Semester – I

17MBP103 MOLECULAR GENETICS 4H – 4C

Instruction Hours / week: L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

UNIT – I

Genetics – historical introduction – Mendelian principles – nucleic acid as genetic material Experimental evidence. The duplex DNA – chemical and physical structure of DNA – circular and super helical DNA - different forms of DNA. DNA replication – enzymology of DNA replication – different modes, models and types of DNA replication – Eukaryotic DNA replication.

UNIT – II

Mutagen, mutagenesis and mutation. Luria Delbruck experiment and its significance. Molecular basis of mutation. Spontaneous and induced mutations. Different types of mutation, mutant detection, mutant selection and carcinogenicity testing. DNA damage – types of damage (deamination, oxidative damage, alkylation, Pyrimidine dimers) – DNA repair mechanism (base excision, nucleotide excision, recombination repair, SOS repair).

UNIT – III

Genetic code: DNA transcription in prokaryotes and eukaryotes. Trancriptional control and modification system – RNA translation in prokaryotes and eukaryotes. Polypeptide synthesis (maturation and processing of RNA) – Translational modification. Regulation of gene expression – Operon model (Lac, Trp, Ara) – Regulation of gene expression in eukaryotes.

UNIT – IV

Genetic recombination in bacteria – conjugation, transformation, transduction. Linkage and genetic mapping. Phage genetics (Replication cycle) – Phage T4 mutants (detection and isolation) — Genetic map of T4 phage.

UNIT – V

Yeast genetics – Life cycle, metabolism, genome and extra chromosomal element. Genetic nomenclature in yeast. Tetrad analysis, Petite mutants (mutant isolation and complementation). Genetic mapping in yeast, *Neurospora* and *Drosophila*.

SUGGESTED READINGS

TEXT BOOKS

1. Malacinski, G.M. (2008). *Freifelder's Essentials of Molecular Biology*. Narosa Publishing House, New Delhi.
2. Verma, P.S., and Agarwal, V.K., (2008). *Cell Biology, Genetics, Molecular Biology and Evolution.* S. Chand & Company Ltd, New Delhi
3. Gardner, E.J., Simmons, M.J., and Snustad, D.P., (2008). *Principles of Genetics*. (8^{th} ed.). John Wiley and Sons, NY.
4. Guthrie, C., and Fink, G., (2002). *Guide to Yeast Genetics and Molecular Cell Biology*. Elsevier Publication, USA.
5. Klug, W.S., Cummings, M.R. Spencer, C.A., and Palladino, M.A., (2009). *Essentials of Genetics*. (7^{th} ed.). Prentice Hall, New Jersey.

6. Maloy, S.R., Cronan Jr, J.E., and Freifelder, D., (2001). *Microbial Genetics*. Narosa Publishing House. New Delhi.
7. Weaver, R.F. (2002). *Molecular Biology*. (2nd ed.). McGraw-Hill, New York.
8. Peter J. Russell, i Genetics – A molecular approach, 7th edition, 2010. Pearson Benjamin Cummings Publishers, Boston, USA.

REFERENCES

1. Alberts. (2008). *Molecular Biology of The Cell*, (5th ed.). Garland Science, Taylor and Francis group, LIC, an Inforrma Science.
2. Griffiths *et al.*, (2002). *Modern genetic analysis*, (2nd ed.). Freeman.
3. Hartl and Jones, (1998). *Genetics-Principles and Analysis*, (4th ed.). Jones & Bartlett.
4. Krebs, E,J., S.T.Kilpatrick and E.S.Goldstein, (2008). *Lewin's Genes X*, (10th ed.). Jones and Bartlett publishers, Canada.
5. Nelson, D., and Cox, M.M., (2008). *Lehninger's Principles of Biochemistry*, (5th ed.). McMillan.
6. Tamarin, R.H. (2001). *Principles of Genetics*. (7th ed.). Wm. C. Brown Publishers. England
7. Turner, P., McLennan, A., Bates, A., and White, M., (2005). *Molecular Biology*. (3rd ed.). Taylor and Francis group.
8. Watson, J.D., Baker, T., Bell, S., Gann, A., Levine, M., and Losick, R., (2008). *Molecular Biology of Genes*. (6th ed.). Pearson Education.

DEPARTMENT OF MICROBIOLOGY

KARPAGAM UNIVERSITY

(Deemed University Established Under Section 3 of UGC Act, 1956)

Eachanari PO, Coimbatore -641 021, India.

I - M.Sc Microbiology (Batch 2017-2019)

Lecture Plan

Unit - I

S. No	Duration	Topic	Reference
1.	2	History of Genetics – Types and Mendelian principles	T1:1 to 10 R1: 3 to 4/ 24-27
2.	2	Experimental evidence on nucleic acid as genetic material	T1: 13 to 15 / 17 to 28 R1: 245 to 247
3.	2	Structure of DNA – Physical and Chemical Nature	T1:20 to 44 R1: 29 to 49
4.	2	Different forms of DNA	T1: 25 to 26
5.	2	DNA replication – Types, models and modes of replication.	T1: 170 to 172 R1: 47 to 48
6.	2	Enzymology of DNA replication	T1: 185 to 187
7.	1	Video presentation on DNA structure and DNA replication	W1
8.	1	Class Test I	-
		Total Hours	**14**

R1: David Freifelder, Microbial Genetics. Narosa Publishing House, 10th edition, 2004. New Delhi, India.

T1: Peter J. Russell, i Genetics – A molecular approach, 7th edition, 2010. Pearson Benjamin Cummings Publishers, Boston, USA.

W1: www.coldspringharborlabs.edu/Mol.Biol.html.

P. SRINIVASAN
Assistant Professor
Department of Microbiology

2017

Unit - I

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 to be generated and overcome before modern genetics could arise.**

1. All life comes from other life. Living organisms are not spontaneouslygenerated from non-living material. Big exception: origin of life.
2. Species concept: offspring arise only when two members of the same species mate. Monstrous hybrids don't exist.
3. Organisms develop by expressing information carried in their hereditary material. As opposed to "preformation", the idea that in each sperm (or egg) is a tiny, fully-formed human that merely grows in size.
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 the results of his 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.
1905	Nettie Stevens, Edmund Wilson	Independently described the behavior of sex chromosomes-XX determines female; XY determines male.
1908	Archibald Garrod	Proposed that some human diseases are due to "inborn errors of metabolism" that result from the lack

Prepared by – Mr. P. Srinivasan, Assistant Professor, Dept. of Microbiology, KAHE.

		of a specific enzyme.
1910	Thomas Hunt Morgan	Proposed a theory of sex-linked inheritance for the first mutation discovered in the fruit fly, Drosophila, white eye. This was followed by the gene theory, including the principle of linkage.
1927	Hermann J. Muller	Used x-rays to cause artificial gene mutations in Drosophila.
1928	Fred Griffith	Proposed that some unknown "principle" had transformed the harmless R strain of *Diplococcus* to the virulent S strain.
1931	Harriet B. Creighton ,Barbara McClintock	Demonstrated the cytological proof for crossing-over in maize.
1941	George Beadle ,Edward Tatum	Irradiated the red bread mold, *Neurospora*, and proved that the gene produces its effect by regulating particular enzymes.
1944	Oswald Avery ,Colin MacLeod, Maclyn McCarty	Reported that they had purified the transforming principle in Griffith's experiment and that it was DNA.
1945	Max Delbruck	Organized a phage course at Cold Spring Harbor Laboratory which was taught for 26 consecutive years. This course was the training ground of the first two generations of molecular biologists
late 1940s	Barbara McClintock	Developed the hypothesis of transposable elements to explain color variations in corn.
1950	Erwin Chargaff	Discovered a one-to-one ratio of adenine to thymine and guanine to cytosine in DNA samples from a variety of organisms.
1951	Rosalind Franklin	Obtained sharp X-ray diffraction photographs of DNA.
1952	Martha Chase ,Alfred Hershey	Used phages in which the protein was labeled with 35S and the DNA with 32P for the final proof that DNA is the molecule of heredity.
1953	Francis Crick ,James Watson	Solved the three-dimensional structure of the DNA molecule.
1958	Matthew Meselson ,Frank Stahl	Used isotopes of nitrogen to prove the semiconservative replication of DNA.

1958	Arthur Kornberg	Purified DNA polymerase I from E. coli, the first enzyme that made DNA in a test tube.
1966	Marshall Nirenberg ,H. Gobind Khorana	Led teams that cracked the genetic code- that triplet mRNA codons specify each of the twenty amino acids.
1970	Hamilton Smith ,Kent Wilcox	Isolated the first restriction enzyme, HindII, that could cut DNA molecules within specific recognition sites.
1972	Paul Berg ,Herb Boyer	Produced the first recombinant DNA molecules.(First cloning)
1973	Joseph Sambrook	Led the team at Cold Spring Harbor Laboratory that refined DNA electrophoresis by using agarose gel and staining with ethidium bromide.
1973	Annie Chang Stanley Cohen	Showed that a recombinant DNA molecule can be maintained and replicated in E. coli.
1975		International meeting at Asilomar, California urged the adoption of guidelines regulating recombinant DNA experimentation.
1977	Fred Sanger	Developed the chain termination (dideoxy) method for sequencing DNA.
1977		The first genetic engineering company (Genentech) is founded, using recombinant DNA methods to make medically important drugs.
1978		Somatostatin became the first human hormone produced using recombinant DNA technology.
1981		Three independent research teams announced the discovery of human oncogenes (cancer genes).
1983	James Gusella	Used blood samples collected by Nancy Wexler and her co-workers to demonstrate that the Huntington's disease gene is on chromosome 4.
1985	Kary B. Mullis	Published a paper describing the polymerase chain reaction (PCR), the most sensitive assay for DNA yet devised.
1988		The Human Genome Project began with the goal of determining the entire

		sequence of DNA composing human chromosomes.
1989	Alec Jeffreys	Coined the term DNA fingerprinting and was the first to use DNA polymorphisms in paternity, immigration, and murder cases.
1989	Francis Collins Lap-CheeTsui	Identified the gene coding for the cystic fibrosis transmembrane conductance regulator protein (CFTR) on chromosome 7 that, when mutant, causes cystic fibrosis.
1990		First gene replacement therapy-T cells of a four-year old girl were exposed outside of her body to retroviruses containing an RNA copy of a normal ADA gene. This allowed her immune system to begin functioning.
1994		FlavrSavr tomatoes, genetically engineered for longer shelf life, were marketed.
1995		The first genome of *Heamophilusinfluenzae*of a free living organism to be sequenced
1996		The genome of *Saccharomyces cerevisiae* is sequenced
1997	Ian Wilmut and Colleagues	The first cloning of a mammal –Dolly the sheep is performed.
2001		First draft sequences of the human genome are released simultaneously by human genome project.
2003		Successful completion of human Genome Project with 99.99% accuracy.
2007		Controversies continue over human and animal cloning, research on stem cells and genetic modifications of crops.

Gregor Mendel

- Documented a particulate mechanism of inheritance through his experiments with garden peas

Mendelian Genetics

- Gregor Johann Mendel (1822-1884)
  - Augustinian monk, Czech Republic
  - Foundation of modern genetics

- Studied segregation of traits in the garden pea (*Pisum sativum*) beginning in 1854
- Published his theory of inheritance in 1865. "Experiments in Plant Hybridization"
- Mendel was "rediscovered" in 1902
- One general idea was that traits from parents came together and blended in offspring.

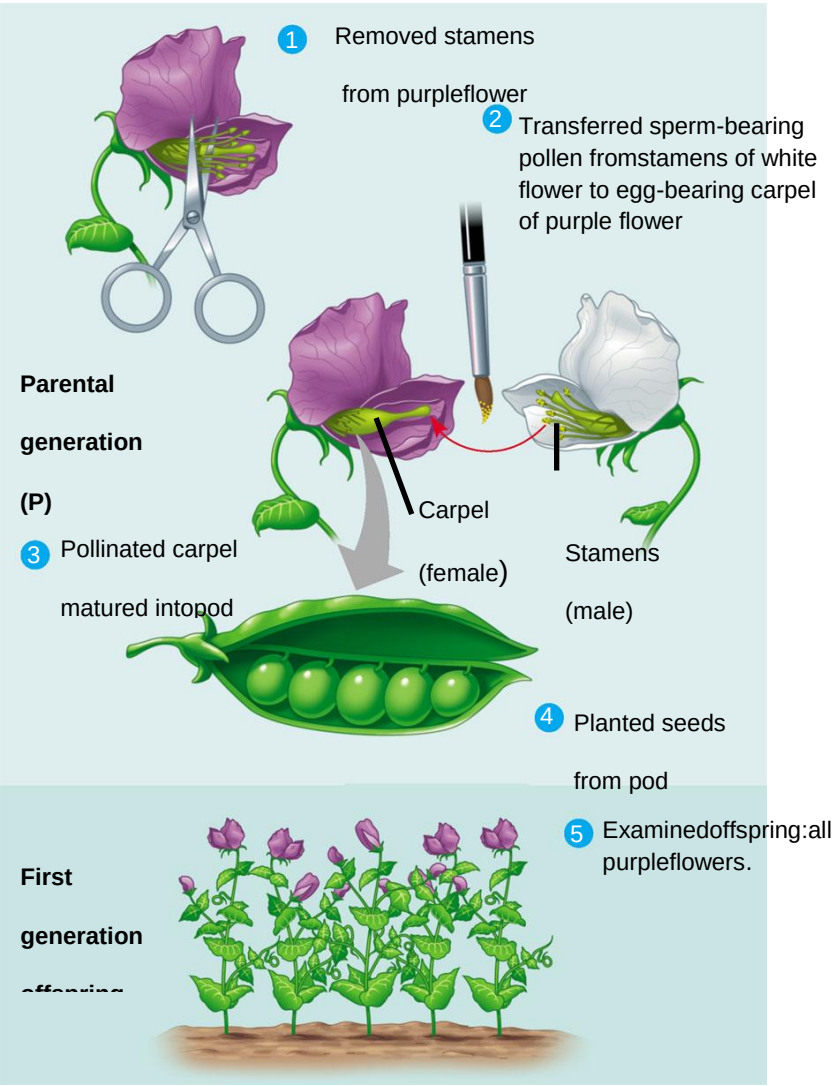
Mendel's Experimental, Quantitative Approach

- Mendel used the scientific approach to identify two laws of inheritance
- Mendel discovered the basic principles of heredity by breeding garden peas in carefully planned experiments
- Mendel chose to work with the garden pea (*Pisum sativum*)

Because they are available in many varieties, easy to grow, easy to get large numbers

- Because he could strictly control mating.

Crossing Pea Plants



Mendel's experimental design

- Statistical analyses:
  - Worked with large numbers of plants
  - counted all offspring
  - made predictions and tested them
- Excellent experimentalist
  - controlled growth conditions
  - focused on traits that were easy to score
  - chose to track only those characters that varied in an "either-or" manner

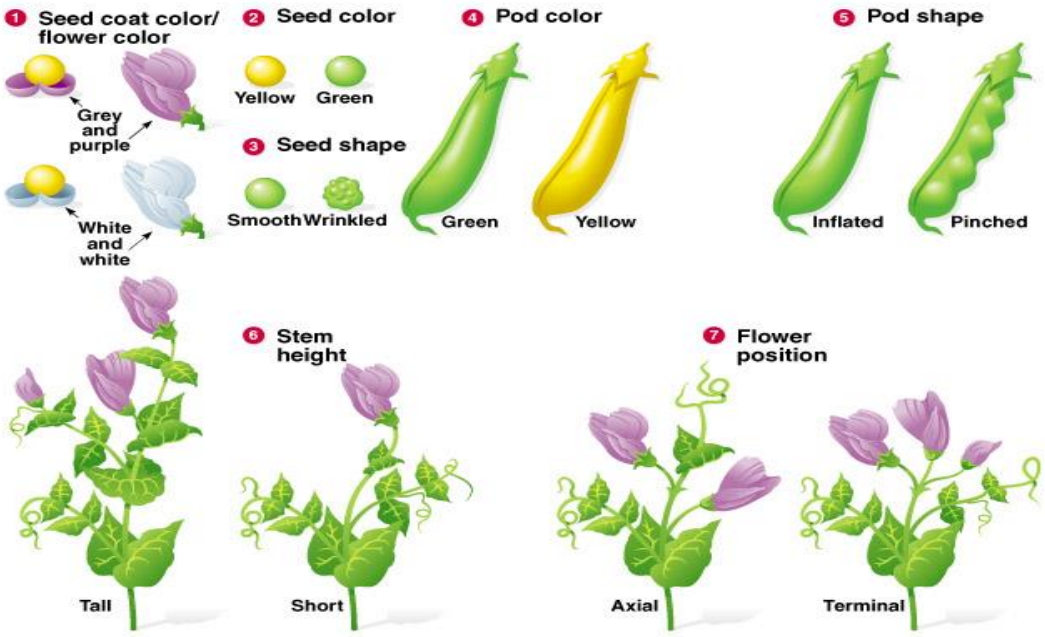


Fig: Mendel's Studied Discrete Traits

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 a 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)
- ***Generations***:

- P = parental generation
- F1 = 1st filial generation, hybrid progeny of the P generation
- F2 = 2nd filial generation, hybrid progeny of the F1 generation (F3 and so on)

Mendel's Experiments

- 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

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

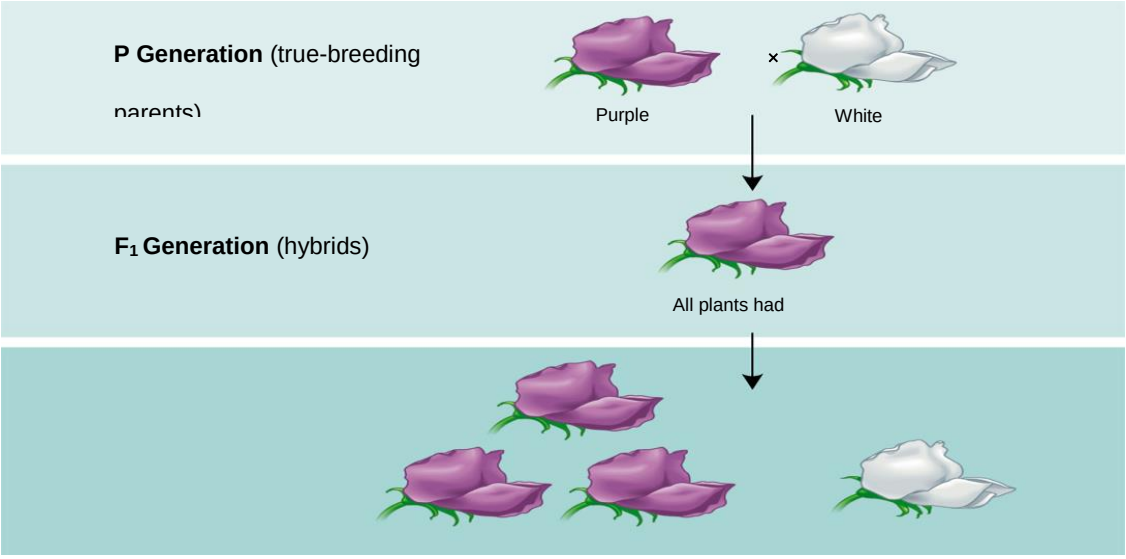
Mendel's Principles

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



Punnett Squares

- A Punnett square is a grid that enables one to predict the outcome of simple geneti crosses

- Proposed by the English geneticist, Reginald Punnett

Mendel's Law Of Segregation

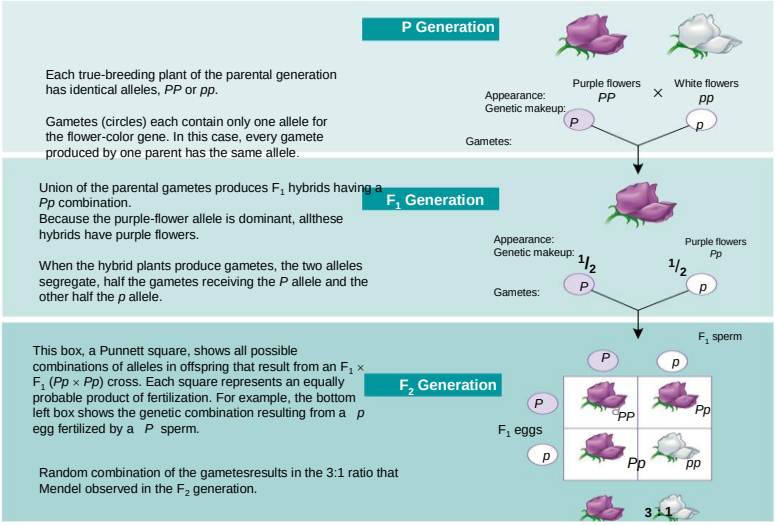
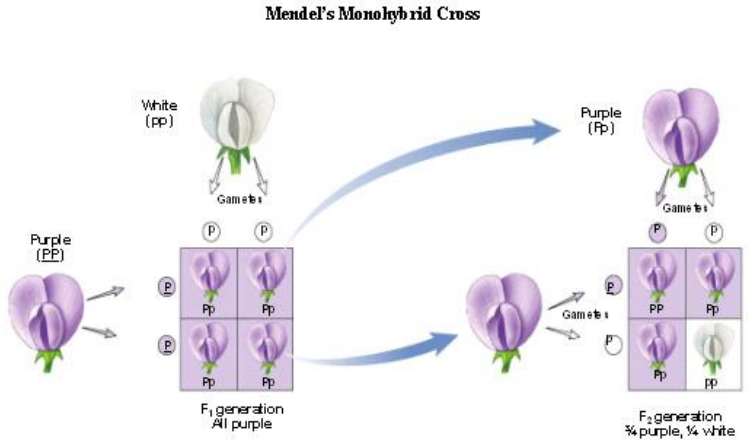


Fig: Mendel's Law Of Segregation

Mendel's Monohybrid Cross

Across of two different true-breeding strains (homozygotes) that differ in a single 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)

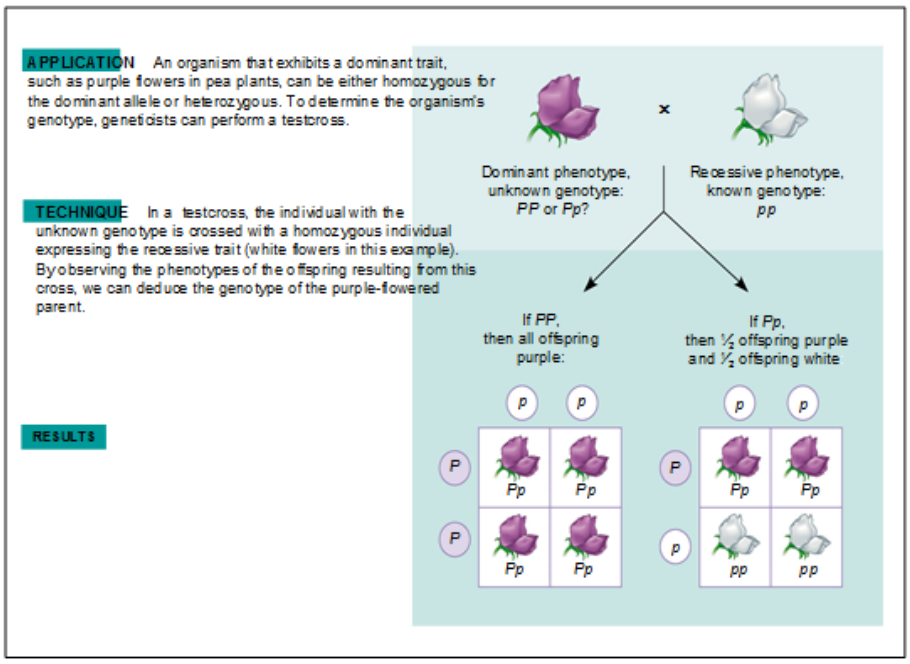


Fig : Test Cross

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 Law of Independent Assortment

- 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

Dihybrid cross

A cross of two different true-breeding strains (homozygotes) that differ in two traits.

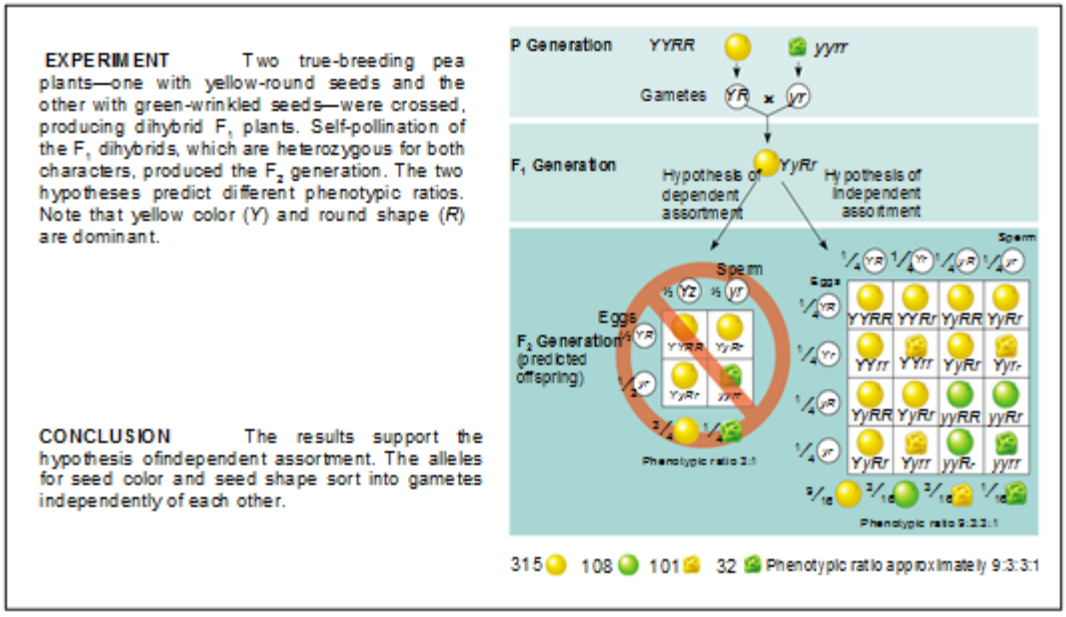


Fig: A Dihybrid Cross

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

Summary of Mendel's Principles

- Mendel's Principle of Uniformity in F1:
  - F1 offspring of a monohybrid cross of true-breeding strains resemble only one of the parents.
  - Smooth seeds (allele S) are completely dominant to wrinkled seeds (alleles).
- Mendel's Law of Segregation:
  - Recessive characters masked in the F1 progeny of two true-breeding strains, reappear in a specific proportion of the F2 progeny.
  - Two members of a gene pair segregate (separate) from each other during the formation of gametes.
- Mendel's Law of Independent Assortment:
  - Alleles for different traits assort independently of one another.
  - Genes on different chromosomes behave independently in gamete production.

Morgans Hypothesis

- Thomas Hunt Morgan in The Fly Room! (Columbia University 1910)
- Fruit Flies (*Drosophila melanogaster*)
- 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 Determination in *Drosophila*

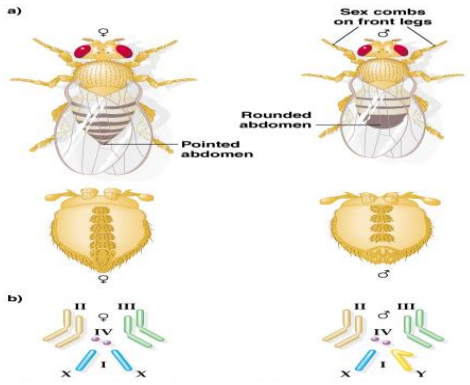
- An X-chromosome-autosome balance system is used.
- Drosophila has three pairs of autosomes, and one pair of sex chromosomes. Like humans, XX is female and XY is male. Unlike humans, Y does not determine sex.
- An XXY fly is female, and an XO fly is male. The sex of the fly results from the ratio of the number of X chromosomes (X) to the number of sets of autosomes (A):
- Dosage compensation in *Drosophila* results in more expression of X-linked genes in males, so the level of transcription equals that from a female's two chromosomes.
- In both humans and fruit flies (*Drosophila melanogaster*) females have two X chromosomes, while males have X and Y

a. Males produce two kinds of gametes with respect to sex chromosomes (X or Y), and are called the heterogametic sex.

b. Females produce gametes with only one kind of sex chromosome (X) and are called the homogametic sex.

c. In some species the situation is reversed, with heterogametic females and homogametic males.

Drosophila melanogaster (fruit fly), an organism used extensively in genetics experiments



Sex Linkage

Morgan (1910) found a mutant white-eyed male fly, and used it in a series of experiments that showed a gene for eye color located on the X chromosome.

- First, he crossed the white-eyed male with a wild-type (red-eyed) female. All F1 flies had red eyes. Therefore, the white-eyed trait is recessive.
- Next, F1 were interbred. They produced an F2 with:3,470 red-eyed flies and 782 white-eyed flies.
- The recessive number is too small to fit Mendelian ratios (explanation discovered later is that white-eyed flies have lower viability).
- All of the F2 white-eyed flies were male.

Character - Traits
Eye colour - Red eye (wild type)
White eye (mutant)

P **Phenotypes** Wild type (red-eyed) **female** x White-eyed **male**
F1 **Phenotypes** All red-eyed
Red eye is dominant to white eye

Hypothesis :A cross between the F1 flies should give us: 3 red eye : 1 white eye

F2	**Phenotypes**	Red eye	White eye
	Numbers	3470 82%	782 18%

A test cross

Phenotypes F1 Red-eyed female x White-eyed male
Expected result 50% red-eyed offspring: 50% white-eyed offspring Regardless of the sex

Observed Results

Red-eyed Males	Red-eyed Females	White-eyed Males	White-eyed Females
132	129	86	88

Genetic diagrams for sex linked genes

Character	Trait	Alleles
Eye colour	Red eye	R
	White eye	r

Genotypes	**Phenotypes**
XRXR	Red-eyed female

XRXr XrXr	Red-eyed female White-eyed female
XRY XrY	Red-eyed male White-eyed male

P **Phenotype** Wild type (red-eyed) female x White-eyed male
Genotypes X^RX^R X^rY
Gametes X^R X^R X^r Y

Fertilisation	**Xr**	**Y**
XR	XRXr	XRY
XR	XRXr	XRY

F1 **Phenotype** **Red-eyed female** x **Red-eyed male**

Genotypes X^RX^r X^RY

Gametes X^R X^r X^R Y

Fertilisation	**XR**	**Y**
XR	XRXR	XRY
Xr	XRXr	XrY

F1 **Phenotypes** (Red-eyed) Females (White-eyed) x (Red-eyed) Males (White-eyed)

Expected **All** **None** 50% 50%

Observed 2459 0 1011 782

This gene has its LOCUS on the X-chromosome. It is said to be SEX-LINKED

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
- Several researchers proposed in the early 1900s that genes are located on chromosomes

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

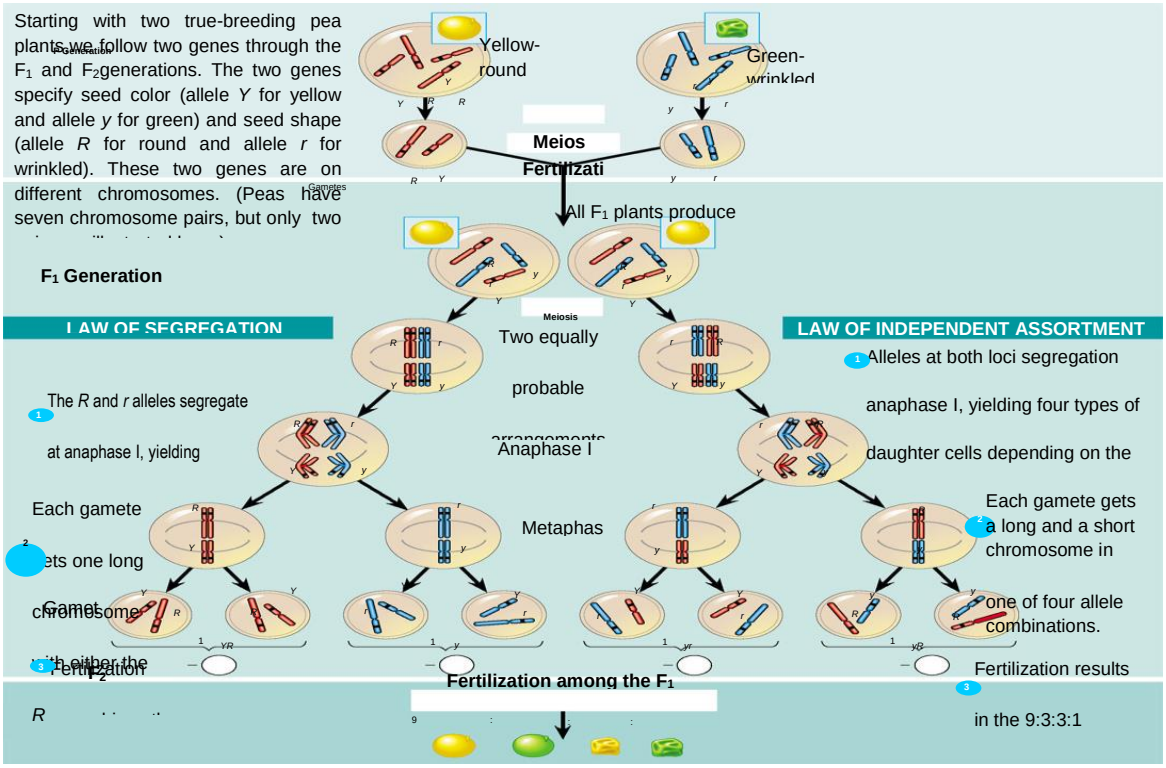


Fig:Chromosome Theory of Inheritance

KARPAGAM ACADEMY OF HIGHER EDUCATION (KARPAGAM UNIVERSITY)
DEPARTMENT OF MICROBIOLOGY
MOLECULAR GENETICS - 17MBP103

Unit I Question
_____ million base pairs of nucleotides are seen in *E.coli*
_____ degrades DNA
Adenine always pair with
Bacteria contains
Basically, flow of genetic material is accompolished by
Bonding between two bases
Chargaff's rule
Chromosomal theory of inheritance was formulated by
Dihybrid ratio
Distance between the two base pairs is
DNA absorbs UV light at _____ wavelength
Enzyme that adds methyl group to the newly formed DNA
Eukaryotic DNA damage or replication errors are corrected during
Eukaryotic DNA replication is a conserved mechanism that restricts DNA replication to
Experiments of Hershey and Chase was based on
Father of genetics
Formation of pre-replicating complex is seen in replication mechanism of
Heat Killed S cells + Live R cells produced
If a free phosphate is found at the 5' end of a DNA strand, what is found at the other end of the same stran
In eukaryotes, the vast majority of DNA synthesis occurs during _____ of the cell cycle
Initiation of replication is carried out by
Initiation of replication occurs
Joining of DNA fragments
Key enzyme in rolling circle replication
Left handed DNA
Length of primer during replication is
Longest DNA is seen in
Mendels pioneer work was with
Nucleoside is
Number of base pairs per helical turn of B form DNA
Okazaki fragments are
Proof reading activity of DNA polymerase is in the direction
Purines are
Repair and insertion of DNA is carried out by
RNA primer is removed by
Semiconservative DNA replication model
Semiconservative mechanism of DNA replication was demonstrated by
Sequencing and molecular characterization of genome

SSB protein helps in
Synthesis of DNA always moves from
The ability to remove incorrectly matched nucleotides or Proof-reading
The contribution of Rosalind Franklin towards structure of DNA was
The DNA of E.coli is _____ times longer than the cell
The enzyme that copies RNA from DNA template
The enzyme that unwinds DNA
The experiments of Avery, McLeod and McCarty was based on
The most common form of DNA is
The most widely studied origin recognition complex of eukaryotes is that of
The negative charge of DNA is due to
The Pioneer work on nucleic acid discovery was carried out by
The replication origin of *E.coli* is approximately
The replication origins of higher eukaryotes are made up of
The size of a major groove is
Thymine in DNA is replaced by _____ in RNA
Transfer of genes from one generation to the next is
Transformation in *Pneumococci* was discovered by
Triple bonding is seen in
Which is involved in synthesis of primer
Which of the following is not associated with DNA replication?
Who proposed the molecular struccture of DNA

Opt 1
64 million base pairs
Polymerase
Guanine
Single circular DNA
Replication
Hydrogen bond
No complementarity
Mendel
3 : 3 : 9 : 1
3.4Å
220 nm
Gyrase
G_1 phase
Never
Virus
Mendel
Prokaryotes
Death in mice + S cells
A hydroxyl group on the 5’ carbon of a deoxyribose sugar
G phase
DnaA
Bidirectionally
DNA ligase
DNA Polymerase-IV
B-DNA
2-10 nucleotides
Human
Monkey
Base + Sugar
13
RNA strands
5' to 3'
A, T
Endonucleases
DNA pol
Daughter molecule contains both from parent
Meselson & Stahl
Genetics

Degradation of protein
3' to 5'
RNA polymerase
X-ray crystallography
1
Dnase
Polymerase
Protein coupling
B-DNA
Bacillus
Deoxyribose Sugar
Friedrick Miescher
245 bp
Different AT-rich regions
34Å
Adenine
Inheritance
Friedrick Griffith
G-T
Ligase
Polymerase
Hershey & Chase

Opt 2
46 million base pairs
Primase
Cytosine
Single linear DNA
Transformation
Hydrophobic bond
Partial complementarity
Miescher
9 : 3 : 3 : 1
34Å
240 nm
Topoisomerase
S phase
Only once per cell cycle
Bacteriophage
Morgan
Plants
Live mice + S cells
A phosphate group on the 3’ carbon of a deoxyribose sugar.
H phase
DnaC
Cross sectionally
Gyrase
DNA Polymerase-III
C-DNA
10-20 nucleotides
Lung fish
Human
Sugar + Phosphate
12
Enzymes
3' to 5'
G, C
Ribozyme
RNA pol
Daughter molecule entirely new
Beedle & Tatum
Molecular biology

Keep the two strands separated after unwinding
5' to 3'
DNA ligase
Electron microscopy
10
Rnase
Ligase
Enzymatic reactions
Z-DNA
Staphylococcus
Phosphate bond
Watson & Crick
425 bp
Similar AT-rich regions
3.4Å
Cytosine
Carrying over
Erwin Chargaff
G-C
Primase
Promoter
Erwin Chargaff

Opt 3
4.6 million base pairs
RNase
Thymine
Double Linear DNA
Transduction
Nitrogen bond
No such rule
Metchinikoff
9 : 3 : 1 : 3
10Å
260 nm
Helicase
G_2 phase
Only twice per cell cycle
Bacteria
Watson
Virus
Death in mice + R cells
A base attached to the 3’ carbon of a deoxyribose sugar
R phase
DnaB
Unidirectionally
RNA polymerase
DNA Polymerase-II
Y-DNA
5-15 nucleotides
Yeast
Garden pea
Base + Phosphate
11
Leading strands
Parallel
C, T
Primase
Terminase
Daughter molecule contains one from parent and one newly synth
Hershy & Chase
Proteomics

Elongation of DNA
Ffrom the centre
DNA polymerase
NMRspectroscopy
1000
DNA polymerase
Gyrase
Synthetic reaction
Y-DNA
Escherichia coli
Hydrogen bond
Griffith
254 bp
Different GC-rich regions
43Å
Guanine
Subheritance
Hershey & Chase
A-T
DNA pol
Primer
Jim Latham

Opt 4	Opt 5	Opt 6
6.4 million base pairs		
DNase		
Uracil		
Double circular DNA		
Conjugation		
Van Der waals		
Complementarity of one strand with the other		
Morgan		
1 : 3 : 3 : 9		
20Å		
280 nm		
Methylase		
R phase		
Only thrice per cell cycle		
Fungi		
McLeod		
Eukaryotes		
Live mice + R cells		
A hydroxyl group on the 3’ carbon of a deoxyribose sugar		
S phase		
DnaE		
Parallely		
DNA polymerase		
DNA Polymerase-I		
Z-DNA		
10-25 nucleotides		
Bacteria		
Mice		
A+T & G+C		
10		
Lagging strands		
Centre		
A, G		
Exonucleases		
Caspase		
Some sections from parent and some newly synthesized		
Avery & McLeod		
Genomics		

Uncoiling of RNA		
Anywhere		
DNA helicase		
Gas chromatography		
100		
RNA polymerase		
Helicase		
DNA binding		
SS-DNA		
Saccharomyces cerevisiae		
Nitrogenous base		
Milstein	Kornberg	
524 bp		
Similar GC-rich regions		
20Å		
Uracil		
Gene transport		
Watson & Crick		
A-C		
rRNA		
RepA protein		
Watson & Crick		

Answer
4.6 million base pairs
DNase
Thymine
Single circular DNA
Replication
Hydrogen bond
Complementarity of one strand with the other
Morgan
9 : 3 : 3 : 1
3.4Å
260 nm
Methylase
G_2 phase
Only once per cell cycle
Bacteriophage
Mendel
Eukaryotes
Death in mice + S cells
A hydroxyl group on the 3’ carbon of a deoxyribose sugar
S phase
DnaA
Bidirectionally
DNA ligase
DNA Polymerase-III
Z-DNA
2-10 nucleotides
Lung fish
Garden pea
Base + Sugar
10
Lagging strands
3' to 5'
A, G
Endonucleases
DNA pol
Daughter molecule contains one from parent and one newly synthesized
Meselson & Stahl
Genomics

Keep the two strands separated after unwinding
5' to 3'
DNA polymerase
X-ray crystallography
1000
RNA polymerase
Helicase
Enzymatic reactions
B-DNA
Saccharomyces cerevisiae
Phosphate bond
Friedrick Miescher
245 bp
Similar AT-rich regions
34Å
Uracil
Inheritance
Friedrick Griffith
G-C
Primase
Promoter
Watson & Crick

DEPARTMENT OF MICROBIOLOGY
KARPAGAM ACADEMY OF HIGHER EDUCATION
KARPAGAM UNIVERSITY

(Deemed University Established Under Section 3 of UGC Act, 1956)
Eachanari PO, Coimbatore -641 021, India.

I - M.Sc Microbiology (Batch 2017-2019)

Lecture Plan

Unit - II

S. No	Duration	Topic	Reference
1.	2	Genetic code and Transcription of DNA and its regulation	T1:115 to 119 R1:124 to 128
2.	2	Regulation of Transcription and its control	T1:87 to 106
3.	2	Translation of RNA and steps involved	T1:111 to 123 R1:122 to 124
4.	2	Post Translational modification	T1:123 to 126 R1:130 to 131
5.	2	Operon concept Lac/Ara operon	T1:516 to 529 R1:143 to 152
6.	1	Trp operon and eukaryotic gene regulation	T1: 516 to 529 R1: 528 ti 529
7.	1	Video presentation of Transcription, Translation and operons	W1
8.	1	Class Test II	-
		Total Hours	**13**

R1: David Freifelder, Microbial Genetics. Narosa Publishing House, 10th edition, 2004. New Delhi, India.

T1: Peter J. Russell, i Genetics – A molecular approach, 7th edition, 2010. Pearson Benjamin Cummings Publishers, Boston, USA.

W1: www.shomusbiology.com/DNAtranscription.index.php

P. SRINIVASAN
Assistant Professor
Department of Microbiology

2017

Unit – II

DNA &RNA : The Genetic Material

The Search for the 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.

Genetic Materials

- Chromosome consists of protein and nucleic acid
- Candidate: Protein v.s. nucleic acid
    - Protein: 20 kinds of amino acid
    - Nucleic acid: 4 kinds of nucleotides
- Complexity of life ◊ very complicated ◊ protein or nucleic acid to account for the level of complexity?

Griffith's Transformation Experiment

- Frederick Griffith's 1928 experiment 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*:
    - S strain is virulent
    - 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.

Prepared by – Mr. P. Srinivasan , Assistant Professor, Dept. of Microbiology, KAHE.

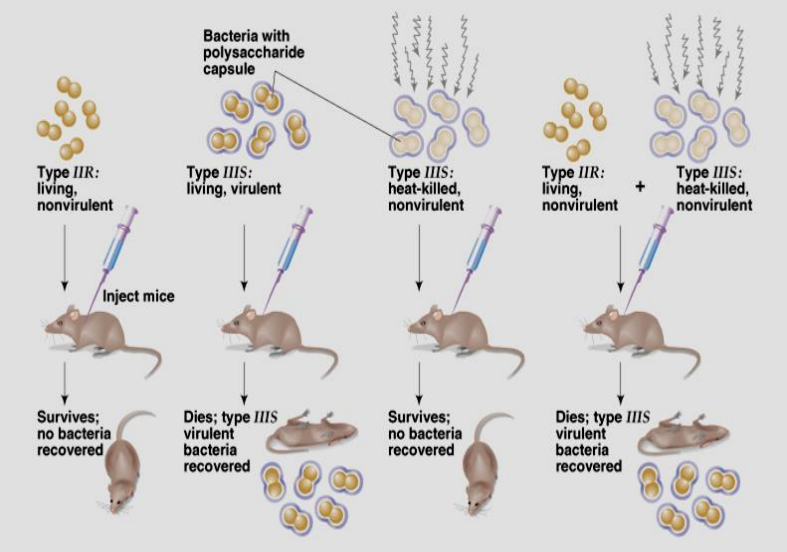


Fig. Griffith's transformation experiment

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.

Prepared by – Mr. P. Srinivasan , Assistant Professor, Dept. of Microbiology, KAHE.

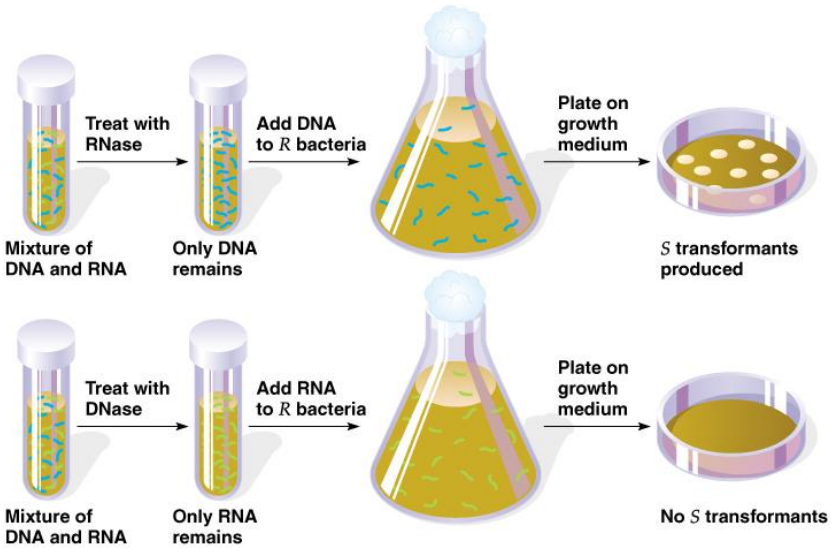
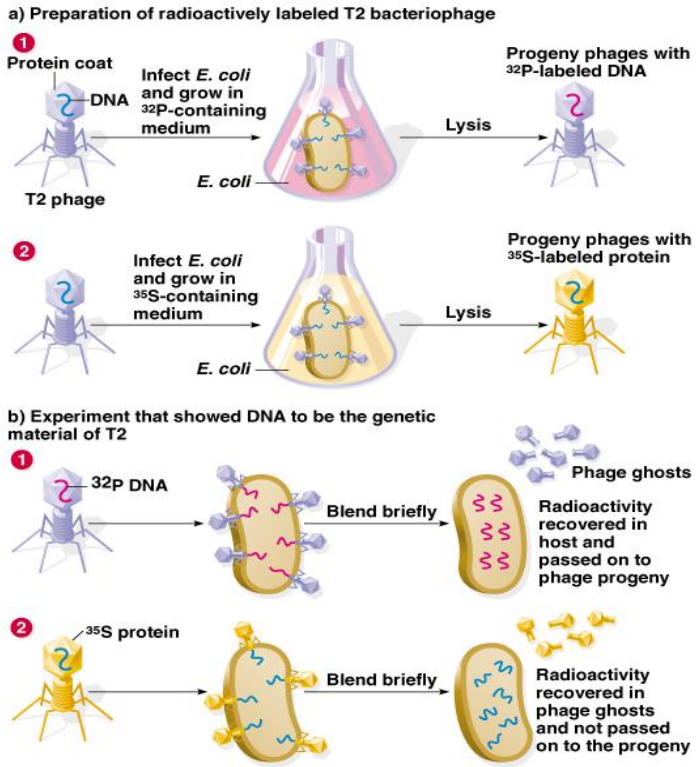


Fig. Experiment that showed that DNA, not RNA, was the transforming principle

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 labeled with ^{35}S, and in the other part, T2 DNA was labeled with ^{32}P. Then each group of labeled 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 ^{35}S -labeled protein was found outside the infected cells, while the ^{32}P -labeled 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.



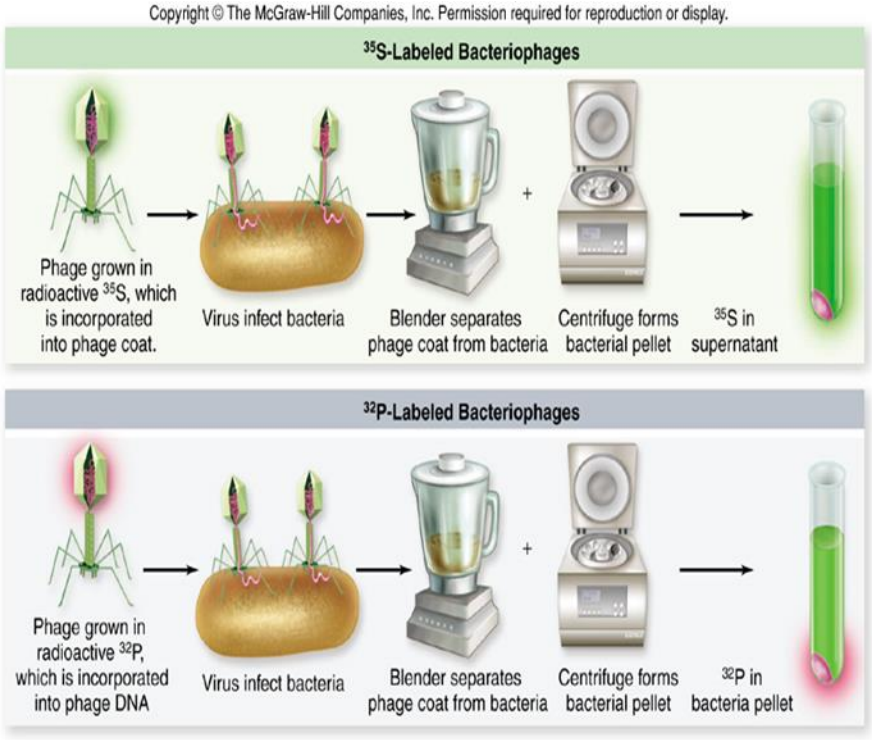


Fig. Hershey-Chase experiment demonstrating DNA is genetic material

The Discovery of RNA as Viral Genetic Material

- TMV (tobacco mosaic virus)
- 1956, A. Gierer and G. Schramm
  - Infected tobacco plant with purified RNA ◊ typical virus-infected lesion
  - RNA treated with RNAase then injected into tobacco ◊ not lesion
- 1957 Heinz Fraenkel-Conrat and B. Singer reconstitue the RNA of one type with the protein of the other type and vice versa and injected to two tobacco plants ◊ the progeny viruses isolated from the resulting lesion were the type specified by the RNA, not by the protein.

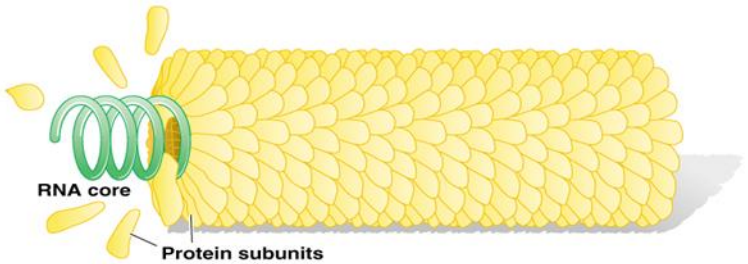


Fig. Typical tobacco mosaic virus (TMV) particle

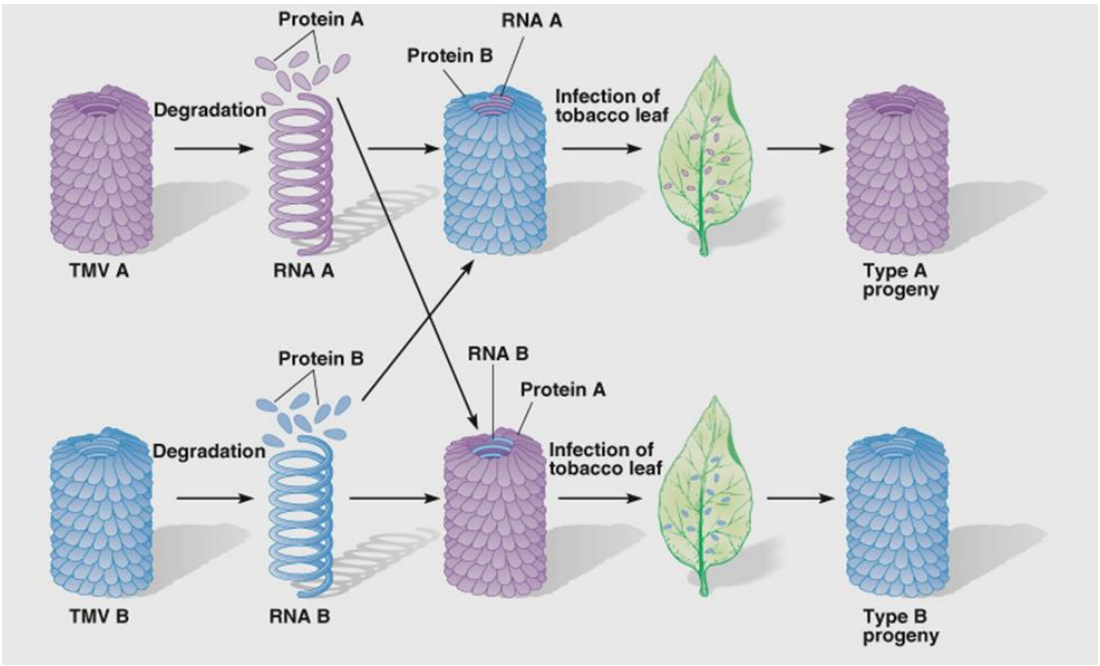


Fig. Demonstration that RNA is the genetic material in tobacco mosaic virus (TMV)

The 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 a hydrogen.

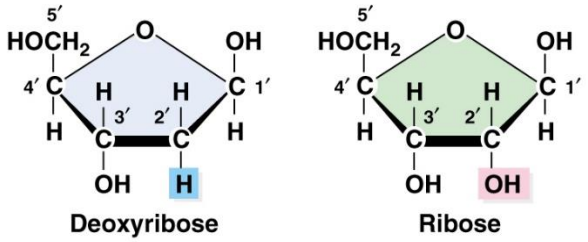


Fig:Structures of deoxyribose and ribose in DNA and RNA

- 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.

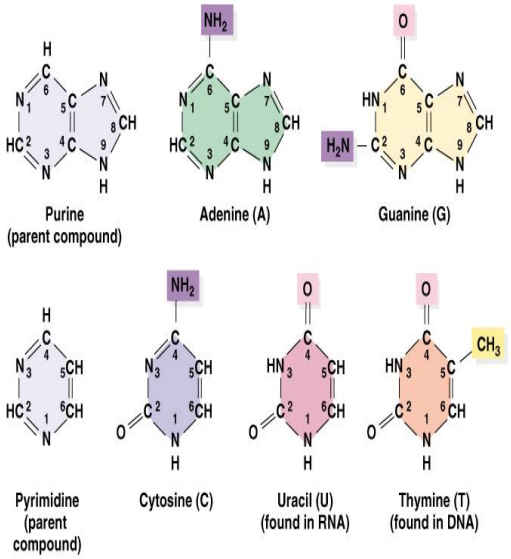


Fig: 2.1 Structures of the nitrogenous bases in DNA and RNA

- 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 a 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.
    - c. Nucleotide examples are shown in Figure 2.2.
- 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.

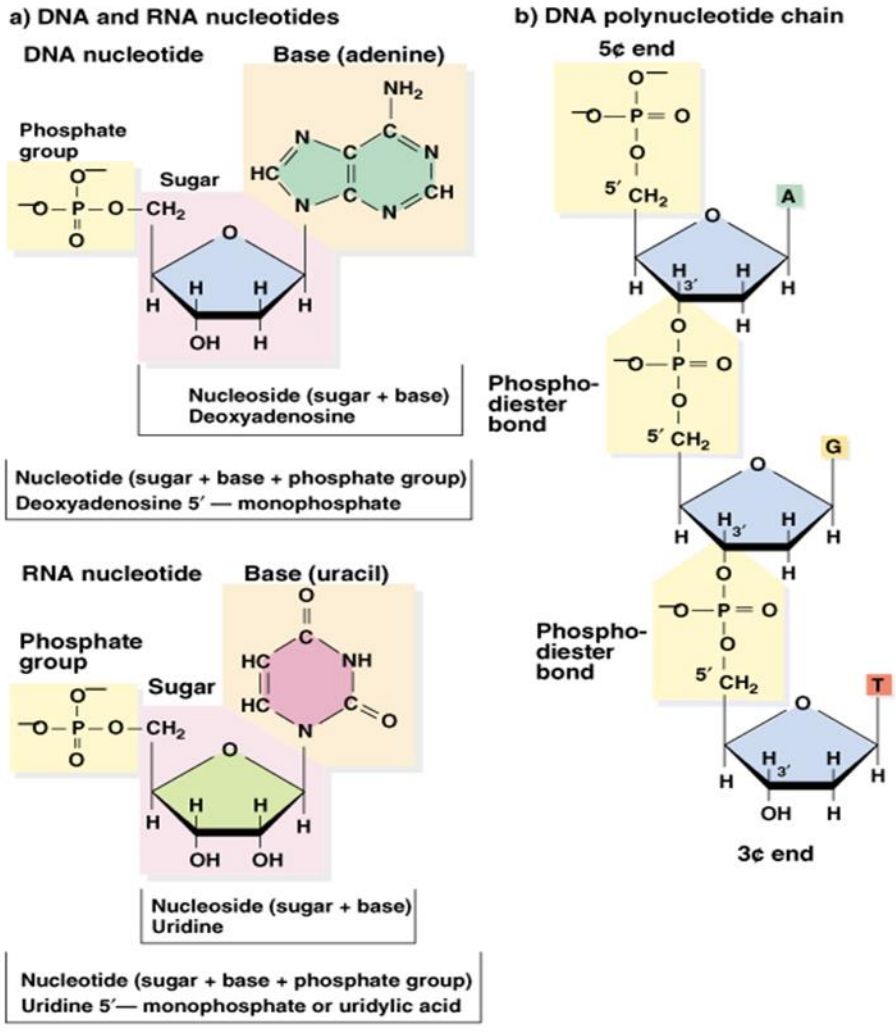


Fig. Chemical structures of DNA and RNA

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 2nm 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.

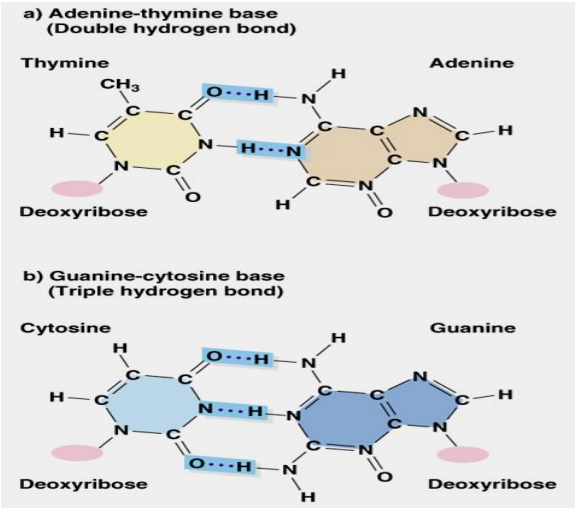


Fig: Complementary Base Pairing

- 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 dsDNA 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.

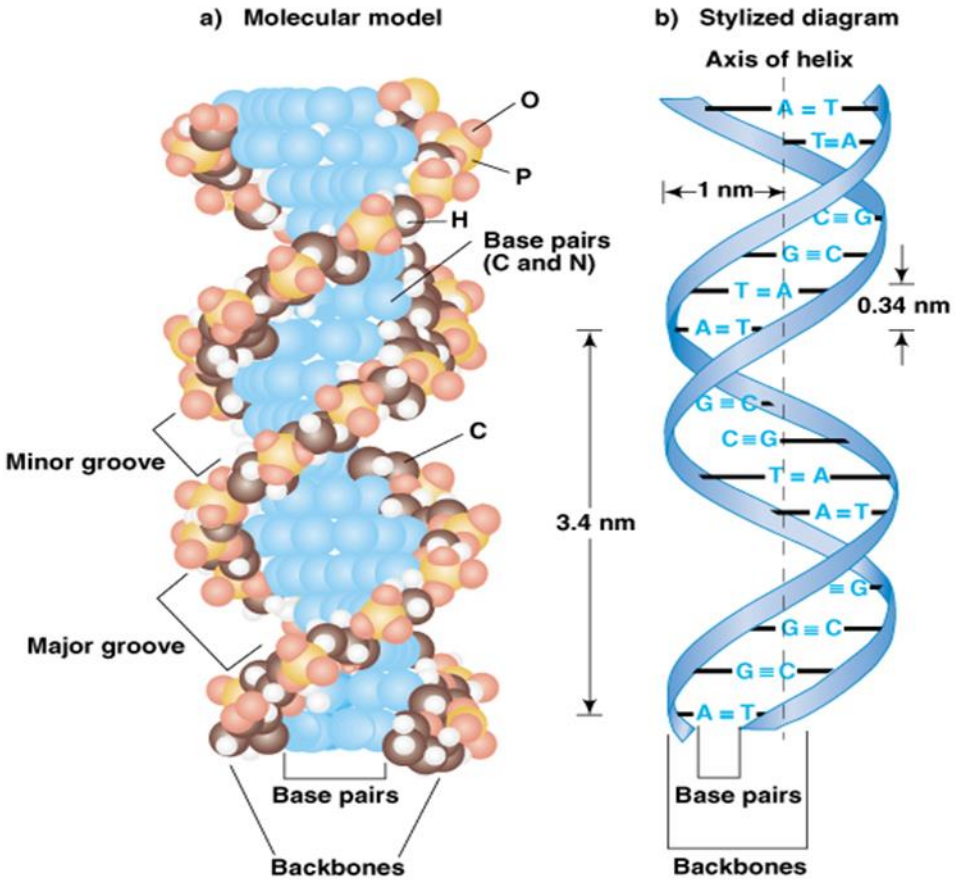


Fig: Physical structure of DNA Double Helix

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

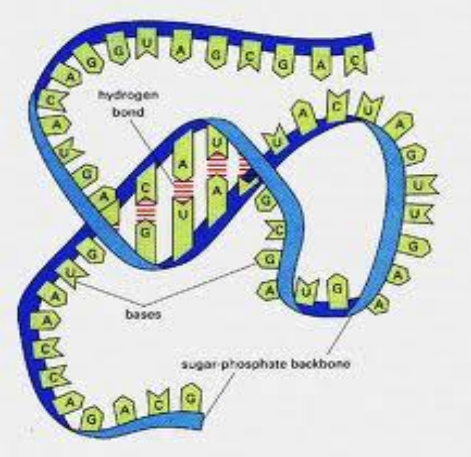


Fig :Structure of RNA

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 a 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 geneticRNA.This type of RNA depends upon the information getting from DNA for its function.

Non GeneticRNAs

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 eukaryotic cells, once precursor mRNA (pre-mRNA) has been transcribed from DNA, it is processed to mature mRNA. This removes its introns—non-coding sections of the pre-mRNA.
- The mRNA is then exported from the nucleus to the cytoplasm, where it is bound to ribosomes and translated into its corresponding protein form with the help of tRNA.
- In prokaryotic cells, which do not have nucleus and cytoplasm compartments, mRNA can bind to ribosomes while it is being transcribed from DNA.

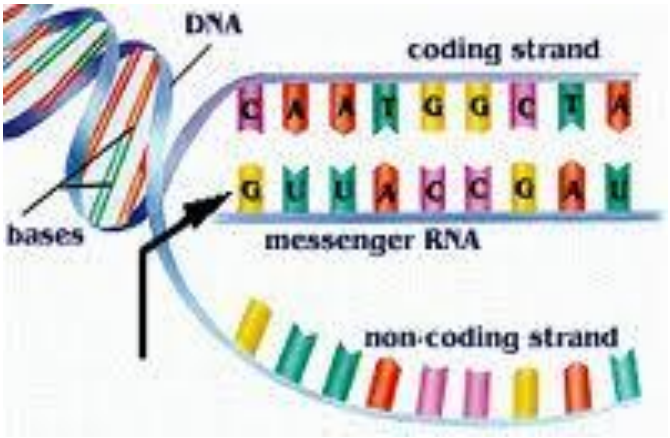


Fig: Messenger RNA

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 bonding.

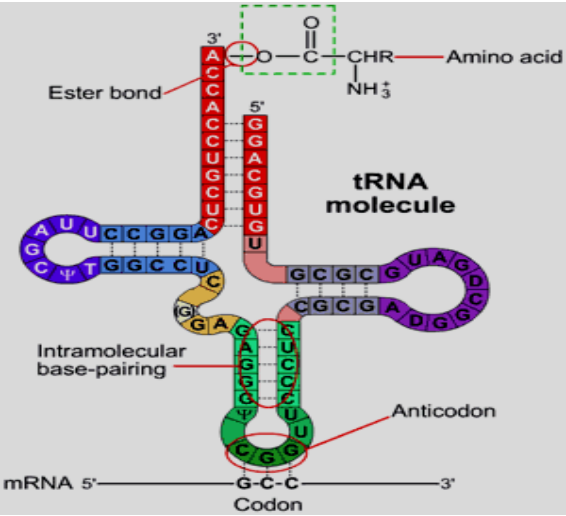


Fig: Structure of tRNA

Ribosomal RNA

- Ribosomal RNA (rRNA) is the catalytic component of the ribosomes.
- Eukaryotic ribosomes contain four different rRNA molecules: 18S, 5.8S, 28S and 5S rRNA.
- 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.
- rRNA is extremely abundant and makes up 80% of the 10 mg/ml RNA found in a typical eukaryotic cytoplasm.

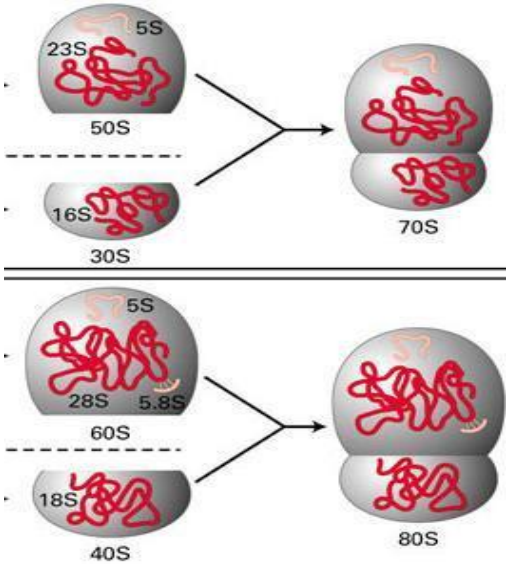


Fig: Prokaryotic and Eukaryotic ribosomes

Difference between RNA & DNA

RNA	DNA
RNA nucleotides contain ribose sugar	DNA contains deoxyribose
RNA has the base uracil	DNA has the base thymine
presence of a hydroxyl group at the 2' position of the ribose sugar.	Lacks of a hydroxyl group at the 2' position of the ribose sugar.
RNA is usually single-stranded	DNA is usually double-stranded

DNA Replication

Genetic information is transferred from parent to progeny organisms by the process of replication of the parent DNA molecules.

Basic rule of DNA replication

- Nucleotide monomers are added one by one to the end of a growing strand by an enzyme called a ***DNA polymerase.***

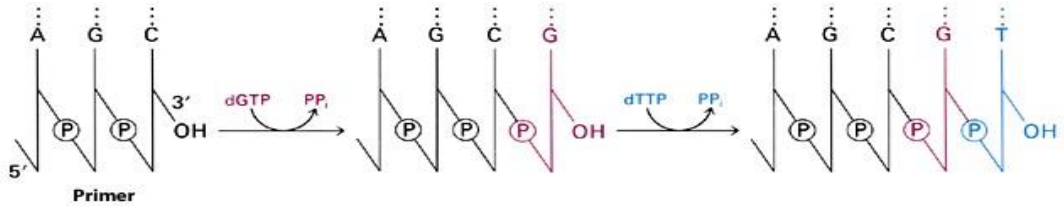
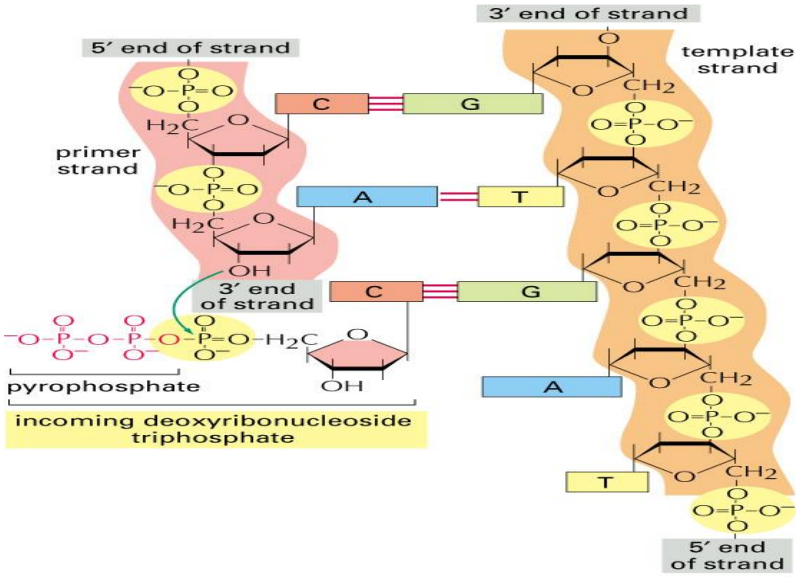


Fig: ***Addition of Nucleotides to a Growing Daughter Strand***

- The sequence of base in each new or ***daughter strand*** is complementary to the base sequence in the old or ***parent strand*** being copied – that is, if there is an adenine in the parent strand, a thymine will be added to the end of the growing daughter strand when the adenine is being copied.



- **Polymerisation:** It is the process of addition of polymers (nucleotides- 4dNTPs) to the end of the growing strand with the help of polymerizing enzyme.

***Four 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 a 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 I* (formerly the *Kornberg enzyme*) (DNA polymerase II & III discovered soon after)

5. *Mg 2+* (optimizes DNA polymeraseactivity)

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**.**

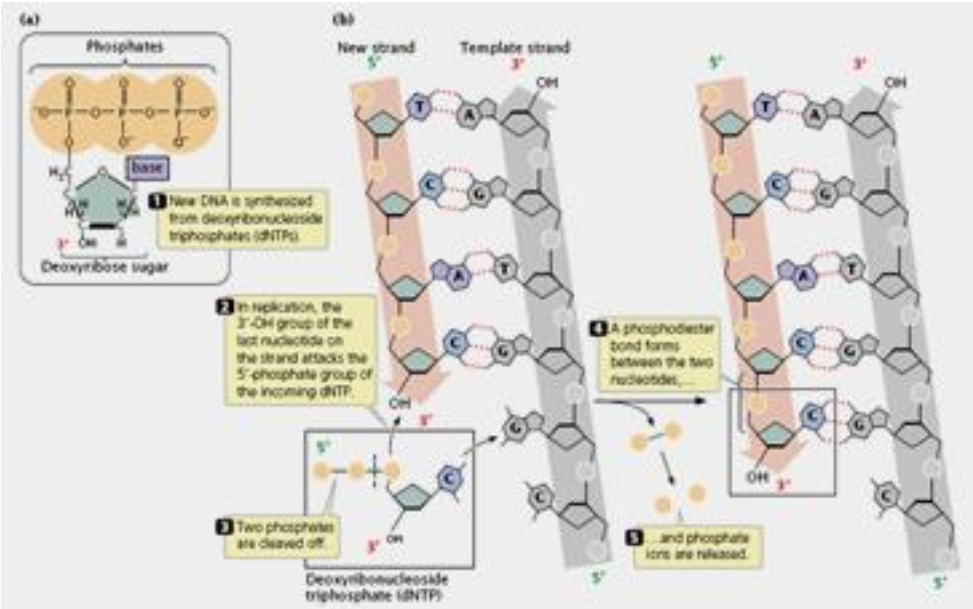


Fig: Polymerization - new DNA is synthesized from deoxyribonucleoside triphosphates (dNTPs).

Enzymology of DNA replication

As replication of the two daughter strands proceeds along the helix there are various types of enzymes involved to carry out replication. They include:

Helicases:

- Unwind double strand DNA at the expense of ATP
- Bacterial DnaB protein
- Activity can be stimulated by DnaG and SSBs
- separates complementary strands of DNA, producing a replication fork

Single-strand DNA binding proteins (SSBs):

- Participate in DNA strand separation but do notcatalyze the strand separation process.
- They bind to single strand DNA as soon as it forms and coat it so that it cannot anneal to reform a double helix.

Topoisomerases:

- Introduce transient single or double stranded breaks into DNA and thereby allow it to change its form, or topology.\

DNA gyrase:

- This is otherwise known as Eco-topoisomerase II which is able to produce negative

superhelicity generated during replication.

DNA polymerases:

Total of 5 different DNAPs have been reported in *E. coli*

- *DNApolymerase I: functions in repair and replication*
- *DNA polymerase II: functions in DNA repair (proven in 1999)*
- *DNA polymerase III: principal DNA replication enzyme*
- *DNA polymerase IV: functions in DNA repair (discovered in 1999)*
- *DNA polymerase V: functions in DNA repair (discovered in 1999)*

DNA polymerase I (102 KD): In 1957,Arthurkornberg showed that the extracts of *E.coli* has five different enzymatic activities,

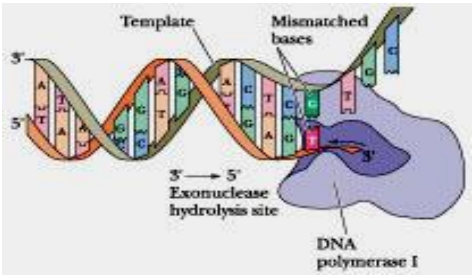


Fig: Proofreading and Editing of DNA polymerase I

- ***5'-3' exonulcease activity*** (remove RNA primers or damage DNA on its path)
- ***3'-5' exonuclease activity*** (proof reading to increase fidelity**)**
- ***Endonuclease activity***(involved in excision repair)
- ***Nick Translation*** (nick or gap moves along moves along the direction of synthesis)It requires 5'-3' activity of DNA pol I

Steps

1. At a nick (free 3' OH) in the DNA the DNA pol I binds and digest nucleotides in a 5'-3' direction
2. The DNA polymerase activity synthesizes a new DNA strand
3. A nick remains as the DNA pol I dissociates from the ds DNA.
4. The nick is closed via DNA ligase

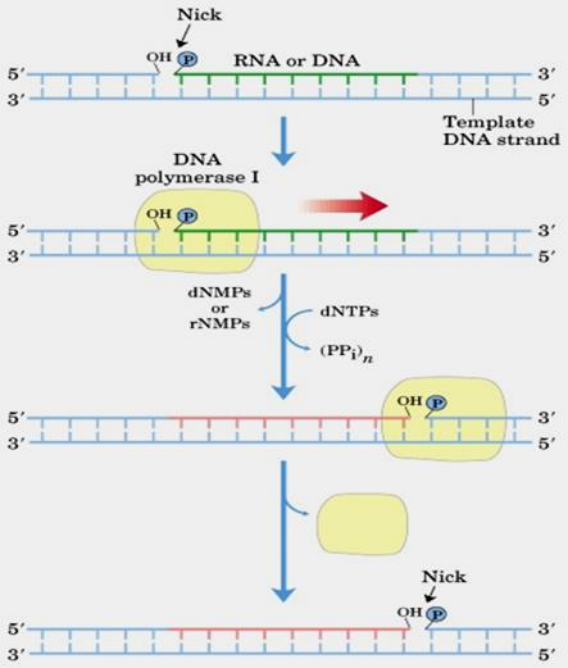


Fig: Nick translation

- ***Strand Displacement***(play a vital role in genetic recombination).

DNA Polymerase II :primary function is repair, but it also can serve as an alternative replicationenzyme if the template is damaged. (DNA p'ase II is essentially a proofreading and repair enzyme)

DNA Polymerase III

- At least 10 different subunits
- "Core" enzyme has three subunits - α, ε, and θ
- Alpha subunit is polymerase
- Epsilon subunit is *3'-5' exonuclease activity*
- Theta function is unknown
- The beta subunit dimer forms a ring around DNA
- Enormous processivity - 5 million bases!
- DNA polymerase III can only add *deoxyribonucleoside triphosphates* to a free 3' end of an existing nucleotide strand

RNA Primase:

- It is otherwise known as RNA polymerase(Dna G protein)
- This enzyme synthesize a single stranded synthetic oligonucleotide RNA primer of 3-5 bases long.
- DNA polymerase III is only able to add DNA nucleotides to a free 3' end on an existing DNA strand

DNA ligase:

- Joins the 5' phosphate of one DNA molecule to the 3' OH of another, using energy in the form of NAD (prokaryotes) or ATP (eukaryotes).
- It prefers substrates that are double-stranded, with only one strand needing ligation, and lacking gaps.
- forms covalent bonds linking together Okazaki fragments
- completing DNA synthesis along the lagging strand

Initiation of Replication

- Replication initiated at specific sites: Origin of Replication (ori)
- Two Types of initiation:
  - ***De novo***:Synthesis initiated with RNA primers. Most common.

  - ***Covalent extension***: synthesis of new strand as an extension of an old strand ("Rolling Circle")

The steps and components involved in the initiation reaction include:

- Binding of dnaA to the four highly conserved 9-mer sequences. About 20-40 molecules of dnaA (52Kdalton monomer) bind to the right end of the 245 bp ori sequence forming a large "nucleosome-like" complex. Nearly 200 base pairs are protected from DNAse I in this complex.
- ATP is bound in two forms to the dnaA complex, both a high and a low affinity bound form.
- In a second step, an open complex is formed in which dnaA now associates with the remaining 60 base pairs of AT rich DNA including the three 13 base pair repeats.
- Conditions required for the open complex are the presence of superhelical density >-0.04, HU protein (or possibly the related IHF - integrative host factor protein),temperature above 21° C.
- This second reaction can be inhibited by the presence of another origin specific DNA binding protein IciA which is a helix-turn-helix protein that binds to the 13mers inhibiting their melting.
- The third step is the formation of the prepriming complex. A complex of dnaB and dnaC protein interacts with the open complex (much like it does with the PriA, B, C complex in X SS—>RF).
- dnaB helicase then continues to open the region begun by dnaA using its helicase activity. This opening requires SSB, DNA gyrase activity as well as ATP for the dnaB helicase. Two dnaB complexes are bound at each end of the opening on what will become the lagging strand of synthesis.
- dnaG (primase) then binds dnaB and primes DNA synthesis by DNA polymerase III holoenzyme at each fork. Although initiated by dnaB-dnaGprimase on the"lagging" strand, the first DNA polymerase II holoenzyme quickly proceeds to the opposite fork, becoming the leading strand enzyme for this opposite fork.

Other factors that influence the rate and specificity of the oriC replication include HU protein, RNA polymerase transcription of nearby regions, ribonuclease H degradation of short non specific RNA synthesis

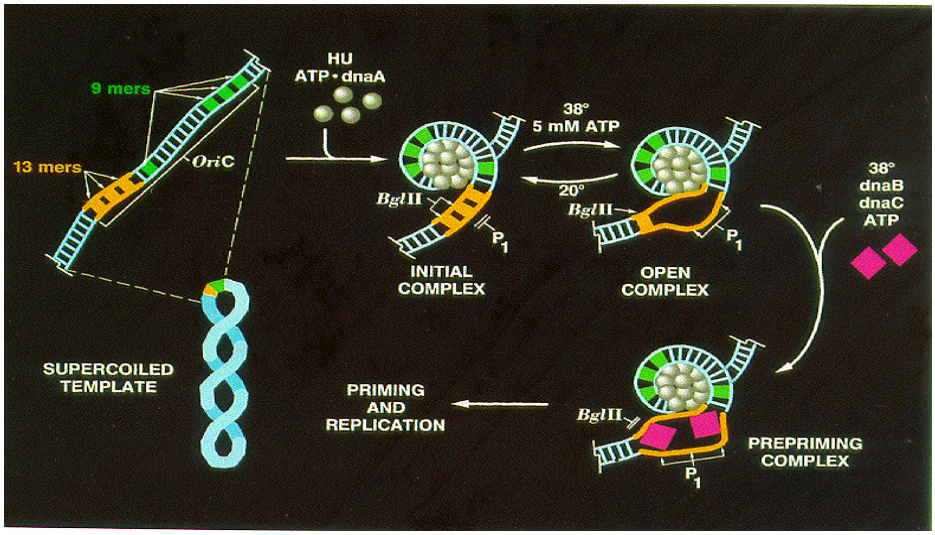


Fig: Initiation of replication

Different modes of DNA replication

Meselson and Stahl Experiment

- *E. coli* are grown in heavy nitrogen (^{15}N) for many generations.
- This caused the nitrogen in the DNA molecule of each cell to contain ^{15}N, a heavier than typical isotope.
- The *E. coli* were then grown for one or two cell divisions in ^{14}N, the lighter and typical isotope.
- DNA was spun in a cesium chloride gradient. *Meselson and Stahl actually invented this technique, called density centrifugation, which now has many other applications, just for the purposes of this experiment.*
- The cesium chloride gradient and centrifugation separates molecules based on their density.
    - The DNA molecules with ^{15}N are more dense than those with ^{14}N, and band below DNA with ^{14}N.
    - If two bands were observed after one division in ^{14}N, there would have been wholly old strands and wholly new strands. This would have been consistent with and meant the replication was conservative.
    - If there was just one band after one division, replication could be either dispersive or semiconservative.

- The result was just one band after one division.
    - If one or a long smear was observed after two divisions in ^{14}N containing medium, dispersive replication would have been the mode.
    - If intermediate weight and light weight molecules were found, semiconcervative would be the mode.
    - This is what was found; the replication was *semiconservative*. This *was* the predicted outcome of Watson and Crick.

Types of Replication

Various types of replication include

- Semi discontinuous replication
- Unidirectional replication
- Bidirectional replication

Semi discontinuous replication

If DNA replicates continuously on both strands in the same direction as the moving fork (ie., both in 5'- 3' and 3'- 5' direction) because of the ant parallel nature of two strands of DNA, one of the strand have free 3'oh group and the other strand would have free 5'p group but Pol-I and Pol-III add nucleotides only to a 3'oh group.Thus the replication takes place by means of one of the following reason which include:

1. There would be another polymerase that add nucleotides to the 5'end that is it would catalyze stand growth in 3-'5' direction, however no such polymerase exist.

2. If the 2 strands both grow in 5'-3' direction but from opposite strands of parent molecule, a significant fraction of the uneplicated molecule could have to be single stranded.

If the 2 strands both grow in 5'-3' direction but not in the same direction as parent molecule. thus this shows that some newly made DNA consist of fragments and this is determined by Okazaki.

Detection of fragments

In 1968,Reijiokazaki demonstrated in *E.Coli*that newly synthesizedDNA is in the form of fragments which latter gets attached to one another to generate continuous strands. There are two predictions in this experiment include,

1. As a result of replication half of the newly synthesized DNA appears first as short pieces, these pieces are detected before they get stitched together. This is done by labeling the short pieces of DNA with radioactive DNA precursor fragments and the labeled fragment is referred as *pulses*.
2. DNA *ligase* which is responsible for stitching the short pieces of DNA synthesized should be eliminated. This is done to detect long pulses of DNA precursors.

Pulse-labeling technique

In this case, a culture of bacterial cells infected with a bacterial virus is given radioactively labeled DNA precursor(Tritiated thymidine ^{3}H-dT). In this case, using sucrose, the DNA molecules never find their equilibrium position because sucrose solutions are much less dense than CsCl solutions and so the molecules are always in motion. only DNA synthesis that has taken place during the time of the pulse will produce radiolabeled molecules that can be located in the gradient.

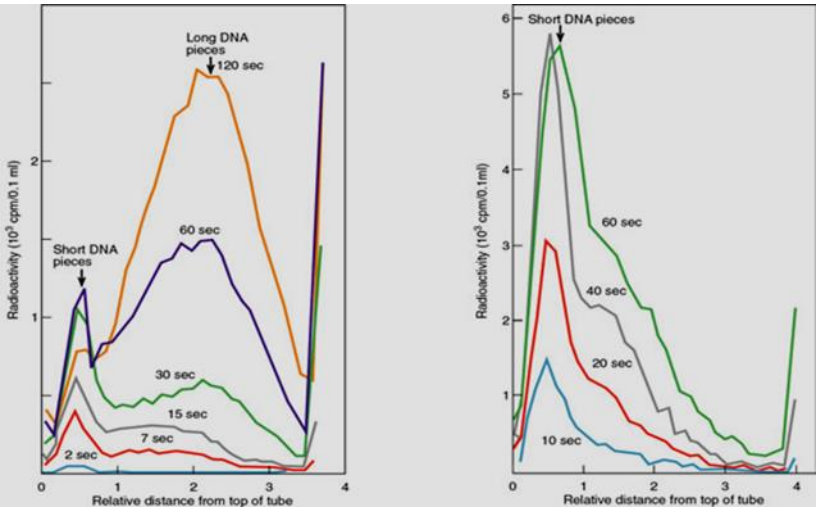


Fig: Demonstration of semidiscontinuous replication ^{3}H labeledokazaki fragments in sucrose density gradients

The resultsshowedvery short times of labeling (short pulses) very short pieces of DNA are found (2 sec, 7 sec, 15 sec). However, with longer and longer times, the pieces of DNA get increasing longer (120 sec). He then tried the same experiment with a mutant virus that was defective in a gene called DNA ligase. We will see that this is the enzyme that joins pieces of DNA together into larger structures. In this case (on the right) the labeled pieces of DNA remained short, even after long times of radiolabeling.The data suggested to Okazaki that DNA replication occurred by the synthesis of small pieces that were later linked together by DNA ligase into larger pieces.

Pulse-chase experiment

In this experiment ,Uninfected bacterial culture was radiolabelled only for a short time, and then followed this by adding a large excess of unlabeled precursor. This resulted in a great decrease in the amount of radiolabel incorporated. The ‘s’ value (sedimentation rate) of radioactive material increases with the time of growth.This is due to the attachment of newly formed DNA with preformed DNA before labeling,the small initial products formed is referred as **Okazaki fragments**.

Okazaki's conclusion

Both the strands replicates with the help of DNA polymerase and this enzyme would make one strand (leading strand) continuously in the 5'-3' direction and the other strand (lagging strand) in order to synthesis in 5'-3' direction is made discontinuously, this discontinuity is due to the synthesis opposite to the direction of the replication fork.

Unidirectional replication

Replication occurs by the separation of DNA strand forming a bubble at the middle of the strand and the new stand synthesis is made. In this replication only 1/4th is active and the DNA replicates from the stationary fork with a defined origin.

Bidirectional replication

In bidirectional replication both fork will be active and the replication proceeds in both the direction with the origin in the middle of each branch point.

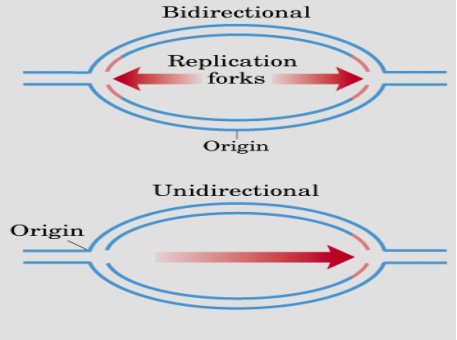


Fig: Bidirectional replication

Events in the replication fork:

- Segments of single-stranded DNA are called template strands.
- Gyrase (a type of topoisomerase) relaxes the supercoiled DNA.
- Initiator proteins and DNA helicase binds to the DNA at the replication fork and untwist the DNA using energy derived from ATP (adenosine triphosphate).(Hydrolysis of ATP causes a shape change in DNA helicase)
- The helicase and SSB moves along the parental strand prepriming it, so that DNA primase (primase is required for synthesis) synthesizes a short RNA primer of 10-12 nucleotides, to which DNA polymerase III adds nucleotides.
- The first step in primer synthesis is the formation of the complex known as preprimosome containing I,n,n',n'',DnaB,DnaC.
- This preprimosome then joins with primase to form primosome.
- The n' protein moves the primosome along the parental strand until the priming site is found with its bound ATP.

- At that time leading strand synthsis starts and advances along the parental strand by nucleotides addition in 5'-3' direction.
- Polymerase III adds nucleotides 5' to 3' on both strands beginning at the RNA primer.
- The RNA primer is removed and replaced with DNA by polymerase I, and the gap is sealed with DNA ligase.
- Single-stranded DNA-binding (SSB) proteins (>200) stabilize the single-stranded template DNA during the process.

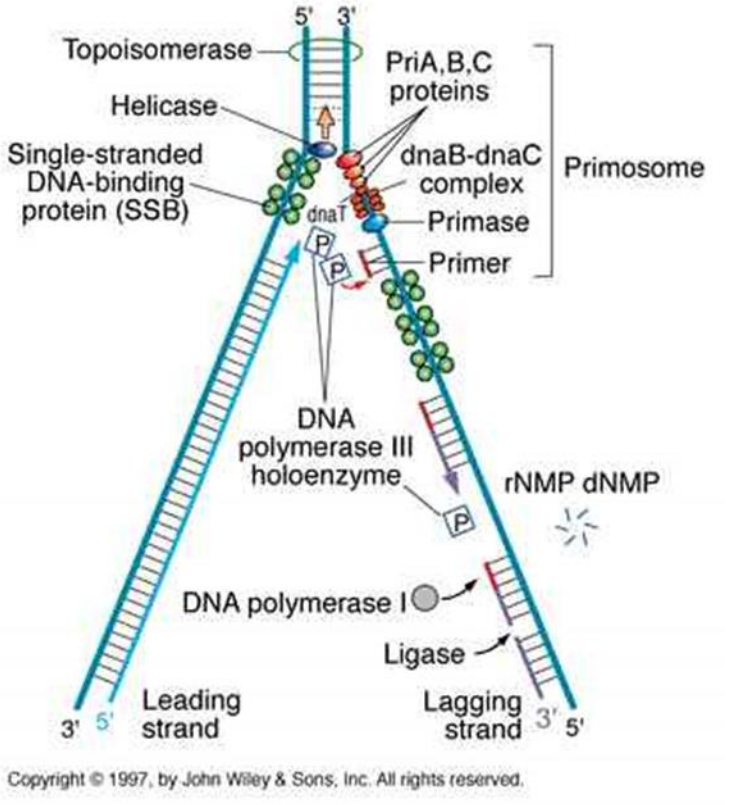
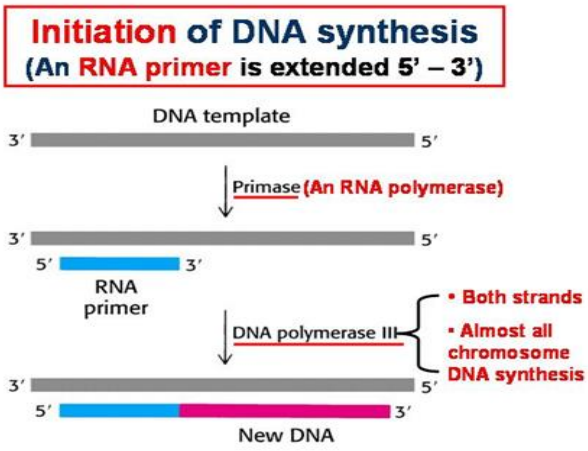
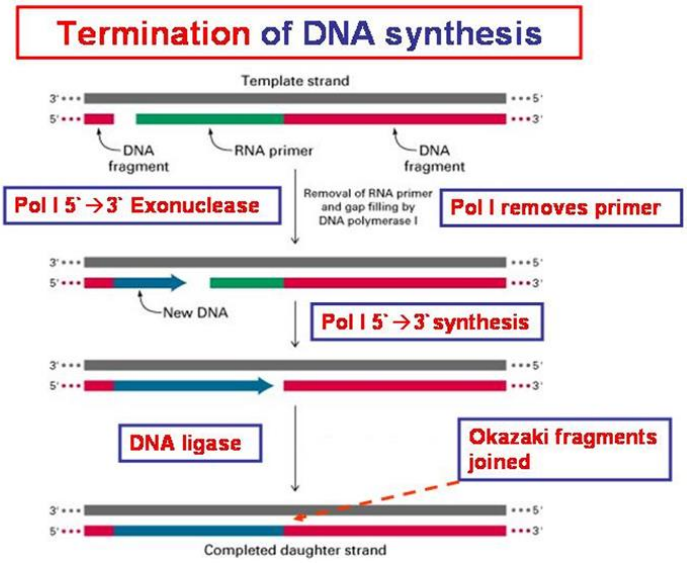


Fig: Events in the replication fork

Mechanism of DNA replication





u

Different models of Replication

Theta model

Pol I also has 5' to 3' exonuclease activity by which it normally removes primers and replaces them with complementary DNA sequences after polymerization has begun. About halfway through the above replication process, the replicative intermediate molecule looks like the Greek letter theta (θ), so is referred to as theta replication .

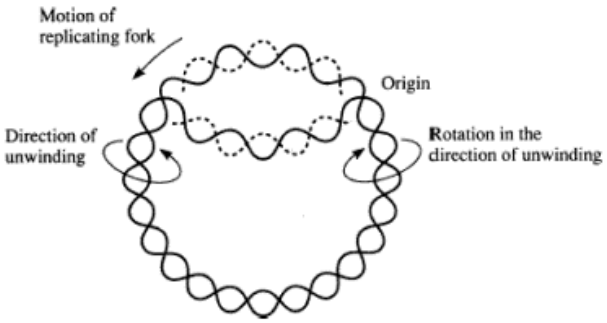


Fig. 10-3. Theta (θ) replication. Newly synthesized DNA is indicated by broken lines. Overwinding of the unreplicated segment (caused by unwinding of the daughter branches) is removed by the nicking action of DNA gyrase.

D loop model

Initially only oneof the parental strands is used as template for synthesis of a new strand. This single new strand displaces the non-template parental strand, forming a displacement loop, or **Dloop**. After replication of the first strand has proceeded about half way round the mitochondrial genome, synthesis of the other strand begins at a second origin and proceeds aroDuisnpdlatcheemgeennot more D. -loops

Rolling Circle model

A rolling circle is a replicative structure in which one strand of a circular duplex is used as atemplate for multiple rounds of replication, generating many copies of that template. When replication proceeds by a rolling circle, replication of one strand of the duplex begins at a nick atthe origin. The newly synthesized strand displaces the original nicked strand, which does not serve as a template for new synthesis. Thus the rolling circle mechanism copies only one strand of the DNA. Elongation proceeds by the replication machinery going around the template multiple times, in a pattern resembling a rolling circle. The large number of copies of a single strand of a phage genome made by the rolling circle are **concatenated**, or connected end-to-end. The single-stranded DNA can be cleaved and ligated to generate unit length genomes, which are packaged into phage particles. This occurs in replication of single-stranded DNA phages such as φX174 or M13.

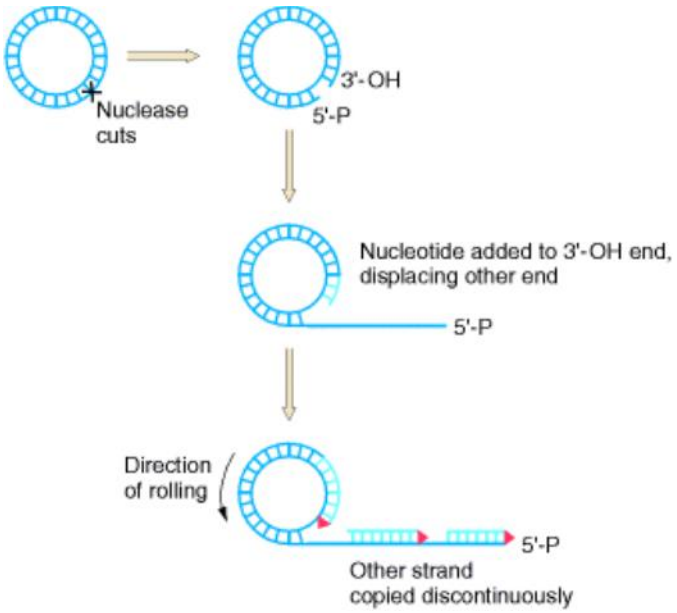


Fig: Rolling circle replication

Looped rolling circle model : The steps in the formation of the ØX primosome involve:

- Coating of the single-stranded ØX174 DNA with *Escherichia coli* SSB DNA binding protein
- Binding of three proteins (PriA, priB and priC) to the primer assembly sequence.
- Formation of a complex of six subunits of dnaB protein coupled with six subunits of dnaC protein.
- Transfer of the complex of dnaB·dnaC to the priA-B-C complex at the primer assembly site via the dnaT gene product. dnaC dissociates at this step and the resulting complex is known as the preprimosome.
- Binding of primase (dnaG) to the preprimosome complex to form the primosome.
- The mature primosome can then proceed in an ATP dependent fashion to traverse the DNA. The primosome can apparently be driven by either the dnaB protein in a 3'-5' direction or by the priA protein in the 5'-> 3' direction.
- Both the dnaB protein and the priA protein in the primosome can serve as a DNA helicase activities.
- The priA protein can also displace SSB from in front of the moving primosome. whilednaB cannot and can only move on naked DNA template.
- During either of these motions, the primase activity can synthesize primers 11 ± 1 nucleotides in length at various sites along the template in a reaction requiring the four rNTPs.
- Once these primers are extended by DNA polymerase III, the SSB protein is permanently displaced from the single-stranded DNA template. Removal of the RNA primers and ultimate sealing of the nicks in the DNA require the combined action of 5' exonuclease of DNA polymerase I and DNA ligase.

Prepared by – Mr. P. Srinivasan , Assistant Professor, Dept. of Microbiology, KAHE.

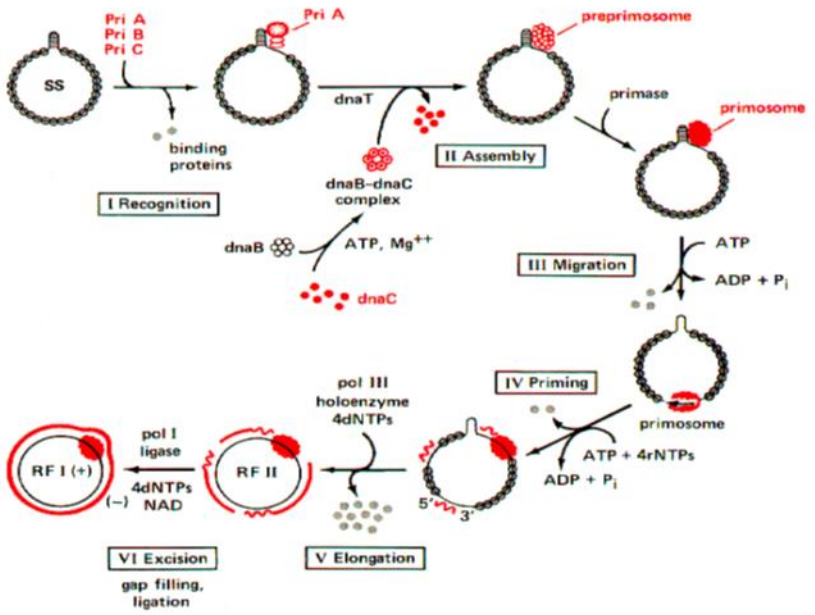


Fig: Looped rolling circle replication

Eukaryotic DNA replication

- **Five DNA polymerases:** Eukaryotic DNA replication is not as well understood as prokaryotic. However, there are at least five separate DNA polymerases, as described below.
- **Separate enzymes for leading and lagging strands:** Leading and lagging strands appear to be synthesized simultaneously in eukaryotic cells, but two separate enzymes are involved, rather than a dimer of a single enzyme. Polymerase alpha is currently believed to be responsible for synthesis of the lagging strand and polymerase delta for the leading strand.
- **DNA repair:** Polymerases beta and eta are also nuclear and are generally thought to be involved in repair.
- **Mitochondrial DNA synthesis:** As we will see in lecture 35, mitochondria contain an independent DNA genome. Polymerase gamma is believed to be involved in mitochondrial DNA synthesis.
- **Replicons:** Because of the great length of the DNA molecules in eukaryotic chromosomes, they have multiple origins of replication. Each unit of DNA replication is referred to as a replicon.

Histone synthesis: Histones are basic proteins that interact with eukaryotic DNA to form stuctural units known as nucleosomes .The synthesis of new histones is tightly linked to DNA synthesis with immediate formation of new nucleosomes.

KARPAGAM ACADEMY OF HIGHER EDUCATION (KARPAGAM UNIVERSITY)
DEPARTMENT OF MICROBIOLOGY
MOLECULAR GENETICS - 17MBP103

Unit II Question
_____ is a non-ionizing radiation
Alkylation is
Alkylation of guanine causes its removal from DNA in a reaction called
An intercalating dye
Bacterium used in Ames test
Biological agents of mutagenesis are
Cancer that results from deletion of a portion of chrosome 13 is
Change of purine to pyrimidine base in an mutation
Chemical agent that resembles thiamine
Chemical mutagens leading to addition of nucleotides to the DNA are
Chromosomal mutation is
Common chemical events that produce spontaneous mutation
Converts amino groups to keto groups by oxidative deamination
Daughter strand repair is also called as
Deficiency in phenylalanine hydroxidase results in
Detection of silent mutations require
Duplication mutation is
Frameshift mutation is caused by
Herman J.Mueller reported results of induced mutations on
Human bladder cancer is brought about by a change in single point mutation of
In bacteria, a small circle of DNA found outside the main chromosome is called
In *E.coli* , parental DNA is methylated at an adenine residue found in the sequence
Mismatch repair cannot take place if there is a mutation in
Most common proto-oncogene implicated in human cancers
Most common repair system is
Most frequently employed technique in the study of mutations
Mutation generating new stop codon are called
Mutation in which a purine base is substituted with another purine base is
Mutation in which there is an amino acid substitution is called
Mutation involving single-base changes are
Mutation resulting from deamination of 5-methylcytosine produces Thymine which pairs with
Mutation that has no detectable effect on the phenotype of a cell
Mutations that result from treatment with mutagens are called
Natural phenomena of changes in chemical structure of nitrogenous bases is called
Naturally occurring mutations are
Nonsense mutation leads to
Nutritional mutans of neurospora are known as
Oncogenes are found in certain
Potent oxidizing agent that can alter structure of purine and pyrimidine

Radiation that causes cross chromosomal mutations in humans
Rapid screening technique for mutagens and carcinogens
Recombinational repair is often due to
Repairing mechanism of _____ depends on absorption of visible light by the enzyme.
Reverse mutation is
Sickle cell anaemia is caused by change in amino acid from
Site that mutates at a rate significantly greater thag statistical probability is referred to as
The function of DNA glycosylase in base excision repair is
The most common error prone repair mechanism is
The result of addition or deletion of one or more base pair in a gene is
Transposition is
Transposons was first reported by
UV induced dimers are separated using light energy by
UV radiation at 260 nm cross-links adjacent thiamine that produces
UV radiation causes
Virus capable of causing mutations is
When a part of chrosome is moved to another chromosome, it is called as
Which of the following biomolecule has self¬repair mechanisms?
Which of the following chemicals induce depurination
Which of the following dimer formation is most common
Xeroderma pigmentosum is a genetic disorder of

Opt 1
Alpha
Addition of methyl or ethyl group
Deamination
Sunset yellow
Salmonella
Transposable elements
Eye cancer
Transition mutation
5-bromothiamine
Thimers
Abberation
Deamination
Hydrochloric acid
Recombination repair
Cancer
Aminoacid analysis
Segments of nucleotides sequences are repeated
Proflavin
Yeast
Valine to glycine
Cosmid
5' TAGC 3'
Helicase
s-rac
SOS
Analysis of phenotypes
Nonsense mutation
Transverse mutation
Missense
Induced mutations
Uracil
Point
Induced mutation
Complementary
Induced
Termination of DNA synthesis
Phototrophs
Bacteria
Free radicals

UV
Aims test
many cytidine dimer and associated large gaps in a strand
DNA helicase
Wild type to mutant
Glutamic acid
Hotspots
Addition of correct base
Mismatch
Frameshift
Movement of a phage
Louise pasteur
Primase
Butane ring
Adenine dimers
Bacteriophage Ru
Point mutation
DNA,RNA and protein
Methyl ethane sulphonate
Cytidine dimer
Skin

Opt 2
UV
Deletion of ethyl and addition of methyl group
Depyrimidation
Safranin
Shigella
Lipids
Bone cancer
Transverse mutation
5-bromoadenine
Base analogs
Change over
Depurination
Nitrous acid
SOS repair
Phenylketonuroa
Peptide analysis
Segments of nucleotides sequences are deleted
Nitrous acid
Drosophila
Isoleucine to leucine
Bacmid
5' ATGC 3'
Polymerase
a-src
Photoreactive
Analysis of genotypes
Misense mutation
General mutation
Nonsense
Point mutations
Adenine
Induced
Uninduced
Conservative
Spontaneous
Termination of protein synthesis
Auxotrophs
Fungi
Water

Visible
Sima test
incorporation of many incorrect nucleotides by DNA polymerase
DNA ligase
Mutant to wild type
Alanine to Leucine
Blackspots
Addition of correct nucleotide
Excision
Base pair substitution
Movement of a virus
Koch
Photolyase
Cyclane ring
Cytosine dimers
Bacteriophage Mu
Induced mutation
DNA and RNA
Guanidine
Uracil dimer
Hair

Opt 3
Gamma
Deletion of methyl and addition of ethyl group
Degradation
India ink
Streptococcus
Bacteria
Skin cancer
General mutation
5-bromoguanine
Alkylating agents
Variation
Dimerization
Sulphuric acid
Photo repair
Melanoma
RNA analysis
Segments of nucleotides sequences are inserted
UV
Fish
Leucine to isoleucine
Transposon
5' CATG 3'
Ligase
r-cas
Mismatch
Analysis of proteins
Point mutation
Transition mutation
Silent
Silent mutations
Cytosine
Silent
Spontaneous
Tautomeric
Nonsense
Termination of cell wall synthesis
Heterotrophs
Viruses
Dyes

Ionozing
Ames test
many thymidine dimer formation and associated large gaps in a st
DNA gyrase
A new gene introduced
Valine to Glutamic acid
Dotspots
Removal of incorrect base
SOS
Misense mutation
Movement of a transposon
Barbara McClintock
Dnase
Butocyclane ring
Guanine dimers
Bacteriophage Nu
Spontaneous mutation
DNA and proteins
Ethyl sulphonate
Thymidine dimer
Nail

Opt 4	Opt 5	Opt 6
Beta		
Deletion of methyl or ethyl group		
Depurination		
Acridine orange		
Staphylococcus		
Carbohydrates		
Lung cancer		
Transformation		
5-bromouracil		
Interchelating agents		
Genetic change		
Isomerization		
Oxalic acid		
Excision repair		
Asthma		
Nucleotide analysis		
Segments of nucleotide sequences are inserted & deleted evenly		
X-rays		
Pea plant		
Glycine to valine		
Plasmid		
5' GATC 3'		
Methylase enzyme		
c-ras		
Excision		
Analysis of both phenotypes and genotypes		
Silent mutation		
Transduction		
Point		
Inverse mutations		
Guanine		
Leaky		
Frameshift		
Telomeric		
Frameshift		
Termination of RNA synthesis		
Isotrophs		
Algae		
Acids		

X-rays		
Sema test		
DNA breaking		
DNA photolyase		
A gene deleted		
Leucine to alanine		
DNA spots		
Removal of phosphodiester bond		
Recombination		
Nonsense mutation		
Movement of a plasmid		
Lister		
Rnase		
Phenyl alanine		
Thiamine dimers		
Bacteriophage Ly		
Translocation mutation		
DNA only		
Dichlor		
Adenosine dimer		
Tongue		

Answer
UV
Addition of methyl or ethyl group
Depurination
Acridine orange
Salmonella
Transposable elements
Eye cancer
Transverse mutation
5-bromouracil
Interchelating agents
Abberation
Depurination
Nitrous acid
Recombination repair
Phenylketonuroa
Nucleotide analysis
Segments of nucleotides sequences are repeated
Proflavin
Drosophila
Glycine to valine
Plasmid
5' GATC 3'
Methylase enzyme
c-ras
Excision
Analysis of phenotypes
Nonsense mutation
Transition mutation
Missense
Point mutations
Adenine
Silent
Induced mutation
Tautomeric
Spontaneous
Termination of protein synthesis
Auxotrophs
Viruses
Free radicals

Ionozing
Ames test
incorporation of many incorrect nucleotides by DNA polymerase
DNA photolyase
Mutant to wild type
Valine to Glutamic acid
Hotspots
Removal of phosphodiester bond
SOS
Frameshift
Movement of transposon
Barbara McClintock
Photolyase
Cyclobutane ring
Thiamine dimers
Bacteriophage Mu
Translocation mutation
DNA only
Methyl ethane sulphonate
Thymidine dimer
Skin

DEPARTMENT OF MICROBIOLOGY
KARPAGAM ACADEMY OF HIGHER EDUCATION
KARPAGAM UNIVERSITY
(Deemed University Established Under Section 3 of UGC Act, 1956)
Eachanari PO, Coimbatore -641 021, India.

I - M.Sc Microbiology (Batch 2017-2019)

Lecture Plan

Unit - III

S. No	Duration	Topic	Reference
1.	2	Genetic recombination – conjugation, transformation and transduction	T1:481 to 506 R1:281 to 317
2.	2	Linkage and Genetic mapping	T1:312 to 314
3.	2	Phage genetics – Lytic and Lysogenic cycle	R1:447 to 450
4.	2	Detection and isolation of phage T4 mutant	T1:456 to 462
5.	1	Gene map of T4 phage	R1:314 to 327
6.	1	Class Test III	-
		Total Hours	**10**

R1: David Freifelder, Microbial Genetics. Narosa Publishing House, 10th edition, 2004. New Delhi, India.

T1: Peter J. Russell, i Genetics – A molecular approach, 7th edition, 2010. Pearson Benjamin Cummings Publishers, Boston, USA.

W1: www.slideshare.com/MolecularBiology-Geneticrecombation.html

P. SRINIVASAN
Assistant Professor
Department of Microbiology

2017

Unit – III
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′- A UG- 3′ codes for methionine**

5′- UCU- 3′ codes for serine

5′ - CCA- 3′ codes for proline

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, procaryotics and eucaryotics.

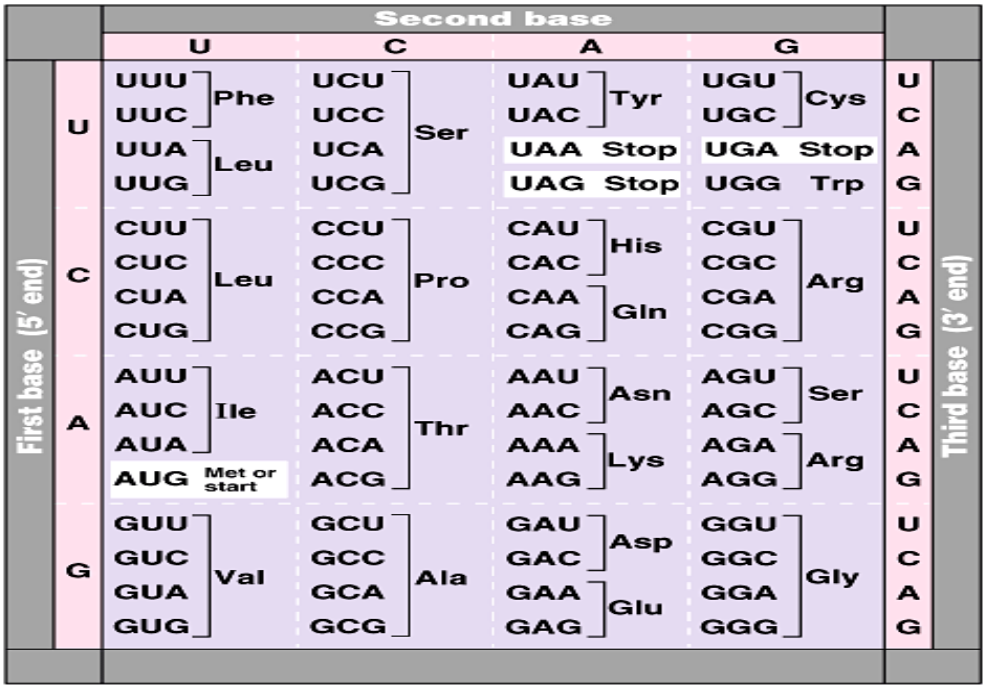
Degeneracy: the genetic code is degenerate i.e. although each codon corresponds to a single amino acid,one amino acid may have more than one codons. e.g arginine has 6 different codons.

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.

Prepared by – Mr. P. Srinivasan, Assistant Professor, Dept. of Microbiology, KAHE.

- **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.

Fig: Table showing characters of Genetic code

Gene organization in chromosomes

The coding potential of human DNA

- human DNA contains 6 x 10^9 base pairs/cell = 6,000,000 kb pairs
- compare to 4700 kb pairs/*E. coli*, a very sophisticated bacterium. Human DNA is more than 1000x bigger!
- If all human DNA coded for proteins, would have enough for roughly 5 million different proteins
- But currently only know ~ 3000 human proteins, and estimates as to how many we truly have range from 10,000 to 100,000

- In fact, **less than 5% of human DNA codes for protein**!
- What does the rest of the DNA do?

Functions of human DNA

- Coding for proteins. Eukaryotic genes are organized in peculiar fashion:
    1. **Exons**: (short for "expressed") -- regions of DNA that code for amino acids.
    2. **Introns**: (short for "intervening" or "interrupting") -- regions of DNA inside a gene, located in between exon regions, but not coding amino acids
    3. When RNA is transcribed from a gene, it initally contains both introns and exons, and cannot be called "messenger RNA" yet because the message is interrupted. Introns must be removed by "cut-and-paste", called **RNA splicing**.
    4. **snRNPs** ("snurps") = **small ribonucleoprotein particles**, found in nucleus. Composed of RNA and a few proteins. snRNPs associate to form a **Spliceosome**, which locates the junction of intron and exon, specifically cuts at this junction, and joins the cut ends of exons to form messenger RNA.
    5. **Ribozymes**: the enzymatic activity of spliceosomes was initially thought to be in the protein. However, now known to be on RNA; first example of catalytic RNA (called **ribozyme** for as opposed to enzyme, which is protein).
    6. Note: almost all genes in eukaryotes contain intron/exon organization. In some cases, amount of intron can be much larger than amount of exon DNA.
    7. Evolutionary importance of introns: since many proteins consist of several domains with different functions,
- **Multigene Families**: some genes are represented by more than one copy, typically for products needed in large quantity by cell.
    1. Example 1: **ribosomal genes** (for ribosomal RNA). Copies of the same gene are clustered together in enormous number (hundreds of thousands of identical gene copies).
    2. Example 2: **histone genes** (for proteins that bind to DNA to make chromatin). Family of histone proteins is represented many times.
- **Pseudogenes**: examples of multigene families where some copies of the gene have mutated to the point where they no longer function at all in the cell.
    1. Example: globin gene family. In humans, find several slightly different globin genes that produce the hemoglobin molecules needed by fetus, embryo, and adult. But also find a cluster of genes nearly identical in base sequence, but never expressed in the life of a human.
    2. Explanation: at some time in evolutionary past, globin genes were duplicated (by gene transposition). One cluster retained the job of making functional hemoglobin. The other cluster mutated so that promoter site no longer could be recognized by RNA polymerase. Result = this gene cluster now serves no purpose, cannot make any RNA or protein, but provides evidence of an evolutionary past. Called a **pseudogene** because it looks like a gene, but doesn't function.
- **Repetitive sequence DNA**. Some regions of DNA contain short sequences repeated many thousands of times = "tandem repeats". No coding function at all.

1. Example 1: **"satellite" DNA**. Sequence such as ACAAACT repeated again and again (producing ...ACAAACTACAAACTACAAACTACAAACTACAAACTACAAACT...). These regions appear to be located where the centromere forms, so this sequence must have mechanical properties that allow recognition by kinetochore and mitotic spindle.
2. Example 2: **"telomeric" DNA**. Sequences such as TTAGGG repeated over and over, 250-1500 times. Found at the ends of linear chromosomes (telomeres) where RNA primase (needed to prime the synthesis of new DNA) cannot work on lagging strand. Telomeric DNA acts like a "cap" on the end of the chromosome. If didn't have this, then DNA would lose a bit every replication, chromosome would gradually get shorter

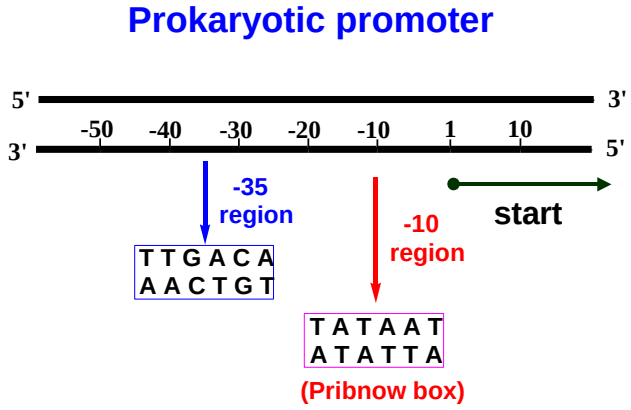
Transcription

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

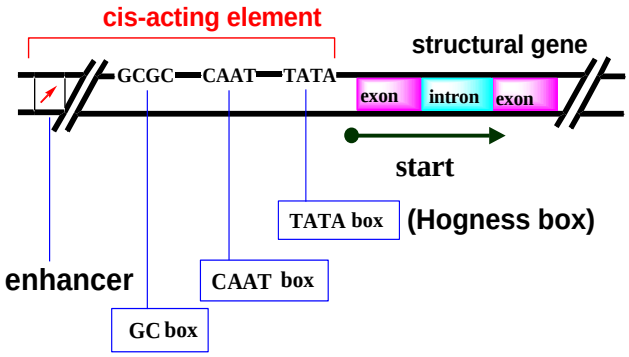
- First step in making proteins
- Process of taking one gene (DNA) and converting into a mRNA strand
- DNA -> RNA
- *Location*:Nucleus of the cell

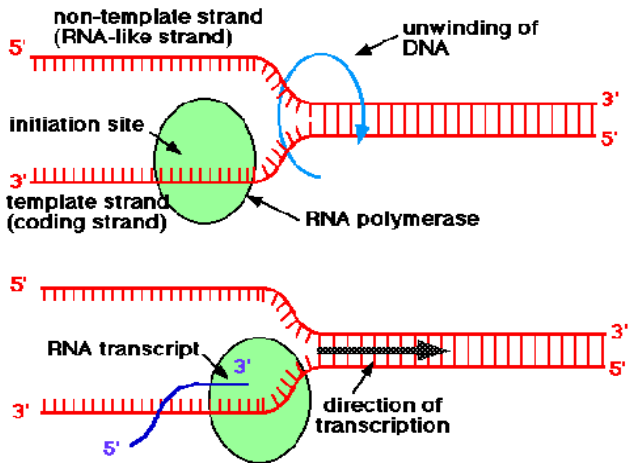
Steps to Transcription

- An enzyme attaches to the promoter (start signal region) of a gene and unwinds the DNA.
  - The -35 region of TTGACA sequence is the recognition site and the binding site of RNA-pol.
  - The -10 region of TATAAT is the region at which a stable complex of DNA and RNA-pol is formed.



Cis-acting element





- One strand acts as a template
- A mRNA copy is made from the DNA template strand by RNA polymerase
- A mRNA copy is made until it reaches the termination (stop signal) sequence
- The two strands of DNA rejoin.

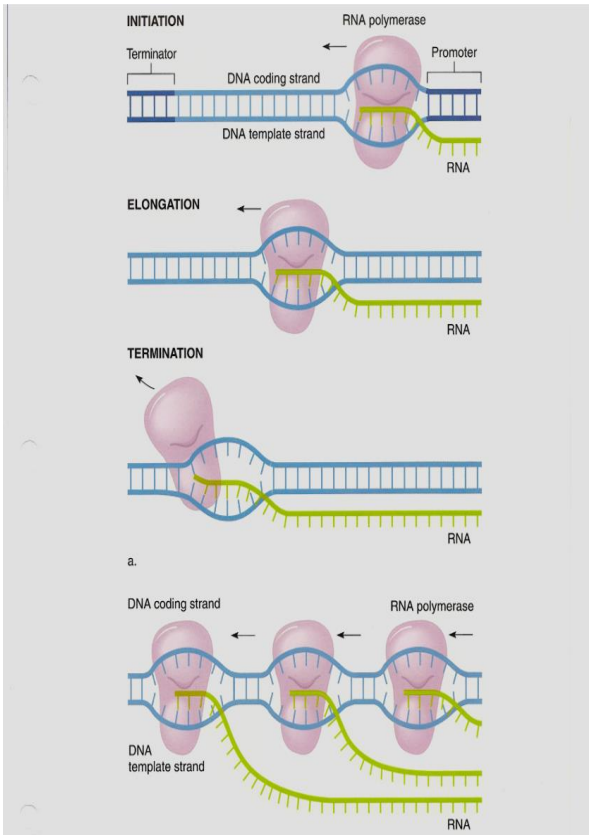


Fig: Mechanism of Transcription

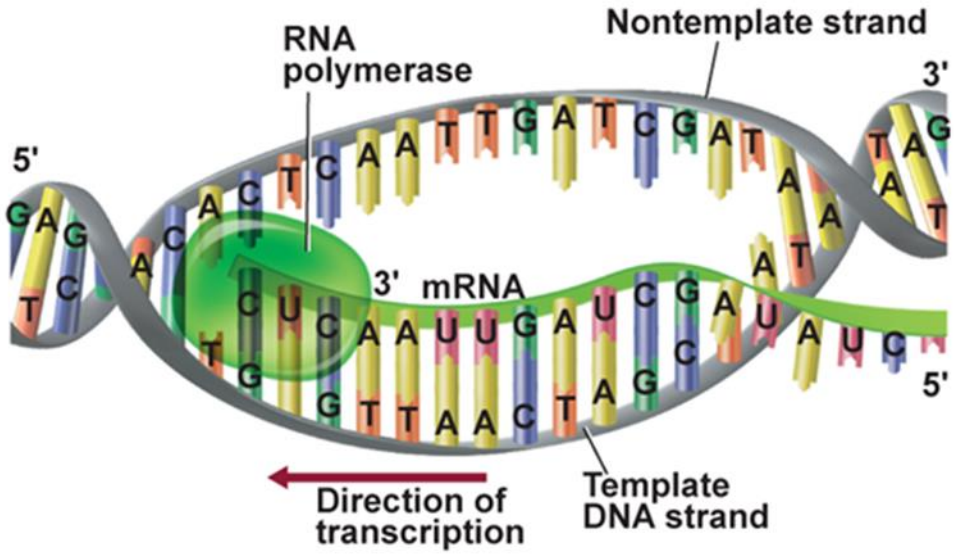


Fig: Initiation of transcription

Post transcriptional modification

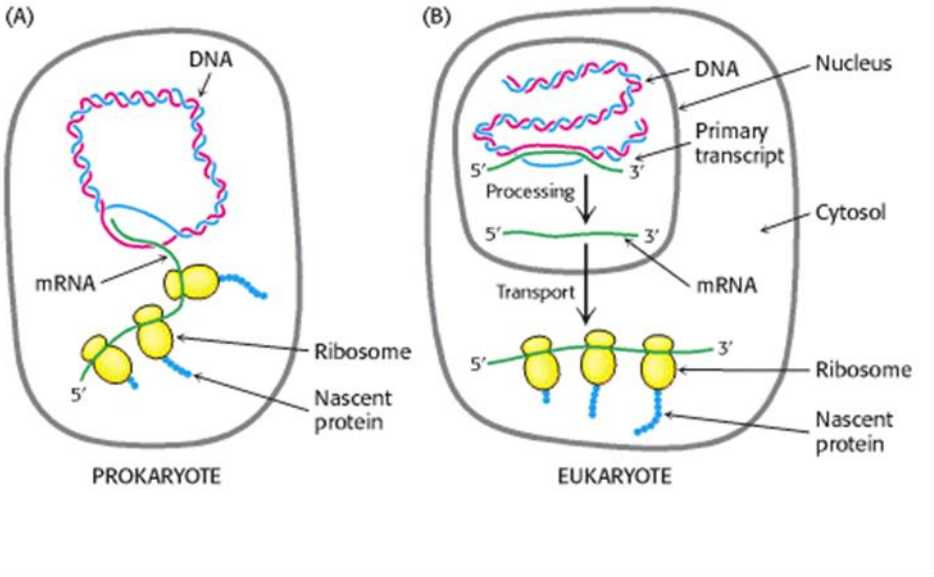


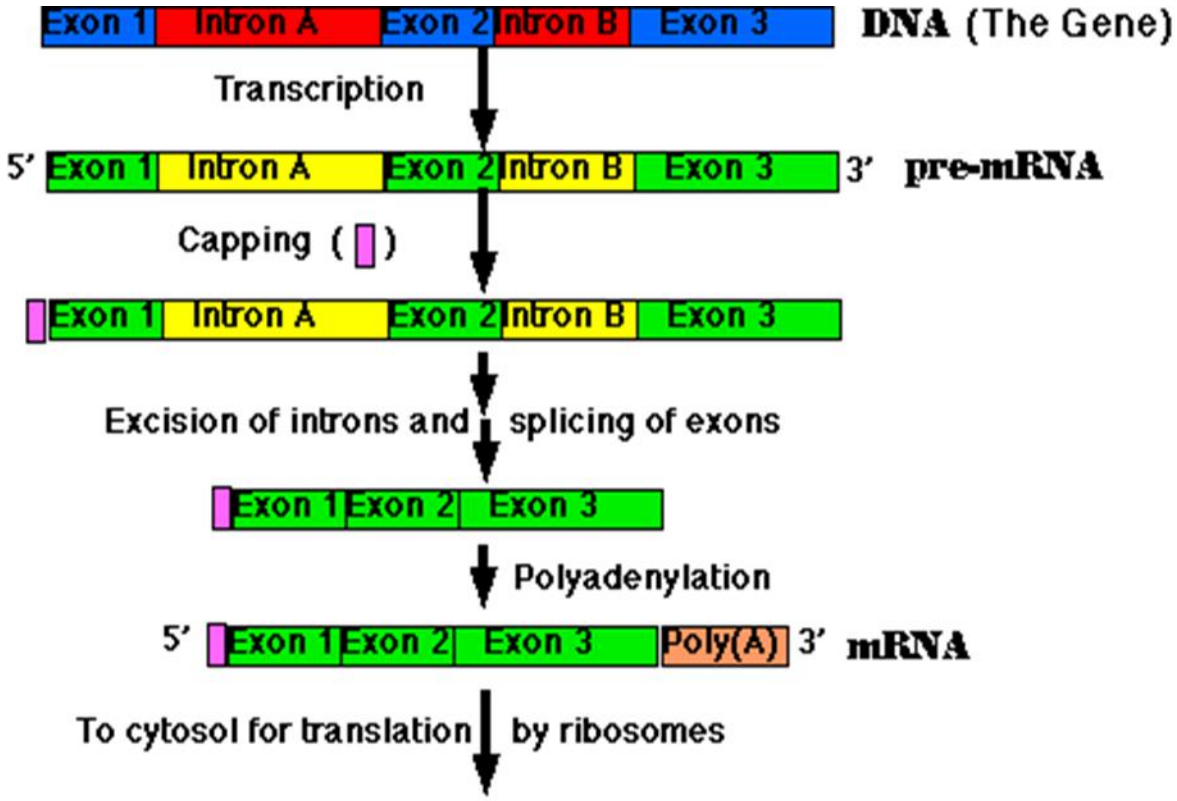
Fig: Post transcriptional modification both in prokaryotes and Eukaryotes

mRNA Processing

- Pre-mRNA – the original sequence of RNA created during transcription
- mRNA reaches the ribosomes
- After transcription the pre-mRNA molecule undergoes processing
    - 5’ cap is added
    - Poly A tail is added to the 3’ end
    - Introns are removed.
    -

RNA Processing

- Takes place in Eukaryotes only
- *Introns*- non-coded sections
- *Exons*- codes for a protein
- Before RNA leaves the nucleus, introns are removed and exons are spliced together
- A cap and poly A tail are added to ends of the sequence
- mRNA leaves the nucleus through the nuclear pores



Translation

- Production of proteins from mRNA
- mRNA goes to the ribosomes in the cytoplasm

Components required for protein synthesis*:*

Amino acids: all amino acids involved in the finished protein must be present at the time of protein synthesis.

Ribosomes: the site of protein synthesis. They are large complexes of protein and rRNA. In human, they consist of two subunits, one large (60S) and one small (40S).

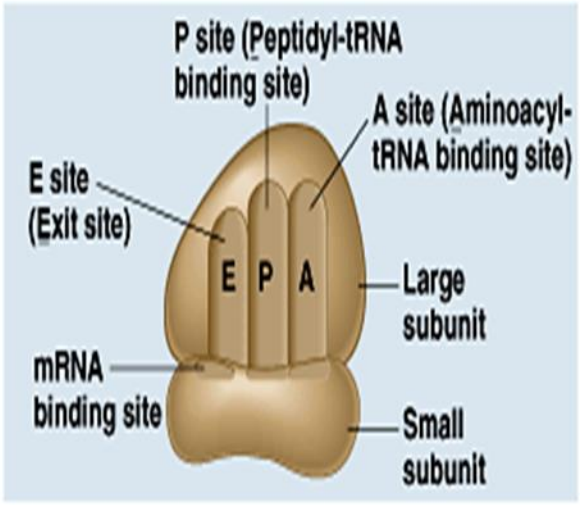
tRNA: at least one specific type of tRNA is required to transfer one amino acid. There about 50 tRNA in human for the 20 amino acids, this means some amino acids have more than one specific tRNA. The role of tRNA in protein synthesis is discussed before. (amino acid attachment and anticodon loop).

aminoacyl-tRNAsynthetase: This is the enzyme that catalyzes the attachment of amino acid with its corresponding tRNA forming aminoacyltRNA

mRNA: that carry code for the protein to be synthesized

protein factors: Initiation, elongation and termination (or release) factors are required for peptide synthesis

ATP and GTP : are required as source of energy.



(b) Schematic model showing binding sites

Steps:

***Initiation*:**

Initiation (start) codon is usually AUG which is the codon of methionine, so the initiator tRNA is methionnyltRNA (Met. tRNA).

- The initiation factors (IF-1, IF-2 and IF-3) binds the Met. tRNA with small ribosomal subunit then to mRNA containing the code of the protein to be synthesized. IFs recognizes mRNA from its 5' cap

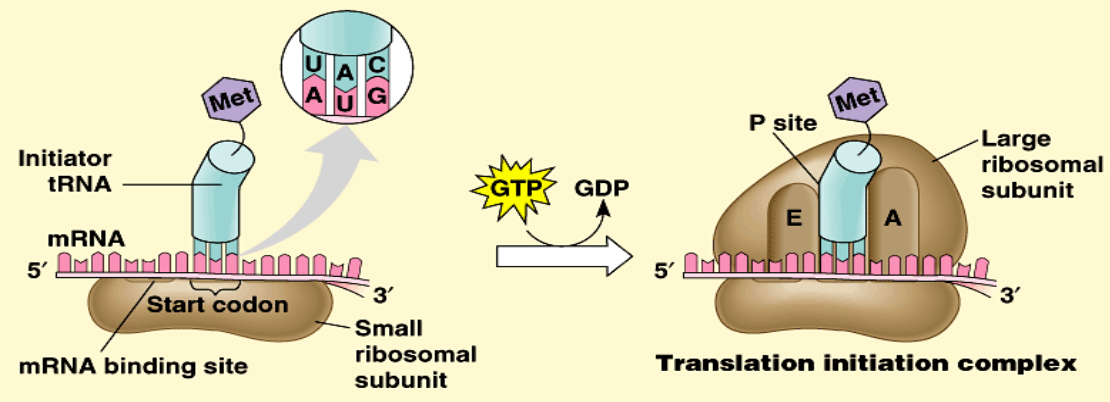


Fig: Initiation of translation

- This complex binds to large ribosomal subunit forming initiation complex in which Met. tRNA is present in P- site of 60 ribosomal subunit. tRNA bind with mRNA by base pairing between codon on mRNA and anticodon on tRNA.
- mRNA is read from 5′ → 3′ direction

- P-site: is the peptidyl site of the ribosome to which methionyltRNA is placed (enter).

Elongation:

Elongation factors (EFs) stimulate the stepwise elongation of polypeptide chain as follow:

- The next aminoacyltRNA (tRNA which carry the next amino acid specified by recognition of the next codon on mRNA) will enter A site of ribosome
  A site or acceptor site or aminoacyltRNAsite :Is the site of ribosome to which each new incoming aminoacyltRNA will enter.

- *Ribosomal peptidyltransferase*, enzyme will transfer methionine from methionyltRNA into A site to form a peptide bond between methionine and the new incoming amino acid to form dipeptidyltRNA.
- Elongation factor-2 (EF-2), (called also, translocase): moves mRNA and dipeptidyltRNA from A site to P site leaving A site free to allow entrance of another new aminoacyltRNA.
- Elongation process continous resulting in the formation of of poly peptide chain.

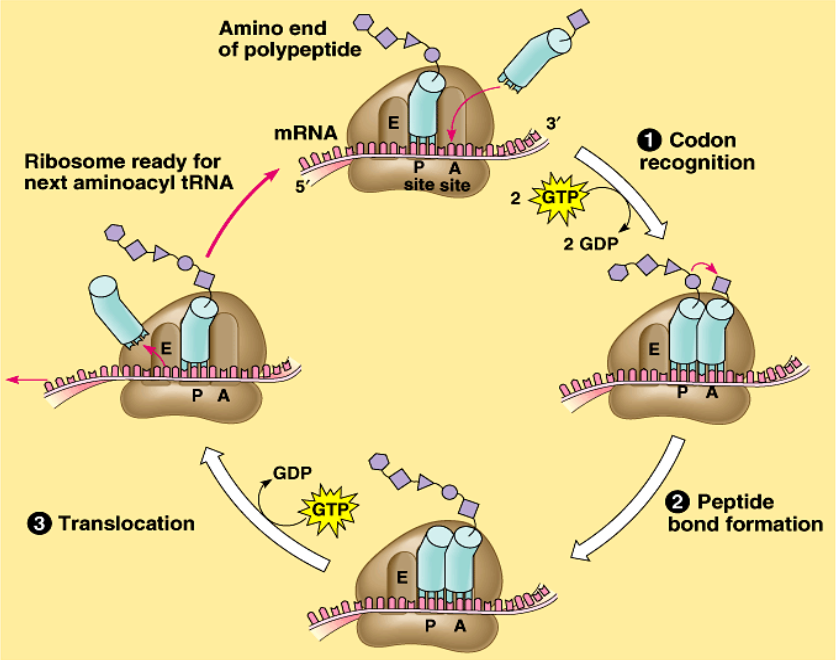


Fig: Repetitive cycle of elongation

***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, RF-2, RF-3. RFs cause the newly synthesized protein to be released from the ribosomal complex and dissociation of ribosomes from mRNA (i.e. cause dissolution of the complex)

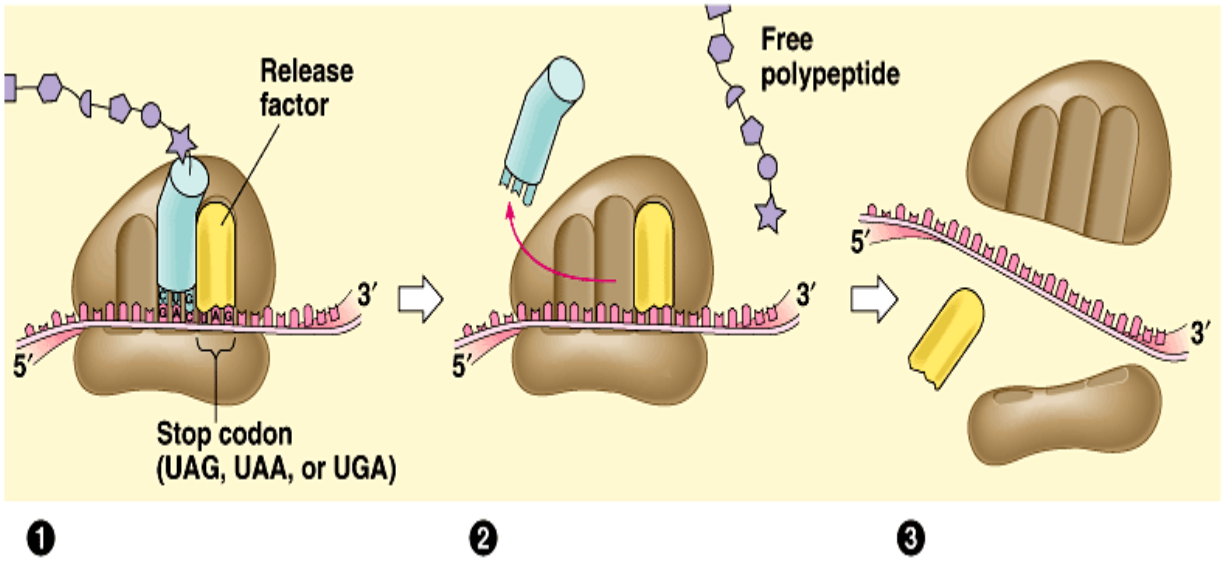


Fig: Termination of transcription

Regulating Gene Expression

- Microbes respond to changing environment
  - Alter growth rate
  - Alter proteins produced
- Must sense their environment
  - Receptors on cell surface
- Must transmit information to chromosome
- Alter gene expression
  - Change transcription rate
  - Change translation rate

Operonic regulation

- Coding vs regulatory sequences
- Regulatory sequences: promoters, operator and activator sequences
- Regulatory proteins: repressors, activators
  - Repressors bind operator sequences, block transcription
    - Induction vsDerepression

- Activator proteins bind sequences near by promoters, facilitate RNA Pol binding, upregulate transcription

Operon

- Multiple genes transcribed from one promoter

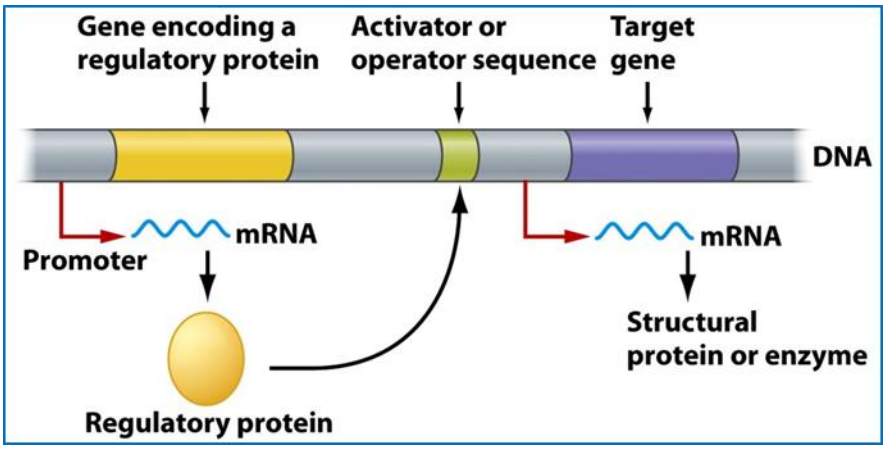


Fig: Structural and regulatory genes of an operon

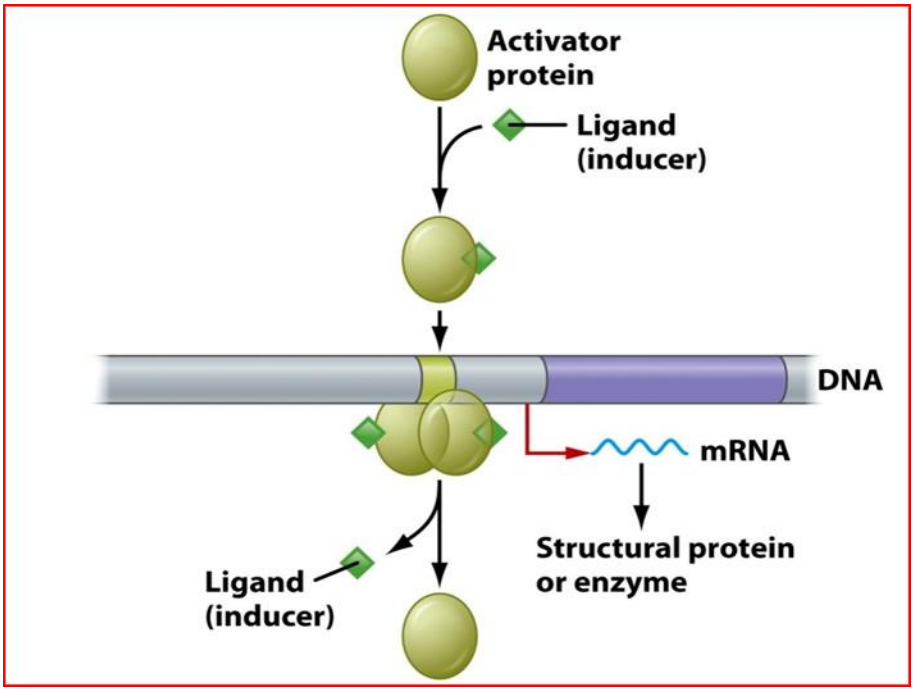


Fig: Operonic regulation

The *E. colilac* Operon

- Lactose (milk sugar) is used for food
  - Cannot pass through plasma membrane
    - Lactose permease allows entry
    - PMF used to bring lactose inside cell
  - Must be converted to glucose to be digested

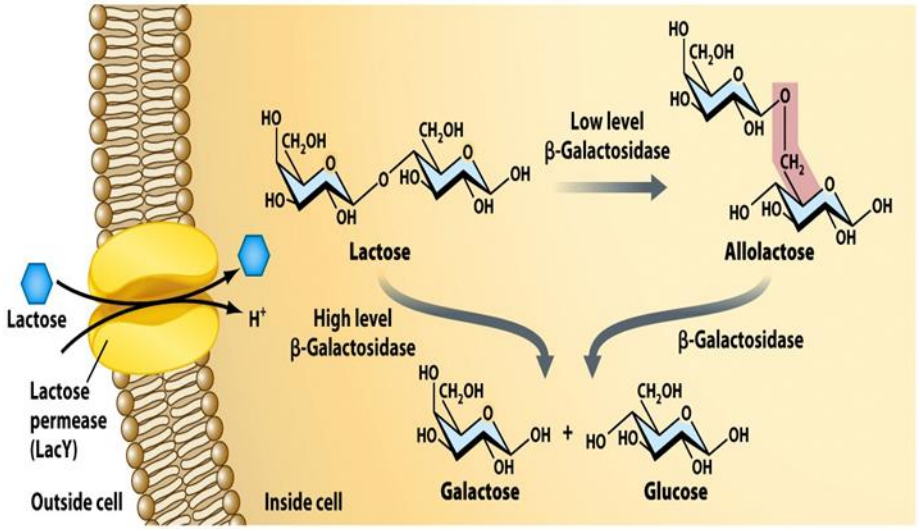


Fig: Lac operon regulation

- β-galactosidase converts lactose to glucose and galactose
- People also make β -galactosidase
- If not, person is lactose-intolerant

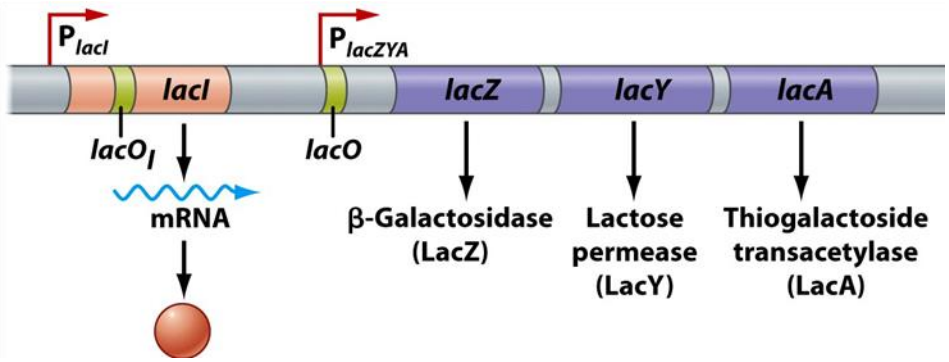


Fig: Structural and regulatory gene of Lac Operon

- The *lacZ* gene encodes b-galactosidase
- The *lacY* gene encodes lactose permease

  - Need both proteins to digest lactose
- Repressor protein LacI blocks transcription
  - Repressor binds to operator
  - Blocks s factor from binding promoter
- Repressor responds to presence of lactose
  - Binds inducer (allolactose) or DNA, not both
  - Add lactose → repressor falls off operator

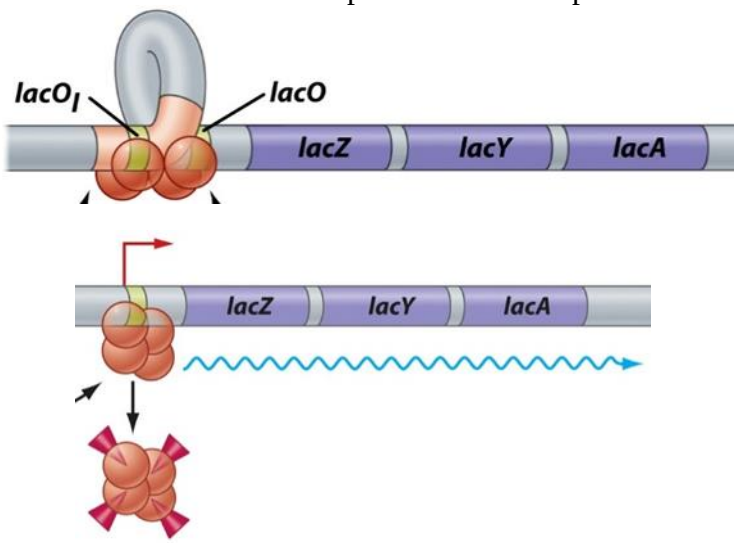


Fig: Allolactose cause operon induction

Activation of the *lac* Operon by cAMP-CRP

- Maximum expression requires cAMP and cAMP receptor protein (CRP)
  - The cAMP-CRP complex binds to the promoter at -60 bp
  - Interacts with RNA pol, increase rate of transcription initiation
- CRP acts as activator only when bound to cAMP

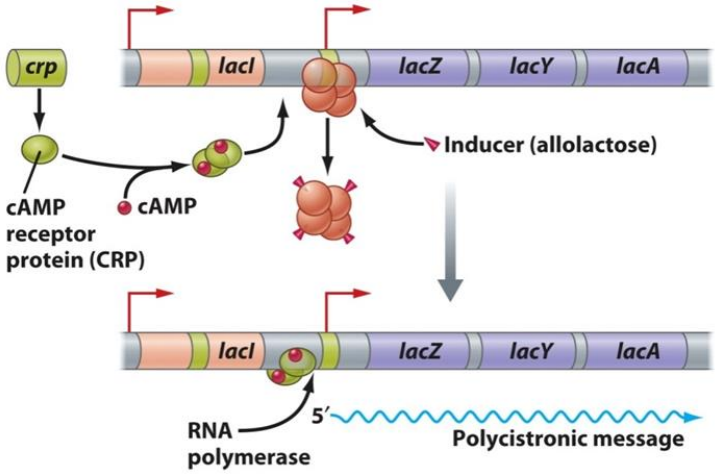


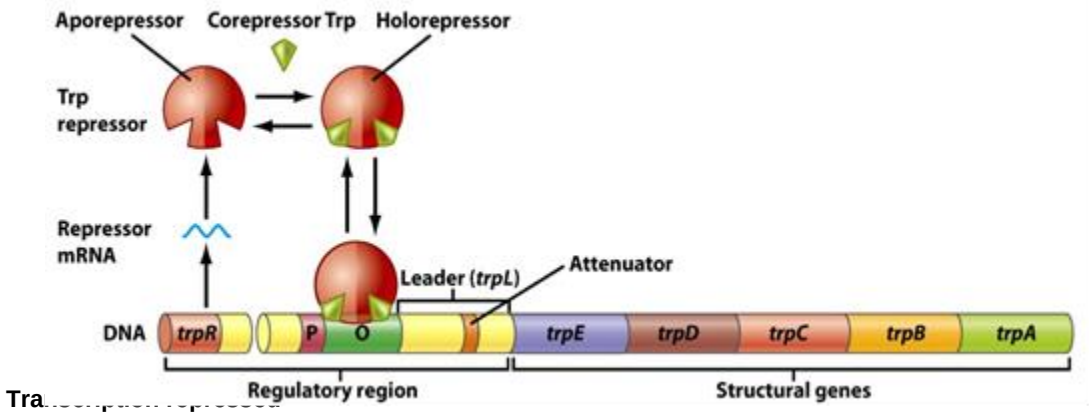
Fig: Catabolite repression

Catabolite Repression

- Two mechanisms involved
  1. High glucose → low cAMP levels → CRP inactive
     - Can't bind operon → low level of *lac* transcription

Trp operon: Repression and Attenuation

- *trp* operon
  - Cell must make the amino acid tryptophan
    - Trp operon codes and regulates biosythetic enzymes
    - When tryptophan is plentiful, cell stops synthesis
- Regulation by two mechanisms
  Repression: Trp repressor must bind tryptophan to bind DNA
    - Opposite of *lac* repressor



***Attenuation*:** a regulatory mechanism in which translation of a leader peptide affects transcription of a downstream structural gene.The attenuator region of the *trp* operon has 2 trp codons and is capable of forming stem-loop structures.

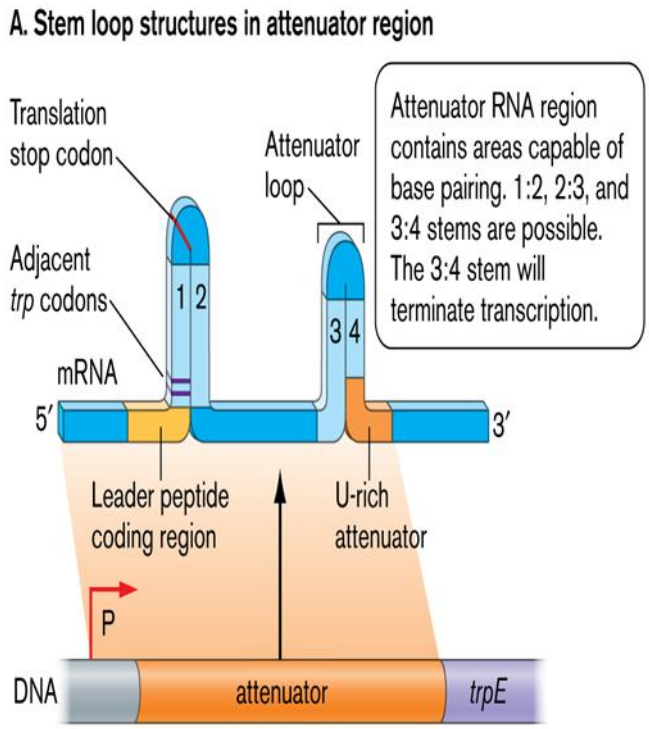
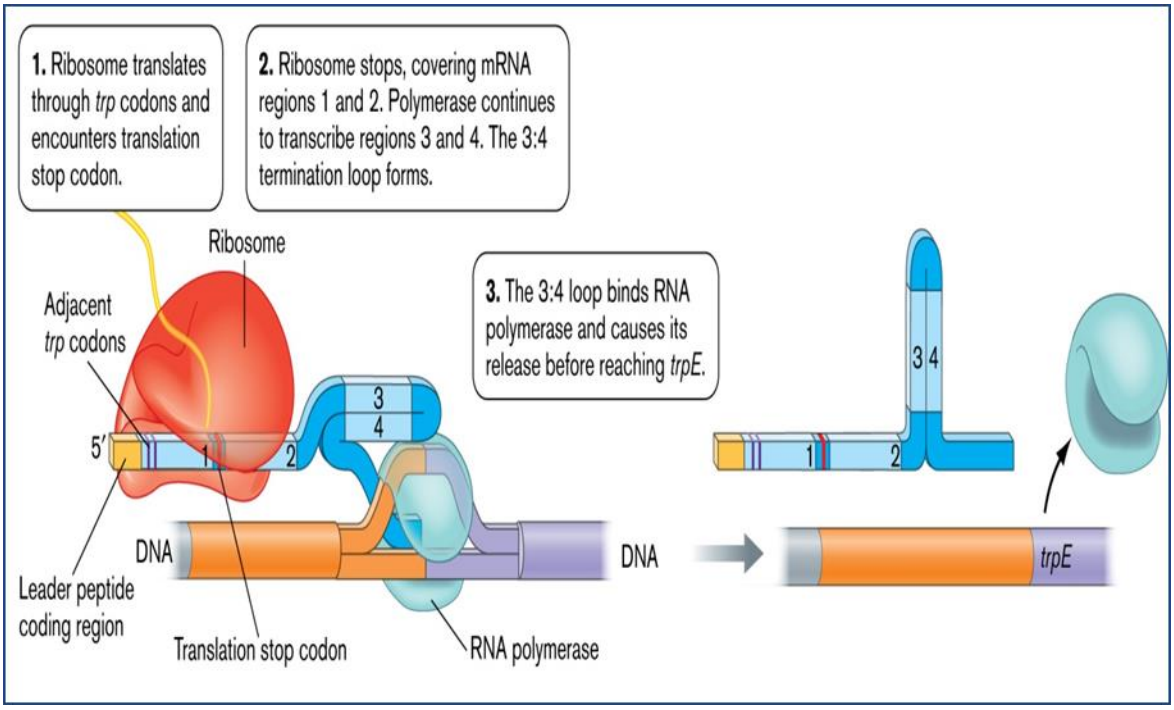
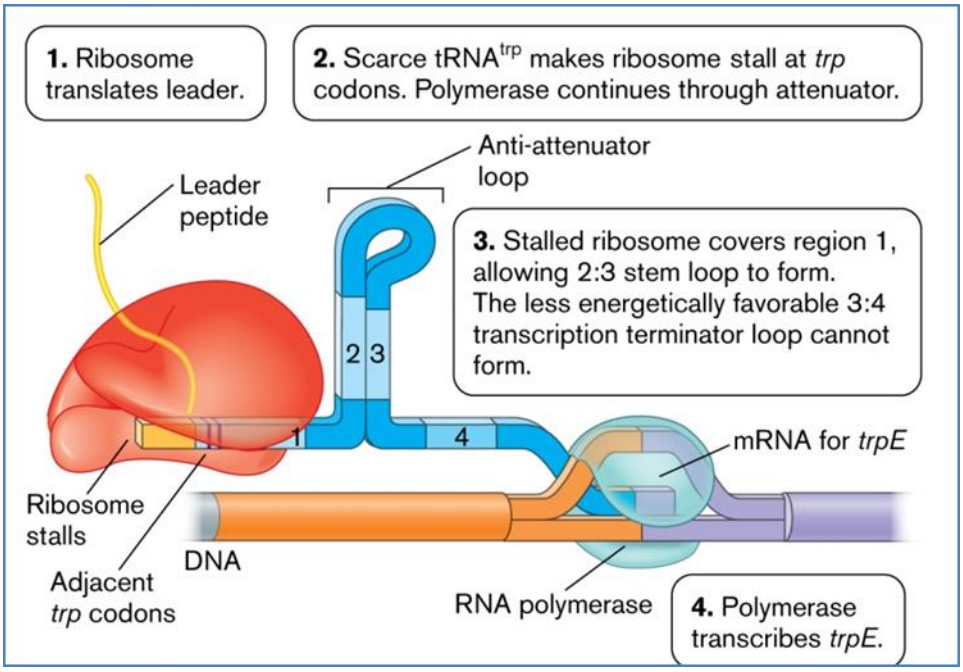


Fig: Transcriptional Attenuation Mechanism of the *trp* Operon





Low tryptophan

Fig: Attenuation control in Trp Operon

Arabinose operon

- Regulation by dual role regulatory protein AraC
- "AraC" acts as repressor to block transcription (no arabinose)
- Acts also as activator when bound to "arabinose" (the inducer)
  - Operators O1, O2 and araI control AraC and AraBAD proteins expression

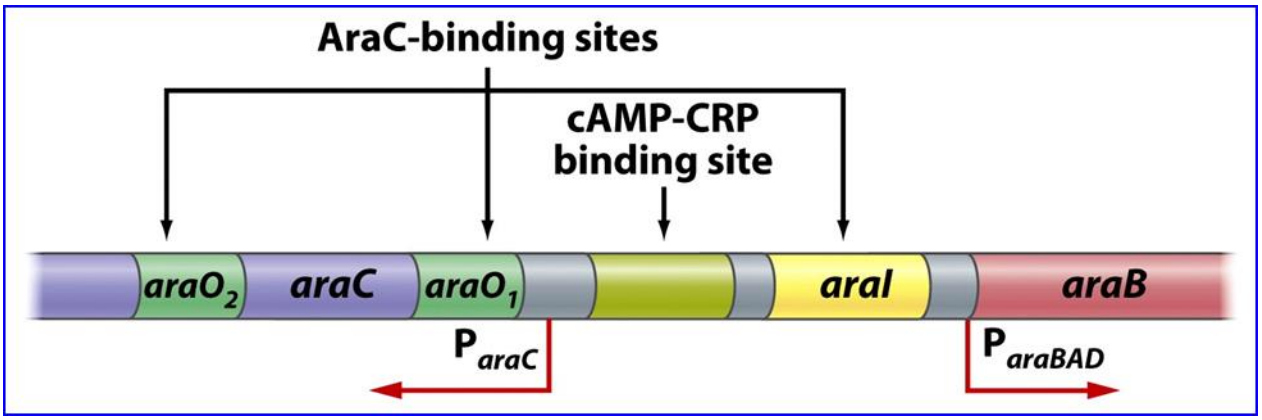
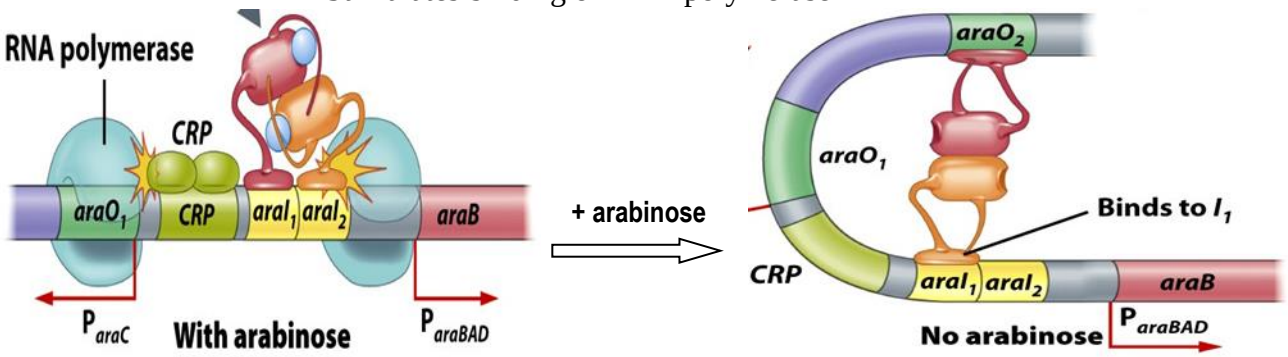


Fig:Structural and regulatory genes of Ara operon

Ara Operon Controls

- ***No arabinose present***
  - "AraC" forms long dimeric conformation, blocks transcription (binding O2, araI1)

- ***Arabinose added***
  - changes AraCdimeric conformation
    - acts as activator
    - Stimulates binding of RNA polymerase



KARPAGAM ACADEMY OF HIGHER EDUCATION (KARPAGAM UNIVERSITY)
DEPARTMENT OF MICROBIOLOGY
MOLECULAR GENETICS - 17MBP103

Unit III Question
_____ is the first amino acid during translation of proteins
2006 Nobel Prize in Physiology & Medicine for studies on molecular basis of eukaryotic transcription
7-methylguanosine cap is an important site
Action of repressor protein in *Lac* operon is called as
Addition of poly A tail to 3' end of mRNA is mediated by the enzyme
All aminoacids have more than one codon except
Allosteric enzymes that are controlled by a molecule other than it's substrate
Amino acid that have largest number of codons
A-site is the ribosomal site most frequently occupied by the
Capping in mRNA is addition of the group
Codon/Anticodon consists of _____ nucleotides
Common method of covalent modification of enzyme in regulation of gene expression is
Confirmational changes in protein is brought about by
Control of gene expression was proposed by
Enzyme activity is regulated by changes in the confirmation of enzymes except
Enzyme that lactose in to glucose and galactose
Genes are located in specialized structures called
In post translational modification of RNAs, trimming is
In prokaryotes, AUG is translated in to
In Rho-independent transcription termination, the termination sequence is usually
In RNA, thiamine is replaced by
In the absence of effector molecule, the enzyme is said to be in
In trp operon, the genes *trp* E & *trp* D codes for
lac operon is an example for
Mammalian mitochondrion not only uses AUG as initiation codon but also
Model example for gene regulation by repression
Molecular weight of egg lysozyme is
Monad & Cohen-Bazire first reported the evidence for the repression of the enzyme
Non codon specifies more than ___ amino acid
Non-coding regions are called as
Operon model that demonstrates both positive and negative control of gene regulation
Other than methionine _____ is the amino acid that appear rarely in proteins
Polyadenylation is
Region that comprise the core prokaryotic promoter
Repressor molecule in lac operon is a
Ribosomal site most frequently occupied by the tRNA carrying the growing peptide chain
rRNA is also called
Sequence of codons in mRNA between a start and a stop sequence is called as
Short sequence of aminoacids are called

Site to which substrate molecules are attached
Stop codon UAA is also called
Stop codons in mammalian mitochodria are
Structure of proteins may be classified into ___ types
The ability of the cell to choose between glucose and other sugars is termed as
The first and best example of control of gene expression was proposed by
The first codon during translation is
The main function of nonsense codons is to
The number of nitrogenous bases codes by 9 amino acids would be
The promoter sequence in eukaryotes is
The stop codons are called as
The termination of transcription is signaled by rich
Transcription initiation site starts from
Transcription is
Translation is
tRNA is responsible for the transfering
tRNA's are matched with their aminoacids by a group of enzymes collectively called as
What are the possible number of codons that can be generated using possible nucleotide combinations
Which is astop codon
Which transports lactose across the cell membrane
Who deciphered the genetic code

Opt 1
Threonine
Arthur Nirenberg
For eukaryotic transcription initiation factor
Positive control
RNA polymerase
Methionine & Tryptophan
Cohesive molecules
Proline
Aminoacyl-rRNA
7-ethylguanosine
4
to methylate the enzyme at a proline residue
Systematic molecules
Beedle & Tatum
Polymerase
Lactosidase
Histone
Removal of excess nucleotides
Methionine
Palindromic sequence
Uracil
Relaxed state
Arginase
Repressible operon
AUA, AUU, AUC
trp operon
19300 daltons
Tryptophan synthase
1
Exons
lac operon
Arginine
Addition of adenosines to 3' end of mRNA
Klenow box
Dimer
A-site
Rnase
Close reading frame
Peptides

Catalytic site
Amber
GAA & GAG
2
Catabolic repression
Khorana & Nirenberg
AGU
Initiate protein synthesis
27
TATAAA
Missense
AT containing inverted repeat
-1
DNA to rRNA
rRNA to protein
Protein
Aminoacyl DNA synthatases
46
UAA
Galactosidase permease
Hershey & Chase

Opt 2
Leucine
Roger D. Kornberg
For prokaryotic translation initiation factor
Negative control
DNA polymerase
Valine & Leucine
Systematic molecules
Cysteine
Aminoacyl-mRNA
7-methylguanosine
6
to phosphorylate the enzyme at a proline residue
Cohesive molecules
Avery & McLeod
Ribozymes
Glucanse
RNA
Removal of excess proteins
N-acetyl-methionine
Paliomic sequence
Adenine
Tense state
Tryptophan synthase
Inducible operon
UAA, UAU, UAC
lac operon
13900 daltons
Gluconase synthetase
2
Introns
ara operon
Tryptophan
Addition of adenosines to 5' end of mRNA
Pribnow box
Trimer
P-site
Ribase
Open reading frame
Proteins

Effector site
Opal
AGA & AGG
3
Catabolic expression
Hershey & Chase
AUG
Elongate protein synthesis
36
TAATAA
Nonsense codons
AC containing inverted repeat
Plus 1
DNA to tRNA
tRNA to protein
Aminoacid
Aminoacyl synthatases
64
AAU
β-galactosidase
Avery & McLeod

Opt 3
Methionine
David Osborne
For eukaryotic translation initiation factor
Neutral control
Rnase
Threonine & Alanine
Effector molecules
Serine
Iminoacyl-tRNA
7-methylcytosine
3
to phosphorylate the enzyme at a serine residue
Affector molecules
Jacob & Monad
Chimozymes
α-galactosidase
Chrosomes
Removal of excess lipids
N-formamyl-aspargine
Panoramic sequence
Cytosine
Free state
Anthranilate isomerase
Mutated operon
AAU, UAU, CAU
ara operon
31900 daltons
Arabinase trimutase
3
Cistrons
gal operon
Glutamic acid
Deletion of adenosines to 3' end of mRNA
TAGTAG box
Tetramer
E-site
Ribulase
Central reading frame
Polypeptides

Alleosteric site
Acre
CGA & AGC
4
Metabolic repression
Avery & McLeod
GUA
Terminate protein synthesis
18
TTGACA
Central codons
GC containing inverted repeat
-10
DNA to mRNA
DNA to protein
Codon
Amino synthatases
20
AUA
Glucanse
Beedle & Tatum

Opt 4	Opt 5	Opt 6
Valine		
Michael Whitney		
For prokaryotic translation initiation factor		
No control		
poly A polymerase		
Lysine & Arginine		
Affector molecules		
Valine		
Aminoacyl-tRNA		
7-ethylcytosine		
9		
to methylate the enzyme at a serine residue		
Effector molecules		
Hershey & Chase		
Nuclease		
β-galactosidase		
Genomes		
Removal of excess carbohydrates		
N-formamyl-methionine		
Pandemic sequence		
Guanine		
Degrading state		
Anthranilate synthase		
Neutral operon		
GUA, GUU, GUC		
gal operon		
91300 daltons		
Tryptophanase		
4		
Positrons		
trp operon		
Threonine		
Deletion of adenosines to 5' end of mRNA		
Polypeptide box		
Pentamer		
G-site		
Ribozyme		
Last reading frame		
Palindromes		

Binding site		
Ochre		
CGG & GCG		
5		
Metabolic expression		
Jacob and Monad		
UGA		
Regulate protein synthesis		
9		
GTTAAA		
Last codons		
CT containing inverted repeat		
Plus 10		
DNA to protein		
mRNA to protein		
Anticodon		
Aminoacyl tRNA synthatases		
30		
AAA		
Glucose permease		
Nirenberg & Khorana		

Answer
Methionine
Roger D. Kornberg
For eukaryotic translation initiation factor
Negative control
poly A polymerase
Methionine & Tryptophan
Effector molecules
Serine
Aminoacyl-tRNA
7-methylguanosine
3
to phosphorylate the enzyme at a serine residue
Effector molecules
Jacob & Monad
Ribozymes
β-galactosidase
Chrosomes
Removal of excess nucleotides
N-formamyl-methionine
Palindromic sequence
Uracil
Tense state
Anthranilate synthase
Inducible operon
AUA, AUU, AUC
trp operon
13900 daltons
Tryptophan synthase
1
Exons
ara operon
Tryptophan
Addition of adenosines to 3' end of mRNA
Pribnow box
Tetramer
P-site
Ribozyme
Open reading frame
Peptides

Catalytic site
Ochre
AGA & AGG
4
Catabolic repression
Jacob and Monad
AUG
Terminate protein synthesis
27
TATAAA
Nonsense codons
GC containing inverted repeat
Plus 1
DNA to mRNA
mRNA to protein
Anticodon
aminoacyl tRNA synthatases
64
UAA
Galactosidase permease
Nirenberg & Khorana

DEPARTMENT OF MICROBIOLOGY
KARPAGAM ACADEMY OF HIGHER EDUCATION
KARPAGAM UNIVERSITY

(Deemed University Established Under Section 3 of UGC Act, 1956)
Eachanari PO, Coimbatore -641 021, India.

I - M.Sc Microbiology (Batch 2017-2019)

Lecture Plan

Unit - IV

S. No	Duration	Topic	Reference
1.	2	Mutagen, Mutagenesis and mutation- Luria Delbruck experiment	T1:133 to 134 R1:212 to 214
2.	2	Spontaneous and induced mutation –Types	T1:136 to 146 R1:214 to 224
3.	2	Mutant detection and test of carcinogenicity	R1:214 to 224 T1: 147 to 148
4.	2	DNA damage and type	R1: 232 to 236
5.	2	DNA repair mechanism and types	T1: 149 to 157
6.	1	Video presentation on enzymology of DNA repair	W1
7.	1	Power point presentation on DNA	W1
8.	1	Class Test IV	-
		Total Hours	**13**

R1: David Freifelder, Microbial Genetics. Narosa Publishing House, 10th edition, 2004. New Delhi, India.

T1: Peter J. Russell, i Genetics – A molecular approach, 7th edition, 2010. Pearson Benjamin Cummings Publishers, Boston, USA.

W1: www.slideshare.com/MolecularBiology-Geneticrecombination.html

P. SRINIVASAN
Assistant Professor
Department of Microbiology

2017

Unit –IV

Molecular basis of spontaneous and induced mutations

Major concepts

- Classification of mutations
- Types of point mutations:
  - Missense mutations: transitions, transversions.
  - Missense mutations may be temperature-sensitive.
  - Nonsense mutations: amber, ochre, opal.
  - Frameshift mutations: deletion, insertion. .
- Spontaneous mutation
  - Tautomers: keto-enoltautomers; amino-iminotautomers.
  - Mispairing due to tautomerization: TG pairs, AC pairs.
  - Mispairing of repeated bases to cause frameshift.
  - Mispairing caused by deamination of cytosine or adenine.
  - Deamination of 5-methylcytosine yields thymine.
- Spontaneous mutation rate: .
  - Bacteria, cultured cells: measured for a specific gene per cell division.
  - Higher animals: measured for a specific gene per gamete per generation.
- Chemical mutagenesis:
  - Mutations caused by base analogues.
  - Mutations caused by alkylation of bases.
  - Intercalation and frameshift mutations.
- Reversion
  - True reversion of missense mutations
  - Second-site reversion: intragenic suppression, intergenic suppression.
  - Frameshift reversion
  - Deletions and other large-scale changes usually exhibit no reversion.
- Molecular interpretation of recessive and dominant phenotypes
  - Recessiveness due to loss of function, amorphicaleles, hypomorphic alleles.
  - Dominance due to gain of function: hypermorphicalleses, antimorphic alleles, neomorphic alleles.
  - Dominance due to haplo-insufficiency, dominant lethals.

Introduction: These notes are unusually long, at least in part because they bring together into one place many aspects of mutation and mutagenesis that have already been introduced at least briefly in previous lectures. As you read through the familiar parts, please do not overlook the substantial amount of new material that has been blended in to provide a more thorough understanding of the overall concepts.

Mutation: A mutation is any change in genetic information relative to a reference "wild-type" genome, including changes that affect expression of genes without altering their coding sequences and changes that do not cause any detectable phenotypic difference (silent mutations).

Prepared by – Mr. P. Srinivasan, Assistant Professor, Dept. of Microbiology, KAHE.

In a complex organism, mutation can occur at many different structural levels and can be classified in many different ways:

1. Magnitude of genetic change: point, gene, chromosomal, genomic mutations.
2. Pattern of inheritance: somatic vs. germ-line; autosomal, sex-linked, dominant, codominant, partially dominant, recessive.
3. Phenotypic properties: morphological (shape, size, quantity, coloration), nutritional (auxotrophic), biochemical, lethal (conditional lethal, dominant lethal), behavioral, silent.
4. Changes in DNA: missense (transitions, transversions), nonsense (amber, ochre, opal), deletion, insertion, frameshift, inversion, duplication, translocation.
5. Conditional: temperature-sensitive, suppresible.
6. Regulatory: increased or decreased expression, altered message processing, stability, or rate of translation.

Point mutations were originally defined as those involving a chromosomal region that was too small for the change to be detected cytologically (particularly in the giant polytene chromosomes of *Drosophila* larval salivary glands). In current usage, point mutations are usually understood to involve only one base pair, but to include both substitutions (transitions and transversions), and the addition or deletion of a single base pair. A point mutation can result in missense (amino acid substitution), nonsense (insertion of a stop codon), or frameshift (either positive or negative).

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.

Chromosomal mutations are defined as those that involve deletion, inversion, duplication, or other changes of a chromosomal region that is large enough so the change can be detected cytologically. By definition, chromosomal mutations are limited entirely to a single chromosome, although there could be more than one chromosomal mutation within a genome.

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**.

The importance of mutation: Genes are stable repositories of the information needed for synthesis of all of the RNA and proteins in a living organism. Survival and stability of each species is dependent on faithful replication of genetic information for use by each new generation. However, a low level of mutational change is highly desirable. Over an extended period of time, mutational changes provide the ability for species to adapt to changing conditions and challenges, and thus serve as the raw material for selective survival and the evolution of more advanced and efficient species, as well as the development of biological diversity.

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. Colored spots in Indian corn are caused by back

mutation of a relatively unstable mutation that is responsible for loss of pigmentation. Cancer is caused by somatic mutations that alter normal cellular growth regulatory mechanisms in a single cell and its direct progeny.

Morphological mutations: Classical genetics was based almost exclusively on the study of mutations that caused affected progeny to be visibly altered. Mendel's original work was done with inbred strains of peas that were true-breeding for particular traits. However, in the years following the rediscovery of Mendel's laws, mutations were generated in wild type stocks by exposure to X-rays or other mutagenic treatments. In order to be detected and studied, the mutations had to be visibly different from the wild-type parental strains. Although the term "morphological" normally refers to structural properties, the term "morphological mutation" is often used more broadly to refer to any visible change, including changes in coloration.

Naming of mutations: When working with classical morphological mutations, it is important to remember that the names given to induced mutations usually describe the recessive phenotype. Thus, a gene named for ***white*** eyes codes for a gene product needed for normal synthesis of pigments in wild-type eyes. Similarly, a gene called ***brown*** codes for a step in the synthesis of vermillion pigment, which, when absent, leaves the eyes with a brown color. This nomenclature can be thoroughly confusing when one begins to analyze the molecular and biochemical mechanisms responsible for classical mutations. It is important to remember that for most classical recessive mutations, the ***wild-type allele*** codes for the protein that must be present and functional to prevent expression of the mutant phenotype.

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.

Lethal mutations: Any mutation that disrupts an essential function needed for survival will be lethal when homozygous. In many cases, heterozygotes can function reasonably normally, and they may be virtually indistinguishable from wild type. In other cases, the heterozygote may have a distinctive phenotype, as in the tailless Manx cat. In such cases, the gene is described as a **dominant lethal**, as discussed in earlier lectures. (Note that a more precise description would refer to the phenotype of the heterozygote as dominant and the lethality as recessive)

Molecular nature of point mutations: Point mutations can occur in a variety of ways (including frameshift mutations, which are discussed separately below). A change in a single base pair that alters a codon and causes an amino acid substitution in the coded protein is called a ***missense*** mutation. If one purine is replaced by another purine or if one pryimidine is replaced by another pyrimidine in the sense strand base sequence (with complementary changes in the antisense strand), the substitution is called a ***transition***. If the substitution involves replacement of a purine with a pyrimidine or a pyrimidine with a purine, it is called a ***transversion***.

Missense mutations: Most base pair substitutions change the amino acid specified by the codon in which they occur. Such mutations are described as ***missense*** mutations because they cause an

amino acid substitution in the coded protein. Depending on the nature of the amino acid substitution and its location within the protein, missense mutations may have a variety of effects, ranging from complete loss of biological activity to reduced activity or temperature-sensitive activity or no funtional effect at all.

Nonsense mutations: Base pair mutations that generate a translation stop codon (TAA, TAG or TGA in the DNA sense strand, transcribed as UAA, UAG or UGA in the mRNA) cause premature termination of translation of the coded protein and are referred to as ***nonsense*** mutations. In some cases, the effects of nonsense mutations can be suppressed by modified tRNA molecules that insert an amino acid with a low efficiency when a stop codon is encountered. Bacterial strains that contain such tRNAs are referred to as ***suppressor*** strains.

Silent mutations: In some cases, base pair substitutions generate a different codon for the same amino acid, with no biological effect whatsoever. This is most likely to happen in the third position (wobble base) of redundant codons for the same amino acid. Such changes are considered to be mutations because they alter the genetic code. However, because they have no phenotypic effect, even at the level of protein amino acid sequence, they are called ***silent*** mutations.

Frameshift mutations: The genetic code is translated three nucleotide bases (one codon) at a time, with no punctuation between the codons. Addition or deletion of a single base pair in the middle of a coding sequence will result in out-of-frame translation of all of the downstream codons, and thus result in a completely different amino acid sequence, which is often prematurely truncated by stop codons (UAG,UAA,UGA) generated by reading the coding sequence out-of-frame. Such mutations, which are a special subclass of point mutations, are referred to as ***frameshift***mutations. Deletion of a single base pair results in moving ahead one base in all of the codons, and is often referred to as a ***positive frameshift***. Addition of one base pair (or loss of two base pairs) shifts the reading frame behind by one base, and is often referred to as a ***negative frameshift***. Note that deletion or addition of three base pairs (or multiples of threes) does not cause a frameshift, but instead results in deletion or addition of one or more amino acids in the coded protein.

Conditional mutations: Some types of mutations exert their phenotypic effects only under certain environmental conditions. Such mutations are called ***conditional mutations.***

Temperature-sensitive *(ts)* mutations are missense mutations that do not seriously affect the biological activity of the coded proteins, but cause them to have a reduced thermal stability. Such proteins become denatured at temperatures that do not affect the corresponding wild-type proteins. However, when the mutant strains are maintained at a lower temperature, the proteins are still able to function reasonably well, and no mutant phenotype is observed. Temperature-sensitive mutations are particularly useful for studying vital functions, such as progression through the cell division cycle. In order to maintain stock cultures of organisms carrying such mutations, it is necessary to be able to expand populations under conditions where the mutations are not expressed phenotypically. Growth at low temperature and analysis of the mutant phenotype at a higher temperature provides such a system.

Prepared by – Mr. P. Srinivasan, Assistant Professor, Dept. of Microbiology, KAHE.

Nonsense suppression: Another approach to conditional mutation that is used extensively in studies on bacterial viruses is to generate ***nonsense*** mutations involving the ***amber*** codon (UAG). Viruses bearing such mutations can often be maintained in ***amber suppressor*** strains of bacteria and then transferred to regular strains to study the phenotypic effects of the mutations. The amber suppressor strains contain an altered transfer RNA that inefficiently reads the UAG codon as coding for an amino acid. If the protein is able to function with that particular amino acid inserted at the location of the amber mutation, the virus is able to replicate, although often with reduced efficiency, in the amber suppressor strain (see page 333 of the textbook).

Permissive and nonpermissive conditions: The conditions that allow growth or function without phenotypic expression of conditional mutations are referred to as ***permissive.*** The conditions that cause phenotypic expression to occur are referred to as ***nonpermissive***. This nomenclature refers primarily to conditions that permit growth or do not permit growth, but can also be used for other types of conditional mutations, such as loss of pigmentation at higher temperatures in Siamese cats and Himalayan rabbits (Figure 7.3 in the textbook). Permissive conditions allow the non-mutant phenotype to be expressed.

Conditional lethal mutations: Conditional mutations that do not allow survival of the organism under nonpermissive conditions are referred to as ***conditional lethal mutations***. Note that many other conditional mutations cause expression of mutant phenotypes at non-permissive temperatures without being lethal. Bleaching of coat color on warmer parts of the bodies of Siamese cats is an example of this.

Historical considerations: The textbook devotes several pages (pp. 390-392) to early studies that pointed the way toward modern genetic concepts of mutation prior to the availability of DNA sequence analysis as a method for determining the exact structure of genes. Some of the key points are summarized briefly below.

Delbruck and Luriafluctuation test: This test was designed to determine whether bacterial mutations were induced by stress conditions, such as bacteriophage infection, or whether they occurred spontaneously and were present prior to exposure to the stress conditions. Delbruck and Luria argued that if mutation was spontaneous, cultures started from small populations and grown up to large numbers in the absence of bacteriophage T1 should exhibit major variability in numbers of phage-resistant cells that they contain, depending on when during the culture history the mutations to phage resistance occurred, This was in fact what they found when the cultures were inoculated onto plates that contained large numbers of phage (Table 14.1). This was one of the final steps in disproving the Lamarkian view that genetic change was induced in response to environmental conditions.

Mechanisms of mutation: This portion of the lecture deals primarily with the mechanisms responsible for point mutations and their reversion or suppression. Strictly speaking, the term reversion should be used only to describe an exact reversal of the original mutational change. Many other secondary changes, either within the same gene, or in other genes can suppress the effects of a mutation. Such changes are called ***intragenicsuppression*** and ***intergenic suppression***, respectively.

Tautomerization: Spontaneous mutations that involve base pair substitutions are caused primarily by configurational changes within the individual bases that result in mispairing. These changes, which are called tautomeric shifts, involve momentary expression of rare alternative molecular configurations that exist in equilibrium with the more common forms. Specifically, proton shifts can convert the amino groups in adenine and cytosine to imino groups, and the keto groups in guanine and thymine to enol groups (Figures 14.7, 14.8)

Transitions: A tautomeric shift in any of the four DNA bases can lead to mispairing of A to C or G to T. The tautomeric state can occur either in the template base or the incoming base. During the next round of DNA synthesis, the mispaired base pairs with its normal partner, resulting in a transition, in which an AT base pair replaces a GC or a GC replaces an AT, with no change in the purine:pyrimidine polarity of the base pair (Figure 14.9). Transitions are the most common type of mutation resulting from spontaneous mispairing due to tautomerization.

Transversions: To achieve a transversion, in which the positions of purine and pyrimidine are reversed in the DNA double helix, two events are thought to be involved, tautomerization of one of the bases and rotation of the other to yield a purine:purine pairing. Based on information from the previous textbook for this course, the frequency of spontaneous transversions, which is lower than that of transitions, appears to be consistent with this interpretation. However, that book also warns that recent studies suggest that the overall picture may be more complex. Our current text does not discuss transversions in much detail. A second possible mechanism for transversions is the formation of an apurinic site (described below), which can result in replacement of the original purine with any of the four bases.

Frameshifts: Spontaneous frameshift mutations are believed to arise primarily from mispairing within long runs of the same base in a coding sequence. Such regions are believed to be one of the causes of mutational "hot spots" that have been observed during fine-structure genetic mapping.

Deamination: Our current textbook discusses deamination of cytosine primarily in terms of mutagenesis by nitric oxide (page 405), but spontaneous deamination also has an important role, particularly in methylated regions of DNA. If a cytosine undergoes oxidative deamination, it becomes uracil, which is capable of pairing with adenine (as in RNA synthesis), but is detected as an anomoly in DNA and may trigger repair mechanisms. However, if 5-methylcytosine is deaminated, it forms thymine, which is a normal DNA base that is not detected by repair systems (other than proofreading of GT mispairing during DNA synthesis). Because of selective methylation of CG sequences in many DNAs, there is a tendancy for all non-essential CG sequences to be converted to TG sequences over time. Methylated CG sequences are thus hot spots for mutation, such that in DNA in general, CG sequences tend to be far less frequent that TG sequences. (Remember that a sequence is always described in 5' to 3' terms, such that CG means 5'-CG-3').

Spontaneous mutation rate: For single-celled organisms ranging from bacteria to cultured mammalian cells, mutation rate is usually measured as the probability of mutation within a specific gene per cell division. For higher animals, the rate is measured in terms of the probability per gamete per generation (remember that each new individual contains the

contributions from two separate gametes). Bacterial rates are typically in the range of 10^{-8} to 10^{-6} per generation. Mammalian (including human) rates for individual easily observed mutations tend to be on the order of 10^{-5} per generation (See Table 14.2).

Chemical mutagenesis: A variety of chemical mutagens have been discovered that act in several distinctly different ways. Many chemicals that are used in modern industry and technology are potentially mutagenic, which includes their ability to cause cancer as a result of somatic mutation. Page 409 of the textbook contains a description of the **Ames test** for carcinogens, which is based on mutagenicity in specially engineered strains of bacterial cells that have been stripped of most of their repair mechanisms, and that must undergo back mutation in a gene for histidine synthesis to be able to form colonies on a selective medium. In some cases, a liver extract is added to simulate metabolic conversion of potential carcinogens into active carcinogens in the human body. The test has been further refined through the use of strains that respond to different types of mutagenic activity (base substitution vs. frameshift).

Base analogues: One of the more popular approaches to experimental mutagenesis is the use of base analogues. These are substances that are sufficiently similar to naturally occurring DNA bases so that their deoxyribonucleotide triphosphates will incorporate into DNA in place of the normal bases. However, they also have anomolous base-pairing properties, leading to an increased rate of mutagenesis. For example, 5-bromouracil (Fig. 14.10) pairs like thymine (5-methyluracil), but undergoes more enoltautomerization, leading to more frequent mispairing with guanine. Similarly, 2-aminopurine normally pairs with thymine, but can also pair with cytosine (see Insights and Solutions #2, page 423). These mispairings lead to an increase in the frequency of transition mutagenesis.

Nitrous acid: Treatment of DNA with nitrous acid leads to deamination of cytosine and adenine, again resulting in transitions, as described above for spontaneous deamination (Figure 14.13).

Alkylating agents: Certain alkylating agents, such as ethyl methane sulfonate (EMS) and ethyl ethane sulfonate (EES) add alkyl groups to purines, which can cause mispairing (Fig.14.11), and also destabilize the bond between the purine and deoxyribose, leaving ***apurinic*** sites. The absence of a base-pairing partner allows any base to be inserted during the next round of DNA synthesis. This frequently leads to transversions (as well as transitions).

Intercalation: Certain flat aromatic molecules, such as acridine orange and proflavin become inserted between base pairs in DNA, which can lead to misalignment during replication and the occurence of frameshift mutation (fig 14.12).

Reversion: As indicated earlier, the term reversion should only be used to describe an exact reversal of a mutation. For a base-substitution mutation (missense or nonsense), this would mean replacement of the substituted base with the original base. For a frameshift, this would mean removal of the inserted base pair or replacement of the deleted base pair. The net result of reversion is to restore the original genetic sequence exactly. Note that complete failure to revert usually indicates that a mutation is the result of a major change, such as a deletion that is incapable of being reversed.

Prepared by – Mr. P. Srinivasan, Assistant Professor, Dept. of Microbiology, KAHE.

Intragenic suppression: Intragenic suppression refers to a second mutation within the same coding unit that reverses the effect of the first mutation without actually correcting it. For example, if correct protein folding depended on interaction of a positive charge with a negative charge and the positive was mutated to negative, function could be restored by mutating the original negative to positive so that there was once again a positive-negative pair to guide the folding. Similarly, a frameshift might be reversed by a nearby second frameshift in the opposite direction, such that only a few non-essential amino acids were altered.

Intergenic suppression: In some cases, a second mutation in another gene can reverse the effects of a mutation. For example, if heterodimer formation is required for function, a complementary change in the second protein could allow proper pairing to occur once again. This is also the presumed mechanism for the intracistronic complementation that is sometimes observed, although in this case, the two changes are are in the same protein, making it intragenic suppression. Another example is suppression of a nonsense mutation by an altered tRNA that reads the stop codon as an amino acid specifying codon (Page 333).

Sickle-cell anemia as an example of a missense mutation: Sickle-cell anemia was identified in 1957 as being caused by a missense mutation resulting in a single amino acid substitution in the beta-globulin subunit of the hemoglobin tetramer (2 alpha + 2 beta subunits). A transversion causes the codon GAG to be changed to GUG (GTG in the DNA). This replaces a glutamic acid with a valine as the sixth amino acid (counting from the N-terminus) in the mature beta-globulin molecule. That substitution causes the hemoglobin to precipitate into fibrous aggregates that distort the shapes of red blood cells under low-oxygen conditions, resulting both in blockage of capillary circulation and breakage of the red blood cells. (Described on pages 371-373 of our textbook)

Heterozygote advantage: One obvious question is why a genetic disease as severe as sickle-cell anemia is present at such a high level in African-American populations. The reason is that in regions of Africa with a high incidence of malaria, individuals who are heterozygous for the altered beta-globulin have a better survival rate due to malaria resistance than individuals who are homozygous for unaltered beta-globulin. Thus, the heterozygotes had enough selective advantage so that the sickle-cell gene became well established in the population even though homozygotes were severely unhealthy and usually experienced early death. Unfortunately, this genetic legacy will persist for many generations, even in the absence of the selective effect of malaria.

Molecular basis for dominance and recessiveness: As we have gained a better understanding of the molecular nature of different types of mutaitons, we have also begun to understand what makes a particular mutation recessive or dominant.

Recessive mutations usually result from partial or complete loss of a wild type function. ***Amorphic*** alleles are those that have completely lost the function. An example would be a mutation in which production of pigment is completely lost in the homozygous state, causing albinism. ***Hypomorphic*** alleles are those in which function is reduced, but not completely lost. An example would be a mutation that causes a partial loss of pigmentation, giving a lighter color when homozygous.

Dominance can be caused in a wider variety of ways. There are three classes of so called gain-of-function alleles. ***Hypermorphic*** alleles are those that cause excess product to be produced. ***Antimorphic*** alleles are those that produce an altered gene product that "poisons" or disrupts the function of the normal gene product. ***Neomorphic*** alleles cause the gene product to be expressed in the wrong types of cells, and can have drastic effects, such as that of the antennapedia gene that coverts the antennae of flies into legs.

Another type of dominance is **haplo-insufficiency**. In this case, loss of a gene product causes a recognizably different phenotype in the heterozygote. This is considered to be a dominant mutation because the presence of one copy of the mutant allele in combination with one copy of the wild-type allele causes an altered phenotype. In many cases, the homozygote is lethal, as in the case of the Manx cat. In cases where the mutation is not lethal when homozygous, haplo-insufficiency is more likely to be called partial dominance, as in the formation of a pink flower by a heterozygote containing one red allele and one white allele.

DNA repair mechanism

This is only a summary and you will need to add details from Chapter 10 o f Madigan, Martino and Parker, Brock, Biology of Microorganisms, 10th editon. P rentice Hall, publishes.

Ionizing radiation causes three types of damage to DNA*:*

- **Single-strand breaks** - mostly sealed by DNA ligase so don't contribute to lethality
- **Double-strand breaks** - often lethal because can't be resealed by ligase so degraded by nucleases
- **Alteration of bases** - this type of oxidative damage is usually lethal because it forms a replication barrier at that site.

A UV radiation - 260 nm is wavelength at which maximum absorption occurs for DNA

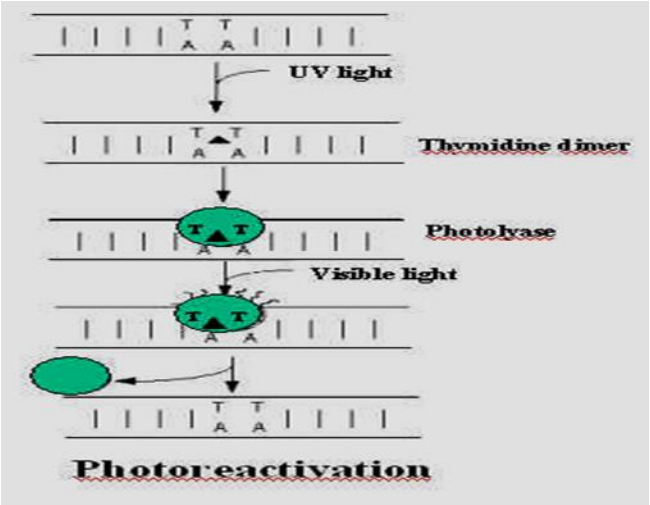
UV - major photoproduct is intrastrand linkage of adjacent pyrimidines, usually thymines, called thymine dimers. Creates distortion in helix and affects replication and transcription.

Pol III can't replicate past T-dimer because if puts in A across from dimer, recognizes the weak H-bonding as a mismatch and proofreads. Tries to put in another A, fails. Causes stuttering of Pol III at this site.

There are four ways to repair of T dimers in *E. coli*:

Photoreactivation (aka Light Repair)

phr gene - codes for deoxyribodipyrimidinephotolyase that, with cofactor folic acid, binds in dark to T dimer. When light shines on cell, folic acid absorbs the light and uses the energy to break bond of T dimer; photolyase then falls off DNA.



Excision Repair

There are 3 different types of repair mechanisms which use different enzymes but none-the-less follow the same basic principle as outlined in the figure below.

AP Repair (Base Excision Repair, BER):

Repair of apurinic and apyrimidinic sites on DNA in which base has been removed. Base removed by radiation or DNA glycosylases which sense and remove damaged bases. *ung* gene codes for uracil-DNA glycosylase which recognizes and removes U in DNA by cleaving the sugar-nitrogen bond to remove the base. AP endonucleases: class I nick at 3' side of AP site and class II nick at 5' side of AP site. Exonuclease removes short region of DNA and DNA Pol I and ligase fill in gap.

UV Damage Repair (also called NER - nucleotide excision repair):
NER differs from BER in several ways.
1.It uses different enzymes.

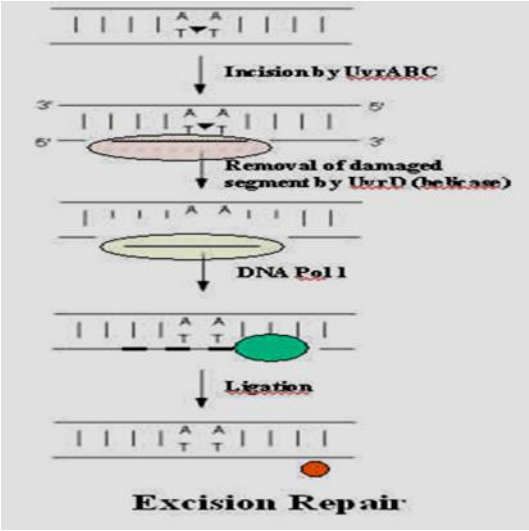
2.Even though there may be only a single "bad" base to correct, its nucleotide is removed along with many other adjacent nucleotides; that is, NER removes a large "patch" around the damage.

3.Excinuclease (an endonuclease; also called correndonuclease [correction endo.]) that can detect T dimer, nicks DNA strand on 5' end of dimer (composed of subunits coded by *uvrA, uvrB* and *uvrC* genes). UvrA protein and ATP bind to DNA at the distortion.

4.UvrB binds to the UvrA-DNA complex and increases specificity of UvrA-ATP complex for irradiated DNA. UvrC nicks DNA 8 bases upstream and 4 or 5 bases downstream of dimer.

5.UvrD (DNA helicase II; same as DnaB used during replication initiation) separates strands to release 12-bp segment.

6.DNA polymerase I now fills in gap in 5'>3' direction and ligase seals. *polA* - encodes DNA pol I; mutant was viable (retained normal 5'>3' exo. activity and only 2% of polymerase activity) so Pol I not major replication enzyme, but mutant also had somewhat increased sensitivity to UV so first suggested that played a role in DNA repair.



Mismatch Repair (MMR): Accounts for 99% of all repairs
Follows behind replication fork. Two ways to correct mistakes made during replication:

- 3'>5' exonuclease - proofreading
- Mismatch repair

Because of methylation. DNA methylase (coded for by *dam* [DNA adenine methylase] locus) methylates 5'-GATC-3' sequence in DNA at A residue. Mismatch from replication recognized by *mutL* and *mutS* gene products. *mutH* gene product nicks DNA strand (progeny strand) on either side of mismatch. DNA helicase II from *mutU* gene (also called *uvrD* gene) unwinds DNA duplex and releases nicked region. Gap filled in by DNA Pol I and ligase.

Postreplicative (Recombinational) Translesion Bypass Repair

If T dimer is not repaired, DNA Pol III can't make complementary strand during replication. Postdimer initiation - skips over lesion and leaves large gap (800 bases). Gap may be repaired by enzymes in recombination system - lesion remains but get intact double helix. RecA - coats ssDNA and causes it to invade dsDNA. When stimulated by presence of ssDNA, it also acts as protease to cleave lambda repressor and acts to cause autocatalysis of LexA repressor. *recA* mutants - very UV-sensitive Now have sister-strand exchange - a type of recombination Translesion bypass. Postreplicative repair is part of SOS response.

SOS Repair

- occurs when cells are overwhelmed by UV damage - this allows the cell to survive but at the cost of mutagenesis.
- response is only triggered when other repair systems fail as they are overwhelmed by the increased amount of damage so that unrepaired DNA accumulates in the cell.
- The accumulation of DNA damage leads to repair induction or W-reactivation (Weigle-reactivation).
- Irradiated lambda phage are more likely to survive in an irradiated rather than. anunirradiated host because SOS system has already been turned on in irradiated host.

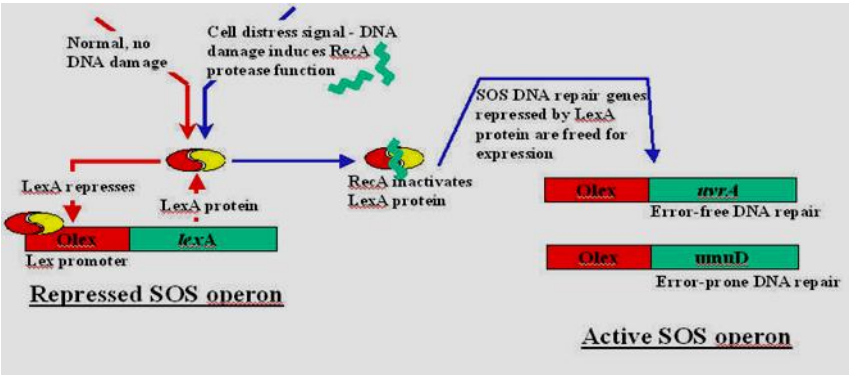


Fig: SOS repair mechanism

KARPAGAM ACADEMY OF HIGHER EDUCATION (KARPAGAM UNIVERSITY)
DEPARTMENT OF MICROBIOLOGY
MOLECULAR GENETICS - 17MBP103

Unit IV Question
_____ involves finding a contiguous series of cloned DNA fragments which contain overlapping portion
_____ refers to a genetic changes in different genomes of same cell.
_____ is a DNA associated protein
A bacterium harboring a prophage is called
A cell carrying an integrated F factor is called an
An example for specialized transducing particle
Avery, MacLeod & McCarty used enzymes and solvents to destroy molecules such as
Bacteriophages were jointly discovered by
Capsule of *Streptococcus pneumoniae* are made up of
Capsules help bacteria in escaping
Cells carrying non-integrated transducing fragments are called _____
Complete linkage has been reported in
Conditions that favor the termination of the lysogenic state
Conjugation can only occur between cells of
Conjugation involves the use of _____for mapping
Conjugation involves the use of _____for mapping
Conjugation is predominant in _____
Conjugational genetic exchange has been - - - - - - - - frequently encountered among gram positive than gr
Crossing over occurs during
Direct way of observing the physical arrangement of markers along the chromosomes
During insertion of lambda DNA in to host, a viral protein called _____ is required along with integra
Experiment on transformation
Genes responsible for antibiotic resistance are mainly transferred across bacterial population by
Genes that cause suppression of mutations in other genes are called -------- genes
Genetic fine structure mapping of T4 was studied by _____
Genetic recombination in phages was discovered by _____
Genome of T4 phage is
Genome of different bacteria suggest that genes have in the past moved from one species to another. This p
Give the full form for Hfr
In conjugation, the donor always carries on
In genetic mapping, the measurement of distance between the genes is expressed as
Integeration of viral nucleic acid in to host chrosome is termed as
Linkage prevents
Map distance is equal to the percentage of _____
Metalloproteins found in all eukaryotes
Methods used to identify the locus of a gene and the distances between genes
Non sex chrosomes are called
Occurs when new DNA does not integrate into the chromosome, not replicated and is eventually lost
Pneumococcal 'S' cells produce _____ colonies during growth on agar plates

Results from inaccurate excision of an integrated prophage with addition of some bacterial genes
Size of T4 phage genome
Specialised transduction is effected by _____
T4 bacteriophages generally parasitizes
T4 is capable of undergoing only a
Tendency of alleles located close together on a chromosome to be inherited together during the meiosis
The Competence of a cell in the process of transformation is aided by
The complex of DNA, RNA and protein is
The first demonstration of bacterial transformation was done with
The frequency at which two genes are - - - - - - - by population of phages can be used to estimate their re
The gene linkage minimize the chances of
The non specific transduction is also called as - - - - - - - - -
The phenomenon in which genes are present on the same chromosomes is
The phenomenon of linkage was first observed in the plant
The process to identify a genetic element that is responsible for a disease is also referred as
The viral genome integrated to the bacterial genome is called
Transfer of a portion of chromosome to a recipient with direct contact is termed
Transfer of DNA from one bacterium to another through the action of viruses
Uptake of DNA molecules from environmental surrounding
Virulence in *Streptococcus pneumoniae* is attributed to
Who coined the term linkage

Opt 1
Physical mapping
Trans type
Protone
Lytic phage
F
No infection
Anything except DNA
Frederick Twort and by Felix d'Herelle
Protein
Inflammation
Specialized
Male Drosophila
Desiccation
F positive types
Interrupted mating
Interrupted mating
Spirochaetes
More
Pachytene
Fluorescence in situ hybridization
Integrase
Monad
Conjugation
Reverse genes
Benzon
Hershey and Rotma
ds DNA
DNA transfer
High fertility recombination
(F-)
Centimorgan
Microphage
Segregation of alleles
Recombinant meiotic product
Zinc fingers
Gene mapping
Rhizomes
Abortive transduction
Smooth

Specialized Transduction
169 kbp
T_2
Bacillus
Lytic cycle
Linkage
$CaCl_2$
Chromatin
Streptococcus pyogenes
Transduced
Cross over
Restricted transduction
Cross over
Lathyrus odoratus
Mapping
Plasmid
Gene expression
Transduction
Transduction
Flagella
Mendel

Opt 2
Chemical mapping
Cis type
Histone
Helper phage
Hfr
T2 phage infects *Staphylococcus*
RNA
Hershey and Chase
Lipid
RBC's
Abortive transductants
Human female
Decomposition
F negative types
Direct mapping
Direct mapping
G+ bacteria
Less
Diplotene
Fluorescence invitro hybridization
Caspase
Griffith
Transformation
Control genes
Mendel
Hershey and Chase
ss DNA
RNA transfer
High fundamental recombination
(F+)
Centimeter
Prophage
Homozygous condition
Reproducible meiotic product
Iron fingers
Chromosomal linkage
Lysosomes
Specialized Transduction
Rough

Generalized Transduction
196 kbp
Mu
E.coli
Lysogenic cycle
Crossing over
$MgCl_2$
Somatin
Staphylococcus aureus
Co transduced
Segregation
Generalized transduction
Segregation
Pisum sativum
Linkage
Capsid
Transformation
Conjugation
Conjugation
Capsules
Morgan

Opt 3
Marker mapping
Same type
Chromotome
Transducing phage
Hfr 1+
Phage P22 infects *Salmonella typhimurium*
Lipids & proteins
Luria and Delbruck
Glycoprotein
Phagocytosis
Generalized
Female Drosophila
Nutrient Media
Same mating types
Contact mapping
Contact mapping
G-bacteria
Very High
Diakinesis
Fluorescence invivo hybridization
Helicase
Morgan
Transduction
Suppressor genes
Colins
Hershey and Wollmer
ds RNA
Gene transfer
High frequency recombination
F neutral
Millimorgan
Prephage
Hybrid formation
Recombinant mitotic product
Lead fingers
Gene walking
Mesosomes
Generalized Transduction
Elongated

Abortive transduction
619 kbp
P_1
Psuedomonas
Both Lytic & Lysogenic cycle
Gene overlapping
KCl
Pigmentin
Streptococcus pneumoniae
Co repressor
Recombination
Non specific transduction
Linkage
Datura
Sequencing
Prophage
Transduction
Transformation
Transformation
Pili
de Vries

Opt 4	Opt 5	Opt 6
Loci mapping		
Different type		
Cistron		
Lysogency		
trans		
Phage lambda infects *E.coli*		
Polysaccharide		
McKay and McCartney		
Polysaccharide		
Antibodies		
Conjugation		
Maize		
Macronutrient		
Opposite mating types		
Linkage		
Linkage		
Cyanobacteria		
Very low		
Haplotene		
Fluorescence in cell hybridization		
Polymerase		
Hersehy		
Gene expression		
Inducer genes		
Bennazir		
Hershey and Singer		
ss RNA		
Protein transfer		
Heavy frequency recombination		
No F		
Millimeter		
Macrophage		
Heterozygous condition		
Reproducible mitotic product		
Copper fingers		
Chromosomal walking		
Autosomes		
Transfusion		
Flat		

Transfusion		
916 kbp		
T_7		
Agrobacterium		
Other cycle		
Recessive genes		
$AgCl_2$		
Fromatin		
Klebsiella pneumoniae		
Co operator		
Assortment		
Specialised transduction		
Assortment		
Mirabilus jalapa		
Genome data mining		
Virion		
Conjugation		
Gene expression		
Gene expression		
Fimbriae		
Correns		

Answer
Physical mapping
Cis type
Histone
Lysogency
Hfr
Phage lambda infects *E.coli*
Anything except DNA
Frederick Twort and by Felix d'Herelle
Polysaccharide
Phagocytosis
Abortive transductants
Male Drosophila
Desiccation
Opposite mating types
Interrupted mating
Interrupted mating
G-bacteria
Less
Pachytene
Fluorescence in situ hybridization
Integrase
Griffith
Conjugation
Suppressor genes
Benzon
Hershey and Rotma
ds DNA
Gene transfer
High frequency recombination
(F+)
Centimorgan
Prophage
Segregation of alleles
Recombinant meiotic product
Zinc fingers
Gene mapping
Autosomes
Abortive transduction
Smooth

Specialized Transduction
169 kbp
T_2
E.coli
Lytic cycle
Linkage
$CaCl_2$
Chromatin
Streptococcus pneumoniae
Co transduced
Recombination
Generalized transduction
Linkage
Lathyrus odoratus
Mapping
Prophage
Conjugation
Transduction
Transformation
Capsules
Morgan

DEPARTMENT OF MICROBIOLOGY
KARPAGAM ACADEMY OF HIGHER EDUCATION
KARPAGAM UNIVERSITY

(Deemed University Established Under Section 3 of UGC Act, 1956)
Eachanari PO, Coimbatore -641 021, India.

I - M.Sc Microbiology (Batch 2017-2019)

Lecture Plan

Unit - V

S. No	Duration	Topic	Reference
1.	1	Introduction to yeast Genetics	R1: 120 to 123
2.	1	Metabolism: genome and extra chromosomal elements	T1:130 to 131 R1: 143 to 152
3.	1	Genetic nomenclature in yeast	R1: 153 to 157
4.	1	Tetrad Analysis	R1:528 to 529
5.	1	Petit mutant -yeast	T1: 122 to 129/W1
6.	1	Petit mutant - Neurospora	T1:122 to 129/W1
7.	1	Petit mutant – Drosophila	T1:122 to 129/W1
8.	1	Class Test V	-
9.	2	Revision of Previous year ESE question paper	-
		Total Hours	**10**

R1: David Freifelder, Microbial Genetics. Narosa Publishing House, 10th edition, 2004. New Delhi, India.

T1: Peter J. Russell, i Genetics – A molecular approach, 7th edition, 2010. Pearson Benjamin Cummings Publishers, Boston, USA.

W1: www.Molecularbiologyfordummies.com/Genetics/index.php

P. SRINIVASAN
Assistant Professor
Department of Microbiology

2017

Unit- V

General Features of Gene Transfer in Bacteria

- Unidirectional
  - Donor to recipient
- Donor does not give an entire chromosome
  - Merozygotes
- Gene transfer can occur between species

Bacterial genome alteration

***Transformation*-** alteration of bacterialDNA by uptake of naked, foreign,DNA from the surrounding environment.

***Transduction*-**DNA transfer via phages

Generalized-random pieces of host DNA gets transfered

Specialized-prophage exits chromosome and carried pieces of host DNA with it

***Conjugation* -**Gene transfer from a donor to a recipient by direct physical contact between cell

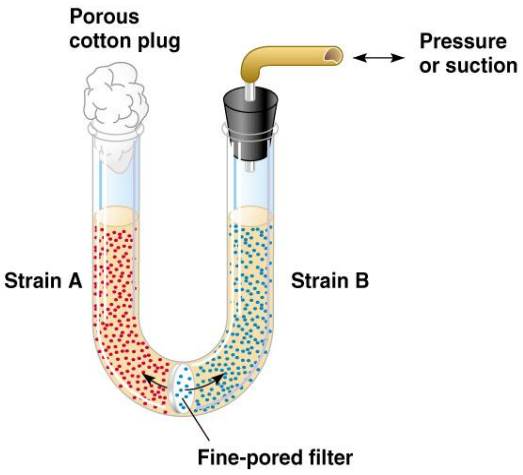


Fig: Davis's U-tube experiment

Transformation

***Definition*:** Gene transfer resulting from the uptake of DNA from a donor.

Factors affecting transformation

- DNA size and state
  - Sensitive to nucleases
- Competence of the recipient (*Bacillus, Haemophilus, Neisseria, Streptococcus*)
  - Competence factor
  - Induced competence

Steps

- Uptake of DNA

Prepared by – Mr. P. Srinivasan, Assistant Professor, Dept. of Microbiology, KAHE.

- Gram +
- Gram -

– Recombination
  - Legitimate, homologous or general
  - recA, recB and recC genes

Significance

– Phase variation in *Neiseseria*
– Recombinant DNA technology

Genetic Mapping in Bacteria by Transformation

Transformation is used to map genes in situations where mapping by conjugation or transduction is not possible.

- Donor DNA is extracted and purified, broken into fragments, and added to a recipient strain of bacteria. Donor and recipient will have detectable differences in phenotype, and therefore genotype.
- If the DNA fragment undergoes homologous recombination with the recipient's chromosome, a new phenotype may be produced. Transformants are detected by testing for phenotypic changes.

Some bacterial cells take up DNA naturally (e.g., Bacillus subtilis), while others require engineered transformation for efficient transfer (e.g., E. coli).

Completed transformation occurs in a small proportion of the cells exposed to new DNA. Bacillus subtilis is an example

- Donor is wild-type (a+). Recipient is mutant (a).
- One of donor DNA strands is degraded, leaving ssDNA with the a+ allele.
- The donor ssDNA pairs with homologous DNA in recipient's chromosome, forming a triple-stranded region.
- A double crossover event occurs, replacing one recipient DNA strand with the donor strand.
- The recipient now has a region of heteroduplex DNA. One strand has the recipient's original a allele and the other strand has the new a+ allele.
- DNA replication will produce one chromosome with the original (a) genotype, and one with the recombinant (a+) genotype.
- The cell with the recombinant genotype is then selected by its phenotypic change.

Prepared by – Mr. P. Srinivasan, Assistant Professor, Dept. of Microbiology, KAHE.

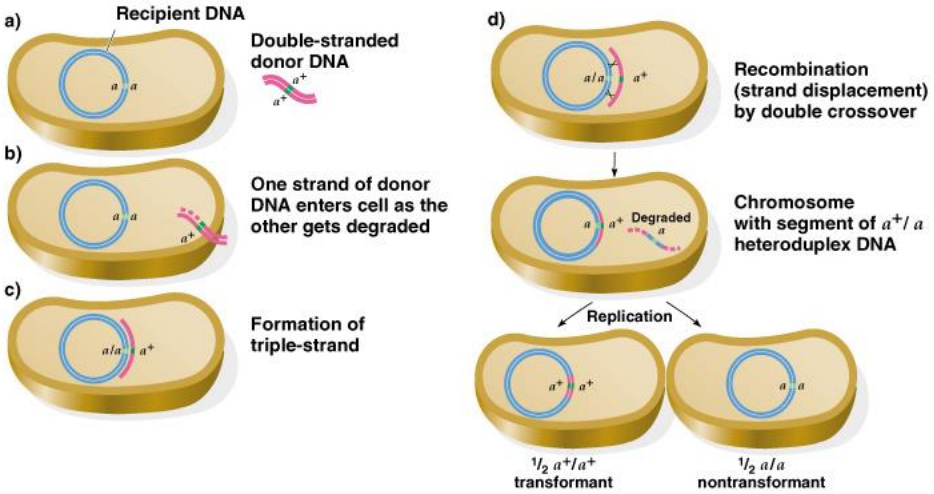


Fig: Process of Transformation

Transformation experiments are used to determine:

- Whether genes are linked (physically close on the bacterial chromosome).
    - Transformation works best with small DNA fragments that hold only a few genes.
    - Cotransformation is an indication that two genes are near each other. It is analyzed mathematically.
        - Experimentally, if cotransformation is more frequent than would be expected randomly (the product of the transformation rates for each gene), the genes must be close together.
        - If the cotransformation rate is close to the transformation rate for each gene alone, the genes are linked.
- The order of genes on the genetic map.
        - Suppose two genes (e.g., p and q) cotransform and are thus linked. One of them (e.g., often cotransformations with another gene (e.g., *o).*
        - Determining the distance between p and *o* involves analyzing their cotransformation frequency.
            - If p and *o* rarely cotransform, the gene order is *p-q-o.*
            - If p and *o* frequently cotransform, the gene order is *p-o-q.*
- The map distance between genes. Recombination frequencies are used to infer map distances

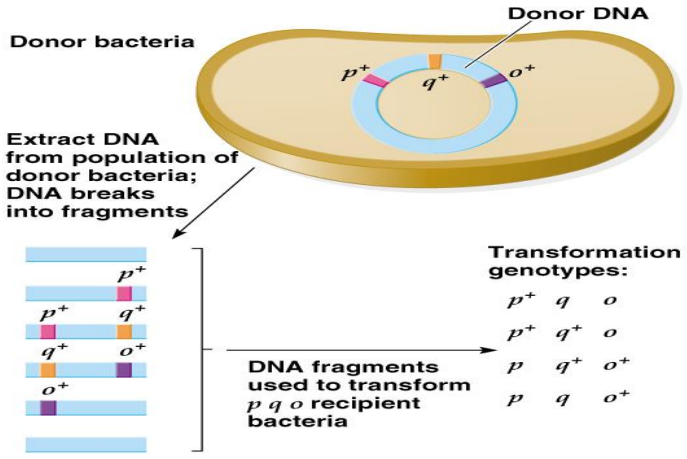


Fig: Mapping by Transformation

Transduction

Definition: Gene transfer from a donor to a recipient by way of a bacteriophage

- Significance
  - Common in Gram+ bacteria
  - Lysogenic (phage) conversion
- Resistant to environmental nucleases
- Types of transduction
  - Generalized - Transduction in which potentially any dornor bacterial gene can be transferred.
  - Specialized - Transduction in which only certain donor genes can be transferred
  -

Generalized Transduction

- Infection of Donor
- Phage replication and degradation of host DNA
- Assembly of phages particles
- Release of phage
- Infection of recipient
- Legitimate recombination

Specialized Transduction

- Lysogenic Phage
- Excision of the prophage
- Replication and release of phage
- Infection of the recipient
- Lysogenization of the recipient
  - Legitimate recombination also possible

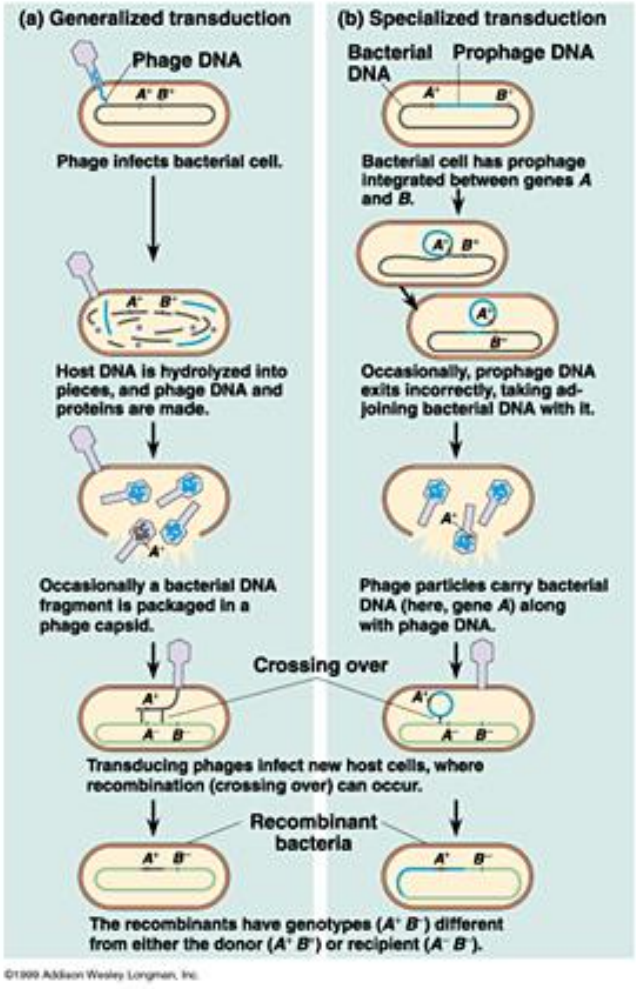


Fig: Process of Transduction

Mapping by Transduction

Generalized Transduction

Generalized transduction was discovered by Lederberg and Zinder (1952), in an experiment with Salmonella typhimurium bacteria

- Experiment was similar to the *E. coli* conjugation experiment, in which bacterial strains are separated by a ifiter to prevent physical contact.
- *S. typhimurium,* unlike *E. coli*, produced recombinants in this experiment. The filterable agent moving genes was the temperate phage P22.

Another example of a generalized transducing phage is P1 in E. coli

- P1 enters and integrates as a prophage.
- If the lysogenic state is not maintained, P1 enters a lytic cycle and produces progeny phages.

- The bacterial chromosome is degraded during lytic infection, and rarely, bacterial DNA is packaged as if it were a P1 chromosome, producing a transducing phage.
- The transducing phage DNA enters the host cell in the normal P1 way, and may be incorporated into the host's chromosome by homologous recombination. The resulting bacteria are transductants.

Transduction experiments use genetic markers to follow gene movement.

- Selectable markers allow detection of low frequency events. For example, auxotrophic recipients can easily be detected if they convert to the donor';s prototrophic phenotype, because they alone can grow on minimal media.
- Other markers in the experiment are called unselected markers.

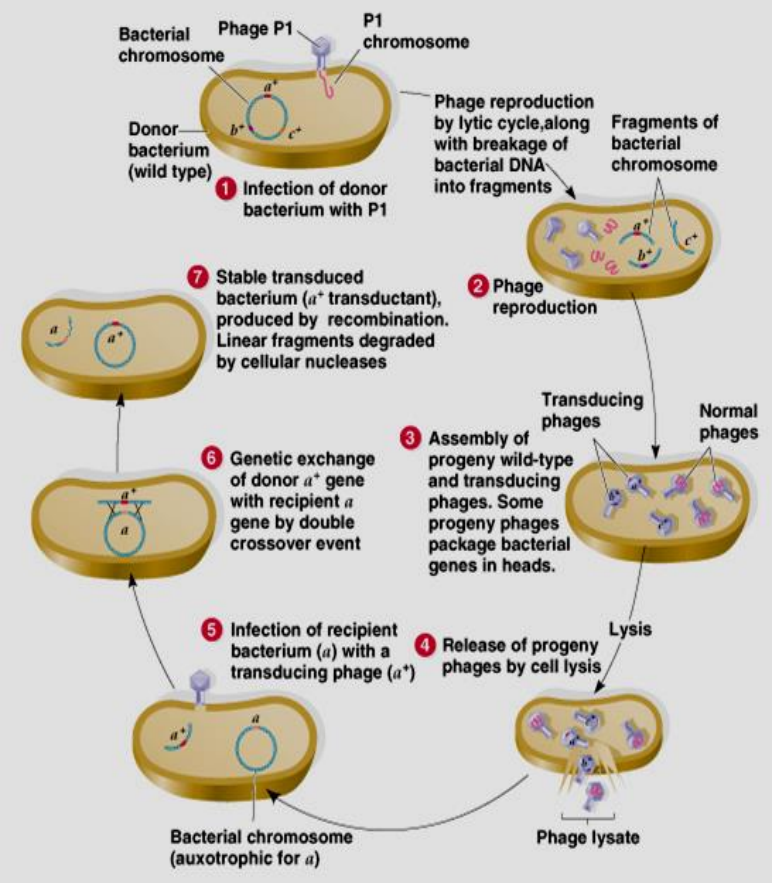


Fig: Generalized transduction between strains of *E. coli*

Closely linked genes are cotransduced at high frequency, allowing a detailed genetic map to be generated. For example:

- P1 was used to map *E. coli* genes.
  - Donor strain is able to grow on minimal medium, and is also resistant to the metabolic poison sodium azide *(leu+ thr+ aziR*).

- Recipient strain can't make leucine or threonine, and is poisoned by sodium azide *(ieu thr azi*S*).*
- P1 lysate grown on donor cells is used to infect recipient cells.
- Transductants can be selected for any of these traits (e.g., *leu*+, and then checked for the unselected markers (e.g., *thr*+ *aziR)*
- For example:
  - Of the l*eu*+ selected transductants, 50% have *aziR* and 2% have *thr*+.
  - Of the *thr*+ selected transductants, 3% have *leu*+, and 0% have *aziR.*
  - This gives the map order: *thr—leu--azi.*
  -

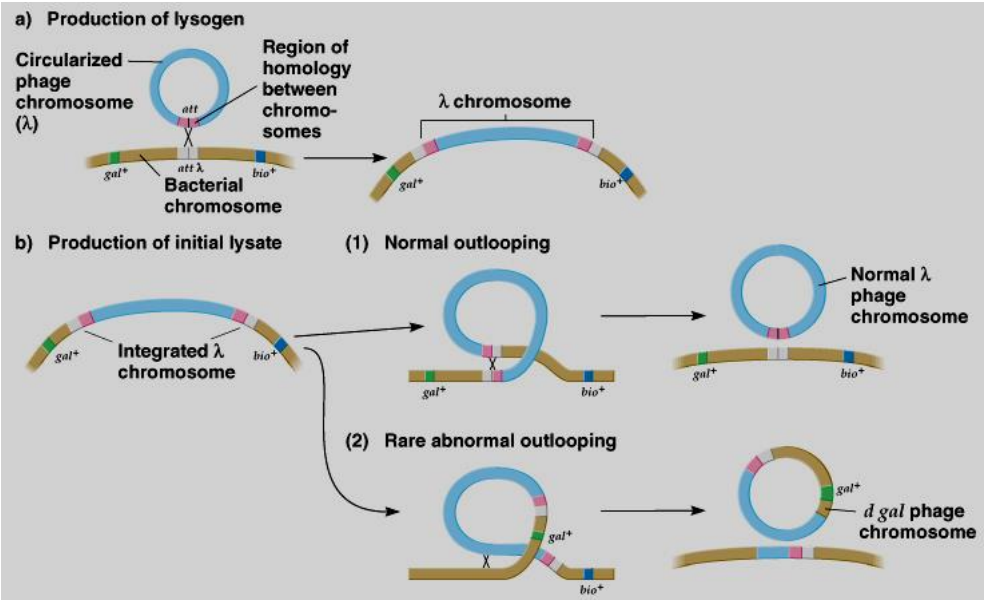
*Map distances are calculated from the cotransduction frequency of gene pairs. It is effective only with genes located near each other on the chromosome***.**

Specialized Transduction

Some phages transduce only certain regions of the chromosome, corresponding with their integration site(s). An example is λ in E. coli

- Phageλ integrates by a single crossover into the *att*λ site on the *E. coil K12* chromosome. The *att*λ site is located between the *gal* and *bio* genes. The prophage is maintained by a phage repressor protein.
- In this example, the *E. coil K12* strain that integrates theλ prophage is *gal*+ (a phenotype easily detected with specific culture medium).
- If this *E. coli K12(λ)* is induced to the lytic cycle, theλ prophage DNA is excised by a single cross-over event.
- Excision is usually precise.
- Rarely excision results in genetic exchange, with a fragment ofλDNA remaining in the *E. coli* chromosome, and some bacterial DNA (e.g., *gal*+) added to the λ chromosome.
- The resulting transducing phage is designated λ*d gal*+ *(d* for defective, since not all phage genes are present).
- λd *gal*+ can replicate and lyse the host cell, since allλgenes are present either on the phage or bacterial chromosome.
- Because transducing phage are only rarely produced, a low- frequency transducing (LFT) lysate results. Infection of *gal* bacterial cells results in two types of transductants
- Unstable transductants result when wild-typeλintegrates first at its normal *att*λsite. λd *gal*+ then integrates into the wild-typeλ, producing a double lysogen with both types ofλ integrated.
- The host bacterium is now heterozygous *(gal*+ */ gal),* and can ferment galactose.
- The transductant is unstable because the wild-typeλcan be induced into the lytic cycle. Both wild-typeλand λd *gal*+ are replicated, producing a high-frequency transducing (HFT) lysate.
- Stable transductants are produced when a cell is infected only by a λd *gal*+ phage, and the *gal*+ allele is recombined into the host chromosome by double cross-over with *gal.*

Specialized transduction is useful for moving specific genes between bacteria, but not for general genetic mapping.



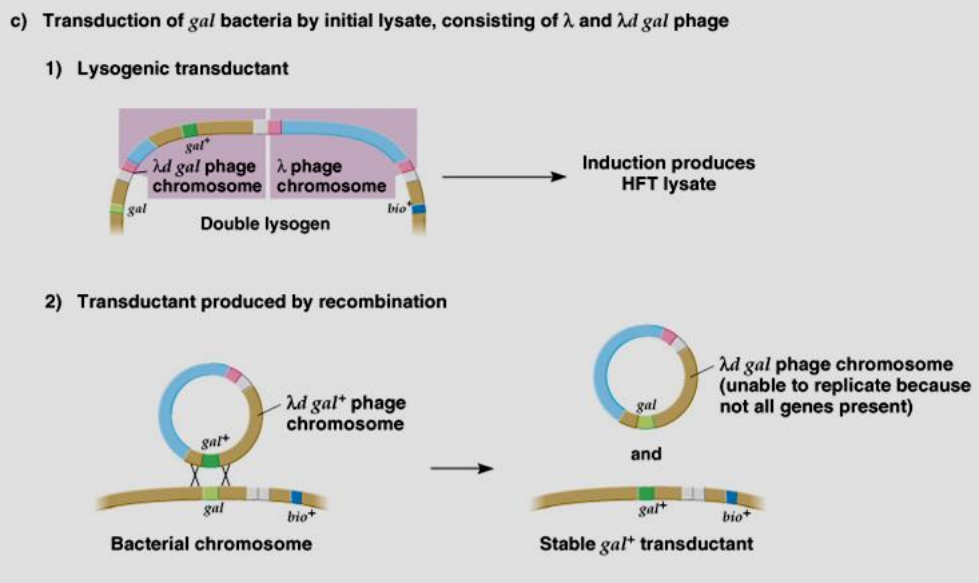


Fig: Specialized transduction by bacteriophage λ

Discovery of Conjugation in *E. coli*

Lederberg and Tatum discovered conjugation (1946) using two E. coli auxotrophic mutant strains:

- Strain A's genotype was met bio thr+ leu+ thi+. It grows on minimal medium supplemented with methionine and biotin.
- Strain B's genotype was met+ bio+ thr leu thi. It grows on minimal medium supplemented with threonine, leucine and thiamine.

- Strains A and B were mixed, and plated onto minimal medium. About 1/106 cells produced colonies with the phenotype met+ bio+ thr+ leu+ thi+ (Figure 14.2).
- Neither strain produced colonies when plated alone onto minimal medium, so the new phenotype resulted from recombination.

Davis tested whether cell-to-cell contact was required:

- Strain A cells were placed on one side of a filter, and strain B on the other. Cells could not move through the filter but molecules moved freely, encouraged by alternating suction and pressure.
- No prototrophic colonies appeared when the cells were plated on minimal medium. This indicates that cell-to-cell contact is required, and the genetic recombination results from conjugation.

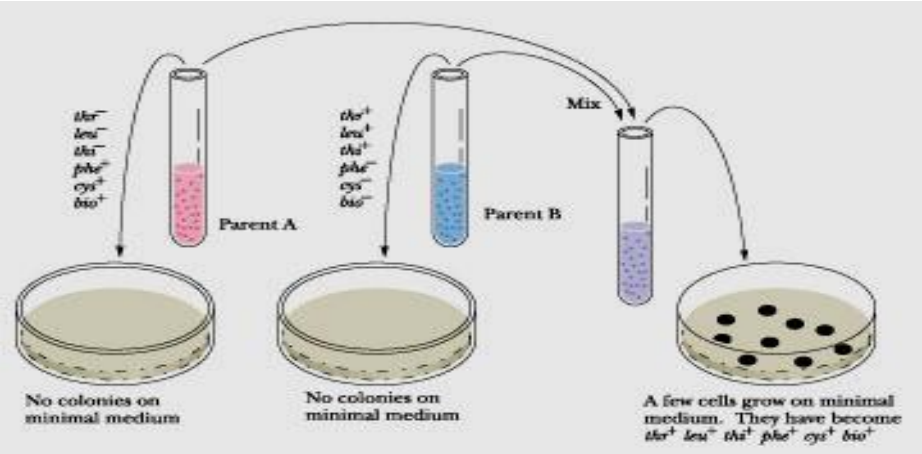


Fig: Cell to cell contact by conjugation a medium of gene transfer

Sexual Conjugation in Bacteria

The transfer of DNA between bacteria takes place via a process known as **sexual conjugation,** a phenomenon unsuspected prior to the Lederberg-Tatum experiment. Bacterial cells sometimes contain, in addition to their chromosome, extrachromosomal DNA molecules called **plasmids** . Plasmids represent "extra" or auxiliary genetic information. Bacterial cells are capable of conjugation if they possess a particular plasmid called the **F factor** (F for fertility). Such F^+, or *donor, cells* have thin, hollow tubes projecting from their surface known as **sex pili** or **F pili** (singular = *pilus*). One or more pili can bind to specific receptors on the surface of cells that lack an F factor (F^-, or *recipient, cells*)The pilus provides a connection between the two cells. Upon conjugation, a single strand of the F factor is passed to the F^- cell, where its complementary strand is synthesized .The recipient F^- cell thus becomes F^+ by virtue of now having a double-stranded F factor plasmid. The F factor plasmid consists of about 94,000 base pairs; about one-third of this DNA is devoted to about 25 genes that function specifically in the transfer of genetic material from F^+ to F^- cells. Among these genes are those necessary for the formation of pili. In reality, the F factor is an infectious agent.

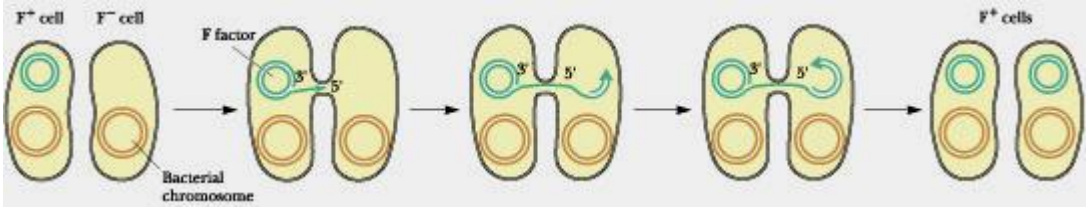


Fig: Physical factors in Bacterial conjugation

Mapping by Conjugation

Chromosomes In 1946, Joshua Lederberg and Edward Tatum discovered that genetic information could be transferred between bacteria. They used two strains of E. *coli* that differed in their growth requirements due to mutations each carried .One strain (*thr*$^-$, *leu*$^-$, *thi*$^-$) required threonine, leucine, and thiamine to grow; the other (*phe*$^-$, *cys*$^-$, *bio*$^-$) required phenylalanine, cystine, and biotin. These two strains were mixed together and spread on the surface of a petri plate of minimal medium lacking any of the required supplements. After a day, a very small number of bacterial colonies were observed to be growing. Somehow, these growing bacteria had acquired functional (wild-type) copies of each of the mutant genes. This remarkable result suggested strongly that the chromosomes of the two different cell types were brought together in a process akin to sexual exchange. In order for the progeny cells (which contain but one chromosome) to have acquired genetic information from the parental strains, **genetic recombination** must have occurred. This represents, in the words of Lederberg and Tatum, "the assortment of genes in new combinations." Apparently, at some point in time, parental DNA molecules must have aligned along regions of homology (sequence similarity), and segments from one of these molecules must have been interchanged with similar segments from the other parents so that some DNA molecules (chromosomes) now carried wild-type *thr*$^+$ *leu*$^+$ *thi*$^+$ *phe*$^+$ *cys*$^+$ *bio*$^+$ gene

Interrupted-mating experiment

Conjugation experiments to map genes begin with appropriate Hfr strains selected from the progeny of F+ X F- crosses.Jacob and Wollman (1950s) used Hfr donor strains with allelic differences from the F- recipient strains, in interrupted-mating experiments.

- Donor: HfrH thr+ leu+ aziR tonR lac+ gal+ strR.
- Recipient: F- thr leu aziS tonS lac gal strS.
- The 2 cell types are mixed in liquid medium at 37°C. Samples are removed at time points and agitated to separate conjugating pairs.
- Selective media are used to analyze the transconjugants. Results in this experiment:
    - The 1st donor genes to be transferred to the F- recipient are thr+ and leu+, and their entry time is set as 0 minutes.
    - At 8 minutes, aziR is transferred, and tonR follows at 10 minutes.
    - At about 17 minutes lac+ transfers, followed by gal+ at about 25 minutes.
- Recombination frequency becomes less at later time points, because more pairs have already broken apart before the sample was taken.

The transfer time for each gene is reproducible, indicating its chromosomal position. A map may be constructed with the distance between genes measured in minutes. (The *E. coli* chromosome map spans about 100 minutes)

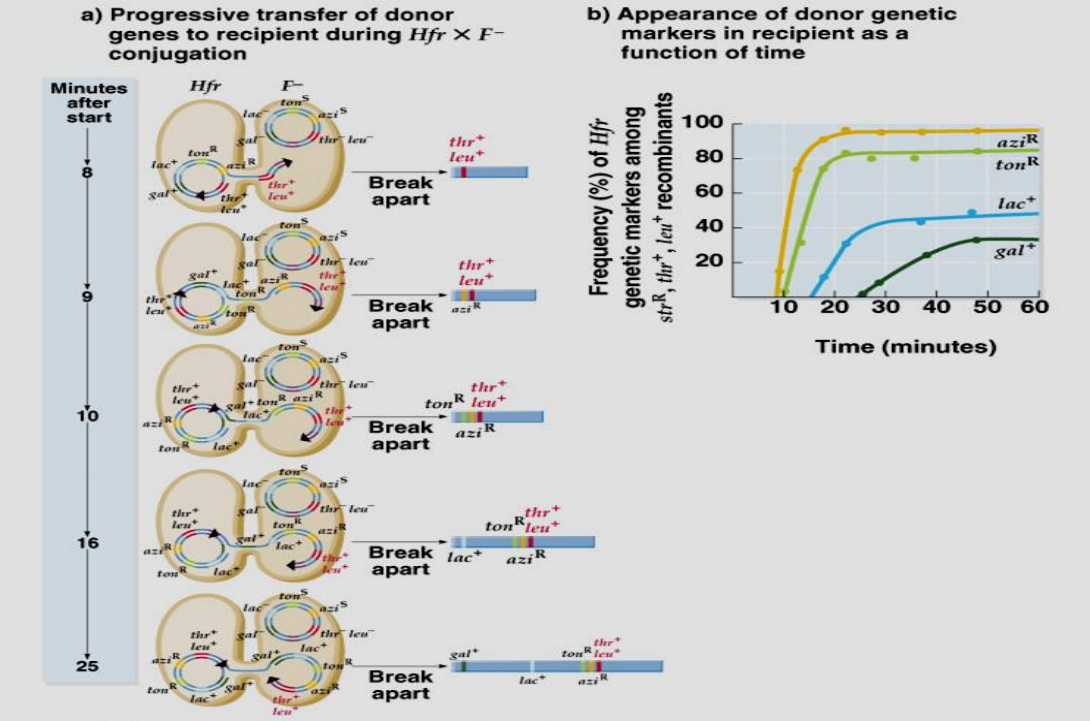


Fig: Interrupted-mating experiment

KARPAGAM ACADEMY OF HIGHER EDUCATION (KARPAGAM UNIVERSITY)
DEPARTMENT OF MICROBIOLOGY
MOLECULAR GENETICS - 17MBP103

Unit V Question
_____ test is used to determine which gene(s) are defective in petite yeast mutants
_____ contains two types of spores of same parental genotype
_____ intial products of meiosis forms two identical spore
_____ refers to a genetic changes in different genomes of same cell
_____determines the number of crossover events and give correct map distance
_____developed an algebriac method to determine the consequence of various number of exchanging
_____distance in map unit is only half the percentage of second division segregated tetrads
A feature of petite is the occurrence of _____ from the circular mitochondrial genome
A new genetic nomenclature for S. cerevisiae transposon
Among haploid and diploid vegetative cells of yeast ,which is mainly used for genetic mapping
An estimation of map between a- & b- can be obtained from number of recombinant ascospore detected by
Arg^{+} is
Cells which contains single copy of chromosome is called _____
Common model organism in studying unicellular eukaryotes/budding yeast
Gene conversion can be either allelic or _____
Gene conversion in yeast may be important in maintaining_____within families
Gene conversion is the _____ transfer of information from one DNA duplex to another
Generation time of yeast takes place at _____
Genetic maps of chromosome are based on the frequencies of _____
Give full form for NPD
Haploid to diploid phase in yeast is brought about by
HFT is
His 3 is an _____ protein.
If single crossover occurs between a- & b- then tetra type ___ results
In *Drosophila* , the meiotic recombination occurs only in _____
In Transcription of yeast genome,_____ makes all RNA to serve as mRNA
In yeast, 22% of the genome is made up of _____ DNA
Intermediate compound responsible for red color of adenine-requiring yeast petite mutants
Map distance is equal to the percentage of
Meiosis in yeast life cycle leads in forming
Method available for locating mutation in *Neurospora crassa* is
Petite phenotypes caused by the absence of, or mutations in, mitochondrial DNA are termed as
Petite yeast mutants are unable to grow on media containing
Pleiotrophy is common in _____
Process by which one DNA sequence replaces a homologous sequence
Random spore analysis gives _____ values
Recombination does not only occur during meiosis, but also as a mechanism for
Sample of ascospores is spread on to the agar medium without leucine and survival was tested using
Sex linkage was explained by _____

Significant feature of sex determination in Drosophila is the presence of abnormal flies called _____
Small pieces of DNA that can insert themselves into chromosomes are known
Tetrad showing second division segregation has _____ products
The ascus burst releasing the ascospores, each of which germinates and divides by mitosis to produce new
The binding of two DNA helices through X-shaped junction called _____
The developing haploid spores are enclosed in a membranous structure called_____
The general mapping function of Haldane is based on _____
The leu2- strain carries a mutation that inactivates leu2 gene which codes for _____
The percentage of recombinant meiotic product is one half of the percentage of _____
The repair of double-strand gaps is an efficient process in yeast known to be
The term genetic linkage was given by ----------
The_____ to a particular chromosome is the first step in genetic mapping
Well characterized Baker's yeast has __ cell
When mutation in single gene affect more than one trait is called _____
Which contains four different ascospores , one of each genotypes
Which of the following is used in density gradient centrifugation?
Yeast are _____
Yeast genome has _____ introns
Yeast genome is _____
Yeast has two mating types, _____ , which show primitive aspects of sex differentiation
Yeasts fail to grow on

Opt 1
Complementation test
Parental haploid
4
Trans type
Genetic analysis
Klebs & Loeffler
Tetromere
Insertion
jumping element
Haploid and diploid
Genetic analysis
A strain not requiring aspargine
Triploid
Saccharomyces cerevisiae
Allergic
Sequence homogeneity
Reciprocal
2hrs 30 min
Reproducible mitotic product
Non-parental dikaryon
Fusion of opposite mating types
High frequency transducing
Transducer
A
Both Female and Male
DNA polymerase
Polygenic
Aminoimidazole
Reproducible mitotic product
Sporangium
Co-segregation
Cytoplasmic petites
Only nitrogen sources
No organism
Gene mutation
Approximate
Repair of single-strand breaks
ELISA
Morgan

Gyno variants
Plasmid
Four meiotic
Vegetative cells
Polytron
Spores
Haldane distribution
Endonuclease
Second division
Seggregation
Morgan
Elemination of mutated type
Irregular
Parental genes
Tetratype
Glucose
Multicellular fungi
233
12,520 kb
a & b
Glucose

Opt 2
Complement fixation test
Parental diploid
8
Same type
Physical analysis
Pastuer & Winogradsky
Telomere
Excertion
Ty elements
Diploid
Physical analysis
A strain requiring aspargine
Haploid and diploid
Cryptococcus Neoformans
Ectopic
Sequence heterogenecity
Non-reciprocal
3 hrs
Reproducible meiotic product
Non-performing data
Fusion of positive mating types
High frequency transcribing
Indicator
B
Female
RNA polymerase
Monogenic
Aminoimidazole ribonucleotide
Recombinant mitotic product
Fragmented mycelium
Co-opression
Energy deficient petites
Only Mineral sources
All organism
Gene repulsion
Accurate
Repair of double-strand breaks
PCR
Mendel

Gynocoid
Transposon
Two Meiotic
Spores
Cholistron
Pycus
Poisson distribution
Beta isoprophylmalate dehydrogenase
Fourth division
Assortment
Meischer
Localization of wild type
Round
Pleiomorphic genes
Parental type
Sucrose
Dicellular
236
1,252 kb
a & α
Lactose

Opt 3
Completed test
Non-parental ditype
16
Cis type
Random spore analysis
Shult & Lindegrin
Centromer
Addition
Tx elements
Haploid
Random spore analysis
A strain not requiring arginine
Diploid
Candida albicans
Endemic
Sequence array
Direct
60 min
Recombinant mitotic product
Non-parental data
Fusion of negative mating types
Height frequency transducing
Selector
T
Male
RNA pol I
Intragenic
Aminoimidazoleribotide
Reproducible meiotic product
Ascus spores
Independent assortment
Mitochondrial petites
Only Lipid Source
Higher organism
Gene transtition
Null
Repair of proteins
Random spore analysis
Primrose

Gyrates
Cosmid
Four mitotic
Ascus
Diptron
Zygote
Twart
Helicase
Primary division
SOS repair
Wilkins
Localization of mutation
Spheroid
Priogenic genes
Parental ditype
Fructose
Acellular
323
1,02,520 kb
b & β
Maltose

Opt 4	Opt 5	Opt 6
Conjugation test		
Parental ditype		
32		
Different type		
Tetrad analysis		
Klug & Cumming		
Primer		
Deletion		
Ta element		
Triploid		
Tetrad analysis		
A strain requiring arginine		
Haploid		
Pitchia pastoris		
Endopic		
Sequence hologenecity		
Indirect		
30 min		
Recombinant meiotic product		
Non-parental ditype		
Fusion of opposite genes		
Heavy frequency transducing		
Repressor		
U		
None		
RNA pol II		
Intergenic		
Aminoimmunoribotide		
Recombinant meiotic product		
Endospores		
Tetrad analysis		
Chrosomal petites		
Only fermentable carbon sources		
Lower organism		
Gene conversion		
Partial		
Repair of RNA		
RPR		
Pastuer		

Gynondromorphs		
Artificial chrosome		
Two mitotic		
Zygote		
Cistron		
Ascus		
Switz		
Ligase		
Tetrad		
Crossing over		
Mendel		
Elimination of wild type		
Disc shaped		
Pleiotrophic genes		
Non-parental ditype		
Agarose		
Unicellular fungi		
326		
15,052 kb		
α & β		
Trehalose		

Answer
Complementation test
Parental diploid
4
Cis type
Tetrad analysis
Shult & Lindegrin
Telomere
Deletion
Ty elements
Haploid
Random spore analysis
A strain not requiring arginine
Haploid
Saccharomyces cerevisiae
Ectopic
Sequence homogeneity
Non-reciprocal
2hrs 30 min
Recombinant meiotic product
Non-parental ditype
Fusion of opposite mating types
High frequency transducing
Indicator
T
Female
RNA pol II
Intergenic
Aminoimidazoleribotide
Recombinant meiotic product
Ascus spores
Co-segregation
Cytoplasmic petites
Only non-fermentable carbon sources
Higher organism
Gene conversion
Approximate
Repair of double-strand breaks
Random spore analysis
Morgan

Gynondromorphs
Transposon
four meiotic
Vegetative cells
Cistron
Ascus
Poisson distribution
Beta isoprophylmalate dehydrogenase
Tetrad
Crossing over
Morgan
Localization of mutation
Spheroid
Pleiotrophic genes
Tetratype
Sucrose
Unicellular fungi
233
12,520 kb
a & α
Lactose

Reg. No. : ----------

[17MBP103]

KARPAGAM UNIVERSITY

(Deemed University Established Under Section 3 of UGC Act 1956)
Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

DEPARTMET OF MICROBIOLOGY

M.Sc., DEGREE FIRST CIA EXAM, (AUGUST – 2017)

MOLECULAR GENETICS

Time: 2 hours **Maximum: 50 marks**

PART–A **(20 x 1 = 20 marks)**

(Answer all questions)

1. _____ enzyme degrades DNA
   a. Polymerase b. Primase
   c. RNase d. DNase
2. In a DNA molecule the Nitrogenous base Adenine always pair with_____.
   a. Guanine b. Cytosine
   c. Thymine d. Uracil
3. Bacteria contains_____.
   a. Chromosome b. Nucleolus
   c. Plasmid d. Plastids
4. Basically, flow of genetic material between two bacteria is accomplished by_____.
   a. Replication b. Transformation
   c. Transduction d. Conjugation.
5. 7-methylguanosine cap is added at_____ site of RNA.
   a. 5` b. 3`
   c. 5`- 3` d. 3`- 5`
6. _____ is a DNA associated supercoiling protein.
   a. Protone b. Histone
   c. Kinetin d. Complement
7. Nucleotide refers to combination of _____
   a. Sugar and Phosphate b. Sugar and dNTPs
   c. dNTPs and Phosphate d. Sugar, dNTPs and Phosphate
8. Bonding between two nitrogenous base is _____.
   a. Hydrogen bond b. Hydrophobic bond
   c. Nitrogen bond d. Van Der waals
9. Chargaff's rule states_____.
   a. No complementarity b. Partial complementarity.
   c. No such rule d. Complementarity of one strand with the other
10. Chromosomal theory of inheritance was formulated by_____.
    a. Mendel b. Miescher
    c. Metchinikoff d. Morgan
11. Dihybrid ratio is _____.

a. 3 : 3 : 9 : 1. b. 9 : 3 : 3 : 1
c. 9 : 3 : 1 : 3 d. 1 : 3 : 3 : 9

12. Distance between the two base pairs is_____.
a. 3.4Å b. 34Å
c. 10Å d. 20Å

13. Codon/Anticodon consists of _____nucleotides
a. 4 b. 6
c. 3 d. 9

14. _____ enzyme plays a key role in the prokaryotic DNA replication.
a. Polymerase I b. Polymerase II
c. Polymerase III d. Polymerase IV

15. Heat Killed S cells + Live R cells produced_____.
a. Death in mice + S cells b. Live mice + S cells
c. Death in mice + R cells d. Live mice + R cells

16. Capping in mRNA is addition of the group_____.
a. 7-ethylguanosine b. 7-methylguanosine
c. 7-methylcytosine d. 7-ethylcytosine

17. DNA absorbs UV light at _____ wavelength.
a. 220 nm b. 240 nm.
c. 260 nm d. 280 nm

18. Enzyme that super coils DNA is _____.
a. Gyrase b. Topoisomerase
c. Helicase d. Methylase

19. Capsule of *Streptococcus pneumoniae* are made up of_____
a. Protein b. Lipid
c. Glycoprotein d. Polysaccharide

20. Amino acid that has AUG as codon is _____.
a. Proline b. Cysteine
c. Serine d. Methionine

PART– B **(3 x 2 = 6 marks)**
(Answer all questions)

21. Write an account on Okazaki fragments.
Okazaki fragments - lagging template strand- 1000 and 2000nucleotides long in prokaryotes-100 to 200 nucleotides long in eukaryotes

22. Write short notes on purines and pyrimidines.
Purine - heterocyclic aromatic organic compound - imidazole ring.
Pyrimidine - heterocyclic aromatic organic compound - two nitrogen atoms at positions 1 and 3 of the six-member ring- isomeric - diazine.

23. Comment of Chargaff's law of base complementarity.
Chargaff's rules - DNA - 1:1 ratio (base Pair Rule) of pyrimidine andpurine bases -the amount of guanine is equal to cytosine and the amount of adenine is equal tothymine. - Austrian born chemist Erwin Chargaff in 1940s.

PART– C **(3 x 8 = 24 marks)**

(Answer all questions)

24. a) Explain the structure and functions of DNA double helix.

The double-helix model of DNA structure - Chargaff's rules- Rosalind Franklin and Maurice Wilkins - James Watson and Francis Crick in 1953 - three-dimensional model - complementary bases - A-DNA, B-DNA, Z-DNA.

(Or)

b) Give an account on mono hybrid and di hybrid cross.

Mendelian inheritance- Gregor Mendel in 1865 and 1866 - re-discovered in 1900- hybridisation experiments with pea plants (*Pisum sativum*) - Mendel's Principles of Heredity- crossed purebred white flower and purple flower pea plants- F_1 & F_2 generation - alleles - homozygous gene - heterozygous gene - Law of Segregation - Law of Independent Assortment - Law of Dominance - Mendelian trait - Non-Mendelian inheritance - incomplete dominance - codominance - multiple alleles - polygenic traits - Punnett square.

25. a) What is replication? Write about bacterial DNA replication process.

DNA Replication - Genetic information - parent to progeny – Polymerisation – dNTPs - DNA template - RNA Primer - DNA polymerase I - Mg 2+- leading strand - lagging strand – enzymology of replication - Initiation of Replication – helicases – topoisomerase – Elongation - Okazaki fragments – Termination - nucleases "proofread"

(Or)

b) Give an account on prokaryotic transcription?

Genetic information - DNA to RNA - synthesis of messenger, transfer, and ribosomal RNAs Promoter recognition - RNA polymerase – Elongation – Termination- Rho-independent Terminator- Rho-Dependent Terminator

26. a) Describe the process of initiation and elongation in prokaryotic translation.

Production of proteins from mRNA – Ribosomes – tRNA - aminoacyl-tRNAsynthetase – mRNA- protein factors- ATP and GTP – Initiation - initiation factors – A site – Elongation - Ribosomal peptidyltransferase - P site translocase – Termination - stop codons - release factors - E site

(Or)

b) Comment on *Lac* operon concept.

Activation of the *lac* Operon by cAMP-CRP - Catabolite Repression - polycistronic – repressor - inducer – operator- lacZ - β-galactosidase – lac Y- lactose permease – lac A- lactose transacetylase

******* (All the Best) *******

Reg. No. : ----------

[17MBP103]

KARPAGAM UNIVERSITY

(Deemed University Established Under Section 3 of UGC Act 1956)
Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

DEPARTMET OF MICROBIOLOGY

M.Sc., DEGREE SECOND CIA EXAM, (OCTOBER – 2017)

MOLECULAR GENETICS

Time: 2 hours **Maximum: 50 marks**

PART–A **(20 x 1 = 20 marks)**

(Answer all questions)

1. Experiments of Hershey and Chase was based on_____.
   a. Virus b. Bacteriophage.
   c. Bacteria d. Fungi
2. Father of genetics_____.
   a. Mendel b. Morgan
   c. Watson d. McLeod
3. Formation of pre-replicating complex is seen in replication mechanism of_____.
   a. Prokaryotes. b. Plants
   c. Virus d. Eukaryotes.
4. _____ converts amino groups to keto groups by oxidative deamination.
   a. Hydrochloric acid b. Nitrous acid
   c. Sulphuric acid d. Oxalic acid
5. Daughter strand repair is also called as_____.
   a. Recombination repair b. SOS repair
   c. Photo repair d. Excision repair
6. Conjugation can only occur between cells of_____.
   a. F positive types b. F negative types
   c. Same mating types d. Opposite mating types
7. Conjugation involves the use of _____for mapping.
   a. Interrupted mating b. Direct mapping
   c. Contact mapping d. Linkage
8. _____gene is involved in SOS repair of DNA.
   a. *recA* b. *lexA*
   c. *lexA* and *recA* d. *Ph1*
9. Duplication mutation is when the segments of nucleotide sequences are_____.
   a. repeated b. deleted
   c. inserted d. inserted and deleted evenly
10. Frameshift mutation is caused by_____.
    a. Proflavin b. Nitrous acid
    c. UV d. X-rays
11. Herman J. Mueller reported results of induced mutations on_____.
    a. Yeast b. Drosophila
    c. Fish d. Pea plant
12. Genes are located in specialized structures called_____.
    a. Histone b. RNA
    c. Chromosomes d. Genomes
13. Generation time of yeast takes place at _____.
    a. 2hrs 30 min b. 3 hrs
    c. 60 min d. 30 min
14. Genetic maps of chromosome are based on the frequencies of _____.
    a. Reproducible mitotic product b. Reproducible meiotic product
    c. Recombinant mitotic product d. Recombinant meiotic product

15. RAPD stands for Random Amplified _____ DNA.
    a. Polymeric b. Parental
    c. Polymorphic d. Phage
16. Change in a single base pair of DNA is called_____ mutation.
    a. Point b. Addition
    c. Deletion d. Inversion
17. _____ among the following is a chemical mutagen.
    a. Ethyl benzoate b. Ethidium Bromide
    c. Caesium fluoride d. 2-mercapto ethanol
18. The distance between two point on a loci of a gene is measured in _____ units.
    a. Centi Morgan b. Branch
    c. Map d. Node
19. _____ score is used to assess the quality of the gene sequence process.
    a. UGMP b. Phred
    c. Tm d. Phy and Psi
20. In a DNA _____ is required for translocation of genes.
    a. Transposases b. STRs
    c. VNTRs d. INS

PART– B **(3 x 2 = 6 marks)**

(Answer all questions)

21. Write an account on Intercalators.
    Insertion of molecules - deoxyribonucleic acid - analyzing DNA - Ligands interact with DNA - ethidium bromide
22. Write short notes on Frame shift mutation.
    Addition or deletion - out-of-frame translation - special subclass of point mutations - *positive frameshift - negative frameshift*
23. Comment on oxidative deamination.
    Deamination - α-keto acids - catabolism of amino acids - glutamate dehydrogenase - monoamine oxidase

PART– C **(3 x 8 = 24 marks)**

(Answer all questions)

24. a) Explain the process of conjugation.
    Conjugation - *Lederberg and Tatum* - Sexual Conjugation in Bacteria - F factor - sex pili - relaxase enzyme - high frequency ofrecombination - Mapping by Conjugation - genetic recombination - Interrupted-mating experiment

(or)

b) Give an account on Transduction and its types.

Gene transfer - donor to a recipient – bacteriophage - Common in Gram+ bacteria - Lysogenic (phage) conversion - Generalized Transduction - Specialized Transduction

25. a) Define Mutation. Write about spontaneous mutation.
    permanent alteration - nucleotide sequence - Spontaneous mutation – Tautomers - Mispairing due to tautomerization - Mispairing of repeated bases - Mispairing caused by deamination- Deamination of 5-methylcytosine - Spontaneous mutation rate

(Or)

b) Elaborately discuss on the chemical mutagens and types

Chemical mutagens - potentially mutagenic - Ames test - Base analogues - deoxyribonucleotide triphosphates - Nitrous acid - deamination - Alkylating agents - ethyl methane sulfonate - alkyl groups to purines – Intercalation - aromatic molecules – acridine

26. a) Describe the process of DNA repair mechanism.

Single-strand breaks- Double-strand breaks - Alteration of bases - DNA repair systems - fix different type of DNA alterations- Direct Repair- Photolyase- O6-alkylguanine alkylguanine alkyltransferase - Excision Repair- Nucleotide Excision Repair System - UV Damage Repair - Mismatch Base Repair- Postreplicative (Recombinational) Translesion Bypass Repair - SOS Repair Mechanism

(or)

b) Comment on genetic map of *Drosophila*.

Gene mapping - Thomas Hunt Morgan - crossing-over

******* (All the Best) *******

Reg. No. : ----------

[17MBP103]

KARPAGAM UNIVERSITY

(Deemed University Established Under Section 3 of UGC Act 1956)
Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

DEPARTMET OF MICROBIOLOGY

M.Sc., DEGREE FIRST CIA EXAM, (AUGUST– 2017)

MOLECULAR GENETICS

Time: 2 hours **Maximum: 50marks**

PART–A (20 x 1 = 20 marks)

(Answer all questions)

1. _____ enzyme degrades DNA
   a. Polymerase b. Primase
   c. RNase d. DNase
2. In a DNA molecule the Nitrogenous base Adenine always pair with_____.
   a. Guanine b. Cytosine
   c. Thymine d. Uracil
3. Bacteria contains_____.
   a. Chromosome b. Nucleolus
   c. Plasmid d. Plastids
4. Basically, flow of genetic material between two bacteria is accomplished by_____.
   a. Replication b. Transformation
   c. Transduction d. Conjugation.
5. 7-methylguanosine cap is added at_____ site of RNA.
   a.5` b. 3`
   c. 5`- 3` d.3`- 5`
6. _____ is a DNA associated supercoiling protein.
   a. Protone b. Histone
   c. Kinetin d. Complement
7. Nucleotide refers to combination of _____
   a. Sugar and Phosphate b. Sugar and dNTPs
   c. dNTPs and Phosphate d. Sugar, dNTPs and Phosphate
8. Bonding between two nitrogenous base is _____.
   a. Hydrogen bond b. Hydrophobic bond
   c. Nitrogen bond d. Van Der waals
9. Chargaff's rule states_____.
   a. No complementarity b. Partial complementarity.
   c. No such rule d. Complementarity of one strand with the other
10. Chromosomal theory of inheritance was formulated by_____.
    a. Mendel b. Miescher
    c. Metchinikoff d. Morgan
11. Dihybrid ratio is _____.
    a. 3 : 3 : 9 : 1. b. 9 : 3 : 3 : 1
    c. 9 : 3 : 1 : 3 d. 1 : 3 : 3 : 9
12. Distance between the two base pairs is_____.
    a. 3.4Å b. 34Å
    c. 10Å d. 20Å
13. Codon/Anticodon consists of _____nucleotides
    a.4 b. 6
    c. 3 d.9

14. _____ enzyme plays a key role in the prokaryotic DNA replication.
    a.Polymerase I b. Polymerase II
    c. Polymerase III d.Polymerase IV
15. Heat Killed S cells + Live R cells produced_____.
    a. Death in mice + S cells b. Live mice + S cells
    c. Death in mice + R cells d. Live mice + R cells
16. Capping in mRNA is addition of the group_____.
    a. 7-ethylguanosine b. 7-methylguanosine
    c. 7-methylcytosine d. 7-ethylcytosine
17. DNA absorbs UV light at _____ wavelength.
    a. 220 nm b. 240 nm.
    c. 260 nm d. 280 nm
18. Enzyme that super coils DNA is _____.
    a. Gyrase b. Topoisomerase
    c. Helicase d. Methylase
19. Capsule of *Streptococcus pneumoniae* are made up of_____
    a. Protein b. Lipid
    c. Glycoprotein d. Polysaccharide
20.Amino acid that has AUG as codon is _____.
    a. Proline b. Cysteine
    c. Serine d. Methionine

PART– B (3 x 2 = 6 marks)

(Answer all questions)

21. Write an account on Okazaki fragments.
22. Write short notes on purines and pyrimidines.
23. Comment of Chargaff's law of base complementarity.

PART– C (3 x 8 = 24 marks)

(Answer all questions)

24. a) Explain the structure and functions of DNA double helix.

(or)

    b) Give an account on mono hybrid and di hybrid cross.

25. a) What is replication? Write about bacterial DNA replication process.

(or)

    b) Give an account on prokaryotic transcription?

26. a) Describe the process of initiation and elongation in prokaryotic translation.

(or)

    b) Comment on *Lac* operon concept.

******* (All the Best) *******

Reg. No. : ----------

[17MBP103]

KARPAGAM UNIVERSITY

(Deemed University Established Under Section 3 of UGC Act 1956)
Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

DEPARTMET OF MICROBIOLOGY

M.Sc., DEGREE SECOND CIA EXAM, (OCTOBER– 2017)

MOLECULAR GENETICS

Time: 2 hours **Maximum: 50marks**

PART–A (20 x 1 = 20 marks)

(Answer all questions)

1. Experiments of Hershey and Chase was based on_____.
   a. Virus b. Bacteriophage.
   c. Bacteria d. Fungi
2. Father of genetics_____.
   a. Mendel b. Morgan
   c. Watson d. McLeod
3. Formation of pre-replicating complex is seen in replication mechanism of_____.
   a. Prokaryotes. b. Plants
   c. Virus d. Eukaryotes.
4. _____ converts amino groups to keto groups by oxidative deamination.
   a. Hydrochloric acid b. Nitrous acid
   c. Sulphuric acid d. Oxalic acid
5. Daughter strand repair is also called as_____.
   a. Recombination repair b. SOS repair
   c. Photo repair d. Excision repair
6. Conjugation can only occur between cells of_____.
   a. F positive types b. F negative types
   c. Same mating types d. Opposite mating types
7. Conjugation involves the use of _____for mapping.
   a. Interrupted mating b. Direct mapping
   c. Contact mapping d. Linkage
8. _____gene is involved in SOS repair of DNA.
   a. *recA* b. *lexA*
   c. *lexA* and *recA* d. *Ph1*
9. Duplication mutation is when the segments of nucleotide sequences are_____.
   a. repeated b. deleted
   c. inserted d. inserted and deleted evenly
10. Frameshift mutation is caused by_____.
    a. Proflavin b. Nitrous acid
    c. UV d. X-rays
11. Herman J. Mueller reported results of induced mutations on_____.
    a. Yeast b. Drosophila
    c. Fish d. Pea plant
12. Genes are located in specialized structures called_____.
    a. Histone b. RNA
    c. Chromosomes d. Genomes
13. Generation time of yeast takes place at _____.
    a. 2hrs 30 min b. 3 hrs
    c. 60 min d. 30 min
14. Genetic maps of chromosome are based on the frequencies of _____.
    a. Reproducible mitotic product b. Reproducible meiotic product
    c. Recombinant mitotic product d. Recombinant meiotic product

15. RAPD stands for Random Amplified _____ DNA.
    a. Polymeric b. Parental
    c. Polymorphic d. Phage
16. Change in a single base pair of DNA is called_____ mutation.
    a. Point b. Addition
    c. Deletion d. Inversion
17. _____ among the following is a chemical mutagen.
    a. Ethyl benzoate b. Ethidium Bromide
    c.Caesium fluoride d. 2-mercapto ethanol
18. The distance between two point on a loci of a gene is measured in _____ units.
    a.Centi Morgan b. Branch
    c. Map d. Node
19. _____ score is used to assess the quality of the gene sequence process.
    a. UGMP b. Phred
    c. Tm d. Phy and Psi
20. In a DNA _____ is required for translocation of genes.
    a. Transposases b. STRs
    c. VNTRs d. INS

PART– B (3 x 2 = 6 marks)

(Answer all questions)

21. Write an account on Intercalators.
22. Write short notes on Frame shift mutation.
23. Comment on oxidative deamination.

PART– C (3 x 8 = 24 marks)

(Answer all questions)

24. a) Explain the process of conjugation.

(or)

b) Give an account on Transduction and its types.

25. a) Define Mutation. Write about spontaneous mutation.

(or)

b) Elaborately discuss on the chemical mutagens and types

26. a) Describe the process of DNA repair mechanism.

(or)

b) Comment on genetic map of *Drosophila*.

******* (All the Best) *******

Reg. No. : ----------

[15MBP103]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

M.SC. DEGREE EXAMINATION, NOVEMBER 2015

FIRST SEMESTER

MICROBIOLOGY

MOLECULAR GENETICS

Time: 3 hours **Maximum: 60marks**

PART–A (20 x 1 = 20 marks)

(Answer all questions)

(Multiple Choice QuestionNo.1 to 20 Online Exam)

1. Distance between the two base pairs is_____.
   a. 3.4Å b. 34Å
   c. 10Å d. 20Å
2. DNA absorbs UV light at _____ wavelength.
   a. 220 nm b. 240 nm.
   c. 260 nm d. 280 nm
3. Enzyme that adds methyl group to the newly formed DNA_____.
   a. Gyrase b. Topoisomerase
   c. Helicase d. Methylase
4. Eukaryotic DNA damage or replication errors are corrected during_____.
   a. G1 phase. b. S phase
   c. G2 phase d. R phase.
5. Chemical agent that resembles thiamine_____.
   a. 5-bromothiamine b. 5-bromoadenine
   c. 5-bromoguanine d. 5-bromouracil
6. Chemical mutagens leading to addition of nucleotides to the DNA are_____.
   a. Thimers b. Base analogs
   c. Alkylating agents d. Interchelating agents
7. Chromosomal mutation is_____.
   a. Abberation b. Change over
   c. Variation d. Genetic change
8. Common chemical events that produce spontaneous mutation_____.
   a. Deamination b. Depurination
   c. Dimerization d. Isomerization
9. A-site is the ribosomal site most frequently occupied by the_____.
   a. Aminoacyl-rRNA b. Aminoacyl-mRNA
   c. Iminoacyl-tRNA d. Aminoacyl-tRNA.
10. Capping in mRNA is addition of the group_____.
    a. 7-ethylguanosine b. 7-methylguanosine
    c. 7-methylcytosine d. 7-ethylcytosine
11. Codon/Anticodon consists of _____nucleotides
    a.4 b. 6
    c. 3 d.9
12. Common method of covalent modification of enzyme in regulation of gene expression is_____.
    a. to methylate at a proline residue b. to phosphorylate at a prolineresidue
    c. to phosphorylate at a serine residue d. to methylate at a serine residue
13. Capsule of *Streptococcus pneumoniae* are made up of_____
    a. Protein b. Lipid
    c. Glycoprotein d. Polysaccharide

14. Capsules help bacteria in escaping_____.
    a. Inflammation b. RBC's
    c. Phagocytosis d. Antibodies
15. Cells carrying non-integrated transducing fragments are called _____.
    a. Specialized b. Abortive transductants
    c. Generalized d. Conjugation
16. Complete linkage has been reported in_____.
    a. Male Drosophila b. Human female
    c. Female Drosophila d. Maize
17. A new genetic nomenclature for *S. cerevisiae* transposon_____.
    a. Jumping element b. Ty elements
    c. Tx elements d. Ta element.
18. Among vegetative cells of yeast, _____ is mainly used for genetic mapping.
    a. Haploid and diploid b. Diploid
    c. Haploid d. Triploid
19. An estimation of map between a- and b- can be obtained from number of recombinant ascospore detected by using _____.
    a. Genetic analysis b. Physical analysis
    c. Random spore analysis d. Tetrad analysis
20. Arg+ is_____
    a. A strain not requiring aspargine b. A strain requiring asparagine
    c. A strain not requiring arginine d. A strain requiring arginine

PART– B (5 x 6 = 30 marks)
(Answer all questions)

21. a) Outline the different forms of DNA.

(or)

b) Explain two experiments that proved DNA as genetic material.

22. a) What are induced mutations? Describe frame shift mutation.

(or)

b) Give an account on types of DNA damage.

23. a) State about the maturation and processing of RNA in bacteria.

(or)

b) Elaborate polypeptide synthesis in bacteria.

24. a) Give a note on types of recombination in bacteria.

(or)

b) Illustrate the genetic map of T4 phage.

25. a) Write note on Tetrad analysis.

(or)

b) Genetic mapping in *Neurospora*.

PART– C (1 x 10 = 10 marks)
(Compulsory Question)

26. Give a detailed account on DNA repair mechanisms.

******* (All the Best) *******

Reg. No. : ----------

[16MBP103]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

M.SC. DEGREE EXAMINATION, NOVEMBER 2016

FIRST SEMESTER

MICROBIOLOGY

MOLECULAR GENETICS

Time: 3 hours **Maximum: 60marks**

PART–A (20 x 1 = 20 marks)

(Answer all questions)

(Multiple Choice QuestionNo.1 to 20 Online Exam)

1. Bonding between two bases is _____.
   a. Hydrogen bond b. Hydrophobic bond
   c. Nitrogen bond d. Van Der waals
2. Chargaff's rule states_____.
   a. No complementarity b. Partial complementarity.
   c. No such rule d. Complementarity of one strand with the other
3. Chromosomal theory of inheritance was formulated by_____.
   a. Mendel b. Miescher
   c. Metchinikoff d. Morgan
4. Dihybrid ratio is _____.

a. 3 : 3 : 9 : 1. b. 9 : 3 : 3 : 1
   c. 9 : 3 : 1 : 3 d. 1 : 3 : 3 : 9.
5. Bacterium used in Ames test is _____.
   a. *Salmonella* b. *Shigella*
   c. *Streptococcus* d. *Staphylococcus*
6. Biological agents of mutagenesis are_____.
   a. Transposable elements b. Lipids
   c. Bacteria d. Carbohydrates
7. Cancer that results from deletion of a portion of chromosome 13 is_____.
   a. Eye cancer b. Bone cancer
   c. Skin cancer d. Lung cancer
8. Change of purine to pyrimidine base is a _____.
   a. Transition mutation b. Transverse mutation
   c. General mutation d. Transformation
9. Addition of poly A tail to 3' end of mRNA is mediated by the enzyme_____.
   a. RNA polymerase b. DNA polymerase
   c. Rnase d. poly A polymerase.
10. All aminoacids have more than one codon except_____.
   a. Methionine and Tryptophan b. ValineandLeucine
   c. Threonine and Alanine d. Lysine and Arginine
11. Allosteric enzymes that are controlled by a molecule other than it's substrate_____.
   a.Cohesive molecules b. Systematic molecules
   c. Effector molecules d.Affector molecules
12. Amino acid that have largest number of codons is _____.
   a. Proline b. Cysteine
   c. Serine d. Valine
13. A cell carrying an integrated F factor is called an_____.
   a. F b. Hfr
   c. Hfr 1+ d. trans

14. An example for specialized transducing particle_____.
    a. No infection
    b. T2 phage infects *Staphylococcus*
    c. Phage P22 infects *Salmonellatyphimurium*
    d. Phage lambda infects *E.coli*
15. Avery, MacLeod and McCarty used enzymes and solvents to destroy molecules such as_____.
    a. Anything except DNA
    b. RNA
    c. Lipids and proteins
    d. Polysaccharide
16. Bacteriophages were jointly discovered by_____.
    a. Frederick Twort and by Felix d'Herelle
    b. Hershey and Chase
    c. Luria and Delbruck
    d. McKay and McCartney
17. _____determines the number of crossover events and give correct map distance.
    a. Genetic analysis
    b. Physical analysis
    c. Random spore analysis
    d. Tetrad analysis.
18._____developed an algebraic method to determine the consequence of various number of exchanging.
    a. KlebsandLoeffler
    b. PastuerandWinogradsky
    c. ShultandLindegrin
    d. Klug and Cumming
19. _____distance in map unit is only half the percentage of second division segregated tetrads.
    a. Tetromere
    b. Telomere
    c. Centromere
    d. Primer
20. A feature of petite is the occurrence of _____from the circular mitochondrial genome
    a. Insertion
    b. Exertion
    c. Addition
    d. Deletion

PART– B (5 x 6 = 30 marks)
(Answer all questions)

21. a) Write down the difference between prokaryotic and eukaryotic DNA replication.

(or)

b) Explain i) Leading and Lagging strands ii) Single-strand DNA-binding protein.

22. a) What are mutagens? Write note on mutagenic agents.

(or)

b) Give a brief account on SOS repair.

23. a) Explain post translational modification of proteins in eukaryotes.

(or)

b) What are transcriptional factors?

24. a) Give an account on detection and isolation of phage T4 mutants.

(or)

b) Describe the lytic cycle of a bacteriophage.

25. a) Write note on Yeast Artificial Chromosome.

(or)

b) Explain about petite mutants.

PART– C (1 x 10 = 10 marks)
(Compulsory Question)

26. Illustrate regulation of gene expression using *Lac* operon model.

******* (All the Best) *******

Reg. No. : ----------

[17MBP103]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

M.SC. DEGREE EXAMINATION, NOVEMBER 2017

FIRST SEMESTER

MICROBIOLOGY

MOLECULAR GENETICS

Time: 3 hours **Maximum: 60marks**

PART–A (20 x 1 = 20 marks)

(Answer all questions)

(Multiple Choice QuestionNo.1 to 20 Online Exam)

1. _____ degrades DNA
   a. Polymerase b. Primase
   c. RNase d. DNase
2. Adenine always pair with_____.
   a. Guanine b. Cytosine.
   c. Thymine d. Uracil
3. Bacteria contains_____.
   a. Single circular DNA b. Single linear DNA
   c. Double Linear DNA d. Double circular DNA
4. Basically, flow of genetic material is accomplished by_____.
   a. Replication. b. Transformation
   c. Transduction d. Conjugation.
5. _____ is a non-ionizing radiation
   a. Alpha b. UV
   c. Gamma d. Beta
6. _____is a selective media for *Nesseria*
   a. PLET b. Blood agar
   c. Mannitol salt agar d. Martin Thayer
7. Alkylation is_____.
   a. Addition of methyl or ethyl group b. Deletion of ethyl and addition of methyl group
   c. Deletion of methyl and addition of ethyl groupd. Deletion of methyl or ethyl group
8. Alkylation of guanine causes its removal from DNA in a reaction called_____.
   a. Deamination b. Depyrimidation
   c. Degradation d. Depurination
9. _____is the first amino acid during translation of proteins.
   a. Threonine b. Leucine
   c. Methionine d. Valine.
10. 2006 Nobel Prize for studies on f eukaryotic transcription was conferred to_____.
   a. Arthur Nirenberg b. Roger D. Kornberg
   c. David Osborne d. Michael Whitney
11. 7-methylguanosine cap is an important site_____.
   a.For eukaryotic transcription initiation factor b. For prokaryotic translation initiation factor
   c. For eukaryotic translation initiation factor d.For prokaryotic translation initiation factor
12. Action of repressor protein in Lac operon is called as_____.
   a. Positive control b. Negative control
   c. Neutral control d. No control
13. _____involves finding a contiguous series of cloned DNA fragments which contain overlapping portions of the genome.
   a. Physical mapping b. Chemical mapping
   c. Marker mapping d. Loci mapping

14. _____ refers to genetic changes in different genomes of same cell.
    a. Trans type b. Cis type
    c. Same type d. Different type
15. _____ is a DNA associated protein.
    a. Protone b. Histone
    c. Chromotome d. Cistron
16. A bacterium harboring a prophage is called_____.
    a. Lytic phage b. Helper phage
    c. Transducing phage d. Lysogency
17. _____ test is used to determine which gene(s) are defective in petite yeast mutants.
    a. Complementation test b. Complement fixation test
    c. Completed test d. Conjugation test.
18._____contains two types of spores of same parental genotype
    a. Parental haploid b. Parental diploid
    c. Non-parental ditype d. Parental ditype
19. _____initial products of meiosis forms two identical spore.
    a. 4 b. 8
    c. 16 d. 32
20. _____refers to a genetic change in different genomes of same cell
    a. Trans type b. Levo type
    c. Cistype d. Dextro type

PART– B (5 x 6 = 30 marks)
(Answer all questions)

21. a) Write an account on Okazaki fragments.

(or)

b) Write short notes on purines and pyrimidines.

22. a) Explain deamination and oxidative damage of DNA.

(or)

b) Describe mutation selection.

23. a) Explain the structure and functions of RNA polymerase.

(or)

b) Give an account on post-transcriptional modification in eukaryotes.

24. a) What is genetic recombination? Write its significance in bacteria.

(or)

b) Give an account on prophage?

25. a) Describe gene mapping in *Drosophila*.

(or)

b) Comment on Yeast as model eukaryotic organism.

PART– C (1 x 10 = 10 marks)

(Compulsory Question)

26. Write a detailed account on *Lac*, *Trp* and *Ara*operon concept.

******* (All the Best) *******