Genetics

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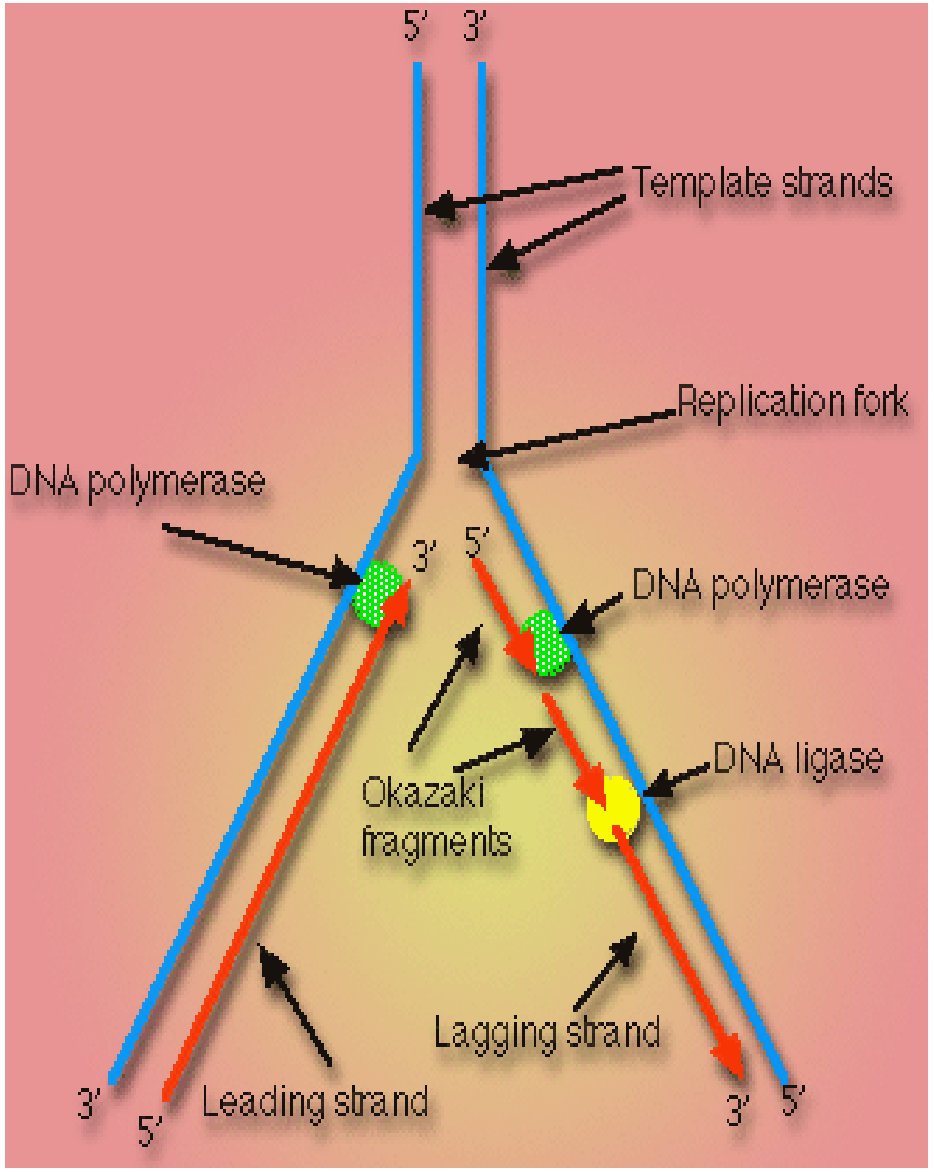
Topic 6: DNA replication

- During DNA replication, the original DNA strands are used as templates for the synthesis of new strands.
- During this process, the two complementary strands of DNA come apart and serve as template strands for the synthesis of two new strands of DNA.
- Before it was known how DNA was replicated, there were two basic models for DNA replication: **conservative** and **semi-conservative** replications.
- In **conservative replication**, the two strands of DNA do not unwind as a brand new copy is produced and the old molecule remains intact.
- In **semi-conservative replication**, the DNA strands unwind and each strand serves as a template for the replication.

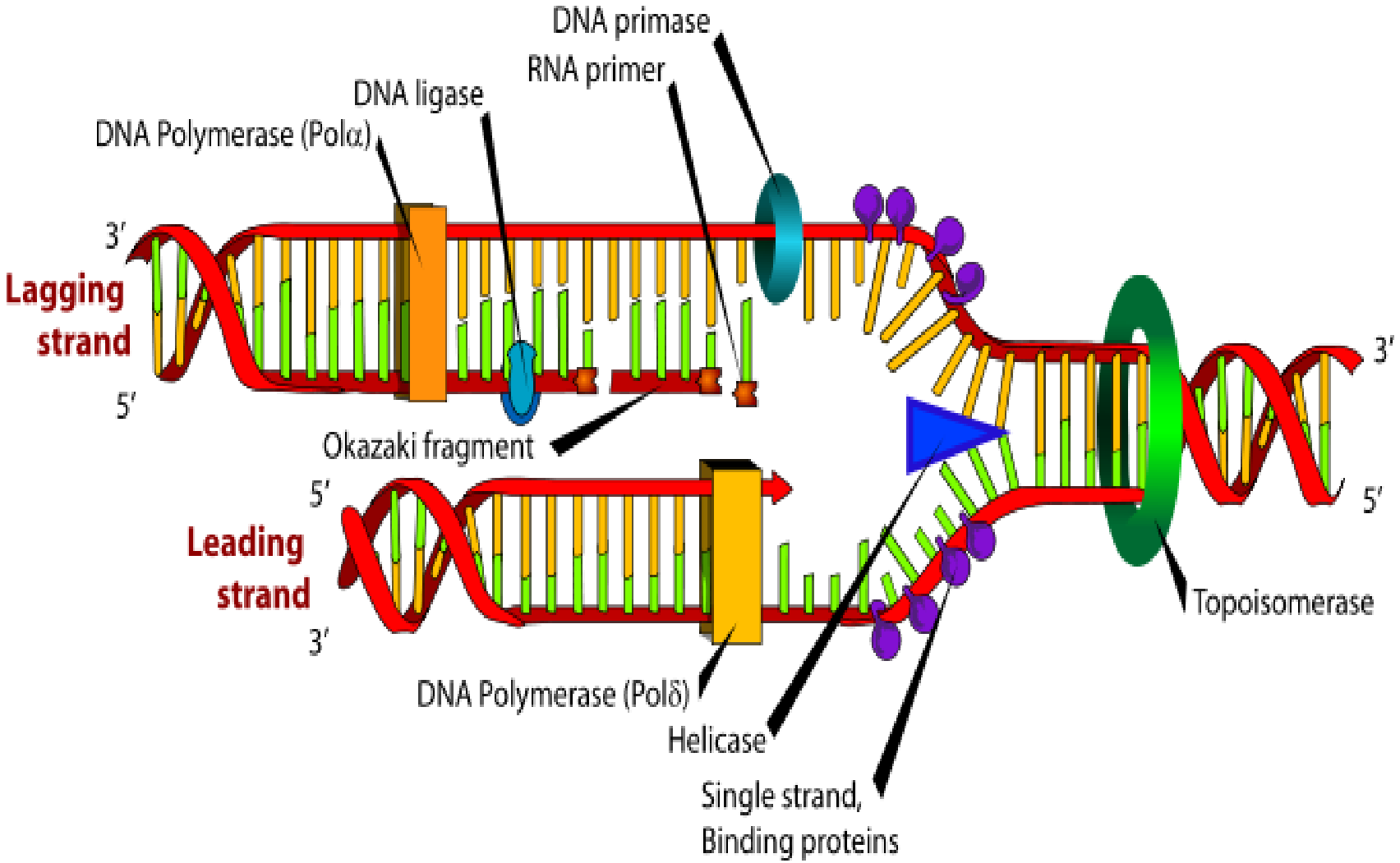
- In 1957, Meselson and Stahl found that DNA followed the **semi-conservative** model.
- DNA replication begins at specific locations in the genome, called "**origins of replication**".
- Unwinding of DNA at the origin, and synthesis of new strands, forms **a replication fork**.
- Many enzymes are necessary to successfully replicate DNA.



Steps of replication in Bacteria

- Much research on DNA replication has focused on the bacterium ***E. coli*** ➔ provided the foundation of our current molecular understanding of DNA replication.

- The bacterial chromosome is **circular.** The site on the bacterial chromosome where DNA synthesis begins is known as the **"origin of replication".** This origin is called ***oriC (245 bp)*** and contains an AT-rich region and DnaA box sequences**.**


DNA primase
RNA primer
DNA ligase
DNA Polymerase (Polα)
3′
Lagging strand
5′
Okazaki fragment
5′
Leading strand
3′
DNA Polymerase (Polδ)
Helicase
Single strand, Binding proteins
Topoisomerase
3′
5′

- The synthesis of new daughter strands is initiated by the binding of DnaA proteins to the DnaA box sequences and proceeds in two directions around the bacterial chromosome (bidirectional replication).

- This means that two replication forks move in opposite directions outward from the origin and meet each other on the opposite side of the chromosome to complete the replication process.

- To act as templates for DNA replication, the two strands of the double helix must separate.

- First, the AT-rich region separates by the action of **DNA-binding proteins**. After separation of the AT-rich region, the enzyme **helicase** starts separating the two strands by breaking the hydrogen bonds between the base pairs.

- Helicase activity generates **supercoiling** ahead of the replication fork. This supercoiling is relaxed by **DNA gyrase** (a topoisomerase enzyme) which travels ahead of the helicase enzyme.

- To keep the two strands unwound, single-strand binding proteins bind to each of the two parental strands and prevent them from reforming a double helix.

- The next step involves the synthesis of a short strand of RNA (not DNA) called an **RNA primer** **(short sequence typically 10 to 12 nucleotides)**. This strand of RNA is synthesized by the linkage of ribonucleotides by an enzyme called **primase**.

- DNA polymerase enzymes link nucleotides to synthesize the daughter strands. In *E. coli*, there are five different polymerases designated:

  polI, polII, polIII, polIV, *and* ***polV.***

- ***PolI*** and ***polIII*** play a role in normal DNA replication while ***polII, polIV*** and ***polV*** play a role in DNA repair and the replication of damaged DNA.
- ***PolI*** consists of a single subunit. Its role is to fill in small regions where RNA primers were located.

- *PolIII* consists of 10 subunits. The α subunit catalyzes the bond formation between adjacent nucleotides. The ε subunit has a 3' to 5' proofreading function (**removes mismatched nucleotides**).

- DNA polymerases can only elongate a strand starting with an RNA primer or existing DNA strand.

- Also DNA polymerases can attach nucleotides only in the 5' to 3' directions (not 3' to 5') . The reason behind this is because 3' is more stable than 5' when attaching a new nucleotides.

- If DNA polymerase ran in the other direction then there is the risk that the phosphate group could break off.

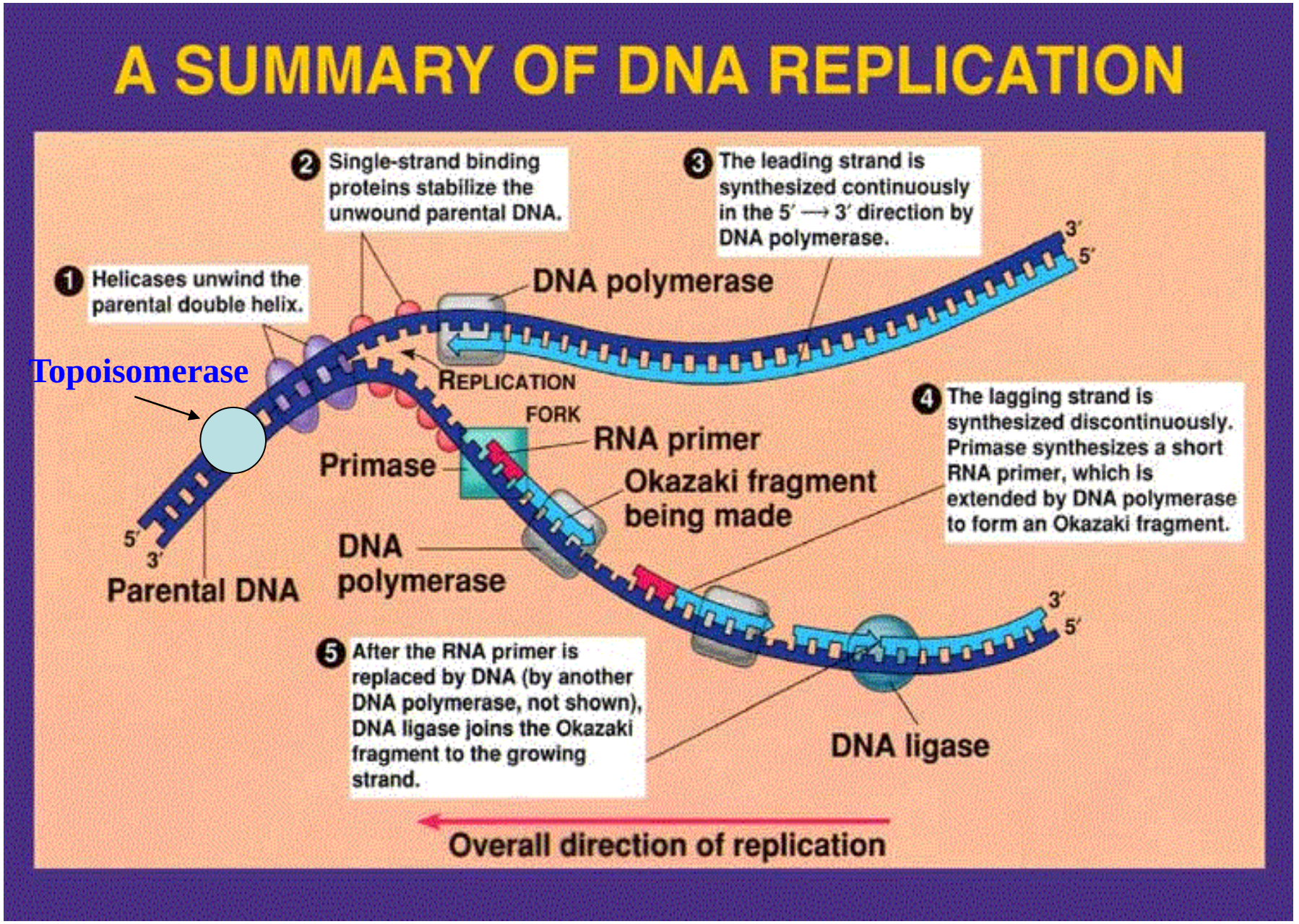
- **DNA polymerase III** (*polIII*) synthesizes one strand continuously (called **the leading strand**) in the direction of the fork but synthesizes the other strand (called **lagging strand**) in fragments in opposite direction of the fork. These fragments are called **Okazaki fragments**.

- In the leading strand, one RNA primer is made at the origin and the *DNA polIII* can attach the nucleotides in 5' to 3' direction as it slides toward the opening of the fork (**at a rate of 750 nucleotides per second**).

- In the lagging strand, the synthesis of DNA is also from 5' to 3' but it occurs in the direction away from the replication fork. In this strand, short segments of DNA are made:

  - in bacteria: 1000 to 2000 nucleotides

  - in Euokaryotes: 100 to 200 nucleotides.
- Each fragment contains a short RNA primer at the 5`end, which is made by DNA primase, the remainder of the fragment is a strand of DNA made by DNA *polIII*.
- In E. coli, the RNA primers are removed by the **DNA *polI*** which then fills the gap by synthesizing DNA. Then DNA **ligase** covalently links the DNA fragments together.


A SUMMARY OF DNA REPLICATION
1 Helicases unwind the parental double helix.
2 Single-strand binding proteins stabilize the unwound parental DNA.
3 The leading strand is synthesized continuously in the 5′ ⟶ 3′ direction by DNA polymerase.
4 The lagging strand is synthesized discontinuously. Primase synthesizes a short RNA primer, which is extended by DNA polymerase to form an Okazaki fragment.
5 After the RNA primer is replaced by DNA (by another DNA polymerase, not shown), DNA ligase joins the Okazaki fragment to the growing strand.
Topoisomerase
DNA polymerase
REPLICATION FORK
RNA primer
Primase
Okazaki fragment being made
DNA polymerase
Parental DNA
DNA ligase
3′
5′
5′
3′
3′
5′
Overall direction of replication

Termination of replication in Bacteria

- Replication is terminated when the two replication forks meet at the terminus sequences.
- On the side of the chromosome opposite to the oriC are **two termination sequences** , *T1* and *T2* (called *ter* sequences).
- A protein known as the ***termination utilization substance*** (*Tus*) binds to the *ter* sequences and stops the movement of the replication forks.
- DNA replication ends when oppositely advancing forks meet at T1 or at T2.

- Finally, DNA ligase covalently links the two daughter strands forming two circular double-stranded DNA molecules.

- When DNA replication is complete, the two circular chromosomes may be interlocked.

- Interlocked circular molecules are called **catenanes**.

- **Topoisomerases** introduce a temporary break into the DNA strands then rejoin them after the strands have been unlocked.

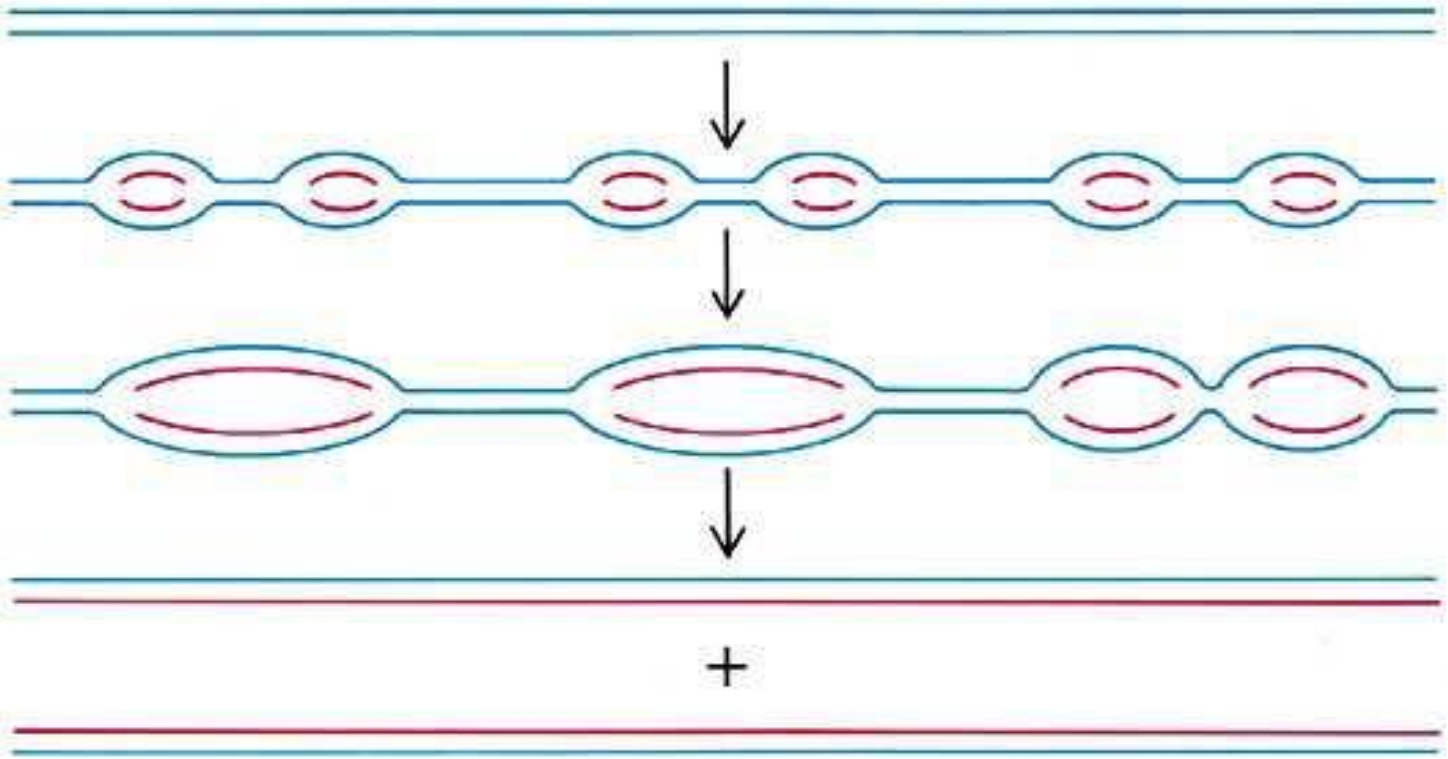
- Mistakes can occur during DNA replication but they are very rare.
- In the case of DNA synthesis by polIII, only one mistake per 100 million nucleotides is made.
- The reasons that there are very few mistakes are:

1. Hydrogen bonding between matched base pairs is more stable than that between mismatched pairs.
2. DNA polymerase is unlikely to catalyze bond formation between adjacent nucleotides if a mismatched base pair is formed.
3. DNA polymerase can identify a mismatched pair and remove it by **exonuclease cleavage** (this ability to remove mismatched pairs is called **the proofreading function** of DNA polymerase).

DNA replication in Eukaryotes

- There are extensive similarities between DNA replication in prokaryotes and Eukaryotes.
- For example, the types of enzymes identified in bacterial DNA replication are also found in Eukaryotes.
- Nevertheless, at the molecular level, DNA replication in Eukaryotes is more complex.
- This is related to the features of Eukaryotic cells (**have larger, linear chromosomes**, **the chromatin is tightly packed in the nucleosomes** and the **cell cycle regulation is more complex).**

- Initiation of replication in Eukaryotes occurs at **multiple origins of replication** (this makes the process **faster**).
- DNA replication proceeds **bidirectionally** from many origins of replication. The **multiple replication forks** eventually meet with each other to complete the replication process.
- In the yeast, several origins have been identified and sequenced. They have been named **ARS elements** (Autonomously Replicating Sequence).
- ARS elements are 100-150 bp in length and have **higher percentage of A and T** than the rest of the DNA.



Multiple origins of replication in eukaryotes

- The **origin recognition complex** (ORC) is a six-subunit protein complex that acts as the **initiator of eukaryotic DNA replication.**
- **ORC** was originally identified in yeast as a protein complex **that binds directly to the ARS elements**.
- Mammalian cells contain over a dozen different DNA polymerases, four of these have the primary function of replicating DNA:

1. Pol α (alpha)
2. Pol δ (delta)
3. Pol ε (epsilon)

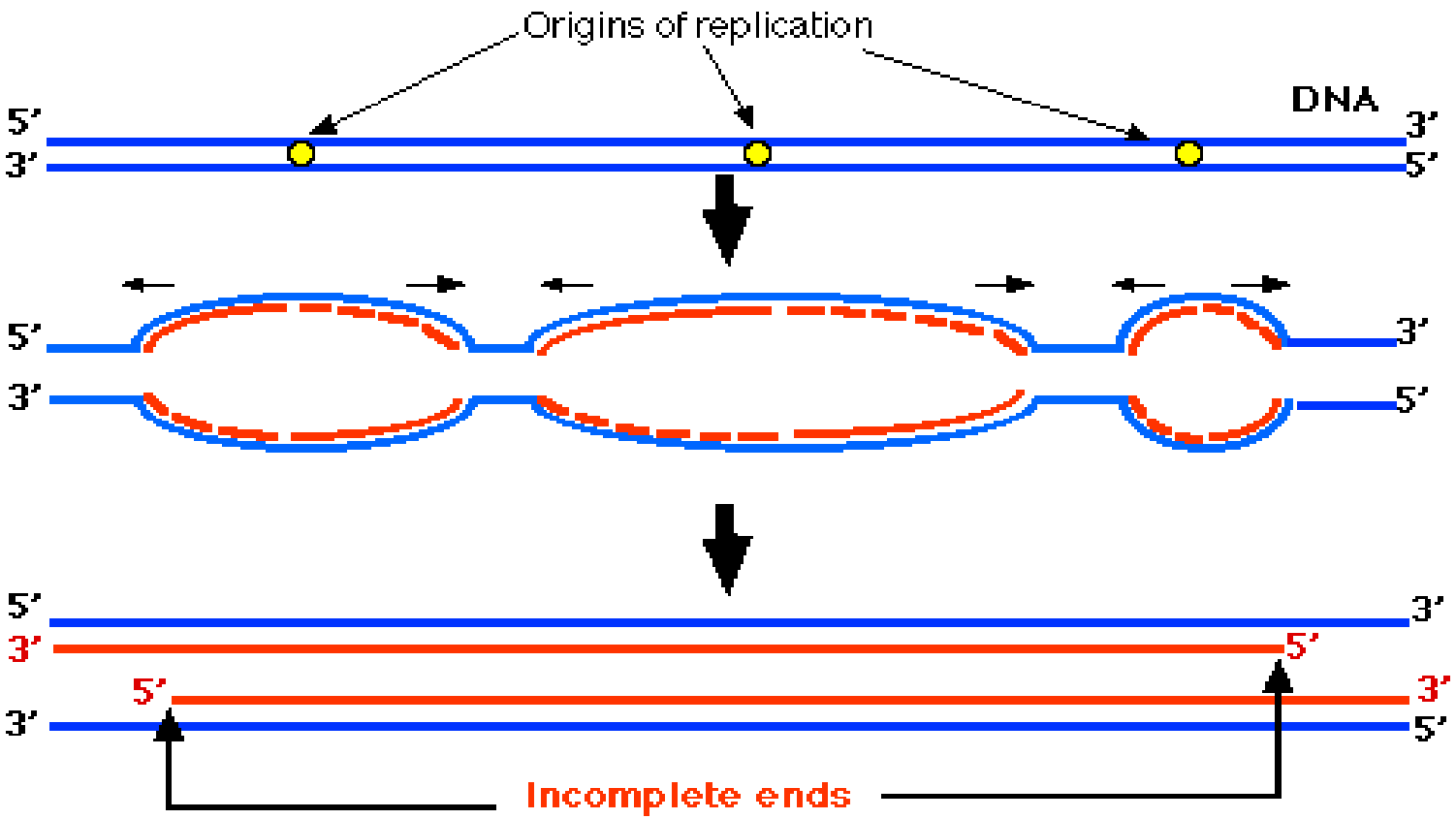
Involved with DNA replication in the nucleus during S phase

4. Pol γ (gamma): replicates mitochondrial DNA

- Another polymerase **(Pol β)** plays an important role in **removing incorrect bases from damaged DNA**.
- Pol α associates with **primase**. This pol α/primase complex synthesizes a short RNA-DNA hybrid of approximately 10 RNA nucleotides followed by 20 to 30 DNA nucleotides.
- This short RNA-DNA strand is then used by DNA pol δ or ε for elongation of the leading and lagging strands.
- The exchange of DNA Pol α for δ or ε is called a **polymerase switch** and only occurs after the RNA-DNA primer has been made.

The ends of eukaryotic chromosomes are replicated by Telomerase

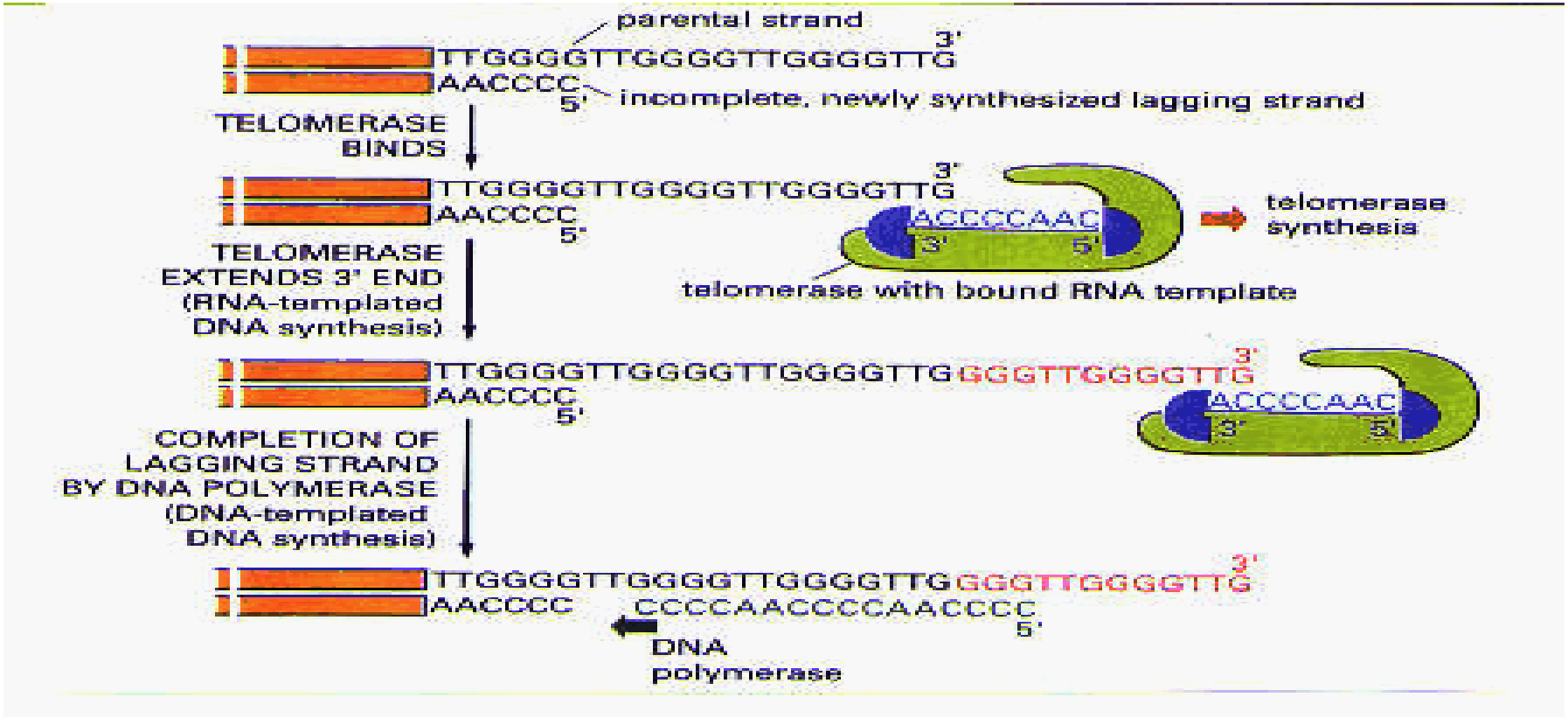
- Linear eukaryotic chromosomes contain telomeres at both ends.
- The term telomere refers to the telomeric sequences within the DNA and the special proteins that are bound to these sequences.
- These consist of repetitive tandem array of **G and T** and a 3`overhang region of 12 to 16 nucleotides.
- One reason why telomeric sequences are needed is because DNA polymerase is unable to replicate the 3`end of the DNA strands.



If this problem is not solved, the linear chromosome will become progressively shorter with each round of replication

- Telomerase is an enzyme that adds telomere repeat sequences to the 3' end of DNA strands.

- By lengthening this strand, DNA polymerase is able to complete the synthesis of the "incomplete ends" of the opposite strand.



This figure shows how telomeres (red) that make up the ends of chromosomes, are formed. Telomere replication requires the action of telomerase (light green), which is a protein–RNA complex that carries an RNA strand (blue) that serves as a template for the formation of the G-rich telomere DNA sequence. After telomere replication, DNA synthesis can continue, and a new DNA strand formed

Nucleosomes containing new histone proteins are quickly formed after DNA replication

- DNA within eukaryotic cells is wrapped around histone proteins to form a nucleosome structure.
- Since replication doubles the DNA, the cell must synthesize more histone proteins to accommodate this increase.
- Like replication of DNA, the synthesis of histones occurs during the S phase of the cell cycle.

The DNA (red) is wrapped around the histone octamer (blue) and both form the nucleosome core particle. This structure is locked in mammals by the linker histone H1 (yellow). The chromatin fiber is further folded into a thicker fiber, the so-called **solenoid** that is 30 nm in diameter.