Enzymes Involved in DNA Replication

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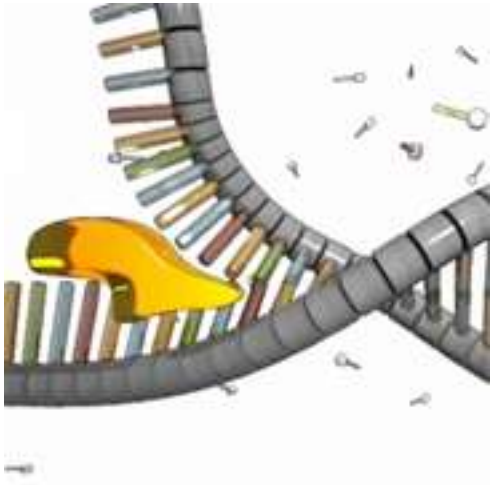
Enzymes

- Helicase
- Topoisomerase

  -> SSB (binding protein)
- RNA Polymerase
- DNA Polymerase
- RNAse H
- DNA Ligase

Helicase

- Also k/a Helix destabilizing enzyme or Unwindase.



- Unwind the DNA helix using hydro-lysis of ATP.

- They are motor proteins that move directionally along the nucleic acid phosphodiester backbone.

Topoisomerase

- Relieve the winding strain generated during unwinding of the DNA by introducing a nick in DNA.
- Topoisomerase I - cut the ssDNA.
- Topoisomerase II – cut the dsDNA.

Single Strand Binding Protein

- Binding protein that binds to ssDNA and prevent the double helix from re-annealing after DNA helicase unwinds it.

- Maintains the strand separation and facilitate the synthesis of nascent DNA.

DNA Primase

- Attaches Primer and provide a starting point to DNA for DNA Polymerase to begin synthesis of new DNA strand.

- It is a type of RNA Polymerase.

DNA Polymerase

- DNA Polymerase III:

  - added nucleotide in 5’ to 3’ direction.

  - consist of three subunit: α, Ɛ, θ

    α- polymerase activity

    Ɛ- exonucleolytic proofreader

    θ- stabilizer for Ɛ.

DNA Polymerase

- DNA Polymerase II:
  - 3’ to 5’ exonuclease activity and DNA repair.
  - encoded by *polB gene.*

- DNA Polymerase I:
  - both 3’ to 5’ & 5’ to 3’ exonuclease activity.
  - processing of okazaki fragments.
  - encoded by *polA gene.*

Ribonuclease H

- RNAse H- endonuclease.

- Removes RNA primer as it possess the capability to cleave the RNA by hydrolytic mechanism; allowing completion of newly synthesized DNA.

*DNA Polymerase-I can also remove the RNA Primer.

Ligase

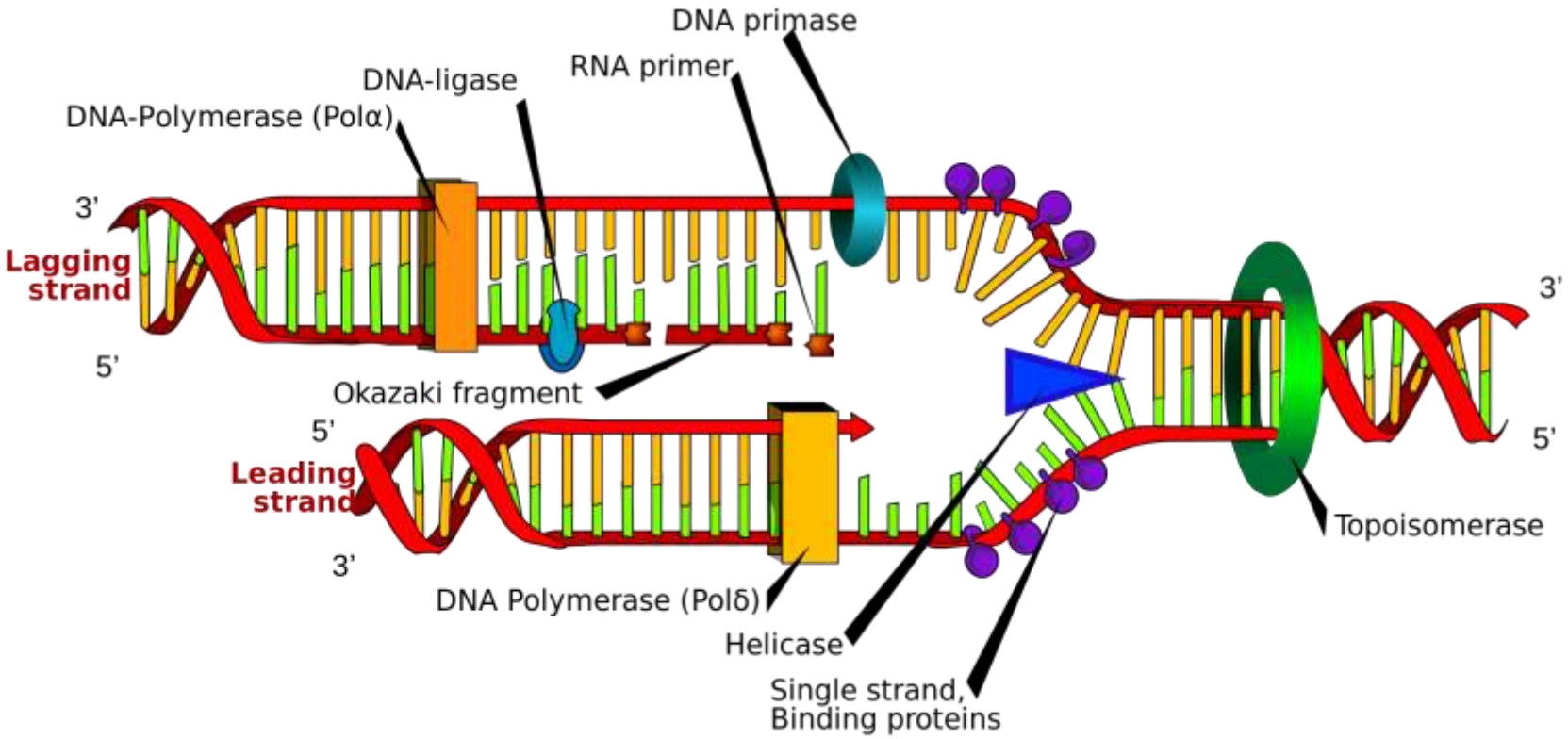
- Joints okazaki fragment of lagging strand by catalyzing the formation of Phosphodiester bond.
- Mechanism:

- Adenylation (Addition of AMP).

-Transfer of AMP to 5’ phosphate of donor.

-Formation of Phosphodiester bond between 5’ phosphate of donor and 3’ hydroxyl of acceptor.

Enzymes


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'

Comparison

- **Prokaryotes-**
  - DNA polymerase – I
  - DNA Polymerase - II
  - DNA Polymerase – III
  - DNA Gyrase
- **Eukaryotes-**
  - DNA Polymerase β
  - DNA Polymerase δ
  - DNA Polymerase α, Ɛ
  - Topoisomerase

Thank you!