**Semester – II**

**19MBU203** **MICROBIAL GENETICS** **(4H –4C)**

**Instruction Hours / week: L: 3 T: 1 P: 0** **Marks:** Internal: **40** External: **60** Total:**100**
**End Semester Exam:** 3 Hours

**COURSE OBJECTIVES**

1. To focus on the basic principles of genetics incorporating the concepts of classical, molecular and population genetics.
2. Compilation is required for recent advances in genetic principles for strong foundation in Biotechnology.

**COURSE OUTCOME (CO'S)**

1. This course provided candidates with basic knowledge and understanding of Molecular Biologywith special reference to microbialgenome.
2. Students undertaking this course will be able to describe the nature of molecular world and its application in modern Microbiologicalsectors.

**Unit I- History of genetics**

Concept of Genetics, Mendelian principles, DNA as a genetic material, Experimental evidence – Chromosomal theory of inheritance. DNA structure, models of DNA, DNA replication, transcription, translation, RNA structure and types.

**Unit II- Plasmids**

Types of plasmids- replication, partitioning, host range, plasmid-incompatibility, amplification, curing and application.

**Unit III- Genetic code**

Genetic code- Operon concept-Lactose, tryptophan.Genetic recombination in bacteria- Conjugation, Transformation-Transduction and its types.Gene Mapping techniques-gene and chromosome walking.

**Unit IV- Mutations**

Mutations and mutagenesis, types of mutations and mutagens.Identification of mutants- Ames test.

**Unit V- Transposons**

Transposons-definition, types of Transposons, mechanism of transposition and application.
Mu transposon and eukaryotic transposable elements.Applications.

**SUGGESTED READINGS**

1. Klug, W.S., Cummings, M.R., Spencer, C., Palladino, M. (2011). Concepts of Genetics, 10th edition, BenjaminCummings.
2. Krebs, J., Goldstein, E., Kilpatrick, S. (2013). Lewin's Essential Genes, 3rd edition, Jones and Bartlett Learning.
3. Pierce, B.A. (2011) Genetics: A Conceptual Approach, 4th edition, Macmillan Higher Education Learning.
4. Watson, J,D., Baker, T.A., Bell, S.P., et al. (2008) Molecular Biology of the Gene, 6thedition, BenjaminCummings.
5. Gardner, E.J., Simmons, M.J., Snustad, D,P. (2008). Principles of Genetics. 8th edition,Wiley-India.
6. Russell, P.J. (2009). Genetics- A Molecular Approach. 3rd edition, BenjaminCummings.
7. Sambrook, J., and Russell, D.W. (2001). Molecular Cloning: A Laboratory Manual. 4thedition, Cold Spring Harbour Laboratorypress.
8. Maloy, S.R, Cronan, J.E., and Friefelder, D. (2004) Microbial Genetics 2nd edition, Jones and Barlett Publishers.

# KARPAGAM ACADEMY OF HIGHER EDUCATION

**CLASS: I BSC Microbiology COURSE NAME: MICROBIAL GENETICS**

**COURSE CODE: 19MBU203 LECTURE PLAN BATCH: 2019-2022**

## UNIT I

| Duration | Topic | Reference |
|---|---|---|
| 01 | Introduction to Genetics, Concept of Genetics | R1:1 to 2; T1:3 to 4 |
| 02 | Mendelian principles | R1: 271 to 272 |
| 01 | DNA as a genetic material | R1: 13 to 19 |
| 01 | Experimental evidence – Chromosomal theory of inheritance | R1: 27 to 35 |
| 01 | DNA structure, models of DNA | R1: 43 to 45; T1: 167 to 169; T1: 170 to 172; R1: 50 to 52; T1: 178 to 181 |
| 01 | DNA replication | |
| 01 | RNA structure and types | R1: 25 to 26; T1: 46 to 49 |
| 1.5 | Transcription | R1: 87- 106, 119-126 |
| 1.5 | Translation | T1:120 to 174 |
| 01 | Unit revision | |
| | **Total hours: 12 h** | |

## UNIT II

| Duration | Topic | Reference |
|---|---|---|
| 01 | Types of plasmids | R1: 20 to 21; T1: 29 to 32 |
| 01 | Plasmid replication | R1: 22 to 24; T1: 39 to 42 |
| 01 | Plasmid partitioning and host range | R1: 25 to 26; T1: 46 to 47<br>R1: 25 to 26; T1: 47 to 49 |
| 01 | Plasmid-incompatibility and amplification | R1: 45 to 47; T1: 170 to 172<br>R1: 47 to 48; T1: 175 to 176 |
| 01 | Curing and application. | T1: 176 to 17 |
| 01 | Unit revision | |
| | **Total hours: 6 h** | |

**UNIT III**

| Duration | Topic | Reference |
|---|---|---|
| 01 | Genetic code | R1: 115 to 117<br>T1: 124 to 126 |
| 01 | Operon concept-Lactose | R1:516 to 526<br>T1:143 to 152 |
| 01 | Operon concept - tryptophan | R1:528 to 529<br>T1:153 to 157 |
| 01 | Genetic recombination in bacteria- Conjugation | R1: 484 to 485<br>T1:331 to 332 |
| 01 | Transformation | R1: 490 to 492<br>T1:311 to 313 |
| 01 | Transduction and its types | |
| 01 | Gene Mapping techniques-gene and chromosome walking | |
| 01 | Unit revision | |
| | **Total hours: 8 h** | |

**UNIT IV**

| Duration | Topic | Reference |
|---|---|---|
| 01 | Mutations | R1:133 to 134<br>T1: 212 to 213 |
| 01 | Mutagenesis | |
| 01 | Types of mutations | R1: 136 to 140<br>T1:214 to 215; 218 to 224 |
| 01 | Types of mutagens | T1:226 to 232 |
| 01 | Identification of mutants- Ames test | R1: 148 to 150<br>T1:232 to 236 |
| 01 | Unit revision | |
| | **Total hours: 6 h** | |

**UNIT V**

| Duration | Topic | Reference |
|---|---|---|
| 01 | Introduction and Transposons- definition | R1: 481 to 482<br>T1: 281 to 308 |
| 01 | Types of Transposons | |
| 01 | Mechanism of transposition | R1: 482 to 485<br>T1:331 to 332 |
| 01 | Mu transposon | R1: 485 to 486<br>T1:334 to 335<br>R1: 490 to 492<br>T1:311 to 313 |
| 01 | Eukaryotic transposable elements | |
| 01 | Applications of Transposons | |
| 01 | Unit revision | |
| 01 | Discussion on previous years question papers | |
| | **Total hours: 8 h** | |

**TEXTBOOKS**

T1: David Freifelder, Microbial Genetics. Narosa Publishing House, 10th edition, 2004. New Delhi, India.

**REFERENCES**

R1: Peter J. Russell, i Genetics – A molecular approach, 7th edition, 2010. Pearson Benjamin Cummings Publishers, Boston, USA.

**WEBSITES**

W1: www.shomusbiology/MolecularBiology/index.conjugationandtransduction %crp/html.

# Unit I

## Syllabus

Concept of Genetics, Mendelian principles, DNA as a genetic material, Experimental evidence – Chromosomal theory of inheritance. DNA structure, models of DNA, DNA replication, transcription, translation, RNA structure and types

## Concept of Genetics

Genetics is a field of biology that studies how traits are passed from parents to their offspring, heredity. A gene is a specific segment of a DNA/RNA molecule that holds the information for one specific protein.

Like the other living organisms, microorganisms have heredity and variable characteristics. Heredity keeps microbial genetic traits relatively stable to ensure the reproduction of the species, while variations produce changes in the microorganisms that are useful for microbial survival and evolution and ultimately lead to the generation of new species.

Microbes are ideally suited for biochemical and genetic studies and have made huge contributions to these fields of science such as the demonstration that DNA is the genetic material, that the gene has a simple linear structure that the genetic code is a triplet code, and that gene expression is regulated by specific genetic processes.

Historical Development

- People have known about inheritance for a long time
  Example - children resemble their parents; domestication of animals and plants, selective breeding for good characteristics
- Despite knowing about inheritance in general, a number of incorrect ideas had generated and overcome before modern genetics could arise.
  1. All life comes from other life. Living organisms are not spontaneously generated from non-living material.
  2. Species concept: offspring arise only when two members of the same species mate.
  3. Organisms develop by expressing information carried in their hereditary material.
  4. The environment can‘t alter the hereditary material in a directed fashion. There is no inheritance of acquired characteristics. Mutations are random events.
  5. Male and female parents contribute equally to the offspring.

Three major events in the mid-1800’s led directly to the development of modern genetics.

| Year | Scientist(s) | Discovery |
|---|---|---|
| 1858 | Charles Darwin, Alfred Russel Wallace | Joint announcement of the theory of natural selection-that members of a population who are better adapted to the environment survive and pass on their traits. |
| 1859 | Charles Darwin | Published The Origin of Species. |
| 1866 | Gregor Mendel | Published theresultsofhis investigations of the inheritance of "factors" in pea plants. |

| | | |
|---|---|---|
| 1900 | Carl Correns, Hugo de Vries, Erich von Tschermak | Mendel's principles were independently discovered and verified, marking the beginning of modern genetics. |
| 1902 | Walter Sutton | Pointed out the interrelationships between cytology and Mendelism, closing the gap between cell morphology and heredity. |

**Note:** Terms in Genetics

- *Character*– a heritable feature, such as flower color
- *Trait*– a variant of a character, such as purple or white flowers
- *Hybrid* – Each trait carries two copies of unit of inheritance, one inherited from the mother and the other from the father
- Alternative forms of traits are called *alleles*
- *Phenotype* – observable characteristic of an organism(morphology, development, biochemical or physiological properties, or behavior)
- *Genotype* – actual gene constitution of a cell, an organism, or an individual (usually with reference to a specific character under consideration)
- *Homozygous* – two alleles of trait are the same (YY or yy)
- *Heterozygous* – two alleles of trait are different (Yy)
- Capitalized traits – dominant phenotypes(YY)
- Lowercase traits– recessive phenotypes(yy)
- Punnett square - is a square diagram that is used to predict the genotypes of a particular cross or breeding experiment. It is named after Reginald C. Punnett, who devised the approach.
- Allele: is a variant form of a gene. Some genes have a variety of different forms, which are located at the same position, or genetic locus, on a chromosome

**Mendelian principles:**

Gregor Mendel (1822–1884) is known as the father of genetics. He studied segregation of traits in the garden pea (*Pisum sativum*) beginning in 1854 and published his theory of inheritance in 1865. He also proposed the key laws of genetics from this work on inheritance of traits in peas in 1866.

Mendel's experiments: Mendel used the scientific approach to identify the laws of inheritance and discovered the basic principles of heredity by breeding garden peas in carefully planned experiments.

Mendel chose to experiment with peas because they possessed four important qualities:

1. Peas had been shown to be true-breeding (all offspring will have the same characteristic generation after generation).
2. Peas exhibit a variety of contrasting traits
    a) Pea shape (round or wrinkled)
    b) Pea colour (green or yellow)
    c) Pod shape (constricted or inflated)
    d) Pod colour (green or yellow)

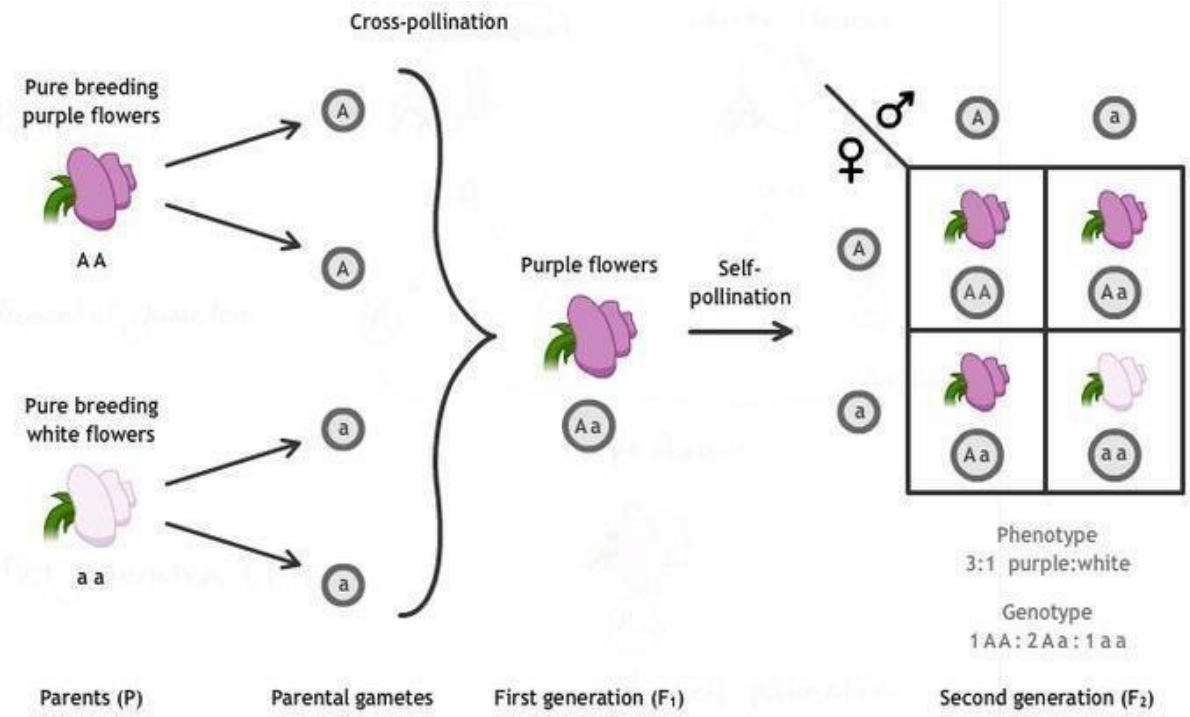
e) Flower colour (purple or white)
f) Plant size (tall or dwarf)
g) Position of flowers (axial or terminal)

3. The shape of the pea flower protected it from foreign pollen. Peas usually reproduce by self-pollination, in which pollen produced by a flower fertilizes eggs in the same flower.
4. Pea plants grow quickly, easy to grow, easy to get large numbers.

- In a typical breeding experiment Mendel mated two contrasting, true-breeding varieties, a process called hybridization
- The true-breeding parents are called the P generation
- The hybrid offspring of the P generation are called the F1 generation
- When F1 individuals self-pollinate the F2 generation is produced

| Pea trait | Dominant trait | | Recessive trait | | Numbers in second generation (F2) | Ratio |
|---|---|---|---|---|---|---|
| **Seeds** | | | | | | |
| Seed shape | Round |  | Wrinkled |  | 5474:1850 | 2.96:1 |
| Seed colour | Yellow |  | Green |  | 6002:2001 | 2.99:1 |
| **Whole plants** | | | | | | |
| Flower colour | Purple |  | White |  | 705:224 | 3.15:1 |
| Flower position | Axial |  | Terminal |  | 651:207 | 3.14:1 |
| Plant height | Tall |  | Short |  | 787:277 | 2.84:1 |
| Pod shape | Inflated |  | Constricted |  | 882:299 | 2.95:1 |
| Pod colour | Green |  | Yellow |  | 428:152 | 2.82:1 |

**Crossing Pea Plants (flower color)**




**Mendel's Observations**

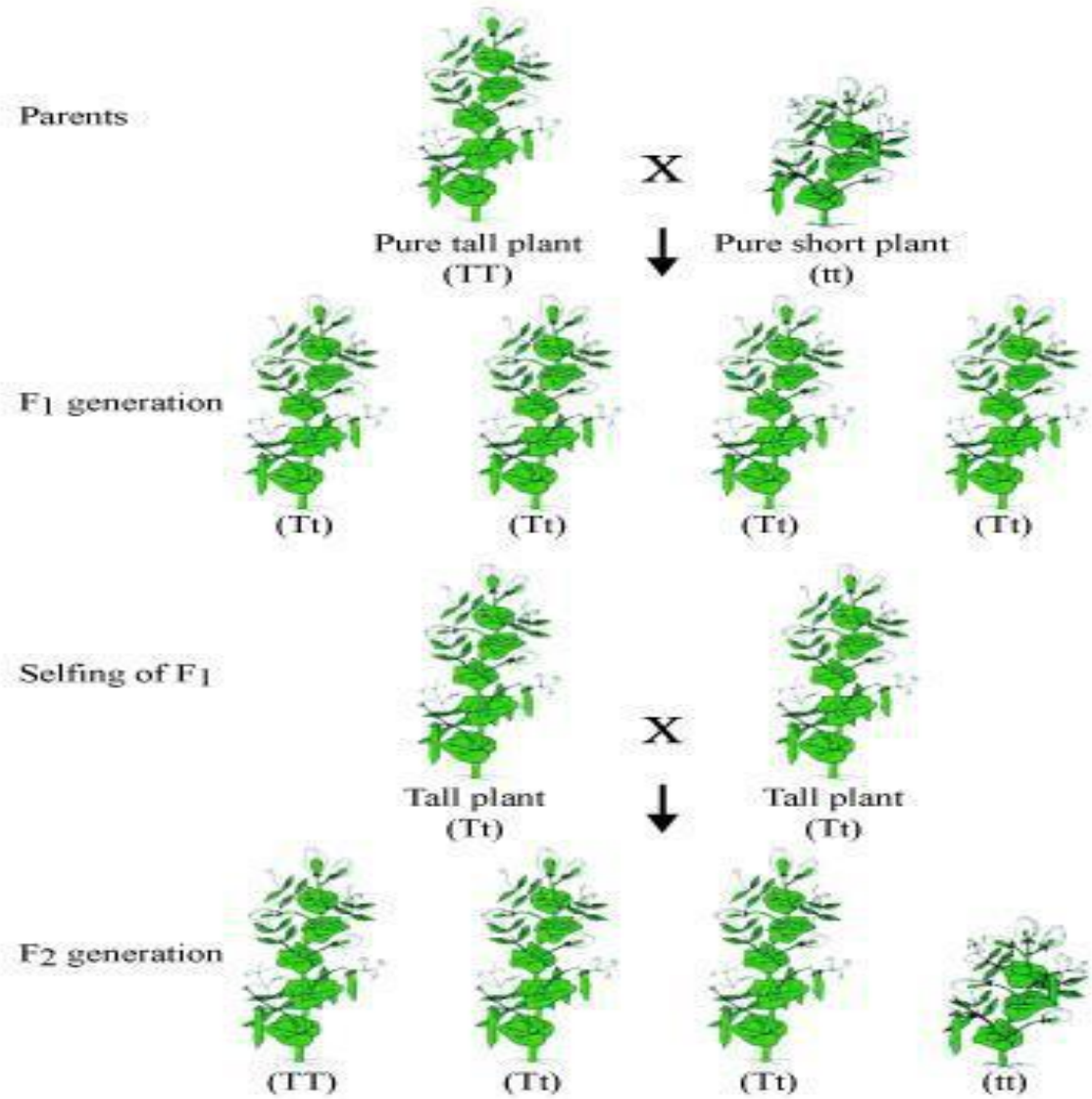
• When Mendel crossed contrasting, true-breeding white and purple flowered pea plants all of the offspring were purple

• When Mendel crossed the F1 plants, many of the plants had purple flowers, but some had white flowers

• A ratio of about three to one, purple to white flowers, in the F2 generation.

**Crossing Pea Plants (plant height)**

| | | |
|---|---|---|
| *P*: | Tall (true breeding) × Dwarf (true beeding) | |
| Factors: | (*TT*) | (*tt*) |
| Gametes: | *T* | *t* |
| $F_1$ hybrid: | Tall × Self *i.e.* Tall (*Tt*) | |
| | (*Tt*) | |
| Gametes: | *T*, *t* | *T*, *t* |
| $F_2$: | 3 Tall: 1 Dwarf | |
| | (*TT*, *Tt*, *tT*) (*tt*) | |




**Mendel's Principles**

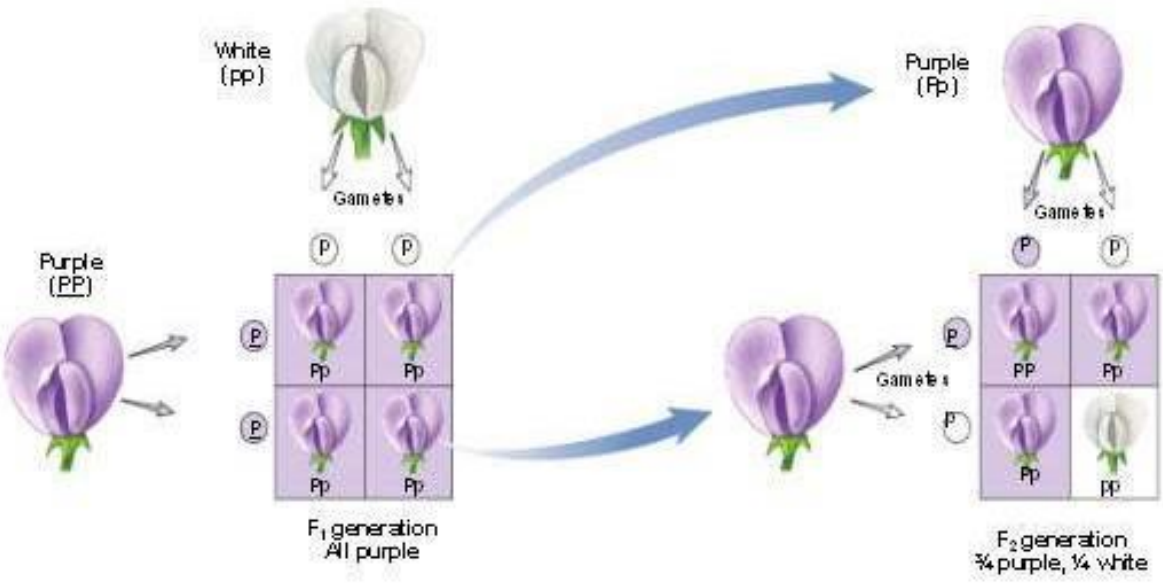
Mendel's Law of Segregation: Two members of a gene pair segregate (separate) from each other during the formation of gametes.
Example: Monohybrid Cross

Mendel's Law of Independent Assortment: Genes on different chromosomes behave independently in gamete production.
Example: Dihybrid Cross

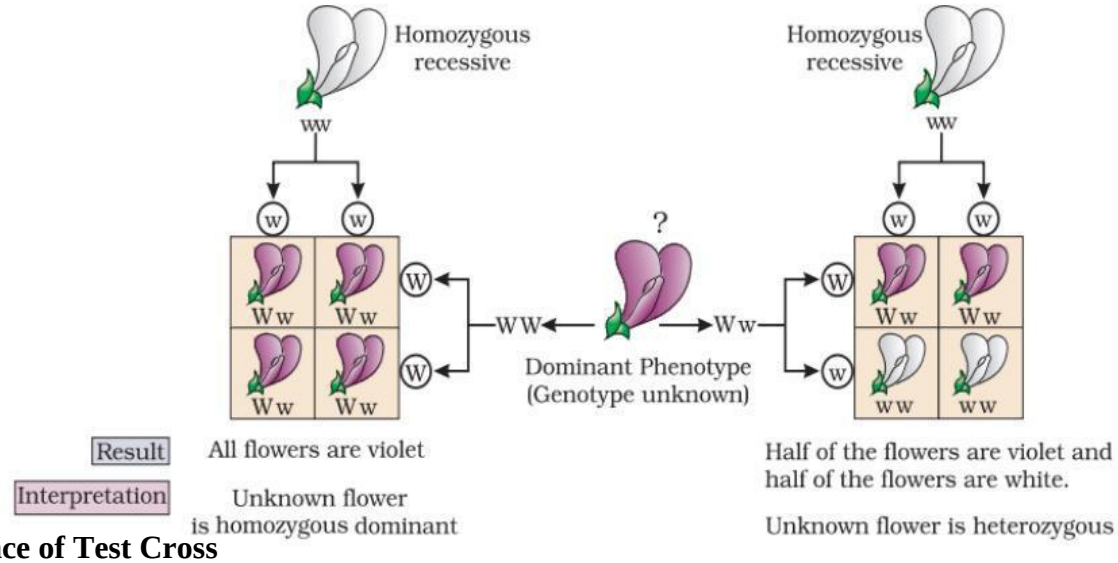
- The law of segregation, describing how individual traits are inherited.
- The law of independent assortment, describing how two or more traits are inherited relative to one another.

Mendel's Monohybrid Cross: A monohybrid cross is a breeding experiment between P generation (parental generation) organisms that differ in a single given trait. The P generation organisms are homozygous for the

given trait, however, each parent possesses different alleles for that particular trait.




**Test Cross:** Mendel devised a system of conducting verification for the results obtained by him. It is known as test cross. It is a cross between F1 plant and the recessive parent. A test cross- conducted for the monohybrid inheritance results in the two opposite characters expressing in a ratio of 1:1. Similarly, a test cross-conducted for the dihybrid inheritance results in the expression of the two parental combinations and the two recombinations appear in the ratio 1:1:1:1.




**Significance of Test Cross**

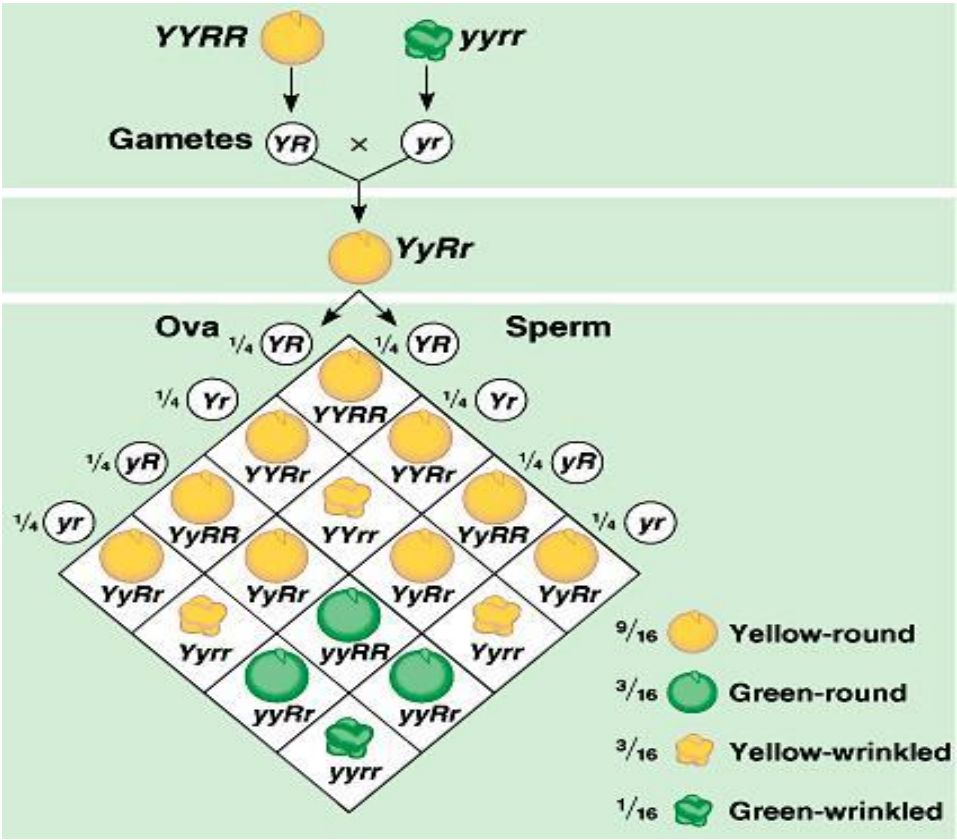
- Test cross can be used to determine the genotype of the F1 plant.
- The test cross can be used to support the idea that the reappearance of the recessive character in the F2 generation is due to the heterozygous condition of the F1 plant.
- The test can be used to verify whether any given pair of characters can be alleles (contrasting characters)

**Back Cross:** If an $F_1$ individual or an individual of $F_2$ or $F_3$ generations is crossed with any one of the parents it is called a back cross

**Mendel's Dihybrid Cross:** A dihybrid cross is a breeding experiment between P generation (parental generation) organisms that differ in two traits. The individuals in this type of cross are homozygous for a specific trait.

- Mendel identified his second law of inheritance by following two characters at the same time

- Mendel was interested in determining whether alleles at 2 different gene loci segregate dependently or independently
- Crossing two, true-breeding parents differing in two characters produces dihybrids in the F1 generation, heterozygous for both characters
- Mendel chose to cross a pea plant that was homozygous and dominant for round (RR), yellow (YY) seeds with a pea plant that was homozygous and recessive for wrinkled (rr), green (yy) seeds.
- Organisms in this initial cross are called the parental, or P generation. The offspring of the RRYY x rryy cross, which is called the F1 generation, were all heterozygous plants with round, yellow seeds and the genotype RrYy.
- Next, Mendel crossed two plants from the F1 generation. This step is the dihybrid cross, and it is represented as:
  RrYy x RrYy




- Mendel observed that the F2 progeny of his dihybrid cross had a 9:3:3:1 ratio and produced nine plants with round, yellow seeds, three plants with round, green seeds, three plants with wrinkled, yellow seeds and one plant with wrinkled, green seeds. From his experiment, Mendel observed that the pairs of traits in the

parental generation sorted independently from one another, from one generation to the next.

**Mendel's conclusions**

- Genes are distinct entities that remain unchanged during crosses
- Each plant has two alleles of a gene
- Alleles segregated into gametes in equal proportions, each gamete got only one allele
- During gamete fusion, the number of alleles was restored to two

The chromosomes present in the diploid cells of the majority of the sexually reproducing animals are of two types: autosomes bearing genes for somatic characters and sex chromosomes bearing genes for sex. Sex chromosomes also carry some genes for non-sexual characters such as colour blindness and haemophilia.

Such genes which are always associated with sex chromosomes are called sex-linked genes. In man and Drosophila the sex chromosomes (X and Y) are unequal in size and shape, X being larger and rod shaped whereas Y is small and slightly curved.

In Mendelian pattern of inheritance, the genes for contrasting characters were located on autosomes but not on the sex chromosomes. There are three types of sex-linked genes depending upon their association with particular chromosome.

They are as follows:

(i) The genes which are located on X-chromosomes are called X-linked genes or sex linked genes.

(ii) The genes which are located on Y chromosomes are called Y-linked genes or holandric genes.

(iii) Certain genes are found to occur in both X and Y chromosomes. Such genes are called incomplete sex-linked genes.

In order to understand the inheritance of character present in sex chromosomes, let us understand transmission of X-chromosome from male individual in Drosophila or in man. The X-chromosome from male individual will always pass to the daughter, while X-chromosomes from female individual will be distributed equally among the daughter and sons.

A character from the father goes to the daughter ($F_1$) and then from daughter to grandson in the next generation ($F_2$). Such type of inheritance is also called as criss-cross inheritance. In this type of inheritance result of the reciprocal crosses are not identical as in case with Mendelian crosses.

**Morgan's Hypothesis**

- Thomas Hunt Morgan provided convincing evidence that chromosomes are the location of Mendel's heritable factors
- Morgan worked with fruit flies
    - Because they breed at a high rate
    - A new generation can be bred every two weeks
    - They have only four pairs of chromosomes

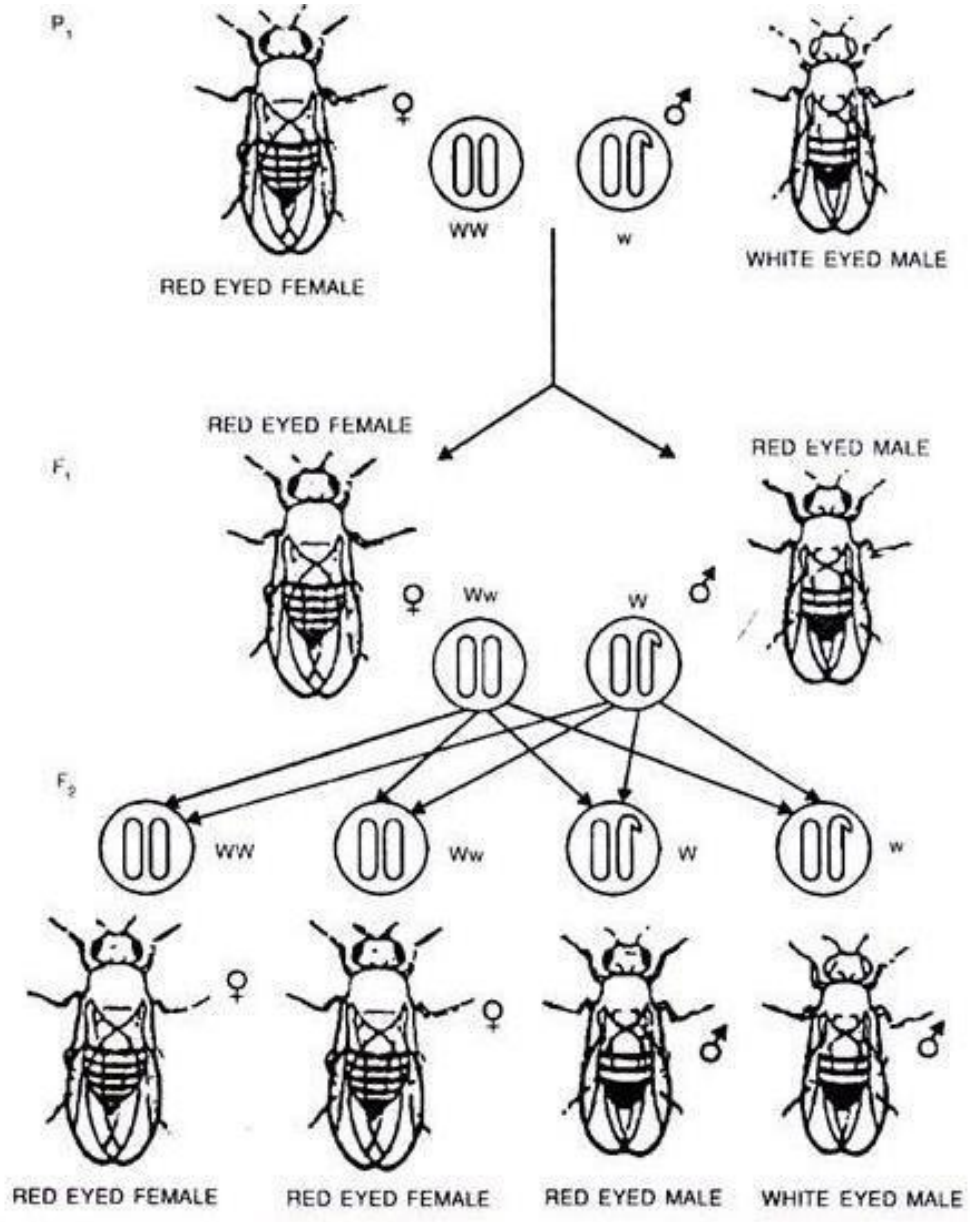
Sex-Linkage in *Drosophila*:

T.H. Morgan (1910) for the first time discovered sex-linkage in *Drosophila melanogaster*. Morgan when experimenting noted the sudden appearance of one white-eyed male (mutant form) in the culture of normal red-eyed *Drosophila*. This white-eyed male was crossed with red eyed female. The $F_1$ flies (both male and female) were all red-eyed indicating that white eye colour is recessive to the normal red eye colour.

Character - Traits

Eye colour - Red eye (wild type)

White eye (mutant)




When these $F_1$ flies were inter-crossed freely, the red-and white-eyed flies appeared in the ratio 3: 1 in the $F_2$ generation. White- eyed flies were male. Among the red eyed flies two-third was female and one-

third was male. The females were all red eyed whereas 50 % males were white eyed and the remaining 50 % males were red eyed.

***X- linked genes***

- In sex linked characteristics the reciprocal crosses do not give the same results
- For X-linked genes fathers do not pass the mutant allele onto their sons
- For X-linked genes fathers pass the mutant allele onto their daughters who are carriers
- Carrier mothers may pass the allele onto their sons (50 % chance)
- Females showing the trait for an X-linked mutant allele can exist but they are rare
- Female carriers may show patches of cells with either trait due to X chromosome inactivation.

**The Chromosomal basis of Inheritance**

***Chromosomal Behavior***

- Mendelian inheritance has its physical basis in the behavior of chromosomes
- The behavior of chromosomes during meiosis was said to account for Mendel‘s laws of segregation and independent assortment

***Chromosome Theory of Inheritance***

- By the beginning of the 20th century, cytologists had observed that chromosome number is constant in all cells of a species, but varies widely between species.
- Sutton and Boveri (1902) independently realized the parallel between Mendelian inheritance and chromosome transmission, and proposed the chromosome theory of inheritance, which states that Mendelian factors (genes) are located on chromosomes.
- Mendelian genes have specific loci on chromosomes
- Chromosomes undergo segregation and independent assortment

**DNA as a genetic material:**

1. Some substance must be responsible for passage of traits from parents to offspring. For a substance to do this it must be:
    a. Stable enough to store information for long periods.
    b. Able to replicate accurately.
    c. Capable of change to allow evolution.
2. In the early 1900s, chromosomes were shown to be the carriers of hereditary information. In eukaryotes they are composed of both DNA and protein, and most scientists initially believed that protein must be the genetic material.

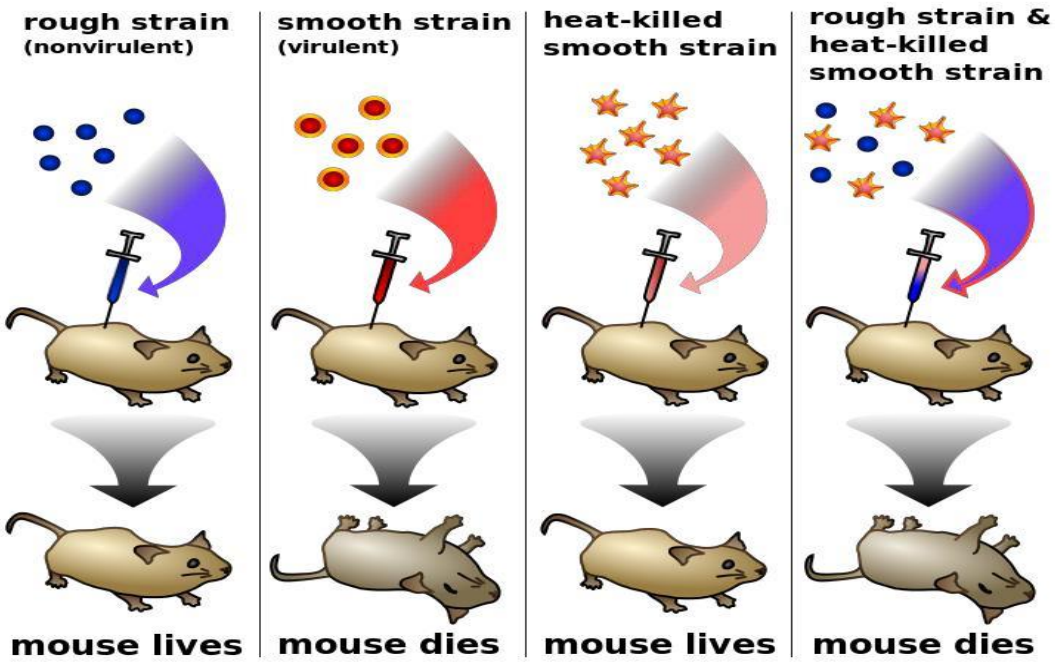
Griffith’s Transformation Experiment

- In 1928, Frederick Griffith‘s experimented with *Streptococcus pneumoniae* bacteria in mice showed that something passed from dead bacteria into nearby living ones, allowing them to change their cell surface.
- There are 2 strains of *Streptococcus pneumoniae*
    - a type III-S strain is virulen

- a type II-R strain is nonvirulent

- Griffith infected mice with these strains hoping to understand the difference between the strains
- He called this agent the transforming principle, but did not know what it was or how it worked.

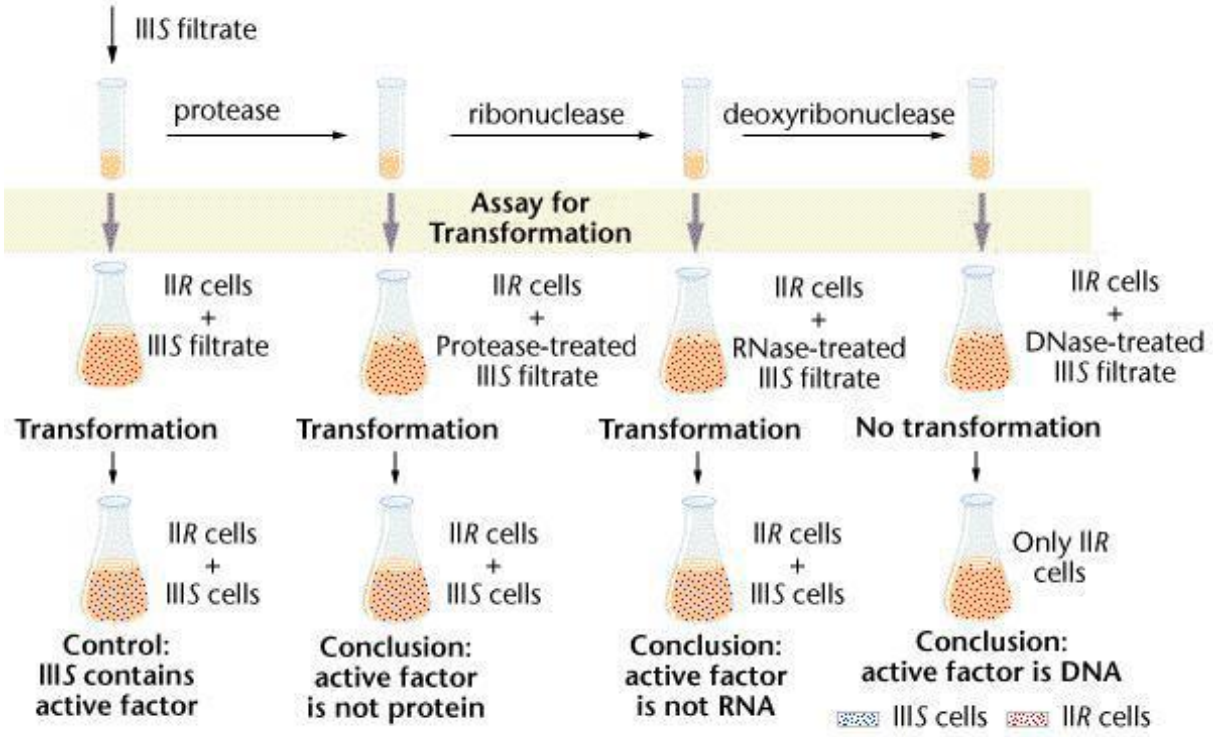
The III-S strain synthesized a polysaccharide capsule that protected itself from the host's immune system, resulting in the death of the host, while the II-R strain did not have that protective capsule and was defeated by the host's immune system.




**Griffith's results:**

- live S strain cells killed the mice
- live R strain cells did not kill the mice
- heat-killed S strain cells did not kill the mice
- heat-killed S strain + live R strain cells killed the mice

**Griffith's conclusion:** Information specifying virulence passed from the dead S strain cells into the live R strain cells. Griffith called the transfer of this information 'transformation'.



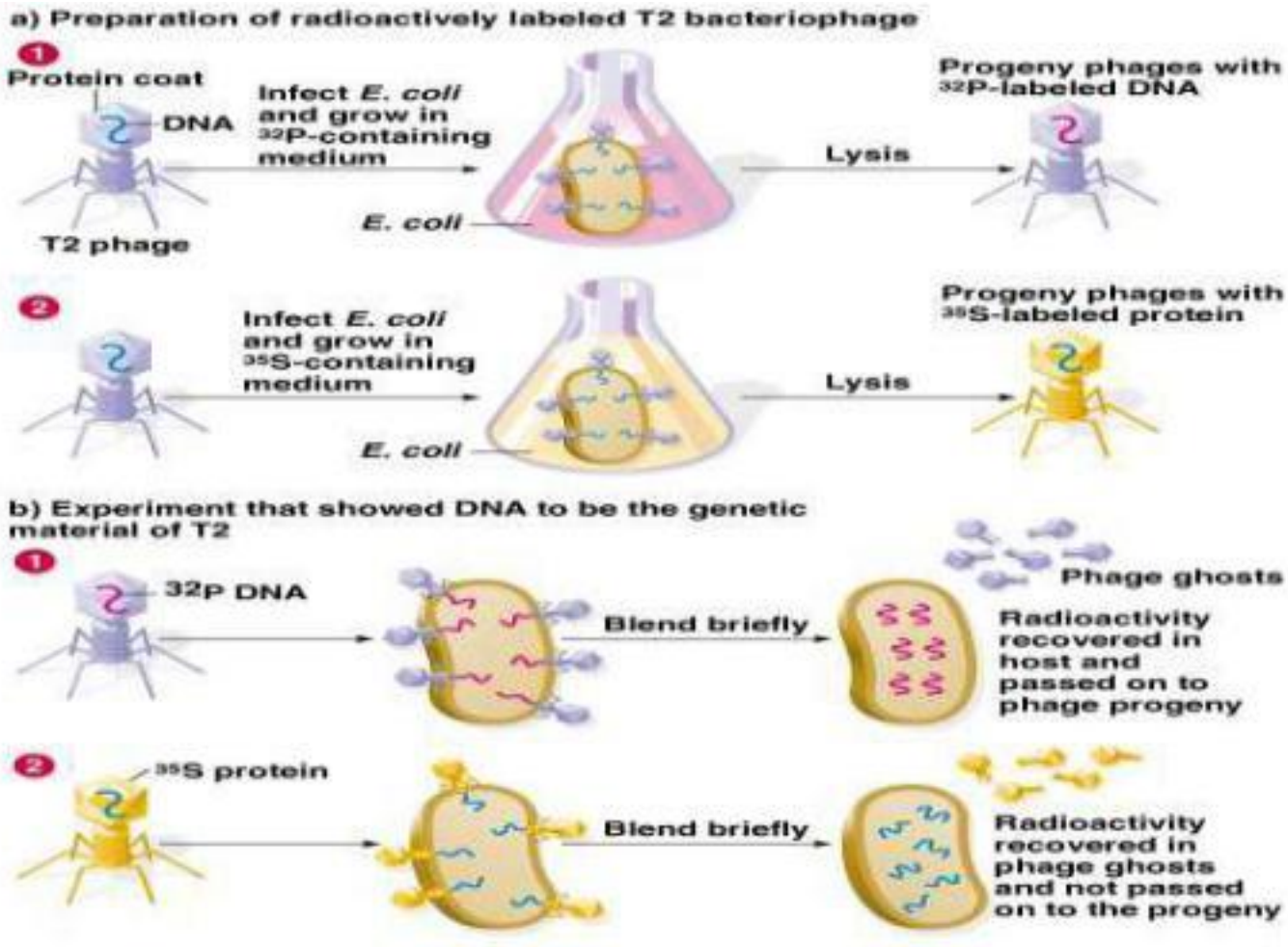

**Avery's Transformation Experiment**

- In 1944, Avery, MacLeod and McCarty published results of a study that identified the transforming principle from *S. pneumoniae*. Their approach was to break open dead cells, chemically separate the components (e.g., protein, nucleic acids) and determine which was capable of transforming live *S. pneumoniae* cells.
- Only the nucleic acid fraction was capable of transforming the bacteria.
- Critics noted that the nucleic acid fraction was contaminated with proteins. The researchers treated this fraction with either RNase or protease and still found transforming activity, but when it was treated with DNase, no transformation occurred, indicating that the transforming principle was DNA

**The Hershey-Chase Bacteriophage Experiment**

- More evidence for DNA as the genetic material came in 1953 with Alfred Hershey and Martha Chase's work on *E. coli* infected with bacteriophage T2.
- The bacteriophage was composed of only DNA and protein
- They wanted to determine which of these molecules is the genetic material that is injected into the bacteria
- In one part of the experiment, T2 proteins were labelled with $S^{35}$, and in the other part, T2 DNA was labelled with $P^{32}$. Then each group of labelled viruses was mixed separately with the *E. coli* host. After a short time, phage attachment was disrupted with a kitchen blender, and the location of the label determined.

- The $S^{35}$-labelled protein was found outside the infected cells, while the $P^{32}$-labelled DNA was inside the *E. coli*, indicating that DNA carried the information needed for viral infection. This provided additional support for the idea that genetic inheritance occurs via DNA.



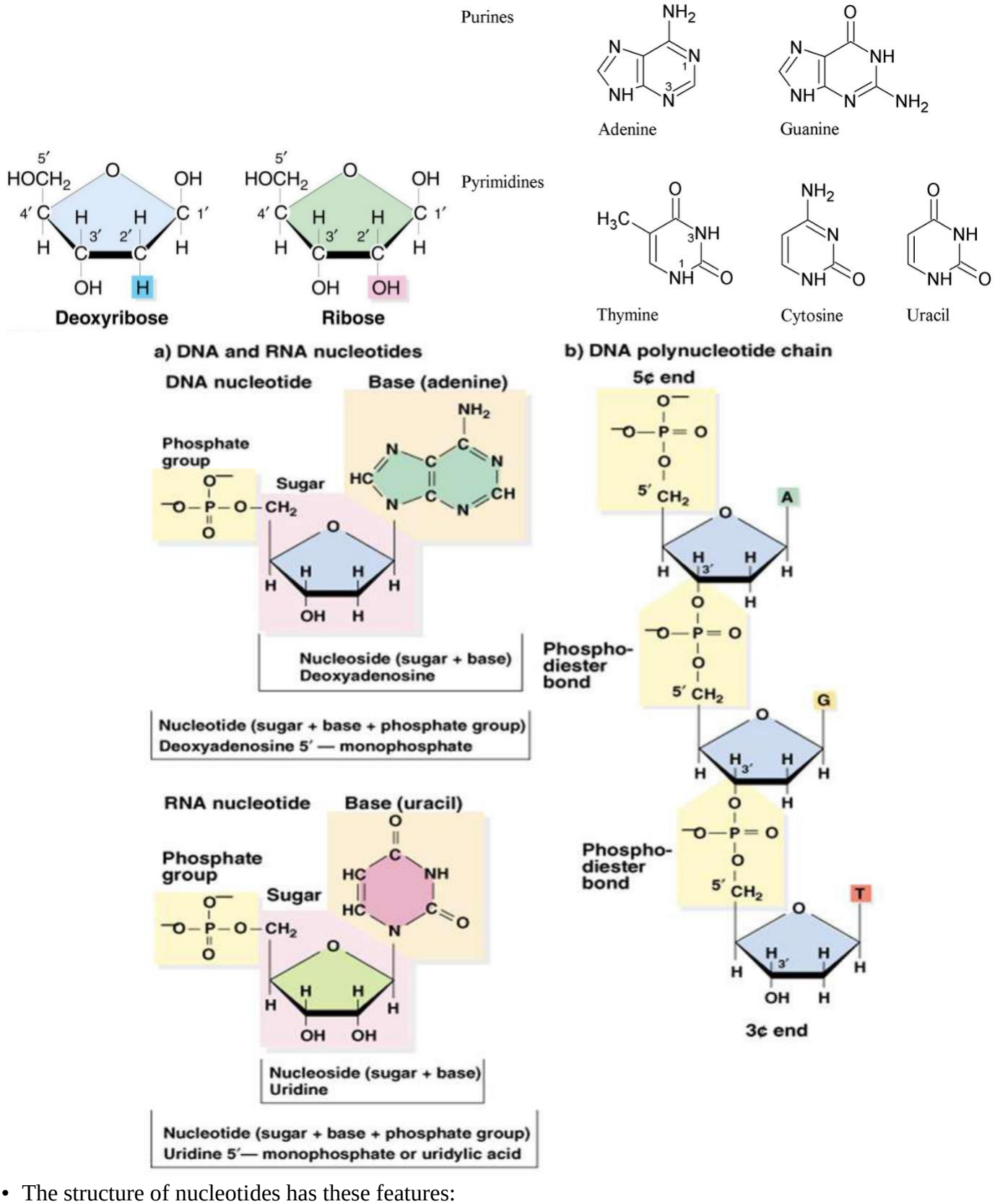

**Composition and Structure of DNA & RNA**

- DNA and RNA are polymers composed of monomers called nucleotides.
- Each nucleotide has three parts:
  a. A pentose (5-carbon) sugar.
  b. A nitrogenous base.
  c. A phosphate group.
- The pentose sugar in RNA is ribose, and in DNA it‘s deoxyribose. The only difference is at the 29 position, where RNA has a hydroxyl (OH) group, while DNA has only hydrogen.

There are two classes of nitrogenous bases:

a. Purines (double-ring, nine-membered structures) include adenine (A) and guanine (G).

b. Pyrimidines (one-ring, six-membered structures) include cytosine (C), thymine (T) in DNA and uracil (U) in RNA.




- The structure of nucleotides has these features:

  a. The base is always attached by a covalent bond between the 1′ carbon of the pentose sugar and nitrogen in the base (specifically, the nine nitrogen in purines and the one nitrogen in pyrimidines).

b. The sugar-base combination is a nucleoside. When a phosphate is added (always to the 5′ carbon of the pentose sugar), it becomes a nucleoside phosphate, or simply nucleotide.

- Polynucleotides of both DNA and RNA are formed by stable covalent bonds (phosphodiester linkages) between the phosphate group on the 5′ carbon of one nucleotide, and the 3′ hydroxyl on another nucleotide. This creates the ―backbone of a nucleic acid molecule.
- The asymmetry of phosphodiester bonds creates 3′-5′ polarity within the nucleic acid chain.

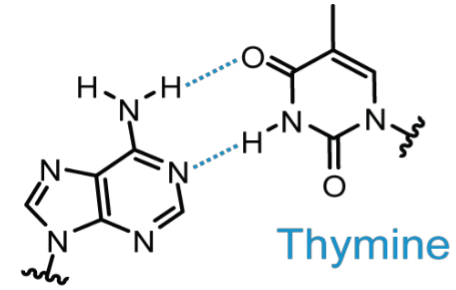
**Structure and Functions of DNA**

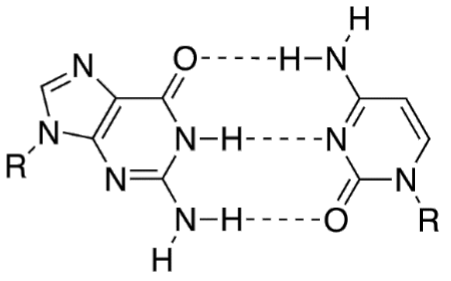
- Determining the 3-dimmensional structure of DNA involved the work of a few scientists:
  – Erwin Chargaff determined that Amount of adenine = amount of thymine; Amount of cytosine = amount of guanine, this is known as Chargaff's Rules
- Rosalind Franklin and Maurice Wilkins
  – Franklin performed X-ray diffraction studies to identify the 3-D structure
  – discovered that DNA is helical
  – discovered that the molecule has a diameter of 2 nm and makes a complete turn of the helix every 3.4 nm
- James Watson and Francis Crick, 1953
  – deduced the structure of DNA using evidence from Chargaff, Franklin, and others
  – proposed a double helix structure

Watson and Crick's three-dimensional model has the following main features:

- It is two polynucleotide chains wound around each other in a right-handed helix.
- The two chains are antiparallel.
- The sugar-phosphate backbones are on the outside of the helix, and the bases are on the inside, stacked perpendicularly to the long axis like the steps of a spiral staircase.

The bases of the two strands are held together by hydrogen bonds with complementary bases on the opposite sugar-phosphate backbone (two for A-T pairs and three for G-C pairs). Individual H-bonds are relatively weak and so the strands can be separated (by heating, for example). Complementary base pairing means that the sequence of one strand dictates the sequence of the other strand.




- The base pairs are 0.34 nm apart, and one full turn of the DNA helix takes 3.4 nm, so there are 10 bp in a complete turn. The diameter of a ds DNA helix is 2 nm.

- Because of the way the bases H-bond with each other, the opposite sugar-phosphate backbones are not equally spaced, resulting in a major and minor groove. This feature of DNA structure is important for protein binding

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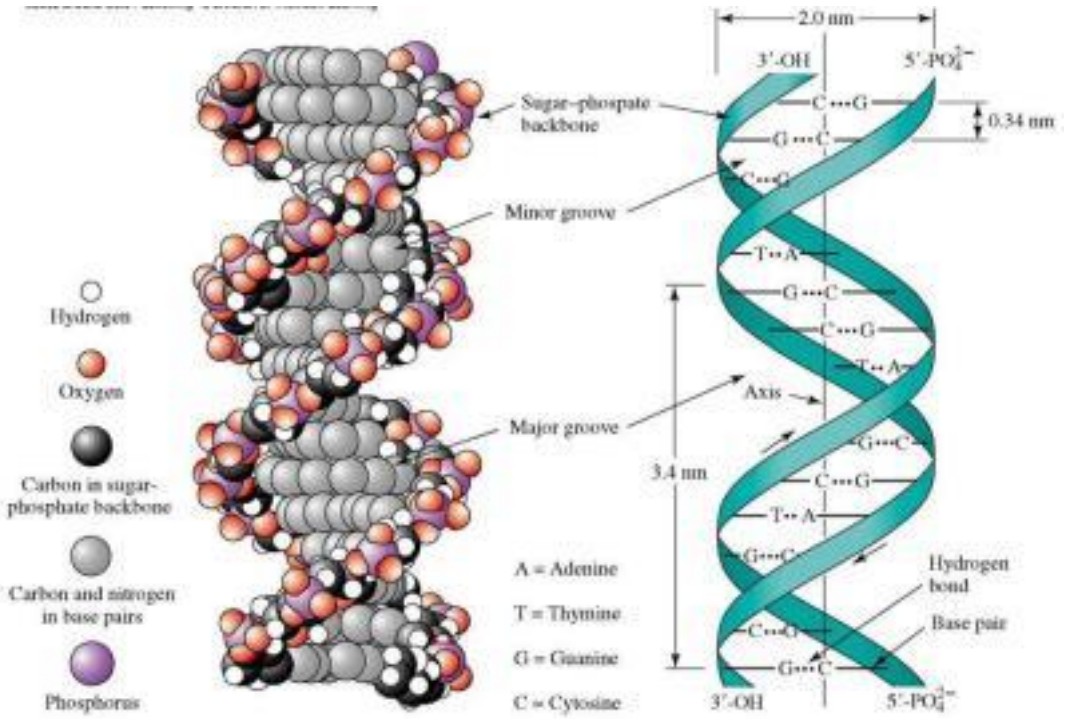



**Different DNA Structures**

X ray diffraction studies show that DNA can exist in different forms.

- A-DNA is the dehydrated form, and so it is not usually found in cells. It is a right- handed helix with 10.9 bp/turn, with the bases inclined 13° from the helix axis. A- DNA has a deep and narrow major groove, and a wide and shallow minor groove.
- B-DNA is the hydrated form of DNA, the kind normally found in cells. It is also a right-handed helix, with only 10.0 bp/turn, and the bases inclined only 2° from the helix axis. B-DNA has a wide major groove and a narrow minor groove, and its major and minor grooves are of about the same depth.
- Z-DNA is a left-handed helix with a zigzag sugar-phosphate backbone that gives it its name. It has 12.0 bp/turn, with the bases inclined 8.8° from the helix axis. Z- DNA has a deep minor groove, and a very shallow major groove. Its existence in living cells has not been proven.

**DNA in the Cell**

All known cellular DNA is in the B form.

A-DNA would not be expected because it is dehydrated and cells are aqueous. Z-DNA has never been found in living cells, although many organisms have been shown to contain proteins that will bind to Z-DNA

**Structure and Functions of RNA**

- RNA is a biologically important type of molecule that consists of a long chain of nucleotide units.
- Each nucleotide consists of a nitrogenous base, a ribose sugar, and a phosphate

**Types of RNA**

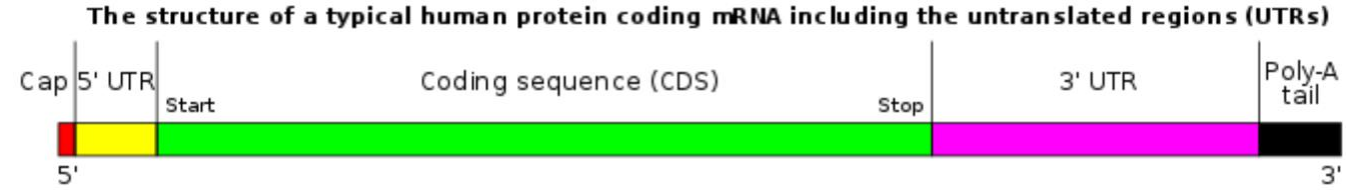
Based on the functions of RNA it is classified into two which include,

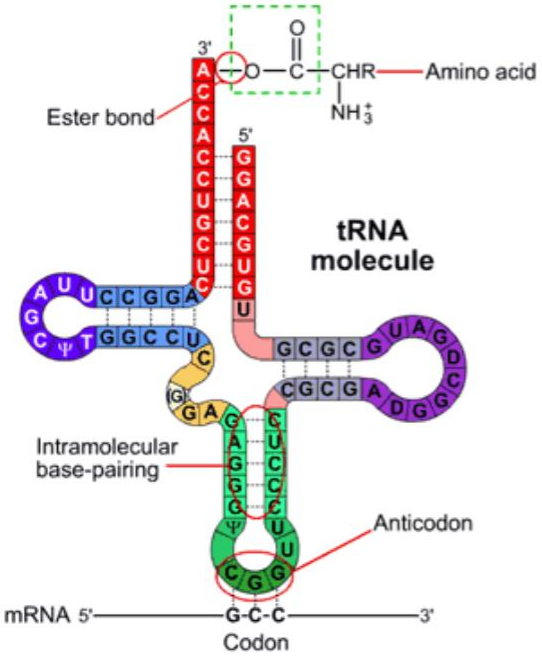
- Genetic RNA- If the RNA is involved in genetic mechanism it is known as Genetic RNA. Such RNA contains information which is normally found in DNA in higher organisms. In other words, RNA has replaced DNA in such cases.
- Non Genetic RNA- In some organisms where genetic information is contained in, and transmitted through DNA, RNA though present in good quantity but it cannot serve as genetic material. So it is known as non genetic RNA. This type of RNA depends upon the information getting from DNA for its function.

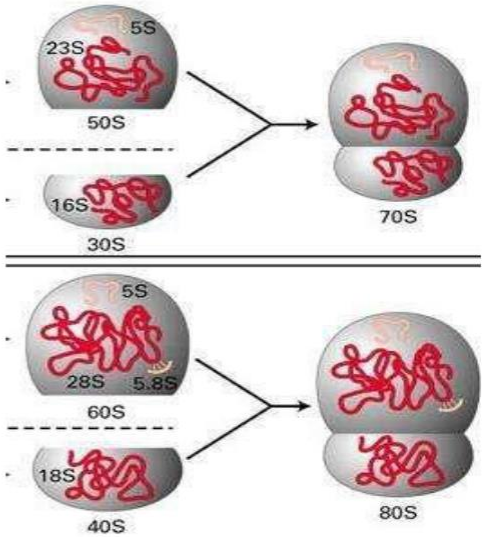
**Non Genetic RNAs**

**(a) Messenger RNA**

- mRNA carries information about a protein sequence to the ribosomes, the protein synthesis factories in the cell.
- It is coded so that every three nucleotides (a codon) correspond to one amino acid.
- In prokaryotic cells, mRNA binds to ribosome's while it is being transcribed from DNA.



**(b) Transfer RNA**

- Transfer RNA (tRNA) is a small RNA chain of about 80 nucleotides that transfers a specific amino acid to a growing polypeptide chain at the ribosomal site of protein synthesis during translation.
- It has sites for amino acid attachment and an anticodon region for codon recognition that site binds to a specific sequence on the messenger RNA chain through hydrogen bondind

**(c) Ribosomal RNA**

- Ribosomal RNA (rRNA) is the catalytic component of the ribosomes.
- rRNA molecules are synthesized in the nucleolus.
- In the cytoplasm, ribosomal RNA and protein combine to form a nucleoprotein called a ribosome.
- The ribosome binds mRNA and carries out protein synthesis. Several ribosomes may be attached to a single mRNA at any time.

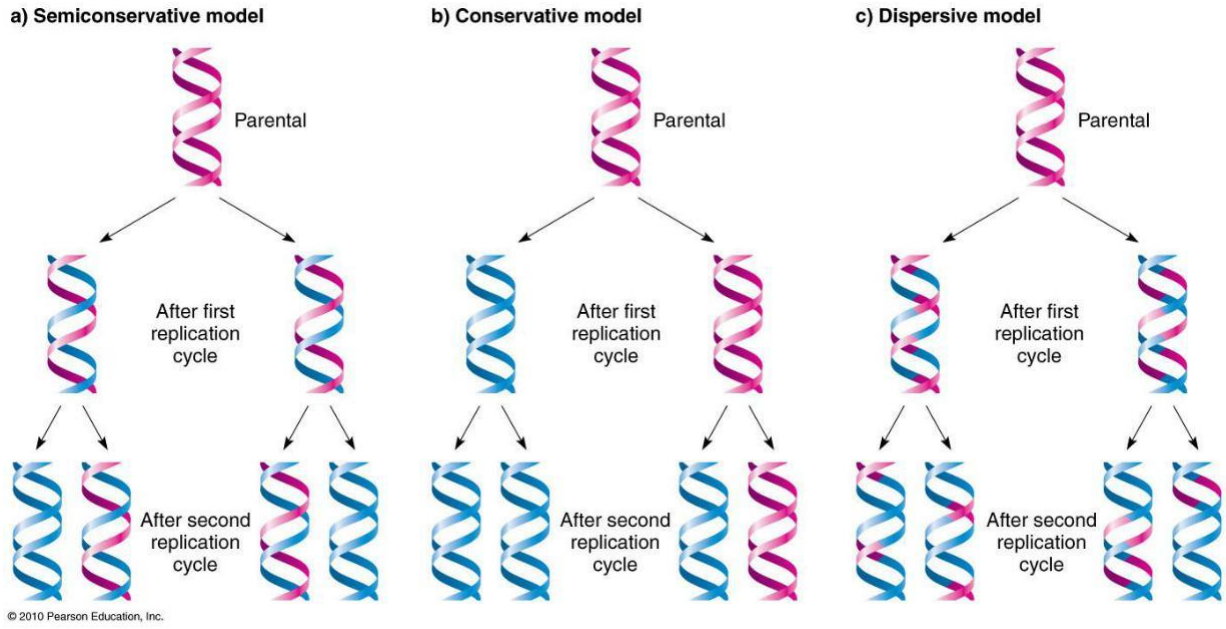
**DNA Replication**

3 possible ways of replication

(i) Semi discontinuous replication: In this model, the two strands of DNA unwind from each other, and each acts as a template for synthesis of a new, complementary strand. This results in two DNA molecules with one original strand and one new strand.

(ii) Unidirectional replication: In this model, DNA replication results in one molecule that consists of both original DNA strands (identical to the original DNA molecule) and another molecule that consists of two new strands (with exactly the same sequences as the original molecule).

(iii) Bidirectional replication: In the dispersive model, DNA replication results in two DNA molecules that are mixtures, or "hybrids," of parental and daughter DNA. In this model, each individual strand is a patchwork of original and new DNA.



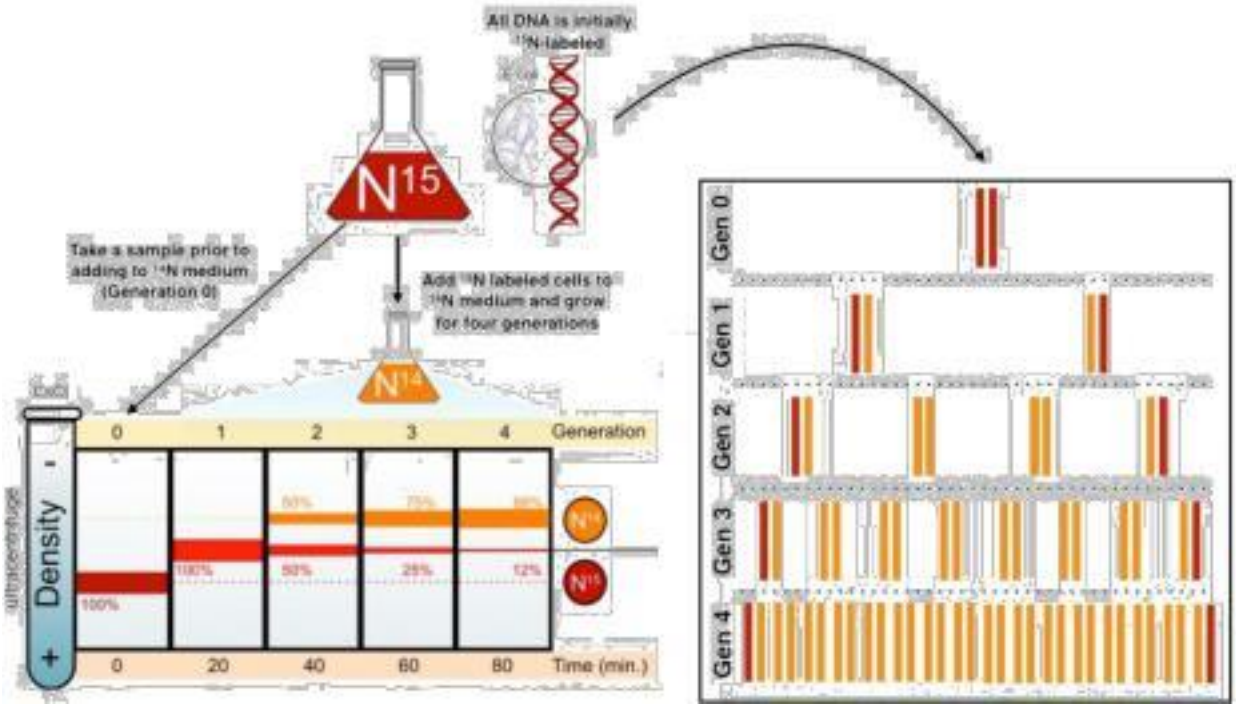
**Meselson–Stahl experiment**

In 1958, Matthew Meselson and Franklin Stahl conducted experiments using *E. coli* bacteria as a model system to understand the DNA replication.

They began by growing *E. coli* in medium, or nutrient broth, containing a "heavy" isotope of nitrogen, $^{15}N$. When grown on medium containing heavy $^{15}N$, the bacteria took up the nitrogen and used it to synthesize new biological molecules, including DNA.

After many generations growing in the $^{15}N$ medium, the nitrogenous bases of the bacteria's DNA were all labeled with heavy $^{15}N$. Then, the bacteria were switched to medium containing a "light" $^{14}N$ isotope and allowed to grow for several generations. DNA made after the switch would have to be made up of $^{14}N$, as this would have been the only nitrogen available for DNA synthesis.

Meselson and Stahl knew how often *E. coli* cells divided, so they were able to collect small samples in each generation and extract and purify the DNA. They then measured the density of the DNA (and, indirectly, its $^{15}N$ and $^{14}N$) using density gradient centrifugation.




The above result is consistent with the semiconservative replication model, which predicts that all DNA molecules will consist of one $^{15}N$-labeled DNA strand and one $^{14}N$-labeled DNA strand.

DNA Polymerisation is the process of addition of nucleotides to the end of the growing strand with the help of *DNA polymerase* enzyme

Five components are required:

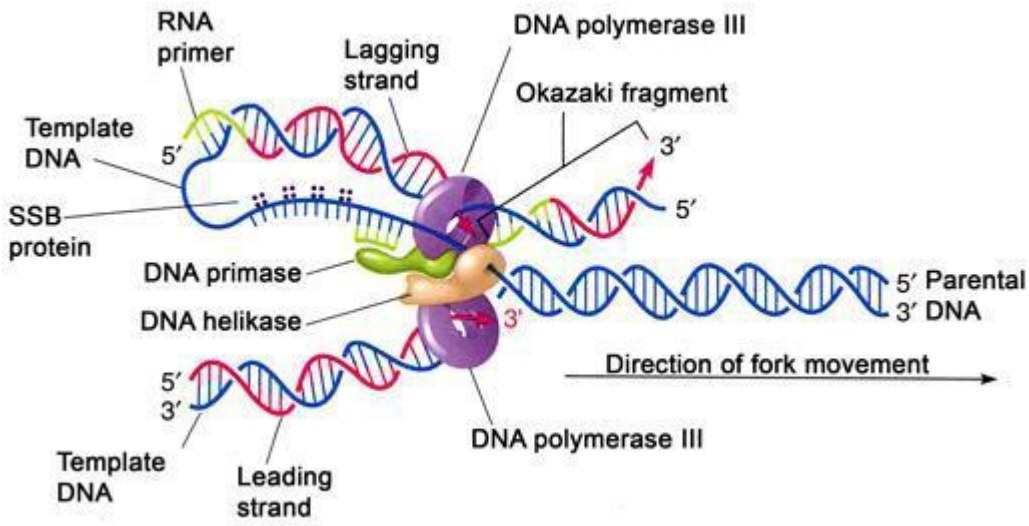
1. dNTPs: dATP, dTTP, dGTP, dCTP (deoxyribonucleoside 5‘-triphosphates) (sugar-base + 3 phosphates)

2. DNA template: It is a strand of DNA that is used as a guide in making a complementary strand of DNA and is base paired with a newly made DNA but is not covalently linked to it.
3. RNA Primer: It is synthetic oligonucleotide RNA hydrogen bonded to the template strand and whose terminal 3‘OH is available for the reaction and are covalently linked to the 5‘P ends of the newly made DNA.
4. DNA polymerase: (formerly the Kornberg enzyme) (DNA polymerase II & III discovered soon after)
5. $Mg^{2+}$ (optimizes DNA polymerase activity)

Thus polymerization consists of a reaction between a 3‘OH group at the end of the growing strand and the 5‘triphosphate group of an incoming nucleotide. When the nucleotide is added it supplies a free 3‘OH group to the growing strand, since each DNA has a 5‘P terminus and 3‘PH terminus, strand growth is said to be proceeding in 5‘ 3‘ direction**.**

General feature of DNA replication

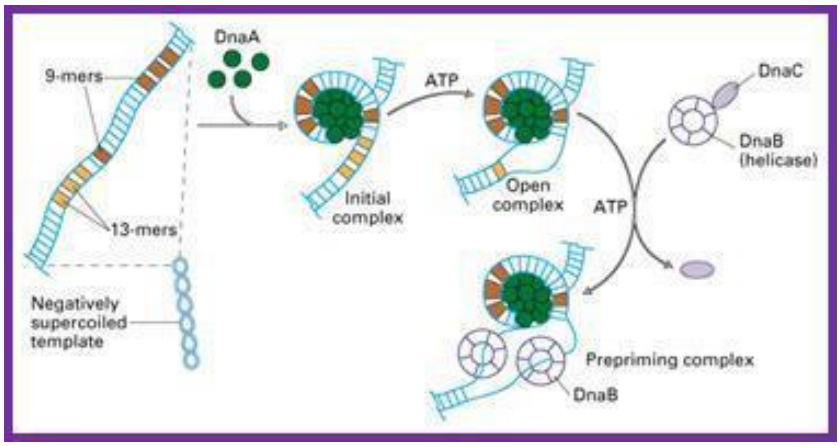
- DNA replication is semi conservative
- It is bidirectional process
- It proceed from a specific point called origin
- It proceed in 5’-3’ direction
- It occur with high degree of fidelity
- It is a multi-enzymatic process




DNA replication occurs by three steps

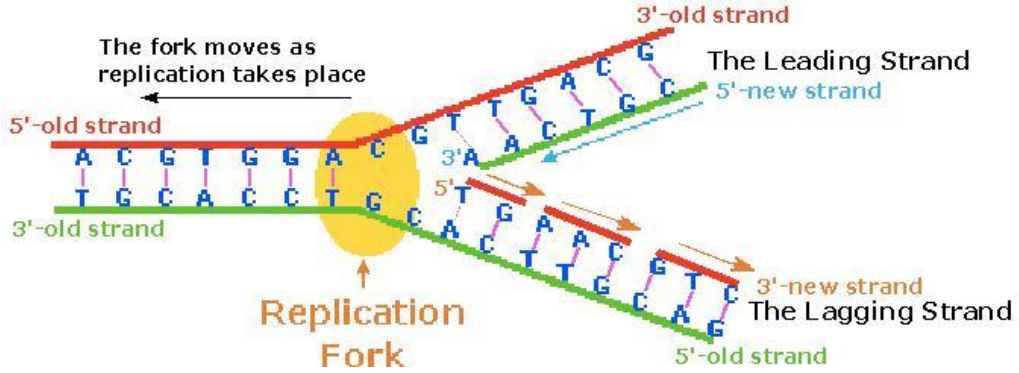
1. Initiation:

DNA replication begins from origin. In *E coli*, replication origin is called OriC which consists of 245 base pair and contains DNA sequences that are highly conserved among bacterial replication origin. Two types of conserved sequences are found at OriC, three repeats of 13 bp (GATRCTNTTNTTTT) and four/five repeats of 9 bp (TTATCCACA) called 13 mer and 9 mer respectively.




- About 20 molecules of Dna A proteins binds with 9 mer repeats along with ATP which causes DNA to wraps around dnaA protein forming initial complex. The dna A protein and ATP trigger the opening of 13 mer repeats froming open complex.
- Two copies of dnaB proteins (helicase) binds to 13 mer repeats. This binding is facilitated by another molecule called dnaC. The dnaB-dnaC interaction causes dnaB ring to open which binds with each of the DNA strand. The hydrolysis of bound ATP release dnaC leaving the dnaB bound to the DNA strand.
- The binding of helicase is key step in replication initiation. dnaB migrates along the single stranded DNA in 5'-3' direction causing unwinding of the DNA.
- The activity of helicase causes the topological stress to the unwinded strand forming supercoiled DNA. This stress is relieved by the DNA topoisomerase (DNA gyrase) by negative supercoiling. Similarly, single stranded binding protein binds to the separated strand and prevents reannaeling of separated strand and stabilize the strand.
- The DNA polymerase cannot initiate DNA replication. So, at first primase synthesize 10±1 nucleotide (RNA in nature) along the 5'-3' direction. In case of *E.coli* primer synthesized by primase starts with ppp-AG-nucleotide. Primer is closely associated with dnaB helicase so that it is positioned to make RNA primer as ssDNA of lagging strand.

2. <u>Elongation:</u>



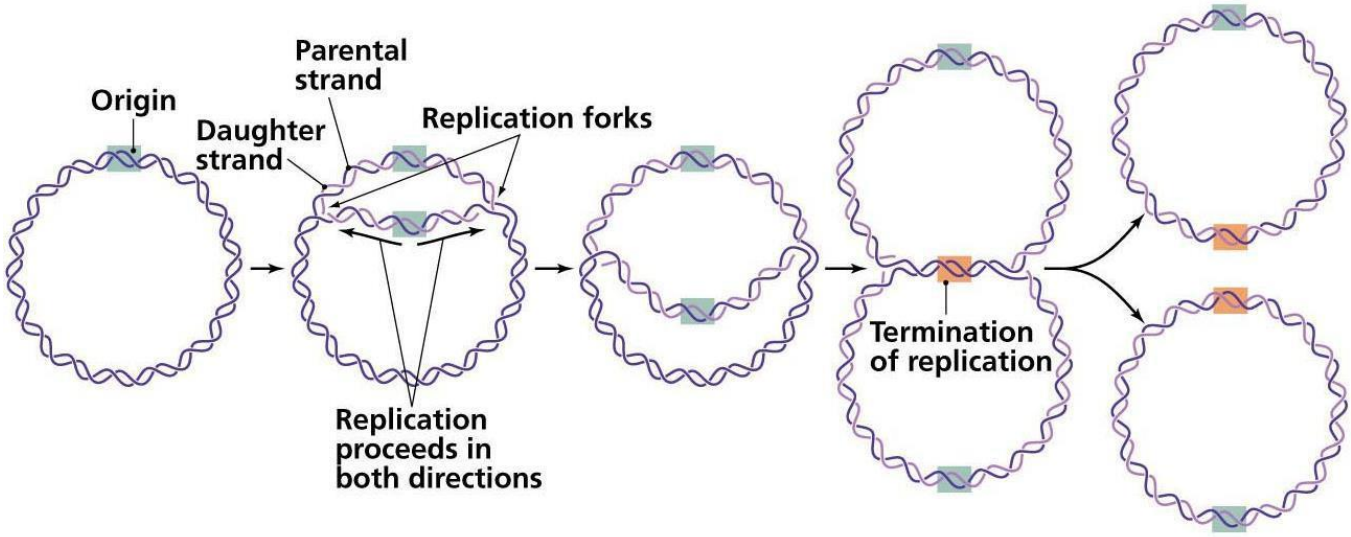
- The complexicity lies in the co-ordination of leading and lagging strand synthesis. Both the strand are synthesized by a single DNA polymerase III dimer which accomplished the looping of template DNA of lagging strand synthesizing Okazaki fragments.
- Helicase (dnaB) and primase (dnaG) constitute a functional unit within replication complex called primosome.
- DNA pol III use one set of core sub unit (Core polymerase) to synthesize leading strand and other set of core sub unit to synthesize lagging strand.
- In elongation steps, helicasein front of primaseand pol III, unwind the DNA at the replication fork and travel along lagging strand template along 5'-3' direction.
- DnaG primase occasionally associated with dnaB helicase synthesizes short RNA primer. A new B-sliding clamp is then positioned at the primer by B-clamp loading complex of DNA pol III.
- When the Okazaki fragments synthesis is completed, the replication halted and the core sub unit dissociates from their sliding clamps and associates with new clamp. This initiates the synthesis of new Okazaki fragments.

Both leading and lagging strand are synthesized co-ordinately and simultaneously by a complex protein moving in 5'-3' direction. In this way both leading and lagging strand can be replicated at same time by a complex protein that move in same direction.

- Every so often the lagging strands must dissociates from the replicosome and reposition itself so that replication can continue.
- Lagging strand synthesis is not completes until the RNA primer has been removed and the gap between adjacent Okazaki fragments are sealed. The RNA primer are removed by exonuclease activity (5'-3') of DNA pol-I and replaced by DNA. The gap is then sealed by DNA ligase using NAD as co-factor.

3. Termination:

- Evantually the two replication fork of circular E. coli chromosome meet at termination recognizing sequences (ter).
- The Ter sequence of 23 bp are arranged on the chromosome to create trap that the replication fork can enter but cannot leave. Ter sequences function as binding site for TUS protein.
- Ter-TUS complex can arrest the replication fork from only one direction. Ter-TUS complex encounter first with either of the replication fork and halt it. The other opposing replication fork halted when it collide with the first one. This seems the Ter-TUS sequences are not essential for termination but it may prevent over replication by one fork if other is delayed or halted by damage or some obstacle.
- When either of the fork encounter Ter-TUS complex, replication halted.
- Final few hundred bases of DNA between these large protein complexes are replicated by not yet known mechanism forming two interlinked (cataneted) chromosome.
- In E. coli DNA topoisomerase IV (type II) cut the two strands of one circular DNA and segrate each of the circular DNA and finally join the strand. The DNA finally transfers to two daughter cells.



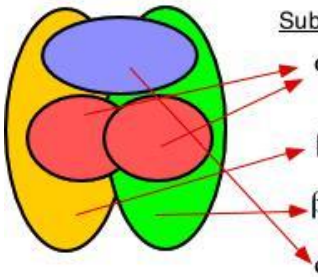

**Transcription**

The synthesis of RNA molecules using DNA strand as the template so that the genetic information can be transferred from DNA to RNA.

- First step in making proteins
- Converting into a mRNA strand
- Location: Nucleus of the cell

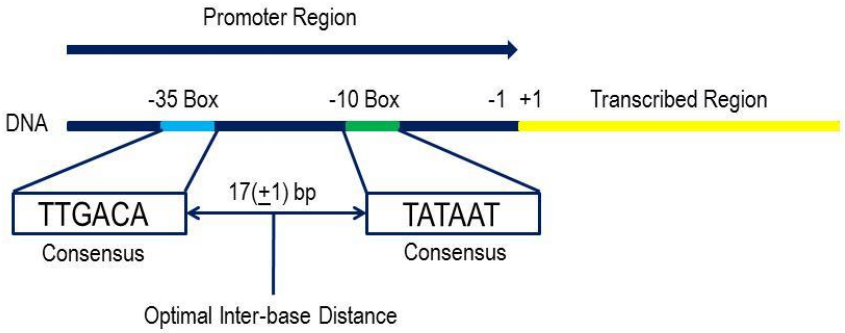
The enzyme involved in transcription is RNA polymerase. Unlike DNA polymerase it can initiate transcription by itself, it does not require primase. More exactly it is a DNA dependent RNA polymerase

**Prokaryotic RNA Polymerase: Holoenzyme Enzyme**



| Subunit | Size | #/Molecule | Function |
|---|---|---|---|
| α | 36.5 kD | 2 | Ability to recognize and to interact with regulatory proteins |
| β | 151 kD | 1 | Synthesis of RNA |
| β' | 155 kD | 1 | Ability to identify DNA template |
| σ | 70 kD | 1 | Ability to identify the promoter region |

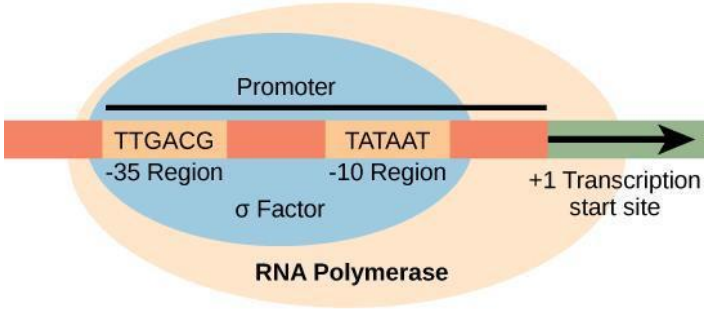
- Promoter sequences are DNA sequences that define where transcription of a gene by RNA polymerase begins. In prokaryotes, the promoter consists of two short sequences at -10 (Pribnow box) and -35 positions on upstream from the transcription start site



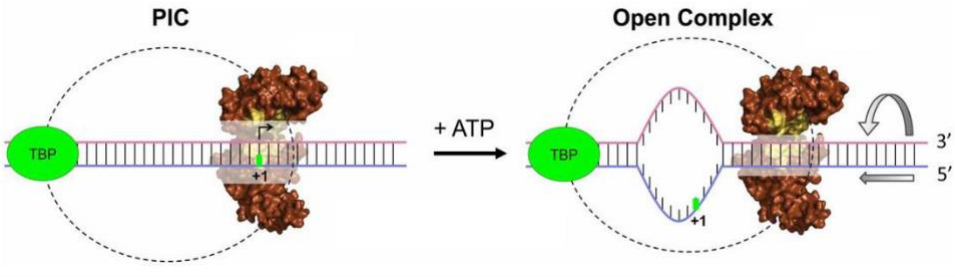

Steps of Transcription: Transcription is an enzymatic process. The mechanism of transcription completes in three major steps

1A. Pre-initiation
1B. Initiation
2. Elongation
3. Termination

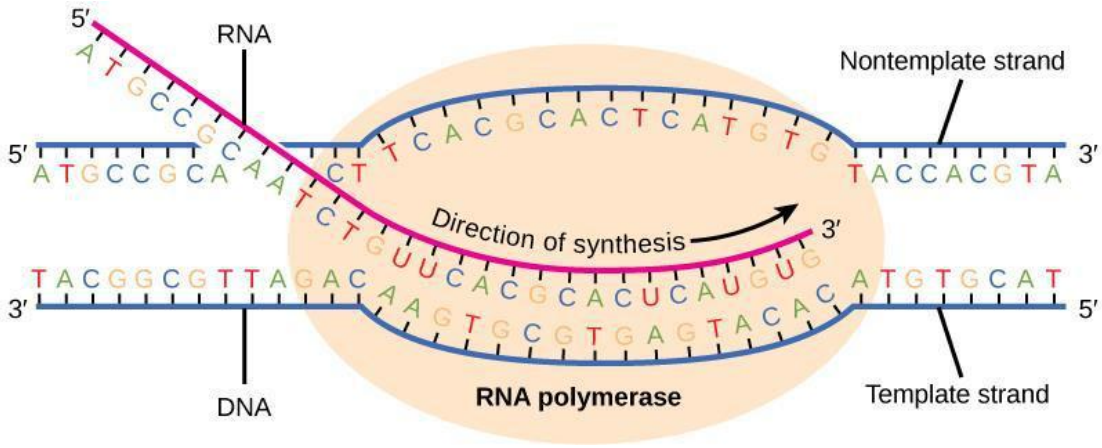
1A. Pre-initiation: The transcription is initiated by binding of $\sigma$ subunit followed by the binding of RNA polymerase core enzyme on promoter.




1B. Initiation: The A–T-rich -10 region facilitates unwinding of the DNA template, and melting of several phosphodiester bonds between 17 base pairs (open reading frame or open complex). The σ subunit dissociates from the polymerase after formation of open complex.



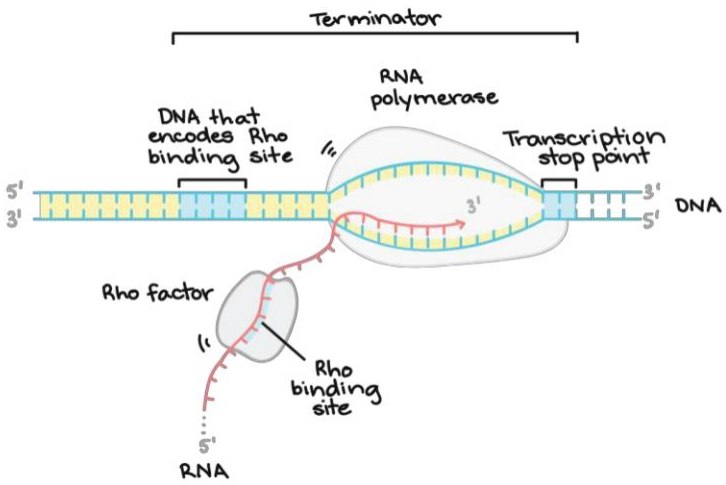

2. Elongation: The dissociation of $\sigma$ subunit allows the core enzyme to proceed along the DNA template (formation of transcription bubble creates pressure which is removed by DNA gyrase enzyme), synthesizing mRNA in the 5' to 3' direction at a rate of approximately 40 nucleotides per second. As elongation proceeds, the DNA is continuously unwound ahead of the core enzyme and rewound behind it.

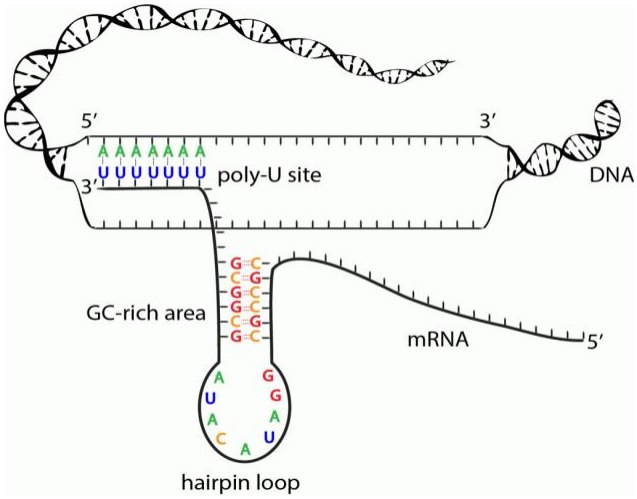



3. Termination: Once a gene is transcribed, the prokaryotic polymerase needs to be dissociate from the DNA template and liberate the newly made mRNA. Depending on the gene being transcribed, there are two kinds of termination signals. One is protein-based (Rho- dependent) and the other is RNA-based (Rho-independent).

Rho- dependent: A specific protein, named p factor (275 KD), binds to the growing RNA (and not to RNA polymerase) or weakly to DNA and in the bound state it acts as ATPase and terminates transcription and releases RNA. The p factor is also responsible for the dissociation of RNA polymerase from DNA.

Rho- independent: Rho-independent termination is controlled by specific sequences in the DNA template strand. As the polymerase nears the end of the gene being transcribed, it encounters a region rich in C–G nucleotides. The mRNA folds back on it and the complementary C–G nucleotides bind together. The result is a stable hairpin that causes the polymerase to stall as soon as it begins to transcribe a region rich in A–T nucleotides. The complementary U–A region of the mRNA transcript forms only a weak interaction with the template DNA that induces enough instability for the core enzyme to break away and liberate the new mRNA transcript.

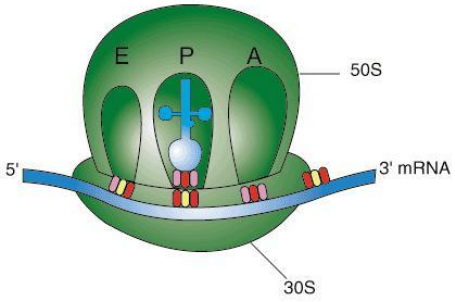


**Translation:**

The process by which proteins are produced with amino acid sequences specified by the sequence of codons in messenger RNA.

- Second step in making proteins
- Synthesis of protein using mRNA
- Location: cytoplasm of the cell
- The translation process requires mRNA, rRNA, ribosomes, 20 kinds of amino acids and their specific tRNAs.
- Codon: A codon is a sequence of three DNA or RNA nucleotides that corresponds with a specific amino acid or stop signal during protein synthesis.
- Ribosomes: A minute particle consisting of RNA and associated proteins found in large numbers in the cytoplasm of living cells. They bind messenger RNA and transfer RNA to synthesize polypeptides and proteins. Prokaryotic ribosomes are composed of 30s of smaller subunit and 70s of larger subunit.
- The larger subunit have 3 sites; A (aminoacyl tRNA) site, P (peptidyl) site and E (exit) site.

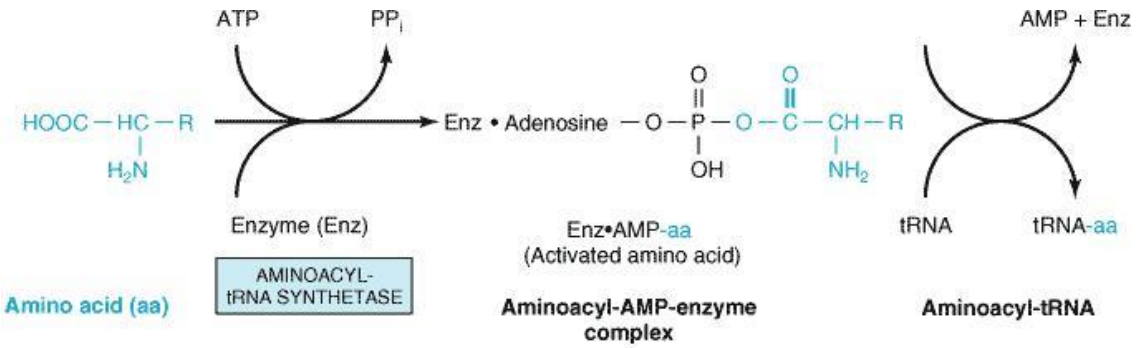



- Shine-Dalgarno (SD) Sequence: They are generally located around 8 bases upstream of the start codon AUG. The smaller subunit of ribosome have specific purine rich sequence those are complementary to Shine-Dalgarno (SD) Sequence.
- Starter codon: AUG ; Stop codons: UAA, UGA, UAG

Steps of Translation: The mechanism of translation completes in three major steps

1A. Activation of amino acids
1B. Initiation
2. Elongation
3.Termination

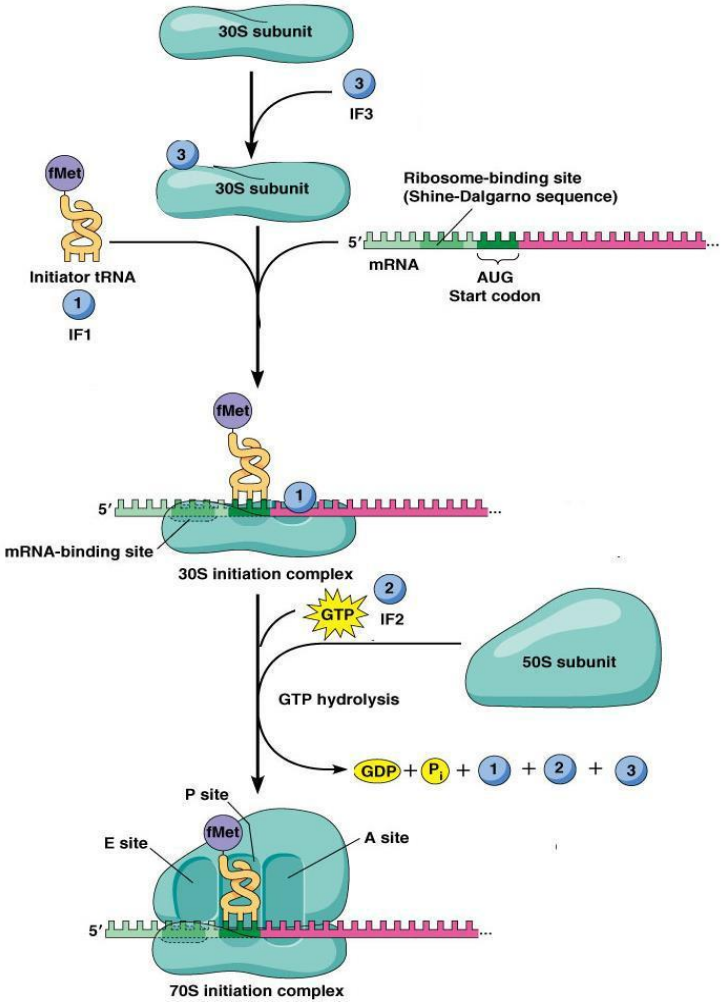
1A. Activation of aminoacyl tRNA:



- The tRNA attached to amino acid is called as charged tRNA
- The -COOH end of amino acid is bound to 3' end of their specific tRNA in the presence of ATP and $Mg^{2+}$.
- AUG acts as starter codon and also codes for methionine. A formyl group is attached to amino acid of tRNA (FMet–$tRNA^{FMet}$) by methionine tRNA transformylase. Now FMet–$tRNA^{FMet}$ recognises AUG codon as initiator codon.

1B. Initiation:

- In the first step, initiation factor-3 (IF-3) binds to 30S ribosomal unit.
- Then mRNA binds to 30S ribosomal subunit in such a way that AUG codon lie on the peptidyl (P) site and the second codon lies on aminoacyl (A) site.
- The tRNA carrying formylated methionine ie. *FMet–tRNA*$^{FMet}$ along with IF-1 is placed at P-site. This specificity is induced by IF-2 with utilization of GTP. The IF-1 prevent binding of *FMet–tRNA*$^{FMet}$ at A-site.
- Shinedalgrno sequence in the mRNA guide correct positioning of AUG codon at P-site of 30S ribosome.
- After binding of *FMet–tRNA*$^{FMet}$ on P-site, IF-3, IF-2 and IF-1 are released so that 50S ribosomal unit bind with 30S forming 70S sibosome.



2. Elongation: Elongation occurs in 3 steps at A and P sites with involvement of elongation factors $T_s$ and $T_u$.

i. Binding of AA-tRNA at A-site:

- The 2nd tRNA carrying next aminoacid comes into A-site and recognizes the codon on mRNA. This binding is facilitated by EF-$T_U$ and utilizes GTP.
- After binding, GTP is hydrolysed and EF-$T_U$-GDP is released
- EF-$T_U$-GDP then and enter into EF-$T_S$ cycle.

ii. Peptide bond formation:

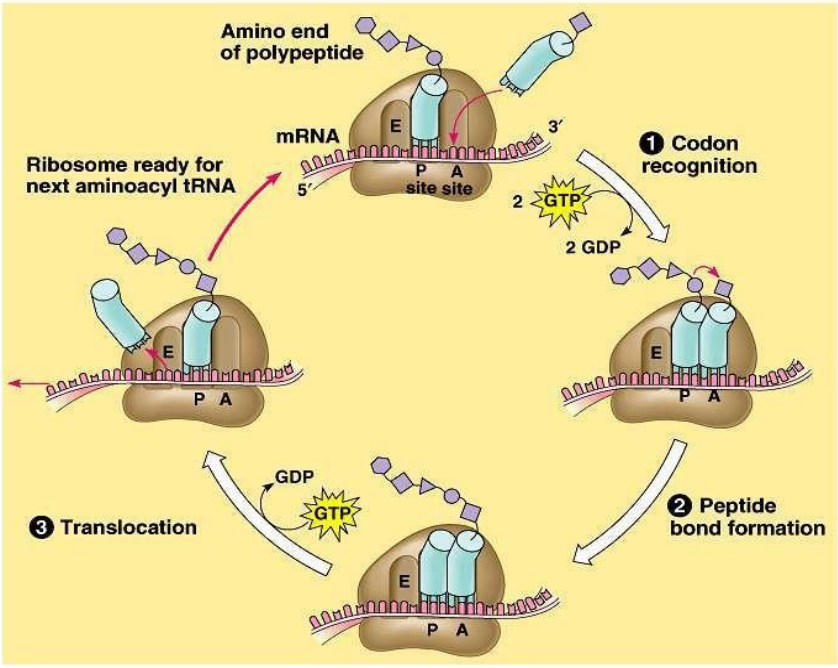
- The amino acid present in t-RNA of P-site i.e., Fmet is transferred to t-RNA of A-site forming peptide bond. This reaction is catalyzed by peptidyltransferase.
- Now, the t-RNA at P-site become uncharged

iii. Ribosome translocation:

- After peptide bond formation ribosome moves one codon ahead along 5'-3' direction on mRNA, so that dipeptide-tRNA appear on P-site and next codon appear on A-site.
- The uncharged tRNA exit from ribosome and enter to cytosol

The ribosomal translocation requires EF-G-GTP (translocase enzyme) which change the 3D structure of ribosome and catalyze 5'-3' movement.

- The codon on A-site is now recognized by other aminoacyl-tRNA as in previous.
- The dipeptide on P-site is transferred to A-site forming tripeptide.
- This process continues giving long polypeptide chain of aminoacids.




3. Termination: This process occurs when one of the three stop codons (UAA, UAG or UGA) enters A site of the ribosome. These codons are recognized by release factors (RFs) which are RF-1 (identifies UAA, UAG stop codons), RF- 2 (identifies UAA, UGA stop codons), RF-3 (involved in GTP hydrolysis). RFs

cause the newly synthesized protein to be released from the ribosomal complex and dissociation of ribosomes from mRNA.

**Possible Questions**

**Part-A (1 mark)**

**Part-B (2 marks)**

1. Define gene.
2. What is meant by homozygous and heterozygous?
3. Write the law of independent assortment.
4. What is meant by okazaki fragment?
5. Draw the diagram of beta helix DNA molecule.
6. Write short notes on leading and lagging strand of DNA.
7. Comment on DNA polymerase I and III.
8. Explain the chargaff‘s equivalence rule
9. Discuss shortly on DNA helicase and SSBP.
10. Define transcription and translation.
11. What is meant by Rho dependant termination?
12. Write about the Shine Dalgarno sequence.
13. Define law of dominance.
14. What is meant by 5` capping?
15. Write any two characteristics of tRNA.
16. Describe the structure of clover leaf model of tRNA molecule.
17. What is meant by codon?
18. Define Genetic code.
19. How is translation terminated?

**Part-C (8 marks)**

20. Write a note on Chromosomal theory of inheritance.
21. Explain the term: Phenotype, Genotype, homozygote and Heterozygote.
22. Explain the term: Dominant trait, Recessive trait, Monohybrid cross, Dihibrid cross, allele.
23. Discuss the Mendel‘s principle of inheritance.
24. With evidences, prove that DNA serves as genetic material.
25. Briefly summarize the experiment of Avery, Macleod, and Mccarty. Why this experiment was important to the development of Microbial genetics.
26. Briefly summarize the experiment of Hershey and Chase.
27. Write the salient features of DNA double helix.
28. Explain the steps involved in transcription.
29. Discuss in detail about the different modes and model of DNA replication.
30. Explain about the process of RNA translation with diagrammatic representation.
31. What are the different forms of DNA? Differentiate.
32. Explain in detail about different types of RNA.

| Sl. No | Question | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | Dihybrid ratio | 3:3:9:1 | 9:3:3:1 | 9:3:1:3 | 1:3:3:9 | 9:3:3:1 |
| 2 | Experiments of Hershey and Chase was based on | Virus | Bacteriophage | Bacteria | Fungi | Bacteriophage |
| 3 | In eukaryotes, the vast majority of DNA synthesis occurs during _____ of the cell cycle | G phase | H phase | R phase | S phase | S phase |
| 4 | Key enzyme in rolling circle replication | DNA Polymerase-IV | DNA Polymerase-III | DNA Polymerase-II | DNA Polymerase-I | DNA Polymerase-III |
| 5 | Mendels pioneer work was with | Monkey | Human | Garden pea | Mice | Garden pea |
| 6 | Okazaki fragments are | RNA strands | Enzymes | Leading strands | Lagging strands | Lagging strands |
| 7 | The ability to remove incorrectly matched nucleotides or Proof-reading | RNA polymerase | DNA ligase | DNA polymerase | DNA helicase | DNA polymerase |
| 8 | The enzyme that unwinds DNA | Polymerase | Ligase | Gyrase | Helicase | Helicase |
| 9 | _____ million base pairs of nucleotides are seen in *E.coli* | 64 million base pairs | 46 million base pairs | 4.6 million base pairs | 6.4 million base pairs | 4.6 million base pairs |
| 10 | Chargaff's rule | No complementarity | Partial complementarity | No such rule | Complementarity of one strand with the other | Complementarity of one strand with the other |
| 11 | Eukaryotic DNA damage or replication errors are corrected during | $G_1$ phase | S phase | $G_2$ phase | R phase | $G_2$ phase |
| 12 | Semiconservative DNA replication model | Daughter molecule contains both from parent | Daughter molecule entirely new | Daughter molecule contains one from parent and one newly synthesized | Some sections from parent and some newly synthesized | Daughter molecule contains one from parent and one newly synthesized |
| 13 | The negative charge of DNA is due to | Deoxyribose | Phosphate bond | Hydrogen bond | Nitrogenous base | Phosphate bond |

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|---|---|---|---|---|---|---|
| | | Sugar | | | | |
| 14 | Thymine in DNA is replaced by _____ in RNA | Adenine | Cytosine | Guanine | Uracil | Uracil |
| 15 | Which is involved in synthesis of primer | Ligase | Primase | DNA pol | rRNA | Primase |
| 16 | _____ degrades DNA | Polymerase | Primase | RNase | DNase | DNase |
| 17 | Enzyme that adds methyl group to the newly formed DNA | Gyrase | Topoisomerase | Helicase | Methylase | Methylase |
| 18 | Heat Killed S cells + Live R cells produced | Death in mice + S cells | Live mice + S cells | Death in mice + R cells | Live mice + R cells | Death in mice + S cells |
| 19 | Initiation of replication occurs | Bidirectionally | Cross sectionally | Unidirectionally | Parallely | Bidirectionally |
| 20 | The replication origin of *E.coli* is approximately | 245 bp | 425 bp | 254 bp | 524 bp | 245 bp |
| 21 | Transformation in *Pneumococci* was discovered by | Friedrick Griffith | Erwin Chargaff | Hershey & Chase | Watson & Crick | Friedrick Griffith |
| 22 | Bacteria contains | Single circular DNA | Single linear DNA | Double Linear DNA | Double circular DNA | Single circular DNA |
| 23 | Who proposed the molecular struccture of DNA | Hershey & Chase | Erwin Chargaff | Jim Latham | Watson & Crick | Watson & Crick |
| 24 | The most common form of DNA is | B-DNA | Z-DNA | Y-DNA | SS-DNA | B-DNA |
| 25 | Sequencing and molecular characterization of genome | Genetics | Molecular biology | Proteomics | Genomics | Genomics |
| 26 | Adenine always pair with | Guanine | Cytosine | Thymine | Uracil | Thymine |
| 27 | Initiation of replication is carried out by | DnaA | DnaC | DnaB | DnaE | DnaA |
| 28 | Which of the following is not associated with DNA replication? | Polymerase | Promoter | Primer | RepA protein | Promoter |
| 29 | Father of genetics | Mendel | Morgan | Watson | McLeod | Mendel |
| 30 | If a free phosphate is found at the 5’ end of a | A hydroxyl group | A phosphate | A base attached to | A hydroxyl group | A hydroxyl group |

| | | | | | | |
|---|---|---|---|---|---|---|
| | DNA strand, what is found at the other end of the same strand? | on the 5’ carbon of a deoxyribose sugar | group on the 3’ carbon of a deoxyribose sugar. | the 3’ carbon of a deoxyribose sugar | on the 3’ carbon of a deoxyribose sugar | on the 3’ carbon of a deoxyribose sugar |
| 31 | The replication origins of higher eukaryotes are made up of | Different AT-rich regions | Similar AT-rich regions | Different GC-rich regions | Similar GC-rich regions | Similar AT-rich regions |
| 32 | Transfer of genes from one generation to the next is | Inheritance | Carrying over | Subheritance | Gene transport | Inheritance |
| 33 | Basically, flow of genetic material is accompolished by | Replication | Transformation | Transduction | Conjugation | Replication |
| 34 | Purines are | A, T | G, C | C, T | A, G | A, G |
| 35 | The DNA of E.coli is _____ times longer than the cell | 1 | 10 | 1000 | 100 | 1000 |
| 36 | SSB protein helps in | Degradation of protein | Keep the two strands separated after unwinding | Elongation of DNA | Uncoiling of RNA | Keep the two strands separated after unwinding |
| 37 | The experiments of Avery, McLeod and McCarty was based on | Protein coupling | Enzymatic reactions | Synthetic reaction | DNA binding | Enzymatic reactions |
| 38 | The contribution of Rosalind Franklin towards structure of DNA was | X-ray crystallography | Electron microscopy | NMRspectroscopy | Gas chromatography | X-ray crystallography |
| 39 | Proof reading activity of DNA polymerase is in the direction | 5' to 3' | 3' to 5' | Parallel | Centre | 3' to 5' |
| 40 | Length of primer during replication is | 2-10 nucleotides | 10-20 nucleotides | 5-15 nucleotides | 10-25 nucleotides | 2-10 nucleotides |
| 41 | Distance between the two base pairs is | 3.4Å | 34Å | 10Å | 20Å | 3.4Å |
| 42 | Formation of pre-replicating complex is seen in replication mechanism of | Prokaryotes | Plants | Virus | Eukaryotes | Eukaryotes |
| 43 | Triple bonding is seen in | G-T | G-C | A-T | A-C | G-C |
| 44 | Repair and insertion of DNA is carried out by | Endonucleases | Ribozyme | Primase | Exonucleases | Endonucleases |

| | | | | | | |
|---|---|---|---|---|---|---|
| 45 | Nucleoside is | Base + Sugar | Sugar + Phosphate | Base + Phosphate | A+T & G+C | Base + Sugar |
| 46 | Left handed DNA | B-DNA | C-DNA | Y-DNA | Z-DNA | Z-DNA |
| 47 | Bonding between two bases | Hydrogen bond | Hydrophobic bond | Nitrogen bond | Van Der waals | Hydrogen bond |
| 48 | DNA absorbs UV light at _____ wavelength | 220 nm | 240 nm | 260 nm | 280 nm | 260 nm |
| 49 | The Pioneer work on nucleic acid discovery was carried out by | Friedrick Miescher | Watson & Crick | Griffith | Milstein | Friedrick Miescher |
| 50 | The enzyme that copies RNA from DNA template | Dnase | Rnase | DNA polymerase | RNA polymerase | RNA polymerase |
| 51 | The size of a major groove is | 34Å | 3.4Å | 43Å | 20Å | 34Å |
| 52 | Chromosomal theory of inheritance was formulated by | Mendel | Miescher | Metchinikoff | Morgan | Morgan |
| 53 | Eukaryotic DNA replication is a conserved mechanism that restricts DNA replication to | Never | Only once per cell cycle | Only twice per cell cycle | Only thrice per cell cycle | Only once per cell cycle |
| 54 | Semiconservative mechanism of DNA replication was demonstrated by | Meselson & Stahl | Beedle & Tatum | Hershy & Chase | Avery & McLeod | Meselson & Stahl |
| 55 | The most widely studied origin recognition complex of eukaryotes is that of | *Bacillus* | *Staphylococcus* | *Escherichia coli* | *Saccharomyces cerevisiae* | *Saccharomyces cerevisiae* |
| 56 | Number of base pairs per helical turn of B form DNA | 13 | 12 | 11 | 10 | 10 |
| 57 | RNA primer is removed by | DNA pol | RNA pol | Terminase | Caspase | DNA pol |
| 58 | Longest DNA is seen in | Human | Lung fish | Yeast | Bacteria | Lung fish |
| 59 | Joining of DNA fragments | DNA ligase | Gyrase | RNA polymerase | DNA polymerase | DNA ligase |
| 60 | Synthesis of DNA always moves from | 3' to 5' | 5' to 3' | Ffrom the centre | Anywhere | 5' to 3' |

# Unit – II

## Syllabus

Types of plasmids- replication, partitioning, host range, plasmid-incompatibility, amplification, curing and application

**Plasmids**

- Plasmid was coined by Lederberg.
- Plasmids are extra chromosomal double-stranded circular DNA molecules that can be found in various bacteria and some eukaryotes
- Plasmids are considered replicons, units of DNA capable of replicating autonomously within a suitable host.
- In their original form, the size of plasmids ranges between 1 and 200 kbp (kilo-base pairs)
- Plasmids often contain genes encoding enzymes that confer a certain selective advantage to the host cell. Such selective advantages include resistance to certain antibiotics
- Plasmids are widely used as cloning vector
- If both strands of DNA are intact circles the molecules are described as covalently closed circles or CCC DNA.
- When isolated from cells, covalently closed circles often have a deficiency of turns in the double helix, such that they have a super coiled configuration
- Cryptic Plasmids do not have genes that contribute to the phenotype of the host cell. They usually have genes to self replicate

Properties of Plasmids:

(i) They are specific to one or a few particular bacteria.
(ii) They replicate independently of the bacterial chromosome.
(iii) They code for their own transfer.
(iv) They act as episomes and reversibly integrate into bacterial chromosome.
(v) They may pick-up and transfer certain genes of bacterial chromosome,
(vi) They may affect certain characteristics of the bacterial cell,

**Types of plasmids**

Plasmids can be categorized into one of two major types –

- Conjugative: Conjugative plasmids contain a set of transfer or *tra* genes which promote sexual conjugation between different cells
- Non-conjugative: Non-conjugative plasmids do not contain *tra* genes

Plasmids can also be categorized on the basis of copies per cell being maintained as

- Relaxed plasmids - multiple copies per cell
- Stringent plasmids - a limited number of copies per cell

Another way to classify plasmids is by function. There are five main classes:

Fertility F-plasmids, which contain *tra* genes. They are capable of conjugation and result in the expression of sex pili

- Resistance (R) plasmids, which contain genes that provide resistance against antibiotics or poisons. Historically known as R-factors, before the nature of plasmids was understood
- Col plasmids, which contain genes that code for bacteriocins, proteins that can kill other bacteria. The best-studied Col plasmid is Col E plasmid.
- Degradative plasmids, which enable the digestion of unusual substances, e.g. toluene and salicylic acid.
- Virulence plasmids, which turn the bacterium into a pathogen.
  Plasmids can belong to more than one of these functional groups

**Natural plasmids and artificial plasmids**

Natural plasmids: They occur naturally in prokaryotes or eukaryotes.

Few examples of naturally occurring plasmids and their characteristics are listed in table below

| Plasmid | Size (kb) | Origin | Host range | Antibiotic resistance | Additional marker genes showing insertional inactivation |
|---|---|---|---|---|---|
| RSF1010 | 8.6 | *E.coli* (strain K-12) | Broad host range | Streptomycin and sulfonamides. | None |
| ColE1 | 6.6 | *E.coli* | Narrow host range | None | Immunity to colicin E1 |
| R100 | 94.2 | *E.coli* | *E.coli* K-12, *Shigella flexneri* 2b | Streptomycin, chloramphenycol, tetracycline | Mercuric (ion) reductase, putative ethidium bromide (EtBr) resistant protein. |

**RSF1010**

- It is a naturally occurring plasmid isolated from *E.coli K-12*.
- This plasmid has broad host range in gram negative bacteria.
- The size of plasmid is 8694bp.
- Antibiotic resistance genes for Streptomycin and sulfonamides have present.
- The replication of RSF1010 starts either bi- or uni-directionally from unique *ori-V*region (2347-2742).

• It cannot initiate transformation independently but can be transferred to host bacterium in presence of helper plasmid.
• Genebank accession no. M28829.

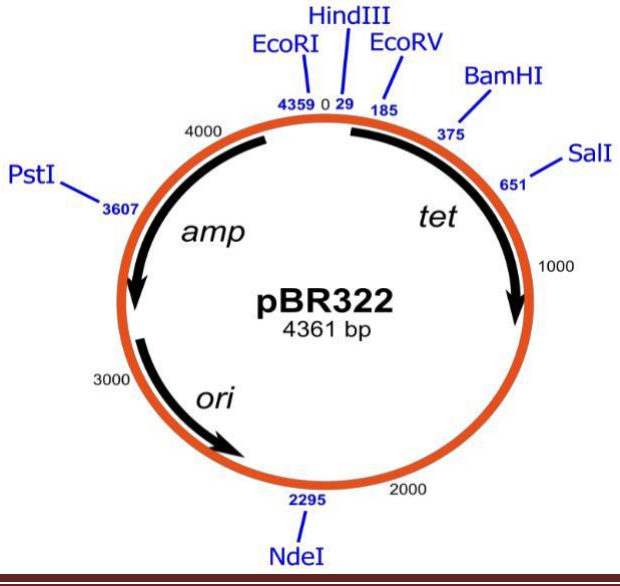
**ColE1:**
• It is a naturally occurring multicopy plasmid obtained from *E.coli (*copy number is around 40).
• The size of this natural plasmid is 6646bp.
• It forms the basis of many artificial vectors used in molecular cloning.

• The natural ColE1 plasmid has genes for colicin E1 production. Colicin is an antibacterial toxin produced under stressed condition. Cells harboring the plasmid will have resistance against the toxin.
• For using in molecular cloning experiment, colicin genes are replaced with selection marker (antibiotic resistant) gene.
• Genebank accession no. M33100.

**Artificial Plasmids:** They are constructed *in-vitro* by re-combining selected segments of two or more other plasmids (natural or artificial) to overcome the limitations of natural vectors. Artificial plasmids vectors are classified into two broad types based on their use:

1. Cloning vector (e.g. *pBR322 plasmid*)
2. Expression vector

Cloning vectors are designed for efficient transfer of foreign DNA into the host.
***pBR322 plasmid:*** is the most popular and most widely used plasmid of 4361 bp; The name pBR denotes the following: p signifies plasmid, B is from Boliver, and R is from Rodriguez, the two initials of the scientist who developed in 1977 in the laboratory.



- It has the replication module of *E. coli* plasmid Col El.
- This module has been incorporated in many other plasmid vectors since it permits plasmid replication even when chromosome replication and cell division are inhibited by amino acid starvation or chloramphenicol.
- Under such conditions, each cell accumulates several thousand copies of the plasmid so that one litre of bacterial culture easily yields a milligram of plasmid DNA.
- It has two selectable markers (tetracycline, tetr, and ampicillin, amp', resistance genes), and unique recognition sites for 12 different restriction enzymes (two unique sites, PstI and PvuI, are located within the amp' gene, and 4, e.g., BamHI, SalI, etc., are within tetr gene).

The presence of restriction sites within the markers tetr and amp permits an easy selection for cells transformed with the recombinant pBR322.

- Insertion of the DNA fragment into the plasmid using restriction enzyme PstI or PvuI places the DNA insert within the gene amp'; this makes amp' nonfunctional.
- Bacterial cells containing such a recombinant pBR322 will be unable to grow in the presence of ampicillin, but will grow on tetracycline.
- Similarly, when restriction enzyme BamHI or SalI is used, the DNA insert is placed within the gene tetr making it nonfunctional.
- Bacterial cells possessing such a recombinant pBR322 will, therefore, grow on ampicillin but not on tetracycline.
- This feature allows an easy selection of a single bacterial cell having recombinant pBR322 from among 108 other types of cells.
- Transformed *E. coli* cells are first plated on an agar medium containing the antibiotic within the resistance gene for which the DNA fragment is not inserted, i.e., for which the bacterial cells having the recombinant DNA are expected to be resistant.
- This eliminates nontransformed bacterial cells; the resulting bacterial colonies will posses either recombinant or unaltered pBR322.
- The colonies so obtained are then replica plated on agar plates containing the other antibiotic (within the resistance gene for which the DNA insert is placed); all the colonies that develop on this plate will contain the unaltered pBR322.
- Therefore, the antibiotic sensitive colonies are identified and recovered from the master plate; these colonies will have the recombinant pBR322. This entire process may take up to 2 days.

Expression vectors have efficient machinery for cloning and expression of foreign gene in the host system.

**Yeast YIp, YEp and YCp vectors**

Yeast expression plasmids used in the lab typically contain all the necessary components to allow shuttling between *E. coli* and yeast cells. Yeast vectors can be grouped into five general classes, based on their mode of replication in yeast: YIp, YRp, YCp, YEp, and YLp plasmids. With the exception of the YLp plasmids (yeast linear plasmids), all of these plasmids can be maintained in *E. coli* as well as in *S. cerevisiae* and thus are referred to as shuttle vectors

**Plasmid replication**

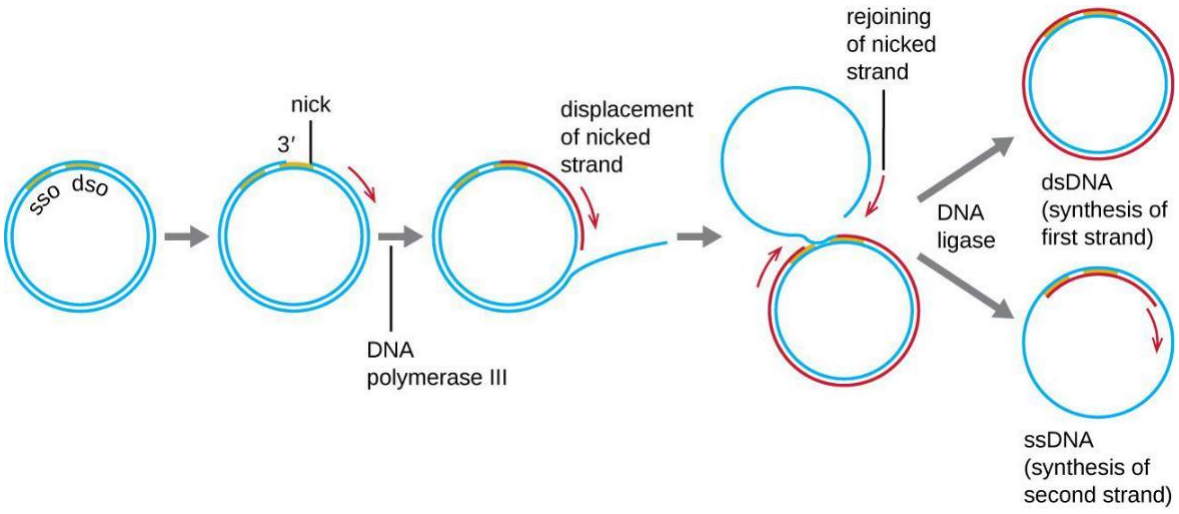
There are three types of plasmid replication namely (i) rolling circle, (ii) Col E1 type and (iii) Iteron contain replication

(i) Rolling circle

- Rolling circle replication mechanism is specific to bacteriophage family M13 and the fertility F factor which encodes for sex pili formation during recombination by means of conjugation.
- Fragments smaller than 10 kbp usually replicate by this replication mechanism as reported in some

gram positive bacteria.

- It allows the transfer of single stranded replication product at a faster rate to the recipient cell through pilus as in case of fertility factor or to the membrane in case of phage.



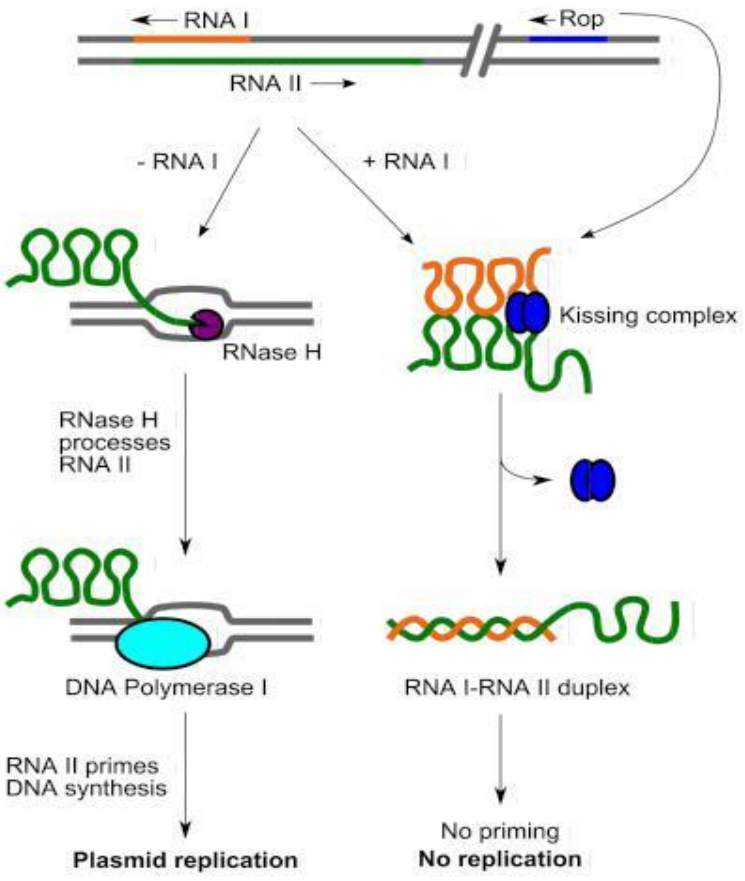

Mechanism: Rolling circle occurs to a covalently closed circular piece of double-stranded DNA. A nick is produced in one of the strands by enzyme creating a 5’ phosphate and a 3’ hydroxyl. Free 3’ hydroxyl will be used by DNA polymerase to make new DNA pushing the old nicked strand off of the template DNA

(ii) Col E1 type replication

- Col E1 replication is a negative regulation mechanism which enables the plasmid to control its own copy numbers by involving RNA type I and RNA type II.
- Col E1 replication is initiated by means of RNA-RNA interactions and does not rely on replication initiation protein encoded by the plasmid to regulate its copy number.

Mechanism: ColE1 replication begins at 555bp upstream from the origin. RNA polymerase initiates transcription of RNAII which acts as a pre-primer and begins the synthesis of the leader strand. The transcript (mRNA) folds into a secondary structure which stabilizes the interaction between the nascent RNA and the origin's DNA. This hybrid is attacked by RNase H, which cleaves the RNA strand, exposing a 3' hydroxyl group. This allows the extension of the leading strand by DNA Polymerase I

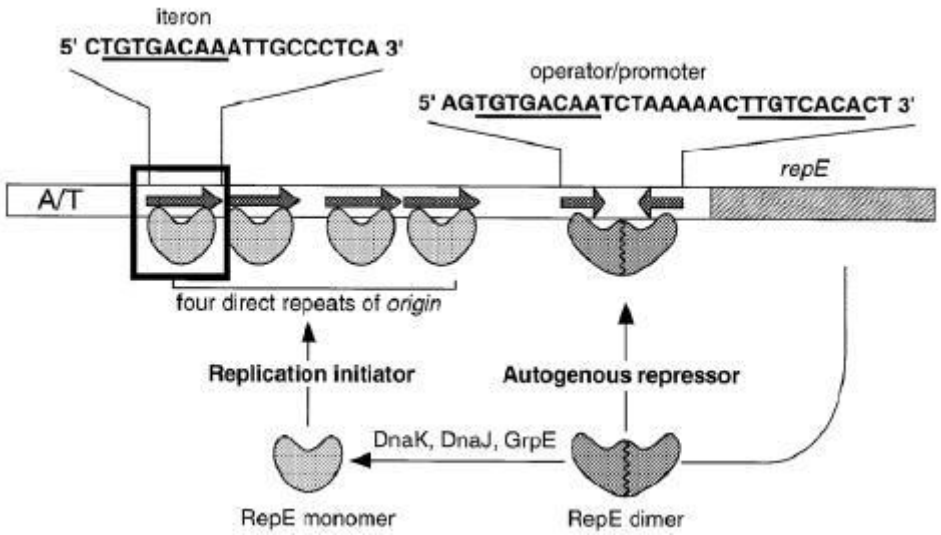
RNAI is a counter-transcript to a section of RNAII and so binds to its 5' end. This alters the folding of RNAII so that the DNA-RNA hybrid is not stabilized and cleavage does not occur. This ensures that at high copy numbers, replication is slowed down due to increased RNA I concentration.



(iii) Iteron-containing replicons

➢ This replicon consists of a gene that encodes Rep protein for plasmid replication initiation, set of direct repeat sequences called iteron, adjacent A=T-rich region and Dna boxes which is a protein required for bacterial chromosome replication initiation. However length of adjacent AT-rich region and number of iterons and DnaA boxes differs in a replicon

Mechanism: Iteron contain replication begins with the binding of Rep proteins to the iteron being organized in the same orientation of the DNA helix. And by binding to the DnaA boxes in the replicon the Rep-DnaA-DNA assembly promotes melting of the strand at the nearby AT-rich region to which host replication factors subsequently gain access and promote leading and lagging strand synthesis in a manner analogous to initiation of replication at the chromosomal origin, oriC. Plasmids copy number is controlled principally at the beginning of replication initiation. The frequency with which initiation of replication of iteron-containing plasmids occurs is modulated in part by sequestration of the origin region in nucleoprotein complexes and intermolecular pairing of complexes on different plasmids, which is referred to as "handcuff ".



Plasmid stability

One of the characteristic features of plasmids is their instability. Plasmid-borne features are often lost from a population at a higher frequency than would be expected for the normal processes of mutation. The extent of this instability varies enormously from one plasmid to another. Naturally- occurring plasmids are usually (but not always) reasonably stable.

Artificially constructed plasmids on the other hand are often markedly unstable. This is usually merely a nuisance on the laboratory scale, but can become a very expensive problem in the industrial use of strains carrying such plasmids. There are three quite distinct phenomena associated with the concept of plasmid stability: (1) plasmid integrity, (2) partitioning at cell division and (3) differential growth rates.

**Partitioning**

As mentioned previously, correct partitioning at cell division is essential if the plasmid is to be maintained in the culture. Although high copy number plasmids can rely principally on random distribution between the two daughter cells, this can be compromised by a tendency for plasmids to form multimeric structures during replication and also by recombination between monomers.

**Plasmid-incompatibility**

Plasmid incompatibility is generally defined as the failure of two coresident plasmids to be stably inherited in the absence of external selection.

Generally, two closely related plasmids cannot coexist in a bacterial cell. In the population of progeny cells derived from a cell containing two such plasmids, the proportion of cells having only one of the two plasmids increases with every cell division. This is known as plasmid incompatibility.

On the other hand, two different unrelated plasmids, e.g. F plasmid and ColEl can exist together without any difficulty, because these plasmids belong to two different incompatibility groups. Whereas, two F-plasmids cannot coexist in the same cell.

One mechanism by which a plasmid already resident in a cell prevents the entry of a second similar plasmid into the same cell is by surface exclusion. For example, an F-plasmid of *E. coli* does not allow entry of another F-plasmid by inhibiting it from leaving the cell where it is already located. The effect is mediated at the surface of the cell whereby the F-DNA cannot come out of the cell.

A different mechanism operates when a cell already has two closely related plasmids, say F1 and F2. We know that the copy number of plasmids is controlled by specific inhibitors coded by the plasmid itself.

As F1 and F2 are two closely related plasmids, it would be expected that their inhibitors would also be closely similar and that replication of both the plasmids would be regulated by the inhibitor produced either by F1 or F2.

During replication, F1 and F2 may be selected at random, so that, during first replication of the plasmid, a cell initially containing one copy of each plasmid may produce two copies of either F1 or F2, so that the

cell has now two copies of either F1 or F2 and one copy of the un-replicated plasmid i.e. 2F1 + F2 or F1 + 2F2. In the second round of plasmid replication, each cell will contain 4 plasmids, but depending on which plasmid is replicated, the combination may be F1 + 3F2, 2F1 + 2F2 or 3F1 + F2.

Now the cell divides to produce two daughter cells, each with 2 plasmids and the plasmid combinations of the daughter cells may be F1 + F1, F1 + F2 or F2 + F2. Thus the probability of progeny cells having either two F1 plasmids or two F2 plasmids is equal to those having two different plasmids i.e. F1 + F2. In other words, the probability of elimination of one plasmid is 50%. Such probability increases with more cell generations.

**Applications of plasmids:**

- To create transgenic organisms by introducing beneficial genes into host cells. For example, the Ti plasmid is used in plant pathology to develop resistance in plants against diseases such as holcus spot on leaves and crown gall tumors. The plasmid is rendered avirulent by curing it, prior to its use as a vector.
- The artificial and cost-effective bulk production of antibiotics can be achieved by incorporating an expression vector for that antibiotic in microbial cells. Similarly, other biomolecules can also be produced.
- They can be used to administer gene therapy, which is a technique used to correct defective genes responsible for disease development.
- They are also used to study the role of various gene and gene products in a biological system. This is achieved by silencing or over expressing a particular gene and observing the effects, if any (gene knockout).

**Possible Questions**

**Part-A (1 mark)**

**Part-B (2 Marks)**

1. Define plasmids.
2. What are F plasmid?
3. What are degradative plasmids?
4. What is meant by R plasmid?
5. Write any two properties of plasmids.
6. Write about the copy number of a plasmid.
7. Mention the three types of plasmid replication.

**Part-C (8 Marks)**

8. Distinguish between natural and artificial plasmid
9. Write briefly about types of plasmids.
10. Explain the method of constructing pBR322
11. Write briefly about plasmid partitioning.
12. Explain the replication mechanism of R plasmids and F plasmids.
13. Write in detail about the application of plasmids.

| Sl. No | Question | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | Chemical agent that resembles thiamine | 5-bromothiamine | 5-bromoadenine | 5-bromoguanine | 5-bromouracil | 5-bromouracil |
| 2 | Duplication mutation is | Segments of nucleotides sequences are repeated | Segments of nucleotides sequences are deleted | Segments of nucleotides sequences are inserted | Segments of nucleotide sequences are inserted & deleted evenly | Segments of nucleotides sequences are repeated |
| 3 | Mutations that result from treatment with mutagens are called | Induced mutation | Uninduced | Spontaneous | Frameshift | Induced mutation |
| 4 | Oncogenes are found in certain | Bacteria | Fungi | Viruses | Algae | Viruses |
| 5 | Mutation generating new stop codon are called | Nonsense mutation | Misense mutation | Point mutation | Silent mutation | Nonsense mutation |
| 6 | UV induced dimers are separated using light energy by | Primase | Photolyase | Dnase | Rnase | Photolyase |
| 7 | The function of DNA glycosylase in base excision repair is | Addition of correct base | Addition of correct nucleotide | Removal of incorrect base | Removal of phosphodiester bond | Removal of phosphodiester bond |
| 8 | Which of the following biomolecule has self¬repair mechanisms? | DNA,RNA and protein | DNA and RNA | DNA and proteins | DNA only | DNA only |
| 9 | Rapid screening technique for mutagens and carcinogens | Aims test | Sima test | Ames test | Sema test | Ames test |
| 10 | Mutation resulting from deamination of 5-methylcytosine produces Thymine which pairs with | Uracil | Adenine | Cytosine | Guanine | Adenine |
| 11 | Converts amino groups to keto groups by oxidative deamination | Hydrochloric acid | Nitrous acid | Sulphuric acid | Oxalic acid | Nitrous acid |
| 12 | In bacteria, a small circle of DNA found outside the main chromosome is called | Cosmid | Bacmid | Transposon | Plasmid | Plasmid |

| | | | | | | |
|---|---|---|---|---|---|---|
| 13 | Biological agents of mutagenesis are | Transposable elements | Lipids | Bacteria | Carbohydrates | Transposable elements |
| 14 | Most common proto-oncogene implicated in human cancers | *s-rac* | *a-src* | *r-cas* | *c-ras* | *c-ras* |
| 15 | Deficiency in phenylalanine hydroxidase results in | Cancer | Phenylketonuroa | Melanoma | Asthma | Phenylketonuroa |
| 16 | Cancer that results from deletion of a portion of chrosome 13 is | Eye cancer | Bone cancer | Skin cancer | Lung cancer | Eye cancer |
| 17 | Mutation in which a purine base is substituted with another purine base is | Transverse mutation | General mutation | Transition mutation | Transduction | Transition mutation |
| 18 | Sickle cell anaemia is caused by change in amino acid from | Glutamic acid | Alanine to Leucine | Valine to Glutamic acid | Leucine to alanine | Valine to Glutamic acid |
| 19 | Which of the following chemicals induce depurination | Methyl ethane sulphonate | Guanidine | Ethyl sulphonate | Dichlor | Methyl ethane sulphonate |
| 20 | Xeroderma pigmentosum is a genetic disorder of | Skin | Hair | Nail | Tongue | Skin |
| 21 | UV radiation causes | Adenine dimers | Cytosine dimers | Guanine dimers | Thiamine dimers | Thiamine dimers |
| 22 | The result of addition or deletion of one or more base pair in a gene is | Frameshift | Base pair substitution | Misense mutation | Nonsense mutation | Frameshift |
| 23 | Repairing mechanism of _____ depends on absorption of visible light by the enzyme. | DNA helicase | DNA ligase | DNA gyrase | DNA photolyase | DNA photolyase |
| 24 | Herman J.Mueller reported results of induced mutations on | Yeast | *Drosophila* | Fish | Pea plant | *Drosophila* |
| 25 | Chromosomal mutation is | Abberation | Change over | Variation | Genetic change | Abberation |
| 26 | _____ is a non-ionizing radiation | Alpha | UV | Gamma | Beta | UV |
| 27 | Mutation involving single-base changes are | Induced mutations | Point mutations | Silent mutations | Inverse mutations | Point mutations |
| 28 | Transposons was first reported by | Louise pasteur | Koch | Barbara | Lister | Barbara |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | McClintock | | McClintock |
| 29 | Nonsense mutation leads to | Termination of DNA synthesis | Termination of protein synthesis | Termination of cell wall synthesis | Termination of RNA synthesis | Termination of protein synthesis |
| 30 | Alkylation is | Addition of methyl or ethyl group | Deletion of ethyl and addition of methyl group | Deletion of methyl and addition of ethyl group | Deletion of methyl or ethyl group | Addition of methyl or ethyl group |
| 31 | Chemical mutagens leading to addition of nucleotides to the DNA are | Thimers | Base analogs | Alkylating agents | Interchelating agents | Interchelating agents |
| 32 | Human bladder cancer is brought about by a change in single point mutation of | Valine to glycine | Isoleucine to leucine | Leucine to isoleucine | Glycine to valine | Glycine to valine |
| 33 | Which of the following dimer formation is most common | Cytidine dimer | Uracil dimer | Thymidine dimer | Adenosine dimer | Thymidine dimer |
| 34 | Daughter strand repair is also called as | Recombination repair | SOS repair | Photo repair | Excision repair | Recombination repair |
| 35 | An intercalating dye | Sunset yellow | Safranin | India ink | Acridine orange | Acridine orange |
| 36 | Most common repair system is | SOS | Photoreactive | Mismatch | Excision | Excision |
| 37 | Virus capable of causing mutations is | Bacteriophage Ru | Bacteriophage Mu | Bacteriophage Nu | Bacteriophage Ly | Bacteriophage Mu |
| 38 | Potent oxidizing agent that can alter structure of purine and pyrimidine | Free radicals | Water | Dyes | Acids | Free radicals |
| 39 | Reverse mutation is | Wild type to mutant | Mutant to wild type | A new gene introduced | A gene deleted | Mutant to wild type |
| 40 | Alkylation of guanine causes its removal from DNA in a reaction called | Deamination | Depyrimidation | Degradation | Depurination | Depurination |
| 41 | In *E.coli*, parental DNA is methylated at an adenine residue found in the sequence | 5' TAGC 3' | 5' ATGC 3' | 5' CATG 3' | 5' GATC 3' | 5' GATC 3' |

| | | | | | | |
|---|---|---|---|---|---|---|
| 42 | Mutation in which there is an amino acid substitution is called | Missense | Nonsense | Silent | Point | Missense |
| 43 | Nutritional mutans of neurospora are known as | Phototrophs | Auxotrophs | Heterotrophs | Isotrophs | Auxotrophs |
| 44 | UV radiation at 260 nm cross-links adjacent thiamine that produces | Butane ring | Cyclane ring | Butocyclane ring | Phenyl alanine | Cyclobutane ring |
| 45 | When a part of chrosome is moved to another chromosome, it is called as | Point mutation | Induced mutation | Spontaneous mutation | Translocation mutation | Translocation mutation |
| 46 | Mismatch repair cannot take place if there is a mutation in | Helicase | Polymerase | Ligase | Methylase enzyme | Methylase enzyme |
| 47 | Common chemical events that produce spontaneous mutation | Deamination | Depurination | Dimerization | Isomerization | Depurination |
| 48 | Bacterium used in Ames test | *Salmonella* | *Shigella* | *Streptococcus* | *Staphylococcus* | *Salmonella* |
| 49 | Naturally occurring mutations are | Induced | Spontaneous | Nonsense | Frameshift | Spontaneous |
| 50 | Recombinational repair is often due to | many cytidine dimer and associated large gaps in a strand | incorporation of many incorrect nucleotides by DNA polymerase | many thymidine dimer formation and associated large gaps in a strand | DNA breaking | incorporation of many incorrect nucleotides by DNA polymerase |
| 51 | The most common error prone repair mechanism is | Mismatch | Excision | SOS | Recombination | SOS |
| 52 | Site that mutates at a rate significantly greater thag statistical probability is referred to as | Hotspots | Blackspots | Dotspots | DNA spots | Hotspots |
| 53 | Transposition is | Movement of a phage | Movement of a virus | Movement of a transposon | Movement of a plasmid | Movement of transposon |
| 54 | Radiation that causes cross chromosomal mutations in humans | UV | Visible | Ionozing | X-rays | Ionozing |
| 55 | Frameshift mutation is caused by | Proflavin | Nitrous acid | UV | X-rays | Proflavin |
| 56 | Change of purine to pyrimidine base in an | Transition | Transverse | General | Transformation | Transverse |

| | | | | | | |
|---|---|---|---|---|---|---|
| | mutation | mutation | mutation | mutation | | mutation |
| 57 | Detection of silent mutations require | Aminoacid analysis | Peptide analysis | RNA analysis | Nucleotide analysis | Nucleotide analysis |
| 58 | Most frequently employed technique in the study of mutations | Analysis of phenotypes | Analysis of genotypes | Analysis of proteins | Analysis of both phenotypes and genotypes | Analysis of phenotypes |
| 59 | Natural phenomena of changes in chemical structure of nitrogenous bases is called | Complementary | Conservative | Tautomeric | Telomeric | Tautomeric |
| 60 | Mutation that has no detectable effect on the phenotype of a cell | Point | Induced | Silent | Leaky | Silent |

# Unit – III

## Syllabus

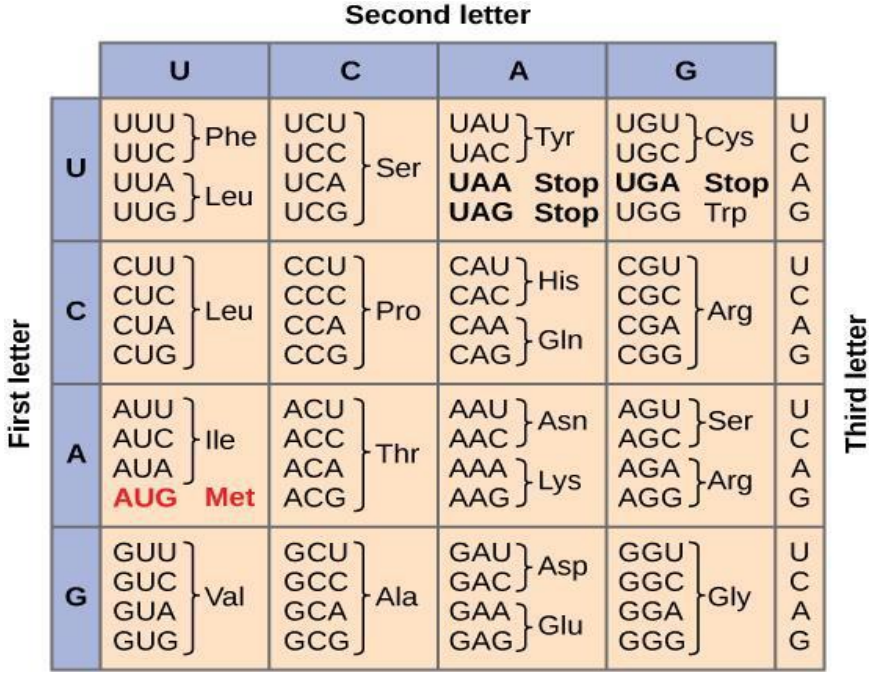
Genetic code- Operon concept-Lactose, tryptophan. Genetic recombination in bacteria- Conjugation, Transformation-Transduction and its types. Gene Mapping techniques-gene and chromosome walking

## Genetic code

- Genetic code is the nucleotide base sequence on DNA (and subsequently on mRNA by transcription) which will be translated into a sequence of amino acids of the protein to be synthesized.
- The code is composed of codons.
- Codon is composed of 3 bases ( e.g. ACG or UAG).
- Each codon is translated into one amino acid.
- The 4 nucleotide bases (A,G,C and U) in mRNA are used to produce the three base codons.
- There are therefore, 64 codons code for the 20 amino acids, and since each codon code for only one amino acids this means that, there are more than one cone for the same amino acid.
- Each triplet is read from 5′ → 3′ direction so the first base is 5′ base, followed by the middle base then the last base which is 3′ base.

Examples: 5′- AUG- 3′ codes for methionine 5′- UCU- 3′ codes for serine

Termination (stop or nonsense) codons: Three of the 64 codons; UAA, UAG, UGA do not code for any amino acid. They are termination codes which when one of them appear in mRNA sequence, it indicates finishing of protein synthesis.



Characters of the genetic code:

- Specificity: the genetic code is specific, that is a specific codon always code for the same amino acid.
- Universality: the genetic code is universal, that is, the same codon is used in all living organisms
- Degeneracy: the genetic code is degenerate i.e. although each codon corresponds to a single amino acid, one amino acid may have more than one codons. e.g arginine has 6 different codons.

Properties:

- **The genetic code is composed of nucleotide triplets.** In other words, three nucleotides in mRNA (a codon) specify one amino acid in a protein.
- **The code is non-overlapping.** This means that successive triplets are read in order. Each nucleotide is part of only one triplet codon.
- **The genetic code is unambiguous.** Each codon specifies a particular amino acid, and only one amino acid. In other words, the codon ACG codes for the amino acid threonine, and only threonine.
- **The genetic code is degenerate.** In contrast, each amino acid can be specified by more than one codon.
- **The code is nearly universal.** Almost all organisms in nature (from bacteria to humans) use exactly the same genetic code. The rare exceptions include some changes in the code in mitochondria, and in a few protozoan species.

**Operon regulation**

- The term "operon" was coined by Jacob and Monod, who characterized the first defined classical operon, the *lac* operon, in *Escherichia coli*.
- An operon is a group of closely linked genes that produces a single messenger RNA molecule in transcription and that consists of structural genes and regulating elements (such as an operator and promoter)

Structural Genes:

1. The gene is connected with transcription or formation of mRNA for synthesis of particular polypeptide.
2. Structural gene functions only when receives complementary nucleotides and RNA polymerase.
3. The gene is moderately long to large depending upon the polypeptide to be synthesized.
4. It functions through the formation of mRNA for structural or enzymatic polypeptide.

Operator Gene:

1. It determines the functioning of structural genes.
2. Operator gene functions only when it is not blocked by repressor.
3. The gene is small.
4. It functions through the presence or absence of repressor.

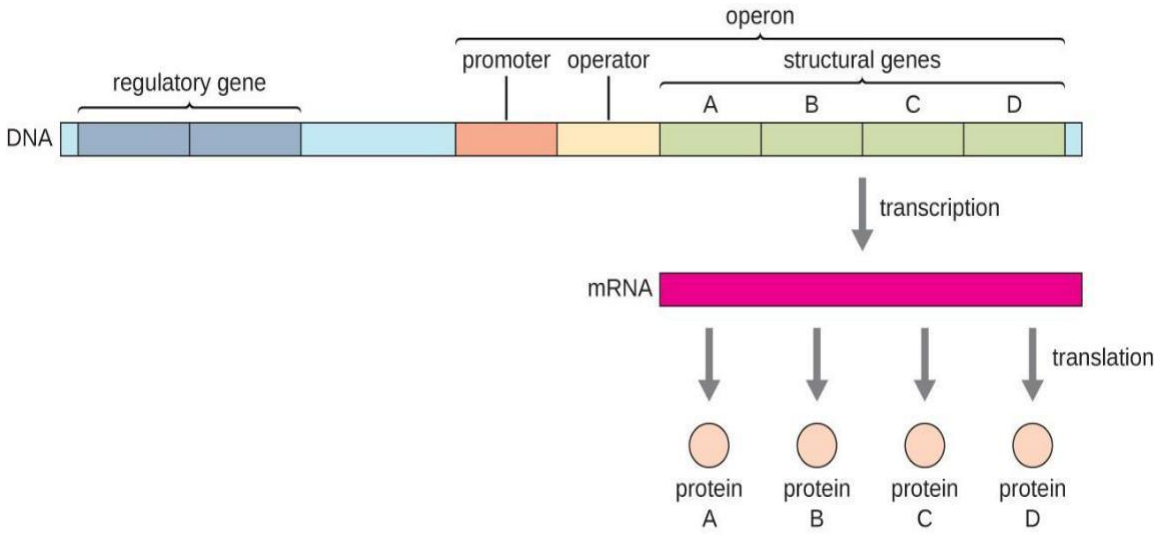
Promoter Gene:

1. It is site for binding of RNA polymerase.

2. It is functional only when operator gene allows passage of RNA-polymerase to structure genes.
3. The gene is small.
4. It functions by providing recognition and binding sites for RNA polymerase.

Regulator Gene:

1. It controls the functioning of operator gene.
2. Regulator gene produces a repressor or Apo repressor for blocking operator gene.
3. It is commonly a large gene.
4. It functions through the formation of an mRNA of represso



**Operons may be inducible or repressible:**

Some operons are usually "off," but can be turned "on" by a small molecule. The molecule is called an **inducer**, and the operon is said to be **inducible**.

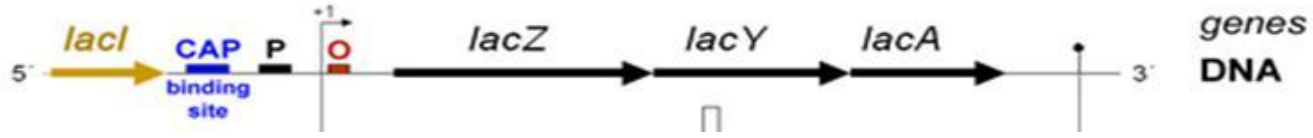
- For example, the *lac* operon is an inducible operon that encodes enzymes for metabolism of the sugar lactose. It turns on only when the sugar lactose is present (and other, preferred sugars are absent). The inducer in this case is allolactose, a modified form of lactose.

Other operons are usually "on," but can be turned "off" by a small molecule. The molecule is called a **co-repressor**, and the operon is said to be **repressible**.

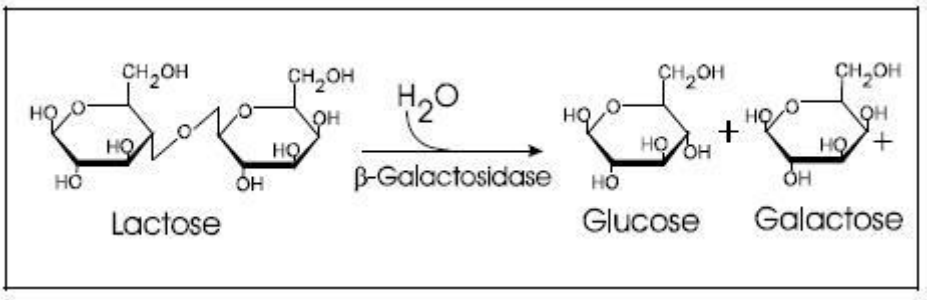
- For example, the *trp* operon is a repressible operon that encodes enzymes for synthesis of the amino acid tryptophan. This operon is expressed by default, but can be repressed when high levels of the amino acid tryptophan are present. The co-repressor in this case is tryptophan.

***Lac* operon:**

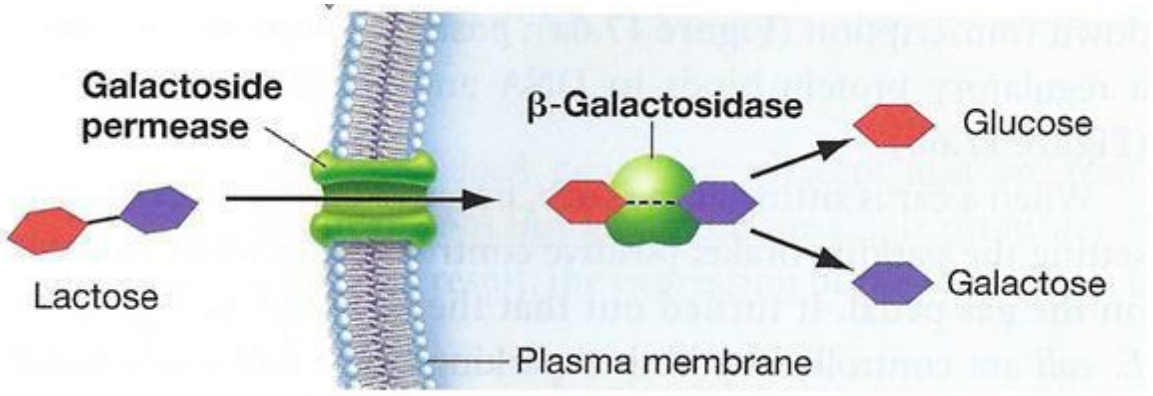
The lac operon, short for lactose operon, is a series of three genes in bacteria that produce the necessary enzymes to obtain energy from lactose. Typically, glucose is the sugar of choice for bacterial organisms, but when this supply runs lows, the lac operon allows the organisms to survive on lactose. The three genes within the lac operon are *lacZ*, *lacY*, and *lacA*.




The function of lacz gene is, which codes for the enzyme **β-galactosidase**; the enzyme breaks the β-galactoside into its components Sugar; E.g: Lactose is cleaved into Glucose and Galactose for further metabolism.

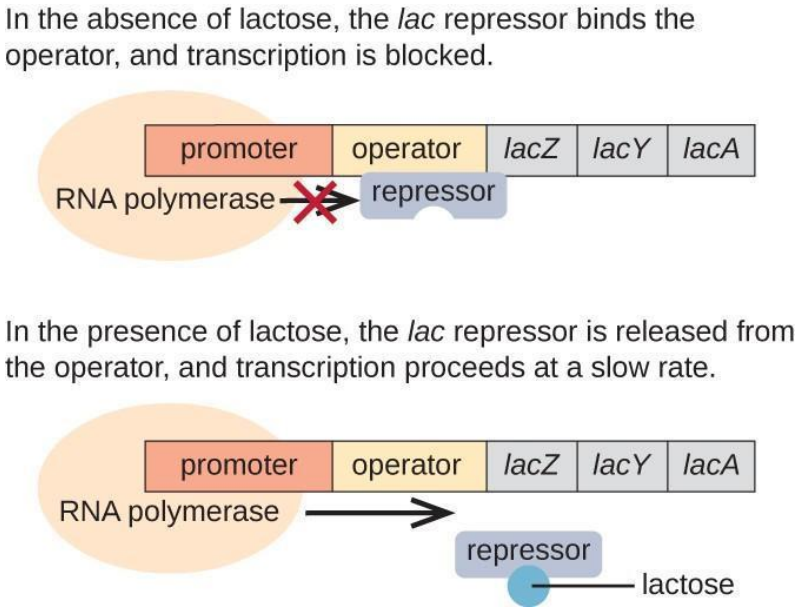



Lac Y codes for the **β-galactoside Permease**; this transports the β-galactosides into the cell.




Lac A codes for **β-galactoside transacetylase**, an enzyme that transfers an acetyl group from acetyl~CoA to β-Galactosides.

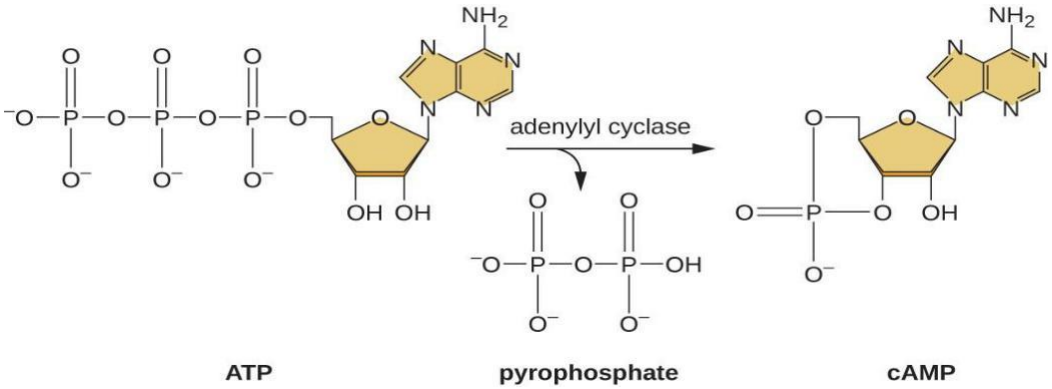
In the absence of lactose, the *lac* repressor is bound to the operator region of the *lac* operon, physically preventing RNA polymerase from transcribing the structural genes. However, when lactose is present, the lactose inside the cell is converted to allolactose. Allolactose serves as an **inducer** molecule, binding to the **repressor** and changing its shape so that it is no longer able to bind to the operator DNA. Removal of the repressor in the presence of lactose allows **RNA polymerase** to move through the operator region and begin transcription of the *lac* structural genes.



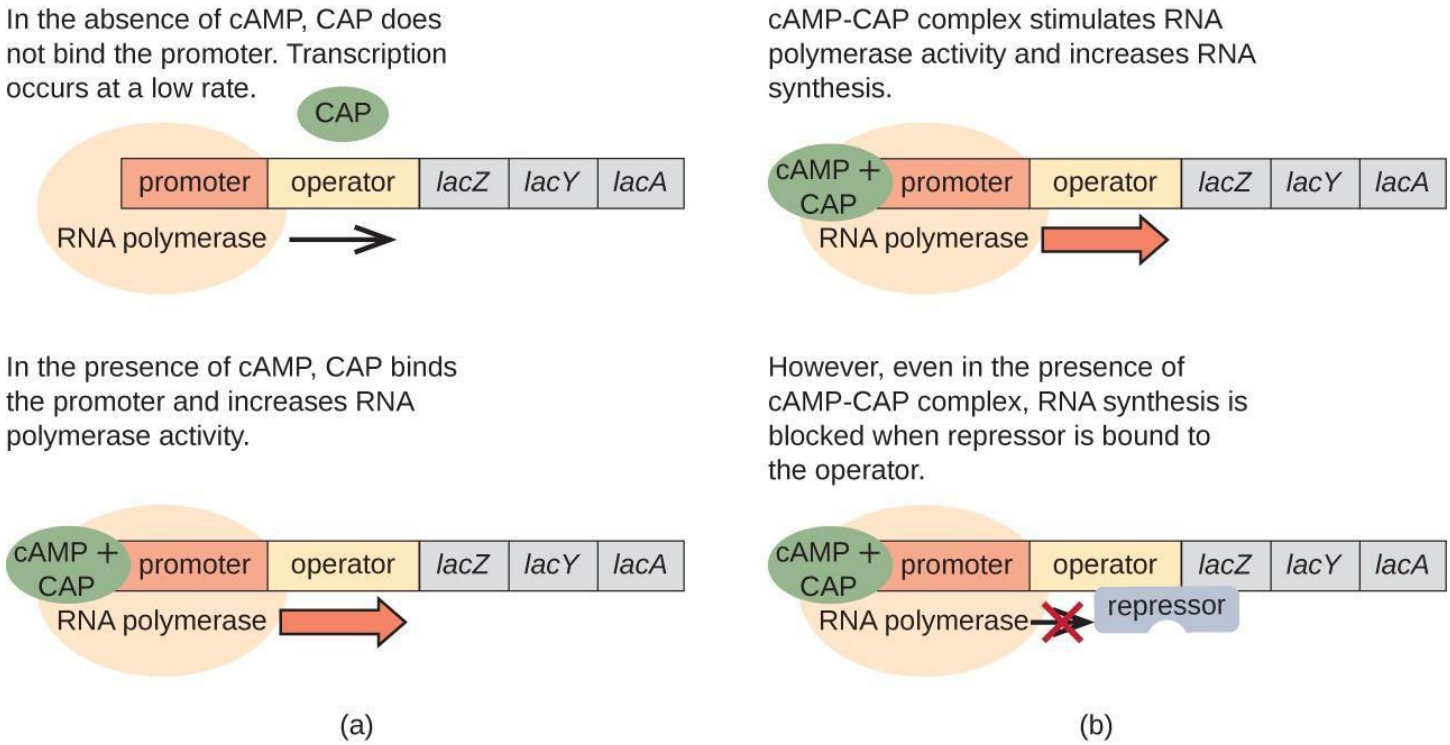



The ability to switch from glucose use to another substrate like lactose is a consequence of the activity of an enzyme called **Enzyme IIA (EIIA)**. When glucose levels drop, cells produce less ATP from catabolism,

and EIIA becomes phosphorylated. Phosphorylated EIIA activates adenylyl cyclase, an enzyme that converts some of the remaining ATP to **cyclic AMP (cAMP)**, a cyclic derivative of AMP and important signaling molecule involved in glucose and energy metabolism in *E. coli*. As a result, cAMP levels begin to rise in the cell.




The *lac* operon also plays a role in this switch from using glucose to using lactose. When glucose is scarce, the accumulating cAMP caused by increased adenylyl cyclase activity binds to **catabolite activator protein (CAP)**, also known as cAMP receptor protein (CRP). The complex binds to the promoter region of the *lac* operon.




(a) In the presence of cAMP, CAP binds to the promoters of operons, like the lac operon, that encode genes for enzymes for the use of alternate substrates. (b) For the lac operon to be expressed, there must be activation by cAMP-CAP as well as removal of the lac repressor from the operator.

In the regulatory regions of these operons, a CAP binding site is located upstream of the RNA polymerase binding site in the promoter. Binding of the CAP-cAMP complex to this site increases the binding ability of RNA polymerase to the promoter region to initiate the transcription of the structural genes. Thus, in the case of the *lac* operon, for transcription to occur, lactose must be present (removing the lac repressor protein) and glucose levels must be depleted (allowing binding of an activating protein).

When glucose levels are high, there is catabolite repression of operons encoding enzymes for the metabolism of alternative substrates. Because of low cAMP levels under these conditions, there is an insufficient amount of the CAP-cAMP complex to activate transcription of these operons.

**Tryptophan operon**

The tryptophan operon is the regulation of transcription of the gene responsible for biosynthesis of tryptophan from chorismate.

Tryptophan operon consists of structural gene and regulatory gene.
I. Regulatory gene are Promoter, Repressor, Operater and Leader sequence.
II. Structural gene are TrpE, TrpD, TrpC, TrpB and TrpA

1. trpE: It enodes the enzyme Anthranilate synthase I
2. trpD: It encodes the enzyme Anthranilate synthase II
3. trpC: It encodes the enzyme N-5'-Phosphoribosyl anthranilate isomerase and Indole-3-glycerolphosphate synthase
4. trpB: It encodes the enzyme tryptophan synthase-B sub unit
5. trpA: It encode the enzyme tryptophan synthase-A sub unit

Tryptophan operon is regulated by following mechanism

I. <u>Repression</u>

<u>a. when tryptophan is absent in cell:</u>

- Repressor gene (trpR) encodes the repressor proten which is originally inactive. In the absence of tryptophan, transcription of structural gene occur for the biosynthesis of tryptophan from chorismite

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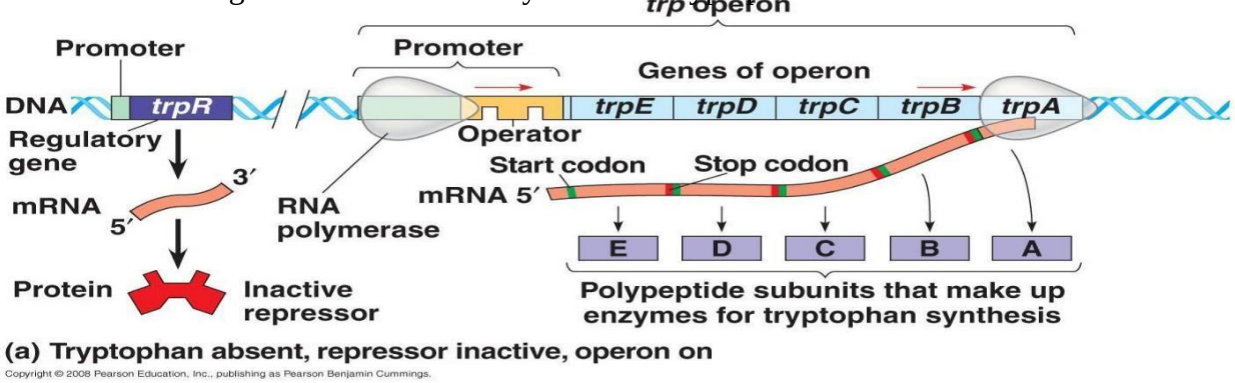
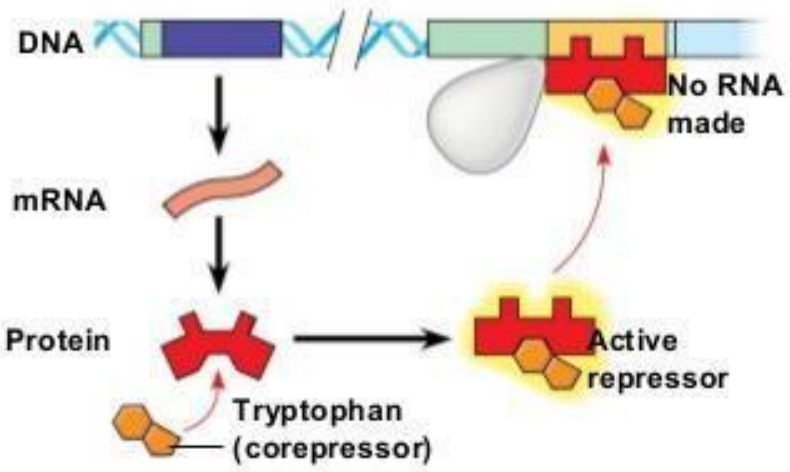


b. when tryptophan is high in cell:

- When tryptophan is high in cell then it binds with repressor protein and change its confirmation so that it become active and bind to the operator near promoter.
- Binding of repressor protein to operator overlaps the promoter, so RNA polymerase cannot bind to the prometer. Hence transcription is halted.
- Since tryptophan is already high in cell, no transcription of structural gene is required for biosynthesis of tryptophan. This is also known as negative regulation.

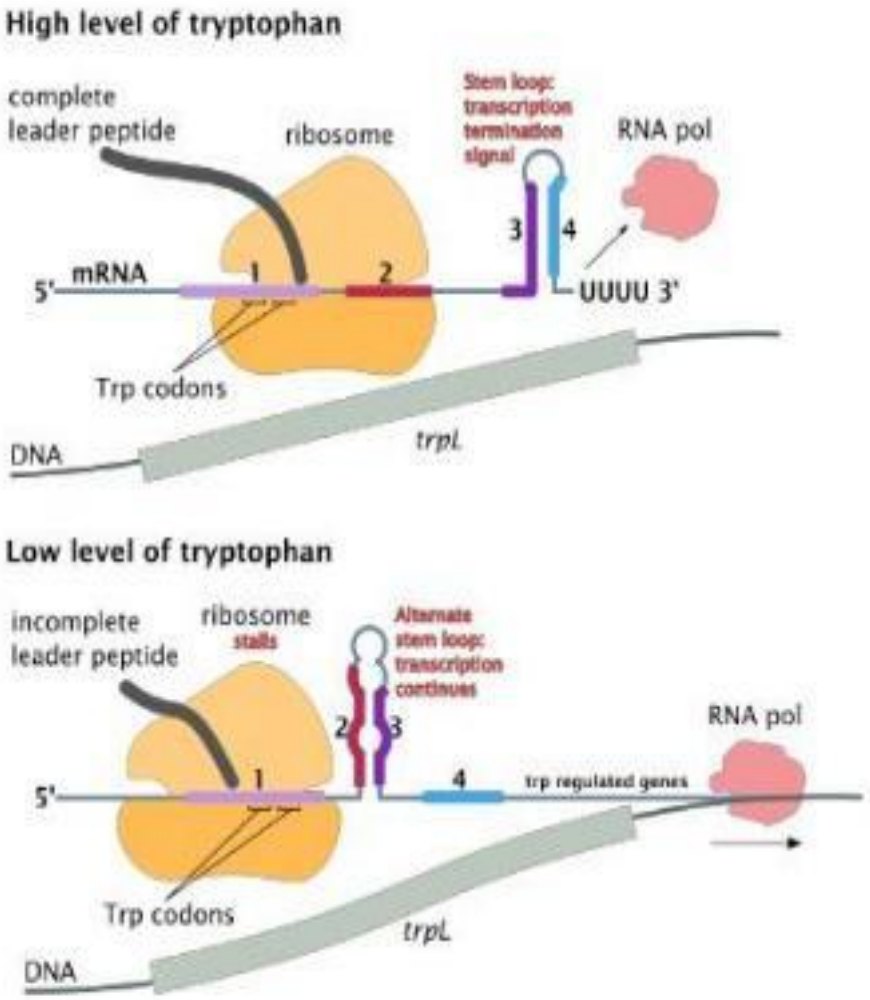


(b) Tryptophan present, repressor active, operon off

II. Attenuation:

- In bacteria, transcription and translation occurs simultaneously. The translation starts before transcription completes. In this attenuation mechanism, rate of translation determines whether transcription continues or terminates. Therefore the attenuation mechanism is only found in bacteria but not in eukaryotic cell.
- Leader sequence (trpL) play important role in attenuation. Leader sequence contains such a nucleotide sequence that mRNA transcribed from it contains four specific region. Region 1, region 2, region 3 and Region 4. Region 3 is complementary to both region 2 and region 4.
- If region 3 and region 4 base pair with each other, they form a loop like structure called attenuator and it function as transcriptional termination. If pairing occur between region 3 and region 2, then no such attenuator form so that transcription continues.
- Region 1 is the most important region that determines whether to form loop between region 2-region 3 or region 3-region 4. The region 1 consists of sequence of 14 codons, out of which two codons are tryptophan codon (codon 10 and 11).
- When tryptophan is high in cell than tRNA crrying tryptophan encodes codon 10 and 11. Such that ribosome encloses the region 2 which is near to the tryptophan codon. Hence region 3 base pair with region 4 to form attenuator as region 2 is not available for pairing. Consequently, transcription is halted.

When tryptophan is low or absent in cell, then translation stops at the position of tryptophan codon. Such that loop between region 2 and region 3 forms. Transcription continues.




**Feedback mechanism of trp operon**

1. When tryptophan is high in cell then transcription of structural gene does not occur.
2. When tryptophan is absent or very low then transcription continues
3. If charismate is high in cell then it favour the transcription of structural gene
4. If charismate is low or absent in cell then it inhibit transcription

**Genetic Recombination in Microbes**

Genetic recombination is the production of offspring with combinations of traits that differ from those found in either parent.

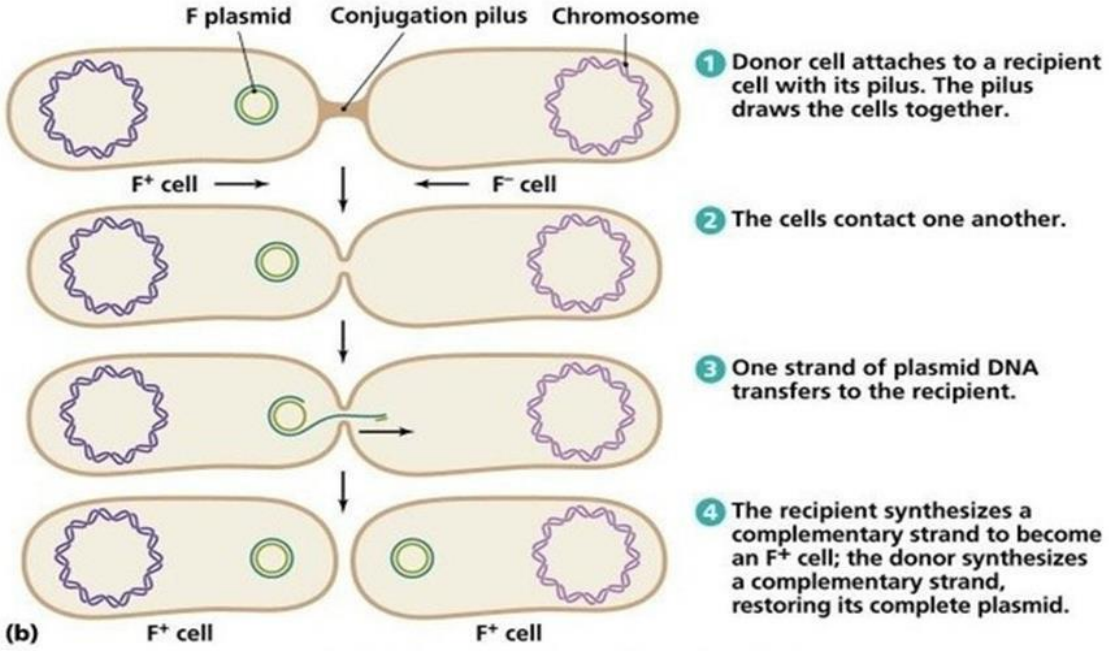
Genetic recombination occurs in bacteria and archaea, which use asexual reproduction. Recombination can be artificially induced in laboratory (in vitro) settings, producing recombinant DNA for purposes including vaccine development. Recombination in organisms with an adaptive immune system is a type of site-specific genetic recombination that helps immune cells rapidly diversify to recognize and adapt to new pathogens.

**Bacterial Conjugation**

Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. It is a mechanism of horizontal gene transfer as are transformation and transduction although these two other mechanisms do not involve cell-to-cell contact. Bacterial conjugation is often regarded as the bacterial equivalent of sexual reproduction or mating since it

involves the exchange of genetic material. During conjugation the donor cell provides a conjugative or mobilizable genetic element that is most often a plasmid or transposon. Most conjugative plasmids have systems ensuring that the recipient cell does not already contain a similar element.

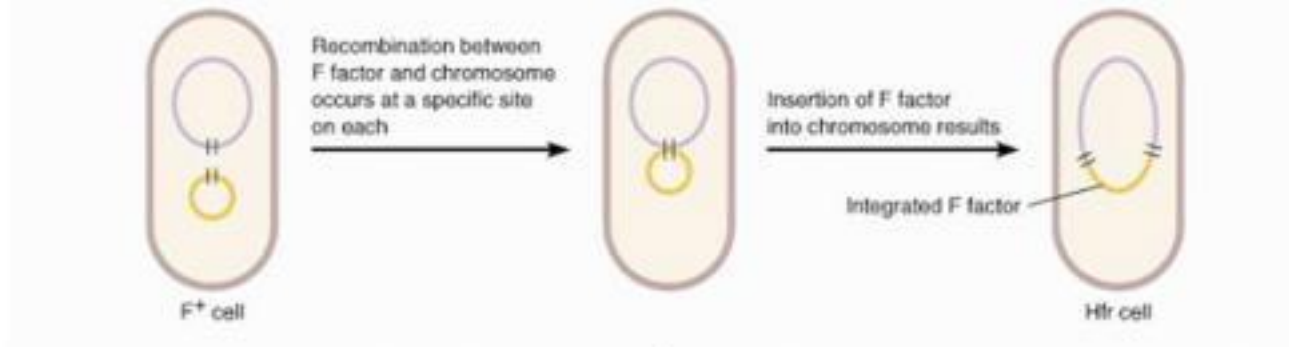
The genetic information transferred is often beneficial to the recipient. Benefits may include antibiotic resistance, xenobiotic tolerance or the ability to use new metabolites. Such beneficial plasmids may be considered bacterial endosymbionts. Other elements, however, may be viewed as bacterial parasites and conjugation as a mechanism evolved by them to allow for their spread. The process was discovered in 1946 by Joshua Lederberg and Edward Tatum.



Donor cell produces pilus.
Pilus attaches to recipient cell and brings the two cells together.
The mobile plasmid is nicked and a single strand of DNA is then transferred to the recipient cell.
Both cells synthesize a complementary strand to produce a double stranded circular plasmid and also reproduce pili; both cells are now viable donor for the F-factor.

The F-plasmid is an episome (a plasmid that can integrate itself into the bacterial chromosome by homologous recombination) with a length of about 100 kb. It carries its own origin of replication, the oriV, and an origin of transfer, or oriT. There can only be one copy of the F- plasmid in a given bacterium, either free or integrated, and bacteria that possess a copy are called F-positive or F-plus (denoted F+). Cells that lack F plasmids are called F-negative or F-minus (F−) and as such can function as recipient cells.

Among other genetic information, the F-plasmid carries a tra and trb locus, which together are about 33 kb long and consist of about 40 genes. The tra locus includes the pilin gene and regulatory genes, which together form pili on the cell surface. The locus also includes the genes for the proteins that attach themselves to the surface of F− bacteria and initiate conjugation.

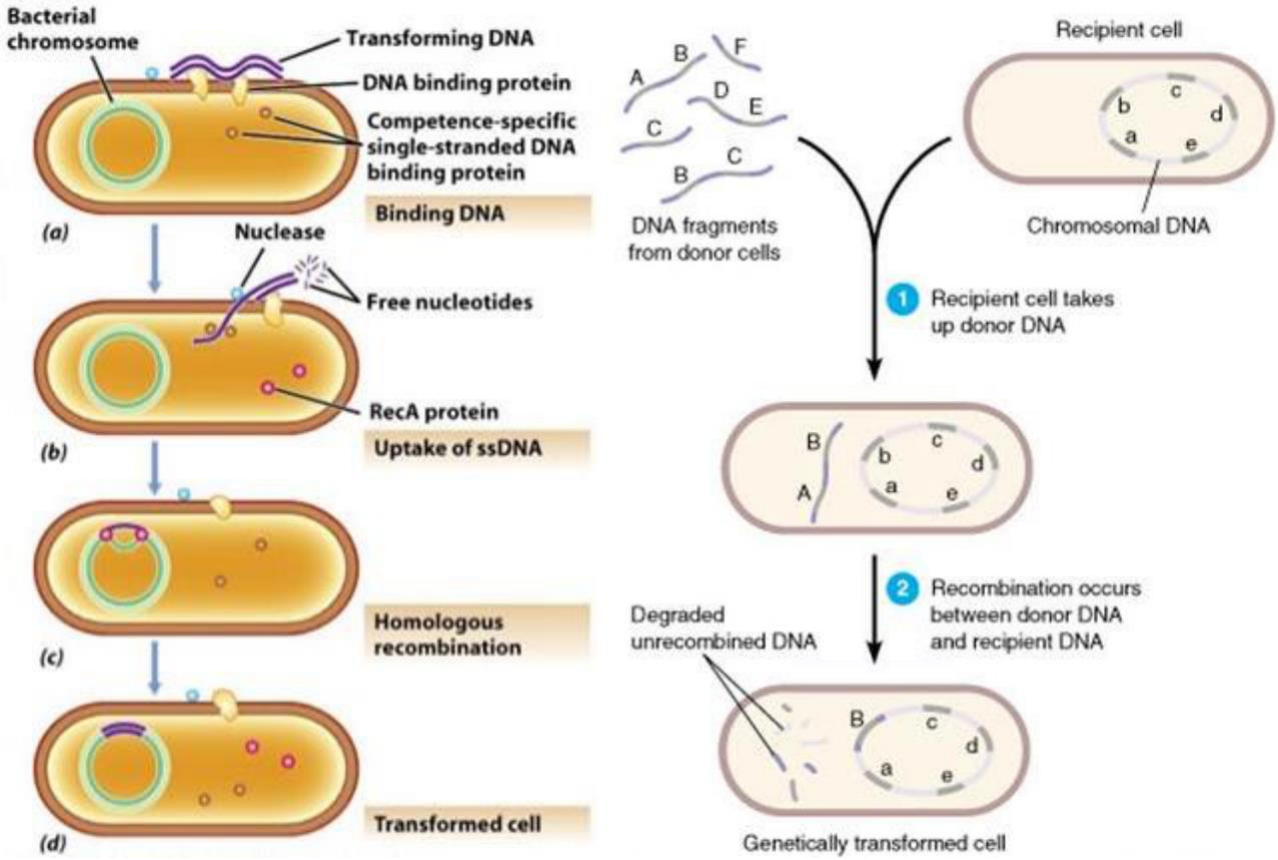


b) When an F factor becomes integrated into the chromosome of an F+ cell, it makes the cell a high frequency of recombination (Hfr) cell.

If the F-plasmid that is transferred has previously been integrated into the donor's genome (producing an Hfr strain ("High Frequency of Recombination") some of the donor's chromosomal DNA may also be transferred with the plasmid DNA. The amount of chromosomal DNA that is transferred depends on how long the two conjugating bacteria remain in contact. In common laboratory strains of *E. coli* the transfer of the entire bacterial chromosome takes about 100 minutes. The transferred DNA can then be integrated into the recipient genome via homologous recombination.

**Transformation**

Transformation in bacteria was first demonstrated in 1928 by British bacteriologist Frederick Griffith and hypothesized that some "transforming principle". In 1944 this "transforming principle" was identified as being genetic by Oswald Avery, Colin MacLeod, and Maclyn McCarty and called "transformation".




Transformation is one of three forms of horizontal gene transfer that occur in nature among bacteria, in which DNA encoding for a trait passes from one bacterium to another and is integrated into the recipient genome by homologous recombination; the other two are transduction, carried out by means of a bacteriophage, and conjugation, in which a gene is passed through direct contact between bacteria. In transformation, the genetic material passes through the intervening medium, and uptake is completely dependent on the recipient bacterium.

Competence refers to a temporary state of being able to take up exogenous DNA from the environment; it may be induced in a laboratory. It appears to be an ancient process inherited from a common prokaryotic ancestor that is a beneficial adaptation for promoting recombinational repair of DNA damage, especially

damage acquired under stressful conditions. Natural genetic transformation appears to be an adaptation for repair of DNA damage that also generates genetic diversity.

**Transduction**

Transduction is the process by which foreign DNA is introduced into a cell by a virus or viral vector. An example is the viral transfer of DNA from one bacterium to another. Transduction does not require physical contact between the cell donating the DNA and the cell receiving the DNA (which occurs in conjugation), and it is DNase resistant.

When viruses, including bacteriophages (viruses that infect bacteria), infect bacterial cells, their normal mode of reproduction is to harness the replicational, transcriptional, and translation machinery of the host bacterial cell to make numerous virions, or complete viral particles, including the viral DNA or RNA and the protein coat. Transduction was discovered by Norton Zinder and Joshua Lederberg at the University of Wisconsin–Madison in 1952 in *Salmonella*.

There are generally different types of recombination events that can lead to this incorporation of bacterial DNA into the viral DNA, leading to two modes of recombination.

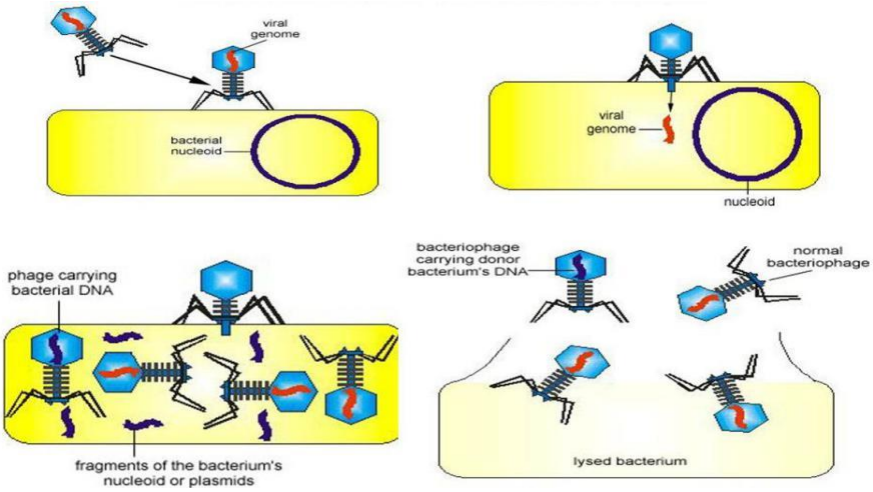
(i) Generalized transduction

Generalized transduction is the process by which any bacterial gene may be transferred to another bacterium *via* a bacteriophage, and very rarely a small number of phages carry the donor (bacterial genome) genome. In essence, this is the packaging of bacterial DNA into a viral envelope. This may occur in two main ways, recombination and headful packaging.

If bacteriophages undertake the lytic cycle of infection upon entering a bacterium, the virus will take control of the cell's machinery for use in replicating its own viral DNA. If by chance bacterial chromosomal DNA is inserted into the viral capsid which is usually used to encapsulate the viral DNA, the mistake will lead to generalized transduction.

If the virus replicates using 'headful packaging', it attempts to fill the nucleocapsid with genetic material. If the viral genome results in spare capacity, viral packaging mechanisms may incorporate bacterial genetic material into the new virion. The new virus capsule now loaded with part bacterial DNA continues to infect another bacterial cell. This bacterial material may become recombined into another bacterium upon infection.

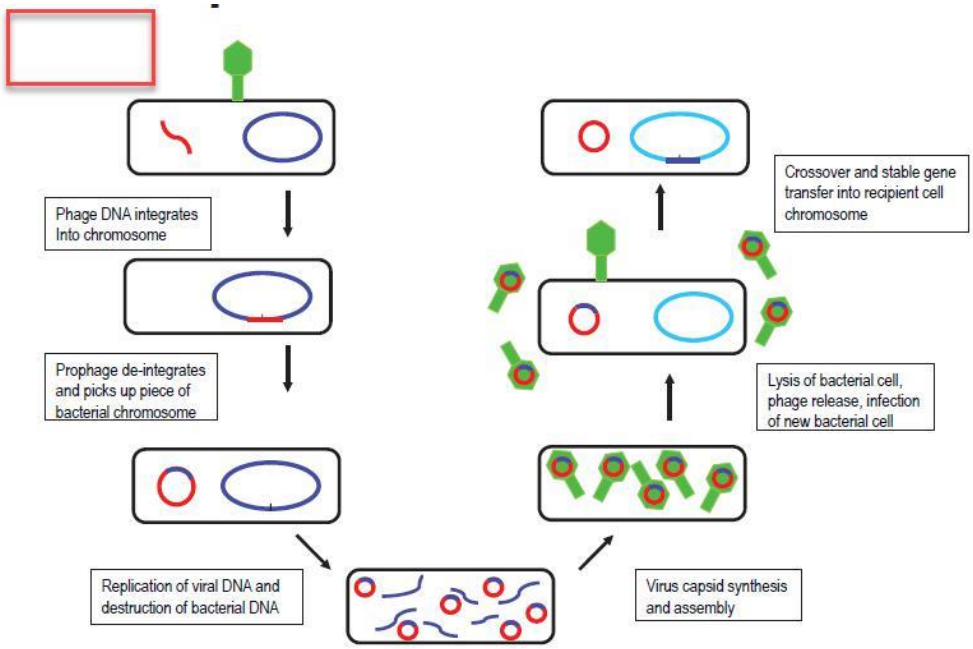
When the new DNA is inserted into this recipient cell it can fall to one of three fates. The DNA will be absorbed by the cell and be recycled for spare parts. If the DNA was originally a plasmid, it will re-circularize inside the new cell and become a plasmid again. If the new DNA matches with a homologous region of the recipient cell's chromosome, it will exchange DNA material similar to the actions in bacterial recombination.




(ii) Specialized transduction

Specialized transduction is the process by which a restricted set of bacterial genes is transferred to another bacterium. The genes that get transferred (donor genes) depend on where the phage genome is located on the chromosome.

Specialized transduction occurs when the prophage excises imprecisely from the chromosome so that bacterial genes lying adjacent to the prophage are included in the excised DNA. The excised DNA is then packaged into a new virus particle, which then delivers the DNA to a new bacterium, where the donor genes can be inserted into the recipient chromosome or remain in the cytoplasm, depending on the nature of the bacteriophage.When the partially encapsulated phage material infects another cell and becomes a "prophage" (is covalently bonded into the infected cell's chromosome), the partially coded prophage DNA is called a "heterogenote".An example of specialized transduction is λ phage in *Escherichia coli.*



**Gene and Chromosome walking:**

- **Gene walking** is basic method of **genetic** engineering that allows identification of unknown regions flanking (e.g. promoter regions) a known DNA sequence.
- Chromosome walking is a method of positional cloning used to find, isolate, and clone a particular allele in a gene library.
- Chromosome Walking was developed by Welcome Bender, Pierre Spierer, and David S. Hogness in the Early 1980's.
- There are nearly half a dozen positional cloning tests that are done prior to a chromosome walk.
- Each clone in the cosmic library has a DNA insert of 50 KB.
- The walking starts at the closest gene that has already been identified, known as a marker gene.
- Once the markers on either side of an unmapped sequence are found, the chromosome walk can begin from one of the markers.
- Each successive gene in the sequence is tested repeatedly, known as overlap restrictions and mapped for their precise location in the sequence.
- Eventually, walking through the genes reaches the mutant gene in an unmapped sequence that binds to a fragment of a gene of that particular disease.
- The testing on each successive clone is complex, time-consuming, and varied by species.
- This series of overlapping clones could for example consist of Bacterial Artificial Chromosomes

Application:

- This technique can be used for the analysis of genetically transmitted diseases, to look for mutations.
- Chromosome Walking is used in the discovery of single-nucleotide polymorphism of different organisms.

Disadvantages:

- There is a limitation to the speed of chromosome walking because of the small size of the fragments that are to be cloned.
- Another limitation is the difficulty of walking through the repeated sequence that are scattered through the gene.
- If the markers were too far away, it simply was not a viable option.
- Additionally, chromosome walking could easily be stopped by unclonable sections of DNA.
- A solution to this problem was achieved with the advent of chromosome jumping (Marx, 1989), which allows the skipping of unclonable sections of DNA.

**Possible Questions**

**Part-A (1 mark)**

**Part-B (2 marks)**

1. Define genetic code.
2. Mention the start and stop codons.
3. Mention any four properties of genetic codons.
4. What are the three enzymes of lac operon?
5. What are regulating elements?
6. What are structural genes?
7. What is conjugation?
8. What is transduction?
9. What is transformation?
10. What is chromosome walking?

**Part-C (8 marks)**

11. Give a detailed account on Genetic Code.
12. Draw the Genetic code table with corresponding amino acids.
13. Discuss the salient features of lac operon.
14. Discuss in detail the tryptophan operon regulation in *E. coli*.
15. Explain the process of chromosome walking.
16. Discuss in detail about conjugation and transformation.
17. How is transduction classified? Explain.

| Sl. No | Question | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | Addition of poly A tail to 3' end of mRNA is mediated by the enzyme | RNA polymerase | DNA polymerase | Rnase | poly A polymerase | poly A polymerase |
| 2 | Confirmational changes in protein is brought about by | Systematic molecules | Cohesive molecules | Affector molecules | Effector molecules | Effector molecules |
| 3 | In Rho-independent transcription termination, the termination sequence is usually | Palindromic sequence | Paliomic sequence | Panoramic sequence | Pandemic sequence | Palindromic sequence |
| 4 | Model example for gene regulation by repression | *trp* operon | *lac* operon | *ara* operon | *gal* operon | *trp* operon |
| 5 | Operon model that demonstrates both positive and negative control of gene regulation | *lac* operon | *ara* operon | *gal* operon | *trp* operon | *ara* operon |
| 6 | Repressor molecule in lac operon is a | Dimer | Trimer | Tetramer | Pentamer | Tetramer |
| 7 | Stop codon UAA is also called | Amber | Opal | Acre | Ochre | Ochre |
| 8 | The first codon during translation is | AGU | AUG | GUA | UGA | AUG |
| 9 | Transcription initiation site starts from | -1 | Plus 1 | -10 | Plus 10 | Plus 1 |
| 10 | What are the possible number of codons that can be generated using possible nucleotide combinations | 46 | 64 | 20 | 30 | 64 |
| 11 | Which transports lactose across the cell membrane | Galactosidase permease | β-galactosidase | Glucanse | Glucose permease | Galactosidase permease |
| 12 | Who deciphered the genetic code | Hershey & Chase | Avery & McLeod | Beedle & Tatum | Nirenberg & Khorana | Nirenberg & Khorana |
| 13 | The stop codons are called as | Missense | Nonsense codons | Central codons | Last codons | Nonsense codons |
| 14 | The ability of the cell to choose between glucose and other sugars is termed as | Catabolic repression | Catabolic expression | Metabolic repression | Metabolic expression | Catabolic repression |
| 15 | Sequence of codons in mRNA between a start and a stop sequence is called as | Close reading frame | Open reading frame | Central reading frame | Last reading frame | Open reading frame |

| | | | | | | |
|---|---|---|---|---|---|---|
| 16 | Polyadenylation is | Addition of adenosines to 3' end of mRNA | Addition of adenosines to 5' end of mRNA | Deletion of adenosines to 3' end of mRNA | Deletion of adenosines to 5' end of mRNA | Addition of adenosines to 3' end of mRNA |
| 17 | Non codon specifies more than ___ amino acid | 1 | 2 | 3 | 4 | 1 |
| 18 | Molecular weight of egg lysozyme is | 19300 daltons | 13900 daltons | 31900 daltons | 91300 daltons | 13900 daltons |
| 19 | *lac* operon is an example for | Repressible operon | Inducible operon | Mutated operon | Neutral operon | Inducible operon |
| 20 | In RNA, thiamine is replaced by | Uracil | Adenine | Cytosine | Guanine | Uracil |
| 21 | Genes are located in specialized structures called | Histone | RNA | Chrosomes | Genomes | Chrosomes |
| 22 | Enzyme activity is regulated by changes in the confirmation of enzymes except | Polymerase | Ribozymes | Chimozymes | Nuclease | Ribozymes |
| 23 | Codon/Anticodon consists of _____ nucleotides | 4 | 6 | 3 | 9 | 3 |
| 24 | Amino acid that have largest number of codons | Proline | Cysteine | Serine | Valine | Serine |
| 25 | 7-methylguanosine cap is an important site | For eukaryotic transcription initiation factor | For prokaryotic translation initiation factor | For eukaryotic translation initiation factor | For prokaryotic translation initiation factor | For eukaryotic translation initiation factor |
| 26 | Capping in mRNA is addition of the group | 7-ethylguanosine | 7-methylguanosine | 7-methylcytosine | 7-ethylcytosine | 7-methylguanosine |
| 27 | _____ is the first amino acid during translation of proteins | Threonine | Leucine | Methionine | Valine | Methionine |
| 28 | Control of gene expression was proposed by | Beedle & Tatum | Avery & McLeod | Jacob & Monad | Hershey & Chase | Jacob & Monad |
| 29 | In prokaryotes, AUG is translated in to | Methionine | N-acetyl-methionine | N-formamyl-aspargine | N-formamyl-methionine | N-formamyl-methionine |
| 30 | In trp operon, the genes *trp*E & *trp*D codes for | Arginase | Tryptophan synthase | Anthranilate isomerase | Anthranilate synthase | Anthranilate synthase |
| 31 | Enzyme that lactose in to glucose and galactose | Lactosidase | Glucanse | α-galactosidase | β-galactosidase | β-galactosidase |
| 32 | The number of nitrogenous bases codes by 9 | 27 | 36 | 18 | 9 | 27 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | amino acids would be | | | | | |
| 33 | The termination of transcription is signaled by rich | AT containing inverted repeat | AC containing inverted repeat | GC containing inverted repeat | CT containing inverted repeat | GC containing inverted repeat |
| 34 | tRNA's are matched with their aminoacids by a group of enzymes collectively called as | Aminoacyl DNA synthatases | Aminoacyl synthatases | Amino synthatases | Aminoacyl tRNA synthatases | aminoacyl tRNA synthatases |
| 35 | Translation is | rRNA to protein | tRNA to protein | DNA to protein | mRNA to protein | mRNA to protein |
| 36 | Stop codons in mammalian mitochodria are | GAA & GAG | AGA & AGG | CGA & AGC | CGG & GCG | AGA & AGG |
| 37 | Ribosomal site most frequently occupied by the tRNA carrying the growing peptide chain | A-site | P-site | E-site | G-site | P-site |
| 38 | Non-coding regions are called as | Exons | Introns | Cistrons | Positrons | Exons |
| 39 | Mammalian mitochondrion not only uses AUG as initiation codon but also | AUA, AUU, AUC | UAA, UAU, UAC | AAU, UAU, CAU | GUA, GUU, GUC | AUA, AUU, AUC |
| 40 | Region that comprise the core prokaryotic promoter | Klenow box | Pribnow box | TAGTAG box | Polypeptide box | Pribnow box |
| 41 | All aminoacids have more than one codon except | Methionine & Tryptophan | Valine & Leucine | Threonine & Alanine | Lysine & Arginine | Methionine & Tryptophan |
| 42 | Site to which substrate molecules are attached | Catalytic site | Effector site | Alleosteric site | Binding site | Catalytic site |
| 43 | The main function of nonsense codons is to | Initiate protein synthesis | Elongate protein synthesis | Terminate protein synthesis | Regulate protein synthesis | Terminate protein synthesis |
| 44 | Which is astop codon | UAA | AAU | AUA | AAA | UAA |
| 45 | The promoter sequence in eukaryotes is | TATAAA | TAATAA | TTGACA | GTTAAA | TATAAA |
| 46 | Structure of proteins may be classified into ___ types | 2 | 3 | 4 | 5 | 4 |
| 47 | rRNA is also called | Rnase | Ribase | Ribulase | Ribozyme | Ribozyme |
| 48 | 7-methylguanosine cap is an important site | For eukaryotic transcription initiation factor | For prokaryotic translation initiation factor | For eukaryotic translation initiation factor | For prokaryotic translation initiation factor | For eukaryotic translation initiation factor |

| | | | | | | |
|---|---|---|---|---|---|---|
| 49 | 2006 Nobel Prize in Physiology & Medicine for studies on molecular basis of eukaryotic transcription | Arthur Nirenberg | Roger D. Kornberg | David Osborne | Michael Whitney | Roger D. Kornberg |
| 50 | Transcription is | DNA to rRNA | DNA to tRNA | DNA to mRNA | DNA to protein | DNA to mRNA |
| 51 | tRNA is responsible for the transfering | Protein | Aminoacid | Codon | Anticodon | Anticodon |
| 52 | Monad & Cohen-Bazire first reported the evidence for the repression of the enzyme | Tryptophan synthase | Gluconase synthetase | Arabinase trimutase | Tryptophanase | Tryptophan synthase |
| 53 | A-site is the ribosomal site most frequently occupied by the | Aminoacyl-rRNA | Aminoacyl-mRNA | Iminoacyl-tRNA | Aminoacyl-tRNA | Aminoacyl-tRNA |
| 54 | Action of repressor protein in *Lac* operon is called as | Positive control | Negative control | Neutral control | No control | Negative control |
| 55 | Other than methionine _____ is the amino acid that appear rarely in proteins | Arginine | Tryptophan | Glutamic acid | Threonine | Tryptophan |
| 56 | In post translational modification of RNAs, trimming is | Removal of excess nucleotides | Removal of excess proteins | Removal of excess lipids | Removal of excess carbohydrates | Removal of excess nucleotides |
| 57 | Common method of covalent modification of enzyme in regulation of gene expression is | to methylate the enzyme at a proline residue | to phosphorylate the enzyme at a proline residue | to phosphorylate the enzyme at a serine residue | to methylate the enzyme at a serine residue | to phosphorylate the enzyme at a serine residue |
| 58 | Short sequence of aminoacids are called | Peptides | Proteins | Polypeptides | Palindromes | Peptides |
| 59 | In the absence of effector molecule, the enzyme is said to be in | Relaxed state | Tense state | Free state | Degrading state | Tense state |
| 60 | Allosteric enzymes that are controlled by a molecule other than it's substrate | Cohesive molecules | Systematic molecules | Effector molecules | Affector molecules | Effector molecules |
| 61 | The first and best example of control of gene expression was proposed by | Khorana & Nirenberg | Hershey & Chase | Avery & McLeod | Jacob and Monad | Jacob and Monad |

## Unit IV

## Syllabus

Mutations and mutagenesis, types of mutations and mutagens. Identification of mutants- Ames test

### Mutations and mutagenesis

- A mutation is the permanent alteration of the nucleotide sequence of the genome of an organism, virus, or extrachromosomal DNA or other genetic elements.
- Mutagenesis is a process by which the genetic information of an organism is changed, resulting in a mutation.
- In a complex organism, mutation can occur at many different structural levels and can be classified in many different ways:
  - Magnitude of genetic change: point, gene, chromosomal, genomic mutations.
  - Pattern of inheritance: somatic vs. germ-line; autosomal, sex-linked, dominant, codominant, partially dominant, recessive.
  - Phenotypic properties: morphological (shape, size, quantity, coloration), nutritional (auxotrophic), biochemical, lethal, behavioral, silent.
  - Changes in DNA: missense (transitions, transversions), nonsense (amber, ochre, opal), deletion, insertion, frameshift, inversion, duplication, translocation.
  - Conditional: temperature-sensitive, suppresible.
  - Regulatory: increased or decreased expression, altered message processing, stability, or rate of translation.

### Types of mutations:

### Spontaneous mutation

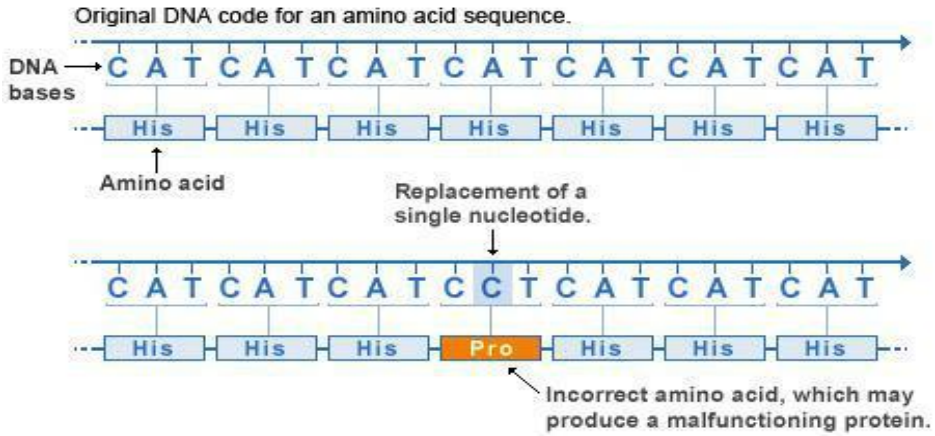
- A mutation which occurred without any known cause is called as a spontaneous mutation.
- It arises due to metabolic errors, replication errors or during development errors.
- Spontaneous mutations are rare and occurred without any reason.
- Larger genes are more prone to spontaneous mutation because as the gene is larger, the chance of error in replication is higher.
- The rate of spontaneous mutation is $10^{-5}$ per gene per generation during replication.

### Induced mutation

- Induced mutation is resulting from exposure of an organism to mutagenic agents.
- The general mutagenic agents are radiation, UV light and chemicals.

**Gene mutations:** are defined as those that occur entirely within one gene (and its upstream regulatory sequences) and may be either point mutations or other small disruptions of normal chromosomal structure that occur entirely within one gene.

**Point mutations:** It occurs as a result of replacement of one nucleotide by other in specific nucleotide sequence of gene. Point mutation brings little phenotypic change.

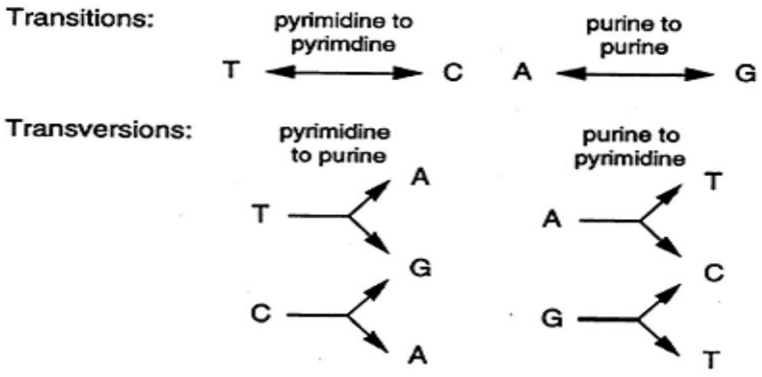



Example of point and missense mutation

**Missense mutations:** This type of mutation is a change in one DNA base pair that results in the substitution of one amino acid for another in the protein made by a gene.

**Transitions:** ***Transitions*** are interchanges of purines (A-G) or pyrimdines (C-T), which involve bases of similar shape.

**Transversion:** Transversions are interchanges between purine and pyrmidine bases, which involve exchange of one-ring and two-ring structures

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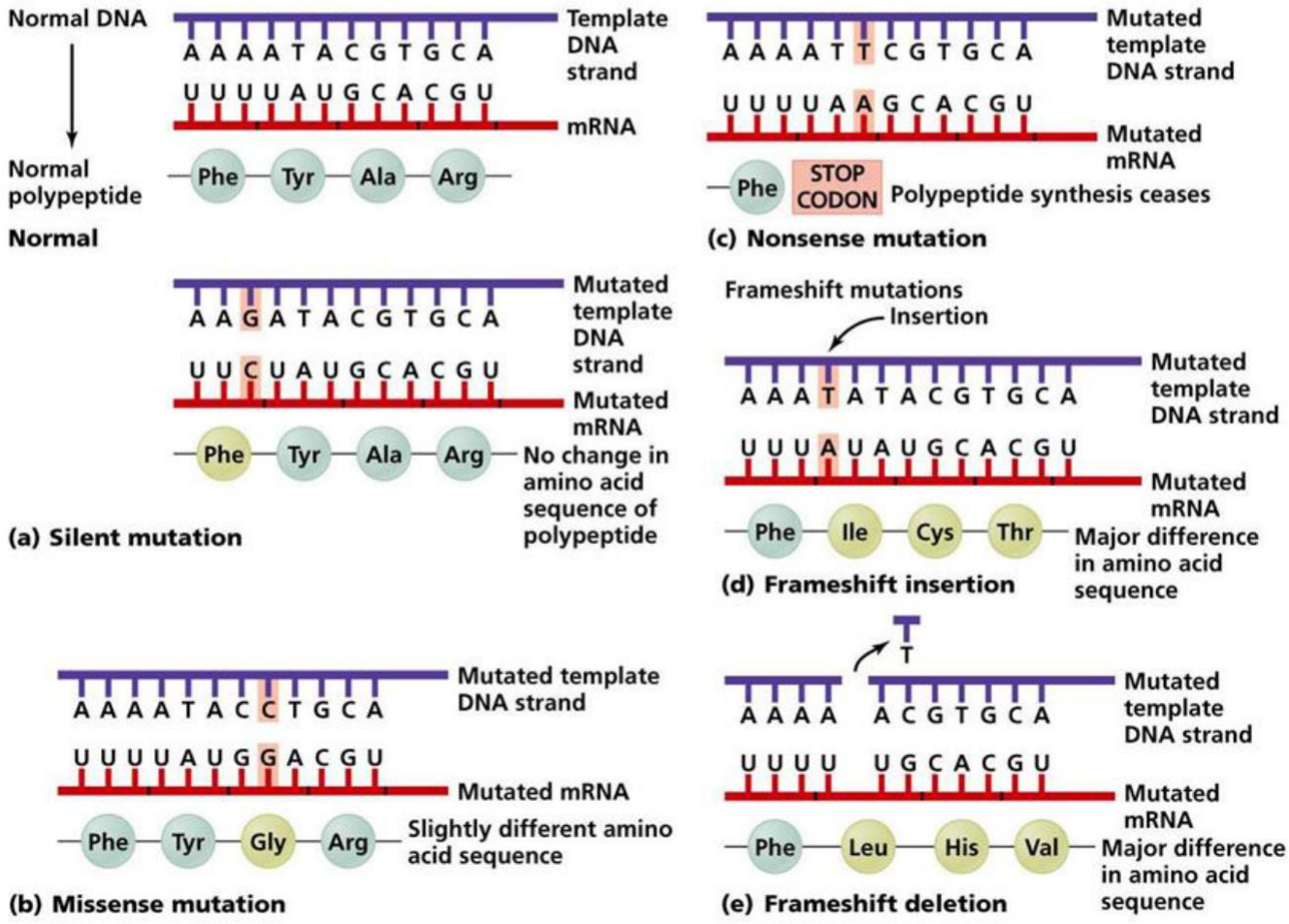
**Nonsense mutations:** A stop codon is added to the premature protein. It stops protein synthesis because a stop codon ends synthesis of protein and results in a premature protein.

**Silent mutations:** It is non-expressive. In silent mutation, the new codon is created from the mutation but it codes for the same amino acid as wild-type.

| | |
|---|---|
| Wild Type DNA | TAC GGG AAA GTC CGT GGC |
| Wild Type mRNA | AUG CCC UUU CAG GCA CCG |
| Amino acids | Met -Pro- Phe- Gln- Ala- Pro |
| Mutated DNA | TAC GGG AAG GTC CGT GGC |
| Mutated mRNA | AUG CCC UUC CAG GCA CCG |
| Amino acids | Met -Pro- Phe- Gln- Ala- Pro |



**Frameshift mutations:** One or 2 nucleotide pairs are inserted into or deleted from the molecule, causing an alteration of the *reading frame.* As the result of this shift, codons downstream of the insertion or deletion site specify an entirely new sequence of amino acids. Depending on where the insertion or deletion occurs in the gene, different effects can be generated. In addition to producing an entirely new polypeptide sequence. immediately after the change, frameshift mutations usually produce a stop or termination codon within a short distance of the mutation. This codon terminates the already altered polypepetide chain. A frame shift in a gene specifying an enzyme usually result in a loss of enzyme activity. If the enzyme is an essential one, the effect on the organism can be disastrous.

**Chromosomal mutation**

At the molecular level, single nucleotide to several hundred nucleotides is altered but at the chromosomal level large segment of the chromosome with one gene to many, are deleted or added.

**Addition:** when some of the extra segment is added to a chromosome, is called as an addition.
**Deletion:** when some segment of an entire chromosome is deleted and loses several genes, it is called as a deletion.

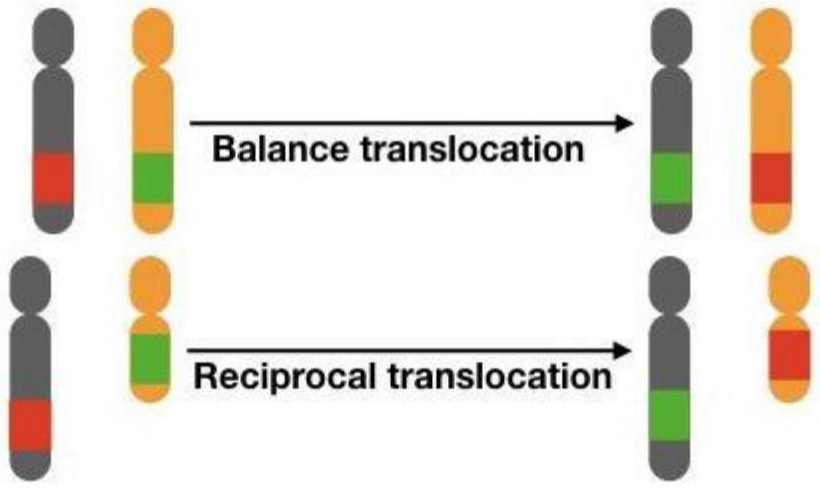
**Duplication:** one segment of a chromosome is duplicated in a manner which alters the structural hierarchy of the entire chromosome and creates chromosomal abnormality is called as a chromosomal duplication.
**Substitution:** when one or more bases in the sequence are replaced by the same number of bases.

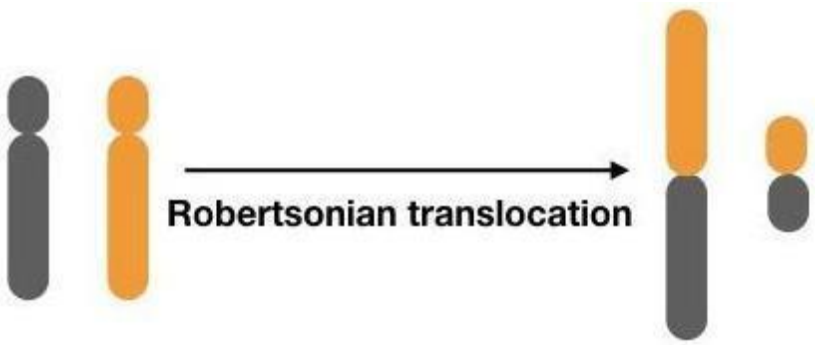
**Translocation:**

Balanced translocation: when two segments (nearly same) are exchanged, resulting in balanced translocation. Here the two segments are approximately the same in size hence it is not possible to detect such type of translocation by cytogenetic analysis.

Reciprocal translocation: translocation occurred between two non-homologous chromosomes, is called as a reciprocal translocation. This type of translocation is most common in nature.

Robertsonian translocation: Robertsonian translocation occurs between two acrocentric chromosomes. Acrocentric chromosomes are small short chromosome with one long q-arm and a short or very smaller p-arm.




Robertsonian translocation

**Inversion**

- A segment of a chromosome is inverted and rearranged back to the parental chromosome, is called as an inversion.
- Here if the inversion occurs in only one arm of the chromosome, this type of inversion is called as a paracentric inversion
- If the inversion occurs between two arms of a chromosome, this type of inversion is called as a pericentric inversion.

**Ring chromosome** when both ends of a chromosome are fused together, it creates a ring-like structure and so-called as ring chromosome. A ring chromosome is generally observed in the long metacentric chromosomes.

**Morphological mutation:** The Genetic mutation which affects the outer characteristic or physical characteristic of an organism is called as a morphological mutation. This type of mutation alters the physical properties like shape, size and colour of an organism.

**Lethal mutation:** A Genetic mutation which causes the death of an organism or affects the survival of an organism is called as a lethal mutation.

**Conditional mutation:** In this type of Genetic mutations, the mutant allele causes mutant phenotype in a certain specific environment while remaining wild type in some other environment.

**Genomic mutations** Genomic mutations are defined as those that involve loss or gain of whole chromosomes, translocation from one chromosome to another or other gross chromosomal rearrangements. Note that both chromosomal and genomic mutations can cause aneuploidy.

**Somatic and germ-line mutations:** The mutations that we normally deal with in genetics are those that occur in the germ-line and are thus passed on to subsequent generations. However, mutations can also occur in somatic cells. Those mutations affect only the immediate progeny of the cells they occur in and are not inherited.

**Nutritional and biochemical mutations:** For microorganisms that can be grown on defined (or semi-defined) culture media, it is possible to select for ***auxotrophic*** mutations that require nutrients that the wild type organisms can make for themselves. Wild type organisms that are able to multiply in a medium lacking such a nutrient are called prototrophs. There are also many ***biochemical*** mutations that affect proteins other than those involved in synthesis of nutrients.

**Types of mutagens**

A mutagen is a physical or chemical agent that changes the genetic material, usually DNA, of an organism and thus increases the frequency of mutations above the natural background level.

There are different types of mutagens: Chemical mutagens, physical mutagens and biological mutagens

(a) Chemical Mutagens: Chemical mutagen are

1. Nitrosoguanidine (NTG)
2. Base **analogue**s: These chemical are morphologically similar to that of normal nitrogen bases. So during replication these molecules are incorporated instead of normal nitrogen bases and hence causes mutation Example; 2-aminopurine is analogue to Adenine, 5-bromourcail is analogue to thymine
3. Simple chemicals: These chemical mutagens react directly with the nitrogenous bases of DNA and chemically modify the DNA causing mutation. Example; Nitrous acid react with nitrogenous bases and remove amino group from purine and pyriminine
4. Alkylating agents (e.g. N-ethyl-N-nitrosourea (ENU): These agents can mutate both replicating and non-replicating DNA. In contrast, a base analog can only mutate the DNA when the analog is incorporated in replicating the DNA. Each of these classes of chemical mutagens has certain effects that then lead to transitions, transversions, or deletions.
5. Methylating agents (e.g. ethane methyl sulfonate (EMS))
6. Polycyclic hydrocarbons (e.g. benzpyrenes found in internal combustion engine exhaust)
7. DNA intercalating agents: The chemical intercalate or slip in between two base pair in Double stranded DNA helix and hence alter the morphology of DNA at that position. Chances of error during replication are higher at this position causing mutation. Examples; Acridine orange, ethidium bromide, proflavin
8. DNA crosslinker (e.g. platinum)
9. Oxidative damage caused by oxygen radicals

(b) Physical mutagens arc non-ionizing and ionizing radiations:

I. Ultraviolet radiation (nonionizing radiation): These radiations excite electrons to a higher energy level. DNA absorbs ultraviolet light. Two nucleotide bases in DNA – cytosine and thymine-are most vulnerable to excitation that can change base-pairing properties. UV light can induce adjacent thymine bases in a DNA strand to pair with each other, as a bulky dimer.

2. Ionizing radiation: These radiations are X- rays. gamma rays etc. DNA has so-called hotspots. where mutations occur up to 100 times more than the normal mutation rate. A hotspot can be at an unusual base, e.g.. 5-methylcytosine. Ionizing radiations attacks on these hot spot and break the DNA.

(c) Biological mutagens:

- Transposons and insertion sequence (IS) elements are biological mutagens.

Examples; mutator gene, bacteriophage MU etc.

Transposons and IS elements are small sequence of DNA that moves from one site to another along DNA strand and causes mutation. Transposons and insertion sequences are also known as jumping gene.These sequence contains gene which codes the enzyme transposase which helps in transposition of these sequence from one site to other.

- IS element are simplest type of transposable element. They are short DNA about 1000 nucleotide long and typically contains inverted repeats of 10-50 base pair. The only gene they poses is the gene for enzyme transposase. IS element are found in both chromosome and plasmid of both bacteria and Archea as well as some bacteriophage.
- Transposons are larger than IS element but similar as IS element because it also has two essential component; contains inverted repeats and encodes transposases.

**Identification of mutants**

Many different systems for detecting mutagen have been developed

(a) Bacterial:

**Ames test**

- Ames test it is a biological assay to assess the mutagenic potential of chemical compounds. It utilizes bacteria to test whether a given chemical can cause mutations in the DNA of the test organism.
- The test was developed by Bruce N. Ames in 1970s to determine if a chemical at hand is a mutagen.
- This test is based on the principle of reverse mutation or back mutation**.** So, the test is also known as bacterial reverse mutation assay.
- Test organism: Ames test uses several strains of bacteria (*Salmonella, E.coli*) that carry mutation. Eg: A particular strain of *Salmonella Typhimurium* carries mutation in gene that encodes histidine. So it is an auxotrophic mutant which loss the ability to synthesize histidine (an amino acid) utilizing the ingredients of culture media. Those strains are known as His- and require histidine in growth media.
- Culturing His- salmonella is in a media containing certain chemicals, causes mutation in histidine encoding gene, such that they regain the ability to synthesize histidine (His+) This is the reverse mutation. Such chemicals responsible to revert the mutation are actually a mutagen. So, this Ames test is used to test mutagenic ability of varieties of chemicals.

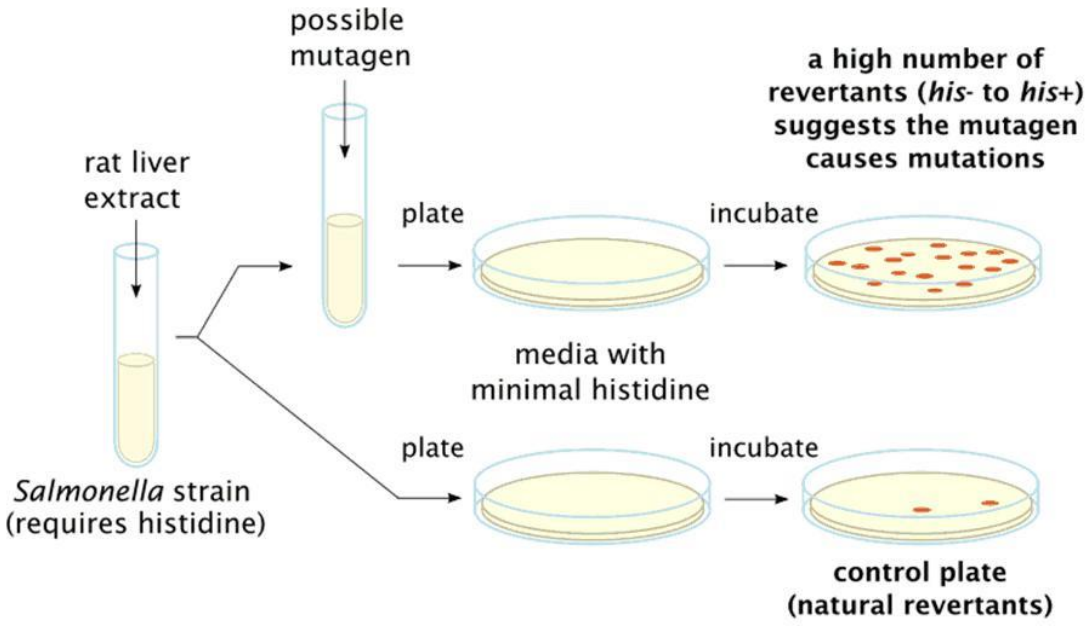
Basic steps involved:

- ✓ Isolate an auxotrophic strain of *Salmonella Typhimurium* for histidine. (ie. His-ve)
- ✓ Prepare a test suspension of his-ve *Salmonella Typhimurium* in a plain buffer with test chemical (let's say 2-aminofluorene). Also add small amount of histidine.

  Ps: small amount of histidine is required for initial growth of bacteria. Once histidine is depleted only those bacteria mutated to gain the ability to synthesize histidine form colony.
- ✓ Also prepare a control suspension of His-ve *Salmonella Typhimurium* but without test chemicals.

- ✓ Incubate the suspensions at 37 °C for 20 min
- ✓ Prepare the two agar plate and spread the suspension on agar plate.
- ✓ Incubate the plates at 37°C for 48 hours.
- ✓ After48 hours count the number of colonies in each plate. The mutagenicity of chemicals is proportional to number of colonies observed. If large number of colonies on test plate is observed in comparison to control, then such chemical are said to be mutagens.

*Very few number of colonies can be seen on control plate also. This may be due to spontaneous point mutation on hisidine encoding gene.




Applications:

1. The practical application of Ames test is to screen chemical mutagens that causes mutation and are carcinogenic to human and animals. Some of the chemicals used as food additive (AF-2), flavoring agent (Safrole) are mutagenic as well carcinogenic.
2. Isoniazid; an anti TB drug is also mutagens.
3. Ames test adopted to use eukaryotic cell culture, yeast cell, as well as animal model to test mutagens. Since, Salmonella is not a best test organism to test mutagens for Human. Certain chemicals initially are not mutagens to human but convert into mutagens when metabolized (acted upon by body enzymes). For example; sodium nitrate (NaNO3) is not mutagens until it is acted upon by HCl in stomach to form Nitrous oxide HNO2 (a potent mutagen).

4. Ames test can detect Suitable mutants in large population of bacteria with high sensitivity.

5. It is test for mutagenicity not carcinogenicity. However, most of the mutagens (more than 90 %) detected by Ames test are responsible to cause cancer.
6. It is a bacterial reverse mutation assay. So the defective gene of bacteria can be mutated into functional gene.

(b) Yeast:

Systems similar to Ames test have been developed in yeast. *Saccharomyces cerevisiae* is generally used. These systems can check for forward and reverse mutations, as well as recombinant events

**Site-directed mutagenesis**

Site-directed mutagenesis (SDM) is a method to create specific, targeted changes in double stranded plasmid DNA. There are many reasons to make specific DNA alterations (insertions, deletions and substitutions), including:

To study changes in protein activity that occur as a result of the DNA manipulation.

- To select or screen for mutations (at the DNA, RNA or protein level) that have a desired property
- To introduce or remove restriction endonuclease sites or tags

- The basic procedure requires the synthesis of a short DNA primer. This synthetic primer contains the desired mutation and is complementary to the template DNA around the mutation site so it can hybridize with the DNA in the gene of interest.
- The mutation may be a single base change (a point mutation), multiple base changes, deletion, or insertion.
- The single-strand primer is then extended using a DNA polymerase, which copies the rest of the gene.
- The gene thus copied contains the mutated site, and is then introduced into a host cell as a vector and cloned.
- Finally, mutants are selected by DNA sequencing to check that they contain the desired mutation.

Applications:

- Used to generate mutations that may produce rationally designed protein that has improved or special properties
- Proteins may be engineered to produce proteins that are tailored for a specific application.

Site-directed mutagenesis has been widely used in the study of protein functions.

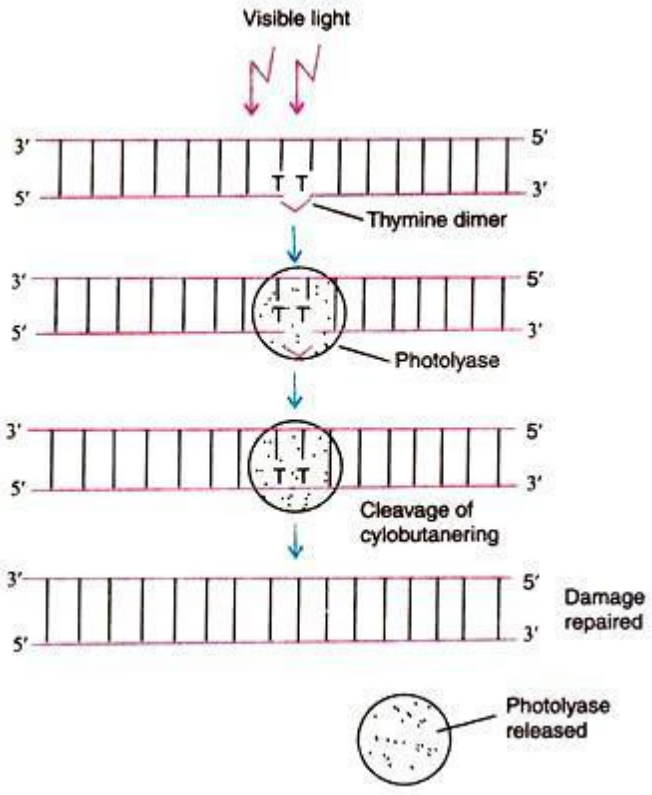
**DNA repair**

- DNA repair is basically a cellular response that rectifies DNA damage by restoring the normal base pairs and the original structure
- The different pathways of DNA repair are

(a) Photo reactivation
(b) Excision repair system: (i) Base excision repair and (ii) Nucleotide excision repair
(c) SOS repair

Photo reactivation

- Ultraviolet light is a physical mutagen and can induce mutation. Ultra violet radiation (254 nm) causes formation of pyrimidine dimers (cyclobutane ring), when two pyrimidine bases occurs together in single strand of DNA.
- Thymine dimer is most common one but cytosine dimer as well as thymine-cytosine may also occurs. Thymine dimer is a state in which two adjacent thymine molecules are chemically joined distorting the structure of DNA, so that impeding transcription and replication process.
- This pyrimidine dimer formation is lethal to the cell unless it is corrected. A repair mechanism known as photo reactivation can repair this mutation.
- When UV radiated population of bacteria is subsequently exposed to visible light of wave length of 300-450nm, the survival rate increases and frequency of mutation decreases. This is due to activation of photo reactivating enzyme photolyase, which splits thymine dimer.
- In the dark, the enzyme bind with thymine dimer and in presence of visible light the enzyme split the thymine dimers.
- Upto 80 % of thymine dimers existing in genome can be photoreactivated.



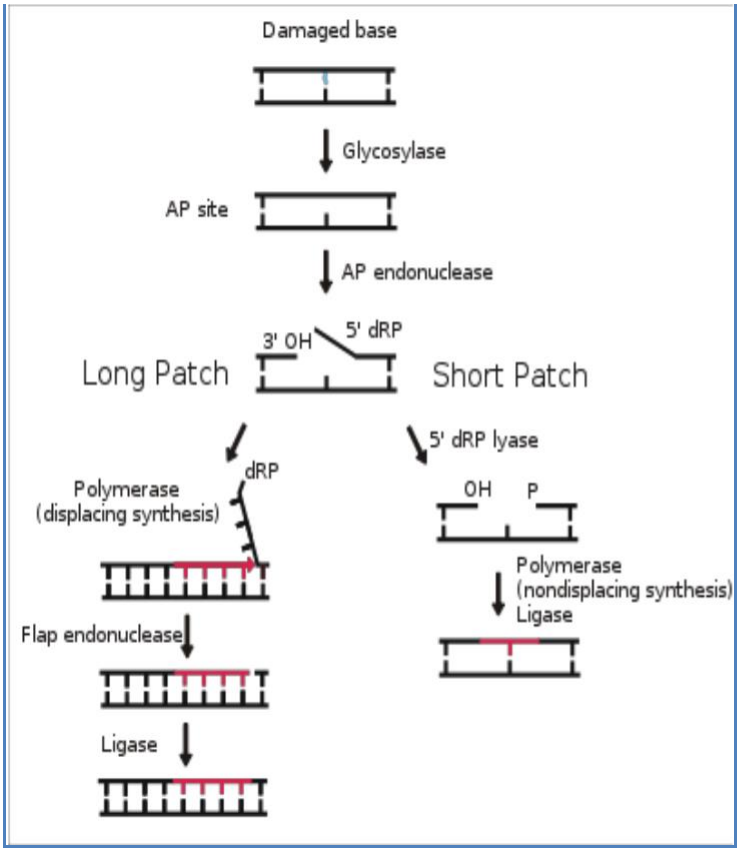
**Excision repair system**

Common Features of Excision-Type Repair Pathways

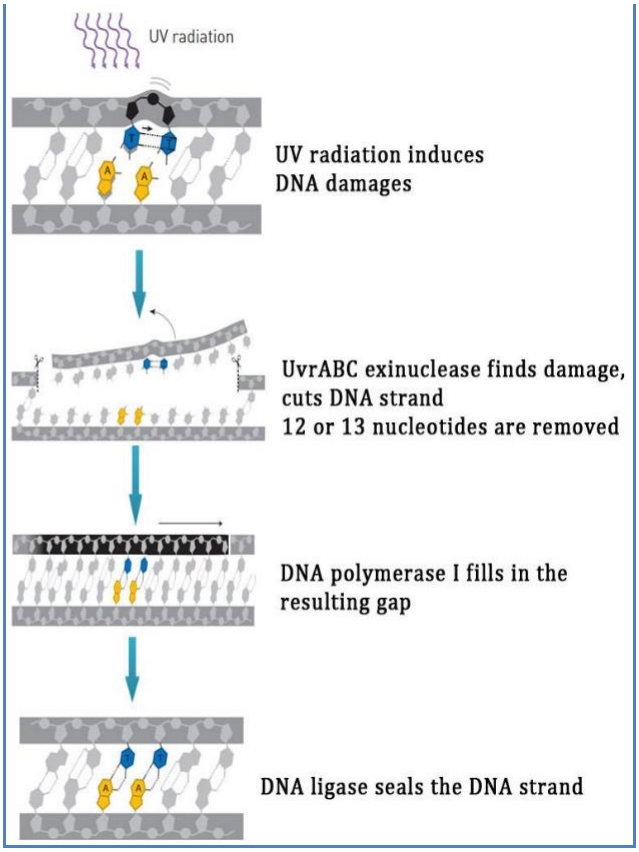
- Recognition: Altered DNA is recognized and bound by a specific damage-recognition protein. This first step recruits other components required for the repair reaction.
- Excision: Damaged base(s), and in some cases adjacent nucleotides, are excised from the strand by exonucleases, resulting in a gapped DNA.
- Resynthesis: The gap is refilled by a DNA polymerase using the complementary strand as a template.

**(i) Base excision repair (BER):**

- Base excision repair involves five basic steps, beginning with the identification and removal of the mutated base from the DNA helix by an enzyme known as DNA glycosylase.
- Next, an enzyme called AP (apurinic/apyrimidinic) endonuclease makes an incision at the abasic site, creating a break, or nick, in the strand of DNA.
- The site is then "cleaned," in which various intermediates produced from the strand break and other lingering chemicals are enzymatically removed in preparation for repair synthesis.
- In the final two steps, one or more nucleotides are synthesized to fill the gap, and the nick in the DNA strand is sealed. (A nucleotide is a base linked to a sugar and phosphate group, which forms the backbone of DNA.)




BER




NER

Nucleotide excision repair (NER):

- This mechanism is used to replace regions of damaged DNA up to 30 bases in length.
- Common causes of such DNA damage include ultraviolet (UV) light, smoking, Ionizing radiation, cancer chemotherapeutic agents, and a variety of chemicals found in the environment.
- The process of nucleotide excision repair is controlled in *Escherichia coli* by the UvrABC endonuclease enzyme complex, which consists of four Uvr proteins: UvrA, UvrB, UvrC, and DNA helicase II (sometimes also known as UvrD in this complex).
- First, a UvrA-UvrB complex scans the DNA, with the UvrA subunit recognizing distortions in the helix, caused for example by pyrimidine dimers.
- When the complex recognizes such a distortion, the UvrA subunit leaves and an UvrC protein comes in and binds to the UvrB monomer and, hence, forms a new UvrBC dimer.
- UvrB cleaves a phosphodiester bond 4 nucleotides downstream of the DNA damage, and the UvrC cleaves a phosphodiester bond 8 nucleotides upstream of the DNA damage and created 12 nucleotide excised segment.
- DNA helicase II (sometimes called UvrD) then comes in and removes the excised segment by actively breaking the hydrogen bonds between the complementary bases.
- The resultant gap is then filled in using DNA polymerase I and DNA ligase.
- The basic excision process is very similar in higher cells, but these cells usually involve many more proteins

- Mismatch repair (MMR)

o During DNA replication mistakes can occur as DNA polymerase copies the two strands. The wrong nucleotide can be incorporated into one of the strands causing a mismatch.

o The *E. coli* MMR system has been well characterized and reconstituted using recombinant proteins.

o In this system, a mismatched base is recognized by a MutS homodimer.

o A MutL homodimer interacts with the MutS-DNA complex, and then a MutH restriction endonuclease is activated by MutL.

o The MMR system needs to discriminate the newly-synthesized/error-containing strand; however, a mismatched base itself contains no such signal.

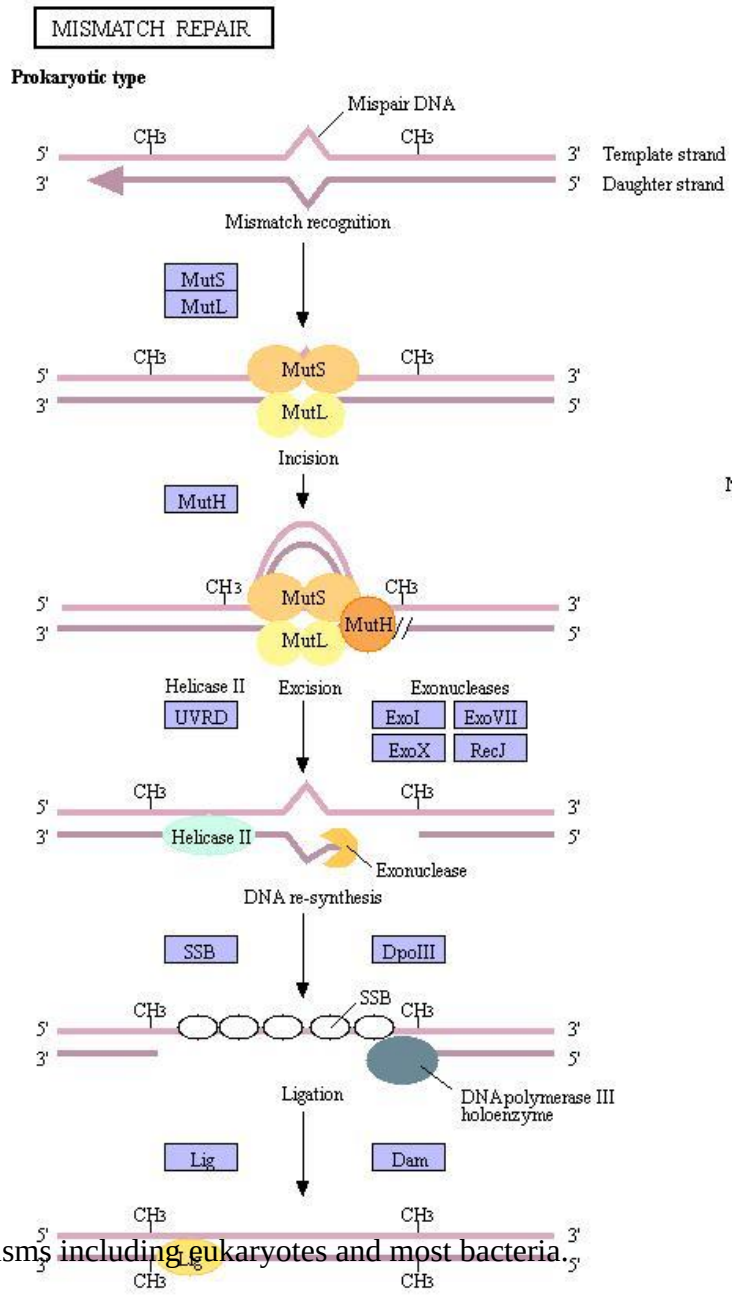
o The *E. coli* MMR system utilizes the absence of methylation at the restriction site to direct the repair to the newly synthesized strand.

o MutH nicks the unmethylated strand at the hemimethylated GATC site to introduce an entry point for the excision reaction.

o The error-containing strand is removed by helicases and exonucleases, and a new strand is synthesized by DNA polymerase III and ligase.

o The absence of methylation serves as a signal for the discrimination of the error-containing strand, and hence, *E. coli* MMR is called methyl-directed MMR.

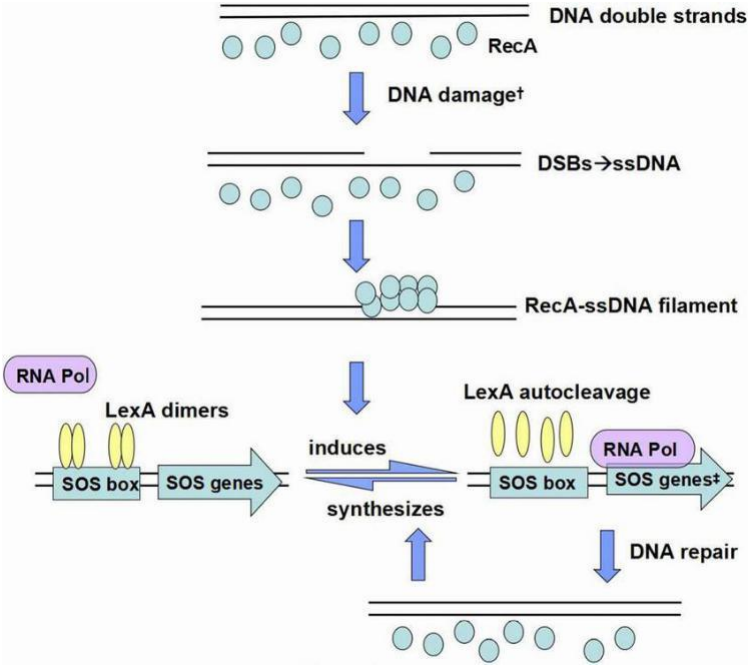
o Although homologues of *E. coli* MutS and MutL exist in almost all organisms, no homologue of *E. coli* MutH has been identified in the majority of organisms including eukaryotes and most bacteria.




**SOS repair**

- SOS response is induced when DNA is damaged or when replication of DNA stops and single stranded DNA accumulates.
- Agents such as UV radiations, methyl methane sulphonate, as well as chemicals that damages DNA induces SOS response. Rec A and Lex A gene are major in SOS response induction.

• lexA encodes lexA protein which is repressor. It binds to SOS box near promotor of SOS gene and prevents gene expression.




• RecA protein is a nucleoprotein and is always high in bacterial cell. It form nucleoprotein filaments on single stranded DNA and protects further damage of DNA. Rec A protein acquire proteases activities and activates self-cleavage of lexA protein from SOS box.
• Now the operator and promoter site become free and facilitates gene expression of SOS box gene

## Possible Questions

### Part A (1 mark)

### Part B (2 marks)

1. What is mutation?
2. What are mutagens?
3. What is mutagenesis?
4. Give an account on induced mutation.
5. Write a note on (i) Transitions (ii) Transversion (iii) Frame – Shift mutations.
6. What are the different types of physical agents that are capable of inducing mutations?

### Part C (8 marks)

7. Briefly explain the types of mutation.
8. What are chemical mutagens? How do they cause mutation?
9. Give a detailed account on site directed mutagenesis and its application.
10. Explain the methodology and principle involved in replica plating technique.
11. Write a note on DNA repair mechanism and add a note on RecA protein in it.
12. Discuss about proof – reading in DNA & mismatch repair.
13. Give an account of excision repair mechanisms.
14. Write a note SOS repair.

# KARPAGAM ACADEMY OF HIGHER EDUCATION

**CLASS: I BSC Microbiology COURSE NAME: MICROBIAL GENETICS**

**COURSE CODE: 19MBU203 UNIT: `IV BATCH: 2019-2022**

| Sl. No | Question | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | Conjugation involves the use of _____for mapping | Interrupted mating | Direct mapping | Contact mapping | Linkage | Interrupted mating |
| 2 | Genetic fine structure mapping of T4 was studied by _____ | Benzon | Mendel | Colins | Bennazir | Benzon |
| 3 | In genetic mapping, the measurement of distance between the genes is expressed as | Centimorgan | Centimeter | Millimorgan | Millimeter | Centimorgan |
| 4 | Methods used to identify the locus of a gene and the distances between genes | Gene mapping | Chromosomal linkage | Gene walking | Chromosomal walking | Gene mapping |
| 5 | T4 bacteriophages generally parasitizes | *Bacillus* | *E.coli* | *Psuedomonas* | *Agrobacterium* | *E.coli* |
| 6 | The non specific transduction is also called as - - - - - - - - - | Restricted transduction | Generalized transduction | Non specific transduction | Specialised transduction | Generalized transduction |
| 7 | Transfer of a portion of chromosome to a recipient with direct contact is termed | Gene expression | Transformation | Transduction | Conjugation | Conjugation |
| 8 | The first demonstration of bacterial transformation was done with | *Streptococcus pyogenes* | *Staphylococcus aureus* | *Streptococcus pneumoniae* | *Klebsiella pneumoniae* | *Streptococcus pneumoniae* |
| 9 | Size of T4 phage genome | 169 kbp | 196 kbp | 619 kbp | 916 kbp | 169 kbp |
| 10 | Map distance is equal to the percentage of _____ | Recombinant meiotic product | Reproducible meiotic product | Recombinant mitotic product | Reproducible mitotic product | Recombinant meiotic product |
| 11 | Genome of different bacteria suggest that genes have in the past moved from one species to another. This phenomenon is called lateral | DNA transfer | RNA transfer | Gene transfer | Protein transfer | Gene transfer |
| 12 | Experiment on transformation | Monad | Griffith | Morgan | Hersehy | Griffith |
| 13 | Conjugational genetic exchange has been - - - - - - - - frequently encountered among gram positive than gram negative bacteria. | More | Less | Very High | Very low | Less |
| 14 | Complete linkage has been reported in | Male Drosophila | Human female | Female | Maize | Male |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | Drosophila | | Drosophila |
| 15 | Bacteriophages were jointly discovered by | Frederick Twort and by Felix d'Herelle | Hershey and Chase | Luria and Delbruck | McKay and McCartney | Frederick Twort and by Felix d'Herelle |
| 16 | Capsules help bacteria in escaping | Inflammation | RBC's | Phagocytosis | Antibodies | Phagocytosis |
| 17 | Genes responsible for antibiotic resistance are mainly transferred across bacterial population by | Conjugation | Transformation | Transduction | Gene expression | Conjugation |
| 18 | The phenomenon of linkage was first observed in the plant | *Lathyrus odoratus* | *Pisum sativum* | *Datura* | *Mirabilus jalapa* | *Lathyrus odoratus* |
| 19 | The Competence of a cell in the process of transformation is aided by | $CaCl_2$ | $MgCl_2$ | KCl | $AgCl_2$ | $CaCl_2$ |
| 20 | The viral genome integrated to the bacterial genome is called | Plasmid | Capsid | Prophage | Virion | Prophage |
| 21 | Pneumococcal 'S' cells produce _____ colonies during growth on agar plates | Smooth | Rough | Elongated | Flat | Smooth |
| 22 | In conjugation, the donor always carries on | (F-) | (F+) | F neutral | No F | (F+) |
| 23 | Occurs when new DNA does not integrate into the chromosome, not replicated and is eventually lost | Abortive transduction | Specialized Transduction | Generalized Transduction | Transfusion | Abortive transduction |
| 24 | Tendency of alleles located close together on a chromosome to be inherited together during the meiosis | Linkage | Crossing over | Gene overlapping | Recessive genes | Linkage |
| 25 | Virulence in *Streptococcus pneumoniae* is attributed to | Flagella | Capsules | Pili | Fimbriae | Capsules |
| 26 | The phenomenon in which genes are present on the same chromosomes is | Cross over | Segregation | Linkage | Assortment | Linkage |
| 27 | Specialised transduction is effected by _____ | $T_2$ | Mu | $P_1$ | $T_7$ | $T_2$ |
| 28 | Integeration of viral nucleic acid in to host chrosome is termed as | Microphage | Prophage | Prephage | Macrophage | Prophage |
| 29 | Genetic recombination in phages was discovered by | Hershey and | Hershey and | Hershey and | Hershey and | Hershey and |

| | | | | | | |
|---|---|---|---|---|---|---|
| | _____ | Rotma | Chase | Wollmer | Singer | Rotma |
| 30 | Conjugation can only occur between cells of | F positive types | F negative types | Same mating types | Opposite mating types | Opposite mating types |
| 31 | An example for specialized transducing particle | No infection | T2 phage infects *Staphylococcus* | Phage P22 infects *Salmonella typhimurium* | Phage lambda infects *E.coli* | Phage lambda infects *E.coli* |
| 32 | _____ involves finding a contiguous series of cloned DNA fragments which contain overlapping portions of the genome | Physical mapping | Chemical mapping | Marker mapping | Loci mapping | Physical mapping |
| 33 | _____ refers to a genetic changes in different genomes of same cell. | Trans type | Cis type | Same type | Different type | Cis type |
| 34 | Conditions that favor the termination of the lysogenic state | Desiccation | Decomposition | Nutrient Media | Macronutrient | Desiccation |
| 35 | Direct way of observing the physical arrangement of markers along the chromosomes | Fluorescence in situ hybridization | Fluorescence invitro hybridization | Fluorescence invivo hybridization | Fluorescence in cell hybridization | Fluorescence in situ hybridization |
| 36 | Metalloproteins found in all eukaryotes | Zinc fingers | Iron fingers | Lead fingers | Copper fingers | Zinc fingers |
| 37 | The gene linkage minimize the chances of | Cross over | Segregation | Recombination | Assortment | Recombination |
| 38 | Transfer of DNA from one bacterium to another through the action of viruses | Transduction | Conjugation | Transformation | Gene expression | Transduction |
| 39 | The frequency at which two genes are - - - - - - - - by population of phages can be used to estimate their relative distance | Transduced | Co transduced | Co repressor | Co operator | Co transduced |
| 40 | Who coined the term linkage | Mendel | Morgan | de Vries | Correns | Morgan |
| 41 | The complex of DNA, RNA and protein is | Chromatin | Somatin | Pigmentin | Fromatin | Chromatin |
| 42 | The process to identify a genetic element that is responsible for a disease is also referred as | Mapping | Linkage | Sequencing | Genome data mining | Mapping |

| | | | | | | |
|---|---|---|---|---|---|---|
| 43 | Cells carrying non-integrated transducing fragments are called _____ | Specialized | Abortive transductants | Generalized | Conjugation | Abortive transductants |
| 44 | Crossing over occurs during | Pachytene | Diplotene | Diakinesis | Haplotene | Pachytene |
| 45 | Avery, MacLeod & McCarty used enzymes and solvents to destroy molecules such as | Anything except DNA | RNA | Lipids & proteins | Polysaccharide | Anything except DNA |
| 46 | A bacterium harboring a prophage is called | Lytic phage | Helper phage | Transducing phage | Lysogency | Lysogency |
| 47 | Genome of T4 phage is | ds DNA | ss DNA | ds RNA | ss RNA | ds DNA |
| 48 | Non sex chrosomes are called | Rhizomes | Lysosomes | Mesosomes | Autosomes | Autosomes |
| 49 | Uptake of DNA molecules from environmental surrounding | Transduction | Conjugation | Transformation | Gene expression | Transformation |
| 50 | Results from inaccurate excision of an integrated prophage with addition of some bacterial genes | Specialized Transduction | Generalized Transduction | Abortive transduction | Transfusion | Specialized Transduction |
| 51 | Give the full form for Hfr | High fertility recombination | High fundamental recombination | High frequency recombination | Heavy frequency recombination | High frequency recombination |
| 52 | Genes that cause suppression of mutations in other genes are called -------- genes | Reverse genes | Control genes | Suppressor genes | Inducer genes | Suppressor genes |
| 53 | Conjugation is predominant in _____ | Spirochaetes | G+ bacteria | G-bacteria | Cyanobacteria | G-bacteria |
| 54 | _____ is a DNA associated protein | Protone | Histone | Chromotome | Cistron | Histone |
| 55 | Conjugation involves the use of _____for mapping | Interrupted mating | Direct mapping | Contact mapping | Linkage | Interrupted mating |
| 56 | During insertion of lambda DNA in to host, a viral protein called _____ is required along with integration host factor (IHF) | Integrase | Caspase | Helicase | Polymerase | Integrase |
| 57 | Linkage prevents | Segregation of alleles | Homozygous condition | Hybrid formation | Heterozygous condition | Segregation of alleles |

| | | | | | | |
|---|---|---|---|---|---|---|
| 58 | T4 is capable of undergoing only a | Lytic cycle | Lysogenic cycle | Both Lytic & Lysogenic cycle | Other cycle | Lytic cycle |
| 59 | A cell carrying an integrated F factor is called an | F | Hfr | Hfr 1+ | trans | Hfr |
| 60 | Capsule of *Streptococcus pneumoniae* are made up of | Protein | Lipid | Glycoprotein | Polysaccharide | Polysaccharide |

## Unit- V

### Syllabus

Transposons-definition, types of Transposons, mechanism of transposition and application.
Mu transposon and eukaryotic transposable elements

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### Transposable element

A transposable element (TE or transposon) is a DNA sequence that can change its position within a genome, sometimes creating or reversing mutations and altering the cell's genetic identity and genome size.

Transposable elements make up a large fraction of the genome and are responsible for much of the mass of DNA in a eukaryotic cell. It has been shown that TEs are important in genome function and evolution. Different insertion sequences range in size from about 800 bp to 2000 bp. The DNA sequence of an IS has inverted repeats (about 10 to 40 bp) at its termini.

### Discovery

Barbara McClintock discovered the first TEs in maize at the Cold Spring Harbor Laboratory in New York. McClintock was experimenting with maize plants that had broken chromosomes. Approximately 90 % of the maize genome is made up of TEs, as is 44 % of the human genome.

### Types of Transposons

Transposable elements represent one of several types of mobile genetic elements. TEs are assigned to one of two classes according to their mechanism of transposition, which can be described as either copy and paste (Class I TEs) or cut and paste (Class II TEs).

A. Class I (retrotransposons)

Class I TEs are copied in two stages: first, they are transcribed from DNA to RNA, and the RNA produced is then reverse transcribed to DNA. This copied DNA is then inserted back into the genome at a new position. The reverse transcription step is catalyzed by a reverse transcriptase, which is often encoded by the TE itself. The characteristics of retrotransposons are similar to retroviruses, such as HIV.

B. Class II (DNA transposons)

DNA transposons are a group of transposable elements that can move in the DNA of an organism via a single or double stranded DNA intermediate. DNA transposons have been found in both prokaryotic and eukaryotic organisms. The cut-and-paste transposition mechanism of class II TEs does not involve an RNA intermediate. The transpositions are catalyzed by several transposase enzymes. Some transposases non-specifically bind to any target site in DNA, whereas others bind to specific target sequences. The transposase makes a staggered cut at the target site producing sticky ends, cuts out the DNA transposon and ligates it into the target site. A DNA polymerase fills in the resulting gaps from the sticky ends and DNA ligase closes the sugar-phosphate backbone. This results in target site duplication and the insertion sites of DNA transposons may be identified by short direct repeats (a staggered cut in the target DNA filled by DNA polymerase) followed by inverted repeats (which are important for the TE excision by transposase).

Autonomous and non-autonomous

Transposition can be classified as either "autonomous" or "non-autonomous" in both Class I and Class II TEs. Autonomous TEs can move by themselves, whereas non- autonomous TEs require the presence of another TE to move. This is often because dependent TEs lack transposase (for Class II) or reverse transcriptase (for Class I). Activator element (Ac) is an example of an autonomous TE, and dissociation elements (Ds) is an example of a non-autonomous TE.

Example

Transposons in bacteria usually carry an additional gene for functions other than transposition, often for antibiotic resistance. In bacteria, transposons can jump from chromosomal DNA to plasmid DNA and back, allowing for the transfer and permanent addition of genes such as those encoding antibiotic resistance (multi-antibiotic resistant bacterial strains can be generated in this way). Bacterial transposons of this type belong to the Tn family. When the transposable elements lack additional genes, they are known as insertion sequences.

**Mechanism of transposition**

The bacterial transposon Tn3 has been extensively studied. Analysis of DNA sequences and its junction with target DNA provides some clue to the mechanism of transposition.

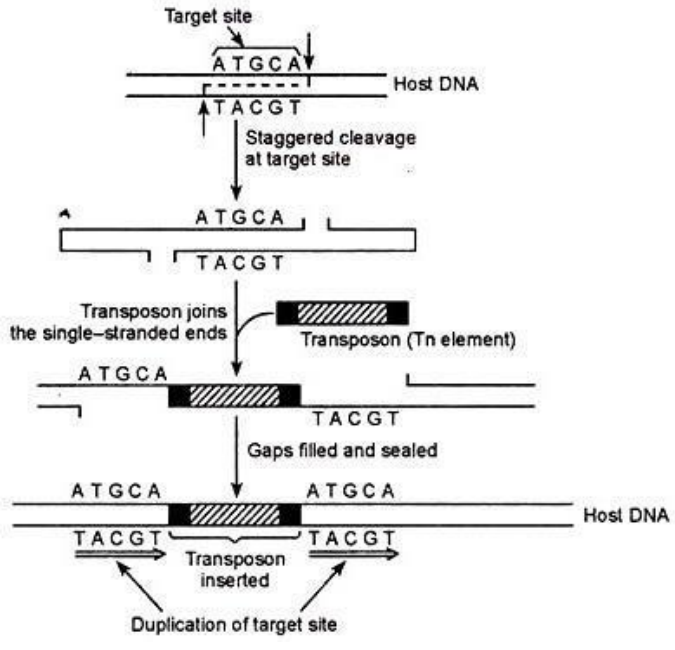
Movement of transposons occurs only when the enzyme transposase recognises and cleaves at either 5′ or 3′ of both ends of transposon, and catalyses at either 5 or 3′ of both ends of transposon at the target site. Depending on transposon, a duplication of 3-12 bases of target DNA occurs at the site where insertion is to be done. One copy remains at each end of the transposon sequence.

After attachment of both ends of transposon to the target site, two replication forks are immediately formed (Fig. 8.34 B-C). From this stage there starts two path for carrying out onward processes.

1. Replicative transposition:

The transposon is duplicated during the process of transposition; one copy of the Tn element remains at the original site while the duplicated copy is inserted at a new site. Such type of transposition is a characteristic of TnA group of transposons (see later).

In this type of transpositions the number of copies of the transposon increases. Two endonucleases are involved in transposition: the enzyme transposase acts on the ends of the original transposon while another enzyme resolvase acts on the duplicated copy.




2. No-Replicative Transposition:

In this case, the transposable element moves from one site into the other. Since there is no

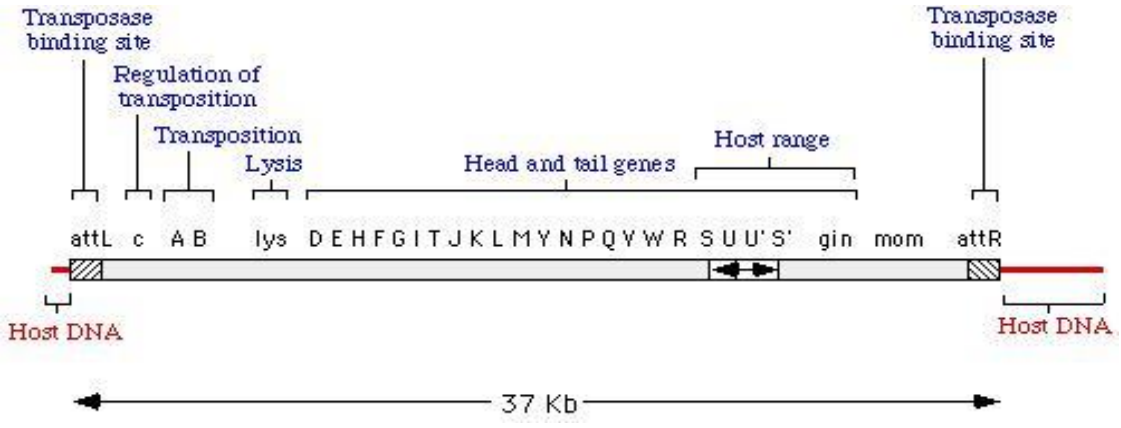
duplication of the transposon, it is lost from the original site.

Thus, for transposition the two enzymes, transposase and resolvase coded by tnpA and tnpR respectively are required. Transposase recognises the ends of transposon and connects them to the target site. Resolvase provides a site-specific recombination function. Transposase recognises the ends of elements and binds to 25 bp long sequence located within 38 bp of the inverted terminal repeat. Transposase also makes the staggered 5 bp breaks in target DNA where transposon is to be inserted.

**Applications:**

- Appropriately, TEs have been an especially useful tool in plant molecular biology. Researchers use them as a means of mutagenesis.
- Sometimes the insertion of a TE into a gene can disrupt that gene's function in a reversible manner, in a process called insertional mutagenesis; transposase-mediated excision of the DNA transposon restores gene function. This produces plants in which neighboring cells have different genotypes.
- TEs are also a widely used tool for mutagenesis of most experimentally tractable organisms. The Sleeping Beauty transposon system has been used extensively as an insertional tag for identifying cancer genes. The Tc1/mariner-class of TEs Sleeping Beauty transposon system awarded Molecule of the Year in 2009is active in mammalian cells and is being investigated for use in human gene therapy. TEs are used for the reconstruction of phylogenies by the means of presence/absence analyses.

**Mu transposon**

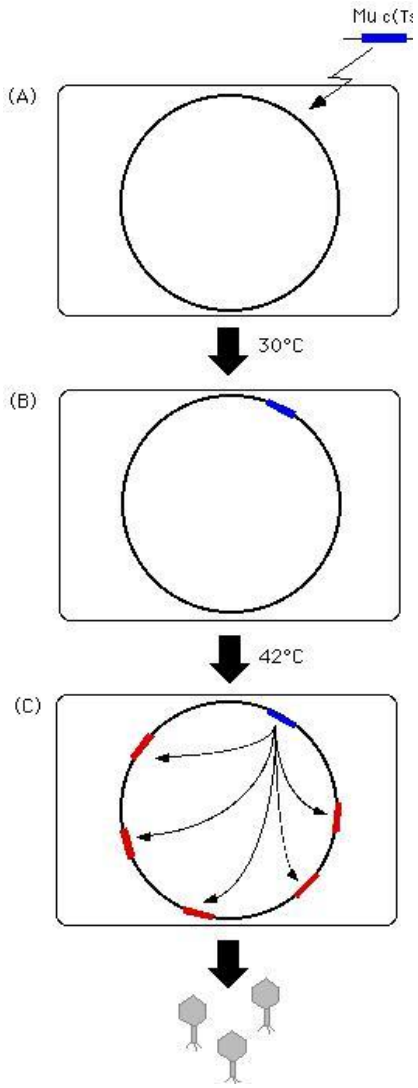



- The A and B gene products encode transposase -- the A protein is required for all transposition events, but the B protein is only required for replicative transposition events. Expression of the transposase genes is repressed by the c gene product. Transposition requires the two ends of Mu, labeled attL and attR (sometimes called MuL and MuR).
- When Mu DNA is packaged into a phage head it includes about 50-150 bp of host DNA at the left end and a variable amount of host DNA on the right end. For wild-type Mu the amount of host DNA on the right end is about 2 Kb but, because of the headful packaging mechanism shown below, the length of host DNA on this end increases if part of the Mu genome is deleted. Each Mu is packaged from a different site in the host genome, so the host DNA on the ends of Mu is unique in every different phage head.

a) When Mu infects a sensitive host, the linear DNA enters the cell and the Mu DNA (i.e. not including the variable sequences of DNA acquired from the previous host) is inserted into the recipient genome via a non- replicative, "cut and paste" mechanism.

b) Lysogens of wild-type Mu are quite stable and are not induced by UV or other DNA damaging agents. However, derivatives of Mu with a temperature sensitive repressor -- Mu c(Ts) -- can be induced by shifting the lysogen to 42 C.

c) When the repressor is inactivated, the A and B proteins are expressed and Mu transposes by a replicative mechanism to 50 - 100 new sites on the chromosome. Meanwhile, late phage gene products are made (including phage heads, tails, lysis proteins, etc). The phage DNA is packaged by a headful mechanism, beginning by cutting the dsDNA in host sequences located about 100 bp from the left end of Mu. The length of Mu DNA is about 37 Kb and about 39 Kb are packaged into each head, so about approximately 2 Kb of host DNA is included on the right end of the packaged DNA. After assembly of the phage, the host is lysed.



## Possible Questions

### Part-A (1 mark)

### Part-B (2 marks)

1. Define Transposable elements.
2. What is meant by composite transposable elements? Give example.
3. What are insertional repeats?
4. Write short notes on resolvase, transposase enzyme.
5. Write any two application of transposons.
6. What is the role of the transposons in plants.

### Part-C (8 marks)

7. Explain about different types of Transposons.
8. What is the difference between composite and non composite transposons? Explain.
9. Discuss on the applications of transposons.
10. Give a detailed account on the role of Mu transposons in gene exchange mechanism.

| Sl. No | Question | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
|---|---|---|---|---|---|---|
| 1 | _____ refers to a genetic changes in different genomes of same cell | Trans type | Same type | Cis type | Different type | Cis type |
| 2 | _____distance in map unit is only half the percentage of second division segregated tetrads | Tetromere | Telomere | Centromer | Primer | Telomere |
| 3 | An estimation of map between a- & b- can be obtained from number of recombinant ascospore detected by using _____ | Genetic analysis | Physical analysis | Random spore analysis | Tetrad analysis | Random spore analysis |
| 4 | Common model organism in studying unicellular eukaryotes/budding yeast | *Saccharomyces cerevisiae* | *Cryptococcus Neoformans* | *Candida albicans* | *Pitchia pastoris* | *Saccharomyces cerevisiae* |
| 5 | Gene conversion is the _____ transfer of information from one DNA duplex to another | Reciprocal | Non-reciprocal | Direct | Indirect | Non-reciprocal |
| 6 | Haploid to diploid phase in yeast is brought about by | Fusion of opposite mating types | Fusion of positive mating types | Fusion of negative mating types | Fusion of opposite genes | Fusion of opposite mating types |
| 7 | In *Drosophila*, the meiotic recombination occurs only in _____ | Both Female and Male | Female | Male | None | Female |
| 8 | Map distance is equal to the percentage of | Reproducible mitotic product | Recombinant mitotic product | Reproducible meiotic product | Recombinant meiotic product | Recombinant meiotic product |
| 9 | Petite phenotypes caused by the absence of, or mutations in, mitochondrial DNA are termed as | Cytoplasmic petites | Energy deficient petites | Mitochondrial petites | Chrosomal petites | Cytoplasmic petites |
| 10 | Random spore analysis gives _____ values | Approximate | Accurate | Null | Partial | Approximate |

| | | | | | | |
|---|---|---|---|---|---|---|
| 11 | Sex linkage was explained by _____ | Morgan | Mendel | Primrose | Pastuer | Morgan |
| 12 | The ascus burst releasing the ascospores, each of which germinates and divides by mitosis to produce new _____ | Vegetative cells | Spores | Ascus | Zygote | Vegetative cells |
| 13 | The general mapping function of Haldane is based on _____ | Haldane distribution | Poisson distribution | Twart | Switz | Poisson distribution |
| 14 | The repair of double-strand gaps is an efficient process in yeast known to be | Seggregation | Assortment | SOS repair | Crossing over | Crossing over |
| 15 | Well characterized Baker's yeast has __ cell | Irregular | Round | Spheroid | Disc shaped | Spheroid |
| 16 | Which of the following is used in density gradient centrifugation? | Glucose | Sucrose | Fructose | Agarose | Sucrose |
| 17 | Yeast genome is _____ | 12,520 kb | 1,252 kb | 1,02,520 kb | 15,052 kb | 12,520 kb |
| 18 | When mutation in single gene affect more than one trait is called _____ | Parental genes | Pleiomorphic genes | Priogenic genes | Pleiotrophic genes | Pleiotrophic genes |
| 19 | The binding of two DNA helices through X-shaped junction called _____ | Polytron | Cholistron | Diptron | Cistron | Cistron |
| 20 | Small pieces of DNA that can insert themselves into chromosomes are known | Plasmid | Transposon | Cosmid | Artificial chrosome | Transposon |
| 21 | Recombination does not only occur during meiosis, but also as a mechanism for | Repair of single-strand breaks | Repair of double-strand breaks | Repair of proteins | Repair of RNA | Repair of double-strand breaks |
| 22 | Petite yeast mutants are unable to grow on media containing | Only nitrogen sources | Only Mineral sources | Only Lipid Source | Only fermentable carbon sources | Only non-fermentable carbon sources |
| 23 | Meiosis in yeast life cycle leads in forming | Sporangium | Fragmented mycelium | Ascus spores | Endospores | Ascus spores |

| | | | | | | |
|---|---|---|---|---|---|---|
| 24 | In yeast, 22% of the genome is made up of _____ DNA | Polygenic | Monogenic | Intragenic | Intergenic | Intergenic |
| 25 | Genetic maps of chromosome are based on the frequencies of _____ | Reproducible mitotic product | Reproducible meiotic product | Recombinant mitotic product | Recombinant meiotic product | Recombinant meiotic product |
| 26 | Gene conversion can be either allelic or _____ | Allergic | Ectopic | Endemic | Endopic | Ectopic |
| 27 | Gene conversion in yeast may be important in maintaining_____within families | Sequence homogeneity | Sequence heterogenecity | Sequence array | Sequence hologenecity | Sequence homogeneity |
| 28 | The leu2- strain carries a mutation that inactivates leu2 gene which codes for _____ | Endonuclease | Beta isoprophylmalate dehydrogenase | Helicase | Ligase | Beta isoprophylmalate dehydrogenase |
| 29 | The percentage of recombinant meiotic product is one half of the percentage of _____ | Second division | Fourth division | Primary division | Tetrad | Tetrad |
| 30 | A new genetic nomenclature for S. cerevisiae transposon | jumping element | Ty elements | Tx elements | Ta element | Ty elements |
| 31 | Among haploid and diploid vegetative cells of yeast ,which is mainly used for genetic mapping | Haploid and diploid | Diploid | Haploid | Triploid | Haploid |
| 32 | His 3 is an _____ protein. | Transducer | Indicator | Selector | Repressor | Indicator |
| 33 | If single crossover occurs between a- & b- then tetra type ___ results | A | B | T | U | T |
| 34 | Pleiotrophy is common in _____ | No organism | All organism | Higher organism | Lower organism | Higher organism |
| 35 | Process by which one DNA sequence replaces a homologous sequence | Gene mutation | Gene repulsion | Gene transtition | Gene conversion | Gene conversion |
| 36 | The term genetic linkage was given by ---------- | Morgan | Meischer | Wilkins | Mendel | Morgan |

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| 37 | The_____ to a particular chromosome is the first step in genetic mapping | Elemination of mutated type | Localization of wild type | Localization of mutation | Elimination of wild type | Localization of mutation |
| 38 | Yeast are _____ | Multicellular fungi | Dicellular | Acellular | Unicellular fungi | Unicellular fungi |
| 39 | Yeast genome has _____ introns | 233 | 236 | 323 | 326 | 233 |
| 40 | Yeast has two mating types, _____ , which show primitive aspects of sex differentiation | a & b | a & α | b & β | α & β | a & α |
| 41 | Yeasts fail to grow on | Glucose | Lactose | Maltose | Trehalose | Lactose |
| 42 | Sample of ascospores is spread on to the agar medium without leucine and survival was tested using | ELISA | PCR | Random spore analysis | RPR | Random spore analysis |
| 43 | _____ test is used to determine which gene(s) are defective in petite yeast mutants | Complementation test | Complement fixation test | Completed test | Conjugation test | Complementation test |
| 44 | _____ contains two types of spores of same parental genotype | Parental haploid | Parental diploid | Non-parental ditype | Parental ditype | Parental diploid |
| 45 | _____determines the number of crossover events and give correct map distance | Genetic analysis | Physical analysis | Random spore analysis | Tetrad analysis | Tetrad analysis |
| 46 | _____developed an algebriac method to determine the consequence of various number of exchanging | Klebs & Loeffler | Pastuer & Winogradsky | Shult & Lindegrin | Klug & Cumming | Shult & Lindegrin |
| 47 | Which contains four different ascospores , one of each genotypes | Tetratype | Parental type | Parental ditype | Non-parental ditype | Tetratype |
| 48 | The developing haploid spores are enclosed in a membranous structure called_____ | Spores | Pycus | Zygote | Ascus | Ascus |

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| 49 | Method available for locating mutation in *Neurospora crassa* is | Co-segregation | Co-opression | Independent assortment | Tetrad analysis | Co-segregation |
| 50 | In Transcription of yeast genome,_____ makes all RNA to serve as mRNA | DNA polymerase | RNA polymerase | RNA pol I | RNA pol II | RNA pol II |
| 51 | Intermediate compound responsible for red color of adenine-requiring yeast petite mutants | Aminoimidazole | Aminoimidazole ribonucleotide | Aminoimidazoleribotide | Aminoimmunoribotide | Aminoimidazoleribotide |
| 52 | $Arg^+$ is | A strain not requiring aspargine | A strain requiring aspargine | A strain not requiring arginine | A strain requiring arginine | A strain not requiring arginine |
| 53 | Cells which contains single copy of chromosome is called _____ | Triploid | Haploid and diploid | Diploid | Haploid | Haploid |
| 54 | _____ intial products of meiosis forms two identical spore | 4 | 8 | 16 | 32 | 4 |
| 55 | A feature of petite is the occurrence of _____ from the circular mitochondrial genome | Insertion | Excertion | Addition | Deletion | Deletion |
| 56 | Generation time of yeast takes place at _____ | 2hrs 30 min | 3 hrs | 60 min | 30 min | 2hrs 30 min |
| 57 | Give full form for NPD | Non-parental dikaryon | Non-performing data | Non-parental data | Non-parental ditype | Non-parental ditype |
| 58 | HFT is | High frequency transducing | High frequency transcribing | Height frequency transducing | Heavy frequency transducing | High frequency transducing |
| 59 | Significant feature of sex determination in Drosophila is the presence of abnormal flies called _____ | Gyno variants | Gynocoid | Gyrates | Gynondromorphs | Gynondromorphs |
| 60 | Tetrad showing second division segregation has _____ products | Four meiotic | Two Meiotic | Four mitotic | Two mitotic | four meiotic |

| Sl. No | Question | Opt 1 | Opt 2 | Opt 3 | Opt 4 | Answer |
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| 1 | Chemical agent that resembles thiamine | 5-bromothiamine | 5-bromoadenine | 5-bromoguanine | 5-bromouracil | 5-bromouracil |
| 2 | Duplication mutation is | Segments of nucleotides sequences are repeated | Segments of nucleotides sequences are deleted | Segments of nucleotides sequences are inserted | Segments of nucleotide sequences are inserted & deleted evenly | Segments of nucleotides sequences are repeated |
| 3 | Mutations that result from treatment with mutagens are called | Induced mutation | Uninduced | Spontaneous | Frameshift | Induced mutation |
| 4 | Oncogenes are found in certain | Bacteria | Fungi | Viruses | Algae | Viruses |
| 5 | Mutation generating new stop codon are called | Nonsense mutation | Misense mutation | Point mutation | Silent mutation | Nonsense mutation |
| 6 | UV induced dimers are separated using light energy by | Primase | Photolyase | Dnase | Rnase | Photolyase |
| 7 | The function of DNA glycosylase in base excision repair is | Addition of correct base | Addition of correct nucleotide | Removal of incorrect base | Removal of phosphodiester bond | Removal of phosphodiester bond |
| 8 | Which of the following biomolecule has self¬repair mechanisms? | DNA,RNA and protein | DNA and RNA | DNA and proteins | DNA only | DNA only |
| 9 | Rapid screening technique for mutagens and carcinogens | Aims test | Sima test | Ames test | Sema test | Ames test |
| 10 | Mutation resulting from deamination of 5-methylcytosine produces Thymine which pairs with | Uracil | Adenine | Cytosine | Guanine | Adenine |
| 11 | Converts amino groups to keto groups by oxidative deamination | Hydrochloric acid | Nitrous acid | Sulphuric acid | Oxalic acid | Nitrous acid |
| 12 | In bacteria, a small circle of DNA found outside the main chromosome is called | Cosmid | Bacmid | Transposon | Plasmid | Plasmid |

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| 13 | Biological agents of mutagenesis are | Transposable elements | Lipids | Bacteria | Carbohydrates | Transposable elements |
| 14 | Most common proto-oncogene implicated in human cancers | *s-rac* | *a-src* | *r-cas* | *c-ras* | *c-ras* |
| 15 | Deficiency in phenylalanine hydroxidase results in | Cancer | Phenylketonuroa | Melanoma | Asthma | Phenylketonuroa |
| 16 | Cancer that results from deletion of a portion of chrosome 13 is | Eye cancer | Bone cancer | Skin cancer | Lung cancer | Eye cancer |
| 17 | Mutation in which a purine base is substituted with another purine base is | Transverse mutation | General mutation | Transition mutation | Transduction | Transition mutation |
| 18 | Sickle cell anaemia is caused by change in amino acid from | Glutamic acid | Alanine to Leucine | Valine to Glutamic acid | Leucine to alanine | Valine to Glutamic acid |
| 19 | Which of the following chemicals induce depurination | Methyl ethane sulphonate | Guanidine | Ethyl sulphonate | Dichlor | Methyl ethane sulphonate |
| 20 | Xeroderma pigmentosum is a genetic disorder of | Skin | Hair | Nail | Tongue | Skin |
| 21 | UV radiation causes | Adenine dimers | Cytosine dimers | Guanine dimers | Thiamine dimers | Thiamine dimers |
| 22 | The result of addition or deletion of one or more base pair in a gene is | Frameshift | Base pair substitution | Misense mutation | Nonsense mutation | Frameshift |
| 23 | Repairing mechanism of _____ depends on absorption of visible light by the enzyme. | DNA helicase | DNA ligase | DNA gyrase | DNA photolyase | DNA photolyase |
| 24 | Herman J.Mueller reported results of induced mutations on | Yeast | *Drosophila* | Fish | Pea plant | *Drosophila* |
| 25 | Chromosomal mutation is | Abberation | Change over | Variation | Genetic change | Abberation |
| 26 | _____ is a non-ionizing radiation | Alpha | UV | Gamma | Beta | UV |
| 27 | Mutation involving single-base changes are | Induced mutations | Point mutations | Silent mutations | Inverse mutations | Point mutations |
| 28 | Transposons was first reported by | Louise pasteur | Koch | Barbara | Lister | Barbara |

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| | | | | McClintock | | McClintock |
| 29 | Nonsense mutation leads to | Termination of DNA synthesis | Termination of protein synthesis | Termination of cell wall synthesis | Termination of RNA synthesis | Termination of protein synthesis |
| 30 | Alkylation is | Addition of methyl or ethyl group | Deletion of ethyl and addition of methyl group | Deletion of methyl and addition of ethyl group | Deletion of methyl or ethyl group | Addition of methyl or ethyl group |
| 31 | Chemical mutagens leading to addition of nucleotides to the DNA are | Thimers | Base analogs | Alkylating agents | Interchelating agents | Interchelating agents |
| 32 | Human bladder cancer is brought about by a change in single point mutation of | Valine to glycine | Isoleucine to leucine | Leucine to isoleucine | Glycine to valine | Glycine to valine |
| 33 | Which of the following dimer formation is most common | Cytidine dimer | Uracil dimer | Thymidine dimer | Adenosine dimer | Thymidine dimer |
| 34 | Daughter strand repair is also called as | Recombination repair | SOS repair | Photo repair | Excision repair | Recombination repair |
| 35 | An intercalating dye | Sunset yellow | Safranin | India ink | Acridine orange | Acridine orange |
| 36 | Most common repair system is | SOS | Photoreactive | Mismatch | Excision | Excision |
| 37 | Virus capable of causing mutations is | Bacteriophage Ru | Bacteriophage Mu | Bacteriophage Nu | Bacteriophage Ly | Bacteriophage Mu |
| 38 | Potent oxidizing agent that can alter structure of purine and pyrimidine | Free radicals | Water | Dyes | Acids | Free radicals |
| 39 | Reverse mutation is | Wild type to mutant | Mutant to wild type | A new gene introduced | A gene deleted | Mutant to wild type |
| 40 | Alkylation of guanine causes its removal from DNA in a reaction called | Deamination | Depyrimidation | Degradation | Depurination | Depurination |
| 41 | In *E.coli*, parental DNA is methylated at an adenine residue found in the sequence | 5' TAGC 3' | 5' ATGC 3' | 5' CATG 3' | 5' GATC 3' | 5' GATC 3' |

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| 42 | Mutation in which there is an amino acid substitution is called | Missense | Nonsense | Silent | Point | Missense |
| 43 | Nutritional mutans of neurospora are known as | Phototrophs | Auxotrophs | Heterotrophs | Isotrophs | Auxotrophs |
| 44 | UV radiation at 260 nm cross-links adjacent thiamine that produces | Butane ring | Cyclane ring | Butocyclane ring | Phenyl alanine | Cyclobutane ring |
| 45 | When a part of chrosome is moved to another chromosome, it is called as | Point mutation | Induced mutation | Spontaneous mutation | Translocation mutation | Translocation mutation |
| 46 | Mismatch repair cannot take place if there is a mutation in | Helicase | Polymerase | Ligase | Methylase enzyme | Methylase enzyme |
| 47 | Common chemical events that produce spontaneous mutation | Deamination | Depurination | Dimerization | Isomerization | Depurination |
| 48 | Bacterium used in Ames test | *Salmonella* | *Shigella* | *Streptococcus* | *Staphylococcus* | *Salmonella* |
| 49 | Naturally occurring mutations are | Induced | Spontaneous | Nonsense | Frameshift | Spontaneous |
| 50 | Recombinational repair is often due to | many cytidine dimer and associated large gaps in a strand | incorporation of many incorrect nucleotides by DNA polymerase | many thymidine dimer formation and associated large gaps in a strand | DNA breaking | incorporation of many incorrect nucleotides by DNA polymerase |
| 51 | The most common error prone repair mechanism is | Mismatch | Excision | SOS | Recombination | SOS |
| 52 | Site that mutates at a rate significantly greater thag statistical probability is referred to as | Hotspots | Blackspots | Dotspots | DNA spots | Hotspots |
| 53 | Transposition is | Movement of a phage | Movement of a virus | Movement of a transposon | Movement of a plasmid | Movement of transposon |
| 54 | Radiation that causes cross chromosomal mutations in humans | UV | Visible | Ionozing | X-rays | Ionozing |
| 55 | Frameshift mutation is caused by | Proflavin | Nitrous acid | UV | X-rays | Proflavin |
| 56 | Change of purine to pyrimidine base in an | Transition | Transverse | General | Transformation | Transverse |

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| | mutation | mutation | mutation | mutation | | mutation |
| 57 | Detection of silent mutations require | Aminoacid analysis | Peptide analysis | RNA analysis | Nucleotide analysis | Nucleotide analysis |
| 58 | Most frequently employed technique in the study of mutations | Analysis of phenotypes | Analysis of genotypes | Analysis of proteins | Analysis of both phenotypes and genotypes | Analysis of phenotypes |
| 59 | Natural phenomena of changes in chemical structure of nitrogenous bases is called | Complementary | Conservative | Tautomeric | Telomeric | Tautomeric |
| 60 | Mutation that has no detectable effect on the phenotype of a cell | Point | Induced | Silent | Leaky | Silent |