ISSN No:-2456-2165

DNA Synthesis and Sequence

[1:] VIVEK SHUKLA
(Btech/Mtech Dual Degree 3rd Year Student atKIIT School of Biotechnology(KSBT), KIIT University, Bhubaneswar, Odisha)

[2:] MANOJ KUMAR
(MSc Botany 1st year Student at Seth MotiLal College, Rajasthan)

Abstract:- *DNA(Deoxyribonucleic acid*) is a chemical molecule which consists of all the necessary information that a living human being must possess. These instructions are then passed on from one generation to another. Our main objective via this article is to explain how DNA can be obtained by many different techniques and methods of analysis. DNA synthesis techniques are becoming a major part of modern day biology and plays a vital role in the field of synthetic biology. Through this review paper we will try to explore the various techniques that are widely used in the synthesis of DNA. We have also discussed the applications of DNA to gene expression. We will also provide a brief introduction and discuss about DNA replication and what are processes responsible for DNA replication.

I. INTRODUCTION

- **Genes** consists of DNA and re responsible for the source of information to makemolecules called proteins.
- In1868, **Friedrich Miescher** secluded a phosphorus-involving material. He discovered that the material consisted of DNA, and a **basic portion(protein)**.Kossel showed that DNA contains four nitrogenous bases A, C, G, and T.
- Levene later showed that DNA comprises of a huge quantity of reoccurring units called **nucleotides;** which **consisted of *a sugar, a phosphate, and a base.***
- He also incorrectly suggested that DNA comprises of a chain of four-nucleotide units, consisting of all the four bases—***"Adenine, Guanine, Cytosine, and Thymine"***—in a fixed sequence(tetra nucleotide theory).

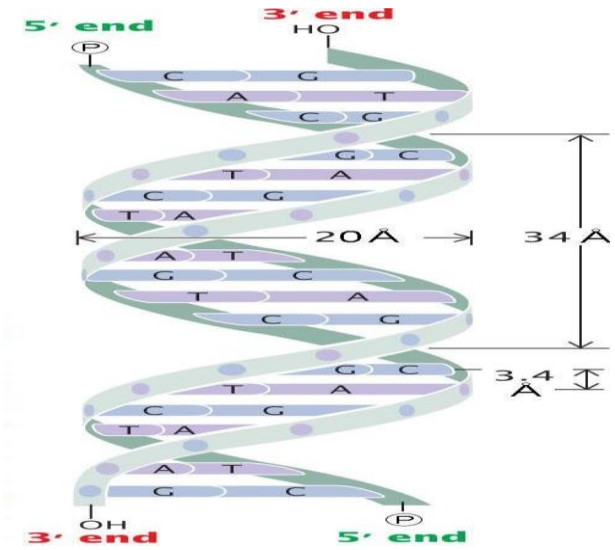

Figure-1: DETAILED ARRANGEMENT

- DNA molecule comprises of two poly nucleotide strands.
- The bases are occupied the interior of the helix, the sugar–phosphate chains are wrapped about its periphery, thus reducing the repulsions among charged phosphate groups
- DNA comprises of two long chains of simple units which are called nucleotides, with backbones made of base, sugars and phosphate groups.
- Each nucleotide is always attached via hydrogen bond to form specific complementary base pairing.
- The aromatic bases have a vander Waals thickness of 3.4Å and are always partially piled upon each other.
- Sugar & Base is together called as nucleoside
- Phosphate, sugar & Base are collectively known as nucleotide
- **Adenine** and **Guanine** are together known in a group as – **Purines**
- **Cytosine** & **Thymine** are together known in a group as - **Pyrimidines.**
- **BASE PAIRING** : Adenine pairs with Thymine with a double bond(A =T) in case ofDNA whereas it pairs with Uracil in case of RNA(A=U), Guanine pairs with Cytosinewith a triple bond(G≡C)
- Major difference between DNA and RNA is the sugar, with the 2-deoxyribose in DNA being substituted by the different pentose sugar ribose in RNA.
- DNA is a negatively charged molecule due to the presence of phosphate group.

DNA as the Genetic Material :-

The genetic material has four criteria.

1. **Information:** comprises of information essential to construct an entire organism.(**codingregions/genes**)
2. **Transmission:** It is carried from parents to offspring during the process of reproduction(**vertical transmission**).
3. **Replication:** It must be copied(**haploid to diploid**).
4. **Variation:** The genetic material must differ in ways that are responsible for the the known phenotypic differences among all species(**recombination**).

Chargaff (late 1940s) –DNA molecules have distinctive basecompositions:-

1. The base formation of DNA is generally different among all genus.
2. DNA specimen extracted from different tissues of the same species have similarcomposition.
3. The base composition of DNA in a given species remains unaltered with an organism's age, nutritional state, or changing environment.
4. Among all genus:

- **The total purine residues = the total pyrimidine residues**
- **Chargaff base pairing rule: [Purines = Pyrimidines]**
- **Applies to ds DNA, important for maintaining a stable DNA structure.**

II. DNA SEQUENCING

It is a method of establishing the nucleic acid **pattern**– that is the arrangement of nucleotides in **DNA**. It consists of any technique that can be taken into consideration in order to predict the arrangement of the four bases: adenine, guanine, cytosine, and thymine.

Different technologies:-

- **Sanger sequencing**.- is performed during low-throughput, targeted, or short-read.
- **Capillary electrophoresis**. Capillary electrophoresis (CE) instruments are efficient in performing both Sanger sequencing and fragment **analysis**.
- **Next-generation sequencing(NGS)**, they are vast-scale techniques that enhance the velocity and lower the expenditure.

DNA sequencing can establish a error-free diagnosis influencing the medical administration of symptoms, or supply treatment options.

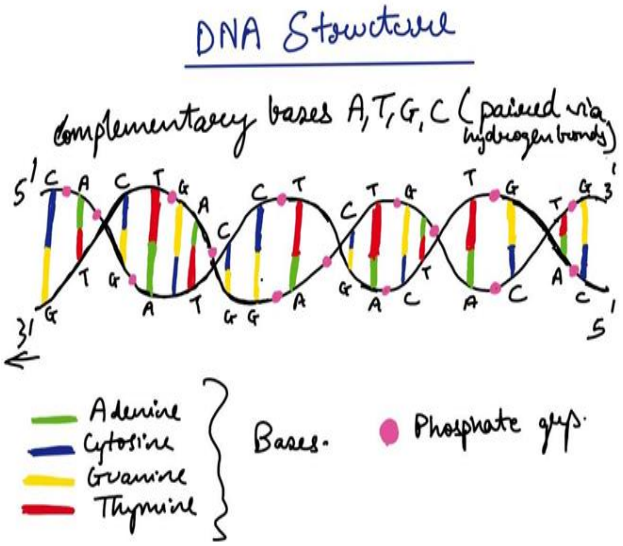

Figure-2:- STRUCTURE OF DNA & COMPLENTARY BASES

(i)SANGER SEQUENCING:-.

This method is established for predicting the pattern of nucleotide bases in a portion of DNA.It was used in the Human Genome Project to predict the pattern of small portions of human DNA. These fragments and constituted larger DNA portions and, ultimately, entire chromosomes. The development of NGS technologies has exaggerated genomics research.. Sanger sequencing remains widely used in the sequencing field as it offers several prominent advantages:

(i)less expensive(ii)accurate

Sanger methods:

(1) The double-stranded DNA (dsDNA) is reduced to two single-stranded DNA(ssDNA).
(2) A primer that harmonizes to one terminal end of the sequence is joined.
(3) Four polymerase solutions consist of four types of dNTPs however only onetype of dNTP is added.
(4) The DNA synthesis reaction starts and the length elongates unless any stopnucleotide is added casually
(5) The consequential DNA portions are reduced into ssDNA.
(6) The reduced portions are discarded via the process of gel electrophoresis andthe sequence is analysed.

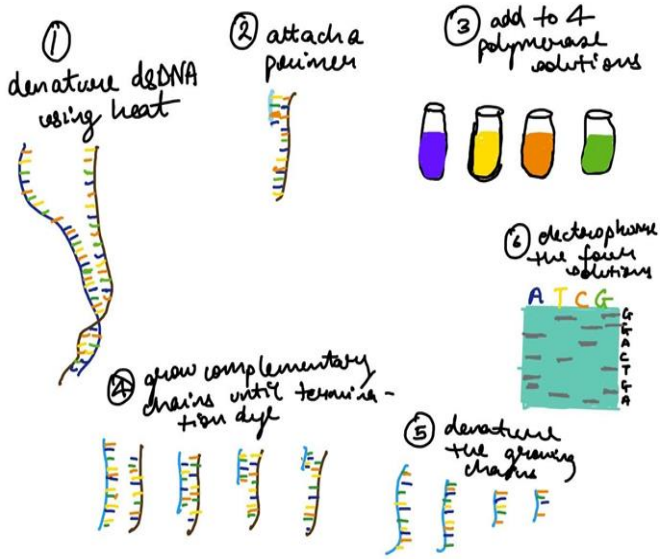

Figure-3:- SANGER SEQUENCING METHODS AND STEPS

Sanger sequencing provides high-value **sequence** for approximately long stretches of DNA (up to about 900 base pairs). It is mostly done to **sequence** separate pieces of DNA, such as bacterial plasmids or DNA transcribed in Polymerase Chain Reaction.

Capillary electrophoresis and fragment analysis:-

Capillary electrophoresis (CE) is an alternative to conventional slab gel **electrophoresis** for the removal of **DNA** portions. The bulk of **DNA** needed for separation is in the nanogram range. Single-base resolution can be obtained on portions up to several hundred base pairs.

Capillary electrophoresis is a method that removes ions based on their electrophoretic mobility with the application of an applied voltage. The electrophoretic mobility is dependent upon the charge of the molecule, the viscosity, and the atom's radius **Capillary electrophoresis** (**CE**) is the most frequently used for separating and detecting short tandem repeat (STR) alleles in forensic **DNA** laboratories spread worldwide.

ISSN No:-2456-2165

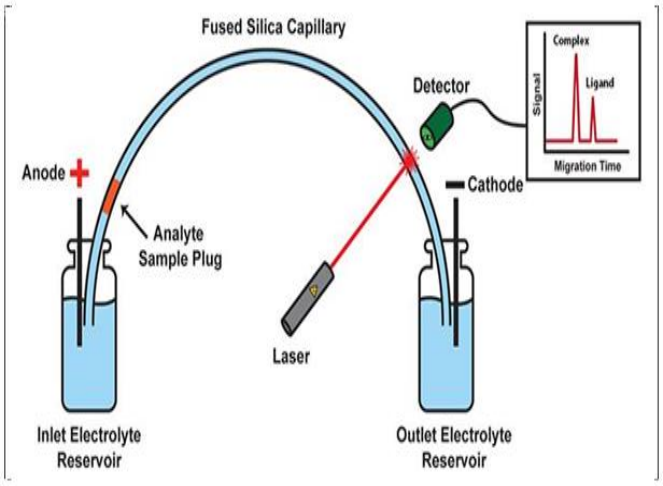

Figure-4:- CAPILLARY ELECTROPHORESIS

ii) Next-generation sequencing(NGS):-

Next generation sequencing (NGS), explains a **DNA sequencing** technology which has modernised research in genomics. The basic **next-generation sequencing** processes comprises of breaking up **DNA** or RNA in numerous portions, inclusion of connectors, and reorganising. It is mostly identical to capillary electrophoresis.

Methods of Next-Generation Sequencing:-

1. Reversible Terminator Sequencing .
2. Single-Molecule Real-Time Sequencing
3. Ion Torrent

The new methods came to be **known as next-generation sequencing** as they could formulate collateral methods to develop high quantity of sequence from numerous samples at very fast rate.

Advantages of NGS include:

Higher sensitivity to track and identify low-frequency variants. Quick replication time for high sample volumes. High genome analysis in details.

III. DNA SYNTHESIS

DNA synthesis is a biological process by which a **DNA** molecule is formed. In the cell, each of the two strands of the **DNA** molecule acts as a template for the **synthesis** of a complementary strand. **DNA** biosynthesis **happens** when a cell divides, in a process called **replication**. It comprises of separation of the **DNA** double helix and thereafter **synthesis** of complementary **DNA** strand, using the parent **DNA** chain as a template. The other strand (lagging strand) also has to be **produced** in segments (Okasaki fragments)

Oligos are **synthesized** from building blocks which replicate natural bases. The process has been automated since the late 1970s and can be **used to** form desired genetic sequences as well as for other **uses in** medicine and molecular biology.

Phosphoramidite-based **synthesis** of oligonucleotides. This **synthesis** process is the **most commonly used** for the **synthesis** of **DNA** oligonucleotides for gene **synthesis**.

The main **difference between** protein **synthesis and DNA replication** is that the protein **synthesis** is the formation of a functional protein molecule based on the information in the genes whereas **DNA replication** is the production of an exact replica of an existing **DNA** molecule.

The most important event **occurring in S phase** is the **replication** of **DNA**. The aim of this process is to produce double the amount of **DNA**, providing the basis for the chromosome sets of the daughter cells

The raw materials for DNA synthesis are the **nucleotides:-**

- deoxyadenosine triphosphate (dATP),
- deoxythymidine triphosphate (dTTP),
- deoxycytidine triphosphate (dCTP), and
- deoxyguanosine triphosphate (dGTP)—collectively referred to as deoxyribonucleoside
- triphosphates (dNTPs) or deoxyribonucleotides

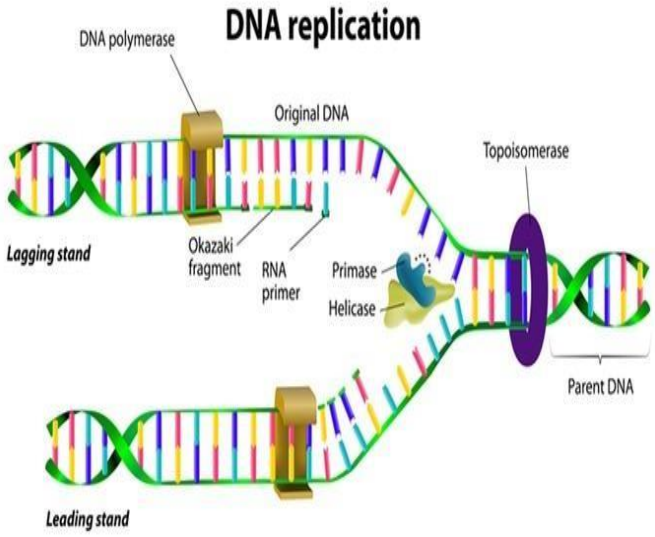

Figure-5:- DNA REPLICATION DIAGRAM

IV. DNA REPLICATION

DNA replication is the biological process of forming two similar replicas of **DNA** from one original **DNA** molecule. **DNA replication** happens in all living organisms acting as the mostnecessary part for biological legacy..

The purpose of **DNA replication** is to produce two identical copies of a **DNA** molecule. This is essentialfor cell division during growth or repair of damaged tissues. **DNA replication** ensures that each new cell receives its own copy of the **DNA**.

ISSN No:-2456-2165

DNA replication is semiconservative:-

Semiconservative replication supports copied DNA to remain loyal to naïve template. The type of RNA molecule which forms ribosomes.

Replication occurs in three major steps:

- The beginning of the double helix and removal of the DNA strands,
- The reading of the template strand, and
- The **gathering** of the new DNA segment. During **separation**, the two strands of the DNA double helix uncoil at a definite location called the origin.

Three models were proposed for DNA replication:-

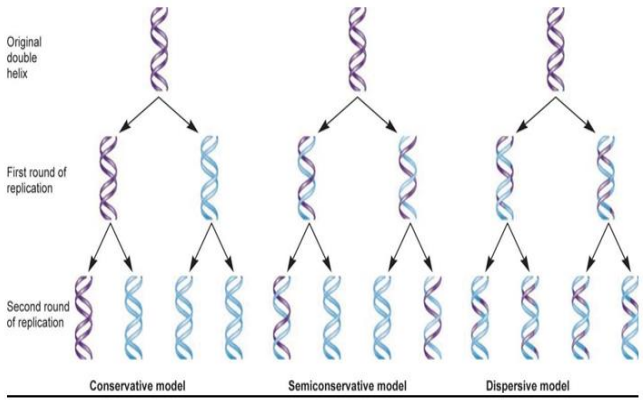

Figure-6:- VARIOUS MODELS OF DNA REPLICATION

The semi conservative model is correct

Protein	Prokaryotic/ eukaryotic	Activity/role
DNA Polymerase I	Prokaryotic	5' to 3' polymerase, 3' to 5' exonuclease, 5' to 3' exonuclease
DNA Polymerase III	Prokaryotic	5' to 3' polymerase, 3' to 5' exonuclease,
DNA Polymerase α	Eukaryotic	5' to 3' polymerase, complexes with primase then begins DNA synthesis from RNA primers, low processivity (~100 nt), no exonuclease activity
DNA Polymerase δ	Eukaryotic	5' to 3' polymerase, 3' to 5' exonuclease (proof reading), high processivity when complexed with PCNA
DNA Polymerase ε	Eukaryotic	5' to 3' polymerase, high processivity, probable regulatory role
DNA Polymerase γ	Eukaryotic	Mitochondrial DNA polymerase (5' to 3')
Primase	Both	RNA polymerase (5' to 3') makes primers
DNA helicase	Both	Untwists DNA
Single stranded Binding Protein (SSBP)	Both	Coats DNA to prevent strands re-annealing
Topoisomerases (type I and II)	Both	Relieves stress of supercoiling (type I) and introduces negative supercoiling (type II)
DNA gyrase	Prokaryotic	Type II topoisomerase
DNA ligase	Both	Seals breaks in the DNA backbone between 3'OH and 5' PO_4 requires energy source
Initiator proteins	Both	Bind at the origin of replication
Telomerase	Eukaryotic	Reverse transcriptase activity (5' to 3") using an endogenous RNA template

Figure-7:-ROLE OF THE ACTIVITY OF THE VARIOUS ENZYMES INOVLVED IN DNA

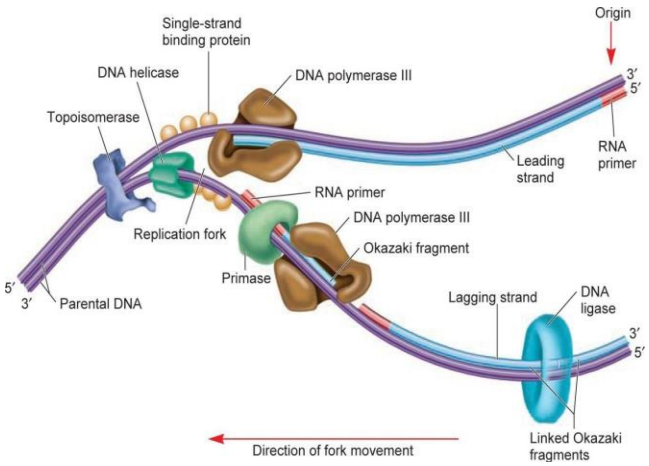

Figure-8:- DIRECTION OF MOVEMENT OF FORK

STAGES IN DNA REPLICATION:-

- Step 1: Replication Fork Formation. Before DNA can be replicated, the double stranded molecule must be ―unwinded‖ into two single strands.
- Step 2: Primer Binding. The leading strand is the simplest to replicate.
- Step 3: Elongation.
- Step 4: Termination.

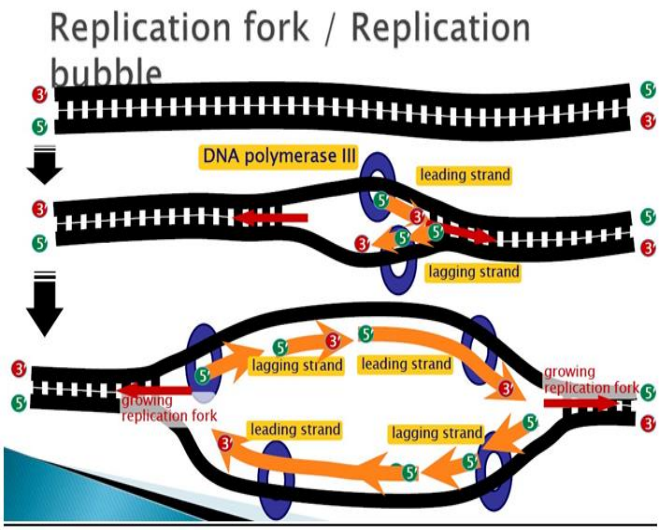

Figure-9:- REPLICATION FORK BUBBLE

Primer Binding:-

Primers are portable pieces of RNA, ribonucleic acid, about five to fifteen nucleotides long. Primase forms a short piece of RNA that is complementary to the template **DNA** strand and forms hydrogen bonds with it. This gives **DNA polymerase** the starting point it needs to initiate synthesis.

Okazaki fragments are small arrangements of DNA nucleotides formed abruptly and then joined together by the enzyme DNA ligase to form the lagging strand during DNA replication. The **Okazaki fragments** are **vital** for DNA synthesis because **there** is no 3' to 5'strand of DNA for the polymerase to use as a perpetual template.

A **primer** is essential to start **DNA** synthesis by providing a 3' end to add nucleotides to. Thisis usually a combination of Primase, a short RNA **primer**, and **DNA Polymerase** alpha, a short **DNA primer**..

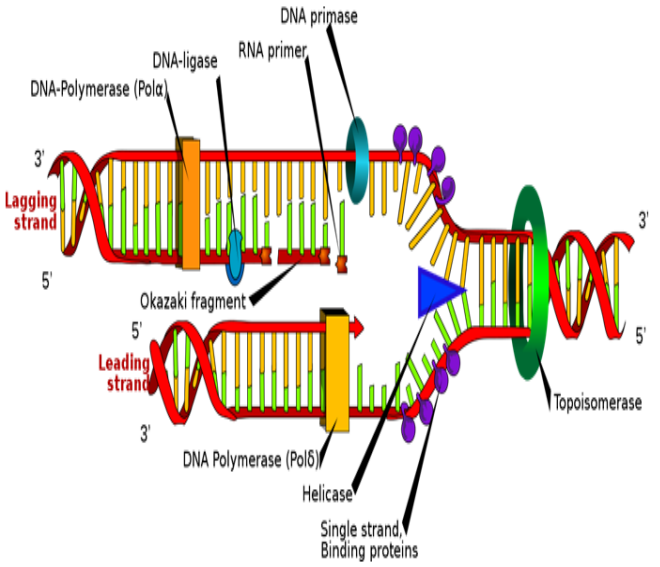

Figure-10:- PRIMER BINDING

ELONGATION:-

During elongation, RNA polymerase "walks" along one strand of DNA, known as the **template strand**, in the 3' to 5' direction. For each nucleotide in the template, RNA polymerase adds a matching (complementary) RNA nucleotide to the 3' end of the RNAstrand.

DNA is interpreted with the help of DNA polymerase only in the 3′ to 5′ direction

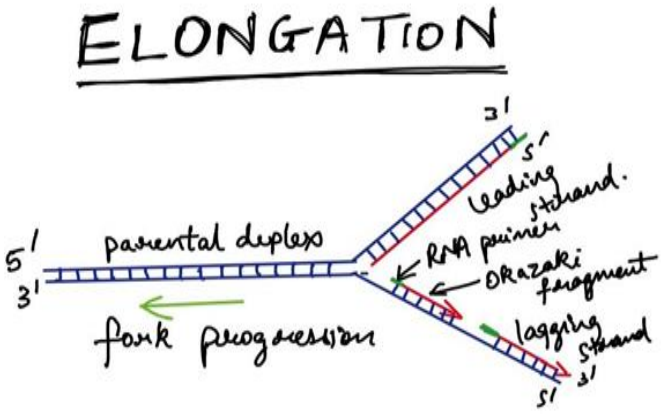

Figure-11:- DNA ELONGATION

TERMINATION:-

Termination of DNA replication initiates as soon as the two **replication** forks coincide with the stretched **DNA**, whereafter the forks intersect until all intervening **DNA** is unwound; any remaininggaps are filled and ligated

The RTP is one of only two well-described proteins known to be involved in arresting DNA replication forks, the other being a protein known as **tus** (termination utilisation substance) from E.coli.

Termination happens because the two replication forks coincide at the identical level of **DNA**, thereplicating forks broadcast until all mediating **DNA** is unwound; any left over gaps are filled and joined.

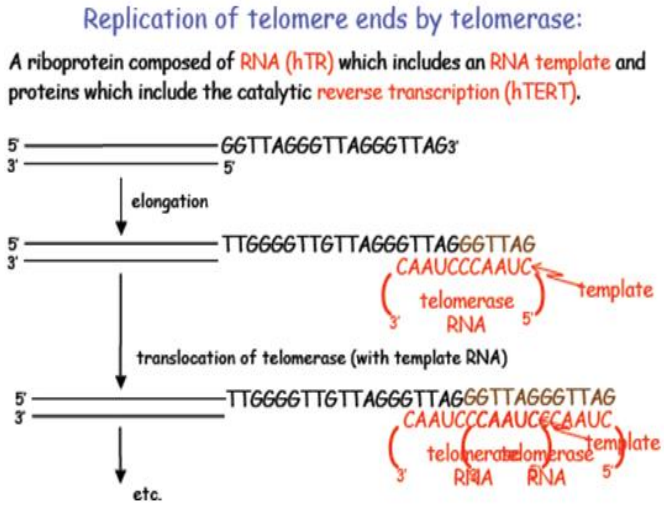

Figure-12:- REPLICATION OF TELOMERE ENDS BY TELOMERASE ENZYME

ISSN No:-2456-2165

Eukaryotic Chromosomes are rod shaped implementing they have terminal endings. These endings give rise to an issue for DNA **replication**. The DNA at the very **end** of the chromosome cannot becompletely copied in each round of **replication**, resulting in a moderate, cautious tallowing of the chromosome.

RNA polymerase continues **transcribing** unless it receives signals to terminate.

Termination occurs when the ribosome reaches a stop codon (UAA, UAG, and UGA). Since there are no tRNA molecules that can recognize these codons, the ribosome recognizes that **translation** iscomplete. The new protein is then released, and the **translation** complex comes apart.

Transcription termination occurs in a reaction integrated to RNA 3′-end processing. Most eukaryotic mRNA precursors are splitted in a site-specific manner in the 3′-UTR, followed by polyadenylation of the upstream cleavage product. Numerous proteins assist these reactions.

THE CENTRAL DOGMA:-

It is pathway of the formation DNA to RNA(in this case mRNA) to Proteins.
1: **Replication Fork Formation**.2: **Primer Binding**.
3: **Elongation**.
4: **Termination**.

Transcription takes place in three steps: **initiation**, **elongation**, and **termination**.

Transcription occurs in the nucleus. It uses DNA as a template to make an RNA molecule. RNA then leaves the nucleus and goes to a ribosome in the cytoplasm, where translation occurs. Translation analyses the genetic code in mRNA and manufactures a protein

- Transcription occurs in three stages:-
- Initiation
- Elongation
- Termination
- Above three steps involve the function of special and specific proteins interactingwith the gene. For example:

- ***A component of RNA polymerase –Sigma factor- helps binding to thepromoter sites to start RNA synthesis at a specific site***
- ***Termination factors (Rho factors in bacteria) are involved in terminationof RNA synthesis.***

Transfer RNA (tRNA) molecule transferring the amino acid methionine binds to the start codon of the mRNA sequence. It includes three steps**: initiation, elongation, and termination**.

"Polymerase chain reaction (PCR)" explains a procedure expeditiously adopted in order to produce numerous copies of a definite DNA sample, permitting researchers to magnify a portion of a DNA and analyse it minutely.

Results in PCR reaction is mostly visualized via gel electrophoresis. Gel electrophoresis process involves separating DNA portions conforming to their proportions. **Applications** includes various research laboratories, forensics, genetic testing, diagnostics, and amplification of genes.

For proper endpoint with fast and productable results, the five key steps to consider:

- DNA isolation.
- Primer designing
- Enzyme selection techniques
- Thermal cycling.
- Amplicon analysis and designing

V. CONCLUSION

DNA Synthesis and sequencing is a technique which is used to figure out the bases in a DNA molecule. It can also be used to study the genome structure. Through this paper we have tried to explain the various DNA sequencing methods and also the various applications of DNA synthesis and sequencing. We have also explained many terminologies regarding the DNA sequencing and synthesis methods. Through this review paper we have also discussed Polymerase Chain Reaction and its applications in brief. New methods in understanding DNA sequencing is the key to the future aspects and applications in the field of molecular biology.

REFERENCES

[1]. https://www.nature.com/scitable/topicpage/dna-replication-and-checkpoint-control-in-s-14202419/#:~:text=In%20the%20eukaryotic%20cell%20cycle,%22%20(the%20mitosis%20 phase)
[2]. https://www.google.com/search?sxsrf=ALeKk0046TW4REFxwRRX119_z3qmqu4iMw:1619014486234&q=How+is+DNA+replication+terminated+in+eukaryotes%3F&sa=X&ved=2ahUKEwif4tn6wo_wAhVDSX0KHfOlALgQzmd6BAgIEAk&biw=1707&bih=771&dpr=1.13
[3]. Methods in Molecular Biology_ ***John M. Walker***
[4]. Recombinant DNA Technology by Keya Chaudhury
[5]. Understanding DNA 3rd Edition by **Chris R. Calladine** Department of Engineering University of Cambridge, Cambridge, UK **Horace R. Drew** CSIRO Division of Molecular Science Sydney Laboratory, Australia **,Ben F. Luisi** Department of Biochemistry University of Cambridge, Cambridge, UK **,Andrew A. Travers** Medical Research Council Laboratory of Molecular Biology Cambridge, UK
[6]. https://www.researchgate.net/post/The-end-replication-problem
[7]. http://biology.hunter.cuny.edu/molecularbio2/Articles%20to%20Read/Lecture%206/DNA%20replication%20in%20eukaryotic%20cells.pdf
[8]. https://www.thoughtco.com/dna-replication-3981005