Meselson & Stahl experiment in 1958 shows the semi-conservative nature of DNA replication:

Using N15 isotope, shows daughter DNA each contain one parent strand and one daughter strand

Replication Origin

- One in prokaryotes - OriC
- Many in eukaryotes - Ori
- A replication bubble forms at the origin, with two replication forks, allow bidirectional replication
- Daughter strand elongate in 5' to 3' direction, antiparallel structure leads to leading and lagging strand

Initiator proteins

- Prokaryotes: DnaA, bind to recognition element next to AT-rich sequence, separate AT rich strands
- Eukaryotes: Origin Recognition Complex (ORC), bind to recognition element.
- Recruit Helicase

Helicase

- Hexameric Ring shape protein
- Unwind and separate two DNA strands using ATP hydrolysis
- Bind to sugar-phosphate backbone

SSB (Prokaryotic single strand binding protein) & RPA (Eukaryotic Replication Protein A)

- Bind to single strand DNA to stabilise it and prevent reannealing
- Interact with other proteins

Topoisomerase

- Prokaryotic Gyrase
- Eukaryotic Topoisomerase I and II, induce single or double stranded break to alleviate torsion stress
- Bind to dsDNA in front of the replication fork

Ligase: seals Okazaki fragments

Primase

- Prokaryotic primase
- Eukaryotic DNA polymerase alpha primase
- Is a RNA polymerase, part of primosome complex, add a 10 bp primer to the origin of replication in 5' to 3' manner.
- Primer later degraded by ribonucleas/polymerase

DNA polymerase

- E.Coli have 5, Eukaryotes have >15.
- Prokaryotic: Pol I(RNA primer removal, DNA repair), Pol III (DNA replication, form holoenzyme)
- Eukaryotic: Pol α (primase synthesis), Pol δ (lagging strand synthesis, MMR), Pol ε(leading strand, MMR)

structure:

- Palm: conserved β-pleated sheet, catalytic site for DNA replication, proofreading
- Fingers: α-helices, interact with incoming dNTP (deoxyribose nucleotide triphosphates), guide them to the palm
- Thumb: α-helix, stabilise replicated dsDNA, maintains template/polymerase position

DNA polymerase action:

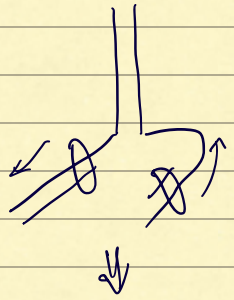
- ssDNA fit through fingers
- Fingers guide dNTP to the next position on ssDNA, close around
- Palm catalyse formation of phosphodiester bond between dNTP and primer
- After formation of bond, finger open, allow dsDNA to move 1 position forward
- Thumb interact dsDNA and stabilise structure

Clamp & Clamp loader

- Prokaryotic: β-sliding clamp + tau/gamma τ/γ loader
- Eukaryotic: Proliferating cell nuclear antigen (PCNA) + Replication factor C (RFC)
- Clamp protein is a conserved ring structure, bind to DNA ensure binding of polymerase to DNA
- Clamp loader is a 5-subunit ring structure, load & unload clamp onto DNA, ATP hydrolysis allow loading + detatch

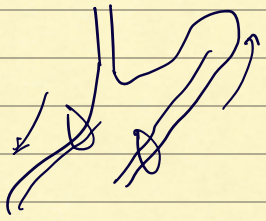
Okazaki fragment and ligase

- DNA polymerase activity stops before next primer in lagging strand
- Another DNA polymerase cleaves rNTP with exonuclease action, replace with dNTP
- Ligase bind and catalyse phosphodiester bond formation



Holoenzyme and trombone model:

- Holoenzyme is a clamp loader + 3 clamp + 3 polymerases
- Allow coupling between leading and lagging strand replication activity.
- In trombone model, leading strand continue to be synthesised in 3' - 5' fashion
- Lagging strand synthesised by the holoenzyme as well, but loop resets onece in a while.

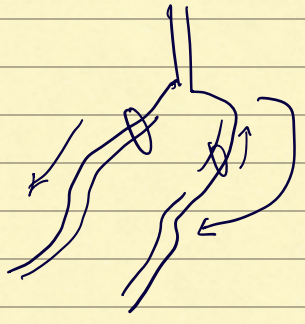


The collective is called the replisome:

Helicase, primase, ssb, topoisomerase, polymerase, clamp loader, clamp, ligase

Replication mechanism

- Initiator bind to origin of replication, initiator complex cause initial unwinding
- Binding of ssb to ssDNA
- Initiator recruit helicase, hydrolyse ATP + separate strands
- Helicase recruit primase
- Primase add 10bp primer, recruit loader
- Loading clamp, recruit holoenzyme and its polymerase
- Holoenzyme move along DNA
- Topoisomerase relieve torsion
- Ligase seal okazaki fragment



Experiment showing nature of DNA replication

9 proteins involved in replication, pro vs eu

Initiator

Helicase

Ssb

Primase

Clamp loader

Clamp

Topoisomerase

Polymerase (structure, action)

Ligase

Replication timeline, mechanism and function of each protein

Trombone model