

β Sliding Clamp Dynamics within *E. Coli* DNA Polymerase III Holoenzyme

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INTRODUCTION

The replicase that duplicates the *E. coli* chromosome, DNA polymerase III holoenzyme (pol III holoenzyme), shares in common with other cellular replicases a multiprotein structure.¹ Why do chromosomal replicases consist of numerous polypeptides? There are several tasks beyond simple polymerization in the duplication of a chromosome, and these jobs require the assistance of "accessory proteins." Some of these extra tasks, and the proteins that perform them, have been identified in several replication systems. Overall, it is remarkable how similar the different systems are. In this report, the functions of accessory proteins in the *E. coli* system will be examined and related to accessory protein function in other systems.

Pol III holoenzyme of *E. coli* consists of 10 nonidentical subunits and has at least 18 polypeptide chains in all.^{2,3} Pol III holoenzyme has several special properties that distinguish it as a replicative polymerase. For example, pol III hydrolyzes ATP to bind tightly to a primed template and utilizes a single-stranded (ss) DNA template that is fully coated with the single-strand DNA binding protein (SSB).^{4,5} After pol III holoenzyme is locked onto DNA by ATP, it is rapid (750 nucleotides/second) and highly processive (>100 kb per binding event) in synthesis without a further requirement for ATP.⁶⁻⁸ Upon encounter with a short heteroduplex in its path, pol III holoenzyme does not displace it but rather slides right over it and reinitiates processive extension without need for more ATP.⁹ The basic mechanisms by which pol III holoenzyme couples ATP to bind DNA, slides over duplex DNA, and achieves a high speed and processivity have been revealed through study of individual subunits of pol III and its subassemblies.

ACCESSORY PROTEIN FUNCTION

The 10 subunits of pol III holoenzyme are listed in TABLE 1. Each of these subunits are now available pure and in quantity through molecular cloning of their genes. Even before most individual subunits were isolated, valuable information was gleaned from subassemblies fractionated from the holoenzyme. For example, all the catalytic functions were found to reside within a three-subunit subassembly purified by Charley McHenry called the core polymerase.¹⁰ Later studies using isolated subunits showed that α is the actual DNA polymerase¹¹ and that the proofreading 3'-5' exonuclease resides in an entirely separate polypeptide, ϵ .¹² This core poly-

TABLE 1. Subunits and Subassemblies of polIII Holoenzyme

Subunit	Mass (kDa)	Gene	Function	Subassembly
α	129	<i>dnaE</i>	DNA polymerase	pol III core pol III' pol III*
ϵ	28	<i>dnaQ</i> , <i>mutD</i>	3'-5' Exonuclease	
θ	8.6	<i>holE</i>	Unknown	
τ	71	<i>dnaX</i>	α Dimerization	
γ	47	<i>dnaX</i>	Binds ATP	γ Complex "clamp loader"
δ	38.7	<i>holA</i>	Binds β	
δ'	37	<i>holB</i>	Induces ATPase of γ	
χ	16.6	<i>holC</i>	Binds SSB-DNA	
ψ	15.2	<i>holD</i>	Unknown	
β	40.6	<i>dnaN</i>	Sliding clamp on DNA	

merase is neither fast nor processive by itself⁷ but becomes fast and processive in the presence of both the β subunit and a five-protein subassembly called the γ complex.¹³⁻¹⁵ Study of the processive polymerase showed it assembled in two steps. First the γ complex and β form a tight "preinitiation complex" on primed DNA, and then, in a second, ATP-independent step, the core polymerase joins the complex to form the processive enzyme (see FIG. 1).¹³⁻¹⁵ Thus, the tight grip of pol III holoenzyme to DNA has nothing to do with the polymerase, but instead resides entirely in the accessory proteins. Structural studies showed the preinitiation complex consists only of a dimer of the β subunit, which restores full processivity to the core polymerase.¹⁶ The γ complex, while not needed during elongation, is needed to place β onto DNA; and study of its function showed that it specifically recognizes a primed template junction that elicits a cryptic ATPase and couples ATP hydrolysis to place the β dimer onto DNA.¹⁷ The γ complex then dissociates from the DNA, leaving only the β dimer, and can act catalytically to place multiple β dimers onto different DNA templates.^{14,16} However, the γ complex within the holoenzyme particle stays on the DNA through its associations with other pol III holoenzyme subunits.^{2,18} Hence, this artificial system, using only this subset of proteins, reveals the ability of the γ complex to act catalytically in assembly of β on DNA.

THE β SLIDING CLAMP

The nature of the contact between DNA and the β dimer has been studied, with the finding that β freely slides on duplex DNA in a bidirectional and ATP-independent fashion.¹⁶ A surprising property of the β -DNA complex was its very tight binding to circular DNA but its complete lack of affinity to linear DNA.¹⁶ The lack of affinity to linear DNA was due to the β dimer sliding off the ends of DNA, as evidenced by ability of a protein bound near the ends to block dissociation of β from linear DNA.¹⁶ Proteins typically bind DNA through direct chemical attractive forces (i.e., hydrogen bonds, ionic interaction, hydrophobic forces), and upon sliding to the end of DNA, such protein would not simply give these attractive forces up to water but would instead stay put on DNA at the end or slide back the other way. Hence, it appeared that β may interact with DNA more like catenated DNA circles do,

through their topology. Catenated rings are tightly bound to each other, but upon cutting one, the other circle slides right off. Hence, it was hypothesized that β may also be bound to DNA topologically and may perhaps be shaped like a doughnut, with DNA through the hole in the middle.¹⁶ These dynamics of β on DNA are consistent with the descriptive term "sliding clamp" that had been coined for the action of the phage T4 accessory proteins (see FIG. 1).^{19,20}

How would a ring-shaped protein clamp confer processivity to a polymerase? The simplest notion is that the β ring would bind directly to the polymerase (FIG. 2). As the polymerase extends DNA, it would pull β along in back by passive diffusion. Then, as the polymerase tries to dissociate from the DNA, the β ring would act as a tether to continuously hold the polymerase down to DNA and make it highly processive. Several lines of evidence now exist for a direct interaction between β and the α subunit, the simplest being that β and α form a direct protein-protein complex in the complete absence of other proteins and DNA.¹⁶

STRUCTURE OF β

Subsequent crystal structure analysis of β showed that the dimer was indeed in the shape of a ring, with a central cavity of sufficient diameter to accept duplex DNA (FIG. 3A).²⁰ The center is lined with 12 α helices, and these are the only helices in the entire molecule. The outside perimeter of β is one continuous layer of antiparallel beta sheet structure all around the ring. The dimer interface is also formed by this same continuous layer of sheet. The 12 α helices of the β dimer are tilted such that they are oriented perpendicular to the phosphate backbone of duplex DNA and are of sufficient length to span the major and minor grooves. Hence, these helices may act as "protein crossbars" to prevent β from entering the grooves of DNA while sliding.

Turned on its side, the β ring is rather thin, approximately one turn of duplex DNA (FIG. 3B). The head-to-tail arrangement of the dimer gives two physically distinct faces: one is rather flat, and the other has several protruding loops. It seems likely that the polymerase will bind only one side of β , as the polymerase subunit, α , is not that much larger than a β dimer. Hence the γ complex must use the 3' terminus of a primed template as a guidepost during assembly of β onto DNA in

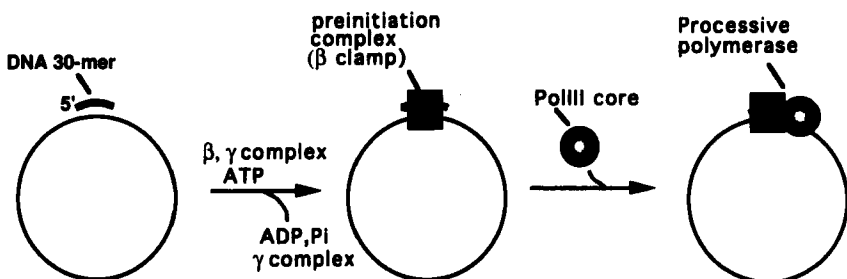


FIGURE 1. The accessory proteins form a protein clamp on DNA. Assembly of the processive polymerase proceeds in two steps. First, the γ complex couples ATP to transfer a β dimer (preinitiation complex) to a primed circular single-strand DNA coated with SSB. In a second step, which is ATP independent, the core polymerase associates with the β clamp, making it highly processive.

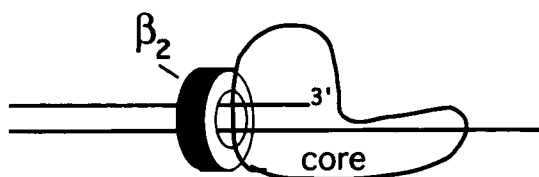


FIGURE 2. The β clamp tethers the core polymerase to DNA. A β clamp bound to DNA by topology is depicted as encircling DNA. The β subunit binds directly to the α subunit of the core polymerase and may slide along in back of core during synthesis, thus continuously tethering the polymerase to DNA for high processivity.

order to orient the correct face of β toward the primer terminus for productive coupling to the polymerase subunit (FIG. 4).

An unexpected feature of β prior to the crystal structure was its high degree of internal symmetry. The dimer has a six-fold appearance (FIG. 3A) even though it is a dimer and its only true axis of symmetry is two-fold. This six-fold appearance derives from the fact that each monomer is composed of three globular domains, which have very nearly the same three-dimensional structure.²⁰ Presumably, β evolved from two successive gene duplication events to produce a fused timer. However, over the course of evolution the amino acid sequences of these domains have diverged beyond recognition of any homology.

The distance between DNA modeled into the center of β and side chains in the central cavity is 5 angstroms, sufficient room for 1–2 layers of water molecules. This distance seems fitting for a protein sliding clamp designed to have a minimum of specific interactions with the DNA.

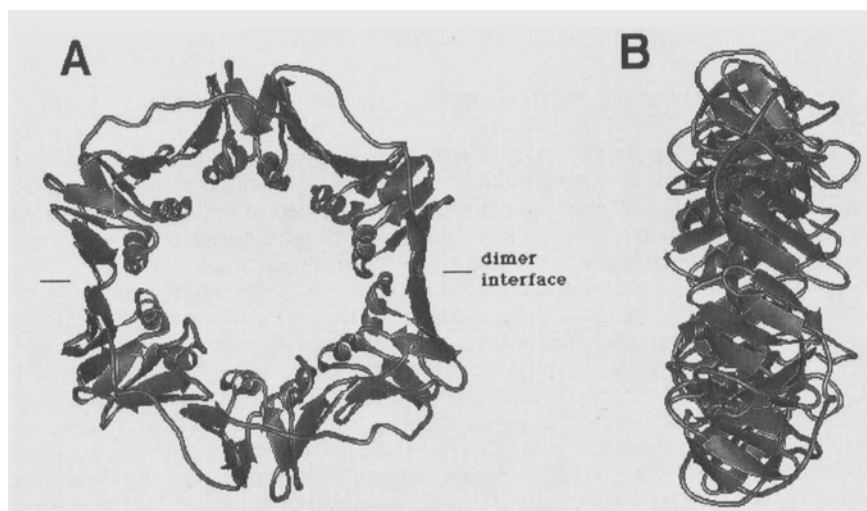


FIGURE 3. Ribbon diagram of the β dimer. (A) Front view. Twelve α helices line the inner cavity of 35-angstrom diameter. The outside of β is a continuous layer of sheet structure even across the dimer interfaces. (B) Side view. The right side has loops that extend further into solution than those on the left side.

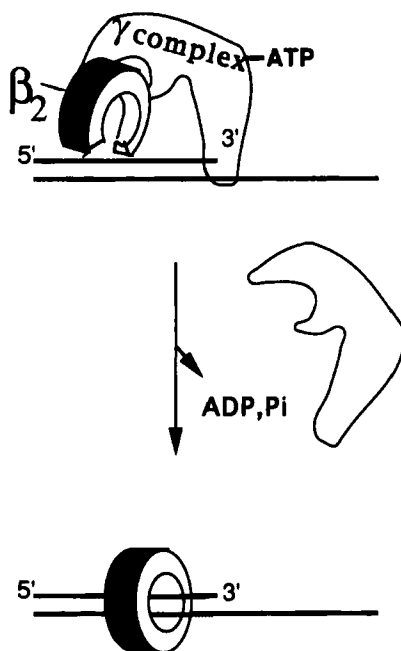


FIGURE 4. The γ complex orients the β clamp using the primer terminus as a guidepost. **Top:** The γ complex recognizes a 3' primer terminus and binds β and ATP. Perhaps one interface of β is opened. **Bottom:** β is closed around DNA upon hydrolysis of ATP, and the γ complex dissociates from DNA.

HOMOLOGY OF Pol III HOLOENZYME TO OTHER REPLICASES

The subunit organization of pol III holoenzyme is similar to that of polymerase δ of humans and yeast and to the replicase of T4 phage. In each of these systems the replicase is composed of three parts (TABLE 2): a polymerase, and several accessory proteins that assort into two categories—a complex of proteins and a single subunit. In each case the accessory complex and single subunit form a tight protein complex on primed DNA in a reaction that consumes ATP, and this complex confers high processivity onto the respective polymerase (see TABLE 2 for references). The amino acid sequence of some subunits in the γ complex show homology to the sequence of subunits in the corresponding RF-C complex of humans and to the gene 44 protein of the T4 phage g44/g62 protein complex.²² The PCNA protein of humans and yeast and also the g45 protein of phage T4 are highly acidic proteins like β , and a case has been made for these proteins having a ring shape from the sequence alignment of their hydrophobic residues with the buried hydrophobic core residues of β .²⁰ Further, Peter von Hippel's lab has visualized the sliding clamp of the phage T4 replicase in the electron microscope; this has similar dimensions to the β subunit, and duplex DNA passes straight through its center.²³ Hence, it would appear that the basic mechanism of achieving highly processive replication has been conserved throughout evolution.

TABLE 2. Common Subunit Structure among Several Chromosomal Replicases

Organism	Polymerase	Accessory Complex "Clamp Loader"	Accessory Subunit "Sliding Clamp"
<i>E. coli</i>	Core ($\alpha\epsilon\theta$)	γ Complex (5 proteins)	β
Phage T4	g43 Protein	g44/62 Complex	g45 Protein
Yeast	Polymerase δ	RF-C (5 proteins)	PCNA
Humans	Polymerase δ	RF-C (A-I) (5 proteins)	PCNA

NOTE: Reviews on the subunit structures of these polymerases are as follows: T4 phage, Refs. 20, 34; *E. coli*, Refs. 1, 35, 36; eukaryotic polymerase δ , Refs. 37–39.

MANIPULATION OF THE β RING DURING CHROMOSOME REPLICATION

The lagging strand of a chromosome is replicated as a series of discontinuous fragments. In *E. coli* these fragments are 1–2 kb each and are formed every second or two. Due to the scarcity of pol III holoenzyme in the cell, the polymerase must be efficiently recycled from the end of a completed fragment onto a new primer for the next Okazaki fragment. However pol III binds tightly to DNA even after full replication of a template,^{18,24,25} so how does it let go? Perhaps the polymerase "knows" when it has finished a template (i.e., fragment) and then opens the β ring, lets DNA go, and rebinds a new primer. However, several studies have shown pol III holoenzyme remains firmly fixed on its product duplex and will not transfer to a new substrate even after it has replicated the initial DNA to the last nucleotide.^{18,24,25}

Recently a specific mechanism for recycling of the polymerase has been described. In this reaction, the polymerase rapidly releases the product duplex DNA, provided one adds to the reaction a second primed DNA that already contains a β clamp.^{15,18} An important feature of this reaction is that the polymerase will not cycle to the new β clamp unless it has first completely replicated the initial template to the last nucleotide. Another important feature of this reaction is that the β clamp first becomes disengaged from the rest of the polymerase and slides away from it, creating a vacancy for the next β clamp.²⁶ This disassembly of β from the polymerase occurs only upon completion of the DNA template. The polymerase remains firmly bound to the product and awaits collision with the new β clamp on the next template, which then extracts the polymerase from the original DNA. The initial β clamp is left behind, sliding along the product duplex.²⁶ The implication of these protein dynamics for replication of the lagging strand is illustrated in FIGURE 5. First, the polymerase extends an Okazaki fragment to completion, and then it disengages its β clamp. After this, the polymerase is handed off to a new β clamp on the next primer through a bimolecular collision reaction, leaving behind its original β clamp. By this route the polymerase is repositioned back near the fork for the next round of processive extension of another Okazaki fragment.

The model of FIGURE 5 is a simplistic version of the replication fork as it shows only events on the lagging strand. In FIGURE 6 the full subunit structure of pol III

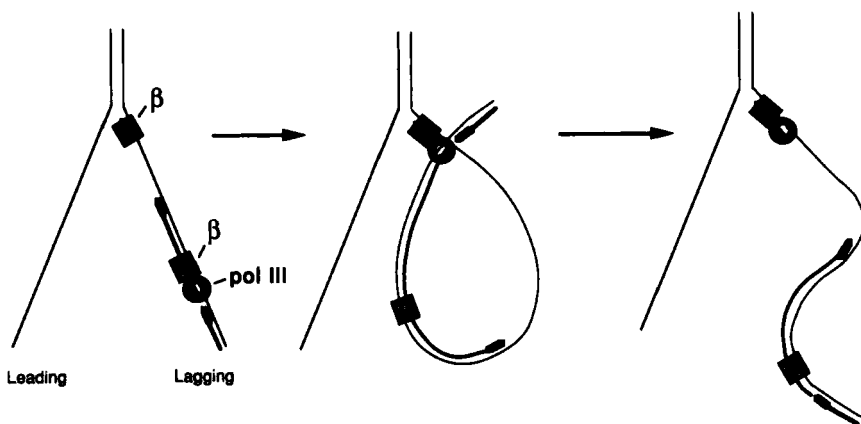


FIGURE 5. Polymerase transfer on the lagging strand. **Left:** A β clamp is placed on an upstream primer as the polymerase extends on Okazaki fragment. **Middle:** Upon complete extension of the fragment, the β clamp is disengaged, and the polymerase is handed off to the upstream clamp in a bimolecular collision. **Right:** Polymerase transfer is complete, resulting in the processive polymerase positioned for extension of the next Okazaki fragment.

holoenzyme is set into the context of the replication fork. The overall subunit organization of pol III holoenzyme is illustrated in the top of the figure (FIG. 6A). Although the τ subunit is not needed to place β onto DNA or to make the core polymerase processive, the τ subunit fulfills the important function of holding all the other proteins together in one particle.³ The τ subunit binds directly to the polymerase subunit α ²⁷ and is a tight dimer, providing it with the ability to bind two core polymerases, as illustrated in FIGURE 6A.^{26,28} It has been hypothesized that a chromosomal replicase should have two polymerases for concurrent replication of both strands of duplex DNA, and therefore the two cores are shown on the leading and lagging strands of a replication fork, and each have a β clamp for processivity.^{29,30} The τ and γ subunits share sequence through a translational frameshift, which generates the truncated product γ that is missing the C-terminal 2/3 of τ , a region needed to bind the core polymerase.³¹⁻³³ The τ subunit is also required to bring the γ complex into the pol III holoenzyme structure through direct contact between τ and γ .³

In FIGURE 6B the lagging polymerase has completed an Okazaki fragment and has disengaged the β clamp. The γ complex then assembles a new β clamp on an upstream RNA primer (FIG. 6C) and delivers it into the lagging strand polymerase (FIGURE 6D), complementing one round of fragment synthesis and resulting in a processive polymerase on the new primer for the next Okazaki fragment. The catalytic action of the γ complex in assembly of multiple β clamps on different primed templates makes an attractive arrangement in which it could load a β dimer onto the RNA primer during the time that the lagging strand polymerase is busy extending an Okazaki fragment. This action could save time in the lagging strand cycle, as an upstream β clamp could be ready and waiting for the polymerase to finish an Okazaki fragment.

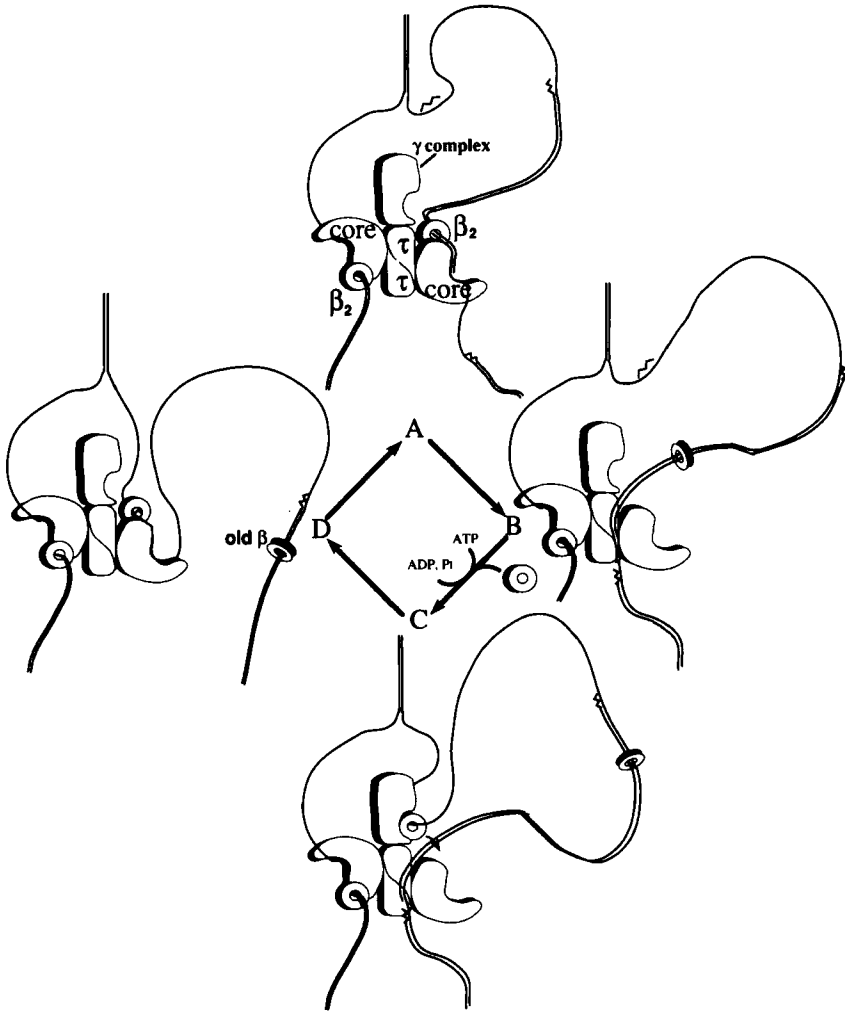


FIGURE 6. Model of subunit dynamics within the holoenzyme structure during chromosome replication. (A) The dimeric polymerase structure of pol III holoenzyme is set into the context of the replication fork. The leading strand is the **left**, and the lagging strand is the **right**. The helicase and primase are not shown but are present at the top of the fork. See text for details. (B) The Okazaki fragment is complete, and the β clamp slides away from the lagging core polymerase. (C) The γ complex places a β dimer on the upstream RNA primer. (D) The lagging polymerase cycles to the new β clamp for the next Okazaki fragment.

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DISCUSSION OF THE PAPER

P. H. VON HIPPEL (*University of Oregon, Eugene, Oreg.*): One thing you implied in a few of your cartoons but didn't say explicitly has to do with the question of the