BIO102-Foundations of Biology (I)

DNA Replication

INDRAPRASTHA INSTITUTE *of*
INFORMATION TECHNOLOGY **DELHI**

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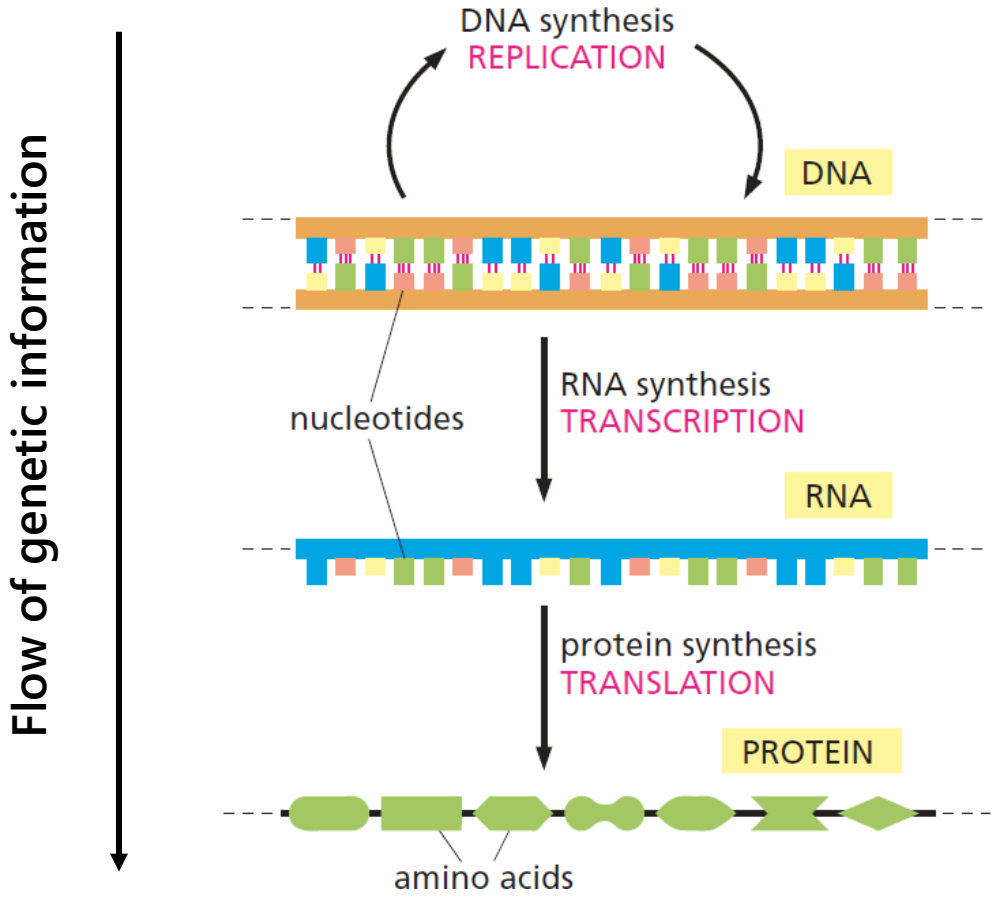
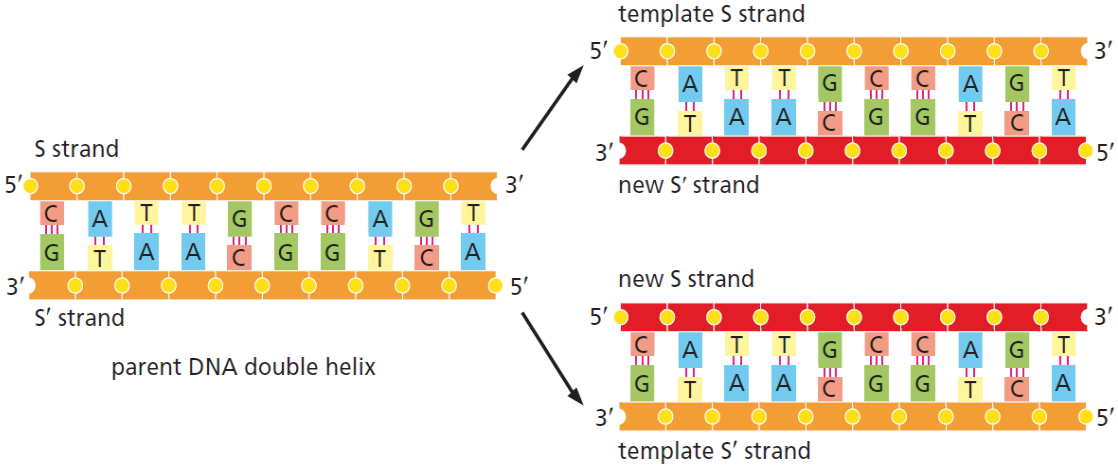
Central Dogma

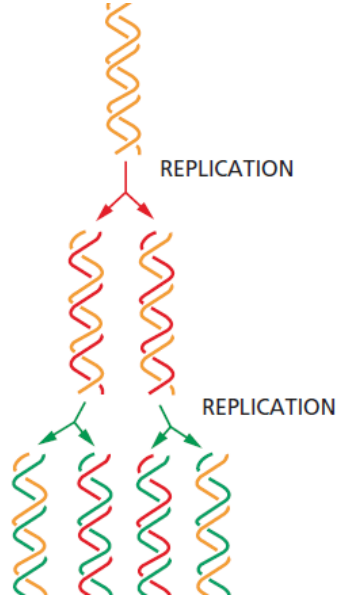


DNA replication is *semiconservative*

At each cell division, a cell must copy its genome with extraordinary accuracy.

Semiconservative Replication



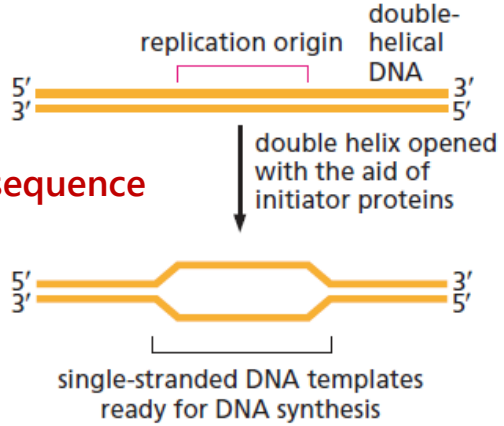


Each parental strand serves as the template for one new strand.

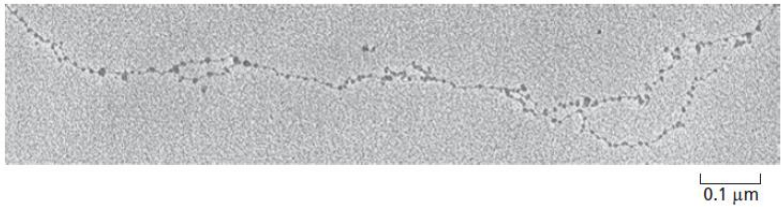
Each of the daughter DNA double helices ends up with one of the original (old) strands plus one strand that is completely new.

DNA synthesis begins at replication origins

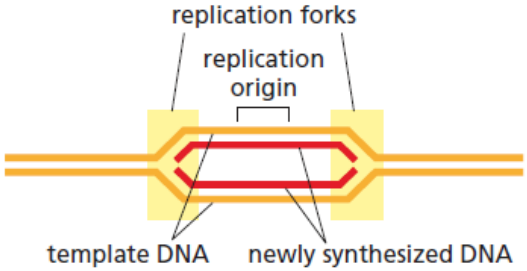
Replication origin



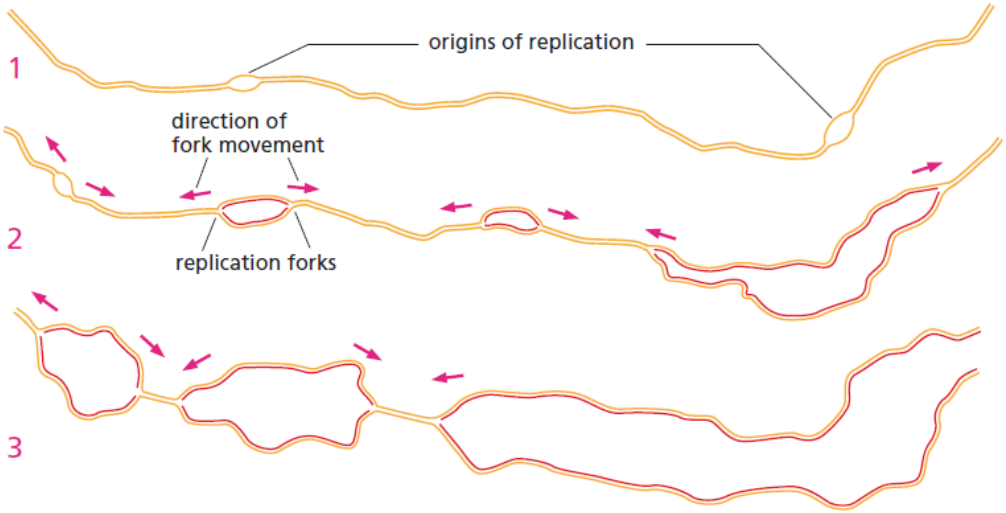
A-T rich sequence



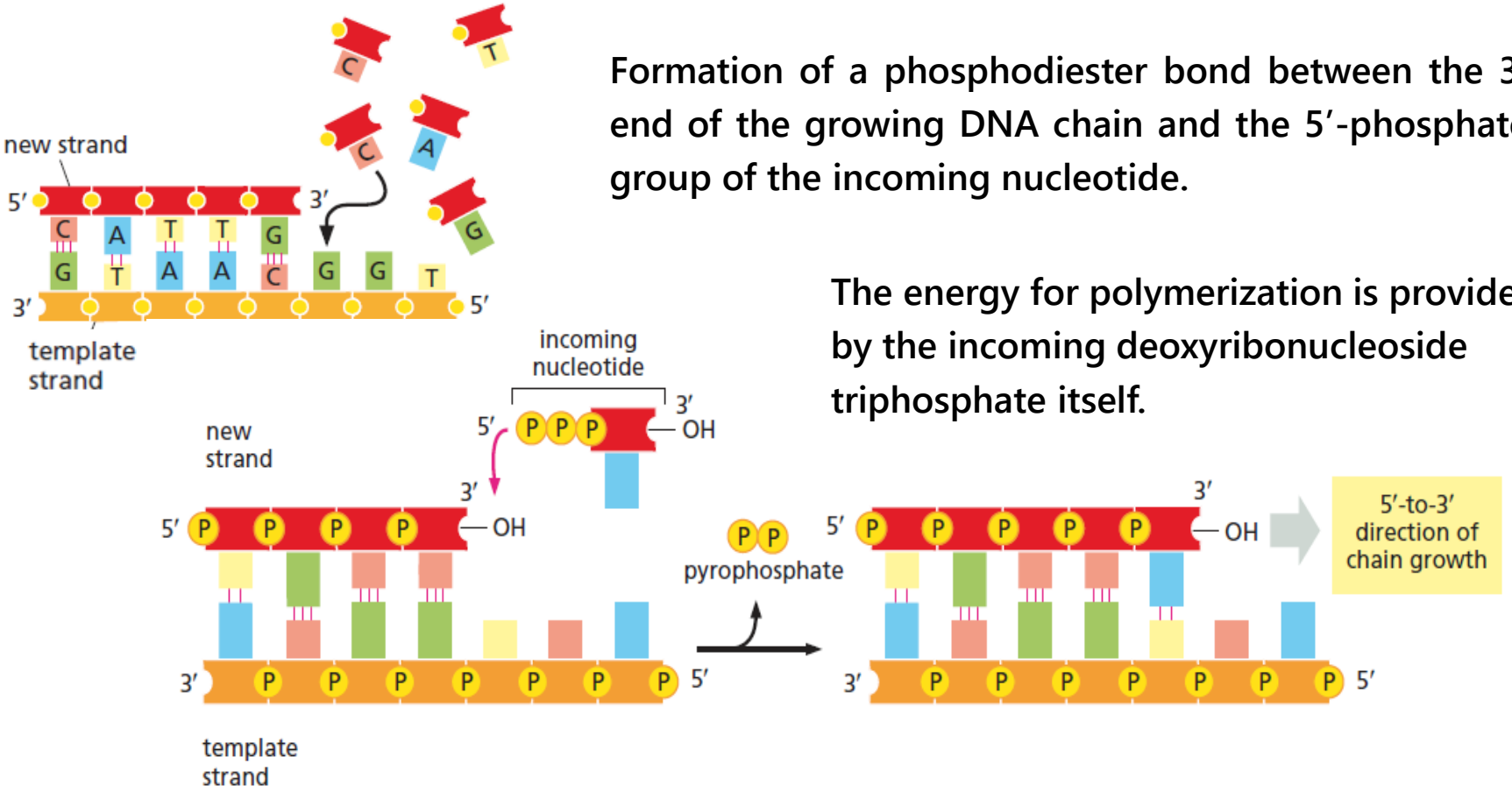
Replication fork



Bidirectional replication



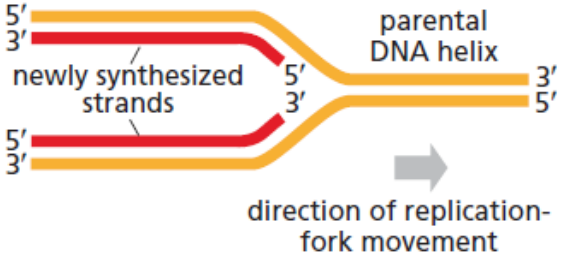
New DNA strand is synthesized in the 5′ to 3′ direction

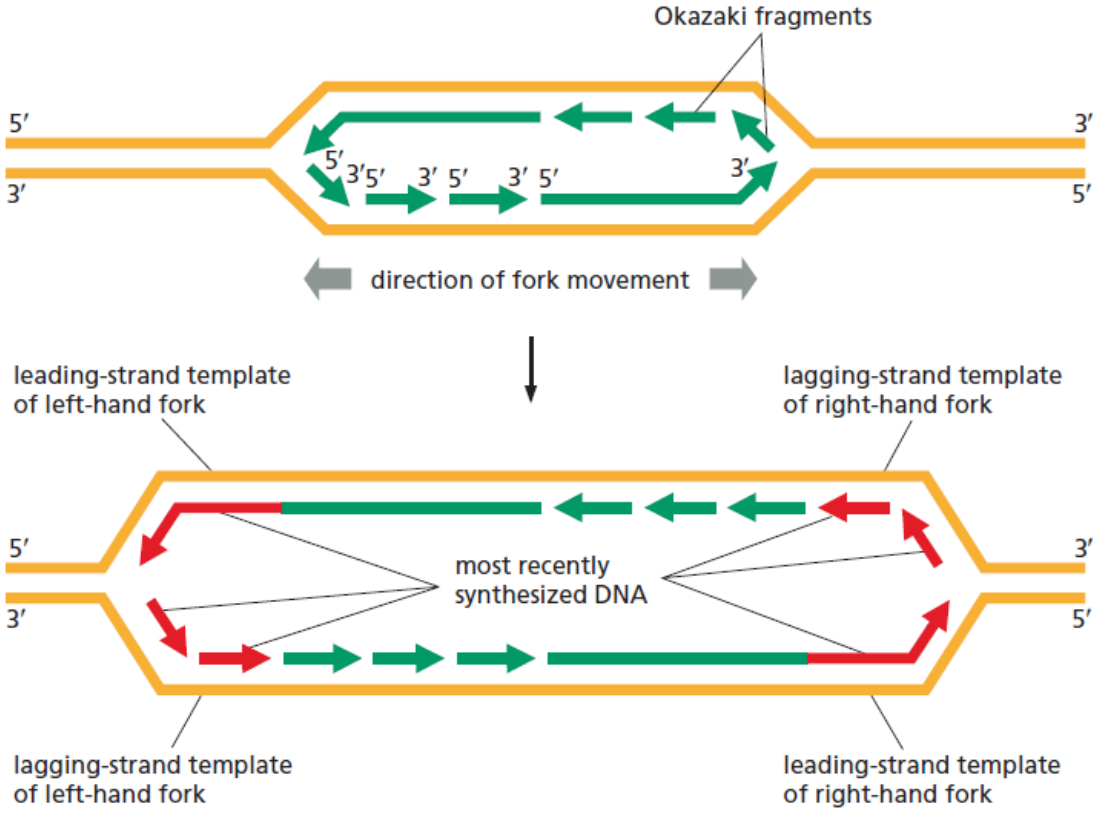


Formation of a phosphodiester bond between the 3′ end of the growing DNA chain and the 5′-phosphate group of the incoming nucleotide.

The energy for polymerization is provided by the incoming deoxyribonucleoside triphosphate itself.

Replication fork is asymmetrical





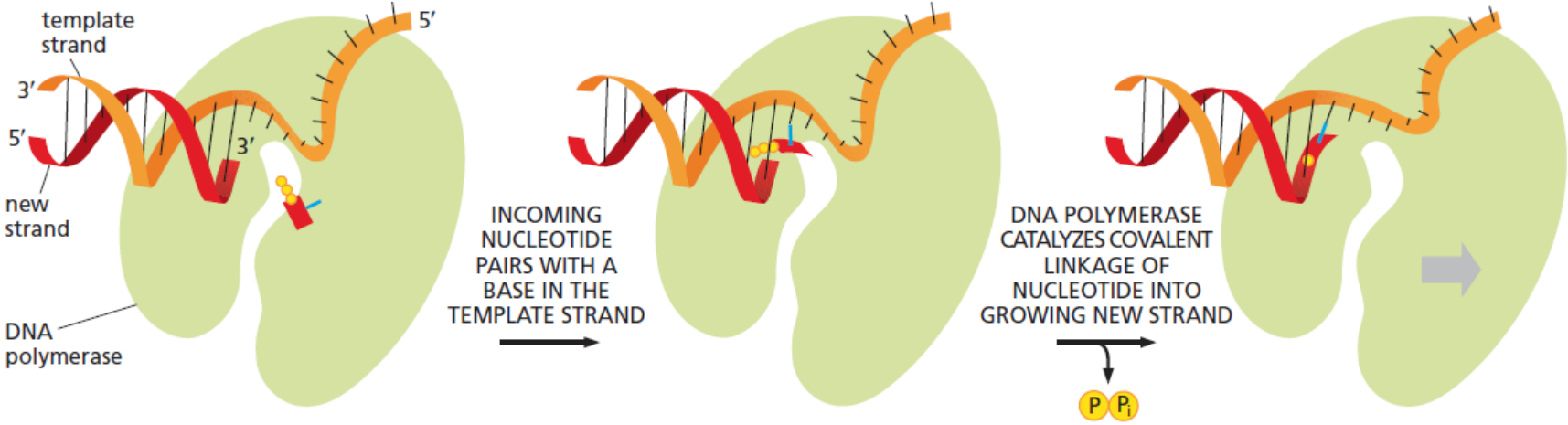
- DNA strand that appears to grow in the incorrect 3′-to-5′ direction is actually made discontinuously, in successive, separate, small pieces.
- The resulting small DNA pieces are called Okazaki fragments.

Replication machinery

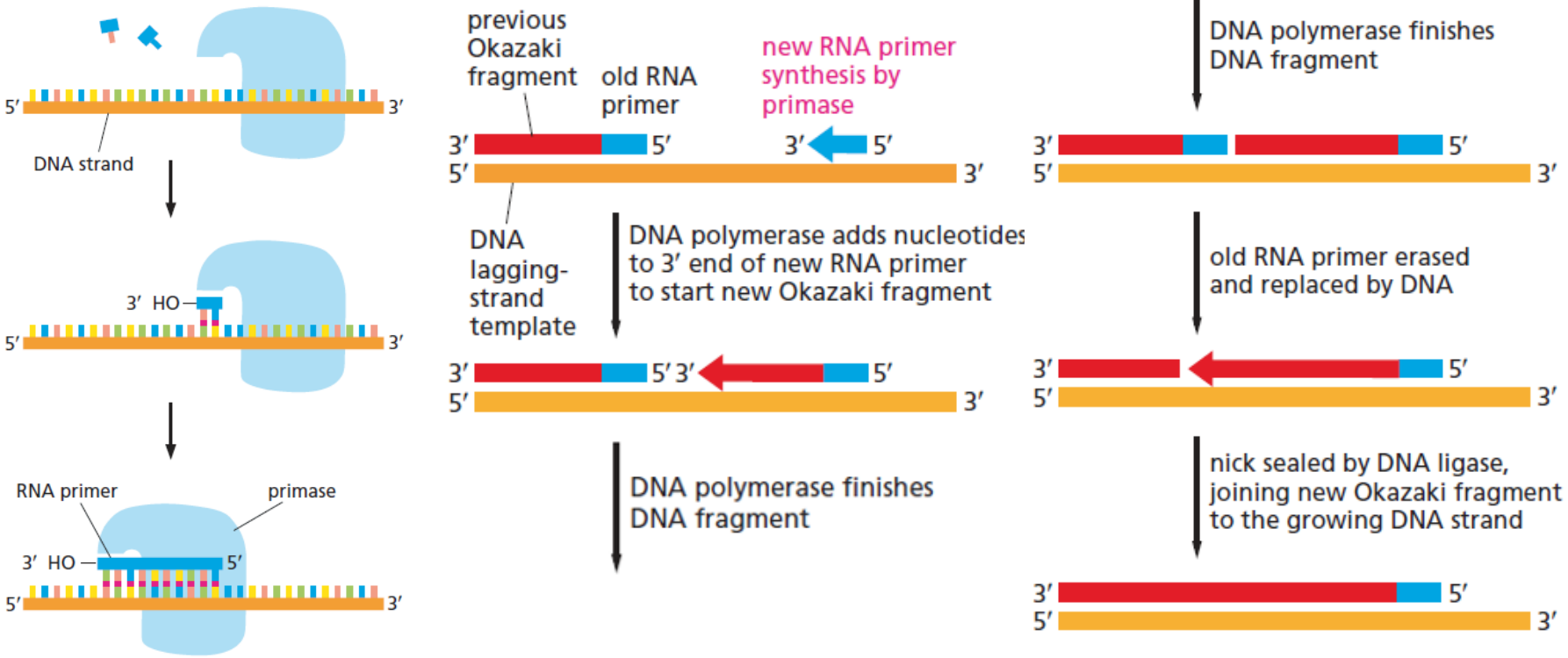
Different proteins coordinate to initiate and complete the process of replication:

- Helicase
- Single strand DNA-binding proteins
- DNA polymerase
- Sliding clamp
- Clamp loader
- Primase
- Nuclease
- Repair polymerase
- DNA ligase

DNA polymerase adds a deoxyribonucleotide to the 3′ end

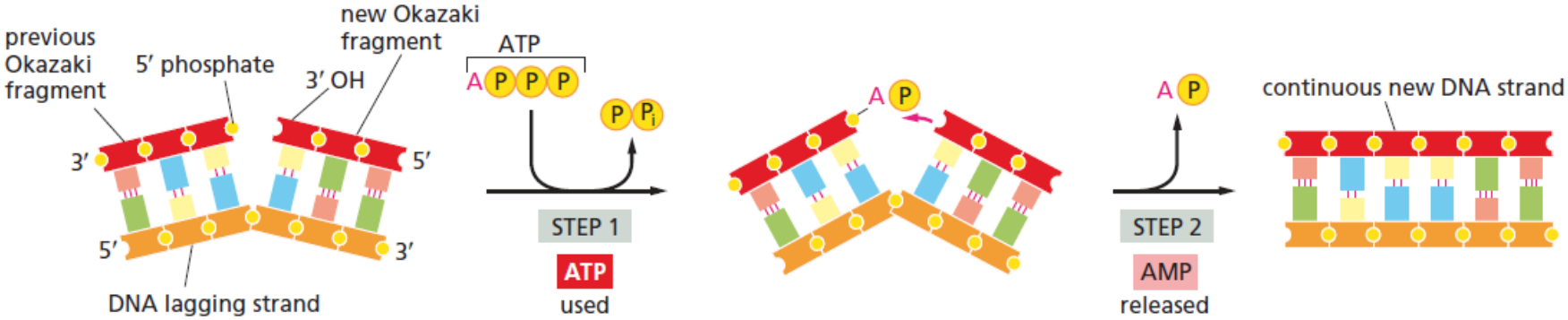


Primase, Nuclease and DNA ligase

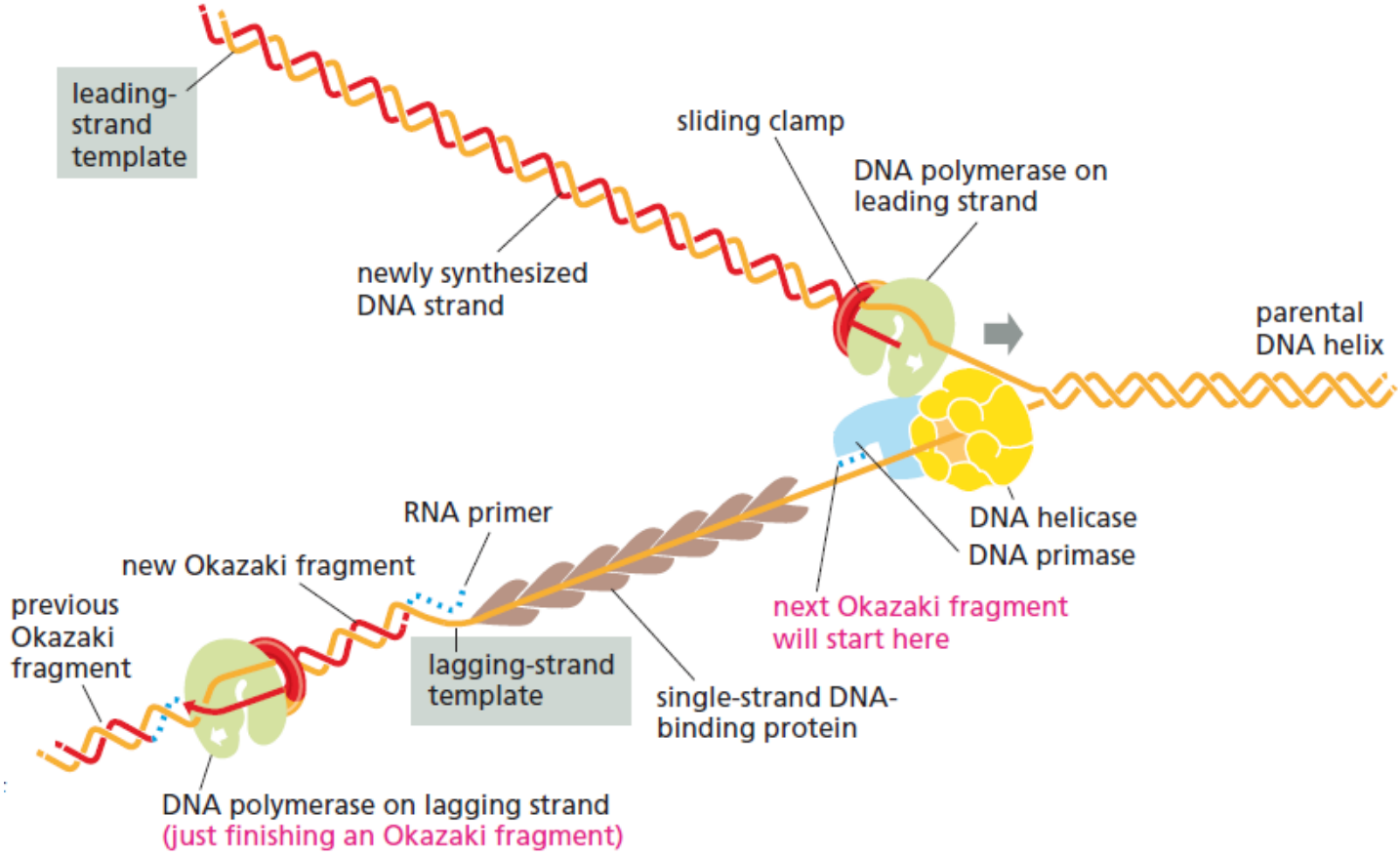


DNA ligase

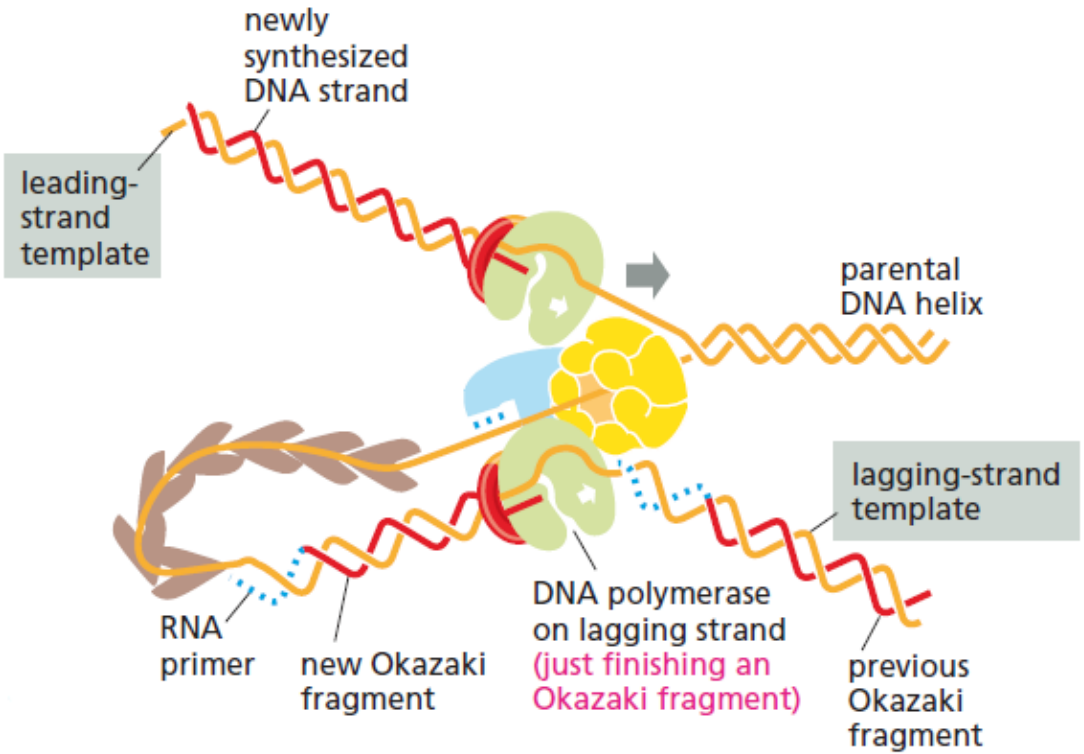
The ligase enzyme uses a molecule of ATP to activate the 5′ end of one fragment (step 1) before forming a new bond with the 3′ end of the other fragment (step 2)



Replication machinery – proteins involved



All proteins work as a big complex



Replication machinery – proteins involved

- DNA helicase sits at the very front of the replication machine where it uses the energy of ATP hydrolysis to propel itself forward, pulling apart the double helix as it speeds along the DNA.
- Single strand DNA-binding proteins cling to the single-stranded DNA exposed by the helicase, transiently preventing the strands from re-forming base pairs and keeping them in an elongated form so that they can serve as efficient templates.
- DNA polymerase catalyzes the addition of nucleotides to the 3’ end of a growing DNA strand, using one of the original, parental DNA strands as a template.
- Sliding clamp keeps DNA polymerase firmly attached to the template while it is synthesizing new strands of DNA. Clamp loader, which hydrolyzes ATP each time it locks a sliding clamp around a newly formed DNA double helix

Replication machinery

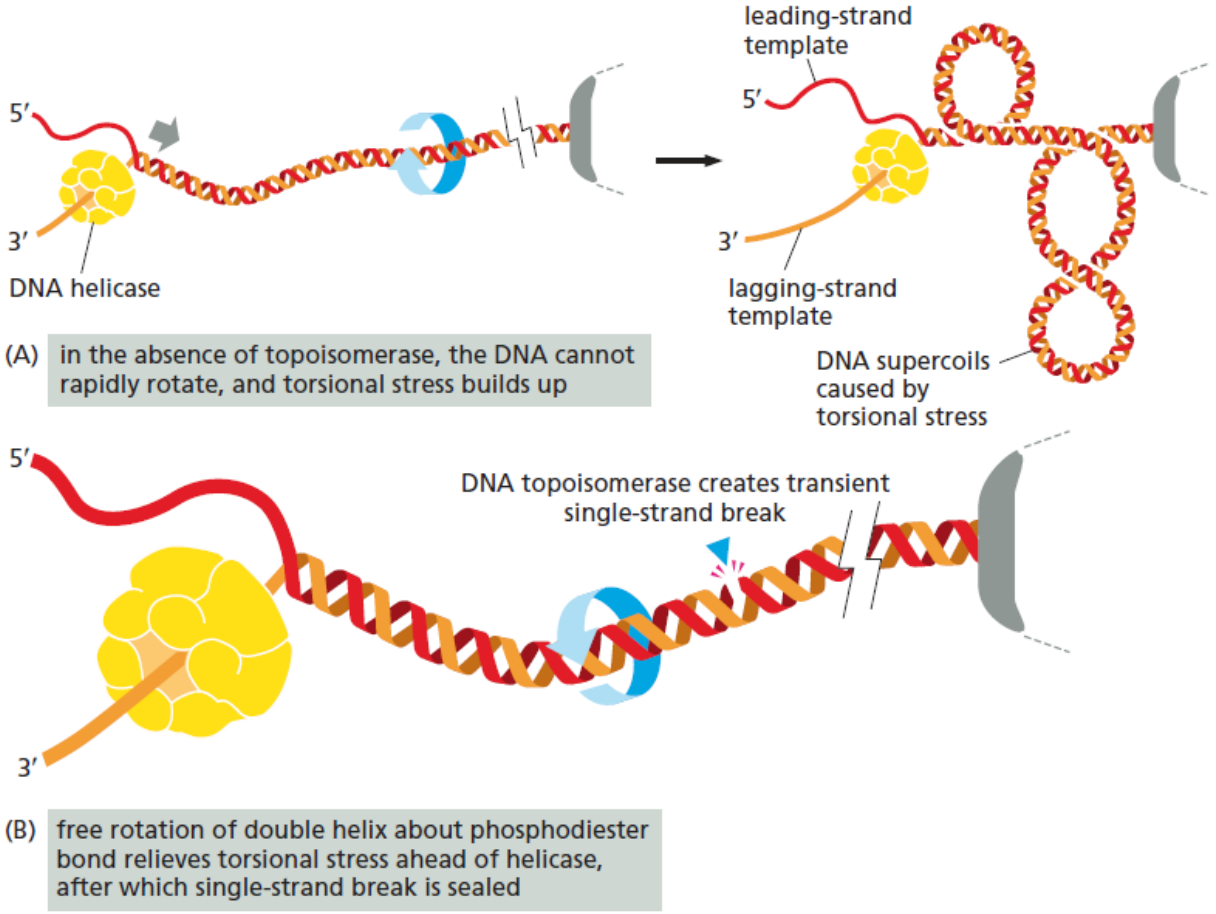
- RNA polymerase called primase helps in synthesizing the primer providing a base-paired 3′ end as a starting point.

To produce a continuous new DNA strand from the many separate pieces of nucleic acid made on the lagging strand, three additional enzymes are needed.

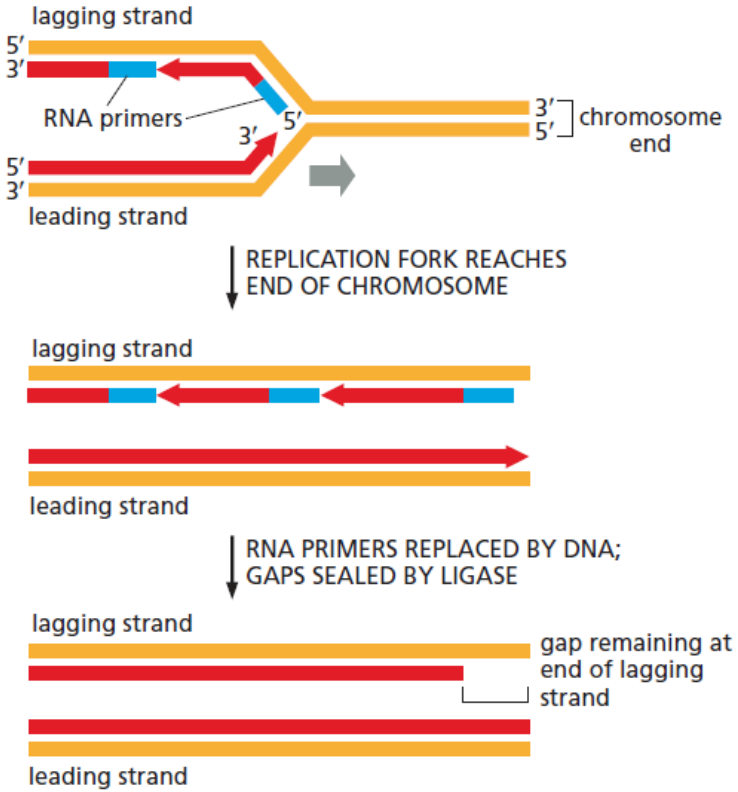
- Nuclease degrades the RNA primer.
- DNA polymerase called a repair polymerase replaces this RNA with DNA (using the end of the adjacent Okazaki fragment as a primer).
- DNA ligase joins the 5′-phosphate end of one DNA fragment to the adjacent 3′-hydroxyl end of the next.

DNA topoisomerase

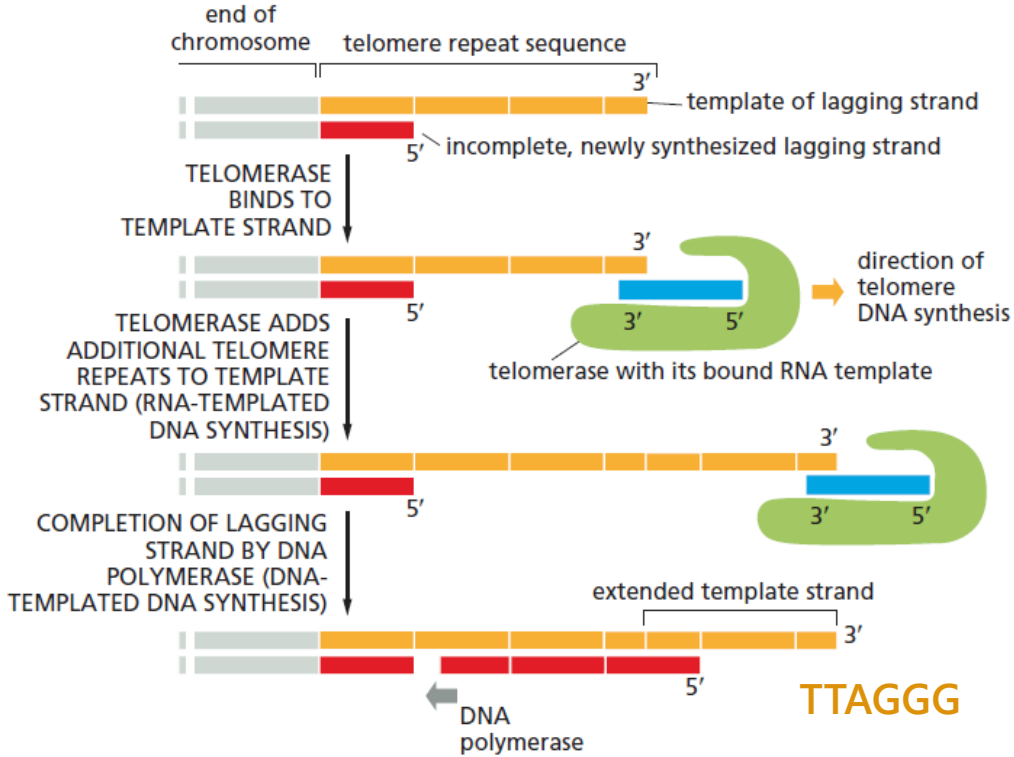
- **DNA topoisomerases** produce transient nicks in the DNA backbone, which temporarily release the torsional tension.
- They then reseal the nick before falling off the DNA.



Telomerase replicates the ends of eukaryotic chromosomes



Telomere and Telomerase



DNA replication should be accurate

Proofreading activity of DNA polymerase