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Polymerases and the Replisome: Machines within Machines

Review

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Synthesis of all genomic DNA involves the highly coordinated action of multiple polypeptides. These proteins assemble two new DNA chains at a remarkable pace, approaching 1000 nucleotides (nt) per second in *E. coli*. If the DNA duplex were 1 m in diameter, then the following statements would roughly describe *E. coli* replication. The fork would move at approximately 600 km/hr (375 mph), and the replication machinery would be about the size of a FedEx delivery truck. Replicating the *E. coli* genome would be a 40 min, 400 km (250 mile) trip for two such machines, which would, on average make an error only once every 170 km (106 miles). The mechanical prowess of this complex is even more impressive given that it synthesizes two chains simultaneously as it moves. Although one strand is synthesized in the same direction as the fork is moving, the other chain (the lagging strand) is synthesized in a piecemeal fashion (as Okazaki fragments) and in the opposite direction of overall fork movement. As a result, about once a second one delivery person (i.e., polymerase active site) associated with the truck must take a detour, coming off and then rejoining its template DNA strand, to synthesize the 0.2 km (0.13 mile) fragments.

In this review we describe our current understanding of the organization and function of the proteins of the replication fork and how these complexes are assembled at origins of replication. Understanding the architecture of DNA polymerases is relevant to RNA polymerases as well, as the core of the polynucleotide polymerization machine appears to be similar for all such enzymes. In the discussion of the replisome, we particularly focus on features shared by the machinery from different organisms.

Replication Forks

The replication fork contains several key activities that can be considered as machines on their own: (1) the specialized polymerases that synthesize new strands; (2) the editing exonuclease associated with the replicative polymerase; (3) the accessory proteins that control interaction of the polymerases with the DNA, and (4) the helicase that melts the DNA double helix to generate the replication fork. These components are functionally conserved in diverse organisms. Table 1 lists the replication proteins that serve similar functions from phage T4, *E. coli*, yeast and human cells (based on the requirements to replicate the SV40 virus). Below, we first outline the recent progress in understanding the activities, architecture, and mechanism of these component machines followed by a discussion of how they communicate with one another within the replisome.

Polymerases: Template-Directed Phosphoryl Transfer Machines

Synthesis of the new DNA strands occurs as a result of a collaboration between the synthetic capacities of multiple polymerases. Two types of polymerases are required: primases, which start chains, and replicative polymerases, which synthesize the majority of the DNA (Kornberg and Baker, 1992). The replication fork, however, contains at least three distinct polymerase activities: a primase and a replicative polymerase for each of the two template strands. In *E. coli*, primase is a single polypeptide, and the replicative polymerase is a dimer of DNA polymerase (pol) III core and several accessory proteins that together form the pol III holoenzyme (reviewed in Marians, 1992; Kelman and O'Donnell, 1995). Similarly, phage T4 has one primase and one replicative polymerase that appears to function as a dimer (Alberts, 1987; Munn and Alberts, 1991). The situation in eukaryotic cells is slightly different (Stillman, 1994). The primase is in a tight complex with a DNA polymerase (pol α) and eukaryotic cells have two distinct replicative polymerases: polymerase δ (pol δ) and polymerase ϵ (pol ϵ).

All the replicative polymerases have one large subunit that contains the polymerase active site and, with the exception of pol α–primase, the same subunit or an associated polypeptide carries a proofreading 3′→5′ exonuclease. The polymerase subunits also interact with proteins that dramatically influence their association with DNA. In *E. coli*, the replicative polymerase is found in a complex with proteins that control polymerase processivity; this holoenzyme, consists of 10 distinct polypeptides (Kelman and O'Donnell, 1995). In contrast, neither the T4 nor the eukaryotic polymerases copurify in a complex with the processivity factors (Alberts, 1987; Stillman, 1994). Therefore, these proteins are called accessory proteins rather than subunits (see Table 1).

Polymerase Architecture. The central feature of all the known polymerase structures is the existence of a large cleft comprised of three subdomains referred to as the fingers, palm, and thumb by virtue of the similarity of the structures to a half-opened right hand (Figure 1; polymerase structures are reviewed in Joyce and Steitz, 1994, 1995; Sousa, 1996). A diverse set of polymerases—including several replicative and repair DNA polymerases from viral, prokaryotic, and eukaryotic sources, reverse transcriptase, and even an RNA polymerase—share this general structure. The palm subdomain, at the bottom of the cleft, contains the active site, including the essential acidic amino acids that bind metal ions involved in catalysis, residues that interact with the primer terminus, and the α-phosphate of the incoming dNTP. The conserved amino acid sequence motifs A and C, found in all nucleic acid polymerases, and motif B, found in the DNA-dependent enzymes, are present within this palm domain where they contribute to the active site (Figure 1A).

The walls of the polymerase cleft are made up of the finger and thumb subdomains. Although less well conserved than the catalytic palm (in some polymerases, these domains are unrelated), these subdomains

Table 1. Proteins that Perform Analogous Functions at Replication Forks

Function	*E. coli*/Phage λ	Phage T4	SV40/Human	Yeast
Helicase	DnaB	gp41	T antigen (SV40 specific)	MCM proteins?
Primase	DnaG primase	gp61	Primase subunit of pol α–primase	Primase subunit of pol α–primase
Polymerase	α subunit of DNA pol III H.E.	gp43	pol δ	pol δ and pol ϵ both involved
Proofreading exonuclease	ϵ subunit of DNA pol III H.E.	Part of gp43 polymerase subunit	Part of polymerase subunit of pol δ	Part of polymerase subunit of both pol ϵ and pol δ
Sliding clamp	β subunit	gp45	PCNA	PCNA
Clamp loader	γ complex	gp44/62	RF-C	RF-C
Single-strand DNA-binding protein	SSB	gp32	RP-A	RP-A

H.E., holoenzyme.

contribute analogous functions in many polymerases (Wang et al., 1997; Kiefer et al., 1998; and reviewed in Joyce and Steitz, 1995; Sousa, 1996). Based on DNA cocrystal structures and modeling studies, the fingers subdomain makes contact with the single-stranded template strand, that has yet to be copied. Part of the fingers domain, along with the palm domain, is involved in binding the incoming substrate dNTP. The thumb subdomain interacts with the template-primer DNA helix (Figure 1B). The recently solved structure of the *B. stearothermophilus* DNA pol I (49% identical in sequence to the *E. coli* enzyme) with a primer-template in the polymerase active site, provides insight into the mechanism by which polymerases interact with DNA in a sequence-independent manner (Kiefer et al., 1998). The polymerase makes extensive interactions with the DNA minor groove of the first four base pairs (with respect to the 3′ primer terminus) of the primer-template helix. Minor groove contacts allow binding to any sequence because the minor groove, in contrast to the major groove, contains a pattern of hydrogen bond donors and acceptors that are independent of the nucleotide sequence, as long as the bases are in proper Watson-Crick pairs.

A central feature of polymerase active sites is the cluster of conserved carboxylates and other polar residues at the base of the cleft in the palm domain (Steitz et al., 1994). These carboxylates anchor two divalent metal ions involved in catalysis. The polymerization reaction proceeds by nucleophilic attack by the 3′ hydroxyl of the primer terminus on the dNTP α-phosphate with release of PPi. One divalent metal ion is thought to promote the deprotonation of the 3′ hydroxyl of the primer strand whereas the other facilitates formation of the pentacovalent transition state at the α-phosphate of the dNTP and the departure of the PPi leaving group. Similar two-metal mechanisms have been proposed to catalyze phosphoryl transfer reactions in numerous other systems including the proofreading exonuclease associated with polymerases and RNaseH domains associated with reverse transcriptases (Joyce and Steitz, 1994). In addition to the acidic amino acids from the palm subdomain, several residues from the fingers subdomain participate directly in catalysis in the pol I family of polymerases. For example, a tyrosine residue in the *B. stearothermophilus* DNA pol I plays a critical function in establishing the geometry of the active site, thereby enforcing the requirement for proper base pairing prior to catalysis (Kiefer et al., 1998).

The close similarity between the structures of DNA

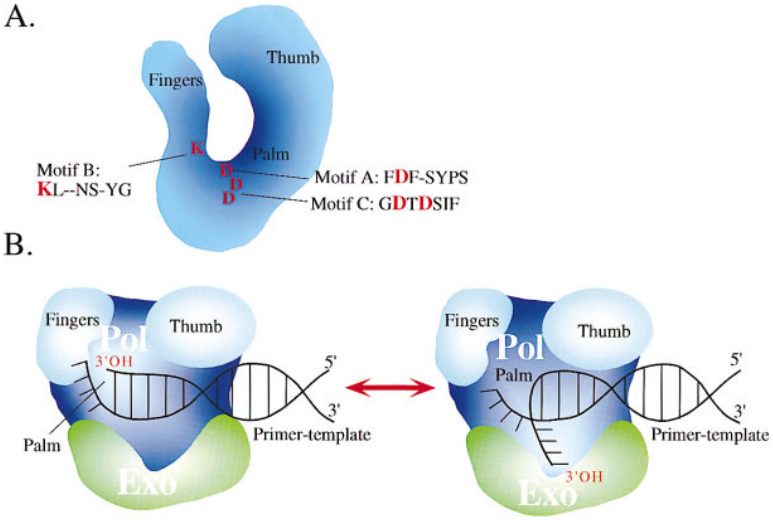

Figure 1. Polymerase Architecture

(A) Side view of a DNA polymerase. The basic shape shown is based on the outline of the fingers, thumb, and palm domains of gp43 from bacteriophage RB69. The exonuclease domain is not shown. Consensus sequences for the conserved polymerase motifs are based on the recent pol α family alignment of Wang et al., 1997.

(B) Top view of a DNA polymerase bound to a primer-template junction in its synthesis (left) and proofreading (right) modes. Pol denotes the polymerase active site; Exo, the 3′→5′ exonuclease active site. This figure was redrawn and modified from Joyce and Steitz, 1995.

pol I and the T7 RNA polymerase clearly indicate that these two proteins arose from a common ancestor (reviewed in Joyce and Steitz, 1995). Furthermore, the palm subdomains of the pol α family polymerase (RB69 gp43), *E. coli* pol I and HIV reverse transcriptase can all be superimposed (Wang et al., 1997). In contrast, the mammalian DNA pol β is distinct, being more similar to the nucleotidyl transferase enzyme family, and it has been argued that the similarities between this protein and the other polymerases are an example of convergent evolution. Comparisons of the structures and sequence motifs present in different polymerases also provide clues to the molecular mechanisms determining the specificity of different family members. For example, specific motifs found in DNA polymerases but not in RNA polymerases correlate with the specificity for dNTPs versus rNTPs and the requirement for a primer (Sousa, 1996; Joyce, 1997).

Editing. The polymerases responsible for the majority of DNA synthesis in phage, prokaryotes, and eukaryotes (phage T4 gp43, DNA pol III holoenzyme, pol δ and pol ϵ) all have an associated proofreading exonuclease. These activities, which preferentially excise a mismatched nucleotide from the primer terminus, contribute about three orders of magnitude to the fidelity of DNA replication (Kornberg and Baker, 1992). The central features of this editing mechanism are likely to be general as many polymerases carry exonuclease domains that are similar in amino acid sequence. Sequence alignments, structural studies, and site-directed mutagenesis indicate that the exonuclease active site and the polymerase active site of these enzymes can be considered largely independent catalytic modules (Joyce and Steitz, 1994). The mechanism of editing is most thoroughly understood for *E. coli* DNA pol I (Freemont et al., 1988; Joyce and Steitz, 1994). Its polymerase and exonuclease active centers are located 30 Å apart but are linked by a shared DNA binding cleft. This arrangement dictates that the 3′ end of the growing chain must switch from the polymerase active site to the editing active site for a mistake to be excised (Figure 1B). This switch reflects a preference of the polymerase active site for a properly base-paired primer terminus; a misincorporation results in a 3′ terminus that is not base paired and therefore slows the forward rate of polymerization. This misincorporation also promotes melting of the primer-template duplex to generate the preferred substrate for the exonuclease, a DNA molecule with the last 4–5 nt at the 3′ end single stranded. Thus, incorporation of a mismatched base simultaneously encourages melting of the duplex to generate the substrate for the exonuclease while inhibiting the polymerase.

Processivity Factors: Sliding Clamps and Clamp Loaders. The exceptional processivity of replicative polymerases is controlled by protein subunits specialized for this function. The replicative polymerases of phage T4, *E. coli*, and eukaryotic cells each have two key processivity factors: (1) the sliding clamp and (2) the clamp loader (Yao et al., 1996).

Sliding clamps are protein rings that encircle the DNA (Kong et al., 1992; Krishna et al., 1994). Examples of sliding clamps include phage T4 gp45, the *E. coli* β subunit of DNA pol III holoenzyme, and the eukaryotic PCNA. The different clamp proteins, although distinct in sequence and multimeric state (a "ring" of β is a dimer, whereas PCNA is a trimer) have very similar folds (Kong et al., 1992; Krishna et al., 1994). The structures of both the β subunit and PCNA reveal that each protein is a doughnut-shaped multimer, with a 35 Å hole, big enough for a duplex DNA to slide through the middle without physically contacting the protein; indeed there is room for one to two layers of water molecules between the inner protein surface and the DNA, which may facilitate sliding (Kong et al., 1992; Krishna et al., 1994). These clamp proteins are topologically linked to, rather than in physical contact with, the DNA (Kuriyan and O'Donnell, 1993). As a result of this mode of DNA interaction, clamp proteins remain stably bound to a circular DNA molecule but rapidly dissociate from the same DNA once it is linearized; dissociation occurs upon DNA cleavage because the protein simply slides off the end of the DNA. By interacting with the polymerase while remaining linked around the DNA, these proteins clamp the enzymatic subunits to the template (Stukenberg et al., 1994).

Because the sliding clamps are closed circles of protein, energy-dependent clamp-loader machines are needed to assemble them onto DNA (Figure 2). The clamp loaders of phage T4 (gp44/62), *E. coli* (the γ complex), and eukaryotic cells (RF-C) each consist of multiple subunits, some of which are DNA-dependent ATPases (O'Donnell et al., 1993). The basic steps involved in loading include: recognition of the primer-template junction, binding the sliding clamp, disruption of the subunit interactions to open the ring, and placement of the ring around the DNA near a primer terminus. The following series of steps have been proposed for the mechanism of clamp loading by the *E. coli* γ complex (Kelman and O'Donnell, 1995): (1) the γ complex binds ATP and undergoes a conformational change to expose the β-binding surface of the γ complex's δ subunit; (2) the δ subunit binds β and opens the β ring; (3) the γ complex then recognizes the primer-template DNA and brings β to the DNA; (4) ATP-hydrolysis or ADP release then reburies the δ subunit in the complex, destablizing the δ–β interaction, thereby causing the β subunit to "snap" shut around the DNA. Interestingly, the γ complex can promote both the loading and unloading of β rings from the DNA. Whether interaction of γ complex with β results in loading or unloading is modulated by the interaction between β and the α subunit of pol III holoenzyme (Naktinis et al., 1996). Because polymerases and the clamp loader interact with the same face of the clamp, β subunits that are complexed with a polymerase are specifically protected from unloading, whereas those free from a polymerase may be unloaded and recycled. Evidence for similar loading schemes has emerged from studies of the T4 and eukaryotic clamp loaders (Tsurimoto and Stillman, 1991; Yao et al., 1996; Young et al., 1996) although the order of the individual steps may differ.

Sequence homology and structure-based alignments indicate that the clamp loader subunits are a family of related proteins that are likely to have similar folds (Guenther et al., 1997). The crystal structure of one of the clamp loader subunits, the δ' protein of the γ complex, reveals that it is a "C"-shaped protein (Guenther et al., 1997). The location of the ATP-binding site in these proteins is positioned such that ATP binding or hydrolysis

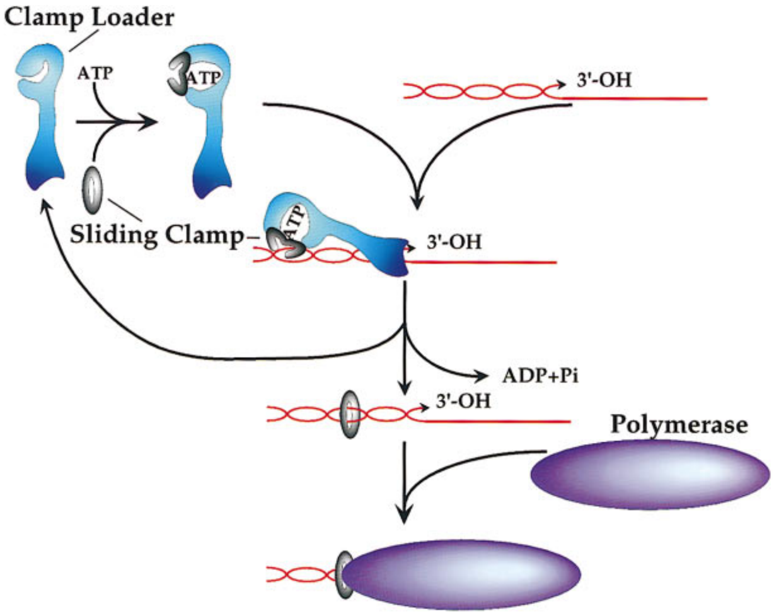

Figure 2. Steps Involved in Loading a Sliding Clamp for Processive DNA Synthesis.

See text for explanation.

could cause a conformational change; that this change results in the mouth of the "C" cycling between open and closed states is an attractive model for a protein that must open protein clamps using the energy of ATP hydrolysis.

Primases. Several features distinguish primases from replicative polymerases. Primases are unique among the polymerases involved in DNA replication in their ability to start the synthesis of new polynucleotide chains (Kornberg and Baker, 1992). Primases initiate chain synthesis at preferred sites on the template DNA; these "start sites" correspond to degenerate trinucleotide sequences (Kornberg and Baker, 1992). Thus, there are many places where primers can be initiated. Nonetheless, this sequence preference clearly distinguishes primases from replicative DNA polymerases. Different primases recognize different sequences. In some primases a zinc finger–like DNA-binding domain is involved in DNA sequence selection (Kusakabe and Richardson, 1996). Most primases can use either deoxy- or ribonucleotides; however, primers are usually RNA because of the larger cellular pools of ribonucleotides. Primases have very limited processivity and usually synthesize chains shorter than 12 nt. In eukaryotic cells, RNA primers are synthesized by the bifunctional pol α–primase and the short RNA primers synthesized by the primase active site are rapidly elongated by the associated DNA polymerase (see below).

Helicases: ATPases that Generate the Fork

The familiar structure of a replication fork as a site where the two strands of a duplex DNA are separated to reveal the single strands of opposite polarity, is generated through the action of a replicative DNA helicase. Although RNA polymerases can melt a DNA duplex, replicative DNA polymerases depend on a separate helicase. Helicases, or proteins with sequence homology to helicases, have been discovered with functions in DNA repair, genetic recombination, or transcription, however, only a subset of helicases are specialized to create replication forks. Recent work on the *E. coli* replicative helicase DnaB, the SV40 T antigen, and the phage helicases from T7 and T4, reveal that these four proteins have a common hexameric architecture and similar biochemical properties including high processivity and synergistic interactions with their cognate replicative DNA polymerases (Egelman, 1996, see below). Even in the absence of a clear sequence relationship, it is likely that these properties will be widespread among helicases that generate replication forks.

Electron microscopy image reconstruction techniques reveal that the hexameric replicative helicases form protein rings that can encircle DNA. The phage T7 helicase, for example, is a hexameric ring with two distinct faces (C6 symmetry, Yu et al., 1996a). Single-stranded DNA passes through the center of this protein ring; a similar arrangement is thought to exist for the DnaB, the T4 helicase and the SV40 T antigen–DNA complexes (Egelman, 1996). Whether one or both strands of DNA enter the ring in each case is not yet clear and may differ among the different helicases. The T7 helicase ring is 130 Å in diameter with a 25 to 30 Å hole and covers about 30 nt of DNA (Yu et al., 1996a). The fact that these helicases can encircle DNA provides a structural explanation for their nearly unlimited processivity in the context of a replication fork. Thus, both the sliding clamps and the hexameric DNA helicases appear to have met the requirement for high processivity by becoming topologically linked to the DNA. Once these helicases associate productively with DNA, helix melting continues until some active process terminates helicase activity. Sequence-specific termination proteins provide such helicase road blocks in bacteria (reviewed in Baker, 1995).

How ATP (NTP) fuels unwinding by the hexameric DNA helicases is not yet clear; however, some basic features of the cycle are emerging (see Lohman and Bjornson, 1996, for a recent review of helicase mechanism). The helicase cycle must involve an ordered series of conformational changes, modulated by ATP (NTP) binding, hydrolysis and release, allowing it to move along the DNA (Marians, 1997). For example, one conformation may interact with the duplex DNA, whereas a second conformation binds the melted single strands; ATP-dependent switching between these conformations may therefore represent the "power stroke" that melts the duplex and propels the helicase forward on the DNA strand. The existence of distinct conformational states of the hexameric helicases is supported by structural and kinetic studies (Bujalowski et al., 1994; San Martin et al., 1995; Egelman, 1996; Yu et al., 1996b). For example, DnaB helicase assumes two distinct protein conformations visible by electron microscopy (Yu et al., 1996b), and exchange between these conformations, controlled by nucleotide cofactors and DNA, may relate to the mechanism of movement. The number of ATP molecules hydrolyzed per unwound base pair has been estimated to be approximately 1–2 for several helicases (see Lohman and Bjornson, 1996). It is unlikely, however, that helicases move with a "step size" of a single base pair (e.g., melting 1 bp during each ATPase cycle); for example, a step size of 5 nt per reaction cycle has been reported for one well-studied enzyme (Lohman and Bjornson, 1996).

The hexameric replicative helicases are members of a helicase superfamily that carry conserved amino acid sequence motifs. Although there are no high-resolution structures of the hexameric helicases, the crystal structures of two other members of this helicase superfamily (the Bacillus PcrA protein and *E. coli* Rep, Subramanya et al., 1996; Korolev et al., 1997) have recently been solved. These structures reveal that most of the residues that make up the conserved helicase motifs form regions involved in DNA and ATP binding (reviewed in Marians, 1997). These DNA- and ATP-binding regions are near each other and connected by secondary structure elements, providing a picture of how a cycle of nucleotide binding and hydrolysis may direct movement along the DNA (Marians, 1997). How related the hexameric helicases will be to these proteins awaits determination of their structures. Nonetheless, the sequence similarity between the different helicases suggests that general features regarding the structural basis for coupling ATP binding, DNA binding, and protein translocation may be similar.

A cellular hexameric helicase with an essential role at the replication fork has not been discovered in eukaryotes. One attractive candidate for the eukaryotic replicative helicase is the complex of MCM proteins, a family of related putative DNA-dependent ATPases first identified as genes required for minichromosome maintenance in yeast (reviewed in Dutta and Bell, 1997). Recent studies indicate that a nonprocessive ($<$30 nt) DNA helicase activity cofractionates with a subset of the human MCM proteins (Mcm4p, Mcm6p, and Mcm7p; Ishimi, 1997). In addition, in vivo observations in *S. cerevisiae* indicate that MCM proteins are loaded at the origin and suggest that they move with the replication fork (Aparicio et al., 1997; Tanaka et al., 1997). Despite these advances, further studies will be required to establish that the DNA helicase activity observed is due to the direct action of the MCM proteins and that these proteins are continuously present at the replication fork.

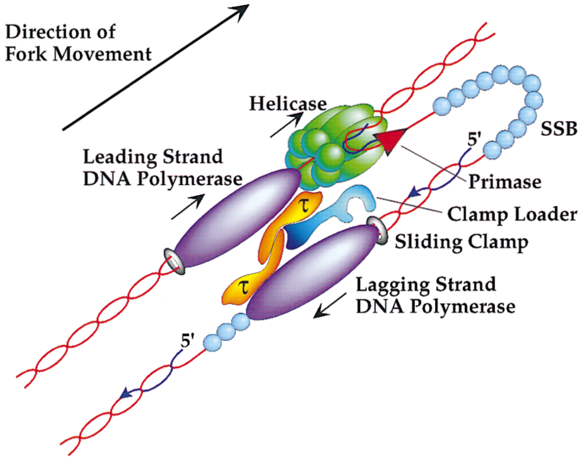
Direction of Fork Movement
Helicase
SSB
5'
Primase
Leading Strand DNA Polymerase
τ
Clamp Loader
Sliding Clamp
τ
Lagging Strand DNA Polymerase
5'

Figure 3. Model of the Replisome Based on Interactions Documented with *E. coli* Proteins

DNA is in red and RNA primers are in blue. Arrows adjacent to the DNA polymerases and DNA helicase indicate their direction of movement relative to their DNA template. See text for further explanation. (Adapted from Kim et al., 1996.)

Interactions at the Replication Fork

Interactions between the helicase, the replicative polymerase and the primase all contribute to the functional integrity of the replication fork. Figure 3 shows a model of a replication fork complex, based principally on work with the *E. coli* replication proteins. Interactions established for *E. coli* replication forks will be described first, followed by a discussion of some of the differences seen with eukaryotic replication forks.

The helicase makes functional contacts with the polymerase in the context of the replication fork (Kim et al., 1996; Yuzhakov et al., 1996). DnaB helicase moves into the fork in the 5′-to-3′ direction on the lagging strand template. In the absence of contact with polymerase, DnaB helicase unwinds about 35 nt of DNA per second. Contact between DnaB and polymerase, mediated by the τ subunit of pol III holoenzyme, increases this unwinding rate more than 10-fold (Kim et al., 1996). This contact also imparts increased processivity to the leading strand polymerase of the holoenzyme dimer (Yuzhakov et al., 1996). A similar synergistic interaction between the phage T4 helicase and polymerase has been demonstrated (Dong et al., 1996).

Protein contact between DnaB helicase and primase is also essential for the function of the replication fork. The polymerase activity of primase must be activated by interaction with DnaB (see Kornberg and Baker, 1992; Tougu and Marians, 1996). Furthermore, unlike DnaB and DNA pol III holoenzyme, primase does not travel as a stable component of the protein complex at the fork, but is recruited from solution for each priming event. Interestingly, primase mutations that alter the primase–DnaB interaction change the size of Okazaki fragments (Tougu and Marians, 1996). Thus, the frequency of the primase–DnaB interaction can dictate the frequency of priming the lagging strand. Primase also interacts with

DNA pol III holoenzyme, again via the τ subunit; this contact stimulates the frequency of priming and limits the size of primers to 12 residues (Zechner et al., 1992).

Communication between DNA pol III holoenzyme subunits, promoted principally by the τ subunit, also couples the activities of the replisome to ensure coordinated synthesis on the two template strands (Kelman and O'Donnell, 1995). Dimerization of the polymerase catalytic core assemblies (core is a tight complex between the polymerase subunit [α], the exonuclease [ϵ], and a third subunit of unknown function [θ]) is promoted by the τ subunit (Onrust et al., 1995). The ability of τ to dimerize core appears to be an essential function in vivo. Interestingly, the τ subunit and the γ subunit (which is part of the clamp loader) are both encoded by the same gene; γ therefore consists of the N-terminal 430 residues of τ. The unique C-terminal portion of τ, corresponding to the pol III core–binding domain, is essential. As τ is also the subunit that interacts with DnaB helicase and primase, it is a central scaffolding subunit in the replisome that enables the cooperative function of the helicase, primase, and polymerase submachines (Yuzhakov et al., 1996). Lack of this coordination during leading and lagging strand synthesis would likely have disastrous consequences. For example, without this interaction, synthesis on one strand could continue unfettered even when the opposite strand polymerase is stalled by a DNA lesion (Kelman and O'Donnell, 1995).

As the *E. coli* replication fork moves at about 1000 nt/s, one strand is synthesized discontinuously in segments (Okazaki fragments) that are 1000–2000 base pairs in length. Thus, a cycle of Okazaki strand synthesis occurs every 1–2 s. This cycle involves (1) recruitment of primase by the interaction with DnaB helicase, (2) primer synthesis, (3) loading of a β clamp on the new primer template junction, (4) transfer of the lagging strand catalytic core of DNA pol III holoenzyme to the new primer terminus, and (5) chain synthesis. The dimeric nature of the holoenzyme facilitates this cycle because the clamp loader and lagging strand polymerase move with the fork (Figure 3; Kelman and O'Donnell, 1995). Completion of an Okazaki fragment is followed by release of the lagging strand core polymerase from its associated sliding clamp (β subunit), allowing it to disengage from the completed fragment in preparation for recycling to the next primer (Stukenberg et al., 1994). There are 20 to 30 times more β subunits than holoenzyme assemblies, allowing β to be loaded on the new primer terminus before the previous Okazaki fragment is complete. The lagging strand polymerase is therefore thought to transfer between β clamps with each cycle without dissociating from the protein complex at the fork.

In contrast to the situation in *E. coli*, the eukaryotic DNA polymerases and accessory proteins are not tightly associated with one another in solution (Sugino, 1995). Nevertheless, studies of SV40 replication provide evidence for the coordinated function of these factors at the fork. All priming of DNA synthesis is mediated by pol α–primase. The primase activity of this enzyme first synthesizes a short RNA primer that is then elongated by the DNA polymerase activity of the same complex to form an RNA–DNA primer about 300 nt long (Tsurimoto et al., 1990; Waga and Stillman, 1994). The fragment length is limited by the low processivity of this enzyme. The resulting primer-template junction is recognized by RF-C (the eukaryotic clamp loader), which loads a PCNA sliding clamp. One of the PCNA-dependent DNA polymerases (pol δ or pol ϵ) utilizes the loaded PCNA to initiate processive synthesis (reviewed in Stillman, 1994). This polymerase switching event probably occurs during all priming events on both the leading and lagging strand. The exact role of the two PCNA-dependent eukaryotic polymerases (ϵ and δ) remains unknown and may differ at different replicons. Studies of SV40 DNA replication indicate that this virus is replicated in the absence of pol ϵ (Stillman, 1994). In contrast, genetic studies of mutant forms of pol δ and pol ϵ in yeast (defective in the proofreading exonuclease) suggest that during chromosomal replication the specific polymerases are dedicated to the leading and lagging strands (Shcherbakova and Pavlov, 1996), although which polymerase acts on which strand has not been determined.

Processing of Fragments into Continuous Strands

A set of proteins distinct from the replisome is required for the processing of Okazaki fragments into a continuous DNA strand. There are three steps in Okazaki fragment processing: removal of the primer, synthesis of DNA across the resulting gap, and DNA ligation. In *E. coli* the first two steps can be performed simultaneously by the nick translation activity of DNA pol I (Kornberg and Baker, 1992). The RNA primers are removed by the action of the 5′→3′ exonuclease domain of pol I (distinct from the editing 3′→5′ exo) and DNA is synthesized by the polymerase active site. RNaseH can also remove most of the RNA primer (all but the ribonucleotide at the RNA–DNA junction). The length of nick translation is presumably limited by the low processivity of pol I.

None of the eukaryotic DNA polymerases carries a 5′→3′ exonuclease (Sugino, 1995). Instead, removal of RNA primers is performed by a separate 5′→3′ exo/endo-nuclease called FEN-1. This enzyme is a nuclease specific for unannealed 5′ single-stranded tails on an otherwise duplex DNA (reviewed in Lieber, 1997). After completion of an Okazakai fragment, a helicase is thought to displace the 5′ end of the RNA-primed strand to generate the substrate for FEN-1. In *S. cerevisiae* this helicase is almost certainly the Dna2 protein, which interacts directly with FEN-1 (Budd and Campbell, 1997). As described above, RNaseH1 may also play a role in primer removal. FEN-1 interacts with PCNA, which likely functions to recruit FEN-1 to its site of action (Li et al., 1995; Chen et al., 1996; Wu et al., 1996). FEN-1-like nucleases also provide another example of replication enzymes with holes in their structures that encircle the polynucleotide chain of their substrates (Ceska et al., 1996). Once the primer is removed, a DNA polymerase fills the gap, leaving a nick appropriate for sealing by DNA ligase. In SV40 replication in vitro, a strong preference is observed for DNA ligase I in this step (there are at least 4 ligases in eukaryotic cells), suggesting that this enzyme is specialized for processing Okazaki fragments (Turchi et al., 1994; Waga and Stillman, 1994).

Assembly of DNA Replication Forks

Although events at replication forks exhibit little sequence specificity, they are assembled at specific chromosomal sites known as origins of replication. The machinery required to assemble the replication fork includes

Table 2. Initiation Proteins

Function	*E. coli*	Phage λ	Phage T4	SV40/Human	Yeast
Initiator protein	DnaA	λ O	none	T antigen	ORC
Loading and remodeling factor(s)	DnaC	λ P, DnaJ, DnaK	gp59	Cellular chaperone?	Cdc6 protein
DNA helicase	DnaB	DnaB	gp41	T antigen	MCM proteins?

proteins that specifically recognize these origin sequences (initiator proteins), proteins required for assembly but not for subsequent steps in DNA synthesis (loading and remodeling factors), and components of the fork machinery (e.g., DNA helicase). Together these factors control the timing and site of assembly of the DNA replication fork during cell division and are the likely targets of cell cycle regulation. As with the components of the replication fork, initiation factors are functionally conserved between prokaryotes, phage, and eukaryotes (Table 2).

Origin Recognition and Initial Melting of the DNA Strands

Initiator proteins, including *E. coli* DnaA protein, λ O protein, SV40 T antigen, and the eukaryotic origin recognition complex (ORC) recognize their cognate origins and form the foundation for all subsequent events during initiation. Once bound to DNA, these proteins frequently have two additional common features: (1) they facilitate the unwinding or distortion of adjacent DNA to provide the entry site for the DNA helicase, and (2) they recruit additional factors involved in both the assembly and function of the replication fork.

Multiple subunits of DnaA, λ O, and T antigen assemble into large complexes at their origins (Borowiec et al., 1990; Kornberg and Baker, 1992). In contrast, ORC is a six-subunit heteromultimer, preassembled in solution, that binds origin DNA as a large complex in the presence of ATP (Bell and Stillman, 1992; Klemm et al., 1997). DnaA and T antigen are also ATP-binding proteins, and although the nucleotide requirement for DNA binding is not absolute, ATP does influence subsequent initiator functions (Sekimizu et al., 1987; Borowiec et al., 1990). Binding of initiators to origins, although critical for initiation, is not sufficient. These proteins bind to DNA sites that are not functional origins (Santocanale and Diffley, 1996; Messer and Weigel, 1997). Furthermore, DnaA and ORC are bound to origins at times of the cell cycle when these origins are not active (Diffley et al., 1994; Cassler et al., 1995). Thus, binding of the initiator protein to the DNA is not the committed step during initiation of replication.

A second critical step in fork assembly is the initial separation of the two strands of DNA for loading the replication machinery and providing the template for DNA synthesis. DnaA, λ O protein, and SV40 T antigen all induce distortion or unwinding of DNA adjacent to their binding sites at the origin (Borowiec et al., 1990). In each case, DNA unwinding occurs at specific AT-rich sequences that are required for origin function. Both T antigen and DnaA protein interact specifically with one strand of the resulting single-stranded regions (Hwang and Kornberg, 1992; SenGupta and Borowiec, 1994). Thus, in addition to their ability to recognize specifically their cognate double-stranded DNA binding sites, initiator proteins can act as single-stranded DNA binding proteins during origin unwinding. In this regard, it is interesting that ORC preferentially interacts with one strand of its double-stranded DNA binding site (Lee and Bell, 1997); however, DNA unwinding promoted by ORC has not been observed.

Recruitment of the Replication Machinery to the Origin

Once bound to the origin DNA, initiator proteins recruit additional factors involved in fork assembly. The primary goal of this recruitment is to bring the DNA helicase to the fork assembly site (Figure 4). For *E. coli* chromosomal and phage λ replication, the helicase is recruited as a complex with a loading factor (DnaC and λ P, respectively; Baker and Wickner, 1992). In addition to escorting the DnaB hexamers to the fork assembly site, these factors maintain the helicase in an inactive state. The gp59 protein plays a similar role as a loading factor for gp41 helicase during initiation of phage T4 replication (Kreuzer and Morrical, 1994). Protein–protein interactions between the DnaB·loading factor complex and the initiator protein, as well as interactions of the loading factor with single-stranded DNA, are important during this assembly stage (Learn et al., 1997).

Although the replicative DNA helicase remains elusive in eukaryotic cells (see above), recent studies of the assembly of proteins at cellular origins suggest a similar set of recruitment steps is involved in fork assembly as in prokaryotes (Figure 4). Both in vitro studies in *Xenopus* extracts and in vivo studies in *S. cerevisiae* cells indicate that the Cdc6 protein (a protein required for entry into S phase and known to interact with ORC; Liang et al., 1995, and reviewed in Dutta and Bell, 1997) and the MCM proteins are assembled onto an ORC–origin complex in an ordered fashion prior to initiation of DNA synthesis (Coleman et al., 1996; Romanowski et al., 1996; Rowles et al., 1996; Aparicio et al., 1997; Donovan et al., 1997; Tanaka et al., 1997). Cdc45 protein, a factor that interacts with both ORC and MCM proteins (reviewed in Dutta and Bell, 1997), also associates with the origin prior to initiation (Aparicio et al., 1997). The recent evidence suggesting that MCM proteins act at replication forks, as well as the finding that Cdc6 protein is required for initiation but not elongation (reviewed in Dutta and Bell, 1997), suggests that Cdc6 protein is analogous to the loading factors λ P and DnaC whereas the MCM proteins may function like DnaB helicase (Figure 4). The role of Cdc45 protein is less clear. However, because it has been implicated as moving with the replication fork (Aparicio et al., 1997), one possibility is that it coordinates the assembly and function of the eukaryotic polymerases, perhaps analogously to the polymerase-coordinating activity of the *E. coli* τ protein.

Replication Complex Remodeling

The assembly of specific, stable replication protein complexes on origin DNA generates an inherent dilemma for the cell. Assembly at the correct sites requires both high

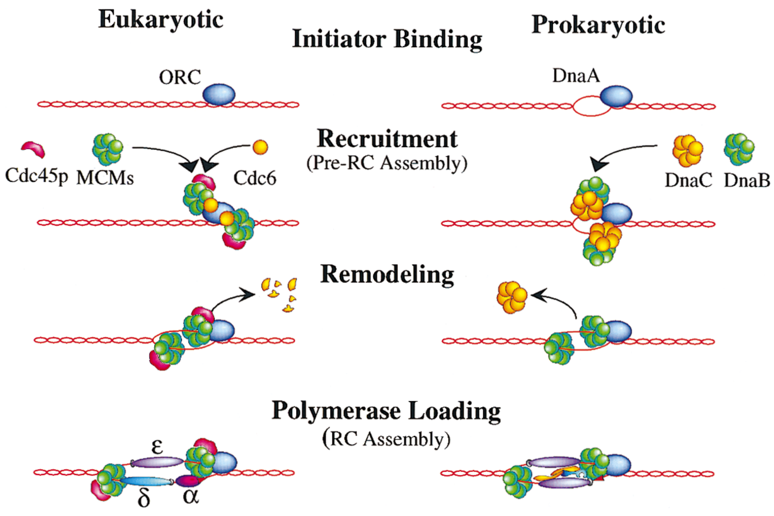

Figure 4. Comparison of the Protein Complexes Formed at Origins during the Initiation of Replication in *E. coli* and Eukaryotic DNA Replication

Pre-RC and RC stand for **p**re-**r**eplicative **c**omplex and **r**eplicative **c**omplex, respectively. The RC is the complex formed when the polymerases are first recruited to the origin. See text for explanation.

specificity and stability; however, DNA replication (or transcription, recombination, or repair) requires that these initial protein–DNA complexes release the polymerases, helicases, and other associated factors to perform their designated functions. Thus, it is important to understand how these complexes are remodeled or disassembled during replication initiation to trigger the transition from a stable origin-bound complex to a mobile replication machine.

During this transition, protein–protein and protein–DNA contacts that were essential to recruit factors to the origin complex must be disrupted. Remodeling of the prereplicative complexes can be controlled by a number of different factors. Initiation of phage λ DNA replication requires molecular chaperones to remodel the replication complex. Separation of the loading factor λ P from the DnaB helicase is catalyzed by the combined action of DnaK (the *E. coli* Hsp70), DnaJ, and GrpE (Alfano and McMacken, 1989; Osipiuk et al., 1993; Wyman et al., 1993). Similarly, the C-terminal region of SV40 T antigen is a DnaJ-like domain, which may recruit cellular chaperones to function during initiation (Campbell et al., 1997; Kelley and Georgopoulos, 1997; Srinivasan et al., 1997). A second method to alter the composition of origin-bound initiation complexes is the alteration of the ATP-bound state of its components. For example, *E. coli* DnaC protein must be in the ATP-bound state to interact with and load DnaB at oriC but appears to exit the complex upon ATP hydrolysis or release (Wahle et al., 1989).

Studies of eukaryotic origin-associated protein complexes suggest that similar remodeling events are coupled to replication initiation. During the process of initiation, the protein components associated with the origins clearly change. These alterations include release or degradation of the putative loading factor, Cdc6 protein, localization of DNA polymerases to the origin, and the eventual disassembly of the origin-associated complex (Diffley et al., 1994; Santocanale and Diffley, 1996; Aparicio et al., 1997; Donovan et al., 1997; Drury et al., 1997; Tanaka et al., 1997). Mechanisms controlling these changes have not been defined; however, there are several interesting possibilities. Modification of one or more of the components of the prereplicative complex (the assembly of proteins localized to eukaryotic origins during G1, Diffley et al., 1994) by cell cycle–regulated kinases is a likely trigger for remodeling (Stillman, 1996). In addition, ATP hydrolysis by ORC or Cdc6 could trigger a change in the protein composition or conformation of the complex at the origin (Zwerschke et al., 1994; Klemm et al., 1997). Degradation of Cdc6 protein at the G1/S transition is another attractive mechanism for remodeling the proteins assembled prior to initiation (Drury et al., 1997).

The first major remodeling step during initiation appears to result in engagement of the helicase at the assembling fork. The mechanism of helicase loading is unclear, but because replicative DNA helicases are thought to encircle one or both strands of the DNA, protein-catalyzed "ring opening" may be required. Helicase loading, in turn, is likely to be critical in recruiting the remainder of the fork components. The same helicase, primase, and polymerase interactions responsible for coordinating the replisome (see above) are also likely to be involved in recruiting components to the assembling fork. Additional interactions between origin binding proteins and polymerases that function specifically during initiation are also likely. For example, eukaryotic viral initiator proteins (which are also helicases) interact directly with and presumably recruit DNA pol α–primase to the origin (Collins and Kelly, 1991; Dornreiter et al., 1992; Collins et al., 1993; Park et al., 1994).

Perspectives

The replisome is a well-characterized example of how protein components communicate with one another to coordinate the action of multiple molecular machines. The molecular structure of numerous replication factors has provided beautiful and insightful explanations of the molecular basis of protein function. Especially stunning are the hand-like structures found in all polymerases and the ring-like architecture of the sliding clamps and helicases. Mechanistic and structural information regarding the DNA replication apparatus provide the foundation for elucidating the molecular mechanisms by which these important machines are regulated. Furthermore, the required transition between a static complex fixed at the origin and the fast-moving replisome illustrates the general importance that remodeling of stable protein–nucleic acid complexes to release mobile enzymatic activities will play in biological regulation. For example, similar transitions must occur during transcription where RNA polymerase first must assemble at specific sites, yet later must sacrifice the sequence-specific DNA contacts and associations with assembly factors to successfully clear the promoter and carry out transcription.

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References

Alberts, B.M. (1987). Prokaryotic DNA replication mechanisms. Philos. Trans. R. Soc. Lond. B *317*, 395–420.

Alfano, C., and McMacken, R. (1989). Heat shock protein-mediated disassembly of nucleoprotein structures is required for the initiation of bacteriophage lambda DNA replication. J. Biol. Chem. *264*, 10709–10718.

Aparicio, O.M., Weinstein, D.M., and Bell, S.P. (1997). Components and dynamics of DNA replication complexes in S. cerevisiae: redistribution of MCM proteins and Cdc45p during S phase. Cell *91*, 59–69.

Baker, T.A. (1995). Replication arrest. Cell *80*, 521–524.

Baker, T.A., and Wickner, S.H. (1992). Genetics and enzymology of DNA replication in Escherichia coli. Annu. Rev. Genet. *26*, 447–477.

Bell, S.P., and Stillman, B. (1992). ATP-dependent recognition of eucaryotic origins of DNA replication by a multiprotein complex. Nature *357*, 128–134.

Borowiec, J.A., Dean, F.B., Bullock, P.A., and Hurwitz, J. (1990). Binding and unwinding—how T antigen engages the SV40 origin of DNA replication. Cell *60*, 181–184.

Budd, M.E., and Campbell, J.L. (1997). A yeast replicative helicase, Dna2 helicase, interacts with yeast FEN-1 nuclease in carrying out its essential function. Mol. Cell. Biol. *17*, 2136–2142.

Bujalowski, W., Klonowska, M.M., and Jezewska, M.J. (1994). Oligomeric structure of Escherichia coli primary replicative helicase DnaB protein. J. Biol. Chem. *269*, 31350–31358.

Campbell, K.S., Mullane, K.P., Aksoy, I.A., Stubdal, H., Zalvide, J., Pipas, J.M., Silver, P.A., Roberts, T.M., Schaffhausen, B.S., and DeCaprio, J.A. (1997). DnaJ/hsp40 chaperone domain of SV40 large T antigen promotes efficient viral DNA replication. Genes Dev. *11*, 1098–1110.

Cassler, M.R., Grimwade, J.E., and Leonard, A.C. (1995). Cell cycle-specific changes in nucleoprotein complexes at a chromosomal replication origin. EMBO J. *14*, 5833–5841.

Ceska, T.A., Sayers, J.R., Stier, G., and Suck, D. (1996). A helical arch allowing single-stranded DNA to thread through T5 5′- exonuclease. Nature *382*, 90–93.

Chen, U., Chen, S., Saha, P., and Dutta, A. (1996). p21Cip1/Waf1 disrupts the recruitment of human Fen1 by proliferating cell nuclear antigen into the DNA replication complex. Proc. Natl. Acad. Sci. USA *93*, 11597–11602.

Coleman, T.R., Carpenter, P.B., and Dunphy, W.G. (1996). The Xenopus Cdc6 protein is essential for the initiation of a single round of DNA replication in cell-free extracts. Cell *87*, 53–63.

Collins, K.L., and Kelly, T.J. (1991). Effects of T antigen and replication protein A on the initiation of DNA synthesis by DNA polymerase alpha-primase. Mol. Cell. Biol. *11*, 2108–2115.

Collins, K.L., Russo, A.A., Tseng, B.Y., and Kelly, T.J. (1993). The role of the 70 kDa subunit of human DNA polymerase alpha in DNA replication. EMBO J. *12*, 4555–4566.

Diffley, J.F.X., Cocker, J.H., Dowell, S.J., and Rowley, A. (1994). Two steps in the assembly of complexes at yeast replication origins in vivo. Cell *78*, 303–316.

Dong, F., Weitzel, S.E., and von Hippel, P.H. (1996). A coupled complex of T4 DNA replication helicase (gp41) and polymerase (gp43) can perform rapid and processive DNA strand-displacement synthesis. Proc. Natl. Acad. Sci. USA *93*, 14456–14461.

Donovan, S., Harwood, J., Drury, L.S., and Diffley, J.F. (1997). Cdc6p-dependent loading of Mcm proteins onto pre-replicative chromatin in budding yeast. Proc. Natl. Acad. Sci. USA *94*, 5611–5616.

Dornreiter, I., Erdile, L.F., Gilbert, I.U., von, W.D., Kelly, T.J., and Fanning, E. (1992). Interaction of DNA polymerase alpha-primase with cellular replication protein A and SV40 T antigen. EMBO J. *11*, 769–776.

Drury, L.S., Perkins, G., and Diffley, J.F.X. (1997). The Cdc4/34/53 pathway targets Cdc6p for proteolysis in budding yeast. EMBO J. *16*, 5966–5976.

Dutta, A., and Bell, S.P. (1997). Initiation of DNA replication in eukaryotic cells. Annu. Rev. Cell Dev. Biol. *13*, 293–332.

Egelman, E.H. (1996). Homomorphous hexameric helicases: tales from the ring cycle. Structure *4*, 759–762.

Freemont, P.S., Friedman, J.M., Beese, L.S., Sanderson, M.R., and Steitz, T.A. (1988). Cocrystal structure of an editing complex of Klenow fragment with DNA. Proc. Natl. Acad. Sci. USA *85*, 8924–8928.

Guenther, B., Onrust, R., Sali, A., O'Donnell, M., and Kuriyan, J. (1997). Crystal structure of the δ' subunit of the clamp loader complex of E. coli DNA polymerase III. Cell *91*, 335–346.

Hwang, D.S., and Kornberg, A. (1992). Opening of the replication origin of Escherichia coli by DnaA protein with protein HU or IHF. J. Biol. Chem. *267*, 23083–23086.

Ishimi, Y. (1997). A DNA helicase activity is associated with an MCM4, -6, and −7 protein complex. J. Biol. Chem. *272*, 24508–24513.

Joyce, C.M. (1997). Choosing the right sugar: how polymerases select a nucleotide substrate. Proc. Natl. Acad. Sci. USA *94*, 1619–1622.

Joyce, C.M., and Steitz, T.A. (1994). Function and structure relationships in DNA polymerases. Annu. Rev. Biochem. *63*, 777–822.

Joyce, C.M., and Steitz, T.A. (1995). Polymerase structures and function: variations on a theme? J. Bacteriol. *177*, 6321–6329.

Kelley, W.L., and Georgopoulos, C. (1997). The T/t common exon of simian virus 40, JC, and BK polyomavirus T antigens can functionally replace the J-domain of the Escherichia coli DnaJ molecular chaperone. Proc. Natl. Acad. Sci. USA *94*, 3679–3684.

Kelman, Z., and O'Donnell, M. (1995). DNA polymerase III holoenzyme: structure and function of a chromosomal replicating machine. Annu. Rev. Biochem. *64*, 171–200.

Kiefer, J.R., Mao, C., Braman, J.C., and Beese, L.S. (1998). Visualizing DNA replication in a catalytically active polymerase crystal at 1.8 angstrom resolution. Nature, in press.

Kim, S., Dallmann, H.G., McHenry, C.S., and Marians, K.J. (1996). Coupling of a replicative polymerase and helicase: a tau-DnaB interaction mediates rapid replication fork movement. Cell *84*, 643–650.

Klemm, R.D., Austin, R.J., and Bell, S.P. (1997). Coordinate binding of ATP and origin DNA regulates the ATPase activity of the origin recognition complex. Cell *88*, 493–502.

Kong, X.P., Onrust, R., O'Donnell, M., and Kuriyan, J. (1992). Three-dimensional structure of the beta subunit of E. coli DNA polymerase III holoenzyme: a sliding DNA clamp. Cell *69*, 425–437.

Kornberg, A., and Baker, T.A. (1992). DNA Replication, Second Ed. (New York: W.H. Freeman and Company).

Korolev, S., Hsieh, J., Gauss, G.H., Lohman, T.M., and Waksman, G. (1997). Major domain swiveling revealed by the crystal structures of complexes of E. coli Rep helicase bound to single-stranded DNA and ADP. Cell *90*, 635–647.

Kreuzer, K.N., and Morrical, S.W. (1994). Initiation of DNA Replication. In Molecular Biology of Bacteriophage T4, J.D. Karam., ed. (Washington, D.C.: ASM Press), 28–42.

Krishna, T.S., Kong, X.P., Gary, S., Burgers, P.M., and Kuriyan, J. (1994). Crystal structure of the eukaryotic DNA polymerase processivity factor PCNA. Cell *79*, 1233–1243.

Kuriyan, J., and O'Donnell, M. (1993). Sliding clamps of DNA polymerases. J. Mol. Biol. *234*, 915–925.

Kusakabe, T., and Richardson, C.C. (1996). The role of the zinc motif in sequence recognition by DNA primases. J. Biol. Chem. *271*, 19563–19570.

Learn, B.A., Um, S.J., Huang, L., and McMacken, R. (1997). Cryptic single-stranded-DNA binding activities of the phage lambda P and Escherichia coli DnaC replication initiation proteins facilitate the transfer of E. coli DnaB helicase onto DNA. Proc. Natl. Acad. Sci. USA *94*, 1154–1159.

Lee, D., and Bell, S.P. (1997). Architecture of the yeast origin recognition complex bound to origins of DNA replication. Mol. Cell. Biol. *17*, 7159–7168.

Li, X., Li, J., Harrington, J., Lieber, M.R., and Burgers, P.M. (1995). Lagging strand DNA synthesis at the eukaryotic replication fork involves binding and stimulation of FEN-1 by proliferating cell nuclear antigen. J. Biol. Chem. *270*, 22109–22112.

Liang, C., Weinreich, M., and Stillman, B. (1995). ORC and Cdc6p interact and determine the frequency of initiation of DNA replication in the genome. Cell *81*, 667–676.

Lieber, M.R. (1997). The FEN-1 family of structure-specific nucleases in eukaryotic DNA replication, recombination and repair. Bioessays *19*, 233–240.

Lohman, T.M., and Bjornson, K.P. (1996). Mechanisms of helicase-catalyzed DNA unwinding. Annu. Rev. Biochem. *65*, 169–214.

Marians, K.J. (1992). Prokaryotic DNA replication. Annu. Rev. Biochem. *61*, 673–719.

Marians, K.J. (1997). Helicase structures: a new twist on DNA unwinding. Structure *5*, 1129–1134.

Messer, W., and Weigel, C. (1997). DnaA initiator—also a transcription factor. Mol. Microbiol. *24*, 1–6.

Munn, M.M., and Alberts, B.M. (1991). The T4 DNA polymerase accessory proteins form an ATP-dependent complex on a primer-template junction. J. Biol. Chem. *266*, 20024–20033.

Naktinis, V., Turner, J., and O'Donnell, M. (1996). A molecular switch in a replication machine defined by an internal competition for protein rings. Cell *84*, 137–145.

O'Donnell, M., Onrust, R., Dean, F.B., Chen, M., and Hurwitz, J. (1993). Homology in accessory proteins of replicative polymerases—E. coli to humans. Nucleic Acids Res. *21*, 1–3.

Onrust, R., Finkelstein, J., Turner, J., Naktinis, V., and O'Donnell, M. (1995). Assembly of a chromosomal replication machine: two DNA polymerases, a clamp loader, and sliding clamps in one holoenzyme particle. III. Interface between two polymerases and the clamp loader. J. Biol. Chem. *270*, 13366–13377.

Osipiuk, J., Georgopoulos, C., and Zylicz, M. (1993). Initiation of lambda DNA replication. The Escherichia coli small heat shock proteins, DnaJ and GrpE, increase DnaK's affinity for the lambda P protein. J. Biol. Chem. *268*, 4821–4827.

Park, P., Copeland, W., Yang, L., Wang, T., Botchan, M.R., and Mohr, I.J. (1994). The cellular DNA polymerase alpha-primase is required for papillomavirus DNA replication and associates with the viral E1 helicase. Proc. Natl. Acad. Sci. USA *91*, 8700–8704.

Romanowski, P., Madine, M.A., Rowles, A., Blow, J.J., and Laskey, R.A. (1996). The Xenopus origin recognition complex is essential for DNA replication and MCM binding to chromatin. Curr. Biol. *6*, 1416–1425.

Rowles, A., Chong, J.P.J., Brown, L., Howell, M., Evan, G.I., and Blow, J.J. (1996). Interaction between the origin recognition complex and the replication licensing system in Xenopus. Cell *87*, 287–296.

San Martin, M., Stamford, N.P., Dammerova, N., Dixon, N.E., and Carazo, J.M. (1995). A structural model for the Escherichia coli DnaB helicase based on electron microscopy data. J. Struct. Biol. *114*, 167–176.

Santocanale, C., and Diffley, J.F.X. (1996). ORC- and Cdc6-dependent complexes at active and inactive chromosomal replication origins in Saccharomyces cerevisiae. EMBO J. *15*, 6671–6679.

Sekimizu, K., Bramhill, D., and Kornberg, A. (1987). ATP activates dnaA protein in initiating replication of plasmids bearing the origin of the E. coli chromosome. Cell *50*, 259–265.

SenGupta, D.J., and Borowiec, J.A. (1994). Strand and face: the topography of interactions between the SV40 origin of replication and T-antigen during the initiation of replication. EMBO J. *13*, 982–992.

Shcherbakova, P.V., and Pavlov, Y.I. (1996). 3′→5′ exonucleases of DNA polymerases epsilon and delta correct base analog induced DNA replication errors on opposite DNA strands in Saccharomyces cerevisiae. Genetics *142*, 717–726.

Sousa, R. (1996). Structural and mechanistic relationships between nucleic acid polymerases. Trends Biochem. Sci. *21*, 186–190.

Srinivasan, A., McClellan, A.J., Vartikar, J., Marks, I., Cantalupo, P., Li, Y., Whyte, P., Rundell, K., Brodsky, J.L., and Pipas, J.M. (1997). The amino-terminal transforming region of simian virus 40 large T and small t antigens functions as a J domain. Mol. Cell. Biol. *17*, 4761–4773.

Steitz, T.A., Smerdon, S.J., Jager, J., and Joyce, C.M. (1994). A unified polymerase mechanism for nonhomologous DNA and RNA polymerases. Science *266*, 2022–2025.

Stillman, B. (1994). Smart machines at the DNA replication fork. Cell *78*, 725–728.

Stillman, B. (1996). Cell cycle control of DNA replication. Science *274*, 1659–1664.

Stukenberg, P.T., Turner, J., and O'Donnell, M. (1994). An explanation for lagging strand replication: polymerase hopping among DNA sliding clamps. Cell *78*, 877–887.

Subramanya, H.S., Bird, L.E., Brannigan, J.A., and Wigley, D.B. (1996). Crystal structure of a DExx box DNA helicase. Nature *384*, 379–383.

Sugino, A. (1995). Yeast DNA polymerases and their role at the replication fork. Trends Biochem. Sci. *20*, 319–323.

Tanaka, T., Knapp, D., and Nasmyth, K. (1997). Loading of an Mcm protein onto DNA replication origins is regulated by Cdc6p and CDKs. Cell *90*, 649–660.

Tougu, K., and Marians, K.J. (1996). The interaction between helicase and primase sets the replication fork clock. J. Biol. Chem. *271*, 21398–21405.

Tsurimoto, T., Melendy, T., and Stillman, B. (1990). Sequential initiation of lagging and leading strand synthesis by two different polymerase complexes at the SV40 DNA replication origin. Nature *346*, 534–539.

Tsurimoto, T., and Stillman, B. (1991). Replication factors required for SV40 DNA replication in vitro. I. DNA structure-specific recognition of a primer-template junction by eukaryotic DNA polymerases and their accessory proteins. J. Biol. Chem. *266*, 1950–1960.

Turchi, J.J., Huang, L., Murante, R.S., Kim, Y., and Bambara, R.A. (1994). Enzymatic completion of mammalian lagging-strand DNA replication. Proc. Natl. Acad. Sci. USA *91*, 9803–9807.

Waga, S., and Stillman, B. (1994). Anatomy of a DNA replication fork revealed by reconstitution of SV40 DNA replication in vitro. Nature *369*, 207–212.

Wahle, E., Lasken, R.S., and Kornberg, A. (1989). The dnaB-dnaC replication protein complex of Escherichia coli. II. Role of the complex in mobilizing dnaB functions. J. Biol. Chem. *264*, 2469–2475.

Wang, J., Sattar, A.K., Wang, C.C., Karam, J.D., Konigsberg, W.H. and Steitz, T.A. (1997). Crystal structure of a pol α family replication DNA polymerase from bacteriophage RB69. Cell *89*, 1087–1099.

Wu, X., Li, J., Li, X., Hsieh, C.L., Burgers, P.M., and Lieber, M.R. (1996). Processing of branched DNA intermediates by a complex of human FEN-1 and PCNA. Nucleic Acids Res. *24*, 2036–2043.

Wyman, C., Vasilikiotis, C., Ang, D., Georgopoulos, C., and Echols, H. (1993). Function of the GrpE heat shock protein in bidirectional unwinding and replication from the origin of phage lambda. J. Biol. Chem. *268*, 25192–25196.

Yao, N., Turner, J., Kelman, Z., Stukenberg, P.T., Dean, F., Shechter, D., Pan, Z.Q., Hurwitz, J., and O'Donnell, M. (1996). Clamp loading, unloading and intrinsic stability of the PCNA, beta and gp45 sliding clamps of human, E. coli and T4 replicases. Genes Cells *1*, 101–113.

Young, M.C., Weitzel, S.E., and von Hippel, P. (1996). The kinetic mechanism of formation of the bacteriophage T4 DNA polymerase sliding clamp. J. Mol. Biol. *264*, 440–452.

Yu, X., Hingorani, M.M., Patel, S.S., and Egelman, E.H. (1996a). DNA is bound within the central hole to one or two of the six subunits of the T7 DNA helicase. Nat. Struct. Biol. *3*, 740–743.

Yu, X., Jezewska, M.J., Bujalowski, W., and Egelman, E.H. (1996b). The hexameric E. coli DnaB helicase can exist in different quaternary states. J. Mol. Biol. *259*, 7–14.

Yuzhakov, A., Turner, J., and O'Donnell, M. (1996). Replisome assembly reveals the basis for asymmetric function in leading and lagging strand replication. Cell *86*, 877–886.

Zechner, E.L., Wu, C.A., and Marians, K.J. (1992). Coordinated leading- and lagging-strand synthesis at the Escherichia coli DNA replication fork. III. A polymerase-primase interaction governs primer size. J. Biol. Chem. *267*, 4054–4063.

Zwerschke, W., Rottjakob, H.W., and Kuntzel, H. (1994). The Saccharomyces cerevisiae CDC6 gene is transcribed at late mitosis and encodes a ATP/GTPase controlling S phase initiation. J. Biol. Chem. *269*, 23351–23356.