KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed to be University Established Under Section 3 of UGC Act 1956)
Coimbatore – 641 021.

LECTURE PLAN
DEPARTMENT OF BIOCHEMISTRY

STAFF NAME: K.DEVAKI
SUBJECT NAME: GENE ORGANISATION, REPLICATION AND REPAIR
SUB.CODE:16BCU401
SEMESTER: IV CLASS: II B.Sc (BC)

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-I	
1	1	**Structure, Genes and genomic organization of DNA- Introduction**	R3: 225-230
2	1	DNA structure, features of the double helix,	R3: 225-230
3	1	Various forms of DNA,	W1
4	1	Denaturation and reassociation of DNA.	R2: 259-260
5	1	**Genes and genomic organization** - Genome sequence	R2:217-224
6	1	Chromosome diversity	R3:141-143
7	1	Definition of a gene, organization of genes in viruses	R3:441-443
8	1	Organization of genes in bacteria	R1:193-202
9	1	Organization of genes in animals and plants	-
10	1	Nucleosome structure and packaging of DNA into higher order structures	R2:938-943
11	1	Revision and QP discussion	-
	Total No of Hours Planned For Unit 1=11		
		UNIT-II	
1	1	**Replication of DNA in Prokaryotes-** The chemistry of DNA synthesis	R2:950-951

2	1	DNA polymerase,	R2:952-955
3	1	The replication fork, origin of replication,	R3:259-263
4	1	Enzymes and proteins in DNA replication	R3:955-957
5	1	Various modes of replication	R1:301-304
6	1	Stages of replication of *E. coli* chromosome,	R2:958-959
7	1	Stages of replication of *E. coli* chromosome,	R2:961-963
8	1	Stages of replication of *E. coli* chromosome	R2:961-963
9	1	Relationship between replication and cell division	W2
10	1	Revision and QP discussion	-
	Total No of Hours Planned For Unit II=10		
		UNIT-III	
1	1	**Replication of DNA in Eukaryotes** Replication in eukaryotes.	R2:964-966
2	1	Comparison of replication in prokaryotes and eukaryotes	R2:966-968
3	1	Inhibitors of DNA replication	W31
4	1	Applications DNA inhibitors in medicine	W31
5	1	Supercoiling of DNA and its importance	R2:930-933 R1:256-261
6	1	Topoisomerases,	R2:933-936
7	1	Critical role of topoisomerases in cell	R1:262-265
8	1	Topoisomerase	R1:262-265
9	1	Topoisomerase inhibitors application in medicine	W32 A1
10	1	Revision and discussion of possible question	-
	Total No of Hours Planned For Unit III=10		
		UNIT-IV	
1	1	**Recombination and transposition of DNA** Homologous recombination-Introduction	R1:316-323

2	1	proteins and enzymes in recombination	R2:978-979
3	1	site-specific recombination	R2:984-988
4	1	serine and tyrosine recombinases	R1:323-325
5	1	biological roles of site-specific recombination	R2:984-988
6	1	transposition	R2:988-990
7	1	three classes of transposable elements,	R1:332-334
8	1	three classes of transposable elements,	R3:477-480
9	1	importance of transposable elements in horizontal transfer of genes	R3:480-481
10	1	importance of transposable elements in evolution.	R3:491-493
11	1	Revision and discussion of possible question	-
	Total No of Hours Planned For Unit IV=11		
		UNIT-V	
1	1	**Molecular basis of mutations and DNA repair**	R3:358-360
2	1	Importance of mutations in evolution of species.	R3:360-366
3	1	Types of mutations - transition, transversions, frame shift mutation	W5
4	1	mutations induced by chemicals	R3:371-377
5	1	mutations induced by radiation,	R3:377-378
6	1	transposable elements, Ames test.	R3:378-382
7	1	Various modes of DNA repair - Replication errors	R2:966-976
8	1	mismatch repair system	R1:348-385
9	1	repair of DNA damage, direct repair,	R3:383-385
10	1	base excision repair, nucleotide excision repair	R2:970-976
11	1	recombination repair,	R1:352-353
12	1	translesion DNA synthesis.	R1:353-354
13	1	Revision and discussion of	-

		possible question	
14	1	Previous year End Semester Exam- QP discussion	-
15	1	Previous year End Semester Exam- QP discussion	-
16	1	Previous year End Semester Exam- QP discussion	-
	Total No of Hours Planned for unit V=16		
Total Planned Hours	**58**		

REFERENCE:

R1: Watson, J.D., Baker, T.A., Bell, S.P., Gann, A., Levine, M. and Losick, R., (2008). Molecular Biology of the Gene 6th ed., Cold Spring Harbor Laboratory Press, Cold spring Harbor (New York), ISBN:0-321-50781 / ISBN:978-0-321-50781-5.

R2: Nelson, D.L. and Cox, M.M., (2013). Lehninger: Principles of Biochemistry 6th ed., W. H. Freeman & Company (New York), ISBN:13: 978-1-4292-3414-6 / ISBN:10-14641-0962-1.

R3: Snustad, D.P., and Simmons, M.J., (2010). Principles of Genetics 5th ed., John Wiley & Sons Asia, ISBN:978-0-470-39842-5.

WEBSITE

W1: www.biology discussion.com.....DNA/forms of DNA

W2: www.ncbi.nlm.nih.gov/pmc/articles/pcm 246144

W31: http://study.com..../inhibition of DNA, rDNA syn...

W32: http:// chemoth/type/topoisomerase....

W5: http//:ghr.nlm.nih.gov/primer/mutation

A1: Topoisomerase 2 inhibitors, (20011), Int.J.Pharm sci and nanotech, 3,1173-1181.

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Unit 1

Structure, Genes and genomic organization of DNA

DNA structure, features of the double helix, various forms of DNA, denaturation and reassociation of DNA. **Genes and genomic organization** - Genome sequence and chromosome diversity, definition of a gene, organization of genes in viruses, bacteria, animals and plants. Nucleosome structure and packaging of DNA into higher order structures.

Unit 2

Replication of DNA in Prokaryotes

The chemistry of DNA synthesis, DNA polymerase, the replication fork, origin of replication, enzymes and proteins in DNA replication, various modes of replication, stages of replication of *E. coli* chromosome, relationship between replication and cell division,

Unit3

Replication of DNA in Eukaryotes

Replication in eukaryotes. Comparison of replication in prokaryotes and eukaryotes. Inhibitors of DNA replication and applications in medicine. Supercoiling of DNA and its importance, topoisomerases, critical role of topoisomerases in cell, topoisomerase inhibitors and their application in medicine.

Unit 4

Recombination and transposition of DNA

Homologous recombination, proteins and enzymes in recombination, site-specific recombination, serine and tyrosine recombinases, biological roles of site-specific recombination, transposition, three classes of transposable elements, importance of transposable elements in horizontal transfer of genes and evolution.

Unit 5

Molecular basis of mutations and DNA repair

Importance of mutations in evolution of species. Types of mutations - transition, transversions, frame shift mutations, mutations induced by chemicals, radiation, transposable elements, Ames test. Various modes of DNA repair - Replication errors and mismatch repair system, repair of DNA damage, direct repair, base excision repair, nucleotide excision repair, recombination repair, translesion DNA synthesis.

REFERENCES

Watson, J.D., Baker, T.A., Bell, S.P., Gann, A., Levine, M. and Losick, R., (2008). Molecular Biology of the Gene 6th ed., Cold Spring Harbor Laboratory Press, Cold spring Harbor (New York), ISBN:0-321-50781 / ISBN:978-0-321-50781-5.

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Snustad, D.P., and Simmons, M.J., (2010). Principles of Genetics 5th ed., John Wiley & Sons Asia, ISBN:978-0-470-39842-5.

TEXT BOOK

Lehninger, L., Nelson, D.L., and Cox, M.M., (2012). Principles of Biochemistry, 6th edition WH Freeman and Company, New York.

Murray, R.K., Bender, D.A., Botham, K.M., and Kennelly, P.J., (2012). Harper's illustrated Biochemistry, 29th Edition. McGraw-Hill Medical. London.

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Donald Voet and Judith Voet ,2004. Biochemistry, John Wiley and Sons,. 2ndEedition. New York

Leubert Stryer, 2009. Biochemistry, W.H. Freeman and Company. New York.

Pamila C. Champ and Richard A. Harvey ,2008. Biochemistry, Lipponcott Company, Philadelphia.

Smith. 2003. Principles of Biochemistry, McGraw– Hill International Book Company, London.

Zubay, G., (2009). Biochemistry, W.C Brown Publishers, Saunders and Company, Philadelphia.

UNIT-I

SYLLABUS

Structure, Genes and genomic organization of DNA

DNA structure, features of the double helix, various forms of DNA, denaturation and reassociation of DNA. **Genes and genomic organization** - Genome sequence and chromosome diversity, definition of a gene, organization of genes in viruses, bacteria, animals and plants. Nucleosome structure and packaging of DNA into higher order structures.

DNA

- DNA is a polymer of deoxyribonucleotides (or simply deoxynucleotides).
- It is composed of monomeric units namely deoxyadenylate (dAMP), eoxyguanylate (dGMP), deoxycytidylate(dCMP) and deoxythymidylate(d TMP) (It may be noted here that some authors prefer to use TMP for deoxythymidylate, since it is found only in DNA).
- The details of the nucleotide structure are given above.

STRUCTURE OF DNA (DNA DOUBLE HELIX)

- The double helical structure of DNA was proposed by James Watson and Francis Crick in 1953 (Nobel Prize, 1962).
- The elucidation of DNA structure is considered as a milestone in the era of modern biology.
- The structure of DNA double helix is comparable to a twisted ladder.
- The salient features of Watson - Crick Model of DNA (now known as B-DNA) are described next.

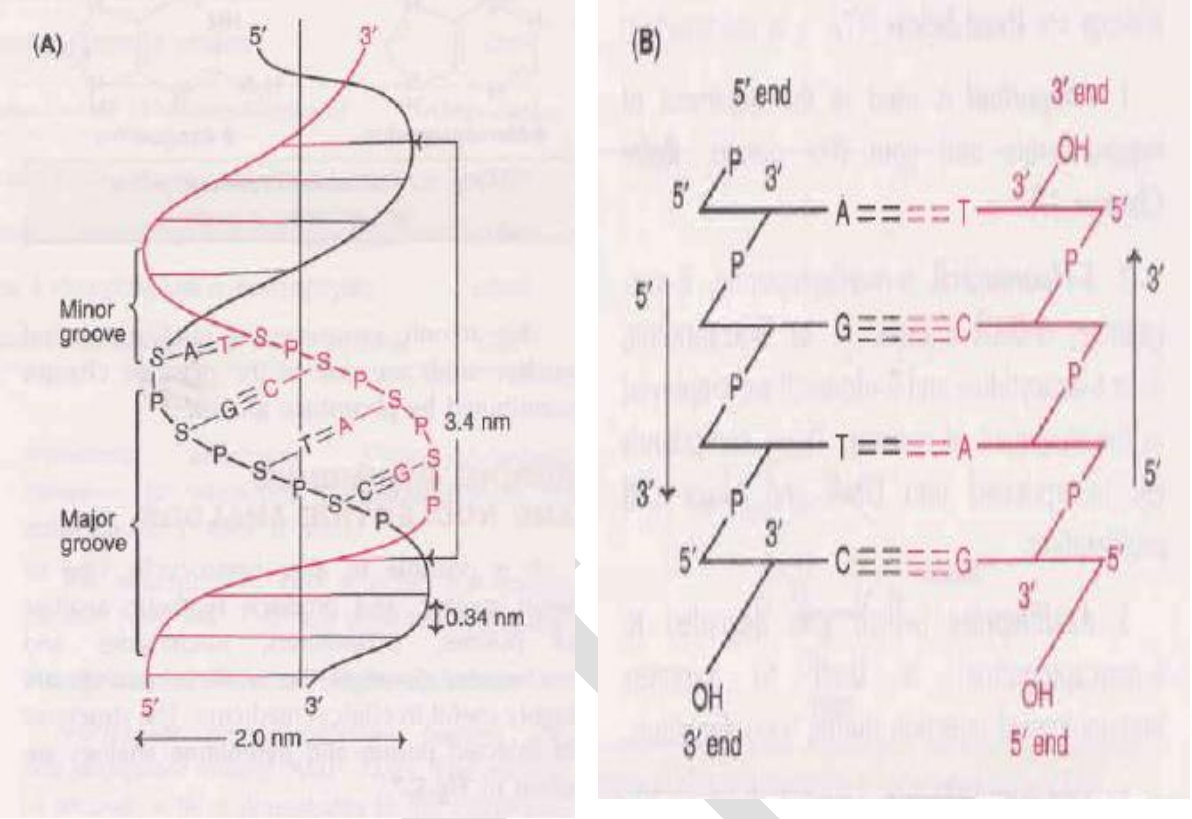

Fig: (A) Watson-Crick model of DNA helix (B) Complementary base pairing in DNA helix.

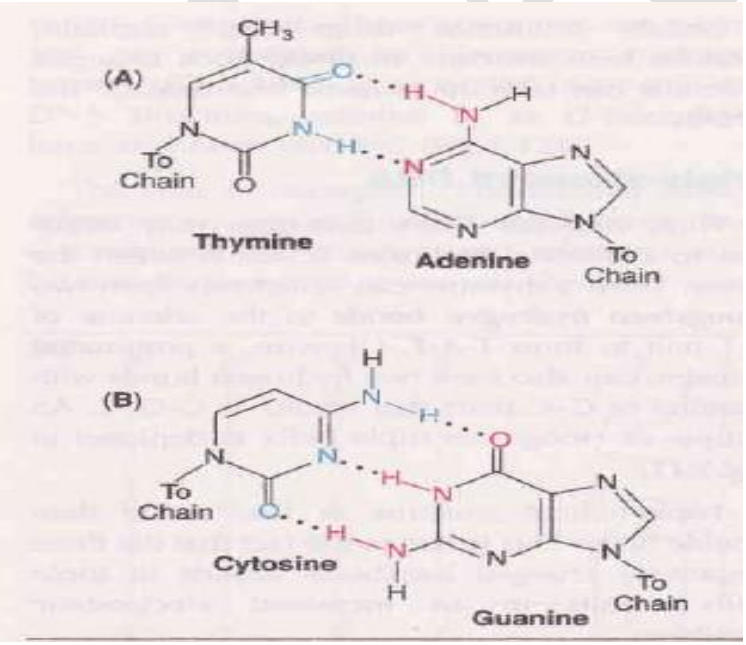

Fig:Complementary base paring in DNA
(A) Thymine pairs with adenine by 2 hydrogen bonds
(B) Cytosine pairs with guanine by 3 hydrogen bonds.

Features of the double helix

- The DNA is a right handed double helix.
- It consists of two polydeoxyribonucleotide chains (strands) twisted around each other on a common axis.
- The two strands are **antiparallel,** i.e., one strand runs in the 5' to 3' direction while the other in 3'to 5'direction.T his is comparable to two parallel adjacent roads carrying traffic in opposite direction.
- The width (or diameter) of a double helix is 20 A^o (2 nm).
- Each turn (pitch) of the helix is 34 A" (3.4 nm) with 10 pairs of nucleotides each pair placed at a distance of about 3.4 A^o.
- Each strand of DNA has a hydrophilic deoxyribose phosphate backbone (3'-5' **phosphor diester bonds**)on the outside (periphery) of the molecule while the hydrophobic bases are stacked inside (core).
- The two polynucleotide chains are not identical but complementary to each other due to base pairing.
- The two strands are held together by **hydrogen bonds** formed by complementary base pairs.
- The A-T pair has 2 hydrogen bonds while G-C pair has 3 hydrogen bonds. The G = C is stronger by about 50% than A=T.
- The hydrogen bonds are formed between a purine and a pyrimidine only. If two purines face each other, they would not fit into the allowable space. And two pyrimidines would be too far to form hydrogen bonds. The only base arrangement possible in DNA structure, from spatial considerations is A-T, T-A, G-C and C-C.
- The **complementary base pairing in DNA helix proves Chargaffs rule**.The content of adenine equals to that of thymine (A = T) and guanine equals to that of cytosine (G = C).
- The genetic information resides on one of the two strands known as template strand or sense strand. The opposite strand is antisense strand.
- The double helix has (wide) major grooves and (narrow) minor grooves along the phosphodiester backbone.Proteins interact with DNA at these grooves, without disrupting the base pairs and double helix.

Various forms of DNA

A, B, Z forms of DNA

B-form

- Most common DNA conformation in vivo
- Narrower, more elongated helix than A.
- Wide major groove easily accessible to proteins
- Narrow minor groove

- Favored conformation at high water concentrations (hydyration of minor groove seems to favor B-form)
- Base pairs nearly perpendicular to helix axis

A-form

- Most RNA and RNA-DNA duplex in this form
- shorter, wider helix than B.
- deep, narrow major groove not easily accessible to proteins
- wide, shallow minor groove accessible to proteins, but lower information content than major groove.
- favored conformation at low water concentrations
- base pairs tilted to helix axis

Z-form

- Helix has left-handed sense
- Can be formed in vivo, given proper sequence and superhelical tension, but function remains obscure.
- Narrower, more elongated helix than A or B.
- Major "groove" not really groove
- Narrow minor groove
- Conformation favored by high salt concentrations, some base substitutions, but requires alternating purine-pyrimidine sequence.
- N2-amino of G H-bonds to 5' PO: explains slow exchange of proton, need for G purine.
- Base pairs nearly perpendicular to helix axis
- GpC repeat, not single base-pair
    - P-P distances: vary for GpC and CpG
    - GpC stack: good base overlap
    - CpG: less overlap.
- Zigzag backbone due to C sugar conformation compensating for G glycosidic bond conformation

The backbone also is not a continuous curve, it "zig-zags" back and forth (hence "Z"-DNA).

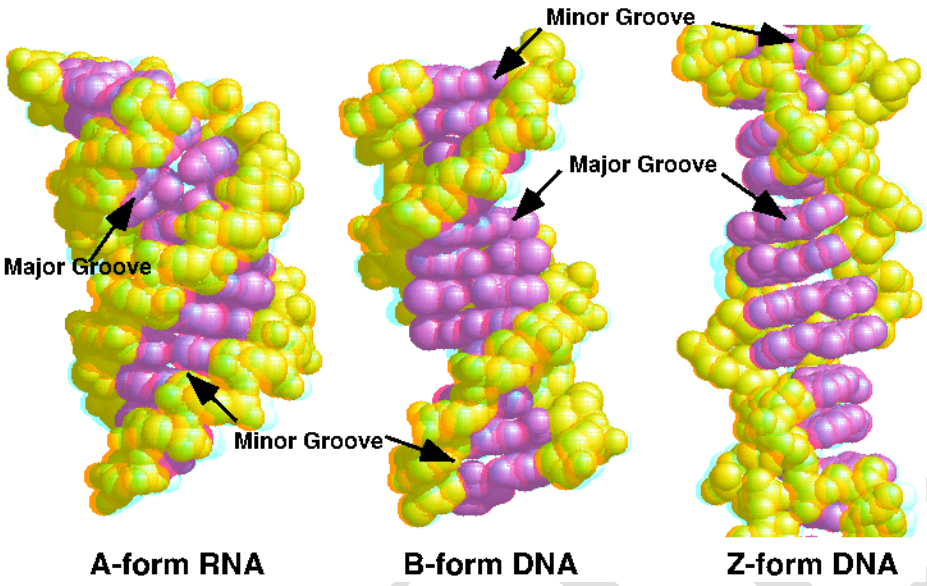

Fig: DNA Structure - A,B, Z forms

The geometry of the DNA forms can be used to describe the differences seen.

	A	B	Z
Helix sense	Right handed	Right-handed	Left handed
Repeating unit	1 bp	1bp	2 bp
Rotation/bp	33.6°	35.9°	60°/2
Mean bp/turn	10.7	10.0	12
Inclination of bp to axis	+19°	-1.2°	-9°
Rise/bp along axis	2.3Å	3.32Å	3.8Å
Pitch/turn of helix	24.6Å	33.2Å	45.6Å
Mean propeller twist	+18°	+16°	0°
Glycosyl angle	anti	anti	C: anti, G: syn
Sugar pucker	C3'-endo	C2'-endo	C: C2'-endo, G: C2'-exo

Diameter	26Å	20Å	18Å

DNA- DENATURATION AND RENATURATION

DENATURATION

The two strands of DNA helix are held together by hydrogen bonds.Disruption of hydrogen bonds (by change in pH or increase in temperature) results in the separation of polynucleotide strands. This phenomenon of loss of helical structure of DNA is known as denaturation.

- The phosphodiester bonds are not broken by denaturation. The loss of helical structure can be measured by increase in absorbance at 260 nm (in a spectrophotometer).
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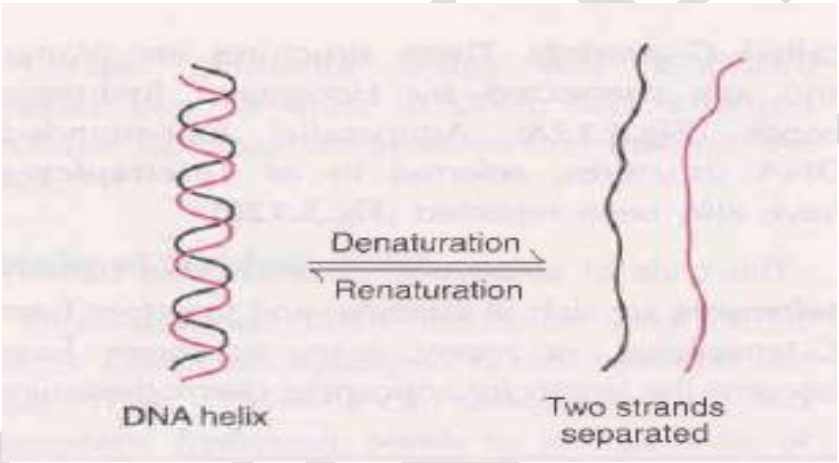


Fig: Denaturation and renaturation of DNA.

- Melting temperature (Tm) is defined as the temperature at which half of the helical structure of DNA is lost.
- As the temperature increases, local unwinding of the double-stranded DNA occurs. This unwinding occurs preferentially in regions where the two strand are held together less strongly(A-T). In these regions the strands separate to form bubbles of single-stranded regions. The DNA sequence in these regions is enriched in A/T base pairs because the interactions between the two strands are weaker in A/T rich regions. In G/C rich regions strands are held together more strongly so they don't unwind until higher temperatures

- Since C-C base pairs are more stable (due to 3 hydrogen bonds) than A-T base pairs(2 hydrogen bonds), the Tm is greater for DNAs with higher C-C content.
- Thus, the Tm is 65°C for 35% G-C content while it is 70°C for 50% G-C content.
- Formamide destabilizes hydrogen bonds of base pairs and, therefore, lowers Tm.This chemical compound is effectively used in recombinant DNA experiments.

RENATURATION:

- Renaturation or reannealing is the process in which the separated complementary DNA strands can form a double helix.
- As the temperature is lowered, the double-stranded form becomes more stable than the single strand in solution, and the DNA renatures.
- The first step is a nucleation event where two complementary regions come into contact. Nucleation is the rate-limiting step in renaturation. Once nucleation occurs, the rest of the molecule zips up very quickly.
- When all of the base interactions are broken, the two strands separate. This is called denaturation. (Local unwinding is not denaturation.)

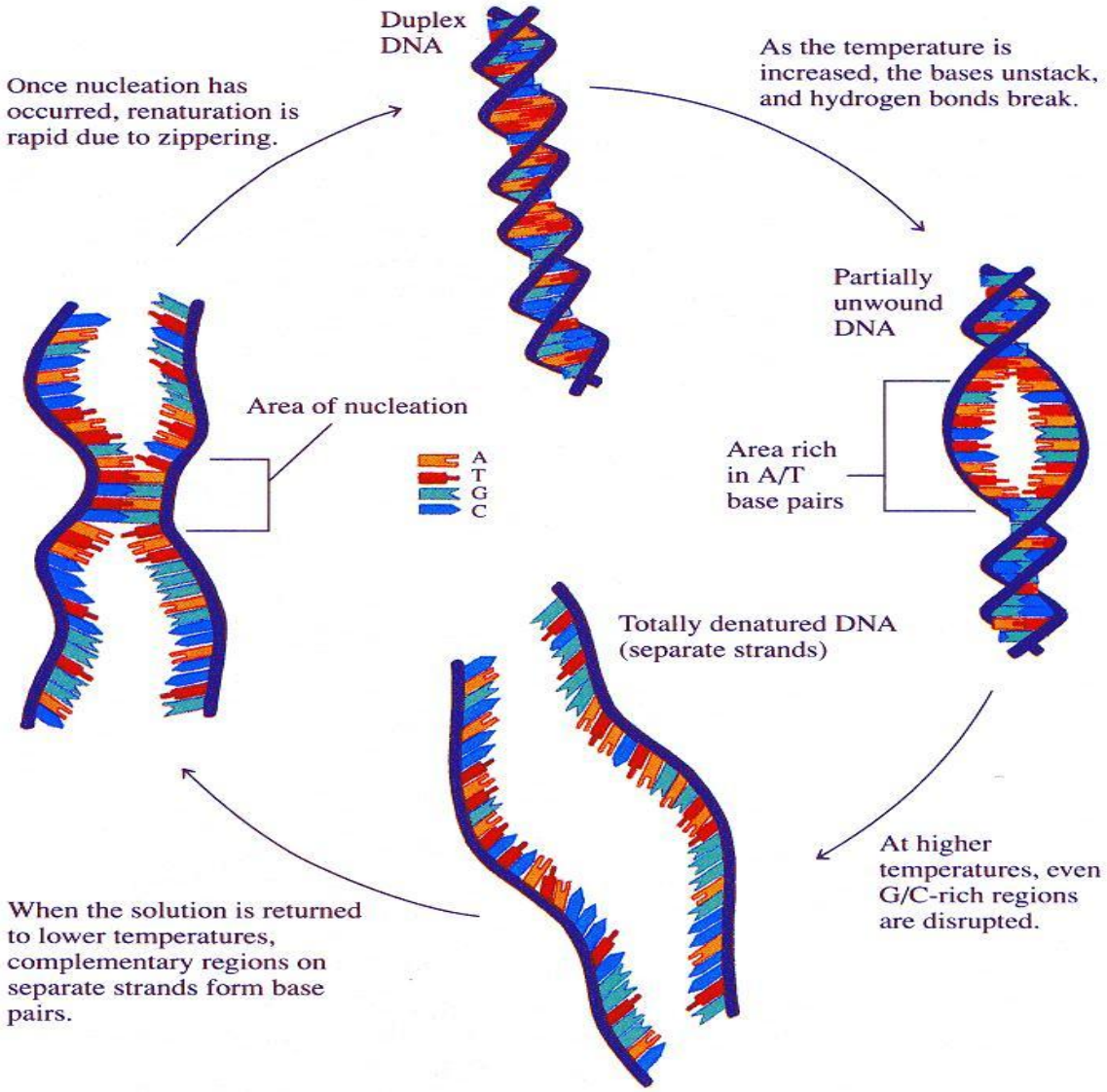


Fig: Steps in DNA Denaturation and Renaturation

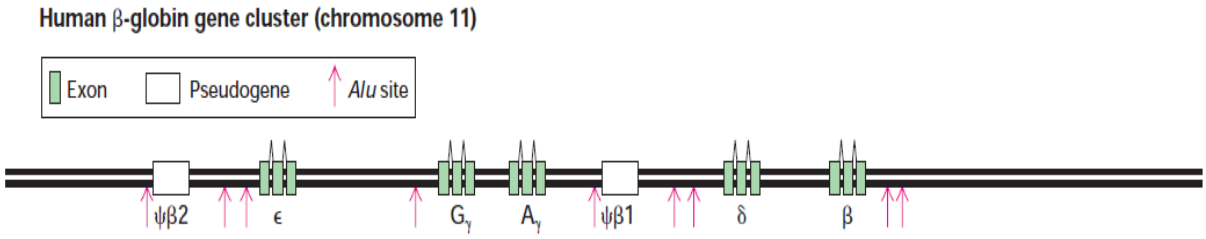
GENES AND GENOMIC ORGANIZATION

GENOME SEQUENCES IN DNA

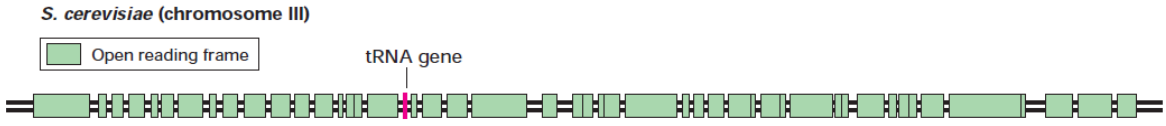
Introduction

- The vertebrates with the greatest amount of DNA per cell are amphibians, which are surely less complex than humans in their structure and behavior.
- The unicellular protozoal species *Amoeba dubia* has 200 times more DNA per cell than humans.

- Many plant species also have considerably more DNA per cell than humans have. For example, tulips have 10 times as much DNA per cell as humans.
- The DNA content per cell also varies considerably between closely related species.
- All insects or all amphibians would appear to be similarly complex, but the amount of haploid DNA in species within each of these phylogenetic classes varies by a factor of 100.
- The genomes of higher eukaryotes contain large amounts of noncoding DNA. For example, only a small portion of the β-globin gene cluster of humans, about 80 kb long, encodes protein.



- Moreover, compared with other regions of vertebrate DNA, the β-globin gene cluster is unusually rich in protein-coding sequences, and the introns in globin genes are considerably shorter than those in many human genes.
- In contrast, a typical 80-kb stretch of DNA from the yeast *S. cerevisiae*, a single-celled eukaryote (Figure 10-3b) contains many closely spaced protein-coding sequences without introns and relatively much less noncoding DNA.



- Of the 94 percent of human genomic DNA that has been sequenced, only ≈1.5 percent corresponds to protein-coding sequences (exons).
- Most human exons contain 50–200 base pairs, although the 3' exon in many transcription units is much longer.
- Human introns vary in length considerably. Although many are ≈90 bp long, some are much longer; their median length is 3.3 kb. Approximately one-third of human genomic

DNA is thought to be transcribed into pre-mRNA precursors, but some 95 percent of these sequences are in introns, which are removed by RNA splicing.

- Different selective pressures during evolution may account, at least in part, for the remarkable difference in the amount of nonfunctional DNA in unicellular and multicellular organisms.
- For example, microorganisms must compete for limited amounts of nutrients in their environment, and metabolic economy thus is a critical characteristic.
- Since synthesis of nonfunctional (i.e., noncoding) DNA requires time and energy, presumably there was selective pressure to lose nonfunctional DNA during the evolution of microorganisms.
- On the other hand, natural selection in vertebrates depends largely on their behavior.
- The energy invested in DNA synthesis is trivial compared with the metabolic energy required for the movement of muscles; thus there was little selective pressure to eliminate nonfunctional DNA in vertebrates.

1.Protein coding genes

There are two groups of protein coding genes. **(i) Solitary genes (ii) Duplicated genes**

(i) Solitary genes

- In multicellular organisms, roughly 25–50 percent of the protein-coding genes are represented only once in the **haploid genome** and thus are termed *solitary* genes.
- A well studied example of a solitary protein-coding gene is the chicken lysozyme gene.
- The 15-kb DNA sequence encoding chicken lysozyme constitutes a simple transcription unit containing four exons and three introns.
- The flanking regions, extending for about 20 kb upstream and downstream from the transcription unit, do not encode any detectable mRNAs.
- Lysozyme, an enzyme that cleaves the polysaccharides in bacterial cell walls, is an abundant component of chicken egg-white protein and also is found in human tears.
- Its activity helps to keep the surface of the eye and the chicken egg sterile.

(ii) Duplicated genes

- Duplicated genes constitute the second group of protein coding genes.
- These are genes with close but nonidentical sequences that generally are located within 5–50 kb of one another. In vertebrate genomes, duplicated genes probably constitute half the protein-coding DNA sequences.
- A set of duplicated genes that encode proteins with similar but nonidentical amino acid sequences is called a **gene family;** the encoded, closely related, homologous proteins constitute a **protein family.**
- A few protein families, such as protein kinases, transcription factors, and vertebrate immunoglobulins, include hundreds of members.
- Most protein families, however, include from just a few to 30 or so members; common examples are cytoskeletal proteins, 70-kDa heat-shock proteins, the myosin heavy chain, chicken ovalbumin, and the α- and β-globins in vertebrates.
- The genes encoding the β-like globins are a good example of a gene family.
- The β-like globin gene family contains five functional genes designated β, δ, Aγ, Gγ, and ε; the encoded polypeptides are similarly designated.
- Two identical β-like globin polypeptides combine with two identical α-globin polypeptides (encoded by another gene family) and four small heme groups to form a hemoglobin molecule All the hemoglobins formed from the different β-like globins carry oxygen in the blood, but they exhibit somewhat different properties that are suited to specific roles in human physiology.
- For example, hemoglobins containing either the Aγor Gγ polypeptides are expressed only during fetal life.
- Because these fetal hemoglobins have a higher affinity for oxygen than adult hemoglobins, they can effectively extract oxygen from the maternal circulation in the placenta.
- The lower oxygen affinity of adult hemoglobins, which are expressed after birth, permits better release of oxygen to the tissues, especially muscles, which have a high demand for oxygen during exercise.

- The different β-globin genes probably arose by duplication of an ancestral gene, most likely as the result of an "unequal crossover" during meiotic recombination in a developing germ cell (egg or sperm).

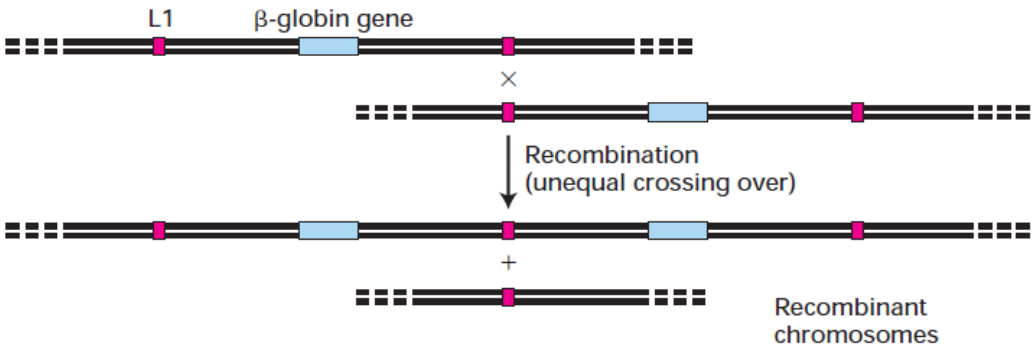


Figure: Gene duplication resulting from unequal crossing over

- Over evolutionary time the two copies of the gene that resulted accumulated random mutations; beneficial mutations that conferred some refinement in the basic oxygen-carrying function of hemoglobin were retained by natural selection, resulting in *sequence drift.*
- Repeated gene duplications and subsequent sequence drift are thought to have generated the contemporary globin-like genes observed in humans and other complex species today.
- Two regions in the human β-like globin gene cluster contain nonfunctional sequences, called **pseudogenes,** similar to those of the functional β-like globin genes.
- Sequence analysis shows that these pseudogenes have the same apparent exon-intron structure as the functional β-like globin genes, suggesting that they also arose by duplication of the same ancestral gene.
- However, sequence drift during evolution generated sequences that either terminate translation or block mRNA processing, rendering such regions nonfunctional even if they were transcribed into RNA.
- Because such pseudogenes are not deleterious, they remain in the genome and mark the location of a gene duplication that occurred in one of our ancestors.

- Other nonfunctional gene copies can arise by reverse transcription of mRNA into cDNA and integration of this intron-less DNA into a chromosome.
- Several different gene families encode the various proteins that make up the cytoskeleton.
- These proteins are present in varying amounts in almost all cells.
- In vertebrates, the major cytoskeletal proteins are the actins, tubulins, and intermediate filament proteins like the keratins.
- We examined the origin of one such family, the tubulin family, in the last chapter (see Figure 9-32). Although the physiological rationale for the cytoskeletal protein families is not as obvious as it is for the globins, the different members of a family probably have similar but subtly different functions suited to the particular type of cell in which they are expressed.

2.Tandemly repeated genes

- In vertebrates and invertebrates, the genes encoding rRNAs and some other noncoding RNAs such as some of the snRNAs involved in RNA splicing occur as tandemly repeated arrays.
- These are distinguished from the duplicated genes of gene families in that the multiple tandemly repeated genes encode identical or nearly identical proteins or functional RNAs.
- Most often copies of a sequence appear one after the other, in a head-to-tail fashion, over a long stretch of DNA.
- Within a tandem array of rRNA genes, each copy is exactly, or almost exactly, like all the others. Although the transcribed portions of rRNA genes are the same in a given individual, the nontranscribed spacer regions between the transcribed regions can vary.
- The tandemly repeated rRNA, tRNA, and histone genes are needed to meet the great cellular demand for their transcripts.
- To understand why, consider that a fixed maximal number of RNA copies can be produced from a single gene during one cell generation when the gene is fully loaded with RNA polymerase molecules.

- If more RNA is required than can be transcribed from one gene, multiple copies of the gene are necessary.
- For example, during early embryonic development in humans, many embryonic cells have a doubling time of ≈24 hours and contain 5–10 million ribosomes.
- To produce enough rRNA to form this many ribosomes, an embryonic human cell needs at least 100 copies of the large and small subunit rRNA genes, and most of these must be close to maximally active for the cell to divide every 24 hours (Table 10-2). That is, multiple RNA polymerases must be loaded onto and transcribing each rRNA gene at the same time.
- All eukaryotes, including yeasts, contain 100 or more copies of the genes encoding 5S rRNA and the large and small subunit rRNAs.
- The importance of repeated rRNA genes is illustrated by Drosophila mutants called bobbed (because they have stubby wings), which lack a full complement of the tandemly repeated **pre-rRNA** genes.
- A bobbed mutation that reduces the number of pre-rRNA genes to less than ≈50 is a recessive lethal mutation.
- Multiple copies of tRNA and histone genes also occur, often in clusters, but generally not in tandem arrays.

Simple sequence DNA

- Besides duplicated protein-coding genes and tandemly repeated genes, eukaryotic cells contain multiple copies of other DNA sequences in the genome, generally referred to as repetitious DNA
- Of the two main types of repetitious DNA, the less prevalent is **simple-sequence DNA**, which constitutes about 3 percent of the human genome and is composed of perfect or nearly perfect repeats of relatively short sequences.
- Simple-sequence DNA is commonly called satellite DNA because in early studies of DNAs from higher organisms using equilibrium buoyant-density ultracentrifugation some simple-sequence DNAs banded at a different position from the bulk of cellular DNA.

These were called satellite bands to distinguish them from the main band of DNA in the buoyant-density gradient.

- Simple-sequence DNAs in which the repeats contain 1–13 base pairs are often called microsatellites.
- Most have repeat lengths of 1–4 base pairs and usually occur in tandem repeats of 150 base pairs or fewer.
- Microsatellites are thought to have originated by "backward slippage" of a daughter strand on its template strand during DNA replication so that the same short sequence is copied twice.
- Microsatellites occasionally occur within transcription units.
- Some individuals are born with a larger number of repeats in specific genes than observed in the general population, presumably because of daughter-strand slippage during DNA replication in a germ cell from which they developed.
- Such expanded microsatellites have been found to cause at least 14 different types of neuromuscular diseases, depending on the gene in which they occur.
- In some cases expanded microsatellites behave like a recessive mutation because they interfere with the function or expression of the encoded gene.
- But in the more common types of diseases associated with expanded microsatellite repeats, myotonic dystrophy and spinocerebellar ataxia, the expanded repeats behave like dominant mutations because they interfere with RNA processing in general in the neurons where the affected genes are expressed.

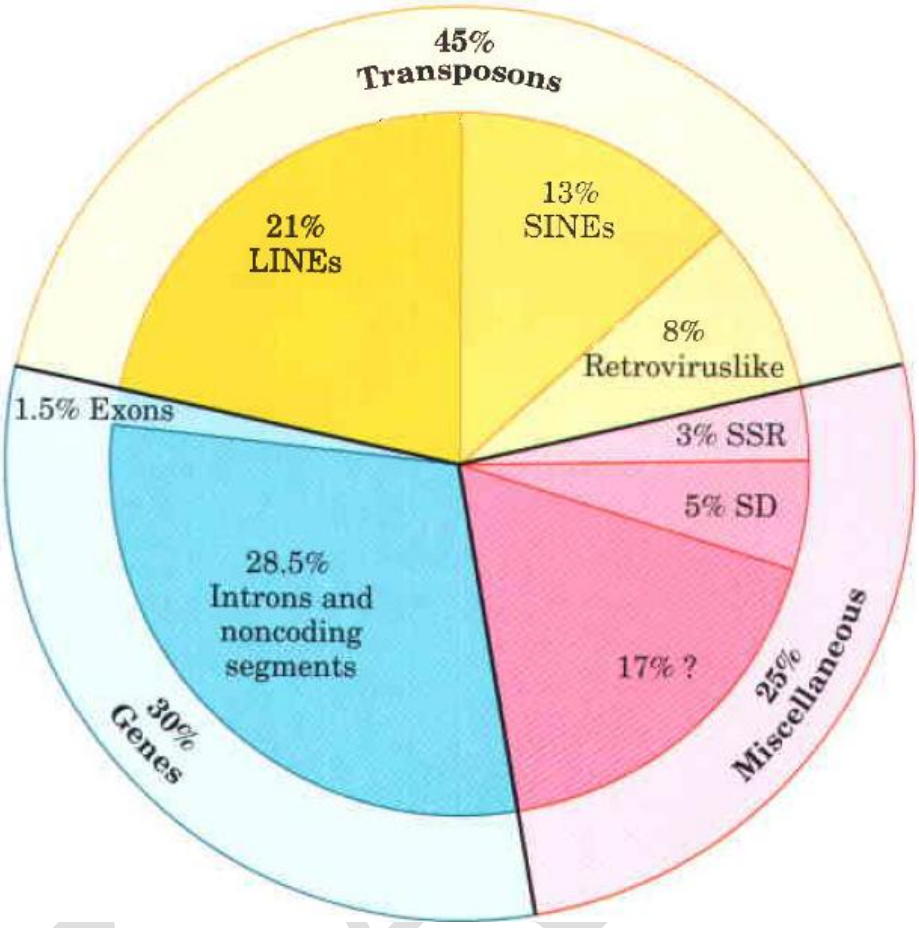


Fig: Types of sequences in human genome

FIGURE **Types of sequences in the human genome.** This pie chart divides the genome into transposons (transposable elements) genes, and miscellaneous sequences. There are four main classes of transposons (three of them shown here). Long interspersed elements (LINEs), 6 to 8 kbp long (1 kbp = 1,000 bp), typically include a few genes encoding proteins that catalyze transposition. The genome has about 850,000 LINEs. Short interspersed elements (SINEs) are about 100 to 300 bp long. Of the 1.5 million in the human genome more than 1 million are Alu elements, so called because they generally include one copy of the recognition sequence for *AluI*, a restriction endonuclease (see Fig. 9–2). The genome also contains 450,000 copies of retrovirus-like transposons, 1.5 to 11 kbp long. Although these are "trapped" in the genome and cannot move from one cell to another, they are evolutionarily related to the retroviruses ( ), which include HIV. A final class of transposons (making up <3% and not shown here) consists of a variety of transposon remnants that differ greatly in length.

About 30% of the genome consists of sequences included in genes for proteins, but only a small fraction of this DNA is in exons (coding sequences). Miscellaneous sequences include simple-sequence repeats (SSR) and large segmental duplications (SD), the latter being segments that appear more than once in different locations.

Molecular Definition of Gene:

In molecular terms, a gene commonly is defined as the entire nucleic acid sequence that is necessary for the synthesis of a functional gene product (polypeptide or RNA). According to this definition, a gene includes more than the nucleotides encoding the amino acid sequence of a protein, referred to as the coding region. A gene also includes all the DNA sequences required for synthesis of a particular RNA transcript. In eukaryotic genes, transcription-control regions known as **enhancers** can lie 50 kb or more from the coding region. Other critical noncoding regions in eukaryotic genes are the sequences that specify 3' cleavage and polyadenylation, known as poly(A) sites, and splicing of primary RNA transcripts, known as splice sites. Mutations in these RNA-processing signals prevent expression of a functional mRNA and thus of the encoded polypeptide.

Although most genes are transcribed into mRNAs, which encode proteins, clearly some DNA sequences are transcribed into RNAs that do not encode proteins (e.g., tRNAs and rRNAs). However, because the DNA that encodes tRNAs and rRNAs can cause specific phenotypes when it is mutated, these DNA regions generally are referred to as tRNA and rRNA genes, even

though the final products of these genes are RNA molecules and not proteins. Many other RNA molecules are also transcribed from non-protein-coding genes.

Monocistronic mRNAs Produced by Eukaryotic Genes and their Introns

Many bacterial mRNAs are polycistronic; that is, a single mRNA molecule (e.g., the mRNA encoded by the trp operon) includes the coding region for several proteins that function together in a biological process. In contrast, most eukaryotic mRNAs are monocistronic; that is, each mRNA molecule encodes a single protein. This difference between polycistronic and monocistronic mRNAs correlates with a fundamental difference in their translation.

Within a bacterial polycistronic mRNA a ribosome binding site is located near the start site for each of the protein coding regions, or cistrons, in the mRNA. Translation initiation can begin at any of these multiple internal sites, producing multiple proteins. In most eukaryotic mRNAs, however, the 5'-cap structure directs ribosome binding, and translation begins at the closest AUG start codon. As a result, translation begins only at this site. In many cases, the primary transcripts of eukaryotic protein-coding genes are processed into a single type of mRNA, which is translated to give a single type of polypeptide.

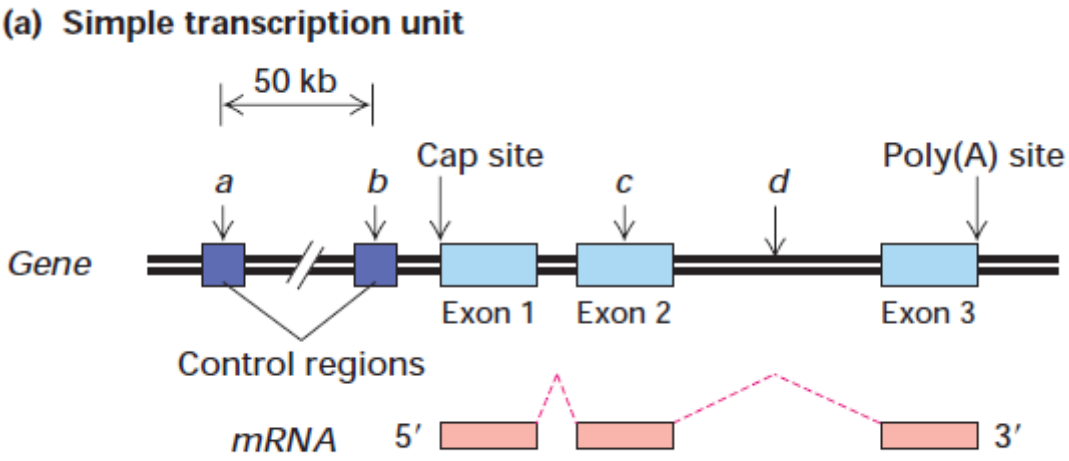
Unlike bacterial and yeast genes, which generally lack introns, most genes in multicellular animals and plants contain introns, which are removed during RNA processing. In many cases, the introns in a gene are considerably longer than the exons. For instance, of the ≈50,000 base pairs composing many human genes encoding average-size proteins, more than 95 percent are present in introns and noncoding 5' and 3' regions. Many large proteins in higher organisms have repeated domains and are encoded by genes consisting of repeats of similar exons separated by introns of variable length. An example of this is fibronectin, a component of the extracellular matrix that is encoded by a gene containing multiple copies of three types of exons.

Simple and Complex Transcription Units in Eukaryotic Genomes

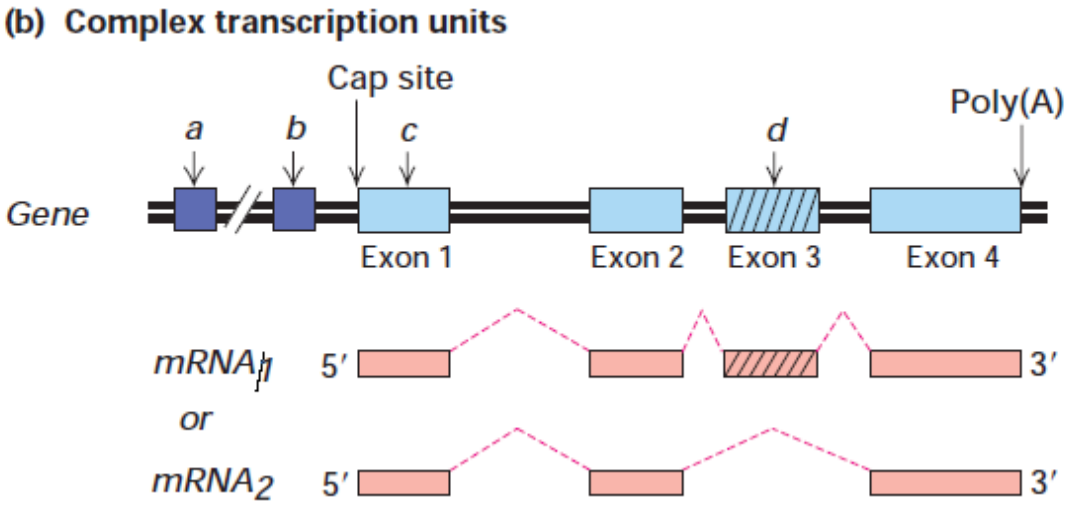
The cluster of genes that form a bacterial operon comprises a single **transcription unit,** which is transcribed from a particular promoter into a single primary transcript. In other words, genes and transcription units often are distinguishable in prokaryotes. In contrast, most

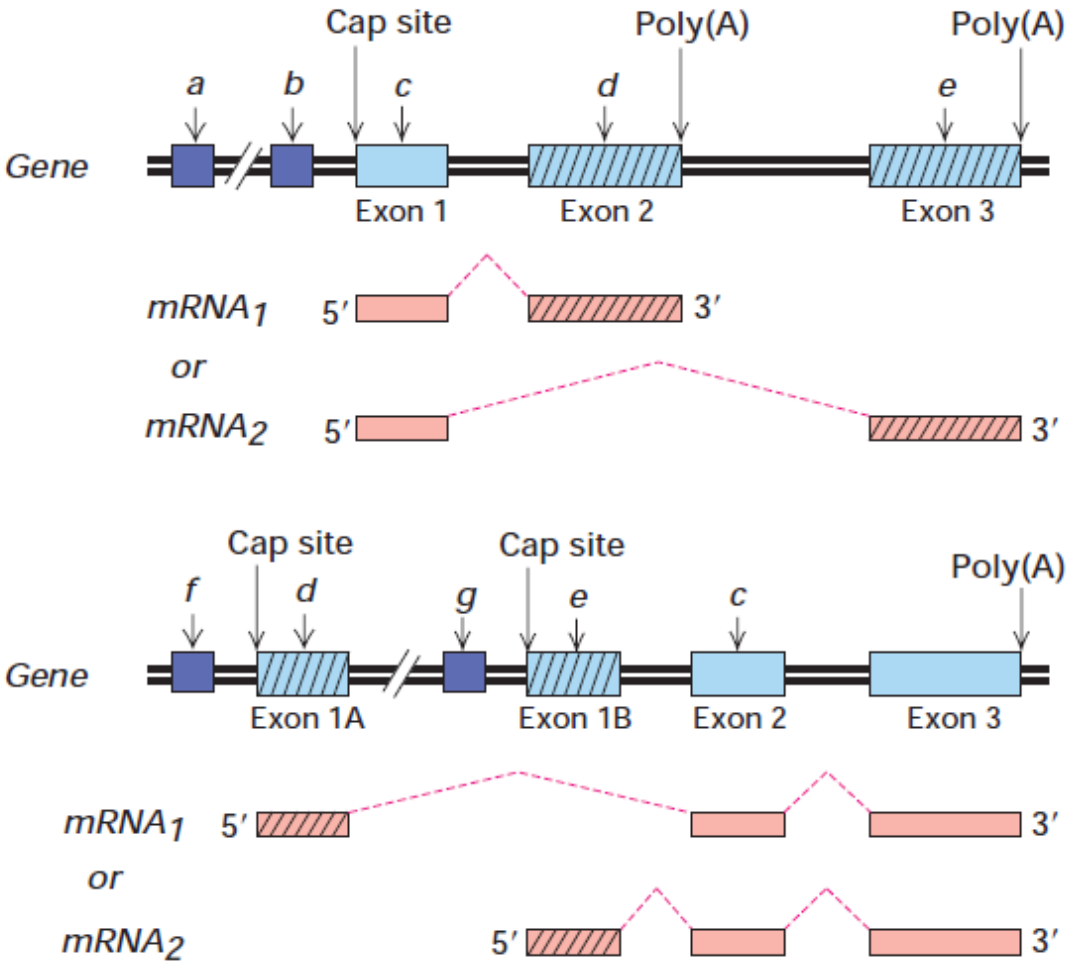
eukaryotic genes and transcription units generally are identical, and the two terms commonly are used interchangeably. Eukaryotic transcription units, however, are classified into two types, depending on the fate of the primary transcript, namely simple transcription unit and complex transcription unit.

The primary transcript produced from a simple transcription unit is processed to yield a single type of mRNA, encoding a single protein.



In the case of complex transcription units, which are quite common in multicellular organisms, the primary RNA transcript can be processed in more than one way, leading to formation of mRNAs containing different exons. Multiple mRNAs can arise from a primary transcript in three ways (Figure):


(b) Complex transcription units
Cap site
Poly(A)
a
b
c
d
Gene
Exon 1
Exon 2
Exon 3
Exon 4
mRNA1
5′
3′
or
mRNA2
5′
3′


Cap site
Poly(A)
Poly(A)
a
b
c
d
e
Gene
Exon 1
Exon 2
Exon 3
mRNA1
5′
3′
or
mRNA2
5′
3′
Cap site
Cap site
Poly(A)
f
d
g
e
c
Gene
Exon 1A
Exon 1B
Exon 2
Exon 3
mRNA1
5′
3′
or
mRNA2
5′
3′

Karpagam Academy of Higher Education
Department of Biochemistry
II BSc Biochemistry
Gene Organisation, Replication and Repair (16BCU401)

UNIT I- POSSIBLE QUESTIONS

2 Mark - Questions

1. Write a note on Repetitive DNA
2. Explain the Single copy DNA
3. Draw the structure of DNA
4. List the salient features of DNA
5. Differentiate A,B and Z DNA
6. Organisation of genes in bacteriophage
7. Give the genetic map of any bacterial genome
8. Give short note on histone proteins
9. What is the basic unit of genome organization? Explain it.
10. What is simple sequence DNA?
11. What is denaturation and denaturation of DNA

Essay type Questions (8 Marks)

1. Explain the structural organization of eukaryotic chromosomes.
2. Give the structure of B DNA and differentiate it from other forms of DNA
3. Describe the genome sequences in DNA
5. Write short notes on
   i) Single copy DNA
   ii) Tandemly repeated genes
   iii) Pseudogenes
6. Describe the process of packaging of DNA into higher order structure
7. Describe the organization of genes in virus and bacteria

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
II B.Sc BIOCHEMISTRY- Fourth Semester
GENE ORGANISATION, REPLICATION AND REPAII (16BCU401)
MULTIPLE CHOICE QUESTIONS
UNIT I

S.No	Unit	Questions	Option 1	Option 2	Option 3	Option 4	Answer
1	I	The macromolecule regarded as the reserve bank of genetic information	Protein	**DNA**	RNA	Polysaccharide	DNA
2	I	The biological information flows from DNA to RNA and from RNA to	lipids	carbohydrates	proteins	nucleotides	proteins
3	I	The total genetic information contained in a DNA is referred to as	gene	genome	Okazaki piece	ribozyme	genome
4	I	The DNA base pairing is based on _____ rules	Chargaff's rule	Arther Kornberg	Stahl & Meselson	McClintock	Chargaff's rule
5	I	Pseudogenes are	Related to non functional genes	Transcribed into mRNA	Translated in to functional proteins	Transcribed into tRNA	Related to non functional genes
6	I	Mobile genetic elements were visualized by	T.H Morgan	Barbara McClintock	G Khorana	C.B Bridge	Barbara McClintock
7	I	Fundamental unit of DNA organization	Replisome	Nucleosome	Primosome	Chromatin	Nucleosome
8	I	Histones are rich in	Arg and Lys	Lys & Gly	Arg & Glu	Arg & Gly	Arg and Lys
9	I	Which histone protein is not a part of core particle of nucleosome?	H1	H2a	H2b	H4	H1
10	I	Which histone protein is involved in the transition between the solenoid form and the extended nucleosome form?	H1	H2a	H2b	H4	H1
11	I	Smaller blocks occur at the end of chromosome arm is called _____	centromere	telomere	blastomeres	blastocyst	telomere
12	I	The functional unit of DNA is	genome	gene	nucleotide	chromosome	nucleotide
13	I	The coiling that cannot be separated except by unwinding is called	supercoiling	negative super coiling	plectonemic coiling	anti parellel coiling	plectonemic coiling
14	I	Self integrating DNA fragments are known as	Transposons	Self posons	Transducers	Transfragments	Transposons
15	I	The molecular chaperone which causes the nucleosome assembly is	Nucleoplasmin	Histone	Hu protein	Ubiquitin	Histone
16	I	Simple sequence DNA is concentrated in	Centromere	Telomere	Blastomeres	Blastocyst	Centromere
17	I	LTRs are absent in	LINES	SINES	LINES AND SINES	Viral retrotransposons	LINES AND SINES
18	I	Chicken lysozyme gene is a good example of	Single copy DNA	Moderately repetitive DNA	Simple sequence DNA	Highly repetitive DNA	Single copy DNA
19	I	Difference in length of simple sequence tandem arrays helps to develop a technique called	Foot printing	Northern blotting	Western	Finger printing	Finger printing
20	I	Important hallmark of IS element is	Short direct repeats	Long direct repeats	Inverted repeats	Tandem repeats	Short direct repeats
21	I	IS element contain _____ enzyme	Helicase	primase	Transposase	topoisomerase	transposase
22	I	The length of the DNA segment present in the nucleosome core particle is	140 bp	200 bp	166 bp	114 bp	166 bp
23	I	The housekeeping genes have	GC box	TATA box	Pribnow box	CAAT box	GC box
24	I	Negative supercoils are removed by	Topoisomerase I	Gyrase	Helicase	Rep protein	Topoisomerase I
25	I	DNA chains differ from each other by one nucleotide can be revolved using	20% agarose	20% polyacrylamide	1% agarose	1% polyacrylamide	1% agarose
26	I	Satellite DNAs are found in the region of	Euchromatin	Heterochromatin	Hypervariable regions	Functional elements	Heterochromatin
27	I	Polytene chromosomes are produced by	Gene inversion	Gene conversion	DNA amplification	Retrotransposons	DNA amplification
28	I	In conjugation DNA is transformed from	Conjugation tube	Bacteriophage	Dead organism	Mice	Dead organism
29	I	Dead organism of pneumococci was completely inactivated by _____ enzymes	DNAase	Amylase	Lipase	Protease	DNAase
30	I	Nonvirulent in pneumococci is imparted by	Polysaccharide	Protein	Lipid	Lipopolysaccharide	Lipopolysaccharide
31	I	In DNA, the genetic information residues in	Purine bases	Pyrimidine bases	Purine and Pyrimidine bases	Sugar	Purine and Pyrimidine bases
32	I	Important characteristic of satellite DNA is, they contain	Repetitive base sequence	GC rich	Unique sequence	AT rich	GC rich
33	I	LINES and SINES come under _____ DNA	Single copy DNA	Moderatively repetitive DNA	Simple sequence DNA	RNA	Moderatively repetitive DNA
34	I	What is the difference between II S and II R strains of bacteria used in Griffith's experiment?	III S strain covers itself with a polysaccharide coat	III S strain covers itself with a protein coat	II R strain covers itself with a protein coat	II R strain covers itself with a polysaccharide coat	III S strain covers itself with a polysaccharide coat
35	I	Avery, Macleod and Mc Carty's experiment was conducted in the year	1928	1974	1964	1938	1974
36	I	To explore the genetic properties of DNA Hershey and Chase used	bacteria	bacterial viruses	fungi	dog	bacterial viruses
37	I	Conjugation was discovered by	Zinder and Ledenberg	Lederberg and Tatum	Frederick Griffith	Hershey and Chase	Lederberg and Tatum
38	I	In conjugation the integration of F factor is mediated by	transposon	IS elements	viruses	simple sequence DNA	IS elements
39	I	The DNA in the chromatin is very tightly associated with proteins called	albumin	globulin	myosin	histones	histones
40	I	The molecular weight of histones is	between 11,000 and 21,000	between 1,000 and 11,000	between 8,000 and 20,000	between 1,000 and 31,000	between 11,000 and 21,000
41	I	Histones are very rich in	acidic amino acids	basic amino acids	neutral amino acids	aromatic amino acids	basic amino acids
42	I	Number of amino acids in H4 histones is	104	108	102	120	102
43	I	The bead of each nucleosome contains	eight histone molecules	ten histone molecules	two histone molecules	eight histone molecules	eight histone molecules
44	I	The number of base pairs serves as linker DNA between nucleosome beads is	200 bp	146bp	54 bp	45 bp	54 bp
45	I	The spacing of the nucleosome beads provides a repeating unit typically of about	200 bp	146bp	54bp	45bp	200 bp
46	I	The total number of base pairs bound tightly around the eight-part histone core are	200 bp	146 bp	54 bp	45 bp	146 bp
47	I	X-ray diffraction analysis of nucleosome reveals that it is a	left-handed solenoidal supercoil	right-handed solenoidal supercoil	left handed double helical supercoil	right handed double helical supercoil	left-handed solenoidal supercoil

48	I	Specific binding sites of histone core to DNA is	A=T rich base pairs	G≡C rich base pairs	equal ratio of both base pairs	1:2 ratio of both the base pairs	A=T rich base pairs
49	I	The SMC protein play a substantial role in linking together sister chromatids is	cohesins	condensins	albumins	globulins	cohesins
50	I	A well studied example of a solitary protein coding gene is	lactate dehydrogenase gene	protein kinase gene	chicken lysozyme gene	Immunoglobulin gene	protein kinase gene
51	I	According to the lac-operon concept, which functional unit of the bacterial gene material is responsible for suppressing the activity of the operator gene in the absence of lactose?	Promoter gene	Repressor protein	Regulator gene	Structural gene	Regulator gene
52	I	The inducer for the lac operon is:	lactose	allolactose	β-galactosidase	galactose	allolactose
53	I	Which of the following genes is not a structural gene of the lac operon?	lacA	lac I	lacY	lacZ	lac I
54	I	Attenuation of the trp operon:	Occurs when transcription is complete before translation begins.	Is mediated by the trp repressor protein.	Occurs in the presence of high levels of tryptophan	Occurs in the presence of high levels of glucose	Occurs in the presence of high levels of tryptophan.
55	I	When both glucose and lactose are present	cyclic AMP is high so transcription occurs	the lac repressor binds with the lactose and transcription occurs	RNA polymerase is able to bind to the operator so transcription occurs	transcription is turned off	transcription is turned off
56	I	The lac repressor	binds to the operator and prevents transcription	binds to the CAP site and prevents transcription	binds to the CAP site and facilitates transcription	binds to the operator and facilitates transcription	binds to the operator and prevents transcription
57	I	In the lactose operon, the molecule that causes the repressor to fall off the promoter is which of the following?	a corepressor	an attenuator	an inducer	a regulatory protein	an inducer
58	I	Satellite DNAs are found in the region of	Euchromatin	Heterochromatin	Hypervariable regions	Functional elements	Heterochromatin
59	I	The total number of base pairs bound tightly around the eight-part histone core are	200 bp	146 bp	54 bp	45 bp	146 bp
60	I	Important characteristic of satellite DNA is, they contain	Repetitive base sequence	GC rich	Unique sequence	AT rich	GC rich
61	I	In the tryptophan operon, tryptophan serves as	corepressor	an inducer	a catalyst	a regulatory protein	corepressor

60	V	The activity of AP endonuclease activity is	Base excision repair	Nucleotide excision repair	Mismatch repair	Double strand break repair	Base excision repair

UNIT-II

SYLLABUS

Replication of DNA in Prokaryotes

The chemistry of DNA synthesis, DNA polymerase, the replication fork, origin of replication, enzymes and proteins in DNA replication, various modes of replication, stages of replication of *E. coli* chromosome, relationship between replication and cell division.

Introduction

An important property of DNA is that it can replicate, or make copies of itself. Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases. This is critical when cells divide because each new cell needs to have an exact copy of the DNA present in the old cell.

The chemistry of DNA synthesis (features)

1. DNA replication always proceed **is 5'→ 3' direction**
2. Replication requires a **template strand** as guiding strand
3. DNA polymerase the enzyme that carryout replication requires **a primer** which provide OH group
4. DNA replication occurs through **semiconservative** mechanism
5. DNA replication starts at origin called, **Ori C**
6. Replication may be unidirectional or **bidirectional**
7. DNA polymerization carried out with **high fidelity**
8. It requires **number of enzymes**

General features of chromosomal replication

DNA replication, the basis for biological inheritance, is a fundamental process that occurs in all living organisms that copies their DNA. This process is "replication" in that each strand of the original double-stranded DNA molecule serves as template for the reproduction of the complementary strand. Therefore, following DNA replication, two identical DNA molecules

have been produced from a single double-stranded DNA molecule. Cellular proofreading and error toe-checking mechanisms ensure near perfect fidelity for DNA replication.

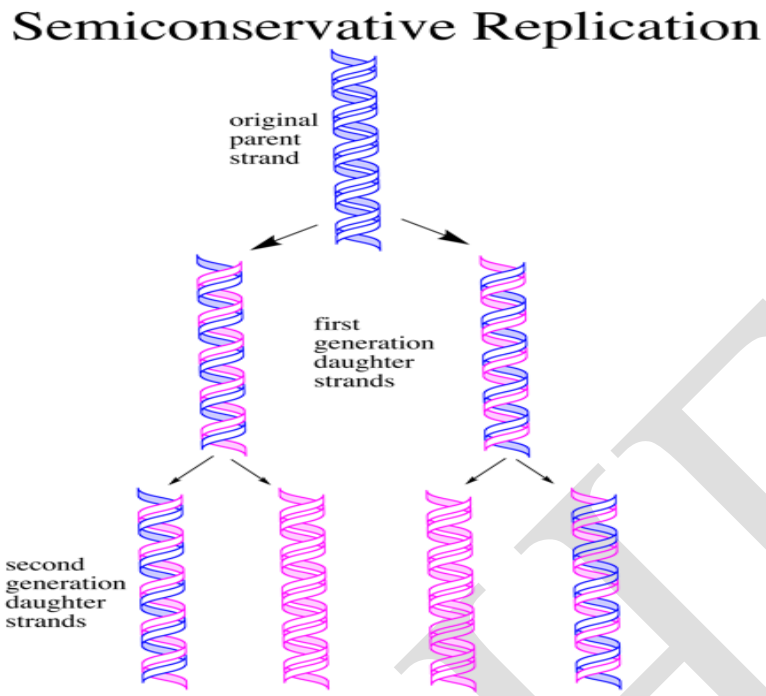
Models proposed for DNA replication

The mechanism of replication was one of three models originally proposed for DNA replication:

- Semi conservative replication describes the mechanism by which DNA is replicated in all known cells. It would produce two copies that each contained one of the original strands and one new strand.
- Conservative replication would leave the two original template DNA strands together in a double helix and would produce a copy composed of two new strands containing all of the new DNA base pairs.
- Dispersive replication would produce two copies of the DNA, both containing distinct regions of DNA composed of either both original strands or both new strands.

Meselson–Stahl experiment to prove semi conservative mechanism

This is an experiment by Matthew Meselson and Franklin Stahl in 1958 which supported the hypothesis that DNA replication was semi conservative. Semi conservative replication means that when the double stranded DNA helix was replicated, each of the two double stranded DNA helices consisted of one strand coming from the original helix and one newly synthesized. It has been called "the most beautiful experiment in biology.



Experimental procedure

Nitrogen is a major constituent of DNA. ^{14}N is by far the most abundant isotope of nitrogen, but DNA with the heavier (but non-radioactive) ^{15}N isotope is also functional.

E. coli were grown for several generations in a medium with ^{15}N. When DNA is extracted from these cells and centrifuged on a salt density gradient, the DNA separates out at the point at which its density equals that of the salt solution. The DNA of the cells grown in 15N medium had a higher density than cells grown in normal ^{14}N medium. After that, E. coli cells with only ^{15}N in their DNA were transferred to a ^{14}N medium and were allowed to divide; the progress of cell division was monitored by measuring the optical density of the cell suspension.

DNA was extracted periodically and was compared to pure ^{14}N DNA and ^{15}N DNA. After one replication, the DNA was found to have close to the intermediate density. Since conservative replication would result in equal amounts of DNA of the higher and lower densities (but no DNA of an intermediate density), conservative replication was excluded. However, this result was consistent with both semiconservative and dispersive replication. Semiconservative replication would result in double-stranded DNA with one strand of ^{15}N DNA, and one of ^{14}N DNA, while dispersive replication would result in double-stranded DNA with both strands

having mixtures of ^{15}N and ^{14}N DNA, either of which would have appeared as DNA of an intermediate density.

The authors continued to sample cells as replication continued. DNA from cells after two replications had been completed was found to consist of equal amounts of DNA with two different densities, one corresponding to the intermediate density of DNA of cells grown for only one division in ^{14}N medium, the other corresponding to DNA from cells grown exclusively in ^{14}N medium. This was inconsistent with dispersive replication, which would have resulted in a single density, lower than the intermediate density of the one-generation cells, but still higher than cells grown only in ^{14}N DNA medium, as the original ^{15}N DNA would have been split evenly among all DNA strands. The result was consistent with the semiconservative replication hypothesis.

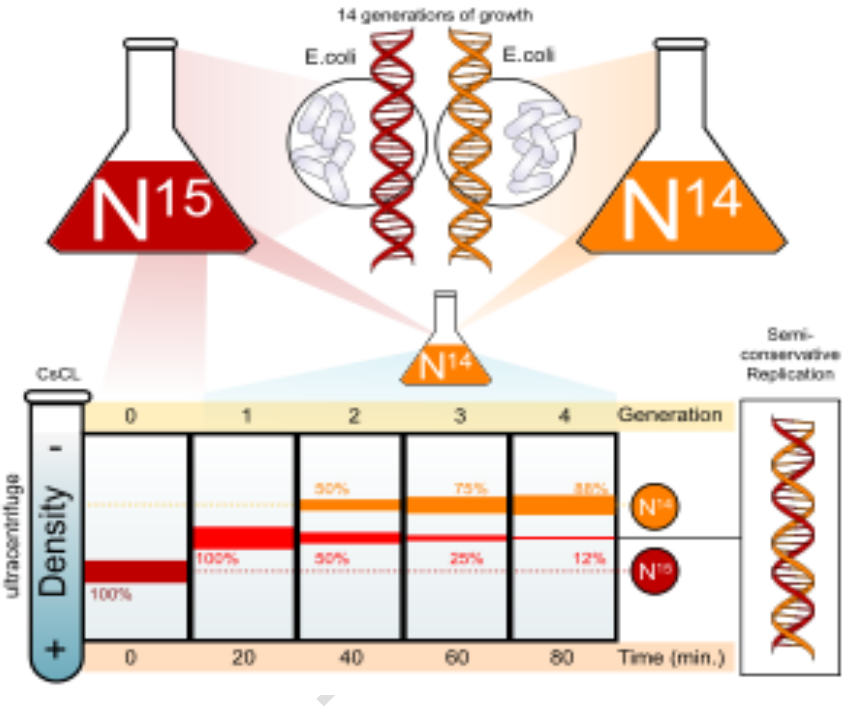
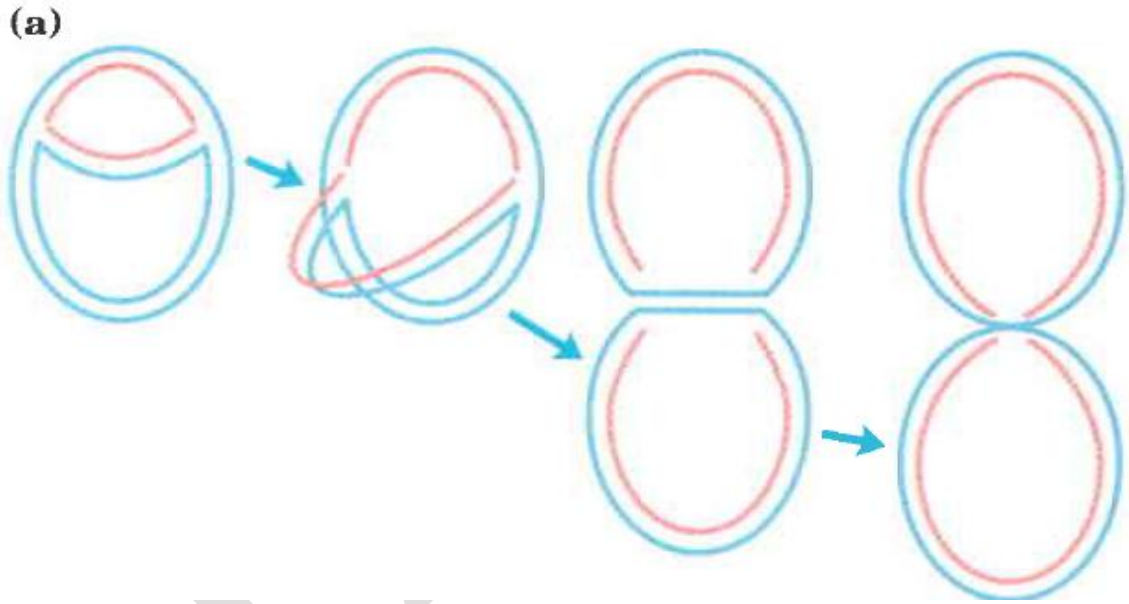


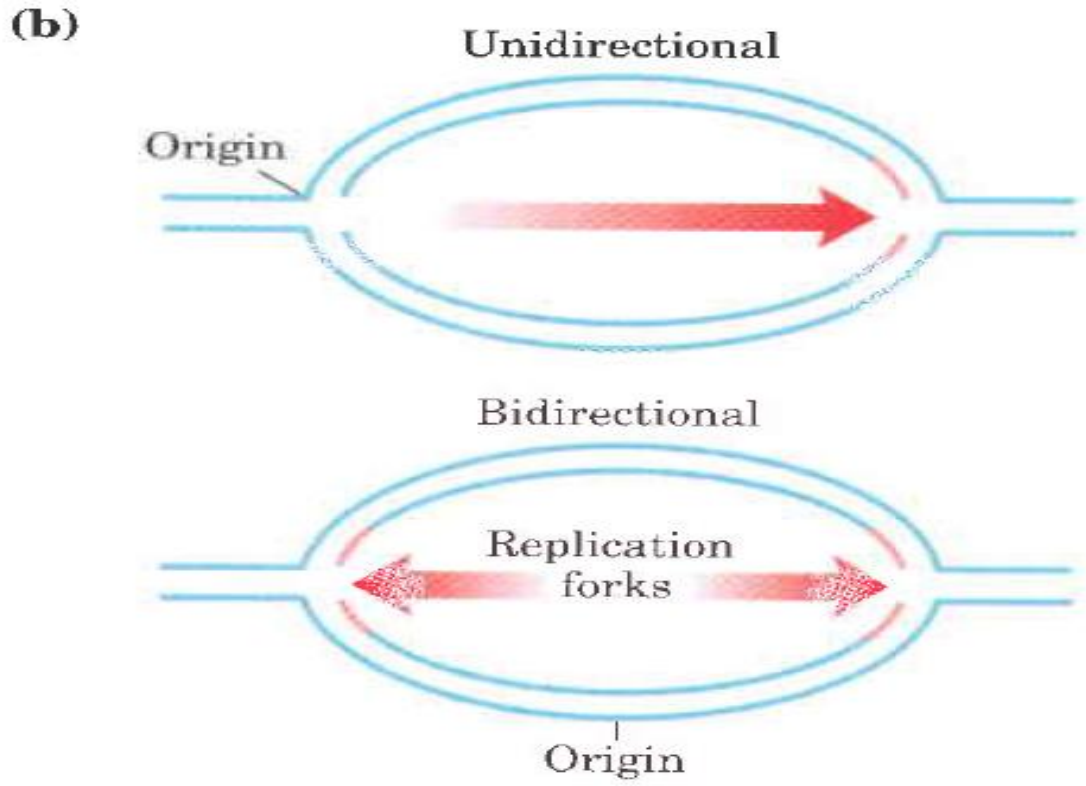
Fig: Meselson–Stahl experiment

Replication forks

An early indication that replication is a highly coordinated process in which the parent strands are simultaneously unwound and replicated was provided by **John Cairns,** using autoradiography He made *E coli* DNA radioactive by growing cells in a medium containing

thymidine labeled with tritium ^{1}H). When the DNA was carefully isolated, spread, and overlaid with a photographic emulsion for several weeks, the radioactive thymidine residues generated "tracks" of silver grains in the emulsion, producing an image of the DNA molecule. These tracks revealed that the intact chromosome of *E. coli* is a single huge circle, 1.2 mm long. Radioactive DNA isolated from cells during replication showed an extra loop (Fig.). Cairns concluded that the loop resulted from the formation of two radlioactive daughter strands, each complementary to a parent strand. One or both ends of the loop are dyrramic points, termed **replication forks**, where parent DNA is being unwound and the separated strands quickly replicated. Cairns's results demonstrated that both DNA strands are replicated simultaneously, and a variation on his experiment (Fig) indicated that replication of bacterial chromosomes is bidirectional, both ends of the loop have active replication forks.





ENZYMOLOGY OF DNA REPLICATION

Enzymes and proteins of DNA Replication

1.Helicase: Unwounds a portion of the DNA Double Helix

2.RNA Primase: Attaches RNA primers to the replicating strands.

3.DNA Polymerase delta (ä): Binds to the 5' - 3' strand in order to bring nucleotides and create the daughter leading strand.

4.DNA Polymerase epsilon (å): Binds to the 3' - 5' strand in order to create discontinuous segments starting from different RNA primers.

5.Exonuclease (DNA Polymerase I): Finds and removes the RNA Primers

6.DNA Ligase: Adds phosphate in the remaining gaps of the phosphate - sugar backbone

7.Nucleases: Remove wrong nucleotides from the daughter strand.

a) primosome is a protein complex responsible for creating RNA primers on single stranded DNA during DNA replication.

The primosome consists of seven proteins: DnaG primase, DnaB helicase, DnaC helicase assistant, DnaT, PriA, Pri B, and PriC. The primosome is utilized once on the leading strand of DNA and repeatedly, initiating each Okazaki fragment, on the lagging DNA strand.

b) Replisome is composed of the following:

2 DNA Pol III enzymes, made up of α, ε and θ subunits.

- the α subunit has polymerization activity.
- the ε subunit has proofreading activity.
- the θ subunit stimulates the ε subunit's proofreading.
- 2 β units which act as sliding DNA clamps, they keep the polymerase bound to the DNA.
- 2 τ units which connect the 2 DNA Pol III enzymes.
- 1 γ unit which acts as a clamp loader for the lagging strand Okazaki fragments, helping the two β subunits to form a unit and bind to DNA. The γ unit is made up of 5 γ subunits.

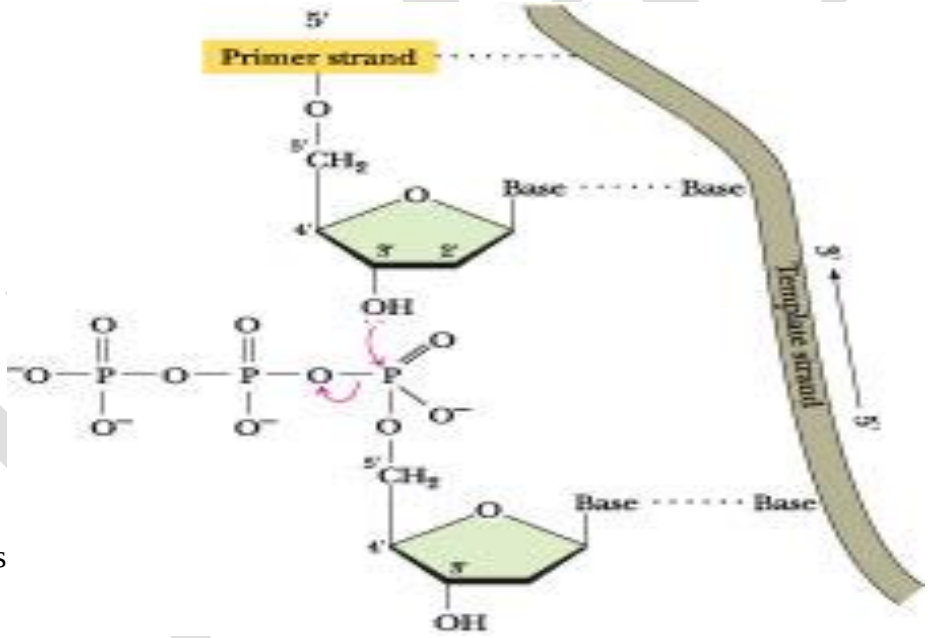
DNA Polymerases—The Enzymes of DNA Replication

All DNA polymerases, whether from prokaryotic or eukaryotic sources, share the following properties: (a) The incoming base is selected within the DNA polymerase active site, as determined by Watson-Crick geometric interactions with its corresponding base in the template strand, (b) chain growth is in the 5' → 3' direction and is antiparallel to the template strand, and (c) DNA polymerases cannot initiate DNA synthesis de novo—all require a primer oligonucleotide with a free 3'-OH to build upon. E. *coli* DNA Polymerases Table compares the properties of the various DNA polymerases in E. *coli*. These enzymes are numbered I, II, and III, in order of their discovery. DNA polymerases I and II function principally in DNA repair; DNA polymerase III is the chief DNA-replicating enzyme of E. *coli*.

Table : Comparison of DNA polymerases

	DNA polymerase		
	I	II	III
Structural gene*	*polA*	*polB*	*polC (dnaE)*
Subunits (number of different types)	1	7	≥10
M_r	103,000	88,000†	791,500
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/s)	16–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

Catalytic function of DNA polymerase



DNA polymerase I joins deoxynucleoside monophosphate units to the 3'-OH carries out a nucleophilic attack on the alpha-phosphoryl group of the incoming dNTP to form a phosphoester bond, and PP_i is released. The subsequent hydrolysis of PP_i by inorganic pyrophosphatase renders the reaction effectively irreversible. The reaction is

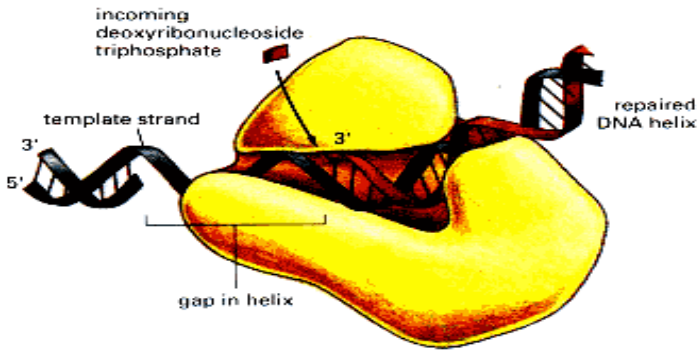
dNTP+pNpNpNpN-3'OH -----→ PP_i+pNpNpNpNpN*-3'OH

Features of DNA polymerase

In 1957, Arthur Kornberg and his colleagues discovered the first DNA polymerase. A **primer** is essential because DNA polymerases can elongate only pre-existing chains; This primer must possess a free 3'-OH end to which an incoming deoxynucleoside monophosphate is added. All four dNTPs are substrates, pyrophosphate (PP_i) is released, and the dNMP is linked to the 3'-OH of the primer chain through formation of a phosphoester bond. The deoxynucleoside monophosphate to be incorporated is chosen through its geometric fit with the template base to form a Watson-Crick base pair. As DNA polymerase I catalyzes the successive addition of deoxynucleotide units to the 3'-end of the primer, the chain is elongated in the 5' - 3' direction, forming a polynucleotide sequence that runs antiparallel to the template but complementary to it. DNA polymerase I can proceed along the **template strand**, synthesizing a complementary strand of about 20 bases before it "falls off" (dissociates from) the template.

Strcuture of DNA polymerase I

E. *coli* DNA polymerase I is a 109-kD protein consisting of a single polypeptide of 928 amino acid residues. In addition to its 5' □ 3' polymerase activity, DNA polymerase I has two other catalytic functions, a 3' □ 5' *exonuclease* (*3'-exonuclease*) activity and a *5' □ 3' exonuclease (5'-exonuclease)* activity. The three distinct catalytic activities of DNA polymerase I reside in separate active sites in the enzyme.



Properties of DNA polymerase I

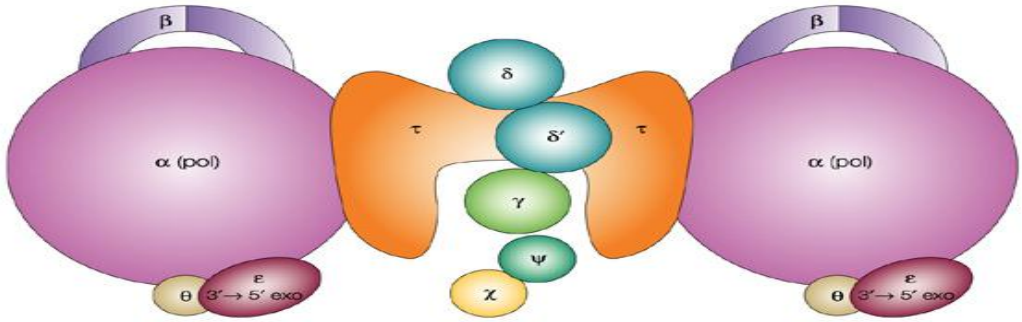
Pol I possesses four enzymatic activities:

1. A 5' → 3' (forward) DNA-Dependent DNA polymerase activity, requiring a 3' primer site and a template strand
2. A 3' → 5' (reverse) exonuclease activity that mediates proofreading

3. A 5' → 3' (forward) exonuclease activity mediating nick translation during DNA repair.
4. A 5' → 3' (forward) RNA-Dependent DNA polymerase activity.

DNA Polymerase III

DNA polymerase III; in its holoenzyme form, responsible for replication of the E. *coli* chromosome.The DNA polymerase III holoenzyme is a multisubunit complex, which consists of 17 polypeptides. It contains four subassemblies. First, the core polymerase consists of three subunits: α(the polymerase); ε(the 3'–5' exonuclease); and θ(the stimulator of the 3'–5' exonuclease). Second, the τsubunit is responsible for dimerization of the core DNA polymerase. Third, the sliding clamp comprises two homodimers of the βsubunit, which provides the ring structure that is needed for processivity. Fourth, five subunits have clamp-loader functions — γ, δ, δ', χ and ψ.



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The replisome is composed of the following:

- 2 DNA Pol III enzymes, each comprising α, ε and θ subunits.
- the α subunit has the polymerase activity.
- the ε subunit as 3'-5' exonuclease activity.
- the θ subunit stimulates the ε subunit's proofreading.
- 2 β units which act as sliding DNA clamps, they keep the polymerase bound to the DNA.
- 2 τ units which acts to dimerize two of the core enzymes (α, ε, and θ subunits).
- 1 γ unit which acts as a clamp loader for the lagging strand Okazaki fragments, helping the two β subunits to form a unit and bind to DNA. The γ unit is made up of 5 γ subunits

which include 3 γ subunits, 1 δ subunit, and 1 δ' subunit. The δ is involved in copying of the lagging strand.

- X and Ψ which form a 1:1 complex and bind to τ.

DNA ligase

DNA ligase seals nicks in double-stranded DNA where a 3'-OH and a 5'-phosphate are juxtaposed. This enzyme is responsible for joining Okazaki fragments together to make the lagging strand a covalently contiguous polynucleotide chain. DNA ligase from eukaryotes and bacteriophage T4 is ATP-dependent; the E. *coli* enzyme requires NAD^+. Both types of DNA ligase act via an adenylylated ε-amino group of a Lys residue. Adenylylation of the 5'-phosphoryl group activates it for formation of a phosphoester bond with the 3'-OH, covalently sealing the sugar-phosphate backbone of DNA.

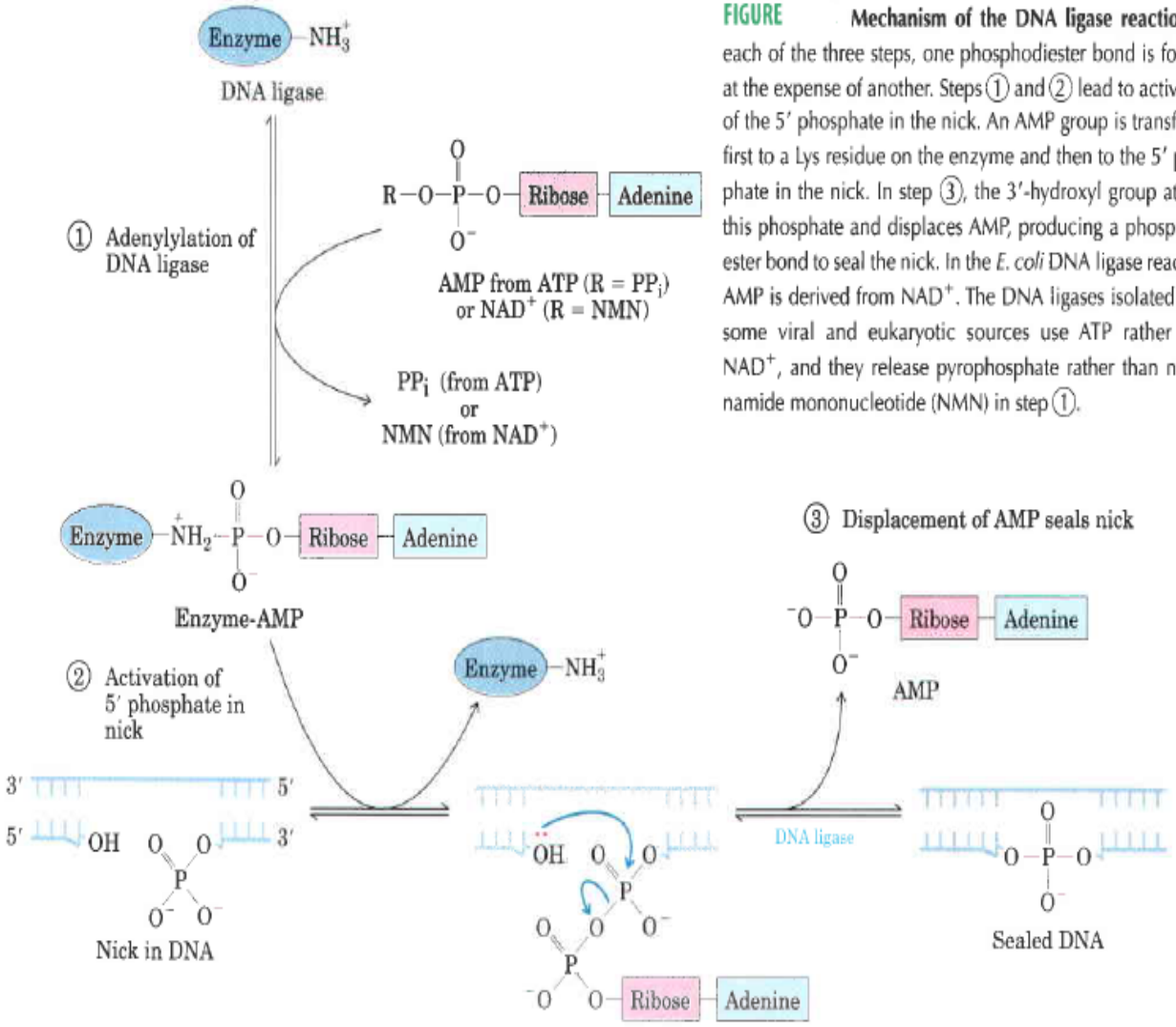


FIGURE **Mechanism of the DNA ligase reaction.** In each of the three steps, one phosphodiester bond is formed at the expense of another. Steps ① and ② lead to activation of the 5′ phosphate in the nick. An AMP group is transferred first to a Lys residue on the enzyme and then to the 5′ phosphate in the nick. In step ③, the 3′-hydroxyl group attacks this phosphate and displaces AMP, producing a phosphodiester bond to seal the nick. In the *E. coli* DNA ligase reaction, AMP is derived from NAD^+. The DNA ligases isolated from some viral and eukaryotic sources use ATP rather than NAD^+, and they release pyrophosphate rather than nicotinamide mononucleotide (NMN) in step ①.

Figure: The mechanism of action of DNA ligases

General Features of a Replication Fork

We now can present a snapshot of the enzymatic apparatus assembled at a replication fork. DNA gyrase (topoisomerase) and helicase unwind the DNA double helix, and the unwound, single-stranded regions of DNA are maintained through interaction with SSB. Primase synthesizes an RNA primer on the lagging strand; the leading strand, which needs priming only once, was primed when replication was initiated. The lagging strand template is looped around, and each replicative DNA polymerase moves 5' -3' relative to its strand, copying template and synthesizing a new DNA strand. Each replicative polymerase is tethered to the DNA by its β -subunit sliding clamp. The DNA pol III complex periodically unclamps and then reclamps.

Downstream on the lagging strand, DNA polymerase I excises the RNA primer and replaces it with DNA, and DNA ligase seals the remaining nick.

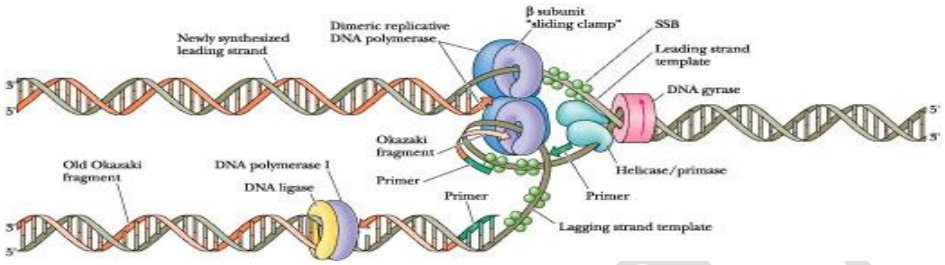


Figure : General features of a replication fork.

Proteins Involved in DNA Replication in E. *Coli*	
Protein	Function
DNA gyrase	Unwinding DNA
SSB	Single-stranded DNA binding
DnaA	Initiation factor
HU	Histone-like (DNA binding)
PriA	Primosome assembly, 3' □ □5' helicase
PriB	Primosome assembly
PriC	Primosome assembly
DnaB	5' ->3' helicase (DNA unwinding)
DnaC	DnaB chaperone
DnaT	Assists DnaC in delivery of DnaB
Primase	Synthesis of RNA primer
DNA polymerase III holoenzyme	Elongation (DNA synthesis)
DNA polymerase I	Excises RNA primer, fills in with DNA
DNA ligase	Covalently links Okazaki fragments
Tus	Termination

VARIOUS MODE OF DNA REPLICATION

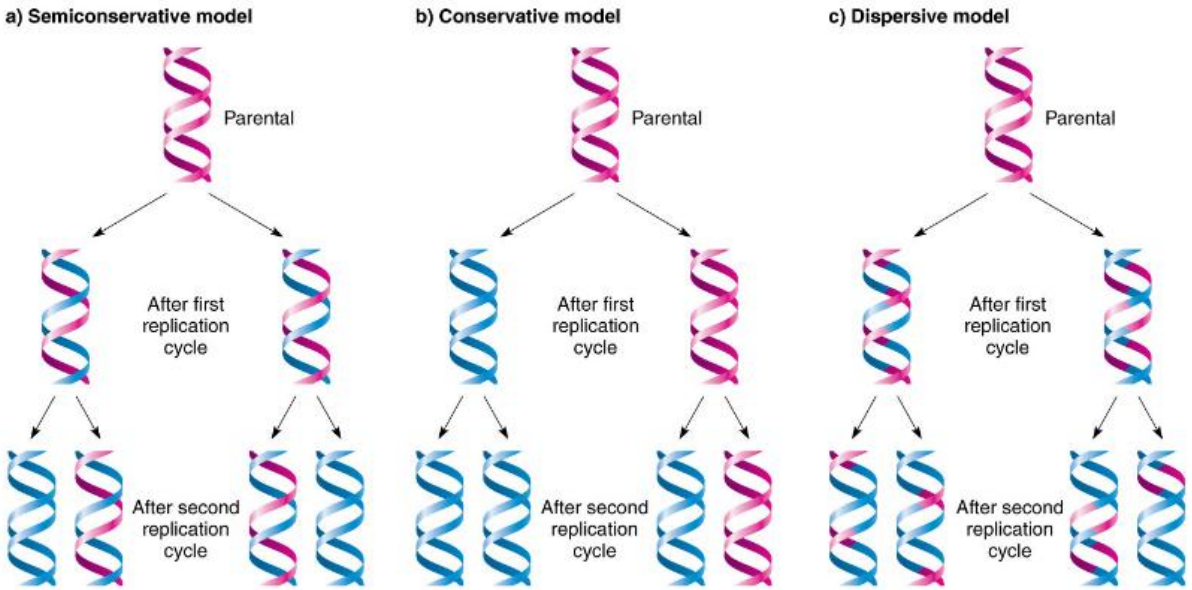
Watson and Crick had proposed that in order to copy itself, DNA would have to open down the center, sort of like a zipper coming apart, so that a new DNA strand could be built on top of the exposed strands. Commonly there are three modes of DNA replication, They are
Conservative, Semi conservative and Dispersive. Each model predicts a different distribution of parent DNA following a round of DNA replication.

In the **semi-conservative** model, the two parental strands separate and each makes a copy of itself. After one round of replication, the two daughter molecules each comprises one old and one new strand. Note that after two rounds, two of the **DNA** molecules consist only of new material, while the other two contain one old and one new strand.

In the **conservative** model, the parental molecule directs synthesis of an entirely new double-stranded molecule, such that after one round of replication, one molecule is conserved as two old strands. This is repeated in the second round.

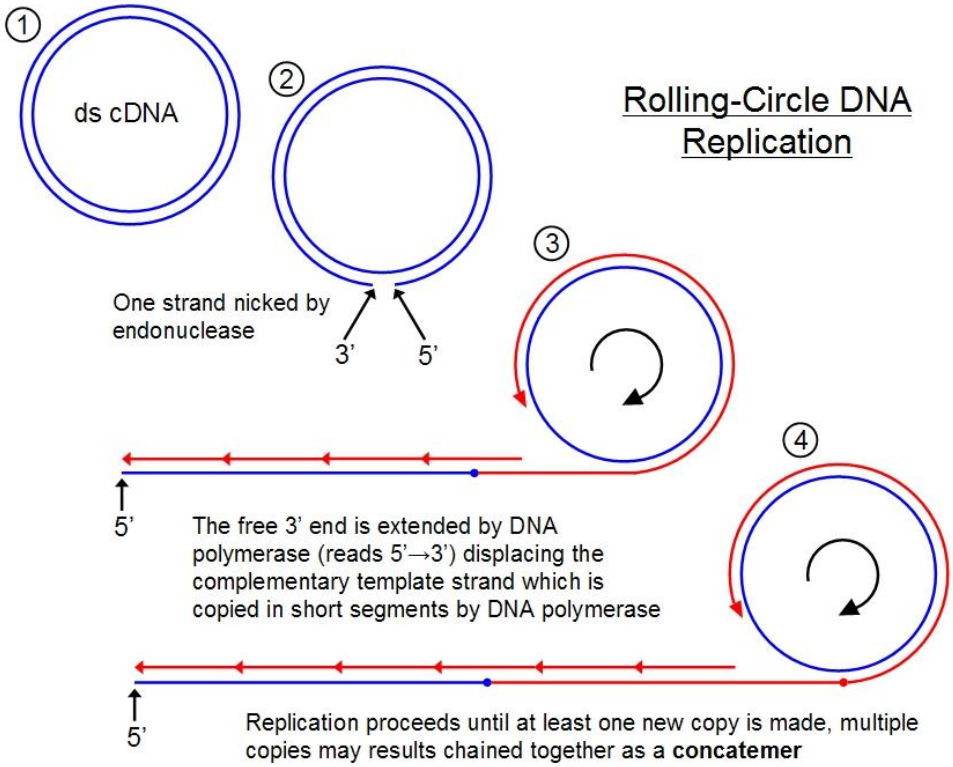
In the **dispersive** model, material in the two parental strands is distributed more or less randomly between two daughter molecules. In the model shown here, old material is distributed symmetrically between the two daughters molecules. Other distributions are possible.

The semi-conservative model is the intuitively appealing model, because separation of the two strands provides two templates, each of which carries all the information of the original molecule. It also turns out to be the correct one (Meselson & Stahl 1958).

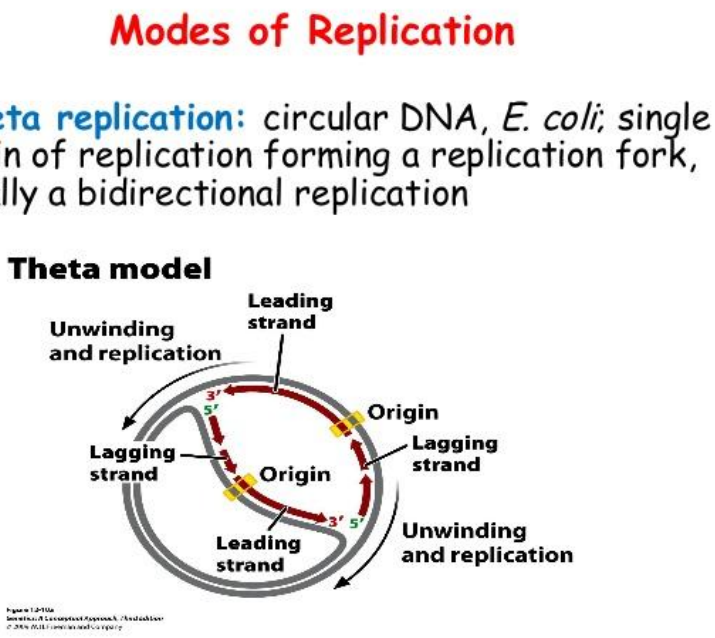


Based on mechanism they are also classified

1)Rolling circle model of recombination



Theta model of replication



STAGES OF REPLICATION -INITIATION, ELONGATION, TERMINATION

INITIATION

The origin of replication (also called the replication origin) is a particular sequence in a genome at which replication is initiated. This can either be DNA replication in living organisms such as prokaryotes and eukaryotes, or RNA replication in RNA viruses, such as double-stranded RNA viruses. DNA replication may proceed from this point bidirectionally or unidirectionally. The specific structure of the origin of replication varies somewhat from species to species, but all share some common characteristics such as high AT content. The origin of replication binds the pre-replication complex, a protein complex that recognizes, unwinds, and begins to copy DNA. The genome of E. coli is contained in a single circular DNA molecule of 4.6 x 106 nucleotide pairs. DNA replication begins at a single origin of replication. Replication origin is known as oriC. In E. coli, the oriC consists of three A-T rich 13-bp repeats and four 9-bp repeats.

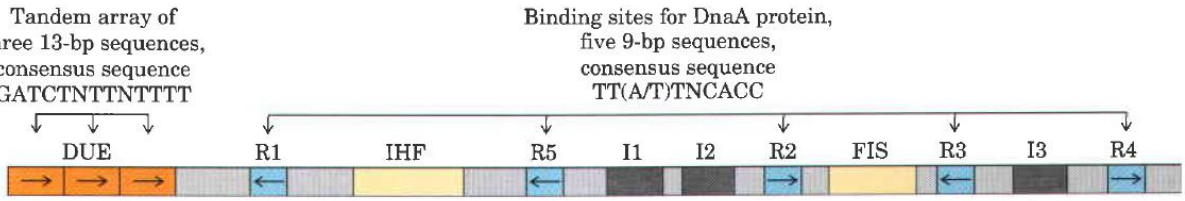


Fig: Ori C

Ten to 20 monomers of the replication protein dnaA bind to the 9 bp(mer) repeats, and the DNA coils around this protein complex forming a protein core. This coiling stimulates the AT rich region in the 13 mer sequence to unwind, allowing copies of the helicase dnaB and its cofactor protein dnaC to bind to each strand of the resulting single-stranded DNA. The dnaB protein forms the basis of the replisome, a complex of enzymes that performs DNA replication.

In a cell, DNA replication begins at specific locations in the genome, called "origins". Unwinding of DNA at the origin, and synthesis of new strands, forms a replication fork. In addition to DNA polymerase, the enzyme that synthesizes the new DNA by adding nucleotides matched to the template strand, a number of other proteins like helicase, primase, SSB and topoisomerase are associated with the fork and assist in the initiation and continuation of DNA synthesis.

Table: Proteins required to initiate DNA replication

Protein	M_r	Number of subunits	Function
DnaA protein	52,000	1	Recognizes ori sequence; opens duplex at specific sites in origin
DnaB protein (helicase)	300,000	6*	Unwinds DNA
DnaC protein	174,000	6*	Required for DnaB binding at origin
HU	19,000	2	Histonelike protein; DNA-binding protein; stimulates initiation
FIS	22,500	2*	DNA-binding protein; stimulates initiation
IHF	22,000	2	DNA-binding protein; stimulates initiation
Primase (DnaG protein)	60,000	1	Synthesizes RNA primers
Single-stranded DNA-binding protein (SSB)	75,600	4*	Binds single-stranded DNA
DNA gyrase (DNA topoisomerase II)	400,000	4	Relieves torsional strain generated by DNA unwinding
Dam methylase	32,000	1	Methylates (5′)GATC sequences at *oriC*

Origins contain DNA sequences recognized by replication initiator proteins (e.g. dnaA in E coli' and the Origin Recognition Complex in yeast).These initiator proteins recruit other proteins to separate the two strands and initiate replication forks.Initiator proteins recruit other proteins to separate the DNA strands at the origin, forming a bubble. Origins tend to be "AT-rich" (rich in adenine and thymine bases) to assist this process.Once strands are separated, RNA primers are created on the template strands.

The initiation of DNA replication is mediated by DnaA, a protein that binds to a region of the origin known as the DnaA box. In E. coli, there are 5 DnaA boxes, each of which contains a highly conserved 9 bp consensus sequence 5' - TTATCCACA - 3'. Binding of DnaA to this region causes it to become negatively supercoiled. Following this, a region of OriC upstream of the DnaA boxes (known as DnaB boxes) become melted. There are three of these regions, and each is 13 bp long, and AT-rich (which facilitates melting because less energy is required to break the five DnaA dimers. DnaC is then released, and the prepriming complex is complete. In order for DNA replication to continue, SSB protein is needed to prevent the single strands of DNA from forming any secondary structures and to prevent them from reannealing, and DNA gyrase is needed to relieve the stress (by creating negative supercoils) created by the action of DnaB helicase. The unwinding of DNA by DnaB helicase allows for primase (DnaG) an RNA polymerase to prime each DNA template so that DNA synthesis can begin.

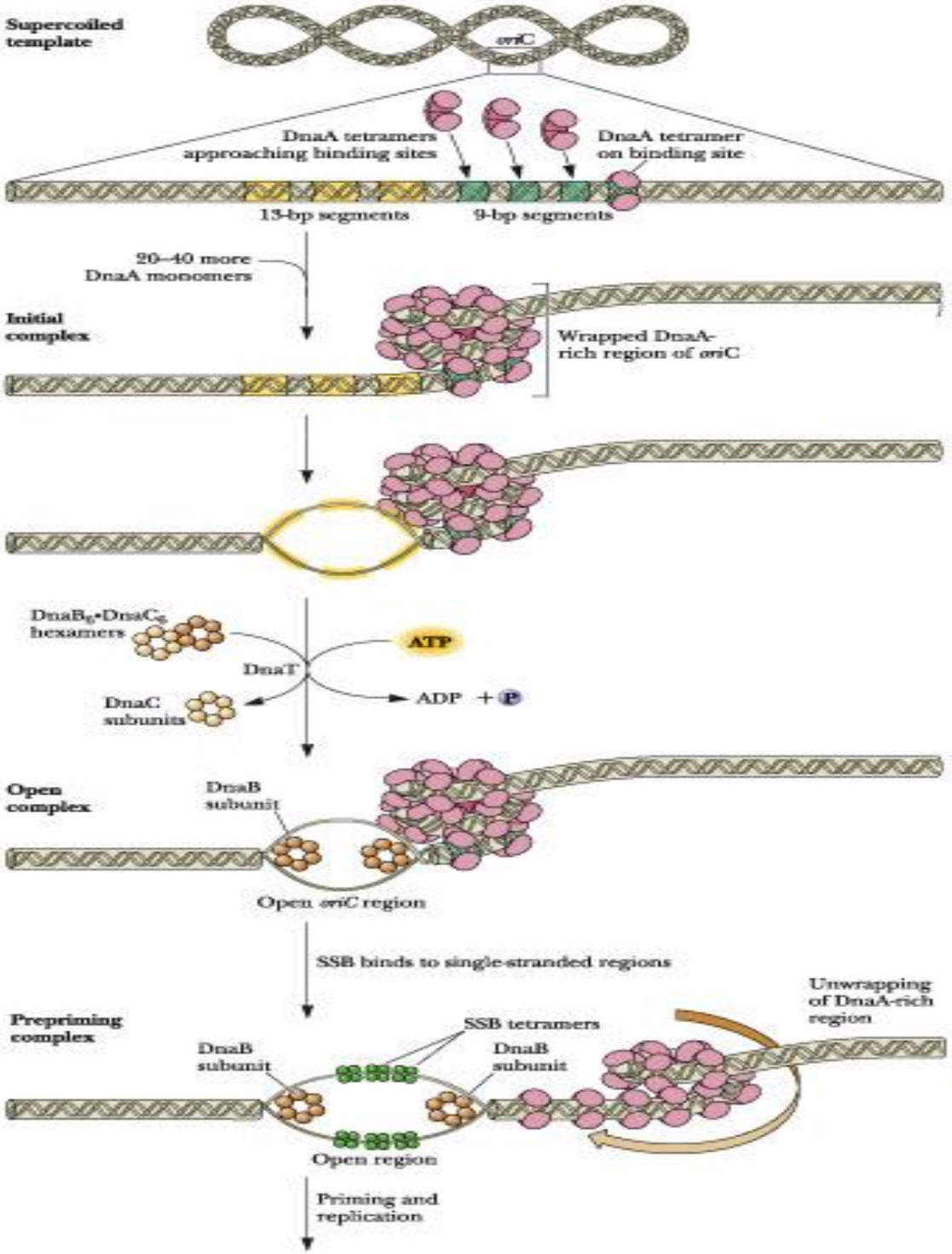


Figure- Initiation of replication

Elongation

The replication fork is a structure that forms within the nucleus during DNA replication. It is created by helicases, which break the hydrogen bonds holding the two DNA strands together. The resulting structure has two branching "prongs", each one made up of a single strand of DNA. These two strands serve as the template for the leading and lagging strands which will be created as DNA polymerase matches complementary nucleotides to the templates; The templates may be properly referred to as the leading strand template and the lagging strand template.

Leading strand

The leading strand is the template strand of the DNA double helix so that the replication fork moves along it in the 3' to 5' direction. This allows the new strand synthesized complementary to it to be synthesized 5' to 3' in the same direction as the movement of the replication fork.

On the leading strand, a polymerase "reads" the DNA and adds nucleotides to it continuously. This polymerase is DNA polymerase III (DNA Pol III) in prokaryotes and presumably Pol ε in eukaryotes.

Lagging strand

The lagging strand is the strand of the template DNA double helix that is oriented so that the replication fork moves along it in a 5' to 3' manner. Because of its orientation, opposite to the working orientation of DNA polymerase III, which moves on a template in a 3' to 5' manner, replication of the lagging strand is more complicated than that of the leading strand.

On the lagging strand, primase "reads" the DNA and adds RNA to it in short, separated segments. In eukaryotes, primase is intrinsic to Pol α. DNA polymerase III or Pol δ lengthens the primed segments, forming Okazaki fragments. Primer removal in eukaryotes is also performed by Pol δ. In prokaryotes, DNA polymerase I "reads" the fragments, removes the RNA using its flap endonuclease domain (RNA primers are removed by 5'-3' exonuclease activity of polymerase I [weaver, 2005], and replaces the RNA nucleotides with DNA nucleotides (this is necessary because RNA and DNA use slightly different kinds of nucleotides). DNA ligase joins the fragments together.

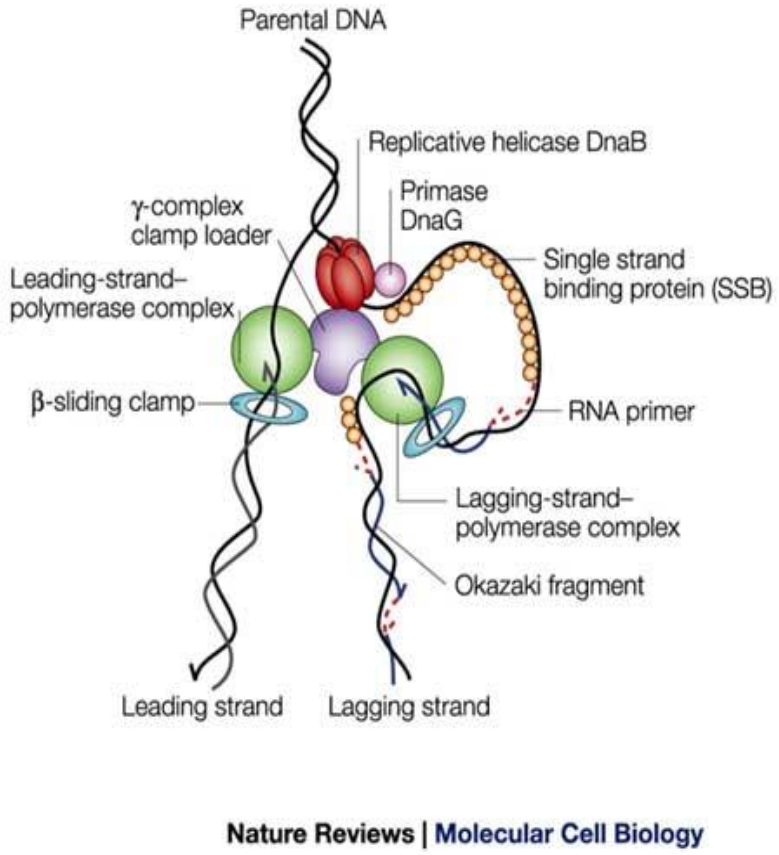


Figure-Elongation of DNA replication

Termination of replication

Located diametrically opposite from *oriC* on the E. *coli* circular map is a terminus region, the **Ter,** or t, locus. The bidirectionally moving replication forks meet here and replication is terminated. The *Ter* region contains a number of short DNA sequences containing a consensus core element 5'-GTGTGTTGT. These *Ter* sequences act as terminators; clusters of three or four *Ter* sequences are organized into two sets inversely oriented with respect to one another. One set blocks the clockwise-moving replication fork, and its inverted counterpart blocks the counterclockwise-moving replication fork. A *Ter* sequence element will impede replication fork progression *only if oriented in the proper direction* with respect to the approaching replication fork and then only if a specific 36-kD replication termination protein, **Tus protein,** is bound to it. Tus protein is a **contrahelicase.** That is, Tus protein prevents the DNA duplex from unwinding by blocking progression of the replication fork and inhibiting the ATP-dependent

DnaB helicase activity. Mutations in either the *Ter* locus or the gene encoding Tus protein do not grossly affect DNA replication, demonstrating that this termination mechanism is not essential.

Final synthesis of both duplexes is completed. Replication usually leaves the circular progeny chromosomes intertwined by 20 to 30 coils about each other, a so-called **catenated** state. In order to disengage the individual duplexes from each other prior to their distribution to daughter cells, double-stranded cuts must be made so that the double helices can pass through one another. Topoisomerase II (DNA gyrase) can catalyze this process.

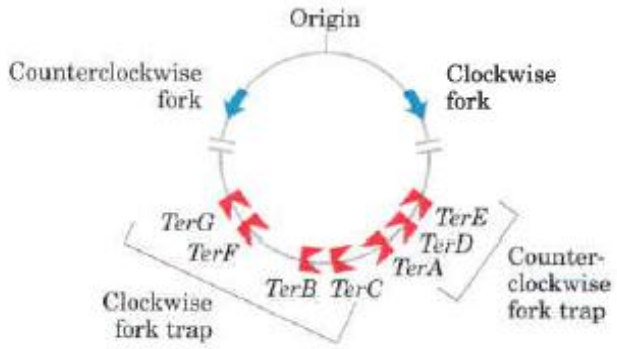
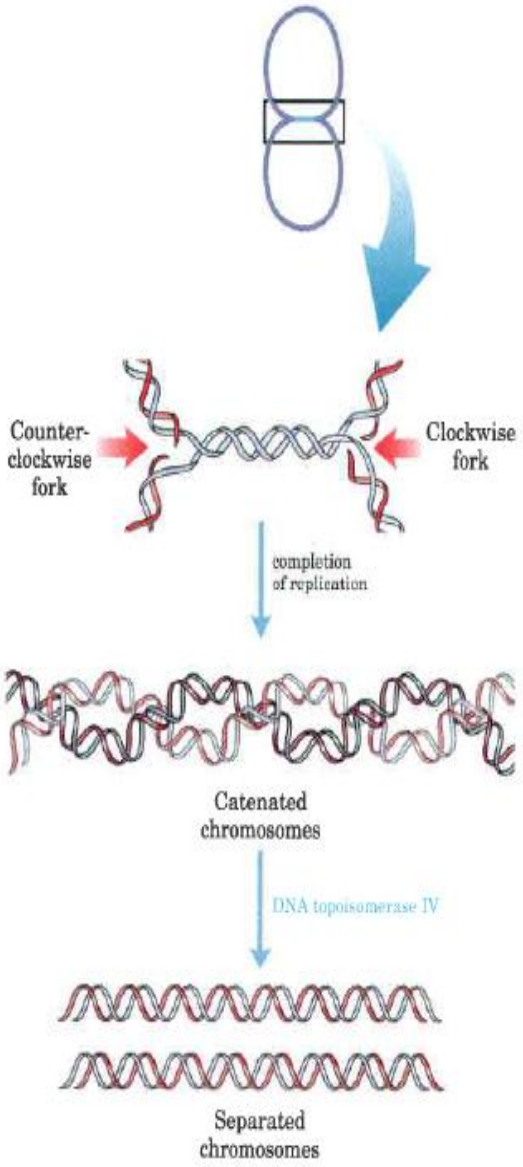


FIGURE **Termination of chromosome replication in *E. coli*.** The Ter sequences (*TerA* through *TerF*) are positioned on the chromosome in two clusters with opposite orientations.



Role of topoisomerases in replication termination.
Replication of the DNA separating opposing replication forks leaves the completed chromosomes joined as catenanes, or topologically interlinked circles. The circles are not covalently linked, but because they are interwound and each is covalently closed, they cannot be separated—except by the action of topoisomerases. In *E. coli*, a type II topoisomerase known as DNA topoisomerase IV plays the primary role in the separation of catenated chromosomes, transiently breaking both DNA strands of one chromosome and allowing the other chromosome to pass through the break.

Relationship between Cell division and Replication

DNA replication is a tightly orchestrated process that is controlled within the context of the cell cycle. Progress through the cell cycle and in turn DNA replication is tightly regulated by the formation and activation of pre-replicative complexs (pre-RCs) which is achieved through the activation and inactivation of cyclin-dependent kinases (Cdks, CDKs). Specifically it is the interactions of cyclins and cyclin dependent kinases that are responsible for the transition from G_1 into S-phase.

During the G_1 phase of the cell cycle there are low levels of CDK activity. This low level of CDK activity allows for the formation of new pre-RC complexes but is not sufficient for DNA replication to be initiated by the newly formed pre-RCs. During the remaining phases of the cell cycle there are elevated levels of CDK activity. This high level of CDK activity is responsible for initiating DNA replication as well as inhibiting new pre-RC complex formation. Once DNA replication has been initiated the pre-RC complex is broken down. Due to the fact that CDK levels remain high during the S phase, G_2, and M phases of the cell cycle no new pre-RC complexes can be formed. This all helps to ensure that no initiation can occur until the cell division is complete.

In addition to cyclin dependent kinases a new round of replication is thought to be prevented through the downregulation of Cdt1. This is achieved via degradation of Cdt1 as well as through the inhibitory actions of a protein known as geminin. Geminin binds tightly to Cdt1 and is thought to be the major inhibitor of re-replication. Geminin first appears in S-phase and is degraded at the metaphase-anaphase transition, possibly through ubiquination by anaphase promoting complex (APC).

Various cell cycle checkpoints are present throughout the course of the cell cycle that determine whether a cell will progress through division entirely. Importantly in replication the G_1, or restriction, checkpoint makes the determination of whether or not initiation of replication will begin or whether the cell will be placed in a resting stage known as G_0. Cells in the G_0 stage of the cell cycle are prevented from initiating a round of replication because the minichromosome maintenance proteins are not expressed. Transition into the S-phase indicates replication has begun

Replication Checkpoint Proteins

In order to preserve genetic information during cell division, DNA replication must be completed with high fidelity. In order to achieve this task, eukaryotic cells have proteins in place during certain points in the replication process that are able to detect any errors during DNA replication and are able to preserve genomic integrity. These checkpoint proteins are able to stop the cell cycle from entering mitosis in order to allow time for DNA repair. Checkpoint proteins are also involved in some DNA repair pathways, while they stabilize the structure of the replication fork

to prevent further damage. These checkpoint proteins are essential to avoid passing down mutations or other chromosomal aberrations to offspring.

Eukaryotic checkpoint proteins are well conserved and involve two phosphatidylinositol 3-kinase-related kinases (PIKKs), ATR and ATM. Both ATR and ATM share a target phosphorylation sequence, the SQ/TQ motif, but their individual roles in cells differ.

ATR is involved in arresting the cell cycle in response to DNA double-stranded breaks. ATR has an obligate checkpoint partner, ATR-interacting-protein (ATRIP), and together these two proteins are responsive to stretches of single-stranded DNA that are coated by replication protein A (RPA). The formation of single-stranded DNA occurs frequently, more often during replication stress. ATR-ATRIP is able to arrest the cell cycle to preserve genome integrity. ATR is found on chromatin during S phase, similar to RPA and claspin.

The generation of single-stranded DNA tracts is important in initiating the checkpoint pathways downstream of replication damage. Once single-stranded DNA becomes sufficiently long, single-stranded DNA coated with RPA are able to recruit ATR-ATRIP.[98] In order to become fully active, the ATR kinase rely on sensor proteins that sense whether the checkpoint proteins are localized to a valid site of DNA replication stress. The RAD9-HUS1-Rad1 (9-1-1) heterotrimeric clamp and its clamp loader RFC are able to recognize gapped or nicked DNA. The RFC clamp loader loads 9-1-1 onto the damaged DNA. The presence of 9-1-1 on DNA is enough to facilitate the interaction between ATR-ATRIP and a group of proteins termed checkpoint mediators, such as TOPBP1 and Mrc1/claspin. TOPBP1 interacts with and recruits the phosphorylated Rad9 component of 9-1-1 and binds ATR-ATRIP, which phosphorylates Chk1. Mrc1/Claspin is also required for the complete activation of ATR-ATRIP that phosphorylates Chk1, the major downstream checkpoint effector kinase. Claspin is a component of the replisome and contains a domain for docking with Chk1, revealing a specific function of Claspin during DNA replication: the promotion of checkpoint signaling at the replisome.

Chk1 signaling is vital for arresting the cell cycle and preventing cells from entering mitosis with incomplete DNA replication or DNA damage. The Chk1-dependent Cdk inhibition is important for the function of the ATR-Chk1 checkpoint and to arrest the cell cycle and allow sufficient time for completion of DNA repair mechanisms, which in turn prevents the inheritance of

damaged DNA. In addition, Chk1-dependent Cdk inhibition plays a critical role in inhibiting origin firing during S phase. This mechanism prevents continued DNA synthesis and is required for the protection of the genome in the presence of replication stress and potential genotoxic conditions. Thus, ATR-Chk1 activity further prevents potential replication problems at the level of single replication origins by inhibiting initiation of replication throughout the genome, until the signaling cascade maintaining cell-cycle arrest is turned off.

Karpagam Academy of Higher Education
Department of Biochemistry
II BSc Biochemistry
Gene Organisation, Replication and Repair (16BCU401)

UNIT II- POSSIBLE QUESTIONS

2 Mark - Questions

1. Define semiconservative mechanism with neat diageam
2. Explain the mechanism that seal the end of DNA
3. Write short notes on okazaki fragments
4. Explain the proof reading activity of DNA polymerase
5. Explain the bidirectional mode of replication
6. Write short notes on i) primosomes ii) Replisomes
7. List the enzymes involved in DNA replication
8. How RNA primers in lagging strand are removed?
9. Give the reaction catalysed by DNA polymetrase
10. Draw the structure of DNA polymerase and mention the role of each subunits
11. What is the relationship between the cell cycle and DNA replication

Essay type Questions (8 Marks)

1. Discuss in detail about the DNA replication in prokaryotes
2. Explain the role of various enzymes involved in DNA replication
3. Draw the structure of OriC and explain events involved in the origin of replication
4. Describe the mechanism of simultaneous synthesis of leading and lagging strand in Prokaryotes

5, Explain the termination of replication in prokaytoes

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
II B.Sc BIOCHEMISTRY- Fourth Semester
GENE ORGANISATION, REPLICATION AND REPAII (16BCU401)
MULTIPLE CHOICE QUESTIONS
UNIT II

S.No	Unit	Questions	Option 1	Option 2	Option 3	Option 4	Answer
1	II	DNA replication occurs at _____ phase of cell cycle	G1	G2	S	M	S
2	II	The process of DNA duplication is	Replication	Transcription	Translation	Reverse transcription	Replication
3	II	An enzyme that catalyses phosphodiester linkages in DNA strands is called	DNA polymerase	DNA ligase	RNA polymerase	Topoisomerase	DNA ligase
4	II	The separation of two DNA strands for replication is brought by	DNA polymerase III	DNA helicase	DNA polymerase I	DNA ligase	DNA helicase
5	II	The synthesis of new DNA (replication) in prokaryotes is catalysed by	DNA polymerase I	DNA polymerase II	DNA polymerase III	DNA topoisomerase	DNA polymerase III
6	II	The proof reading activity of the newly synthesized DNA is present on the enzyme	DNA helicase	DNA polymerase I	DNA polymerase II	DNA polymerase III	DNA polymerase I
7	II	The problem of supercoils during DNA replication is overcome by a group of enzymes called	DNA topoisomerases	DNA ligases	DNA polymerases	DNA helicases	DNA topoisomerases
8	II	The enzyme responsible for the replication of mitochondrial DNA	DNA polymerase α	DNA polymerase β	DNA polymerase γ	DNA polymerase δ	DNA polymerase γ
9	II	Okazaki fragments are initiated with	DNA primer	RNA primer	DNA template	RNA template	RNA primer
10	II	Watson and Crick elucidated ds DNA structure by using	NMR spectroscopy	X-ray diffraction	Circular dichroism	IR and Raman spectroscopy	X-ray diffraction
11	II	Single strand binding protein binds to single strand DNA	to prevent replication	to repair base pairs	to initiate transcription	to prevent reformation of duplex state	to prevent reformation of duplex state
12	II	Rolling circle replication is otherwise called as	θ replication	σ replication	D-loop replication	L-loop replication	σ replication
13	II	The DNA replication is discontinuous was proved by	Messelson-Stahl	Reigi Okazaki	Albert Lehninger	Arthur Kornberg	Reigi Okazaki
14	II	RNA primers are removed by	DNA polymerase I	DNA polymerase II	DNA polymerase III	DNA topoisomerase	DNA polymerase I
15	II	Primase initiates the following activities except	leading strand synthesis	replication	Okazaki fragments	transcription	transcription
16	II	The Klenow fragment exhibits the activity of	5'-3' exonuclease	polymerase and 3'-5' exonuclease	polymerase and 5'-3' exonuclease	an endo nuclease	polymerase and 3'-5' exonuclease
17	II	Ori C is rich in _____sequence	GC	AT	ATGC	GATC	AT
18	II	Dna A protein recognize and binds to	4 9 bp repeats in ter C	13 bp AT rich segment	4 9 bp repeats in Ori C	RNA polymerase	4 9 bp repeats in Ori C
19	II	Which of the following enzymes are used to join bits of DNA?	DNA ligase	DNA polymerase	Primase	Endonuclease	DNA ligase
20	II	Semi conservative replication of DNA was first demonstrated in	*Escherichia coli*	*Streptococcus pneumoniae*	*Salmonella typhi*	*Drosophila melanogaster*	*Escherichia coli*
21	II	Mode of DNA replication in *E. coli* is	Conservative and unidirectional	Semiconservative and unidirectional	Conservative and bidirectional	Semiconservative and bidirectional	Semiconservative and bidirectional
22	II	True replication of DNA is possible due to	Hydrogen bonding	Phosphate backbone	Complementary base pairing rule	Phosphodiesterase	Complementary base pairing rule
23	II	DNA synthesis can be specifically measured by estimating the incorporation of radiolabeled	Uracil	Thymine	Adenine	Deoxyribose sugar	Thymine
24	II	The elongation of the leading strand during DNA synthesis	Progresses away from the replication fork	Occur in 3'-5' direction	Produce Okazaki fragment	Depend on the action of DNA polymerase	Depend on the action of DNA polymerase
25	II	Eukaryotes differ from prokaryote in mechanism of DNA replication due to:	Different enzyme for synthesis of lagging and leading strand	Use of DNA primer rather than RNA primer	Unidirectional rather than bidirectional replication	Discontinuous rather than semidiscontinuous replication	Discontinuous rather than semidiscontinuous replication
26	II	During the replication of DNA, the synthesis of DNA on lagging strand takes place in segments, these segments are called	Satellite segments	Double helix segments	Kornberg segments	Okazaki segments	Okazaki segments
27	II	Which of the following reactions is required for proofreading during DNA replication by DNA polymerase III?	3' - 5' exonuclease activity	5' - 3' exonuclease activity	3' - 5' endonuclease activity	5' - 3' endonuclease activity	3' - 5' endonuclease activity
28	II	In the rolling circle method of replication	the 5' tail of DNA is nicked	RNA is nicked	one strand of DNA in the circle is nicked	both strands of DNA in the circle are nicked	one strand of DNA in the circle is nicked
29	II	All of the following are differences between eukaryotic and prokaryotic DNA replication except	the type and number of polymerases involved in DNA synthesis	multiple vs. single replication origins	the rate of DNA synthesis	the ability to form a replication fork	the ability to form a replication fork
30	II	In the Meselson -Stahl experiment, which mode of replication can be eliminated based on data derived after one generation of replication?	Dispersive	Semiconservative	Conservative	all three modes	Conservative
31	II	The discovery of Okazaki fragments suggested that DNA synthesis is	discontinuous	continuous	3 ' to 5'	semiconservative	discontinuous
32	II	A replicating prokaryotic chromosome has --- ------ replication forks	One	Many	Three	Two	Two
33	II	A replicating eukaryotic chromosome has ---- --------- replication forks	One	Many	Three	Two	Many
34	II	Which molecule serves to destabilize the DNA helix in order to open it up, creating a replication fork?	DNA helicase	DNA ligase	DNA polymerase	SSBPs	DNA helicase
35	II	For DNA Replication, unwinding of DNA is done by	Helicase	Ligase	Hexonuclease	Topoisomerase	Helicase
36	II	In vivo synthesis of DNA is _____	3' to 5'	5' to 3'	both 3' to 5' and 5' to 3'	neither 3' to 5' nor 5' to 3'	5' to 3'
37	II	Which of the following forms of DNA can serve as a template for DNA polymerase	Partially double stranded DNA	Circular double stranded DNA	Intact double stranded DNA	Circular single stranded DNA	Partially double stranded DNA
38	II						
39	II	In which phase of the cell cycle does DNA replication occur?	G0	G1	S	G2	S
40	II	The enzyme responsible for initiating DNA replication in prokaryotes is	DNA polymerase I	DNA polymerase III	Polymerase beta	Primase	Primase
41	II	The enzyme responsible for continuing DNA replication in prokaryotes, once it is initiated is:	DNA polymerase I	DNA polymerase III	Polymerase beta	Polymerase delta	DNA polymerase III
42	II	The enzyme _____ unzips and unwinds the DNA molecule.	DNA polymerase	helicase	primase	DNA ligase	helicase
43	II	Looped rolling circle mode of DNA replication is seen in	E. coli	Chloroplast	θx174	Mitochondria	θx174

44	II	DNA replication results in:	2 completely new DNA molecules	2 DNA molecules that each contain a strand of the original	1 new DNA molecule, 1 old molecule is conserved	1 new molecule of RNA	2 DNA molecules that each contain a strand of the original
45	II	During replication, what enzyme adds complimentary bases?	helicase	synthesase	replicase	polymerase	polymerase
46	II	Helicase opens up the DNA by breaking _____ bonds between the complementary strands.	hydrophobic	hydrogen	phosphodiester	ionic	hydrogen
47	II	Ligase forms a bond between	two segments of single stranded DNA.	two molecules of double-stranded DNA.	two segments of single stranded RNA.	two molecules of double-stranded RNA.	two segments of single stranded DNA.
48	II	DNA polymerase can add new nucleotides to the free _____ of a nucleotide strand.	1' end	3' end	5' end	1' end and 3' end	3' end
49	II	Which of the following possesses both 5'-3' and 3'-5' exonuclease activity?	Kornberg enzyme	DNA polymerase III	Taq DNA polymerase	DNA gyrase	Kornberg enzyme
50	II	The enzyme that catalyzes the synthesis of DNA is called	DNA polymerase	DNA gyrase	DNA ligase	Helicase	DNA polymerase
51	II	Which of the following repairs nicked DNA by forming a phosphodiester bond between adjacent nucleotides?	Helicase	DNA gyrase	Topoisomerases	DNA ligase	DNA ligase
52	II	Proofreading activity to maintain the fidelity of DNA synthesis	occurs after the synthesis has been completed	is a function of the 3'-5' exonuclease activity of the DNA polymerases	requires the presence of an enzyme separate from the DNA polymerases	occurs in prokaryotes but not eukaryotes	is a function of the 3'-5' exonuclease activity of the DNA polymerases
53	II	Both strands of DNA serve as templates concurrently in	replication	excision repair	mismatch repair	Transcription	replication
54	II	In E.coli, which enzyme synthesizes the RNA primer for Okazaki fragments?	DnaA	DnaC	DnaG	DnaB	DnaG
55	II	During which of the following process a new copy of a DNA molecule is precisely synthesized?	Transformation	Transcription	Translation	Replication	Replication
56	II	DNA gyrase is inhibited by	tetracycline	nalidixic acid	tetracycline and nalidixic acid	Cephalosporin	nalidixic acid
57	II	DNA replication rates in prokaryotes are approximately of the order of	10 bases per second	1,00 bases per second	1,000 bases per second	10,000 bases per second	1,000 bases per second
58	II	What DNA sequence is required for termination of replication in E. coli?	oriC	ter	Tus	Fork	ter
59	II	What is the major difference between T1 and T2 ter sequences?	Tus binds to T1 but not T2.	Tus binds to T2 but not T1.	T1 stops counterclockwise-moving forks, T2 stops clockwise-moving forks.	T2 stops counterclockwise-moving forks, T1 stops only clockwise-moving forks.	T1 stops counterclockwise-moving forks, T2 stops clockwise-moving forks.
60	II	What enzyme performs decatenation?	Polymerase	Topoisomerase IV	Telomerase	Decatenase	Topoisomerase IV

UNIT-III

SYLLABUS

Replication of DNA in Eukaryotes

Replication in eukaryotes. Comparison of replication in prokaryotes and eukaryotes. Inhibitors of DNA replication and applications in medicine. Supercoiling of DNA and its importance, topoisomerases, critical role of topoisomerases in cell, topoisomerase inhibitors and their application in medicine.

EUKARYOTIC DNA REPLICATION

The mechanism of eukaryotic DNA replication is similar to that of prokaryotic DNA replication. However, eukaryotic DNA replication requires special consideration due to differences in DNA sizes, unique linear DNA end structures called **telomeres**, and distinctive DNA packaging that involves complexes with histones. Unlike prokaryotes, most eukaryotes are multicellular organisms, except for the unicellular eukaryotes such as yeast, flagellates, and ciliates. Therefore, DNA replication in eukaryotes is a highly regulated process and usually requires extracellular signals to coordinate the specialized cell divisions in different tissues of multicellular organisms. External signals are delivered to cells during the G_1 phase of the cell cycle and activate the synthesis of cyclins. Cyclins form complexes with cyclin-dependent kinases (CDK), which, in turn, stimulate the synthesis of S phase proteins such as DNA polymerases and thymidylate synthase. These complexes prepare cells for DNA replication during the S phase.

Initiation

DNA replication is initiated from specific sequences called origins of replication, and eukaryotic cells have multiple replication origins. Initiation of DNA replication in eukaryotes begins with the binding of the origin recognition complex (ORC) to origins of replication during the G_1 phase of the cell cycle.

Association of the origin recognition complex (ORC) with a replication origin is required to recruit both cell division cycle 6 protein (Cdc6) and chromatin licensing and DNA replication factor 1 protein (Cdt1), which initiate the assembly of the **pre-replicative complexes (pre-RC).** Both Cdc6 and Cdt1 proteins associate with the already bound ORC independently from each other. The ORC, Cdc6, and Cdt1 together are required for the stable association of the minichromosome maintenance (Mcm 2-7) complex proteins with replicative origins during G_1 phase of the cell cycle

Pre-RCs formed during the G_1 phase are converted to the **initiation complex** during cell cycle transition from G_1 to S by the action of two kinases: cyclin-dependent kinase (CDK) and Dbf4-dependent kinase (DDK). Formation of an initiation complex, which includes helicase activity, unwinds the DNA double helix at the origin site (Figure).

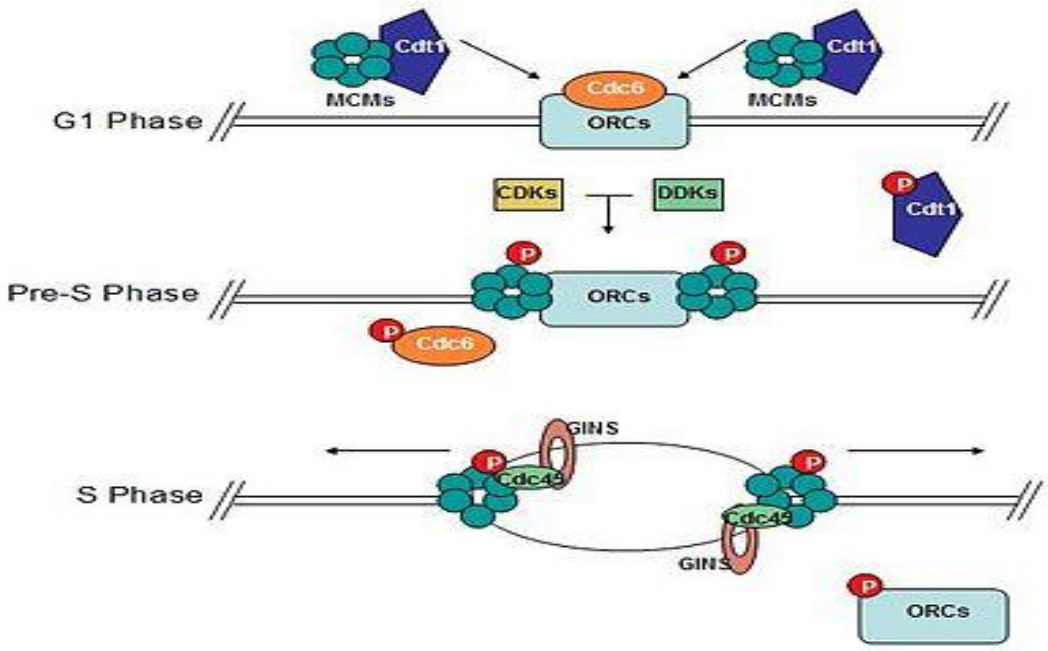


Diagram of the formation of the pre-replicative complex transforming into an active replisome

At the transition of the G_1 stage to the S phase of the cell cycle, S phase–specific cyclin-dependent protein kinase (CDK) and Cdc7/Dbf4 kinase (DDK) transform the pre-RC into an active replication fork. During this transformation, the pre-RC is disassembled with the loss of Cdc6, creating the initiation complex. In addition to the binding of the Mcm proteins, cell division cycle 45 (Cdc45) protein is also essential for initiating DNA replication.

Elongation

Once the initiation complex is formed and the cells pass into the S phase, the complex then becomes a replisome. The eukaryotic replisome complex is responsible for coordinating DNA replication.

After the replicative helicase has unwound the parental DNA duplex, exposing two single-stranded DNA templates, replicative polymerases are needed to generate two copies of the parental genome. DNA polymerase function is highly specialized and accomplish replication on specific templates and in narrow localizations. At the eukaryotic replication fork, there are three

distinct replicative polymerase complexes that contribute to DNA replication: Polymerase α, Polymerase δ, and Polymerase ε. These three polymerases are essential for viability of the cell.

Because DNA polymerases require a primer on which to begin DNA synthesis, polymerase α (Pol α) acts as a replicative primase. Pol α is associated with an RNA primase and this complex accomplishes the priming task by synthesizing a primer that contains a short 10 nucleotide stretch of RNA followed by 10 to 20 DNA bases. Importantly, this priming action occurs at replication initiation at origins to begin leading-strand synthesis and also at the 5' end of each Okazaki fragment on the lagging strand.

However, Pol α is not able to continue DNA replication and must be replaced with another polymerase to continue DNA synthesis. Polymerase switching requires clamp loaders and it has been proven that normal DNA replication requires the coordinated actions of all three DNA polymerases: Pol α for priming synthesis, Pol ε for leading-strand replication, and the Pol δ, which is constantly loaded, for generating Okazaki fragments during lagging-strand synthesis. In eukaryotes, Okazaki fragments generated during lagging-strand synthesis are shorter than those in *E. coli* (up to 200 bases in eukaryotes versus up to 2000 bases long in *E. coli*). Also, eukaryotic DNA replication is initiated by forming many replication forks at multiple origins to complete DNA replication in the time available during the S phase of a cell cycle.

These free nucleotides are added to an exposed 3'-hydroxyl group on the last incorporated nucleotide. In this reaction, a pyrophosphate is released from the free dNTP, generating energy for the polymerization reaction and exposing the 5' monophosphate, which is then covalently bonded to the 3' oxygen. Additionally, incorrectly inserted nucleotides can be removed and replaced by the correct nucleotides in an energetically favorable reaction. This property is vital to proper proofreading and repair of errors that occur during DNA replication.

Many replisome factors including Claspin, And1, replication factor C clamp loader and the fork protection complex are responsible for regulating polymerase functions and coordinating DNA synthesis with the unwinding of the template strand by Cdc45-Mcm-GINS complex. As the DNA is unwound the twist number decreases. To compensate for this the writhe number increases, introducing positive supercoils in the DNA. These supercoils would cause DNA replication to halt if they were not removed. Topoisomerases are responsible for removing these supercoils ahead of the replication fork.

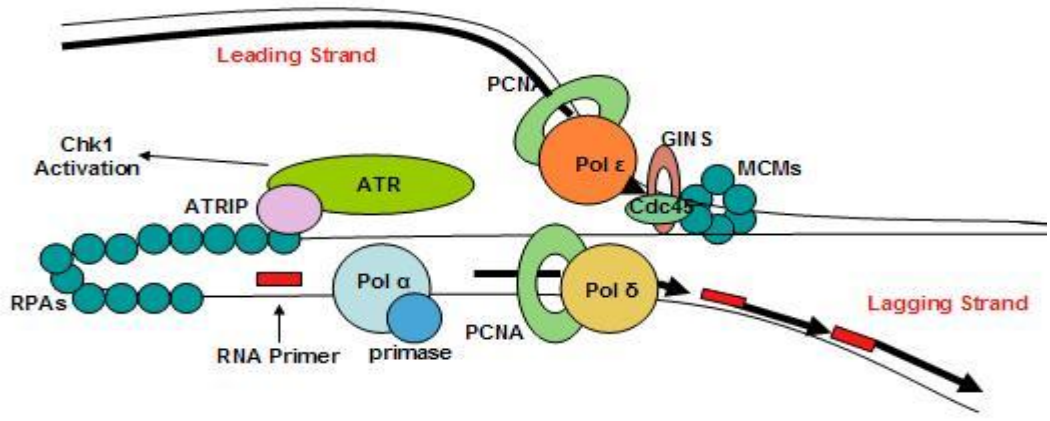


Fig: Eukaryotic replisome complex and associated proteins

The replication fork is the junction between the newly separated template strands, known as the leading and lagging strands, and the double stranded DNA. Since duplex DNA is antiparallel, DNA replication occurs in opposite directions between the two new strands at the replication fork, but all DNA polymerases synthesize DNA in the 5' to 3' direction with respect to the newly synthesized strand. Further coordination is required during DNA replication. Two replicative polymerases synthesize DNA in opposite orientations. Polymerase ε synthesizes DNA on the "leading" DNA strand continuously as it is pointing in the same direction as DNA unwinding by the replisome. In contrast, polymerase δ synthesizes DNA on the "lagging" strand, which is the opposite DNA template strand, in a fragmented or discontinuous manner.

The discontinuous stretches of DNA replication products on the lagging strand are known as Okazaki fragments and are about 100 to 200 bases in length at eukaryotic replication forks. The lagging strand usually contains longer stretches of single-stranded DNA that is coated with single-stranded binding proteins, which help stabilize the single-stranded templates by preventing a secondary structure formation. In eukaryotes, these single-stranded binding proteins are a heterotrimeric complex known as replication protein A (RPA).

Each Okazaki fragment is preceded by an RNA primer, which is displaced by the procession of the next Okazaki fragment during synthesis. RNase H recognizes the DNA:RNA hybrids that are created by the use of RNA primers and is responsible for removing these from the replicated strand, leaving behind a primer:template junction. DNA polymerase α, recognizes these sites and elongates the breaks left by primer removal. In eukaryotic cells, a small amount of the DNA segment immediately upstream of the RNA primer is also displaced, creating a flap structure. This flap is then cleaved by endonucleases. At the replication fork, the gap in DNA after removal of the flap is sealed by DNA ligase I, which repairs the nicks that are left between the 3'-OH and

5'phosphate of the newly synthesized strand. Owing to the relatively short nature of the eukaryotic Okazaki fragment, DNA replication synthesis occurring discontinuously on the lagging strand is less efficient and more time consuming than leading-strand synthesis. DNA synthesis is complete once all RNA primers are removed and nicks are repaired.

Proliferating Cell Nuclear Antigen

DNA polymerases require additional factors to support DNA replication. DNA polymerases have a semiclosed 'hand' structure, which allows the polymerase to load onto the DNA and begin translocating. This structure permits DNA polymerase to hold the single-stranded DNA template, incorporate dNTPs at the active site, and release the newly formed double-stranded DNA. However, the structure of DNA polymerases does not allow a continuous stable interaction with the template DNA.

To strengthen the interaction between the polymerase and the template DNA, DNA sliding clamps associate with the polymerase to promote the processivity of the replicative polymerase. In eukaryotes, the sliding clamp is a homotrimer ring structure known as the proliferating cell nuclear antigen (PCNA). The PCNA ring has polarity with surfaces that interact with DNA polymerases and tethers them securely to the DNA template. PCNA-dependent stabilization of DNA polymerases has a significant effect on DNA replication because PCNAs are able to enhance the polymerase processivity up to 1,000-fold. PCNA is an essential cofactor and has the distinction of being one of the most common interaction platforms in the replisome to accommodate multiple processes at the replication fork, and so PCNA is also viewed as a regulatory cofactor for DNA polymerases

Replication Factor C

PCNA fully encircles the DNA template strand and must be loaded onto DNA at the replication fork. At the leading strand, loading of the PCNA is an infrequent process, because DNA replication on the leading strand is continuous until replication is terminated. However, at the lagging strand, DNA polymerase δ needs to be continually loaded at the start of each Okazaki fragment. This constant initiation of Okazaki fragment synthesis requires repeated PCNA loading for efficient DNA replication.

PCNA loading is accomplished by the replication factor C (RFC) complex. The RFC complex is composed of five ATPases: Rfc1, Rfc2, Rfc3, Rfc4 and Rfc5. RFC recognizes primer-template junctions and loads PCNA at these sites. The PCNA homotrimer is opened by RFC by ATP hydrolysis and is then loaded onto DNA in the proper orientation to facilitate its association with the polymerase. Clamp loaders can also unload PCNA from DNA; a mechanism needed when replication must be terminated.

Stalled replication fork

DNA replication at the replication fork can be halted by a shortage of deoxynucleotide triphosphates (dNTPs) or by DNA damage, resulting in replication stress. This halting of replication is described as a **stalled replication fork**. A fork protection complex of proteins stabilizes the replication fork until DNA damage or other replication problems can be fixed. Prolonged replication fork stalling can lead to further DNA damage. Stalling signals are deactivated if the problems causing the replication fork are resolved.

Termination

Termination of eukaryotic DNA replication requires different processes depending on whether the chromosomes are circular or linear. Unlike linear molecules, circular chromosomes are able to replicate the entire molecule. However, the two DNA molecules will remain linked together. This issue is handled by decatenation of the two DNA molecules by a type II topoisomerase. Type II topoisomerases are also used to separate linear strands as they are intricately folded into a nucleosome within the cell.

As previously mentioned, linear chromosomes face another issue that is not seen in circular DNA replication. Due to the fact that an RNA primer is required for initiation of DNA synthesis, the lagging strand is at a disadvantage in replicating the entire chromosome. The 3'-5' action of DNA polymerase along the parent strand leaves a short single-stranded DNA (ssDNA) region at the 3' end of the parent strand when the Okazaki fragments have been repaired. Since replication occurs in opposite directions at opposite ends of parent chromosomes, each strand is a lagging strand at one end. Over time this would result in progressive shortening of both daughter chromosomes. This is known as the end replication problem.

The end replication problem is handled in eukaryotic cells by telomere regions and telomerase. Telomeres extend the 3' end of the parental chromosome beyond the 5' end of the daughter strand. This single-stranded DNA structure can act as an origin of replication that recruits telomerase. Telomerase is a specialized DNA polymerase that consists of multiple protein subunits and an RNA component. The RNA component of telomerase anneals to the single stranded 3' end of the template DNA and contains 1.5 copies of the telomeric sequence. Telomerase contains a protein subunit that is a reverse transcriptase called telomerase reverse transcriptase or TERT. TERT synthesizes DNA until the end of the template telomerase RNA and then disengages. This process can be repeated as many times as needed with the extension of the 3' end of the parental DNA molecule. This 3' addition provides a template for extension of the 5' end of the daughter strand by lagging strand DNA synthesis. Regulation of telomerase activity is handled by telomere-binding proteins.

In G_1 phase of the cell cycle, many of the DNA replication regulatory processes are initiated. In eukaryotes, the vast majority of DNA synthesis occurs during S phase of the cell cycle, and the entire genome must be unwound and duplicated to form two daughter copies. During G_2, any damaged DNA or replication errors are corrected. Finally, one copy of the genomes is segregated to each daughter cell at mitosis or M phase. These daughter copies each contain one strand from the parental duplex DNA and one nascent antiparallel strand.

Comparision of replication in prokaryotes and eukaryotes

Similarities Between Prokaryotic and Eukaryotic DNA Replication

The steps for DNA replication are generally the same for all prokaryotic and eukaryotic organisms. Unwinding the DNA is accomplished by an enzyme named DNA helicase. Manufacturing new DNA strands is orchestrated by enzymes called polymerases.

Both types of organisms also follow a pattern called semi-conservative replication. In this pattern, the individual strands of DNA are manufactured in different directions, producing a leading and a lagging strand. Lagging strands are created by the production of small DNA fragments called Okazaki fragments that are eventually joined together. Both types of organisms also begin new DNA strands with a small primer of RNA.

Differences Between Prokaryotic and Eukaryotic DNA Replication

Differences between prokaryotic and eukaryotic DNA replication are largely related to contrasts in size and complexity of the DNA and cells of these organisms. The average eukaryotic cell has 25 times more DNA than a prokaryotic cell.

In prokaryotic cells, there is only one point of origin, replication occurs in two opposing directions at the same time, and takes place in the cell cytoplasm. Eukaryotic cells on the other hand, have multiple points of origin, and use unidirectional replication within the nucleus of the cell. Prokaryotic cells possess one or two types of polymerases, whereas eukaryotes have four or more.

Difference between replication in eukaryotes and prokaryotes

Important features

1. Origin(ARS,replisome)

In prokaryotic cells, there is only one point of origin, replication occurs in two opposing directions at the same time, and takes place in the cell cytoplasm. Eukaryotic cells on the other

hand, have multiple points of origin, and use **unidirectional replication** within the nucleus of the cell.

2. Polymerase - alpha(α,), delta and eata (-€)

Prokaryotic cells possess one or two types of polymerases, whereas eukaryotes have four or more.

3.Telemere synthesis

Eukaryotes also have a distinct process for replicating the telomeres at the ends of their chromosomes Because eukaryotes have linear chromosomes, DNA replication often fails to synthesize to the very end of the chromosomes (telomeres), resulting in telomere shortening. This is a normal process in somatic cells. With their circular chromosomes, prokaryotes have no ends to synthesize.

4. Rate of replication

Replication also happens at a much faster rate in prokaryotic cells, than in eukaryotes. Some bacteria take only 40 minutes, while animal cells such as humans may take up to 400 hours.

5. Time of replication

Lastly, the short replication in prokaryotes occurs almost continuously, but eukaryotic cells only undergo DNA replication during the S-phase of the cell cycle.

6. Regulation of replication

Within eukaryotes, DNA replication is controlled within the context of the cell cycle. As the cell grows and divides, it progresses through stages in the cell cycle; DNA replication occurs during the S phase (Synthesis phase). The progress of the eukaryotic cell through the cycle is controlled by cell cycle checkpoints. Progression through checkpoints is controlled through complex interactions between various proteins, including cyclins and cyclin-dependent kinases.

The G1/S checkpoint (or restriction checkpoint) regulates whether eukaryotic cells enter the process of DNA replication and subsequent division. Cells which do not proceed through this checkpoint are quiescent in the "G0" stage and do not replicate their DNA.

Inhibitors of DNA replication

DNA replication inhibitors are commonly used as anticancer and antiviral agents. Drugs inhibit DNA synthesis by two mechanisms that are generally associated: 1/ direct interference with molecules required for DNA polymerization or/and initiation of replication; and 2/ checkpoint response(s). They are

1. Nucleotide Triphosphate Inhibitors
2. Chain Elongation Inhibitors
3. Antiviral Drugs that Block Chain Elongation
4. DNA Polymerase Inhibitors
5. DNA Template damaging drugs
6. DNA Topoisomerase Inhibitors
7. Cdk Inhibitors and Checkpoint Inhibitors

1.Nucleotide Triphosphate Inhibitors

Several approaches in developing cancer therapeutics that bear chemical similarity to the various "building blocks" of nucleic acids and inhibit the formation of functional nucleotide triphosphates needed to synthesize either DNA or RNA. Many of these agents have been labeled **"antimetabolites**" because of their structural similarities to naturally occurring metabolites.These include the **antifolates (e.g., methotrexate), pyrimidines like 5-Fluorouracil (5-FU), and purines like 6- mercaptopurine and 6-thioguanine**. All of the agents inhibit DNA synthesis and affect the S-phase of the cell cycle. Thus rapidly growing cancers theoretically should be potentially the most responsive. They also share toxicities toward the most rapidly growing normal "host" cells (e.g., hematopoietic cells - white blood cells, red blood cells, and platelets; gastrointestinal mucosal cells and hair) such that common side effects are produced (e.g. myelosuppression, anemia, thrombocytopenia, diarrhea, and hair loss).

2.Chain Elongation Inhibitors

these drugs share a similar site of action, inhibiting chain elongation of the deoxyribonucleotide strand being synthesized. Chain elongation inhibitors are useful not only as anticancer drugs, but also as antivirals. Example:

Cytosine Arabinoside is essentially a "pure" S- phase inhibitor

Gemcitabine (2, 2-difluoroeoxycytidine; dFdC) is a relatively new pyrimidine antimetabolite drug analog of deoxycytidine (dC)

3.Antiviral Drugs that Block Chain Elongation

Acyclovir (Ac) is an antimetabolite analog of 2'-deoxyguanosine

Ganciclovir is a purine nucleoside-like antimetabolite

4.DNA Polymerase Inhibitors

Aphidicolin (APH) is a specific inhibitor of eukaryotic and viral encoded replicative DNA polymerases.

Foscarnet interferes with the polymerization step by complexing with the pyrophosphate binding site of DNA polymerase, preventing cleavage of pyrophosphate from nucleoside triphosphates, thereby blocking elongations

5. DNA Template damaging drugs

Three main DNA template lesions induced by anticancer agents can block replication fork progression: DNA adducts, DNA strand breaks and DNA-protein crosslinks. They are

- DNA alkylating agents
- Methylmethanesulfonate
- Cisplatin, carboplatin and oxaliplatin:
- Nitrogen mustards (Melphalan, Chlorambucil and Cyclophosphamide) and nitrosoureas (CCNU, BCNU):

The activity of alkylating agents is correlated with inhibition of DNA synthesis, which is due to alteration of the nucleic acid template rather than to inactivation of DNA polymerase or other enzymes responsible for DNA synthesis

6. DNA Topoisomerase Inhibitors

Top1 inhibitors: camptothecins;Trapping of Top1 cleavage complexes by endogenous DNA lesions and carcinogenic adducts

Top2 inhibitors: etoposide and anthracyclines;and Trapping of Top2 cleavage complexes by endogenous DNA lesions and carcinogenic adducts

7. Cdk Inhibitors and Checkpoint Inhibitors

The cyclin-dependent kinase (Cdk) inhibitors, **flavopiridol and roscovitine** are in clinical trials as anticancer agents

Caffeine was the first cell cycle checkpoint abrogator described

DNA Topoisomerase Inhibitors

DNA topoisomerase inhibitors are **commonly used as anticancer drugs**. The two main classes of clinical topoisomerase inhibitors target topoisomerases I and II.

Seven topoisomerase genes are encoded in the human nuclear genome. The enzymes have been numbered in the order of their discovery except for the most recent enzyme, mitochondrial topoisomerase I (Top1mt). Vertebrate cells contain two Top1 (Top1 for the nuclear genome and Top1mt for the mitochondrial genome), two Top2 (Top2□ and □) and two Top3 (Top3□ and □ □ . The seventh topoisomerase is Spo11, whose expression is restricted to germ cells. Top3□ forms heterodimers with BLM (the gene product deficient in Bloom syndrome) and is mechanistically related to the resolution of post-replicative hemicatenanes and recombination intermediates. Top1's belong to the family of the tyrosine recombinases (which includes □-integrase, Flp and Cre recombinases), and Top2 is related to bacterial gyrase and Topo IV, which are the target of quinolone antibiotics. Topoisomerases and tyrosine recombinases nick and religate DNA by forming a covalent enzyme-DNA intermediate between a specific enzyme catalytic tyrosine residue for each enzyme and the DNA via a DNA phosphodiester bond. Topoisomerases have also been classified in two groups depending whether they cleave and

religate one strand (type I) or both strands (type II) of the DNA duplex. Type I enzymes include Top1 and Top3 and type II, Top2 and Spo11.

Top1 inhibitors: camptothecins;Trapping of Top1 cleavage complexes by endogenous DNA lesions and carcinogenic adducts

Top1 relaxes DNA supercoiling processively by nicking the DNA and allowing rotation of the broken strand around the Top1-bound DNA strand. Once the DNA is relaxed, Top1 religates the breaks by reversing its covalent binding . Under normal condition, the cleavage intermediates are transient. Religation is much faster than cleavage. Camptothecins and non-camptothecin Top1 inhibitors trap Top1 cleavage complexes by binding at the enzyme-DNA interface. Hence Top1 inhibitors represent a paradigm for "interfacial inhibitors". Interfacial inhibition has recently accounts for the molecular mechanism of inhibition of many natural products that block specific conformational states of macromolecular complexes. Aphidicolin and Top2 inhibitors have been proposed to follow the interfacial inhibition paradigm. Interfacial inhibition is a special case of uncompetitive inhibition.

Two camptothecin derivatives are used in cancer therapy: hycamtin (Topotecan®) and CPT-11 (Irinotecan; Camptosar®). CPT-11 is an inactive prodrug. It needs to be converted to its active metabolite SN-38. Hence, it is preferable to use Topotecan and the parent drug, camptothecin for pharmacological studies. Top1 cleavage complexes can also be trapped by endogenous and frequent DNA lesions including abasic sites, mismatches, oxidized bases, and carcinogenic DNA adducts.

Top1 cleavage complexes on the leading strand are converted into replication double- strand breaks (Rep-DSB) by replication "run-off". These Rep-DSB activate the ATM-Chk2 pathway with hyperphosphorylation of histone H2AX (-H2AX formation) and phosphorylation of BLM on threonine 99. They also induce the rapid and sustained phosphorylation of RPA2 at least in part by DNA-PK. Top1 cleavage complexes also activate the ATR-Chk1 pathway by mechanisms that still remain to be elucidated. Camptothecins are now commonly used to induce Rep-DSB in vertebrate and yeast cells. They complement hydroxyurea and aphidicolin as pharmacological tools to "collapse" replication forks. It can be argued that the nature of the Rep-DSB are better defined in the case of camptothecins than for hydroxyurea or aphidicolin. The repair of the Top1-DNA adducts needs to take place for replication restart and camptothecins can be used to study the genetic and molecular pathways implicated in this repair.

Top2 inhibitors: etoposide and anthracyclines;and Trapping of Top2 cleavage complexes by endogenous DNA lesions and carcinogenic adducts

Top2 enzymes functions as homodimers and generate DSB. These breaks are staggered by 4 base pairs and are generated by the covalent linkage of each monomer to the 5'-end of the broken DNA. By contrast to Top1, which allows controlled rotation (swiveling) of the broken DNA around the intact strand, Top2 catalyzes the passage of an intact duplex through the broken DNA held within the Top2 complex. This strand passage reaction allows reactions that are specific for Top2 such as decatenation of duplex circles (and its reverse reaction, catenation) and knotting/unknotting. Decatenation is essential at the end of replication to allow the segregation of newly replicated chromatin domains.

Etoposide, doxorubicin, anthracyclines and other Top2 inhibitors trap the cleavage intermediate. This type of inhibition is probably mechanistically similar to Top1 trapping by camptothecin. The Top2 inhibitors are therefore candidate interfacial inhibitors by trapping Top2 cleavage complexes by forming ternary complexes with Top2 and the cleaved duplex.

As in the case of Top1, base alterations including oxidative lesions and carcinogenic adducts, as well as DNA single-strand break can interfere the DNA religation step, and therefore trap Top2 cleavage complexes under physiological condition (i.e. during normal cell cycle in the absence of drug/pharmacological inhibitors).

Top2 cleavage complexes produce large protein-DNA adducts. Each enzyme subunit covalently linked to DNA has a molecular mass of 170 kDa (Top2□) or 180 kDa (Top2□). There is no evidence that heterodimers □ /□ form unde physiological conditions. The trapped Top2 homodimer complexes can alter DNA replication by forming steric blocks on the DNA template. They also can interfere with other DNA metabolic processes including transcription and probably DNA repair, and chromatin remodeling.

Supercoling of DNA

Supercoiling means the coiling of a coil.DNA is coiled in the form of a double helix, wrth both strands of the DNA coiline around an axis The further coiling of that axis upon itself produces DNA supercoiling As detailed below, DNA supercoiling is generally a manifestation of structural strain. When there is no net bending of the DNA axis upon itself, the DNA is said to be in a relaxed state.

DNA supercoiling refers to the over- or under-winding of a DNA strand, and is an expression of the strain on that strand. Supercoiling is important in a number of biological processes, such as compacting DNA, and by regulating access to the genetic code, affects gene expression. Additionally, certain enzymes such as topoisomerases are able to change DNA topology to facilitate functions such as DNA replication or transcription. Mathematical expressions are used to describe supercoiling by comparing different coiled states to relaxed B-form DNA.

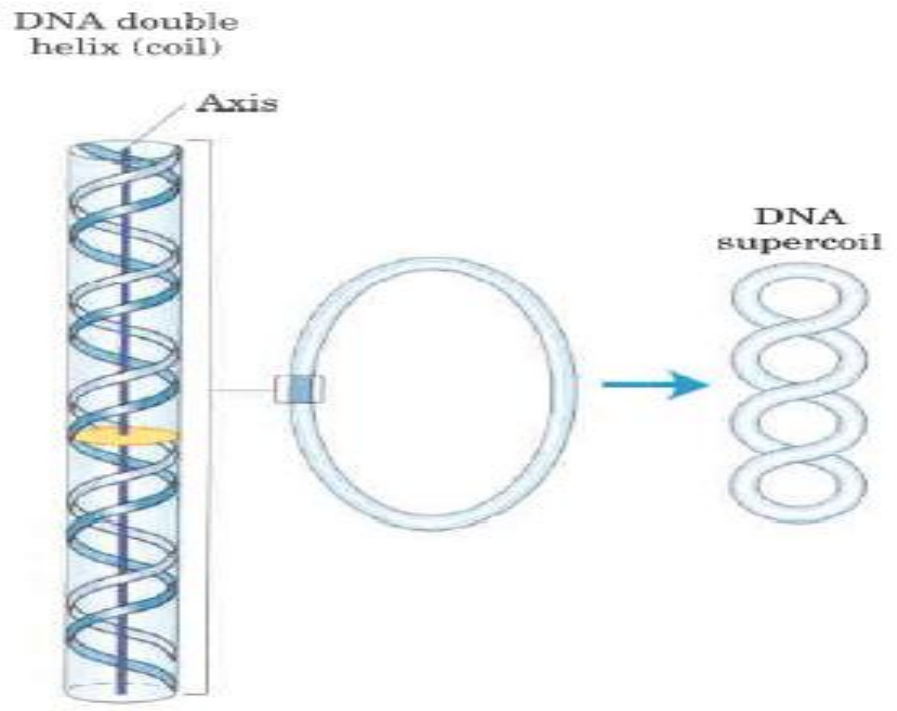


FIGURE **Supercoiling of DNA.** When the axis of the DNA double helix is coiled on itself, it forms a new helix (superhelix). The DNA superhelix is usually called a supercoil.

Linking Number, Twist and Writhe

The fundamental topological parameter of a covalently closed circular DNA is called the linking number (Lk). Assume that one DNA strand is the edge of an imaginary surface and count the number of times that the other DNA strand crosses this surface (Figure). The algebraic sum of all intersections (which accounts for a sign of every intersection) is the Lk. The only way to change Lk is to introduce a break in one or both DNA strands, rotate the two DNA strands relative to each other and seal the break. This is precisely the role of DNA topoisomerases.

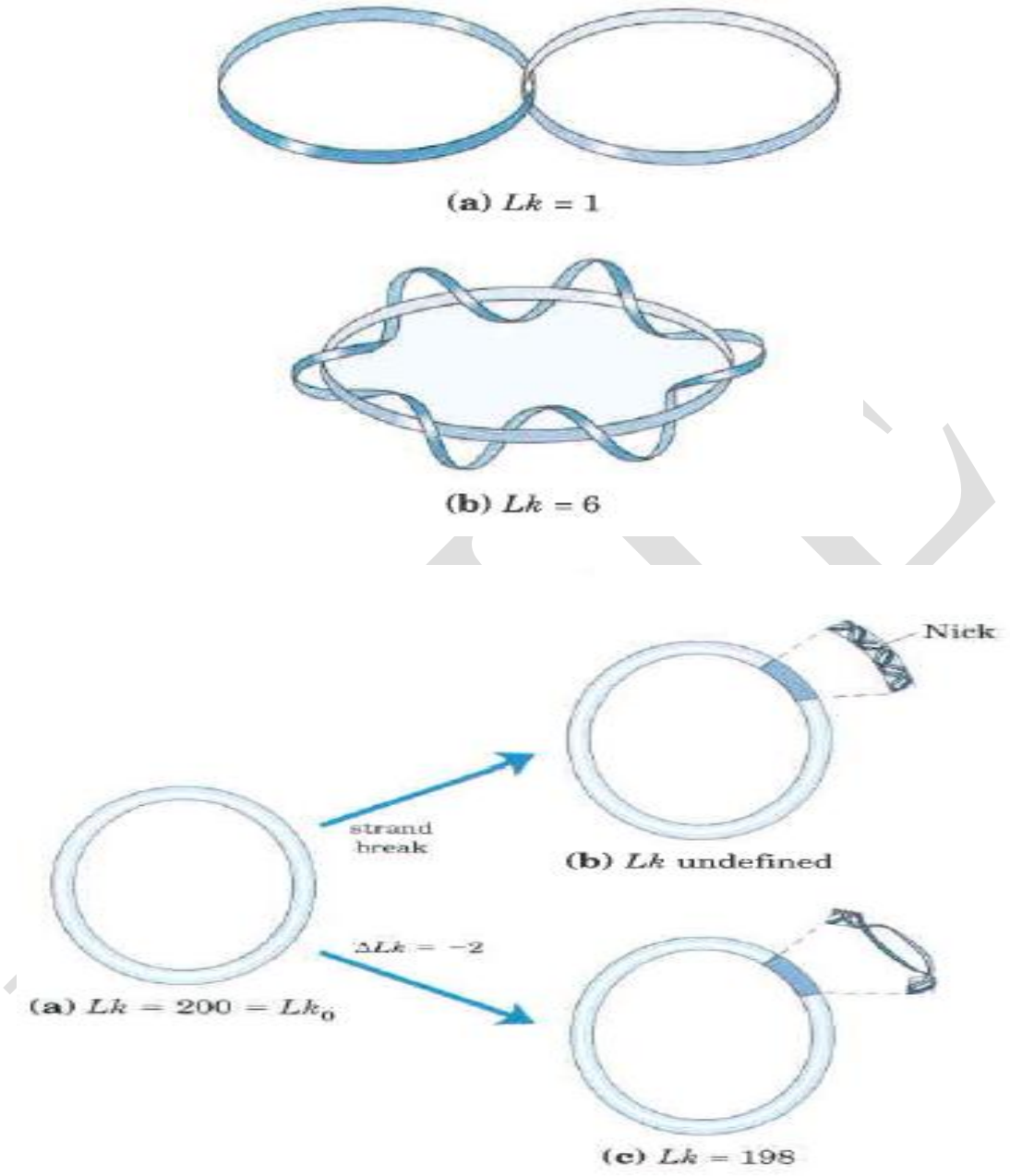


FIGURE **Linking number applied to closed-circular DNA molecules.** A 2,100 bp circular DNA is shown in three forms: **(a)** relaxed, $Lk = 200$; **(b)** relaxed with a nick (break) in one strand, Lk undefined; and **(c)** underwound by two turns, $Lk = 198$. The underwound molecule generally exists as a supercoiled molecule, but underwinding also facilitates the separation of DNA strands.

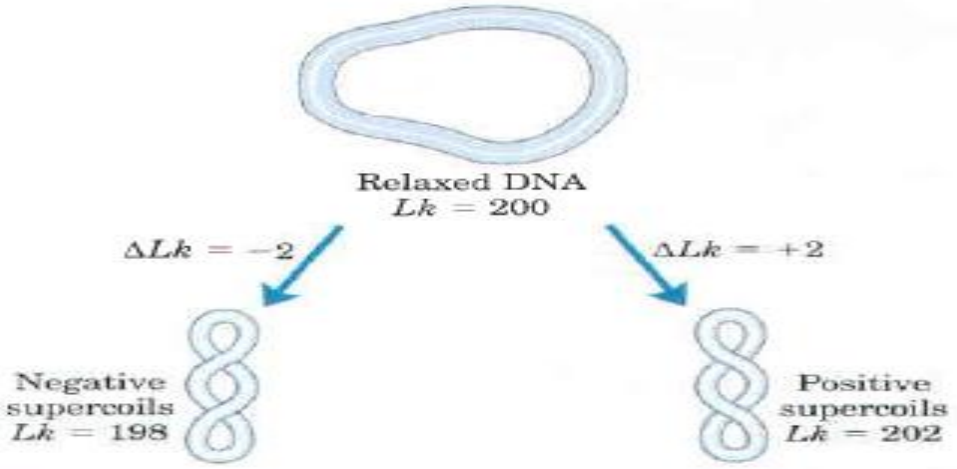
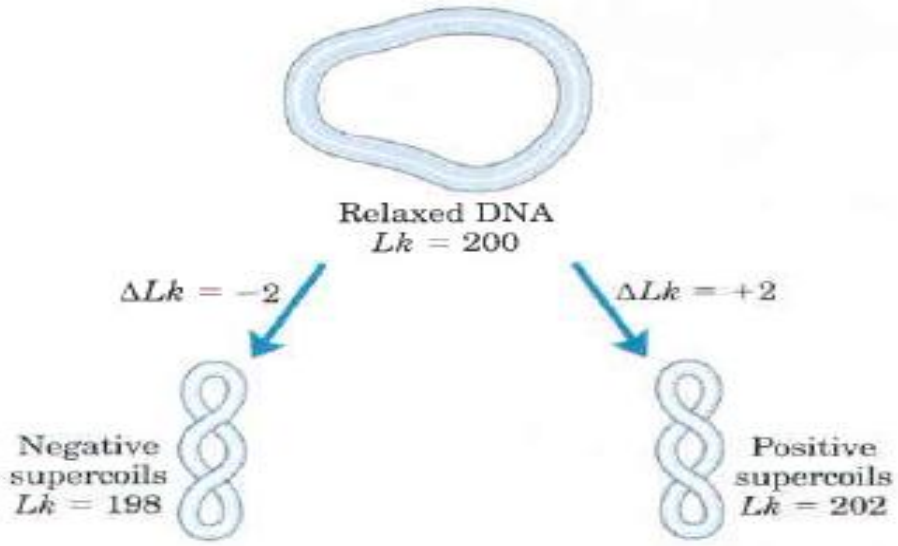


FIGURE **Negative and positive supercoils.** For the relaxed DNA molecule of Figure 24–16a, underwinding or overwinding by two helical turns (Lk = 198 or 202) will produce negative or positive supercoiling, respectively. Note that the DNA axis twists in opposite directions in the two cases.



Linking number can be broken down into two structural componentst,Twist (Tw) and writhe (Wr) (Fig). These are more difficult to describe than linking number, but writhe may be thought of as a measure of the coiling of the helix axis, and twist as determining the local twisting or

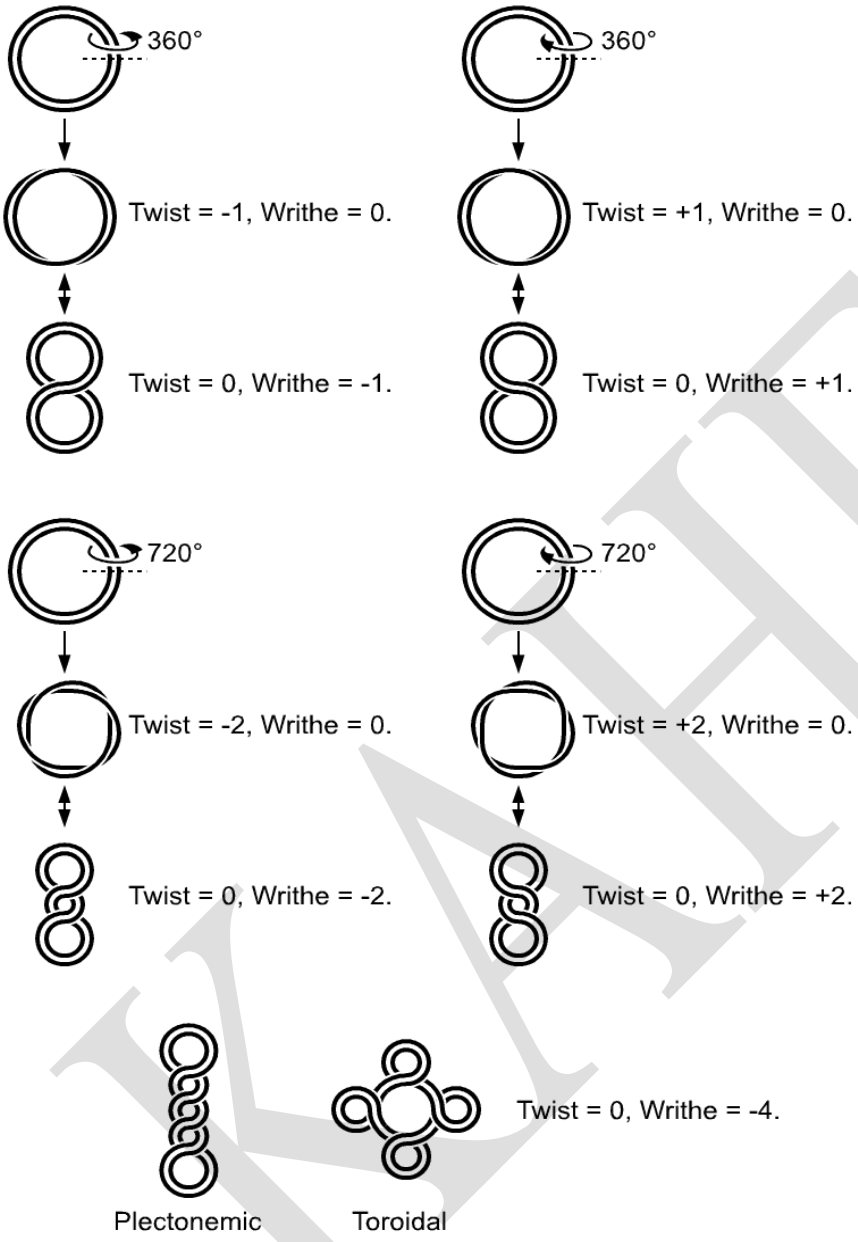
spatial relationship of neighboring base pairs. When the linking nurnber changes some of the resulting strain is usually compensated for by writhe (supercoiling) and some by changes in **twist**, giving rise to the equation

Lk: Tw + Wr

Tw and Wr need not be integers. Twist and writhe are geometric rather than topological properties, because they may be changed by deformation of a closed-circular DNA molecule.

Another characteristic of a circular DNA is called **twist**, or Tw. Tw is the total number of helical turns in circular DNA under given conditions. Since DNA is a right- handed helix with 10.5 base pairs (bp) per turn, Tw is a large positive number for any natural DNA

Writhing (Wr) is the third important characteristic of circular DNA, describing the spatial pass of the double helix axis, i.e. the shape of the DNA molecule as a whole. Wr can be of any sign, and usually its absolute value is much smaller than that of Tw.


360°
Twist = -1, Writhe = 0.
Twist = 0, Writhe = -1.
360°
Twist = +1, Writhe = 0.
Twist = 0, Writhe = +1.
720°
Twist = -2, Writhe = 0.
Twist = 0, Writhe = -2.
720°
Twist = +2, Writhe = 0.
Twist = 0, Writhe = +2.
Twist = 0, Writhe = -4.
Plectonemic
Toroidal

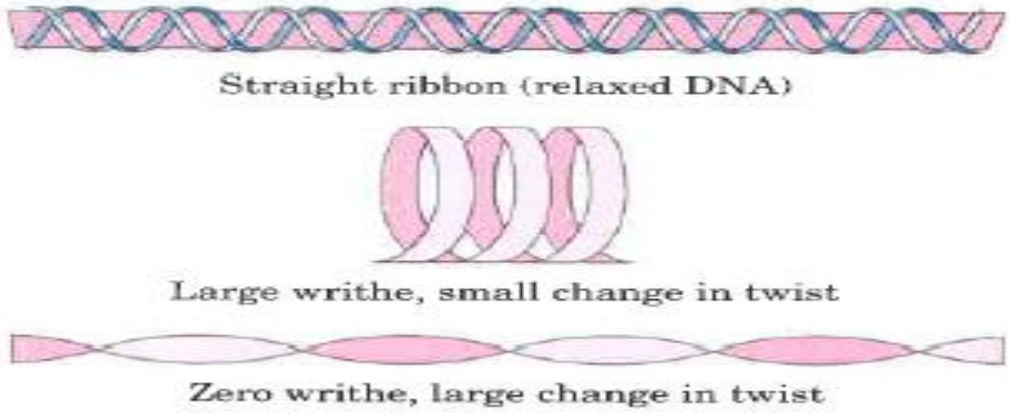


Fig: Ribbon model for illustrating twist and writhe

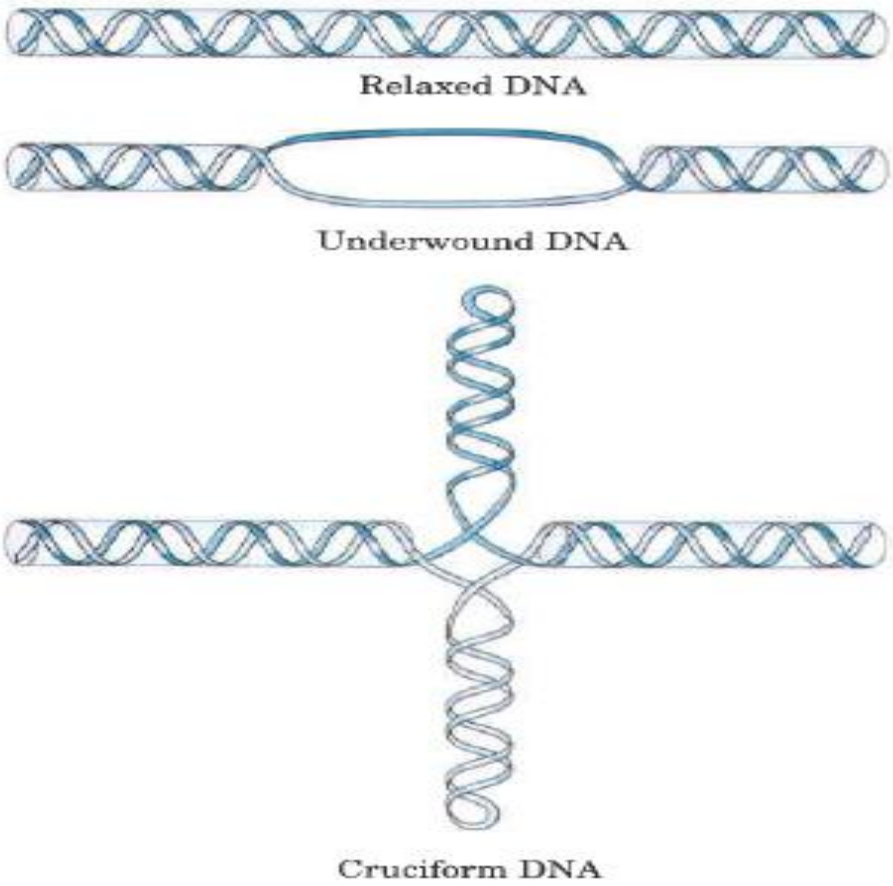


FIGURE Promotion of cruciform structures by DNA underwinding. In principle, cruciforms can form at palindromic sequences but they seldom occur in relaxed DNA because the linear DNA accommodates more paired bases than does the cruciform structure. Underwinding of the DNA facilitates the partial strand separation needed to promote cruciform formation at appropriate sequences.

DNA Compaction Requires specific form of supercoiling

Supercoiled DNA molecules are uniform in a number of respects. The supercoils are right-handed in a negatively supercoiled DNA molecule (Fig.), and they tend to be extended and narrow rather than compacted, often with multiple branches (Fig). At the super helical densities normally encountered in cells, the length of the supercoil axis, including branches, is about 4 0o/of the length of the DNA. This type of supercoiling is referred to a **splectonemic** (from the Greek plektos, "twisted," and nema, "thread"). This term can be applied to any structure with strands intertwined in some simple and regular way, and it is a good description of the general structure of supercoiled DNA in solution.

Plectonemic supercoiling, the form observed in isoIated DNAs in the laboratory, does not produce sufflcient compaction to package DNA in the cell. A second form of supercoiling, **solenoidal** can be adopted by an underwound DNA. Instead of the extended right-handed supercoils characteristic of the plectonemic form, solenoidal supercoiling involves tight left-handed turns, similar to the shape taken up by a garden hose neatly wrapped on a reel. Although their structures are dramatically different, plectonemic and solenoidal supercoiling are two forms of negative supercoiling that can be taken up by the same segment of underwound DNA. The two forms are readily interconvertible. Although the plectonemic form is more stable in solution, the solenoidal form can be stabilized by protein binding and is the form found in chromatin. It provides a much greater degree of compaction. Solenoidal supercoiling is the mechanism by which underwinding contributes to DNA compaction.

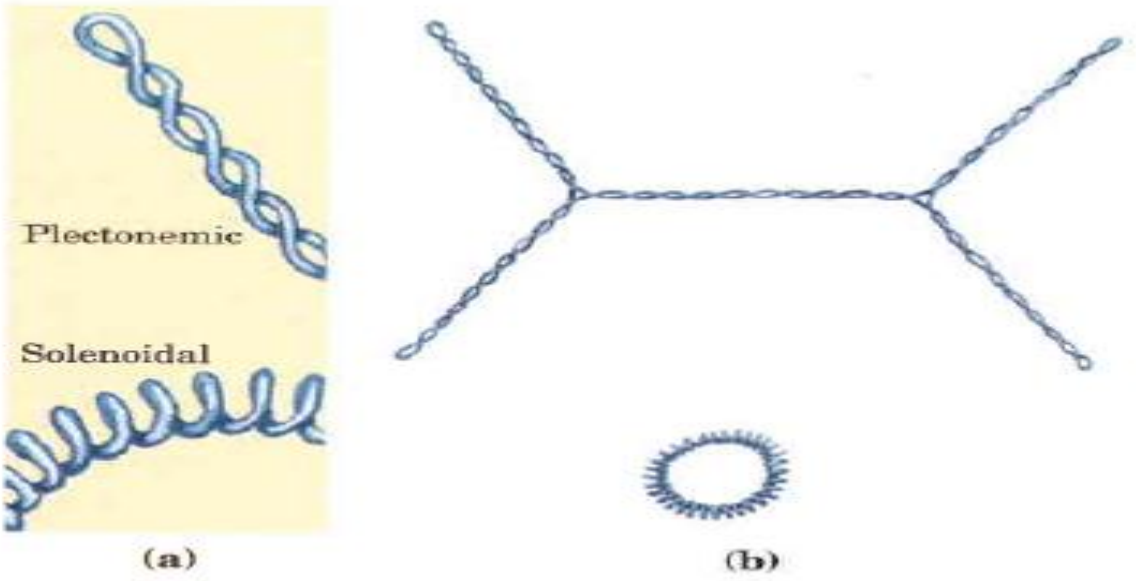


FIGURE **Plectonemic and solenoidal supercoiling. (a)** Plectonemic supercoiling takes the form of extended right-handed coils. Solenoidal negative supercoiling takes the form of tight left-handed turns about an imaginary tubelike structure. The two forms are readily interconverted, although the solenoidal form is generally not observed unless certain proteins are bound to the DNA. **(b)** Plectonemic (top) and solenoidal supercoiling of the same DNA molecule, drawn to scale. Solenoidal supercoiling provides a much greater degree of compaction.

In a "relaxed" double-helical segment of B-DNA, the two strands twist around the helical axis once every 10.4–10.5 base pairs of sequence. Adding or subtracting twists, as some enzymes can do, imposes strain. If a DNA segment under twist strain were closed into a circle by joining its two ends and then allowed to move freely, the circular DNA would contort into a new shape, such as a simple figure-eight. Such a contortion is a **supercoil**. The noun form "supercoil" is often used in the context of DNA topology.

Positively supercoiled (overwound) DNA is transiently generated during DNA replication and transcription, and, if not promptly relaxed, inhibits (regulates) these processes. The simple figure eight is the simplest supercoil, and is the shape a circular DNA assumes to accommodate one too many or one too few helical twists. The two lobes of the figure eight will appear rotated either clockwise or counterclockwise with respect to one another, depending on whether the helix is over- or underwound. For each additional helical twist being accommodated, the lobes will show one more rotation about their axis. As a general rule, the DNA of most organisms is negatively supercoiled.

Lobal contortions of a circular DNA, such as the rotation of the figure-eight lobes above, are referred to as *writhe*. The above example illustrates that twist and writhe are interconvertible. Supercoiling can be represented mathematically by the sum of twist and writhe. The twist is the number of helical turns in the DNA and the writhe is the number of times the double helix crosses over on itself (these are the supercoils). Extra helical twists are positive and lead to positive supercoiling, while subtractive twisting causes negative supercoiling. Many topoisomerase enzymes sense supercoiling and either generate or dissipate it as they change DNA topology. DNA of most organisms is negatively supercoiled.

In part because chromosomes may be very large, segments in the middle may act as if their ends are anchored. As a result, they may be unable to distribute excess twist to the rest of the chromosome or to absorb twist to recover from underwinding—the segments may become *supercoiled*, in other words. In response to supercoiling, they will assume an amount of writhe, just as if their ends were joined.

Supercoiled DNA forms two structures; a plectoneme or a toroid, or a combination of both. A negatively supercoiled DNA molecule will produce either a one-start left-handed helix, the toroid, or a two-start right-handed helix with terminal loops, the plectoneme. Plectonemes are typically more common in nature, and this is the shape most bacterial plasmids will take. For larger molecules it is common for hybrid structures to form – a loop on a toroid can extend into a plectoneme. If all the loops on a toroid extend then it becomes a branch point in the plectonemic structure. DNA supercoiling is important for DNA packaging within all cells, and seems to also play a role in gene expression.

Functions

1.Genome packaging

DNA supercoiling is important for DNA packaging within all cells. Because the length of DNA can be thousands of times that of a cell, packaging this genetic material into the cell or nucleus (in eukaryotes) is a difficult feat. Supercoiling of DNA reduces the space and allows for DNA to be packaged. In prokaryotes, plectonemic supercoils are predominant, because of the circular chromosome and relatively small amount of genetic material. In eukaryotes, DNA supercoiling exists on many levels of both plectonemic and solenoidal supercoils, with the solenoidal supercoiling proving most effective in compacting the DNA. Solenoidal supercoiling is achieved with histones to form a 10 nm fiber. This fiber is further coiled into a 30 nm fiber, and further coiled upon itself numerous times more.

DNA packaging is greatly increased during nuclear division events such as mitosis or meiosis, where DNA must be compacted and segregated to daughter cells. Condensins and cohesins

are *structural maintenance of chromosome* (SMC) proteins that aid in the condensation of sister chromatids and the linkage of the centromere in sister chromatids. These SMC proteins induce positive supercoils.

Supercoiling is also required for DNA/RNA synthesis. Because DNA must be unwound for DNA/RNA polymerase action, supercoils will result. The region ahead of the polymerase complex will be unwound; this stress is compensated with positive supercoils ahead of the complex. Behind the complex, DNA is rewound and there will be **compensatory** negative supercoils. Topoisomerases such as DNA gyrase (Type II Topoisomerase) play a role in relieving some of the stress during DNA/RNA synthesis.

2. Control gene expression

Specialized proteins can unzip small segments of the DNA molecule when it is replicated or transcribed into RNA. Simply twisting DNA can expose internal bases to the outside, without the aid of any proteins. Also, transcription itself contorts DNA in living human cells, tightening some parts of the coil and loosening it in others. That stress triggers changes in shape, most notably opening up the helix to be read. It is hypothesized that these structural changes might trigger stress elsewhere along its length, which in turn might provide trigger points for replication or gene expression.

TOPOISOMERASE

The DNA in the nucleus of a cell contains all the information it requires to carry out life's processes: growth, development, maintenance, reproduction and protection. With all this information, it is no wonder that the length of a cell's DNA is far greater than that of the cell compartment that contains it - the DNA in a single human diploid cell contains over 7 billion base pairs divided into 46 chromosomes, which would extend over 2 meters in length if stretched end to end. Yet this massive volume of DNA can be condensed to yield highly compact chromosomes through the tight packing of the DNA: first the DNA is wound around protein nucleosomes like beads on a string, then the beads are coiled into a helical structure, and finally the helical structure is itself supercoiled into a highly-packed coiled-coil structure. This highly compact structure allows the DNA to be safely stored in the nucleus, and to be divided up during cell division without damaging the DNA.

However, such tightly wound DNA does not permit molecules to gain access to individual genes in order to transcribe copies of them, as required for protein synthesis. To overcome this problem, cells use specialised proteins to unwind the DNA in specific regions when it needs access to it, while keeping the rest of the DNA molecule tightly wound and out of harms way. Once the DNA is uncoiled, the DNA double helix itself needs to be unwound to

separate it into two individual strands so the information it contains can be accessed. The proteins that carry out this job are collectively known as DNA topoisomerases.

DNA Topoisomerases, Unravelling DNA Strands

DNA topoisomerases are ubiquitous enzymes found in all cell types from viruses to man. These enzymes act to regulate DNA supercoiling by catalysing the winding and unwinding of DNA strands. They do this by making an incision that breaks the DNA backbone, so they can then pass the DNA strands through one another, swivelling and relaxing/coiling the DNA before resealing the breaks. DNA topoisomerases can be divided into two groups based on the number of strands that they break.

Class I DNA Topoisomerases

 Break one strand of a DNA helix.
 Topoisomerases I, III and V.
 ATP independent (except for reverse gyrase).
 Primarily responsible for relaxing positively supercoiled (over-wound) and/or negatively supercoiled (under-wound) DNA, while reverse gyrase can introduce positive supercoils into DNA.
 Mechanism involves rotating the broken strand around the intact strand to relax (unwind) the strain on the DNA helix, followed by resealing the ends of the broken strand.
 Play an important role in DNA replication and transcription (topoisomerase I), and recombination (topoisomerase III).
 Subclasses:

1) Type IA enzymes:

 Bacterial topoisomerase I
 Topoisomerase III
 Reverse gyrase

2) Type IB enzymes:

 Eukaryotic and eukaryal viral topoisomerase I
 Archaeal topoisomerase V

Class II DNA Topoisomerases

 Break two strands of a DNA helix.
 Topoisomerases II (gyrase), IV and VI.
 ATP dependent.
 Responsible for relaxing DNA (topoisomerase IV), as well as introducing either negative (topoisomerase II).

 Mechanism involves passing an intact DNA helix through the gap made by the broken DNA helix, then resealing the strands.

 Play an important role in chromosome condensation (topoisomerase II) and in the segregation of daughter chromosomes during cell division (topoisomerase IV).

 Subclasses:

1) Type IIA enzymes:

 Eukaryotic and eukaryal viral topoisomerase II

 Gyrase (bacterial topoisomerase II)

 Topoisomerase IV

2) Type IIB enzymes:

 Archaeal topoisomerase VI

Topoisomerase critical role

Topoisomerases are enzymes that participate in the overwinding or underwinding of DNA. The winding problem of DNA arises due to the intertwined nature of its double-helical structure. During DNA replication and transcription, DNA becomes overwound ahead of a replication fork. If left unabated, this torsion would eventually stop the ability of DNA or RNA polymerases involved in these processes to continue down the DNA strand.

In order to prevent and correct these types of topological problems caused by the double helix, topoisomerases bind to DNA and cut the phosphate backbone of either one or both the DNA strands. This intermediate break allows the DNA to be untangled or unwound, and, at the end of these processes, the DNA backbone is resealed again. Since the overall chemical composition and connectivity of the DNA do not change, the DNA substrate and product are chemical isomers, differing only in their global topology, resulting in the name for these enzymes. Topoisomerases are isomerase enzymes that act on the topology of DNA.

Bacterial topoisomerases and human topoisomerases proceed via similar mechanisms for managing DNA supercoils.

There are three main types of topology:

- Supercoiling
- Knotting
- Catenation

Outside of the essential processes of replication or transcription, DNA must be kept as compact as possible, and these three states help this cause. However, when transcription or replication occurs, DNA must be free, and these states seriously hinder the processes. In addition, during replication, the newly replicated duplex of DNA and the original duplex of DNA become intertwined and must be completely separated in order to ensure genomic integrity as a cell

divides. As a transcription bubble proceeds, DNA ahead of the transcription fork becomes overwound, or positively supercoiled, while DNA behind the transcription bubble becomes underwound, or negatively supercoiled. As replication occurs, DNA ahead of the replication bubble becomes positively supercoiled, while DNA behind the replication fork becomes entangled forming precatenanes. One of the most essential topological problems occurs at the very end of replication, when daughter chromosomes must be fully disentangled before mitosis occurs. Topoisomerase IIA plays an essential role in resolving these topological problems.

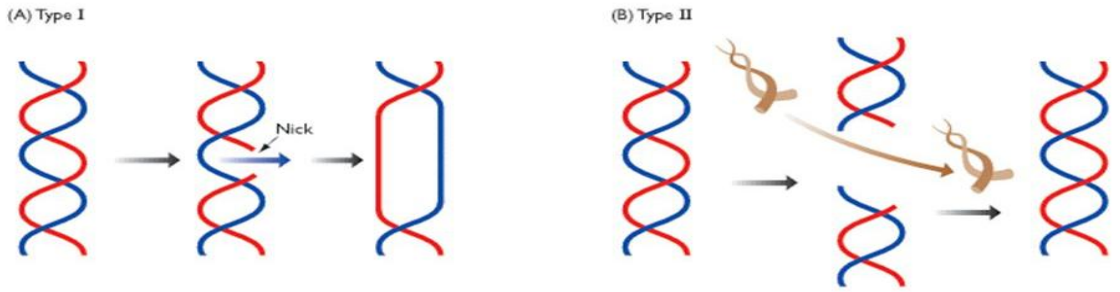
Classes

Topoisomerases can fix these topological problems and are separated into two types depending on the number of strands cut in one round of action:[5] Both these classes of enzyme utilize a conserved tyrosine. However these enzymes are structurally and mechanistically different. For a video of this process click here.

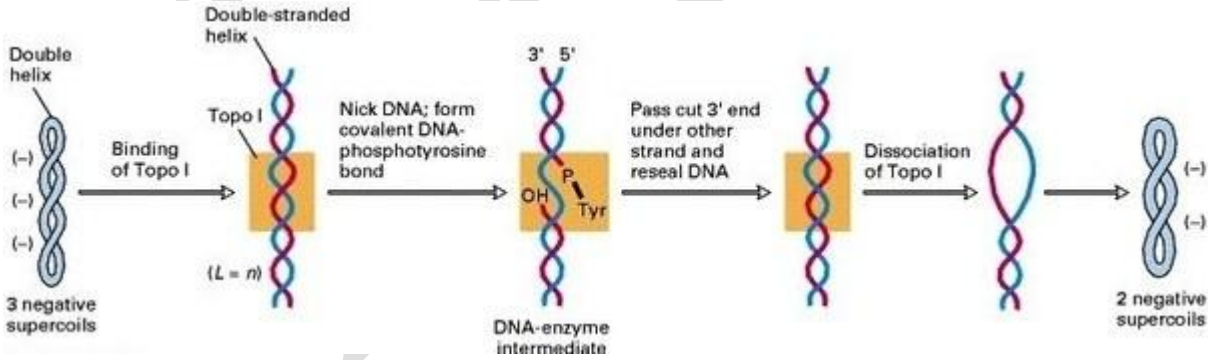
- A type I topoisomerase cuts one strand of a DNA double helix, relaxation occurs, and then the cut strand is re-ligated. Cutting one strand allows the part of the molecule on one side of the cut to rotate around the uncut strand, thereby reducing stress from too much or too little twist in the helix. Such stress is introduced when the DNA strand is "supercoiled" or uncoiled to or from higher orders of coiling. Type I topoisomerases do not require ATP for hydrolysis are subdivided into three subclasses:
    - Type IA topoisomerases, which share many structural and mechanistic features with the type II topoisomerases. Examples of type IA topoisomerases include prokaryotic Topoisomerase I and III, eukaryotic Topoisomerase IIIα and Topoisomerase IIIβ and Reverse Gyrase. Like type II topoisomerases, type IA topoisomerases form a covalent intermediate with the 5' end of DNA.
    - Type IB topoisomerases, which utilize a controlled rotary mechanism. Examples of Type IB topoisomerases include Eukaryotic and eukaryal viral Topoisomerase I. In the past, type IB topoisomerases were referred to as eukaryotic topoisomerase I, but IB topoisomerases are present in all three domains of life. Type IB topoisomerases form a covalent intermediate with the 3' end of DNA.
    - Type IC topoisomerase (also called Topoisomerase V) has been identified.[6] While it is structurally unique from type IA and IB topoisomerases, It shares a similar mechanism with type IB topoisomerase.
- A type II topoisomerase cuts both strands of one DNA double helix, passes another unbroken DNA helix through it, and then re-ligates the cut strands. Type II

topoisomerases utilize ATP hydrolysis and are subdivided into two subclasses which possess similar structure and mechanisms:

- Type IIA topoisomerases which include eukaryotic and eukaryal viral Topoisomerase IIα and Topoisomerase IIβ, bacterial gyrase, and topoisomerase IV.
- Type IIB topoisomerases, which include Topoisomerase VI found in archaea.



The mode of action of Type I and Type II DNA topoisomerases. (A) A Type I topoisomerase makes a nick in one strand of a DNA molecule, passes the intact strand through the nick, and reseals the gap. (B) A Type II topoisomerase makes a double-stranded break in the double helix, creating a gate through which a second segment of the helix is passed.



Functions

The double-helical configuration of DNA strands makes them difficult to separate, which is required by helicase enzymes if other enzymes are to transcribe the sequences that encode proteins, or if chromosomes are to be replicated. In circular DNA, in which double-helical DNA is bent around and joined in a circle, the two strands

are topologically linked, or knotted. Otherwise identical loops of DNA, having different numbers of twists, are topoisomers, and cannot be interconverted without the breaking of DNA strands. Topoisomerases catalyze and guide the unknotting or unlinking of DNA[2] by creating transient breaks in the DNA using a conserved tyrosine as the catalytic residue.[1]

The insertion of (viral) DNA into chromosomes and other forms of recombination can also require the action of topoisomerases.

Topologically linked circular molecules, are catenanes, adopt a positive supercoiled form during the process of replication of circular plasmids. The unlinking of catenanes is performed by type IIA topoisomerases, which were recently found to be more efficient unlinking positive supercoiled DNA. The conformational properties of negative vs. positive supercoiled catenanes affects their features in respect to their corresponding enzymatic reaction catalyzed by topoisomerases. Experiments have demonstrated that positive supercoiled DNA provides a sharp DNA bend in the first bound DNA segment, which allows the topoisomerase to bind successfully and therefore carry on its enzymatic reaction to the following segment in an specific inside-to-outside matter. On the other hand, negative supercoiled DNA does not provide such bend and the access of the enzyme to the first segment is nearly impossible, therefore inhibiting unlinking.

Critical role of topoisomerase/Functions of DNA Topoisomerases

There are a number of different types of topoisomerases, each specialising in a different aspect of DNA manipulation.

1.Accessing DNA

During transcription and DNA replication, the DNA needs to be unwound in order for the transcription/replication machinery to gain access to the DNA so it can be copied or replicate, respectively. Topoisomerase I can make single-stranded breaks to allow these processes to proceed.

2. Removing DNA Supercoils

During transcription and DNA replication, the DNA helix can become either over-wound or under-wound. For instance, during DNA replication, the progress of the replication fork generates positive supercoils ahead of the replication machinery and negative supercoils behind it. Such tensional problems also exist when transcribing DNA to make an RNA copy for protein synthesis. During these processes, the DNA can be supercoiled to such an extent that if left unchecked it could impede the progress of the protein machinery involved. This is prevented by topoisomerase I, which makes single-stranded nicks to relax the helix.

3.Strand Breakage during Recombination

Before the chromosomes separate from one another during cell division, they are able to exchange genetic information through a process known as recombination, where physical pieces of DNA on one chromosome can be swapped for DNA on the matching sister chromosome in order to shuffle the genetic information. Topoisomerase III can introduce single-strand breaks that are required for DNA to be exchanged by adjacent chromosomes.

4.Chromosome Condensation

During the cell cycle, chromosomes must be alternatively condensed and decondensed at specific stages. Topoisomerase II (gyrase) acts as a molecular motor, using the energy gained from ATP hydrolysis to introduce tight supercoils into the DNA helix in order to condense the chromosome. Because this process must be highly regulated, topoisomerase II can form molecular complexes with important cell cycle regulators (such as p53, TopBP1, 14-3-3 epsilon, and Cdc2) to ensure that chromosome condensation occurs at the correct time in the cell cycle.

5.Disentangling Intertwined DNA

During cell division, once the chromosomes have been replicated, they must separate and travel to opposite ends of the cell to become part of two separate daughter cells. Topoisomerases IV acts to disentangle the replicated daughter strands by making double-strand breaks that allow one duplex to pass through the other.

Topoisomerase inhibitors application in medicine(Clinical significance)- Topoisomerases as Drug Targets

Many drugs operate through interference with the topoisomerases . The broad-spectrum fluoroquinolone antibiotics act by disrupting the function of bacterial type II topoisomerases. These small molecule inhibitors act as efficient anti-bacterial agents by hijacking the natural ability of topoisomerase to create breaks in chromosomal DNA.

Some chemotherapy drugs called topoisomerase inhibitors work by interfering with mammalian-type eukaryotic topoisomerases in cancer cells. This induces breaks in the DNA that ultimately lead to programmed cell death (apoptosis). This DNA-damaging effect, outside of its potential curative properties, may lead to secondary neoplasms in the patient.

Topoisomerase I is the antigen recognized by Anti Scl-70 antibodies in scleroderma.

Topoisomerases have been the focus for the treatment of certain diseases. Bacterial gyrase (topoisomerase II) and topoisomerase IV are the targets of two classes of antibiotic drugs:

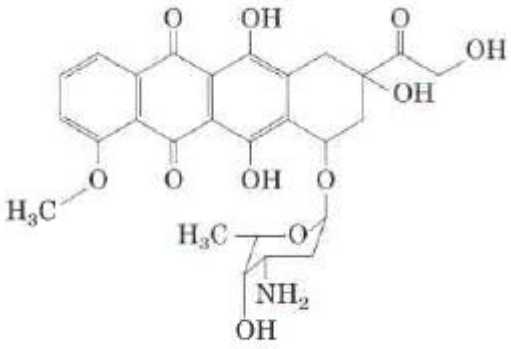
quinolones and coumarins. These antibiotics are used to treat an assortment of different diseases, such as pneumonia, tuberculosis and malaria, by inhibiting DNA replication in the bacteria responsible.

Eukaryotic topoisomerases I and II are the targets of an increasing number of anti-cancer drugs that act to inhibit these enzymes by blocking the reaction that reseals the breaks in the DNA. Often the binding of the drug is reversible, but if a replication fork runs into the blocked topoisomerase, then a piece of the gapped DNA strand not bound by the topoisomerase could be released, creating a permanent breakage in the DNA that leads to cell death. Most of these inhibitors are selective against either topoisomerase I or II, but some can target both enzymes.

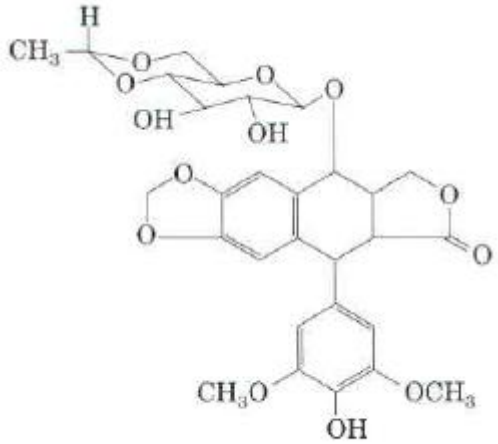
Topoisomerase I inhibitors induce single-strand breaks into DNA, and can work by a variety of mechanisms. Some drugs, such as camptothecins, inhibit the dissociation of topoisomerase and DNA, leading to replication-mediated DNA damage, which can be repaired more efficiently in normal cells than in cancer cells (deficient for DNA repair). Topoisomerase I inhibitors can also cause gene inactivation through chromatid aberrations.

Topoisomerase II inhibitors, such as anthracyclines, are amongst the most widely used anti-cancer agents. These drugs are potent inducers of double strand breaks in DNA, and can cause arrest in the cell cycle at the G2 stage, the latter occurring by disrupting the interaction between topoisomerase II and regulators of the cell cycle, such as Cdc2. Topoisomerase II inhibitors can cause a wide range of chromosomal aberrations, and can act by either stabilising topoisomerase II-DNA complexes that are easily cleaved, or by interfering with the catalytic activity of the enzyme, both resulting in double-strand breaks in the DNA.

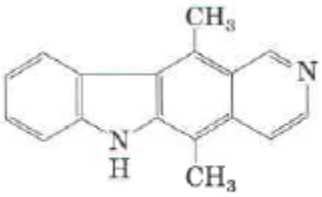
There are also dual inhibitors that target both topoisomerase I and II, which increases the potency of the anti-cancer effect. These drugs work by a variety of means: by recognising structural motifs present on both enzymes, by linking separate topoisomerase inhibitors together into a hybrid drug, or by using inhibitors that bind to and intercalate DNA



Doxorubicin



Etoposide



Ellipticine

Karpagam Academy of Higher Education
Department of Biochemistry
II BSc Biochemistry
Gene Organisation, Replication and Repair (16BCU401)

UNIT III- POSSIBLE QUESTIONS

2 Mark - Questions

1. Mention the role of telomerase
2. Give the role of topoisomerase in DNA replication
3. Initiation of eukaryotic replication
4. Add note on pre replicative complex
5. What are the classification of topoisomerase? Mention their role
6. List any two topoisomerase inhibitor and mention their role
7. Add note on supercoling of DNA
8. Define the terms Linking number, writhe and twist and their relationship
9. Differentiate the plectonemic and solenoidal coiling of DNA
10. List any four difference between the prokaryotic and eukaryotic replication

Essay type Questions (8 Marks)

1. Compare and contrast the eukaryotic and prokaryotic replication
2. Describe the events in initiation of DNA synthesis of eukaryotes
3. Discuss the termination process of the eukaryotic replication
4. Give a quick review on DNA synthesis in eukaryotes
5. Describe the inhibitors of DNA replication and its application in medicine
6. Describe the supercoiling of DNA and its importance
7. Describe the types and application of topoisomerase

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
II B.Sc BIOCHEMISTRY- Fourth Semester
GENE ORGANISATION, REPLICATION AND REPAII (16BCU401)
MULTIPLE CHOICE QUESTIONS
UNIT III

S.No	Unit	Questions	Option 1	Option 2	Option 3	Option 4	Answer
1	III	Unique linear DNA end structure present in eukaryptoea is	Centromere	Telomere	Anomer	Octamer	Telomere
2	III	Dna replication ineukaryotes occur at _____phase of cell cycle	M	S	G1	G2	S
3	III	Pre replication complex is formed during	M	S	G1	G2	G1
4	III	_____ protein involved in cell cycle control	cdc6 protein	Dna B	Dna A	Dna C	cdc6 protein
5	III	_____ complex is responsible for coordinating DNA replication in eukaryotes	Primosome	Replisome	Chromosome	centrosome	Replisome
6	III	An enzyme associated with primer synthesis in DNA replication is	Pol α	Pol δ	Pol β	Pol ε	Pol α
7	III	Enzyme involve din DNA leading stand synthesis in eukaryote is	Pol α	Pol δ	Pol β	Pol ε	Pol ε
8	III	Enzyme involved in lagging strand synthesis in eukaryote is	Pol α	Pol δ	Pol β	Pol ε	Pol δ
9	III	Length of Okazaki fragment in eukaryote is	1000	800	600	200	200
10	III	The replication protein A(RPA) function like	SSB	Helicase	topoisomerase	Ligase	SSB
11	III	Enzyme that remove RNA primer in eukaryote	RNase H	Rnase P	DNA helicase	DNase	RNase H
12	III	Processivity of DNA polymerase is increased by	ARS	RPA	PCNA	RFC	PCNA
13	III	Clamp loader in eukarotic replication is	ARS	RPA	PCNA	RFC	PCNA
14	III	PCNA loading function is achieved with the help of	ARS	RPA	PCNA	RFC	RFC
15	III	Catenane in eukaryote is removed by	Type II Topoisomerase	Type I Topoisomerase	Type IV Topoisomerase	Type III Topoisomerase	Type II Topoisomerase
16	III	Negative supercoils are removed by	Topoisomerase I	Gyrase	Helicase	Rep protein	Topoisomerase I
17	III	DNA chains differ from each other by one nucleotide can be revolved using	20% agarose	20% polyacrylamide	1% agarose	1% polyacrylamide	1% agarose
18	III	End replication in DNA is carried out by	Topoisomerase I	Gyrase	Helicase	Telomerase	Telomerase
19	III	Telemerase have inbuilt _____ enzyme activity	polymerase	Ligase	Reverse transcriptase	Helicase	Reverse transcriptase
20	III	In which phase of cell cycle damaged DNA replicaation errors are rectified	M	S	G1	G2	G2
21	III	Eukaryotic replication occur at	Cytoplasm	Nucleus	Golgi complex	Endoplasmic reticulum	Nucleus
22	III	_____ inhibit formation of functional nucleotides	Antimetabolites	Antibiotics	antisubstrates	antioxidants	Antimetabolites
23	III	Example for antifolate drug is	5 Fluoro uracil	6 mercapto purine	cytosine	Methotrexate	Methotrexate
24	III	Example for pyrimidine analogue is	5 Fluoro uracil	6 mercapto purine	cytosine	Methotrexate	5 Fluoro uracil
25	III	Example for purine analogue is	5 Fluoro uracil	6 mercapto purine	cytosine	Methotrexate	6 mercapto purine
26	III	Among the following which is chain elongation inhibitor	cytosine	Methotrexate	Arabinoside	lactose	Arabinoside
27	III	Antiviral drug that inhibit chain elongation is	Methotrexate	Dihydrofolate	Acyclovir	Viridin	Acyclovir
28	III	Among the following which is DNA polymerase inhibitor	Acyclovir	6 mercapto purine	Colchicine	Aphidicolin	Aphidicolin
29	III	DNA template damaging drugs cause all except	DNA adduct	DNA cross links	Dna strand breaks	Photolysis	Photolysis
30	III	Among the following which is DNA template damaging drug	DNA alkylating drug	DNA intercalating drug	DNA aromatisation drug	DNA acidifying drug	DNA alkylating drug
31	III	_____ topoisomerase inhibitors are commonly used as anticancer drug	Type I and II	Type I and III	Type IV and II	Type III and II	Type I and II
32	III	Topoisomerase I inbitor among the following is	Camphothecin	Etiopside	Anthra cyclin	Doxorubicin	Camphothecin
33	III	All are Topoisomerase II inhibitor except	Camphothecin	Etiopside	Anthra cyclin	Doxorubicin	Camphothecin
34	III	If there is no net bending in DNA , the DNA is said to be	Relaxed	Supercoiled	Negatively coiled	Postive coiled	Relaxed
35	III	Coiling of coil is known as	knotting	Catenane	Supercoiling	superwinding	Supercoiling
36	III	The algebric sum of all intersection of DNA structure is	Writhe	Twist	Linking number	Joining number	Linking number
37	III	Underwinding of DNA introduce ____ coil	Positive supercoil	Negatice supetrcoil	Neutral supercoil	No supercoil	Negatice supetrcoil
38	III	Overwinding of DNA introduce ____ coil	Positive supercoil	Negatice supetrcoil	Neutral supercoil	No supercoil	Positive supercoil
39	III	In Dna caompaction_____ coiling plays an important role	Splectonemic	Plectonemic	Telomeric	Metameric	Splectonemic
40	III	Solenoidal supercoiling can be adopted by ___ DNA	Under wound	Superwound	over wound	lowerwound	Under wound
41	III	_____ form of DNA is stable in solution	Splectonemic	Plectonemic	Telomeric	Metameric	Plectonemic
42	III	_____ coils are produced during DNA replication	Positive supercoil	Negatice supetrcoil	Neutral supercoil	No supercoil	Positive supercoil
43	III	Supercoiling in DNA is sensed and rectified by which enzyme	Topoisomerase	Gyrase	Helicase	Telomerase	Topoisomerase
44	III	In eukaryotes, _____ provides most efficient DNA compaction	Solenoid	Plectonemic	Teloid	Tetrad	Solenoid
45	III	Which of the following is the SMC protein	Condensin	Cohesin	Clathrin	Cadherin	Condensin
46	III	Condensation of sister chromatids and linkage to centromere is catalysed by	Condensin and Cohesin	Colchicine and Cohesin	Condensin and Colchicine	Condensin and Cadherin	Condensin and Cohesin
47	III	Regulation of supercoiling is achieved by	Topoisomerase	Gyrase	Helicase	Telomerase	Topoisomerase
48	III	Topological problem during DNA replication is mainly relieved by	Topoisomerase II	Gyrase	DNA Pol III	Telomerase	Topoisomerase II
49	III	One strand bteak is produced by	Topoisomerase I	Topoisomerase II	Topoisomerase III	Topoisomerase IV	Topoisomerase I
50	III	Double stand break is maily introduced by	Topoisomerase I	Topoisomerase II	Topoisomerase III	Topoisomerase IV	Topoisomerase II
51	III	The following are the functions of topoisomerase except	Strand breakage	Dna super coiling	Recombination	Repair	Repair
52	III	Topoisomerase inhibitor that inhibit bacterial replication is	Acycovir	Gangliovir	Statin	Coumarin	Coumarin

53	III	Topoisomerase inhibitor II cause cell cycle arrest at _____ stage	M	S	G1	G2	G2
54	III	Replication factor C is a complex of _____ proteins	5	3	2	6	**5**
55	III	Halting of replication fork is described as _____ replication fork	Halted	Stallled	Inhibited	blocked	Stallled
56	III	PCNA referes to	poly cell nuclear antigen	Proliferating cell nuclear antigen	Polymerase cell nuclear antigen		Proliferating cell nuclear antigen
57	III	Total number of helical turns in circular DNA under given condition is	Twist	Writhe	Linking number	Loading number	Twist
58	III	ARS refres to	Autosomal replicating sequence	Autonomously replicating sequences	Autonomous repairing sequence	Autonomous reading sequence	Autonomously replicating sequences
59	III	SMC referes to	Structural maintenance chromosome	Sequence maintenance chromosome	Similarity maintenance chromosome	Sister chromatid maintenance chromosome	Structural maintenance chromosome
60	III	ORC binds with orogin of replication during _____ phase of celll cycle	M	S	G1	G2	G1

UNIT-IV
SYLLABUS

Recombination and transposition of DNA

Homologous recombination, proteins and enzymes in recombination, site-specific recombination, serine and tyrosine recombinases, biological roles of site-specific recombination, transposition, three classes of transposable elements, importance of transposable elements in horizontal transfer of genes and evolution.

HOMOLOGOUS RECOMBINATION

Proteins and enzymes in recombination

Rec A protein

RecA protein is required for homologous recombination mediated by both the RecBCD and RecFOR pathways in E. coli. In addition to promoting genetic recombination, RecA plays an important role in the recombinational repair of DNA damage, and the induction of the SOS system in response to DNA damage. The *E. coli* RecA protein has multiple biochemical functions: it binds to both single- and double-stranded DNA, it promotes pairing and exchange of homologous DNA, it catalyzes ATP hydrolysis, and it promotes the proteolytic cleavage of several proteins including LexA, UmuD, and the lambda cI repressor. RecA is highly conserved between bacteria. Homologs of RecA have also been found in Archae (RadA and RadB), Eukarya (Rad51, Rad57, Rad55 and DMC1), and phage (e.g., the phage T4 UvsX protein). The RecA protein family website provides links to recA sequences from a variety of organisms.

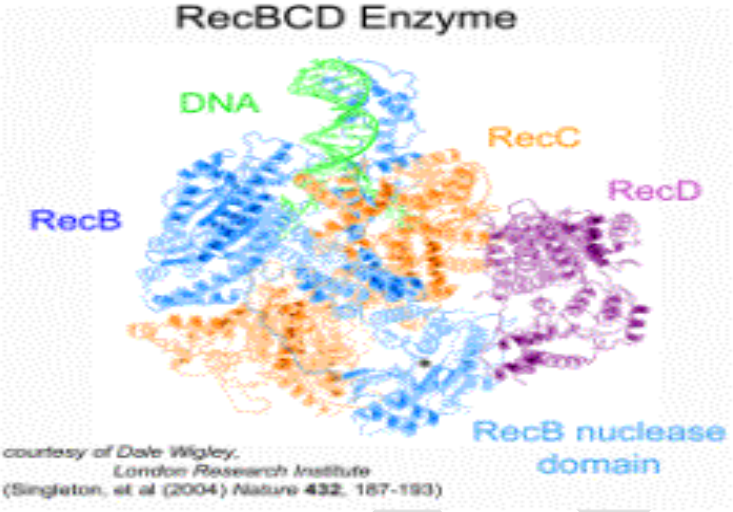
RecBCDEnzyme

RecBCD, also known as Exonuclease V, is an enzyme of the E. coli bacterium that initiates recombinational repair from potentially lethal double strand breaks in DNA which may result from ionizing radiation, replication errors, endonucleases, oxidative damage, and a host of other factors.[1] The RecBCD enzyme is both a helicase that unwinds, or separates the strands of, DNA and a nuclease that makes single-stranded nicks in DNA.

Structure

The enzyme complex is composed of three different subunits called RecB, RecC, and RecD and hence the complex is named RecBCD (Figure 1). Before the discovery of the *recD* gene,[3] the enzyme was known as "RecBC." Each subunit is encoded by a separate gene:

gene	chain	protein	Function
RecB	beta	P08394	3'-5' helicase, nuclease
RecC	gamma	P07648	Likely recognizes Chi (*c*rossover *h*otspot *i*nstigator)
RecD	alpha	P04993	5'-3' helicase



Function

Both the RecD and RecB subunits are helicases, i.e., energy-dependent molecular motors that unwind DNA (or RNA in the case of other proteins). The RecB subunit in addition has a nuclease function.[4] Finally, RecBCD enzyme (perhaps the RecC subunit) recognizes a specific sequence in DNA, 5'-GCTGGTGG-3', known as Chi (sometimes designated with the Greek letter χ).

RecBCD is unusual amongst helicases because it has two helicases that travel with different rates[5] and because it can recognize and be altered by the Chi DNA sequence.[6][7] RecBCD avidly binds an end of linear double-stranded (ds) DNA. The RecD helicase travels on the strand with a 5' end at which the enzyme initiates unwinding, and RecB on the strand with a 3' end. RecB is slower than RecD, so that a single-stranded (ss) DNA loop accumulates ahead of RecB (Figure 2). This produces DNA structures with two ss tails (a shorter 3' ended tail and a longer 5' ended tail) and one ss loop (on the 3' ended strand) observed by electron microscopy.[8] The ss tails can anneal to produce a second ss loop complementary to the first one; such twin-loop structures were initially referred to as "rabbit ears."

Holliday model for recombination

The widely accepted model for DNA crossover was first proposed by Robin Holliday in 1964. It involves several steps as illustrated in the following figure.

The Holliday model of DNA crossover.

(a) Two homologous DNA molecules line up (e.g., two nonsister chromatids line up during meiosis).

(b) Cuts in one strand of both DNAs.

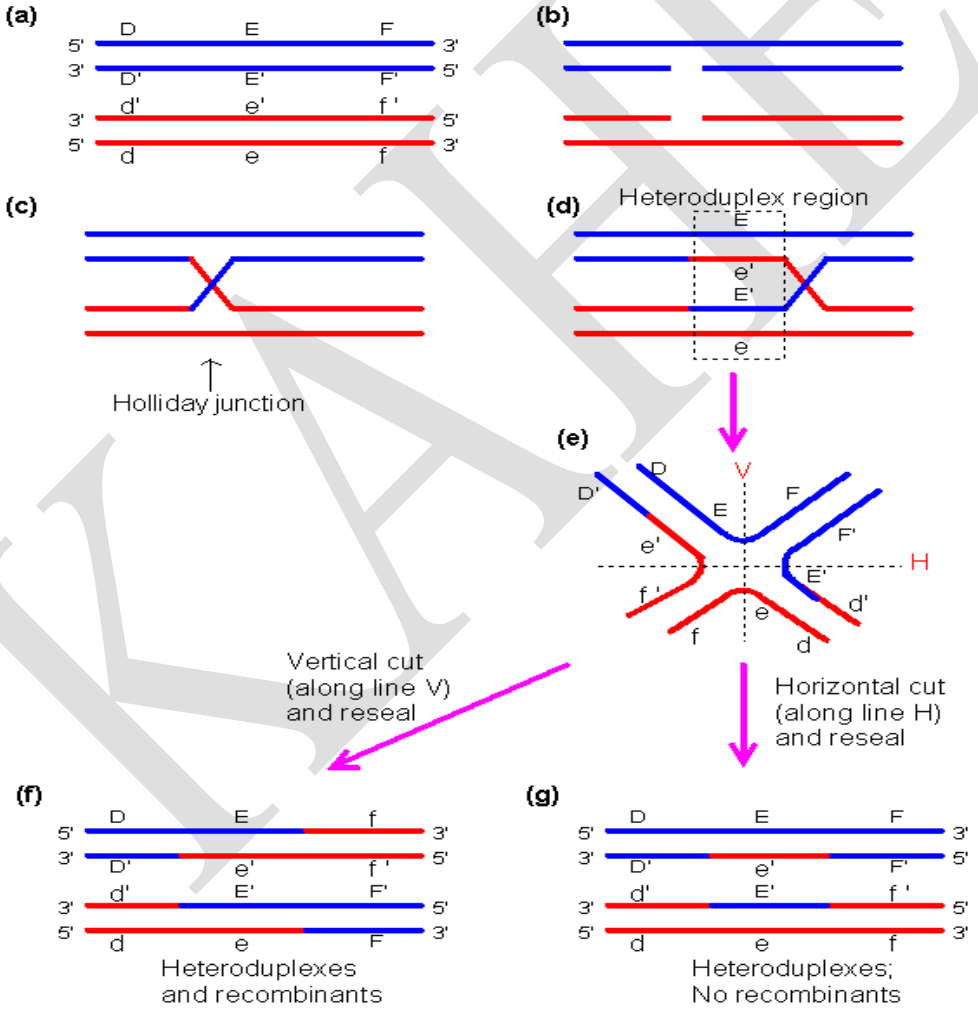
(c) The cut strands cross and join homologous strands, forming the Holliday structure (or Holliday junction).

(d) Heteroduplex region is formed by branch migration.

(e) Resolution of the Holliday structure. Figure 8-D-2e is a different view of the Holliday junction than Figure 8-D-2d. DNA strands may be cut along either the vertical line or horizontal line.

(f) The vertical cut will result in crossover between f-f' and F-F' regions. The heteroduplex region will eventually be corrected by mismatch repair.

(g) The horizontal cut does not lead to crossover after mismatch repair. However, it could cause gene conversion.



The detailed mechanism of homologous recombination was mainly obtained from the study of *E. coli*. Although bacteria do no undergo meiosis, homologous recombination could

occur during or immediately after DNA replication. It may also occur in a mating process called conjugation.

In *E.coli*, the recombination is initiated by the enzyme RecBCD, consisting of three subunits: RecB, RecC and RecD. This enzyme has both helicase and nuclease activities. The enzyme first uses its helicase activity to unwind DNA. When it hits the Chi site (with sequence GCTGGTGG), one of the exposed strand will be cut by its nuclease activity. The reason why this special site is called the "Chi site" is because the Greek letter (chi) looks like a crossover point. The Chi site is the position of the Holliday junction and also the position of chiasma.

After DNA strands are cut by RecBCD, the strand invasion is catalyzed by RecA proteins, which can wrap around single stranded DNA and direct it to form the Holliday structure.

Finally, the branch migration is catalyzed by RuvA and RuvB. The Holliday structure is resolved by the protein RuvC.

Meselson and Radding model

Meselson And Radding Model - The original Meselson and Radding model (1975) for the mechanisms of strand transfer bas been elaborated by Radding (1978). The mechanisms involved are strand displacement, uptake, loop cleavage, assimilation, isomerization and branch migration.

The Meselson-Radding model of meiotic recombination explains how the regions of heteroduplex DNA on both sides of the initial recombination event are not necessarily the same length, providing an advantage over the Holiday model of recombination.

Firstly, a nick is made in one strand of one of the chromosomes (in the following, each line represents a single strand of a double helix)

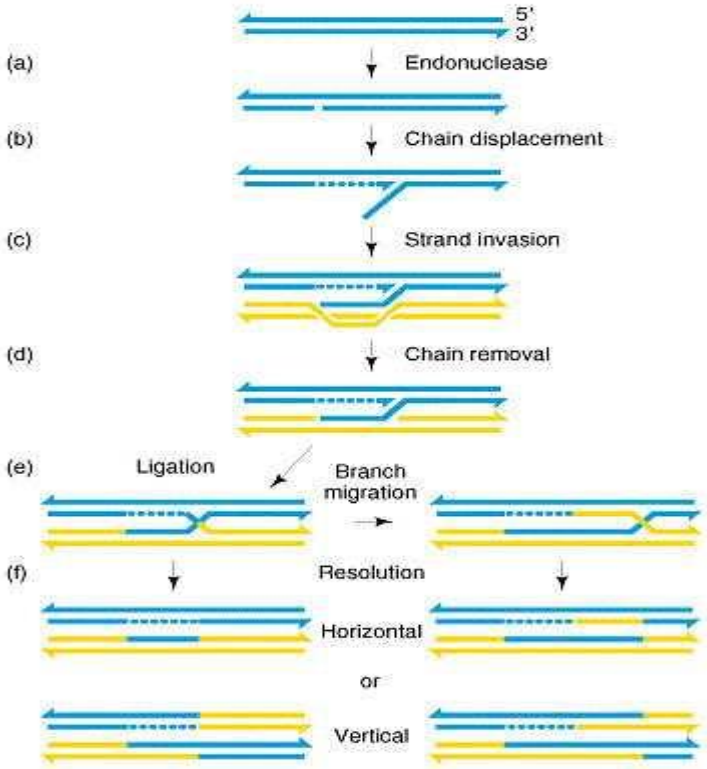
Secondly, a new strand is produced, displacing the original one on one side of the nick (the new strand is represented by c, but is identical to a)

Thirdly, the displaced a strand invades the other chromosome (this requires RecA)

Strand migration occurs

The junction may be resolved in one of two ways. One produces non-recombinant chromosomes with a heteroduplex region. The other produces recombinant chromosomes.

(Type 1 - non-recombinant)



SITE SPECIFIC RECOMBINATION

In general recombination, DNA rearrangements occur between DNA segments that are very similar in sequence. Although these rearrangements can result in the exchange of alleles between chromosomes, the order of the genes on the interacting chromosomes typically remains the same. A second type of recombination, called site-specific recombination, can alter gene order and also add new information to the genome. Site-specific recombination moves specialized nucleotide sequences, called *mobile genetic elements*, between nonhomologous sites within a genome. The movement can occur between two different positions in a single chromosome, as well as between two different chromosomes.

Mechanism

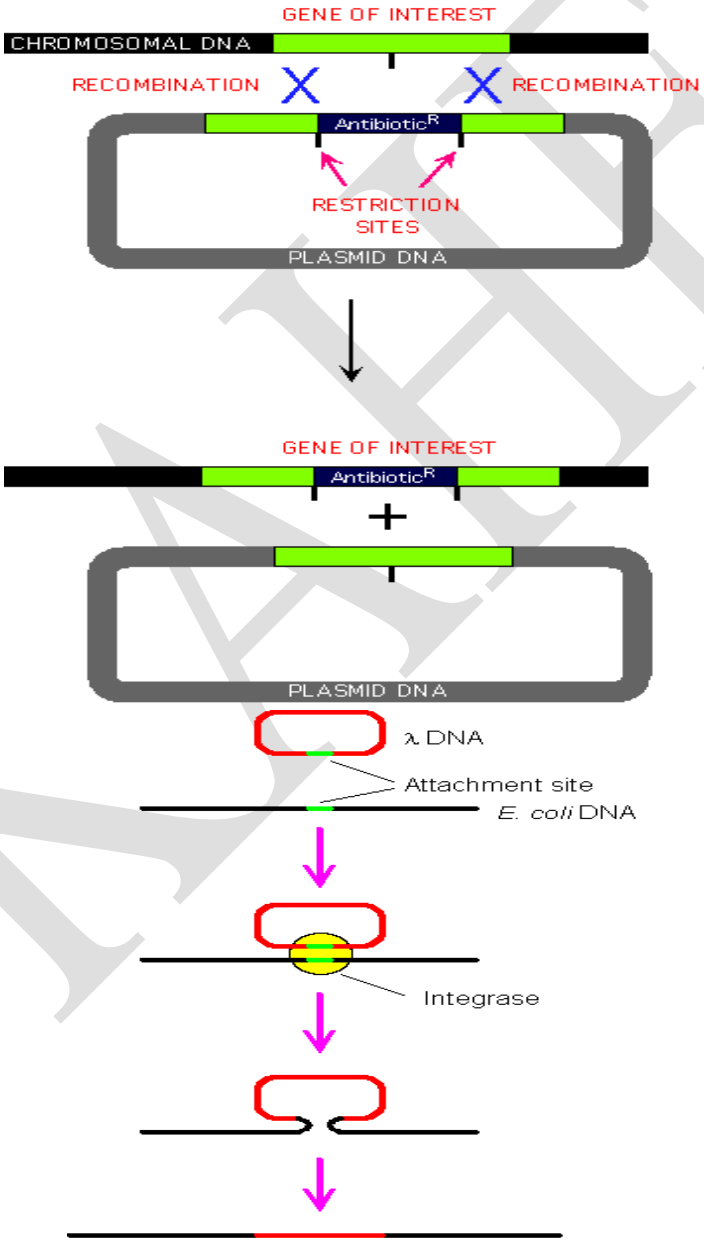
Site-specific recombination differs from general recombination in that short specific sequences which are required for the recombination, are the only sites at which recombination occurs. These reactions invariably require specialized proteins to recognize these sites and to catalyze the recombination reaction at these sites.

Site-specific recombination occurs at a specific DNA sequence. The first example was found in the integration between lambda DNA and E. coli DNA. Both of them contain a sequence, 5'-TTTATAC-3', called the attachment site, which allows the two DNA molecules to attach together by base pairing. Once attached, the enzyme integrase catalyzes two single strand

breaks as in the Holliday model. After a short branch migration, the integrase exerts a second strand cuts on two other strands. Resolution of two Holliday junctions completes the integration process.

The steps and features of the general recombination reaction, however, still apply:

- strand exchange
- formation of a Holliday intermediate
- branch migration
- resolution.



Using General Recombination in a Site-specific Event (biological role of site specific recombination)

Construction of interposon mutants

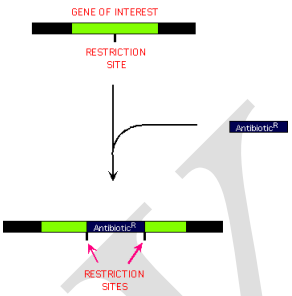
Whenever one is studying any particular gene, one invariably wants to know what happens if the gene cannot be expressed *in vivo*. To do this, one could use site-specific mutagenesis to place very specific base pair changes within a coding region. However, this is both difficult and requires a lot of information about the system to know exactly what sort of chnages must be made.

A much easier way to do this is simply to insert a large piece of DNA in the middle of the gene. This should block expression of the gene since transcription is unlikely to proceed all the way through the inserted DNA, and, even if it did, the mRNA that is made would not be translated into a functional protein.

If you're going to do this, then you need a way to know that the piece of DNA which you wish to insert really has been inserted. If the piece of DNA to be inserted codes for an antibiotic resistance, then you can select for its presence quite easily.

The following diagrams outline the procedure that is followed:

Cloning the antibiotic resistance piece of DNA into the middle of his gene was straightforward and only involved standard cloning techniques.



If we now transform or conjugate the plasmid into the bacterium of choice, at this point we have two copies of the target gene in the cell, but

- one of them has an antibiotic resistance element in the middle of it, and
- if, we are studying an organism other than *E. coli*, it is on a plasmid that cannot replicate.

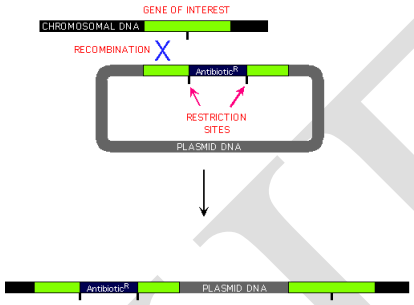
(Note that it is not always possible to transform bacteria with DNA. If transformation is not possible then conjugation or electroporation can be used to get DNA into the bacterium.)

Now if we select for cells that grow on media containing the antibiotic, they cannot do so unless the antibiotic resistance gene is maintained in the cell. However, if it is located on a plasmid that cannot replicate, it must integrate into the chromosome in order to be maintained and expressed.

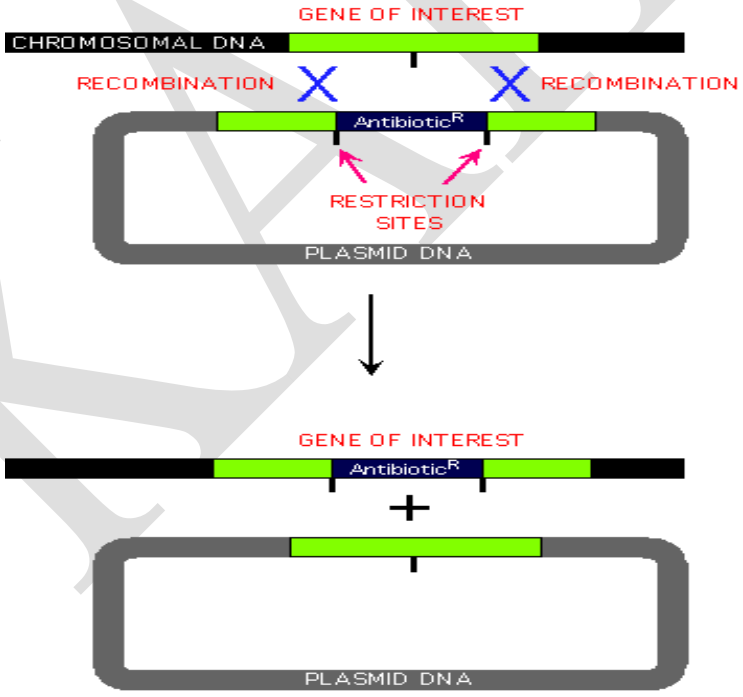
This can only happen if there is a region of homology in the plasmid which can recombine with the host chromosome.

There are, in fact, two such regions - one on either side of the antibiotic resistance gene, i.e. the two halves of the original target gene.

A single recombination on one side only will result in the integration of the whole plasmid containing the interrupted target gene. This would leave us with two copies of the RNA-binding protein gene in the cell:



Recombination on both sides would get rid of the wild-type copy of the gene. However, this can be difficult to find unless specially designed plasmid vectors are used that force the second recombination event to take place.



So this Interposon mutagenesis requires a site-specific recombination event in the sense that the recombination events must occur only in the range of places determined by a cloned piece of

DNA. However, the mechanism by which this recombination takes place is still general homologous recombination.

SERINE AND TYROSINE RECOMBINASE

Based on amino acid sequence homology and mechanistic relatedness most site-specific recombinases are grouped into one of two families:

the tyrosine recombinase family

the serine recombinase family.

The names stem from the conserved nucleophilic amino acid residue that they use to attack the DNA and which becomes covalently linked to it during strand exchange. Early members of the serine recombinase family were known as resolvase / DNA invertases, while the founding member of the tyrosine recombinases, lambda- integrase, using attP/B recognition sites) differs from the now well known enzymes such as Cre (from the P1 phage) and FLP (from yeast *S. cerevisiae*) while famous serine recombinases include enzymes such as: gamma-delta resolvase (from the Tn*1000* transposon), Tn3 resolvase (from the Tn3 transposon) and *φ*C31 integrase (from the *φ*C31 phage).

Although the individual members of the two recombinase families can perform reactions with same practical outcomes, the two families are unrelated to each other, having different protein structures and reaction mechanisms. Unlike tyrosine recombinases, serine recombinases are highly modular as was first hinted by biochemical studies, and later shown by crystallographic structures Knowledge of these protein structures could prove useful when attempting to reengineer recombinase proteins as tools for genetic manipulation

Mechanism

Although the basic chemical reaction is the same for both tyrosine and serine recombinases, there are marked differences. Tyrosine recombinases, such as Cre or Flp, cleave one DNA strand at a time at points that are staggered by 6-8bp, linking the 3’ end of the strand to the hydroxyl group of the tyrosine nucleophile. Strand exchange then proceeds via a crossed strand intermediate analogous to the Holliday junction in which only one pair of strands has been exchanged

The mechanism and control of serine recombinases is much less well understood. This group of enzymes was only discovered in the mid-1990s and is still relatively small. The now classical members gamma-delta and Tn3 resolvase, but also new additions like φC31-, Bxb1-, and R4 integrases, cut all four DNA strands simultaneously at points that are staggered by 2bp. During cleavage, a protein-DNA bond is formed via a transesterification reaction in which a phosphodiester bond is replaced by a phosphoserine bond between a 5’ phosphate at the cleavage site and the hydroxyl group of the conserved serine residue (S10 in resolvase).

It is still not entirely clear how the strand exchange occurs after the DNA has been cleaved. However, it has been shown that the strands are exchanged while covalently linked to the protein, with a resulting net rotation of 180°.The most quoted (but not the only) model accounting for these facts is the "subunit rotation model". Independent of the model, DNA duplexes are situated outside of the protein complex, and large movement of the protein is needed to achieve the strand exchange. In this case the recombination sites are slightly asymmetric, which allows the enzyme to tell apart the left and right ends of the site. When generating products, left ends are always joined to the right ends of their partner sites, and vice versa. This causes different recombination hybrid sites to be reconstituted in the recombination products. Joining of left ends to left or right to right is avoided due to the asymmetric "overlap" sequence between the staggered points of top and bottom strand exchange, which is in stark contrast to the mechanism employed by tyrosine recombinases

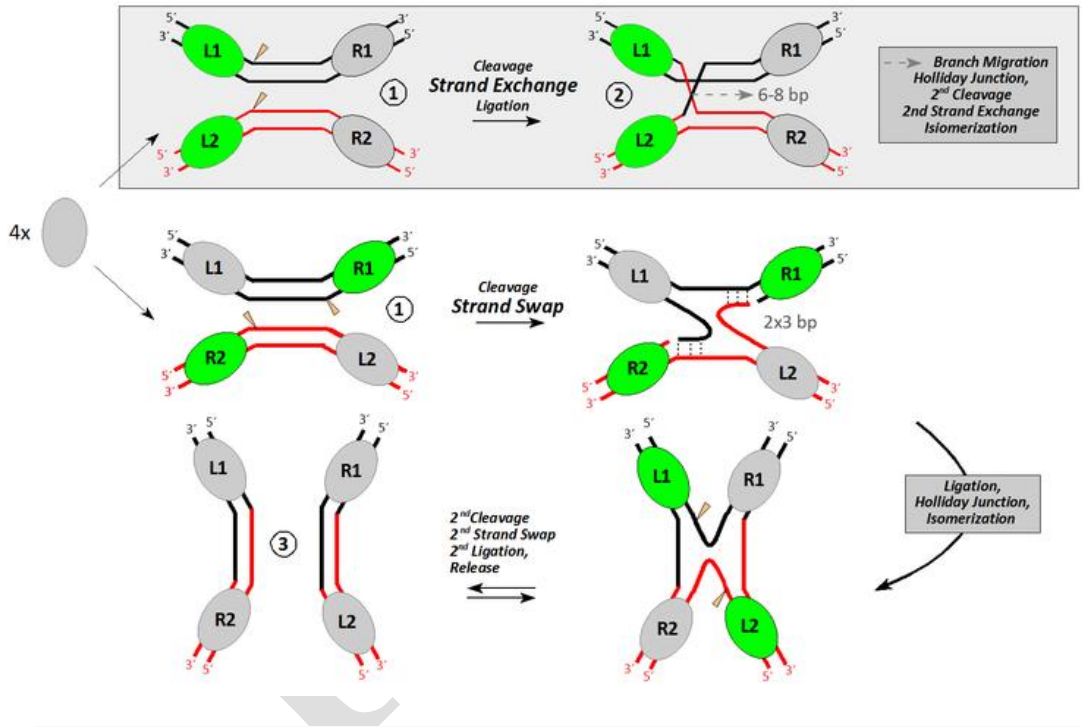


Fig: **Tyr-Recombinases: Details of the crossover step** Top: Traditional view including strand-exchange followed by branch-migration (proofreading). The mechanism occurs in the framework of a synaptic complex (1) including both DNA-sites in parallel orientation. While branch-migration explains the specific homology requirements and the reversibility of the process in a straightforward manner, it could not be reconciled with the motions recombinase subunits have to undergo in three dimensions. Bottom: Current view. Two simultaneous strand-swaps, each depending on the complementarity of three successive bases at (or close to) the edges of the 8 bp spacer (dashed lines indicate base-pairing). Didactic complications arise from the fact that, in

this model, the synaptic complex must accommodate both substrates in an anti-parallel orientation. This synaptic complex (1) arises from the association of two individual recombinase subunits ("protomers"; gray ovals) with the respective target site. Its formation depends on inter-protomer contacts and DNA bending, which in turn define the subunits (green) with an active role during the first crossover reaction. Both representations illustrate only one half of the respective pathway. These parts are separated by a Holliday-junction/isomerization step before the product (3) can be released

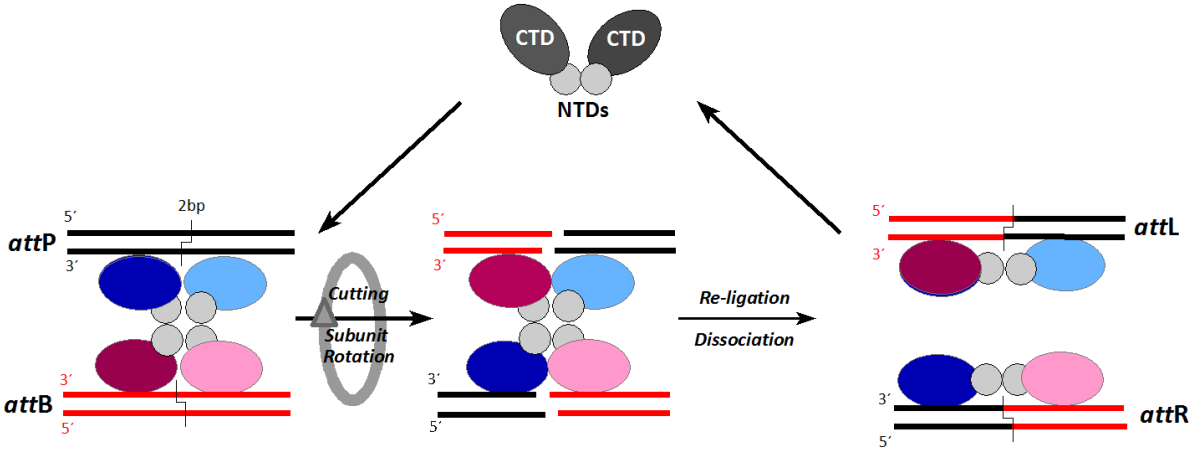


Fig: Ser-Recombinases: The (essentially irreversible) subunit-rotation pathway. Contrary to Tyr-recombinases, the four participating DNA-strands are cut in synchrony at points staggered by only 2 bp (leaving little room for proofreading). Subunit-rotation (180°) permits the exchange of strands while covalently linked to the protein partner. The intermediate exposure of double-strand breaks bears risks of triggering illegitimate recombination and thereby secondary reactions. Here, the synaptic complex arises from the association of pre-formed recombinase dimers with the respective target sites (CTD/NTD, C-/N-terminal domain). Like for Tyr-recombinases, each site contains two arms, each accommodating one protomer. As both arms are structured slightly differently, the subunits become conformationally tuned and thereby prepared for their respective role in the recombination cycle. Contrary to members of the Tyr-class the recombination pathway converts two different substrate sites (attP and attB) to site-hybrids (attL and attR). This explains the irreversible nature of this particular recombination pathway, which can only be overcome by auxiliary "recombination directionality factors" (RDFs).

Applications of site specific recombination

mechanism of DNA recombination can be utilized in different biological contexts to bring about integration, excision (deletion) and inversion of DNA segments.

The reaction catalysed, for instance, by Cre-recombinase may lead to excision of the DNA segment flanked by the two sites (Fig. A), but may also lead to integration or inversion of the orientation of the flanked DNA segment (Fig. B). What the outcome of the reaction will be is dictated mainly by the relative location and the orientation of sites that are to be recombined, but also by the innate specificity of the site-specific system in question. Excisions and inversions occur if the recombination takes place between two sites that are found on the same molecule (intramolecular recombination), and if the sites are in the same (direct repeat) or in an opposite orientation (inverted repeat), respectively. Insertions, on the other hand, take place if the recombination occurs on sites that are situated on two different DNA molecules (intermolecular recombination), provided that at least one of these molecules is circular. Most site-specific systems are highly specialised, catalysing only one of these different types of reaction, and have evolved to ignore the sites that are in the 'wrong' orientation.

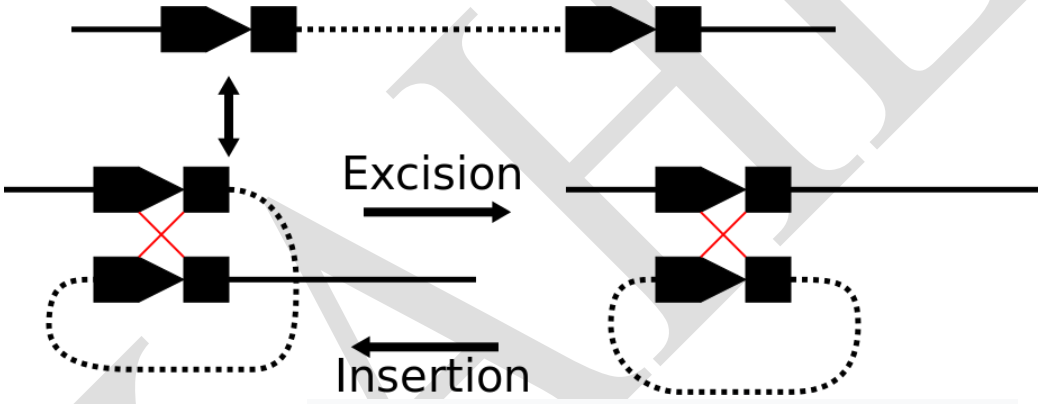


Fig A: *Reversible insertion and excision by the Cre-lox system*

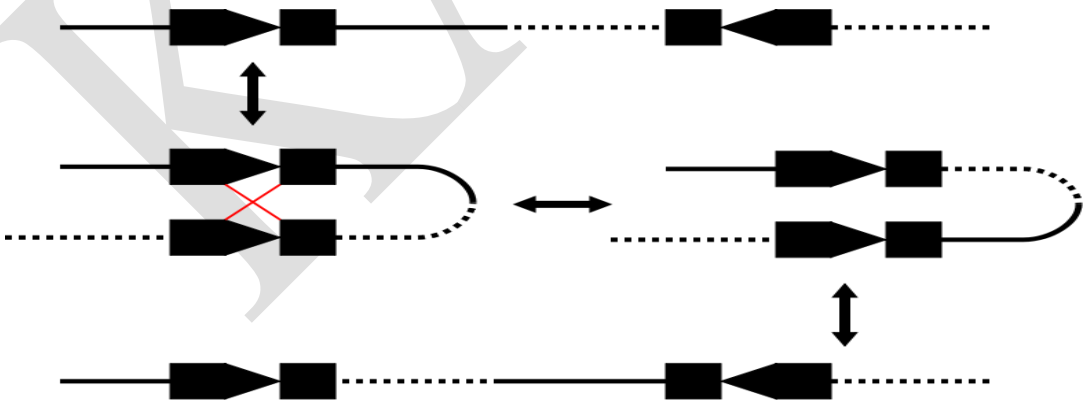


Fig B : *Inversion by the Cre-lox system*

Since Cre and Flp have extremely simple reaction requirements, these recombination systems have been reconstituted in a variety of organisms-bacteria, fungi, plants, nematodes, flies and

animals. Cre and Flp can be placed under regulatable promoters for conditional or tissuespecific expression. An important first step in applying site-specific recombination in a genetic context of interest is the introduction of the target site or sites at the desired locale(s). Once this has been accomplished, the rest of the experimental steps are quite straightforward

1. One of the most useful applications of site-specific recombination in basic biology has been in tracking the lineage of cells during development.
2. **2.** One can also delete a particular gene at a given stage of development, and follow the consequence of the deletion during further development
3. One can induce the expression of a gene at a desired point in development via site-specific recombination.

Transposition

Transposition is the process by which genetic elements move between different locations of the genome, whereas site-specific recombination is a reaction in which DNA strands are broken and exchanged at precise positions of two target DNA loci to achieve determined biological function. Both types of recombination are represented by diverse genetic systems which generally encode their own recombination enzymes. These enzymes, generically called transposases and site-specific recombinases, can be grouped into several families on the basis of amino acid sequence similarities, which, in some cases, are limited to a signature of a few residues involved in catalysis. The well characterized site-specific recombinases are found to belong to two distinct groups, whereas the transposases form a large super-family of enzymes encompassing recombinases from both prokaryotes and eukaryotes. In spite of important differences in the catalytic mechanisms used by these three classes of enzymes to cut and rejoin DNA molecules, similar strategies are used to coordinate the biochemical steps of the recombination reaction and to control its outcome.

Although transposition and site-specific recombination seem to be fundamentally distinct processes that often have very different biological outcomes, increasing evidence indicates that they are related in many ways. In general recombination, genetic material is exchanged through a cascade of events involving multiple proteins assembled in different enzymatic complexes. In contrast, both categories of specialised recombination systems utilise relatively simple recombination machineries in which one (or sometimes two) enzyme, generically referred to as the transposase and site-specific recombinase, catalyses the essential DNA breakage and joining reactions. These proteins act at specific DNA sequences which are characteristic for each genetic element.

Mobile DNA elements (transposons)

- Mobile genetic elements range in size from a few hundred to tens of thousands of nucleotide pairs, and they have been identified in virtually all cells that have been examined. Some of these elements are viruses in which site-specific recombination is used to move their genomes into and out of the chromosomes of their host cell.

- It is the second type of repetitious DNA in eukaryotic genomes, termed interspersed repeats (also known as moderately repeated DNA, or intermediate-repeat DNA).
- They are composed of a very large number of copies of relatively few sequence families.
- These sequences, which are interspersed throughout mammalian genomes, make up ≈25–50 percent of mammalian DNA (≈45 percent of human DNA).
- Because moderately repeated DNA sequences have the unique ability to "move" in the genome, they are called **mobile DNA elements** (or transposable elements).
- Although mobile DNA elements, ranging from hundreds to a few thousand base pairs in length, originally were discovered in eukaryotes, they also are found in prokaryotes.
    - The process by which these sequences are copied and inserted into a new site in the genome is called **transposition.**
- Mobile DNA elements (or simply mobile elements) are essentially molecular symbionts and have no specific function in the biology of their host organisms, but exist only to maintain themselves.
- For this reason, Francis Crick referred to them as "selfish DNA."
- When transposition of eukaryotic mobile elements occurs in germ cells, the transposed sequences at their new sites can be passed on to succeeding generations.
- In this way, mobile elements have multiplied and slowly accumulated in eukaryotic genomes over evolutionary time.
- Since mobile elements are eliminated from eukaryotic genomes very slowly, they now constitute a significant portion of the genomes of many eukaryotes.
- Transposition also may occur within a somatic cell; in this case the transposed sequence is transmitted only to the daughter cells derived from that cell.
- In rare cases, this may lead to a somatic-cell mutation with detrimental phenotypic effects, for example, the inactivation of a tumor suppressor gene.
- In addition to moving themselves, all types of mobile genetic elements occasionally move or rearrange neighboring DNA sequences of the host cell genome. These movements can cause deletions of adjacent nucleotide sequences, for example, or can carry these sequences to another site. **In this way, site-specific recombination, like general recombination, produces many of the genetic variants upon which evolution depends.**
- The translocation of mobile genetic elements gives rise to spontaneous mutations in a large range of organisms including humans; in some, such as the fruit fly *Drosophila*, these elements are known to produce most of the mutations observed. Over time, site-specific recombination has thereby been responsible for a large fraction of the important evolutionary changes in genomes.

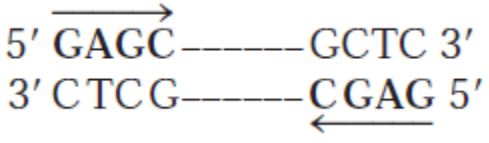
Mobile Genetic Elements Can Move by Either Transpositional or Conservative Mechanism

Unlike general recombination, site-specific recombination is guided by recombination enzymes that recognize short, specific nucleotide sequences present on one or both of the recombining DNA molecules. Extensive DNA homology is not required for a recombination event. Each type of mobile element generally encodes the enzyme that mediates its own movement and contains special sites upon which the enzyme acts. Many elements also carry other genes. For example, viruses encode coat proteins that enable them to exist outside cells, as well as essential viral enzymes. The spread of mobile elements that carry antibiotic resistance genes is a major factor underlying the widespread dissemination of antibiotic resistance in bacterial populations.

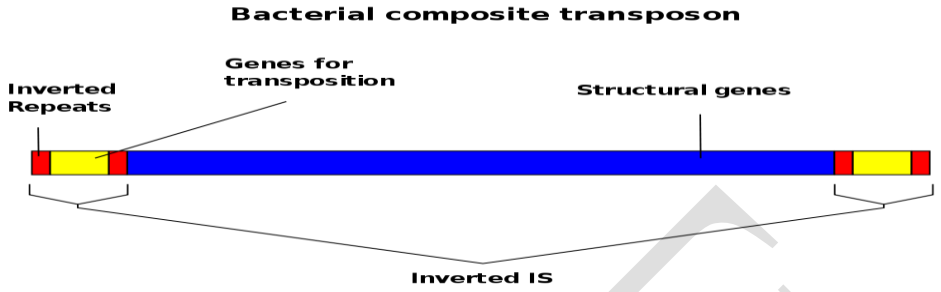
Major types of mobile elements

Bacterial Insertion Sequences

- Certain *E.coli* mutations caused by the spontaneous insertion of a DNA sequence, ≈1–2 kb long, into the middle of a gene. these inserted stretches of DNA are called *insertion sequences*, or *IS elements*.
- So far, more than 20 different IS elements have been found in *E. coli* and other bacteria.
- Transposition of an IS element is a very rare event, occurring in only one in 10^5–10^7 cells per generation, depending on the IS element.
- Many transpositions inactivate essential genes, killing the host cell and the IS elements it carries.
- Therefore, higher rates of transposition would probably result in too great a mutation rate for the host cell to survive.
- However, since IS elements transpose more or less randomly, some transposed sequences enter nonessential regions of the genome (e.g., regions between genes), allowing the cell to survive.
- At a very low rate of transposition, most host cells survive and therefore propagate the symbiotic IS element.
- IS elements also can insert into plasmids or lysogenic viruses, and thus be transferred to other cells. When this happens, IS elements can transpose into the chromosomes of virgin cells.
- An inverted repeat, usually containing ≈50 base pairs, invariably is present at each end of an insertion sequence. In an inverted repeat the 5'→ 3'sequence on one strand is repeated on the other strand, as:



Between the inverted repeats is a region that encodes transposase, an enzyme required for transposition of the IS elements



The following is the figure of common mobile genetic element found in bacteria(IS3, Tn3, Tn10)

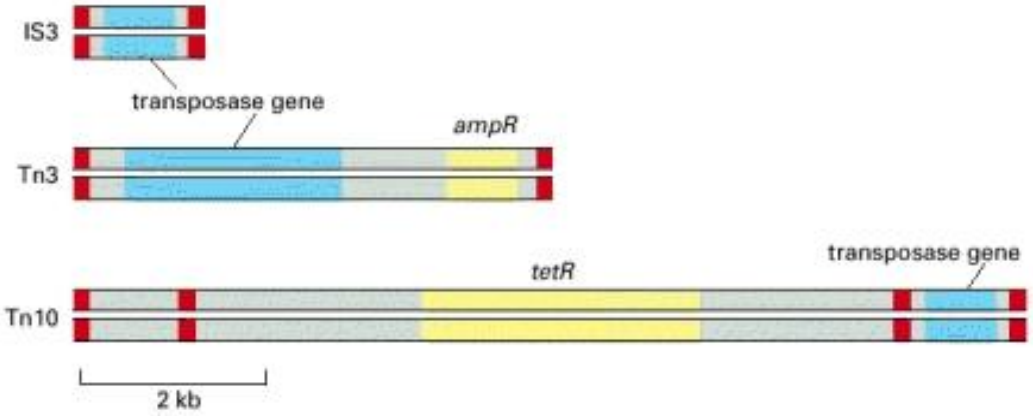


Figure : Three of the many types of mobile genetic elements found in bacteria

Each of these DNA elements contains a gene that encodes a *transposase*, an enzyme that conducts at least some of the DNA breakage and joining reactions needed for the element to move. Each mobile element also carries short DNA sequences (indicated in *red)* that are recognized only by the transposase encoded by that element and are necessary for movement of the element. In addition, two of the three mobile elements shown carry genes that encode enzymes that inactivate the antibiotics ampicillin *(ampR)* and tetracycline *(tetR).* The transposable element Tn10, shown in the bottom diagram, is thought to have evolved from the chance landing of two short mobile elements on either side of a tetracyclin-resistance gene; the wide use of tetracycline as an antibiotic has aided the spread of this gene through bacterial populations. The three mobile elements shown are all examples of *DNA-only transposons.*

Mechanism of transposition

Transpositional site-specific recombination can proceed via either of two distinct mechanisms, each of which requires specialized recombination enzymes and specific DNA sites. (1) **Transpositional site-specific recombination** usually involves breakage reactions at the ends of the mobile DNA segments embedded in chromosomes and the attachment of those ends at one of many different nonhomologous target DNA sites. It does not involve the formation of heteroduplex DNA. (2) **Conservative site-specific recombination** involves the production of a very short heteroduplex joint, and it therefore requires a short DNA sequence that is the same on both donor and recipient DNA molecules

Transpositional Site-specific Recombination Can Insert Mobile Genetic Elements into Any DNA sequence

Transposons, also called transposable elements, are mobile genetic elements that generally have only modest target site selectivity and can thus insert themselves into many different DNA sites. In transposition, a specific enzyme, usually encoded by the transposon and called a *transposase*, acts on a specific DNA sequence at each end of the transposon—first disconnecting it from the flanking DNA and then inserting it into a new target DNA site. There is no requirement for homology between the ends of the element and the insertion site.

Most transposons move only very rarely (once in 10^5 cell generations for many elements in bacteria), and for this reason it is often difficult to distinguish them from nonmobile parts of the chromosome. In most cases, it is not known what suddenly triggers their movement.

On the basis of their structure and transposition mechanisms, transposons can be grouped into three large classes (Table). Those in the first two of these classes use virtually identical DNA breakage and DNA joining reactions to translocate. However, for the *DNA-only transposons*,the mobile element exists as DNA throughout its life cycle: the translocating DNA segment is directly cut out of the donor DNA and joined to the target site by a transposase.

In contrast, *retroviral-like retrotransposons* move by a less direct mechanism. An RNA polymerase first transcribes the DNA sequence of the mobile element into RNA. The enzyme reverse transcriptase then transcribes this RNA molecule back into DNA using the RNA as a template, and it is this DNA copy that is finally inserted into a new site in the genome. For historical reasons, the transposase-like enzyme that catalyzes this insertion reaction is called an *integrase* rather than a transposase.

The third type of transposon in Table also moves by making a DNA copy of an RNA molecule that is transcribed from it. However, the mechanism involved for these *nonretroviral*

retrotransposons is distinct from that just described in that the RNA molecule is directly involved in the transposition reaction.

CLASS DESCRIPTION AND STRUCTURE	GENES IN COMPLETE ELEMENT	MODE OF MOVEMENT	EXAMPLES
DNA-only transposons			
short inverted repeats at each end	encodes transposase	moves as DNA, either excising or following a replicative pathway	P element (*Drosophila*) Ac-Ds (maize) Tn3 and IS1 (*E.coli*) Tam3 (snapdragon)
Retroviral-like retrotransposons			
directly repeated long terminal repeats (LTRs) at ends	encodes reverse transcriptase and resembles retrovirus	moves via an RNA intermediate produced by promoter in LTR	Copia (*Drosophila*) Ty1 (yeast) THE-1 (human) Bs1 (maize)
Nonretroviral retrotransposons			
Poly A at 3′ end of RNA transcript; 5′ end is often truncated	encodes reverse transcriptase	moves via an RNA intermediate that is often produced from a neighboring promotor	F element (*Drosophila*) L1 (human) Cin4 (maize)

These elements range in length from 1000 to about 12,000 nucleotide pairs; each family contains many members, only a few of which are listed here. In addition to transposable elements, there are selected viruses that can move in and out of host cell chromosomes; these viruses are related to the first two classes of transposons.

DNA-only Transposons Move By DNA Breakage and Joining Mechanisms

Many DNA-only transposons move from a donor site to a target site by cut-and-paste transposition, using the mechanism outlined. Each subunit of a transposase recognizes the same specific DNA sequence at an end of the element; the joining together of these two subunits to form a dimeric transposase creates a DNA loop that brings the two ends of the element together. The transposase then introduces cuts at both ends of this DNA loop to expose the element termini and remove the element completely from its original chromosome complete the reaction, the transposase catalyses a direct attack of the element's two DNA termini on a target DNA molecule, breaking two phosphodiester bonds in the target molecule as it joins the element and target DNAs together

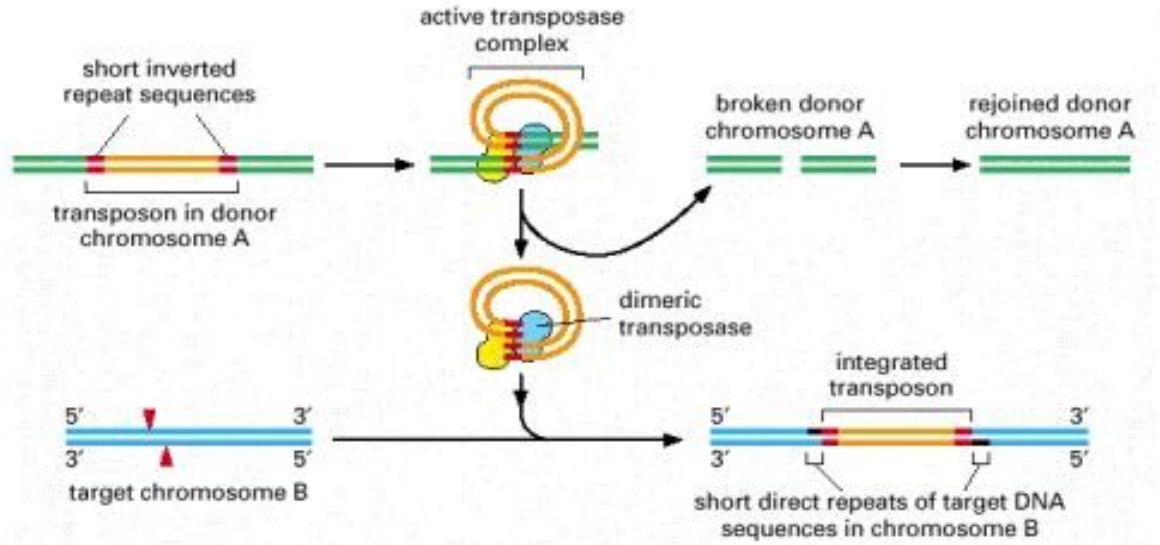


Figure : Cut-and-paste transposition

DNA-only transposons can be recognized in chromosomes by the "inverted repeat DNA sequences" *(red)* at their ends. Experiments show that these sequences, which can be as short as 20 nucleotides, are all that is necessary for the DNA between them to be transposed by the particular transposase enzyme associated with the element. The cut-and-paste movement of a DNA-only transposable element from one chromosomal site to another begins when the transposase brings the two inverted DNA sequences together, forming a DNA loop. Insertion into the target chromosome, catalyzed by the transposase, occurs at a random site through the creation of staggered breaks in the target chromosome *(red arrowheads).* As a result, the insertion site is marked by a short direct repeat of the target DNA sequence, as shown. Although the break in the donor chromosome *(green)* is resealed, the breakage-and-repair process often alters the DNA sequence, causing a mutation at the original site of the excised transposable element

Because the breaks made in the two target DNA strands are staggered *(red arrowheads* in), two short, single-stranded gaps are initially formed in the product DNA molecule, one at each end of the inserted transposon. These gaps are filled-in by a host cell DNA polymerase and DNA ligase to complete the recombination process, producing a short duplication of the adjacent target DNA sequence. These flanking direct repeat sequences, whose length is different for different transposons, serve as convenient markers of a prior transpositional site-specific recombination event.

When a cut-and-paste DNA-only transposon is excised from the donor chromosome, a double-strand break is created in the vacated chromosome. This break can be perfectly "healed" by

a homologous end-joining reaction. Alternatively, the break can be resealed by a nonhomologous end-joining reaction; in this case, the DNA sequence that flanked the transposon is often altered, producing a mutation at the chromosomal site from which the transposon was excised (see Figure).

Some DNA-only transposons move using a variation of the cut-and-paste mechanism called *replicative transposition*. In this case, the transposon DNA is replicated and a copy is inserted at a new chromosomal site, leaving the original chromosome intact. Although the mechanism used is more complex, it is closely related to the cut-and-paste mechanism just described; indeed, some transposons can move by either pathway.

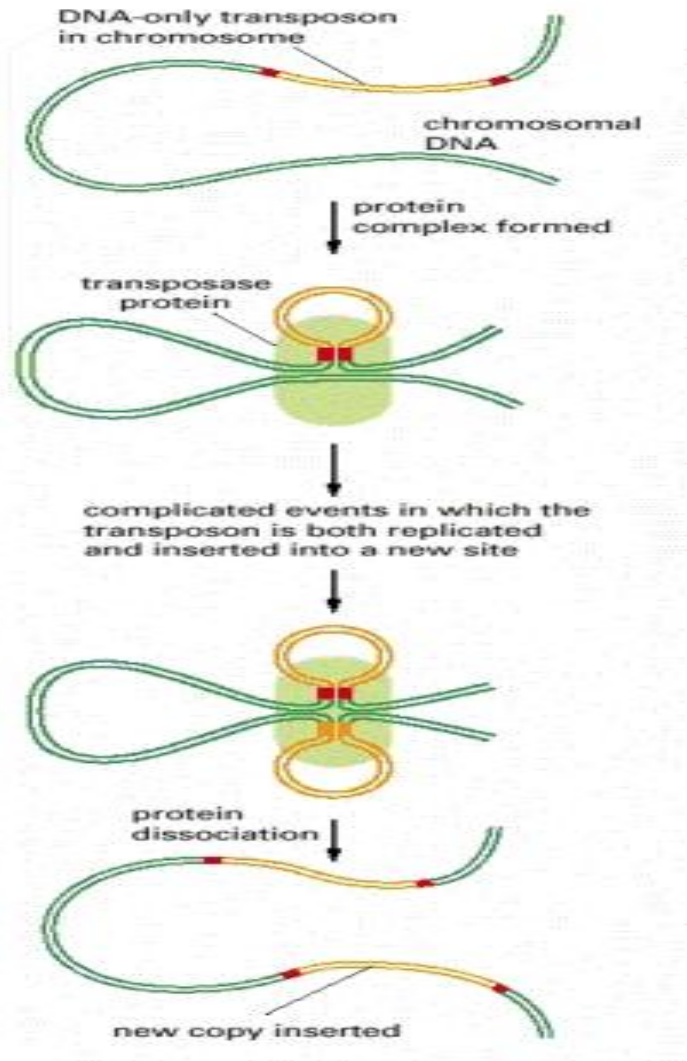


Figure : Replicative transposition

In the course of replicative transposition, the DNA sequence of the transposon is copied by DNA replication. The end products are a DNA moleculethat is identical to the original donor and a target DNA molecule that has a transposon inserted into it. In general, a particular DNA-only transposonmoves either by the cut-and-paste or by the replicative pathway outlined here. However, the two mechanisms have many enzymatic similarities, and a few transposons can move by either pathway

Some Viruses Use Transpositional Site-specific Recombination to Move Themselves into host cell chromosomes

Certain viruses are considered mobile genetic elements because they use transposition mechanisms to integrate their genomes into that of their host cell. However, these viruses also encode proteins that package their genetic information into virus particles that can infect other cells. Many of the viruses that insert themselves into a host chromosome do so by employing one of the first two mechanisms listed in Table 5-3. Indeed, much of our knowledge of these mechanisms has come from studies of particular viruses that employ them.

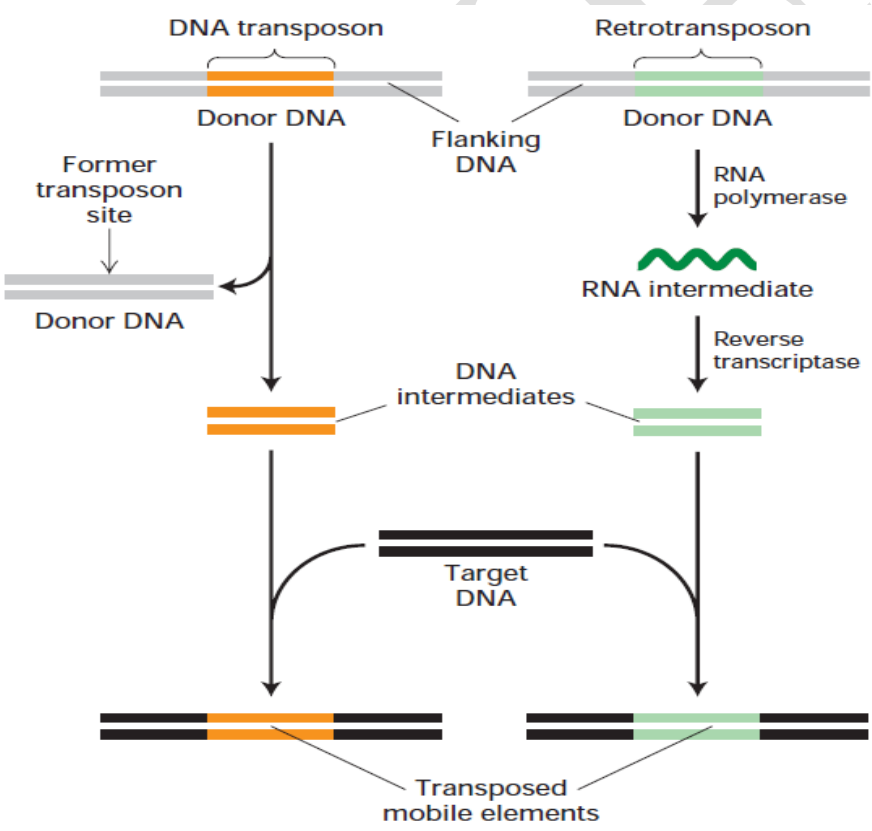
A virus that infects a bacterium is known as a bacteriophage. The *bacteriophage Mu* not only uses DNA-based transposition to integrate its genome into its host cell chromosome, it also uses the transposition process to initiate its viral DNA replication. The Mu transposase was the first to be purified in active form and characterized; it recognizes the sites of recombination at each end of the viral DNA by binding specifically to this DNA, and closely resembles the transposases just described.

Transposition also has a key role in the life cycle of many other viruses. Most notable are the **retroviruses**, which include the AIDS virus, called HIV, that infects human cells. Outside the cell, a retrovirus exists as a single-stranded RNA genome packed into a protein capsid along with a virus-encoded reverse transcriptase enzyme. During the infection process, the viral RNA enters a cell and is converted to a double-stranded DNA molecule by the action of this crucial enzyme, which is able to polymerize DNA on either an RNA or a DNA template (Figures 5-73 and 5-74). The term *retrovirus* refers to the fact that these viruses reverse the usual flow of genetic information, which is from DNA to RNA (see Figure).

Movement of mobile elements

- Barbara McClintock discovered the first mobile elements in maize (corn) during the 1940s.
- She characterized genetic entities that could move into and back out of genes, changing the phenotype of corn kernels.
- Her theories were very controversial until similar mobile elements were discovered in bacteria, where they were characterized as specific DNA sequences, and the molecular basis of their transposition was deciphered.

- Mobile elements are classified into two categories: (1) those that transpose directly
- as DNA and (2) those that transpose via an RNA intermediate transcribed from the mobile element by an RNA polymerase and then converted back into double-stranded DNA by a **reverse transcriptase** .
- Mobile elements that transpose through a DNA intermediate are generally referred to as **DNA transposons.**
- Mobile elements that transpose to new sites in the genome via an RNA intermediate are called **retrotransposons** because their movement is analogous to the infectious process of retroviruses. Indeed, retroviruses can be thought of as retrotransposons that evolved genes encoding viral coats, thus allowing them to transpose between cells.

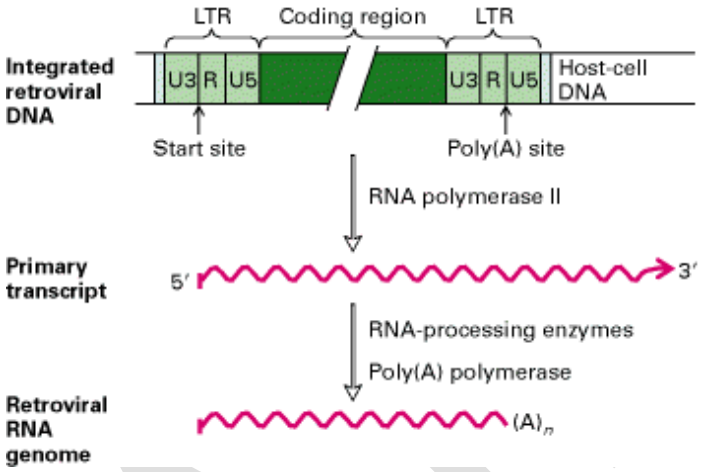


The general structure of viral retrotransposons found in eukaryotes

General structure of eukaryotic viral retrotransposon

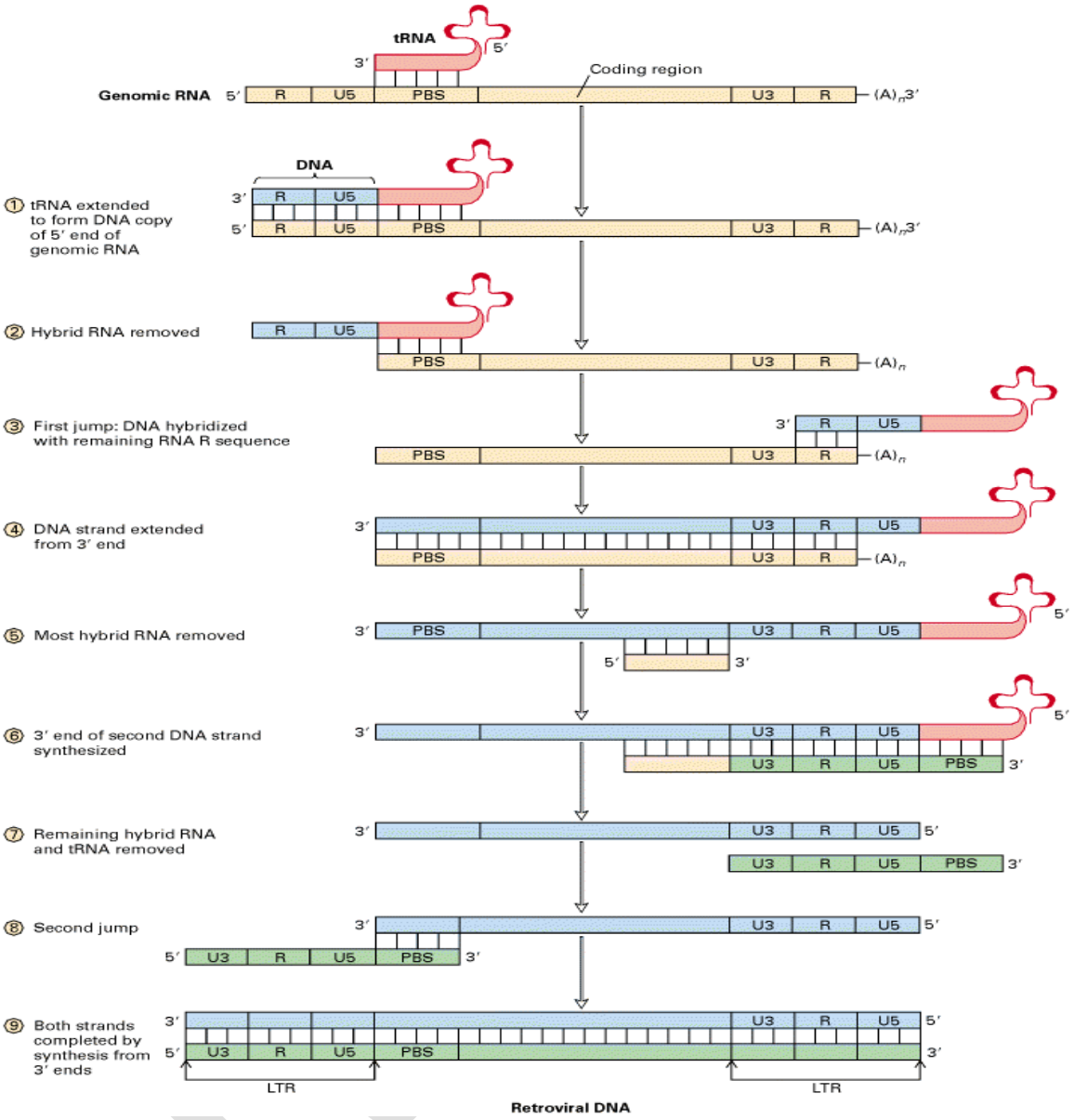
- 5 and 3 direct repeats typical of all mobile elements.

- The central protein-coding region is flanked by two long terminal repeats (LTRs) 250- to 600-bp, which are element-specific direct repeats.
- LTRs, the hallmark of these mobile elements, also are present in retroviral DNA.
- LTRs are characteristic of integrated retroviral DNA and are critical to the life cycle of retroviruses.
- Like other mobile elements, integrated retrotransposons have short target-site direct repeats at their 3 and 5 ends.
- The protein-coding region constitutes 80 percent or more of a retrotransposon.



Generation of retroviral genomic RNA from integrated retroviral DNA

- The short direct repeat sequences (light blue) of target-site DNA are generated during integration of the retroviral DNA into the host-cell genome.
- The left LTR directs cellular RNA polymerase II to initiate transcription at the first nucleotide of the left R region. The resulting primary transcript extends beyond the right LTR.
- The right LTR, now present in the RNA primary transcript, directs cellular enzymes to cleave the primary transcript at the last nucleotide of the right R region and to add a poly(A) tail, yielding a retroviral RNA genome.
- The genomic RNA is packaged in the virion with a retrovirus-specific cellular tRNA hybridized to a complementary sequence near its 5 end called the primer-binding site (PBS).
- The retroviral RNA has a short direct-repeat terminal sequence (R) at each end. The overall reaction is catalyzed by reverse transcriptase, which catalyzes polymerization of deoxyribonucleotides and digestion of the RNA strand in a DNA-RNA hybrid.
- The entire process yields a double-stranded DNA molecule that is longer than the template RNA and has a long terminal repeat (LTR) at each end.
- The PBS and R regions are actually much shorter than the U5 and U3 regions, and the central coding region is very much longer (7500 nucleotides) than the other regions.



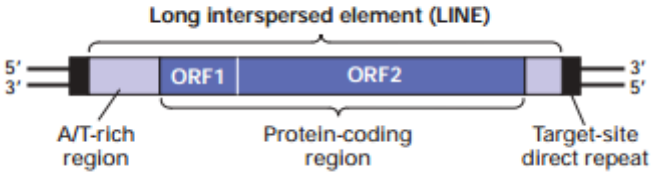
Generation of LTRs during reverse transcription of retroviral genomic RNA

Nonviral Retrotransposons

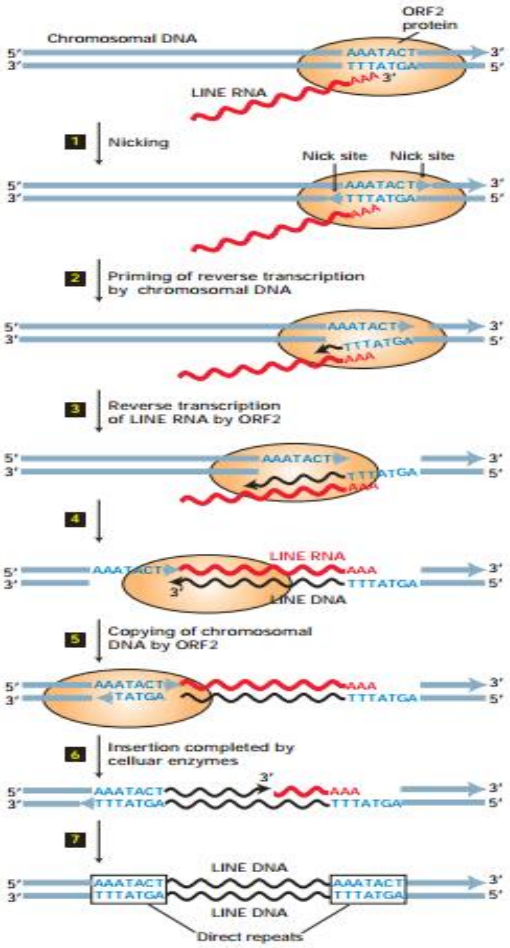
- The most abundant mobile elements in mammals are retrotransposons that lack LTRs, sometimes called nonviral retro-transposons.
- There are two classes of nonviral retro transposons.
  - (i) long interspersed elements (LINEs)and
  - (ii) short interspersed elements (SINEs).
- In humans, full-length LINEs are ≈6 kb long, and SINEs are ≈300 bp long.
- Repeated sequences with the characteristics of LINEs have been observed in protozoans, insects, and plants, and they are particularly abundant in the genomes of mammals.

- SINEs also are found primarily in mammalian DNA.
- They transpose through an RNA intermediate.
- Human DNA contains three major families of LINE sequences that are similar in their mechanism of transposition, but differ in their sequences: L1, L2, and L3. Only members of the L1 family transpose in the contemporary human genome.
- LINE sequences are present at ≈900,000 sites in the human genome, accounting for a staggering 21 percent of total human DNA.

The general structure of a complete LINE



- LINEs usually are flanked by short direct repeats, and contain two long open reading frames (ORFs).
- ORF1, ≈1 kb long, encodes an RNA-binding protein.
- ORF2, ≈4 kb long, encodes a protein that has a long region of homology with the reverse transcriptases of retroviruses and viral retrotransposons, but also exhibits DNA endonuclease activity.
- Since LINEs do not contain LTRs, their mechanism of transposition through an RNA intermediate differs from that of LTR retrotransposons.
- ORF1 and ORF2 proteins are translated from a LINE RNA.
- Transcription by RNA polymerase II is directed by promoter sequences at the left end of integrated LINE DNA.
- LINE RNA is polyadenylated by the same post-transcriptional mechanism that polyadenylates other mRNAs.
- The LINE RNA then is transported into the cytoplasm, where it is translated into ORF1 and ORF2 proteins.
- Multiple copies of ORF1 protein then bind to the LINE RNA, and ORF2 protein binds to the poly(A) tail.
- The LINE RNA is then transported back into the nucleus as a complex with ORF1 and ORF2. ORF2 then makes staggered nicks in chromosomal DNA on either side of any A/T-rich sequence in the genome (step 1).



- Reverse transcription of LINE RNA by ORF2 is primed by the single-stranded T-rich sequence generated by the nick in the bottom strand, which hybridizes to the LINE poly(A) tail (step 2). ORF2 then reverse-transcribes the LINE RNA (step 3) and then continues this new DNA strand, switching to the single-stranded region of the upper chromosomal strand as a template (steps 4 and 5).
- Cellular enzymes then hydrolyze the RNA and extend the 3' end of the chromosomal DNA top strand, replacing the LINE RNA strand with DNA (step 6).
- Finally, 5'and 3'ends of DNA strands are ligated, completing the insertion (step 7).
- These last steps (6 and 7) probably are catalyzed by the same cellular enzymes that remove RNA primers and ligate Okazaki fragments during DNA replication.
- The complete process results in insertion of a copy of the original LINE retrotransposon into a new site in chromosomal DNA.
- A short direct repeat is generated at the insertion site because of the initial staggered cleavage of the two chromosomal DNA strands (step 1).

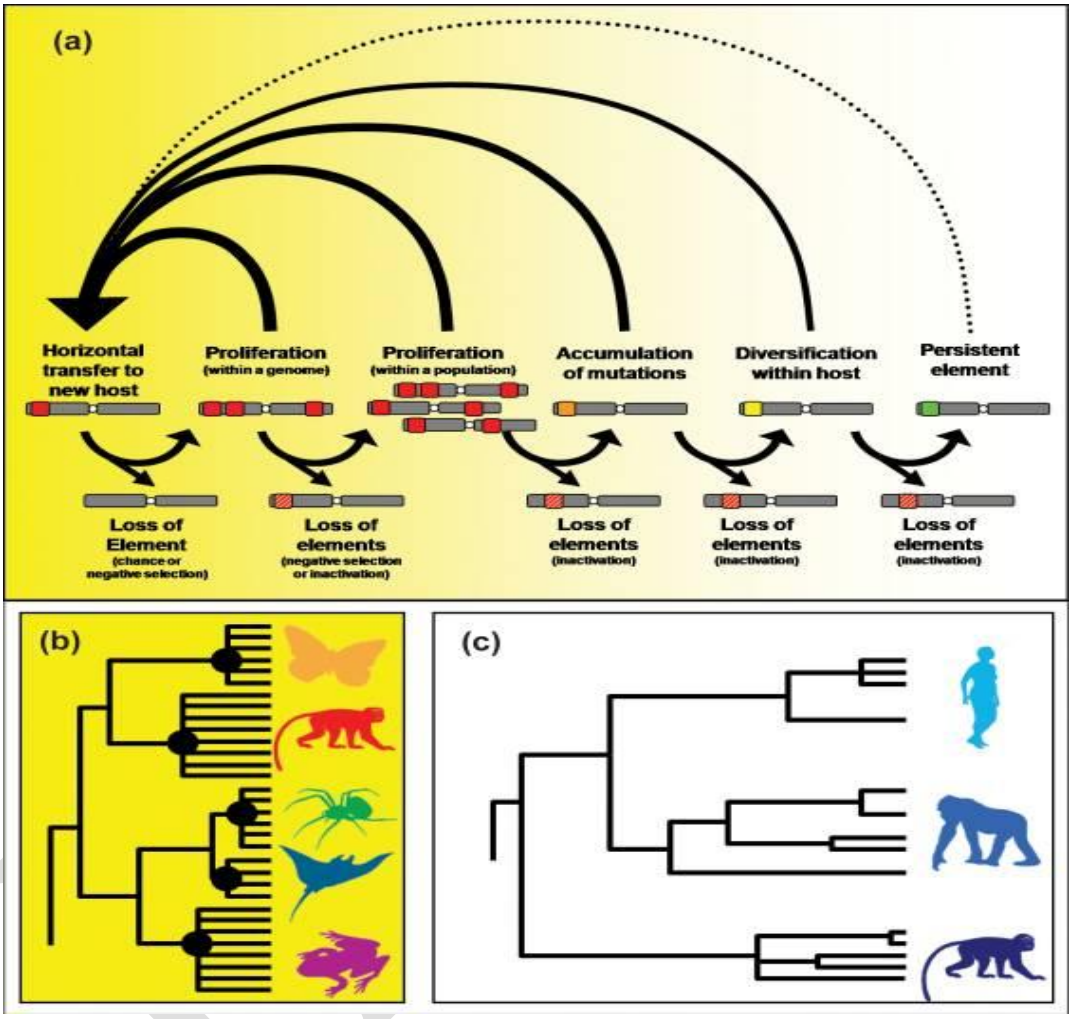
SINEs

- The second most abundant class of mobile elements in the human genome, SINEs constitute ≈13 percent of total human DNA.
- Varying in length from about 100 to 400 base pairs, these retrotransposons do not encode protein, but most contain a 3' A/T-rich sequence similar to that in LINEs.
- SINEs are transcribed by RNA polymerase III, the same nuclear RNA polymerase that transcribes genes encoding tRNAs, 5S rRNAs, and other small stable RNAs
- Most likely, the ORF1 and ORF2 proteins expressed from full length LINEs mediate transposition of SINEs by the retrotransposition mechanism.
- SINEs occur at about 1.6 million sites in the human genome.
- Of these, ≈1.1 million are Alu elements, so named because most of them contain a single recognition site for the restriction enzyme AluI.
- Alu elements exhibit considerable sequence homology with and may have evolved from 7SL RNA, a component of the signal-recognition particle.
- This abundant cytosolic ribonucleoprotein particle aids in targeting certain polypeptides, as they are being synthesized, to the membranes of the endoplasmic reticulum.
- Alu elements are scattered throughout the human genome at sites where their insertion has not disrupted gene expression: between genes, within introns, and in the 3'untranslated regions of some mRNAs.
- For instance, nine Alu elements are located within the human β-globin gene cluster.
- The overall frequency of L1 and SINE retrotranspositions in humans is estimated to be about one new retro-transposition in very eight individuals, with ≈40 percent being L1 and 60 percent SINEs, of which ≈90 percent are Alu elements.
- Similar to other mobile elements, most SINEs have accumulated mutations from the time of their insertion in the germ line of an ancient ancestor of modern humans.
- Like LINEs, many SINEs also are truncated at their 5'end.

Horizontal transfer is the passage of genetic material between genomes by means other than parent-to-offspring inheritance. Although the transfer of genes is thought to be crucial in prokaryotic evolution, few instances of horizontal gene transfer have been reported in multicellular eukaryotes; instead, most cases involve transposable elements. With over 200 cases now documented, it is possible to assess the importance of horizontal transfer for the evolution of transposable elements and their host genomes.

A fundamental difference in the genomic composition of multicellular eukaryotes compared to prokaryotes is that genes represent a minor and relatively static component of most eukaryotic genomes. Instead, most eukaryotic genomes are littered with non-coding DNA and transposable elements (TEs), discrete segments of DNA capable of moving from one locus to another and often duplicating themselves in the process. Not only are TEs the single most abundant entity of

large eukaryotic genomes (e.g. about half of the human genome and 85% of the maize genome; [3,4]), they are also one of their most dynamic components. The movement and accumulation of TEs introduces a prolific source of raw genomic and epigenomic variation among lineages that has both an immediate and lasting influence on the evolutionary trajectory of the host species



a) Simplified model of the lifecycle of TE families and the importance of horizontal transfer

Grey bars represent chromosomes, colored squares represent TEs (with the original sequence in red, mutated in orange, diversified in yellow, persistent in green, and inactivated by orange and red lines). Arrows represent transitions between stages (not all possibilities are illustrated). (b and c) Expected phylogenetic patterns of TEs found among hosts when HTT is frequent (b) versus rare (c). Hypothetical TE phylogenies are depicted with black lines, black circles at nodes illustrate episodes of HTT, and host species are shown in colored silhouettes on the right of each tree with color similarity indicative of their phylogenetic relatedness.

Karpagam Academy of Higher Education
Department of Biochemistry
II BSc Biochemistry
Gene Organisation, Replication and Repair (16BCU401)

UNIT IV- POSSIBLE QUESTIONS

2 Mark - Questions

1. List the proteins and enzymes of homologous recombination
2. Define homologous recombination
3. Differentiate the homologous recombination and heterologous recombination
4. Add note on site specific recombination
5. Narrate about mobile DNA elements
6. Write about the three classes of transposable element
7. How transposable elements helps in evolution
8. Ad note on Rec BCD enzyme
9. Explain the structure of DNA only transposons
10. Write short note on viral trasposans.
11. Give note on bacterial IS elements
12. What is Rec A protein ? Explain its role in various process
13. Write about site specific recombination?

Essay type Questions (8 Marks)

1. Explain the Holliday model of recombination in detail
2. With a neat diagram discuss the events in site specific recombination
3. Describe the role of various enzymes and proteins in recombination
4. Explain the various classes of transposable elements
5. Explain the mechanism of transposition in DNA only transposons
6. Explain the mechanism of transposition in retro viral tranasposons
7. Explain the mechanism of transposition in non retro viral trnasposons

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
II B.Sc BIOCHEMISTRY- Fourth Semester
GENE ORGANISATION, REPLICATION AND REPAII (16BCU401)
MULTIPLE CHOICE QUESTIONS
UNIT IV

S.No	Unit	Questions	Option 1	Option 2	Option 3	Option 4	Answer
1	IV	The role of Rec A gene in homologous recombination is	DNA pairing	Nicking of strands	To provide energy	DNA synthesis	DNA pairing
2	IV	Rec A protein has an important function in	SOS repair	Recombination	SOS repair and Recombination	Base excision repair	SOS repair and Recombination
3	IV	Rec BCD initiates recombination by making	ss nicks	ds nicks	exchange process	mispairing	ss nicks
4	IV	The Holliday model provides a molecular basis for	the association between aberrant segregation and crossing over	the activation of transcription	the repression of early transcription	DNA repair	the association between aberrant segregation and crossing over
5	IV	Rec BCD protein is a multifunctional protein with an	endonuclease activity	exonuclease activity	polymerase activity	ligase activity	endonuclease activity
6	IV	Rec A performs all of the following except	it coats the ss region and polymerizes	it catalyses the *in vitro* formation of Holliday structures	It participates in DNA repair by regulating SOS response	It is involved in the DNA replication	It is involved in the DNA replication
7	IV	Rec BCD is also known as	Exonuclease N	Exonuclease B	Exonuclease V	Exonuclease D	Exonuclease V
8	IV	Rotation of the Holliday structure at the crossover site forms a	rotational isomer	Circular isomer	Crosslinking isomer	exchange isomer	rotational isomer
9	IV	Rotational isomer is also called as	Holliday structure	Radding structure	Circular structure	Rotational structure	Holliday structure
10	IV	Who gave basis of most popular current models for molecular events of recombination?	Robin Holliday	Matthew Messelson and Charles Radding	Arthur Kornberg	Messelson Stahl	Robin Holliday
11	IV	Who suggested mechanism for creating cross-strand Holliday structure?	Matthew Meselson and charles Radding	Robin Holliday	Arthur Kornberg	Messelson Stahl	Robin Holliday
12	IV	LTRs are absent in	LINES	SINES	LINES AND SINES	Viral retrotransposons	LINES AND SINES
13	IV	Important hallmark of IS element is	Short direct repeats	Long direct repeats	Inverted repeats	Tandem repeats	Short direct repeats
14	IV	IS element contain _____ enzyme	Helicase	primase	Transposase	topoisomerase	transposase
15	IV	LINES and SINES come under _____ DNA	Single copy DNA	Moderatively repetitive DNA	Simple sequence DNA	RNA	Moderatively repetitive DNA
16	IV	In site specific recombination _____ elements are moved	Operon	Mobile genetic elements	Promotor	Viral DNA	Mobile genetic elements
17	IV	Moleuclar Symbionts are	Virus	Provirus	Mobile genetic elements		Mobile genetic elements
18	IV	Selfish DNA is	Viral DNA	Bacterial DNA	Rec A protein	Transposons	Transposons
19	IV	Mobile genetic elements are also known as	Transposons	Tranducers	Conjugators	ligators	Transposons
20	IV	Enzyme involved in transposition is	Ligase	Transposase	Invertase	Mutase	Transposase
21	IV	On basis of transposition mechanism, the transposons are classifeid into	3	5	6	2	**3**
22	IV	In DNA only transposons, the mobile genetic elemnet exiast as _____	DNA	RNA	Protein	Polysacchride	DNA
23	IV	Specific enzyme present in retroviral transposons for transposition mechanism is __	DNA polymerase	Reverse transcriptase	RNA polymerase	Topoisomerase	Reverse transcriptase
24	IV	Transposase is also called as	Integrase	helicase	Rec A protein	Ruv protein	Integrase
25	IV	Rec BCD enzyme has the following activity	Exonuclease II	Exonuclease I	Exonuclease IV	Exonuclease V	Exonuclease V
26	IV	Major enzyme in E.coli that intiate recombinational repair is	Exonuclease II	Exonuclease I	Exonuclease IV	Exonuclease V	Exonuclease V
27	IV	RecBCD enzyme have the following activities	Helicase, nuclease	Helicase,Topoisomerase	nuclease,Topoisomerase	Topoisomerase,Polymerase	Helicase, nuclease
28	IV	RecBCD enzyme recognise a specific sequence in DNA called	Pie	Chi	Delta	Theta	Chi
29	IV	Number of subunits present in Rec BCD enzyme is	5	3	6	2	3
30	IV	Among the following which increase branch migration	Ruv A & Ruv B	Rec BCD enzyme	Rec A protein	Rec D protein	Ruv A & Ruv B
31	IV	Holiday structure is resolved by the protein	Ruv A	Ruv B	Ruv C	Ruv D	Ruv C
32	IV	Site specific base pair changes with in the coding region of DNA is introduced by	Recombinational repair	Homologous Recombination	Heterologous Recombination	Site specific recombination	Site specific recombination
33	IV	_____ recombination can alter gene and also add new information to the genome	Recombinational repair	Homologous Recombination	Heterologous Recombination	Site specific recombination	Site specific recombination
34	IV	Mobile genetic elments are moved between the non homologous site with in genome by	Recombinational repair	Homologous Recombination	Heterologous Recombination	Site specific recombination	Site specific recombination
35	IV	Abundant repeated DNA sequence found in vertebate genome are mostly derived from	Viral DNA	Mobile genetic elements	Bacterial DNA	Rec A protein	Mobile genetic elements
36	IV	The trnas;location of mobile genetic elements give rise to _____ Mutation	Induced	frmae shift	Spontaneous	Forced	Spontaneous
37	IV	Mobile DNA eelements are also known as	Trnasducers	Conjugators	Transposons	Replicon	Transposons
38	IV	The process by which the the mobile genetic are copied and inserted into a new site is called _____	Transposition	Homologous Recombination	Heterologous Recombination	Site specific recombination	Transposition
39	IV	Conversion of DNAto RNA And to cDNA is used in transposition of	DNA only transposons	Retro viral transposons	Non retro viral transposons	RNA only tranposons	Retro viral transposons
40	IV	DNA copy of RNA molecule is produced in recombination of	DNA only transposons	Retro viral transposons	Non retro viral transposons	RNA only tranposons	Non retro viral transposons
41	IV	Cut and paste mechanism is followed by _____-	DNA only transposons	Retro viral transposons	Non retro viral transposons	RNA only tranposons	DNA only transposons
42	IV	P element of Drosophila is an example of _____ transposons	DNA only transposons	Retro viral transposons	Non retro viral transposons	RNA only tranposons	DNA only transposons
43	IV	Copia element of Drosophila is an example of _____ transposons	DNA only transposons	Retro viral transposons	Non retro viral transposons	RNA only tranposons	Retro viral transposons
44	IV	F element of Drosophila is an example of _____ transposons	DNA only transposons	Retro viral transposons	Non retro viral transposons	RNA only tranposons	Non retro viral transposons
45	IV	_____ trnasposition was the first identified transposons	Chi	Pie	Mu	Theta	Mu
46	IV	The genetic material of AIDS virus is	RNA	DNA	Protein	Polysaccharide	RNA
47	IV	The genetic material of retro virus is	RNA	DNA	Protein	Polysaccharide	RNA
48	IV	_____ repressor inhibits trnascription of all genes SOS genes	Rec A	Rec BCD	Lex B	Lex A	Lex A

49	IV	Extensive DNA damage induce _____ response	ROS	SOS	RES	SES	SOS
50	IV	One of the regulatory protein of SOS response is	Rec B	Rec BCD	Lex B	Lex A	Lex A
51	IV	One of the regulatory protein of SOS response is	Rec A	Rec BCD	Lex B	Lex C	Rec A
52	IV	_____ protein provide functional link between DNA damage and induction of SOS response	Rec A	Rec BCD	Lex B	Lex C	Rec A
53	IV	_____ protein bindg to single strand DNA and facilitate of Lex A repressor	Rec A	Rec B	Rec C	Rec D	Rec A
54	IV	DNA lesions and cross links can result from _____	Chemicals	Virus	ionising radiation	Bacteria	ionising radiation
55	IV	Extensive DNA damage in bacteria induce _____ response	ROS	SOS	RES	SES	SOS
56	IV	In error prone translesion DNA synthesis, DNA repair is	More accurate with less mutation	Less accurate with high mutation	Moderately accurate with high mutation	Accurate with no mutation	Less accurate with high mutation
57	IV	Among the following which is SOS response protein	Dna G ans Dna A	Uvr A and Uvr B	Ruv A & Ruv B	Ruv C & Ruv D	Uvr A and Uvr B
58	IV	Specialised DNA polymerase in volved in replication of Dna lesion is	DNA Pol I	DNA Pol II	DNA Pol III	DNA Pol V	DNA Pol V
59	IV						
60	IV						

UNIT-V
SYLLABUS

Molecular basis of mutations and DNA repair

Importance of mutations in evolution of species. Types of mutations - transition, transversions, frame shift mutations, mutations induced by chemicals, radiation, transposable elements, Ames test. Various modes of DNA repair - Replication errors and mismatch repair system, repair of DNA damage, direct repair, base excision repair, nucleotide excision repair, recombination repair, translesion DNA synthesis.

MOLECULAR BASIS OF MUTATIONS AND DNA REPAIR

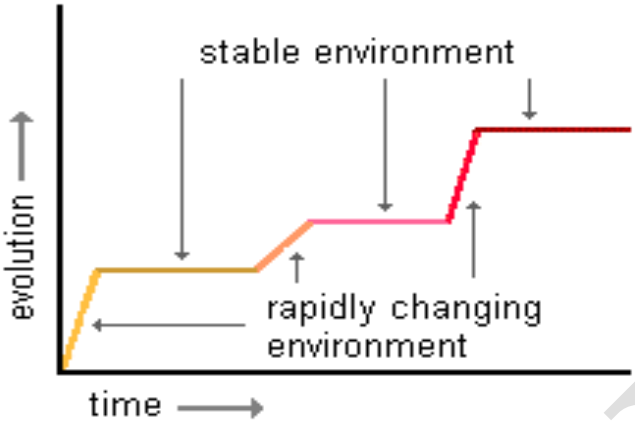
Importance of mutations in evolution of species (Micro and Macro evolution)

Throughout most of the 20th century, researchers developing the synthetic theory of evolution primarily focused on **microevolution** , which is slight genetic change over a few generations in a population. Until the 1970's, it was generally thought that these changes from generation to generation indicated that past species evolved gradually into other species over millions of years. This model of long term gradual change is usually referred to as **gradualism** or phyletic gradualism . It is essentially the 19th century Darwinian idea that species evolve slowly at a more or less steady rate. A natural consequence of this sort of **macroevolution** would be the slow progressive change of one species into the next in a line, as shown by the graph on the right.

Beginning in the early 1970's, this model was challenged by Stephen J. Gould, Niles Eldredge, and a few other leading paleontologists . They asserted that there is sufficient fossil evidence to show that some species remained essentially the same for millions of years and then underwent short periods of very rapid, major change. Gould suggested that a more accurate model in such species lines would be **punctuated equilibrium**

The punctuated, or rapid change periods, were presumably the result of major environmental changes in such things as predation pressure, food supply and climate. During these times, natural selection can favor varieties that were previously at a comparative disadvantage. The result can be an accelerated rate of change in gene pool frequencies in the direction of the varieties that become the most favored by the new environmental conditions. It would be expected that long severe droughts, major volcanic eruptions, and the beginning and ending of ice ages would be likely triggers for rapid evolution. In such stressful situations, populations would be expected to initially diminish and become isolated. Genetic drift would then potentially speed up the rate of evolution. If by chance nature favored successful adaptations, the population would again increase in numbers as a radically changed species. Conversely, if it

favored maladaptive variations, the population would decrease in numbers further and possibly even become extinct.



Random mutations provide variations that help a species survive. Mutations in regulator genes in particular can quickly result in radically new variations in the organization of the body and its important structures. As a consequence, changes in these genes can result in a greater likelihood that at least some individuals will have variations that will allow them to survive during times of extinction level events. In this situation, subsequent generations would be significantly changed from the generations before the period of severe natural selection. In other words, regulator genes probably play an important part in the rapid change phases of punctuated evolution. Short-lived species with quick generation replacement times usually evolve at a faster rate than do large, long-lived species. This is because new genetic variations normally appear each generation as a consequence of mutation in sex cells. Those variations may be selected for or against depending on the environment at the time. As a consequence, quicker reproductive cycles generally result in speeded up species divergence. It is not surprising that there are far more species of insects and microscopic organisms than species of large trees and big animals such as elephants, horses, and humans.

Tropical species also generally evolve at a faster rate than do those from colder temperate climates. Subsequently, tropical forests are more diverse ecosystems than forests in colder regions. This is probably because warm environments promote shorter generation times and higher mutation rates.

A relatively new but extremely important factor in affecting rates of evolution has been people. There are now nearly 7 billion of us, and our numbers are growing rapidly. We have already severely changed most environments on our planet to suit our needs. In addition, we are the super predator around the globe, bringing many species to the brink of extinction and beyond. As a consequence, humans have dramatically altered natural selection. The surviving animal and

plant species have responded to this pressure in a variety of ways. For instance, fish species that are heavily exploited by people now usually have smaller bodies as adults and begin to reproduce at an earlier age. It is also likely that because humans increasingly live in urban environments and rely on ever more technology, the evolution of our species has accelerated and changed in ways that are yet to be discovered.

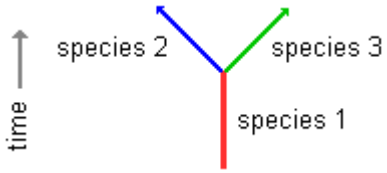
It is apparent that the evolutionary history of life on this planet is extremely complicated. Different species have evolved at different rates and those rates have changed through time in response to complex patterns of interaction with other species and other environmental factors. In addition, it is clear that most species lines have already become extinct as a result of their inability to adapt to changed conditions.

Origin of Species

Where do new species come from? That is a key question that the biological sciences have been asking for more than 200 years. Charles Darwin gave us part of the answer in his explanation of natural selection. The remainder came as a result of Gregor Mendel's experiments with basic genetic inheritance and the 20th century discoveries of the other natural processes that can cause evolution. We now know that evolution can occur in two different patterns: adaptive radiation and successive speciation.

Adaptive radiation is the progressive diversification of a species into two or more species as groups adapt to different environments. Natural selection is usually the principle mechanism driving adaptive radiation. The initial step is the separation of a species into distinct breeding populations. This usually happens as a result of geographic or social isolation. Over time, the gene pools of the isolated populations diverge from each other by gradually acquiring different mutations and sometimes as a result of random genetic drift. When the populations are in dissimilar environments, environmental stresses are often not the same. As a result, nature selects for different traits existing within the gene pools of the now cut off populations. Over time, the populations genetically diverge enough so that they can no longer reproduce with each other. At this point, they have become separate species and usually continue to diverge in subsequent generations. In intermediate stages, the two newly or about to be separated species may be able to interbreed and produce children, but most of them are likely to be sterile. This is the case with the offspring of female horses and male donkeys--i.e., mules. Eventually, however, species genetically diverge so much that they are unable to produce any offspring. This is the case with sheep and cattle. The process of adaptive radiation results in a branching evolutionary pattern known as **cladogenesis** .

Adaptive radiation resulting in cladogenesis



The evolution of species by **successive speciation** occurs within a single evolutionary line without the branching of adaptive radiation. This takes place when the members of a species consist of a single breeding population for many generations. Descendant generations experience continuous spontaneous mutations and new directions of natural selection as the environment changes. This results in progressive changes in the gene pool frequencies of the population. At any one time, all members of the population are the same species. However, as generations subsequently replace each other, the gene pool is transformed--i.e., it evolves. Eventually, the changes are great enough that if descendants could go back in time to mate with their distant ancestors, the genetic differences would prevent them from producing fertile offspring. In other words, they would be different species. The process of successive speciation results in a non-branching evolutionary pattern known as **anagenesis** .

Successive speciation resulting in anagenesis

In the real world, the patterns of evolution can be very complex and changing. Both adaptive radiation and successive speciation can go on simultaneously.

MUTATION

Mutation-Definition

A mutation is a permanent change in the sequence of DNA. In order for an observable effect, mutations must occur in gene exons or regulatory elements. Changes in the non-coding regions of DNA (introns and junk DNA) generally do not affect function.

Causes of mutations

Mutations can be caused by external (exogenous) or endogenous (native) factors or they may be caused by errors in the cellular machinery. Physical or chemical agents that induce mutations in DNA are called mutagens and are said to be mutagenic.

Exogenous factors: environmental factors such as sunlight, radiation, and smoking can cause mutations.

Endogenous factors: errors during DNA replication can lead to genetic changes as can toxic by-products of cellular metabolism.

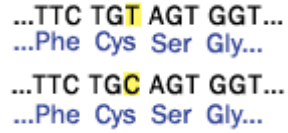
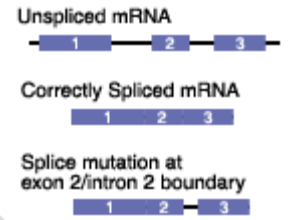
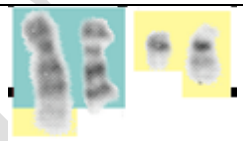
Consequences of mutations

Mutations can be advantageous and lead to an evolutionary advantage of a certain genotype. Mutations can also be deleterious, causing disease, developmental delays, structural abnormalities, or other effects.

TYPES OF MUTATIONS:

There are several classes of mutations

Type	Description	Example
1. Deletion	Genetic material is removed or deleted. A few bases can be deleted (as shown on the left) or it can be complete or partial loss of a chromosome (shown on right).	...TCGGAATCGC... ...TCGCGC...
2. Frameshift	The insertion or deletion of a number of bases that is not a multiple of 3. This alters the reading frame of the gene and frequently results in a premature stop codon and protein truncation.	...ACT TTT CAT AGT... ...Thr Phe His Ser... ...ACT TTT TCA TAG T... ...Thr Phe Ser Stop
3. Insertion	When genetic material is put into another region of DNA. This may be the insertion of 1 or more bases, or it can be part of one chromosome being inserted into another, non-homologous chromosome.	...TTGAAAACGCTG... ...TTGAAAAACGCTG...
4. Missense	A change in DNA sequence that changes the codon to a different amino acid. Not all missense mutations are deleterious, some changes can have no effect. Because of the ambiguity of missense mutations, it is often difficult to interpret the consequences of these mutations in causing disease.	...ACT CAG AAC... ...Thr Gln Asn... ...ACT CGG AAC... ...Thr Arg Asn...
5. Nonsense	A change in the genetic code that results in the coding for a stop codon rather than an amino acid. The shortened protein is generally non-function or its function is impeded.	...ATA CGA GCT... ...Ile Arg Ala... ...ATA TGA GCT... ...Ile Stop
6. Point	A single base change in DNA sequence. A point mutation may be silent, missense, or nonsense.	...CGTAATCCTCGA... ...CGTAGTCCTCGA...

7. Silent	A change in the genetic sequence that does not change the protein sequence. This can occur because of redundancy in the genetic code where an amino acid may be encoded for by multiple codons.	
8. Splice Site	A change in the genetic sequence that occurs at the boundary of the exons and introns. The consensus sequences at these boundaries signal where to cut out introns and rejoin exons in the mRNA. A change in these sequences can eliminate splicing at that site which would change the reading frame and protein sequence.	
9. Translocation	A structural abnormality of chromosomes where genetic material is exchanged between two or more non-homologous chromosomes.	

Mutations caused by chemicals and radiation

Some of the chemical mutagens and mutagenesis are given in Table , and described below:

Table : Different types of chemical mutagens

Class of Chemical	*Chemical Mutagens*
Acridines	Ethyleneimine (EI)
Mustard	Nitrogen mustard
	Sulphur mustard
Nitrosamines	Diethylnitrosamine (DMN)
	Diethylsulphonate (DES)
	Nitrosomethylurea (NMU)
Epoxide	Ethyleneoxide (EO)
	Diepoxybutane (DEB)
Alkyl sulphonates	Diethylsulphonate (DES)
	Methylmethanesulphonate (MMS)
	Ethylmethanesulphonate (EMS)
Others	Nitrous acid
	Maleic hydrazide
	Hydroxylamine

i. Base Analogues:

A base analogue is a chemical compound similar to one of the four bases of DNA. It can be incorporated into a growing polynucleotide chain when normal process of replication occurs.' These compounds have base pairing properties different from the bases. They replace the bases and cause stable mutation.

A very common and widely used base analogue is 5-bromouracil (5-BU) which is an analogue of thymine. The 5-BU functions like thymine and pairs with adenine (Fig. 9.6A).

The 5-BU undergoes tautomeric shift from keto form to enol form caused by bromine atom. The enol form can exist for a long time for 5-BU than for thymine (Fig. 9.6B). If 5-BU replaces a thymine, it generates a guanine during replication which in turn specifies cytosine causing G: C pair (Fig. 9.6A).

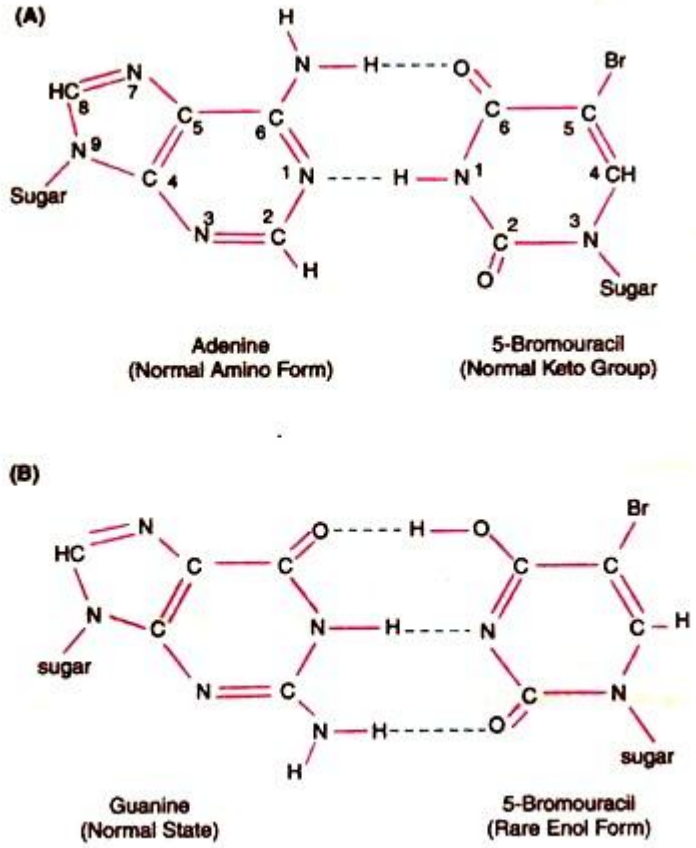


Fig. 9.6 : Mutagenesis by base analogue 5-bromouracil. A, the keto form of 5-BU pairs with adenine; B, 5-BU is tautomarised to enol form and pairs with guanine rather than adenine.

During the replication, keto form of 5-BU substitutes for T and the replication of an initial AT pair becomes an A: BU pair (Fig. 9.7A). The rare enol form of 5-BU that pairs with G is the first mutagenic step of replication. In the next round of replication G pairs with C. Thus, the transition is completed from AT→GC pair.

The 5-BU can also induce the conversion of GC to AT. The enol form infrequently acts as an analogue of cytosine rather than thymine. Due to error, GC pair is converted into a G: BU pair which in turn becomes an AT pair (Fig. 9.7B). Due to such pairing properties 5-BU is used in

chemotherapy of viruses and cancer. Because of pairing with guanine it disturbs the normal replication process in microorganisms.

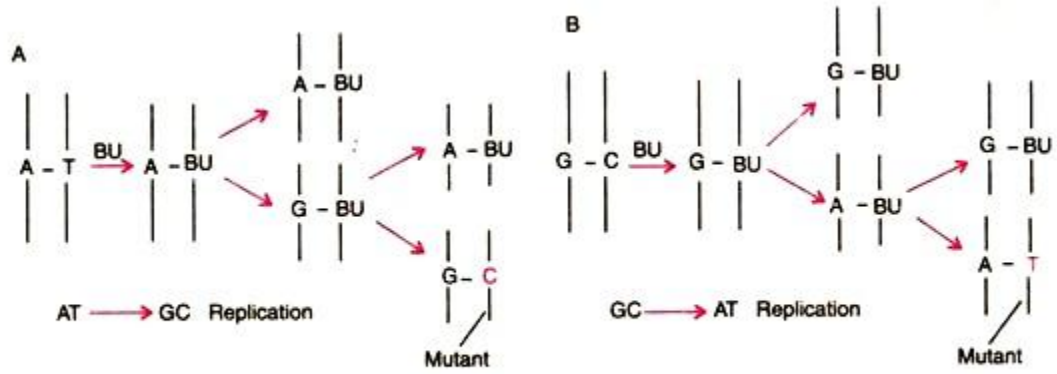


Fig. 9.7 : Mechanism of 5-bromouracil (BU)-induced mutagenesis. A, AT→GC replication; B, GC→AT replication.

The 5-bromodeoxyuridine (5-BDU) can replace thymidine in DNA molecule. The 2-amino-purine (2-AP) and 2, 6-di-amino-purine (2, 6-DAP) are the purine analogues. The 2-AP normally pairs with thymine but it is able to form a single hydrogen bond with cytosine resulting in transition of AT to GC. The 2-AP and 2, 6-DAP are not as effective as 5-BU and 5-BDU.

ii. Chemicals Changing the Specificity of Hydrogen Bonding:

There are many chemicals that after incorporation into DNA change the specificity of hydrogen - bonding. Those which are used as mutagens are nitrous oxide (HNO_2), hydroxylamine (HA) and ethyl-methane-sulphonate (EMS).

(a) Nitrous Oxide (HNO_2):

Nitrous oxide converts the amino group of bases into keto group through oxidative deamination. The order of frequency of deamination (removal of amino group) is adenine > cytosine > guanine.

(b) Deamination of Adenine:

Deamination of adenine results in formation of hypoxanthine, the pairing behaviour of which is like guanine. Hence, it pairs with cytosine instead of thymine replacing AT pairing by GC pairing (Fig. 9.8A).

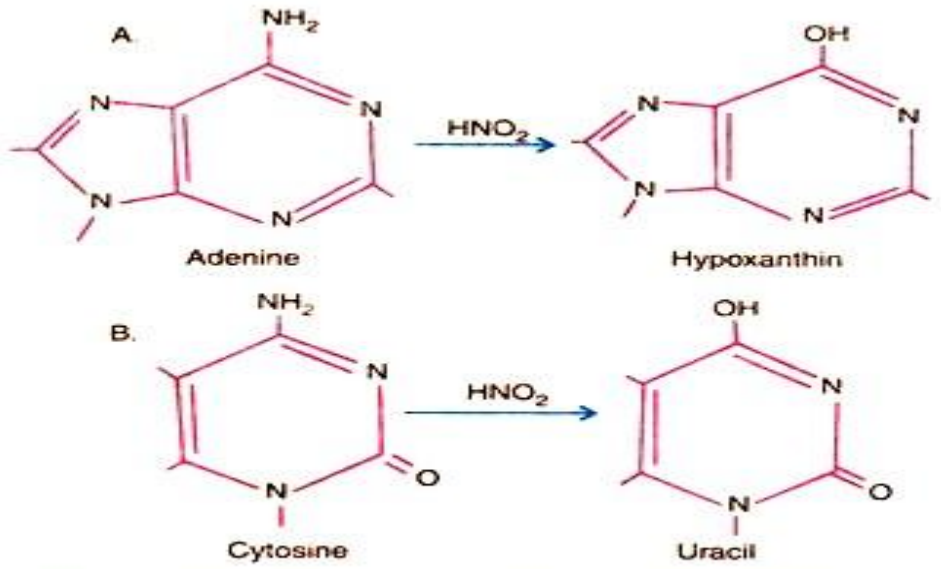


Fig. 9.8 : Deamination by nitrous oxide of adenine into hypoxanthin (A), and cytosine into uracil (B).

(c) Deamination of Cytosine:

Deamination of cytosine results in formation of uracil by replacing – NH_2 group with -OH group. The affinity for hydrogen bonding of uracil is like thymine; therefore, C-G pairing is replaced by U-A pairing (Fig. 9.8B).

(d) Deamination of Guanine:

Deamination of guanine results in formation of xanthine, the later is not mutagenic. Xanthine behaves like guanine because there is no change in pairing behaviour. Xanthine pairs with cytosine. Therefore, G-C pairing is replaced by X-C pairing.

(e) Hydroxylamine (NH_2OH):

It hydroxylates the C_4 nitrogen of cytosine and converts into a modified base via deamination which causes to base pairs like thyamine. Therefore, GC pairs are changed into AT pairs.

iii. Alkylating Agents:

Addition of an alkyl group to the hydrogen bonding oxygen of guanine (N_7 position) and adenine (at N_3 position) residues of DNA is done by alkylating agents. As a result of alkylation, possibility of ionization is increased with the introduction of pairing errors. Hydrolysis of linkage of base-sugar occurs resulting in gap in one chain.

This phenomenon of loss of alkylated base from the DNA molecule (by breakage of bond joining the nitrogen of purine and deoxyribose) is called depurination. Depurination is not always mutagenic. The gap created by loss of a purine can effectively be repaired.

Following are some of the important widely used alkylating agents:

(a) Dimethyl sulphate (DMS)

(b) Ethyl methane sulphonate (EMS) -$CH_3CH_2SO_3CH_3$

(c) Ethyl ethane sulphonate (EES) -$CH_3CH_2SO_3CH_2CH_3$

EMS has the specifity to remove guanine and cytosine from the chain and results in gap formation. Any base (A,T,G,C) may be inserted in the gap. During replication chain without gap will result in normal DNA. In the second round of replication gap is filled by suitable base.

If the correct base is inserted, normal DNA sequence will be produced. Insertion of incorrect bases results in transversion or transition mutation. Another example is methyl nitrosoguanidine that adds methyl group to guanine causing it to mispair with thyamine. After subsequent replication, GC is converted into AT transition.

iv. Intercalating Agents:

There are certain dyes such as acridine orange, proflavine and acriflavin which are three ringed molecules of similar dimensions as those of purine pyrimidine pairs (Fig. 9.9). In aqueous solution these dyes can insert themselves in DNA (i.e. intercalate the DNA) between the bases in adjacent pairs by a process called intercalation.

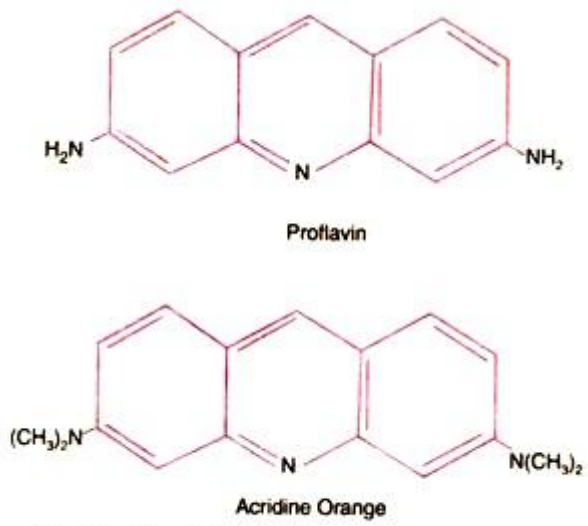


Fig. 9.9 : Chemical structure of two mutagenic acridine derivatives.

Therefore, the dyes are called intercalating agents. The acridines are planer (flat) molecules which can be intercalated between the base pairs of DNA; distort the DNA and results deletion

or insertion after replication of DNA molecule. Due to deletion or insertion of intercalating agents, there occur frameshift mutations (Fig. 9.10).

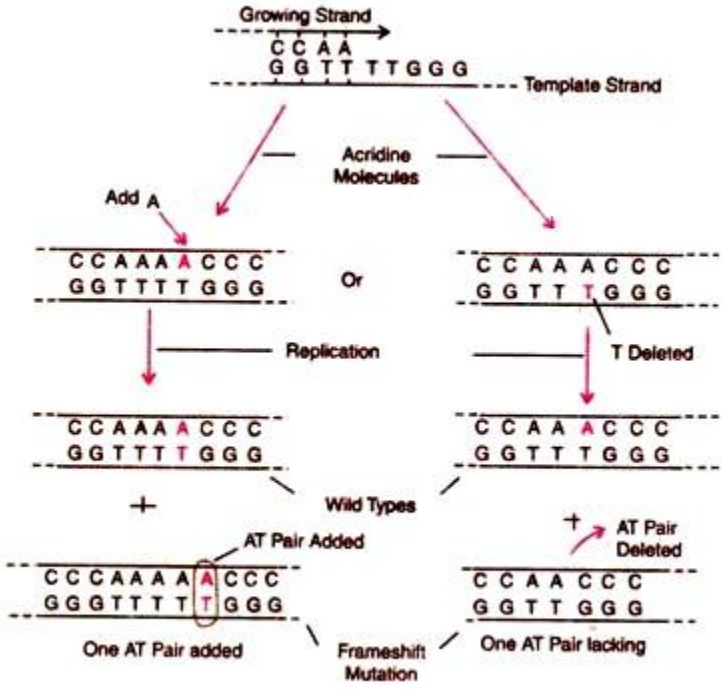


Fig. 9.10 : Mechanism of intercalation of an acridine molecule in the replication fork.

2. PHYSICAL MUTAGENS:

i. Radiations as Mutagens:

Radiation is the most important among the physical mutagens. Radiations damaging the DNA molecules fall in the wavelength range below 340 nm and photon energy above 1 electro-volt (eV). The destructive radiation consists of ultraviolet (UV) rays, X-rays, γ-rays, alpha (α) rays, beta (β) rays, cosmic rays, neutrons, etc.

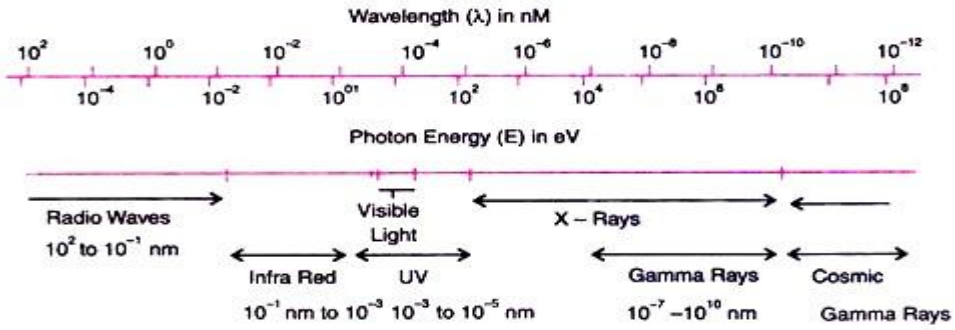


Fig. 9.11 : Wavelengths and photon energy of various radiations.

Radiation induced damage can be categorized into the three broad types: lethal damage (killing the organisms), potentially lethal damage (can be lethal under certain ordinary conditions) and sub-lethal damage (cells do not die unless radiation reaches to a certain threshold value). The effect of damage is at molecular level.

In a live cell radiation damage to proteins, lipoproteins, DNA, carbohydrates, etc. is caused directly by ionization/excitation, or indirectly through highly reactive free radicals produced by radiolysis of cellular water. DNA stores genetic information's so a damage to it assumes great dimension. It can perpetuate genetic effects and, therefore, the cellular repair system is largely devoted to its welfare.

When the bacteria are exposed to radiation they gradually lose the ability to develop colonies. This gradual loss of viability can be expressed graphically by plotting the surviving colonies against the gradually increasing exposure time

ii. Ultraviolet (UV) Radiation:

UV radiation causes damage in the DNA duplex of the bacteria and phages. The UV rays are absorbed and cause excitation of macromolecules. The absorption maxima of nucleic acid = (280 nm) and protein (260 nm) are more or less similar. The DNA molecule is the target molecule for UV rays but not the proteins. However, absorption spectrum of RNA is quite similar to that of DNA.

The excited DNA leads to cross-linking, single strand breaks and base damage as minor lesion and generation of nucleotide dimer as a major one. Purines are generally more radio – resistant than the pyrimidine of the latter, thymine is more reactive than cytosine.

Hence, the ratio of thymine-thymine (TT), thymine-cytosine (TC), cytosine-cytosine (CC) dimer (Fig. 9.14) is 10:3:3, respectively. A few dimers of TU and UU also appear. The initial step in pyrimidine dimerization is known to be hydration of their 4: 5 bonds.

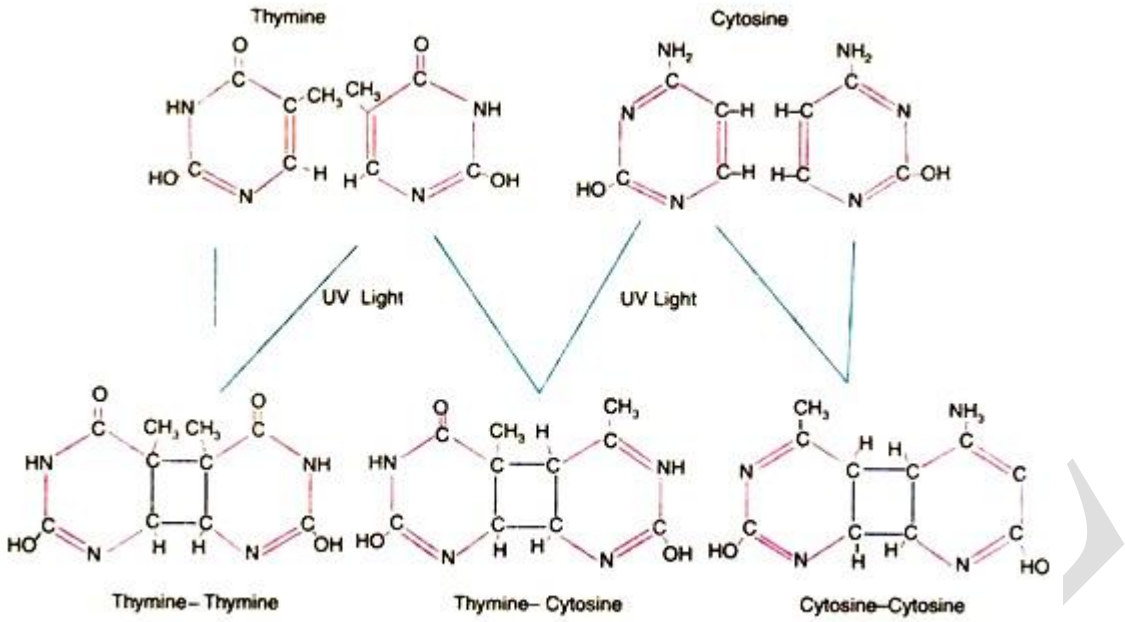


Fig. 9.14 : Formation of pyrimidine dimer induced by UV radiation.

Formation of thymine-thymine (TT) dimer causes distortion of DNA helix because the thymines are pulled towards one another. The distortion results in weakening of hydrogen-bonding to adenines in the opposing strand. This structural distortion inhibits the advance of replication fork.

ii. The X-Rays:

The X-rays cause breaking of phosphate ester linkages in the DNA. This breakage occurs at one or more points. Consequently, a large number of bases are deleted or rearranged in the DNA molecule.

The X-rays may break the DNA either in one or both strands. If breaks occur in both strands, it becomes lethal. The DNA segment between the two breaks is removed resulting in deletion.

Mutation caused by transposable elements

Transposon mutagenesis, or **transposition mutagenesis**, is a biological process that allows genes to be transferred to a host organism's chromosome, interrupting or modifying the function of an extant gene on the chromosome and causing mutation. Transposon mutagenesis is much more effective than chemical mutagenesis, with a higher mutation frequency and a lower chance of killing the organism.

They induce mutation by inserting into other genes and by promoting DNA rearrangements(Recombination). Genetic aberration can also be caused by recombination between different copies of a transposable element

Examples: Approximately haly of the spontaneous mutations in Drosophila

Human genetic diseases

The colour of grapes

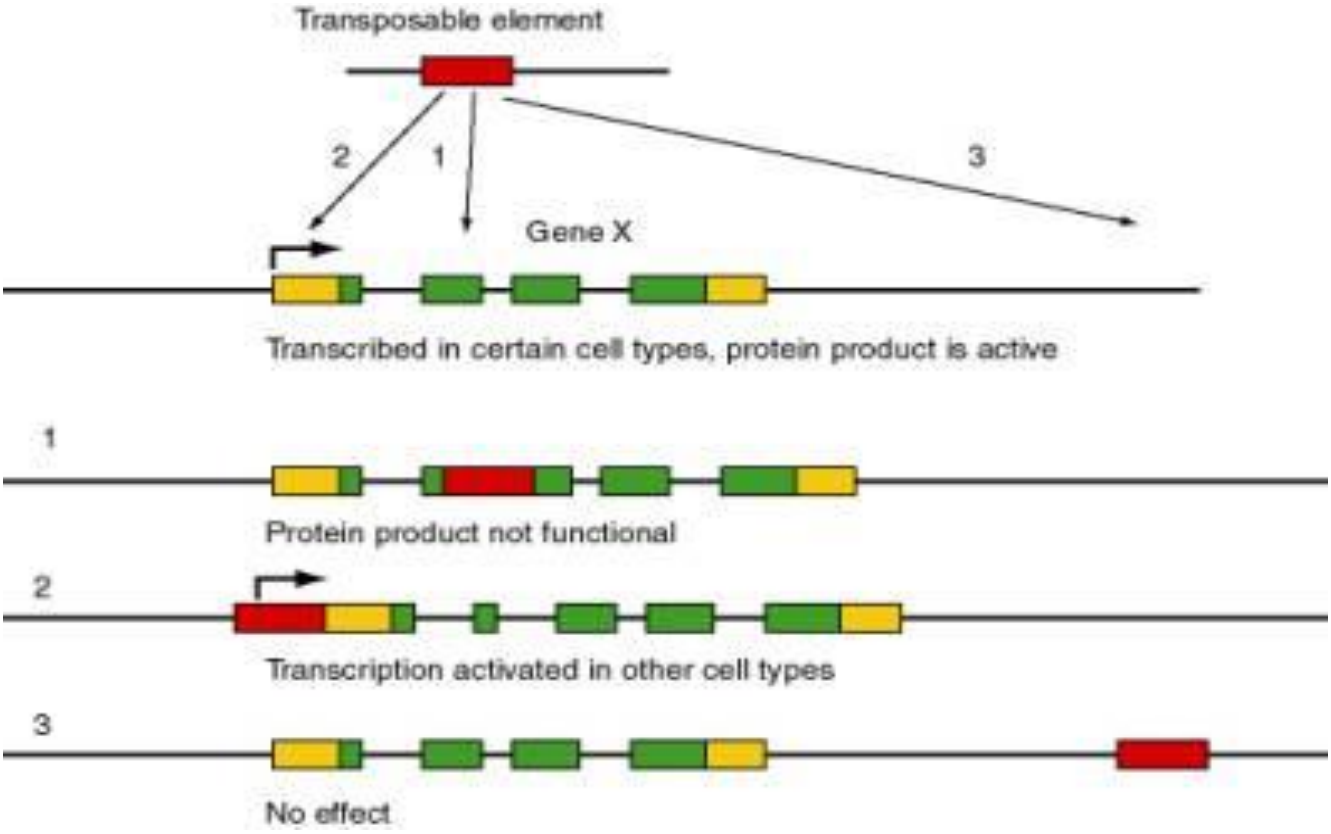


Fig: Genetic changes caused by transposons

DNA damage

Types of damage

DNA damage can be subdivided into two main types:

1. Endogenous damage such as attack by reactive oxygen species produced from normal metabolic byproducts (spontaneous mutation), especially the process of oxidative deamination; also includes replication errors

2. Exogenous damage caused by external agents such as

1. Ultraviolet [UV 200-300nm] radiation from the sun
2. other radiation frequencies, including x-rays and gamma rays

3. Hydrolysis or thermal disruption
4. Certain plant toxins
5. Human-made mutagenic chemicals, especially aromatic compounds that act as DNA intercalating agents
6. Cancer chemotherapy and radiotherapy
7. Viruses

The replication of damaged DNA before cell division can lead to the incorporation of wrong bases opposite damaged ones. Daughter cells that inherit these wrong bases carry mutations from which the original DNA sequence is unrecoverable (except in the rare case of a back mutation, for example, through gene conversion).

Sources of damage

There are five main types of damage to DNA due to endogenous cellular processes:

1. Oxidation of bases [e.g. 8-oxo-7,8-dihydroguanine (8-oxoG)] and generation of DNA strand interruptions from reactive oxygen species,

2. Alkylation of bases (usually methylation), such as formation of 7-methylguanine, 1-methyladenine, 6-O-Methylguanine

3. Hydrolysis of bases, such as deamination, depurination and depyrimidination.

4. "Bulky adduct formation" (i.e. benzo[a]pyrene diol epoxide-dG adduct)

5. Mismatch of bases, due to errors in DNA replication, in which the wrong DNA base is stitched into place in a newly forming DNA strand, or a DNA base is skipped over or mistakenly inserted.

Damage caused by exogenous agents comes in many forms. Some examples are:

1. UV-B light causes crosslinking between adjacent cytosine and thymine bases creating pyrimidine dimers. This is called direct DNA damage.

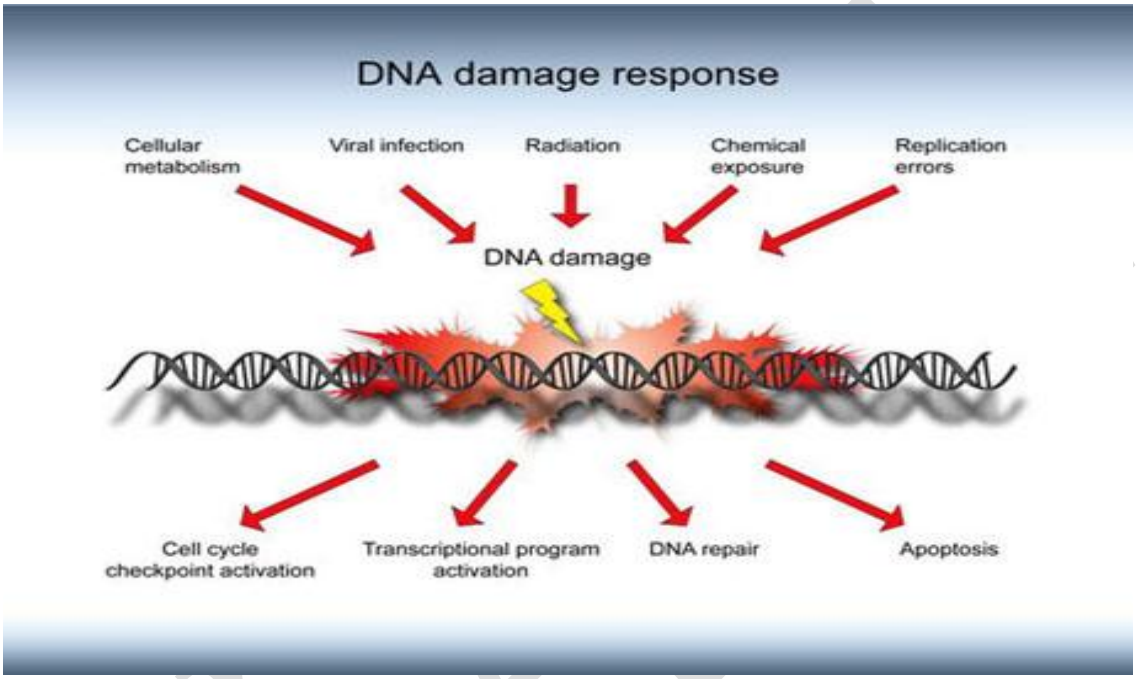
2. UV-A light creates mostly free radicals. The damage caused by free radicals is called indirect DNA damage.

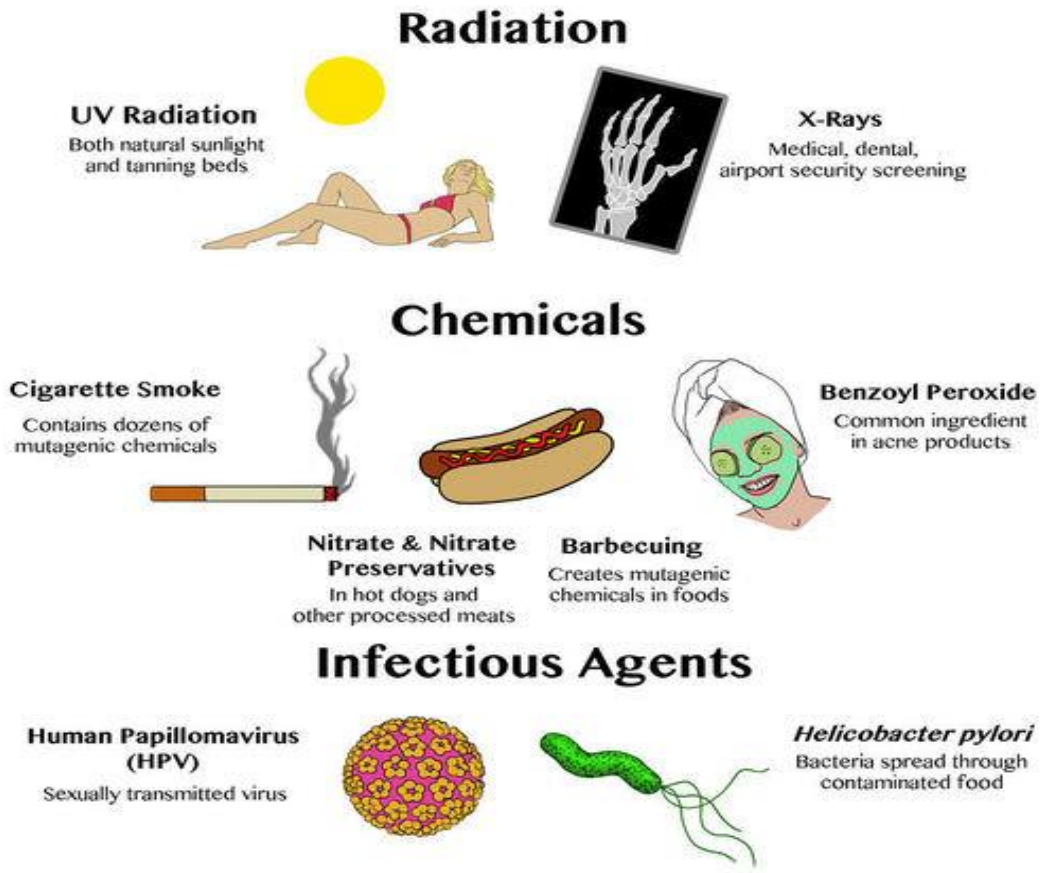
3. Ionizing radiation such as that created by radioactive decay or in cosmic rays causes breaks in DNA strands. Low level ionizing radiation may induce irreparable DNA damage (leading to replicational and transcriptional errors needed for neoplasia or may trigger viral interactions) leading to pre-mature aging and cancer.

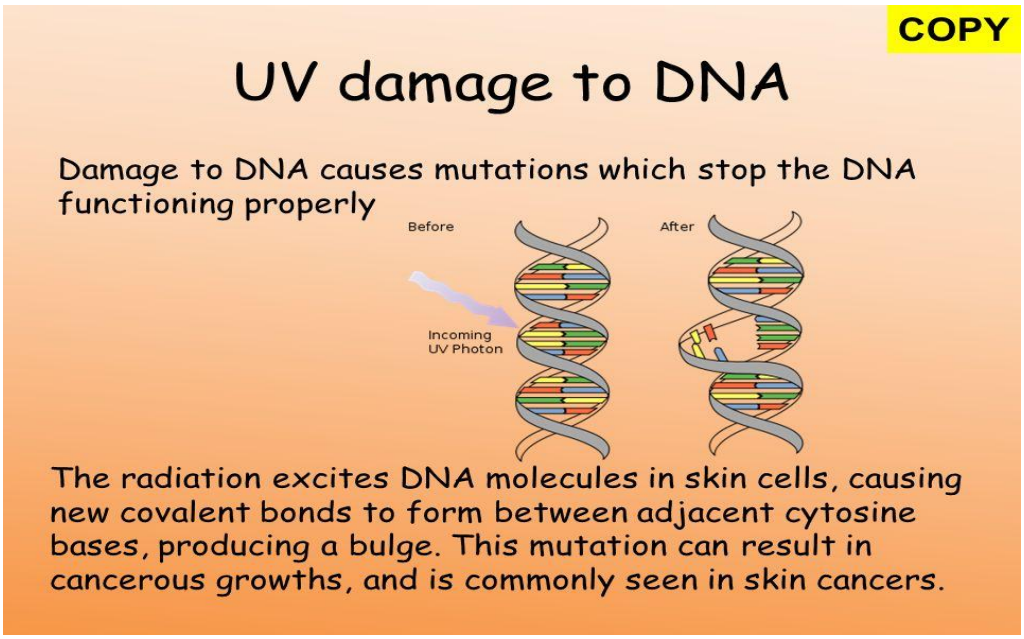
4. Thermal disruption at elevated temperature increases the rate of depurination (loss of purine bases from the DNA backbone) and single strand breaks. For example, hydrolytic depurination is seen in the thermophilic bacteria, which grow in hot springs at 40-80 °C. The rate of depurination (300 purine residues per genome per generation) is too high in these species to be repaired by normal repair machinery, hence a possibility of an adaptive response cannot be ruled out.

5. Industrial chemicals such as vinyl chloride and hydrogen peroxide, and environmental chemicals such as polycyclic hydrocarbons found in smoke, soot and tar create a huge diversity of DNA adducts- ethenobases, oxidized bases, alkylated phosphotriesters and Crosslinking of DNA just to name a few.

UV damage, alkylation/methylation, X-ray damage and oxidative damage are examples of induced damage. Spontaneous damage can include the loss of a base, deamination, sugar ring puckering and tautomeric shift.



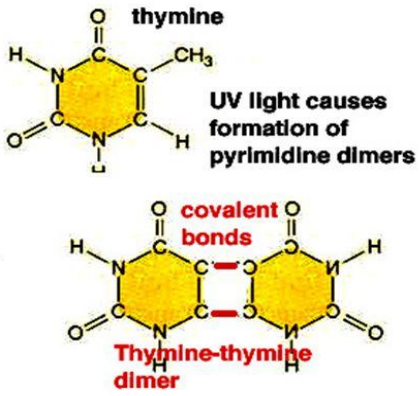

Radiation
UV Radiation
Both natural sunlight and tanning beds
X-Rays
Medical, dental, airport security screening
Chemicals
Cigarette Smoke
Contains dozens of mutagenic chemicals
Benzoyl Peroxide
Common ingredient in acne products
Nitrate & Nitrate Preservatives
In hot dogs and other processed meats
Barbecuing
Creates mutagenic chemicals in foods
Infectious Agents
Human Papillomavirus (HPV)
Sexually transmitted virus
Helicobacter pylori
Bacteria spread through contaminated food


COPY
UV damage to DNA
Damage to DNA causes mutations which stop the DNA functioning properly
Before
After
Incoming UV Photon
The radiation excites DNA molecules in skin cells, causing new covalent bonds to form between adjacent cytosine bases, producing a bulge. This mutation can result in cancerous growths, and is commonly seen in skin cancers.

15

Radiation

- UV light at 265 nm
  - causes thymine dimers; covalent connections between adjacent thymines. Hurried repair makes mistakes.
- Ionizing radiation
  - short wavelength, high energy radiation, e.g. x-rays, gamma radiation.
  - Causes ss, ds breaks in DNA.



http://academic.brooklyn.cuny.edu/biology/bio4fv/page/molecular%20biology/mutation-prym-dimers.jpeg

AMES TEST

The **Ames test** is a widely employed method that uses bacteria to test whether a given chemical can cause mutations in the DNA of the test organism. More formally, it is a biological assay to assess the mutagenic potential of chemical compounds.[1] A positive test indicates that the chemical is mutagenic and therefore may act as a carcinogen, because cancer is often linked to mutation. The test serves as a quick and convenient assay to estimate the carcinogenic potential of a compound because standard carcinogen assays on mice and rats are time-consuming (taking two to three years to complete) and expensive.

Principle

Ames test is developed by **Bruce N. Ames** in 1970s to test for determining if the chemical is mutagens. This test is based on the principle of **reverse mutation or back mutation**. So, the test is also known as **bacterial reverse mutation assay.**

- **Test organism:** Ames test uses several strains of bacteria (*Salmonella*, *E.coli*) that carry mutation. Eg A particular strain of *Salmonella* Typhimurium carry mutation in gene that encodes histidine. So it is an auxotrophic mutant which loss the ability to synthesize histidine (an amino acid) utilizing the ingredients of culture media. Those strains are known as **His-** and require histidine in growth media.

- Culturing His- salmonella is in a media containing certain chemicals, causes mutation in histidine encoding gene, such that they regain the ability to synthesize histidine **(His+)** This is the reverse mutation. Such chemicals responsible to revert the mutation is actually a mutagen. So, this Ames test is used to test mutagenic ability of varieties of chemicals.

Procedure:

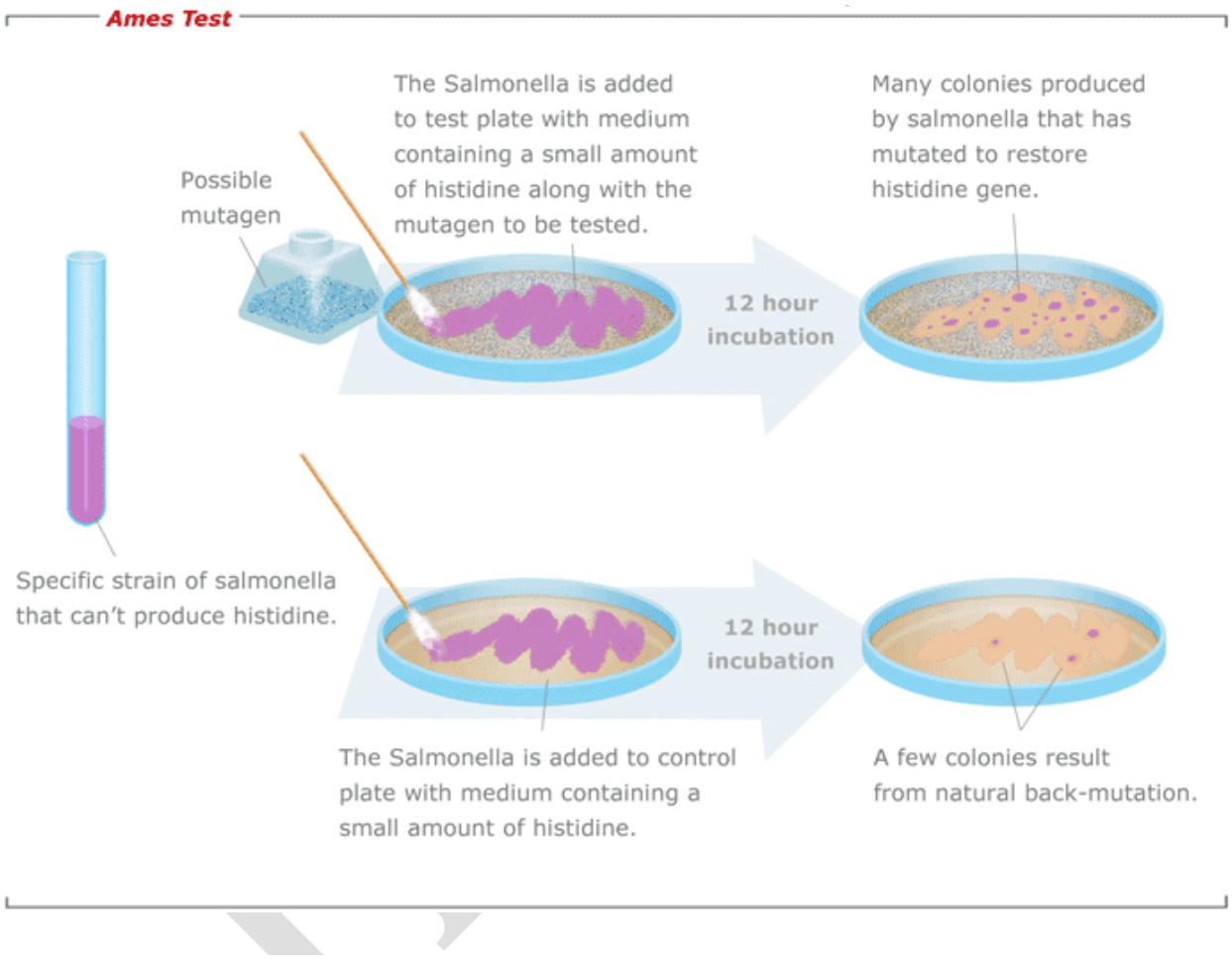


figure: Ames test

i) Isolate an auxotrophic strain of *Salmonella* Typhimurium for histidine. (ie. His-ve)

ii) Prepare a test suspension of his-ve *Salmonella* Typhimurium in a plain buffer with test chemical (let's say 2-aminofluorene). Also add small amount of histidine.

Ps: small amount of histidine is required for initial growth of bacteria. Once histidine is depleted only those bacteria mutated to gain the ability to synthesize histidine form colony.

iii) Also prepare a control suspension of His-ve *Salmonella* Typhimurium but without test chemicals.

iv) Incubate the suspensions at 37°C for 20 minutes

v) Prepare the two agar plate and spread the suspension on agar plate.

vi) Incubate the plates at 37°C for 48 hours.

vii) After48 hours count the number of colonies in each plate. The mutagenicity of chemicals is proportional to number of colonies observed. If large number of colonies on test plate is observed in comparison to control, then such chemical are said to be mutagens.

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

Application:

1. The practical application of Ames test is to screen chemical mutagens that causes mutation and are carcinogenic to human and animals. Some of the chemicals used as food additive (AF-2), flavoring agent (Safrole) are mutagenic as well carcinogenic.
2. Isoniazid; an anti TB drug is also mutagens.
3. Ames test adopted to use eukaryotic cell culture, yeast cell, as well as animal model to test mutagens. Since, Salmonella is not a best test organism to test mutagens for Human. Certain chemicals initially are not mutagens to human but convert into mutagens when metabolized (acted upon by body enzymes). For example; sodium nitrate (NaNO3) is not mutagens until it is acted upon by HCL in stomach to form Nitrous oxide HNO2 (a potent mutagen).
4. Ames test can detects Suitable mutants in large population of bacteria with high sensitivity.
5. It is test for mutagenicity not carcinogenicity. However, most of the mutagens (more than 90%) detected by Ames test are responsible to cause cancer.
6. It is a bacterial reverse mutation assay. So the defective gene of bacteria can be mutated into functional gene.

VARIOUS MODES OF DNA REPAIR
DNA REPAIR MECHANISMS

Repair of **single strand break**

When only one of the two strands of a double helix has a defect, the other strand can be used as a template to guide the correction of the damaged strand. In order to repair damage to one of the two paired molecules of DNA, there exist a number of excision repair mechanisms that remove the damaged nucleotide and replace it with an undamaged nucleotide complementary to that found in the undamaged DNA strand.

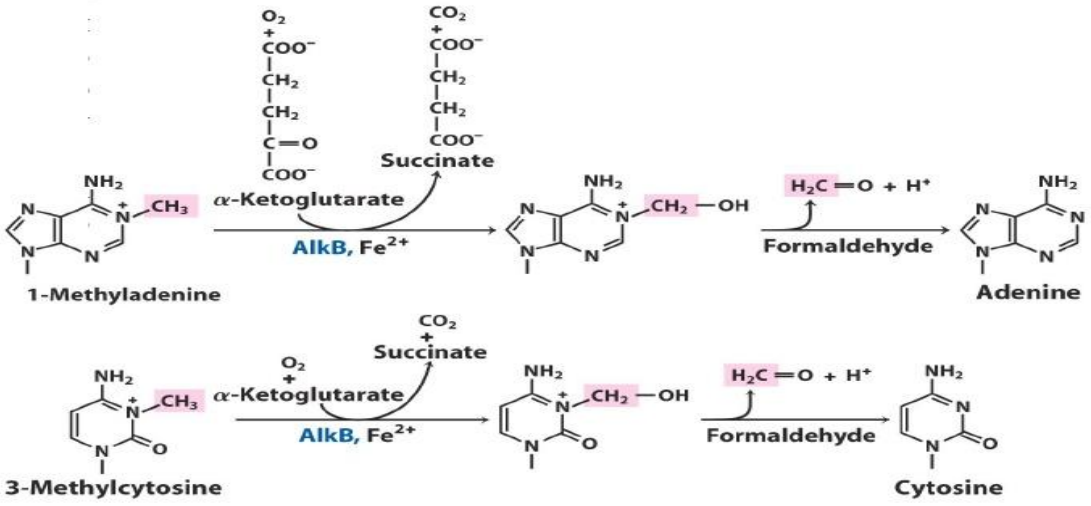
They are

1. Direct reversal
2. Base exicision repair
3. Nucleotide excision repair
4. Mismatch repair

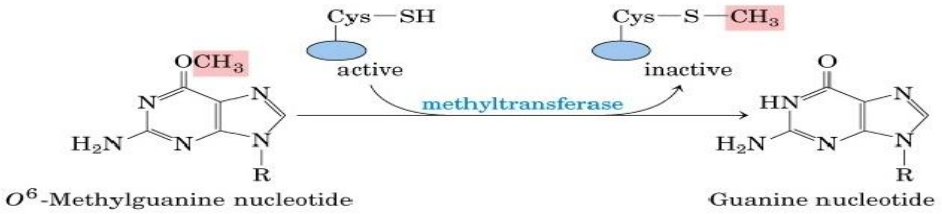
1.Direct reversal

Cells are known to eliminate three types of damage to their DNA by chemically reversing it. These mechanisms do not require a template, since the types of damage they counteract can only occur in one of the four bases. Such direct reversal mechanisms are specific to the type of damage incurred and do not involve breakage of the phosphodiester backbone. The formation of pyrimidine dimers upon irradiation with UV light results in an abnormal covalent bond between adjacent pyrimidine bases. The photoreactivation process directly reverses this damage by the action of the enzyme photolyase, whose activation is obligately dependent on energy absorbed from blue/UV light (300–500 nm wavelength) to promote catalysis. Another type of damage, methylation of guanine bases, is directly reversed by the protein methyl guanine methyl transferase (MGMT), the bacterial equivalent of which is called ogt. This is an expensive process because each MGMT molecule can only be used once; that is, the reaction is stoichiometric rather than catalytic. A generalized response to methylating agents in bacteria is known as the adaptive response and confers a level of resistance to alkylating agents upon sustained exposure by upregulation of alkylation repair enzymes. The third type of DNA damage reversed by cells is certain methylation of the bases cytosine and adenine.

Direct repair of alkylated bases by AlkB.



Direct Repair: Reversal of O6 methyl G to G by methyltransferase



2.Base excision repair (BER)

This repairs damage to a single base caused by oxidation, alkylation, hydrolysis, or deamination. The damaged base is removed by a DNA glycosylase. The "missing tooth" is then recognised by an enzyme called AP endonuclease, which cuts the Phosphodiester bond. The missing part is then resynthesized by a DNA polymerase, and a DNA ligase performs the final nick-sealing step.

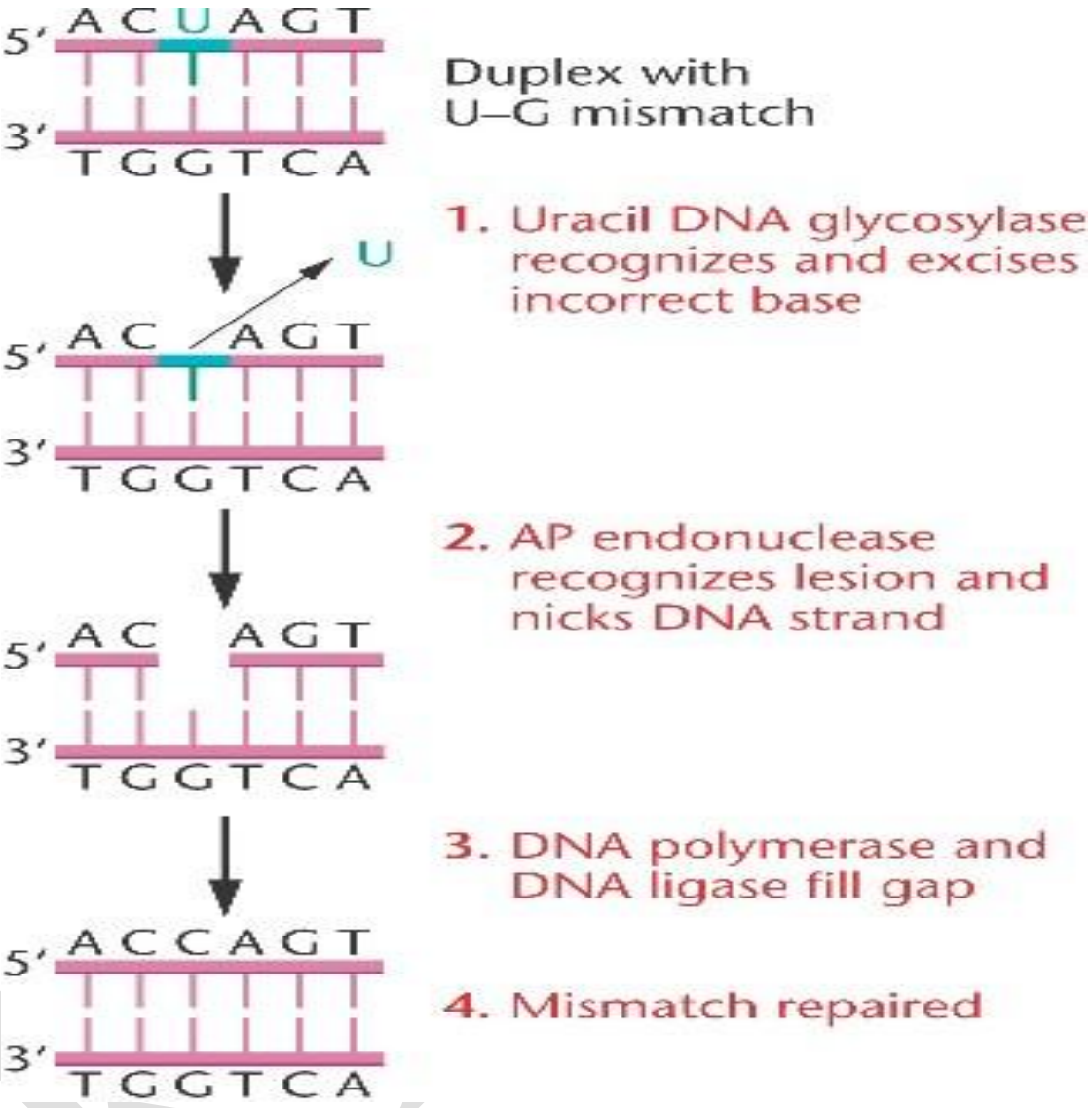
Steps in base excision repair in *E. coli*.

A base pair **mismatch** is recognized by DNA glycosylase, which removes the base from the sugar, creating an apurinic/apyrimidinic (**AP**) site.

AP endonuclease makes a cut in the phosphodiester backbone at the **AP** site.

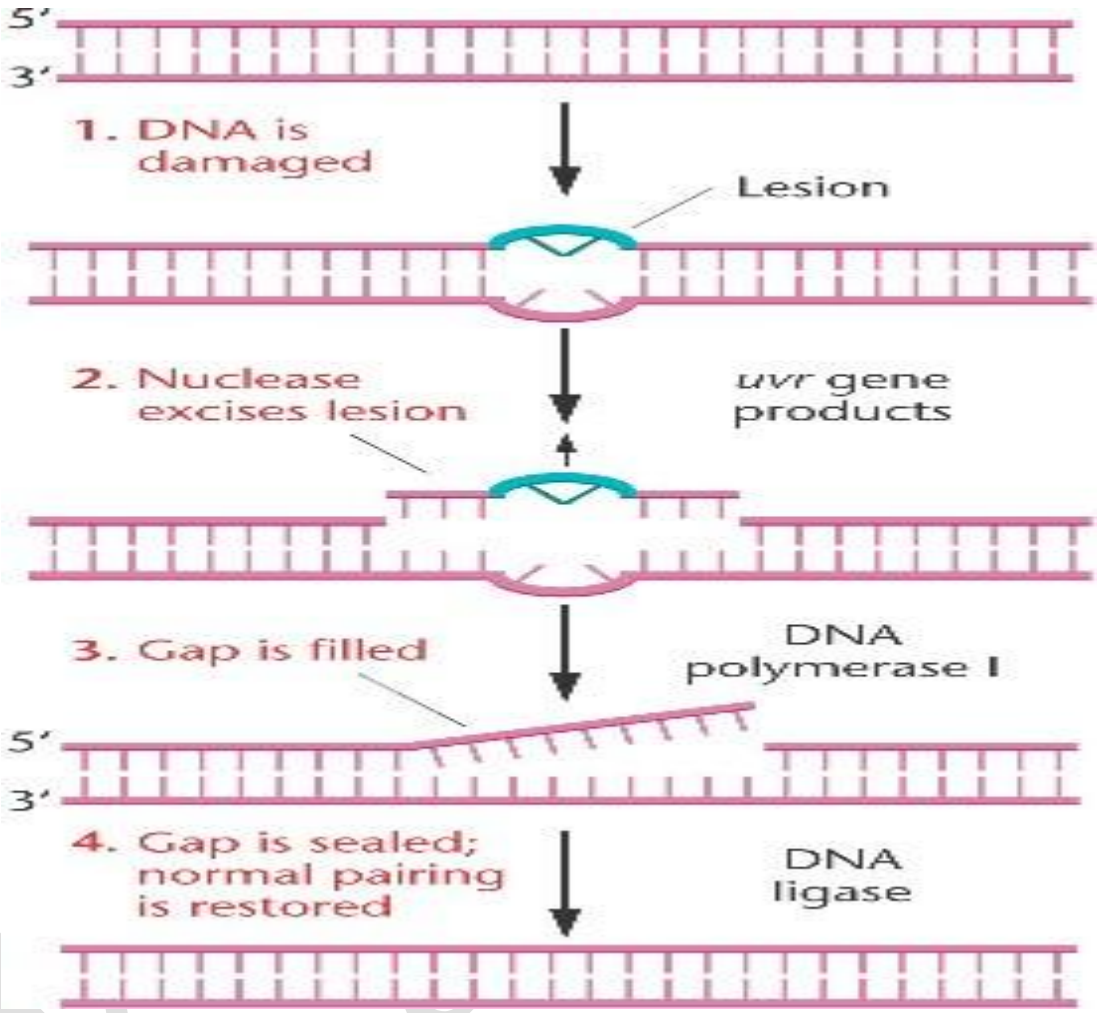
The gap is filled by DNA polymerase I and DNA ligase

The error is corrected.



3.Nucleotide excision repair (NER),

This recognizes bulky, helix-distorting lesions such as pyrimidine dimers and 6,4 photoproducts. A specialized form of NER known as transcription-coupled repair deploys NER enzymes to genes that are being actively transcribed.

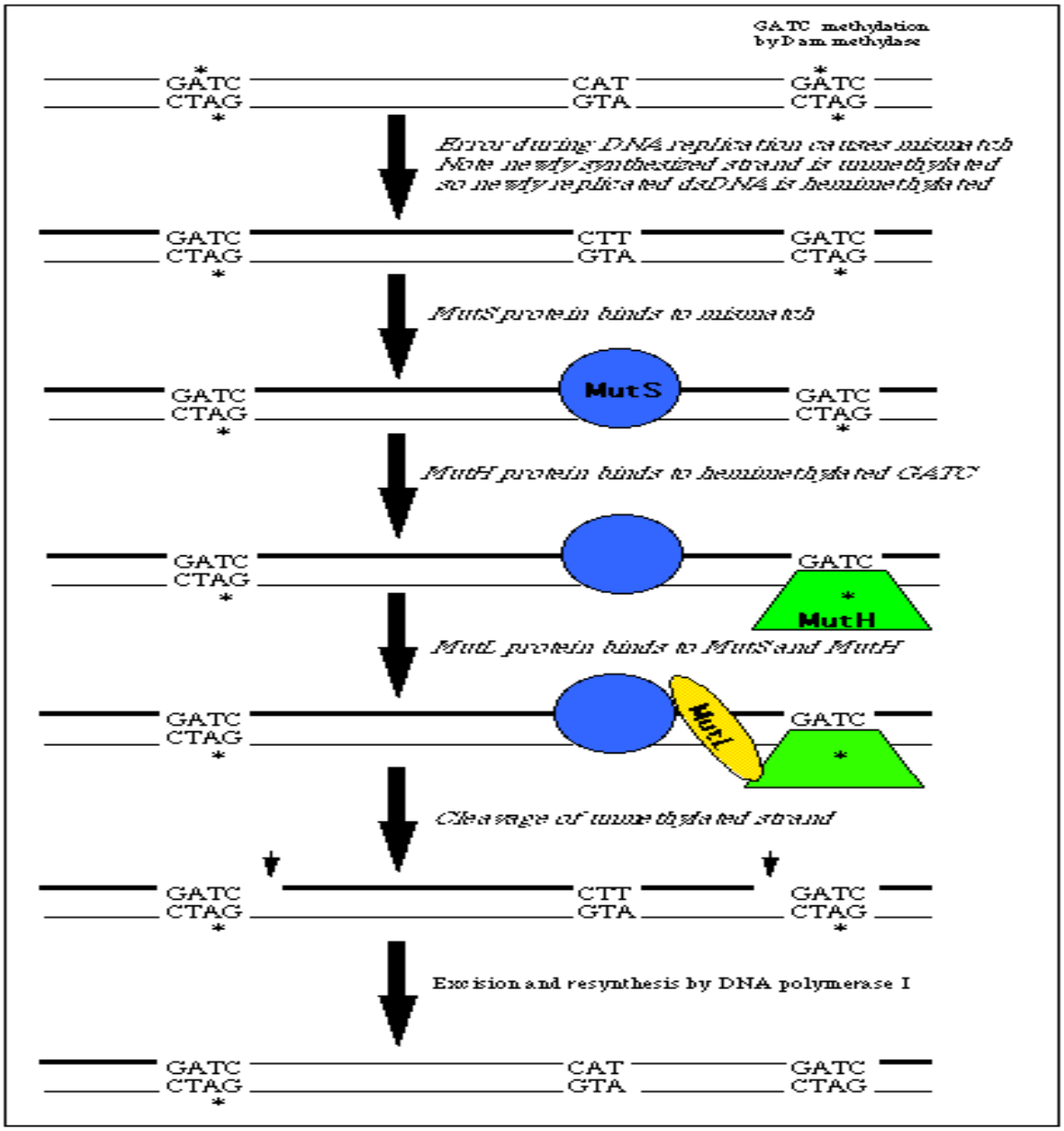


1. Damaged DNA is recognized by **uvr** (ultraviolet repair) proteins.
2. A number of nucleotides is clipped out around the **lesion** by a **nuclease**.
3. The **gap** is filled by **DNA polymerase I** and **DNA ligase**
4. The error is corrected.

4. Mismatch repair (MMR)

Errors of DNA replication and recombination that result in mispaired (but undamaged) nucleotides are corrected by this mechnism

Mismatch repair of newly replicated DNA by the MutSLH proteins is depicted in the diagram.



Repair of double strand break

Homologous recombination requires the presence of an identical or nearly identical sequence to be used as a template for repair of the break. The enzymatic machinery responsible for this repair process is nearly identical to the machinery responsible for chromosomal crossover during meiosis. This pathway allows a damaged chromosome to be repaired using a sister chromatid (available in G2 after DNA replication) or a homologous chromosome as a template. DSBs caused by the replication machinery attempting to synthesize across a single-strand break or

unrepaired lesion cause collapse of the replication fork and are typically repaired by recombination.This involves the protein Rec A and Rec BCD enzymes.

5. Recombinational repair

recombinational DNA repair is critical for the survival of UV radiation-damaged cells. It accounts for about 50% of the survival of UV irradiated *Escherichia coli* (see below). It is a very complicated process that requires two DNA duplexes, and the exchange of a strand of DNA from one DNA duplex to the other

Most DNA repair mechanisms rely on the redundant information inherent to the structure of the DNA double helix to fix the damage: The base that is mismatched, modified, adducted, or fragmented is removed to generate a single-stranded gap, which is then filled in by DNA polymerase, using the complementary strand as template, and ligated. Lesions that affect both strands of the duplex cannot be repaired by this general reaction mechanism. Such lesions can be rectified only by retrieving the lost information from a homologous duplex in diploid organisms, or from the sister chromatid in haploid organisms such as Escherichia coli, which under exponential growth conditions is partially diploid for a major fraction of its chromosome. Three types of lesion affect both strands and are repaired by recombinational repair: post-replication gaps across from a lesion, interstrand cross-links, and double-strand breaks (Fig. 1).

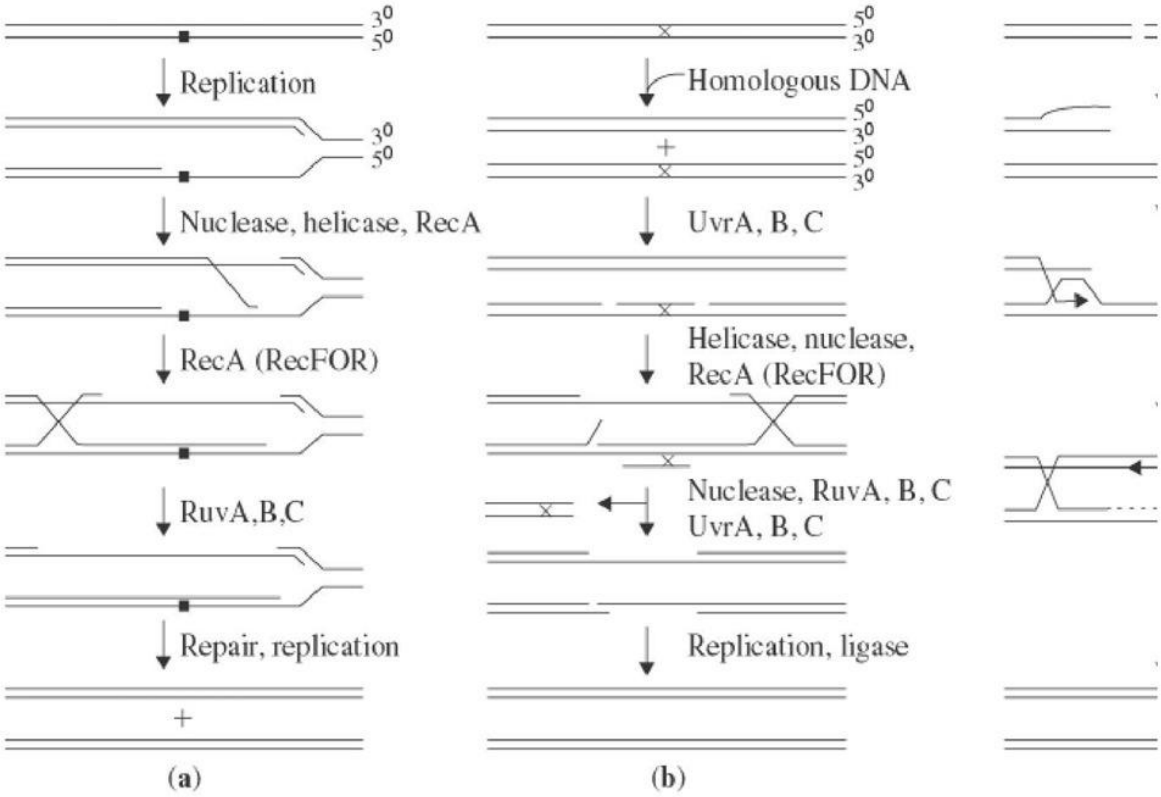


Figure 1. Mechanisms of recombinational repair. (a) In post-replication repair a lesion in one strand leads to gap formati gap is invaded by the complementary strand from the sister duplex. Following further processing by nucleases and DNA junction is formed which is resolved by RuvABC resolvase. The remaining lesion in the duplex is then removed by excis link repair, (A)BC excinuclease makes dual incisions in one strand, and the cross-linked oligomer is displaced by the rec generates a Holliday structure and a "dangling" oligomer cross-linked to the duplex. This structure is recognized as a mo excinuclease and is released by dual incisions. The Holliday structure is resolved and the gaps resulting from recombinat in by polymerases and ligated. (c) In double-strand break the RecBCD helicase/nuclease unwinds the duplex from both s generates a structure which can be processed by the RecA strand transfer activity.

1. Post-replication Repair

When the E. coli replication machinery encounters certain nucleotide adducts, such as pyrimidine dimers, it stops replicating and reinitiates about 1000 base pairs beyond the adduct, generating a single-stranded gap that contains a damaged nucleotide (1). At the same time, a normal duplex is produced from the complementary strand. Thus, replication of a damaged duplex gives rise to one duplex with two normal strands and one partial duplex with a lesion in one strand and a gap in the other. The duplex with the defect is repaired by a process that involves both recombination and excision repair. The RecA protein forms a helical filament at the post-replication gap and promotes homologous pairing with the intact sister duplex. This is followed by reciprocal strand exchange, so that the gap is "transferred" from the damaged duplex to the undamaged duplex, concomitant with the formation of a Holliday intermediate (2, 3). The latter is resolved by a resolvase encoded by the ruvABC or rusA genes. Filling in the gap by DNA polymerase, using the intact strand as template, yields two uninterrupted duplexes, one of which still contains a damaged base which can now be eliminated by a conventional excision repair reaction (4).

2. Interstrand Cross-link Repair

Many carcinogenic or chemotherapeutic chemicals, including formaldehyde, nitrous acid, nitrogen and sulfur mustards, mitomycin C, cisplatin, and psoralen plus light, cross-link the two strands of the duplex covalently. Such cross-links prevent separation of the two strands and hence constitute absolute blocks for transcription and replication. Because of this effect on strand separation, crosslinks constitute a more challenging lesion to cellular repair machinery than the post-replication gaps. Neither strand of the cross-linked DNA can be replicated to generate an intact duplex and a doubly damaged duplex that could recombine to produce two contiguous duplexes. Instead, the cross-link must be processed by the excision repair machinery. The mechanism of interstrand cross-link repair is relatively well-understood in E. coli (9). First, the (A)BC excinuclease incises the ninth phosphodiester bond 5′ of the cross-linked base, as well as the third phosphodiester bond 3′, in one strand only. Second, the resulting "excised" oligomer is displaced by RecA, which may form a filament at the gap and promote strand invasion of the homologous duplex. The recombination reaction generates a gapped duplex from the initially undamaged DNA and a triple-strand intermediate, in which the cross-linked oligomer (12-mer) is

flipped out of the duplex. This latter structure is recognized as a form of monoadduct by the (A)BC excinuclease, which cuts out the dangling oligomer and produces a 12-nucleotide gap in one strand. The end-product of dual incision-recombination-dual incision reactions is to generate two duplexes, each with about a 12-nucleotide gap in one strand. These gaps are filled and ligated to complete repair

3. Double-Strand Break Repair

he basic mechanism of double-strand break repair in E. coli appears to be quite similar to the mechanism of cross-link repair. Again, RecA aided by RecBCD helicase/nuclease promotes strand invasion from the site of the double-strand break, using the homologous duplex as a target. The proposed reaction pathway generates two Holliday junctions, which are resolved by the RuvABC or rusA resolvase systems (15, 16). In mammalian cells, the double-strand break repair does not appear to employ homologous recombination. Instead, the DNA-dependent protein kinase (DNA-PK) complex appears to play a crucial role in restoring the continuity of the broken duplex. The complex consists of a Ku70/Ku80 heterodimer, which has affinity for DNA ends, binds to the site of a double-strand break and recruits the DNA-PK catalytic subunit (DNA-PKcs), a 470-kDa protein with serine/threonine kinase activity to the site of the double-strand break.

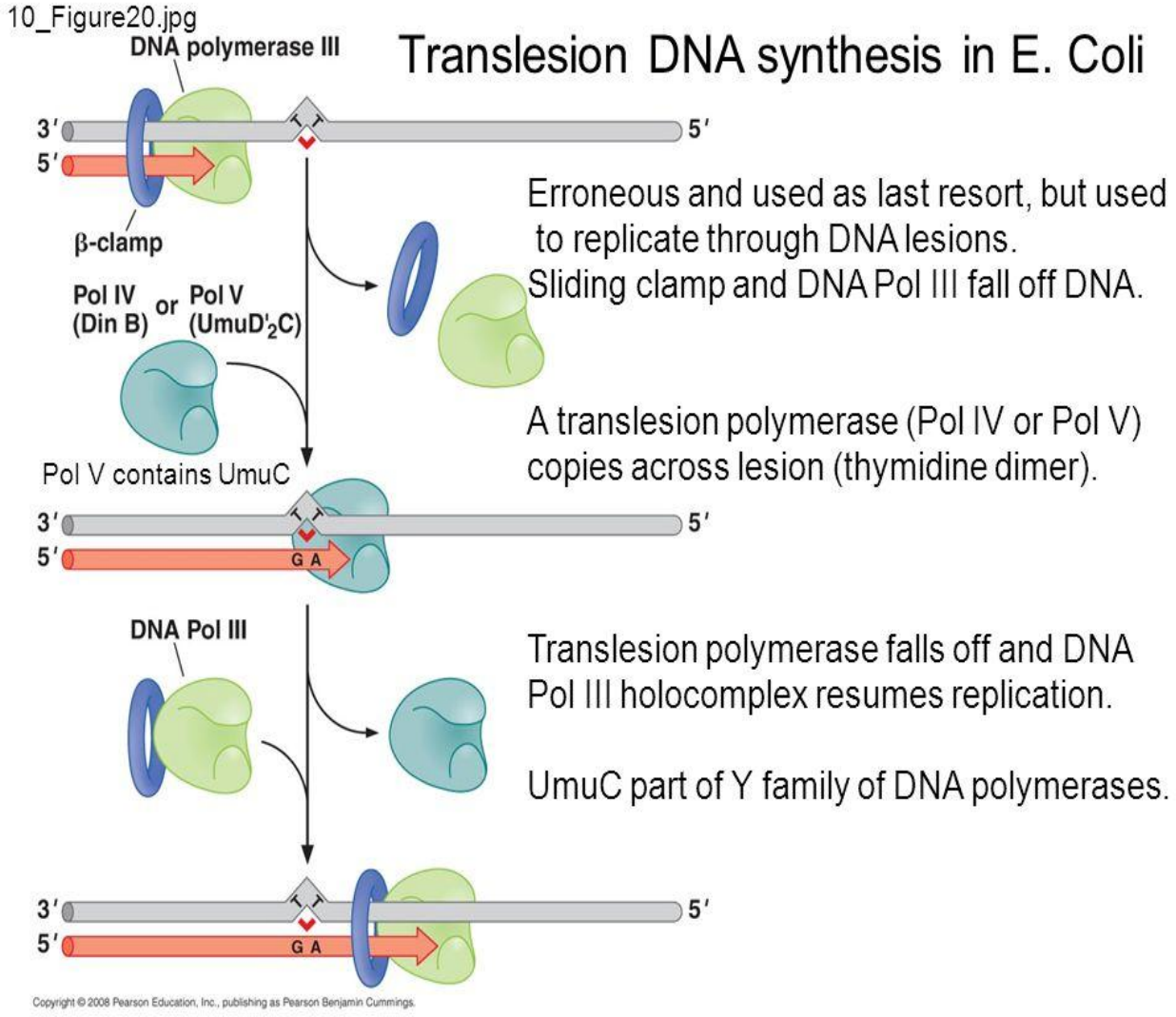
4. **Translesion DNA synthesis**

In certain types of s uch as double-strand breaks, double-strand cross-links ,or lesions in a single-stranded DNA,t he complementary strand is itself damaged or is absent.Double-strand breaks and lesions in single-stranded DNA most often arise when a replication fork encounters an unrepaired DNA lesion. Such lesions and DNA cross-links can also result from ionizing r dration and oxidative reactions.

At a stalled bacterial replication fork, there are two avenues fo r repair. In the absence of a second strand,the information required for accurate repair must come from a separate homologous chromosome. The repair system thus involves homologous genetic recombination.

This recombinational DNA repair is considered in detail already. Under some conditions, a second repair pathway, error-prone translesion DNA synthesis (often abbreviated TLS), becomes available w hen this pathway is active, DNA repair becomes signiflcantly less accurate and a high mutation rate can result. In bacteria,error-prone translesion DNA synthesis is part of a cellular stress response to extensive DNA damage known, Appropriately enough, as the SOS response. Some SOS proteins, such as the umuA and umuB proteins already described, are normaliy present in the cell but are induced to higher levels as part of the SOS response. Addrtional SOS proteins participate in the pathway for error-prone repair; these include the UmuC and UmuD proteins. The UmuD protein is cleaved in an SoS-regulated process to a shorter form called UmuD', which forms a complex with UmuC to create a specialized DNA polymerase, DNA polymerase V, that can replicate past many of the DNA lesions that would

normally block replication. Proper base pairing is often impossible at the site of sucha lesion, so this translesion replication is error-prone.



Karpagam Academy of Higher Education
Department of Biochemistry
II BSc Biochemistry
Gene Organisation, Replication and Repair (16BCU401)

UNIT V- POSSIBLE QUESTIONS

2 Mark - Questions

1. How mutation helps in the evolution of species?
2. What is mutation? List the types of mutations
3. Differentiate transition and transversion mutation
4. What are chemical mutagens? Give the role of any one mutagen in mutation process
5. Give the role of transposable elements in mutation process
6. What is the importance of Ames test?
7. What are the types of DNA damages?
8. List the various types of DNA repair
9. Add note recombinational repair
10. What do you mean by translesion DNA synthesis?
11. List the DNA damages caused by radiation
12. What are intercalating agents? Describe their role in mutation

Essay type Questions (8 Marks)

1. Explain the role of glycosylase and ligase in DNA repair
2. Explain the photo reactivation repair of DNA and removal of DNA adduct
3. Explain the various types of mutation in detail.
4. Explain the importance and mechanism of Post replication repair and SOS response
5. Explain the mechanism of UV repair and mismatch repair
6. Describe the DNA damages caused by chemicals and radiation

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KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
II B.Sc BIOCHEMISTRY- Fourth Semester
GENE ORGANISATION, REPLICATION AND REPAII (16BCU401)
MULTIPLE CHOICE QUESTIONS
UNIT I

S.No	Unit	Questions	Option 1	Option 2	Option 3	Option 4	Answer
1	I	The macromolecule regarded as the reserve bank of genetic information	Protein	**DNA**	RNA	Polysaccharide	DNA
2	I	The biological information flows from DNA to RNA and from RNA to	lipids	carbohydrates	proteins	nucleotides	proteins
3	I	The total genetic information contained in a DNA is referred to as	gene	genome	Okazaki piece	ribozyme	genome
4	I	The DNA base pairing is based on _____ rules	Chargaff's rule	Arther Kornberg	Stahl & Meselson	McClintock	Chargaff's rule
5	I	Pseudogenes are	Related to non functional genes	Transcribed into mRNA	Translated in to functional proteins	Transcribed into tRNA	Related to non functional genes
6	I	Mobile genetic elements were visualized by	T.H Morgan	Barbara McClintock	G Khorana	C.B Bridge	Barbara McClintock
7	I	Fundamental unit of DNA organization	Replisome	Nucleosome	Primosome	Chromatin	Nucleosome
8	I	Histones are rich in	Arg and Lys	Lys & Gly	Arg & Glu	Arg & Gly	Arg and Lys
9	I	Which histone protein is not a part of core particle of nucleosome?	H1	H2a	H2b	H4	H1
10	I	Which histone protein is involved in the transition between the solenoid form and the extended nucleosome form?	H1	H2a	H2b	H4	H1
11	I	Smaller blocks occur at the end of chromosome arm is called _____	centromere	telomere	blastomeres	blastocyst	telomere
12	I	The functional unit of DNA is	genome	gene	nucleotide	chromosome	nucleotide
13	I	The coiling that cannot be separated except by unwinding is called	supercoiling	negative super coiling	plectonemic coiling	anti parellel coiling	plectonemic coiling
14	I	Self integrating DNA fragments are known as	Transposons	Self posons	Transducers	Transfragments	Transposons
15	I	The molecular chaperone which causes the nucleosome assembly is	Nucleoplasmin	Histone	Hu protein	Ubiquitin	Histone
16	I	Simple sequence DNA is concentrated in	Centromere	Telomere	Blastomeres	Blastocyst	Centromere
17	I	LTRs are absent in	LINES	SINES	LINES AND SINES	Viral retrotransposons	LINES AND SINES
18	I	Chicken lysozyme gene is a good example of	Single copy DNA	Moderately repetitive DNA	Simple sequence DNA	Highly repetitive DNA	Single copy DNA
19	I	Difference in length of simple sequence tandem arrays helps to develop a technique called	Foot printing	Northern blotting	Western	Finger printing	Finger printing
20	I	Important hallmark of IS element is	Short direct repeats	Long direct repeats	Inverted repeats	Tandem repeats	Short direct repeats
21	I	IS element contain _____ enzyme	Helicase	primase	Transposase	topoisomerase	transposase
22	I	The length of the DNA segment present in the nucleosome core particle is	140 bp	200 bp	166 bp	114 bp	166 bp
23	I	The housekeeping genes have	GC box	TATA box	Pribnow box	CAAT box	GC box
24	I	Negative supercoils are removed by	Topoisomerase I	Gyrase	Helicase	Rep protein	Topoisomerase I
25	I	DNA chains differ from each other by one nucleotide can be revolved using	20% agarose	20% polyacrylamide	1% agarose	1% polyacrylamide	1% agarose
26	I	Satellite DNAs are found in the region of	Euchromatin	Heterochromatin	Hypervariable regions	Functional elements	Heterochromatin
27	I	Polytene chromosomes are produced by	Gene inversion	Gene conversion	DNA amplification	Retrotransposons	DNA amplification
28	I	In conjugation DNA is transformed from	Conjugation tube	Bacteriophage	Dead organism	Mice	Dead organism
29	I	Dead organism of pneumococci was completely inactivated by _____ enzymes	DNAase	Amylase	Lipase	Protease	DNAase
30	I	Nonvirulent in pneumococci is imparted by	Polysaccharide	Protein	Lipid	Lipopolysaccharide	Lipopolysaccharide
31	I	In DNA, the genetic information residues in	Purine bases	Pyrimidine bases	Purine and Pyrimidine bases	Sugar	Purine and Pyrimidine bases
32	I	Important characteristic of satellite DNA is, they contain	Repetitive base sequence	GC rich	Unique sequence	AT rich	GC rich
33	I	LINES and SINES come under _____ DNA	Single copy DNA	Moderatively repetitive DNA	Simple sequence DNA	RNA	Moderatively repetitive DNA
34	I	What is the difference between II S and II R strains of bacteria used in Griffith's experiment?	III S strain covers itself with a polysaccharide coat	III S strain covers itself with a protein coat	II R strain covers itself with a protein coat	II R strain covers itself with a polysaccharide coat	III S strain covers itself with a polysaccharide coat
35	I	Avery, Macleod and Mc Carty's experiment was conducted in the year	1928	1974	1964	1938	1974
36	I	To explore the genetic properties of DNA Hershey and Chase used	bacteria	bacterial viruses	fungi	dog	bacterial viruses
37	I	Conjugation was discovered by	Zinder and Ledenberg	Lederberg and Tatum	Frederick Griffith	Hershey and Chase	Lederberg and Tatum
38	I	In conjugation the integration of F factor is mediated by	transposon	IS elements	viruses	simple sequence DNA	IS elements
39	I	The DNA in the chromatin is very tightly associated with proteins called	albumin	globulin	myosin	histones	histones
40	I	The molecular weight of histones is	between 11,000 and 21,000	between 1,000 and 11,000	between 8,000 and 20,000	between 1,000 and 31,000	between 11,000 and 21,000
41	I	Histones are very rich in	acidic amino acids	basic amino acids	neutral amino acids	aromatic amino acids	basic amino acids
42	I	Number of amino acids in H4 histones is	104	108	102	120	102
43	I	The bead of each nucleosome contains	eight histone molecules	ten histone molecules	two histone molecules	eight histone molecules	eight histone molecules
44	I	The number of base pairs serves as linker DNA between nucleosome beads is	200 bp	146bp	54 bp	45 bp	54 bp
45	I	The spacing of the nucleosome beads provides a repeating unit typically of about	200 bp	146bp	54bp	45bp	200 bp
46	I	The total number of base pairs bound tightly around the eight-part histone core are	200 bp	146 bp	54 bp	45 bp	146 bp
47	I	X-ray diffraction analysis of nucleosome reveals that it is a	left-handed solenoidal supercoil	right-handed solenoidal supercoil	left handed double helical supercoil	right handed double helical supercoil	left-handed solenoidal supercoil

48	I	Specific binding sites of histone core to DNA is	A=T rich base pairs	G≡C rich base pairs	equal ratio of both base pairs	1:2 ratio of both the base pairs	A=T rich base pairs
49	I	The SMC protein play a substantial role in linking together sister chromatids is	cohesins	condensins	albumins	globulins	cohesins
50	I	A well studied example of a solitary protein coding gene is	lactate dehydrogenase gene	protein kinase gene	chicken lysozyme gene	Immunoglobulin gene	protein kinase gene
51	I	According to the lac-operon concept, which functional unit of the bacterial gene material is responsible for suppressing the activity of the operator gene in the absence of lactose?	Promoter gene	Repressor protein	Regulator gene	Structural gene	Regulator gene
52	I	The inducer for the lac operon is:	lactose	allolactose	β-galactosidase	galactose	allolactose
53	I	Which of the following genes is not a structural gene of the lac operon?	lacA	lac I	lacY	lacZ	lac I
54	I	Attenuation of the trp operon:	Occurs when transcription is complete before translation begins.	Is mediated by the trp repressor protein.	Occurs in the presence of high levels of tryptophan	Occurs in the presence of high levels of glucose	Occurs in the presence of high levels of tryptophan.
55	I	When both glucose and lactose are present	cyclic AMP is high so transcription occurs	the lac repressor binds with the lactose and transcription occurs	RNA polymerase is able to bind to the operator so transcription occurs	transcription is turned off	transcription is turned off
56	I	The lac repressor	binds to the operator and prevents transcription	binds to the CAP site and prevents transcription	binds to the CAP site and facilitates transcription	binds to the operator and facilitates transcription	binds to the operator and prevents transcription
57	I	In the lactose operon, the molecule that causes the repressor to fall off the promoter is which of the following?	a corepressor	an attenuator	an inducer	a regulatory protein	an inducer
58	I	Satellite DNAs are found in the region of	Euchromatin	Heterochromatin	Hypervariable regions	Functional elements	Heterochromatin
59	I	The total number of base pairs bound tightly around the eight-part histone core are	200 bp	146 bp	54 bp	45 bp	146 bp
60	I	Important characteristic of satellite DNA is, they contain	Repetitive base sequence	GC rich	Unique sequence	AT rich	GC rich
61	I	In the tryptophan operon, tryptophan serves as	corepressor	an inducer	a catalyst	a regulatory protein	corepressor

60	V	The activity of AP endonuclease activity is	Base excision repair	Nucleotide excision repair	Mismatch repair	Double strand break repair	Base excision repair