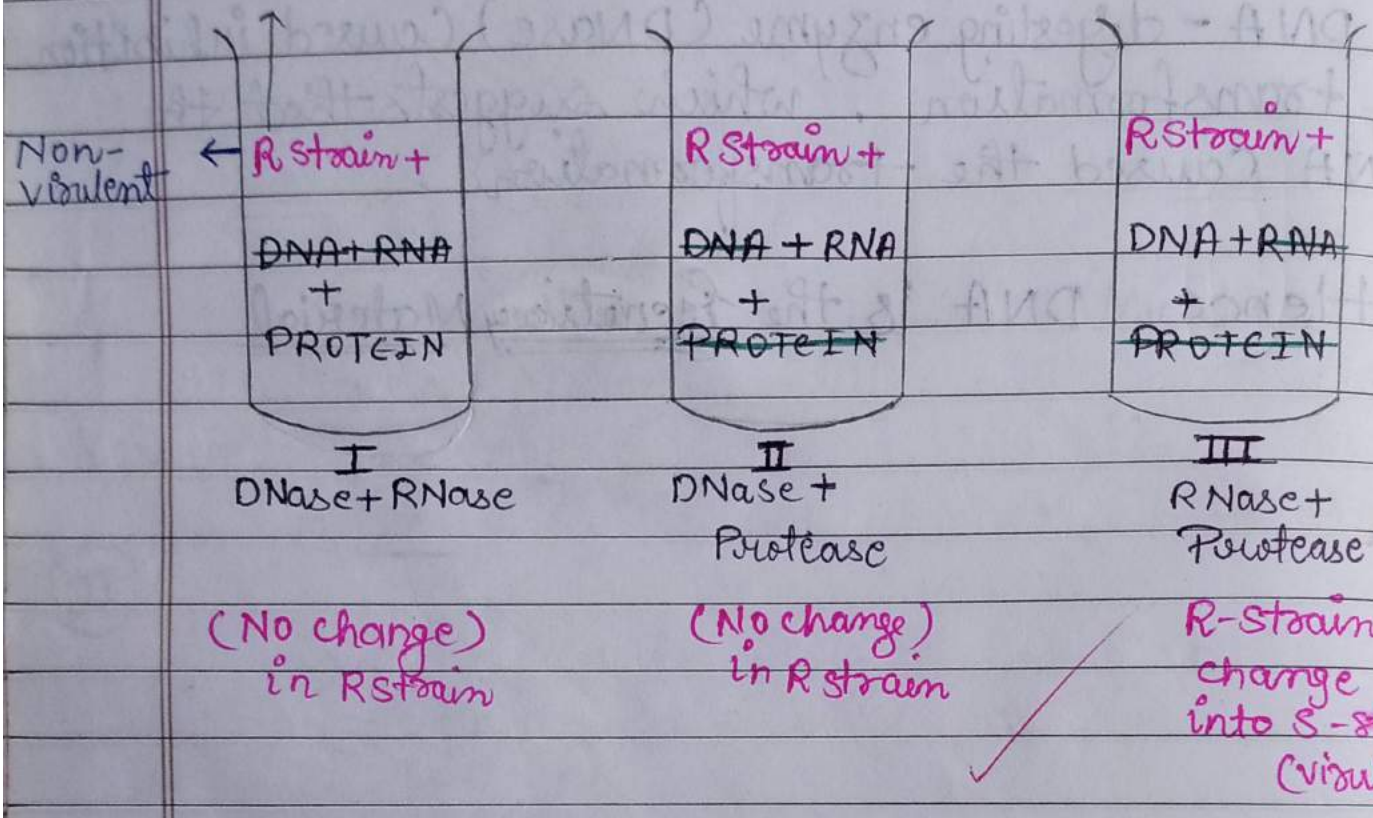
→ It was proved by Oswald Avery, Colin Macleod and Maclyn McCarty. (1933-44)

→ Scientist Suppose Genetic material

- Protein
- RNA
- DNA

→ Proteases → Protein digest
RNase → RNA "
DNase → DNA "

They Purified biochemical from heated-S strain
i.e. Protein, RNA, DNA
Heat Killed S-strain.



Conclusion —
So they Concluded that ~~a Gen~~ DNA is a hereditary material because in the presence of it transformation takes place not in the presence of RNA, Protein

The Genetic Material is DNA

Hershey and chase experiment

It is also known as Blending experiment

Experiment with T2 bacteriophages

Viruses that infect bacteria and replicate inside it

- The function of DNA & protein could be found out by labelling them with radioactive tracers DNA contain Phosphorus (^{32}P) but not Sulphur.
- Similarly protein of phage contains Sulphur (^{35}S) but not phosphorus.

Now Some bacteriophages were grown in radioactive phosphorus medium. So that there DNA will be radioactive but protein will not be radioactive because protein does not contain phosphorus.

Similarly Some were grown in the radioactive Sulphur medium. So that the protein coat will be radioactive but DNA will not be the radioactive one.

Bacteriophage with radioactive DNA attached to bacteria (E-coli)

① Landing
② Pinning
③ penetrate

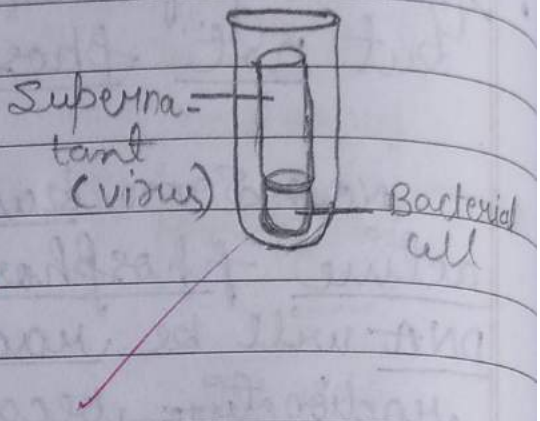
Three Steps were followed.

- Infection – Both types of labelled phages were allowed to infect normally cultured bacteria in separate experiments.
- Blending – These bacterial cells were agitated in a blender to break the contact b/w virus & bacteria.

- Centrifugation – The virus particles were separated from the bacteria by spinning them in a centrifuge.

Result

Bacterial cells showed the presence of radioactivity, no radioactivity detected in supernatant



Bacteriophages with radioactive protein attach to bacteria

- Similarly, bacteria was infected.
- Agitated in a blender to separate phage particles from the E. Coli bacteria cells.
- Centrifugation separated the phages particles as a supernatant.

Result

Bacteria that were infected with viruses that had Radioactive proteins were not radioactive. This indicates that Protein did not enter the bacteria from the virus.

Therefore, DNA is the Genetic material that is passed from Virus to bacteria

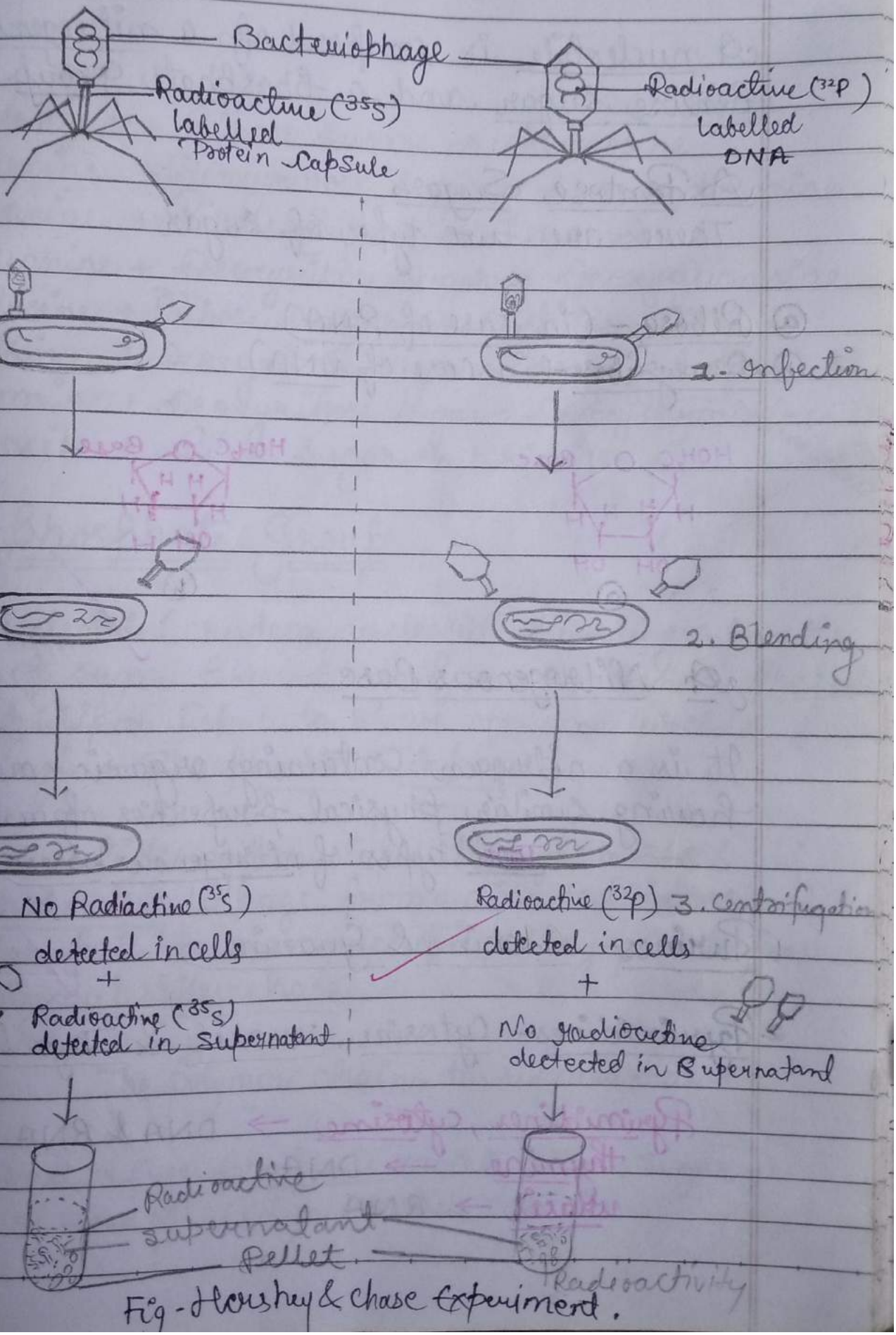


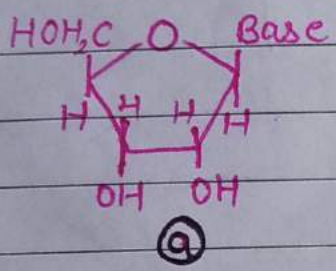
Fig - Hershey & Chase Experiment.

Structure of a Polynucleotide chain
DNA | RNA

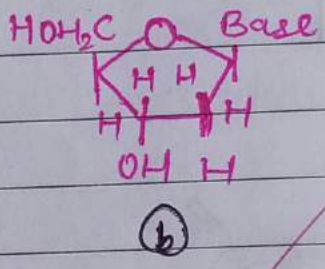
A nucleotide is composed of a nitrogenous base, pentose sugar and a phosphate group.

A Pentose Sugar
There are two types of sugar

(a) Ribose - (in case of RNA)
(b) Deoxyribose - (in case of DNA)



(a)



(b)

A Nitrogenous Base

It is a nitrogen containing organic molecule having similar physical properties of a base.

Two types of nitrogenous bases.

1. Purines - Adenine & Guanine
2. Pyrimidines - Cytosine, Uracil & Thymine

Pyrimidines, cytosine → DNA & RNA
thymine → DNA
uracil → RNA

Nucleoside is formed, when a nitrogenous base is linked to a Pentose Sugar through N-Glycosidic linkage.

- Adenine + Ribose Sugar → Adenosine
- Adenine + Deoxyribose Sugar → Deoxyadenosine
- Guanine + Ribose Sugar → Guanosine
- Guanine + Deoxyribose Sugar → Deoxyguanosine
- Cytosine + Ribose Sugar → Cystidine
- Cytosine + Deoxyribose Sugar → Deoxycystidine
- Thymine + Deoxyribose Sugar → Deoxythymidine
- Uracil + Ribose Sugar → Uridine.

A Phosphate Group

* A nucleotide (or deoxynucleotide depending upon the type of Sugar present) is formed, when a Phosphate Group is linked to 5' OH of a nucleoside through a Phosphoester linkage.

* Two nucleotides when linked through a 3'-5' phosphodiester linkage, from a dinucleotide. In a similar fashion, more nucleotides may join to form a Polynucleotides chain.

The polymer chain, thus formed has -

i) one end + free phosphate moiety ribose sugar = 5' end of Polynucleotide chain.

ii) one end The other end + free hydroxyl group = 3' end of Polynucleotide chain.

Sugar and Phosphates form → Backbone

Discoveries Related to Structure and Composition of DNA

i) Friedrich Meischer (1869) Said that DNA is an acidic present in Nucleus. → termed it Nuclein later renamed as nucleic acid due to its acidic properties by Altman (1899).

Substance

ii) Levene (1910) found DNA contain Phosphoric acid & deoxyribose Sugar
He characterised 4 types of nucleotides present in DNA

iii) Erwin chargaff (1950) formulated the bases & other contents of DNA called Chargaff rule. It states that for a double-Stranded DNA ratio b/w adenine (A) & Thymine (T) & Guanine (G) & Cytosine (C) are Constant & equal to one.

$$i.e. \frac{A+T}{G+C} = 1$$

(v) James watson & Francis crick (1953)
Proposed the double helix model for DNA structure on basis of X-ray diffraction data produced by Maurice Wilkins & Rosalind Franklin in same year.
important feature → Complementry base pairing. (If sequence of base in 1 strand is known, the sequence of other base in other strand can be easily predicted).
DNA acts as a template for synthesis of a new strand, daughter DNA produced indentical to parental DNA molecule.

Types of Linkge or Bond

- N-glycosidic linkage
  A nitrogenous base is linked to pentose sugar through N-glycosidic linkage to form a nucleoside.
- Phosphoester linkage
  Phosphate group is linked to 5' oh of nucleoside through phosphester linkage to form nucleotide.
- Phosphodiester linkage
  Two nucleotides are linked through 3'-5' phosphodiester to form a dinucleotide.

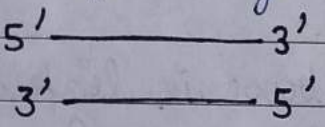
watson and crick Model of DNA

They proposed a very simple but famous double helix model for the structure of DNA.
This was based on the observation of Erwin Chargaff.

Salient features of the double helix structure of DNA are-

1. DNA Consist of two polynucleotide chains. The backbone is Constituted by sugar - phosphate and bases project inside.

2. Two chains of DNA run in antiparallel fashion with 5' → 3' polarity in other chain.



3. These bases in two strands are paired through hydrogen bonds.

4. Adenine forms 2H-bonds with thymine & vice versa

5. Similarly, Guanine is bonded with Cytosine with 3H-bonds

6. As a result always a Purine (A, G) Comes opposite to Pyrimidine (C, T, U).

7. Each turn of double helix or the pitch of helix is 3.4 nm. It has approx 10 bp in each turn.

8. Percentage Calculation of bases is done by -
$A + T = 100 - (G+C)$

9. Plane of 1 base pair stacks over the other in double helix. This provides the stability to helical structure, in addition to H-bond.

The length of DNA in E-coli is 1.36 mm, while in human it is 2.2 m

Packaging of DNA Helix

The length of DNA in humans can be calculated as
Distance b/w two consecutive base pair is
$0.34\ nm = 0.34 \times 10^{-9}\ m$
Total number of base pair in a DNA helix in a typical mammalian cell = 6.6×10^9
∴ length of this DNA double helix
$= 6.6 \times 10^9 \times 0.34 \times 10^{-9}\ m$
$= 2.2\ m$ (approx.)

* The DNA inside of a cell is organized so that it fits well within the small size of a cell.

Packaging of DNA in Prokaryotes

- In prokaryotes, DNA is not scattered throughout the cell although they do not have defined nucleus.
- DNA is organized into loops held by proteins which have +ve charge.

- Nucleoid region where DNA is present. or (Circular DNA)

DNA Packaging in Eukaryotes

- In eukaryotes, the DNA occurs inside nucleus. There are set of basic & +vely proteins is called histones

- Histones are rich in lysine and ~~ag~~ arginine which carry +ve charge.

- There are 5 types of histones proteins H_1, H_2A, H_2B, H_3, H_4 Four of them (H_2A, $H2B$ H_3 H_4) occur in pair to form Histone octamer or nucleosome or core of nucleosome. DNA connecting the two adjacent nucleosome is called Linker DNA It has H_1.

- Due to all of those phosphate groups along DNA backbone, results it has a -ve charge & is attracted +vely charged molecules Such as histone

- The -vely charged DNA is wrapped around the +vely charged histone octamer to form a Structure called Nucleosome.

- The Condensed DNA or the nucleosome Constitute repeating unit of a structure in nucleus called Chromatin

- Nucleosomes in chromatin are seen as beads-on-string under electron microscope.
- Nucleosome further coils to form solenoid / chromatin fibre
- The packaging of chromatin at higher level requires additional set of proteins that collectively are referred as Non histone chromosomal (NHC) protein

Beads-on-a string structure

then a more compact structure

* Chromatin is differentiated into two regions on the basis of staining behaviour in a typical nucleus

Heterochromatin	Euchromatin
• It is darkly stained region	• Light stained region
• Densely packed	• Loosely packed
• Transcriptionally it is inactive	• Transcriptionally active
• Present in Eukaryotes	• Present in : Eukaryotes & Prokaryotes
• Contains more DNA	• Contains less DNA

RNA WORLD

In some viruses RNA is the genetic material.
eg. Tobacco Mosaic viruses, QB bacteriophages etc.

* Why DNA is consider more stable than RNA?

i. Free 2'OH of RNA make it more labile and easily degradable. Therefore DNA in comparison is more stable.

ii. Presence of thymine at the place of uracil also confers additional stability to DNA.

- RNA was the first genetic material.
- Walter gilbert (1986) hypothesized that once there was a RNA world.
- RNA used to act as a genetic material as well as catalyst.
- There are some important biochemicals reactions in living system that are catalyzed by RNA Catalyst (ie ribozymes) & not by protein enzymes.

Nitrogenous bases in RNA are of 2 types
1. Purines - (adenine & guanine)
2. Pyrimidines - (cytosine & uracil)

Ribose Sugar + Nitrogenous base → Ribonucleoside
Ribonucleoside + Phosphate group → Ribonucleotide

CENTRAL DOGMA

Francis Crick (1958) → proposed the central dogma of molecular biology which explains the one way of flow the synthesis of RNA from DNA And the process is called transcription. while Synthesis of protein from RNA called translation.

* DNA $\xrightarrow{\text{Transcription}}$ mRNA $\xrightarrow{\text{Translation}}$ Polypeptide
* DNA $\xrightarrow{\text{Replication}}$ DNA $\xrightarrow{\text{Transcription}}$ mRNA $\xrightarrow{\text{Translation}}$ Polypeptide

Temin (1970) → Some viruses show reverse transcription the synthesis of DNA from an RNA template. A class of RNA viruses called retroviruses, are characterized by the presence of an RNA-dependent DNA polymerase (reverse transcriptase)

Replication of DNA

- It is a process of reproducing or copy of Genetic mate
- Watson & Crick had immediately proposed scheme for DNA replication while proposing the double helical structure of the DNA
- In replication 2 strands of DNA separate & both separated strands acts as a template strand.

Semiconservative DNA replication

The scheme suggested that the two strands would separated and acts as template for the synthesis of new complementary strand. After the completion of replication each DNA molecule would have one parental and one newly synthesized strand this scheme was termed as semiservation DNA replication

Experimental Proof for Replication of DNA

MESELSON AND STAL'S EXPERIMENT
(Matthew Meselson & Franklin Stahl)

The Semi-Conservative model, in which each strand of DNA serves as a template to make a new, Complementary strand, seemed most likely based on DNA's structure.

In 20 minute new generation [E. Coli

Date: Page: 155

- They began by growing E. Coli in medium, or nutrient broth, containing a "heavy" isotope of nitrogen N^{15}
- The bacteria took up the nitrogen & used it to synthesize new biological molecules, including DNA.
- The bacteria were switched to medium containing a "light" N^{14} isotope and allowed to grow for several generations.
- Then measured the density of the DNA N^{15} & N^{14} using density gradient Centrifugation.
- A dense solution of CsCl on centrifugation forms density gradient bands of solution of lower density at top that increase gradually towards bottom with highest density.

Results of the Experiment

When DNA from the first generations of E. Coli was analyzed, it produced the pattern of bands. ~~shown in the figure.~~

Thus the DNA that was extracted from culture after 1st generation i.e. just after 20 min had a hybrid or intermediate density DNA extracted from the culture after another generation i.e 2nd generation or 40 min was composed of equal amounts of this hybrid.

★ Similar experiments on 'Vicia faba' (faba beans) were conducted by Taylor and colleagues in (1958), involving use of radioactive thymidine. The results were that the DNA in chromosomes also replicate Semiconservatively.

Enzymes for DNA Replication
OR
Replication Mechanism
(DNA - Dependent DNA Polymerase)

- The process of replication in living cells requires a set of enzymes.
- The main enzyme is referred to as DNA-dependent DNA Polymerase.
- It has the ability to polymerize some 2000 bp per sec.
- Any mistakes during replication would result into mutation!
- In Prokaryotes – there are 3 types of DNA polymerases. i.e, DNA polymerase – I, II, III.
- In Eukaryotes – 5 different polymerase. i.e. DNA polymerase – $\alpha, \beta, \gamma, \delta$ & ε.

- These enzymes also help in removing mismatched nucleotides by a mechanism called Proof reading

NOTE → E. Coli has about 4.6×10^6 bp in its DNA and takes around 18 minutes to Completely replicate its Genome.

Process of DNA Replication

Replication is an energy expensive process. deoxyribonucleoside triphosphates (dNTPs) serve the dual purpose
i) acting as a substrate
ii) providing energy (from two terminal phosphates)

DNA replication Completes in following steps.

i) Origin of replication iii) Formation of Primer strand
ii) unwinding of helix iv) Elongation of new strand

i Origin of replication

- Replication begins at a particular region of DNA which is called Origin of replication
- It is called Ori-C in E Coli.
- Prokaryotes have single origin of replication, on the other hand eukaryotes have several thousands. origin of replication.

ii. unwinding of helix

- It is brought by enzymes helicase, which is ATP dependent.

- Replication fork – unwinding of DNA molecule into strands results in the formation of Y-shaped structure called replication fork.

iii. Formation of Primer Strands.

Enzyme known as Primase synthesize a short primer strand of RNA.

★ DNA replication would not occur without enzyme that catalyze various steps in the process. Enzyme that participate in the eukaryotic DNA replication process include.

DNA helicase – unwinds & separates double stranded DNA as it moves along the DNA. it forms the replication fork by breaking hydrogen bonds between nucleotide pairs in DNA.

Primase – Primase initiates the process by creating small RNA segments called RNA primers.

DNA Polymerase – it extends the primed segments by adding free nucleotides.

Toposomerase – unwinds & rewinds DNA strands to prevent the DNA from becoming tangled or supercoiled.

DNA ligase – Joins DNA fragments togther by forming phosphodiester bonds between nucleotides.

iv Elongation of New Strand.

Continous Synthesis (leading strand)

- DNA polymerase catalyses polymerization only in the direction. That is 5' → 3'

- This creates some additional complication at the replication fork. Consequently the replication is continuous on one template strand with polarity 3' → 5'. It is known as leading daughter strand

Discontinuous Synthesis (lagging strand)

- The replication is discontinuous in the form of Okazaki Fragments on the other template strand with polarity 5' → 3' It is known as lagging daughter Strand

- Primase initiates the process by creating small RNA segments called RNA Primase. DNA polymerase extends the primed segments by adding free nucleotides. RNA primers are removed and replaced with DNA.

- The discontinuously synthesized fragments are later joined by enzyme called DNA ligase

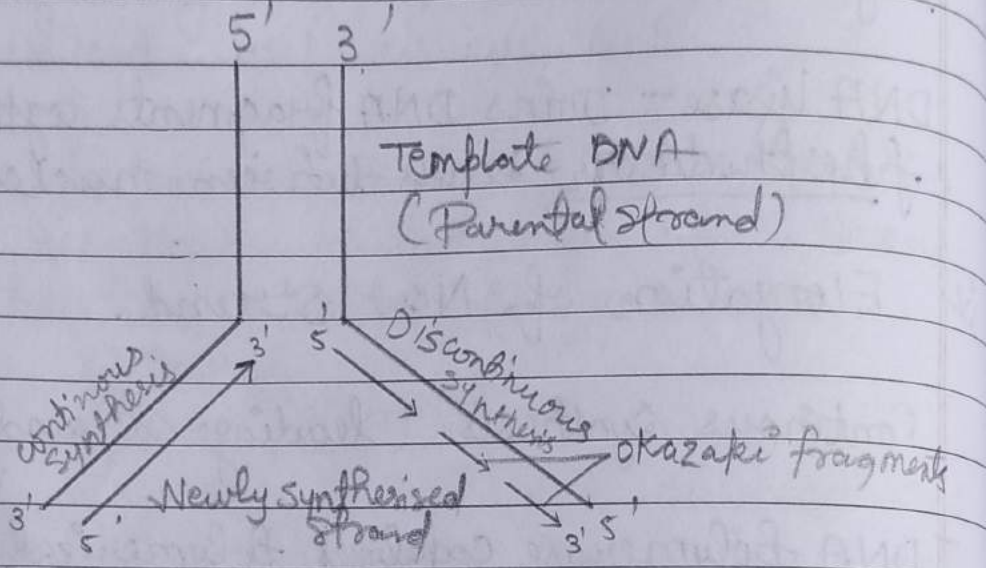


Fig- Replicating fork of DNA

Transcription

The process of copying genetic information from one strand of the DNA into RNA is known as transcription.

Why both the strands of DNA not being copied during transcription.

- If both strands act as a template. They would code for RNA molecule with different sequences & in turn, if they code for proteins would be different. Hence one segment of DNA would be coding for two different proteins & this would complicate the genetic information transfer machinery.

- Two RNA molecules, if produced simultaneously would be complementary to each other, hence would

form a double stranded RNA. This would prevent the translation of RNA into proteins.

Transcription unit

The segment of DNA that takes part in transcription is called transcription unit.

It has 3 Components

1. A promoter
2. The structural gene
3. A terminator.

- Since the two strands have opposite polarity & the DNA dependent RNA polymerase also catalyses the polymerizational in only one direction that 5'-3'

- The strand that has the polarity 3'-5' act as template & is called template strand or non-coding strand.

- The other strand with polarity 5'-3' and the sequence same as RNA, except thymine at the place of uracil is displayed during transcription & this strand is called coding strand or sens strand or non template strand.

The DNA strand over which RNA replication takes place has 3 regions:-

1- Promoter - It is present at the 3' end of the template strand of DNA, σ factor recognise this site & RNA polymerase starts adding nucleotide.

2- Structural Gene - All along this region RNA polymerase adds the nucleotide & elongate the RNA strand.

3- Terminator - It is the region which is recognize by ρ factor & result in the termination of RNA polymerization & the RNA strand releases.

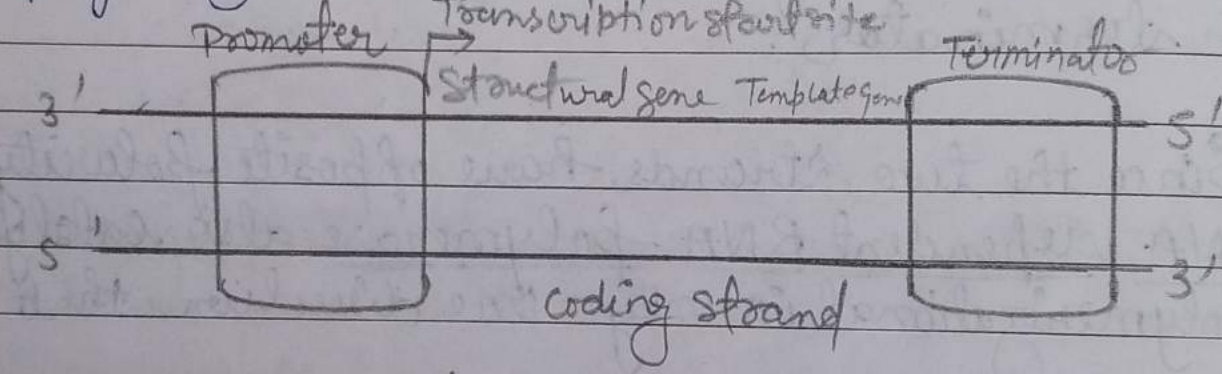


Fig - Schematic Structure of a transcription unit

Transcription in Prokaryotes

There are three types major types of RNA's in bacteria

1. mRNA
2. tRNA
3. rRNA

All three RNA's are needed to Synthesis a protein in a cell.

2. mRNA - It provides the template for protein synthesis to occur.

2. tRNA - It brings aminoacid in front of mRNA & reads the Genetic Code.

3. rRNA - It plays structural & catalytic role during translation i.e, Protein Synthesis

Transcription in Prokaryotes or bacteria include three Steps.

1- Initiation - when RNA Polymerase binds to the Promoter region & initate the transcription this is called initiation. It uses nucleoside triphosphate as Substrate and polymerises in template.

2- Elongation - when RNA polymerase start and recognize the promoter region. This will elongate the template strand Complementary.

3- Termination - once the RNA polymerase reaches the terminator region the RNA falls off. so also the RNA polymerase. This results in the termination of transcription

Transcription in Eukaryotes

★ The structural genes in a transcription unit could be said to as monocistronic (mostly in eukaryotes) or polycistronic (mostly in bacterias & prokaryotes)

The Structural Genes in eukaryotes are split into two regions.

1- **EXons**— the regions or expressed sequences are defined as exons.

2. **Introns** – the regions which do not code for Proteins

Due to the presence of Introns

Primary RNA – transcripts became non-functional

RNA Splicing

In some genes the protein-coding section of the DNA (exons) are interrupted by non-coding regions (Introns). RNA splicing removes the introns from Pre mRNA to produce the final set of instruction.

Types of RNA Polymerase

1. RNA polymerase I → transcription of r-RNA
2. RNA polymerase II → transcription of m-RNA
3. RNA polymerase III → transcription of t-RNA

The hnRNA undergoes two additional process i.e, post transcriptional modifications.

Capping and Tailing Processes

Additional modification to the 5' & 3' end of eukaryotic pre-mRNA

Capping

- It is the addition of a cap on 1st nucleotide's 5' end of Pre-mRNA
- A 7-methylguanosine cap is added to the 5' end.
- It is formed by methyl guanosine triphosphate.
- In addition, initiation factors involved in protein synthesis recognize the cap to help initiate translation by ribosomes.

Tailing

- Tailing is basically addition of poly (A) adenine tail to the 3' end of pre-mRNA.

- mRNA Stability.
- Multiple A (adenine) Group is being added to the 3' end.

5'cap Pre- mRNA

Step for Capping

1st [Free triphosphate Group is replaced by another Structure called CAP i.e. 7-methylguanosine cap]

2nd [The Cap is added by the enzyme (guanyl transferase)]

3rd [The 5' Cap protects the nascent mRNA from degradation & assists in ribosome binding during translation]

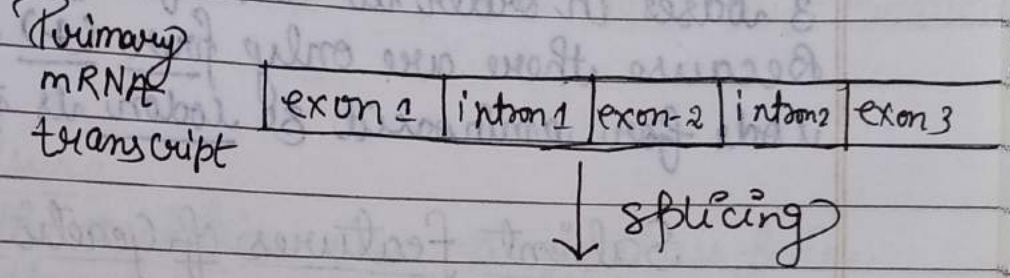
4th [The cap plays a role in the ribosomal recognition of messenger RNA during translation into a protein]

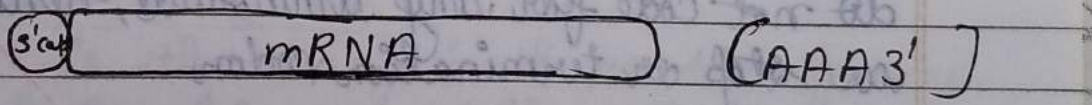
SPLICING

In eukaryotes before the mRNA can be translated

into proteins, non-coding portions of the sequence, called introns, must be removed & protein-coding parts, called exons joined by RNA splicing to produce a mature mRNA.

- Intron sequences in mRNA do not encode functional proteins





Steps for tailing

1st — RNA processing at the opposite end of the transcript comes in the form of a string of adenine bases attached to the end of the synthesized RNA chain

2nd — This string of adenine is called the "Poly A Tail"

3rd — The addition of the adenines is catalyzed by the enzyme Poly(A) Polymerase which recognize the sequences AAUAAA as a signal for the addition.

Genetic Code

The relationship b/w the sequence of nucleotides on mRNA and sequence of aminoacid in the polypeptide is called Genetic Code.

George Gamow suggested that the code must be of 3 bases in order to code for 20 aminoacid. Because there are only four (4) bases which code for aminoacid so codon is triplet

Salient features of Genetic Code

- The codons are triplet out of 64 codons 61 codes for 20 amino acids and 3 codons (UAA, UGA, UAG) do not code for any aminoacids, hence, function as stop or termination codon.
- One codon codes for only one particular aminoacid, hence the code is unambiguous and specific.
- Some aminoacids are coded by more than one codon-hence, the code is degenerate.
- The codon is read on mRNA in a contiguous fashion that is without punctuations and thus the code is commaless.
- Genetic code is nearly universal.
- AUG is a codon with dual functions. It codes for the amino methionine (met) & also acts as an initiater codon.

The Genetic Code and Mutation

Mutation is defined as the sudden inheritable change in the genetic material.

It can be of the following two major type

(1) Point Mutation (2) Frame shift Mutation

(1) Point Mutation

It is the mutation in a single base pair, which is replaced by another base.

e.g. :- Sickle cell anemia.

(2) Frame-shift mutation

It is the change in the reading frame because of insertion or deletion of base pairs.

i) Insertion → It is the addition of one or more nucleotide in the DNA Segment.

ii) Deletion → It is the removal of one or more nucleotide from the DNA Segment.

★ tRNA (transfer RNA) – The adaptor molecule.

- Francis Crick proposed the presence of an adaptor molecule which could read the code on one end and on the other end would bind to the specific aminoacids.

- It carries the aminoacid to the ribosomes to make protein.

- There are specific tRNA for each codon & aminoacid.

Structure of t-RNA

The Secondary structure of t-RNA is clover leaf like but the three-dimensional tertiary structure depicts it as a compact inverted L-Shaped molecule.

t-RNA has five arms or loops.

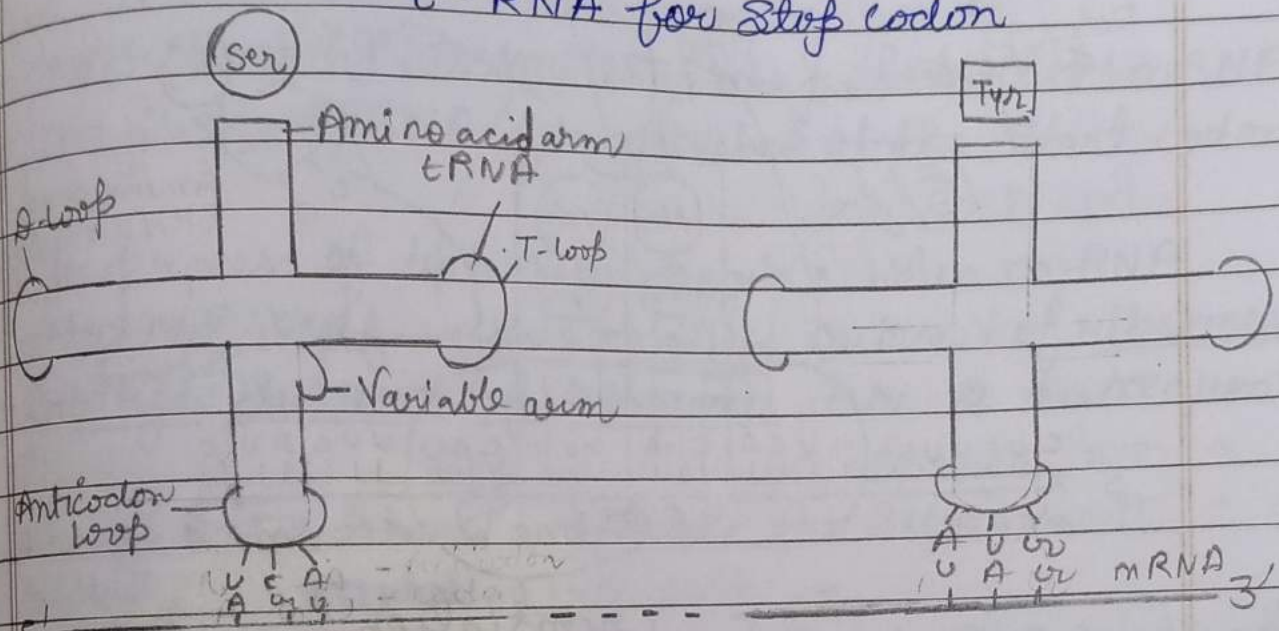
- Anticodon loop - which has bases complementary to the code.

- Amino acid acceptor ~~or~~ end - to which amino acid binds.

- T loop - which help in binding to ribosome.

- D loop - which helps in binding aminoacyl Synthetase.

- Variable loop – a specific t-RNA for initiation is called initiator t-RNA. There is no t-RNA for Stop codon



TRANSLATION

Translation is the process of Synthesis of Protein from mRNA with the help of ribsomes

There are four stages of Protein Synthesis

1. Activation of Amino acids
2. Initiation
3. Elongation
4. Termination

- Amino acids foom Protein by Polymerization
- Peptide bond formation takes place b/w amino acids & t-RNA.
- Energy for bond formation is provided by ATP

A·A ① ATP + enzyme (Aminoacylation of Aminoacid)

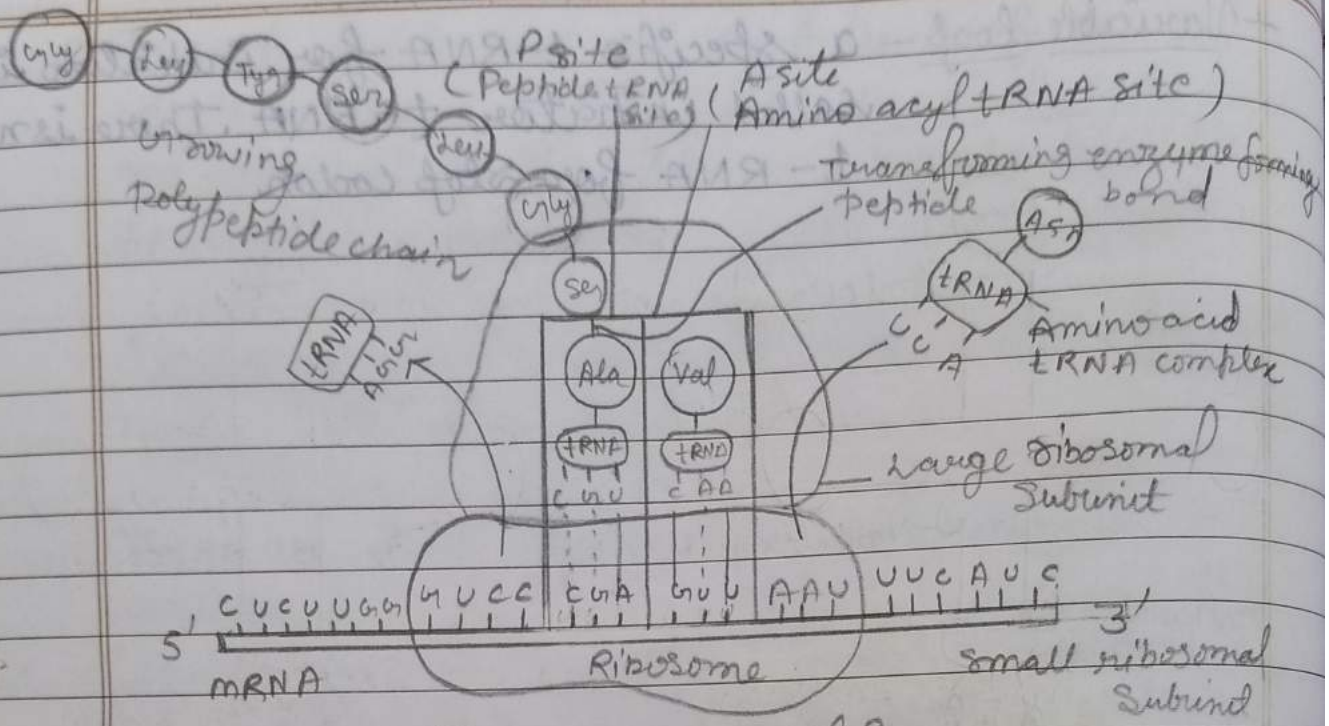


Fig - Translation

① Activation of amino acids

- Amino acid activation refers to the attachment of an amino acid to its Transfer RNA (tRNA)

- Aminoacids are present in the cytoplasm and they are activated in the presence of ATP by Aminoacetyl t-RNA Synthetase enzyme. This process is known as aminoacylation

- High energy bond will be from b/w aminoacids & t-RNA. This energy is given in the form of ATP, Stability of bond is less which can be easily broken.

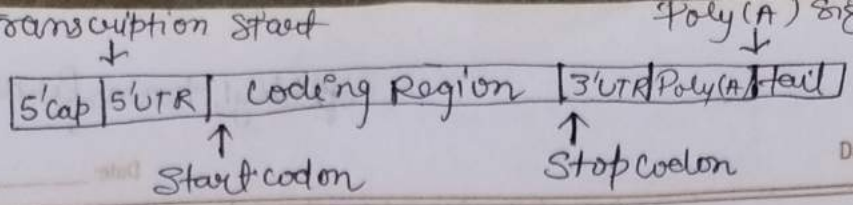
- Once the aminoacylation is done it is said to be 'aa-tRNA or Charged t-RNA.

② Initiation

- The ribosome assembles around the target mRNA. The first t-RNA is attached at the Start codon
- The process of Initiation begins when m-RNA attached itself with smaller Subunit of ribsomes & large Subunit of ribosomes has 2 Subsequent Sites → P- Site & A- Site
- At P- site the complementary t-RNA complex & read the Code of m-RNA & established temporary hydrogen bond with it

Steps of chain initiation

1. mRNA binds to small ribosomal Subunit at binding Site
2. tRNA carrying 'met' binds to Start Codon ↑ P-Site of ribosome
3. Large ribosomal Subunit attaches.
4. tRNA with anticodon corresponding to the next Codon attaches. ← A-site of ribosome.
5. Peptide bond form b/w amino acids.



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Translational unit

It is the Sequence of RNA flanked by the Start Codon (AUG) and the Stop Codon in mRNA. It Codes for a pef polypeptide that has to be produced.

UTR untranslated region

- There are some parts present on mRNA which does not code for any amino acid.
- These pairs are present at 5' end & 3' end of mRNA these are called UTR.

Elongation

- The tRNA transfers an amino acid to the RNA corresponding to the next Codon. The ribosome then moves (translocates) to the next mRNA Codon to Continue the process, creating an amino acid chain.

- It's the addition of amino acids by the formation of Peptide bonds. Elongation is just what it Sounds like, a chain of amino acids grows longer & longer as more amino acids are added on. This will eventually create the polypeptide.

Termination

when a Stop codon is reached, the ribosome releases the polypeptide.

Regulation of Gene expression

- Regulation of gene expression means controlling the amount & time of formation of gene products according to the requirement of the cell.

- Gene expression can be regulated.
  - During transcription (transcriptional control)
  - During translation (translational control)
  - After translation (post-translational control)

In Eukaryotes

1- transcriptional level- A primary transcript is formed.

2- Processing level- Regulation of Splicing

3- Transport of mRNA- From nucleus to the cytoplasm.

4- Translational level- Gene expression regulated by controlling the rate of initation of transcription.

In Prokaryotes

The accessibility of promoter region of prokaryotic DNA in many cases is regulated by the interaction of proteins with sequences termed as operators

OPERON MODEL

(1961) French Scientists Lwoff, Jacob, & monad developed the Concept of operon to explain the regulatory mechanism in prokaryotes.

The Lac OPERON

An operon is a unit of prokaryotic gene expression which includes coordinately regulated (Structural Gene) & Control elements which are recognized by regulatory gene product

Components of operon

1 Promoter – The sequence of DNA where RNA polymerase binds & initiates transcription of structural gene.

2- Operator – The sequence of DNA adjacent to promoter where specific repressor protein binds it's called operator.

3- Regulatory Gene – The gene that codes for the repressor protein that binds to the operator & Suppresses its activity as a result of which transcription will be Switched off (i Gene)

4- Structural Gene - The fragment of DNA which transcribe mRNA for polypeptide synthesise. There are three structural gene in lac operon (z, y, a)

5- Inducer - The substrate that prevents the repressor from binding to the operator it is called an inducer.

Functional of lac operon

- Repressor is synthesized by I Gene & it is functional all the time.
- Repressor binds to the operator of lac operon.
- RNA Polymerase is prevented from transcribing the template strand of DNA.
- Lactose is used as a inducer in lac operon.
- Gene z code for β-Galactosidase breaks lactose down into glucose & galactose while the other two proteins Permease & transacetylase aid in the metabolic process.
- Gene a codes for a transacetylase.
- Gene y - Codes for permease that increase the permeability of the cell to β-Galactose.

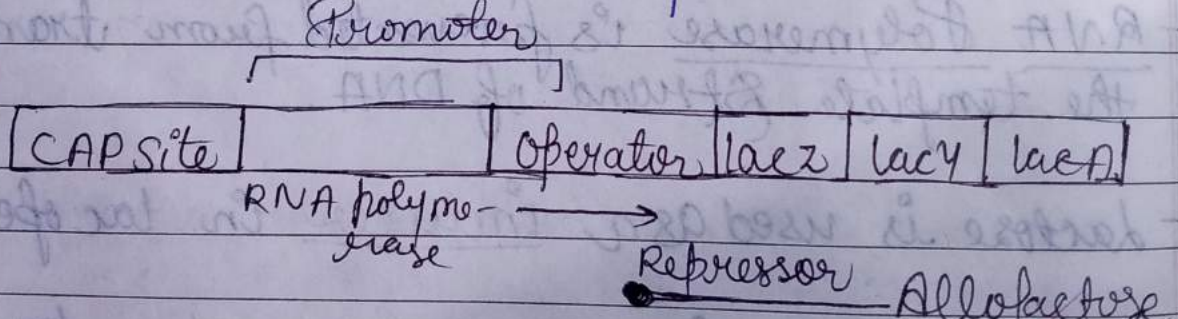
When lactose is absent

When lactose are present in the cell, it will bind to sites on the repressor protein, changing its conformation & rendering it inactive.

As the repressor protein detaches from the operator, RNA polymerase can bind to the promoter, transcription can occur, & the three lactose degradation genes can be synthesized.

With lactose

Allolactose (rearranged lactose) bind to the lac repressor & makes it let go of the operator. RNA polymerase can now transcribe the operon. Let go of the operator. RNA polymerase can now transcribe the operon.



HUMAN GENOME PROJECT

- It is also called MEGA project.
- The total cost of the project was 9 billion US dollars & an enormous amount of data was

13 yrs 1990-2003

generated, therefore it is called mega Project

- Human Genome Project was an undertaking by many countries to acquire complete knowledge of the organisation, Structure and functions of the human genome.

- The human Genome project was a 13 yers project by US department of energy and the National Institute of health. It was launched in 1900 & complete in 2003.

- HGP was closely associated with development of a new area in biology termed as ~~bain~~ bioinformatics.

Goals of Human Genome Project.

1 - To dentify all the approx 20,000-25000 gene in human DNA.

2 - TO determine the Sequences of the 3 billion base pair that makes up human DNA.

3 - To store this information in data base.

4 - To develop improvised tools for data analysis.

5 - To transfer related technologies to other Sectors such as industries.

6- To address the ethical, legal & Social issues (ELSI) that may arise from the Project.

Important features of Human Genome

1- The human genome contains 3164.7 million nucleotide bases.

2- The average gene consist of 3000 bases, but gene size very greatly. (the largest human gene is dystrophin containing 2.4 million bases)

3- The total number of genes in the genome is estimated at 30,000 and all 99.9% nucleotide bases are exactly the same in all people.

4- Functions of about 50% of the discovered genes are still unknown.

5- Less than ~~20% of the Genes (2968) and~~ 2% of the genes of the genome codes for proteins.

6- Chromosome 1 has most Genes (2968) and they has the fewest (231)

7- Repeated Sequences (AT-AT-AT or GC-GC-GC)

make up very large portion of the human Genome.

6- Scientists have identified about 1.4 million locations where single base DNA differentes (SNPs single nucleotide polymorphism, pronounced as 'snips') occur on humans.

Benefits of HGP

i- The knowledge about effects of the DNA variations among individuals can leads to discovery of new way in diagnosis, treatment & prevention of various diseases affecting mankind.

ii- It can help in learning about DNA sequences of non-human organisms that can lead to an understanding of their natural capabilities.

Methodologies of HGP

1- Expressed Sequence Tags (ESTs) — this method focuses on identifying all the genes that are expressed as RNA.

2- Sequence an annotation — the method involves sequencing the whole set of genome and then assigning functions to the different regions in the sequence.

DNA Fingerprinting

DNA fingerprinting was invented in 1984 by Professor. Sir Alec Jeffreys after he realised you could detect variations in human DNA.

DNA fingerprinting is a method used to identify an individual from a sample of DNA by looking at unique patterns in their DNA

Seven Steps to understanding DNA fingerprinting

1- Extracting DNA from cells (DNA Isolation)
2- Cutting up the DNA using an enzyme (..
3- separating the DNA fragments in gel.
4- Transferring the DNA onto paper.
5- Adding the radioactive probe.
6- Setting up the X-ray film.
7- observe the dark bands.

Polymorphism

In a population, if an inheritable mutation is observed at high frequency it is referred to as DN polymorphism.

Technique of DNA Fingerprinting

1- The first step of DNA fingerprinting was to extract DNA from a sample of human material usually blood.

2- Molecular 'Scissors' called restriction enzymes were used to cut the DNA : Thus resulted in thousands of pieces of DNA with a variety of different lengths.

3- These pieces of DNA were then separated according to size by a process called gel electrophoresis

i) An electric current was applied which pulled the -vely charged DNA through the gel

ii) The shorter pieces of DNA moved through the gel easiest & therefore fastest. It is more difficult for the longer pieces of DNA to move through the gel so they travelled slower.

iii) As a result, by the time the electric current was switched off, the DNA pieces had been separated in order of size the smallest DNA molecules were furthest away from where the original sample was loaded on to the gel.

4- Once the DNA had been Sorted, the pieces of DNA were transferred or blotted out of the fragile gel on to a robust piece of nylon membrane and then 'unzipped' to produce Single strands of DNA.

5. Next the nylon membrane was incubated with radioactive probes.

6- The probes only attach to the pieces of DNA that they are complementary to - in this case they attach to the minisatellite in the Genome. This is called hybridization. They are observed as dark bands on X-ray films.

Application of DNA Fingerprinting

i) used as a tool in fosensic investigations.

ii) To Settle ~~paterni~~ paternity disputes.

iii) To Study evolution by determining the genetic diversities among population