Zoo-342 Molecular biology
Lecture 2

DNA replication

DNA replication

- DNA replication is the process in which one doubled-stranded DNA molecule is used to create two double-stranded molecules with identical DNA sequences (Figure 1).
- DNA replication occurs during the S phase of the eukaryotic cell cycle.

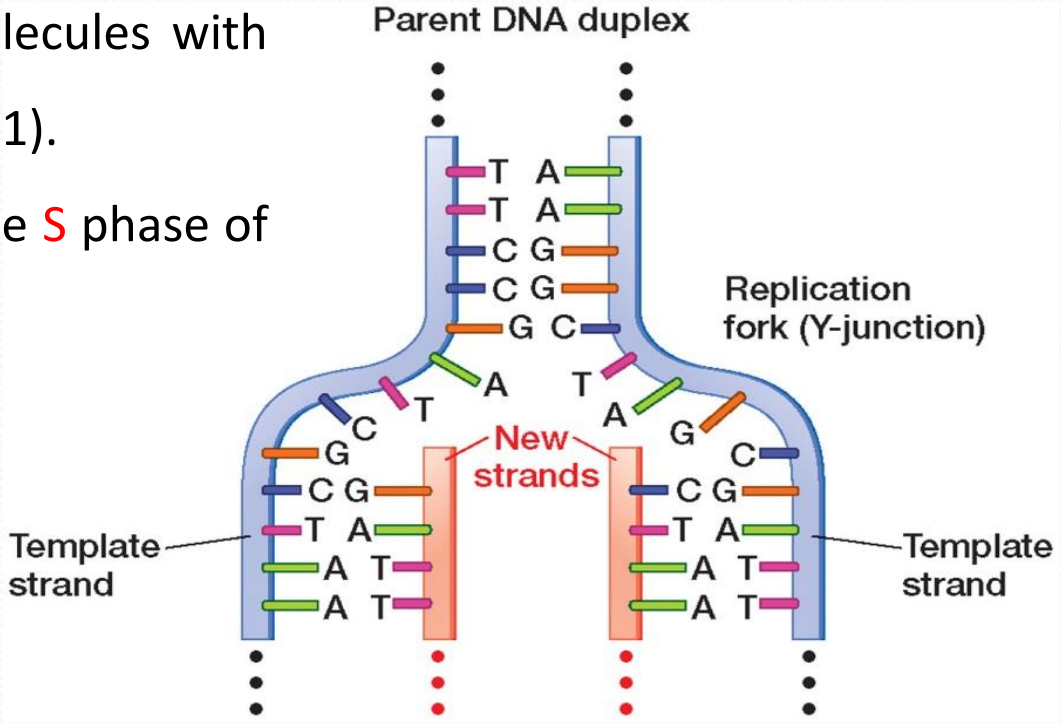


Figure 1: Base complementary provides a mechanism for accurate DNA replication

Three alternative models for DNA replication (Figure 2)**:**

1. **Semiconservative replication** (Watson and Crick model): Each strand of the parental molecule serves as a template for the synthesis of a new strand (complementary strand).

- The double helix of each daughter DNA molecule contains one strand from the parental DNA molecule and one newly synthesized strand.

2. **Conservative replication**: The parent DNA molecule is conserved. The resulting daughter DNA molecules consists of two strands from the parental DNA molecule and other are all new strands.

3. **Dispersive replication**: The double helix of each daughter DNA molecule consists of strands each containing segments of both parental DNA and newly synthesized strand.

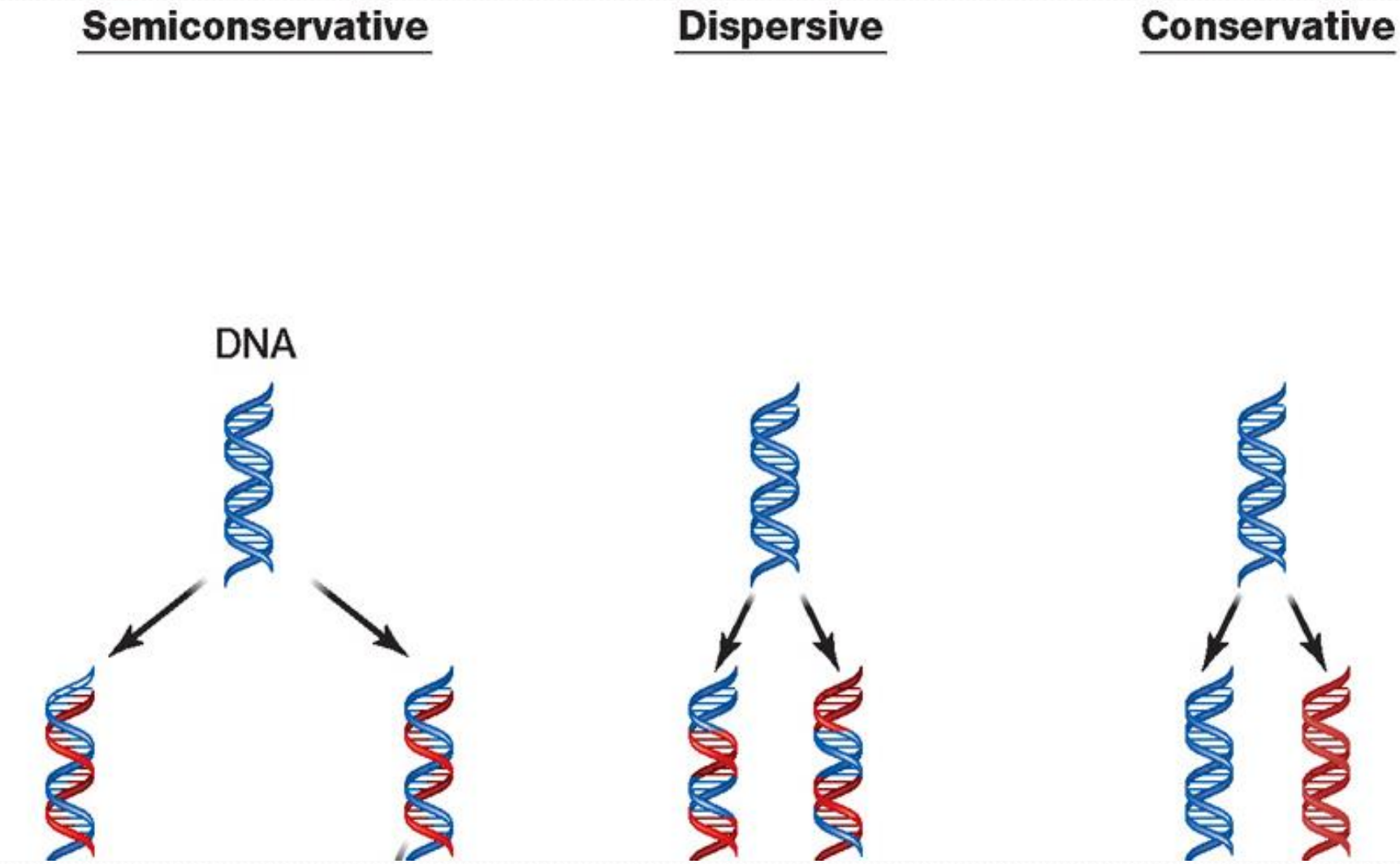
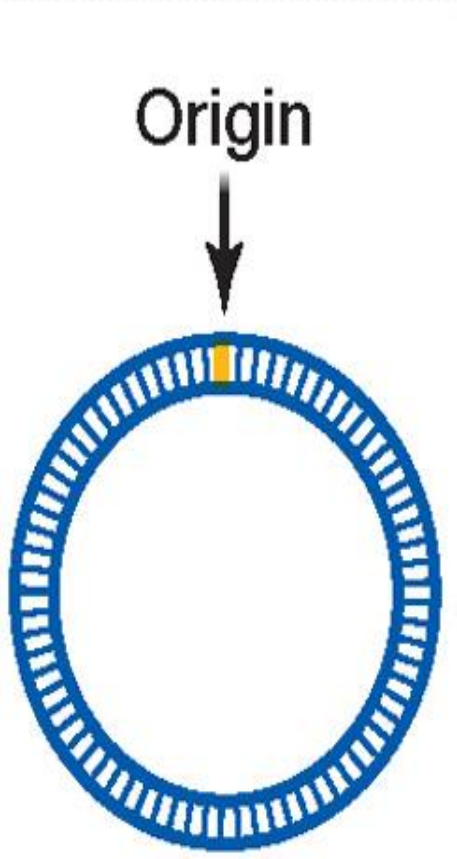


Figure 2: The three alternative models for DNA replication. Gold lines represent the newly synthesized strand

Sites of initiation of DNA replication in prokaryotes and eukaryotes:

- Each mammalian chromosome is composed of many replication sites or origins of replication (**replicons)**.
- In comparison, the *Escherichia coli* (prokaryotes), chromosome is composed of only one replicon (Figure 3).
- Multiple origins allow eukaryotes to replicate their larger quantities of DNA in a relatively short time.
- The basic process of DNA replication is the same in both prokaryotes and eukaryotes, except that eukaryotic DNA molecule consists of multiple replicons relative to the single replicon in the prokaryotic genome.



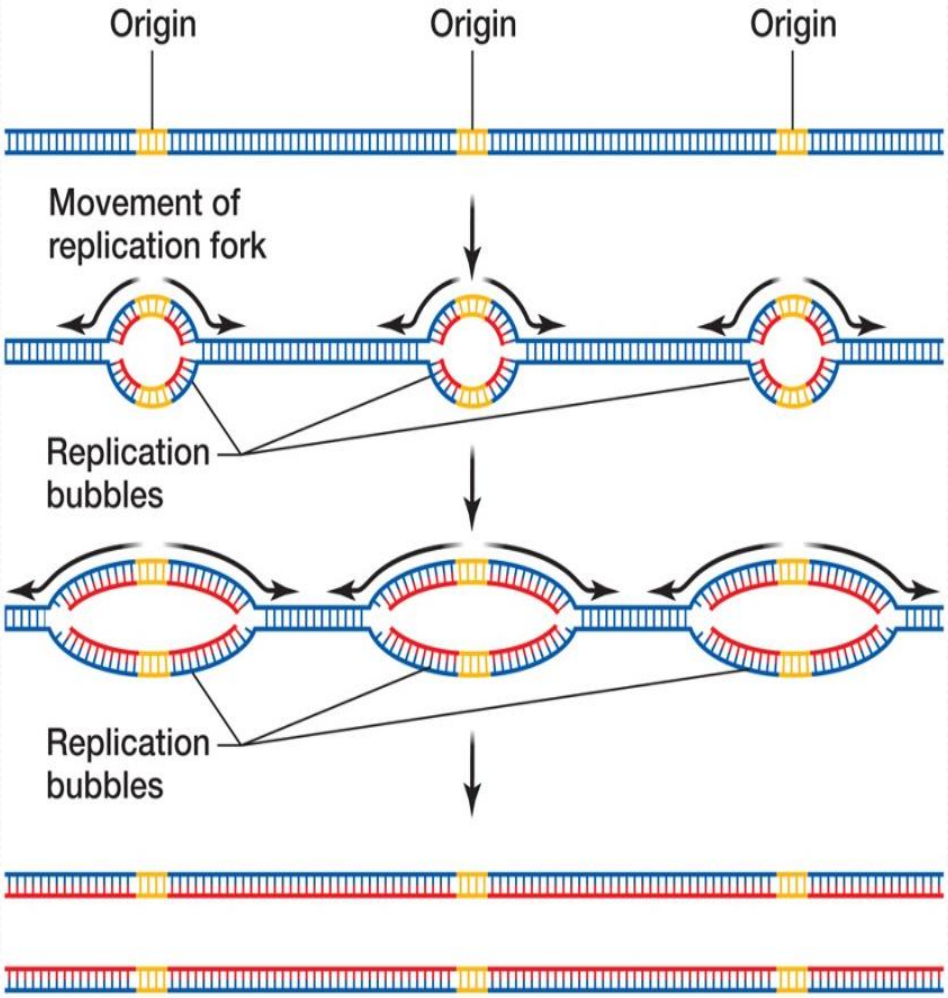
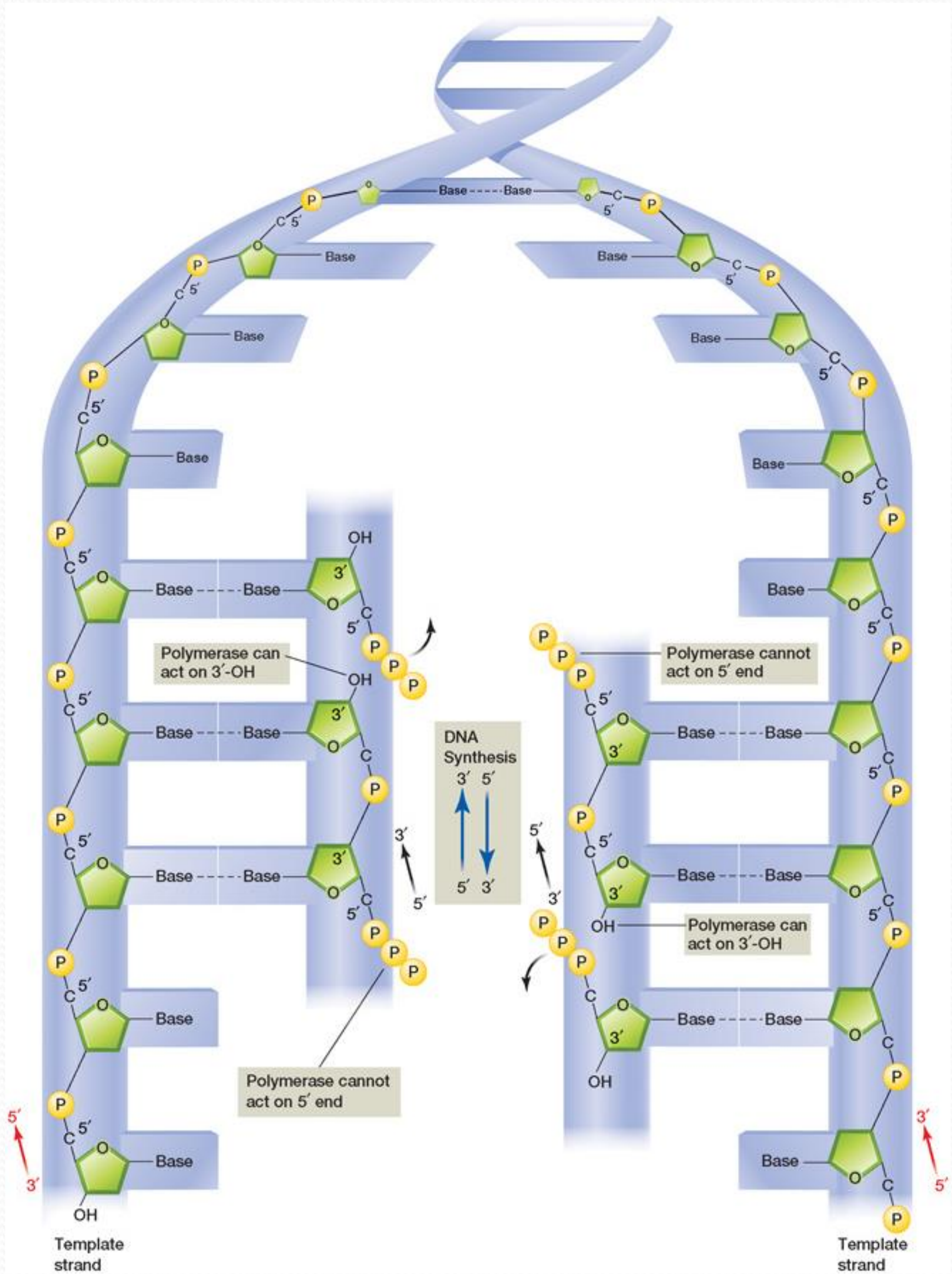


Figure 3: Origin of DNA replication in prokaryotic and eukaryotic chromosomes

Initiation of DNA replication:

- The replication of DNA begins at a specific site (a sequence of nucleotides) called **the origin of replication**.
- **Helicase** unwind the double-stranded DNA helix and **single-strand binding proteins** (ssb protein) react with the single-stranded regions of the DNA to prevent the strands from annealing until the new strand is synthesized.
- **DNA polymerase III** is the major enzyme involved in DNA replication.
- RNA polymerase, called a **primase** adds ribonucleotides in 5' to 3' direction into an RNA primer, a sequence of about 10 nucleotides, complementary to the parent DNA.
- DNA polymerase III can then add deoxyribonucleotides to the 3-prime end (Figure 4) to synthesize the new complementary strand of DNA.

Figure 4: During DNA replication, new nucleotides can be added to the free 3'-OH end of the new synthesized DNA strand, not to the 5'-PO_4



DNA replication fork:

- DNA replication begins when the enzyme helicase unwind a segment of the DNA and breaks (open up) the hydrogen bonds between the two complementary strands of DNA.
- DNA polymerase III can only add new nucleotide to a free 3' end of a growing chain. Synthesis of one strand of the DNA, called the leading strand, proceeds continuously in the 5' to 3' direction (Figure 5).
- Synthesis of the complementary strand, called the lagging strand is more complex. This strand must replicate away from the replication fork. RNA primase attaches to the DNA and synthesizes several short RNA primers (Figures 6 and 7). The replication occurs in short segments called Okazaki fragments. The size of Okazaki fragments is 1500 nucleotides in prokaryotes and 150 nucleotides in eukaryotes.

- DNA polymerase III then adds deoxyribonucleotides to the 3-prime end of the RNA primer.
- DNA polymerase I removes the RNA primer and replaces it with DNA.
- The enzyme DNA ligase forms a phosphodiester bond between the short DNA strands.
- During DNA replication, the leading strand is synthesized continuously and it is possible on the template strand that is orientated 3'-5' toward the replication fork, while lagging strand is synthesized discontinuously and takes place on the template strand that is orientated 5'-3' toward the replication fork (Figure 7).

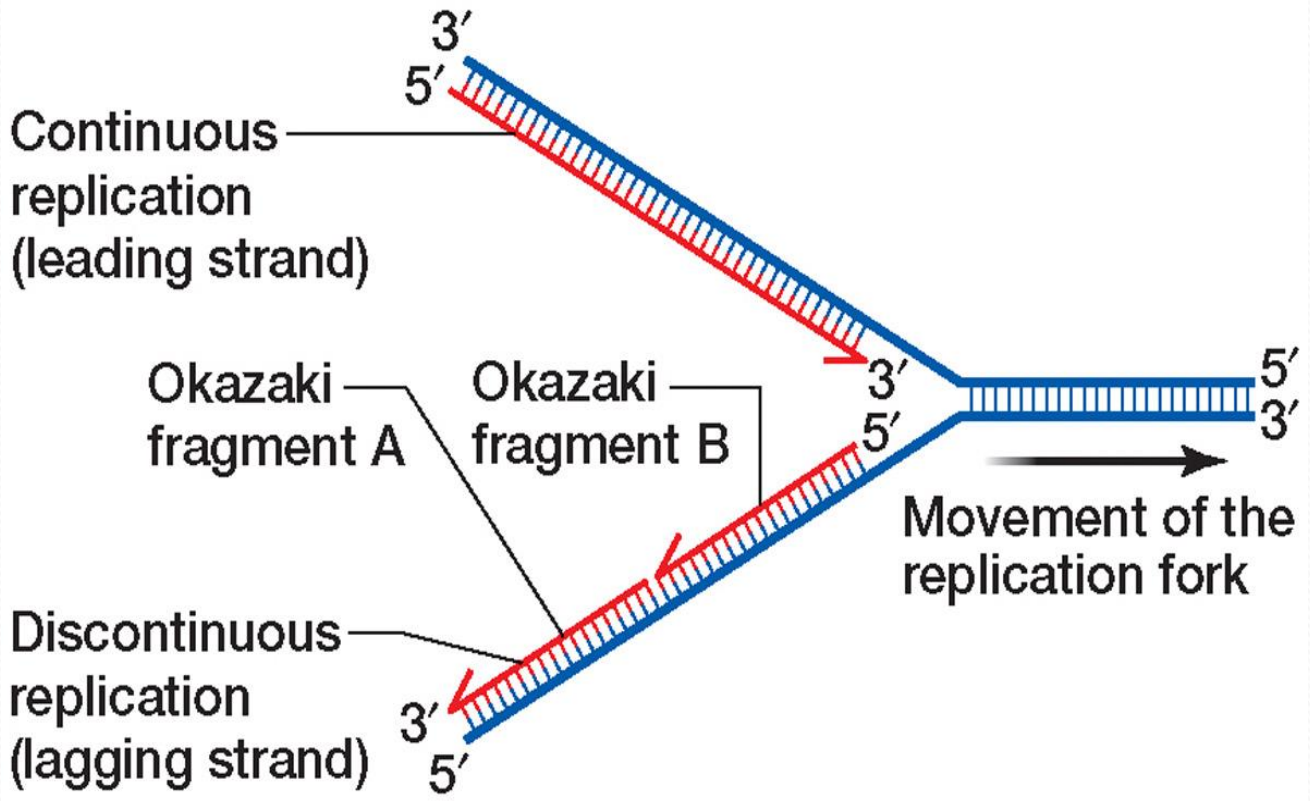


Figure 5: Continuous and discontinuous model of DNA replication

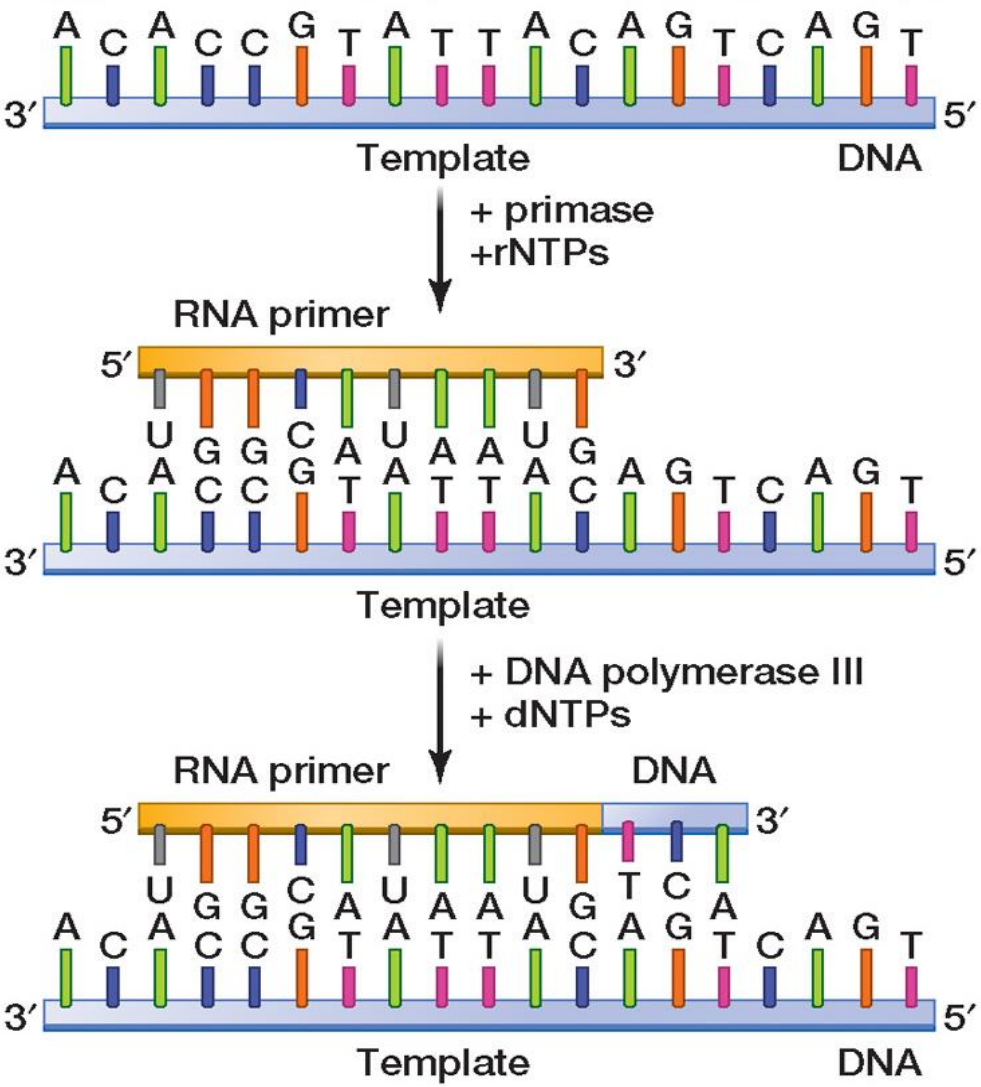


Figure 6: Generation of RNA primer

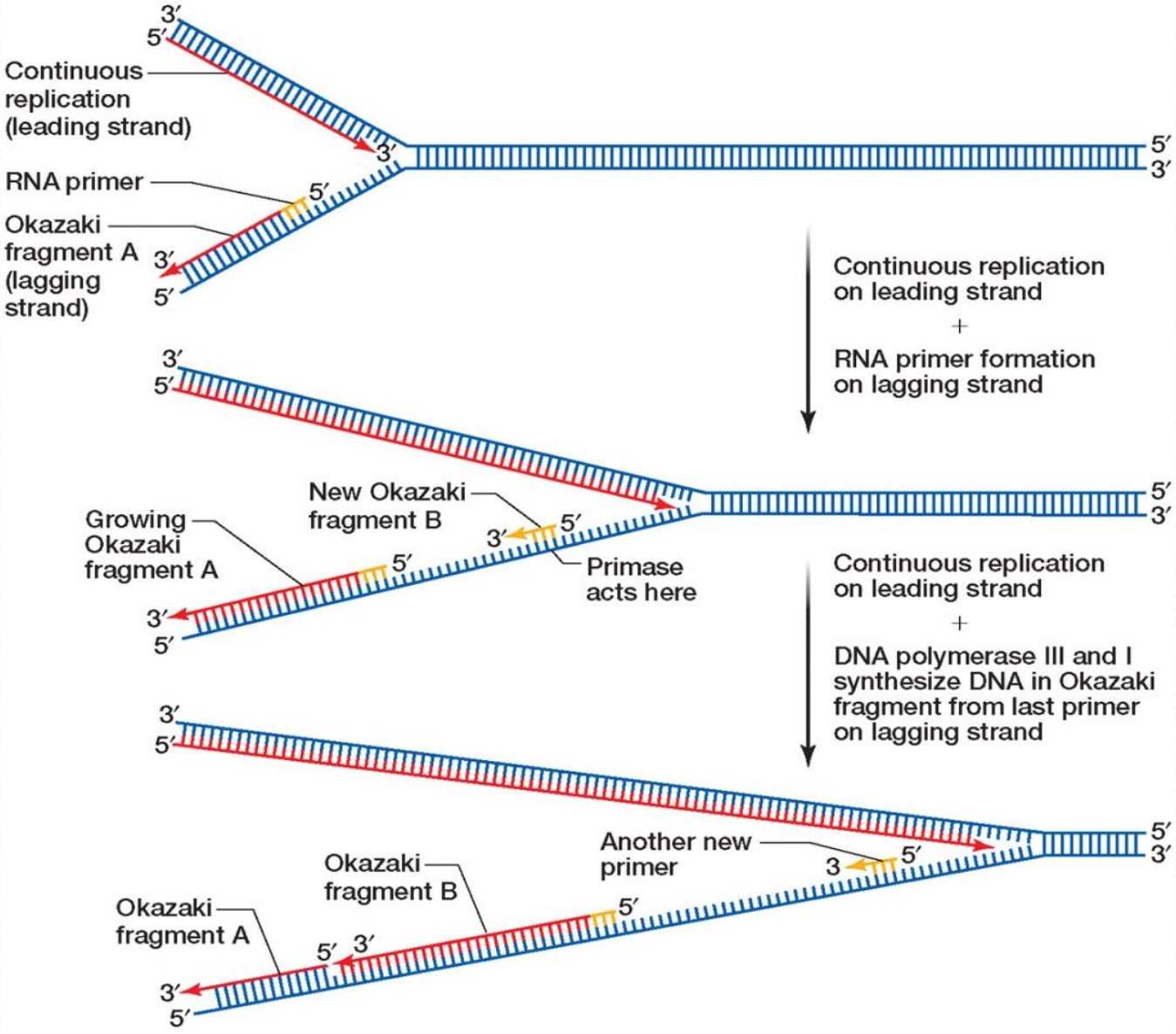


Figure 7: Primer formation and elongation create Okazaki fragments during discontinuous DNA replication

Differences between DNA replication in prokaryotes and eukaryotes

	Prokaryotes	Eukaryotes
Site of DNA replication	In the cytoplasm	In the nucleus
The number of origin replication (replicon)	One	Several (many)
The number of nucleotides in the replicon	100-200 nucleotides	Each replicon is formed of approximately 150 nucleotides
The number of replication forks	Two	Many
Types of DNA polymerase	Leading and lagging strands synthesize by DNA polymerase III	Leading strand is synthesized by DNA polymerase delta (δ), while lagging strand is synthesized by DNA polymerase alpha (α).
Size of Okazaki fragments	Large, approximately 1500 nucleotides	Short, approximately 150 nucleotides

http://highered.mheducation.com/sites/0072987760x/student_view0/chapter9/structural_basis_of_dna_replication.html

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