17MBU203 **MICROBIAL GENETICS** **Semester – II (4H – 4C)**

Instruction Hours / week: L: 4 T: 0 P: 0 **Marks:** Internal: **40 External: 60** Total: **100** **End Semester Exam:** 3 Hours

SCOPE

The application of molecular genetics has completely transformed the biotechnology industry, with new possibilities ranging from the treatment of human diseases to the development of new forms of crops. Molecular genetics looks set to be the most promising and exciting science of the next few decades.

OBJECTIVES

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

Unit I

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

Unit II

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

Unit III

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

Unit IV

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

Unit V

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

SUGGESTED READINGS

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

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act, 1956)
Coimbatore -641 021.

LECTURE PLAN
DEPARTMENT OF MICROBIOLOGY

STAFF NAME : Dr. P. SRINIVASAN
SUBJECT NAME: MICROBIAL GENETICS SUB.CODE:17MBU203
SEMESTER : II CLASS: I B.Sc (MB)

S. No	Duration	Topic to be Covered	Reference
		UNIT - I	
1.	1	Introduction to Genetics	R1:1 to 2 T1:3 to 4
2.	1	Historical developments in Genetics – Classical and Modern Genetics	R1:2 to 3 T1: 4 to 10; 485 to 486
3.	1	Mendelian Genetics – Principles and law involved	R1: 271 to 272
4.	1	Basic concepts of Genetics – DNA, Genes and Chromosomes	R1: 4 to 5 T1: 13 to 22
5.	1	DNA as genetic material	R1: 13 to 14
6.	1	Experiments involved in the search of genetic materials – Griffith's Experiment, Avery's Experiments, and The Hershey –Chase Experiment	R1: 15 to 19
7.	1	Chromosomal as genetic material	R1: 27 to 28
8.	1	Chromosomal Theory of inheritance	R1: 28 to 35
9.	2	DNA replication – Basic rules of nucleic acid replication.	R1: 43 to 44 T1: 167 to 169
10.	2	Semi conservative mode of DNA replication-The Messelson and Stahl Experiment.	R1: 44 to 45 T1: 170 to 172
11.	2	Modes of DNA replication-Discontinuous, Bidirectional, Rolling circle.	R1: 50 to 52 T1: 178 to 181
12.	2	Transcription, Translation and regulation in microbes	R1: 87 to106, 119-126 T1:120 to 174
13.	2	The composition and structure of RNA, Types of RNA and its function.	R1: 25 to 26 T1: 46 to 47 R1: 25 to 26 T1: 47 to 49
14.	1	Unit Revision and Possible Questions	-
		Total Hours planned for Unit I	**19**
		UNIT - II	
1.	1	The composition and structure of plasmid.	R1: 20 to 21 T1: 29 to 32
2.	1	The composition and structure of plasmid.	
3.	1	Different forms of plasmid replication.	R1: 22 to 24

4.	1	Different forms of plasmid replication.	T1: 39 to 42
5.	1	Copy number and partitioning of plasmids.	R1: 25 to 26 T1: 46 to 47
6.	1	Copy number and partitioning of plasmids.	
7.	1	Plasmid and host ranges.	R1: 25 to 26 T1: 47 to 49
8.	1	Plasmid and host ranges.	
9.	1	Plasmid incompatibility	R1: 45 to 47 T1: 170 to 172
10.	1	Plasmid incompatibility	
11.	1	Plasmid amplification	R1: 47 to 48 T1: 175 to 176
12.	1	Plasmid amplification	
13.	1	Curing and application	T1: 176 to 177
14.	1	Curing and application	
15.	1	Unit Revision and Possible Questions	-
		Total Hours planned for Unit II	**15**
		UNIT - III	
1.	1	Introduction to Genetic Code, Role of Codons in cell function.	R1: 115 to 117 T1: 124 to 126
2.	1	Introduction to Genetic Code, Role of Codons in cell function.	R1: 115 to 117 T1: 124 to 126
3.	2	Wobble Hypothesis, Arrangement of genetic codes into amino acids.	R1: 117 to 119 T1: 126 to 128
4.	2	Operon concept of gene expression-LAC operon	R1:516 to 526 T1:143 to 152
5.	2	Gene expression-Arabinose and Tryptophan operon	R1:528 to 529 T1:153 to 157
6.	2	Genetic recombination- an overview.	R1: 481 to 482 T1: 281 to 308
7.	2	Mapping of genes in bacteria by conjugation.	R1: 484 to 485 T1:331 to 332
8.	1	Transfer of Hfr genes between cells.	R1: 485 to 486 T1:334 to 335
9.	1	Genetic mapping of genes by transformation	R1: 490 to 492 T1:311 to 313
10.	1	Genetic mapping of genes by transduction and types	
11.	1	Gene and chromosome walking	
12.	1	Video presentation on working principle of centrifugation	W1
13.	1	Unit Revision and Possible Questions	-
		Total Hours planned for Unit III	**18**
		UNIT - IV	
1.	1	Mutation and mutagenesis.	R1:133 to 134 T1: 212 to 213
2.	1	Mutagens responsible for DNA mutation.	R1: 134 to 135 T1:213 to 214
3.	1	Biochemical basis of mutants.	R1: 135 to 136 T1:213 to 214
4.	2	Types of mutation – spontaneous.	R1: 136 to 139 T1:214 to 215

5.	2	Types of mutation-Induced mutation.	R1: 139 to 140 T1:218 to 224
6.	1	Mutagenesis by base analogues.	T1:226 to 227
7.	2	Mutagenesis by chemical mutagens.	T1:228 to 229
8.	1	Mutagenesis by intercalating substances.	T1:229 to 230
9.	1	Mutator genes and mutagenesis; selection.	T1:231 to 232
10.	1	Detection of mutation – Ames test.	R1: 148 to 150 T1:232 to 236
11.	1	Unit Revision and Possible Questions	-
		Total Hours planned for Unit IV	**14**
UNIT - V			
1.	1	Transposons and its types	R1: 481 to 482 T1: 281 to 308
2.	1	Mechanism of transposition and application	R1: 482 to 485 T1:331 to 332
3.	1	Mechanism of transposition and application	
4.	1	Mu transposon and eukaryotic transposable elements and application.	R1: 485 to 486 T1:334 to 335
5.	1	Mu transposon and eukaryotic transposable elements and application.	R1: 490 to 492 T1:311 to 313
6.	1	Unit Revision and Possible Questions.	-
7.	3	Revision of previous year question papers.	-
		Total Hours planned for Unit V	**9**
Total Planned Hours	**75**		

TEXTBOOKS

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

REFERENCES

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

WEBSITES

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

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 spontaneously generated 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 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.

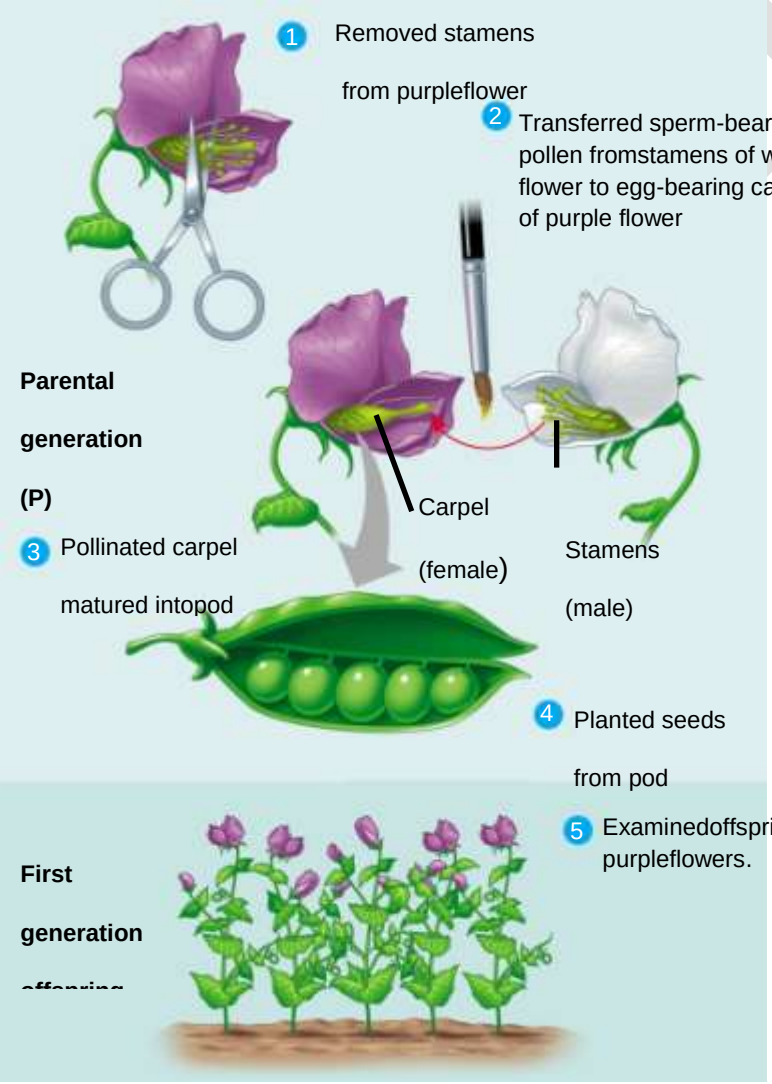
Mendel's Experimental, Quantitative Approach

- 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

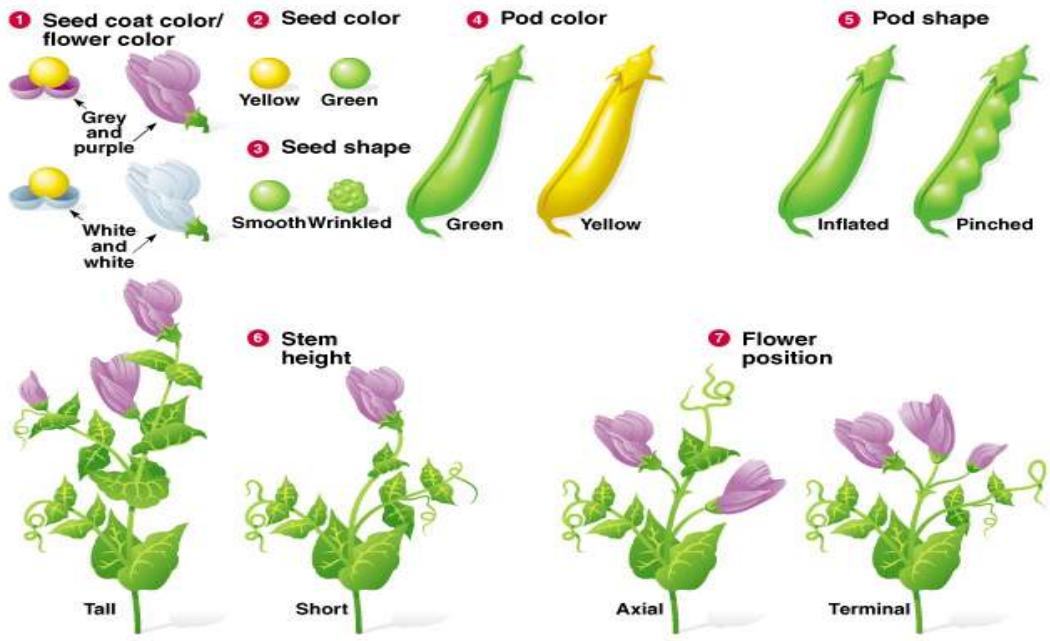


Fig: Mendel's Studied Discrete Traits

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

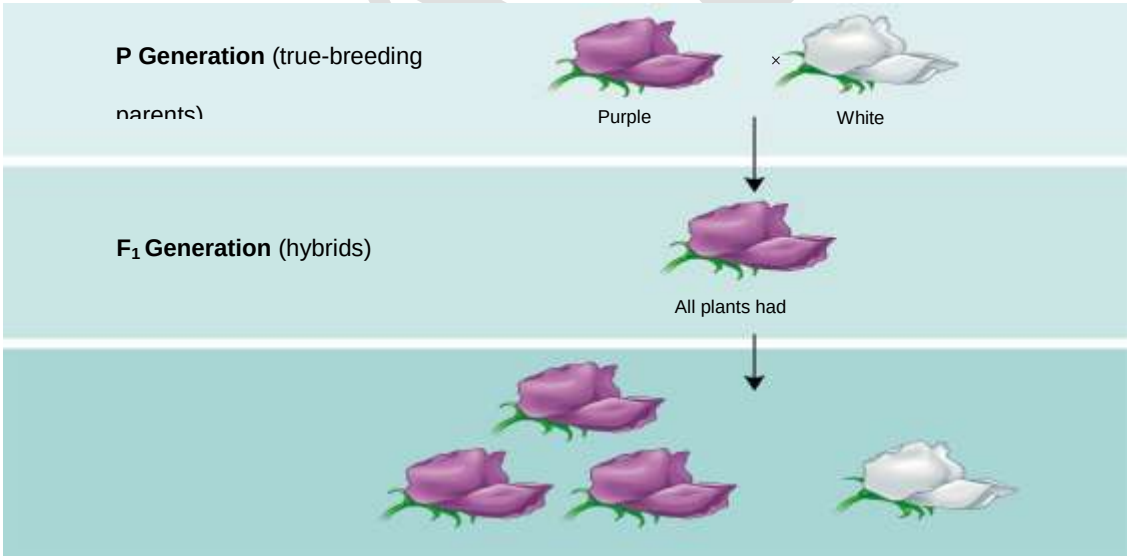
Mendel's Principles

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

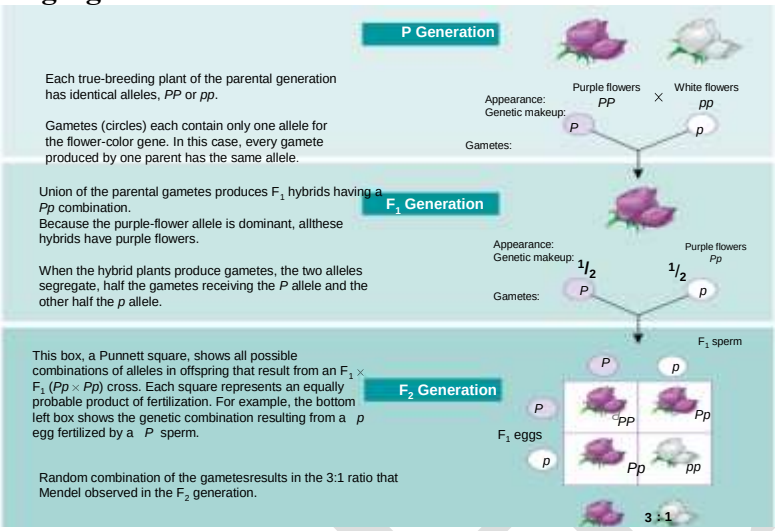
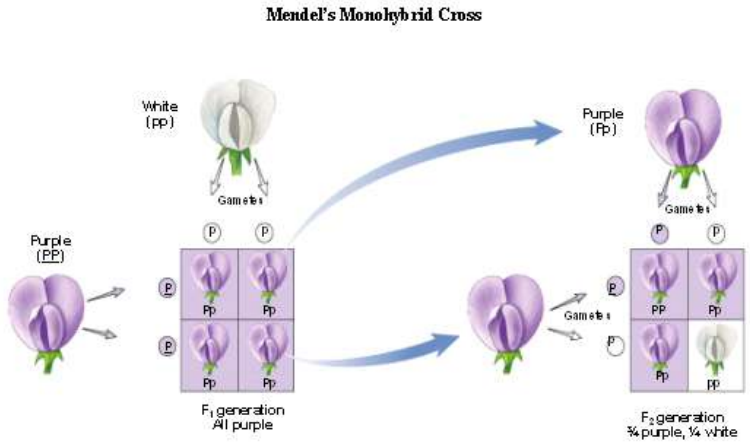


Fig: Mendel's Law Of Segregation

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)

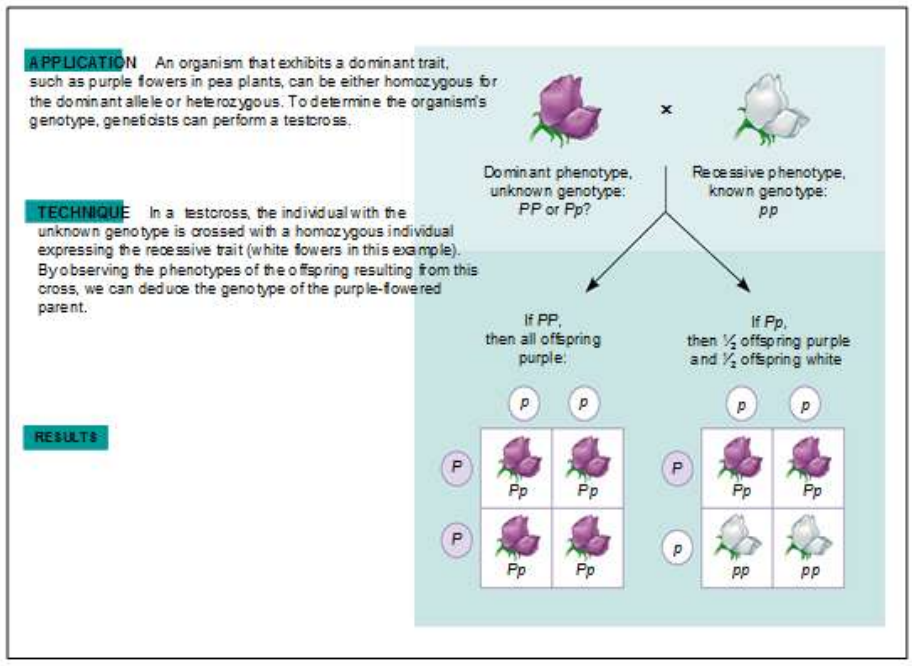


Fig : Test Cross

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.

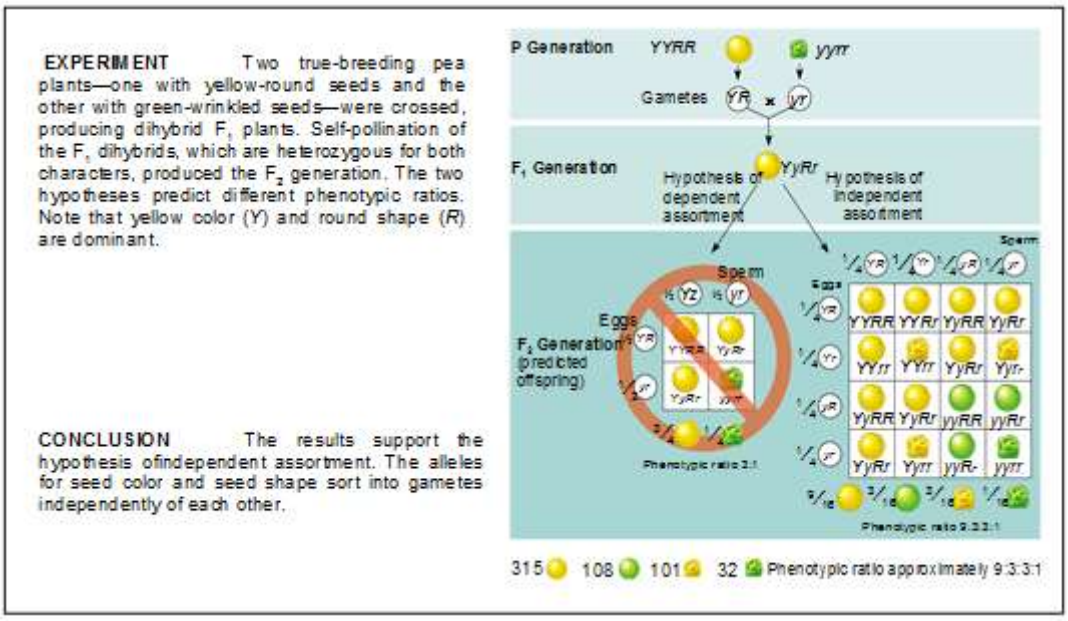


Fig: A Dihybrid Cross

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*

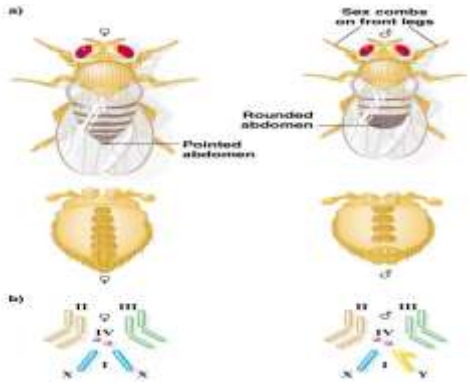
- An X-chromosome-autosome balance system is used.
- Drosophila has three pairs of autosomes, and one pair of sex chromosomes. Like humans, XX is female and XY is male. Unlike humans, Y does not determine sex.
- An XXY fly is female, and an XO fly is male. The sex of the fly results from the ratio of 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	Red-eyed female
XrXr	White-eyed female
XRY	Red-eyed male
XrY	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

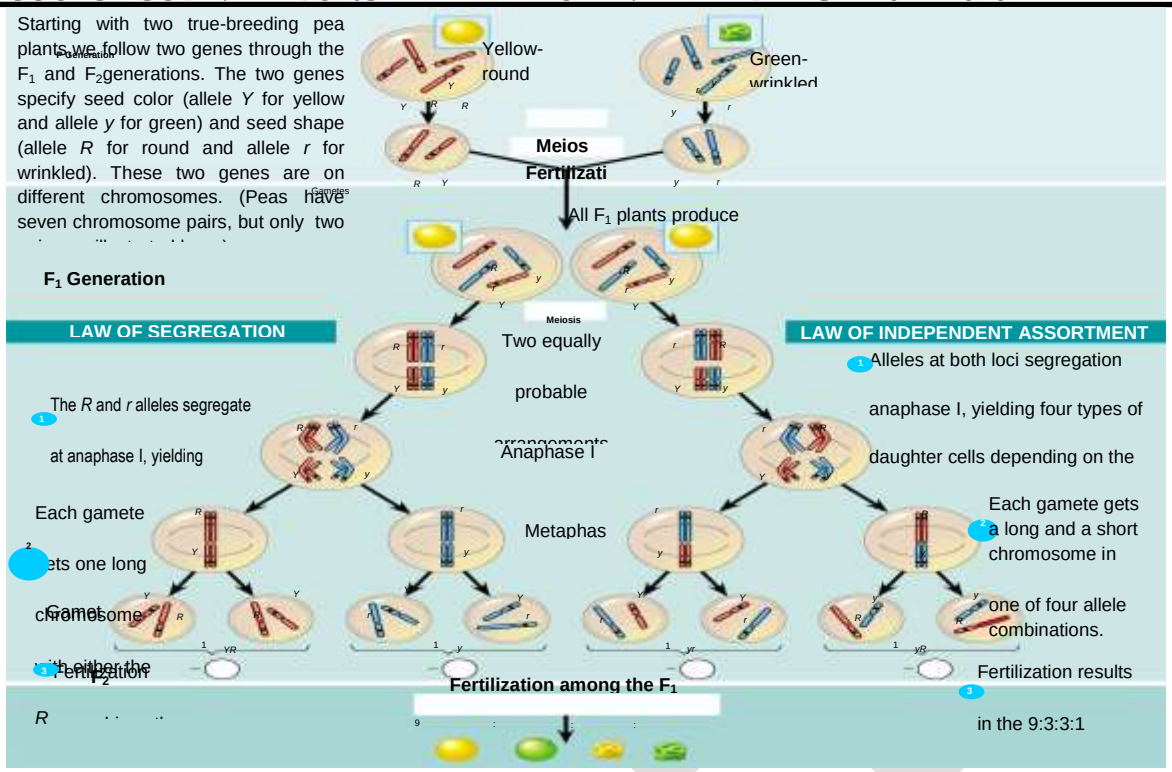


Fig:Chromosome Theory of Inheritance

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.

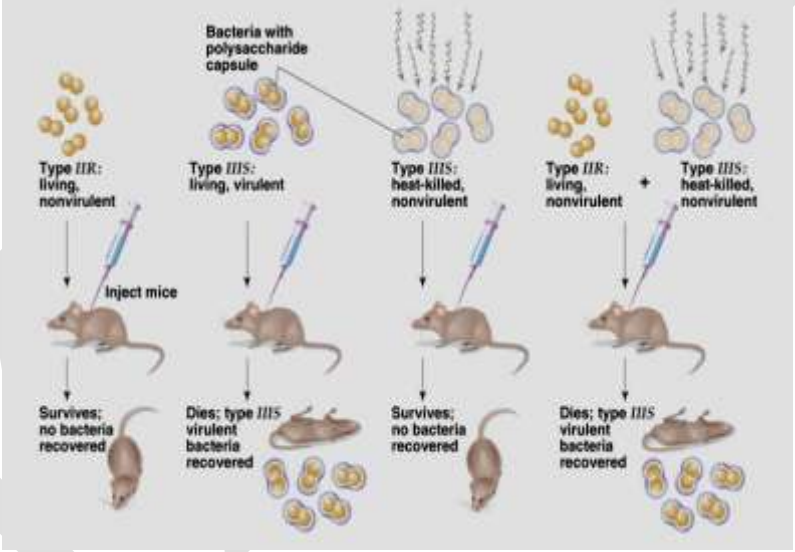


Fig. Griffith's transformation experiment

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.

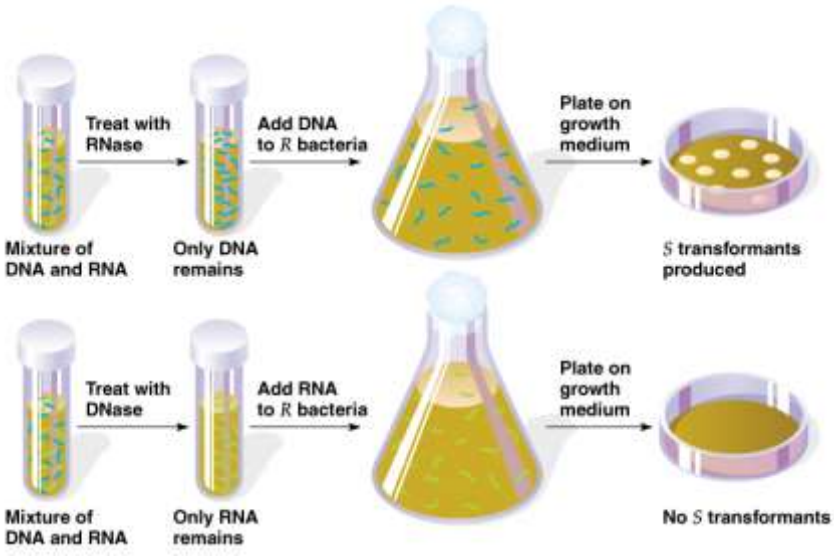
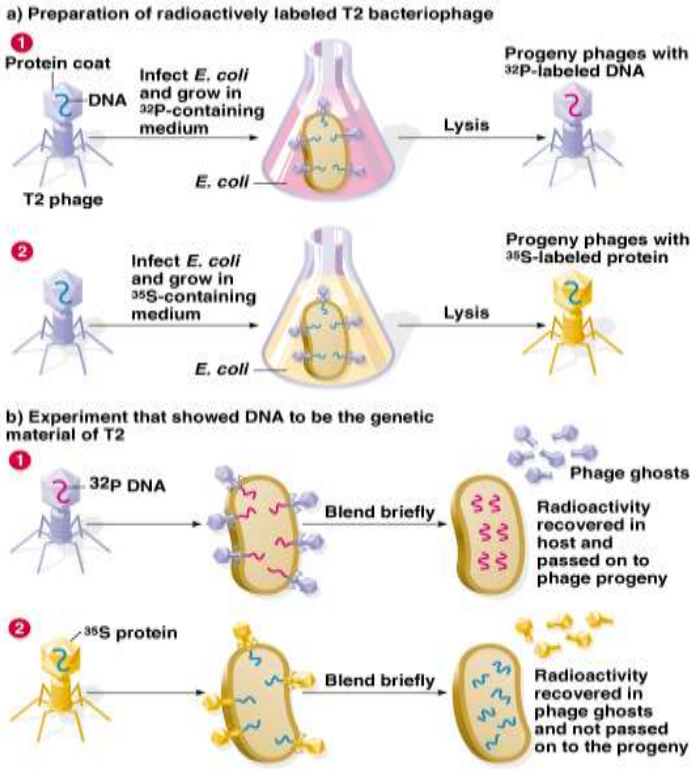


Fig. Experiment that showed that DNA, not RNA, was the transforming principle

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.



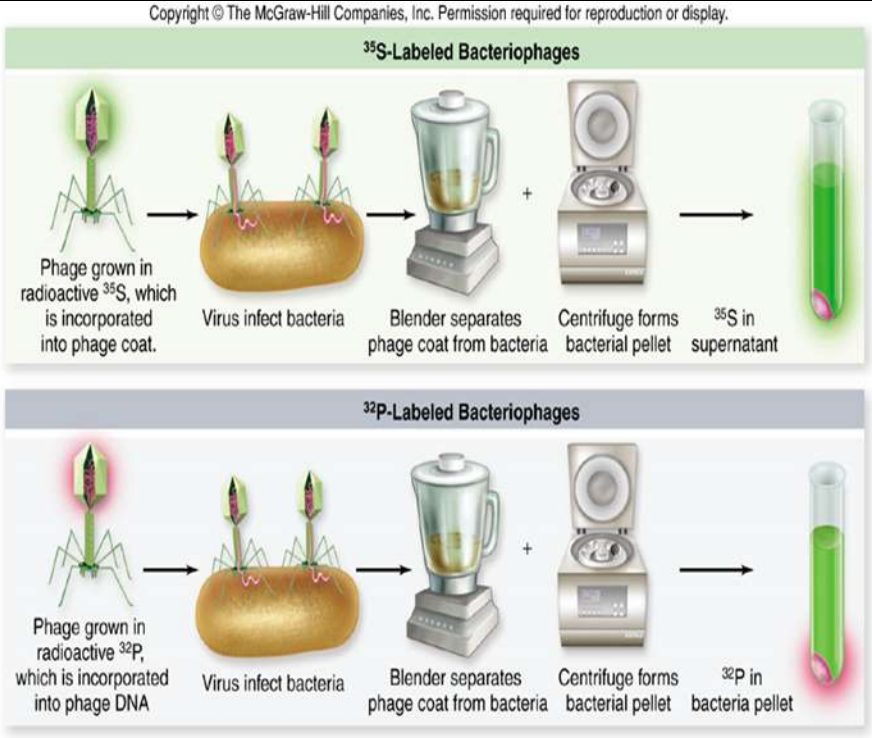


Fig. Hershey-Chase experiment demonstrating DNA is genetic material

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.

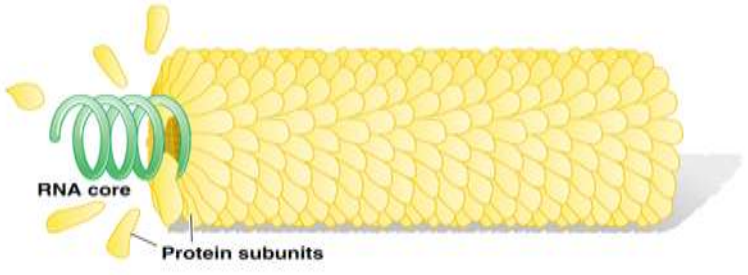


Fig. Typical tobacco mosaic virus (TMV) particle

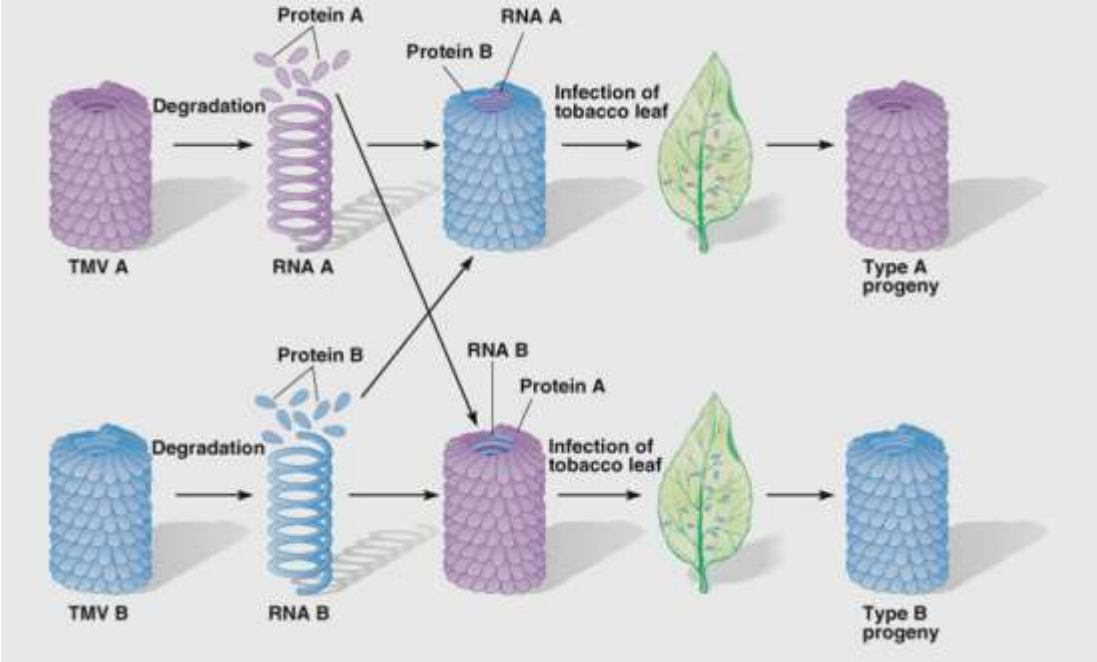


Fig. Demonstration that RNA is the genetic material in tobacco mosaic virus (TMV)

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.

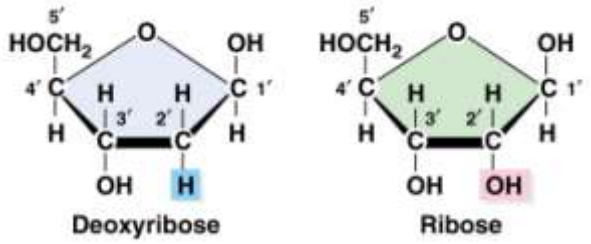


Fig:Structures of deoxyribose and ribose in DNA and RNA

- 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.

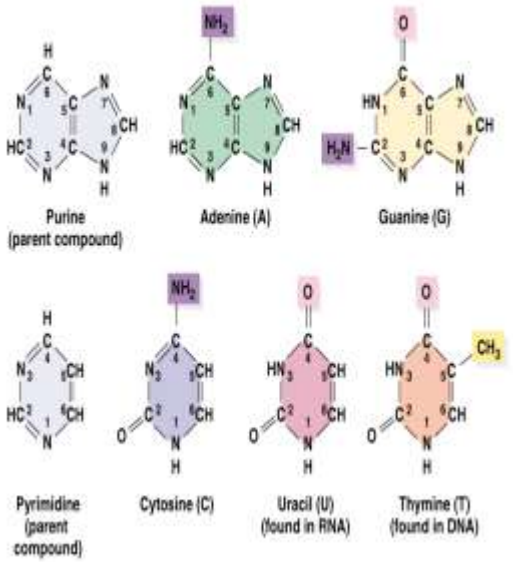


Fig: 2.1 Structures of the nitrogenous bases in DNA and RNA

- 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.

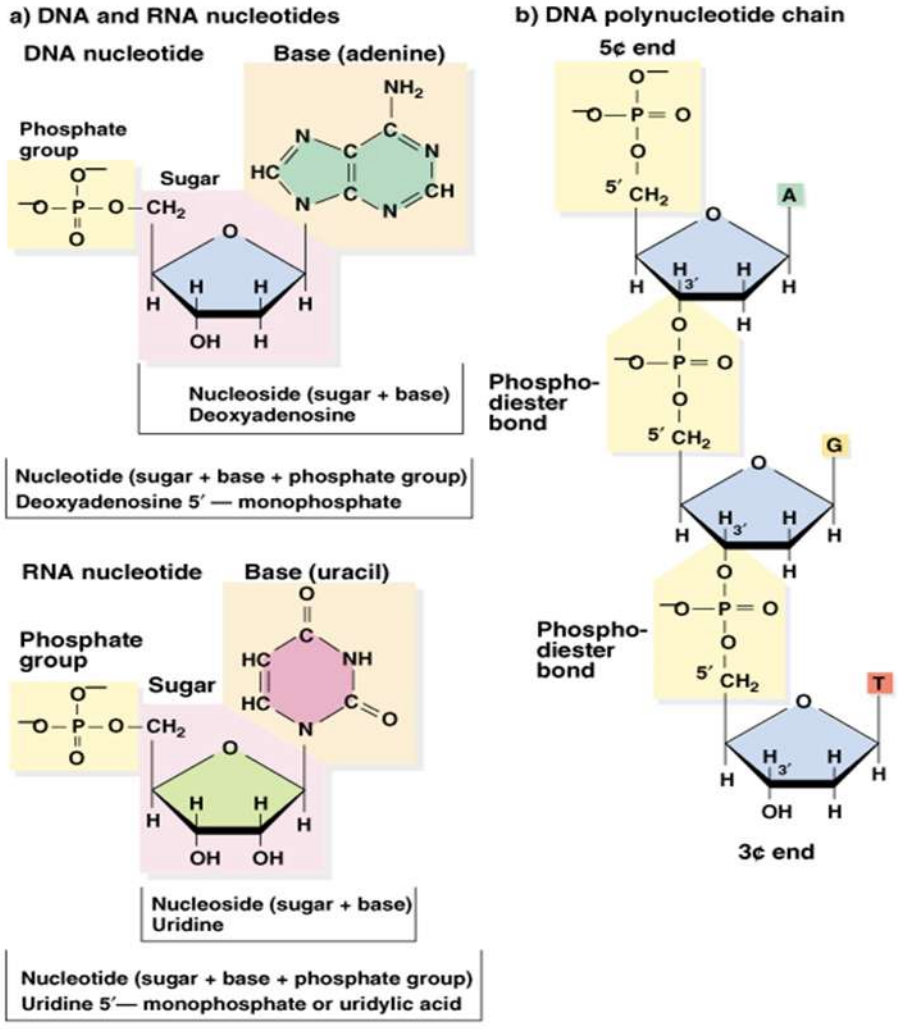


Fig. Chemical structures of DNA and RNA

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.

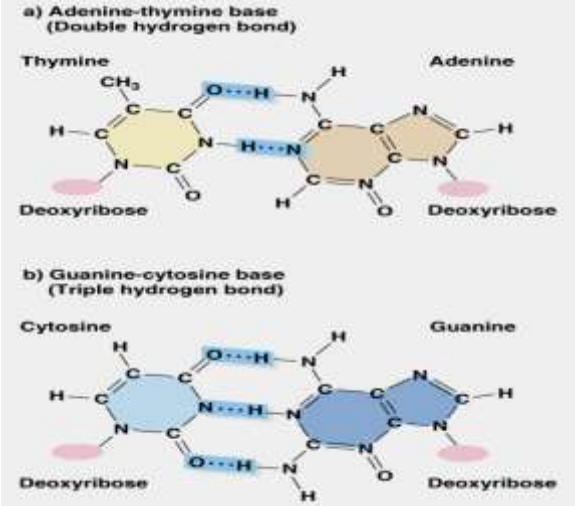


Fig: Complementary Base Pairing

- 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.

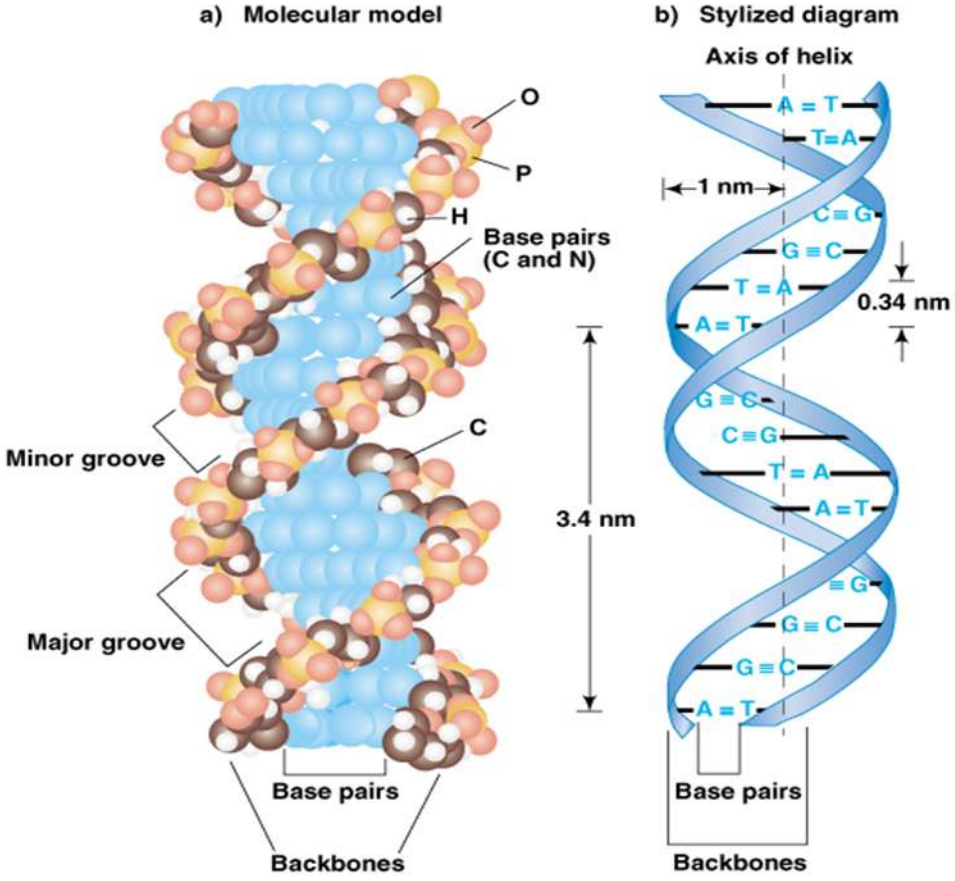


Fig: Physical structure of DNA Double Helix

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

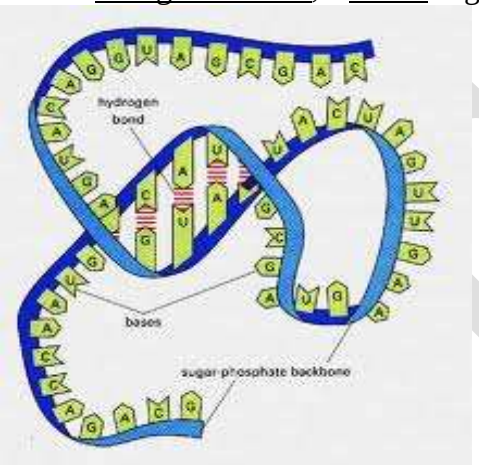


Fig :Structure of RNA

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.

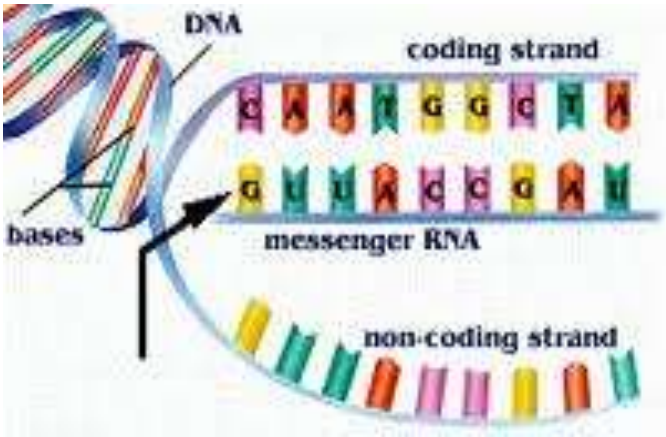


Fig: Messenger RNA

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.

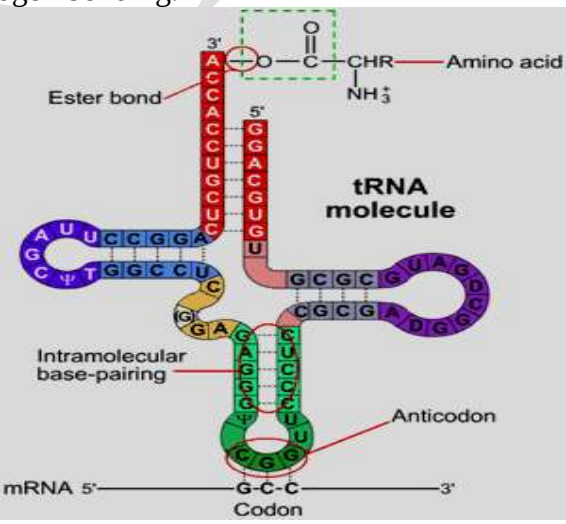


Fig: Structure of tRNA

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.

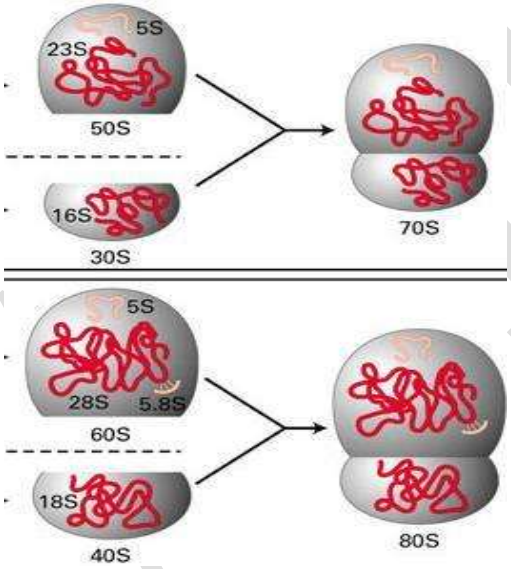


Fig: Prokaryotic and Eukaryotic ribosomes

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.***

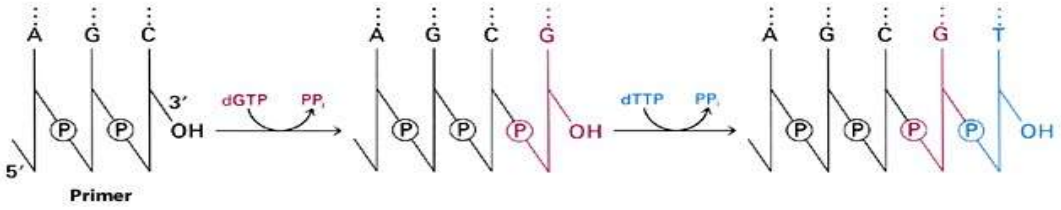
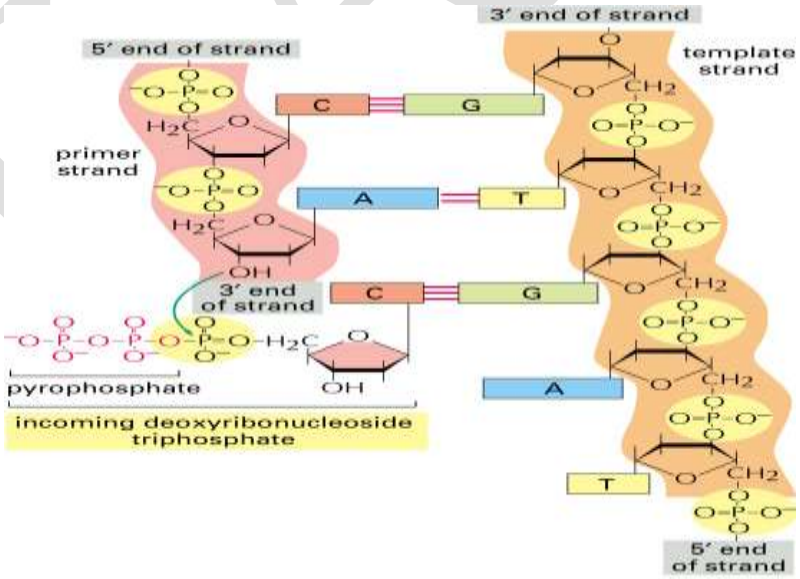


Fig: ***Addition of Nucleotides to a Growing Daughter Strand***

- 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**.**

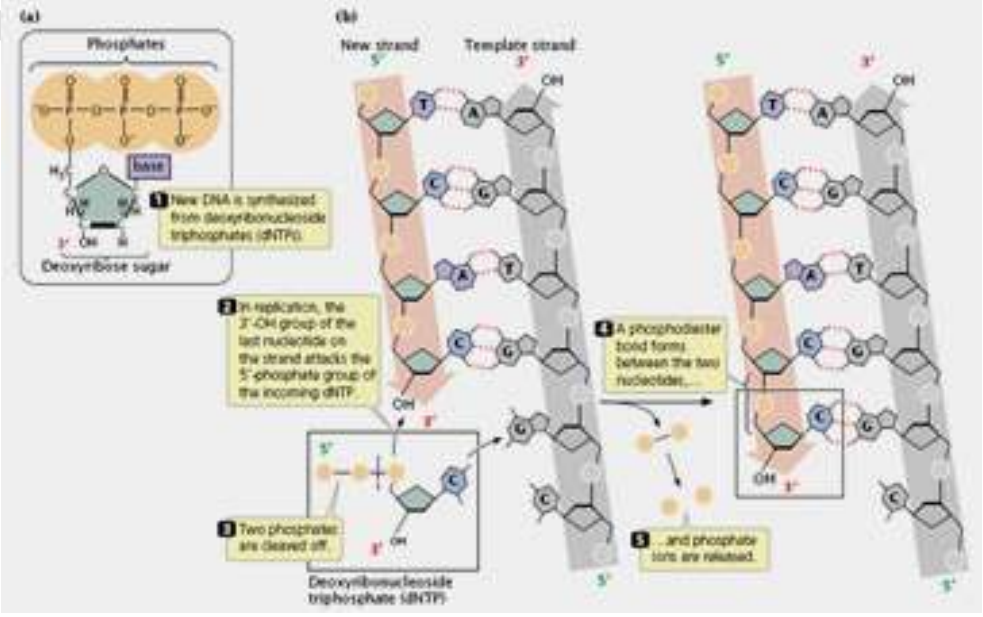


Fig: Polymerization - new DNA is synthesized from deoxyribonucleoside triphosphates (dNTPs).

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,

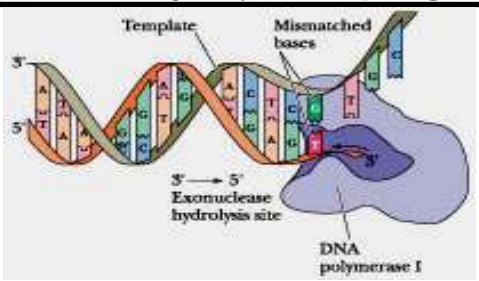


Fig: Proofreading and Editing of DNA polymerase I

- ***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

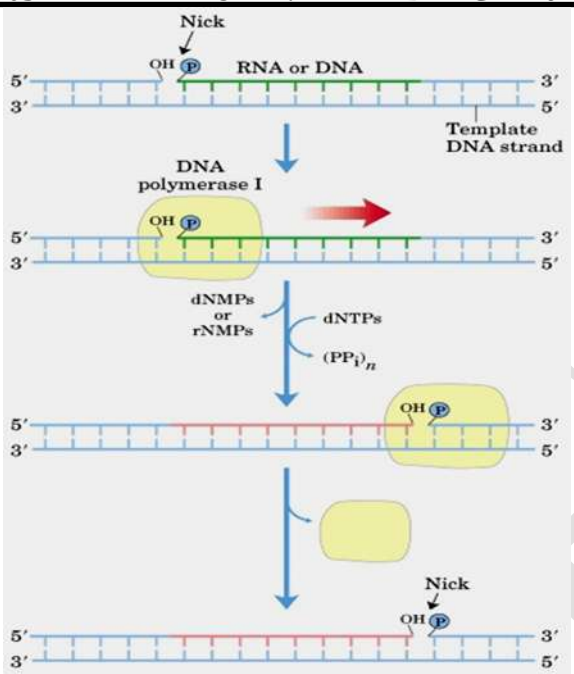


Fig: Nick translation

- ***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

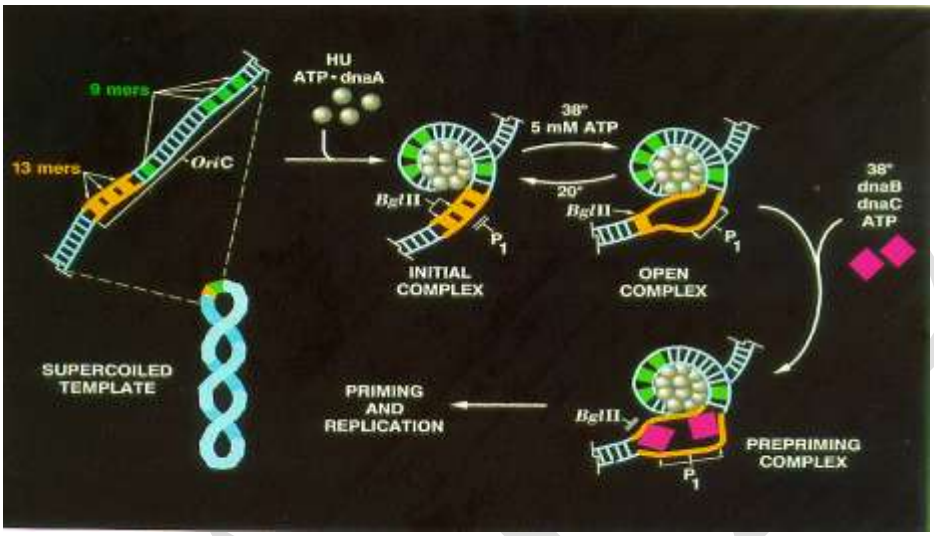


Fig: Initiation of replication

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.

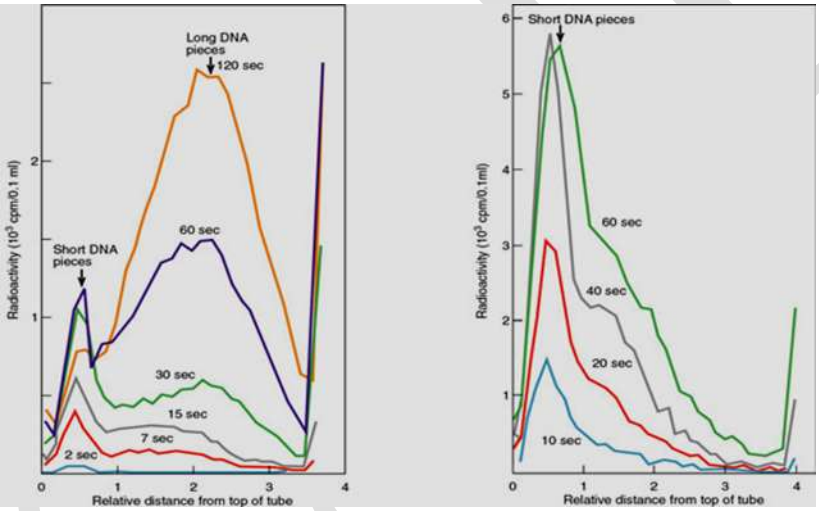


Fig: Demonstration of semidiscontinuous replication ^{3}H labeledokazaki fragments in sucrose density gradients

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 $1/4^{th}$ 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.

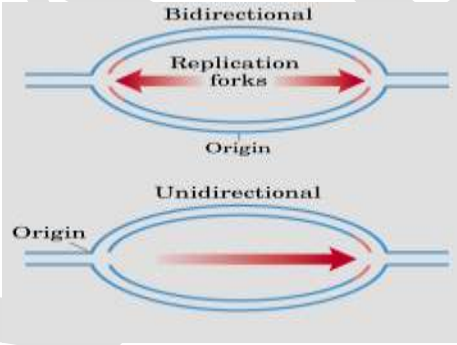


Fig: Bidirectional replication

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.

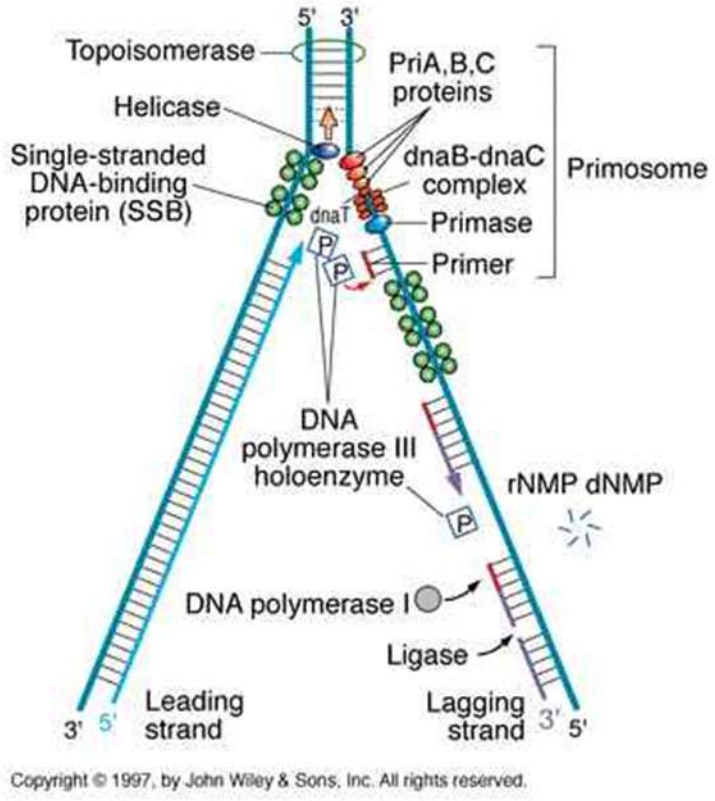
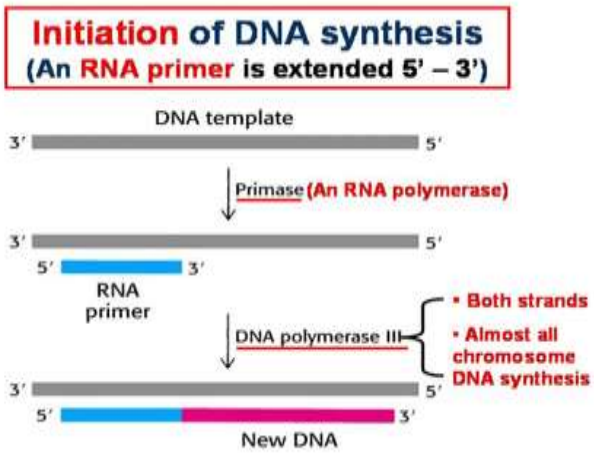
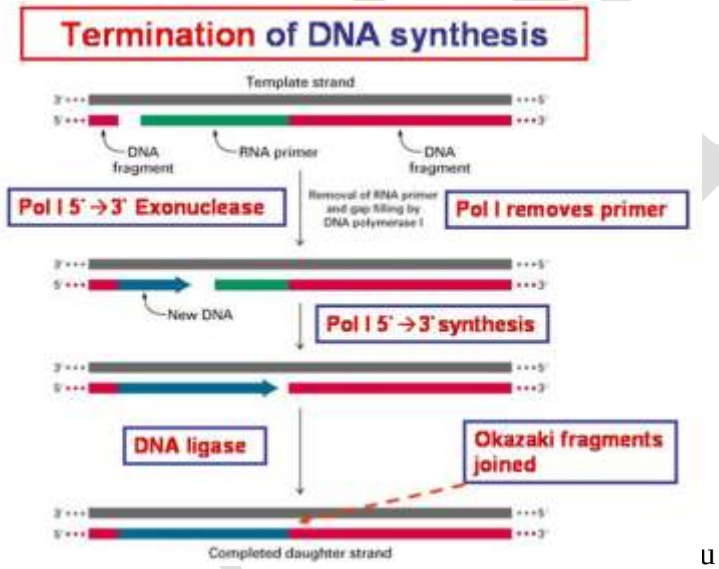


Fig: Events in the replication fork

Mechanism of DNA replication





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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 .

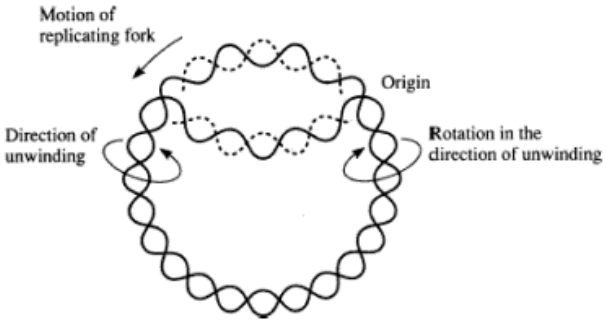


Fig. 10-3. Theta (θ) replication. Newly synthesized DNA is indicated by broken lines. Overwinding of the unreplicated segment (caused by unwinding of the daughter branches) is removed by the nicking action of DNA gyrase.

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.

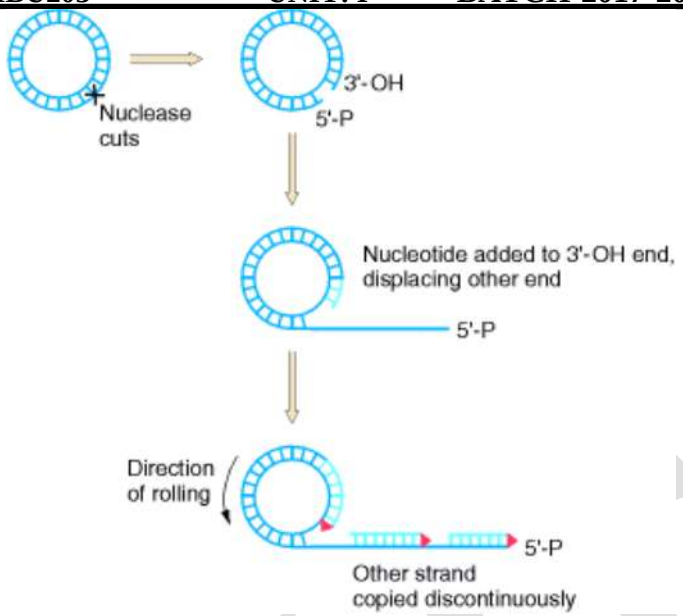


Fig: Rolling circle replication

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.

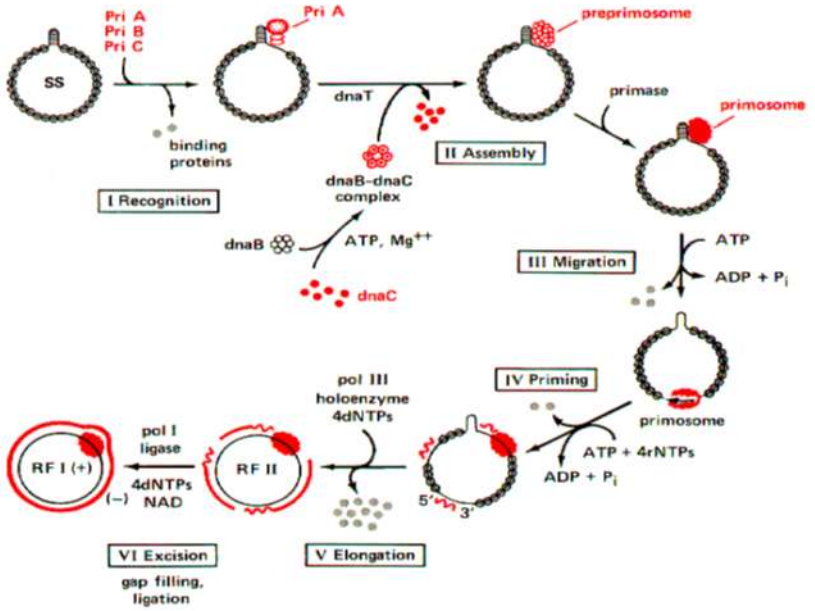


Fig: Looped rolling circle replication

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.

Transcription

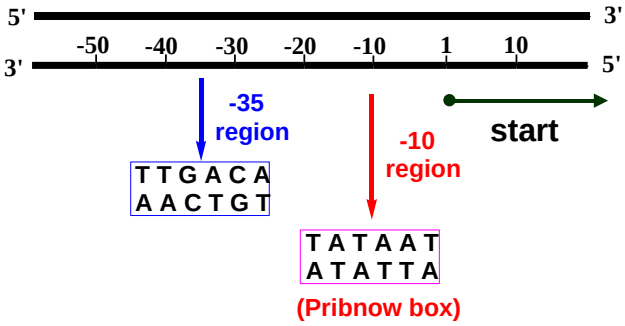
The synthesis of RNA molecules using DNA strands as the templates so that the genetic information can be transferred from DNA to RNA.

- 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

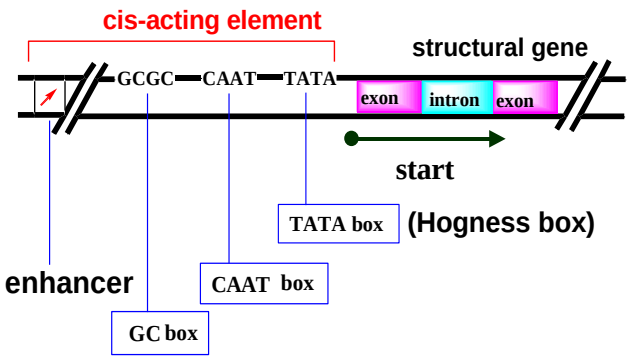
- An enzyme attaches to the promoter (start signal region) of a gene and unwinds the DNA.
  - The -35 region of TTGACA sequence is the recognition site and the binding site of RNA-pol.
  - The -10 region of TATAAT is the region at which a stable complex of DNA and RNA-pol is formed.

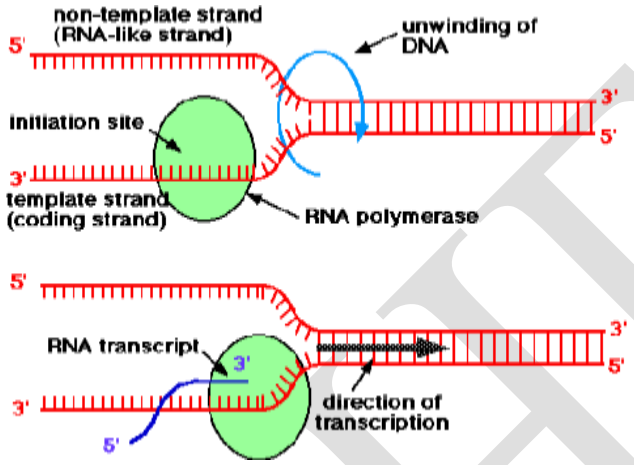
Prokaryotic promoter



Consensus sequence

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.

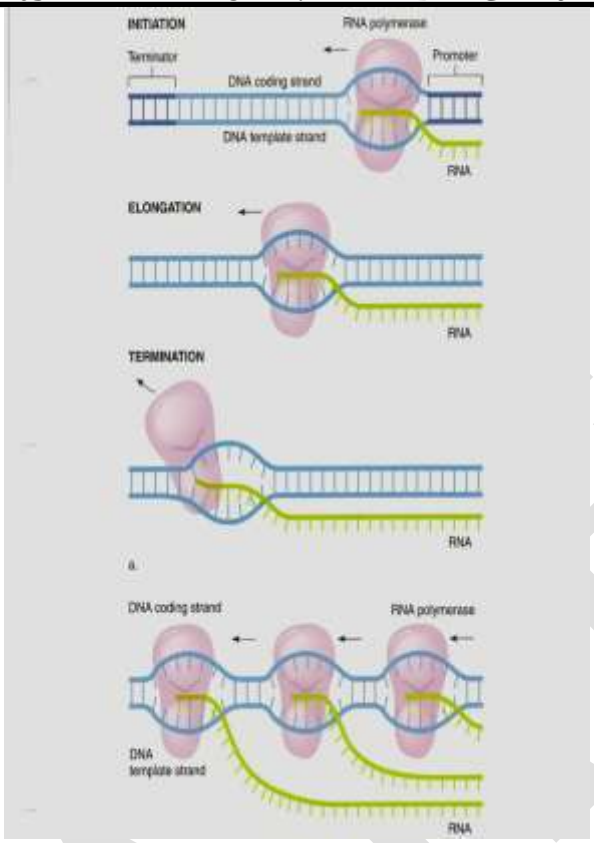


Fig: Mechanism of Transcription

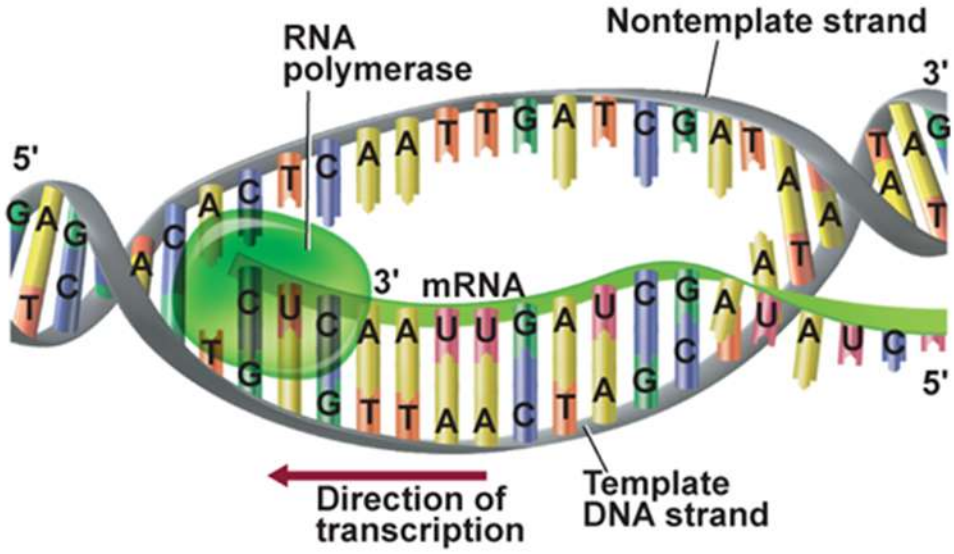


Fig: Initiation of transcription

Post transcriptional modification

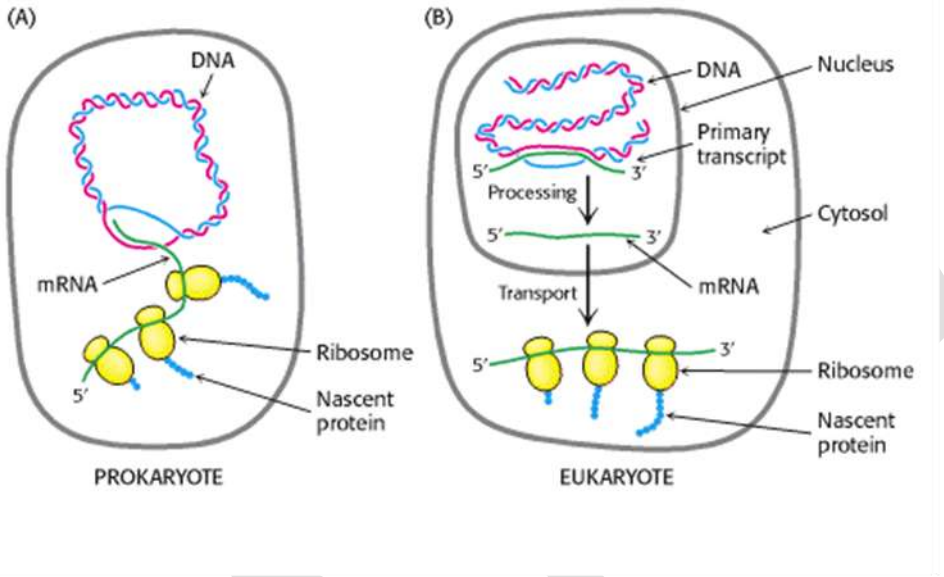


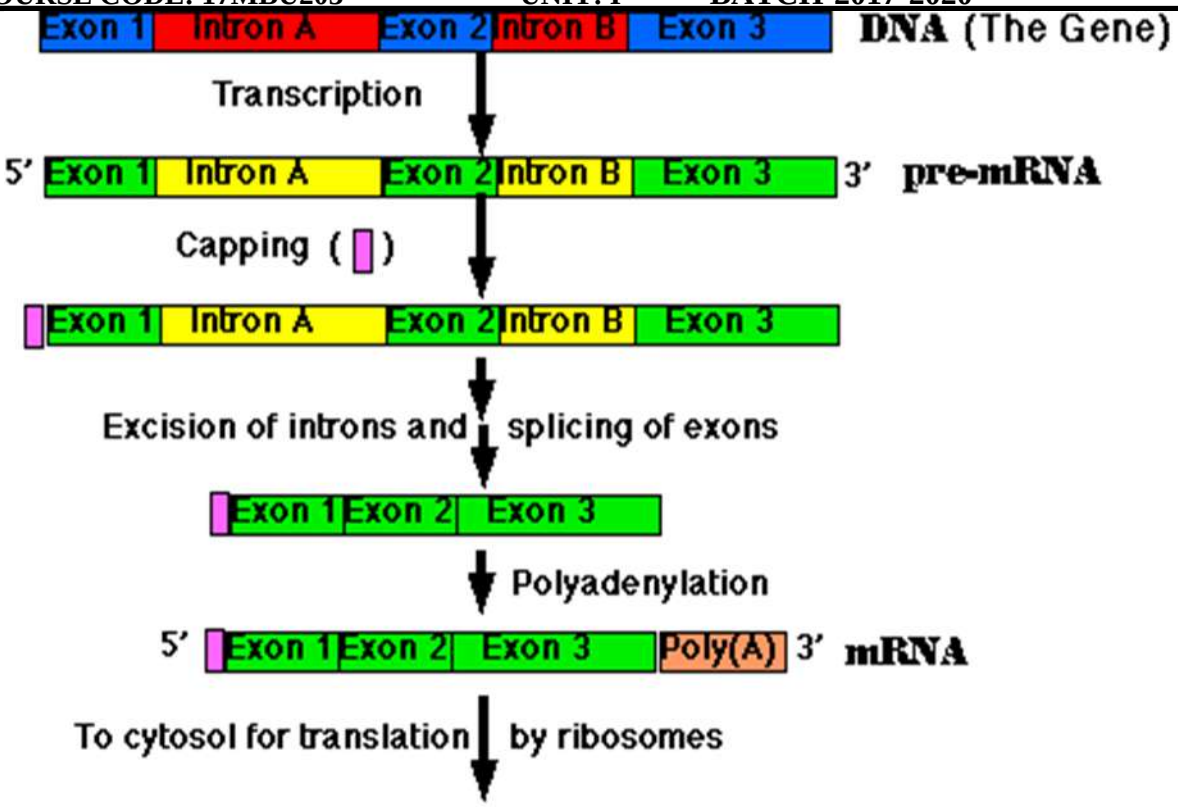
Fig: Post transcriptional modification both in prokaryotes and Eukaryotes

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).

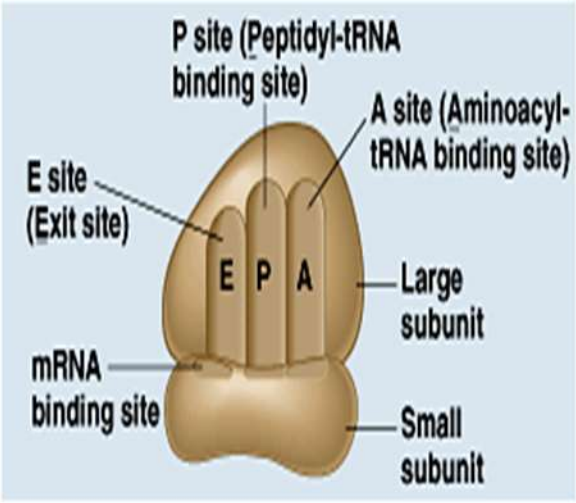
tRNA: at least one specific type of tRNA is required to transfer one amino acid. There about 50 tRNA in human for the 20 amino acids, this means some amino acids have more than one specific tRNA. The role of tRNA in protein synthesis is discussed before. (amino acid attachment and anticodon loop).

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

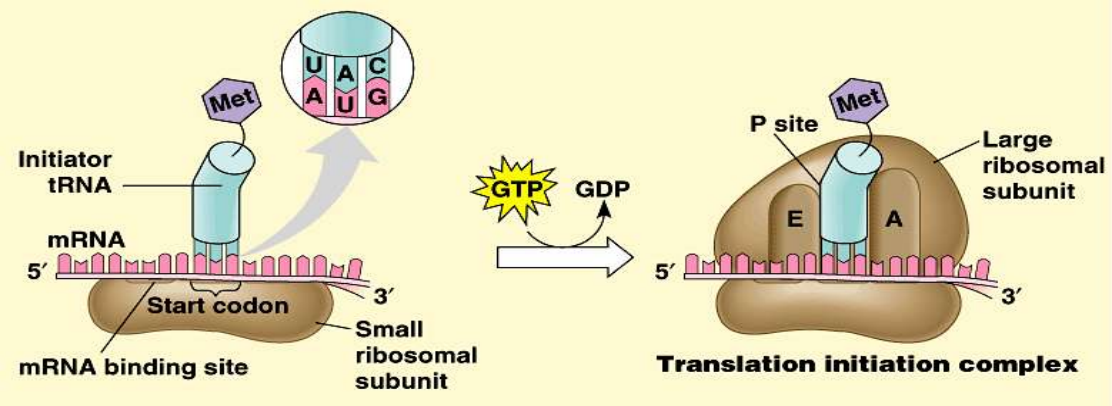


Fig: Initiation of translation

- 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.

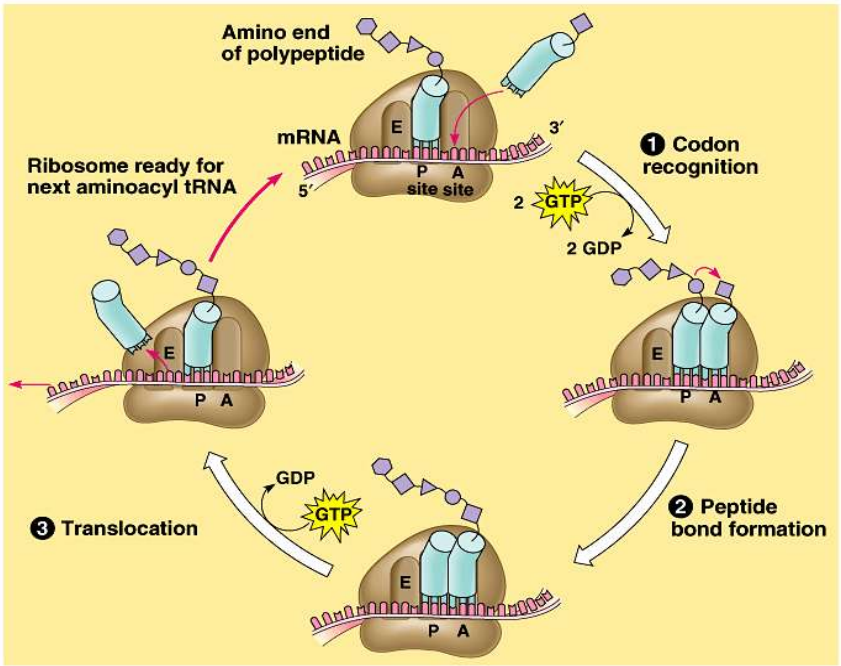


Fig: Repetitive cycle of elongation

***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)

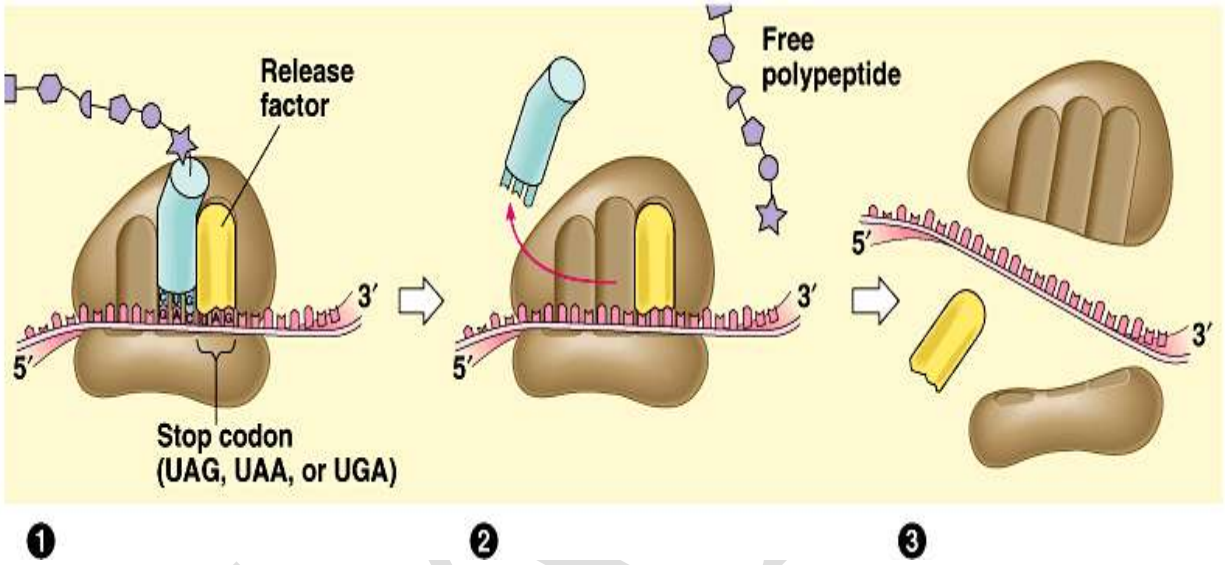


Fig: Termination of translation

Possible Questions

Two marks

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

Eight marks

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

DEPARTMENT OF MICROBIOLOGY,KARPAGAM ACADEMY OF HIGHER EDUCATION
MICROBIAL GENETICS (17MBU203)

Unit I Question
Dihybrid ratio
Experiments of Hershey and Chase was based on
In eukaryotes, the vast majority of DNA synthesis occurs during _____ of the cell cycle
Key enzyme in rolling circle replication
Mendels pioneer work was with
Okazaki fragments are
The ability to remove incorrectly matched nucleotides or Proof-reading
The enzyme that unwinds DNA
_____ million base pairs of nucleotides are seen in *E.coli*
Chargaff's rule
Eukaryotic DNA damage or replication errors are corrected during
Semiconservative DNA replication model
The negative charge of DNA is due to
Thymine in DNA is replaced by _____ in RNA
Which is involved in synthesis of primer
_____ degrades DNA
Enzyme that adds methyl group to the newly formed DNA
Heat Killed S cells + Live R cells produced
Initiation of replication occurs
The replication origin of *E.coli* is approximately
Transformation in *Pneumococci* was discovered by
Bacteria contains
Who proposed the molecular struccture of DNA
The most common form of DNA is
Sequencing and molecular characterization of genome
Adenine always pair with
Initiation of replication is carried out by
Which of the following is not associated with DNA replication?
Father of genetics
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
The replication origins of higher eukaryotes are made up of
Transfer of genes from one generation to the next is
Basically, flow of genetic material is accompolished by
Purines are
The DNA of E.coli is _____ times longer than the cell
SSB protein helps in
The experiments of Avery, McLeod and McCarty was based on
The contribution of Rosalind Franklin towards structure of DNA was
Proof reading activity of DNA polymerase is in the direction
Length of primer during replication is

Distance between the two base pairs is
Formation of pre-replicating complex is seen in replication mechanism of
Triple bonding is seen in
Repair and insertion of DNA is carried out by
Nucleoside is
Left handed DNA
Bonding between two bases
DNA absorbs UV light at _____ wavelength
The Pioneer work on nucleic acid discovery was carried out by
The enzyme that copies RNA from DNA template
The size of a major groove is
Chromosomal theory of inheritance was formulated by
Eukaryotic DNA replication is a conserved mechanism that restricts DNA replication to
Semiconservative mechanism of DNA replication was demonstrated by
The most widely studied origin recognition complex of eukaryotes is that of
Number of base pairs per helical turn of B form DNA
RNA primer is removed by
Longest DNA is seen in
Joining of DNA fragments
Synthesis of DNA always moves from

Opt 1
3 : 3 : 9 : 1
Virus
G phase
DNA Polymerase-IV
Monkey
RNA strands
RNA polymerase
Polymerase
64 million base pairs
No complementarity
G_1 phase
Daughter molecule contains both from parent
Deoxyribose Sugar
Adenine
Ligase
Polymerase
Gyrase
Death in mice + S cells
Bidirectionally
245 bp
Friedrick Griffith
Single circular DNA
Hershey & Chase
B-DNA
Genetics
Guanine
DnaA
Polymerase
Mendel
A hydroxyl group on the 5’ carbon of a deoxyribose sugar
Different AT-rich regions
Inheritance
Replication
A, T
1
Degradation of protein
Protein coupling
X-ray crystallography
5' to 3'
2-10 nucleotides

3.4Å
Prokaryotes
G-T
Endonucleases
Base + Sugar
B-DNA
Hydrogen bond
220 nm
Friedrick Miescher
Dnase
34Å
Mendel
Never
Meselson & Stahl
Bacillus
13
DNA pol
Human
DNA ligase
3' to 5'

Opt 2
9 : 3 : 3 : 1
Bacteriophage
H phase
DNA Polymerase-III
Human
Enzymes
DNA ligase
Ligase
46 million base pairs
Partial complementarity
S phase
Daughter molecule entirely new
Phosphate bond
Cytosine
Primase
Primase
Topoisomerase
Live mice + S cells
Cross sectionally
425 bp
Erwin Chargaff
Single linear DNA
Erwin Chargaff
Z-DNA
Molecular biology
Cytosine
DnaC
Promoter
Morgan
A phosphate group on the 3’ carbon of a deoxyribose sugar.
Similar AT-rich regions
Carrying over
Transformation
G, C
10
Keep the two strands separated after unwinding
Enzymatic reactions
Electron microscopy
3' to 5'
10-20 nucleotides

34Å
Plants
G-C
Ribozyme
Sugar + Phosphate
C-DNA
Hydrophobic bond
240 nm
Watson & Crick
Rnase
3.4Å
Miescher
Only once per cell cycle
Beedle & Tatum
Staphylococcus
12
RNA pol
Lung fish
Gyrase
5' to 3'

Opt 3
9 : 3 : 1 : 3
Bacteria
R phase
DNA Polymerase-II
Garden pea
Leading strands
DNA polymerase
Gyrase
4.6 million base pairs
No such rule
G_2 phase
Daughter molecule contains one from parent and one newly synthesized
Hydrogen bond
Guanine
DNA pol
RNase
Helicase
Death in mice + R cells
Unidirectionally
254 bp
Hershey & Chase
Double Linear DNA
Jim Latham
Y-DNA
Proteomics
Thymine
DnaB
Primer
Watson
A base attached to the 3’ carbon of a deoxyribose sugar
Different GC-rich regions
Subheritance
Transduction
C, T
1000
Elongation of DNA
Synthetic reaction
NMRspectroscopy
Parallel
5-15 nucleotides

10Å
Virus
A-T
Primase
Base + Phosphate
Y-DNA
Nitrogen bond
260 nm
Griffith
DNA polymerase
43Å
Metchinikoff
Only twice per cell cycle
Hershy & Chase
Escherichia coli
11
Terminase
Yeast
RNA polymerase
Ffrom the centre

Opt 4	Opt 5	Opt 6
1 : 3 : 3 : 9		
Fungi		
S phase		
DNA Polymerase-I		
Mice		
Lagging strands		
DNA helicase		
Helicase		
6.4 million base pairs		
Complementarity of one strand with the other		
R phase		
Some sections from parent and some newly synthesized		
Nitrogenous base		
Uracil		
rRNA		
DNase		
Methylase		
Live mice + R cells		
Parallely		
524 bp		
Watson & Crick		
Double circular DNA		
Watson & Crick		
SS-DNA		
Genomics		
Uracil		
DnaE		
RepA protein		
McLeod		
A hydroxyl group on the 3’ carbon of a deoxyribose sugar		
Similar GC-rich regions		
Gene transport		
Conjugation		
A, G		
100		
Uncoiling of RNA		
DNA binding		
Gas chromatography		
Centre		
10-25 nucleotides		

20Å		
Eukaryotes		
A-C		
Exonucleases		
A+T & G+C		
Z-DNA		
Van Der waals		
280 nm		
Milstein	Kornberg	
RNA polymerase		
20Å		
Morgan		
Only thrice per cell cycle		
Avery & McLeod		
Saccharomyces cerevisiae		
10		
Caspase		
Bacteria		
DNA polymerase		
Anywhere		

Answer
9 : 3 : 3 : 1
Bacteriophage
S phase
DNA Polymerase-III
Garden pea
Lagging strands
DNA polymerase
Helicase
4.6 million base pairs
Complementarity of one strand with the other
G_2 phase
Daughter molecule contains one from parent and one newly synthesized
Phosphate bond
Uracil
Primase
DNase
Methylase
Death in mice + S cells
Bidirectionally
245 bp
Friedrick Griffith
Single circular DNA
Watson & Crick
B-DNA
Genomics
Thymine
DnaA
Promoter
Mendel
A hydroxyl group on the 3’ carbon of a deoxyribose sugar
Similar AT-rich regions
Inheritance
Replication
A, G
1000
Keep the two strands separated after unwinding
Enzymatic reactions
X-ray crystallography
3' to 5'
2-10 nucleotides

3.4Å
Eukaryotes
G-C
Endonucleases
Base + Sugar
Z-DNA
Hydrogen bond
260 nm
Friedrick Miescher
RNA polymerase
34Å
Morgan
Only once per cell cycle
Meselson & Stahl
Saccharomyces cerevisiae
10
DNA pol
Lung fish
DNA ligase
5' to 3'

Unit – II

Plasmids and phages provide an important extra dimension to the flexibility ofthe organism's response to changes in its environment, whether those changes arehostile (e.g. the presence of antibiotics) or potentially favourable (the availabilityof a new substrate). This extra dimension therefore consists of characteristics thatare peripheral to the replication and production of the basic structure of the cell –they are the optional extras. Their role in contributing these additional characteristicsis particularly significant because of the relative ease with which they can betransferred between strains or between different species.

Some bacterial characteristics are determined by plasmids

Antibiotic resistance

The most widely studied plasmid-borne characteristic is that of drug resistance.Many bacteria can become resistant to antibiotics by acquisition of a plasmid,although plasmid-borne resistance to some drugs such as nalidixic acid andrifampicin does not seem to occur. (In those cases, resistance usually occurs bymutation of the gene that codes for the target protein). The antibiotic resistancegenes themselves are many and varied, ranging from plasmid-encoded betalactamaseswhich destroy penicillins to membrane proteins which reduce theintracellular accumulation of tetracycline. The ability of plasmids to be transferredfrom one bacterium to another, even sometimes between very differentspecies, has contributed greatly to the widespread disseminationof antibiotic resistance genes. Bacteria can become resistant to a number ofseparate antibiotics, either by the acquisition of several independent plasmids orthrough acquiring a single plasmid with many resistance determinants on it. Someexamples will be discussed later in this chapter. Transposons arethought to have played a major role in the development of drug resistanceplasmids, by promoting the movement of the genes responsible between differentplasmids or from the chromosome of a naturally resistant organism onto aplasmid.

It should be appreciated that other mechanisms of antibiotic resistance alsooccur and that such resistance is not always due to plasmids: indeed many of thebacteria that are currently causing problems of hospital cross-infection are eitherinherently resistant or owe their antibiotic resistance to chromosomal genes.

Colicins and bacteriocins

Another property conferred by some plasmids that has been widely studied is theability to produce a protein which has an antimicrobial action, usually againstonly closely-related organisms. One group of such proteins, produced by strainsof E. coli, are capable of killing other E. coli strains, and are hence referred to ascolicins, and the strains that produce them are colicinogenic. (These terms aremore familiar than the general ones, bacteriocin and bacteriocinogenic). The colicin gene is carried on a plasmid (knownas a Col plasmid), together with a second gene that confers immunity to the actionof the colicin, thus protecting the cell against the lethal effects of its own product.One particular Col plasmid, ColE1, is of special

importance because of thedetailed information that is available concerning its replication and control and also because most of the commonly used *E. coli* cloningvectors are based on ColE1 or a close relative.

Virulence determinants

The previous chapter discussed how bacteriophages can carry genes that code fortoxins and that the presence of the phage is necessary for pathogenicity. In somebacterial species toxin genes are carried on plasmids rather than phages. Forexample, some strains of *E. coli* are capable of causing a disease that resemblescholera (although milder). These strains produce a toxin known as LT (labiletoxin – to distinguish it from a different, heat-stable, toxin known as ST). The LTtoxin is closely related to the cholera toxin, but whereas the gene in *V. cholerae* iscarried by a prophage, the LT gene in *E. coli* is found on a plasmid.

Plasmids can also carry other types of genes that are necessary for (or enhance)virulence. One of the most dramatic examples of this is the 70-kb virulenceplasmid of *Yersinia* species. This plasmid which is found in species of *Yersinia*(including *Yersinia pestis*, the causative organism of plague) has been aptlydescribed as a mobile arsenal since it encodes an integrated system which allowsthese bacteria to inject effector proteins into cells of the immune response todisarm them, to disrupt their communications or even to kill them.

Plasmids in plant-associated bacteria

A different type of pathogenicity is seen with the plant pathogen *Agrobacteriumtumefaciens*, which causes a tumour-like growth known as a crown gall in someplants. Again, it is only strains that carry a particular type of plasmid (known as aTi plasmid, for Tumour Inducing) that are pathogenic; in this case however,pathogenicity is associated with the transfer of a specific part of the plasmidDNA itself into the plant cells. This phenomenon has additional importancebecause of its application to the genetic manipulation of plant cells.Members of the genus Rhizobium also 'infect' plants, although in this case therelationship is symbiotic rather than pathogenic. These bacteria form nodules onthe roots of leguminous plants. Under these conditions the bacteria are able to fixnitrogen and supply the plant with a usable source of reduced nitrogen, a processof considerable ecological and agricultural importance. The genes necessary forboth nodulation and nitrogen fixation are carried by plasmids.

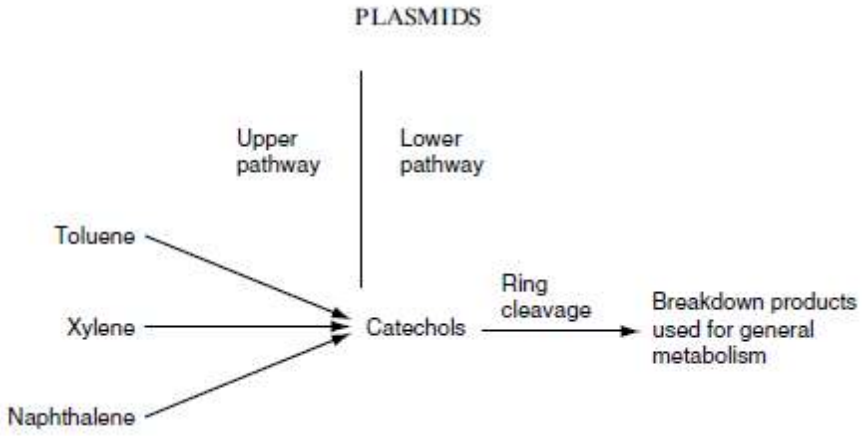
Metabolic activities

Plasmids are capable of expanding the host cell's range of metabolic activities in avariety of other ways. For example, a plasmid that carries genes for the fermentationof lactose, if introduced into a lactose non-fermenting strain, will convert it toone that is able to utilize lactose. Such plasmids can cause problems in diagnosticlaboratories where organisms are often identified on the basis of a limited set ofbiochemical characteristics. Commonly the potentially pathogenic *Salmonella*genus is differentiated from the (usually) non-pathogenic *E. coli* species primarilybecause of the inability of Salmonella to ferment lactose. In some cases, thedetection of

serious epidemics of Salmonella infections has been delayed becausethe causative agent had acquired a lactose-fermenting plasmid.A large number of other genes have also been found on plasmids, includingthose for fermentation of other sugars such as sucrose, hydrolysis of urea, orproduction of H2S. Many of these were initially identified because of the confusionthey caused in biochemical identification tests.

Bacterial species	Disease	Virulence gene(s)	Location
Corynebacterium diphtheriae	Diphtheria	Toxin	Phage
Streptococcus pyogenes	Scarlet fever	Toxin	Phage
Vibrio cholerae	Cholera	Toxin	Phage
Shigella spp.	Dysentery	Invasion/adhesion	Plasmid
Yersinia enterocolitica	Gastroenteritis	Yops (outer membrane proteins)	Plasmid
Clostridium botulinum	Botulism	Toxin	Phage
Clostridium tetani	Tetanus	Toxin	Plasmid
Escherichia coli	Gastroenteritis	Enterotoxins	Plasmids
Escherichia coli	Gastroenteritis	Adhesion	Plasmids

Some plasmids and their roles



Pathways involved in plasmid activity

Biodegradation and bioremediation

Another type of plasmid-mediated metabolic activity is the ability to degradepotentially toxic chemicals. One such plasmid, pWWO, obtained from Pseudomonasputida, encodes a series of enzymes that convert the cyclic hydrocarbonstoluene and xylene to benzoate and a secondoperon responsible for the degradation of benzoate, via ring cleavage of a catecholintermediate, into metabolic intermediates that can be used for energyproduction and

biosynthesis (lower pathway – see Figure 5.1). This organismcan therefore grow using toluene as a sole carbon source. The enzymes of theupper pathway are specialized; other plasmids code for upper pathway enzymeswith different specificities, enabling the organism to convert other chemicals intobenzoate and catechol derivatives which can be degraded by the lower pathwayenzymes. Plasmid-mediated degradation includes naphthalene and camphor, aswell as chlorinated aromatic compounds such as 3-chlorobenzoate and the herbicide2,4-D (dichlorophenoxyacetic acid).The ability to degrade environmentally damaging chemicals is potentiallyuseful in clearing up polluted sites (bioremediation). There is therefore considerableinterest in extending the range of chemicals that can be degraded by microorganisms,both by modification of existing pathways and also by screeningbacteria isolated from contaminated sites for novel activities. The usefulness ofsuch strains is also potentiated by plasmids which confer resistance to toxic metalions, notably copper and mercury.

Molecular properties of plasmids

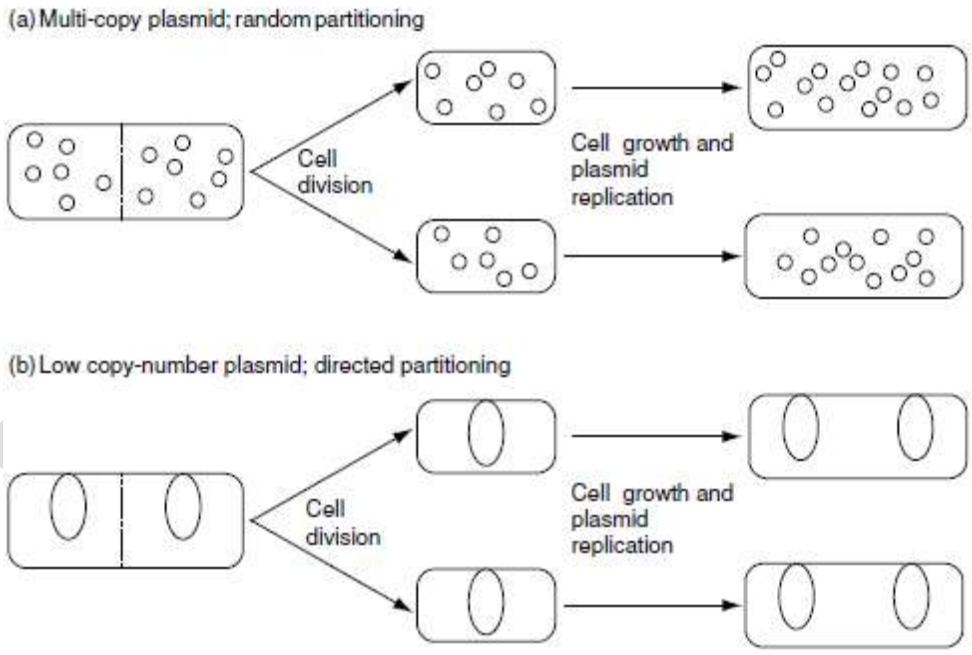
Bacterial plasmids in general exist within the cell as circular DNA molecules witha very compact conformation, due to supercoiling of the DNA. In many cases,they are quite small molecules, just fewkilobases in length, but in someorganisms, notably members of the genus Pseudomonas, plasmids up to severalhundred kilobases are common. However, it is worth noting that the standardmethods for isolating plasmids (see below) are geared to the separation of smallcovalently closed circular DNA, and the occurrence of large plasmids, or alternativeforms such as linear plasmids, may be underestimated.It is convenient to regard plasmids from E. coli as consisting of two types. Thefirst group, of which ColE1 is the prototype, are relatively small (usually less than10 kb), and are present in multiple copies within the cell. Their replication is notlinked to the processes of chromosomal replication and cell division (hence thehigh copy number), although there are some controls on plasmid replication. Replication of these plasmids can continue undercertain conditions (such as inhibition of protein synthesis) that prevent chromosomereplication, giving rise to a considerable increase in the number of copies ofthe plasmid per cell. This phenomenon, known as plasmid amplification, is veryuseful for isolating the plasmid concerned.The second group of plasmids, exemplified by the F plasmid, are larger (typicallygreater than 30 kb; F itself is about 100 kb) and are present in only one ortwo copies per cell. This is because their replication is controlled in essentially thesame manner as that of the chromosome; hence when a round of chromosomereplication is initiated, replication of the plasmid will occur as well. It followstherefore that plasmids of this type cannot be amplified. In general, these largeplasmids are able to promote their own transfer by conjugation.

The existence of these two groups can be rationalized on the basis of theirdifferent survival strategy. Members of the first group rely on their high copynumber to ensure that, at cell division, if the plasmid molecules partition randomlybetween the two cells, then each daughter cell is virtually certain to containat least one copy of the plasmid. For example, with a plasmid that ispresent in 50 copies per cell, the chance of one daughter cell not receiving anycopies of the plasmid is as low as 1 in 1015.However, high copy number imposes a size constraint. Replication of a plasmidimposes a metabolic burden that is related to the size and copy number

of theplasmid. The greater the burden, the greater the selective pressure in favour ofthose cells that do not possess the plasmid. Hence it is logical that high copynumber plasmids will also be small. ColE1, for example, is 6.4 kb in size. If thereare 30 copies per cell, this represents about 4 per cent of the total DNA of the cell.

The F plasmid on the other hand (c. 100 kb), if it were to be present at a similarcopy number, would add nearly 70 per cent to the total DNA content whichwould inevitably make the cell grow much more slowly and any cells that had lostthe plasmid would have a marked selective advantage.But the information required to establish conjugation in *E. coli* is quite extensive(see Chapter 6). With the F plasmid for example about 30 kb (out of 100 kb)consists of genes required for plasmid transfer. It follows therefore that a smallplasmid will not be able to carry all the information needed for conjugativetransfer.

The second type of plasmid has evolved a different strategy. Firstly, linking replication of the plasmid to that of the chromosome ensuresthat there are at least two copies of the plasmid available when the cell divides.



Plasmid partition

Secondly, random partitioning will not be sufficient to ensure that each of thedaughter cells receives a copy; so the plasmid must be distributed betweenthe progeny in a directed manner, in much the same way as the copies of thechromosome are distributed. The ability to transfer by conjugation provides aback-up mechanism since any plasmid-free cells that arise in the population byfailure of the partitioning mechanism will then be able to act as recipients fortransfer of the plasmid.It is necessary to be aware that this picture, although useful, is a highlysimplified one, and there are many exceptions, even in E. coli. There are

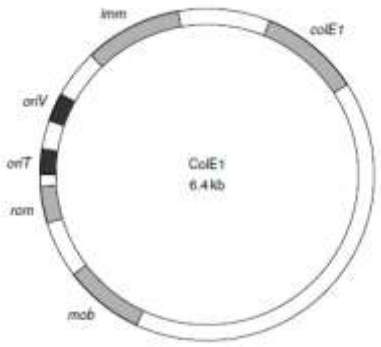
numerousexamples of small plasmids that have a low copy number, although none of themare conjugative, and some examples of larger plasmids that exist in multiplecopies. In addition, in other organisms the picture is less clear; for example inStreptomyces it seems that quite small plasmids are able to promote their owntransfer by conjugation.

Plasmid replication

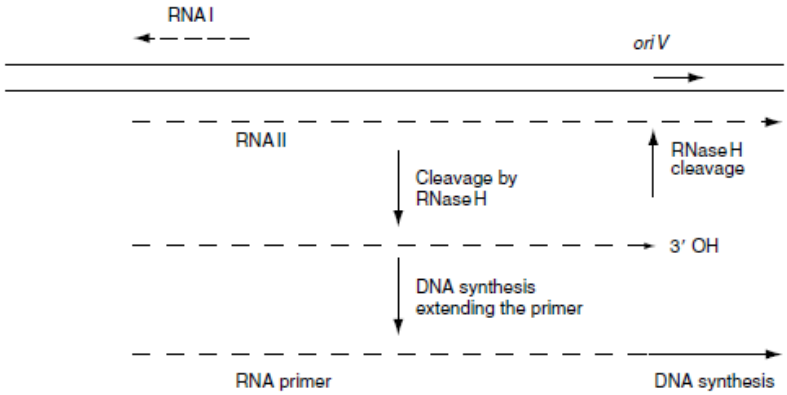
In order to understand the reasons for the different behaviour of plasmidsas described above, we need to look at the mechanisms of plasmid replicationand how it is controlled. This should be compared with the description ofchromosome replication in Chapter 1. Many plasmids are replicated as doublestrandedcircular molecules. The overall picture with such plasmids is basicallysimilar to that of the chromosome, in that replication starts at a fixed pointknown as oriV (the vegetative origin, to distinguish it from the point at whichconjugative transfer is initiated, oriT), and proceeds from this point, either in onedirection or in both directions simultaneously until the whole circle is copied.However there are some aspects of replication that differ from that of thechromosome, especially for the multicopy plasmids. Two examples that havebeen studied intensively are ColE1 and R100. Other plasmids with quite differentmodes of replication are dealt with later on.

Replication of ColE1

The colicinogenic plasmid ColE1 is a comparatively small (6.4 kb)plasmid that carries just the genes for production of colicin E1, and immunity toit, together with functions involved in plasmid maintenance. This is probably thebest understood of all plasmids. Replication starts with the production of anRNA primer (RNA II), starting from a site 555 bp upstream from oriV. Transcription occurs through the origin (oriV), and RNA II is cutat a specific site by RNase H



Genetic map of the plasmid ColE1. colE1, imm: genes for production of,and immunity to, colicin E1; mob codes for a nuclease required for mobilization; romcodes for a protein required for effective control of copy number; oriT: origin of conjugaltransfer; oriV: origin of replication



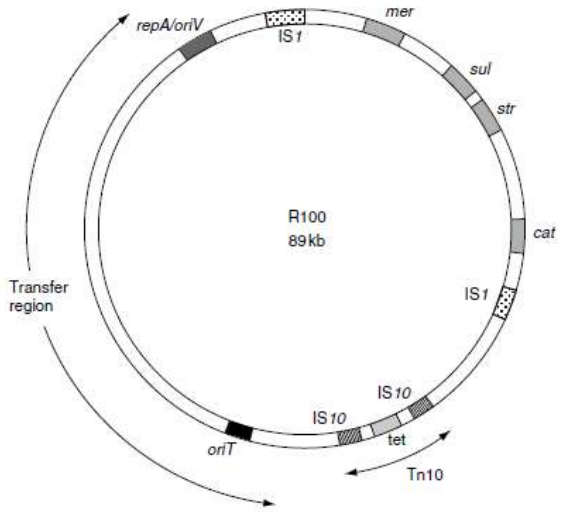
Replication of ColE1 plasmid: Structure and control of the origin of replication of the ColE1 plasmid.RNA II, after cleavage by RNaseH, acts as a primer for DNA synthesis. RNA I binds toRNA II and prevent RNase cleavage and hence prevents initiation of replicationan RNA–DNA hybrid). DNA synthesis then occurs by addition of deoxynucleotidesto the 30 OH end of the RNA primer.

The RNA primer is known as RNA II because there is another RNA molecule produced from the same region, which is called RNA I. This is transcribed fromthe opposite strand to RNA II, and is complementary to the first 108 bases ofRNA II. The presence of RNA I is inhibitory to replication, because binding ofRNA I to RNA II prevents cleavage of RNA II by RNaseH, due to interferencewith the secondary structure of RNA II. So, although the copy number is high,replication is still controlled to some extent. An additional gene that controlsreplication is the rom (or rop) gene, which codes for a protein that facilitates theinteraction of RNA I and RNA II. Derivatives of ColEI in which the rom gene hasbeen deleted have a higher copy number.

The ColE1 plasmid is non-conjugative, that is, it is not able to transfer itselffrom one cell to another. However, in common with many other non-conjugativeplasmids, it can be transferred by conjugation if the cell carries a compatibleconjugative plasmid. This effect, which involves the mob and oriT sites, is known as mobilization

Replication of R100

R100 is a low copy number, conjugative, resistance plasmid, which contains about89 kb of DNA; it confers resistance to four different antibiotics (tetracycline,chloramphenicol, streptomycin and sulphonamides), as well as to mercury salts.



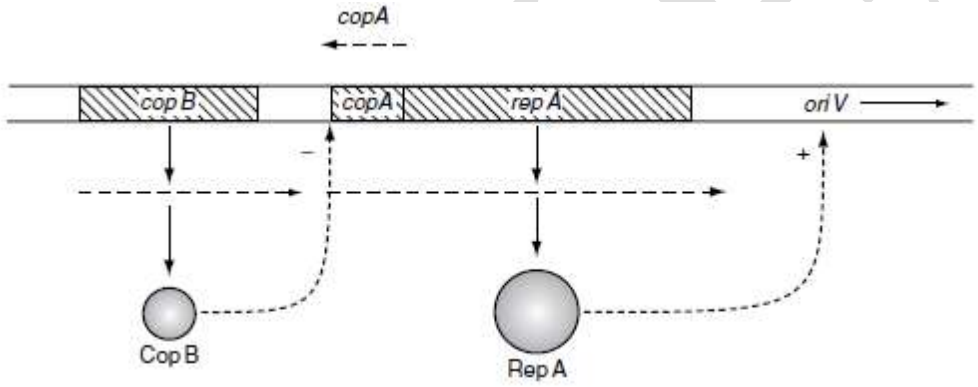
Genetic map of the conjugative E. coli plasmid R100. Resistance genes: cat,chloramphenicol (chloramphenicol acetyltransferase); mer, mercuric ions; str, streptomycin;sul, sulphonamides; tet, tetracycline. Other sites: oriT, origin of conjugative transfer;repA/oriV, replication functions and origin of replication. IS1, IS10 are insertionsequences, Tn10 is a transposon

It is immediately apparent that allthe genes required for conjugative transfer, comprising nearly half of the plasmid,are clustered together, adjacent to the origin of replication (oriV), while theantibiotic resistance determinants are all found on the right hand half of thediagram. These large antibiotic resistance plasmids are commonly organized inthis way, which is thought to reflect their evolution by sequential addition ofresistance genes to a basic replicon, i.e. it started as a cryptic plasmid comprisingjust the transfer region and origin of replication, to which the various resistancegenes have been added (individually or in blocks).One mechanism for acquiring extra resistance genes is shown by the tetracyclineresistance determinant (tet). This is flanked by two copies of an insertion sequence(IS10); this combination results in a mobile structure known as a transposon(Tn10 in this case) that can move from one DNA site to another. Tn10 hastherefore presumably transposed into R100 from another plasmid. R100 alsocontains two copies of a different insertion sequence, IS1. Transposons andinsertion sequences play a key role in the evolution of plasmids,

Replication of R100 occurs from a single origin. In addition to the origin ofreplication, a gene known as repA (adjacent to oriV) is required for the initiation ofreplication from oriV (Figure 5.6). Plasmid copy number is controlled by twogenes that regulate the production of the RepA protein. One of these, copB, codesfor a protein that represses transcription of the repA gene. When the plasmid firstenters a bacterial cell, the absence of CopB allows expression of RepA and so thereis a burst of replication, until the level of CopB builds up to repress this promoter.From then on, expression of RepA occurs at a low level from the copB promoter.

The second regulatory gene, copA, then regulates expression of RepA. This genecodes for an 80–90-nucleotide untranslated RNA molecule. The copA gene iswithin the region of DNA

that is transcribed for production of RepA, butis transcribed in the opposite direction (it is an antisense RNA). The copA RNAis therefore complementary to a short region of the repA transcript, and will bindto it, interfering with translation of the RepA protein. When the plasmid replicates,the number of copies of the copA gene is doubled and the amount of thecopA RNA will therefore increase; this causes a marked reduction in furtherreplication initiation, until cell division restores the original copy number.R100 is unable to co-exist with other related plasmids such as R1; this incompatibilityis one way of classifying plasmids (see below). R100 and R1 belong tothe IncFII group of plasmids. The copA gene is responsible for the incompatibilityof R100 and R1. The sequence of the copA gene is very similar in these twoplasmids and the products are interchangeable. The R100 copA RNA will thereforeinhibit replication of R1 and vice versa, which results in one or the otherplasmid being lost at cell division. It should be noted however that with otherplasmids there are different causes of incompatibility.



Replication control of the plasmid R100. The RepA protein is needed forinitiation of replication. Transcription of repA is repressed by CopB and translation of therepA mRNA is inhibited by the antisense copA RNA

Plasmids like R100 also contain a region known as the par locus (for partitioning)that is necessary for accurate partitioning of plasmid copies at cell division.This sequence can only act in cis, i.e. it must be present on the plasmid itself andnot on some other plasmid. (Genes that are able to have an effect on other DNAmolecules are said to be active in trans, while those that can only affect the sameDNA molecule are cis-acting). It is likely that all low copy number plasmids havea par sequence (or some other mechanism for ensuring accurate partitioning),while plasmids such as ColE1 rely primarily on high copy number to ensure thateach daughter cell receives a copy of the plasmid.

Plasmid stability

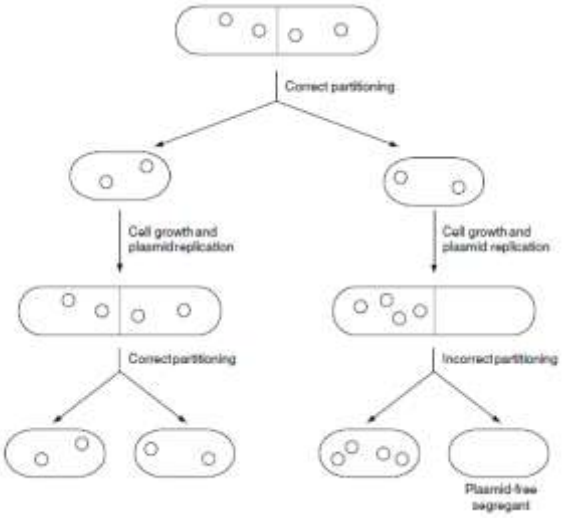
One of the characteristic features of plasmids is their instability. Plasmid-bornefeatures are often lost from a population at a higher frequency than wouldbe expected for the normal processes of mutation. The extent of this instabilityvaries enormously from one plasmid to another. Naturally-occurring plasmidsare usually (but not always) reasonably stable: selection will tend to operate

in that way, and in addition in isolating the strain and looking for the plasmidthe more stable plasmids will tend to be selected. Unstable ones will be harderto find.

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

Partitioning

As mentioned previously, correct partitioning at cell division is essential if theplasmid is to be maintained in the culture (Figure 5.15). Although high copynumber plasmids can rely principally on random distribution between the twodaughter cells, this can be compromised by a tendency for plasmids to formmultimeric structures during replication and also by recombination betweenmonomers. Furthermore, since a dimer contains two origins of replication, itwill be expected to replicate more efficiently than a monomer; multimers wouldreplicate even more efficiently. This could potentially lead to what is known as a‘dimer catastrophe’, in which the proportion of dimers, and higher multimers,increases to an extent that threatens the sustained maintenance of the plasmid.It is worth pausing here to examine why it should matter whether the plasmid ispresent as monomers, dimers or multimers. The models for the control of plasmidcopy number work essentially by counting the number of replication origins, rather than the number of molecules. So, a plasmid with a copy number of 30would be present as 30 monomers, or 15 dimers or 10 trimers and so on. (In realitythere would be a heterogeneous mixture of different sizes). So the existence of asubstantial proportion of these plasmids as multimers will reduce the effectivecopy number when it comes to cell division and hence increase the likelihood thatone of the daughter cells will not receive a copy of the plasmid.



Plasmid segregation through failure of partitioning

The main mechanism for countering this effect involves site-specific recombination.or example, ColE1 contains a site known as cer which is a target for theaction of the proteins XerC and XerD. In a dimer, there are two copies of the cersequence and the Xer proteins will catalyse recombination between them – itbreaks both DNA molecules, crosses them over, and rejoins them – thus resolvingthe dimeric structure into two monomers. (XerC and XerD areactually host proteins, which carry out a similar function in resolving anychromosome dimers produced accidentally during replication). The importanceof this system is demonstrated by the marked instability that results from deletionof the cer locus of ColE1.

Low copy-number plasmids cannot rely on random partitioning. As well as anactive partitioning mechanism, some plasmids supplement their partitioningsystem with an ability to kill any cells that have lost the plasmid (post-segregationalkilling). These systems consist of two components: a stable, long-lived toxinand an unstable factor that either prevents expression of the toxin or acts as anantidote to it.

Possible Questions

Two marks

1. Define plasmids.
2. What are natural plasmids? Give example.
3. What are artificial plasmids? Give example.
4. Define linkers.
5. What are restriction enzymes?
6. Differentiate endo and exonuclease.
7. Write about ligase.
8. How are plamids named? Give example.
9. Draw the diagram of pBR322
10. Draw the diagram of pUC18 vector.
11. What are F plasmid?
12. What is meant by R plasmid?
13. What are co-integrative plasmid?
14. Define a cosmid.
15. What are phagemids?
16. Write any two properties of plasmids.
17. Write short notes of Ti plasmid.
18. What is meant by cell competency?
19. Write about the copy number of a plasmid.
20. What is meant by Hfr cells?

Eight marks

21. Distinguish between natural and artificial plamid.
22. Explain the method of constructing pBR322
23. How is plasmid DNA isolated from bacterial cell?
24. Write briefly about plasmid partitioning?
25. Explain about the replication mechanism of R plasmids and F plasmids?
26. Write in detail about the application of plasmids.

DEPARTMENT OF MICROBIOLOGY,KARPAGAM ACADEMY OF HIGHER EDUCATION
MICROBIAL GENETICS (17MBU203)

Unit II Question
Chemical agent that resembles thiamine
Duplication mutation is
Mutations that result from treatment with mutagens are called
Oncogenes are found in certain
Mutation generating new stop codon are called
UV induced dimers are separated using light energy by
The function of DNA glycosylase in base excision repair is
Which of the following biomolecule has self¬repair mechanisms?
Rapid screening technique for mutagens and carcinogens
Mutation resulting from deamination of 5-methylcytosine produces Thymine which pairs with
Converts amino groups to keto groups by oxidative deamination
In bacteria, a small circle of DNA found outside the main chromosome is called
Biological agents of mutagenesis are
Most common proto-oncogene implicated in human cancers
Deficiency in phenylalanine hydroxidase results in
Cancer that results from deletion of a portion of chrosome 13 is
Mutation in which a purine base is substituted with another purine base is
Sickle cell anaemia is caused by change in amino acid from
Which of the following chemicals induce depurination
Xeroderma pigmentosum is a genetic disorder of
UV radiation causes
The result of addition or deletion of one or more base pair in a gene is
Repairing mechanism of _____ depends on absorption of visible light by the enzyme.
Herman J.Mueller reported results of induced mutations on
Chromosomal mutation is
_____ is a non-ionizing radiation
Mutation involving single-base changes are
Transposons was first reported by
Nonsense mutation leads to
Alkylation is
Chemical mutagens leading to addition of nucleotides to the DNA are
Human bladder cancer is brought about by a change in single point mutation of
Which of the following dimer formation is most common
Daughter strand repair is also called as
An intercalating dye
Most common repair system is
Virus capable of causing mutations is
Potent oxidizing agent that can alter structure of purine and pyrimidine
Reverse mutation is
Alkylation of guanine causes its removal from DNA in a reaction called
In *E.coli* , parental DNA is methylated at an adenine residue found in the sequence

Mutation in which there is an amino acid substitution is called
Nutritional mutans of neurospora are known as
UV radiation at 260 nm cross-links adjacent thiamine that produces
When a part of chrosome is moved to another chromosome, it is called as
Mismatch repair cannot take place if there is a mutation in
Common chemical events that produce spontaneous mutation
Bacterium used in Ames test
Naturally occurring mutations are
Recombinational repair is often due to
The most common error prone repair mechanism is
Site that mutates at a rate significantly greater thag statistical probability is referred to as
Transposition is
Radiation that causes cross chromosomal mutations in humans
Frameshift mutation is caused by
Change of purine to pyrimidine base in an mutation
Detection of silent mutations require
Most frequently employed technique in the study of mutations
Natural phenomena of changes in chemical structure of nitrogenous bases is called
Mutation that has no detectable effect on the phenotype of a cell

DEPARTMENT OF MICROBIOLOGY,KARPAGAM AC

MICROBIAL GENETICS (17MBU203)

Opt 1
5-bromothiamine
Segments of nucleotides sequences are repeated
Induced mutation
Bacteria
Nonsense mutation
Primase
Addition of correct base
DNA,RNA and protein
Aims test
Uracil
Hydrochloric acid
Cosmid
Transposable elements
s-rac
Cancer
Eye cancer
Transverse mutation
Glutamic acid
Methyl ethane sulphonate
Skin
Adenine dimers
Frameshift
DNA helicase
Yeast
Abberation
Alpha
Induced mutations
Louise pasteur
Termination of DNA synthesis
Addition of methyl or ethyl group
Thimers
Valine to glycine
Cytidine dimer
Recombination repair
Sunset yellow
SOS
Bacteriophage Ru
Free radicals
Wild type to mutant
Deamination
5' TAGC 3'

Missense
Phototrophs
Butane ring
Point mutation
Helicase
Deamination
Salmonella
Induced
many cytidine dimer and associated large gaps in a strand
Mismatch
Hotspots
Movement of a phage
UV
Proflavin
Transition mutation
Aminoacid analysis
Analysis of phenotypes
Complementary
Point

Opt 2
5-bromoadenine
Segments of nucleotides sequences are deleted
Uninduced
Fungi
Misense mutation
Photolyase
Addition of correct nucleotide
DNA and RNA
Sima test
Adenine
Nitrous acid
Bacmid
Lipids
a-src
Phenylketonuroa
Bone cancer
General mutation
Alanine to Leucine
Guanidine
Hair
Cytosine dimers
Base pair substitution
DNA ligase
Drosophila
Change over
UV
Point mutations
Koch
Termination of protein synthesis
Deletion of ethyl and addition of methyl group
Base analogs
Isoleucine to leucine
Uracil dimer
SOS repair
Safranin
Photoreactive
Bacteriophage Mu
Water
Mutant to wild type
Depyrimidation
5' ATGC 3'

Nonsense
Auxotrophs
Cyclane ring
Induced mutation
Polymerase
Depurination
Shigella
Spontaneous
incorporation of many incorrect nucleotides by DNA polymerase
Excision
Blackspots
Movement of a virus
Visible
Nitrous acid
Transverse mutation
Peptide analysis
Analysis of genotypes
Conservative
Induced

Opt 3
5-bromoguanine
Segments of nucleotides sequences are inserted
Spontaneous
Viruses
Point mutation
Dnase
Removal of incorrect base
DNA and proteins
Ames test
Cytosine
Sulphuric acid
Transposon
Bacteria
r-cas
Melanoma
Skin cancer
Transition mutation
Valine to Glutamic acid
Ethyl sulphonate
Nail
Guanine dimers
Misense mutation
DNA gyrase
Fish
Variation
Gamma
Silent mutations
Barbara McClintock
Termination of cell wall synthesis
Deletion of methyl and addition of ethyl group
Alkylating agents
Leucine to isoleucine
Thymidine dimer
Photo repair
India ink
Mismatch
Bacteriophage Nu
Dyes
A new gene introduced
Degradation
5' CATG 3'

Silent
Heterotrophs
Butocyclane ring
Spontaneous mutation
Ligase
Dimerization
Streptococcus
Nonsense
many thymidine dimer formation and associated large gaps in a strand
SOS
Dotspots
Movement of a transposon
Ionozing
UV
General mutation
RNA analysis
Analysis of proteins
Tautomeric
Silent

Opt 4	Opt 5	Opt 6
5-bromouracil		
Segments of nucleotide sequences are inserted & deleted evenly		
Frameshift		
Algae		
Silent mutation		
Rnase		
Removal of phosphodiester bond		
DNA only		
Sema test		
Guanine		
Oxalic acid		
Plasmid		
Carbohydrates		
c-ras		
Asthma		
Lung cancer		
Transduction		
Leucine to alanine		
Dichlor		
Tongue		
Thiamine dimers		
Nonsense mutation		
DNA photolyase		
Pea plant		
Genetic change		
Beta		
Inverse mutations		
Lister		
Termination of RNA synthesis		
Deletion of methyl or ethyl group		
Interchelating agents		
Glycine to valine		
Adenosine dimer		
Excision repair		
Acridine orange		
Excision		
Bacteriophage Ly		
Acids		
A gene deleted		
Depurination		
5' GATC 3'		

Point		
Isotrophs		
Phenyl alanine		
Translocation mutation		
Methylase enzyme		
Isomerization		
Staphylococcus		
Frameshift		
DNA breaking		
Recombination		
DNA spots		
Movement of a plasmid		
X-rays		
X-rays		
Transformation		
Nucleotide analysis		
Analysis of both phenotypes and genotypes		
Telomeric		
Leaky		

Answer
5-bromouracil
Segments of nucleotides sequences are repeated
Induced mutation
Viruses
Nonsense mutation
Photolyase
Removal of phosphodiester bond
DNA only
Ames test
Adenine
Nitrous acid
Plasmid
Transposable elements
c-ras
Phenylketonuroa
Eye cancer
Transition mutation
Valine to Glutamic acid
Methyl ethane sulphonate
Skin
Thiamine dimers
Frameshift
DNA photolyase
Drosophila
Abberation
UV
Point mutations
Barbara McClintock
Termination of protein synthesis
Addition of methyl or ethyl group
Interchelating agents
Glycine to valine
Thymidine dimer
Recombination repair
Acridine orange
Excision
Bacteriophage Mu
Free radicals
Mutant to wild type
Depurination
5' GATC 3'

Missense
Auxotrophs
Cyclobutane ring
Translocation mutation
Methylase enzyme
Depurination
Salmonella
Spontaneous
incorporation of many incorrect nucleotides by DNA polymerase
SOS
Hotspots
Movement of transposon
Ionozing
Proflavin
Transverse mutation
Nucleotide analysis
Analysis of phenotypes
Tautomeric
Silent

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.

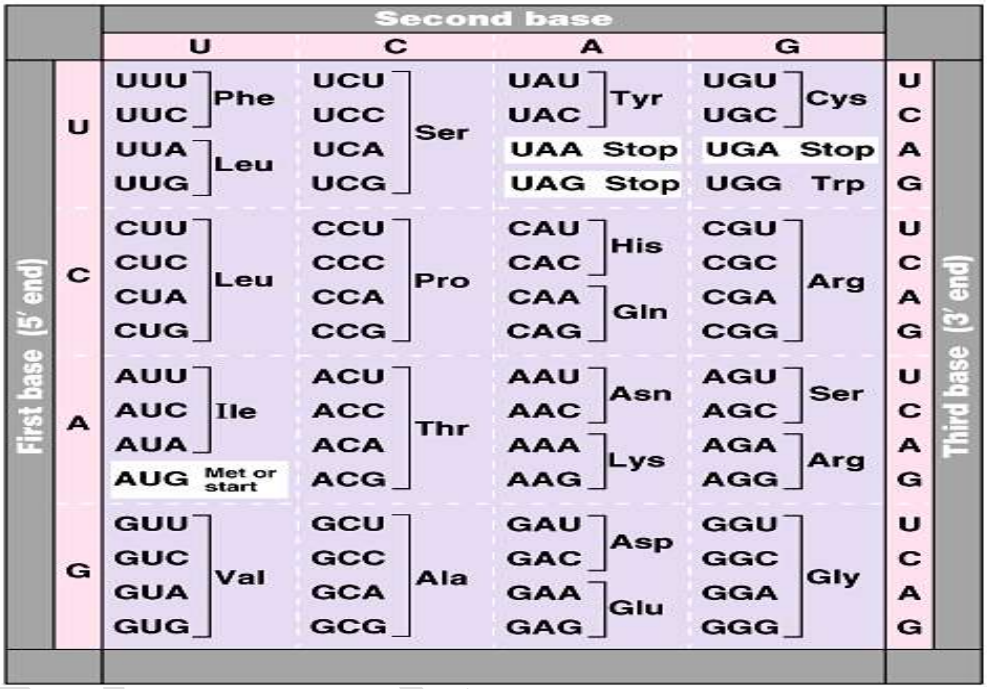
Universality: the genetic code is universal, that is, the same codon is used in all living organisms, procaryotics and eucaryotics.

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.
- **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

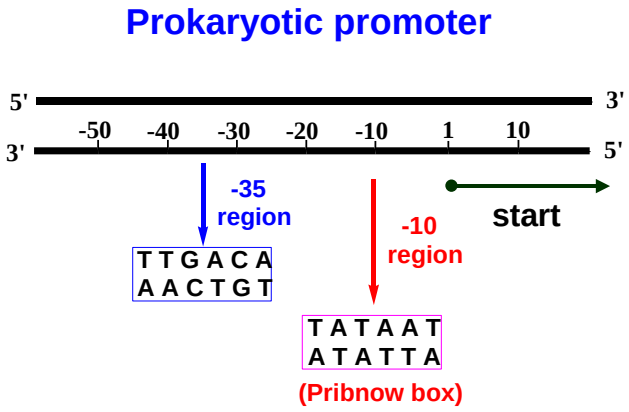
Transcription

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

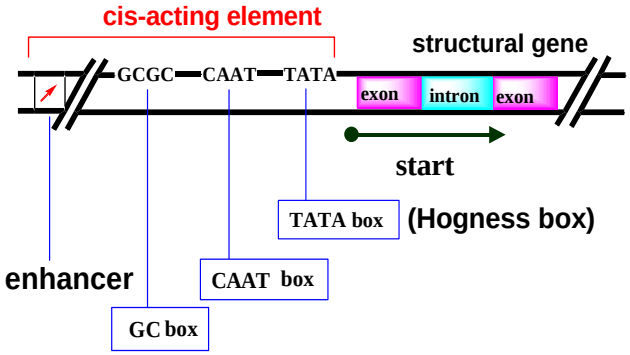
- First step in making proteins
- Process of taking one gene (DNA) and converting into a mRNA strand
- DNA -> RNA
- *Location*:Nucleus of the cell

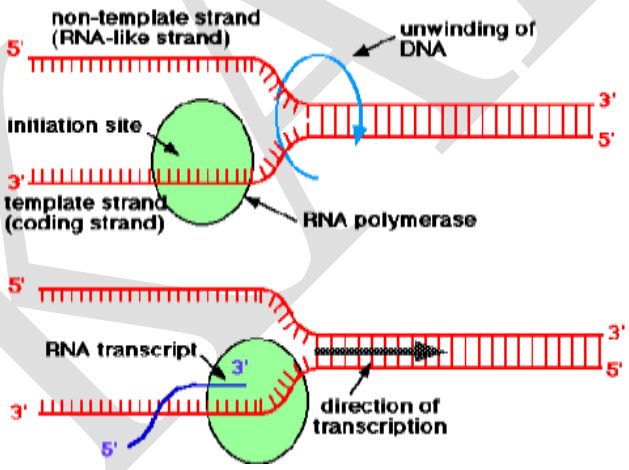
Steps to Transcription

- 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.

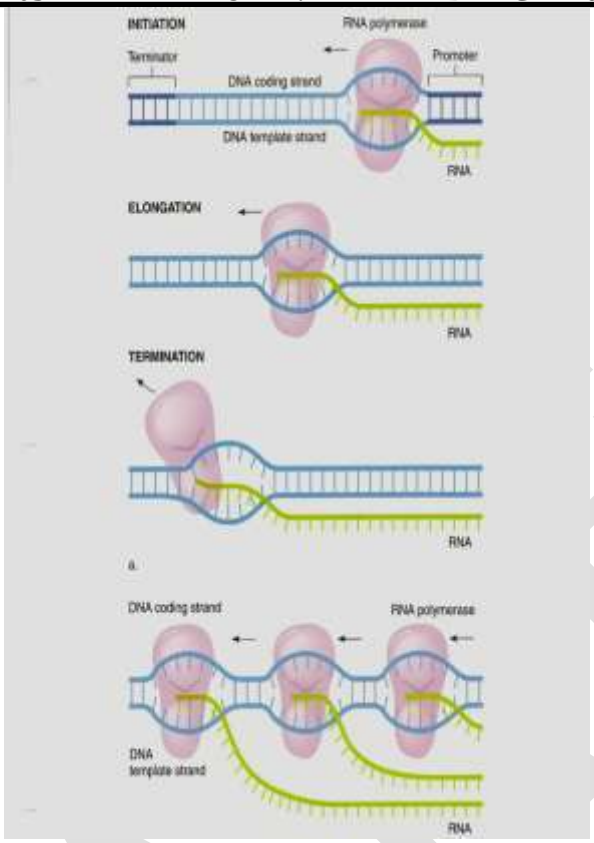


Fig: Mechanism of Transcription

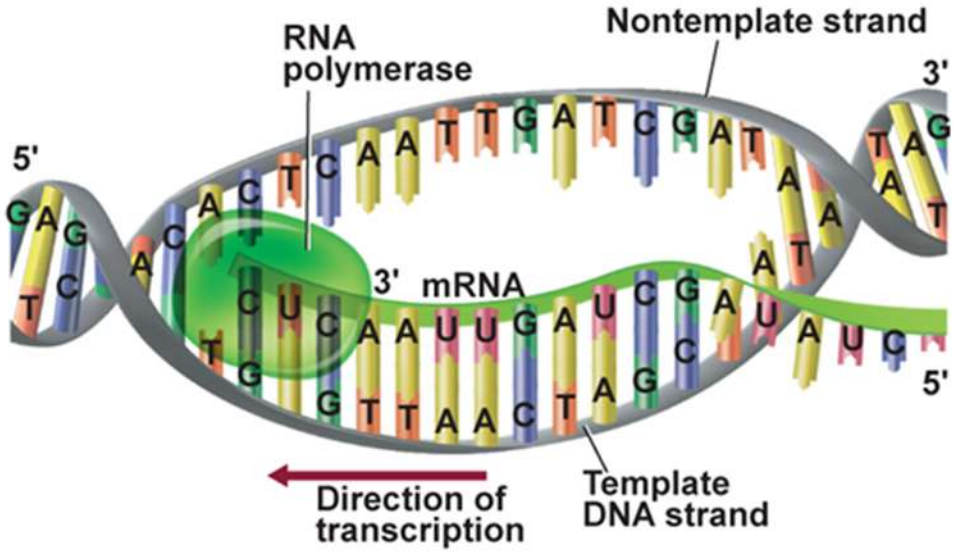


Fig: Initiation of transcription

Post transcriptional modification

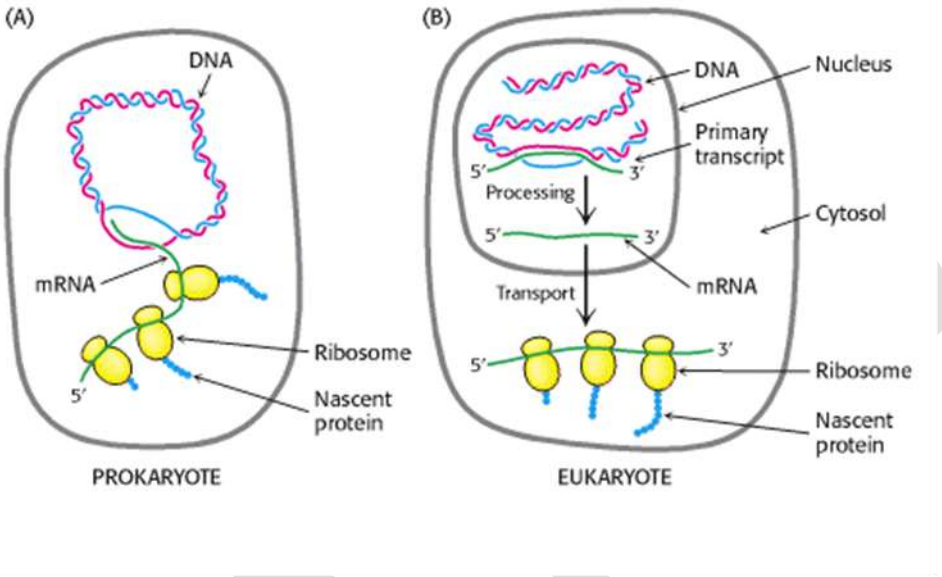


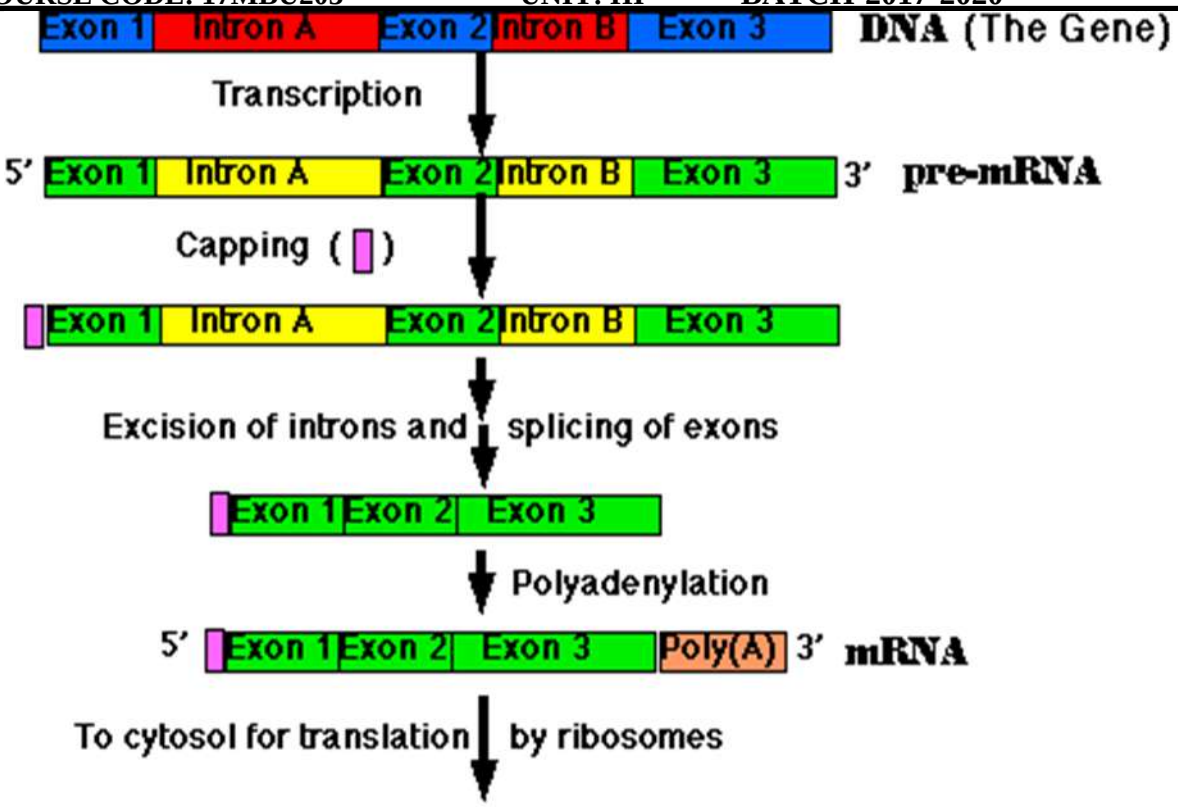
Fig: Post transcriptional modification both in prokaryotes and Eukaryotes

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).

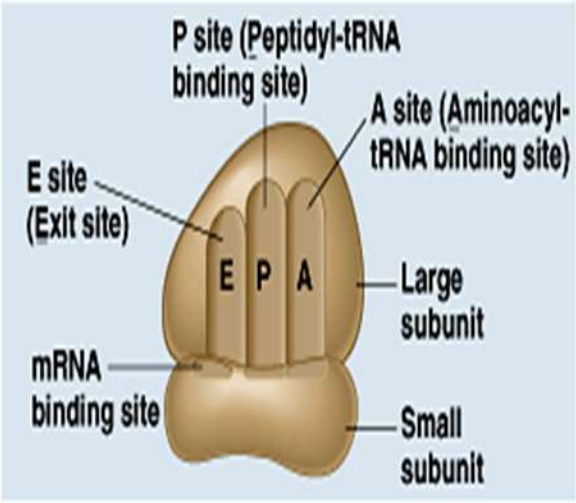
tRNA: at least one specific type of tRNA is required to transfer one amino acid. There about 50 tRNA in human for the 20 amino acids, this means some amino acids have more than one specific tRNA. The role of tRNA in protein synthesis is discussed before. (amino acid attachment and anticodon loop).

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

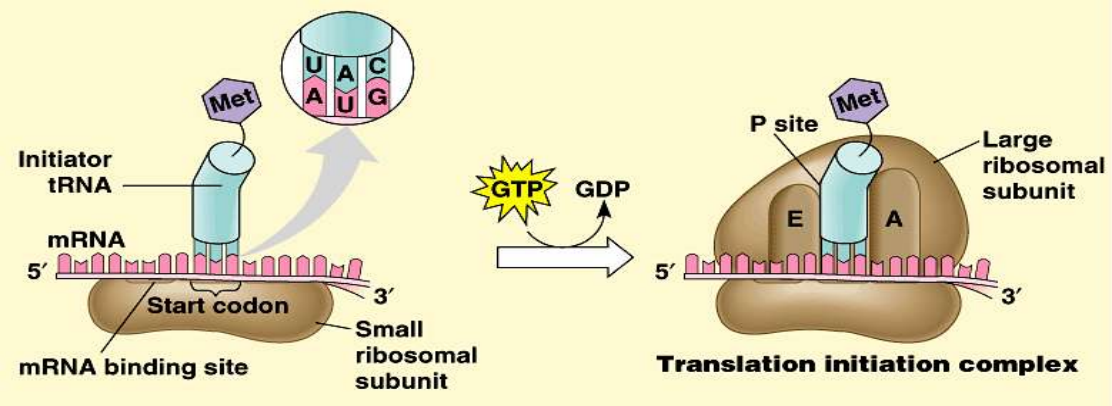


Fig: Initiation of translation

- 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.

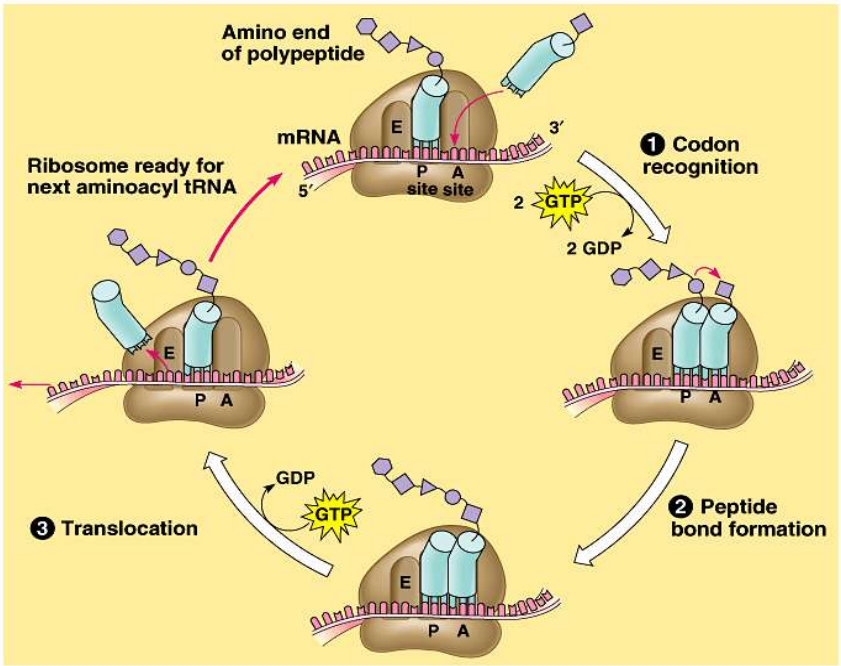


Fig: Repetitive cycle of elongation

***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)

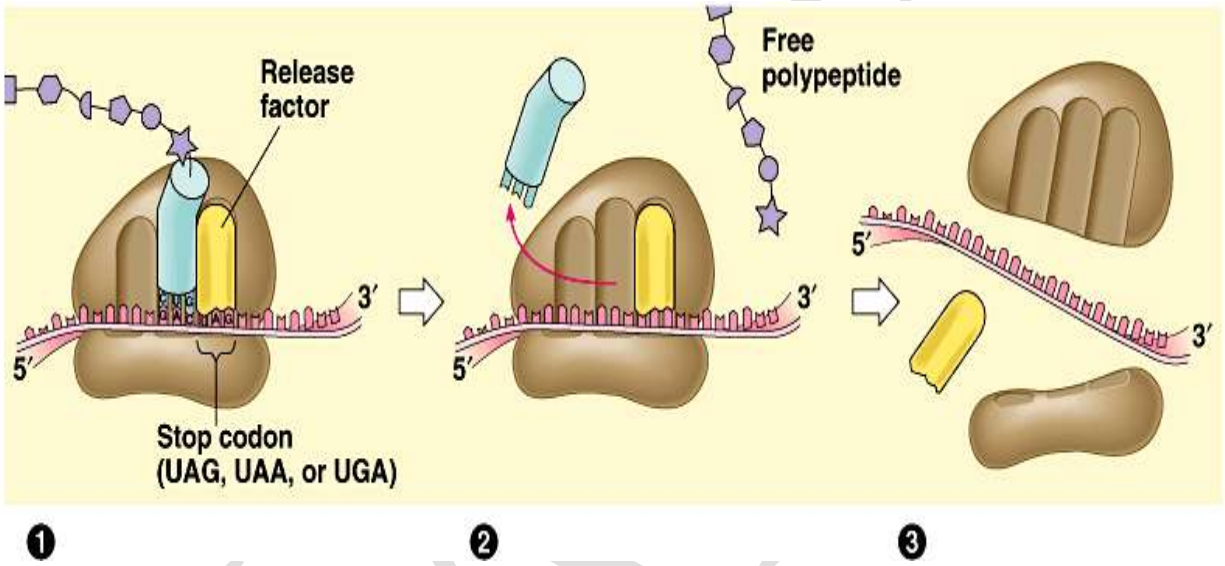


Fig: Termination of transcription

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

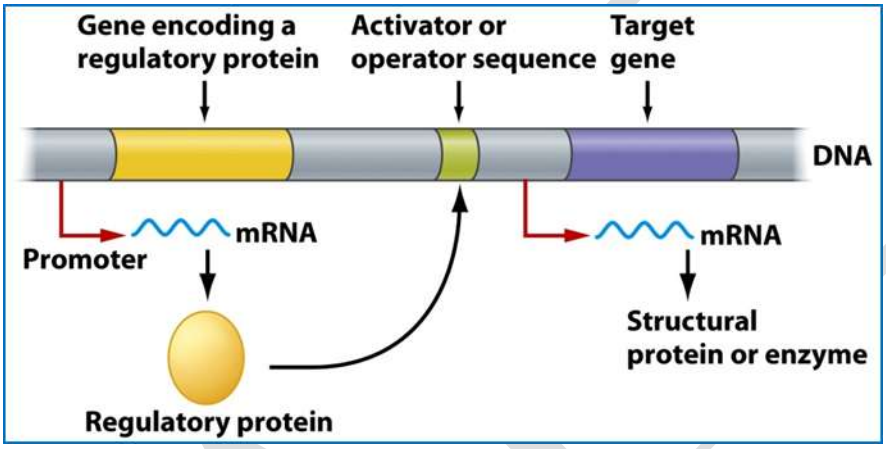


Fig: Structural and regulatory genes of an operon

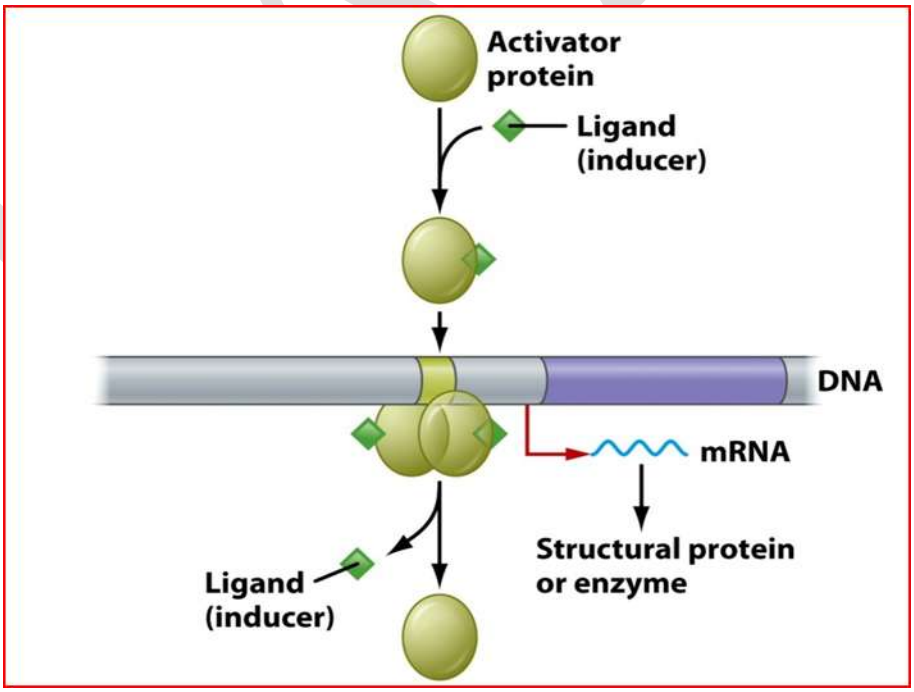


Fig: Operonic regulation

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

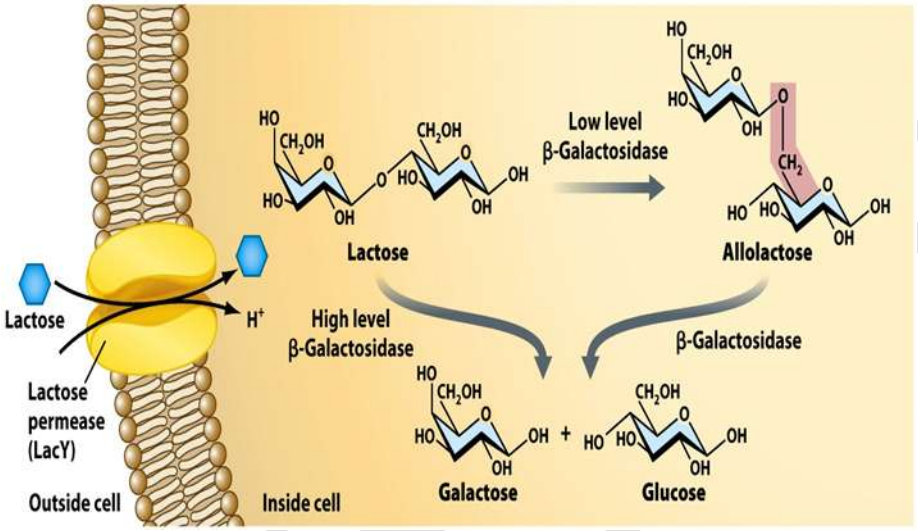


Fig: Lac operon regulation

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

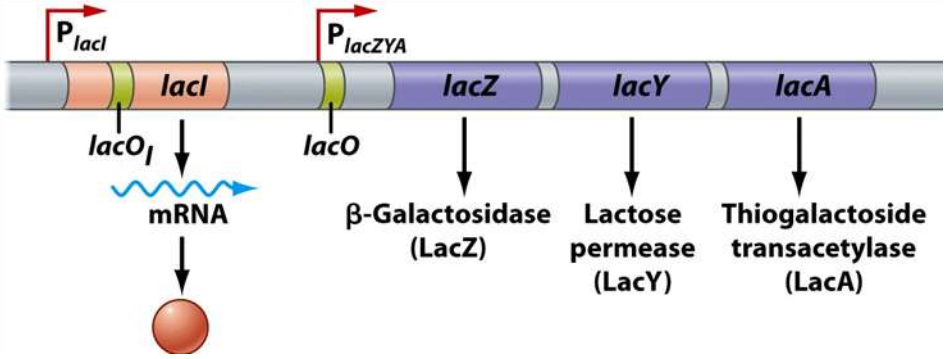


Fig: Structural and regulatory gene of Lac Operon

- 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

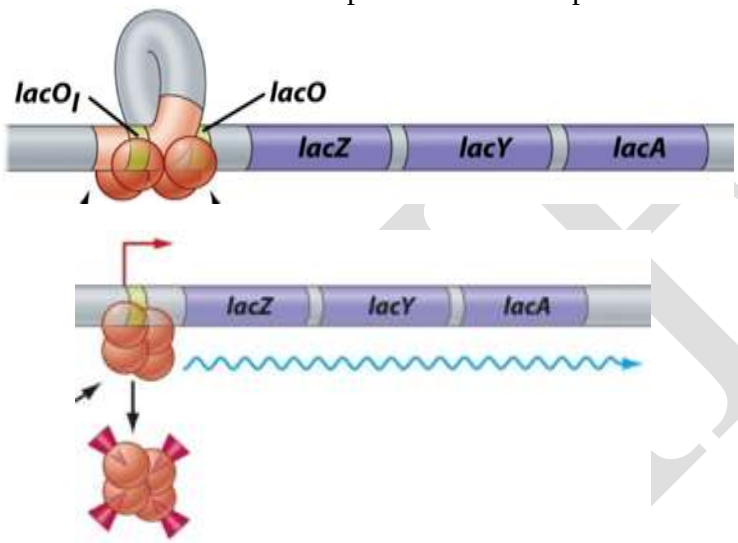


Fig: Allolactose cause operon induction

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

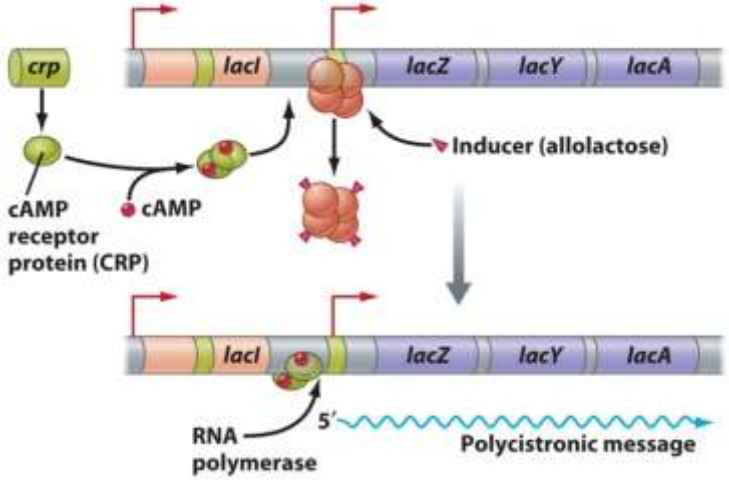


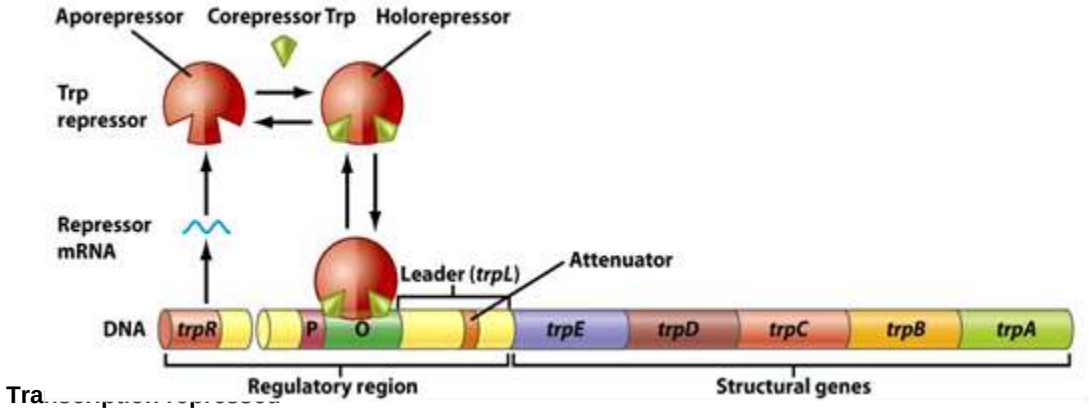
Fig: Catabolite repression

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



Tra...

***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.

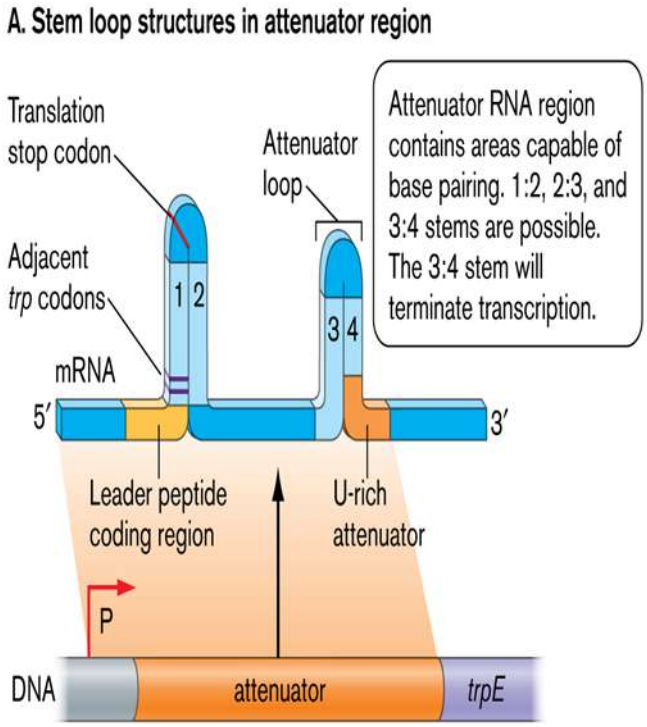
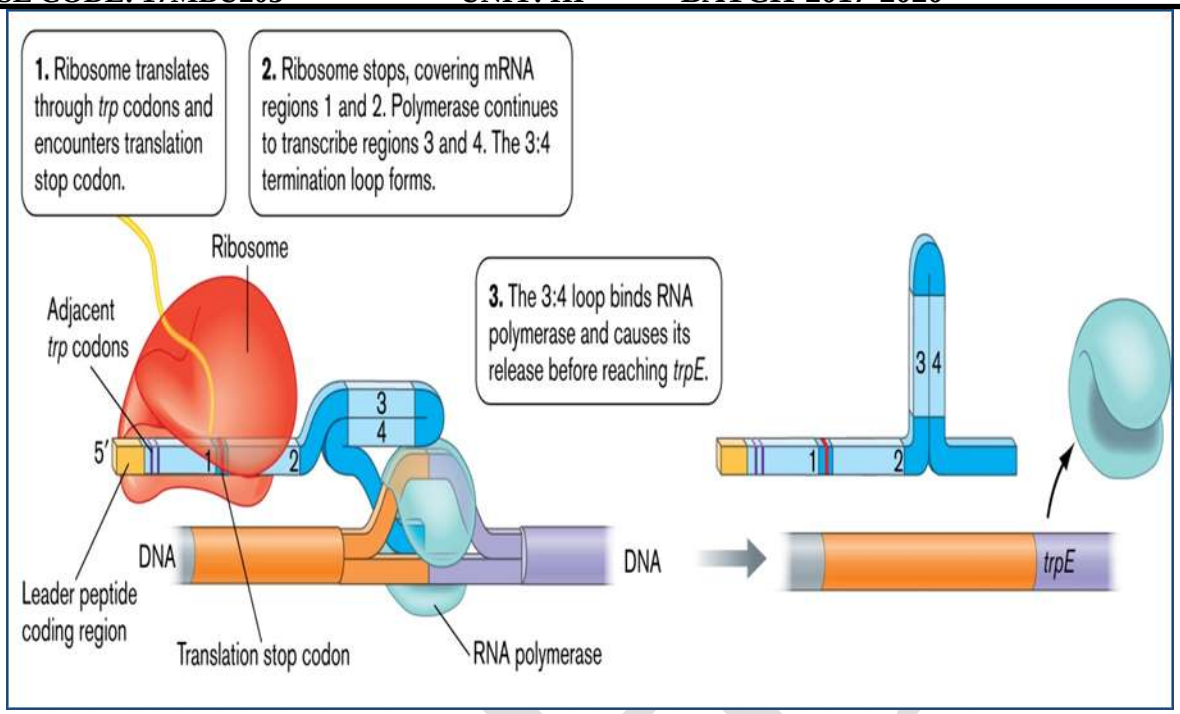
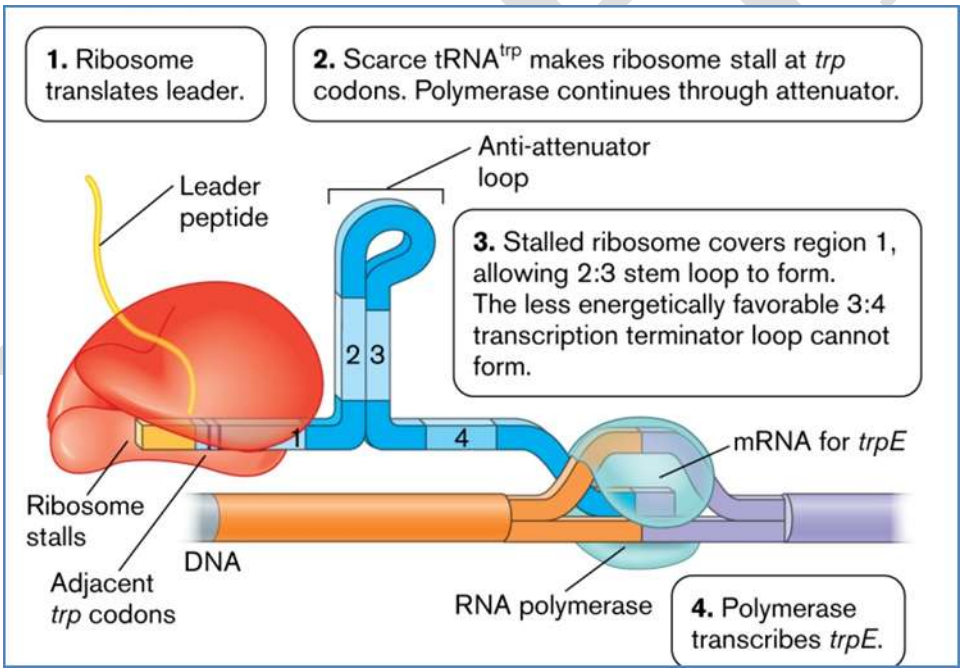


Fig: Transcriptional Attenuation Mechanism of the *trp* Operon





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

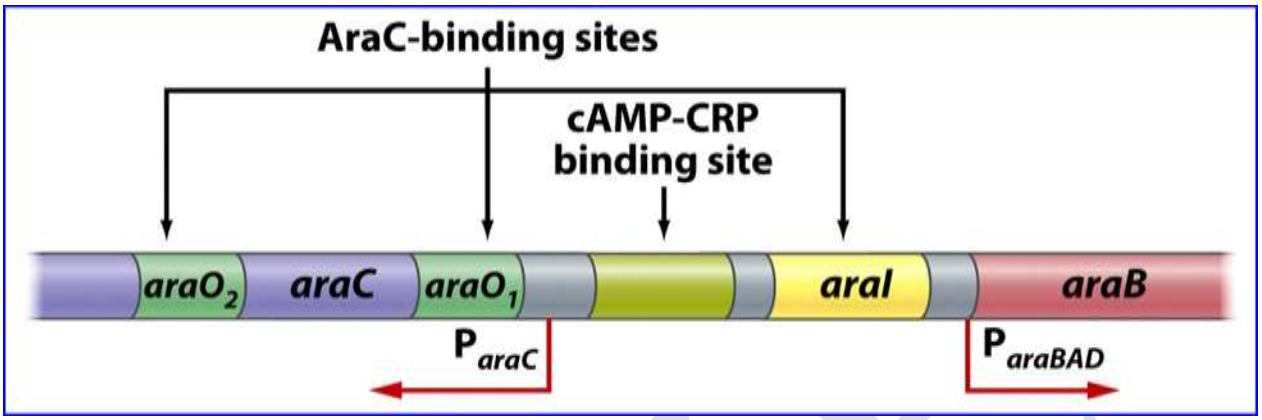
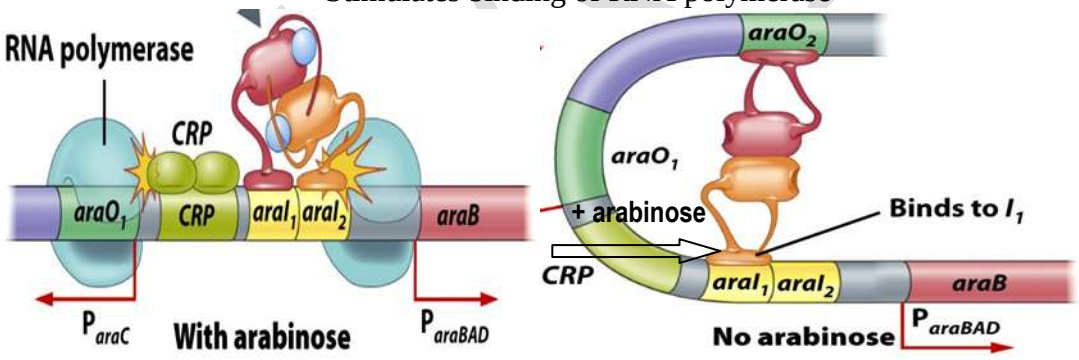


Fig:Structural and regulatory genes of Ara operon

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



Genetic Recombination in Microbes

Genetic recombination is the production of offspring with combinations of traits that differ from those found in either parent. In eukaryotes, genetic recombination during meiosis can lead to a novel set of genetic information that can be passed on from the parents to the offspring. Most recombination is naturally occurring.During meiosis in eukaryotes, genetic recombination involves the pairing of homologous chromosomes. This may be followed by information transfer between the chromosomes. The information transfer may occur without physical exchange (a section of genetic material is copied from one chromosome to another, without the donating chromosome being changed); or by the breaking and rejoining of DNA strands, which forms new molecules of DNA.

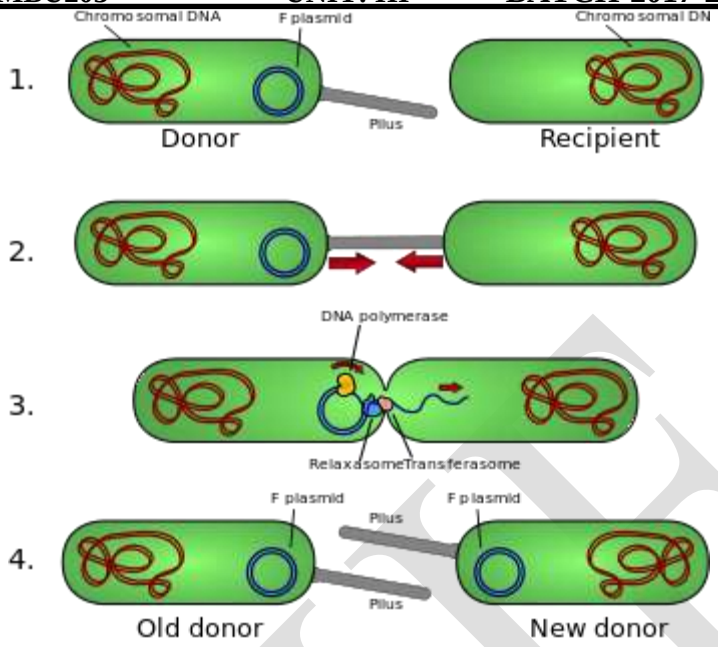
Recombination may also occur during mitosis in eukaryotes where it ordinarily involves the two sister chromosomes formed after chromosomal replication. In this case, new combinations of alleles are not produced since the sister chromosomes are usually identical. In meiosis and mitosis, recombination occurs between similar molecules of DNA (homologs). In meiosis, non-sister homologous chromosomes pair with each other so that recombination characteristically occurs between non-sister homologues. In both meiotic and mitotic cells, recombination between homologous chromosomes is a common mechanism used in DNA repair.

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

Bacterial Conjugation

Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. It is a mechanism of horizontal gene transfer as are transformation and transduction although these two other mechanisms do not involve cell-to-cell contact.Bacterial conjugation is often regarded as the bacterial equivalent of sexual reproduction or mating since it involves the exchange of genetic material. However, it is not sexual reproduction, since no exchange of gamete occurs. During conjugation the donor cell provides a conjugative or mobilizable genetic element that is most often a plasmid or transposon. Most conjugative plasmids have systems ensuring that the recipient cell does not already contain a similar element.

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



Conjugation diagram

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

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

Among other genetic information, the F-plasmid carries a tra and trb locus, which together are about 33 kb long and consist of about 40 genes. The tra locus includes the pilin gene and regulatory genes, which together form pili on the cell surface. The locus also includes the genes for the proteins that attach themselves to the surface of F− bacteria and initiate conjugation. Though there is some debate on the exact mechanism of conjugation it seems that the pili are not the structures through which DNA exchange occurs. This has been shown in experiments where the pilus are allowed to make contact, but then are denatured with SDS and yet DNA transformation still proceeds. Several proteins coded for in the tra or trb locus seem to open a channel between the bacteria and it is thought that the traD enzyme, located at the base of the pilus, initiates membrane fusion.

When conjugation is initiated by a signal the relaxase enzyme creates a nick in one of the strands of the conjugative plasmid at the oriT. Relaxase may work alone or in a complex of over a dozen proteins known collectively as a relaxosome. In the F-plasmid system the relaxase enzyme is called TraI and the relaxosome consists of TraI, TraY, TraM and the integrated host factor IHF. The nicked strand, or T-strand, is then unwound from the unbroken strand and transferred to the recipient cell in a 5'-terminus to 3'-terminus direction. The remaining strand is replicated either independent of conjugative action (vegetative replication beginning at the oriV) or in concert with conjugation (conjugative replication similar to the rolling circle replication of lambda phage). Conjugative replication may require a second nick before successful transfer can occur. A recent report claims to have inhibited conjugation with chemicals that mimic an intermediate step of this second nicking event.

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

A cell culture that contains in its population cells with non-integrated F-plasmids usually also contains a few cells that have accidentally integrated their plasmids. It is these cells that are responsible for the low-frequency chromosomal gene transfers that occur in such cultures. Some strains of bacteria with an integrated F-plasmid can be isolated and grown in pure culture. Because such strains transfer chromosomal genes very efficiently they are called Hfr (high frequency of recombination). The *E. coli* genome was originally mapped by interrupted mating experiments in which various Hfr cells in the process of conjugation were sheared from recipients after less than 100 minutes (initially using a Waring blender). The genes that were transferred were then investigated.Since integration of the F-plasmid into the *E. coli* chromosome is a rare spontaneous occurrence, and since the numerous genes promoting DNA transfer are in the plasmid genome rather than in the bacterial genome, it has been argued that conjugative bacterial gene transfer, as it occurs in the *E. coli* Hfr system, is not an evolutionary adaptation of the bacterial host, nor is it likely ancestral to eukaryotic sex.

Transformation

Transformation in bacteria was first demonstrated in 1928 by British bacteriologist Frederick Griffith.Griffith was interested in determining whether injections of heat-killed bacteria could be used to vaccinate mice against pneumonia. However, he discovered that a non-virulent strain of *Streptococcuspneumoniae* could be made virulent after being exposed to heat-killed virulent strains. Griffith hypothesized that some "transforming principle" from the heat-killed strain was responsible for making the harmless strain virulent. In 1944 this "transforming principle" was identified as being genetic by Oswald Avery, Colin MacLeod, and Maclyn McCarty. They isolated DNA from a virulent strain of *S. pneumoniae* and using just this DNA were able to make a harmless strain virulent. They called this uptake and incorporation of DNA by bacteria "transformation" (See Avery-MacLeod-McCarty experiment) The results of Avery et

al.'s experiments were at first skeptically received by the scientific community and it was not until the development of genetic markers and the discovery of other methods of genetic transfer (conjugation in 1947 and transduction in 1953) by Joshua Lederberg that Avery's experiments were accepted.

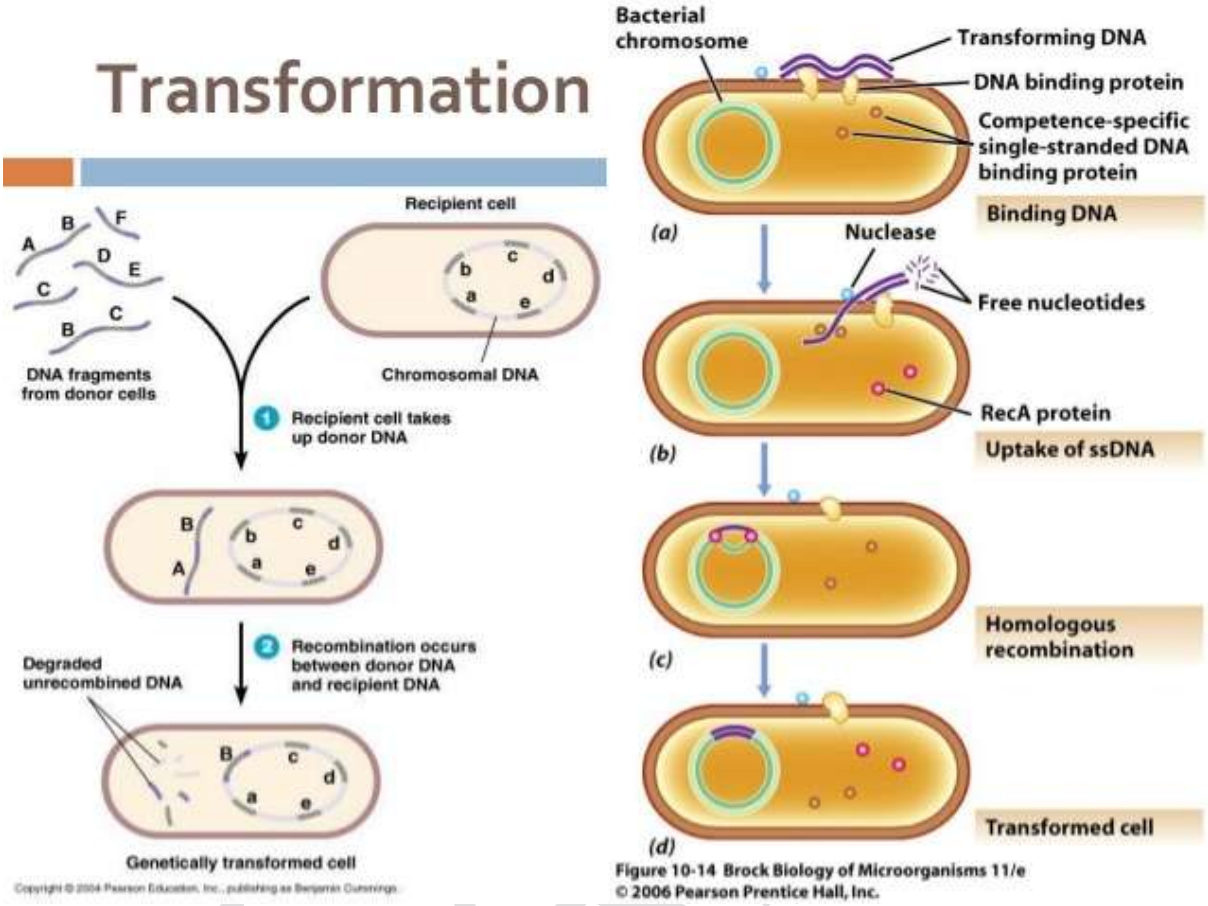
It was originally thought that Escherichia coli, a commonly used laboratory organism, was refractory to transformation. However, in 1970, Morton Mandel and Akiko Higa showed that *E. coli* may be induced to take up DNA from bacteriophage λ without the use of helper phage after treatment with calcium chloride solution. Two years later in 1972, Stanley Norman Cohen, Annie Chang and Leslie Hsu showed that $CaCl_2$ treatment is also effective for transformation of plasmid DNA. The method of transformation by Mandel and Higa was later improved upon by Douglas Hanahan.The discovery of artificially induced competence in *E. coli* created an efficient and convenient procedure for transforming bacteria which allows for simpler molecular cloning methods in biotechnology and research, and it is now a routinely used laboratory procedure.

Transformation using electroporation was developed in the late 1980s, increasing the efficiency of in-vitro transformation and increasing the number of bacterial strains that could be transformed. Transformation of animal and plant cells was also investigated with the first transgenic mouse being created by injecting a gene for a rat growth hormone into a mouse embryo in 1982. In 1907 a bacterium that caused plant tumors, *Agrobacterium tumefaciens*, was discovered and in the early 1970s the tumor-inducing agent was found to be a DNA plasmid called the Ti plasmid. By removing the genes in the plasmid that caused the tumor and adding in novel genes, researchers were able to infect plants with *A. tumefaciens* and let the bacteria insert their chosen DNA into the genomes of the plants. Not all plant cells are susceptible to infection by *A. tumefaciens*, so other methods were developed, including electroporation and micro-injection. Particle bombardment was made possible with the invention of the Biolistic Particle Delivery System (gene gun) by John Sanford in the 1980s.

Definitions

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

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



Transformation process in bacteria

Transformation has been studied in medically important Gram-negative bacteria species such as *Helicobacter pylori, Legionella pneumophila, Neisseria meningitidis, Neisseria gonorrhoeae, Haemophilus influenzae* and *Vibrio cholerae*. It has also been studied in Gram-negative species found in soil such as *Pseudomonas stutzeri, Acinetobacter baylyi,* and Gram-negative plant pathogens such as *Ralstonia solanacearum* and *Xylella fastidiosa*. Transformation among Gram-positive bacteria has been studied in medically important species such as *Streptococcus pneumoniae, Streptococcus mutans, Staphylococcus aureus* and *Streptococcussanguinis* and in Gram-positive soil bacterium *Bacillussubtilis*.It has also been reported in at least 30 species of Proteobacteria distributed in the classes alpha, beta, gamma and epsilon. The best studied Proteobacteria with respect to transformation are the medically important human pathogens *Neisseria gonorrhoeae* (class beta), *Haemophilus influenzae* (class gamma) and *Helicobacter pylori* (class epsilon).

Transduction

Transduction is the process by which foreign DNA is introduced into a cell by a virus or viral vector. An example is the viral transfer of DNA from one bacterium to another. Transduction does not require physical contact between the cell donating the DNA and the cell

receiving the DNA (which occurs in conjugation), and it is DNase resistant (transformation is susceptible to DNase). Transduction is a common tool used by molecular biologists to stably introduce a foreign gene into a host cell's genome (both bacterial and mammalian cells).

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

Transduction by bacteriophages

The packaging of bacteriophage DNA has low fidelity and small pieces of bacterial DNA, together with the bacteriophage genome, may become packaged into the bacteriophage genome. At the same time, some phage genes are left behind in the bacterial chromosome.There are generally three types of recombination events that can lead to this incorporation of bacterial DNA into the viral DNA, leading to two modes of recombination.

Generalized transduction

Generalized transduction is the process by which any bacterial gene may be transferred to another bacterium via a bacteriophage, and very rarely a small number of phages carry the donor(bacterial genome)genome,(1 phage in 10.000 ones carry the donor genome). In essence, this is the packaging of bacterial DNA into a viral envelope. This may occur in two main ways, recombination and headful packaging.

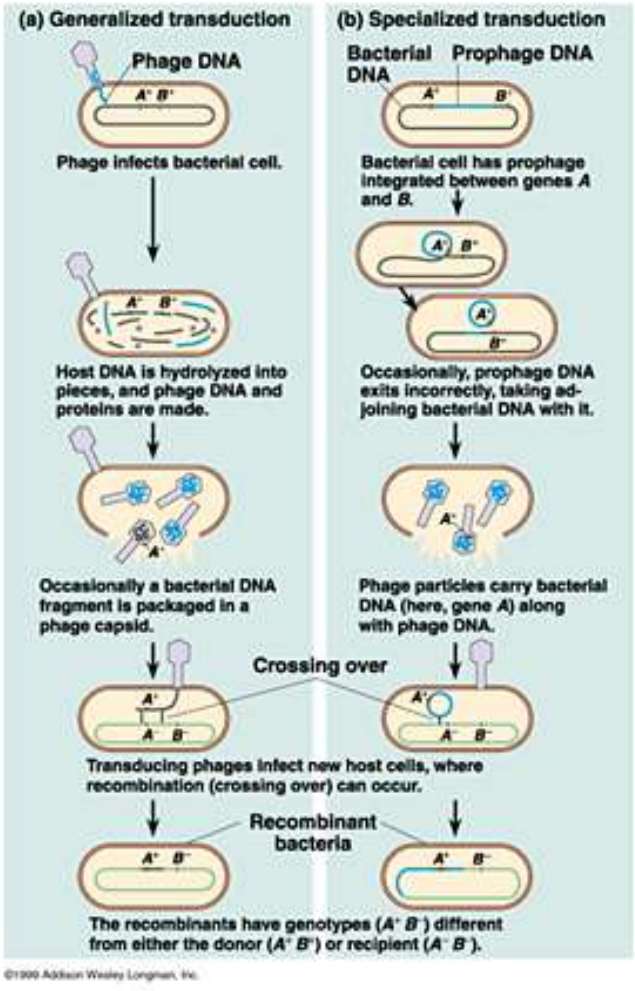
If bacteriophages undertake the lytic cycle of infection upon entering a bacterium, the virus will take control of the cell’s machinery for use in replicating its own viral DNA. If by chance bacterial chromosomal DNA is inserted into the viral capsid which is usually used to encapsulate the viral DNA,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

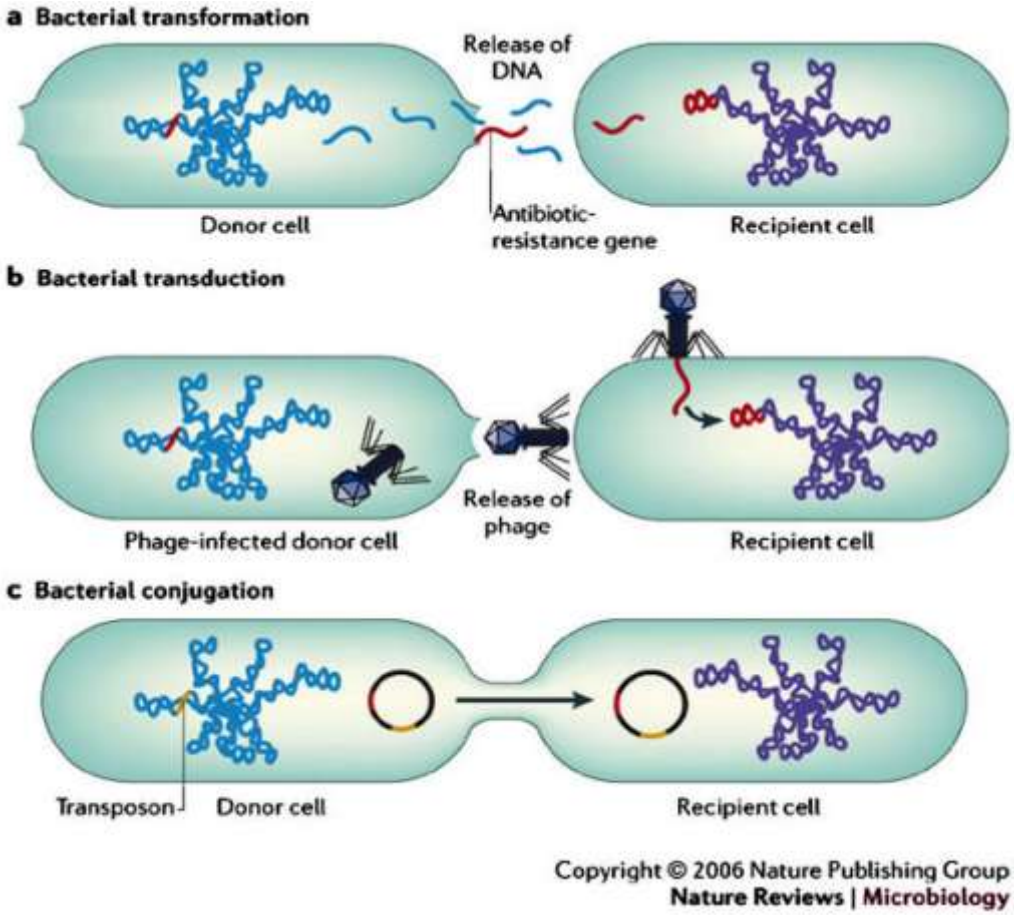
If the virus replicates using 'headful packaging', it attempts to fill the nucleocapsid with genetic material. If the viral genome results in spare capacity, viral packaging mechanisms may incorporate bacterial genetic material into the new virion. The new virus capsule now loaded with part bacterial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

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

Specialized transduction

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





Comparison between different gene transfer mechanism in bacteria

Transfection

Transfection is the process of deliberately introducing naked or purified nucleic acids into eukaryotic cells.It may also refer to other methods and cell types, although other terms are often preferred: "transformation" is typically used to describe non-viral DNA transfer in bacteria and non-animal eukaryotic cells, including plant cells. In animal cells, transfection is the preferred term as transformation is also used to refer to progression to a cancerous state (carcinogenesis) in these cells. Transduction is often used to describe virus-mediated gene transfer into eukaryotic cells.

The word transfection is a blend of trans- and infection. Genetic material (such as supercoiled plasmid DNA or siRNA constructs), or even proteins such as antibodies, may be transfected.Transfection of animal cells typically involves opening transient pores or "holes" in the cell membrane to allow the uptake of material. Transfection can be carried out using calcium phosphate (i.e. tricalcium phosphate), by electroporation, by cell squeezing or by mixing a cationic lipid with the material to produce liposomes which fuse with the cell membrane and

deposit their cargo inside.Transfection can result in unexpected morphologies and abnormalities in target cells.

Nonviral methods

Chemical-based transfection

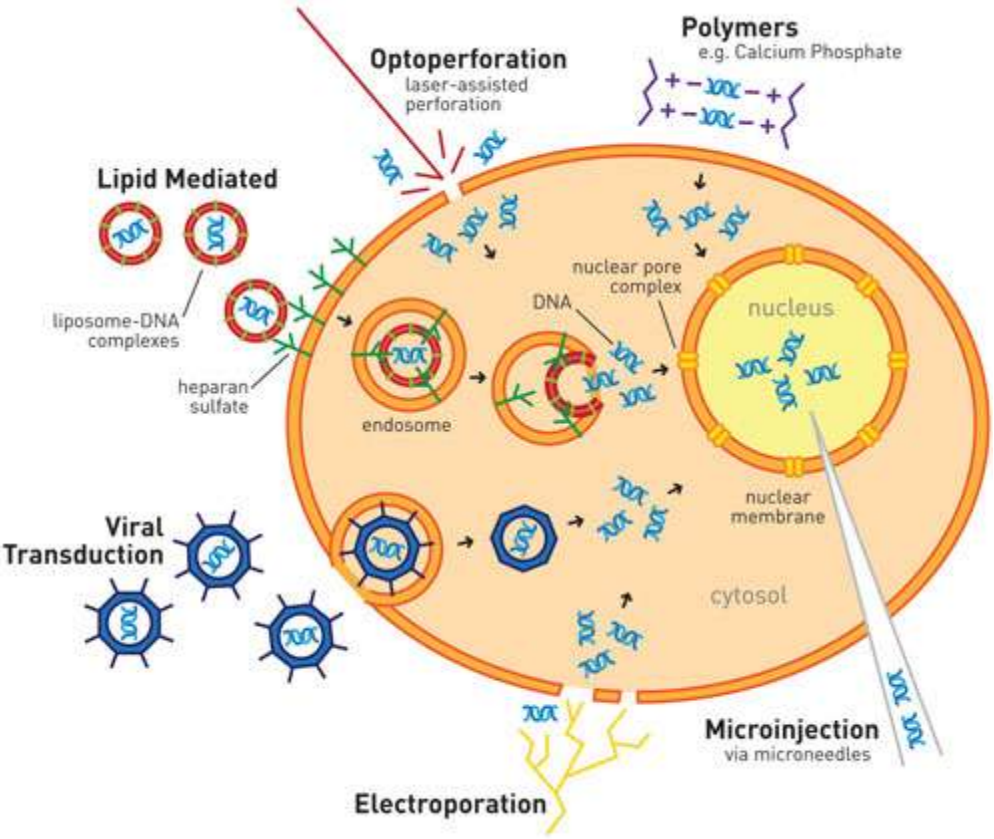
Chemical-based transfection can be divided into several kinds: cyclodextrin, polymers, liposomes, or nanoparticles.

- One of the cheapest methods uses **calcium phosphate**, originally discovered by F. L. Graham and A. J. van der Eb in 1973. HEPES-buffered saline solution (HeBS) containing phosphate ions is combined with a calcium chloride solution containing the DNA to be transfected. When the two are combined, a fine precipitate of the positively charged calcium and the negatively charged phosphate will form, binding the DNA to be transfected on its surface. The suspension of the precipitate is then added to the cells to be transfected (usually a cell culture grown in a monolayer). By a process not entirely understood, the cells take up some of the precipitate, and with it, the DNA. This process has been a preferred method of identifying many oncogenes.
- Other methods use **highly branched organic compounds**, so-called dendrimers, to bind the DNA and get it into the cell.
- Another method is the use of **cationic polymers** such as DEAE-dextran or polyethylenimine (PEI). The negatively charged DNA binds to the polycation and the complex is taken up by the cell via endocytosis.
- **Lipofection** (or liposome transfection) is a technique used to inject genetic material into a cell by means of liposomes, which are vesicles that can easily merge with the cell membrane since they are both made of a phospholipid bilayer. Lipofection generally uses a positively charged (cationic) lipid (cationic liposomes or mixtures) to form an aggregate with the negatively charged (anionic) genetic material. This transfection technology performs the same tasks as other biochemical procedures utilizing polymers, DEAE-dextran, calcium phosphate, and electroporation. The efficiency of lipofection can be improved by treating transfected cells with a mild heat shock.
- **Fugene** is a series of widely used proprietary non-liposomal transfection reagents capable of directly transfecting a wide variety of cells with high efficiency and low toxicity.

Non Chemical Methods

- **Electroporation** (gene electrotransfer) is a popular method, where transient increase in the permeability of cell membrane is achieved when the cells are exposed to short pulses of an intense electric field.
- **Cell squeezing** is a method invented in 2012 by Armon Sharei, Robert Langer and Klavs Jensen at MIT. It enables delivery of molecules into cells via cell membrane deformation. It is a high throughput vector-free microfluidic platform for intracellular delivery. It reduces the possibility of toxicity or off-target effects as it does not rely on exogenous materials or electrical fields.[20]

- **Sonoporation** uses high-intensity ultrasound to induce pore formation in cell membranes. This pore formation is attributed mainly to the cavitation of gas bubbles interacting with nearby cell membranes since it is enhanced by the addition of ultrasound contrast agent, a source of cavitation nuclei.



Different methods of transfection

- **Optical transfection** is a method where a tiny (~1 μm diameter) hole is transiently generated in the plasma membrane of a cell using a highly focused laser. This technique was first described in 1984 by Tsukakoshi et al., who used a frequency tripled Nd:YAG to generate stable and transient transfection of normal rat kidney cells.[21] In this technique, one cell at a time is treated, making it particularly useful for single cell analysis.
- **Protoplast fusion** is a technique in which transformed bacterial cells are treated with lysozyme in order to remove the cell wall. Following this, fusogenic agents (e.g., Sendai virus, PEG, electroporation) are used in order to fuse the protoplast carrying the gene of interest with the target recipient cell. A major disadvantage of this method is that bacterial components are non-specifically introduced into the target cell as well.

- **Impalefection** is a method of introducing DNA bound to a surface of a nanofiber that is inserted into a cell. This approach can also be implemented with arrays of nanofibers that are introduced into large numbers of cells and intact tissue.
- **Hydrodynamic delivery** is a method used in mice and rats, but to a lesser extent in larger animals, in which DNA most often in plasmids (including transposons) can be delivered to the liver using hydrodynamic injection that involves infusion of a relatively large volume in the blood in less than 10 seconds; nearly all of the DNA is expressed in the liver by this procedure.

Viral methods

DNA can also be introduced into cells using viruses as a carrier. In such cases, the technique is called viral transduction, and the cells are said to be transduced. Adenoviral vectors can be useful for viral transfection methods because they can transfer genes into a wide variety of human cells and have high transfer rates. Lentiviral vectors are also helpful due to their ability to transduce cells not currently undergoing mitosis.

Gene and Chromosome walking

Primer walking is a sequencing method of choice for sequencing DNA fragments between 1.3 and 7 kilobases. Such fragments are too long to be sequenced in a single sequence read using the chain termination method. This method works by dividing the long sequence into several consecutive short ones. The DNA of interest may be a plasmid insert, a PCR product or a fragment representing a gap when sequencing a genome. The term "primer walking" is used where the main aim is to sequence the genome. The term "chromosome walking" is used instead when the sequence is known but there is no clone of a gene. For example, the gene for a disease may be located near a specific marker such as an RFLP on the sequence.

The fragment is first sequenced as if it were a shorter fragment. Sequencing is performed from each end using either universal primers or specifically designed ones. This should identify the first 1000 or so bases. In order to completely sequence the region of interest, design and synthesis of new primers (complementary to the final 20 bases of the known sequence) is necessary to obtain contiguous sequence information.

Process

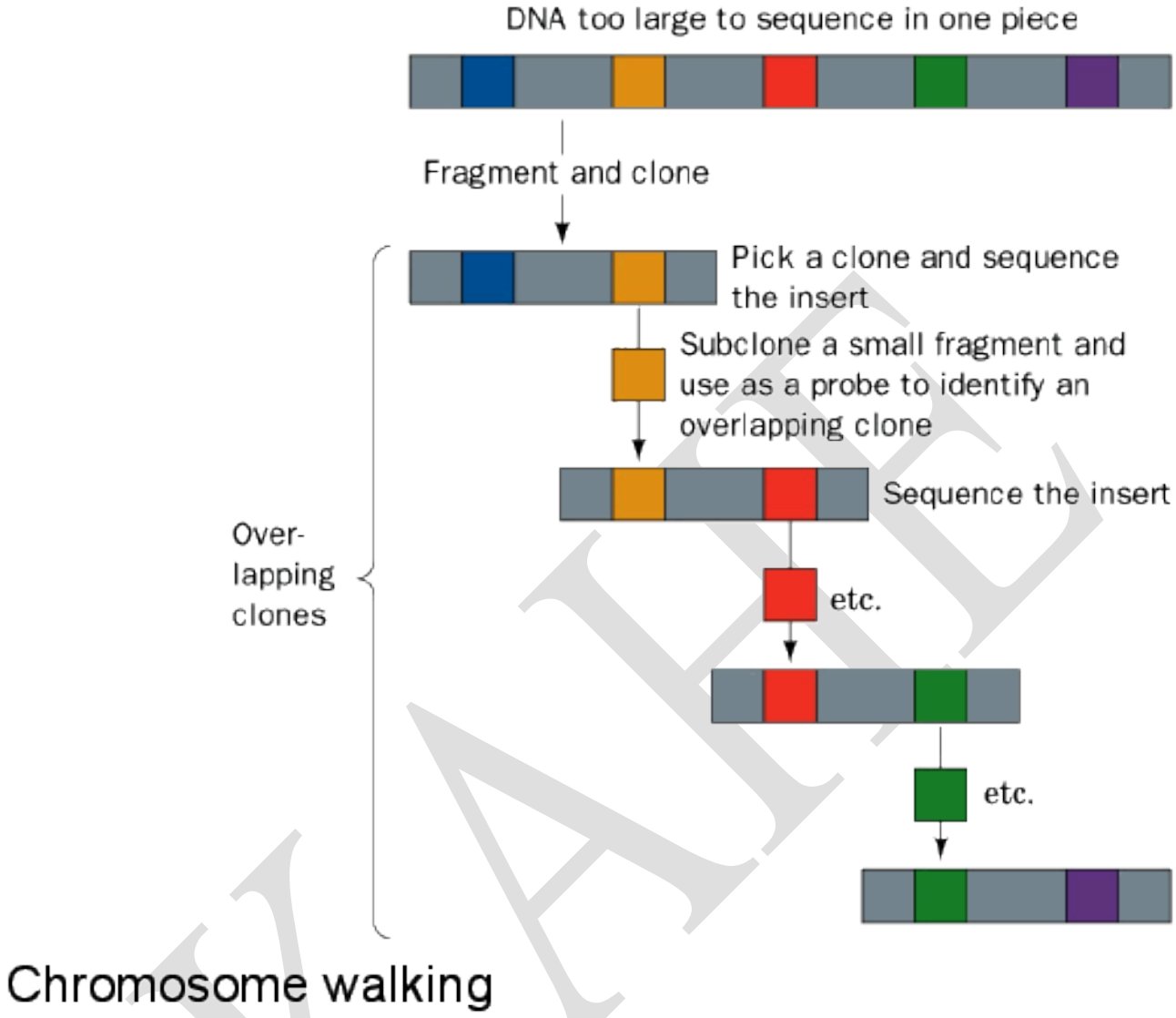
The overall process is as follows:

A primer that matches the beginning of the DNA to sequence is used to synthesize a short DNA strand adjacent to the unknown sequence, starting with the primer (see PCR). The new short DNA strand is sequenced using the chain termination method.The end of the sequenced strand is used as a primer for the next part of the long DNA sequence, hence the term "walking".The method can be used to sequence entire chromosomes (hence "chromosome walking"). Primer walking was also the basis for the development of shotgun sequencing, which uses random primers instead of specifically chosen ones.

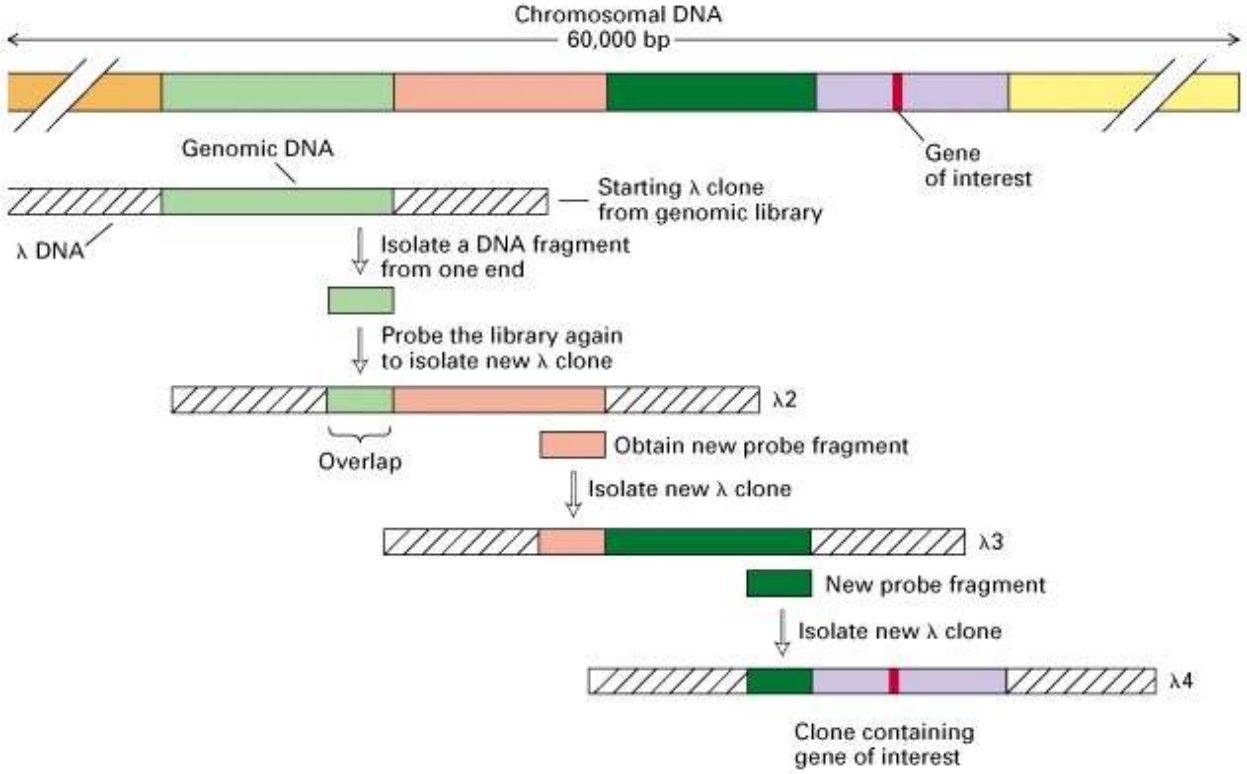
Chromosome jumping is a tool of molecular biology that is used in the physical mapping of genomes. It is related to several other tools used for the same purpose, including chromosome walking.Chromosome jumping is used to bypass regions difficult to clone, such as those containing repetitive DNA, that cannot be easily mapped by chromosome walking, and is useful in moving along a chromosome rapidly in search of a particular gene.

Chromosome jumping allows more rapid movement through the genome compared to other techniques, such as chromosome walking, and can be used to generate genomic markers with known chromosomal locations.

Chromosome jumping enables two ends of a DNA sequence to be cloned without the middle section. Genomic DNA may be partially digested using restriction endonucleases and with the aid of DNA ligase, the fragments are circularized. From a known sequence, a primer is designed to sequence across the circularized junction. This primer is used to jump 100 kb-300 kb intervals: a sequence 100 kb away would have come near the known sequence on circularization. Thus, sequences not reachable by chromosome walking can be sequenced. Chromosome walking can be used from the new jump position (in either direction) to look for gene-like sequences, or additional jumps can be used to progress further along the chromosome.



Simplified Schematics of chromosome walking



Process of Chromosome walking

Possible Questions

Two marks

1. Define genetic code.
2. What is meant by wobble hypothesis?
3. Write about the start and stop codons.
4. What are the three enzymes of lac operon?
5. Write about Shine Dalgarno sequence.

Eight marks

6. Give a detailed account on Genetic Code.
7. Draw the Genetic code table with corresponding amino acids.
8. Write in detail about transcription process?
9. Describe about post-transcriptional modifications?
10. Explain the translation process involved in protein synthesis?
11. Give a detailed account on Translation.
12. Write a note on post-translational modifications.
13. Discuss the salient features of lac operon.
14. Discuss in detail the tryptophan operon regulation in *E. coli*.
15. Explain about the Ara operon with neat sketch.
16. Explain the process of chromosome walking.
17. Discuss in detail about conjugation, transduction, transformation.
18. Write in detail about the methods involved in the tranfections.
19. How is transduction classified? Explain.
20. How is competent cell prepared? Explain.

DEPARTMENT OF MICROBIOLOGY,KARPAGAM ACADEMY OF HIGHER EDUCATION
MICROBIAL GENETICS (17MBU203)

Unit III Question
Addition of poly A tail to 3' end of mRNA is mediated by the enzyme
Confirmational changes in protein is brought about by
In Rho-independent transcription termination, the termination sequence is usually
Model example for gene regulation by repression
Operon model that demonstrates both positive and negative control of gene regulation
Repressor molecule in lac operon is a
Stop codon UAA is also called
The first codon during translation is
Transcription initiation site starts from
What are the possible number of codons that can be generated using possible nucleotide combinations
Which transports lactose across the cell membrane
Who deciphered the genetic code
The stop codons are called as
The ability of the cell to choose between glucose and other sugars is termed as
Sequence of codons in mRNA between a start and a stop sequence is called as
Polyadenylation is
Non codon specifies more than ___ amino acid
Molecular weight of egg lysozyme is
lac operon is an example for
In RNA, thiamine is replaced by
Genes are located in specialized structures called
Enzyme activity is regulated by changes in the confirmation of enzymes except
Codon/Anticodon consists of _____ nucleotides
Amino acid that have largest number of codons
7-methylguanosine cap is an important site
Capping in mRNA is addition of the group
_____ is the first amino acid during translation of proteins
Control of gene expression was proposed by
In prokaryotes, AUG is translated in to
In trp operon, the genes *trp* E & *trp* D codes for
Enzyme that lactose in to glucose and galactose
The number of nitrogenous bases codes by 9 amino acids would be
The termination of transcription is signaled by rich
tRNA's are matched with their aminoacids by a group of enzymes collectively called as
Translation is
Stop codons in mammalian mitochodria are
Ribosomal site most frequently occupied by the tRNA carrying the growing peptide chain
Non-coding regions are called as
Mammalian mitochondrion not only uses AUG as initiation codon but also
Region that comprise the core prokaryotic promoter

All aminoacids have more than one codon except
Site to which substrate molecules are attached
The main function of nonsense codons is to
Which is astop codon
The promoter sequence in eukaryotes is
Structure of proteins may be classified into ___ types
rRNA is also called
7-methylguanosine cap is an important site
2006 Nobel Prize in Physiology & Medicine for studies on molecular basis of eukaryotic transcription
Transcription is
tRNA is responsible for the transfering
Monad & Cohen-Bazire first reported the evidence for the repression of the enzyme
A-site is the ribosomal site most frequently occupied by the
Action of repressor protein in *Lac* operon is called as
Other than methionine _____ is the amino acid that appear rarely in proteins
In post translational modification of RNAs, trimming is
Common method of covalent modification of enzyme in regulation of gene expression is
Short sequence of aminoacids are called
In the absence of effector molecule, the enzyme is said to be in
Allosteric enzymes that are controlled by a molecule other than it's substrate
The first and best example of control of gene expression was proposed by

Opt 1	Opt 2
RNA polymerase	DNA polymerase
Systematic molecules	Cohesive molecules
Palindromic sequence	Paliomic sequence
trp operon	*lac* operon
lac operon	*ara* operon
Dimer	Trimer
Amber	Opal
AGU	AUG
-1	Plus 1
46	64
Galactosidase permease	β-galactosidase
Hershey & Chase	Avery & McLeod
Missense	Nonsense codons
Catabolic repression	Catabolic expression
Close reading frame	Open reading frame
Addition of adenosines to 3' end of mRNA	Addition of adenosines to 5' end of mRNA
1	2
19300 daltons	13900 daltons
Repressible operon	Inducible operon
Uracil	Adenine
Histone	RNA
Polymerase	Ribozymes
4	6
Proline	Cysteine
For eukaryotic transcription initiation factor	For prokaryotic translation initiation factor
7-ethylguanosine	7-methylguanosine
Threonine	Leucine
Beedle & Tatum	Avery & McLeod
Methionine	N-acetyl-methionine
Arginase	Tryptophan synthase
Lactosidase	Glucanse
27	36
AT containing inverted repeat	AC containing inverted repeat
Aminoacyl DNA synthatases	Aminoacyl synthatases
rRNA to protein	tRNA to protein
GAA & GAG	AGA & AGG
A-site	P-site
Exons	Introns
AUA, AUU, AUC	UAA, UAU, UAC
Klenow box	Pribnow box

Methionine & Tryptophan	Valine & Leucine
Catalytic site	Effector site
Initiate protein synthesis	Elongate protein synthesis
UAA	AAU
TATAAA	TAATAA
2	3
Rnase	Ribase
For eukaryotic transcription initiation factor	For prokaryotic translation initiation factor
Arthur Nirenberg	Roger D. Kornberg
DNA to rRNA	DNA to tRNA
Protein	Aminoacid
Tryptophan synthase	Gluconase synthetase
Aminoacyl-rRNA	Aminoacyl-mRNA
Positive control	Negative control
Arginine	Tryptophan
Removal of excess nucleotides	Removal of excess proteins
to methylate the enzyme at a proline residue	to phosphorylate the enzyme at a proline residue
Peptides	Proteins
Relaxed state	Tense state
Cohesive molecules	Systematic molecules
Khorana & Nirenberg	Hershey & Chase

Opt 3	Opt 4	Opt 5
Rnase	poly A polymerase	
Affector molecules	Effector molecules	
Panoramic sequence	Pandemic sequence	
ara operon	*gal* operon	
gal operon	*trp* operon	
Tetramer	Pentamer	
Acre	Ochre	
GUA	UGA	
-10	Plus 10	
20	30	
Glucanse	Glucose permease	
Beedle & Tatum	Nirenberg & Khorana	
Central codons	Last codons	
Metabolic repression	Metabolic expression	
Central reading frame	Last reading frame	
Deletion of adenosines to 3' end of mRNA	Deletion of adenosines to 5' end of mRNA	
3	4	
31900 daltons	91300 daltons	
Mutated operon	Neutral operon	
Cytosine	Guanine	
Chrosomes	Genomes	
Chimozymes	Nuclease	
3	9	
Serine	Valine	
For eukaryotic translation initiation factor	For prokaryotic translation initiation factor	
7-methylcytosine	7-ethylcytosine	
Methionine	Valine	
Jacob & Monad	Hershey & Chase	
N-formamyl-aspargine	N-formamyl-methionine	
Anthranilate isomerase	Anthranilate synthase	
α-galactosidase	β-galactosidase	
18	9	
GC containing inverted repeat	CT containing inverted repeat	
Amino synthatases	Aminoacyl tRNA synthatases	
DNA to protein	mRNA to protein	
CGA & AGC	CGG & GCG	
E-site	G-site	
Cistrons	Positrons	
AAU, UAU, CAU	GUA, GUU, GUC	
TAGTAG box	Polypeptide box	

Threonine & Alanine	Lysine & Arginine	
Alleosteric site	Binding site	
Terminate protein synthesis	Regulate protein synthesis	
AUA	AAA	
TTGACA	GTTAAA	
4	5	
Ribulase	Ribozyme	
For eukaryotic translation initiation factor	For prokaryotic translation initiation factor	
David Osborne	Michael Whitney	
DNA to mRNA	DNA to protein	
Codon	Anticodon	
Arabinase trimutase	Tryptophanase	
Iminoacyl-tRNA	Aminoacyl-tRNA	
Neutral control	No control	
Glutamic acid	Threonine	
Removal of excess lipids	Removal of excess carbohydrates	
to phosphorylate the enzyme at a serine residue	to methylate the enzyme at a serine residue	
Polypeptides	Palindromes	
Free state	Degrading state	
Effector molecules	Affector molecules	
Avery & McLeod	Jacob and Monad	

Opt 6	Answer
	poly A polymerase
	Effector molecules
	Palindromic sequence
	trp operon
	ara operon
	Tetramer
	Ochre
	AUG
	Plus 1
	64
	Galactosidase permease
	Nirenberg & Khorana
	Nonsense codons
	Catabolic repression
	Open reading frame
	Addition of adenosines to 3' end of mRNA
	1
	13900 daltons
	Inducible operon
	Uracil
	Chrosomes
	Ribozymes
	3
	Serine
	For eukaryotic translation initiation factor
	7-methylguanosine
	Methionine
	Jacob & Monad
	N-formamyl-methionine
	Anthranilate synthase
	β-galactosidase
	27
	GC containing inverted repeat
	aminoacyl tRNA synthatases
	mRNA to protein
	AGA & AGG
	P-site
	Exons
	AUA, AUU, AUC
	Pribnow box

	Methionine & Tryptophan
	Catalytic site
	Terminate protein synthesis
	UAA
	TATAAA
	4
	Ribozyme
	For eukaryotic translation initiation factor
	Roger D. Kornberg
	DNA to mRNA
	Anticodon
	Tryptophan synthase
	Aminoacyl-tRNA
	Negative control
	Tryptophan
	Removal of excess nucleotides
	to phosphorylate the enzyme at a serine residue
	Peptides
	Tense state
	Effector molecules
	Jacob and Monad

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). 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.

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. This was one of the final steps in disproving the Lamarkian view that genetic change was induced in response to environmental conditions.

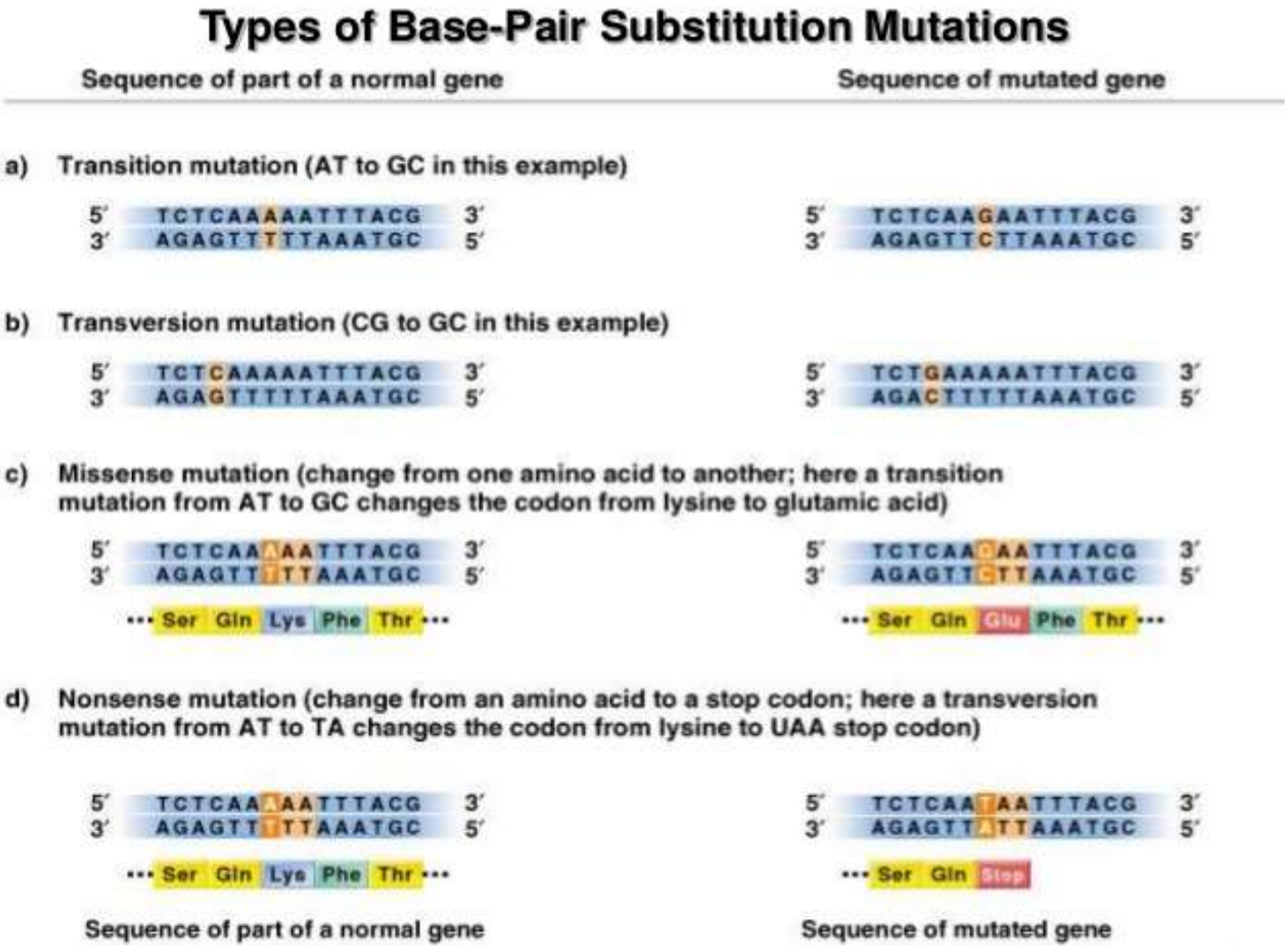
Mechanisms of mutation: This portion of the lecture deals primarily with the mechanisms responsible for point mutations and their reversion or suppression. Strictly speaking, the term reversion should be used only to describe an exact reversal of the original mutational change. Many other secondary changes, 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.

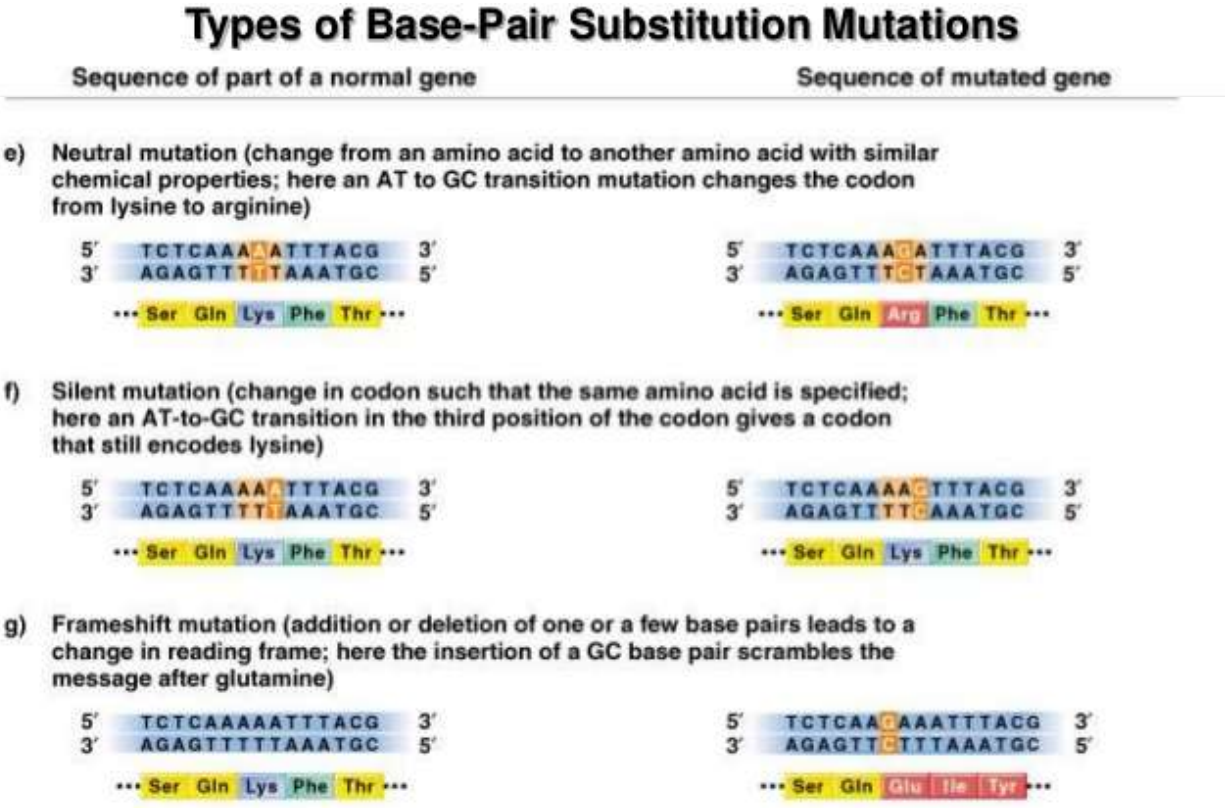
Transitions: A tautomeric shift in any of the four DNA bases can lead to mispairing of A to C or G to T. The tautomeric state can occur either in the template base or the incoming base. During the next round of DNA synthesis, the mispaired base pairs with its normal partner, resulting in a transition, 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, 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.



Types of spontaneous mutation – a comparative schema



Deamination: Our current textbook discusses deamination of cytosine primarily in terms of mutagenesis by nitric oxide, 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.

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 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). 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.

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, 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.

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.

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.

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.

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.

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.

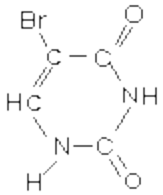
Recessive mutations usually result from partial or complete loss of a wild type function. ***Amorphic*** alleles are those that have completely lost the function. An example would be a mutation in which production of pigment is completely lost in the homozygous state, causing albinism. ***Hypomorphic*** 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.

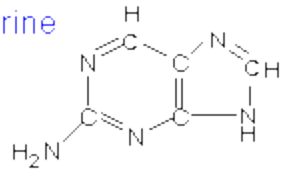
Another type of dominance is **haplo-insufficiency**. In this case, loss of a gene product causes a recognizably different phenotype in the heterozygote. This is considered to be a dominant mutation because the presence of one copy of the mutant allele in combination with one copy of the wild-type allele 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.

I. Base analogs

5-Bromouracil

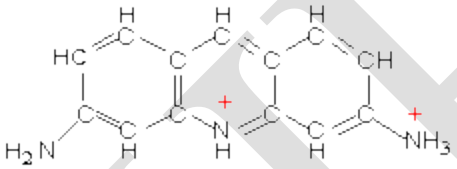


2-Aminopurine



II. Acridines

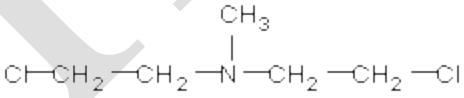
2,8,-Diamino acridine
(Proflavin)



III. Alkylating agents

Di-(2-chloroethyl)sulfide (Sulfur mustard)

Di-(2-chloroethyl)methylamine
(Nitrogen mustard)



Ethylmethane sulfonate (EMS)

IV. Deaminating agents

Nitrous acid HNO_2

V. Miscellaneous

Hydroxylamine NH_2OH

Free radicals

Chemical mutagen types

DNA repair mechanism

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

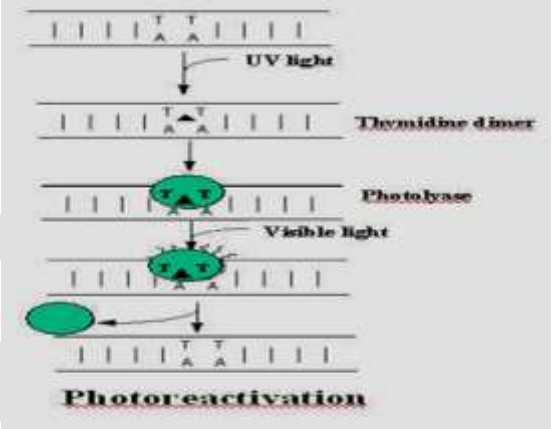
Ionizing radiation causes three types of damage to DNA:

- **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.

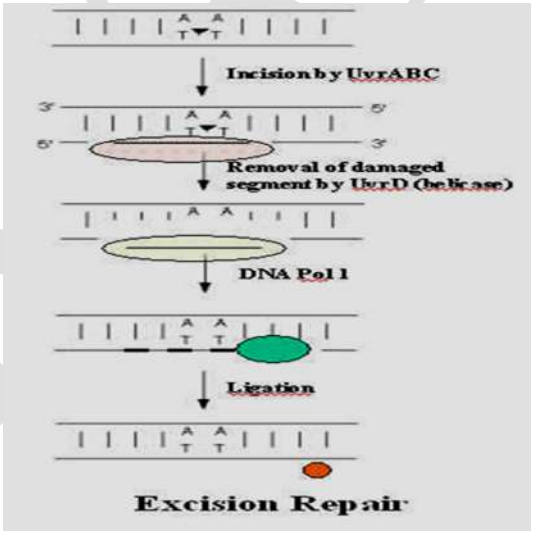
2.Even though there may be only a single "bad" base to correct, its nucleotide is removed along with many other adjacent nucleotides; that is, NER removes a large "patch" around the damage.

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]

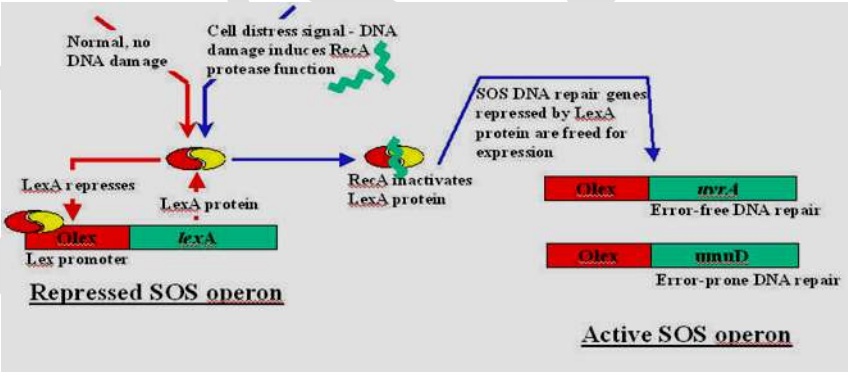
locus) methylates 5'-GATC-3' sequence in DNA at A residue. Mismatch from replication recognized by *mutL* and *mutS* gene products. *mutH* gene product nicks DNA strand (progeny strand) on either side of mismatch. DNA helicase II from *mutU* gene (also called *uvrD* gene) unwinds DNA duplex and releases 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.



SOS repair mechanism

Possible Questions

Two marks

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

Eight marks

6. Explain Delbruck experiments by highlighting its significance?
7. Briefly explain the types of mutation?
8. What are chemical mutagens? How do they cause mutation?
9. Describe in detail about the molecular basis of mutation?
10. Give a detailed account on site directed mutagenesis and its application?
11. How will you detect and select a mutant and explain the role of replica plating in mutation?
12. Explain the methodology and principle involved in replica plating technique?
13. Discuss the consequences of DNA damage in bacteria?
14. Write a note on DNA repair mechanism and add a note on RecA protein in it?
15. Write a note base anologue and acridine induced changes in DNA?
16. Discuss about proof – reading in DNA & mismatch repair?
17. Write a note recombination repair mechanism?
18. Give an account of excision repair mechanisms?
19. Write a note SOS repair?
20. Explain tautomerism.

DEPARTMENT OF MICROBIOLOGY,KARPAGAM ACADEMY OF HIGHER EDUCATION
MICROBIAL GENETICS (17MBU203)

Unit IV Question
Conjugation involves the use of _____for mapping
Genetic fine structure mapping of T4 was studied by _____
In genetic mapping, the measurement of distance between the genes is expressed as
Methods used to identify the locus of a gene and the distances between genes
T4 bacteriophages generally parasitizes
The non specific transduction is also called as - - - - - - - - -
Transfer of a portion of chromosome to a recipient with direct contact is termed
The first demonstration of bacterial transformation was done with
Size of T4 phage genome
Map distance is equal to the percentage of _____
Genome of different bacteria suggest that genes have in the past moved from one species to another. This
Experiment on transformation
Conjugational genetic exchange has been - - - - - - - - frequently encountered among gram positive than gr
Complete linkage has been reported in
Bacteriophages were jointly discovered by
Capsules help bacteria in escaping
Genes responsible for antibiotic resistance are mainly transferred across bacterial population by
The phenomenon of linkage was first observed in the plant
The Competence of a cell in the process of transformation is aided by
The viral genome integrated to the bacterial genome is called
Pneumococcal 'S' cells produce _____ colonies during growth on agar plates
In conjugation, the donor always carries on
Occurs when new DNA does not integrate into the chromosome, not replicated and is eventually lost
Tendency of alleles located close together on a chromosome to be inherited together during the meiosis
Virulence in *Streptococcus pneumoniae* is attributed to
The phenomenon in which genes are present on the same chromosomes is
Specialised transduction is effected by _____
Integeration of viral nucleic acid in to host chrosome is termed as
Genetic recombination in phages was discovered by _____
Conjugation can only occur between cells of
An example for specialized transducing particle
_____ involves finding a contiguous series of cloned DNA fragments which contain overlapping portio
_____ refers to a genetic changes in different genomes of same cell.
Conditions that favor the termination of the lysogenic state
Direct way of observing the physical arrangement of markers along the chromosomes
Metalloproteins found in all eukaryotes
The gene linkage minimize the chances of
Transfer of DNA from one bacterium to another through the action of viruses
The frequency at which two genes are - - - - - - - - by population of phages can be used to estimate their re
Who coined the term linkage
The complex of DNA, RNA and protein is

The process to identify a genetic element that is responsible for a disease is also referred as
Cells carrying non-integrated transducing fragments are called _____
Crossing over occurs during
Avery, MacLeod & McCarty used enzymes and solvents to destroy molecules such as
A bacterium harboring a prophage is called
Genome of T4 phage is
Non sex chrosomes are called
Uptake of DNA molecules from environmental surrounding
Results from inaccurate excision of an integrated prophage with addition of some bacterial genes
Give the full form for Hfr
Genes that cause suppression of mutations in other genes are called -------- genes
Conjugation is predominant in _____
_____ is a DNA associated protein
Conjugation involves the use of _____for mapping
During insertion of lambda DNA in to host, a viral protein called _____ is required along with integra
Linkage prevents
T4 is capable of undergoing only a
A cell carrying an integrated F factor is called an
Capsule of *Streptococcus pneumoniae* are made up of

Opt 1	Opt 2
Interrupted mating	Direct mapping
Benzon	Mendel
Centimorgan	Centimeter
Gene mapping	Chromosomal linkage
Bacillus	*E.coli*
Restricted transduction	Generalized transduction
Gene expression	Transformation
Streptococcus pyogenes	*Staphylococcus aureus*
169 kbp	196 kbp
Recombinant meiotic product	Reproducible meiotic product
DNA transfer	RNA transfer
Monad	Griffith
More	Less
Male Drosophila	Human female
Frederick Twort and by Felix d'Herelle	Hershey and Chase
Inflammation	RBC's
Conjugation	Transformation
Lathyrus odoratus	*Pisum sativum*
$CaCl_2$	$MgCl_2$
Plasmid	Capsid
Smooth	Rough
(F-)	(F+)
Abortive transduction	Specialized Transduction
Linkage	Crossing over
Flagella	Capsules
Cross over	Segregation
T_2	Mu
Microphage	Prophage
Hershey and Rotma	Hershey and Chase
F positive types	F negative types
No infection	T2 phage infects *Staphylococcus*
Physical mapping	Chemical mapping
Trans type	Cis type
Desiccation	Decomposition
Fluorescence in situ hybridization	Fluorescence invitro hybridization
Zinc fingers	Iron fingers
Cross over	Segregation
Transduction	Conjugation
Transduced	Co transduced
Mendel	Morgan
Chromatin	Somatin

Mapping	Linkage
Specialized	Abortive transductants
Pachytene	Diplotene
Anything except DNA	RNA
Lytic phage	Helper phage
ds DNA	ss DNA
Rhizomes	Lysosomes
Transduction	Conjugation
Specialized Transduction	Generalized Transduction
High fertility recombination	High fundamental recombination
Reverse genes	Control genes
Spirochaetes	G+ bacteria
Protone	Histone
Interrupted mating	Direct mapping
Integrase	Caspase
Segregation of alleles	Homozygous condition
Lytic cycle	Lysogenic cycle
F	Hfr
Protein	Lipid

Opt 3	Opt 4	Opt 5	Opt 6
Contact mapping	Linkage		
Colins	Bennazir		
Millimorgan	Millimeter		
Gene walking	Chromosomal walking		
Psuedomonas	*Agrobacterium*		
Non specific transduction	Specialised transduction		
Transduction	Conjugation		
Streptococcus pneumoniae	*Klebsiella pneumoniae*		
619 kbp	916 kbp		
Recombinant mitotic product	Reproducible mitotic product		
Gene transfer	Protein transfer		
Morgan	Hersehy		
Very High	Very low		
Female Drosophila	Maize		
Luria and Delbruck	McKay and McCartney		
Phagocytosis	Antibodies		
Transduction	Gene expression		
Datura	*Mirabilus jalapa*		
KCl	$AgCl_2$		
Prophage	Virion		
Elongated	Flat		
F neutral	No F		
Generalized Transduction	Transfusion		
Gene overlapping	Recessive genes		
Pili	Fimbriae		
Linkage	Assortment		
P_1	T_7		
Prephage	Macrophage		
Hershey and Wollmer	Hershey and Singer		
Same mating types	Opposite mating types		
Phage P22 infects *Salmonella typhimurium*	Phage lambda infects *E.coli*		
Marker mapping	Loci mapping		
Same type	Different type		
Nutrient Media	Macronutrient		
Fluorescence invivo hybridization	Fluorescence in cell hybridization		
Lead fingers	Copper fingers		
Recombination	Assortment		
Transformation	Gene expression		
Co repressor	Co operator		
de Vries	Correns		
Pigmentin	Fromatin		

Sequencing	Genome data mining		
Generalized	Conjugation		
Diakinesis	Haplotene		
Lipids & proteins	Polysaccharide		
Transducing phage	Lysogency		
ds RNA	ss RNA		
Mesosomes	Autosomes		
Transformation	Gene expression		
Abortive transduction	Transfusion		
High frequency recombination	Heavy frequency recombination		
Suppressor genes	Inducer genes		
G-bacteria	Cyanobacteria		
Chromotome	Cistron		
Contact mapping	Linkage		
Helicase	Polymerase		
Hybrid formation	Heterozygous condition		
Both Lytic & Lysogenic cycle	Other cycle		
Hfr 1+	trans		
Glycoprotein	Polysaccharide		

Answer
Interrupted mating
Benzon
Centimorgan
Gene mapping
E.coli
Generalized transduction
Conjugation
Streptococcus pneumoniae
169 kbp
Recombinant meiotic product
Gene transfer
Griffith
Less
Male Drosophila
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$CaCl_2$
Prophage
Smooth
(F+)
Abortive transduction
Linkage
Capsules
Linkage
T_2
Prophage
Hershey and Rotma
Opposite mating types
Phage lambda infects *E.coli*
Physical mapping
Cis type
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Fluorescence in situ hybridization
Zinc fingers
Recombination
Transduction
Co transduced
Morgan
Chromatin

Mapping
Abortive transductants
Pachytene
Anything except DNA
Lysogency
ds DNA
Autosomes
Transformation
Specialized Transduction
High frequency recombination
Suppressor genes
G-bacteria
Histone
Interrupted mating
Integrase
Segregation of alleles
Lytic cycle
Hfr
Polysaccharide

Unit- V

Transposable element

A transposable element (TE or transposon) is a DNA sequence that can change its position within a genome, sometimes creating or reversing mutations and altering the cell's genetic identity and genome size. Transposition often results in duplication of the same genetic material. Barbara McClintock's discovery of these jumping genes earned her a Nobel Prize in 1983.

Transposable elements make up a large fraction of the genome and are responsible for much of the mass of DNA in a eukaryotic cell. It has been shown that TEs are important in genome function and evolution. In Oxytricha, which has a unique genetic system, these elements play a critical role in development. Transposons are also very useful to researchers as a means to alter DNA inside a living organism.

There are at least two classes of TEs: Class I TEs or retrotransposons generally function via reverse transcription, while Class II TEs or DNA transposons encode the protein transposase, which they require for insertion and excision, and some of these TEs also encode other proteins.

Discovery

Barbara McClintock discovered the first TEs in maize (Zea mays) at the Cold Spring Harbor Laboratory in New York. McClintock was experimenting with maize plants that had broken chromosomes.

In the winter of 1944–1945, McClintock planted corn kernels that were self-pollinated, meaning that the silk (style) of the flower received pollen from its own anther. These kernels came from a long line of plants that had been self-pollinated, causing broken arms on the end of their ninth chromosomes. As the maize plants began to grow, McClintock noted unusual color patterns on the leaves. For example, one leaf had two albino patches of almost identical size, located side by side on the leaf. McClintock hypothesized that during cell division certain cells lost genetic material, while others gained what they had lost. However, when comparing the chromosomes of the current generation of plants with the parent generation, she found certain parts of the chromosome had switched position. This refuted the popular genetic theory of the time that genes were fixed in their position on a chromosome. McClintock found that genes could not only move, but they could also be turned on or off due to certain environmental conditions or during different stages of cell development.

McClintock also showed that gene mutations could be reversed. She presented her report on her findings in 1951, and published an article on her discoveries in Genetics in November 1953 entitled "Induction of Instability at Selected Loci in Maize".Her work would be largely dismissed and ignored until the late 1960s–1970s when it would be rediscovered after TEs were found in bacteria. She was awarded a Nobel Prize in Physiology or Medicine

in 1983 for her discovery of TEs, more than thirty years after her initial research. Approximately 90% of the maize genome is made up of TEs,as is 44% of the human genome.

Classification

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

Class I (retrotransposons)

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

Retrotransposons are commonly grouped into three main orders:

- TEs with long terminal repeats (LTRs), which encode reverse transcriptase, similar to retroviruses
- Long interspersed nuclear elements (LINEs, LINE-1s, or L1s), which encode reverse transcriptase but lack LTRs, and are transcribed by RNA polymerase II
- Short interspersed nuclear elements do not encode reverse transcriptase and are transcribed by RNA polymerase III

[Note : Retroviruses can also be considered TEs. For example, after conversion of retroviral RNA into DNA inside a host cell, the newly produced retroviral DNA is integrated into the genome of the host cell. These integrated DNAs are termed proviruses. The provirus is a specialized form of eukaryotic retrotransposon, which can produce RNA intermediates that may leave the host cell and infect other cells. The transposition cycle of retroviruses has similarities to that of prokaryotic TEs, suggesting a distant relationship between the two].

Class II (DNA transposons)

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

Cut-and-paste TEs may be duplicated if their transposition takes place during S phase of the cell cycle, when a donor site has already been replicated but a target site has not yet

been replicated.Such duplications at the target site can result in gene duplication, which plays an important role in genomic evolution.Not all DNA transposons transpose through the cut-and-paste mechanism. In some cases, a replicative transposition is observed in which a transposon replicates itself to a new target site (e.g. helitron).Class II TEs comprise less than 2% of the human genome, making the rest Class I.

Autonomous and non-autonomous

Transposition can be classified as either "autonomous" or "non-autonomous" in both Class I and Class II TEs. Autonomous TEs can move by themselves, whereas non-autonomous TEs require the presence of another TE to move. This is often because dependent TEs lack transposase (for Class II) or reverse transcriptase (for Class I). Activator element (Ac) is an example of an autonomous TE, and dissociation elements (Ds) is an example of a non-autonomous TE. Without Ac, Ds is not able to transpose.

Example

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

Applications

The first TE was discovered in maize (Zea mays) and is named dissociator (Ds). Likewise, the first TE to be molecularly isolated was from a plant (snapdragon). Appropriately, TEs have been an especially useful tool in plant molecular biology. Researchers use them as a means of mutagenesis. In this context, a TE jumps into a gene and produces a mutation. The presence of such a TE provides a straightforward means of identifying the mutant allele relative to chemical mutagenesis methods.

Sometimes the insertion of a TE into a gene can disrupt that gene's function in a reversible manner, in a process called insertional mutagenesis; transposase-mediated excision of the DNA transposon restores gene function. This produces plants in which neighboring cells have different genotypes. This feature allows researchers to distinguish between genes that must be present inside of a cell in order to function (cell-autonomous) and genes that produce observable effects in cells other than those where the gene is expressed.

TEs are also a widely used tool for mutagenesis of most experimentally tractable organisms. The Sleeping Beauty transposon system has been used extensively as an insertional tag for identifying cancer genes. The Tc1/mariner-class of TEs Sleeping Beauty transposon system, awarded Molecule of the Year in 2009is active in mammalian cells and is being investigated for use in human gene therapy. TEs are used for the reconstruction of phylogenies by the means of presence/absence analyses.

Tn3 transposon

The Tn3 transposon is a 4957 base pair mobile genetic element, found in prokaryotes. It encodes three proteins:

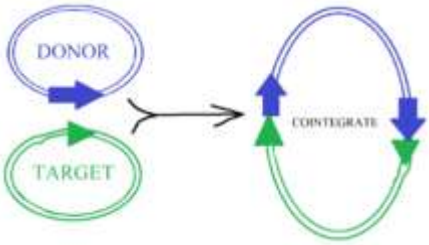
- β-lactamase, an enzyme that confers resistance to β-lactam antibiotics (and is encoded by the gene Bla).
- Tn3 transposase (encoded by gene tnpA)
- Tn3 resolvase (encoded by gene tnpR)

Initially discovered as a repressor of transposase, resolvase also plays a role in facilitating Tn3 replication (Sherratt 1989).The transposon is flanked by a pair of 38bp inverted repeats.

Mechanism of replication

Step 1 – Replicative integration

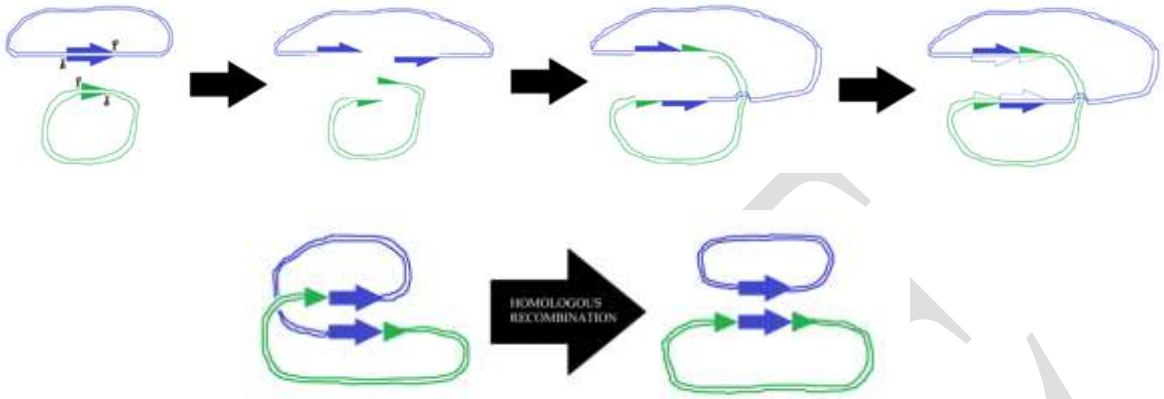
This first stage is catalysed by transposase. The plasmid containing the transposon (the donor plasmid) fuses with a host plasmid (the target plasmid). In the process, the transposon and a short section of host DNA are replicated. The end product is a 'cointegrate' plasmid containing two copies of the transposon.



Step 2 – Resolution

- To separate the host and target molecules Tn3 resolvase executes site-specific recombination between the old and new copy of transposon at a specific site called res, which is present in each copy of the transposon
- Res is 114 bp long and it consists of 3 sub-sites, namely sites I, II and III. Each of these sites is of different lengths (28, 34 and 25bp, respectively) and they are unevenly spaced with 22bp separating sites I and II and only 5bp between sites II and III. The sites consist of 6bp inverted repeat motifs flanking a central sequence of variable length.

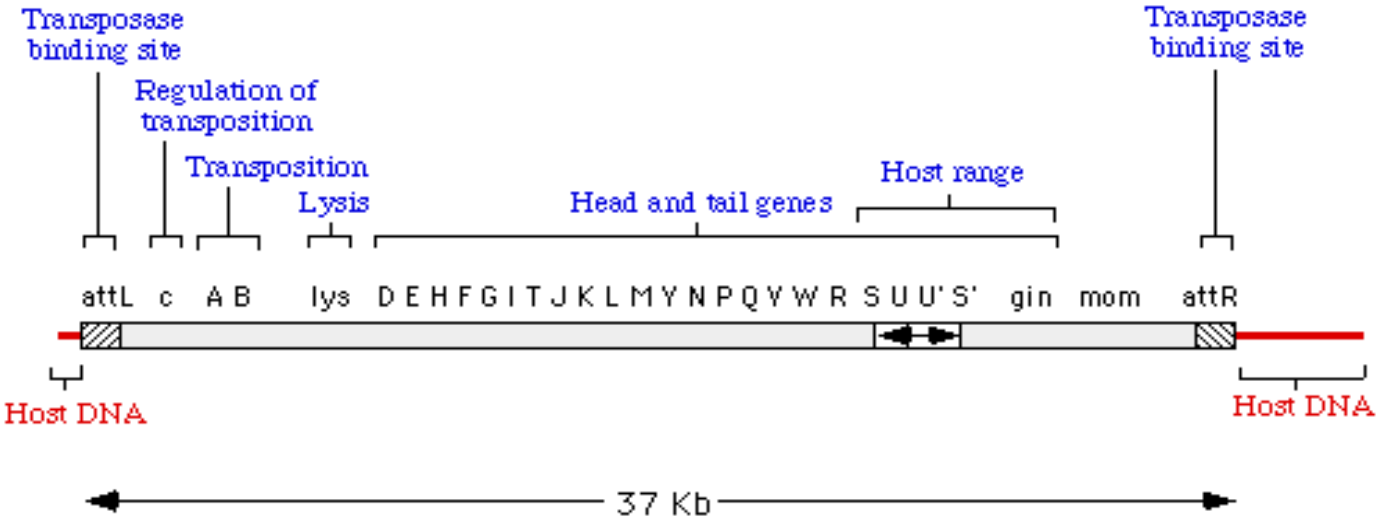
- These motifs act as binding sites for resolvase, so that each site binds a resolvase dimer but with varying affinity and probably a slightly different protein-DNA complex architecture. All three sub-sites are essential for recombination.



Process of resolution

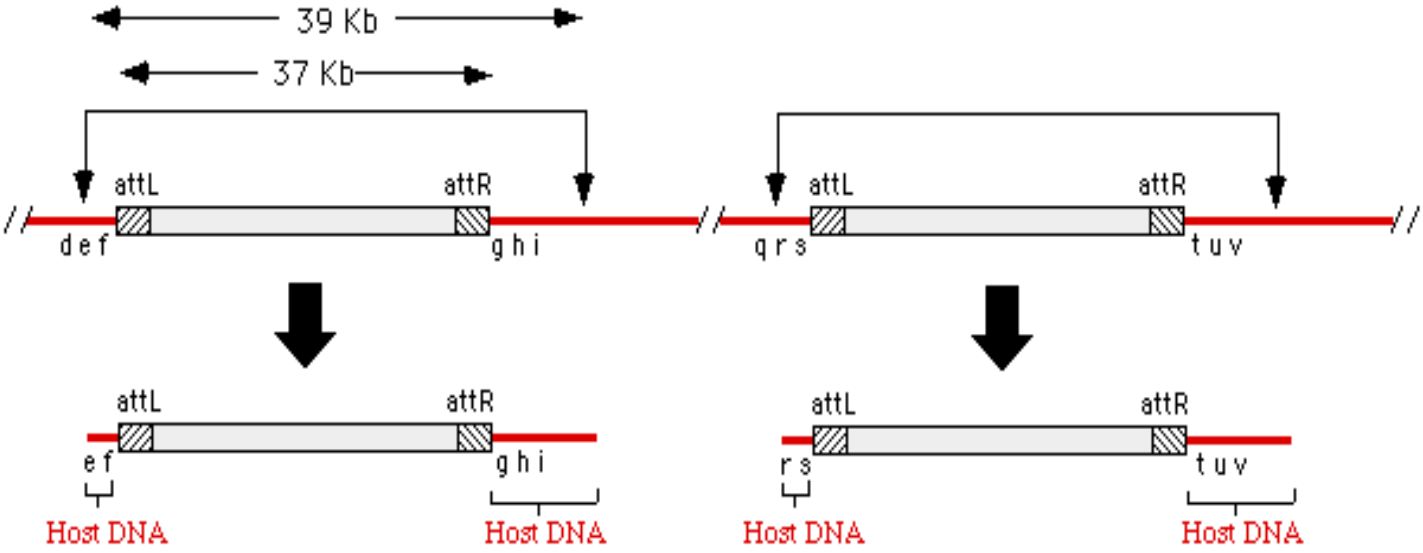
Mu transposon

Phage Mu is a phage that reproduces by transposition. A simplified Schema of the Mu genetic map is shown below.



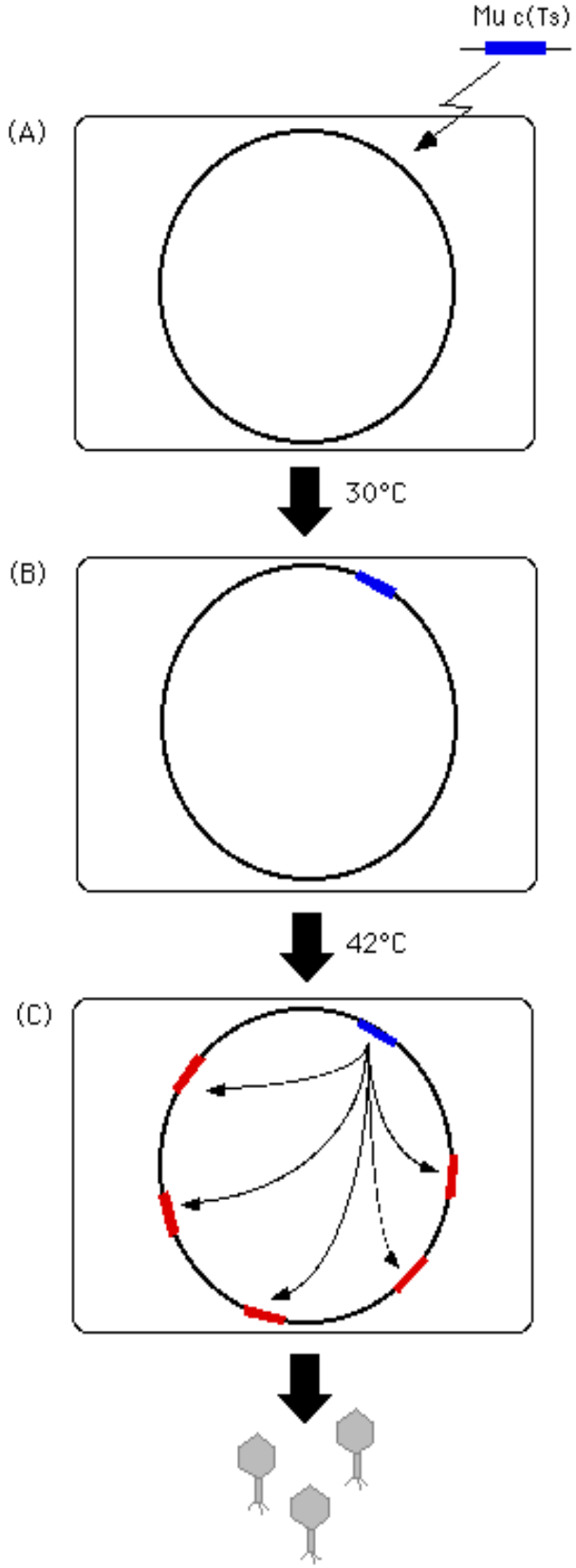
Features of Mu phage

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



Mechanism of transposition in Mu phage.

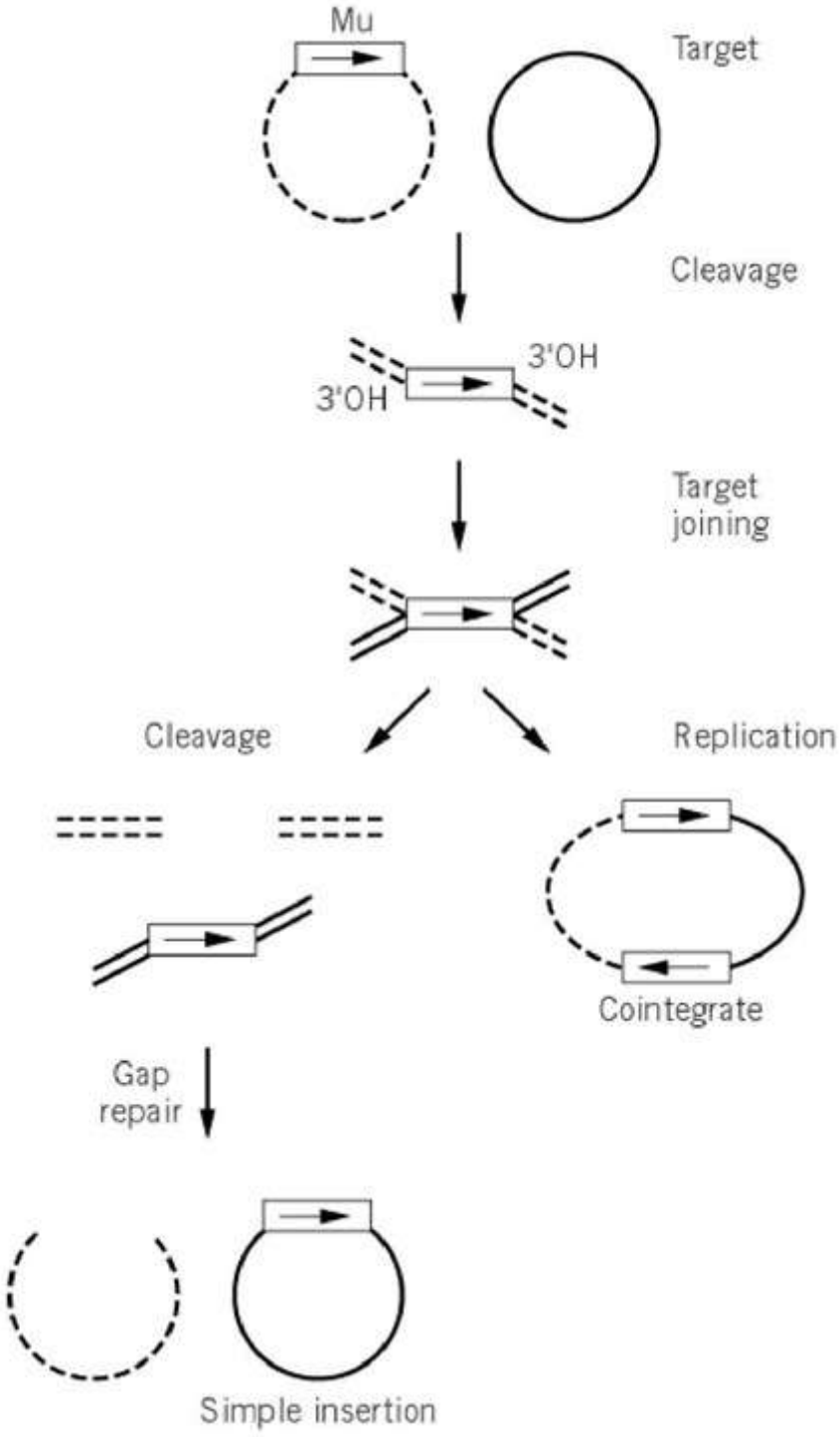
The life cycle of phage Mu is shown in the as below.



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

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

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


Mu
Target
Cleavage
3'OH
3'OH
Target
joining
Cleavage
Replication
Cointegrate
Gap
repair
Simple insertion

Possible Questions

Two marks

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

Eight marks

8. Explain about different types of Transposons.
9. What is the difference between composite and non composite transposons? Explain.
10. Comment of Tn3 transposon.
11. Explain in detail about the Tn10 transposons.
12. Discuss on the applications of transposons.
13. Give a detailed account on the role of Mu transposons in gene exchange mechanism.

DEPARTMENT OF MICROBIOLOGY,KARPAGAM ACADEMY OF HIGHER EDUCATION
MICROBIAL GENETICS (17MBU203)

Unit V Question
_____ refers to a genetic changes in different genomes of same cell
_____distance in map unit is only half the percentage of second division segregated tetrads
An estimation of map between a- & b- can be obtained from number of recombinant ascospore detected by
Common model organism in studying unicellular eukaryotes/budding yeast
Gene conversion is the _____ transfer of information from one DNA duplex to another
Haploid to diploid phase in yeast is brought about by
In *Drosophila* , the meiotic recombination occurs only in _____
Map distance is equal to the percentage of
Petite phenotypes caused by the absence of, or mutations in, mitochondrial DNA are termed as
Random spore analysis gives _____ values
Sex linkage was explained by _____
The ascus burst releasing the ascospores, each of which germinates and divides by mitosis to produce new
The general mapping function of Haldane is based on _____
The repair of double-strand gaps is an efficient process in yeast known to be
Well characterized Baker's yeast has __ cell
Which of the following is used in density gradient centrifugation?
Yeast genome is _____
When mutation in single gene affect more than one trait is called _____
The binding of two DNA helices through X-shaped junction called _____
Small pieces of DNA that can insert themselves into chromosomes are known
Recombination does not only occur during meiosis, but also as a mechanism for
Petite yeast mutants are unable to grow on media containing
Meiosis in yeast life cycle leads in forming
In yeast, 22% of the genome is made up of _____ DNA
Genetic maps of chromosome are based on the frequencies of _____
Gene conversion can be either allelic or _____
Gene conversion in yeast may be important in maintaining_____within families
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 _____
A new genetic nomenclature for S. cerevisiae transposon
Among haploid and diploid vegetative cells of yeast ,which is mainly used for genetic mapping
His 3 is an _____ protein.
If single crossover occurs between a- & b- then tetra type ___ results
Pleiotrophy is common in _____
Process by which one DNA sequence replaces a homologous sequence
The term genetic linkage was given by ----------
The_____ to a particular chromosome is the first step in genetic mapping
Yeast are _____
Yeast genome has _____ introns
Yeast has two mating types, _____ , which show primitive aspects of sex differentiation
Yeasts fail to grow on

Sample of ascospores is spread on to the agar medium without leucine and survival was tested using
_____ test is used to determine which gene(s) are defective in petite yeast mutants
_____ contains two types of spores of same parental genotype
_____determines the number of crossover events and give correct map distance
_____developed an algebriac method to determine the consequence of various number of exchanging
Which contains four different ascospores , one of each genotypes
The developing haploid spores are enclosed in a membranous structure called_____
Method available for locating mutation in *Neurospora crassa* is
In Transcription of yeast genome,_____ makes all RNA to serve as mRNA
Intermediate compound responsible for red color of adenine-requiring yeast petite mutants
Arg^{+} is
Cells which contains single copy of chromosome is called _____
_____ intial products of meiosis forms two identical spore
A feature of petite is the occurrence of _____ from the circular mitochondrial genome
Generation time of yeast takes place at _____
Give full form for NPD
HFT is
Significant feature of sex determination in Drosophila is the presence of abnormal flies called _____
Tetrad showing second division segregation has _____ products

Opt 1	Opt 2
Trans type	Same type
Tetromere	Telomere
Genetic analysis	Physical analysis
Saccharomyces cerevisiae	*Cryptococcus Neoformans*
Reciprocal	Non-reciprocal
Fusion of opposite mating types	Fusion of positive mating types
Both Female and Male	Female
Reproducible mitotic product	Recombinant mitotic product
Cytoplasmic petites	Energy deficient petites
Approximate	Accurate
Morgan	Mendel
Vegetative cells	Spores
Haldane distribution	Poisson distribution
Seggregation	Assortment
Irregular	Round
Glucose	Sucrose
12,520 kb	1,252 kb
Parental genes	Pleiomorphic genes
Polytron	Cholistron
Plasmid	Transposon
Repair of single-strand breaks	Repair of double-strand breaks
Only nitrogen sources	Only Mineral sources
Sporangium	Fragmented mycelium
Polygenic	Monogenic
Reproducible mitotic product	Reproducible meiotic product
Allergic	Ectopic
Sequence homogeneity	Sequence heterogenecity
Endonuclease	Beta isoprophylmalate dehydrogenase
Second division	Fourth division
jumping element	Ty elements
Haploid and diploid	Diploid
Transducer	Indicator
A	B
No organism	All organism
Gene mutation	Gene repulsion
Morgan	Meischer
Elemination of mutated type	Localization of wild type
Multicellular fungi	Dicellular
233	236
a & b	a & α
Glucose	Lactose

ELISA	PCR
Complementation test	Complement fixation test
Parental haploid	Parental diploid
Genetic analysis	Physical analysis
Klebs & Loeffler	Pastuer & Winogradsky
Tetratype	Parental type
Spores	Pycus
Co-segregation	Co-opression
DNA polymerase	RNA polymerase
Aminoimidazole	Aminoimidazole ribonucleotide
A strain not requiring aspargine	A strain requiring aspargine
Triploid	Haploid and diploid
4	8
Insertion	Excertion
2hrs 30 min	3 hrs
Non-parental dikaryon	Non-performing data
High frequency transducing	High frequency transcribing
Gyno variants	Gynocoid
Four meiotic	Two Meiotic

Opt 3	Opt 4	Opt 5	Opt 6
Cis type	Different type		
Centromer	Primer		
Random spore analysis	Tetrad analysis		
Candida albicans	*Pitchia pastoris*		
Direct	Indirect		
Fusion of negative mating types	Fusion of opposite genes		
Male	None		
Reproducible meiotic product	Recombinant meiotic product		
Mitochondrial petites	Chrosomal petites		
Null	Partial		
Primrose	Pastuer		
Ascus	Zygote		
Twart	Switz		
SOS repair	Crossing over		
Spheroid	Disc shaped		
Fructose	Agarose		
1,02,520 kb	15,052 kb		
Priogenic genes	Pleiotrophic genes		
Diptron	Cistron		
Cosmid	Artificial chrosome		
Repair of proteins	Repair of RNA		
Only Lipid Source	Only fermentable carbon sources		
Ascus spores	Endospores		
Intragenic	Intergenic		
Recombinant mitotic product	Recombinant meiotic product		
Endemic	Endopic		
Sequence array	Sequence hologenecity		
Helicase	Ligase		
Primary division	Tetrad		
Tx elements	Ta element		
Haploid	Triploid		
Selector	Repressor		
T	U		
Higher organism	Lower organism		
Gene transtition	Gene conversion		
Wilkins	Mendel		
Localization of mutation	Elimination of wild type		
Acellular	Unicellular fungi		
323	326		
b & β	α & β		
Maltose	Trehalose		

Random spore analysis	RPR		
Completed test	Conjugation test		
Non-parental ditype	Parental ditype		
Random spore analysis	Tetrad analysis		
Shult & Lindegrin	Klug & Cumming		
Parental ditype	Non-parental ditype		
Zygote	Ascus		
Independent assortment	Tetrad analysis		
RNA pol I	RNA pol II		
Aminoimidazoleribotide	Aminoimmunoribotide		
A strain not requiring arginine	A strain requiring arginine		
Diploid	Haploid		
16	32		
Addition	Deletion		
60 min	30 min		
Non-parental data	Non-parental ditype		
Height frequency transducing	Heavy frequency transducing		
Gyrates	Gynondromorphs		
Four mitotic	Two mitotic		

Answer
Cis type
Telomere
Random spore analysis
Saccharomyces cerevisiae
Non-reciprocal
Fusion of opposite mating types
Female
Recombinant meiotic product
Cytoplasmic petites
Approximate
Morgan
Vegetative cells
Poisson distribution
Crossing over
Spheroid
Sucrose
12,520 kb
Pleiotrophic genes
Cistron
Transposon
Repair of double-strand breaks
Only non-fermentable carbon sources
Ascus spores
Intergenic
Recombinant meiotic product
Ectopic
Sequence homogeneity
Beta isoprophylmalate dehydrogenase
Tetrad
Ty elements
Haploid
Indicator
T
Higher organism
Gene conversion
Morgan
Localization of mutation
Unicellular fungi
233
a & α
Lactose

Random spore analysis
Complementation test
Parental diploid
Tetrad analysis
Shult & Lindegrin
Tetratype
Ascus
Co-segregation
RNA pol II
Aminoimidazoleribotide
A strain not requiring arginine
Haploid
4
Deletion
2hrs 30 min
Non-parental ditype
High frequency transducing
Gynondromorphs
four meiotic

Reg. No._____

[17MBU203]

KARPAGAM ACADEMY OF HIGHER EDUCATION

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

DEPARTMET OF MICROBIOLOGY

B.Sc., DEGREE FIRST CIA EXAM, (JANUARY– 2018)

MICROBIAL GENETICS

Time: 2 hours **Total : 50 Marks**

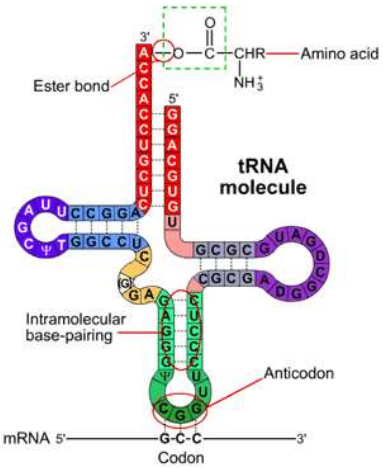
PART –A (Answer all the questions) 20 x 1 = 20 marks

1. Who is called father of Genetics?
   a) Gregor Johan Mendel b) Beedle and Tatum
   c) Morgan d) Walter Sutton and Theoder Boveri
2. Experiment of Hershey and Chase was based on_____.
   a) Virus b) Bacteriophage
   c) Bacteria d) Fungi
3. Bonding between two Nitrogenous bases present in opposite DNA strands are_____.
   a) Hydrogen Bonds b) Phosphodiester bond
   c) Covalent Bonds d) Nitrogen bond
4. Distance between the two base pairs is_____.
   a) 34Å b) 3.4Å
   c) 10Å d) 20Å
5. The classic β-helix DNA of Watson and Crick is in_____ form.
   a) A form b) B form
   c) Z form d) E form
6. During DNA synthesis, DNA polymerase can add nucleotides to which end_____.
   a) 3’ end b) 5’ end
   c) 2’ end d) 4’ end
7. Okazaki fragments are present in _____.
   a) RNA strands b) Enzymes
   c) Leading strands d) Lagging strands
8. The enzyme that unwinds dsDNA into ssDNA during replication is_____.
   a) Polymerase b) Ligase
   c) Gyrase d) Helicase
9. The process in which genetic information is copied to an RNA molecule is called_____.
   a) Transformation b) Translocation
   c) Transcription d) Translation
10. In RNA synthesis, Adenine of DNA pairs with_____ in RNA.
    a) Uracil b) Thymine
    c) Guanine d) Cytosine
11. Which of the following is the best description of a gene?
    a) A molecule of DNA b) A nucleotide
    c) A sequence of amino acids d) An unit of DNA that codes for RNA

12. A-site is the ribosomal site most frequently occupied by the
    a) Aminoacyl-rRNA b) Aminoacyl-tRNA
    c) Aminoacyl-mRNAs d) Aminoacyl-rRNA
13. _____is an example for stop codon.
    a) GUA b) AAU
    c) UAA d) CUA
14. Thymine in DNA is replaced by_____in RNA
    a) Adenine b) Cytosine
    c) Guanine d) Uracil
15. _____possesses anti-codons which are complimentary to the mRNA codons.
    a) DNAs b) tRNAs
    c) mRNAs d) rRNAs
16. The CCA end of a tRNA molecule is called_____
    a) Anti-codon arm b) Acceptor arm
    c) Variable arm d) Extra arm
17. Extra Chromosomal, double stranded, circular DNA molecule present in Bacteria is called _____.
    a) Cosmid b) plasmid
    c) Bacmid d) Phagemid
18. Drug resistance in bacteria is associated with _____.
    a) F – plasmid b) R – plasmid
    c) Ti – plasmid d) col – plasmid
19. Col-plasmid produces _____.
    a) Colicin b) Vaccine
    c) Toxin d) Trypsin
20. Ti plasmid is responsible for_____
    a) Transformation b) Tumor induction
    c) Transcription d) Trans-illumination

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

21. What are the components of Nucleotide?
    Sugar + Phosphate + Nitrogen base
22. Draw the clover leaf model of t-RNA and write any two properties.

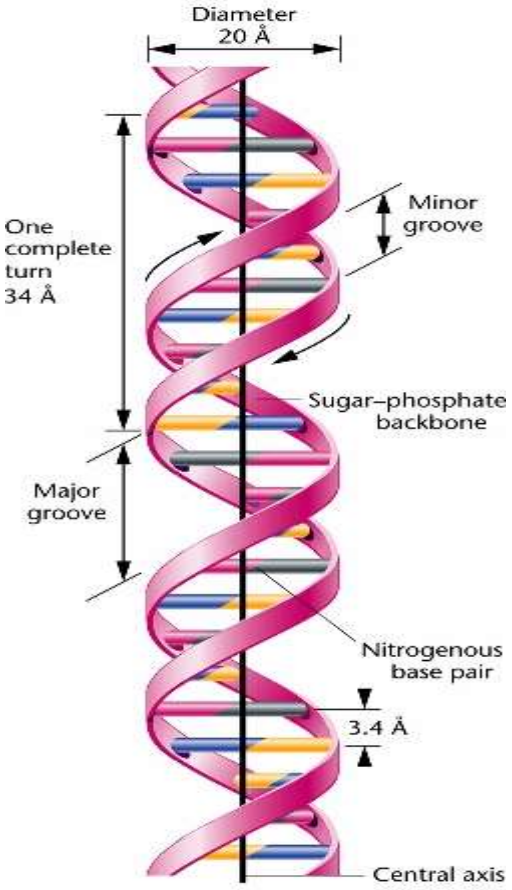


23. Define plasmid with an example?

    Extrachromosomal Closed Circular DNA. Eg. pBR322.

PART C (Answer all the questions) **3 x 8 = 24 marks**

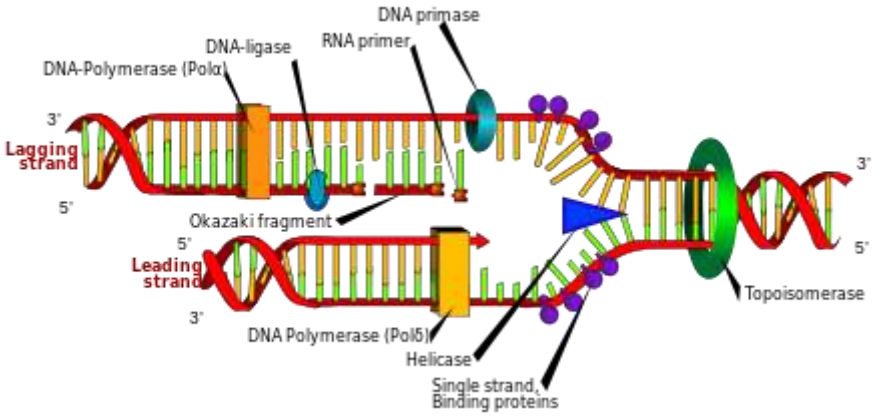
24. **a)** Discuss in detail about DNA structure.



DNA-nucleoside-nucleotide-deoxy ribose sugar-hydrogen bond- phosphodiester bond-ATGC- base complementarity- antiparallelity-Chargaff's rule- Rosalind Franklin-Watson and Crick- Groove conformation.

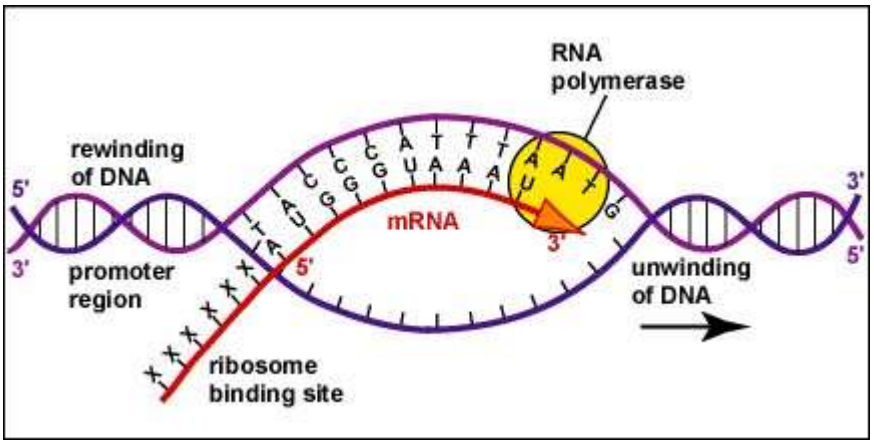
or

b) Explain DNA replication with neat sketch .



Origin of replication-dnaA protein- topoisomerase III- helicase- primase- DNA Polymerase III-dNTPs- Leading strand- Lagging Strand- Okazaki Fragment-Primosome- Replisome – Replication fork- 3` addition of bases- Intiation- Elogation- Termination- DNA ligase- DNA Polymerase I.

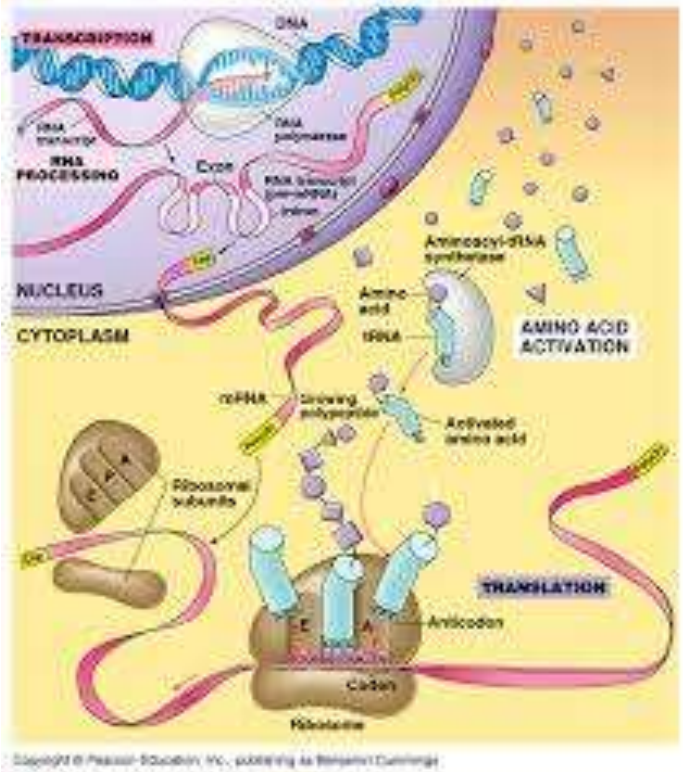
25. **a)** Describe in detail about Transcription.



DNA- Initiation – Initiation complex – upstream and Downstream sequencses- RNA Polymerase- sigma factor- base addition- Elongation- Termination- Rho dependent and Rho Independent termination- RNA stem and loop formation-Post translational RNA modification- 5`capping of RNA- Poly adenylation at 3` end- migration of RNA to cytoplasm- conformational change of tRNA – synthesis of rRNA and Ribosome.

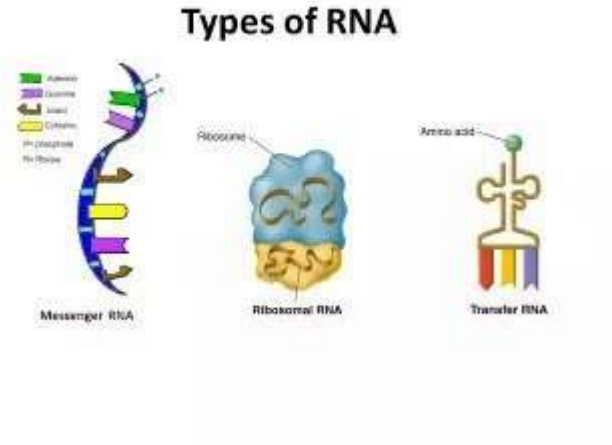
or

b) Write a detailed note on Translation.



Translation- definition- initiation – intiation factor – 30 S ribosome- 50S ribosome- formation of intiation complex- Shine Dalgarno Sequence- AUG start codon- initiation complex formation- aminoacyl tRNA synthase- formation of fMet tRNA- sliding of ribosomal machinery- formation of A site, P site, E site- Elongation- codon - elongation factor-synthesis of polypeptide chain-peptidyl transferase-termination- stop codon (UAA,UAG,UGA)-Apurinic site- post translational modification- degree of organization of proteins.

26. **a)** Describe types of RNA and their functions



RNA- Definition- types –mRNA-rRNA-tRNA-snRNA-hnRNA-miRNA- properties of RNA-single stranded-Base modification to Uracil- 5` capping-3` poly adenylation-clover leaf model of tRNA-TUC arm-DHU arm- anticodon arm-ACC acceptor arm-extra arm-Ribosomal protein + rRNA conformational change.

or

b) Explain shortly: a) F-plasmid b) R-plasmid c) col-plasmid d) Ti-plasmid

F-plasmid-fertility plasmid-sex pilus-Hfr cells- beta cells-competency-conjugation

R-plasmid-resistant plasmid-TetR-AmpR-Mip Resistance in MTB-Marker gene- Dissociation of resistance using molecular siccors.

Col Plasmid- colicin-definitions-bacteriocin-alpha, beta, gamma- nature of activity of colicin-antagonistic activity- dominance.

Ti plasmid- *A. tumefaciens*- Tra gene- Opine gene- virulence-tumor induction-BT plants.

****

(All the Best)

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

[16MBU203]

KARPAGAM ACADEMY OF HIGHER EDUCATION

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

Eachanari post, Coimbatore – 641 021, Tamil Nadu, India

B.Sc. DEGREE EXAMINATION, APRIL 2017

MICROBIOLOGY

SECOND SEMESTER

MICROBIAL GENETICS

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

PART-A 20x 1=20 marks

(Multiple Choice Question No 1 to 20 Online Exam)

1. Father of genetics is _____
   a. Mendel b. Newton
   c. Morgan d. Boveri
2. DNA structure identified by Watson &crick is
   a. Z-DNA c.C –DNA
   c. B-DNA d.T-DNA
3. In nucleic acids _____cause intra strand pairing between nucleotides bases
   a. Hydrogen bond c.Hydrophobic bond
   c. Nitrogen bond d.Van Der waals
4. The process in which genetic information is copied to an RNA molecule is called
   a. Transformation c.Transduction
   c. Translation d.Transcription
5. In molecule of tRNA, three of the unpaired bases constitute
   a. codon c.An Anti codon
   c. clover leaf configuration d.termination region
6. Extra Chromosomal, double stranded, circular DNA molecule present in Bacteria
   a. cosmid b. plasmid
   c. bacmid d. phagemid
7. Bacterial conjugation is mediated by_____
   a. F Plasmid b.R Plasmid
   c. Ti Plasmid d. Col Plasmid
8. Drug resistance in bacteria is associated with
   a. R-plasmid b. F-plasmid
   c. Col-plasmid d. Ti-plasmid
9. _____is an example for stop codon.
   a. GUA b. AAU
   c. UAA d. CUA
10. Lactose operon model was proposed by_____
    a.Watson and crick b. Beedle and Tatum
    c. Jacob and Monad d. Zinder and Lederberg
11. Hfr is strands for_____
    a. High Frequency Recombination b. Heat frequency transformation
    c. High fluent resistance d. High Fluent regeneration
12. Transfer of genetic material into bacteria by mediation of bacteriophage is_____
    a. Conjugation b. Transformation
    c. Mutation d. Transduction

13. The complete set of genes or genetic material present in a cell or organism is termed as
    a. Genome b. Genotype
    c. Genosis d. Generation
14. _____ is the process by which a DNA base pair or a chromosome change occur
    a. Mediation b. Mutation
    c. Maturation d. Molting
15. Mutation that result from treatment with mutagens is called_____
    a. Spontaneous b. Frame shift
    c. Induced d. Nonsense
16. _____ is a protocol for identifying mutagenic chemical and physical agents
    a. Ames Test b. Agar test
    c. Amino test d. Allele test
17. _____are segments of DNA that can move around to different positions in the genome of a single cell
    a. Transducers b. Transposons
    c. Transformers d. Translucents
18. The ability of genes to change position on chromosomes
    a. Transformation b. Transposition
    c. Transduction d. Transaction
19. _____ are ideal for the study of genetics with reference to genes causes diseases in human
    a. Drosophila b. Dragon
    c. Saccharomyces d. House Fly
20. _____ is example of a eukaryotic transposable element in Yeast
    a. Tr element b. Tg element
    c. Ty element d. T element

PART-B **5x 2=10 marks**

(Answer All the Questions)

21. Differentiate Nucleoside and Nucleotide.
22. Describe shortly R-plasmid.
23. What is an Anti-codon?
24. What is mutation and give an example?
25. Define transposons.

PART-C **5x 6=30 marks**

(Answer All the Questions by choosing either 'a' or 'b')

26. a. Explain Mendel's principles of Genetics.
    (Or)
    b. Describe the structure of DNA.
27. a. Describe about Ti plasmids.
    (Or)
    b. List out the applications of Plasmids.
28. a. Explain Genetic code with clear table.
    (Or)
    b. Describe Transformation.
29. a. Describe different types of Mutations.
    (Or)
    b. Give a detailed account on chemically induced mutation.
30. a. Write a detailed note on Prokaryotic transposable elements.
    (Or)
    b. Explain the uses of transposons.

Set II **Reg. No. : ----------**

[15MBU203]

KARPAGAM ACADEMY OF HIGHER EDUCATION

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

Eachanari post, Coimbatore – 641 021, Tamil Nadu, India

B.Sc. DEGREE EXAMINATION, APRIL 2016

MICROBIOLOGY

SECOND SEMESTER

MICROBIAL GENETICS

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

PART-A **20x 1=20 marks**

(Multiple Choice Question No 1 to 20 Online Exam)

1. Mendel's pioneer work was with_____
   a. Garden Pea b. Evening Primrose
   c. Lotus d. Daffodil
2. Adenine always base pairs with
   a. Guanine b. Cytosine
   c. Thymine d. Uracil
3. Synthesis of DNA always moves from
   a. 3' to 5' b. 5' to 3'
   c. From the first codon d. From polymerase
4. Synthesis of protein from m-RNA is_____
   a. Transformation b. Translation
   c. Replication d. Transcription
5. _____is the example of virulence plasmid
   a. Ti-plasmid b. R-Plasmid
   c. Yeast plasmid d. F-plasmid
6. Borellia consist_____
   a. Super coiled plasmid b. Linear plasmid
   c. Phagmid d. Cosmid
7. Plasmid can be used as _____ in rDNA technology
   a. Molecular Vector b. Donor
   c. Recipient d. Inducer
8. Plasmid mediated gene transfer is _____
   a. Transformation b. Transduction
   c. Replication d. Conjugation
9. Uptake of DNA fragments by a bacterial cell from surrounding medium
   a. Transformation b. Transduction
   c. Translation d. Transcription
10. Virus which kills bacteria
    a. Bacteriophage b. Virophage
    c. Mycophage d. Phycopage
11. Which of the following is a process of phage replication
    a.Lytic and Lysogenic cycles b. Carbon cycle
    c.Photosynthetic cycle d. Replication cycle
12. Lactose is hydrolysed by _____
    a. Lactate dehydrogenase b. Lipid dehydrogenase
    c. Lactate synthetase d. Lactate enolase

13. _____ is the sudden change is the genetic material of an organism
    a. Mediation b. Mutation
    c. Maturation d. Molting
14. The complete set of genes or genetic material of E. coli is called as
    a. E. coli Genome b. E. coli Genotype
    c. E. coli proteins d. E. coli proteome
15. Chemical used to induce Mutation are _____
    a. Mutagens b. Enhancers
    c. Repressors d. Catalyzers
16. One of the carcinogenicity testing
    a. Ames Test b. Agar test
    c. Amino test d. Allele test
17. In Genetics, TE is strands for _____
    a. Transducer Element b. Transposable Element
    c. Transforming Element d. Translucent Element
18. The Ac/Ds transposable element system of
    a. Maize b. Drosophila
    c. Yeast d. Bacteria
19. _____ can be used to introduce a piece of foreign DNA into a genome
    a. DNA transposons b. Bacterial Chromosome
    c. Yeast Genome d. Pea plants
20. The ability of genes to change position on chromosomes
    a. Transformation b. Transposition
    c. Transduction d. Transaction

PART-B **5x 2=10 marks**

(Answer All the Questions)

21. Write about the Purines and Pyrimidines.
22. Describe shortly F-plasmid.
23. Explain Bacteriophage and their role in genetics.
24. Write a short note on Mutagenesis with an example.
25. Explain shortly the use of transposons.

PART-C **5x 6=30 marks**

(Answer All the Questions by choosing either 'a' or 'b')

26. a. Explain in detail about DNA replication.
    (Or)
    b. Give a detailed account on Transcription.
27. a. Explain: i) R-plasmid ii) Col-plasmid iii) Ti-plasmid.
    (Or)
    b. Describe plasmid replication and partitioning.
28. a. Write in detail about Bacterial transformation.
    (Or)
    b. Explain transduction.
29. a. Give a detailed account on induced mutations.
    (Or)
    b. Describe about Saccharomyces genome.
30. a. Write a detailed note on eukaryotic transposable elements.
    (Or)
    b. Explain the P-elements of Drosophila.