Semester – I

19MBP103 **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: 19MBP103 **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.

Genetics & Its Types:

Almost every human trait and disease has a genetic component, whether inherited or influenced by behavioral factors such as exercise. Genetic components can also modify the body's response to environmental factors such as toxins. Understanding the underlying concepts of human genetics and the role of genes, behavior, and the environment is important for appropriately collecting and applying genetic and genomic information and technologies during clinical care. It is important in improving disease diagnosis and treatment as well. This chapter provides fundamental information about basic genetics concepts, including cell structure, the molecular and biochemical basis of disease, major types of genetic disease, laws of inheritance, and the impact of genetic variation.

Types:

- Molecular genetics
- Developmental genetics
- Population genetics
- Quantitative genetics
- Phylogenetics

Mendelian Principles:

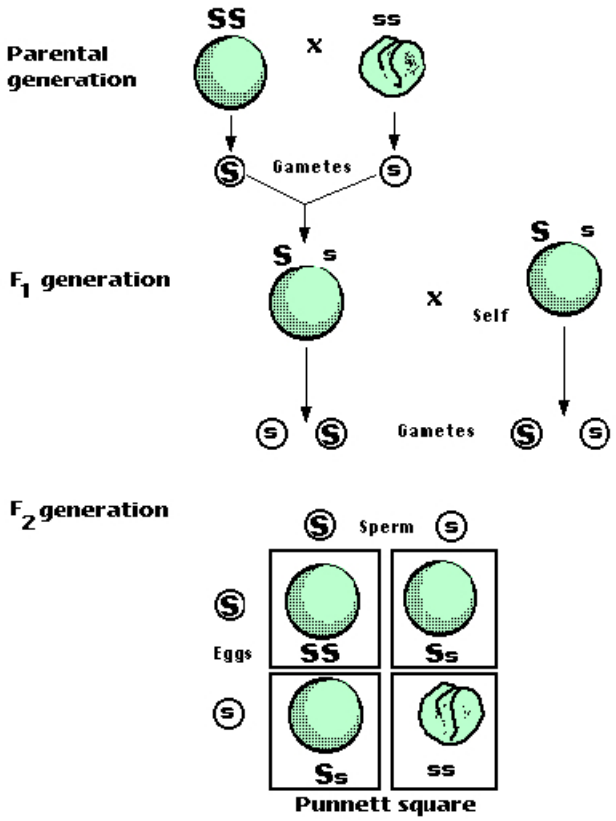
In the 1860's, an Austrian monk named Gregor Mendel introduced a new theory of inheritance based on his experimental work with pea plants. Prior to Mendel, most people believed inheritance was due to a blending of parental 'essences', much like how mixing blue and yellow paint will produce a green color. Mendel instead believed that heredity is the result of discrete units of inheritance, and every single unit (or **gene**) was independent in its actions in an individual's genome. According to this Mendelian concept, inheritance of a trait depends on the passing-on of these units. For any given trait, an individual inherits one gene from each parent so that the individual has a pairing of two genes. We now understand the alternate forms of these units as '**alleles**'. If the two alleles that form the pair for a trait are

identical, then the individual is said to be **homozygous** and if the two genes are different, then the individual is **heterozygous** for the trait.

Based on his pea plant studies, Mendel proposed that traits are always controlled by single genes. However, modern studies have revealed that most traits in humans are controlled by multiple genes as well as environmental influences and do not necessarily exhibit a simple Mendelian pattern of inheritance(see "Mendel's Experimental Results").

Mendel's Experimental Results:

Mendel carried out breeding experiments in his monastery's garden to test inheritance patterns. He selectively cross-bred common pea plants (*Pisum sativum*) with selected traits over several generations.



After crossing two plants which differed in a single trait (tall stems vs. short stems, round peas vs. wrinkled peas, purple flowers vs. white flowers, etc), Mendel discovered that the next generation, the "F1" (first filial generation), was comprised entirely of individuals exhibiting only one of the traits. However, when this generation was interbred, its offspring, the "F2" (second filial generation), showed a 3:1 ratio- three individuals had the same trait as one parent and one individual had the other parent's trait.

- Mendel then theorized that genes can be made up of three possible pairings of heredity units, which he called 'factors': AA, Aa, and aa. The big 'A' represents the dominant factor and the little 'a' represents the recessive factor. In Mendel's crosses, the starting plants were homozygous AA or aa, the F1 generation were Aa, and the F2 generation were AA, Aa, or aa. The interaction between these two determines the physical trait that is visible to us.
- Mendel's Law of Dominance predicts this interaction; it states that when mating occurs between two organisms of different traits, each offspring exhibits the trait of one parent only. If the dominant factor is present in an individual, the dominant trait will result. The recessive trait will only result if both factors are recessive.

Mendel's Laws of Inheritance:

Mendel's observations and conclusions are summarized in the following two principles, or laws.

Law Of Segregation:

The Law of Segregation states that for any trait, each parent's pairing of genes (alleles) split and one gene passes from each parent to an offspring. Which particular gene in a pair gets passed on is completely up to chance.

Law Of Independent Assortment:

The Law of Independent Assortment states that different pairs of alleles are passed onto the offspring independently of each other. Therefore, inheritance of genes at one location in a genome does not influence the inheritance of genes at another location.

Evidences of Nucleic Acids as Genetic Material:

Nucleic acids (DNA and RNA) act as genetic material in all organisms and viruses: 1. Evidences from Bacteria 2. Evidence from Bacteriophages 3. Evidence from Bacterial Conjugation 4. Evidence from RNA Viruses.

1. Evidences from Bacteria:

For the first time, an English Health officer, Frederick Griffith (1928) gave an experimental evidence that the DNA was the genetic material. He took two types of a bacterial strain, pneumococci (Streptococcus pneumonia) that causes pneumonia in humans and other animals.

There were two types of pneumococci, type II and type III. Each type exists in two forms RII, SII, and RIII, SIII forms where 'R' represents the rough, non-capsulated and non- virulent form and 'S' represent the smooth, encapsulated and virulent form.

After injection of smooth (virulent) strain, mice were killed (Fig. 5.24). When boiled smooth strain was injected, the mice were not affected and no pneumococci could be recovered from the mice. Upon injection of a mixture of heat killed smooth strain and live rough (non-virulent) strain, the mice were killed and live virulent bacteria could be recovered from blood of dead mice.

Griffith called this change of non-virulent strain into virulent strain as transformation, because the virulent strain transformed the non-virulent strain into the virulent strain. This is called Griffith's transformation experiment (Fig. 5.24). The phenomenon of bacterial transformation is called "Griffith effect".

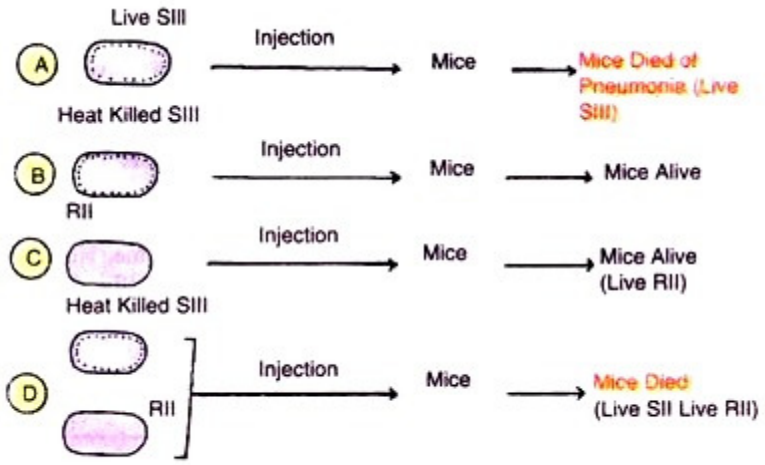


Fig. 5.24 : Griffith's transformation experiment.

Identification of transforming substance:

Griffith could not identify the nature of transforming substance. Oswald T. Avery, C.M. Mac Leod and M.J. Mc Carty (1944), set out experiments to identify the transforming principle. They destroyed cell constituents in extract of virulent pneumococci SIII using enzyme that hydrolyzed DNA, RNA, proteins and polysaccharides.

They treated the non-virulent strain separately with bacterial extracts as below:

RII Cells + Purified SIII cell polysaccharide → R colonies

RII Cells + Purified SIII cell protein → R colonies

RII Cells -H Purified SIII cell RNA → R colonies

RII Cells + Purified SIII cell DNA → SIII COLONIES

RII Cells + Purified SIII cell extract + protease → SIII colonies

RII Cells 4- Purified SIII cell extract h- RNase → SIII colonies

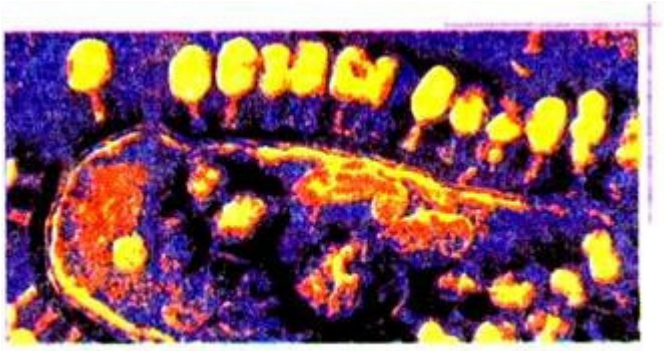
They concluded that a cell free and highly purified RNA extract of SIII strain could bring about transformation of RII strain into SIII strain. However, this effect was lost when the extract was treated with deoxy-ribonuclease.

Therefore, DNA is the genetic material and carries sufficient information for tons-formation. Thereafter, the transforming material (DNA) was also confirmed in several bacteria such as Bacillus subtilis, Haemophilus influenzae, Shigella para-dysenteriae, etc.

2. Evidence from Bacteriophages:

Alfred D. Hershey and Martha Chase (1952) carried out several experiments in bacteriophage T_2 that proved the DNA to be the genetic material of T_2. They prepared a culture medium for the growth of E. coli that contained phosphorus and sulphur.

To this culture medium known amount of isotopic phosphorus (^{32}P) and sulphur (^{35}S) was amended. Cell suspension of E. coli was poured onto the growth medium. After bacterial growth ^{32}P and ^{35}S were incorporated into the bacterial cell.



Electron micrograph of T4 virus particles infecting a host bacterial cell.

These cells were allowed to get infected by T_2 bacteriophage. T_2 was multiplied in E. coli cells. The ^{32}Pwas incorporated into DNA molecule and ^{35}S into capsid protein of T_2 phage. Since DNA lacks sulphur, therefore, ^{35}S was not incorporated into DNA. By using this method they labelled differentially the phage DNA and coat protein with the isotopes. These radiolabelled phage particles were recovered from E. coli by centrifugation method.

In another experiment, non-radiolabelled E. coli cells were allowed to be infected by radiolabelled bacteriophages. After absorption the coat protein remained outside and only radiolabelled DNA was injected. From the infected E. coli cells, the viral particles were separated from the host through agitation.

The amount of ^{32}P and ^{35}S in bacteriophage, particles, E. coli cells and medium was estimated. The ^{32}p was estimated with the bacterial cells and ^{35}S with protein coat left outside the bacterial wall i.e. the growth medium. This experiment shows that the genetic material of T_2 phage resides in DNA but not in their protein.

For this work A.D. Hershey shared Nobel Prize in medicine for 1969 with M. Delbruck and S.E. Luria. Furthermore, this experiment was repeated by several other workers by taking different bacteriophages. In all the cases, DNA was found to give evidence of genetic material.

3. Evidence from Bacterial Conjugation:

Conjugation is one of the methods of transfer of DNA from donor bacterium to the recipient bacterium. Lederberg and Tatum (1946) demonstrated when F^+ (bacterium containing fertility factor i.e. male) strain of E. coli cells is mixed with F^- (cells devoid of fertility factor i.e. female); the F^- cells were converted to F^+ cells.

F^+ cells + F^- cells → F^+ cells

The F^+ factor is a segment of plasmid DNA. It is evident from this experiment that DNA is the genetic material.

4. Evidence from RNA Viruses:

In 1956, A. Gierer and S. Schramm put example that when healthy tobacco leaves are inoculated by RNA purified from tobacco mosaic virus, lesions developed on healthy leaves which have like those obtained from tobacco mosaic plants.

Like Hershey and Chase, another experiment was done by H.F. Conract and B. Singler in 1957. They separated RNA from protein coat of TMV and reconstituted them. One group contained parental RNA and protein from mutant TMV, and the other had RNA from the mutant TMV and protein coat from the parental TMV.

The reconstituted viruses were allowed to infect healthy tobacco leaves. After infection from the lesions the TMV particles were recovered. In all the cases the progeny TMV particles contained parental RNA type, but not parental protein type.

Physical and Chemical structure of DNA:

Deoxyribonucleic acid (DNA) IS the genetic information of most living organisms (a contrario, some viruses, called retroviruses, use ribonucleic acid as genetic information).

- DNA can be copied over generations of cells: DNA replication
- DNA can be translated into proteins: DNA transcription into RNA, further translated into proteins ,
- DNA can be repaired when needed: DNA repair .
- Ribonucleic acids (RNAs) are described in another chapter (mRNA, r-RNA, t-RNA...)
- DNA is a polymere, made of units called nucleotides (or mononucleotides).
- Nucleotides also have other functions: (energy carriers: ATP, GTP; cellular respiration: NAD, FAD; signal transduction: cyclic AMP; coenzymes: CoA, UDP; vitamins: nicotinamide mononucleotide, Vit B2).

Using the protein nomenclature, we could speak in terms of primary, secondary, tertiary and quaternary structures of the molecule:

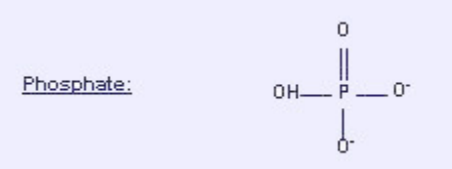
I Primary Structure Of The Molecule:

Covalent Backbone And Bases Aside:

- A nucleoside is made of a sugar + a nitrogenous base.
- A nucleotide is made of a phosphate + a sugar + a nitrogenous base. In DNA, the nucleotide is a deoxyribonucleotide (in RNA, the nucleotide is a ribonucleotide).

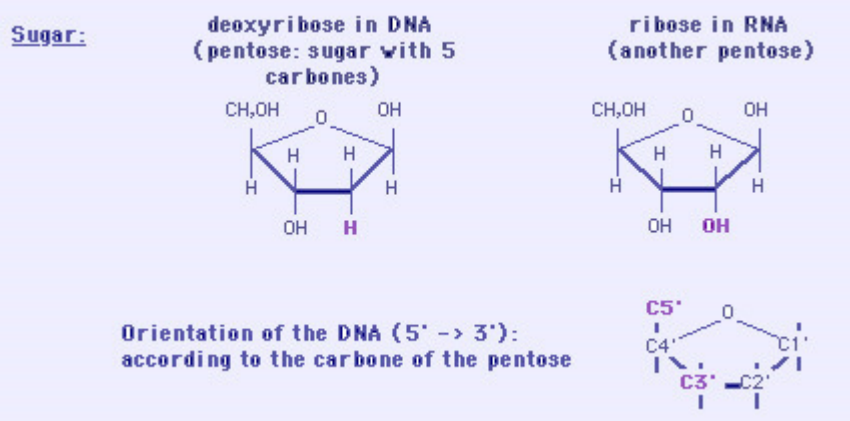
I-1 Phosphoric acid

Gives a phosphate group.



I-2 Sugar:

Deoxyribose, which is a cyclic pentose (5-carbon sugar). Note: the sugar in RNA is a ribose. Carbons in the sugar are noted from 1' to 5'. A nitrogen atom from the nitrogenous base links to C1' (glycosidic link), and the phosphate links to C5' (ester link) to make the nucleotide. The nucleotide is therefore: phosphate - C5' sugar C1' - base.

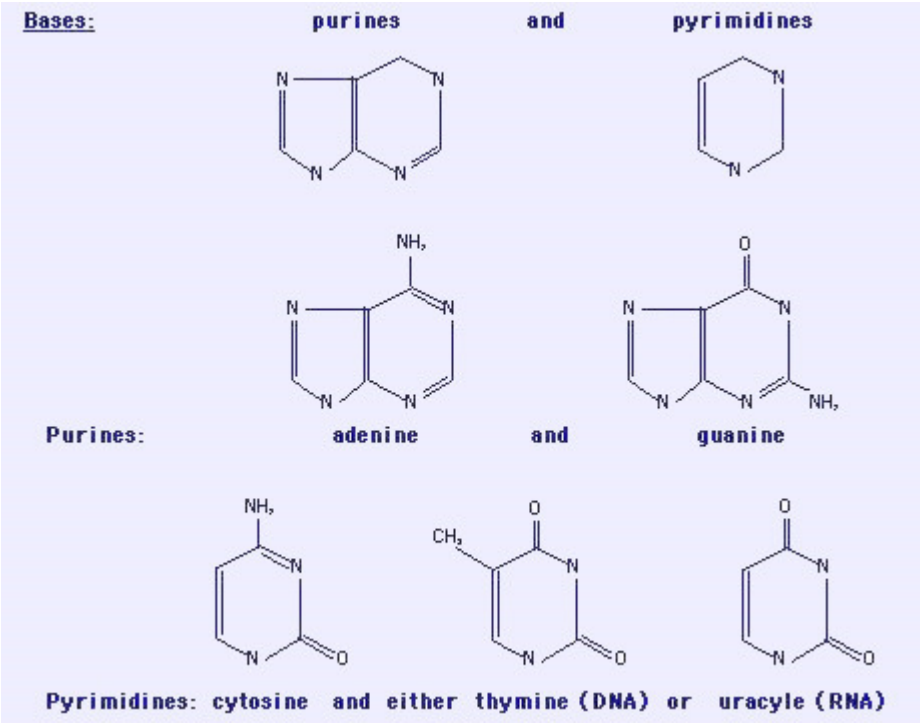


I-3 Nitrogenous bases:DNA can be copied over generations of cells: DNA replication

- Aromatic heterocycles; there are purines and pyrimidines.

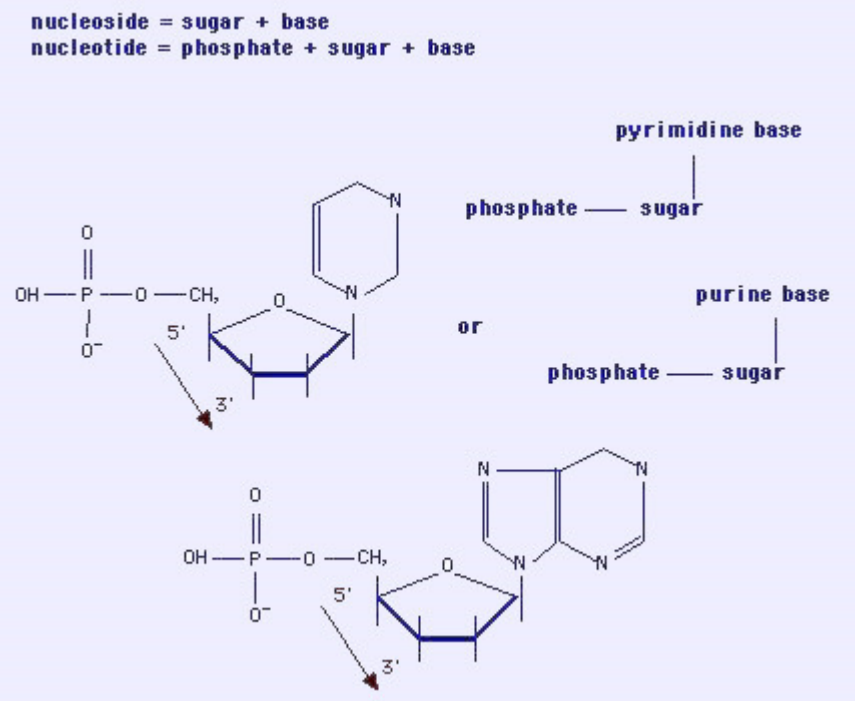
- Purines: adenine (A) and guanine (G).
- Pyrimidines: cytosine (C) and thymine (T) (Note: thymine is replaced by uracyle (U) in RNA).

Note: other nitrogenous bases exist, in particular methylated bases derived from the above mentioned; methylation of the bases has a functional role (see chapter ad hoc).



Glossary:

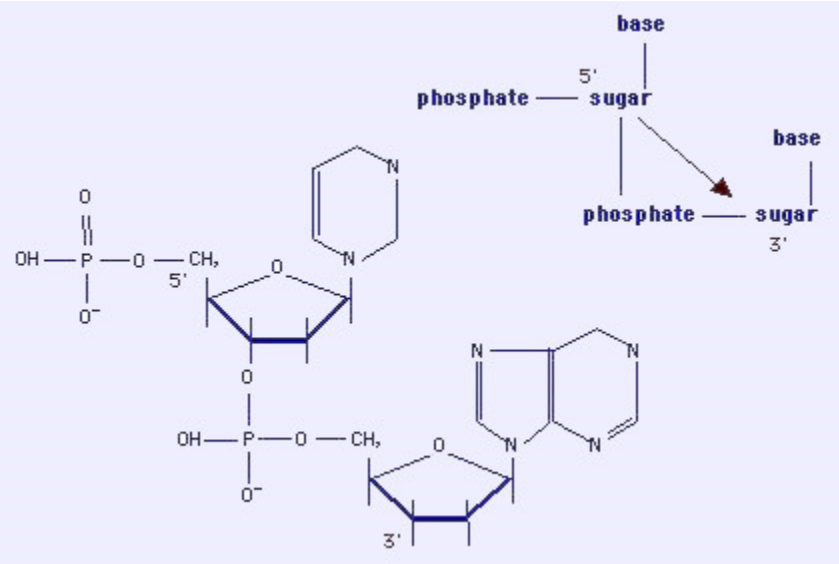
- Nucleoside names: deoxyribonucleosides in DNA: deoxyadenosine, deoxyguanosine, deoxycytidine, deoxythymidine in DNA (ribonucleosides in RNA: adenosine, guanosine, cytidine, uridine).
- Nucleotide names: deoxyribonucleotides in DNA: deoxyadenylic acid, deoxyguanylic acid, deoxycytidylic acid, deoxythymidylic acid (ribonucleotides in RNA: adenylic acid, guanylic acid, cytidylic acid, uridylic acid).



II Secondary and tertiary structures of the molecule - Three-dimentional conformation of DNA

II.1 Dinucleotides

Dinucleotides form from a phosphodiester link between 2 mononucleotides. The phosphate of a mononucleotide (in C5' of its sugar) being linked to the C3' of the sugar of the previous mononucleotide. Then, we start with a phosphate, a 5' sugar (+base) and the 3' of this sugar, linked to a second phosphate - 5' sugar, which 3' is free for next step. The link - and the orientation of the molecule- is therefore 5' -> 3'. Polynucleotides are made of the successive addition of monomeres in a general 5' -> 3' configuration. The backbone of the molecule is made of a succession of phosphate-sugar (nucleotide n) - phosphate-sugar (nucleotide n+1), and so on, covalently linked, the bases being aside.

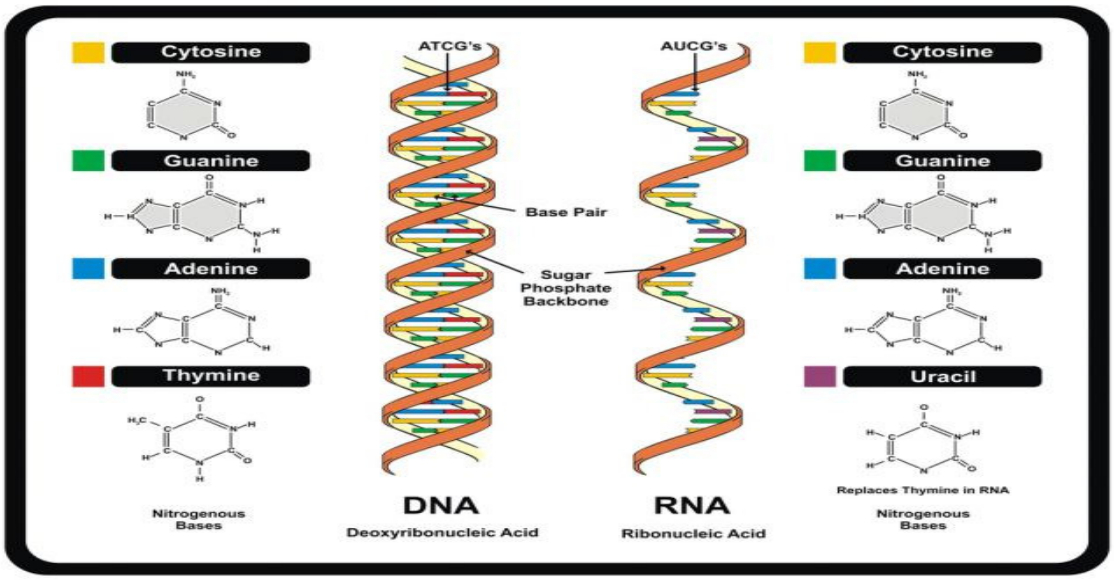


DNA is made up of molecules called nucleotides. Each nucleotide contains a phosphate group, a sugar group and a nitrogen base. The four types of nitrogen bases are adenine (A), thymine (T), guanine (G) and cytosine (C). The order of these bases is what determines DNA's instructions, or genetic code. Human DNA has around 3 billion bases, and more than 99 percent of those bases are the same in all people, according to the U.S. National Library of Medicine (NLM).

Similar to the way the order of letters in the alphabet can be used to form a word, the order of nitrogen bases in a DNA sequence forms genes, which in the language of the cell, tells cells how to make proteins. Another type of nucleic acid, ribonucleic acid, or RNA, translates genetic information from DNA into proteins.

Nucleotides are attached together to form two long strands that spiral to create a structure called a double helix. If you think of the double helix structure as a ladder, the phosphate and sugar molecules would be the sides, while the bases would be the rungs. The bases on one strand pair with the bases on another strand: adenine pairs with thymine, and guanine pairs with cytosine.

DNA molecules are long — so long, in fact, that they can't fit into cells without the right packaging. To fit inside cells, DNA is coiled tightly to form structures we call chromosomes. Each chromosome contains a single DNA molecule. Humans have 23 pairs of chromosomes, which are found inside the cell's nucleus.



DNA Forms:

Main Forms of DNA:

The most common form of DNA which has right handed helix and proposed by Watson and Crick is called B-form of DNA or B-DNA. In addition, the DNA may be able to exist in other forms of double helical structure. These are A and C forms of double helix which vary from B- form in spacing between nucleotides and number of nucleotides per turn, rotation per base pair, vertical rise per base pair and helical diameter (Table 5.3).

Table 5.3 : Forms of double stranded DNA helix

Parameters	*Forms of DNA*				
	A	*B*	*C*	*D*	*Z*
Conditions	75% rel. Humidity, Na^+ K^+, Cs^+ ions	92% rel. humidity low ions	66% rel. humidity Li^+ ions	—	low high salt conc.
Base pair per turn	11	10	9.33	8	12 (6 dimers)
Rotation per bp	+32.7°	+36.0°	+38.6°	—	-30.0°
Vertical rise per bp	2.56Å	3.38Å	3.32Å	3.03Å	3.71Å
Helical diameter	23Å	20Å	19Å	—	18Å
Pitch of the helix	28.15Å	34Å	31Å	—	45 Å
Tilt of bp Sugar puckering	20.2Å	6.3°	-7.8°	-16.7°	7°

1. The B-Form of DNA (B-DNA):

Structure of B-form of DNA has been proposed by Watson and Crick. It is present in every cell at a very high relative humidity (92%) and low concentration of ions. It has antiparallel double helix, rotating clockwise (right hand) and made up of sugar- phosphate back bone combined with base pairs or purine-pyrimidine.

The base pairs are perpendicular to longitudinal axis of the helix. The base pairs tilt to helix by 6.3°. The B-form of DNA is metabolically stable and undergo changes to A, C or D forms depending on sequence of nucleotides and concentration of excess salts.

2. The A-Form of DNA (A-DNA):

The A-form of DNA is found at 75% relative humidity in the presence of Na+, K+ or Cs+ ions. It contains eleven base pairs as compared to ten base pairs of B-DNA which tilt from the axis of helix by 20.2°. Due to this displacement the depth of major groove increases and that of minor groove decreases. The A-form is metastable and quickly turns to the D-form.

3. The C-Form DNA (C-DNA):

The C-form of DNA is found at 66% relative humidity in the presence of lithium (Lit+) ions. As compared to A- and B-DNA, in C-DNA the number of base pairs per turn is less i.e. 28/3 or 9 1/3. The base pairs show pronounced negative tilt by 7.8°.

4. The D-Form of DNA (D-DNA):

The D-form of DNA is found rarely as extreme vanants. Total number of base pairs per turn of helix is eight. Therefore, it shows eight-fold symmetry. This form is also called poly (dA-dT) and poly (dG-dC) form. There is pronounced negative tilt of base pairs by 16.7° as compared to C form i.e. the base pairs are displaced backwardly with respect to the axis of DNA helix.

5. The Z-Form of DNA (Z-DNA) or Left Handed DNA:

In 1979, Rich and coworkers at MIT (U.S.A.) obtained Z-DNA by artificially synthesizing d (C-G) 3 molecules in the form of crystals. They proposed a left handed (synistral) double helix model with zig-zag sugar-phosphate back bone running in antiparallel direction.Therefore, this DNA has been termed as Z-DNA. The Z-DNA has been found in a large number of living organisms including mammals, protozoans and several plant species.

There are several similarities with B-DNA in having:

- Double helix,
- Two antiparallel strands, and
- Three hydrogen bonds between G-C pairing.

In addition, the Z-DNA differs from the B-DNA in the following ways:

- The Z-DNA has left handed helix, while the B-DNA has right handed helix.
- The Z-DNA contains zig-zag sugar phosphate back bone as compared to regular back bone of the B-DNA.

6. Single Stranded (ss) DNA:

Almost all the organisms contain double stranded DNA except a few viruses such as bacteriophage φ × 174 which consists of single stranded circular DNA. It becomes double stranded only at the time of replication.

The differences of ssDNA from the dsDNA are as below:

- The dsDNA absorbs wavelength 2600 Å of ultra violet light constantly from 0 to 80°C, thereafter rise sharply, whereas in ssDNA absorption of UV light increases steadily from 20° to 90°C.
- The dsDNA resists the action of formaline due to closed reactive site, while the ss DNA does not resist it due to exposed reactive sites.
- Base pair composition in dsDNA is equal i.e. A=T and G=C, in ssDNA the composition of A, T, G, C is in proportion of 1:1.33:0.98:0.75.
- The dsDNA always remains in linear helical form, while the ssDNA remains in circular form; however, it becomes double stranded only during replication (i.e. replicative form).

7. Circular and Super Helical DNA:

Almost in all the prokaryotes and a few viruses, the DNA is organised in the form of closed circle. The two ends of the double helix get covalently sealed to form a closed circle. Thus, a closed circle contains two unbroken complementary strands. Sometimes one or more nicks or breaks may be present on one or both strands, for example DNA of phage PM2 (Fig. 5.7 A).

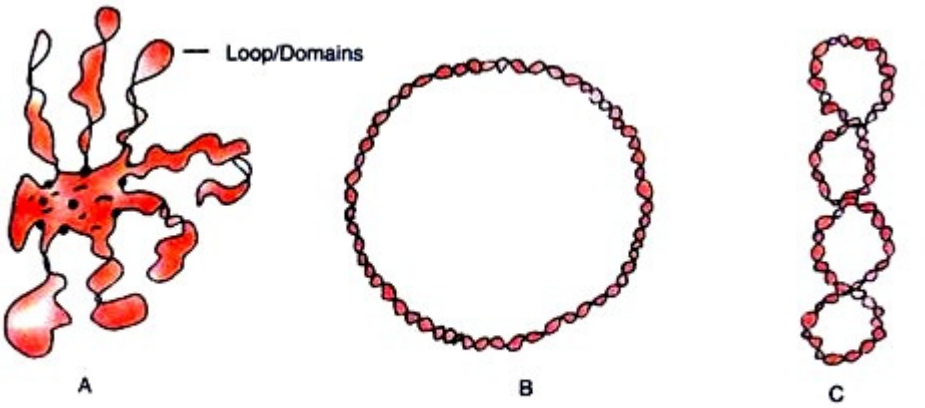


Fig. 5.7 : The forms of DNA. A, Nucleoids of *E.coli*; B, a closed, circular bacterial DNA; C, twisted supercoils of double stranded DNA.

Besides some exceptions, the covalendy closed circles are twisted into super helix or super coils (Fig.5.7 B) and is associated with basic proteins but not with histones found complexed with all eukaryotic DNA.

This histone like proteins appear to help the organization of bacterial DNA into a coiled chromatin structure with the result of nucleosome like structure, folding and super coiling of DNA, and association of DNA polymerase with nucleoids. Several histones like DNA binding proteins have been described in bacteria (Table 5.4).

These nucleoid-associated proteins include HU proteins, IHF, protein H1, Fir A, H-NS and Fis. In archaeobacteria (e.g. Archaea) the chromosomal DNA exists in protein-associated form. Histone like proteins has been isolated from nucleoprotein complexes in Thermoplasma acidophilurn and Halobacterium salinanim.

Thus, the protein associated DNA and nucleosome like structures are detected in a variety of bacteria. If the helix coils clockwise from the axis the coiling is termed as positive or right handed coiling. In contrast, if the path of coiling is anticlockwise, the coil is called left handed or negative coil.

Enzymology of DNA Replication:

Replication of double stranded DNA molecule is a complex pro-cess involving a number of enzymes. For DNA replication to occur, the following events should take place

- Temporary separation of the two parental strands.
- Stabilization of the single stranded DNA molecule.
- Initiation of daughter strand synthesis.

- Elongation of the daughter strands.
- Termination of the reaction.

All the stages are individual enzymatic activities and do not func-tion independently and are contained in a discrete multiprotein struc-ture called the replisome. Enzymes that are able to synthesize new DNA strands on a template strand are called DNA polymerases.

The enzymes that polymerize nucleotides into a growing strand of DNA are called as polymerases. There are three known enzymes in*E.coli*

- DNA Polymerase I
- DNA Polymerase II
- DNA Polymerase III

In a simple model of DNA replication, according to the rule of complementarity, nucleotides will be synthesized on both the strands on the replication fork. During DNA replication polymerization pro-ceeds from 5' to 3' direction. Since both strands are running in oppo-site direction one new strand has to be replicated in the 5' to 3' direc-tion and the other in the 3' to 5' direction. However, all the known polymerases synthesise nucleotides only in the 5' to 3' direction. Evi-dence from autoradiography suggests that there are 2 types of replica-tion

- Continuous replication
- Discontinuous replication

The discontinuous form of replication takes place on the comple-mentary strand in short segments in a backward direction. These short segments are called as Okazaki fragments, named after R. Okazaki who first saw them. The length of Okazaki fragments in prokaryotes is 1500 nucleotides and 150 in eukaryotes. The strand that is synthesized continuously is called as leading strand. The discontinuous strand is called as lagging strand.

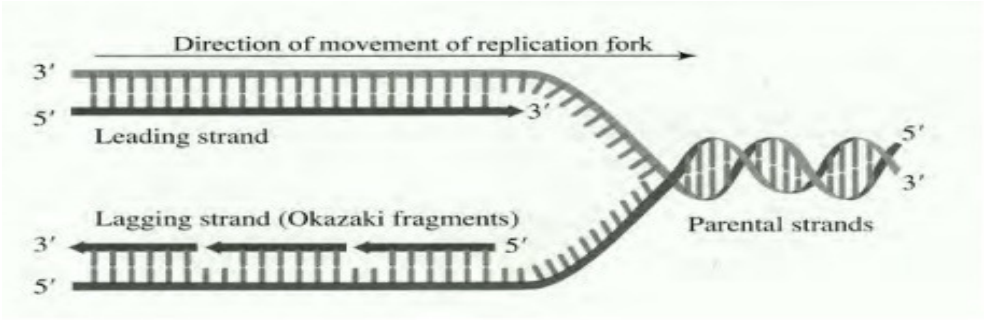
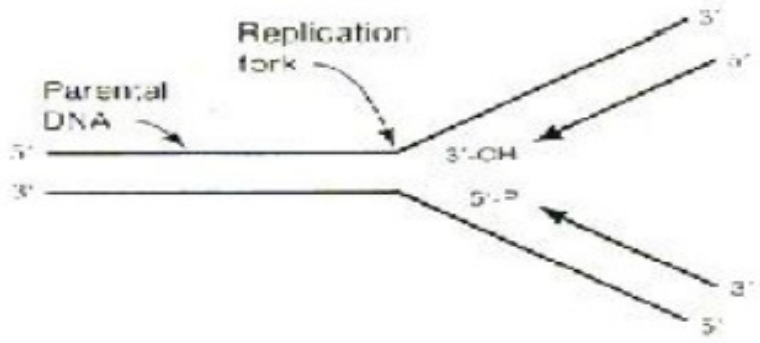


Fig. 35-12 The leading and lagging strands of DNA synthesis

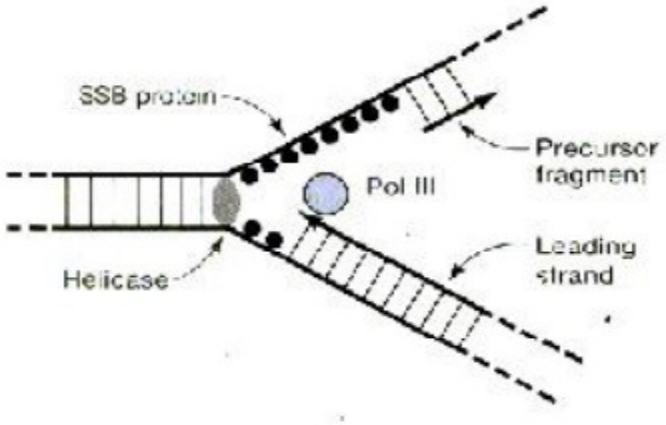
The list of major proteins necessary for DNA replication in *E.coli*is tabulated:

Helicase	Starts unwinding of DNA double helix
DNA gyrase	Assists unwinding
SSB Protein	Stabilize single strand of DNA
Primase	Synthesis of RNA primer
DNA Pol III	Elongation of chain by DNA synthesis
DNA Pol I	Removal of RNA primer and fill in gap with DNA
DNA ligase	Closes last phosphodiester gap

The combined effect of helicase and gyrase results in the forma-tion of a replication fork. (Figure 35-13



The helicase enzyme, (also called as unwinding protein and rep protein) recognizes and binds to the origin of replication and catalyses separation of the two DNA strands by breaking the hydrogen bonds between base pairs. DNA gyrase, a topoisomerase, assists unwinding of DNA strands by inducing supercoiling. The exposed single strands of DNA is stabilized and protected from hydrolytic cleavage of phosphodiester bonds. The SSB proteins (Single Stranded DNA Bind-ing protein) perform this protective role.



The various events of replication with essential enzymes and proteins:

The separated polynucleotide strands are used as templates for the synthesis of complementary strands. The next step is the initiation of DNA synthesis. All of the known DNA polymerases can extend a deoxyribonucleotide chain from a free 3' – OH end, but none can initiate synthesis. The DNA Polymerase III requires primer with a free3'-hydroxyl end. The primer is short stretch of RNA (4 to 10 nucle-otides) complementary to the DNA template.

RNA synthesis is catalysed by an enzyme called primase. The action of primase is required only once for the initiation of the leading strand of DNA where as each okazaki fragment must be initiated by the action of primase.

The DNA synthesis is catalysed by Polymerase III and can pro-ceed from 3'-hydroxyl group. The RNA primer is removed from theDNA by the 5' - - > 3' nuclease action of DNA Polymerase I and byRNAase H after the second synthesis is computed.

When the primer is removed, there will be a gap. DNA Poly-merase I is likely to be involved in filling the gap.

Both leading and lagging strand are extended in the 5' - - > 3' di-rection. The leading strand proceed in the direction of the advancing replication fork Synthesis of the lagging strand continues in the oppo-site direction until it meets the fragment previously synthesized.

Once the RNA has been removed and replaced, the adjacent Okazaki fragments must be linked together. The 3'-OH end of one fragment will be adjacent to the 5' phosphate end of the previous frag-ment. The gap is filled by the enzyme DNA ligase by forming the final phosphodiester bond.

Termination of the replication process occurs when the two rep-lication forks meet in the circular *E.coli* chromosome.

Types of DNA Replication

Three modes of DNA replication have been reported which are:

1. Semiconservative replication,
2. (2) Conservative replication and
3. (3) Dispersive replication.

Semiconservative DNA Replication:

Two strands of the original DNA are separated, and each is duplicated by the synthesis of the complementary strand, thus producing two exact replica of the parent DNA. Each of the two molecules thus contains one original strand and one newly synthesized strand. Thus, only one of the two strands of the original DNA is conserved. This mode of replication is known as semiconservative mode of replication.

Conservative Replication:

When complementary polynucleotide chains are synthesized, and a exact replica of parental DNA is formed. But the two newly created strands then come together and the parental strands reassociate. The original helix is conserved.

Dispersive Replication:

Here the parental strands are dispersed in to two new double helices following replication. Hence each strand consists of both old and new DNA. This mode will involve cleavage of the parental strands during replication. It is the most complex of the three possibilities and is, therefore, considered to be least likely to occur Unidirectional and bidirectional DNA replication According to John Cairns the DNA synthesis starts at a fixed point on the chromosome and proceeds in one direction. It was subsequently realized that Cairns results could be interpreted in terms of bidirectional replication also.

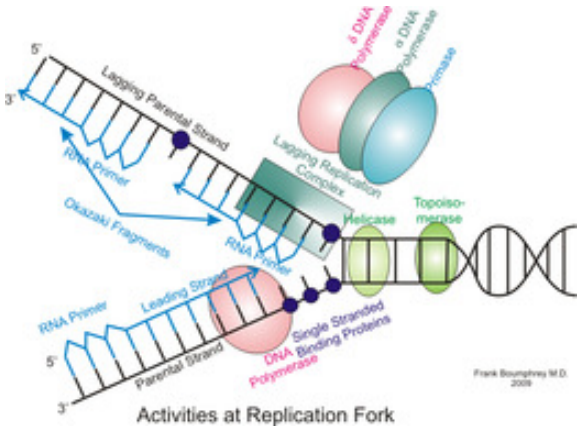
Additional evidences supporting bidirectional replication were obtained from (1) autoradiography, (2) electron microscopy and (3) genetic studies. In view of the above evidences, it is now known that DNA replication can be

unidirectional or bidirectional, depending upon whether the replication from the point of origin proceeds only in one direction or proceeds in both the directions.

Eukaryotic DNA replication:

It is a conserved mechanism that restricts DNA replication to once per cell cycle. Eukaryotic DNA replication of chromosomal DNA is central for the duplication of a cell and is necessary for the maintenance of the eukaryotic genome.

DNA replication is the action of DNA polymerases synthesizing a DNA strand complementary to the original template strand. To synthesize DNA, the double-stranded DNA is unwound by DNA helicases ahead of polymerases, forming a replication fork containing two single-stranded templates. Replication processes permit the copying of a single DNA double helix into two DNA helices, which are divided into the daughter cells at mitosis. The major enzymatic functions carried out at the replication fork are well conserved from prokaryotes to eukaryotes, but the replication machinery in eukaryotic DNA replication is a much larger complex, coordinating many proteins at the site of replication, forming the replisome



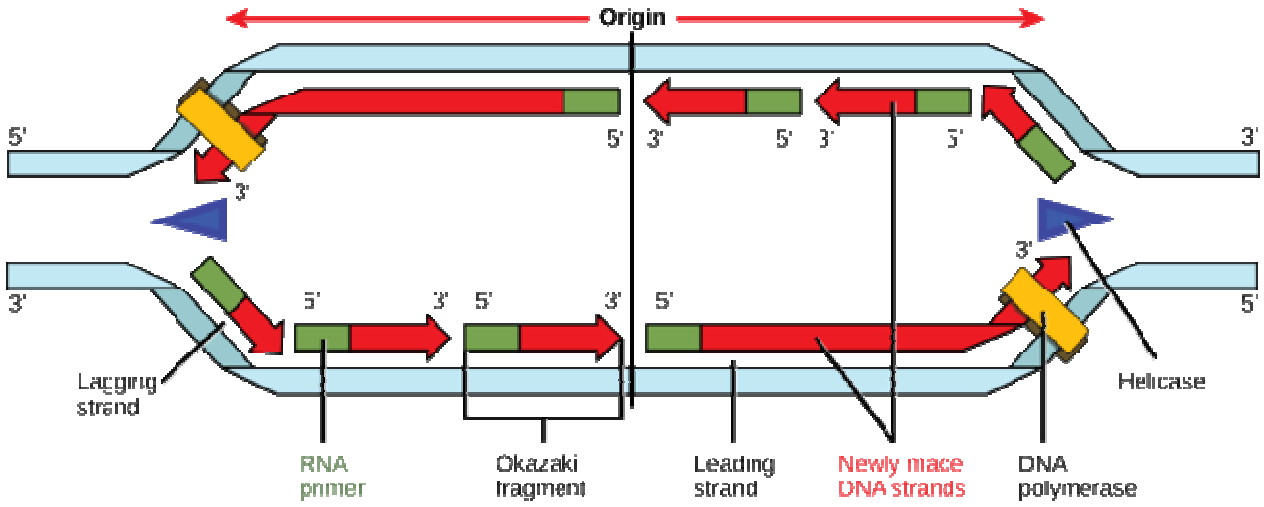
DNA replication is a very complicated process that involves several enzymes and other proteins. It occurs in three main stages: initiation, elongation, and termination.

Initiation

Eukaryotic DNA is bound to proteins known as histones to form structures called nucleosomes. During initiation, the DNA is made accessible to the proteins and enzymes involved in the replication process. There are specific chromosomal locations called origins of replication where replication begins. In some eukaryotes, like yeast, these locations are defined by having a specific sequence of basepairs to which the replication initiation proteins bind. In other eukaryotes, like humans, there does not appear to be a consensus sequence for their origins of replication. Instead, the replication initiation proteins might identify and bind to specific modifications to the nucleosomes in the origin region.

Certain proteins recognize and bind to the origin of replication and then allow the other proteins necessary for DNA replication to bind the same region. The first proteins to bind the DNA are said to “recruit” the other proteins.

Two copies of an enzyme called helicase are among the proteins recruited to the origin. Each helicase unwinds and separates the DNA helix into single-stranded DNA. As the DNA opens up, Y-shaped structures called replication forks are formed. Because two helicases bind, two replication forks are formed at the origin of replication; these are extended in both directions as replication proceeds creating a replication bubble. There are multiple origins of replication on the eukaryotic chromosome which allow replication to occur simultaneously in hundreds to thousands of locations along each chromosome.



Replication Fork Formation:

A replication fork is formed by the opening of the origin of replication; helicase separates the DNA strands. An RNA primer is synthesized by primase and is elongated by the DNA polymerase. On the leading strand, only a single RNA primer is needed, and DNA is synthesized continuously, whereas on the lagging strand, DNA is synthesized in short stretches, each of which must start with its own RNA primer. The DNA fragments are joined by DNA ligase (not shown).

Elongation

During elongation, an enzyme called DNA polymerase adds DNA nucleotides to the 3′ end of the newly synthesized polynucleotide strand. The template strand specifies which of the four DNA nucleotides (A, T, C, or G) is added at each position along the new chain. Only the nucleotide complementary to the template nucleotide at that position is added to the new strand.

DNA polymerase contains a groove that allows it to bind to a single-stranded template DNA and travel one nucleotide at at time. For example, when DNA polymerase meets an adenosine nucleotide on the template strand, it adds a thymidine to the 3′ end of the newly synthesized strand, and then moves to the next nucleotide on the template strand. This process will continue until the DNA polymerase reaches the end of the template strand.

DNA polymerase cannot initiate new strand synthesis; it only adds new nucleotides at the 3′ end of an existing strand. All newly synthesized polynucleotide strands must be initiated by a specialized RNA polymerase called primase.

Primase initiates polynucleotide synthesis and by creating a short RNA polynucleotide strand complementary to template DNA strand. This short stretch of RNA nucleotides is called the primer. Once RNA primer has been synthesized at the template DNA, primase exits, and DNA polymerase extends the new strand with nucleotides complementary to the template DNA.

Eventually, the RNA nucleotides in the primer are removed and replaced with DNA nucleotides. Once DNA replication is finished, the daughter molecules are made entirely of continuous DNA nucleotides, with no RNA portions.

The Leading and Lagging Strands

DNA polymerase can only synthesize new strands in the 5′ to 3′ direction. Therefore, the two newly-synthesized strands grow in opposite directions because the template strands at each replication fork are antiparallel. The "leading strand" is synthesized continuously toward the replication fork as helicase unwinds the template double-stranded DNA.

The "lagging strand" is synthesized in the direction away from the replication fork and away from the DNA helicase unwinds. This lagging strand is synthesized in pieces because the DNA polymerase can only synthesize in the 5′ to 3′ direction, and so it constantly encounters the previously-synthesized new strand. The pieces are called Okazaki fragments, and each fragment begins with its own RNA primer.

Termination

Eukaryotic chromosomes have multiple origins of replication, which initiate replication almost simultaneously. Each origin of replication forms a bubble of duplicated DNA on either side of the origin of replication. Eventually, the leading strand of one replication bubble reaches the lagging strand of another bubble, and the lagging strand will reach the 5′ end of the previous Okazaki fragment in the same bubble.

DNA polymerase halts when it reaches a section of DNA template that has already been replicated. However, DNA polymerase cannot catalyze the formation of a phosphodiester bond between the two segments of the new DNA strand, and it drops off. These unattached sections of the sugar-phosphate backbone in an otherwise full-replicated DNA strand are called nicks.

Once all the template nucleotides have been replicated, the replication process is not yet over. RNA primers need to be replaced with DNA, and nicks in the sugar-phosphate backbone need to be connected.

The group of cellular enzymes that remove RNA primers include the proteins FEN1 (flap endonulcease 1) and RNase H. The enzymes FEN1 and RNase H remove RNA primers at the start of each leading strand and at the start of each Okazaki fragment, leaving gaps of unreplicated template DNA. Once the primers are removed, a free-floating DNA polymerase lands at the 3′ end of the preceding DNA fragment and extends the DNA over the gap. However, this creates new nicks (unconnected sugar-phosphate backbone).

In the final stage of DNA replication, the enyzme ligase joins the sugar-phosphate backbones at each nick site. After ligase has connected all nicks, the new strand is one long continuous DNA strand, and the daughter DNA molecule is complete.

Questions:

Long answer questions:

1. Explain Mendelin Principles and his experiments
2. Write about Physical and chemical structure of DNA
3. What are the different forms of DNA
4. Brief notes about Enzymology og DNA
5. Explain various stages of Eukaryotic Replication.

Short answer questions:

1. What is Genetic material?
2. Notes on Genes and Alleles
3. Short notes on Circular DNA
4. What is Dominance and recessive genes?

Unit I Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
Key enzyme in rolling circle replication	DNA Polymerase-IV	DNA Polymerase-III	DNA Polymerase-II	DNA Polymerase-I	DNA Polymerase-III
Left handed DNA	B-DNA	C-DNA	Y-DNA	Z-DNA	Z-DNA
Length of primer during replication is	2-10 nucleotides	10-20 nucleotides	5-15 nucleotides	10-25 nucleotides	2-10 nucleotides
Longest DNA is seen in	Human	Lung fish	Yeast	Bacteria	Lung fish
Mendels pioneer work was with	Monkey	Human	Garden pea	Mice	Garden pea
Nucleoside is	Base + Sugar	Sugar + Phosphate	Base + Phosphate	A+T & G+C	Base + Sugar
Number of base pairs per helical turn of B form DNA	13	12	11	10	10
Okazaki fragments are	RNA strands	Enzymes	Leading strands	Lagging strands	Lagging strands
_____ 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
_____ degrades DNA	Polymerase	Primase	RNase	DNase	DNase
Adenine always pair with	Guanine	Cytosine	Thymine	Uracil	Thymine
Bacteria contains	Single circular DNA	Single linear DNA	Double Linear DNA	Double circular DNA	Single circular DNA

Basically, flow of genetic material is accompolished by	Replication	Transformation	Transduction	Conjugation	Replication
Bonding between two bases	Hydrogen bond	Hydrophobic bond	Nitrogen bond	Van Der waals	Hydrogen bond
Chargaff's rule	No complementarity	Partial complementarity	No such rule	Complementarity of one strand with the other	Complementarity of one strand with the other
Chromosomal theory of inheritance was formulated by	Mendel	Miescher	Metchinikoff	Morgan	Morgan
Formation of pre-replicating complex is seen in replication mechanism of	Prokaryotes	Plants	Virus	Eukaryotes	Eukaryotes
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
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
In eukaryotes, the vast majority of DNA synthesis occurs during _____ of the cell cycle	G phase	H phase	R phase	S phase	S phase
Initiation of replication is carried out by	DnaA	DnaC	DnaB	DnaE	DnaA
Initiation of replication occurs	Bidirectionally	Cross sectionally	Unidirectionally	Parallely	Bidirectionally
Joining of DNA fragments	DNA ligase	Gyrase	RNA polymerase	DNA polymerase	DNA ligase

Dihybrid ratio	3 : 3 : 9 : 1	9 : 3 : 3 : 1	9 : 3 : 1 : 3	1 : 3 : 3 : 9	9 : 3 : 3 : 1
Distance between the two base pairs is	3.4Å	34Å	10Å	20Å	3.4Å
DNA absorbs UV light at _____ wavelength	220 nm	240 nm	260 nm	280 nm	260 nm
Enzyme that adds methyl group to the newly formed DNA	Gyrase	Topoisomerase	Helicase	Methylase	Methylase
Eukaryotic DNA damage or replication errors are corrected during	G_1 phase	S phase	G_2 phase	R phase	G_2 phase
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
Experiments of Hershey and Chase was based on	Virus	Bacteriophage	Bacteria	Fungi	Bacteriophage
Father of genetics	Mendel	Morgan	Watson	McLeod	Mendel
Proof reading activity of DNA polymerase is in the direction	5' to 3'	3' to 5'	Parallel	Centre	3' to 5'
Purines are	A, T	G, C	C, T	A, G	A, G
Repair and insertion of DNA is carried out by	Endonucleases	Ribozyme	Primase	Exonucleases	Endonucleases
RNA primer is removed by	DNA pol	RNA pol	Terminase	Caspase	DNA pol

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
Semiconservative mechanism of DNA replication was demonstrated by	Meselson & Stahl	Beedle & Tatum	Hershy & Chase	Avery & McLeod	Meselson & Stahl
Sequencing and molecular characterization of genome	Genetics	Molecular biology	Proteomics	Genomics	Genomics
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
Synthesis of DNA always moves from	3' to 5'	5' to 3'	Ffrom the centre	Anywhere	5' to 3'
The ability to remove incorrectly matched nucleotides or Proof-reading	RNA polymerase	DNA ligase	DNA polymerase	DNA helicase	DNA polymerase
The contribution of Rosalind Franklin towards structure of DNA was	X-ray crystallography	Electron microscopy	NMRspectroscopy	Gas chromatography	X-ray crystallography
The DNA of E.coli is _____ times longer than the cell	1	10	1000	100	1000
The enzyme that copies RNA from DNA template	Dnase	Rnase	DNA polymerase	RNA polymerase	RNA polymerase
The enzyme that unwinds DNA	Polymerase	Ligase	Gyrase	Helicase	Helicase

The experiments of Avery, McLeod and McCarty was based on	Protein coupling	Enzymatic reactions	Synthetic reaction	DNA binding	Enzymatic reactions
The most common form of DNA is	B-DNA	Z-DNA	Y-DNA	SS-DNA	B-DNA
The most widely studied origin recognition complex of eukaryotes is that of	*Bacillus*	*Staphylococcus*	*Escherichia coli*	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*
The negative charge of DNA is due to	Deoxyribose Sugar	Phosphate bond	Hydrogen bond	Nitrogenous base	Phosphate bond
The Pioneer work on nucleic acid discovery was carried out by	Friedrick Miescher	Watson & Crick	Griffith	Milstein	Friedrick Miescher
The replication origin of *E.coli* is approximately	245 bp	425 bp	254 bp	524 bp	245 bp
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
The size of a major groove is	34Å	3.4Å	43Å	20Å	34Å
Thymine in DNA is replaced by _____ in RNA	Adenine	Cytosine	Guanine	Uracil	Uracil
Transfer of genes from one generation to the next is	Inheritance	Carrying over	Subheritance	Gene transport	Inheritance
Transformation in *Pneumococci* was discovered by	Friedrick Griffith	Erwin Chargaff	Hershey & Chase	Watson & Crick	Friedrick Griffith
Triple bonding is seen in	G-T	G-C	A-T	A-C	G-C
Which is involved in synthesis of primer	Ligase	Primase	DNA pol	rRNA	Primase

Which of the following is not associated with DNA replication?	Polymerase	Promoter	Primer	RepA protein	Promoter
Who proposed the molecular structure of DNA	Hershey & Chase	Erwin Chargaff	Jim Latham	Watson & Crick	Watson & Crick

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 Sonsense) 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:

1. **The genetic code is composed of nucleotide triplets.** In other words, three nucleotides in mRNA (acodon) specify one amino acid in a protein.
2. **The code is non-overlapping.** This means that successive triplets are read in order. Each nucleotideis part of only one triplet codon.

3. **The genetic code is unambiguous.** Each codon specifies a particular amino acid, and only one aminoacid. In other words, the codon ACG codes for the amino acid threonine, and only threonine.
4. **The genetic code is degenerate.** In contrast, each amino acid can be specified by more than one codon.
5. **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

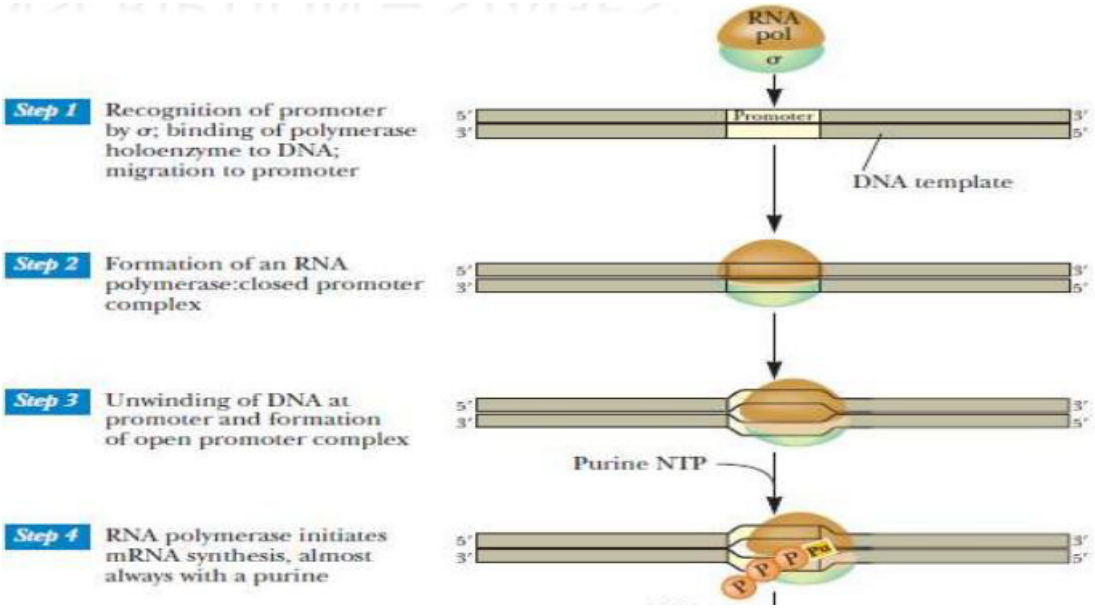
Transcription: Synthesis of RNA using DNA as a template

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

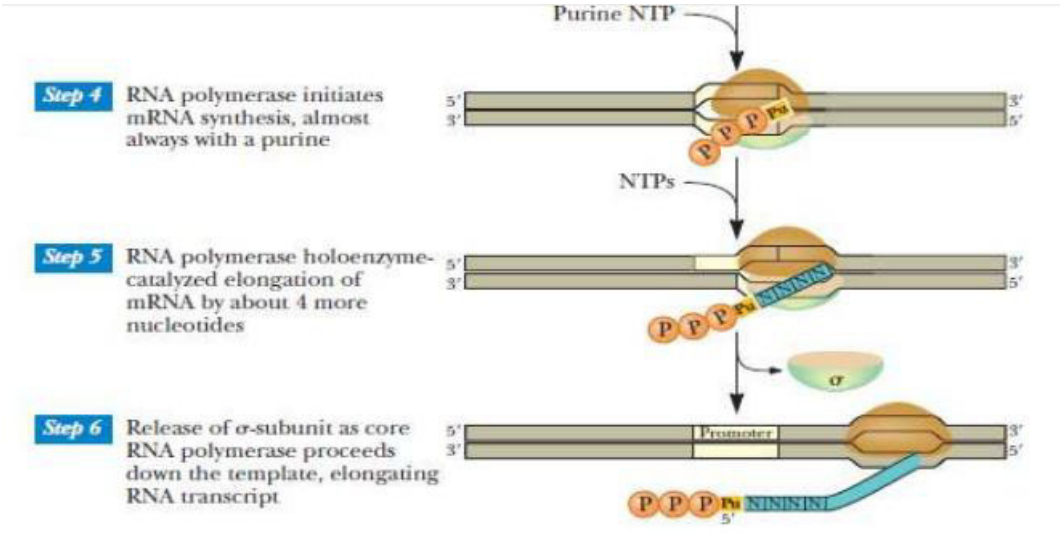
Steps to Transcription

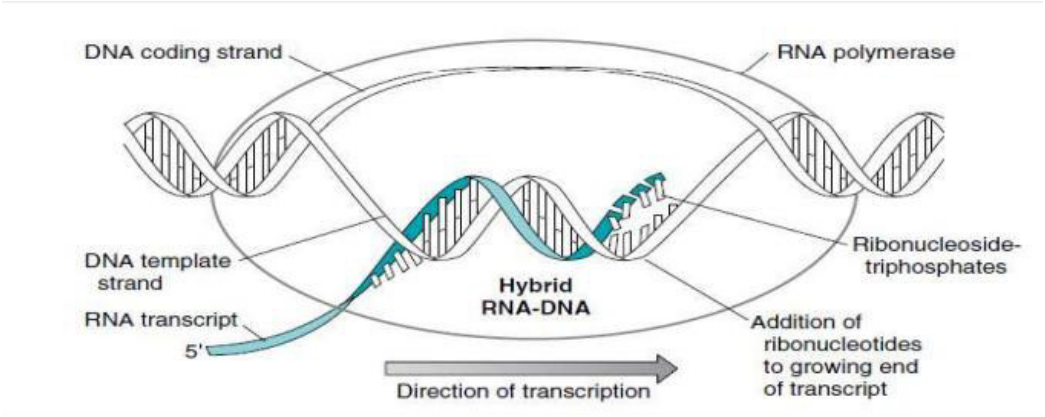
INITIATION
ELONGATION
TERMINATION

INITIATION:

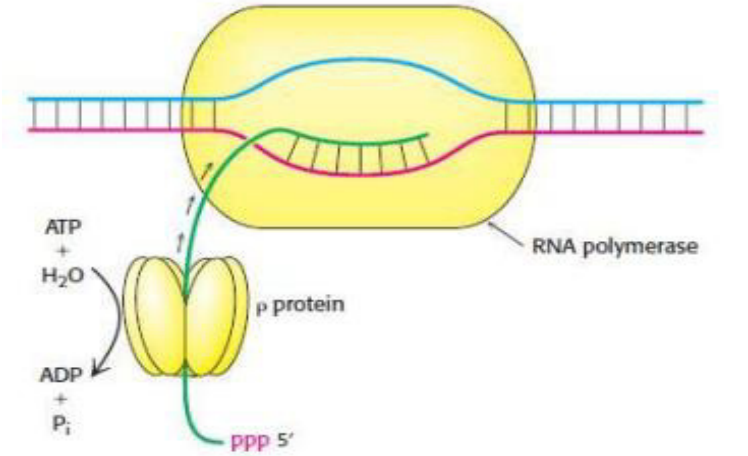


ELONGATION

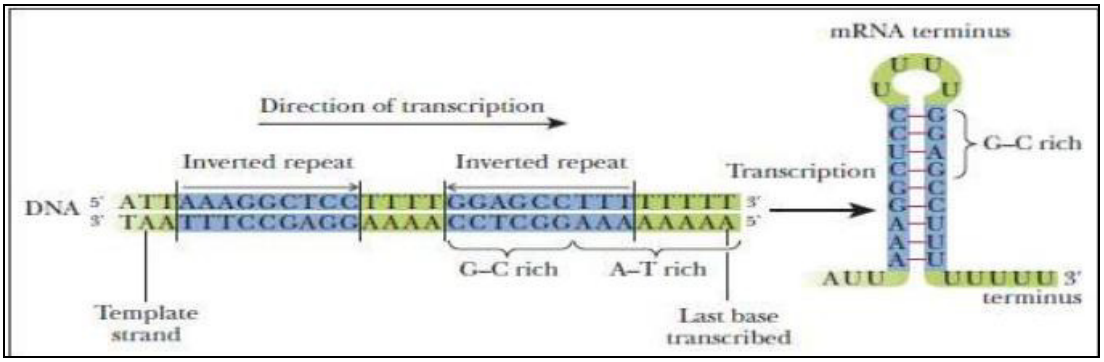




TERMINATION
Two Mechanisms
Rho (p) dependent and Rho independent



Rho dependent



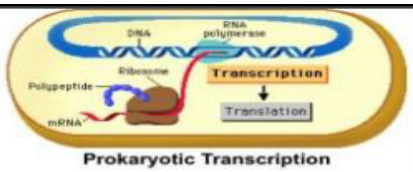
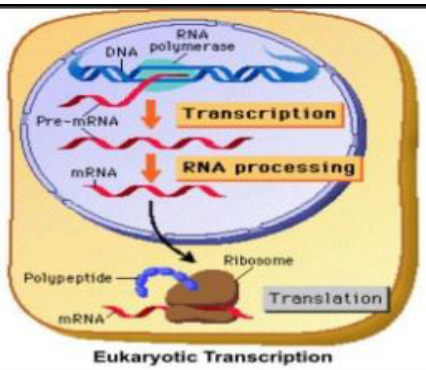
Rho independent

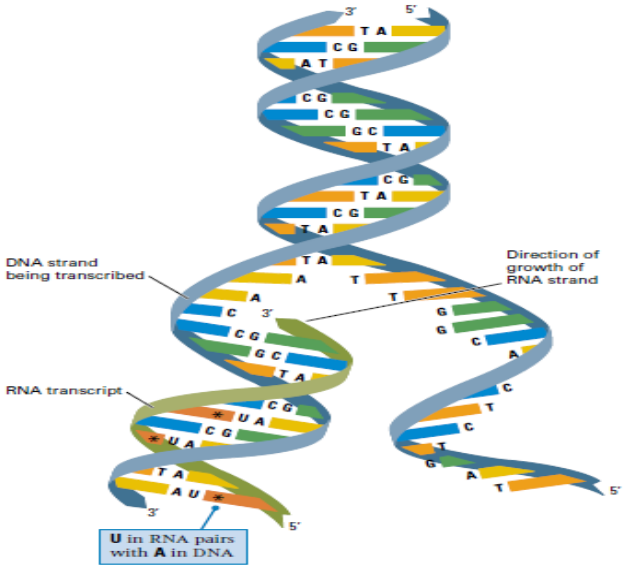
Main differences in prokaryotic and eukaryotic transcription

RNA Polymerase

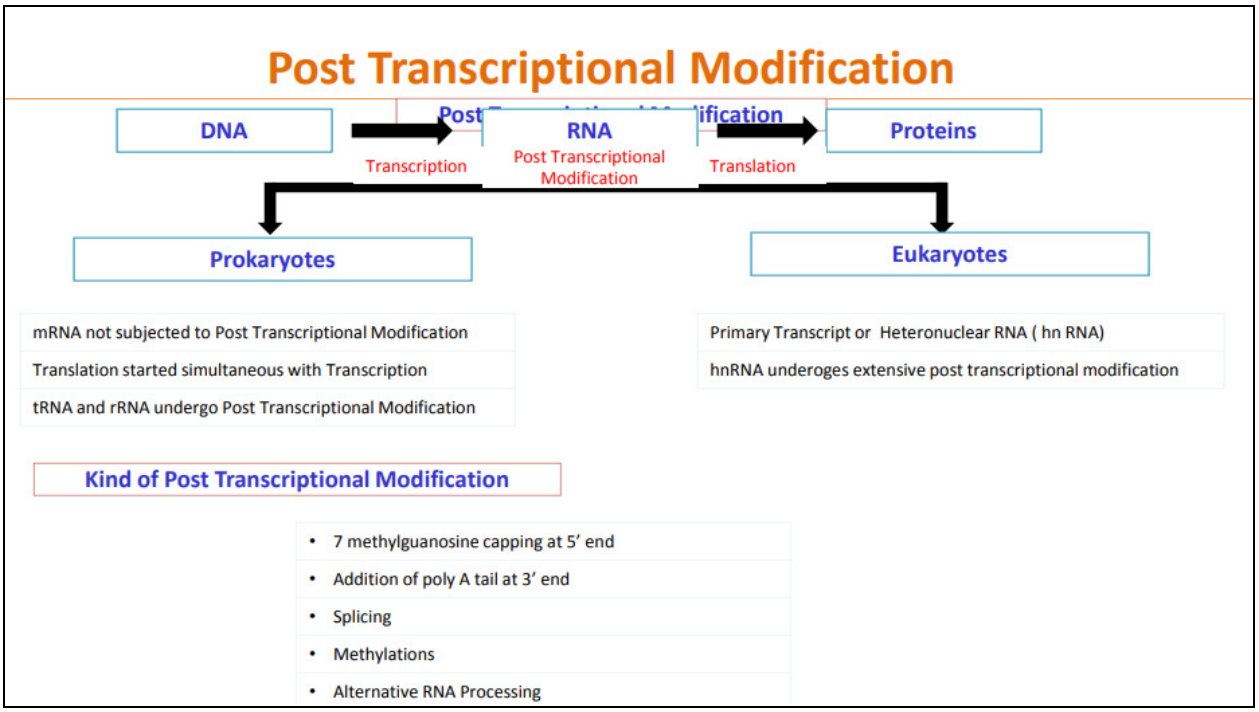
Promoter sites Enhancers,

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

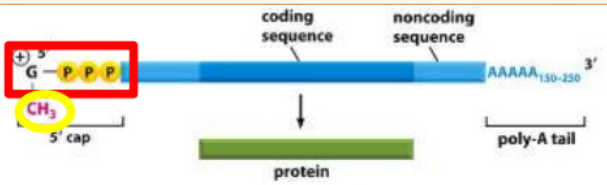


Post transcriptional modification both in prokaryotes and Eukaryotes

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

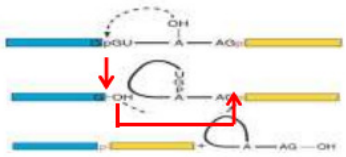
RNA Processing

- 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

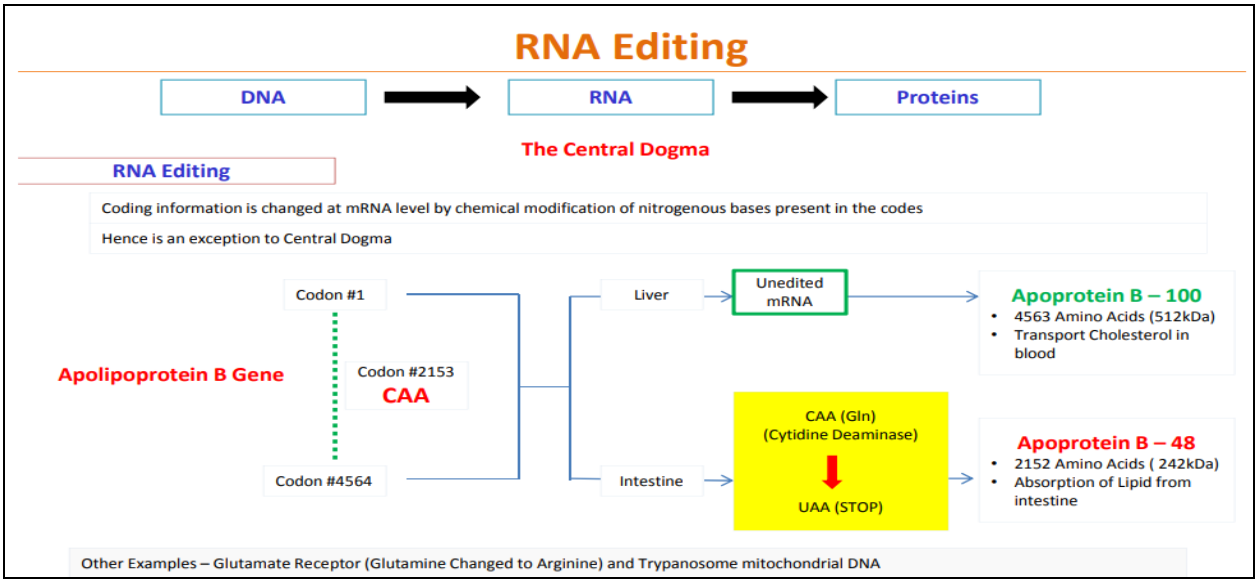
Alternative 5′ Donor — 5′ Donor Site of certain exons is changed

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.

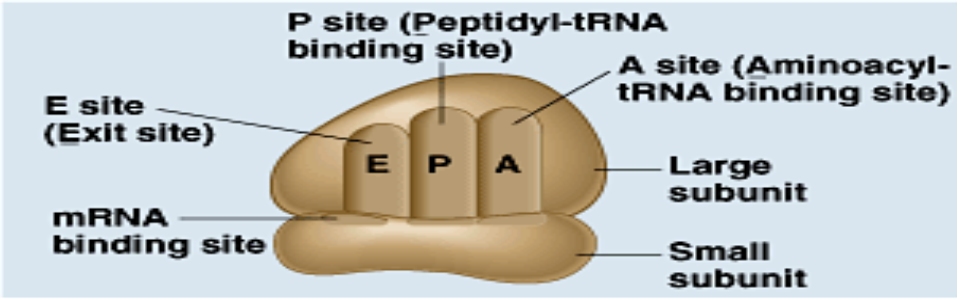
Ribosomes: the site of protein synthesis. They are large complexes of protein and rRNA. In human, they consist of two subunits, one large (60S) and one small (40S).

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.



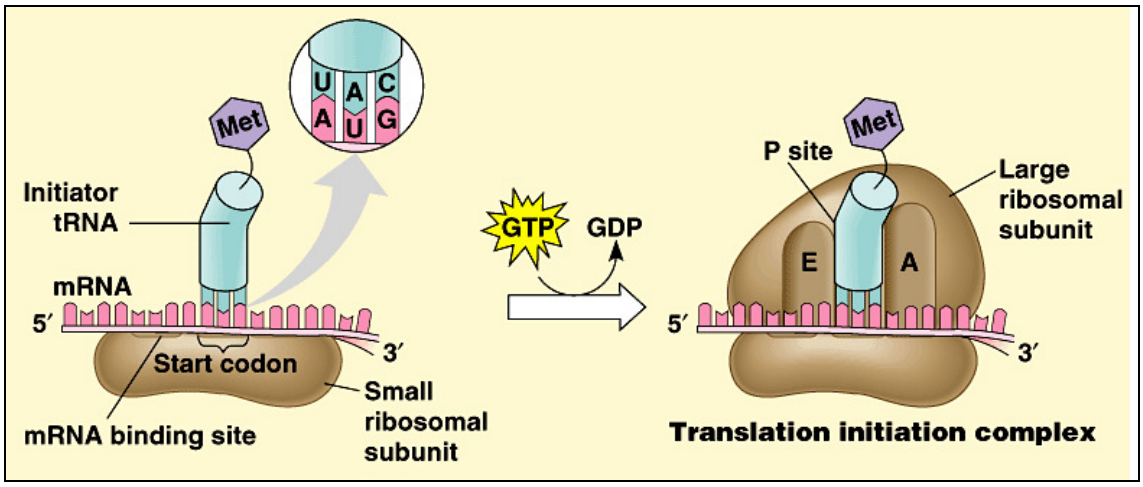
(b) Schematic model showing binding sites

Steps:

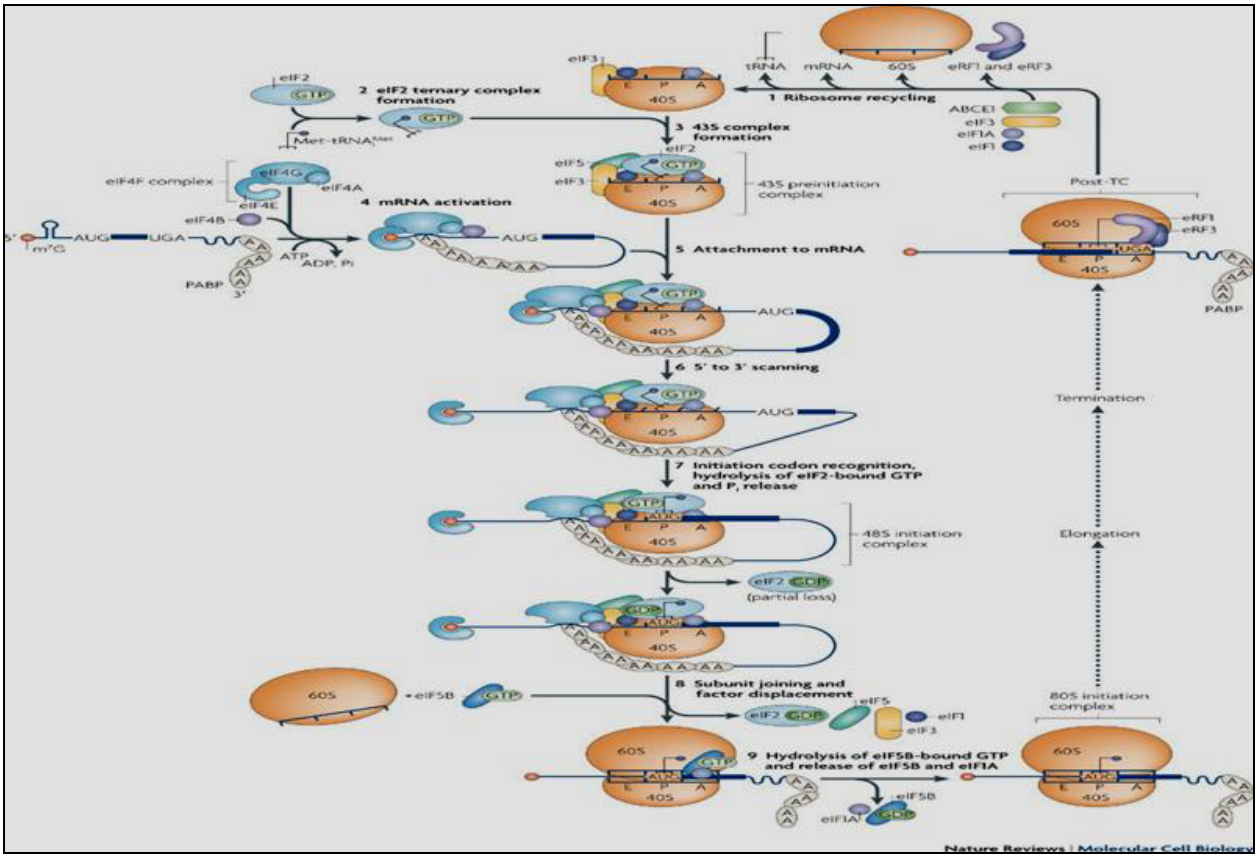
***Initiation*:**

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

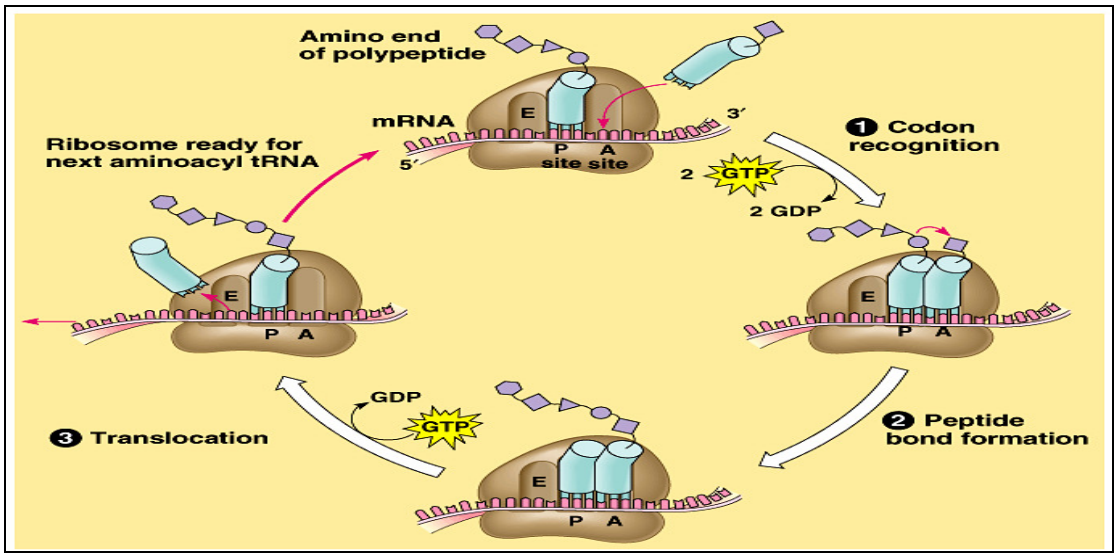
Inactive 80S ribosomal subunit is separated 40S and 60S ribosomal subunits, and result in the formation of an 80S ribosomal initiation complex, in which Met-tRNAMeti is base paired with the initiation codonin the ribosomal P-site and which is competent to start the translation elongation stage. These 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.

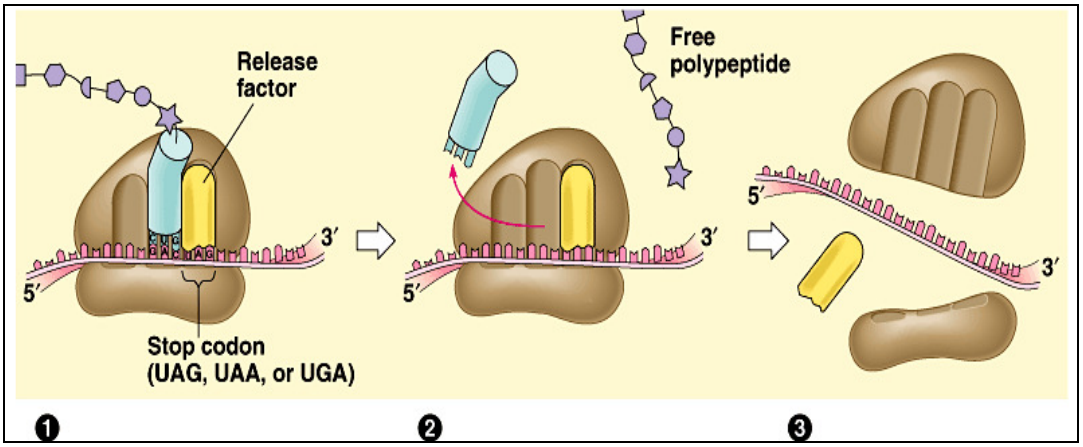


Repetitive cycle of elongation

\

***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

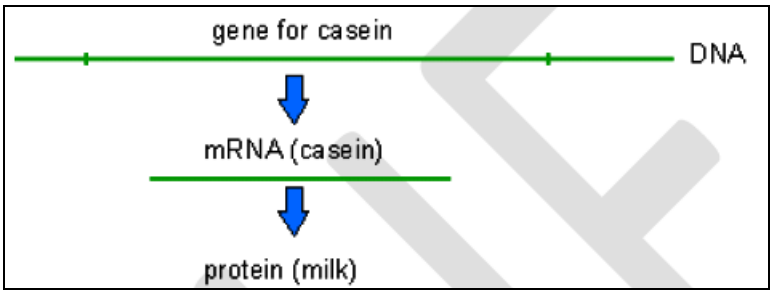
Ribonucleases are enzymes that destroy mRNA.

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.

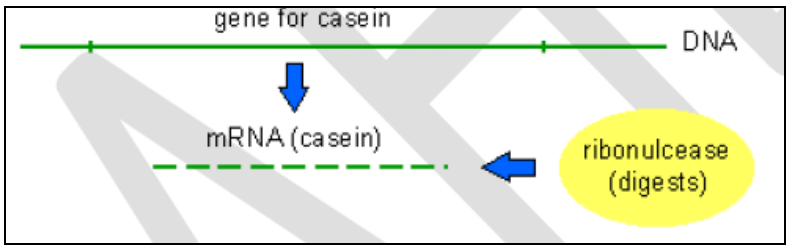
Hormones stabilize certain mRNA transcripts.

Example:

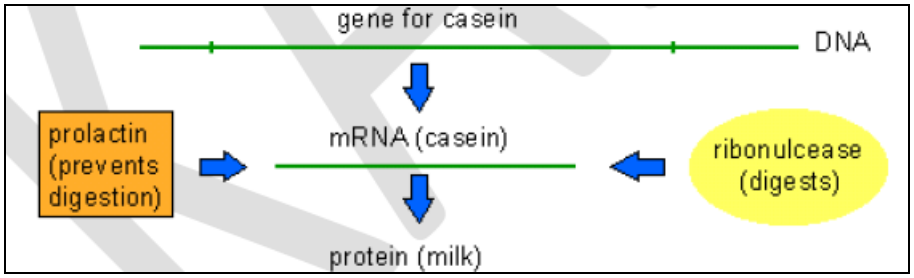
Prolactin is a hormone that promotes milk production because it affects the length of time the mRNA for casein (a major milk protein) is available.



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

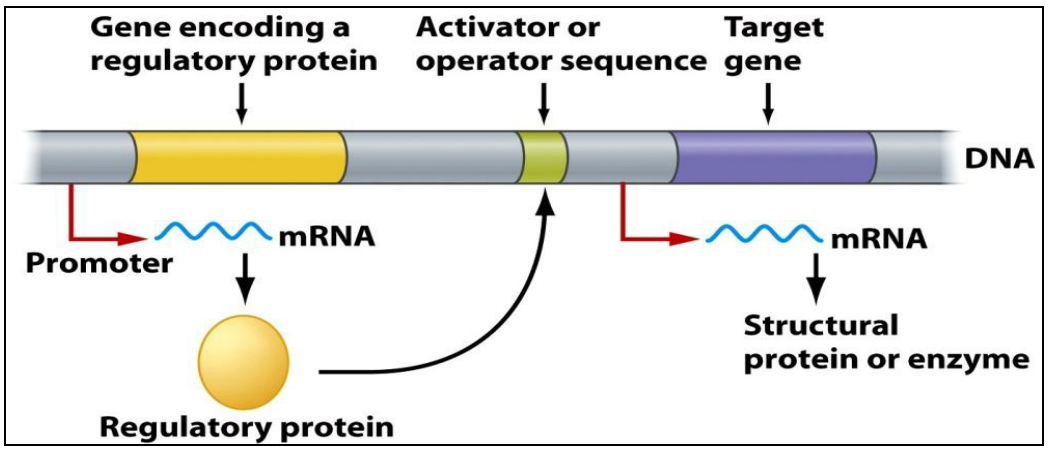
- Microbes respond to changing environment
- Alter growth rate
- Alter proteins produced
- Must sense their environment
- Receptors on cell surface
- Must transmit information to chromosome
- Alter gene expression
- Change transcription rate
- Change translation rate

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

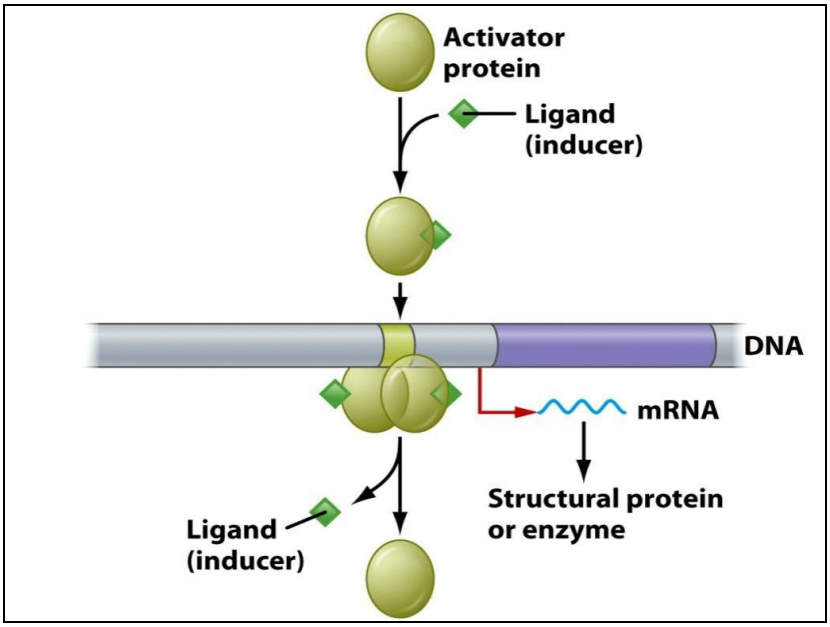
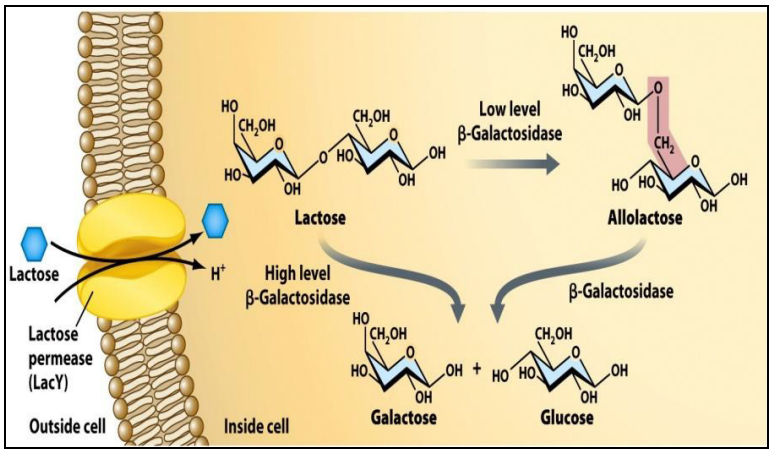


Fig: Operonic regulation

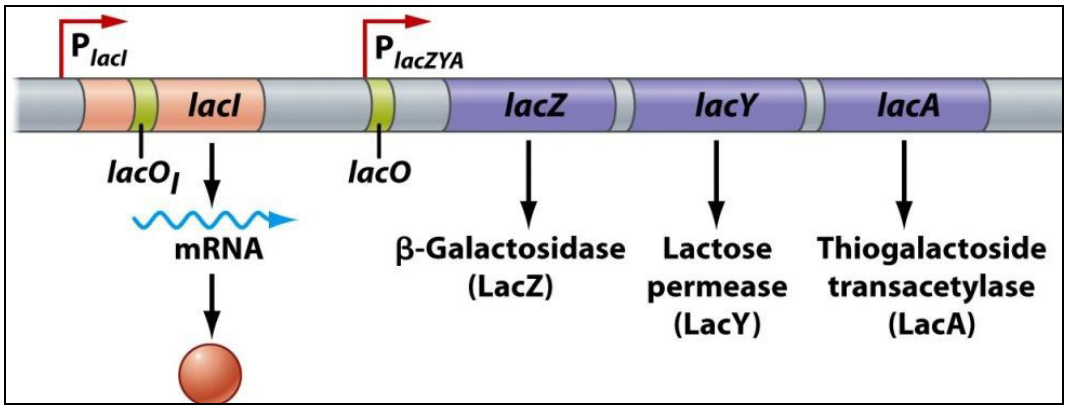
The *E. coli lac* Operon

- 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



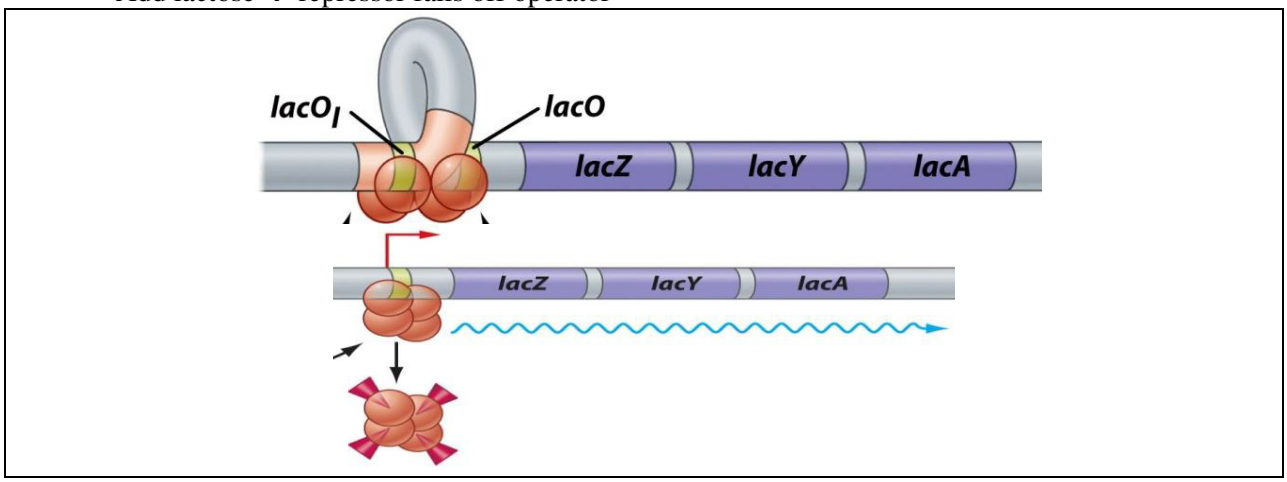
Lac operon regulation

- 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

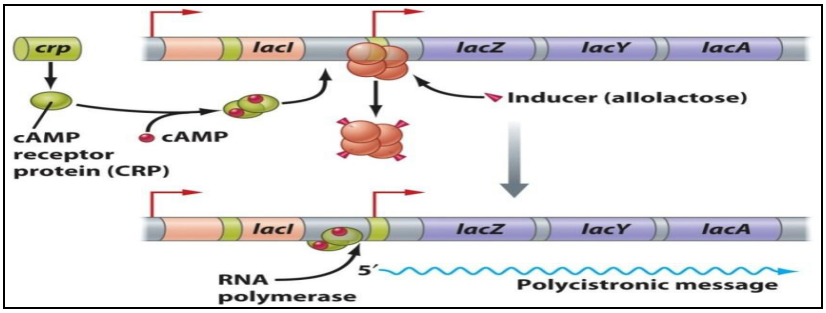
- The *lacZ* gene encodes b-galactosidase
- The *lacY* gene encodes lactose permease
- Need both proteins to digest lactose
- Repressor protein LacI blocks transcription
- Repressor binds to operator
- Blocks s factor from binding promoter
- Repressor responds to presence of lactose
- Binds inducer (allolactose) 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



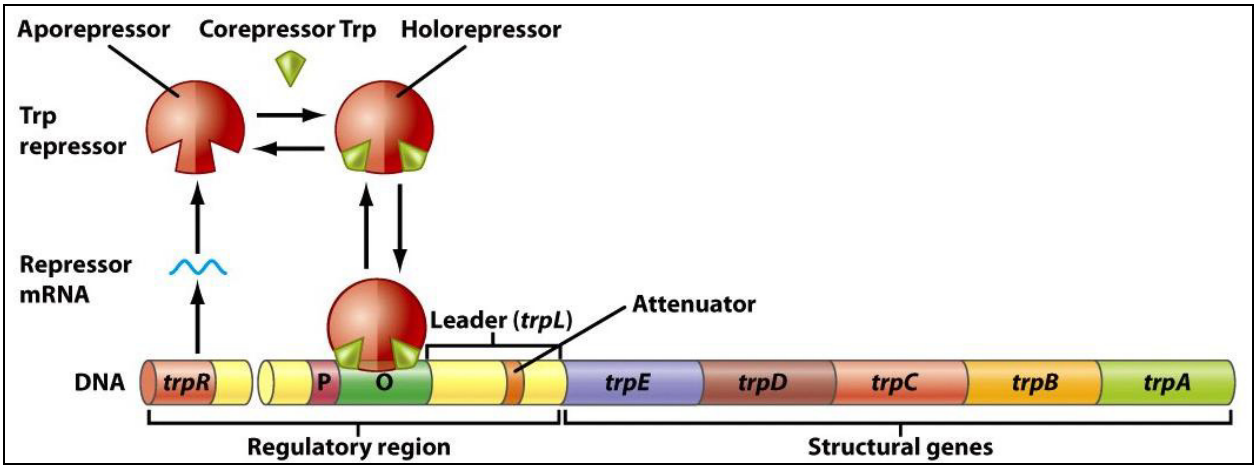
Catabolite repression

Catabolite Repression

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

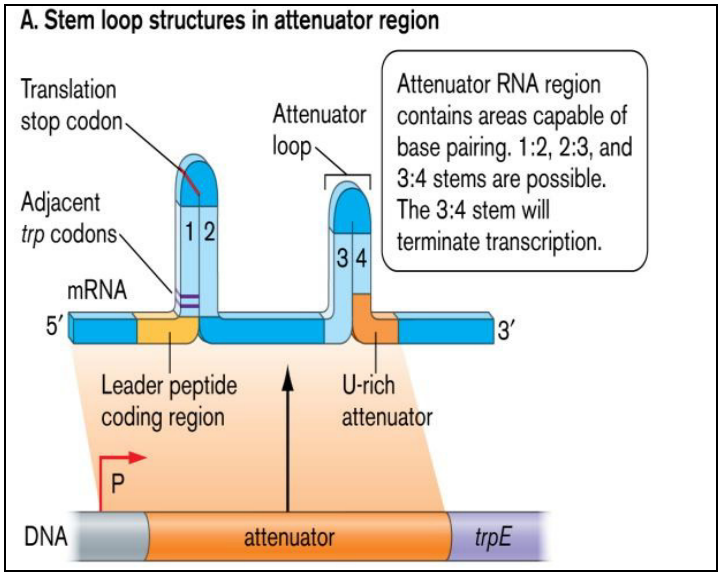
Trp operon: Repression and Attenuation

- *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

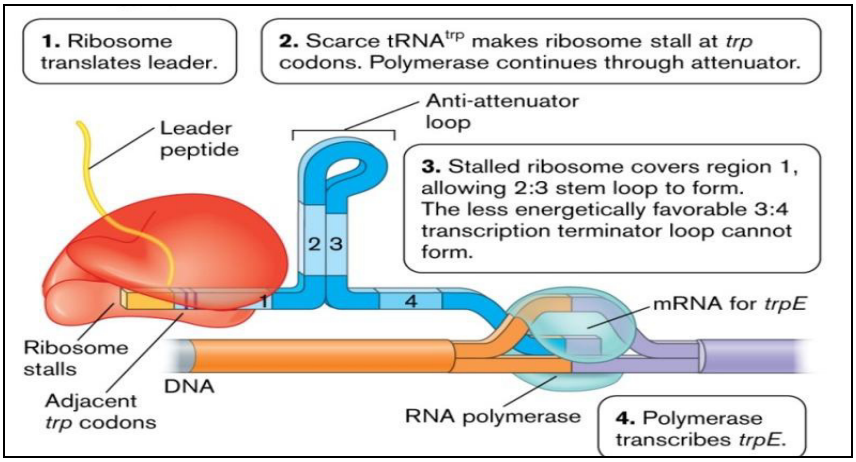


Transcriptions repressed

***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.



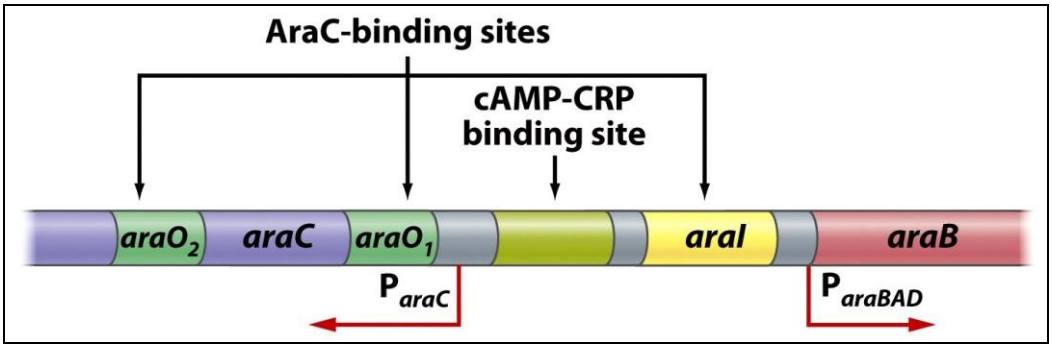
Transcriptional Attenuation Mechanism of the *trp* Operon



Attenuation control in Trp Operon

Arabinose operon

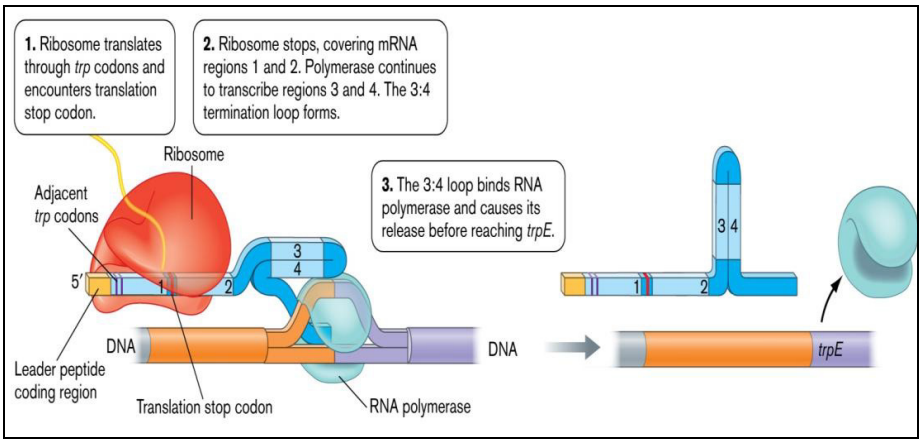
- Regulation by dual role regulatory protein AraC
- "AraC" acts as repressor to block transcription (no arabinose)
- Acts also as activator when bound to "arabinose" (the inducer)
- Operators O1, O2 and araI control AraC and AraBAD proteins expression



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 109 base pairs/cell = 6,000,000 kb pairs . compare to 4700 kb pairs/*E. coli*, a very sophisticated bacterium. Human DNA is more than 1000x bigger. If all human DNA coded for proteins, would have enough for roughly 5 million different proteins

But currently only know ~ 3000 human proteins, and estimates as to how many we truly have range from

10,000 to 100,000. In fact, less than 5% of human DNA codes for protein.

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 "cutand-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.

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.

Unit:II

Syllabus

Mendel'sLaws:Dominance,Segregation,independent,assortment,deviation from Mendelian inheritance,Rediscovery of Mendel's principle,chromosome theory of inheritance:Allele, multiple alleles,pseudoallele,complementation test,Extension of Mendelian genetics:Allelic interaction,concept of dominace,recessiveness,Incomplete dominace and co –dominance.Epistasis,penetrance and expressivity

MULTIPLE ALLELES WITH EXAMPLE:

Multiple alleles is a type of non-Mendelian inheritance pattern that involves more than just the typical two alleles that usually code for a certain characteristic in a species. With multiple alleles, that means there are more than two phenotypes available depending on the dominant or recessive alleles that are available in the trait and the dominance pattern the individual alleles follow when combined together.

Gregor Mendel only studied traits in his pea plants that showed simple or complete dominance and had only two alleles that could contribute to any one trait the plant showed. It wasn't until later that it was discovered that some traits can have more than two alleles that code for their phenotypes. This allowed many more phenotypes to be visible for any given trait while still following Mendel's Laws of Inheritance.

Most of the times, when multiple alleles come into play for a trait, there is a mix of types of dominance patterns that occur. Sometimes, one of the alleles is completely recessive to the others and will be masked by any of those that are dominant to it. Other alleles may be co-dominant together and show their traits equally in the phenotype of the individual.

There are also some cases where some alleles exhibit incomplete dominance when put together in the genotype. An individual with this type of inheritance connected to its multiple alleles will show a blended phenotype that mixes both of the alleles' traits together.

Examples of Multiple Alleles

The human ABO blood type is a good example of multiple alleles. Humans can have red blood cells that are of type A (I^A), type B (I^B), or type O (i). These three different alleles can be combined in different ways following Mendel's Laws of Inheritance. The resulting genotypes make either type A, type B, type AB, or type O blood.

Type A blood is a combination of either two A alleles ($I^A I^A$) or one A allele and one O allele ($I^A i$). Similarly, type B blood is coded for by either two B alleles ($I^B I^B$) or one B allele and one O allele ($I^B i$). Type O blood can only be obtained with two recessive O alleles (ii). These are all examples of simple or complete dominance.

Type AB blood is an example of co-dominance. The A allele and the B allele are equal in their dominance and will be expressed equally if they are paired together into the genotype $I^A I^B$. Neither the A allele or the B allele is dominant over each other, so each type is expressed equally in the phenotype giving the human an AB blood type.

EPISTASIS:

Definition

Epistasis is the interaction between genes that influences a phenotype. Genes can either mask each other so that one is considered "dominant" or they can combine to produce a new trait. It is the conditional relationship between two genes that can determine a single phenotype of some traits. At each locus are two alleles that dictate phenotypes.

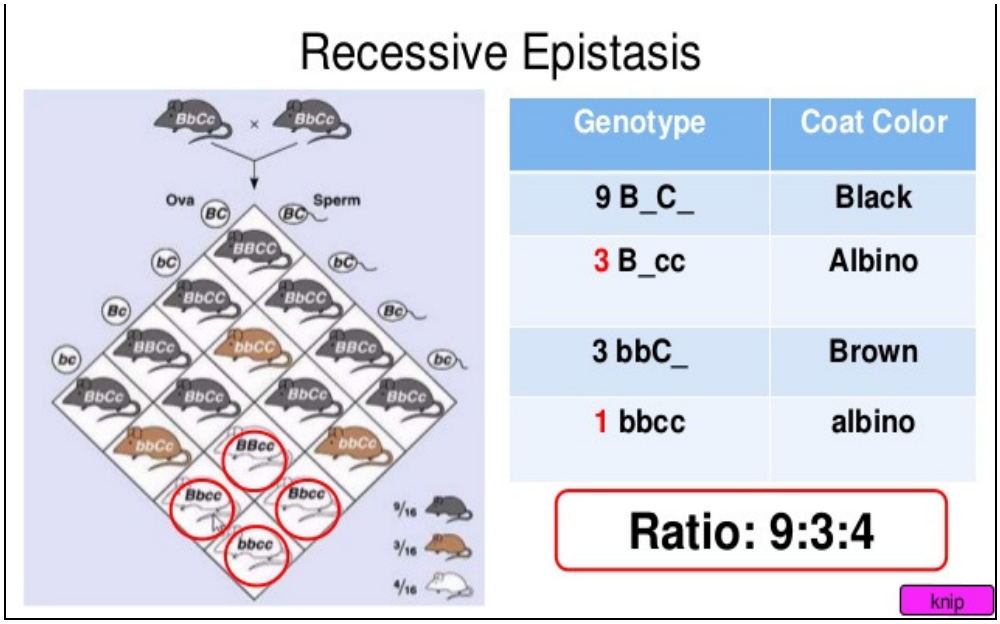
They can affect one another in such a way that, regardless of the allele of one gene, it is recessive to one dominant allele of the other. An alternative expression to epistasis involves a ratio chart or table. With two genes there are four total alleles, meaning there are 16 pairs that can be made. These 16 allele pairs translate to 16 phenotypes. However, not all the combinations are different due to dominant and recessive characteristics of the dominant and recessive alleles. With four alleles, a 4×4 chart can be used as a visual representation of the 16 different allele combinations.

Types of Epistasis:

There are six common types of epistasis gene interactions: **dominant, dominant inhibitory, duplicate dominant, duplicate recessive, polymeric gene interaction, and recessive**. When a dominant allele masks the expression of both dominant and recessive alleles at another locus, it is referred to as **Dominant epistasis** or simple epistasis. **Example :** *Cucurbita pepo* (Summar squash)

When it is a recessive allele that masks the expression, it is called **Recessive epistasis**. Some genes can also mask other genes by suppression. This is referred to as dominant inhibitory

or suppression epistasis because the gene is acting as a suppressor, or a factor that prevents the expression of another allele. **Example**: Coat color of mice.

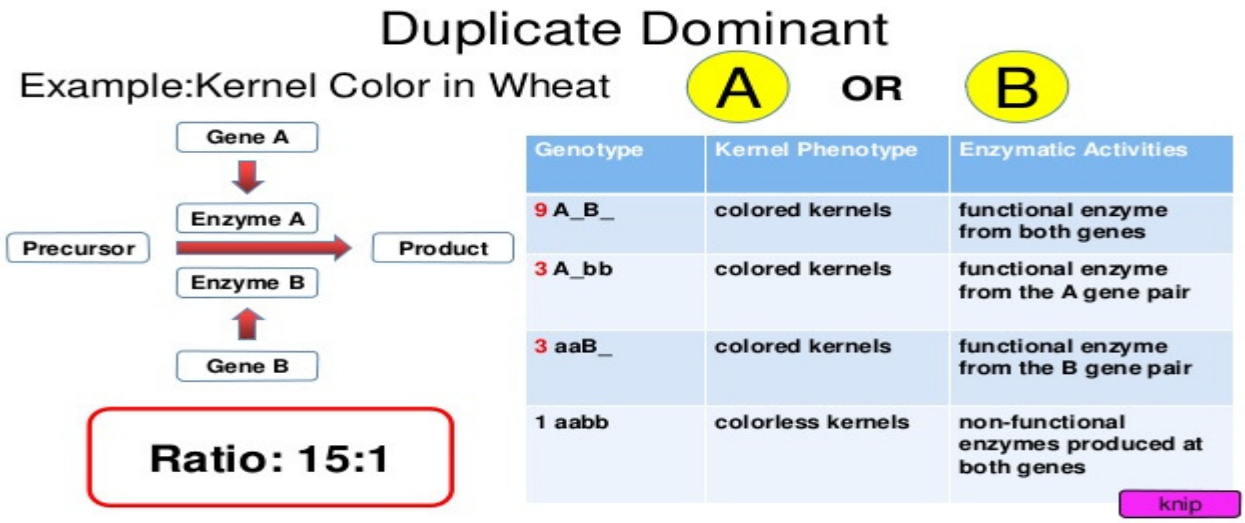


Duplicate types of epistasis depend on two loci. When there is a dominant allele masking the expression of recessive alleles at two loci, this is known as **duplicate dominant epistasis** or duplicate gene action. When there is a recessive allele masking the expression of dominant alleles at two loci, this is known as **duplicate recessive epistasis.**

It is also known as complementary gene action because both genes are required in order for the correct phenotype to be present.

Example: 1.Kernel color of the wheat for duplicate dominant.

2. Production of Anthocyanin for duplicate recessive.



Dominant Recessive Epistasis:

Certain genes have the ability to suppress the expression of a gene at a second locus of chromosomes is called Dominant Recessive Epistasis.

Example: Malvidin production in *Primula* plant.

GENE & ALLELE : DEFINITION:

A gene is the basic physical and functional unit of heredity. Genes are made up of DNA. Some genes act as instructions to make molecules called proteins. However, many genes do not code for proteins. In humans, genes vary in size from a few hundred DNA bases to more than 2 million bases. The Human Genome Project estimated that humans have between 20,000 and 25,000 genes.

Every person has two copies of each gene, one inherited from each parent. Most genes are the same in all people, but a small number of genes (less than 1 percent of the total) are slightly different between people. **Alleles** are forms of the same gene with small differences in their sequence of DNA bases. These small differences contribute to each person's unique physical features.

PSEUDOALLELE:

Pseudoallelelism is a state in which two genes with similar functions are located so close to one another on a chromosome that they are genetically linked. This means that the two genes (pseudoalleles) are nearly always inherited together.

Since the two genes have related functions, they may appear to act as a single gene. In rare cases, the two linked pseudoalleles can be separated, or recombined. One hypothesis is that pseudoalleles are formed as a result of gene duplication events, and the duplicated genes can undergo gene evolution to develop new functions.

Characteristic of pseudoalleles:

1. These are closely linked allele within which crossing over occur.
2. They affect the same character.

Example: Red eye colour of *Drosophila* has different mutants like white and apricot. They affect pigmentation i.e., affect the same character. So, they are allelic. They can undergo recombination, i.e., they are nonallelic.

COMPLIMENTAION TEST:

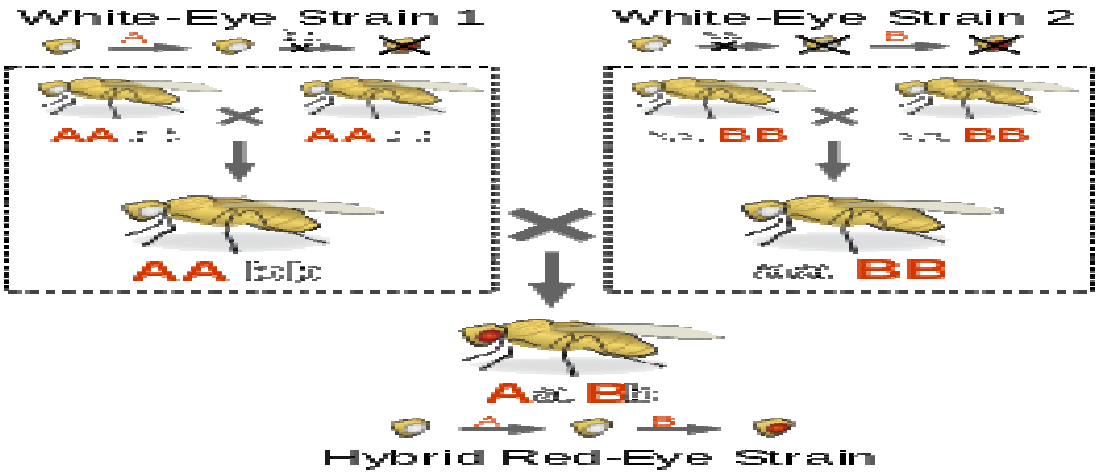
Complementation test, also called **cis-trans test**, in genetics, test for determining whether two mutations associated with a specific phenotype represent two different forms of the same gene (alleles) or are variations of two different genes. The complementation test is relevant for recessive traits (traits normally not present in the phenotype due to masking by a dominant allele).

In instances when two parent organisms each carry two mutant genes in a homozygous recessive state, causing the recessive trait to be expressed, the complementation test can determine whether the recessive trait will be expressed in the next generation.

When two mutations occur in different genes, they are said to be complementary, because the heterozygote condition rescues the function otherwise lost in the homozygous recessive state. Hence, the term *complementation test* is used to describe the process to test for gene function in recessive allelism.

The alternative name *cis-trans test* describes the two central components of the test. The terms *cis* and *trans* refer to the relationship of the two mutations, with *cis* used to describe mutations occurring on the same chromosome and *trans* used to describe mutations occurring on different chromosomes. The cis portion of the complementation test

essentially acts as a control and involves creating heterozygotes (one mutated chromosome and one wild-type, or normal, chromosome) such that one parent bears both mutations. In the cis test, a functional protein is always produced regardless of whether both mutations are on the same gene or on different genes. The trans test involves creating heterozygotes with different mutations from different parents. In this case a functional protein is produced only if the mutations are on different genes.Example includes: Eye colour of *Drosophila.*



TEST CROSS:

Definition:

A test cross is a way to explore the genotpye of an organism. Early use of the test cross was as an experimental mating test used to determine what alleles are present in the genotype. An organism's genetic makeup is called its genotype, and it reflects all of the alleles, or forms of the gene, that are carried by the organism. Consequently, a test cross can help determine whether a dominant phenotype is homozygous or heterozygous for a specific allele.

POLYGENIC INHERITANCE:

Polygenic inheritance refers to a single characteristic that is controlled by more than two genes (also called **Multifactorial inheritance**). It describes the inheritance of traits that are determined by more than one gene. These genes, called **polygenes**, produce specific traits when they are expressed together. Polygenic inheritance differs from Mendelian inheritance patterns, where traits are determined by a single gene. Polygenic traits have many possible phenotypes (physical characteristics) that are determined by interactions among several alleles.

Examples of polygenic inheritance in humans include traits such as skin color, eye color, hair color, body shape, height, and weight.

INCOMPLETE DOMINACE & CO-DOMINANCE:

When one allele for a specific trait does not completely dominate over the other allele, and therefore the phenotype produced is the complete mixture of both dominant and recessive allele is known as **Incomplete Dominance**.

While in **Co-Dominance** both the allele for a specific trait are equally expressed. The resultant phenotype will express both the character equally of both participating alleles.

Penetrance and Expressivity:

Penetrance refers to the probability of a gene or trait being expressed. In some cases, despite the presence of a dominant allele, a phenotype may not be present. One example of this is polydactyly in humans (extra fingers and/or toes). A dominant allele produces polydactyly in humans but not all humans with the allele display the extra digits. “Complete” penetrance means the gene or genes for a trait are expressed in all the population who have the genes. “Incomplete” or ‘reduced’ penetrance means the genetic trait is expressed in only part of the population

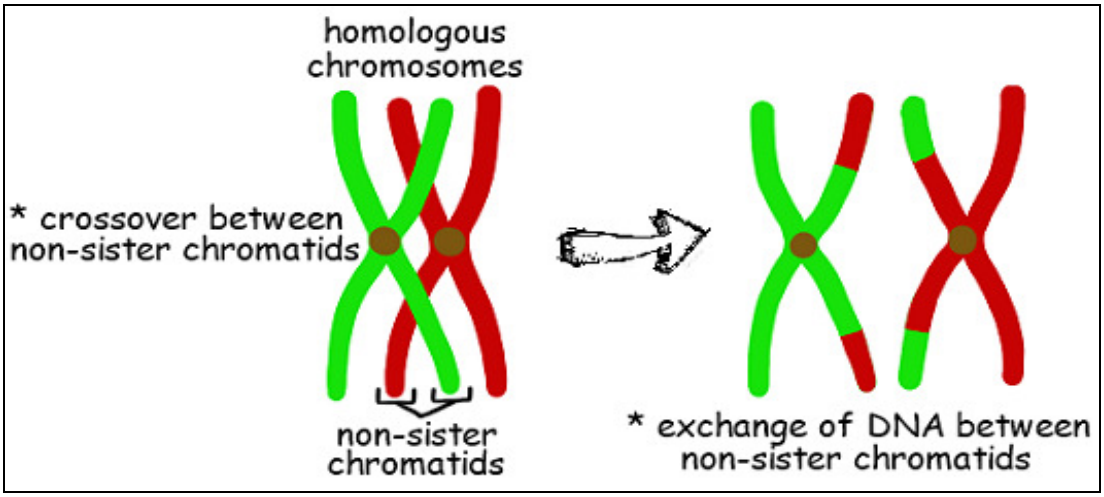
Expressivity on the other hand refers to variation in phenotypic expression when an allele is penetrant. Back to the polydactyly example, an extra digit may occur on one or more appendages. The digit can be full size or just a stub. Hence, this allele has reduced penetrance as well as variable expressivity. Variable expressivity refers to the range of signs and symptoms that can occur in different people with the same genetic condition.

Linked Genes :

Linked genes are genes that are likely to be inherited together because they are physically close to one another on the same chromosome. During meiosis, chromosomes are recombined, resulting in gene swaps between homologous chromosomes. If genes are close together, the chances of being recombined are higher than if they are far away from each other. It is impossible for linked genes to be on different chromosomes.

Cross Over and Gene Mapping:

Crossovers occur when homologous chromosomes are aligned: chromatids from two different chromosomes can exchange segments as in Fig. 3. In Fig. 3a, chromatids from two homologous chromosomes come in contact at an equivalent point along their lengths. In Fig. 3b, the two chromatids have separated after exchanging the segments between the contact point and the tips of the chromatids.



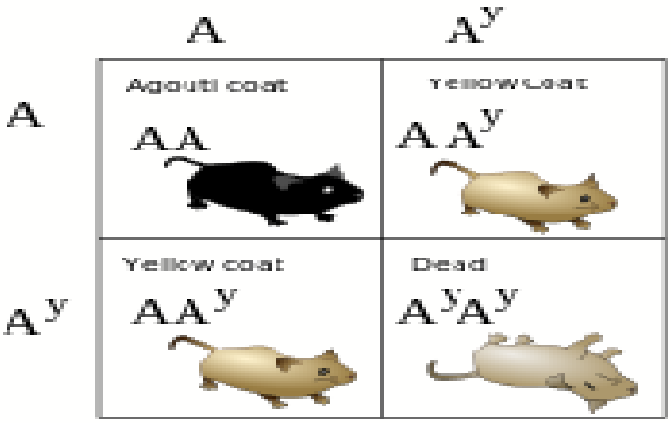
Gene mapping:

Gene mapping is the sequential allocation of loci to a relative position on a chromosome. Genetic maps are species-specific and comprised of genomic markers and/or genes and the genetic distance between each marker. These distances are calculated based on the frequency of chromosome crossovers occurring during meiosis, and not on their physical

location on the chromosome. There are existing dense genetic marker maps available for humans, and the introduction of next-generation sequencing technologies is facilitating increased construction of genetic maps for other species. Genetic maps are a necessary tool for mapping of disease genes or trait loci, a method also commonly known as linkage mapping.

Lethal Alleles:

"Lethal alleles" as the name indicates are alleles that are responsible for the death of an organism, where the gene involved is an essential gene. These essential genes are important for survival. Lethal alleles are produced when mutation in a usual allele distorts the function of an essential gene. This in turn results in a phenotype, which when expressed, is fatal to the organism carrying them. One example is the gene having the alleles expressing the coat color in mouse.



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Question:

Long answer question:

1. Expline Mendel's law
2. Chromosome theory of inheritance
3. Explain multiple alleles with examples
4. Extension of Mendelian genetics
5. Explain Epistasis.
6. Breief about Penetrance and Expressivity.

Short answer question:

1. What is gene ?
2. What is Allele?
3. Explain co-dominance
4. What is incomplete dominance?
5. What is penetrance?

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.

Genetic Recombination in Bacteria:

The following points highlight the three main processes involved in the genetic recombination of bacteria. The processes are: 1. Conjugation 2. Transformation 3. Transduction.

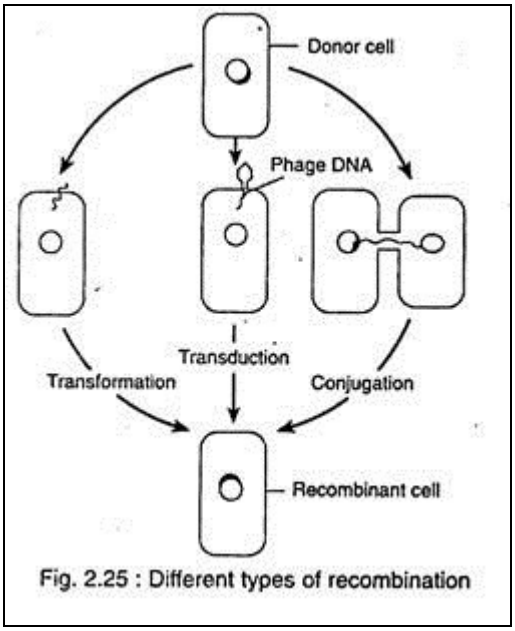


Fig. 2.25 : Different types of recombination

Process # 1. Conjugation:

In this process, the exchange of genetic material takes place through a conjugation tube between the two cells of bacteria. The process was first postulated by Joshua Lederberg and Edward Tatum (1946) in Escherichia coli. They were awarded the Nobel Prize in 1958 for their work on bacterial genetics. Later on, it has also been demonstrated in Salmonella, Vibrio and Pseudomonas there are two mating types of bacteria, one is. male type or F^+ or donor cell, which donates some DNA. The other one is female type or F^- or recipient cell, which receives DNA. Later, after receiving DNA, the recipient cell may behave as donor cell i.e., F^+ type. The F-factor is the fertility factor, sex-factor or F-plasmid present in the cell of F^+ i.e., donor cell or male type. The plasmid takes part in conjugation is called episome. In this process, two cells of opposite mating type i.e., F^+ and F^- become temporarily attached with each other by sex pilus (Fig. 2.26). The sex pilus has a hole of 2.5 pm diameter through which DNA can pass from donor to

recipient cell. The F-factor or F-plasmid is a double stranded DNA loop, present in the cytoplasm; apart from the nucleoid. The F-factor contains about 20 genes.

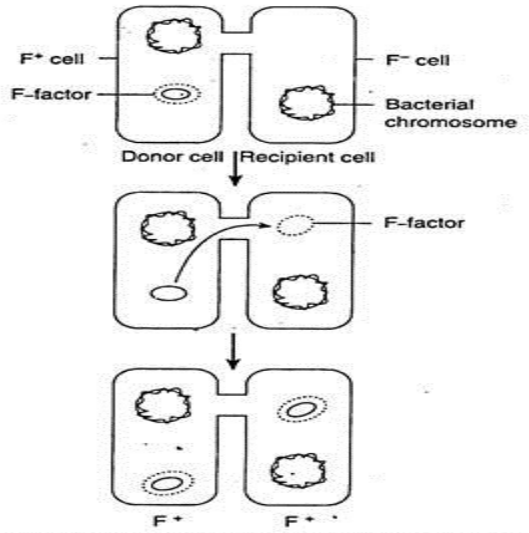


Fig. 2.26 : Recombination by F-factor in conjugation

After the establishment of conjugation tube, the F-factor prepares for replication by the rolling circular mechanism. The two strands of F- factor begin to separate from each other and one of them passes to the recipient i.e., F^- cell.

After reaching in F^- cell, enzymes synthesise a complementary strand that forms a double helix, which bends into a loop. The conversion process is thus completed. In the donor cell i.e., in F^+, a new DNA strand also forms to complement the left over DNA strand of the F-factor.

There is another type of conjugation where passage of nucleoid DNA takes place through conjugation tube. Strains of bacteria are known as Hfr (high frequency of recombination) strain. William Hayes discovered such strains of E. coli in 1950s. The Hfr factor is also called episome. In Hfr strain, the F-factor is attached with the nucleoid DNA i.e., the bacterial chromosome.

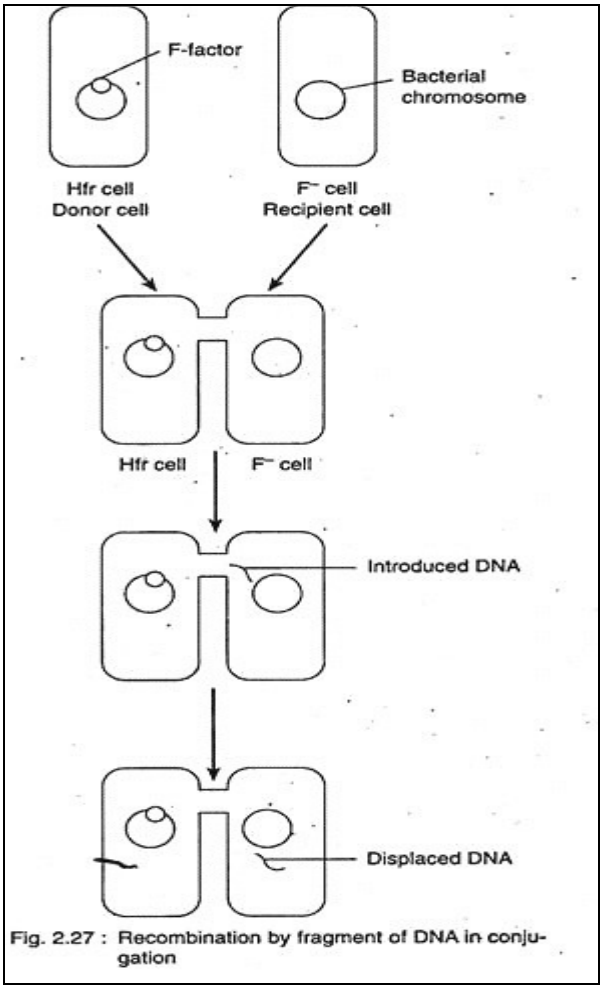


Fig. 2.27 : Recombination by fragment of DNA in conjugation

In this process, Hfr and F⁻ cells become attached with each other by sex pilus (Fig. 2.27). At the point of attachment of F-factor, the bacterial chromosome opens and a copy of one strand is formed by the rolling circular mechanism.

A portion of single stranded DNA then passes into the recipient cell through pilus. Due to agitation in medium, the conjugation tube may not survive for long time because of broken pilus.

Thereby, the total length of transfer DNA may not be able to take entry to the recipient cell.

The behaviour of the transferred DNA depends on the presence and absence of F-factor:

- If F-factor is indeed transferred, then it usually remains detached from the chromosome of recipient cell and enzymes synthesise a complementary DNA strand. The factor then forms a loop and exists as a plasmid, thereby the recipient cell becomes a donor.

- If F-factor remains at the rear end of the transfer DNA during its entry to the recipient cell, the F-factor may not be able to take entry due to broken pilus and only a portion with new genes (Fig. 2.27) takes up the entry. Thereby, the F^- strain remains as recipient one. In F^- strain, genetic recombination takes place between donor fragment and recipient DNA.

- Sometimes, if the F-factor gets free from the Hfr cell and maintains an independent status, then the Hfr cell converts to a F^+ cell. Sometimes during the leaving of F-factor from the bacterial chromosome, it takes a segment of chromosomal DNA. The F-factor with segment of chromosomal DNA is called F'-factor.

Later on, during conjugation, when this F'-factor is transferred, the recipient cell receives some chromosomal DNA from the donor cell. This process is called sexduction. In this process, the recipient cell receives a portion of chromosomal DNA which duplicates with the existing one for a specific function, thereby the recipient cell is a partial diploid
.

Process # 2. Transformation:

It is a kind of genetic recombination where only the carrier of genes, i.e., the DNA molecules of donor cell, pass into the recipient cell through the liquid medium:
It was described by Frederick Griffith (1928), an English bacteriologist. He had done his experiment with laboratory mice and two types of Diplococcus pneumoniae, the pneumonia causing organism. One type has rough (R) non-capsulated cells and another one with smooth (S) capsulated cells. The R-type is non-pathogenic, while the S-type is pathogenic.

The process of transformation is mentioned below (Fig. 2.28):

- When live non-pathogenic (R-type) cells are injected in mice, the mice remain alive.
- When dead pathogenic (S-type) cells are injected in mice, the mice also remain alive.'
- When pathogenic (S-type) cells are injected in mice, they suffer from pneumonia and died.
- When live non-pathogenic (R-type) cells are mixed with dead pathogenic (S-type) cells and are injected in mice, they also suffered from pneumonia and died. On isolation of dead tissue of mice, the smooth (S) qapsulated cells are found on agar.

The above experiment indicates the conversion of R-type to S-type, called transformation.
Later, James L. Alloway (1932), transformed the rough type cells to smooth type, by using the fragments from dead smooth-type cells and confirmed Griffith's work.

Further, Oswald T. Avery, Colin M. MacLeod and Maclyn N. McCarty (1944) also found that DNA isolated from the fragments could induce the transformation. Their experimental result was the first proof of DNA as the genetic material in living organism. The possible mechanism of transformation can be explained (Fig. 2.29).

The transformation takes place in a few cell of the mixed population. It is an important method of genetic recombination. A few donor cells break apart and an explosive release and fragmentation of DNA take place. A fragment of double stranded DNA (10-20 genes) then gets attached with the recipient cell for entry (Fig. 2.29).

During entry one strand of the fragment becomes dissolved by enzyme leaving the second strand, which then passes to the recipient cell through cell wall and cell membrane.

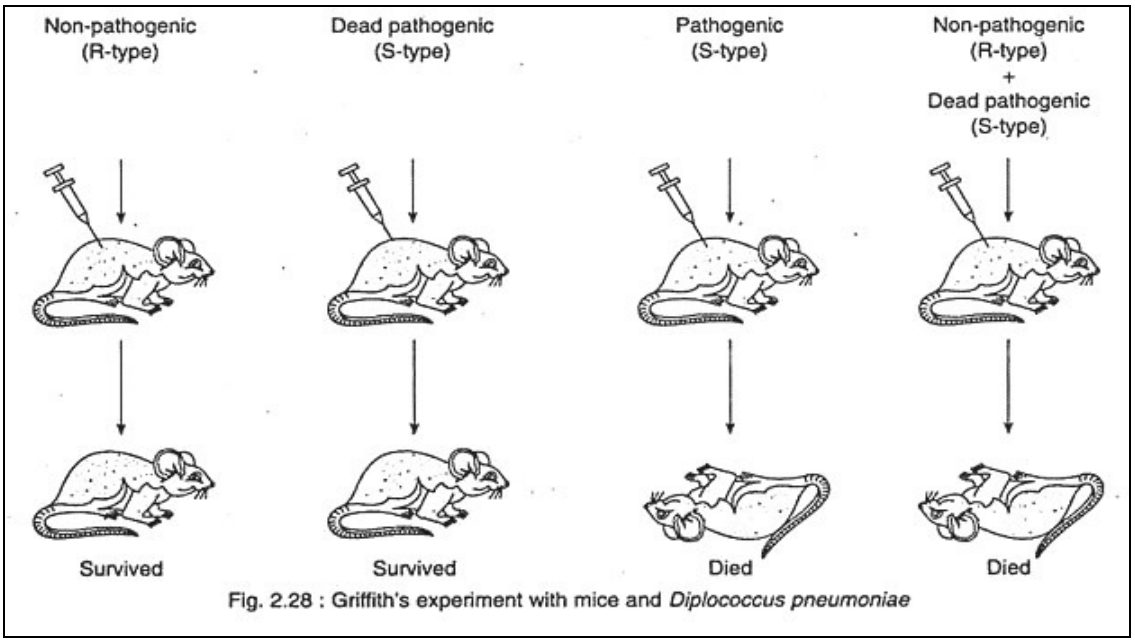


Fig. 2.28 : Griffith's experiment with mice and *Diplococcus pneumoniae*

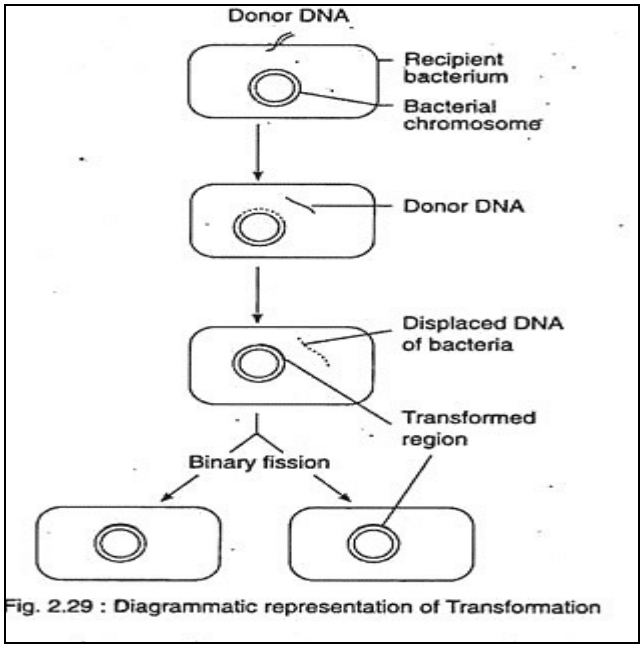


Fig. 2.29 : Diagrammatic representation of Transformation

After entry, a portion of single strand of double stranded DNA of recipient cell gets displaced by enzyme and then replaced by the DNA of donor cell. The displaced DNA is then dissolved by other enzyme. Thus the recipient cell becomes transformed which will display its own as well as the characters of the newly incorporated DNA.

Detailed mechanism of transformation, with especial emphasis on natural and induced competence and DNA uptake:

Thus the transformation takes place by horizontal gene transfer through uptake of free DNA by other bacteria. This transformation takes place either spontaneously by taking DNA from the environment, i.e., Natural, or by forced uptake under laboratory condition i.e., Artificial process.

A. Natural Transformation:

During natural transformation, free naked fragments of double stranded DNA of donor cell become attached to the surface of the recipient cell. The free double stranded ON A molecules may be available in the medium by lysis or natural decay of bacteria (Fig. 2.30).

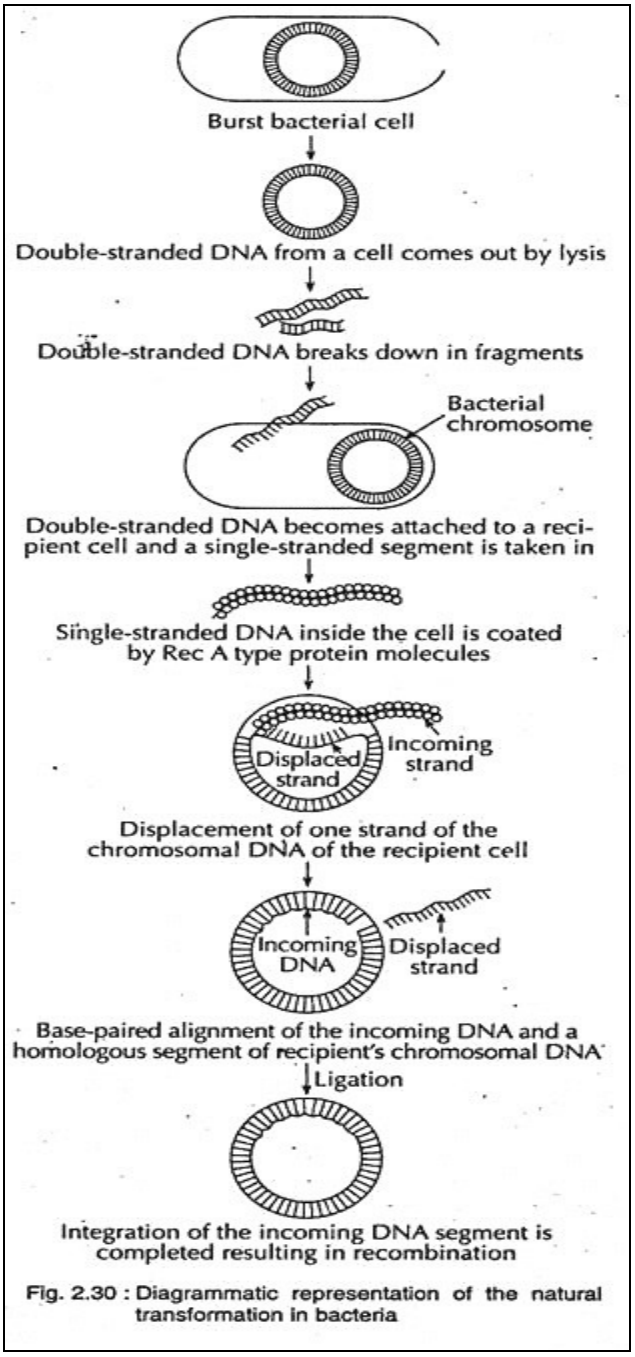


Fig. 2.30 : Diagrammatic representation of the natural transformation in bacteria

After attachment of donor double stranded DNA with the surface of recipient bacterium, one strand is digested by the bacterial nuclease and the remaining one strand is then taken in by an energy-requiring transport system. This uptake of DNA takes place during late logarithmic phase of growth.
During this process, Rec A type of protein plays an important role. The Rec A protein binds with the single stranded DNA and forms a coating around the DNA (Fig. 2.30). The coated single stranded DNA and DNA of recipient cell then move close to each other to get homologous sequence.
After reaching at proper place, the Rec A protein actively displaces one strand of chromosomal DNA of recipient cell. The process requires hydrolysis of ATP to get energy. The incoming DNA strand is then integrated with one strand of bacterial DNA by base pairing and ligation takes place by DNA ligase.
The displaced DNA strand of recipient cell is then digested by cellular DNase activity. Any mismatch between the two strands of new region is corrected by them. Thus the transformation is completed. If the introduced single stranded DNA fails to recombine with the recipient DNA, it is digested by cellular DNase and gets lost.

B. Artificial Transformation:

The E. coli, an ideal material for research is not transformed naturally. Later, it has been discovered that the transformation in E. coli can be done by special physical and chemical treatments. This can be done by exposure of E. coli to high voltage electric field and also by high concentration of $CaCI_2$. Under such condition, the bacterial cells are forced to take up foreign DNA. This type of transformation is called artificial.

During this process, the recipient bacterial cells are able to take up double stranded DNA fragments. Physical or chemical treatment forces the recipient bacterial cell to receive exogenous DNA. The foreign DNA is then integrated with the chromosome by homologous recombination, mediated by Rec A protein. The Rec A protein catalyses the annealing of two DNA segments and exchange of homologous region.

Process # 3. Transduction:

It is a special method of genetic recombination where genetic material is transferred from the donor to the recipient cell through a non- replicating bacteriophage — temperate bacteriophage. This was discovered by Joshua Leaderberg and Nortor Zinder (1952) during their research with Salrv onella typhimurium.

In this process, a small fragment of bacterial DNA is incorporated into an attacking bacteriophage (i.e., virus which infect bacteria) and when this bacteriophage infects a new bacterial cell, it transfers the genetic material into it, and thus genetic recombination takes place.

Transduction are of two types:

- Specialised transduction, and
- Generalized transduction.

Specialised Transduction:

In this process, the bacteriophage gets attached to a bacterial cell wall at the receptor site and the nucleic acid of bacteriophage is transferred into the cytoplasm of the host cell. The phage does not cause the lysis of the host bacterium. In the bacterial cell, the phage nucleic acid codes for the synthesis of specific proteins, the repressor proteins.

The repressor proteins prevent the virus to produce the material require for its replication. In the bacterial cell, the viral DNA may exist as a fragment in the cytoplasm or it may attach itself to the chromosome, known as prophage. The bacterial cell which carries the prophage is called lysogenic and the phenomenon where the phage DNA and bacterium exist together is called lysogeny.
The bacterial cell may remain lysogenic for many generations and during this period the viral DNA replicates many times together with the bacterial chromosome.

However, in course of time, the phage stops the synthesis of repressor proteins in the bacterial cell, and then the synthesis of phage components starts. Now the phage DNA separates from the bacterial chromosome and starts the synthesis of phage proteins.

During this separation, a number of genes of the bacterium get attached to it. These attached genes keep on replicating along with the phage DNA and later on it develops into phage particles, those come out from the bacterial cell by bursting.

When the new phage particle infects a new bacterial cell, the attached bacterial genes present along with phage particle enters in the chromosome of the new bacterium and causes recombination.

Thus the new bacterial cell contains its own genes and several genes from the parent bacterial cell. This type of transduction is known as specialised transduction, which is an extremely rare event.

Generalised Transduction:

This process of transduction is more common than specialized transduction. Here the prophage particle is present in the cytoplasm of the infected bacterial cell. In this process, the phage DNA starts synthesising new phages.

During this process chromosome of bacterial cell gets fragmented and some of the fragments become attached with the DNA of some new phage particle, while others remain with phase DNA.

When the newly formed phage with fragment of bacterial chromosome in its DNA attacks a new bacterium, the gene of the parent bacterium is transferred to the new bacterium and causes recombination. This type of transduction is called generalised transduction. This type of transduction is also rare.

Difference between transformation and transduction

Transformation	Transduction
1. Transfer of genetic material takes place from donor to recipient bacterium through the liquid medium.	Transfer of genetic material takes place from donor to recipient bacterium through a Bacteriophage.
2. The enzyme deoxyribonuclease can completely check the process.	The deoxyribonuclease has no effect on trans-

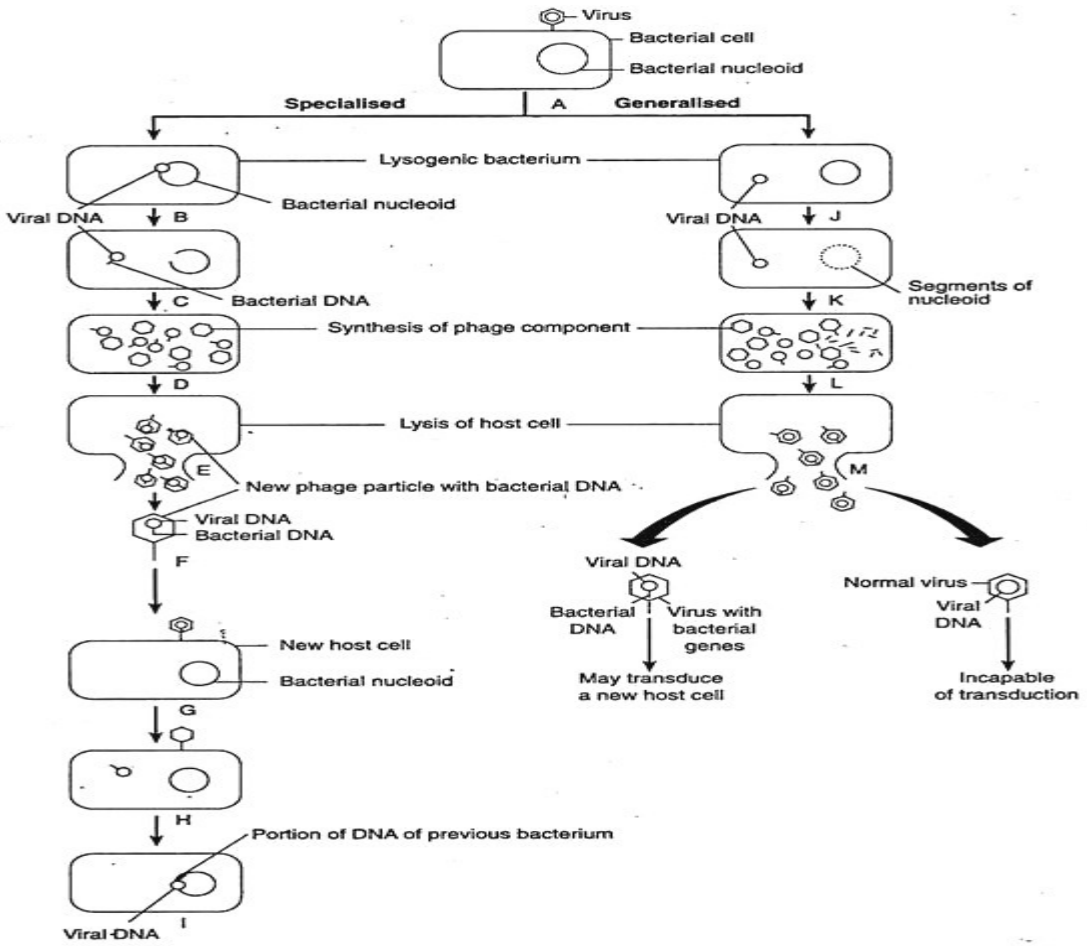


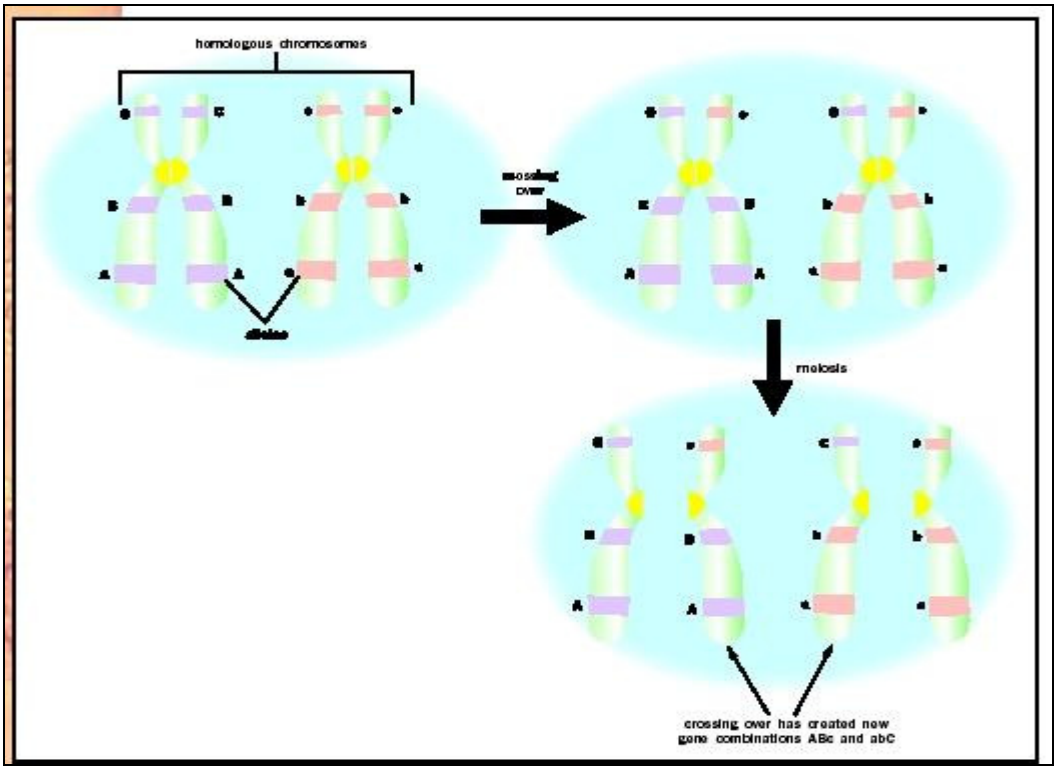
Fig. 2.32 : Diagrammatic representation of Transduction

Linkage and Genetic mapping:

Linkage refers to the presence of two different genes on the same **chromosome** . Two genes that occur on the same chromosome are said to be linked, and those that occur very close together are tightly linked. Study of linkage provides information about the relative position of genes on chromosomes, allowing the construction of chromosome maps.

Linkage in Fruit Flies:

An example of using linkage to explore gene position is provided by inheritance of eye color and body color in fruit flies, both of which are located on the X chromosome. This example begins with purebred (homozygous) parents, one yellow-bodied and red-eyed, the other grey-bodied and white-eyed. They mate to produce all **heterozygous** daughters, who carry the yellow-red combination on one **homologous** chromosome and the grey-white combination on the other. When the heterozygotes create gametes, the eye-color alleles cannot assort independently from the body-color alleles because they are linked. Some crossing over can occur, though. As in humans, male fruit flies carry only one X chromosome, and so will show exactly what alleles are present on their X. When one counts the male offspring, approximately 49.5 percent are yellow-bodied and red-eyed, 49.5

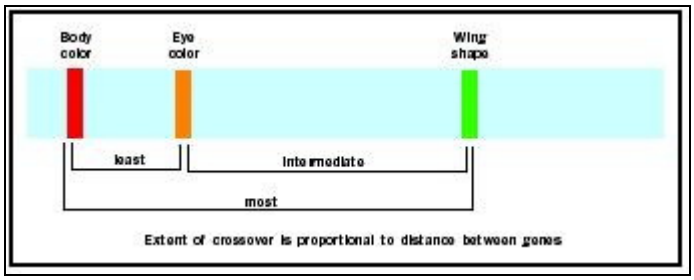


Crossing over between homologous chromosomes creates new combinations of alleles.

percent are grey-bodied and white-eyed, 0.5 percent are yellow-bodied and white-eyed, and 0.5 percent are grey-bodied and red-eyed. This indicates very tight linkage—close proximity—of the two genes.
In this example, the yellow-body allele and the white-eye allele are said to be "out of phase" in the parental strains. The most frequent pair of gamete types are described as "parental types" because they retain the alleles for the two genes as transmitted by the original parent strains. The two gamete types that are less frequent are the "recombinant types," which results only from an exchange or crossover of homologous chromosomes in the interval between the genes.

Gene Mapping

As an undergraduate in 1913, A. H. Sturtevant wrote a brilliant paper that extended linkage analysis into gene mapping. Sturtevant analyzed numerous linkage experiments in the fruit fly, each using two genes. For instance, a similar experiment with body color and wing shape shows many more outof-phase offspring, indicating the wing-shape gene is further from the bodycolor.



Three fruit fly genes on the same chromosome show different levels of separation during crossover, proportional to the distance between them.

Gene than the eye-color gene is. Another experiment showed an intermediate number of out-of-phase offspring for eye color and wing shape. This allowed Sturtevant to reason that the body-color gene and wing shape gene are furthest apart, with eye color in between them.

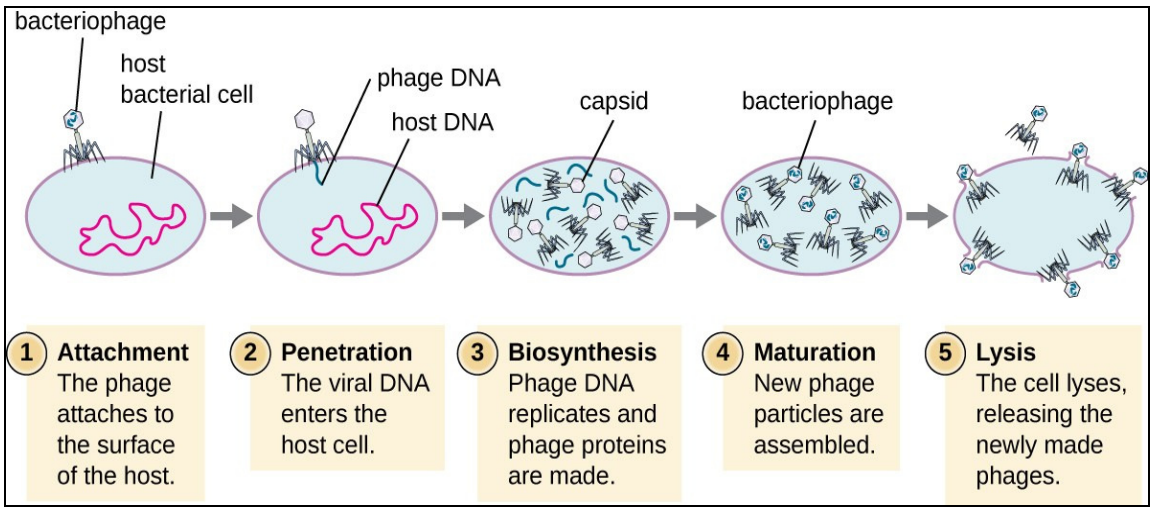
Extension of this technique allowed the distance between genes to be expressed as map units. One map unit is defined as the effective distance needed to obtain a 1 percent recombination between linked alleles. The map unit is also called the centiMorgan (cM), to honor T. H. Morgan, Sturtevant's teacher and one of the founders of chromosomal genetics. Because crossing over is not equally likely between any two points, map units do not correspond directly to number of **nucleotides** along the DNA double helix.
Sturtevant's work helped show that the chromosome is a linear sequence of genes. Gene mapping determines the position and order of genes relative to other genes along the chromosome. A well-marked linkage group extends from markers located at one end of the chromosome to those in the middle, and on to markers located at the other end. The number of linkage groups for an organism is equal to its number of homologous chromosome pairs.

Phage genatics: Replication cycle:

Bacteriophages, **viruses** that infect **bacteria** , are useful in the study of how genes function. The attributes of bacteriophages include their small size and simplicity of genetic organization.
The most intensively studied **bacteriophage** is the phage called lambda. It is an important model system for the latent infection of mammalian cells by **retroviruses** , and it has been widely used for **cloning** purposes. Lambda is the prototype of a group of phages that are able to infect a cell and redirect the cell to become a factory for the production of new virus particles.During infection a phage attaches to a bacterium and inserts its genetic material into the cell. After that a phage usually follows one of two life cycles, lytic (virulent) or lysogenic (temperate). Lytic phages take over the machinery of the cell to make phage components. They then destroy, or lyse, the cell, releasing new phage particles. Lysogenic phages incorporate their nucleic acid into the chromosome of the host cell and replicate with it as a unit without destroying the cell. Under certain conditions lysogenic phages can be induced to follow a lytic cycle.

The Lytic Cycle:

During the **lytic cycle** of virulent phage, the bacteriophage takes over the cell, reproduces new phages, and destroys the cell. T-even phage is a good example of a well-characterized class of virulent phages. There are five stages in the bacteriophage lytic cycle (see Figure 1). **Attachment** is the first stage in the infection process in which the phage interacts with specific bacterial surface receptors (e.g., lipopolysaccharides and OmpC protein on host surfaces). Most phages have a narrow host range and may infect one species of bacteria or one strain within a species. This unique recognition can be exploited for targeted treatment of bacterial infection by phage therapy or for phage typing to identify unique bacterial subspecies or strains. The second stage of infection is entry or **penetration**. This occurs through contraction of the tail sheath, which acts like a hypodermic needle to inject the viral genome through the cell wall and membrane. The phage head and remaining components remain outside the bacteria.

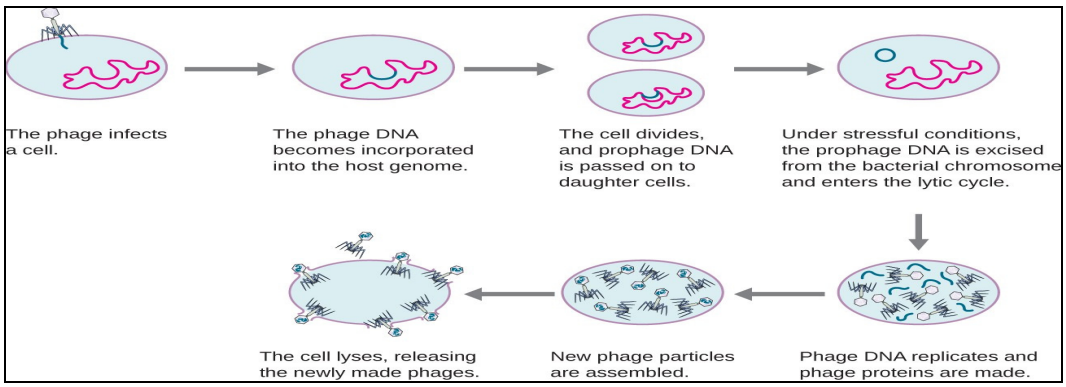


The third stage of infection is **biosynthesis** of new viral components. After entering the host cell, the virus synthesizes virus-encoded endonucleases to degrade the bacterial chromosome. It then hijacks the host cell to replicate, transcribe, and translate the necessary viral components (capsomeres, sheath, base plates, tail fibers, and viral enzymes) for the assembly of new viruses. Polymerase genes are usually expressed early in the cycle, while capsid and tail proteins are expressed later. During the **maturation** phase, new virions are created. To liberate free phages, the bacterial cell wall is disrupted by phage proteins such as holin or lysozyme. The final stage is release. Mature viruses burst out of the host cell in a process called **lysis** and the progeny viruses are liberated into the environment to infect new cells.

The Lysogenic Cycle:

In a **lysogenic cycle**, the phage genome also enters the cell through attachment and penetration. A prime example of a phage with this type of life cycle is the lambda phage. During the lysogenic cycle, instead of killing the host, the phage genome integrates into the bacterial chromosome and becomes part of the host. The integrated phage genome is called a **prophage**. A bacterial host with a prophage is called a **lysogen**. The process in which a bacterium is infected by a temperate phage is called **lysogeny**. It is typical of temperate phages to be latent or inactive within the cell. As the bacterium replicates its chromosome, it also replicates the phage's DNA and passes it on to new daughter cells during reproduction. The presence of the phage may alter the phenotype of the bacterium, since it can bring in extra genes (e.g., toxin genes that can increase bacterial virulence). This change in the host phenotype is called **lysogenic conversion** or **phage conversion**.

Some bacteria, such as *Vibrio cholerae* and *Clostridium botulinum*, are less virulent in the absence of the prophage. The phages infecting these bacteria carry the toxin genes in their genome and enhance the virulence of the host when the toxin genes are expressed. In the case of *V. cholera*, phage encoded toxin can cause severe diarrhea; in *C. botulinum*, the toxin can cause paralysis. During lysogeny, the prophage will persist in the host chromosome until **induction**, which results in the excision of the viral genome from the host chromosome. After induction has occurred the temperate phage can proceed through a lytic cycle and then undergo lysogeny in a newly infected cell .



Phage T4 Mutant:

One type of mutation in the T4 bacteriophage identified by researchers in phage genetics by the 1950s was known as *r* (for *rapid*), which caused the phage to destroy bacteria more quickly than normal. These could be spotted easily because they would produce larger plaques rather than the smaller plaques characteristic of the wild type virus. Through genetic mapping, the researchers had identified specific regions in the T4 chromosome, called the *r*I, *r*II, and *r*III loci, associated with the *r* mutants. In 1952, while performing experiments with *r*II mutants, Seymour Benzer found a strain that did not behave normally.

LUNA (1947) and LUIUA and DULBECCO (1949) ob served that bacterial cells infected by more than one UV-irradiated phage particle produced a burst of viable progeny phage with a higher probability than expected if the irradiated phage survived independently of each other. This "multiplicity reactivation" demonstrated that irradiated phage particles could cooperate to come back to life. LUNA and DULBECCO offered a simple, well defined hypothesis for the phenomenon. They proposed that T2 phage (a close relative of T4) are made of functionally distinct subunits of equal UV sensitivity. A phage particle is "killed" by UV when any one of its subunits is "hit." An infected cell will produce progeny phage if, among the several infecting particles, there is at least one un-hit subunit of each type.

Production and screening of mutants:

The procedure described by Tessman (34) was used to obtain phage carrying mutations in nonessential genes. In the control tube, without hydroxylamine, the phage titer decreased only 25% during incubation for 72 hr at 37 C, whereas the titer in the tube with hydroxylamine decreased logarithmically to a survival of about 0.1% during a similar incubation. Survivors included large and small plaque-formers, in addition to the apparently normal plaque-formers. One hundred plaques, including all plaque morphologies observed, were isolated and screened as described below. Plaques produced by surviving phage were picked with toothpicks which were then placed in 1 ml of growth medium to give phage titers of 2-bout 5 X 106.

These phage isolates were replated at a dilution giving 10 to 200 plaques per plate and were repicked in an attempt to eliminate phage with lethal mutations which might be replicated in cells infected with the viable isolates. Only one isolate was lost during this step. This second set of isolates was then used to produce lysates with higher titers by growing in E. coli B at 30 C. One more isolate was lost during this step, leaving 98 isolates to be screened as described below.

The lysates described above were screened for presumed nuclease deficiency by comparing phage synthesis in the presence and absence of HU. Lysates were diluted to 1010 phage/ml and 0.1 ml of each was added to a 4-ml culture of E. coli (4 X 108 cells/ml) containing 0.01 M HU in a 50-ml Erlenmeyer flask. A similar addition was made to a culture without HU, and both were shaken at 37 C for 30 min and were then titered for phage. The value for +HU titer/- HU titer obtained with wild-type phage under these conditions was 0.10 to 0.12.

Isolates which had a value of less than 0.09 were then analyzed more carefully to identify those which make no phage in the presence of HU. Lysates were diluted to about 5 X 108 phage/ ml, and 0.1 ml of each was added to a 5-ml culture of E. coli (5 X 103 cells/ml) in a 50-ml Erlenmeyer flask. These were shaken at 37 C for 1 min, and then 0.1 ml was diluted into 10 ml of fresh medium to minimize reinfection after lysis. After 0.5 min, 5 ml of this solution was transferred to a similar flask containing 4.4 mg of HU (final concentration of 0.012 M). Samples were removed from each flask at various times after infection and titered for phage. Phage which replicated in the absence of HU but failed to induce net phage synthesis in the presence of HU were designated nuclease deficient (nd).

When 100 isolates from the heavily mutagenized phage stock were tested for their ability to replicate in the presence of HU, four isolates clearly lacked this ability but could replicate in its absence. These isolates were purified as described above and have been designated nd for their presumed nuclease deficiency. Figure 1 shows a typical growth curve for wild-type T4 and one of these nd mutants, nd28. Whereas nd28 replicates as well as T4 in E. coli B in the absence of HU, there is no net phage production in cells infected with nd28 for up to 40 min after infection in the presence of HU. The mutation is not an am mutation since the same effect occurs when E. coli CR63 is used as the host. Double mutants were isolated from crosses of nd28 with various am mutants

Purification and mapping:

E. coli strains B/5, S/6/5, and CR63, obtained from R. S. Edgar, were grown in H-broth (7) for these experiments. For genetic purification, backcrosses were done at 30 C in E. coli B/5 by using wild-type/nid ratios of 4 as described by Edgar, except that antiserum was not used. The nd mutants were backcrossed to wild-type six times, and these purified mutants were used for all studies described below, unless otherwise indicated.

Progeny were tested for genotype by plating on S/6/5 indicator, incubating at 30 C overnight, resuspending single plaques in 2 ml of H-broth, and testing the ability of each to grow in the presence and absence of HU. Clonal stocks were then prepared from nd isolates for subsequent backcrosses. One mutant with the izd phenotype, designated nd28, has been carefully mapped. Mapping this mutant was facilitated by the observation that it produces a considerably smaller plaque than does wild-type T4. Reconstruction experiments indicated that, whereas it was possible to score large plaques unambiguously as nd+, approximately 5% of the small plaques scored as nd were actually found to be nd+. It is likely, therefore, that the map intervals derived from these data are slightly underestimated. Crosses of nd28 with am mutants were performed at 30 C in E. coli strain CR63.

The procedure employed was as described by Edgar (6). Superinfection exclusion was prevented by adding KCN; unadsorbed phage were inactivated with antiserum. All adsorption tubes and growth tubes were aerated by slow bubbling. Total progeny were scored on CR63 indicator; wild-type (nd+am+) recombinants were scored by enumerating large plaques on S/6/5 indicator.

The relative efficiency of plating on CR63 and S/6/5 was determined in each experiment, and the titers determined were corrected accordingly. Double mutants (nd am) were also identified among the progeny on the CR63 plates from the above crosses by screening for the am mutation on E. coli B and then testing these am mutants for the presence of nd as indicated above. All double mutants obtained in

this way were tested for complementation with am mutations in genes 30, 42-46, and 56 as described by Wiberg .

Questions:

Long answer questions:

1. Explain genetic recombination in Bacteria.
2. Brief about Linkage in chromosomes
3. Genetic mapping with examples.
4. Account on replication cycle of phage.
5. Phage T4 Mutants detection and isolation.

Short anwer questions:

1. What is Recombination in Bacteria
2. What is linkage in chromosome
3. Lytic cycle- short notes
4. Charactertics of bacteriophage.

Unit III Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
Confirmational changes in protein is brought about by	Systematic molecules	Cohesive molecules	Affector molecules	Effector molecules	Effector molecules
Control of gene expression was proposed by	Beedle & Tatum	Avery & McLeod	Jacob & Monad	Hershey & Chase	Jacob & Monad
Enzyme activity is regulated by changes in the confirmation of enzymes except	Polymerase	Ribozymes	Chimozymes	Nuclease	Ribozymes
Enzyme that lactose in to glucose and galactose	Lactosidase	Glucanse	α-galactosidase	β-galactosidase	β-galactosidase
Genes are located in specialized structures called	Histone	RNA	Chrosomes	Genomes	Chrosomes
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
In prokaryotes, AUG is translated in to	Methionine	N-acetyl-methionine	N-formamyl-aspargine	N-formamyl-methionine	N-formamyl-methionine

In Rho-independent transcription termination, the termination sequence is usually	Palindromic sequence	Paliomic sequence	Panoramic sequence	Pandemic sequence	Palindromic sequence
A-site is the ribosomal site most frequently occupied by the	Aminoacyl-rRNA	Aminoacyl-mRNA	Iminoacyl-tRNA	Aminoacyl-tRNA	Aminoacyl-tRNA
Capping in mRNA is addition of the group	7-ethylguanosine	7-methylguanosine	7-methylcytosine	7-ethylcytosine	7-methylguanosine
Codon/Anticodon consists of _____ nucleotides	4	6	3	9	3
Common method of covalent modification of enzyme in regulation of gene expression is	to methylate the enzyme at a proline residue	to phosphorylate the enzyme at a proline residue	to phosphorylate the enzyme at a serine residue	to methylate the enzyme at a serine residue	to phosphorylate the enzyme at a serine residue
Short sequence of aminoacids are called	Peptides	Proteins	Polypeptides	Palindromes	Peptides
Site to which substrate molecules are attached	Catalytic site	Effector site	Alleosteric site	Binding site	Catalytic site

Stop codon UAA is also called	Amber	Opal	Acre	Ochre	Ochre
Stop codons in mammalian mitochodria are	GAA & GAG	AGA & AGG	CGA & AGC	CGG & GCG	AGA & AGG
Structure of proteins may be classified into ___ types	2	3	4	5	4
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
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
The first codon during translation is	AGU	AUG	GUA	UGA	AUG
In RNA, thiamine is replaced by	Uracil	Adenine	Cytosine	Guanine	Uracil
In the absence of effector molecule, the enzyme is said to be in	Relaxed state	Tense state	Free state	Degrading state	Tense state

In trp operon, the genes *trp*E & *trp*D codes for	Arginase	Tryptophan synthase	Anthranilate isomerase	Anthranilate synthase	Anthranilate synthase
lac operon is an example for	Repressible operon	Inducible operon	Mutated operon	Neutral operon	Inducible operon
Mammalian mitochondrion not only uses AUG as initiation codon but also	AUA, AUU, AUC	UAA, UAU, UAC	AAU, UAU, CAU	GUA, GUU, GUC	AUA, AUU, AUC
Model example for gene regulation by repression	*trp* operon	*lac* operon	*ara* operon	*gal* operon	*trp* operon
Molecular weight of egg lysozyme is	19300 daltons	13900 daltons	31900 daltons	91300 daltons	13900 daltons
Monad & Cohen-Bazire first reported the evidence for the repression of the enzyme	Tryptophan synthase	Gluconase synthetase	Arabinase trimutase	Tryptophanase	Tryptophan synthase
Non codon specifies more than ___ amino acid	1	2	3	4	1
Non-coding regions are called as	Exons	Introns	Cistrons	Positrons	Exons

Operon model that demonstrates both positive and negative control of gene regulation	*lac* operon	*ara* operon	*gal* operon	*trp* operon	*ara* operon
Other than methionine _____ is the amino acid that appear rarely in proteins	Arginine	Tryptophan	Glutamic acid	Threonine	Tryptophan
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
Region that comprise the core prokaryotic promoter	Klenow box	Pribnow box	TAGTAG box	Polypeptide box	Pribnow box
Repressor molecule in lac operon is a	Dimer	Trimer	Tetramer	Pentamer	Tetramer
Ribosomal site most frequently occupied by the tRNA carrying the growing peptide chain	A-site	P-site	E-site	G-site	P-site
rRNA is also called	Rnase	Ribase	Ribulase	Ribozyme	Ribozyme
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

_____ is the first amino acid during translation of proteins	Threonine	Leucine	Methionine	Valine	Methionine
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
7-methylguanosine cap is an important site	For eukaryotic transcription initiation factor	For prokaryotic translation initiation factor	For eukaryotic translation initiation factor	For prokaryotic translation initiation factor	For eukaryotic translation initiation factor
Action of repressor protein in *Lac* operon is called as	Positive control	Negative control	Neutral control	No control	Negative control
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
All aminoacids have more than one codon except	Methionine & Tryptophan	Valine & Leucine	Threonine & Alanine	Lysine & Arginine	Methionine & Tryptophan
Allosteric enzymes that are controlled by a molecule other than it's substrate	Cohesive molecules	Systematic molecules	Effector molecules	Affector molecules	Effector molecules

Amino acid that have largest number of codons	Proline	Cysteine	Serine	Valine	Serine
tRNA is responsible for the transfering	Protein	Aminoacid	Codon	Anticodon	Anticodon
tRNA's are matched with their aminoacids by a group of enzymes collectively called as	Aminoacyl DNA synthatases	Aminoacyl synthatases	Amino synthatases	Aminoacyl tRNA synthatases	aminoacyl tRNA synthatases
What are the possible number of codons that can be generated using possible nucleotide combinations	46	64	20	30	64
Which is astop codon	UAA	AAU	AUA	AAA	UAA
Which transports lactose across the cell membrane	Galactosidase permease	β-galactosidase	Glucanse	Glucose permease	Galactosidase permease
Who deciphered the genetic code	Hershey & Chase	Avery & McLeod	Beedle & Tatum	Nirenberg & Khorana	Nirenberg & Khorana
Translation is	rRNA to protein	tRNA to protein	DNA to protein	mRNA to protein	mRNA to protein

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
Transcription initiation site starts from	-1	Plus 1	-10	Plus 10	Plus 1
The main function of nonsense codons is to	Initiate protein synthesis	Elongate protein synthesis	Terminate protein synthesis	Regulate protein synthesis	Terminate protein synthesis
The number of nitrogenous bases codes by 9 amino acids would be	27	36	18	9	27
The promoter sequence in eukaryotes is	TATAAA	TAATAA	TTGACA	GTTAAA	TATAAA
The stop codons are called as	Missense	Nonsense codons	Central codons	Last codons	Nonsense codons
Transcription is	DNA to rRNA	DNA to tRNA	DNA to mRNA	DNA to protein	DNA to mRNA

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).

Mutation:

DNAPermanent, heritable *alterations in the base sequence of DNA*

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 theirgenetic material (i.e. relatively few mutations occured) while distantly related organisms show bigger differences in their.

Mutagens:

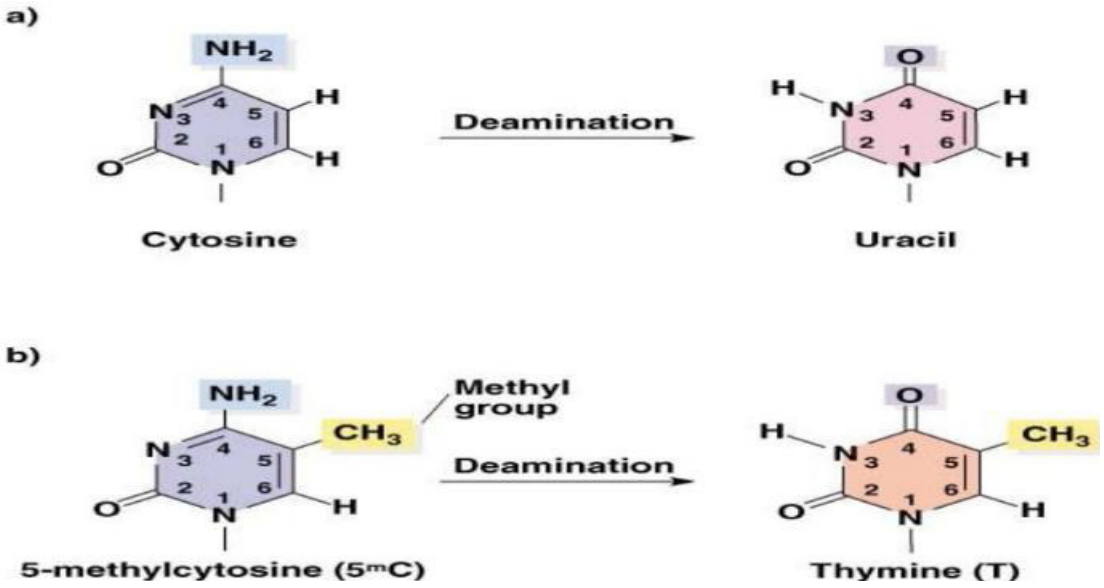
Agents that cause DNA damage that can be converted to mutations.

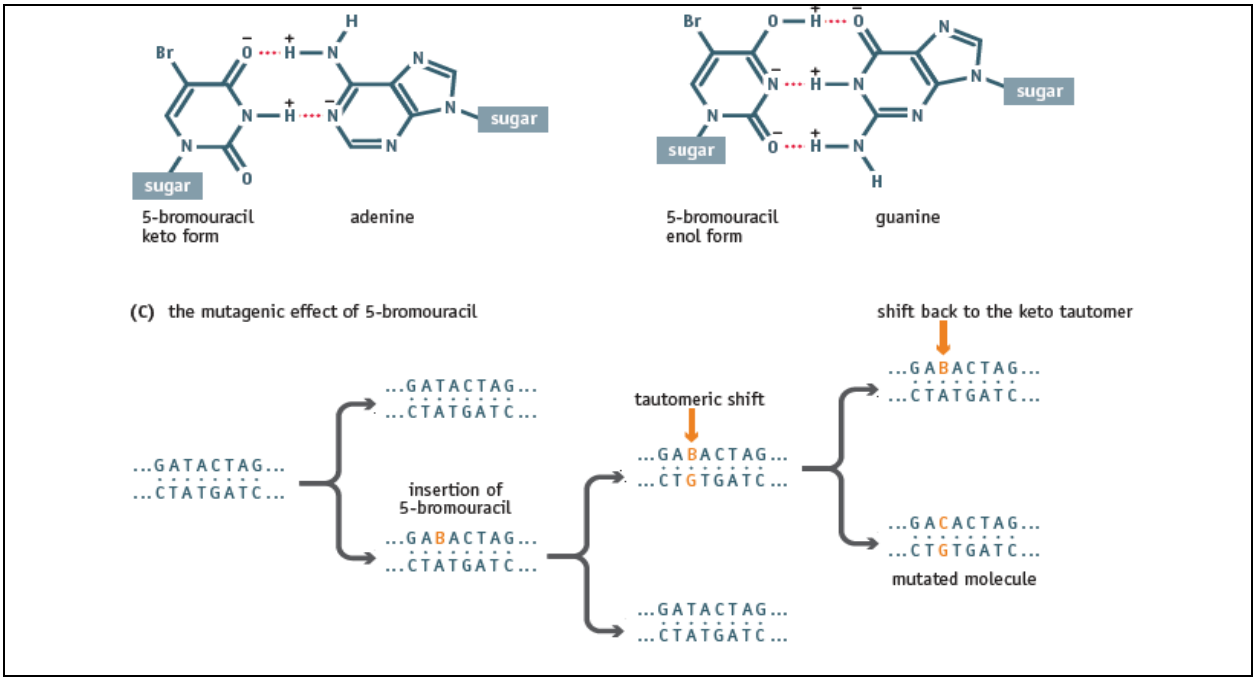
Physical mutagens responsible for mutation

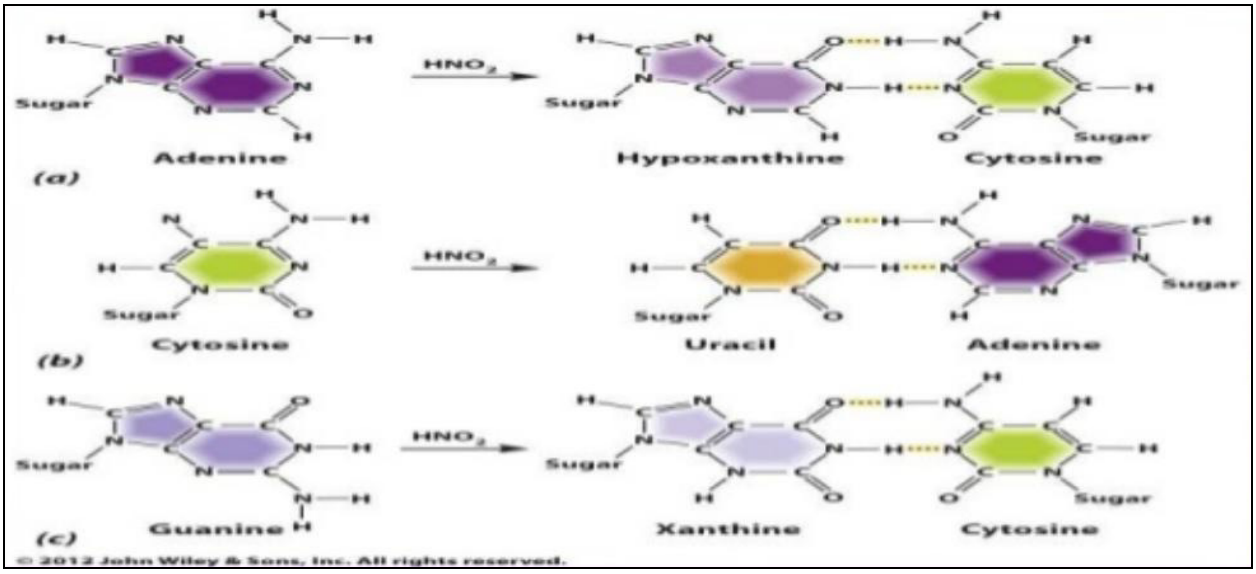
- ***High-energy ionizing radiation***: X-rays and g-rays → strand breaks and base/sugar Destruction
- ***Nonionizing radiation*** : UV light → pyrimidine dimmers

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

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.

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):

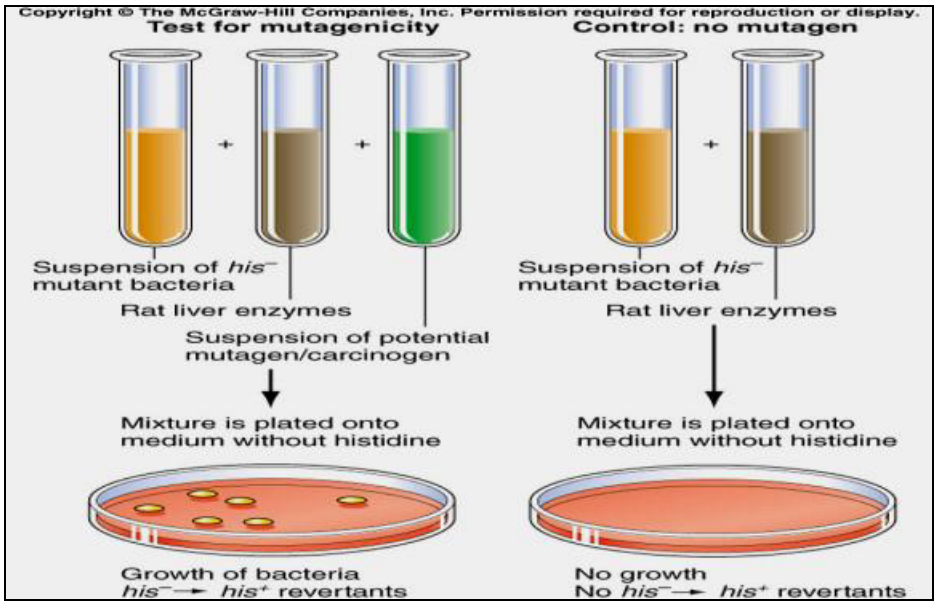
An *E. coli* population started from one cell should show different patterns resistance depending on which theory is correct.

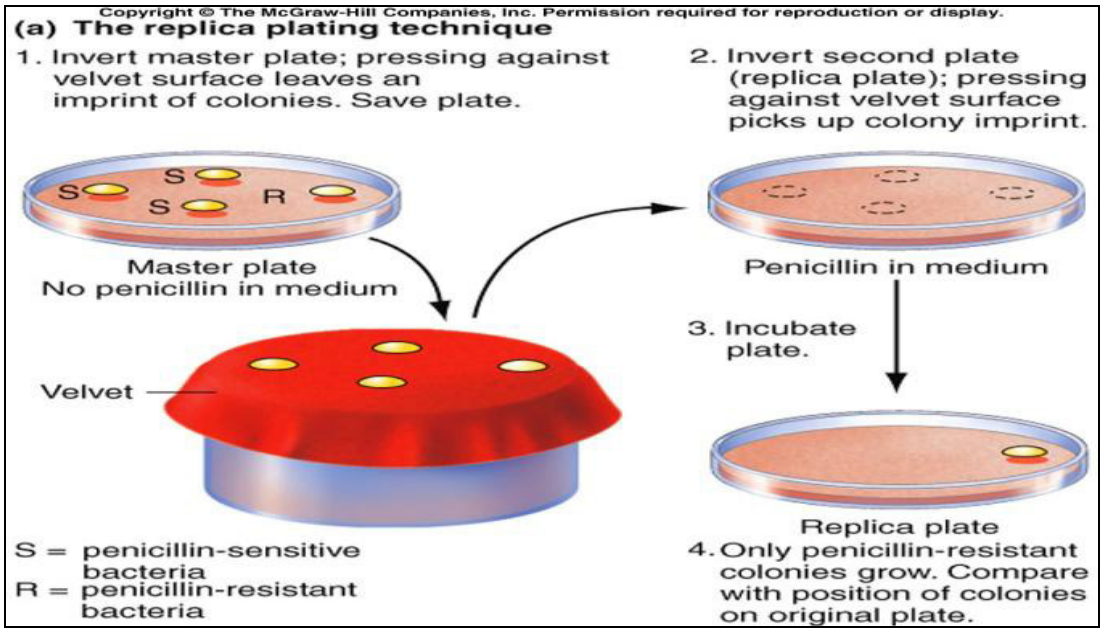
- Adaptive theory states that cells are induced to become resistant when T1 is added; proportion of resistant cells should be the same for all cultures with the same genetic background.
- Mutation theory states that random events confer resistance to T1; duplicate cultures with the same genetic background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

Luria-Delbruck Fluctuation Test:

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:

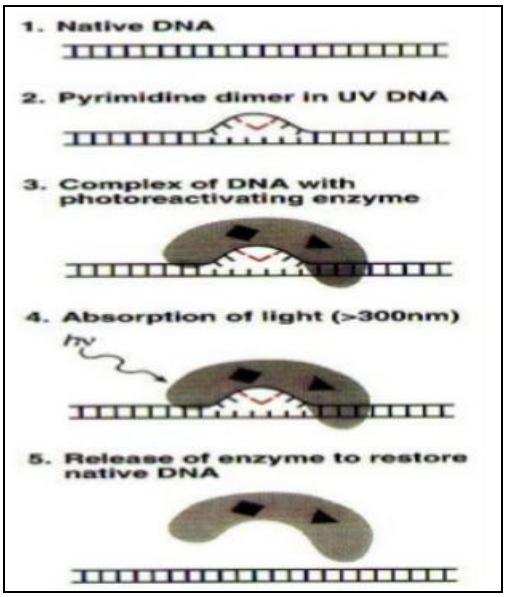
- Radiations Highly reactive oxygen radicals produced during normal cellular respiration as well as by other biochemical pathways
- Ionizing radiation such as gamma rays and x-rays o Ultraviolet rays, especially the UV-C rays (~260nm) that are absorbed strongly by DNA but also the longer-wavelength UV-B 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:

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

- Base excision repair (BER)
- Nucleotide excision repair (NER),
- Mismatch repair (MMR) and
- 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

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.

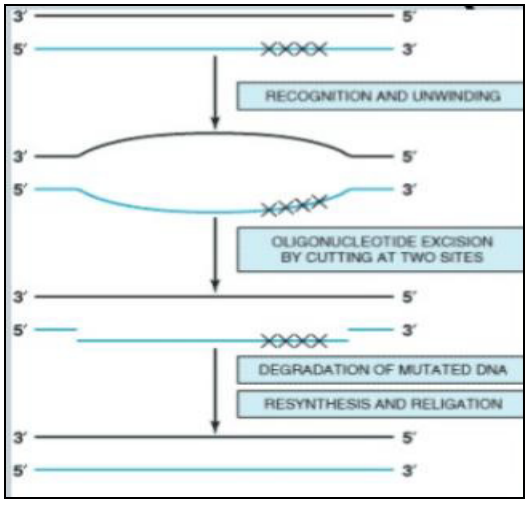
Finally, the DNA polymerase and a ligase catalyze the incorporation of a specific deoxyribonucleotide into the repaired site, enabling correct base pairing

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.

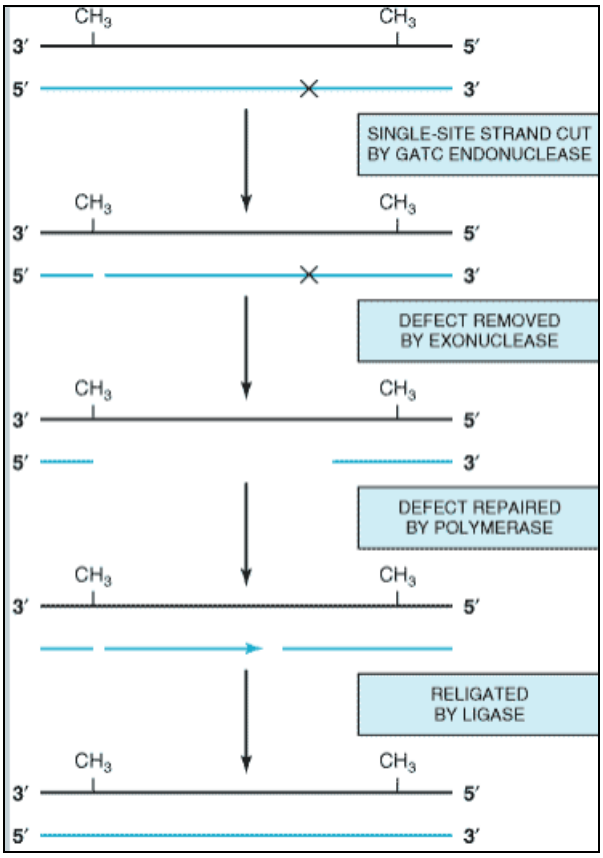


- These are repaired by a process called nucleotide excisionrepair
- NER is a much more complex biochemical process than BER, especially in eukaryotic cells.
- Several gene products are required in a multiple step process, during which the ordered assembly of DNA proteins provides an enzymatic complex 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 lesionrecognition 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
- 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 doublestrand 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:

1. Burkitt's lymphoma
2. Philadelphia chromosome in chronic myelogenous leukemia(CML),
3. B-cell leukemia.

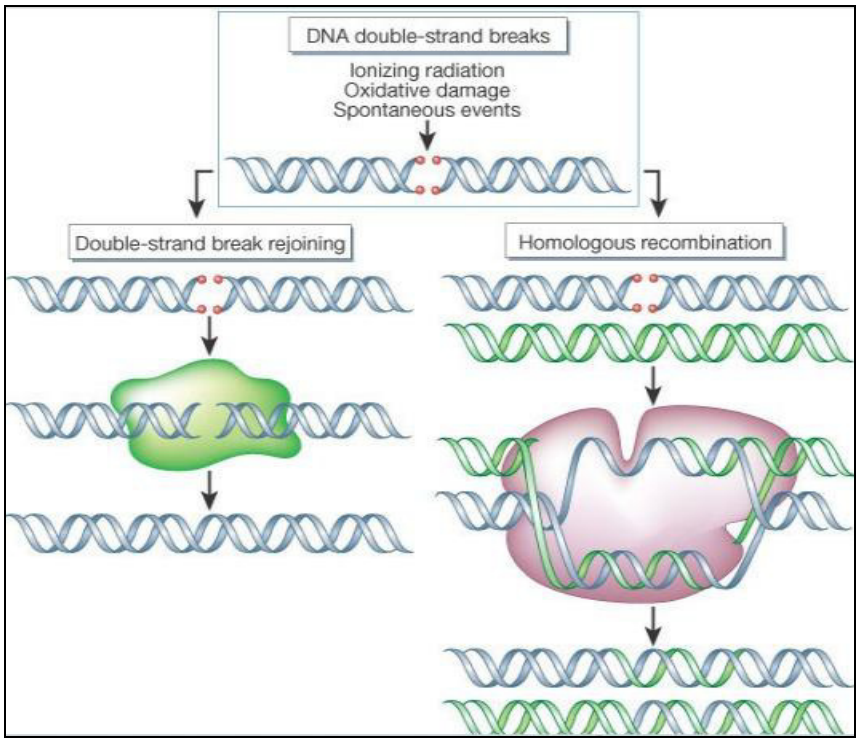
2) Homologous Recombination.

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).

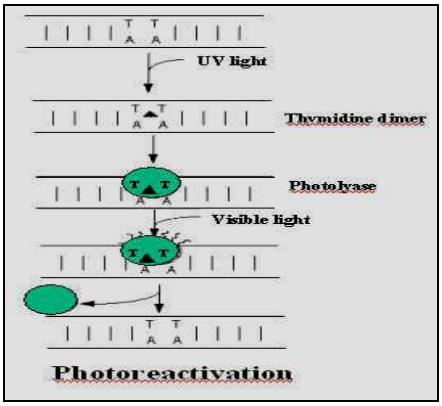
Two of the proteins used in homologous recombination are encoded by the genes BRCA1 and BRCA2.

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 (Weiglereactivation). 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.

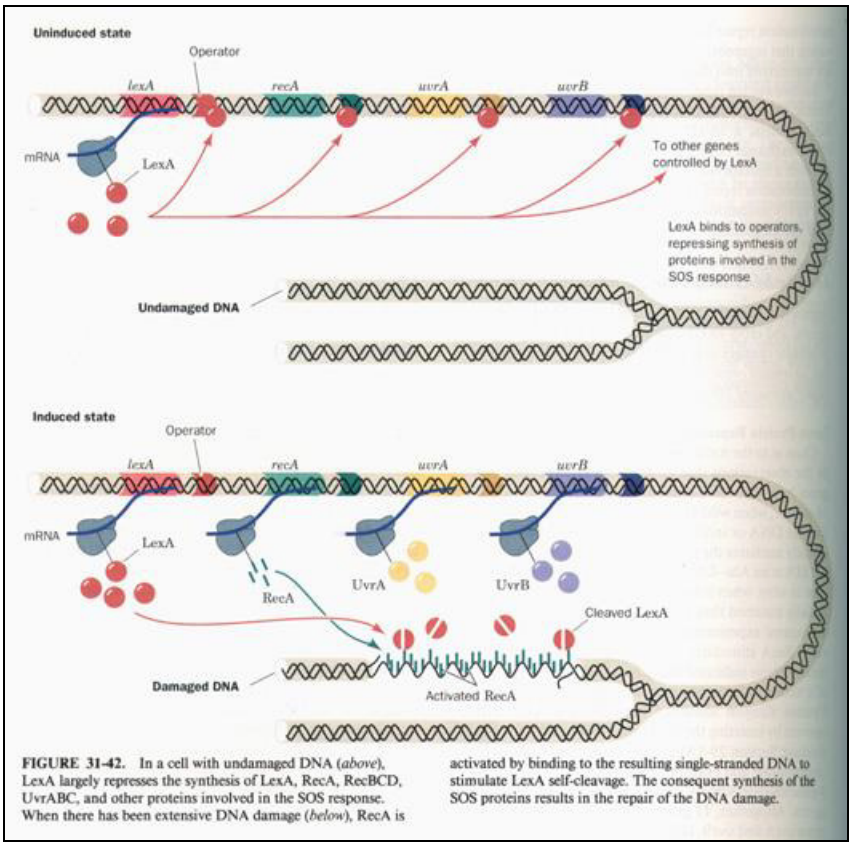


FIGURE 31-42. In a cell with undamaged DNA (*above*), LexA largely represses the synthesis of LexA, RecA, RecBCD, UvrABC, and other proteins involved in the SOS response. When there has been extensive DNA damage (*below*), RecA is activated by binding to the resulting single-stranded DNA to stimulate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

UNIT: IV
Syllabus

Rules of extra nuclear inheritance,Organelle heredity-Chloroplast mutation in *Chlamydomonas,* mitochondrial mutation in *Saccharomyces*, Menternal effects-Shell coiling in *Limnaea peregre* infection heredity-Kappa partical in *Paramecium*. Pedigree analysis, lod scorefor linkage testing, karyotypes, genetic disorders. Polygenic ingeritance, heritability and its measurements, QTL mapping.

Extranuclear Inheritance :

Extranuclear inheritance or cytoplasmic inheritance is the transmission of genes that occur outside the nucleus. It is found in most eukaryotes and is commonly known to occur in cytoplasmic organelles such as mitochondria and chloroplasts or from cellular parasites like viruses or bacteria.

Organelle Heredity:

Mitochondria are organelles which function to transform energy as a result of cellular respiration. Chloroplasts are organelles which function to produce sugars via photosynthesis in plants and algae. The genes located in mitochondria and chloroplasts are very important for proper cellular function, yet the genomes replicate independently of the DNA located in the nucleus, which is typically arranged in chromosomes that only replicate one time preceding cellular division. The extranuclear genomes of mitochondria and chloroplasts however replicate independently of cell division. They replicate in response to a cell's increasing energy needs which adjust during that cell's lifespan. Since they replicate independently, genomic recombination of these genomes is rarely found in offspring, contrary to nuclear genomes in which recombination is common. Mitochondrial diseases are inherited from the mother, not from the father: mitochondria with their mitochondrial DNA from the mother's egg cell are incorporated into the zygote and passed to daughter cells, whereas those from the sperm are not.

Parasites:

Extranuclear transmission of viral genomes and symbiotic bacteria is also possible. An example of viral genome transmission is perinatal transmission. This occurs from mother to fetus during the perinatal period, which begins before birth and ends about 1 month after birth. During this time viral material may be passed from mother to child in the bloodstream or breastmilk. This is of particular concern with mothers carrying HIV or Hepatitis C viruses.[2][3] Symbiotic cytoplasmic bacteria are also inherited in organisms such as insects and protists.

Types:

Three general types of extranuclear inheritance exist.

- Vegetative segregation results from random replication and partitioning of cytoplasmic organelles. It occurs with chloroplasts and mitochondria during mitotic cell divisions and results in daughter cells that contain a random sample of the parent cell's organelles. An example of vegetative segregation is with mitochondria of asexually replicating yeast cells.
- Uniparental inheritance occurs in extranuclear genes when only one parent contributes organellar DNA to the offspring. A classic example of uniparental gene transmission is the maternal inheritance of human mitochondria. The mother's mitochondria are transmitted to the offspring at fertilization via the egg. The father's mitochondrial genes are not transmitted to the offspring via the sperm. Very rare cases which require further investigation have been reported of paternal mitochondrial inheritance in humans, in which the father's mitochondrial genome is found in offspring.[6] Chloroplast genes can also inherit uniparentally during sexual reproduction. They are historically thought to inherit maternally, but paternal inheritance in many species is increasingly being identified. The mechanisms of uniparental inheritance from species to species differ greatly and are quite complicated. For instance, chloroplasts have been found to exhibit maternal, paternal and biparental modes even within the same species.
- Biparental inheritance occurs in extranuclear genes when both parents contribute organellar DNA to the offspring. It may be less common than uniparental extranuclear inheritance, and usually occurs in a permissible species only a fraction of the time. An example of biparental mitochondrial inheritance is in the yeast *Saccharomyces cerevisiae*. When two haploid cells of opposite mating type fuse they can both contribute mitochondria to the resulting diploid offspring.

Mutant Mitochondria:

Poky is a mutant of the fungus Neurospora crassa that has extranuclear inheritance. Poky is characterized by slow growth, a defect in mitochondrial ribosome assembly and deficiencies in several cytochromes. The studies of poky mutants were among the first to establish an extranuclear mitochondrial basis for inheritance of a particular genotype. It was initially found, using genetic crosses, that poky is maternally inherited. Subsequently, the primary defect in the poky mutants was determined to be a deletion in the mitochondrial DNA sequence encoding the small subunit of mitochondrial ribosomal RNA

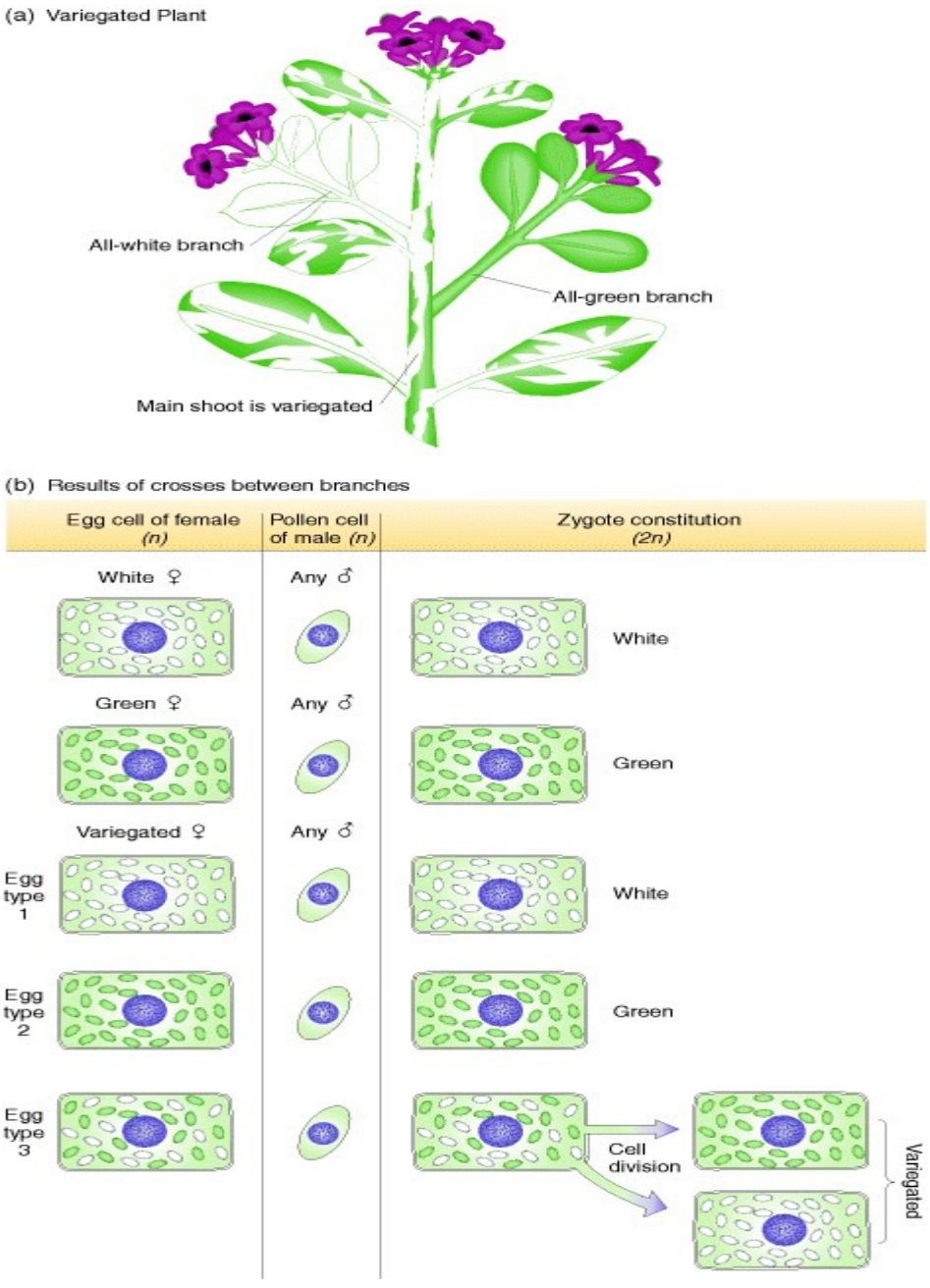
Inheritance of Organelle Genes:

Mitochondria and chloroplasts are specialized organelles located in the cytoplasm. They contain a defined subset of the total cell genome .These genes show their own special mode of inheritance. In a cross, both parents contribute equally to the nuclear genome of the zygote. However, the cytoplasmic contribution of the male and the female parent is generally unequal; the egg contributes the bulk of the cytoplasm and the sperm essentially none. Because organelles reside in the cytoplasm, the organelle genes generally show strictly maternal inheritance. In other words, essentially none of the organelle DNA in the zygote is from the male parent. A simple example is seen in the inheritance of the *Neurospora* slow-growing mutant poky, which is caused by a defect in one of the mitochondrial genes. *Neurospora* can be crossed in such a way that one parent acts as the maternal and the other the paternal parent. In the cross of a poky female with a normal male, the progeny are all poky (FIG1), the precise inheritance pattern expected from a mitochondrial gene.

Maps of yeast and human mtDNAs. Each map is shown as two concentric circles corresponding to the two strands of the DNA helix. Note that the mutants used in yeast mtDNA analysis are shown opposite their corresponding structural genes. Green = exons and

Explanation of the different results from reciprocal crosses of poky and normal *Neurospora.* The parent contributing most of the cytoplasm of the progeny cells is called *female.* Brown shading represents cytoplasm with the poky determinants. The nuclear.

Figure 4-30 on page 120 demonstrates maternal inheritance in the four-o'clock plant. The color of the chloroplasts in this plant determines the color of the various branches. Variegated branches are mosaics of all-green and all-white cells. Flowers can come from green, white, or variegated branches, but when crossed, it is the egg cell that determines branch color in the resulting plant. For example, if the egg cell comes from a flower on the white branch, regardless of the origin of the pollen, the resulting plant will have white branches, thus showing maternal inheritance. The variegated zygotes (bottom of Figure 4-30) are cytoplasmic mixtures of two chloroplast types, and, interestingly, these two types undergo cytoplasmic segregation during cell division, yielding the distinct green and white sectors that cause the variegation in the branches.


(a) Variegated Plant
All-white branch
All-green branch
Main shoot is variegated
(b) Results of crosses between branches
Egg cell of female (n)
Pollen cell of male (n)
Zygote constitution (2n)
White ♀
Any ♂
White
Green ♀
Any ♂
Green
Variegated ♀
Any ♂
Egg type 1
White
Egg type 2
Green
Egg type 3
Cell division
Variegated

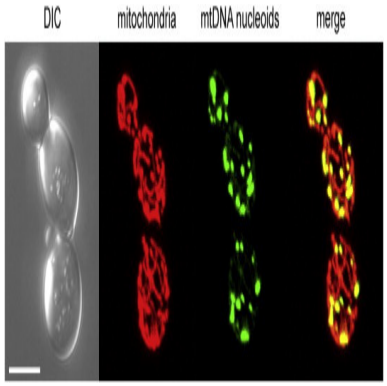


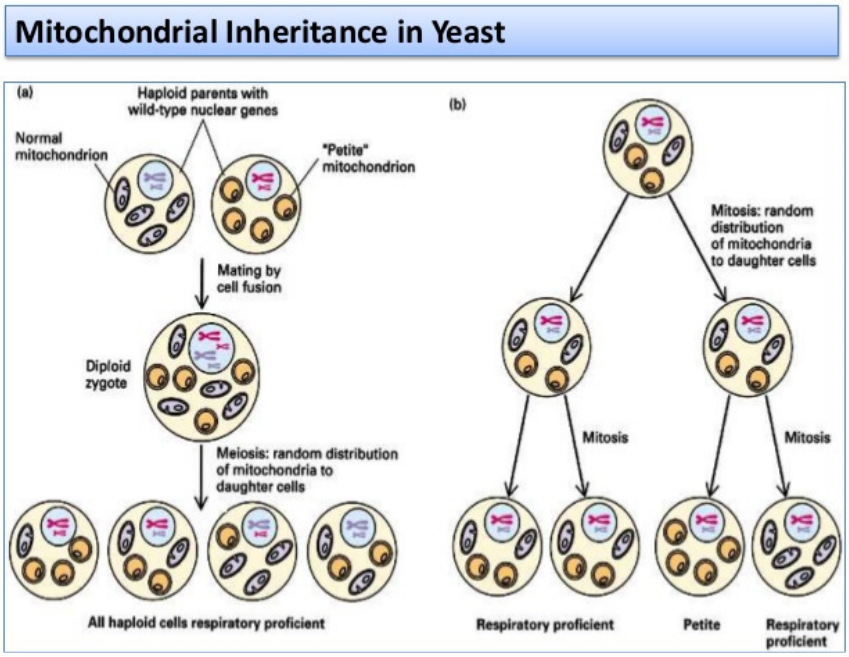
Figure 4-30

Leaf variegation in *Mirabilis jalapa,* the four-o'clock plant. (a) Flowers may form on any branch (varie-gated, green, or white), and these flowers may be used in crosses. (b) In crosses between flowers on different-colored branches, color of the zygote and resulting plant is deter-mined by maternal inheritance. The first two crosses shown here exhibit strict maternal inheritance. If the maternal branch is variegated, three types of zygotes can result, depending on whether the egg cell contains only white, only green, or both green and white chloroplasts. Maternal inheritance still applies in all three types, but in the variegated zygotes, a process of cytoplasmic segregation during subsequent cell divisions produces a mosaic of all-green and all-white cells, hence, a variegated plant.

Mitochondrial Mutation in Yeast:

The yeast *Saccharomyces cerevisiae* can mutate to the respiratory-incompetent petite colony form. The mutation is probably caused by damage to, or loss of, the yeast's mitochondrial DNA, for petite mutants often lack mitochondrial DNA, possess it in abnormal amounts or with abnormal buoyant density. Some of the agents, such as acrifiavine or ethidium bromide, which induce the petite mutation interfere with mitochondrial DNA synthesis whereas ethidium bromide also causes or permits degradation of *Saccharomyces cerevisiae* mitochondrial DNA. We have observed that nalidixate (50 µg/ml.), an inhibitor of DNA synthesis, can prevent or delay petite mutation induced by ethidium bromide[4]. A similar effect has been observed by Hollenberg and Borst using a higher nalidixate concentration

The mitochondrial genome is packaged into protein–DNA complexes. These structures are called nucleoids by analogy to DNA-organizing structures in bacteria, even though mtDNA packaging proteins probably are of eukaryotic origin . *S. cerevisiae* has about 10–40 nucleoids per cell which are anchored to the mitochondrial inner membrane and evenly spaced along the mitochondrial reticulum (Fig. 1). Each nucleoid contains several mtDNA copies . The major DNA-binding protein of yeast nucleoids is the non-histone high mobility group protein Abf2 . Abf2 plays a major role in packaging of mtDNA, protects it against nuclease attack and chemical damage, and binds and stabilizes recombination intermediates . Additional nucleoid components are the proteins required for DNA replication, transcription, repair, and recombination . Other proteins that were found in nucleoids include the mitochondrial chaperonin Hsp60, which was proposed to be required for nucleoid division , the citric acid cycle enzyme aconitase, which was suggested to couple mtDNA maintenance with cell metabolism , and various other heat shock proteins, metabolic enzymes, and proteins of unknown function.



The mitochondrial genome is packaged into protein–DNA complexes. These structures are called nucleoids by analogy to DNA-organizing structures in bacteria, even though mtDNA packaging proteins probably are of eukaryotic origin . *S. cerevisiae* has about 10–40 nucleoids per cell which are anchored to the mitochondrial inner membrane and evenly spaced along the mitochondrial reticulum . Each nucleoid contains several mtDNA copies . The major DNA-binding protein of yeast nucleoids is the non-histone high mobility group protein Abf2 . Abf2 plays a major role in packaging of mtDNA, protects it against nuclease attack and chemical damage, and binds and stabilizes recombination intermediates . Additional nucleoid components are the proteins required for DNA replication, transcription, repair, and recombination . Other proteins that were found in nucleoids include the mitochondrial chaperonin Hsp60, which was proposed to be required for nucleoid division , the citric acid cycle enzyme aconitase, which was suggested to couple mtDNA maintenance with cell metabolism, and various other heat shock proteins, metabolic enzymes, and proteins of unknown function .

Surprisingly little is known about the cellular mechanisms of mtDNA segregation in yeast cells. During its sexual life cycle two haploid yeast cells of opposite mating type fuse to form a diploid zygote. If the parental cells contribute different mitochondrial genomes the zygote contains a mixture of mtDNAs with different genotypes, a state termed heteroplasmy. However, within few cell divisions the mtDNAs unmix, and cells become homoplasmic . Genetic evidence suggests that only a small fraction of the

mtDNA pool is transferred from the zygote to the bud, and that the position of the bud determines which parental cell contributes its mtDNA. Cells that bud from the mid-point of the zygote inherit mtDNA from both parents, whereas those that bud from either end preferentially inherit mtDNA from only one parent . Furthermore, examination of fluorescently labeled nucleoids in zygotes indicated that nucleoids are anchored within the organelle and remain localized in distinct parts of the cell .

Thus, it is thought that diffusion of mtDNA within the organelle is limited. Instead, it is actively transported into the bud by a yet poorly characterized nucleoid segregation apparatus . Presumably, similar mtDNA segregation mechanisms are active in zygotes and vegetatively growing cells.

S. cerevisiae has been used extensively to study the molecular mechanisms of organelle inheritance.During mitotic growth yeast cells multiply by asymmetric cell division, a process termed budding. At the beginning of each cell cycle cells become polarized and select a site for bud emergence. Growth is initially restricted to the bud tip and then switches to even expansion over the entire bud surface. As the bud reaches the size of the mother cell, growth is directed to the bud neck, and a septum is formed that separates the daughter cell from its mother. Correct organelle partitioning is achieved by active and directed transport of organelles to the growing bud concomitant with retention of a portion of the organelles in the mother cell . Actin cables that consist of bundles of actin filaments provide the tracks for directed transport processes during cell growth. These cables are assembled by formins, conserved proteins that are located at the bud tip or bud neck and associate with the plus ends of actin filaments. Thus, polarized actin cables initially extend from the growing bud deep into the mother cell. When the bud grows larger formins are relocated from the bud tip to the bud neck and assemble cables that emanate from the bud neck and extend into the mother and daughter .

Immediately after bud emergence mitochondria enter the bud to ensure inheritance of the organelle . Mounting evidence suggests that bud-directed mitochondrial movement along actin cables is driven by myosin motor proteins.

Chloroplast mutation in clamydomonas:

The single chloroplast of the alga *Chlamydomonas reinhardtii* contains at least 100 copies of the chloroplast chromosome. It is not known how the chloroplast (or cell) becomes homoplasmic for a mutation that arises in one of these copies. Under suitable selection conditions, clones with chloroplast mutations for streptomycin resistance induced by methyl methanesulfonate can be recovered with direct plating after mutagenesis. Using an adaptation of the LURIA-DELBRÜCK fluctuation test, mutagenized cultures grown on nonselective liquid medium for seven to nine doublings show negligible proliferation of cells capable of forming such mutant colonies. In contrast, cells among the same cultures with reduced

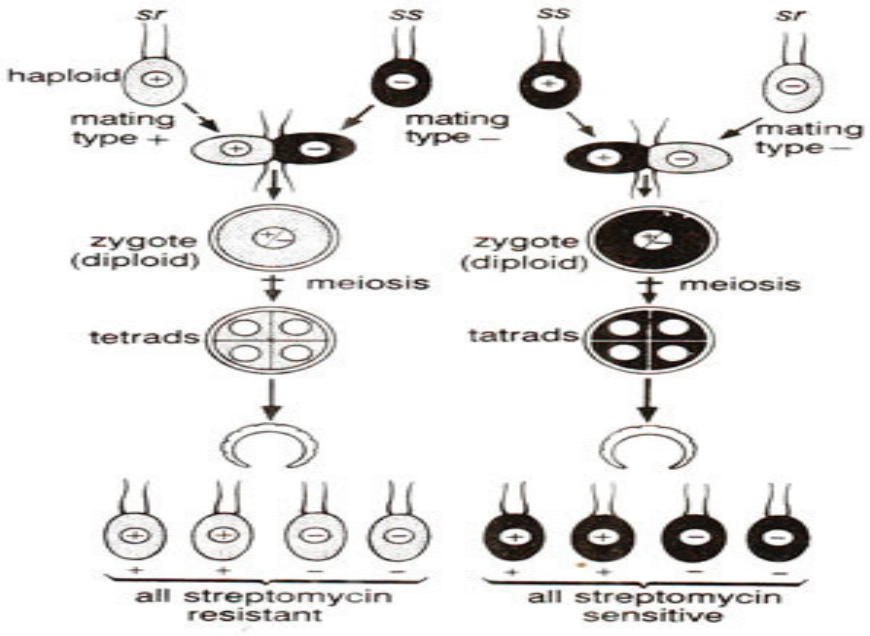
nuclear mutations conferring streptomycin resistance reveal considerable clonal propagation prior to plating on selection medium. Reconstruction growth-rate experiments show no reduced growth of cells with chloroplast mutations relative to either wild-type cells or to those with nuclear mutations.

All eukaryotic cells so far examined have multiple copies of their chloroplast A and/or mitochondrial chromosomes. Despite this fact, cell lines homoplasmic for a chloroplast or mitochondrial mutation can appear among the mitotic descendants of at least some cells that initially contained single mutations in only one of these copies. An important step toward understanding how homoplasmic lines originate is to establish the pattern(s) by which an induced mutant allele is transmitted to daughter cells during vegetative division. If there is good reason to believe that the mutant allele, or the cells containing it, are not at a significant replicative disadvantage under the conditions of growth employed, we can ask if this allele initially segregates in a random or nonrandom fashion. With random segregation, copies of the mutant allele would sometimes go to the same daughter cell and sometimes to both daughter cells; with nonrandom segregation, one or the other pattern would prevail for the first several divisiom following induction of the mutation. The haploid unicellular alga Chlamydomonas reinhardtii is well suited for investigating the induction and vegetative transmisson of chloroplast mutations. It possesses only a single chloroplast, so that complications associated with multiple organelles can be avoided. Also, non-Mendelian mutations for streptomycin resistance in this alga provide a convenient chloroplast genetic marker. All such mutations, so far mapped, are localized in one of four loci on a single nonMendelian linkage group , and there is substantial evidence that this linkage group resides in the chloroplast .

Chloroplast mutations for streptomycin resistance in this alga confer moderate to high-level resistance to streptomycin on solid medium and are thus phenotypically distinct from Mendelian (nuclear) streptomycin-resistant mutants, which confer only low-level resistance to this antibiotic. The correlation between high-level resistance (at least 500 pg/ml streptomycin sulfate) and inheritance pattern was originally described by SAGER (1954) and has since been confirmed with numerous streptomycin-resistant mutations that have either arisen spontaneously or were induced with the alkylating mutagens nitrosoguanidine (MNNG) or methyl methanesulfonate (MMS). In addition, certain chloroplast streptomycin-resistant mutants have been recovered after MNNG mutagenesis that are reported to be sensitive to high levels of antibiotic .

However, using screening conditions similar to those employed in this study, we have reexamined one of these mutants and routinely detect slight but positive growth on 500 pg/ml of antibiotic, whereas nuclear streptomycin-resistant mutants on the same plates always score as sensitive . In the present study, the mutations resistant to low and high levels of streptomycin are designated str-50 and str-500, respectively. Chloroplast mutants for streptomycin resistance induced with MNNG or MMS can be recovered on low levels of streptomycin (50 to 100 pg/ml) with plating directly after mutagenesis,

but these mutants cannot normally be recovered on the concentration of streptomycin (500 pg/ml) to which they are ultimately resistant. The apparent linear relationship between MNNG dose and the recovery of both nuclear and chloroplast mutants on low levels of antibiotic suggests that one mutational event is sufficient for their detection under such selection conditions . In this study, the transmission of chloroplast (str-500) , and nuclear (str-50) -coded, streptomycin-resistant mutations during the first several cell divisions following their induction with MMS. Mutagenesis was performed on wild-type haploid cells harvested from cultures synchronized by growth under alternating 12-hr light, 12-hr dark periods. At the cell-cycle stage employed (near the onset of light), cells had just completed division and contain about 100 copies of the chloroplast chromosome per cell. This estimate is based on the average cellular content of chloroplast DNA in such cells being about 2.2 X 10-14g and on the molecular weight of individual chloroplast DNA molecules being about 1.3 x lo8 daltons. The transmission of chloroplast and nuclear mutations during vegetative cell division was followed by an adaptation of the classical fluctuation test . Starting cell concentrations and culture numbers were employed to insure that several cultures received one cell with an induced chloroplast or nuclear streptomycin-resistance mutation, but that no cultures were likely to receive more than one. The results show little evidence for the proliferation of cells, preceding action of the antibiotic, that are capable of forming chloroplast mutant colonies on low-level streptomycin agar medium. In contrast, within many of the same culture tubes, there was a marked proliferation of cells capable of forming nuclear mutant colonies.



[G-) FIGURE 1 : Proposed segregation of chloroplast mutations during vegetative cell division of C. reinhardtii. Wild-type chromosomes are represented by white circles and mutant chromosomes by black circles. The single chloroplast of C. reinhardtii has 60 to 100 chromosomes, SO that 6 to 7 divisions would be required before the segregation of a cell homoplasmic for a chloroplast mutation. 1954; RYAN, FRIED and SCHWARTZ 1954). With such a model, segregation of the mutant allele to more than one daughter cell would usually be delayed until cells become homoplasmic, or nearly so, for this mutation. If all mutant and wild-type alleles replicate once with each chloroplast (or cell) doubling and if the partition of chloroplast chromosomes is numerically equal, then the length of this delay in cell doublings (N) would be related to the average number of chromosomes per chloroplast

Snail shell coiling and maternal effects:

The embryo is formed when a female gamete unites with a male gamete. In the vast majority of species, the female gamete is physically larger than the male gamete and provides the cytoplasm for the developing embryo. Within this cytoplasm are factors that were released by the nuclear genes of the female. Those factors may have specific effects upon the developing embryo. The female cytoplasm also contributes the mitochondria for all species as well as the chloroplast for plant species. These two organelles contain DNA and control certain traits in the offspring. Those phenotypes that are controlled by nuclear factors found in the cytoplasm of the female are said to express a maternal effect. Those phenotypes controlled by organelle genes exhibit maternal inheritance.

The classic phenotype which exhibits maternal effects is coiling direction of snail shells. The coiling phenotype that is seen in the offspring is controlled by the genotype of the mother. The following crosses were made between pure line snails, and the following results were seen. By convention, the female is always given first.

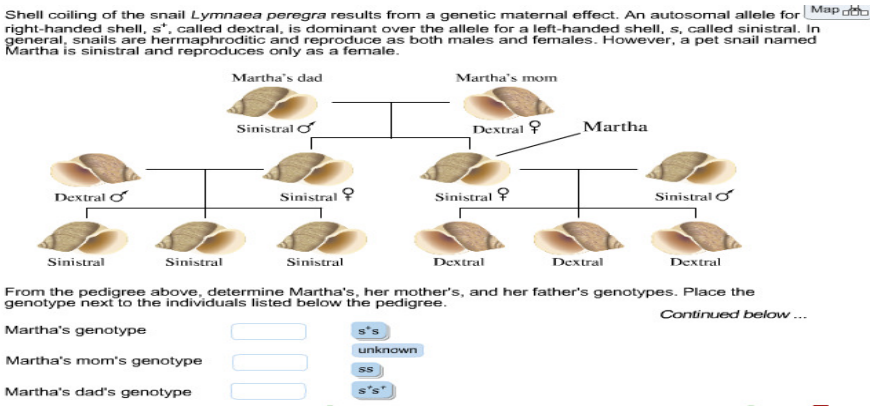
	Cross 1	Cross 2
Parents	Right coil x Left coil	Left coil x Right coil
F1	All right coil	All left coil
F2	All right coil	All right coil
F3	3 right coil : 1 left coil	3 right coil : 1 left coil

These results at first glance appear to be at odds with Mendel's laws. First, the F1 phenotype is not the same for both crosses. With other experiments, the results of reciprocal crosses (complementary crosses were the phenotypes of female and male are reversed in the initial parental cross) were equivalent, but with this experiment it appears that the female controls the phenotype. Yet, the F2 appears to contradict this hypothesis because the left- and right-coiled F1 individuals produced all right progeny. Furthermore, the 3:1 Mendelian ratio is not seen in the F2, but rather appears in the F3 generation.

How can this result be explained? First, let's look for results that are familar. The F3 ratio of 3 right:1 left for both crosses suggests that right-coiled shells are dominant to left-coiled shells. If this is the case, then we can assign the following genotypes to the pure lines:

- Right-coiled shell: *s+s+*
- Left-coiled shell: *ss*

The next observation is that the phenotype of the F1 generation is always that of the female parent. One hypothesis would suggest that the genotype of the female controls the genotype of its offspring. Can these result be confirmed in the subsequent generations? If the genotypes we assigned to the parents are correct, then the genotype of F1 individuals from each cross are s+s (from s+s+ x ss and ss x s+s+). If the female genotype does control the phenotype of its offspring, then we would predict that all the F2 snails would have right coils. This is the exact result that is seen. But what would the genotypes of the F2 snails be? If we intermate snails with the genotype s+s the genotypic ratio should be 3 *s+_* to 1 ss. These genotypes would not be expressed as a phenotype until the F3 generation. These are the results that were obtained. A general conclusion from all traits that express a maternal effect is that the normal Mendelian ratios are expressed one generation than expected.



Kappa Particles in Paramecium:

One of the most striking and spectacular cases of cytoplasmic inheritance occurs in paramecium aurelia. In 1938, T.M. Sonneborn reported that some strains contain kappa particles in the cytoplasm and are known as "Killers ". Kappa particles are about 2 μ x in diameter and contain DNA and protein.
Individuals not possessing Kappa particles are sensitive and are killed by a poison 'paramecin' which is secreted by Killer individuals. The secretion paramecin is harmless to the killers. The different killer strains have different means of killing their victims.

Most of them do not kill their mates. But there are some strains that instead of killing from a distance by secretion, kill their mates through close contact. The killer character has a nuclear as well as cytoplasmic basis.

The existence and increase of Kappa particles is determined by the presence of a nuclear dominant gene K. The animals that are homozygous for recessive 'k' are sensitive to killing and they cannot themselves become killers. Animals that are homozygous for dominant K or heterozygous in normal cytoplasm are potential killers.

They are actual killers when their cytoplasms contain kappa particles which in turn produce the lethal poison. In animals of genotype KK or Kk, kappa particles are transmitted from cell to cell; once they have been lost from a cell, they do not again develop by themselves.

The individuals with genotype kk may also contain Kappa particles of some sort in the cytoplasm, although this state is unstable and eventually the particles disappear.

Paramecium generally reproduces by conjugation method, a system of parasexual reproduction (Fig. 18.6) and autogamy (Fig. 18.6). When a killer strain of paramecium aurelia with genotype KK conjugates with sensitive strain having genotype kk, the ex-conjugants are all heterozygous (Kk).

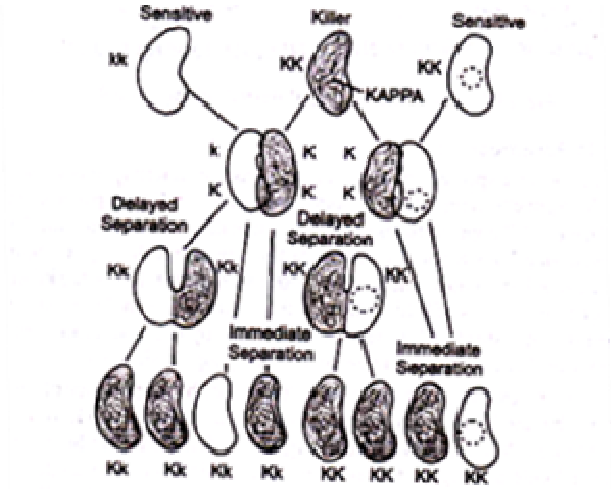


Fig. 18.6 Inheritance of Kappa particle in *Paramecium*.

The genotype Kk of hetrozygous ex-conjugants suggests that they should be identical and killers. But, this is not always the case and Kk hetrozygotes are equally divided into killers and sensitive (Fig. 18.6). If the conjugation is accomplished in a short duration normally no exchange of cytoplasm takes place between the killers and sensitive individuals.

When autogamy takes place in hetrozygotes 8 nuclei are formed after meiosis and mitosis divisions and then 7 of the 8 haploid nuclei degenerate and the remaining nucleus undergoes mitosis and the two identical nuclei so formed fuse to form a homozygous diploid. Thus all the sensitive hetrozygotes produce only sensitive offspring and the killer hetrozygotes produce only killer offspring.

Since a heterozygote Kk after autogamy does not produce sensitive and killer types, this pattern of inheritance is Non-Mendelian which confirms cytoplasmic basis of killer trait. The inheritance of Kappa was at first considered a good example of cytoplasmic inheritance.

These particles are not true cell organelles like the plastids or mitochondria. Closer studies, however, have shown that kappa particles are infectious and resemble bacterium caedobacter taeniospiralis.

Their transmission in cytoplasm from cell to cell is, therefore, more correctly compared with the transmission of parasitic micro-organisms. The toxic substance produced by killer paramecia is diffusible

in liquid medium. This is evident from the fact that when the killers were allowed to remain in a fluid medium for a time and were then replaced by sensitive individuals, the latter were killed.

The toxic substance had no effect on the killer strain. The fact that kappa particles can be maintained only in the animals with gene K is no rigid argument against the interpretations. Individuals with genotype KK will be sensitive if there are no Kappa particles in the cytoplasm.

When such sensitive cells are placed in concentrated suspension of disintegrated killer animals, some of them acquire kappa from the suspension and are changed to killers. Kappa is subject to mutation and if killer Paramecia is exposed to high temperature.

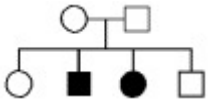
Pedigre Analysis:

Pedigree Analysis is a tabular representation of a family history by taking a particular disease or character into consideration.

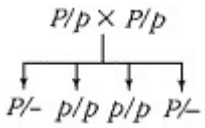
In humans, controlled crosses cannot be made, so geneticists must resort to scrutinizing family records in the hope that informative matings have been made that can be used to deduce dominance and distinguish autosomal from X-linked inheritance. The investigator traces the history of some variant phenotype back through the history of the family and draws up a family tree, or pedigree. The clues in the pedigree have to be interpreted differently depending on whether one of the contrasting phenotypes is a rare disorder or whether both phenotypes of a pair are common morphs of a polymorphism. The genetic disorders of human beings can be dominant or recessive phenotypes and can be either autosomal or X-linked

Autosomal Recessive Disorders

The unusual phenotype of a recessive disorder is determined by homozygosity for a recessive allele, and the unaffected phenotype is determined by the corresponding dominant allele. In Chapter 3 we saw that phenylketonuria (PKU) is a recessive phenotype. PKU is determined by an allele that we can call *p*, and the normal condition by *P*. Therefore, sufferers of this disease are of genotype *p/p*, and unaffected people are either *P/P* or *P/p*. What patterns in a pedigree would reveal such an inheritance? Two key points are that generally the disease appears in the progeny of unaffected parents and that the affected progeny include both males and females equally. When we know that both male and female phenotypic proportions are equal, we can assume that we are dealing with autosomal inheritance, not X-linked inheritance. The following typical pedigree illustrates the key point that affected children are born to unaffected parents:



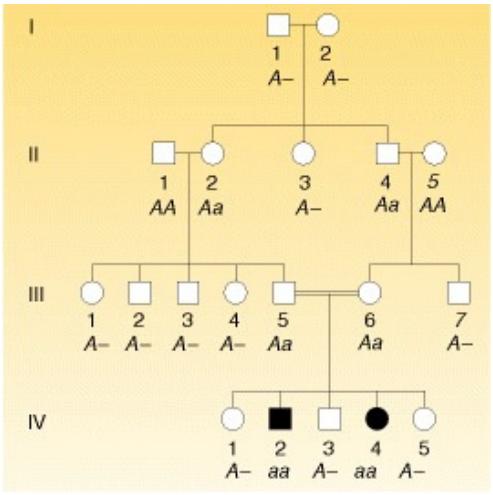
From this pattern we can immediately deduce autosomal inheritance, with the recessive allele responsible for the exceptional phenotype (indicated by shading). Furthermore, we can deduce that the parents must both be heterozygotes, *P/p*. (Both must have a *p* allele because each contributed one to each affected child, and both must have a *P* allele because the people are phenotypically normal.) We can identify the genotypes of the children (in the order shown) as *P/–*, *p/p*, *p/p*, and *P/–*. Hence, the pedigree can be rewritten



Notice another interesting feature of pedigree analysis: even though Mendelian rules are at work, Mendelian ratios are rarely observed in single families because the sample sizes are too small. In the above example, we see a 1:1 phenotypic ratio in the progeny of what is clearly a monohybrid cross, in which we might expect a 3:1 ratio. If the couple were to have, say, 20 children, the ratio would undoubtedly be something like 15 unaffected children and 5 with PKU (the expected monohybrid 3:1 ratio), but in a sample of four any ratio is possible and all ratios are commonly found.

In the case of a rare recessive allele, in the population most of these alleles will be found in heterozygotes, not in homozygotes. The reason is a matter of probability: to conceive a recessive homozygote, both parents must have had the *p* allele, but to conceive a heterozygote all that is necessary is one parent with the allele. The formation of an affected individual usually depends on the chance union of unrelated heterozygotes, and for this reason the pedigrees of autosomal recessives look rather bare, generally with only siblings of one cross affected.

Inbreeding (mating between relatives) increases the chance that a mating will be between two heterozygotes. An example of a cousin marriage is shown in Figure 4-18. Individuals III-5 and III-6 are first cousins and produce two children. You can see from the figure that an ancestor who is a heterozygote may produce many descendants who are also heterozygotes. Matings between relatives thus run a higher risk of producing abnormal homozygous recessives than do matings between nonrelatives. It is for this reason that first cousin marriages are responsible for a large portion of recessive diseases in human populations.



Pedigree of a rare recessive phenotype determined by a recessive allele*a*. Gene symbols normally are not included in pedigree charts, but genotypes are inserted here for reference. Note that individuals II-1 andII-5 marry into the family; they are assumed to be normal because the heritable condition under scrutiny is rare. Note also that it is not possible to be certain of the genotype in some individuals with normal phenotype; such individuals are indicated by A–

Albinism is another rare condition that is inherited in a Mendelian manner as an autosomal recessive phenotype in many animals, including humans. The striking "white" phenotype is caused by a defect in an enzyme that synthesizes melanin, the pigment responsible for most black and brown coloration of animals. In humans, such coloration is most evident in hair, skin, and retina, and its absence in albinos (who have the homozygous recessive genotype *a/a*) leads to white hair, white skin, and eye pupils that are pink because of the unmasking of the red hemoglobin pigment in blood vessels in the retina.

In pedigree analysis, the main clues for identifying an autosomal dominant disorder are that the phenotype tends to appear in every generation of the pedigree and that affected fathers and mothers transmit the phenotype to both sons and daughters. Again, the representation of both sexes among the affected offspring argues against X-linked inheritance. The phenotype appears in every generation because generally the abnormal allele carried by an individual must have come from a parent in the previous generation. (Abnormal alleles can arise de novo by mutation. This is relatively rare, but must be kept in mind as a possibility.) A typical pedigree for a dominant disorder . Once again, notice that Mendelian ratios are not necessarily observed in families. As with recessive disorders, individuals bearing one copy of the rare allele (*A/a*) are much more common than those bearing two copies (*A/A*), so most affected people are heterozygotes, and virtually all matings involving dominant disorders are *A/a* × *a/a*. Therefore, when the progeny of such matings are totaled, a 1:1 ratio is expected of unaffected (*a/a*) to affected individuals (*A/a*).

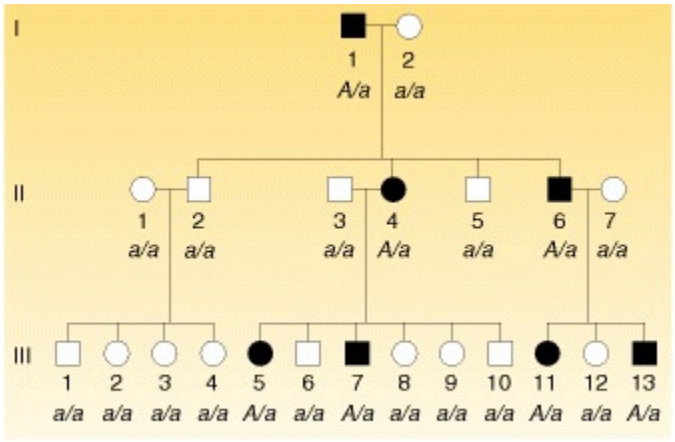


Figure 4-19

Pedigree of a dominant phenotype determined by a dominant allele *A*. In this pedigree, all the genotypes have been deduced.

Huntington's disease is an example of an autosomal dominant disorder. The phenotype is one of neural degeneration, leading to convulsions and premature death. However, it is a late-onset disease, the symptoms generally not appearing until after the person has begun to have children. Each child of a carrier of the abnormal allele stands a 50 percent chance of inheriting the allele and the associated disease. This tragic pattern has led to a drive to find ways of identifying people who carry the abnormal allele before they experience the onset of the disease. The discovery of the molecular nature of the mutant allele, and of neutral DNA mutations that act as "markers" close to the affected allele on the chromosome, has revolutionized this sort of diagnosis.

MESSAGE

Pedigrees of autosomal dominant disorders show affected males and females in each generation and also show affected men and women transmitting the condition to equal proportions of their sons and daughters.

LOD score for Linkage testing:

The LOD score (logarithm (base 10) of odds), developed by Newton Morton, is a statistical test often used for linkage analysis in human, animal, and plant populations. The LOD score compares the likelihood of obtaining the test data if the two loci are indeed linked, to the likelihood of observing the same data purely by chance. Positive LOD scores favour the presence of linkage, whereas negative LOD scores indicate that linkage is less likely. Computerised LOD score analysis is a simple way to analyse complex family pedigrees in order to determine the linkage between Mendelian traits (or between a trait and a marker, or two markers).

The method is described in greater detail by Strachan and Read. Briefly, it works as follows:

1. Establish a pedigree
2. Make a number of estimates of recombination frequency
3. Calculate a LOD score for each estimate
4. The estimate with the highest LOD score will be considered the best estimate

The LOD score is calculated as follows:

LOD=Z=$\log_{10}$ Probability of birth sequence with a given lnkage value=$\log_{10}$ $(1-\theta)^{NR}\times\theta^{R}$

Probability of birth sequence with no linkage $(0.5^{(NR+R)})$

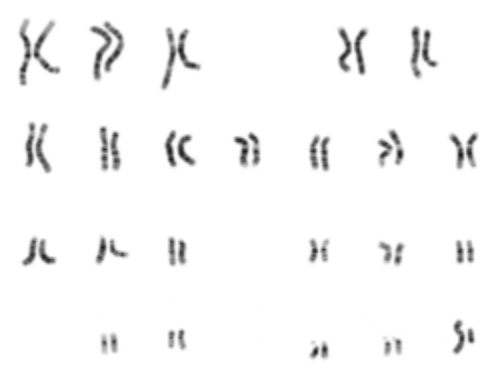
NR denotes the number of non-recombinant offspring, and R denotes the number of recombinant offspring. The reason 0.5 is used in the denominator is that any alleles that are completely unlinked (e.g. alleles on separate chromosomes) have a 50% chance of recombination, due to independent assortment. 'θ' is the recombinant fraction, i.e. the fraction of births in which recombination has happened between the studied genetic marker and the putative gene associated with the disease. Thus, it is equal to R / (NR + R)

By convention, a LOD score greater than 3.0 is considered evidence for linkage, as it indicates 1000 to 1 odds that the linkage being observed did not occur by chance. On the other hand, a LOD score less than -2.0 is considered evidence to exclude linkage. Although it is very unlikely that a LOD score of 3 would be obtained from a single pedigree, the mathematical properties of the test allow data from a number of pedigrees to be combined by summing their LOD scores. A LOD score of 3 translates to a *p*-value of approximately 0.05,[9] and no multiple testing correction (e.g. Bonferroni correction) is required.

Karyotype

Karyotyping is the process by which cytogeneticists take photographs of chromosomes in order to determine the chromosome complement of an individual, including the number of chromosomes and any abnormalies. The term is also used for the complete set of chromosomes in a species or in an individual organism and for a test that detects this complement or measures the number.

Karyotypes describe the chromosome count of an organism and what these chromosomes look like under a light microscope. Attention is paid to their length, the position of the centromeres, banding pattern, any differences between the sex chromosomes, and any other physical characteristics. The preparation and study of karyotypes is part of cytogenetics.



Karyogram of human male using Giemsa staining

The study of whole sets of chromosomes is sometimes known as *karyology*. The chromosomes are depicted (by rearranging a photomicrograph) in a standard format known as a *karyogram* or *idiogram*: in pairs, ordered by size and position of centromere for chromosomes of the same size.

The basic number of chromosomes in the somatic cells of an individual or a species is called the *somatic number* and is designated *2n*. In the germ-line (the sex cells) the chromosome number is *n* (humans: n = 23). Thus, in humans 2n = 46.

So, in normal diploid organisms, autosomal chromosomes are present in two copies. There may, or may not, be sex chromosomes. Polyploid cells have multiple copies of chromosomes and haploid cells have single copies.

The study of karyotypes is important for cell biology and genetics, and the results may be used in evolutionary biology (*karyosystematics*) and medicine. Karyotypes can be used for many purposes; such as to study chromosomal aberrations, cellular function, taxonomic relationships, and to gather information about past evolutionary events.

Observations On Karyotypes

Staining

The study of karyotypes is made possible by staining. Usually, a suitable dye, such as Giemsa,[19] is applied after cells have been arrested during cell division by a solution of colchicine usually in metaphase or prometaphase when most condensed. In order for the Giemsa stain to adhere correctly, all chromosomal proteins must be digested and removed. For humans, white blood cells are used most frequently because they are easily induced to divide and grow in tissue culture. Sometimes observations may be made on non-dividing (interphase) cells. The sex of an unborn fetus can be determined by observation of interphase cells (see amniotic centesis and Barr body).

Observations

Six different characteristics of karyotypes are usually observed and compared:

1. Differences in absolute sizes of chromosomes. Chromosomes can vary in absolute size by as much as twenty-fold between genera of the same family. For example, the legumes *Lotus tenuis* and *Vicia faba* each have six pairs of chromosomes, yet *V. faba* chromosomes are many times larger. These differences probably reflect different amounts of DNA duplication.
2. Differences in the position of centromeres. These differences probably came about through translocations.
3. Differences in relative size of chromosomes. These differences probably arose from segmental interchange of unequal lengths.
4. Differences in basic number of chromosomes. These differences could have resulted from successive unequal translocations which removed all the essential genetic material from a chromosome, permitting its loss without penalty to the organism (the dislocation hypothesis) or through fusion. Humans have one pair fewer chromosomes than the great apes. Human chromosome 2 appears to have resulted from the fusion of two ancestral chromosomes, and many of the genes of those two original chromosomes have been translocated to other chromosomes.
5. Differences in number and position of satellites. Satellites are small bodies attached to a chromosome by a thin thread.
6. Differences in degree and distribution of heterochromatic regions. Heterochromatin stains darker than euchromatin. Heterochromatin is packed tighter. Heterochromatin consists mainly of genetically inactive and repetitive DNA sequences as well as containing a larger amount of Adenine-Thymine pairs. Euchromatin is usually under active transcription and stains much lighter as it has less affinity for the giemsa stain Euchromatin regions contain larger amounts of Guanine-Cytosine pairs. The staining technique using giemsa staining is called G banding and therefore produces the typical "G-Bands".

A full account of a karyotype may therefore include the number, type, shape and banding of the chromosomes, as well as other cytogenetic information.

Variation is often found:

1. between the sexes,
2. between the germ-line and soma (between gametes and the rest of the body),
3. between members of a population (chromosome polymorphism),
4. in geographic specialization, and
5. in mosaics or otherwise abnormal individuals.

Human karyotype

human karyotype (male)

The normal human karyotypes contain 22 pairs of autosomal chromosomes and one pair of sex chromosomes (allosomes). Normal karyotypes for females contain two X chromosomes and are denoted 46,XX; males have both an X and a Y chromosome denoted 46,XY. Any variation from the standard karyotype may lead to developmental abnormalities.

Autosomal dominant

Only one mutated copy of the gene will be necessary for a person to be affected by an autosomal dominant disorder. Each affected person usually has one affected parent. The chance a child will inherit the mutated gene is 50%. Autosomal dominant conditions sometimes have reduced penetrance, which means although only one mutated copy is needed, not all individuals who inherit that mutation go on to develop the disease. Examples of this type of disorder are Huntington's disease, neurofibromatosis type 1, neurofibromatosis type 2, Marfan syndrome, hereditary nonpolyposis colorectal cancer, hereditary multiple exostoses (a highly penetrant autosomal dominant disorder), Tuberous sclerosis, Von Willebrand disease, and acute intermittent porphyria. Birth defects are also called congenital anomalies.

Diversity and evolution of Karyotypes

Although the replication and transcription of DNA is highly standardized in eukaryotes, the same cannot be said for their karyotypes, which are highly variable. There is variation between species in chromosome number, and in detailed organization, despite their construction from the same macromolecules. This variation provides the basis for a range of studies in evolutionary cytology.In some cases there is even significant variation within species.

Genetic disorder

A genetic disorder is a genetic problem caused by one or more abnormalities formed in the genome. Most genetic disorders are quite rare and affect one person in every several thousands or millions. The earliest known genetic condition in a hominid was in the fossil species *Paranthropus robustus,* with over a third of individuals displaying Amelogenesis imperfecta.

Genetic disorders may be hereditary or non-hereditary, meaning that they are passed down from the parents' genes. However, in some genetic disorders, defects may be caused by new mutations or changes to the DNA. In such cases, the defect will only be passed down if it occurs in the germline. Genetic disorders can be monogenic, multifactoral, or chromosomal.

Single-Gene

A single-gene (or monogenic) disorder is the result of a single mutated gene. Over 6000 human diseases are caused by single-gene defects.[9] Single-gene disorders can be passed on to subsequent generations in

several ways. Genomic imprinting and uniparental disomy, however, may affect inheritance patterns. The divisions between recessive and dominant types are not "hard and fast", although the divisions between autosomal and X-linked types are (since the latter types are distinguished purely based on the chromosomal location of the gene). For example, achondroplasia is typically considered as a dominant disorder, but children with two genes for achondroplasia have a severe skeletal disorder of which achondroplasics could be viewed as carriers. Sickle-cell anemia is also considered as a recessive condition, but heterozygous carriers have increased resistance to malaria in early childhood, which could be described as a related dominant condition.

Autosomal recessive

Two copies of the gene must be mutated for a person to be affected by an autosomal recessive disorder. An affected person usually has unaffected parents who each carry a single copy of the mutated gene and are referred to as "carriers". Each parent with a defective gene normally do not have symptoms. Two unaffected people who each carry one copy of the mutated gene have a 25% risk with each pregnancy of having a child affected by the disorder. Examples of this type of disorder are Albinism, Medium-chain acyl-CoA dehydrogenase deficiency, cystic fibrosis, sickle-cell disease, Tay–Sachs disease, Niemann-Pick disease, spinal muscular atrophy, and Roberts syndrome. Certain other phenotypes, such as wet versus dry earwax, are also determined in an autosomal recessive fashion.

X-linked dominant

X-linked dominant disorders are caused by mutations in genes on the X chromosome. Only a few disorders have this inheritance pattern, with a prime example being X-linked hypophosphatemic rickets. Males and females are both affected in these disorders, with males typically being more severely affected than females. Some X-linked dominant conditions, such as Rett syndrome, incontinentia pigmenti type 2, and Aicardi syndrome, are usually fatal in males either *in utero* or shortly after birth, and are therefore predominantly seen in females. Exceptions to this finding are extremely rare cases in which boys with Klinefelter syndrome (47,XXY) also inherit an X-linked dominant condition and exhibit symptoms more similar to those of a female in terms of disease severity. The chance of passing on an X-linked dominant disorder differs between men and women. The sons of a man with an X-linked dominant disorder will all be unaffected (since they receive their father's Y chromosome), and his daughters will all inherit the condition. A woman with an X-linked dominant disorder has a 50% chance of having an affected fetus with each pregnancy, although in cases such as incontinentia pigmenti, only female offspring are generally viable.

X-linked recessive

X-linked recessive conditions are also caused by mutations in genes on the X chromosome. Males are more frequently affected than females, and the chance of passing on the disorder differs between men and women. The sons of a man with an X-linked recessive disorder will not be affected, and his daughters will carry one copy of the mutated gene. A woman who is a carrier of an X-linked recessive disorder (X^RX^r)

has a 50% chance of having sons who are affected and a 50% chance of having daughters who carry one copy of the mutated gene and are therefore carriers. X-linked recessive conditions include the serious diseases hemophilia A, Duchenne muscular dystrophy, and Lesch-Nyhan syndrome, as well as common and less serious conditions such as male pattern baldness and red-green color blindness. X-linked recessive conditions can sometimes manifest in females due to skewed X-inactivation or monosomy X (Turner syndrome).

Mutifactorial Disorder:

Genetic disorders may also be complex, multifactorial, or polygenic, meaning they are likely associated with the effects of multiple genes in combination with lifestyles and environmental factors. Multifactorial disorders include heart disease and diabetes. Although complex disorders often cluster in families, they do not have a clear-cut pattern of inheritance. This makes it difficult to determine a person's risk of inheriting or passing on these disorders. Complex disorders are also difficult to study and treat, because the specific factors that cause most of these disorders have not yet been identified. Studies which aim to identify the cause of complex disorders can use several methodological approaches to determine genotype-phenotype associations. One method, the genotype-first approach, starts by identifying genetic variants within patients and then determining the associated clinical manifestations. This is opposed to the more traditional phenotype-first approach, and may identify causal factors that have previously been obscured by clinical heterogeneity, penetrance, and expressivity.

On a pedigree, polygenic diseases do tend to "run in families", but the inheritance does not fit simple patterns as with Mendelian diseases. But this does not mean that the genes cannot eventually be located and studied. There is also a strong environmental component to many of them (e.g., blood pressure).

Chromosomal Disorder:

A chromosomal disorder is a missing, extra, or irregular portional of chromosomal DNA. It can be from an atypical number of chromosome or a structural abnormality in one or more chromosome. An example of these disorder is Trisomy 21 (Down syndrome), in which there is an extra copy of chromosome 21.

Heritability and its measurements:

Amount of phenotypic (observable) variation in a population that is attributable to individual genetic differences. Heritability, in a general sense, is the ratio of variation due to differences between genotypes to the total phenotypic variation for a character or trait in a population. The concept typically is applied in behaviour genetics and quantitative genetics, where heritability estimates are calculated by using either correlation and regression methods or analysis of variance (ANOVA) methods.

Heritability is expressed as $H^2 = Vg/Vp$, where H is the heritability estimate, Vg the variation in genotype, and Vp the variation in phenotype. Heritability estimates range in value from 0 to 1. If $H = 1$, then all variation in a population is due to differences or variation between genotypes (i.e., there is no environmentally caused variation). If $H = 0$, there is no genetic variation; in this case all variation in the population comes from differences in the environments experienced by individuals.

Heritability is commonly used in twin studies in the field of behaviour genetics. The methodology is based on the fact that identical twins (monozygotic, or one-egg twins) share 100 percent of their genes in common and nonidentical, or fraternal, twins (dizygotic, or two-egg twins) are similar to other siblings (i.e., brothers and sisters) in that they share 50 percent of their genes in common. The correlation between identical twins is expected to be equal to 1.0 and that of fraternal twins to be 0.50. In the field of quantitative genetics, the concept of heritability is used to partition observable phenotypic variation between individuals into genetic and environmental components.

There are several drawbacks to the use of heritability estimates. First, heritability is not a measurement of how sensitive a character or trait might be to a change in environment. For example, a trait may have complete heritability ($H = 1$) yet be altered drastically by environmental change. This can be seen in certain genetic disorders of metabolism, such as phenylketonuria and Wilson disease, where heritability of phenotypic outcomes equals 1.0 but effective treatment is possible through dietary interventions. A second problem with heritability estimates is that they measure variation only within populations. In other words, a heritability estimate cannot be used to determine the causes of differences between populations, nor can it be used to determine the extent to which an individual's phenotype is determined by genes versus environment.

Quantitative Trait Locus

A quantitative trait locus (QTL) is a locus (section of DNA) which correlates with variation of a quantitative trait in the phenotype of a population of organisms.[1] QTLs are mapped by identifying which molecular markers (such as SNPs or AFLPs) correlate with an observed trait. This is often an early step in identifying and sequencing the actual genes that cause the trait variation.

For organisms whose genomes are known, one might now try to exclude genes in the identified region whose function is known with some certainty not to be connected with the trait in question. If the genome is not available, it may be an option to sequence the identified region and determine the putative functions of genes by their similarity to genes with known function, usually in other genomes. This can be done using BLAST, an online tool that allows users to enter a primary sequence and search for similar sequences within the BLAST database of genes from various organisms. It is often not the actual gene underlying the phenotypic trait, but rather a region of DNA that is closely linked with the gene.

Another interest of statistical geneticists using QTL mapping is to determine the complexity of the genetic architecture underlying a phenotypic trait. For example, they may be interested in knowing whether a phenotype is shaped by many independent loci, or by a few loci, and do those loci interact. This can provide information on how the phenotype may be evolving.

In a recent development, classical QTL analyses were combined with gene expression profiling i.e. by DNA microarrays. Such expression QTLs (eQTLs) describe cis- and trans-controlling elements for the expression of often disease-associated genes.[17] Observed epistatic effects have been found beneficial to identify the gene responsible by a cross-validation of genes within the interacting loci with metabolic pathway- and scientific literature databases.

Analysis of variance

The simplest method for QTL mapping is analysis of variance (ANOVA, sometimes called "marker regression") at the marker loci. In this method, in a backcross, one may calculate a t-statistic to compare the averages of the two marker genotype groups. For other types of crosses (such as the intercross), where there are more than two possible genotypes, one uses a more general form of ANOVA, which provides a so-called F-statistic. The ANOVA approach for QTL mapping has three important weaknesses. First, we do not receive separate estimates of QTL location and QTL effect. QTL location is indicated only by looking at which markers give the greatest differences between genotype group averages, and the apparent QTL effect at a marker will be smaller than the true QTL effect as a result of recombination between the marker and the QTL. Second, we must discard individuals whose genotypes are missing at the marker. Third, when the markers are widely spaced, the QTL may be quite far from all markers, and so the power for QTL detection will decrease.

Interval mapping

Lander and Botstein developed interval mapping, which overcomes the three disadvantages of analysis of variance at marker loci.[18] Interval mapping is currently the most popular approach for QTL mapping in experimental crosses. The method makes use of a genetic map of the typed markers, and, like analysis of variance, assumes the presence of a single QTL. In interval mapping, each locus is considered one at a time and the logarithm of the odds ratio (LOD score) is calculated for the model that the given locus is a true QTL. The odds ratio is related to the Pearson correlation coefficient between the phenotype and the marker genotype for each individual in the experimental cross

The term ‘interval mapping’ is used for estimating the position of a QTL within two markers (often indicated as ‘marker-bracket’). Interval mapping is originally based on the maximum likelihood but there are also very good approximations possible with simple regression.

The principle for QTL mapping is: 1) The Likelihood can be calculated for a given set of parameters (particularly QTL effect and QTL position) given the observed data on phenotypes and marker genotypes. 2) The estimates for the parameters are those where the likelihood are highest. 3) A significance threshold can be established by permutation testing.

Question:

Long answer question:

1. Explain Orgenelle heredity
2. Chloroplast mutation in *Chamydomonas*
3. Mitochondrial mutation in *saccharomyces*.
4. Explain Meternal effect with example.
5. Account on kappa particles in *Paramecium*.
6. Pedigree Analysis.

Short answer question:

1. What is Pedigree
2. LOD score for linkage
3. Karyotypes
4. Polygenic inheritance
5. Qlt mapping.

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

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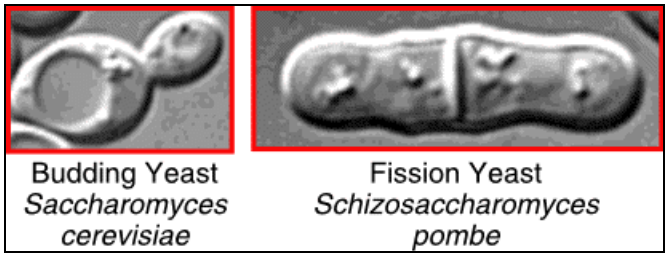
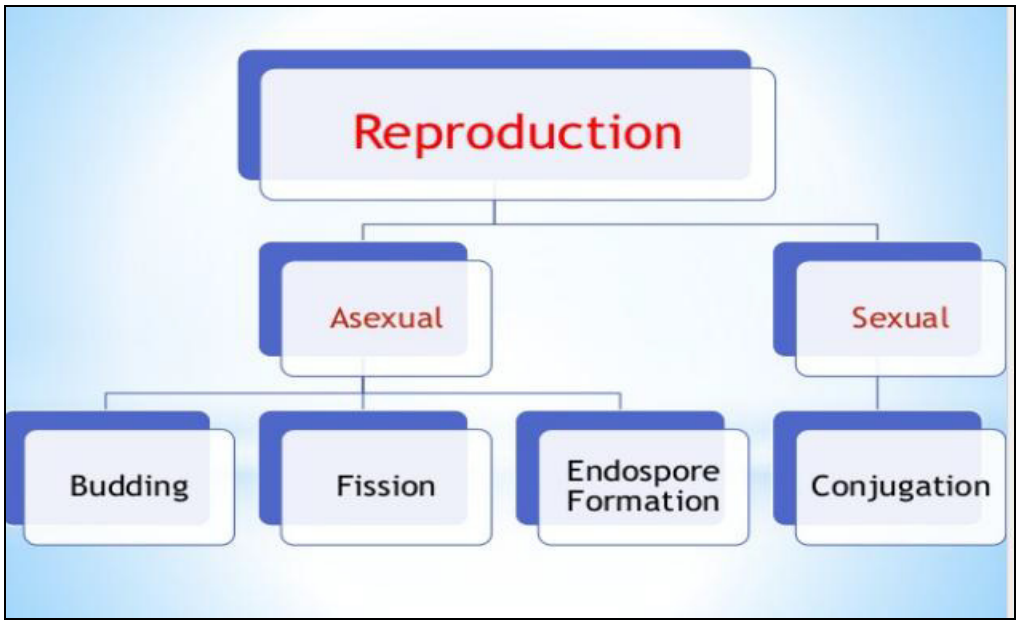


***Saccharomyces cerevisiae* is a eukaryote:**

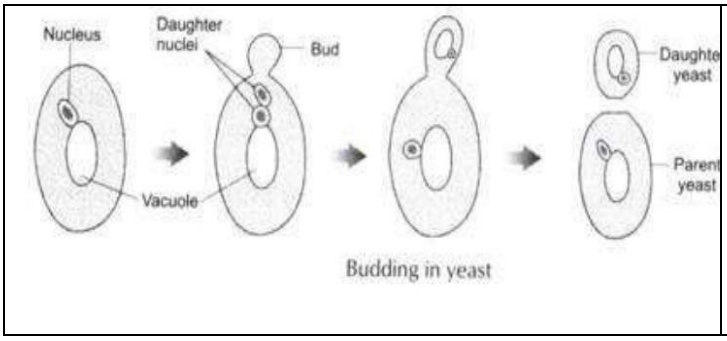
- Belongs to fungi, ascomycetes
- Unicellular organism with ability to produce pseudohyphae
- *S. cerevisiae* divides by budding (hence: budding yeast) while *Schizosaccharomyces pombe* divides by fission (hence: fission yeast)
- Budding results in two cells of unequal size, a mother (old cell) and a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

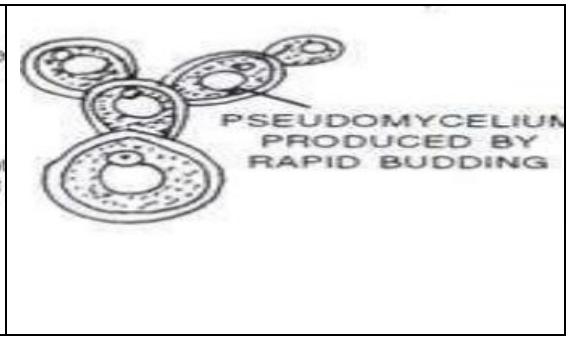
- Vacuole as storage and hydrolytic organelle
- Secretory pathway with endoplasmic reticulum, Golgi apparatus and secretory vesicles
- Peroxisomes for oxidative degradation
- Mitochondria for respiration.

Life cycle of yeasts:



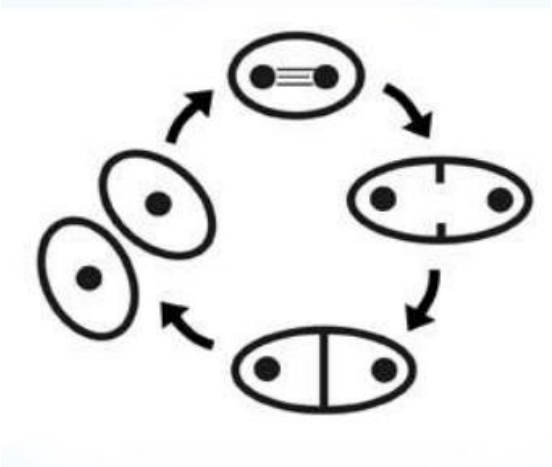
Budding: Most common method, Under favourable condition the yeast cell gives rise to a tiny outgrowth that gradually increase in size. The nucleus divides amitotically, one of which migrates into the newly forme outgrowth and forms a bud. The bud seperates from parent and becomes independent.





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

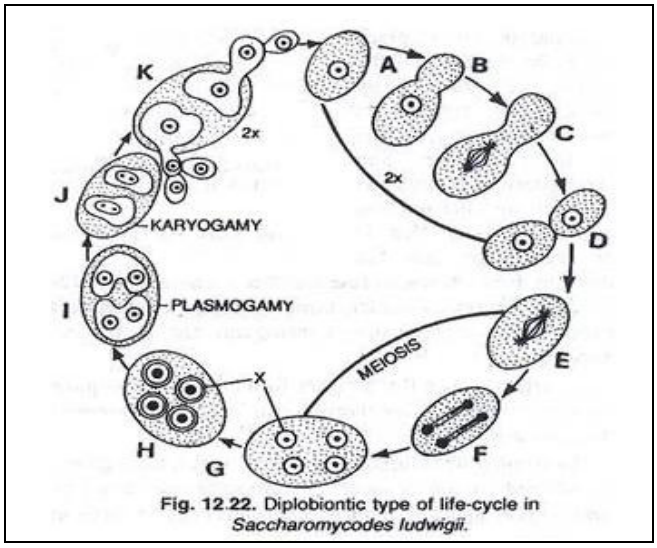


Fig. 12.22. Diplobiontic type of life-cycle in *Saccharomycodes ludwigii.*

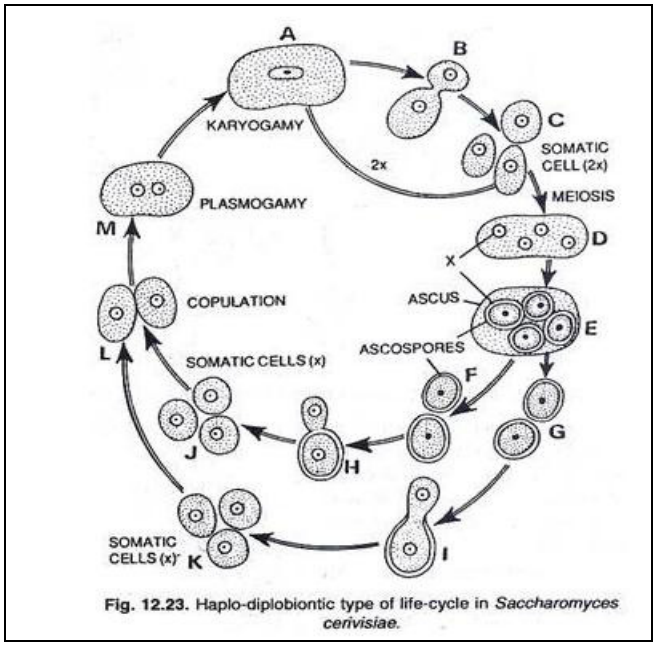
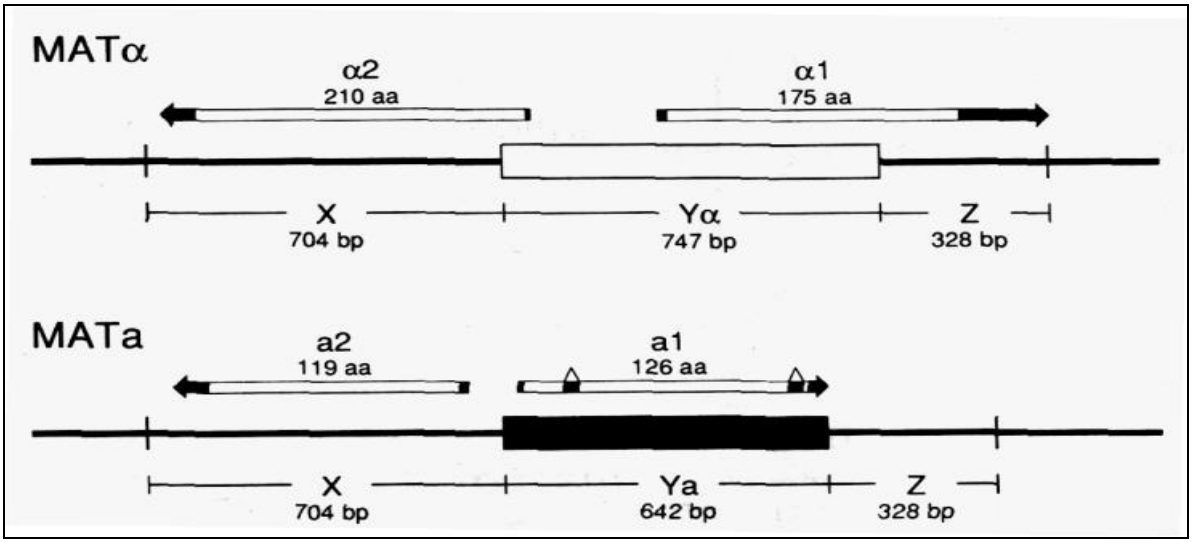


Fig. 12.23. Haplo-diplobiontic type of life-cycle in *Saccharomyces cerivisiae.*

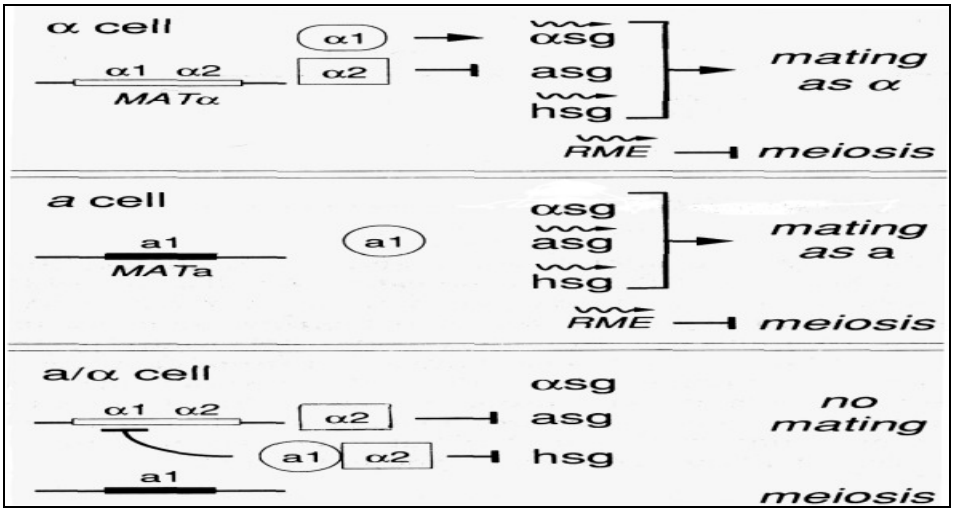
Genetic determination of yeast cell type:

- 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 alphaspecific 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 adiploid, 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 uncharacterized 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 localization
- 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 targeted
- 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 caneasily 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.

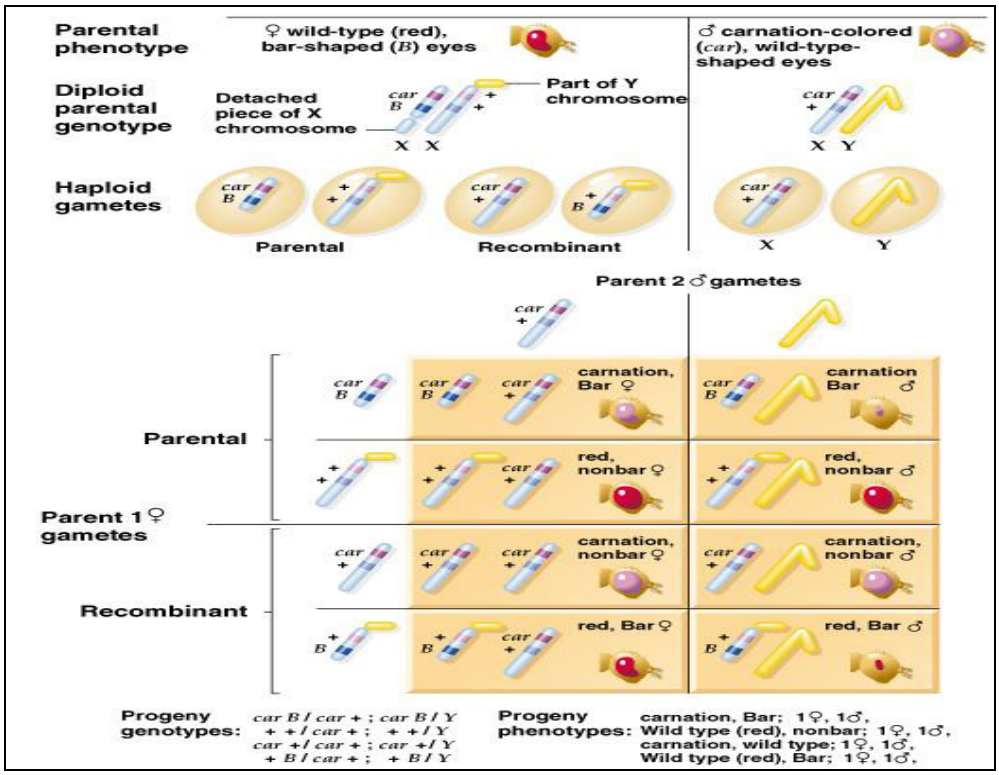
Complementation groups

- 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:

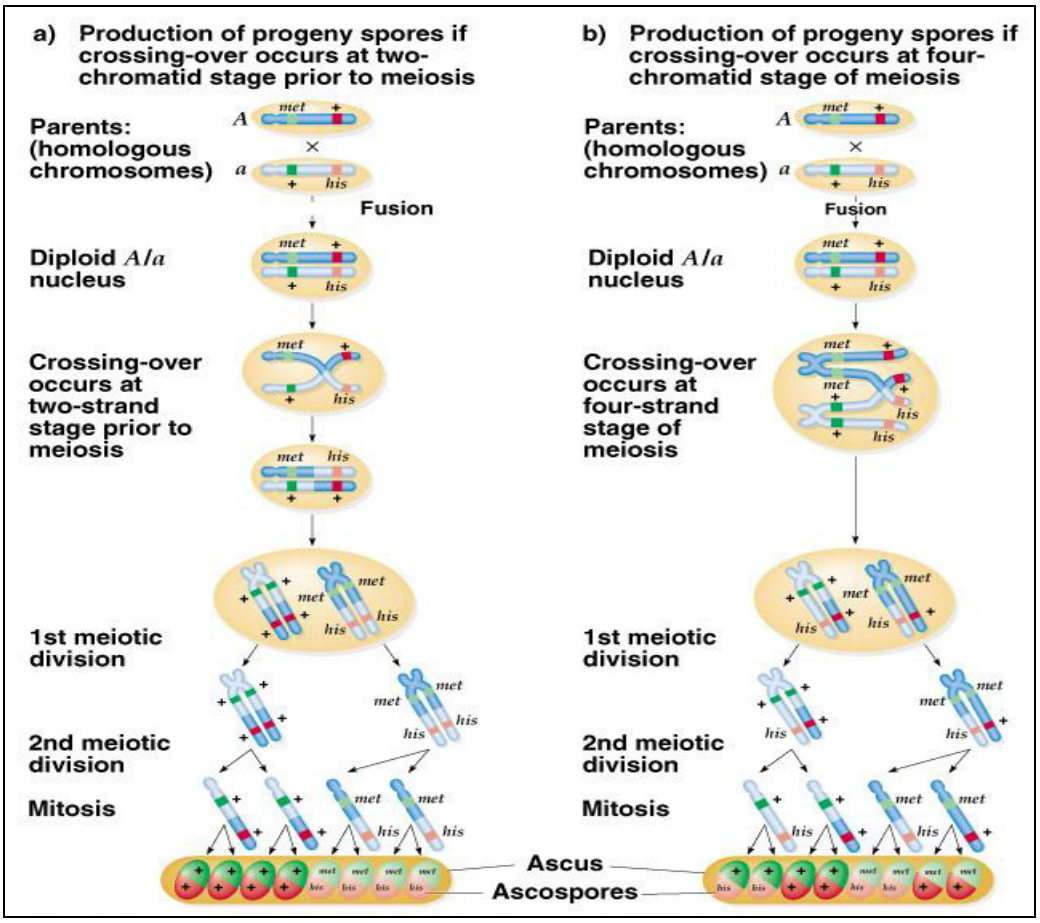
- Tetrad refers to the four haploid gametes produced by meiosis.

- 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):

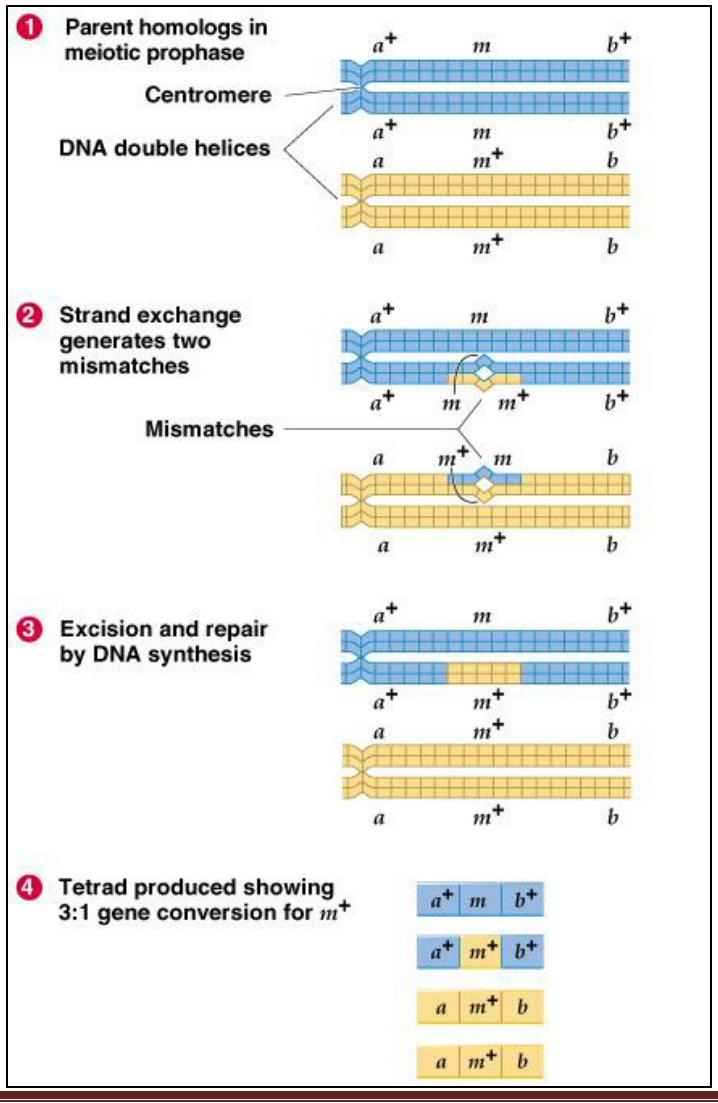
- Yeast (*Saccharomyces cerevisiae*)
- Green algae (*Chlamydomonas reinhardtii*)
- Orange bread mold (*Neurospora crassa*)

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

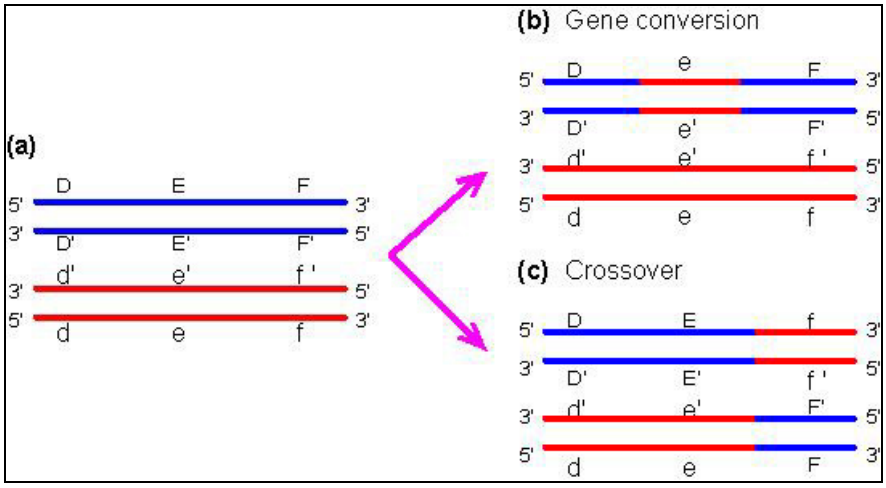


Gene conversion:

- 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).

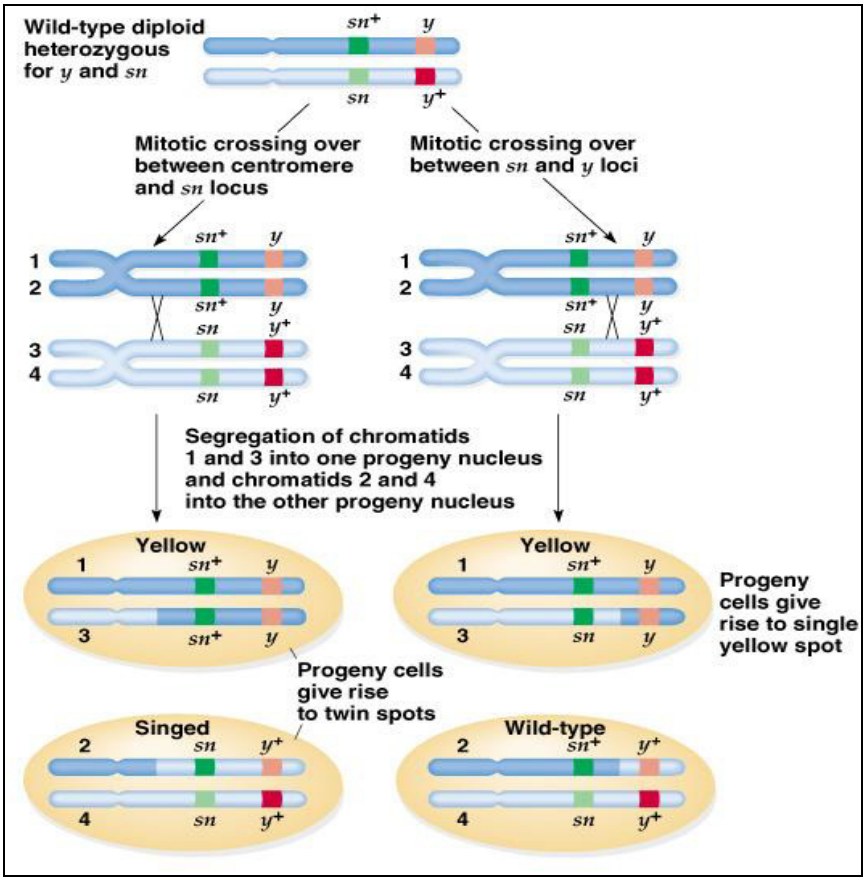


Gene conversion vs crossing over:



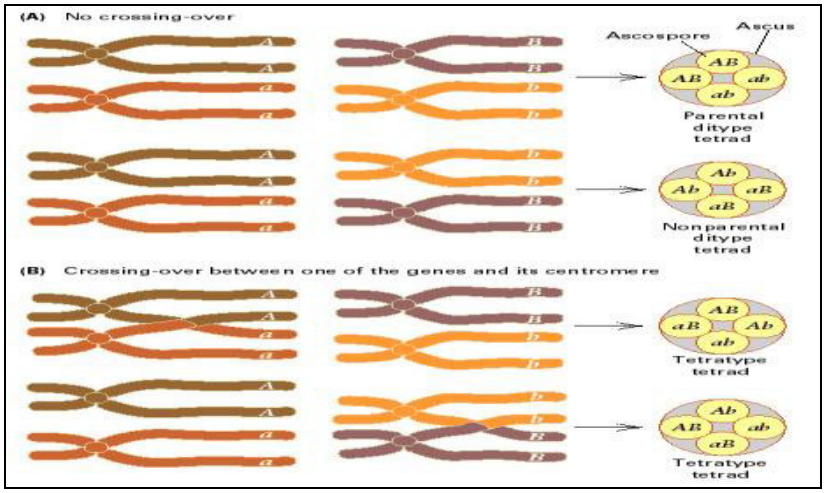
Mitotic recombination:

- 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.



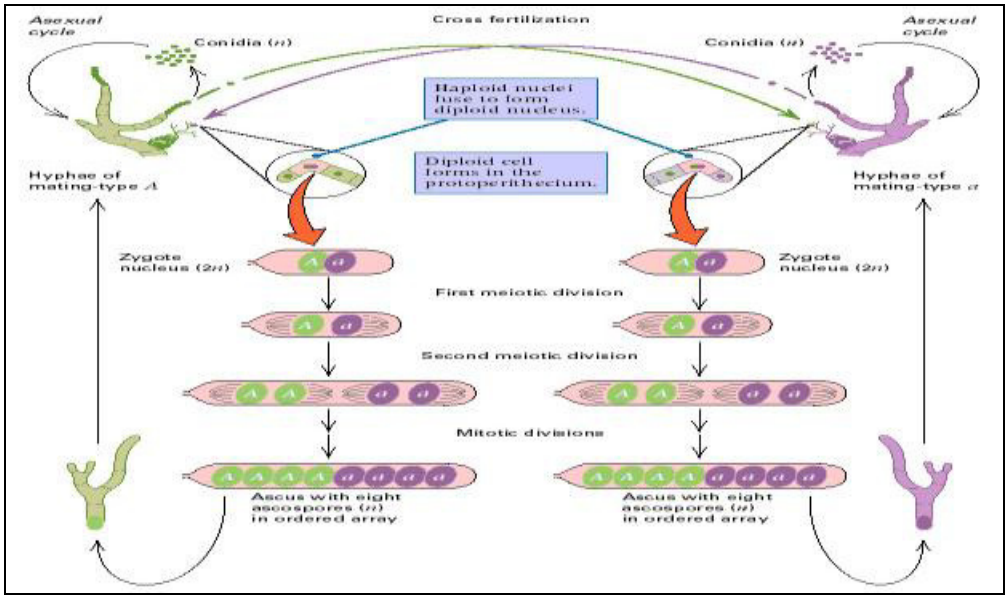
Mapping in *Neurospora*

- 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 o 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 junctionresolving Enzyme.

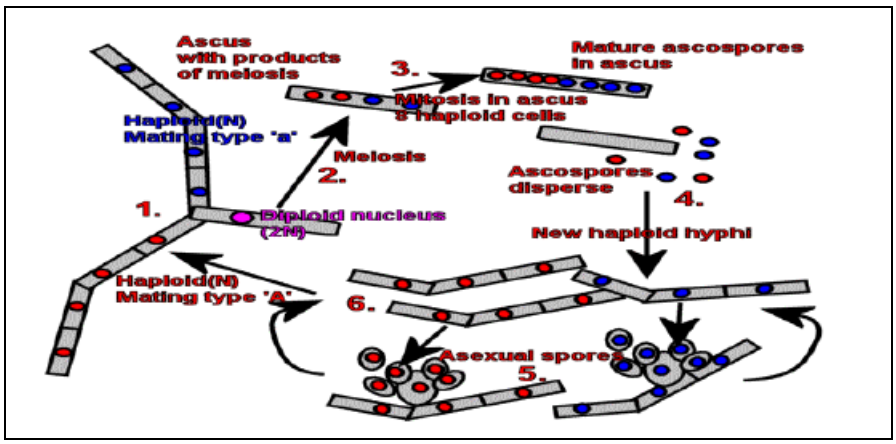


Fig:Neurospora Life Cycle- advantages for genetics

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.

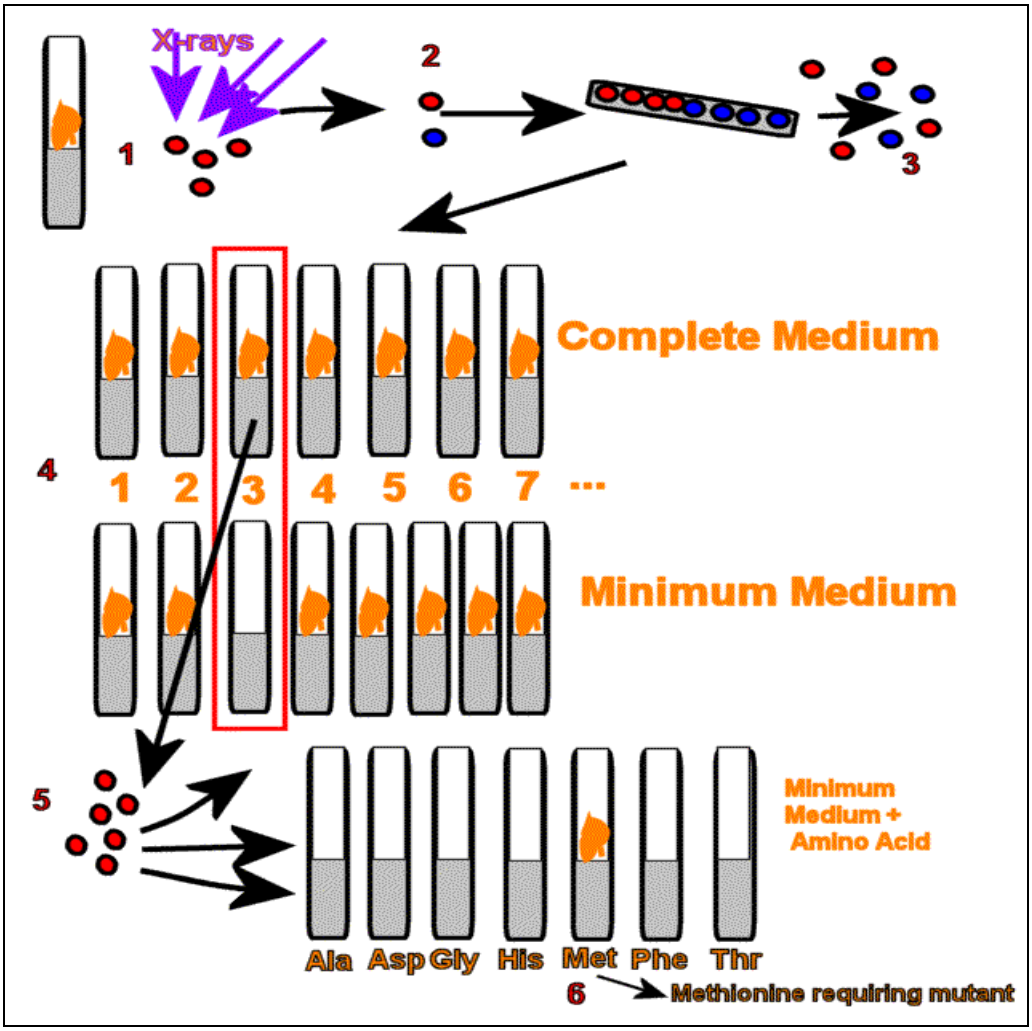


Fig: Beadle and Tatum's Experimental Techniques

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

Unit V:
Sylabus

Structural organization of chromosomes-centromere, telomeres and repitive DNA, Packaging of DNA molecules into chromosomes, concept of Euchromatin and Hetrochromatin, Normal and abnormal karyotypes of human chromosomes, Chromosome banding, Giant chromosome, Polytene and Lampbrush chromosomes, Variations in chromosome structure: deletion, inversion, translocation, Variations in chromosome numbers and structural abnormalities: Klinefelter syndrome, Turner syndrome, Down syndrome.

Structural Organization of chromosomes:

Centromere, structure in a chromosome that holds together the two chromatids (the daughter strands of a replicated chromosome). The centromere is the point of attachment of the kinetochore, a structure to which the microtubules of the mitotic spindle become anchored. The spindle is the structure that pulls the chromatids to opposite ends of the cell during the cell division processes of mitosis and meiosis. Once separated, each chromatid becomes a chromosome. Thus, when the cell divides, both daughter cells have complete sets of chromosomes.

The **centromere** is the specialized DNA sequence of a chromosome that links a pair of sister chromatids (a dyad). During mitosis, spindle fibers attach to the centromere via the kinetochore. Centromeres were first thought to be genetic loci that direct the behavior of chromosomes.

The physical role of the centromere is to act as the site of assembly of the kinetochores – a highly complex multiprotein structure that is responsible for the actual events of chromosome segregation – i.e. binding microtubules and signalling to the cell cycle machinery when all chromosomes have adopted correct attachments to the spindle, so that it is safe for cell division to proceed to completion and for cells to enter anaphase.

There are, broadly speaking, two types of centromeres. "Point centromeres" bind to specific proteins that recognize particular DNA sequences with high efficiency. Any piece of DNA with the point centromere DNA sequence on it will typically form a centromere if present in the appropriate species. The best characterised point centromeres are those of the budding yeast, *Saccharomyces cerevisiae*. "Regional centromeres" is the term coined to describe most centromeres, which typically form on regions of preferred DNA sequence, but which can form on other DNA sequences as well. The signal for formation of a regional centromere appears to be epigenetic. Most organisms, ranging from the fission yeast *Schizosaccharomyces pombe* to humans, have regional centromeres.

Positions

Each chromosome has two arms, labeled *p* (the shorter of the two) and *q* (the longer). Many remember that the short arm 'p' is named for the French word "petit" meaning 'small', although this explanation was shown to be apocryphal. They can be connected in either metacentric, submetacentric, acrocentric or telocentric manner

Metacentric

These are X-shaped chromosomes, with the centromere in the middle so that the two arms of the chromosomes are almost equal.

A chromosome is metacentric if its two arms are roughly equal in length. In a normal human karyotype, five chromosomes are considered metacentric: chromosomes 1, 3, 16, 19, and 20. In some cases, a metacentric chromosome is formed by balanced translocation: the fusion of two acrocentric chromosomes to form one metacentric chromosome.

Submetacentric

If arms' lengths are unequal, the chromosome is said to be submetacentric. Their shape is L shape.[11]

Acrocentric

If the p (short) arm is so short that it is hard to observe, but still present, then the chromosome is acrocentric (the "acro-" in acrocentric refers to the Greek word for "peak"). The human genome includes five acrocentric chromosomes: 13, 14, 15, 21, 22.The Y chromosome is also acrocentric

In an acrocentric chromosome the p arm contains genetic material including repeated sequences such as nucleolar organizing regions, and can be translocated without significant harm, as in a balanced Robertsonian translocation. The domestic horse genome includes one metacentric chromosome that is homologous to two acrocentric chromosomes in the conspecific but undomesticated Przewalski's horse. This may reflect either fixation of a balanced Robertsonian translocation in domestic horses or, conversely, fixation of the fission of one metacentric chromosome into two acrocentric chromosomes in Przewalski's horses. A similar situation exists between the human and great ape genomes; in this case, because more species are extant, it is apparent that the evolutionary sequence is a reduction of two acrocentric chromosomes in the great apes to one metacentric chromosome in humans (see Karyotype#Aneuploidy).[11]

Strikingly, harmful translocations in disease context, especially unbalanced translocations in blood cancers, more frequently involve acrocentric chromosomes than non-acrocentric chromosomes. Although the cause is not known, this probably relates to the physical location of acrocentric chromosomes within the nucleus. Acrocentric chromosomes are usually located in and around the nucleolus, so in the center of the nucleus, where chromosomes tend to be less densely packed than chromosomes in the nuclear periphery.Consistently, chromosomal regions that are less densely packed are also more prone to chromosomal translocations in cancers.

Telocentric

A telocentric chromosome's centromere is located at the terminal end of the chromosome. A telocentric chromosome has therefore only one arm. Telomeres may extend from both ends of the chromosome, their shape is similar to letter "i" during anaphase. For example, the standard house mouse karyotype has only telocentric chromosomes. Humans do not possess telocentric chromosomes.

Subtelocentric

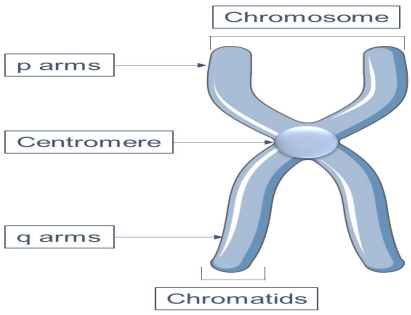
If the chromosome's centromere is located closer to its end than to its center, it may be described as subtelocentric.

Holocentric

With **holocentric** chromosomes, the entire length of the chromosome acts as the centromere. Examples of this type of centromere can be found scattered throughout the plant and animal kingdoms, with the most well-known example being the nematode *Caenorhabditis elegans*.

Acentric

If a chromosome lacks a centromere, it is said acentric. The macronucleus of ciliates for example contains hundreds of acentric chromosomes. Chromosome-breaking events can also generate acentric chromosomes or acentric fragments.

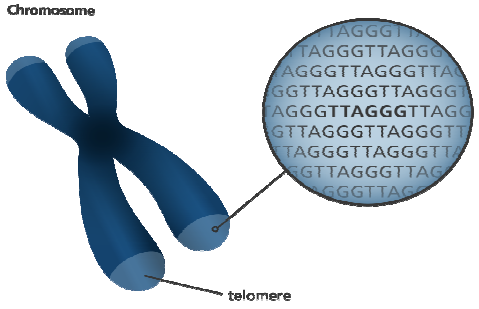


Telomeres

Thess are distinctive structures found at the ends of our chromosomes. They consist of the same short DNA sequence repeated over and over again.

- Telomeres are sections of DNA[?] found at the ends of each of our chromosomes[?].
- They consist of the same sequence of bases [?]repeated over and over.

- In humans the telomere sequence is TTAGGG.
- This sequence is usually repeated about 3,000 times and can reach up to 15,000 base pairs[?] in length.



Telomeres serve three major purposes:

- They help to organise each of our 46 chromosomes in the nucleus[?] (control centre) of our cells[?].
- They protect the ends of our chromosomes by forming a cap, much like the plastic tip on shoelaces. If the telomeres were not there, our chromosomes may end up sticking to other chromosomes.
- They allow the chromosome to be replicated properly during cell division[?]:
- Every time a cell[?] carries out DNA replication[?] the chromosomes are shortened by about 25-200 bases (A, C, G, or T) per replication.
- However, because the ends are protected by telomeres, the only part of the chromosome that is lost, is the telomere, and the DNA is left undamaged.

- Without telomeres, important DNA would be lost every time a cell divides (usually about 50 to 70 times).

- This would eventually lead to the loss of entire genes?.

What happens to telomeres as we age?

- Each time a cell divides, 25-200 bases are lost from the ends of the telomeres on each chromosome.
- Two main factors contribute to telomere shortening during cell division?:
    - The "end replication problem" during DNA replication: Accounts for the loss of about 20 base pairs? per cell division.
    - Oxidative stress: Accounts for the loss of between 50-100 base pairs per cell division. The amount of oxidative stress in the body is thought to be affected by lifestyle factors such as diet, smoking and stress.
- When the telomere becomes too short, the chromosome reaches a 'critical length' and can no longer be replicated.
- This 'critical length' triggers the cell to die by a process called apoptosis?, also known as programmed cell death.

How is telomere length maintained?

- Telomerase? is an enzyme? that adds the TTAGGG telomere sequence to the ends of chromosomes.
- Telomerase is only found in very low concentrations in our somatic cells?. Because these cells do not regularly use telomerase they age leading to a reduction in normal function.
- The result of ageing cells, is an ageing body.

- Telomerase is found in high levels in germline? cells (egg and sperm) and stem cells. In these cells telomere length is maintained after DNA replication and the cells do not show signs of ageing.
- Telomerase is also found in high levels in cancer? cells. This enables cancer cells to be immortal and continue replicating themselves. If telomerase activity was switched off in cancer cells, their telomeres would shorten until they reached a 'critical length'. This would, prevent the cancer cells from dividing uncontrollably to form tumours.
- The action of telomerase allows cells to keep multiplying and avoid ageing.

Use of telomeres in medicine

- Research on telomeres and the role of telomerase could uncover valuable information to combat ageing and fight cancer.
- The medical relevance of telomeres is uncertain.
- Human cells cultured in the lab have been observed to stop dividing when telomerase is inactivated, because the length of telomeres is not maintained after cell division.
- The cells then enter a state of inactivity called senescence. However, once telomerase is reactivated, the cells are able to continue dividing.
- If telomerase can be used to help human cells live forever, it may also be possible to mass produce cells for transplantation. These cells could help to treat a range of conditions, from severe burns to diabetes?.

Telomeres and ageing

- Mice models lacking the enzyme telomerase were found to show signs of premature ageing.
- However, it is not certain whether telomere shortening is responsible for ageing in humans or whether it is just a sign of ageing, like grey hair.
- There are several indications that telomere length is a good predictor of lifespan.

- Newborn babies tend to have telomeres ranging in length from around 8,000 to 13,000 base pairs. It has been observed that this number tends to decline by around 20-40 base pairs each year. So, by the time someone is 40 years old they could have lost up to 1,600 base pairs from their telomeres.
- However, looking at the bigger picture, the overall shortening of our telomeres is not significant, even in very old people.
- Cells that divide rapidly, such as germ cells? and stem cells?, are among the few cell types in our bodies containing active telomerase.
- This means that in these cells telomere length is maintained or even lengthened over time.
- However, there are a number of other factors that have an effect on the length of our telomeres that all need to be considered, such as smoking and obesity.

Telomeres and cancer

- Telomeres and telomerase present a number of potential targets for the design of new cancer therapies.
- Cancer cells contain active telomerase to enable them to become 'immortal' and continue dividing uncontrolled.
- Cancer is a disease characterised by the rapid and uncontrolled division of cells.
- Without telomerase activity, these cells would become inactive, stop dividing and eventually die.
- Drugs that inhibit telomerase activity, or kill telomerase-producing cells, may potentially stop and kill cancer cells in their tracks.
- However, blocking telomerase activity could affect cells where telomerase activity is important, such as sperm, eggs, platelets and immune cells.
- Disrupting telomerase in these cell types could affect fertility, wound healing and the ability to fight infections.
- However, telomerase activity in somatic cells is very low. These cells would therefore be largely unaffected by anti-telomerase therapy.

- Scientists hope this would result in fewer side effects for the patient, compared to current cancer therapies.
- Telomere biology is incredibly important in human cancer and scientists are working hard to understand the best way to exploit their knowledge of it to advance the treatment of cancer.

Repetitive DNA:

DNA sequences that are repeated in the genome. These sequences do not code for protein. One class termed highly repetitive DNA consists of short sequences, 5-100 nucleotides, repeated thousands of times in a single stretch and includes satellite DNA. Another class termed moderately repetitive DNA consists of longer sequences, about 150-300 nucleotides, dispersed evenly throughout the genome, and includes what are called Alu sequences and transposons.

Functions

Debates regarding the potential functions of these elements have been long standing. Controversial references to 'junk' or 'selfish' DNA were put forward early on, implying that repetitive DNA segments are remainders from past evolution or autonomous self-replicating sequences hacking the cell machinery to proliferate. Originally discovered by Barbara McClintock, dispersed repeats have been increasingly recognized as a potential source of genetic variation and regulation. Together with these regulatory roles, a structural role of repeated DNA in shaping the 3D folding of genomes has also been proposed.This hypothesis is only supported by a limited set of experimental evidence. For instance in human, mouse and fly, several classes of repetitive elements present a high tendency for co-localization within the nuclear space, suggesting that DNA repeats positions can be used by the cell as a genome folding map.

Tandem repeats in human disease

Tandem repeat sequences, particularly trinucleotide repeats, underlie several human disease conditions. Trinucleotide repeats may expand in the germline over successive generations leading to increasingly severe manifestations of the disease. The disease conditions in which expansion occurs include Huntington's disease, fragile X syndrome, several spinocerebellar ataxias, myotonic dystrophy and Friedrich ataxia. Trinucleotide repeat expansions may occur through strand slippage during DNA replication or during DNA repair synthesis.

major categories of **repeated sequence** or **repeats**:

Tandem repeats: copies which lie adjacent to each other, either directly or inverted

Satellite DNA - typically found in centromeres and heterochromatin

- Minisatellite - repeat units from about 10 to 60 base pairs, found in many places in the genome, including the centromeres
- Microsatellite - repeat units of less than 10 base pairs; this includes telomeres, which typically have 6 to 8 base pair repeat units
- Interspersed repeats (aka. interspersed nuclear elements)
- Transposable elements
- DNA transposons
- retrotransposons
- LTR-retrotransposons (HERVs)
- non LTR-retrotransposons
- SINEs (**S**hort **I**nterspersed **N**uclear **E**lements)
- LINEs (**L**ong **I**nterspersed **N**uclear **E**lements)

In primates, the majority of LINEs are LINE-1 and the majority of SINEs are Alu's. SVAs are hominoid specific.

In prokaryotes, CRISPR are arrays of alternating repeats and spacers.

DNA in Chromosomes

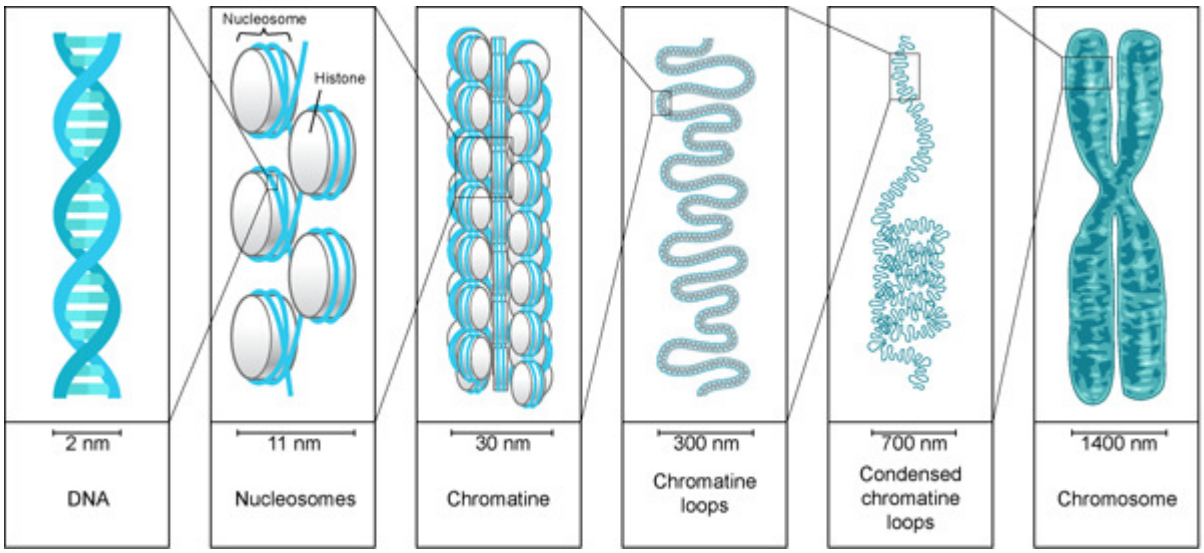
Chromosomes are made up of long pieces of double-stranded DNA twisted and condensed into a compact package. If left uncondensed, the strands of DNA would be about two meters each, far too long to fit inside your cells. A person's entire DNA is separated into 22 matched pairs of chromosomes, plus two sex chromosomes, for a total of 46. Along the length of the DNA, some of the regions code for proteins, while others do not. The protein-coding sections are your genes, so each chromosome is home to hundreds or thousands of genes.

Packaging Chromosomes

Specialized proteins bind to the DNA and help fold it properly so that it condenses into the tight configuration required to make chromosomes without getting tangled. The condensed DNA must also be configured so that enzymes can reach each part of it for repair, transcription, and translation. The basic DNA double helix is wound around histone proteins, and these DNA-protein complexes then fold into structures called nucleosomes. A strand of nucleosomes winds into a fiber called chromatin, which is about 30 nanometers in diameter and visible in an electron microscope. A chromosome is made up of tightly packed chromatin strands.

Packed Like Sardines

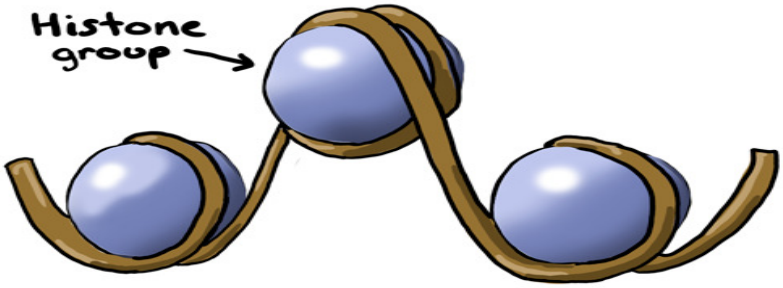
The double helix of DNA is highly negatively charged due to all the negatively charged phosphates in the backbone. All that negative charge must be counterbalanced by a positive charge, and the cell makes proteins called **histones** that bind DNA and aid in DNA's packaging. Histones are positively charged proteins that wrap up DNA through interactions between their positive charges and the negative charges of DNA. Double-stranded DNA loops around 8 histones twice, forming the **nucleosome**, which is the building block of **chromatin packaging**.



DNA can be further packaged by forming coils of nucleosomes, called **chromatin fibers**. These fibers are condensed into chromosomes during **mitosis**, or the process of **cell division**. However, packaging of **chromatin** into **chromosomes** that we are most familiar with occurs only during a few stages of mitosis. Most of the time, DNA is loosely packaged.

Histones: DNA Tupperware™

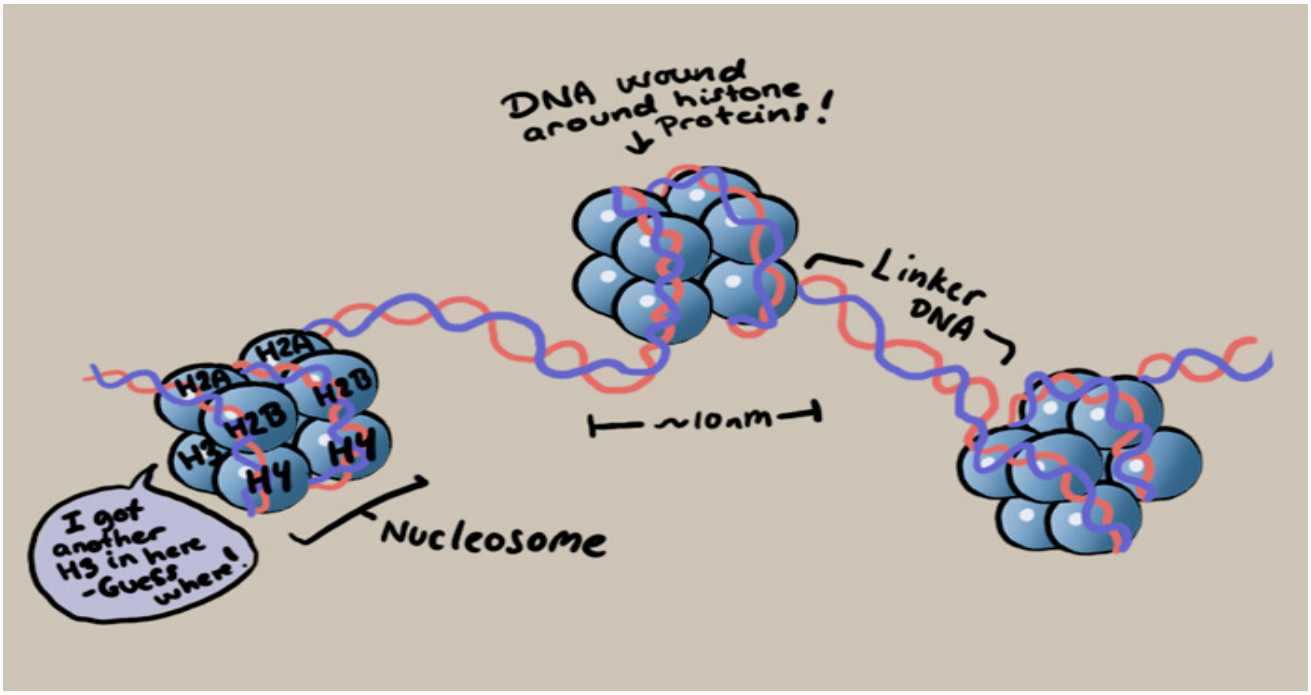
Histones are positively charged proteins that facilitate the packing of DNA into condensed **chromatin fibers**. They are basically the Tupperware™ of DNA packaging, and they come in many kitchen-friendly colors. Histones have many arginine and lysine amino acids that easily bind to the negatively charged DNA, based on Paula Abdul's principle that **opposites attract**. Just kidding on that last part. DNA is highly negatively charged because of the phosphate group of each nucleotide is negatively charged.



Histones are divided into two groups:

- **Corehistones**
- **Linkerhistones**

Core histones are H2A, H2B, H3, and H4, where two H3/H4 dimers (H3 and H4 hooked together) and two H2A/H2B dimers (these two hooked together) form the octamer (all eight of these guys together). **Linker** histone H1 basically locks the DNA in place onto the **nucleosome** and can be removed for **transcription** while linker histone H5 is a variant of H1 predominantly used in birds.



Is that confusing? Well, it gets worse. H1, H2A, H2B, H3, H4, and H5 are all names that define **families** of proteins. Individual histone proteins are specific for certain types of DNA or certain cell types. Just as H5 is the avian version of H1, there are individual histone proteins that package certain regions of DNA, or package DNA in specific **tissue types**. Just like you would not put a giant pot of chili in small Tupperware containers (or maybe you would…we try not to judge, but seriously?), specific histones are important for specific parts of DNA.

One important aspect of histones is that they can be changed to alter how much packing the DNA is capable of. There are several modifications that affect how well DNA is packaged. The three major types of modifications can be seen in the following table.

Modification	Modification Structure (R = chemical functional group)	Charge	Effect
Methylation	R-CH_3	Neutral	Increases packing
Acetylation	R-$COCH_3$	Negative	Decreases packing

PhosphorylationR-PO NegativeDecreases packing

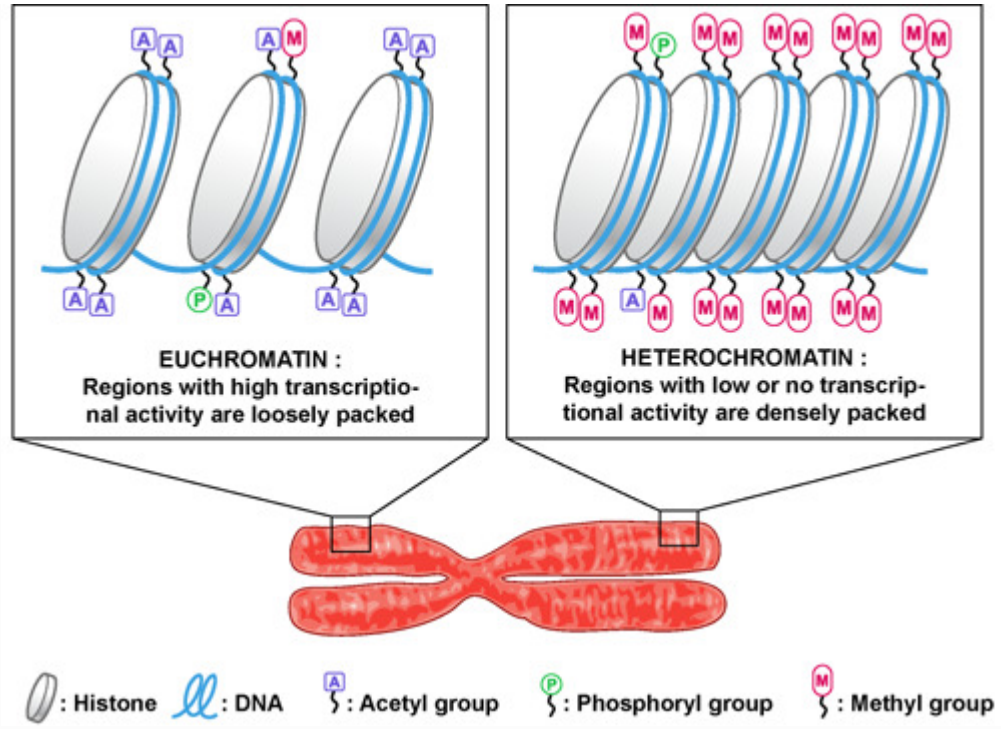
Normally, histones are positively charged molecules, and the addition of methyl groups (**methylation**) makes them more **hydrophobic** (water-hating). Hydrophobic molecules tend to stick together, and increasing **histone methylation will** cause the histones to pack even more tightly than usual.

Acetylation (adding an acetyl group) and **phosphorylation** (adding a phosphate group) make the histones more negatively charged because acetyl and phosphoryl groups are negative. They are "glass is half empty" molecules. By making histones more negatively charged, their grip on DNA will be much looser because DNA is also negatively charged. Similar charges (negative and negative) repel one another.

Lockup: DNA Edition

One of the perks of packaging DNA is that you can separate it into things you use a lot and things you do not. Unless you are a maniacal hoarder, every fall, you put away your summer clothes for things more winter-appropriate. In the same way, certain parts of DNA are only important for certain times. However, some things you need year-round, like shoes, so there is no point in putting those things away. The cell does the same thing with DNA.

Regions that are necessary for making proteins and are important for the cell are loosely packed and called **euchromatin**. By having a loose packing of DNA in euchromatin, proteins involved in **transcription** can easily get in and make RNA (see Genes to Proteins section for more detail). On the other hand, some regions of DNA you do not need except for special occasions, like that velvet suit you have that you never wear. These regions are called **heterochromatin** and are tightly packed through DNA as well as through good ol' **histone methylation**.

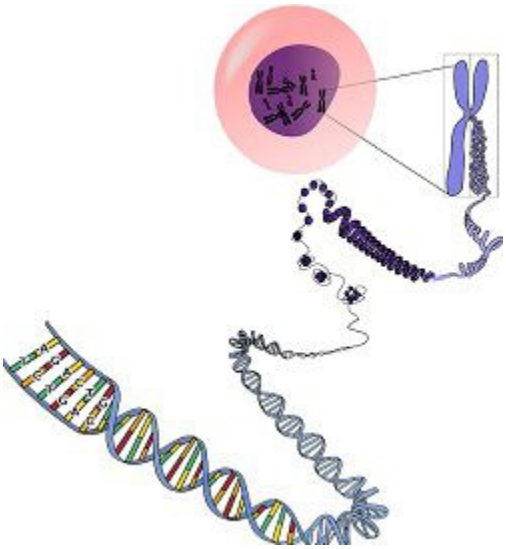


Enzymes that add **acetyl groups** to histones are called **histone acetyltransferases (HATs)** while those that remove acetyl groups are called **histone deacetylases (HDACs)**. Enzymes that add methyl groups are called **histone methyltransferases (HMTs)**. Activity of these enzymes affects whether or not regions of DNA are tightly packed, and unable to **transcribe**, or are loosely packed and therefore, highly transcribed.

Histone methylation is a tricky concept, though, because usually, **histone methylation** goes along with methylation of cytosines in DNA, called **DNA methylation**. Together, these processes create regions of DNA that cannot be transcribed. However, sometimes, methylation of positively charged amino acids in histones promotes **transcriptional activation**, but only when DNA is not methylated. The methylation of DNA and modifications of histones that affect **transcription** are the focus of study called **epigenetics**.

When the cell is undergoing the process of **mitosis**, **chromatin packing** is important, and this packing is done by packing DNA into condensed **chromatin fibers** to the point where they are the recognized **chromosomes** that we know and love…or "really like," if you are unprepared to make that kind of commitment. These chromosomes divide into **daughter cells**, and after mitosis is complete, the DNA is unpacked so **transcription** can occur again. Therefore, we can think of mitosis like a big DNA moving day. The packing starts with HDACs and HMTs tightening the packaging, and once mitosis is completed, HATs and **phosphoryltransferases (HPTs)** reduce the packaging.

Heterochromatin and Euchromatin:



The major difference between heterochromatin and euchromatin is that **heterochromatin** is such part of the chromosomes, which is a firmly packed form and are **genetically inactive**, while **euchromatin** is an uncoiled (loosely) packed form of chromatin and are **genetically active**.

When the non-dividing cells of the nucleus were observed under the light microscope, it exhibited the two regions, on the ground of concentration or intensity of staining. The **dark stained** areas are said as heterochromatin and **light stained** areas are said as euchromatin.

Around **90%** of the total human genome is euchromatin. They are the parts of chromatin and participate in the protection of DNA in the genome present inside the nucleus. **Emil Heitz** in the year 1928, coined the term Heterochromatin and Euchromatin.

By focussing on the few more points, we will be able to understand the difference between both types of chromatin. Given below is the comparison chart along with the brief description of them.

Content: Heterochromatin Vs Euchromatin

1. Comparison Chart
2. Definition
3. Key Differences
4. Conclusion

Comparison Chart

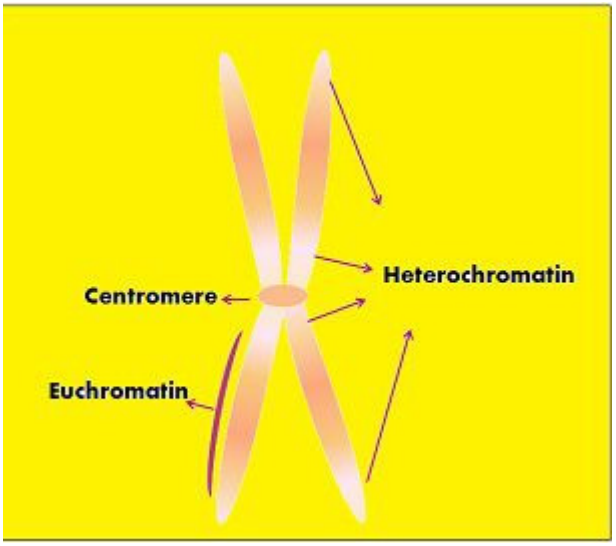
BASIS FOR COMPARISON	HETEROCHROMATIN	EUCHROMATIN
Meaning	The tightly packed form of DNA in the chromosome is called as heterochromatin.	The loosely packed form of DNA in the chromosome is called as euchromatin.
DNA density	High DNA density.	Low DNA density.
Kind of stain	Stained dark.	Lightly stained.
Where they are present	These are found at the periphery of the nucleus in eukaryotic cells only.	These are found in the inner body of the nucleus of prokaryotic as well as in eukaryotic cells.
Transcriptional activity	They show little or no transcriptional activity.	They actively participate in the process of transcription.
Other features	They are compactly coiled.	They are loosely coiled.
	They are late replicative.	They are early replicative.
	Regions of heterochromatin are sticky.	Regions of euchromatin are non-sticky.
	Genetically inactive.	Genetically active.
	Phenotype remains unchanged of	Variation may be seen, due

BASIS FOR COMPARISON	HETEROCHROMATIN	EUCHROMATIN
	an organism.	to the affect in DNA during the genetic process.
	It permits the gene expression regulation and also maintains the structural integrity of the cell.	It results in genetic variations and permits the genetic transcription.

Definition of Heterochromatin

The area of the chromosomes which are **intensely stained** with DNA-specific strains and are relatively condensed is known as **heterochromatin**. They are the **tightly packed** form of DNA in the nucleus.

The organization of heterochromatin is so highly compact in the way that these are inaccessible to the protein which is engaged in gene expression. Even the chromosomal crossing over is not possible due to the above reason. Resulting them to be transcriptionally as well as genetically inactive.



Heterochromatin is of two types: Facultative heterochromatin and constitutive heterochromatin. The genes which get silenced through the process of **Histone methylation or siRNA** through **RNAi** are called

as **facultative heterochromatin**. Hence they contain inactive genes and is not a permanent character of every nucleus of the cells.

While the **repetitive and structurally functional genes** like telomeres or centromeres are called as **Constitutive heterochromatin**. These are the continuing nature of the cell's nucleus and contains no gene in the genome. This structure is retainable during the interphase of the cell.

The **main function** of the heterochromatin is to protect the DNA from the endonuclease damage; it is due to its compact nature. It also prevents the DNA regions to get accessed to proteins during gene expression.

Definition of Euchromatin

That part of chromosomes, which are **rich in gene** concentrations and are loosely packed form of chromatin is called as **euchromatin**. They are active during transcription.

Euchromatin covers the maximum part of the dynamic genome to the inner of the nucleus and is said that euchromatin contains about **90% of the entire human genome**.

To allow the transcription, some parts of the genome containing active genes are loosely packed. The wrapping of DNA is so loose that DNA can become readily available. The structure of euchromatin resembles the nucleosomes, which consist of histones proteins having around 147 base pairs of DNA wrapped around them.

Euchromatin actively participates in transcription from DNA to RNA. The **gene regulating mechanism** is the process of transforming euchromatin into heterochromatin or vice versa.

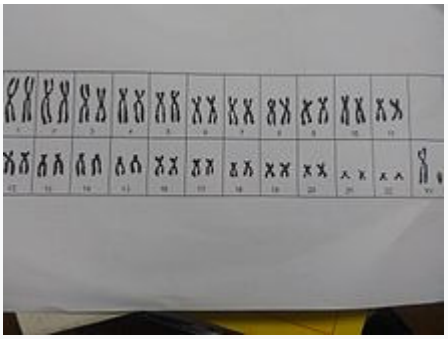
The active genes present in euchromatin gets transcribed to make mRNA whereby further encoding the functional proteins is the **main function** of euchromatin. Hence they are considered as genetically and transcriptionally active. **Housekeeping** genes are one of the forms of euchromatin.

Conclusion

From the above information regarding chromatin – their structure and types. We can say that only Euchromatin is vigorously involved in the transcription process although heterochromatin and its types do not play such significant role.

Constitutive heterochromatin contains the satellite DNA, and it surrounds the centromere, and facultative heterochromatin is disbanded. So apparently it can be said that the eukaryotic cells and their inner structure are relatively complex.

Human karyotype[edit]



human karyotype (male)

The normal human karyotypes contain 22 pairs of autosomal chromosomes and one pair of sex chromosomes (allosomes). Normal karyotypes for females contain two X chromosomes and are denoted 46,XX; males have both an X and a Y chromosome denoted 46,XY. Any variation from the standard karyotype may lead to developmental abnormalities.

Diversity and Evoution of Karyotype:

Although the replication and transcription of DNA is highly standardized in eukaryotes, the same cannot be said for their karyotypes, which are highly variable. There is variation between species in chromosome number, and in detailed organization, despite their construction from the same macromolecules. This variation provides the basis for a range of studies in evolutionary cytology.

In some cases there is even significant variation within species. In a review, Godfrey and Masters conclude:

In our view, it is unlikely that one process or the other can independently account for the wide range of karyotype structures that are observed ... But, used in conjunction with other phylogenetic data, karyotypic fissioning may help to explain dramatic differences in diploid numbers between closely related species, which were previously inexplicable.[23]

Although much is known about karyotypes at the descriptive level, and it is clear that changes in karyotype organization has had effects on the evolutionary course of many species, it is quite unclear what the general significance might be.

We have a very poor understanding of the causes of karyotype evolution, despite many careful investigations ... the general significance of karyotype evolution is obscure.

Changes during development

Instead of the usual gene repression, some organisms go in for large-scale elimination of heterochromatin, or other kinds of visible adjustment to the karyotype.

- Chromosome elimination. In some species, as in many sciarid flies, entire chromosomes are eliminated during development.
- Chromatin diminution (founding father: Theodor Boveri). In this process, found in some copepods and roundworms such as *Ascaris suum*, portions of the chromosomes are cast away in particular cells. This process is a carefully organised genome rearrangement where new telomeres are constructed and certain heterochromatin regions are lost. In *A. suum*, all the somatic cell precursors undergo chromatin diminution.
- X-inactivation. The inactivation of one X chromosome takes place during the early development of mammals (see Barr body and dosage compensation). In placental mammals, the inactivation is random as between the two Xs; thus the mammalian female is a mosaic in respect of her X chromosomes. In marsupials it is always the paternal X which is inactivated. In human females some 15% of somatic cells escape inactivation,[and the number of genes affected on the inactivated X chromosome varies between cells: in fibroblast cells up about 25% of genes on the Barr body escape inactivation.[30]

Number of chromosomes in a set

A spectacular example of variability between closely related species is the muntjac, which was investigated by Kurt Benirschke and his colleague Doris Wurster. The diploid number of the Chinese muntjac, *Muntiacus reevesi*, was found to be 46, all telocentric. When they looked at the karyotype of the closely related Indian muntjac, *Muntiacus muntjak*, they were astonished to find it had female = 6, male = 7 chromosomes.

They simply could not believe what they saw ... They kept quiet for two or three years because they thought something was wrong with their tissue culture ... But when they obtained a couple more specimens they confirmed

The number of chromosomes in the karyotype between (relatively) unrelated species is hugely variable. The low record is held by the nematode *Parascaris univalens*, where the haploid n = 1; and an ant: *Myrmecia pilosula*.[32] The high record would be somewhere amongst the ferns, with the adder's tongue fern *Ophioglossum* ahead with an average of 1262 chromosomes. Top score for animals might be the shortnose sturgeon *Acipenser brevirostrum* at 372 chromosomes. The existence of supernumerary or B chromosomes means that chromosome number can vary even within one interbreeding population; and aneuploids are another example, though in this case they would not be regarded as normal members of the population.

Fundamental number

The fundamental number, *FN*, of a karyotype is the number of visible major chromosomal arms per set of chromosomes Thus, FN ≤ 2 x 2n, the difference depending on the number of chromosomes considered single-armed (acrocentric or telocentric) present. Humans have FN = 82, due to the presence of five acrocentric chromosome pairs: 13, 14, 15, 21, and 22 (the human Y chromosome is also acrocentric). The fundamental autosomal number or autosomal fundamental number, *FN* or *AN*, of a karyotype is the number of visible major chromosomal arms per set of autosomes (non-sex-linked chromosomes).

Ploidy

Ploidy is the number of complete sets of chromosomes in a cell.

- Polyploidy, where there are more than two sets of homologous chromosomes in the cells, occurs mainly in plants. It has been of major significance in plant evolution according to Stebbins The proportion of flowering plants which are polyploid was estimated by Stebbins to be 30–35%, but in grasses the average is much higher, about 70%.Polyploidy in lower plants (ferns, horsetails and psilotales) is also common, and some species of ferns have reached levels of polyploidy far in excess of the highest levels known in flowering plants.
- Polyploidy in animals is much less common, but it has been significant in some groups.
- Polyploid series in related species which consist entirely of multiples of a single basic number are known as euploid.
- Haplo-diploidy, where one sex is diploid, and the other haploid. It is a common arrangement in the Hymenoptera, and in some other groups.
- Endopolyploidy occurs when in adult differentiated tissues the cells have ceased to divide by mitosis, but the nuclei contain more than the original somatic number of chromosomes. In the *endocycle* (endomitosis or endoreduplication) chromosomes in a 'resting' nucleus undergo reduplication, the daughter chromosomes separating from each other inside an *intact* nuclear membrane.
  In many instances, endopolyploid nuclei contain tens of thousands of chromosomes (which cannot be exactly counted). The cells do not always contain exact multiples (powers of two), which is why the simple definition 'an increase in the number of chromosome sets caused by replication without cell division' is not quite accurate.
  This process (especially studied in insects and some higher plants such as maize) may be a developmental strategy for increasing the productivity of tissues which are highly active in biosynthesis.
  The phenomenon occurs sporadically throughout the eukaryote kingdom from protozoa to humans; it is diverse and complex, and serves differentiation and morphogenesis in many ways.
- See palaeopolyploidy for the investigation of ancient karyotype duplications.

Aneuploidy[edit]

Aneuploidy is the condition in which the chromosome number in the cells is not the typical number for the species. This would give rise to a chromosome abnormality such as an extra chromosome or one or more chromosomes lost. Abnormalities in chromosome number usually cause a defect in development. Down syndrome and Turner syndrome are examples of this.

Aneuploidy may also occur within a group of closely related species. Classic examples in plants are the genus *Crepis*, where the gametic (= haploid) numbers form the series x = 3, 4, 5, 6, and 7; and *Crocus*, where every number from x = 3 to x = 15 is represented by at least one species. Evidence of various kinds shows that trends of evolution have gone in different directions in different groups.[50] Closer to home, the great apes have 24x2 chromosomes whereas humans have 23x2. Human chromosome 2 was formed by a merger of ancestral chromosomes, reducing the number.

Chromosomal polymorphism

Some species are polymorphic for different chromosome structural forms. The structural variation may be associated with different numbers of chromosomes in different individuals, which occurs in the ladybird beetle *Chilocorus stigma*, some mantids of the genus *Ameles*,the European shrew *Sorex araneus*.There is some evidence from the case of the mollusc *Thais lapillus* (the dog whelk) on the Brittany coast, that the two chromosome morphs are adapted to different habitats.

Species trees

The detailed study of chromosome banding in insects with polytene chromosomes can reveal relationships between closely related species: the classic example is the study of chromosome banding in Hawaiian drosophilids by Hampton L. Carson.

In about 6,500 sq mi (17,000 km^2), the Hawaiian Islands have the most diverse collection of drosophilid flies in the world, living from rainforests to subalpine meadows. These roughly 800 Hawaiian drosophilid species are usually assigned to two genera, *Drosophila* and *Scaptomyza*, in the family Drosophilidae.

The polytene banding of the 'picture wing' group, the best-studied group of Hawaiian drosophilids, enabled Carson to work out the evolutionary tree long before genome analysis was practicable. In a sense, gene arrangements are visible in the banding patterns of each chromosome. Chromosome rearrangements, especially inversions, make it possible to see which species are closely related.

The results are clear. The inversions, when plotted in tree form (and independent of all other information), show a clear "flow" of species from older to newer islands. There are also cases of colonization back to older islands, and skipping of islands, but these are much less frequent. Using K-Ar dating, the present islands date from 0.4 million years ago (mya) (Mauna Kea) to 10mya (Necker). The oldest member of the Hawaiian archipelago still above the sea is Kure Atoll, which can be dated to 30 mya. The archipelago itself (produced by the Pacific plate moving over a hot spot) has existed for far

longer, at least into the Cretaceous. Previous islands now beneath the sea (guyots) form the Emperor Seamount Chain.

All of the native *Drosophila* and *Scaptomyza* species in Hawai'i have apparently descended from a single ancestral species that colonized the islands, probably 20 million years ago. The subsequent adaptive radiation was spurred by a lack of competition and a wide variety of niches. Although it would be possible for a single gravid female to colonise an island, it is more likely to have been a group from the same species

Using Karyograms to Detect Chromosomal Abnormalities

Today, G-banded karyograms are routinely used to diagnose a wide range of chromosomal abnormalities in individuals. Although the resolution of chromosomal changes detectable by karyotyping is typically a few megabases, this can be sufficient to diagnose certain categories of abnormalities. For example, aneuploidy, which is often caused by the absence or addition of a chromosome, is simple to detect by karyotype analysis. Cytogeneticists can also frequently detect much more subtle deletions or insertions as deviations from normal banding patterns. Likewise, translocations are often readily apparent on karyotypes.

When regional changes in chromosomes are observed on karyotypes, researchers often are interested in identifying candidate genes within the critical interval whose misexpression may cause symptoms in patients. This search process has been greatly facilitated by the completion of the Human Genome Project, which has correlated cytogenetic bands with DNA sequence information. Consequently, investigators are now able to apply a range of molecular cytogenetic techniques to achieve even higher resolution of genomic changes. Fluorescence *in situ* hybridization (FISH) and comparative genomic hybridization (CGH) are examples of two approaches that can potentially identify abnormalities at the level of individual genes.

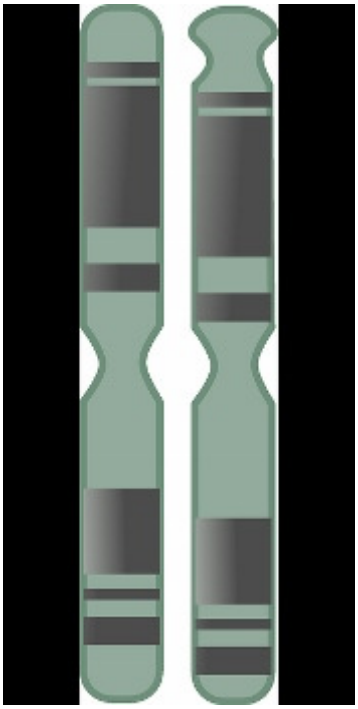
Molecular cytogenetics is a dynamic discipline, and new diagnostic methods continue to be developed. As these new technologies are implemented in the clinic, we can expect that cytogeneticists will be able to make the leap from karyotype to gene with increasing efficiency.

What is Chromosome Banding

You may talk about your genes from time to time - 'Oh, I have the gene for that.' But how do you see your genes? A gene is a functional unit of DNA, and your DNA is organized onto chromosomes. **Chromosome banding** is a little like tie-dying your chromosomes.

A **chromosome** is a unit of tightly-packed DNA. DNA has to wrap tightly around itself, because you have quite a lot of it. In fact, if you unrolled all the DNA in a single one of your cells, it would be about three meters long. Humans have 46 chromosomes - 23 from Mom and 23 from Dad.

In **chromosome banding**, we treat chromosomes with chemicals to stain them and learn about a chromosome by how it stains. There are several different types of stains we can use.



Banded chromosomes

There are several types of **chromosome banding**. Here, we will list a few of the most common types.

- **G-banding** uses a stain called Giemsa stain. G-banding gives you a series of light and dark stripes along the length of the chromosome. We will discuss G-banding in the most detail, because you will likely see G-banding if you take a genetics class.
- **Q-banding** uses a stain called quinacrine. Q-banding yields a fluorescent pattern. It is similar in pattern to G-banding, but glows yellow.
- **C-banding** only stains the centromeres. Centromeres are little constricted portions of chromosomes. That's where sister chromatids (two copies of the same chromosome) will attach to each other when the cell is getting ready to divide.
- **R-banding** is the opposite of C-banding. R-banding stains non-centromeric regions.

Giemsa Stain

G-banding is useful because the patterns of stripes on the chromosomes are unique enough that you should be able to confidently identify each chromosome.

Giemsa staining was named after the German scientist **Gustav Giemsa**, who worked in the early part of the 20th century. Giemsa's immediate goal was to find a stain that would work on *Plasmodium*, the parasite that causes malaria. Giemsa stain, however, was quickly found to have many uses. Dr. Giemsa lamented the fact that he would be known for his staining procedure rather than for his work on tropical diseases.

Giemsa stain is a mixture of a stain called methylene blue and one called azure, which form a type of stain called an eosin compound. Researchers will typically wash a sample in Giemsa stain for around seven minutes. You would typically stain chromosomes during the early parts of the cell cycle (prophase or metaphase), because the chromosomes are partially but not fully condensed.

Karyotypes

A **karyotype** is a profile of a person's chromosomes, organized by size. Scientists will use a karyotype to identify any abnormalities that may lead to a genetic disorder. For instance, people who have Down syndrome carry an extra copy of Chromosome 21. Having an extra chromosome makes it hard for cells to properly regulate how much protein to make. Down syndrome is a developmental disorder that is characterized by intellectual disability and distinctive facial features such as a flat face, abnormal ears, large tongue, and upward-slanting eyes. People with Down syndrome are prone to medical complications including respiratory problems, heart defects, hearing loss, and leukemia.

Kinds of Chromosomes: Lampbrush, Polytene :

I] Lampbrush chromosomes:

These are the largest known chromosomes found in the yolk rich oocytic nuclei of certain vertebrates such as fishes, amphibians, reptiles and birds.

They can be seen with naked eye and are characterized by fine lateral loops, arising from the chromomeres, during first prophase (diplotene) of meiosis.

These loops give it a brush-like appearance; that is why these are called lampbrush chromosomes first discovered by Flemming in 1882 and were described in shark oocytes by Ruckert (1892). Lampbrush chromosomes of certain urodele oocytes may reach upto 5900µ in length.

It consists of longitudinal axis formed by a single DNA molecule along which several hundred bead-like chromomeres are distributed in a linear fashion. From each chromomere there emerge two symmetrical lateral loops (one for each chromatid), which are able to expand or contract in response to various environmental conditions.

About 5 to 10% of the DNA is in the lateral loops. Loop formation reduces the mass of the corresponding chromomeres, implying a spinning out of chromomere material into the lateral strands. The centromeres also have the appearance of elongate Feulgen-positive chromomeres but they characteristically lack lateral loops.

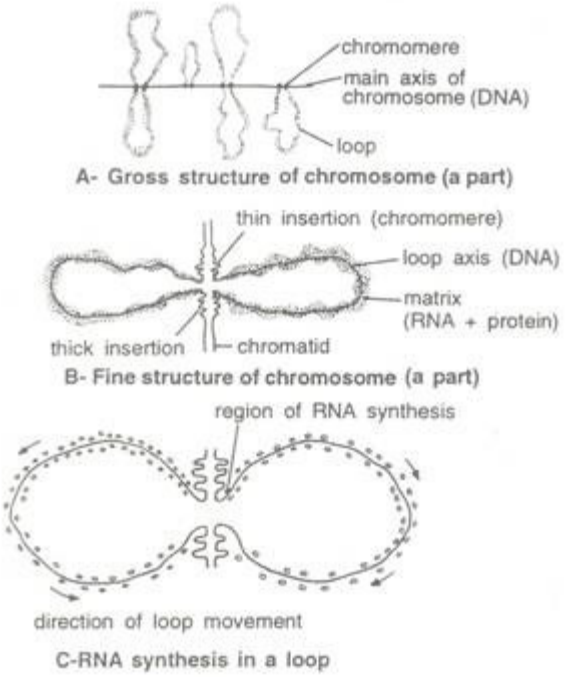


Fig. 11. Lampbrush chromosome.

Lampbrush chromosomes can be dissected in (toto) from oocyte nucleus. Individual chromosomes are liable to stretching. With extreme stretching, chromomeres begin to separate transversely into two halves, so that the paired loops form double stranded bridges. The axis between chromomeres is also double, which can be seen in certain special regions where two elements separate longitudinally and bear single loops (Callan, 1955).

These experiments indicate that each chromomere possesses four quadrants separated by both a transverse and a longitudinal line of division (Fig. C). Callan (1963) regards it as that the entire chromatid pair is made up of two continuous strands, which lie parallel to one another in the interchromomere

regions, are tightly folded in the chromomeres, and separate as single, unfolded fibres in the loops. Each of the two fibres would correspond to one conventional metaphase chromatid.

There is fundamental similarity in the organization of amphibian lmpbrush chromosomes and dipteran giant polytene chromosomes: in both cases, very long single fibres correspond to single chromatids and are partly but not completely extended. The substructure of the salivary chromosome 'puffs' also bear some similarity to that of the lampbrush lateral loops.

Lateral loops are formed of DNA, in chromomeres regions DNA is tightly folded and transcriptionally inactive. In lateral loops RNA synthesis is intense. Each loop in turn has an axis formed by a single DNA molecule, which is coated by a matrix of nascent RNA and proteins. The matrix is asymmetrical, being thicker at one end of the loop. RNA synthesis starts at the thinner end and progresses toward the thicker end.

Functions of Lampbrush chromosomes,

(a) Synthesis of RNA:

Functions of lampbrush chromosomes involve synthesis of RNA and protein by their loops. RNA is synthesized only at the thin insertion and then carried around the loops to the thick insertion. There it may be either destroyed or released into nucleus.

(b) Formation of yolk material:

There are some probabilities that lampbrush chromosomes help in the formation of certain amount of yolk material for the egg.

[II] Polytene chromosomes:

These are also giant chromosomes but relatively smaller than lampbrush chromosomes, found in the larvae of certain dipterans. Such banded chromosomes occur in the larval salivary glands, midgut epithelium, and rectum and Malpighian tubules of various genera (Drosophila, Sciara, Rhynchosciara, and Chironomus). In these larvae the salivary glands contain salivary cells so large in size that they can easily be seen with the lens power of a dissecting microscope.

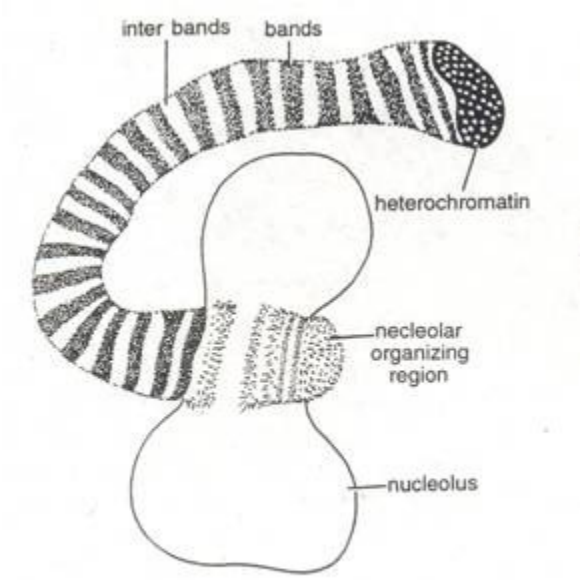


Fig. 12. Structure of a polytene chromosome of *Cecidomyia serotinae* showing nucleolar part.

Nuclei of these cells are much larger than those of ordinary cells being generally about 25μ in diameter, and chromosomes in nuclei are so large that they are 50 to 200 times as large as chromosomes in other body cells of the organism.

Ultrastructure of giant polytene (poly=many, tene=strands) chromosomes:

It was first investigated by Beermann and Bahr (1954), who observed numerous fine fibrils in the Balbiani rings of Chironomus and estimated that each chromosome contains 1000 to 2000 separate strands (corresponding to the degree of ploidy).

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Later Gay (1956) observed strands 200 to 500 A in diameter in sectioned Drosophila salivary chromosomes. The individual fibres in band and interband regions are similar in appearance, but the

fibres in the bands exhibit a considerable degree of metaphase-like folding and are much more tightly packed.

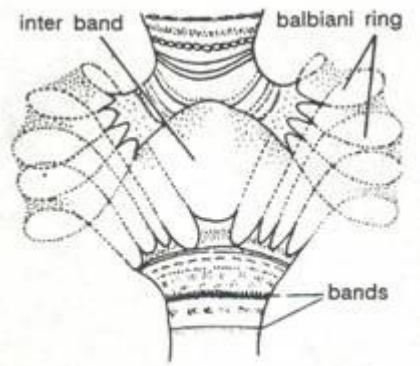


Fig. 13. Balbiani ring of a polytene chromosome.

Polytene chromosomes get their name from the fact that they are formed by many parallel chromatids, often more than a thousand strands, which do not separate from one another following duplication. Along each chromatid strand some regions of chromatin are tightly coiled and other regions are less coiled, with the result that polytene chromosomes appear to consist of light and dark bands when observed under a microscope.

During larval development, specific areas on polytene chromosomes become uncoiled, forming localized regions called 'pufs'. Puffs represent regions of active RNA synthesis (transcription). In the puff individual fibres remain continuous across the puff and they become extended as short lateral loops (Bahr, 1954). DNA is concentrated almost entirely in the bands. Protein and RNA is also found in puffs. Puffing is due to the uncoiling of chromosome fibres which are usually closely folded or coiled in the dense band regions. These fibres then project in the form of loops.

Structural Variations in Chromosomes :

The following point highlight the five main types of structural variation in chromosomes. The types are: 1. Deletion or Deficiency 2. Duplications 3. Translocations 4. Inversions .

Type # 1. Deletion or Deficiency:

A deficiency means deletion of a small portion of a chromosome resulting in loss of one or more genes. A deficiency originates from breakage occurring at random in both chromatids of a chromosome (called chromosome break), or only in one chromatid (chromatid break).

The breakage may be caused by various agents such as radiation, chemicals, drugs or viruses at any time during the cell cycle, either in somatic or in germ cells. Depending upon its location, a deletion may be terminal when a single break occurs near the end of the chromosome; or interstitial when two breaks occur in a middle portion of the chromosome

Each break produces two raw ends which may behave in one of following three ways:

(a) There might be reunion of the broken ends called restitution so that the original chromosome structure is restored;

(b) The broken ends may not unite giving rise to a chromosomal segment without a centromere which is eventually lost during cell division;

(c) If two single breaks occur in two different chromosomes in a cell, the deleted segment of one chromosome may unite with the raw broken end on the other chromosome; this is called exchange union.

Fate of a Deleted Fragment:

If the fragment does not have a centromere (acentric), then at metaphase it will not be able to get attached to spindle fibres and move towards a pole with other centric chromosomes. It will remain at the centre of the cell and will not be included within any of the two daughter nuclei. It will be free in the cytoplasm and will eventually be lost (Fig. 12.1). In this way, the cell will lose one or more genes contained in the deleted fragment.

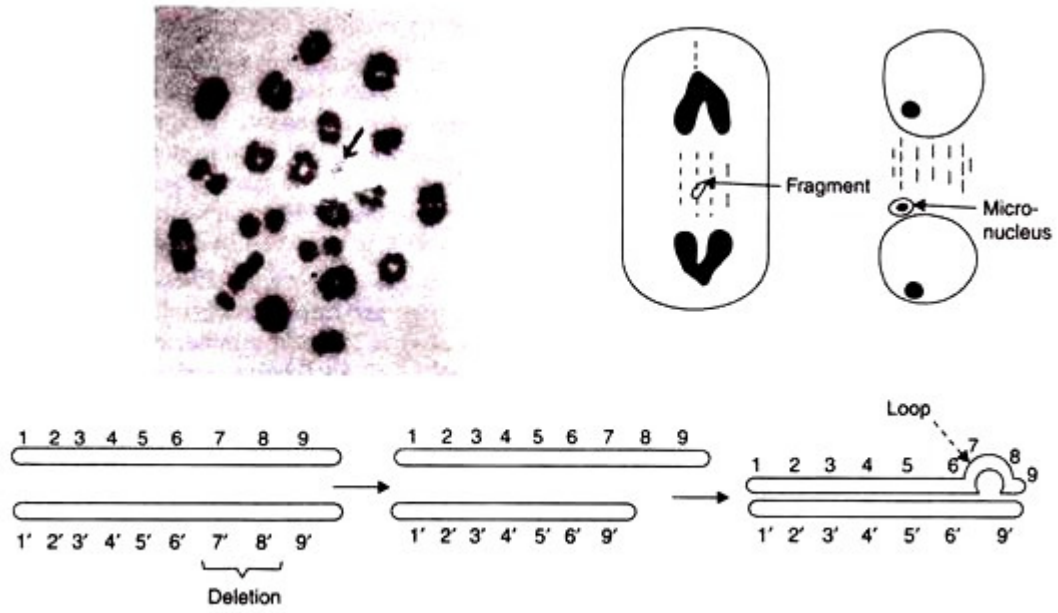


Fig. 12.1 Acentric chromosome fragment (arrow) at diakinesis.

A diploid cell has a homologue of the chromosome which has lost a segment. The corresponding segment of the intact homologue will have alleles of the genes that the cell has lost. Such a cell is said to be heterozygous for a deficiency. A very small deficiency in the heterozygous state is viable, but if homozygous it is lethal. When a deletion is large it is lethal even in the heterozygous state.

If a deletion occurs in cells of the germ line, then 50% of the gametes formed will have a deleted chromosome and 50% gametes would be normal. This would result in half the offspring with phenotypic abnormalities related to the genes carried on a small deleted fragment.

If the deficiency occurs in a developing embryo, some cells would have normal chromosomes and other cells would have the deficiency. This could produce a mosaic individual with two different phenotypes.

Detection of Deficiency:

The occurrence of a deficiency can sometimes be inferred from the results of a genetic cross when a rare recessive phenotype unexpectedly appears in the progeny. Consider a cross between two

parents DD and dd where D controls the dominant expression of a trait, and d is the recessive allele. The F1 is expected to show the dominant trait and have the genotype Dd.

If on the contrary, some F1 individuals show the recessive phenotype, one explanation could be sought in a deletion of the chromosomal segment bearing gene D. Since other interpretations are also possible, it is best to confirm the occurrence of deficiency from a cytological study of the chromosomes as described below.

Deficiencies are best observed in preparations of homologously paired chromosomes at meiotic prophase either in large sized plant chromosomes or in polytene chromosomes. Normally during pachytene homologous chromosomes are intimately synapsed throughout their length.

If one of the homologues is deficient over a small length, the corresponding portion of the second homologue has nothing to pair with. It therefore, forms a loop (Fig. 12.1), which is clearly visible in cytological preparations and is clear-cut proof that deficiency has occurred.

Type # 2. Duplications:

A duplication involves attachment of a chromosomal fragment resulting in addition of one or more genes to a chromosome. Whenever there is a duplication in a chromosome, there is a corresponding deletion in another chromosome.

Following types of duplications are known (Fig. 12.3: the diagrams are self-explanatory):

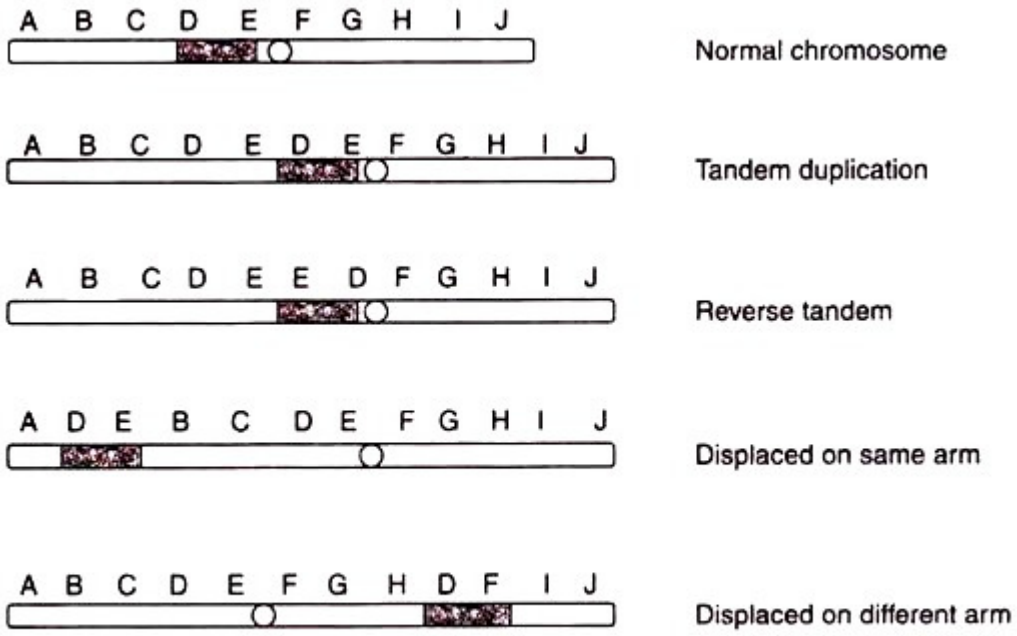


Fig. 12.3 Diagrams showing different types of duplications.

The phenotypic effect produced by a duplication is illustrated by the attached-X females in Drosophila. Consider such flies which are homozygous for some recessive sex-linked traits. It is found that when a fly receives a fragment of an X chromosome carrying the wild type allele from its male parent, then only the dominant phenotype is expressed.

The recessive alleles of the same gene although present in the homozygous condition, are not able to express themselves. Evidently the presence of a single dominant allele in a duplication is enough to produce the wild type phenotype.

The origin of duplications can be traced to unequal crossing over during meiosis. Normally homologous chromosomes are paired in a perfect manner so that identical loci lie exactly opposite each other.

The mechanism ensures that after crossing over between non-sister chromatids, equal exchange products are formed. If paired chromosomes are misaligned, it is not possible for exchange to take place between exactly opposite locations on two chromatids.

Instead, exchange occurs between adjacent points on two chromatids so that one resulting chromatid will have a duplication, the other a deletion. Such an exchange is called unequal crossing over. A gamete that receives a chromosome with a duplication will be diploid for some genes. When it fertilises a normal gamete, the zygote will have three sets of those genes that are present in the duplicated segment.

Bar eyes is a dominant X-linked trait in Drosophila females which provides a range of interesting phenotypes resulting from duplication. In a homozygous wild type female there is a large oval compound eye (non-bar) with about 779 facets.

The Bar trait reduces the eye to a vertical bar with very few facets. Bridges analysed the salivary gland chromosomes of Drosophila and found that the Bar gene (B) was present on a region designated 16A of the X chromosome.

When the band in the 16A region is present in duplicate in one X chromosome of the female (i.e. heterozygous for the duplication B/X), it results in an elongated Bar-shaped eye, smaller than the wild type (+/+) due to the presence of only 358 facets.

When a female is homozygous for the duplication (B/B), the Bar-shaped eye is further reduced in size and has 68 facets. If there is unequal crossing over in a female homozygous for Bar (B/B), it results in one chromatid where the 16A region (Bar locus) is present in triplicate, and the second chromatid with only one Bar locus.

Such a heterozygous triplicate condition produces a phenotype known as ultra-bar (B^u) with only 45 facets. If the triplicate condition becomes homozygous (B^U/B^U), the result is a very small eye with only 25 facets (Fig. 12.4). Unequal crossing over is also responsible for a rare human haemoglobin known as haptoglobin.

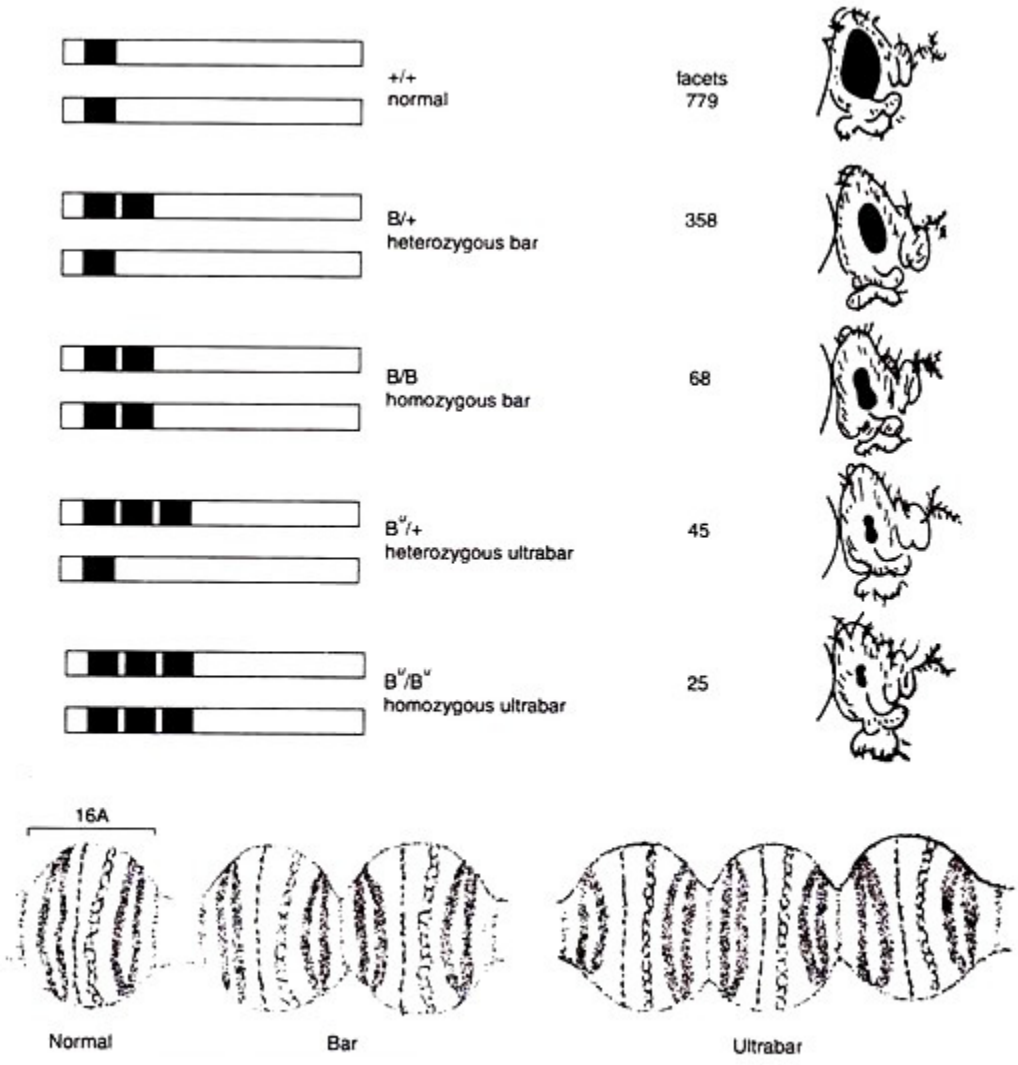


Fig. 12.4 Diagrams showing correlation between duplication of region 16A and inheritance of bar eye trait in *Drosophila*. Below is enlargement of 16A region of the chromosome.

The Bar locus in Drosophila provides an explanation for position effect. According to this phenomenon the expression of a gene becomes altered when the position of the gene is physically changed. Cytologically, a duplication is identified by the same method as deficiency, since in the heterozygous condition the extra fragment forms a loop in one of the two homologues.

Type # 3. Translocations:

Sometimes a segment of a chromosome becomes detached and unites with another non-homologous chromosome. Such an inter-chromosomal rearrangement is called translocation.

The rearrangements are of following types (Fig. 12.5):

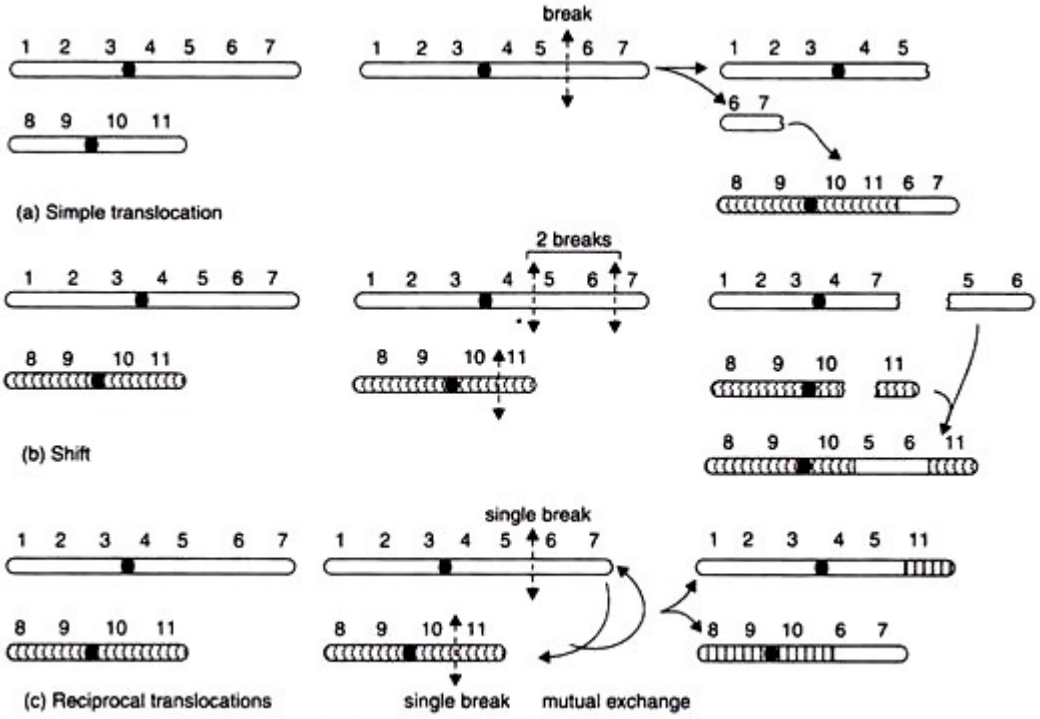


Fig. 12.5 The various types of translocations.

a. Simple Translocation:

A single break occurs in a chromosome, and the broken fragment becomes attached to the end of another chromosome. However, due to the presence of "non-sticky" telomeres at the unbroken ends of a chromosome, such a terminal attachment of a segment does not take place.

b. Shifts:

In this type three breaks are involved. Two breaks occur in a chromosome to produce an interstitial fragment. This fragment becomes inserted into one of the arms of another non-homologous chromosome in which a single break has produced two "sticky" ends.

c. Reciprocal Translocations:

These are the most frequent and extensively studied translocations. A single break occurs in each of the two non-homologous chromosomes followed by a mutual exchange of the broken fragments. This results in two new chromosomes each having one segment of the other chromosome.

d. Multiple Translocations:

Sometimes more than two pairs of non-homologous chromosomes may be involved in a translocation as observed Drosophila and Oenothera. In 1930 Stern studied a multiple translocation system in Drosophila in which a segment of the Y chromosome became attached to the X chromosome. At the same time a reciprocal translocation occurred between the X and chromosome IV. This resulted in a female with 9 chromosomes instead of 8.

e. Half Translocations:

When the nucleus containing two broken chromosomes is small, the broken ends are not widely separated in space and have better chance of undergoing reciprocal exchange. This is true for the small compact nucleus in the head of a sperm.

Type # 4. Inversions:

Inversions result when there are two breaks in a chromosome and the detached segment becomes reinserted in the reversed order. They are classified into two types depending upon the inclusion or absence of the centromere within the inverted segment.

Thus when both breaks occur in one arm of the chromosome it leads to a paracentric inversion; when a break occurs in each of the two arms, the centromere is included in the detached segment and leads to a pericentric inversion.

CHRO.MOSOME	
CHRO.SOMOME	Paracentric inversion
CHOM.OROSOME	Pericentric inversion

Meiosis is normal in inversion homozygotes. In heterozygotes pairing between homologous chromosomes is affected in the region of the inverted segment. Consequently, there is a suppression of recombination and fertility is impaired.

Genetic disorders:

Klinefelter syndrome

Klinefelter syndrome (KS) is a condition that occurs in males when they have an extra X chromosome. Some males with KS have no obvious signs or symptoms while others may have varying degrees of cognitive, social, behavioral, and learning difficulties. Adults with Klinefelter syndrome may also have primary hypogonadism (decreased testosterone production), small and/or undescendent testes (cryptorchidism), enlarged breast tissue (gynecomastia), tall stature, and/or inability to have biological children (infertility), as well as an abnormal opening of the penis (hypospadias), and an small penis (micropenis). KS is not inherited, but usually occurs as a random event during the formation of reproductive cells (eggs and sperm) that results in the presence of one extra copy of the X chromosome in each cell (47,XXY). KS treatment is based on the signs and symptoms present in each person.[1][2][3] Life expectancy is usually normal and many people with KS have normal life. There is a very small risk of developing breast cancer and other conditions such as a chronic inflammatory disease called systemic lupus erythematosus.[3]

In some cases, there is more than one X chromosome in each cell (for example, 48,XXXY or 49,XXXXY). These conditions, which are often called "variants of Klinefelter" syndrome usually have more serious problems (intellectual disability, skeletal problems, and poor coordination) than classic Klinefelter syndrome (47,XXY).[3]

Last updated: 2/14/2018

Symptoms

Listen

The signs and symptoms of Klinefelter syndrome (KS) vary among affected people. Some men with KS have no symptoms of the condition or are only mildy affected. In these cases, they may not even know that they are affected by KS. When present, symptoms may include:[1][2][3]

- Small, firm testicles
- Delayed or incomplete puberty with lack of secondary sexual characteristics resulting in sparse facial, body, or sexual hair a high-pitched voice and body fat distribution resulting in a rounder, lower half of the body, with more fat deposited in the hips, buttocks and thigh instead of around the chest and abdomen
- Breast growth (gynecomastia)
- Reduced facial and body hair
- Infertility
- Tall stature
- Abnormal body proportions (long legs, short trunk, shoulder equal to hip size)
- Learning disablity
- Speech delay
- Crypthochirdism
- Opening (meatus) of the urethra (the tube that carries urine and sperm through the penis to the outside) on the underside of the penis (hypospadias) instead of the tip of the head of the penis
- Social, psychologic and behavioral problems
- Whether or not a male with KS has visible symptoms depends on many factors, including how much testosterone his body makes, if he is mosaic (with both XY and XXY cells), and his age when the condition is diagnosed and treated.[1] Some people have a slightly increased risk of developing breast cancer, a rare extragonadal germ cell tumor, lung disease, varicose veins and osteoporosis as well as some autoimmune disorders such as systemic lupus erythematosus, rheumatoid arthritis and Sjogren's syndrome.[2][4]

  Some people with features of Klinefelter syndrome have more than one extra X chromosome in each cell (such as 48,XXXY or 49,XXXXY). In these cases, known as "variants of Klinefelter syndrome", the signs and symptoms can be more severe and may include:[1][2][4]

- Intellectual disability
- Distinctive facial features
- Skeletal abnormalities
- Poor coordination

- Severe speech difficulties
- Behavioral problems
- Heart defects
- Teeth problems.

CauseKlinefelter syndrome usually occurs as a random event during the formation of reproductive cells (eggs and sperm). An error in cell division called nondisjunction results in a reproductive cell with an abnormal number of chromosomes. For example, an egg or sperm cell may gain one or more extra copies of the X chromosome as a result of nondisjunction. If one of these atypical reproductive cells contributes to the genetic makeup of a child, the child will have one or more extra X chromosomes in each of the body's cells.[2]

Most often, Klinefelter syndrome is caused by a single extra copy of the X chromosome, resulting in a total of 47 chromosomes per cell. Males normally have one X chromosome and one Y chromosome in each cell (46, XY), while females have two X chromosomes (46, XX). People with Klinefelter syndrome usually have two X chromosomes and one Y chromosome (47, XXY). Some people with Klinefelter syndrome have the extra X chromosome in only some of their cells; these people are said to have mosaic Klinefelter syndrome.[2]

It is estimated that about half of the time, the cell division error occurs during development of the sperm, while the remainder are due to errors in egg development. Women who have pregnancies after age 35 have a slightly increased chance of having offspring with this syndrome.[5]

The features of Klinefelter syndrome are due to the extra copies of genes on the extra X chromosome, which can alter male sexual development.

some people with features of Klinefelter syndrome have conditions known as "variants of Klinefelter syndrome" where there is more than one extra sex chromosome in each cell (48,XXXY, 48,XXYY and 49,XXXXY).[5]
Last updated: 2/14/2018

Inheritance:

klinefelter syndrome is not inherited, but usually occurs as a random event during the formation of reproductive cells (eggs and sperm). An error in cell division called nondisjunction can result in reproductive cells with an abnormal number of chromosomes. For example, an egg or sperm cell may gain one or more extra copies of the X chromosome as a result of nondisjunction. If one of these reproductive cells contributes to the genetic makeup of a child, the child will have one or several extra X chromosomes in each of the body's cells.

Turner syndrome:

Turner syndrome, a condition that affects only females, results when one of the X chromosomes (sex chromosomes) is missing or partially missing. Turner syndrome can cause a variety of medical and developmental problems, including short height, failure of the ovaries to develop and heart defects.

Turner syndrome may be diagnosed before birth (prenatally), during infancy or in early childhood. Occasionally, in females with mild signs and symptoms of Turner syndrome, the diagnosis is delayed until the teen or young adult years.

Girls and women with Turner syndrome need ongoing medical care from a variety of specialists. Regular checkups and appropriate care can help most girls and women lead healthy, independent lives.

Symptoms

Signs and symptoms of Turner syndrome may vary among girls and women with the disorder. For some girls, the presence of Turner syndrome may not be readily apparent, but in other girls, a number of physical features and poor growth are apparent early. Signs and symptoms can be subtle, developing slowly over time, or significant, such as heart defects.

Before birth

Turner syndrome may be suspected prenatally based on prenatal cell-free DNA screening — a method to screen for certain chromosomal abnormalities in a developing baby using a blood sample from the mother — or prenatal ultrasound. Prenatal ultrasound of a baby with Turner syndrome may show:

- Large fluid collection on the back of the neck or other abnormal fluid collections (edema)
- Heart abnormalities
- Abnormal kidneys

At birth or during infancy

Signs of Turner syndrome at birth or during infancy may include:

- Wide or weblike neck
- Low-set ears
- Broad chest with widely spaced nipples
- High, narrow roof of the mouth (palate)
- Arms that turn outward at the elbows
- Fingernails and toenails that are narrow and turned upward
- Swelling of the hands and feet, especially at birth
- Slightly smaller than average height at birth
- Slowed growth
- Cardiac defects
- Low hairline at the back of the head
- Receding or small lower jaw
- Short fingers and toes

In childhood, teens and adulthood

The most common signs in almost all girls, teenagers and young women with Turner syndrome are short stature and ovarian insufficiency due to ovarian failure that may have occurred by birth or gradually during childhood, the teen years or young adulthood. Signs and symptoms of these include:

- Slowed growth
- No growth spurts at expected times in childhood
- Adult height significantly less than might be expected for a female member of the family
- Failure to begin sexual changes expected during puberty
- Sexual development that "stalls" during teenage years
- Early end to menstrual cycles not due to pregnancy
- For most women with Turner syndrome, inability to conceive a child without fertility treatment.
- Causes

Most people are born with two sex chromosomes. Boys inherit the X chromosome from their mothers and the Y chromosome from their fathers. Girls inherit one X chromosome from each parent. In girls who have Turner syndrome, one copy of the X chromosome is missing, partially missing or altered.

The genetic alterations of Turner syndrome may be one of the following:

- **Monosomy.** The complete absence of an X chromosome generally occurs because of an error in the father's sperm or in the mother's egg. This results in every cell in the body having only one X chromosome.
- **Mosaicism.** In some cases, an error occurs in cell division during early stages of fetal development. This results in some cells in the body having two complete copies of the X chromosome. Other cells have only one copy of the X chromosome.
- **X chromosome abnormalities.** Abnormal or missing parts of one of the X chromosomes can occur. Cells have one complete and one altered copy. This error can occur in the sperm or egg with all cells having one complete and one altered copy. Or the error can occur in cell division in early fetal development so that only some cells contain the abnormal or missing parts of one of the X chromosomes (mosaicism).

- **Y chromosome material.** In a small percentage of Turner syndrome cases, some cells have one copy of the X chromosome and other cells have one copy of the X chromosome and some Y chromosome material. These individuals develop biologically as female, but the presence of Y chromosome material increases the risk of developing a type of cancer called gonadoblastoma.

Effect of the chromosomal errors

The missing or altered X chromosome of Turner syndrome causes errors during fetal development and other developmental problems after birth — for example, short stature, ovarian insufficiency and heart defects. Physical characteristics and health complications that arise from the chromosomal error vary greatly.

Risk factors

The loss or alteration of the X chromosome occurs randomly. Sometimes, it's because of a problem with the sperm or the egg, and other times, the loss or alteration of the X chromosome happens early in fetal development.

Family history doesn't seem to be a risk factor, so it's unlikely that parents of one child with Turner syndrome will have another child with the disorder.

Complications

Turner syndrome can affect the proper development of several body systems, but varies greatly among individuals with the syndrome. Complications that can occur include:

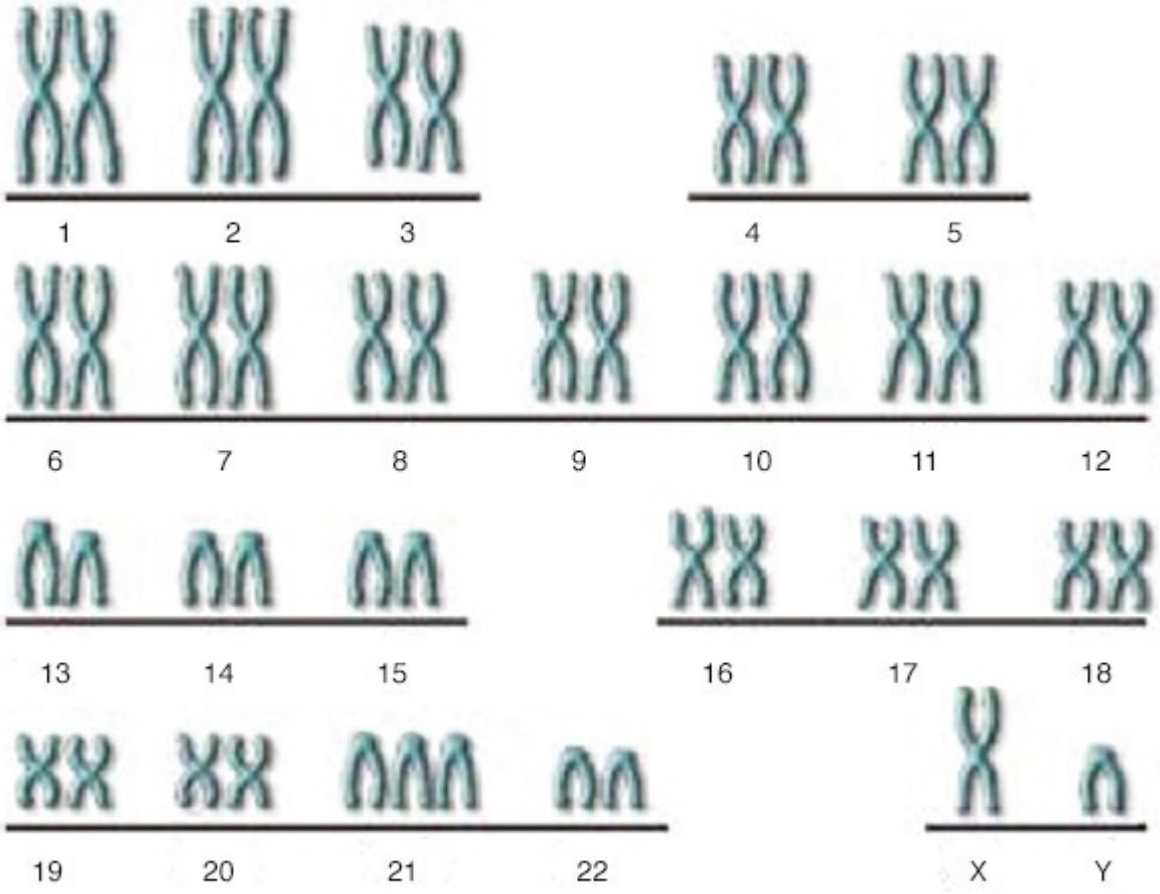
- **Heart problems.** Many infants with Turner syndrome are born with heart defects or even slight abnormalities in heart structure that increase their risk of serious complications. Heart defects often include problems with the aorta, the large blood vessel that branches off the heart and delivers oxygen-rich blood to the body.

- **High blood pressure.** Women with Turner syndrome have an increased risk of high blood pressure — a condition that increases the risk of developing diseases of the heart and blood vessels.
- **Hearing loss.** Hearing loss is common with Turner syndrome. In some cases, this is due to the gradual loss of nerve function. An increased risk of frequent middle ear infections can also result in hearing loss.
- **Vision problems.** Girls with Turner syndrome have an increased risk of weak muscle control of eye movements (strabismus), nearsightedness and other vision problems.
- **Kidney problems.** Girls with Turner syndrome may have some malformation of the kidneys. Although these abnormalities generally don't cause medical problems, they may increase the risk of high blood pressure and urinary tract infections.
- **Autoimmune disorders.** Girls and women with Turner syndrome have an increased risk of an underactive thyroid (hypothyroidism) due to the autoimmune disorder Hashimoto's thyroiditis. They also have an increased risk of diabetes. Some women with Turner syndrome have gluten intolerance (celiac disease) or inflammatory bowel disease.
- **Skeletal problems.** Problems with the growth and development of bones increase the risk of abnormal curvature of the spine (scoliosis) and forward rounding of the upper back (kyphosis). Women with Turner syndrome are also at increased risk of developing weak, brittle bones (osteoporosis).
- **Learning disabilities.** Girls and women with Turner syndrome usually have normal intelligence. However, there is increased risk of learning disabilities, particularly with learning that involves spatial concepts, math, memory and attention.
- **Mental health issues.** Girls and women with Turner syndrome may have difficulties functioning well in social situations and have an increased risk of attention-deficit/hyperactivity disorder (ADHD).
- **Infertility.** Most women with Turner syndrome are infertile. However, a very small number of women may become pregnant spontaneously, and some can become pregnant with fertility treatment.

- **Pregnancy complications.** Because women with Turner syndrome are at increased risk of complications during pregnancy, such as high blood pressure and aortic dissection, they should be evaluated by a cardiologist before pregnancy.

Down syndrome:

Overview



The genetic basis of Down syndrome

Down syndrome is a genetic disorder caused when abnormal cell division results in an extra full or partial copy of chromosome 21. This extra genetic material causes the developmental changes and physical features of Down syndrome.

Down syndrome varies in severity among individuals, causing lifelong intellectual disability and developmental delays. It's the most common genetic chromosomal disorder and cause of learning disabilities in children. It also commonly causes other medical abnormalities, including heart and gastrointestinal disorders.

Better understanding of Down syndrome and early interventions can greatly increase the quality of life for children and adults with this disorder and help them live fulfilling lives.

Symptoms

Each person with Down syndrome is an individual — intellectual and developmental problems may be mild, moderate or severe. Some people are healthy while others have significant health problems such as serious heart defects.

Children and adults with Down syndrome have distinct facial features. Though not all people with Down syndrome have the same features, some of the more common features include:

- Flattened face
- Small head
- Short neck
- Protruding tongue
- Upward slanting eye lids (palpebral fissures)
- Unusually shaped or small ears
- Poor muscle tone

- Broad, short hands with a single crease in the palm
- Relatively short fingers and small hands and feet
- Excessive flexibility
- Tiny white spots on the colored part (iris) of the eye called Brushfield's spots
- Short height

Infants with Down syndrome may be average size, but typically they grow slowly and remain shorter than other children the same age.

Intellectual disabilities

Most children with Down syndrome have mild to moderate cognitive impairment. Language is delayed, and both short and long-term memory is affected.

Causes

Human cells normally contain 23 pairs of chromosomes. One chromosome in each pair comes from your father, the other from your mother.

Down syndrome results when abnormal cell division involving chromosome 21 occurs. These cell division abnormalities result in an extra partial or full chromosome 21. This extra genetic material is responsible for the characteristic features and developmental problems of Down syndrome. Any one of three genetic variations can cause Down syndrome:

- **Trisomy 21.** About 95 percent of the time, Down syndrome is caused by trisomy 21 — the person has three copies of chromosome 21, instead of the usual two copies, in all cells. This is caused by abnormal cell division during the development of the sperm cell or the egg cell.
- **Mosaic Down syndrome.** In this rare form of Down syndrome, a person has only some cells with an extra copy of chromosome 21. This mosaic of normal and abnormal cells is caused by abnormal cell division after fertilization.

- **Translocation Down syndrome.** Down syndrome can also occur when a portion of chromosome 21 becomes attached (translocated) onto another chromosome, before or at conception. These children have the usual two copies of chromosome 21, but they also have additional genetic material from chromosome 21 attached to another chromosome.
- There are no known behavioral or environmental factors that cause Down syndrome.

Is it inherited?

Most of the time, Down syndrome isn't inherited. It's caused by a mistake in cell division during early development of the fetus.

Translocation Down syndrome can be passed from parent to child. However, only about 3 to 4 percent of children with Down syndrome have translocation and only some of them inherited it from one of their parents.

When balanced translocations are inherited, the mother or father has some rearranged genetic material from chromosome 21 on another chromosome, but no extra genetic material. This means he or she has no signs or symptoms of Down syndrome, but can pass an unbalanced translocation on to children, causing Down syndrome in the children.

Risk factors

Some parents have a greater risk of having a baby with Down syndrome. Risk factors include:

- **Advancing maternal age.** A woman's chances of giving birth to a child with Down syndrome increase with age because older eggs have a greater risk of improper chromosome division. A woman's risk of conceiving a child with Down syndrome increases after 35 years of age. However, most children with Down syndrome are born to women under age 35 because younger women have far more babies.
- **Being carriers of the genetic translocation for Down syndrome.** Both men and women can pass the genetic translocation for Down syndrome on to their children.

- **Having had one child with Down syndrome.** Parents who have one child with Down syndrome and parents who have a translocation themselves are at an increased risk of having another child with Down syndrome. A genetic counselor can help parents assess the risk of having a second child with Down syndrome.
- Complications

- People with Down syndrome can have a variety of complications, some of which become more prominent as they get older. These complications can include:

- **Heart defects.** About half the children with Down syndrome are born with some type of congenital heart defect. These heart problems can be life-threatening and may require surgery in early infancy.
- **Gastrointestinal (GI) defects.** GI abnormalities occur in some children with Down syndrome and may include abnormalities of the intestines, esophagus, trachea and anus. The risk of developing digestive problems, such as GI blockage, heartburn (gastroesophageal reflux) or celiac disease, may be increased.
- **Immune disorders.** Because of abnormalities in their immune systems, people with Down syndrome are at increased risk of developing autoimmune disorders, some forms of cancer, and infectious diseases, such as pneumonia.
- **Sleep apnea.** Because of soft tissue and skeletal changes that lead to the obstruction of their airways, children and adults with Down syndrome are at greater risk of obstructive sleep apnea.
- **Obesity.** People with Down syndrome have a greater tendency to be obese compared with the general population.
- **Spinal problems.** Some people with Down syndrome may have a misalignment of the top two vertebrae in the neck (atlantoaxial instability). This condition puts them at risk of serious injury to the spinal cord from overextension of the neck.
- **Leukemia.** Young children with Down syndrome have an increased risk of leukemia.

- **Dementia.** People with Down syndrome have a greatly increased risk of dementia — signs and symptoms may begin around age 50. Having Down syndrome also increases the risk of developing Alzheimer's disease.
- **Other problems.** Down syndrome may also be associated with other health conditions, including endocrine problems, dental problems, seizures, ear infections, and hearing and vision problems.

Questions:

Long answer questions:

1. Explain about centromeres and telomeres.
2. Packaging of DNA molecules into chromosome
3. Concept of Euchromatin and heterochromatin.
4. Chromosome banding.
5. Giant and lumpbrush chromosome.
6. Variation in chromosome structure.
7. Genetic disorders.

Short answer questions:

1. Centromeres
2. Euchromatin
3. Polytene chromosome
4. Inversion of chromosome
5. Down syndrome.