The Sliding Clamp of DNA Polymerase III Holoenzyme Encircles DNA

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DNA polymerases that duplicate chromosomes are remarkably processive multiprotein machines. These replicative polymerases remain in continuous association with the DNA over tens to hundreds of kilobases. What is the chemical basis of their strong grip to the template? The mystery behind the high processivity of the replicative polymerase of the *Escherichia coli* chromosome, DNA polymerase III holoenzyme, lies in a ring-shaped protein that acts as a sliding clamp for the rest of the machinery. A ring-shaped sliding clamp is likely to be general for replicative polymerases, being formed by the PCNA protein of yeast and humans and the gene 45 protein of T4 phage.

A short summary of the subunit organization of four replicative polymerases, presented below, is necessary to appreciate the similarity between the prokaryotic and eukaryotic systems. Then the detailed mechanism by which the *E. coli* DNA polymerase III holoenzyme achieves its high processivity will be described, followed by a consideration of whether other polymerases spanning the evolutionary spectrum utilize a common mechanism.

SUBUNIT STRUCTURE

The subunit structure of the T4 phage, *E. coli*, yeast, and human replicative polymerases is presented in Kornberg and Baker (1991) and in several recent reviews (see Table 1 for references). During purification, these multiprotein polymerases generally dissociate into three components: a DNA polymerase, a multisubunit accessory protein complex, and a single subunit accessory protein (Table 1). The polymerase components of the two prokaryotic replicases are the single subunit bacteriophage T4 gene 43 protein and the *E. coli* core polymerase, which is a heterotrimer of α (polymerase), ϵ (3'-5' exonuclease) and θ . Yeast and humans utilize two polymerases, pol δ and pol ϵ , which both interact with the same set of accessory proteins. These prokaryotic and eukaryotic polymerases are not highly processive

on their own; for this they require their respective accessory proteins. The accessory protein complex of T4 is the two subunit gene 44/62 protein complex, in *E. coli* it is the five subunit γ complex, and in yeast and humans it is the five subunit RF-C complex. In each system the accessory protein complex is a DNA dependent ATPase that recognizes a primer-template junction. The single accessory subunit of T4 phage is the gene 45 protein, in *E. coli* it is β subunit, and in eukaryotes it is proliferating cell nuclear antigen (PCNA). These single accessory subunits have no inherent catalytic activity, but they stimulate the ATPase activity of their respective accessory protein complex.

THE PREINITIATION COMPLEX

The *E. coli* accessory proteins, γ complex plus β subunit, hydrolyze ATP to form a tight protein clamp (termed a "preinitiation complex") on primed single-stranded (ss) DNA coated with ssDNA binding protein (Figure 1) (Wickner, 1976; O'Donnell, 1987; Maki and Kornberg, 1988). The core polymerase then associates with the preinitiation complex to form the highly processive holoenzyme. The accessory proteins of phage T4, yeast, and humans also hydrolyze ATP to form a preinitiation complex on primed ssDNA followed by association of the polymerase (Hockensmith *et al.*, 1986; Munn, 1986; Burgers, 1991; Capson *et al.*, 1991; Lee *et al.*, 1991a,b; Munn and Alberts, 1991a,b; Tsurimoto and Stillman, 1991). In the T4 system, the accessory proteins have been referred to as a "sliding clamp" implying they act to clamp the gene 43 polymerase to DNA and slide with it (Huang *et al.*, 1981). Detailed studies in the *E. coli* system, summarized below, have shown the preinitiation complex does indeed act as a sliding clamp.

THE β SLIDING CLAMP OF DNA POLYMERASE III

In the *E. coli* system, the γ complex can be separated from the preinitiation complex by gel filtration leaving

Table 1. Subunit structure of the replicative polymerases of *Escherichia coli*, phage T4, yeast, and humans

	Polymerase ^a	Multiple accessory complex	Single accessory subunit
Phage T4	43 protein	44/62 complex	45 protein
<i>E. coli</i>	pol III core (α , ϵ , θ)	γ complex (γ , δ , δ' , χ , ψ)	β
Yeast	pol δ , pol ϵ	RF-C	PCNA
Humans	pol δ , pol ϵ	RF-C, A-1 ^b	PCNA

Reviews on the subunit structures of these polymerases are as follows: T4 phage (Nossal and Alberts, 1983; Marians, 1992), *E. coli* (Kornberg, 1988; McHenry, 1991; O'Donnell, 1992), eukaryotic pol δ (Downey *et al.*, 1990; Hurwitz *et al.*, 1990), and eukaryotic pol ϵ (Hübscher and Thömmes, 1992).

^a Two molecules of core are held together in the holoenzyme by the τ accessory protein proposed to be for concurrent replication of leading and lagging strands (Kornberg and Baker, 1991). The two polymerases of yeast and humans, pol δ and pol ϵ , are thought to be needed to replicate the leading and lagging strands.

^b The human accessory complex is referred to as either RF-C (Tsurimoto and Stillman, 1990) or Activator 1 (Lee *et al.*, 1991a).

only a dimer of β on primed DNA. This dimer retains the capacity to confer highly processive synthesis onto the core polymerase (Wickner, 1976; Maki and Kornberg, 1988; Stukenberg *et al.*, 1991). Hence the γ complex is not required during processive elongation. The role of the γ complex is to recognize primed DNA and to couple ATP hydrolysis to clamp β onto DNA (Onrust *et al.*, 1991). Only the γ and δ subunits are essential to clamp β onto DNA, although the other subunits of the γ complex modulate the activity of the γ and δ subunits (O'Donnell and Studwell, 1990; Onrust *et al.*, 1991).

Several β clamps can be transferred by one γ complex onto a single circular plasmid DNA molecule containing one nick (Stukenberg *et al.*, 1991). These β clamps freely diffuse along the plasmid DNA and will slide off the end of this DNA if the circular plasmid is cut with a restriction enzyme. The sliding motion of β is bidirectional,

independent of ATP, and occurs only on duplex DNA (Stukenberg *et al.*, 1991). Why does β , once transformed by the γ complex into a strong DNA binding protein, readily slide off linear DNA? To explain this behavior it was hypothesized that the β dimer is shaped like a ring and encircles the duplex like a doughnut (Stukenberg *et al.*, 1991). This β ring could then slide off linear DNA like a washer off the end of a steel rod.

How does the β clamp confer processivity onto the core polymerase? The β subunit binds directly to the polymerase subunit (α) and therefore, can tether the polymerase to DNA for processive syntheses. As the polymerase extends the 3' terminus it simply pulls the β sliding clamp along.

STRUCTURAL ARCHITECTURE OF β

X-ray analysis of β shows that it has the shape of a ring (Figure 2A) (Kong *et al.*, 1992). The central cavity is of sufficient diameter to accommodate duplex DNA and is lined with 12 α helices that are supported by a single continuous layer of β sheet structure all around the outside. Although the β ring is uninterrupted and looks like a single molecule, it is actually a head-to-tail dimer. The width of the β clamp is approximately that of one turn of the DNA helix, and the head-to-tail arrangement creates physically distinct front and back faces (Figure 2B). The "A" face in Figure 2B is rather flat and is more negatively charged than the "B" face, which is characterized by several loops. The γ complex likely opens and closes the β ring around DNA and orients the correct face toward the primer terminus for interaction with the polymerase. Each β monomer is composed of three domains that have the same three-dimensional structure giving the β dimer a six-fold repeat appearance. This high degree of symmetry in the β ring could help promote smooth sliding along the symmetrical DNA duplex.

The β clamp is negatively charged overall, but the α helices lining the central cavity are positively charged, perhaps to stabilize the β clamp on DNA. Ionic inter-

Figure 1. Two-stage assembly of *E. coli* polymerase III holoenzyme. In the first stage, the β subunit and γ complex couple ATP hydrolysis to formation of a "preinitiation complex" clamp on ssDNA primed with a synthetic oligonucleotide and coated with single-strand DNA binding protein (SSB). In the second stage, the polymerase core assembles with the preinitiation complex to form the highly processive holoenzyme.

Figure 2. Structure of the β dimer at 2.5-Å resolution. Only the α -carbon atoms of the polypeptide backbone are shown. (A) Front view. Inner and outer diameters of the β ring are shown below the structure. Arrows denote the dimer interface. (B) Side view. Duplex DNA (B form) has been computer modeled through the central cavity and the width is shown below. The head-to-tail dimer results in structurally distinct faces, "A" and "B".

action between β and DNA must be mediated by water because the inside diameter of the β ring is too large for direct contact between the basic side chains and the DNA at the center. The lack of direct contact between β and DNA may facilitate the sliding motion.

DO T4, YEAST, AND HUMANS HAVE A RING-SHAPED POLYMERASE ACCESSORY PROTEIN?

The functional analogue of β is the PCNA protein in yeast and humans, and the 45 protein in phage T4 (Table 1). Do PCNA and 45 protein also form ring-shaped structures? PCNA and 45 protein are only 2/3 the size of β and thus can accommodate only two of the three domains in a β monomer. However, molecular weight measurements of yeast PCNA and the 45 protein indicate that they are trimers (Bauer and Burgers, 1988; Jarvis *et al.*, 1989) and thereby have the mass required for a six domain ring-like β . The β , PCNA, and 45 protein lack significant amino acid sequence homology; however, the three domains of β , which have the same structure, show no obvious homology either. The hydrophobic core residues in each domain of β (residues inaccessible to water) consist of conserved hydrophobic amino acids, and both PCNA and 45 protein sequences can be aligned with β such that these hydrophobic core residues are conserved throughout the length of PCNA and 45 protein (Kong *et al.*, 1992).

Although this type of sequence alignment between β , PCNA, and 45 protein suggests structural similarity among them, the question of whether these are ring proteins can only be answered by further experimentation. Recently, the T4 accessory proteins have been visualized on a nicked plasmid DNA molecule by cryo-

electron microscopy (Gogol *et al.*, 1992). They appear as a tall thin cylinder with duplex DNA through the middle, and they often are present in clusters of up to 20 on one plasmid, suggesting that they slide. Their dimensions, 80 Å tall and 30 Å wide, are similar to β . The preinitiation complex of the T4, *E. coli*, and human replicases all protect 20–30 nucleotides from nucleases on the duplex portion of a primer template, further substantiating the similarities among these systems (Munn, 1986; Munn and Alberts, 1991a,b; Tsurimoto and Stillman, 1991; Reems and McHenry, 1992).

WHY IS THE CLAMP SEPARATE FROM THE POLYMERASE?

The answer to this question is by no means clear. However, it seems likely that there are advantages in having a separate clamp. For example, the lagging strand is synthesized as a series of short fragments, and in *E. coli* the polymerase must complete a fragment then dissociate from it and reassociate with a new primer made by primase all within a second. Biochemical studies show that the polymerase core rapidly dissociates from its β clamp, but only after DNA synthesis is complete, and then it reassociates with another β clamp on a new primed template (O'Donnell, 1987; Studwell *et al.*, 1990). Hence, rapid disassembly/reassembly of the polymerase with separate β clamps may be used as a mechanism by which a highly processive polymerase can rapidly recycle on and off DNA during synthesis of the numerous lagging strand fragments.

Another possible reason for evolution of a separate clamp protein may be for its use in other protein machines. Genetic studies of phage T4 show that the 45 protein is needed not only for DNA replication but also

Figure 3. Double use of the sliding clamp to activate transcription and for processive replication. The T4 polymerase accessory protein sliding clamp acts at a distance to activate transcription of T4 late genes (Herendeen *et al.*, 1989). The essential nick for activation of late promoters may be generated during replication of the lagging strand.

for transcription of late genes that are expressed only after DNA replication. Geiduschek's group has reconstituted the transcriptional enhancement by the T4 accessory proteins in vitro (Herendeen *et al.*, 1989). Through several elegant experiments they have determined that the T4 sliding clamp assembles at a nick to enhance transcription initiation of distant late gene promoters, apparently by sliding to the promoter for direct contact with RNA polymerase. The essential nick must be on the nontranscribed strand for transcription enhancement, implying that the same face of the sliding clamp that interacts with DNA polymerase also interacts with RNA polymerase. At a recent Keystone meeting on replication, Bruce Alberts suggested a scheme similar to that in Fig. 3 in which RNA polymerase is activated by sliding clamps behind the replication fork. These sliding clamps may assemble on the nicks between lagging strand fragments or may be the clamps generated during recycling of the polymerase on the lagging strand (see previous paragraph). Eukaryotic viruses such as SV40, adenovirus, herpes simplex virus, and vaccinia virus also require replication for late gene transcription. In this regard, it is interesting to note that the ETL protein of the insect baculovirus (*Autographa californica*) is highly homologous to PCNA (42% identity), and genetic studies show that it is also required for late viral gene transcription (O'Reilly *et al.*, 1989). Hence, replication dependent activation of late genes in eukaryotic viruses is very likely mediated by a sliding clamp of PCNA. Perhaps even the cell takes advantage of sliding clamps to activate genes after replication of the chromosome.

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