Chapter 5
The DNA Replication Machine: Structure and Dynamic Function

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Abstract In all cell types, a multi-protein machinery is required to accurately duplicate the large duplex DNA genome. This central life process requires five core replisome factors in all cellular life forms studied thus far. Unexpectedly, three of the five core replisome factors have no common ancestor between bacteria and eukaryotes. Accordingly, the replisome machines of bacteria and eukaryotes have important distinctions in the way that they are organized and function. This chapter outlines the major replication proteins that perform DNA duplication at replication forks, with particular attention to differences and similarities in the strategies used by eukaryotes and bacteria.

Keywords Replisome · Helicase · DNA polymerase · Primase · Sliding clamp · Clamp loader

Introduction

Cellular DNA genomes are duplicated by a multiprotein machinery referred to as a replisome (Kornberg and Baker 1992). Replisomes of bacteria, archaea and eukaryotes utilize a core set of five distinctive activities that we refer to here as the "core" replisome factors (Bell and Labib 2016; Yao and O'Donnell 2019). The core replisome factors of Escherichia coli and Saccharomyces cerevisiae, representative of bacteria and eukaryotes respectively, are listed in Table 5.1. The core replisome factors are: (1) a hexameric helicase, that encircles one strand of DNA and uses ATP

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Table 5.1 The five core replisome factors of bacteria and eukaryotes

Cell type	Helicase	Primase	DNA polymerase	Clamp	Clamp loader
Bacteria (E. coli)	DnaB (Homohexamer)	Primase (Monomer)	Pol III (α Pol; ε exo; θ)	Beta homodimer	Tau complex $\tau_3\delta\delta'\chi\psi$
Eukaryote (S. cerevisiae)	CMG: Cdc45, Mcm 2, 3, 4, 5, 6, 7, and GINS (Psf1, 2, 3, and Sld5)	Pol α-primase (Pol 1, Pol 12, Pri 1, Pri 2)	Pol ε: Pol 2 (Pol 2, Dpb2, Dpb3, Dpb4) Pol δ: Pol 3, Pol 31, Pol 32	PCNA (homotrimer)	RFC (RFC 1,2,3,4,5)

to motor along the ssDNA and unwind the duplex, (2) DNA polymerases, which polymerize DNA and contain a 3'–5' proofreading exonuclease, (3) a primase that synthesizes RNA (or RNA-DNA) primers to initiate elongation by DNA polymerases, (4) a sliding clamp ring that encircles double-strand (ds) DNA (or RNA-DNA) and tethers DNA polymerases to a primed DNA for highly processive synthesis, and (5) a clamp loader complex that uses ATP to open and close the circular sliding clamp around DNA. There are two other replication proteins that are important to genome duplication in all cell types but are not always considered part of the replisome machine. One of these replication proteins is the single-strand (ss) DNA binding protein (e.g. bacterial SSB (Single-Strand Binding protein) and eukaryotic RPA (Replication Protein A)) that coat ssDNA to melt hairpin blocks to polymerization and also to protect ssDNA from nucleases. The other replication protein is a DNA topoisomerase(s) that removes helical turns from dsDNA, needed for progression of the replisome. DNA topoisomerases are thought to act separate (and ahead) of replication forks. The archaeal replisome is quite similar to the eukaryotic replisome as the archaeal replication machinery is evolutionarily related to the eukaryotic replisome, and interested readers are referred to reviews on archaeal replication (Makarova et al. 2012; Makarova et al. 2014).

A comparison of the subunit composition of the core replisome factors of bacteria (E. coli) and eukaryotes (S. cerevisiae) shows that the eukaryotic core factors are multi-subunit complexes giving eukaryotic replisomes many more distinct polypeptides compared to bacterial replisomes (Table 5.1). For example, the eukaryotic replicative helicase is composed of 11 subunits and is referred to as CMG for its 3 subcomponents, the Cdc45 (Cell Division Cycle 45) protein, the Mcm (Mini Chromosome Maintenance) 2–7 heterohexamer motor, and the 4 subunit GINS complex (Psf 1,2,3 and Sld 5) (Ilves et al. 2010; Moyer et al. 2006). In contrast, the DnaB helicase of the E. coli replisome is a single polypeptide that forms a homohexamer (Kornberg and Baker 1992; LeBowitz and McMacken 1986). The eukaryotic primase is the four-subunit DNA polymerase alpha-primase that contains a DNA polymerase (Pol 1) and RNA primase (Pri 1/2) in addition to the Pol 12 subunit and generates a RNA-DNA primer of about 25 nucleotides (Conaway and Lehman 1982). In contrast, the E. coli primase is only a single subunit that makes RNA primers of 10-12

nucleotides in length (Kornberg and Baker 1992). The eukaryotic replisome utilizes two distinct DNA polymerases, epsilon and delta, for replication of the leading and lagging strands, respectively; each are multisubunit complexes (Burgers and Kunkel 2017). The E. coli replisome utilizes identical DNA polymerase III enzymes for both strands, and while the E. coli polymerase III is composed of separate DNA polymerase and 3'–5' exonuclease subunits, the corresponding replicative DNA polymerase of most bacteria is a single subunit that contains both polymerase and exonuclease activities in the same polypeptide chain (Kornberg and Baker 1992; McHenry 2011). The sliding clamp of eukaryotes is the homotrimeric PCNA (Proliferating Cell Nuclear Antigen), and in bacteria it is the dimeric beta, but each form rings with similar dimensions and molecular weight (Itsathitphaisarn et al. 2012; Kong et al. 1992). The eukaryotic clamp loader, RFC (Replication Factor C), is a heteropentamer of proteins that each share homology to AAA + proteins (ATPases Associated with diverse Activities), and the E. coli clamp loader also contains five AAA + subunits (Jeruzalmi et al. 2001; Kelch et al. 2012). The E. coli SSB is a homotetramer and each subunit contains an OB (Oligosaccharide Binding) fold, while eukaryotic RPA is a heterotrimer that also uses four OB folds to bind ssDNA (Antony and Lohman 2019; Chen and Wold 2014). The eukaryotic replisome contains several "accessory" factors that travel with the replisome and have no functional counterpart in bacteria (Gambus et al. 2006; Gambus et al. 2009). Although we focus on the core replisome factors is this review, we discuss a few of the eukaryotic replisome accessory factors that have significant impact on undisturbed replisome action. The known eukaryotic replisome accessory factors include: the Ctf4 (Chromosome Transmission Fidelity) homotrimer (metazoan AND1), the Mcm10 (Mini Chromosome Maintenance) initiation factor, the Mrc1 (Mediator of Replication Checkpoint) mediator of the S-phase DNA checkpoint response (metazoan CLASPIN), the Tof1-Csm3 (Topoisomerase 1 interacting Factor and Chromosome Segregation in Meiosis) paused fork stabilizing subunits (metazoan TIM-TIPPIN), and the FACT (FAcilitates Chromatin Transcription) nucleosome mobility factor (Gambus et al. 2006; Gambus et al. 2009). Of these replisome accessory factors, only Mcm10 is essential to S. cerevisiae cell viability.

Evolution and Structure of the Core Replisome Components

The advent of genomic sequencing revealed the surprising finding that major components of bacterial and eukaryotic replisomes have non-homologous sequences and thus are presumed to have evolved independently (Table 5.2) (Forterre et al. 2004;

Table 5.2 Structural folds in the non-homologous core replisome factors of bacteria and eukaryotes

Component	Bacteria	Eukaryote
Helicase	RecA fold	AAA + fold
Primase	Toprim fold	Pol X fold
DNA Polymerase	Polymerase III	Polymerases ε and δ

Koonin et al. 2015; Yao and O'Donnell 2016). This stands in contrast to other major information pathways in which there exists homology between bacterial and eukaryotic ribosomes and RNA polymerase subunits. The three core replisome factors that lack homology are as follows. Bacterial helicase (e.g. E. coli DnaB) uses a RecA fold for ATPase driven function, while the MCM motors of eukaryotic CMG utilize a AAA + fold for ATP binding and hydrolysis. DNA polymerases assort into distinct non-homologous sequence families (Steitz 1999; Yang and Woodgate 2007), and the eukaryotic leading and lagging strand DNA polymerases epsilon and delta are in the B-family of polymerases, while the bacterial replicative polymerase (Pol III/PolC) is in the C-family of polymerases. The bacterial primase is derived from a topoisomerase fold (Aravind et al. 1998; Podobnik et al. 2000), while the RNA primase in the eukaryotic DNA polymerase alpha-primase has the folding pattern of the DNA polymerase X family (Kilkenny et al. 2013; Kirk and Kuchta 1999).

Helicase

Replicative hexameric ring shaped helicases in both bacteria and eukaryotes are composed of 6 motor subunits that each have a N-terminal and a C-terminal domain (Fig. 5.1a), giving the hexameric ring an appearance of two stacked rings on side view (Fig. 5.1a) (O'Donnell and Li 2018). Thus the hexamer consists of an N-tier ring and a C-tier ring. The ATP binding sites are located in the C-terminal domain in

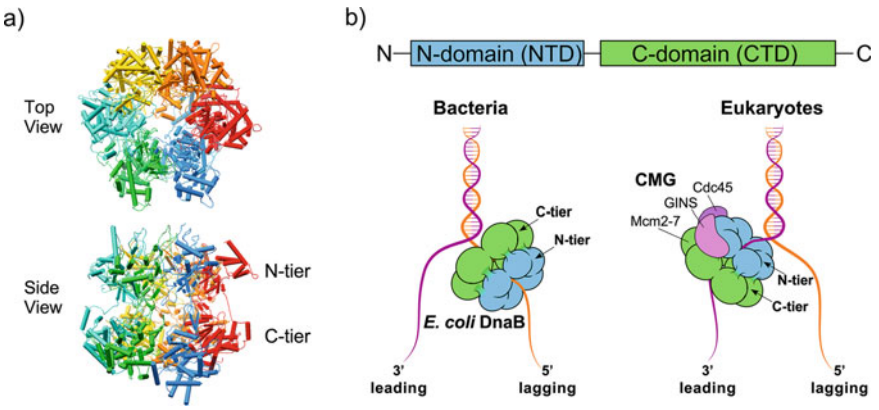

Fig. 5.1 Replicative helicases are hexameric rings with two tiers. **a** Top and side views of the eukaryotic Mcm2-7 heterohexamer of the CMG helicase; the GINS and Cdc45 subunits are omitted for clarity (PDB 3JC7). The side view shows that the two domains of each subunit give the helicase the appearance of two stacked rings, forming a N-tier and a C-tier. **b** Top: Each subunit of replicative hexameric helicases have an N-terminal and C-terminal domain. The ATP sites are located in the C-terminal domain for both bacteria and eukaryotes. Bottom: Hexameric helicases of eukaryotes and bacteria encircle opposite strands because they track in opposite directions on ssDNA. Note also that the eukaryotic helicase tracks N-tier first while the bacterial helicase tracks C-tier first

both E. coli DnaB and the eukaryotic Mcm2–7 motors of CMG, and the ATP sites are positioned at subunit interfaces of the C-tier ring in which residues from both neighboring subunits are required for ATP hydrolytic activity (Enemark and Joshua-Tor 2008; Lyubimov et al. 2011; O'Donnell and Li 2018). The interfacial ATP sites may enable intramolecular communication within the ring during unwinding action. Both the bacterial DnaB and eukaryotic CMG function by steric exclusion, in which they translocate along a ssDNA and act as a wedge at a forked junction to separate the strands; one strand is excluded to the outside of the helicase ring and the other strand is pulled through the central channel of the helicase ring by loops within the motors in the C-terminal domains (Ahnert and Patel 1997; Fu et al. 2011; Hacker and Johnson 1997; Lee et al. 2014). Interestingly, the bacterial and eukaryotic replicative helicases translocate on ssDNA in opposite directions. The RecA based bacterial helicase travels 5'–3', placing it on the lagging strand while AAA + based eukaryotic CMG travels 3'–5', placing it on the leading strand (Fig. 5.1b). Furthermore, the bacterial helicase travels C-tier first (Itsathitphaisarn et al. 2012), while the eukaryotic CMG travels N-tier first (Georgescu et al. 2017). For further information about the detailed structure and mechanism of hexameric helicases the reader is referred to reviews that focus on this subject (Enemark and Joshua-Tor 2008; Lyubimov et al. 2011; O'Donnell and Li 2018).

DNA Polymerase

All known DNA polymerases are shaped as a right hand, having thumb, fingers and palm sub-domains, even though DNA polymerases can be assorted into at least six different families that do not share homology to one another (Steitz 1999; Yang and Woodgate 2007). Many (but not all) DNA polymerases, such as the replicative DNA polymerases, also contain a proofreading 3'–5' exonuclease that removes mismatched nucleotides inserted by mistake. The bacterial replicative polymerase is the sole member of the C-family of DNA polymerases. The eukaryotic DNA polymerases delta and epsilon are in the B-family and thus share homology to one another. The leading strand DNA polymerase epsilon is unique in that it contains two DNA polymerases in its Pol 2 subunit (Tahirov et al. 2009). The N-terminal polymerase domain in Pol 2 of DNA polymerase epsilon is the active DNA polymerase, while the C-terminal polymerase is inactive (Tahirov et al. 2009). The active N-terminal domain of Pol 2 in DNA polymerase epsilon is unique in having an extra sub-domain (P-domain) that joins with the thumb/finger/palm sub-domains to completely encircle the primed template (Hogg et al. 2014). Interestingly, genetic studies reveal that the inactive C-terminal polymerase of Pol 2 is essential, while the N-terminal active polymerase of Pol 2 is not required for cell viability, although the mutant cells grow very slow (Dua et al. 1999; Kesti et al. 1999). It is thought that the catalytic N-terminal half of Pol 2, if deleted, can be substituted by DNA polymerase delta (Dua et al. 1999; Kesti et al. 1999). The essential, but inactive C-terminal polymerase in

Pol 2 is thought to serve an essential structural role, and recent studies indicate that it is important for the assembly of CMG at an origin (Goswami et al. 2018).

Primase

Primases of bacteria and eukaryotes are highly diverged and bear little similarity in sequence or structure. It is still unresolved why the eukaryotic DNA polymerase alpha-primase contains a DNA polymerase and functions with its priming subunits to make a hybrid RNA-DNA primer. Interestingly, a recent study showed that mutating the polymerase active site in Pol 1, the DNA polymerase of polymerase alpha-primase, still gave full replication activity in a single-molecule study, implying RNA primers can be used instead of RNA-DNA hybrid primers (Lewis et al. 2020). However, whether cells require DNA polymerase activity within polymerase alpha-primase for cell viability is not yet known.

Clamps and Clamp Loaders

Unlike the other core factors, the clamp and clamp loaders of bacteria and eukaryotes share the same common ancestor (Kelch et al. 2012; Yao and O'Donnell 2016). The beta and PCNA clamps are essentially superimposable, having the same secondary and tertiary 6 domain structure, with the exception that each monomer of dimeric bacterial beta has three domains (Kong et al. 1992) and each monomer of trimeric PCNA has two domains (Fig. 5.2a) (Gulbis et al. 1996). The clamp loader subunits of bacteria and eukaryotes are heteropentamers that share sequence homology to one another, and display homology from bacteria to eukaryotes (Cullmann et al. 1995; O'Donnell et al. 1993). Clamp loader pentamers form a gapped ring that bind dsDNA in the central chamber (Fig. 5.2b) (Kelch et al. 2011, 2012). Clamp loaders operate by binding/opening their respective clamp and then bind primed DNA in the center of the pentamer ring, thereby positioning the DNA through the clamp (Fig. 5.2b). The DNA induces a conformation change in the clamp loader that activates the ATP sites for hydrolysis, which ejects the clamp loader and enables the clamp to snap shut around DNA. For a more in-depth discussion of the mechanism of clamp loaders, the reader is referred to reviews on the subject (Kelch 2016; Kelch et al. 2012; Yao and O'Donnell 2012).

One may ask why the clamp and clamp loader are conserved while the other core replisome factors have distinct structures and evolutionary heritage? Clamps and clamp loaders are well known to be involved in numerous processes, not just replication (De Biasio and Blanco 2013; Georgescu et al. 2015a; Yao and O'Donnell 2016). Hence, while they were discovered in DNA replication systems, they may have originally evolved for function in other DNA metabolic reactions, and then were recruited later in evolution for DNA replication. For example, use of a clamp

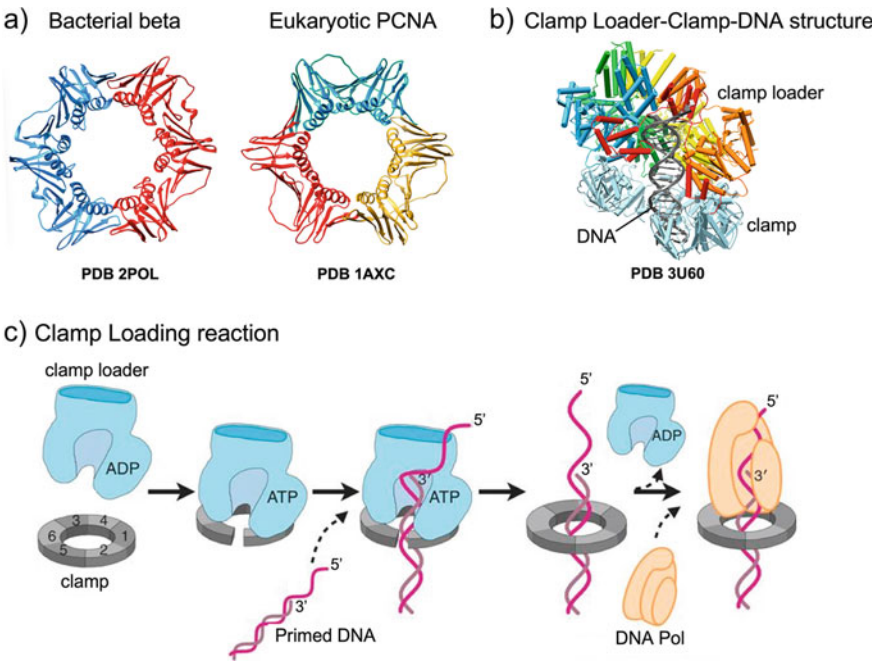

Fig. 5.2 Sliding clamps and clamp loaders. **a** Representative sliding clamps of bacteria (E. coli beta, PDB 2POL) and eukaryotes (human PCNA, PDB 1AXE) are shown in ribbon representation. **b** The clamp loader of T4 bacteriophage bound to its clamp and primed DNA. **c** Steps in the clamp loading reaction. ATP binding to the clamp loader enables it to bind/open the clamp, which then binds primed DNA in the central chamber of the clamp loader, positioning dsDNA through the clamp. ATP hydrolysis ejects the clamp loader, enabling the clamp to close and bind to the DNA polymerase. Panel c is reproduced with permission from Fig. 5.2 in (Jeruzalmi et al. 2001; Kelch et al. 2012)

that binds a variety of different DNA metabolic proteins will enhance their effective concentration on DNA, and thus the cell would not need to produce as much of the protein for its activity on DNA. Specifically, the effective local concentration of a clamp-interacting protein relative to DNA will be greatly enhanced by proximity to DNA via interaction with a mobile sliding clamp that tethers it to DNA.

Considering that bacteria and eukaryotic replisomes evolved separately, why do they have similar core replisome factors? One possible explanation is that genome replication of the Last Universal Common Ancestor (LUCA) cell may have been very different from modern day cells. The LUCA genome could possibly have even been RNA. Viruses and bacteriophage have a plethora of different replication strategies, providing precedent for unique DNA replication processes (Kornberg and Baker 1992). For example, LUCA may have replicated a duplex genome one strand at a time (e.g. first duplicating one strand and then doubling back to replicate the other). This "sequential strand replication" strategy is observed in some dsDNA and RNA viruses today, and can circumvent the need for both primase and helicase.

Primase is circumvented by sequential strand replication by protein priming by phi29 bacteriophage, priming by cleavage of a terminal hairpin by adeno-associated virus, and tRNA priming in retroviruses (Kornberg and Baker 1992). The need for a helicase can be circumvented using a strand displacing DNA polymerase. For example, phi29 bacteriophage replicates its dsDNA genome using a strand displacing polymerase instead of a helicase.

Taking these speculations a step further, it is possible that cells eventually evolved a large enough genome to require a mechanism to rapidly replicate both strands at the same time to be more fit, or competitive with other cells. Rapid and simultaneous replication of both strands of the duplex requires an RNA primase for semi-discontinuous replication, and an ATP driven DNA helicase dedicated to rapid unwinding of the duplex genome. If these requirements for cellular fitness occurred after the split of bacteria and eukaryotes from LUCA, it could possibly explain why bacteria utilize a helicase and primase that share no homology to their eukaryotic counterparts.

Organization of Replisomes

The apparent distinct evolutionary heritage of helicase, primase and DNA polymerases of eukaryotic and bacterial replisomes presents the possibility that these components are organized differently in their respective replisome machines. Thus, we summarize here the current state of knowledge about the organization of the core replisome factors in bacterial and eukaryotic cell types, using E. coli to represent bacteria and S. cerevisiae to represent eukaryotes.

Bacterial Replisome

The bacterial homohexameric replicative helicase (e.g. E. coli DnaB) unwinds DNA 5'–3' and is thus located on the lagging strand (LeBowitz and McMacken 1986). A direct connection of E. coli polymerase III to the replicative DnaB helicase has yet to be observed, and if it exists it is sufficiently weak to avoid detection. However, three of the clamp loading subunits (tau) contain C-terminal appendages that bind both the DnaB helicase and DNA polymerase III (Dallmann et al. 2000; Gao and McHenry 2001; O'Donnell et al. 2001). Thus, the clamp loader is the organizing center of the E. coli replisome, connecting the helicase to the DNA polymerases (Fig. 5.3) (O'Donnell et al. 2001). The primase transiently binds the DnaB helicase for function, and extends primers in the opposite direction of helicase unwinding (Kornberg and Baker 1992). The E. coli primase acts distributively in the replisome, coming on and off the replisome to prime the lagging strand at intervals of 1-2 kb (Tougu and Marians 1996). The lagging strand DNA polymerase III, held to DNA by its beta clamp, is capable of processive synthesis of an Okazaki fragment but

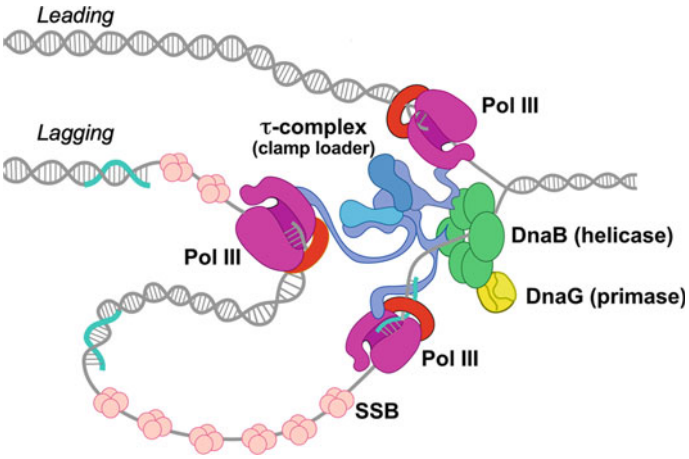

Fig. 5.3 Organization of the core replisome factors of the E. coli replisome. The leading strand DNA polymerase III is held to DNA by the beta clamp. The τ-complex clamp loader contains 3 t subunits, each of which contain extensions that bind a DNA polymerase III molecule and connect to the DnaB helicase hexamer that encircles the lagging strand. Two molecules of DNA polymerase III-beta on the lagging strand. Primase transiently binds the helicase to initiate synthesis of the lagging strand RNA primers onto which the central clamp loader repeatedly loads beta clamps as RNA primers are formed. Extension of a lagging strand primer results in formation of a 1-2 kb Okazaki fragment DNA loop. The SSB tetramer binds the lagging strand ssDNA to protect it from nucleases and to remove secondary structures that would normally impede DNA polymerase movement

has an intrinsic mechanism that ejects DNA polymerase III from the beta clamp and DNA upon completing a segment of DNA, enabling it to recycle to a new RNA primed site while remaining bound to the clamp loader/replisome (O'Donnell 1987; Stukenberg et al. 1994). This polymerase recycling mechanism will be discussed further in the next section. Structural studies of the bacterial cellular replisome have yet to be reported. Hence, specific details about the organization of the replisome factors relative to one another in 3D space is not yet known. However, the structure of the T7 bacteriophage replisome has recently been determined (Gao et al. 2019), and has similarity to the eukaryotic replisome structure, as will be described below.

Considering that leading and lagging strand DNA polymerases are structurally connected, it was originally thought that the leading and lagging strand polymerases would also be functionally coupled (Kornberg and Baker 1992). Specifically, if a lagging strand polymerase were to stall, the leading strand polymerase would also stop (and the converse). Indeed, early in vitro studies of the T4 phage and T7 phage replisomes indicated functional coupling of the two DNA polymerases (Lee et al. 1998; Salinas and Benkovic 2000). But studies in E. coli using both ensemble and single-molecule methods showed that the leading/lagging strand polymerases within the replisome are not functionally coupled, even though they are structurally attached (McInerney and O'Donnell 2004; Yao et al. 2009). A recent study has re-confirmed these actions in the E. coli system (Graham et al. 2017).

Eukaryotic Replisome

The CMG helicase tracks 3'–5' and thus encircles the leading strand, the opposite strand that bacterial DnaB helicase encircles (Ilves et al. 2010; Moyer et al. 2006). Furthermore, the leading strand polymerase epsilon directly binds to the replicative CMG helicase (Fig. 5.4) (Langston et al. 2014). This stands in contrast to the E. coli DNA polymerases which lack direct connection to the DnaB helicase, and require the clamp loader to mediate their connection. Also, unlike E. coli, the eukaryotic DNA polymerase alpha-primase forms an indirect contact to CMG mediated by the Ctf4 trimer replisome accessory factor (Simon et al. 2014; Sun et al. 2015). Furthermore, recent single-molecule studies demonstrate that DNA polymerase delta travels with the replisome for several kilobases (Lewis et al. 2020). These observations infer that numerous lagging strand DNA loops occur during replication of multiple Okazaki fragments in one DNA polymerase delta binding event, especially considering the small 100–200 bp length of eukaryotic Okazaki fragments (Smith and Whitehouse 2012). A strong interaction between the lagging strand DNA polymerase delta and individual replisome factors is not yet observed, and it seems possible that the connection of polymerase delta to the replisome may require multiple weak contacts that sum to form a strong connection. A previously identified contact between the Pol 32 subunit of DNA polymerase delta and the Pol 1 subunit of DNA polymerase alpha-primase (Huang et al. 1999; Johansson et al. 2004) has been shown to be functionally relevant in the single-molecule studies (Lewis et al. 2020).

Decades of textbook illustrations of the replisome show the helicase at the prow of the replication fork and the DNA polymerases trailing behind. However, structural

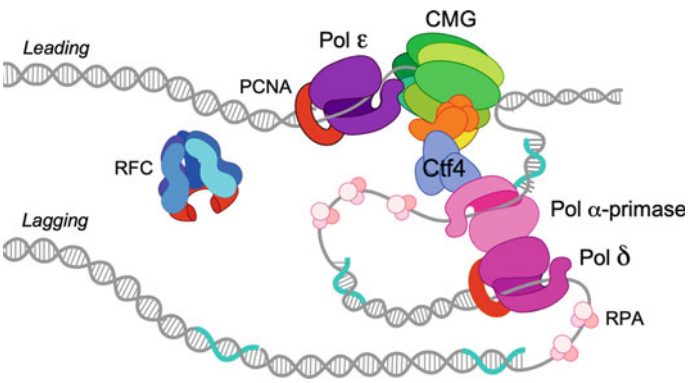

Fig. 5.4 Organization of the core replisome factors of the S. cerevisiae replisome. The leading strand polymerase epsilon is held to DNA by the PCNA clamp and by interaction with the CMG helicase that encircles the leading strand. The Ctf4 scaffolding trimer holds the lagging strand DNA polymerase alpha-primase to CMG helicase. The lagging strand DNA polymerase delta-PCNA complex travels with the replisome, resulting in 100-200 nucleotide Okazaki fragment DNA loops. The RPA heterotrimer binds the lagging strand ssDNA, protecting it from nucleases and melting secondary structures that impede polymerase progression

studies of the eukaryotic replisome reveal the leading strand DNA polymerase epsilon is on the opposite side of CMG from the lagging strand DNA polymerase alpha-primase, revealing that one polymerase travels ahead of the helicase and one travels behind the helicase (Fig. 5.4) (Sun et al. 2015). Further study revealed the orientation of CMG on DNA, and that polymerase alpha primase travels ahead of CMG while polymerase epsilon tracks in back of CMG (Georgescu et al. 2017). This anti-intuitive view of the lagging and leading strand DNA polymerases acting either in front or behind the helicase generalizes to the structure of the T7 bacteriophage replisome (Gao et al. 2019; Li and O'Donnell 2019). While the T7 bacteriophage does not use a clamp/clamp loader, it utilizes a hexameric helicase, and the structural studies reveal the identical leading and lagging strand polymerases are located on opposite faces of the helicase.

Now that the eukaryotic replisome structure is coming into view, one can compare and contrast several salient details of replisome architecture between E. coli and S. cerevisiae (compare Figs. 5.3 and 5.4). The directionality of the helicases places them on opposite strands, DnaB acts 5'–3'on the lagging strand and eukaryotic CMG acts 3'–5' on the leading strand. The CMG helicase organizes the eukaryotic replisome, binding the leading polymerase epsilon directly and the lagging DNA polymerase alpha-primase through the Ctf4 scaffolding factor. In E. coli, the leading and lagging strand DNA polymerases connect indirectly to the helicase through their mutual binding to the clamp loader that organizes the replisome. Bacterial primase does not form a stable attachment to the replisome, while eukaryotic DNA polymerase alpha-primase is attached to the replisome through the Ctf4 scaffolding factor (Gambus et al. 2009; Yuan et al. 2019). While eukaryotes utilize two distinct DNA polymerases for the two daughter strands, E. coli uses identical DNA polymerases. The bacterial primase synthesizes a RNA primer, while the eukaryotic DNA polymerase alpha-primase synthesizes a hybrid RNA-DNA primer. The eukaryotic CMG helicase is sandwiched, ahead and behind, by leading and lagging strand DNA polymerases, and DNA polymerases sandwiching the helicase generalizes to T7 bacteriophage, but whether this generalizes to bacterial cellular replisome is not yet certain.

Despite these differences, there are numerous similarities. The helicase motors in bacteria and eukaryotes are circular hexamers. The DNA polymerases in both cell types are tethered to DNA by circular clamps, loaded on DNA by a clamp loader pentamer. Furthermore, numerous Okazaki fragments can be synthesized by a single replisome in both bacteria and eukaryotes, indicating that the lagging strand polymerase stays attached to the replisome during multiple cycles of the lagging strand polymerase coming on/off DNA to extend Okazaki fragments in the opposite direction of fork movement. This "recycling" of the lagging strand polymerase is a fundamental problem for replisome action and is the subject of the next section.

Clamps Mediate Polymerase Recycling During Okazaki Fragment Synthesis

The antiparallel architecture of dsDNA, and unidirectional action of DNA polymerases require the lagging strand DNA polymerase to extend DNA in the opposite direction of the leading strand DNA polymerase. This action requires repeated priming and synthesis of the lagging strand in sections (Okazaki fragments) (Kornberg and Baker 1992). In vitro studies have elucidated an unanticipated mechanism by which this process is performed, as illustrated in Fig. 5.5.

Bacteria

In E. coli the repeated extension of RNA primers is accomplished by a DNA polymerase recycling process in which the same DNA polymerase is re-utilized numerous times by hopping among beta clamps, illustrated in Fig. 5.5 (Johnson and O'Donnell 2005). Specifically, while DNA polymerase III-beta is extending an Okazaki fragment, a new beta clamp is assembled on the next RNA primer by the clamp loader within the replisome. DNA polymerase III contains an inherent mechanism whereby it binds its beta clamp tightly during synthesis, but rapidly disengages from its beta

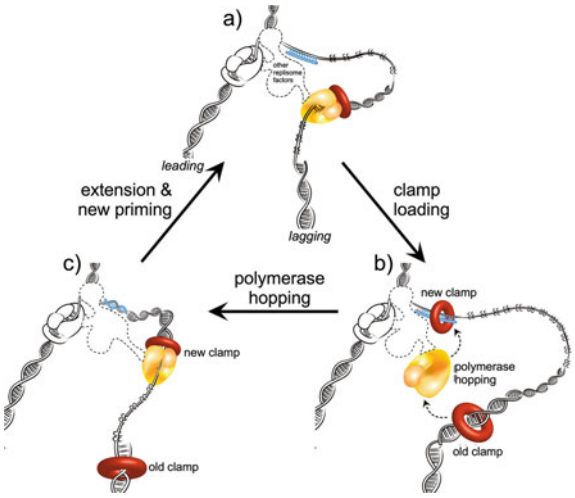

Fig. 5.5 Lagging strand polymerase recycling by hopping among sliding clamps. **a** The lagging strand DNA polymerase is attached to the leading strand DNA polymerase by connections with other replisome factors. The lagging strand is primed while the lagging strand polymerase extends a previously formed primer. **b** and **c** When the Okazaki fragment is complete, the lagging strand polymerase hops to a new clamp placed on the next primed site, leaving the old clamp behind on DNA

clamp upon finishing a section of DNA (O'Donnell 1987). Thus when polymerase III releases its beta clamp upon completing extension of an Okazaki fragment, it rapidly comes off DNA and binds a new beta clamp at the next primed site for rapid and processive extension of the next Okazaki fragment (Stukenberg et al. 1994). This rapid release and recycling process is referred to as "collision release".

Further work in the T4 phage and E. coli systems revealed that not all lagging strand DNA polymerase recycling events require the complete extension of an Okazaki fragment, and that sometimes the DNA polymerase is "signaled" to recycle from one clamp to a new clamp before an Okazaki fragment is extended to completion (Kurth et al. 2013; Li and Marians 2000; Yang et al. 2004). While the signal of this "signal release" mechanism is not clear, the process of disengaging the strong DNA polymerase-clamp connection while extending DNA would require significant energy. Torsional strain during replication contains more than sufficient energy to disrupt the strong polymerase-clamp connection, and experimental evidence exists that the extensive energy generated by supercoiling during fork advance can force the dissociation of polymerase from its clamp before an Okazaki fragment is complete (Kurth et al. 2013).

Single-molecule assays have shown that recycling to new clamps by a lagging strand polymerase that remains attached to the replisome can operate for at least 86 kb of fork progression, during which multiple Okazaki fragments of 1 kb or less are generated (Yao et al. 2009). Polymerase hopping among sliding clamps (O'Donnell 1987; Stukenberg et al. 1994) is the accepted process by which E. coli DNA polymerase III recycles from one Okazaki fragment to the next. This process appears to be at work in eukaryotes as well, and is described in more detail in the next section.

Eukaryotes

The lagging strand specific eukaryotic DNA polymerase delta-PCNA clamp is highly processive during replication of a > 5 kb primed substrate, and despite the non-homology in bacterial and eukaryotic DNA polymerases, DNA polymerase delta demonstrates collision release from its PCNA clamp upon completing DNA much as observed for E. coli DNA polymerase III and illustrated in Fig. 5.5 (Langston and O'Donnell 2008). However, unlike the E. coli system a strong and direct connection of polymerase delta to other replisome components has not been detected, and therefore it is generally thought that the eukaryotic lagging strand polymerase delta is used stoichiometrically—one DNA polymerase delta molecule for each Okazaki fragment. As described earlier in this review, a recent single-molecule study of the eukaryotic replisome reveals that a single molecule of DNA polymerase delta remains with the replisome for numerous Okazaki fragments, and therefore is efficiently recycled during replication (Lewis et al. 2020). Interestingly, when additional DNA polymerase delta is present in solution there occurs an exchange of polymerase delta from

solution with polymerase delta in the replisome. DNA polymerases epsilon and alpha-primase also undergo a similar exchange rate when either of these DNA polymerases is present in excess (Lewis et al. 2020). This result is consistent with observations of protein exchange within bacterial replisomes that depends on excess unbound protein (Mueller et al. 2019). Regardless, the single molecule studies utilizing an 18 kb DNA in the absence of excess solution phase proteins reveals that polymerase delta can be held into the replisome for numerous Okazaki fragments that requires DNA looping as illustrated in Fig. 5.5, especially considering that each eukaryotic Okazaki fragment is only 100–200 bp in length (Lewis et al. 2020; Smith and Whitehouse 2012). One connection point of DNA polymerase delta is through DNA polymerase alpha-primase (Huang et al. 1999), consistent with polymerase delta function in extending lagging strand primed sites into full length Okazaki fragments (Lewis et al. 2020).

It is unclear why DNA looping is utilized in DNA replication, either bacterial or eukaryotic. Perhaps DNA looping on the lagging strand is simply a consequence of a processive lagging strand polymerase that is held to the replication fork. Whether there are other advantages to a DNA looping process for lagging strand synthesis will remain for future studies.

Assembly and Escape of Replicative Helicases from the Origin

The goal of an origin, in both bacteria and eukaryotes, is to load two helicases onto the DNA to form bidirectional replication forks. In bacteria this is a comparatively simple process because they often have one circular chromosome with only one origin, and the firing of the origin is not strictly regulated. For example, in rich growth media the single origin in the E. coli chromosome can fire more than one time before cell division takes place (Kornberg and Baker 1992). Unlike E. coli, eukaryotes contain numerous origins within each of several linear chromosomes, and unregulated firing of these numerous origins could lead to genome instability. In fact, eukaryotic origins are highly regulated, being "licensed" in the G1 phase of the cell cycle, constraining them to fire once and only once during the ensuing S phase of the cell cycle and preventing re-initiations that could result in a tangle of DNA segments having different copy numbers (Blow and Laskey 1988). Many studies on this subject have led to the finding of numerous proteins and two cell cycle kinases that are required for initiation at eukaryotic origins (Bell and Labib 2016). Study of the licensing step in G1 phase revealed that two Mcm2–7 hexamers are loaded onto dsDNA at S. cerevisiae origins (Evrin et al. 2009; Remus et al. 2009). Upon entering S-phase, origin initiation factors assemble GINS and Cdc45 onto each Mcm2–7 to form two CMG helicases. This two-step process, separated into two cell cycle stages, ensures than an origin only fires once per cell cell cycle.

Bacterial Helicase

The objective of the bacterial origin is to load two helicases onto DNA. However, while eukaryotic CMG is assembled around dsDNA and must melt dsDNA to switch onto ssDNA, the bacterial helicase is directly loaded onto a ssDNA bubble formed by origin binding proteins (e.g. DnaA protein) (Bramhill and Kornberg 1988a, b). Also, unlike eukaryotic CMG, the bacterial replicative hexameric helicase tracks C-tier first (Itsathitphaisarn et al. 2012; Lyubimov et al. 2011). In many bacteria the helicase, like E. coli DnaB helicase, exists as a hexameric ring that use a protein (e.g. E. coli DnaC) that acts as a "ring breaker" to open and close the DnaB ring around the ssDNA, but some bacteria have proteins that act as "ring makers" that assemble the hexameric helicase around ssDNA from monomeric subunits (Davey and O'Donnell 2003). In either case, once the helicase ring is assembled and encircles ssDNA it can proceed to unwind dsDNA by the steric exclusion process. It is interesting to note that the two E. coli DnaB helicases assembled at the open ssDNA bubble of an origin face one another and must pass each another during initial origin unwinding (Fang et al. 1999). As described below, this aspect mimics the two eukaryotic CMG helicases that pass one another in the final stages of bidirectional origin unwinding.

Eukaryotic Helicase

Origins in eukaryotes require numerous proteins that act as chaperonin assembly factors to assemble the 11 subunit CMG helicase at the G1/S phase boundary. At this boundary, there exist numerous factors and kinases that modify and assemble head-to-head CMGs to ensure that an origin fires once and only once per cell cycle. Most of these factors are needed to assemble the 11-subunit CMG helicase, but do not travel with the replication fork (Bell and Labib 2016). It has long been thought that eukaryotic CMG tracks C-tier first like the bacterial hexameric helicase (Bell and Labib 2016). This view is promoted by the fact that the two CMGs that are formed at an origin are oriented N-tier to N-tier, suggesting they could simply move apart in opposite directions to form bidirectional replication forks if they traveled C-tier first.

Despite the attractive view of CMGs moving C-tier first from an origin, a C-tier first orientation of CMG unwinding, when modeled into the cryo-EM replisome structure, would require the leading strand polymerase epsilon to ride at the top of the replisome during replication, and thus the unwound DNA would need to loop back from the bottom of CMG to the top of the replisome to reach polymerase epsilon, a distance of approximately 120 Angstroms and would leave the ssDNA vulnerable to nuclease attack (Sun et al. 2015). Use of a strategy to determine a high resolution cryo-EM 3D structure of CMG acting at a replication fork revealed that CMG tracks N-tier first along ssDNA, which contradicted the long-held view that CMG travels C-tier first (Georgescu et al. 2017). This new "N-tier first orientation of CMG" showed

that the two CMGs at an origin are directed inward toward one another, not outward. While inward directed CMGs would block one another, and may initially seem a stand-off, this view of an inward direction of CMGs also offers a new way to look at origin initiation (Georgescu et al. 2017). Specifically, if each CMG binds one strand while surrounding dsDNA, the two opposite facing CMGs will block one another from moving, but would pull on opposite strands. If they have sufficient force, they could rip the DNA strands apart by pulling them through their respective rings while the two CMG rings stand still. In fact, CMG has been demonstrated to track on only one strand while encircling the duplex (Langston and O'Donnell 2019). Interestingly, earlier studies of E. coli DnaB and eukaryotic Mcm4,6,7 showed that they could rip DNA duplex structures apart, but the exact function of this observation was not understood at the time (Kaplan et al. 2003; Kaplan and O'Donnell 2002). Recent work using CMG and a 150 bp linear ARS1 origin DNA showed that two head-on CMGs can in fact rip, or shear, the DNA strands apart provided that Mcm10 is also present (Langston and O'Donnell 2019). Mcm10 binds tightly to CMG and enhances helicase activity over 10-fold (Langston et al. 2017). Furthermore, in vivo studies document that Mcm10 is required for head-on CMGs to unwind the origin (Kanke et al. 2012; van Deursen et al. 2012; Watase et al. 2012). Hence, two head-on CMGs that track on opposite strands may explain how head-to-head CMGs + Mcm10 unwind dsDNA at origins (Fig. 5.6).

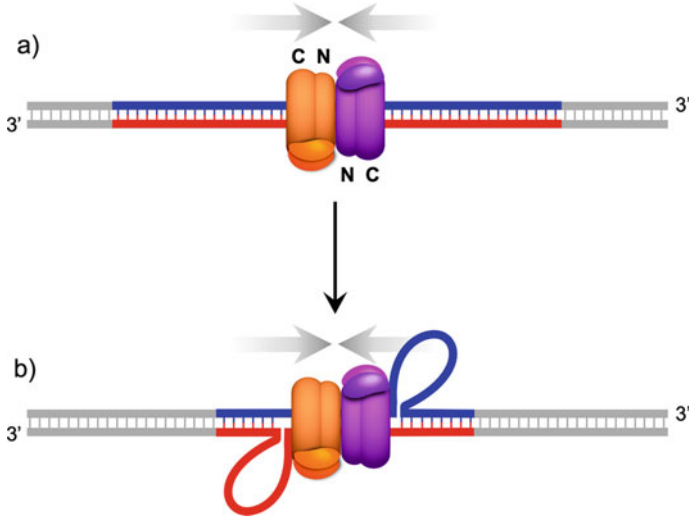

Fig. 5.6 Head-to-head CMGs shear dsDNA to ssDNA. Two CMGs are oriented head-to-head (N-tier to N-tier) on dsDNA at eukaryotic origins. The CMGs track N-first on DNA and thus the two CMGs are directed inward toward one another. Furthermore, each CMG tracks on one one strand of the duplex and therefore pull the two strands in opposite directions. Experiments show that the pulling force is sufficient to shear apart the two strands of a 150 bp ARS1 origin of yeast (Langston and O'Donnell 2019)

Once CMGs that encircle dsDNA unwind an origin, they must transition to ssDNA to become bone fide replicative helicases acting at replication forks (Fig. 5.7). The mechanism of this piece of the initiation puzzle has been determined from a single-molecule study (Wasserman et al. 2019). In the single-molecule study, CMG + Mcm10 could load onto linear ssDNA that is blocked by optical traps on either end. This indicates that CMG contains a ssDNA gate to enable it to get onto ssDNA, because both ends of the DNA are blocked by beads in the optical trap. The inference of a ssDNA gate in CMG is also consistent with earlier studies demonstrating that CMG can melt a 5' tailed oligonucleotide from circular M13 ssDNA (Ilves et al. 2010). Cryo-EM structures of CMG show no openings in the Mcm2-7 ring (Abid Ali et al. 2016; Yuan et al. 2016) and therefore one may presume the ssDNA gate is transient. The presence of a ssDNA gate in CMG also has implications to DNA repair, to which the reader is referred to the original article (Wasserman et al. 2019).

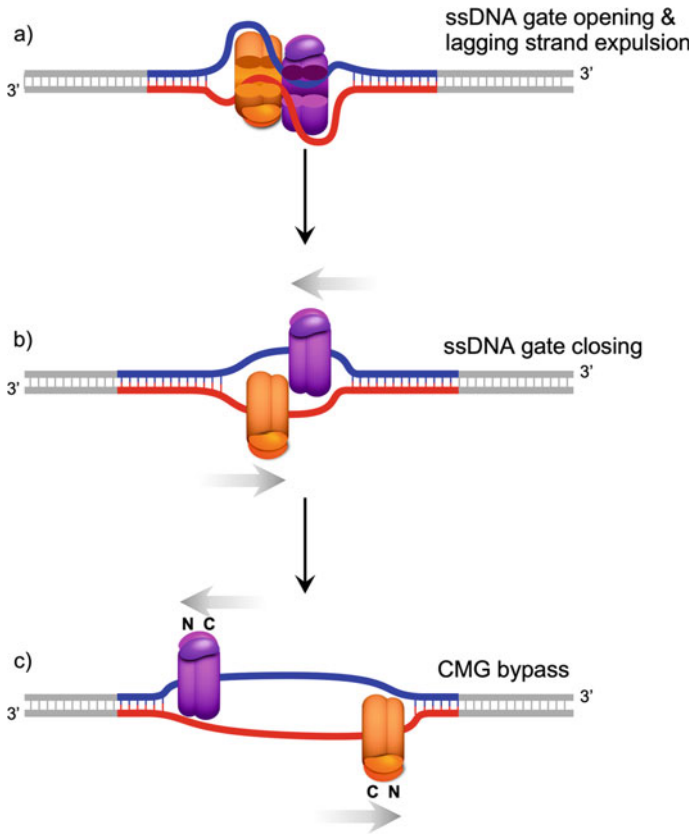

Fig. 5.7 A ssDNA gate in CMG enables it to transition from dsDNA to ssDNA. CMG is assembled onto dsDNA at origins, but must transition onto ssDNA for steric exclusion helicase action at replication forks. CMG is demonstrated to have a ssDNA gate that allows it to exclude one strand after initial unwinding of dsDNA. See text for details

The CMG does not load onto dsDNA, consistent with the Mcm2–5 dsDNA entry gate of Mcm2–7 being shut tight by the Cdc45/GINS accessory factors (Abid Ali et al. 2016; Yuan et al. 2016). Hence, the ssDNA gate is presumed to be located between Mcm subunits other than the Mcm2/5 interface, and identification of this ssDNA gate awaits future studies.

Assembly of Bacterial and Eukaryotic Replisomes

Bacterial Replisome

Two DnaB helicases assemble onto the ssDNA bubble formed by origin binding factors (E. coli DnaA/DnaC), and a further small amount of unwinding (< 100 bp) by the DnaB helicases enables primase to form RNA primed sites (Bramhill and Kornberg 1988b; Fang et al. 1999). The DnaB helicase also recruits the clamp loader through direct interaction (Kim et al. 1996). These actions only require the two bidirectional helicases to unwind less than 100 bp at an origin to enable priming of both strands and to assemble two replisomes (Fang et al. 1999). Replisome assembly is straightforward because the E. coli clamp loader tightly binds three DNA polymerase III molecules to form a tightly associated assembly containing one clamp loader and three DNA polymerase III's (McInerney et al. 2007). Thus, once DnaB recruits a primase to form a primed site, the clamp loader within the clamp loader-3 polymerase III assembly only needs to place clamps onto the primed DNA and bind the helicase and clamp for assembly of a leading/lagging strand replisome. After assembly of the helicase onto ssDNA, the replisome assembly process occurs spontaneously and without need of catalytic assembly factors (McInerney et al. 2007).

Eukaryotic Replisome

The fact that eukaryotes have different DNA polymerases for the leading and lagging strands might suggest the need for certain chaperonin factors and/or kinases to correctly assemble it, much like the assembly of CMG helicase at an origin. The process of asymmetric assembly of the replicative polymerases has been recapitulated in vitro (Georgescu et al. 2014; Georgescu et al. 2015b). The process involves two suppression reactions that eliminate a DNA polymerase from the replication fork if they load onto the wrong strand. The asymmetric polymerase assembly process also involves two major recruitment interactions that are specific for each DNA polymerase. The recruitment mechanism that locates DNA polymerase delta to the lagging strand is due to its much stronger affinity to the PCNA clamp compared to the affinity of DNA polymerase epsilon to PCNA (Schauer et al. 2017). The suppression mechanism that prevents polymerase epsilon from function on the lagging strand is

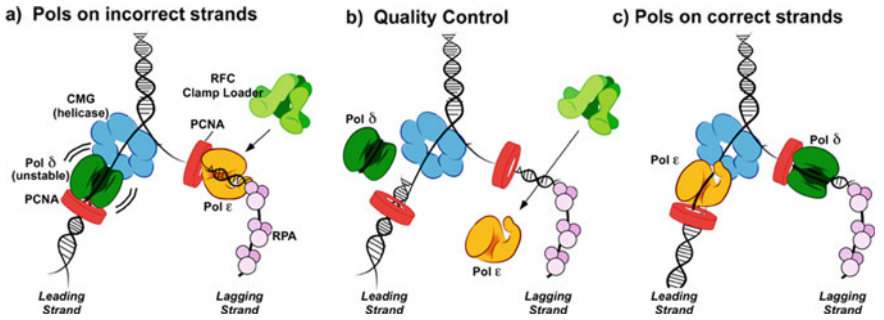

Fig. 5.8 Quality control processes that direct DNA polymerases epsilon and delta to their respective strands. Panels **a** and **b** When a DNA polymerase loads onto the wrong strand, it is unstable. Thus DNA polymerase delta undergoes collision release from PCNA-DNA upon colliding with CMG on the leading strand. The RFC clamp loader out-competes polymerase epsilon on lagging strand primed sites. **c** When a polymerase assembles on the correct strand, it is stabilized and retained. Thus polymerase epsilon binds to CMG and is held to the leading strand, and CMG also prevents RFC from displacing polymerase epsilon from a leading strand primed site. Polymerase delta binds tightly to PCNA at a primed site on the lagging strand, preventing it from becoming displaced by the RFC clamp loader. Reproduced with permission from Fig. 5.8 in (Schauer et al. 2017)

its sensitivity to inhibition by the RFC clamp loader, which competes DNA polymerase epsilon off a primer terminus (Schauer et al. 2017). This RFC suppression does not occur on the leading strand because CMG binds DNA polymerase epsilon and protects it from RFC inhibition, thus enabling PCNA to be loaded onto the leading strand for use by DNA polymerase epsilon (Schauer et al. 2017). The mechanism that locates DNA polymerase epsilon to the leading strand is also two-fold. Firstly, DNA polymerase epsilon is recruited to the leading strand by specific interaction with CMG that encircles leading strand ssDNA (Langston et al. 2014). Once polymerase epsilon is complexed to CMG, polymerase delta cannot displace it from the leading strand (Georgescu et al. 2014; Georgescu et al. 2015b). But if DNA polymerase delta arrives at the leading strand before DNA polymerase epsilon, DNA polymerase delta self-ejects from DNA and PCNA upon colliding with CMG (Georgescu et al. 2014; Schauer et al. 2017). Taken together the studies indicate that assembly of an asymmetric eukaryotic replisome is inherent in the core factors and does not require outside assembly factors as illustrated in Fig. 5.8. Suppression reactions for polymerase occupancy are unprecedented for bacterial systems, because the leading and lagging strand DNA polymerases are identical.

Summary and Future Outlook

Reconstitution of the bacterial and eukaryotic replisomes reveal the main structural and biochemical differences by which the replisome machine functions in these two cell types. The non-homologous sequences and structures of the helicase, primase

and DNA polymerase core replisome factors of bacteria and eukaryotes is compelling evidence that they evolved separately. However, as explained here, there are uncanny similarities between assembly and function of bacterial and eukaryotic replisomes when viewed with a wide-angle lens. Specifically, the origins have the same goal of loading two bidirectional helicases onto DNA. Both the bacterial and eukaryotic helicases are hexamers with N- and C-tiers. But bacterial and eukaryotic hexameric helicases act on opposite strands setting the ground work for a distinct organization of their respective replisomes. In bacteria, the clamp loader binds the helicase and joins it to the leading and lagging strand DNA polymerases. In eukaryotes the helicase directly binds the leading strand DNA polymerase, and binds the lagging strand DNA polymerase indirectly through other replisome proteins. In both cases, two replisomes spontaneously assemble once the helicases are loaded onto a bidirectional origin. Another similarity is that in both cell types, the mechanism of polymerase recycling by hopping among DNA sliding clamps for synthesis of lagging strand Okazaki fragments appears to be conserved, although future studies are need to confirm if this is true.

These generalities are comforting for the idea that an understanding of replisome action is at hand, but there are still many mysteries that are yet to be solved. The reason that the eukaryotic replisome uses different DNA polymerases for leading and lagging strand DNA synthesis is unknown, while bacteria utilize identical enzymes for the same purpose. Also, yet to be determined is the reason that eukaryotes use a RNA-DNA primer to initiate DNA synthesis by the replicative DNA polymerases epsilon and delta. Could it be that the RNA portion is all that is normally used, as in bacteria and its phages? Perhaps the Pol 1 subunit activity of polymerase alpha-primase is only required for certain regions of the genome. Another remaining mystery is why the eukaryotic helicase has so many subunits. Presumably the extra subunits are needed after the initial licensing step to close the gap between Mcm2 and Mcm5, needed for assembly of the Mcm2–7 ring onto dsDNA in G1 phase. However, these accessory subunits also bind DNA polymerase epsilon and the Ctf4 scaffolding factor, and thus appear central to direct the leading and lagging strand DNA polymerases to their respective strands. It also remains possible that the multisubunit structure of CMG is used for regulation, such as in response to the S phase replication checkpoint which involves modification of some of the CMG subunits (Ilves et al. 2012; Labib and De Piccoli 2011).

Eukaryotic replisomes must also deal with nucleosomes, epigenetic marks, sister chromosome cohesion, and DNA repair. These aspects have not been dealt with in this review. While it is obvious that replication must interface with these important processes, there is still very little known about the mechanistic details. For example, it has been shown in budding yeast that mutations in histone binding sites in DNA polymerase epsilon, Pol 1 of DNA polymerase alpha-primase, and an N-terminal region of Mcm2 result in differential transfer of parental nucleosomes to either the leading or lagging daughter strand, depending on the mutant protein (Gan et al. 2018; He et al. 2017; Petryk et al. 2018; Yu et al. 2018). These findings imply there are different pathways for epigenetic marks to be transferred to one strand or the other during genome replication. The mechanistic basis for these pathways

are unknown, and remain important open questions for future studies with high potential for an impact on disease and on developmental biology. While there is very little is known about how replication interfaces with these important processes, a mechanistic understanding of these aspects of chromatin structure and replication are very important to our understanding of development, cancer, and ultimately a cure for certain human health and diseases.

References

Abid Ali F, Renault L, Gannon J, Gahlon HL, Kotecha A, Zhou JC, Rueda D, Costa A (2016) Cryo-EM structures of the eukaryotic replicative helicase bound to a translocation substrate. Nat Commun 7:10708. https://doi.org/10.1038/ncomms10708

Ahnert P, Patel SS (1997) Asymmetric interactions of hexameric bacteriophage T7 DNA helicase with the 5'- and 3'-tails of the forked DNA substrate. J Biol Chem 272(51):32267–32273

Antony E, Lohman TM (2019) Dynamics of E. coli single stranded DNA binding (SSB) protein-DNA complexes. Semin Cell Dev Biol 86:102–111. https://doi.org/10.1016/j.semcdb.2018.03.017

Aravind L, Leipe DD, Koonin EV (1998) Toprim–a conserved catalytic domain in type IA and II topoisomerases, DnaG-type primases, OLD family nucleases and RecR proteins. Nucleic Acids Res 26(18):4205–4213. https://doi.org/10.1093/nar/26.18.4205

Bell SP, Labib K (2016) Chromosome duplication in saccharomyces cerevisiae. Genetics 203(3):1027–1067. https://doi.org/10.1534/genetics.115.186452

Blow JJ, Laskey RA (1988) A role for the nuclear envelope in controlling DNA replication within the cell cycle. Nature 332(6164):546–548. https://doi.org/10.1038/332546a0

Bramhill D, Kornberg A (1988a) Duplex opening by dnaA protein at novel sequences in initiation of replication at the origin of the E. coli chromosome. Cell 52(5):743–755

Bramhill D, Kornberg A (1988b) A model for initiation at origins of DNA replication. Cell 54(7):915–918. https://doi.org/10.1016/0092-8674(88)90102-x

Burgers PMJ, Kunkel TA (2017) Eukaryotic DNA Replication Fork. Annu Rev Biochem 86:417–438. https://doi.org/10.1146/annurev-biochem-061516-044709

Chen R, Wold MS (2014) Replication protein A: single-stranded DNA's first responder: dynamic DNA-interactions allow replication protein A to direct single-strand DNA intermediates into different pathways for synthesis or repair. Bioessays 36(12):1156–1161. https://doi.org/10.1002/bies.201400107

Conaway RC, Lehman IR (1982) A DNA primase activity associated with DNA polymerase alpha from Drosophila melanogaster embryos. Proc Natl Acad Sci U S A 79(8):2523–2527

Cullmann G, Fien K, Kobayashi R, Stillman B (1995) Characterization of the five replication factor C genes of Saccharomyces cerevisiae. Mol Cell Biol 15(9):4661–4671

Dallmann HG, Kim S, Pritchard AE, Marians KJ, McHenry CS (2000) Characterization of the unique C terminus of the Escherichia coli tau DnaX protein. Monomeric C-tau binds alpha AND DnaB and can partially replace tau in reconstituted replication forks. J Biol Chem 275(20):15512–15519. https://doi.org/10.1074/jbc.M909257199 M909257199 [pii]

Davey MJ, O'Donnell M (2003) Replicative helicase loaders: ring breakers and ring makers. Curr Biol 13(15):R594–596. https://doi.org/10.1016/s0960-9822(03)00523-2

De Biasio A, Blanco FJ (2013) Proliferating cell nuclear antigen structure and interactions: too many partners for one dancer? Adv Protein Chem Struct Biol 91:1–36. https://doi:B978-0-12-411637-5.00001-9 [pii] https://doi.org/10.1016/b978-0-12-411637-5.00001-9

Dua R, Levy DL, Campbell JL (1999) Analysis of the essential functions of the C-terminal protein/protein interaction domain of Saccharomyces cerevisiae pol epsilon and its unexpected ability to support growth in the absence of the DNA polymerase domain. J Biol Chem 274(32):22283–22288

Enemark EJ, Joshua-Tor L (2008) On helicases and other motor proteins. Curr Opin Struct Biol 18(2):243–257. https://doi:S0959-440X(08)00011-0 [pii] https://doi.org/10.1016/j.sbi.2008.01.007

Evrin C, Clarke P, Zech J, Lurz R, Sun J, Uhle S, Li H, Stillman B, Speck C (2009) A double-hexameric MCM2-7 complex is loaded onto origin DNA during licensing of eukaryotic DNA replication. Proc Natl Acad Sci U S A 106(48):20240–20245. https://doi:0911500106 [pii] https://doi.org/10.1073/pnas.0911500106

Fang L, Davey MJ, O'Donnell M (1999) Replisome assembly at oriC, the replication origin of E. coli, reveals an explanation for initiation sites outside an origin. Mol Cell 4(4):541–553

Forterre P, Filee J, Myllykallio H (2004) Origin and evolution of DNA and DNA replication machineries. In: de Pouplana LR (ed) The genetic code and the origin of life. Landes Bioscience, pp. 145–168

Fu YV, Yardimci H, Long DT, Ho TV, Guainazzi A, Bermudez VP, Hurwitz J, van Oijen A, Scharer OD, Walter JC (2011) Selective bypass of a lagging strand roadblock by the eukaryotic replicative DNA helicase. Cell 146(6):931–941. https://doi:S0092-8674(11)00889-0 [pii] https://doi.org/10.1016/j.cell.2011.07.045

Gambus A, Jones RC, Sanchez-Diaz A, Kanemaki M, van Deursen F, Edmondson RD, Labib K (2006) GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. Nat Cell Biol 8(4):358–366. https://doi.org/10.1038/ncb1382

Gambus A, van Deursen F, Polychronopoulos D, Foltman M, Jones RC, Edmondson RD, Calzada A, Labib K (2009) A key role for Ctf4 in coupling the MCM2-7 helicase to DNA polymerase alpha within the eukaryotic replisome. EMBO J 28(19):2992–3004. https://doi.org/10.1038/emboj.2009.226

Gan H, Serra-Cardona A, Hua X, Zhou H, Labib K, Yu C, Zhang Z (2018) The Mcm2-Ctf4-Polalpha Axis Facilitates Parental Histone H3-H4 Transfer to Lagging Strands. Mol Cell 72(1):140–151 e143. https://doi.org/10.1016/j.molcel.2018.09.001

Gao D, McHenry CS (2001) tau binds and organizes Escherichia coli replication through distinct domains. Partial proteolysis of terminally tagged tau to determine candidate domains and to assign domain V as the alpha binding domain. J Biol Chem 276(6):4433–4440. https://doi.org/10.1074/jbc.M009828200

Gao Y, Cui Y, Fox T, Lin S, Wang H, de Val N, Zhou ZH, Yang W (2019) Structures and operating principles of the replisome. Science 363(6429). https://doi.org/10.1126/science.aav7003

Georgescu RE, Langston L, Yao NY, Yurieva O, Zhang D, Finkelstein J, Agarwal T, O'Donnell ME (2014) Mechanism of asymmetric polymerase assembly at the eukaryotic replication fork. Nat Struct Mol Biol 21(8):664–670. https://doi:nsmb.2851 [pii] https://doi.org/10.1038/nsmb.2851

Georgescu R, Langston L, O'Donnell M (2015a) A proposal: Evolution of PCNA's role as a marker of newly replicated DNA. DNA Repair (Amst) 29:4–15. https://doi:S1568-7864(15)00027-0 [pii] https://doi.org/10.1016/j.dnarep.2015.01.015

Georgescu RE, Schauer GD, Yao NY, Langston LD, Yurieva O, Zhang D, Finkelstein J, O'Donnell ME (2015b) Reconstitution of a eukaryotic replisome reveals suppression mechanisms that define leading/lagging strand operation. Elife 4:e04988. https://doi.org/10.7554/eLife.04988

Georgescu R, Yuan Z, Bai L, de Luna Almeida Santos R, Sun J, Zhang D, Yurieva O, Li H, O'Donnell ME (2017) Structure of eukaryotic CMG helicase at a replication fork and implications to replisome architecture and origin initiation. Proc Natl Acad Sci U S A 114(5):E697–E706. https://doi.org/10.1073/pnas.1620500114

Goswami P, Abid Ali F, Douglas ME, Locke J, Purkiss A, Janska A, Eickhoff P, Early A, Nans A, Cheung AMC, Diffley JFX, Costa A (2018) Structure of DNA-CMG-Pol epsilon elucidates the roles of the non-catalytic polymerase modules in the eukaryotic replisome. Nat Commun 9(1):5061. https://doi.org/10.1038/s41467-018-07417-1

Graham JE, Marians KJ, Kowalczykowski SC (2017) Independent and Stochastic Action of DNA Polymerases in the Replisome. Cell 169(7):1201–1213 e1217. https://doi.org/10.1016/j.cell.2017.05.041

Gulbis JM, Kelman Z, Hurwitz J, O'Donnell M, Kuriyan J (1996) Structure of the C-terminal region of p 21(WAF1/CIP1) complexed with human PCNA. Cell 87(2):297–306. https://doi.org/10.1016/S0092-8674(00)81347-1

Hacker KJ, Johnson KA (1997) A hexameric helicase encircles one DNA strand and excludes the other during DNA unwinding. Biochemistry 36(46):14080–14087. https://doi.org/10.1021/bi971644v bi971644v [pii]

He H, Li Y, Dong Q, Chang AY, Gao F, Chi Z, Su M, Zhang F, Ban H, Martienssen R, Chen YH, Li F (2017) Coordinated regulation of heterochromatin inheritance by Dpb3-Dpb4 complex. Proc Natl Acad Sci U S A 114(47):12524–12529. https://doi.org/10.1073/pnas.1712961114

Hogg M, Osterman P, Bylund GO, Ganai RA, Lundstrom EB, Sauer-Eriksson AE, Johansson E (2014) Structural basis for processive DNA synthesis by yeast DNA polymerase varepsilon. Nat Struct Mol Biol 21(1):49–55. https://doi.org/10.1038/nsmb.2712

Huang ME, Le Douarin B, Henry C, Galibert F (1999) The Saccharomyces cerevisiae protein YJR043C (Pol32) interacts with the catalytic subunit of DNA polymerase alpha and is required for cell cycle progression in G2/M. Mol Gen Genet 260(6):541–550. https://doi.org/10.1007/s004380050927

Ilves I, Petojevic T, Pesavento JJ, Botchan MR (2010) Activation of the MCM2-7 helicase by association with Cdc45 and GINS proteins. Mol Cell 37(2):247–258. https://doi.org/10.1016/j.molcel.2009.12.030

Ilves I, Tamberg N, Botchan MR (2012) Checkpoint kinase 2 (Chk2) inhibits the activity of the Cdc45/MCM2-7/GINS (CMG) replicative helicase complex. Proc Natl Acad Sci USA 109(33):13163–13170. https://doi:1211525109 [pii] https://doi.org/10.1073/pnas.1211525109

Itsathitphaisarn O, Wing RA, Eliason WK, Wang J, Steitz TA (2012) The hexameric helicase DnaB adopts a nonplanar conformation during translocation. Cell 151(2):267–277. https://doi:S0092-8674(12)01117-8 [pii] https://doi.org/10.1016/j.cell.2012.09.014

Jeruzalmi D, O'Donnell M, Kuriyan J (2001) Crystal structure of the processivity clamp loader gamma (gamma) complex of E. coli DNA polymerase III. Cell 106(4):429-441. https://doi:S0092-8674(01)00463-9 [pii]

Johansson E, Garg P, Burgers PM (2004) The Pol32 subunit of DNA polymerase delta contains separable domains for processive replication and proliferating cell nuclear antigen (PCNA) binding. J Biol Chem 279(3):1907–1915. https://doi.org/10.1074/jbc.M310362200 M310362200 [pii]

Johnson A, O'Donnell M (2005) Cellular DNA replicases: components and dynamics at the replication fork. Annu Rev Biochem 74:283–315. https://doi.org/10.1146/annurev.biochem.73.011303.073859

Kanke M, Kodama Y, Takahashi TS, Nakagawa T, Masukata H (2012) Mcm10 plays an essential role in origin DNA unwinding after loading of the CMG components. EMBO J 31 (9):2182–2194. https://doi:emboj201268 [pii] https://doi.org/10.1038/emboj.2012.68

Kaplan DL, O'Donnell M (2002) DnaB drives DNA branch migration and dislodges proteins while encircling two DNA strands. Mol Cell 10(3):647–657

Kaplan DL, Davey MJ, O'Donnell M (2003) Mcm4,6,7 uses a "pump in ring" mechanism to unwind DNA by steric exclusion and actively translocate along a duplex. J Biol Chem 278(49):49171–49182. https://doi.org/10.1074/jbc.M308074200 M308074200 [pii]

Kelch BA (2016) Review: the lord of the rings: Structure and mechanism of the sliding clamp loader. Biopolymers 105(8):532–546. https://doi.org/10.1002/bip.22827

Kelch BA, Makino DL, O'Donnell M, Kuriyan J (2011) How a DNA polymerase clamp loader opens a sliding clamp. Science 334(6063):1675–1680. https://doi.org/10.1126/science.1211884

Kelch BA, Makino DL, O'Donnell M, Kuriyan J (2012) Clamp loader ATPases and the evolution of DNA replication machinery. BMC Biol 10:34. https://doi.org/10.1186/1741-7007-10-34

Kesti T, Flick K, Keranen S, Syvaoja JE, Wittenberg C (1999) DNA polymerase epsilon catalytic domains are dispensable for DNA replication, DNA repair, and cell viability. Mol Cell 3(5):679–685. https://doi:S1097-2765(00)80361-5 [pii]

Kilkenny ML, Longo MA, Perera RL, Pellegrini L (2013) Structures of human primase reveal design of nucleotide elongation site and mode of Pol alpha tethering. Proc Natl Acad Sci USA 110(40):15961–15966. https://doi.org/10.1073/pnas.1311185110

Kim S, Dallmann HG, McHenry CS, Marians KJ (1996) Coupling of a replicative polymerase and helicase: a tau-DnaB interaction mediates rapid replication fork movement. Cell 84(4):643–650. https://doi:S0092-8674(00)81039-9 [pii]

Kirk BW, Kuchta RD (1999) Arg304 of human DNA primase is a key contributor to catalysis and NTP binding: primase and the family X polymerases share significant sequence homology. Biochemistry 38(24):7727–7736. https://doi.org/10.1021/bi990247c

Kong XP, Onrust R, O'Donnell M, Kuriyan J (1992) Three-dimensional structure of the beta subunit of E. coli DNA polymerase III holoenzyme: a sliding DNA clamp. Cell 69(3):425–437. https://doi.org/10.1016/0092-8674(92)90445-I

Koonin EV, Dolja VV, Krupovic M (2015) Origins and evolution of viruses of eukaryotes: The ultimate modularity. Virology 479–480:2–25. https://doi:S0042-6822(15)00085-9 [pii] https://doi.org/10.1016/j.virol.2015.02.039

Kornberg A, Baker TA (1992) DNA Replication. Freeman, New York, W.H

Kurth I, Georgescu RE, O'Donnell ME (2013) A solution to release twisted DNA during chromosome replication by coupled DNA polymerases. Nature 496(7443):119–122. https://doi:nature11988 [pii] https://doi.org/10.1038/nature11988

Labib K, De Piccoli G (2011) Surviving chromosome replication: the many roles of the S-phase checkpoint pathway. Philos Trans R Soc Lond B Biol Sci 366(1584):3554–3561. https://doi:366/1584/3554 [pii] https://doi.org/10.1098/rstb.2011.0071

Langston LD, O'Donnell M (2008) DNA polymerase delta is highly processive with proliferating cell nuclear antigen and undergoes collision release upon completing DNA. J Biol Chem 283(43):29522–29531. https://doi.org/10.1074/jbc.M804488200

Langston LD, O'Donnell ME (2019) An explanation for origin unwinding in eukaryotes. Elife 8. https://doi.org/10.7554/eLife.46515

Langston LD, Zhang D, Yurieva O, Georgescu RE, Finkelstein J, Yao NY, Indiani C, O'Donnell ME (2014) CMG helicase and DNA polymerase epsilon form a functional 15-subunit holoenzyme for eukaryotic leading-strand DNA replication. Proc Natl Acad Sci U S A 111(43):15390–15395. https://doi:1418334111 [pii] https://doi.org/10.1073/pnas.1418334111

Langston LD, Mayle R, Schauer GD, Yurieva O, Zhang D, Yao NY, Georgescu RE, O'Donnell ME (2017) Mcm10 promotes rapid isomerization of CMG-DNA for replisome bypass of lagging strand DNA blocks. eLife 6:e29118. https://doi.org/10.7554/eLife.29118

LeBowitz JH, McMacken R (1986) The Escherichia coli dnaB replication protein is a DNA helicase. J Biol Chem 261(10):4738–4748

Lee J, Chastain PD, 2nd, Kusakabe T, Griffith JD, Richardson CC (1998) Coordinated leading and lagging strand DNA synthesis on a minicircular template. Mol Cell 1(7):1001–1010. https://doi:S1097-2765(00)80100-8 [pii]

Lee SJ, Syed S, Enemark EJ, Schuck S, Stenlund A, Ha T, Joshua-Tor L (2014) Dynamic look at DNA unwinding by a replicative helicase. Proc Natl Acad Sci USA 111(9):E827–835. https://doi:1322254111 [pii] https://doi.org/10.1073/pnas.1322254111

Lewis JS, Spenkelink LM, Schauer GD, Yurieva O, Mueller SH, Natarajan V, Kaur G, Maher C, Kay C, O'Donnell ME, van Oijen AM (2020) Tunability of DNA Polymerase Stability during Eukaryotic DNA Replication. Mol Cell 77(1):17–25 e15. https://doi.org/10.1016/j.molcel.2019.10.005

Li X, Marians KJ (2000) Two distinct triggers for cycling of the lagging strand polymerase at the replication fork. J Biol Chem 275(44):34757–34765. https://doi.org/10.1074/jbc.M006556200

Li H, O'Donnell ME (2019) DNA replication from two different worlds. Science 363(6429):814–815. https://doi.org/10.1126/science.aaw6265

Lyubimov AY, Strycharska M, Berger JM (2011) The nuts and bolts of ring-translocase structure and mechanism. Curr Opin Struct Biol 21(2):240–248. https://doi.org/10.1016/j.sbi.2011.01.002
Makarova KS, Koonin EV, Kelman Z (2012) The CMG (CDC45/RecJ, MCM, GINS) complex is a conserved component of the DNA replication system in all archaea and eukaryotes. Biol Direct 7:7. https://doi:1745-6150-7-7 [pii] https://doi.org/10.1186/1745-6150-7-7
Makarova KS, Krupovic M, Koonin EV (2014) Evolution of replicative DNA polymerases in archaea and their contributions to the eukaryotic replication machinery. Front Microbiol 5:354. https://doi.org/10.3389/fmicb.2014.00354
McHenry CS (2011) Bacterial replicases and related polymerases. Curr Opin Chem Biol 15(5):587–594. https://doi:S1367-5931(11)00125-6 [pii] https://doi.org/10.1016/j.cbpa.2011.07.018
McInerney P, O'Donnell M (2004) Functional uncoupling of twin polymerases: mechanism of polymerase dissociation from a lagging-strand block. J Biol Chem 279(20):21543–21551. https://doi.org/10.1074/jbc.M401649200 M401649200 [pii]
McInerney P, Johnson A, Katz F, O'Donnell M (2007) Characterization of a triple DNA polymerase replisome. Mol Cell 27(4):527–538. https://doi.org/10.1016/j.molcel.2007.06.019
Moyer SE, Lewis PW, Botchan MR (2006) Isolation of the Cdc45/Mcm2-7/GINS (CMG) complex, a candidate for the eukaryotic DNA replication fork helicase. Proc Natl Acad Sci USA 103(27):10236–10241. https://doi.org/10.1073/pnas.0602400103
Mueller SH, Spenkelink LM, van Oijen AM (2019) When proteins play tag: the dynamic nature of the replisome. Biophys Rev 641–651. https://doi.org/10.1007/s12551-019-00569-4
O'Donnell ME (1987) Accessory proteins bind a primed template and mediate rapid cycling of DNA polymerase III holoenzyme from Escherichia coli. J Biol Chem 262(34):16558–16565
O'Donnell M, Jeruzalmi D, Kuriyan J (2001) Clamp loader structure predicts the architecture of DNA polymerase III holoenzyme and RFC. Curr Biol 11(22):R935–946. https://doi:S0960-9822(01)00559-0 [pii]
O'Donnell ME, Li H (2018) The ring-shaped hexameric helicases that function at DNA replication forks. Nat Struct Mol Biol 25(2):122–130. https://doi.org/10.1038/s41594-018-0024-x
O'Donnell M, Onrust R, Dean FB, Chen M, Hurwitz J (1993) Homology in accessory proteins of replicative polymerases–E. coli to humans. Nucleic Acids Res 21(1):1–3. https://doi.org/10.1093/nar/21.1.1
Petryk N, Dalby M, Wenger A, Stromme CB, Strandsby A, Andersson R, Groth A (2018) MCM2 promotes symmetric inheritance of modified histones during DNA replication. Science 361(6409):1389–1392. https://doi.org/10.1126/science.aau0294
Podobnik M, McInerney P, O'Donnell M, Kuriyan J (2000) A TOPRIM domain in the crystal structure of the catalytic core of Escherichia coli primase confirms a structural link to DNA topoisomerases. J Mol Biol 300(2):353–362. https://doi.org/10.1006/jmbi.2000.3844
Remus D, Beuron F, Tolun G, Griffith JD, Morris EP, Diffley JF (2009) Concerted loading of Mcm2-7 double hexamers around DNA during DNA replication origin licensing. Cell 139(4):719–730. https://doi:S0092-8674(09)01303-8 [pii] https://doi.org/10.1016/j.cell.2009.https://doi.org/10.015
Salinas F, Benkovic SJ (2000) Characterization of bacteriophage T4-coordinated leading- and lagging-strand synthesis on a minicircle substrate. Proc Natl Acad Sci USA 97(13):7196–7201. https://doi.org/10.1073/pnas.97.13.7196
Schauer G, Finkelstein J, O'Donnell M (2017) In vitro assays for eukaryotic leading/lagging strand DNA replication. Bio Protoc 7(18). https://doi.org/10.21769/BioProtoc.2548
Simon AC, Zhou JC, Perera RL, van Deursen F, Evrin C, Ivanova ME, Kilkenny ML, Renault L, Kjaer S, Matak-Vinkovic D, Labib K, Costa A, Pellegrini L (2014) A Ctf4 trimer couples the CMG helicase to DNA polymerase alpha in the eukaryotic replisome. Nature 510(7504):293–297. https://doi:nature13234 [pii] https://doi.org/10.1038/nature13234
Smith DJ, Whitehouse I (2012) Intrinsic coupling of lagging-strand synthesis to chromatin assembly. Nature 483(7390):434–438. https://doi.org/10.1038/nature10895
Steitz TA (1999) DNA polymerases: structural diversity and common mechanisms. J Biol Chem 274(25):17395–17398. https://doi.org/10.1074/jbc.274.25.17395

Stukenberg PT, Turner J, O'Donnell M (1994) An explanation for lagging strand replication: polymerase hopping among DNA sliding clamps. Cell 78(5):877–887. https://doi:http://dx.doi.org/S0092-8674(94)90662-9

Sun J, Shi Y, Georgescu RE, Yuan Z, Chait BT, Li H, O'Donnell ME (2015) The architecture of a eukaryotic replisome. Nat Struct Mol Biol 22(12):976–982. https://doi.org/10.1038/nsmb.3113

Tahirov TH, Makarova KS, Rogozin IB, Pavlov YI, Koonin EV (2009) Evolution of DNA polymerases: an inactivated polymerase-exonuclease module in Pol epsilon and a chimeric origin of eukaryotic polymerases from two classes of archaeal ancestors. Biol Direct 4:11. https://doi:1745-6150-4-11 [pii] https://doi.org/10.1186/1745-6150-4-11

Tougu K, Marians KJ (1996) The interaction between helicase and primase sets the replication fork clock. J Biol Chem 271(35):21398–21405

van Deursen F, Sengupta S, De Piccoli G, Sanchez-Diaz A, Labib K (2012) Mcm10 associates with the loaded DNA helicase at replication origins and defines a novel step in its activation. EMBO J 31(9):2195–2206. https://doi:emboj201269 [pii] https://doi.org/10.1038/emboj.2012.69

Wasserman MR, Schauer GD, O'Donnell ME, Liu S (2019) Replication Fork Activation Is Enabled by a Single-Stranded DNA Gate in CMG Helicase. Cell 178(3):600–611 e616. https://doi.org/10.1016/j.cell.2019.06.032

Watase G, Takisawa H, Kanemaki MT (2012) Mcm10 plays a role in functioning of the eukaryotic replicative DNA helicase, Cdc45-Mcm-GINS. Curr Biol 22(4):343–349. https://doi.org/10.1016/j.cub.2012.01.023

Yang W, Woodgate R (2007) What a difference a decade makes: insights into translesion DNA synthesis. Proc Natl Acad Sci USA 104(40):15591–15598. https://doi:0704219104 [pii] https://doi.org/10.1073/pnas.0704219104

Yang J, Zhuang Z, Roccasecca RM, Trakselis MA, Benkovic SJ (2004) The dynamic processivity of the T4 DNA polymerase during replication. Proc Natl Acad Sci USA 101(22):8289–8294. https://doi.org/10.1073/pnas.0402625101

Yao NY, O'Donnell M (2012) The RFC clamp loader: structure and function. Subcell Biochem 62:259–279. https://doi.org/10.1007/978-94-007-4572-8_14

Yao NY, O'Donnell ME (2016) Evolution of replication machines. Crit Rev Biochem Mol Biol 51(3):135–149. https://doi.org/10.3109/10409238.2015.1125845

Yao NY, O'Donnell ME (2019) Machinery of DNA replication. In: Roberts G, Watts A (eds) Encyclopedia of biophysics. Springer, Berlin, Heidelberg. doi:https://doi.org/10.1007/978-3-642-35943-9

Yao NY, Georgescu RE, Finkelstein J, O'Donnell ME (2009) Single-molecule analysis reveals that the lagging strand increases replisome processivity but slows replication fork progression. Proc Natl Acad Sci USA 106(32):13236–13241. https://doi:0906157106 [pii] https://doi.org/10.1073/pnas.0906157106

Yu C, Gan H, Serra-Cardona A, Zhang L, Gan S, Sharma S, Johansson E, Chabes A, Xu RM, Zhang Z (2018) A mechanism for preventing asymmetric histone segregation onto replicating DNA strands. Science 361(6409):1386–1389. https://doi.org/10.1126/science.aat8849

Yuan Z, Bai L, Sun J, Georgescu R, Liu J, O'Donnell ME, Li H (2016) Structure of the eukaryotic replicative CMG helicase suggests a pumpjack motion for translocation. Nat Struct Mol Biol 23(3):217–224. https://doi.org/10.1038/nsmb.3170

Yuan Z, Georgescu R, Santos RLA, Zhang D, Bai L, Yao NY, Zhao G, O'Donnell ME, Li H (2019) Ctf4 organizes sister replisomes and Pol alpha into a replication factory. Elife 8. https://doi.org/10.7554/eLife.47405