Published in final edited form as:

Curr Opin Cell Biol. 2009 June ; 21(3): 336–343. doi:10.1016/j.ceb.2009.02.008.

Replisome structure and conformational dynamics underlie fork progression past obstacles

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Abstract

Replisomes are multiprotein complexes that unzip the parental helix and duplicate the separated strands during genome replication. The antiparallel structure of DNA poses unique geometric constraints to the process, and the replisome has evolved unique dynamic features that solve this problem. Interestingly, the solution to duplex DNA replication has been co-opted to solve many other important problems that replisomes must contend with during the duplication of long chromosomes. For example, along its path the replisome will encounter lesions and DNA-bound proteins. Recent studies show that the replisome can circumvent lesions on either strand, using the strategy normally applied to the lagging strand synthesis. Circumventing lesions can also be assisted by other proteins that transiently become a part of the replisome. The replisome must also contend with DNA-binding proteins and recent studies reveal a fascinating process that enables it to bypass RNA polymerase without stopping.

Introduction

This review focuses on some of the many exciting discoveries in the field of DNA replication during the past three years. The replication machinery is relatively well conserved in all branches of life, and therefore a discovery made in one system, bacterial or eukaryotic, often generalizes. The similarities suggest that the replication machinery was already developed in the ancestor cell common to all modern cell types. This may not be too surprising given the central role of DNA replication to life.

The core activities at a replication fork are illustrated in Figure 1a for the *Escherichia coli* system, one of the classic systems for replication studies [1-4]. The replicative helicase, DnaB, is a homohexamer that encircles the lagging strand; it uses ATP to translocate along ssDNA acting as a wedge to separate the parental duplex. DnaG primase transiently associates with the helicase to synthesize short 10-12 nucleotide (ntd) RNA primers to initiate the synthesis of Okazaki fragments on the lagging strand. Two copies of DNA polymerase III (Pol III) attach to a central clamp loader apparatus. This enables it to synthesize both the leading and lagging strands at the same time. Highly processive elongation is conferred upon Pol III by a ring-shaped sliding clamp protein that tethers Pol III to DNA during elongation. Sliding clamps are opened and closed around DNA by the clamp loader in an ATP-driven reaction. The *E. coli* 'replisome' is organized by the two τ subunits within the clamp loader, which have C-terminal extensions that bind to both Pol III and DnaB (see Figure 1).

Two classic bacteriophage systems for replication studies include the T4 and T7 phage [1-3]. T4 phage utilizes a similar strategy as *E. coli*, and the T7 phage also has a coupled twin replisome, except the polymerases are processive without clamps and a clamp loader. The major eukaryotic systems are yeast, *Drosophila*, *Xenopus*, and human. The replication proteins of eukaryotes from yeast to human are very similar; they contain components analogous to those of *E. coli* plus additional factors, reflecting the greater complexity and regulatory needs of a eukaryotic cell [5]. Archaeal replication proteins share more similarity to eukaryotes than to bacteria. Major differences between eukaryotes and bacteria include the use of six different subunits to form the helicase (MCM2–7), and the MCM ring encircles the leading strand instead of the lagging strand. In addition, instead of one type of polymerase for both strands, eukaryotes utilize two different DNA polymerases, Pol ϵ and Pol δ , for leading and lagging strand synthesis. The core components at the replication fork in these different classic systems are listed in Figure 1b.

This review will focus on exciting new advances in replisome structure and function made in these systems during the past three years. We refer the reader to earlier reviews that cover earlier work and apologize in advance for the omission of the many important discoveries that cannot be explained in this brief review [2-5].

Crystal structures of replisome components

The structures of many of the components of the replisome have been solved. The remarkable structures of sliding clamps from *E. coli* (β subunit), T4 phage (gp45), yeast, and human (PCNA) were determined over 10 years ago [6-9]. Crystal structures of clamp loader machines from *E. coli* (γ complex) and yeast (RFC), and an EM reconstruction of an archaeal RFC-PCNA-DNA complex are equally fascinating, if not more so, and have also been published over three years ago [10-12]. Below we describe a few of the structures solved during the last few years.

Structure of C-family polymerases

Bacterial replicases are large DNA polymerases that constitute a unique family referred to as the C-family [13]. The C-family was the last DNA polymerase family to be determined structurally, presumably because of their large size (120-170 kDa) and complexity. The structure of the bacterial Pol III α subunit was determined for two Gram-negative organisms, *E. coli* and *Thermus aquaticus* [14,15], and for one Gram-positive bacterium, *Geobacillus kaustophilus* [16]. The α subunit contains many structural features unique to this class, but at its heart it is sculpted in the shape of a right hand like all other DNA polymerases, consisting of palm, thumb, and fingers subdomains [17]. The fingers domain is highly extended and harbors a sequence that binds to the β clamp (Figure 2a). The N-terminal region of α contains a PHP domain which, in *T. aquaticus*, is a proofreading 3'-5' exonuclease [18]. The PHP domain in *E. coli* α appears to have lost catalytic function during evolution, and has recruited a separate protein for the proofreading task, the ϵ subunit.

The architecture of the palm reveals an unexpected surprise. The palm domain of the α subunit contains the polymerase active site and is the most structurally conserved feature of all DNA polymerases [17]. Indeed, with the sole exception of the X-family polymerases (i.e. Pol β), the chain fold of the palm domain from diverse polymerase families is the same. Surprisingly, the palm domain of the α subunit is structurally analogous to the X-family. X-family polymerases include Pol β and nucleotidyl transferases, enzymes that typically dissociate from DNA after incorporating only one or two nucleotides (i.e. distributive). Indeed *E. coli* Pol III α subunit is distributive. Distributive synthesis contrasts with the high

processivity needed to replicate long chromosomes. The sliding clamp processivity factor may have evolved to solve this problem; it confers high processivity to the replicase.

Cocrystal of the β clamp with a primed site

Sliding clamps are ring-shaped homo-oligomers that encircle DNA. The recent structure of the *E. coli* β clamp bound to a primed DNA demonstrates that clamps have intrinsic specificity for a primed template junction (Figure 2b) [19 ••]. The clamp binds the duplex and to the template single-strand (ss) DNA; this double site DNA binding underlies specificity for a primed site. The ssDNA interaction may serve to hold a clamp at the primed site after it is assembled on DNA by the clamp loader, where it is needed for use by the DNA polymerase. Single molecule studies by Ted Laurence of β sliding on DNA support this view [20 ••].

The β clamp is sharply tilted on DNA, about 22° relative to the C2 axis of symmetry. The structure of *T. aquaticus* α subunit bound to DNA can be docked surprisingly well onto the tilted β -DNA structure, suggesting that the tilted stance of the clamp is maintained during synthesis (see Figure 1c) [21 ••]. The direct interaction of the clamp with DNA predicts it does not slide freely, and single molecule observations of β sliding on DNA reveal a diffusion constant 100-1000 times slower than free diffusion [20 ••]. Despite the friction during sliding, the clamp still diffuses faster than nucleotides can be incorporated by the polymerase and therefore is not a drag on elongation speed.

The eukaryotic helicase

Much of the recent excitement in helicase function stems from the identification of the eukaryotic replicative helicase. The eukaryotic replicative helicase has long been suspected to be the MCM complex, a ring-shaped hexamer composed of six different, but homologous, proteins (MCM2-7). Until recently the MCM2-7 complex has not displayed helicase activity, although helicase activity has been demonstrated for a homohexameric archaeal MCM, as well as a subcomplex consisting of the eukaryotic MCM4, 6, and 7 subunits [22-26]. Very recent studies indicate that eukaryotic MCM2-7 interacts tightly with two other factors, the four-subunit GINS complex and the Cdc45 protein, to form the CMG complex (Cdc45-MCM-GINS), and the Botchan lab has demonstrated that the CMG complex derived from *Drosophila* displays helicase activity [27 ••]. Even more recently, the Schwacha lab has established conditions under which the yeast MCM2-7 complex shows helicase activity in the absence of the Cdc45 and GINS proteins [28 ••]. An exciting recent crystal structure of the viral E1 helicase by the Joshua-Tor group implies a mechanism for MCM action [29 ••]. The E1 protein is the replicative helicase of the eukaryotic papilloma virus; it is a hexamer and is related in sequence to the MCMs.

Lagging strand replication

The idea that polymerases function in pairs was hypothesized about 40 years ago by Bruce Alberts working in the T4 system [30]. The lagging strand is replicated as a series of Okazaki fragments in the direction opposite fork progression. A twin polymerase that replicates the leading and lagging strands at the same time implies that lagging strand DNA will form a loop that grows as an Okazaki fragment is extended. The loop will collapse when the polymerase finishes the Okazaki fragment. A new loop is then reset upon starting the next Okazaki fragment. These 'trombone loops' have recently been verified by EM in an elegant study by Nancy Nossal in collaboration with Jack Griffith's group [31 ••].

The lagging strand polymerase makes numerous Okazaki fragments. Therefore the polymerase on the lagging strand must recycle, or come on and off DNA, every time it completes one loop and starts a new one. However, the polymerase is tethered to DNA by a

clamp, raising the question of how an enzyme attached to DNA by a ring is able to recycle from one Okazaki fragment to the next (reviewed in [32]). Studies in the *E. coli* system demonstrate the existence of two related mechanisms for polymerase recycling (Figure 3). In one process, referred to as 'collision release' the polymerase extends a fragment to completion and collides with the 5'-terminus of the previous fragment [33,34]. The completion of DNA triggers Pol III to eject from β , leaving the clamp on DNA. This frees Pol III to bind a new clamp, assembled on a new RNA primer by the clamp loader, to start the next fragment. The second mechanism is similar to the first except that Pol III releases from β before the Okazaki fragment is finished [35]. This second process is sometimes referred to as 'premature release' or 'signaling release', as the study of premature release in the T4 system by the Benkovic lab suggests that release may be signaled by new clamps or priming [36]. The van Oijen group has recently applied clever single molecule studies to the mechanism of lagging strand replication in the T7 system [37]. They observe both collision and premature release, and obtain evidence that the production of the RNA primer by primase signals premature release [38 ••].

Eukaryotes contain two different replicases, Pol δ and Pol ϵ , both of which utilize the PCNA clamp (reviewed in [39]). It is presumed that one polymerase functions on the leading strand and the other on the lagging strand. Using an innovative approach, the Kunkel group has recently resolved the ambiguity regarding which polymerase operates on which strand. They replaced the wt gene for the catalytic subunit of Pol δ with a mutant gene that produces a Pol δ with a high error rate; they find that mutations are targeted to the lagging strand [40 ••]. The result places Pol δ on the lagging strand, and thus Pol ϵ operates on the leading strand. Biochemical studies of yeast Pol δ show that it is exceedingly stable on DNA with PCNA, and highly processive [41]. Despite its tight grip to PCNA and DNA, Pol δ undergoes collision release from the PCNA clamp upon finishing a section of DNA [41].

Replisome dynamics upon encountering obstacles

Replisome skipping over DNA lesions—Cellular studies indicate that lesions are left behind the replication fork in ssDNA gaps, where they can be repaired later by homologous recombination [42,43]. Mechanistic studies reveal how lesions are skipped by the replisome. Skipping over lesions requires a new primed site ahead of the blocking lesion so that the polymerase can jump to it. Keeping in mind how polymerase hops among clamps on the lagging strand, it is easy to conceive of how the replisome can skip over lagging strand blocks. Namely, a blocked polymerase can undergo premature release, leaving its clamp behind on DNA, and the associate with a new clamp assembled on an RNA primer produced by primase.

Until recently there has been no known mechanism for priming the leading strand. Experiments in the Ken Marian's laboratory have now demonstrated that primase can form RNA primers on the leading strand ahead of a stalled Pol III [44 ••]. Transfer of Pol III from a blocked site to a new leading strand primed site probably occurs in a similar fashion as lagging strand recycling. Specifically, the clamp loader attaches a clamp to the new primed site to which the stalled Pol III hops for continued extension.

A recent study of the *E. coli* replicase demonstrates that a three DNA polymerase replicase readily assembles *in vitro*, and each of the three Pol III molecules is capable of synthetic action at the same time [45 ••]. A triple polymerase replisome may confer an advantage in circumventing blocking lesions. Specifically, the 'unused third polymerase' will be available to extend the new primed site placed ahead of the blocked enzyme.

Cells contain specialized DNA polymerases that can bypass certain template lesions (reviewed in [46]). These translesion synthesis (TLS) polymerases have low fidelity and

often lack a 3'-5' exonuclease activity, which enables them to extend DNA past a lesion. TLS polymerases therefore push a replication fork past a lesion, and thus increase cell viability in the face of DNA damage, although they usually produce a mutation. TLS polymerases function with the sliding clamp, and studies in the T4 system demonstrate that polymerases efficiently trade places with one another on the clamp [47 ••]. Polymerase exchange occurs through an intermediate in which two polymerases bind the same clamp, as illustrated in Figure 4b [48 ••].

Collisions between the replisome and a transcribing RNA polymerase—

Cellular studies in bacteria show that a transcribing RNA polymerase that moves in the same direction as a replication fork does not inhibit replication. Most transcription units are in fact organized in the direction of replication fork movement, suggesting a selective pressure for colinearity of transcription with replication. However, the collisions of replication forks with a colinear RNA polymerase are essentially inevitable, since replication forks move 10-20 times faster than RNA polymerase.

Recent *in vitro* studies reveal a remarkable mechanism by which the replisome continues past a colinear transcribing RNA polymerase [49 ••]. The replisome displaces the RNA polymerase, but the mRNA is retained and is used by the leading strand polymerase to continue chain elongation (Figure 4c). Presumably the clamp loader loads β on the exposed mRNA 3'-terminus, and the leading polymerase hops to a new clamp, similar to events during lagging strand replication. Future studies are required to understand how the collisions of the replisome with a head-on RNA polymerase are resolved.

Conclusions

The last few years have witnessed numerous important advances in the field of DNA replication, and this review only focuses on some of these that relate in particular to the replication fork. We now know the detailed atomic structure of the main actors at a replication fork. Despite these detailed structures many questions remain about how they function. For example, the mechanism of the clamp loading apparatus is still relatively unknown, and the mechanism of hexameric helicases is still uncertain. Furthermore, how the various pieces fit together into a replisome also await future studies. Replication is intricately intertwined with other complex processes including repair, recombination, and transcription. The complex interplay between these different multiprotein machineries is only now coming into focus. Despite the huge body of information on these processes, accumulated over several decades of intensive research, there is still a long road ahead before we understand how these complex machineries interdigitate with one another. However long the road may be, it is sure to be a highly rewarding path to follow.

Acknowledgments

The authors are grateful for the grant (GM38839) from the NIH.

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Figure 1.

Replisome structure at the *E. coli* replication fork. (a) DnaB helicase encircles the lagging strand, and primase targets DnaB for RNA primer synthesis. The ssDNA is coated with SSB (not shown). Two Pol III molecules are connected through contact with C-terminal extensions of the two τ subunits of the central clamp loader, which also bind DnaB. The two Pol III core molecules each interact with a β clamp for processive synthesis. Owing to the antiparallel duplex, the connection of the two polymerases results in forming a 'trombone loop' during lagging strand synthesis. (b) Components of replisomes in different replication systems.

Figure 2.

Structures of the bacterial polymerase and the β sliding clamp. (a) The Pol III α subunit from *E. coli* (pdb code, 2HNH), reproduced with permission from [14]. (b) The *E. coli* β clamp on primed DNA (pdb code, 3BEP), reproduced with permission from [19 ••]. (c) Model of *T. aquaticus* Pol III α subunit bound to DNA (pdb code, 3e0d), docked onto the *E. coli* β -DNA complex (pdb code, 3BEP), along with *E. coli* Pol IV attached to β (pdb code, 1UNN). Reproduced with permission from [21 ••].

Figure 3. Polymerase recycling paths on the lagging strand. The lagging strand polymerase undergoes ‘collision release’ upon completing an Okazaki fragment, leaving the clamp on DNA (top path), and undergoes ‘premature release’ when it is signaled to dissociate from the clamp before completing an Okazaki fragment (bottom path).

Figure 4.

The replisome circumvents blocks on the leading strand. **(a)** Pol III stalls at a template lesion and hops to a new clamp on an RNA primer synthesized by primase. **(b)** A TLS polymerase switches with Pol III on the clamp and extends DNA over the template lesion, producing a mutation. **(c)** Codirectional encounters of the replisome with RNA polymerase result in the dissociation of the RNA polymerase and recruitment of the mRNA to continue synthesis.