Dynamics in Replisome Architecture and Dynamics in Escherichia coli

The field of DNA replication was launched upon discovery of the first DNA polymerase by Kornberg and Lehman (1, 2). DNA polymerase I displayed the novel and highly exciting property of template-directed enzymatic action, and like a split personality, DNA polymerase I could also degrade DNA, from either direction. Escherichia coli is now known to contain five different DNA polymerases. The chromosomal replisome is DNA polymerase III, while the damage-inducible DNA polymerases II, IV, and V play roles in DNA repair. DNA polymerase I is involved in both replication and repair and remains the most advanced model for the structure and function of DNA polymerase action.

DNA polymerase III (pol III) functions in the context of a multiprotein apparatus called DNA pol III holoenzyme (reviewed in (3–5)). pol III holoenzyme contains 10 different proteins, which assort into three major functional units: 1) pol III core, 2) the β sliding clamp, and 3) the γ/τ complex clamp loader. The holoenzyme contains two copies of the pol III core, which are connected by the attachment to one clamp loader. The holoenzyme functions within the context of a dynamic replisome containing pol III holoenzyme, a hexameric DNA helicase, DNA primase, and SSB. During replisome function, contacts between these proteins are in a constant state of change. This review briefly summarizes the architecture and dynamic behavior of the E. coli replisome.

The Clamp and Clamp Loader

The β sliding clamp is a homodimer in the shape of a ring, which encircles DNA (Fig. 1A). The β clamp slides on DNA and binds the pol III core, thereby acting as a mobile tether and converting the normally distributive pol III core into a highly processive and rapid polymerase capable of incorporating 500–1,000 nucleotides per second (Fig. 1C). The crystal structure of the β dimer reveals a 6-fold pseudo symmetry that arises from a domain that is repeated three times in the monomer giving the dimer a 6-fold appearance (6). The eukaryotic PCNA clamp and phage T4 gp45 clamp are also six-domain rings, but the monomeric unit contains only two domains and trimerizes to form a six-domain ring (7).

Clamps do not self-assemble onto DNA but require a multiprotein clamp loader, which harnesses the energy of ATP hydrolysis to open and close the clamp around DNA (Fig. 1B). The E. coli γ/τ complex clamp loader contains five subunits that are essential for clamp loading activity, three γ protomers and one copy each of δ and δ'. The small γ and δ subunits are not required for clamp loading, but they stabilize the clamp loader and stimulate its activity at elevated ionic strength. The γ, and δ' clamp loading subunits are members of the AAA + family (ATPases associated with a variety of cellular functions) (8). The subunits consist of three domains, and a AAA + region of homology is localized to the first two domains (9). Many AAA + proteins are homohexamers arranged in a symmetric circle (10, 11). The γ6δδ' pentamer forms an asymmetric circle, and there is a gap in place of the “missing sixth subunit” (Fig. 1B) (12). The C-terminal domains form an uninterrupted collar that ties the pentamer together.

The β clamp docks onto the AAA + domains of γ6δδ' (Fig. 1, B and C). This placement comes from the structure of δ-B1 (13). Replacing δ in γ6δδ' with δ-B1 indicates that β interacts with the other subunits, confirmed biochemically (14). When δ binds to β it opens the clamp (15, 16). However, ATP binding is required for the γ complex to adopt a conformation allowing it to interact with β (17), and thus the unliganded crystal structure is in an inactive conformation. The β-δ, crystal structure reveals that the β dimer is under tension, and when the interface is distorted by δ, the clamp springs open (18).

The AAA + domains of the γ complex are arranged in a spiral (7, 12). Structural studies of the Saccharomyces cerevisiae RFC clamp loader-ATPγS-PCNA complex also show this spiral arrangement. The RFC-ATPγS spiral has a steeper helical pitch than γ complex and closely matches the pitch of B-DNA for DNA interaction (19). DNA fits inside the clamp loader where the AAA + domains form a central chamber and two α helices on each subunit track the minor groove of DNA modeled inside. Several polar and basic side chains on and near these helices are conserved in prokaryotic and eukaryotic clamp loaders. Mutations of these conserved residues in either γ or δ' significantly reduce ability of γ complex to bind DNA (19).

The Open Clamp is a Spiral Lockwasher

Fluorescent energy transfer studies in the T4 phage replication system indicate that the gp45 clamp protomers open out-of-plane with a left-handed helical pitch (20). In the E. coli and S. cerevisiae systems, the right-handed helical arrangement of γ6δδ' and RFC subunits is compatible with a right-handed opening of the clamp, thereby allowing it to dock onto the other clamp loading subunits. Indeed, molecular simulations of PCNA indicate that it springs open out-of-plane with a strong tendency to form a right-handed helix (21). In addition, electron microscopic reconstruction of an archael RFC-PCNA-ATPγS-DNA complex provides direct visual evidence for an open clamp in a right-handed helix (22). DNA is guided from the central chamber of the clamp loader into the clamp docked below (see Fig. 1C). DNA binding is followed by hydrolysis of ATP to eject the clamp loader. Clamp loader ejection is necessary as the pol III core binds the same face of the clamp loader, and only one of these complexes can interact with β at a given time (23, 24).

Clamp loader structures suggest how specificity for a given site is gained (7, 12). Duplex DNA enters into the open clamp and the central chamber of the clamp loader through the gap between δ and δ'. However, the C-terminal domains of the clamp loader form a tight collar with no interruption and thus act as a cap that DNA cannot penetrate. To fit into the clamp loader, DNA must make a sharp bend at the cap and exit out the gap in the side of the clamp loader. A continuous duplex cannot bend abruptly and thus is unable to fit, but ssDNA at a primed site provides flexibility for bending, and thus this structure can be accommodated (e.g. Fig. 1C).

pol III Holoenzyme and Architecture of the Replisome

E. coli pol III holoenzyme is organized by the τ subunit (see Fig. 2A; reviewed in Refs. 3–5). τ is encoded by the same gene as γ, but yacks the C-terminal 24 kDa of τ due to truncation by a translational frameshift. The C-terminal region unique to γ, referred to as γτ, binds to the pol III core. The pol III core consists of three subunits, α (DNA polymerase), ε (3'-5' exonuclease), and θ. Within the holoenzyme two copies of τ replace two γ subunits to form a γεδδ'τψ ψ clamp loader, which in turn binds two pol III cores for leading and lagging strand replication (Fig. 2A). The C termini of the φ subunits protrude from the collar and contain many prolines and polar residues. Hence, the τ region is likely connected to the γ clamp loading domains by a flexible linker to form a pol III holoenzyme containing two cores with one clamp loader suspended from the flexlinkers between the γ hexamers. The single clamp loader can bind β clamps onto the leading and lagging strands for both DNA pol III cores (25).

The φ region connects pol III holoenzyme to the replicative helicase, DnAB (26). DnAB is a circular homohexamer, similar to the homohexameric helicases of the T4 and T7 phages (27–30). DnAB helicase encircles ssDNA and tracks along it during unwinding action; its direction of movement corresponds to the direction of the replisome provided DnAB encircles the lagging strand (Fig. 2B). Unwinding is achieved by steric exclusion in which one strand is excluded from the inside channel, while the tracking strand is retained on the inside of the ring, thus forcing the duplex apart as DnAB moves (30–32).

It is hypothesized that pol III holoenzyme is functionally asymmetric since leading and lagging strand processes are so different, and evidence for asymmetry has been gleaned from studies using ATPγS (33–35). Pol III holoenzyme is also structurally asymmetric, as defined by the clamp loader, which has odd numbers of subunits (36, 37). The DnAB helicase adds asymmetry to the replisome due to its placement on the lagging strand and has been demonstrated to impose asymmetric function on the two polymerases of pol III holoenzyme (25).
FIGURE 1. The β clamp and γ complex clamp loader. A, protomers of the E. coli β dimer are colored differently, and DNA is modeled into the center. Adapted with permission from Kong et al. (7). B, E. coli γ3δδ'. The C-terminal domains form the collar, which mediates tight pentameric contacts. The AAA+ region encompass the two N-terminal domains of each subunit. DNA passes through the gap between δ and δ'. Adapted with permission from Jeruzalmi et al. (13). C, DNA binds in the central chamber of the clamp loader (left). ATP hydrolysis ejects the clamp loader (middle). pol III associates with the β clamp for processive synthesis (right).

FIGURE 2. pol III holoenzyme and replisome architecture. A, arrangement of proteins within pol III holoenzyme is illustrated to the left. Subunit names and their mass are indicated in the table to the right. B, replisome architecture; see text under "Replisome Dynamics" for details.
Interestingly, DnaB can also encircle both strands of duplex DNA and tracks along the duplex with force fueled by ATP (38). Although DnaB does not unwind DNA in this mode, it displaces proteins and catalyzes branch migration of Holliday junctions. It is interesting to note that the eukaryotic helicase, the MCM heterohexamer (reviewed in Refs. 39–41), also tracks on both ssDNA and double-stranded DNA, like DnaB (42, 43). During bi-directional replication the two replication forks are thought to be held together in a replication factory in prokaryotes and possibly eukaryotes as well (44, 45). The replication the two replication forks are thought to be held together in a replication factory in prokaryotes and possibly eukaryotes as well (44, 45). The replication factory are functionally uncoupled (48).

Replisome Dynamics

DNA can be threaded through the replisome as illustrated in Fig. 2B. The leading strand is excluded from the inside of the DnaB ring. The leading pol III core—β continuously extends DNA in the direction of unwinding. Due to the antiparallel structure of DNA, the lagging strand is chemically extended in the opposite direction of the replication fork. Yet the lagging pol III core must physically move with the fork due to its connection to the leading polymerase and helicase. Hence, the lagging strand must form a loop to accommodate these opposite motions, as hypothesized in the “trombone model” of replication (49).

Discontinuous lagging strand fragments are 1–3 kb in length, and primase initiates each fragment by forming a short RNA primer. Primase must interact with DnaB for activity, which ensures that priming occurs at the replication fork junction (45). Primase acts in a fully distributive fashion (50). As Okazaki fragments are produced every few seconds, primase action is highly dynamic, coming on and off DNA for each priming event.

pol III core held to DNA by a protein ring fits nicely for continuous replication of the leading strand. However, discontinuous synthesis on the lagging strand requires the pol III core to hop from a finished fragment to start the next Okazaki fragment every few seconds. How can pol III core rapidly dissociate from a completed fragment when it is held tight to DNA by a clamp? This dilemma is solved by a processivity switch (51, 52). When the lagging pol III core finishes an Okazaki fragment, it rapidly dissociates from the clamp (and DNA). The clamp loader repeatedly loads β clamps onto RNA primers, which allows the lagging pol III core to reassociate with a new clamp for extension of the next fragment (Fig. 3A). The need for polymerase to be processive, yet rapidly recycle, may underlie one reason that replicative polymerases have evolved to function with clamps.

Separation of pol III from β is achieved by the τ, portion of τ subunit (53), pol III—β separation is regulated by DNA; primed DNA turns τ off and blocks it from separating pol III from β. But when synthesis is complete, τ separates pol III from β, leaving the clamp on DNA (53). This stoichiometric use of one β clamp for each Okazaki fragment results in a build-up of used clamps on the lagging strand, consistent with the high copy number of β (∼300/cell) relative to pol III (10–20/cell) (25). The β clamp also interacts with pol I and ligase (54), which are needed to replace the RNA with DNA and seal the finished fragments. Hence, leftover β clamps may mark spots on DNA where these enzymes are needed.

Okazaki fragments outnumber β by about 10-fold, and therefore β clamps must be recycled. The high stability of β on DNA (t1/2 − 1 h at 37 °C) implies that clamps are actively removed from DNA. The γ complex clamp loader is capable of unloading clamps, but the more likely clamp unloader is δ, which is in excess over other clamp loading subunits in the cell (55). δ cannot load β onto DNA but can open β and is highly active in unloading β from DNA.

Physically Coupled but Mechanically Uncoupled Polymerases

Do the two DNA polymerases "communicate" their replication status to one another? For example, if the lagging polymerase becomes blocked (i.e. by a lesion), does the leading polymerase also stop? Studies in the T4 and T7 phage replication systems, which also utilize twin DNA polymerases, demonstrate strict polymerase coupling (56, 57). Shut-down of one DNA polymerase results in cessation of the other. However, in the E. coli system leading strand synthesis continues unabated upon blocking the lagging polymerase (58, 59). In fact, continued fork progression frees the lagging polymerase from the stall site and

FIGURE 3. Protein trafficking on sliding clamps.
A, lagging pol III core hops among β clamps. Left diagram, lagging pol III core is extending an Okazaki fragment. Right diagram, the lagging pol III core finishes the fragment and releases from β. Also, the γ/τ clamp loader places β on a new primed site. Bottom diagram, lagging pol III core binds a new clamp for the next fragment. B, pol IV and pol III bind the same β toolbelt (left), pol III stalls at a lesion (middle), allowing pol IV to take control of the primed site (right). pol III regains the DNA when moving conditions are reestablished.
allows replication of the lagging strand to continue. Hence, the two cores are functionally uncoupled. Blocks on the leading strand also lead to uncoupling, but the replication fork halts its advance within 1 kb (59).

Why do the phage replicates strictly couple synthesis of the two strands, while the E. coli replicase does not? One may speculate that rapid duplication of the cellular chromosome is too important to wait for repair of damaged nucleotides on the lagging strand; these can be left behind and repaired later. Due to the smaller size of phage genomes, blocking lesions may only be encountered in a fraction of replicating molecules, allowing chromosomes containing stalled forks to simply be discarded. In this scenario, tight polymerase coupling may act as a fidelity mechanism rather than a strategy for efficient replication.

### Protein Trafficking on Clamps

The $\beta$ clamp interacts with many different proteins, including all five E. coli DNA polymerases, MutS, ligase, and UvrB. These various proteins bind to the hydrophobic site in $\beta$ to which $\delta$ binds (54, 60–62). E. coli $\alpha$-polymerase subunit contains two regions that bind $\beta$ (62). The extreme C-terminal sequence binds the hydrophobic site on $\beta$, and the second region is about 200 residues internal to the C-terminus (53, 62). Mutations in either region alter the affinity of $\alpha$ for $\beta$. The structure of the C-terminal domain of pol IV bound to $\beta$ also reveals two different sites of interaction with $\beta$ (63). The extreme C-terminal residues of pol IV bind to one of the hydrophobic pockets in the $\beta$ dimer, while an internal region of pol IV binds at an edge of the $\beta$ ring.

The fact that sliding clamps bind numerous proteins has led to speculation that multiple proteins may bind the clamp at the same time (64, 65). In this “toolbelt” hypothesis, the clamp brings together factors that are needed sequentially on DNA. Toolbelt function has been demonstrated for E. coli $\beta$ in which pol IV and pol III form a ternary complex with $\beta$ (66). pol IV is a low fidelity $\gamma$-family polymerase enabling it to bypass lesions that block the replicase (67–69). When pol III stalls, pol IV rapidly gains control of $\beta$ and the primed template. When the block is relieved, pol III regains the primed template. These protein dynamics limit the action of the low fidelity Pol IV to the locale of the block.

### Concluding Remarks

Structural and biochemical studies provide detailed insight into the dynamic workings of the E. coli replisome. Typical of scientific inquiry, these advances leave us with yet more questions. For example, how do moving replication forks handle the myriad array of lesions and DNA bound proteins encountered during synthesis? How do the many proteins that bind $\beta$ coordinate their action on the clamp? What role does DnA play while it encircles double-strand DNA? How are the forks in a replication factory held together, and what other proteins are present? How do replication enzymes meld their action with repair and recombination processes, such as must occur during replication restart after DNA damage? How do replication fork proteins coordinate with topoisomerases during synthesis and upon termination of the chromosome? These are only some of the many exciting questions that remain for the future.

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Mike O'Donnell

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