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Review

Ubiquitylation of DNA polymerase λ

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ABSTRACT

DNA polymerase (pol) λ, one of the 15 cellular pols, belongs to the X family. It is a small 575 amino-acid protein containing a polymerase, a dRP-lyase, a proline/serine rich and a BRCT domain. Pol λ shows various enzymatic activities including DNA polymerization, terminal transferase and dRP-lyase. It has been implicated to play a role in several DNA repair pathways, particularly base excision repair (BER), non-homologous end-joining (NHEJ) and translesion DNA synthesis (TLS). Similarly to other DNA repair enzymes, pol λ undergoes posttranslational modifications during the cell cycle that regulate its stability and possibly its subcellular localization. Here we describe our knowledge about ubiquitylation of pol λ and the impact of this modification on its regulation.

1. DNA polymerase λ, a multitask repair enzyme

Seven DNA polymerase (pol) families have been defined based on sequence homologies. They are called family A, B, C, D, X, Y and reverse transcriptase (RT) [1,2]. The eukaryotic pols can be divided into the five families A, B, X, Y and RT. Pol λ belongs to the X family of DNA pols, which comprises pol β, pol μ and terminal deoxyribonucleotidyl transferase in addition to pol λ [1].

Pol λ is the product of the POLL gene, localized on chromosome 10 in humans and chromosome 19 in mice and is composed of 575 amino acid residues (the murine form having 573 residues) [3]. The C-terminal part of pol λ shows the typical 'right-hand' folding with a palm, finger, thumb and an additional 8 kDa dRP-lyase containing subdomain. The first 230 N-terminal amino acids compose the BRCT and the proline/serine rich domain [4,5] (Fig. 1A). The BRCT domain is believed to be important for protein/protein interactions with components of non-homologous end-joining (NHEJ) such as XRCC4/DNA ligase IV [6]. A possible role of the non-enzymatic proline/serine-rich domain might be modulation of pol λ fidelity, since pre-steady state kinetic studies suggested that this domain contributes to its accuracy [7]. Pol λ possesses multiple activities (reviewed in [2]): in addition to template dependent DNA polymerization it displays dRP-lyase, as well as template-independent terminal deoxynucleotidyl transferase and polynucleotide synthetase activities (Fig. 1B). Also, it efficiently adds DNA bases to a RNA primer [8]. The dRP-lyase activity of pol λ hints towards an involvement in base excision repair (BER) [9] and in vitro experiments suggest that pol λ participates in double strand break DNA repair (DSBR) via NHEJ [10]. In addition, biochemical evidence supports a prominent role of pol λ in the correct repair of oxidative DNA lesions such as 8-oxo-guanine [11–13] and 2-hydroxy-adenine [14], and it seems to perform those functions in cooperation with the auxiliary proteins replication protein A (RP-A) and proliferating cell nuclear antigen (PCNA) (see below). Only in the presence of these two auxiliary proteins the remarkably accurate incorporation opposite 8-oxo-G can be achieved, since the bias of C versus A incorporation increases over 1200 (see Ref. [12] and Table 1 therein). Moreover, pol λ isolated from calf thymus tissue was shown to efficiently bypass apurinic/apyrimidinic (AP) sites [15]. A polymorphic variant of pol λ (R438W) was found to affect the homologous recombination (HR) pathway and sister chromatin exchanges, suggesting that pol λ also has a function in HR [16].

Biochemical studies in Suo's laboratory suggested that an increase in gap size results in lower accuracy for pol λ [17]. The observed decrease in the fidelity appears to be regulated by non-enzymatic N-terminal domains (also see above). Moreover, dCTP was the preferred misincorporated base for full-length pol λ and its N-terminal domain truncation mutants. Their results also indicated that pol λ catalyzes nucleotide incorporation with the

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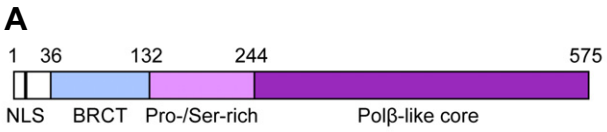

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Activities	Functions
DNA polymerase	DNA synthesis (TLS, BER, NHEJ, HR)
Terminal transferase	V(D)J recombination, NHEJ
dRP lyase	Short-patch BER

Fig. 1. Structure and functions of DNA polymerase λ. For details see text and references therein.

Table 1
Post-translational modifications of BER proteins.[a]

Protein	Modification
TDG	Sumoylation, acetylation
OGG1	Phosphorylation, acetylation
APE1	Phosphorylation, acetylation, ubiquitylation
MUTYH	Phosphorylation
Pol β	Phosphorylation, acetylation, methylation, ubiquitylation
Pol λ	Phosphorylation, ubiquitylation
Pol δ	Phosphorylation, sumoylation, ubiquitylation
Pol ε	Phosphorylation
PCNA	Phosphorylation, acetylation, sumoylation, ubiquitylation
Fenl	Phosphorylation, acetylation
DNA lig I	Phosphorylation
DNA lig III	Phosphorylation
XRCC1	Phosphorylation, sumoylation, ubiquitylation

[a] For details see [31,32] and references therein.

highest combination of efficiency and accuracy when the DNA substrate contains a single-nucleotide gap.

Non-overlapping functions of pols μ, λ, and terminal deoxyribonucleotidyl transferase have been described during immunoglobulin V(D)J recombination in vivo [18]. Pol λ knockout mice are viable and fertile and display a normal hypermutation pattern [19]. Pol $\lambda^{-/-}$ mouse embryonic fibroblasts were shown to be more sensitive to oxidative DNA damage and this phenotype was further enhanced when combined with inactivation of the closely related enzyme pol β, suggesting backup functions these two proteins in the repair of DNA oxidative lesions [20,21]. Furthermore, ionizing radiation sensitivity has been seen in pol λ knockout cells [22].

The pol X family is well characterized on the structural level (reviewed in [23]). Figuratively speaking a pol resembles a human right hand consisting of a palm fingers and a thumb (reviewed in [24]). In general, structural motions of the whole enzyme are observed upon binding of the dNTP's into the active site located in the palm domain. In contrast to this general feature of pols, pol λ was found not to require that subdomain motion (e.g. in fingers and thumb) for catalysis [25], thus making this enzyme particular within all the pol families. When the binary (pol λ/template/primer) and the ternary (pol λ/template/primer/dNTP) complexes were compared, it was found that the essential Asp427, Asp429 and Asp490 possess the same positions whether or not an incoming dNTP was present. Key amino-acids are the Tyr505 and the Phe506 [26] that form the contact with the minor groove of the correctly positioned DNA [25]. Further, it was shown that pol λ can generate single-base deletions during DNA synthesis [27]. This feature was explained on the structural level to be due to DNA strand repositioning induced by the dNTP catalysis, thus controlling the strand slippage [28]. Finally, when the catalytically active form of pol λ was bound to the template/primer with an extrahelical template nucleotide upstream of the active site, pol λ generated strand slippage mutations [29]. In other studies using a mass spectrometry-based protein footprinting approach a solution-phase protein conformational change in pol λ was found [30]. The discrepancy between this observation and the previous structural studies might be due to the fact that the crystallographic structural studies were performed with the catalytic domain only, while the solution studies were carried out with full-length pol λ also containing the BRCT and the proline/serine-rich domains.

In summary, pol λ is a multifunctional enzyme with important functions in BER, NHEJ and translesion DNA synthsis (TLS), the pathways that evolved to reduce the mutational burden in a cell. Unique structural features of pol λ reflected in its enzymatic activity, might be essential for its role as a multifunctional DNA repair pol.

2. Regulation by posttranslational modifications of BER proteins in general and DNA polymerase λ in particular

At present little is known about regulation of BER and its components in the cell. Post-translational modifications (PTM) of BER proteins offer an intriguing possibility to ensure that the components involved act at the right time at the chromatin in the nucleus (reviewed in Refs. [31,32]). PTM's likely involved in this regulation are phosphorylation, acetylation, sumoylation, mono- and polyubiquitylation as well as methylation (Table 1). Dianov and his group showed that BER components are targeted for destruction by the E3 ubiquitin ligase CHIP under normal conditions. However, when DNA damage occurs, those components undergo stabilization to increase the cellular capacity to perform BER [33]. In their work, Dianov and his group found that proteins such as pol β, XRCC1 and DNA ligase III are stabilized when they are bound to chromatin, forming an active repair complex. But when they are not attached to chromatin, meaning that they are not engaged in DNA repair, those components are polyubiquitinated by the concerted action of the E3 ubiquitin ligases CHIP and Mule, and consequently degraded. In a "preview" in the same issue of Mol. Cell Sobol concluded, that "the next goal is now to assess the crosstalk between PTM's, the ability to form productive repair complexes and the stability of these complexes" [34]. Here the concept was put forward that a single PTM, such as phosphorylation, might positively or negatively influence the enzyme's function and constitute a signal for further PTM's.

Initial data concerning the regulation of pol λ in vivo came from a study performed in our laboratory in 2005 when, in a proteomic search for novel interaction partners of pol λ by affinity chromatography, we found cyclin dependent kinase 2 (Cdk2) to interact with pol λ [35]. We showed that pol λ can be phosphorylated in vitro by several Cdk/cyclin complexes, including Cdk2/cyclin A, in its proline-serine rich domain (Fig. 1). Phosphorylation by Cdk2/cyclin A did not affect any biochemical properties of pol λ but the level of this PTM was decreased when pol λ interacted with PCNA, the ring-like moving platform that can interact with 10 different pols (reviewed in [2]). Finally, the phosphorylation-pattern of pol λ in vivo reflected the presence of Cdk2-cyclin A during the cell cycle. In a follow-up work, we could further demonstrate that pol λ was phosphorylated at four distinct sites, among which phosphorylation at Thr553 had a strongest impact on its stability [36].

3. Ubiquitylation of DNA polymerase λ and its implications in repair of 8-oxo-G

When we further investigated the impact of the Thr553 phosphorylation on the stability of pol λ, we found that an increase in the phosphorylation positively correlates with the levels of