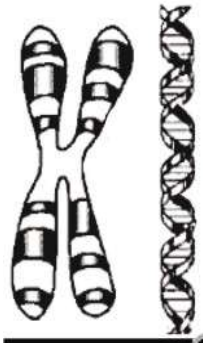


Chapter 20

Chromosomes and DNA

20.0 CHROMOSOMES

Introduction

Chromosomes are thread like structures that appear inside the nucleus at the time of cell division.

Discovery

Chromosomes were first discovered by German embryologist Walther Fleming in 1882 when he was examining the rapidly dividing cells of Salamander larvae.

After this discovery chromosome have been found in the cells of all eukaryotes.

Number of Chromosomes

Number of chromosomes varies from species to species e.g.

Organism	Chromosomes
Penicillin	one pair
Ferns	more than 500 pairs
Mosquito	6
Honey bee	32
Corn	20
Sugar cane	80
Frog	26
Mouse	40
Human	46 chromosomes or 23 pairs

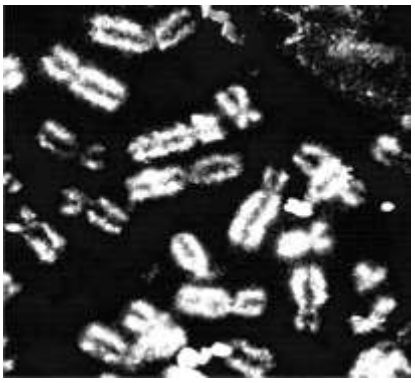


Fig 20.1 Human chromosomes

Genes on Chromosomes

Genes are located on chromosomes e.g. in man each of 46 chromosomes contain hundreds or thousands of genes.

Genes determine developmental processes body features and function.

Importance of Chromosomes

(i) Possession of all chromosomes is essential for survival.

(ii) Missing of a part or whole chromosome leads to serious consequences even death.

20.1 TYPES OF CHROMOSOMES

Karyotypes

Total chromosomes (particular array) of an individual is called karyotype.

It includes following characteristics due to which chromosomes may widely differ.

- Appearance
- Size
- Staining properties
- Location of centromere
- Relative length of arms on sides of centromere
- Karyotypes show marked differences among species and even among individuals of same species.

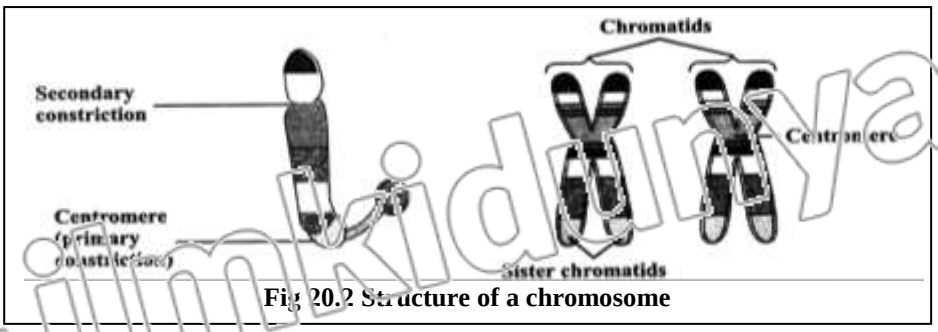


Fig 20.2 Structure of a chromosome

Types

Chromosomes are divided into four types on basis of position of centromere. These types are:

(i) Telocentric

(ii) Acrocentric

(iii) Submetacentric

(iv) Metacentric

These chromosomes acquire different shapes at the time of anaphase during the cell division. The usual shapes are i, j and v.

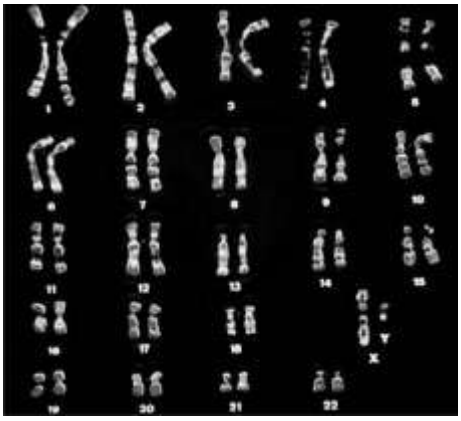


Fig. 20.3 A human karyotype

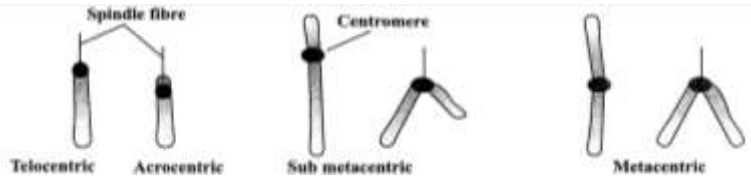


Fig 20.4 Shapes of a chromosomes depend upon the location of centromeres

QUESTIONS RELATED TO ABOVE ARTICLE

What are chromosomes? What do you know about their types?

Describe types of chromosomes on the basis of centromere. (LHR 2017)

20.2 COMPOSITION OF CHROMOSOME

Chemical Composition

Chromosomes are composed of two important components:

1) DNA

2) Proteins

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

1) DNA

(i) It is about 40%.

(ii) It is very long double stranded fiber that extends unbroken through the entire length of chromosome.

(iii) Numerous nucleotides of DNA are present on single chromosome e.g. a typical human chromosome contains about 140 million ($1.4x10^8$) nucleotide in its DNA.

(iv) Chromosome through DNA contains all the information e.g. information of one human chromosome would fill about 280 printed books of 1000 pages each, if each nucleotide corresponds to a word and each page has about 500 words on it.

(v) If strand of DNA from a single chromosome were laid out in a straight line it would be about 5cm long.

2) Proteins

(i) Histone proteins are present in chromosome.

(ii) Histones are positively charged due to abundance of basic amino acids (arginine and lysine) on it.

Physical Structure

Typically, a chromosome is made of two important components:

(i) Centromere which is central part also called primary constriction.

(ii) Chromatids, which are two in number, also called arms.

Sometimes a secondary constriction is also present.

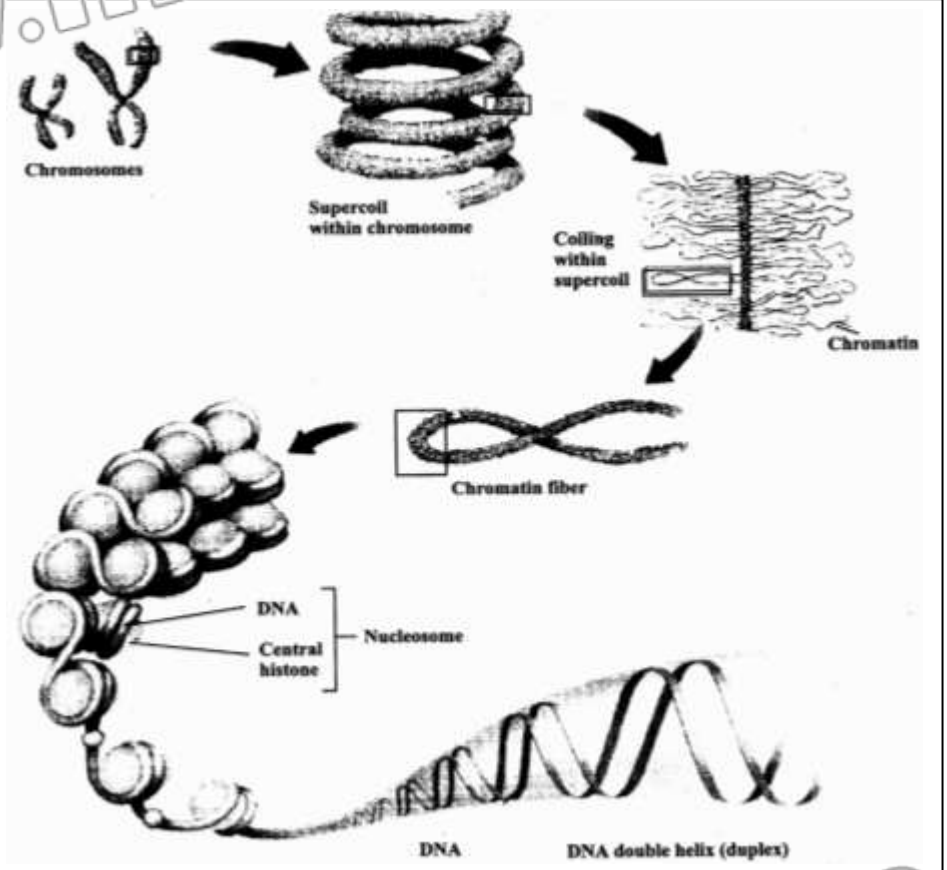


Fig 20.5 Levels of eukaryotic chromosomal organization

Structural Features

(i) Condensed portions and non-Condensed portions

Heterochromatin

- These are highly condensed portions of chromatin.
- They remain permanently condensed.
- Their DNA is never expressed.

Euchromatin

- These are portions other than heterochromatin.
- It is condensed only during cell division when compact packaging facilitates the movement of chromosomes.
- At times other than division, it is present in open configuration and its genes can be expressed.

(ii) Supercoils

Each chromatid is made of higher order coils called supercoils.

(iii) Coils

Turned fibers present within the supercoil are called coils, which are in actual case chromatin fibers. This coiling helps DNA to be present in small space of nucleus.

(iv) Nucleosome

It is the basic unit of chromosome or chromatin fibers.

- In it DNA duplex is coiled around a core of eight histone proteins.
- Nucleosomes are repeated after 200 nucleotides.
- Positively charged histones are linked with negative charged phosphate groups of DNA. The histone cores thus act as magnetic forms that promote and guide the coiling of DNA.

QUESTIONS RELATED TO ABOVE ARTICLE

Write a note on chemical composition of chromosomes. (FSD 2019)

20.3 CHROMOSOMAL THEORY OF INHERITANCE

Work of Karl Correns

He was the scientist who first suggested central role of chromosomes in heredity in 1900.

Work of Walter Sutton

Chromosomal theory of inheritance was first formulated by American Walter Sutton in 1902.

Statement

According to this theory chromosomes are involved in inheritance of characters.

Evidence In Favour of **Sutton's Theory**.

These are based on Mendel's work.

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

(ii) Diploid individuals have two copies of each pair of homologous chromosomes while gametes have only one. This observation was consistent with Mendel's model.

(iii) Chromosomes segregate during meiosis and each pair of homologue orients on the metaphase plate independently of every other pair.

Drawbacks of Theory

According to Mendel, genes (factors) for each trait are located on chromosome which are assorted independent of each other. Mendel's model and Sutton's theory do not explain 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.

Work of T.H. Morgan

Thomas Hunt Morgan studied fruit fly, *Drosophila melanogaster*, in 1910. Normal eye colour of drosophila is red but one day a mutant white eyed male appeared. Morgan worked on it and discovered sex chromosomes and sex linkage.

Experiment

Morgan crossed mutant male to a normal female. All F_1 progeny had red eyes. He then crossed red eyed flies from F_1 generation with each other. Of the 4252 F_2 progeny Morgan examined 782(18%) had white eyes. Ratio of red eyes to white eye in F_2 progeny was greater than **3:1**. All the white eyed F_2 flies were male.

Conclusion

- It is not explaining law of segregation truly.
- Perhaps, it was impossible for a white eyed female fly to exist for some unknown reason.

Experiment-2

In order to test above idea Morgan performed a test cross.
He crossed female F_1 progeny with original white eyed male.
He obtained both white eyed and red eyed males and females in a 1:1:1:1 ratio.

Conclusion:

This experiment shows that females could have white eyes.

Explanation

Appearance of white eyed female in second cross and not in first cross give a clue about sex involvement. Morgan's experiments explain following points.

(i) Gene causing white eye trait in Drosophila resides on X chromosome. It is absent from Y chromosome.

(ii) White eye trait is recessive to red eye trait.

(iii) Trait determined by gene located on X-chromosome is sex linked.

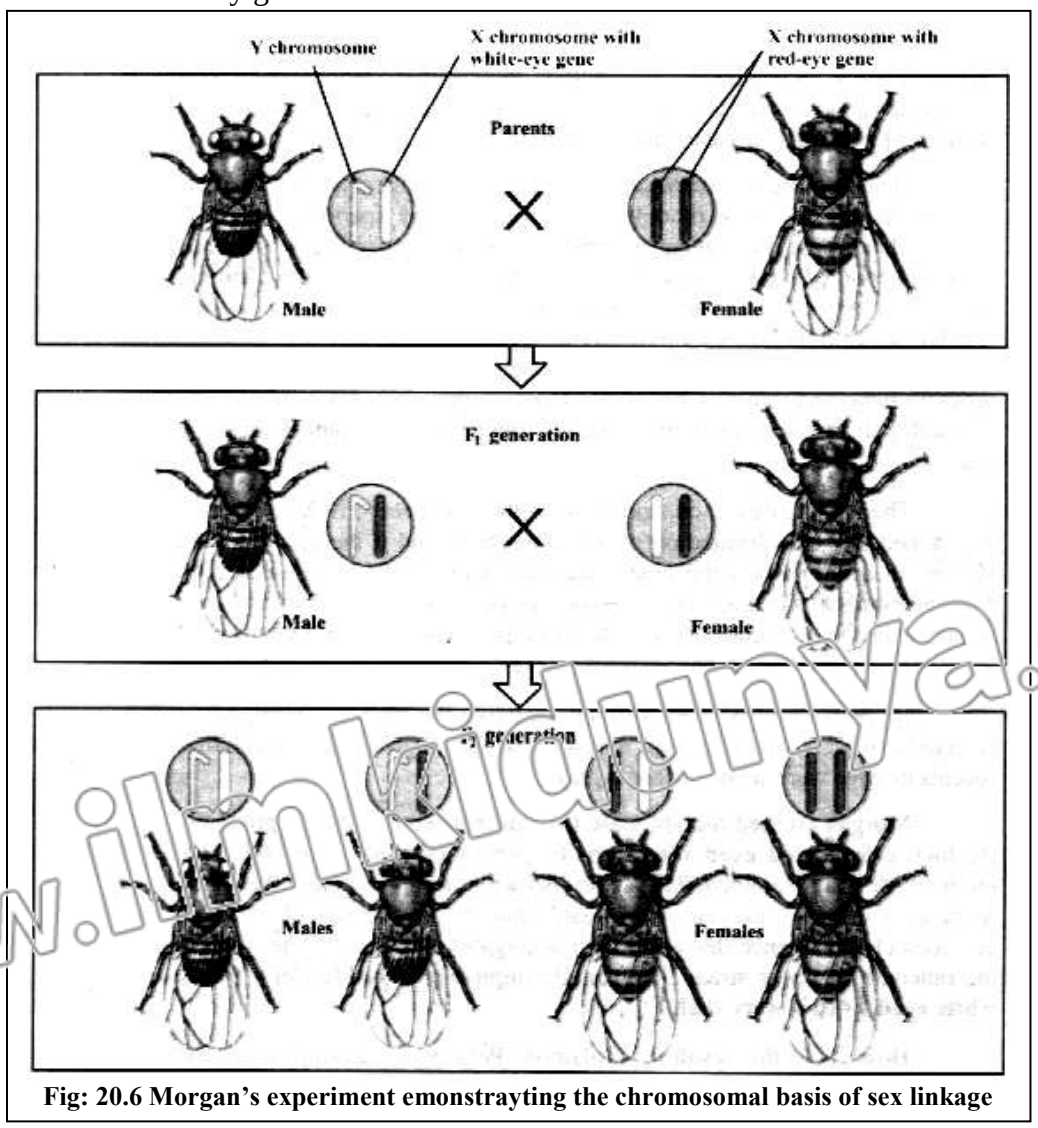


Fig: 20.6 Morgan's experiment emonstrayting the chromosomal basis of sex linkage

Importance

Morgan's experiments presented the first clear evidence that genes determining Mendelian traits do indeed reside on the chromosomes as Sutton had proposed. The segregation of the white eye trait has one-to-one correspondence with the segregation of X-chromosomes. In other words, Mendelian traits such as eye color in *Drosophila* assort independently because chromosomes do so.

QUESTIONS RELATED TO ABOVE ARTICLE

State chromosomal theory of inheritance. Also explain different evidences to support this theory.

20.4 DNA AS HEREDITARY MATERIAL

Work of Frederick Griffith (1928)

Frederick Griffith was a British microbiologist who provided first evidence about hereditary nature of DNA.

He used *Streptococcus pneumoniae* bacteria. There are two types of *S. pneumoniae;*

(i) S form is virulent and contains polysaccharide coat necessary for virulence.

(ii) R form is non-virulent. It lacks an enzyme needed to manufacture polysaccharide coat.

He performed following experiments.

(i) When he infected mice with virulent strain of *S. pneumonae* bacteria S form, it died of blood poisoning.

(ii) When he infected similar mice with mutant strain of *S. pneumonae* that lacked the virulent strains polysaccharides coat R form, it did not cause the death.

(iii) He injected dead bacteria of S-virulent strain into the mice, the mice remained perfectly healthy.

(iv) As a control, he injected mice with a mixture containing dead S bacteria of virulent strain and live coatless R bacteria although each of them did not harm mice separated but their mixture caused death of mice. In blood of dead mice live S bacteria were found.

Transformation

"It is the transfer of genetic material from one cell to another altering genetic make up of the recipient cell."

From these experiments he concluded that some 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.

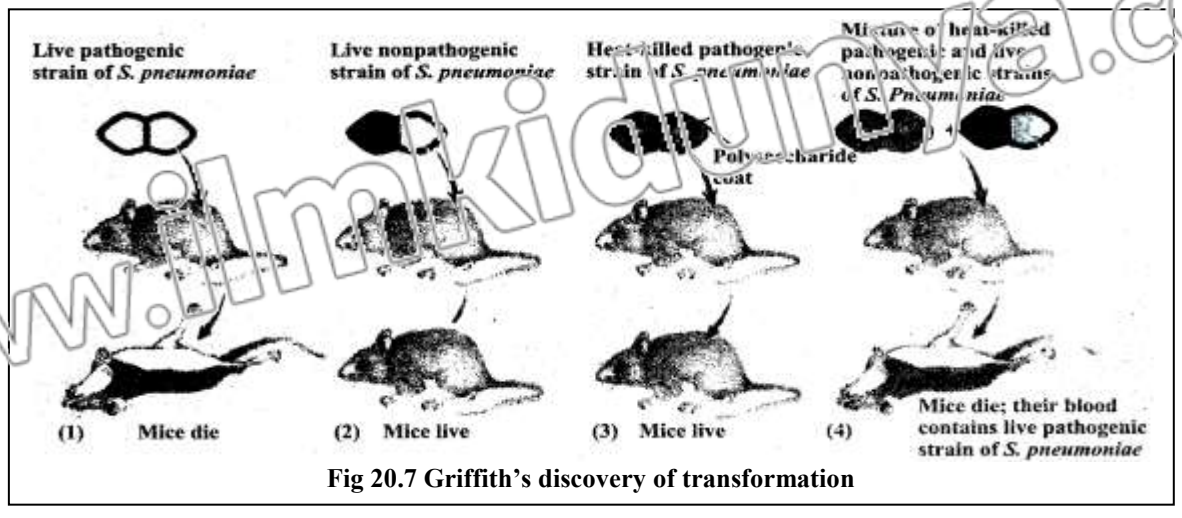


Fig 20.7 Griffith's discovery of transformation

Work of Avery, Macleod and McCarty (1944)

They discovered agent responsible for transforming streptococcus. They performed following experiments.

(i) They prepared mixture of dead S streptococcus and live R streptococcus and removed much of the protein (99.98%) by applying protein digesting enzyme.
Transforming activity was not reduced.

(ii) They removed much of RNA by applying RNA digesting enzyme.
Transforming activity was still present.

(iii) They removed DNA by applying DNAase.
At that time transforming activity was lost.

Work of Hershey and Chase (1952)

They performed experiment with bacteriophages (T_2) supporting Avery's conclusion.

(i) In an experiment, they labeled viruses with radioisotope ^{32}P being incorporated into newly synthesized DNA of growing phage.

(ii) In other experiment, they labeled viruses with radioisotope ^{35}S being incorporated into the amino acids of newly synthesized protein coats.

After labeled viruses were permitted to infect bacteria, bacterial cells were agitated violently in a blender to remove the protein coats of the infecting viruses from the surface of bacteria. This procedure removes nearly the entire ^{35}S label from the bacteria.

However, ^{32}P label had transferred to the interior of the bacteria and was found in viruses subsequently released for the infected bacteria. Hence the hereditary information injected into the bacteria that specified the new generation of viruses was DNA and not protein.

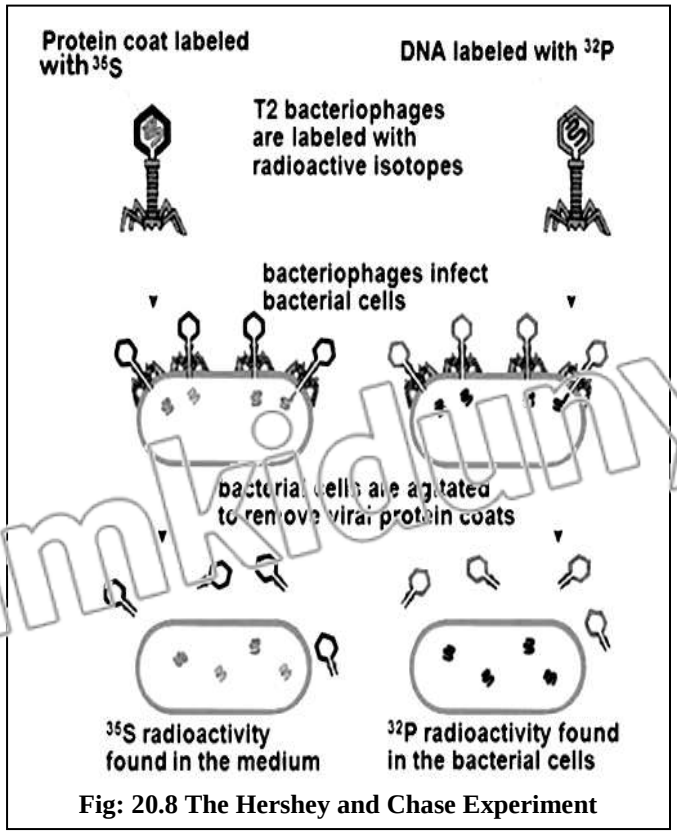


Fig: 20.8 The Hershey and Chase Experiment

QUESTIONS RELATED TO ABOVE ARTICLE

Describe Griffith's experiment to prove DNA as hereditary material.
Prove that DNA is the heredity material. (LHR 2017)
Discuss the experiment of Frederick Griffith (Transformation). (GRW 2018)
Describe how Hershey and chase prove that DNA is the heredity material. (MTN 2019, DGK 2019)
How did Hershay and Chase determine which components of bacterial viruses contain the virus of hereditary information? (Exercise Question i)

20.4.1 Chemical Nature of DNA

Work of Friedrich Miescher

In 1869, right after four years of publishing of Mendel's work a German Chemist Friedrich Miescher discovered DNA.

Miescher extracted a white substance from the nuclei of human cells and fish sperm. As this substance was extracted from nuclei so he called it as nuclein.

After sometime, this substance was called nucleic acid due to its acidic nature.

Work of P.A. Levene

Basic structure of DNA was determined by a biochemist P.A Levene in 1920.

According to Levene, DNA and RNA molecules are made of repeating units called nucleotide. A nucleotide is made of three components.

Composition of Nucleotide

(i) Phosphate group (PO_4)
(ii) 5-carbon sugar (pentose)
(iii) Nitrogen bases

- Nitrogen Bases are:

(a) Purines i.e. Adenine (A) and Guanine (G).
(b) Pyrimidine i.e. Cytosine (C) and Thymine (T) in DNA and Uracil (U) in RNA instead of Thymine.

In nucleotide nitrogen base is attached to carbon number 1of pentose sugar.

- Sugar is:

(a) De-oxyribose in DNA.
(b) Ribose in RNA.

- Phosphate develops phosphodiester linkage.

Formation of Nucleotide

- Levene concluded that DNA and RNA molecules are made of repeating units called nucleotides.
- In a nucleotide, nitrogen base is attached at carbon 1 of a pentose sugar.
- Phosphate group is attached to carbon number 5 of the sugar.
- In a polynucleotide, phosphate is attached to carbon 3 of one sugar and carbon 5 of other.
- Linkage is covalent bond developed by dehydration synthesis involving removal of water
- Resulting polymer still has reacting phosphate group (5) at one end.

Work of Erwin Chargaff

Erwin Chargaff showed that in DNA amount of adenine is always equal to thymine and amount of guanine is always equal to cytosine.

It also implies that there is always equal proportion of purine (A+G) and pyrimidine (C+T).

Work of Franklin and Wilkins

X-ray diffraction analysis of DNA was done by a British chemist Rosalind Franklin in laboratory of British biochemist Maurice Wilkins.

He bombarded a molecule of DNA with a beam x-rays. These rays were bent or diffracted. He recorded diffraction pattern on photographic film and indicated following points.

(i) It is a three-dimensional molecule.

(ii) It is helix shaped. Diameter of helix is 2nm and of complete helical turn is every 3.4nm.

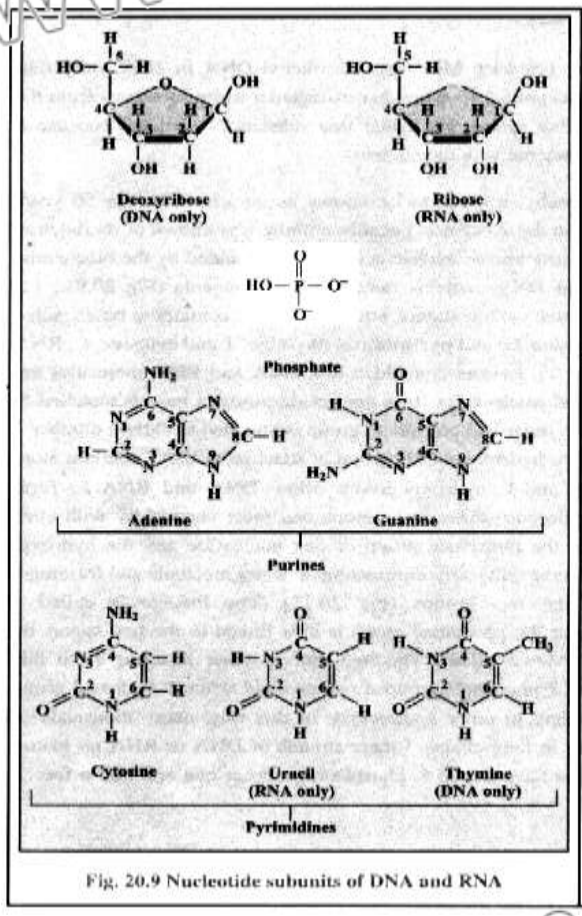


Fig. 20.9 Nucleotide subunits of DNA and RNA

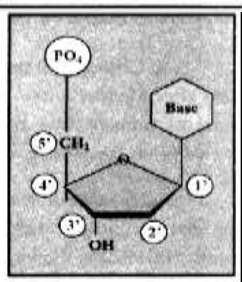


Fig. 20.10 Numbering the carbon atoms in a nucleotide.

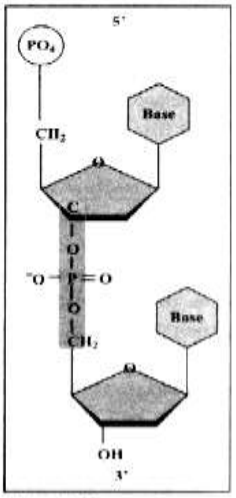


Fig. 20.11
A Phosphodiester bond

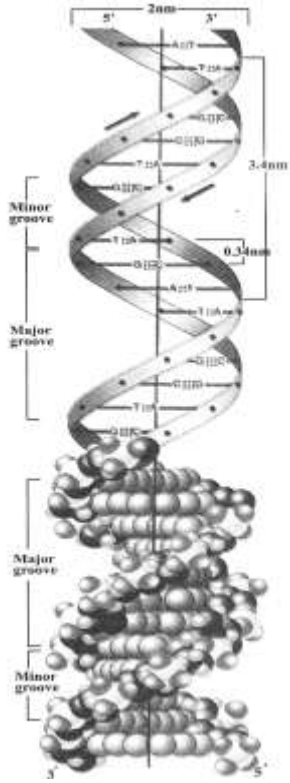


Fig: 20.12X-ray diffraction patterns suggested the double helical shape of DNA

QUESTIONS RELATED TO ABOVE ARTICLE

What is the three dimensional shape of DNA? How does three dimensional shape of

Write a note on chemical nature of DNA. (SWL 2021)

Discuss chemical nature of DNA with reference to nucleoside and nucleotide composition. (LHR 2022)

DNA fit with Chargaff's observations on the proportions of purines and pyrimidines in DNA. (Exercise Question ii)

20.4.2 Double Helical Structure of DNA (Watson and Crick's Model)

Presentation

Watson and crick two young researchers in University of Cambridge worked on helical structure of DNA molecule.

Salient Features

(i) DNA is a simple double helix with the basis of two strands pointed inward towards each other forming base pair.

(ii) Base pairs always contain purines which are large pointing towards pyrimidine's which are small.

(iii) Diameter of DNA molecule always remains constant which is 2nm.

(iv) A duplex DNA molecule is composed of two antiparallel strands, one chain running 3' to 5' and other 5' to 3'.

(v) Base pair are planar (flat) and stack 0.34 nm apart due to hyperphobic interactions between bases.

(vi) Two helices are stabilized with hydrogen bonds. Adenine forms two hydrogen bonds with thymine while guanine forms three hydrogen bonds with cytosine consequently adenine and thymine always occur in same proportion in any DNA molecules as well as guanine and cytosine because of this base pairing.

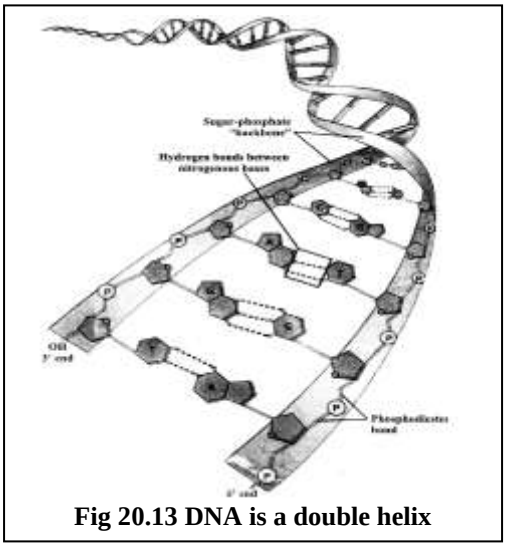


Fig 20.13 DNA is a double helix

QUESTIONS RELATED TO ABOVE ARTICLE

Write a note on Watson and Crick model of DNA. **(GRW 2019, LHR 2021, GRW 2021)**

Describe important features of Watson-Crick model of DNA structure. (DGK 2022)

20.4.3 DNA Replication

As cell reproduces DNA also replicates.

Models of DNA Replication

Three methods had been presented to explain DNA replication. These are

1) Semi-conservative method

2) Conservative method

3) Dispersive method

1) Semi-Conservative Model

It is most accepted model and was presented by Watson and Crick.

According to this method:

i) During unzipping of DNA molecule, the two strands of the duplex separate out each acting as a model or mold/ template.

ii) Appropriate complementary nucleotides get assembled on the exposed single strands to form two daughter complexes with the same sequences.

In this process by separation of two strands, primary structure has been conserved while secondary structure has been disrupted. It means that sequence of original duplex is conserved, the duplex itself is not.

2) Conservative Model

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

3) Dispersive 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.

20.4.4 The Meselson-Stahl Experiment

Introduction

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

Experiment

Step I – Growth of Bacteria in Artificial Medium

They grew bacteria in a medium containing heavy isotope of nitrogen, N^{15}, which became incorporated into the bases of the bacterial DNA. 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}. Then they transferred the bacteria from the N^{15} medium to the N^{14} medium and collected the DNA at various intervals.

Step II – Ultracentrifugation

They dissolved the DNA in Cesium Chloride and then spun it at a very high speed in an ultracentrifuge. DNA strands of different densities got separated. The enormous centrifugal forces generated by the ultracentrifuge 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 CsCl 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.

Observations

- The DNA collected immediately after the transfer was all-dense.
- 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.
- After the second round of replication, two density classes of DNA were observed, one intermediate and one equal to that of N^{14}-DNA.

Interpretations

Meselson and Stahl interpreted their results as follows:

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

Conclusion

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

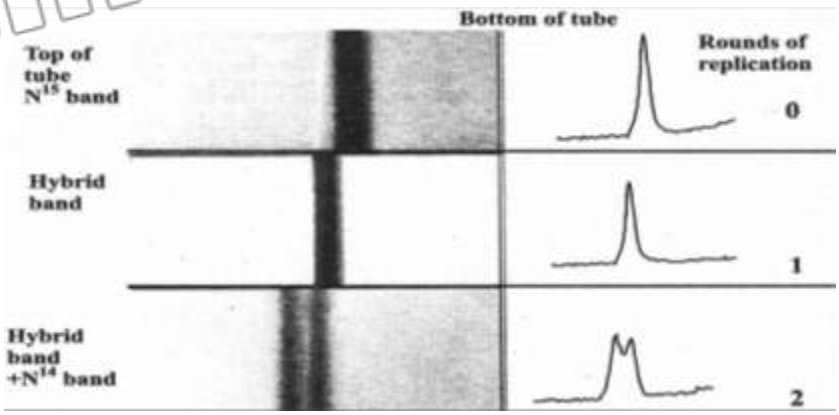


Fig 20.14 The key result of the Meselson and Stahl experiment. The bands on the left side of the finger shown N^{15} DNA which heavier and is present towards the bottom of the tube. The middle bond is a hybrid DNA band of N^{15} and N^{14} and hence lies above the N^{15} band. This is after first round of replication. In the second round of replication, two bands are visible one at the level of hybrid band and the other lighter band which is N^{14} band.

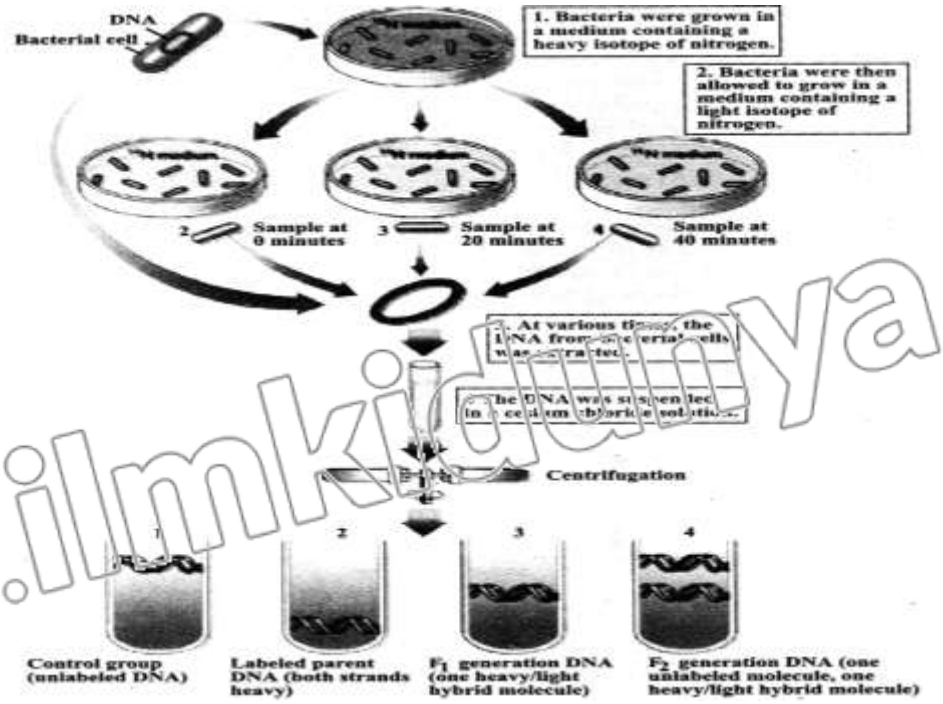


Fig 20.15 The Meselson and Stahl experiment evidence demonstrating Semi-conservative replication.

QUESTIONS RELATED TO ABOVE ARTICLE

Give Meselson-Stahl experiment to show that replication of DNA is semi conservative.

Explain Meselson – Stahl experiment for DNA replication. (GRW 2021, DGK 2019)

How did Meselson and Stahl show that DNA replication is semi-conservative? (MTN 2019, RWP 2019, MTN 2021, SGD 2021, RWP 2021, MTN 2022)

How did Meselson and Stahl show that DNA replication is semi conservative? (Exercise Question iii)

20.4.5 The Replication Process

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

Enzymes Involved

Enzymes that have important role in DNA replication are;

1) Helicase
2) Primase
3) DNA polymerase
4) Ligase

1) Helicase

It is involved to unzip/ open double helix of DNA.

2) Primase

It constructs an RNA primer, a sequence of about 10 RNA nucleotides complementary to the parent DNA template.

The RNA nucleotides in the primers are then replaced by DNA nucleotides.

3) DNA Polymerase

It catalyzes the addition of nucleotides to the growing complementary strands of DNA.

There are three DNA polymerase namely I, II and III in bacteria.

DNA polymerase I is a relatively small enzyme that plays a supporting role in DNA replication.

The true *E. coli* replicating enzyme is DNA polymerase III. Different features of it are as following:

- It is 10 times larger and far more complex in terms of structure.
- Enzyme is a dimer and catalyzes replication of one DNA strand.
- Polymerase III progressively threads the DNA through the enzyme complex moving at a rapid rate, some 1000 nucleotides/ second.
- It can add nucleotide to a chain of nucleotides that is already paired with the parent strand.
- It cannot initiate synthesis on its own.
- DNA polymerase III recognizes the primer and adds DNA nucleotides to it to construct the DNA strands.
- It can add nucleotides only to the 3' end of a DNA strand. It means that replication always proceeds 5' ⟶ 3' direction on a growing DNA strand.

Mechanism

Because the two parent strands of a DNA molecules are antiparallel, the new strands are oriented in opposite directions and therefore the new strands must be elongated by different mechanisms.

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

ii) ***Lagging strand***, which elongates away from the replication fork, is synthesized discontinuously as a series of short segments that are later connected. These segments are called Okazaki fragments. These fragments are;

- 100-200 nucleotides long in eukaryotes.
- 1000-2000 nucleotides long in prokaryotes.

Each Okazaki fragment is synthesized by DNA polymerase III in 5' ⟶ 3' direction, beginning at the replication fork and moving away from it.

iii) When the polymerase reaches the 5' end of the lagging strand, another enzyme, DNA ligase, attaches the fragment to the lagging strand.

iv) The DNA is further unwound, new RNA primers are constructed, and DNA polymerase III then jumps ahead 1000-2000 nucleotides (towards the replication fork) to begin constructing another Okazaki fragment.

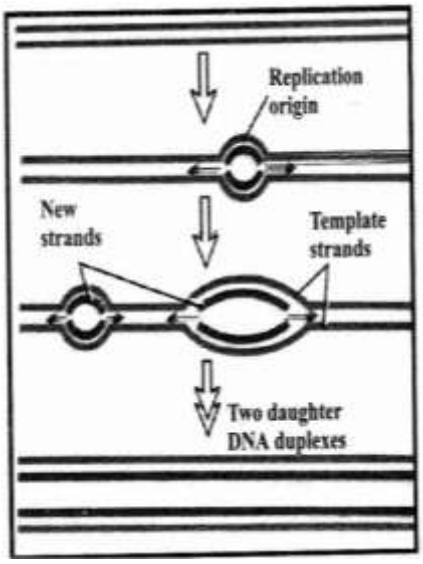


Fig 20.16 Origins of replication

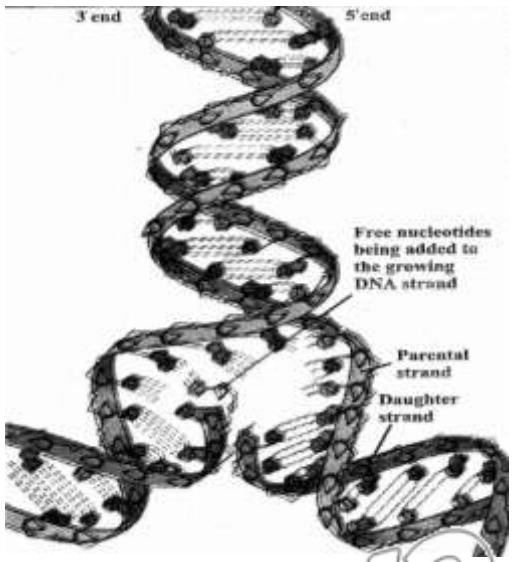


Fig 20.17 Replication of DNA

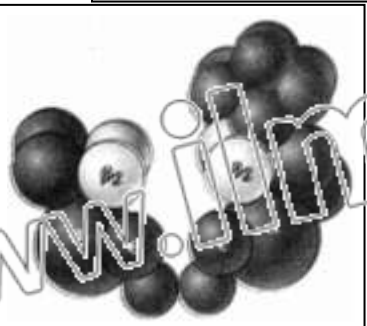


Fig 20.18 Molecular structure of DNA polymerase III complex

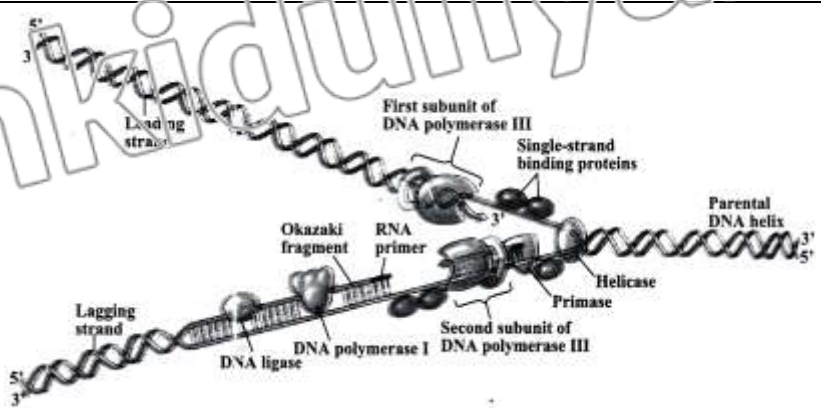


Fig 20.19 A DNA replication fork

QUESTIONS RELATED TO ABOVE ARTICLE

Sketch DNA replication fork and label. (No description)

Explain the process of DNA replication with the help of a diagram. (LHR 2018)

Describe the replication process of DNA. (FSD 2021, FSD 2022)

What is the basis for the requirement that the leading and lagging strands be replicated by different mechanisms? (Exercise Question iv)

20.5 WHAT IS GENE?

HISTORY OF DISCOVERY OF GENE AND ITS ACTION

Work of Garrod and Bateson

Archibald Garrod and William Bateson concluded in 1902 that certain diseases among their parents were more prevalent in particular families.

Investigation of Garrod

Garrod investigated that 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.

Conclusion

From this Garrod concluded that patient suffering from alkaptonuria lacked enzyme necessary to catalyze this breakdown. This enzyme deficiency was considered to be due to inherited disease.

Garrod's findings somewhat showed that information encoded within the DNA of chromosomes acts to specify particular enzymes.

Work of Beadle and Tatum

George Beadle and Edward Tatum from Stanford University in 1941 provided definitive evidence that genes are involved in production of enzymes, while working on *Neurospora*.

Experiment

(i) They exposed *Neurospora* spores to x-rays expecting some changes in their DNA. These changes were mutation and such organisms with changes were mutant.

(ii) They allowed the progeny of irradiated spores to grow on a defined medium containing all of the nutrients necessary for growth.

(iii) They placed subcultures of individual fungal cells on a minimal medium to test irradiated spore for metabolic deficiencies by mutations. Minimal medium contained only sugar, ammonia, salts, a few vitamins and water.

(iv) Cells that had lost the ability to make other compounds necessary for growth were not able to survive on this medium.

Further they isolated many growth relations of DNA with enzyme deficient mutants and concluded that a gene is involved in synthesizing an enzyme.

Confirmation

Later researchers confirmed this by adding various chemicals to minimal medium. This procedure pin pointed the nature of biochemical deficiency that strain had.

For example, addition of arginine permitted several mutant strains, dubbed arginine mutants to grow. Further arginine was found in three areas on chromosome.

Conclusion

From all investigations especially of arginine, Beadle and Tatum found that there was a specific site for each enzyme on chromosome. Different strains obtained after irradiation showed that for each strain there was change at one site.

Beadle and Tatum concluded that genes produce their effects by specifying the structure of enzymes and that each gene encodes the structure of one enzyme. They called this relationship one gene one enzyme hypothesis.

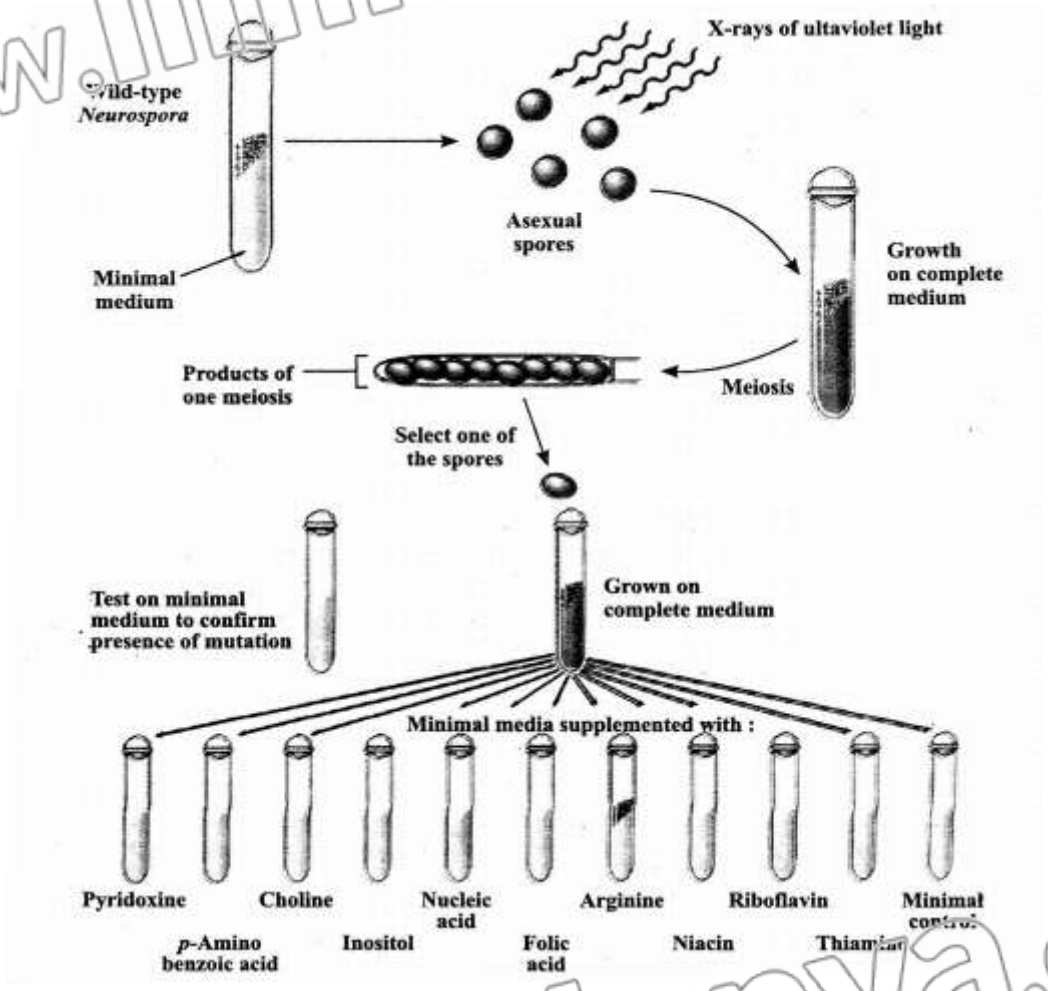


Fig 20.20 Beadle and Tatum's procedure for isolating nutritional mutants in Neurospora

QUESTIONS RELATED TO ABOVE ARTICLE

Describe the work of Beadle and Tatum on Neurospora.

What hypothesis did beadle and Tatum test in their experiment on Neurospora?
(LHR 2018)

Explain work of Beadle and Tatum on Neurospora with help of a figure.
(LHR 2019, SWL 2019)

What hypothesis did Beadle and Tatum test in their experiments on Neurospora?
(Exercise Question v)

20.5.1 One Gene-One Polypeptide Hypothesis

As many enzymes contain multiple protein or polypeptide subunits, each encoded by a separate gene, the relationship is today more commonly referred to as one gene one polypeptide.

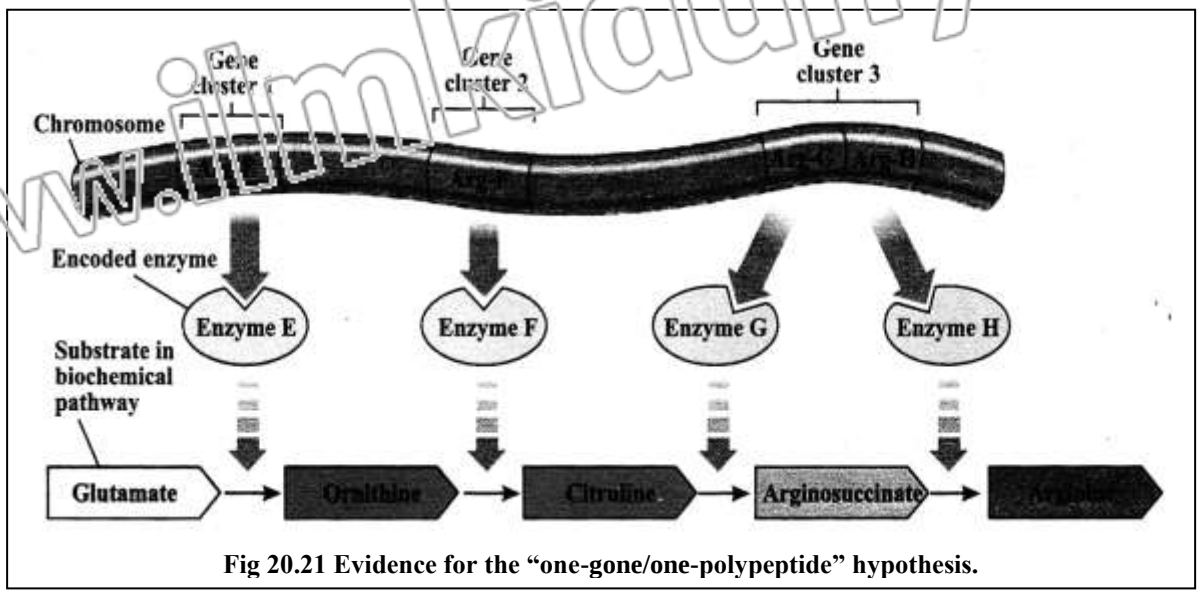


Fig 20.21 Evidence for the "one-gone/one-polypeptide" hypothesis.

QUESTIONS RELATED TO ABOVE ARTICLE

Explain briefly one - gene / one polypeptide hypothesis.

20.5.2 How DNA Encodes Proteins Structure?

Work of F. Sanger

In 1953, an English biochemist Frederick Sanger described the complete sequence of amino acids of insulin. Sanger's work showed that proteins have definable sequence of amino acids.

Work of Vernon Ingram

Vernon Ingram in 1956 discovered the molecular basis of sickle cell anemia.

Cause

He showed that sickle cell anemia is caused by a change from glutamic acid to valine at single position in the protein. Alleles of the gene encoding hemoglobin differed only in their specification of this one amino acid in the hemoglobin amino acid chain.

For example, the critical change leading to sickle cell disease is a mutation that replaces a single thymine with an adenine at position that codes for glutamic acid converting the position to valine.

Conclusion

These studies show that the characteristic of sickle cell anemia and 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 is dictated by the order of nucleotides in a particular region of chromosomes.

The sequence of nucleotides that determines the amino acid sequence of a protein is called a ***gene***.

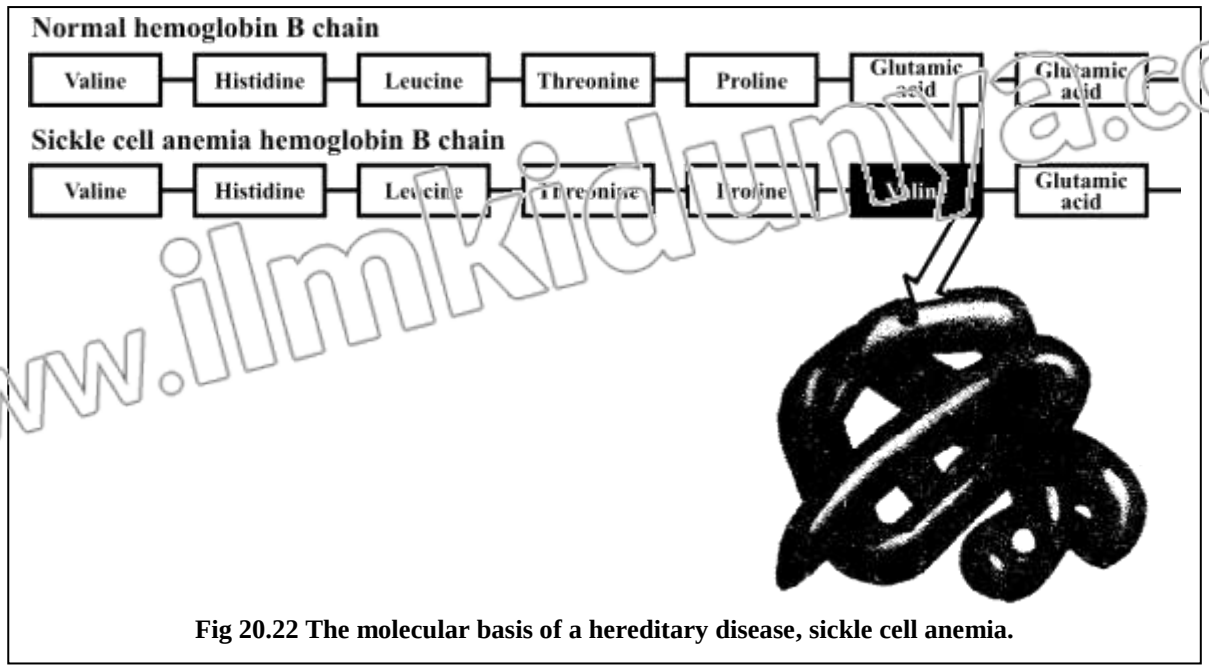


Fig 20.22 The molecular basis of a hereditary disease, sickle cell anemia.

20.6 CELLS USE RNA TO MAKE PROTEIN

ROLE OF RNA IN PROTEIN SYNTHESIS

All organisms use basic mechanism (central dogma) of reading and expressing genes.

Genetic information resides in DNA and then flows into RNA and then into proteins

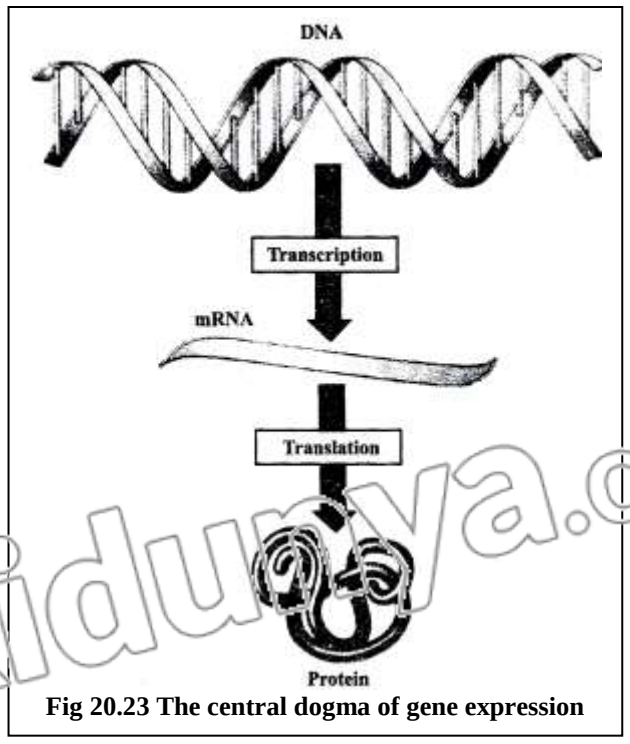


Fig 20.23 The central dogma of gene expression

Mechanism of Central Dogma

Two major steps are involved which collectively represent gene expression.

First Step

First step is transcription during which information is transferred from DNA to mRNA

(i) Enzyme RNA polymerase binds to a particular biding site called promoter located upstream of gene and initiates transcription.

(ii) The enzyme moves along the strand onto gene and mRNA is synthesized.

(iii) At other end of gene (stop signal) enzyme disengages itself from DNA and releases the newly assembled RNA chains.

This chain is a complementary transcript of the gene from which it is copied.

Second Step

Second step is translation during which

(i) Information is transferred from mRNA to protein.

(ii) Ribosomes are attached to mRNA at which amino acids are arranged in sequence to manufacture polypeptide chain.

Types of RNA

(i) Ribosomal RNA (rRNA)

It is found in ribosomes. During translation it provides the site where polypeptides are assembled.

(ii) Transfer RNA (tRNA)

They transport amino acids to ribosomes and arrange them at correct place in sequence to build and elongate polypeptide chain.

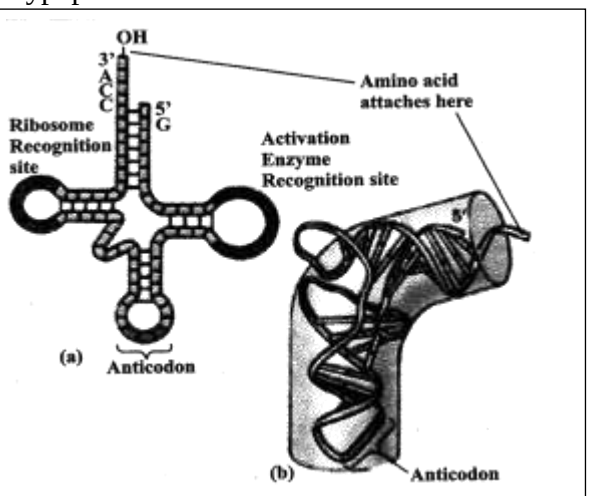


Fig: 20.24 The structure of tRNA
(a) two-dimensional schematic
(b) three dimensional structure

Human cells contain about 45 different kinds of tRNA molecules.

(iii) Messenger RNA (mRNA)

These are long strands of RNA that are transcribed from DNA and that travel to the ribosomes to direct precisely which amino acids are assembled into polypeptides.

QUESTIONS RELATED TO ABOVE ARTICLE

What is the role of RNA in protein synthesis?

20.6.1 Transcription

Definition

The process through which an RNA copy of the DNA sequence encoding the gene is produced with the help of enzyme RNA polymerase.

Features of RNA polymerase

- There is only one type of RNA in prokaryote which is responsible for the synthesis of all the three types of RNAs i.e. rRNA, mRNA and tRNA.
- On the other hand, there are three types of RNA polymerases namely RNA polymerase I, which synthesize rRNA, RNA polymerase II, which synthesizes mRNA and RNA polymerase III which synthesizes tRNA.
- The RNA polymerase enzymes synthesize RNA from 5' ⟶ 3' direction.

Mechanism of Transcription

Only one of the two strands of DNA is transcribed.

- The strand, which is transcribed is called *template strand or antisense strand.*
- The opposite strand is called *coding strand or sense strand.*

Transcription starts at the RNA polymerase binding site called *promoter* on the DNA template strand.

- In prokaryote within promoter, there are two binding sites. These are;
  TTGACA also called -35 sequence
  TATAAT sequence also called -10 sequence.
- In eukaryotes, these sites are;
  TTGACA at -75 sequence.
  TATAAT at -25 sequence.

Different steps involved during transcription are as follows;

i) The binding of RNA polymerase to the promoter is the first step in gene transcription.

ii) One of the subunits of RNA polymerase, sigma factor, is responsible for correct initiation of transcription process. Once the transcription has started, the sigma factor is released and the remaining part of enzyme (core enzyme) moves over the template strand and completes the transcription of the gene.

iii) The DNA strands open up at the place where enzyme is attached to the template strand forming transcription bubble.

iv) The transcription bubble moves down the DNA, leaving the growing strand protruding from the bubble.

v) The stop sequences 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.

vi) 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.

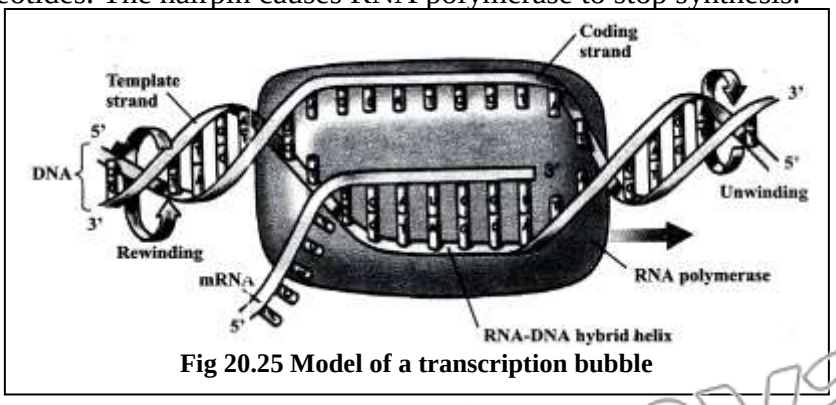


Fig 20.25 Model of a transcription bubble

Fate of mRNA

- In prokaryote (bacteria), the newly synthesized mRNA is directly released into the cytoplasm.
- In eukaryotes, it has to travel large distance from inside the nucleus to ribosomes outside in the cytoplasm. The eukaryotic mRNA is therefore modified in several ways to aid this journey. A cap and a tail are added so that the molecule may remain stable during long journey to ribosomes.

i) The cap is in form of 7 methyl GTP, which is linked 5' to 5' with the first nucleotide.

ii) Tail is in the form of poly A tail linked to 3' end of the RNA.

These caps and tails save the mRNA from variety of nucleases and phosphatases.

QUESTIONS RELATED TO ABOVE ARTICLE

Discuss the process of transcription.
(GRW 2017, BWP 2019, SGD 2019, MTN 2021, BWP 2022)

What is transcription? How it is carried out in cells. (LHR 2022)

Write a note on transcription along with a neat diagram. (SGD 2022)

20.7 GENETIC CODE

It is the code which specifies a particular amino acid in a polypeptide chain/protein.

Triplet Code

The genetic code is a triplet code.

Triplet code is a combination of three nucleotides, which specify a particular amino acid.

Reading of triplet code occurs continuously without punctuation between the three nucleotide units.

Thus, there are three nucleotides in a codon, because;

- A two nucleotide codon would not yield enough combinations to code for 20 different amino acids that commonly occur in proteins. With four DNA nucleotides (G, C, T and A) only 4^2 or 16 pairs of nucleotides could be formed.
- The same nucleotides can be arranged in 4^3 or 64 different combination of three, more than enough to code for the 20 amino acids.

Testing of Genetic Code

After Crick's initial experiments, Marshal Nirenberg, Philip Leader and Hargobind Khorana tested all the 64 codons by making artificial mRNAs and triplet codons and using them to synthesize a protein or aminoacyl-tRNA complexes in cell free systems.

Examples of Genetic Codes

The full genetic code was determined during mid 60s.

- Out of 64 codons, three codons UAA, UAG and UGA do not code for any amino acid and hence are known as nonsense codon. These codons are usually present at the end of the gene and hence are also called stop codons.
- Every gene starts with initiation codon AUG, which encodes the amino acid methionine.

Table 20.1 The Genetic Code

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

Universality of Genetic Code

The genetic code is universal. It is same in almost all the organisms.

For example, AGA specifies arginine in bacteria, in human and all other organisms whose genetic code has been studied.

Because of the universality of codon, the genes can be transferred from one organism to another and be successfully transcribed and translated in their new host.

Non-Universality of Genetic Code

The study of genetic code of mitochondrial DNA showed that genetic code is not all universal.

For example

- UGA codon is normally a stop codon but in mitochondria, it reads as tryptophan.
- AUA was read as methionine instead of isoleucine and AGA and AGG for termination of protein synthesis is instead of arginine.

Thus, it appears that genetic code is not quite universal.

QUESTIONS RELATED TO ABOVE ARTICLE

Why genetic code called as a triplet code? How it is established and proved? Explain different types of genetic codes.

Write a note on genetic code. **(LHR 2021, RWP 2022)**

20.8 TRANSLATION

Definition

Process by which decoding of mRNA occurs by tRNA arranging amino aids in sequence is called translation.

Role of tRNA

tRNA molecules carry amino acids to ribosome/polysome. tRNA contains anticodon, by which it is arranged on specific codon of mRNA transferring amino acid to ribosome to form polypeptide chain.

Mechanism of Translation

Different steps occurring during translation are as follows.

1) Formation of Aminoacyl-tRNA

An activating enzyme called aminoacyl-tRNA synthetase attaches particular tRNA to specific amino acid.

For 20 common amino acids, there are approximately 20 such enzymes.

Joined structure formed by combination of tRNA and amino acid is called aminoacyl-tRNA.

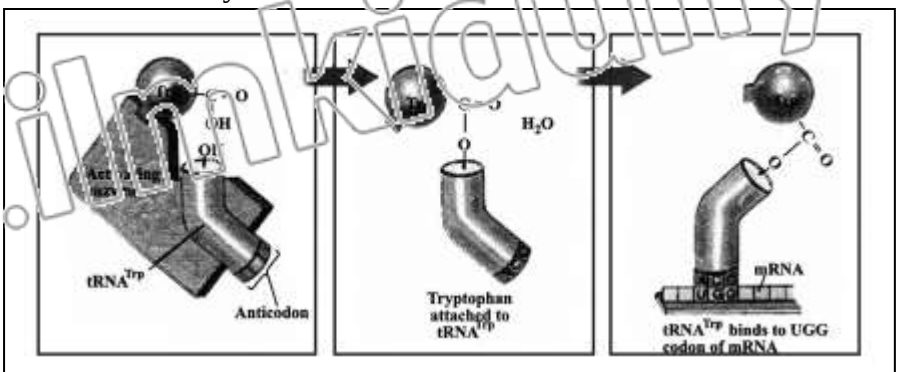


Fig 20.26 Activating enzymes "read" the genetic code

2) Formation of Initiation Complex

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

i) Fist tRNA molecule carrying a chemically modified methionine (N-formyl methionine) binds to small ribosomal subunit. This is done by a protein called initiation factor. tRNA is attached on P site (peptidyl site) where peptide bond will develop.

ii) Two other sites also develop alongwith P site on ribosome. These are

- A site (aminoacyl site) for next aminoacyl-tRNA.
- E site (exit site) for exit of empty tRNA.

iii) All these changes give rise to initiation complex.

iv) Another initiation factor guides this initiation complex to AUG on mRNA.

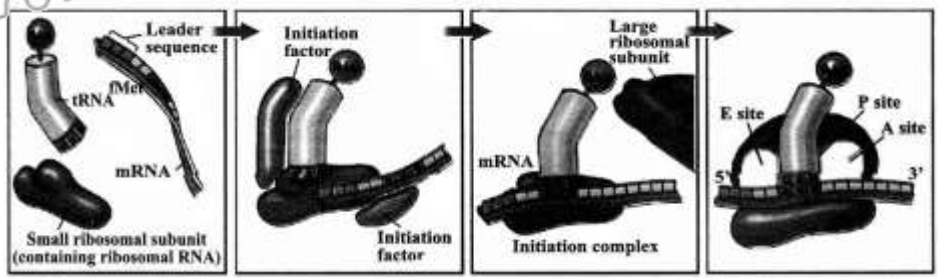


Fig 20.27 Formation of the initiation complex

3) Elongation of Polypeptide Chain

i) Initiation complex has formed and the large ribosomal subunit binds tRNA molecule with appropriate anticodon.

ii) Another protein called elongation factor assist in binding it to the exposed mRNA codon to A site.

iii) The two amino acids, which now lie adjacent to each other, undergo a chemical reaction. This reaction is catalyzed by large ribosomal subunit, which released during initial methionine from its tRNA and is attached to second amino acid through peptide bond.

iv) Another elongation factor translocate ribosome in 5' – 3' direction on mRNA to next codon. This movement translocates the initial tRNA to the E site and ejects it from the ribosome, repositioning the polypeptide chain to the P site and exposed the next codon on the mRNA at the A site.

v) When tRNA molecule recognizing that codon appears, it binds to the codon at the A site, placing its amino acid adjacent to growing chain. The chain then transfers to the new amino acid.

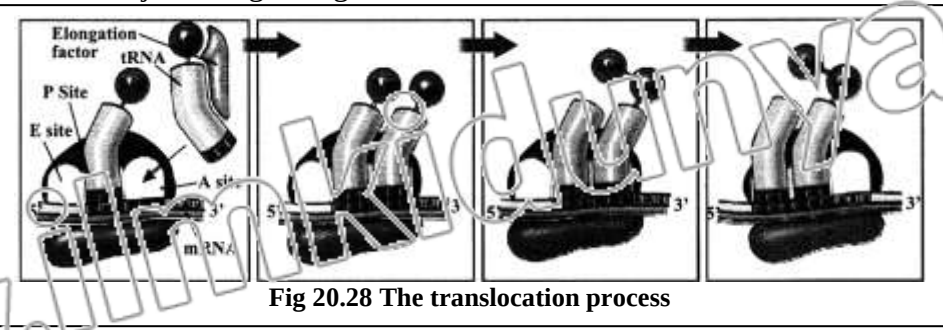


Fig 20.28 The translocation process

vi) Entire process is repeated again.

4) Termination of Polypeptide Chain

Elongation of polypeptide chain continues until a chain-terminating nonsense codon is exposed (e.g. UAA).

Nonsense codons do not bind to tRNA but they are recognized by release factor proteins called release factors. These release factors release the newly polypeptide from the ribosomes.

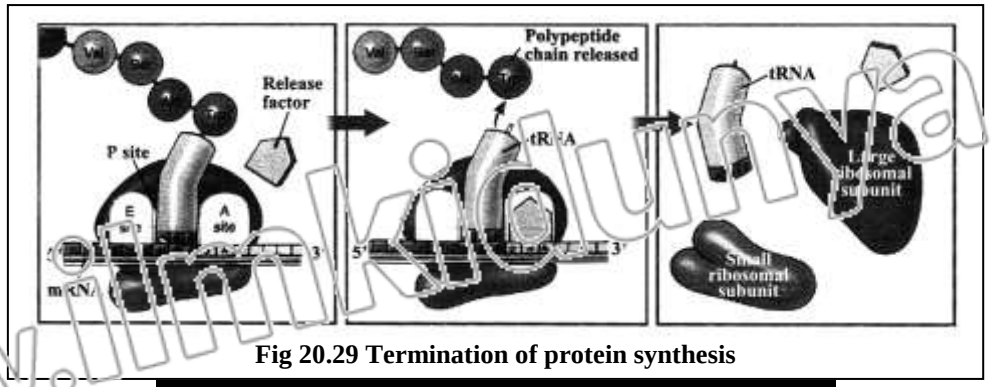


Fig 20.29 Termination of protein synthesis

QUESTIONS RELATED TO ABOVE ARTICLE

Discuss the process of initiation of translation along charging of tRNA.
Describe the process of translation in prokaryotes. (LHR 2019)
How cells use RNA to make Proteins? (BWP 2021)
Explain process of translation. (FSD 2021)

20.9 MUTATIONS

Definition

A permanent change in cell's DNA is called mutation.

The cells of eukaryotes contain an enormous amount of DNA. If the DNA in all of the cells of an adult human were lined up end to end, it would stretch nearly 100 billion kilometer, 60 times the distance from Earth to Jupiter.

Causes of Mutation

Changes in DNA occur due to:

i) Mistake in replication e.g. spontaneous pairing error.

ii) Damage to genetic message e.g. by mutagens usually radiations or chemicals. Mutation due to chemicals is important in industrial societies.

Mutations in Somatic and Germ Line Cells

- Mutation in somatic cells do not pass onto offsprings and so have little evolutionary consequence.
- Mutations in germ line cells are passed on to subsequent generations thus providing the raw material from which natural selection produces evolutionary changes.

Classification of Mutations

Mutation can be classified as:

1) Chromosomal aberration
2) Point mutation

1) Chromosomal Aberration

These are mega-changes and involves:

- Presence of extra chromosome from diploid number
- Loss of a chromosome
- Deletion
- Insertions
- Inversions

Examples

Common examples due to chromosomal aberrations are Down's syndrome, Klinefelter's syndrome etc.

2) Point Mutations

These are mutational changes that affect only message producing alterations in the sequence of DNA nucleotide.

Most of the point mutations involve:

- Alterations in coding sequence of one or more base pairs.
- Spontaneous pairing errors during DNA replication or by mutagens usually chemicals or radiations.

Examples

Sickle cell anemia and phenylketonuria are well known examples of point mutation.

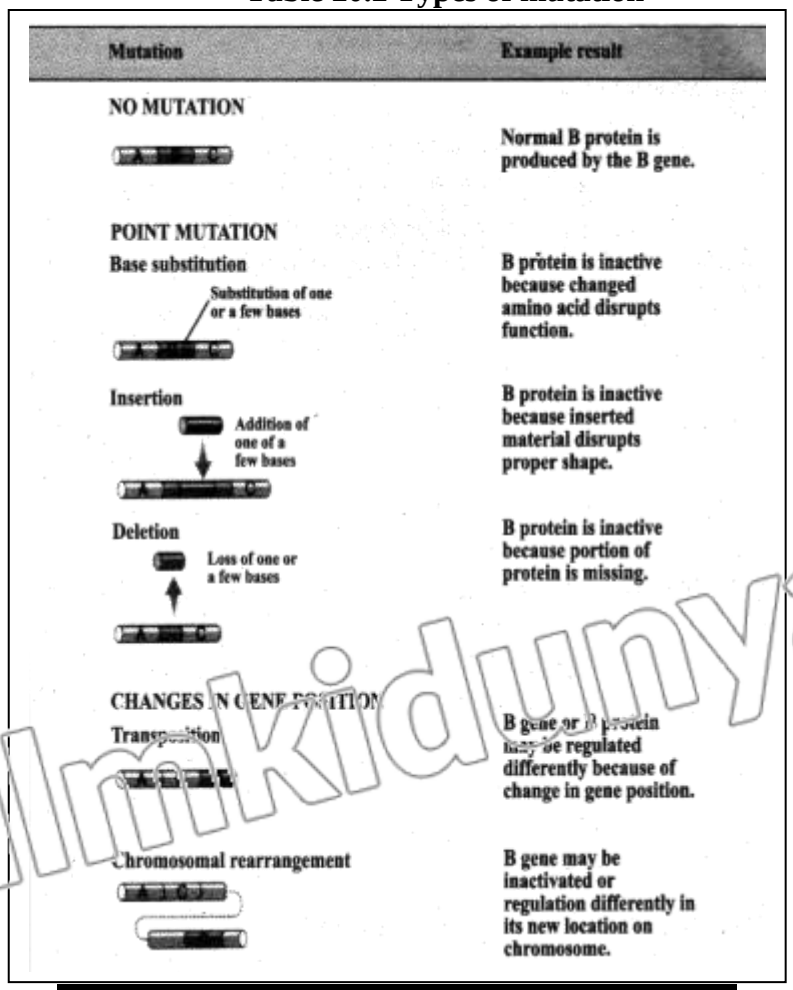
i) Sickle Cell Anemia

Point mutation in it changes glutamic acid to valine at position 6 from N terminal end in hemoglobin β chain. It alters the tertiary structure of hemoglobin and it fails to carry oxygen.

ii) Phenylketonuria

In it phenylalanine is not degraded due to defective enzyme phenylalanine hydroxylase. Phenylalanine consequently accumulates in the brain cells leading to mental retardation as the brain fails to develop in infancy. This disorder is because of point mutation.

Table 20.2 Types of mutation



QUESTIONS RELATED TO ABOVE ARTICLE

Write a note on mutation.

What are mutations? How are they classified? (SWL 2022)

KEY POINTS

F_1 generation

First filial generation. The offspring produced as a result of cross between two parents is called F_1 generation.

F_2 generation

Second filial generation. The cross between progeny of F_1 is called F_2 cross. Their progeny is called F_2 generation.

X – Ray diffraction

It is a technique in which crystals of a compound like DNA are formed. X – rays are cpassed from these crystals. These are scattered in different directions. The pattern of scattering is measured. A model of a compound can be formed from this pattern.

Okazaki fragment

These are small fragment of DNA. These are synthesized on lagging strand. This strand is 3' to 5', while the direction of replication is 5' to 3'. So replication takes place in fragments in lagging strand.

Minimal medium

It is special medium contain, vitamin biotin, salts, sugars and agar. Minimal medium is used for culturing of micro-organisms.

Central dogma

The central dogma is the process by which a gene is read and expressed. It has two steps: transcription and translation.

Gene expression

The expression of gene of a character in the form of synthesis of specific proteins is called gene expression.

Codes and anticodon

Codes are present on DNA and mRNA. While anticodons are present on tRNA. The anticodon is opposite of code of mRNA. The tRNA recognize its code on mRNA with the help of its anticodon and get attached to it.

Aminoacyl-tRNA synthetase

An enzyme that catalyzes the linkage of tRNA molecule to its corresponding amino acids to form aminoacyl-tRNA synthetase complex during protein synthesis, one of which exists for each of the twenty amino acids.

EXERCISE

Q 1 Fill in the blanks.

i) Particular tRNA molecule become attached to specific amino acids through the action of activating enzymes called _____.

ii) _____ is the transfer of genetic material from one cell to another and can alter the genetic make up of the recipient cell.

iii) In a bacteria, a subunit of RNA polymerase called _____ recognizes -10 sequence in the promoter and binds RNA polymerase there.

iv) A typical human chromosome contains about _____ nucleotides in its DNA.

v) Miescher extracted a white substance from the nuclei of human cells and fish sperm and called this substance _____.

Ans i) Aminoacyl-tRNA synthetase
ii) Transformation
iii) Sigma factor
iv) 140 million
v) Nuclein

Q 2 Write whether the statement is true or false and write the correct statement if false.

i) The strand of DNA that is not transcribed is called the coding strand. **(True)**

ii) TATAAT sequence called -35 sequence is part of promoter, where transcription actually starts. **(False)**
TATAAT sequence called -10 sequence is part of promoter, where transcription actually starts.

iii) Rosalind Franklin carried out an x-ray diffraction analysis of DNA. **(True)**

iv) The base pairs in DNA helix are planar and stack 34 nm apart as a result of hydrophobic interactions. **(False)**
The base pairs in DNA helix are planar and stack 0.34 nm apart as a result of hyperphobic interactions.

Q 3 Encircle the correct answer from the multiple choices.

i) mRNA is synthesized by:
(a) DNA polymerase
(b) RNA polymerase
(c) RNA ligase
(d) None of above

ii) Which of the following are nonsense codons?
(a) AUG (b) UAA
(c) CUA (d) All of above

iii) Enzymes are responsible for assembly of:
(a) Nucleic acid
(b) Protein
(c) Carbohydrates
(d) All a, b, c

iv) In bacteria, the newly synthesized mRNA is released in:
(a) Nucleus
(b) Cytoplasm
(c) Mitochondria
(d) In b and c

Answer Key	
1	b
2	b
3	d
4	b

Q 4 Short Questions

i) What are three major classes of RNA?

Ans. There are three classes of RNA:

(i) mRNA: It carries information for protein synthesis from genes to ribosomes

(ii) tRNA: It transfers amino acids to ribosomes for proteins synthesis

(iii) rRNA: It is a major types of RNA and is the integral component of ribosomes.

ii) What is the function of RNA polymerase in transcription?

Ans. It is involved in formation of RNA i.e. transfer of genetic message from DNA to RNA, according to which proteins are synthesized. It forms the transcription bubble.

iii) How did Crick and his colleagues determined that how many nucleotides are used to specify each amino acid?

Ans. There are four nucleotides while 20 amino acids are involved in formation of most of the proteins.

If only one nucleotide (of A, T, C or G) is involved, then they can specify only four amino acids.

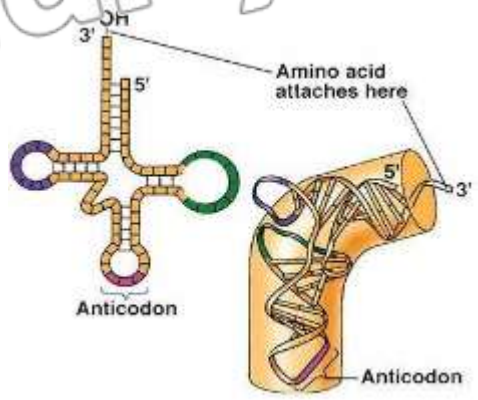
If two nucleotides are involved for one amino acids (e.g. AA, GC, AT) then they can specify only 16 amino acids.

If three nucleotides are involved to specify one amino acid (e.g. AAG, AUG etc) then they can specify 64 amino acids which is sufficient.

Thus from it they concluded that genetic code is actually triplet code.

iv) What is anticodon?

Ans. Triplet code present on tRNA is called anticodon. It binds with triplet codon on mRNA during translation.



Q 5 Extensive Questions.

i) How did Hershay and Chase determine which components of bacterial viruses contain the virus of hereditary information?

Ans (see article 20.4)

ii) What is the three dimensional shape of DNA? How does three dimensional shape of DNA fit with Chargaff's observations on the proportions of purines and pyrimidines in DNA?

Ans (see article 20.4.1)

iii) How did Meselson and Stahl show that DNA replication is semiconservative?

Ans (see article 20.4.4)

iv) What is the basis for the requirement that the leading and lagging strands be replicated by different mechanisms?

Ans (see article 20.4.5)

v) What hypothesis did Beadle and Tatum test in their experiments on *Neurospora*?

(Ans) (see article 20.4.5)