Semester – I

18MBP103 **MOLECULAR GENETICS** **4H – 4C**

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

End Semester Exam: 3 Hours

COURSE OBJECTIVES

The emergence of Molecular Genetics has revolutionized large areas of modern biological and biochemical research work and has had a huge impact on the biotechnology industry. Molecular genetics is concerned with the development of biochemical and genetic techniques for handling the complex nucleic acids that constitute genetic material.

COURSE OUTCOME

This course allows the candidate to recollect the basics of Molecular Genetics and apply a cognitive thinking on the application oriented sectors of Molecular Biology. Students would be able to practically apply this knowledge in different sectors with possibilities ranging from the treatment of human diseases to the development of novel medicines.

UNIT – I

Genetics and its types – History; Mendelian principles – nucleic acid as genetic material Experimental evidence. Structure of DNA – chemical and physical structure of DNA – circular and super helical DNA - different forms of DNA. DNA replication – enzymology of DNA replication – different modes, models and types of DNA replication – Eukaryotic DNA replication.

UNIT – II

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

UNIT – III

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

UNIT – IV

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

UNIT – V

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

SUGGESTED READINGS

TEXT BOOKS

1. Malacinski, G.M. (2008). *Freifelder's Essentials of Molecular Biology*. Narosa Publishing House, New Delhi.
2. Verma, P.S., and Agarwal, V.K., (2008). *CellBiology, Genetics, Molecular Biology and Evolution.* S. Chand & Company Ltd, New Delhi
3. Gardner, E.J., Simmons, M.J., and Snustad, D.P., (2008). *Principles of Genetics*. (8th ed.). John Wiley and Sons, NY.
4. Guthrie, C., and Fink, G., (2002). *Guide to Yeast Genetics and Molecular Cell Biology*. Elsevier Publication, USA.
5. Klug, W.S., Cummings, M.R. Spencer, C.A., and Palladino, M.A., (2009). *Essentials of Genetics*. (7th ed.). Prentice Hall, New Jersey.
6. Maloy, S.R., Cronan Jr, J.E., and Freifelder, D., (2001). *Microbial Genetics*. Narosa Publishing House. New Delhi.
7. Weaver, R.F. (2002). *Molecular Biology*. (2nd ed.). McGraw-Hill, New York.

REFERENCES

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

WEBLINK

1. http://www.biologydiscussion.com/bacteria/genetic-recombination-of-bacteria-with-diagram/47074
2. http://www2.csudh.edu/nsturm/CHEMXL153/RegulationofGeneExpression.htm

   https://www.youtube.com/watch?v=8_f-8ISZ164.

Semester – I

18MBP112 **BASIC PRACTICAL – II** **4H – 2C**

Instruction Hours / week:L: 0 T: 0 P: 4 **Marks:** Internal: **40** External: **60** Total: **100**
End Semester Exam: 9 Hours

COURSE OBJECTIVES

The contents of this course would enable the student

- To acquire practical knowledge on the different molecular mechanism of gene transfer, mutations and separation of nucleic acids.
- To understand the molecular mechanism of compound separation and isolation using chromatography techniques.

COURSE OUTCOME

A student undertaking this course will be learning the principles behind the molecular techniques which would enable him to work in competent molecular biology based laboratories.

EXPERIMENTS

1. Spontaneous Mutation – gradient plate technique
2. Induced Mutagenesis-chemical and physical - UV
3. Replica plating technique.
4. Transformation in Bacteria
5. Bacterial Conjugation
6. Induction of Lac operon
7. Measurement of growth-one step growth curve using a T even phage
8. Titration of phages (T4)
9. Nuclear staining for nucleic acid identification.
10. Spectrophotometric estimation of protein – BSA
11. Protein Purification using microfiltration.
12. Analysis of amino acid by Paper chromatography
13. Analysis of amino acid by Thin layer chromatography
14. Purification of proteins by column chromatography
15. Analysis of amino acid by HPLC – Demonstration

SUGGESTED READINGS

REFERENCES

1. Arora, B., and Arora, D.R., (2007). *Practical Microbiology*, (1st ed.). CBS Publishers and Distributors, Bangalore.
2. Benson, H.J. (1998). *Microbiological Application (Laboratory Manual in General Microbiology)*, (7th ed.). WCB.
3. Palanivelu, P. (2004). *Analytical Biochemistry and Separation Techniques*, (3rd ed.). Twenty First Century Publication, Madurai.
4. Chakraborthy, P., and Pal, N.K., (2008). *Manual of Practical Microbiology and Parasitology*, New Central Book Agency (P) Ltd, India.
5. Gaud, R.S., and Gupta, G.D., (1999). *Practical Microbiology*, 1st Ed.). NiraliPrakashan, Pune.

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2018 onwards)
DEPARTMENT OF MICROBIOLOGY

SUBJECT : Molecular Genetics **SEMESTER: I**
SUBJECT CODE: 18MBP103 **CLASS : I M.Sc.Microbiology**

LECTURE PLAN
DEPARTMENT OF MICROBIOLOGY

S.No	Lecture Duration Hour	Topics to be Covered	Support Material/Page Nos
		UNIT-I	
1	2	History of Genetics Types and Mendelian principles	T1:16 to 39 R1: 3 to 4/ 24-27
2	2	Nucleic acid as genetic material- Experimental evidence	R1: 245 to 247
3	2	chemical and physical structure of DNA	T1:194 to 204 R1: 29 to 49
4	2	Different forms of DNA	T1: 204 to 211
5	1	Enzymology of DNA replication	T1: 216 to 218
6	3	DNA replication – Types, models and modes of replication.	T1: 228 to 229 R1: 47 to 48
7	1	Video presentation on DNA structure and DNA replication	W1
10	1	Recapitulation and Discussion of Important Questions and test	-
	Total No Of Hours Planned For Unit 1=14		
		UNIT-II	
1	2	Genetic code and DNA transcription in prokaryotes	T1:224 to 248 R1:124 to 128
2	2	DNA transcription in eukaryotes	T1:250 to 255
3	2	Translation of RNA and steps involved	T1:111 to 123 R1:122 to 124

4	1	Post Translational modification	T1:255 to 263 R1:130 to 131
5	2	Operon concept Lac/Ara operon	T1:353 to 354 R1:130 to 131
6	2	Trp operon and eukaryotic gene regulation	T1.:360-363
7	1	Video presentation of Transcription, Translation and operons	W1
8	1	Recapitulation and Discussion of Important Questions	T1:516 to 529 R1:143 to 152
	Total No Of Hours Planned For Unit II=14		
		UNIT-III	
1	1	Genetic recombination in bacteria – conjugation	T1.149
2	2	transformation, transduction	T1.147 and 156
3	2	Linkage and genetic mapping	T1.103 to 134
4	1	Phage genetics (Replication cycle)	T1.154
5	1	Phage T4 mutants (detection and isolation)	W2
6	1	Genetic map of T4 phage.	T1.Pg:563-567
9	1	Recapitulation and Discussion of Important Questions	-
	Total No Of Hours Planned For Unit III=9		
		UNIT-IV	
1	2	Mutagen, Mutagenesis and mutation- Luria Delbruck experiment	T: 449 to 472 R1:212 to 214
2	2	Spontaneous and induced mutation –Types	T1:448 to 464 R1:214 to 224
3	2	Mutant detection and test of carcinogenicity	R1:214 to 224 T1: 449 to 456
4	2	DNA damage and type	R1: 232 to 236
5	2	DNA repair mechanism and types	T1: 472 to 477
6	1	Video and power point presentation	W3
7	1	Class test	

	Total No Of Hours Planned For Unit IV=12		
		UNIT-V	
1	1	Introduction to yeast Genetics and life cycle	R1: 120 to 123
2	1	Metabolism: genome and extra chromosomal elements	T1:130 to 131 R1: 143 to 152
3	1	Genetic nomenclature in yeast	R1: 153 to 157
4	1	Tetrad Analysis	R1:528 to 529
5	1	Petit mutant -yeast	T1:122 to 291
6	1	Petit mutant - Neurospora	T2.Pg:494
7	1	Petit mutant – Drosophila	T1:122 to 129/W1
8	1	Class Test	-
11	1	Discussion of Previous ESE Question Papers.	-
12	**Total no of Hours Planned for unit V=12**		
Total Planned Hours		**48**	

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

T1: Robert H. Tamarin, Principals of geneticsWm. C. Brown Publishers, 5th edition. Dubuque.

W1: https://www.youtube.com/watch?v=o_-6JXLYS-k
https://www.youtube.com/watch?v=dKubyIRiN84

W2:https://www.researchgate.net/profile/Robert_Villafane/publication/23642168_Construction_of_Phage_Mutants/links/02e7e529edea728efb000000/Construction-of-Phage- 8lI7E8X9XPKaJw&_iepl

W3: https://fac.ksu.edu.sa/sites/default/files/DNA_Damage_and_Repair.ppt

SUGGESTED READINGS:

1. Malacinski, G.M. (2008). *Freifelder's Essentials of Molecular Biology*. Narosa Publishing House, New Delhi.
2. Verma, P.S., and Agarwal, V.K., (2008). *CellBiology, Genetics, Molecular Biology and Evolution.* S. Chand & Company Ltd, New Delhi

3. Gardner, E.J., Simmons, M.J., and Snustad, D.P., (2008). *Principles of Genetics*. (8th ed.). John Wiley and Sons, NY.
4. Guthrie, C., and Fink, G., (2002). *Guide to Yeast Genetics and Molecular Cell Biology*. Elsevier Publication, USA.
5. Klug, W.S., Cummings, M.R. Spencer, C.A., and Palladino, M.A., (2009). *Essentials of Genetics*. (7th ed.). Prentice Hall, New Jersey.
6. Maloy, S.R., Cronan Jr, J.E., and Freifelder, D., (2001). *Microbial Genetics*. Narosa Publishing House. New Delhi.
7. Weaver, R.F. (2002). *Molecular Biology*. (2nd ed.). McGraw-Hill, New York.

UNIT – I
Syllabus

Genetics and its types – History; Mendelian principles – nucleic acid as genetic material Experimental evidence. Structure of DNA – chemical and physical structure of DNA – circular and super helical DNA - different forms of DNA. DNA replication – enzymology of DNA replication – different modes, models and types of DNA replication – Eukaryotic DNA replication.

Historical Development

The **history of genetics** dates from the classical era with contributions by Hippocrates, Aristotle and Epicurus. Modern biology began with the work of the Augustinian friar Gregor Johann Mendel. His work on pea plants, published in 1866, what is now Mendelian inheritance. Some theories of heredity suggest in the centuries before and for several decades after Mendel's work.

The year 1900 marked the "rediscovery of Mendel" by Hugo de Vries, Carl Correns and Erich von Tschermak, and by 1915 the basic principles of Mendelian genetics had been applied to a wide variety of organisms—most notably the fruit fly *Drosophila melanogaster*. Led by Thomas Hunt Morgan and his fellow "drosophilists", geneticists developed the Mendelian model, which was widely accepted by 1925. Alongside experimental work, mathematicians developed the statistical framework of population genetics, bringing genetic explanations into the study of evolution.

With the basic patterns of genetic inheritance established, many biologists turned to investigations of the physical nature of the gene. In the 1940s and early 1950s, experiments pointed to DNA as the portion of chromosomes (and perhaps other nucleoproteins) that held genes. A focus on new model organisms such as viruses and bacteria, along with the discovery of the double helical structure of DNA in 1953, marked the transition to the era of molecular genetics. In the following years, chemists developed techniques for sequencing both nucleic acids and proteins, while others worked out the relationship between the two forms of biological molecules: the genetic code. The regulation of gene expression became a central issue in the 1960s; by the 1970s gene expression could be controlled and manipulated through genetic engineering. In the last decades of the 20th century, many biologists focused on large-scale genetics projects, sequencing entire genomes.

Brief history of genetics

1866- E. Haeckel proposed that the nucleus contains the factors necessary for heredity.

1871- F. Miescher described some of the chemical property of DNA (C29H49N9P3O22). At that time proteins were supposed to be the hereditary material.

1989 - Altmann names "nucleic acids". 1903 - Walter Sutton proposes that chromosomes contain genetic material.

1908 – All 4 bases in DNA now characterised, (incl. T & C) in roughly equal amounts.

1909 - Enzymes found to be made from proteins. Archibold Garrod proposes chromosomes affects enzymes.

1915 - Phosphate "backbone" proposed to connect DNA bases together.

1927 - Ribose sugar associated with DNA characterised.

1928 - F. Griffith experiments with rough (R) and smooth (S) strains of Streptococcus pneumoniae in mice.

1941 - Beadle and Tatum propose "one gene, one enzyme" hypothesis.

1944 - Avery, MacLeod, McCarty show DNA is "transforming" agent (e.g., genetic material).

1952 - Alfred Hershey & Martha Chase demonstrate that DNA contains genetic material.

1953 - Postulation of a complimentary, double helical structure for DNA (by Watson and Crick).

1955 - Chargaff & Davidson publish exhaustive set of three volumes on "The Nucleic Acids", describing in great detail their physical properties and characterisation.

1956 -Genetic experiments support hypothesis that genetic messages of DNA are conveyed by its sequence of bp.

1958 - Meselson and Stahl demonstrate that DNA replicates semiconservatively. Isolation of the first enzyme (DNA polymerase I) by A. Kornberg

1959 - Discovery of RNA polymerase.

1960 - Discovery of messenger RNA.

1961 - The triplet nature of the genetic code is discovered.

Monad and Jacob propose operon model of gene regulation

1965 - Appreciation that genes conveying antibiotic resistance in bacteria are often carried on small bits of extrachromosomal DNA (plasmids).

1966 - Establishment of complete genetic code. 1967 - Isolation of the enzyme DNA ligase.

1970 - Isolation of the first restriction enzyme.

1970 - Temin and Baltimore report the discovery of reverse transcriptase in retroviruses.

1972 - Use of ligase to link together restriction fragments. First recombinant molecules generated.

1973 - Eukaryotic genes are cloned in bacterial plasmids.

1976 - Retroviral oncogenes are identified as the causative agents of transformation.

1977 - DNA sequencing becomes possible.

Interrupted genes are discovered and splicing of their transcripts is inferred.

1978 - Production of first human hormone (somatostatin) using recombinant DNA methods.

1979 - Cellular oncogenes are discovered by transfection.

1981 - Catalytic activity of RNA is discovered. Transgenic mice and flies are obtained by introducing new DNA into the germ line.

1983 - First version of "GenBank" created for storage of DNA sequences.

1986 - Proposal of Intramolecular Triplex structure for certain purine rich DNA sequences.

1989 - Polymerase Chain Reaction (PCR) technique first used.

1995 - First BACTERIAL genomes completely sequenced. (Haemophilus influenzae and Mycoplasma genitalium).

1996 - Genome of first EUKARYOTE completely sequenced. (Saccharomyces cerevisiae, 13,000,000 bp on 16 chromosomes).

1997 - Dolly the Sheep cloned. E.coli genome sequenced.

2002/03 - Genome of Pasmodium (30 MB), Caenorhabditis elegans (100 Mb), Arabidopsis thaliana (100 Mb), Drosophila melanogaster (120 Mb), Mus musculus (300 Mb) have been completely sequenced.

2004 - The Homo sapiens genome (3000 MB) is sequenced.

2009 – Full Genome sequencing.

Heredity

- What genetic principles account for the transmission of traits from parents to offspring?
- One possible explanation of heredity is a "blending" hypothesis - The idea that genetic material contributed by two parents mixes in a manner analogous to the way blue and yellow paints blend to make green
- An alternative to the blending model is the "particulate" hypothesis of inheritance: the gene idea - Parents pass on discrete heritable units, genes

Gregor Mendel

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

Mendelian Genetics

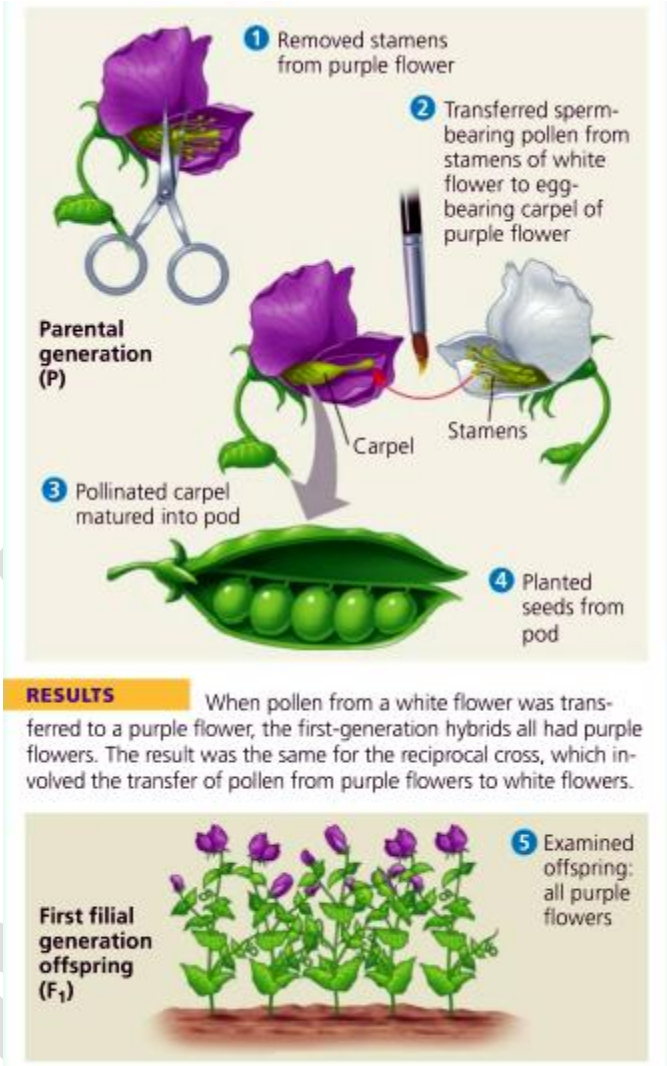
- Gregor Johann Mendel (1822-1884)
  - Augustinian monk, Czech Republic
  - Foundation of modern genetics
  - Studied segregation of traits in the garden pea (*Pisum sativum*) beginning in 1854
  - Published his theory of inheritance in 1865. "Experiments in Plant Hybridization"
  - Mendel was "rediscovered" 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.

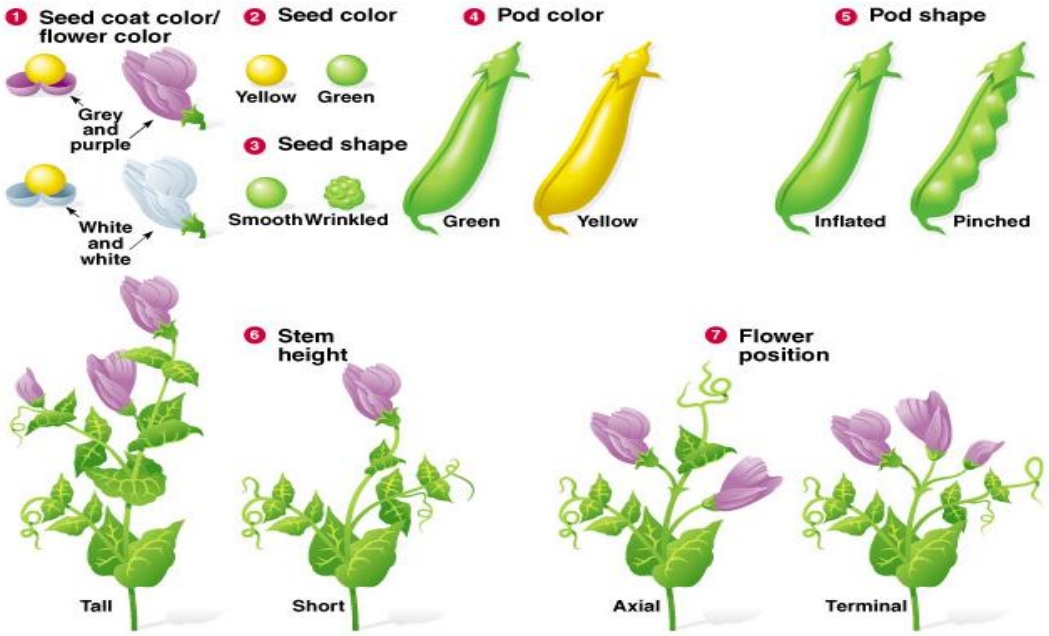
Crossing Pea Plants



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



Mendel's Studied Discrete Traits

Table 14.1 The Results of Mendel's F_1 Crosses for Seven Characters in Pea Plants

Character	Dominant Trait	x	Recessive Trait	F_2 Generation Dominant:Recessive	Ratio
Flower color	Purple	×	White	705:224	3.15:1
Flower position	Axial	×	Terminal	651:207	3.14:1
Seed color	Yellow	×	Green	6,022:2,001	3.01:1
Seed shape	Round	×	Wrinkled	5,474:1,850	2.96:1
Pod shape	Inflated	×	Constricted	882:299	2.95:1
Pod color	Green	×	Yellow	428:152	2.82:1
Stem length	Tall	×	Dwarf	787:277	2.84:1

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)

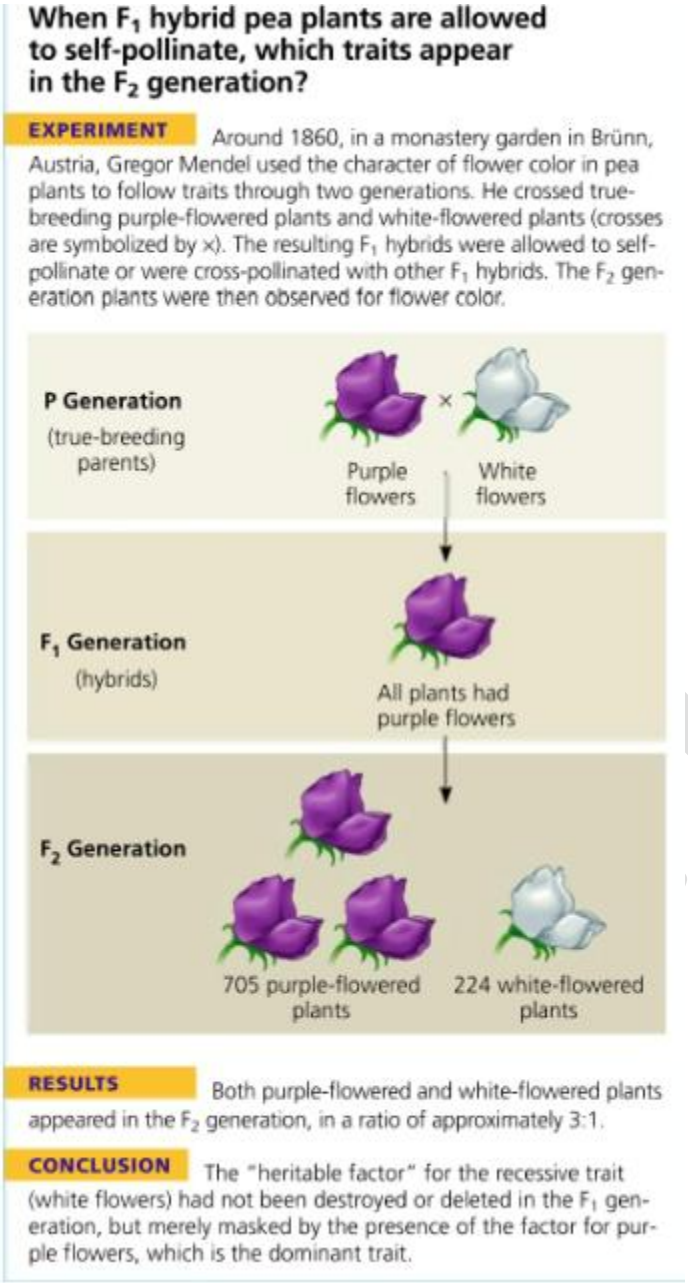
- **Lowercase traits**– recessive phenotypes(yy)
- ***Generations***:
    - P = parental generation
    - F1 = 1st filial generation, hybrid progeny of the P generation
    - F2 = 2nd filial generation, hybrid progeny of the F1 generation (F3 and so on)

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

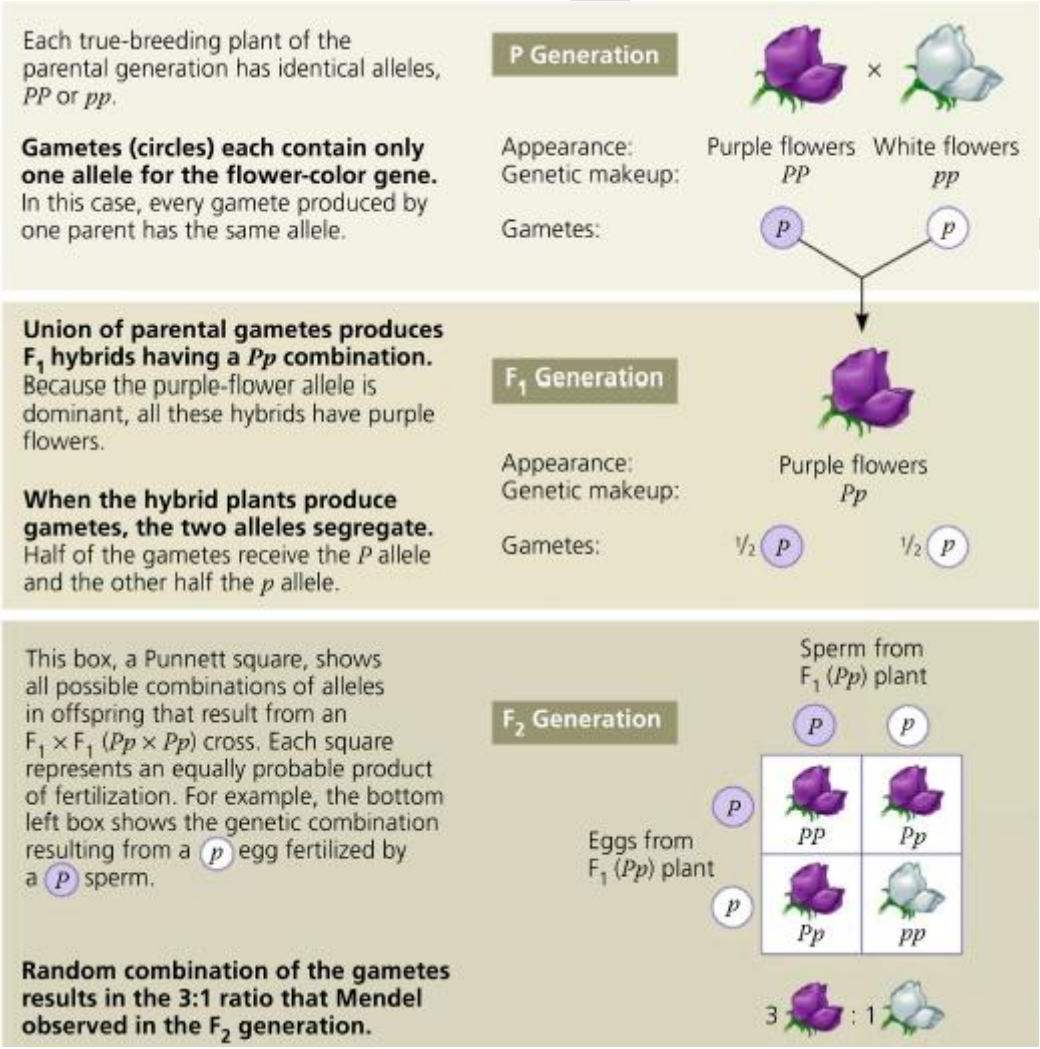
Mendel's Law of Independent Assortment: Genes on different chromosomes behave independently in gamete production.

Example: Dihybrid Cross

Punnett Squares

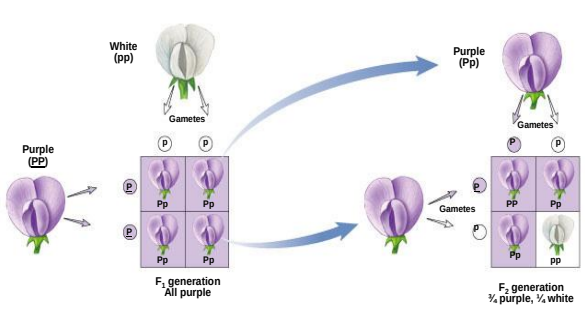
- A Punnett square is a grid that enables one to predict the outcome of simple genetic crosses
- Proposed by the English geneticist, Reginald Punnett

Mendel's Law Of Segregation



Monohybrid Cross

A cross of two different true-breeding strains (homozygotes) that differ in a single trait

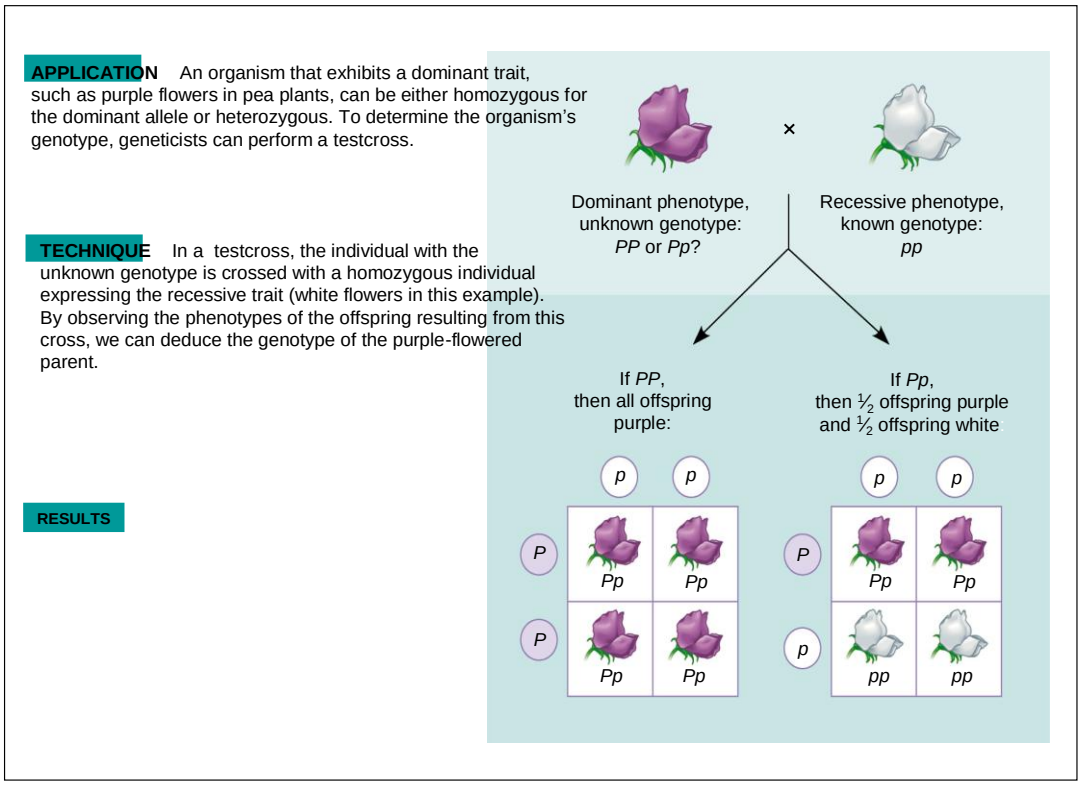


Test Cross

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)



Test Cross

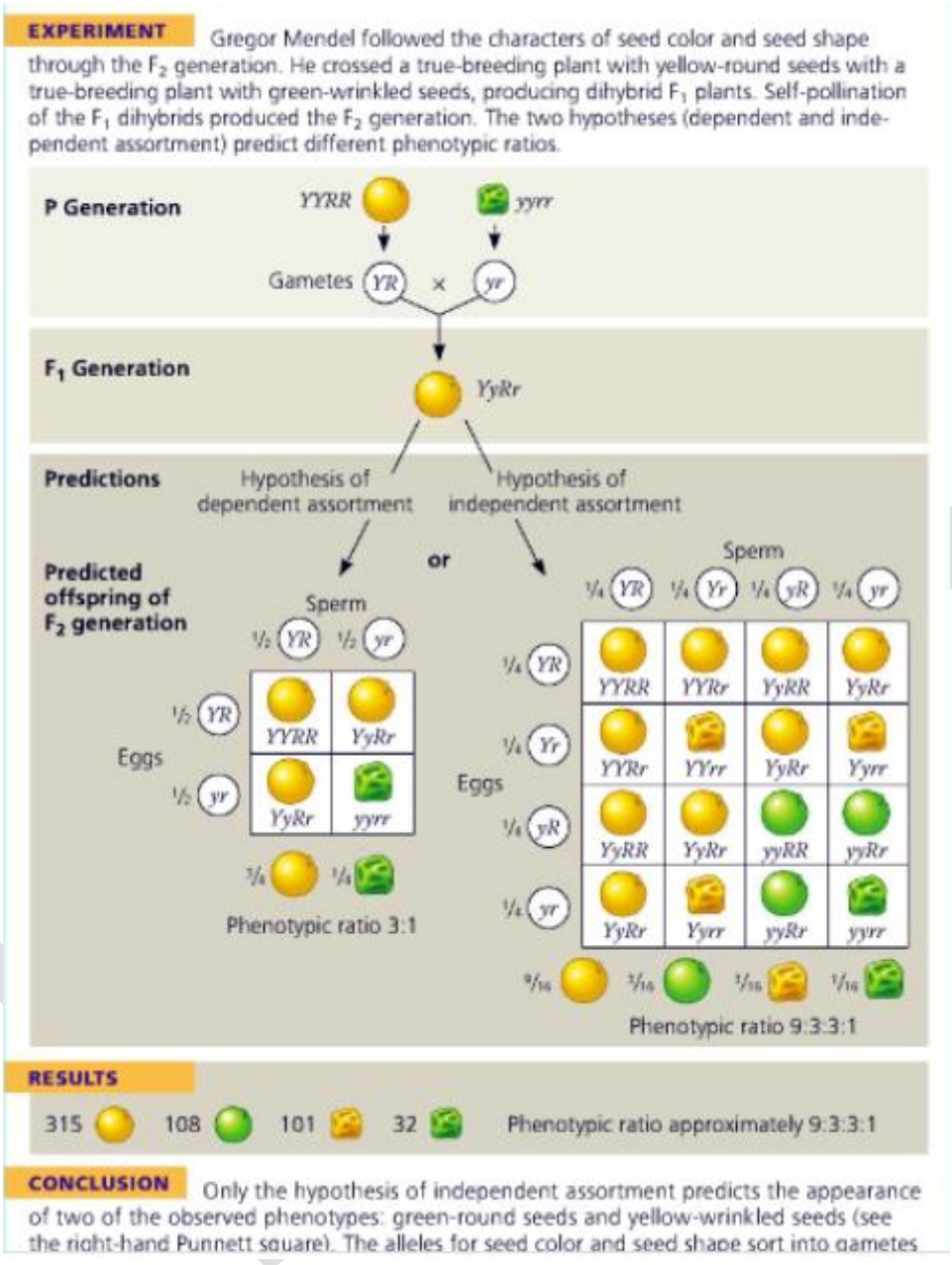
Back Cross

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.



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.

DNA & RNA : The Genetic Material

Genetic Materials

A living cell is composed of several inorganic and organic components. Among them, one will obviously act as genetic material responsible for controlling hereditary characters. Identification of this genetic material remained controversial for a long time.

The genetic material must be capable of storing hereditary information and replicate with high efficiency in successive cell generations forming the basis for transmission of hereditary characteristics it controls. Gene action through a series of chemical reactions results in the ultimate expression of the characteristics within the organism. The genetic material does undergo occasional heritable changes called mutation.

The Search for the Genetic Material

- 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.

- 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.

properties:

a. To control the innumerable diversities in the characteristics of organism available in nature, the genetic material must show a very wide diversity in form.

b. Since phenotype character is the final expression of a chain of reactions initiated at the gene level, obviously the genetic material must be a chemically unique entity.

Before 1900 several biologists proposed that hereditary material must be in the chromosome of the cell nucleus. In 1903, Sutton and Bovery postulated that genes were located in chromosome.

- 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.

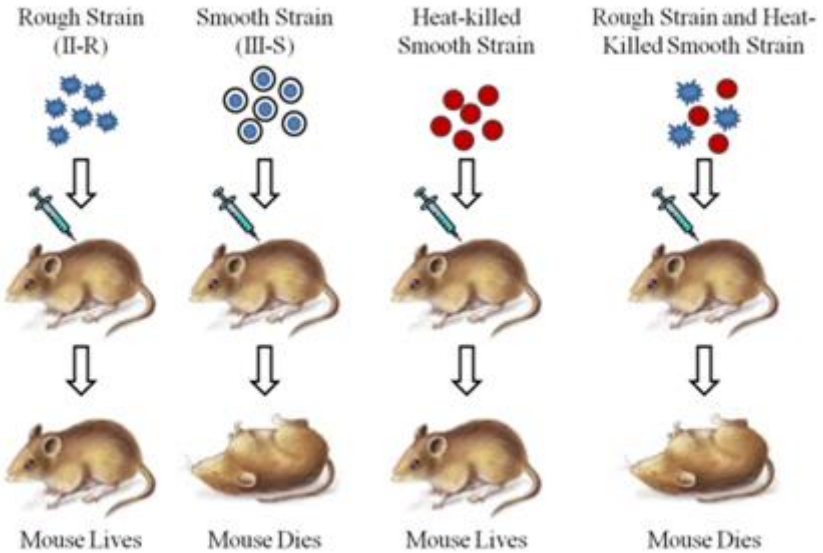


Fig. Griffith's transformation experiment

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

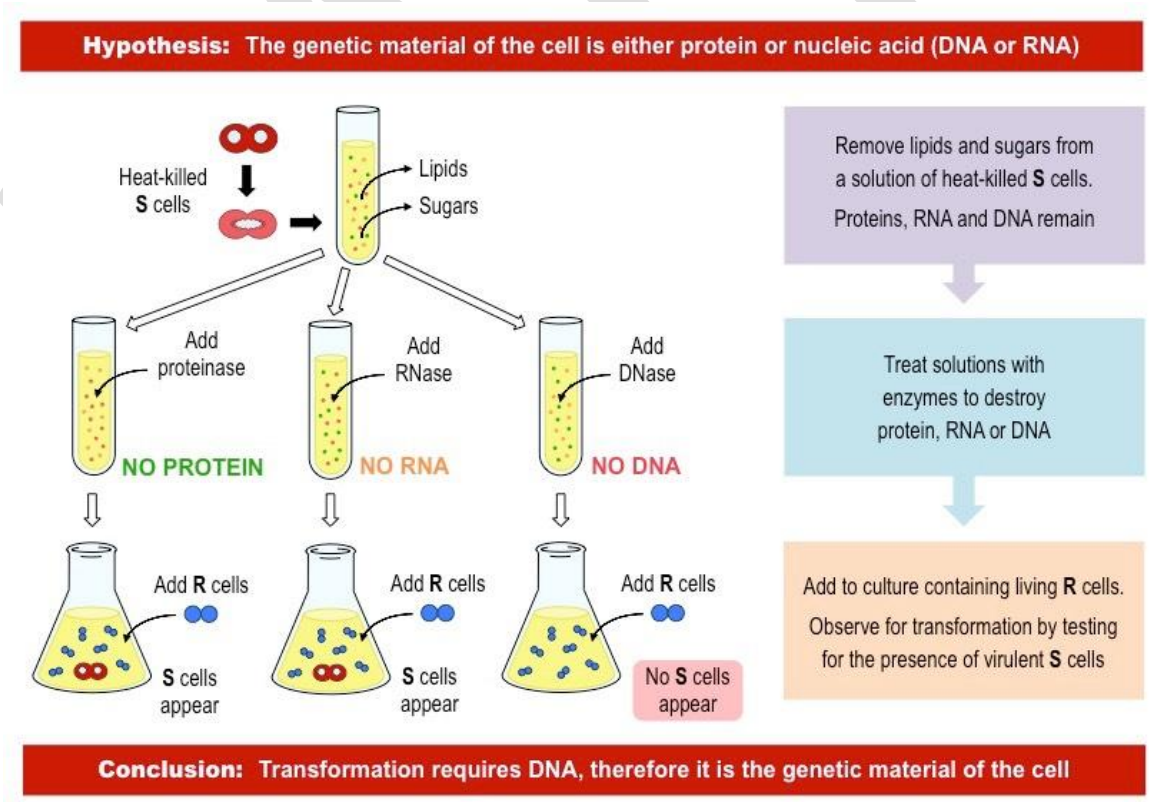
Griffith's conclusion:

- 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

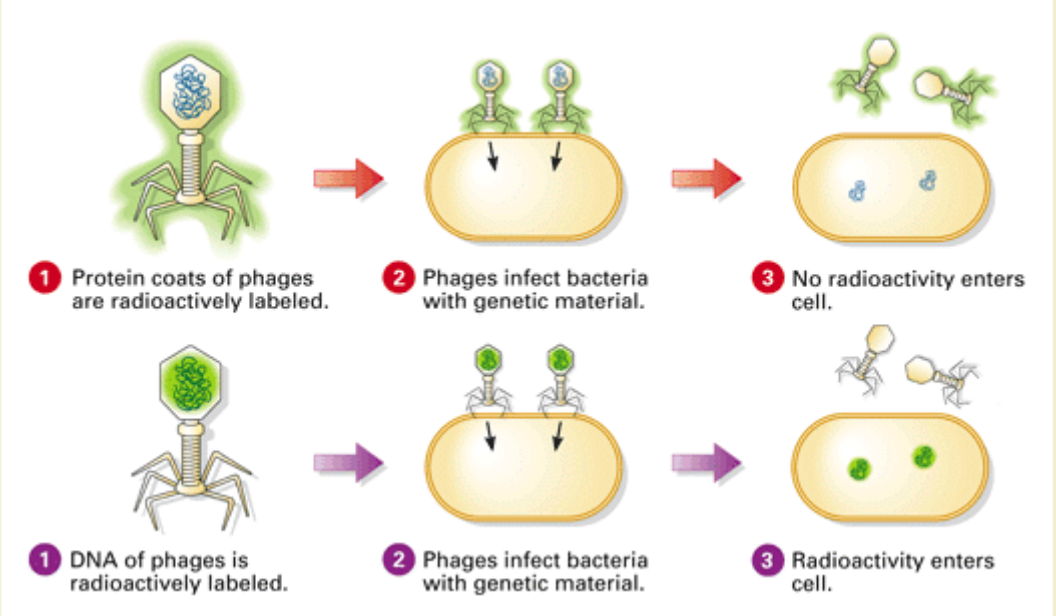
- In 1944, Avery, MacLeod and McCarty published results of a study that identified the transforming principle from *S. pneumoniae*. Their approach was to break open dead cells, chemically separate the components (e.g., protein, nucleic acids) and determine which was capable of transforming live *S. pneumoniae* 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.



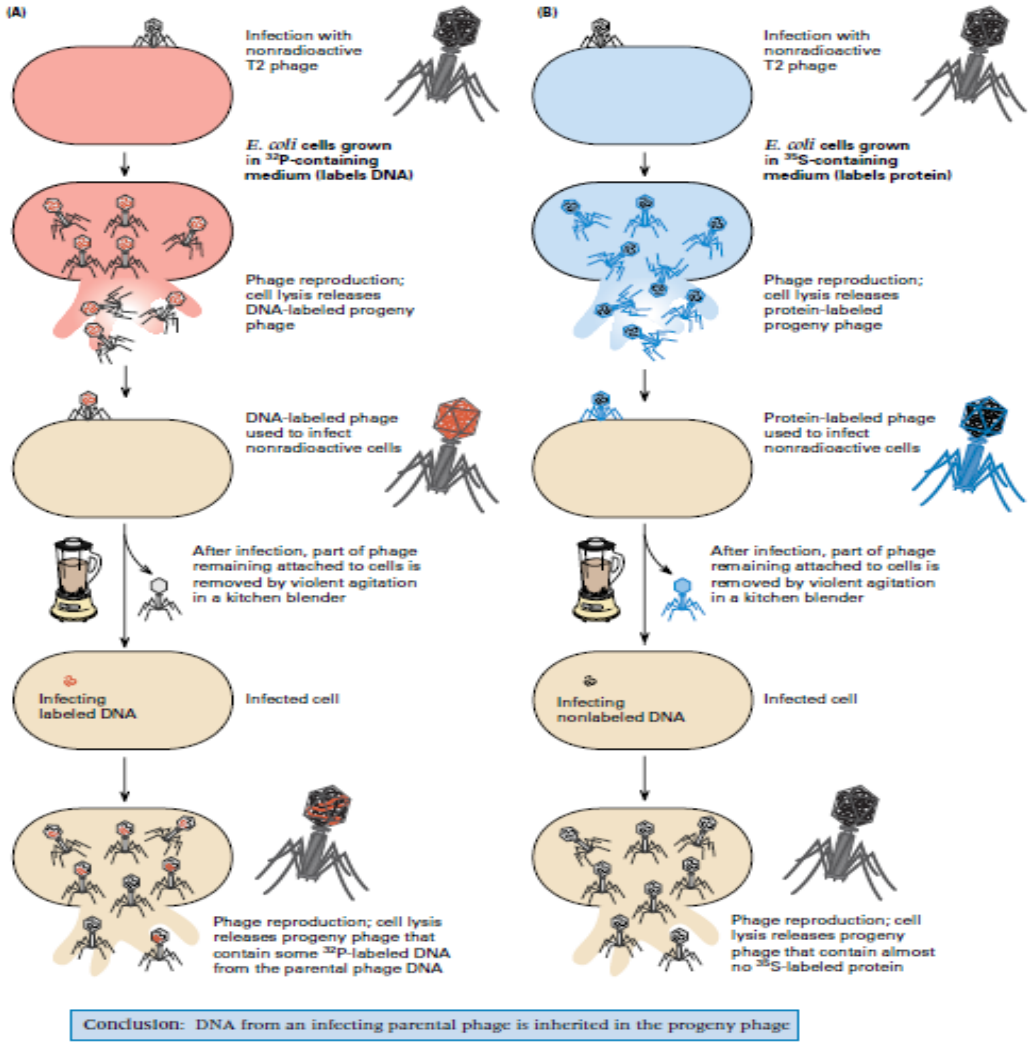
Experiment that showed that DNA, not RNA, was the transforming principle

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.

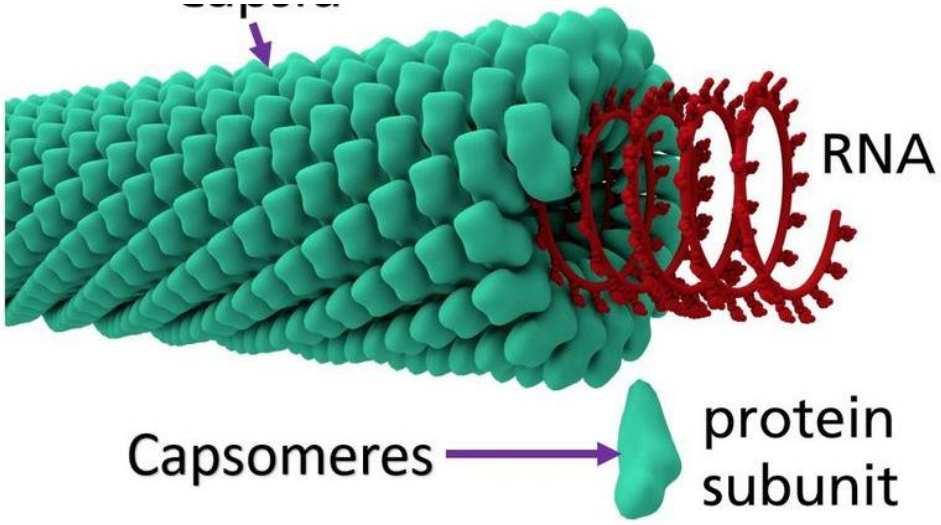


Hershey-Chase experiment demonstrating DNA is genetic material

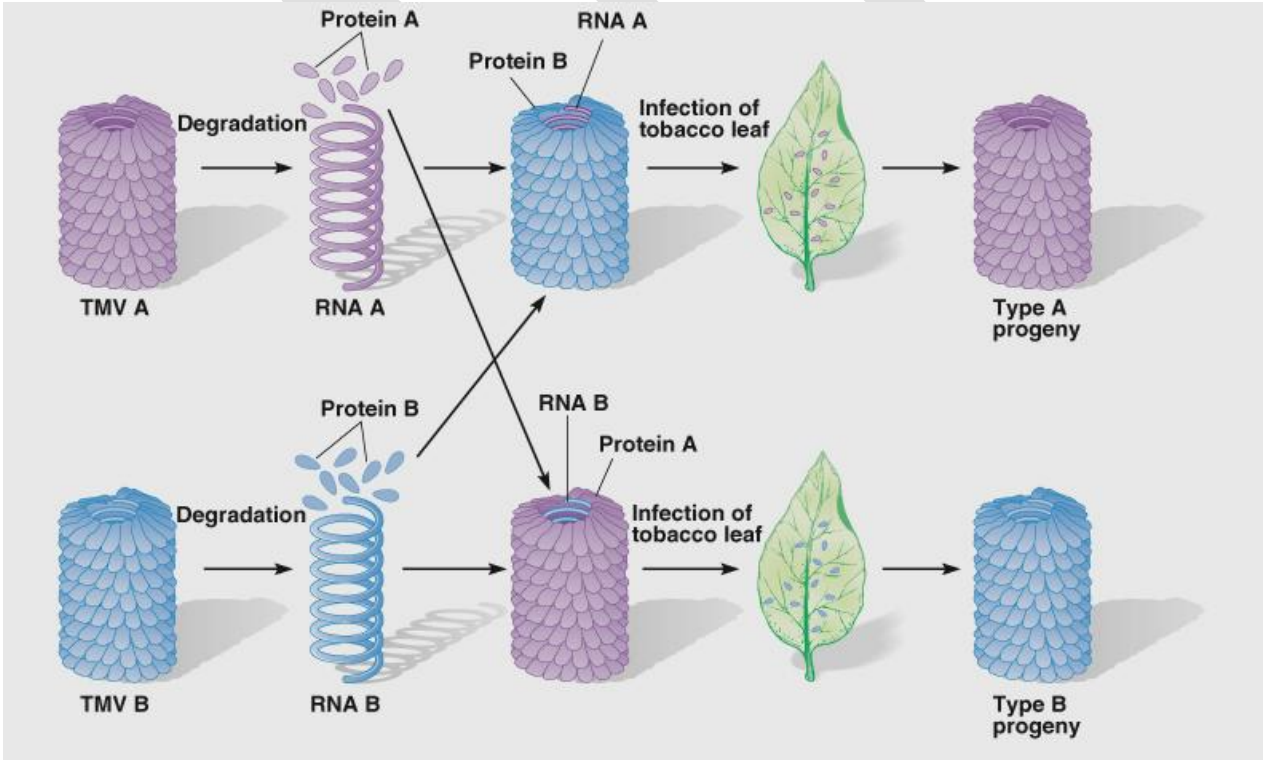


The Discovery of RNA as Viral Genetic Material

- 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.



Typical tobacco mosaic virus (TMV) particle

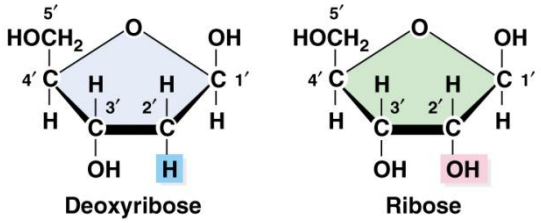


Demonstration that RNA is the genetic material in tobacco mosaic virus (TMV)

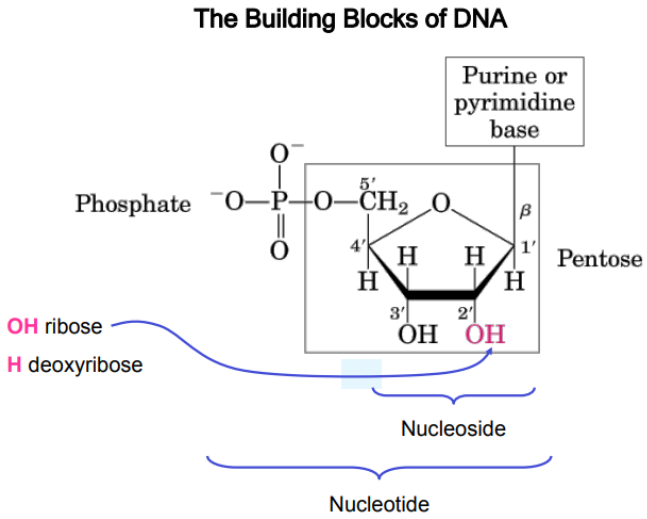
The Composition and Structure of DNA & RNA

DNA and RNA are polymers composed of monomers called nucleotides.

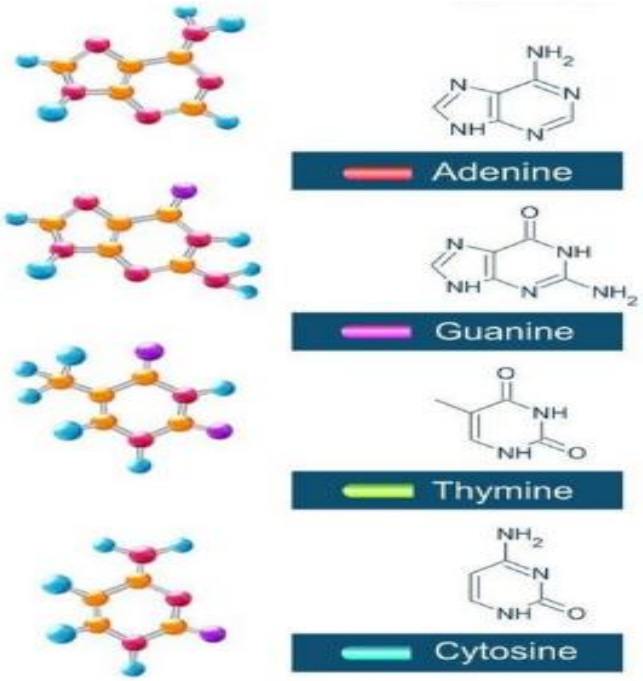
- Each nucleotide has three parts:
  - A pentose (5-carbon) sugar.
  - A nitrogenous base.
  - A phosphate group.
- The pentose sugar in RNA is ribose, and in DNA it's deoxyribose. The only difference is at the 29 position, where RNA has a hydroxyl (OH) group, while DNA has only a hydrogen.



Structures of deoxyribose and ribose in DNA and RNA



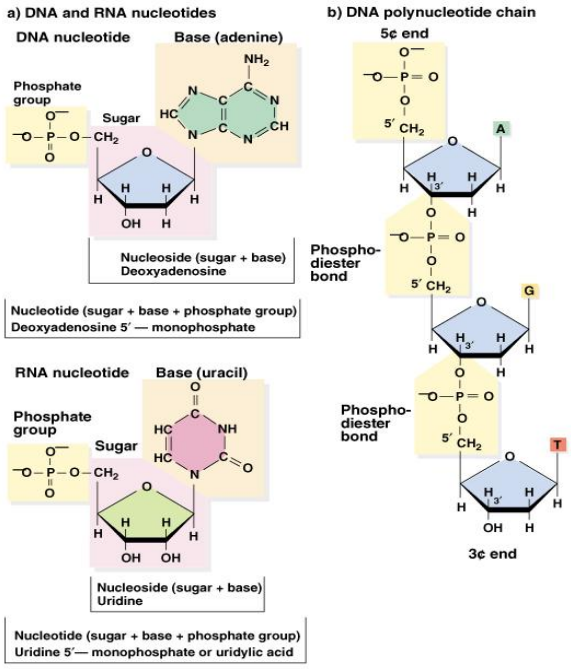
- There are two classes of nitrogenous bases:
  - Purines (double-ring, nine-membered structures) include adenine (A) and guanine (G).
  - Pyrimidines (one-ring, six-membered structures) include cytosine (C), thymine (T) in DNA and uracil (U) in RNA.



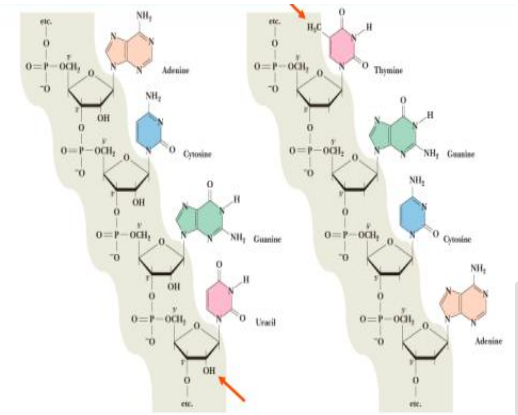
Structures of the nitrogenous bases in DNA and RNA

The structure of nucleotides has these features:

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



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



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. 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.

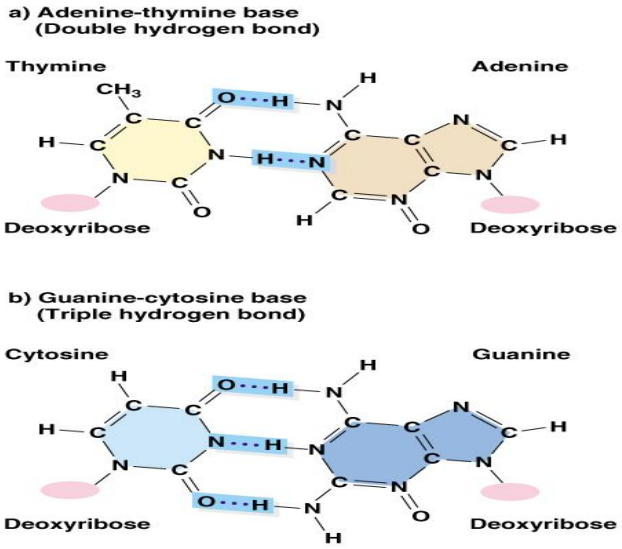
Watson and Crick's three-dimensional model has the following main features:

It is two polynucleotide chains wound around each other in a right-handed helix.

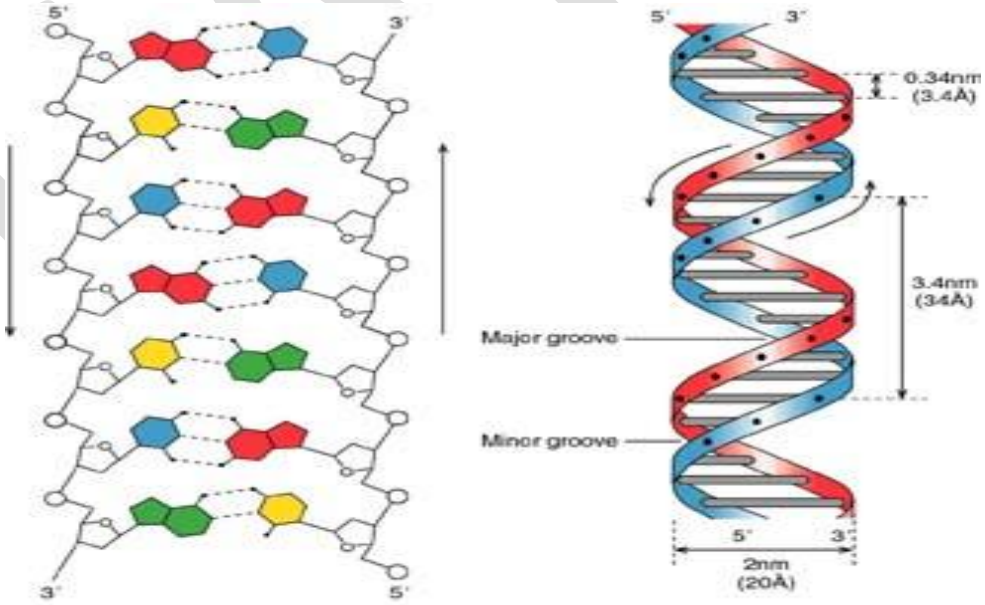
The two chains are antiparallel.

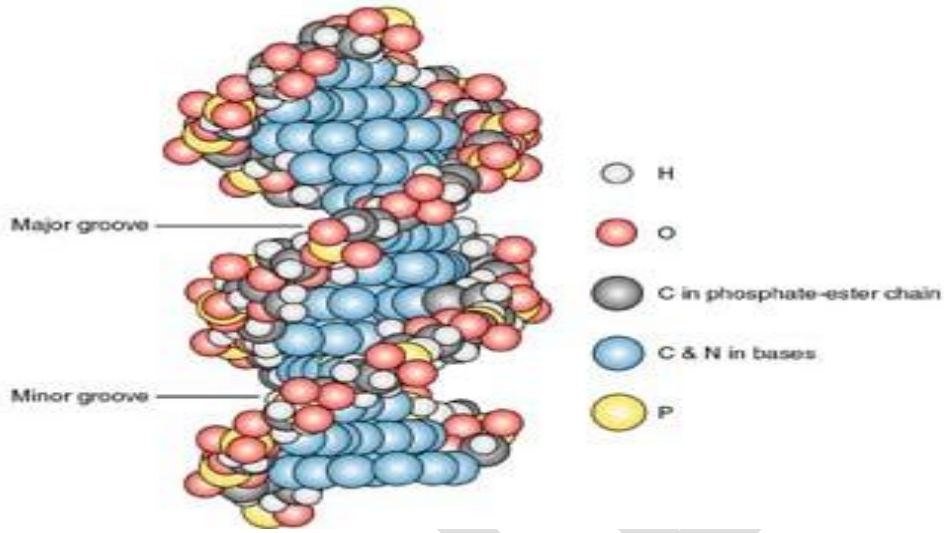
The bases of the two strands are held together by hydrogen bonds with complementary bases on the opposite sugar-phosphate backbone(two for A-T pairs and three for G-C pairs). Individual H-bonds are relatively weak and so the strands can be separated (by heating, for example). Complementary base pairing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

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.





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.

Table 12.1

Comparison of the Structural Properties of A-, B-, and Z-DNA

Double Helix Type	**A**	**B**	**Z**
Overall proportions	Short and broad	Longer and thinner	Elongated and slim
Rise per base pair	2.3 Å	3.32 Å ± 0.19 Å	3.8 Å
Helix packing diameter	25.5 Å	23.7 Å	18.4 Å
Helix rotation sense	Right-handed	Right-handed	Left-handed

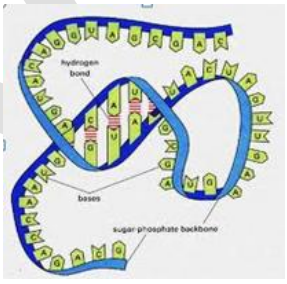
Base pairs per helix repeat	1	1	2
Base pairs per turn of helix	~11	~10	12
Mean rotation per base pair	33.6°	35.9° ± 4.2°	260°/2
Pitch per turn of helix	24.6 Å	33.2 Å	45.6 Å
Base-pair tilt from the perpendicular	+19°	-1.2° ± 4.1°	-9°
Base-pair mean propeller twist	+18°	116° ± 7°	~0°
Helix axis location	Major groove	Through base pairs	Minor groove
Major groove proportions	Extremely narrow but very deep	Wide and with intermediate depth	Flattened out on helix surface
Minor groove proportions	Very broad but shallow	Narrow and with intermediate depth	Extremely narrow but very deep
Glycosyl bond conformation	anti	anti	anti at C, syn at G

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



Structure of RNA

Types of RNA

Type	Abbr	Function	Distribution
Messenger RNA	mRNA	Codes for protein	All organisms
Ribosomal RNA	rRNA	Translation	All organisms
Transfer RNA	tRNA	Translation	All organisms

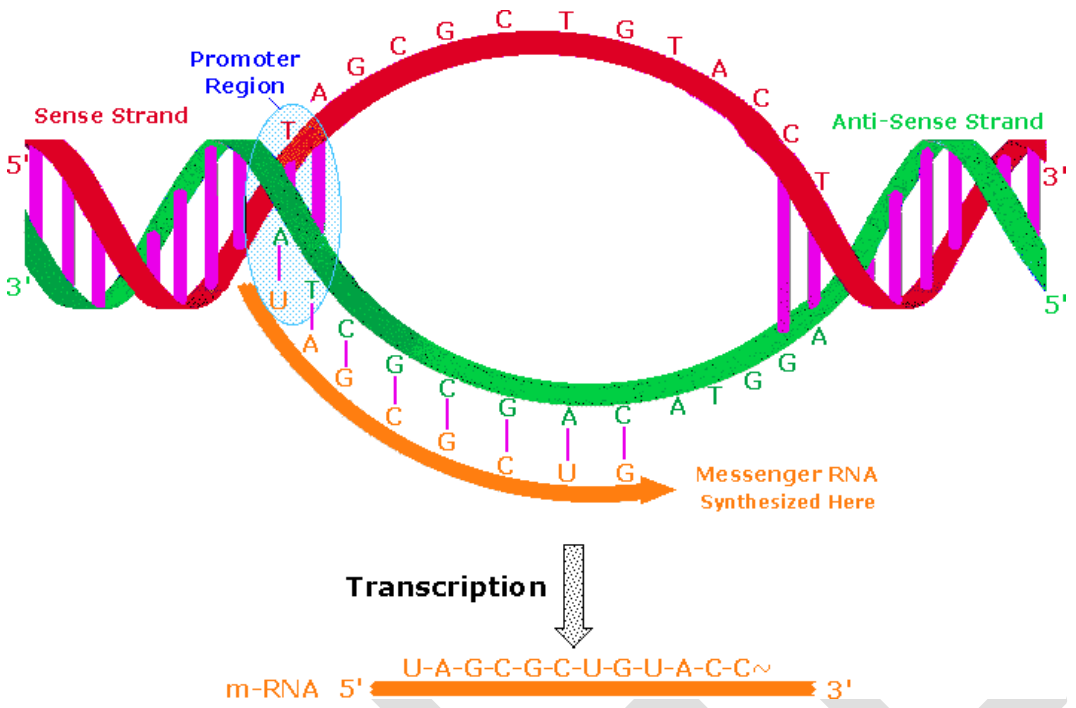
Based on the functions of RNA it is classified into two which include,

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

Messenger RNA

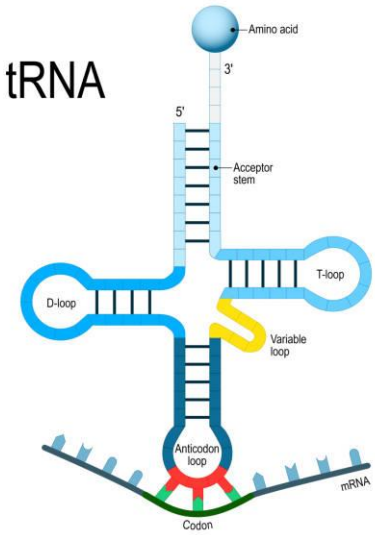
mRNA carries information about a protein sequence to the ribosomes, the protein synthesis factories in the cell. It is coded so that every three nucleotides (a codon) correspond to one amino acid. In eukaryotic cells, once precursor mRNA (pre-mRNA) has been transcribed from DNA, it is processed to mature 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.



Messenger RNA

Transfer RNA

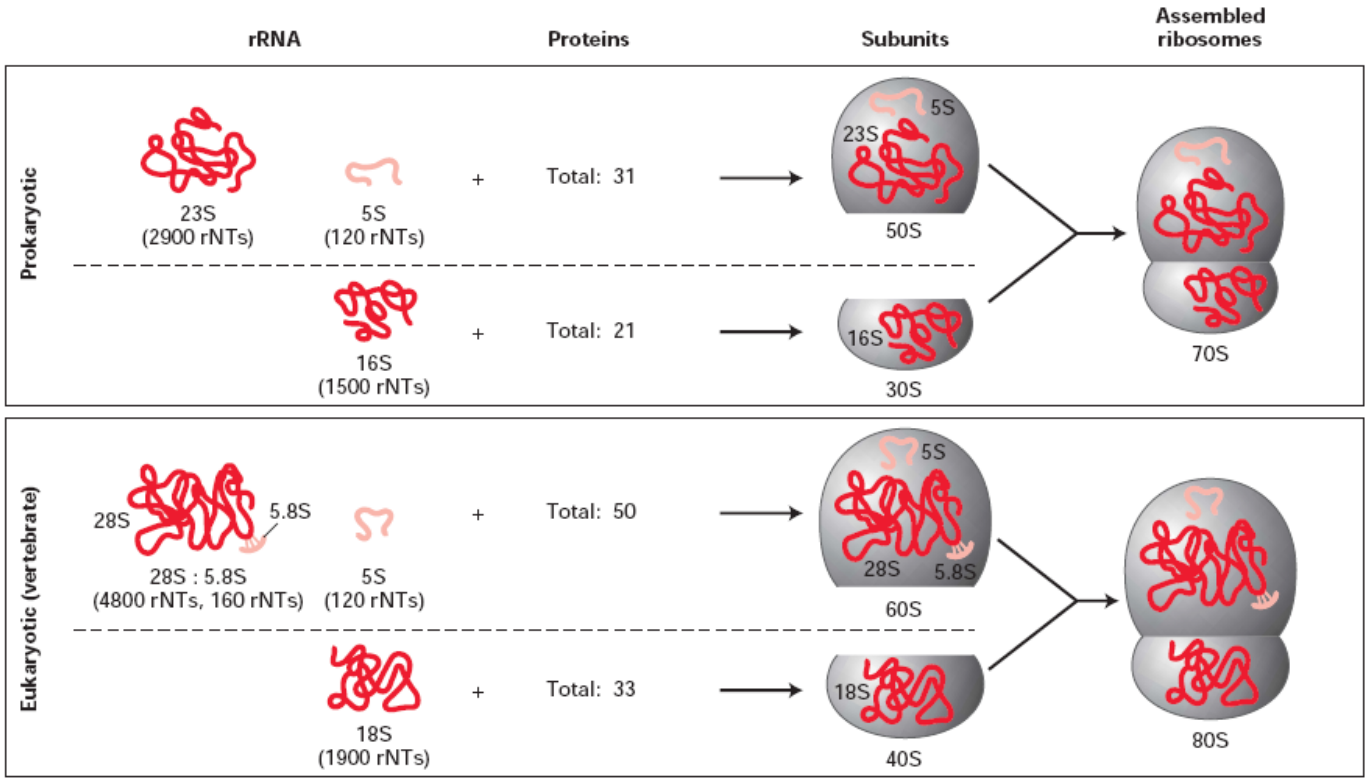
Transfer RNA (tRNA) is a small RNA chain of about 80 nucleotides that transfers a specific amino acid to a growing polypeptide chain at the ribosomal site of protein synthesis during translation. It has sites for amino acid attachment and an anticodon region for codon recognition that site binds to a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



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.



Prokaryotic and Eukaryotic ribosomes

Types of non-genetic RNA and their functions

There are three types of non-genetic RNA.

(1) mRNA or messenger RNA, (2) rRNA or ribosomal RNA and (3) tRNA or transfer RNA.

(1) mRNA (Messenger RNA) : This is called messenger RNA because it carries information for protein synthesis from the DNA to the ribosomes in the cytoplasm (the site of protein synthesis). m-RNA constitutes about 3-5% of the total RNA. It is produced on theDNA strand. The process is called transcription. Hence, the base sequence of mRNA is complementary to that of the DNA strand. The bases on the mRNA strand are organized into triplets. Each triplet

consists of a sequence of three consecutive bases (nucleotides) and is called a codon (code word). Each codon specifies one amino acid. The sequence of codons on the mRNA strand is called themRNA language. It indicates the sequence of amino acids for the synthesis of a protein. It begins with the codon AUG (initiation codon or starting codon) and ends with either UAA, UAG or UGA (stop codons). The single-stranded mRNA molecule is always straight (Fig. 8.10) and therefore, base pairing is totally absent in mRNA.

Role of m-RNA in protein synthesis

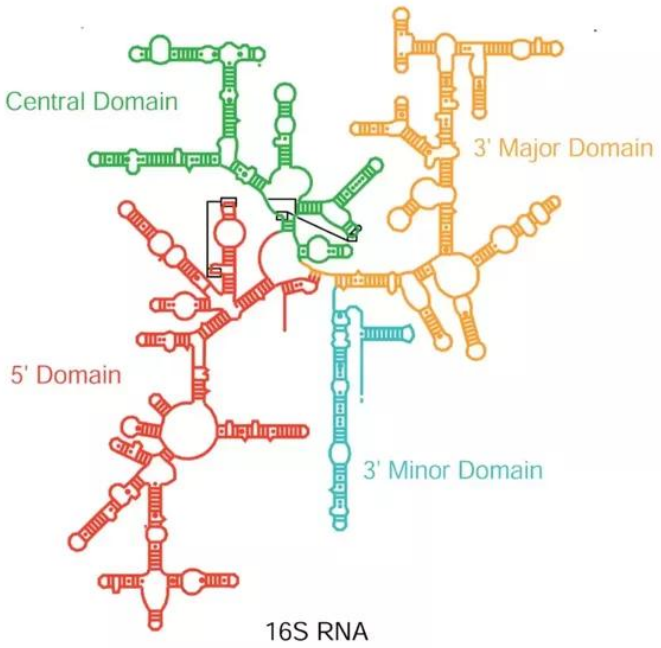
- ✓ Represents the sequence of codons from the DNA strand (transcription).
- ✓ Brings the sequence to the ribosomes (site of protein synthesis) in the cytoplasm.
- ✓ Provides the sequence for the synthesis of specific protein from the amino acids with the help of t-RNA (translation).

rRNA (Ribosomal RNA) : r RNA forms about 80% of the total RNA. It is present in the ribosomes in the cell cytoplasm (site of protein synthesis) and hence called rRNA. The single-stranded molecule of rRNA is variously folded and twisted upon itself in certain regions. In such folded regions, complementary bases form pairs and are joined by hydrogen bonds.

Role of rRNA in protein synthesis: The role of rRNA in protein synthesis is not yet very clearly known but it is known to complex with various protiens. The resulting structure is a ribosome, and this complex reads the coded sequence in mRNA to link amino acids together into particular protiens.

- ➢ It provides proper binding sites for the mRNA of the ribosomes.
- ➢ It orients the mRNA in such a way that its nitrogen base triplets or codons are properly read or translated.
- ➢ It also releases tRNA after the transfer of activated amino acid.
- ➢ It protects the mRNA strand from the action of enzymes (nucleases).
- ➢ It protects the growing (nascent) polypeptide chain from proteolytic enzymes.

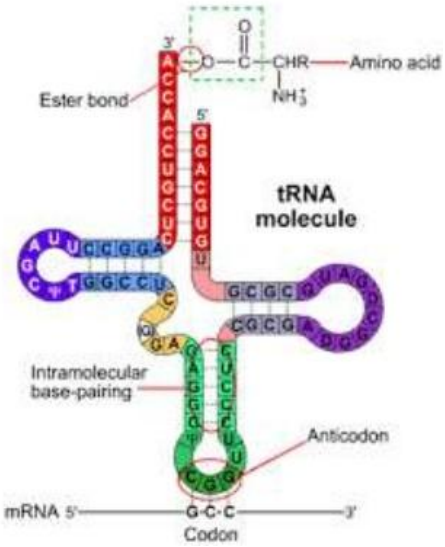
tRNA (Transfer RNA) : It is the smallest of all the types of RNA. About 10 to 20% of the total RNA of the cell is of this type. tRNA strand is folded upon itself forming loops. It results in either a clover leaf pattern or hair pin pattern (Figure 8.12). One end of the strand has guanine, while the other end carries the CCA combination of nitrogen bases. A triplet of nitrogen bases called anticodon is present on one of the loops. The anticodon pairs with the complementary codon on the mRNA molecule. The tRNA molecules carry amino acids to the mRNA during the process of protein synthesis. Each type of the amino acid is carried by a specific tRNA molecule. tRNA is synthesized on the DNA template. It has complementary base pairs in folded regions.



tRNA

tRNA contain 5 main arms or loops:

- Acceptor
- Anticodon arm
- DHU arm
- TΨC arm
- Extra arm



Role of tRNA in protein synthesis

- tRNA carries the required specific amino acids from cell cytoplasm to the ribosome (site of protein synthesis).
- Each type of amino acid is carried by a specific type of tRNA.
- In the ribosome, tRNA helps to arrange the amino acids in their proper sequence for the synthesis of a protein. This is done with the help of the codons on the mRNA and the matching (complementary) anticodons on the tRNA (translation).

Difference between RNA & DNA

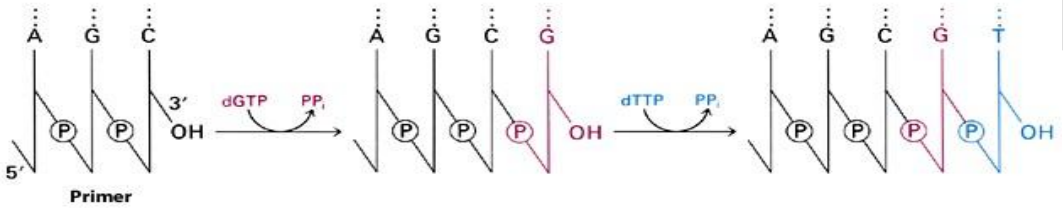
RNA	DNA
RNA nucleotides contain ribose sugar	DNA contains deoxyribose
RNA has the base uracil	DNA has the base thymine
presence of a hydroxyl group at the 2' position of the ribose sugar.	Lacks of a hydroxyl group at the 2' position of the ribose sugar.
RNA is usually single-stranded	DNA is usually double-stranded

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

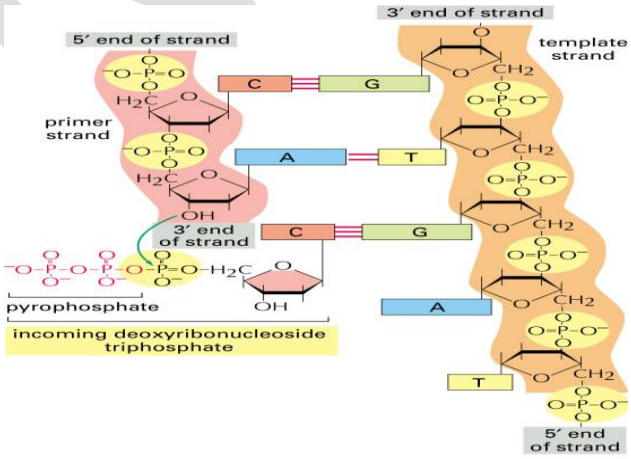
Nucleotide monomers are added one by one to the end of a growing strand by an enzyme called a ***DNA polymerase.***



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.



5'→3 nucleotide addition

***Four components are required*:**

I. *dNTPs*: dATP, dTTP, dGTP, dCTP (deoxyribonucleoside 5'-triphosphates) (sugar-base + 3 phosphates).

II. *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.

III. *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.

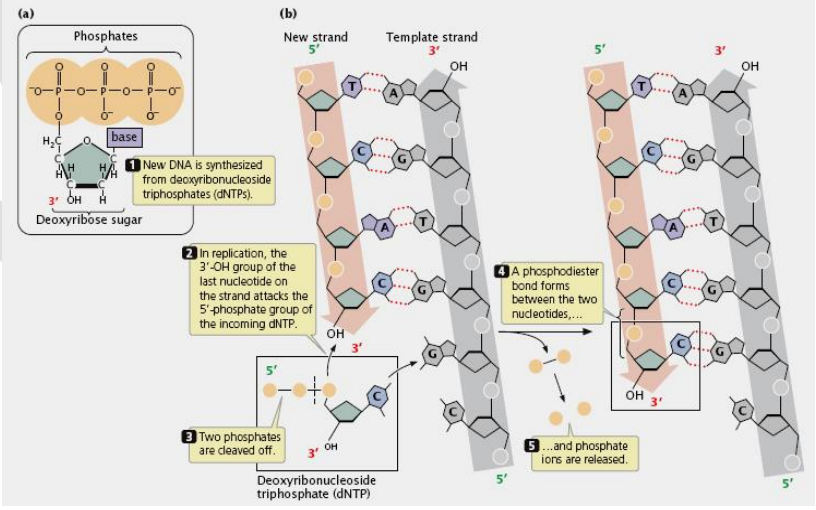
IV. *DNA polymerase I* (formerly the *Kornberg enzyme*) (DNA polymerase II & III discovered soon after).

V. *Mg 2+* (optimizes DNA polymerase activity)

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

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. DNA exists in the nucleus as a condensed, compact structure. To prepare DNA for replication, a series of proteins aid in the unwinding and separation of the double-stranded DNA molecule. These proteins are required because DNA must be single-stranded before replication can proceed. They include:



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.

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 not catalyze 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.

DNA polymerases:

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

DNA polymerase I: functions in repair and replication

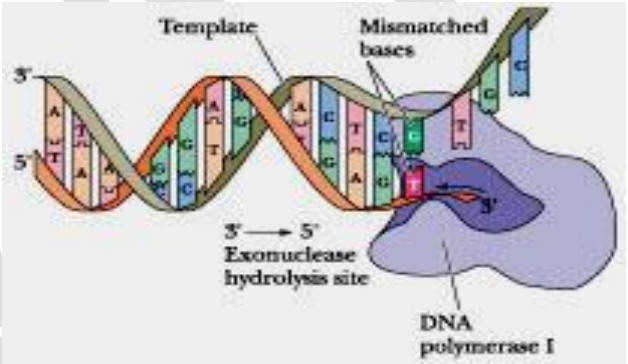
DNA polymerase II: functions in DNA repair (proven in 1999)

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,Arthur kornberg showed that the extracts of *E.coli* has five different enzymatic activities, *3'-5' exonuclease activity* (proof reading to increase fidelity**)**



Proofreading and Editing of DNA polymerase I

5'-3' exonulcease activity (remove RNA primers or damage DNA on its path)

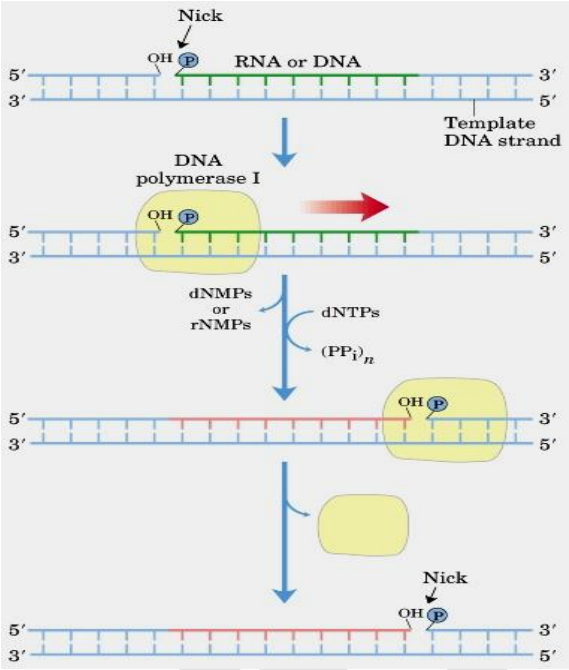
Endonuclease activity(involved in excision repair)

Nick Translation (nick or gap moves along moves along the direction of synthesis) It requires 5'-3' activity of DNA pol I

Steps

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

- The nick is closed via DNA ligase



Nick translation

DNA Polymerase II :

Primary function is repair, but it also can serve as an alternative replication

Enzyme 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

- The requirement for a free 3' hydroxyl group is fulfilled by the RNA primers that are synthesized at the initiation sites by these enzymes.

DNA ligase:

- Nicks occur in the developing molecule because the RNA primer is removed and synthesis proceeds in a discontinuous manner on the lagging strand.
- The final replication product does not have any nicks because DNA ligase forms a covalent phosphodiester linkage between 3'-hydroxyl and 5'-phosphate groups.
- 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.

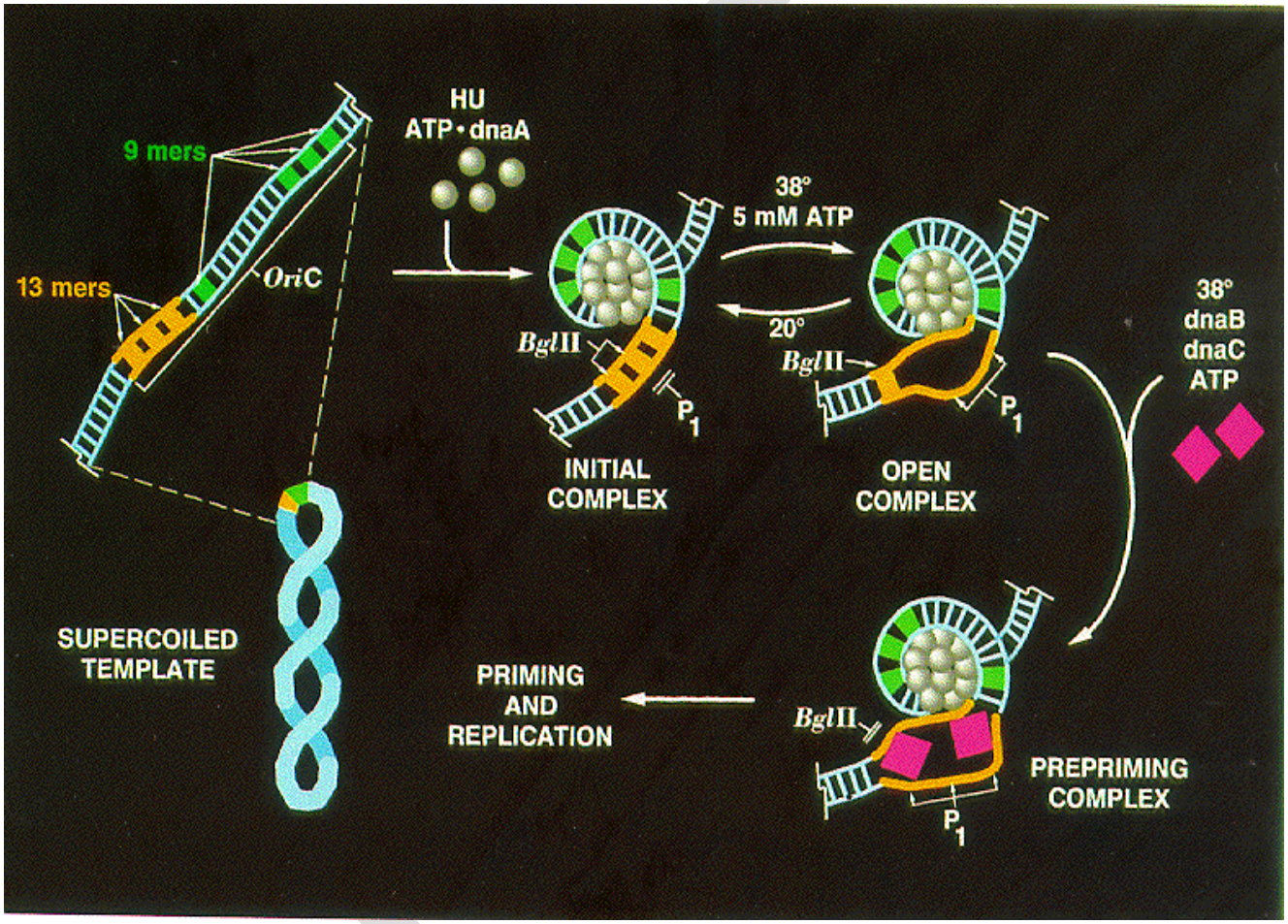
- ***Covalent extension***: synthesis of new strand as an extension of an old strand ("Rolling Circle")

The steps and components involved in the initiation reaction include:

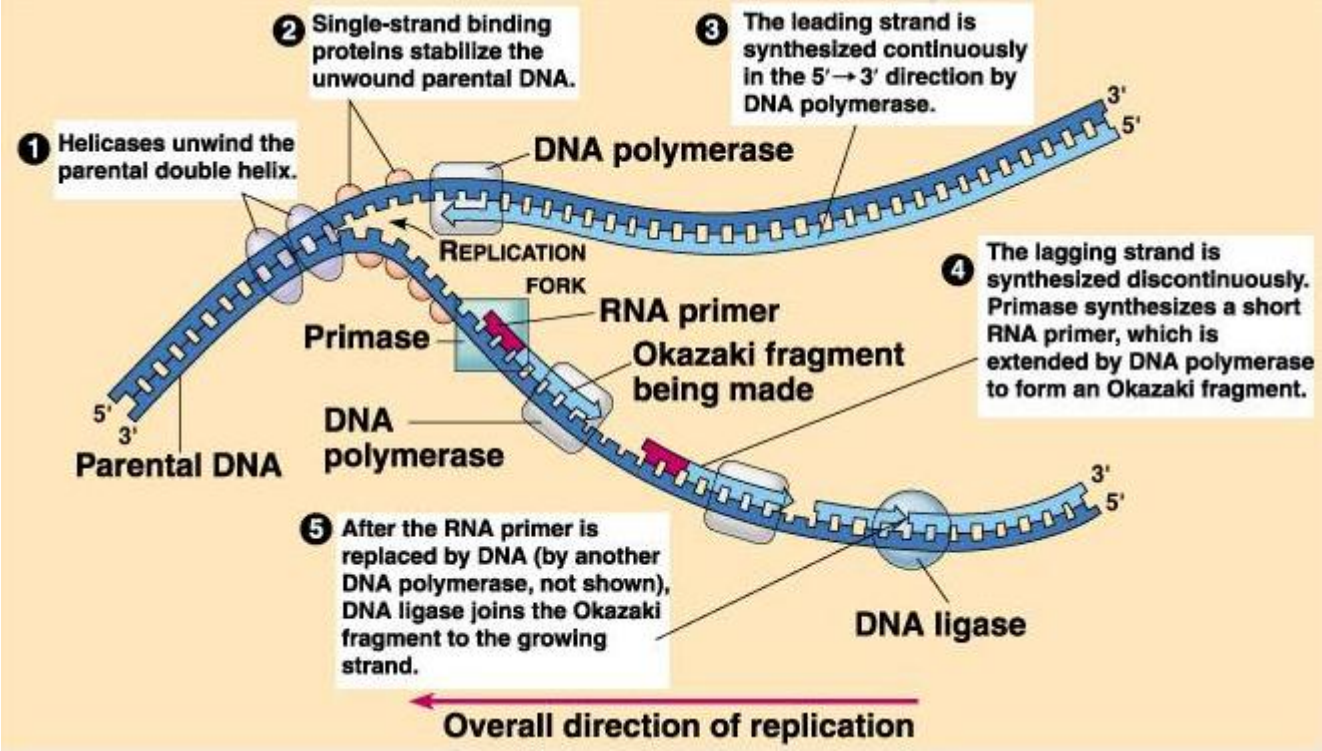
- Binding of dnaA to the four highly conserved 9-mer sequences. About 20-40 molecules of dnaA (52Kdalton monomer) bind to the right end of the 245 bp ori sequence forming a large "nucleosome-like" complex. Nearly 200 base pairs are protected from DNAse I in this complex.
- ATP is bound in two forms to 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-dnaG primase 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



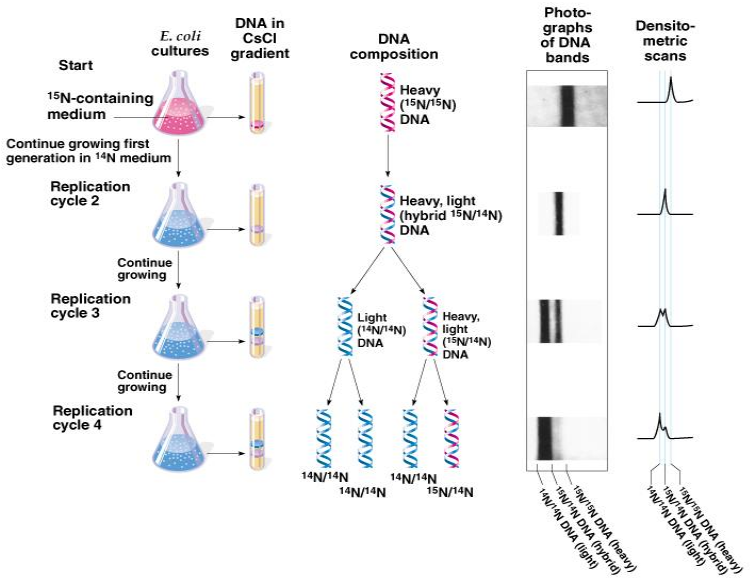
Different modes of DNA replication



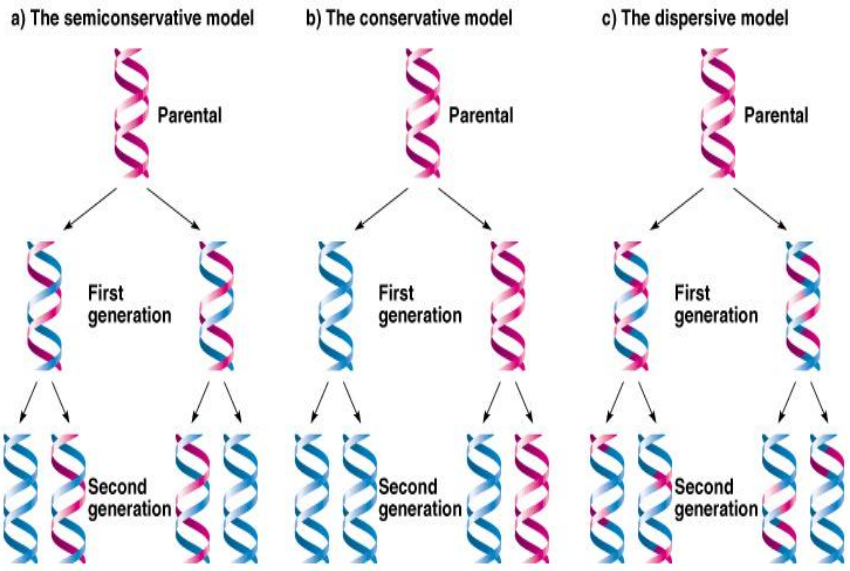
Meselson and Stahl Experiment

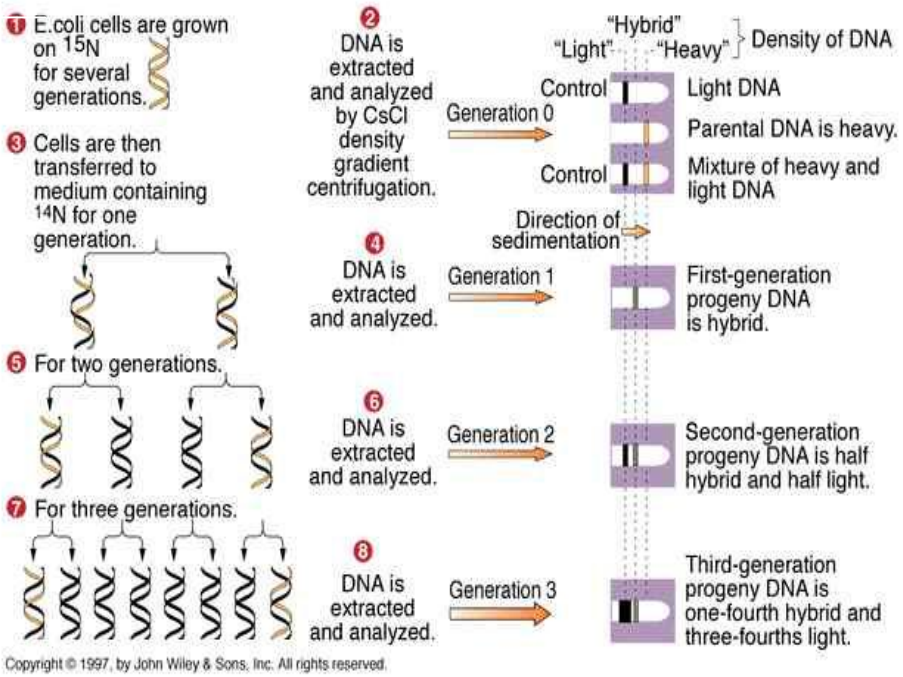
- *E. coli* are grown in heavy nitrogen (^{15}N) for many generations.
- This caused the nitrogen in the DNA molecule of each cell to contain ^{15}N, a heavier than typical isotope.
- The *E. coli* were then grown for one or two cell divisions in ^{14}N, the lighter and typical isotope.
- DNA was spun 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.



Different modes of replication





Meselson and Stahl Experiment

Types of Replication

Various types of replication include

- Semi discontinuous replication
- Unidirectional replication
- Bidirectional replication

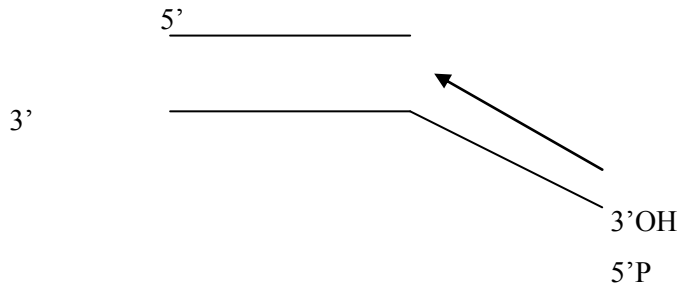
Semi discontinuous replication

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:

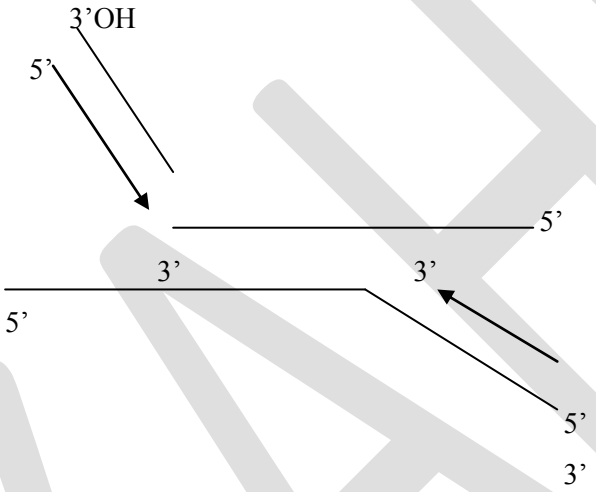
There would be another polymerase that add nucleotides to the 5'end that is it would catalyze stand growth in 3-'5' direction, however no such polymerase exist.

3' OH

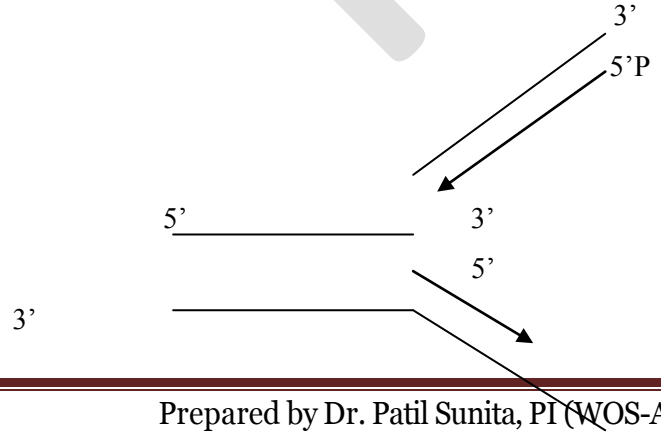
5'P



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.



3'OH
5'P

Detection of fragments

In 1968,Reiji okazaki demonstrated in *E.Coli* that newly synthesized DNA 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,

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*.

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.

The results showed very 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

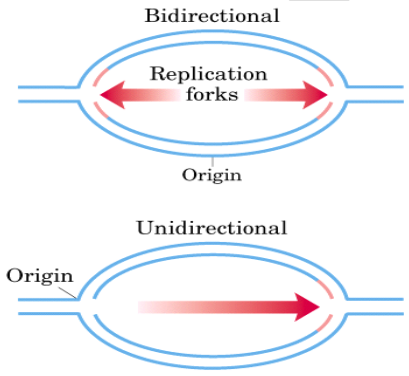
Both the strands replicates with the help of DNA polymerase and this enzyme would make one strand (leading strand) continuously in the 5'-3' direction and the other strand (lagging strand) in order to synthesis in 5'-3' direction is made discontinuously, this discontinuity is due to the synthesis opposite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

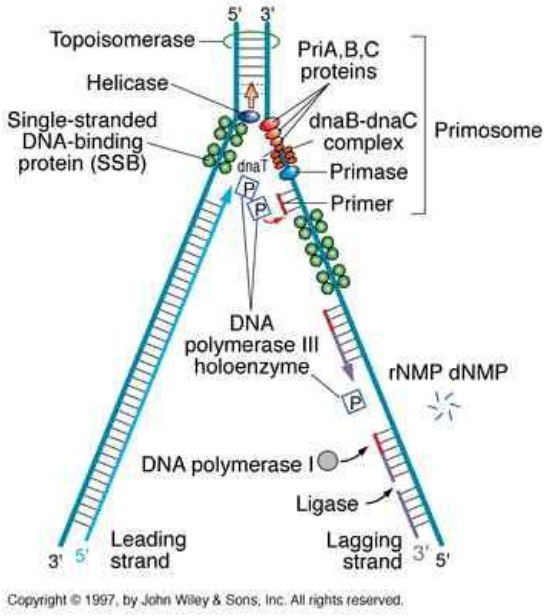


Events in the replication fork:

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.



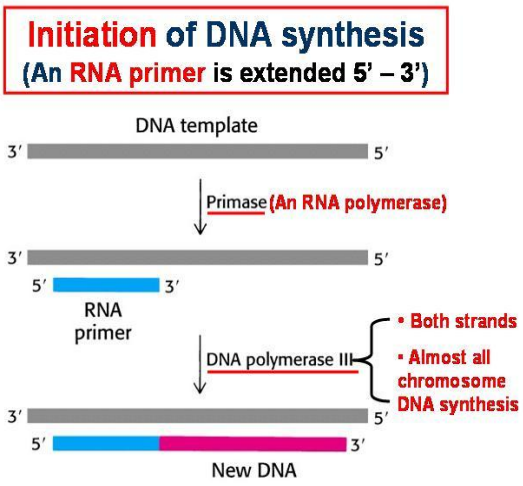
Events in the replication fork

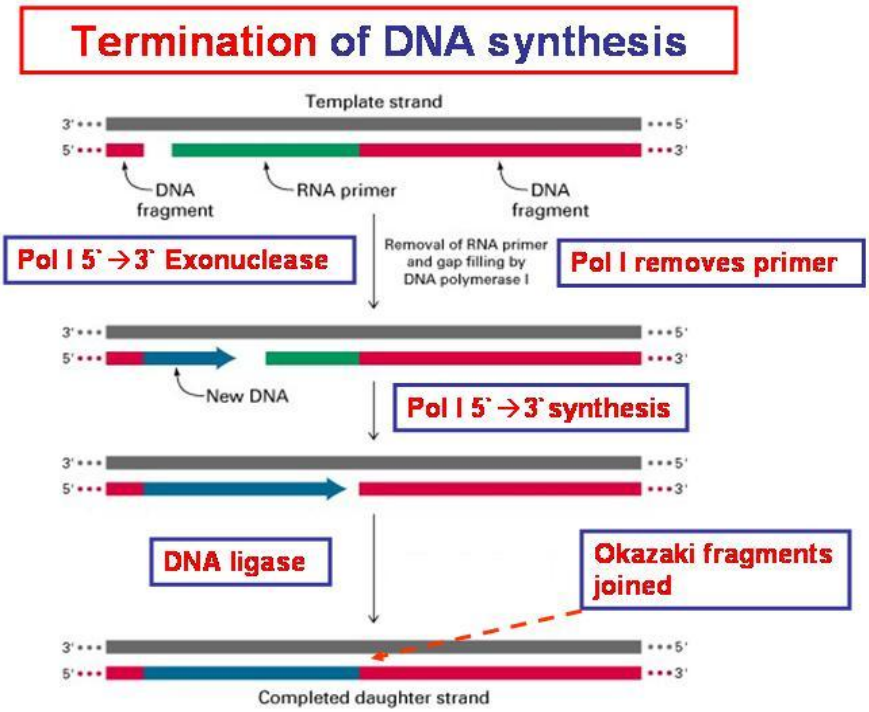
Mechanism of DNA replication

Initiation: this involves the assembly of a replication fork (bubble) at an origin of replication sequence of DNA found at a specific site of the circular chromosome of a bacterium. The fork is generated by a complex of proteins called a primosome.

Elongation: this is the addition of bases by another complex of proteins called the replisome. Parental strands unwind and daughter strands are synthesized.

Termination: the duplicated chromosomes separate from each other.

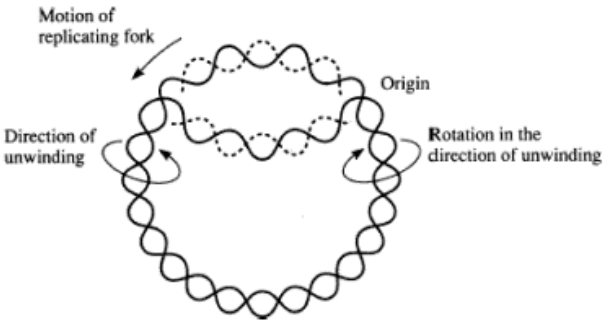




Different models of Replication

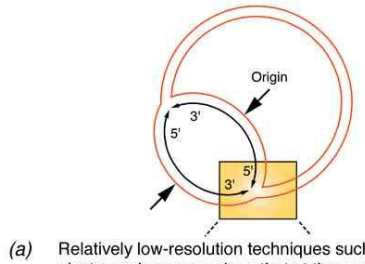
Theta model

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 .

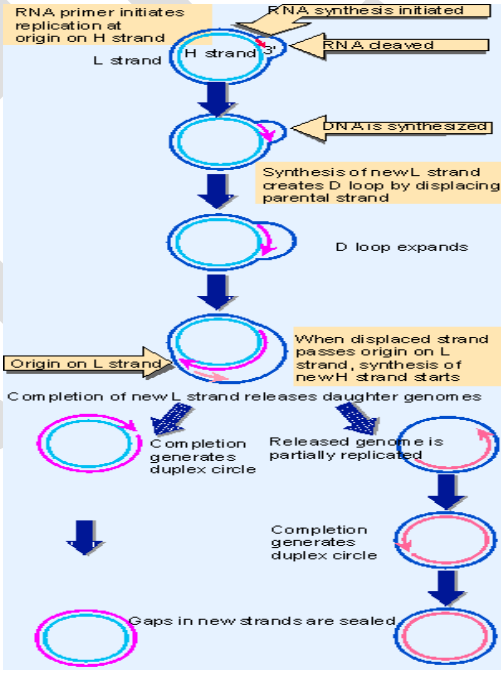


D loop model

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 **D loop**. 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 aroDuisnpdl atcheem geennot more D. -loops



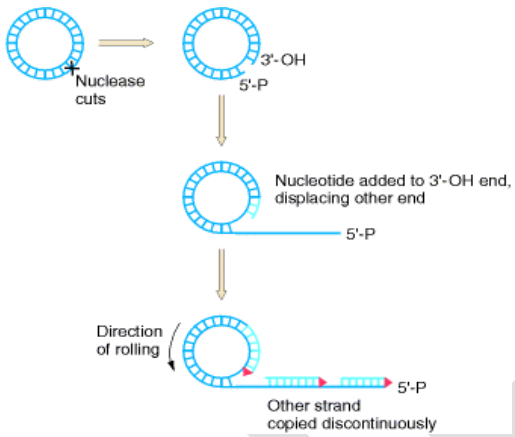
(a) Relatively low-resolution techniques such as autoradiography and electron microscopy show that at the macromolecular level both nascent DNA chains are extended in the same overall direction at each replication fork.



***Rolling Circle model*:**

A rolling circle is a replicative structure in which one strand of a circular duplex is used as a template 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 at the 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

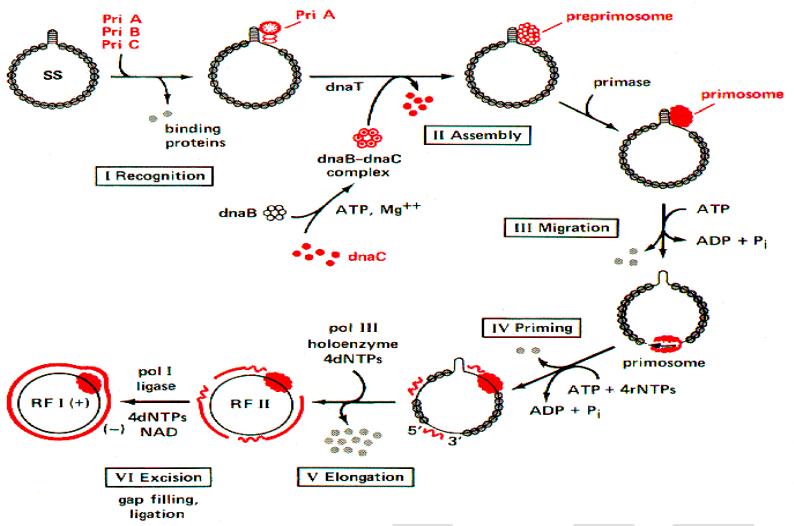
by the rolling circle are **concatenated**, or connected end-to-end. The single-stranded DNA can be cleaved and ligated to generate unit length genomes, which are packaged into phage particles. This occurs in replication of single-stranded DNA phages such as φX174 or M13.



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. while dnaB 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.



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.

Eukaryotic DNA Polymerase

DNA Polymerase alpha and delta replicate the DNA.

DNA polymerase alpha is associated with initiation, and delta extends the nascent strands.

DNA polymerase epsilon and beta are used for repair.

DNA polymerase gamma is for replication of mitochrondrial DNA.

Questions

Long Answer questions

1. Explain: a. The principle of inheritance 2. Mendel's law of segregation
2. Explain Lac operon concept.
3. Describe the double helix model of DNA.
4. Describe the process of DNA replication with help of diagram.
5. Write an account on Okazaki fragments.
6. Write short notes on purines and pyrimidines.
7. Write down the difference between prokaryotic and eukaryotic DNA replication.
8. Explain i) Leading and Lagging strands ii) Single-strand DNA-binding protein.
9. Outline the different forms of DNA.
10. Explain the Mendelian Principles and its importance
11. Describe the events occurring in a replication fork with neat diagram.

Sort answer quesions

1. Distinguish between heterozygous and homozygous
2. Briefly describe the process of DNA Replication
3. Illustrate detailed structure of DNA with suitable diagram.
4. Explain monohybrid inheritance with suitable cross as example.
5. Write the function of DNA ligase?
6. Name the enzyme involved in DNA replication
7. Name the nitrogen base present only in RNA
8. What are the different modes of DNA replication?
9. Mention the different types of RNA.
10. Name the nucleotides of DNA.
11. With the help of Griffith's transformation principle prove DNA is the genetic material.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I MSc Microbiology **COURSE NAME**: Molecular Genetics

COURSE CODE: 18MBP103 **BATCH**-2018-2019

Multiple choice questions

UNIT I

S. No.	Unit I Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	Father of genetics	Mendel	Morgan	Watson	McLeod	Mendel
2	Adenine always pair with	Guanine	Cytosine	Thymine	Uracil	Thymine
3	Bonding between two bases	Hydrogen bond	Hydrophobic bond	Nitrogen bond	Van Der waals	Hydrogen bond
4	Mendels pioneer work was with	Monkey	Human	Garden pea	Mice	Garden pea
5	_____ million base pairs of nucleotides are seen in *E.coli*	64 million base pairs	46 million base pairs	4.6 million base pairs	6.4 million base pairs	4.6 million base pairs
6	_____ degrades DNA	Polymerase	Primase	RNase	DNase	DNase
7	DNA is replicated:	Conservatively	Distributively	Semi-conservatively	Dispersively	Conservatively
8	Bacteria contains	Single circular DNA	Single linear DNA	Double Linear DNA	Double circular DNA	Single circular DNA
9	Basically, flow of genetic material is accompolished by	Replication	Transformation	Transduction	Conjugation	Replication

10	Which of the following sugar is found in RNA	2- deoxy Ribose	b) 3-deoxy Ribose	c) D- Ribose	d) D- Xylulose	D- Ribose
11	Chargaff's rule	No complementarity	Partial complementarity	No such rule	Complementarity of one strand with the other	Complementarity of one strand with the other
12	Chromosomal theory of inheritance was formulated by	Mendel	Miescher	Metchinikoff	Morgan	Morgan
13	Dihybrid ratio	3 : 3 : 9 : 1	9 : 3 : 3 : 1	9 : 3 : 1 : 3	1 : 3 : 3 : 9	9 : 3 : 3 : 1
14	Distance between the two base pairs is	3.4Å	34Å	10Å	20Å	3.4Å
15	DNA absorbs UV light at _____ wavelength	220 nm	240 nm	260 nm	280 nm	260 nm
16	Enzyme that adds methyl group to the newly formed DNA	Gyrase	Topoisomerase	Helicase	Methylase	Methylase
17	Eukaryotic DNA damage or replication errors are corrected during	G_1 phase	S phase	G_2 phase	R phase	G_2 phase
18	Eukaryotic DNA replication is a conserved mechanism that restricts DNA replication to	Never	Only once per cell cycle	Only twice per cell cycle	Only thrice per cell cycle	Only once per cell cycle
19	Experiments of Hershey and Chase was based on	Virus	Bacteriophage	Bacteria	Fungi	Bacteriophage
20	Formation of pre-replicating complex is seen in replication mechanism of	Prokaryotes	Plants	Virus	Eukaryotes	Eukaryotes
21	Heat Killed S cells + Live R cells produced	Death in mice + S cells	Live mice + S cells	Death in mice + R cells	Live mice + R cells	Death in mice + S cells

22	If a free phosphate is found at the 5’ end of a DNA strand, what is found at the other end of the same strand?	A hydroxyl group on the 5’ carbon of a deoxyribose sugar	A phosphate group on the 3’ carbon of a deoxyribose sugar.	A base attached to the 3’ carbon of a deoxyribose sugar	A hydroxyl group on the 3’ carbon of a deoxyribose sugar	A hydroxyl group on the 3’ carbon of a deoxyribose sugar
23	In eukaryotes, the vast majority of DNA synthesis occurs during _____ of the cell cycle	G phase	H phase	R phase	S phase	S phase
24	Initiation of replication is carried out by	DnaA	DnaC	DnaB	DnaE	DnaA
25	Initiation of replication occurs	Bidirectionally	Cross sectionally	Unidirectionally	Parallely	Bidirectionally
26	Joining of DNA fragments	DNA ligase	Gyrase	RNA polymerase	DNA polymerase	DNA ligase
27	Key enzyme in rolling circle replication	DNA Polymerase-IV	DNA Polymerase-III	DNA Polymerase-II	DNA Polymerase-I	DNA Polymerase-III
28	Left handed DNA	B-DNA	C-DNA	Y-DNA	Z-DNA	Z-DNA
29	Length of primer during replication is	2-10 nucleotides	10-20 nucleotides	5-15 nucleotides	10-25 nucleotides	2-10 nucleotides
30	Longest DNA is seen in	Human	Lung fish	Yeast	Bacteria	Lung fish
31	Which of the following is not associated with DNA replication?	Polymerase	Promoter	Primer	RepA protein	Promoter
32	Who proposed the molecular struccture of DNA	Hershey & Chase	Erwin Chargaff	Jim Latham	Watson & Crick	Watson & Crick

33	Nucleoside is	Base + Sugar	Sugar + Phosphate	Base + Phosphate	A+T & G+C	Base + Sugar
34	Number of base pairs per helical turn of B form DNA	13	12	11	10	10
35	Okazaki fragments are	RNA strands	Enzymes	Leading strands	Lagging strands	Lagging strands
36	Proof reading activity of DNA polymerase is in the direction	5' to 3'	3' to 5'	Parallel	Centre	3' to 5'
37	Purines are	A, T	G, C	C, T	A, G	A, G
38	Repair and insertion of DNA is carried out by	Endonucleases	Ribozyme	Primase	Exonucleases	Endonucleases
39	RNA primer is removed by	DNA pol	RNA pol	Terminase	Caspase	DNA pol
40	Semiconservative DNA replication model	Daughter molecule contains both from parent	Daughter molecule entirely new	Daughter molecule contains one from parent and one newly synthesized	Some sections from parent and some newly synthesized	Daughter molecule contains one from parent and one newly synthesized
41	Semiconservative mechanism of DNA replication was demonstrated by	Meselson & Stahl	Beedle & Tatum	Hershy & Chase	Avery & McLeod	Meselson & Stahl
42	Sequencing and molecular characterization of genome	Genetics	Molecular biology	Proteomics	Genomics	Genomics
43	SSB protein helps in	Degradation of protein	Keep the two strands separated after unwinding	Elongation of DNA	Uncoiling of RNA	Keep the two strands separated after unwinding
44	Synthesis of DNA always moves from	3' to 5'	5' to 3'	Ffrom the centre	Anywhere	5' to 3'
45	The ability to remove	RNA	DNA ligase	DNA polymerase	DNA helicase	DNA polymerase

	incorrectly matched nucleotides or Proof-reading	polymerase				
46	The contribution of Rosalind Franklin towards structure of DNA was	X-ray crystallography	Electron microscopy	NMRspectroscopy	Gas chromatography	X-ray crystallography
47	The DNA of E.coli is _____ times longer than the cell	1	10	1000	100	1000
48	The enzyme that copies RNA from DNA template	Dnase	Rnase	DNA polymerase	RNA polymerase	RNA polymerase
49	The enzyme that unwinds DNA	Polymerase	Ligase	Gyrase	Helicase	Helicase
50	The experiments of Avery, McLeod and McCarty was based on	Protein coupling	Enzymatic reactions	Synthetic reaction	DNA binding	Enzymatic reactions
51	The most common form of DNA is	B-DNA	Z-DNA	Y-DNA	SS-DNA	B-DNA
52	The most widely studied origin recognition complex of eukaryotes is that of	*Bacillus*	*Staphylococcus*	*Escherichia coli*	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*
53	The negative charge of DNA is due to	Deoxyribose Sugar	Phosphate bond	Hydrogen bond	Nitrogenous base	Phosphate bond
54	The Pioneer work on nucleic acid discovery was carried out by	Friedrick Miescher	Watson & Crick	Griffith	Milstein Kornberg	Friedrick Miescher
55	The replication origin of *E.coli* is approximately	245 bp	425 bp	254 bp	524 bp	245 bp
56	The replication origins of higher eukaryotes are made up of	Different AT-rich regions	Similar AT-rich regions	Different GC-rich regions	Similar GC-rich regions	Similar AT-rich regions

57	The size of a major groove is	34Å	3.4Å	43Å	20Å	34Å
58	Thymine in DNA is replaced by _____ in RNA	Adenine	Cytosine	Guanine	Uracil	Uracil
59	Transfer of genes from one generation to the next is	Inheritance	Carrying over	Subheritance	Gene transport	Inheritance
60	Transformation in *Pneumococci* was discovered by	Friedrick Griffith	Erwin Chargaff	Hershey & Chase	Watson & Crick	Friedrick Griffith
61	Triple bonding is seen in	G-T	G-C	A-T	A-C	G-C
62	Which is involved in synthesis of primer	Ligase	Primase	DNA pol	rRNA	Primase

UNIT-II

Syllabus

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

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.

First base (5′ end)	Second base: U	Second base: C	Second base: A	Second base: G	Third base (3′ end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met or start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Table showing characters of Genetic code

Transcription: Synthesis of RNA using DNA as a template

- First step in making proteins
- BASIC REQUIREMENTS: Template, Substrate, Enzyme
- *Location*:Nucleus of the cell

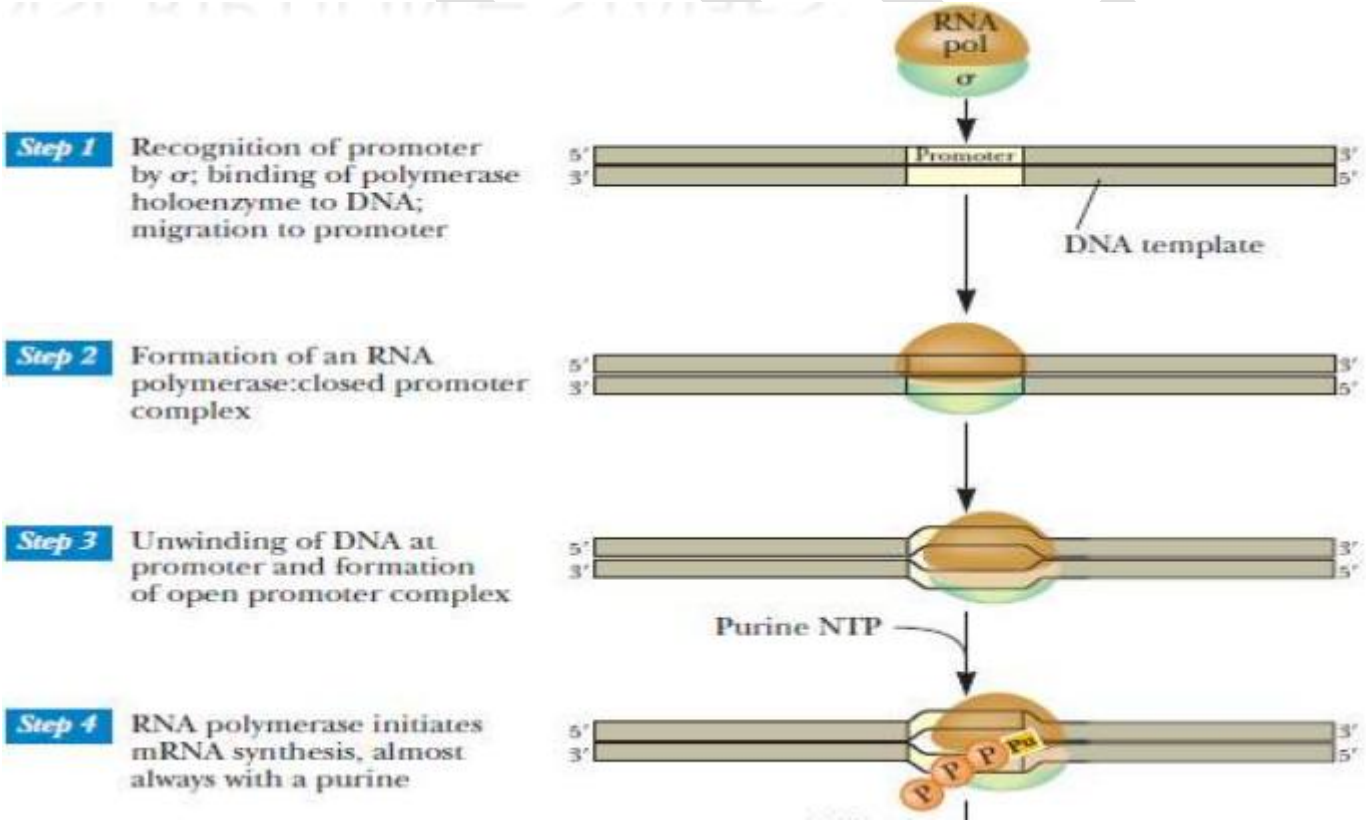
Steps to Transcription

INITIATION

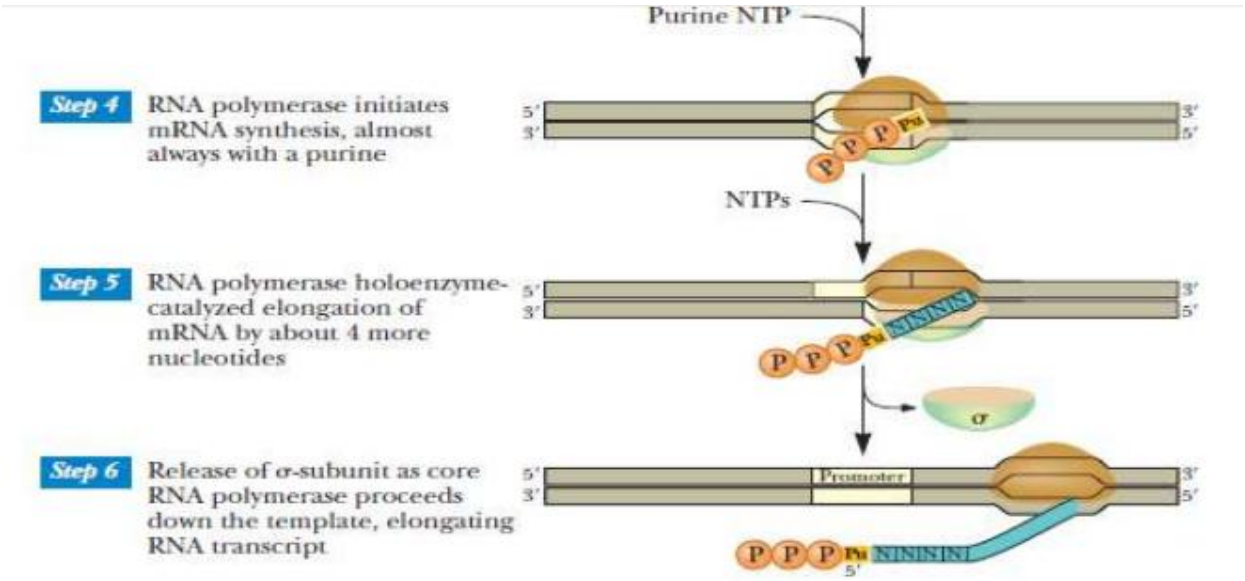
ELONGATION

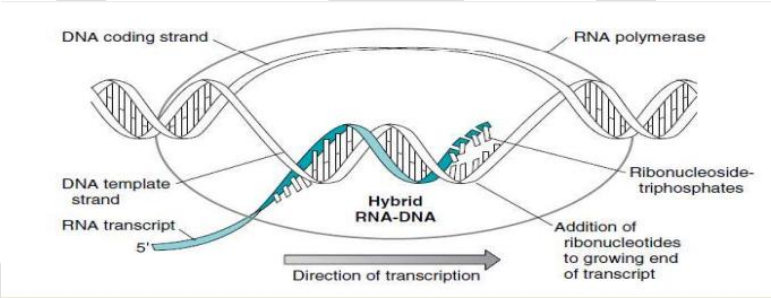
TERMINATION

INITIATION



ELONGATION

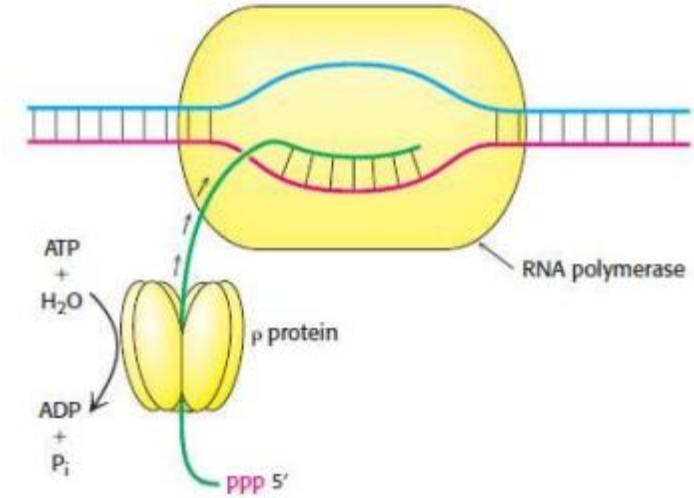




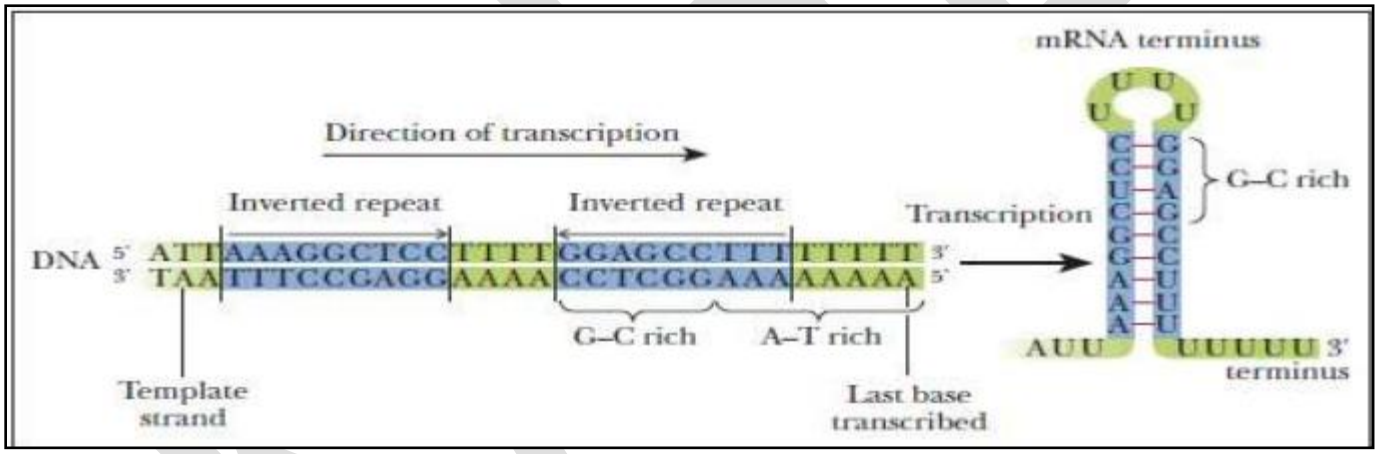
TERMINATION

Two mechanisms

Rho (p) dependent and Rho independent



Rho dependent



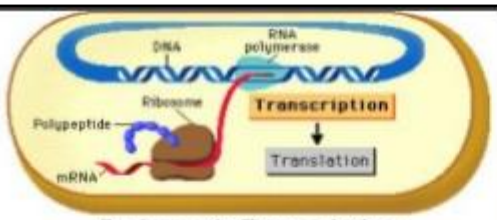
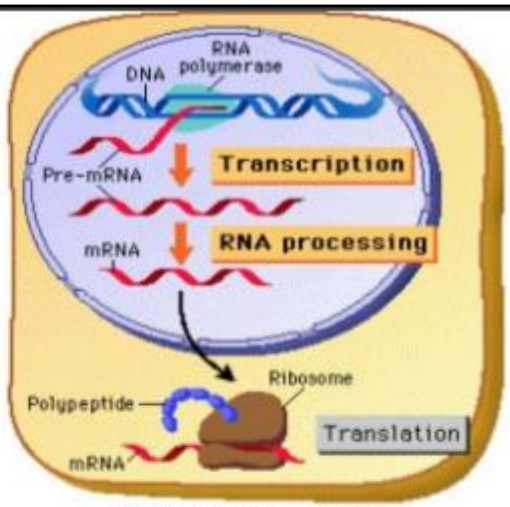
Rho independent

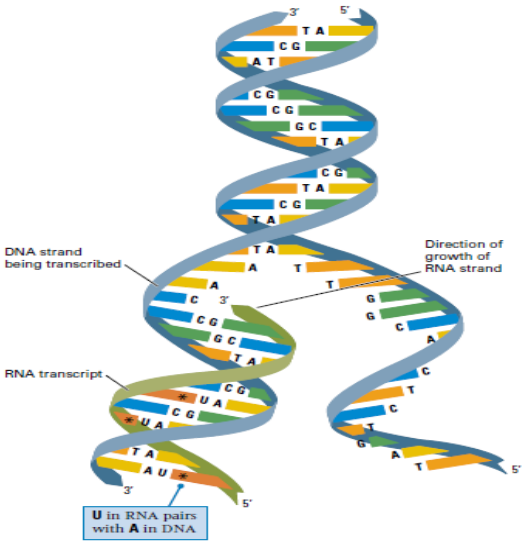
Main differences in prokaryotic and eukaryotic transcription

RNA Polymerase

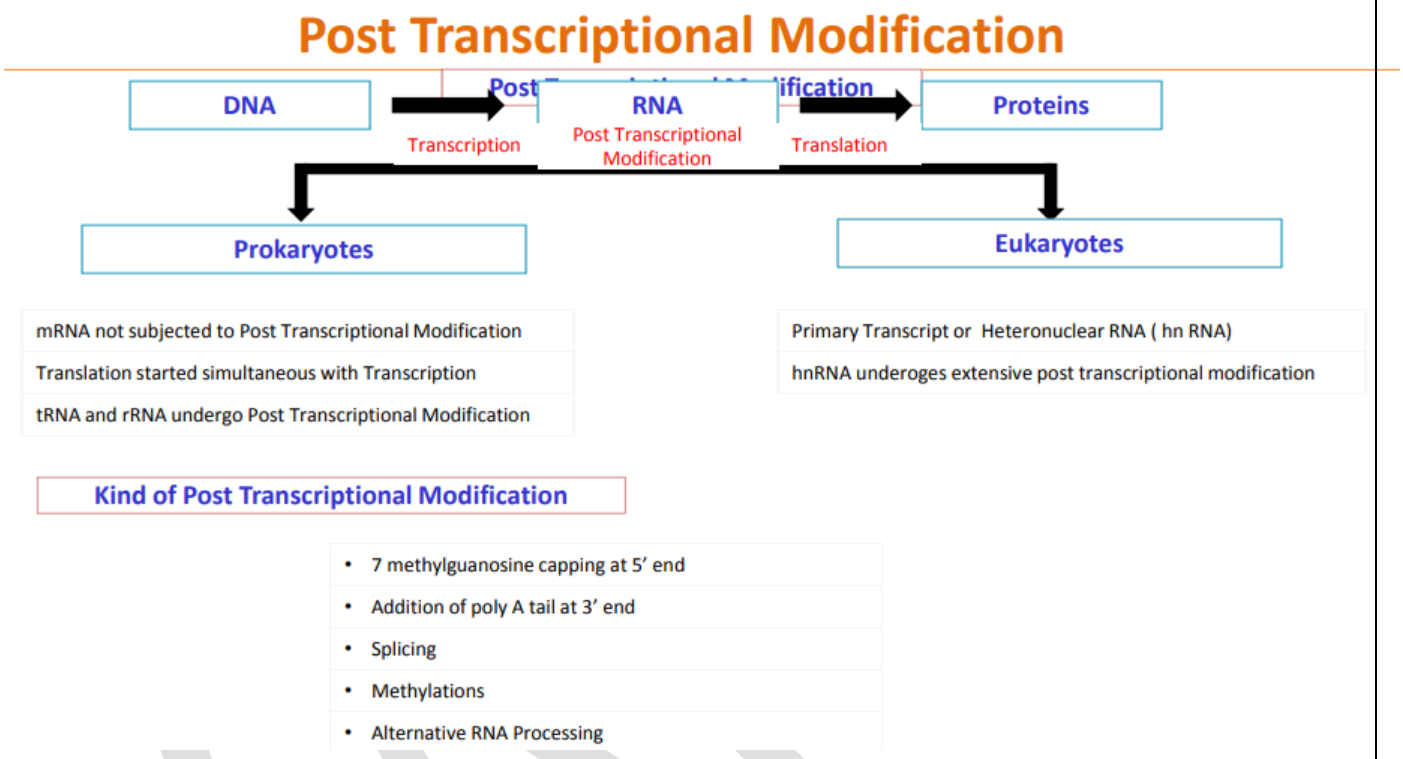
Promoter sites Enhancers,

Silencers and TF

	 Prokaryotic Transcription	 Eukaryotic Transcription
1	Coupled transcription-translation is the rule.	Coupled transcription translation is not possible.
2	Occurs in the cytoplasm.	Occurs in the nucleus.
3	There is no definite phase for its occurrence.	Take place in the G1 and G2 phases of cell cycle.
4	A single RNA polymerase synthesises all the three types of RNA (mRNA, tRNA, rRNA)	The RNA polymerases I, II and III synthesizes rRNA, mRNA and tRNA respectively.



Post transcriptional modification

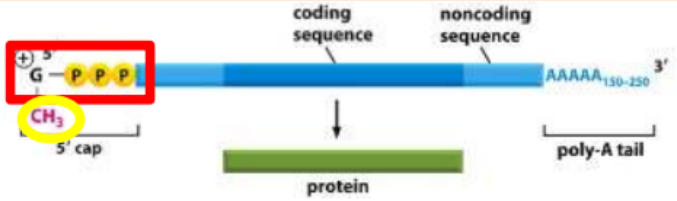


Post transcriptional modification both in prokaryotes and Eukaryotes

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.

Post Transcriptional Modification (Methylation & Poly A)



7 methyl guanosine 5' Capping

- **In Nucleus**
  - Guanosine Triphosphate attached to 5' End by 5'-5' unusual linkage by enzyme Guanylyltransferase
- **In Cytosol**
  - Methylation with methyl group from S Adenosyl Methionine using guanine 7 methyltransferase
- **Functions**
  - Initiation of Translation
  - Stabilize the mRNA
  - Prevents attack of 5'->3' exonuclease

Addition of Poly A Tail at 3' End

- **In Nucleus**
  - Poly A Tail added at 3' by **polyadenylate polymerase**
  - The Length of Poly A Tail can be upto 200 adenine bases
- **Functions**
  - Poly A Tail and its binding protein PAB-1 is required for efficient initiation of protein synthesis
  - Stabilize the mRNA
  - Prevents attack of 3' to 5' exonuclease

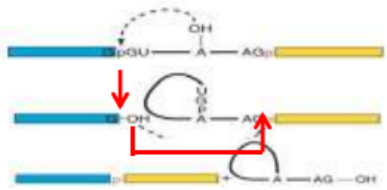
RNA Processing

- 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

Post Transcriptional Modification - Splicing

Joining Together of EXONS (After Removal of Introns)



Binding of SnRNP brings neighboring exons into correct alignment for splicing

Nucleophilic attack at 5' end of intron

Lariat Formation and Cut at 3' End of Intron

Ligation of 3' endo of Exon 1 to 5' End of Exon 2

Self Splicing – Certain hrRNA has Self Spicing activity because of Ribozyme Activity

Spliceosomes	Primary Transcript + 5 snRNA (U1 / U2/ U4 / U5 / U6) and more than 60 Proteins (Ribonucleoproteins or RNP)
Small Nuclear RNA (Sn RNA)	Uracil Rich RNA which acts as Enzyme (Ribozyme). U6 is essential U7 is SnRNA involved in production of correct 3' ends of histone mRNA that lacks Poly A tail
SnRNP Complex (SNURPs)	SnRNA + RNP = SNURPs (Clinical Correlation :- SLE antibodies against SNURPs)

Faulty Splicing Can lead to aberrant proteins .

Example – Incorrect splicing of Beta Globin mRNA is responsible for some cases of Beta Thalassemia

Methylation & Alternative Splicing

Methylations	Methylation of N7 of adenine and 2' Hydroxyl group of ribose in cytosol
Alternative Splicing	A mechanism of producing a diverse set of proteins from a limited set of genes. ways in different tissues The Pre mRNA from same gene is spliced in two or more alternative ways in different tissues

Selective Splicing -	Selective Inclusion or exclusion of exons

Alternative 5' Donor	5' Donor Site of certain exons is changed

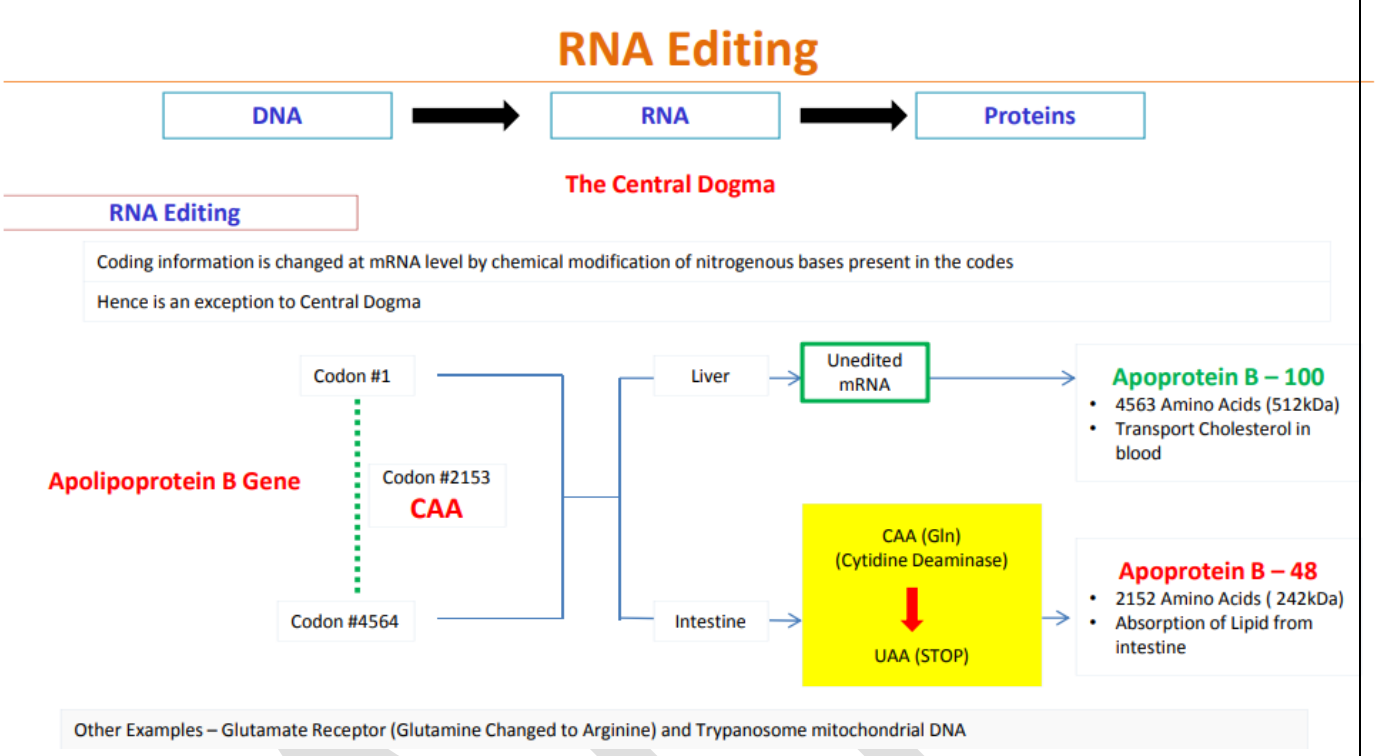
Alternative Polyadenylation	Different Site is used for Polyadenylation

Alternative 3' Acceptor	3' Acceptor site of certain exons is changed

Application of Alt. Splicing

Generation of Membrane bound or Secretory IgG by alternative polyadenylation site

Production of several tissue specific isoforms of tropomyosin from single mRNA Transcript



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).

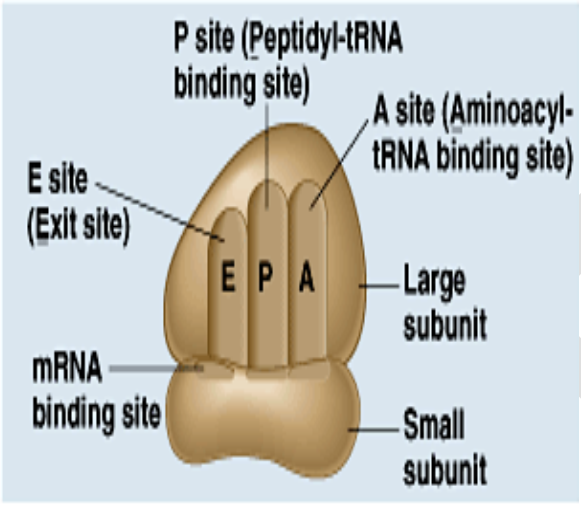
tRNA: at least one specific type of tRNA is required to transfer one amino acid. There about 50 tRNA in human for the 20 amino acids, this means some amino acids have more than one specific tRNA. The role of tRNA in protein synthesis is discussed before. (amino acid attachment and anticodon loop).

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

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.



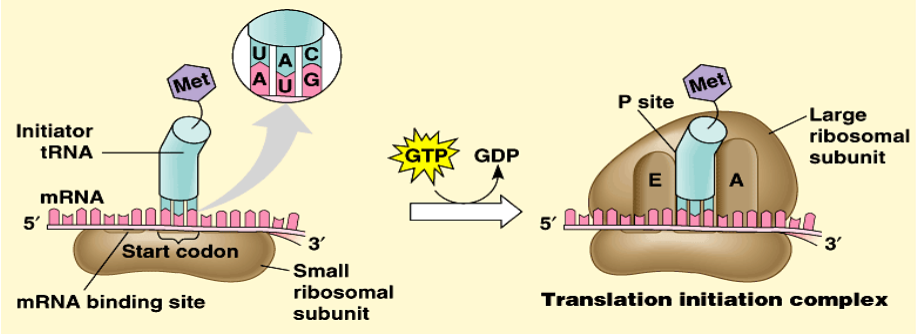
(b) Schematic model showing binding sites

Steps:

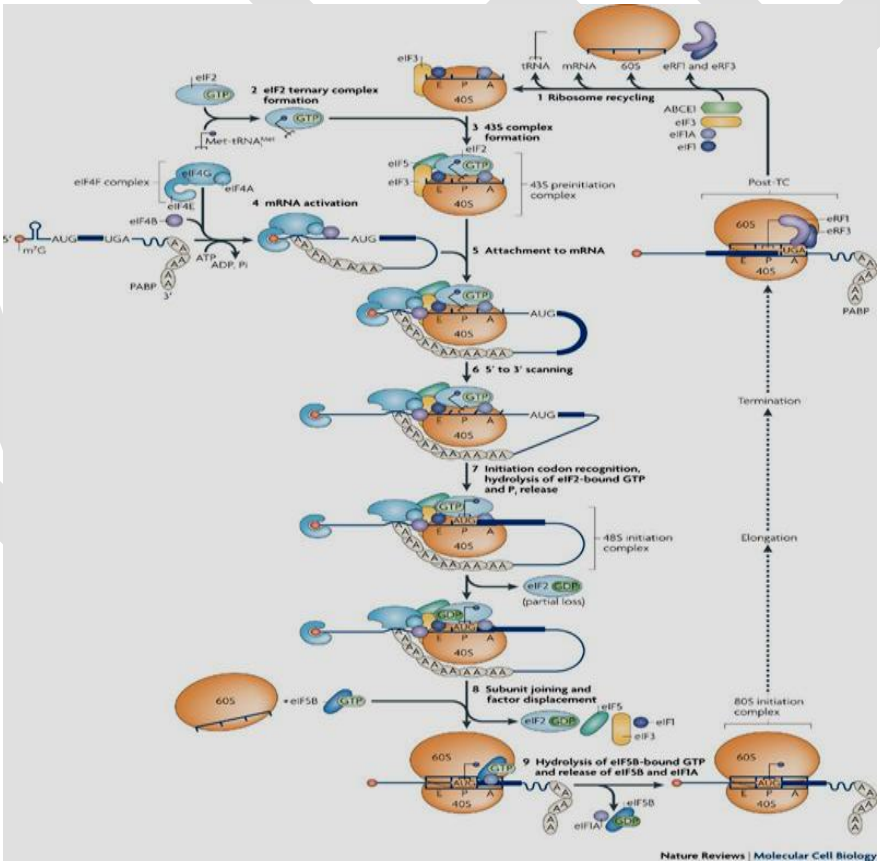
***Initiation*:**

Initiation (start) codon is usually AUG which is the codon of methionine, so the initiator tRNA is methionnyl tRNA (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
- 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 methionyl tRNA is placed (enter).



Initiation of translation in prokaryotes



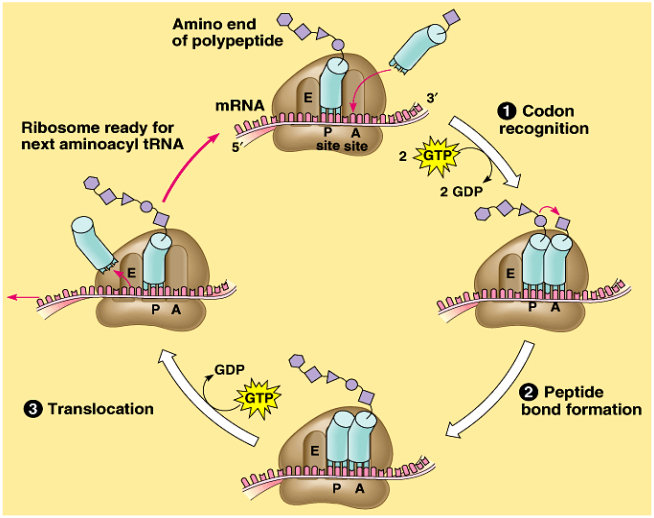
Initiation of translation in eukaryotes

Inactive 80S ribosomal subunit is separated 40S and 60S ribosomal subunits, and result in the formation of an 80S ribosomal initiation complex, in which Met-tRNAMeti is base paired with the initiation codon in the ribosomal P-site and which is competent to start the translation elongation stage. These stages are: eukaryotic initiation factor 2 (eIF2)–GTP–Met-tRNAMeti ternary complex formation (2); formation of a 43S preinitiation complex comprising a 40S subunit, eIF1, eIF1A, eIF3, eIF2–GTP–Met-tRNAMeti and probably eIF5 (3); mRNA activation, during which the mRNA cap-proximal region is unwound in an ATP-dependent manner by eIF4F with eIF4B (4); attachment of the 43S complex to this mRNA region (5); scanning of the 5′ UTR in a 5′ to 3′ direction by 43S complexes (6); recognition of the initiation codon and 48S initiation complex formation, which switches the scanning complex to a 'closed' conformation and leads to displacement of eIF1 to allow eIF5-mediated hydrolysis of eIF2-bound GTP and Pi release (7); joining of 60S subunits to 48S complexes and concomitant displacement of eIF2–GDP and other factors (eIF1, eIF3, eIF4B, eIF4F and eIF5) mediated by eIF5B (8); and GTP hydrolysis by eIF5B and release of eIF1A and GDP-bound eIF5B from assembled elongation-competent 80S ribosomes (9). Translation is a cyclical process, in which termination follows elongation and leads to recycling (1), which generates separated ribosomal subunits. The model omits potential 'closed loop' interactions involving poly(A)-binding protein (PABP), eukaryotic release factor 3 (eRF3) and eIF4F during recycling , and the recycling of eIF2–GDP by eIF2B. Whether eRF3 is still present on ribosomes at the recycling stage is unknown.

Elongation:

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

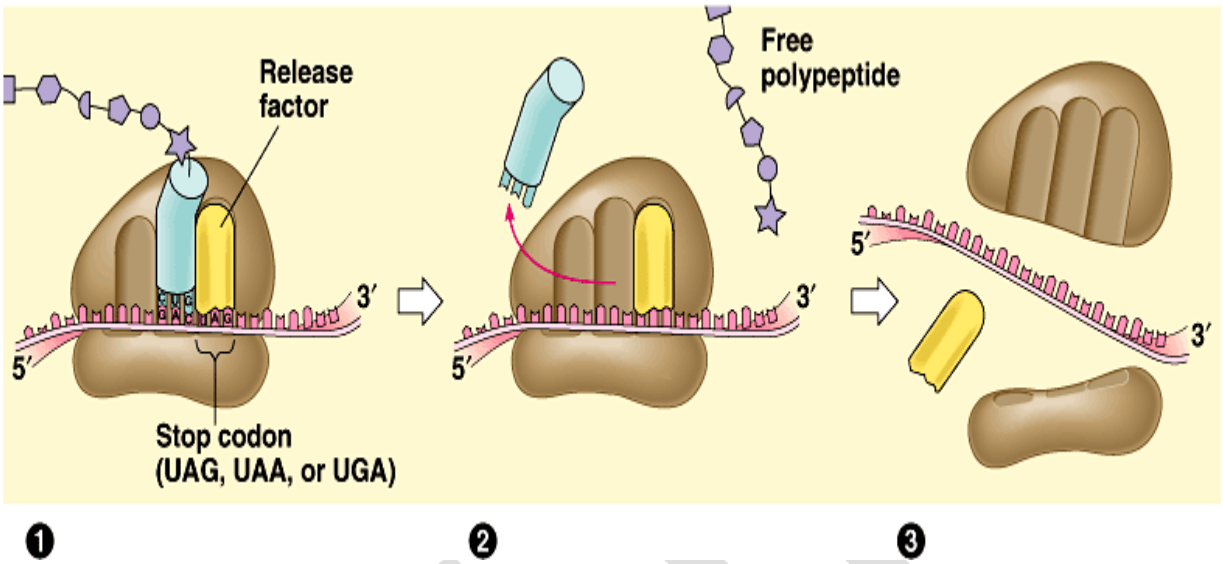
- The next aminoacyl tRNA (tRNA which carry the next amino acid specified by recognition of the next codon on mRNA) will enter A site of ribosome
- A site or acceptor site or aminoacyl tRNA site :Is the site of ribosome to which each new incoming aminoacyl tRNA will enter.
- *Ribosomal peptidyl transferase*, enzyme will transfer methionine from methionyl tRNA into A site to form a peptide bond between methionine and the new incoming amino acid to form dipeptidyl tRNA.
- Elongation factor-2 (EF-2), (called also, translocase): moves mRNA and dipeptidyl tRNA from A site to P site leaving A site free to allow entrance of another new aminoacyl tRNA.
- Elongation process continous resulting in the formation of of poly peptide chain.



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)



Termination of translation

Posttranscriptional Control

These mechanisms control or regulate mRNA after it has been produced.

Differential Removal of Introns

This can produce variations in the mRNA produced. Different mRNA may have different introns removed.

Differential removal of introns enables a gene to code for more than one different protein. An average human gene is thought to code for 3 different proteins.

For example, experiments using radioactive labeling show that calcitonin produced by the hypothalamus is different from that produced by the thyroid. In each case, the same gene produces the protein.

Speed of Transport of mRNA Through the Nuclear Pores

Evidence suggests that this time may vary.

Longevity of mRNA

Messenger RNA can last a long time. For example, mammalian red blood cells eject their nucleus but continue to synthesize hemoglobin for several months. This indicates that mRNA is available to produce the protein even though the DNA is gone.

Ribonucleases

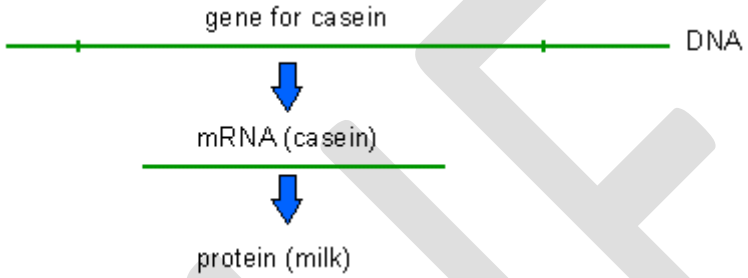
Ribonucleases are enzymes that destroy mRNA.

Messenger RNA has noncoding nucleotides at either end of the molecule. These segments contain information about the number of times mRNA is transcribed before being destroyed by ribonucleases.

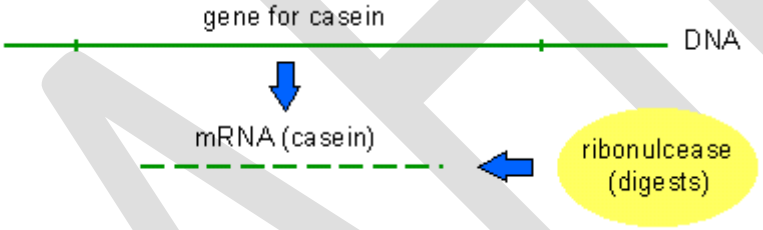
Hormones stabilize certain mRNA transcripts.

Example

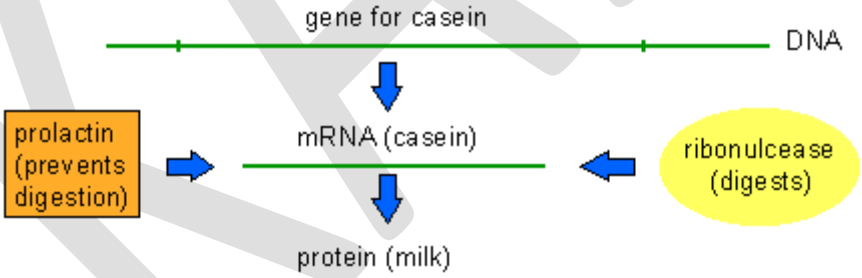
Prolactin is a hormone that promotes milk production because it affects the length of time the mRNA for casein (a major milk protein) is available.



Ribonucleases destroy the mRNA.



Prolactin is a hormone that prevents destruction of the mRNA.



Translational Control

These mechanisms prevent the synthesis of protein. They often involve protein factors needed for translation.

Preventing Ribosomes From Attaching

Proteins that bind to specific sequences in the mRNA and prevent ribosomes from attaching can prevent translation of certain mRNA molecules.

Initiation Factors

Initiation factors are proteins that enable ribosomes to attach to mRNA. These factors can be produced when certain proteins are needed. For example, the eggs of many organisms contain mRNA that is not needed until after fertilization. At this time, an initiation factor is activated.

Posttranslational Control

These mechanisms act after the protein has been produced.

Protein Activation

Some proteins are not active when they are first formed. They must undergo modification such as folding, enzymatic cleavage, or bond formation.

Example: Bovine proinsulin is a precursor to the hormone insulin. It must be cleaved into 2 polypeptide chains and about 30 amino acids must be removed to form insulin.

Feedback Control

Some enzymes in a metabolic pathway may be negatively inhibited by products of the pathway.

Modification of DNA

Gene Amplification

In *Drosophila* (fruit flies), the chorion (eggshell) gene is copied many times in certain cells of the oviduct. These cells make large quantities of the protein needed to surround the egg. In other cells of the body, there is only one copy of this gene.

The Immunoglobin Genes

Immunoglobins (antibodies) are proteins that are used to defend the body against foreign invaders. They are able to do this because they have a shape that matches a shape found on the invader, allowing it to become attached. Particles that have antibodies attached are quickly destroyed by other cells in the immune system.

Our bodies contain millions of different antibodies, each produced by a type of white blood cell called a lymphocyte. A single lymphocyte can produce only one specific kind of antibody, thus, there are millions of different kinds of lymphocytes.

The genes that code for these antibodies differ from one lymphocyte to the next because when the lymphocytes are produced, different regions of the DNA are deleted so that each lymphocyte has a somewhat different version of the genes involved.

Regulating Gene Expression

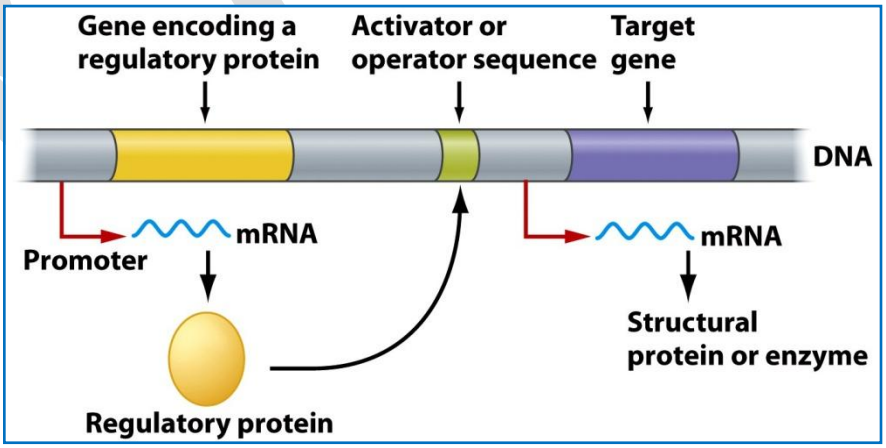
- Microbes respond to changing environment
- Alter growth rate
- Alter proteins produced
- Must sense their environment
- Receptors on cell surface
- Must transmit information to chromosome
- Alter gene expression
- Change transcription rate
- Change translation rate

Operonic regulation

- Coding vs regulatory sequences
- Regu**la**tory sequences: promoters, operator and activator sequences
- Regulatory proteins: repressors, activators
- Repressors bind operator sequences, block transcription
- Induction vs Derepression
- Activator proteins bind sequences near by promoters, facilitate RNA Pol binding, upregulate transcription

Operon

- Multiple genes transcribed from one promoter



Structural and regulatory genes of an operon

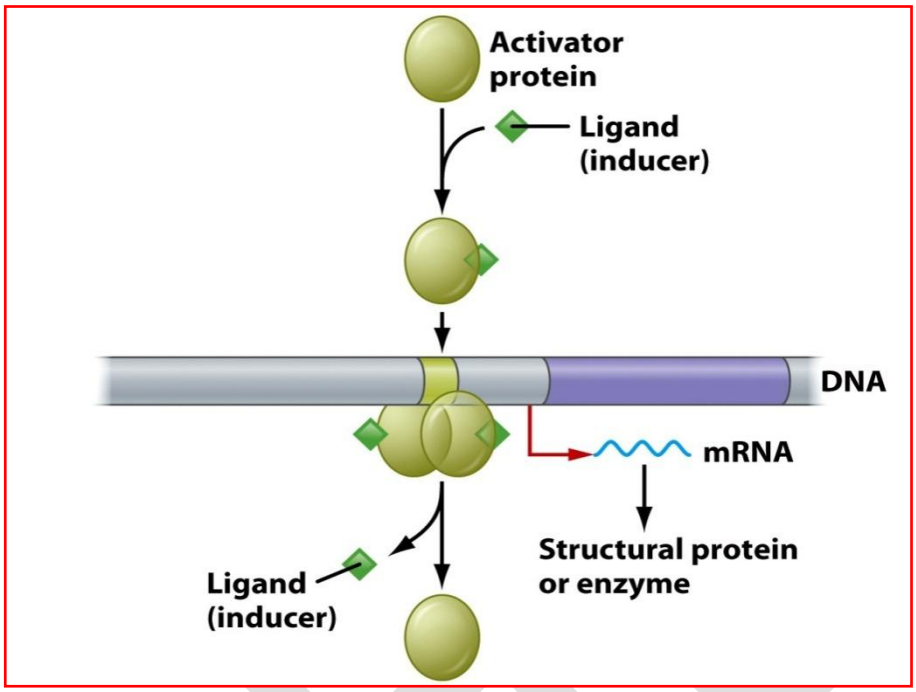
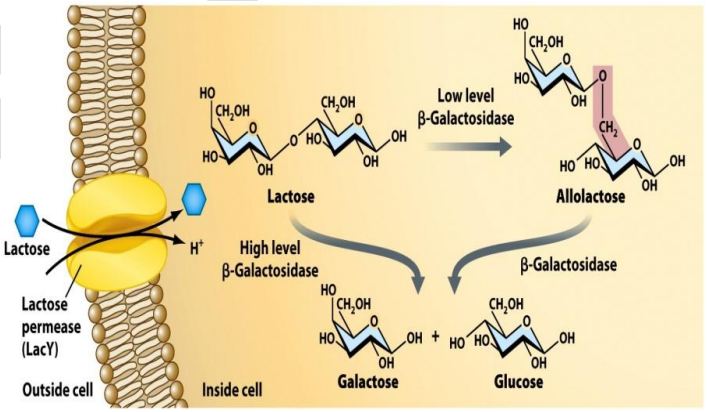


Fig: Operonic regulation

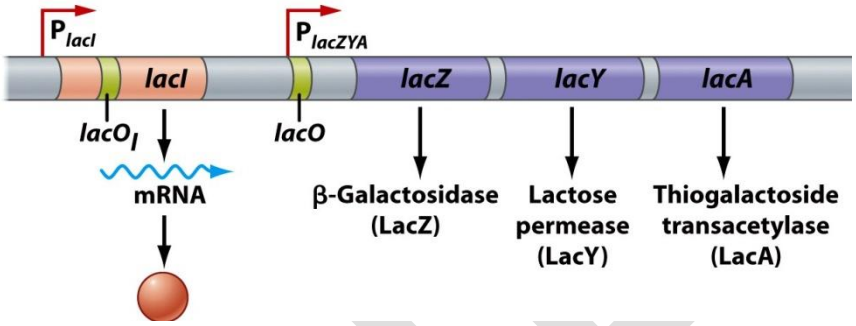
The *E. coli lac* Operon

- 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



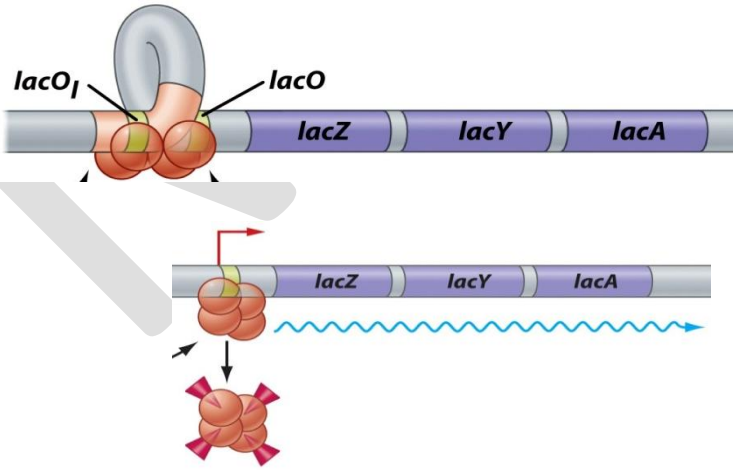
Lac operon regulation

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



Structural and regulatory gene of Lac Operon

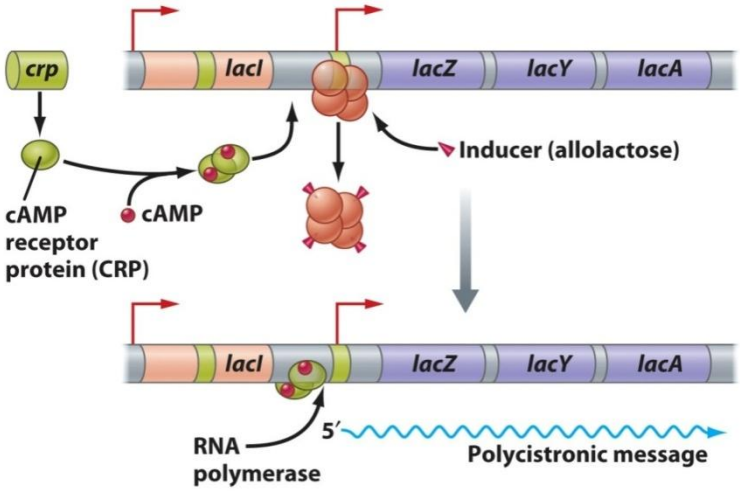
- The *lacZ* gene encodes b-galactosidase
- The *lacY* gene encodes lactose permease
  - Need both proteins to digest lactose
- Repressor protein LacI blocks transcription
  - Repressor binds to operator
  - Blocks s factor from binding promoter
- Repressor responds to presence of lactose
  - Binds inducer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



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



Catabolite repression

Catabolite Repression

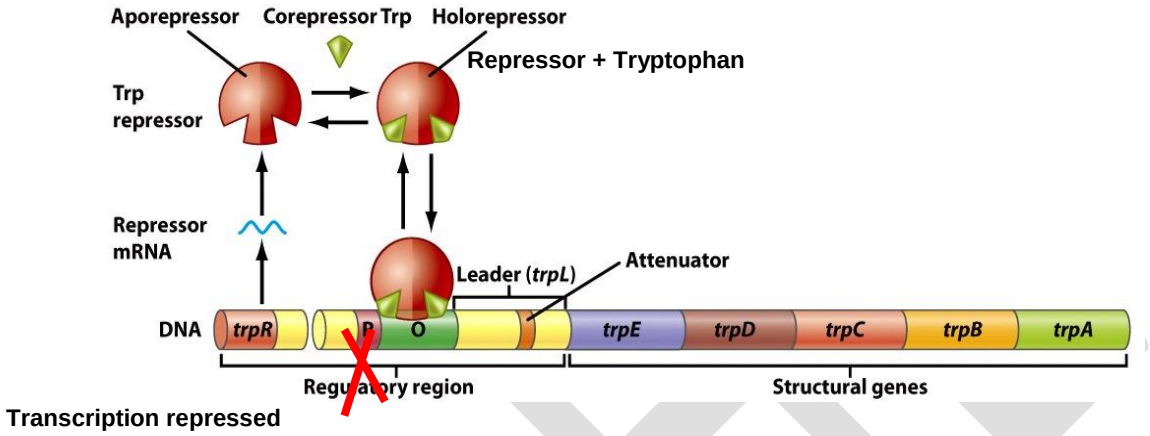
- Two mechanisms involved
- High glucose → low cAMP levels → CRP inactive
  - Can't bind operon → low level of *lac* transcription

Trp operon: Repression and Attenuation

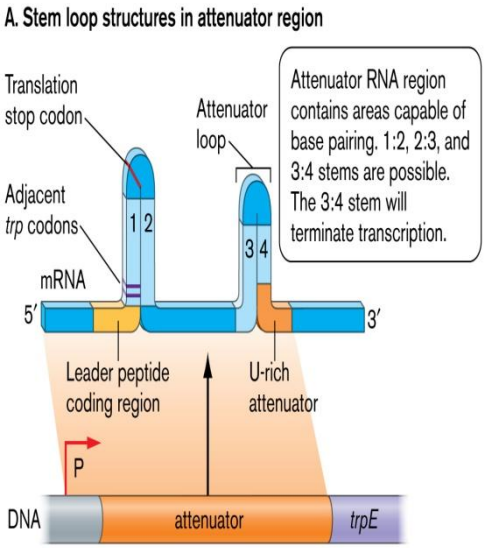
- *trp* operon
  - Cell must make the amino acid tryptophan
    - Trp operon codes and regulates biosythetic enzymes

- When tryptophan is plentiful, cell stops synthesis

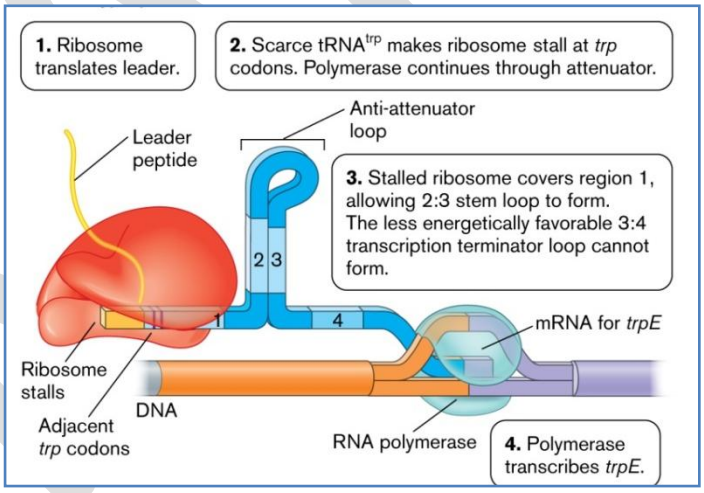
- Regulation by two mechanisms
- ***Repression***: Trp repressor must bind tryptophan to bind DNA
- Opposite of *lac* repressor



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



Transcriptional Attenuation Mechanism of the *trp* Operon

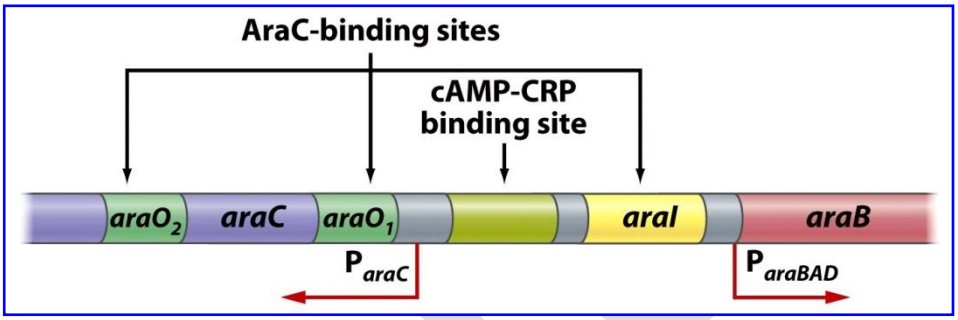


Attenuation control in Trp Operon

Arabinose operon

- Regulation by dual role regulatory protein AraC

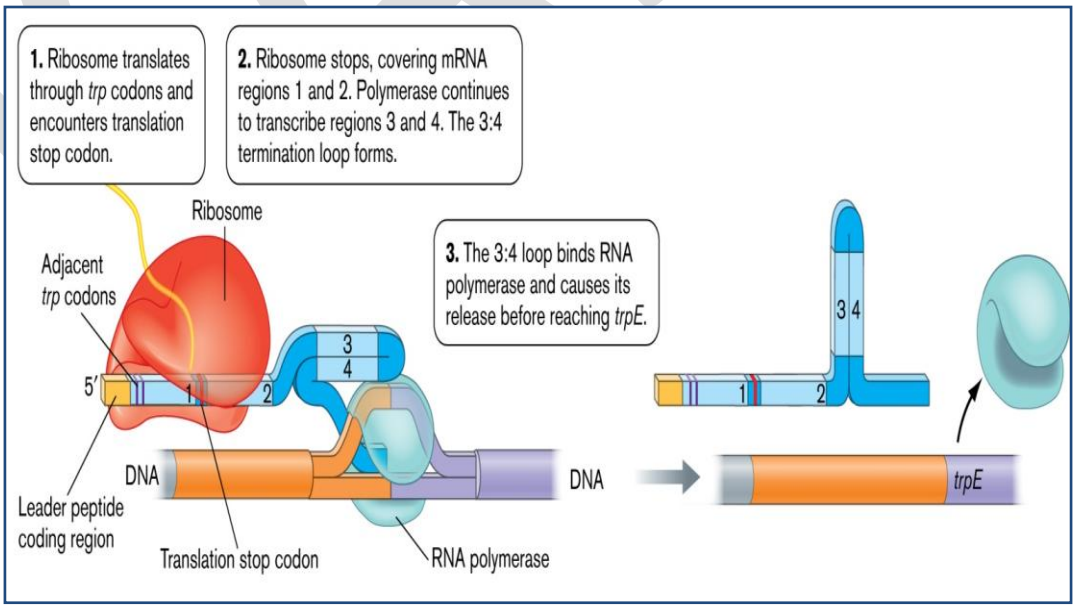
- "AraC" acts as repressor to block transcription (no arabinose)
- Acts also as activator when bound to "arabinose" (the inducer)
- Operators O1, O2 and araI control AraC and AraBAD proteins expression



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 AraC dimeric conformation
- acts as activator
- Stimulates binding of RNA polymerase



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, **l**ess than 5% of human DNA codes for protein.

Functions of human DNA

- Coding for proteins. Eukaryotic genes are organized in peculiar fashion:
- **Exons**: (short for "expressed") -- regions of DNA that code for amino acids.
- **Introns**: (short for "intervening" or "interrupting") -- regions of DNA inside a gene, located in between exon regions, but not coding amino acids
- 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**.
- **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.
- **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).
- 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.
- 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.
- 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).
- 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.
- 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.
- 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.
- 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.
- 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

Questions

Long Answer questions

1. Explain the structure and functions of RNA polymerase.
2. Give an account on post-transcriptional modification in eukaryotes.
3. State about the maturation and processing of RNA in bacteria.
4. Elaborate polypeptide synthesis in bacteria.
5. How is mRNA synthesized from template DNA
6. Outline *Trp* operon model.

7. Outline *Lac* operon model.

Short Answer questions

1. Mention the initiator and terminator codons.
2. Explain any two properties of genetic code.
3. With reference to transcription define splicing and capping.
4. Define translation
5. Lactose is termed as inducer in lac operon. Give reason.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I MSc Microbiology **COURSE NAME**: Molecular Genetics

COURSE CODE: 18MBP103 **BATCH**-2018-2019

Multiple choice questions

UNIT II

Sr. No.	Unit III Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	In prokaryotes, AUG is translated in to	Methionine	N-acetyl-methionine	N-formamyl-aspargine	N-formamyl-methionine	N-formamyl-methionine
2	In Rho-independent transcription termination, the termination sequence is usually	Palindromic sequence	Paliomic sequence	Panoramic sequence	Pandemic sequence	Palindromic sequence
3	In RNA, thiamine is replaced by	Uracil	Adenine	Cytosine	Guanine	Uracil
4	In the absence of effector molecule, the enzyme is said to be in	Relaxed state	Tense state	Free state	Degrading state	Tense state
5	In trp operon, the genes *trp*E & *trp*D codes for	Arginase	Tryptophan synthase	Anthranilate isomerase	Anthranilate synthase	Anthranilate synthase
6	*lac* operon is an example for	Repressible operon	Inducible operon	Mutated operon	Neutral operon	Inducible operon
7	Mammalian mitochondrion not only uses AUG as	AUA, AUU, AUC	UAA, UAU, UAC	AAU, UAU, CAU	GUA, GUU, GUC	AUA, AUU, AUC

	initiation codon but also					
8	Model example for gene regulation by repression	*trp* operon	*lac* operon	*ara* operon	*gal* operon	*trp* operon
9	Molecular weight of egg lysozyme is	19300 daltons	13900 daltons	31900 daltons	91300 daltons	13900 daltons
10	Monad & Cohen-Bazire first reported the evidence for the repression of the enzyme	Tryptophan synthase	Gluconase synthetase	Arabinase trimutase	Tryptophanase	Tryptophan synthase
11	Non codon specifies more than ___ amino acid	1	2	3	4	1
12	Non-coding regions are called as	Exons	Introns	Cistrons	Positrons	Exons
13	Operon model that demonstrates both positive and negative control of gene regulation	*lac* operon	*ara* operon	*gal* operon	*trp* operon	*ara* operon
14	_____ is the first amino acid during translation of proteins	Threonine	Leucine	Methionine	Valine	Methionine
15	2006 Nobel Prize in Physiology & Medicine for studies on molecular basis of eukaryotic transcription	Arthur Nirenberg	Roger D. Kornberg	David Osborne	Michael Whitney	Roger D. Kornberg
16	7-methylguanosine	For eukaryotic	For prokaryotic	For eukaryotic	For	For eukaryotic

	cap is an important site	transcription initiation factor	translation initiation factor	translation initiation factor	prokaryotic translation initiation factor	translation initiation factor
17	Action of repressor protein in *Lac* operon is called as	Positive control	Negative control	Neutral control	No control	Negative control
18	Addition of poly A tail to 3' end of mRNA is mediated by the enzyme	RNA polymerase	DNA polymerase	Rnase	poly A polymerase	poly A polymerase
19	All aminoacids have more than one codon except	Methionine & Tryptophan	Valine & Leucine	Threonine & Alanine	Lysine & Arginine	Methionine & Tryptophan
20	Allosteric enzymes that are controlled by a molecule other than it's substrate	Cohesive molecules	Systematic molecules	Effector molecules	Affector molecules	Effector molecules
21	Amino acid that have largest number of codons	Proline	Cysteine	Serine	Valine	Serine
22	A-site is the ribosomal site most frequently occupied by the	Aminoacyl-rRNA	Aminoacyl-mRNA	Iminoacyl-tRNA	Aminoacyl-tRNA	Aminoacyl-tRNA
23	Capping in mRNA is addition of the group	7-ethylguanosine	7-methylguanosine	7-methylcytosine	7-ethylcytosine	7-methylguanosine
24	Codon/Anticodon consists of _____ nucleotides	4	6	3	9	3
25	Common method of covalent modification of	to methylate the enzyme at a proline	to phosphorylate the enzyme at a proline residue	to phosphorylate the enzyme at	to methylate the enzyme at a serine	to phosphorylate the enzyme at a serine residue

	enzyme in regulation of gene expression is	residue		a serine residue	residue	
26	Confirmational changes in protein is brought about by	Systematic molecules	Cohesive molecules	Affector molecules	Effector molecules	Effector molecules
27	Control of gene expression was proposed by	Beedle & Tatum	Avery & McLeod	Jacob & Monad	Hershey & Chase	Jacob & Monad
28	Enzyme activity is regulated by changes in the confirmation of enzymes except	Polymerase	Ribozymes	Chimozymes	Nuclease	Ribozymes
29	Enzyme that lactose in to glucose and galactose	Lactosidase	Glucanse	α-galactosidase	β-galactosidase	β-galactosidase
30	Genes are located in specialized structures called	Histone	RNA	Chrosomes	Genomes	Chrosomes
31	In post translational modification of RNAs, trimming is	Removal of excess nucleotides	Removal of excess proteins	Removal of excess lipids	Removal of excess carbohydrates	Removal of excess nucleotides
32	Transcription initiation site starts from	-1	Plus 1	-10	Plus 10	Plus 1
33	Transcription is	DNA to rRNA	DNA to tRNA	DNA to mRNA	DNA to protein	DNA to mRNA
34	Translation is	rRNA to protein	tRNA to protein	DNA to protein	mRNA to protein	mRNA to protein
35	tRNA is responsible for the transfering	Protein	Aminoacid	Codon	Anticodon	Anticodon
36	tRNA's are matched with their aminoacids by a	Aminoacyl DNA synthatases	Aminoacyl synthatases	Amino synthatases	Aminoacyl tRNA synthatases	aminoacyl tRNA synthatases

	group of enzymes collectively called as					
37	What are the possible number of codons that can be generated using possible nucleotide combinations	46	64	20	30	64
38	Which is astop codon	UAA	AAU	AUA	AAA	UAA
39	Which transports lactose across the cell membrane	Galactosidase permease	β-galactosidase	Glucanse	Glucose permease	Galactosidase permease
40	Who deciphered the genetic code	Hershey & Chase	Avery & McLeod	Beedle & Tatum	Nirenberg & Khorana	Nirenberg & Khorana
41	The first codon during translation is	AGU	AUG	GUA	UGA	AUG
42	The main function of nonsense codons is to	Initiate protein synthesis	Elongate protein synthesis	Terminate protein synthesis	Regulate protein synthesis	Terminate protein synthesis
43	The number of nitrogenous bases codes by 9 amino acids would be	27	36	18	9	27
44	The promoter sequence in eukaryotes is	TATAAA	TAATAA	TTGACA	GTTAAA	TATAAA
45	The stop codons are called as	Missense	Nonsense codons	Central codons	Last codons	Nonsense codons
46	The termination of transcription is signaled by rich	AT containing inverted repeat	AC containing inverted repeat	GC containing inverted repeat	CT containing inverted repeat	GC containing inverted repeat
47	Other than	Arginine	Tryptophan	Glutamic acid	Threonine	Tryptophan

	methionine _____ is the amino acid that appear rarely in proteins					
48	Polyadenylation is	Addition of adenosines to 3' end of mRNA	Addition of adenosines to 5' end of mRNA	Deletion of adenosines to 3' end of mRNA	Deletion of adenosines to 5' end of mRNA	Addition of adenosines to 3' end of mRNA
49	Region that comprise the core prokaryotic promoter	Klenow box	Pribnow box	TAGTAG box	Polypeptide box	Pribnow box
50	Repressor molecule in lac operon is a	Dimer	Trimer	Tetramer	Pentamer	Tetramer
51	Ribosomal site most frequently occupied by the tRNA carrying the growing peptide chain	A-site	P-site	E-site	G-site	P-site
52	rRNA is also called	Rnase	Ribase	Ribulase	Ribozyme	Ribozyme
53	Sequence of codons in mRNA between a start and a stop sequence is called as	Close reading frame	Open reading frame	Central reading frame	Last reading frame	Open reading frame
54	Short sequence of aminoacids are called	Peptides	Proteins	Polypeptides	Palindromes	Peptides
55	Site to which substrate molecules are attached	Catalytic site	Effector site	Alleosteric site	Binding site	Catalytic site
56	Stop codon UAA is also called	Amber	Opal	Acre	Ochre	Ochre
57	Stop codons in	GAA & GAG	AGA & AGG	CGA & AGC	CGG & GCG	AGA & AGG

	mammalian mitochodria are					
58	Structure of proteins may be classified into ___ types	2	3	4	5	4
59	The ability of the cell to choose between glucose and other sugars is termed as	Catabolic repression	Catabolic expression	Metabolic repression	Metabolic expression	Catabolic repression
60	The first and best example of control of gene expression was proposed by	Khorana & Nirenberg	Hershey & Chase	Avery & McLeod	Jacob and Monad	Jacob and Monad

UNIT III

Syllabus

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

Gene Transfer

Gene transfer is defined simply as a technique to efficiently and stably introduce foreign genes into the genome of target cells.

• The directed desirable gene transfer from one organism to another and the subsequent stable integration & expression of foreign gene into the genome is referred as genetic transformation.

• Stable transformation occur when DNA is integrated into host genome and is inherited in subsequent generations.

• The transferred gene is known as transgene and the organism that develop after a successful gene transfer is known as transgenic.

General Features of Gene Transfer in Bacteria

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

Bacterial genome alteration

CONJUGATION, TRANSFORMATION, TRANSDUCTION

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

Transduction-DNA transfer via phages

Generalized-random pieces of host DNA gets transfered

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

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

For the first time JOSHUA LEDERBERG & EDWARD L.TATUM in 1946 presented the evidence for bacterial conjugation i.e. a process of transfer of genetic material by cell-to-cell contact.

METHODOLOGY

¬ Requires the presence of a special plasmid called the F plasmid.

¬ Bacteria that have a F plasmid are referred to as F+ or male. Those that do not have an F plasmid are F- or female.

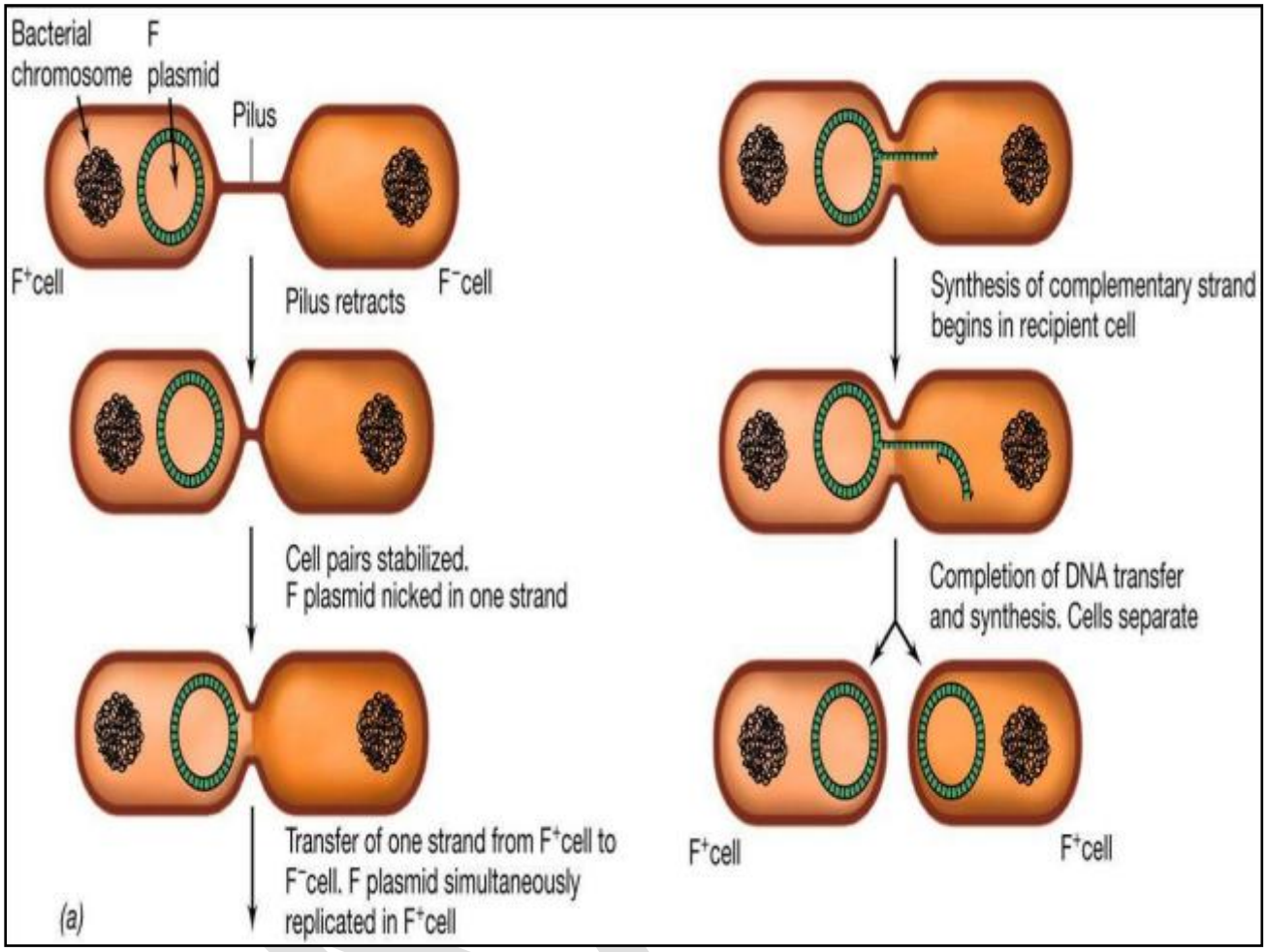
¬ The F plasmid consists of 25 genes that mostly code for production of sex pilli.

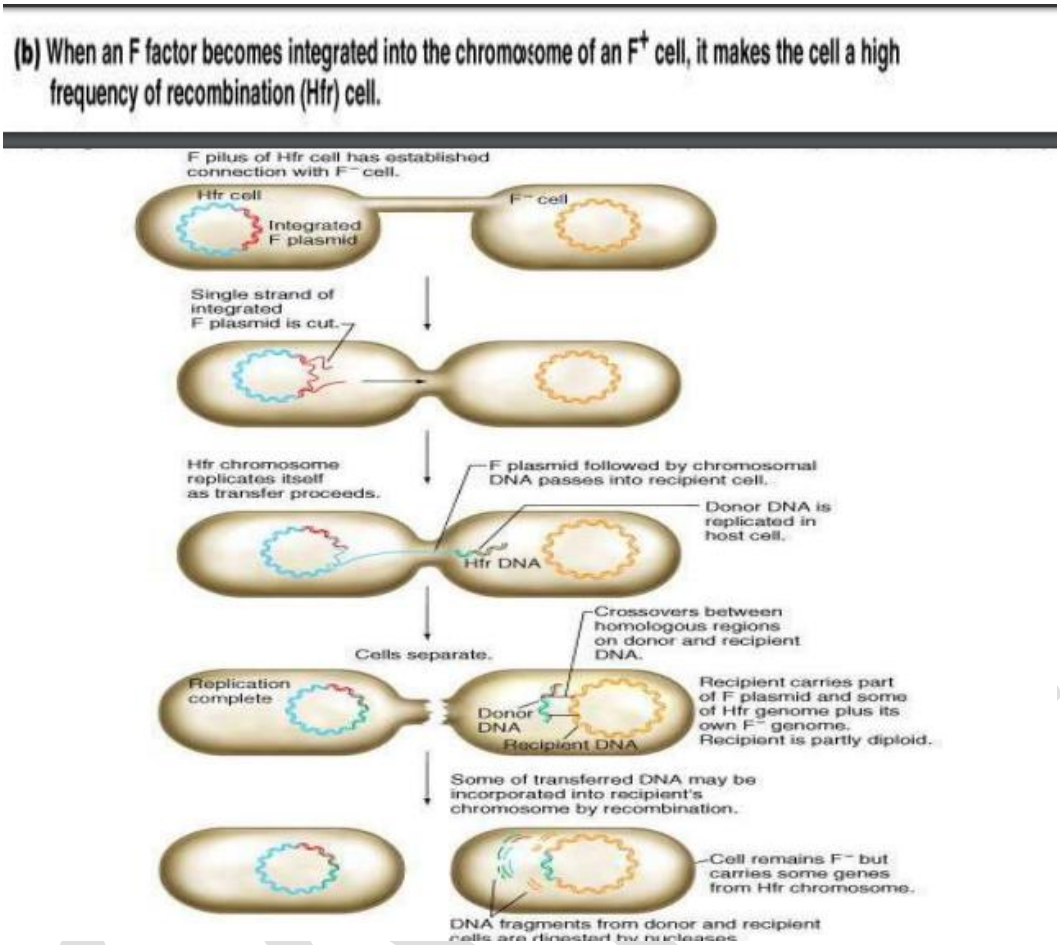
¬ A conjugation event occurs when the male cell extends his sex pilli and one attaches to the female.

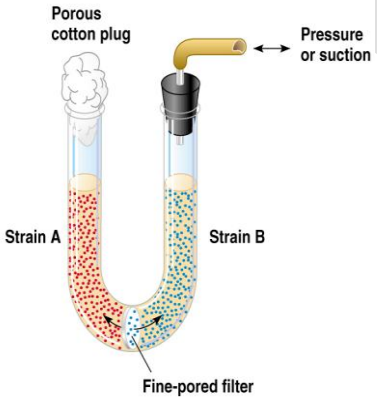
¬ This attached pilus is a temporary cytoplasmic bridge through which a replicating F plasmid is transferred from the male to the female.

¬ When transfer is complete, the result is two male cells.

¬ When the F+ plasmid is integrated within the bacterial chromosome, the cell is called an Hfr cell (high frequency of recombination cell).







Davis's U-tube experiment

Transformation

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

Bacterial transformation was first discovered by GRIFFITH in 1928 between the two strains of Streptococcus (Diplococcus) pneumonia which causes pneumonia in humans and mice.

Factors affecting transformation

DNA size and state

Sensitive to nucleases

Competence of the recipient (*Bacillus, Haemophilus, Neisseria, Streptococcus*)

Competence factor

Induced competence

Steps

Uptake of DNA

Gram +

Gram -

Recombination

Legitimate, homologous or general

recA, recB and recC genes

Significance

Phase variation in *Neiseseria*

Recombinant DNA technology

Genetic Mapping in Bacteria by Transformation

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

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

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

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

Donor is wild-type (a+). Recipient is mutant (a).

One of donor DNA strands is degraded, leaving ssDNA with the a+ allele.

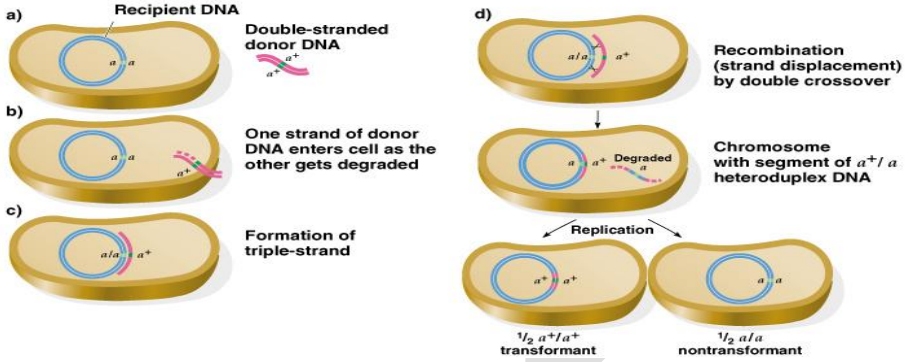
The donor ssDNA pairs with homologous DNA in recipient's chromosome, forming a triple-stranded region.

A double crossover event occurs, replacing one recipient DNA strand with the donor strand.

The recipient now has a region of heteroduplex DNA. One strand has the recipient's original a allele and the other strand has the new a+ allele.

DNA replication will produce one chromosome with the original (a) genotype, and one with the recombinant (a+) genotype.

The cell with the recombinant genotype is then selected by its phenotypic change.

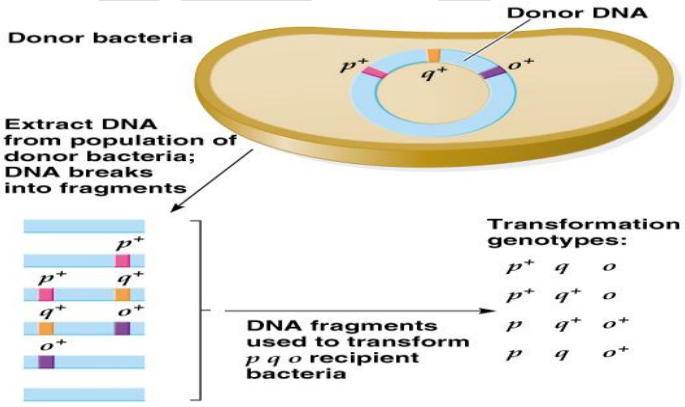


Process of Transformation

Transformation experiments are used to determine:

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

- The map distance between genes. Recombination frequencies are used to infer map distances



Mapping by Transformation

Transduction

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

Significance

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

Generalized Transduction

Transfer of DNA from one bacterium to another via Bacteriophage.

The phenomenon of transduction was first discovered by ZINDER & LEDERBERG in 1952. He discovered this while searching for sexual conjugation in Salmonella species.

Transduction happens through either the lytic cycle or the lysogenic cycle.

If the lysogenic cycle is adopted, the phage chromosome is integrated (by covalent bonds) into the bacterial chromosome, where it can remain dormant for thousands of generations.

If the lysogen is induced (by UV light for example), the phage genome is excised from the bacterial chromosome and initiates the lytic cycle, which culminates in lysis of the cell and the release of phage particles. The lytic cycle leads to the production of new phage particles which are released by lysis of the host.

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

Specialized Transduction

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

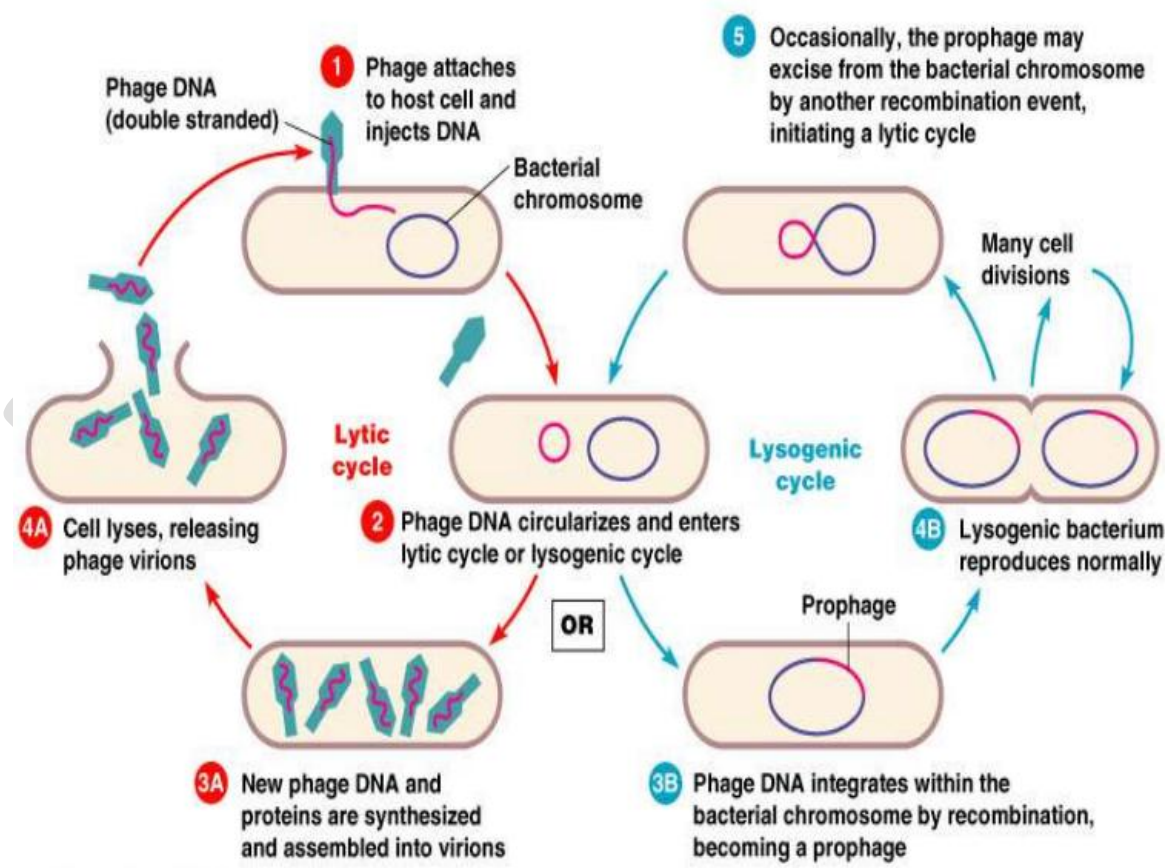


Fig: Process of Transduction

Mapping by Transduction

Generalized Transduction

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

.

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

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

- P1 enters and integrates as a prophage.
- If the lysogenic state is not maintained, P1 enters a lytic cycle and produces progeny phages.
- The bacterial chromosome is degraded during lytic infection, and rarely, bacterial DNA is packaged as if it were a P1 chromosome, producing a transducing phage.
- The transducing phage DNA enters the host cell in the normal P1 way, and may be incorporated into the host's chromosome by homologous recombination. The resulting bacteria are transductants.

Transduction experiments use genetic markers to follow gene movement.

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

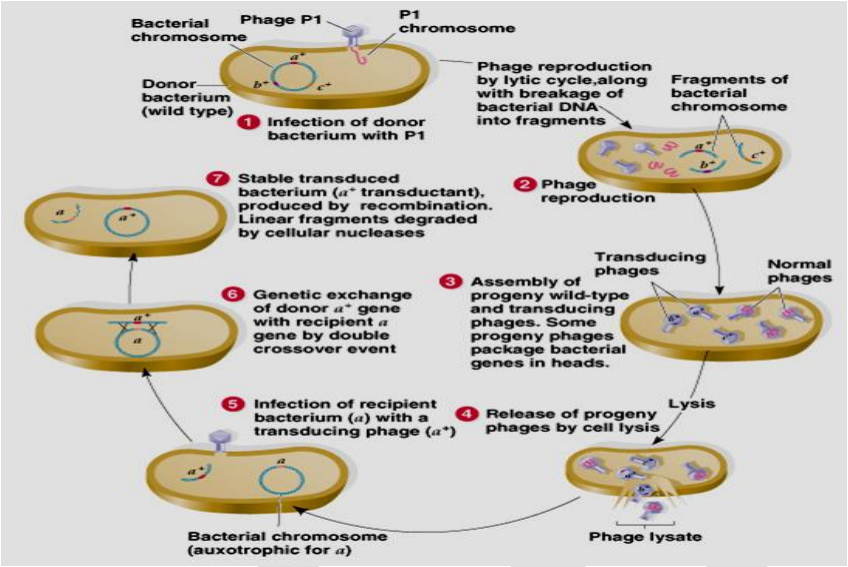


Fig: Generalized transduction between strains of *E. coli*

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

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

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

Specialized Transduction

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

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

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

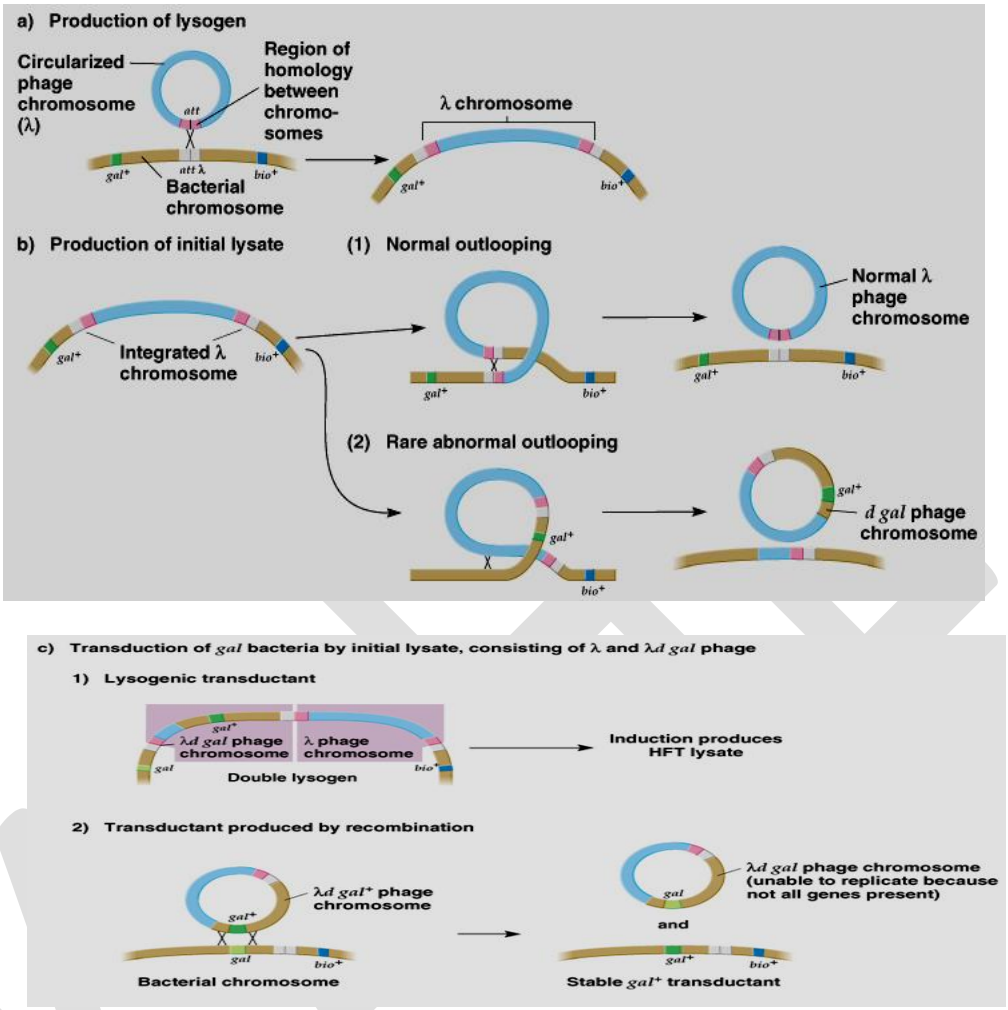


Fig: Specialized transduction by bacteriophage λ

Discovery of Conjugation in *E. coli*

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

- Strain A's genotype was met bio thr+ leu+ thi+. It grows on minimal medium supplemented with methionine and biotin.
- Strain B's genotype was met+ bio+ thr leu thi. It grows on minimal medium supplemented with threonine, leucine and thiamine.
- Strains A and B were mixed, and plated onto minimal medium. About 1/106 cells produced colonies with the phenotype met+ bio+ thr+ leu+ thi+ (Figure 14.2).

- Neither strain produced colonies when plated alone onto minimal medium, so the new phenotype resulted from recombination.

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

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

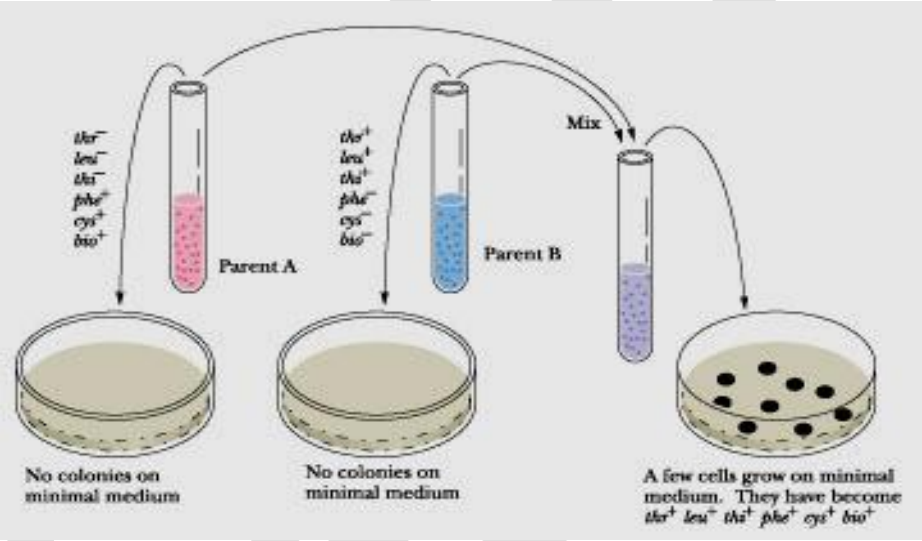


Fig: Cell to cell contact by conjugation a medium of gene transfer

Sexual Conjugation in Bacteria : The transfer of DNA between bacteria takes place via a process known as **sexual conjugation,** a phenomenon unsuspected prior to the Lederberg-Tatum experiment. Bacterial cells sometimes contain, in addition to their chromosome, extrachromosomal DNA molecules called **plasmids** . Plasmids represent "extra" or auxiliary genetic information. Bacterial cells are capable of conjugation if they possess a particular plasmid called the **F factor** (F for fertility). Such F^+, or *donor, cells* have thin, hollow tubes projecting from their surface known as **sex pili** or **F pili** (singular = *pilus*). One or more pili can bind to specific receptors on the surface of cells that lack an F factor (F^-, or *recipient, cells*)The pilus provides a connection between the two cells. Upon conjugation, a single strand of the F factor is passed to the F^- cell, where its complementary strand is

synthesized .The recipient F^- cell thus becomes F^+ by virtue of now having a double-stranded F factor plasmid. The F factor plasmid consists of about 94,000 base pairs; about one-third of this DNA is devoted to about 25 genes that function specifically in the transfer of genetic material from F^+ to F^- cells. Among these genes are those necessary for the formation of pili. In reality, the F factor is an infectious agent.

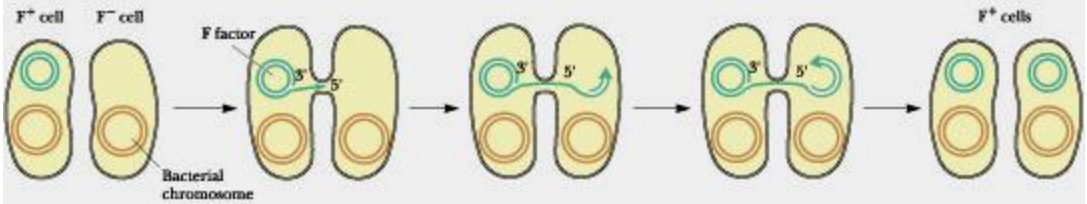


Fig: Physical factors in Bacterial conjugation

Mapping by Conjugation

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

Interrupted-mating experiment

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

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

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

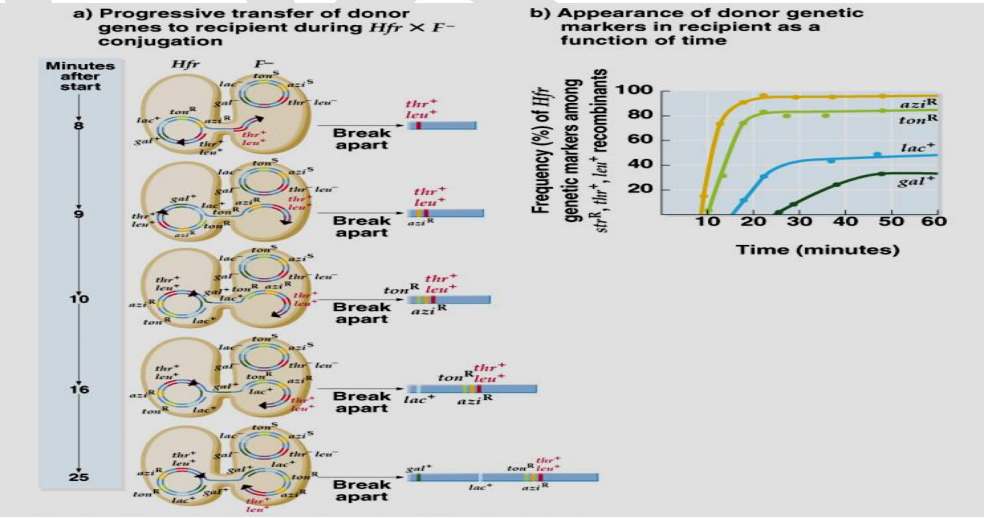


Fig: Interrupted-mating experiment

Linkage and genetic mapping:

A linkage map is a representation, in the form of a table or a graphic, of the position of genes (or markers) within a linkage group. The map positions are inferred from estimates of recombination frequencies between genes.

Genetic Mapping Functions

A genetic mapping function (mf) describes the mathematical relation between recombination frequency (*r*) and map distance (*x*). The map distance between two markers is the average number of recombination events per meiosis between those markers. The exact relation *f* between *r* and *x* (*r* = *f*(x)) depends on the degree on interference between recombination events, in adjacent intervals. In the absence of interference recombination events in adjacent intervals (and as a result, in any pair of intervals) are independent.

(http://www.generationcp.org/capcorner/spain_qtl_2005/genetic_linkage_maps.pdf)

Phage T4 life cycle: (http://flylib.com/books/en/2.643.1.136/1/)

Figure 11.7 The phage T4 lytic cascade falls into two parts : early and quasi-late functions are concerned with DNA synthesis and gene expression; late functions are concerned with particle assembly

Bacteriophage T4 Genome:

Genome of T4 ds DNA, linear, 168,903bp encodes over 250 different proteins have a unique base, 5-hydroxymethylcytosine instead of cytosine. The glucosylated DNA is resistant to virtually all restriction endonucleases of the host T4 DNA is circularly permuted Cut from a circular,

but in each case the cut was at a different place.Terminal repeats of 3000-6000bp. DNA in each virion has repeated sequences at each end .A headful mechanism -the packaging mechanism involves cutting DNA from a long DNA molecule containing several genome equivalents linked end-to-end the concatemer is cut after the head is full, cut not a specific sequence (irrespective of the sequence).The packaging mechanism of T4 DNA-a meadful mechanism. (http://202.204.115.67/jpkch/jpkch/2008/wswx/chapter%209.htm#9)

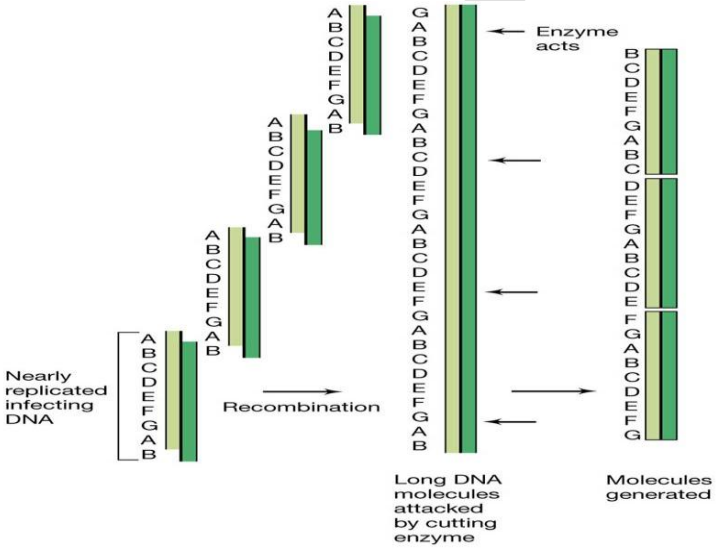


Figure :Generation in T4 of virus length DNA molecules with permuted sequences by a cutting enzyme, which cuts off constant lengths of DNA irrespective of the sequence

Phage T4 has one of the larger genomes (165 kb), organized with extensive functional grouping of genes. Genes that are numbered are *essential* : a mutation in any one of these loci prevents successful completion of the lytic cycle. Genes indicated by three-letter abbreviations are *nonessential* , at least under the usual conditions of infection. We do not really understand the inclusion of many nonessential genes, but presumably they confer a selective advantage in some of T4's habitats. (In smaller phage genomes, most or all of the genes are essential.) (http://flylib.com/books/en/2.643.1.136/1/)

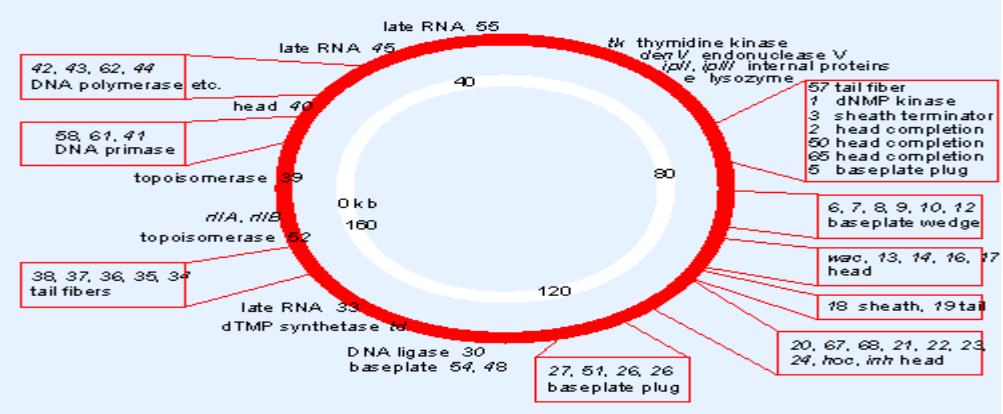


Fig: The map of T4 is circular. There is extensive clustering of genes coding for components of the phage and processes such as DNA replication, but there is also dispersion of genes coding for a variety of enzymatic and other functions. Essential genes are indicated by numbers . Nonessential genes are identified by letters . Only some representative T4 genes are shown on the map

Genome of T4 ds DNA, linear, 168,903bp encodes over 250 different proteins have a unique base, 5-hydroxymethylcytosine instead of cytosine.The glucosylated DNA is resistant to virtually all restriction endonucleases of the host.T4 DNA is circularly permuted cut from a circular, but in each case the cut was at a different place. Terminal repeats of 3000-6000bp DNA in each virion have repeated sequences at each end. A headful mechanism-the packaging mechanism involves cutting DNA from a long DNA molecule containing several genome equivalents linked end-to-end the concatemer is cut after the head is full, cut not a specific sequence(irrespective of the sequence)

Questions

Long answer questions

1. Explain deamination and oxidative damage of DNA.
2. Describe mutation selection.
3. What are mutagens? Write note on mutagenic agents.
4. Give a brief account on SOS repair.

5. Give an account on types of DNA damage.
6. What are induced mutations? Describe frame shift mutation.
7. What are induced mutations? Describe frame shift mutation.
8. Describe any two DNA repair mechanism. Describe any two DNA repair mechanism.
9. Outline the Luria Delbruck experiment.

Short answer questions

1. What are the major types of DNA damage repair
2. List the names of the three types of excision repair
3. State one reason why the process of DNA repair is important
4. Enlist types of DNA damage.
5. Define Mutagen, mutagenesis and mutation
6. What causes DNA mutations

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I MSc Microbiology **COURSE NAME**: Molecular Genetics

COURSE CODE: 18MBP103 **BATCH**-2018-2019

Multiple choice questions

UNIT III

Sr No.	Unit IV Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	Conjugation can only occur between cells of	F positive types	F negative types	Same mating types	Opposite mating types	Opposite mating types
2	Conjugation involves the use of _____for mapping	Interrupted mating	Direct mapping	Contact mapping	Linkage	Interrupted mating
3	Conjugation involves the use of _____for mapping	Interrupted mating	Direct mapping	Contact mapping	Linkage	Interrupted mating
4	Conjugation is predominant in _____	Spirochaetes	G+ bacteria	G-bacteria	Cyanobacteria	G-bacteria
5	Conjugational genetic exchange has been - - - - - - - - - frequently encountered among gram positive than gram negative bacteria.	More	Less	Very High	Very low	Less
6	Crossing over occurs during	Pachytene	Diplotene	Diakinesis	Haplotene	Pachytene

7	Direct way of observing the physical arrangement of markers along the chromosomes	Fluorescence in situ hybridization	Fluorescence invitro hybridization	Fluorescence invivo hybridization	Fluorescence in cell hybridization	Fluorescence in situ hybridization
8	During insertion of lambda DNA in to host, a viral protein called _____ is required along with integration host factor (IHF)	Integrase	Caspase	Helicase	Polymerase	Integrase
9	Experiment on transformation	Monad	Griffith	Morgan	Hersehy	Griffith
10	Genes responsible for antibiotic resistance are mainly transferred across bacterial population by	Conjugation	Transformation	Transduction	Gene expression	Conjugation
11	_____ involves finding a contiguous series of cloned DNA fragments which contain overlapping portions of the genome	Physical mapping	Chemical mapping	Marker mapping	Loci mapping	Physical mapping
12	_____ refers to a genetic changes in different genomes of same cell.	Trans type	Cis type	Same type	Different type	Cis type
13	_____ is a DNA associated protein	Protone	Histone	Chromotome	Cistron	Histone
14	A bacterium harboring a prophage is called	Lytic phage	Helper phage	Transducing phage	Lysogency	Lysogency
15	A cell carrying an integrated F factor is	F	Hfr	Hfr 1+	trans	Hfr

	called an					
16	An example for specialized transducing particle	No infection	T2 phage infects *Staphylococcus*	Phage P22 infects *Salmonella typhimurium*	Phage lambda infects *E.coli*	Phage lambda infects *E.coli*
17	Avery, MacLeod & McCarty used enzymes and solvents to destroy molecules such as	Anything except DNA	RNA	Lipids & proteins	Polysaccharide	Anything except DNA
18	Bacteriophages were jointly discovered by	Frederick Twort and by Felix d'Herelle	Hershey and Chase	Luria and Delbruck	McKay and McCartney	Frederick Twort and by Felix d'Herelle
19	Capsule of *Streptococcus pneumoniae* are made up of	Protein	Lipid	Glycoprotein	Polysaccharide	Polysaccharide
20	Capsules help bacteria in escaping	Inflammation	RBC's	Phagocytosis	Antibodies	Phagocytosis
21	Cells carrying non-integrated transducing fragments are called _____	Specialized	Abortive transductants	Generalized	Conjugation	Abortive transductants
22	Complete linkage has been reported in	Male Drosophila	Human female	Female Drosophila	Maize	Male Drosophila
23	Conditions that favor the termination of the lysogenic state	Desiccation	Decomposition	Nutrient Media	Macronutrient	Desiccation
24	The complex of DNA, RNA and protein is	Chromatin	Somatin	Pigmentin	Fromatin	Chromatin
25	The first demonstration of bacterial transformation was	*Streptococcus pyogenes*	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Klebsiella pneumoniae*	*Streptococcus pneumoniae*

	done with					
26	The frequency at which two genes are - - - - - - - - by population of phages can be used to estimate their relative distance	Transduced	Co transduced	Co repressor	Co operator	Co transduced
27	The gene linkage minimize the chances of	Cross over	Segregation	Recombination	Assortment	Recombination
28	The non specific transduction is also called as - - - - - - - - -	Restricted transduction	Generalized transduction	Non specific transduction	Specialised transduction	Generalized transduction
29	The phenomenon in which genes are present on the same chromosomes is	Cross over	Segregation	Linkage	Assortment	Linkage
30	The phenomenon of linkage was first observed in the plant	*Lathyrus odoratus*	*Pisum sativum*	*Datura*	*Mirabilus jalapa*	*Lathyrus odoratus*
31	The process to identify a genetic element that is responsible for a disease is also referred as	Mapping	Linkage	Sequencing	Genome data mining	Mapping
32	The viral genome integrated to the bacterial genome is called	Plasmid	Capsid	Prophage	Virion	Prophage
33	Transfer of a portion of chromosome to a recipient with direct contact is termed	Gene expression	Transformation	Transduction	Conjugation	Conjugation

34	Transfer of DNA from one bacterium to another through the action of viruses	Transduction	Conjugation	Transformation	Gene expression	Transduction
35	Uptake of DNA molecules from environmental surrounding	Transduction	Conjugation	Transformation	Gene expression	Transformation
36	Virulence in *Streptococcus pneumoniae* is attributed to	Flagella	Capsules	Pili	Fimbriae	Capsules
37	Who coined the term linkage	Mendel	Morgan	de Vries	Correns	Morgan
38	Genes that cause suppression of mutations in other genes are called -------- genes	Reverse genes	Control genes	Suppressor genes	Inducer genes	Suppressor genes
39	Genetic fine structure mapping of T4 was studied by _____	Benzon	Mendel	Colins	Bennazir	Benzon
40	Genetic recombination in phages was discovered by _____	Hershey and Rotma	Hershey and Chase	Hershey and Wollmer	Hershey and Singer	Hershey and Rotma
41	Genome of T4 phage is	ds DNA	ss DNA	ds RNA	ss RNA	ds DNA
42	Genome of different bacteria suggest that genes have in the past moved from one species to another. This phenomenon is	DNA transfer	RNA transfer	Gene transfer	Protein transfer	Gene transfer

	called lateral					
43	Give the full form for Hfr	High fertility recombination	High fundamental recombination	High frequency recombination	Heavy frequency recombination	High frequency recombination
44	In conjugation, the donor always carries on	(F-)	(F+)	F neutral	No F	(F+)
45	In genetic mapping, the measurement of distance between the genes is expressed as	Centimorgan	Centimeter	Millimorgan	Millimeter	Centimorgan
46	Integeration of viral nucleic acid in to host chrosome is termed as	Microphage	Prophage	Prephage	Macrophage	Prophage
47	Linkage prevents	Segregation of alleles	Homozygous condition	Hybrid formation	Heterozygous condition	Segregation of alleles
48	Map distance is equal to the percentage of _____	Recombinant meiotic product	Reproducible meiotic product	Recombinant mitotic product	Reproducible mitotic product	Recombinant meiotic product
49	Metalloproteins found in all eukaryotes	Zinc fingers	Iron fingers	Lead fingers	Copper fingers	Zinc fingers
50	Methods used to identify the locus of a gene and the distances between genes	Gene mapping	Chromosomal linkage	Gene walking	Chromosomal walking	Gene mapping
51	Non sex chrosomes are called	Rhizomes	Lysosomes	Mesosomes	Autosomes	Autosomes
52	Occurs when new DNA does not integrate into the chromosome, not replicated and is eventually lost	Abortive transduction	Specialized Transduction	Generalized Transduction	Transfusion	Abortive transduction
53	Pneumococcal 'S' cells produce _____	Smooth	Rough	Elongated	Flat	Smooth

	colonies during growth on agar plates					
54	Results from inaccurate excision of an integrated prophage with addition of some bacterial genes	Specialized Transduction	Generalized Transduction	Abortive transduction	Transfusion	Specialized Transduction
55	Size of T4 phage genome	169 kbp	196 kbp	619 kbp	916 kbp	169 kbp
56	Specialised transduction is effected by _____	T_2	Mu	P_1	T_7	T_2
57	T4 bacteriophages generally parasitizes	*Bacillus*	*E.coli*	*Psuedomonas*	*Agrobacterium*	*E.coli*
58	T4 is capable of undergoing only a	Lytic cycle	Lysogenic cycle	Both Lytic & Lysogenic cycle	Other cycle	Lytic cycle
59	Tendency of alleles located close together on a chromosome to be inherited together during the meiosis	Linkage	Crossing over	Gene overlapping	Recessive genes	Linkage
60	The Competence of a cell in the process of transformation is aided by	$CaCl_2$	$MgCl_2$	KCl	$AgCl_2$	$CaCl_2$

UNIT IV
Syllabus

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

Reasons

- ✓ Spontaneous errors in DNA replication or meiotic recombination
- ✓ A consequence of the damaging effects of physical or chemical mutagens on DNA

Some consequences of gene mutations on protein-level:

• neutral and missense mutation: exchange of the encoded amino acid

• frameshift mutation: the reading frame will be shifted

• nonsense mutation: change to stop codon

• chain elongation: stop codon changes to amino acid

• silent mutation: no change in amino acid (synonymous codon)

• Molecular phylogenetic hypotheses suppose that closely related organisms show high similarity in their genetic material (i.e. relatively few mutations occured) while distantly related organisms show bigger differences in their

Mutagens: Agents that cause DNA damage that can be converted to mutations.

Physical mutagens responsible for mutation

- ➢ ***High-energy ionizing radiation***: X-rays and g-rays → strand breaks and base/sugar destruction
- ➢ ***Nonionizing radiation*** : UV light→ pyrimidine dimers

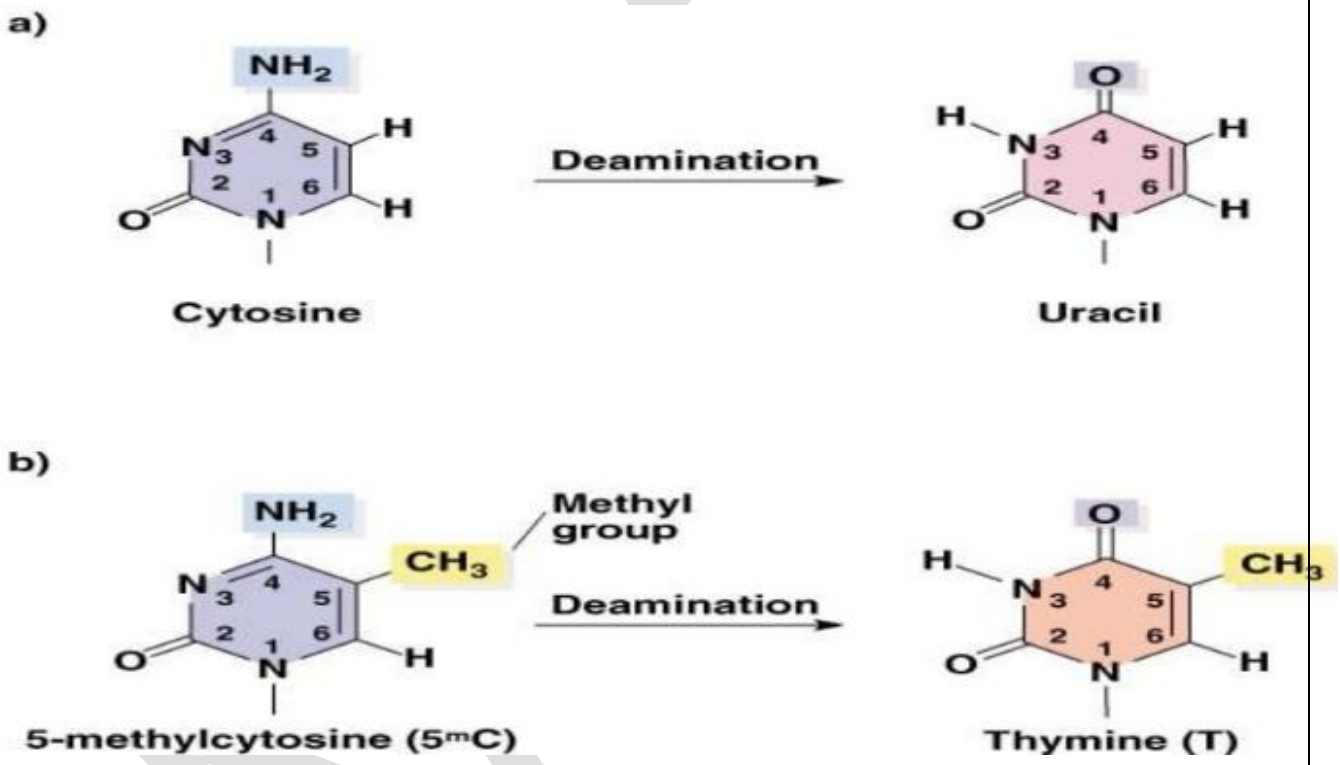
Chemical mutagens responsible for mutation

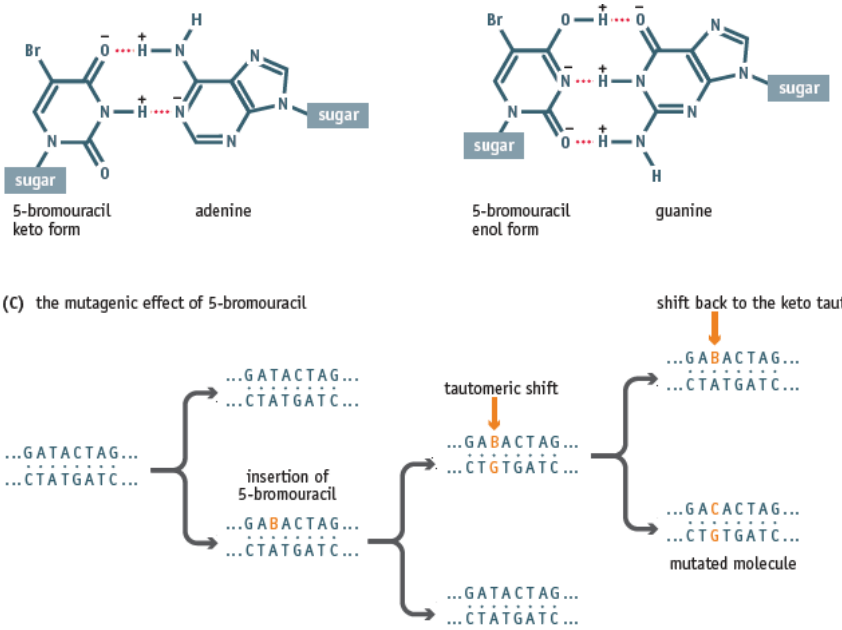
- ◆ ***Base analogs***: direct mutagenesis
- ◆ ***Nitrous acid***: deaminates C to produce

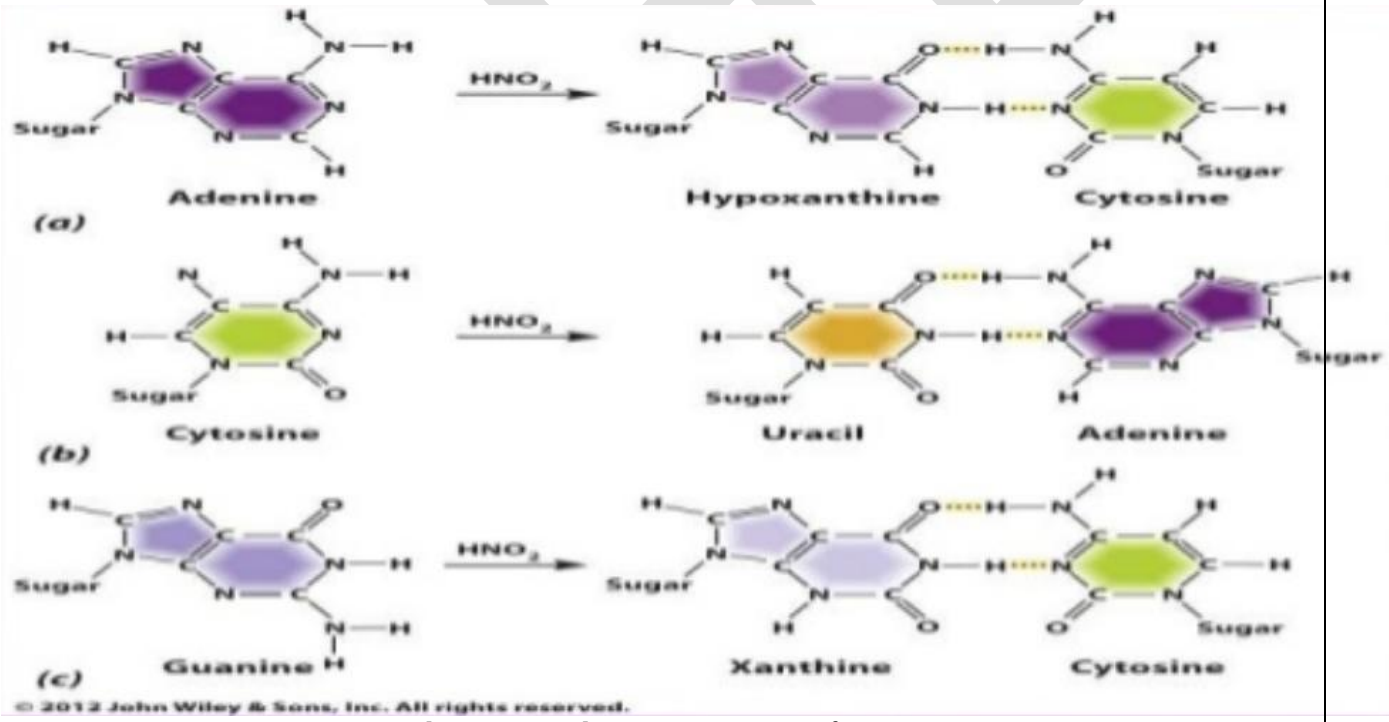
- ***Alkylating agents***: Nucleotide modification caused by electrophilic alkylating agents such as methylmethane sulfonate and ethylnitrosourea
- **DNA intercalating agent**
- **DNA crosslink agent**
- **Reactive oxygen species (ROS)**

· *Base analogs*: derivatives of the normal bases incorporated in DNA, altering base pairing properties.

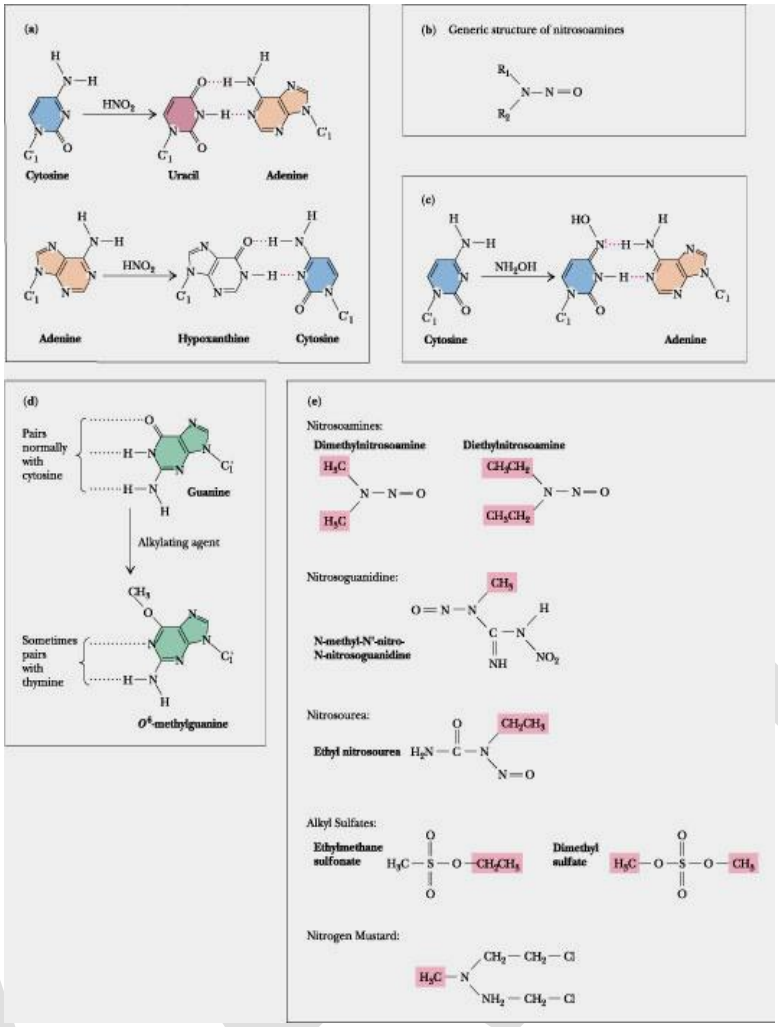
· *Nitrous acid*: deaminates C to produce U, resulting in G·C→ A·U

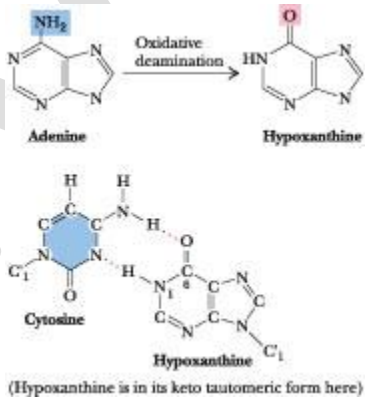






Nitrous Acid Causes Oxidative Deamination of Bases





(Hypoxanthine is in its keto tautomeric form here)

Agent	Action	Result
Base analogs		
5-Bromouracil	Incorporated like T; occasional faulty pairing with G	AT pair → GC pair; occasionally GC → AT
2-Aminopurine	Incorporated like A; faulty pairing with C	AT → GC; occasionally GC → AT
Chemicals reacting with DNA		
Nitrous acid (HNO_2)	Deaminates A and C	AT → GC and GC → AT
Hydroxylamine (NH_2OH)	Reacts with C	GC → AT
Alkylating agents		
Monofunctional (for example, ethyl methane sulfonate)	Puts methyl on G; faulty pairing with T	GC → AT
Bifunctional (for example, nitrogen mustards, mitomycin, nitrosoguanidine)	Cross-links DNA strands; faulty region excised by DNase	Both point mutations and deletions
Intercalative dyes		
Acridines, ethidium bromide	Inserts between two base pairs	Microinsertions and microdeletions
Radiation		
Ultraviolet	Pyrimidine dimer formation	Repair may lead to error or deletion
Ionizing radiation (for example, X-rays)	Free-radical attack on DNA, breaking chain	Repair may lead to error or deletion

Mutagenesis: The molecular process in which the mutation is generated

Direct mutagenesis:The stable, unrepaired base with altered base pairing properties in the DNA is fixed to a mutation during DNA replication.

Indirect mutagenesis:The mutation is introduced as a result of an error-prone repair.

Molecular Basis of mutation

Table 7-1 Gene Mutations at the Molecular Level

Type of mutation	Result and example(s)
Forward mutations	
Single-nucleotide-pair (base-pair) substitutions	
At DNA level	
Transition	Purine replaced by a different purine, or pyrimidine r pyrimidine: A·T ⟶ C·G C·G ⟶ A·T C·G ⟶ T
Transversion	Purine replaced by a pyrimidine, or pyrimidine replace A·T ⟶ C·G A·T ⟶ T·A G·C ⟶ T· T·A ⟶ G·C T·A ⟶ A·T C·G ⟶ A
At protein level	
Silent mutation	Triplet codes for same amino acid: AGG ⟶ CGG both code for Arg

Types of Mutation

- Substitution, deletion, or insertion of a base pair.
- Chromosomal deletion, insertion, or rearrangement.
- Somatic mutations occur in somatic cells and only affect the individual in which the mutation arises.
- Germ-line mutations alter gametes and passed to the next generation.

SOMATIC MUTATIONS

- Arise in the somatic cells.
- Passed on to other cells through the process of mitosis.
- Effect of these mutations depends on the type of the cell in which they occur & the developmental stage of the organism.

• If occurs early in development, larger the clone of the mutated cells.

GERM LINE MUTATION-
• They occur in the cells that produce gametes
• Passed on to future generations
• In multicellular organisms, the term mutation is generally used for germ line mutations

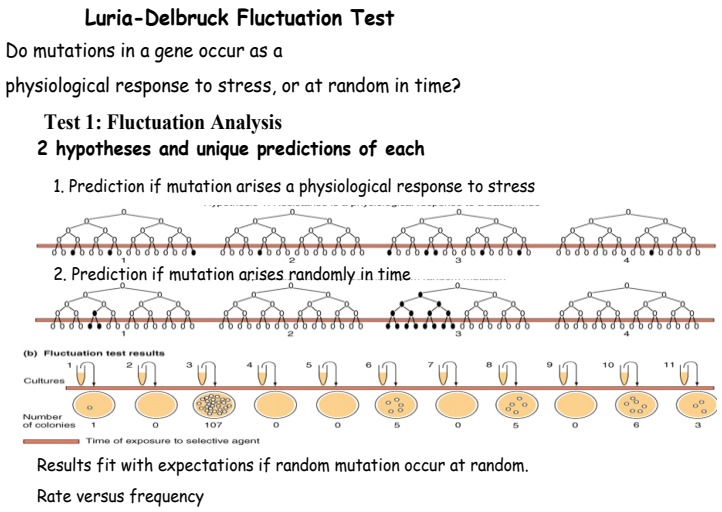
Mutations are quantified in two ways:

- Mutation rate = probability of a particular type of mutation per unit time (or generation).
- Mutation frequency = number of times a particular mutation occurs in a population of cells or individuals.
- Experimental test of Lamarck's "inheritance of acquired traits"

Salvador Luria and Max Delbrück (1943)

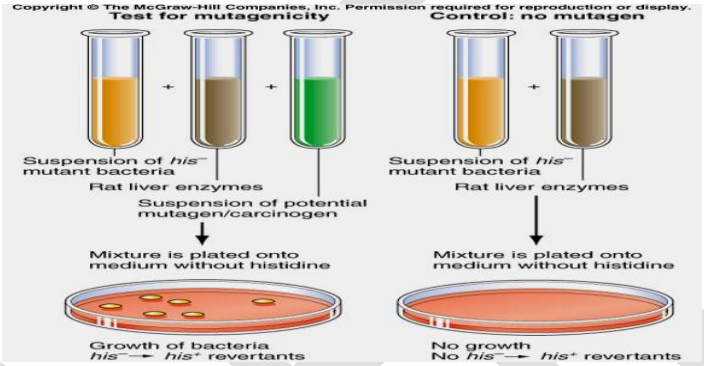
An *E. coli* population started from one cell should show different patterns resistance depending on which theory is correct.

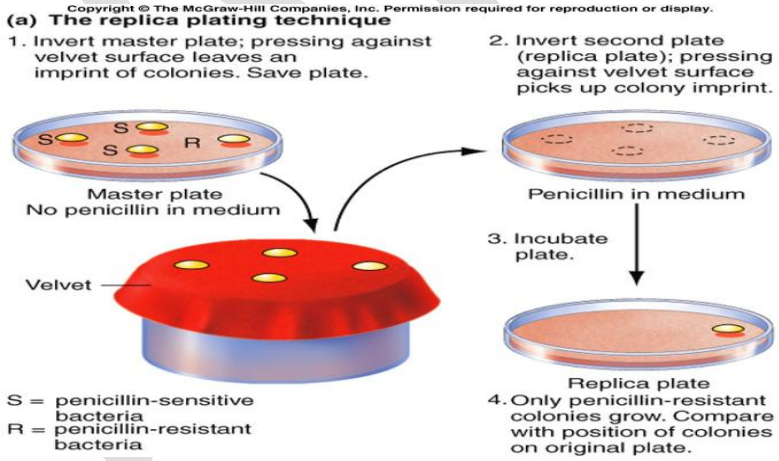
- Adaptive theory states that cells are induced to become resistant when T1 is added; proportion of resistant cells should be the same for all cultures with the same genetic background.
- Mutation theory states that random events confer resistance to T1; duplicate cultures with the same genetic background should show different numbers of T1 resistant cells.



Ames test:

In the 1970s a scientist named, Bruce Ames, discovered a procedure that tests carcinogens in compounds. This procedure became to be known as the Ames Test. Studies show that carcinogens are easily detectable in microorganisms. Therefore, a bacterium Salmonella typhimurium was used. This organism cannot survive without the amino acid histidine. In the procedure, the bacteria were given very little histidine in order to detect its mutation ability. This mutation ability tells us if the compound given to the bacteria is carcinogenic. If the Salmonella mutates, then the compound is carcinogenic. If the Salmonella does not mutates, then the compound is not carcinogenic.





Carcinogenicity test

DNA repair mechanism

.DNA in the living cell is subjected to many chemical alterations.

• The genetic information encoded in the DNA has to remain uncorrupted

• Any chemical changes must be corrected.

• A failure to repair DNA produces a mutation.

Agents that Damage DNA

• Radiations Highly reactive oxygen radicals produced during normal cellular respiration as well as by other biochemical pathways

Ionizing radiation such as gamma rays and x-rays o Ultraviolet rays, especially the UV-C rays (~260 nm) that are absorbed strongly by DNA but also the longer-wavelength UV-B that penetrates the ozone shield

Chemicals in the environment - Aromatic hydrocarbons, including some found in cigarette smoke

Plant and microbial products, e.g. the Aflatoxin produced in moldy peanuts

Chemicals used in chemotherapy, especially chemotherapy of cancers.

DNA Repair DNA repair can be grouped into two major functional categories:

A) Direct Damage reversal

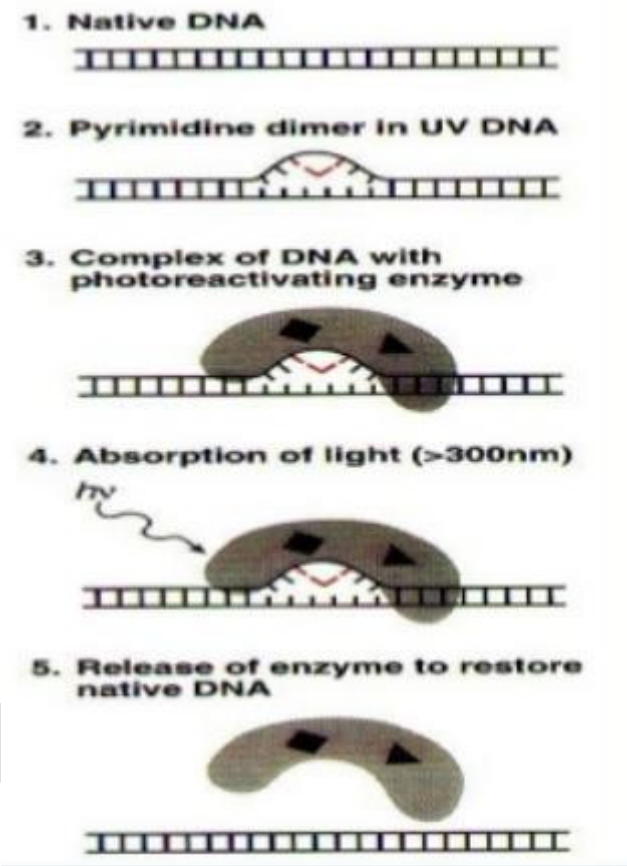
B) Excision of DNA damage

A) The direct reversal of DNA damage is by far the simplest repair mechanism that involves a single polypeptide chain, with enzymatic properties which binds to the damage and restores the DNA genome to its normal state in a single-reaction step. The major polypeptides involved in this pathway are:

i) DNA photolyases, the enzymes responsible for removing cyclobutane pyrimidine dimers from DNA in a light-dependent process called as photo reactivation

ii) 6-methylguanine-DNA methyltransferase I and II (MGMT), also called DNAalkyltransferases, remove the modified bases like 6alkylguanine and 4alkylthymine.

• The photolyase protein is not found in all living cells. However, the DNA alkyl transferases are widespread in nature.



B) Excision of DNA damage

i) Base excision repair (BER)

ii) Nucleotide excision repair (NER),

iii) Mismatch repair (MMR) and

iv) Strand break repairs.

• In these reactions a nucleotide segment containing base damage, double-helix distortion or mispaired bases is replaced by the normal nucleotide sequence in a new DNA polymerase synthesis process.

• All of these pathways have been characterized in both bacterial and eukaryotic organisms

i) Base Excision Repair (BER)

• BER is initiated by DNA glycosylases, which catalyze the hydrolysis of the N-glycosidic bonds, linking particular types of chemically altered bases to the deoxyribosephosphate backbone.

• DNA damage is excised as free bases, generating sites of base loss called apurinic or apyrimidinic (AP) sites.

• The AP sites are substrates for AP endonucleases.

• These enzymes produce incisions in duplex DNA as a result of the hydrolysis of a phosphodiester bond immediately 5' or 3' to each AP site.

• The ribose-phosphate backbone is then removed from the DNA through the action of a specific exonuclease called deoxy ribophosphodiesterase or dRpase.

• Finally, the DNA polymerase and a ligase catalyze the incorporation of a specific deoxyribonucleotide into the repaired site, enabling correct base pairing

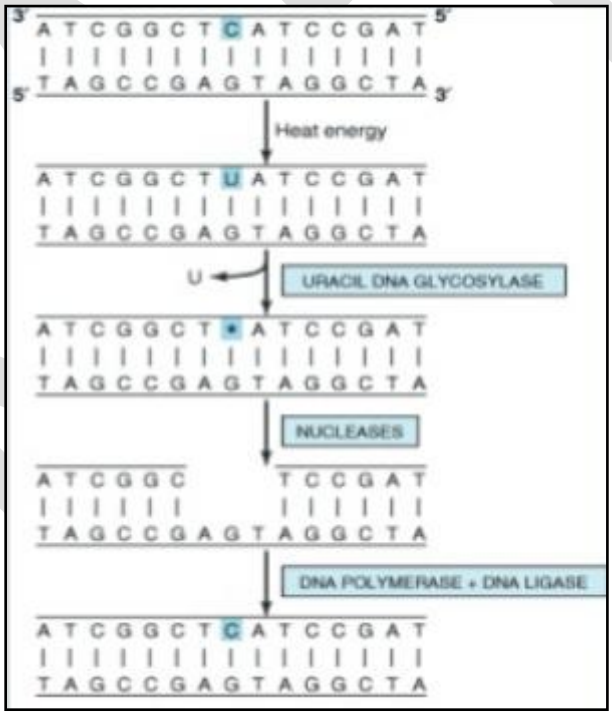
• The enzyme uracil DNA glycosylase removes the uracil created by spontaneous deamination of cytosine in the DNA.

• An endonuclease cuts the backbone near the defect

• An endonuclease removes a few bases

• The defect is filled in by the action of a DNA polymerase and

• The strand is rejoined by a ligase.

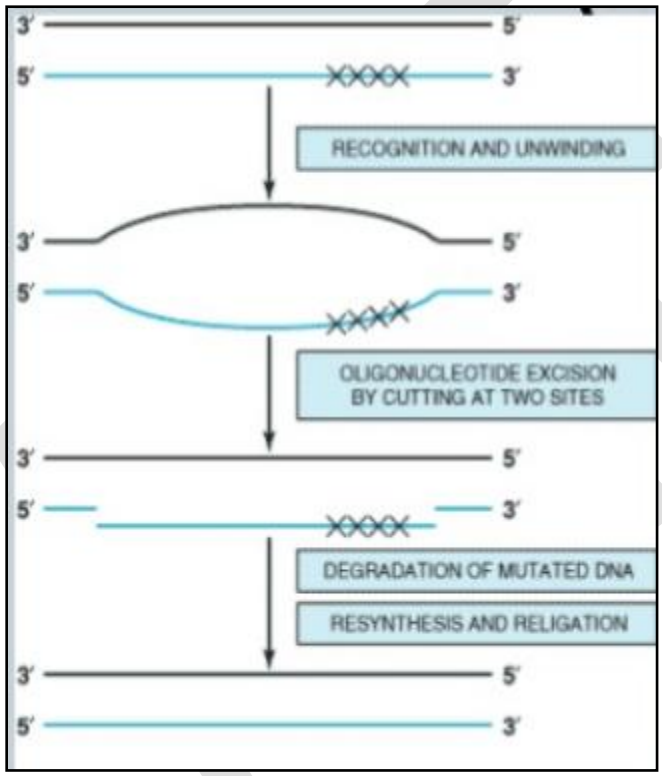


ii) Nucleotide excision repair (NER)

• This mechanism is used to replace regions of damaged DNA up to 30 bases in length.

• Common causes of such DNA damage include ultraviolet (UV) light, which induces the formation of cyclobutane pyrimidine-pyrimidine dimers, and smoking, which causes formation of benzo[a]pyrene-guanine adducts.

• Ionizing radiation, cancer chemotherapeutic agents, and a variety of chemicals found in the environment cause base modification, strand breaks, cross-linkage between bases on opposite strands or between DNA and protein, and numerous other defects.



• These are repaired by a process called nucleotide excisionrepair

• NER is a much more complex biochemical process than BER, especially in eukaryotic cells.

• Several gene products are required in a multiple step process, during which the ordered assembly of DNA proteins provides an enzymatic complex that discriminates damaged from undamaged DNA

• In eukaryotic cells the enzymes cut between the third to fifth phosphodiester bond 3' from the lesion, and on the 5' side the cut is somewhere between the twenty-first and twentyfifth bonds. Thus, a fragment of DNA 27–29 nucleotides long is excised. After the strand is removed it is replaced, again by exact base pairing, through the action of yet another polymeras e,and the ends are joined to the existing strands by DNA ligase.

• In Escherichia coli there are three specific proteins, called UvrA, B and C, involved in lesion recognition and endonuclease incision.

• This fragment is released by UvrD helicase action, generating a gap that is finally submitted to repair synthesis

• Nucleotide-excision repair proceeds most rapidly in cells whose genes are being actively transcribed on the DNA strand that is serving as the template for transcription.

• If RNA polymerase II, tracking along the template (antisense) strand), encounters a damaged base, it can recruit other proteins, to make a quick fix before it moves on to complete transcription of the gene.

iii) Mismatch repair (MMR)

• Mismatch repair corrects errors made when DNA is copied

For example, a C could be inserted opposite an A, or the polymerase could slip or stutter and insert two to five extra unpaired bases.

• Specific proteins scan the newly synthesized DNA, using adenine methylation within a GATC sequence as the point of reference

• The template strand is methylated, and the newly synthesized strand is not.

• This difference allows the repair enzymes to identify the strand that contains the errant nucleotide which requires replacement.

• If a mismatch or small loop is found, a GATC endonuclease cuts the strand bearing the mutation at a site corresponding to the GATC.

• An exonuclease then digests this strand from the GATC through the mutation, thus removing the faulty DNA. This can occur from either end if the defect is bracketed by two GATC sites.

• This defect is then filled in by normal cellular enzymes according to base pairing rules Biochemistry for Medics-Lecture Notes 19 20. 20. iii) Mismatch repair (MMR)

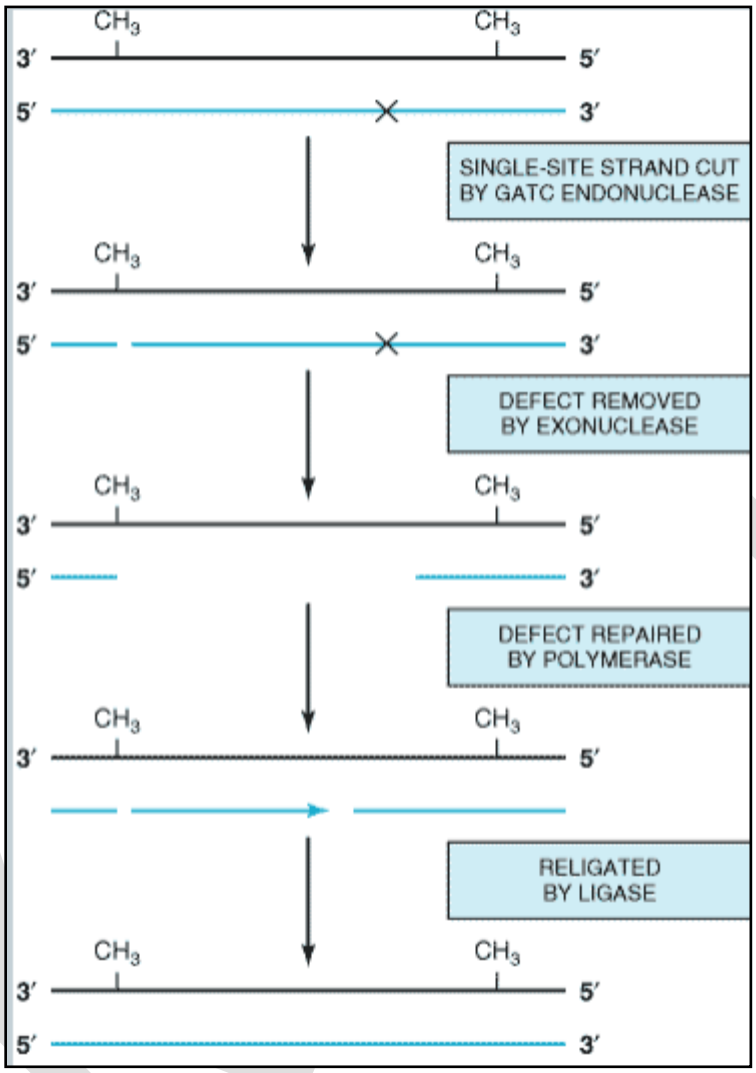
• This mechanism corrects a single mismatch base pair (eg, C to A rather than T to A) or a short region of unpaired DNA.

• The defective region is recognized by an endonuclease that makes a single-strand cut at an adjacent methylated GATC sequence.

• The DNA strand is removed through the mutation, replaced, and religated.

• In E coli, three proteins (Mutt S, Mutt L, and Mutt H) are required for recognition of the mutation and nicking of the strand. Other cellular enzymes, including ligase, polymerase, and SSBs, remove

and replace the strand. The process is more complicated in mammalian cells, as about six proteins are involved in the first steps. Faulty mismatch repair has been linked to hereditary nonpolyposis colon cancer (HNPCC), one of the most common inherited cancers.



B) Repairing Strand Breaks

• Ionizing radiation and certain chemicals can produce both single-strand breaks (SSBs) and double-strand breaks (DSBs) in the DNA backbone. i) Single-Strand Breaks (SSBs)

• Breaks in a single strand of the DNA molecule are repaired using the same enzyme systems that are used in Base-Excision Repair (BER).

• There are two mechanisms by which the cell attempts to repair a complete break in a DNA molecule:

1) Direct joining of the broken ends. This requires proteins that recognize and bind to the exposed ends and bring them together for ligating. This type of joining is also called Nonhomologous End-Joining (NHEJ). A protein called Ku is essential for NHEJ.

• Errors in direct joining may be a cause of the various translocations that are associated with cancers. Examples: • Burkitt's lymphoma • Philadelphia chromosome in chronic myelogenous leukemia (CML), • B-cell leukemia

2) Homologous Recombination. Here the broken ends are repaired using the information on the intact

• sister chromatid, or on the

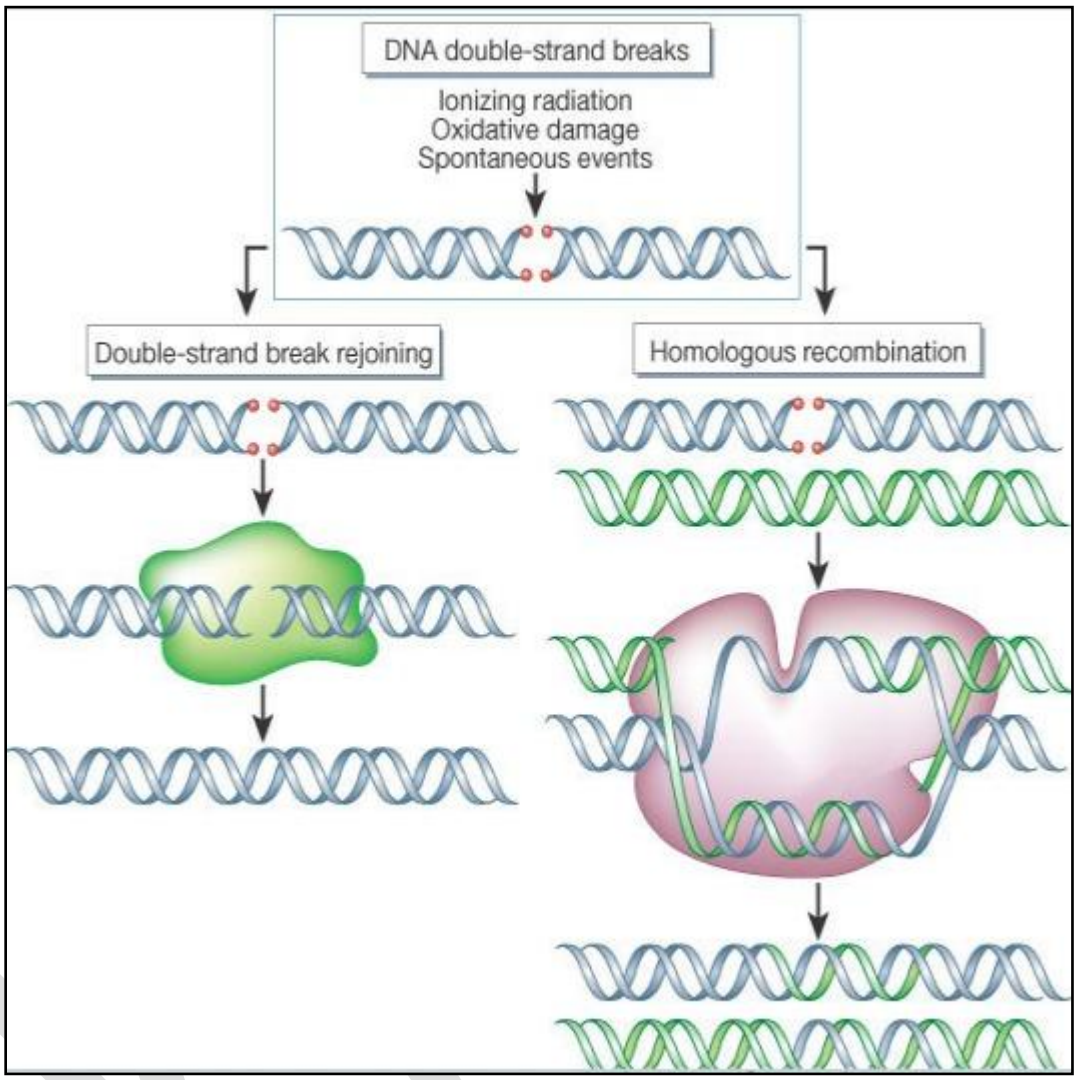
• homologous chromosome

• same chromosome if there are duplicate copies of the gene on the chromosome oriented in opposite directions (head-to-head or back-to-back).

• Two of the proteins used in homologous recombination are encoded by the genes BRCA1 and BRCA2.

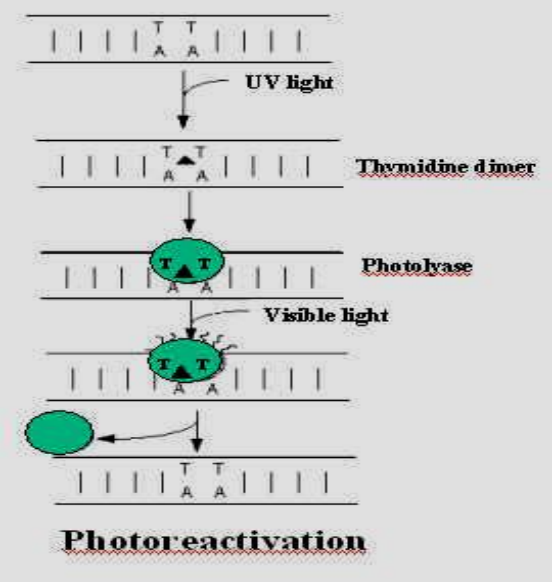
• Inherited mutations in these genes predispose women to breast and ovarian cancers.

Meiosis also involves DSBs Recombination between homologous chromosomes in meiosis I also involves the formation of DSBs and their repair. Meiosis I with the alignment of homologous sequences provides a mechanism for repairing damaged DNA



Photoreactivation (aka Light Repair)

phr gene - codes for deoxyribodipyrimidine photolyase 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



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. an unirradiated host because SOS system has already been turned on in irradiated host.

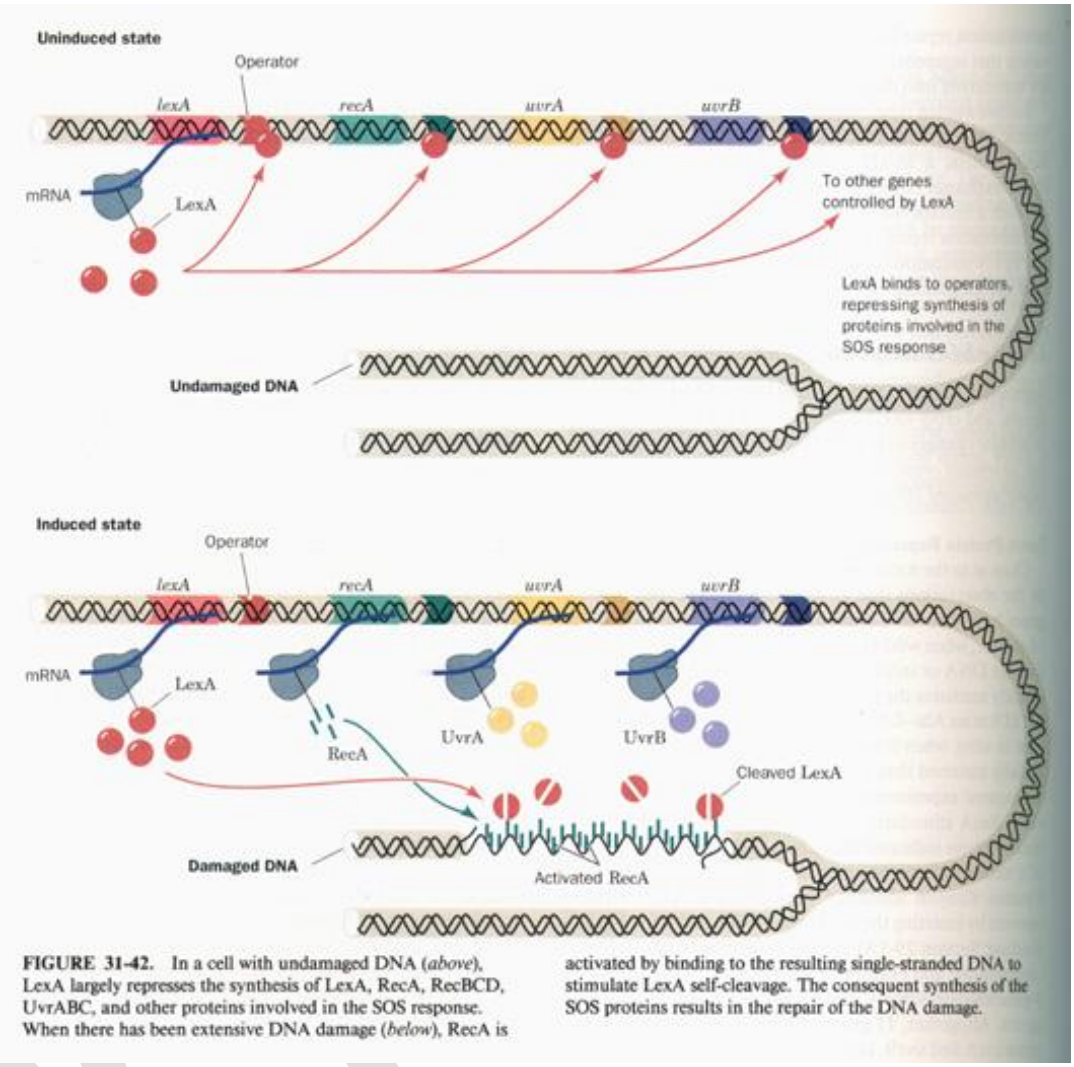


FIGURE 31-42. In a cell with undamaged DNA (*above*), LexA largely represses the synthesis of LexA, RecA, RecBCD, UvrABC, and other proteins involved in the SOS response. When there has been extensive DNA damage (*below*), RecA is activated by binding to the resulting single-stranded DNA to stimulate LexA self-cleavage. The consequent synthesis of the SOS proteins results in the repair of the DNA damage.

SOS repair mechanism

Questions

Long answer questions

1. What is genetic recombination? Write its significance in bacteria.
2. Give an account on Transduction.
3. Describe the lytic cycle of a bacteriophage.
4. Give a note on recombination in bacteria by conjugation.

5. Give a note on recombination in bacteria by conjugation.
6. Explain genetic recombination in bacteria?
7. Write note on conjugation in bacteria.
8. Elaborate genetic recombination in bacteria.

Short answer questions

1. Define the terms recombination and give methods of recombination.
2. Draw genetic map of T4 phage
3. Write a note on lysogenic cycle

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I MSc Microbiology **COURSE NAME**: Molecular Genetics

COURSE CODE: 18MBP103 **BATCH**-2018-2019

Multiple choice questions

UNIT IV

Sr. No	Unit II Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	Sickle cell anaemia is caused by change in amino acid from	Glutamic acid	Alanine to Leucine	Valine to Glutamic acid	Leucine to alanine	Valine to Glutamic acid
2	Site that mutates at a rate significantly greater thag statistical probability is referred to as	Hotspots	Blackspots	Dotspots	DNA spots	Hotspots
3	The function of DNA glycosylase in base excision repair is	Addition of correct base	Addition of correct nucleotide	Removal of incorrect base	Removal of phosphodiester bond	Removal of phosphodiester bond
4	The most common error prone repair mechanism is	Mismatch	Excision	SOS	Recombination	SOS
5	The result of addition or deletion of one or more base pair in a gene is	Frameshift	Base pair substitution	Misense mutation	Nonsense mutation	Frameshift
6	Transposition is	Movement of a phage	Movement of a virus	Movement of a transposon	Movement of a plasmid	Movement of transposon

7	Transposons was first reported by	Louise pasteur	Koch	Barbara McClintock	Lister	Barbara McClintock
8	UV induced dimers are separated using light energy by	Primase	Photolyase	Dnase	Rnase	Photolyase
9	UV radiation at 260 nm cross-links adjacent thiamine that produces	Butane ring	Cyclane ring	Butocyclane ring	Phenyl alanine	Cyclobutane ring
10	UV radiation causes	Adenine dimers	Cytosine dimers	Guanine dimers	Thiamine dimers	Thiamine dimers
11	Virus capable of causing mutations is	Bacteriophage Ru	Bacteriophage Mu	Bacteriophage Nu	Bacteriophage Ly	Bacteriophage Mu
12	When a part of chrosome is moved to another chromosome, it is called as	Point mutation	Induced mutation	Spontaneous mutation	Translocation mutation	Translocation mutation
13	Which of the following biomolecule has self¬repair mechanisms?	DNA,RNA and protein	DNA and RNA	DNA and proteins	DNA only	DNA only
14	Which of the following chemicals induce depurination	Methyl ethane sulphonate	Guanidine	Ethyl sulphonate	Dichlor	Methyl ethane sulphonate
15	Which of the following dimer formation is most common	Cytidine dimer	Uracil dimer	Thymidine dimer	Adenosine dimer	Thymidine dimer
16	Xeroderma pigmentosum is a genetic disorder of	Skin	Hair	Nail	Tongue	Skin
17	_____ is a non-ionizing radiation	Alpha	UV	Gamma	Beta	UV
18	Alkylation is	Addition of methyl or ethyl group	Deletion of ethyl and addition of methyl group	Deletion of methyl and addition of ethyl group	Deletion of methyl or ethyl group	Addition of methyl or ethyl group

19	Alkylation of guanine causes its removal from DNA in a reaction called	Deamination	Depyrimidation	Degradation	Depurination	Depurination
20	An intercalating dye	Sunset yellow	Safranin	India ink	Acridine orange	Acridine orange
21	Bacterium used in Ames test	*Salmonella*	*Shigella*	*Streptococcus*	*Staphylococcus*	*Salmonella*
22	Biological agents of mutagenesis are	Transposable elements	Lipids	Bacteria	Carbohydrates	Transposable elements
23	Cancer that results from deletion of a portion of chrosome 13 is	Eye cancer	Bone cancer	Skin cancer	Lung cancer	Eye cancer
24	Change of purine to pyrimidine base in an mutation	Transition mutation	Transverse mutation	General mutation	Transformation	Transverse mutation
25	Chemical agent that resembles thiamine	5-bromothiamine	5-bromoadenine	5-bromoguanine	5-bromouracil	5-bromouracil
26	Chemical mutagens leading to addition of nucleotides to the DNA are	Thimers	Base analogs	Alkylating agents	Interchelating agents	Interchelating agents
27	Chromosomal mutation is	Abberation	Change over	Variation	Genetic change	Abberation
28	Common chemical events that produce spontaneous mutation	Deamination	Depurination	Dimerization	Isomerization	Depurination
29	Converts amino groups to keto groups by oxidative deamination	Hydrochloric acid	Nitrous acid	Sulphuric acid	Oxalic acid	Nitrous acid
30	Daughter strand repair is also called as	Recombination repair	SOS repair	Photo repair	Excision repair	Recombination repair
31	Deficiency in phenylalanine	Cancer	Phenylketonuroa	Melanoma	Asthma	Phenylketonuroa

	hydroxidase results in					
32	Detection of silent mutations require	Aminoacid analysis	Peptide analysis	RNA analysis	Nucleotide analysis	Nucleotide analysis
33	Duplication mutation is	Segments of nucleotides sequences are repeated	Segments of nucleotides sequences are deleted	Segments of nucleotides sequences are inserted	Segments of nucleotide sequences are inserted & deleted evenly	Segments of nucleotides sequences are repeated
34	Natural phenomena of changes in chemical structure of nitrogenous bases is called	Complementary	Conservative	Tautomeric	Telomeric	Tautomeric
35	Naturally occurring mutations are	Induced	Spontaneous	Nonsense	Frameshift	Spontaneous
36	Nonsense mutation leads to	Termination of DNA synthesis	Termination of protein synthesis	Termination of cell wall synthesis	Termination of RNA synthesis	Termination of protein synthesis
37	Nutritional mutans of neurospora are known as	Phototrophs	Auxotrophs	Heterotrophs	Isotrophs	Auxotrophs
38	Oncogenes are found in certain	Bacteria	Fungi	Viruses	Algae	Viruses
39	Potent oxidizing agent that can alter structure of purine and pyrimidine	Free radicals	Water	Dyes	Acids	Free radicals
40	Radiation that causes cross chromosomal mutations in humans	UV	Visible	Ionozing	X-rays	Ionozing
41	Rapid screening technique for mutagens and carcinogens	Aims test	Sima test	Ames test	Sema test	Ames test
42	Recombinational repair is often due to	many cytidine dimer and	incorporation of many incorrect	many thymidine dimer formation	DNA breaking	incorporation of many incorrect

		associated large gaps in a strand	nucleotides by DNA polymerase	and associated large gaps in a strand		nucleotides by DNA polymerase
43	Repairing mechanism of _____ depends on absorption of visible light by the enzyme.	DNA helicase	DNA ligase	DNA gyrase	DNA photolyase	DNA photolyase
44	Reverse mutation is	Wild type to mutant	Mutant to wild type	A new gene introduced	A gene deleted	Mutant to wild type
45	Frameshift mutation is caused by	Proflavin	Nitrous acid	UV	X-rays	Proflavin
46	Herman J.Mueller reported results of induced mutations on	Yeast	*Drosophila*	Fish	Pea plant	*Drosophila*
47	Human bladder cancer is brought about by a change in single point mutation of	Valine to glycine	Isoleucine to leucine	Leucine to isoleucine	Glycine to valine	Glycine to valine
48	In bacteria, a small circle of DNA found outside the main chromosome is called	Cosmid	Bacmid	Transposon	Plasmid	Plasmid
49	In *E.coli*, parental DNA is methylated at an adenine residue found in the sequence	5' TAGC 3'	5' ATGC 3'	5' CATG 3'	5' GATC 3'	5' GATC 3'
50	Mismatch repair cannot take place if there is a mutation in	Helicase	Polymerase	Ligase	Methylase enzyme	Methylase enzyme
51	Most common proto-oncogene implicated in human cancers	*s-rac*	*a-src*	*r-cas*	*c-ras*	*c-ras*
52	Most common repair	SOS	Photoreactive	Mismatch	Excision	Excision

	system is					
53	Most frequently employed technique in the study of mutations	Analysis of phenotypes	Analysis of genotypes	Analysis of proteins	Analysis of both phenotypes and genotypes	Analysis of phenotypes
54	Mutation generating new stop codon are called	Nonsense mutation	Misense mutation	Point mutation	Silent mutation	Nonsense mutation
55	Mutation in which a purine base is substituted with another purine base is	Transverse mutation	General mutation	Transition mutation	Transduction	Transition mutation
56	Mutation in which there is an amino acid substitution is called	Missense	Nonsense	Silent	Point	Missense
57	Mutation involving single-base changes are	Induced mutations	Point mutations	Silent mutations	Inverse mutations	Point mutations
58	Mutation resulting from deamination of 5-methylcytosine produces Thymine which pairs with	Uracil	Adenine	Cytosine	Guanine	Adenine
59	Mutation that has no detectable effect on the phenotype of a cell	Point	Induced	Silent	Leaky	Silent
60	Mutations that result from treatment with mutagens are called	Induced mutation	Uninduced	Spontaneous	Frameshift	Induced mutation

UNIT V

Syllabus

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

The yeast *Saccharomyces cerevisiae*:

Saccharomyces cerevisiae, commonly known as Baker's yeast, may be found as a harmless and transient digestive commensal and coloniser of mucosal surfaces of normal individuals. The anamorphic state of *S. cerevisiae* is sometimes referred to as *Candida robusta.* This species is phylogenetically closely related to *Candida glabrata* and shares many clinical and microbiological characteristics to this species

Habitat

Yeast lives on fruits, flowers and other sugar containing substrates

Yeast copes with a wide range of environmental conditions:

- Temperatures from freezing to about 55°C are tolerated
- Yeasts proliferate from 12°C to 40°C
- Growth is possible from pH 2.8-8.0
- Almost complete drying is tolerated (dry yeast)
- Yeast can still grow and ferment at sugar concentrations of 3M (high osmoti pressure)
- Yeast can tolerate up to 20% alcohol

  Saccharomyces cerevisiae is the main organism in wine productionbesides other yeasts; reason is the enormous fermentation capacity, low pH and high ethanol tolerance

  Saccharomyces cerevisiae (carlsbergensis) is the beer yeastbecause it ferments sugar to alcohol even in the presence of oxygen, lager yeast ferments at 8°C

  Saccharomyces cerevisiae is the yeast used in bakingbecause it produces carbon dioxide from sugar very rapidly
- *Saccharomyces cerevisiae* is used to produce commercially important proteins because it can be genetically engineered, it is regarded as safe and fermentation technology is highly advanced
- *Saccharomyces cerevisiae* is used for drug screening and functional analysis because it is a eukaryote but can be handled as easily as bacteria

- *Saccharomyces cerevisiae* is the most important eukaryotic cellular model system because it can be studied by powerful genetics and molecular and cellular biology; many important features of the eukaryotic cell have first been discovered in yeast Hence *S. cerevisiae* is used in research that aims to find out features and mechanisms of the function of the living cell AND in to improve existing or to generate new biotechnological processes

Other important yeasts

- *Schizosaccharomyces pombe*, the fission yeast; important model organisms in molecular and cellular biology; used for certain fermentations
- *Kluyveromyces lactis*, the milk yeast; model organism some biotech importance due to lactose fermentation
- *Candida albicans*, not a good model since it lacks a sexual cycle; but studied intensively because it is human pathogen
- *Saccharomyces carlsbergensis* and *Saccharomyces bayanus* are species closely related to *S. cerevisiae*; brewing and wine making
- *Pichia stipidis, Hansenula polymorpha, Yarrovia lipolytica* have smaller importance for genetic studies (specilaised features such as peroxisome biogenesis are studied), protein production hosts

- Filamentous fungi, a large group of genetic model organisms in genera like *Cryptococcus, Aspergillus, Neurospora....*, biotechnological importance, includes human pathogens. Also *S. cerevisiae* can grow in a filamentous form.

Budding Yeast *Saccharomyces cerevisiae*

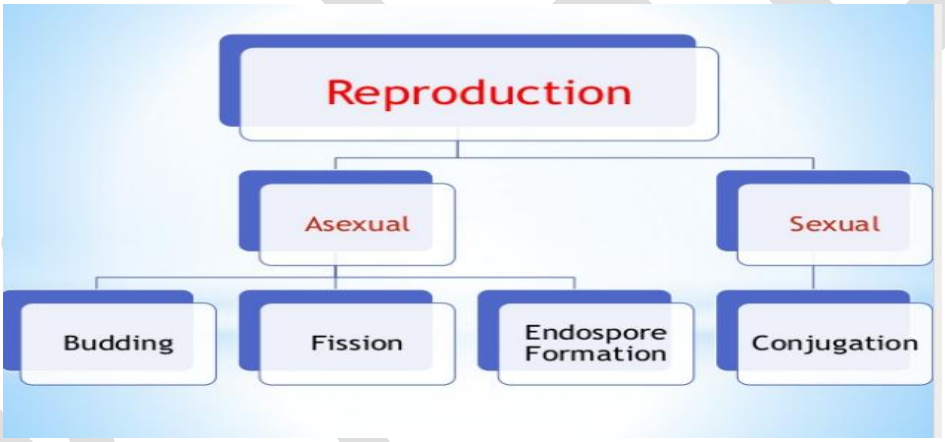
Fission Yeast *Schizosaccharomyces pombe*

***Saccharomyces cerevisiae* is a eukaryote**

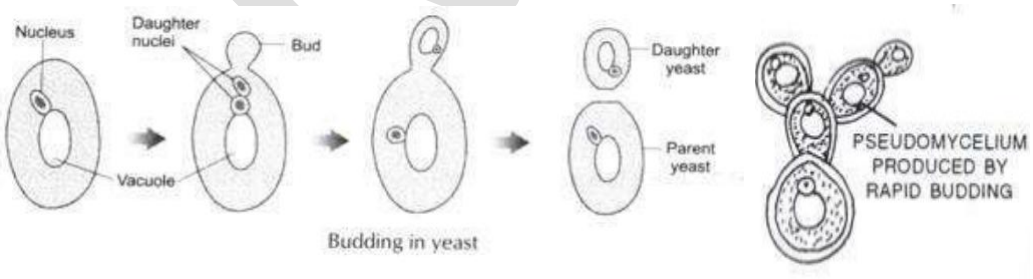
- Belongs to fungi, ascomycetes
- Unicellular organism with ability to produce pseudohyphae
- *S. cerevisiae* divides by budding (hence: budding yeast) while *Schizosaccharomyces pombe* divides by fission (hence: fission yeast)
- Budding results in two cells of unequal size, a mother (old cell) and a daughter (new cell)

- Yeast life is not indefinite; yeast cells age and mothers die after about 30-40 dividions
- Cell has a eukaryotic structure with different organelles:
- Cell wall consisting of glucans, mannans and proteins
- Periplasmic space with hydrolytic enzymes
- Plasma membrane consisting of a phospholipid bilayer and many different proteins
- Nucleus with nucleolus
- Vacuole as storage and hydrolytic organelle
- Secretory pathway with endoplasmic reticulum, Golgi apparatus and secretory vesicles
- Peroxisomes for oxidative degradation
- Mitochondria for respiration

Life cycle of yeasts

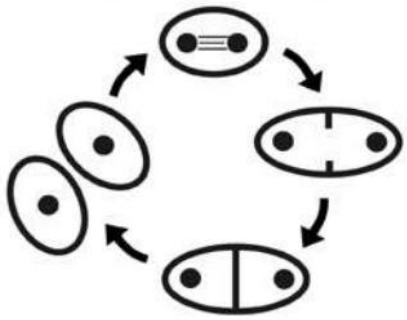


Budding *Most common method *Under favourable condition the yeast cell gives rise to a tiny outgrowth that gradually increase in size. The nucleus divides amitotically, one of which migrates into the newly formed outgrowth and forms a bud. The bud seperates from parent and becomes independent.



Sometimes due to repeated budding yeast cell appears in one or more chains and called pseudomycelium.

Fission *Occurs only in some yeast cells *Yeast cell elongates and its nucleus divides into two. Two nucleus move apart and are seperated by a transverse wall. Cell wall is formed in the middle and the yeast cell divides into two cells each having a nucleus.



Endospore formation *Under unfavourable condition *Thick walled, can withstand adverse conditions. *The protoplast divides into four parts, each becomes surrounded by a thick wall.

Yeast has a sex life

- Yeast cells can proliferate both as haploids (1n, one copy of each chromosome) and as diploids (2n, two copies of each chromosome); 2n cells are 1.2-fold bigger
- Haploid cells have one of two mating types: a or alpha (a)
- Two haploid cells can mate to form a zygote; since yeast cannot move, cells must grow towards each other (shmoos)
- The diploid zygote starts dividing from the junction
- Under nitrogen starvation diploid cells undergo meiosis and sporulation to form an ascus with four haploid spores
- Thus, although yeast is unicellular, we can distinguish different cell types with different genetic programmes:
  - Haploid MATa versus MATalpha
  - Haploid versus Diploid (MATa/alpha)
  - Spores
  - Mothers and daughters

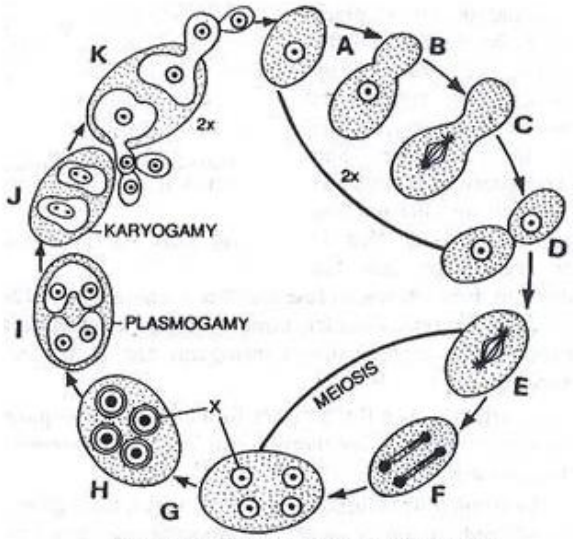


Fig. 12.22. Diplobiontic type of life-cycle in *Saccharomycodes ludwigii*.

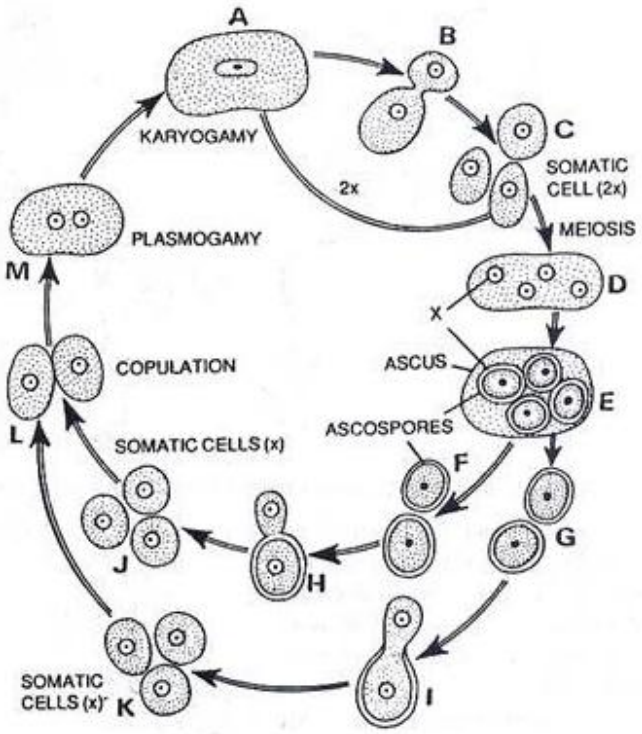
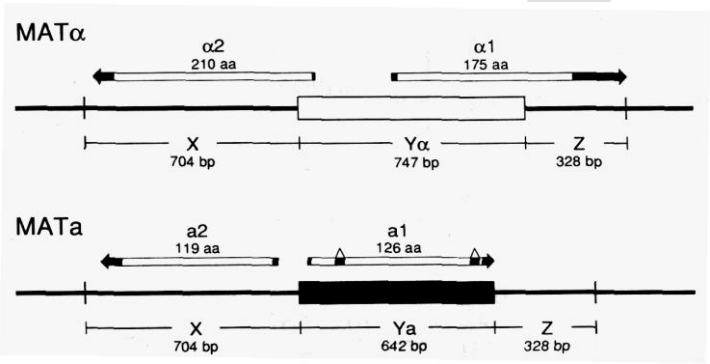


Fig. 12.23. Haplo-diplobiontic type of life-cycle in *Saccharomyces cerivisiae*.

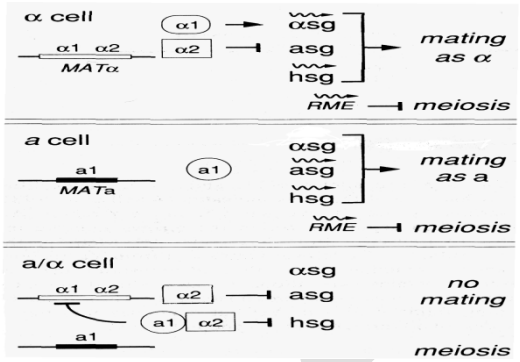
Genetic determination of yeast cell type

- The mating type is determined by the allele of the mating type locus *MAT* on chromosome III
- The mating type locus encodes regulatory proteins, i.e. transcription factors
- The *MATa* locus encodes the a1 transcriptional activator (a2 has no known function)
- The *MATalpha* locus encodes the alpha1 activator and the alpha2 repressor
- The mating type locus functions as a master regulator locus: it controls expression of many genes



Gene expression that determines the mating type

- In alpha cells the alpha1 activator stimulates alpha-specific genes and the alpha2 repressor represses a-specific genes
- In a cells alpha-specific genes are not activated and a-specific genes are not repressed (they use a different transcriptional activitor to become expressed)
- In diploid cells the a1/alpha2 heteromeric repressor represses expression of alpha1 and hence alpha-specific genes are not activated. A-specific genes and haploid-specific genes are repressed too.
- One such haploid-specific gene is *RME*, encoding the repressor of meiosis. Although it is not expressed in diploids the meiosis and sporulation programme will only start once nutrients become limiting
- Taken together, cell type is determined with very few primary transcription factors that act individually or in combination.
- This is a fundamental principle and is conserved in multicellular organisms for the determination of different cell types: homeotic genes (in fact, a1 is a homeobo factor)



Haploids and dipoids in nature and laboratory

- In nature, yeast cells always grow as diploids, probably because this increases their chance to survive mutation of an essential gene (because there is another copy)
- Under nitrogen starvation, diploid cells sporulate and then haploid spores germinate, provided that they have received functional copies of all essential genes
- This often means that only a single spore (if any) of a tetrad survives
- How to make sure that this single spore can find a mating partner to form a diploid again? The answer is mating type switch!
- After the first division the mother cell switches mating type and mates with its daughter to form a diploid, which then of course is homozygous for all genes and starts a new clone of cells
- If mating type can be switched and diploid is the prefered form, why then sporulate and have mating types?
- There are probably several reasons: (1) Spores are hardy and survive very harsh conditions (2) Sporulation is a way to ”clean” the genome from accumulated mutations (3) Meiosis is a way to generate new combinations of alleles, which may turn out to be advantageous, i.e. better than the previous one (4) Sometimes cells may find a mating partner from a different tetrad and form a new clone, with possibly advantageous allele combination
- In order to do yeast genetics and to grow haploid cells in the laboratory, mating type switch must be prevented: all laboratory strains are HO mutants and can not switch
- So how does this mysterious switch of sex work?
- Yeast genetics: the genetic material
- The *S. cerevisiae* nuclear genome has 16 chromosomes

- In addition, there is a mitochondrial genome and a plasmid, the 2micron circle
- The yeast chromosomes contain centromeres and telomeres, which are simpler than those of higher eukaryotes
- The haploid yeast genome consists of about 12,500 kb and was completely sequenced as early 1996 (first complete genome sequence of a eukaryote)
- The yeast genome is predicted to contain about 6,200 genes, annotation is, however, still ongoing
- There is substantial ”gene redundancy”, which originates from an ancient genome duplication
- This means that there are many genes for which closely related homologue exist, which often are differentially regulated
- The most extreme example are sugar transporter genes; there are more than twenty
- Roughly 1/3 of the genes has been characterised by genetic analysis, 1/3 shows homology hinting at their biochemical function and 1/3 is not homologous to other genes or only to other uncharacterised genes
- Only a small percentage of yeast genes has introns, very few have more than one; mapping of introns is not complete
- The intergenic space between genes is only between 200 and 1,000bp
- The largest known regulatory sequences are spread over about 2,800bp (*MUC1/FLO11*
- Yeast genome analysis
- A joint goal of the yeast research community: determination of the function of each and every gene
- For this, there are several large projects and numerous approaches
- Micro array analysis: simultaneous determination of the expression of all genes
- Micro array analysis to determine the binding sites in the genome for all transcription factors
- Yeast deletion analysis: a complete set of more than 6,000 deletion mutants is available for research
- Various approaches to analyse the properties of these mutants
- All yeast genes have been tagged to green fluorescent protein (GFP) to allow protein detection and microscopic localisation
- Different global protein interaction projects are ongoing

Yeast genetics: nomenclature

Yeast genes are given 3 letter names with one or two digits after them, such as CDC33. Classically, yeast gene names were given for the phenotype of the mutant. Thus ste genes such as ste2, ste3, etc., confer a sterile

phenotype and his3 mutants require histidine. Genes can also be named after the proteins or RNAs they encode, an example being CMD1, which encodes calmodulin. As you can see, we use the italic (or underscore) to denote genes and upper case to denote wild-type. Loss of function mutants are lower case. Known alleles are given after a hyphen. A dominant mutant is usually upper case with an allele designation, such as DAF1-1. Corresponding proteins are capitalized as proper nouns, such as Kex2. Phenotypes are usually indicated as follows: STE+ and TS+ mean not sterile and not temperature sensitive. ts- and ste- mean temperature sensitive and sterile. The plus means okay for that phenotype and the minus means the particular strain has the phenotype.

Table 4.1. Genetic nomenclature, using *ARG2* as an example

Gene symbol	Definition
ARG$^+$	A wild-type alleles controlling arginine requirement
ARG2	A locus or dominant allele
arg2	A locus or recessive allele confering an arginine requirement
arg2$^-$	Any *arg2* allele confering an arginine requirement
ARG2$^+$	The wild-type allele
arg2-9	A specific allele or mutation
Arg	A strain not requiring arginine
Arg$^-$	A strain requiring arginine
Arg2p	The protein encoded by *ARG2*
Arg2 protein	The protein encoded by *ARG2*
ARG2 mRNA	The mRNA transcribed from *ARG2*
arg2-Δ1	A specific complete or partial deletion of *ARG2*
ARG2::LEU2	Insertion of the functional *LEU2* gene at the *ARG2* locus, and *ARG2* remains functional and dominant
arg2::LEU2	Insertion of the functional *LEU2* gene at the *ARG2* locus, and *arg2* is or became nonfunctional
arg2-10::LEU2	Insertion of the functional *LEU2* gene at the *ARG2* locus, and the specified *arg2-10* allele which is nonfunctional
cyc1-arg2	A fusion between the *CYC1* and *ARG2* genes, where both are nonfunctional
P*CYC1-ARG2*	A fusion between the *CYC1* promoter and *ARG2*, where the *ARG2* gene is functional

Phenotypes are sometimes denoted by cognate symbols in roman type and by the superscripts + and -. For example, the independence and requirement for arginine

- Yeast genes have names consisting of three letters and up to three numbers: *GPD1, HSP12, PDC6*...Usually they are meaningful (or meaningless) abbreviations
- Wild type genes are written with capital letters in italics: *TPS1, RHO1, CDC28*...
- Recessive mutant genes are written with small letters in italics: *tps1, rho1, cdc28*
- Mutant alleles are designated with a dash and a number: *tps1-1, rho1-23, cdc28-2*

- If the mutation has been constructed, i.e. by gene deletion, this is indicated and the genetic marker used for deletion too: *tps1D::HIS3*
- The gene product, a protein, is written with a capital letter at the beginning and not in italics; often a ”p” is added at the end: Tps1p, Rho1p, Cdc28p
- Many genes have of course only be found by systematic sequencing and as long as their function is not determined they get a landmark name: *YDR518C, YML016W*..., where
  - Y stands for ”yeast”
  - The second letter represents the chromosome (D=IV, M=XIII....)
  - L or R stand for left or right chromosome arm
  - The three-digit number stands for the ORF counted from the centromere on that chromosome arm
  - C or W stand for ”Crick” or ”Watson”, i.e. indicate the strand or direction of the ORF
  - Some genes do not follow this nomenclature: you heard already about: *HO, MATa, MATa*

Yeast genetics: making mutants

- Mutations that enhance or abolish the function of a certain protein are extremely useful to study cellular systems
- The phenotype of mutations (i.e. the properties of the mutant) can tell a lot about the function of a gene, protein or pathway
- This approach is valid even with the genome sequenced and even with the complete deletion set available: point mutations can have different properties than deletion mutants
- Random versus targetted mutations
  - In random mutagenesis one tries to link genes to a certain function/role; this identifies new genes or new functions to known genes
  - Hence in random mutagenesis usually the entire genome is targetted
  - Random mutagenesis is also possible for a specific protein (whose genes is then mutated *in vitro*); in this case one wishes to identify functional domains
  - In targetted mutagenesis one knocks out or alters a specific gene by a combination of *in vitro* and *in vivo* manipulation
- Induced versus spontaneous mutations
  - Mutations can be induced by treating cells with a mutagen; this can of course give multiple hits per cell

- Spontaneous mutations "just occur" at a low frequency and it is likely that there is only one hit per cell

Yeast genetics: finding mutants

Screening versus selection

- For that, one usually plates many cells and tries to find mutants because they are unable to grow on a certain medium after replica-plating or because they develop a colour
- For screening, mutations are usually induced to increase their frequency
- Still: screening requires hundreds of perti dishes and commonly more than 10,000 clones to be scored

➢ To develop a new selection system is the art of genetic analysis

- When selecting for mutants one has established a condition under which the mutant phenotype confers a growth advantage
- In other words, the intellectual challenge is to design conditions and /or strains such that the mutant grows, but the wild type does not
- A smart screening system allows one to go for spontaneous mutations, because up to 108 cells can easily be spread on one plate
- When screening for mutants one tests clone by clone to find interesting mutants
- Selection systems are often based on resistance to inhibitors
- We try to train our students to watch out for any such opportunity to find conditions that allow to select for new mutants with interesting properties to advance the understanding of the system under study

characterising mutants

- Once mutants have been identified they need to be characterised and the genes affected have to be identified; this requires the following steps
- A detailed phenotypic analysis, i.e. testing also for other phenotypes than the one used in screening/selection
- Establishing if a mutant is dominant or recessive
- Placing the mutants into complementation groups. Usually one complemetation group is equivalent to one gene
- Cloning the gene by complementation.
- Dominant and recessive mutations

- The dominant or recessive character is revealed by crossing the mutant with the wild type to form a diploid cell
- Such diploids are heterozygous, because one chromosome carries the wild type allele and the other one the mutant allele of the gene affected
- A mutation is dominant when the mutant phenotype is expressed in a heterozygous diploid cell. The diploid has the same phenotype as the haploid mutant
- A mutation is recessive when the wild type phenotype is expressed in a heterozygous diploid cell. The diploid has the same phenotype as the wild type
- A dominant character can have a number of important reasons, which may reveal properties of the gene product's function:
  - The mutations leads to a gain of function, e.g. a regulatory protein functions even without its normal stimulus
  - The gene product functions as a homo-oligomere and the non-functional monomere causes the entire complex to become non-functional
  - The gene dosis of one wild type allele is insufficient to confer the wild type phenotype, i.e. there is simply not enough functional gene product (this is rare)
- The recessive character of a mutation is usually due to loss of function of the gene product
- This means that recessive mutations are far more common, because it is simpler to destroy a function than to generate one
- Further genetic analysis of the mutant depends on the dominant/recessive character, that is one reason why this step is taken first
- In addition, it is useful to do a tetrad analysis of the diploid in order to test that the mutant phenotype is caused by a single mutation, i.e. that the phenotype segregates 2:2 in at least ten tetrads studied; this is important when mutations have been induced by mutagenesis

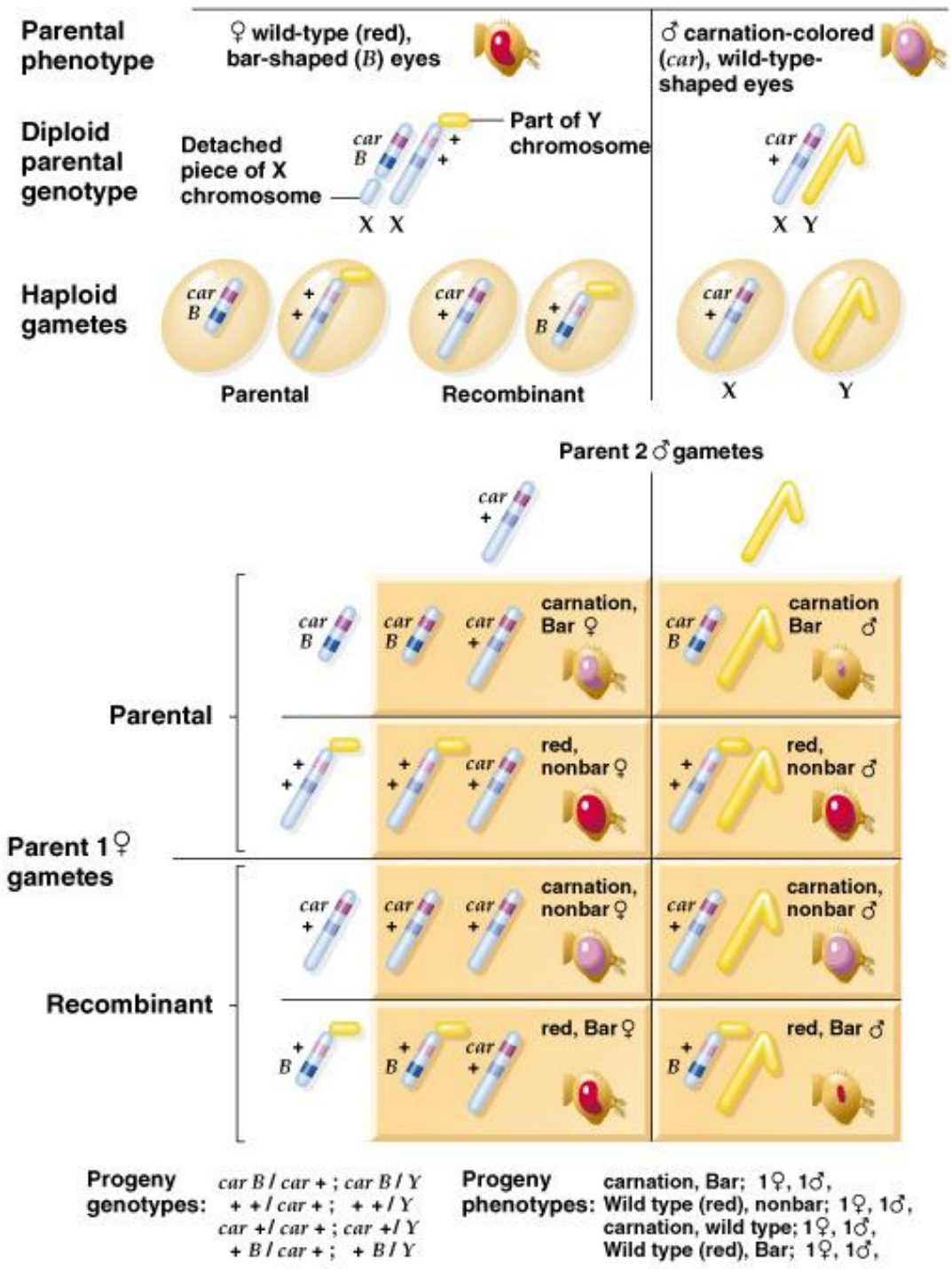
Complementation groups

- After selection or screening for mutants with a certain phenotype and after determination of the dominant/recessive character of the underlying mutation one would like to know if all mutants isolated are affected in the same or in different genes
- For recessive mutations, this is done by a complementation analysis

- This requires that mutants with different mating types are available for generation of diploids (this can be achieved by making the mutants already in two strains with opposite mating type and complementing markers)
- These mutants are then allowed to form diploids in all possible combination; for instance if one has 12 mutants with mating type a and 9 with mating type alpha 9x12=108 crosses are possible
- If two haploid mutants have recessive mutations in one and the same gene the resulting diploid should have the mutant phenotype too
- If two haploids have recessive mutations in two different genes (confering the same phenotype) then the diploid should have wild type phenotype, i.e. the mutations complement each other
- Hence, *mut1* and *mut2* represent two different complementation groups representing most likely different genes

Stern's *Drosophila*Experiment

Recombinant phenotypes and recombinant cytological features show perfect correlation



Molecular mechanism for crossing-over (Robin Holliday, 1960s):

1. Homologous chromosomes "recognize" and align.

2. Single strands of each DNA (one on each chromosome) break and anneal to the opposite chromosome forming Holliday intermediate.
3. As chromosome ends pull apart, branch point migrations occur to create a 4-arm intermediate structure.
4. 4-arm intermediate is cut by by endonucleases in one of 2 planes.
5. DNA seals the gaps.
6. Model predicts that physical exchange between two gene loci at the ends of the chromosomes should occur about 50% of the time. One pattern (intermediate cut in one plane) yields the parental arrangement, the other (cut in the other plane) is recombinant.
7. Holliday model of chromosome recombination

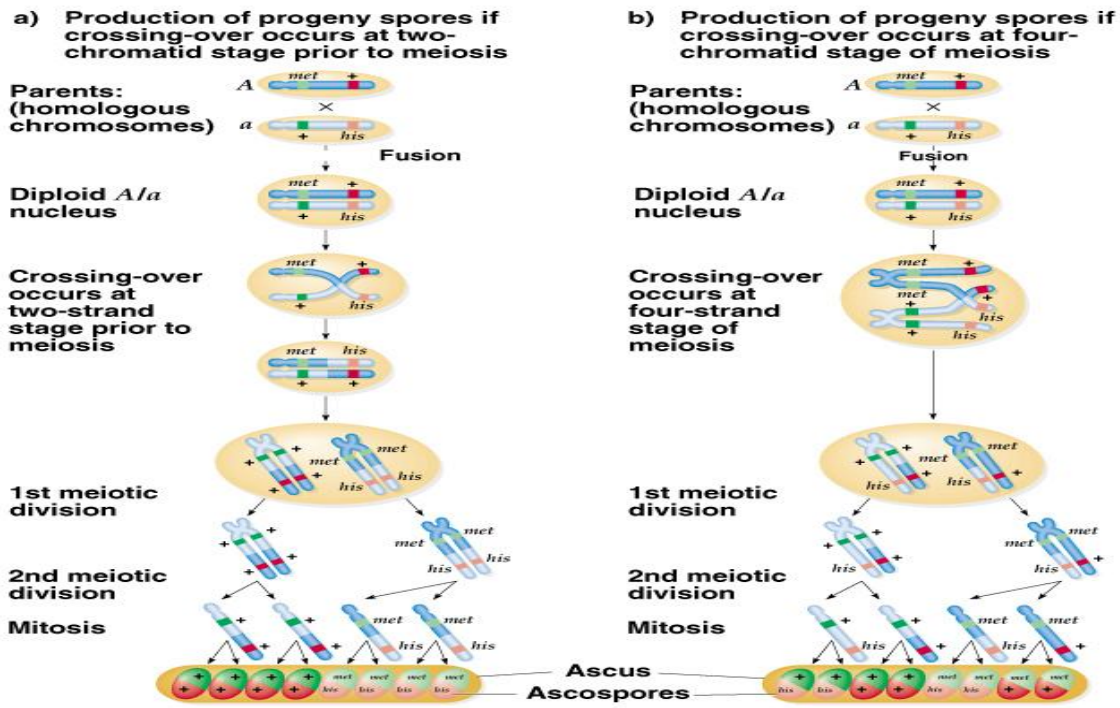
Constructing genetic maps:

- Number (%) of genetic recombinants produced reflects gene linkage relationships.
- Recombination experiments can be used to generate genetic maps.
- Perform a test cross to determine that genes are linked.
- Select a test cross including a homozygous recessive (*or homozygous dominant*) individual for all genes involved.
  - If loci are not linked and the second parent is a heterozygote, all 4 phenotypes will occur in equal numbers in the F1 (2 loci) and the ratio of parentals to recombinants will be 1:1.
  - Compare observed and expected using a goodness of fit test and significant *P*-value < 0.05.
  - Significant deviations from these ratios indicate linkage
- Two arrangements of alleles exist for an individuals heterozygous at two loci.
- *cis* *trans*
- w+m+ or w+m
- w m w m+
- Cross-over of *cis* results in *trans* and vice versa.
- Frequency of recombinants (%) is a characteristic of each gene pair, regardless of cis or trans arrangements.
- Sturtevant (1913) recognized that recombination frequencies could be used to create a map.
- 1% cross-over rate = 1 map unit (mu) or centiMorgan (cM).
- Map units (mu) and centiMorgans (cM) are relative measures.

Tetrad analysis of haploid eukaryotes:

- Tetrad refers to the four haploid gametes produced by meiosis.
- Tetrad may be ordered (e.g., *Neurospora*) or unordered (e.g., yeast).
- In haploid organisms, phenotype corresponds directly with genotype of each member of the tetrad (no dominance or recessiveness).
- Haploidy simplifies interpretation of results and linkage mapping.
- Three of the most common organisms used for tetrad analysis (each have asexual and sexual mating types):
  - Yeast (*Saccharomyces cerevisiae*)
  - Green algae (*Chlamydomonas reinhardtii*)
  - Orange bread mold (*Neurospora crassa*)

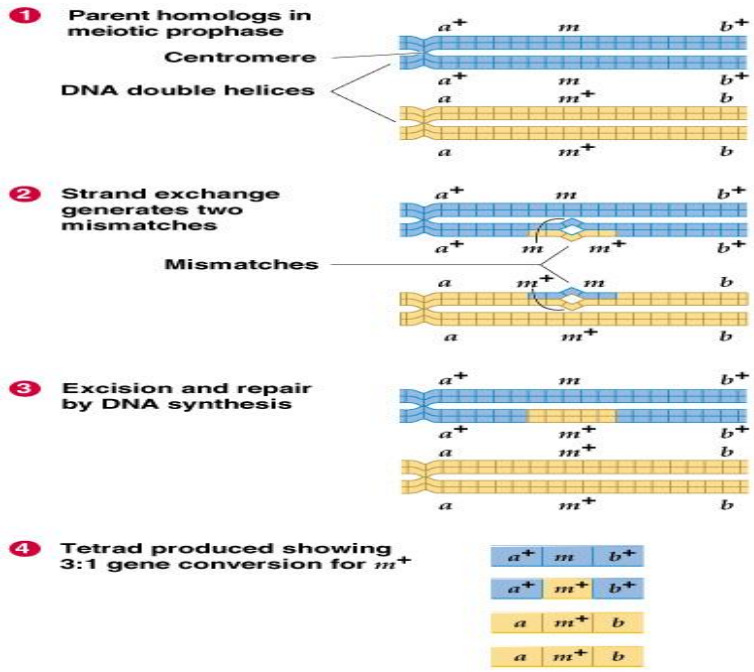
Crossing-over occurs at the 4-chromatid stage, and not the 2-chromatid stage



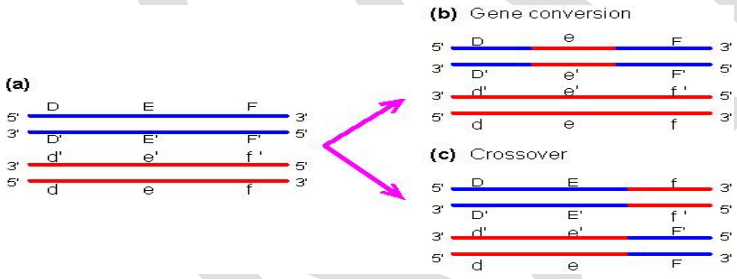
Gene conversion:

- Process by which DNA sequence information is transferred from one DNA helix (which remains unchanged) to another DNA helix, whose sequence is altered.
- Gene conversion is a type of Non-Mendelian Inheritance.
- Evidence for conversion occurs when tetrad gamete genotype ratios are 3:1 or 1:3 instead of 4:4, 2:4:2, or 2:2:2:2.

- Example: m+/m+ x m/m ⇒ m+/m+/m+/m
- Can be caused by mismatch during a recombination event, mismatch is excised (using exonuclease) and replaced (w/DNA polymerase).

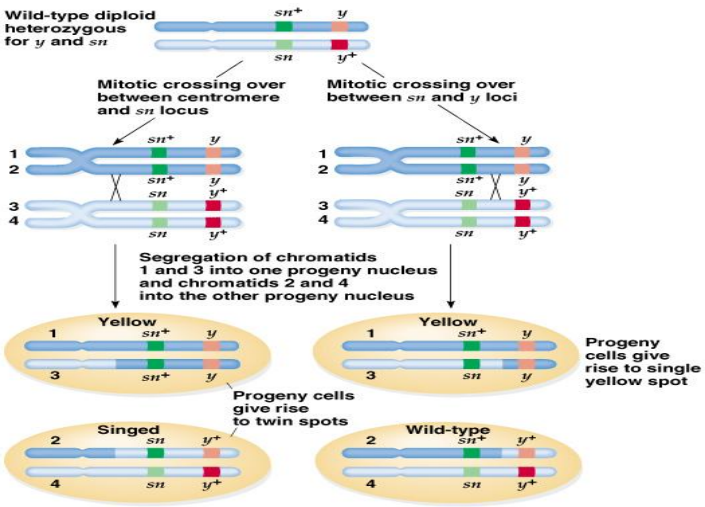


Gene conversion vs crossing over:



Mitotic recombination:

- Crossing-over sometimes occurs in mitosis (i.e., somatic cells)!
- First observed by Curt Stern (1936) in heterozygous *Drosophila* carrying sex-linked mutations for yellow body color (y+/y) and singed bristles (sn/sn+).
- Mitotic cross-over occurred in heterozygotes and appeared as a mosaic of two different phenotypes in the same individual.
- Possibly explained by non-disjunction, but mosaic regions were always adjacent, and therefore likely to be products of the same genetic event.



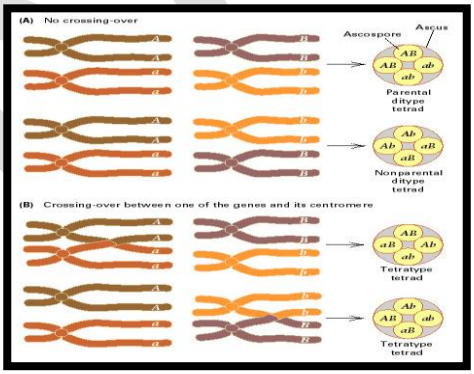
Mapping in *Neurospora*

- In Neurospora, meiotic cell division produces four ascospores; each contains a single product of meiosis
- Analysis of ascus tetrads shows recombination of unlinked genes
- Tetrad analysis shows products of single and double 2, 3 and 4 strand cross-overs of linked genes
- In tetrads when two pairs of alleles are segregating, 3 possible patterns of segregation:

  -parental ditype (PD): two parental genotypes

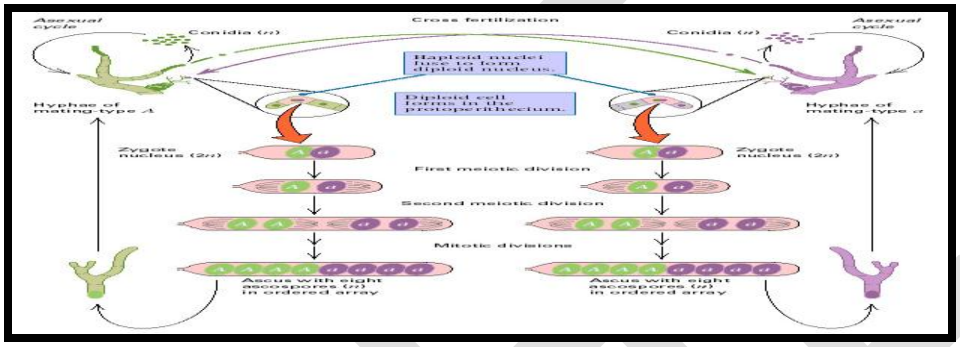
  -nonparental ditype (NPD): only recombinant combinations

  -tetratype (TT): all four genotypes observed



Neurospora: Meiotic Segregation

- Products of meiotic segregation can be identified by tetrad analysis
- Meiosis I segregation in the absence of cross-overs produces 2 patterns for a pair of homologous chromo-somes
- Meiosis II segregation after a single cross-over produces four possible patterns of spores



Genetic nomenclature:

Tetrad Analysis

- Unlinked genes produce parental and nonparental ditype tetrads with equal frequency
- Linked genes produce parental ditypes at much higher frequency than nonparental ditype
- Gene conversion = identical alleles produced by heteroduplex mismatch repair during recombination
- Homologous recombination:
- single-strand break in homologues pairing of broken strands occurs
- branch migration: single strands pair with alternate homologue
- nicked strands exchange places and gaps are sealed to form recombinant by Holliday junction-resolving enzyme

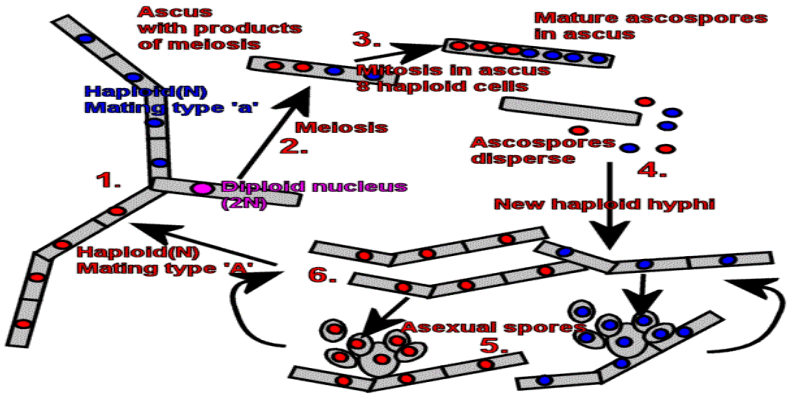


Fig:Neurospora Life Cycle- advantages for genetics

The mold Neurospora has a life cycle well suited to its use as a model organism in genetics. First of all, the organism spends most of its life cycle as a haploid organism. This means it is possible to study the expression of genes without worrying about dominance or recessive alleles.. Any mutations should be easy to detect since mutations will not be masked by another allele. Next, the fungus has alternate mating strains, here called type A and type a. Mating can only take place between different mating strains and the result is a diploid cell in a long sac. The diploid cell undergoes meiosis producing four haploid cells.

The sac or ascus is the next advantage of Neurospora because the results of segregation during metaphase 1 are kept in order. For instance notice that the two haploid cells resulting from each mating type are together in the ascus. These haploid cells undergo one cycle of mitosis in the ascus leading to 8 spores(called ascospores) in order in the ascus.. Scientists have been able to exploit this arrangement to help them screen for mutants and also to do crossover studies with this fungus.

Another advantage of neurospora is that in addition to ascospores, the fungus also produces asexual spores((5) in sacs called conidia. These spores allow scientists to isolate what amount to clones of any interesting Neurospora genotypes.

Next, the life cycle of Neurospora, is quite rapid requiring about 2 weeks, allowing scientists to rapidly conduct

experiments.Finally, wild type Neurospora require a very simple chemical diet. Thus, one could screen for mutants by their inability to grow on this so called minimal medium.

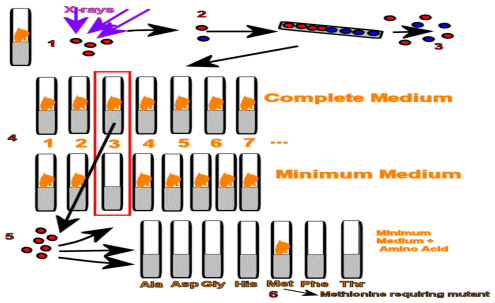


Fig: Beadle and Tatum's Experimental Techniques

The general steps in working with Neurospora and related fungi mutants.

Conidia (asexual spores) obtained from wild type Neurospora are irradiated with x rays (1). Then these asexual spores are germinated and cultured on maximal media to produce Neurospora carrying possible mutations resulting from the x rays. These Neurospora are crossed with wild type Neurospora to produce asci containing segregated products of meiosis. The ascosposres are isolated (3) and grown on complete media. Many hundreds of tubes are used for this step (4). Once the cultures are mature, asexual spores are isolated for each tube in step 4. These spores are then grown on minimal medium. Failure of a specific spore to grow on minimum medium indicates the presence of a mutant unable to synthesize a required compound from the raw materials in the minimum medium.

Questions

Long answer questions

1. Comment on Yeast as model eukaryotic organism.
2. Describe gene mapping in *Drosophila*.
3. Explain about petite mutants.
4. Write note on Yeast Artificial Chromosome.
5. Genetic mapping in *Yeast*.
6. Describe genetic nomenclature in yeast.
7. Write short notes on yeast genetics.
8. Explain the life cycle of budding yeast.

Short answer questions

1. Explain haplobiotic life cycle of yeast.
2. Write note on petit mutant
3. Write a note on genetic nomenclature of yeast

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: I MSc Microbiology **COURSE NAME**: Molecular Genetics

COURSE CODE: 18MBP103 **BATCH**-2018-2019

Multiple choice questions

UNIT V

Sr No.	Unit V Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	Significant feature of sex determination in Drosophila is the presence of abnormal flies called _____	Gyno variants	Gynocoid	Gyrates	Gynondromorphs	Gynondromorphs
2	Small pieces of DNA that can insert themselves into chromosomes are known	Plasmid	Transposon	Cosmid	Artificial chrosome	Transposon
3	Tetrad showing second division segregation has _____ products	Four meiotic	Two Meiotic	Four mitotic	Two mitotic	four meiotic
4	The ascus burst releasing the ascospores, each of which germinates and divides by mitosis to produce new _____	Vegetative cells	Spores	Ascus	Zygote	Vegetative cells

5	The binding of two DNA helices through X-shaped junction called _____	Polytron	Cholistron	Diptron	Cistron	Cistron
6	The developing haploid spores are enclosed in a membranous structure called_____	Spores	Pycus	Zygote	Ascus	Ascus
7	The general mapping function of Haldane is based on _____	Haldane distribution	Poisson distribution	Twart	Switz	Poisson distribution
8	The leu2- strain carries a mutation that inactivates leu2 gene which codes for _____	Endonuclease	Beta isoprophylmalate dehydrogenase	Helicase	Ligase	Beta isoprophylmalate dehydrogenase
9	The percentage of recombinant meiotic product is one half of the percentage of _____	Second division	Fourth division	Primary division	Tetrad	Tetrad
10	The repair of double-strand gaps is an efficient process in yeast known to be	Seggregation	Assortment	SOS repair	Crossing over	Crossing over
11	The term genetic linkage was given by ----------	Morgan	Meischer	Wilkins	Mendel	Morgan
12	The_____ to a particular chromosome is the first step in genetic mapping	Elemination of mutated type	Localization of wild type	Localization of mutation	Elimination of wild type	Localization of mutation
13	Well characterized	Irregular	Round	Spheroid	Disc shaped	Spheroid

	Baker's yeast has __ cell					
14	When mutation in single gene affect more than one trait is called _____	Parental genes	Pleiomorphic genes	Priogenic genes	Pleiotrophic genes	Pleiotrophic genes
15	Which contains four different ascospores , one of each genotypes	Tetratype	Parental type	Parental ditype	Non-parental ditype	Tetratype
16	Which of the following is used in density gradient centrifugation?	Glucose	Sucrose	Fructose	Agarose	Sucrose
17	Yeast are _____	Multicellular fungi	Dicellular	Acellular	Unicellular fungi	Unicellular fungi
18	Yeast genome has _____ introns	233	236	323	326	233
19	Yeast genome is _____	12,520 kb	1,252 kb	1,02,520 kb	15,052 kb	12,520 kb
20	Yeast has two mating types, _____ , which show primitive aspects of sex differentiation	a & b	a & α	b & β	α & β	a & α
21	Yeasts fail to grow on	Glucose	Lactose	Maltose	Trehalose	Lactose
22	Give full form for NPD	Non-parental dikaryon	Non-performing data	Non-parental data	Non-parental ditype	Non-parental ditype
23	Haploid to diploid phase in yeast is brought about by	Fusion of opposite mating types	Fusion of positive mating types	Fusion of negative mating types	Fusion of opposite genes	Fusion of opposite mating types
24	HFT is	High frequency transducing	High frequency transcribing	Height frequency transducing	Heavy frequency transducing	High frequency transducing

25	His 3 is an _____ protein.	Transducer	Indicator	Selector	Repressor	Indicator
26	If single crossover occurs between a- & b- then tetra type ___ results	A	B	T	U	T
27	In *Drosophila*, the meiotic recombination occurs only in _____	Both Female and Male	Female	Male	None	Female
28	In Transcription of yeast genome,_____ makes all RNA to serve as mRNA	DNA polymerase	RNA polymerase	RNA pol I	RNA pol II	RNA pol II
29	In yeast, 22% of the genome is made up of _____ DNA	Polygenic	Monogenic	Intragenic	Intergenic	Intergenic
30	Intermediate compound responsible for red color of adenine-requiring yeast petite mutants	Aminoimidazole	Aminoimidazole ribonucleotide	Aminoimidazoleribotide	Aminoimmunoribotide	Aminoimidazoleribotide
31	Map distance is equal to the percentage of	Reproducible mitotic product	Recombinant mitotic product	Reproducible meiotic product	Recombinant meiotic product	Recombinant meiotic product
32	Meiosis in yeast life cycle leads in forming	Sporangium	Fragmented mycelium	Ascus spores	Endospores	Ascus spores
33	Method available for locating mutation in *Neurospora crassa* is	Co-segregation	Co-opression	Independent assortment	Tetrad analysis	Co-segregation
34	Petite phenotypes caused by the absence	Cytoplasmic petites	Energy deficient	Mitochondrial petites	Chrosomal petites	Cytoplasmic petites

	of, or mutations in, mitochondrial DNA are termed as		petites			
35	Petite yeast mutants are unable to grow on media containing	Only nitrogen sources	Only Mineral sources	Only Lipid Source	Only fermentable carbon sources	Only non-fermentable carbon sources
36	Pleiotrophy is common in _____	No organism	All organism	Higher organism	Lower organism	Higher organism
37	Process by which one DNA sequence replaces a homologous sequence	Gene mutation	Gene repulsion	Gene transtition	Gene conversion	Gene conversion
38	Random spore analysis gives _____ values	Approximate	Accurate	Null	Partial	Approximate
39	Recombination does not only occur during meiosis, but also as a mechanism for	Repair of single-strand breaks	Repair of double-strand breaks	Repair of proteins	Repair of RNA	Repair of double-strand breaks
40	Sample of ascospores is spread on to the agar medium without leucine and survival was tested using	ELISA	PCR	Random spore analysis	RPR	Random spore analysis
41	Sex linkage was explained by _____	Morgan	Mendel	Primrose	Pastuer	Morgan
42	A new genetic nomenclature for S. cerevisiae transposon	jumping element	Ty elements	Tx elements	Ta element	Ty elements
43	Among haploid and diploid vegetative cells of yeast ,which is mainly used for genetic	Haploid and diploid	Diploid	Haploid	Triploid	Haploid

	mapping					
44	An estimation of map between a- & b- can be obtained from number of recombinant ascospore detected by using _____	Genetic analysis	Physical analysis	Random spore analysis	Tetrad analysis	Random spore analysis
45	Arg^+ is	A strain not requiring aspargine	A strain requiring aspargine	A strain not requiring arginine	A strain requiring arginine	A strain not requiring arginine
46	Cells which contains single copy of chromosome is called _____	Triploid	Haploid and diploid	Diploid	Haploid	Haploid
47	Common model organism in studying unicellular eukaryotes/budding yeast	*Saccharomyces cerevisiae*	*Cryptococcus Neoformans*	*Candida albicans*	*Pitchia pastoris*	*Saccharomyces cerevisiae*
48	Gene conversion can be either allelic or _____	Allergic	Ectopic	Endemic	Endopic	Ectopic
49	Gene conversion in yeast may be important in maintaining_____within families	Sequence homogeneity	Sequence heterogenecity	Sequence array	Sequence hologenecity	Sequence homogeneity
50	Gene conversion is the _____ transfer of information from one DNA duplex to another	Reciprocal	Non-reciprocal	Direct	Indirect	Non-reciprocal
51	Generation time of yeast takes place at	2hrs 30 min	3 hrs	60 min	30 min	2hrs 30 min

	_____					
52	Genetic maps of chromosome are based on the frequencies of _____	Reproducible mitotic product	Reproducible meiotic product	Recombinant mitotic product	Recombinant meiotic product	Recombinant meiotic product
53	_____ test is used to determine which gene(s) are defective in petite yeast mutants	Complementation test	Complement fixation test	Completed test	Conjugation test	Complementation test
54	_____ contains two types of spores of same parental genotype	Parental haploid	Parental diploid	Non-parental ditype	Parental ditype	Parental diploid
55	_____ intial products of meiosis forms two identical spore	4	8	16	32	4
56	_____ refers to a genetic changes in different genomes of same cell	Trans type	Same type	Cis type	Different type	Cis type
57	_____determines the number of crossover events and give correct map distance	Genetic analysis	Physical analysis	Random spore analysis	Tetrad analysis	Tetrad analysis
58	_____developed an algebriac method to determine the consequence of various number of exchanging	Klebs & Loeffler	Pastuer & Winogradsky	Shult & Lindegrin	Klug & Cumming	Shult & Lindegrin
59	_____distance in map unit is only half the percentage of second	Tetromere	Telomere	Centromer	Primer	Telomere

	division segregated tetrads					
60	A feature of petite is the occurrence of _____ from the circular mitochondrial genome	Insertion	Excertion	Addition	Deletion	Deletion