

DNA POLYMERASE III HOLOENZYME: Structure and Function of a Chromosomal Replicating Machine

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ABSTRACT

DNA polymerase III holoenzyme contains two DNA polymerases embedded in a particle with 9 other subunits. This multisubunit DNA polymerase is the *Escherichia coli* chromosomal replicase, and it has several special features that distinguish it as a replicating machine. For example, one of its subunits is a circular protein that slides along DNA while clamping the rest of the machinery to the template. Other subunits act together as a matchmaker to assemble the ring onto DNA. Overall, *E. coli* DNA polymerase III holoenzyme is very similar in both structure and function to the chromosomal replicases of eukaryotes, from yeast all the way up to humans. This review summarizes our present knowledge about the function of the 10 subunits of this replicating machine and how they coordinate their actions for smooth duplication of chromosomes.

INTRODUCTION

The main function of DNA polymerase III holoenzyme (holoenzyme) is duplication of the *E. coli* chromosome, although it acts in other areas of DNA metabolism as well (1). Holoenzyme shares special features with replicases of eukaryotes, viruses, prokaryotes, and their phages, which distinguishes holoenzyme from single-subunit polymerases such as DNA polymerase I (Pol I). Among these features are a multisubunit structure, the requirement for ATP to clamp tightly to DNA, the rapid speed of DNA synthesis, and a remarkably high processivity, such that the enzyme remains bound to DNA for thousands of polymerization events (1, 2). Replicases of most systems share amino acid sequence homology to holoenzyme. Hence, holoenzyme is likely to serve as a faithful guide to understanding the basics of replicase action in other systems.

Holoenzyme functions at the point of the replication fork with other proteins. Replication of the chromosome entails separation of the duplex DNA by helicase and topoisomerase, followed by semidiscontinuous synthesis of DNA at a speed of about 1 kilobase (kb) per second (3). The discontinuous strand (lagging) is synthesized by holoenzyme acting with a priming apparatus for repeated initiation and extension of 2000–4000 Okazaki fragments. These fragments are only 1–2 kb in length, and therefore each is completed within 1–2 s. The intracellular scarcity of holoenzyme [10–20 molecules (4)] necessitates rapid recycling upon completing one fragment and transfer to a new primer for the next fragment. Holoenzyme is clamped tightly to DNA by a sliding-clamp subunit that completely encircles the duplex (5, 6), but despite this tight grip to DNA, holoenzyme has a novel mechanism allowing it to rapidly cycle on and off DNA for action on the lagging strand (7–9).

There have been several reviews on holoenzyme in the past few years (2,

10–14), and this review is an update since the last in this series (12). The outline of how holoenzyme functions at a replication fork is presented; the reader is referred to recent reviews for more information (2, 10, 15–17, 17a). Holoenzyme also functions in repair and mutagenesis, and excellent reviews on these subjects have appeared recently (18–21).

THE HOLOENZYME PARTICLE

DNA polymerase III was first identified as the chromosomal replicase on the basis that extracts of temperature-sensitive mutants in the essential *dnaE* gene contained temperature-sensitive DNA polymerase III activity (18–24). Initial purification of DNA polymerase III utilized a template DNA that was nicked and gapped by nuclease action, and probably led to purification of the three-subunit subassembly that is now called DNA polymerase III core (core) (25). Subsequent studies utilized primed circular single-stranded (ss) DNA genomes of bacteriophages M13, G4, and ϕ X174 as templates, which led to purification of holoenzyme and its subassemblies (26–33).

These early studies were hampered by the low abundance of holoenzyme in *E. coli*. There are only 10–20 copies of holoenzyme in the cell, and purification of one mg to near homogeneity requires 7400-fold enrichment from 2–3 kg of cells (29). Despite its scarcity, study of holoenzyme and its subassemblies outlined many important features of this replicating machine. For example, holoenzyme was found to be exceedingly rapid in DNA synthesis—approximately 750 nucleotides/s—consistent with the observed rate of fork movement in *E. coli* (34) and much faster than the 10–20 nucleotides/s of Pol I (35). This rapid rate results from the high processivity of holoenzyme, which extends a chain for several thousand nucleotides without dissociating from the template even once (36, 37). In contrast, Pol I dissociates rapidly from DNA, extending a primer only 10–50 nucleotides for each template-binding event (34). Holoenzyme is also distinguished from Pol I in a requirement for ATP hydrolysis (26, 30). The ATP is only needed initially by holoenzyme to clamp onto a primed template; afterward holoenzyme is rapid and processive without additional ATP (38, 39). Upon encounter with a duplex region, holoenzyme simply diffuses over the duplex, searches out the next 3' end, and reinitiates processive extension without additional ATP (40).

Identification of all the genes encoding the 10 subunits of holoenzyme has been completed recently, the proteins overproduced and purified, and the holoenzyme reconstituted from them. In Table 1 the 10 different subunits are listed in an order that explains which subunits are present in the various subassemblies of holoenzyme. The core polymerase consists of the α , ϵ , and θ subunits (41). The Pol III' subassembly contains two cores and a dimer of τ (42, 43). The presence of two polymerases in one molecular structure sup-

Table 1 DNA Polymerase III holoenzyme subunits and subassemblies

| Subunit | Gene | Mass (kDa) | Function | Subassembly | |
|------------|---------------------------|------------|---|---------------------|-----------|
| α | <i>dnaE</i> | 129.9 | DNA polymerase |] core |] PolIII' |
| ϵ | <i>dnaQ</i> , <i>mutD</i> | 27.5 | Proofreading 3'-5' exonuclease | | |
| θ | <i>holE</i> | 8.6 | Stimulates ϵ exonuclease | | |
| τ | <i>dnaX</i> | 71.1 | Dimerizes core, DNA-dependent ATPase | | PolIII* |
| γ | <i>dnaX</i> | 47.5 | Binds ATP |] γ -complex |] PolIII* |
| δ | <i>holA</i> | 38.7 | Binds to β | | |
| δ' | <i>holB</i> | 36.9 | Cofactor for γ ATPase and stimulates clamp loading | | |
| χ | <i>holC</i> | 16.6 | Binds SSB | | |
| Ψ | <i>holD</i> | 15.2 | Bridge between χ and γ | | |
| β | <i>dnaN</i> | 40.6 | Sliding clamp on DNA | | |

ports the hypothesis that replicative polymerases act in pairs for coordinated replication of both strands of a duplex chromosome (discussed later). The Pol III* assembly contains 9 different subunits; it lacks only β (44). The polymerase activity of each of these subassemblies can be distinguished on the basis of adding either spermidine, ssDNA-binding protein (SSB protein), ethanol, or salt to the assays (36, 45). In general the polymerases become more processive as their subunit complexity increases, but the very high speed and processivity of holoenzyme absolutely requires the β clamp (36, 45). The five-subunit γ complex is a matchmaker that couples ATP hydrolysis to load β clamps on primed DNA (7, 31, 46).

THE CORE POLYMERASE

Core contains the DNA polymerase and proofreading exonuclease activities (47). There are approximately 40 molecules of core in the cell, and therefore only half are assembled into holoenzyme (47). The three subunits of core are tightly associated and cannot be resolved short of denaturation. Individual subunits are provided through use of the genes. Study of α showed it to be the DNA polymerase (8 nucleotides/s), but it lacked exonuclease activity (47, 48). The isolated ϵ subunit is a potent 3'-5' exonuclease (49), consistent with the *dnaQ/mutD* mutator phenotype (50, 51). The α and ϵ subunits form a tight 1:1 complex, resulting in increases in both polymerase activity (34) and exonuclease activity (52). The rate of digestion of ssDNA by ϵ is similar to that of core, but hydrolysis of double-stranded (ds) DNA by ϵ requires α for significant activity (52). Presumably the primer template-recognition site of α brings ϵ in contact with a basepaired 3' end. The function of θ has yet to be identified, except for a slight stimulation of ϵ activity on a mismatched T-G basepair (53). The θ subunit binds ϵ but not α , suggesting a linear α -to- ϵ -to- θ arrangement in core, and structural analysis shows a single copy of each subunit (53).

Core synthesizes DNA at a rate of approximately 20 nucleotides/s and is processive for 11 nucleotides (36), similar to Pol I. However, on a singly primed ssDNA viral template, core is the weakest polymerase known. It cannot extend a unique primer full circle around a natural template no matter how much core is added or how long one waits (54). Presumably some DNA structures are absolute barriers to chain extension by core.

Ironically, core becomes the fastest polymerase in the presence of its accessory proteins (discussed below). In the absence of ϵ , α is stimulated by the accessory proteins, but the processivity drops to 500–1500 nucleotides, and the intrinsic speed is half that of core (34). With accessory proteins, the $\alpha\epsilon$ complex is as fast and processive as core (34). Hence, ϵ has effects on the speed and processivity of holoenzyme, not just fidelity. On the other hand, θ

has no effect on the efficiency of $\alpha\epsilon$ (53). These results are consistent with the growth defect of *dnaQ* (ϵ) mutants and lack thereof in *holE* (θ) null mutants (53) (described in the GENETICS section).

THE β DNA SLIDING CLAMP

The ATP-activated grip of holoenzyme to primed DNA is inherent in the accessory proteins, γ complex and β subunit. The γ complex can be resolved from the holoenzyme only by harsh treatment (29), but some γ complex exists in free form and can be purified alone (56). Presumably, γ complex was the active ingredient in elongation factor II (30). In contrast, the β subunit departs from holoenzyme easily and can be separated on a phosphocellulose column [used to be called copol III* (26, 27) and elongation factor I (30)]. The intracellular abundance of β (300 dimers per cell) made its purification possible without having to resort to resolving it from purified holoenzyme (57). Early studies using partially pure preparations indicated that γ complex coupled ATP to the assembly of β onto DNA (31). A reinvestigation of this reaction using pure proteins and primed DNA coated with SSB protein confirmed the earlier observation. One dimer of β is chaperoned to DNA in an ATP-dependent reaction catalyzed by γ complex in the absence of core to form the "preinitiation complex" (7, 46). In a second stage, the core assembles with the preinitiation complex to form the "initiation complex" in a reaction that does not require ATP (7, 31). Hence, holoenzyme has two components that recognize a primer terminus: the core polymerase and the accessory proteins themselves.

The γ complex has only weak affinity for ss and ds DNA, although it does bind to ssDNA coated with SSB protein (described later). The γ complex easily departs into solution after it places β onto primed DNA. This " β -only" preinitiation complex retains the capacity to restore highly efficient synthesis onto core (see Figure 1) (5). Following departure from the β -DNA complex, the γ complex is still active and is able to place multiple β dimers on DNA, accounting for the high specific activity of the γ complex (5, 29, 56).

The γ complex can place β onto a singly nicked plasmid (RF II), and upon linearizing the circular plasmid with a restriction enzyme, β dissociates from DNA, implying β has mobility on DNA and can slide off over ends (5). This behavior of β on DNA allowed reasoning of the nature of the β -DNA interaction. Since the affinity of β to DNA depends on the geometry of the DNA molecule, β must likewise be bound to DNA by virtue of its protein topology (i.e. by encircling the DNA like a doughnut). If the main attraction of β to DNA were through chemical forces (i.e. ionic, hydrophobic, or hydrogen bonds), as is the case with all other DNA-binding proteins before β , then upon reaching the end, β would have remained bound to DNA rather than give up its tight chemical grip.

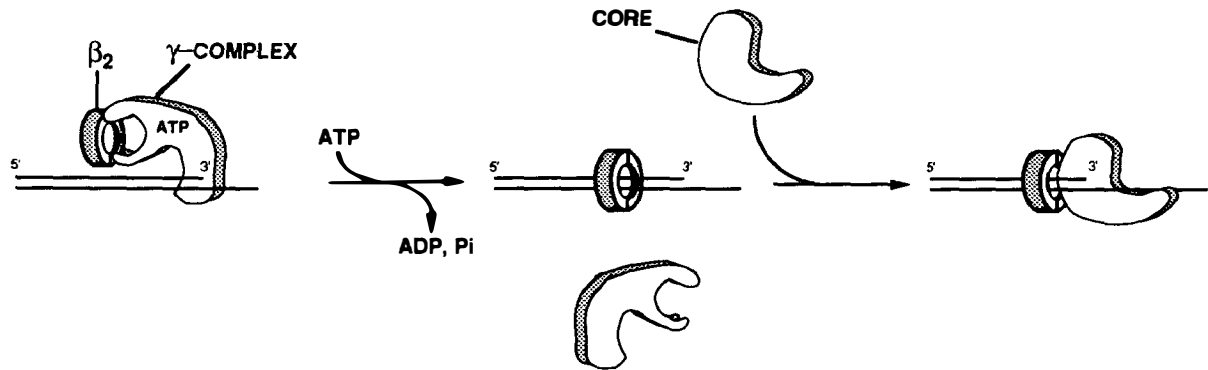


Figure 1 Two-stage assembly of a processive polymerase. The γ complex recognizes a primed template and couples hydrolysis of ATP to assemble β onto DNA. The γ complex easily dissociates from DNA and can resume its action in loading β clamps on other DNA templates. In a second step, core assembles with the β clamp to form a processive polymerase.

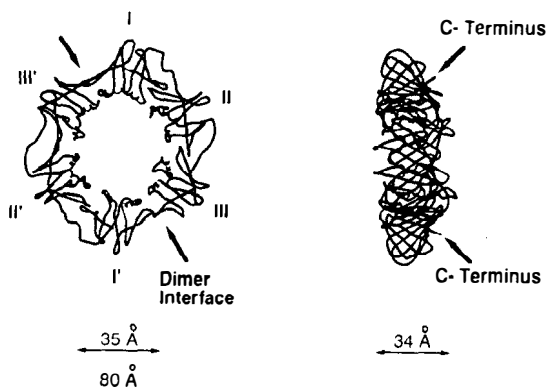


Figure 2 Structure of the β subunit. *Left*—The central cavity is lined with 12 α helices, and the outside perimeter is one continuous layer of sheet structure, which also forms the intermolecular boundaries (arrows). The sixfold appearance stems from three globular domains that compose each monomer. These domains have the same polypeptide chain-folding pattern. The six domains are labeled I, II, and III on one monomer and I', II', and III' on the other monomer. *Right*—The β dimer is turned 90° relative to the view on the left. The thickness of the β ring is approximately equal to one turn of β -form DNA. The two C termini extrude from the same face of the ring (arrows). Dimensions of the inner and outer diameters of the ring and the thickness of β are shown below the diagrams.

A simple mechanism by which a β “sliding-clamp” confers processivity to core is by directly binding core, thus tethering it to DNA; the clamp would be passively pulled along with core during polymerization. Consistent with this notion, β binds to core through the α subunit even in the absence of DNA (5, 58, 59).

The β subunit as a sliding-clamp doughnut was confirmed by X-ray structure analysis (6). The β appeared as a ring-shaped head-to-tail dimer with a central cavity of sufficient diameter to accommodate duplex DNA (Figure 2). The central cavity is lined with 12 α helices, and the ring is encased by one continuous layer of antiparallel pleated sheet along the outside. The β dimer has a six-fold appearance even though it only has a true two-fold rotational axis of symmetry. The apparent six-fold symmetry derives from a three-fold repetition of a globular domain in the monomer (six domains in the dimer). The three domains have no significant amino acid homology, yet they are nearly superimposable.

The 12 α helices lining the central cavity have a common tilt and lie perpendicular to the phosphate backbone of duplex DNA. Hence, the helices may act as crossbars to prevent β from entering the grooves of DNA and facilitate the sliding motion. Further, β is quite acidic ($pI = 5.2$) and would be

repelled by DNA, but there is a net positive surface potential inside the cavity. There is room for 1–2 layers of water molecules between the DNA and the α helices, which may insulate β from local interaction with DNA. Those that are interested in other features of the β structure are referred to several reviews (60–62).

THE γ COMPLEX MATCHMAKER

The β dimer does not assemble onto DNA by itself. The γ complex is a molecular matchmaker that hydrolyzes ATP to load β clamps onto DNA. The γ complex is composed of five different subunits in the stoichiometry $\gamma_2\delta_1\delta'_1\chi_1\psi_1$ (56, 63). The δ and δ' subunits, originally thought to be related by proteolysis, are distinct proteins encoded by different genes (64–67). Interestingly, the δ' amino acid sequence shows homology to γ and τ (64, 67, 68). The γ , τ , and δ' subunits are further characterized by their appearance as doublets on an SDS polyacrylamide gel (64, 67, 69). The physical basis and the function of this microheterogeneity are not known.

The γ complex can be fragmented into a $\gamma\chi\psi$ complex (125.8 kDa) and a $\delta\delta'$ complex (75.6 kDa), and $\delta\delta'$ can be further resolved into δ and δ' (69). In early studies using partially pure fractions, the γ complex activity (elongation factor II) was subdivided into two factors, one of 125 kDa (called DnaZ protein) and one of 63 kDa (called elongation factor III) (31, 32). Presumably these factors were $\gamma\chi\psi$ and $\delta\delta'$, respectively.

The genes encoding each subunit of γ complex have now been identified (64–67). The proteins have been overproduced, purified (65, 70), and used to reconstitute the γ complex in abundance (63). No one subunit alone can assemble β onto DNA (8, 69, 74). At low ionic strength, a combination of γ and δ assembles β onto DNA, but the reaction is feeble; the δ' subunit is needed for an efficient reaction (65, 69, 75). The χ and ψ subunits are also needed at an ionic strength commensurate with that inside the cell (69).

Role of ATP

The γ complex has weak DNA-dependent ATPase activity and is stimulated by β (75). The best effector is a primed template. The only subunit of γ complex with an exact match to an ATP binding site motif is γ (76, 77), and γ binds ATP with a K_d of 2 μ M (78). The γ subunit lacks significant hydrolysis activity even in the presence of DNA (75, 78). Significant DNA-dependent ATPase activity of γ requires δ and δ' , implying that the $\gamma\delta\delta'$ complex recognizes the DNA template (65, 75). ATP is crosslinked to δ upon irradiation with UV light (79), and the δ sequence shows a close match to an ATP site sequence (64, 66). However, evidence against a role for ATP binding in δ action, at least in β assembly, has been gained by replacing the Lys of the putative ATP-binding

site in δ with an Ala. The γ complex constituted using the mutated δ is as active as wild-type γ complex in assembly of β on DNA, and in DNA-dependent ATPase activity (H Xiao, M O'Donnell, unpublished). Mutation of the ATP binding site of γ and subsequent constitution into the γ complex destroys the ATPase activity and ability to assemble β onto DNA. Further, ATP binding site mutants of γ and τ , expressed from a plasmid, fail to complement a conditional lethal *dnaX* strain (J Walker, personal communication).

Holoenzyme hydrolyzes two molecules of ATP upon forming an initiation complex on primed DNA (39). Presumably the action here lies with the γ complex in assembling β onto DNA. That two ATP are hydrolyzed indicates that each γ protomer hydrolyzes one ATP during assembly of β onto DNA (43, 78). τ is also a DNA-dependent ATPase, however, and may contribute to the observed hydrolysis (75, 78, 80).

The K_d for interaction of Pol III* with β is approximately 1 nM in the presence of ATP; in the absence of ATP the interaction is undetectable (81). The γ complex also binds β in an ATP-dependent manner (V Naktinis, M O'Donnell, unpublished). Study of individual subunits of γ complex shows that only δ interacts with β (63). The δ -to- β interaction does not depend on ATP. A simple mechanism to explain the ATP dependence of the γ complex- β interaction and the lack of an ATP requirement for the δ - β interaction is that δ is buried within γ complex and ATP induces a conformational change that presents δ for interaction with β (Figure 3).

Addition of a large excess of β to holoenzyme circumvents the need for ATP in forming a processive polymerase (82, 83). This interesting observation implies that ATP is not needed for the β ring to open and close around DNA. However, these studies were performed using linear templates, and β may have threaded over a DNA end without opening. Indeed, ATP-independent thread-

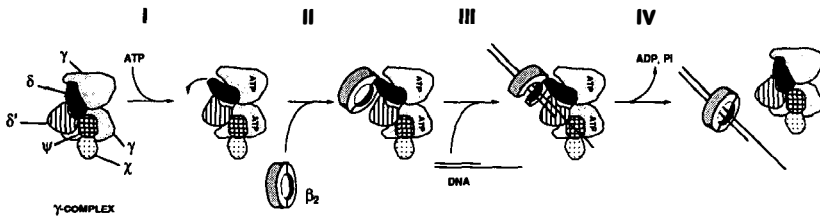


Figure 3 Putative action of γ complex in assembly of a β clamp on DNA. The diagram of γ complex is consistent with the known stoichiometry and contacts between the subunits (γ - γ , γ - ψ , γ - δ' , δ - δ' , and χ - ψ). In the first diagram the surface of δ that interacts with β is buried to explain its inability to bind β in the absence of ATP. Upon binding (or hydrolyzing) ATP, a conformational change exposes δ (step I) for binding β (step II). The γ complex then recognizes a primed template, thus bringing β into proximity with DNA (step III). In step IV, hydrolysis of ATP (or loss of ADP, P_i) sequesters δ back within γ complex, severing the δ - β contact and allowing β to snap shut around DNA and γ complex to dissociate. The β subunit is shown as opening at one interface and then reclosing; other possibilities exist, however, as discussed in the text.

ing of proliferating cell nuclear antigen (PCNA) (the eukaryotic homolog of β) over DNA ends has been observed (84). Further studies on the ATP-independent stimulation of holoenzyme by excess β are necessary to determine what insight the reaction provides into the clamp-loading mechanism.

Interaction of γ Complex with SSB Protein

The γ complex binds ssDNA coated with SSB protein, but not naked ssDNA (85). Study of individual subunits of γ complex showed that only χ interacts with SSB protein (Z Kelman, M O'Donnell, unpublished). The affinity of χ for SSB protein was strengthened approximately eightfold by the presence of ssDNA. The χ -to-SSB protein contact is sensitive to ionic strength and may underlie the known salt sensitivity of holoenzyme initiation complex formation (86). Holoenzyme is more resistant to potassium glutamate than to any other salt (86), consistent with potassium glutamate as the physiological osmolyte of *E. coli* (87).

A clue to further roles of the χ -to-SSB-protein contact may be taken from study of mutant SSB proteins (reviewed in 88, 88a, 89). One SSB protein mutant, *SSB-113*, has a pleiotropic phenotype including defects in replication and recombination. The *SSB-113* is a missense mutant in which the penultimate amino acid at the C terminus, Pro176, is replaced with Ser. *SSB-113* binds ssDNA as tightly as wild-type SSB protein, leading to the suggestion that the C terminus of SSB protein may interact with proteins. Study of χ and *SSB-113* shows χ does not interact with *SSB-113*, implying that χ may be involved in one or more of the *SSB-113* phenotypes (Z Kelman, M O'Donnell, unpublished).

Mechanism of the γ Complex Clamp Loader

A mechanism by which γ complex may assemble β around DNA is hypothesized in Figure 3. Upon binding (or hydrolysis) of ATP by the γ complex, δ is presented for interaction with β . The γ complex recognizes a primed template, possibly aided by the χ -to-SSB protein contact. The interaction of γ complex with both DNA and β , positions β near the primer terminus where it can be assembled around the DNA.

Exactly how the β ring is opened and closed around DNA and how ATP hydrolysis is coupled to the process are unknown. Three possible mechanisms are: (a) Only one interface of the β dimer is opened and closed around DNA (as in Figure 3), (b) both interfaces are opened followed by reforming the dimer around DNA, and (c) the DNA is cut and rejoined after being threaded through the β ring.

A rapid monomer-dimer equilibrium for β ($K_d = 35$ nM) has been reported in the presence of magnesium (90, 91), suggesting the β dimer is inherently unstable and implying that β may come apart at both interfaces during assembly

on DNA. In another study, however, the rate of subunit exchange between β dimers was slow, with a half-life of 3 h at 37°C, suggesting the dimer is quite stable (92). Further studies using β and γ complex are needed to define the mechanism of β assembly onto DNA.

THE τ SUBUNIT

The length of DNA needed to code for the combined mass of τ (71 kDa) and γ (47 kDa) is 3.2 kb. However, the region of DNA expressing both τ and γ is only 2.1 kb (93, 94). Further study showed γ is formed from the same gene that encodes τ (*dnaX*) by an efficient translational frameshift, which produces γ in amounts equal to those of τ (95–97). As a result, γ is the N-terminal 430 residues of τ followed by a unique C-terminal Glu. One may consider that the holoenzyme is composed of two populations: those with γ and those with τ . Examination reveals, however, that each holoenzyme molecule contains both γ and τ (98).

The τ subunit is a DNA-dependent ATPase of ill-defined function (80). From studies using pure subunits, a “ τ complex” ($\tau\delta\delta'\chi\psi$) can be assembled and is active in loading β clamps on DNA (65, 69). Whether τ serves such a role in holoenzyme action is not known. Inability to isolate a τ complex from cell lysates suggests that τ complex is not present *in vivo*, and thus that τ ATPase may be put to another task.

The τ and γ subunits are the only subunits of Pol III* with oligomeric structure. The τ dimer binds two molecules of core (42, 43). The γ subunit does not bind core, and therefore the C-terminal sequence unique to τ is responsible for the τ -core interaction. Indeed, mutation of the C-terminal region of τ destroys cell viability, suggesting that the ability of τ to dimerize core is an essential function (99).

ASYMMETRIC STRUCTURE OF HOLOENZYME

Synthesis of the leading strand and synthesis of the lagging strand are quite different. The leading-strand polymerase need only remain clamped to DNA continuously, but the lagging strand is synthesized discontinuously as a series of fragments. Thus the lagging-strand polymerase must repeatedly be clamped and unclamped from DNA to cycle from one fragment to the next. The hypothesis, that replicative polymerases act in pairs for simultaneous synthesis of both strands of duplex DNA (100, 101), was extended by McHenry by suggesting that the accessory proteins may be distributed asymmetrically relative to the two polymerases to confer distinctive properties for leading and lagging strands (102).

Evidence for functional asymmetry in holoenzyme was obtained from assays

using the ATP analog, ATP γ S (102). In the presence of ATP γ S, one-half the amount of holoenzyme is clamped onto primed DNA relative to use of ATP. After using ATP to clamp holoenzyme onto DNA, treatment with ATP γ S released one half of the enzyme. It was hypothesized that of the two polymerases in the holoenzyme, one could use ATP γ S to clamp onto DNA, and the other was dissociated from DNA by ATP γ S.

Evidence for asymmetry in holoenzyme structure has also been obtained. The τ dimer binds two core polymerases tightly ($K_d < 17$ nM); the simplest arrangement imaginable is one core on each τ protomer (42, 43). The τ subunit also binds the γ complex (described below), leading to an organization of subunits illustrated at the bottom of Figure 4. In Figure 4, the τ dimer is assumed to be in the common isologous arrangement, in which each core- τ protomer unit is related to the other by a two-fold axis of rotation (i.e. τ is symmetric relative to the two polymerases). The γ complex is an asymmetric structure, because four of its subunits are present in a single copy (56, 63). Hence, γ complex imposes an asymmetry about the two core polymerases (as shown in Figure 4). Consistent with the holoenzyme structure in Figure 4, the composition of Pol III* showed a total of 14 polypeptides in the following composition: $\alpha_2\epsilon_2\theta_2\tau_2\gamma_2\delta_1\delta'_1\chi_1\psi_1$ (63). This composition is consistent with Pol III* containing a Pol III' assembly ($\alpha_2\epsilon_2\theta_2\tau_2$) bound to one γ complex ($\gamma_2\delta_1\delta'_1\chi_1\psi_1$).

Although the subunit composition of Pol III* suggests one γ complex bound to Pol III', the Pol III* could not be formed by mixing Pol III' with γ complex even at high concentration (28 μ M each) (63). Since a "tau complex" ($\tau\delta\delta'\chi\psi$) can be constituted (63, 65, 71), it was thought that perhaps Pol III* would assemble upon mixing "tau complex," γ complex, and core; this still did not result in Pol III*, however (63). Further study showed that the assembly of Pol III* relies on adding subunits in a defined order (63). The resulting Pol III* remains associated even when diluted to 30 nM and therefore, once formed, Pol III* does not easily fall apart (J Turner, M O'Donnell, unpublished). An essential contact needed to form Pol III* is interaction of γ with τ to form a $\gamma_2\tau_2$ tetramer (63). The γ -to- τ contact is inhibited if the $\delta\delta'\chi\psi$ subunits are added to γ or τ before mixing γ with τ (63). After the $\gamma_2\tau_2$ tetramer forms, the two core polymerases and the $\delta\delta'\chi\psi$ subunits can be assembled onto it (see Figure 4).

Study of how δ , δ' , χ , and ψ inhibit the γ -to- τ contact showed that δ and δ' , but not χ or ψ , are the culprits (63). The δ' subunit inhibits the γ -to- τ contact if it is present on both γ and τ , but if δ' is present on only γ or τ , the γ -to- τ contact is productive and Pol III* assembly is enabled. Hence, in Figure 4, δ' is shown near the interface of the $\gamma_2\tau_2$ tetramer, such that binding of one δ' occludes a second molecule of δ' . This restriction of only one δ' on either γ or τ to enable Pol III* assembly is consistent with the stoichiometry of one δ'

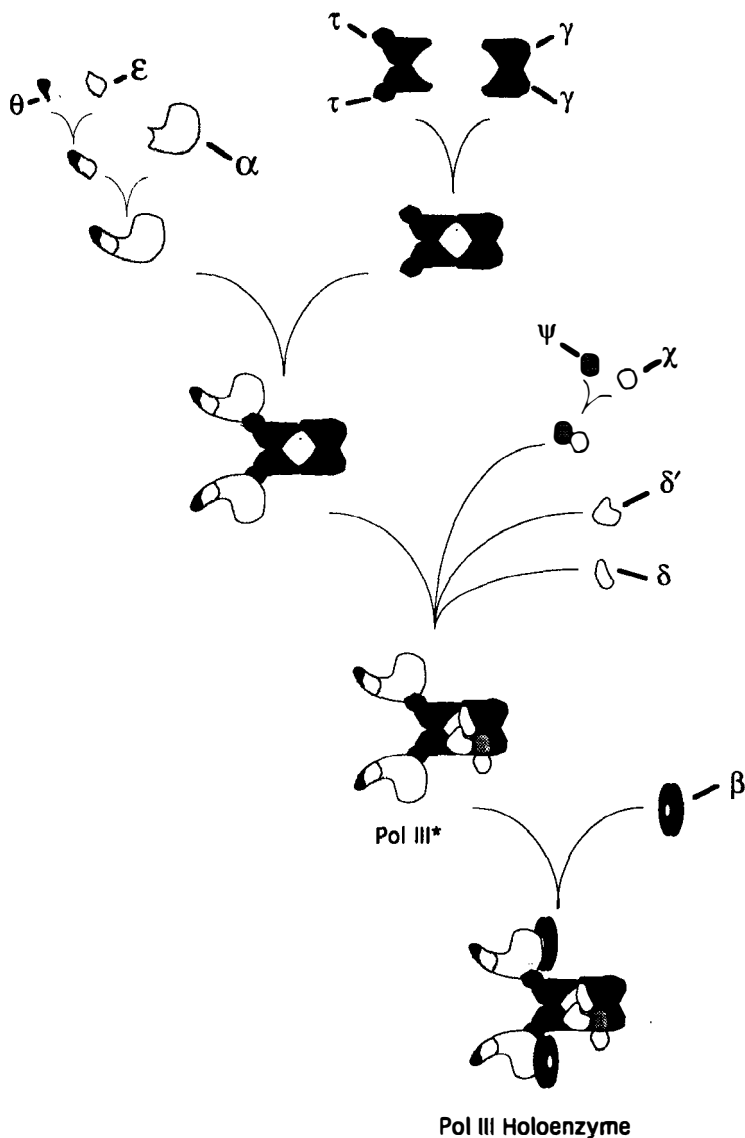


Figure 4 Assembly of the asymmetric holoenzyme. Organization of the 10 different subunits within the holoenzyme particle. The τ dimer and γ dimer are each shown in an isologous arrangement and the γ - τ heterotetramer is also shown as isologous. Each core polymerase is shown as a linear arrangement of α - ϵ - θ . The two core polymerases are attached through α to the τ dimer. The single-copy subunits, δ , δ' , χ , and ψ , assemble onto the γ - τ heterotetramer and must be added in order (see text for details). The δ' is positioned near the τ and γ interface to explain the observation that only one δ' is accommodated in the heterotetramer. The ability to form a $\delta\delta'$ complex is reflected in the contact of δ to δ' . The χ subunit binds ψ , which in turn binds γ (or τ). Two β dimers are shown bound to the two cores. Reflected in the final structure are the strong intersubunit contacts within holoenzyme, identified as α - ϵ , ϵ - θ , τ - α , δ - δ' , χ - ψ , γ - ψ , τ - ψ , and τ - γ (34, 43, 53, 63, 65, 69, 71).

in Pol III*, and also with the single δ in Pol III*, as it has been shown that δ - δ' forms a 1:1 complex (65). The δ subunit also inhibits the γ -to- τ contact if added to the reaction early; if δ is added after the γ -to- τ contact is established, assembly of Pol III* proceeds. This phenomenon explains why Pol III' and γ complex do not assemble to form Pol III* and may even be a useful mechanism to keep some Pol III' and γ complex as separate entities. Perhaps Pol III' and/or γ complex have separate roles in other DNA metabolic pathways, such as in repair or recombination.

Since the δ , δ' , χ , and ψ subunits can be added after mixing τ with γ , their position on either γ or τ is ambiguous. The presence of core on τ decreases the association rate of these subunits with τ , and thus should bias their association toward γ (V Naktinis, M O'Donnell, unpublished). This kinetic bias may explain why Pol III', purified from *E. coli* lysates, does not contain the $\delta\delta'\chi\psi$ subunits (42). It is still possible, however, that in the holoenzyme, the single-copy $\delta\delta'\chi\psi$ subunits are functional with both halves of the $\gamma_2\tau_2$ tetramer. Further, the δ' subunit displays weak, but detectable, clamp-loading activity with τ , but not with γ , thereby presenting the possibility of two clamp loaders in Pol III* consisting of $\tau\delta'$ and $\gamma\delta$ (65, 69).

A slightly different subunit arrangement and stoichiometry were suggested in an earlier study in which core was proposed to be dimerized by θ , and the τ dimer was proposed to bind only one core and γ the other (44). The dimerization of core by θ was indicated by a larger species of core polymerase when concentrated to 18 μM . However, later studies using reconstituted core at 73 μM showed it was only a monomer ($\alpha_1\epsilon_1\theta_1$) (53). Evidence that τ is located on one core and γ on the other lies in an observation that τ and γ complex compete for binding to core and β on primed ssDNA coated with SSB protein (44). The competition between τ and γ complex may have been, however, for sites on the template, since both τ and γ complex bind ssDNA coated with SSB protein (85, 103).

DNA footprinting studies show the holoenzyme protects approximately 30 nucleotides of the duplex portion of a primer template (J Reems, C McHenry, personal communication). Finer analysis using chemical crosslinking agents attached to specific nucleotides on the primer strand show α crosslinks to position -13, γ crosslinks to position -18, and β at position -22; no subunit crosslinks to position -27 (J Reems, C McHenry, personal communication). Fluorescence energy transfer between a fluorophore on β (Cys333) and a fluorophore on DNA (3 nucleotides back from the primer terminus) indicates a distance of 65 Å between them (104).

The arrangement of subunits within holoenzyme and how they are oriented on DNA may be learned from future work by several approaches, including crosslinking, fluorescence energy transfer, neutron scattering, 2D crystals in the electron microscope, and 3D crystals analyzed by X rays.

DNA POLYMERASE III HOLOENZYME AS A REPLICATING MACHINE

Exchange of β from γ Complex to Core

The γ complex must bind β to assemble it on a primer terminus, and core must interact with β for processivity. Since both core and γ complex recognize a primed template junction, they may interact with the same face of the β ring. Comparison of gene sequences encoding β from seven different bacteria shows that the most conserved residues lie on only one face: the face containing the two C termini (see Figure 2) (Z Kelman, M O'Donnell, unpublished). Consistent with this face as a site of interaction, point mutants in four of the five C-terminal residues of β inactivate β in replication assays and also prevent β from binding γ complex (V Naktinis, M O'Donnell, unpublished). Surprisingly, each of the C-terminal point mutations also prevented β from binding core, indicating that core and γ complex bind to β at the same place.

Why do core and γ complex have overlapping binding sites on β ? The γ complex not only loads β onto DNA, but also unloads β clamps from DNA (9, 103). Hence, the competitive arrangement could ensure that while core is using β to extend DNA, it prevents γ complex from unloading β from DNA.

Studies using subassemblies (γ complex, core, and β) invoke the idea that only core and β are present on DNA during chain elongation, since γ complex acts catalytically. In fact, the overlapping binding site of γ complex and core on the β dimer is consistent with this view. Studies using the entire holoenzyme, however, show that γ complex remains with core and β on DNA (44, 105). In the holoenzyme, τ acts as a bridge between core and γ complex to hold them together (103). This arrangement may allow β to be repositioned from γ complex to core as illustrated in Figure 5. Positioning the catalytic clamp-loading activity of γ complex at a replication fork, through constant association with the holoenzyme, would be advantageous for the multiple initiation events on the lagging strand (described below).

Cycling of Holoenzyme on the Lagging Strand

The picture of a polymerase with a sliding clamp riding behind it fits nicely with continuous synthesis of the leading strand. On the lagging strand, however, the DNA is synthesized discontinuously in a series of short Okazaki fragments (1). Each fragment is only 1–2 kb, and at a speed near 1 kb/s, the polymerase will finish a fragment within a second or two and must rapidly recycle to the next RNA primer. The β clamp holding the polymerase tight to DNA would conceptually hinder rapid recycling of polymerase. One strategy to overcome this difficulty would be to produce 4000 molecules of holoenzyme, one for each Okazaki fragment. Because there are only 10–20 molecules

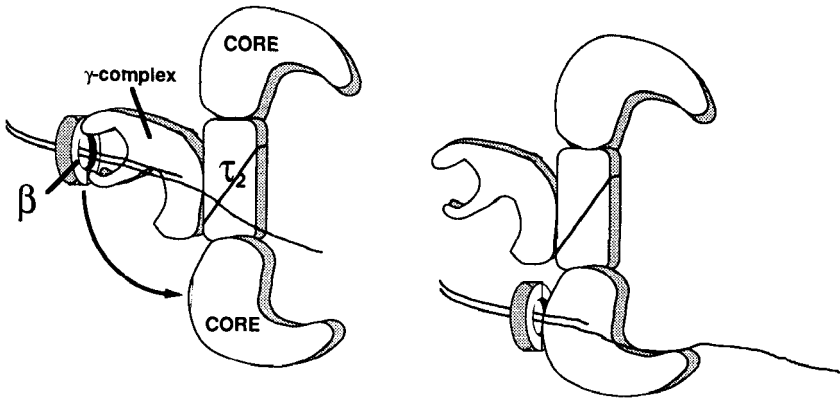


Figure 5 Core and γ complex interact with the same face of the β ring. The holoenzyme contains two core polymerases bound to a τ dimer, and one γ complex clamp loader (see Figure 4). The γ complex interacts with the C termini of the β dimer and presumably orients this face of β toward the primed site. Core interacts with some of the same C-terminal residues on β as the γ complex does. (Hence, after γ complex loads β on DNA, the core may swing into position with the β clamp.) In the holoenzyme, γ complex is held to DNA with core and β through interaction with τ .

of holoenzyme in a cell (4), however, there must be a specialized mechanism for rapid polymerase recycling.

The fact that holoenzyme is held to DNA by a ring-shaped protein suggests that holoenzyme may solve the recycling problem by sliding back along the lagging strand until it regains its position at the fork and captures the next primer. This would require holoenzyme to slide over the duplex fragment it had just finished, and over the gap of ssDNA separating it from the fork. Study of holoenzyme diffusion on DNA showed that holoenzyme slides on duplex DNA, but not on ssDNA, whether SSB protein is present or not (40). These results at first seem inconsistent with a β ring having a central cavity large enough to accommodate duplex DNA, and therefore also ssDNA (at least if SSB protein is not present). β can only slide over a short stretch of ssDNA (up to 25 nucleotides), however; β cannot slide over a 1-kb stretch of ssDNA (with or without SSB protein) (5). Presumably, ssDNA has secondary structure, such as hairpins, that block β sliding.

The mechanism of holoenzyme cycling to new primed sites has been found to lie in the ability of this highly processive enzyme to switch rapidly to a distributive mode in a novel process of partial disassembly of its multisubunit structure and then reassembly (7–9, 103). Prior to completing a template, Pol III* remains stably associated with its β clamp ($t_{1/2} \sim 5$ min), but upon completing a template, Pol III* rapidly dissociates from DNA (in less than 1 s),

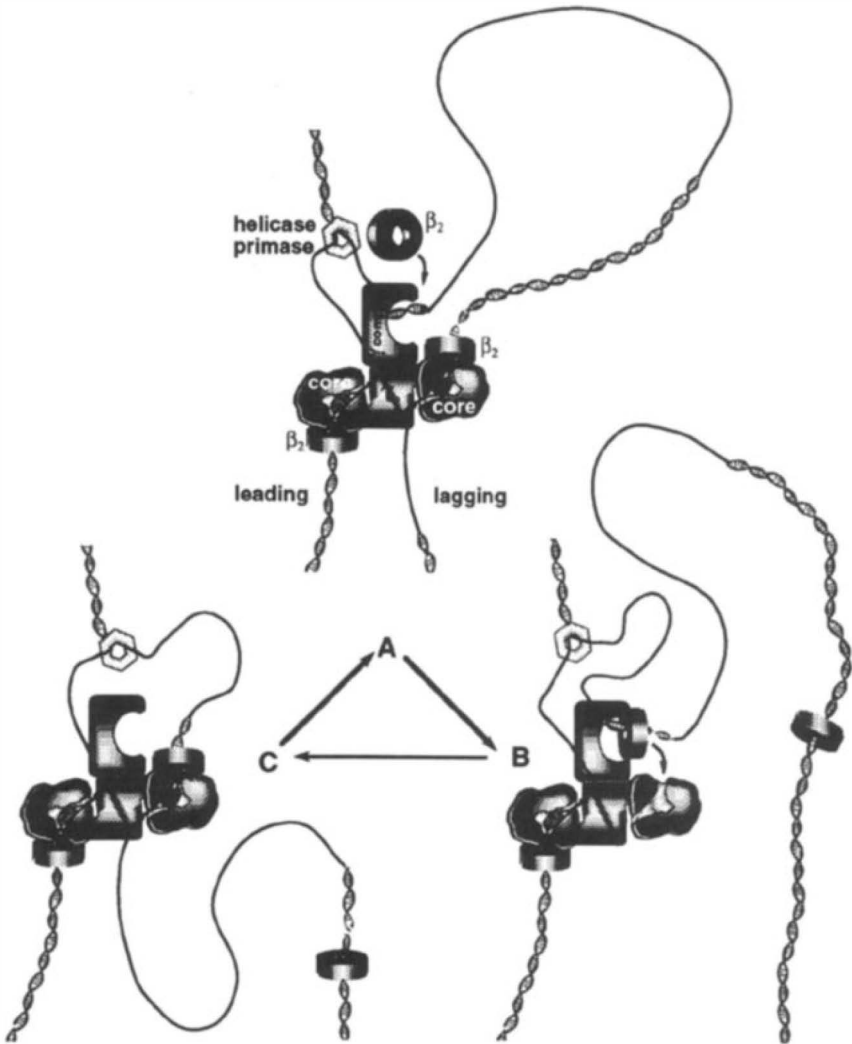


Figure 6 Proposed action of holoenzyme at a replication fork. The helicase and primase are shown as a hexamer surrounding the duplex at the forked junction. The holoenzyme structure is placed at a replication fork with one core polymerase on each strand. The γ complex is asymmetrically disposed relative to the two cores such that it points toward the lagging strand to load β clamps on primers repeatedly to initiate processive extension of Okazaki fragments. (A) As the lagging-strand polymerase extends an Okazaki fragment, the γ complex assembles a β clamp onto an RNA primer. (B) Upon completing an Okazaki fragment, the core disengages its β clamp, creating a vacancy for the new β clamp. (C) The new β clamp falls into place with the lagging-strand core polymerase to start the next Okazaki fragment. (Reproduced from 9)

leaving the β ring behind. Once off the completed DNA, Pol III* rapidly associates with a new β clamp at another primed site. The dynamics of these proteins on DNA imply that at the replication fork, the Okazaki fragment is extended to the very last nucleotide and then Pol III* rapidly dissociates from its β clamp and cycles to the upstream RNA primer (but only after assembly of a new β clamp on the new RNA primer).

Earlier studies concluded that Pol III* required a second β clamp on another DNA molecule to induce Pol III*'s dissociation from a completed template (8). It is now evident, however, that Pol III* does not require assistance to disengage its β clamp after completing a template (9). The earlier observations that holoenzyme remained bound to replicated DNA were likely explained by the presence of too little salt in the analysis (8, 105). At low ionic strength Pol III* binds DNA nonspecifically (5, 9).

The implication of this mechanism of polymerase recycling at a replication fork fits nicely with the overall structure of holoenzyme. In Figure 6, the holoenzyme is placed into the context of a moving replication fork and each core polymerase is shown with a β sliding clamp for processive elongation of both strands. In proceeding from diagram A to B, the γ complex assembles a β ring around a new primed site at the fork. Also in going from diagram A to B, the lagging-strand core completes an Okazaki fragment to a nick, thereby effecting its release from the β clamp and DNA. Polymerase release of the β clamp results in a vacancy in the binding site for β on the core polymerase, a logical prerequisite for association of this core with a new β clamp on the upstream RNA primer. In proceeding from diagram B to C, the lagging-strand core cycles to the new β clamp to initiate processive extension of the next Okazaki fragment.

This entire cycle of events must occur within a second or two. Can β clamps be assembled fast enough to account for a new clamp on every Okazaki fragment (i.e. 1 clamp/s)? Experiments performed at intracellular concentrations of β , DNA, γ complex, and potassium glutamate have shown that one β clamp is assembled on DNA every one-half second (9). Hence β clamp assembly appears rapid enough to account for a new β clamp for each Okazaki fragment, especially considering that the effective concentration of γ complex would be very high at a replication fork due to being held near the DNA by its presence in the holoenzyme structure.

The polymerase transfer mechanism entails stoichiometric use of β for each Okazaki fragment, consistent with the cellular abundance of β relative to holoenzyme. There are approximately 10 times more Okazaki fragments produced during chromosome replication than there are β dimers in the cell, however. Pertinent to this point is the finding that Pol III* not only loads β clamps onto DNA, but also can remove them from DNA for use at new primed sites (9).

Significant insight into the workings of holoenzyme at a replication fork have been obtained from studies using a rolling-circle system (108–112). In the rolling-circle assay the holoenzyme is present with the helicase (DnaB protein) and primase (DnaG protein) (plus or minus the other primosomal proteins, PriA–C, DnaT, and DnaC) to produce a unidirectional replication fork that peels off a long lagging strand as the fork is advanced multiple times around a circular duplex (1). This assay has been exploited to determine the processivity of proteins during fork movement and to characterize the effect on leading- and lagging-strand synthesis of different concentrations of nucleotides, salt, and proteins. Lowering the concentration of β decreased the efficiency of primer utilization on the lagging strand, a result consistent with stoichiometric consumption of one β clamp per Okazaki fragment (108, 109). Further, under some conditions, the final number of Okazaki fragments was greater than the total amount of β in the assay, consistent with eventual recycling of β clamps. Omission of τ significantly reduces replication, consistent with its structural role in dimerizing core (K Marians, personal communication). It is known that τ can replace γ in action with the δ , δ' , χ , and ψ subunits in loading β clamps onto DNA (65, 69), consistent with the ability to omit γ without significant effect (K Marians, personal communication).

Another important observation in the rolling-circle system is that at a low concentration of core, Okazaki fragments are not extended to completion, suggesting that primase can induce premature release of the lagging polymerase (112). A polymerase release mechanism such as this would be a useful backup mechanism to effect the removal of a stalled holoenzyme at a site of DNA damage.

Coordination of Leading and Lagging Strands

Coordinated synthesis of the leading and lagging strands is probably necessary to survival. The issue at stake is the ability to stop one strand if the other strand is stalled, such as upon encounter with a damaged site. For example, if the leading polymerase were to continue unabated while the lagging polymerase was immobilized at a lesion, the lagging-strand template would continue to be spooled out as ssDNA. There are approximately 800 SSB protein tetramers in a cell, and therefore about 50 kb of ssDNA can be coated, after which the exposed ssDNA would be available for nuclease attack. An ssDNA scission would be difficult, if not impossible, to repair. Presumably coordinated synthesis of the two strands occurs, as DNA-damaging agents lead to cessation of replication.

It seems reasonable to expect a dimeric polymerase to be at the root of the mechanism of strand coordination. Perhaps the proximity of the two polymerases facilitates allosteric communication between them, as suggested (12). Or, since polymerases travel in spiral paths when forming a spiral duplex product

(or the DNA spirals in back of the polymerase), perhaps stopping one polymerase prevents spiraling of the other. The mechanism of strand coordination is an important area for future studies.

It should be noted that a dimeric polymerase does not solve the kinetic barrier to polymerase cycling (i.e. rapid dissociation of a processive polymerase from DNA for cycling to the next primer). Although a dimeric structure would result in holding the lagging polymerase at the fork, and thereby increase its effective concentration for action on the lagging strand, dissociation reactions are independent of concentration. As discussed above, holoenzyme has a specific mechanism for rapidly dissociating from DNA upon completing a template (7-9).

COMPARISON OF HOLOENZYME TO OTHER REPLICASES

Holoenzyme can be thought of as three components: a polymerase (core), a sliding clamp (β), and a clamp loader (γ complex). At this level of resolution, the replicases of eukaryotes (Pol δ) and phage T4 are similar to holoenzyme (reviewed in 2).

The replicase of each system has these three activities of *E. coli* holoenzyme (Table 2). The clamp loader of Pol δ is the five-subunit RF-C (also called A1), and the clamp is PCNA (reviewed in 113). In T4, the clamp loader is the gene 44/62 protein complex (g44/62p) and the clamp is the gene 45 protein (g45p) (reviewed in 114). Interestingly, the sequences of all the subunits of the RF-C complex are homologous to one another (115, 116), as are the γ/τ and δ' subunits of γ complex (64, 67). The *E. coli* γ/τ and δ' subunits are also homologous to the human RF-C subunits and to T4 g44p, implying that the mechanism of clamp loading (and unloading) is common to all these systems (68).

Table 2 Comparison of the three-component structure of replicases from *E. coli*, eukaryotes, and T4 phage

| | <i>E. coli</i> | Eukaryotes | T4 phage |
|------------------------------|----------------------------------|------------------------------|---------------------------------|
| Polymerase/ exonuclease | core (3 subunits) | Pol δ (2 subunits) | g43p (1 subunit) |
| Clamp loader (matchmaker) | γ complex (5 subunits) | RF-C complex (5 subunits) | g44/62p complex (2 subunits) |
| Sliding clamp | β | PCNA | g45p |

The monomer mass of PCNA and of g45p is only 2/3 the mass of β , but their native mass is similar to that of β due to their trimeric aggregation state (117, 118). On the basis of the six-domain structure of the β dimer (three domains per monomer), it was hypothesized that PCNA and g45p trimers form rings of six domains, two per monomer (6). Consistent with this hypothesis, human PCNA, like β , slides on DNA and falls off over DNA ends (N Yao, Z Kelman, M O'Donnell, unpublished). Further, yeast PCNA self-loads over the ends of linear DNA, but not on circular DNA (84). In the T4 system, cryoelectron microscopy studies showed that the accessory proteins form a sliding clamp on DNA having similar dimensions as β (119). Also, studies of transcriptional activation by the T4 accessory proteins showed that they track along DNA (120–122). Recent protein-DNA crosslinking studies demonstrate that indeed all three clamps (g45p, β , and PCNA) track along DNA (123).

The crystal structure of yeast PCNA shows just how similar it is to *E. coli* β . The inner and outer diameters of these rings are the same, as is the six-domain structure. In fact, the topologies of the polypeptide chain-folding patterns of the two PCNA domains are the same as those of the three domains of β (123a).

A major difference between *E. coli* holoenzyme and eukaryotic Pol δ is that Pol δ is not organized into a twin polymerase, and the RF-C clamp loader is not physically connected to Pol δ in solution. Hence, at the current state of knowledge, the human system lacks the equivalent of the *E. coli* τ subunit for organizing its polymerases and clamp loader into one particle. Likewise, the T4 system lacks the equivalent of τ , and its clamp loader appears to act separately from the polymerase.

Polymerase action in cycling among Okazaki fragments during lagging-strand replication has been examined in the T4 and T7 systems. The T4 polymerase remains stably associated with its sliding clamp on a primed template, but rapidly disengages from its sliding clamp upon completing synthesis (124, 125). Hence, the T4 and *E. coli* systems behave similarly. Rolling-circle assays in the T4 system show that the leading and lagging strands continue even when the reaction is diluted, and therefore the lagging polymerase must be processive (126). Direct interaction between two T4 polymerase molecules suggests that the lagging polymerase binds the leading polymerase and thereby remains with the replication fork as it cycles among Okazaki fragments (127).

Studies in the T7 system also show rapid cycling of polymerase during lagging-strand replication (128). The T7 polymerase is composed of two subunits: gene 5 protein (the polymerase) and thioredoxin (the processivity factor); it lacks a clamp loader. Hence, the T7 replicase may employ a different mechanism for processivity and cycling than do the replicases of *E. coli*, T4, and eukaryotes. It is conceivable, however, that processivity and cycling in

the T7 system share the basic principles of the other replicases. For example, the T7 polymerase may have a cavity in which a duplex fits, and thioredoxin may seal the cleft, trapping DNA inside. Polymerase cycling may possibly be achieved by partial or complete separation of the two subunits upon completing an Okazaki fragment, followed by reforming the T7 holoenzyme at the next primed site. The herpes simplex replicase is also a highly processive, two-subunit enzyme that lacks a clamp loader, like the T7 polymerase (129, 130).

ARE POLYMERASE SLIDING CLAMPS USED BY OTHER PROTEINS?

Besides the use of β by Pol III*, the β clamp also increases the processivity of DNA polymerase II (Pol II) (131, 132), an enzyme implicated in DNA repair (133, 134). The fact that β can be harnessed by two different DNA polymerases suggests that its use may generalize to yet other enzymes. For example, the β clamp may participate in recombination and repair, or in cell-cycle processes such as cell division and checkpoint control.

The hypothesis that DNA polymerase clamps may be harnessed by other enzymatic machineries is strengthened by the observation that clamps of other systems also interact with proteins besides the replicative polymerase (Table 3). PCNA is utilized by two DNA polymerases, δ and ϵ (135). The T4 g45p interacts with RNA polymerase (modified with g33p and g55p), specifically activating it on late gene promoters (120–122). Human PCNA forms a complex with cyclins, their associated kinases, and p21 (137, 138). Subsequent studies have shown that the p21 kinase inhibitor binds directly to PCNA and thereby inactivates Pol δ (139, 140). PCNA was also shown to interact with Gadd45, a protein that is induced upon DNA damage (140a).

HOLOENZYME IN REPAIR AND MUTAGENESIS

Holoenzyme also functions in mismatch repair and replication recovery after exposure to DNA-damaging agents (18–21). During correction of a mis-

Table 3 Multiple proteins interact with sliding clamps of prokaryotic and eukaryotic DNA polymerases

| Clamp | Interacts with |
|------------------------|---|
| <i>E. coli</i> β | Pol III, Pol II |
| T4 g45p | g43p (pol), RNA polymerase |
| human PCNA | Pol δ , Pol ϵ , p21 cell-cycle kinase inhibitor, Gadd45 |

match, several repair enzymes coordinate their actions to recognize the mismatch, scan the DNA to the nearest methylated site, nick the opposite strand, and excise the DNA strand all the way back to remove the mismatch. This gap is then filled in specifically by holoenzyme; no other DNA polymerase can substitute. Replication recovery occurs after cells are exposed to DNA-damaging agents; replication is stopped, but after a lag it starts up again. The predominant pathway of replication recovery is replication restart, in which it is believed that the replication machinery stops at a lesion and then synthesis is restarted past the lesion by a priming event, leaving the lesion behind for repair enzymes to act upon later. Another pathway, called targeted mutagenesis, requires RecA protein, UmuC protein, and a proteolytic form of UmuD protein (UmuD'). These proteins are hypothesized to assemble into a "mutasome" at the site of the lesion to help holoenzyme past the damaged site, resulting in an error (thus the term "targeted"). In the absence of these other factors, the holoenzyme has been shown to dissociate from DNA upon encountering a lesion, and it has been suggested that the UmuC and D' proteins may act by stabilizing the association of holoenzyme to DNA at a lesion (141–144). Further biochemical studies are needed to define these events. The recent development of an *in vitro* system for lesion bypass requiring RecA, UmuC, UmuD', and holoenzyme holds promise toward this end (145).

A new observation that may be pertinent to the mutagenic pathway is damage-dependent induction of a shorter version of β , called β^* . β^* comprises the C-terminal 2/3 of β , and hence each monomer contains two domains instead of three. Characterization of β^* showed it behaves as a trimer, presumably forming a six-domain ring (like PCNA and g45p), and it stimulates DNA synthesis by Pol III* (Z Livneh, personal communication). Surprisingly, β^* , in the absence of γ complex, converts core to a more salt-resistant form that is not inhibited by SSB protein. It is proposed that β^* may function in repair and mutagenesis, perhaps working specifically with core polymerase instead of Pol III*.

Another pathway for UV-induced mutagenesis is independent of replication and requires the repair genes *uvrA*, *B*, and *C*. An *in vitro* system for this pathway has been developed that depends on the UvrA, B, and C endonuclease, helicase II, and holoenzyme (146, 147). Presumably the error is caused by two closely opposed cylobutyl dimers such that only one is excised and the other is present in the repair gap, thus constraining the polymerase to cross the lesion as it fills the gap. Only holoenzyme is mutagenic in this assay; Pol I and Pol II are not, consistent with *in vivo* observations. The lack of a requirement for β suggests that a subassembly of the holoenzyme may perform this function.

GENETICS OF HOLOENZYME SUBUNITS

Five holoenzyme subunits are encoded by conditional lethal genes: α by *dnaE*, ϵ by *dnaQ*, β by *dnaN*, and γ/τ by *dnaX* (1). The remaining five subunit genes have been identified recently: the genes encoding δ , δ' , χ , ψ , and θ (*hola-E*, respectively) (53, 64–67, 70–73, 148). Genetic knockout experiments of *hola* (δ) and *holB* (δ') show these genes to be essential for cell viability, consistent with the important roles of δ and δ' in assembly of β on DNA (R Krishnan, J Carter, D Berg, C McHenry, personal communication). Knockout of the *holC* gene (χ) is tolerated, but only small colonies form at 37°C and they fail to grow at 42°C (R Maurer, personal communication). Both of these phenotypes are partially corrected upon blocking induction of the SOS response. Another phenotype of *holC* cells was revealed upon study of mutations in recombination genes (*ruvA*, *B*, and *C*, and *recG*), which show no significant phenotype alone, but cannot tolerate interruption of *holC* (the *ruvA*, *holC* double mutant is suppressible by the *ruv* suppressor, *rus-1*) (R Maurer, personal communication). These results imply that χ may function in recombination as well as replication. Mutations of *holD* (ψ) have yet to be performed.

Studies of genes encoding subunits of core showed that a *dnaQ* (ϵ) null mutant shows not only a mutator phenotype, but also a severe growth defect (55, 149), consistent with the requirement of ϵ for holoenzyme to realize its full speed and processivity (34). The growth defect in the *dnaQ* null mutant is suppressible by a mutation in *dnaE*, presumably producing a more efficient α (150). A mutation in α (*dnaE173*) increases the spontaneous mutation frequency 1000-fold, and therefore α is also an important determinant of fidelity (151). Interallelic complementation of conditional lethal *dnaE* alleles is consistent with the presence of two core polymerases in the holoenzyme (152). It is tempting to speculate that one allele is defective on the lagging strand and the other is defective on the leading strand, thus explaining how the two alleles may complement. The function of θ has not been identified, other than a slight stimulation of ϵ in removal of a mismatch (53). Consistent with the subtle function of θ , a deletion of *holE* has no noticeable phenotype (55).

The frameshift site in the chromosomal *dnaX* gene has been mutated such that τ is produced but γ is not (99). These “ γ -less” cells are viable, suggesting that γ is not essential (unless an undetectable but sufficient amount of γ is produced in these cells) (99). Presumably the τ subunit binds $\delta\delta'\chi\psi$ in γ -less cells to substitute for the γ function. Indeed a γ -less form of Pol III*, comparable to Pol III* in activity, can be reconstituted from individual subunits and appears to be present in γ -less cells, although its purification was defeated by proteolysis (99). In the same study, deletion of C-terminal residues in τ , lacking in γ ,

were found to be essential to cell viability. The unique property of τ , lacking in γ , is the ability to bind and dimerize core.

It seems likely that holoenzyme subunit genes will be regulated in accordance with the physiological state of the cell (12). Consistent with this notion, an element located within the coding sequence of *dnaX* has been shown to effect expression of the gene (153). The sequence of the element suggests that the binding factor may be *purO*, a regulator that binds operators involved specifically in purine synthesis, indicating that expression of holoenzyme is tied to the production of nucleotides.

THE FUTURE

The past few years have seen several significant advances in our knowledge of holoenzyme structure and function. All the genes have been identified, proving that all 10 subunits are distinct and are not proteolytic versions of larger subunits. Also, each subunit has been obtained in quantity, and binding studies show that each of them forms a complex with at least one other subunit, with consequences that can be assayed biochemically. Hence, none of these 10 proteins were spurious contaminants in holoenzyme preparations. Further, the holoenzyme particle can be reconstituted from them. The molecular basis underlying the tight grip of holoenzyme to DNA has been explained by the β sliding clamp encircling DNA; this clamp is pulled along by core while passively locking the polymerase to the template. The sliding clamp also explains how the polymerase binds tightly to DNA yet rapidly cycles off DNA upon finishing one fragment to start another. Holoenzyme demonstrates such action by recognizing the completion of the template and then hopping off its current sliding clamp and onto a new sliding clamp.

Despite this knowledge about the structure and function of holoenzyme, it is fair to say that only 3 of the 10 subunits—the polymerase, the exonuclease, and the clamp—have well-defined functions. The mechanism of the γ complex in loading the β clamp—especially the roles of ATP binding and hydrolysis, and the individual functions of the five different subunits—is still relatively obscure. The function of the ATPase activity of τ is still uncertain, and the role of θ is completely unknown. Why Pol III* releases the β clamp only upon finishing a template, and how the leftover clamps are recycled, also lack a detailed explanation. The holoenzyme is asymmetric structurally, but the extent to which this is manifested in function on leading and lagging strands remains for future study.

The imaginable responsibilities of a replicase are far more numerous than are the subunits in the holoenzyme; there is plenty for these proteins to do. Studies of how the holoenzyme interfaces with other replication proteins such as those that activate the origin, advance the replication fork, and terminate

the chromosome have only just begun. Likewise, the roles of holoenzyme and its subassemblies in other processes such as recombination, repair, and mutagenesis have yet to be determined.

The availability of individual subunits in quantity, in addition to the ability to reconstitute the several subcomplexes as well as the entire holoenzyme, provides fertile ground for detailed structural studies, especially X-ray crystallography and examination of 2-D crystals in the electron microscope. It is now abundantly clear that the replicase of eukaryotes and T4 are similar in function to the *E. coli* holoenzyme. Besides the functional similarities, close to half the mass of each of these holoenzymes can be predicted to have similar three-dimensional structure from the homology in sequences among 275 kDa of the holoenzyme (γ_2 , τ_2 , δ'_1), 136 kDa of the T4 holoenzyme (g44p tetramer), and almost all of the 280-kDa five-subunit RF-C complex of humans. The shape of the β subunit tells a lot about its function. Perhaps proteins that work on DNA structures, rather than on specific sequences, reflect their function in their shape. It will be very interesting to see the visual appearance of the other holoenzyme subunits, especially the non-enzymatic ones.

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