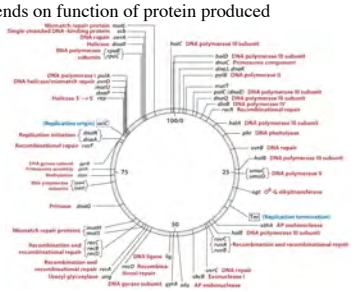
Lectures 5b, 5c

DNA replication and repair

Chapter 25:
DNA Metabolism

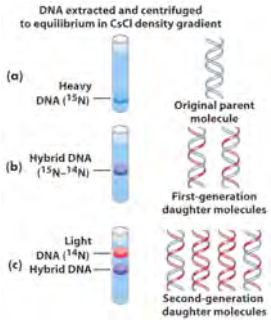
Genetic map = identifies relative positions of genes on chromosomes.

Nomenclature: dnaB = gene for DnaB protein; first 3 letters in small script, depends on function of protein produced

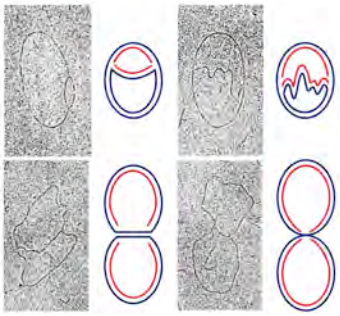


Meselson & Stahl's Experiment: DNA of bacteria grown in ^{15}N then allowed to replicate in normal ^{14}N medium.

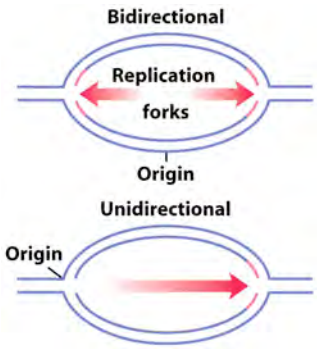
Density centrifugation showed that replication was semi-conservative



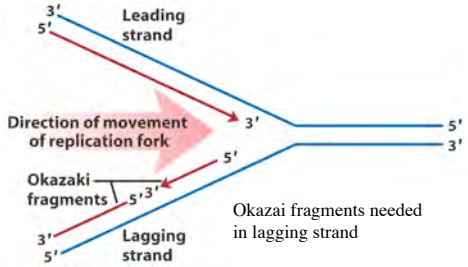
Replication in most bacteria starts at an "origin", proceeds bidirectionally - Theta formation- until it reaches the opposite side of the DNA circle where two distinct daughter DNA separate.



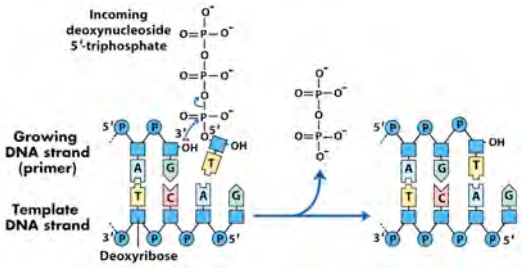
Bidirectional vs unidirectional

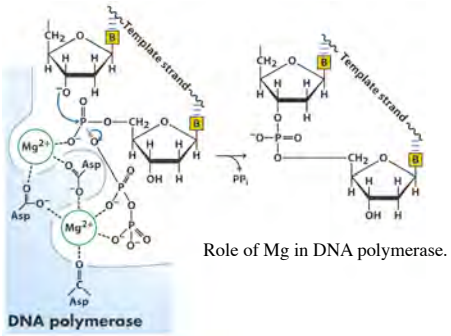


The template strand is read in the 3'-- > 5' direction.
Synthesis of new strand is in the 5'-- > 3' direction.

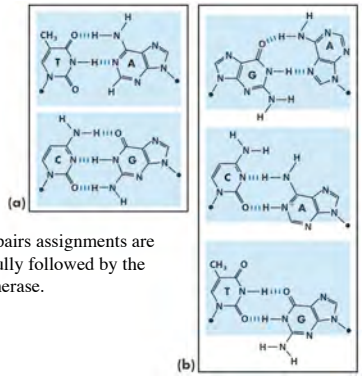


Okazai fragments needed in lagging strand





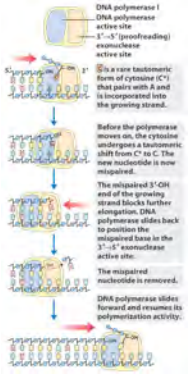
Role of Mg in DNA polymerase.

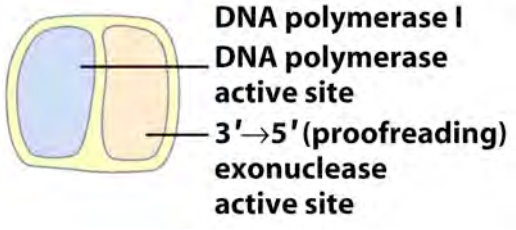


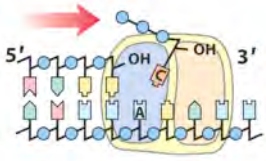
Base pairs assignments are faithfully followed by the polymerase.

DNA polymerases have 3'-> 5' proof reading ability

Pol I for example:

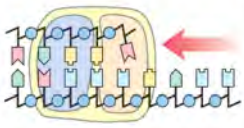




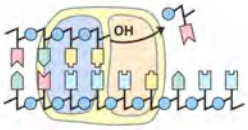


Ç is a rare tautomeric form of cytosine (C*) that pairs with A and is incorporated into the growing strand.

Before the polymerase moves on, the cytosine undergoes a tautomeric shift from C* to C. The new nucleotide is now mispaired.

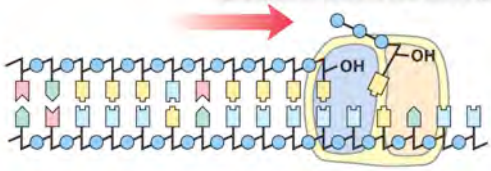


The mispaired 3′-OH end of the growing strand blocks further elongation. DNA polymerase slides back to position the mispaired base in the 3′→5′ exonuclease active site.



The mispaired nucleotide is removed.

DNA polymerase slides forward and resumes its polymerization activity.



There are several DNA polymerases: I , II, and III

TABLE 25–1 Comparison of DNA Polymerases of *E. coli*

	DNA polymerase		
	I	II	III
Structural gene*	*polA*	*polB*	*polC* (*dnaE*)
Subunits (number of different types)	1	7	≥10
M_r	103,000	88,000†	791,500
3′→5′ Exonuclease (proofreading)	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	No
Polymerization rate (nucleotides/s)	16-20	40	250-1,000
Processivity (nucleotides added before polymerase dissociates)	3-200	1,500	≥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*.

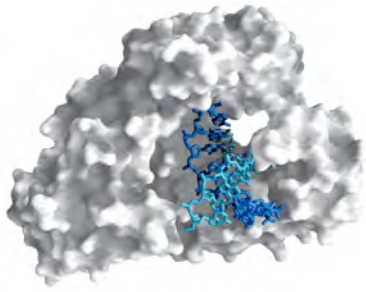
†Polymerization subunit only. DNA polymerase II shares several subunits with DNA polymerase III, including the β, γ, δ, δ′, χ, and ψ subunits (see Table 25-2).

TABLE 25–2 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	*polC* (*dnaE*)	Polymerization activity	Core polymerase
ε	2	27,500	*dnaQ* (*mutD*)	3′→5′ Proofreading exonuclease	
θ	2	8,600	*holE*		
τ	2	71,100	*dnaX*	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	*dnaX*′	Clamp loader	
δ	1	38,700	*holA*	Clamp opener	
δ′	1	36,900	*holB*	Clamp loader	
χ	1	16,600	*holC*	Interaction with SSB	
ψ	1	15,200	*holD*	Interaction with γ and χ	
β	4	40,600	*dnaN*	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 γ subunit has the same amino acid sequence as the γ subunit. The γ subunit is generated by a translational frameshifting mechanism (see Box 27-1) that leads to premature translational termination.

Large Klenow fragment of Pol I retains polymerization and proof reading activity. Shows DNA deep in active site.

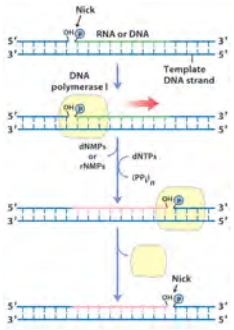


Pol I's 5'->3' proof-reading (exonuclease) activity.

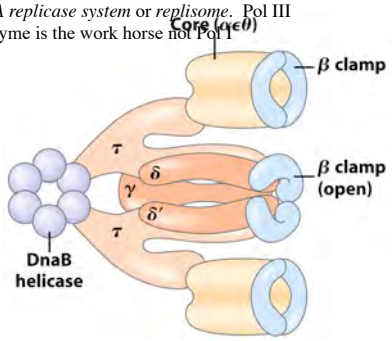
Note: Exonuclease = hydrolyzes DNA at one end of strand.

Endonuclease = hydrolyzes DNA "inside" the strand

n.b. nick is "spliced" by ligase (not shown)



Replication requires about 20 enzymes. Whole thing is the *DNA replicase system* or *replisome*. Pol III holoenzyme is the work horse not Pol I



What are the enzymes/proteins provide the functions needed in a replisome?

Helicases = separation of DNAstrands (to access DNA template)

Topoisomerases= relieve topological stress of strand separation

DNA binding proteins (ssb)= stabilize separated single strands

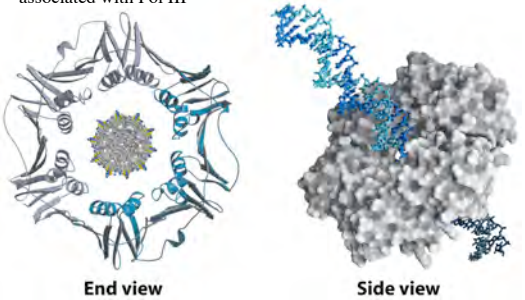
Primases = lay down RNA primers (short segments) for Pol III

Pol III holoenzyme = for most of the replication work (incl O.F.)

Pol I = completes Okazaki f's (O.F.), removes RNA primers

Beta clamp = enhances Pol III processivity,prevents dissociation

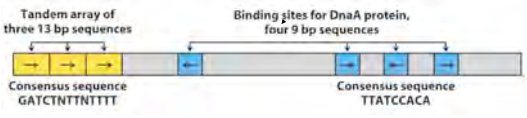
The is the beta clamp involved in the replication fork, and associated with Pol III



The 3 stages of replication are: initiation, synthesis, termination. For initiation need an origin:

oriC = origin of replication in E coli = Highly conserved sequences which signal start of the replication process.

5 repeats not 4



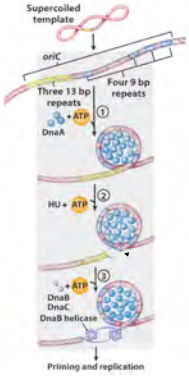
Step I: Initiation unwinding at the origin.

N.b. Know proteins and their roles.

There is a region called DNA unwinding element (DUE), rich in A=T base pairs, which denatures as result of binding of DnaA, HU.

Helicase (DnaB) is helped by DnaC

N.b. initiation is only step regulated in replicn



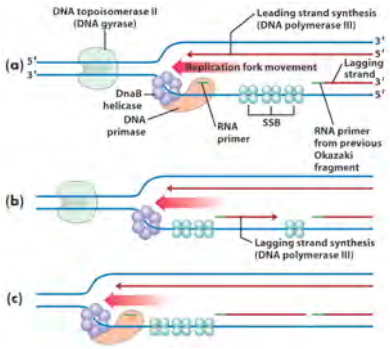
DUE

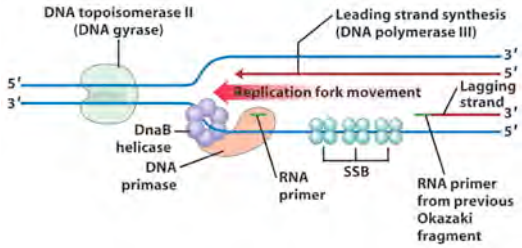
TABLE 25-3 Proteins Required to Initiate Replication at the *E. coli* Origin

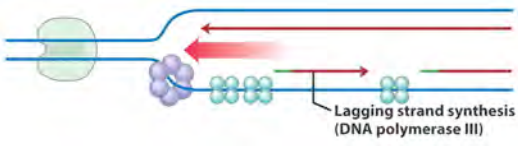
Protein	M_r	Number of subunits	Function
DnaA protein	52,000	1	Recognizes ori sequence; opens duplex at specific sites in origin
DnaB protein (helicase)	300,000	6*	Unwinds DNA
DnaC protein	29,000	1	Required for DnaB binding at origin
HU	19,000	2	Histonelike protein; DNA-binding protein; stimulates initiation
Primase (DnaG protein)	60,000	1	Synthesizes RNA primers
Single-stranded DNA-binding protein (SSB)	75,600	4*	Binds single-stranded DNA
RNA polymerase	454,000	5	Facilitates DnaA activity
DNA gyrase (DNA topoisomerase II)	400,000	4	Relieves torsional strain generated by DNA unwinding
Dam methylase	32,000	1	Methylates (5′)GATC sequences at *ori*C

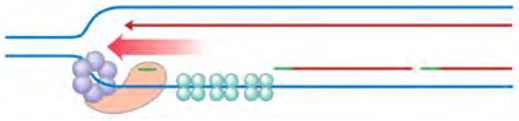
*Subunits in these cases are identical.

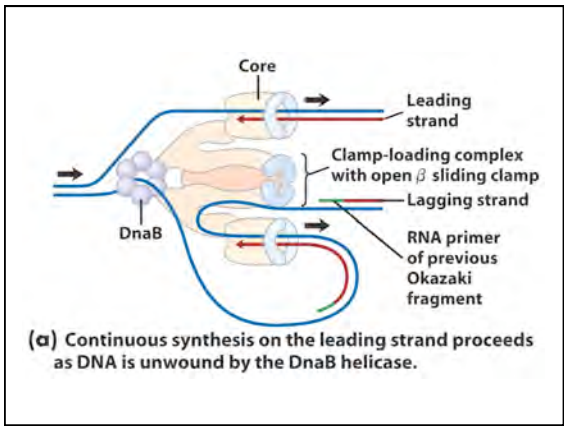
Step 2: elongation (note the synthesis of Okazaki fragments):



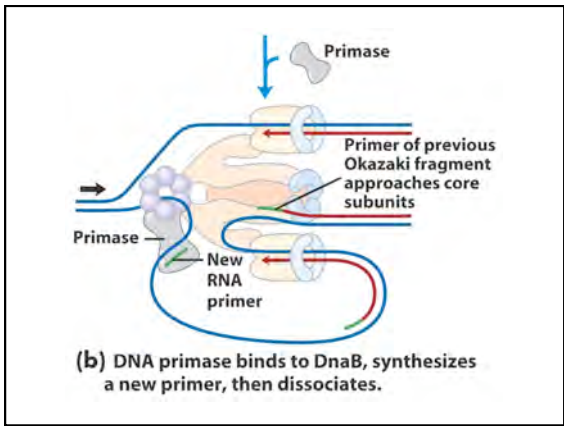




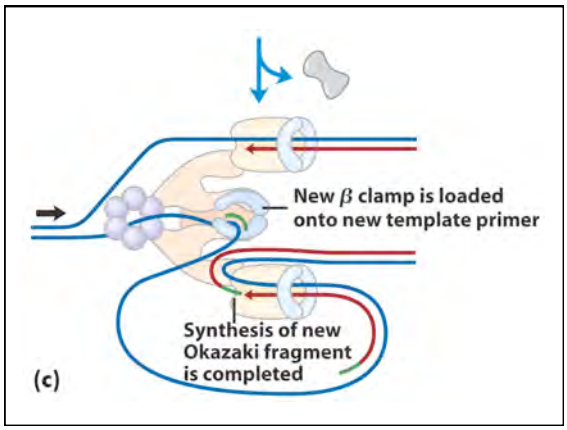


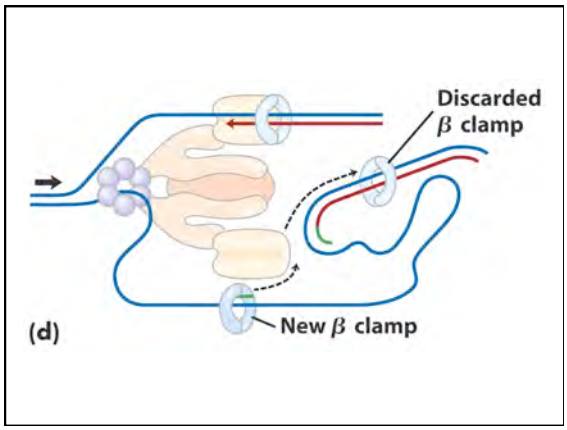


(a) Continuous synthesis on the leading strand proceeds as DNA is unwound by the DnaB helicase.



(b) DNA primase binds to DnaB, synthesizes a new primer, then dissociates.





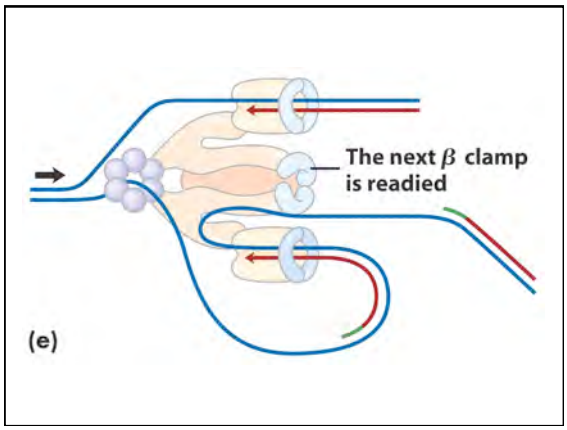
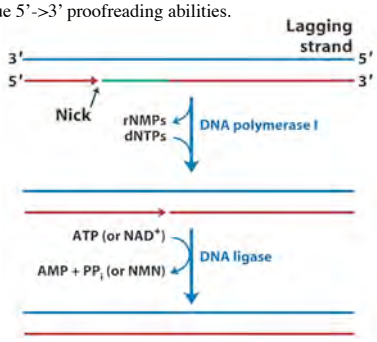


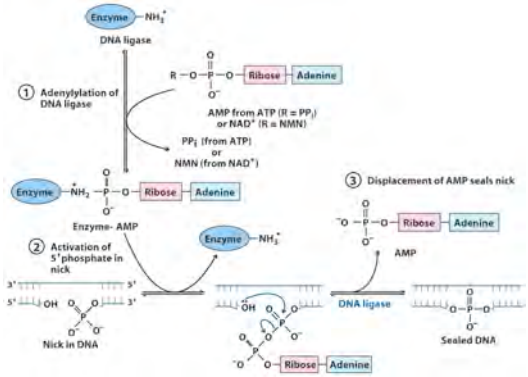
TABLE 25-4 Proteins at the *E. coli* Replication Fork

Protein	M_r	Number of subunits	Function
SSB	75,600	4	Binding to single-stranded DNA
DnaB protein (helicase)	300,000	6	DNA unwinding; primosome constituent
Primase (DnaG protein)	60,000	1	RNA primer synthesis; primosome constituent
DNA polymerase III	791,500	17	New strand elongation
DNA polymerase I	103,000	1	Filling of gaps; excision of primers
DNA ligase	74,000	1	Ligation
DNA gyrase (DNA topoisomerase II)	400,000	4	Supercoiling

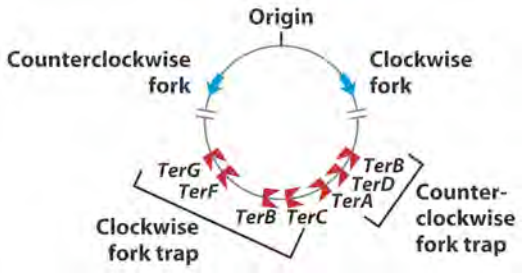
Maxfield New Kennedy, A. (1982) *Supplement to DNA Replication*. Table 511-2. W. H. Freeman and Company, New York.

DNA Pol I acts to remove the RNA primer by means of its unique 5'->3' proofreading abilities.

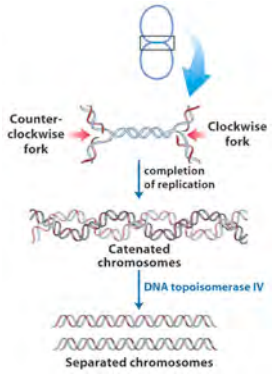




Step 3: Termination requires *Ter* sequences that "trap" fork. Ter binds to *Tus* protein which arrests fork from only 1 direction



When forks "collide", termination ends. 2 duplex DNAs are separated by topoisomerase IV



Replication involves replication systems tethered (bound) to membrane.