

Outline: Chapter 25.1- DNA replication

1. Properties of DNA replication
 - Semiconservative, simultaneous, bidirectional
 - 5'-3' synthesis: semidiscontinuous
2. Enzymes of DNA replication
 - DNA polymerases: bacterial example
 - Structure and mechanism
 - What is the source of fidelity?
 - What other enzymes participate?
3. Regulation of DNA replication
 - Initiation, elongation and termination

Properties of DNA replication

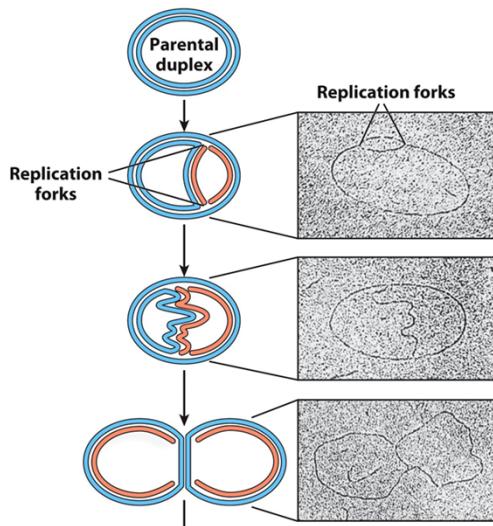
DNA replication is **semi-conservative**:

- Each strand serves as the template for synthesis of a new strand
- Meselson-Stahl experiment (1957): growth in $^{15}\text{N}/^{14}\text{N}$, only hybrid DNA observed after one generation

Both DNA strands are replicated simultaneously

Growth of *E. coli* with ^{3}H -thymidine

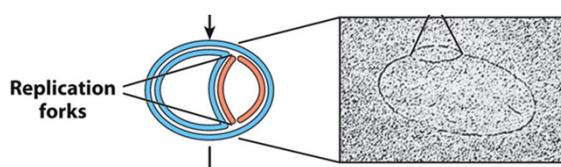
Visualize " Θ structures" on film



Cairns, J. Cold Spring Harb Symp Quant Biol 1963 28:43-46

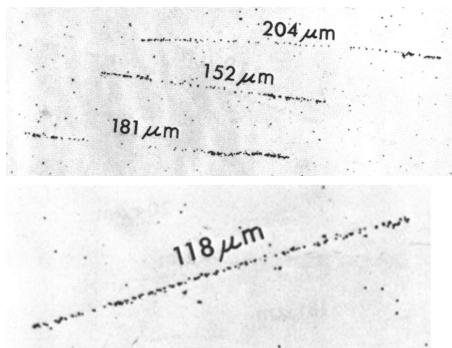
Is DNA synthesis unidirectional or bidirectional?

Either is consistent with replication "bubbles" observed earlier



Prescott, PNAS 1972

- "light" label with ^{3}H , then increase specific activity for certain times
- heavy tracks at both ends indicate bidirectionality



DNA replication only occurs in 5'-3' direction

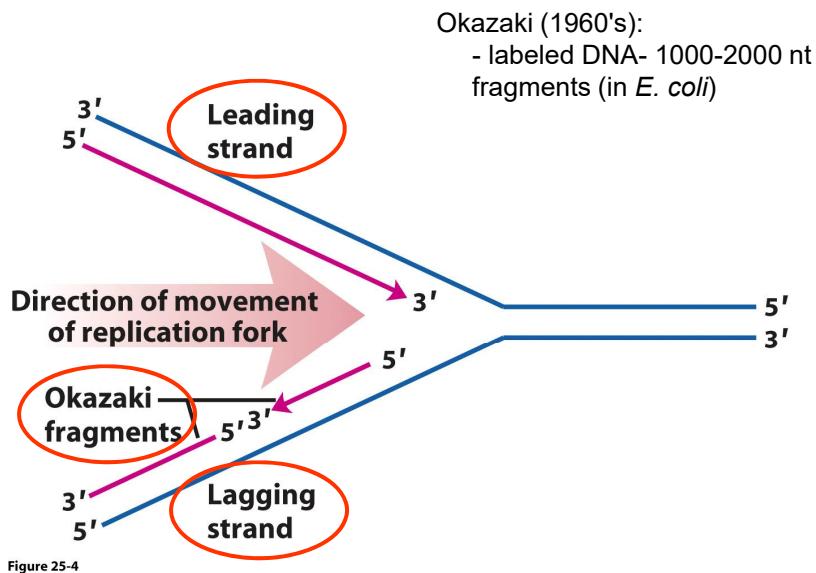


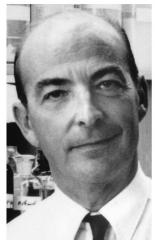
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The first DNA polymerase: *E. coli* Pol I

Arthur Kornberg:



Arthur Kornberg, 1918–2007
Unnumbered 25 p079
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- Conditions for DNA polymerization in extracts
dTTP better than dTMP or dT
add DNA to mixture, improve activity
- Purified **DNA polymerase I** (1957)

Not the major replicative enzyme in *E. coli*!

DNA polymerase mechanism: two metal ion catalysis

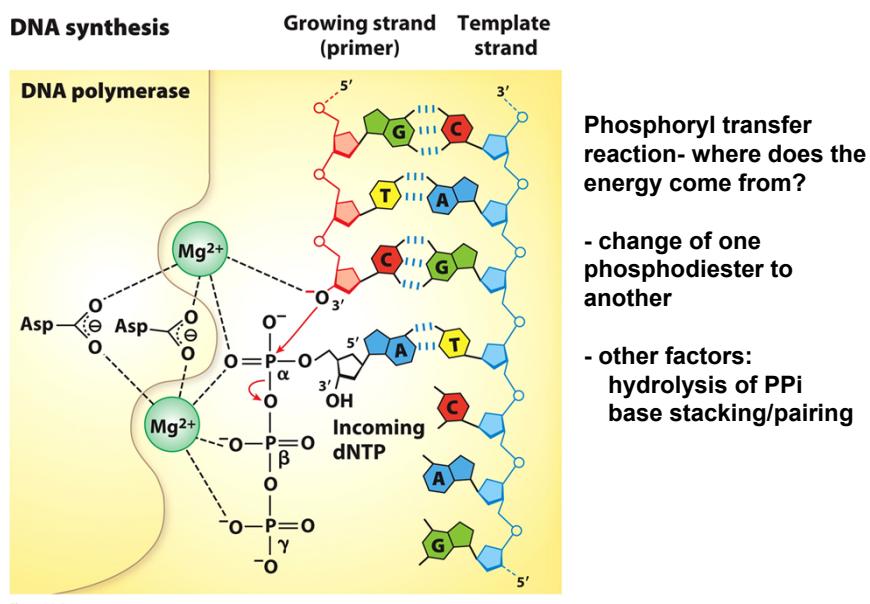
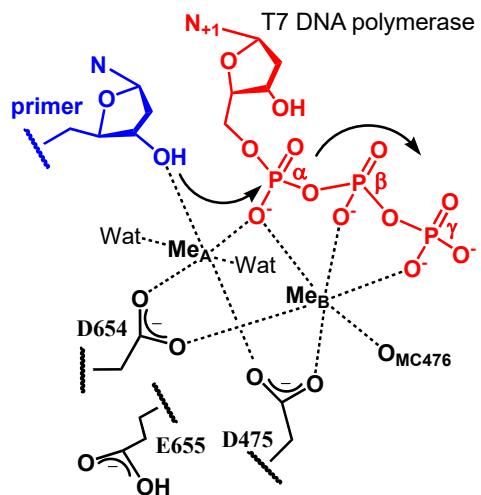


Figure 25-5a
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DNA polymerase mechanism: two metal ion catalysis

Important catalytic groups:

- Metal A
- Metal B
- 2-3 carboxylates



No protein residues directly in chemistry

DNA polymerase structure: analogy to human hand

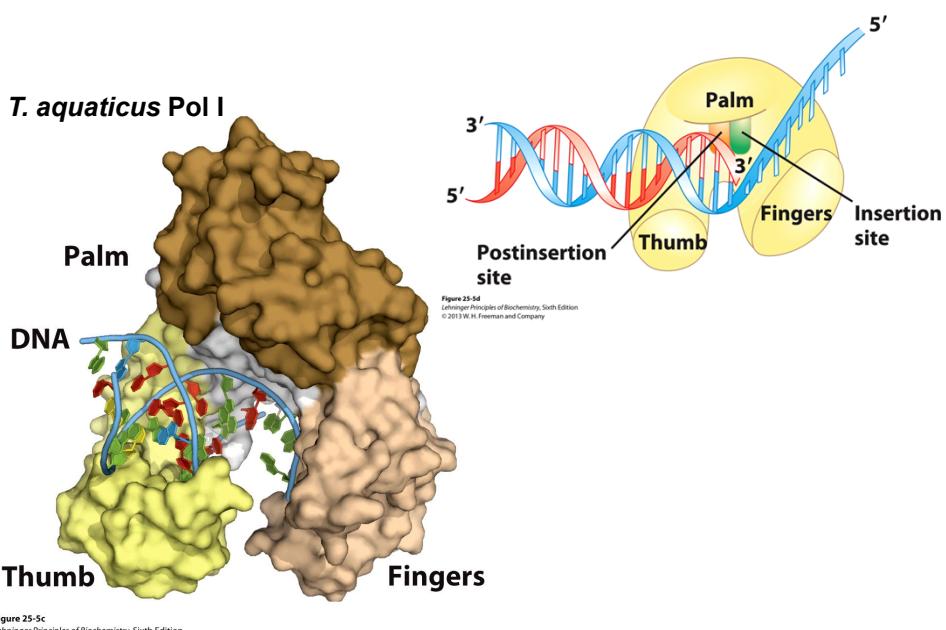


Figure 25-5c
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Other requirements for replication

DNA polymerases require a **template**:

- Kornberg's original observation that DNA stimulates ("activated" DNA)
- First example of templated biological reaction
- Basis for semi-conservative replication

DNA polymerases require a **primer**:

- Require nucleic acid with free 3'-OH
- Can't start from free NTP

Fidelity of replication process is essential

In vivo error rate: 1 error/ 10^9 - 10^{10} bp replicated

Similar numbers measured for:
Bacteria, fungi, humans...

How to keep this number constant?

- 1) Balance pools of dNTPs
- 2) DNA polymerase accuracy
 - select and incorporate right nucleotide
 - proofreading activities
- 3) Post-replicative repair

DNA polymerase fidelity

1. Geometric selection for correct size and shape

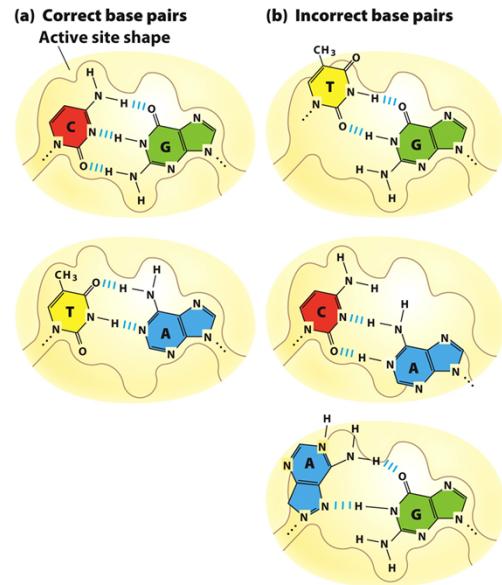
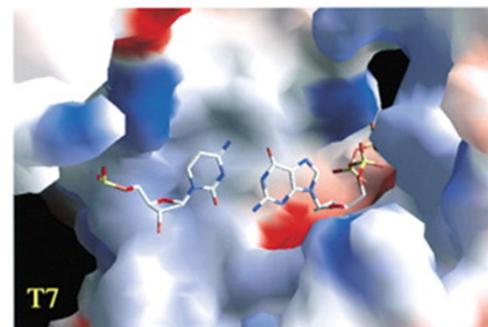


Figure 25-6
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DNA polymerase fidelity

1. Geometric selection for correct size and shape

- Active site complementarity
- local A-DNA conformation



Ling et al, Cell 107: 91-102

2. Conformational changes and other kinetic effects

DNA polymerases exhibit proofreading activities

In vitro polymerase error rate:

- 1 error/ 10^4 - 10^6 nucleotide additions
- Still not enough to account for 10^{-9} to 10^{-10} in vivo

Many DNA polymerases exhibit **3'-5' exonuclease activity**

- **exonuclease**: only cleaves at ends of nucleic acid
- **endonuclease**: cleaves at internal positions

If incorrect nucleotide has been added: **kinetic pause**

- gives the enzyme time to move the incorrect nucleotide to the **SEPARATE 3'-5' exo site**

3'-5' proofreading exonuclease activity

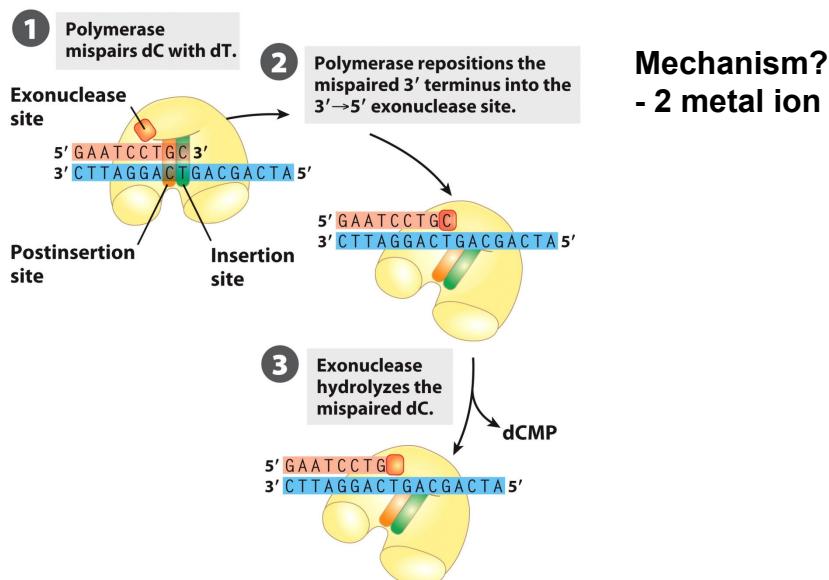
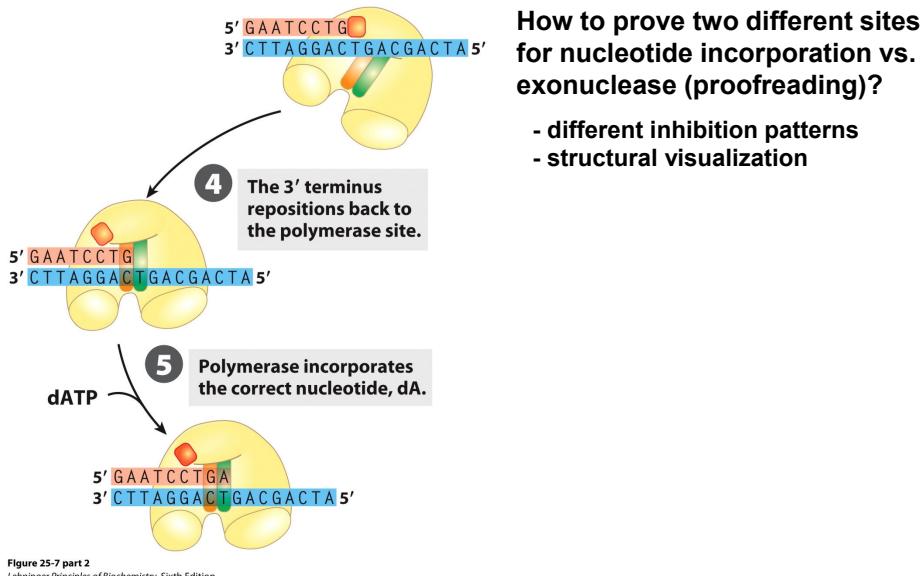


Figure 25-7 part 1
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3'-5' proofreading exonuclease activity



E. coli encodes 5 DNA polymerases

Pol I:

- too slow for known replication reaction
- low processivity
- Pol I polymerase defective mutant = VIABLE
- Contains unusual **5'-3' exonuclease activity**

Pol II: DNA repair

Pol III: The major replicative polymerase

Pol IV, Pol V: Repair polymerases

E. coli encodes 5 DNA polymerases

TABLE 25-1 Comparison of Three DNA Polymerases of *E. coli*

	DNA polymerase		
	I	II	III
Structural gene*	<i>polA</i>	<i>polB</i>	<i>polC (dnaE)</i>
Subunits (number of different types)	1	7	≥ 10
<i>M_r</i>	103,000	88,000 [†]	791,500
3'→5' Exonuclease (proofreading)	Yes	Yes	Yes
5'→3' Exonuclease	Yes	No	No
Polymerization rate (nucleotides/s)	10–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	$\geq 500,000$

*For enzymes with more than one subunit, the gene listed here encodes the subunit with polymerization activity. Note that *dnaE* is an earlier designation for the gene now referred to as *polC*.

[†]Polymerization subunit only. DNA polymerase II shares several subunits with DNA polymerase III, including the β , γ , δ , δ' , χ , and ψ subunits (see Table 25–2).

Table 25-1
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DNA Pol III is a complex multiprotein assembly

TABLE 25-2 Subunits of DNA Polymerase III of *E. coli*

Subunit	Number of subunits per holoenzyme	M _r of subunit	Gene	Function of subunit	
α	2	129,900	<i>polC (dnaE)</i>	Polymerization activity	
ϵ	2	27,500	<i>dnaQ (mutD)</i>	3'→5' Proofreading exonuclease	{ Core polymerase
θ	2	8,600	<i>holE</i>	Stabilization of ϵ subunit	
τ	2	71,100	<i>dnaX</i>	Stable template binding; core enzyme dimerization	{ Clamp-loading (γ) complex that loads β subunits on lagging strand at each Okazaki fragment
γ	1	47,500	<i>dnaX*</i>	Clamp loader	
δ	1	38,700	<i>holA</i>	Clamp opener	
δ'	1	36,900	<i>holB</i>	Clamp loader	
χ	1	16,600	<i>holC</i>	Interaction with SSB	
ψ	1	15,200	<i>holD</i>	Interaction with γ and χ	
β	4	40,600	<i>dnaN</i>	DNA clamp required for optimal processivity	

*The γ subunit is encoded by a portion of the gene for the τ subunit, such that the amino-terminal 66% of the τ subunit has the same amino acid sequence as the γ subunit. The γ subunit is generated by a translational frameshifting mechanism (p. 1111) that leads to premature translational termination.

Table 25-2
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The β clamp: increases processivity

Structure of the β clamp

Increases processivity by preventing dissociation from the DNA

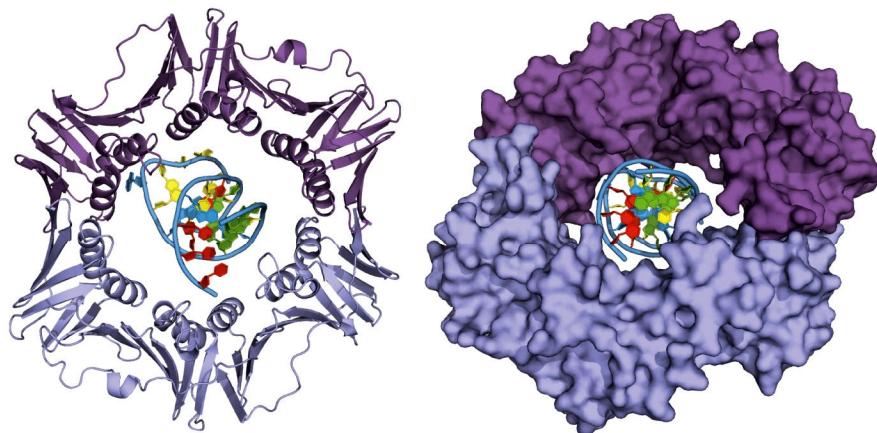


Figure 25-9b
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The DNA Pol III holoenzyme

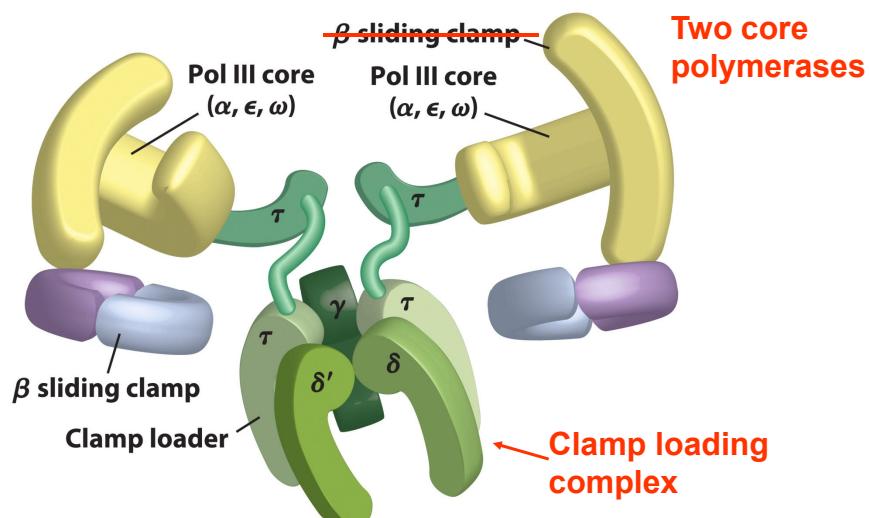


Figure 25-9a
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Set up for simultaneous replication of both strands

Two core polymerase domains, each can replicate one strand

Need some other proteins

- **DnaB helicase:** ATP-dependent, unwind dsDNA
- **Topoisomerase:** relieve stress (**DNA gyrase**, topo II)
- **ssDNA binding proteins:** prevent reannealing at fork
- **Primase:** synthesize (usually) RNA primer
- **DNA ligase:** seal the ends of final product

Lagging vs. leading strand synthesis

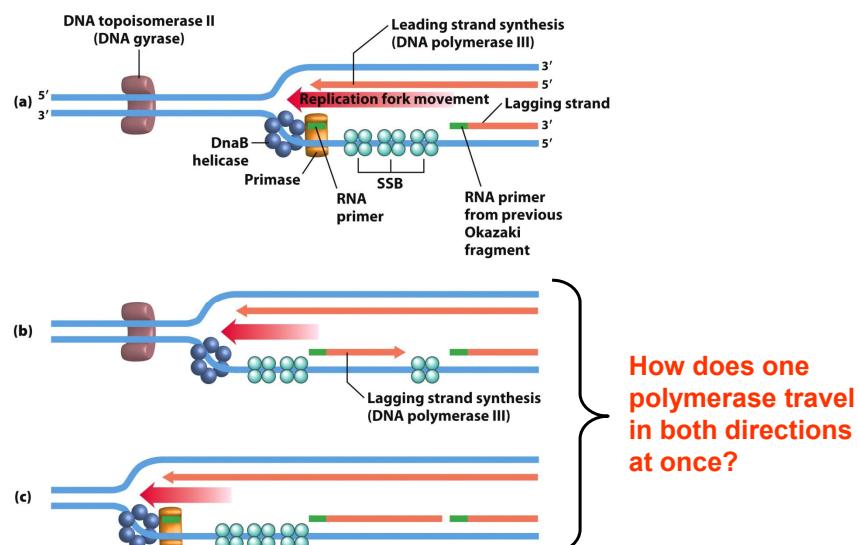


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Looping mechanism for Pol III replication

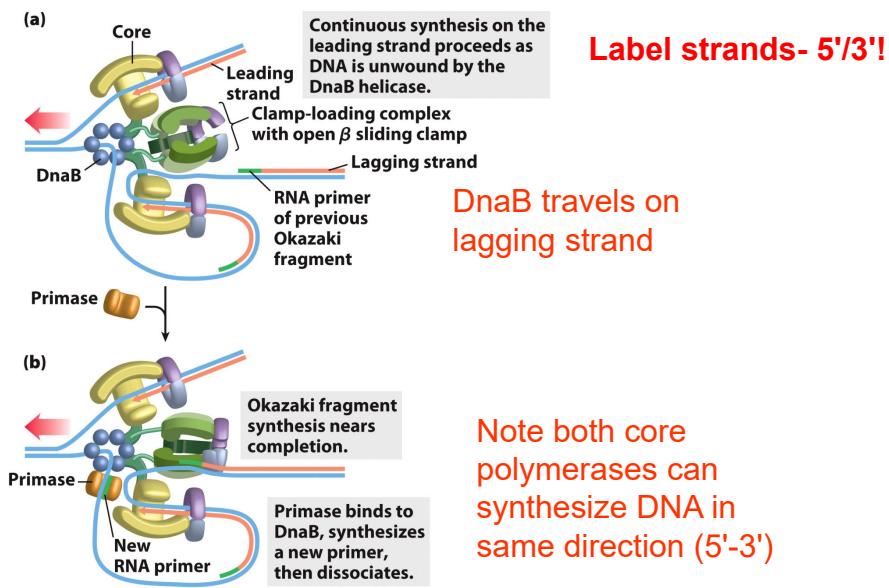


Figure 25-13 part 1
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Looping mechanism for Pol III replication

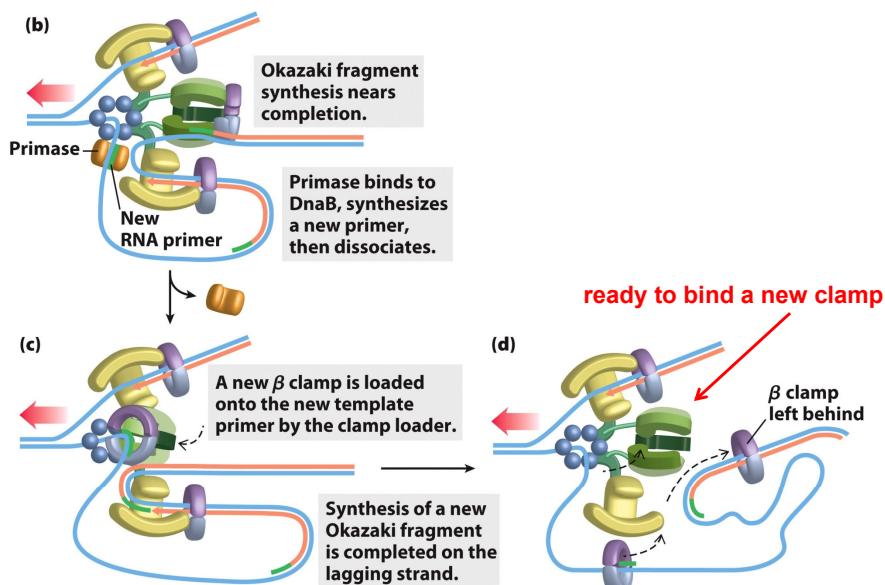


Figure 25-13 part 3
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Loading the clamp requires energy

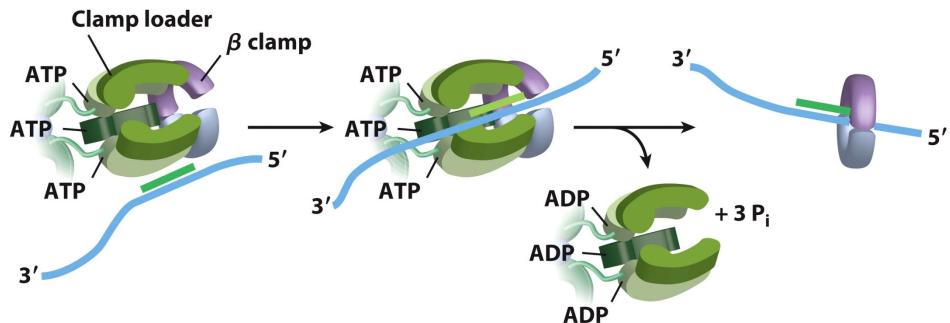


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Hydrolysis of 3 ATP allows clamp to close around the DNA

The final steps: remove primer and seal the nick

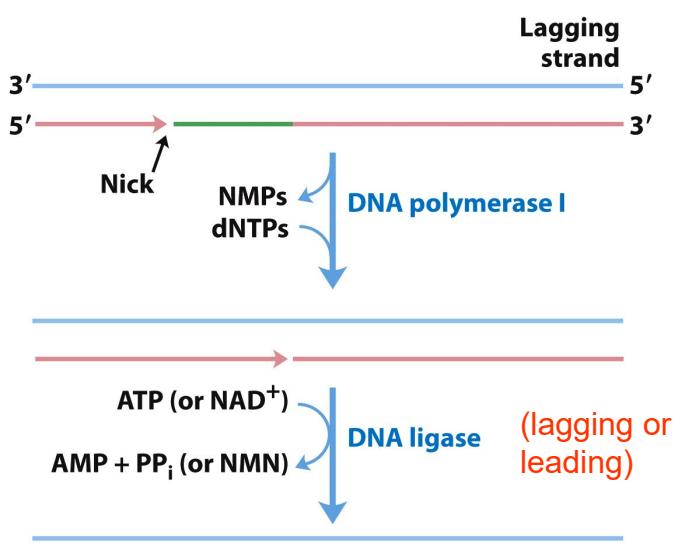


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Specialized role for DNA Pol I in nick translation

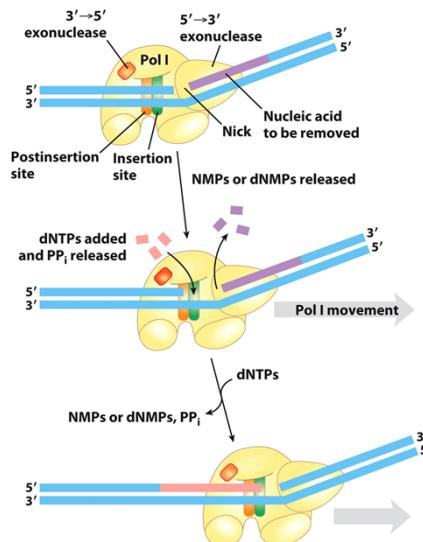


Figure 25-8
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-removal of primers that are used to initiate lagging strand synthesis

- mutants in 5'-3' exo activity are not viable

Comparison: *E. coli* vs eukaryotic replication

Homologous versions of most of the proteins:

<u>E. coli</u>	<u>Humans</u>
β-clamp	PCNA (proliferating cell nuclear antigen)
clamp loader	RFC (replication factor C)
SSB	RPA (replication protein A)
DnaB	MCM (minichromosome maintenance)
DnaA	ORC (origin recognition complex)
Primase	pol α
Pol III	pol δ

At least 15 known polymerases in human cells

Human DNA polymerases

<u>Greek name</u>	<u>HUGO name</u>	<u>family</u>	<u>Other names</u>	<u>Proposed main function(s)</u>
α (alpha)	POLA	B	<i>POL1</i>	DNA replication
β (beta)	POLB	X		Base excision repair
γ (gamma)	POLG	A	<i>MIP1</i>	Mitochondrial replication
δ (delta)	POLD1	B	<i>POL3</i>	DNA replication
ϵ (epsilon)	POLE	B	<i>POL2</i>	DNA replication
ζ (zeta)	POLZ	B	<i>REV3</i>	Bypass synthesis
η (eta)	POLH	Y	<i>RAD30, XPV</i>	Bypass synthesis
θ (theta)	POLQ	A	<i>mus308, eta</i>	DNA repair
ι (iota)	POLI	Y	<i>RAD30B</i>	Bypass synthesis
κ (kappa)	POLK	Y	<i>DinB1, theta</i>	Bypass synthesis
λ (lambda)	POLL	X	<i>POL4, beta2</i>	Base excision repair
μ (mu)	POLM	X		Non-homologous end joining
σ (sigma)	POLS	X	<i>TRF4, kappa</i>	Sister chromatid cohesion
	REV1L	Y	<i>REV1</i>	Bypass synthesis
	TDT	X		Antigen receptor diversity
Plus at least 2 more...				

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Need to coordinate DNA synthesis with cell growth

E. coli:

1000 nt/sec, 4.7×10^6 bp = 40 min/genome

yet doubling time = 25 min

How?- time frequency of initiation

Humans:

50 nt/sec, 1×10^8 bp/chromosome = 15 days/one chromosome

yet 8 hours to replicate, 24 hours to divide

How?- timing and number of origins

Where does replication start?

Replication does not originate at random sequences:

- Analysis of gene frequency in rapidly dividing cultures
- A single unique site is the origin of replication

Replicon: DNA that is replicated under the control of a single origin

- The *E. coli* origin: *oriC*
- Eukaryotic origins are much more complicated...

E. coli replication initiation is regulated

OriC sequence:

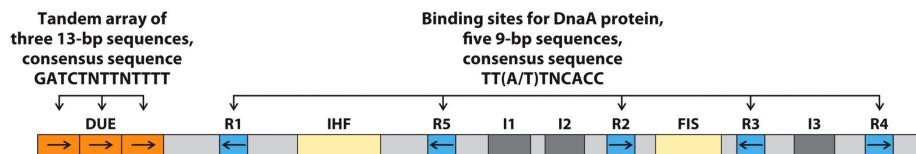


Figure 25-10
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1. Hemimethylation of GATC sites

- 11 GATC in oriC (vs. 1 expected at random)
- immediately after replication: hemimethylated
- lag in Dam methylase activity: can't reinitiate until acts

E. coli replication initiation is regulated

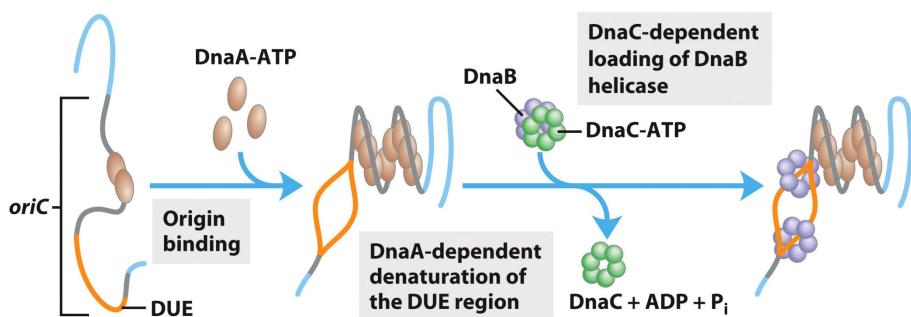


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2. Control of initiation mass through DnaA (AAA+ ATPase)

Release of ADP and rebinding of ATP is slow
ADP-bound enzyme has low affinity for some sites in oriC,
falls off
control of DnaA synthesis also plays role

Where does replication stop?

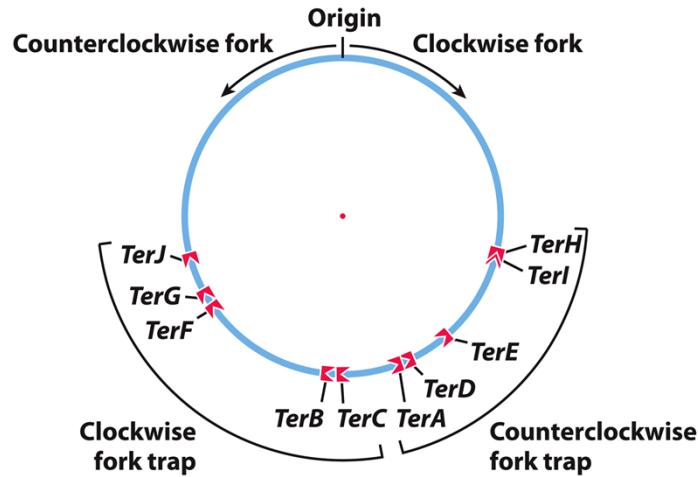


Figure 25-17
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Most forks just meet and fall off;
When is this necessary?