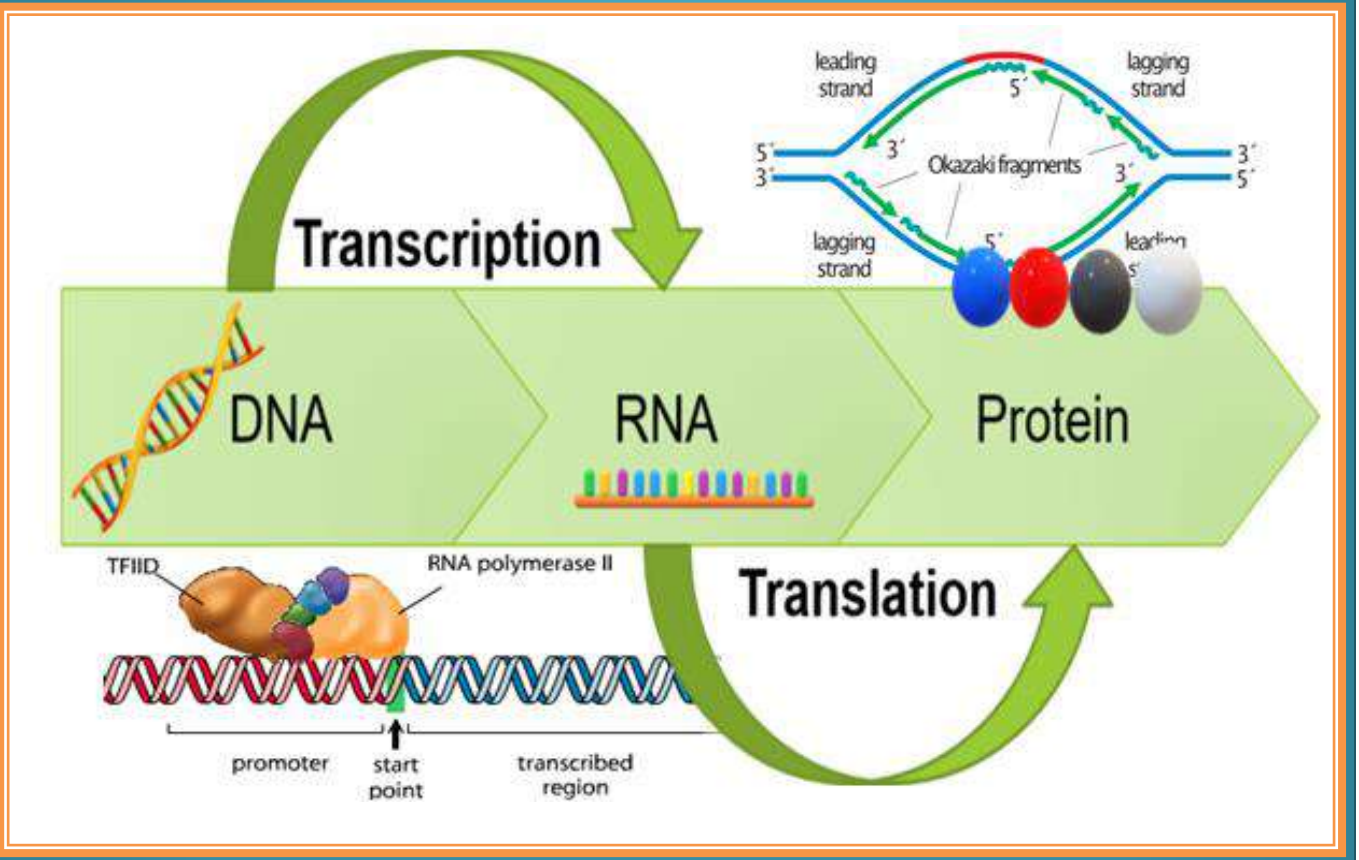
Karnataka State Open University

Mukthagangotri, Mysore-570006

M.Sc. Biotechnology

CBCS Mode

Second Semester



Molecular Biology

MBTDSC- 2.2 **BLOCKS- I, II, III and IV** **UNITS - 1 To 16**

M.Sc. in Biotechnology

SECOND SEMESTER

CBCS MODE

MBTDSE 2. 2 Molecular Biology

Hard Core

(Blocks -I, II, IIIand IV)

MBTDSE 2.2. Molecular Biology: Credits -4

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PUBLISHER

The Registrar, Karnataka State Open University, Mukthagangotri, Mysore-570006

Developed by Academic Section, KSOU, Mysore.

Printed and Published on behalf of Karnataka State Open University, Mysore-570006 by **Registrar (Administration)**

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INTRODUCTION TO MOLECULAR BIOLOGY

Molecular biology is the rapidly changing, dynamic and no doubt it is exciting field fueled by the ability to transfer genetic information between organisms for creating a useful product most exciting and dynamic area of science. Molecular biology also contributes to our understanding of what human beings are and how they fit into this universe. A biological phenomenon is better understood only with the through knowledge at the molecular level. The advancement in medicine and the pharmaceutical industry for the production of new drugs and finding new cures is greatly dependent upon the advancement in the field of molecular biology. Proteins and nucleic acids which carry, transmit, and express the genetic information that defines each living organism are the primary subjects of molecular biology. Understanding these complex macromolecules by different techniques is the heart of molecular biology

Molecular biotechnology is the use of laboratory techniques to study and modify nucleic acids and proteins for applications in human and animal health, agriculture, and the environment. Human genome project has thrown light for various opportunities to create new medicines and treatments, as well as approaches to improve existing medicines.

Molecular biotechnology has applications in development and improvement of drugs, vaccines, therapies, and diagnostic tests that will improve human and animal health and also in plant and animal agriculture, aquaculture, chemical and textile manufacturing, forestry, and food processing. Molecular Biotechnology provides excellent employment opportunities.

In the text of this course maximum attempt has been made to provide complete information on different aspects of molecular biology.All the units have been brought up to date by collecting information from different sources and modified in keeping pace with the learning interest and potential of Open University Students.

Each Unit begins with clearly stated learner-oriented objectives followed by terms important for thorough understanding of the text. Every unit at the end includes key words to easily remember the subject and questions to help the readers to self evaluate their grasp of the concepts.The complete format of self learning material of molecular biologyshould definitely help in creating interest and better learning of different aspects of Biotechnology.The content of this book is organized into 4 blocks, each block with 4 units.The Block I consists of four units (1-4) and describes the History of molecular biology topology, Replication of DNA .The Block II consists of four units (5-8) and deals with prokaryotic and Eukaryotic transcription, structural organization, nuclear export of RNA's and mRNA stability. Block III consists of units (9-12). Unit 9, 10 and 11 explainstranslational Regulation, Translation, Protein localization and. Unit 12 deals with Regulation of gene expression in Prokaryotes: operon concept and different operons.

Block IV consists of units (13-16). In Unit 13 Regulatory elements in prokaryotes, regulation of gene expression are discussed, Unit 14 deals with Regulation of gene expression in Eukaryotes, Unit 15 explains Mechanism of regulation by transcription factors , hormonal regulation of gene expression, post transcriptional control and In unit 16 Antisense RNA and ribozymes and their applications are discussed.

Constructive suggestions, comments and criticism for the improvement of this book are most welcomed.

Dr. N. G. Raju, Chairman,

Department of Biotechnology, KSOU, Mysore.

Block- I

UNIT- 1:

History of molecular biology, DNA and RNA as genetic materials, Experiments of Griffith Avery, MacLeod and McCarty, Hershey and Chase, Lederberg and Tatum, Topoisomerases

Structure of the Unit

1.0. Objectives

After studying this unit you will be able to

- understandthe History of molecular biology.
- discuss about DNA and RNA as genetic materials
- Explain the experiments of Griffith Avery. MacLeod and McCarthy
- differentiate between Hershey and Chase. Lederberg and Tatum experiments.
- summarize the functions of topoisomerases

1.1. Introduction

Molecular biology as a discipline was defined with the convergence of biochemical and genetic techniques. Genetic engineering and recombinant DNA are technologies that made it possible to manipulate genetic material and introduce it into cells. Paul berg received a share of the 1980 Nobel Prize in Chemistry solely for discovering that this technique might be done and then executing it in a test tube for the first time (the other half was shared by Walter Gilbert and Frederick Sanger for studies that enabled efficient DNA sequencing). A comprehension of the methods used to produce the data from which concepts and principles are inferred is essential to comprehending molecular biology because it is an experimental science. All aspects of biological research that investigate cellular mechanisms will employ or utilize various molecular biology tools.

A lot of the focus of molecular biology is on DNA and RNA that are vital to the life process. Scientists and molecular biologists study how molecules function within cells and how they interact with one another. This is why the sciences of genetics, biochemistry, and biophysics are also strongly tied to this one. In addition to researching molecules, scientists can discover how to control them through studying molecular biology. Because of this, molecular biology is a crucial component of a lot of modern research and cutting-edge science. If molecular biology is of interest to you as a subject of study, you probably want to know where molecular biologists typically work. You won't be surprised to find that a lot of molecular biologists wind up working in laboratories. Here, they can carry out experiments, conduct research, and study molecular samples. That being said, molecular biologists can also work in an office setting. In these types of

office jobs, biologists will work with data to try and closely inspect the findings of experiments. It's important to note that whether working in an office or a lab, It's always important to follow safety precautions because molecular biologists may work closely with poisonous substances and potentially dangerous materials. Learn the fundamentals of recombinant DNA technology, often known as DNA cloning, in this unit as we cover some frequently utilised approaches. We'll also delve into more recent genome-wide technologies that enable researchers to examine gene expression on a bigger scale and CRISPR-based methods for editing the genome with unmatched accuracy.

1.2. History of molecular biology

The 1930s saw the fusion of several, in the past separate biological and physical sciences, including physics, biochemistry, genetics, microbiology, and virology. Many physicists and chemists also became interested in what would become molecular biology in the hope of understanding life at its most basic level

Modern molecular biology makes an effort to understand the phenomena of life by starting with the macromolecular characteristics that give rise to such phenomena. The molecular biologist is particularly interested in two classes of macromolecules: 1) Nucleic acids, among which deoxyribonucleic acid (or DNA), the building block of genes is the most well-known; and 2) Proteins, the metabolic building blocks of living things. Therefore, characterising the structure, function, and interactions between these two categories of macromolecules serves as one definition of the field of molecular biology. We may date the so-called "molecular revolution" using these rather constrained criteria, or at the very least, we can create a timeline of its most important advancements.

Although molecular biology is crucial to the present study of life, its origins date back to the 1930s and 1940s, and it became institutionalised in the 1950s and 1960s. Therefore, it should not come as a surprise that many philosophical issues related to molecular biology also happen to be deeply entwined with contemporary events. The four components of molecular biology's development are as follows

1.3. DNA and RNA as genetic materials

Can you identify the chromosomal segment that carries the genetic information? Even though numerous researchers found that the variables causing trait inheritance come from

within the organisms, the answer to this question remained a mystery. None of them recognised the DNA as genetic material. It took a while to establish that DNA serves as the genetic material, even though Mendel introduced the concepts of inheritance in 1865 and Meischer discovered the nucleon at the same time. Humans and nearly all other animals have DNA, also known as deoxyribonucleic acid, as their genetic material. Nearly every cell in a person's body has the same DNA.

What is DNA?

All prokaryotic and eukaryotic cells as well as many viruses include "Deoxyribonucleic Acid," or DNA, which serves as the genetic material that codes the genetic information for the transmission of inherited features. DNA is an organic macromolecule that is made up of two polynucleotide chains that coil around one another to form a double helix structure. DNA contains the genetic material that all living things, including many viruses, need to develop, function, grow, and reproduce.

Properties of Genetic Material

- It should be capable of storing genetic information.
- It should be able to replicate extremely effectively over several generations.
- It ought to serve as the foundation for the transfer of any controlled hereditary traits.
- It must participate in gene activity, which leads to the final expression of the traits within the organism.
- It needs to be able to experience a mutation that gives different creatures different traits.
- It must exhibit a huge variety of forms.

RNA as genetic materials

RNA, also known as ribonucleic acid, is a complex substance with a high molecular weight that plays a role in cellular protein production and, in some viruses, takes the place of DNA (deoxyribonucleic acid) as a carrier of genetic information. The building blocks of RNA are ribose nucleotides, which are nitrogenous bases added to a ribose sugar and joined together by phosphodiester bonds to form strands of various lengths. Adenine,

guanine, cytosine, and uracil are the nitrogenous bases found in RNA, which take the role of thymine in DNA.

Viruses' genomes can be made of DNA or RNA. RNA serves as the genetic material for the majority of plant viruses. Using the tobacco mosaic virus (TMV), Fraenkel-Conrat (1957) performed tests to show that some viruses employ RNA as their genetic material.TMV is a tiny virus made up of one spring-like RNA molecule enclosed in a cylindrical protein coat. On the basis of variations in the chemical makeup of their protein coatings and variations in the symptoms on the tobacco leaves, different strains of TMV can be distinguished. TMV's RNA and proteins can be separated by applying the proper chemical processes. Fraenkel-Conrat conducted experiments to demonstrate that RNA serves as the genetic material in the absence of DNA.In one experiment, the TMV's protein and RNA components were kept apart and used to independently infect tobacco leaves. It was noted that there were no symptoms on the leaf and no virus propagation in the case of protein subunits. The infection was caused by an RNA component, and the tobacco leaf displayed symptoms. The TMV gene was also gained in new progeny. The other experiment involved taking two TMV strains (type A and type B), each of which caused distinct symptoms (one caused spots in a random pattern, the other caused rings to form). There, the protein and RNA components were divided, and chimaera (hybrid) viruses were created by combining type A RNA with type B protein, and vice versa. These hybrid or chimaera viruses were employed to infect tobacco leaves. It was shown that the symptoms on the leaf were consistently associated with the viral strain from which the RNA was extracted. The same strain also included recent offspring. It was discovered that the progeny viruses created when the hybrid or reconstituted viruses were rubbed into living tobacco leaves were always phenotypically and genotypically similar to the parental type from which the RNA had been obtained. These tests demonstrated that the genetic material for TMV is contained in the RNA, not the protein.

1.4. Frederick Griffith: Bacterial transformation

The Streptococcus pneumonia bacteria and mice were used in a number of investigations by British bacteriologist Frederick Griffith in 1928. Griffith was working on a pneumonia vaccine rather than trying to isolate the genetic material. Griffith utilised two related bacterial strains identified as R and S in his research.

R strain. The R bacteria developed colonies, or clusters of related bacteria, with sharp edges and a rough look when cultured in a petri dish (hence the abbreviation "R"). The R bacteria were non virulent, which means that when they were put into a mouse, they did not make the mouse sickness.

S strain.S bacteria generated rounded, smooth colonies (hence the abbreviation "S"). The bacteria's polysaccharide, or sugar-based, coat is what gave the surface its smooth appearance. The S bacteria became more virulent as a result of this coat's protection against the mouse immune system (capable of causing disease). Live S bacteria injections resulted in pneumonia and death in mice.Griffith tried injecting mice with heat-killed S bacteria as part of his investigations (that is, S bacteria that had been heated to high temperatures, causing the cells to die). Unsurprisingly, the mice were not harmed by the heat-killed S bacteria.

The experiment, however, took an unexpected turn when a mouse was injected with a mixture of safe R bacteria and safe S bacteria that had been heat-killed. The mouse not only developed pneumonia and passed away, but when Griffith examined the mouse's blood, he discovered that it still contained live S bacteria!

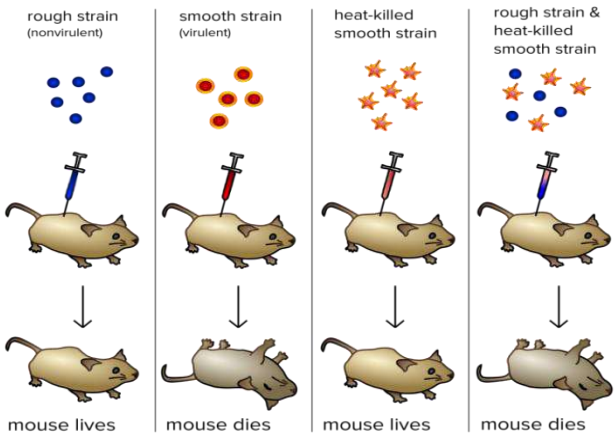

Fig.1.1. Diagram illustrating Frederick Griffith's experiment with S and R bacteria.

A rough strain (non-pathogenic). When a mouse is given an injection of this strain, the mouse survives. Without strain (pathogenic). When a mouse is injected with this strain, the mouse develops pneumonia and passes away. Smooth strain died due to heat. A mouse survives after smooth cells that have been heated to death are put into it.

Smooth strain that has been killed by heat. When these two cell types are combined and put into a mouse, the rodent develops pneumonia and expires. Adapted from Madeleine Price Ball's "Griffith experiment" (CC0/public domain). Griffith came to the conclusion that the heat-killed S bacteria must have given out a "transforming principle" that the R-strain bacteria may have absorbed, allowing them to "transform" into smooth-coated bacteria and become virulent.

1.5. Avery, McCarty, and MacLeod: Identifying the transforming principle

In 1944, Oswald Avery, Maclyn McCarty, and Colin MacLeod, three researchers from Canada and the United States, set out to determine Griffith's "transforming principle." By demonstrating that DNA, not proteins, can alter a cell's characteristics, Oswald Avery, Colin MacLeod, and Maclyn McCarty were able to shed light on the chemical makeup of genes.

While researching the pneumonia-causing bacteria Streptococcus pneumonia, Avery, MacLeod, and McCarty discovered DNA to be the "transforming principle. The bacteriologists were curious about the differences between two strains of Streptococci that Frederick Griffith had discovered in 1923 one, the S (smooth) strain, has a polysaccharide coat and creates colonies on a lab plate that are smooth and shiny; the other, the R (rough) strain, lacks the coat and produces colonies that look rough and irregular. The relatively harmless R strain is deficient in an enzyme required to produce the capsule present in the dangerous S strain.

Griffith had found a way to change the R strain into the virulent S strain. The mice developed pneumonia and died after receiving injections of both heat-killed S strain cells and R strain cells. Griffith discovered live, deadly S type bacteria in their blood. The bacteria from the R strain had been "converted" into the S form by the S strain extract. Over the following 15 years, Avery and members of his group conducted intermittent studies on metamorphosis. They started working together in earnest to clarify the "transforming principle" and comprehend its chemical makeup in the early 1940s.

Bacteriologists believed that a protein might be the transforming factor. Alcohol precipitation of the transforming principle demonstrated that it was not a carbohydrate like the polysaccharide coat itself. However, proteases—enzymes that break down proteins—were found by Avery and McCarty to not eliminate the transforming principle.

Neither did the lipid-digesting enzymes known as lipases. They discovered that the transforming substance had a lot of nucleic acids, but ribonuclease, which breaks down RNA, did not render it inactive. The transforming principle has a large molecular weight, they discovered as well. DNA had been extracted. This was the substance capable of causing an organism to transform permanently and inheritedly.Until then, biochemistshad assumed that deoxyribonucleic acid was a relatively unimportant, structuralchemical in chromosomes and those proteins, with their greater chemical complexity, transmitted genetic traits.

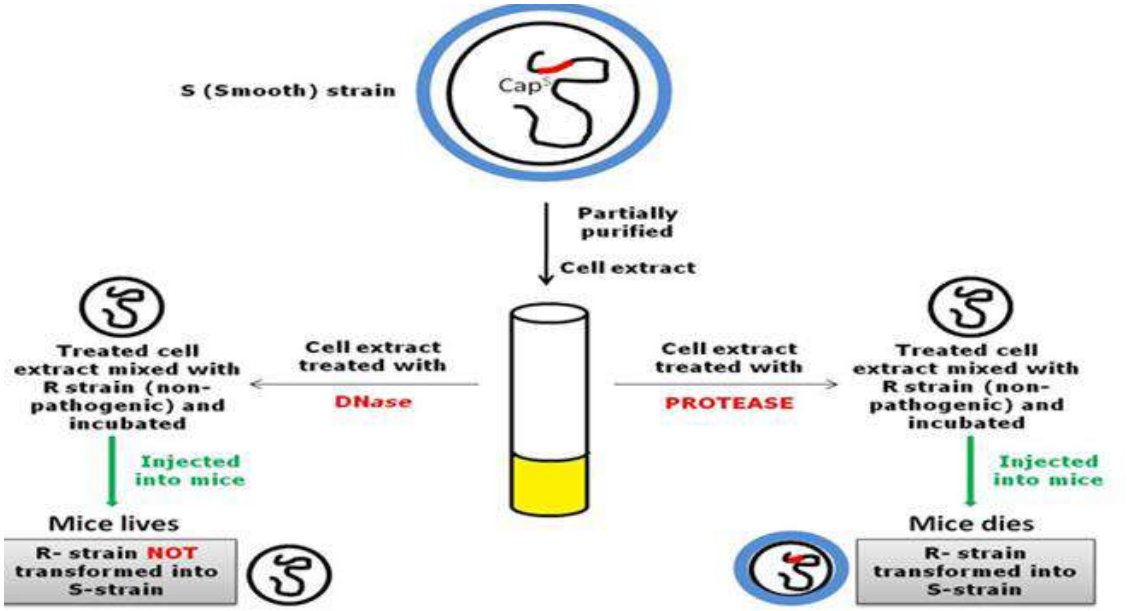

Fig.1.2.S strainand R strain Bacterial culture experiment.(Courtecy. Thomasione)

Because of this possibility, debate over DNA's role continued until 1952, when Alfred Hershey and Martha Chase used a different approach to conclusively identify DNA as the genetic material.

1.6. Check Your Progress- 1

1**.**…….. ……..has a polysaccharide coat and produces smooth, shiny colonies on a lab plate

2**.**…….lacks the coat and produces colonies that look rough and irregular.

3**.** Avery and McCarty observed that proteases - enzymes that degrade……

4**.** ……..British bacteriologist Frederick Griffith conducted a series of experiments using *Streptococcus pneumoniae* bacteria and mice

5**.**……organic macromolecule that is composed of two polynucleotide chains

1.7. The Hershey-Chase experiments

In 1952 A. D. Hershey and Martha Chase, two geneticists, added to the evidence (seven years after Avery's proof that genes were DNA). They utilised the T2 DNA virus, which infects E. coli (and so is a bacteriophage). The essential components of the infection cycle of DNA bacteriophages like T2 are depicted in Figure 1.3. The virions adhere to the host cell's surface (a). The DNA core is injected into the cell by the capsid proteins (b). Once within the cell, some of the bacteriophage genes, known as the "early" genes, are translated and transcribed by the host's ribosomes, tRNA, and other components to create enzymes that multiply the phage DNA and inhibit (or even completely destroy) the host DNA.

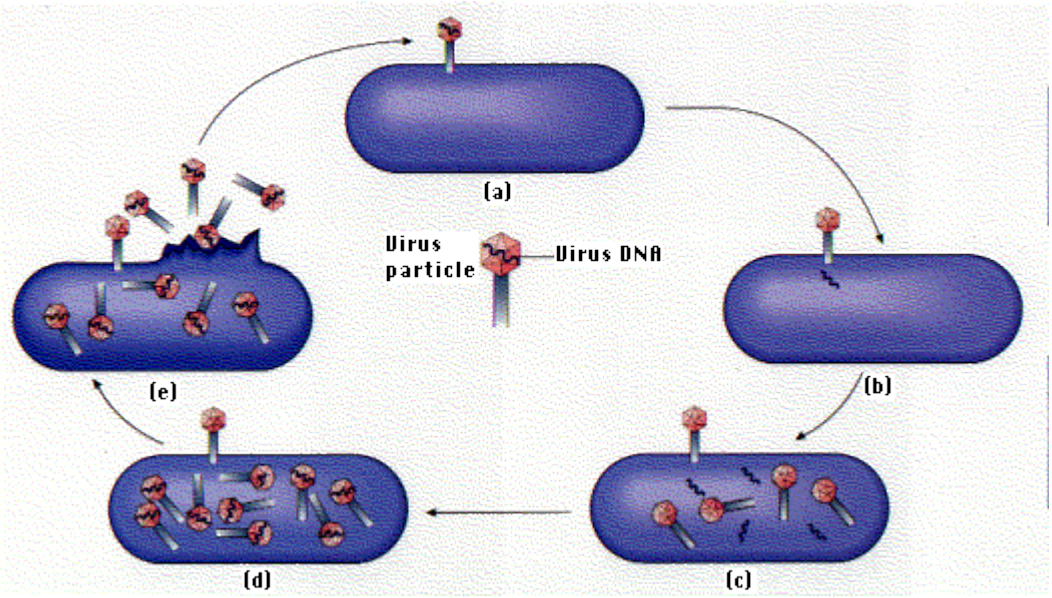

Fig.1.3. the Hershey - Chase Experiment.(Courtecy.Thomasione)

Other genes (the "late" genes) are transcribed and translated to create the proteins of the capsid as more copies of the phage DNA accumulate (c). Complete virions are created by assembling the DNA core material with the capsid proteins (d). Lysozyme molecules are produced by the transcription and translation of a further "late" gene. The peptidoglycan

wall is attacked by the lysozyme (from the inside, of course). Eventually, the cell bursts and unleashes the virions it has stored inside, ready to infect new host cells (e).

In radioactive culture medium, bacteria will create bacteriophages that are radioactive in and of them. Two of the amino acids—cysteine and methionine—contain sulphur, therefore if radioactive sulphur atoms (35S) are present; they will be absorbed into the protein coats of the bacteriophages (Figure1.3). However, since DNA doesn't contain any sulphur atoms, it won't be radioactive. As a result of the large number of phosphorus atoms in DNA, radioactive phosphorus (32P) causes the DNA to become radioactive but not the proteins.

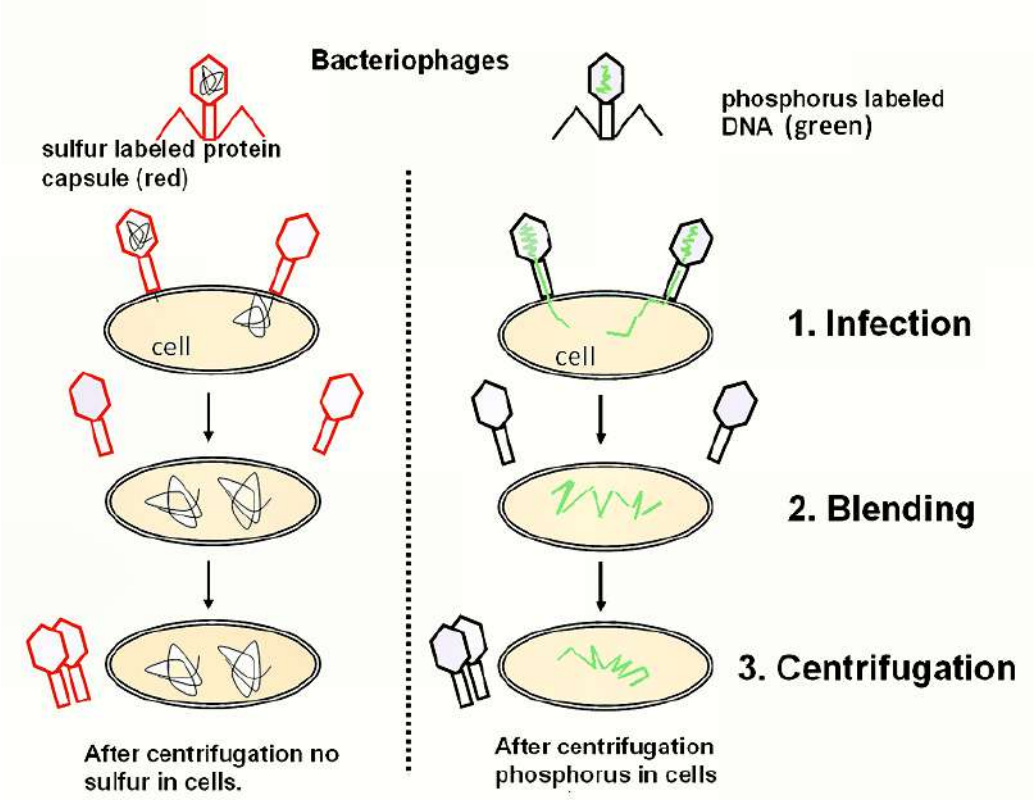

Fig.1.4. Diagram of the Hershey Chase experiment. (Courtecy.GFDL;Thomasione)

Hershey and Chase discovered that when nonradioactive bacteria were exposed to bacteriophages bearing the radioactive 32P, all the infected cells became radioactive and that a large portion of the radioactivity was transferred to the bacteriophages' offspring. However, hardly little radioactivity could be found in the infected cells when the bacteria were infected with bacteriophages labelled with 35S and subsequently the virus coats were removed (by spinning them in an electric blender). These tests demonstrated that the

DNA portion of bacteriophages enters the bacterial cell while the protein portion stays outside. However, the DNA that was injected is what can control the development of fresh virus particles with protein coatings. So here is further proof that **genes are DNA**.

1.8. Lederberg and Tatum

Bacteria weren't good candidates for genetic research in the past because it was known that they were completely cloned from their parent cells. However, Lederberg and Tatum asserted that bacteria use sex to transfer DNA. Conjugation and transduction process are the two hypotheses Lederberg and Tatum (1946) proposed for the mating process in Escherichia coli (E. coli). Genetic material is directly transferred from one bacterium to another during conjugation. Lederberg and Tatum conducted studies to support their claim and show how the process works.They used two auxotroph strains of E. coli K12, the first of which was able to synthesise threonine, leucine, and thiamine but not methionine and biotin, and the second of which was able to do so but no other amino acids. Generalized and specialised transductions are the two types of transduction. Pages can carry any host gene in generalised transduction, but in specialised transduction, only a specific host gene can be conveyed. In 1952, Lederberg and Zinder employed the same methodology that had been used to identify the conjugation procedure for E coli to explore the conjugation process in Salmonella typhimurium. Two distinct strains were used, one of which exhibited phe, trp, and Tyr, while the other was met. After the strains were plated separately, no wild-type cells were seen; however, when the two strains were combined, wild-type cells started to appear roughly 1 in 105 times more frequently. Thus, they draw the conclusion that the circumstances are comparable to those of E. coli recombination and that E. coli utilised transduction as part of their mating process. It's interesting to note that Morton and Akiko Higa confirmed in 1970 that E. coli can undergo transformation via an artificial approach.

1.9. Topoisomerase

Topoisomerase is an essential enzyme that supports transcription, chromosome segregation, DNA replication, and recombination. While researching on Escherichia coli in the 1970s, J.C. Wang made the initial discovery of it. It was a topoisomerase of type I. It aids in altering DNA topology, as the name implies. It has the ability to alter how much DNA is unwound. Given that enzyme solely interacts with DNA strands, it is also known

as DNA topoisomerase. It doesn't work on RNA.It breaks the phosphodiester bond found in the DNA strands' core. As the enzyme departs, the linkages are created once more. Some Key Phrases. Twist (Tw) is the total number of times the DNA strands have been wound around themselves. Writhe (Wr) The number of times the DNA double helix has crossed itself in total is what represents the DNA supercoils. The linking number is the sum of all the twists and turns in DNA.

Topoisomerase Types

There are two types of topoisomerases

Type I Topoisomerase

Type II Topoisomerase

Type I Topoisomerase

Type I Topoisomerase Definition

One strand of DNA is cleaved by a type of topoisomerase called type I topoisomerase. It is not an enzyme that depends on ATP (exception: Reverse Gyrase). Basically, it adds one to the connecting number. Note: Odd types of topoisomerases come under type I and even types under type II.

Type I Topoisomerase Structure

There is the presence of multiple varying domains in the type IA. It can be from I to IV.Top rim domain is contained in domain I. HTH (Helix-Turn-Helix) is present in domains III and IV. The tyrosine residues are present in the HTH of domain III. It appears like a lock with all three domains present at bottom of the topoisomerase structure. Type IB contains active site (tyrosine) bind with C-terminal domain, N-terminal domain, capping, and catalytic lobe.

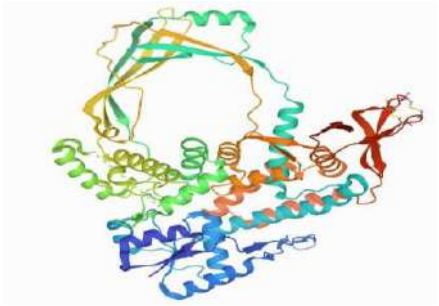

Fig.1.5. Topoisomerase

Structure of Full-Length Topoisomerase I from *Thermotogamaritima* in monoclinic crystal form. Image Source: (RCSB PDB).

Type I Topoisomerase Functions

They are involved in the removal of DNA supercoils during biological processes like transcription and replication. Support for DNA relaxation

They help in strand breaking during recombination.

They participate in the condensation of the chromosomes as well.

Topoisomerase I work to prevent intertwining of the DNA strands during mitosis.

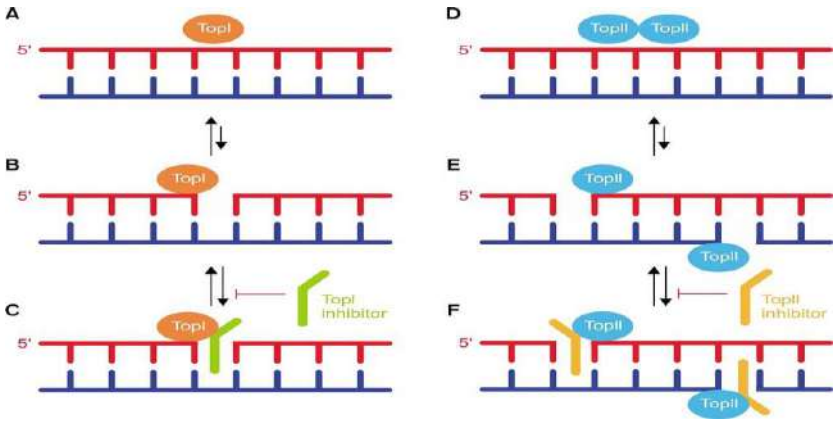

Fig.1.6.Topoisomerase I and Topoisomerase II.Image Source :(Young HoSeo 2015.)

Gyrase: It is found in bacteria and some eukaryotes. It introduces negative supercoiling decreasing the linking number by two.

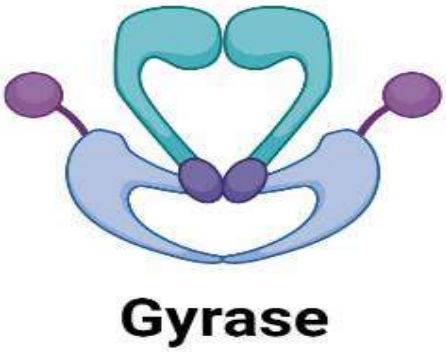

Fig.1.7. Gyrase.

Type IIB topoisomerases

It includes Topo VI which can be found in archaea and some plants.

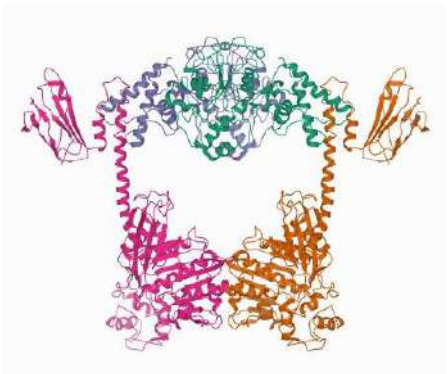

Fig.1.8. Crystal structures of the topoisomerase VI holoenzymes from *Methanosarcinamazei*. Image Source: RCSB PDB.

Topoisomerase Inhibition

Topoisomerase inhibitors are substances that can stop topoisomerase from doing its action. They may obstruct the DNA's ligation process, resulting in broken strands inside the cell and cell death by apoptosis. Drug development for bacterial infection uses the topoisomerase inhibition approach. It consists of antibiotics like novobiocin and coumermycin of the class coumarins that kill bacteria by interfering with their ability to bind ATP. Additionally, it comprises antibiotics from the quinolone class that stop the final step of the topoisomerase working mechanism—the ligation of damaged DNA strands. Topoisomerase in humans may be inhibited by chemotherapy drugs used to treat cancer. They have the ability to stabilise the intermediate formed when DNA phosphate and topoisomerase bind together.

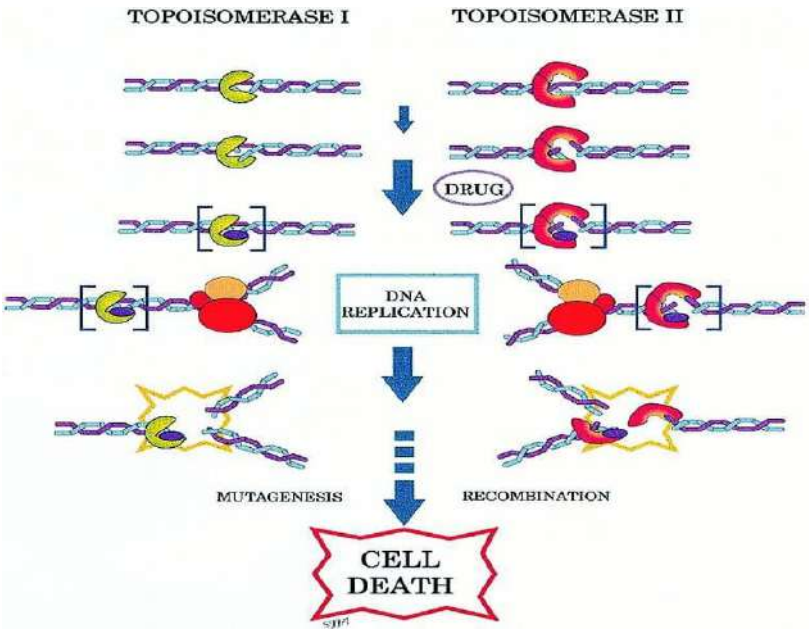

Fig.1.9. Topoisomerase Inhibition. Image Source: Vtvu.

1.10. Summary

Molecular biology is the study of living things at their molecular level,

The study of molecular biology will establish a strong foundation on the fundamental importance of macromolecular mechanisms such as replication, transcription, translation and other cellular functions. The more commonly used molecular biology techniques include- Polymerase Chain Reaction, Electrophoresis, Restriction Digestion, Blotting, Cloning, etc.

Nucleic acids, such as DNA and RNA, as well as protein production in cells, are significant subjects studied in this course. A subfield of biology called molecular biology is closely related to the fields of biochemistry, cell biology, genetics, and genomics. Monod and Jacob also showed, at the start of the 1960s, how particular proteins, known as regulative proteins, attach to DNA at the borders of the genes and regulate the transcription of these genes into messenger RNA; they govern the "expression" of the genes.

The fundamental science that supports molecular biology as a topic of study is genuinely addressed by this timeline. Although it is an oversimplified representation of molecular biology, the so-called Central Dogma of Molecular Biology, which states that genetic material is translated into RNA and subsequently into proteins, is at its core, offers a useful starting point for comprehension of the science. However, this image is being updated in light of newly discovered roles for RNA. The Central Dogma, however, has been the foundation for a revolution in the biological sciences, with the exception of a few footnotes.

In recent years, a lot of work has been done in computational biology and bioinformatics at the intersection of molecular biology and computer science. The most well-known branch of molecular biology as of the early 2000s was molecular genetics, which is the study of gene structure and function.

More and more biological disciplines are focusing on molecules, either directly (as in cell biology and developmental biology) or indirectly (as in evolutionary biology, where fields like population genetics and phylogenetic use molecular biology techniques to infer historical characteristics of populations or species). Additionally, biophysics has a lengthy history of investigating proteins "from the ground up."

Furthermore, the study of protein folding and structures has long been a popular field in molecular biology. The study of protein folding began in 1910 with a well-known paper by Henrietta Chick and C. J. Martin, who demonstrated that the flocculation of a protein consisted of two distinct processes: the protein's precipitation from solution was preceded by a different process known as denaturation, during which the protein became significantly less soluble, lost its enzymatic activity, and increased in chemical reactivity.

Later, Linus Pauling supported William Astbury's initial assertion that hydrogen bonding primarily stabilised protein structure (1933). Interestingly, Pauling's flawed assumption about H-bonds led to his accurate models for the alpha helix and beta sheet, which make up the secondary structure of proteins. Since then, every chemical and physical characteristic of proteins that could be determined has been used to study how proteins fold and preserve structures, and as of 2006, the Protein Data Bank contains close to 40,000 atomic-resolution structures of proteins.

You may have heard that some biologists have called the era from the 1960's until now the "golden age of molecular biology," and now you know a little bit why that is so.

1.11. Check your progress-2

1. ……..is found in bacteria and some eukaryotes
2. …….....is an essential enzyme that aids in the DNA replication process
3. Before, bacteria were known that it is fully cloned from the parent cell so bacteria were not suitable for ……
4. Many medications work by interfering with type II topoisomerases ……..
5. Some chemical components can suppress the action of topoisomerase and are called …….

1.12. Glossary

1. **DNA**: Deoxyribose nucleic acid
2. **RNA**: Ribose nucleic acid
3. **S Strain:** Smooth strain
4. **R Strain:** Rough strain
5. **Gyrase:** It is found in bacteria and some eukaryotes. It introduces negative supercoiling decreasing the linking number by two.
6. **Topoisomerase:** is an essential enzyme that aids in the DNA replication process,

7. **Rough strain:** (non-pathogenic). When this strain is injected into a mouse, the mouse lives
8. **Smooth strain:** (pathogenic). When this strain is injected into a mouse, the mouse gets pneumonia and dies
9. **Heat-killed smooth strain**. When heat-killed smooth cells are injected into a Mouse, the mouse lives.

1.13. Questions for self-study

1. Explain the history of molecular biology
2. Write an account of DNA and RNA as Genetic materials?
3. Describe the experiments of Griffith Avery. MacLeod and McCarty
4. What are Topoisomerases? Discuss the types and significance of topoisomerases?

1.14. Answers to check your progress 1 and 2

1. **S (smooth) strain,**
2. **R (rough) strain**
3. **Proteins**
4. **in 1928,**
5. **DNA is an**
6. **Gyrase**
7. **Topoisomerase**
8. **Genetic study**
9. **in bacteria**
10. **Topoisomerase inhibitors.**

1.15. References

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Unit-2

DNA topology: Closed and super coiled DNA.DNA topoisomerases.DNaseexo and endonucleases. Restriction endonucleases

Structure of the Unit

2.0. Objectives

After studying this unit you will be able to

- understandthe DNA topology of closed and super coiled DNA.
- discuss about DNA Topoisomerase and their functions
- explain about exo and endo nucleases
- describe the Restriction endonucleases and their types

2.1. Introduction

In essence, DNA is a very long, double-stranded rope that is coiled around the other two strands. As a result, practically every significant nucleic acid process is significantly influenced by the topological characteristics of the genetic material, such as DNA under- and over winding, knotting, and tangling. Although DNA topology is important, teaching it can be conceptually challenging since it needs students to visualise three-dimensional interactions. The reader will learn about DNA topology in this article, which also provides demonstrations and useful teaching strategies for this "knotty" subject. Additionally, topoisomerases, the enzymes that control the topological state of DNA in the cell, will be covered. These ubiquitous enzymes perform a number of critical cellular functions. Topoisomerases ensure genomic stability during this catalytic event by creating covalent phosphotyrosyl bonds between the active site residues and the freshly created DNA termini. For cells to survive, topoisomerases are necessary. These enzymes have the ability to cut genetic material, but they can also fragment the genome. Some of the most frequently prescribed anticancer and antibacterial medications now being used in clinical

settings take advantage of this latter property of topoisomerases. Last but not least, topoisomerase activity has been connected to the development of particular subtypes of leukaemia in addition to treating cancer.

2.2. DNA Topology

Notes on DNA Topology Jeremy Kahn the best source is "DNA Topology" by Bates and Maxwell. DNA's helical structure has important implications: Due to the length, stiffness, and helix structure of DNA, both long-distance communication and interference between processes are possible. The following influences DNA transcription, replication, and recombination: DNA Topography One of the most challenging ideas in molecular biology is DNA topology. It is essential to all DNA exchanges yet is frequently disregarded. Only because the cell has invested a significant amount of machinery in solving topological difficulties can we get away with this.Does the driver of a Lexus know all the modifications that the several on-board computers make? Topology is the study of the characteristics of materials that are independent of changes in shape and instead depend on connectivity between portions. Examples include the four-color map theorem (seven colours for a doughnut) or the observation that a donut and a coffee cup with a single handle are topologically similar but different from one another in terms of geometry and gastronomy. When a donut is bit into, it becomes topologically equivalent to a spherical. Imagine a piece of Silly Putty that has been Teflon-coated and is either a cup or a donut. Our model for DNA is the rubber hose. We can define topological invariants and geometric variables when the two strands are ligated to form a closed (topological) circle. The meaning of a linking number is the quantity of intersections between the two strands. Regardless of shape changes, it is always an integer. Must be broken and rejoined at least once in order to alter. "Topoisomers" are the several shapes that the same molecule can take when given different linking numbers.

2.3. Closed and super coiled DNA

If we have four DNA molecules of the same length with different topologies, each molecule migrates differently in agarosegel.Suppose the first DNA is closely circular with no supercoiling, and the second DNA molecule has both strands twisted around each other which means linear double-stranded DNA. The third DNA molecule is coiled around each other with some writhes and the fourth one is supercoiled.In each case, DNA

molecules migrate differently. The more compactly supercoiled DNA migrates faster in the gel. Hence the fourth one (which is supercoiled and compact) migrates faster as compared with other DNA (Lane C and D: the band at the base).

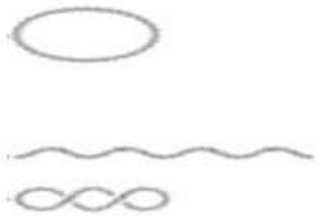

Fig.1.1.the image represents the migration of different DNA molecules.Credit: "Molecular biology of the gene", 7th edition by Watson

The first one, circular DNA does not have any supercoiling hence it migrates slower than any other DNA and remains last (lane A and D).The more compact the DNA is, it can easily pass through agarose pores. So sequentially, circular DNA remains last, linear DNA cover more distance than circular DNA and coiled DNA covers more distance than linear DNA. At last supercoiled DNA cover more distance than any other DNA, and run faster.Topological properties especially the linking number of DNA are very important properties of DNA if it is not maintained properly *in vivo*, DNA cannot be packed properly on the chromosome which will result in epigenetic alteration and failure in repcation.The linking number of the DNA can be calculated using the formula given above, feel free to comment here and let me know how linking numbers can be useful for the diagnosis of disease.

Super coiled DNA

The DNA of a cell as we all know is very compacted, inferring a higher level of structural organization. The folding mechanism of DNA packs the cellular DNA so that the information inside the DNA remains accessible.We need to examine the basic structure of DNA carefully better to understand DNA replication and transcriptional process. We need to have prior knowledge about a crucial property of DNA structure that is supercoiling.Supercoiling implies the further coiling of a coiled structure. Say, for example, a cord of a telephone is normally a coiled wire. The wire between the body of the telephone and the receiver frequently incorporates at least one supercoils. The two DNA strands wrap around each other to produce the DNA double-helical structure. The

coiling in the axis of DNA causes supercoiling. Supercoiling in DNA for the most part, is a sign of underlying structural strain. When there is no resultant DNA axial bending, the DNA is considered in relaxed state.

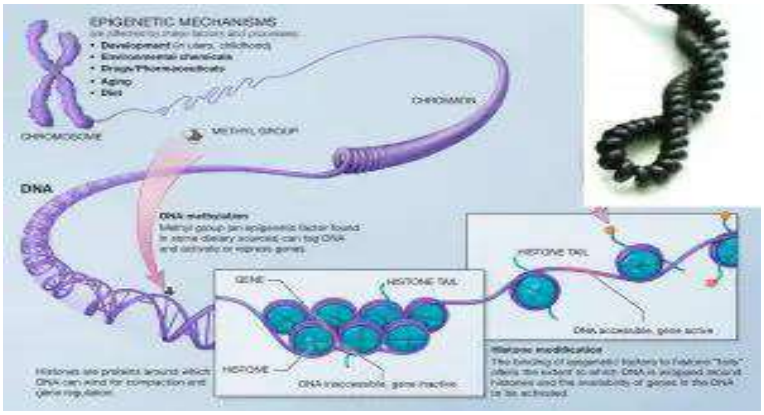

Fig.1.2. DNA Supercoiling, levels of supercoiling found in DNA. Briefly, it resembles with the coiling of telephone wire. commons.wikimedia

We may have anticipated that the process of compaction of DNA included some supercoiling. Maybe less unsurprising is that replication and transcription of DNA additionally influence and are influenced by supercoiling. The replication and transcription both require detachment of DNA strands and helical unwinding of DNA.

- That DNA would itself twist and become supercoiled in compactly packed DNA in the cell would appear sensible, at that point, and maybe even insignificant.
- However, several circular DNA molecules remain exceptionally supercoiled even after their extraction and purification.
- This suggests, supercoiling is intrinsic property of DNA .It happens in every cell DNA and is exceptionally regulated by every cell.
- Various measurable properties of supercoiling have been standardized, and the investigation of supercoiling has given numerous insights into the structure of DNA and its function.
- This investigation is based on the ideas from topology and the investigation of properties of an object that doesn't change under dynamic conditions.

For DNA, consistent deformations incorporate conformational changes because of thermal motion or protein interaction or other chemical agents; intermittent deformations include DNA strand breakage. For a circular DNA topological properties are not affected by structural changes in the DNA strands if strand breaks are not present. Topological properties are disturbed exclusively by sugar-phosphate backbone breaking and re-joining of either of the DNA strands. We currently examine the fundamental characteristics and physical basis of supercoiling.Dear learners please refer the DNA topoisomerase in the unit 1

2.4. Check your progress -1

1. …… Helical nature of DNA has profound implications
2. DNA as the genetic material is a topic of intense interest in the……..
3. The double-strand DNA model was postulated by ……..
4. DNA supercoiling arises mostly in …….
5. The first determined DNA structure of short DNA molecules that were investigated using ……..

2.5. DNaseExo and endonucleases

The exonuclease cuts DNA on both ends (3' and 5'), whereas the endonuclease cleaves DNA from the inside. In genetics, recombinant DNA technology, and genetic engineering, nucleases play a vital role for a variety of applications. They are utilised in numerous assays because of their extraordinary ability to break DNA or RNA. We are accustomed to employing restriction endonuclease, one of the common forms of endonuclease, in our laboratory. At its recognition site, the restriction endonuclease cleaves DNA. We use different restriction digesting enzymes, which are endonucleases in nature, to create different cleaves or restriction sites. Nucleases in cells cleave DNA to carry out a variety of tasks, such as repairing DNA. It cleaves the DNA and relaxes the tension at the location of DNA damage to help the polymerase activity. We are aware that unrepaired cleaved DNA can have a negative impact on human health, but DNA cleaving is just as crucial as DNA synthesis. The two most prevalent forms of nucleases are endonuclease and exonuclease. Both share certain commonalities and distinctions. In this essay, we'll talk about some of the parallels and distinctions between the two.

Endonuclease vsexonuclease

Inside is "Endo," outside is "Exo." While the exonuclease cuts the DNA on the ends, the endonuclease cleaves or cuts the DNA from within or between the sequence. Nuclease has the ability to cut DNA; it does this by releasing the phosphodiester bonds holding nearby nucleotides to one another and separating the two. What does "inside" or "outside" mean? Observe the photo below.

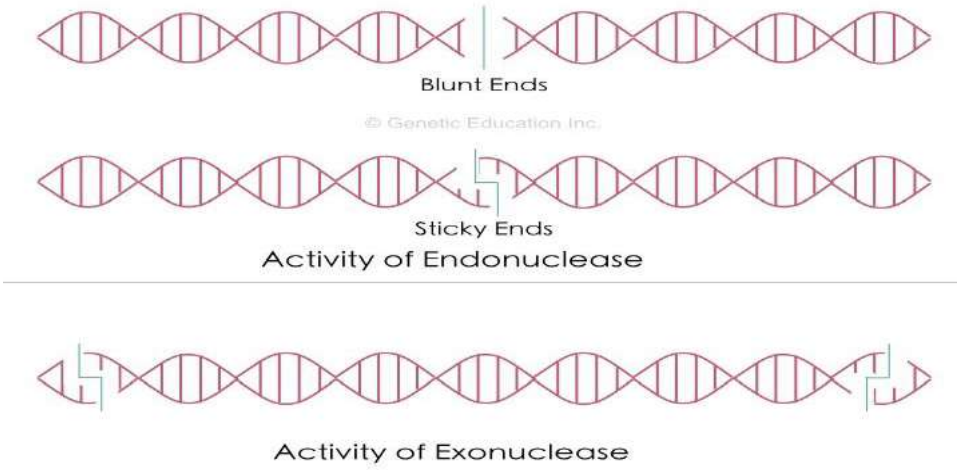

Fig.1.3. Activity of Exonuclease

The difference between exonuclease and endonuclease, and the formation of blunt and sticky ends. The polynucleotide chain is cut by the endonuclease between or away from ends. In contrast, the exonuclease cleaves DNA at either the 3' OH or 5' P ends or both. But there's a good explanation for that. The endonuclease does not require free 3' or 5' to begin its action, but "Exo" one does not. To initiate and catalyse the process, the exonuclease needs free ends of either a 5' phosphate or 3' hydroxyl group. The specificity is another another obvious distinction between endonucleases and exonucleases. The endonucleases are exceedingly sequence-specific and have a high level of specificity. Only at the precise location of recognition did it cut the DNA.

Examples of restriction endonucleases are explained restriction endonucleases using the following examples: How does restricted digestion function? It cannot function until it discovers its precise recognition site. Typically, the recognition site is 6 nucleotides long. The exonuclease, on the other hand, operates arbitrarily. The action required determines its purpose. Since the endonuclease cuts through the DNA, as we already stated, oligonucleotide chains are the result of the enzymatic activity. While the exonuclease's end products are nucleotide monomers.

Exonucleases do not have a lag period since they operate at random, unlike some endonucleases, such as restriction enzymes, which experience a lag period before

beginning their actions. Endonuclease produces either blunt or sticky ends for nucleic acids, depending on the type of enzyme recognition sites. Exonucleases, however, only produce Sticky ends. The endonuclease plays a crucial role in a cell by defending it against pathogen invasion. The endonuclease locates foreign nucleic acids (DNA or RNA) at the cell entry, attaches to them, and then cleaves them. In doing so, it eliminates germs. Sadly, the exonuclease plays no substantial part in preventing the invasion of pathogens. The typical example of an endonuclease is a restriction endonuclease like Hind III, EcoR1, and BamH1. In addition, S1 nuclease and DNase are endonucleases. The DNA polymerase I domain and snake venom are two examples of exonucleases. Let's now look at some commonalities between the two.

Endonuclease vsExonuclease: Similarities

Both belong to the nuclease class, and their primary job is to cleave nucleic acid. Both have the ability to split DNA and RNA. The two split the DNA by severing the phosphodiester bond tying the neighbouring nucleotides together.

Applications: Endonuclease and exonuclease have various applications for, genetic engineering, gene editing, and recombinant DNA technologies. Common restriction and endonuclease enzymes can be used as a marker and in the construction of osis. Restriction fragment length polymorphism- RFLP is the marker based on the use of restriction endonuclease.On a gel, different length fragments can be distinguished using the RFLP marker. It can also be used in conjunction with the PCR, or polymerase chain reaction, to screen for particular disease-related mutations. Plasmid DNA restriction sites are created using The REase. On the other hand, site-directed mutagenesis and SNP detection tests employ exonucleases. The creation of sticky ends, nested deletion in double-stranded DNA, nick-site extension, and the elimination of primers before DNA sequencing are some of its applications.

Various commercially available nucleases that are sold commercially come from prokaryotes like bacteria. and put to many other uses. There are numerous endonucleases and exonucleases available today for use in various applications. Have you ever conducted an experiment or utilised a lab that used exonuclease or endonuclease? Tell me in the comments section below. Additionally, if you want to utilise it and need any procedure, please comment below and let me know.

2.6. Restriction endonucleases

A bacterial enzyme that can cut DNA at a specific place after identifying a specific base sequence or recognition sequence in DNA (the restriction site).

There are two types of nucleases:

Exonucleases - These enzymes remove nucleotides from the ends of DNA.

Endonucleases, often known as molecular scissors, are a subclass of nucleases, a group of enzymes that cut DNA at specified locations. Hamilton Smith, Werner Arber, and Daniel Nathans shared the Nobel Prize for discovering restriction endonuclease. The first restriction enzyme to be identified was Hind *II*.TheHaemophilus influenza bacterium provided the source. Numerous microbial species have been found to manufacture hundreds of restriction enzymes.

These enzymes hydrolysis the recognised sequence and each has a unique restriction site. Palindromic sequences are generally recognised and cut. A palindrome is a sequence of base pairs that reads the same on two complementary strands, for example, GAATTC- complementary base pairs will be CTTAAG. A palindrome is a word that spells the same when read from the backward or forward direction, for example, MALAYALAM.

Sticky/staggered ends are produced after the DNA sequence has been cut with restriction enzymes; these ends are necessary for the intended gene to properly bind. By selecting the proper restriction enzyme, we may precisely cut DNA at the location where the desired gene must be put.REs eliminate non-self DNA (DNA of other organisms e.g. viral DNA is destroyed by bacteria, by cutting it due to methylase activity).They are naturally present in microorganisms and are used as a part of their defence mechanism to prevent viruses from entering; for this reason, the term restriction is used. They are able to remove alien genes.

Methylase enzyme

The enzymes known as methylase enzymes are found in bacteria. These enzymes identify bacteria's own DNA sequence and add a methyl group to it, methylating the sequence.This methylation sequence can be distinguished from foreign DNA and is therefore preserved because bacterial REs do not break it down.Since bacteria do not methylate alien DNA (non-self DNA), REs produced by bacteria destroy foreign DNA.Restriction endonuclease subtypes. The primary categories of restriction endonuclease are as follows

Type I Type II Type III

They differ from each other in their mode of action. Type II restriction enzymes are used in r-DNA technology because they can be used *in vitro* to identify and cleave within specific DNA sequences usually having 4-8 nucleotides.More than 350 different type II endonucleases with 100 different recognition sequences are known. Types I and III exonucleases behave similarly to one another. Both the restriction activity and the methylate activity are included in a single large enzyme complex. They will still make a cut a few base pairs away from the restriction point even after identifying the same sequence. These are the names of exonucleases.

Type II (Endonucleases)

The restriction activity and methylase activity are different i.e. they are not present in single enzyme complex. Type II RE's will identify the sequence in a DNA and cut within sequence at that specific site called as restriction site. Hence the name endonucleases. Widely used in recombinant DNA technology, as here the need is to cut DNA at a particular site.

Nomenclature

According to an established protocol, restriction endonucleases are given names that specifically pertain to the bacterium from which they were isolated. The first letter of the enzymes, which is italicised, denotes the genus, followed by the first two letters, which are also italicised, of the species, the strain of the organism, and then a roman numeral denoting the order of discovery. Below are a couple of instances.

Eco*R I** is from *Escherichia* (E) *coli* (co) strain Ry 13 (R) and first endonuclease (I) to be discovered.Hind* III** is from *Haemophilus* (H) *influenzae* (in) strain Rd (d) and the third endonuclease (III)***BamH1*** is from *Bacillus* (B) *amyloliquefaciens* (am) strain (H) and is the 1st enzyme

Recognition Sequence

The DNA is cut by restriction endonucleases at the recognition site. Since each restriction enzyme recognises a unique short DNA sequence, or restriction site, it can cut both DNA strands simultaneously at certain locations within that site. Palindromic restriction sites predominate.

Restriction Enzymes Indicating the Restriction Site

a. EcoRI → G|AATTC
b. EcoRV → GAT|ATC
c. HinDIII → A|AGCTT
d. XmaI → C|CCGGG

Note: The bar between the sequences depicts the restriction sites.

2.7. Summary

Topoisomerases are real magicians in the world of DNA, according to James Wang (Wang, 1996). The enzyme's ability to modify DNA topology and loosen its structural Restraints are what make it magical. Because it makes the DNA-topoisomerase I covalent complex visible while it is typically fleeting and rarely detectable, CPT can be thought of as a magician's tool. By occupying the interface between the two components, this plant alkaloid interacts closely with the labile complex. If the changed genome is not properly repaired after CPT modifies the topoisomerase I engine, the cell quickly becomes ill and dies. Since topoisomerase I's proper operation is crucial for the rapid proliferation of cancer cells, the presence of a toxin like CPT always results in the death of cancer cells, unless they have developed resistance mechanisms. This idea was used to create the anticancer medications topotecan (Hycamtin®, $180 million in 2004) and irinotecan (Camptosar®, $1,450 million in 2005), two of the pharmaceutical industry's most sought blockbusters. Both medications were introduced in 1996. The CPT vein has been consistently exploited over the past ten years, and many CPT derivatives have advanced to clinical trials. That said, their success is somewhat limited, since no other CPT derivative has been approved by the FDA or EMEA since 1996. However, the mine is not closed, and both CPT and non-CPT topoisomerase I inhibitors remain the subject of intense medicinal chemistry efforts. Beyond the pharmaceutical aspect, The CPT has also made significant contributions to our understanding of the fundamental role of topoisomerase I in all metabolic processes involving DNA, including cell division, survival, and death. These processes include transcription, replication, recombination, DNA repair, chromatin assembly, and chromosome segregation. Gene targeting, which has gained widespread acceptance at the molecular level, has also been linked to CPT and could pave the way for a novel, non-cytotoxic cancer chemotherapy that uses a CPT derivative. The chemotherapeutic approach for cancer is likely to continue to focus on camptothecin in some capacity.

2.8. Check your progress-2

6. .....are necessary for normal replication because

7. With respect to supercoiling, and depending on supercoiling, gene expression may be.....

8.Exonuclease cuts DNA on.....

9. In bacteria there are enzymes called as.....

10..... is from *Escherichia* (E) *coli*

2.9. Glossary

1.**Endonuclease**: cleaves the Polynucleotide chain in between or away from ends

2. **In bacteria:** there are enzymes called as methylase enzymes.

3. **Type II RE's:** will identify the sequence in a DNA and cut within sequence at that specific site called as restriction site. Hence the name endonucleases.

4. **Eukaryotes:** like humans and other multicellular organisms DNA molecules are linear and also are very long

5. ***Hind* III:** is from *Haemophilus* (H) *influenzae*

6. **Recognition Sequence:** *Recognition site is the site where the DNA is cut by restriction endonucleases*

7.**Restriction endonucleases:** are named by a standard procedure,

8.**Exonuclease:** cuts DNA on both ends

9. **DNA:** is predominantly in the B-form although some special sequences form slightly different structures even under normal conditions

2.10. Questions for self-study

1. Explain about super coiled DNA

2. What are restriction endonucleases? And mention the types of endonucleases

3. Discuss the applications of exo and endonucleases

4. Describe the type 1 and type 11 restriction endonucleases

5. What are restriction enzymes?

2.11. Answers to check your progress 1 and 2

1. DNA Topology

2. 21st century

3. Watson and Crick in 1953

4. Prokaryotic cells

5. X-ray crystallography

6. Topoisomerases

7. Affected

8. Both ends

9. methylase enzymes.

10.*Eco***R I**

2.12. References for further studies

1. Levine, C., Hiasa, H., & Marians, K. J. (1998). DNA gyrase and topoisomerase IV: biochemical activities, physiological roles during chromosome replication, and drug sensitivities. *BiochimicaetBiophysicaActa*, *1400*(1-3), 29–43. https://doi.org/10.1016/s0167-4781(98)00126-2
2. Wang JC (June 2002). Cellular roles of DNA topoisomerases: a molecular perspective. *Nat. Rev. Mol. Cell Biol*. **3** (6): 430–40. doi:10.1038/nrm831
3. Sharma A; Hanai R; Mondragón A (August 1994). Crystal structure of the amino-terminal fragment of vaccinia virus DNA topoisomerase I at 1.6 A resolution. *Structure*. **2** (8): 767–77. doi:10.1016/s0969-2126(94)00077-8
4. Anticoagulant enoxaparin & intra-venous immunoglobulin : Export restriction by Directorate General of Foreign Trade (DGFT)Recombinant DNA technology: Pharmaceutical Biotechnology Theory NotesPolymerase chain reaction PCR / Gene amplification
5. GCP Obligations: Investigators, Sponsors, Monitors

UNIT- 3

Replication of DNA: Enzymes in DNA replication, DNA Pol I.II.III. Replication in viruses replication in prokaryotes

Structure of the unit

3.0. Objectives

After studying this unit you will be able to

- understand the Enzymes of DNA Replication
- discuss about the Mechanism of DNA Replication
- explain about Replication fork formation and its function
- describe the DNA replication Okazaki fragments
- why is DNA replication impartant?

3.1. Introduction

It is a unique and complex process that takes place in both prokaryotes and eukaryotes. Do you **k**now how many chromosomes you have? How does the number of chromosomes in a particular organism remain constant? It is because of the DNA Replication process that takes place during the S-phase (synthetic phase) of the cell division (mitosis or meiosis) in each and every cell.

Replication occurs in three major steps: the opening of the double helix and separation of the DNA strands, the priming of the template strand, and the assembly of the new DNA segment. During separation, the two strands of the DNA double helix uncoil at a specific location called the origin. Several enzymes and proteins then work together to prepare, or prime, the strands for duplication. Finally, a special enzyme called DNA polymerase organizes the assembly of the new DNA strands.

3.2. Replicationof DNA

This is a complex process that takes place during cell division, (interphase, and S phase) whereby DNA makes copies (duplicates) before the cell divides through mitosis and meiosis.DNA replication is a **semiconservative process** where a parental strand (template) is used to synthesize a new complementary daughter strand using several protein elements which include enzymes and RNA molecules. DNA replication process

uses **DNA polymerase** as the main enzyme for catalysing the joining of deoxyribonucleoside 5′-triphosphates (dNTPs) forming a growing chain of DNA.

Other proteins are also involved for initiation of the process and copying of DNA, along with proofreading capabilities to ensure the replication process takes place accurately. Therefore DNA replication is a process that produces identical helices of DNA from a single strand of the DNA molecule.DNA replication is an essential mechanism in enhancing cell growth, repair, and reproduction of an organism.

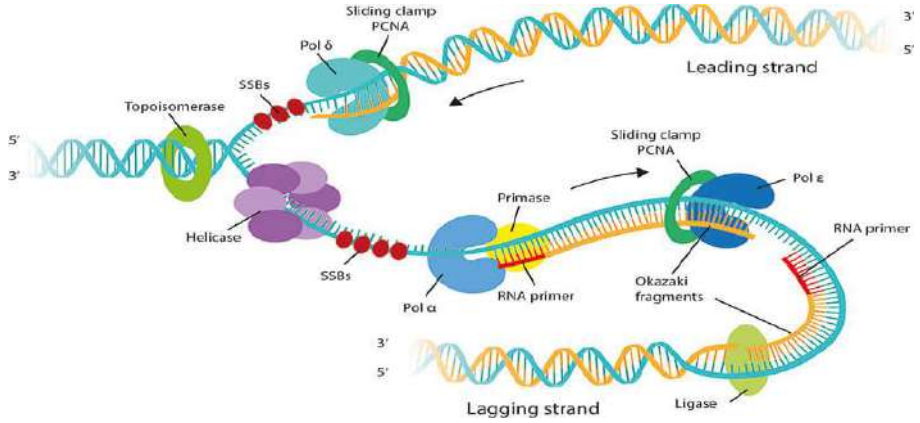

Fig.1.1. the mechanism of DNA replication. Image Source MBInfo © 2018 National University of Singapore.

The mechanism of DNA replication

Opening of the double-stranded helical structure of DNA and separation of the strands Priming of the template strands Assembly of the newly formed DNA segments. During the separation of DNA, the two strands uncoil at a specific site known as the **origin**. With the involvement of several enzymes and proteins, they prepare (prime) the strands for duplication. At the end of the process, DNA polymerase enzyme starts to organize the assembly of the new DNA strands. These are the general steps of DNA replication for all cells but they may vary specifically, depending on the organism and cell type.

Enzymes play a major role in DNA replication because they catalyse several important stages of the entire process.DNA replication is one of the most essential mechanisms of a cell's function and therefore intensive research has been done to understand its processes. The mechanism of DNA replication is well understood in *Escherichia coli*, which is also similar to that in eukaryotic cells. In E.coli, DNA replication is initiated at the oriClocus (oriC), to which DnaA protein binds while hydrolyzing of ATP takes place.

3.3. Enzymes inDNA Replication

DNA polymerase

DNA polymerases are enzymes used for the synthesis of DNA by adding nucleotide one by one to the growing DNA chain. The enzyme incorporates complementary amino acids to the template strand.

DNA polymerase is found in both prokaryotic and eukaryotic cells. They both contain several different DNA polymerases responsible for different functions in DNA replication and DNA repair mechanisms.

DNA Helicase enzyme

This is the enzyme that is involved in unwinding the double-helical structure of DNA allowing DNA replication to commence.

It uses energy that is released during ATP hydrolysis, to break the hydrogen bond between the DNA bases and separate the strands.

This forms two replication forks on each separated strand opening up in opposite directions.

At each replication fork, the parental DNA strand must unwind exposing new sections of single-stranded templates.

The helicase enzyme accurately unwinds the strands while maintaining the topography on the DNA molecule.

DNA primase enzyme

This is a type of RNA polymerase enzyme that is used to synthesize or generate RNA primers, which are short RNA molecules that act as templates for the initiation of DNA replication.

DNA ligase enzyme

This is the enzyme that joins DNA fragments together by forming phosphodiester bonds between nucleotides.

Exonuclease

These are a group of enzymes that remove nucleotide bases from the end of a DNA chain.

Topoisomerase

This is the enzyme that solves the problem of the topological stress caused during unwinding.

They cut one or both strands of the DNA allowing the strand to move around each other to release tension before it rejoins the ends.

And therefore, the enzyme catalysts the reversible breakage it causes by joining the broken strands.

Topoisomerase is also known as DNA gyrase in *E. coli.*

Telomerase

This is an enzyme found in eukaryotic cells that adds a specific sequence of DNA to the telomeres of chromosomes after they divide, stabilizing the chromosomes over time.

DNA Replication Steps/Stages

1. Initiation
2. Elongation
3. Termination

Initiation

This is the stage where DNA replication is initiated.DNA synthesis is initiated within the template strand at a specific coding region site known as **origins**.The origin sites are targeted by the **initiator proteins**, which recruit additional proteins that help in the replication process to form a replication complex around the DNA origin.There are several origin sites on which DNA replication is initiated and they are all known as **replication forks.**The formed replication complex contains the DNA helicase enzyme whose function is to unwind the double helix, exposing the two strands, which act as templates for replication.The mechanism of DNA helicase enzyme is by hydrolyzing the ATP that is used to form the bonds between the nucleobases, thus breaking the bond that holds the two strands.Additionally, during initiation DNA primase enzyme synthesizes small RNA primers that kick-start the function of DNA polymerase.DNA polymerase enzyme functions by growing the new DNA daughter strand.

Elongation

This is the phase where the DNA polymerase grows the new DNA daughter strand by attaching to the original unzipped template strand and the initiating short RNA primer.The DNA polymerase is able to synthesize a new strand that matches the template, by extending the primer via the addition of free nucleotides to the 3′ end.

One of the templates reads in the 3′ to 5′ direction, and therefore, the DNA polymerase synthesizes the new strand in the 5′ to 3′ direction, which is known as the **leading strand.**Along the template strand, DNA primase synthesizes a short RNA primer at the beginning of the template in the 5′ to 3′ direction, which initiates the DNA polymerase

to continue synthesizing new nucleotides, extending the new DNA strand.The other template (5′ to 3′) is elongated in an antiparallel direction, by the addition of short RNA primers which are filled with other joining fragments, forming the newly formed **lagging strand.** These short fragments are known as the **Okazaki fragments.**The synthesis of the lagging strand is discontinuous since the newly formed strand is disjointed.The RNA nucleotides from the short RNA primers must be removed and replaced by DNA nucleotides, which are then joined by the DNA ligase enzyme.

Termination

After the synthesis and extension of both the continuous and discontinued stands, an enzyme knows as exonuclease removes all RNA primers from the original strands.

The primers are replaced with the right nucleotide bases.While removing the primers, another type of exonuclease proofread the new stands, checking, removing, and replacing any errors formed during synthesis.DNA ligase enzyme joins the Okazaki fragments to form a single unified strand. The ends of the parent strand consist of a repetition of DNA sequences known as telomeres which act as protective caps at the ends of chromosomes preventing the fusion of nearby chromosomes. The telomeres are synthesized by a special type of DNA polymerase enzyme known as telomerase. It catalyzes the telomere sequences at the end of the DNA.On completion, the parent and complementary strand coil into a double helical shape, producing two DNA molecules each passing one strand from the parent molecule and one new strand.

3.4. Check your progress-1

1. …….is a type of RNA polymerase enzyme that is used to Synthesize or generate RNA primers
2. …….this is the stage where DNA replication is initiated
3. ……..These is a group of enzymes that remove nucleotide bases from The end of a DNA chain.
4. ……..is a complex process that takes place during cell division
5. …….are bound to each other in strands via phosphodiester bonds Forming a sugar-phosphate backbone.

DNA Replication Okazaki fragments

The two DNA strands run in opposite or antiparallel directions, and therefore to continuously synthesize the two new strands at the replication fork requires that one

strand is synthesized in the 5'to3′ direction while the other is synthesized in the opposite direction, 3'to 5′.However, DNA polymerase can only catalyze the polymerization of the dNTPs only in the 5'to 3'direction.This means that the other opposite new strand is synthesized differently. But how?By the joining of discontinuous small pieces of DNA that are synthesized backward from the direction of movements of the replication fork. These small pieces or fragments of the new DNA strand are known as the **OkasakiFragments.**TheOkasaki fragments are then joined by the action of DNA ligase, which forms an intact new DNA strand known as the lagging strand.

The lagging phase is not synthesized by the primer that initiates the synthesis of the leading strand.

Instead, a short fragment of RNA serves as a primer (RNA primer) for the initiation of replication of the lagging strand.

RNA primers are formed during the synthesis of RNA which is initiated de novo, and an enzyme known as primase synthesizes these short fragments of RNA, which are 3-10 nucleotides long and complementary to the lagging strand template at the replication fork.

The Okazaki fragments are then synthesized by the extension of the RNA primers by DNA polymerase.

However, the newly synthesized lagging strand is that it contains an RNA-DNA joint, defining the critical role of RNA in DNA replication.

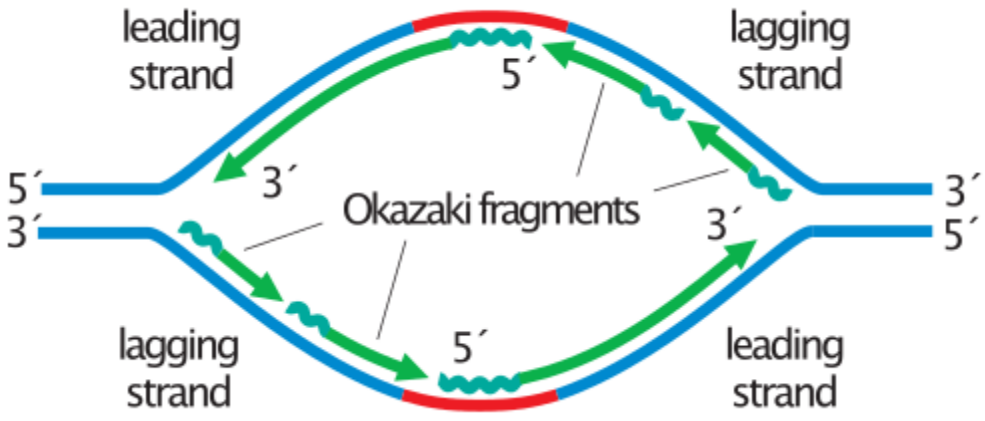

Fig.1.2. Okazaki fragments. Image Source: David O Morgan.

Replication Fork Formation and its function

The replication fork is the site of active DNA synthesis, where the DNA helix unwinds and single strands of the DNA replicates. Several sites of origin represent the replication forks.The replication fork is formed during DNA strand unwinding by the helicase enzyme which exposes the origin of replication. A short RNA primer is synthesized by primase and elongation done by DNA polymerase.The replication fork moves in the direction of the new strand synthesis. The new DNA strands are synthesized in two orientations, i.e 3′ to 5′ direction which is the leading strand, and the 5′ to 3′ orientation which is the lagging strand.The two sides of the new DNA strand (leading and lagging strand) are replicated in two opposite directions from the replication fork.

Therefore the replication fork is bi-directional.

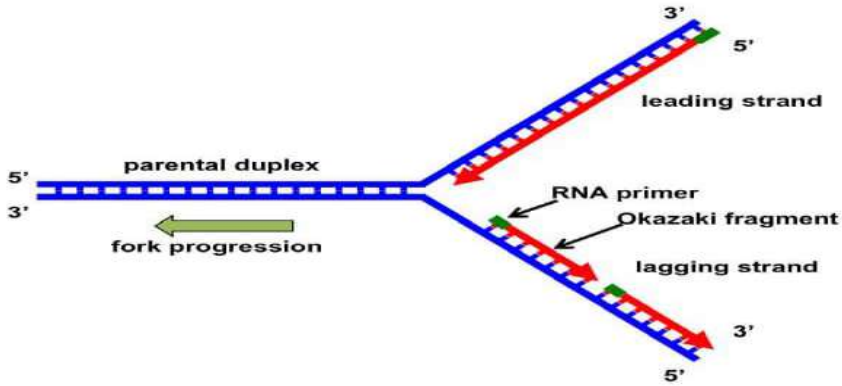

Fig.1.3.DNA Replication Fork. Image Source: MDPI (Adam R. Leman and Eishi Noguchi).

Leading Strand

The leading strand is the new DNA strand that is continuously synthesized by the DNA polymerase enzyme.It is the simplest strand that is synthesized during replication.The synthesis starts after the DNA strand has unzipped and separated. This generates a short piece of RNA known as a **primer**, by the DNA primaseenzyme.The primer binds to the 3′ end (start) of the strand, thus initiating the synthesize of the new strand (leading strand).The synthesis of the leading strand is a continuous process.

The Lagging Strand

This is the template strand (5′ to 3′) that is synthesized in a discontinuous manner by RNA primers. During the synthesis of the leading strand, it exposes small, short strands, or templates that are then used for the synthesis of the Okasaki fragments.

The Okasaki fragments synthesize the lagging strand by the activity of DNA polymerase which adds the pieces of DNA (the Okasaki fragments) to the strand between the primers. The formation of the lagging strand is a discontinuous process because the newly formed strand (lagging strand) is the fragmentation of short DNA strands.

3.5 DNA Pol I.II.III

There are three basic types of DNA polymerase. They are the following

DNA polymerase 1 (Pol 1): It is the repair polymerase; the most abundant polymerase. It proofreads to check for any mistakes during the replication process. It plays an important role in DNA replication. It is also helpful in molecular biology research.

DNA polymerase 2 (Pol 2):it plays an important role in DNA repair, but its main role is its ability to direct polymerase activity during the replication process.

DNA polymerase 3 (Pol 3): It belongs to the family C polymerase and is primarily involved in the DNA replication in E. coli. It is useful in prokaryotic DNA replication. It is also called holoenzyme and plays an important component of the replisome.

3.6. Replication in Viruses

Populations of viruses do not grow through cell division because they are not cells. Instead, they use the machinery and metabolism of a host cell to produce new copies of themselves. After infecting a host cell, a virion uses the cell's ribosomes, enzymes, ATP, and other components to replicate. Viruses vary in how they do this. For example:Some RNA viruses are translated directly into viral proteins in ribosomes of the host cell. The host ribosomes treat the viral RNA as though it were the host's own mRNA.Some DNA viruses are first transcribed in the host cell into viral mRNA. Then the viral mRNA is translated by host cell ribosomes into viral proteins.In either case, the newly made viral proteins assemble to form new virions. The virions may then direct the production of an enzyme that breaks down the host cell wall. This allows the virions to burst out of the cell. The host cell is destroyed in the process. The newly released virus particles are free to infect other cells of the host.

Replication of RNA Viruses

An **RNA virus** is a virus that has RNA as its genetic material. Their nucleic acid is usually single-stranded RNA, but may be double-stranded RNA. Important human pathogenic RNA viruses include the Severe Acute Respiratory Syndrome (SARS) virus, Influenza virus, and Hepatitis C virus. Animal RNA viruses can be placed into different groups depending on their type of replication.

- Some RNA viruses have their genome used directly as if it were mRNA. The viral RNA is translated directly into new viral proteins after infection by the virus.
- Some RNA viruses carry enzymes which allow their RNA genome to act as a template for the host cell to a form viral mRNA.
- **Retroviruses** use DNA intermediates to replicate. **Reverse transcriptase**, a viral enzyme that comes from the virus itself, converts the viral RNA into a complementary strand of DNA, which is copied to produce a double stranded molecule of viral DNA. This viral DNA is then transcribed and translated by the host machinery, directing the formation of new virions. Normal transcription involves the synthesis of RNA from DNA; hence, reverse transcription is the *reverse* of this process. This is an exception to the central dogma of molecular biology.

3.7. Replication in Prokaryotes

The prokaryotic chromosome is a circular molecule with a less extensive coiling structure than eukaryotic chromosomes. The eukaryotic chromosome is linear and highly coiled around proteins. While there are many similarities in the DNA replication process, these structural differences necessitating some differences in the DNA replication process in these two life forms. DNA replication in prokaryotes has been extensively studied, so we will learn the basic process of prokaryotic DNA replication, then focus on the differences between prokaryotes and eukaryotes.

How does the replication machinery know where to start? It turns out that there are specific nucleotide sequences called **origins of replication** where replication begins. *E.*

coli has a single origin of replication on its one chromosome, as do most prokaryotes (**Figure 1**). The origin of replication is approximately 245 base pairs long and is rich in AT sequences. This sequence of base pairs is recognized by certain proteins that bind to this site. An enzyme called **helicase** unwinds the DNA by breaking the hydrogen bonds between the nitrogenous base pairs. ATP hydrolysis is required for this process because it requires energy. As the DNA opens up, Y-shaped structures called **replication forks** are formed (**Figure 1**). Two replication forks are formed at the origin of replication and these get extended bi-directionally as replication proceeds. **Single-strand binding proteins** (Figure 2) coat the single strands of DNA near the replication fork to prevent the single-stranded DNA from winding back into a double helix.

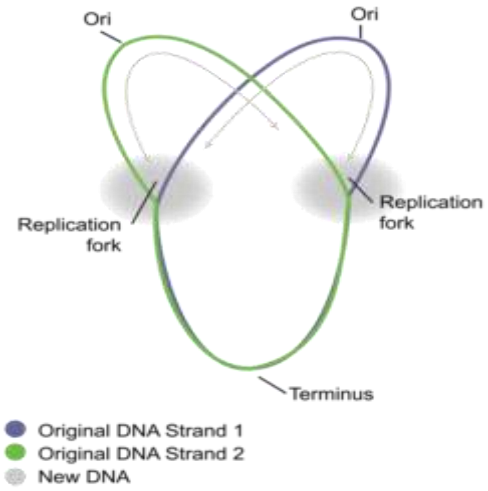

Fig.1.4. DNA replication in prokaryotes, which have one circular chromosome.

The next important enzyme is **DNA polymerase III**, also known as DNA pol III, which adds nucleotides one by one to the growing DNA chain (Figure 2). The addition of nucleotides requires energy; this energy is obtained from the nucleotides that have three phosphates attached to them. ATP structurally is an adenine nucleotide which has three phosphate groups attached; breaking off the third phosphate releases energy. In addition to ATP, there are also TTP, CTP, and GTP. Each of these is made up of the corresponding nucleotide with three phosphates attached. When the bond between the phosphates is broken, the energy released is used to form the phosphodiester bond between the incoming nucleotide and the existing chain.

In prokaryotes, three main types of polymerases are known: DNA pol I, DNA pol II, and DNA pol III. DNA pol III is the enzyme required for DNA synthesis; DNA pol I is used later in the process and DNA pol II is used primarily required for repair (this is another

irritating example of naming that was done based on the order of discovery rather than an order that makes sense).

DNA polymerase is able to add nucleotides only in the 5′ to 3′ direction (a new DNA strand can be only extended in this direction). It requires a free 3′-OH group (located on the sugar) to which it can add the next nucleotide by forming a phosphodiester bond between the 3′-OH end and the 5′ phosphate of the next nucleotide. This essentially means that it cannot add nucleotides if a free 3′-OH group is not available. Then how does it add the first nucleotide? The problem is solved with the help of a **primer** that provides the free 3′-OH end. Another enzyme, **RNA primase**, synthesizes an RNA primer that is about five to ten nucleotides long and complementary to the DNA. RNA primase does not require a free 3′-OH group. Because this sequence primes the DNA synthesis, it is appropriately called the primer. DNA polymerase can now extend this RNA primer, adding nucleotides one by one that are complementary to the template strand Fig.2

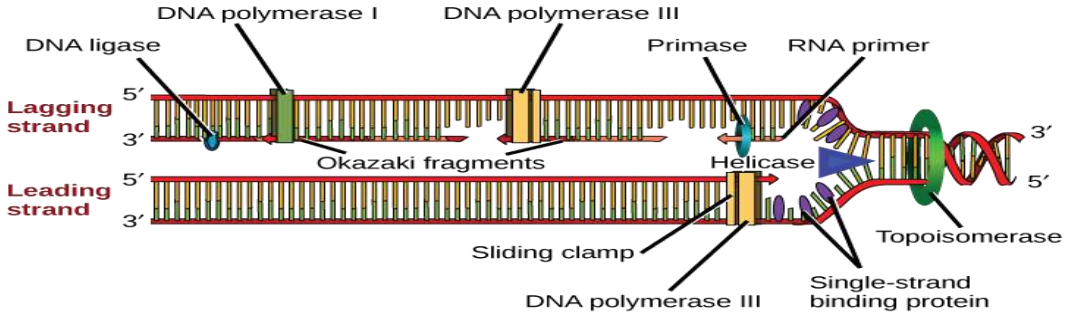

Fig.1.5. a replication fork is formed when helicase separates the DNA strands at the origin of replication.

The DNA tends to become more highly coiled ahead of the replication fork. Topoisomerase breaks and reforms DNA's phosphate backbone ahead of the replication fork, thereby relieving the pressure that results from this supercoiling. Single-strand binding proteins bind to the single-stranded DNA to prevent the helix from re-forming. Primase synthesizes an RNA primer. DNA polymerase III uses this primer to synthesize the daughter DNA strand. On the leading strand, DNA is synthesized continuously, whereas on the lagging strand, DNA is synthesized in short stretches called Okazaki fragments. DNA polymerase I replaces the RNA primer with DNA. DNA ligase seals the gaps between the Okazaki fragments, joining the fragments into a single DNA molecule.

The replication fork moves at the rate of 1000 nucleotides per second. DNA polymerase can only extend in the 5′ to 3′ direction, which poses a slight problem at the replication fork. As we know, the DNA double helix is anti-parallel; that is, one strand is in the 5′ to 3′ direction and the other is oriented in the 3′ to 5′ direction. One strand, which is complementary to the 3′ to 5′ parental DNA strand, is synthesized continuously towards the replication fork because the polymerase can add nucleotides in this direction. This continuously synthesized strand is known as the **leading strand**. The other strand, complementary to the 5′ to 3′ parental DNA is extended away from the replication fork, in small fragments known as **Okazaki fragments**, each requiring a primer to start the synthesis. Okazaki fragments are named after the Japanese scientist who first discovered them. The strand with the Okazaki fragments is known as the **lagging strand**.

The leading strand can be extended by one primer alone, whereas the lagging strand needs a new primer for each of the short Okazaki fragments. The overall direction of the lagging strand will be 3′ to 5′, and that of the leading strand 5′ to 3′. A protein called the **sliding clamp** holds the DNA polymerase in place as it continues to add nucleotides. The sliding clamp is a ring-shaped protein that binds to the DNA and holds the polymerase in place. **Topoisomerase** prevents the over-winding of the DNA double helix ahead of the replication fork as the DNA is opening up; it does so by causing temporary nicks in the DNA helix and then resealing it. As synthesis proceeds, the RNA primers are replaced by DNA pol I, which breaks down the RNA and fills the gaps with DNA nucleotides. The nicks that remain between the newly synthesized DNA (that replaced the RNA primer) and the previously synthesized DNA are sealed by the enzyme **DNA ligase** that catalyzes the formation of phosphodiester linkage between the 3′-OH end of one nucleotide and the 5′ phosphate end of the other fragment.

(Lisa’s note: I think this process is almost impossible to visualize from reading text. I strongly recommend that you watch a couple of animations / videos like the one available here. There are additional links in Blackboard)

Once the chromosome has been completely replicated, the two DNA copies move into two different cells during cell division. The process of DNA replication can be summarized as follows.

Table 1: The enzymes involved in prokaryotic DNA replication and the functions of each. Prokaryotic DNA Replication: Enzymes and Their Function

Enzyme/protein	Specific Function
DNA pol I	Exonuclease activity removes RNA primer and replaces with newly synthesized DNA
DNA pol II	Repair function
DNA pol III	Main enzyme that adds nucleotides in the 5′-3′ direction
Helicase	Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases
Ligase	Seals the gaps between the Okazaki fragments to create one continuous DNA strand
Primase	Synthesizes RNA primers needed to start replication
Sliding Clamp	Helps to hold the DNA polymerase in place when nucleotides are being added
Poisomerase	Helps relieve the stress on DNA when unwinding by causing breaks and then resealing the DNA binding
Single-strand proteins (SSB)	Binds to single-stranded DNA to avoid DNA rewinding back.

DNA replication has been extremely well-studied in prokaryotes, primarily because of the small size of the genome and large number of variants available. *Escherichia coli* has 4.6 million base pairs in a single circular chromosome, and all of it gets replicated in approximately 42 minutes, starting from a single origin of replication and proceeding around the chromosome in both directions. This means that approximately 1000 nucleotides are added per second. The process is much more rapid than in eukaryotes.

3.8. Summary

The prokaryotic chromosome is a circular molecule with a less extensive coiling structure than eukaryotic chromosomes. The eukaryotic chromosome is linear and highly coiled around proteins. While there are many similarities in the DNA replication process, these structural differences necessitating some differences in the DNA replication process in these two life forms. DNA replication in prokaryotes has been extensively studied, so we

will learn the basic process of prokaryotic DNA replication, then focus on the differences between prokaryotes and eukaryotes.

How does the replication machinery know where to start? It turns out that there are specific nucleotide sequences called **origins of replication** where replication begins. *E. coli* has a single origin of replication on its one chromosome, as do most prokaryotes The origin of replication is approximately 245 base pairs long and is rich in AT sequences. This sequence of base pairs is recognized by certain proteins that bind to this site. An enzyme called **helicase** unwinds the DNA by breaking the hydrogen bonds between the nitrogenous base pairs. ATP hydrolysis is required for this process because it requires energy. As the DNA opens up, Y-shaped structures called **replication forks** are formed two replication forks are formed at the origin of replication and these get extended bi-directionally as replication proceeds. **Single-strand binding proteins** coat the single strands of DNA near the replication fork to prevent the single-stranded DNA from winding back into a double helix.

3.9. Check your progress-2

6. ……. are viruses that infect bacteria. They bind to surface Receptor molecules of the bacterial cell
7. ……. prevents the over-winding of the DNA double helix
8. DNA unwinds at the origin of ……..
9. The prokaryotic chromosome is a circular molecule with a less extensive coiling Structure than ……...
10. ……..Main enzyme that adds nucleotides in the 5′-3′ direction

3.10. Glossary

1. **DNA**: is a nucleic acid that is made up of three components
2. **Termination:** After the synthesis and extension of both the continuous and discontinued stands
3. **DNA replication:** This is a complex process that takes place during cell division,

4. **Telomerase :**This is an enzyme found in eukaryotic cells that adds a specific sequence of DNA
5. **DNA structure:** defines the basic genetic makeup of our body
6. **DNA ligase enzyme:** This is the enzyme that joins DNA fragments together by forming phosphodiester bonds between nucleotides
7. **Structural model of DNA**:was initially proposed by James Watson and Francis Click
8. **Leading strand** the leading strand is the new DNA strand that is continuously synthesized by the DNA polymerase enzyme.
9. **Initiation:**This is the stage where DNA replication is initiated.

3.11. Questions for self-study

1. What is DNA replication? explain the Mechanism of DNA replication
2. Discuss about DNA replication enzymes and proteins
3. Describe the Steps DNA replication?
4. Explain about DNA replication Okazaki fragments
5. Give an account of Prokaryotic and Eukaryotic DNA Replication

3.12. Answers to check your progress 1 and 2

1. **DNA primase enzyme**
2. **Initiation**
3. **Exonuclease**
4. **Replication**
5. **Nucleotides**
6. **Bacteriophages**
7. **Topoisomerase**
8. **Replication.**
9. **Eukaryotic chromosomes**
10. **DNA POL III** .

3.13. References for further reading

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UNIT-4

Eukaryotic DNA replication: eukaryotic polymerases. Role of others proteins and enzymes in replication. Inhibitors of replication

Structure of the Unit

4.0. Objectives

After studying this unit you will be able to

- understand the Features of Eukaryotic DNA Replication
- discuss about Replication in Eukaryotes
- explain about Eukaryotic DNA Polymerases
- describe the inhibitors of replication

4.1. Introduction

DNA replication is the process by which an organism duplicates its **DNA** into another copy that is passed on to daughter cells. Replication occurs before a cell divides to ensure that both cells receive an exact copy of the parent's genetic material. Our understanding of replication in eukaryotes is not as extensive as that in prokaryotes, owing to the higher level of complexity in eukaryotes and the consequent difficulty in studying the processes. Even though many of the principles are the same, eukaryotic replication is more complicated in three basic ways: there are multiple origins of replication, the timing must be controlled to that of cell divisions, and more proteins and enzymes are involved.

Replication is bi-directional and originates at multiple origins of replication (Ori C) in eukaryotes. DNA replication uses a semi-conservative method that results in a double-stranded. DNA with one parental strand and a new daughter strand. It occurs only in the S phase and at many chromosomal origins. Takes place in the cell nucleus. Synthesis occurs only in the 5′to 3′direction.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. Eukaryotic cells possess five types of polymerases involved in the replication process.

Helicases: Unwind the DNA helix at the start of replication.

SSB proteins: Bind to the single strands of unwound DNA to prevent reformation of the DNA helix during replication.

4.2. Eukaryotic DNA Replication

Replication of each linear DNA molecule in a chromosome starts at many origins, one every 30–300 kb of DNA depending on the species and tissue, and proceeds bi-

directionally from each origin.At each origin, a replication bubble forms consisting of two replication forks moving in opposite directions. The DNA replicated under the control of a single origin is called a replicon. DNA synthesis proceeds until replication bubbles merge together.At the origin, enzymes unwind the double helix making its components accessible for replication. The helix is unwound by helicase to form a pair of replication forks.The unwound helix is stabilized by SSB proteins and DNA topoisomerases.The RNA primers required are made by DNA polymerase α which carries a primasesubunit.DNA polymerase α initiate synthesis of the lagging strand, making first the RNA primer and then extending it with a short region of DNA.DNA polymerase δ then synthesizes the rest of the Okazaki fragment.The leading strand is synthesized by DNA polymerase δ.The leading strand is synthesized continuously in the 5′to 3′ direction while the lagging strand is synthesized discontinuously in the 5′to 3′ direction through the formation of Okazaki fragments.At the completion of synthesis, DNA ligase seals the breaks between the Okazaki fragments as well as around the primers to form continuous strands. Refer in the unit 3 remaining process of replication

4.3. Eukaryotic Polymerase

Eukaryotic cells contain five different DNA polymerases; α, β, γ, δ and ε.DNA polymerases α and δ replicate chromosomal DNA, DNA polymerases β and ε repair DNA, and DNA polymerase γ replicates mitochondrial DNA.DNA polymerase α and δ synthesize the lagging strand, via Okazaki fragments.The RNA primers are synthesized by DNA polymerase α which carries a primasesubunit.DNA polymerase δ synthesizes the leading strand.Telomerase, a DNA polymerase that contains an integral RNA that acts as its own primer, is used to replicate DNA at the ends of chromosomes (telomeres).

DNA topoisomerase I: Relaxes the DNA helix during replication through creation of a nick in one of the DNA strands.

DNA topoisomerase II: Relieves the strain on the DNA helix during replication by forming supercoils in the helix through the creation of nicks in both strands of DNA.

DNA ligase: Forms a 3′-5′phosphodiester bond between adjacent fragments of DNA.

Eukaryotic DNA polymerase Enzymes

For all species, the process of DNA replication requires the use of multiple different DNA polymerase enzymes. The primary function of these unique multi subunit enzymes is to ensure optimal DNA stability for an accurate replication process.

- Functions of DNA polymerase enzymes
- Types of DNA polymerase enzymes
- Non-replicative/specialized DNA polymerase enzymes

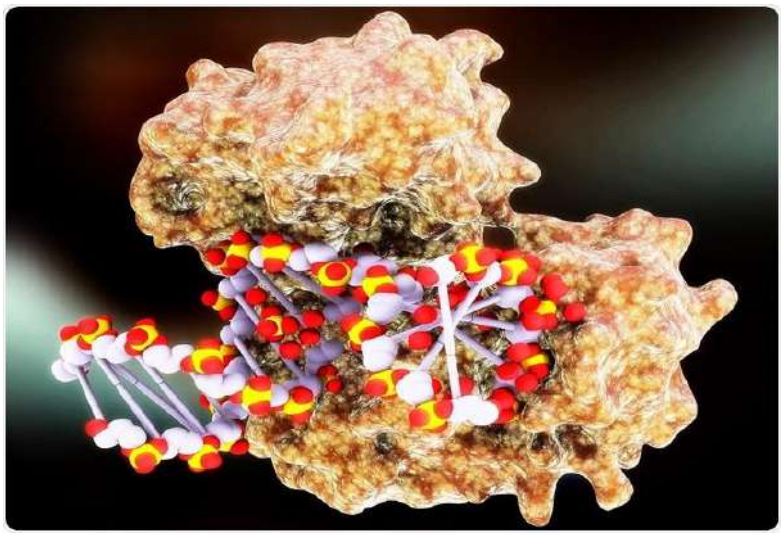

Fig.1.1. Polymerase enzyme

Functions of DNA polymerase enzymes

Since Arthur Kornberg was awarded the Nobel Prize in 1959 for determining the roles of DNA polymerases during DNA replication, it has been widely accepted that the DNA polymerases involved in this process require a single-stranded template to construct a new DNA strand. In addition to DNA polymerase, DNA replication also requires several other enzymes including a helicase to unwind the double-stranded template DNA, as well as a primase to assemble a short RNA primer.The DNA polymerase enzymes involved in the eukaryotic DNA replication belong to the B family of DNA polymerases, whereas those enzymes that function in bacteria belong to families A and C, and those of archaea belong to families B and D.It has been determined that all replicative DNA polymerases contain additional domains that function to facilitate interactions with other proteins, 3’ to 5’ exonucleolytic proofreading and several other important biological processes. During

replication, DNA polymerases are involved in copying both DNA strands from the template strand at each replication fork.

4.4. Check your progress-1

1. DNA replication requires the use of multiple different DNA ……
2. Replication is bi-directional and originates at multiple ……..
3. The DNA polymerase enzymes involved in the eukaryotic DNA replication belong to the B family of ……..
4. Replication of each linear DNA molecule in a chromosome starts a……
5. DNA polymerases are involved in copying both DNA strands from the template strand at each ……

Types of DNA polymerase enzymes

Replisomes are large multiprotein assemblies that contain DNA polymerases, helicases, primases, sliding clamps and other important structures involved in DNA replication. Within the highly complicate replisome structure of eukaryotic cells, three different B-family DNA polymerases exist, of which include the multisubunit polymerases Pol α, Pol δ and Pol ε. In addition to carrying out DNA synthesis, B-family DNA polymerases are also responsible for DNA repair and recombination metabolic processes.The functions of Pol α, which is comprised of four different subunits, is to synthesize a considerable amount of DNA, as well as associate with DNA primase to give the Pol α–DNA primase complex. This complex is comprised of four subunits that include the POLA, which is the catalytic subunit, POLA2, which is the regulatory subunit, and the two primase subunits PRIM1 and PRIM2.The Pol α–DNA primase complex creates RNA-DNA primers. Note that Pol α does not exhibit any proofreading activity; therefore, any errors made by this enzyme will be corrected by other mechanisms, of which include those performed by Pol ε and Pol δ.

The synthesis of DNA on both the leading and lagging strands is accomplished by both Pol ε and Pol δ. Both Pol ε and Pol δ are highly accurate and processive enzymes that utilize their exonuclease activities for proofreading processes during replication. Pol δ, which is a three subunit enzyme, is encoded by the POLD1 gene, whereas Pol ε, a four subunit enzyme, is encoded by the POLE gene.

Non-replicative/specialized DNA polymerase

While replicative DNA polymerases only function during cell division, non-replicative or specialized DNA polymerases are used throughout the lifecycle of the cell. The emergence of specialized DNA polymerases has provided insight into how these enzymes play a role in preventing replicative stress and its devastating consequences during DNA replication.These monomeric enzymes primarily belong to the Y family, of which include Pol η, ι, κ and Rev1 polymerases; however, additional enzymes of the B-family, such as Pol, and the X family, such as Pol θ, fall into this category of specialized DNA polymerases.Pol of the B-family plays a role in the embryonic development of mammals, whereas Pol θ of the X family has been described for its role in lesion bypass, base excision repair mechanisms and somatic hypermutation.

The DNA polymerases of the Y family exhibit a wide variety of cellular functions, many of which are involved in maintaining different types of stability within the cell. For example, Pol η and κ play a role in maintaining the stability of fragile sites (FS) in mammalian cells, which are considered to be the most sensitive components of the genome. In fact, the sensitivity of common fragile sites (CFS) on chromosomes to experience stress after replication has been linked with causing cancer-specific chromosomal rearrangements. The X family of no replicative polymerases, which can be found in all species, particularly vertebrates that carry all four types of these polymerases, include Pols β, λ and μ, as well as the homolog deoxyribonucleotidyltransferase (TdT).The X family polymerases contain highly conserved regions that include two helix-hairpin-helix domains that are essential to their interaction with DNA. One of these motifs is found in a domain that interacts with downstream DNA and the other is found in the thumb domain which works together with the primer strand to help begin the process of DNA replication.

Pol β, which is encoded by the POLB gene in humans, has been shown to participate in different DNA repair and damage tolerance pathways. More specifically, Pol β performs short patch repair of damaged DNA by fixing alkylated, oxidized or abasic sites that have formed as a result of DNA damage. Although this polymerase does not exhibit any proofreading activity on its own, its precise selectivity for correct nucleotides that reduces the chance of errors to occur when in use.Pol λ and Pol μ, which are encoded by the

POLL and POLM genes, respectively, are involved in the rejoining of breaks that have occurred in double strands of DNA that occur following exposure to hydrogen peroxide, as in the case of Pol λ, or ionizing radiation, as in the case of Pol μ, which is an enzyme that is predominantly found in lymphoid tissue.

4.5. Role of other Proteins and Enzymes involved in the replication

A number of enzymes and proteins are associated with the **replication fork** to help in the initiation and continuation of DNA synthesis, most prominently; DNA polymerase synthesizes the new strands by adding nucleotides that complement each (template) strand. DNA replication occurs during the **S-phase** of **interphase**. At the replication fork, many replication enzymes assemble on the DNA into a complex molecular machine called the replisome. The following is a list of major DNA replication enzymes that participate in the replisome.

DNA Helicase:

- Helicase enzyme opens up the DNA double helix by **breaking hydrogen bond** between two strands of DNA and provide single template strand.
- DNA-B is a primary replicative Helicase it binds and **moves on lagging strand** in 5' to 3' direction unwinding the duplex as it goes.
- Helicase requires **ATP** as energy source

Single Stranded Binding Proteins (SSB proteins):

- SSB proteins binds to both separated single stranded DNA and prevent the DNA double helix from **re-annealing** after helicase unwinds.
- SSB proteins are maintaining the strand separation and facilitating the synthesis of the nascent strand.

Topoisomerase:

DNA Topoisomerase is a nuclease enzyme that **breaks** *a* **phosphodiester bond** in a DNA strand.The function of Topoisomerase is **relaxes** the **DNA** from its super coiled nature.

DNA Gyrase: This enzyme is used to make sure the double stranded areas out side of the replication fork do not **supercoil**, DNA Gyrase is one type of topoisomerase.

Primase: Primase provides a starting point of RNA (or DNA) for DNA polymerase to begin synthesise of the new DNA strand. Because DNA polymerase requires **free 3'-OH group** for bind to DNA for starting replication.

DNA polymerase: DNA dependent DNA polymerase enzyme that can synthesise a new strand on a DNA tamplate.DNA polymerase has different types in Prokaryotes and Eukaryotes.

Prokaryotic DNA polymerase

Prokaryotes has 3 types of DNA polymerase, these are

- DNA pol-I,
- DNA pol-II and
- DNA pol-III.

DNA polymerase I –It is made up of one subunits. It has 3' to 5' and 5' to 3' exonucleaseactivity.Function - DNA repair, Gap filling and synthesis of new lagging strand.

DNA polymerase II -It is made up of 7 subunits. It has only 3' to 5' exonucleaseactivity.Function - DNA repair and DNA proof reading.

DNA polymerase III -It is made up of at least 10 subunits. It has 3' to 5' exonucleaseactivity.Function - This is the main replication enzyme in Prokaryotes.

Eukaryotic DNA polymerase

Eukaryotes have 5 types of DNA polymerase which are DNA polymerase α, β, γ, δ and ε.

DNA polymerase α -It has no any exonucleaseactivity.*Function* - DNA replication in the nucleus.

DNA polymerase β -It has no any exonucleaseactivity.*Function* - DNA replication and ***base excision repair***.

DNA polymerase γ -It has 3' to 5' exonucleaseactivity.*Function* - DNA replication in **Mitochondria**.

DNA polymerase δ -It has 3' to 5' exonucleaseactivity.*Function* - Synthesis of lagging strand during DNA replication.

DNA polymerase ε -It has 3' to 5' exonucleaseactivity.*Function* - Synthesis of leading strand during DNA replication.

Beta Clamp Proteins: Beta clamps are the protein which ***prevents*** elongating DNA polymerase from dissociating from the DNA parent strand. It helps hold the DNA polymerase in place on the DNA.

DNA Ligase: DNA Ligase Catalyse the *joining* of ends of two DNA chains by forming phosphodiester bond between 3'-OH group at one end of DNA strand and and 5'-Phosphate group at the end of other DNA strand.DNA ligase joins the Okazaki fragments of two lagging strand.

Telomerase: Lengthens the telomeric DNA by adding repetitive nucleotide sequence to the ends of eukaryotic chromosomes. This allows germ cells and stem cells to avoid the **Hay flick limit** on cell division.

4.6. Inhibitors of Replication

Dauromycin and Adriamycin: They are synthetic chemotherapeutic agents and are inhibitors of both DNA replication and transcription in prokaryotes. These are presumably act by interfering with the passage of both DNA and RNA polymerase. They have planar aromatic ring system which gets intercalated between GC pairs of the double helical structure. Thus, they prevent its replication and transcription

Adriamycin is otherwise known as doxorubicin. Doxorubicin is a cytotoxic anthracycline antibiotic isolated from cultures of Streptomyces peucetiusvar.caesius

Doxorubicin consists of a naphthacenequinone nucleus linked through a glycosidic bond at ring atom 7 to an amino sugar, daunosamine. Doxorubicinbinds to nucleic acids, presumably by specific intercalation of the planar anthracycline nucleus with the DNA double helix. The anthracycline ring is lipophilic, but the saturated end of the ring system contains abundant hydroxyl groups adjacent to the amino sugar, producing a hydrophilic center. The molecule is amphoteric, containing acidic functions in the ring phenolicgroups and a basic function in the sugar amino group. It binds to cell membranes as well asplasma proteins.

It is an antibiotic produced by streptomyces and inhibitsreplication and transcription. It acts by intercalating its phenoxazone ring between two successive GC pairs in duplex DNA. Actinomycin D has two identical pentapeptides which have unusual

composition of D-Valine and Sarcosine which stabilizes this intercalating interaction. It is an antibiotic produced by streptomyces and inhibitsreplication and transcription. It acts by intercalating its phenoxazone ring between two successive GC pairs in duplex DNA. Actinomycin D has two identical pentapeptides which have unusual composition of D-Valine and Sarcosine which stabilizes this intercalating interaction.

It was the first antibiotic shown to have anti-cancer activity, but is not normally used as such, as it is highly toxic, causing damage to genetic material. Actinomycin-D is marketed under the trade name Dactinomycin. Actinomycin-D is one of the older chemotherapy drugs which have been used in therapy for many years. It is a clear, yellow liquid which is administered intravenously and most commonly used in treatment of a variety of cancers

Ethidium bromide and proflavin

They inhibit both replication and transcription by intercalation. As with most fluorescentcompounds, it is aromatic. The main portion of the molecule is a tricyclic structure with aniline (aminobenzene) groups on either side of a pyridine (a six-atom, nitrogen-containing, aromaticring). The dibenzopyridine structure is known as a phenanthridine. The reason for ethidiumbromide's intense fluorescence after binding with DNA is probably not due to rigid stabilization of the phenyl moiety, because the phenyl ring has been shown to project outside the intercalated bases. In fact, the phenyl group is found to be almost perpendicular to the plane of the ringsystem, as it rotates about its single bond to find a position where it will about the ring systemminimally. Instead, the hydrophobic environment found between the base pairs is believed to beresponsible. By moving into this hydrophobic environment and away from the solvent, theethidiumcation is forced to shed any water molecules that were associated with it. As water is a highly efficient fluorescent quencher, the removal of these water molecules allows the ethidium to fluoresce. This property is used to identify the presence of DNA in gel und UV light

Novobiocin and oxolinic Acid

Prokaryotic DNA gyrases are specifically inhibited by two classes of antibiotics. One of these classes includes the Streptomyces-derived novobiocin and the other contains the clinically useful synthetic antibacterial agent oxolinic acid. Both classes of

antibiotics profoundly inhibit bacterial DNA replication and transcription, thereby demonstrating the importance of properly supercoiled DNA in these processes. Studies using antibiotic-resistant E.Coli mutants have demonstrated that oxolinic acid associates with DNA gyrase A subunit and novobiocin binds to its B subunit. When DNA gyrase is incubated with DNA and oxolinic acid, and subsequently denatured with guanidinium chloride, it's A subunits remain covalently linked to the 5'-endsof both cut strands through phosphotyrosine linkages. Apparently oxolinic acid interferes with gyrase action by blocking the strand breaking-re-joining process. Novobiocin, on the other hand, prevents ATP from binding to the enzyme.

Aphidicolin

Aphidicolin inhibits DNA pol α of eukaryotes, so it inhibits eukaryotic replication. Aphidicolin also inhibits DNA pol δ and DNA pol Ɛ. Thus Aphidicolin inhibits both leading and lagging strand synthesis in eukaryotes.

Rifamycin

Rifammycin inhibits RNA polymerase, so RNA primer for leading strand synthesis is not available. Thus replication inhibited. The rifamycins area group of antibiotics which are synthesized either naturally by the bacterium

Amycolatopsismediterranei,or artificially. The rifamycin group includes the "classic" rifamycin drugs as well as the rifamycin derivatives Rifampicin, Rifabutin and Rifapentine. The biological activity ofrifamycins relies on the inhibition of DNA-dependent RNA synthesis. This is due to the highaffinity of rifamycins to prokaryotic RNA polymerase. Crystal structure data of the antibiotic bound to RNA polymerase indicates that rifamycin blocks synthesis by causing strong stericclashes with the growing oligonucleotide. If rifamycin binds the polymerase after the chainelongation process has started, no effect is observed on the biosynthesis, which is consistent with a model that suggests rifamycin physically blocks the chain elongation. In addition, rifamycinsshowed potency towards tumors. This is due to their inhibition of the enzyme reversetranscriptase, which is essential for tumor persistence. However, rifamycins potency proved to bemild and this never leads to their introduction to

4.7. Summary

Replication in eukaryotes follows the same general outline as replication in prokaryotes, with the most important difference being the presence of histone proteins complexes to eukaryotic DNA. Different proteins are used, and the system is more complex than it is in prokaryotes. Replication is controlled so that it occurs only once during a cell-division cycle, during the S phase. Five different DNA polymerases are present in eukaryotes: α, β, γ, δ, and ε. Polymerase δ is the principal synthesizer of DNA and is the equivalent of Pol III in prokaryotes. The workload of copying DNA in eukaryotes is divided among multiple different types of DNA polymerase enzymes. Major families of DNA polymerases across all organisms are categorized by the similarity of their protein structures and amino acid sequences. The first families to be discovered were termed A, B, C, and X, with families Y and D identified later. Family B polymerases in eukaryotes include Pol α, which also functions as a primase at the replication fork, and Pol δ and ε, the enzymes that do most of the work of DNA replication on the leading and lagging strands of the template, respectively. Other DNA polymerases are responsible for such tasks as repairing DNA damage, copying mitochondrial and plastid DNA, and filling in gaps in the DNA sequence on the lagging strand after the RNA primers are removed.

4.8. Check your progress-2

6. Beta clamps are the protein which ***prevents*** elongating……..
7……It is made up of at least 10 subunits.
8. ……..It is made up of 7 subunits. It has only **3' to 5'** exonuclease Activity
9. DNA dependent DNA polymerase enzyme that can synthesise a new strand on a ……
10. Replicative DNA polymerases only function during …….

4.9. Glossary

1. **Eukaryotic replication** : follows many of the same principles as prokaryotic DNA
2. **DNA topoisomerase I:** Relaxes the DNA helix during replication through creation of a nick in one of the DNA strands.

3. **DNA topoisomerase II:** Relieves the strain on the DNA helix during replication by forming supercoils in the helix through the creation of nicks in both strands of DNA.
4. **DNA ligase:** Forms a 3′-5′phosphodiester bond between adjacent fragments of DNA.
5. **Helicases:** Unwind the DNA helix at the start of replication.
6. **SSB proteins:** Bind to the single strands of unwound DNA to prevent reformation of the DNA helix during replication.

4.10. Questions for self-study

1. Explain the features of Eukaryotic DNA Replication
2. Discuss about Replication in Eukaryotes
3. Describe the Eukaryotic DNA Polymerases
4. Describe the inhibitors of replication
5. How are eukaryotic polymerase related to prokaryotic ones?

4.11. Answers for check your progress 1 and 2

1. **Polymerase enzymes.**
2. **Origins of replication**
3. **DNA polymerases**
4. **Many origins,**
5. **Replication fork.**
6. **DNA polymerase**
7. **DNA polymerase III**
8. **DNA polymerase II**
9. **DNA template.**
10. **Cell division**

4.12. References

1. Levine, C., Hiasa, H., & Marians, K. J. (1998). DNA gyrase and topoisomerase IV: biochemical activities, physiological roles during chromosome replication, and drug sensitivities. *Biochimica et BiophysicaActa*, *1400*(1-3), 29–43. https://doi.org/10.1016/s0167-4781(98)00126-2