Chapter 20

CHROMOSOMES AND DNA

CHROMOSOMES:

Chromosomes are thread like structure that appear inside the nucleus at the time o cell division.

Discovery:

They were **first observed** by the German embryologist Walther Fleming in 1882, when he was examining the rapidly dividing cells of salamander larvae.

Number of Chromosomes in Different Organisms:

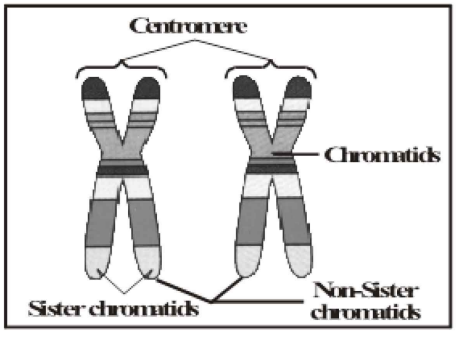
Chromosomes are found in the cells of all eukaryotes. Their number however varies from species to species. **Penicillium**, a fungus, has only one pair of or 2 chromosomes, while some ferns have more than 500 pairs or 1000 chromosomes. A **mosquito** has 3N, **honeybee** 16 pairs, **corn** 10 pairs, **sugarcane** 40, **frog** 13 and a **mouse** has 20 chromosomes. **Human** cells have 46 chromosomes, consisting of 23 pairs.

Function of Chromosomes:

Each chromosomes contains hundreds or thousand of genes that play important roles in determining how a person's body develops and functions. The possession of all the chromosomes is therefore essential for survival. Missing of a part or whole of chromosome leads to serious consequences, and usually death occurs.

Structure of a typical chromosome:

Typically a chromosome is made of chromatids (two replicas), centromere (primary constriction) and a secondary constriction.



Karyotype:

Chromosome may widely differ in appearance. They vary in size, staining properties, the location of centromere, the relative length of the two arms on both sides of centromere and the position of constricted regions along the arms.

Definition:

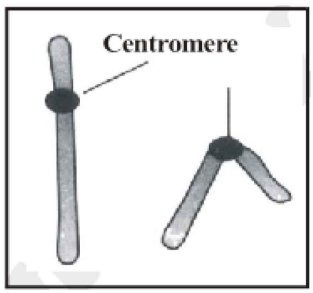
The particular array of chromosomes that an individual possesses is called its Karyotype. Karyotypes show marked differences among species and sometimes even among individuals of the same species.

Types of Chromosomes:

The chromosomes are usually classified on the basis of position of centermere. They are divided into four (4) groups, depending upon the location of centromere between the middle and tip of the chromosomes. These chromosomes. acquire different shapes at the time of anaphase during cell division. The usual shapes are i,j and v.

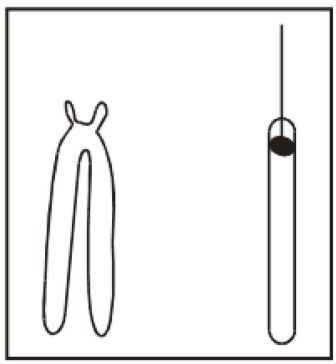
Telocentric:

These are the chromosomes which have centromere at one end. The arms are located on one side only. They appear 'i' shaped during division.



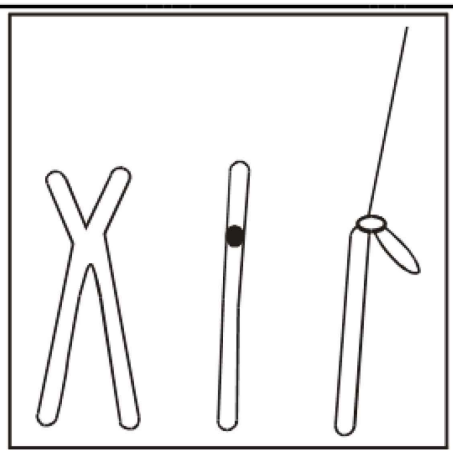
Acrocentric:

They have centromere very close to one end. The arms of one side are very short. They also appear 'i' shaped during cell division.



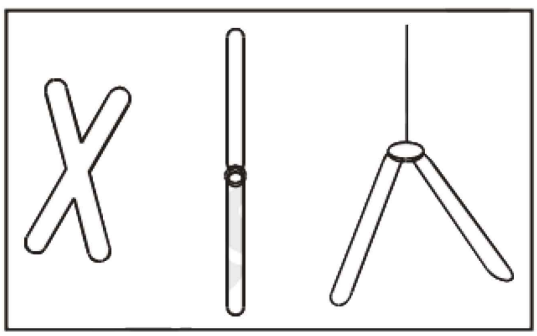
Sub Metacentric:

They have centromere displaced from the center. The arms of both the sides are clearly unequal. They appear 'J' shaped.



Metacentric:

These are the chromosomes which have centromere in the center. The arms of both sides are equal or almost equal. They appear 'V' shaped.



Composition of Chromosomes:

Chromosomes are composed of the following substances.

DNA:

` It exists in the form of a single long double helical molecule. 40% of each chromosomes is DNA.

Protein:

Most chromosomes contain about 60% protein. These are of special type known as 'Histones'. Histones are **positively charged** (most proteins are negativley charged) due to an abundance of the basic amino acids, **aginine** and **lysine**. They are thus strongly attracted to the negatively charged phosphate groups of the DNA.

RNA:

A significant amount of RNA is also associated with chromosomes, because these are the sites of synthesis of all types of RNA.

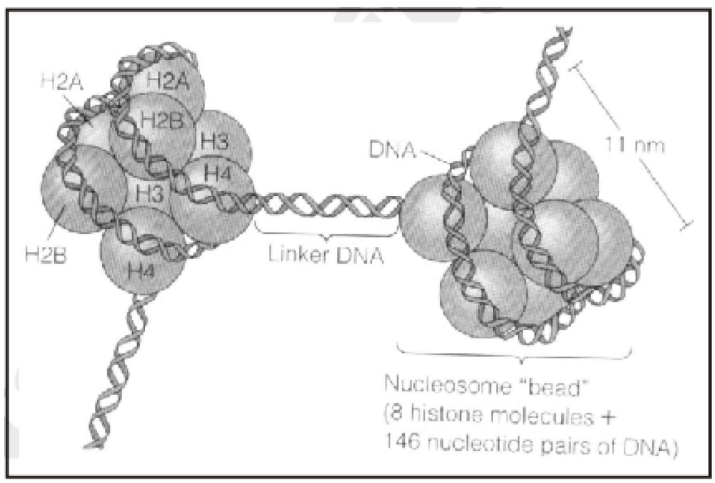
The DNA of a chromosomes is one very, double stranded fiber that extends unbroen through the entire length of the chromosome. A typical human chromosome contains

about 140 million (1.4×10^8) nucleotides in its DNA. The amount of information one chromosome contains would fill about 280 printed books of 1000 pages each, if each nucleotide corresponds to a word and each page had about 500 words on it. Further more, if the strand of DNA from a single chromosome were laid out in a straight line, it would be about 5centimetres long. Fitting such a strand into a small space of nucleus is nature's marvel – and that's only 1 of 46 chromosomes. In the cell, however, the DNA is coiled; allowing it to fit into a much smaller space than would otherwise be possible.

Nucleosomes:

These are small bead like structures formed by the attachment of DNA and histone proteins.

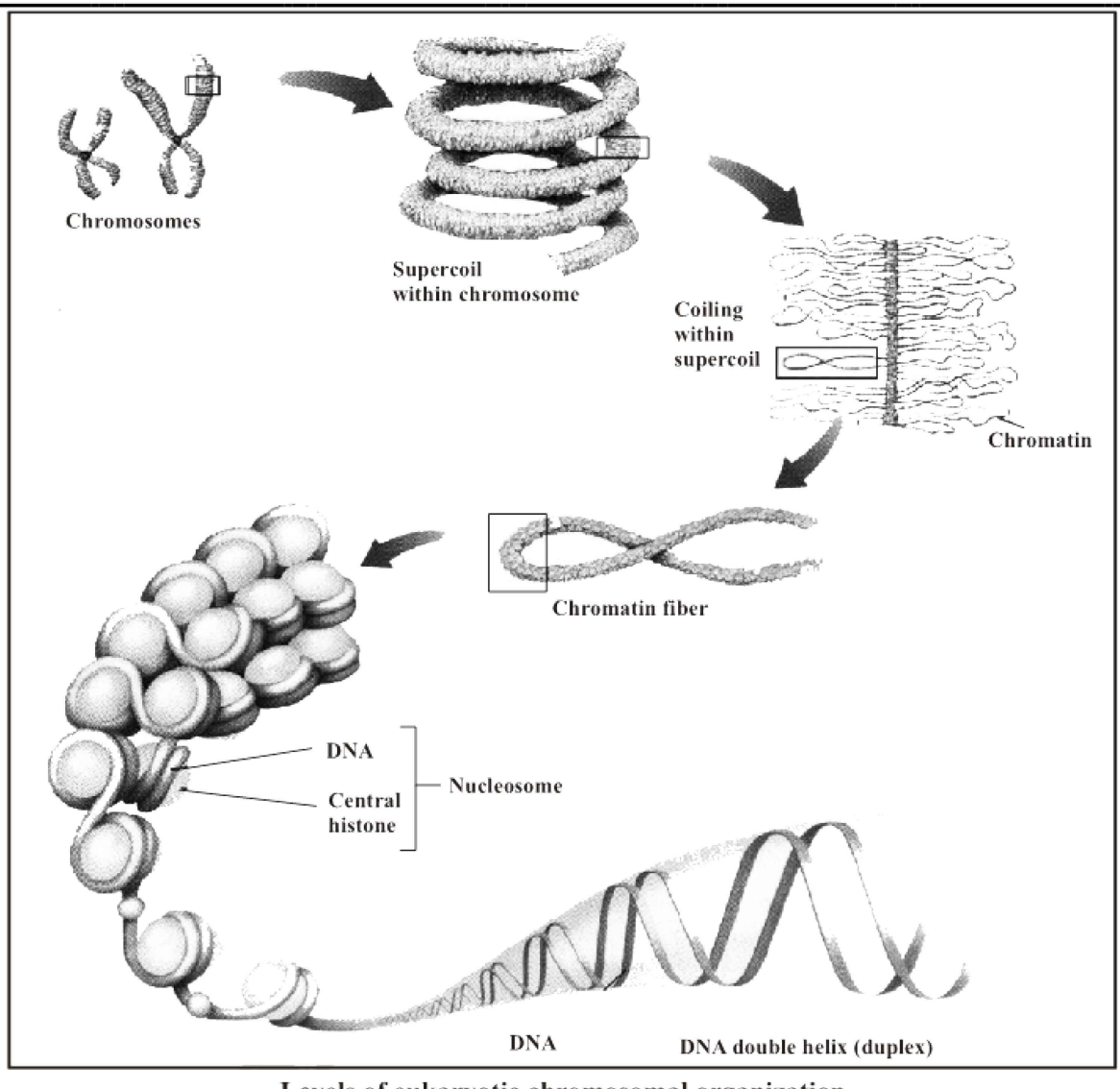
If a eukaryotic chromatin is examined with an electron microscope, it resembles a string of beads. Every 200 nucleotides, the DNA duplex is coiled around a core of eight histone proteins (histone actamere) forming a complex known as a nucleosome. Each nucleosome is about ***10nm*** in diameter.



Condensation:

At the onset of division, the thin long chromatin condenses (becomes thicker and shorter) into rod like chromosomes. Condensation involves coiling and super coiling of chromatin thread.

The histone cores act as magnetic forms that promote and guide the coiling of the DNA. Further coiling occurs when the string of nucleosomes wraps up into higher order coils called supercoils.



Levels of eukaryotic chromosomal organization

Heterchromatin:

Portions of the chromatin that remain highly condensed are called heterochromatin. Some of these portions remain permanently condensed, so that their DNA is never expressed.

Euchromatin:

The part of the chromatin, which is condensed only during cell division, is called euchromatin. At all other times, euchromatin is present in an open configuration and its genes can be expressed. The compact packaging, during division facilitates the movement of the chromosomes.

THE CHROMOSOMAL THEORY OF INHERITANCE

Statement:

According to this theory, " the genes are physical units located on chromosomes".

Contribution of Karl Correns:

A central role for chromosomes in heredity was first suggested in 1900, by the German geneticist Karl Correns. He, in one of the papers announced the rediscovery of Mendel's work.

Contribution of Walter Sutton:

The chromosomal theory of inheritance was first formulated by the American scientist **Walter Sutton in 1902**.

Several Pieces of evidence supported Sutton's theory:

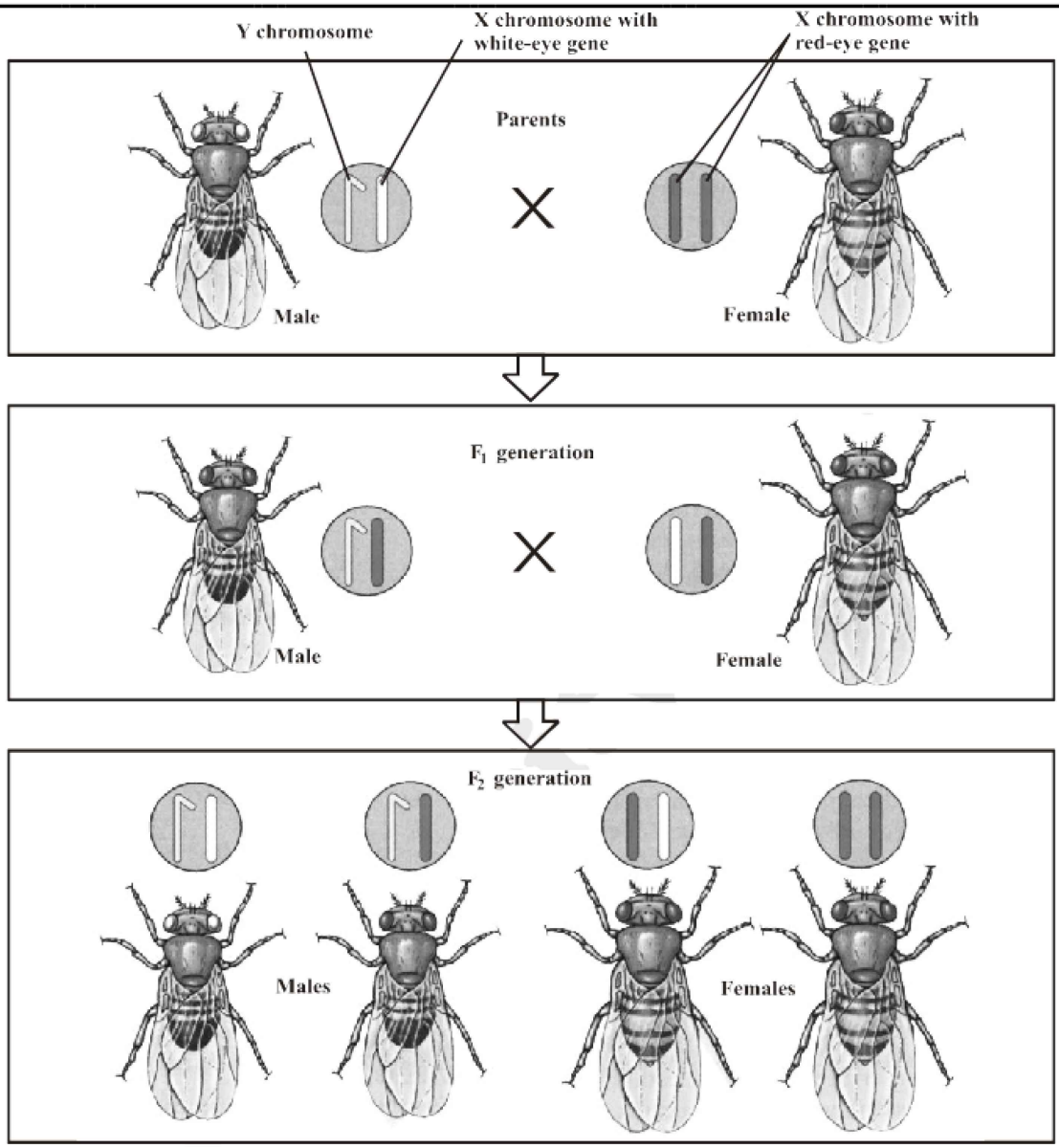
(i) Reproduction involves the initial union of only two cells, egg and sperm. If Mendel's model was correct, then the two gametes must make equal hereditary contributions. Sperm, however, contain little cytoplasm, suggesting that the hereditary material must reside within the nuclei of the gametes.

(ii) The diploid individuals have two copies of each chromosome (in the form of homologous pair), while gametes have only one. This observation was consistent with Mendel's model, in which diploid individuals have two copies of each heritable gene and gametes have one.

(iii) Chromosomes segregate during meioses, and each pair of homologous chromosomes orients on the metaphase plate independent of every other pair.

Problem with the Theory

There is however open problem with this theory. If Mendelian characters are determined by genes located on the chromosomes, and if independent assortment of Mendelian traits reflects the independent assortment of chromosomes in meiosis, why does the number of characters that assort independently in a given kind of organism often greatly exceed the number of chromosome pairs the organism possesses? This is led many early researchers to have serious reservations about Sutton's theory.



Morgan's experiment demonstrating the chromosomal basis of sex linkage.

DNA as Hereditary Material

A large number of scientists contributed to the discovery of genetic material. A few most important works are mentioned here.

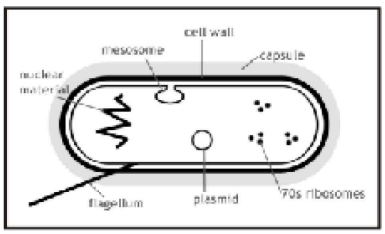
Contribution of Griffith

The first evidence of hereditary nature of DNA was provided by a British microbiologist Frederick. Griffith (1928) who made some unexpected observations while experimenting with pathogenic bacteria.

He was working on two related strains of ***Streptococcus pneumonia*** bacteria (previously known as ***Pneumococcus***)

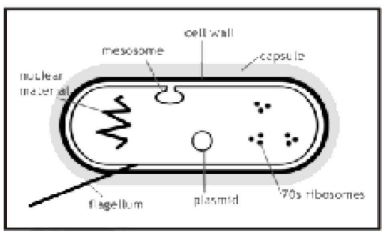
i.e. Virulent strain and mutant strain.

(A) S-Type Bacteria



These bacteria has a capsule of polysaccharides, formed smooth colonies on culture dish and are virulent (pathogenic).

(B) R-Type Bacteria



These bacteria had lost the gene for capsule by mutation, form rough colonies on culture dish and are non virulent.

Experimental Trials on Mice

(i) When he injected mice with a virulent strain of *streptococcus pneumonia* bacteria, the mice died of blood poisoning caused by pathogenic bacteria.

(ii) When he injected similar mice with a mutant strain of S. *pneumonia* that lacked the virulent polysaccharide coat, the mice showed no ill effects. The coat was apparently necessary for virulence.

(iii) To determine whether the polysaccharide coat itself had a toxic effect, Griffith injected dead bacteria of the virulent S strain into the mice; the mice remained perfectly healthy.

(iv) As a control, he injected mice with a mixture containing dead bacteria of the virulent, S strain and live coatless R bacteria, each of which by itself did not harm the mice.

Unexpectedly, the mice developed the disease symptoms and many of them died.

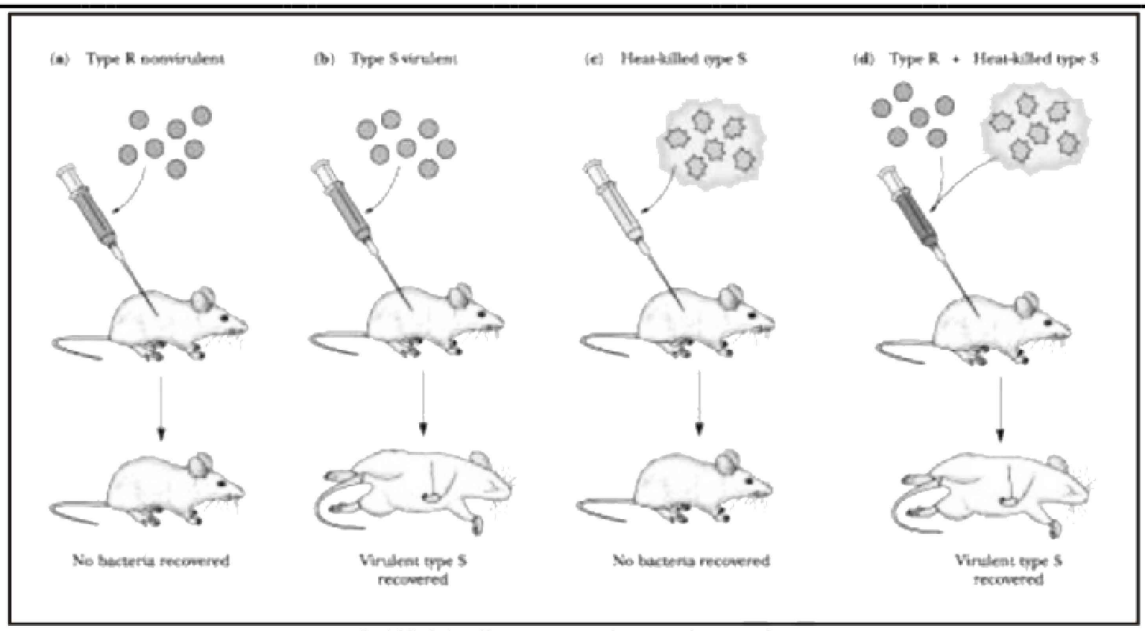
Observations

The blood of the dead mice was found to contain high levels of live, virulent streptococcus type S bacteria, which had surface proteins characteristic of the live (previously R) strain.

Somehow, the information specifying the polysaccharide coat had passed from the dead, virulent S bacteria to the live, coatless R bacteria in the mixture, permanently transforming the coatless R bacteria into the virulent S variety. This process was known as Transformation.

Transformation

The transfer of genetic material from one cell to another.



Griffith's discovery of transformation

Contribution of Avery McLeod and McCarty:

In 1944, in a classic series of experiments Oswald Avery along with Colin MacLeod and Maclyn McCarty characterized what they referred to as the Transforming principle.

- They first prepared mixture of dead S-type Streptococcus and line R streptococcus that Griffith had used. Then they removed s much of the protein as they could from their preparation, eventually achieving 99.98% purity.
- Despite removal of nearly all the protein, the transforming activity was not reduced.
- Moreover, the properties of transforming principle resembled that of DNA.
- The protein digesting enzymes of RNA digesting enzymes did not affect the transforming activity, but the DNA digesting enzyme (Dnose) destroyed all the transforming activity.

Conclusion:

The above experiments concluded that the gene for capsule formation is composed of DNA.

EXPERIMENT OF HERSHEY AND MARTHA CHASE

In 1952 **Alfred Hershey and Martha Chase** experimented with bacteriophages T_2 and provided additional 9evidence supporting Avery's conclusion. They produced two types of viruses.

Phage Labelled with P^{32}

These viruses were provided with radio (radioactive) isotope ^{32}P, which was incorporated into the newly synthesized DNA of growing phage.

Phage Labelled with S^{35}

These viruses were grown on a medium containing ^{35}S, a radioactive isotope of sulphur, which is incorporated into the amino acids of newly synthesized protein coats.

Exposure of bacteria to labelled viruses:

Now are bacterial culture was exposed to ^{32}P labelled viruses and other culture was exposed to ^{35}S labelled viruses.

Removal of protein coats:

The bacterial cells were agitated violently to remove any outside material (hte protein coats) of the infecting viruses from the surfaces of the bacteria.

Testing of the presence of label:

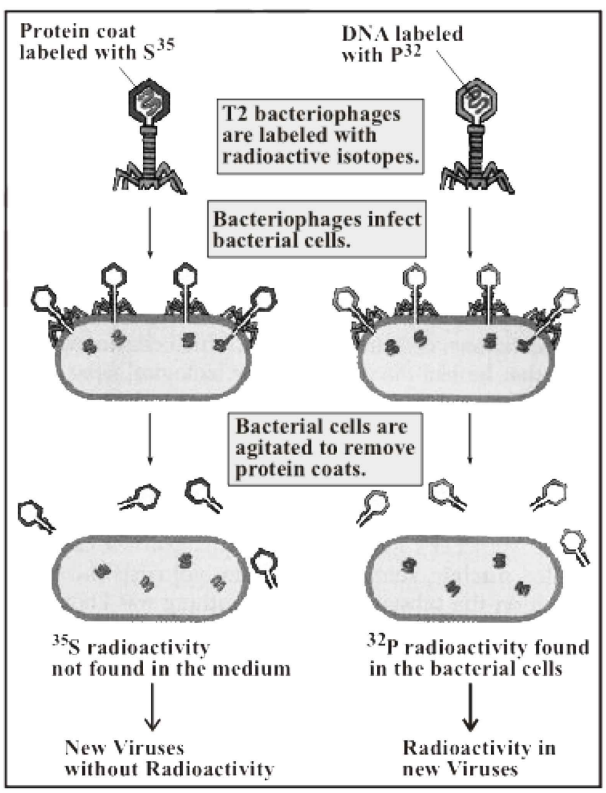
When bacteria were tested for the presence of label it was found that one group of bacteria had lost nearly all of ^{35}S label. However, ^{32}P label was present in bacteria of the other group.

Reason:

It is because ^{35}S was only present in protein coat while ^{32}P was present in DNA that had transferred to the interior of the bacteria. It was confirmed when viruses released from ^{32}P culture were labelled while3viruses released from ^{35}S culture were unlabelled.

Conclusion:

new viruses are produced inside the host cells, and only DNA enters into the bacterial cells. Thus it was proved that the hereditary information injected into the bacteria that specified the new generation of viruses was DNA and not protein.



CHEMICAL NATURE OF DNA

Discovery of DNA:

A German Chemist, **Friedrich Miescher**, discovered DNA in 1869, only four years after Mendel's work was published. Miescher extracted a white substance from the **nuclei of human cells and fish sperm**. He called this substance **"nuclein"** because it seemed to be specifically associated with the nucleus.

Since nuclein was acidic, later on, it came to be known as **nucleic acid.**

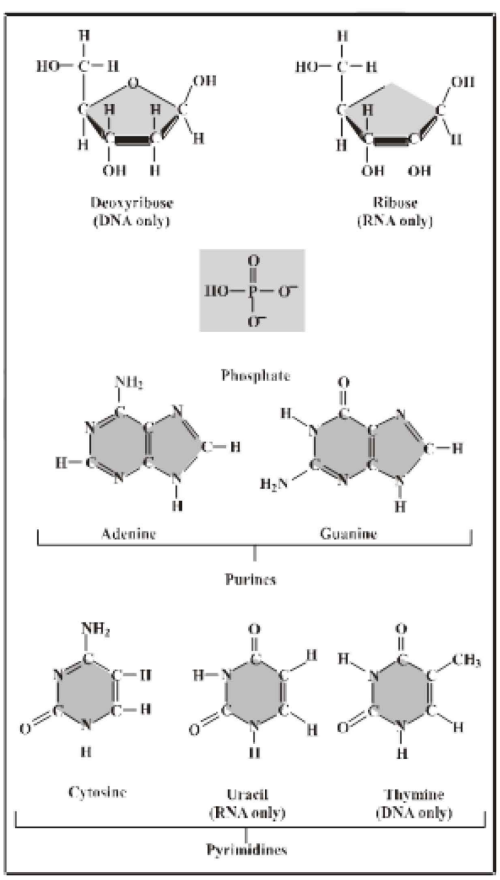
Work of P.A. Levene:

In 1920's, the basic structure of nucleic acids was determined by the biochemist P.A. levene, who found that DNA contains three main components:

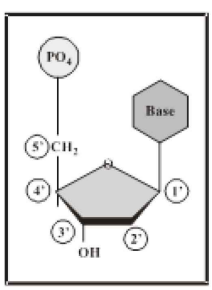
1. Phosphate (PO_4) groups,
2. Five carbon sugar (a pentose), Deoxyribose,
3. Nitrogen containing bases called purines (**adenin, A, and guanine,** G) and pyrimidines (**thymine, T and cytosine, C, RNA** contains **Uracil, U** instead of T).

Structure of a Typical Nucleotide:

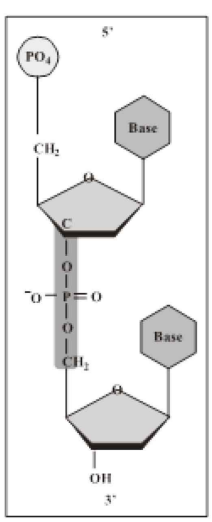
DNA and RNA molecules are made of repeating units called nucleotides. In a nucleotide, nitrogen base is attached to carbon number 1 of a pentose sugar and phosphate group is attached to carbon number 5 of the sugar. In addition a free hydroxyl (-OH) group is attached to the carbon atom number 3 of sugar.



Nucleotide subunits of DNA and RNA



Numbering the carbon atoms n a nucleotide.



A Phosphodiester bond.

FORMATION OF PLYNUCLEOTIDE CHAIN

Phosephodiester Linkage:

The reaction between the phosphate group of one nucleotide and the hydroxyl group of another is a **dehydration synthesis**, eliminating a water molecule and forming a covalent bond that links the two groups. The linkage is called a phosphodiester bond because the phosphate group is now linked to the two sugars by means of pair of ester (P-O-C) bonds.

Polynucleotide chain:

In this way many thousands of nucleotides can join together in long chains.

5' and 3' ends:

Linear strands of DNA or RNA no matter how long, will almost always have a free 5' phosphate group at one end and a free 3' hydroxyl group at the other.

WORK OF CHARGAFF

Erwin chargaff showed that the amount of adenine in DNA always equals the amount of thymine, and the amount of guanine always equals the amount of Cytosine. It implies that there is always equal proportion of the two pairs, i.e., A equal T and G equals C.

Relative amounts of bases in DNA from various organisms

(on percentage basis).

Source of DNA	Adenine	Guanine	Thymine	Cytosine
Man	30.9	19.9	29.4	19.8
Sheep	29.3	21.4	28.3	21.0
Wheat	27.3	22.7	27.1	22.8
Yeast	31.3	18.7	32.9	17.1

WORK OF WILKINS AND FRANKILIN

They made **X-ray diffraction studies** of DNA. In this analysis a molecule is bombarded with a beam of X-rays. When individual rays encounter atoms their path is bent or diffracted and the diffraction pattern is recorded on the photographic film. When carefully analyzed this pattern gives three-dimensional structure of a molecule.

Rosalind Franklin prepared this X-rays diffraction pattern of DNA fibers. The diffraction pattern prepared suggested that the DNA molecule had a shape of a helix with a diameter of **2 nm** and complete **helical turn every 3.4 nm**.

DOUBLE HELICAL STRUCTURE OF DNA (WASTON – CRICK'S MODEL)

In 1953, James Watson and Francis crick, two young researchers in university of Cambridge, proposed structure of the DNA molecule. Important features of the model are as follows:

(i) The DNA molecule is a simple double helix, with the nitrogenous bases of two strands pointed inward, toward each other, forming base pairs.

(ii) Base pairs always consist of purines, which are large, pointing toward pyrimidines which are small, keeping the diameter of the molecule a constant 2 nm.

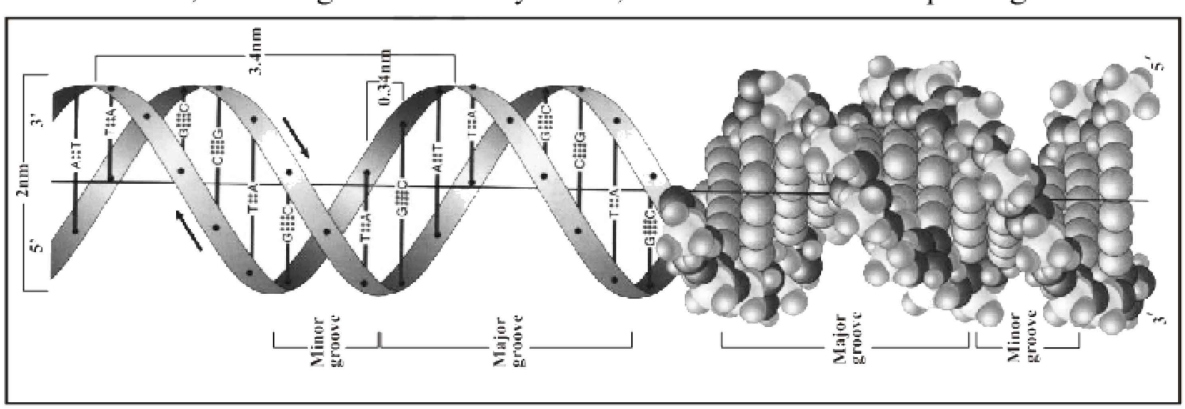
(iii) Because hydrogen bonds exist between the bases in a base pair, the double helix is stabilized as a duplex.

(iv) DNA molecule is compsed of two antiparallel strands, one chain running 3' or 5' and the other 5' to 3'.

(v) The base pairs are planar (horizontal) and stack 0.34nm apart as a result of hydrophobic interactions contributing to the overally stability of the molecule.

(vi) In the double helix adenine forms two hydrogen bonds with thymine while guanine forms three hydrogen bonds with cytosine. (Adenine will not form proper hydrogen bonds with Cytosine and Guanine will not form hydrogen bonds with thymine).

(vii) Adenine and thymine will always occur in the same proportion in any DNA molecule, as well guanine and cytosine, because of this base pairing.



Explain the different models of DNA Replication.

DNA Replication:

Definition:

Formation of DNA from DNA is called Replication.

Models of DNA replication:

There are different models proposed for DNA replication.

1. Semi-Conservative Replication:

The Watson-Crick model immediately suggested that the basis for copying the genetic information is **complementarity**.

If one were to unzip the molecule (open the helix), one would need only to assemble the appropriate complementary nucleotides on the exposed single strands to form two daughter complexes with the same sequences.

This form of DNA replication is called semi-conservative, because while the sequence of the original duplex is conserved after one round of replication, the duplex itself is not. Instead, each strand of the duplex becomes part of another duplex.

In **semi-conservative replication,** the two strands of the duplex separate out each acting as a **model** or **mold**, along which new nucleotides are, arranged thus giving rise to two new duplexes. In this process by separation of two strands, primary structure has been conserved, whereas the secondary structure has been disrupted.

2. Conservative Model:

The Conservative model stand that the **parental** double **helix** would remain **intact** and generate DNA copies consisting of entirely new molecules.

3. Disperssive Model:

The dispersive model predicted that parental DNA would become completely dispersed and that each strand of all the daughter molecules would be a mixture of old and new DNA.

The Meselson – Stahl Experiment:

The **three hypothesis** of **DNA replication** were evaluated by Mathew Meselson and **Franklin Stahl** of the **California Institute** of Technology in **1958**.

1. Growth of Bacteria in N^{18} medium:

They grew bacteria in a medium containing heavy isotopes of nitrogen, N^{15}, which became incorporated into the N – bases of the bacterial DNA.

2. Transfer of Bacteria in N^{14} medium:

After several generations, the DNA of these bacteria was denser than that of bacteria grown in a medium containing the lighter isotope of nitrogen, N^{14}.

Meselson and Stahl then transferred the bacteria form the N^{15}, medium to the N^{14} medium and collected the DNA at various intervals.

3. Collection of sample from N^{14}:

They dissolved the DNA in Cesium Chloride and then spun it at a very speed in an ultra-centrifuge.

DNA Strands of different densities got separated.

The enormous centrifugal forces generated by the ultra centrifuge caused the cesium ions to migrate towards the bottom of the centrifuge tube, creating a gradient of CsCl, and thus of density.

Each DNA floats or sinks in the gradient until it reaches the position where its density exactly matches the density of cesium there.

Because N^{15} strands are denser than N^{14} strands, they migrate farther down the tubes to a denser region of the cesium chloride gradient.

a. Zero Round of replication:

Result:

The DNA collected immediately after the transfer was all dense.

b. First Round of DNA Replication (after 20 min):

Result:

However, after the bacteria completed their first round of DNA replication in the N^{14} medium, the density of their DNA had decreased to a value intermediate between N^{14} --- DNA and N^{15} --- DNA.

c. Second Round of Replication (after 40 min):

Result:

After the second round of replication, two density classes of DNA were observed one intermediate and one equal to that of N^{14} – DNA.

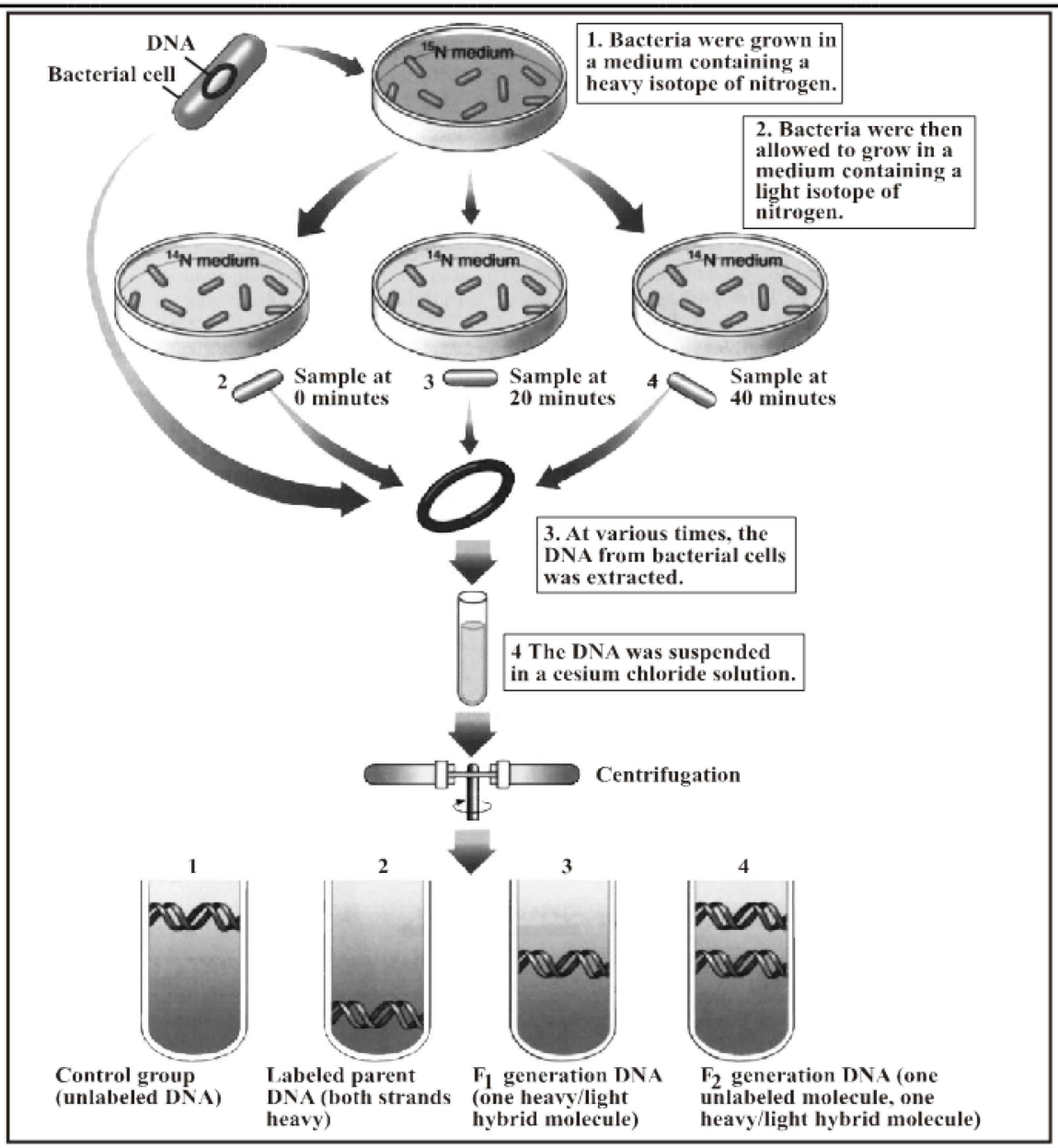
Interpretation of Results:

Meselson and Stahl interpreted their results as follows:

(i) After the first round of replication, each daughter DNA duplex was a hybrid possessing one of the heavy strands of parent molecule and one light strand.

(ii) When this hybrid duplex replicated, it contributed one heavy strand to form another hybrid duplex and one light strand to form a light duplex.

This experiment clearly confirmed the prediction of the Watson – Crick model that DNA replicates in a semi-conservative manner.



This meselson and Stahl experiment: Evidence demonstrating semi – conservative replication.

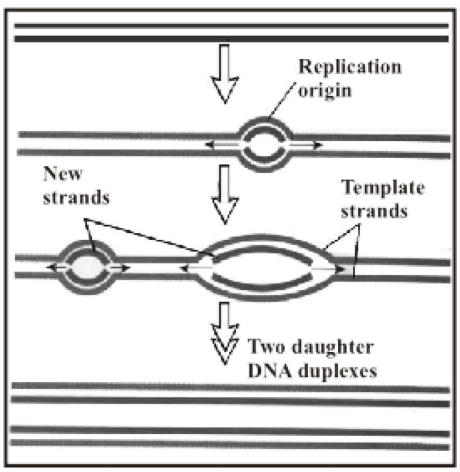
THE REPLICATION PROCESS

Definition:

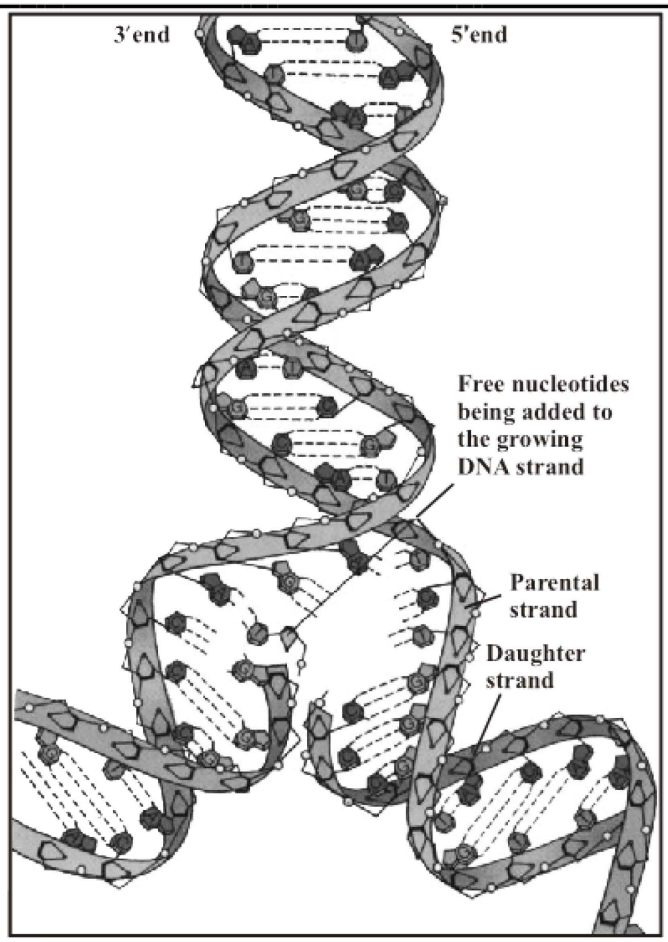
Formation of DNA from DNA is called Replication of DNA.

Replication Site:

The DNA replication begins at one or more sites on the DNA molecule, where there is a specific sequence of nucleotides.



Origins of replication



Replication of DNA

Helicases:

These enzymes are required to unwind DNA double helix. Two strands in a duplex are separated.

Single Strand Binding Proteins (SSBP):

The two separated single strands are stabilized by single-stand binding proteins, which bind to the exposed single strands protecting them from cleavage and preventing them from rewinding.

DNA POLYMERASES

DNA Polymerases are the enzymes which begin a complex process that catalyzes the addition of nucleotides in the growing complementary strands of DNA.

There are these DNA polymerases namely I, II and III in bacteria.

1. DNA polymerase I:

DNA polymerase I is a relatively small enzyme that plays supporting role in DNA replication. It links a few nucleotides usually to full the gaps etc.

2. DNA polymerase II:

DNA polymerase II plays a role in DNA repair.

3. DNA polymerase III:

The true E, coli replicating enzyme is DNA polymerase III, which is 10 times larger and far more complex in structure than the other enzymes.

The enzyme is a dimer and catalyzes replication of one DNA strand.

DNA polymerase progressively threads the DNA through the enzyme complex moving it at a rapid rate of some 1000 nucleotides/second.

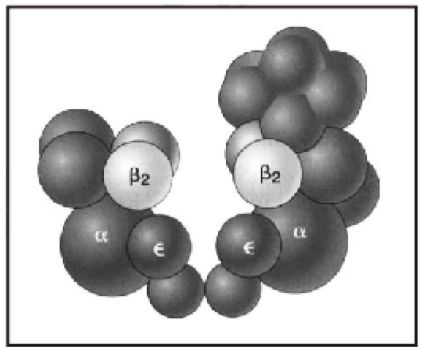
Attachment of Primer:

The DNA polymerase III can add nucleotides only to a chain of necleotides that is already paired with the parental strads, Hence DNA polymerase **cannot initiate** synthesis on its own.

An enzyme, **primase**, constructs an RNA primer, a sequence of about 10 construct the DNA strands. The RNA nucleotides in the primers are later on replaced by DNA nucleotides.

Direction of Addition of Nucleotides:

DNA polymerase III can add nucleotides only to the 3' end of a DNA strand; this means that replication always procedes 5' → 3' direction on a growing DNA strand.



Anti-parallel strands of DNA:

Because the two parent strands of a DNA molecule are anti-parallel, the new strands must elongate by different mechanisms.

The Leading Strand:

The leading strand, which elongates **towards the replication fork**, is built up simply by adding nucleotides continuously to its growing 3' end.

The Lagging Strand:

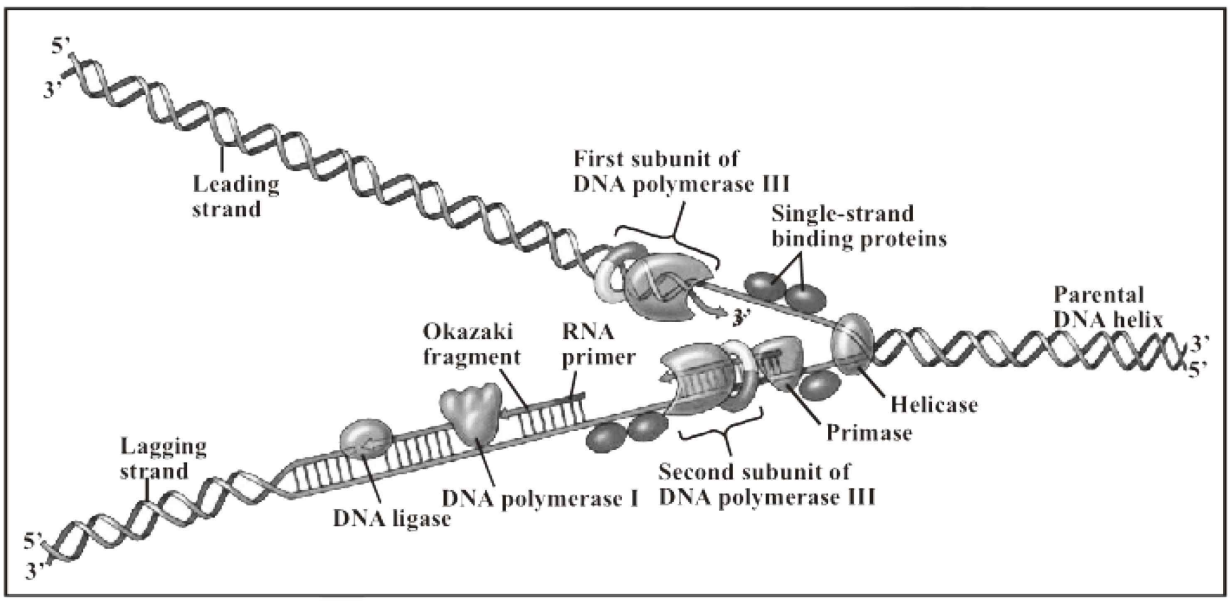
In contrast, the lagging strand, which elongates away from the replication fork. is synthesized discontinuously as a series of short segments that are later connected together.

Okazaki Fragments:

These are small segments of DNA, which are about 100 – 200 nucleotides long in eukaryotes and 1000 – 2000 nucleotides long in prokaryotes. Each Okazaki fragment is synthesized by DNA polymerase III in 5' → 3' direction, beginning at the replication fork (from a primer) and moving away from it.

DNA Ligase:

When the polymerase reaches the 5' end of the adjacent fragment of the lagging strand, the enzyme, **DNA ligase**, attaches the fragment to the 5' end of the next fragment of the lagging strand. The DNA is further unwound, new RNA primers are constructed, and DNA polymerase III then jumps ahead 1000 – 2000 nucleotides (toward the replication fork) to begin constructing another Okazaki fragment. This process continues up to the completion of replication.



WHAT IS A GENE

Each gene produces its effect by controlling the synthesis of a particular enzyme. This is called **one-gene one-enzyme hypothesis**. Since each enzyme is formed of polypeptides so this hypothesis may be known as **one-gene one-polypeptide** hypothesis.

Work of Garrod and Bateson:

Archibald Garrod and William Bateson concluded in 1902 that certain metabolic diseases among their patients were more prevalent in particular families. By examining several generations of these families, Garrod found that some of the diseases behaved as if they were the product of simple recessive alleles.

Alkaptonuria:

In Alkaptonuria the patients produced urine that contained **homogentisic acid**. This substance oxidized rapidly when exposed to air, turning the urine black. In normal individuals, homogentisic acid is broken down into simpler substances.

With considerable insight Garrod concluded that patients suffering from Alkaptonuria lacked the enzyme necessary to catalyze this breakdown.

He speculated that **many other inherited diseases might also reflect enzyme defriciencies.**

Conclusion:

From Garrod's findings, it is concluded that the information present within the DNA of chromosomes acts to specify particular enzymes.

CENTRIBUTION OF BEADLE AND TATUM

In 1941 a series of experiments were conducted by Stanford university geneticist **George Beadle** and **Edward Tatum** who provided clear evidence on the functions of genes.

Neurospora (Wild type):

They selected wild type of neurospora (a fungus known as red bread mold for their experiments, which could grow on minimal medium.

Minimal Medium:

It was the medium that provided a few substances for the growth of the mold. It contained only **sugar, ammonia, salts, a few vitamins and water**. Neurospora can synthesize all of the other substances for itself through metabolic pathways, because it contains normal enzymes.

Exposing of spores to X-rays:

Beadle and Tatum exposed spores of **Neurospora** to X-rays, expecting that DNA in some of these spores would experience damage in their ability to make compounds needed for normal growth.

DNA changes of this kind are called **mutations** and the organisms that have undergone such changes are called **mutants**.

Growth of Irradiated Spores:

Initially, they allowed the progeny of the irradiated spores to grow in a complete medium containing all of the nutrients necessary for growth, so that any growth deficient mutants resulting from the irradiation could take up the required substance from the medium and would be kept alive.

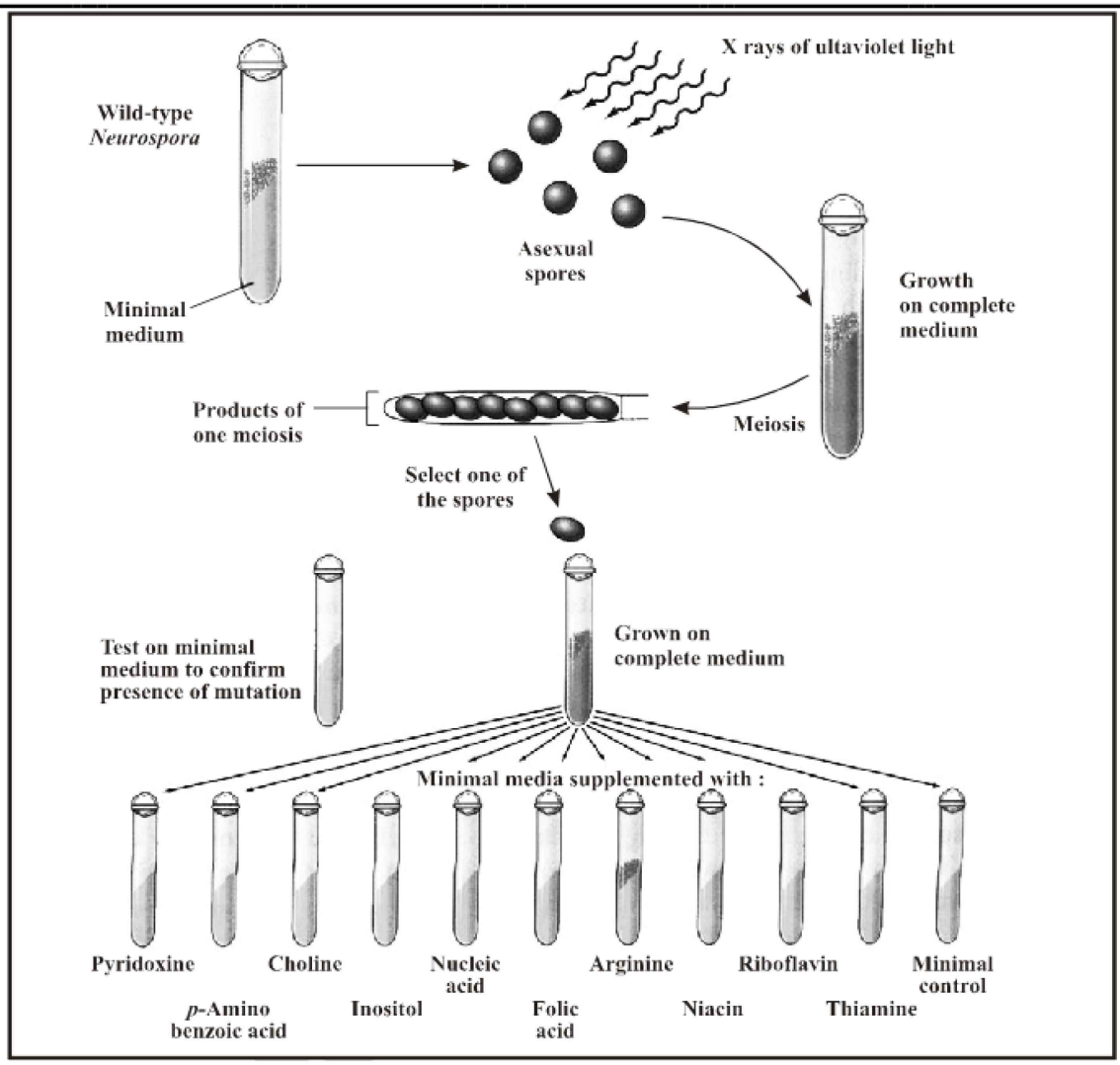
DETERMINATION OF MUTANTS

To determine whether any of the progeny of the irradiated spores had mutation causing metabolic deficiencies, Beadle and Tatum placed subcultures of individual fungal cells on a "minimal" medium.

Cells that had lost the ability to make other compounds necessary for growth would not survive on such a medium.

They added one by one, various chemicals to the minimal medium in an attempt to find one that would enable a given mutant strain to grow.

Among various chemicals they tried, the addition of arginine, permitted several mutant strains, dubbed (named as) **arg** mutants, to grow.

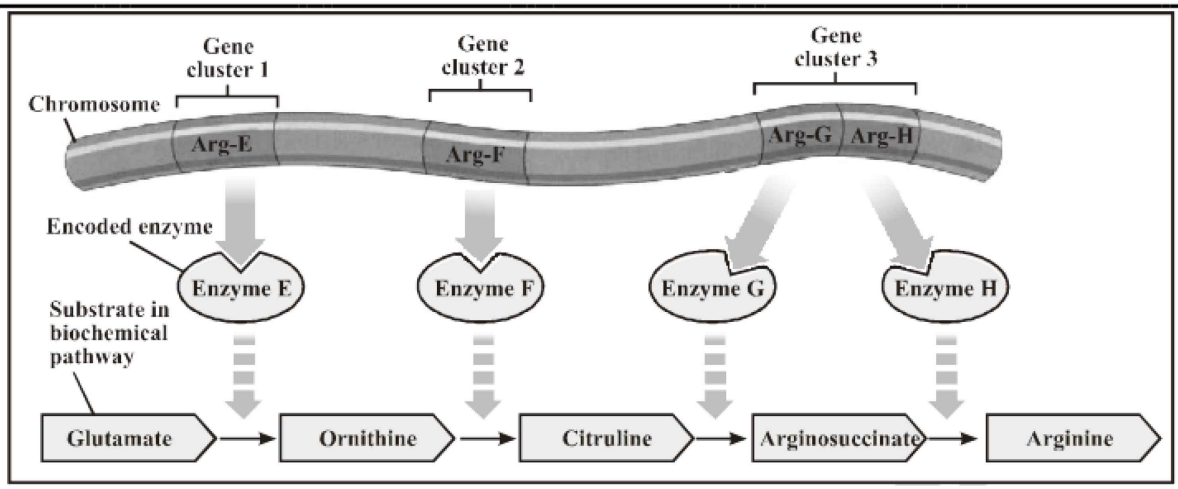


Beadle and Tatum's procedure for isolating nutritional mutants in *Neurospora*.

Mutation in Chromosomes:

When their chromosomal positions were located, the **arg** mutations were found to cluster in three areas.

- Beadle and Tatum studied each enzyme in the arginine biosynthetic pathway.
- They found that a mutation in gene cluster 1 made defect in enzyme E that controlled the conversion of **Gluamate into Ornithine**.
- A mutation in gene cluster 2 made defect in enzyme F that controlled the conversion of ornithine to citrulline.
- Similarly a mutation in gene cluster 3 made defect in enzyme F and enzyme H which controlled the conversion of citrolline to argininosuccinate and argginino succinate into arginine respectively.
- Thus, each mutant had a defect in a single enzyme, caused by a mutation at a single site on one chromosome.



Conclusion:

Beadle and Tatum concluded that genes produce their effect by specifying the structure of enzymes and that each gene encodes the structure of one enzyme.

They called this relationship **one gene one polypeptide**.

One-Gene One-Polypeptide:

Because many enzymes contain many polypeptide subunits, each encoded by a separate gene, the relationship is today more commonly refferred to as " one gene one-polypeptide".

HOW DNA-ENCODES PROTEIN STRUCTURE

DNA contains information for the maintenance of amino acid sequence in proteins. The sequence of nucleotides that determines the amino acid sequence in a protein is called a gene.

CENTRIBUTION OF SANGER

In 1953, an English biochemist Frederick Sanger, described the complete sequence of amino acids of insulin. Sanger's achievement was significant, as it indicated that proteins consist of fixed sequences of amino acids. Soon it was revealed that all enzymes and other proteins are strings of amino acids arranged in a certain definite order.

WORK OF VERNON INGRAM

Vernon Ingram in 1956, working at Cambridge University, discovered the molecular basis of sickle cell anaemia, (a protein defect inherited).

By analyzing the structure of normal and sickle cell heamoglobin, Ingram, showed that sickle cell anaemia is caused by a change from glutamic acid to valine at a single position in the protein.

The two alleles of the gene differed only in their specification of this one amino acid in the haemoglobin amino acid chain.

Conclusion:

These experiments finally brought us to a clear understanding that genes maintain sequence of amino acids in polypeptide chains. The characteristics of sickle cell anaeamia and most other hereditary traits are defined by changes in protein structure brought about by an alteration in the sequence of amino acids that make up the protein.

This sequence in turn is dictated by the order of nucleotides in particular region of chromosomes. For example, in sickle cell disease, a single thymine replaces an adenine in the DNA. As result, instead of glutamic acid, it now contains valine in the protein (GAA, GAG---glumtamic acid; GTA, GTG---valine).

THE CENTRAL DOGMA

Definition:

The mechanism of reading and expressing genes is referred to as central dogma. The genetic information resides in DNA. According to this information, protein is synthesized. It completes in two steps.

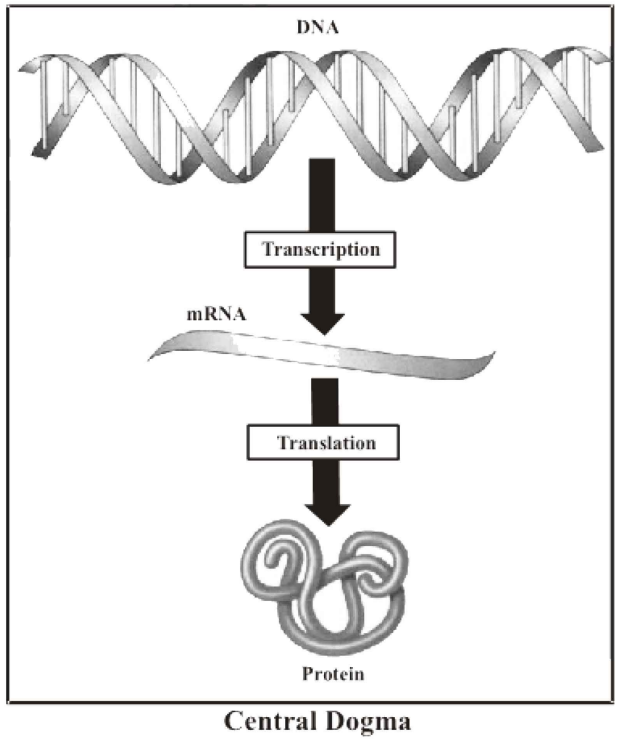
1. Transcription
2. Translation

1. Transcription:

Formation of RNA from DNA.

Initiation:

Transcription is initiated when the enzyme RNA polymerease binds to a particular binding site called a promoter (page) located at the beginning of the gene. The enzyme then moves along the strand into the gene and mRNA is synthesized.



Central Dogma

Stop signal:

At stop signal on the other end of gene, the enzyme disengages itself from the DNA and releases the newly assembled RNA chain. This chain is a complementary transcript of the gene from which it was copied.

2. Translation:

Definition:

Formation of protein from RNA.

It is the second step is the transfer of information form **RNA to proteins**, which occurs when the information contained in the mRNA is translated into amino acid sequence in the polypeptide chain.

The two steps of central Dogma taken together describe the mechanism of gene expression.

RNA & ITS TYPES

The whole process of gene expression requires the help of various kings of RNA molecules.

Types of RNA:

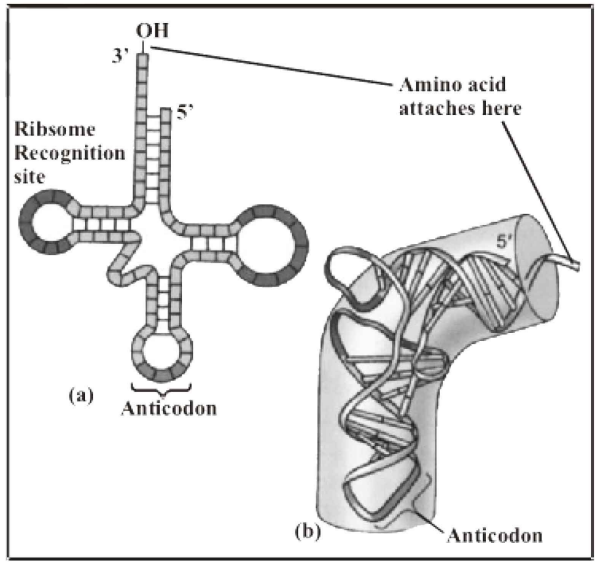
There are three different types of RNA:

(i) Ribosomal RNA (rRNA):

The class of RNA found in ribosome is called ribosomal RNA (r RNA). During translation, rRNA provides the site where polypeptides are assembled.

(ii) Transfer RNA (tRNA):

Transfer RNA moleocules transport specific amino acids to the ribosomes for use in building the polypeptides and position each amino acid at the correct place on the elongating polypeptide chain. Each tRNA has a sequence of three bases, called anticodon, which is complementary to codon of mRNA. Human cells contain about 45 different kinds of tRNA molecules (each having different type of anticodon).



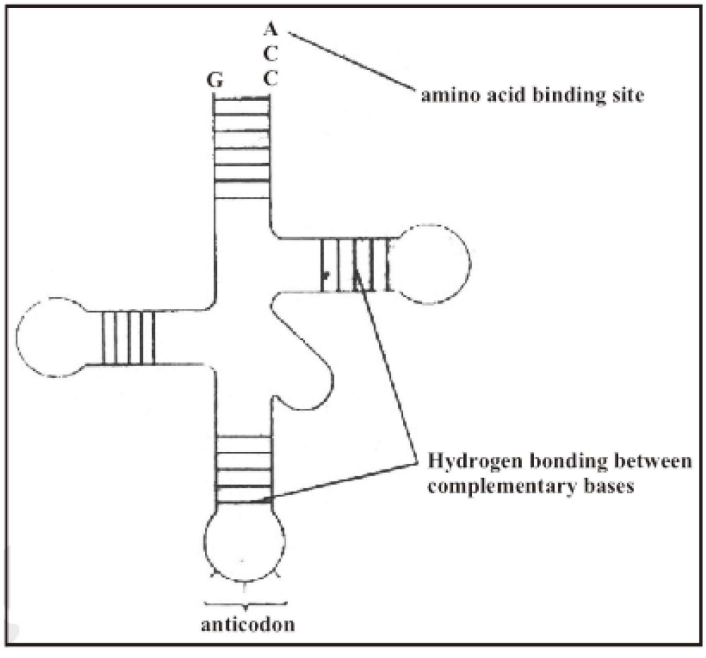
(iii) Messenger RNA (mRNA):

Messenger RNA has long strand of RNA that is transcribed from DNA and that travels to the ribosomes to direct the sequence of amino acids in polypeptides. A sequence of three nuleotides in mRNA, which specifies the position of an amino acid, is called a triplet codon (page).

TRANSCRIPTION

Definition:

This is the process in which an RNA copy of the DNA sequence encoding the gene is produced with the help of an enzyme, RNA polymerase.



Process of Transcription:

The process of transcription can be divided into three stages

1. Initiation
2. Elongation
3. Termination

1. INITIATION:

Template and coding strands' of DNA:

Only one of the two strands of DNA is transcribed. This strand is called template strand or the **antisense-strand**. The opposite strand is known as coding or sense strand.

RNA polymerase:

This is an enzyme which synthesizes the RNA against a template of DNA.

Features of RNA polymerase:

- The RNA polymerase enzyme of synthesizes RNA from 5' → 3' direction.
- There is only one type of RNA polymerase in prokaryotes, which is responsible for the synthesis of all the synthesis of all the three types of RNAs *viz*, rRNA, mRNA and tRNA.
- In eukaryotes, threr are three types of RNA polymerases namely

  RNA polymerase I, which synthesizes rRNA.

  RNA polymerase II, which synthesizes mRNA.

  RNA polymerase III, which synthesizes tRNA.

Promoter:

- This is an area before a gene on the DNA template strand where RNA polymerase binds to start transcription.
- In prokaryotes within promoter there are two binding sites **TTGACA** also called –35 sequence and **TATAAT** also called –10 sequence. These specific sites have affinity for the attachment of RNA polymerase.
- In eukaryotes these sites are known as –25 and –75.

Singma factor:

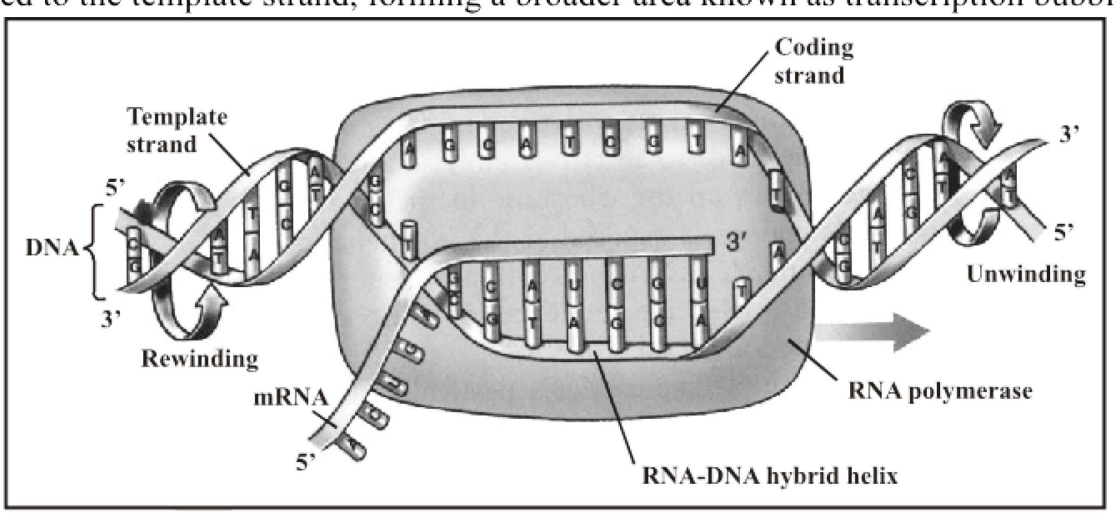
It is a small protein of RNA polymerase, which is responsible for correct recognition of binding site and initiation process. It guides the attachment of the enzyme on promoter site. Once the transcription has started the sigma factor is released.

Core enzyme:

The part of enzyme without the sigma factor is known as core enzyme. It moves over the template strand and completes the transcription process.

Transcription Bubble:

At the start transcription, the DNA duplex opens up at the place where enzyme is attached to the template strand, forming a broader area known as transcription bubble.



Model of a transcription bubble.

2. ELONGATION:

The transcription bubble moves down the DNA, leaving the growing strand protruding from the bubble. Ribounucleotides are linked in a chain against DNA template. The RNA polymerase copies the DNA sequence accurately because only the complementary nucleotides are linked (G against C and U against A).

3. TERMINATION:

The stop sequence at the end of the gene terminate the synthesis of mRNA. The simplest stop signal is a series of GC base pairs followed by a series of AT base pairs. The RNA formed in this region forms a GC hairpin followed by four or more U ribonucleotides. The hairpin causes RNA polymerase to stop synthesis and detach from the template.

POST TRANSCRIPTIONAL MODIFICATIONS

In Prokaryotes:

In bacteria the newly synthesized mRNA is directly released into the cytoplasm, where it guides the synthesis of polypeptide chain.

In Eukaryotes:

In eukaryotes mRNA has to travel long distance from nucleus to ribosomes present in the cytoplasm. The eukaryotic mRNA is therefore modified in several ways to protect it from variety of nucleases and phosphatases during this journey.

Addition of Cap and Tail:

A cap and a tail are added so that the molecule mat remain stable during long journey to ribosomes.

(a) Addition of a Cap:

The tail is in the form of poly a linked to 3' end of the mRNA. It is 100 – 2000 nucleotides long. While cap in 7 methyl GTP.

These capsand tails save the mRNA from a variety of nucleases and phosphatases in nucleoplasm and cytoplasm.

GENETIC CODE

Definition:

Genetic code is a linear sequence of nucleotides in DNA (arranged in triplets), which specifies the sequence of amino acids in a polypepetide chain.

Characteristic

Discovery of Genetic code:

- The sequence of bases maintains the sequence of amino acids in proteins.
- There are only four kinds of bases and twenty kinds of amino acids.
- If only one base served as a code for an amino acid we could have only four codons.
- The minimum number of bases that could be involved in amino acid selection seemed to be three. This is the reason; genetic code is called the triplet code.
- After Cricks initial experiments **Marshall Nirenberg, Philip Leader** and **Har Gobind Khorana** tested all the 64 codons by making artificial mRNAs and triplet codons and using them to synthesize proteins or aminoacyl-tRNA complexes in cell free systems.
- The full genetic code was determined during mid 60s.

Table: The Genetic code

First letter	Second Letter U		Second Letter C		Second Letter A		Second Letter G		Third letter
U	UUU	Phenylalanine	UCU	Serine	UAU	Tryosine	UGU	Cysteine	U
	UUC		UCC		UAC		UGC		C
	UUA	Leucine	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Tryptophan	G
C	CUU	Leucine	CCU	Proline	CAU	Histidine	CGU	Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Isoleucine	ACU	Treonine	AAU	Asparagine	AGU	Serine	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lysine	AGA	Arginine	A
	AUG	Methionine; Start	ACG		AAG		AGG		G
G	GUU	Valine	GCU	Alanine	GAU	Aspartate	GGU	Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glutamate	GGA		A
	GUG		GCG		GAG		GGG		G

CHARACTERISTICS OF GENETIC CODE

1. Genetic code is in the form of triplet codons. A codon is a sequence of three nucleotides in mRNA, which determines the position of an amino acid in a polypeptide chain.
2. Most of the amino acids are specified by more than one codons.
3. The reading occurs continuously without punctuation between the adjacent triplets.
4. Every genestarts with codon AUG, which normally encodes the amino acid methionine. However, when AUG codon is present at the beginning of mRNA in prokaryotes, it will code for the incorporation of N-formyl methionine (fMet) and will start protein synthesis. (AUG at any other position will code for normal methionine (Met).
5. Out of 64 codons, three codons UAA, UAG and UGA do not code for any amino acid and hence are known as non-sense codons. These codons are usually present at the end of the gene and hence they are also called stop codons.
6. The Genetic code is universal. It is the same in almost all the organisms. For example AGA specifies arginine in bacteria, in humans and all other organisms whose genetic code has been studied. Because of the universality of codon the genes can be transferred from one organisms to another and be successfully transcribed and translated in their new host.

7. The study of genetic code of mitochondral DNA showed that genetic code is not that unversal.

For example UGA codon is normally a stop codon but in mitochondria it codes for tryptophan. Likewise AUA was read as Methionine rather than Isoleucine.

Thus it appears that genetic code is not quite universal.

TRANSLATION

Definition:

This is a process in which protein is synthesized by the ribosome according to the message present in mRNA.

Requirements for Translation:

(i) Amino acyl-tRNA

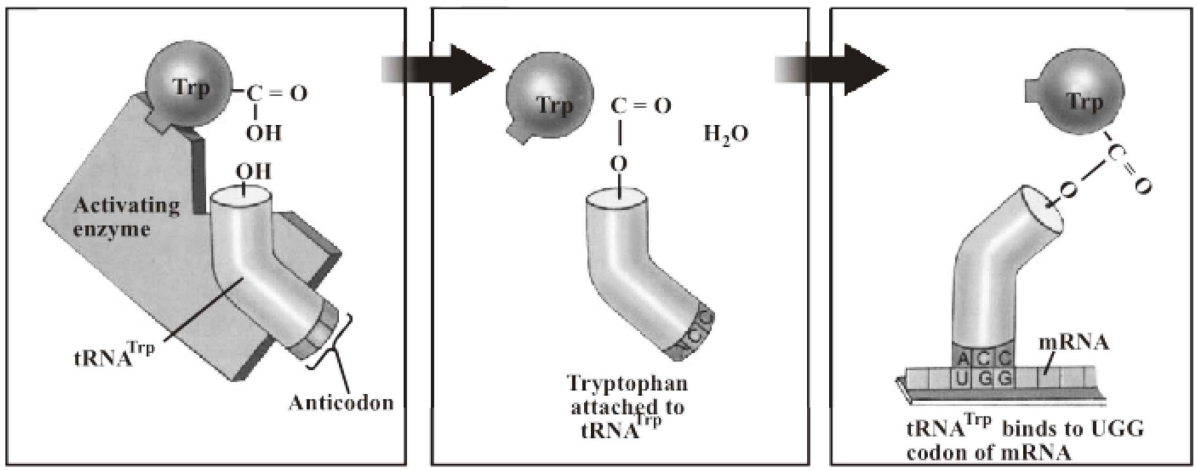
(ii) Ribosomes

(iii) Enzymes and other factors.

(iv) mRNA

(i) Amino acyl-tRNA:

Every tRNA molecule carries an amino acid at its 3' end. The structure formed in known as amino acyl-tRNA. An enzyme amino acyl-tRNA synthetase (also known as activating enzyme), catalyses this reaction. There is a different tRNA and different enzyme for every different amino acid.



(ii) Ribosomes:

These are the factories for protein synthesis. Each ribosome has three sites named as A, P and E site.

A site (amino acyl site):

It is the site of attachment of new amino acyl-tRNA molecules.

P site (Peptidyl site):

It is the site where peptide linkage will form between the two adjacent amino acids.

E site (Exit site):

It is the site of removal of vacant tRNA molecules, which have given their respective amino acids to the growing polypeptide chain.

STEPS OF TRANSLATION

The process of translation, in prokaryotes, can be divided into three stages:

1. Initiation complex
2. Initiation
3. Elongation
4. Termination

1. INITIATION COMPLEX:

(i) Formation of initiation complex:

In prokaryotes, polypeptide synthesis begins with the formation of initiation complex.

Attachment f-Met tRNA to Ribosome:

First a tRNA molecule carrying a chemically modified methionine (called N-formyl methionine) binds to the small ribosomal subunit.

Initiation factors:

Protein called intiation factor (IF) position bonds will form.

Attachment of initiation complex to mRNA:

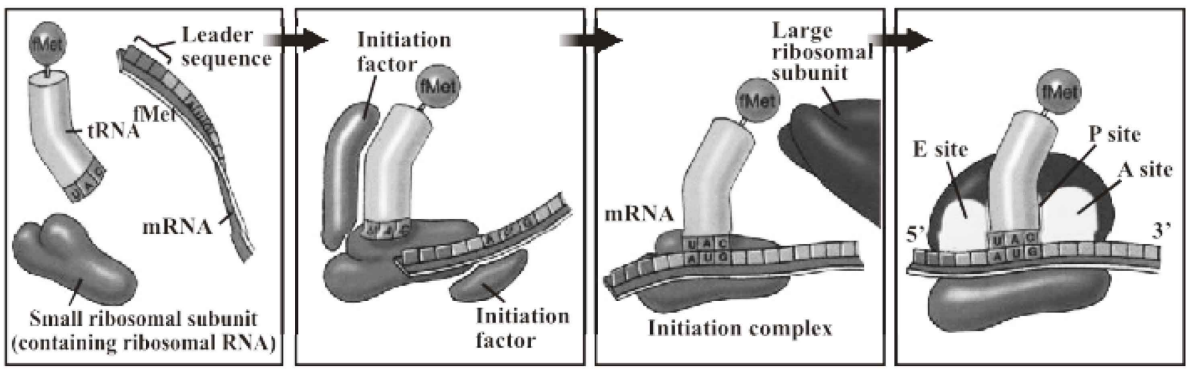
This intiation complex, guided by another initiation factor, binds to initiation codon "AUG" on the mRNA.

2. INITIATION

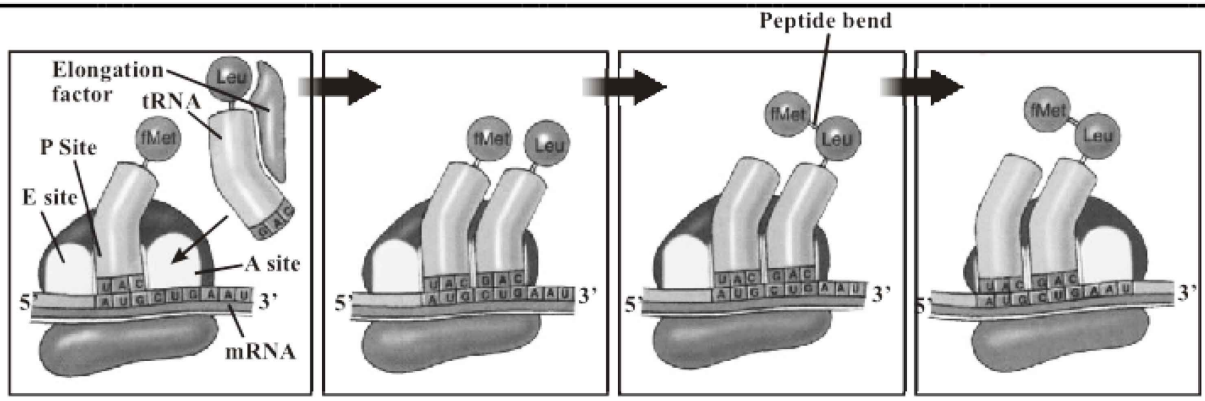
Attachment of large subunit:

After the inintiation complex has formed, the large ribosome subunit bind to small subunit.

Initiation factors are released and reutilized.



Formation of the initiation complex



The translocation process

3. ELONGATION:

(i) Binding of new aminoacyl tRNA:

When a tRNA molecule with the appropriate anticodon appear, proteins called elongation factors (EF) assist in binding it to the exposed mRNA codon at the A site.

(ii) Formation of peptide bond:

The two amino acids undergo a chemical reaction, catalyzed by the large ribosomal subunit, which release the initial methionine from its tRNA and attaches it instead by a peptide bond to the second amino acid.

(iii) Translocation and liberation of free tRNA:

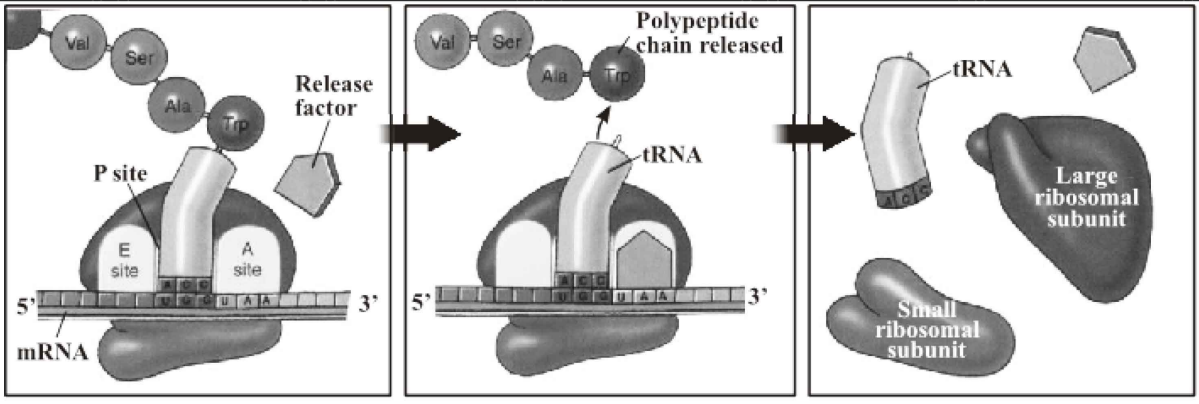
The ribosome now moves (translocates) there more nucleotides (a codon) along the mRNA molecule in the 5' → 3' direction, guided by other elongation factors. This movement translocates the initial tRNA to the E site and ejects (releases) it from the ribosome. It also repositions the growing polypepetide chain (at this point containing two amino acids) to the P site, and exposes the next codon on the mRNA at the A site.

(iv) Growing Polypeptide Chain:

When a tRNA molecule recognizing that codon appears it binds to the codon at the site, placing its amino acid adjacent to the growing chain. The chain is then transferred to the new amino acid, (by the formation of peptide bond) and the entire process is repeated.

4. TERMINATION:

Elongation continues in this fashion untill a chain – terminating non-sense codon is exposed (for example UAA, UGA or UAG). There is no tRNA for these non-sense codons, but they are recognized by release factors (RF). The release factors are the proteins that release the newly made polypeptide from the ribosomes.



Termination of protein synthesis

MUTATIONS

Definition:

A change in the tiny part of the DNA is called mutation.

Explanation:

Changes in the DNA occur due to two reasons.

A mistake in replication of Damage to the genetic message.

The mutation in somatic cells do not pass on to offspring and so have little evolutionary consequence than germ line changes.

The mutation in germ line cell is passed to subsequent generations thus providing the raw material from which natural selection produces evolutionary change.

CLASSIFICATION OF MUTATIONS

Mutations can broadly be classified as:

1. **Chromosomal aberrations**
2. **Point mutations**

1. CHROMOSOMAL ABRRATIONS:

These are further divided into two kinds:

(i) Change in number of chromosomes

(ii) Change in structure of chromosomes

(i) Change in Chromosomal Number:

Some chromosomal aberrations involve presence of one or more extra chromosomes or loss of a chromosome from the diploid number.

The changes appear as a result of unseparation of homologous chromosomes during meiosis, which is termed as **non-disjunction of chromosomes**. The non-disjunction may lead to **Down's syndrome, klinefelter's syndrome** etc (chapter 21).