Multiple roles for kinases in DNA replication

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DNA replication is carried out by the replisome, which includes several proteins that are targets of cell-cycle-regulated kinases. The phosphorylation of proteins such as replication protein A, DNA polymerase- α and - δ , replication factor C, flap endonuclease 1 and DNA ligase I leads to their inactivation, suggesting that phosphorylation is important in the prevention of re-replication. Moreover, the phosphorylation of several of these replication proteins has been shown to block their association with the 'moving platform'—proliferating cell nuclear antigen. Therefore, phosphorylation seems to be a crucial regulator of replisome assembly and DNA replication, although its precise role in these processes remains to be clarified.

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Introduction

DNA replication guarantees the duplication of the genome and requires the concerted action of many enzymes (Bell & Dutta, 2002). This process is initiated by the assembly of the origin-recognition complex (ORC) at replication origins. The ORC recruits the pre-replicative complex (cell division cycle 6 (Cdc6), Cdc10-dependent transcript 1 (Cdt1) and the minichromosome maintenance (Mcm) proteins) to replication origins during the G1 phase. This complex is activated by cyclin-dependent kinases (Cdks) and the cell division cycle 7–dumb bell former 4 (Cdc7–Dbf4) complex in S phase to promote the initiation of DNA replication. For example, the helicase activity of the Mcm subcomplex unwinds the DNA, which in turn attracts replication protein A (RPA). RPA stabilizes the single-stranded (ss)DNA. After DNA polymerase- α (Pol- α)-dependent synthesis of an RNA/DNA primer on the ssDNA (Fig. 1, step 1), replication factor C (RFC) induces a DNA-polymerase switch, from Pol- α to Pol- δ . To accomplish this, RFC binds to the primer and loads proliferating cell nuclear antigen (PCNA) (Fig. 1, step 2) onto the DNA, which, in turn, recruits Pol- δ (Fig. 1, step 3). Whereas the Pol- δ holoenzyme (comprising PCNA, RFC and Pol- δ) carries out processive DNA synthesis on the leading strand, it performs discontinuous assembly on the lagging strand. The latter process involves first extending the RNA/DNA primers that are

produced by Pol- α to create Okazaki fragments. When Pol- δ encounters the previous RNA/DNA primer during the elongation step of the lagging strand, it performs strand displacement to release the primer (Fig. 1, step 4). Next, PCNA binds flap endonuclease 1 (Fen1), which then cuts the displaced RNA/DNA (Fig. 1, step 5), possibly in conjunction with the endonuclease Dna2. Finally, PCNA recruits DNA ligase I (LigI) to seal the DNA strand (Fig. 1, step 6). Because the length of an Okazaki fragment is less than 200 bases, this process occurs more than 2×10^7 times per round of replication in the human genome.

Many of the eukaryotic DNA replication proteins have been shown to be targets for phosphorylation. In this review, we summarize the progress in understanding the role of this modification in regulating the activities of these proteins.

Functional consequences of phosphorylation

The activation of pre-replicative complexes (consisting of the ORC, Cdc6, Cdt1 and Mcm2–Mcm7) at origins of DNA replication at the beginning of S phase occurs due to the action of many Cdks. It is also aided by the Cdc7–Dbf4 complex, although the extent of involvement of this complex is not well understood. However, as Cdks have been implicated not only in the initiation of DNA replication, but also in the prevention of re-replication of the genome (Bell & Dutta, 2002), the phosphorylation of Cdk targets seems to have both positive and negative effects on DNA replication (Jallepalli & Kelly, 1997).

Consistent with the dual role of phosphorylation in the control of DNA replication, the phosphorylation of replication proteins is not restricted to the initiation step. The ssDNA binding protein RPA, which is composed of three subunits (RPA1, RPA2 and RPA3), is phosphorylated twice during the cell cycle. The first time is during S phase, at which point RPA2 (Din *et al.*, 1990), specifically, is phosphorylated by the Cdk2 kinase (Dutta & Stillman, 1992), while it is bound to ssDNA (Fotedar & Roberts, 1992). This event has been shown to result in the inhibition of simian virus 40 (SV40) DNA replication *in vitro* (Pan *et al.*, 1995). RPA2 is also phosphorylated by Cdk1–cyclin A and Cdk1–cyclin B in late S phase, which leads to dissociation of the RPA trimer (Treuner *et al.*, 1999). Surprisingly, mutations that prevent phosphorylation of RPA2 have no effect on SV40 DNA replication (Henricksen *et al.*, 1994), although a truncated RPA2 that lacks the Cdk phosphorylation site has a cell proliferation phenotype in yeast (Philipova *et al.*, 1996).

The p180 and p68 subunits of the heterotetrameric Pol- α have also been reported to be phosphorylated at different stages of the cell cycle in many organisms, including humans (Nasheuer *et al.*, 1991). The p68 subunit is phosphorylated during S phase in *Saccharomyces cerevisiae* (Foiani *et al.*, 1995), and the p180 subunit during late

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S phase in *Schizosaccharomyces pombe* (Park *et al.*, 1995). Both subunits also seem to be modified at the G2–M phase transition by Cdc2 (Voitenleitner *et al.*, 1997). SV40 DNA-replication experiments showed that Pol- α is stimulated on Cdk2–cyclin-E-mediated phosphorylation of both p180 and p68, and showed that, in contrast, Cdk2–cyclin A and Cdk1–cyclin A each have an inhibitory effect on both subunits (Voitenleitner *et al.*, 1999). Nevertheless, in non-viral systems, phosphorylation of p68 by Cdk2–cyclin A stimulates Pol- α activity *per se*, whereas phosphorylation of p180 inhibits the Pol- α initiation activity by blocking any interaction with the large T antigen (Schub *et al.*, 2001). In summary, the control of Pol- α might occur as follows: first, before the cell enters S phase, the 180-kDa subunit of Pol- α is phosphorylated by Cdc7–Dbf4, an enzyme complex that is important for the initiation of DNA replication (Jares *et al.*, 2000). Second, at the G1–S phase transition, the 180-kDa subunit is phosphorylated by Cdk2–cyclin E. Third, Cdk2–cyclin E also stimulates the initiation of replication during early S phase, and Cdk2–cyclin A kinase competes with protein phosphatase 2A, which until this point ensures that the p68 subunit remains inactive (Schub *et al.*, 2001). This results in an overall increase in phosphorylation and activation of the p68 subunit. Finally, later in S phase, Cdk2–cyclin A and Cdk1–cyclin A phosphorylate p180 to prevent re-initiation.

RFC induction of the polymerase switch, an ATP-dependent process that occurs after the RNA/DNA primer is synthesized, is another event that requires phosphorylation. Maga *et al.* (1997) have shown that the calmodulin-dependent protein kinase II (CaMKII) phosphorylates the PCNA binding region of the large RFC subunit

p140 *in vitro*, thus blocking the PCNA–RFC interaction. CaMKII activity is required for the G1–S phase transition of the cell cycle, and therefore might be involved in signalling downstream of Cdk activation (Morris *et al.*, 1998; Rasmussen & Rasmussen, 1995). Furthermore, the RFC subunit p140 may be phosphorylated by Cdk1 (Koundrioukoff *et al.*, 2000), as also suggested by the recent discovery of a replication complex that includes RFC, cyclin A, cyclin B1, Cdk1 and Cdk2 (Frouin *et al.*, 2002).

The recruitment of heterotetrameric Pol- δ on PCNA loading onto DNA may also require phosphorylation. The large subunit of Pol- δ (p125) is known to be phosphorylated during the cell cycle, preferentially during the S phase (Zeng *et al.*, 1994). Although *in vitro* studies have failed to show a difference in activity when this catalytic subunit is phosphorylated by Cdk2–cyclin kinases, recent data suggest that the p60 subunit is phosphorylated *in vivo*, as well as being a substrate for Cdk2–cyclin E, Cdk2–cyclin A and Cdk1–cyclin A *in vitro* (Ducoux *et al.*, 2001). Furthermore, phosphorylation might have an effect on the interaction between PCNA and the Pol- δ holoenzyme (Frouin *et al.*, 2002).

Several findings have also suggested a regulatory role for Cdks in the recruitment of the endonucleases Dna2 and Fen1 during the maturation of Okazaki fragments on the lagging strand. For example, yeast Dna2 is phosphorylated by Cdc7–Dbf4 *in vitro* (Y.S. Seo, personal communication), and it has been postulated that the cyclin-A-dependent kinase might regulate the function of Fen1 (G.H., S.K. & U.H., unpublished data). Also, phosphorylation of Fen1 by Cdk2–cyclin A and Cdk1–cyclin A seems to reduce its

Fig. 1 | Eukaryotic DNA replication. Step 1: primer synthesis by DNA polymerase- α (Pol- α); step 2: replication factor C (RFC) displacement of DNA polymerase and recruitment of proliferating cell nuclear antigen (PCNA); step 3: elongation by the newly recruited DNA polymerase- δ holoenzyme (Pol- δ); step 4: strand displacement by Pol- δ ; step 5: cutting of the 5' displaced flap by flap endonuclease 1 (Fen1); and step 6: sealing by DNA ligase I (LigI). For details, see main text.

Table 1 | Phosphorylation of replication proteins

Protein	Subunit	Kinase	Effect on DNA synthesis
RPA	p34	Cdk2–cyclin A Cdk1–cyclin A DNA-PK	Inhibition; dissociation of the RPA complex
Pol- α	p180	Cdk2–cyclin E	Stimulation
	p68	Cdk2–cyclin A and Cdk1–cyclin A	Inhibition
RFC	p68	Cdc7–Dbf4	Unknown
	p140	CamkII	Inhibition; PCNA binding blocked
Pol- δ	p140	Cdks	Unknown
	p125 p66	Cdk2–cyclins Cdk2–cyclin E Cdk2–cyclin A and Cdk1–cyclin A	Unknown PCNA binding blocked?
Fen1		Cdk2–cyclin E Cdk2–cyclin A and Cdk1–cyclin A	No effect Inhibition; downregulation of endonuclease activity and PCNA binding blocked
LigI		CkII Cdks	Downregulation Unknown
Dna2		Cdc7–Dbf4	Stimulation of its nuclease activity ¹

¹Y.S. Seo, personal communication. CaMKII, calmodulin-dependent kinase II; Cdc7, cell division cycle 7; Cdk1/2, cyclin-dependent kinase 1/2; CKII, casein kinase II; Dbf4, dumb bell former 4; Dna2, an endonuclear protein; DNA-PK, DNA-dependent serine/threonine protein kinase; Fen1, flap endonuclease 1; LigI, ligase I; PCNA, proliferating cell nuclear antigen; Pol- α/δ , DNA polymerase- α/δ ; RFC, replication factor C; RPA, replication protein A.

endo- and exonuclease activities and prevents its PCNA binding (G.H., S.K. & U.H., unpublished data). These findings suggest that phosphorylation first functions to inactivate Fen1 and subsequently blocks its PCNA binding ability. It has also been proposed that phosphorylation regulates LigI activity, which is required to seal the nicks left by the endonuclease (Bell & Dutta, 2002). *In vitro* studies have shown that LigI is phosphorylated by casein kinase II (CKII; Rossi *et al.*, 1999), a ubiquitous serine/threonine kinase (Allende & Allende, 1995) that is thought to be involved in a Cdk pathway, at the end of S phase (Russo *et al.*, 1992). Moreover, LigI is a substrate for Cdks *in vitro* (Koundrioukoff *et al.*, 2000), and PCNA recruitment of LigI to replication factories—the nuclear structures that contain the replication machinery (Montecucco *et al.*, 1998)—has also been found to be cell-cycle regulated, with the PCNA interaction with LigI being blocked after S phase (Rossi *et al.*, 1999). It is possible that LigI phosphorylation by Cdks and CKII prevents its interaction with PCNA, resulting in the release of LigI from the replication machinery.

Synopsis of Cdk activity in the cell cycle

As is the case for its well-described control over cell-cycle progression, the Cdk protein family has a central role in regulating DNA replication through the successive activation of different family members (Nigg, 1995). The activation of different Cdks through the G1–G2 phases of the cell cycle proceeds as follows: complexes of D-type cyclins and Cdk4–Cdk6 are required for G1 progression; the association of Cdk2 and cyclin E is required for

the G1–S phase transition; the activity of the Cdk2–cyclin A complex is needed for progression through S phase; and finally, A- and B-type cyclins associate with Cdk1 to promote the start of mitosis. Throughout this progression, Cdks are required for replication initiation (Bell & Dutta, 2002), as well as being key enzymes in DNA synthesis. Table 1 gives a summary of Cdk substrates and the biological consequences of their phosphorylation. Further evidence that Cdks are involved in replication is that Cdk2–cyclin A is localized to replication foci (Cardoso *et al.*, 1993), and that cyclin A, cyclin B and Cdk2, together with Cdk1, have been found in replication complexes (Fotedar & Roberts, 1991; Frouin *et al.*, 2002; Jaumot *et al.*, 1994). Other kinases, such as CaMKII and CKII, phosphorylate RFC and LigI (Maga *et al.*, 1997; Prigent *et al.*, 1992; Rossi *et al.*, 1999), respectively, and each of these kinases has been shown to be involved in a pathway that includes Cdks. CaMKII acts after the Cdks are activated in G1, and its inhibition does not lead to Cdk inactivation (Rasmussen & Rasmussen, 1995). CKII is phosphorylated by Cdk1 (Litchfield *et al.*, 1992) and, although it can also phosphorylate Cdk1 (Russo *et al.*, 1992), most of the data suggest that the Cdks probably act upstream of CKII and CaMKII.

Phosphorylation, at certain times, activates some replication proteins. However, the following data make a case for the idea that phosphorylation also functions to inhibit the activity of replication proteins. First, the RPA complex dissociates after phosphorylation late in the cell cycle (Treuner *et al.*, 1999). Second, Pol- α is inhibited by the phosphorylation of its p180 subunit during the later stages of the cell cycle (Schub *et al.*, 2001). Third, the interactions between PCNA and RFC (Maga *et al.*, 1997), Fen1 (G.H., S.K. & U.H., unpublished data), LigI (Rossi *et al.*, 1999), and possibly Pol- δ (Ducoux *et al.*, 2001), are blocked after each of these PCNA-binding proteins is phosphorylated. Therefore, phosphorylation might control DNA replication as follows (Fig. 2): Cdks first inhibit Pol- α activity on DNA; CaMKII and Cdks act in concert to dissociate the Pol- δ holoenzyme originally loaded onto the DNA by RFC–PCNA; CKII and the Cdks disrupt the interactions between PCNA and Fen1 and LigI, ending the synthesis of the lagging strand; and finally, RPA phosphorylation causes its dissociation from the ssDNA. This makes sense because when replication is complete, no ssDNA remains and the presence of RPA is not required. This suggests that the consequences of phosphorylation events that have a negative impact on replication could have a common basis: the disruption of interactions between other replication proteins and PCNA. This idea is appealing as, although PCNA itself does not seem to be phosphorylated, it exists in a complex *in vivo* with Cdks (Xiong *et al.*, 1992) and also with the phosphatase regulator Cdc25C (Kawabe *et al.*, 2002). The ‘moving platform’ PCNA might therefore function as a partner to kinases and Cdc25C in the regulation of DNA replication by controlling the phosphorylation state of incoming target proteins. The localization of replication proteins to replication factories is a dynamic process (Dimitrova & Gilbert, 2000; Leonhardt *et al.*, 2000; Montecucco *et al.*, 2001), and Cdk–cyclin complexes may co-ordinate the recruitment of replication proteins to the fork while they are associated with PCNA. Furthermore, RPA and Pol- α are not localized to chromatin when a mitotic Cdk is active (Adachi & Laemmli, 1994; Desdouets *et al.*, 1998), illustrating again that kinases probably control the organization of the replication fork.

Fig. 2 | Overview of cell-cycle-regulated kinases that phosphorylate the DNA replication machinery during S phase. Different cyclin-dependent kinase (Cdk)-cyclin combinations, DNA-dependent serine/threonine protein kinase (DNA-PK), cell division cycle 7-dumb bell former 4 (Cdc7-Dbf4), calmodulin-dependent kinase II (CaMKII) and casein kinase II (CKII) target different DNA replication proteins, such as replication protein A (RPA), DNA polymerase- α (Pol- α), replication factor C (RFC), DNA polymerase- δ (Pol- δ), flap endonuclease 1 (Fen1) and DNA ligase I (LigI) for phosphorylation (arrows). Different Cdk-cyclin combinations, CaMKII and CKII are involved in the same pathway (double arrow), although their precise relationships in this pathway remain unclear, as denoted by the broken arrows. Phosphorylation blocks the interactions between DNA replication proteins and PCNA (marked with a red X).

The multifunctional protein RPA may be an example of a typical component of a complex phosphorylation pathway. The direct and/or indirect activity of Cdk2-cyclin A leads to RPA2 phosphorylation. This process is carried out by a DNA-dependent serine/threonine protein kinase (DNAPK) (Fotedar & Roberts, 1992) that is activated when it is bound to DNA, and is involved in DNA repair and recombination (Smith & Jackson, 1999). The combined kinase activities of Cdk2 and DNAPK lead to hyperphosphorylation of RPA2 and to dissociation of the RPA heterotrimer (Treuner *et al.*, 1999). The fact that phosphorylation of RPA2 occurs even in cells with defective checkpoints, such as those from patients with ataxia telangiectasia, suggests that this is a late response to DNA damage. A plausible model for the mechanism of action of DNAPK might be that its hyperphosphorylation of RPA2 helps to redirect RPA towards DNA repair. Finally, RPA has also been implicated in homologous recombination (McIlwraith *et al.*, 2000), which is a DNA-repair pathway that removes double-stranded breaks. This last example clearly shows that phosphorylation of RPA is involved in the quality control of DNA, as well as in the regulation of both the cell cycle and DNA replication.

Future directions

The regulation of DNA replication by cell-cycle-regulated kinases has been established clearly, and the list of new kinases and phosphorylation-targeted replication proteins continues to grow. The task ahead is to define the functional consequences of phosphorylation during DNA replication. Novel methods that are developed

for functional genomics will help to dissect this extremely complex network of regulation and to more accurately define the protein complexes that are involved in DNA replication (such as for RPA, as discussed above). Once the pieces of this puzzle are assembled, the next goal will be to determine how and when each phosphorylation modification occurs during the cell cycle. This is especially important if the control of phosphorylation is considered to be part of the framework of checkpoint control mechanisms (Norbury & Hickson, 2001), and will lead to an understanding of how DNA lesions activate the DNA damage response proteins through phosphorylation.

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REFERENCES

- Adachi, Y. & Laemmli, U.K. (1994) Study of the cell cycle-dependent assembly of the DNA pre-replication centres in *Xenopus* egg extracts. *EMBO J.*, **13**, 4153–4164.
- Allende, J.E. & Allende, C.C. (1995) Protein kinases. 4. Protein kinase CK2: an enzyme with multiple substrates and a puzzling regulation. *FASEB J.*, **9**, 313–323.
- Bell, S.P. & Dutta, A. (2002) DNA replication in eukaryotic cells. *Annu. Rev. Biochem.*, **71**, 333–374.
- 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.

- Desdouets, C., Santocanale, C., Drury, L.S., Perkins, G., Foiani, M., Plevani, P. & Diffley, J.F. (1998) Evidence for a Cdc6p-independent mitotic resetting event involving DNA polymerase α . *EMBO J.*, **17**, 4139–4146.
- 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.
- Din, S., Brill, S.J., Fairman, M.P. & Stillman, B. (1990) Cell-cycle-regulated phosphorylation of DNA replication factor A from human and yeast cells. *Genes Dev.*, **4**, 968–977.
- Ducoux, M., Urbach, S., Baldacci, G., Hubscher, U., Koundrioukoff, S., Christensen, J. & Hughes, P. (2001) Mediation of proliferating cell nuclear antigen (PCNA)-dependent DNA replication through a conserved p21(Cip1)-like PCNA-binding motif present in the third subunit of human DNA polymerase δ . *J. Biol. Chem.*, **276**, 49258–49266.
- Dutta, A. & Stillman, B. (1992) Cdc2 family kinases phosphorylate a human cell DNA replication factor, RPA, and activate DNA replication. *EMBO J.*, **11**, 2189–2199.
- Foiani, M., Liberi, G., Lucchini, G. & Plevani, P. (1995) Cell cycle-dependent phosphorylation and dephosphorylation of the yeast DNA polymerase α -primase B subunit. *Mol. Cell. Biol.*, **15**, 883–891.
- Fotedar, R. & Roberts, J.M. (1991) Association of p34cdc2 with replicating DNA. *Cold Spring Harb. Symp. Quant. Biol.*, **56**, 325–333.
- Fotedar, R. & Roberts, J.M. (1992) Cell cycle regulated phosphorylation of RPA-32 occurs within the replication initiation complex. *EMBO J.*, **11**, 2177–2187.
- Frouin, I., Montecucco, A., Biamonti, G., Hübscher, 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.
- Henricksen, L.A., Umbricht, C.B. & Wold, M.S. (1994) Recombinant replication protein A: expression, complex formation, and functional characterization. *J. Biol. Chem.*, **269**, 11121–11132.
- Jallepalli, P.V. & Kelly, T.J. (1997) Cyclin-dependent kinase and initiation at eukaryotic origins: a replication switch? *Curr. Opin. Cell Biol.*, **9**, 358–363.
- Jares, P., Donaldson, A. & Blow, J.J. (2000) The Cdc7/Dbf4 protein kinase: target of the S phase checkpoint? *EMBO Rep.*, **1**, 319–322.
- Jaumot, M., Grana, X., Giordano, A., Reddy, P.V., Agell, N. & Bachs, O. (1994) Cyclin/cdk2 complexes in the nucleus of HeLa cells. *Biochem. Biophys. Res. Commun.*, **203**, 1527–1534.
- Kawabe, T., Suganuma, M., Ando, T., Kimura, M., Hori, H. & Okamoto, T. (2002) Cdc25C interacts with PCNA at G2/M transition. *Oncogene*, **21**, 1717–1726.
- Koundrioukoff, S., Jonsson, Z.O., Hasan, S., de Jong, R.N., van der Vliet, P.C., Hottiger, M.O. & Hübscher, 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.
- 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**, 271–280.
- Litchfield, D.W., Luscher, B., Lozeman, F.J., Eisenman, R.N. & Krebs, E.G. (1992) Phosphorylation of casein kinase II by p34cdc2 *in vitro* and at mitosis. *J. Biol. Chem.*, **267**, 13943–13951.
- Maga, G., Mossi, R., Fischer, R., Berchtold, M.W. & Hübscher, U. (1997) Phosphorylation of the PCNA binding domain of the large subunit of replication factor C by Ca^{2+} /calmodulin-dependent protein kinase II inhibits DNA synthesis. *Biochemistry*, **36**, 5300–5310.
- McIlwraith, M.J., Van Dyck, E., Masson, J.Y., Stasiak, A.Z., Stasiak, A. & West, S.C. (2000) Reconstitution of the strand invasion step of double-strand break repair using human Rad51 Rad52 and RPA proteins. *J. Mol. Biol.*, **304**, 151–164.
- 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.
- Morris, T.A., DeLorenzo, R. J. & Tombes, R. M. (1998) CaMK-II inhibition reduces cyclin D1 levels and enhances the association of p27kip1 with Cdk2 to cause G1 arrest in NIH 3T3 cells. *Exp. Cell Res.*, **240**, 218–227.
- Nasheuer, H.P., Moore, A., Wahl, A.F. & Wang, T.S. (1991) Cell cycle-dependent phosphorylation of human DNA polymerase α . *J. Biol. Chem.*, **266**, 7893–7903.
- Nigg, E.A. (1995) Cyclin-dependent protein kinases: key regulators of the eukaryotic cell cycle. *Bioessays*, **17**, 471–480.
- Norbury, C.J. & Hickson, I.D. (2001) Cellular responses to DNA damage. *Annu. Rev. Pharmacol. Toxicol.*, **41**, 367–401.
- Pan, Z.Q., Park, C.H., Amin, A.A., Hurwitz, J. & Sancar, A. (1995) Phosphorylated and unphosphorylated forms of human single-stranded DNA-binding protein are equally active in simian virus 40 DNA replication and in nucleotide excision repair. *Proc. Natl Acad. Sci. USA*, **92**, 4636–4640.
- Park, H., Davis, R. & Wang, T.S. (1995) Studies of *Schizosaccharomyces pombe* DNA polymerase α at different stages of the cell cycle. *Nucleic Acids Res.*, **23**, 4337–4344.
- Philipova, D., Mullen, J.R., Maniar, H.S., Lu, J., Gu, C. & Brill, S.J. (1996) A hierarchy of SSB protomers in replication protein A. *Genes Dev.*, **10**, 2222–2233.
- 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.
- Rasmussen, G. & Rasmussen, C. (1995) Calmodulin-dependent protein kinase II is required for G1/S progression in HeLa cells. *Biochem. Cell Biol.*, **73**, 201–207.
- 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.
- Russo, G.L., Vandenberg, M.T., Yu, I.J., Bae, Y.S., Franza, B.R. Jr & Marshak, D.R. (1992) Casein kinase II phosphorylates p34cdc2 kinase in G1 phase of the HeLa cell division cycle. *J. Biol. Chem.*, **267**, 20317–20325.
- Schub, O., Rohaly, G., Smith, R.W., Schneider, A., Dehde, S., Dornreiter, I. & Nasheuer, H.P. (2001) Multiple phosphorylation sites of DNA polymerase α -primase cooperate to regulate the initiation of DNA replication *in vitro*. *J. Biol. Chem.*, **276**, 38076–38083.
- Smith, G.C. & Jackson, S.P. (1999) The DNA-dependent protein kinase. *Genes Dev.*, **13**, 916–934.
- Treuner, K., Findeisen, M., Strausfeld, U. & Knippers, R. (1999) Phosphorylation of replication protein A middle subunit (RPA32) leads to a disassembly of the RPA heterotrimer. *J. Biol. Chem.*, **274**, 15556–15561.
- Voitenleitner, C., Fanning, E. & Nasheuer, H.P. (1997) Phosphorylation of DNA polymerase α -primase by cyclin A-dependent kinases regulates initiation of DNA replication *in vitro*. *Oncogene*, **14**, 1611–1615.
- 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.
- Xiong, Y., Zhang, H. & Beach, D. (1992) D type cyclins associate with multiple protein kinases and the DNA replication and repair factor PCNA. *Cell*, **71**, 505–514.
- Zeng, X.R., Hao, H., Jiang, Y. & Lee, M.Y. (1994) Regulation of human DNA polymerase δ during the cell cycle. *J. Biol. Chem.*, **269**, 24027–24033.

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