Structural basis for the molecular interactions in DNA damage tolerances

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DNA damage tolerance (DDT) is a cell function to avoid replication arrest by DNA damage during DNA replication. DDT includes two pathways, translesion DNA synthesis (TLS) and template-switched DNA synthesis (TS). DDT is regulated by ubiquitination of proliferating cell nuclear antigen that binds to double-stranded DNA and functions as scaffold protein for DNA metabolism. TLS is transient DNA synthesis using damaged DNA as a template by error-prone DNA polymerases termed TLS polymerases specialized for DNA damage. TS, in which one newly synthesized strand is utilized as an undamaged template for replication by replicative polymerases, is error-free process. Thus, DDT is not inherently a repair pathway. DDT is a mechanism to tolerate DNA damage, giving priority to DNA synthesis and enabling finish of DNA replication for cell survival and genome stability. DDT is associated with cancer development and thus is of great interest in drug discovery for cancer therapy. This review article describes recent progress in structural studies on protein-protein and protein-DNA complexes involved in TLS and TS, providing the molecular mechanisms of interactions in DDT.

Key words: DNA damage tolerance, translesion DNA synthesis, template-switching, protein-protein interaction, crystal structure

Genomic DNA is constantly damaged by various internal and external factors. In fact, it has been roughly estimated that an individual cell can suffer up to one million DNA damages per day. The majority of DNA lesions stall replicative polymerases, such as DNA polymerase (Pol) δ or Polε, resulting in the arrest of DNA replication. This replication arrest causes lethal effects such as genomic instability and cell-death. To avoid replication arrest by DNA damage and to restart DNA synthesis at damaged site, cells employ DNA damage tolerance (DDT) [1]. DDT is not inherently a repair pathway. DDT is a mechanism to tolerate DNA damage, enabling finish of DNA replication for cell survival and genome stability. DNA damages are repaired by appropriate DNA repair pathways after replication. Thus, DDT is also termed post replication repair. DDT can be divided into two different pathways that continue to synthesize DNA, translesion and template-switched DNA syntheses (Fig. 1). Translesion DNA synthesis (TLS) is transient DNA synthesis using a damaged template by error-prone DNA polymerases specialized for DNA damage. These DNA polymerases are known as TLS polymerases. TLS is an inherently error-prone DNA synthesis. Template-switched DNA synthesis (template-switching, TS), also known as the damage avoidance path-

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replicative polymerase at the damage site. In the second step, the extender polymerase, Polζ composed of REV3 and REV7 subunits, extends additional nucleotides. Then, a replicative polymerase restarts DNA synthesis. In these processes, PCNA functions as a scaffold protein to tether DNA polymerases on dsDNA. In fact, inserter polymerases contain a PCNA binding motif termed PCNA interacting protein box (PIP-box) (Fig. 2a). Structural studies on PCNA in complex with peptides of inserter polymerases revealed the molecular mechanisms to regulate interactions of inserter polymerases with PCNA [6]. Furthermore, structural studies on Polζ and REV1 provided implication for recruitment of extender polymerase and mechanism of polymerase switching [7–13].

1-1. Mechanism to regulate interactions of inserter polymerases with PCNA

PCNA forms a ring-shaped homo trimer and binds dsDNA within the central pore, thereby functioning as a “sliding clamp” on DNA [14,15]. In combination with its DNA binding function, PCNA is now known as a bona fide hub protein that physically interacts with more than hundreds of proteins involved in DNA metabolism, including DNA replication, repair, recombination, and sister-chromatid cohesion, and that provides a molecular platform to stimulate the functions of partner proteins [4]. Proteins that interact with PCNA contain PIP-box [16,17]. The consensus sequence of canonical PIP-box is defined as Q-xx-[L/I/M]-xx-[F/Y]-[F/Y], where x is any residue (Fig. 2b). Namely, glutamine, hydrophobic, and two aromatic residues are conserved in the canonical PIP-box at position 1, 4, 7, and 8, respectively (Fig. 2b), and side chains of these residues bind to the outer surface of the ring structure of PCNA (Fig. 2c–e). Polδ, a replicative DNA polymerase, consists of four subunits, p125, p50, p66, and p12 subunits in human. Of these, p66 subunit contains the canonical PIP-box (Fig. 2b). The p66 subunit of Polδ is hereafter simply abbreviated as “Polδ” for clarification, unless otherwise noted. Crystal structure of PCNA in complex with the canonical PIP-box peptide of Polδ is shown in Figure 2d [18]. The side chain of Q456 (position 1) binds to the hydrophilic pocket of PCNA, and I459 (position 4), F461 (position 7), and F463 (position 8) bind to the hydrophobic pockets of PCNA (Fig. 2d, e). Distinct from Polδ, the glutamine residue at position 1 is not conserved in inserter polymerases, Polη, Polι, and Polκ that have non-canonical PIP-boxes (Fig. 2b). In the absence of DNA damage, DNA synthesis by TLS polymerases is not adequate, because TLS is frequently error-prone process. Therefore, binding affinity of non-canonical of PIP-boxes of inserter polymerases for PCNA is supposed to be lower than that of replicative polymerases. Crystal structures of PCNA in complex with the non-canonical PIP-box peptide of Polδ is shown in Figure 2d [18]. The side chain of Q456 (position 1) binds to the hydrophilic pocket of PCNA, and I459 (position 4), F461 (position 7), and F463 (position 8) bind to the hydrophobic pockets of PCNA (Fig. 2d, e). Distinct from Polδ, the glutamine residue at position 1 is not conserved in inserter polymerases, Polη, Polι, and Polκ that have non-canonical PIP-boxes (Fig. 2b). In the absence of DNA damage, DNA synthesis by TLS polymerases is not adequate, because TLS is frequently error-prone process. Therefore, binding affinity of non-canonical of PIP-boxes of inserter polymerases for PCNA is supposed to be lower than that of replicative polymerases. Crystal structures of PCNA in complex with the non-canonical PIP-boxes of Polη, Polι, and Polκ revealed a structural basis of low affinity of the non-canonical PIP-boxes for PCNA [6].

Structures of the non-canonical PIP-boxes of inserter
polymerases binding to PCNA are shown in Figure 2f. Interestingly, the side chain of M701 at position 1 of Polη inserts into the hydrophilic pocket of PCNA as observed in the conserved glutamine residue of the canonical PIP-box such as Polδ (Fig. 2f, left panel). The interaction between M701 of Polη and PCNA is van der Waals contact, which could be weaker than electrostatic interactions observed in Q456 of Polδ. Unexpectedly, the lysine residues of Polι and Polκ at position 1 have no contact with the pocket of PCNA (Fig. 2f, center and right panels). M701Q mutant of Polη interacts with PCNA by roughly 4 times stronger than the wild-type of Polη [6], suggesting that the low affinity of inserter polymerases for PCNA might depend on interactions between the residue at position 1 of PIP-box and the hydrophilic pocket of PCNA. In the first step of TLS, a replicative polymerase is replaced with an inserter polymerase. To switch DNA polymerases and facilitate DNA synthesis by inserter polymerases, increase of their affinity for PCNA would be required. As mentioned above, the mono-ubiquitination of PCNA initiates TLS pathway. The ubiquitination of PCNA could increase affinity of inserter polymerases for PCNA. Consistent with this, Polη, Polι, and Polκ contain one or two ubiquitin binding domains, UBM or UBZ (Fig. 2a).

1-2. Mechanism of recruitment of extender polymerase and polymerase switching

In the second step of TLS, an inserter polymerase is replaced with the extender polymerase, Polζ. As briefly mentioned above, Polζ consists of two subunits, REV3 and REV7 [19,20]. REV3 is the catalytic subunit of Polζ that belongs to B-family DNA polymerase, but lacks 3–5’ exonuclease activity. REV3 can extend a primer strand with terminal mismatches. REV7 can switch with PCNA by roughly 4 times stronger than the wild-type of Polη [6], suggesting that the low affinity of inserter polymerases for PCNA might depend on interactions between the residue at position 1 of PIP-box and the hydrophilic pocket of PCNA. In the first step of TLS, a replicative polymerase is replaced with an inserter polymerase. To switch DNA polymerases and facilitate DNA synthesis by inserter polymerases, increase of their affinity for PCNA would be required. As mentioned above, the mono-ubiquitination of PCNA initiates TLS pathway. The ubiquitination of PCNA could increase affinity of inserter polymerases for PCNA. Consistent with this, Polη, Polι, and Polκ contain one or two ubiquitin binding domains, UBM or UBZ (Fig. 2a). Therefore, affinity of inserter polymerases for PCNA might be regulated by the residue at position 1 of PIP-box and the ubiquitination of PCNA.

Figure 2 (a) Domain architectures of human Polη, Polι, and Polκ. Polη and Polκ have PIP-box in their C-termini, and Polι has PIP-box in the internal region. Polη has a UBD termed the UBM domain, which is a CCHH-type Zn-finger domain. Polι has two UBZ domains, which are CCHC-type Zn-finger domains. Polκ has two UBZ domains termed the UBM domains. Polη, Polι, and Polκ have RIR that has conserved two phenylalanines (FF) similar to PIP-box. (b) Amino acid sequence of the canonical and non-canonical PIP-boxes. Positions of amino acid residues (1–8) are indicated above sequences. Consensus sequence of the canonical PIP-boxes are shown on the top. Conserved residues are highlighted by black backgrounds. Specific residues of non-canonical PIP-boxes of TLS polymerases are highlighted by green, blue, and pink backgrounds. (c) Overall structure of PCNA in complex with a PIP-box peptide. Crystal structure of human PCNA in complex with PIP peptide is shown as a representative structure of PIP-box bound to PCNA (PDB ID: 2ZVM). PIP-box binding site of PCNA and K164 subjected to ubiquitination in DTT are indicated by dot circles and arrows, respectively. (d) Structure of the canonical PIP-box of Polδ bound to PCNA. Polδ bound to PCNA is shown by silver tube (PDB ID: 1U76). Conserved residues within the canonical PIP-box (Q456, I459, F462, and F463) are shown by stick representation. PCNA is shown by surface representation colored in pale cyan. (e) Schematic drawing of interaction between the canonical PIP-box and PCNA. Residues “h” and “a” correspond to those of (b) (f) Structures of the non-canonical PIP-boxes of Polη, Polι, and Polκ bound to PCNA. Polη (PDB ID: 2ZVK), Polι (PDB ID: 2ZVM), and Polκ (PDB ID: 2ZVL) are shown in the left, center, and right panels, respectively. PCNA is shown by surface representation colored in pale cyan. TLS polymerases are shown by green tube. Residues at positions 1, 4, 7, and 8 are shown by stick representations.
REV7 are indicated. Relative cell survival of three genotypes, Wildtype, REV7-/-, and REV7(Y63A/W171A) indicate. In vitro binding assay using numerous alanine mutants of REV7 firstly confirmed a formation of ternary complex of REV1-REV7-REV3 and revealed that L186, Q200, and Y202 are crucial for REV1 binding (Fig. 3d) [7]. Intriguingly, these residues located on the C-terminal β-strands of REV7 (Fig. 3b). Because of the REV3 binding resulting in a knot structure, the C-terminal region of REV7 could be intrinsically disordered without the REV3 binding, implying a sequential binding of REV7, REV3, and REV1. Subsequently, crystal structures of protein complexes including REV1-CTD revealed details of REV7-REV1 interaction and provided structural basis for polymerase switching [8–13]. The interactions between REV7 and REV3 complex revealed by crystal structures were consistent with previous biochemical results [7, 10, 11]. Namely, L186 of REV7 binds to the hydrophobic pocket formed by L1203, Y1244, and L1248 of REV1, and these van der Waals contacts were essential for the interaction between REV7 and REV1. Y202 and Q200 of REV7 are also significantly involved in the interaction in hydrophobic and hydrophilic manners, respectively (Fig. 4a) [11]. As described above, REV1-CTD binds the inserter polymerases such as Polh, Poln, and Polk (Fig. 1) [22]. Structural studies revealed that the inserter binding site of REV1 was not overlapped with the REV7 binding site [8, 9], enabling the formation of an inserter-extender complex (Fig. 4b) [10].
such as Polη, Polι, and Polκ. The inserter-REV1 complex facilitates to insert a nucleotide on damaged site. The extender polymerase, Polζ consisting of REV7 and REV3, is recruited on the primer end through the interaction between REV1 and REV7. Formation of a quaternary complex consisting of an inserter and extender mediated by REV1-CTD facilitates switching from an inserter to the extender polymerases and then Polζ extends a few additional nucleotides. After that, a replicative polymerase restarts DNA replication.

2. Mechanism of an early step in TS

DDT includes two pathways, TLS and TS, but how one pathway is chosen over the other remains a mystery. TLS appears to be a rapid process, because it is performed by several TLS polymerases and does not require structural change of the replication fork. In contrast, TS is a much more complicated process including regression of the stalled replication fork, DNA synthesis using switched template, and reversal of the regressed fork (Fig. 1). Recently, a mechanism of an early step in TS is getting clarified by structural studies on HLTF, a crucial protein that has activities of E3 ubiquitin ligase and DNA helicase [28–30]. The domain architecture of human HLTF is shown in Figure 5a. Previously, a bioinformatics has suggested that the N-terminal HIRAN domain of HLTF has DNA binding activity [31]. The crystal structure of the HIRAN domain of human HLTF in complex with dsDNA revealed the DNA recognition mechanism of HLTF in an early step of TS [28].

The HIRAN domain of HLTF adopts a β-barrel structure composed of six β-strands flanked with two α-helices (Fig. 5b), resulting in an OB-fold structure that contains oligonucleotide-binding proteins. HLTF HIRAN domain has a concave surface that interacts with the two bases of the primer stand of dsDNA. Interestingly, the 3’-end of the primer strand is specifically bound to a pocket of the domain. Structural details of interaction between HLTF HIRAN domain and DNA is shown in Figure 5b, left panel. D94 of HLTF recognizes 3’-OH of primer strand and it binds to DNA so that it unwinds the dsDNA by two tyrosine residues (Y72 and Y93) that pinch two bases of 3’-end of the primer strand (Cyt12 and Cyt13). Based on the structure, a mechanism of an early step in TS is proposed, as follows (Fig. 5b). HLTF binds to the 3’end of the primer strand of the stalled replication fork through the interaction between the HIRAN domain and DNA. HLTF and RAD18 could perform poly-ubiquitination of K164 of PCNA, whereas the mechanism underlying poly-ubiquitination of PCNA by RAD18 and HLTF is controversial [32,33]. The poly-ubiquitinated PCNA could be recognized by ZRANB3, a DNA helicase that contains two PCNA-binding motifs and a poly-ubiquitin binding domain, thereby ZRANB3 might be recruited to the damaged site and the fork regression to switch template strand might be facilitated.
To address the mechanism behind orchestration of TLS polymerases, structural studies of a full-length TLS polymerase and mono-ubiquitinated PCNA are required. Recently, low resolution structures of Polη and mono-ubiquitinated PCNA by electron microscopy revealed domain arrangements in their complex bound to DNA [38]. To date, many structures of catalytic domain of inserter polymerases have revealed structural basis for the mechanisms of nucleotide insertion at atomic resolution. In contrast, structural information about the extender, Polζ, is very limited in an overall structure of yeast Polζ by electron microscopy at low resolution [39]. High resolution structures of Polζ and its DNA complex are needed to resolve the molecular mechanism of the extension step in TLS.

Structural studies on the HIRAN domain of HLTF have shed light on the mechanisms of an early step of TS in which the HIRAN domains could be involved. But, structural detail of the molecular mechanism behind overall TS pathway is still an open question. Very recently, a structural study on ZRANB3 provided basis for functions as a structure-specific DNA nuclease and PCNA interactions [40]. However, a function as DNA helicase remains unclear. Further structural and biochemical studies on HLTF and ZRANB3 are urgently required to address challenges accompanying TS.

Conflict of Interest

The authors declare no conflict of interests.

Author Contributions

H. H., A. H., K. H., and S. K. drafted the manuscript and prepared figure representations.

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