DNA as the Genetic Material

In the early studies of the heredity it was evident that the phenotypic characteristics are nothing but the expression of the information stored in the hereditary material i.e. chromosome because –

1. Only chromosomes are sent to the next generation by parents
2. Chromosomes carry genes
3. They has fixed number and morphology in a specific organism
4. Any change in the chromosome resulted in to the change in the organism

As it was clarified that the morphology of the organism is determined by the chromosomes then it was certain question that what the nature of the material controlling the phenotypes is? The chemical analysis of chromosomes revealed that they are made up of proteins (Histones) and nucleic acid (Deoxyribonucleic Acid: DNA and Ribonucleic Acid: RNA). Therefore, it was the common thought that genes controlling phenotypic expression are either protein or nucleic acid in nature. Earlier molecular biologists have considered proteins as genes because they are more complex than the DNA or RNA and proteins are responsible for the specific trait (character) of any organism.

The controversy about the genetic material was arose after 1949, when A. Mirsky and H. Ris reported that all the organism has the same amount of the DNA whereas different proteins in different types of the cells of the same individual. This evoked a thought that as proteins are different and DNA is unique and same all over the cells of an organism, the genetic material must be DNA and not the protein.

To clarify the exact nature of the genetic material (protein or DNA), variety of experiments were performed by researchers. Some of the milestone contributions are –

1. Transformation Experiment by Griffith
2. Phage Labeling Experiment by Hershey and Chase
3. Bacterial Conjugation Experiment by Laderberg and Tatum
4. Exception to DNA as a genetic material by Conrat and Singer

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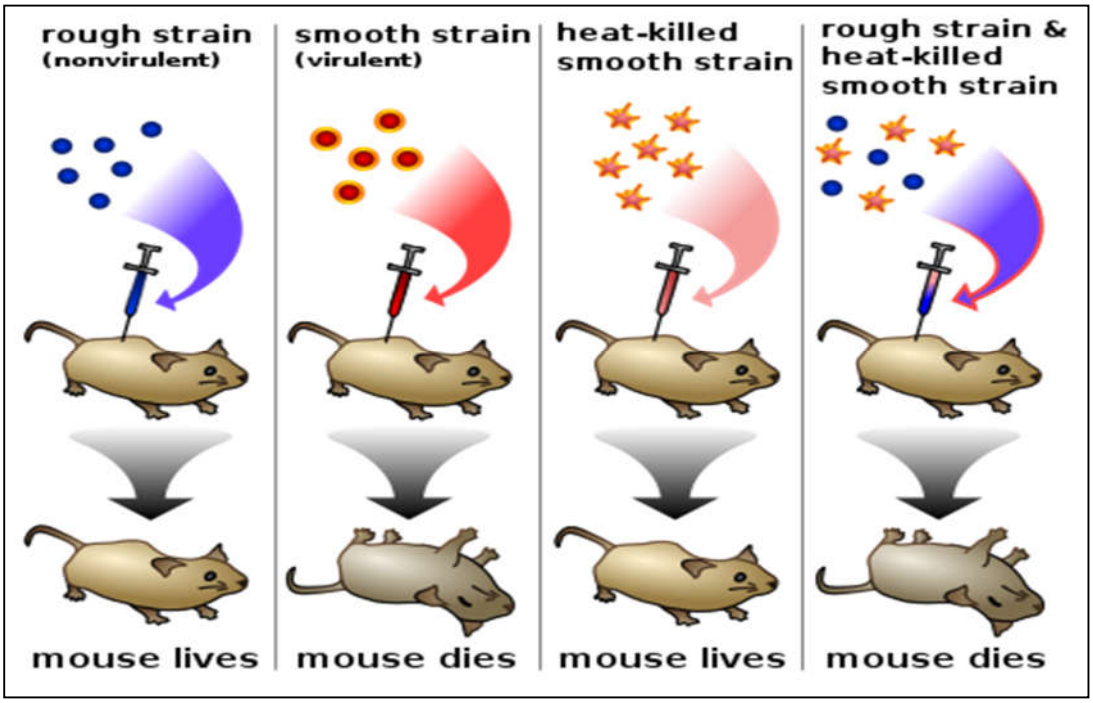
1. Transformation Experiment by Griffith

Griffith's experiment, conducted in 1928 by Frederick Griffith, was one of the first experiments suggesting that bacteria are capable of transferring genetic information through a process known as transformation

Griffith used two strains of *Pneumococcus* (which infects mice), a type III-S (smooth) and type II-R (rough) strain. The III-S strain covers itself with a polysaccharide capsule that protects it from the host's immune system, resulting in the death of the host, while the II-R strain doesn't have that protective capsule and is defeated by the host's immune system. A German bacteriologist, Fred Neufeld, had discovered the three pneumococcal types (Types I, II, and III) and discovered the Quellung reaction to identify them in vitro. Until Griffith's experiment, bacteriologists believed that the types were fixed and unchangeable, from one generation to another.

In this experiment, bacteria from the III-S strain were killed by heat, and their remains were added to II-R strain bacteria. While neither alone harmed the mice, the combination was able to kill its host. Griffith was also able to isolate both live II-R and live III-S strains of *pneumococcus* from the blood of these dead mice. Griffith concluded that the type II-R had been "transformed" into the lethal III-S strain by a "transforming principle" that was somehow part of the dead III-S strain bacteria.



Today, we know that the "transforming principle" Griffith observed was the DNA of the III-S strain bacteria. While the bacteria had been killed, the DNA had survived the heating process and was taken up by the II-R strain bacteria. The III-S strain DNA contains the genes that form the protective polysaccharide capsule. Equipped with this gene, the former II-R strain bacteria were now protected from the host's immune system and could kill the host. The exact nature of the transforming principle (DNA) was verified in the experiments done by Avery, McLeod and McCarty and by Hershey and Chase.

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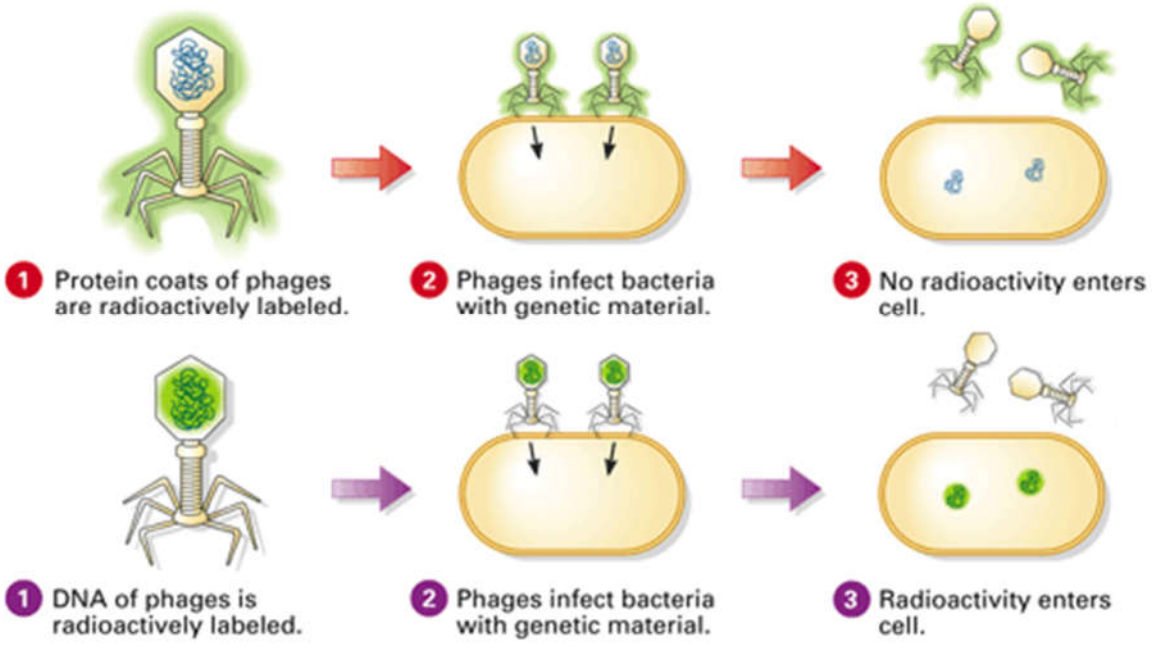
2. Phage Labeling Experiment by Hershey and Chase

The **Hershey-Chase experiments** were a series of experiments conducted in 1952 by Alfred Hershey and Martha Chase, confirming that DNA was the genetic material, which had first been demonstrated in the 1944 Avery-MacLeod-McCarty experiment. While DNA had been known to biologists since 1869, most assumed at the time that proteins carried the information for inheritance.

Hershey and Chase conducted their experiments on the T2 phage, a virus whose structure had recently been shown by electron microscopy. The phage consists only of a protein shell containing its genetic material. The phage infects a bacterium by attaching to its outer membrane and injecting its genetic material, causing the bacterium's genetic machinery to produce more viruses, leaving its empty shell attached to the bacterium.



In a first experiment, they labeled the DNA of phages with radioactive Phosphorus-32 (the element phosphorus is present in DNA but not present in any of the 20 amino acids from which proteins are made). They allowed the phages to infect *E. coli*, then removed the protein shells from the infected cells with a blender and separated the cells and viral coats by using a centrifuge. They found that the radioactive tracer was visible only in the pellet of bacterial cells and not in the supernatant containing the protein shells.

In a second experiment, they labeled the phages with radioactive Sulfur-35 (Sulfur is present in the amino acids cysteine and methionine, but not in DNA). After separation, the radioactive tracer then was found in the protein shells, but not in the infected bacteria, supporting the hypothesis that the genetic material which infects the bacteria is DNA.

Clearly, this experiment has proved that the genetic material was DNA and not protein Hershey shared the 1969 Nobel Prize in Physiology or Medicine for his “discoveries concerning the genetic structure of viruses.”

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The Structure of DNA

In 1869, a Swiss doctor, Freidrich Miescher isolated nuclei from pus cell in waste surgical bandages. He found that nuclei contained a novel phosphorus bearing substance, which he named as nuclein. Nuclein is mostly chromatin, a complex of deoxyribonucleic acid (DNA) and chromosomal proteins. A range of experimental techniques mostly cytochemistry suggested that either DNA or protein or both must be the genetic material. The characteristics of all living organisms, including humans, are essentially determined by information contained within **DNA** that they inherit from their parents. Fisher – 1880 suggested only Purine as component of DNA. But Levene – 1910, discovered that DNA is Composed of Four Different Ribonucleotides (A,T,G,C). John Buchanan & GreenBerg in 1950 clarified the linkages in single nucleotide. The molecular structure of DNA can be imagined as a zipper with each tooth represented by one of four letters **(A, C, G,** or **T)** and with opposite teeth forming one of two pairs, either AT or GC. The information contained in DNA is the sequence of letters along the zipper. For example, the sequence ACGCT represents different information than the sequence AGTCC in the same way that the word "POST" has a different meaning from "STOP" or "POTS," even though they use the same letters. The traits of a human being are the result of information contained in the DNA code. Living organisms that look different or have different characteristics also have different DNA sequences. The more different the organisms, the more different are the DNA sequences.

In 1951, the then 23-year old biologist James Watson traveled from the United States to work with Francis Crick, an English physicist at the University of Cambridge. Crick was already using the process of X-ray crystallography to study the structure of protein molecules. Together, Watson and Crick used X-ray crystallography data, produced by Rosalind Franklin and Maurice Wilkins at King's College in London, to decipher DNA's structure.

This is what they already knew from the work of many scientists, about the DNA molecule:

1. DNA is made up of subunits which scientists called nucleotides.
2. Each nucleotide is made up of a sugar, a phosphate and a base.
3. There are 4 different bases in a DNA molecule:

   adenine (a purine)

   cytosine (a pyrimidine)

   guanine (a purine)

   thymine (a pyrimidine)

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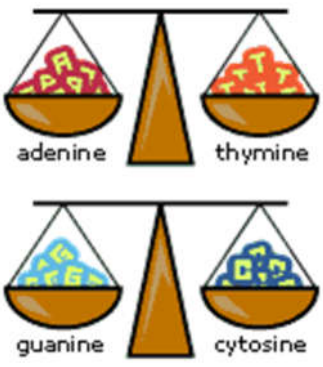
4. The number of purine bases equals the number of pyrimidine bases
5. The number of adenine bases equals the number of thymine bases
6. The number of guanine bases equals the number of cytosine bases
7. The basic structure of the DNA molecule is helical, with the bases being stacked on top of each other

Working with nucleotide models made of wire, Watson and Crick attempted to put together the puzzle of DNA structure in such a way that their model would account for the variety of facts that they knew described the molecule. Once satisfied with their model, they published their hypothesis, entitled "Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid" in the British journal Nature (April 25, 1953. volume 171:737-738.) It is interesting to note that this paper has been cited over 800 times since its first appearance!

Studies of DNA had revealed much about its chemical and physical nature, but Watson believed that its function could not be understood fully until its structure was known. Crick and Watson used the results from previous studies and X-ray diffraction data from Maurice Wilkins and Rosalind Franklin to help them determine DNA's molecular structure. By 1953 they had built a model which incorporated all known features of DNA, and proposed the double helix structure which is commonly referred to as the Watson-Crick model of DNA. Crick, Watson and Wilkins were awarded a Nobel prize for this work in 1962.

Watson Crick Model of DNA - Watson and Crick proposed the DNA model by using all the information that was available at that time. They used the data obtained from experiments carried out on DNA by Chargaff, and Maurice Wilkins and Rosalind Franklin.



Chargaff Rule - The base-pairing mystery had been partly solved by the biochemist Erwin Chargoff some years earlier. In 1949 he showed that even though different organisms have different amounts of DNA, the amount of adenine always equals the amount of thymine. The same goes for the pair guanine and cytosine. For example, human DNA contains about 30 percent each of adenine and thymine, and 20 percent each of guanine and cytosine.

With this information at hand Watson was able to figure out the pairing rules. On the 21st of February 1953 he had the key insight, when he saw that the adenine-thymine bond was exactly as long as the cytosine-guanine bond. If the bases were paired in this way, each rung

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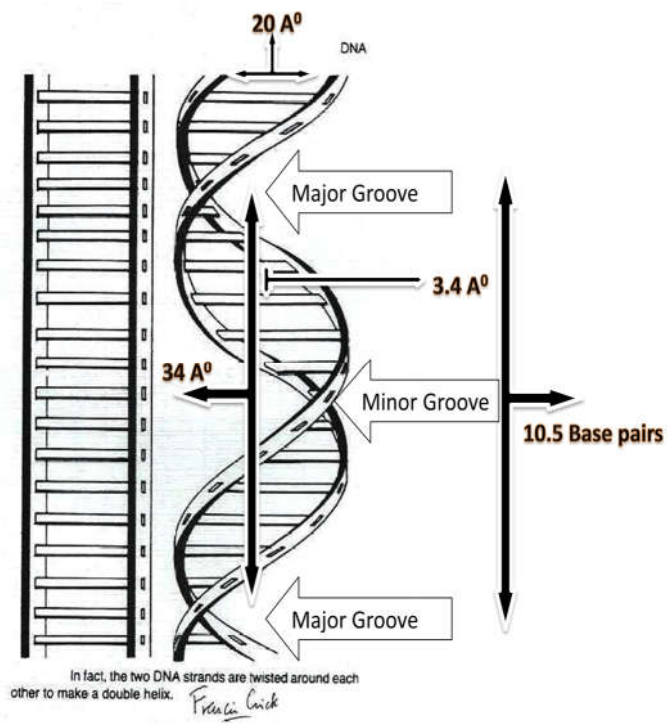
of the twisted ladder in the helix would be of equal length, and the sugar-phosphate backbone would be smooth.

Chargaff rule states that the number of purines is always equal to the number of pyrimidines in a given DNA. The relationship is that the number of adenine residue equals the number of thymine, and the number of guanines equals the number of cytosine that is A = T and G = C. Thus we can say A + G = C + T.

X-ray diffraction studies X-ray diffraction studies by M. Wilkins and R. Franklin suggested that DNA could be a helix with two regular periodicities of 3.4 A^0 and 34 A^0 along the axis of the molecules.

Based upon the above facts, Watson and Crick proposed the famous DNA structure model. The important features of this model are:

1. The DNA molecule is a double helix with single polynucleotides (phosphates, sugar, base) running in opposite directions
2. The double helix is right-handed. This means that if the double helix is a spiral staircase that you were climbing up, the base would be on your right hand side.
3. The double helix has two different grooves. The helix is not absolutely regular. A major (-22A) and a minor (-12A) groove can be distinguished. This feature is important in the interaction between the double helix and the proteins involved in DNA replication and in expression.



4. The nitrogenous bases are stacked towards the inside of the helix. The experimental evidence also indicated that the sugar-phosphate backbone of the molecules is on the outside, with the bases inside the helix.

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5. Bases of the two polynucleotides interact by hydrogen bonding. This is the explanation of Chargaff's base pairs. An adenine residue in one of the polynucleotides is always adjacent to a thymine in the other strand; similarly guanine is always adjacent to cytosine. These two pairs of bases, and no other combinations are able to form hydrogen bonds between each other. These hydrogen bonds are the only attractive forces between the two polynucleotides of the double helix and serve to hold the structure together.
6. Ten base pairs occur per turn of the helix. The double helix executes a turn every ten base pairs (abbreviated as 10 bp). The height or pitch of the helix is 34 A^0. The bases are stacked one on top of the other like a pile of plates. The space between the two base pairs is 3.4 A^0 and has an angle of 36°.
7. The diameter of the helix is 20 A^0.

Chemical Composition of DNA

Chemical analyses by scientists revealed the general chemical composition of nucleic acids (DNA and RNA): they are composed of nucleotides. A nucleotide consists of a phosphate group, a five-carbon sugar (deoxyribose in DNA and ribose in RNA), and a nitrogenous base bonded together.

Each nucleotide in a DNA molecule has one of four nitrogenous bases: adenine, guanine, thymine, and cytosine. The first two are called purine bases because their structure consists of two rings of atoms. The latter two are known as pyrimidine bases, since they have a single ring of atoms. RNA has three of the same nucleotides, but instead of thymine, RNA has uracil, another pyrimidine base. RNA will come back later, but for the remainder of this section and the next few sections, we will discuss only DNA.

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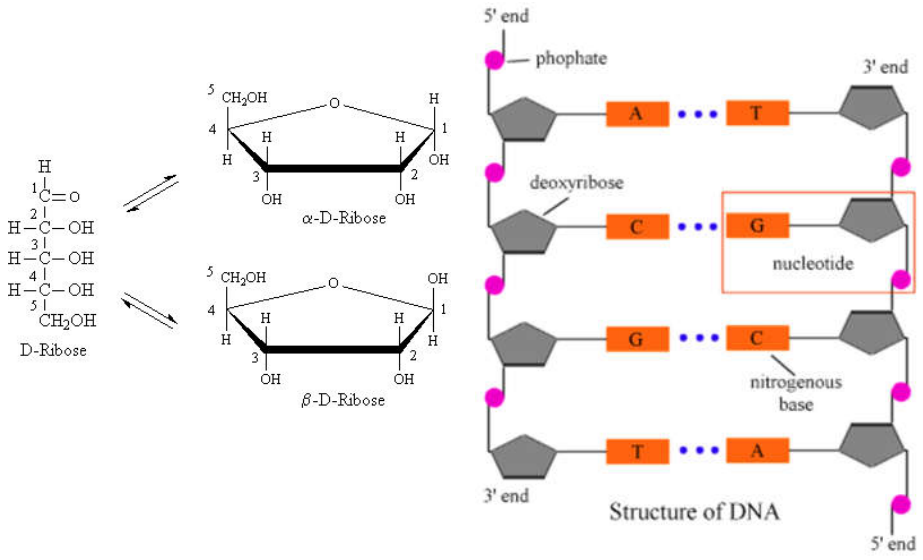

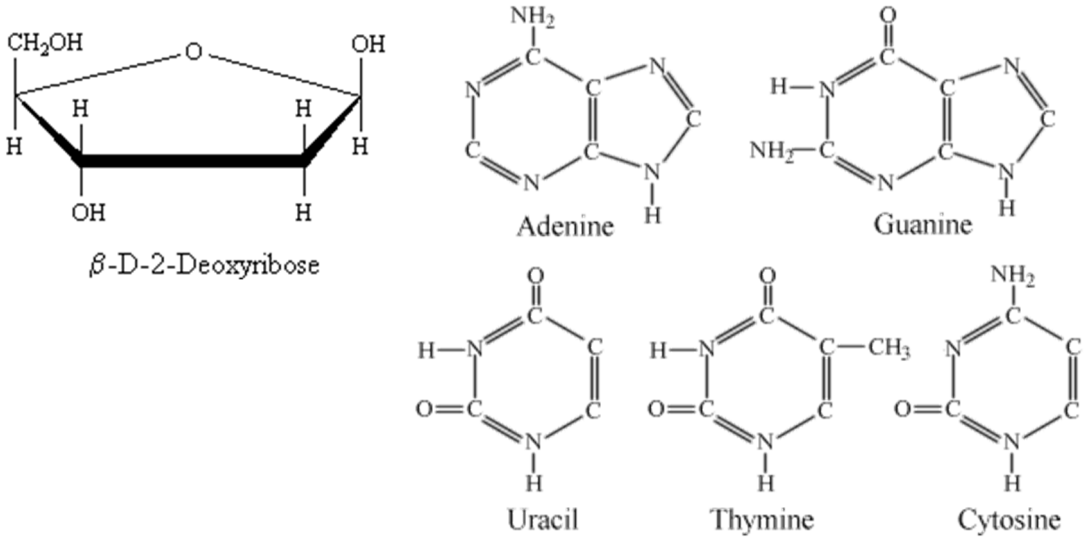

Pentose Sugar of DNA –

In DNA, the sugar component of the nucleotide is a pentose called 2 '-deoxyribose base. Pentose sugars contain five carbon atoms and can exist in two forms (i) straight chain or Fischer structure and (ii) the ring or Haworth structure. In DNA, pentose sugar exists in ring form and is 2-deoxyribose type.

The name 2-deoxyribose indicates that the standard ribose structure has been altered by replacement of the hydroxyl group (-OH) attached to carbon atom number 2' with a hydrogen group (-H)**.** The carbon atoms are always numbered in the same way with the carbon of the carbonyl group (-C=O), occurring at one end of the chain form numbered 1. It is important to remember the numbering of the carbons because it is used to indicate at which positions on the sugar other components of the nucleotide are attached.

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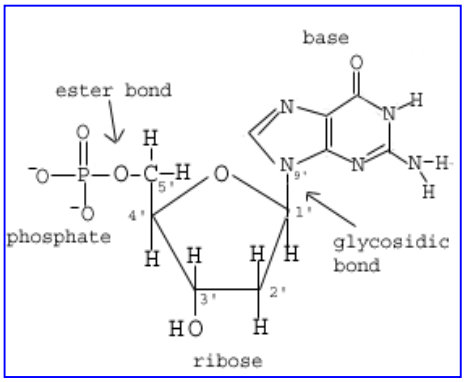
Nitrogen Bases of DNA - A **nitrogenous base** is an organic compound that owes its property as a base to the lone pair of electrons of a nitrogen atom. Notable nitrogenous bases include pyrimidine and purine bases, the nucleobases (building blocks of DNA and RNA): adenine, guanine, thymine, cytosine, and uracil. Adenine and thymine/guanine and cytosine are complementary to each other. Adenine pairs only with thymine, and cytosine pairs only with guanine. **However, in RNA, adenine pairs with uracil, replacing thymine in the RNA strand.** Adenine and guanine are purines, and cytosine and thymine are pyrimidines. The nitrogenous bases hydrogen bond between opposing DNA strands to form the rungs of the "twisted ladder" or double mount helix of DNA. It can also be noted that Nitrogenous bases are more likely to be modified in RNA than DNA, and the variety of modifications is larger.

These are rather complex single or double ring structures containing nitrogen and are attached to the carbon of the sugar.

Nitrogen bases are of two types based on the number of rings –

Purines

Purines are double ringed nitrogenous bases which are linked to sugar by using nitrogen present at 9' -position. Adenine and guanine come under this group.



Pyrimidines

Pyrimidines are single ringed nitrogen bases which are linked to sugar by using nitrogen present at 1' -position. Thymine and cytosine come under this group.

The scientific names according to IUPAC nomenclature system are as follows -

1. Adenine (6-aminopurine)
2. Guanine (2-amino 6-oxypurine)
3. Cytosine (4-amino 2-oxypyrimidine)
4. Thymine (2,4-oxy S-methyl pyrimidine)
5. Uracil (2,4-oxypyrimidine)

Phosphoric Acid Component of DNA - A molecule comprising the sugar joined to a base is called a nucleoside. This is converted into a nucleotide by attachment of a phosphoric acid group to the 5 ' -carbon of the sugar. Phosphate group is attached by phosphodiester bond. An

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ester is an organic compound formed from an alcohol and acid. In the case of a nucleotide, the alcohol group is the 5 ' hydroxyl of the sugar and the acid is phosphoric acid.

Hence we call the ester as phosphodiester, as a single phosphoric acid is linked to two sugars present on different nucleotides. Thus, the bond is called as phosphodiester bond. To be precise, we should call this bond as 3'-5 ' phosphodiester bond so that there is no confusion about which carbon atoms in the sugar participate in the bond. Up to three individual phosphate groups can be attached in series giving a nucleotide monophosphate (NMP), nucleotide diphosphate (NDP) and nucleotide triphosphate (NTP). The individual phosphate groups are designated α, β and γ with the α phosphate being the one attached directly to the sugar.

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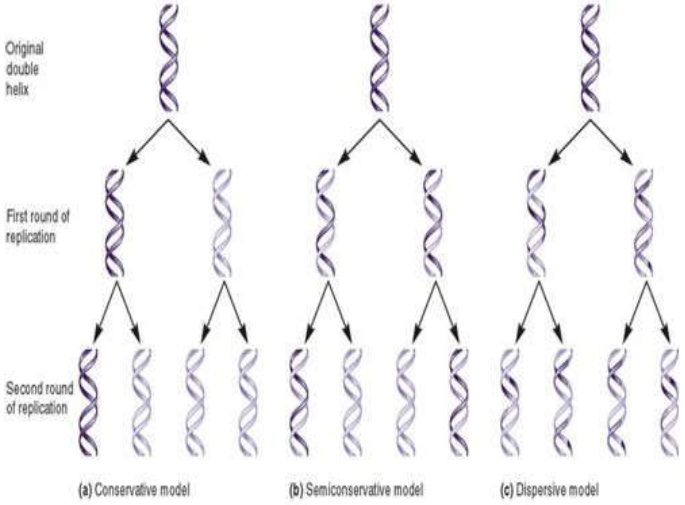
DNA REPLICATION

Replication is the production of copies of the DNA (Genetic Material) during cell division. The cell division is the very important growth process requires the duplication of the genetic material to transfer it to the next generation of the cell with great accuracy.

The in vitro study of DNA replication was pioneered by Arthur Kornberg for which he honoured by Nobel Prize in 1959. Watson and Crick hinted that the replication of double helix could takes place to form a new DNA.

In the late 1950s, three different modes were proposed for the replication of DNA



Conservative model

Both parental strands stay together after DNA replication

Semiconservative model

When the double stranded DNA helix was replicated, each of the two double stranded DNA helices consisted of one strand coming from the original helix and one newly synthesized.

Dispersive model: Parental and daughter DNA are interspersed in both strands following replication

The widely accepted mode of replication is Semiconservative mode of replication. The Meselson–Stahl experiment was an experiment by Matthew Meselson and Franklin Stahl in 1958 which supported the hypothesis that DNA replication was semiconservative. It has been called "the most beautiful experiment in biology.

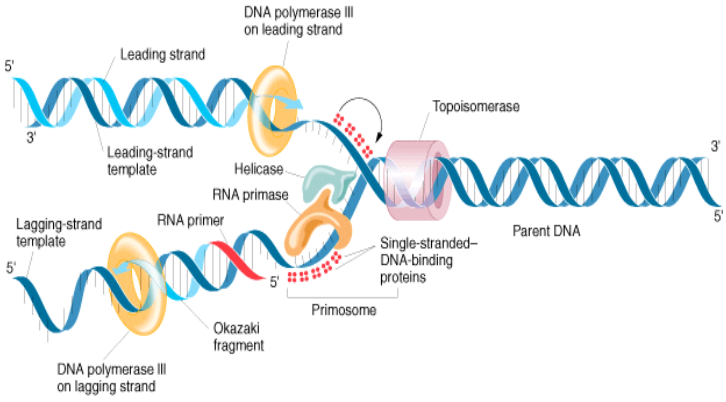
DNA REPLICATION MECHANISM

Auto radiographic evidences leads us to believe that replication occurs simultaneously on both the strands

- Continuous Replication (Leading)
- Discontinuous Replication (Lagging)

Steps in replication –

I. Unwinding DNA
II. Primer Formation
III. Elongation
IV. Proofreading
V. Primer Removal
VI. Ligation



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The minimal activities required at a replication fork are the following: a DNA helicase must unwind the parental template; a primase must synthesize short oligoribonucleotides that serve as primer for synthesis of the Okazaki fragments on the lagging strand, a single-stranded DNA-binding protein that coats the single-stranded lagging-strand template and interacts with other replication proteins; and a DNA polymerase must synthesize the nascent leading and lagging strands.

I. Unwinding DNA

As double stranded DNA is coiled and forming a helical structure, it has to be relaxed or opened to copy it accurately. The unwinding of DNA is carried out by the enzyme **Helicase**, which breaks the hydrogen bonds between the strands and separate them. This generates positive supercoiling ahead of each replication fork. **Topoisomerase**, is the enzyme which relaxed strand by removing twist (supercoiling) in it. **Single-strand binding proteins** (SSBs) bind to the separated DNA strands to keep them apart.

II. Primer Formation

DNA polymerase is the enzyme which catalyzes the formation of the DNA strand but this enzyme cannot initiate the polymerization of DNA. Therefore, the initiation of new strand sysnthesis is carried out by the enzyme called as **RNA primase (DNA dependent RNA polymerase).** The primase enzyme polymerize the RNA strand on both the DNA strand having direction of 3' to 5' and 5' to 3'. A short (10 to 12 nucleotides) RNA primers are synthesized.

III. Elongation

Once the RNA primer initiated the replication, the DNA polymerase takes over the charge of polymerization and it polymerize DNA for both the strands. The strands having direction of 3' to 5' and 5' to 3' has independent mechanisms. They are –

- **Continuous Replication (Leading)**

Parental strand with 3' to 5' and new strand with 5' to 3' has continuous formation of the new strand called as leading strand. The DNA polymerase III enzyme polymerizes nucleotides by adding new nucleotide to the 3' end of the previous nucleotide without any break.

- **Discontinuous Replication (Lagging)**

DNA polymerase III has limitation that, it can add or polymerize DNA in the direction of 5' to 3'. Therefore, the parental strand with direction of 5' to 3' cannot continuously polymerized. It will use only 5' to 3' direction to synthesize DNA. Hence, when DNA replication fork moved forward in the replication of the leading strand for approximately 30 base pairs, it will start DNA synthesis in the same direction as that of the leading strand i.e. 5' to 3'

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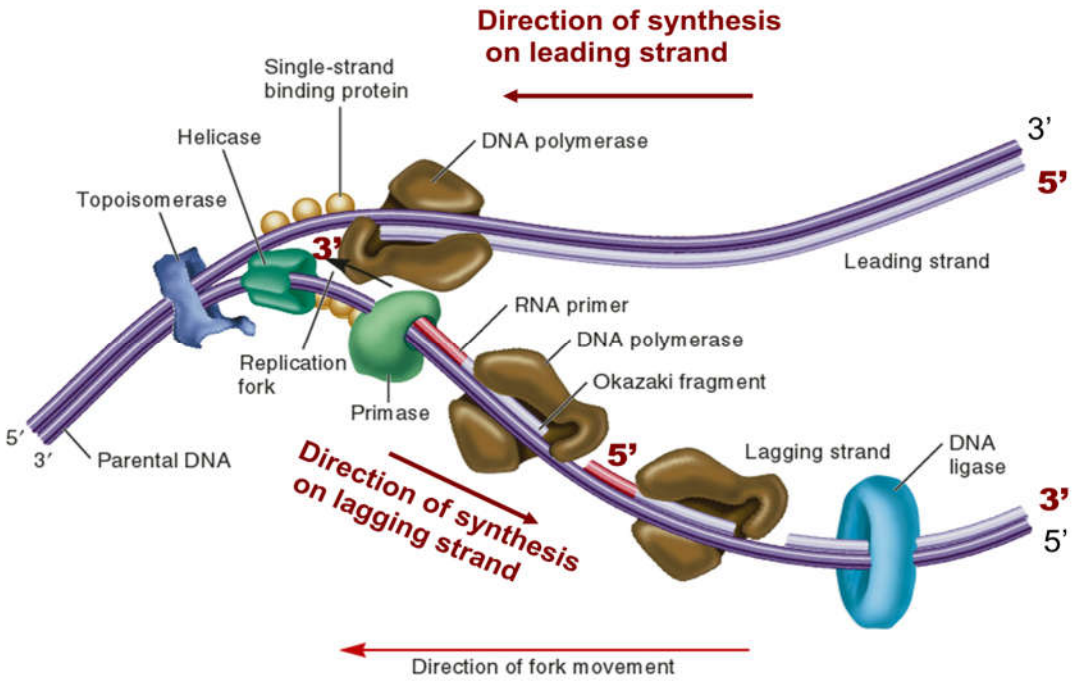

IV. Proofreading

If any mismatched base is inserted in the new strand by mistake, **the DNA polymerase I worked as exonuclease enzyme to remove the incorrect base**. In the replication mechanism the chance of one error per addition of 10 million nucleotides may be possible. This error is also removed in the proofreading mechanism.

V. Primer Removal

After the complete polymerization (synthesis) of DNA is carried out the RNA strand, which was synthesized in the beginning of the replication has to be removed to have the complete new strand of DNA. Again the DNA polymerase III comes in action and removes the RNA strand and synthesize the DNA strand in place of removed RNA.

VI. Ligation

The lagging strand used to be synthesized in patches and it creates gaps in the **Okazaki Fragments.** These gaps are sealed by enzyme DNA ligase.

Because of these events, one parental strand gives two daughter strands containing one new and one parental strand.

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NUCLEOSOME MODEL

The organization of the genome is very important task for all the living organisms. The genomic DNA is huge in size and length e.g. Human cell contain ~3 billion BP genome stretching upto 1 meter in length if aligned strait. From the person with average weight of 60 Kg with ~60 million cells if entire DNA combined and stretched then it covers distance of 2X10 ^{11} Km. which comparable to distance between Sun – Earth i.e. 1.5X10^{8} Km. To accommodate this gigantic DNA nature has adopted the DNA packaging process in which DNA is super coiled (Coiling of coil) to form beaded string model of chromatin. Each DNA strand wraps around groups of small protein molecules called **histones**, forming a series of bead-like structures, called **nucleosomes**, connected by the DNA strand (as illustrated in Figure). Under the microscope, uncondensed chromatin has a "beads on a string" appearance. The string of nucleosomes, already compacted by a factor of six, is then coiled into an even denser structure known as a **solenoid** that compacts the DNA by a factor of 40. The solenoid structure then coils to form a hollow tube. This complex compression and structuring of DNA serves several functions. The overall negative charge of the DNA is neutralized by the positive charge of the histone molecules, the DNA takes up much less space, and inactive DNA can be folded into inaccessible locations until it is needed.

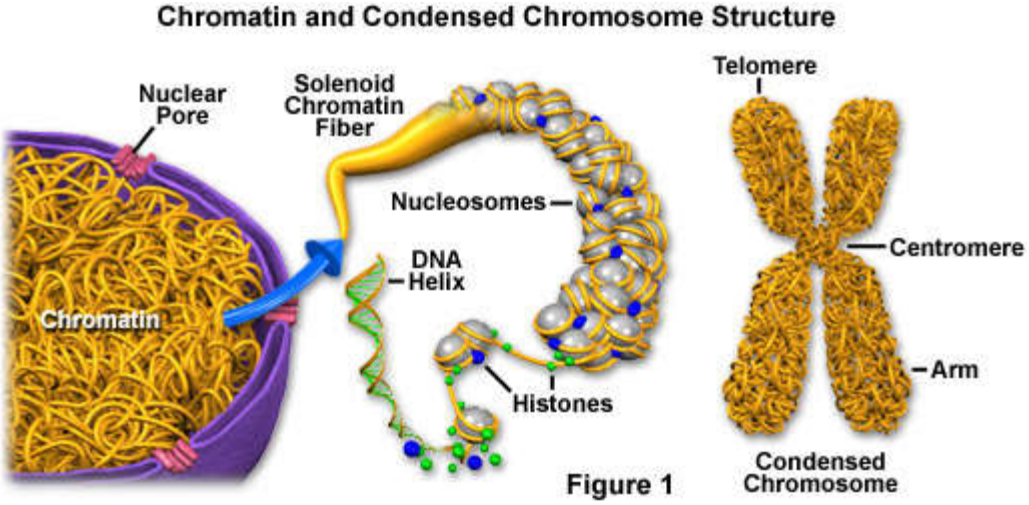

Figure 1

Packed inside the nucleus of every human cell is nearly 6 feet of DNA, which is subdivided into 46 individual molecules, one for each chromosome and each about 1.5 inches long. Collecting all this material into a microscopic cell nucleus is an extraordinary achievement of packaging. For DNA to function when necessary, it can't be haphazardly crammed into the nucleus or simply wound up like a ball of string. Consequently, during interphase, DNA is combined with proteins and organized into a precise, compact structure, a dense string-like fiber called chromatin, which condenses even further into chromosomes during cell division.

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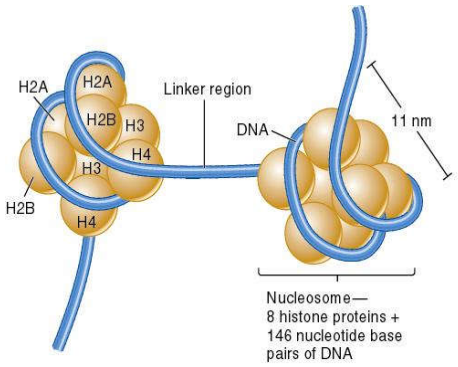
NUCLEOSOME

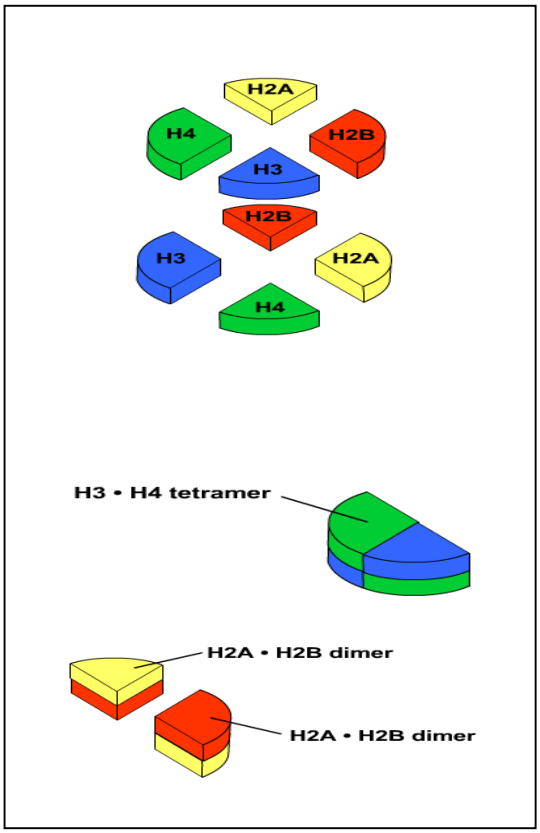


In 1975, P. M. Outdet used the term "Nucleosome" for the particles seen by earlier worker in Electron Microscope. Because the total length of cellular DNA is up to a hundred thousand times a cell's length, the packing of DNA is crucial to cell architecture. During interphase, when cells are not dividing, the genetic material exists as a nucleoprotein complex called **chromatin,** which is dispersed through much of the nucleus. Further folding and compaction of chromatin during mitosis produces the visible **metaphase chromosomes.** The chemical analysis of the chromatin revealed that it consists of the DNA and proteins (Basic proteins: Histones & Non Histone Proteins).

The **histones are** a family of small, basic proteins present in all eukaryotic nuclei. The five major types of histone proteins— termed ***H1, H2A, H2B, H3,*** **and** ***H4***—are rich in positively charged basic amino acids, which interact with the negatively charged phosphate groups in DNA. The amino acid sequences of four histones (H2A, H2B, H3, and H4) are remarkably similar among distantly related species.

TYPES OF HISTONES –

Type	MW	Amino acids richness
H 2 A	13960	129 (Lys, Arg)
H 2 B	13774	125 (Lys)
H 3	15273	135 (arg)
H 4	11236	102 (agr, gly)
H 1	21130	223 (lys)



Structure of Nucleosomes The DNA component of nucleosomes is much less susceptible to nuclease digestion than is the linker DNA between them. If nuclease treatment is carefully controlled, all the linker DNA can be digested, releasing individual nucleosomes with their DNA component. A nucleosome consists of a protein core with DNA

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wound around its surface like thread around a spool. The core is an octamer containing two copies each of histones H2A, H2B, H3, and H4.

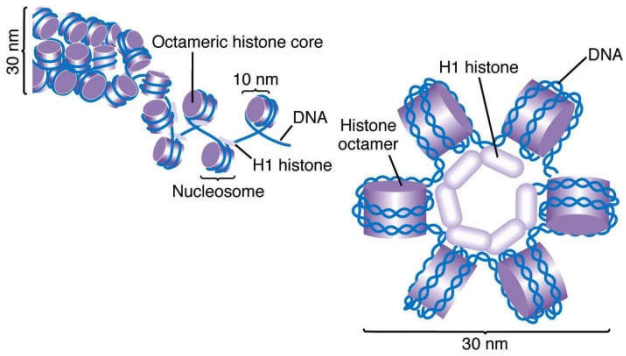
X-ray crystallography has shown that –

- The octameric histone core is a roughly diskshaped molecule made of interlocking histone subunits.
- Dimensions: Height – 6 nm, and Diameter – 11 nm
- Nucleosomes contain 147 base pairs of DNA wrapped slightly less than two turns around the protein core.
- The length of the linker DNA is more variable among species, ranging from about 15 to 55 base pairs.
- Histones are forming heterodimer

  H 2 A and H 2 B forms two Dimers and H 3 and H 4 forms one Tetramer

SOLENOID

A solenoid (Greek **solen** "pipe, channel" + combining form of Greek **eidos** "form, shape") is a coil wound into a tightly packed helix. The term was invented by French physicist **André-Marie Ampère** to designate a helical coil.



In case of DNA Packaging the solenoid defines "the packing of DNA as a 30 nm fiber of chromatin and results from the helical winding of at least five nucleosome strands".

In eukaryotic cells, 146 bp of DNA are wrapped approximately 1.65 times around a histone octamer (each histone consists of 2 H2A, H2B dimers, and H3, H4 tetramer) which together are called a nucleosome. Histone H1, which is not part of the binding histones, tightens the DNA bound to the eight protein complex. **The nucleosomes, which at this point resemble beads on a string, are further compacted into a helical shape via the NH2 terminal protein interactions of the octameric histones, called a solenoid.**

DNA packed into solenoids, unlike DNA in nucleosome form, is not transcriptionally active. With more packing, solenoids are able to become increasingly more packed, forming chromosomes. Around 1200 bps can be accommodated in the solenoid. At this point, solenoids coil around each other to form a loop (anywhere from 20 to 80,000 base pairs),

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followed by a rosette (consisting of six connected loops), then a coil, and at last, two chromatids. The end result is the metaphase chromosome. The completely condensed chromatin has a diameter of up to 600 nm.

SATELITE DNA

Satellite DNA consists of highly repetitive DNA, and is so called because repetitions of a short DNA sequence tend to produce a different frequency of the nucleotides adenine, cytosine, guanine and thymine, and thus have a different density from bulk DNA - such that they form a second or 'satellite' band when genomic DNA is separated on a density gradient. The buoyant density ρ of a molecule in CsCl is defined as the density of the saltsolution where that molecule is found, at sedimentation equilibrium.

Satellite DNA, together with Minisatellite and Microsatellite DNA constitute the Tandem repeats.

Some types of satellite DNA in humans are:

Type Size of repeat unit (bp) Location α (alphoid DNA) 171 All chromosomes β 68 Centromeres of chromosomes 1, 9, 13, 14, 15, 21, 22 and Y Satellite 1 25-48 Centromeres and other regions in heterochromatin of most chromosomes Satellite 2 5 Most chromosomes Satellite 3 5 Most chromosomes

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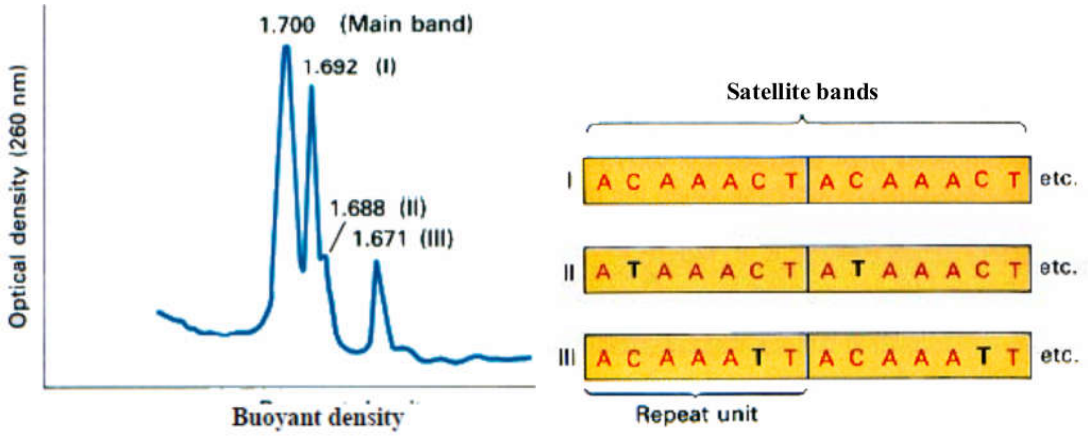

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Some highly repetitive DNA has a base composition that is different from that of bulk chromosomal DNA. In a CsCl density gradient this DNA bands at a position different from bulk DNA. It is called satellite DNA

A repeated pattern can be between 1 base pair long (a mononucleotide repeat) to several thousand base pairs long, and the total size of a satellite DNA block can be several megabases without interruption. Most satellite DNA is localized to the telomeric or the centromeric region of the chromosome. The nucleotide sequence of the repeats is fairly well conserved across a species. However, variation in the length of the repeat is common. For example, minisatellite DNA is a short region (1-5kb) of 20-50 repeats. The difference in how many of the repeats is present in the region (length of the region) is the basis for DNA fingerprinting.

Some highly repetitive DNA has a base composition that is different from that of bulk chromosomal DNA. In a CsCl density gradient this DNA bands at a position different from bulk DNA. It is called satellite DNA.

Applications:

1. Understanding nuclear genome
2. Hybridizations Studies
3. Dissociation / Reassociations studies
4. DNA fingerprinting
5. Evolutionary studies
6. Base composition (GC richness / Tm value)
7. Naturally associating (symbiotic or parasitic) species studies.

REPETITIVE DNA

When the chromosomes of an organism such as wheat or pine is dissected at the molecular level, stretches of nucleotide sequence that occur once or only a few times - including the genes - represent as little as 5% of the DNA. Most plant and animal genomes consist largely of repetitive DNA - perhaps 30 sequence motifs, typically one to 10 000 nucleotides long, present many hundreds or thousands of times in the genome, which may be located at a few defined chromosomal sites or widely dispersed.

In the study of DNA sequences, one can distinguish two main types of **repeated sequence**:

- Tandem repeats:
    - Satellite DNA,
    - Minisatellite,
    - Microsatellite;

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- Interspersed repeats:
    - SINEs (**S**hort **IN**terspersed **E**lements),
    - LINEs (**L**ong **IN**terspersed **E**lements).

The majority of LINEs are LINE-1 and the majority of SINEs are Alu's.

In prokaryotes, CRISPR are arrays of alternating repeats and spacers.

Species over wide taxonomic groupings have similar genes and arrangements of genes along the chromosomes - they show conserved synteny. However, knowledge of synteny - provided by high-density, marker-saturated genetic maps and genomic DNA sequence data - tells us relatively little about the large-scale physical organization of the chromosomes and the repetitive DNA elements that make up the bulk of most genomes.

Repetitive DNA, with different selective pressures from those acting on genes and evolutionarily successful multigene modules, may show extensive differences in sequence motifs and abundance even between closely related species. The repetitive DNA in the genome is also important for evolutionary, genetic, taxonomic and applied studies.

An excellent database of various repetitive sequences from plants is maintained by Jiri Macas at the Czech Academy of Sciences, Budejovice. Not surprisingly, many of these (probably about 50) are from Triticeae and many although reported under their original species (particularly rye) are also present in other species.

TRANSPOSABLE ELEMENT

"A transposable element is a bit of DNA that can move from place to place in an organism's genome.... This DNA fragment is excised from one site and inserted at another site. The movement of a transposable element can generate mutations or chromosomal rearrangements and thus affect the expression of other genes."

Barbara McClintock in 1953 (1983 Nobel Prize), was the first scientist to predict that transposable elements, mobile pieces of the genetic material (DNA), were present in eukaryotic genomes. She performed her work on corn and specifically followed seed color phenotypes.

These elements are also called as Jumping genes / Mobile genetics elements / Controlling elements/ Transposons / Insertion Sequences / Selfish DNA / Roving genes / Cassettes

They are of following types:

1. **Autonomous:** where transposase gene is functional and carry out mobilization and
2. **Non autonomous:** where transposase gene is not functional

Structural Features:

Transposable element possess following special features:

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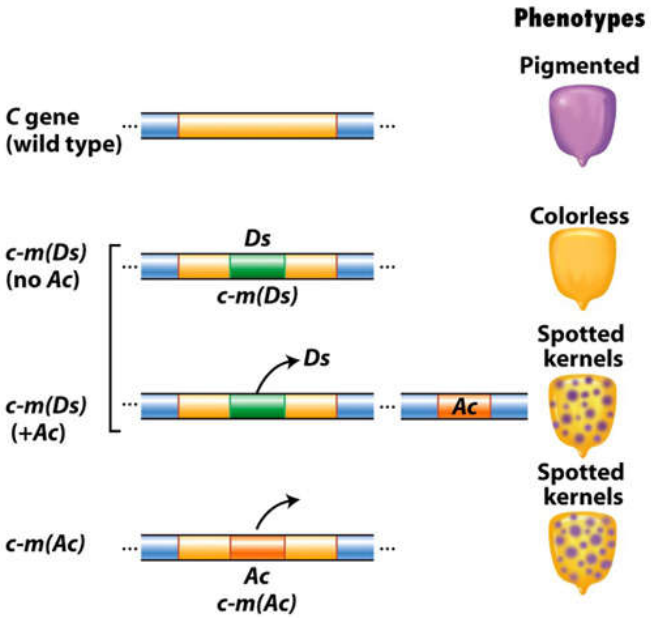
1. It has transposes gene to facilitate transposition.
2. Genes are sandwiched by Inverted Repeats at both the ends.
3. Restriction sites are found at many regions.

AC – DS System

In particular, Barbara was analyzing genes that control the color of the aleurone layer of the endosperm. Remember that this tissue is triploid (3n). The genes that she was following were located on the **short arm of chromosome 9** of corn and were involved in the development of the color of the seed.

The grains in the maize kernel are variedly coloured, Patched and white. This variation in colour is due to:

a. pigments produced by gene leading to the **coloured grains**
b. Gene function blocked by insertion of Ds element in between lead to loss of pigment and hence **white in colour**
c. Removal of the Ds element with the help of Ac element lead to formation of **Patched colouration** in seeds
d. Hence the differential colours are due to actions of Ac and Ds elements in Maize.



Top row: Wild type pigmented kernel.

Second row: Ds is inserted into pigment gene (C) permanently, disabling it. By itself, it can't move. It's stuck. Ds is a non-autonomous element.

Third row: Ds and Ac both present, Ds can now excise from the C gene in some cells (i.e., it can transpose) during development, creating developmental fields that can produce pigment. This is because Ac has provided the elements needed for Ds to transpose.

Fourth row: Ac is inserted into pigment gene, but not permanently, as it can provide the elements that allow its removal from the gene. Ac is an autonomous element.

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GENE CONCEPT

Gene is the basic functional unit of life. Just as atoms are the functional units in chemistry, so are genes in biology. The atom combines in n number of ways to form the massive, complex structures like mineral ore, etc. Similarly the genes combine in n forms to form complex individuals.

Just like the atoms combine and exchange electrons from one group to the other to form complex atoms, genes also combine with one another to form complex genes, thus providing a scope for complex gene structure formation. **The concept of gene was first introduced by Mendel and he termed them as hereditary factors or elements.**

This concept was purely hypothetical and did not carry any experimental evidence. **Wilhelm Johansen coined the term gene in 1909 to describe heritable factors responsible for the transmission and expression of a given biological character**, but without reference to any particular theory of inheritance. But evidence generated from experimental work carried out with higher plants suggest genes as the units of inheritance, controlling one phenotypic trait.

Based on this data, Morgan in 1926 proposed the particulate gene theory in which he stated that genes are corpuscular and arranged in a linear order on chromosomes like beads on a string. According to the pre 1940 beads-on-a-string concept, the gene was the basic unit of inheritance defined by three criteria function, recombination and mutation pair. Since it clearly does not make sense to call each nucleotide pair a gene, emphasis has been shifted to the original definition of the gene as the unit of function. Thus, Mendel's unit of inheritance co relates more directly with the gene as a unit of function than with the unit of structure or single nucleotide pair.

1. The unit of function means that a fragment or unit of genetic material controls the inheritance of one unit character or attribute of phenotype.

2. The unit of structure could be operationally defined in two ways

    i. As the unit of inheritance not sub divisible by recombination.

    ii. The smallest unit of genetic material capable of independent mutation.

The classical view was that all three criteria defined the same basic unit of inheritance. But this theory was discarded with advances in the DNA structure. Then Sutton introduced a new gene concept, which was elaborated by Muller. This concept was known as classical gene structure. It states that

1. Genes determine physical as well as physiological characteristics.

2. Genes are present on the chromosome, and on a single chromosome there are many genes.

3. Genes occupy a specific position on a chromosome which is called as locus or loci and genes are arranged in single linear order.

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MODERN GENE CONCEPT –

According to our current molecular concept, the gene is the functional unit of inheritance coding for one polypeptide chain and operationally defined by the cis trans or complementation test.

The other two criteria, recombination and mutation, define the unit of structure, which is equivalent to the single nucleotide pair gene, emphasis has been shifted to the original definition of the gene as the unit of function.

Thus Mendel's unit of inheritance co relates more directly with the gene as a unit of function than with the unit of structure or single nucleotide pair.

MODERN DEFINITION OF GENE

After the discovery of DNA, its parallel behaviour with that of chromosomes and proper understanding of most of the molecular phenomena which may interplay in the determination of a phenotypic trait. **Seymour Benzer** (October 15, 1921 – November 30, 2007) proposed the modern concept of gene. He has been defined gene as follows:

1. **Cistron** The portion of DNA specifying a single polypeptide chain is termed as cistron, which is a synonym for the termed, the gene of physiological function. The term cistron has been coined by Seymour Benzer. Haemoglobin, therefore, would require two cistrons for its globin protein fraction, one each for the α and β chains. A cistron for α -chain has at least 141 X 3-423 nucleotides and the citron for the β-chain 146 X 3=438 nucleotides.

2. **Muton** There are many positions or sites within a cistron where mutations can occur. Therefore, the gene as a unit of mutation is smaller. i.e., it consists of fewer nucleotides than a cistron. Benzer coined the word muton to that smallest length of DNA capable of mutational change.

Thus, a muton can be defined as the smallest unit of genetic material which when changed or mutated produces a phenotypic effect. A muton may thus be delimited to a single nucleotide or some part of nucleotide. Different forms of a mutationality defined genes are called homoalleles. For example, in bacteria muton may be nucleotide pair and in cistron for haemoglobin the muton may be single nucleotid.

3. Recon Sometimes crossing over or recombination occure in a cistron and this provides still, other sub-divisional concept of the cistron, namely the recon. A recon is the smallest unit of DNA capable of recombination or of being integrated by transformation in bacteria.

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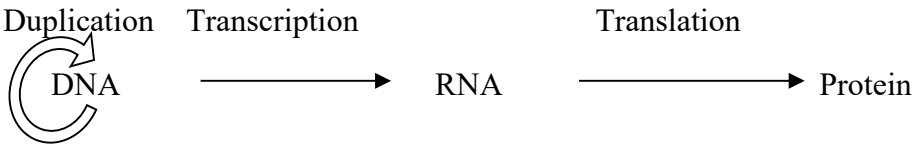
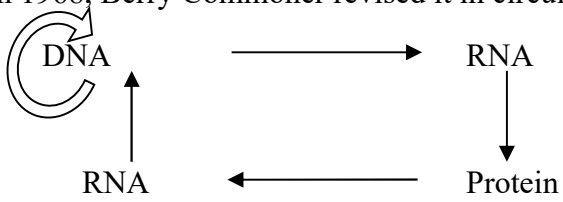
EXPLAIN MECHANISM OF PROTEIN SYNTHESIS (CENTRAL DOGMA):

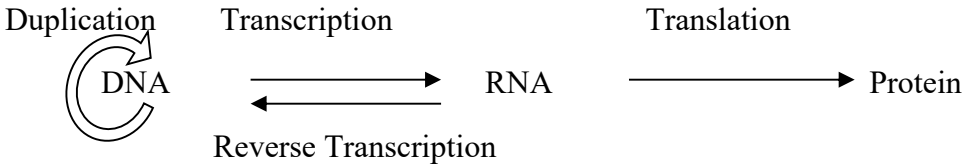
Protein synthesis is the key physiological process in all living organisms. All the metabolic processes requires the protein (enzymes) hence this process is very important in living systems. The process involves central dogma of molecular biology. Crick, 1958 proposed that genetic information flows from nucleic acid to the protein through two-step process as follows –



In 1968, Berry Commoner revised it in circular flow of information.



Later in 1970, Temin reported enzyme Reverse Transcriptase, which confirmed by Baltimore and concept of "Central Dogma Reverse" or "Teminism" as illustrated below –



The process of protein synthesis requires following material –

1. Amino acids
2. Ribosomes
3. m RNA
4. t RNA
5. Enzymes
6. ATP for energy
7. GTP for peptide bond synthesis
8. Factors for initiation, elongation or termination
9. Various inorganic ions

The protein synthesis is carried out in following two major steps –

A) Transcription
B) Translation

A) TRANSCRIPTION

The transcription is further has three steps

i. Initiation

The double stranded DNA is copied by the enzyme RNA polymerase in the form of RNA. The strand with direction of 3' – 5' is read by the enzyme, hence called as "sense strand" and other 5' – 3' strand is known as "antisense strand". RNA polymerase is multimeric protein

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having five subunits (α, α', β, β' and ω). The additional subunit assisting transcription initiation is σ (sigma) temporarily with RNA polymerase.

RNA polymerase initiates transcription in following steps –

a) **Template binding:** It binds to the promoter region and forms the closed complex with the help of sigma factor.
b) **Formation of Open complex:** The sigma factor changes its confirmation and position a little bit and the coiled double stranded DNA is opened at the core enzyme by degradation of the hydrogen bonds between two strands of DNA.
c) **Abortive Initiation:** RNA polymerase synthesize (polymerize) RNA with the help of ribonucleotides up to 9 – 10 bases and abort this stretch. The sigma factor releases from the enzyme and transcription elongation will be started.

ii. Elongation

The elongation is carried out by core enzymes only. The core enzyme moves along the DNA polymerizing RNA by using nucleoside triphosphate which is complementary with the DNA template strand. The RNA polymerase enzyme opens DNA in front (α subunit) and rewind it at the end portion (α') subunit.

iii. Termination

Termination is carried out by two mechanisms –

a) Rho (ρ) independent termination: Transcription is terminated at a specific sequences at the end of the gene. These sequences are called as inverted repeats. They are having complementary bases of each other from reverse direction

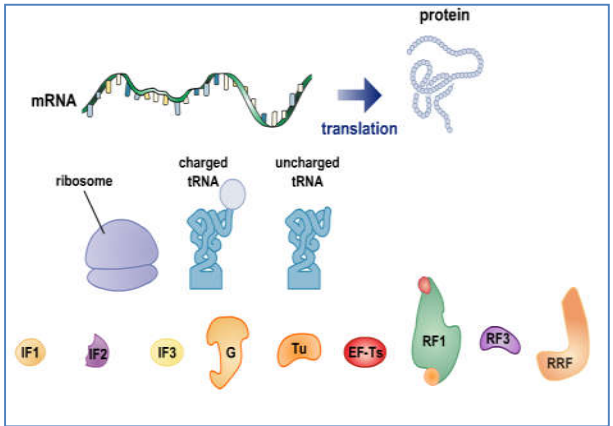
e.g. ATGCGCTA- **TAGCGCAT**

b) Rho (ρ) dependent termination:

If the inverted repeats will not be there to terminate the transcription the hexameric, ring shaped specialized protein called rho (ρ) terminate the transcription by using energy from ATP.

On termination RNA polymerase enzyme leaves DNA and newly synthesized RNA will be ready for further process of translation.

B) TRANSLATION



The translation requires all requirements shown in figure and has following three major steps –

i. Initiation

Translation initiation has many events to be carried out simultaneously

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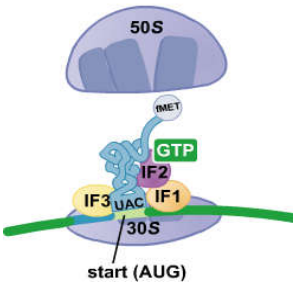


a) **Formation on 30 S initiation complexes** by interaction of initiation factor (IF)-3, 30 S ribosomal subunit and mRNA. IF-1 occupies the A (acceptor / amino acid) site of 30 S subunit and IF-2 guides incoming charged tRNA to the P (Peptydyl) site.

b) **Activation of amino acid:** 20 standard amino acids are present in the cytoplasm in inactive state. The specific amino acid is activated by the specific enzyme (aminoacyl synthetase) and ATP.

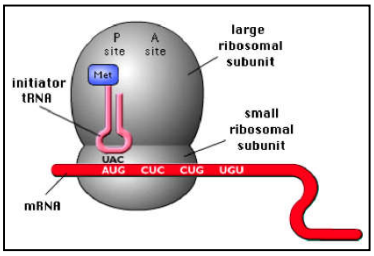
AA + ATP $\longrightarrow$ **AA~AMP-Enzyme + PP (pyrophosphate)**

Aminoacyl synthetase Aminoacyl adenylate enzyme complex

c) **tRNA charging:** Amino acid residue of the amino acyladenylate is transferred to 3-OH group of the adenine base of tRNA (having CCA bases at the 3' end)

AA~AMP-Enzyme + tRNA $\longrightarrow$ **AA- tRNA + AMP + Enzyme**

d) **Formation of 70S initiation complex:** Once the charged tRNA arrived at P site of 30S subunit of ribosome initiation factors releases from the assembly and 50S subunit land on the initiation complex and forms the 70S initiation complex.

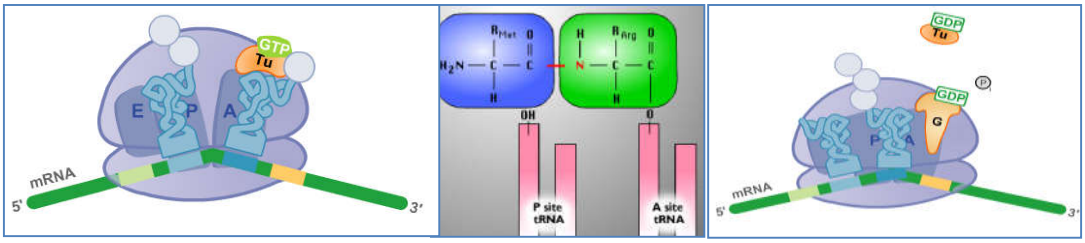


ii. Elongation

a) Entry of next AA-tRNA: As per the codon in the sequence of mRNA next charged tRNA is brought by elongation factor (EF)-Tu, which is associated by GTP.

b) Formation of peptide bond: The chain of amino acid on tRNA from P site will establish peptide bond between last amino acid in the chain and the incoming amino acid on the tRNA from A site with the help of enzyme peptydyl transferase.

c) Translocation of t RNA: EF-Ts is the factor which pushes the loaded tRNA from A site to P site and empty tRNA from P site to E site.



iii. Termination

Termination of the translation is under control of the stop codons (UAA, UGA, UAG). These codons are recognized by the Termination/Release Factors (RF). They are of two types – Class-I and Class-II,

Class-I and Class-II release factors enters in the A site on the arrival of stop codon in it. GGQ sequence on the Class-I release factor hydrolyze the peptide chain from the tRNA in P site and release the entire peptide from the ribosome.

After releasing the peptide ribosome, mRNA dissociates from each other and Ribosome Recycling Factor (RRF) recycle ribosome by disassembling it.

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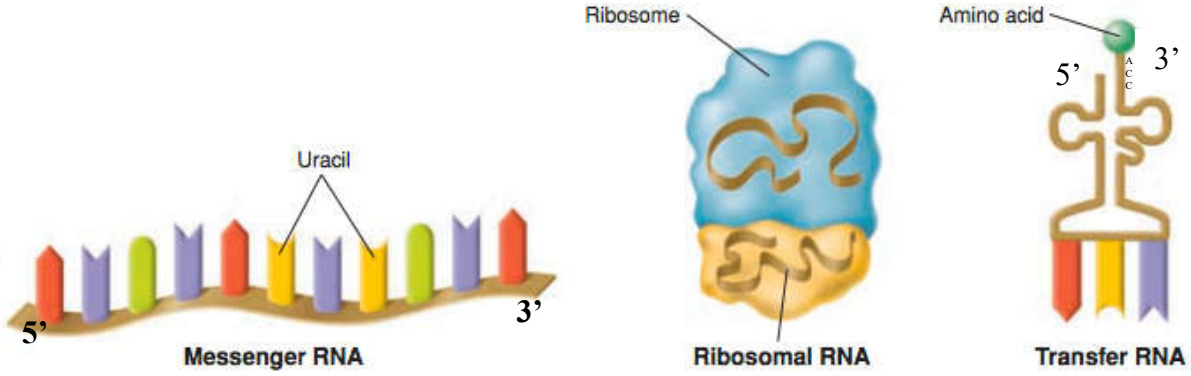
TYPES OF RNA



Types of RNA The three main types of RNA are messenger RNA, ribosomal RNA, and transfer RNA. Ribosomal RNA is combined with proteins to form ribosomes.

Messenger RNA:

Messenger RNA (mRNA) is synthesized from a gene segment of DNA which ultimately contains the information on the primary sequence of amino acids in a protein to be synthesized. The genetic code as translated is for m-RNA not DNA. The messenger RNA carries the code into the cytoplasm where protein synthesis occurs.

Ribosomal RNA:

In the cytoplasm, ribsomal RNA (rRNA) and protein combine to form a nucleoprotein called a ribosome. The ribosome serves as the site and carries the enzymes necessary for protein synthesis. In the graphic on the left, the ribosome is shown as made from two sub units, 50S and 30 S. There are about equal parts rRNA and protein. The far left graphic shows the complete ribosome with three tRNA attached.

The ribosome attaches itself to m-RNA and provides the stabilizing structure to hold all substances in position as the protein is synthesized. Several ribosomes may be attached to a single RNA at any time. In upper right corner is the 30S sub unit with mRNA and tRNA attached.

Transfer RNA:

Transfer RNA (tRNA) contains about 75 nucleotides, three of which are called anticodons, and one amino acid. The tRNA reads the code and carries the amino acid to be incorporated into the developing protein.

There are at least 20 different tRNA's - one for each amino acid. The basic structure of a tRNA is shown in the left graphic. Part of the tRNA doubles back upon itself to form several double helical sections. On one end, the amino acid, phenylalanine, is attached. On the

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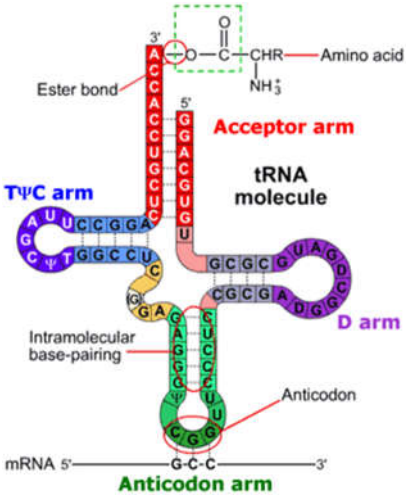
opposite end, a specific base triplet, called the anticodon, is used to actually "read" the codons on the mRNA.



The tRNA "reads" the mRNA codon by using its own anticodon. The actual "reading" is done by matching the base pairs through hydrogen bonding following the base pairing principle. Each codon is "read" by various tRNA's until the appropriate match of the anticodon with the codon occurs.

In this example, the tRNA anticodon (AAG) reads the codon (UUC) on the mRNA. The UUC codon codes for phenylalanine which is attached to the tRNA. Remember that the codons read from the mRNA make up the genetic code as read by humans.

Small nuclear ribonucleic acid (snRNA), also commonly referred to as U-RNA, is a class of small RNA molecules that are found within the nucleus of eukaryotic cells. The length of the an average snRNA is approximately 150 nucleotides. They are transcribed by either RNA polymerase II or RNA polymerase III, and studies have shown that their primary function is in the processing of pre-mRNA (hnRNA) in the nucleus. They have also been shown to aide in the regulation of transcription factors (7SK RNA) or RNA polymerase II (B2 RNA), and maintaining the telomeres.

Ribozyme (ribonucleic acid enzyme) is an RNA molecule that is capable of performing specific biochemical reactions, similar to the action of protein enzymes. The 1981 discovery of ribozymes demonstrated that RNA can be both genetic material (like DNA) and a biological catalyst (like protein enzymes), and contributed to the RNA world hypothesis, which suggests that RNA may have been important in the evolution of prebiotic self-replicating systems. Also termed catalytic RNA

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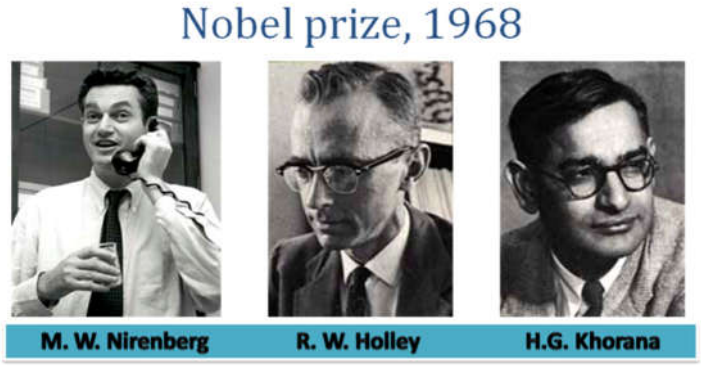
PROPERTIES OF GENETIC CODE

The **genetic code** is the set of rules by which information encoded in genetic material (DNA or RNA sequences) is translated into proteins (amino acid sequences) by living cells. Specifically, the code defines a mapping between tri-nucleotide sequences called **codons**, and amino acids; every triplet of nucleotides in a nucleic acid sequence specifies a single amino acid. Because the vast majority of genes are encoded with exactly the same code, this particular code is often referred to as the canonical or standard genetic code, or simply *the* genetic code. For example, in humans, protein synthesis in mitochondria relies on a genetic code that varies from the canonical code.

The genetic code is elucidated by Nirenberg, Khorana and Holley in the year of 1959.



The genetic code has following properties –

2. Triplet Nature
3. Non Overlapping
4. Non Punctuating
5. Degeneracy (Synonyms)
6. Non Ambiguous
7. Stop codons
8. Universal
9. Wobbling

1. Triplet Nature

Codon is also called as mini messenger as it carries information for one amino acid. Each codon consist of three bases e.g. UUU for Phenyl alanine. The logical reasoning was proposed by **George Gamov (1954)** about the possible size of a codon. The simplest possible size is singlet (a code of one nucleotide) where one nucleotide will specify one amino acid. Here coding of only 4 amino acids by 4 bases (A, T, G & C) is the possibility. A doublet code

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(a code of two nucleotides) may specify only 16 amino acids of the standard 20 (4x4 bases). Whereas triplet code (a code of three nucleotides) could specify 64 amino acids (4x4x4 bases). Therefore, to code 20 amino acids 64 codons must be there and hence the codon size is triplet. The first experimental proof in this support was provided by **Crick and coworker** in 1961.

2. **Non Overlapping**

The codon are read by triplets without any overlapping. Codons are purely non overlapping. Change in a base can affect more than one codon

One base overlapping

	CAG	CAG	CAG	CAG		4 Codons
	Gln	Gln	Gln	Gln		
One	CAG	**G**CA	**A**GC	**C**AG	**G**CA	**A**G 6 Codons
	Gln	Ala	Ser	Gln	Ala	Ser

3. **Non Punctuating**

Genetic code is non punctuating, there is no punctuating codon in between. The reading frame of m RNA could not have any break during translation. Punctuation in codon may be lethal. It is called as comma less language

4. **Degeneracy (Synonyms)**

One amino acid is coded by many (determined) number of codons. The Codons are degenerate, they do not posses independent coding by them only, the same amino acid can be coded by other codon. These codon are called as Synonymous Codons.

Synonymous Codons

Six	-	Ser, Arg, Leu
Four	-	Val, Pro, Thr, Ala, Gly
Three	-	Ile
Two	-	Phe, Tyr, Asn, His, Gln, Asp, Lys, Glu, Cys,
One	-	Met, Trp

Eight groups of dictionary coding just one amino acid. Based on the degeracy codon groups in the code (families) are classified as -

I. **Unmixed families –** Needs reading only only first two bases

II. **Mixed families –** Group is coding two different amino acid or stop codon

5. **Non Ambiguous**

A particular codon will always code for a specific amino acid. There is no ambiguity (doubt) in the meaning of the codon.

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Exceptions

AUG – N – Formyl Metheonine

GUG – Valine, Methionine

UGA can code for selenocysteine and UAG can code for pyrrolysine Selenocysteine is now viewed as the 21st amino acid, and pyrrolysine is viewed as the 22nd.

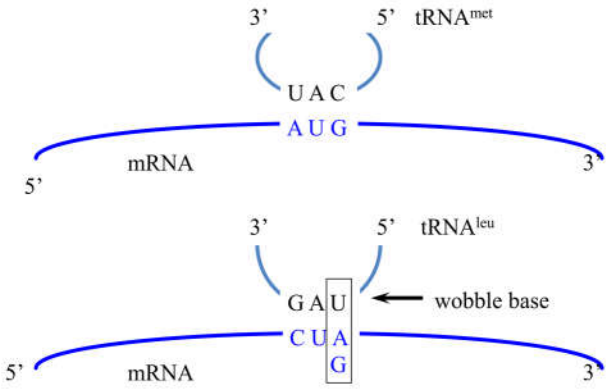
6. Stop codons

Three codons from the dictionary are working as stop codons. They are UAG, UAA & UGA. They do not code for any amino acid. These codons are not read by any tRNA (via there anticodons) but they are read by specific proteins called release facors (RF-1, RF-2 & RF-3)

7. Universal

All the living organisms are having same meaning of the code. The genetic code is valid for microbes, plants and animals. i.e. AGA codon is coding for arginine in all the organisms. In 1980, discrepancies in the code were thought and clarified that organeller genomes are having different meaning. Bonitz (1980) Proposed new genetic code for mitochondrial DNA, Ciliated Protozoans, Mycoplasms, etc.

8. Wobbling



Wobble hypothesis proposed by Dr. F. H. C. Crick (1965) According to this hypothesis, only the first two bases of the codon have a precise pairing with the bases of the anticodon of mRNA, while the pairing between the third bases of codon and anticodon may Wobble (non specific).

The pairing in the third base is ambiguous. Thus a single tRNA can pair (bind) with more than one mRNA codon differing in only the third base. For example the anticodon ICG of tRNA can read codons AGC and AGU of mRNA.

The pairing between the first two bases is normal, while between G and U is against the normal pairing pattern. This unusual bonding between G and U is called Wobble pairing. Same tRNA can recognize more than one codon UCX, UAA, UUG (Leu) by Same t RNA

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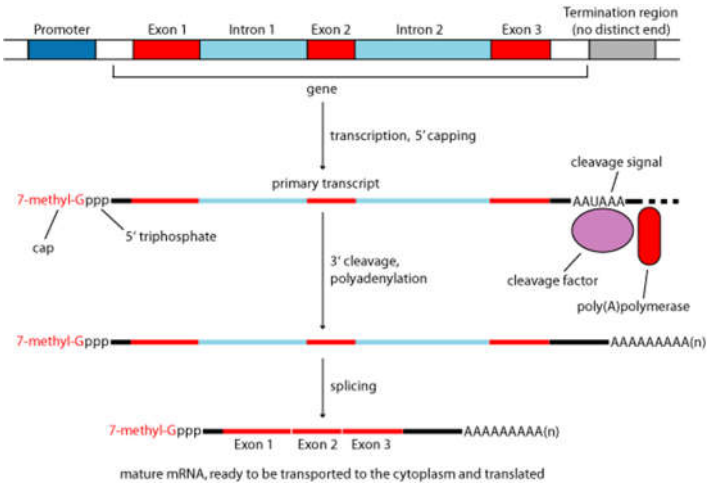
RNA PROCESSING

The initial primary transcript synthesized by RNA polymerase II during transcription undergoes several processing steps before a functional mRNA is produced. It happens only in eukaryotic cells and called as RNA processing, which includes three major processes:

1. **5′ capping,**
2. **3′ polyadenylation**,
3. **RNA splicing**

Processing occurs in the nucleus, and the functional mRNA produced is transported to the cytoplasm.

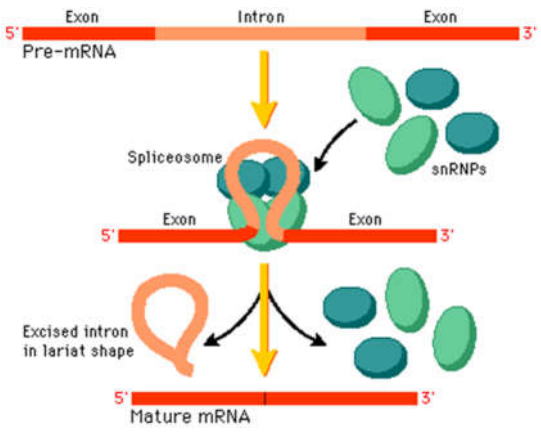


1. **5’ Capping:** The 5′-Cap Is Added to Nascent RNAs Shortly after Initiation by RNA Polymerase II. After nascent RNA molecules produced by RNA polymerase II reach a length of 25 – 30 nucleotides, 7-methylguanosine is added to their 5′ end. This initial step in RNA processing is catalyzed by a **dimeric capping enzyme**, which associates with the phosphorylated Carboxyl-Terminal tail Domain (CTD) of RNA polymerase II.

2. **3’ Tailing:** Pre-mRNAs are **Cleaved** at specific 3′ sites and rapidly **Polyadenylated**. A cleavage and polyadenylation specificity factor (CPSF), composed of four different polypeptides, and cleavage stimulatory factor (CStF), binds to the CPSF-RNA complex. And finally, a **poly (A) polymerase** (PAP) binds to the complex before cleavage can occur. This requirement for PAP binding links cleavage and polyadenylation, so that the free 3′ ends generated are rapidly polyadenylated.

   Following cleavage at the poly(A) site, polyadenylation proceeds in two phases. Addition of the first 12 or so A residues occurs slowly, followed by rapid addition of up to 200 – 250 more A residues. The rapid phase requires the binding of multiple copies of a **poly(A)-binding protein** to protect Poly A Tail.

3. **RNA Splicing:** During the final step in formation of a mature, functional mRNA, the introns (non coding region) are removed and exons are spliced together. Eukaryotic pre-mRNAs typically include introns. Introns are removed by RNA processing in which the intron is looped out and cut away from the exons by snRNPs, and the exons are spliced together to produce the translatable mRNA. The steps of pre-mRNA splicing (intron removal) are as follows:



   a. The intron loops out as snRNPs (small nuclear ribonucleoprotein particles, complexes of snRNAs and proteins) bind to form the spliceosome.
   b. The intron is excised, and the exons are then spliced together
   c. The resulting mature mRNA may then exit the nucleus and be translated in the cytoplasm.

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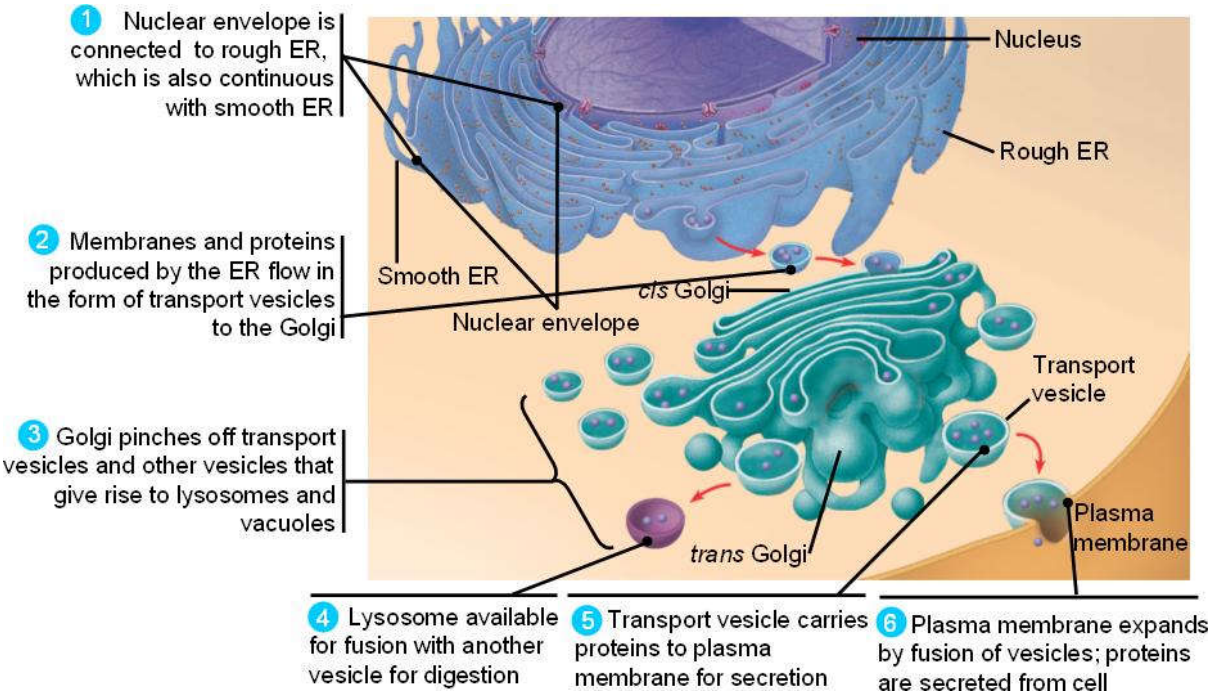
ENDOMEMBRANE SYSTEM

The endomembrane system is composed of the different membranes that are suspended in the cytoplasm within a eukaryotic cell. These membranes divide the cell into functional and structural compartments, or organelles.

In eukaryotes the organelles of the endomembrane system include: Nuclear Envelope, Endoplasmic Reticulum, Golgi Apparatus, Lysosomes, Vacuoles, Vesicles, Endosomes and the Cell Membrane.

The system is defined more accurately as the set of membranes that form a single functional and developmental unit, either being connected directly, or exchanging material through vesicle transport. Importantly, the endomembrane system does not include the membranes of mitochondria and chloroplasts.

The nuclear envelope is a membrane containing two layers, that encompasses the contents of the nucleus. The endoplasmic reticulum (ER) is a synthesis and transport organelle that branches into the cytoplasm in plant and animal cells. The Golgi apparatus is a series of multiple compartments where molecules are packaged for delivery to other cell components or for secretion from the cell. Vacuoles, which are found in both plant and animal cells (though much bigger in plant cells), are responsible for maintaining the shape and structure of the cell as well as storing waste products. A vesicle is a relatively small, membrane-enclosed sac that stores or transports substances. The plasma membrane, also referred to as the cell membrane, is a protective barrier that regulates what enters and leaves the cell.



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RIBOSOME AS TRANSLATION MACHINE

In genetics, translation is the process by which mRNA is decoded and translated to produce a polypeptide sequence, otherwise known as a protein. This process is preceded by the transcription of DNA to RNA. This method of synthesizing proteins is directed by RNA and accomplished with the help of Ribosome. In translation, a cell decodes the mRNA's genetic message and assembles the brand new polypeptide chain accordingly.

Ribosomes help coordinate the binding of tRNA anticodons with mRNA codons during translation. Ribosome can be like molecular machine that can decode RNA and use the information to build a polypeptide that contains a precise sequence of amino acid. The decoding process of the ribosome may be compared to a language translation machine that converts one language to another.

So, the ribosome can be viewed as translating the language of the mRNA code into sensible protein sequences that conduct the activites of the cell. In eukaryotes, the ribosomal subunits are produced in the nucleolus of the cell.

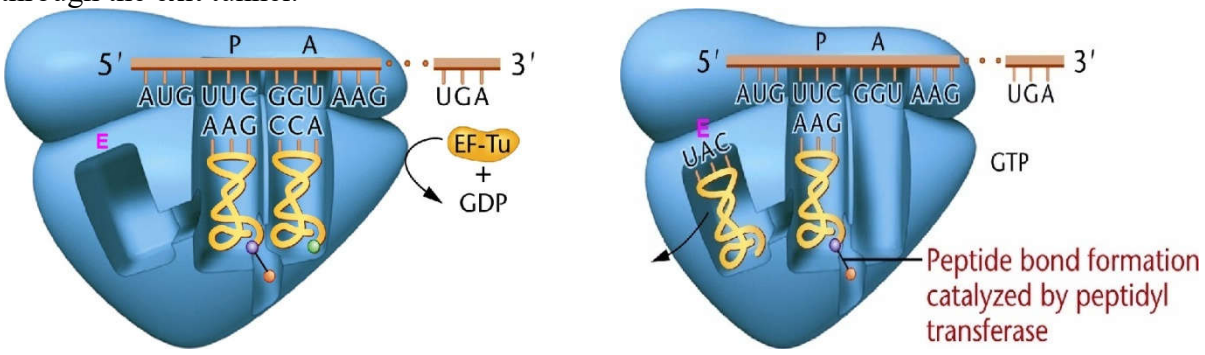
A ribosome is made up of two subunits: the large and small subunits. These subunits are made up of proteins and RNA molecules called ribosomal RNA (rRNA).

The smaller subunit agrees to the selection of correct aminoacyl tRNAs by facilitating base-pairing between mRNA codons and tRNA anticodons, while the larger subunit contains both the peptidyl transfer during protein elongation and hydrolysis of the petidyl tRNA during termination.

The structure of the ribosome consists of a binding site for mRNA and three binding sites for tRNA:

1. For tRNA, the **P site (peptidyl-tRNA site)** carries the growing polypeptide chain, while
2. **A site (aminoacyl-tRNA site)** holds the tRNA that carries the next amino acid that is to be added to the growing chain.
3. **E site (exit site)** is the site where discharged tRNAs leave the ribosome.

The ribosome holds these two components close together and positions the new amino acid in a way that allows the addition of new amino acids to the carboxyl end of the growing polypeptide chain. As the chain gets longer, it goes through an **exit tunnel** in the large ribosomal subunit. Once the chain is completed, it is released to the cytosol of the cell through the exit tunnel.



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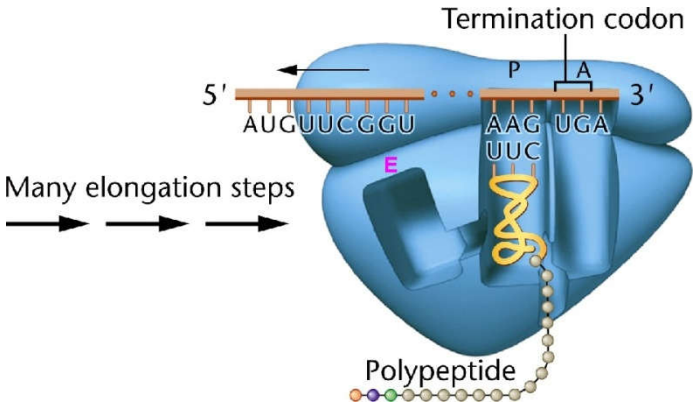

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