

# Chapter 2

## DNA Replication Fork Proteins

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### Summary

DNA replication is a complex mechanism that functions due to the co-ordinated interplay of several dozen protein factors. In the last few years, numerous studies suggested a tight implication of DNA replication factors in several DNA transaction events that maintain the integrity of the genome. Therefore, DNA replication fork proteins have also to be considered as part of a general process aiming at replicating and protecting the genome in order to allow the correct function of a cell and of its eventual daughter cells. This is illustrated by several DNA repair pathways such as base excision repair, nucleotide excision repair, double-strand break repair, and mismatch repair. Furthermore, several of the replication proteins have also been shown to be essential in sensing and transducing DNA damages through the checkpoint cascade pathways. This review will summarize the properties of DNA replication proteins that function exclusively at the replication fork.

**Key words:** DNA replication fork, DNA polymerase, DNA helicase, Proliferating cell nuclear antigen, Replication protein A, Replication factor C, Flap endonuclease 1, Dna2, DNA ligase I.

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### 1. Introduction

DNA replication is a complex mechanism which necessitates the co-ordinated interplay of dozens of different proteins (1–3). Due to the antiparallel nature of the DNA and the 5'→3' polymerization direction of any DNA polymerase (pol) known, one strand, called the leading strand, is synthesized continuously, and the other, called the lagging strand, discontinuously in short pieces of about 200 bases. These pieces are called Okazaki fragments. The successful processing of the 20 million Okazaki fragments at the lagging strand requires at least 23 polypeptides (4). The complex replication machinery is assembled at the so-called replication forks, which are gathered in 'nuclear replication factories'. The molec-

ular bases of the replisome have been established several years ago thanks to the SV40 in vitro model system, that used nuclear extracts from different sources to define the factors involved (5). Later, functional replisomes have been reconstituted with purified proteins, allowing depicting more accurately the mechanisms involved in DNA replication. The replisomal proteins can be divided into two main categories: first, the polymerases and second the ‘accessory’ proteins (*see Table 1*). The polymerases catalyse the DNA polymerization, whereas the accessory proteins

**Table 1**  
**Proteins and their functions at the replication fork**

Protein	Function at the replication fork
Origin recognition complex	Forms the pre-replicative complex at the origin of replication
Cdt 1	Licensing cofactor
Cdc 6	Licensing ATPase
Cdc 45	Connector to pol $\alpha$ /primase and to GINS
GINS proteins	Connector to MCM DNA helicase and pol $\alpha$ /primase
MCM 10	Recruits the MCM DNA helicase and pol $\alpha$ /primase to chromatin
And-1/CTF4	Recruits pol $\alpha$ /primase to chromatin
MCM DNA helicase	Opens ds DNA in front of DNA polymerases
Replication protein A	Stabilizes ss DNA created by the MCM DNA helicase
DNA polymerase $\alpha$ /primase	Initiator DNA polymerase, can synthesize short RNA and DNA pieces
DNA polymerase $\epsilon$	Elongating DNA polymerase, likely the leading strand
DNA polymerase $\delta$	Elongating DNA polymerase, likely the lagging strand
3'→5' exonuclease	Proofreading for DNA polymerases, part of the DNA polymerases $\epsilon$ and $\delta$
Replication factor C	Loader of proliferating cell nuclear antigen, responsible for DNA polymerase switch, bridge for the replicative DNA polymerases $\epsilon$ and $\delta$
Proliferating cell nuclear antigen	Moving platform for DNA polymerases and other replication proteins (replication factor C, flap endonuclease 1, DNA ligase I)
Flap endonuclease 1	Removes initiator RNA and DNA at the lagging strand of the replication fork
Dna2 endonuclease	Removes initiator RNA and DNA at the lagging strand of the replication fork that is covered by replication protein A
DNA topoisomerases I and II	Solve topological problems of the DNA in advance and after DNA replication
DNA ligase I	Ligates DNA pieces

are indispensable for assembly and functioning of the replisome at the DNA replication fork. All these polypeptides are spatially arranged and temporally co-ordinated in order to achieve complete bi-directional replication of the replicon.

To accomplish highly accurate cellular DNA replication the replication proteins ORC (origin recognition complex), Cdc6 (cell division cycle), Cdt1 (Cdc10-dependent target), Mcm2–Mcm7 (minichromosome maintenance), Cdc45, replication protein A (RP-A), the GINS complex, proliferating cell nuclear antigen (PCNA), replication factor C (RF-C), topoisomerases, and various DNA polymerases, flap endonuclease 1 (Fen1), Dna 2 endonuclease (Dna 2), DNA ligase I as well as their regulatory protein kinases, CDK and DDK (cyclin- and Dbf4-dependent kinases) have to cooperate (1–3).

The details are as follows (as a review *see* **ref. 6**). First, ORC binds to origin DNA and cooperates with the proteins Cdc6 and Cdt1 to load the MCM complex, consisting of the proteins Mcm2–Mcm7, onto chromatin to form the pre-replicative complex in G1 of the cell cycle. The activation of the pre-replicative complex by CDKs and DDK allows the loading of Mcm10, RP-A, and Cdc45 onto chromatin, which is followed by the formation of the initiation complex, as well as the activation of the replicative MCM DNA helicase. In this early phase, before DNA synthesis by pol  $\alpha$ /primase can start, the following proteins fulfil the so-called *connector* functions (7, 8): the MCM10 protein, the And-1/Ctf4 protein, the Cdc45 protein, and the four-subunit GINS protein. This allows the helicase activity of the MCM complex to unwind the DNA, which attracts the RP-A thereby stabilizing the single-strand (ss) DNA. After the pol  $\alpha$ /primase dependent synthesis of an RNA/DNA primer of 30 nucleotides on the ssDNA (**Fig. 1, step 1**), a DNA polymerase switch from pol  $\alpha$  to pol  $\delta$  and/or pol  $\epsilon$  is induced by RF-C. To accomplish this RF-C binds to the primer and loads PCNA (**Fig. 1, step 2**) that recruits the replicative polymerase (**Fig. 1, step 3**). Subsequently, DNA replication takes place on two strands: the leading strand, where pol  $\epsilon$  holoenzyme (PCNA, RF-C, and pol  $\epsilon$ ) performs processive DNA synthesis and the lagging strand, where pol  $\alpha$ /primase produces an RNA/DNA primer on Okazaki fragments, which are extended by the pol  $\delta$  holoenzyme (PCNA, RF-C, and pol  $\delta$ ). During the elongation step of the lagging strand, when pol  $\delta$  encounters the previous RNA/DNA primer, the pol  $\delta$  holoenzyme performs strand displacement to release the RNA/DNA primer (**Fig. 1, step 4**). Next, PCNA binds Fen1 which cuts the displaced RNA/DNA (**Fig. 1, step 5**) possibly in conjunction with Dna 2. Finally, PCNA recruits DNA ligase I to seal the DNA strand (**Fig. 1, step 6**).

DNA replication has to be performed in an accurate manner. To achieve this, the fidelity of DNA synthesis is controlled by several mechanisms: (1) by the intrinsic mechanisms of steric and

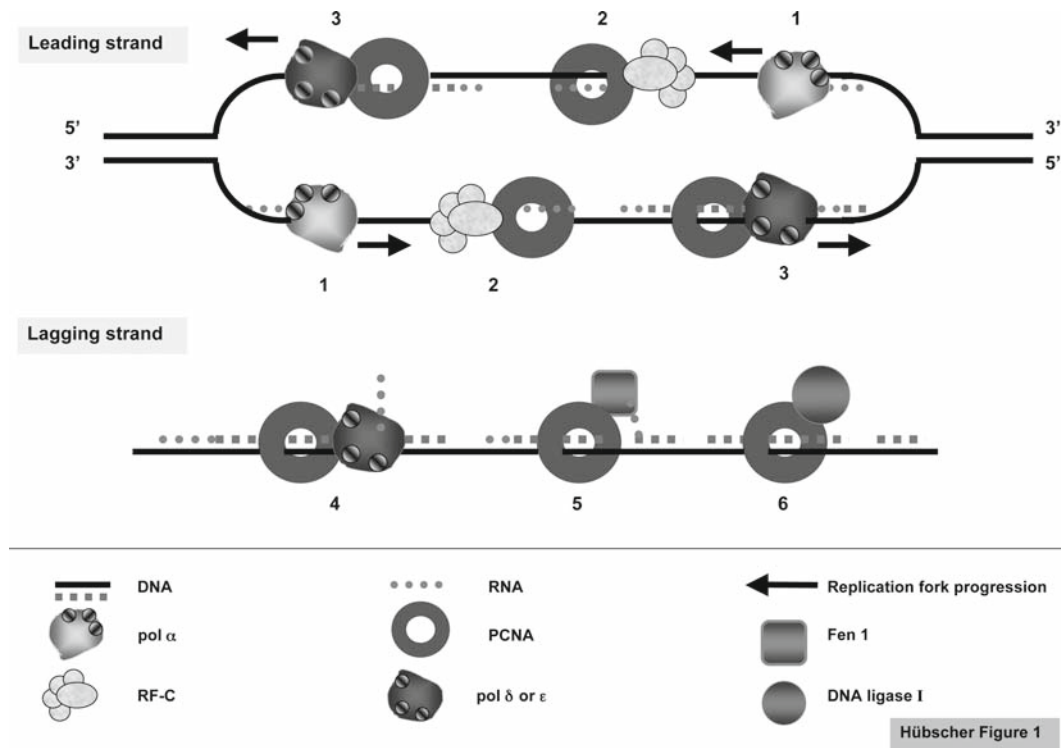


Fig. 1. Eukaryotic DNA replication. (1) primer synthesis by pol  $\alpha$ /primase; (2) DNA polymerase switch by RF-C and loading of PCNA; (3) elongation by pol  $\epsilon$  (leading strand) or  $\delta$  holoenzyme (lagging strand); (4) strand displacement by pol  $\delta$  on the lagging strand; (5) cutting of the 5' displaced flap by Fen1; (6) sealing by DNA ligase I. For details *see* text.

energetic exclusion of non-complementary base pairing (base selection) of pol  $\alpha$ , pol  $\delta$ , and pol  $\epsilon$  that operate at the fork, (2) by the proofreading activity of the 3'  $\rightarrow$  5' exonucleases of pol  $\delta$  and pol  $\epsilon$  or possibly by exogenous exonucleases, such as TREX and (3) by post-replication DNA mismatch repair (9).

Finally before, during and after DNA replication topological constraints occur at the DNA. They are relieved by DNA topoisomerases, whereby DNA topoisomerase I cuts one strand and DNA topoisomerase II two strands. These enzymes will not be summarized in this overview (10).

## 2. Components of the Replication Machinery

### 2.1. MCM DNA Helicase, the DNA Unwinder

The heterohexameric complex Mcm2–Mcm7 is constituting the replicative DNA helicase (11). This complex is first loaded onto chromatin as an inactive DNA helicase in the G1 phase of the cell cycle. It forms there the so-called pre-replicative complexes. In a

so far yet not understand way the MCM DNA helicase is subsequently activated during the initiation of replication phase. Two kinases play an important role: first, the cyclin dependent kinase (CDK) and second, the Cdc7 kinase (Cdc7). Since the isolated MCM complex is inactive as a DNA helicase, it is speculated that activation includes post-translational modification (e.g. phosphorylation) followed by association with other factors (12). Among them Cdc45 forms a tight complex with the MCM complex at the replication fork and appears to be required for unwinding of the DNA at least in the initiation phase of replication. It is likely that other proteins are required to co-ordinate the MCM helicase to the replication machinery. A novel heterotetrameric protein complex, called GINS (9), was found to have an essential role in the establishment, the progression, and the co-ordination of the replication fork. The name GINS derives from studies in *Saccharomyces cerevisiae* where the four essential genes Sld5-Psf1-Psf2-Psf3 were termed GINS from the Japanese language 'Go-Ischi-Ni-San' (5-1-2-3). Since GINS also physically and functionally interacts with the initiator pol  $\alpha$ /primase it can perfectly fulfil a connector function between the MCM DNA helicase and the start of RNA/DNA synthesis by pol  $\alpha$ /primase (13).

## **2.2. Replication Protein A, More Than Single-Strand DNA Protector**

Another important component of the DNA replication machinery is the single-strand binding protein called RP-A (14). It covers and protects ssDNA during replication fork progression. RP-A is involved in the regulation of several steps of DNA replication. First, it has been shown that RP-A acts as a 'fidelity clamp' for pol  $\alpha$ /primase (15). Second, it can modulate the strand displacement synthesis of pol  $\delta$  (16) and, third, it can regulate the sequential action of the two flap nucleases Fen1 and Dna 2, both involved in Okazaki fragment processing (17).

Studies about DNA repair mechanisms showed that RP-A plays a major role in co-ordinating DNA repair mechanisms and is therefore more than a simple 'protecting' protein for ssDNA (18). Indeed, ssDNA is the most commonly generated structure upon DNA damage or during DNA repair. Therefore binding of RP-A to ssDNA brings it to an excellent strategic position for modulation of DNA metabolic processes. It is therefore not surprising that RP-A was found to have an important role in early checkpoint signalling (18).

In summary RP-A protects ssDNA from nucleolytic degradation, can prevent hairpin formation, and prevents DNA re-annealing until the DNA metabolic events are successfully terminated (e.g. DNA replication, different DNA repair pathways, and DNA recombination).

## **2.3. DNA Polymerase $\alpha$ /Primase, the Initiating DNA Polymerase**

As already indicated pol  $\alpha$ /primase has an essential role early in DNA replication. In all eukaryotic organisms the pol  $\alpha$ /primase complex consists of four subunits, with molecular masses of 165,

67–86, 58, and 48 kDa (2). The p48 and p58 subunits form the heterodimeric primase. Pol  $\alpha$ /primase also plays an important role in co-ordinating DNA replication, DNA repair, and cell-cycle checkpoints. It couples mitosis to the completion of DNA replication and to the repair of DNA damage, and is essential for the arrest of yeast cells in G1 prior to the start of S phase. Furthermore, CDKs phosphorylate and regulate pol  $\alpha$ /primase during the cell cycle. A direct role of pol  $\alpha$ /primase in DNA repair and DNA recombination is still under discussion, but published data suggested that the leading-strand replicative polymerases are required for double-strand breaks' repair in yeast by homologous recombination (19). In DNA replication, on the other hand, pol  $\alpha$ /primase, soon after replication has been initiated at the origin, synthesizes first on the leading strand the initiation primer, consisting of 30 nucleotides (ten nucleotides of RNA and 20 nucleotides of DNA). During this synthesis RF-C binds the double-stranded (ds) DNA from the 5'-side and, as soon as 30 nucleotides are reached, a DNA polymerase switch from pol  $\alpha$ /primase to pol  $\delta$  and/or pol  $\epsilon$  occurs (20, 21). A similar scenario is envisaged on the frequent initiation and elongation of the Okazaki fragments on the lagging strand of the replication fork.

#### **2.4. Replication Factor C, the Moving Platform Loader and DNA Polymerase Switcher**

RF-C was first identified as an essential factor required for simian virus 40 DNA replication (2). It is a heteropentameric protein consisting of subunits of 128, 40, 38, 37, and 36 kDa. Studies from many laboratories have identified RF-C as an ATPase machine that can act as a matchmaker for the moving platform PCNA (reviewed in *ref.* 22). RF-C binds preferentially to template/primer junctions and there either to the 5' or to the 3' ends. By binding to the 3' end RF-C can cover up to 20 nucleotides of the primer. RF-C first binds ATP and then the template/primer junction (21). PCNA can then bind to the RF-C/ATP/template/primer complex. RF-C can then transiently open the PCNA ring, thus loading it onto the template/primer junction. ATP hydrolysis is not required for this step. Upon ATP hydrolysis by the RF-C ATPase activity, the PCNA/RF-C/DNA likely undergoes a conformational change, resulting in the release of RF-C from the DNA (reviewed in *ref.* 23). This is the time when the moving platform is handed over to the polymerases. In replication this is either pol  $\epsilon$  or pol  $\delta$ . A similar mechanism is feasible for many other pols, such as the translesion polymerases ( $\eta$ ,  $\kappa$ ,  $\iota$ ,  $\zeta$ ) and the DNA repair polymerases ( $\beta$ ,  $\lambda$ ) (24).

RF-C has another important function in DNA replication. As mentioned earlier, it can also bind to 5' end of the primer/template. Pol  $\alpha$ /primase starts DNA replication on both strands by first synthesizing an RNA of 10 nucleotides which is followed by DNA synthesis. RF-C bound to the 5' end somehow clamps down as soon as 30 nucleotides are synthesized by pol  $\alpha$ /primase, thus allowing pol  $\delta$  or  $\epsilon$  to take over (21). This step is called

the DNA polymerase switch from the initiating pol  $\alpha$ /primase to the bulk synthesizing pols  $\delta$  or  $\epsilon$ .

**2.5. Proliferating Cell Nuclear Antigen, the Moving Platform with a Great Promiscuity**

The identification of PCNA as a processivity factor for replicative polymerases placed it at the heart of the replisome (25, 26). However, additional roles for this protein started to emerge in co-ordinating the complex network of interactions occurring at the replication fork. Our current view of DNA replication in eukaryotes predicts that pol  $\alpha$ /primase synthesizes the first RNA/DNA primer on the leading strand (*see* earlier). Then, together with the DNA polymerase switch initiated by RF-C, pol  $\epsilon$  with its processivity factor PCNA performs continuous leading-strand synthesis, whereas pol  $\alpha$ /primase is involved in RNA priming and discontinuous DNA synthesis at the lagging strand. However, completion of Okazaki fragment synthesis requires the action of a processive pol  $\delta$  holoenzyme (pol  $\delta$ , RF-C, and PCNA). Thus, both initiation of leading-strand DNA replication and discontinuous lagging-strand synthesis require a DNA polymerase switch. PCNA has been shown to play a central role in co-ordinating this process. In fact, PCNA loading at the 3'-OH end of the nascent DNA strand by the clamp-loader RF-C triggers the displacement of pol  $\alpha$ /primase and subsequent recruitment of pol  $\epsilon$  or pol  $\delta$  for processive synthesis. Re-binding of pol  $\alpha$ /primase is prevented by the presence of PCNA bound at the 3'-OH end, which functions as a specific recruiting signal for the more processive pols  $\delta$  and  $\epsilon$ . Synthesis of an Okazaki fragment is terminated when pol  $\delta$  or  $\epsilon$  holoenzyme meets the 5'-end of the RNA portion of the previously synthesized fragment and performs strand displacement synthesis. Finally, specialized proteins are recruited that remove the RNA part, fill the gap, and ligate the two adjacent fragments. The two PCNA-binding proteins Fen1 and DNA ligase I are involved in this process. Moreover, PCNA has been shown to stimulate Fen1 activity. In vitro re-constitution of the Okazaki fragment maturation process showed that competition for PCNA binding among pol  $\delta$ , Fen1, and DNA ligase I co-ordinates the ordered action of these enzymes. When pol  $\delta$  holoenzyme encounters the 5'-end of the previous fragment, it performs strand displacement synthesis in conjunction with the helicase/endonuclease Dna 2. This structure is bound by the ssDNA-binding protein RP-A, which triggers dissociation of pol  $\delta$  from PCNA. With the recruitment of Fen1, the PCNA/Fen1 complex efficiently removes the flap. This is followed by binding of DNA ligase I to PCNA to ensure the reconstitution of the integrity of the dsDNA.

**2.6. DNA Polymerase  $\epsilon$ , the Likely Leading-Strand Replicase**

Pol  $\epsilon$  is composed of four subunits of 256, 78, 24, and 22 kDa in mammalian and fission yeast cells and is essential for cell viability and is required for chromosomal DNA replication in *Saccharomyces cerevisiae* (2). In addition to DNA replication, pol  $\epsilon$  is thought to



be involved in DNA repair and cell-cycle checkpoint control. Pol  $\epsilon$  from *Saccharomyces cerevisiae* consists of four polypeptides (Pol2, Dpb2, Dpb3, and Dpb4) and the genes encoding the 256-kDa catalytic and 78-kDa subunits are essential for growth, as is the catalytic subunit of *Schizosaccharomyces pombe* pol  $\epsilon$ . Requirement of the catalytic subunit has been taken to reflect the need for polymerase activity of pol  $\epsilon$  in replication in yeast cells. Recent experiments suggest that pol  $\epsilon$  participates in DNA replication of the leading strand (27). On the other hand, the question concerning the role of pol  $\epsilon$  in replication has become intriguing because the catalytic polymerase domain within the 256-kDa polypeptide is apparently not essential in budding yeast, but mutants are severely affected in growth (28). In addition to DNA replication and viability, cells that lack the catalytic polymerase domain are also proficient in DNA repair and recombination (29).

### **2.7. DNA Polymerase $\delta$ , the Likely Lagging-Strand Replicase**

Pol  $\delta$  exists as a heterotetrameric enzyme with subunits of 125, 67, 50, and 12 kDa and possesses a wide range of functions (2): it is needed (1) in DNA replication (lagging strand) (2) in several DNA repair events (long-patch base excision repair, nucleotide excision repair, mismatch repair), (3) in translesion synthesis, (4) possibly in cell-cycle control, because different temperature-sensitive mutants in the large 125-kDa subunit of *Schizosaccharomyces pombe* pol  $\delta$  exhibited a typical 'cell division cycle' terminal phenotype and finally (5) in meiotic recombination, where a novel allele of the *Saccharomyces cerevisiae* *POL3* gene was identified whose mutant strain produces shorter-strand exchange intermediates and fewer crossover products during meiotic recombination (reviewed in (1–3)).

Beside polymerase activity, pol  $\delta$  possesses, as does pol  $\epsilon$ , an intrinsic 3'→5' exonuclease activity in the catalytic subunit (p125) (30). Yeast *POL3* mutant strains lacking the exonuclease activity of pol  $\delta$  have a strong mutator phenotype. *POL3* mutations in regions outside of the exonuclease domain elevate the frequency of deletions likely reflecting an increased frequency of DNA polymerase slippage. Moreover, reduction in the expression level of wild-type pol  $\delta$  results in a similar mutator phenotype and in increased sensitivity to the DNA-damaging agent methyl methane sulphonate (31). These results might suggest that both the quantity and the quality of pol  $\delta$  are important in ensuring genome stability. The 3'→5' exonuclease activity of pol  $\delta$  besides acting as a proofreader has additional biological roles in Okazaki fragment maturation and mismatch repair. Defective pol  $\delta$  proofreading causes cancer susceptibility in mice (32, 33).

### **2.8. Flap Endonuclease 1 and Dna 2, the Trimmers at the Lagging Strand**

Fen1 is a key enzyme for maintaining genetic stability in eukaryotic genomes (34, 35). Haploinsufficiency of Fen1 leads to rapid progression of tumours in mice (36). Fen1 has an essential role in DNA replication, where it participates in the removal of initiator



nucleic acid (RNA and DNA) during Okazaki fragment processing. Moreover, its involvement in DNA repair events, such as base excision repair and non-homologous-end joining of ds DNA breaks, attributes to Fen1 a paramount role in preventing a situation in the genome that leads to unwanted genetic exchanges and, eventually, to a cancerous phenotype (34, 35).

The completion of DNA synthesis at the lagging strand requires the removal of RNA primers from Okazaki fragments prior to gap filling synthesis and ligation. Strand displacement by pol  $\delta$  of the downstream Okazaki fragment is followed by the endonuclease activity of Fen1, which removes the entire RNA-containing 5' flap (37). Despite the well established *in vitro* roles of Fen1, genetic analysis in yeast revealed that null mutants Fen1 are not lethal, suggesting that an additional enzymatic activity may be required for the removal of RNA (34, 35). Genetic and biochemical studies revealed that the endonuclease Dna 2 is involved in Okazaki fragment processing, since it physically and genetically interacts with Rad27p (the budding yeast homologue of Fen1) and with proteins involved in elongation or maturation of Okazaki fragments. Okazaki fragment processing requires RP-A which mediates the sequential action of the Dna 2 and Fen1 to remove the RNA/DNA primers (38). First, DNA synthesis catalysed by pol  $\delta$  holoenzyme displaces the RNA containing 5'-terminus of the downstream Okazaki fragment. Second, RP-A rapidly forms an initial complex with the nascent displaced structure and third recruits Dna 2 to form a ternary complex. Access and cleavage by Fen1 at this step is inhibited by RP-A. After cleavage of the RNA-containing segment by Dna 2, producing a short flap DNA product (Dna 2 cleaves endonucleolytically 5–7 nt before the junction), further processing by the action of Fen1 occurs, which is loaded onto the DNA through protein-protein interactions with PCNA (39). RP-A appears to play a critical role in regulating the extent of unwinding of the Okazaki fragment by preventing uncontrolled displacement by pol  $\delta$ . Otherwise this will lead to extensive degradation of a pre-existing Okazaki fragment and the formation of long ss DNA, potentially forming secondary structures that would be resistant to cleavage by Fen1.

It was also found that the 3'→5' exonuclease activity of pol  $\delta$  can substitute for Fen1 in processing Okazaki fragments *in vivo*, and this led to the hypothesis that another pathway for Okazaki fragment maturation exists in eukaryotic cells (40–42). Fen1 appears to be the main degradation force, and the activity of Dna 2 becomes crucial in cases where strand displacement proceeds to an extent that they can neither be cleaved by Fen1 nor realigned by the 3'→5' exonuclease activity of pol  $\delta$ . Short flaps are processed either by Fen1 alone or together with pol  $\delta$ . The 3'→5' exonuclease seems to be required under conditions

of Fen1 deficiency by degrading back the strand that pol  $\delta$  had synthesized to allow the displaced strand to re-hybridize to the template, thus producing a proper nick for ligation by DNA ligase I (40).

Fen1 is recruited to the site of DNA synthesis by PCNA, which can stimulate Fen1 by stabilizing its interaction to the cleavage site and must be located below the 5' flap. The essential PCNA-binding domain was mapped to a region near the basic C-terminus of Fen1 which contains the consensus motif Qxx-LxxFF (43). Two regions in PCNA, one located in the interdomain connector loop (IDCL) and the second near the C-terminus, are important for binding to Fen1. In the absence of DNA, the IDCL of PCNA mediates binding to Fen1; however, once PCNA has encircled DNA, proper function of Fen1 requires interaction with the C-terminus of PCNA (44, 45).

### **2.9. DNA Ligase I, the DNA Connector**

DNA strand breaks can be produced as a consequence of normal DNA transactions. Examples of DNA breaks in normal cell metabolism are, first, Okazaki fragments, that are generated by discontinuous lagging-strand DNA synthesis, and, second, programmed site-specific DNA ds breaks, such as those made in some types of immune cells undergoing immunoglobulin gene rearrangements. DNA strand breaks can be also generated directly by DNA-damaging agents or as a consequence of DNA lesion removal by one of the excision repair pathways. DNA ligase I is conserved in all eukaryotes and required in DNA replication for the ligation of Okazaki fragments during lagging-strand synthesis and for long-patch base excision repair (46). The full-length cDNA of human DNA ligase I encodes a 102-kDa protein of 919 amino acid residues. The active site lysine residue, which binds ATP, is located at position 568 (46).

DNA ligase I acts in DNA replication and other cell processes as a part of a carefully co-ordinated process involving the interaction of several different proteins. Its main function is in lagging-strand synthesis in eukaryotes, where DNA ligase I finally seals the nick. PCNA, well known as a processivity factor for polymerases (*see* PCNA section), also interacts with many other replication and repair factors and is responsible for recruiting these proteins to replication or repair foci. Among them DNA ligase I interacts with the interdomain connector loop (IDCL) of PCNA through its PCNA-binding peptide (PIP) located into the first 20 amino acids of DNA ligase I (47). There are contradictory data in literature about the functional consequences of DNA ligase I/PCNA interactions: on the one hand, an inhibitory effect of PCNA on DNA ligase I was found (47, 48), while, on the other hand, DNA ligase I activity could be stimulated by interaction with PCNA (49). The recruitment of DNA ligase I to replication foci and to the DNA damage sites, as well as the efficient joining

of Okazaki fragments and long-patch base excision repair completion, is directly dependent on the interaction between DNA ligase I and PCNA. Association between RF-C and DNA ligase I was described involving both the N- and C-terminal domains of DNA ligase I. RF-C inhibited DNA joining by DNA ligase I, but the addition of PCNA decreased the inhibition by RF-C (50). Moreover, it was suggested that the pairwise interactions among RF-C, PCNA, and DNA ligase I could co-ordinate the joining step of Okazaki fragments processing and of long-patch base excision repair (50).

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### 3. The Replication Ensemble Plays Together in the Replisome

So far the replication proteins, with respect to their individual function or their mutual interaction, were described side by side. It is, however, conceivable that they all have to interact and display their physiological roles when they are needed in the replisome. The current loop model is based on an initial idea of Bruce Alberts in which the lagging strand forms a loop thus allowing the unidirectional movement of the two replicative pols  $\epsilon$  and  $\delta$  (1–3, 51). **Figure 2** shows a possible scenario for this ‘trombone model’.

First, at the origin of replication, the activated hexameric MCM DNA helicase encircles the lagging strand and melts upon hydrolysis of ATP the DNA in the direction of DNA replication. Second, pol  $\alpha$ /primase initiates at the leading strand (for simplicity not shown in **Fig. 2**) and, third, replication of the leading strand is started by the DNA polymerase switch by RF-C to pol  $\epsilon$  (27, 52). Fourth, pol  $\alpha$ /primase is also acting on the lagging strand to frequently initiate the Okazaki fragments where, fifth, RF-C frequently (every 200 bases) switches to pol  $\delta$  to complete Okazaki fragment DNA synthesis. In this model RF-C bridges the two replicative pols  $\delta$  and  $\epsilon$  as well as the MCM DNA helicase. RP-A is bound to the ssDNA on the loop forming lagging strand. In this continuous dynamic of the MCM DNA helicase and the three pols  $\alpha$ ,  $\delta$ , and  $\epsilon$  other factors such as Cdc45 and the GINS complex are likely to be required.

This takes also into account a recent work suggesting a three-DNA polymerase model (51, 53). This model predicts that three polymerases act simultaneously at the replication fork. The authors suggest that two polymerases act at the leading and lagging strand, respectively. The third polymerase acts as a reserve enzyme to overcome obstacles that occur to the replication fork. This has not only the advantage to be dynamic, especially at the lagging strand, but would also allow other components to enter during replication if required. In other words, translesion polymerases and DNA repair

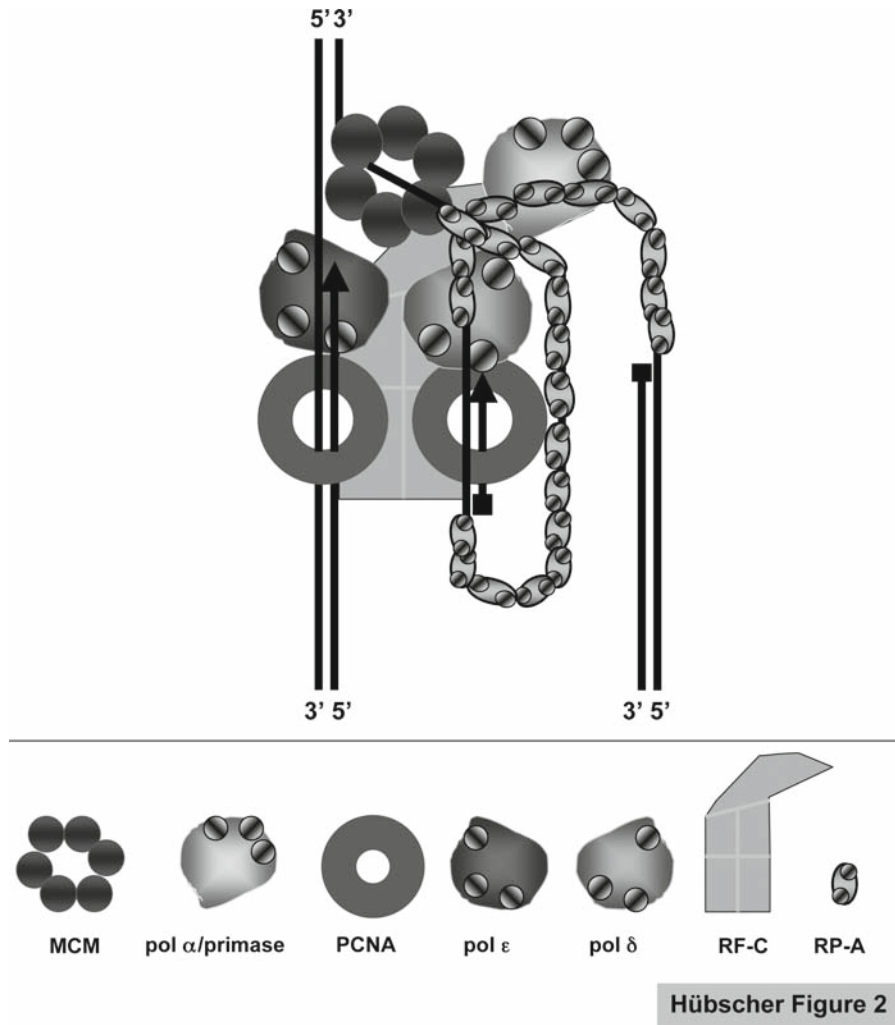


Fig. 2. Simplified scenario of how the eukaryotic replisome might work. The model represents a 'momentum' situation at the replication fork. The hexameric MCM DNA helicase encircles the lagging strand and melts the DNA; pol  $\epsilon$  replicates the leading strand and pol  $\delta$  the lagging strand. RF-C might bridge the two replicative pols  $\epsilon$  and  $\delta$  as well as the MCM DNA helicase. Pol  $\alpha$ /primase is on the lagging strand to frequently initiate the Okazaki fragments and RP-A is bound to the ss DNA on the lagging strand that forms a loop. *Thick line*: initiator RNA, *thin line*: DNA. Further proteins not shown include Cdc45 and the GINS complex. The maturation of the Okazaki fragments by Fen1, DNA2, and DNA ligase I is omitted. For details *see text*.

polymerases might replace the replicative polymerases when DNA is damaged (54). The signal for this might come from PCNA in the replisome that is transiently post-translationally modified by phosphorylation, monoubiquitination, polyubiquitination, and sumoylation, depending on the damage occurring during DNA replication (25, 26).

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