MOLECULAR BASIS OF INHERITANCE

RNA WORLD

GENETIC MATERIAL :

It is the substance that controls the inheritance of traits from one generation to the next generation and is also able to express its effect through the formation and functioning of the traits.

PROPERTIES OF GENETIC MATERIAL :

As a genetic material, A molecule must fulfill the following criteria.

(i) Hereditary information must be present in the coded form in the genetic material.

(ii) The genetic material should be able to replicate and then transmitted faithfully to the next generation.

(iii) The genetic material should also be capable of variations, i.e., mutations and recombinations. These variations should be stable and inheritable.

(iv) The genetic material should be able to generate its own kind and also new kinds of molecules.

(v) Genetic material must be able to express its effect in the form of Mendelian characters.

These requirements are found in DNA thus, DNA is now recognized as genetic material.

DNA AS GENETIC MATERIAL :

(1) Direct evidences **(2) Indirect evidences**

(1) DIRECT EVIDENCES :

(i) Transformation :

It is the conversion in the genetic constitution of an organism by picking up genes present in the remains of its dead relatives.

The transformation experiments conducted by **Frederick Griffith** in 1928. He used two strains of bacterium **Diplococcus pneumoniae (Streptococcus pneumoniae).**

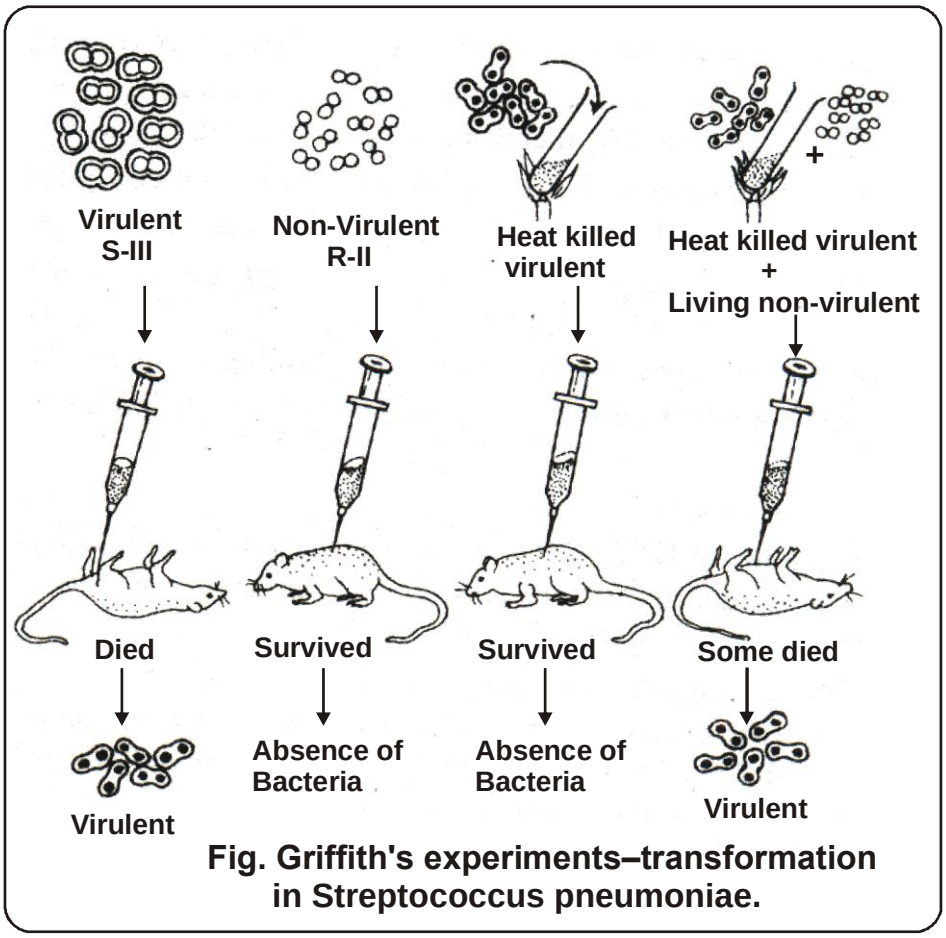


Fig. Griffith's experiments–transformation in Streptococcus pneumoniae.

(a) Smooth (S) or capsulated type which have a capsule. These bacteria are of **virulent** strain and cause **pneumonia.**

(b) Rough (R) or non-capsulated in which capsule is absent. These bacteria are of **non virulent** strain and do not cause pneumonia.

The experiment described in following four parts.

(a) When S- type bacteria injected into mice. The latter died as a result of pneumonia caused by bacteria.

(b) When R- type bacteria injected into mice. The latter lived and pneumonia was not produced.

(c) S- type bacteria which normally cause disease were heat killed and then injected into the mice. The mice lived and pneumonia was not caused.

(d) The mixed solution of Rough type bacteria (living) and smooth type heat-killed bacteria (both known not to cause disease) injected into mice.Some mice died due to pneumonia and virulent smooth type living bacteria could also be recovered from their bodies.

The fourth part of the experiment indicates that some R-type bacteria (non-virulent) were transformed into S- type of bacteria (virulent). The phenomenon is called **Griffith effect or transformation.**

Later, **Avery, Macleod and McCarty (1944)** repeated the experiment **in vitro** to identify transforming substance. They proved that this substance is in fact DNA.

They purified biochemicals from the **killed S-type bacteria** into three components – **DNA, carbohydrate and protein**.

DNA fraction was further divided into two parts: one with deoxyribonuclease or **DNase** and the **other without it.** The four components were then added to separate culture tubes containing R-type bacteria. After some time they were then analysed for bacteria.

Only DNA of S-type can changed R-type of bacteria into S-type. Therefore, the character or gene of virulence is located in DNA. Thus they proved that the chemical to be inherited is DNA and it forms the **chemical or molecular basis of heredity.**

(ii) Multiplication of Bacteriophage (Transduction) :

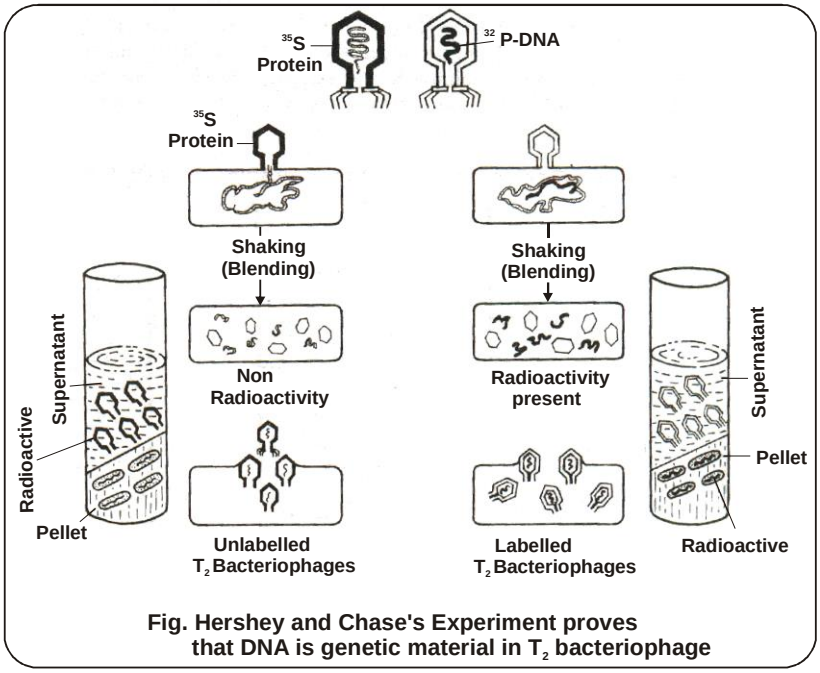


Fig. Hershey and Chase's Experiment proves that DNA is genetic material in T_2 bacteriophage

The transfer of genetic material from one bacterium to another through bacteriophage is called **transduction**. T_2 is a **Bacteriophage** which infects **E. coli.**

Hershey and Chase (1952) used radioactive phosphorus ^{32}P & radio-active sulphur ^{35}S for their experiment and proved that DNA is a genetic matarial.

DNA

Structure of DNA (Deoxyribonucleic acid) :

J.D. Watson and F.H.C. Crick (1953) proposed double helical structure of DNA based on the results of **M.H.F.Wilkins and co-workers.** All these three persons were awarded Nobel Prize in 1962 for this work.

The following are some of the characteristic features of double helical structure of DNA.

(1) **Each nucleotide** consists of **sugar, phosphate and a nitrogenous base**. Many such nucleotides are linked by phosphodiester bonds to form a polynucleotide chain or strand.

(2) **Phospho diester bonds** are formed between 5'carbon of sugar of one nucleotide and 3' carbon of sugar of the next nucleotide.

(3) **Nitrogenous base** is attached to **1' carbon of sugar**. At this place **purine base** is attached by its **9' position** and **pyrimidine** by its **3' position**.

(4) Polynucleotide strand is made of backbone of sugar and phosphate forming its long axis and bases at right angles to it.

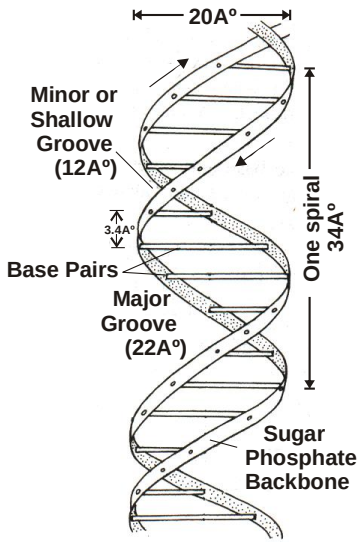


Fig. Structure of DNA- coiling in double helix of DNA

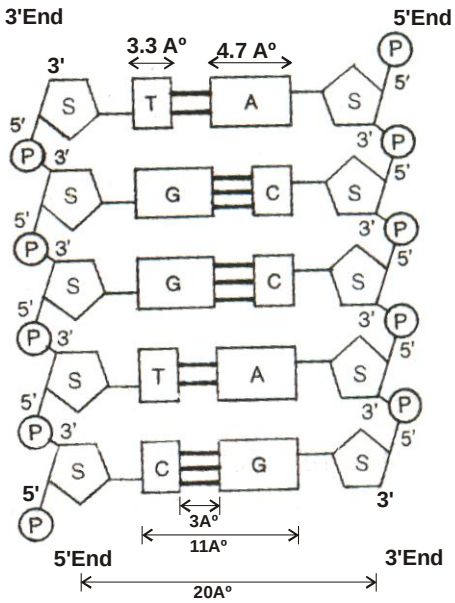


Fig. Arrangement of various Components of DNA

Chargaff (1950) made observations on the base and other contents of DNA. These observations or generalizations are called Chargaffs rule.

(i) Purine and pyrimidine base pairs are in equal amount, that is, adenine + guanine =thymine + cytosine.

(ii) Molar amount of purine-adenine is always equal to the molar amount of pyrimidine thymine. Similarly, guanine is equalled by cytosine.

(iii)Sugar deoxyribose and phosphate occur in equimolar proportions.

(iv)The ratio of A + T/G + C is constant for a species.

(5) Chargaffs rule states that in natural DNAs the base ratio AT is always close to unity and the GC ratio also to always close to unity indicated that A always pairs with T and G pairs with C. A and T, G and C, therefore,are complementary base pairs.

(6) Thus, if one DNA strand has A, the other would have T and if it has G, the other, would have C. Therefore, if the base sequence of one strand is CAT TAG GAC, the base sequence of other strand would be GTA ATC CTG. Hence, the two poly nucleotide strands are called complementary to one another.

(7) Two such complementary strands are joined with one another by hydrogen bonds between their complementary nitrogenous bases. There are three hydrogen bonds between cytosine and guanine and two hydrogen bonds between adenine and thymine.

(8) The two polynucleotide chains are helically coiled around the same axis in such a way that these can separate from one another only by uncoiling. Helical coiling is supposed to be right handed. Such a form of DNA is now called B-DNA

(9) The two chains or strands are antiparallel, i.e., they run in opposite directions in relation to their sugar molecules. Their 5'p 3' OH phosphodiester linkages are in opposite directions

(10) **Double standed DNA molecule has a diameter of 20Aº.**

(11) **The helix makes one complete turn every 34 Aº along its length.**

(12) **There are 10 nucleotides per turn of helix. Thus the distance between two neighbouring base pairs is 3.4 Aº.** Since the discovery of DNA structure, some other forms of DNA have also been recognised. These forms have been classified considering

(i) the number of base pairs per turn of helix

(ii) the distance of base pairs along the helical axis. Accordingly,

besides commonly known B-DNA, other forms are A, C (sometimes D and E) and Z DNA. Some important similarities and differences among different types of DNA are given in.

S.No.		B	Z	A	C	D
1	Handedness of helix	Right handed	Left handed	Right handed	Right handed	Right handed
2	Pitch of helix per turn	34 Å	46 Å	25 Å	30 Å	24 Å
3	Diameter of helix	20 Å	18 Å (thinnest)	23 Å (widest)	19 Å	—
4	Stability	Stable and Physiologically active form	Unstable	Unstable	Unstable	Unstable
5	Base pairs per turn of helix	10	12 (6 dimers)	11	9.33	8
6	Distance between two base pairs	3.4Å	3.8 Å	2.5 Å	3.3 Å	3.03 Å
7	Repeating unit	Mononucleotide	Dinucleotide	Mononucleotide	Mononucleotide	Mononucleoide

RAN

RNA was the first genetic material. There are evidences to suggest that essential life processes, such as metabolism, translation, splicing etc. evolved around RNA. RNA used to act as a genetic material as well as a catalyst there are some important biochemical reactions in living systems that are catalysed by RNA catalysts and not by protein enzymes (e.g., splicing)RNA being a catalyst was reactive and hence unstable. Therefore, DNA has evolved from RNA with chemical modifications that make it more stable. DNA being double stranded and having complementary strand further resists changes by evolving a process of repair. RNA is adapter, structural molecule and in some cases catalytic. Thus RNA is better material for transmission of information.

STRUCTURE OF RNA (RIBONUCLEIC ACID) :

RNA or ribonucleic acid is present in all the living cells. It is found in the cytoplasm as well as nucleus. Sugar in RNA is ribose sugar. Phosphoric acid is similar to that present in DNA. Purine bases are adenine and guanine but pyrimidine bases are cytosine and uracil (thymine being replaced by uracil).

TYPES OF RNA :

RNA is generally involved in protein synthesis but in some viruses, it also serves as a genetic material. Therefore two major types of RNA are as follows.

(a) **Genetic RNA** (b) **Non-genetic RNA.**

(a) **Genetic RNA : H. Frankle-Conrat (1957)** showed that RNA present in TMV (Tabacco Mosaic Virus) is genetic material. RNA acts as a genetic material in most plant viruses.

(b) **Non-genetic RNA :** This type of RNA is present in cells where DNA is genetic material. Non-genetic RNA is synthesized on DNA template. It is of following three types.

(i) **Messenger RNA (mRNA) :** It carries genetic information present in DNA. mRNA constitutes about 5-10% of the total RNA present in the cell. The molecular weight varies from 25,000 to 1,00,000.

(ii) Ribosomal RNA (rRNA) : It is most stable type of RNA and is found associated with ribosomes. It forms about 80% of the total cell RNA. The molecular weight varies from 35,000 to 10,00,000.

(iii)Transfer RNA (tRNA) : It is also known as soluble RNA (sRNA).

These are the smallest molecules which carry amino acids to the site of protein synthesis.There are approximately 80 bases. These constitute about 10-15% of the total cell RNA. The molecular weight of tRNA varies from 23,000 to 30,000.

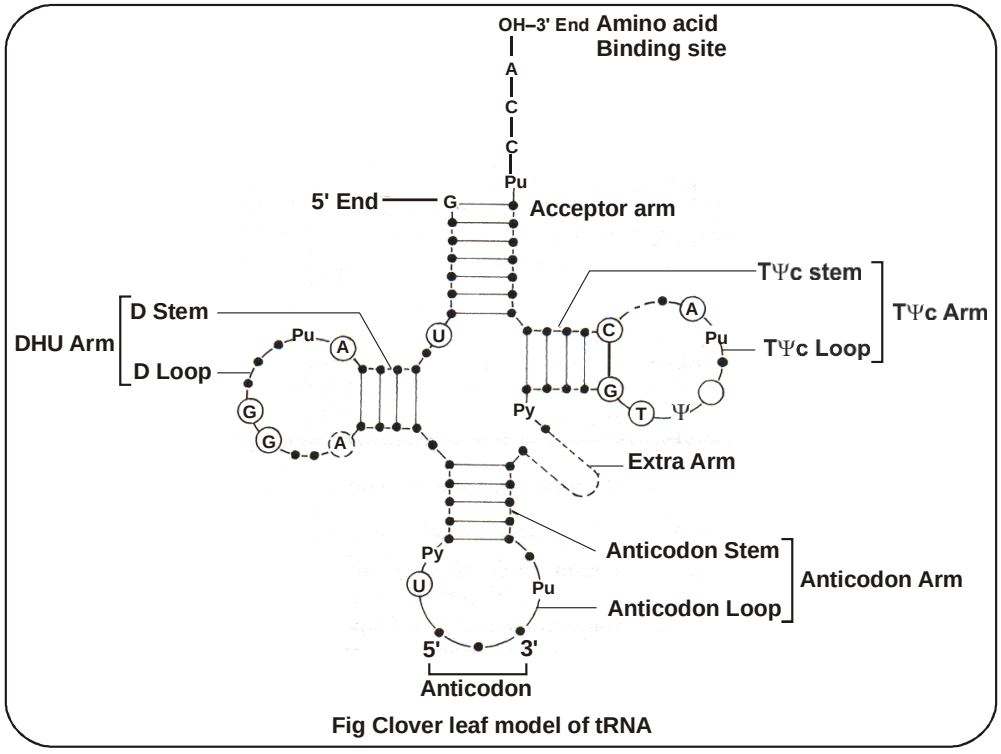


Fig Clover leaf model of tRNA

STRUCTURE OF T-RNA :

Two dimentional clover leaf model of t-RNA was proposed by Holley, (1965). tRNA molecule appears like a clover leaf being folded with three or more double helical regions, each having loop.

(i) Anticodons loop : It has 7 bases out of which three bases form anticodon (nodoc) for recognising and attaching to the codon of mRNA.

(ii) AA-Binding Site : It is amino acid binding site. The site lies at the 3' end opposite the anticodon and has CCA–OH group. The 5' end bears G. Amino acid or AA-binding site and anticodon are the two recognition sites of tRNA .

(iii)T Ψ C Loop : It has 7 bases out of which Ψ (Pseudouridine) and rT (ribothymidine) are unusual bases.The loop is the site for attaching to ribosome.

(iv)DHU Loop : The loop contains 8– 12 bases. It is largest loop and has dihydroxyuridine. It is binding site for aminoacyl synthetase enzyme.

(v) Extra Arm : It is a variable side arm or loop which lies between T Ψ C loop and anticodon. It is not present in all tRNAs. The exact role of arm is not known. The **three dimensional structure** of this **tRNA** was, however, found to be characteristic **L-shaped** by **Kim (1973).**

S.No	DNA	RNA
1	It usually occurs inside nucleus and some cell organelles.	Very little RNA occurs inside nucleus. Most of it is found in the cytoplasm.
2	DNA is the genetic material.	RNA is not the genetic material except in certain viruses, e.g. Reovirus.
3	It is double stranded with the exception of some viruses (e.g. psi × 174)	RNA is single stranded except in certain viruses, double stranded Reovirus.
4	DNA contains over a million nucleotides.	Depending upon the type, RNA contains 70-12000 nucleotides.
5	DNA is of only two types; intra-nuclear and extra-nuclear.	There are at least three types of RNAs– mRNA, rRNA and tRNA.
6	It contains deoxyribose sugar.	It contains ribose sugar.
7	Nitrogen base thymine occurs in DNA alongwith three others–adenine, cytosine and guanine.	Thymine is replaced by uracil in RNA The other three are similar – adenine, cytosine and guanine.
8	It replicates to form new DNA molecules. DNA transcribes genetic information to RNA.	It cannot normally replicate itself. RNA translates the transcribed message for forming polypeptides.
9	DNA control metabolism and genetics. including variation.	It only controls metabolism under instruction from DNA.
10	Purine and pyrimdine bases are in equal number.	There is no proportionality between number of purine and pyrimidine bases.

SEMICONSERVATIVE DNA REPLICATION (MESSELSON AND STAHL'S EXPERIMENT)

SEMI CONSERVATIVE MODE OF DNA REPLICATION

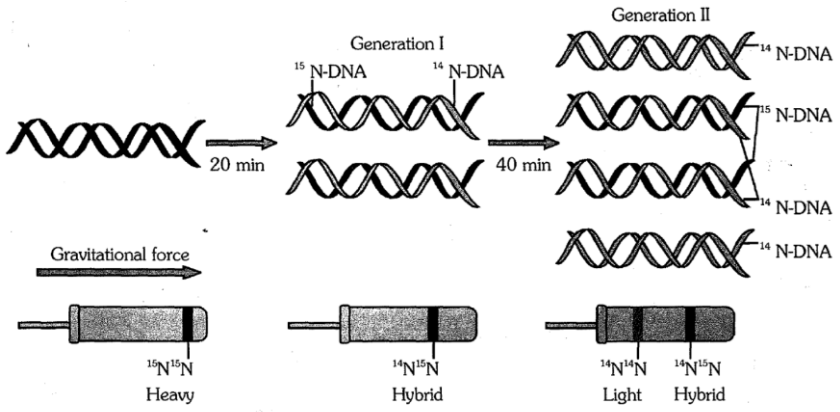
Semi conservative mode of D.N.A. replication was first proposed by Watson & Crick. Later on it was experimentally proved by Meselson & Stahl (1958) in E- Coli and Taylor in Viciafaba (1958). To prove this method, Taylor used Radiotracer Technique in which Radioisotopes (tritiated thymidine $= {}_1H^3$ were used. Meselson and Stahl used heavy isotope of nitrogen (N^{15}).

Matthew Meselson and Franklin Stahl performed the following experiment in 1958 :

(i) They grew E. coli in a medium containing $^{15}NH_4Cl$ (^{15}N is the heavy isotope of nitrogen) as the only nitrogen source for many generations. The result was that ^{15}N was incorporated into newly synthesized DNA (as well as other nitrogen containing compounds). This heavy DNA molecule could be distinguished from the normal DNA by centrifugation in a cesium chloride (CsCl) density gradient (Please note that ^{14}N is not a radioactive isotope, and it can be separated from MN only based on densities).

(ii) Then they transferred the cells into a medium with normal HNH1Cl and took samples at various definite time intervals as the cells multiplied and extracted the DNA that remained as double-stranded helices. The various samples were separated independently on CsCl gradients to measure the densities of DNA.

(iii) Thus, the DNA that was extracted from the culture one generation after the transfer from ^{15}N to ^{14}N medium [that is after 20 minutes; E. coli divides in 20 minutes] had a hybrid or intermediate density. 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.



Separation of DNA by Centrifugation

MECHANISM OF DNA REPLICATION :

(1) Origin of Replication :

It starts at a particular place called **origin of replication** or **Ori**. In prokaryotes replication starts at one point & entire DNA strand takes part in replication thus it contains **single replicon** while in Eukaryotes **several replicons** present.

DNA replicaton is **bidirectional, semidiscontinuous and semiconservative** in eucaryotes .

(2) Activation of Deoxyribonucleotides :

The phosphorylated nucleotides (deAMP, deGMP, deCMP , deTMP) are found in inactivated form. they react with ATP in the presence of **phosphorylase enzyme** & converted in to active deATP, deGTP, deCTP , deTTP.

(3) Exposure of DNA helix :

Helicase enzyme acts over the ori site of DNA template and unwinds the two strands of DNA.

SSB (single stranded binding) Protein prevents the recoiling of uncoiled DNA strands.

Topoisomerase cause nicking of one strand of DNA (for removing coils) and resealing the same. Along with **Topoisomerase**, **bacteria** possess another enzyme called **DNA Gyrase** which can introduce negative supercoils.

Whole of the DNA does not open in one stretch due to very high energy requirement but the point of separation proceeds slowly from one end to other . It gives the appearance of Y-shaped structure called **replication fork.**

(4) RNA Primer :

It is small strand of RNA (5–10 nucleotide). It is synthesized at 5'end of new strand with help of enzyme **Primase**. Formation of RNA primer constitutes the initiation phase of synthesis because without the presence of RNA primer, DNA polymerase can not add nucleotides.

MECHANISM OF DNA REPLICATION

Replication of DNA :

The synthesis of DNA from DNA is called Replication.

It usually occurs during **S-phase** of cell cycle.

DNA performs two types of functions

(A) Autocatalytic : DNA synthesizes new DNA by replication,

(B) Heterocatalyic : DNA helps in the synthesis of other substances like RNA, protein.
According to **Delbruck**, DNA replication is of three types.

(1) Dispersive : Old structure undergoes fragmentation. Fragments synthesise complementary structures, both of which assemble randomly to form two replicase.

(2) Conservative : A New structure is formed over the template of old structure (conserved)

(3) Semiconservative : One half is parent structure and one half new structure in each replica. It was firstly suggested by **watson and crick (1953).**

Taylor (1957) discovered semiconservative nature of DNA replication by the use **H^3 radioactive thymidine** in **broad been (Vicia faba).**

Meselson and stahl (1958) proved that DNA replication is **semiconservative.** They performed an experiment on **E. coli.**

They cultured E. coli bacteria in culture medium containing $N^{15}H_4Cl$ (Heavy isotope of N as N^{15}) for several generations.

Now they introduced labelled Bacteria in another culture medium contain N^{14} H_4 Cl (Normal N^{14}).

Now they used **density gradient centrifugation** method with **$CsCl_2$ (ceasium chloride)** to examine DNA of its offsprings.

They found that **DNA** was **intermediate type** in **first generation** in which one strand was heavy (containing N^{15}) and other strand was light (containing N^{14}).

Second generation of bacteria contained two types of DNA, **50% light ($N^{14}N^{14}$)** and **50% intermediate ($N^{15}N^{14}$).**

In **third generation** of bacteria contained **25% intermediate ($N^{15}N^{14}$)** and **75% light ($N^{14}N^{14}$)** in **1 : 3 ratio** and **fourth generation** bacteria contained **12.5% $N^{15}N^{14}$** and **87.5% $N^{14}N^{14}$ DNA** in **1 : 7 ratio.**

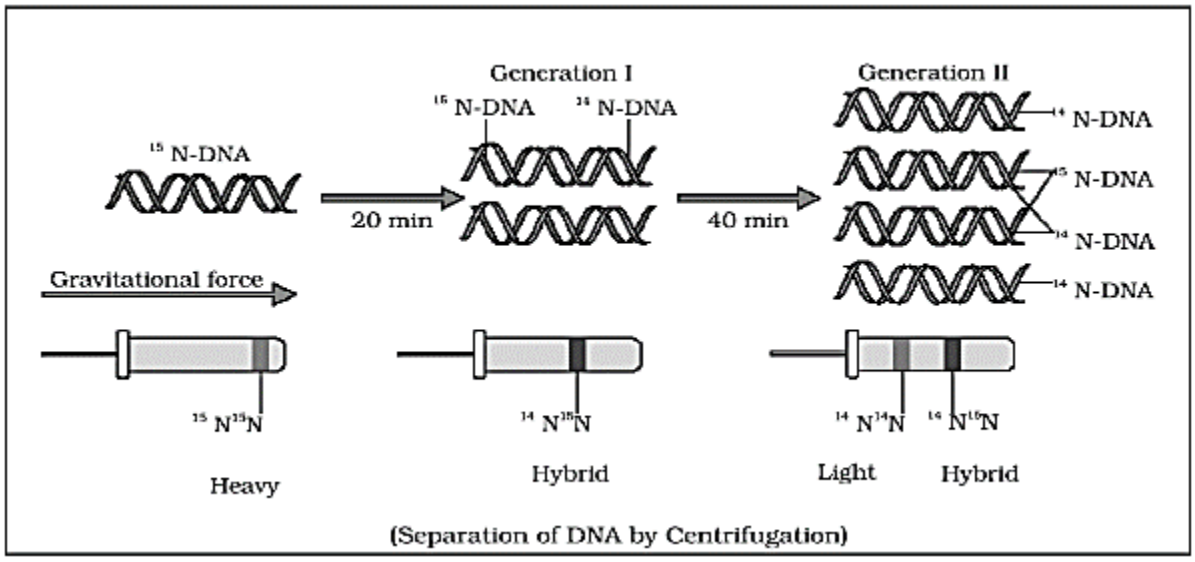


Figure : Semi-conservative replication of DNA (Results of experiment of Meselson & Stahl)

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Point of remember

In **eukaryotes**, the **function** of **primase** is carried out by **enzyme DNA polymerase.**

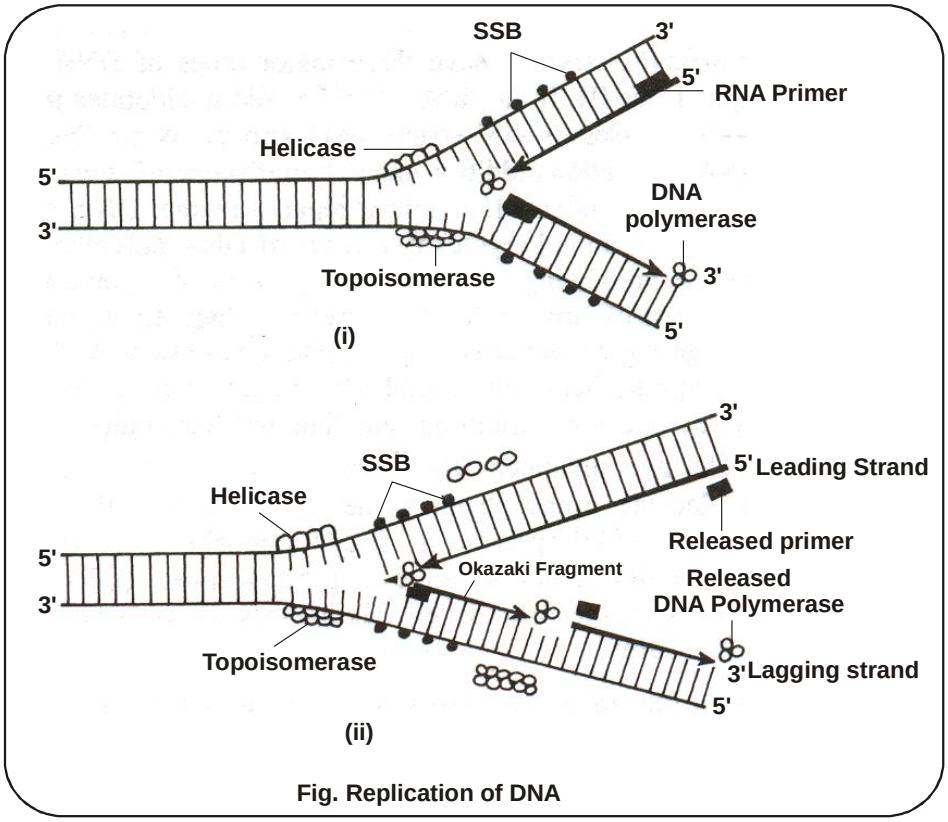


Fig. Replication of DNA

DNA POLYMERASE :

(5) Prokaryotes possess three types of DNA synthesising enzymes called **DNA polymerases III, II and I** they add nucleotides in **5'3' direction** on **3'5' strand.** DNA replication is mainly

performed by DNA polymerase III. DNA polymerase I is major repair enzyme where as polymerase II is minor repair enzyme.

In **eukaryotes five types of DNA polymerases (α, β, , δ, ε)** have been reported. out of them α, δ, ε are major enzymes. According to **sugino et al, DNA polymerase** acts at both the leading and lagging strands and initiates DNA synthesis along with primase activity while **DNA polymerase δ, ε** are involved in **elongation** of the **leading and lagging strands** respectively.

(6) Base Pairing :

Two separated strands of DNA in the replication fork function as template.

Deoxyribnucleoside triphosphates come to lie opposite the nitrogen bases of exposed DNA templates – deTTP opposite-A, deCTP opposite G, deATP opposite T and deGTP opposite C.

With the help of **pyrophosphatase** enzyme the two extra phosphates present on the dexyribonucleotides separate. Energy is released in this process that is utillized for base pairing.

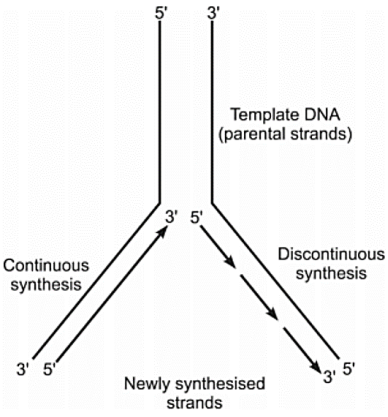
Energy is used in establishing hydrogen bonds between the free nucleotides and nitrogen bases of templates.

(7) Chain formation :

It requires **DNA polymerase III** in **prokaryotes** and **polymerase** in **eukaryotes**. **DNA polymerase III** is a complex enzyme having **seven subunits (α, β,, γ, , θ, τ).**

In the presence of Mg^{++}, ATP/ GTP, TPP and DNA polymerase -III, the adjacent nucleotides attached to

nitrogen bases of each template DNA strand establish **phosphodiester bonds** and get linked to form replicated DNA strand. Two strands of DNA run antiparalled to each other.



Replication on one DNA template is continuous in **5'3' direction** due to opening of its 3' end this newly formed strand is called **leading strand**.

On the second DNA template the replication of DNA is **discontinuous** due to opening of small stretch of fork at a time. Small fragments deposite with the help of RNA primer. these fragements are called **okazaki fragements (1000 - 2000 nucleotides in prokaryotes and 100–200 in eukaryotes).**

After deposition of each okazaki fragment RNA primer is released and gap is filled by the activity of DNA polymerase thus the new strand is formed called **Lagging strand**.

After deposition of bases **DNA Ligase** enzyme seals these bases.

Thus one strand grows continuously while the other strand is formed discontinuously hence DNA replication is **semidiscontiuous**.

(8) Proof reading and DNA repair:

Sometimes wrong base is deposited in the strand. **DNA poymerase III** is able to check this error and removes the wrong base. It allows addition of proper base but DNA polymerase III can not distinguish **uracil** from **thymine** such an error is corrected by number of enzymes.

DNA polymerase I removes the wrong base and attaches the correct base in the strand in Prokaryotes where as **DNA polymerase β** in eukaryotes.

ONE GENE–ONE ENZYME HYPOTHESIS :

Archibald garrod (1909) : He stated that diseases inherited from parents are inborn error of metabolism.These are due to defect in a single enzyme that catalyzes a particular reaction.

Beadle and Tatum, (1948) Proposed **One gene–One enzyme hypothesis**. They conducted experiments on the nutrition of **pink mould (Neurospora crassa).** This fungus grows on simple nutrient medium and has the ability to synthesize all its cellular components. Such an organism is called **prototroph.**

An organism that is unable to synthesize a particular cellular component such as an amino acid or coenzyme is called **auxotroph**.

Beadle and tatum produced **arginine** (an amino acid) auxotrophs (mutants of Neurospora unable to synthesize arginine).

Arginine synthesis passes through the following path-

Ammonia + Sugar Ornithine Citrulline Arginine

They found that any step of this metabolic chain could be blocked by a mutation in a specific enzyme catalyzing the reaction, each enzyme representing a different gene product. Thus Beadle and Tatum reached a conclusion that each gene regulates the synthesis of a single enzyme. This laid the foundation of biochemical genetics. Beadle and tatum were awarded Nobel Prize in 1958.

ONE GENE–ONE POLYPEPTIDE HYPOTHESIS :

Yanofsky et al (1965) : proposed one gene–one polypeptide hypothesis He stated that a structural gene regulates the synthesis of a single polypeptide. Haemoglobin is composed of two and two polypeptides synthesised by two separate genes.

ONE GENE–ONE FUNCTION HYPOTHESIS :

A **gene or cistron** (It is a part of DNA composed of stretch of deoxyribonucleotides that codes for a biochemical controlling other cistrons, rRNA, tRNA or polypeptide through mRNA) performs one function, **structural or regulatory**.