

Human DNA

3.3×10^9 bp of nucleotides in the DNA of haploid chromosomes

Nucleotides

N-glycosidic linkage-

Nitrogenous base is attached to OH of 1'C of deoxyribose or ribose sugar

Phosphoester linkage-

Phosphate group attached to OH of 5'C of deoxyribose or ribose sugar

Polynucleotide

Two nucleotides are joined by 3'-5' phosphodiester linkage. OH of 3'C of pentose sugar of one nucleotide is attached to the phosphate group of 5'C of another nucleotide

5' end of
polynucleotides

Free Phosphate
group at 5'C of the
pentose sugar

3' end of
polynucleotides

Free OH group at
3'C of the pentose
sugar


Friedrich Miescher

Identified DNA in 1869

Named it 'Nuclein'

Present in nucleus and
acidic

Maurice Wilkins and
Rosalind Franklin

Obtained DNA images from
X-ray crystallography

James Watson and
Francis Crick

Proposed the double-
helix model of DNA in
1953

Erwin Chargaff

Elucidated that the DNA
contains equal amounts
of adenine and thymine
and in the same way
guanine and cytosine

DNA double helix

Antiparallel strands, coiled in a right-handed fashion

Backbone- sugar-phosphate

Bases are bonded by hydrogen bonds and projected inside

Adenine bonds to thymine by two hydrogen bonds

Guanine bonds to cytosine by three hydrogen bonds

Pitch- 3.4 nm and 10 bp in each turn

Central Dogma

Proposed by Francis Crick in 1958

The flow of genetic information from DNA → RNA → Protein

Nucleoid

Found in prokaryotes

The region where DNA is concentrated within a cell associated with positively charged proteins

Histones

Positively charged basic proteins, rich in lysine and arginine

eight molecules of histones associated with DNA to form a nucleosome

Nucleosome

DNA associated with histones octamer

200 bp of DNA in each nucleosome

Appear as beads on chromatin threads

Euchromatin

Loosely packed and lightly stained part of chromatin

Shows active transcription

Heterochromatin

Densely packed and darkly stained part of chromatin

Inactive for transcription

Transforming principle

Frederick Griffith in 1928

He concluded that there was some transforming principle in the heat-killed S-strain, which transformed R strain

Streptococcus pneumoniae to virulent

Avery, MacLeod and McCarty

Demonstrated that the DNA caused the bacterial transformation

They showed that proteases and RNAases did not affect transformation, whereas DNAase inhibit transformation

Alfred Hershey and Martha Chase

Unequivocal proof of DNA as genetic material in 1952

They worked on bacteriophages

Radioactive ^{35}S labelled- protein capsule

Radioactive ^{32}P labelled- DNA

RNA as a genetic material

Present in many viruses known as retrovirus

E.g. TMV, QB bacteriophage, etc.

ds RNA- reovirus, etc.

Semiconservative DNA replication

Proposed by Watson and Crick

Experimentally proved by Matthew Meselson and Franklin Stahl in 1958 using ^{15}N isotope in the growing medium for *E.coli*

Taylor and colleagues worked on *Vicia faba* using radioactive thymidine

DNA-dependent DNA polymerase

Main enzyme for replication

Polymerisation in $5' \rightarrow 3'$ direction

DNA ligase

Join the DNA strand, which is synthesised discontinuously during replication

DNA helicase

Unwind DNA at the origin of replication forming replication fork

Transcription

Synthesis of RNA from DNA
Template strand- 3'→5' DNA
Coding strand- 5'→3' DNA, has the same sequence as newly synthesised RNA strand

DNA-dependent RNA polymerase

Catalyses transcription
Polymerisation in 5'→3' direction

Transcription unit

Reference point with respect to the coding strand ($5' \rightarrow 3'$, DNA)

Promoter- present at 5' end of a structural gene, RNA polymerase binds here

Terminator- at 3' end of the coding strand

Cistron

A segment of DNA coding for polypeptide

Monocistronic- eukaryotes

Polycistronic- prokaryotes

Exons

Coding sequence of DNA expressed in mature and processed RNA

Introns

Interrupted sequence which are spliced in mature RNA

Translation

Formation of polypeptide from RNA

mRNA- template for protein synthesis

tRNA- adapter molecule, brings amino acids and reads codon on mRNA

rRNA- structural and catalytic role

Eukaryotic transcription

RNA polymerase I - 28S, 18S, 5.8S rRNA

RNA polymerase II - hnRNA (mRNA precursor)

RNA polymerase III - tRNA, 5S rRNA, snRNA

hnRNA processing

Splicing - removal of introns and joining together of exons

Capping - addition of methyl guanosine triphosphate at 5' end

Tailing - addition of adenylate residues (200-300) at 3' end

Marshall Nirenberg

Deciphered first the 64 triplet codons for 20 amino acids present in protein

Start codon

AUG

Codes for Methionine

Stop codons

UAA, UAG, UGA

They do not code for any amino acids

tRNA

Structure- 2° structure like clover leaf, 3° like inverted L

Anticodon loop- complementary mRNA codon for specific codon

Acceptor arm- binds to amino acid

DHU loop, T ψ C loop and variable loop

Function- Adapter molecule, brings amino acid for translation and reads codon

Occurs in cytoplasm

Translation

First step is charging of tRNA, aminoacylation or binding of tRNA to amino acid

Peptide bond formation takes place between the amino acid of growing chain and newly brought amino acid in two sites present in large subunit of ribosomes

Ribozyme

Catalytic RNA

28S rRNA in bacteria

Untranslated regions (UTR)

Regions on mRNA, which are not translated

Present on both 5' and 3' ends

Required for efficient translation

Francois Jacob and Jacque Monod

First elucidated transcriptionally regulated system in *lac* operon

Operon

Present in prokaryotes

Contains multiple genes, which are regulated by a promoter and an operator

E.g. *lac* operon, *val* operon, *his*, *ara*, *trp*, operon, etc.

lac operon

i gene- repressor (negative regulation)

Structural genes-

z - beta-galactosidase (β -gal)

y - permease

a - transacetylase

Inducer - lactose or allolactose
Started in 1990, Completed in 2003

Human genome project (HGP)

3×10^9 base pairs (haploid)

20,000-25,000 genes

Largest gene- Dystrophin gene of the X chromosome (2.4 million bp)

Smallest gene- SRF/TDY gene of the Y chromosome (14 bp)

SNPs

Single nucleotide polymorphism

Single base differences exist at 1.4 million places in the human genome

Sequence annotation

Assigning different regions of genome according to function

Expressed Sequence Tags (ESTs)

Identifying all the genes, which are expressed as RNAs

DNA Fingerprinting

Developed by Sir Alec Jeffreys

Involves identifying of repeated DNA sequences

Satellite DNA forms smaller peaks and shows a high degree of polymorphism

VNTR

Variable number of tandem repeats

Mini-satellite

Number and position differs from person to person

Flanked by restriction sites

Southern blotting

Transfer of electrophoresis separated DNA to nitrocellulose or nylon membrane and detection by probe hybridisation

Used to detect DNA sample in blood or tissue