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1.1. Introduction

DNA Replication is the process by which DNA makes a copy of itself during cell division. DNA is made up of a double helix of two complementary strands. During replication, these strands are separated. Each strand of the original DNA molecule then serves as a template for the production of its counterpart.

What is need of replication?

DNA carries all of the genetic information for life. One enormously long DNA molecule forms haploid set of the chromosomes of an organism, 23 of them in a human. The fundamental living unit is the single cell. A cell gives rise to many more cells through serial repetitions of a process known as cell division. Before each division, new copies must be made of each of the many molecules that form the cell, including the duplication of all DNA molecules, so that it can be equally distributed between two daughter cells.

When DNA replicates during cell cycle?

In prokaryotes DNA replication occurs during C-stage of cell cycle (I→C→D Model) while in eukaryotes it occurs during S-phase of cell cycle (G_1→S→G_2→M).

What are similarities in replication of prokaryotes and eukaryotes?

Basic mechanisms of DNA replication are similar across organisms from prokaryotes to multicellular eukaryotes

- Origin of replication usually contains AT rich sequences.
- Replication is bidirectional from replication origin.
- Replication is semi-conservative process
- Replication occurs in 5'→3 direction and is template dependent process
- Replication is semi-discontinuous process.
- DNA polymerase cannot initiate replication, its needs free 3'OH group for extension of strand.

What are differences in replication of prokaryotes and eukaryotes?

- In prokaryotes DNA is always accessible to replication machinery but in eukaryotes it not directly accessible, it is bound to nucleosome.
- In prokaryotes, often there is single origin from where replication starts while eukaryotes, has multiple origin from replication can be initiated.
- Prokaryotic chromosomal DNA replication stops at one specific locus while in case of eukaryotes at stops at all those locus where two replication fork meets.
- Multiple rounds of replication are possible during prokaryotic cell division while in eukaryotes only one round of replication is possible per cell division.
- For linear chromosomes, which are most common in eukaryotes, a special problem arises during replication of chromosomal ends (telomeres).

1.2. Basic Concepts of Replication

What are possible modes of replication?

Theoretically, the template dependent DNA replication model can be explained by three modes: semi-conservative, conservative and dispersive (**Figure 1**).

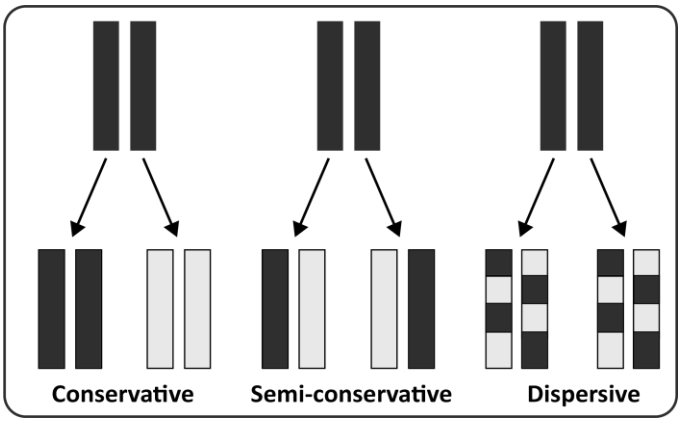

Figure 1: Possible modes of DNA replication

1. **Semi-conservative replication:** In this model, the two strands of DNA unwind from each other, and each acts as a template for synthesis of a new, complementary strand. This results in two DNA molecules with one original strand and one new strand. This is the correct mode of DNA replication in biological organism.

2. **Conservative replication:** In this model, DNA replication results in one molecule that consists of both original DNA strands (identical to the original DNA molecule) and another molecule that consists of two new strands (with exactly the same sequences as the original molecule). This mode does not exist in biological organisms.

3. **Dispersive replication:** In the dispersive model, DNA replication results in two DNA molecules that are mixtures, or "hybrids," of parental and daughter DNA. In this model, each individual strand is a patchwork of original and new DNA. This mode also does not exist in biological organisms.

How we can say that mode of DNA replication is semi-conservative?

In semi-conservative mode of DNA replication two parental strands unwind and are used for synthesis of new strands following the rule of complimentary base pairing. The semi-conservative mechanism of DNA replication can be best explained by a classic experiment conducted by **M. Meselson and W. F. Stahl,** outlined in **Figure 2**. Their experiment significantly explains differences between the semiconservative, conservative, and dispersive modes of replication.

First Meselson and Stahl, labelled DNA in a bacterium by flowing cells in medium containing either ^{14}N nitrogen or the **heavier isotope**, ^{15}N (It has an additional neutron than the naturally occurring ^{14}N but not radioactive). Furthermore, they isolated pure DNA from these organisms, and subjected it to CsCl density gradient centrifugation leading separation of light (^{14}N) and heavy (^{15}N) forms of DNA to different locations in the centrifuge tube **(Figure 2).**

To determine density of the strands a technique known as **density-gradient centrifugation** was used. Density-gradient centrifugation involves spinning a solution of **cesium chloride (CsCl)** – a heavy metal salt – and the DNA samples in an ultracentrifuge at high speed for several hours. Eventually, there occurs an equilibrium between centrifugal force and diffusion, such that there is formation of a bottom to top gradient of CsCl. DNA forms a band at a concentration of CsCl having density in the gradient equal to that of the density of DNA. The bands are detected by checking for the **absorbance** under ultraviolet light at a wavelength of **260 nm** where DNA has maximum absorption.

After growing for many generations in ^{15}N containing medium, Meselson and Stahl transferred the bacteria to an only ^{14}N contacting medium. They found that DNA replicated in the ^{14}N medium for one generation was of **intermediate density** between light (^{14}N) and heavy (^{15}N). In the next generation, only DNA of intermediate and light density was present. The results shown in **figure 2a** are consistent only with semiconservative replication.

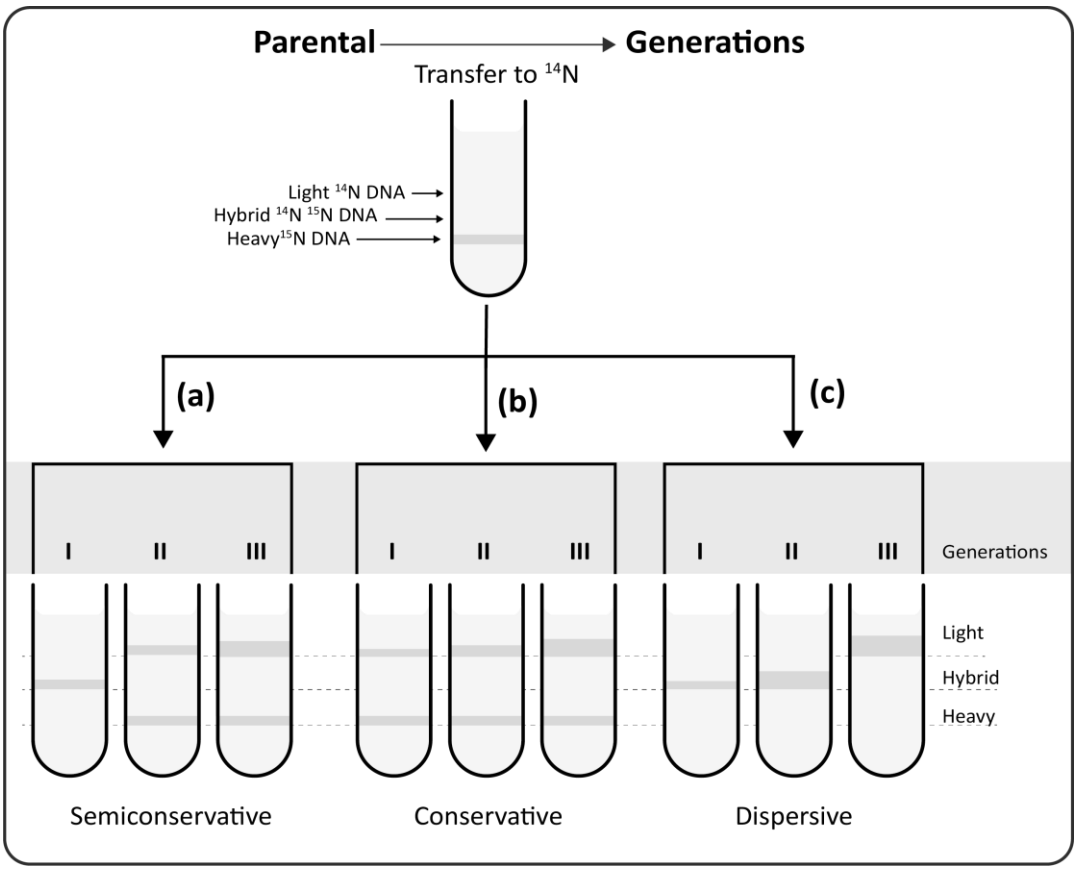

Figure 2: Experiment demonstrating that DNA replication is bacteria is (a) semiconservative. (b) and (c) Results if DNA replication has been conservative or dispersive

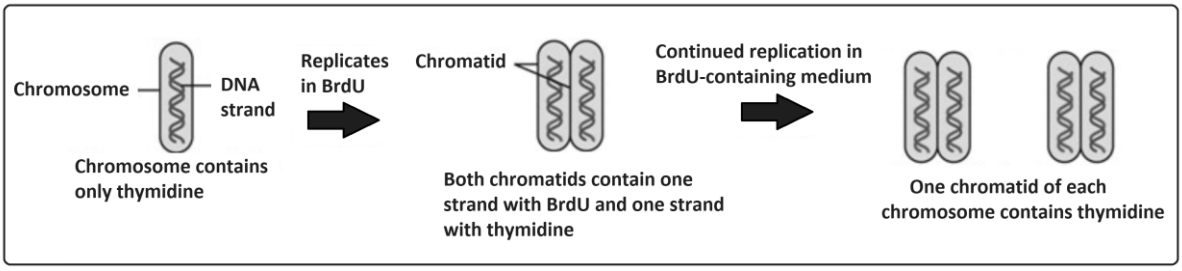

Figure 3: Experimental demonstration that DNA replication occurs semi conservatively in eukaryotes

If replication would be conservative, then there would be presence of **two bands** after first generation an original ^{15}N (heavy) double helix and a new ^{14}N (light) double helix. And also the original DNA would have continued to show up as a ^{15}N (heavy) band throughout the experiment. If the method of replication had been dispersive, there would be **multiple-banding** patterns, depending on the degree of dispersiveness.

How we can say that replication is also semi-conservative in eukaryotes?

DNA replication is semi-conservative in eukaryotes was demonstrated by **J. Herbert.** Experiments were performed to explain the mechanism as show in **figure 3**. Cultured mammalian cells were allowed to multiply in the presence of **bromodeoxyuridine** (BrdU); **BrdU** is thus gets incorporated in newly produced strands at the place of thymidine. After replication, a chromosome, which is having two chromatids are produced. After one round of replication in BrdU, both chromatids of each chromosome contained BrdU (**Figure 3**).

After two rounds of replication in BrdU, one chromatid of each chromosome was composed of two BrdU-containing strands, whereas the other chromatid was a hybrid consisting of a BrdU-containing strand and a thymidine-containing strand (**Figure 3**). The thymidine containing strand is the original parental strand which was present prior to addition of BrdU.

What is proof that DNA replication is bidirectional?

J. Cairns in 1963 verified that DNA replication is semi-conservative using the technique of **autoradiography.** In this technique there involves the exposure of radioactive emissions on a photographic film. Quantification of radioactive material can be done by counting the silver colored dots on photographic film. Cairns extracted DNA from *E.coli* culture grown on **tritium labeled thymine.**

Autoradiographs were made for the radiolabelled DNA. By analysis of DNA at different time points during replication (it takes approximately 42 minutes to replicate the entire genome), Cairns showed that replication of the circular genome was **bidirectional.** There is an origin of replication (a point where replication starts). From origin local unwinding (melting) of DNA starts. There is a formation of replication "**eye**" or "**bubble**" that contains two replication forks that proceed in opposite directions around the circular DNA. During replication, the chromosome looks like the Greek letter theta **(θ)** by electron microscopy. Replication intermediates are thus termed "**theta structures**." Cairns' findings have subsequently been verified by both autoradiographic and genetic analysis (**Figure 4**).

What is direction of DNA synthesis?

The new strand of DNA is synthesized from 5' to 3' during semiconservative replication. New nucleotides are added one at a time to the 3' hydroxyl end of the DNA chain, forming new phosphodiester bond. The 3'-5' parental strand act as template.

What is substrate for DNA synthesis?

Deoxynucleoside 5' triphosphates (dNTPs) are the building blocks of DNA from which the terminal two phosphates are lost during phosphodieseter bond formation, making the reaction essentially irreversible.

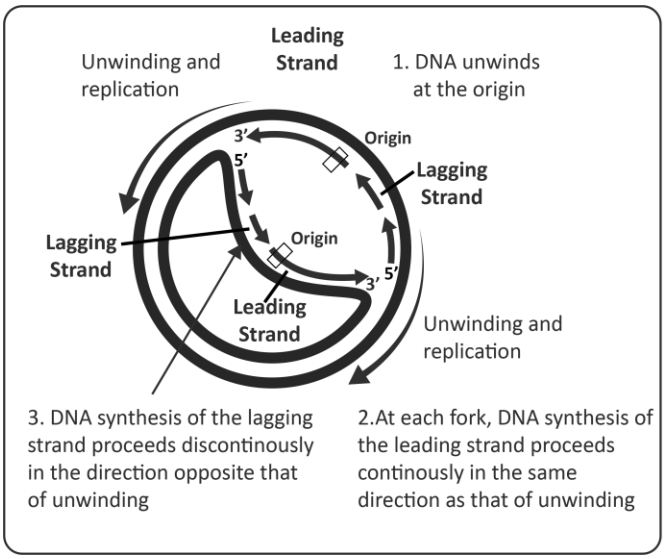

Figure 4: Theta replication in *E. coli*

Why DNA polymerase need template: primer Junction for DNA synthesis?

The process of replication is **template dependent** that is the sequential addition of nucleotides in new strand is complementary to that of the parental strand.

DNA polymerases are the key enzymes involved in DNA replication. Initially **Arthur Kornberg** and his colleagues purified an enzyme from bacterial extracts that incorporated radioactively labeled DNA precursors into an acid-insoluble polymer identified as DNA. The enzyme was later on named as DNA polymerase (and later, after the discovery of additional DNA-polymerizing enzymes, it was named **DNA polymerase I**).

DNA polymerase I requires the presence of template DNA and all four **deoxyribonucleoside triphosphates** (dTTP, dATP, dCTP, and dGTP) for the reaction to proceed. Kornberg used radiolabeled dNTPs and an unlabeled template DNA. The newly synthesized, radioactively labeled DNA had the same base composition as the original unlabeled DNA, which strongly suggested that the original DNA strands had served as templates for the polymerization reaction.

With increasing knowledge of DNA replication it became clear that DNA replication was more complex than previously thought. An intact, double-stranded DNA molecule, for example, does not stimulate incorporation. A completely single stranded, circular DNA or linear molecule must prove itself a best template since replication requires a separated single stranded DNA, but it was found that no replication was initiated from these templates. Even double stranded DNA cannot be utilized by DNA polymerase for DNA synthesis. A DNA strand must fulfill some structural requirements for being template strand.

Later on it became clear that single stranded circular DNA cannot serve as template because the DNA polymerase enzyme can only add nucleotides to a pre-existing free 3' –OH end of nucleotides hydrogen bonded to template strand. Thus, the enzyme DNA polymerase whether prokaryotic or eukaryotic; needs a **primer**. A primer is a strand of nucleotides/deoxyribonucleotides that provides a free 3' –OH end. Both the prokaryotic as well as eukaryotic DNA polymerase have two basic requirements that is a **template strand** to copy and a **primer strand with free 3'OH** group to which can be extended by adding nucleotides.

DNA molecules that do not fulfill these two basic requirements fail to promote DNA synthesis. Linear single stranded DNA has a free 3'-OH end but lacks a template while circular single strand of DNA can act as template but lacks a free 3'-OH end. Partially double stranded DNA satisfies both the requirements and thus promotes nucleotide incorporation (**Figure 5**).

DNA replication is semi-discontinuous?

Besides with being semi-conservative, DNA replication is semi-discontinuous. The major form of replication that occurs in nuclear DNA (eukaryotes), some viruses (e.g. the papovavirus SV40), and bacteria is called **semi-discontinuous** DNA replication. Term semi-discontinuous implies that on one strand DNA replication runs without any pause while on other strand synthesis takes place in the form of fragments. This is due to the fact that, DNA polymerase can only add nucleotides in the 5' →3' direction.

Synthesis of complimentary strands require that DNA synthesis proceeds in opposite direction, while the double helix is progressively unwinding and replicating in only one direction. one of the DNA strands is continuously synthesized in the same direction as the advancing replication fork and is called **leading strand** whereas the other strands is synthesized discontinuously in segments and is referred to as **lagging strands**. (**Figure 6**).

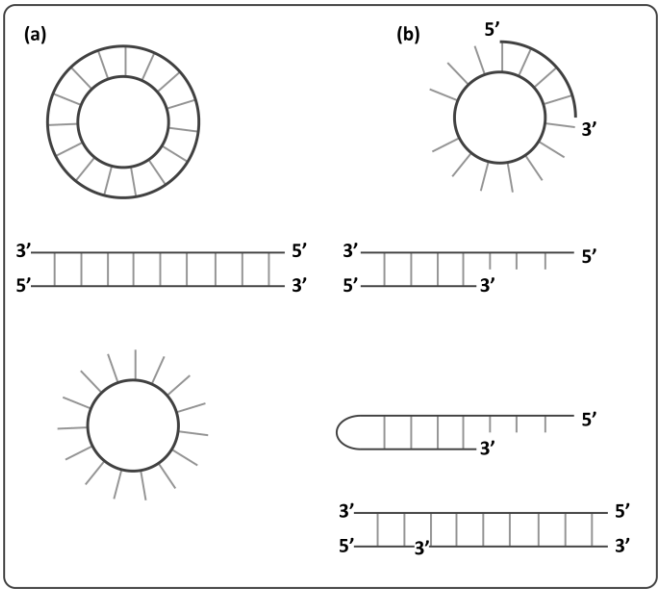

Figure 5: Templates and non-templates for DNA polymerase activity (a) Examples of DNA structures that do allow DNA synthesis (b) Examples of DNA structures that stimulates DNA synthesis. The molecules that allow DNA synthesis by DNA polymerase contain a single stranded template strand to copy and a primer strand with a 3'OH on which new nucleotides are added.

Continuous DNA Synthesis occurs on leading strand

Two strands of DNA run are **antiparallel** (i.e., running in opposite directions). Thus, problem might have been occurred since DNA polymerase can only add nucleotides in

the **5' → 3' direction**. To avoid this, two strands are replicated in 5' to 3' direction by different mechanisms. After primer binding on **3'→5' template** strand replication takes place in continuous manner without any pause.

This strand is called as "**leading strand**". Synthesis of leading strand is in the direction of proceeding replication fork. Thus, the polymerase gets easy access of single stranded template DNA. DNA polymerase on leading strand is highly processive i.e., it does not get released until it meets up a replication fork running in opposite direction or entire strand is replicated (**Figure 6**).

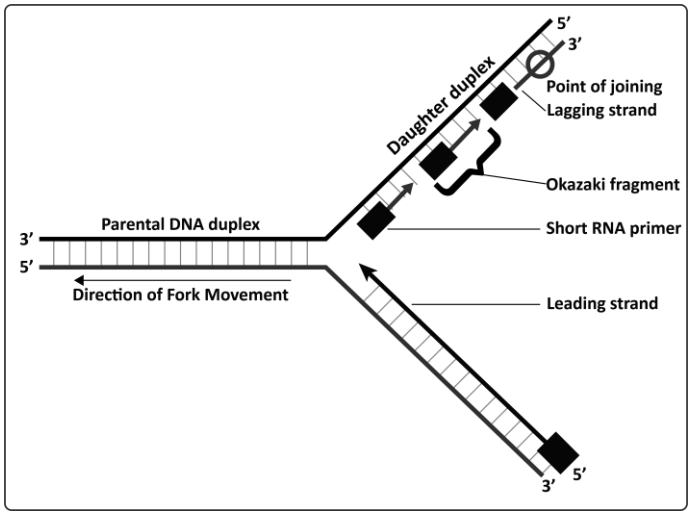

Figure 6: Semi-discontinuous DNA synthesis

Why on Lagging Strand DNA Replication Is Discontinuous?

The **lagging strand** is the strand of new DNA whose direction of synthesis is opposite to the direction of the growing replication fork (**Figure 6**). On the **"lagging strand"**, DNA replication does not occurs as a continuous stretch but it takes place in the form of short fragments.

Since the direction of polymerase (also the direction of synthesis) is opposite to that of the direction of migration of replication fork, the DNA is copied in short segments (1000–2000 nt in prokaryotes and 100–200 nt in eukaryotes). These short segments are called as "**Okazaki fragments**" as they were first described by **Reiji** and **Tuneko Okazaki** in 1969.

Replication of lagging strand in bacteria requires the repetition of four basic steps: primer synthesis by primase, elongation by DNA polymerase III, primer removal with gap filling by DNA polymerase I, and joining of the Okazaki fragments by DNA ligase. Despite these extra steps, synthesis of both new strands occurs concurrently. Nucleotides are added to the leading and lagging strands at the same time and rate, by two DNA polymerases, one for each strand.

1.3. Proteins/ Enzymes Involved In Replication:

Some fundamental properties of replication are conserved from *E. coli* to humans while specific enzymes and other proteins are the major distinguishing characters (**Table 1**).

Table 1: Important proteins involved in replication of *E.coli* and eukaryotes

***E. coli* Protein**	**Eukaryotic Protein**	**Function**
DnaA	ORC proteins	Recognization of origin of replication
Gyrase	Topoisomerase II	Relieves positive supercoil ahead of replication fork
DnaB	Mcm	DNA helicase that unwinds parental duplex
DnaC	Cdc6, Cdt1	Load DNA helicase to origin
SSB	RPA	Maintain DNA in single stranded state
γ-complex	RFC	Clamp loader, load clamps on to the DNA, Subunit of DNA polymerase
Pol III core	pol δ/ε	Major replicating enzymes, synthesize leading and lagging strand
β clamp	PCNA	Increase processivity of DNA polymerase; works with pol III or pol δ/ε
Primase	Primase	Synthesize RNA primer
-	pol α	Extend RNA primer with short DNA oligonucleotides
DNA ligase	DNA ligase	Seals Okazaki fragments into continuous strand
pol I	FEN-1	Removes RNA primers; pol I of *E.coli* also fills gap with DNA

What are initiator proteins?

The proteins which recognize replication origin and assist in loading of helicase to origin are referred as initiator proteins. The *E. coli* replication origin (***oriC***) is recognized by ***DnaA*** protein while yeast replication origins (**ARS**) is recognized by origin recognition complex (**ORC 1-6**).

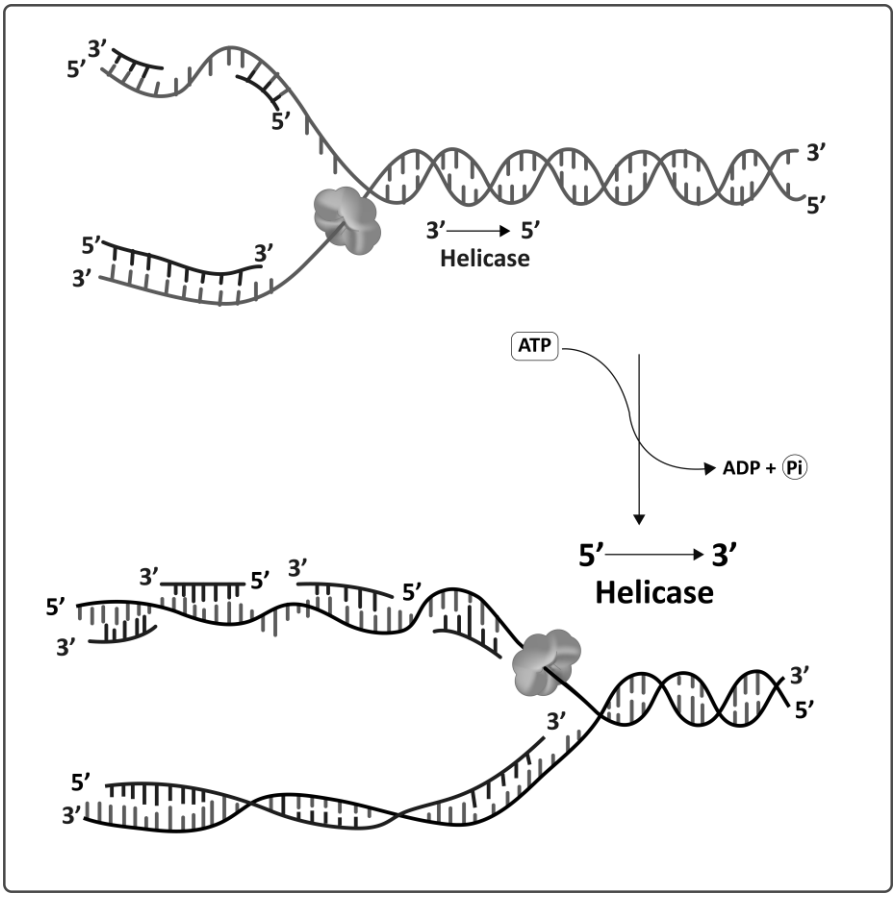

Figure 7: Polarity of helicase 3′→5′ template for leading strand, 5′→3′ template for lagging strand

What are functions of helicase loader?

Helicase loaders recognize proteins bound at replication origin (*DnaA/ORC*) and recruits helicase (**DnaB/MCM**) to origin. The role of helicase loader is prokaryotes is performed by ***DnaC*** protein while in eukaryotes same function is done by ***cdt1*** and ***cdc6*** proteins.

How DNA duplexes unwind at replication forks?

Unwinding or separation is done by specialized enzymes called as **helicases** in prokaryotes as well as in eukaryotes. DNA helicases are the enzymes that use **ATP** as energy to separate (melt) the DNA duplex in the direction of replication fork. They progressively catalyze the transition from double stranded DNA to single stranded DNA. The role of helicase in *E. coli* is done by ***DnaB*** while in eukaryotes it is performed by ***MCM 2-7*** proteins.

What is polarity of helicases?

The *E.coli* helicases **(*DnaB*) migrates in 5′→3′ direction** bound to the lagging strand and unwinds DNA. Whereas helicases that are functional in eukaryotic system like **SV40 T antigen** or the **MCM2-7** move in **3′→5′ direction**, thus are bound on leading strand template during DNA replication (**Figure 7**).

How Single-Stranded DNA-Binding Proteins (SSB/RP-A) stabilize ssDNA before Replication?

Once the helicase has passed replication origin, the two separated strands may reanneal together as there is high degree of complementarity. Thus, to avoid pairing amongst themselves, these single strands must be kept occupied by some other protein until the DNA polymerase adds complementary sequences.

To stabilize the separated strands, ssDNA-binding proteins **(SSBs in bacteria** and **RPA in yeast)** rapidly bind to the separated strands. Binding of one SSB promotes the binding of another SSB to the immediately adjacent ssDNA. This is called '**cooperative binding'** and occurs because SSB molecules bound to immediately adjacent regions of ssDNA also bind to each other. Cooperative binding ensures that ssDNA is rapidly coated by SSB as it emerges from the DNA helicase. Once coated with SSBs, ssDNA is held in an elongated state that facilitates its use as a template for DNA or RNA primer synthesis. SSBs interact with ssDNA in a **sequence-independent manner**. SSBs primarily contact ssDNA through **electrostatic interactions** with the phosphate backbone and **stacking interactions** with the DNA bases.

What are total number of DNA polymerases?

All the known DNA polymerases can add nucleotides in 5'→3' direction. They add nucleotides at 3'OH of a growing strand of DNA. There are **five** DNA polymerases in bacteria as compared to the **fourteen** DNA polymerases in eukaryotes and perform the similar overall function as the eukaryotic DNA polymerases. (**Table 2**).

Bacterial DNA dependent DNA polymerases

DNA polymerase I: The very first polymerase purified and characterized from any organism was DNA polymerase I. Although it is not the major DNA polymerase involved in prokaryotic DNA replication; it is the most abundant polymerase in *E.coli.* It is involved in **nucleotide excision repair pathway** and **primer removal** followed by **gap filling** between two Okazaki fragments.

On enzymatically cleaved by the protease **subtilisin**, DNA polymerase I give two fragments.

- The larger fragment called as the **Klenow fragment** that has **5' →3' polymerase** activity **and 3'→5' exonuclease activity (proof reading).**
- The other smaller fragment has **5'→3' exonuclease** activity which is used for the **primer removal**.

The Klenow fragment is also widely used for labeling DNA by a technique called "**random priming**". Klenow fragment has unique ability to start replication at a nick (broken phosphodiester bond) in the DNA sugar–phosphate backbone. This property has been used in the laboratory to make radio-labeled DNA by a technique called "**nick translation**".

DNA polymerase III: It is very surprising that though being the most abundant polymerase in *E.coli,* DNA polymerase I is not the major replicative polymerase. In fact, a much less abundant enzyme, DNA polymerase III, catalyzes genome replication. DNA polymerase III is a holoenzyme complex made up of **10** different polypeptide subunits. **The *α*-subunit** has the **replicase** (5' →3' polymerase) activity and the ***ε*-subunit** has the **proofreading activity** (3'→5' exonuclease). DNA polymerase III also plays a role in **nucleotide excision repair pathways**.

DNA polymerases II: DNA Pol II is single subunit protein encoded by the *polB* (*dinA*) gene. The enzyme has 5'→3' DNA synthesis capability as well as 3'→5' exonuclease proofreading activity. It is involved in replication on lagging strand and **base excision repair mechanisms**.

DNA polymerases IV and V: DNA polymerases IV and V are members of **Y family DNA polymerases** that mediate **translesion DNA synthesis**. These polymerases are known for their ability to bypass the DNA damage that has blocked the DNA replication by DNA polymerase III. These polymerases are **error prone** enzymes and thus may play a role in **adaptive mutagenesis**.

Table 2: List of DNA polymerase in prokaryotes and eukaryotes

Prokaryotic (*E. coli*) DNA polymerases			**Eukaryotic DNA polymerases**		
	Sub Units	**Function**		**Sub Units**	**Function**
Pol I	1	RNA primer removal, DNA repair	Pol α	4	Primer synthesis during DNA replication
Pol II (Din A)	1	DNA repair	Pol β	1	Base excision repair
Pol III core	3	Chromosome replication	Pol γ	3	Mitochondrial DNA replication and repair
Pol III holoenzyme	9	Chromosome replication	Pol δ	2-3	Lagging strand synthesis; nucleotide and base excision repair
Pol IV (DinB)	1	DNA repair, translesion synthesis	Pol ε	4	Leading strand synthesis; nucleotide and base excision repair
Pol V (UmuC, $UmuD_2$'C)	3	Translesion synthesis	Pol Θ	1	DNA repair of cross links
			Pol σ	1	TLS
			Pol λ	1	Meiosis associated DNA repair
			Pol μ	1	Somatic hypermutation
			Pol κ	1	TLS
			Pol η	1	Relatively accurate TLS past cis-syn cyclobutane dimers
			Pol ι	1	TLS, somatic hypermutaion
			Rev l	1	TLS

DNA polymerase IV, also called **DinB**, is encoded by the ***dinB*** gene. DNA polymerase V, also known as the **$UmuD_2'C$** complex, is encoded by the ***umuDC*** operon.

Eukaryotic DNA dependent DNA polymerase:
In eukaryotes there are four replicative DNA polymerases that are involved in DNA replication; out of the hour, three are DNA polymerase α, δ and DNA polymerase ε involved in nuclear DNA replication. DNA polymerase α initiates DNA replication, **DNA polymerase δ** works on **lagging strand** and **DNA polymerase ε** works on **leading strand.**

DNA polymerase γ is involved in **mitochondrial and chloroplast DNA replication**. Rest of the DNA polymerases are involved in repair processes as shown in table 2. All eukaryotic DNA polymerase has 5'→3' polymerase activity but 3'→5' proof reading (exonuclease) activity is present only in DNA polymerase α, γ, δ and ε.

What is rate of DNA replication?
The rate of bacterial DNA replication is around **500-1000 nt/second** and mammalian DNA polymerases can add around **50-100 nt/second.** The reason for slow rate of polymerization in eukaryotes is that DNA is not readily accessible for DNA replication (It is bound with histone proteins).

How DNA polymerase distinguish ribonucleotides from dexoyribonucleotides?
Although ribonucleoside triphosphates (rNTPs) are present at approximately 10-fold higher concentration than deoxyribo-nucleoside triphosphates (dNTPs) in the cell but they are incorporated into DNA at a rate that is more than 1000-fold lower than dNTPs. This is because DNA polymerase efficiently discriminates between rNTPs and dNTPs, because its nucleotide binding pocket cannot accommodate a 2'-OH on the incoming nucleotide.

Why no DNA polymerase has evolved which can initiate the replication?
DNA polymerases cannot initiate DNA synthesis is on their own. DNA polymerases can extend the pre-existing strand but they cannot initiate synthesis. An exception to this is **DNA polymerase α,** the eukaryotic polymerase which has associated sub-unit which acts as primase involved in primer synthesis. During primase activity proof reading cannot be performed by DNA polymerase α.

If DNA polymerase would have started replication process, then during addition of first two deoxyribonucleotides, proof reading cannot be performed as there is no DNA to back track. This lack of proofreading would have decreased fidelity of DNA replication process. So, it better evolutionary decision that DNA replication is initiated by **Primase** which add RNA primer without proof reading. A primer is usually a short RNA chain which must be synthesized on the DNA template before DNA polymerase can start elongation of a new DNA chain. DNA polymerases recognize and add dNTPs to the free 3'-hydroxyl group at the end of the primer. Later on same RNA primer is removed and replaced by DNA with efficient proof reading of DNA polymerase I.

How Primase initiates DNA synthesis?
As soon as DNA at origin of replication is opened, the enzymes helicase and primase are recruited for replication initiation. For addition of nucleotide by DNA polymerase a free 3'OH end is needed. This free 3'-OH end is provided by a short sequence of nucleotides which is referred as primer. The first step in DNA replication is thus synthesis of primers. **Primase** is a specialized **RNA polymerase** dedicated to making short RNA primers (5–10 nucleotides long) on an ssDNA template. Primase binds to single-stranded DNA that is coated with single-stranded binding proteins. Thus, **a DNA dependent RNA polymerase** can act as primase. On leading strand, primer is added only once. While Priming occurs more frequently on lagging strand since DNA replication is semi-discontinuous.The bacterial primers are short RNA stretches of around 5–10 nucleotides that are synthesized by a primase that then hands off replication directly to DNA polymerase III. However in eukaryotes, the primase complex contains four subunits: two subunits that function as a primase, bound in a complex with the DNA polymerase α catalytic subunit and an accessory subunit. **(Figure 8)**

The polymerase α–primase complex synthesizes a stretch of 10–30 nucleotides of RNA, which the polymerase α subunit of the complex elongates with a short stretch of DNA. After this primer synthesis, the replicative polymerases δ or ε take over and carry out the rest of the elongation phase of DNA replication. This handover from polymerase α–primase to polymerase δ on lagging strand or polymerase ε on leading strand is known as '**polymerase switching'**. Polymerase switch occurs each time a new DNA strand is started on both the leading and the lagging strands (**Figure 9**).

What is clamp loader?
Clamp loaders are the special class of protein complexes that open up the clamp and also place it on the DNA. The **γ, δ, and δ' subunit of DNA polymerase constitute** the clamp loader in prokaryotes while in eukaryotes the protein **Replication Factor C (RFC)** is clamp loader. The RFC consists of five different protein subunits, called **Rfc1–5**. Clamp loader recruits sliding clamp (β-clamp/PCNA) at the primer DNA junction at the double stranded stretch near the free 3' end which is going to be elongated. The sliding clamp in turn recruits the replicative polymerase at the time of