Molecular Basis of Inheritance

I. Introduction

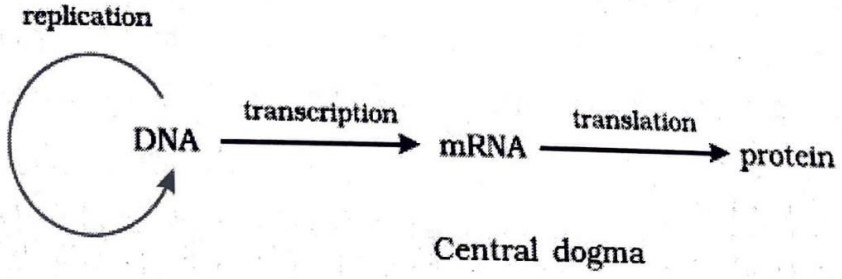
1. **NUCLEOSIDE:** A compound consisting of a pentose sugar and a nitrogenous base is known as **nucleoside**.
2. **NUCLEOTIDE:** A compound consisting of a nucleoside linked to a phosphate group is known as **nucleotide**.
3. **POLYNUCLEOTIDE CHAIN:** Many nucleotides linked together through 3"-5' phosphodiester linkage forms polynucleotide chain.
4. **NITROGENOUS BASES:** There are two types of nitrogenous bases namely Purines and Pyrimidines
5. **PURINES:** Adenine and Guanine.
6. **PYRIMIDINES:** cytosine, Uracill and Thymine. Nitrogenous bases present in DNA are A,GT,C and in RNA are A,GU,C.

IL Structure of DNA

1. **DNA** is a long polymer of **Deoxyribonucleotides.**
2. The length of DNA is usually defined as number of nucleotides present in it.
3. Bacteriophage lambda has 48502 base pairs.
4. *Escherichia coli* has 4.6 x 106 bp, and
5. Haploid content of human DNA is 3.3 x 10° bp.
6. **The Double helix Structure of DNA** was proposed by James Watson and Francis Crick in 1953.
7. It is made of two polynucleotide chains.
8. The two chains have anti-parallel polarity.
9. The bases in two strands are paired through hydrogen bond forming base pairs (bp), A=T and G=C.
10. The two chains are coiled in a right-handed fashion.

D The pitch of the helix is 3.4 nm, roughly 10 bp in each turn and the distance between a bp in a helix is approximately equal to 0.34 nm.

12. **Chargaff's rule:** The ratio between adenine and thymine and guanine and cytosine are constant\/\/S and equals one.
13. ***Francis Crick*** proposed **central dogma of molecular biology**



III. Packaging of DNA

1. **Histones:** A set of positivity charged, basic proteins associated with DNA.
2. They are rich in basic amino acids like **lysine and arginine**.
3. **Histone octamer:** Histones organized to form a unit of eight molecules.
4. **Nucleosome:** A negatively charged DNA wrapped around positively charged histone octamer is known as **Nucleosome**.
5. Nucleosome is the basic unit of DNA packaging in eukaryotes.
6. A typical nucleosome consists of 200 bp.
7. **Chromatin:** The Mass of genetic material composed of DNA and proteins that condense to form chromosomes is known as Chromatin.
8. **Euchromatin:** In a typical nucleus, some region of chromatin are loosely packed, transcriptionally active and stains light and are referred to as Euchromatin.
9. **Heterochromatin:** The chromatin that is more densely packed, transcriptionally inactive and stains dark is referred to as Heterochromatin.
10. **Non histone chromosomal (NHC)proteins:** The packaging of chromatin at higher level requires additional set of proteins that collectively are referred to as **NHC proteins**.

IV. Search for genetic material

1. **Transforming principle:** When heat killed S and live R bacteria are mixed, the R strain bacteria gets transformed by the heat killed S strain.
2. In 1928, Frederick Griffith in a series of experiments with Streptococcus pneumoniae (bacteria) witnessed a miraculous transformation (DNA) in bacteria.
3. He used bacteria of two strains S-strain which is virulent and R-strain which is Non virulent.
4. **Transformation:** Transfer of genetic material from one bacteria to other is known as transformation.
5. **Biochemical characterisation of transforming principle:** *Avery, Macleod, Mc carty* discovered that DNA alone from 'S' bacteria caused 'R' bacteria to become transformed.
6. **Hershey and Chase**(1952) worked on bacteriophages.
7. They grew some viruses on a medium that contained radioactive phosphorus and some others on medium that contained radioactive sulphur.
8. Viruses grown in the presence of radioactive phosphorus contained radioactive DNA.
9. Viruses grown on radioactive sulphur contained radioactive protein.

V. Properties of genetic material

1. A molecule that can act as a genetic material must fulfil the following criteria.
   - **(i)** It should be able to generate its replica (Replication).
   - **(ii)** It should chemically and structurally be stable.
   - **(iii)** It should provide the scope for slow changes (mutation) that are required for evolution.
   - **(iv)** It should be able to express itself in the form of 'Mendelian Characters'.

VI. RNA world

1. **RNA was the first genetic material.**
2. RNA acts as a genetic material as well as a catalyst (**Ribozyme**).

VII. Replication

1. **Replication:** Duplication of genetic material is known as Replication.
2. In Eukaryotes replication occurs in S phase of cell cycle.
3. **Semiconservative DNA replication:** Semiconservative replication would produce two copies that each contained one of the original strands(old) and one entirely new strand.
4. The Experimental Proof for semi conservative mode was given by Matthew Meselson and Franklin Stahl (1958).
5. They grew E. coli in a medium containing $^{15}NH_4$,Cl (^{15}IN is the heavy isotope of nitrogen).
6. Then they transferred the cells into a medium with normal "$^{14}NH_4$,Cl".
7. Heavy DNA molecule could be distinguished from the normal DNA by centrifugation in a caesium chloride (CSCI) density gradient.
8. The DNA that was extracted from the culture one generation after the transfer from 'N to 14N medium [that is after 20 minutes] had a hybrid or intermediate density.
9. DNA extracted from the culture after another generation [that is after 40 minutes, II generation] was composed of equal amounts of this hybrid DNA and of 'light' DNA.
10. Taylor and colleagues in 1958 performed on Vicia faba to prove that the DNA in chromosomes also replicate semi conservatively.
11. **The main enzyme is referred to as DNA-dependent DNA polymerase** that synthesizes dows DNA molecules always in 5'→3' direction.
12. **Deoxyribonucleoside triphosphate** serve dual purposes i.e act as substrates and they provide energy for polymerisation reaction.
13. **Replication fork:** The junction where the double-stranded DNA splits apart into 2 single strands is called **Replication fork.**
14. **Leading strand:** The template with polarity 3-5, the replication is continuous is known as leading stand.
15. **Lagging strand:** The template with polarity 5→3, the replication is **discontinuous is known as lagging strand**.
16. **DNA ligase:** The enzyme that joins the discontinuous fragments of the DNA (Okazaki fragment).
17. **Helicase:** An enzyme that catalyses the unwinding of the DNA helix during DNA replication.
18. **Origin of replication (ORI):** A definite region in E.coli DNA where the replication originates.

VIII. Transcription

1. **Transcription:** The process of copying genetic information from one strand of the DNA into RNA is termed as transcription.
2. Transcription unit consists of promotor, structural gene and terminator.
3. **DNA dependent RNA polymerase** also catalyse the polymerisation in only one direction, that is 5' $\rightarrow$3`.
4. **Template strand:** The stand that has the polarity 3-5 acts as a template and is also referred to as **template strand**.
5. **Coding strand:** The other strand which does not code for anything is referred to as **coding strand**.
6. The **promoter** and **terminator** flank the **structural gene** in transcription unit.

IX. Transcription unit and the gene

1. **Cistron:** A segment of DNA coding for a polypeptide is called as **Cistron**.
2. **Monocistronic:** Structural gene in a transcription unit could be said as **monocistronic**.
3. It is seen mostly in Eukaryotes.
4. **Polycistronic:** Structural gene in a transcription unit mostly in **bacteria or prokaryotes** is called **polycistronic**.

5. **Exons:** The coding sequences or expressed sequences that appear in mature or processed RNA are called **exons**.
6. **Introns:** Intervening sequences that interrupt the exons and do not appear in mature or processed RNA are called **introns**.

X. Process of transcription

1. **Initiation:** RNA polymerase binds to promoter and initiates transcription (sigma factor).
2. **Elongation**: RNA polymerase uses Nucleoside triphosphates as substrate and polymerises in a template depended fashion following the rule of complementarity
3. Termination: Once the polymerase reaches the termination region which is recognized by Rho factor, the nascent RNA falls off, so also the RNA polymerase results in termination.
4. There are three types of RNA polymerase.
5. The RNA polymerase I transcribes **rRNA** As (28S, 18S, 8S).
6. RNA polymerase III is responsible for transcription **tRNA, 5 srRNA and snRNAs** (small nuclear RNAs).
7. The RNA polymerase II transcription precursor of mRNA, the **Heterogenous nuclear RNA** (hnRNA).

XI. Post transcriptional modifications

1. **Splicing-**The primary transcripts contain both exons and introns and are non- functional, so it is subjected to a process called **splicing**.
2. The introns are removed and exons are joined in a defined order.
3. **Capping-**An unusual nucleotide (methyl guanosine triphosphate) is added to the s^1-end of hnRNA is known as **capping**.
4. **Tailing:** Adenylate residues (200-300) are added at 3'-end in a template independent manner is called as **tailing**.

XII. Genetic code

1. One codon codes for only one amino acid, hence it is **unambiguous and specific**.
2. Same amino acids coded by more than one coden, hence the code is **degenerate**.
3. AUG has dual functions. It codes for methionine (met), and it also acts as **initiator codon.**
4. **UAA, UAG and UGA** are terminator codons.

XIII. Mutations

1. **Point mutation**: Single base pair substitution is known as point mutation.
2. In sickle cell anaemia, a change of amino acid residue glutamate to valine at 6th position of beta globin chain is seen.
3. **Frame-shift insertion or deletion mutations:** Insertion or deletion of three or its multiple bases insert or delete one or multiple codon hence one or multiple amino acids, and reading frame remains unaltered from that point onwards.

XIV. tRNA/SRNA/Adaptor molecule

1. The structure of tRNA is clover leaf or inverted L shape.
2. The tRNA has an **anticodon** loop that has bases complementary to the genetic code.
3. It also has **an amino acid acceptor** end at 3^1 to which it binds amino acids activated in the presence of ATP and linked to their cognate tRNA.

XV. Translation

1. **Translation:** The process of polymerisation of amino acid to form a polypeptide is called as translation.
2. **Aminoacylation of tRNA:** It is the first phase where amino acids are activated in the presence of ATP and linked to their cognate tRNA.
3. Process of translation involves Initiation, elongation and termination.
4. **Initiation: The ribosome binds to the mRNA at the start codon (AUG) that is recognised only by the initiator tRNA.**
5. **Elongation: complexes composed of an amino acid linked to tRNA, sequentially bind to the appropriate codon in mRNA by forming complementary base pairs with the tRNA anticodon.**
6. **Termination:** At the end, a release factor binds to the stop codon, terminating translation and releasing the complete polypeptide from the ribosome.
7. **Untranslated regions (UTR):** An mRNA also has some additional sequences that are not translated and are referred to as untranslated regions.

XVI. Regulation of gene expression

1. **Regulation:** Regulation of gene expression refers to a very broad term that may occur at various levels. 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.
2. **Operon:** In Lac operon, a polycistronic structural gene is regulated by a common promoter & regulatory gene such arrangement is very common in bacteria, & is referred to as operon.
3. **Lac operon: It was proposed by Francois Jacob and Jacque Monod.**
4. **It consists of** one regulatory gene 'i' gene inhibitor coding for repressor protein and three structural genes z, y, a.
5. z gene codes for β-galactosidase that hydrolysis lactose into galactose and glucose.
6. y gene codes for permease which increases permeability of cell to β- galactosidase and
7. a gene codes for transacetylase.
8. Inducer: Lactose is the substrate for the enzyme beta-galactosidase, and it regulates switching on & off of the operon. Hence, it is termed as inducer.
9. Negative regulation: Regulation of lac operon by repressor in referred to as negative regulation.

XVII. Human Genome Project (HGP)

1. **Human genome project:** The mega project to sequence all the genes in human genome, which was closely associated with the rapid development of a new area in biology called bioinformatics.

XVIII. Methodologies

1. **Expressed sequence tags (ESTs)**- An approach focused on identifying all the genes that are expressed as RNA is called Expressed sequence tags (EST).
2. A blind approach of simply sequencing the whole set of genome that contained all the coding & non-coding sequence & later assigning different regions in the sequence.

XIX. Salient features of human genome

1. The human genome contains 3164.7 million nucleotide bases.
2. The average gene consists of 3000 bases.
3. The largest known human gene being dystrophin at 2.4 million bases.
4. The total number of genes is estimated at 30,000-much lower than previous estimates of 80,000 to 1,40,000 genes.
5. The functions are unknown for over 50 per cent of the discovered genes.
6. Less than 2 per cent of the genome codes for proteins.
7. Repeated sequences make up very large portion of the human genome.
8. Chromosome 1 has most genes (2968), and the Y has the fewest (231).
9. Scientists have identified about 1.4 million locations where single base DNA differences (SNPs-single nucleotide polymorphism, pronounced as "snips") occur in humans.

XX. DNA Fingerprinting

1. DNA Fingerprinting was initially developed by *Alec Heffereys.*
2. **Repetitive** DNA is a sequence with small stretch of DNA repeated many times.
3. **Satellite DNA:** The bulk DNA forms a major peak and the other small peaks are referred to as satellite DNA. It does not code for any protein.
4. **Polymorphism:** It is variation at genetic level that arises due to mutations.
5. **DNA POLYMORPHISM:** If an inheritable natation is observed in a population at high frequency, it is referred to as DNA polymorphism
6. **VNTR:** A satellite DNA as probe that shows very high degree of polymorphism. It was called as Variable Number of Tandem Repeats (VNTR).
7. The VNTR belongs to a class of satellite DNA referred to as mini satellite.
8. The technique of DNA fingerprinting involves-isolation of DNA, digestion of DNA by restriction endonucleases, separation of DNA fragments by electrophoresis, transferring (blotting) of separated DNA fragments to synthetic membranes, such as nitrocellulose or nylon, hybridisation using labelled VNTR probe, and detection of hybridised DNA fragments by **autoradiography**.

XXI. Application of DNA finger printing

1. In forensic science in determining population and genetic diversities.
2. Solving maternity and paternity issues.