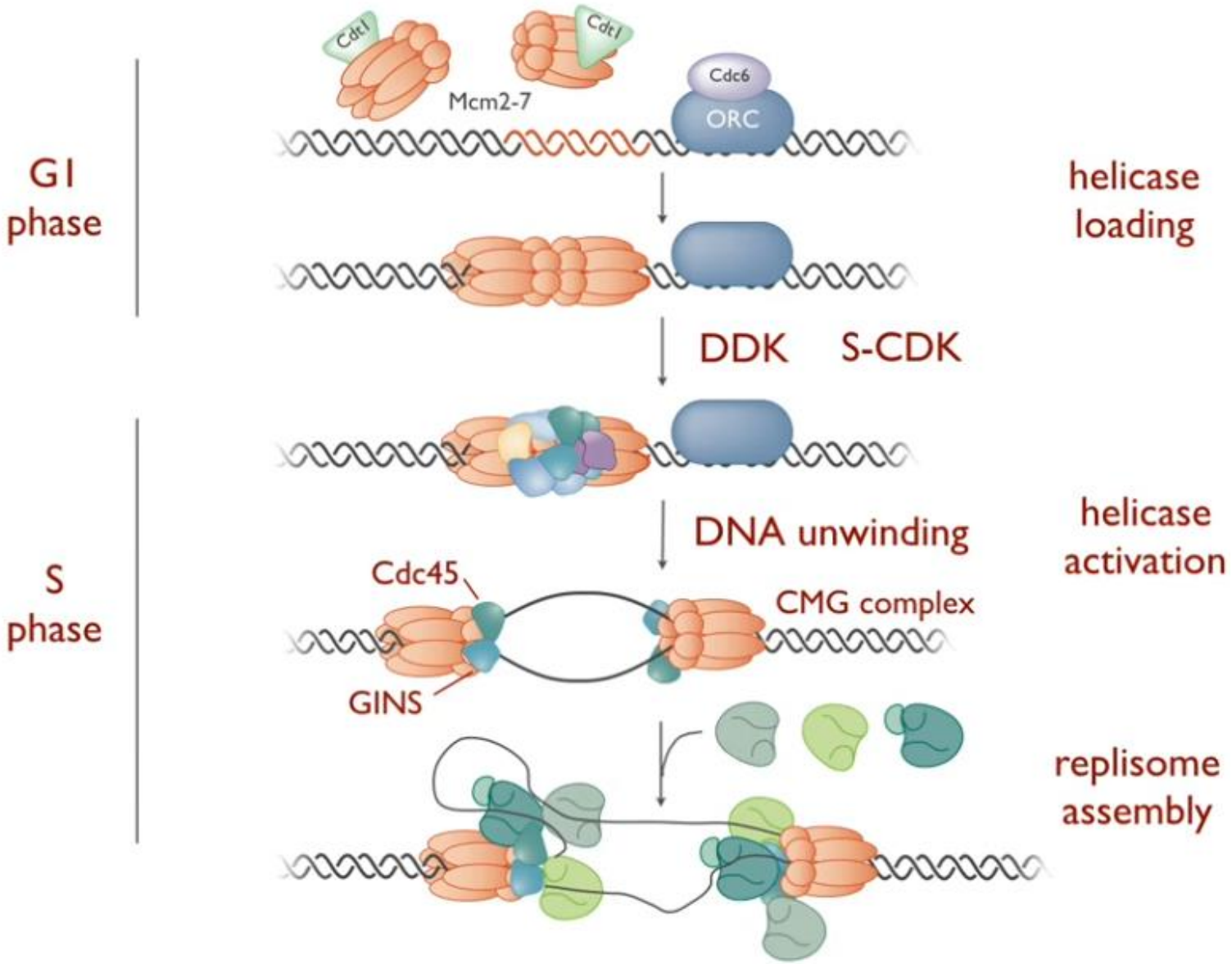
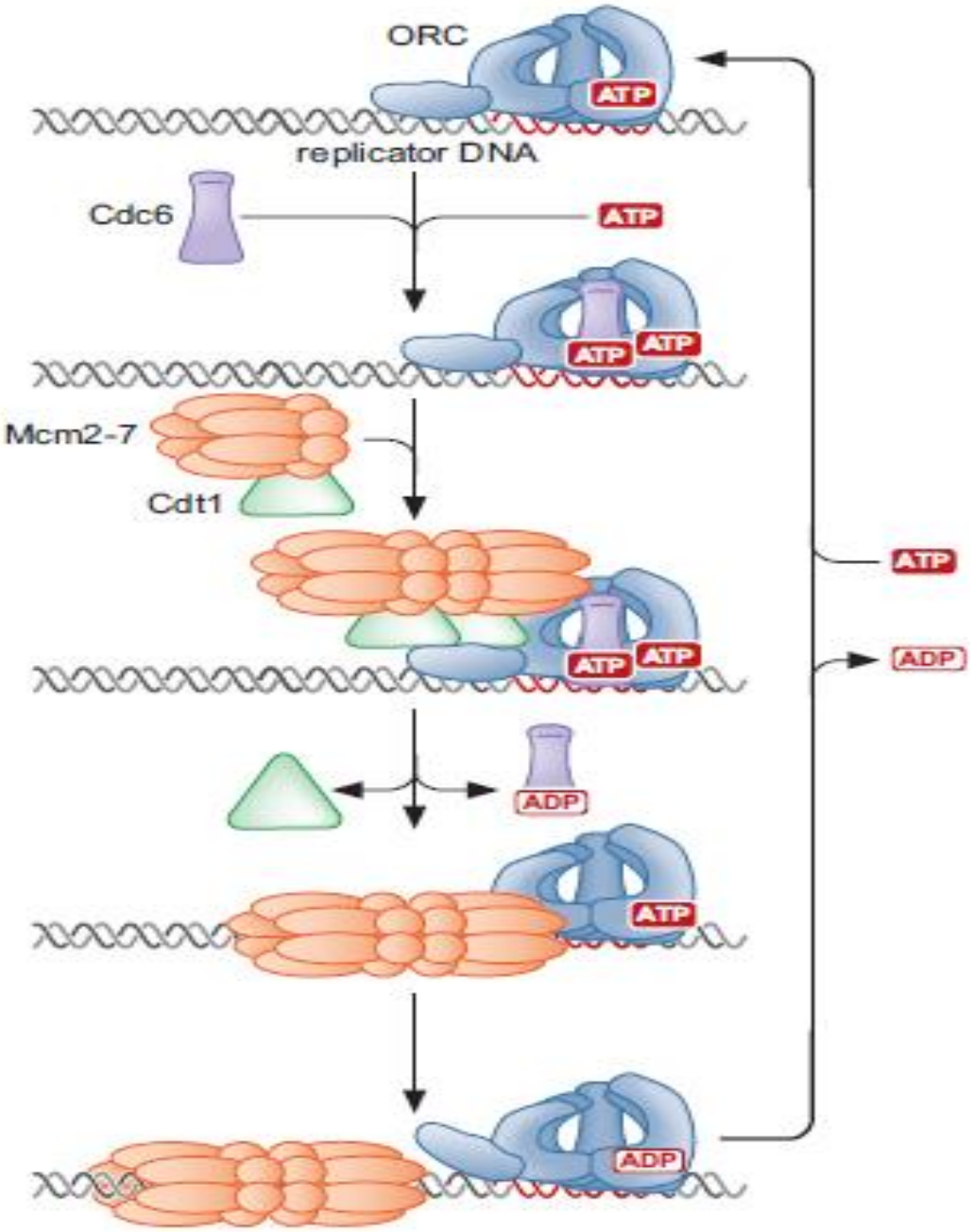
Events of Eukaryotic DNA Replication Initiation




ORC
ATP
replicator DNA
Cdc6
ATP
ATP
ATP
Mcm2-7
Cdt1
ATP
ATP
ATP
ADP
ADP
ATP
ADP

- In G_1 phase of the cell cycle, many of the DNA replication regulatory processes are initiated.

- Initiation of DNA replication in eukaryotes begins with the binding of the **origin recognition complex (ORC)** to origins of replication during the G_1 phase of the cell cycle.
- **Origin recognition complex (ORC)** is a multi-subunit DNA binding complex (6 subunits) that binds in all eukaryotes in an ATP-dependent manner to origins of replication.
- The subunits of this complex are encoded by the ORC1, ORC2, ORC3, ORC4, ORC5 and ORC6 genes.

- The ORC complex then serves as a platform for forming much more complicated **pre-replicative complexes (pre-RCs).**
- The pre-RC formation involves the ordered assembly of many replication factors including:

✓ **the origin recognition complex (ORC) ,**

✓ **Cdc6 protein** (**cell division cycle 6**),

✓ **Cdt1 protein** (**Chromatin licensing and DNA replication factor 1**), and

✓ **minichromosome maintenance proteins (Mcm2-7)** (**heterohexamer of the six MCM proteins (MCM2-7**).

- Pre-RC assembly during G1 is required for **replication licensing** of chromosomes prior to DNA synthesis during S phase.

- ORC, Cdc6, and Cdt1 are all required to load the six protein minichromosome maintenance (Mcm 2-7) complex onto the DNA. (It is thought that the Cdc6p-Cdt1 complex uses ATP hydrolysis to thread DNA through the central hole of the MCM doughnut).

- Pre-RCs formed during the G_1 phase are converted to the **initiation complex** during cell cycle transition from G_1 to S by the action of two kinases: **cyclin-dependent kinase (CDK)** and **Dbf4-dependent kinase (DDK)**. i.e. Once the pre-RC is formed, activation of the complex is triggered by two kinases, cyclin-dependent kinase (CDK) and Dbf4-dependent kinase (DDK) that help transition the pre-RC to the initiation complex prior to the initiation of DNA replication.

- Formation of an initiation complex, which includes helicase activity, unwinds the DNA double helix at the origin site.

Eukaryotic helicase loading

- Loading of the eukaryotic replicative DNA helicase is an ordered process that is initiated by the association of the ATP-bound origin recognition complex (ORC) with the replicator.(The initiation of DNA replication is directed by specific DNA sequences called replicators).
- Once bound to the replicator, ORC recruits **ATP-bound Cdc6** and **two copies of the Mcm2-7 helicase** bound to a second **helicase loading protein, Cdt1**.
- This assembly of proteins triggers ATP hydrolysis by Cdc6, resulting in the loading of a head-to-head dimer of the Mcm2-7 complex encircling double-stranded origin DNA and the release of Cdc6 and Cdt1 from the origin.

- Eukaryotic helicase loading does not lead to the immediate unwinding of origin DNA. Instead, helicases that are loaded during G1 are **only** activated to unwind DNA and initiate replication **after cells pass from the G1 to the S phase** of the cell cycle.
- Loaded helicases are activated by two protein kinases: CDK (cyclin dependent kinase) and DDK (Dbf4-dependent kinase). These kinases are activated when cells enter S phase. Once activated, DDK targets the loaded helicase, and CDK targets two other replication proteins. Phosphorylation of these proteins results in the **Cdc45 and GINS proteins** binding to the Mcm2-7 helicase.

- Importantly, Cdc45 and GINS strongly stimulate the Mcm2-7 ATPase and helicase activities and together form the **Cdc45–Mcm2-7–GINS (CMG) complex**, **which is the active form of the Mcm2-7DNA helicase.**

B-Synthesis of the two DNA strands:

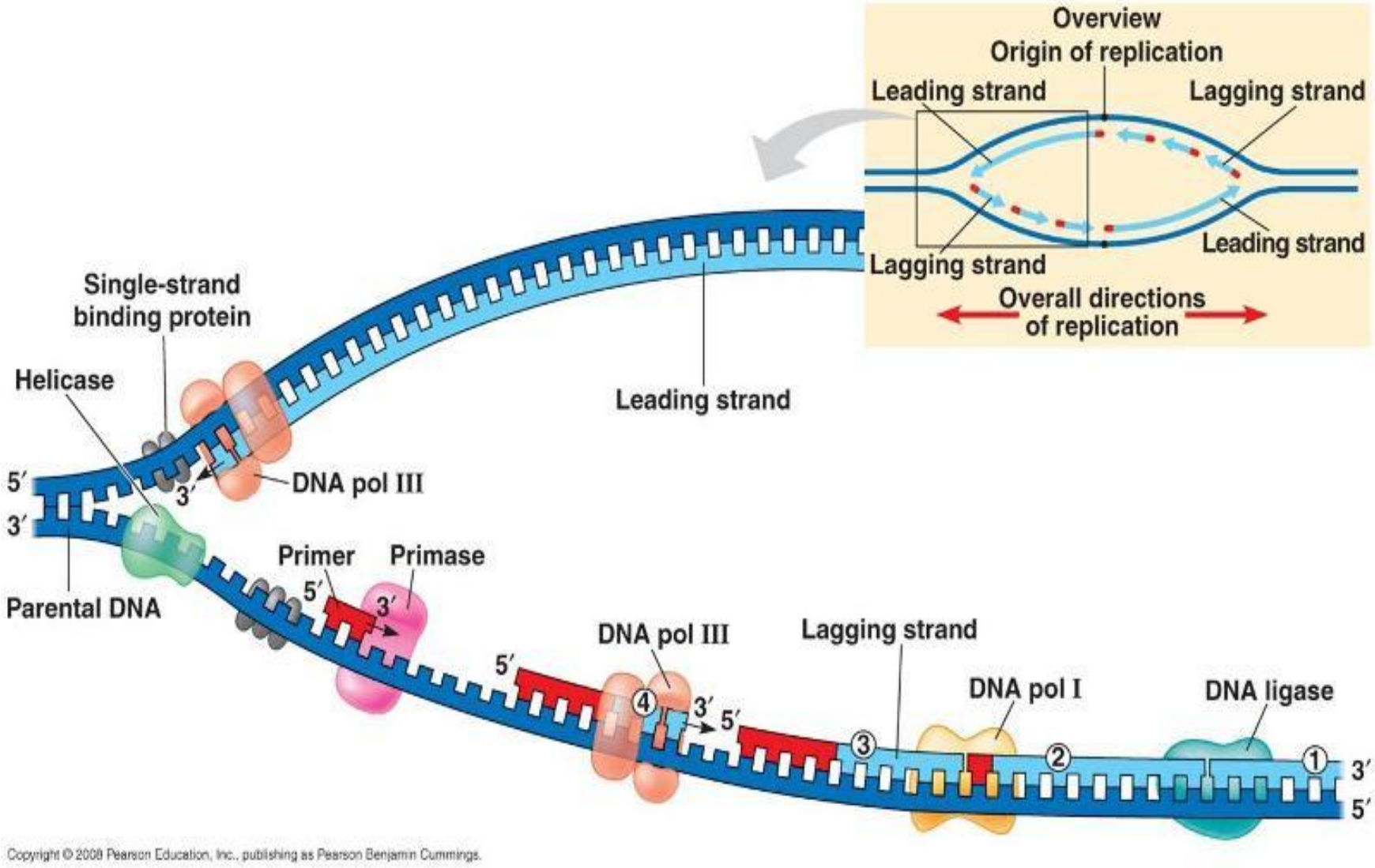
- **DNA polymerase III** enzyme is responsible for the synthesis of both new DNA strands. The enzyme synthesizes the new DNA strands **only in the 5\→3\ direction**, and it cannot start DNA synthesis without the presence of **RNA primers.**

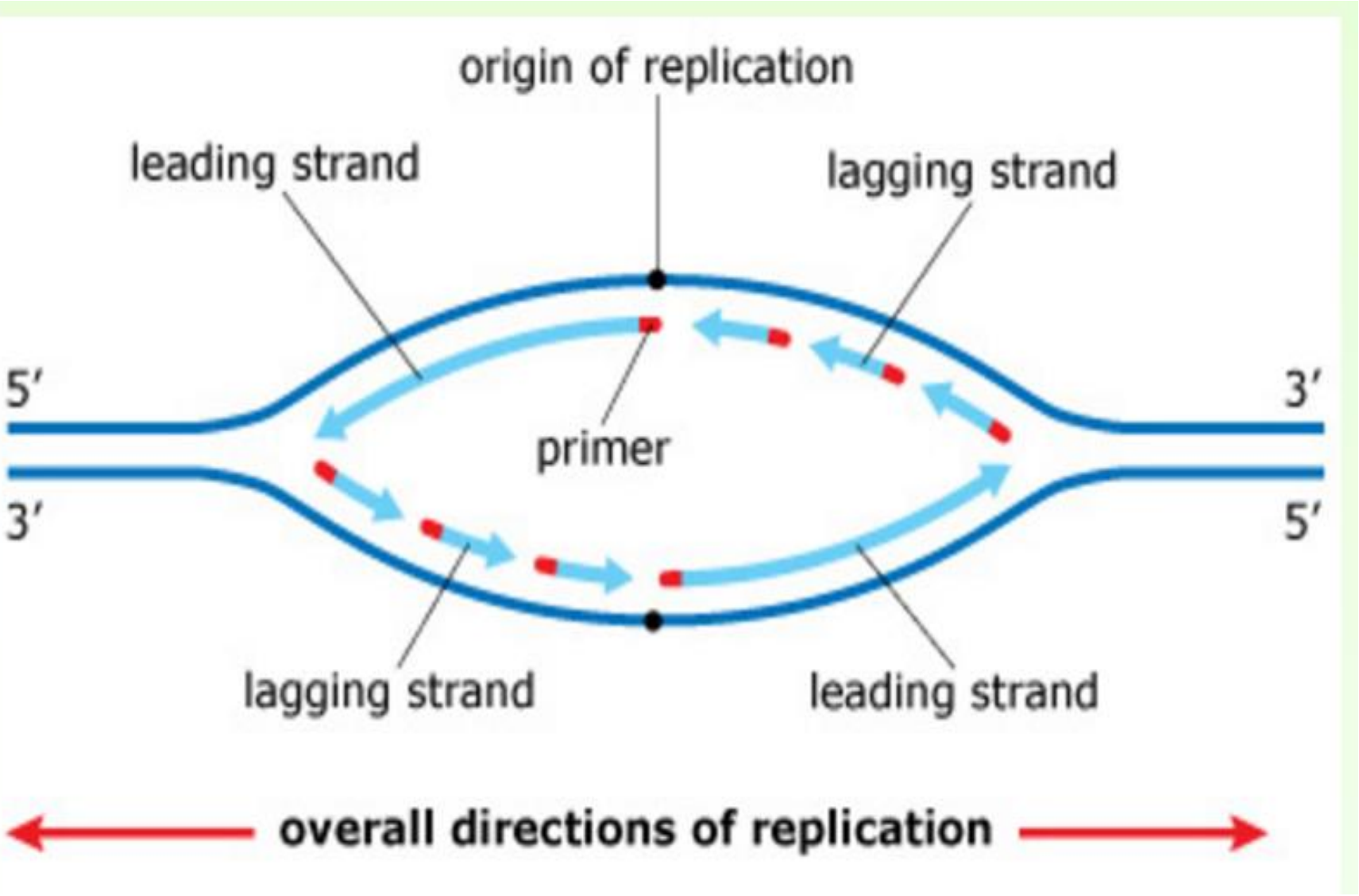
Synthesis of RNA primers:

- Primers are short RNA molecules about 5-10 nucleotides in length and are complementary to a segment of the DNA strand. Primers are synthesized in the direction of 5\→3\ direction by **primase** (**RNA polymerase**) enzyme using ribonucleotide triphosphate (ATP, GTP, CTP, **UTP**).

Synthesis of the DNA strand:

- **DNA polymerase III** synthesizes DNA in the 5\→3\ direction by using deoxyribonucleotide triophosphate (d ATP, d GTP ,d CTP & d ATP) to form the new strands in a **complementary sequence to that of the parent DNA according to base paring rule**. The first added deoxyribonucleotide triphosphate will form **phosphate diester bond with the OH at the 3\ end of the RNA primer with removal of pyrophosphate** .The next added deoxyribonucleotide triphosphate form phosphodiester bond with the previously added one with hydrolysis of pyrophosphate **to provide the energy** for the reaction.




origin of replication
leading strand
lagging strand
5′
3′
primer
3′
5′
lagging strand
leading strand
overall directions of replication

DNA synthesis in two different directions:

Leading strand

Lagging strand

- **Leading strand:** The strand that is being copied **in the direction of the advancing replication fork** is called the leading strand and is synthesized continuously. It is **complementary to the parent strand that has the direction of 3\→5**

- **Lagging strand:** The strand that is being copied **in the direction away from the replication fork** is synthesized discontinuously, with small fragments of DNA being copied near the replication fork. It is **complementary to the parent strand that has the direction of 5\→3** These short stretches of discontinuous DNA, termed **Okazaki fragments**, are eventually joined (ligated) to become a single, continuous strand. The new strand of DNA produced by this mechanism is termed the lagging strand**.(Many RNA primers are needed for synthesis of the lagging strand)**

Excision of RNA primers and their replacement by DNA

- DNA polymerase III continues to synthesize DNA on the lagging strand until it is becomes very close to the next RNA primer. When this occurs, **DNA polymerase I** excise the RNA primer (has a 5` exonuclease activity)and the gap filled by DNA nucleotides. **DNA ligase** enzyme connects the DNA fragments.

Proofreading of newly synthesized DNA:

- To ensure replication fidelity, DNA polymerase III has, in addition to its 5\→3\ polymerase activity, a "**proofreading**" activity (**3`→5 ` exonuclease**). As each nucleotide is added to the chain, DNA polymerase III checks to make certain the added nucleotide is, in fact, correctly matched to its complementary base on the template. If it is not, the 3\→5\ exonuclease activity corrects the mistake.