As each newly formed segment of the lagging strand approaches the 5′ end of the adjacent Okazaki fragment (the one just completed), *E. coli* DNA polymerase I takes over. Unlike polymerase III, polymerase I has 5′ → 3′ *exonuclease activity*, which removes the RNA primer of the adjacent fragment; the polymerization activity of polymerase I simultaneously fills in the gap between the fragments by addition of deoxyribonucleotides. Finally, another critical enzyme, DNA ligase, joins adjacent completed fragments (Figure 12-9c).

E. coli DNA Polymerase III Catalyzes Nucleotide Addition at the Growing Fork

Three DNA polymerases (I, II, and III) have been purified from *E. coli* (Table 12-1). In addition to its role in filling the gaps between Okazaki fragments, DNA polymerase I probably is the most important enzyme for gap filling during DNA repair. DNA polymerase II functions in the inducible SOS response discussed later; this polymerase also fills gaps and appears to facilitate DNA synthesis directed by damaged templates. Our discussion here focuses on DNA polymerase III, which catalyzes chain elongation at the growing fork in *E. coli*.

The DNA polymerase III holoenzyme is a very large (>600 kDa), highly complex protein composed of 10 different polypeptides. The so-called *core polymerase* is composed of three subunits. The α subunit contains the active site for nucleotide addition, and the ϵ subunit is a 3′ → 5′ exonuclease that removes incorrectly added (mispaired) nucleotides from the end of the growing chain. (This "proofreading" activity of DNA polymerase III is described later.) The function of the θ subunit is not known.

The central role of the remaining subunits is to convert the core polymerase from a *distributive* enzyme, which falls off the template strand after forming relatively short stretches of DNA containing 10–50 nucleotides, to a *processive* enzyme, which can form stretches of DNA containing up to 5×10^5 nucleotides without being released from the template. This latter activity is necessary for efficient synthesis of both leading and lagging strands. The key to the processive nature of DNA polymerase III is the ability of the β subunit to form a donut-shaped dimer around duplex DNA and then associate with and hold the catalytic core poly-

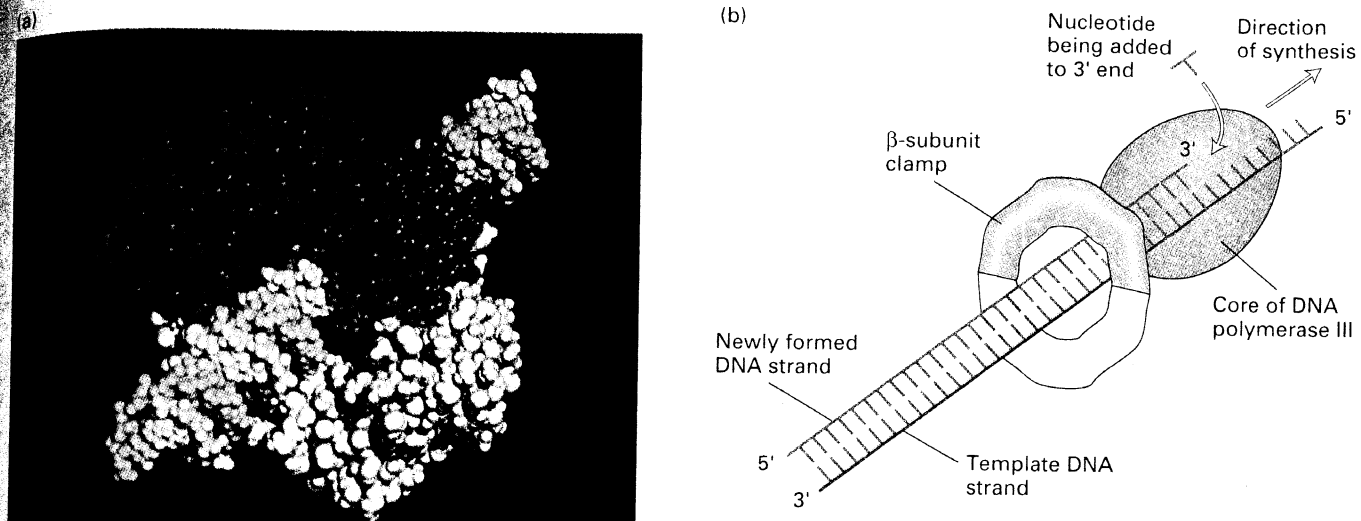
TABLE 12-1 Properties of DNA Polymerases

E. coli	I	II	III		
Polymerization: 5′ → 3′	+	+	+		
Exonuclease activity:					
3′ → 5′	+	+	+		
5′ → 3′	+	−	−		
Synthesis from:					
Intact DNA	−	−	−		
Primed single strands	+	−	−		
Primed single strands plus single-strand-binding protein	+	−	+		
In vitro chain elongation rate (nucleotides per minute)	600	?	30,000		
Molecules present per cell	400	?	10–20		
Mutation lethal?	+	−	+		
Mammalian Cells*	**α**	**β†**	**γ**	**δ**	**ϵ**
Polymerization: 5′ → 3′	+	+	+	+	+
Exonuclease proofreading activity:‡ 3 → 5′	−	−	+	+	+
Synthesis from:					
RNA primer	+	−	−	+	?
DNA primer	+	+	+	+	+
Associated DNA primase	+	−	−	−	−
Sensitive to aphidicolin (inhibitor of cell DNA synthesis)	+	−	−	+	+
Cell location:					
Nuclei	+	+	−	+	+
Mitochondria	−	−	+	−	−

**Yeast DNA polymerase I, II, and III are equivalent to polymerase α, β, and δ, respectively. I and III are essential for cell viability.*

†Polymerase β is most active on DNA molecules with gaps of about 20 nucleotides and is thought to play a role in DNA repair.

‡FEN1 is the eukaryotic 5′ → 3′ exonuclease that removes RNA primers; it is similar in structure and function to the domain of E. coli *polymerase I that contains the 5′ → 3′ exonuclease activity.*



▲ FIGURE 12-10 A β-subunit dimer tethers the core of *E. coli* DNA polymerase III to DNA, thereby increasing its processivity. (a) Space-filling model based on x-ray crystallographic studies of the dimeric β subunit binding to a DNA duplex. Two β subunits (red and yellow) form a donut-like clamp that remains tightly bound to a closed circular DNA molecule, but readily slides off the ends of a linear DNA molecule. (b) Schematic diagram of proposed association of the core polymerase (green) with the β subunit clamp at the primer-template terminus. This interaction keeps the core from "falling off" the template and positions it near the point of nucleotide addition, increasing the processivity of the core polymerase by more than a thousandfold. [Part (a) from X-P. Kong et al., 1992, *Cell* **69**:425; part (b) adapted from S. Kim et al., 1996, *Cell* **84**:643.]

MEDIA CONNECTIONS
Nucleotide Polymerization by DNA Polymerase

merase near the 3′ terminus of the growing strand (Figure 12-10). Once tightly associated with the DNA, the β-subunit dimer functions like a "clamp," which can slide freely along the DNA, like a ring on a string, as the associated core polymerase moves. In this way, the active sites of the core polymerase remain near the growing fork and the processivity of the enzyme is maximized. Remarkably, of the six remaining subunits, five (γ, δ, δ′, χ, and ψ) form the so-called γ complex, which mediates two essential tasks: (1) loading of the β-subunit clamp onto the duplex DNA–primer substrate in a reaction that requires hydrolysis of ATP and (2) unloading of the β-subunit clamp after a strand of DNA has been completed. Loading and unloading of the β-subunit clamp requires opening the clamp ring, but exactly how the γ complex accomplishes this feat is not known. The final subunit (τ) acts to dimerize two core polymerases and, as summarized in the next section, is essential for coordinating the synthesis of the leading and lagging strands at each growing fork.

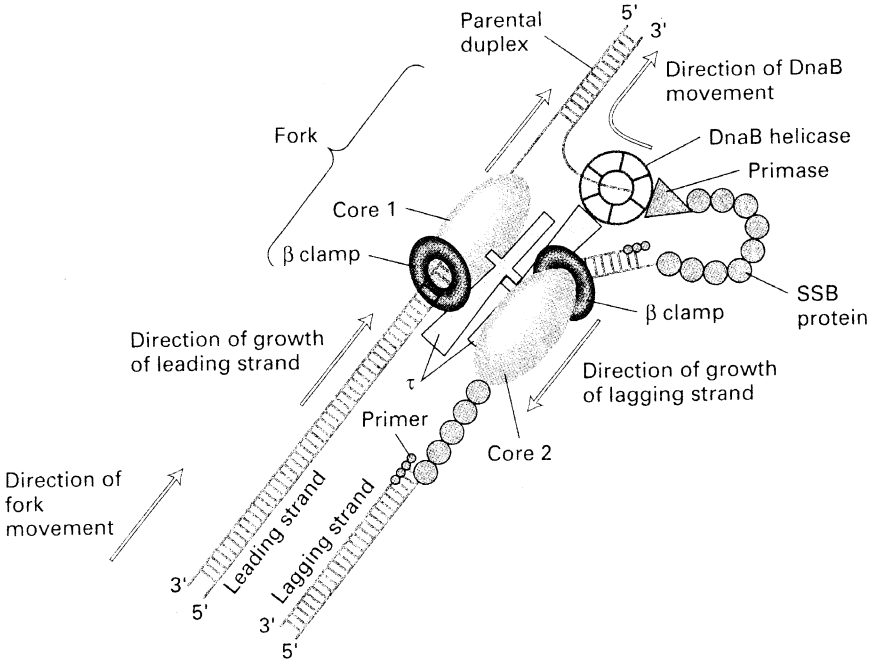
The Leading and Lagging Strands Are Synthesized Concurrently

Once the prepriming complex and an RNA primer are formed at the *E. coli* replication origin, chain elongation to yield the leading strand proceeds with little difficulty. As we've seen, however, lagging-strand synthesis proceeds discontinuously from multiple primers. Two molecules of core DNA polymerase III are bound at each growing fork; one adds nucleotides to the leading strand, and the other adds nucleotides to the lagging strand. Coordination between elongation of the leading and lagging strand is essential; otherwise one template strand would be incorporated into a duplex with a newly synthesized complementary strand while large parts of the other template strand would remain single-stranded.

Figure 12-11 shows how this coordination is achieved. The two core-polymerase molecules at the fork are linked together by a τ-subunit dimer. The core polymerase synthesizing the leading strand moves, together with its β-subunit clamp, along its template in the direction of the movement of the fork, elongating the leading strand. It follows closely the movement of the DnaB helicase bound to the lagging-strand template as the helicase melts the duplex DNA at the fork. Since this core-polymerase molecule remains attached to the DNA template, leading-strand synthesis occurs continuously.

The other core-polymerase molecule, which elongates the lagging strand, moves with its β-subunit clamp in the direction opposite to that of the fork movement. As elongation of the lagging strand proceeds, the size of the DNA "loop" between this core polymerase and the fork increases. One way to see this is to imagine the core 2 polymerase fixed in space, linked to core 1; double-stranded DNA newly synthesized by core 2 would be "pushed" into the loop. Eventually the core polymerase synthesizing the lagging strand will complete an Okazaki fragment; it then dissociates from the DNA template, but the τ-subunit dimer continues to tether it to the fork-protein complex. Simultaneously, a primase binds to a site adjacent to the DnaB helicase on the single-stranded segment of the lagging-strand template and initiates synthesis of

MEDIA CONNECTIONS
Coordination of Leading and Lagging Strand Synthesis



▲ FIGURE 12-11 Schematic model of the relationship between *E. coli* replication proteins at a growing fork. (1) A single DnaB helicase moves along the lagging-strand template toward its 3′ end, thereby melting the duplex DNA at the fork. (2) One core polymerase (core 1) quickly adds nucleotides to the 3′ end of the leading strand as its single-stranded template is uncovered by the helicase action of DnaB. This leading-strand polymerase, together with its β-subunit clamp, remains bound to the DNA, synthesizing the leading strand continuously. (3) A second core polymerase (core 2) synthesizes the lagging strand discontinuously as an Okazaki fragment (see Figure 12-9b). The two core polymerase molecules are linked via a dimeric τ protein. (4) As each segment of the single-stranded template for the lagging strand is uncovered, it becomes coated with SSB protein and forms a loop. Once synthesis of an Okazaki fragment is completed, the lagging-strand polymerase dissociates from the DNA but the core remains bound to the τ-subunit dimer. The released core polymerase subsequently rebinds with the assistance of another β clamp in the region of the primer for the next Okazaki fragment. See the text for additional details. [Adapted from A. Kornberg, 1988, *J. Biol. Chem.* **263**:1; S. Kim et al., 1996, *Cell* **84**:643.]

another RNA primer. The resulting DNA-primer complex attracts another β clamp to this segment of the lagging-strand template, followed by re-binding of the core polymerase, which is still attached to the fork complex. This polymerase molecule then proceeds to elongate the RNA primer to form another Okazaki fragment. As mentioned earlier, as each Okazaki fragment nears completion, the RNA primer of the previous fragment is removed by the 5′ → 3′ exonuclease activity of DNA polymerase I. This enzyme also fills in the gaps between the lagging-strand fragments, which then are ligated together by DNA ligase (see Figure 12-9b).

Although the two core polymerase molecules are linked by the τ-subunit dimer, they are oriented in opposite directions (see Figure 12-11). Thus, the 3′ growing ends of both the leading and lagging strands are close together but offset from each other. For this reason, the point in the template at which the lagging strand is being copied is displaced from the point in the template at which leading-strand copying is occurring. Nonetheless, the two core polymerases can add deoxyribonucleotides to the growing strands at the same time and rate, so that leading- and lagging-strand synthesis occurs concurrently.

One τ subunit also contacts the DnaB helicase at the fork. Experiments with purified replication proteins have shown that this interaction increases the normally slow unwinding rate of the helicase (≈35 bp/s) over tenfold, thereby enabling the fork to move at rates up to 1000 bp/s. Thus, there is a physical and functional link between the two major replication machines at the fork—the two core polymerases and the primosome complex of primase and DnaB. By closely coordinating all the events depicted in Figures 12-9 and 12-11, the growing fork moves 500–1000 bp/s while both strands are being replicated.

Eukaryotic Replication Machinery Is Generally Similar to That of *E. coli*

As in *E. coli*, researchers investigating DNA replication in eukaryotes initially concentrated on characterizing the different DNA polymerases present in eukaryotic cells (see Table 12-1). This work was followed by development of in vitro systems for copying small chromosomes from animal viruses (e.g., SV40) whose replication is dependent almost entirely on host-cell proteins. As a result of these studies, the SV40