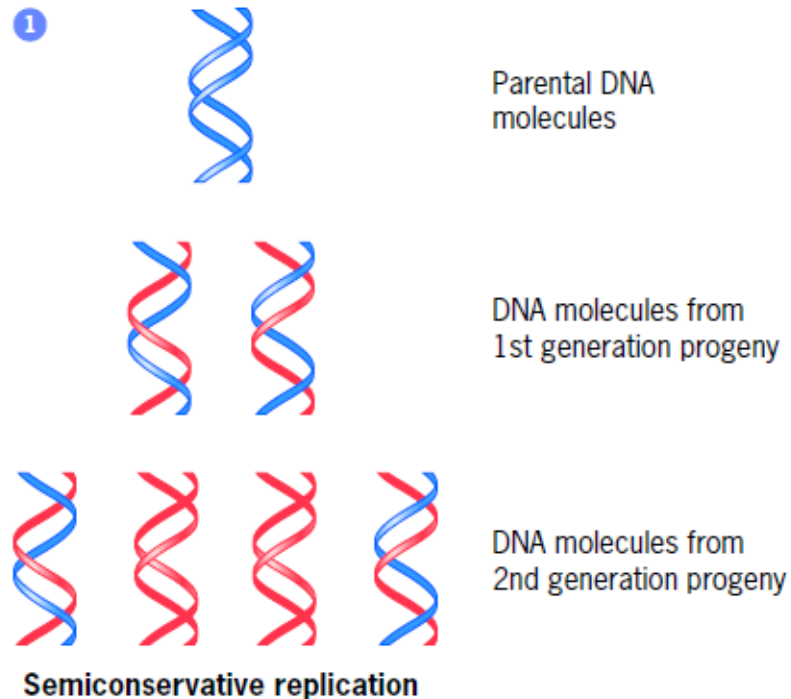


DNA REPLICATION

- DNA replication is the process by which DNA makes a copy of itself during cell division.
- It is semi-conservative, bidirectional and discontinuous (Semi-conservative mechanism was demonstrated by Meselson and Stahl in 1958)



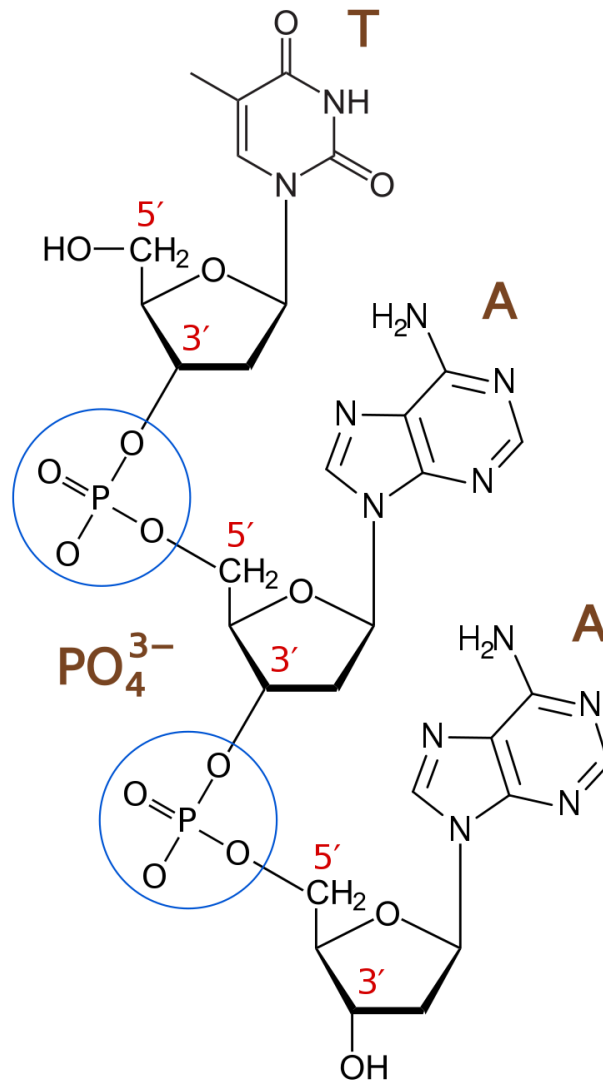
(Source: Cell and Molecular Biology- Concepts and Experiments- Gerald Karp. 7th Edition Wiley)

- DNA replication is an essential mechanism in enhancing cell growth, repair, and reproduction of an organism.

OVERVIEW OF STRUCTURE OF DNA

- The structural model of DNA was initially proposed by James Watson and Francis Crick.
- They found that DNA is a double-helical structure with two paired DNA strands with complementary nucleotide sequences.
- The double-stranded DNA molecule has two parallel spiral nucleic acid chains that are twisted into a double helix shape. The twisting gives the DNA its compactness.
- DNA is made up of millions of nucleotides that are molecules composed of deoxyribose sugar with a phosphate group and a nucleobase that is attached to it.
- Each nucleotide is tightly base paired with a complementary nucleotide on the opposite strand, i.e. with nucleobases Adenine (A) paired with Thymine (T) or Guanine (G) paired with cytosine (C) – by Hydrogen Bonds (A=T and G=C)

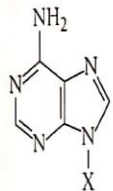
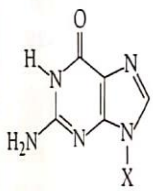
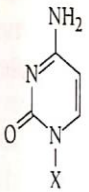
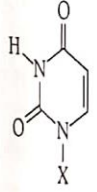
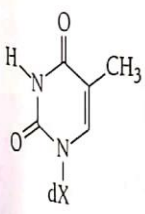
- Nucleotides are bound to each other in strands by phosphodiester bonds forming a sugar-phosphate backbone. The bond is a linkage between the third carbon atom on the deoxyribose sugar designated as the 3' (three prime) and the fifth carbon atom of another sugar on the next nucleotide designated as the 5' (five prime), i.e. between phosphate and two hydroxyl groups of adjacent nucleotides.



Source: https://en.wikipedia.org/wiki/File:Phosphodiester_Bond_Diagram.svg

Phosphodiester bond formation between nucleotides (5' position and 3' position)

Table 3-1. Names and Abbreviations of Nucleic Acid Bases, Nucleosides, and Nucleotides

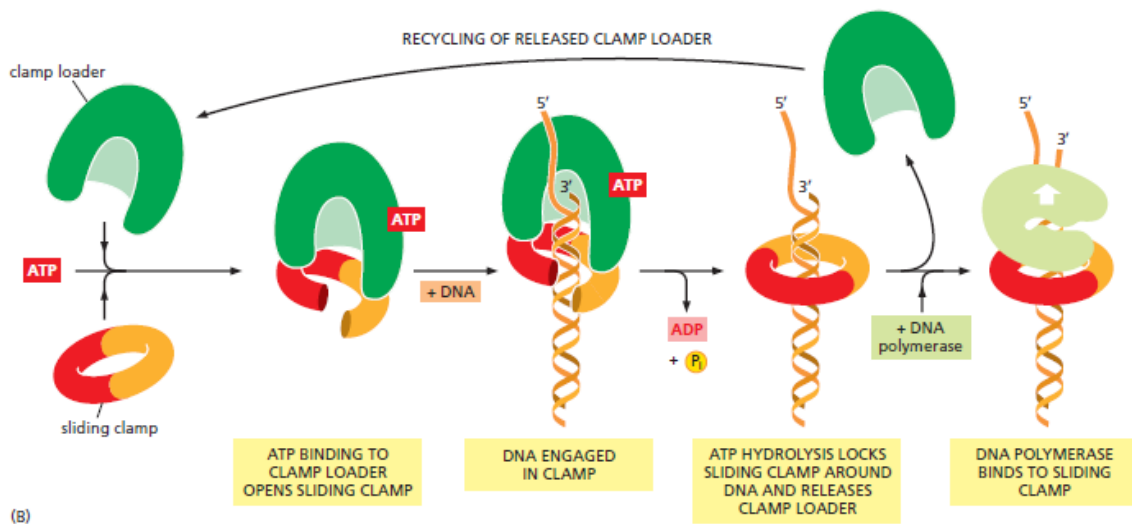
Base Formula	Base (X = H)	Nucleoside (X = ribose ^a)	Nucleotide ^b (X = ribose phosphate ^a)
	Adenine Ade A	Adenosine Ado A	Adenylic acid Adenosine monophosphate AMP
	Guanine Gua G	Guanosine Guo G	Guanlylic acid Guanosine monophosphate GMP
	Cytosine Cyt C	Cytidine Cyd C	Cytidylic acid Cytidine monophosphate CMP
	Uracil Ura U	Uridine Urd U	Uridylic acid Uridine monophosphate UMP
	Thymine Thy T	Deoxythymidine dThd dT	Deoxythymidylic acid Deoxythymidine monophosphate dTMP

- DNA fits within the nucleus by being closely packed into tight coils known as chromatins.
- The process takes place during cell division, (Interphase - S phase).
- The chromatins condense to form the chromosomes during cell division.

Before DNA replication, the chromatins loosen up giving the replication machinery access to the DNA strands.

DNA replication requires the cooperation of many proteins /enzymes

- **DNA polymerase** and **DNA primase** to catalyse nucleotide triphosphate polymerization, i.e. add nucleotides to 3'end (5' to 3'). Primase adds primers.
- **DNA helicases** and **single-strand DNA-binding (SSB) proteins** respectively to help in opening up the DNA helix and prevent from breakage and renaturing
- **DNA ligase** and an enzyme that degrades RNA primers to seal together the discontinuously synthesized lagging strand DNA fragments;
- **DNA topoisomerases/gyrase** to help to relieve helical winding and DNA tension problems.
- **DNA clamp** is a protein which prevents elongating DNA polymerases from dissociating from the DNA parent strand (beta subunits of the holoenzyme - DNA polymerase).



Source: Molecular Biology of The Cell. Bruce Alberts et al. 6th Edition. Garland Science

- **DNA-dependent DNA polymerase:** It helps in the polymerization and catalyzes the whole process of DNA replication with the support of other enzymes. DNA polymerase is of three types:
 - **DNA Polymerase I:** It is a DNA repair enzyme. It is involved in three activities:
 - 5'-3' polymerase activity
 - 5'-3' exonuclease activity
 - 3'-5' proof reading exonuclease activity
 - It fills the gap between the lagging strand fragments
 - **DNA Polymerase II:** It is responsible for primer extension and proofreading.

- DNA Polymerase III : It is responsible for in vivo DNA replication. It has two subunits, one each for a strand. The alpha subunit is the actual polymerase.
- Helicase: Helicase is the enzyme which unzips the DNA strands by breaking the hydrogen bonds between them. Thus, it helps in the formation of the replication fork.
- Ligase: It is the enzyme which joins the discontinuous DNA strands by forming phosphodiester bonds between 3'OH and 5' PO₄
- Primase: This enzyme helps in the synthesis of short RNA primers complementary to the DNA template strand.
- Single-stranded Binding Proteins: It binds to single-stranded DNA and protects it from forming secondary structures and thus keeping the strand unwound.

- *In vivo*, the DNA polymerase III holoenzyme dimer, the primosome and the DNA helicases are believed to be physically associated in a large complex called a **replisome**.
- Each strand in a parental duplex DNA acts as a template for synthesis of a daughter strand and remains base paired to the new strand, forming a daughter duplex.
- New strands are formed in the 5' to 3' direction.
- Replication begins at a sequence called an origin of replication. Each eukaryotic chromosomal DNA molecule contains multiple replication origins.
- DNA replication generally occurs by a bidirectional mechanism in which a replication fork forms at an origin and synthesis of each strand moves in opposite directions, with both template strands being copied at the replication fork.
- At a replication fork, one daughter strand (the leading strand) is elongated continuously.
- The other daughter strand (the lagging strand) is formed as a series of discontinuous Okazaki fragments from primers synthesized every few hundred nucleotides.
- The energy for polymerization comes from the hydrolysis of the dNTPs and the resulting pyrophosphate.

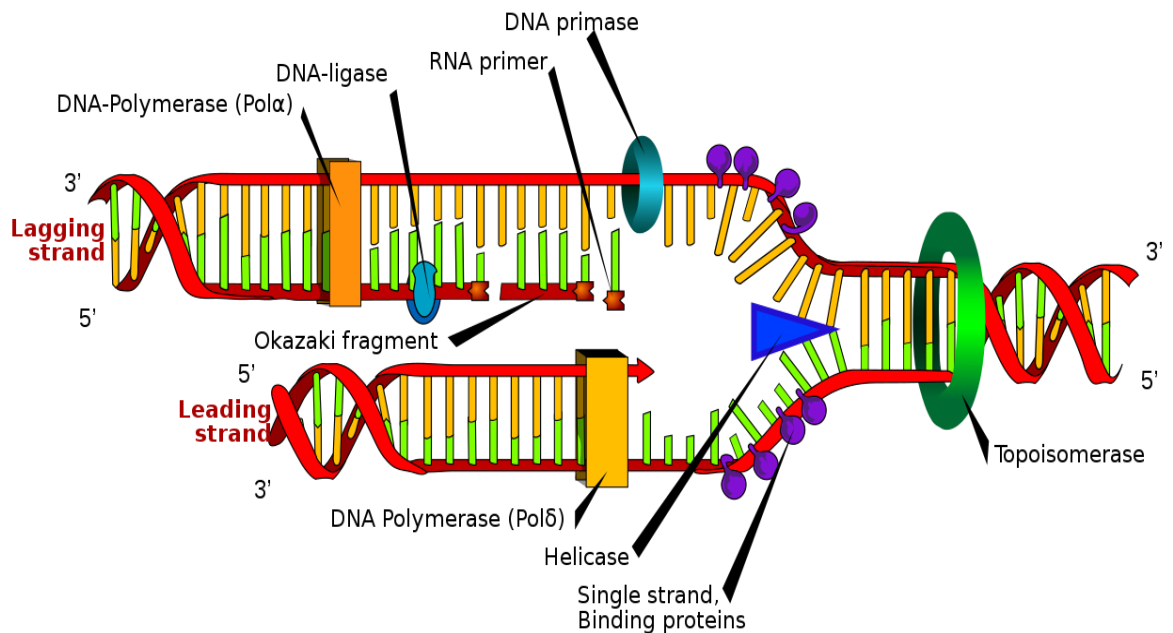
Leading strand

- The leading strand is the strand of nascent DNA which is synthesized in the same direction as the growing replication fork. This sort of DNA replication is **continuous**.

Lagging strand

- The lagging strand is the strand of nascent DNA whose direction of synthesis is opposite to the direction of the growing replication fork. Because of its orientation, replication of the lagging strand is more complicated as compared to that of the leading strand. As a consequence, the DNA polymerase on this strand is seen to "lag behind" the other strand.
- The lagging strand is synthesized in short, separated segments. On the lagging strand *template*, a primase "reads" the template DNA and initiates synthesis of a short complementary RNA primer.

- A DNA polymerase extends the primed segments, forming **Okazaki Fragments**. This sort of DNA replication is **discontinuous**.
- The RNA primers are then removed and replaced with DNA, and the fragments of DNA are joined together by DNA ligase.
- All origins contain AT-rich sequences where the strands initially separate. AT-rich regions are more easily opened than GC-rich.



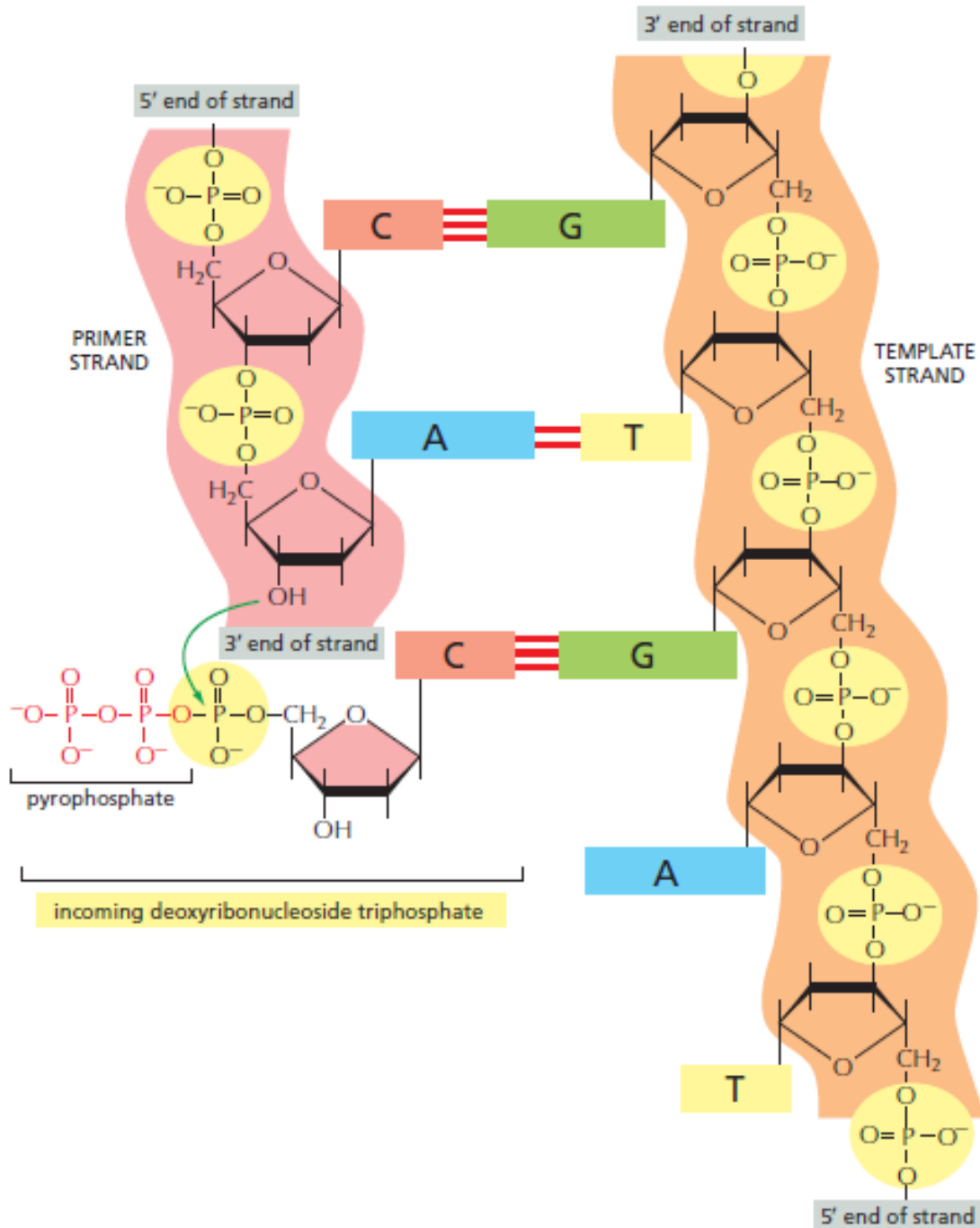
Source: https://en.wikipedia.org/wiki/File:DNA_replication_en.svg

Steps involved in DNA Replication

INITIATION

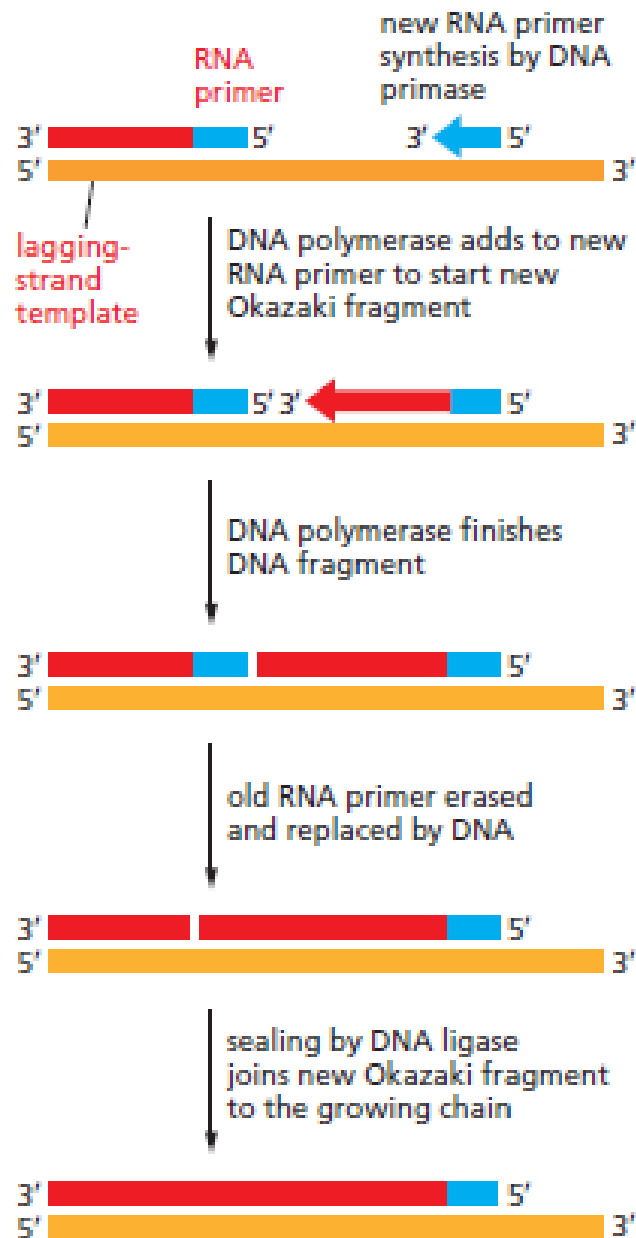
- The two strands of DNA unwind at the origin of replication.
- Helicase opens the DNA and replication fork is formed at the point of separation of strands.
- The DNA is coated by the single-strand binding proteins around the replication fork to prevent rewinding of DNA.
- Topoisomerase prevents the supercoiling of DNA and release the tension.
- RNA primers are synthesised by primase complementary to the DNA strand.
- Initiation in prokaryotes is at a single site OriC.
- There are multiple origin sites in eukaryotic DNA
- In *E.coli*. the primary initiator protein is DnaA
- In Yeast, this is the Origin Recognition Complex (ORC)

- ❑ Sequences used by initiator proteins tend to be "AT-rich" (rich in adenine and thymine bases), because A-T base pairs have two hydrogen bonds (rather than the three formed in a C-G pair) and thus are easier to strand-separate.
- ❑ In eukaryotes, the origin recognition complex that is formed, catalyses the assembly of initiator proteins into the pre-replication complex.



ELONGATION

- ❑ DNA polymerase III starts adding nucleotides at the end of the primers.
- ❑ The leading and lagging strands continue to elongate with the formation of Okazaki fragments in the lagging strand.

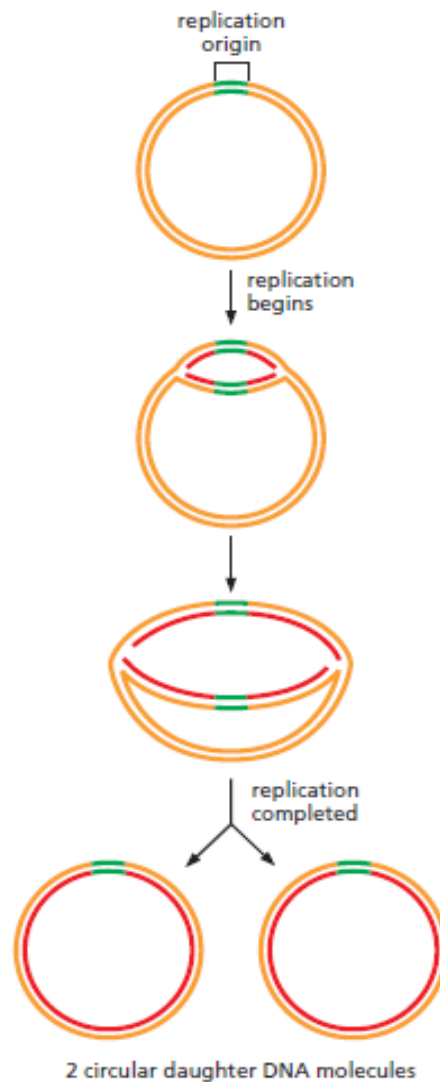


TERMINATION

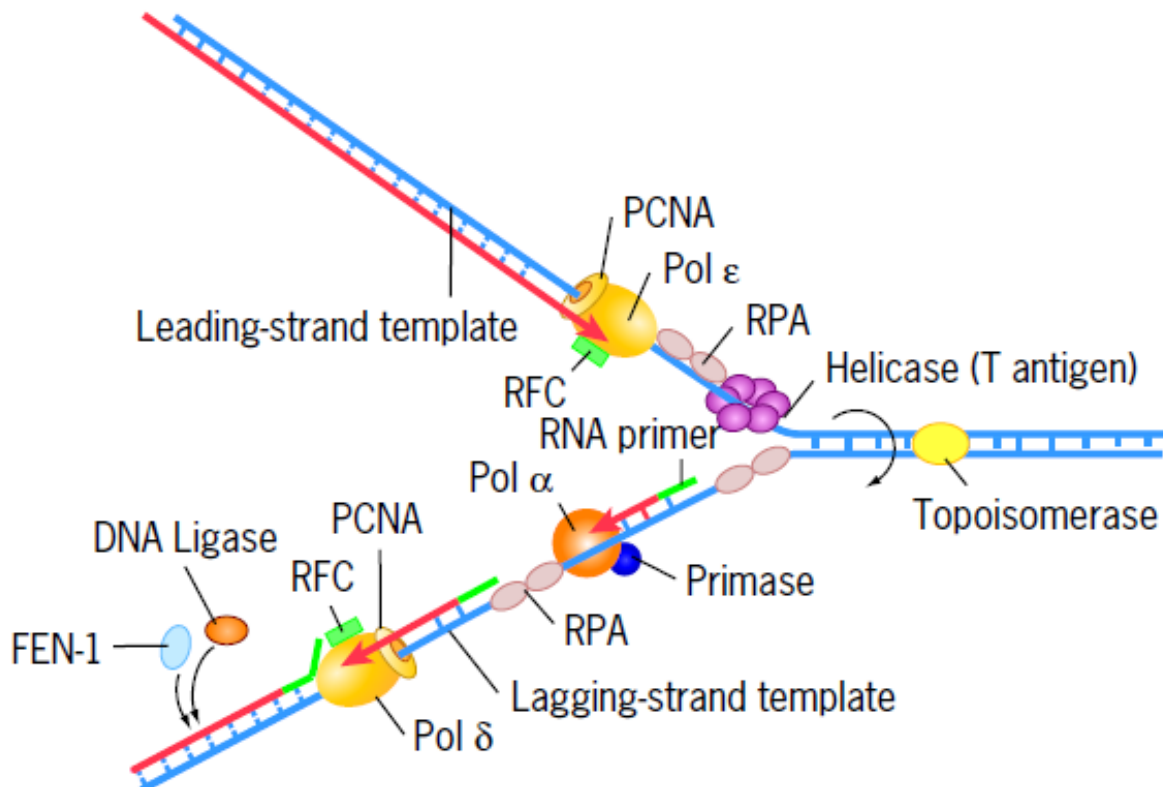
- ❑ The primers are removed and the gaps are filled with DNA Polymerase I and sealed by ligase.
- ❑ Termination occurs when a termination site sequence in the DNA is reached, and a protein binds to this sequence to physically stop DNA replication, the DNA replication terminus site-binding protein, Ter protein, an inhibitor of DnaB helicase which works along with a Tus factor (forming a complex).

Prokaryotic and Eukaryotic DNA Replication (differences)

- The DNA replication in eukaryotes is similar to the DNA replication in prokaryotes with a little difference.
- All prokaryotic chromosomes and many bacteriophage and viral DNA molecules are circular.



- Cells of higher organisms may have a thousand times as much DNA as this bacterium, yet their polymerases incorporate nucleotides into DNA at much slower rates. To accommodate these differences, eukaryotic cells replicate their genome in small portions, termed *replicons*. Each replicon has its own origin from which replication forks proceed outward in both directions
- The initiation process is more complex in eukaryotes than prokaryotes. The initiator protein complex in bacteria is DnaA-ATP, Helicase is DnaB, Topoisomerase is DNA gyrase.
- Prokaryotic chromosomes replicate as single units called replicons while in eukaryotes, there are multiple origin of replication present.
- In contrast to prokaryotes, eukaryotic replicons can only initiate once per cell cycle.
- A pre-replication complex is made with other initiator proteins.
- Origin of replication is OriC in prokaryotes
- The process is entirely the same but the enzymes used are different, e.g. in eukaryotes, the polymerization process is carried out by the enzyme Pol δ (delta Polymerase helped by Polymerase alpha and epsilon), whereas in prokaryotes it is done by DNA Pol III having two subunits (helped by Polymerase I and II)



(Source: Cell and Molecular Biology- Concepts and Experiments- Gerald Karp. 7th Edition Wiley)

Replication fork in eukaryotes

Comparison of Prokaryotic and Eukaryotic Proteins in Replication

(Source: Cell and Molecular Biology- Concepts and Experiments- Gerald Karp. 7th Edition Wiley)

Table 13.1 Some of the Proteins Required for Replication

<i>E. coli</i> protein	Eukaryotic protein	Function
DnaA	ORC proteins	Recognition of origin of replication
Gyrase	Topoisomerase I/II	Relieves positive supercoils ahead of replication fork
DnaB	Mcm	DNA helicase that unwinds parental duplex
DnaC	Cdc6, Cdt1	Loads helicase onto DNA
SSB	RPA	Maintains DNA in single-stranded state
γ -complex	RFC	Subunits of the DNA polymerase holoenzyme that load the clamp onto the DNA
pol III core	pol δ/ϵ	Primary replicating enzymes; synthesize entire leading strand and Okazaki fragments; have proofreading capability
β clamp	PCNA	Ring-shaped subunit of DNA polymerase holoenzyme that clamps replicating polymerase to DNA; works with pol III in <i>E. coli</i> and pol δ or ϵ in eukaryotes
Primase	Primase	Synthesizes RNA primers
DNA ligase	pol α	Synthesizes short DNA oligonucleotides as part of RNA-DNA primer
pol I	DNA ligase	Seals Okazaki fragments into continuous strand
	FEN-1	Removes RNA primers; pol I of <i>E. coli</i> also fills gap with DNA

Regulation of DNA Replication

- Regulatory mechanisms for DNA replication are central to the control of the cell-cycle in **Eukaryotic** cells.
- Replication of the eukaryotic genome occurs only once during the S-phase of each cell cycle.
- The signal transduction pathway is the biochemical mechanism by which the growth factor signal to grow is communicated from the outside of the cell into the nucleus to ultimately cause that cell to begin replication and growth.
- The genes that encode elements of the signal transduction pathway are proto-oncogenes, genes that when altered can cause cancer.
- Eukaryotic DNA replication is regulated at various stages to ensure all chromosomes replicate once and only once per cell cycle.
- Cell cycle regulation by protein phosphorylation ensures that pre-RC assembly can only occur in G1 phase, whereas helicase activation and loading can only occur in S phase.
- In **Prokaryotes**, the replication origin and the initiator protein DnaA are the main targets for regulation of chromosome replication (as in bacteria).
- The origin bears multiple DnaA binding sites, while DnaA contains ATP/ADP-binding and DNA-binding domains. When enough ATP-DnaA has accumulated in the cell, an active initiation complex can be formed at the origin resulting in strand opening and recruitment of the replicative helicase.
- In *Escherichia coli*, oriC activity is directly regulated by DNA methylation and specific oriC-binding proteins. Regulations by oriC-binding proteins are also conserved in bacteria.
- DnaA activity is regulated by proteins that stimulate ATP-DnaA hydrolysis, yielding inactive ADP-DnaA. Regulation of DnaA gene expression is also important for initiation and is conserved in bacteria.
- The S phase CDK complexes stimulate the onset of organized DNA synthesis. The machinery ensures that each chromosome is replicated only once.

(CDK complexes are specific protein kinases made up of a regulatory subunit and a catalytic subunit. The regulatory subunits are called cyclins and the catalytic subunits are called cyclin-dependent kinases (CDKs)).

- A novel mammalian kinase, Cdc7-ASK (Activator of S phase Kinase), plays a key role at the entry into S phase as a molecular switch for DNA replication. This kinase is specially activated during S phase and triggers DNA replication by phosphorylating an essential DNA helicase component of the replication complex.
- Certain proto-oncogenes and tumour suppressor genes regulate the passage of cells from G1 to S phase.

Further Reading

Cell and Molecular Biology. P.K. Gupta. Rastogi Publications

Cell Biology, Genetics, Mol.Biology, Evolution and Ecology. P.S. Verma. S.Chand Publication

Molecular Biology of The Cell. Bruce Alberts et al. 6th Edition. Garland Science

Cell and Molecular Biology- Concepts and Experiments- Gerald Karp. 7th Edition Wiley