

TOPIC 7.1: DNA REPLICATION

Replication Enzymes

Helicase:

- Helicase separates the DNA strands to form a replication fork (breaks the hydrogen bonds between complementary base pairs)
- Single stranded binding proteins prevent strands re-annealing

DNA Gyrase:

- DNA gyrase reduces the torsional strain created by helicase
- It prevents the DNA from supercoiling as it is being unwound

DNA Primase:

- DNA primase generates a short RNA primer on each strand
- Primers provide an initiation point for DNA polymerase III (DNA pol III can only add nucleotides to 3'-end of a primer)

DNA Polymerase III:

- Free nucleotides (dNTPs) line up opposite complementary bases
- DNA polymerase III covalently joins free nucleotides together

Okazaki Fragments:

- DNA strands are *antiparallel*, so replication occurs bidirectionally (replication always occurs in a 5' → 3' direction on each strand)
- Synthesis is continuous on the leading strand (*towards fork*) and is discontinuous on the lagging strand (*away from fork*)
- Discontinuous segments are called Okazaki fragments

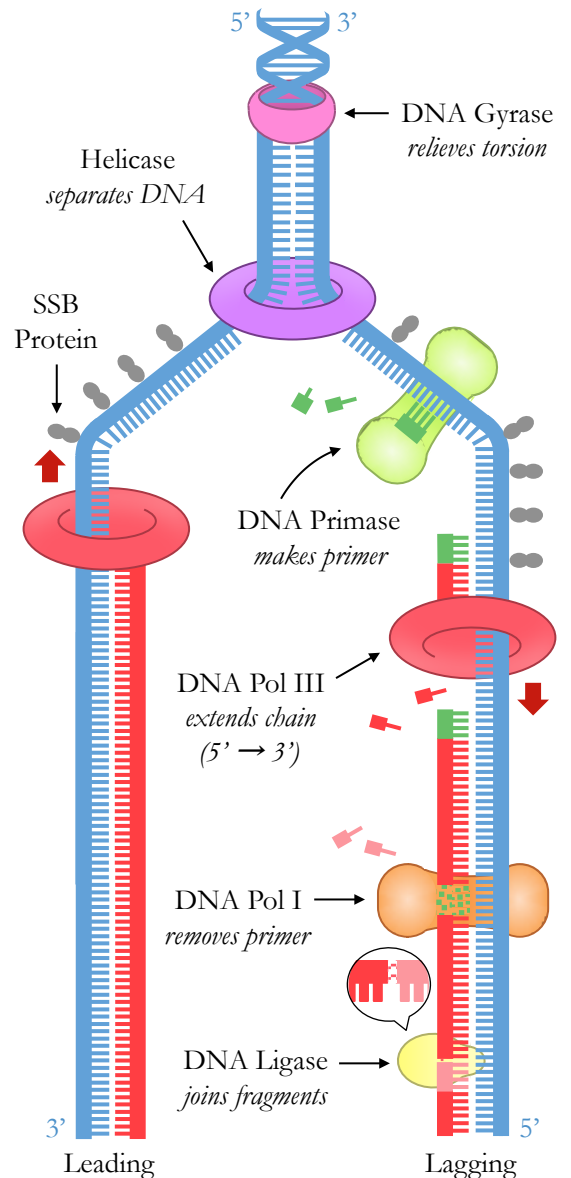
DNA Polymerase I:

- DNA pol I removes RNA primers and replaces them with DNA

DNA Ligase:

- DNA ligase covalently joins the Okazaki fragments together

Replication Process



DNA Sequencing

Sequencing is a technique by which the nucleotide base order of a DNA sequence is elucidated (typically via Sanger method)

- Dideoxynucleotides (ddNTPs) lack the 3'-hydroxyl group needed to form covalent bonds (they terminate replication)
- Four PCR mixtures are prepared – each with stocks of normal bases **and one** dideoxynucleotide (ddA, ddT, ddG, ddC)
- Whenever the dideoxynucleotide is randomly incorporated, the DNA sequence is terminated at that base position
- Because a complete PCR cycle generates millions of sequences, every base position is likely to have been terminated
- These sequences are separated by gel electrophoresis to determine base sequence (according to ascending sequence length)
- Automated machines can determine the sequence quickly if fluorescent labeling of the dideoxynucleotides has occurred

