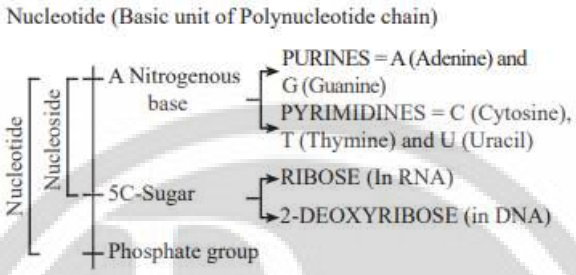
Molecular Basis of Inheritance

- DNA is a long polymer of deoxyribonucleotides. The length of DNA usually defined as number of nucleotides is also characteristic of an organism.

Structure of Polynucleotide Chain



- Uracil is present in RNA; Thymine (5-methyl uracil) in DNA

CENTRAL DOGMA OF MOLECULAR BIOLOGY

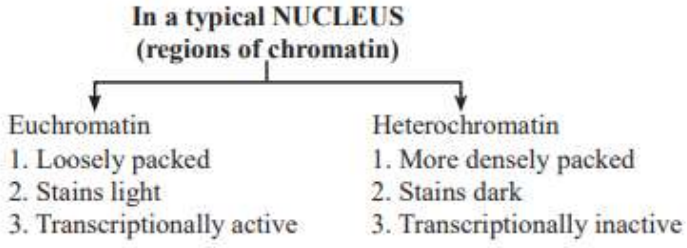
Proposed by FRANCIS CRICK.

- Only applicable to dsDNA.

In some viruses, the flow of information is in reverse direction, i.e. from RNA to DNA. It is called reverse of central dogma.

PACKAGING OF DNA HELIX

- In prokaryote (E.coli), the DNA (negatively charged) is held with some proteins (positive charges) in the "Nucleoid". The DNA in nucleoid is organised in large loops held by proteins.
- In eukaryotes, it is much more complex.
  - Histone Octamer: Positively charged set of basic proteins, Histone (rich in lysine and arginine) are organised to form a unit of eight molecules, called Histone octamer.
  - Nucleosome: Negatively charged DNA is wrapped around positively charged histone octamer. A typical nucleosome contains 200 bp of DNA helix.
- Nucleosomes constitute the repeating unit of a structure in nucleus called chromatin, thread like stained bodies seen in nucleus.
- Nucleosomes in chromatin are seen as beads-on-string structure under electron microscope. Packaged to form chromatin fibers that are further coiled and condensed at metaphase stage to form chromosomes.



SEARCH FOR GENETIC MATERIAL

Transforming Principle

- In 1928, Frederick Griffith, in a series of experiments with Streptococcus pneumoniae, witnessed a miraculous transformation in Bacteria.
- Griffith concluded that R-strain was somehow transformed by heat-killed S-strain. It must be due to the transfer of genetic material (transforming principle).

Biochemical Nature of Transforming Principle

- Oswald Avery, Colin MacLeod and Maclyn McCarty (1933-44) discovered that DNA is the genetic material.
- DNA of S bacteria caused R bacteria to become transformed.
- Proteases and RNAse did not affect transformation but DNAase inhibit transformation.

Genetic Material is DNA

The Unequivocal proof that DNA is the genetic material came from the experiments of Alfred Hershey and Martha Chase (1952), on bacteriophages, using radioactive phosphorus 32P and sulphur 35S in separation medium with E. coll.

RNA WORLD

- RNA was the first genetic material.
- The essential life processes like metabolism, translation, splicing evolved around RNA.
- RNA used to act as a genetic material as well as a catalyst, so was reactive and hence unstable. (DNA has evolved from RNA with chemical modifications that make it more stable.)

REPLICATION

- Watson and Crick proposed a scheme for replication of DNA while proposing the double helix structure of DNA.
- Semi conservative DNA replication: Two strands would separate and act as a template for the synthesis of new complementary strands. After completion of replication, each DNA molecule would have one parental and one newly synthesised strand.

EXPERIMENTAL PROOF

- Semi-conservative DNA replication was shown first in Escherichia coli, then in higher organisms like plants and human cells.
- Matthew Meselson and Franklin Stahl, performed the experiment (1958) to prove semi conservative nature of DNA by using normal 14N and heavy 15N isotope of Nitrogen.
- Taylor and colleagues (1958) used radioactive thymidine and Vicia faba (Faba beans) to prove that DNA in chromosomes also replicate semi-conservatively.

TRANSCRIPTION

- Process of copying genetic information from one strand of DNA into RNA.
- Principle of complementarity governs transcription (except, adenine forms pair with uracil instead of thymine). In transcription, only a segment of DNA and only one of the two strands is copied into RNA.

TRANSCRIPTION UNIT AND GENE

- Cistron is defined as a segment of DNA coding for polypeptide.
- The structural gene is monocistronic (mostly in eukaryotes) or polycistronic (mostly in bacteria or prokaryotes).
- In eukaryote, genes are split between coding sequences or Exons, which appear in mature RNA and Introns or intervening sequence.
- Regulatory sequences are defined as regulatory genes, even though they do not code for any RNA or protein.
- In bacteria, mRNA does not require any processing, so transcription and translation are coupled.
- In eukaryotes there are three RNA polymerases in the nucleus, and capping and tailing is required to form mature RNA.

GENETIC CODE

- Genetic code should be triplet.
- 61 codons code for amino acids and 3 codons are stop codons.
- The code is degenerate, contiguous and universal.
- AUG has dual function. It codes for methionine and act as initiator codon.
- UAA, UAG and UGA- Stop/terminator codons.

tRNA-Adapter Molecule

- Francis Crick postulated the presence of an adapter molecule that would read the code and bind to specific amino acid.
- tRNA has an anti-codon loop that has bases complementary to the code and it also has an amino acid acceptor end to which it binds to amino acids, tRNAs are specific for each amino acid.
- For initiation, there is a specific tRNA that is called initiator tRNA. There are no tRNAs for stop codons.
- Secondary structure of tRNA looks like a cloverleaf, though the actual structure is a compact molecule which looks like inverted L.

TRANSLATION

- Translation refers to the process of polymerisation of amino acids to form a polypeptide. The order and sequence of amino acids are defined by the sequence of bases in the mRNA.
- A translational unit in mRNA is flanked by a start condon (AUG) and the stop codon.
- Untranslated regions, (UTRs) are present at both 5'-end (before start codon)and at 3'-end (after stop codons). UTRs are required for efficient translation.
- The ribosome moves from codon to codon along the mRNA. Amino acids are added one by one and translated into polypeptide sequences.

REGULATION OF GENE EXPRESSION

- Gene expression results in formation of a polypeptide. It can be regulated at several levels. In eukaryotes, the regulation could be exerted at
  1. Transcriptional level (Formation of primary transcript).
  2. Processing level (Regulation of splicing).
  3. Transport of mRNA from nucleus to cytoplasm.
  4. Translational level.

Lac OPERON

- Francois Jacob and Jacque Monod were the first to elucidate a transcriptionally regulated system, the lac operon (lac refers to lactose), a polycistronic structural gene regulated by a common promoter and regulatory gene called operon.

Lac operon consists of :

- One regulatroy gene i.e., i (i refers to inhibitor) codes for repressor, three structural genes (z, y and a), z-for β-galactosidase (β-gal), y-for permease and gene a codes for transacetylase.
  ™Lactose is the substrate of β-galactosidase and it regulates switching on/off of operon, so called inducer.
  ™lac operon is negative regulated and is inducible.

HUMAN GENOME PROJECT - (HGP)

- Launched in 1990, a 13 year project was co-ordinated by U.S. department of energy and National Institute of Health, Welcome trust (UK), Japan, France, Germany, China participated. It was completed in 2003.
- Human genome has approximately 3 × 109 bp and the cost of sequencing in the beginning was US $3 per bp, i.e. 9 billion US dollars. HGP lead to the rapid development of a new area in biology called bioinformatics.
- Many non-human model organisms like bacteria, Yeast, Caenorhabditis elegans, Drosophila, plant (rice and Arabidopsis) have also been sequenced.

METHODOLOGIES

- Expressed sequence tags (ESTs) identification: Focussed on identifying all genes that expressed as RNA.
- Sequence annotation: sequencing the whole genome containing coding and non-coding sequences, needing vectors like BAC (Bacterial artificial chromosomes) and YAC (Yeast Artificial Chromosomes).

DNA FINGERPRINTING

- 99.9% base sequence among humans is same. 0.1% differences in sequence of DNA make every individual unique in their phenotype.
- It involves identifying difference in repetitive DNA, a small stretch of DNA repeated many times, called satellite DNA.
- Depending on base composition (A:Tor G:C rich), length of segment and number of repetitive units, the satellite DNA is classified into micro-statellites and mini satellites. They do not code for any proteins. They form large portion of human genome and show high degree of polymorphism and form the basis of DNA fingerprinting.
- Polymorphisms are inheritable from parent to child so DNA fingerprinting solves paternity disputes.
- The technique of DNA finger printing was initially developed by Alec Jeffreys.

Steps = Isolating the DNA (I)
↓
Digestion by restriction endonuclease (II)
↓
Separating of DNA fragments by electrophoresis (III)
↓
Blotting on nitrocellulose or Nylon membrane(IV).
↓
Hybridisation using labelled VNTR probes (V).
↓
Detection of hybridised DNA fragments by autoradiography (VI)

Significance

- VNTR are called mini-satellite, a small DNA sequence arranged tandomly in many copies. The size of VNTR varies from 0.1 to 20 kb. After hybridisation with VNTR probe, the autoradiogram gives many bands of differing sizes. These bands give a characteristic pattern for an individual DNA. It differs from individual to individual in a population except in monozygotic twins.
- The sensitivity of the technique has been increased by use of polymerase chain reaction (PCR).
- DNA fingerprinting has much wider application in determining population and genetic diversities. Currently, many different probes are used to generate DNA fingerprints.