Genes and Environment, Vol. 34, No. 2 pp. 63–69 (2012)

Review

Human DNA Polymerase η and Its Regulatory Mechanisms

Chikahide Masutani[1]

Department of Genome Dynamics, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan

(Received February 6, 2012; Revised March 2, 2012; Accepted March 2, 2012)

Defects in DNA polymerase (Pol) η result in a cancer-prone and UV-sensitive inherited syndrome, a variant form of xeroderma pigmentosum, suggesting that Polη plays a vital role in preventing UV-induced skin cancers. In fact, Polη can catalyze translesion synthesis (TLS) past prominent UV-induced lesions efficiently and accurately. However, Polη is intrinsically an error-prone DNA polymerase, like other TLS polymerases. Biochemical, structural and physiological studies revealed that Polη and other TLS polymerases participate in multiple mutagenic mechanisms, including somatic hypermutation of immunoglobulin genes. Protein-protein interactions between Polη and PCNA, TLS polymerases, RAD18 and DNA repair proteins, as well as their posttranslational modifications, have been shown to be important for regulating Polη.

Key words: translesion synthesis, protein-protein interaction, posttranslational modification

Identification of *POLH*, The Gene Responsible for XP-V

Xeroderma pigmentosum (XP) is an autosomal recessive genetic disorder characterized by sunlight sensitivity, cutaneous and ocular deterioration, premature malignant skin neoplasms, and an increased incidence of skin cancer after sunlight exposure. XP has been classified into eight complementation groups, XP-A to XP-G, and XP-V. Unlike XP-A to XP-G, cells from XP-V patients are proficient in nucleotide excision repair (NER) but display abnormal DNA replication, including a reduced ability to elongate nascent UV-irradiated DNA strands (1). It has also been demonstrated that extracts from XP-V cells lack lesion bypass replication activity in an *in vitro* DNA replication system when a plasmid containing a cyclobutane pyrimidine dimer (CPD) was used (2–5). Using an improved *in vitro* DNA replication system, we purified an XP-V correcting protein from HeLa cell extracts and found that the purified protein displayed DNA polymerase activity capable of replicating past CPDs (6). cDNA cloning revealed that the responsible DNA polymerase is the product of one of two human homologues of the *S. cerevisiae* gene, *RAD30* (7). Johnson *et al.* independently identified the same gene as the human counterpart of yeast *RAD30* (8). The gene, which we then termed *POLH*, is mutated in XP-V patients, though no mutations were found in the *POLI* gene, another human homologue of *RAD30* (9).

Properties of DNA Polymerase η

The *POLH* gene encodes DNA polymerase (Pol) η, which can catalyze translesion synthesis (TLS) past CPDs as efficiently as undamaged DNA templates. Fourteen DNA template-dependent DNA polymerases have been identified in human cells and classified into four families (Table 1). Polη belongs to the Y-family of polymerases with Polι, Polκ, and REV1. The Y-family

Table 1. Human DNA template-dependent DNA polymerases

Polymerase	Gene	Family	Functions
Polα	*POLA*	B	DNA replication priming
Polβ	*POLB*	X	BER, meiotic recombination
Polγ	*POLG*	A	Mitochondrial DNA replication and repair
Polδ	*POLD*	B	DNA replication and repair
Polε	*POLE*	B	DNA replication and repair
Polζ	*REV3*	B	TLS
Polη	*POLH*	Y	TLS, SHM, HR
Polι	*POLI*	Y	TLS, BER
Polκ	*POLK*	Y	TLS, NER
Polθ	*POLQ*	A	TLS, SHM
Polλ	*POLL*	X	V(D)J recombination
Polμ	*POLM*	X	V(D)J recombination
Polν	*POLN*	A	Interstrand crosslink repair
REV1	*REV1*	Y	TLS, SHM

BER: base excision repair, TLS: translesion DNA synthesis, SHM: somatic hypermutation, HR: homologous recombination.

[1]Correspondence to: Chikahide Masutani, Department of Genome Dynamics, Research Institute of Environmental Medicine, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8601, Japan. Tel: +81-52-789-3871, Fax: +81-52-789-3890, E-mail: masutani@riem.nagoya-u.ac.jp

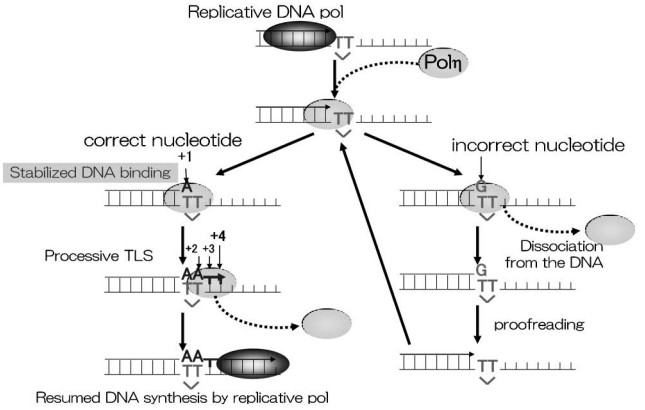

Fig. 1. A model for TLS past TT-CPDs by Polη with biased fidelity (modified from ref. 17). Polη can be loaded in place of the arrested replicative DNA polymerase at TT-CPDs. Polη is a low processive polymerase and easily dissociates from the template-primer DNA. (left) However, if correct nucleotides are incorporated at positions +1, +2, or +3 opposite of the TT-CPD template, Polη stably binds to the template-primer DNA and processively performs TLS until the +4 position. This is the minimum reaction needed to resume replicative DNA polymerase-catalyzed DNA synthesis. (right) Alternatively, if incorrect nucleotides are incorporated, Polη easily dissociates from the template-primer DNA, and the resulting products are preferentially proofread rather than elongated. As a result, bypassed products contain the correct sequences.

polymerases are conserved from bacteria to humans (10) and are thought to catalyze TLS across DNA lesions (11), like other polymerases such as Polθ and Polζ.

Structural analyses of Y-family DNA polymerases revealed that they have a spacious active site that facilitates lesion bypass (12,13). Biochemical analyses showed that Y-family DNA polymerases lack exonucleolytic proofreading activity, have low processivity, and can catalyze TLS, making them intrinsically error-prone. In fact, human Polη can generate base substitutions with error rates of 10^{-2} to 10^{-3} when used by itself to replicate undamaged DNA (14). Polη has an ability to preferentially incorporate adenines opposite the damaged thymines of *cis-syn* TT-CPDs, but misincorporations may also take place (15,16). It should be noted that replicative DNA polymerases such as Polδ and Polα are unable to incorporate nucleotides opposite of the TT-CPDs and, more importantly, they also cannot extend primers until two more nucleotides have been incorporated beyond the CPD (17). This means that, for lesion bypass, nucleotides must be incorporated by TLS polymerases beyond the lesion in addition to the initial incorporation of a nucleotide opposite the lesion. Polη has a unique DNA binding property that allows it to synthesize DNA (Fig. 1). Polη preferentially binds to template-primer DNAs consisting of TT-CPD templates and primers whose 3′ ends are correct nucleotides situated opposite to the TT of CPDs and opposite of the next nucleotide beyond the CPD. Thus, Polη can replicate past TT-CPDs processively without dissociating from the template primer when the correct nucleotides are incorporated. However, Polη easily dissociates from the template primer if incorrect nucleotides are incorporated. As a result, replicated DNA contains correct nucleotides opposite of the lesion, meaning that Polη bypasses CPDs with biased fidelity (17,18). Structural analyses of human Polη at four consecutive steps during DNA synthesis through TT-CPD and two nucleotides beyond the CPD showed that Polη acts as a molecular splint to stabilize damaged DNA in a normal conformation (19). Both biochemical and structural analyses indicate that Polη catalyzes efficient and accurate bypass across CPD lesions and prevents skin cancer (19,20) but cannot bypass another major UV-induced lesion, the 6–4 photoproduct. Conversely, NER which is abrogated in XP-A to XP-G cells efficiently removes 6–4 photoproducts and prevents skin cancer but removes CPD inefficiently (21). Therefore, both TLS and NER are important for UV damage tolerance in humans. It has been shown that Polη contributes to global genome repair of UV-induced lesions during S phase (22), further suggesting that there may be crosstalk between TLS and NER.

In addition to UV-irradiation, XP-V cells are also sensitive to cisplatin (23). Polη can bypass cisplatin adducts *in vitro* (24) and, together with Polζ, catalyzes TLS past cisplatin adducts in human cells (25). Polη also contributes to TLS across lesions such as 8-oxoguanine, thymine glycols, acetylaminofluorene adducts, benzo[*a*]pyrene-7,8-diol-9,10-epoxide (BPDE) adducts (16,25–28). However, unlike CPD bypass, in which Polη alone acts as the main polymerase, Polη may be one of multiple polymerases that cells use to bypass these non-CPD lesions, resulting in errors (29). Thus, depending on the lesions generated, cells may use various mechanisms to select the appropriate polymerase.

Interaction with Monoubiquitinated PCNA

Human Polη consists of 713 amino acids. The N-terminal half contains residues that are conserved among Y-family DNA polymerases and constitute the hand-like structure that is common to DNA polymerases and an additional unique Y-family domain, called the little finger or PAD (12,13). A molecule consisting of only the N-terminal 511 amino acids was originally identified to exhibit the XP-V correcting DNA polymerase activity (6), and the N-terminal 432 residues exhibited basal DNA polymerase activity (30). Thus, the C-terminal residues are dispensable for DNA polymerase activity and likely contain regulatory elements as depicted in Fig. 2. The nuclear localization signal and PCNA interaction peptide (PIP) sequences are located close to the C-terminus and are important for the cellular localization of Polη and nuclear foci formation with PCNA af-

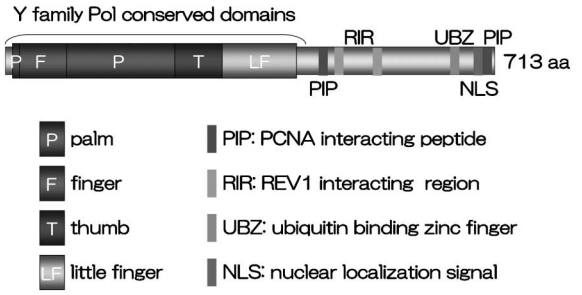

Fig. 2. Domain structures of human Polη.

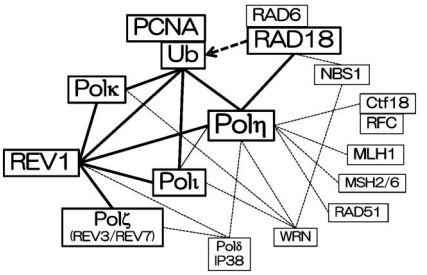

Fig. 3. Networks among Polη and related proteins.

ter UV-irradiation, respectively (31). An ubiquitin-binding zinc finger (UBZ) domain is required for the interaction with monoubiquitinated PCNA and for its relocalization to damaged chromatin (32,33). Another PIP-like domain is also reported to be located upstream of the UBZ domain (34). The ability of Polη mutants to rescue the UV-sensitivity of XP-V cells was only partially reduced with PIP mutations. Since foci formation was almost completely abolished by mutations in the C-terminal PIP domain, it is thought that nuclear foci formation is not required for TLS by Polη. More confusingly, some point mutations in the UBZ domain severely affected the ability of XP-V cells to cope with UV-induced DNA damage, while a version of Polη lacking its C-terminus (including the UBZ sequence) but retaining an intact PIP-like motif was able to promote cellular survival in response to UV (30). The interaction of Polη with monoubiquitinated PCNA via these domains could be important for its function, although some recent reports suggest that cells are able to activate and relocate Polη independently of PCNA monoubiquitination (35–38). Thus, the role of these domains and the relevance of the interaction with monoubiquitinated PCNA still need to be elucidated.

Interaction with TLS Proteins

Human Polη interacts with REV1 through two domains located in the C-terminus (39,40). Mutations in these domains disrupted the Polη-REV1 interaction but did not affect the ability of Polη to catalyze TLS past CPDs and promote the survival of XP-V cells after UV damage (41). REV1 is a member of the Y-family of DNA polymerases and is known to play a crucial role in UV-induced mutagenesis with Polζ. Interestingly, REV1-interaction defective Polη only partially suppressed spontaneous mutations in XP-V cells, whereas it suppressed UV-induced mutations completely (41). REV1 interacts with Y-family polymerases Polι and Polκ, as well as Polη and Polζ, through its C-terminus (39,40,42,43) and is thought to play a central role in TLS polymerase switching. Together with the observations that the Polη-REV1 interaction stimulates the accumulation of endogenous REV1 to UV-damaged DNA sites (41) and that Polι foci formation is enhanced by Polη (44), cells may preferentially recruit Polη to lesions first and then switch to other TLS polymerases. Since REV1 forms foci in response to UV-irradiation independently of Polη (41,45), and REV1 and other TLS polymerases interact with monoubiquitinated PCNA directly, each polymerase could be recruited to arrested replication forks independently of each other. However, among all of the TLS polymerases, only Polη interacts with RAD18, which is the primary ubiquitin ligase responsible for PCNA monoubiquitination. RAD18-Polη interaction is crucial for guiding Polη to arrested replication forks after UV-irradiation (46). Polη also interacts with and is stimulated by a PCNA loader complex, Ctf18-RFC (47). A Polδ interacting protein, PDIP38, also interacts with Polη, REV1 and Polζ (48). Thus, it is likely that the replication and translesion machinery are connected in various ways in order to tolerate DNA lesions. Protein-protein interactions among Polη and related proteins are depicted in Fig. 3.

Interaction with DNA Repair Proteins

Several reports may suggest that defects in the crosstalk between TLS and other DNA response mechanisms contribute to diseases associated with genomic instability.

Nijmegen breakage syndrome (NBS) is characterized by high sensitivity to ionizing radiation and predisposition to malignancies, and cells from NBS patients exhibited defects in double-stranded DNA break repair and checkpoint controls (49). Since a reduction of the responsible gene product, NBS1, sensitized human cells to UV, NBS1 is likely also involved in the UV damage response (50,51). Recently, NBS1 was found to interact with and recruit RAD18 to damaged chromatin and regulate Polη-catalyzed TLS past UV-induced lesions (52).

Werner syndrome (WRN) is characterized by premature aging and cancer predisposition (53). Cells from WRN patients showed a reduced proliferative life span, genomic instability, and sensitivity to 4-nitroquinoline 1-oxide and crosslinking agents. The responsible gene product, WRN, is a RecQ family DNA helicase associ-

ated with exonuclease activity. WRN interacts with and stimulates the polymerase activities of TLS polymerases Polη, Polι and Polκ (54). WRN also regulates TLS as the WRN-NBS1 interaction is important for RAD18-mediated PCNA ubiquitination (55,56).

Hereditary nonpolyposis colon cancer (HNPCC) is linked with mutations in mismatch repair genes including MLH1, MSH2 and MSH6 (57). Human Polη interacts with MLH1 and MSH2/MSH6 through its N-terminus and C-terminus, respectively (58,59). In addition to using mismatch repair to correct errors during DNA replication, mismatch repair proteins also contribute to other mechanisms including somatic hypermutation of immunoglobulin genes (60). Interestingly, MSH2 and MSH6, but not MLH1, participate in somatic hypermutation (61), indicating that mismatch repair proteins have separable functions. The mutation spectrum of somatic hypermutation in MSH2- or MSH6-deficient mice, in which mutations at A:T in immunoglobulin genes were drastically reduced, was similar to that of Polη-deficient mice, as described below. A:T somatic hypermutations also require RAD18 (62) and PCNA monoubiquitination (63), although PCNA ubiquitination-independent mechanisms have also been considered (38). *In vitro* reconstitution experiments demonstrating that MSH2/MSH6 stimulates Polη activity (58) suggests that the interaction between Polη and MSH2/MSH6 may be involved in the somatic hypermutation of immunoglobulin genes. More recently, however, the involvement of MSH2/MSH6, Polη, and monoubiquitinated PCNA in the oxidative stress response has also been reported (64). Thus, the interaction between Polη and mismatch repair proteins may be involved in the repair of oxidative stress-induced DNA lesions.

Regulation of Polη by Posttranslational Modifications

Posttranslational modifications of Polη have been reported. Lysines in the NLS located at the C-terminus of Polη were found to be monoubiquitinated, which prevents the interaction between Polη and PCNA (65). An E3 ubiquitin ligase, Pirh2, binds to Polη, catalyzes its monoubiquitination, and suppresses TLS (66). Thus, monoubiquitination at its C-terminus inactivates Polη after TLS is completed. On the other hand, ATR- or PKC-mediated phosphorylation of Polη at C-terminal residues activates Polη (67,68). Phosphorylation of RAD18 by the protein kinase Cdc7 also regulates Polη-RAD18 interaction and Polη activation (69).

An E3 ubiquitin ligase Mdm2 interacts with and promotes polyubiquitination of Polη and its proteasomal degradation (70). In *C. elegans*, degradation of Polη is mediated by the Cul4-Ddb1-Cdt2 pathway, while SUMOylation of Polη counteracts this proteolysis (71). Thus, proteolytic degradation and its regulation can control TLS. Molecular chaperone HSP90 is also reported to regulate the stability of Polη in human cells (72).

In Vivo Functions of Polη

Polη-deficient mice are viable, fertile and do not show any apparent spontaneous physiological defects under normal conditions. However, fibroblasts from these mice showed enhanced sensitivity to UV, and all of the Polη-deficient mice developed skin tumors after UV-irradiation. These results confirm that Polη prevents UV-induced cell death and skin cancer by catalyzing the accurate bypass of CPDs in mice as well as in humans (73,74).

In addition to accurate TLS, Polη participates in somatic hypermutation of immunoglobulin genes as an error-prone enzyme (75). In peripheral blood lymphocytes from XP-V patients, the decrease of the A:T mutation of the immunoglobulin variable gene, was observed, although the overall mutation frequency was normal (76–79). Similar results were observed in Polη-deficient mice (80,81). It is proposed that multiple DNA polymerases, including Polη, Polθ, and REV1, participate in somatic hypermutation, and that Polη is the main mutator at A and T residues. In chicken DT40 cells, disruption of Polη causes a decrease in the frequency of immunoglobulin gene conversion and double-strand break-induced homologous recombination (82). Human Polη has also shown an ability to synthesize DNA from D-loop recombination intermediates, which is stimulated by interaction with the recombination protein RAD 51 (83). Together, these data suggest that Polη may also play an important role in homologous recombination, though the related phenotypes have not been addressed in XP-V patients nor Polη-knockout animals so far.

Conclusions

Human Polη is the gene product of XP-V and plays an invaluable role in preventing UV-induced skin cancers by catalyzing efficient and accurate translesion synthesis across UV-induced CPD lesions. Polη is also involved in the somatic hypermutation of immunoglobulin genes. However, a lack of Polη does not cause immunodeficiency since multiple error-prone DNA polymerases participate in somatic hypermutation. Considering that Polη and other TLS polymerases are ubiquitously expressed in organs, Polη and other TLS polymerases likely have multiple functions that have not yet been identified. In addition, posttranslational modifications of PCNA are known to regulate TLS and another DNA damage tolerance mechanism called template switch. Protein-protein interactions between TLS polymerases, PCNA and its modifiers, and other DNA damage response players, and their regulatory mechan-

isms have also been identified. A greater understanding of the physiological relevance and regulatory mechanisms of TLS in DNA damage tolerance could lead to the development of new ways to treat cancers and other diseases.

Acknowledgements: This work was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by the Mitsubishi Foundation and by Takeda Science Foundation.

References

1 Lehmann AR, Kirk-Bell S, Arlett CF, Paterson MC, Lohman PH, de Weerd-Kastelein EA, Bootsma D. Xeroderma pigmentosum cells with normal levels of excision repair have a defect in DNA synthesis after UV-irradiation. Proc Natl Acad Sci USA. 1975; 72: 219–23.

2 Cordeiro-Stone M, Zaritskaya LS, Price LK, Kaufmann WK. Replication fork bypass of a pyrimidine dimer blocking leading strand DNA synthesis. J Biol Chem. 1997; 272: 13945–54.

3 Ensch-Simon I, Burgers PMJ, Taylor J-S. Bypass of a site-specific *cis-syn* thymine dimer in an SV40 vector during *in vitro* replication by HeLa and XPV cell-free extracts. Biochemistry. 1998; 37: 8218–26.

4 Svoboda DL, Briley LP, Vos J-MH. Defective bypass replication of a leading strand cyclobutane thymine dimer in xeroderma pigmentosum variant cell extracts. Cancer Res. 1998; 58: 2445–8.

5 Cordonnier AM, Lehmann AR, Fuchs RP. Impaired translesion synthesis in xeroderma pigmentosum variant extracts. Mol Cell Biol. 1999; 19: 2206–11.

6 Masutani C, Araki M, Yamada A, Kusumoto R, Nogimori T, Maekawa T, Iwai S, Hanaoka F. Xeroderma pigmentosum variant (XP-V) correcting protein from HeLa cells has a thymine dimer bypass DNA polymerase activity. EMBO J. 1999; 18: 3491–501.

7 Masutani C, Kusumoto R, Yamada A, Dohmae N, Yokoi M, Yuasa M, Araki M, Iwai S, Takio K, Hanaoka, F. The *XPV* (xeroderma pigmentosum variant) gene encodes human DNA polymerase η. Nature. 1999; 399: 700–4.

8 Johnson RE, Kondratick CM, Prakash S, Prakash L. *hRAD30* mutations in the variant form of xeroderma pigmentosum. Science 1999; 285: 263–5.

9 McDonald JP, Tissier A, Frank EG, Iwai S, Hanaoka F, Woodgate R. DNA polymerase ι and related rad30-like enzymes. Philos Trans Soc Lond. B Biol Sci. 2001; 356: 53–60.

10 Ohmori H, Friedberg EC, Fuchs RPP, Goodman MF, Hanaoka F, Hinkle D, Kunkel TA, Lawrence CW, Livneh Z, Nohmi T, Prakash L, Prakash S, Todo T, Walker GC, Wang Z, Woodgate R. The Y-family of DNA polymerases. Mol Cell. 2001; 8: 7–8.

11 Lange SS, Takata K, Wood RD. DNA polymerases and cancer. Nature RevCancer. 2011; 11: 96–110.

12 Yang W. Damage repair DNA polymerases Y. Curr Opin Struct Biol. 2003; 13: 23–30.

13 Prakash S, Johnson RE, Prakash L. Eukaryotic translesion synthesis DNA polymerases: specificity of structure and function. Annu Rev Biochem. 2005; 74: 317–53.

14 Matsuda T, Bebenek K, Masutani C, Hanaoka F, Kunkel TA. Low fidelity DNA synthesis by human DNA polymerase η. Nature 2000; 404: 1011–3.

15 Johnson RE, Washington MT, Prakash S, Prakash L. Fidelity of human DNA polymerase η. J Biol Chem. 2000; 275: 7447–50.

16 Masutani C, Kusumoto R, Iwai S, Hanaoka F. Mechanisms of accurate translesion synthesis by human DNA polymerase η. EMBO J. 2000; 19: 3100–9.

17 Kusumoto R, Masutani C, Simmyo S, Iwai S, Hanaoka F. DNA binding properties of human DNA polymerase η: Implication for polymerase switching. Genes Cells. 2004; 9: 1139–50.

18 McCulloch SD, Kokoska RJ, Masutani C, Iwai S, Hanaoka F, Kunkel TA. Preferential *cis-syn* thymine dimer bypass by DNA polymerase η occurs with biased fidelity. Nature. 2004; 428: 97–100.

19 Biertumpfel C, Zhao Y, Kondo Y, Ramon-Maiques S, Gregory M, Lee JY, Masutani C, Lehmann AR, Hanaoka F, Yang W. Structure and mechanism of human DNA polymerase η. Nature. 2010; 465: 1044–9.

20 Silverstein TD, Johnson RE, Jain R, Prakash L, Prakash S, Aqqarwal AK. Structual basis for the suppression of skin cancers by DNA polymerase η. Nature. 2010; 465: 1039–1043.

21 Naegeli H, Sugasawa K. The xeroderma pigmentosum pathway: decision tree analysis of DNA quality. DNA Repair. 2011; 10: 673–83.

22 Auclair Y, Rouget R, Belisle JM, Costantino S, Drobetsky EA. Requirement for functional DNA polymerase η in genome-wide repair of UV-induced DNA damage during S phase. DNA Repair. 2010; 9: 754–64.

23 Albertella MR, Green CM, Lehmann AR, O'Connor MJ. A role for polymerase η in the cellular tolerance to cisplatin-induced damage. Cancer Res. 2005; 65: 9799–806.

24 Vaisman A, Masutani C, Hanaoka F, Chaney SG. Efficient translesion replication past oxaliplatin and cisplatin GpG adducts by human DNA polymerase η. Biochemistry. 2000; 39: 4575–80.

25 Shachar S, Ziv O, Avkin S, Adar S, Wittschieben J, Reissner T, Charney S, Friedberg EC, Wang Z, CArell T, Geacintov N, Livneh Z. Two-polymerase mechanisms dictate error-free and error-prone translesion synthesis in mammals. EMBO J. 2009; 28: 383–93.

26 Kusumoto R, Masutani C, Iwai S, Hanaoka F. Translesion synthesis by human DNA polymerase η across thymine glycol lesions. Biochemistry. 2002; 41: 6090–9.

27 Chiapperino D, Cai M, Sayer JM, Yagi H, Kroth H, Masutani C, Hanaoka F, Jerina DM, Cheh AM. Error-prone translesion synthesis by human DNA polymerase η on DNA containing deoxyadenosine adducts of 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene. J Biol Chem. 2005; 280: 39684–92.

28 Lee DH, Pfeifer GP. Translesion synthesis of 7,8-dihydro-8-oxo-2′-deoxyguanosine by DNA polymerase η *in vivo*. Mutat Res. 2008; 641: 19–26.

29 Livneh Z, Siv O, Shachar S. Multiple two-polymerase mechanisms in mammalian translesion DNA synthesis. Cell Cycle. 2010; 9: 729–35.

30 Acharya N, Yoon J-H, Hurwitz J, Prakash L, Prakash S. DNA polymerase h lacking the ubiquitin-binding domain promotes replicative lesion bypass in human cells. Proc Natl Acad Sci USA. 2010; 107: 10401–5.
31 Kannouche P, Broughton BC, Volker M, Hanaoka F, Mullenders LH, Lehmann AR. Domain structure, localization, and function of DNA polymerase η, defective in xeroderma pigmentosum variant cells. Genes Dev. 2001; 15: 158–72.
32 Kannouche PL, Wing J, Lehmann AR. Interaction of human DNA polymerase η with monoubiquitinated PCNA: a possible mechanism for the polymerase switch in response to DNA damage. Mol Cell. 2004; 14: 491–500.
33 Bienko M, Green CM, Crosetto N, Rudolf F, Zapart G, Coull B, Kannouche P, Wider G, Peter M, Lehmann AR, Hofmann K, Dikic I. Ubiquitin-binding domains in Y-family polymerases regulate translesion synthesis. Science. 2005; 310: 1821–4.
34 Acharya N, Yoon JH, Gali H, Unk I, Haracska L, Johnson RE, Hurwitz J, Prakash L, Prakash S. Roles of PCNA-binding and ubiquitin-binding domains in human DNA polymerase η in translesion synthesis. Proc Natl Acad Sci USA. 2008; 105: 17724–9.
35 Sabbioneda S, Gourdin AM, Green CM, Zotter A, Giglia-Mari G, Houtsmuller A, Vermeulen W, Lehmann AR. Effect of proliferating cell nuclear antigen ubiquitination and chromatin structure on the dynamics properties of the Y-family DNA polymerases. Mol Biol Cell. 2008; 19: 5193–202.
36 Schmutz V, Janel-Bintz R., Wagner J, Biard D, Shiomi N, Fuchs R, Cordonnier AM. Role of the ubiquitin-binding domain of Polη in Rad18-independent translesion DNA synthesis in human extracts. Nucl Acids Res. 2010; 38: 6456–65.
37 Hendel A, Krijger PH, Diamant N, Goren Z, Langerak P, kim J, Rei?ner T, Lee K-y, Geacintov NE, CArell T, Myung K, Tateishi S, D'Andrea A, Jacobs H, Livneh Z. PCNA ubiquitination is important, but not essential for translesion DNA synthesis in mammalian cells. PLoS Genet. 2011; 7: e1002262.
38 Krijger PHL, van den Berk PCM, Wit N, Langerak P, Jansen JG, Reynaud CA, de Wind N, Jacobs H. PCNA ubiquitination-independent activation of polymerase η during somatic hypermutation and DNA damage tolerance. DNA Repair. 2011; 10: 1051–9.
39 Ohashi E, Murakumo Y, Kanjo N, Akagi J, Masutani C, Hanaoka F, Ohmori H. Interaction of hREV1 with three human Y-family DNA polymerases. Genes Cells. 2004; 9: 523–31.
40 Tissier A, Kannouche P, Reck MP, Lehmann AR, Fuchs RP, Cordonnier A. Co-localization in replication foci and interaction of human Y-family members, DNA polymerase polη and REV1 protein. DNA Repair. 2004; 3: 1503–14.
41 Akagi J, Masutani C, Kataoka K, Kan T, Ohashi E, Mori T, Ohmori H, Hanaoka F. Interaction with DNA polymerase η is required for nuclear accumulation of REV1 and suppression of spontaneous mutations in human cells. DNA Repair. 2009; 8: 585–99.
42 Murakumo Y, Ogura Y, Ishii H, Numata S, Ichihara M, Croce CM, Fishel R, Takahashi M. Interaction in the error-prone postreplication repair proteins hREV1, hREV3, and hREV7. J Biol Chem. 2001; 276: 35644–51.
43 Guo C, Fischhaber PL, Luk-Paszyc MJ, Masuda Y, Zhou J, Kamiya K, Kisker C, Friedberg EC. Mouse Rev1 protein interacts with multiple DNA polymerases involved in translesion DNA synthesis. EMBO J. 2003; 22: 6621–30.
44 Kannouche P, Fernandez de Henestrosa AR, Coull B, Vidal A, Gray C, Zicha D, Woodgate R, Lehmann AR. Localization of DNA polymerase η and ι to the replication machinery is tightly coordinated in human cells. EMBO J. 2002; 21: 6246–56.
45 Andersen PL, Xu F, Ziola B, MaGregor WG, Xiao W. Sequential assembly of translesion DNA polymerases at UV-induced DNA damage sites. Mol Cell Biol. 2011; 22: 2373–83.
46 Watanabe K, Tateishi S, Kawasuji M, Tsurimoto T, Inoue H, Yamaizumi M. Rad18 guides polη to replication stalling sites through physical interaction and PCNA monoubiquitination. EMBO J. 2004; 23: 3886–96.
47 Shiomi Y, Masutani C, Hanaoka F, Kimura H, Tsurimoto T. A second proliferating cell nuclear antigen loader complex, Ctf18-replication factor C, stimulates DNA polymerase η activity. J Biol Chem. 2007; 282: 20906–14.
48 Tissier A, Janel-Bintz R, Coulon S, Klaile E, Kannouche P, Fuchs RP, Cordonnier AM. Crosstalk between replicative and translesional DNA polymerase: PDIP38 interacts directly with Polη. DNA Repair. 2010; 9: 922–8.
49 Matsuura A, Kobayashi J, Tauchi H, Komatsu K. Nijimegen breakage syndrome and DNA double strand break repair by NBS1 complex. Adv Biophys. 2004; 38: 65–80.
50 Zhong H, Bryson A, Eckersdorff M, Ferguson DO. Rad50 depletion impacts upon ATR-dependent DNA damage responses. Hum Mol Genet. 2005; 14: 2685–93.
51 Biard DS. Untangling the relationships between DNA repair pathways by scilencing more than 20 DNA repair genes in human stable clones. Nucl Acids Res. 2007; 35: 3535–50.
52 Yanagihara H, Kobayashi J, Tateishi S, Kato A, Matsuura S, Tauchi H, Yamada K, Takezawa J, Sugasawa K, Masutani C, Hanaoka F, Weemaes CM, Mori T, Zou L, Komatsu K. NBS1 recruits RAD18 via a RAD6-like domain and regulates Polη-dependent translesion DNA synthesis. Mol Cell. 2011; 43: 788–97.
53 Bohr VA. Deficient DNA repair in the human progeroid disorder, Werner syndrome. Mutat Res. 2005; 577: 252–9.
54 Kamath-Loeb AS, Lan L, Nakajima S, Yasui A, Loeb LA. Werner syndrome protein interacts functionally with translesion DNA polymerases. Proc Natl Acad Sci USA. 2007; 104: 10394–9.
55 Cheng WH, von Kobbe C, Opresko PL, Arthur LM, Komatsu K, Seidman MM, Carney JP, Bohr VA. Linkage between Werner syndrome protein and the Mre11 complex via Nbs1. J Biol Chem. 2004; 279: 21169–76.
56 Kobayashi J, Okui M, Asaithamby A, Burma S, Chen BP, Tanimoto K, Matsuura S, Komatsu K, Chen DJ. WRN participates in translesion synthesis pathway through interaction with NBS1. Mech Ageing Dev. 2010; 131: 636–44.

57 Lynch HT, Lynch PM, Lanspa SJ, Snyder CL, Lynch JF, Boland CR. Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. Clin Genet. 2009; 76: 1–18.

58 Wilson TM, vaisman A, Martomo SA, Sullivan P, Lan L, Hanaoka F, Yasui A, Woodgate R, Gearhart PJ. MSH2-MSH6 stimulates DNA polymerase η, suggesting a role for A:T mutations in antibody genes. J Exp Med. 2005; 201: 637–45.

59 Kanao R, Hanaoka F, Masutani C. A novel interaction between human DNA polymerase η and MutLα. Biochem Biophys Res Commun. 2009; 389: 40–5.

60 Jiricny J. The multifacedted mismatch-repair system. Nat Rev Mol Cell Boil. 2006; 7: 335–46.

61 Saribasak H, Rajagopal D, Maul RW, Gearhart PJ. Hijacked DNA repair proteins and unchained DNA polymerases. Philos Trans R Soc Lond Biol Sci. 2009; 364: 605–11.

62 Bachl J, Ertongur I, Jungnickel B. Involvement of Rad18 in somatic hypermutation. Proc Natl Acad Sci USA. 2006; 103: 12081–6.

63 Langerak P, Nygren AO, Krijger PH, van den Berk PC, Jacobs H. A/T mutagenesis in hypermutated immunoglobulin genes strongly depends on PCNAK164 modification. J Exp Med. 2007; 204: 1989–98.

64 Zlatanau A, Despras E, Braz-Petta T, Boubakour-Azzouz I, Pouvelle C, Stewart GS, Nakajima S, Yasui A, Ischenko AA, Kannouche P. The hMsh2-hMsh6 complex acts in concert with monoubiquitinated PCNA and Polη in response to oxidative DNA damage in human cells. Mol Cell. 2011; 43: 649–62.

65 Bienko M, Green CM, Sabbioneda S, Crosetto N, Matic I, Hibbert RG, Begovic T, Niimi A, Mann M, Lehmann AR, Dikic I. Regulation of translesion synthesis DNA polymerase η by monoubiquitination. Mol Cell. 2010; 37: 396–407.

66 Jung YS, Hakem A, Hakem R, Chen X. Pirh2 E3 ubiquitin ligase monoubiquitinates DNA polymerase η to suppress translesion DNA synthesis. Mol Cell Biol. 2011; 31: 3997–4006.

67 Chen YW, Cleaver JE, Hatahet Z, Honkanen RE, Chang JY, Yen Y, Chou KM. Human DNA polymerase η activity and translocation is regulated by phosphorylation. Proc Natl Acad Sci USA. 2008; 105: 16578–83.

68 Gohler T, Sabbioneda S, Green CM, Lehmann AR. ATR-mediated phosphorylation of DNA polymerase η is needed for efficient recovery from UV damage. J Cell Boil. 2011; 192: 219–27.

69 Day TA, Palle K, Barkley LR, Kakusho N, Zou Y, Tateishi S, Verreault A, Masai H, Vaziri C. Phosphorylated Rad18 directs DNA polymerase η to sites of stalled replication. J Cell Biol. 2010; 191: 953–66.

70 Jung YS, Qian Y, Chen X. DNA polymerase η is targeted by Mdm2 for polyubiquitination and proteasomal degradation in response to ultraviolet irradiation. DNA Repair. 2012; 11: 177–84.

71 Kim S-H, Michael WM. Regulated proteolysis of DNA polymerase η during the DNA-damage response in *C.elegans*. Mol Cell. 2008; 32: 757–66.

72 Sekimoto T, Oda T, Pozo FM, Murakumo Y, Masutani C, Hanaoka F, Yamashita T. The molecular chaperone Hsp90 regulates accumulation of DNA polymerase η at replication stalling sites in UV-irradiated cells. Mol Cell. 2010; 37: 79–89.

73 Lin Q, Clark AB, McCulloch SD, Yuan T, Bronson RT, Kunkel TA, Kucherlapati R. Increased susceptibility to UV-induced skin carcinogenesis in polymerase η-deficient mice. Cancer Res. 2006; 66: 87–94.

74 Ohkumo T, Kondo Y, Yokoi M, Tsukamoto T, Yamada A, Sugimoto T, Kanao R, Higashi Y, Kondoh H, Tatematsu M, Masutani C, Hanaoka F. Ultraviolet B radiation induces epithelial tumors in mice lacking Polη and mesenchymal tumors in mice deficient for Polι. Mol Cell Biol. 2006; 26: 7696–706.

75 Seki M, Gearhart PJ, Wood RD. DNA polymerases and somatic hypermutation of immunoglobulin genes. EMBO Rep. 2005; 6: 1143–8.

76 Zeng X, Winter DB, Kasmer C, Kreamer KH, Lehmann AR, Gearhart PJ. DNA polymerase η is an A-T mutator in somatic hypermutation of immunoglobulin variable genes. Nat Immunol. 2001; 2: 537–41.

77 Faili A, Aoufouchi S, Weller S, Vuillier F, Stray A, Sarasin A, Reynaud CA, Weill JC. DNA polymerase η is involved in hypermutation occurring during immunoglobulin class switch recombination. J Exp Med. 2004; 199: 165–270.

78 Zeng X, Negrete GA, Kasmer C, Yang WW, Gearhart PJ. Absence of DNA polymerase η reveals targeting of C mutations on the nontranscribed strand in immunoglobulin switch regions. J Exp Med. 2004; 199: 917–24.

79 Mayorov VI, Rogozin IB, Adkison LR, Gearhart PJ. DNA polymerase η contributes to strand bias of mutations of A versus T in immunoglobulin genes. J Immunol. 2005; 174: 7781–6.

80 Delbos F, De Smet A, Faili A, Aoufouchi S, Weill JC, Reynaud CA. Contribution of DNA polymerase η to immunoglobulin gene hypermutation in the mouse. J Exp Med. 2005; 201: 1191–6.

81 Martomo SA, Yang WW, Wersto RP, Ohkumo T, Kondo Y, Yokoi M, Masutani C, Hanaoka F, Gearhart PJ. Different mutation signatures in DNA polymerase η- and MSH2-deficient mice suggest separate roles in antibody diversification. Proc Natl Acad Sci USA. 2005; 102: 8656–61.

82 Kawamoto T, Araki K, Sonoda E, Yamashita YM, Harada KK, Kikuchi K, Masutani C, Hanaoka F, Nozaki K, Hashimoto N, Takeda S. Dual roles for DNA polymerase η in homologous DNA recombination and translesion DNA synthesis. Mol Cell. 2005; 20: 793–9.

83 Mcllwraith MJ, Vaisman A, Liu Y, Fanning E, Woodgate R, West SC. Human DNA polymerase η promotes DNA synthesis from strand invasion intermediates of homologous recombination. Mol Cell. 2005; 20: 783–92.