EMBO reports

review

DNA replication: a complex matter

Isabelle Frouin[1,2], Alessandra Montecucco[1], Silvio Spadari[1] & Giovanni Maga[1+]

[1]Istituto di Genetica Molecolare, Consiglio Nazionale delle Ricerche, Pavia, Italy, and [2]Institute of Veterinary Biochemistry and Molecular Biology, University of Zürich-Irchel, Zürich, Switzerland

In eukaryotic cells, the essential function of DNA replication is carried out by a network of enzymes and proteins, which work together to rapidly and accurately duplicate the genetic information of the cell. Many of the components of this DNA replication apparatus associate with other cellular factors as components of multiprotein complexes, which act cooperatively in networks to regulate cell cycle progression and checkpoint control, but are distinct from the pre-replication complexes that associate with the origins and regulate their firing. In this review, we summarize current knowledge about the composition and dynamics of these large multiprotein complexes in mammalian cells and their relationships to the replication factories.

EMBO reports **4**, 666–670 (2003)
doi:10.1038/sj.embor.embor886

The DNA synthesome and the SV40 system paradigm

Reconstitution of the complete replication process of plasmid DNA containing the viral SV40 *ori* originally identified DNA polymerase (Pol)-α/primase, Pol-δ, replication protein A (RPA), replication factor C (RFC), proliferating cell nuclear antigen (PCNA), Flap endonuclease 1 (Fen1), RNase H1 and DNA ligase I (LigI) as the essential components of the eukaryotic replisome (Waga & Stillman, 1994). Since these initial studies were performed, larger (18S–21S) multiprotein complexes that contain replication proteins (known as 'multiprotein replication complexes' or 'DNA synthesomes') have been isolated from the extracts of a variety of eukaryotic cells (for a review, see Malkas, 1998). Although the exact protein composition of the DNA synthesomes varies (see Supplementary information online, Table 1), the fact that most of their components are shared argues that the differences are not due to the extraction procedure used. The functional relationships of the components of the DNA synthesome have not been addressed in detail because of their numbers. However, the finding that a smaller DNA replication-competent (RC) multiprotein complex in calf thymus contains at least Pol-α/primase, Pol-δ and RFC (Maga & Hubscher, 1996) suggests that an asymmetric complex of two different Pol enzymes could represent the minimal 'core' of the mammalian DNA synthesome, and that it thereby resembles the Pol III holoenzyme in *Escherichia coli*. More recently, a complex of not only DNA replication proteins, but also cell-cycle regulatory factors including cyclin A, cyclin B1, cyclin-dependent protein kinase 2 (Cdk2) and Cdk1, was purified from nuclear extracts of HeLa cells that were synchronized at different stages of the cell cycle (Frouin *et al*., 2002). This finding raises the possibility that there are direct links between the synthesome and control of the cell cycle at the level of the replication apparatus, which we explore in this review.

The mechanics of the DNA replication machine

Our current view of DNA replication in eukaryotes, which comes mainly from *in vitro* studies using purified components of the DNA synthesome, is that both the initiation of leading strand DNA replication and discontinuous lagging strand synthesis require a switch from the use of the Pol-α to either the Pol-δ or Pol-ε enzyme, as well as continuous recycling of the processivity factor PCNA by the enzymatic clamp-loader complex RFC (Waga & Stillman, 1994). PCNA has been shown to have a central role in the coordination of the order of events at the replication fork. In fact, the loading of PCNA by RFC at the 3′-OH end of the nascent DNA strand triggers the displacement of Pol-α and the subsequent recruitment of Pol-δ or Pol-ε for processive synthesis (Yuzhakov *et al*., 1999; Maga *et al*., 2000). When, during the generation of Okazaki fragments, the Pol-δ or Pol-ε holoenzyme meets the 5′-end of the RNA portion of the previously synthesized fragment, it generates a displaced strand that forms a flap structure. This structure is bound by the single-stranded DNA-binding protein RPA, which triggers dissociation of Pol-δ from PCNA. This in turn favours the recruitment of the endonuclease Fen1, a specialized PCNA-binding protein (Maga *et al*., 2001), and probably also the binding of the helicase/nuclease Dna2 (Bae *et al*., 2001). The PCNA/Fen1/Dna2 complex then efficiently removes the flap structure. This is followed by binding of LigI to PCNA, which reconstitutes the integrity of the double-stranded DNA (Ayyagari *et al*., 2003; Jin *et al*., 2003). Thus, an ordered sequence of association and dissociation events that involve different proteins ensures the coordinated action of the various components of the DNA synthesome.

Complex dynamics: the replication factories

Fluorescence microscopy of incorporated biotin-labelled dUTP or bromodeoxyuridine (BrdU) has been used to show that, in mammalian cells, newly synthesized DNA localizes to discrete subnuclear sites, known as 'replication foci' or 'replication sites' (Nakayasu & Berezney, 1989; O'Keefe *et al*., 1992). The colocalization of replication factors

[1]Istituto di Genetica Molecolare, Consiglio Nazionale delle Ricerche, via Abbiategrasso 207, I-27100 Pavia, Italy
[2]Institute of Veterinary Biochemistry and Molecular Biology, University of Zürich-Irchel, Winterthurerstrasse 190, CH-8050 Zürich, Switzerland
[+]Corresponding author. Tel: +39 0382 546355; Fax: +39 0382 422286;
E-mail: maga@igm.cnr.it

Submitted 28 February 2003; accepted 21 May 2003

Table 1 | Factors recruited to the replication factories

Proliferating cell nuclear antigen (Hozak *et al*., 1993)
Replication protein A (Cardoso *et al*., 1993)
DNA polymerase-α (Hozak *et al*., 1993)
DNA polymerase-ε (Fuss & Linn, 2002)
DNA ligase I (Montecucco *et al*., 1995)
DNA topoisomerase II-α (Niimi *et al*., 2001)
DNA-C5-Methyltransferase (Chuang *et al*., 1997; Leonhardt *et al*., 1992)
Histone deacetylase (Rountree *et al*., 2000)
Chromatin assembly factor (Krude, 1995)
Uracil DNA glycosylase (Otterlei *et al*., 1999)
DNA polymerase-η (Kannouche *et al*., 2002)
DNA polymerase-ι (Kannouche *et al*., 2002)
MRE11 (Maser *et al.*, 2001)
MSH3/MSH9 (Kleczkowska *et al*., 2001)
hMYH (Boldogh *et al*., 2001)
Cdk2/Cyclin A (Cardoso *et al*., 1993)

in these foci gave rise to the idea that replication takes place in 'replication factories' that assemble at these sites (Hozak *et al*., 1993). Considering the number of factories present in a nucleus, the length of the genome, and the rate of synthesis along a bidirectional replication fork, it has been calculated that an average factory in early S phase must contain approximately 5–6 replicons that are spatially distributed in a small nuclear volume (Jackson & Pombo, 1998; Ma *et al*., 1998). It has been suggested that these factories correspond to the DNA polymerase-containing 'megacomplexes' that have been solubilized from the nuclear matrix (Tubo & Berezney, 1987; Cook, 1999). The number and size of the replication factories vary throughout S phase according to a programme that corresponds to the replication of various portions of the genome. For example, at the onset of S phase, replication factories are associated with transcribed genes (Hassan *et al*., 1994), whereas the appearance of mid- and late S-phase factories coincides with the replication of the more numerous silent, heterochromatic regions (O'Keefe *et al*., 1992). Thus, the active replication forks in each factory must range from a few units in early S phase, to many thousands in late S phase.

Leonhardt and co-workers studied the dynamics of replication factories in living cells in greater detail by imaging stable cell lines that express a green fluorescent protein (GFP)–PCNA fusion protein (Leonhardt *et al*., 2000). On the basis of their studies, they proposed that replication factories are stably anchored in the nucleus and that changes in their distribution occur through their gradual, coordinated assembly and disassembly throughout S phase. According to this model, the large replication factories that can be visualized in late S phase do not originate from the coalescence of several small factories, but rather are assembled *de novo* throughout S phase. In the past few years, the use of confocal microscope analyses has shown that, in addition to replication enzymes, replication factories contain an ever-growing list of factors involved in DNA metabolism and cell cycle control (Table 1). Interestingly, early and late S-phase factories differ not only in size, but also in their composition. For instance, Rountree and co-workers found that the histone deacetylase HDAC2 colocalizes with late, but not with early S-phase replication factories (Rountree *et al*., 2000), which may be functionally linked to its role in establishing the hypoacetylated status of the heterochromatin.

The complex matter of complex regulation

The data discussed above indicate that pools of DNA replication proteins are likely to be organized into DNA synthesomes of differing composition and that they accumulate to form discrete subnuclear structures, the 'replication factories'. Here, we explore the mechanisms that control the dynamic behaviour of the different pools.

Extensive mutation analyses of human LIGI and DNA methylase (Chuang *et al*., 1997; Montecucco *et al*., 1998) have shown that a short sequence of homology between these proteins is sufficient to target a GFP fusion to replication factories. This sequence, known as the replication factory targeting sequence (RFTS), corresponds to the first 20 amino acids of LIGI and, in this protein, is the binding site for PCNA, an essential component of the DNA synthesome. Any mutation in this sequence that affects its interaction with PCNA also abrogates its recruitment to the replication factories. The same PCNA binding site is also required *in vivo* for the activity of LIGI during the maturation of Okazaki fragments (Levin *et al.*, 2000). Thus, the interaction with PCNA is required both to localize LIGI to replication factories and to tether the enzyme to the template during DNA synthesis (Tom *et al.*, 2001). Several other proteins that interact with PCNA through the RFTS have also been found to colocalize with the replication factories, indicating that PCNA is a general recruiting factor. In addition, two distinct PCNA binding sites have been described in the large subunit of RFC (RFC1). The first, domain B, maps to the carboxyl terminus of RFC1 and is also present in the other four subunits of RFC (Fotedar *et al*., 1996). The second, found at the RFC1 amino terminus (residues 1–24), shares homology with the human LIGI RFTS (Montecucco *et al*., 1998). As this site is unecessary for the assembly of the RFC complex, as well as for its *in vitro* replication activity (Uhlmann *et al*., 1997), it is tempting to speculate that the site has a role in the recruitment of the complex to the replication factories.

Studies of the localization of PCNA, using fluorescence recovery after photobleaching (FRAP) of a GFP–PCNA fusion, revealed that there is little, if any, turnover of this protein at replication sites (Sporbert *et al.*, 2002). This is consistent with the resistance of replication-factory-associated PCNA to *in situ* extraction and with its suggested recruiting activity. It is conceivable that PCNA has a role in increasing the local concentration of replication factors, providing the optimal conditions for DNA replication as well as for post-replicative events, such as DNA methylation, histone modification and DNA repair. We favour a model in which each replication factory contains two pools of PCNA molecules with distinct roles: one is directly engaged in DNA replication, and the other recruits reserves of replication factors into the vicinity of active replicons (Fig. 1). This model might explain the presence of enzymes that are expected to be involved in DNA repair, recombination or chromatin remodelling on newly synthesized DNA, but are not required for DNA synthesis *per se* (Table 1). As numerous replication factors are also involved in repair pathways, the factory model could provide links between synthesis and repair pathways. According to an alternative model, however, the PCNA that remains at replication foci after *in situ* extraction would be directly bound to DNA and engaged in the synthesis of the Okazaki fragments of several synchronously replicating replicons (Sporbert *et al.*, 2002).

Post-translational modifications of replication factors are likely to occur in the dynamic processes described above. For example, two immunologically distinct Pol-α/primase subpopulations have been described in mammalian cells (Dehde *et al.*, 2001). These differ mainly in terms of the phosphorylation status of p68, one of the four

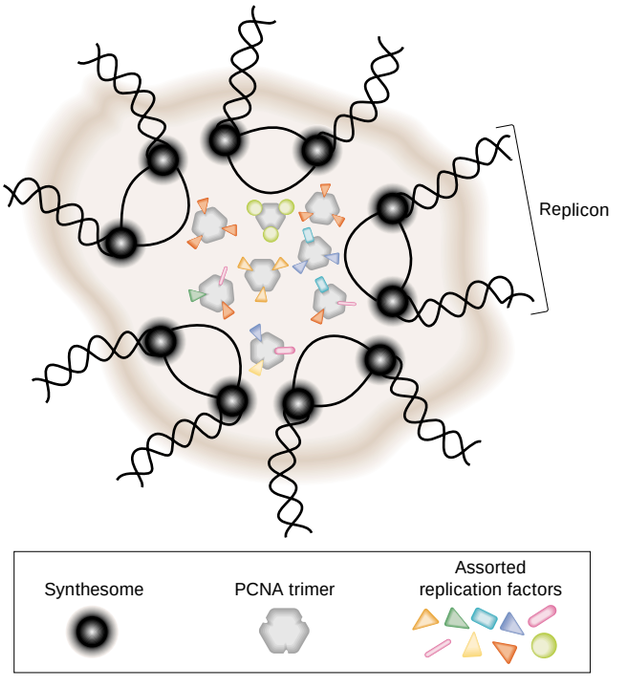

Fig. 1 | Model of a DNA replication factory. This shows five activated replicons in a single factory (shaded area). Each replicon contains a replication bubble and two replication forks. At each fork, a DNA synthesome (dark grey circle) is bound to DNA and contains all the proteins required for DNA synthesis, including proliferating cell nuclear antigen (PCNA; not shown). However, additional replication factors (coloured symbols) accumulate in the factory in the vicinity of the replicons, as they bind to PCNA trimers (hexagons) that are not associated directly with replicating DNA. PCNA trimers may bind either three molecules of the same replication factor or different combinations of replication factors.

subunits of the Pol-α/primase, whose modification by the Cdk2/cyclin A complex inhibits the ability of the Pol-α/primase tetramer to initiate SV40 DNA replication at the origin (Voitenleitner *et al*., 1999). By using monoclonal antibodies that are selective for the two enzyme populations, Dehde and co-workers showed that only the phosphorylated enzyme co-immunoprecipitates with cyclin A, which acts as a regulatory subunit to Cdk2, and that it colocalizes with the replication factories. By contrast, the hypophosphorylated form co-immunoprecipitates with cyclin E and with protein phosphatase 2A (PP2A), and its distribution at the G1/S transition partially overlaps with that of MCM2, a constituent of the pre-replication complexes. On the basis of these observations, the authors propose that the hypophosphorylated enzyme initiates DNA replication at origins, and the phosphorylated form synthesizes the primers for the lagging strand of the replication fork. However, a more recent analysis (Ott *et al.*, 2002) has shown that phosphorylation of p68 by Cdk2/cyclin A inhibits not only the initiation of SV40 DNA replication at the origin, but also priming and elongation on RPA-saturated M13 single-stranded DNA. The authors speculate that as Cdk2/cyclin A activity rises during S phase, modification of the phosphorylation sites of p68, which are clustered, may increase progressively, shutting down enzyme activity as DNA replication is completed. The two main questions raised by this finding are whether phosphate turnover regulates polymerase activity during S phase and whether the phosphorylated form, detectable at replication factories, is catalytically active.

Human LIGI has also been found to exist *in vivo* in two forms that differ in their level of phosphorylation (Rossi *et al.*, 1999). LIGI is phosphorylated on Ser 66, which is located in a casein kinase II (CKII) consensus site, in a cell-cycle-dependent manner. After dephosphorylation in early G1, the level of phosphorylated protein remains minimal during the rest of this phase, before increasing progressively during S phase and peaking in the G2 and M phases. The analysis of epitope-tagged LIGI mutants showed that the dephosphorylation of Ser 66 requires both nuclear localization and the PCNA-binding site. Confocal microscopy analysis with a selective antibody showed that the enzyme fraction phosphorylated at Ser 66 during S phase is associated with the replication factories (Rossi *et al*., 1999). The phosphorylation status of LIGI at this site is also regulated in response to DNA damage, and the dispersal of the factories in response to damage is followed by dephosphorylation at this site (Montecucco *et al.*, 2001; Rossi *et al*., 2002). *In vitro*, LIGI is also a substrate for cyclin-dependent protein kinases (Prigent *et al.*, 1992; Koundrioukoff *et al*., 2000). The aforementioned colocalization of LIGI and Cdk2/cyclin A in a replication-competent multiprotein complex (Frouin *et al*., 2002) and at replication factories (Cardoso *et al*., 1993; Montecucco *et al.*, 1995) is consistent with an ability of Cdk2/cyclin A to modify LIGI *in vivo*. However, whether LIGI is indeed an *in vivo* substrate of Cdk2/cyclin A and what the biological relevance of this might be remains to be determined.

Cdk/cyclin complexes might have an additional role in the regulation of replication complex association with chromatin during the cell cycle (Frouin *et al*., 2002). As shown in Fig. 2, the DNA synthesome can exist in two states, a chromatin-free state (which may be predominant at the G1/S transition) and a chromatin-bound state (which may be enriched during S phase). Chromatin binding initially seems to be repressed by the association of Cdk2 with the complex. Before the onset of mitosis, however, the Cdk2/cyclin A complex is replaced with Cdk1/cyclin A and Cdk1/cyclin B complexes, which might repress DNA replication more efficiently. Indeed, Cdk1/cyclin B maintains the complex in an inactive state to prevent unscheduled re-replication of DNA.

The stable association of cyclin A with replication complexes during S phase in the absence of a Cdk indicates an alternative, Cdk-independent function for this protein, as has been suggested for the repression of transcription during S phase (Kim & Kaelin, 2001). This hypothesis supports the so-called Cdk-driven 'replication switch' model (Kelly & Brown, 2000), which predicts that cyclin/Cdk complexes function both to activate initiation complexes and to inhibit the initiation of the assembly of additional complexes.

The DNA synthesome and checkpoint control

Progression through the cell cycle is dependent on quality-control mechanisms, known as checkpoints, that monitor the correct execution of essential tasks (for example, the completion of DNA synthesis) before allowing the cell to proceed to the next phase of the cycle. The intra-S-phase (or S- phase-specific) checkpoint, in particular, ensures that mitosis occurs only after DNA replication is complete. Central to this particular checkpoint is a protein kinase, called Mec1 in yeast and ATR in higher eukaryotes (for a review, see Melo & Toczyski, 2002).

Two recent studies indicate that, in mammalian cells, the ordered assembly and disassembly of replication factories may be monitored

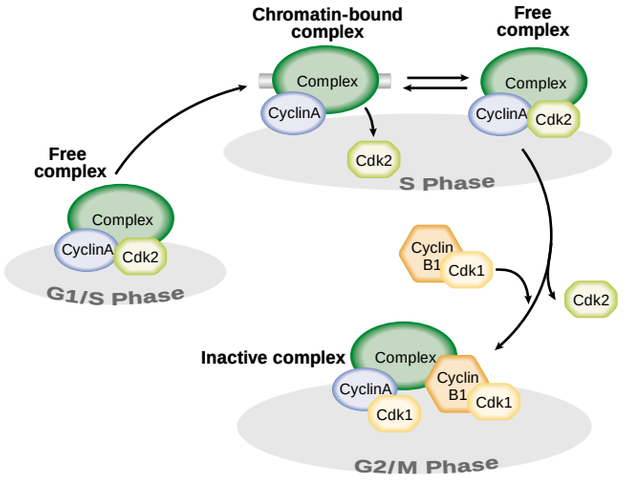

Fig. 2 | Dynamics of the DNA synthesome. Chromatin binding of the DNA synthesomes might be regulated by cyclin-dependent protein kinase (Cdk)/cyclin complexes. At the G1/S-phase transition (left side of panel), the DNA synthesomes are maintained in a free or chromatin-unbound state through their association with Cdk2/cyclin A complexes. In S phase (centre, top of panel), two kinds of DNA synthesome exist: the one that is not associated with the chromatin contains both cyclin A and Cdk2, and the one that is chromatin-associated contains cyclin A but not Cdk2. The equilibrium between these two forms is regulated by the association–dissociation dynamics of Cdk2 (as indicated by the black arrows). During the G2 phase (right lower side of panel), Cdk2 is replaced by Cdk1 and both cyclin B and cyclin A associate with the free complex. This prevents further association with chromatin, thus shifting the equilibrium towards the chromatin-unbound state. It is not yet known whether such an inactive complex is targeted for degradation during M phase or is re-used in the subsequent cell cycle.

by the intra-S-phase checkpoint pathway. In the first study, Dimitrova & Gilbert (2000) reported that in mammalian cells with stalled replication forks (as a result of treatment with aphidicolin), the addition of inhibitors of this checkpoint resulted in the initiation of replication at later-replicating chromosomal domains. Furthermore, they showed that this response involves the redistribution of PCNA and RPA from early to late replication factories in the absence of detectable DNA synthesis. Thus, checkpoint control is required for the maintenance of replication factory integrity and to allow time for the rescue of DNA replication at stalled forks. In the second study (Montecucco *et al.*, 2001), cells whose replication was arrested by treatment with a DNA-damaging agent (etoposide) were shown to rely on the same checkpoint for the disassembly of replication factories. Inhibition of the checkpoint pathway was found to abrogate the disassembly of replication factories that was otherwise induced by the damage.

Evidence that more directly links the components of the DNA synthesome with the checkpoint proteins comes from two sources. At the beginning of S phase, RNA primer synthesis by Pol-α/primase on the template strand seems to be required for the 'licensing' of the chromatin, which makes it competent to pass the checkpoint. In addition, the chromatin association, on which ATR relies for its control of the replication checkpoint, depends on RNA primer synthesis by Pol-α/primase (Hekmat-Nejad *et al.*, 2000). Recent studies in *Xenopus* have shown that the association on chromatin between ATR and Hus1 (Xhus1), another protein that is essential for the DNA replication checkpoint, is dependent on the presence of RPA and Pol-α/primase (You *et al.*, 2002). These results led to a model in which the DNA synthesome is recruited to the unwound origin after the initiation of DNA replication, and the presence of Pol-α/primase and RPA then promotes the association between ATR and Hus1. This renders the chromatin competent to initiate the checkpoint response. In this scenario, the DNA synthesome loaded at the fork has an active role in sensing and transducing signals from and to the checkpoint-control pathways during S phase.

Conclusion and perspectives

Eukaryotic DNA replication is a highly dynamic process that requires the coordinated and tightly regulated action of many molecular machines. Eukaryotic cells must assemble many protein complexes (referred to here as DNA synthesomes) at hundreds of origins of replication, and must activate them according to a strict temporal programme. The combination of genetic and biochemical approaches that have been used so far has begun to reveal the mechanisms that underlie these intricate networks of interactions, as well as the connections between the DNA synthesome and the cell cycle and checkpoint machineries. However, further work is required to delineate a precise map of the voyage of the DNA synthesome through the nucleus.

Supplementary information is available at *EMBO reports* online (www.emboreports.org).

ACKNOWLEDGEMENTS

The work in the authors' laboratory has been partially supported by the Consiglio Nazionale delle Ricerche (CNR) Target Project on Biotechnology (to S.S.), the CNR Agenzia 2000 (to S.S. and G.M.), the EU project No. QLK3-CT-202-02071-REPBIOTECH (to G.M.) and the Associazione Italiana per la Ricerca sul Cancro (to A.M.).

REFERENCES

Ayyagari, R., Gomes, X.V., Gordenin, D.A. & Burgers, P.M. (2003) Okazaki fragment maturation in yeast. I. Distribution of functions between FEN1 and DNA2. *J. Biol. Chem.*, **278**, 1618–1625.

Bae, S.H., Bae, K.H., Kim, J.A. & Seo, Y.S. (2001) RPA governs endonuclease switching during processing of Okazaki fragments in eukaryotes. *Nature,* **412**, 456–461.

Boldogh, I., Milligan, D., Lee, M.S., Bassett, H., Lloyd, R.S. & McCullough, A.K. (2001) hMYH cell cycle-dependent expression, subcellular localization and association with replication foci: evidence suggesting replication-coupled repair of adenine:8-oxoguanine mispairs. *Nucleic Acids Res.*, **29**, 2802–2809.

Cardoso, M.C., Leonhardt, H. & Nadal-Ginard, B. (1993) Reversal of terminal differentiation and control of DNA replication: cyclin A and cdk2 specifically localize at subnuclear sites of DNA replication. *Cell*, **74**, 979–992.

Chuang, L.S.-H., Ian, H.-I., Koh, T.-W., Ng, H.-H., Xu, G. & Li, B.F.L. (1997) Human DNA-(cytosine-5) methyltransferase–PCNA complex as a target for p21WAF1. *Science*, **277**, 1996–2000.

Cook, P.R. (1999) The organization of replication and transcription. *Science*, **284**, 1790–1795.

Dehde, S., Rohaly, G., Schub, O., Nasheuer, H.-P., Bohn, W., Chemnitz, J., Deppert, W. & Dornreiter, I. (2001) Two immunologically distinct human DNA polymerase α-primase subpopulations are involved in cellular DNA replication. *Mol. Cell. Biol.*, **21**, 2581–2593.

Dimitrova, D.S. & Gilbert, D.M. (2000) Temporally coordinated assembly and disassembly of replication factories in the absence of DNA synthesis. *Nature Cell Biol.*, **2**, 686–694.

Fotedar, R., Mossi, R., Fitzgerald, P., Rousselle, T., Maga, G., Brickner, H., Messier, H., Kasibhatla, S., Hübscher, U. & Fotedar, A. (1996) A conserved domain of the large subunit of replication factor C binds PCNA and acts like a dominant negative inhibitor of DNA replication in mammalian cells. EMBO J., 15, 4423–4433.

Frouin, I., Montecucco, A., Biamonti, G., Hubscher, U., Spadari, S. & Maga, G. (2002) Cell cycle-dependent dynamic association of cyclin/Cdk complexes with human DNA replication proteins. *EMBO J.*, **21**, 2485–2495.

Fuss, J. & Linn, S. (2002) Human DNA polymerase ε colocalizes with proliferating cell nuclear antigen and DNA replication late, but not early, in S phase. *J. Biol. Chem.*, **277**, 8658–8666.

Hassan, A., Errington, R., White, N., Jackson, D. & Cook, P. (1994) Replication and transcription sites are colocalized in human cells. *J. Cell Sci.*, **107**, 425–434.

Hekmat-Nejad, M., You, Z., Yee, M.C., Newport, J.W. & Cimprich, K.A. (2000) *Xenopus* ATR is a replication-dependent chromatin-binding protein required for the DNA replication checkpoint. *Curr. Biol.*, **10**, 1565–1573.

Hozak, P., Hassan, A.B., Jackson, D.A. & Cook, P.R. (1993) Visualization of replication factories attached to a nucleoskeleton. *Cell*, **73**, 361–373.

Jackson, D. & Pombo, A. (1998) Replicon clusters are stable units of chromosome structure: evidence that nuclear organization contributes to the efficient activation and propagation of S phase in human cells. *J. Cell Biol.*, **140**, 1285–1295.

Jin, Y.H., Ayyagari, R., Resnick, M.A., Gordenin, D.A. & Burgers, P.M. (2003) Okazaki fragment maturation in yeast. II. Cooperation between the polymerase and 3′–5′-exonuclease activities of Pol δ in the creation of a ligatable nick. *J. Biol. Chem.*, **278**, 1626–1633.

Kannouche, P., Fernandez de Henestrosa, A.R., Coull, B., Vidal, A.E., Gray, C., Zicha, D., Woodgate, R. & Lehmann, A.R. (2002) Localization of DNA polymerases η and ι to the replication machinery is tightly co-ordinated in human cells. *EMBO J.*, **21**, 6246–6256.

Kelly, T.J. & Brown, G.W. (2000) Regulation of chromosome replication. *Annu. Rev. Biochem.*, **69**, 829–880.

Kim, T.Y. & Kaelin, W.G. Jr (2001) Differential control of transcription by DNA-bound cyclins. *Mol. Biol. Cell*, **12**, 2207–2217.

Kleczkowska, H.E., Marra, G., Lettieri, T. & Jiricny, J. (2001) hMSH3 and hMSH6 interact with PCNA and colocalize with it to replication foci. *Genes Dev.*, **15**, 724–736.

Koundrioukoff, S., Jonsson, Z.O., Hasan, S., de Jong, R.N., van der Vliet, P.C., Hottiger, M.O. & Hubscher, U. (2000) A direct interaction between proliferating cell nuclear antigen (PCNA) and Cdk2 targets PCNA-interacting proteins for phosphorylation. *J. Biol. Chem.*, **275**, 22882–22887.

Krude, T. (1995) Chromatin assembly factor 1 (CAF-1) colocalizes with replication foci in HeLa cell nuclei. *Exp. Cell Res.*, **220**, 304–311.

Leonhardt, H., Page, A.W., Weier, H.U. & Bestor, T.H. (1992) A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei. *Cell*, **71**, 865–873.

Leonhardt, H., Rahn, H.-P., Weinzierl, P., Sporbert, A., Cremer, T., Zink, D. & Cardoso, M.C. (2000) Dynamics of DNA replication factories in living cells. *J. Cell Biol.*, **149**, 271–279.

Levin, D., McKenna, A., Motycka, T., Matsumoto, Y. & Tomkinson, A. (2000) Interaction between PCNA and DNA ligase I is critical for joining of Okazaki fragments and long-patch base-excision repair. *Curr. Biol.*, **10**, 919–922.

Ma, H., Samarabandu, J., Devdhar, R.S., Acharya, R., Cheng, P., Meng, C. & Berezney, R. (1998) Spatial and temporal dynamics of DNA replication sites in mammalian cells. *J. Cell Biol.*, **143**, 1415–1425.

Maga, G. & Hübscher, U. (1996) DNA replication machinery: functional characterization of a complex containing DNA polymerase α, DNA polymerase δ, and replication factor C suggests an asymmetric DNA polymerase dimer. *Biochemistry*, **35**, 5764–5777.

Maga, G., Stucki, M., Spadari, S. & Hübscher, U. (2000) DNA polymerase switching: I. Replication factor C displaces DNA polymerase α prior to PCNA loading. *J. Mol. Biol.*, **295**, 791–801.

Maga, G., Villani, G., Tillement, V., Stucki, M., Locatelli, G.A., Frouin, I., Spadari, S. & Hübscher, U. (2001) Okazaki fragment processing: modulation of the strand displacement activity of DNA polymerase δ by the concerted action of replication protein A, proliferating cell nuclear antigen and flap endonuclease-1. *Proc. Natl Acad. Sci. USA*, **4**, 14298–14303.

Malkas, L.H. (1998) DNA replication machinery of the mammalian cell. *J. Cell Biochem.*, **30–31** (Suppl.), 18–29.

Maser, R.S., Mirzoeva, O.K., Wells, J., Olivares, H., Williams, B.R., Zinkel, R.A., Farnham, P.J. & Petrini, J.H. (2001) Mre11 complex and DNA replication: linkage to E2F and sites of DNA synthesis. *Mol. Cell. Biol.*, **21**, 6006–6016.

Melo, J. & Toczyski, D. (2002) A unified view of the DNA-damage checkpoint. *Curr. Opin. Cell Biol.*, **14**, 237–245.

Montecucco, A., Savini, E., Weighardt, F., Rossi, R., Ciarrocchi, G., Villa, A. & Biamonti, G. (1995) The N-terminal domain of human DNA ligase I contains the nuclear localisation signal and directs the enzyme to sites of DNA replication. *EMBO J.*, **14**, 5379–5386.

Montecucco, A., Rossi, R., Levin, D.S., Gary, R., Park, M.S., Motycka, T.A., Ciarrocchi, G., Villa, A., Biamonti, G. & Tomkinson, A.E. (1998) DNA ligase I is recruited to sites of DNA replication by an interaction with proliferating cell nuclear antigen: identification of a common targeting mechanism for the assembly of replication factories. *EMBO J.*, **17**, 3786–3795.

Montecucco, A., Rossi, R., Ferrari, G., Scovassi, A.I., Prosperi, E. & Biamonti, G. (2001) Etoposide induces the dispersal of DNA ligase I from replication factories. *Mol. Biol. Cell*, **12**, 2109–2118.

Nakayasu, H. & Berezney, R. (1989) Mapping replicational sites in the eucaryotic cell nucleus. *J. Cell Biol.*, **108**, 1–11.

Niimi, A., Suka, N., Harata, M., Kikuchi, A. & Mizuno, S. (2001) Co-localization of chicken DNA topoisomerase IIα, but not β, with sites of DNA replication and possible involvement of a C-terminal region of α through its binding to PCNA. *Chromosoma*, **110**, 102–114.

O'Keefe, R.T., Henderson, S.C. & Spector, D.L. (1992) Dynamic organization of DNA replication in mammalian cell nuclei: spatially and temporally defined replication of chromosome-specific α-satellite DNA sequences. *J. Cell Biol.*, **116**, 1095–1110.

Ott, R., Rehfuess, C., Podust, V., Clark, J. & Fanning, E. (2002) Role of the p68 subunit of human DNA polymerase α-primase in simian virus 40 DNA replication. *Mol. Cell. Biol.*, **22**, 5669–5678.

Otterlei, M., Warbrick, E., Nagelhus, T.A., Haug, T., Slupphaug, G., Akbari, M., Aas, P.A., Steinsbekk, R., Bakke, O. & Krokan, H.E. (1999) Post-replicative base excision repair in replication foci. *EMBO J.*, **18**, 3834–3844.

Prigent, C., Lasko, D.D., Kodama, K., Woodgett, J.R. & Lindahl, T. (1992) Activation of mammalian DNA ligase I through phosphorylation by casein kinase II. *EMBO J.*, **11**, 2925–2933.

Rossi, R., Villa, A., Negri, C., Scovassi, I., Ciarrocchi, G., Biamonti, G. & Montecucco, A. (1999) The replication factory targeting sequence/PCNA-binding site is required in G(1) to control the phosphorylation status of DNA ligase I. *EMBO J.*, **18**, 5745–5754.

Rossi, R., Montecucco, A., Donzelli, M., Denegri, M., Biamonti, G. & Scovassi, A.I. (2002) DNA ligase I is dephosphorylated during the execution step of etoposide-induced apoptosis. *Cell Death Differ.*, **9**, 89–90.

Rountree, M.R., Bachman, K.E. & Baylin, S.B. (2000) DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. *Nature Genet.*, **25**, 269–277.

Sporbert, A., Gahl, A., Ankerhold, R., Leonhardt, H. & Cardoso, M. (2002) DNA polymerase clamp shows little turnover at established replication sites but sequential *de novo* assembly at adjacent origin cluster. *Mol. Cell*, **10**, 1355–1365.

Tom, S., Henricksen, L., Park, M. & Bambara, R. (2001) DNA ligase I and proliferating cell nuclear antigen form a functional complex. *J. Biol. Chem.*, **276**, 24817–24825.

Tubo, R. & Berezney, R. (1987) Identification of 100 and 150 S DNA polymerase α-primase megacomplexes solubilized from the nuclear matrix of regenerating rat liver. *J. Biol. Chem.*, **262**, 5857–5865.

Uhlmann, F., Cai, J., Gibbs, E., O'Donnel, M. & Hurwitz, J. (1997) Deletion analysis of the large subunit p140 in human replication factor C reveals regions required for complex formation and replication activities. *J. Biol. Chem.*, **272**, 10058–10064.

Voitenleitner, C., Rehfuess, C., Hilmes, M., O'Rear, L., Liao, P.-C., Gage, D.A., Ott, R., Nasheuer, H.-P. & Fanning, E. (1999) Cell cycle-dependent regulation of human DNA polymerase α-primase activity by phosphorylation. *Mol. Cell. Biol.*, **19**, 646–656.

Waga, S. & Stillman, B. (1994) Anatomy of a DNA replication fork revealed by reconstitution of SV40 DNA replication *in vitro*. *Nature*, **369**, 207–212.

You, Z., Kong, L. & Newport, J. (2002) The role of single-stranded DNA and polymerase α in establishing the ATR, Hus1 DNA replication checkpoint. *J. Biol. Chem.*, **277**, 27088–27093.

Yuzhakov, A., Kelman, Z., Hurwitz, J. & O'Donnell, M. (1999) Multiple competition reactions for RPA order the assembly of the DNA polymerase δ holoenzyme. *EMBO J.*, **18**, 6189–6199.