

Molecular Machines of the Cell

Replisome mechanics: insights into a twin DNA polymerase machine

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Chromosomal replicases are multicomponent machines that copy DNA with remarkable speed and processivity. The organization of the replisome reveals a twin DNA polymerase design ideally suited for concurrent synthesis of leading and lagging strands. Recent structural and biochemical studies of *Escherichia coli* and eukaryotic replication components provide intricate details of the organization and inner workings of cellular replicases. In particular, studies of sliding clamps and clamp-loader subunits elucidate the mechanisms of replisome processivity and lagging strand synthesis. These studies demonstrate close similarities between the bacterial and eukaryotic replication machineries.

Introduction

Replication is carried out in a semiconservative manner as each DNA strand is copied in a 5' to 3' direction to create two newly formed daughter strands [1]. The antiparallel conformation of DNA therefore presents major organizational challenges for the replication machinery as the replisome moves in a unidirectional fashion along the separated single strands. These challenges are overcome by the evolution of a dynamic multicomponent holoenzyme complex that employs a dual DNA polymerase design to carry out concurrent continuous and discontinuous synthesis of leading and lagging strands, respectively.

Much insight into how replicases carry out leading and lagging strand synthesis is provided by recent biochemical and structural studies of replication components from bacteria, eukaryotes, archaea and bacteriophage T4 [2–5]. These studies include detailed biochemical and structural analyses of sliding clamps, which reveal ring-shaped proteins that confer speed and processivity to the replisome by tethering DNA polymerases to their respective templates. Studies of bacterial and eukaryotic multisubunit clamp loaders elucidate the mechanisms by which these dynamic machines use the energy of ATP to assemble sliding clamps around duplex DNA at primed sites [5]. Biochemical analyses of the *Escherichia coli* replication fork further characterize the clamp loader as a protein trafficking component that is essential for lagging strand synthesis and the overall organization of the replication machinery [2,3]. Last, biochemical studies characterize the *E. coli* replicative helicase, which uses the energy of ATP to unwind duplex

DNA ahead of the replication fork [6,7]. Owing to space limitations, we focus our attention on the well-defined replication system of *E. coli* and present certain parallel findings from the eukaryotic system. The brevity of this review does not permit a detailed discussion of replication initiation, which is reviewed elsewhere (see Refs [1,8–10]).

Mechanics of leading and lagging strand synthesis

Cellular replicases move along DNA in a unidirectional manner while copying both strands of the double helix. The antiparallel conformation of DNA therefore raises questions as to how the replication machinery simultaneously copies both strands of DNA since the direction of nucleic acid synthesis can only occur in a 5' to 3' direction. Biochemical studies indicate that cellular replicases overcome this dilemma by synthesizing leading and lagging strands in a continuous and discontinuous manner, respectively.

Figure 1 illustrates how leading and lagging strands are extended by cellular replicases. Replicative DNA polymerases initiate DNA synthesis by extending the 3' end of a RNA primer. Short RNA primers, ~12 nucleotides in length, are synthesized by a specialized RNA polymerase called primase [11]. As illustrated in Figure 1, extension of the leading strand requires only one or a few priming events and is therefore thought to occur in a continuous fashion. This widely accepted view is based on replication assays performed *in vitro* with highly purified proteins. A recent *in vivo* study of replication intermediates, however, suggests that the process of leading strand synthesis might be frequently interrupted [12]. Moreover, occasional inactivation of the replication fork due to DNA damage or other impediments along the leading strand result in additional priming events, which are necessary for replication restart (reviewed in Refs [13–16]). Further studies are required to determine exactly how often leading strand synthesis is interrupted under normal growth conditions.

By contrast, lagging strand synthesis requires multiple primed sites because this strand must be synthesized as a series of discontinuous fragments. Importantly, the lagging strand is thought to form a loop that results in the directional alignment of lagging strand synthesis with the movement of the replisome [17] (Figure 1, right). Synthesis of a lagging strand fragment proceeds until the 5' end of the next downstream primer is encountered, resulting in the dissociation of the lagging strand polymerase [18,19]. These relatively short (1–3 kb) RNA-primed lagging strand fragments are referred to as Okazaki fragments. The 5'

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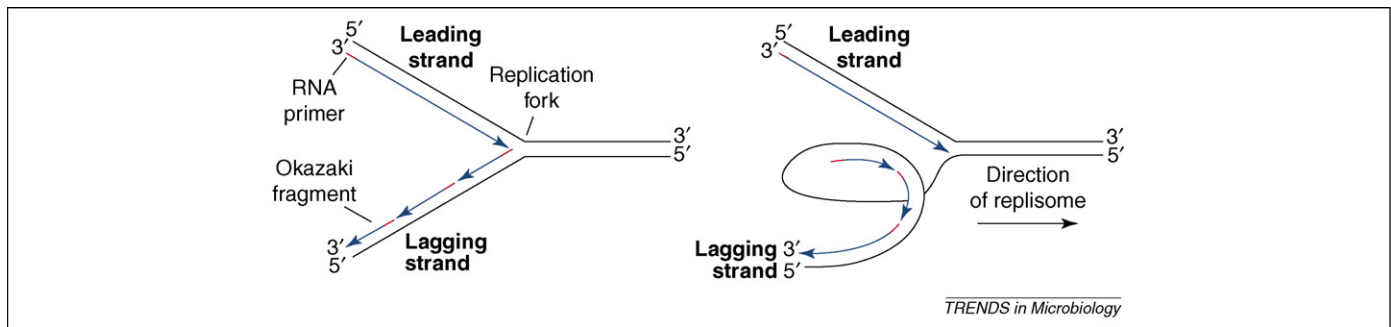


Figure 1. Organization of leading and lagging strand synthesis. DNA polymerase initiates DNA synthesis by extending an RNA primer (red). Leading strand synthesis requires only one or a few primers because this strand is copied in a continuous fashion. Lagging strand synthesis requires multiple primers for discontinuous synthesis. The lagging strand is thought to form a loop, which results in the directional alignment of the replisome with the lagging strand polymerase (right panel). Daughter strands are indicated in blue.

terminal RNA portion of each Okazaki fragment is later replaced with DNA by DNA polymerase I, and the resulting nicks between adjacent DNA segments are joined together by ligase.

Replisome architecture

Evidence as to how replicases employ clamps and clamp loaders to carry out concurrent leading and lagging strand

synthesis efficiently is mainly derived from biochemical and structural studies of *E. coli* and bacteriophage T4 replication systems (reviewed in Refs [2,20]). Because our current structural understanding of the *E. coli* replication fork is more advanced, we base our discussion of replisome architecture on the bacterial system, which is presented in Figure 2. The *E. coli* replicase, DNA polymerase III (Pol III) holoenzyme, is a multicomponent

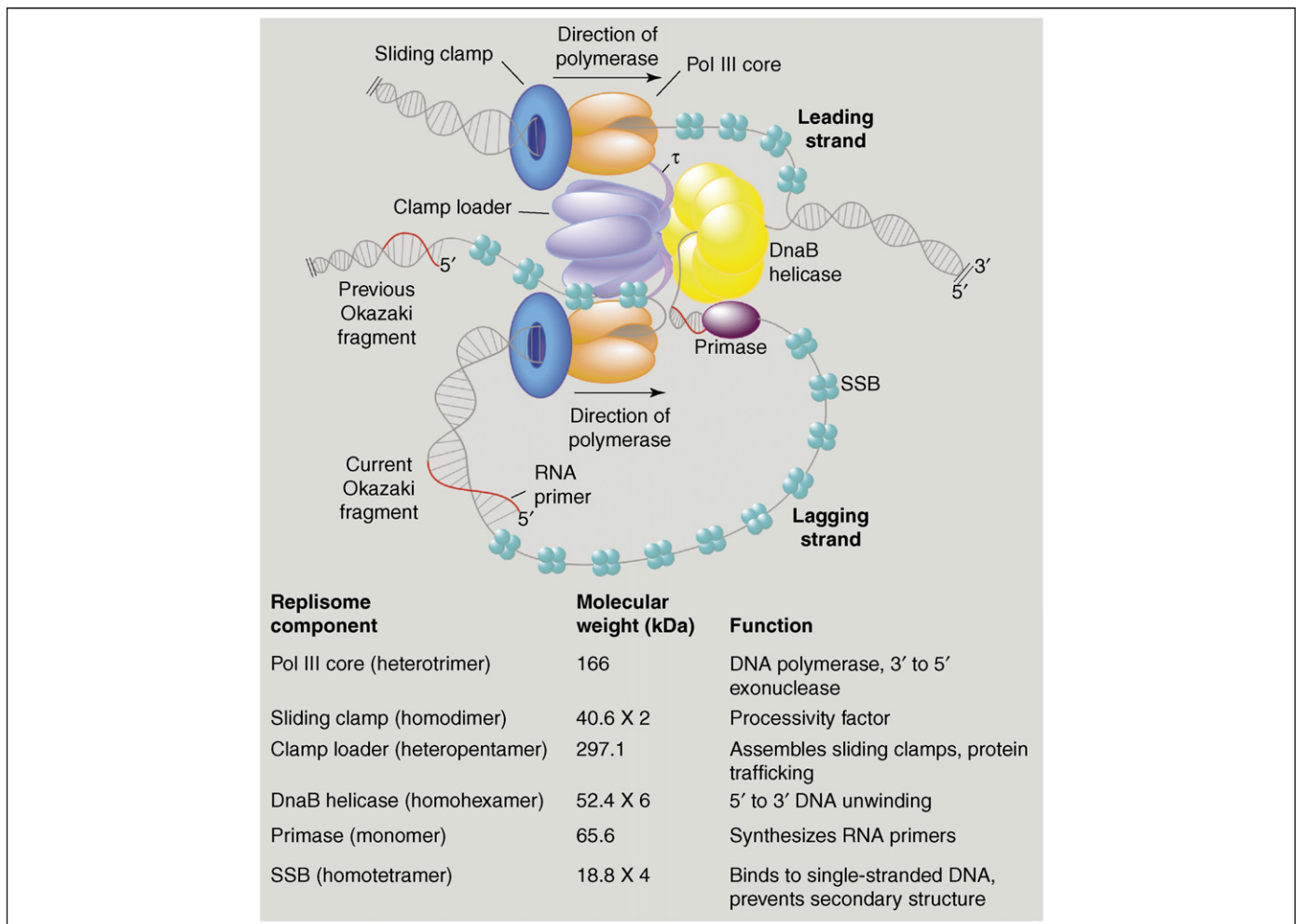


Figure 2. Organization of the *Escherichia coli* replicase. The *E. coli* replisome uses twin Pol III core enzymes (orange) to copy leading and lagging strands. Sliding clamps (dark blue) confer processivity to the Pol III core by tethering the polymerase to DNA. The clamp loader (light purple) uses the energy of ATP hydrolysis to assemble sliding clamps on DNA at primed sites. The clamp loader crosslinks leading and lagging strand polymerases and binds to the replicative helicase (yellow). The replicative helicase unwinds duplex DNA ahead of the replication fork and stimulates primase activity. Primase (dark purple) synthesizes short RNA primers. SSB (turquoise) prevents secondary structure formation of single-stranded DNA.

complex consisting of ten subunits that can be categorized into three major components: (i) the Pol III core (orange in Figure 2); (ii) the β -clamp (dark blue in Figure 2); and (iii) the γ complex clamp loader (light purple in Figure 2). The Pol III core contains DNA polymerase and 3' to 5' exonuclease proofreading activity. The β -clamp confers processivity to the polymerase by holding the Pol III core onto DNA. The clamp loader assembles β -clamps onto DNA at primed sites and functions in the organization of proteins involved in lagging strand synthesis. Other components of the replication machinery include the replicative DnaB helicase (yellow in Figure 2), which unwinds duplex DNA ahead of the fork, and primase (dark purple in Figure 2), which synthesizes RNA primers. We begin our review with discussion of the Pol III core.

DNA polymerase III core

As illustrated in Figure 2, the *E. coli* replication machinery employs twin DNA polymerases for concurrent leading and lagging strand synthesis. The two Pol III cores are linked together by the multisubunit ATPase clamp loader (γ complex; light purple). The Pol III core is a heterotrimer composed of α , ϵ and θ subunits [3]. The α subunit is responsible for DNA polymerase activity [1,2]. The ϵ subunit Exhibits 3' to 5' exonuclease activity and functions in proofreading the DNA product to ensure high fidelity replication [1,2]. The function of the θ subunit remains unclear.

The α polymerase subunit is a member of the C family of DNA polymerases, which are exclusively found in eubacteria and share no sequence similarity to other DNA polymerases [21]. Eukaryotic replicative DNA polymerases δ and ϵ , however, are among the B family of DNA polymerases [21]. Thus, it is reasonable to assume that replicative DNA polymerases from eubacteria and eukaryotes could have evolved independently. Recent crystallographic studies indicate that the organization of the α subunit active site is similar to DNA polymerases of the X family, which are involved in RNA editing and DNA repair [22,23]. Intriguingly, a region near the active site of the α subunit is thought to resemble a structural element within the active site of canonical nucleotide polymerases of the A family (i.e. DNA polymerase I, Taq DNA polymerase, T7 RNA polymerase) [22]. Thus, although the geometry of the catalytic domain of α is dissimilar to DNA polymerases of the A family, it remains possible that all polymerases share a distant relative.

The Pol III core replicates DNA at a slow rate (<20 nucleotides s^{-1}) and exhibits low processivity (<10 base pairs per DNA binding event) in the absence of the β -clamp [1,2]. Coupling of the Pol III core to the ring-shaped β -clamp confers a large increase in catalytic rate and processivity [24].

E. coli and eukaryotic sliding clamps

Sliding clamps are ring-shaped proteins that confer processivity onto the replisome by tethering replicative DNA polymerases to their respective template strands. The tether is achieved as the *E. coli* β -clamp encircles duplex DNA immediately behind Pol III while binding to the α subunit of the polymerase core [24] (Figure 2). As replication ensues, β slides along DNA while remaining

bound to the Pol III core, thereby increasing the speed (1 kb s^{-1}) and processivity ($>50 \text{ kb}$) of the Pol III core. The eukaryotic sliding clamp, proliferating cell nuclear antigen (PCNA), functions in an identical manner and stimulates the processivity of the eukaryotic replicative DNA polymerase δ [2,8,9].

Crystallographic studies of β and PCNA sliding clamps reveal similar symmetrical ring structures with sufficient space within their central channel to accommodate duplex DNA [25–27] (Figure 3). Both β and PCNA have a sixfold 'pseudosymmetry' formed by repetition of a globular subdomain. Although similar in structure, β and PCNA are organized somewhat differently: β is a three-domain protein that dimerizes to form a six-domain ring while PCNA contains only two domains per protomer and trimerizes to form a six-domain ring.

The protomers of β and PCNA are arranged in a head-to-tail fashion resulting in two distinct planes or 'faces' on each ring. The face of β and PCNA from which the C-termini protrude, referred to as the C-terminal face, is involved in intermolecular interactions [2,26,28] (Figure 3). Biochemical studies indicate that the intermolecular binding site on the C-terminal face of β is essential for proteins to bind during clamp loading, lagging strand synthesis and polymerase switching during translesion synthesis (reviewed in Ref. [2]). β and PCNA interact with proteins involved in recombination, repair and cell cycle control, indicating that sliding clamps have a wide role in DNA metabolic processes [29].

The mechanics of clamp loading

Clamp loaders are heteropentameric complexes that use the energy of ATP to assemble sliding clamps around DNA

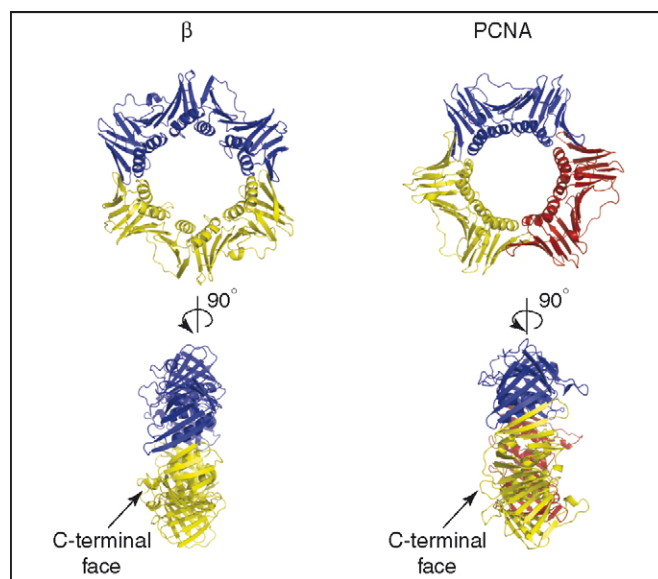


Figure 3. Structures of *Escherichia coli* and eukaryotic sliding clamps β and PCNA. Sliding clamps form ring-shaped structures that encircle duplex DNA and confer processivity to replicative DNA polymerases. β is composed of a homodimer with each protomer containing three subdomains (left). PCNA is composed of a homotrimer with each protomer containing two subdomains (right). The C-terminal face of β and PCNA is the molecular surface that is involved in intermolecular interactions. Protomers are indicated in blue, yellow and red. Protein Data Bank codes: β , 2POL; PCNA, 1PLO. Figure reproduced, with permission, from *Nature Reviews Molecular Cell Biology* (Ref. [5]; <http://www.nature.com/reviews>). © (2006) Macmillan Magazines Ltd.

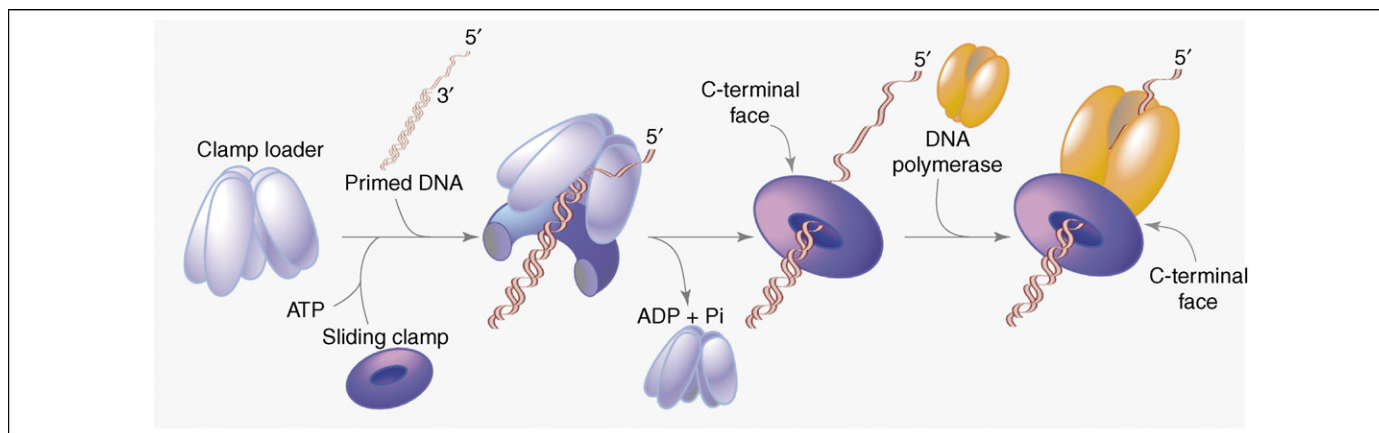


Figure 4. The clamp-loading process. The multisubunit AAA+ clamp loader uses the energy of ATP hydrolysis to assemble sliding clamps at primed sites. In the presence of ATP, the clamp loader binds to and opens a sliding clamp. The clamp loader selectively binds to primer-template junctions, which stimulates ATP hydrolysis and results in dissociation of the clamp loader from the clamp and closure of the sliding clamp around DNA. DNA polymerase is recruited to the primer-template junction by the sliding clamp assembled onto the RNA primer.

at primed sites [30,31]. A general overview of the clamp loading process is illustrated in Figure 4. In the presence of ATP, the clamp loader binds to and opens the ring-shaped sliding clamp. The ATP-bound clamp-loader complex binds to a primer-template junction, which stimulates the hydrolysis of ATP. ATP hydrolysis results in dissociation of the clamp loader from DNA. Finally, DNA polymerase is recruited to the primer-template junction as it binds to the sliding clamp, thus enabling the initiation of DNA synthesis at the 3' primer terminus.

The *E. coli* clamp-loader complex

Insight into the organization and mechanism of the clamp loading process has come from crystallographic and biochemical studies of the *E. coli* γ -complex clamp loader. The 'minimal' clamp loader is composed of five subunits ($\gamma_3, \delta, \delta'$) and is sufficient for clamp assembly [31]. The clamp loader also includes additional subunits (χ, Ψ) that are not required for the clamp loading reaction. These small subunits bind to single-stranded DNA binding protein (SSB), which coats single-stranded DNA, and they also facilitate handoff of an RNA primer between primase and the clamp loader [32–35]. The clamp loader at the replication fork also includes two τ subunits, which replace two γ subunits within the complex (discussed later). Structural analysis of the minimal clamp loader reveals a spiral-shaped subunit organization [31] (Figure 5a). The three γ subunits are located adjacent to one another and flanked by the δ and δ' subunits. The γ subunits exhibit ATPase activity and are therefore referred to as the 'motor'. γ is a member of the AAA+ (ATPases associated with a variety of cellular activities) family of proteins, which commonly exhibit molecular remodeling functions [36]. The δ subunit is referred to as the 'wrench' owing to its ability to independently bind to and 'crack' open the β -clamp, resulting in dissociation of β from DNA [37,38]. Last, the δ' subunit is thought to function as a rigid body within the complex and is therefore referred to as the 'stator' [31]. ATP binding induces a conformational change within the clamp loader that enables it to bind to β [39,40]. In this state, the 'wrench' binds to a hydrophobic patch near the β dimer interface, which becomes distorted, and results in the open state of the clamp [41].

The clamp loader contains three ATP sites that are formed at the subunit interfaces and include a conserved intermolecular SRC (Ser-Arg-Cys) motif, which is common among all known clamp-loader complexes (Figure 5a). The 'arginine finger' of the SRC motif binds to the triphosphate moiety of ATP and is essential for catalytic activity [42,43]. Each arginine finger is provided by a subunit that is adjacent to the subunit that binds to ATP. This intermolecular ATP site architecture is common to AAA+ oligomers and is thought to confer cooperativity or 'communication' between subunits. The ATP sites in γ complex hydrolyze ATP in a sequential order, which presumably coordinates this spiral-shaped assembly to close the β -clamp around DNA [42].

Further insight into the mechanism of clamp assembly comes from structural analysis of the β - δ complex, which includes the δ wrench bound to a monomeric mutant β [41]. Comparison of monomeric β (in a β_1 - δ_1 complex) and β_2 indicate that rigid-body motions of the globular domains in β result in opening of the clamp to enable DNA entry into the center of the ring [41].

The clamp loader associated with the *E. coli* replication fork has the subunit composition $\gamma, \tau_2, \delta, \delta', \chi, \Psi$. Thus, two γ subunits are replaced by two τ subunits. τ and γ are encoded by the same DNA sequence; τ represents the full-length gene product whereas γ is truncated owing to a translational frameshift [44,45]. Therefore, the γ complex clamp loader might exist in multiple states; for example, it could contain three γ subunits or one γ and two τ subunits. The γ sequences are necessary for clamp loading action, while the 24-kDa C-terminal sequence of τ that is not present in γ contains two additional domains: one binds to the Pol III core and the other binds to the replicative helicase, DnaB [46,47] (Figure 2). The two τ subunits within the clamp loader crosslink the two Pol III cores in the holoenzyme for coordinated leading and lagging strand synthesis [48–50] (Figure 2). The asymmetric subunit organization of the clamp loader is thought to confer distinct characteristics to the two Pol III cores [3,51]. Thus, the respective activities of leading and lagging strand polymerases might be assigned as a result of their orientation within the replisome. In addition, recent biochemical studies indicate that the replicative clamp

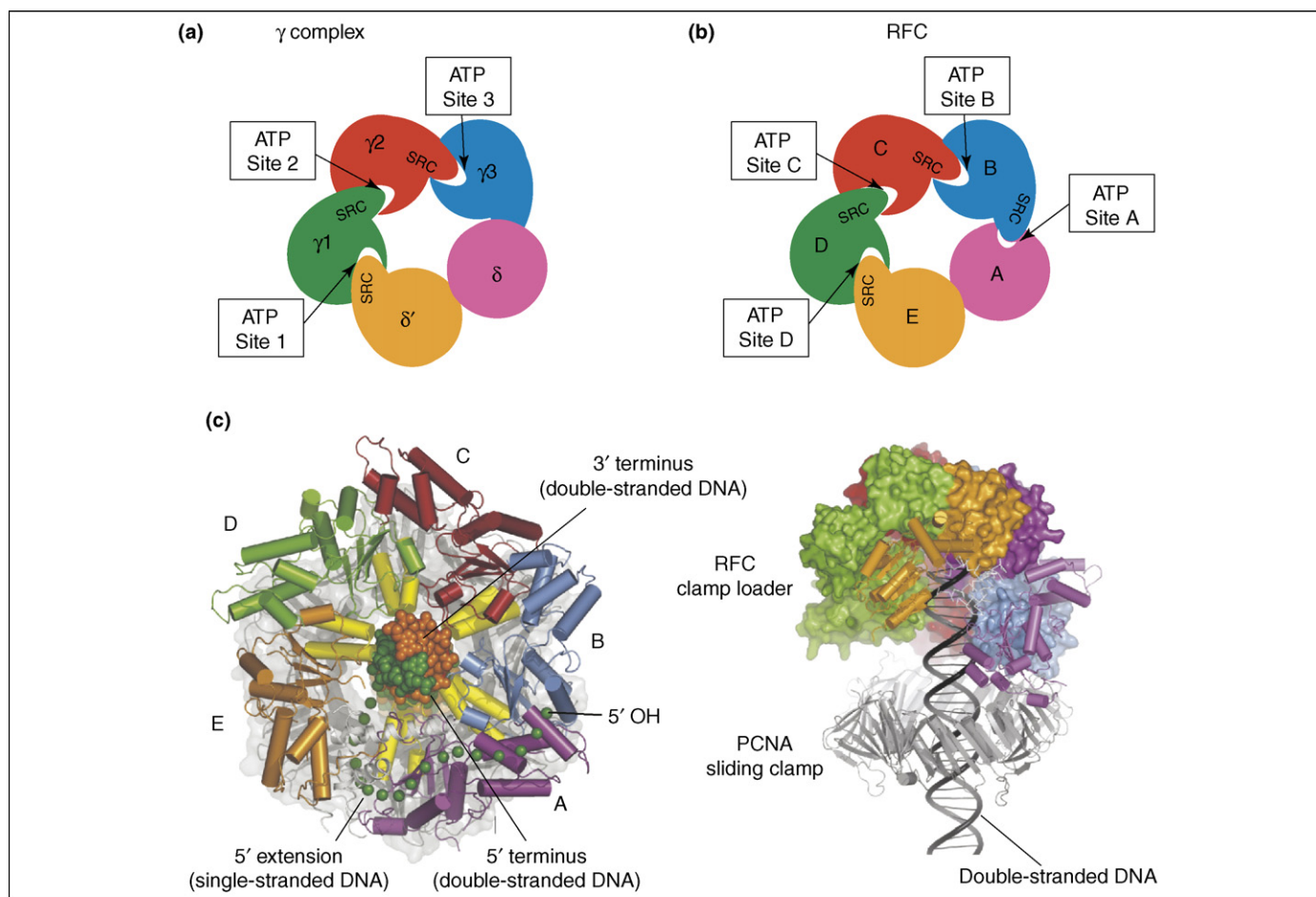


Figure 5. Organization of *Escherichia coli* and eukaryotic clamp loaders. The γ complex and RFC clamp loaders have similar spiral-shaped subunit architectures. (a) The γ complex is composed of five subunits ($\delta, \delta', \gamma_3$) and includes three ATP sites. (b) RFC is composed of five subunits (A, B, C, D, E) and includes four ATP sites. The ATP sites of γ complex and RFC are located at subunit interfaces and contain an arginine finger embedded in a conserved SRC motif. Parts (a) and (b) adapted, with permission, from reference [42]. (c) Model of the RFC–PCNA complex bound to primed DNA. Top (left) and side (right) views of the co-crystal structure of the RFC–PCNA complex are shown modeled in complex with primed DNA. The top and side portions of RFC are removed for clarity in the left and right panels, respectively. RFC–PCNA Protein Data Bank code: 1SXJ. Part (c) adapted, with permission, from *Nature* (Ref. [30]; <http://www.nature.com>). © (2004) Macmillan Magazines Ltd.

loader exhibits essential protein trafficking functions during lagging strand synthesis (discussed later).

The eukaryotic clamp-loader complex

The eukaryotic replication factor C (RFC) clamp loader is structurally and functionally similar to the *E. coli* γ complex (Figure 5b). The five subunits of RFC are encoded by distinct genes but are homologous to one another [52]. The subunits of RFC belong to the AAA+ family of ATPases, and contain the same chain fold as γ , δ and δ' . Furthermore, the ATP sites of RFC are similarly located at subunit interfaces and each uses an arginine finger contained within an SRC motif. RFC and γ complex, however, differ in their respective number of ATP sites. RFC contains an additional ATP site located at the interface of its right two terminal subunits, A and B. The function of this site is currently unclear.

Further insight into the mechanism of clamp loading by RFC is provided by structural analysis of the RFC–PCNA–ATP γ S complex [30]. The structure reveals a closed planar PCNA ring that contacts RFC subunits A, B and C. The ring might be closed as a result of mutations introduced for structural analysis of the RFC–PCNA complex or it might represent an intermediate in the clamp loading reaction.

Molecular simulations suggest that PCNA opens to form a right-handed spiral, consistent with electron microscopy reconstruction of an archaeal RFC–PCNA complex [53,54]. The five RFC subunits also form a right-handed spiral. Modeling of the open spiral form of PCNA onto the RFC structure shows a good fit to the spiral RFC, indicating that PCNA binds to all five subunits of RFC [53]. This model agrees with biochemical studies demonstrating that RFC subunits D and E open PCNA [55]. The RFC–PCNA structure also indicates that the right-handed spiral arrangement of RFC closely resembles the pitch of double-stranded DNA. Modeling suggests that duplex DNA binds within the central cavity of RFC [30] (Figure 5c). This view is supported by mutagenesis of basic residues within the central cavity of RFC, which results in a lower affinity of the clamp loader for DNA [55]. Similar studies of the *E. coli* γ complex indicates that the bacterial clamp loader also binds DNA inside a central chamber, thus positioning DNA through the open ring below [56] (Figure 4). Studies of ATP interaction with RFC demonstrate that the nucleotide affects the affinity of RFC for DNA and PCNA [57,58], and hydrolysis of ATP presumably results in dissociation of the clamp loader from DNA, enabling the ring to close as observed in studies of the *E. coli* γ complex [59,60].

Replicative helicase

The process of DNA replication requires unwinding of the double helix ahead of the replication fork. This function is performed by the *E. coli* replicative helicase, DnaB, which converts the energy of ATP into the mechanical work of translocation and strand separation. DnaB is a ring-shaped homohexamer that encircles the lagging strand as it unwinds DNA with a 5' to 3' polarity [6] (Figure 2). The connection of the Pol III holoenzyme to DnaB,

mediated by τ , greatly stimulates DnaB helicase activity [61]. DnaB also binds to primase and stimulates its activity at the replication fork.

A ring-shaped heterohexameric complex, MCM2–7, is thought to function as the replicative helicase in eukaryotes (reviewed in Refs [9,10,62,63]). The MCM4,6,7 subcomplex can encircle single-stranded DNA and unwind duplex DNA with a 3' to 5' polarity [64,65]. This action would place the MCM helicase on the leading strand.

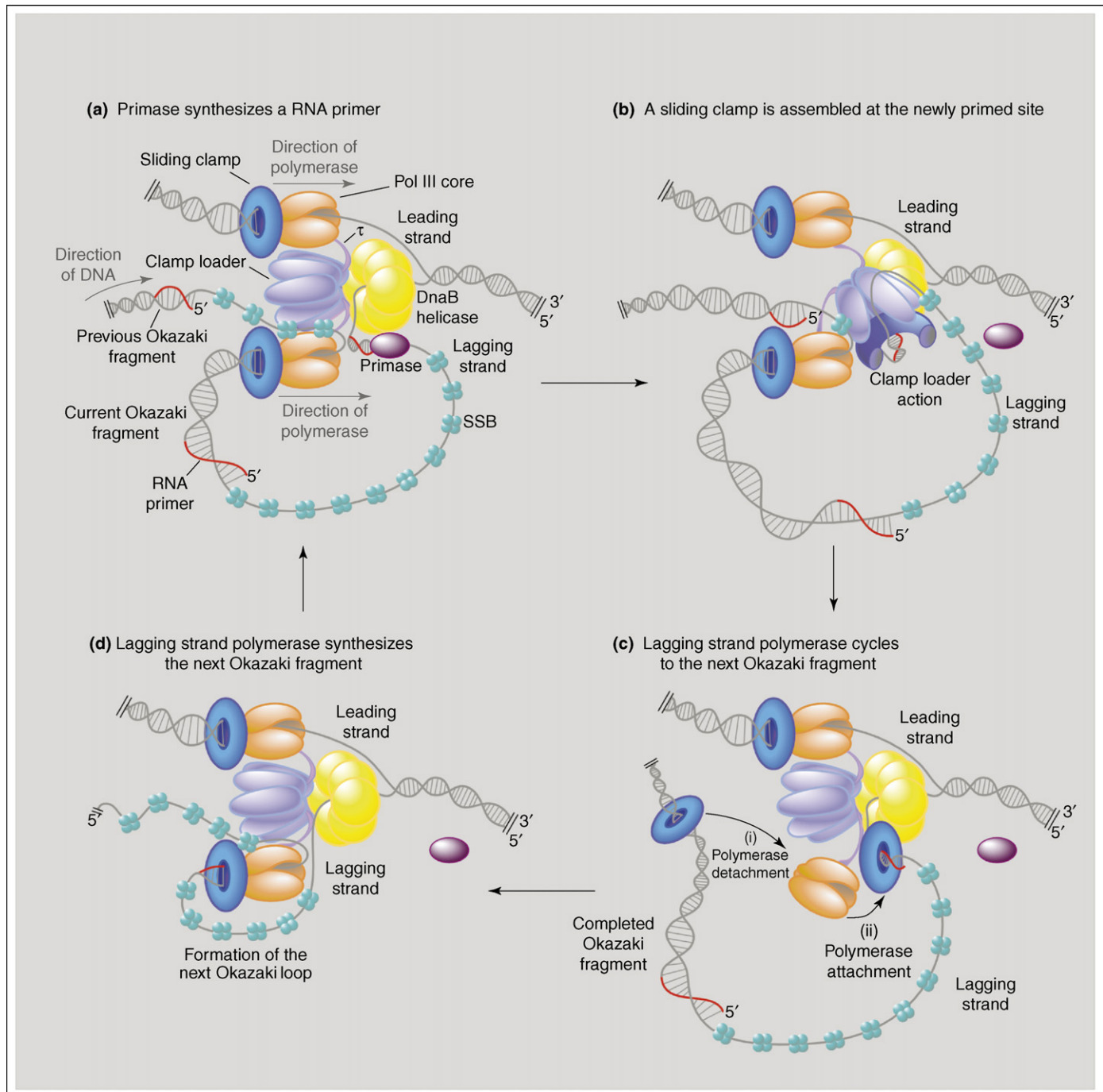


Figure 6. Protein dynamics at the *Escherichia coli* replication fork. Leading strand synthesis is a continuous process that requires only one or a few priming events. The lagging strand is synthesized in multiple discontinuous DNA segments called Okazaki fragments. (a) An Okazaki fragment is initiated when primase synthesizes a short RNA primer along the lagging strand. Primase dissociates from DNA upon completion of primer synthesis. (b) The γ complex clamp loader uses the energy of ATP to 'crack' open and assemble the β -clamp at the newly primed site. The clamp loader dissociates from DNA upon completion of clamp assembly leaving β behind at the 3' primer terminus. (c) The lagging strand polymerase dissociates from β upon completion of an Okazaki fragment and cycles to the β -clamp assembled at the newly primed site. The β -clamp is left behind on the completed Okazaki fragment. (d) The lagging strand polymerase initiates DNA synthesis of the Okazaki fragment. (See Figure 2 for the function of each replisome component.)

However, the MCM4,6,7 subcomplex can also encircle double-stranded DNA (like DnaB) and track along it using ATP [66]. This action has led to suggestions that the MCM complex can function on duplex DNA. Interestingly, helicase activity is only observed with MCM4,6,7. Although the MCM2–7 complex seems to be inactive as a helicase [64], each MCM protein is required for the progression of the replisome [67]; MCM2, 3 and 5 are thought to have regulatory functions [68]. Finally, MCM proteins become phosphorylated in S phase and might activate the complex or disassemble it to the MCM4,6,7 complex [10].

Replisome dynamics during fork progression

As described earlier, leading strand synthesis proceeds in a continuous and processive manner. The leading strand polymerase therefore uses only one or a few sliding clamps as it copies the entire leading strand. Lagging strand synthesis, however, occurs in a discontinuous fashion – the lagging strand polymerase is recycled among several clamps to synthesize multiple Okazaki fragments. Protein dynamics during lagging strand synthesis are illustrated in Figure 6.

As described earlier, Okazaki fragments are initiated when primase synthesizes short RNA primers along the lagging strand (Figure 6a). Primase activity is stimulated by DnaB, which binds to primase as it unwinds DNA ahead of the replication fork. As the replisome progresses, the clamp loader repeatedly assembles β -clamps at newly synthesized primed sites [35] (Figure 6b). Once β is assembled on DNA, the Pol III core establishes a tight grip onto the clamp, thus competing off the clamp loader by binding an overlapping site on β [69]. When the lagging strand polymerase finishes an Okazaki fragment, it ‘bumps’ into the 5’ end of the previously extended Okazaki fragment, whereupon it dissociates from β and is recruited to a clamp assembled at a new primed site [18,70] (Figure 6c,d). The Pol III core can also dissociate from β before completing the synthesis of an Okazaki fragment [19]. Premature release is necessary to recycle a stalled polymerase and might be used to ensure continued replisome movement in the face of DNA damage [13].

Concluding remarks and future perspectives

Recent structural and biochemical studies of bacterial and eukaryotic replisome components have significantly advanced our understanding of how cellular replicases perform concurrent leading and lagging strand synthesis. The bacterial replisome uses twin DNA polymerases in which each polymerase performs either leading or lagging strand synthesis. Crystallographic and biochemical studies elucidate the organization of bacterial and eukaryotic sliding clamps and clamp loaders and provide insights into the mechanisms of replication processivity and the clamp-loading reaction. Furthermore, biochemical studies of the *E. coli* replication fork uncover the dynamic process of lagging strand synthesis and demonstrate functional coupling with helicase DnaB at the head of the replication fork.

Although our current understanding of the organization and mechanism of the bacterial replisome is rather advanced, several important questions remain unanswered

Box 1. Unanswered questions and future directions

- What are the detailed mechanisms of clamp-loader action and polymerase function with sliding clamps?
- How does the replication machinery interface with proteins involved in recombinational repair?
- What are the consequences of a replication-fork encounter with protein–DNA complexes, such as repressors and DNA-compaction agents?
- How does the eukaryotic replication fork function?

(Box 1). What is the exact mechanism of the clamp loading process? How is DNA organized within the cavity of the sliding clamp? How exactly does the polymerase connect with the β -clamp on DNA and then disconnect from the clamp upon finishing an Okazaki fragment?

Importantly, the progression of the replication machinery is often arrested owing to DNA damage. Lesions within the lagging strand have little or no effect on the progression of the replisome because the lagging strand polymerase is cycled among multiple Okazaki fragments. Lesions within the leading strand, however, result in replication fork inactivation as a result of uncoupling of the leading strand polymerase from the replicative DnaB helicase. Several mechanisms have evolved to facilitate the restart of inactivated replication forks (i.e. recombinational repair, translesion synthesis, reactivation downstream from a leading strand block). How the replication machinery interdigitates its action with various recombinational repair processes is an important avenue for future investigation.

The replisome can also be arrested by protein–nucleic acid complexes that are bound tightly to the DNA. What is the fate of the replisome following a collision with a repressor or other protein–DNA complexes? Do these collisions result in replication fork inactivation or does the replication machinery use helicases or other factors to remove protein–nucleic acid blocks?

Finally, our current understanding of the architecture and mechanisms of the eukaryotic replication machinery is very limited. The eukaryotic replicase seems to employ two different DNA polymerases at the replication fork. Are they physically coupled together by other proteins? Eukaryotes also seem to require many additional replication proteins that have no known counterpart in bacteria. Some of these additional proteins can be presumed to fulfill one or more roles that are novel to eukaryotic replication forks. The MCM2–7 complex is thought to be the eukaryotic replicative helicase, yet this activity has not been demonstrated. Future investigations promise exciting developments towards a detailed understanding of the organization and mechanisms of the eukaryotic replication machinery.

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