Phosphorylation of human DNA polymerase \( \lambda \) by the cyclin-dependent kinase Cdk2/cyclin A complex is modulated by its association with proliferating cell nuclear antigen

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ABSTRACT

DNA polymerase (Pol) \( \lambda \) is a member of the Pol X family and possesses four different enzymatic activities, being DNA polymerase, terminal transferase, deoxyribose phosphate lyase and polynucleotide synthetase, all localized in its C-terminal region. On the basis of its biochemical properties, Pol \( \lambda \) has been implicated in various DNA repair pathways, such as abasic site translesion DNA synthesis, base excision repair and non-homologous end joining of double strand breaks. However, its role in vivo has not yet been elucidated. In addition, Pol \( \lambda \) has been shown to interact with the replication clamp proliferating cell nuclear antigen (PCNA) in vitro and in vivo. In this work, we searched by affinity chromatography for novel partners and we identified the cyclin-dependent kinase Cdk2 as novel partner of Pol \( \lambda \). Pol \( \lambda \) is phosphorylated in vitro by several Cdk/cyclin complexes, including Cdk2/cyclin A, in its proline-serine-rich domain. While the polymerase activity of Pol \( \lambda \) was not affected by Cdk2/cyclin A phosphorylation, phosphorylation of Pol \( \lambda \) was decreased by its interaction with PCNA. Finally, Pol \( \lambda \) is also phosphorylated in vivo in human cells and this phosphorylation is modulated during the cell cycle.

INTRODUCTION

Cell cycle progression is regulated by a family of cyclin-dependent kinases (Cdks) which phosphorylate and activate proteins that execute events critical to cell cycle progression. For activity, Cdks require association with a cyclin and phosphorylation by a Cdk activating kinase (CAK) at a conserved threonine residue (1). Each phase of the cell cycle is characterized by the expression of different Cdk/cyclin complexes that phosphorylate and regulate downstream substrates. In vertebrates, Cdk4/6/cyclin D complexes are active throughout the G1 phase, Cdk2/cyclin E at the G1/S boundary, Cdk2/cyclin A during S phase and Cdk1/cyclin A and Cdk1/cyclin B during the G2/M transition. Studies using knockout mice (2–4) revealed that neither Cdk2 nor cyclin E is essential for mitotic cell division. Cyclin E seems to be important for the control of endoreplication and for quiescent cells which re-enter in cell cycle. Furthermore, Cdk2 is essential in the regulation of the meiotic cell cycle, suggesting a novel tissue-specific function for cyclins and Cdks.

Human DNA polymerase (Pol) \( \lambda \) belongs to the Pol X family based on sequence homology with Pol \( \beta \), Pol \( \mu \) and terminal deoxynucleotidyl transferase (5). Pol \( \lambda \) contains a nuclear localization signal (residues 1–35), a BRCA1 C-terminal domain (BRCT, residue 36–132), a proline-serine-rich region (residues 133–243) and a Pol \( \beta \)-like core region (residues 244–575). Pol \( \lambda \) has been well characterized in vitro and it possesses four different enzymatic activities: DNA polymerase, terminal transferase, deoxyribose phosphate lyase and polynucleotide synthetase, all localized in its C-terminal region (6–8). On the basis of its biochemical properties, Pol \( \lambda \) has been proposed to be involved in various DNA repair pathways, such as abasic site translesion DNA synthesis (9,10), base excision repair (11,12), repair of oxidant DNA damage (13) and non-homologous end joining of double strand breaks (DSBs) (14,15). In addition, Pol \( \lambda \) has been shown to interact with the replication clamp proliferating cell nuclear antigen (PCNA) (9,16) and with the DNA repair protein ligase IV/XRCC4 (17). Mapping of Pol \( \lambda \) interaction with PCNA identified a helix–hairpin–helix motif localized in its Pol \( \beta \)-like core region (16). The major sites of interaction between PCNA and many of its partners are the interdomain

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The sequence of the d73mer is: 5′-GATCGGGAGGGTAG-GAAATTTGAGGATAGGTTGAGTGGAGA-TAGTGGAGGTTAGTATGGTGGATA3′. The sequence complementary to the 17mer primer is underlined.

MATERIALS AND METHODS

Chemicals

[γ-32P]ATP (3000 mCi/mmol) and unlabeled ATP were purchased from Amersham Biosciences, DNA oligonucleotides from Microsynth Gmbh (Balghach, Switzerland). All other reagents were from Merck, Fluka or Sigma.

Enzymes and proteins

Recombinant human wild-type Pol λ was expressed and purified as described by Ramadan et al. (7). Human recombinant PCNA was purified as previously described. The SHV43 mutant of human PCNA was generated, expressed in *Escherichia coli* and purified as described previously (21). The pGEX-3X anticyclic E, Cdk2/cyclin A, Cdk1/cyclin A complexes (22) were gifts from H. P. Nasheuer (Jena, Germany). Histone H1 was purchased from Roche.

DNA substrate

The sequence of the d73mer is: 5′-GATCGGGAGGGTAG-GAAATTTGAGGATAGGTTGAGTGGAGA-TAGTGGAGGTTAGTATGGTGGATA3′. The sequence complementary to the 17mer primer is underlined.

Cell culture

HeLa cells were grown as monolayers in DMEM (Gibco) supplemented with 10% fetal bovine serum (Gibco), 10 μg/ml antibiotics (penicillin and streptomycin) at 37°C in a humidified atmosphere containing 5% CO2. Synchronization of HeLa cells at the G1/S border was obtained by growing cells in nocodazole (40 ng/ml) for 20 h. After nocodazole removal, cells were washed twice with phosphate-buffered saline (PBS), harvested immediately (M phase) or were further cultured in complete medium for 4 h (G1 phase). Cells were subsequently harvested and washed three times with PBS and were pelleted by low-speed centrifugation (250 g, 5 min, 4°C), and the cell pellets stored at −80°C until further use.

Preparations of cell extracts for DNA polymerase λ

Western blots

HeLa cells were washed twice with cold PBS. Then, 500 μl of Laemmli buffer without β-mercaptoethanol were added to lyse the cells. The lysed cells were scraped off the dish and the DNA was shared by using a syringe. β-mercaptoethanol (1.25%) and bromophenol blue (0.005%) were added and the samples were incubated for 5 min at 95°C.

Affinity chromatography

His-Pol λ or BSA were covalently bound to a Hitrap NHS-activated Sepharose High performance column (Amersham Biosciences) according to the manufacturer’s instructions. HeLa cells were lysed for 15 min in a high salt buffer [100 mM HEPES, pH 7.5, 400 mM NaCl, 1 mM DTT, 2 mM phenylmethylsulfonyl fluoride (PMSF), 20% glycerol, 0.5% NP-40, 10 mM glycerophosphate, 1 mM Na3VO4, 1 mM NaF, 2 μg/ml leupeptin, 1 μg/ml pepstatin and 1 μg/ml bestatin] and centrifuged for 10 min at 10000 g at 4°C, and the supernatant was kept as the total extract. The total extract was diluted 1:4 with dilution buffer (100 mM HEPES, pH 7.5, 1 mM DTT, 2 mM PMSF, 20% glycerol, 10 mM glycerophosphate, 1 mM Na3VO4, 1 mM NaF, 2 μg/ml leupeptin, 1 μg/ml pepstatin and 1 μg/ml bestatin). Aliquots containing 40 μg of the diluted extract were loaded in parallel onto either a BSA (control) or a Pol λ column. The columns were washed with 2 column volumes with a buffer containing 50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 10 mM MgCl2, 10% glycerol, 1 mM PMSF, 1 mM DTT and 0.05% NP-40. Subsequently, the bound proteins were eluted with the above mentioned buffer containing, respectively, 150, 350 and 750 mM NaCl. Fractions (20 μl) were analyzed by western blot by using different antibodies.

Antibodies

Antibodies against Cdk2 (sc-748) and PCNA (clone PC10) were purchased from Santa Cruz Biotechnology and α-tubulin (T-6199) from Sigma.

Immunoprecipitation experiments

Total extracts from HeLa cells were prepared as described above. An aliquot of 1.5 mg of total extract was diluted 1:4 in buffer A (10 mM Tris–HCl, pH 7.5, 2.5 mM MgCl2, 0.5% NP-40, 1 mM Na3VO4, 1 mM NaF, 1 mM PMSF, 2 μg/ml leupeptin, 1 μg/ml pepstatin and 1 μg/ml bestatin) to yield a final concentration of 100 mM NaCl and were then incubated with 2 μg of antibodies against Cdk2 or 2 μg of rabbit IgG (Vector Laboratories) as a negative control for 3 h at 4°C. Then, 25 μl of protein G-Sepharose (Amersham Biosciences) coated with BSA were added for 1 h at 4°C. The samples were centrifuged for 30 min at 13000 g at 4°C and the pellets washed three times for 10 min at 4°C with a buffer containing 50 mM Tris–HCl, pH 8, 50 mM NaCl, 75 mM KCl, 0.1% NP-40, 2 μg/ml leupeptin, 1 μg/ml pepstatin and 1 μg/ml...
bestatin. After immunoprecipitation the proteins were heated 5 min at 95°C in Laemmli buffer and analyzed by SDS–PAGE and western blot.

**Pull-down assay**

Pull-down experiments were performed by incubating 750 ng of GST-Cdk2 with His-tagged Pol λ bound to Ni²⁺-NTA beads or with Ni²⁺-NTA beads alone as a control, for 2 h at 4°C in 40 mM Tris–HCl, pH 7.5, 100 mM NaCl, 0.1% (v/v) NP-40, 5 mM imidazole, 2 μg/ml leupeptin, 1 μg/ml pepstatin and 1 μg/ml bestatin. After washing four times with the same buffer, the beads were heated for 5 min at 95°C in Laemmli buffer and the co-precipitated proteins analyzed by western blot using the corresponding antibodies.

**Enzymatic assays**

**Kinase assay.** Phosphorylation was carried out in a final volume of 15 μl containing kinase buffer [50 mM Tris–HCl, pH 7.5, 10 mM MgCl₂, 0.5 μM [γ⁻³²P]ATP (3000 mCi/mmol)], 66.6 μM ATP, in the presence of substrate (Histone H1 or His-Pol λ) and 40 ng of purified Cdk/cyclin complexes. Reactions were carried out for 20 min at 37°C, and the proteins separated on a 10% SDS–PAGE. The gel was stained by Coomassie brilliant blue, dried and exposed to an X-ray film. The incorporation of [γ⁻³²P]ATP in Pol λ polypeptide was determined by cutting out the band corresponding to Pol λ after Coomassie staining, and by measuring the radioactivity by liquid scintillation counting. Background was determined by cutting out the band corresponding to Pol λ incubated in the reaction mixture in the absence of Cdk/cyclin complex and subtracted from the obtained measurements. Pnols of incorporated radioactivity were calculated according to the specific activity of the [γ⁻³²P]ATP used in the assay.

**DNA polymerase assay**

Product analysis by His-Pol λ in the linear kinetic region was performed using a sequencing gel. The reaction mixture included in a final volume of 10 μl: 50 mM Tris–HCl, pH 7.5, 50 mM NaCl, 1 mM MnCl₂ and 10 μM of each unlabeled dNTPs, 20 fmol [32P]5'-end-labeled 17mer primer annealed to the d73mer template (see above). Reactions were incubated for 15 min at 37°C, stopped by addition of sequencing gel loading buffer [95% (v/v) formamide, 20 mM EDTA, pH 8.0] and heated for 5 min at 95°C. The reaction products were resolved on a 10% polyacrylamide, 7 M urea gel. The gels were dried and exposed to an X-ray film.

**Phosphatase treatment**

HeLa cells were grown as previously described, washed once with ice-cold PBS, scraped off the dish and collected in ice-cold PBS. The cells were spun down, the packed cell volume (PCV) was estimated and the cells were resuspended in 1 PCV of buffer [50 mM Tris–HCl pH 7.5, 150 mM NaCl, 1% (v/v) Triton X-100, 0.5 mM PMSF, 2 μg/ml leupeptin, 1 μg/ml pepstatin and 1 μg/ml bestatin]. After incubation on ice for 15 min the samples were centrifuged for 10 min at 10,000 g at 4°C, and the supernatants kept as cell lysates. The phosphatase assay was carried out in a volume of 20 μl containing 25 μg of cell lysate, the λ-phosphatase mixture (50 mM Tris–HCl, pH 7.5, 0.1 mM Na₂EDTA, 5 mM DTT, 0.01% Brij 35 and 2 mM MnCl₂) and 400 U of λ-phosphatase (New England Biolabs). Incubation was at 30°C for 45 min. Two negative controls were added one without λ-phosphatase and the other with λ-phosphatase in the presence of the phosphatase inhibitors EDTA (5 mM) and Na₃VO₄ (1 mM). Then, 2× Laemmli buffer was added, the samples were heated at 90°C for 2 min and loaded on a 10% SDS–PAGE gel. The shift of the phospho-band was analyzed by western blot by using the corresponding antibodies.

**RESULTS**

DNA polymerase λ interacts with Cdk2 in vitro and in vivo

First, we searched for novel partners of Pol λ by affinity chromatography. His-Pol λ or BSA were covalently bound on HiTrap NHS-activated Sepharose HP columns. Total extracts of HeLa cells were prepared as described in Materials and Methods and loaded in parallel onto the two affinity columns. Bound proteins were eluted successively by 150, 350 and 750 mM NaCl. The purification was carried out essentially by following the PCNA elution, a protein known to interact with Pol λ in vitro (9) and in vivo (16). The elution profiles of BSA and Pol λ are shown in Figure 1A. Proteins binding unspecifically were eluted in peak 1 at low NaCl concentrations from the Pol λ and the BSA columns. Two additional peaks were exclusively present in the Pol λ column elution. They contained proteins eluted at higher NaCl concentration, suggesting that they interact specifically with Pol λ. The eluted fractions were tested by western blot by using different antibodies. We first confirmed that, as expected, PCNA specifically bound to the Pol λ column (data not shown). Next, we found that the cyclin-dependent kinase Cdk2 was eluted in peak 2 (fractions 24–28) from the Pol λ column (Figure 1B), whereas it bound only unspecifically to the BSA column (peak 1). We further confirmed this interaction in vitro by a His pull-down experiment. For this, GST-Cdk2 was incubated with His-Pol λ bound to Ni-beads or with Ni-beads alone as a negative control (Figure 1C). Western blot analysis against Cdk2 showed a specific co-precipitation of Pol λ with Cdk2, suggesting a direct interaction of these two proteins. Finally, the interaction was tested in vivo by immunoprecipitation. Anti-Cdk2 or control IgG bound to protein G-Sepharose was incubated with HeLa cells total extracts. After immunoprecipitation, western blot against Pol λ showed that Pol λ was co-immunoprecipitated with Cdk2 only when the anti-Cdk2 antibody was used (Figure 1D). In summary, these results indicate that Cdk2 can physically interact with Pol λ in vitro and in vivo.

DNA polymerase λ is phosphorylated by the Cdk2/cyclin E, Cdk2/cyclin A and Cdk1/cyclin A complexes in vitro

Next, we searched for potential Cdk phosphorylation sites in the amino acid sequence of Pol λ. We found three serines (amino acids 167, 177 and 230, respectively) which could be part of consensus sites for Cdk phosphorylation. They are all located in the proline-serine-rich domain of Pol λ. These sites are specific to Pol λ, since they do not occur in the sequence of any of the other Pol X family members
In order to verify whether Pol λ could be a substrate for Cdk-dependent phosphorylation, kinase assays with different Cdk/cyclin complexes were performed in the presence of recombinant Pol λ and [γ-32P]ATP. As shown in Figure 2B, a [32P]labeled band of the expected molecular weight for Pol λ appeared after incubation with the Cdk2/cyclin E, Cdk2/cyclin A and Cdk1/cyclin A complexes. Histone H1 was used as a positive control. The identity of the phosphorylated band with Pol λ was confirmed by SDS–PAGE and immunoblot analysis (data not shown, and Figures 1D and 3B, left panel). In summary, these results suggest that Pol λ is a target for phosphorylation by these three Cdk/cyclin complexes in vitro.

The proline-serine-rich domain of DNA polymerase λ is the target of phosphorylation by Cdk2/cyclin A

In order to map the phosphorylation domains of Pol λ, kinase assays were performed by using deletion mutants of Pol λ (illustrated in Figure 3A). Figure 3B (left panel) shows a Coomassie staining of the samples after the phosphorylation reaction separated by SDS–PAGE. The same gel was then exposed to an X-ray film to reveal the phosphorylated products. As shown in Figure 3B (right panel), Pol λ, deleted for the BRCT domain, was still phosphorylated by Cdk2/cyclin A, whereas the mutant missing both the BRCT and proline-serine-rich domain was not. This result supported our findings that the three potential sites of phosphorylation are located in the proline-serine-rich domain (see Figure 2A) and suggested that this domain of Pol λ is the target for Cdk2/cyclin A.

Phosphorylation of DNA polymerase λ does not affect its polymerase activity and is regulated by its interaction with PCNA

Furthermore, the effect of Pol λ phosphorylation on its polymerase activity was tested. For this, His-Pol λ was
phosphorylated in vitro by Cdk2/cyclin A in the presence of ATP. Preliminary experiments showed that the degree of Pol λ phosphorylation was between 56 and 84% considering that it can be phosphorylated on one to three sites (see Materials and Methods). This suggested that any effect due to phosphorylation of Pol λ could be visualized by the sensitive method of product analysis. As negative controls, His-Pol λ was either incubated in the presence of Cdk2/cyclin A without ATP, or in the presence of ATP without Cdk2/cyclin A. The Pol λ polymerase activity was then tested on a 5′-labeled d17:d73mer primer-template and the elongation products were visualized on a polyacrylamide denaturating gel. DNA synthesis by Pol λ was stimulated when it was incubated with ATP, either alone (lanes 7–9) or in combination with Cdk2/cyclin A (Figure 4A, lanes 1–3), compared with the reactions where ATP was omitted (lanes 4–6, lanes 10–12). Stimulation was greater when Pol λ was incubated in the presence of ATP alone (lanes 7–9), suggesting that the observed stimulation was rather due to the presence of ATP than to phosphorylation. This effect could be explained by the fact that Pol λ, similarly to Pol μ (24), is able to incorporate ribonucleotides opposite a DNA template (K. Ramadan and U. Hübischer, unpublished data). In this case, ATP could be used as a nucleotide substrate by Pol λ, thus explaining the increased product elongation observed upon addition of ATP (Figure 4A, lanes 7–9). Moreover, the slight decrease in the effect of ATP in the presence of Cdk2/cyclin A may be due to its consumption occurring upon Pol λ phosphorylation (Figure 4A, lanes 1–3). From these results, we concluded that phosphorylation of Pol λ by Cdk2/cyclin A does not directly affect its polymerase activity. In addition, by testing the effect of phosphorylation on Pol λ terminal deoxynucleotidyl transferase activity, we showed that phosphorylation did not affect this activity (data not shown).

Since Pol λ and PCNA have been shown to interact in vivo and in vitro (9,16), and PCNA is known to interact with Cdk/cyclin complexes (19), we next tested the effect of PCNA on
phosphorylation of Pol λ. Incubation of Pol λ with PCNA during phosphorylation by Cdk2/cyclin A in vitro resulted in a decrease in Pol λ phosphorylation (Figure 4B, upper panel). However, when the same experiment was performed by using the SHV43 PCNA mutant (18), no decrease of the Pol λ phosphorylation was observed (Figure 4B, lower panel). In both cases, Pol λ phosphorylation was abolished by addition of the specific Cdk inhibitor p21, confirming that phosphorylation was due to Cdk2/cyclin A. It has been shown that the PCNA mutant SHV43, in which the residues S43, H44 and V45 in the hydrophobic pocket on the C-side of the trimer were changed to A (18), is no more able to interact with Pol λ (16) but can still bind Cdk2 (25). Thus, this result suggested that the physical interaction of PCNA with Pol λ prevented phosphorylation by Cdk2/cyclin A, suggesting a possible regulation of the Pol λ phosphorylation status via its interaction with PCNA.

DNA polymerase λ is phosphorylated during the cell cycle

Having shown that Pol λ can be phosphorylated by Cdk/cyclin complexes in vitro, the next step was to test whether Pol λ is phosphorylated during the cell cycle. For this, HeLa cells were synchronized at the G1/S transition by double thymidine block, and subsequently released in normal medium. Samples were collected at four different time points: 3 h (corresponding to mid-S phase), 6 h (late S phase), 9 h (G2 phase) and 12 h (G2/M phase). Synchronization in M phase was performed by treatment of the cells with nocodazole. Cells released in normal medium progressed in G1 phase and were collected to mid-S phase, 6 h (late S phase), 9 h (G2 phase) and 12 h (G2/M phase). Synchronization in M phase was performed by treatment of the cells with nocodazole. Cells released in normal medium progressed in G1 phase and were collected to mid-S phase, 6 h (late S phase), 9 h (G2 phase) and 12 h (G2/M phase).
the presence of the phosphatase only one form of Pol λ was visible (Figure 5B, lane 1), corresponding to the faster migrating form, as revealed by comparison with reaction without phosphatase (lane 2) or in the presence of phosphatase inhibitors (lane 3), suggesting that Pol λ is phosphorylated in vivo and that its phosphorylation status is modulated during cell cycle.

**DISCUSSION**

In our efforts to better understand the Pol λ function, we searched for partners by affinity chromatography and we found Cdk2 as a novel interacting partner of Pol λ. Analysis of the amino acid sequence of Pol λ revealed the presence of three potential phosphorylation sites (S167, S177 and S230) for Cdk in its proline-serine-rich domain and we showed that this domain is the target of phosphorylation of Pol λ by the Cdk2/cyclin A complex. This finding is not unexpected, since in other proteins proline-rich domains have been shown to be targets of post-translational modifications and to be involved in the regulation of protein function. Moreover, we found that Pol λ is phosphorylated during the cell cycle and exists in two forms in S phase with a majority of hypophosphorylated form, whereas it is present exclusively in its hyperphosphorylated form from G2 to M phase (Figure 5). The different phosphorylation status of Pol λ could be linked with different Pol λ functions in vivo. Interestingly, Pol λ is mainly present in the hypophosphorylated form in the S phase of the cell cycle when PCNA is also abundant. This is in agreement with our findings that the presence of PCNA reduces Pol λ phosphorylation in vitro and suggests that this regulation may also happen in vivo.

In terms of physiological role, it has been shown that PCNA stimulated translesion synthesis of Pol λ past an abasic site as it did for Pol τ, Pol χ and Pol η but by a different mechanism of interaction. Thus, it is tempting to speculate that the interplay among Cdk2/cyclin A, PCNA and Pol λ, revealed by our study, may act in regulating the ‘switch’ between the replicative Pol δ and the translesion Pol λ through direct competition for PCNA, yielding to elongation of the nascent DNA strand past abasic sites. This model was supported by the finding that Pol λ interacts with PCNA at the same site as Pol δ and many other partners and that PCNA could promote bypass of abasic sites during DNA replication (9,16). Moreover, we showed that phosphorylation of Pol λ does not influence its polymerase activity, suggesting that phosphorylation of Pol λ rather regulates its interaction with cellular factors (e.g. PCNA) than its intrinsic activity.

In summary, our results allow to hypothesize that regulation of Pol λ phosphorylation by PCNA during the cell cycle may be important in modulating the interaction of Pol λ with other protein partners, or its recruitment to specific sites on chromatin. The effect of Cdk/cyclin phosphorylation on several replication proteins has been studied and seems to be specific for each protein [reviewed in (26)]. For example, phosphorylation has an inhibitory or stimulatory effect on the activity on Pol α depending on which Cdk/cyclin complexes phosphorylate it (27). In other cases, phosphorylation has been shown to regulate interaction between proteins, such as Fen 1 (28) or DNA ligase I (29,30), with PCNA. In addition, phosphorylation of replication protein A (RP-A) by Cdk1/cyclin A and Cdk1/cyclin B in late S phase leads to the dissociation of the RP-A trimer (31).

The interaction between Cdk2 and Pol λ also fits with the proposed role of Pol λ in non-homologous end joining (14,15). In fact, Cdk2 has been recently shown to regulate DNA DSB repair when associated to cyclin A1. Cyclin A1 is a second A-type cyclin abundantly expressed in testis (32,33). Since Pol λ is also known to be abundant in testis (34) one could propose a role of Pol λ in DSB repair in germ cells. Additional observations of Cdk2 localization at the telomeric ends of chromosome from leptotene to diplotene stage of meiosis (35) could support a role of Pol λ by its terminal transferase activity (7) in germ cells. In addition, our data are supported by the recent findings that Pol λ is selectively involved in NHEJ processing DNA with complementary overhangs, which occur upon DNA damage, whereas Pol μ seems to be mainly involved in NHEJ of DNA with non-complementary ends (36). In this context, phosphorylation of Pol λ may be one of the factors influencing selectively the recruitment of Pol λ on places where its activity is needed.

In conclusion, we showed for the first time that Pol λ is post-translationally modified in human cells, and that this modification is regulated during the cell cycle, opening a way for a better understanding of the Pol λ function in vivo.

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