DNA polymerase III

Enzyme used during replication
Multisubunit protein
High processivity

DNA polymerase III = DNA Pol III

- Discovered in extracts of *polA*$^{-}$ cells, i.e. lacking DNA Pol I
- DNA Pol III is the replicative polymerase
- Loss-of-function mutations in the genes encoding its subunits block DNA replication (*dna* mutants)
- Highly processive
- Multiple subunits
- Also discovered DNA Pol II in *polA*$^{-}$ extracts (role in DNA repair)

DNA Pol III: Low abundance but high processivity

Comparison	Pol I	Pol III core	Pol III holo
molecules per cell	400	40	10
nts polymerized min^{-1} (molecule enz)$^{-1}$	600	9000	42,000
processivity [nts polymerized per initiation]	3-188	10	$>10^5$
5' to 3' polymerase	+	+	+
3' to 5' exo, proofreading	+	+	+
5' to 3' exo	+	-	-

Processivity

- Amount of polymerization catalyzed by an enzyme each time it binds to a template.
- Measured in nucleotides polymerized per initiation
- High processivity of DNA Pol III results from activities of non-polymerase subunits

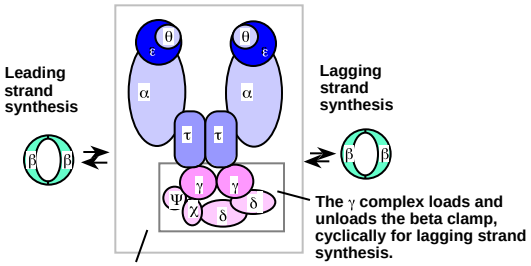
Subunits of DNA Pol III and functions

Functional component	Subunit	Mass (kDa)	Gene	Activity
Core polymerase	α	129.9	*polC=dnaE*	5' to 3' polymerase
	ε	27.5	*dnaQ=mutD*	3'-5' exonuclease
	θ	8.6		Stimulates ε exonuclease
Linker protein	τ	71.1	*dnaX*	Dimerizes cores
Clamp loader (aka γ complex) (ATPase)	γ	47.5	*dnaX*	Binds ATP
	δ	38.7		Binds to β
	δ'	36.9		Binds to γ and β
	χ	16.6		Binds to SSB
	ψ	15.2		Binds to χ and γ
Sliding clamp	β	40.6	*dnaN*	Processivity factor

Synergism among subunits

- Activities of polymerase (α) and 3' to 5' exonuclease (ε) are higher in the core (αεθ) than in individual subunits.
- The γ complex has ca. 6 subunits that work together to load and unload the clamp (β2) for processive synthesis

Subassemblies have distinct functions



Pol III* subassembly lacks the beta sliding clamp.

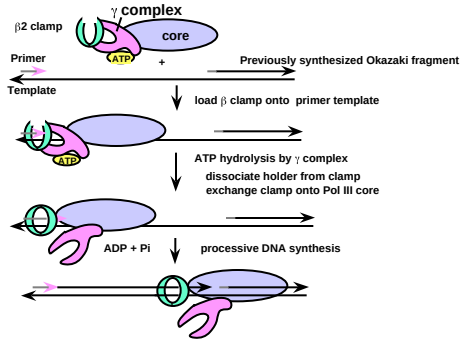
Processivity factor beta2: Sliding clamp

- The β subunit forms a homodimer.
- The structure of this homodimer is a ring.
- The ring encloses DNA, thereby clamping the DNA Pol III holoenzyme to the template.
- An enzyme that is clamped on cannot come off easily, and thus will be highly processive.

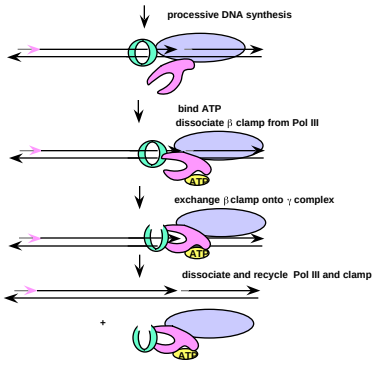
Gamma complex: Clamp loader/unloader

- The γ complex ($\gamma_2\delta\delta'\chi\psi$) loads the β dimer clamp onto a primer-template.
  - Bind the clamp (β dimer) onto the loader (γ complex): need ATP
  - Exchange the clamp from the loader to the core: need ATP hydrolysis
  - Unload the clamp when polymerase reaches a previously synthesized Okazaki fragment: need ATP
    - The ATP-bound form of the γ complex can bind the clamp
    - The ADP-bound form releases the clamp

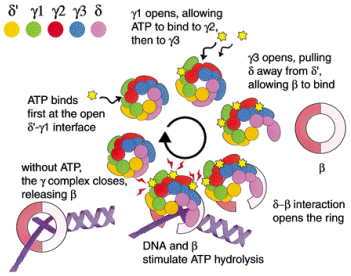
Loading the beta2 clamp



Unloading the beta2 clamp



Model for gamma complex loading beta clamp

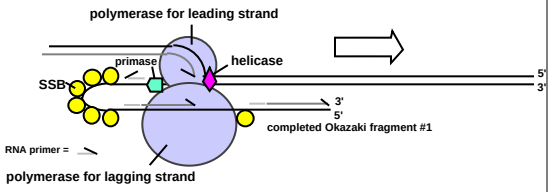


Jeruzalmi, O'Donnell and Kuriyan (2001) Cell 106: 429-441

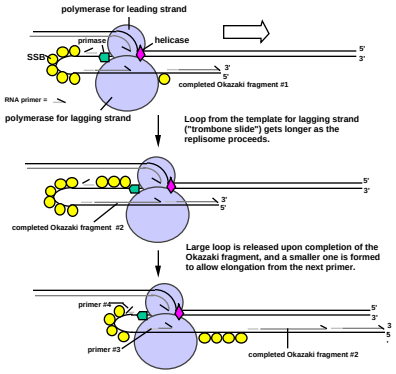
Asymmetric dimer of DNA PolIII: simultaneous replication of both strands of DNA

- The 2 catalytic cores of DNA Pol III are joined by the tau subunits to make an asymmetric dimer.
- Model: one holoenzyme synthesizes both strands at a replication fork.
  - One core synthesizes the leading strand
  - Other synthesizes the lagging strand.
  - If the template for lagging strand synthesis is looped around the enzyme, then both strands are synthesized in the direction of fork movement.

One holoenzyme, 2 templates



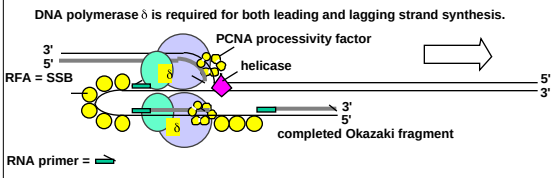
Simultaneous replication of both strands of DNA



Eukaryotic replicative DNA polymerases

- Nuclear DNA replication:
  - ♦ α: primase plus low processivity polymerase
  - ♦ δ: both leading and lagging strand synthesis
  - ♦ ε: may be used in lagging strand synthesis

Eukaryotic DNA polymerases in replication



PCNA is homologous to beta2 clamp

Mammalian PCNA is a trimer, each monomer of which has two similar domains.

The beta subunit of *E. coli* DNA Pol III is a dimer, each monomer of which has three similar domains.

The domains in each are very similar, and the net result is a ring of 6 domains. The two proteins are structurally homologous and have similar functions.

Similarities between bacterial and eukaryotic replication machinery

Function	*E. coli* Pol III	Eukaryotic
Leading and lagging strand synthesis	asymmetric dimer	polymerase δ
Sliding clamp	β subunit	PCNA
Clamp loader	γ-complex	RFC
Primase	DnaG	polymerase α
Single strand binding	SSB	RFA
Swivel (Maintain DNA topology)	Gyrase (Topo II)	Topo I or II

Additional eukaryotic DNA polymerases

- Nuclear repair: DNA polymerases β and ε
- Mitochondrial DNA replication: γ
- Plus:
  - Reverse transcriptase
  - Terminal deoxynucleotidyl transferase
  - Telomerase

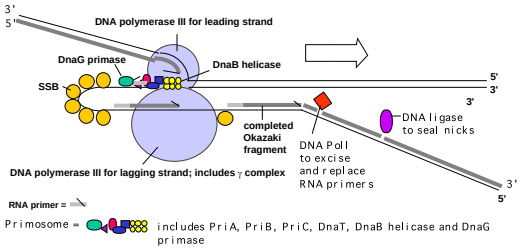
Enzymes other than polymerases needed for replication

Helicases

Ligases

Primosome

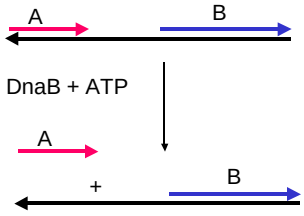
Model for replication fork movement in *E. coli*



DNA helicases

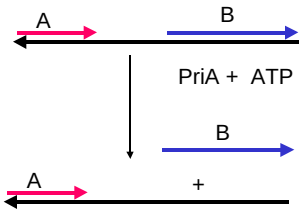
- Unwind the DNA duplex as the replication fork moves.
- Use ATP: Hydrolyze 2 ATPs to 2ADP+2Pi for every base pair that is unwound.
- In addition, helicases move along single stranded DNA with a specific polarity; referred to as *tracking*.

Assay for helicase movement, #1



Displacement of A shows that DnaB moved 5' to 3' *along the single-stranded DNA*.

Assay for helicase movement, #2



Displacement of B shows that PriA moved 3' to 5' *along the single-stranded DNA*.

Single-stranded binding protein (SSB)

- Encoded by the *ssb* gene in *E. coli*.
- Loss-of-function mutants in *ssb* have a quick-stop phenotype for DNA synthesis. They are also defective in repair and recombination.
- Binds cooperatively to single-stranded DNA to prevent reannealing to the complementary strand.
- SSB is a homo-tetramer, monomer is 74 kDa
- Eukaryotic RFA (analog to SSB) is a heterotrimer.

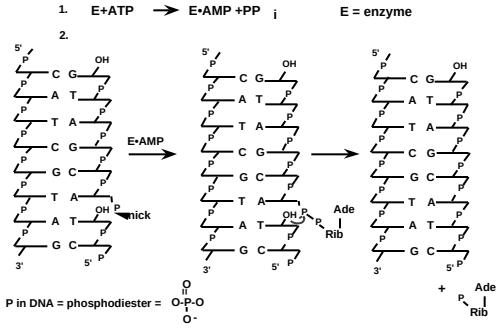
Topoisomerases

- Topoisomerase I: relaxes DNA
  - Transient break in one strand of duplex DNA
  - *E. coli*: nicking-closing enzyme
  - Calf thymus Topo I
- Topoisomerase II: introduces negative superhelical turns
  - Breaks both strands of the DNA and passes another part of the duplex DNA through the break; then reseals the break.
  - Uses energy of ATP hydrolysis
  - *E. coli*: gyrase

DNA ligases

- Join together the Okazaki fragments during lagging strand synthesis
- Tie together a nick

Mechanism of DNA ligase



2 step mechanism for ligase

- First: Enzyme is modified by addition of AMP (from ATP or NADH).
- Second: Adenylylated enzyme transfers AMP to the 5' phosphate at the nick.
  - The 5' phosphate is activated by addition of the AMP
  - The 3' OH is a nucleophile and attacks the adenylylated 5' end of the chain
  - Forms a new phosphodiester bond and sealing the nick.

Primase

- Synthesizes short oligo*ribo*nucleotides from which DNA polymerases can begin synthesis.
- Does not itself require a primer.
- *E. coli* primase is DnaG, 60 kDa
- Acts within a large primosome.

Primers made by DnaG

- Primers can be as short as 6 nt, as long as 60 nt.
- Can substitute dNTPs for rNTPs in all except 1st and 2nd positions
  - Make hybrid primers with dNMPs and rNMPs interspersed.
- Primase binds to CTG
  - T serves as template for 1st nucleotide of primer.

Assembly and migration of the primosome

Primosome has many proteins

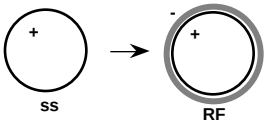
Pre-priming complex:

Protein	gene	function
PriA	*priA*	helicase, 3' to 5' movement, site recognition
PriB	*priB*	
PriC	*priC*	
DnaT	*dnaT*	needed to add DnaB-DnaC complex to preprimosome
DnaC	*dnaC*	forms complex with DnaB
DnaB	*dnaB*	helicase, 5' to 3' movement, is a hexamer DNA dependent ATPase.

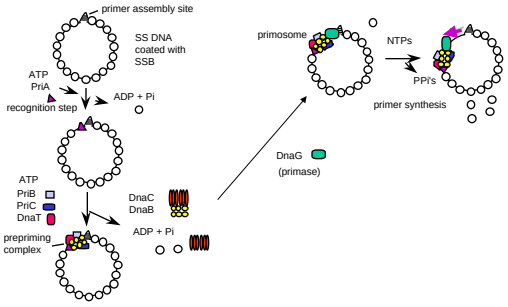
Primase = DnaG

Assay for assembly and migration of the primosome

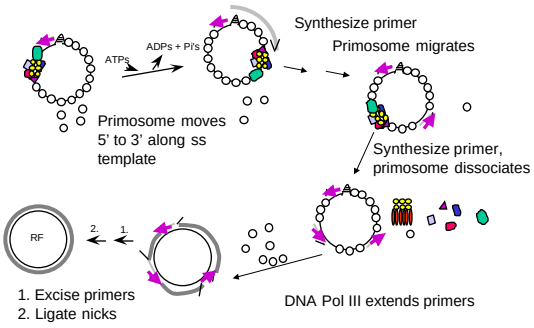
Convert single stranded (ss) φX174 to duplex, replicative form (RF)



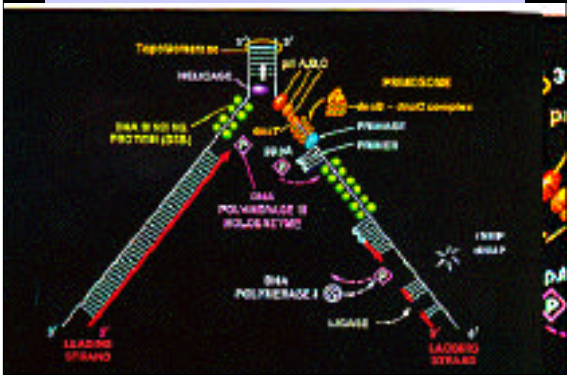
Steps in priming and synthesis



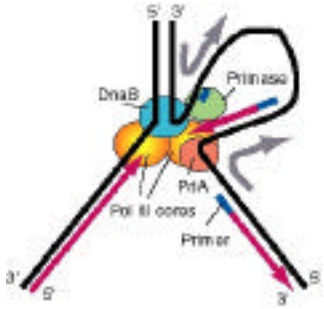
Primosome movement



Replication fork, Kornberg diagram



Activities of DnaB and PriA in replisome



"Sewing machine" model

Types of enzymes at the replication fork

- Helicases to unwind DNA (Rep, helicase II, helicase III, DnaB, PriA)
- SSB to stabilize the unwound DNA
- Topoisomerases to relieve tension and provide a swivel
- Leading strand: DNA polymerase III holoenzyme (1 of the cores + beta2 clamp)

Types of enzymes at the replication fork, #2

- Lagging strand:
- Primosome
  - PriA, PriB, PriC, DnaT, DnaB, DnaG (primase)
  - DnaC complexed with DnaB when not on DNA
- "Half" DNA polymerase III holoenzyme
  - core, gamma complex, beta2 clamp
- DNA polymerase I
  - remove primers and fill in with DNA
- DNA ligase to seal the remaining nicks

Rate of fork movement

- *E. coli*: 50,000 bp per min
- Plants and animals: 1000 to 3000 bp per min
- Need many origins for replication of large genomes