Translesion synthesis : cellular and organismal functions

Temviriyanukul, P.

CHAPTER 7

PERSPECTIVES

In this thesis, I have characterized the role of genes involved in TLS at the genome of mammalian cells in response to DNA-damaging agents, using mouse embryonic fibroblast (MEFs) and mice with defined mutations in these genes. To this aim, I have developed a toolbox to study multiple aspects of TLS of the damaged genome, including bypass of different types of lesions, replication fork progression, mutagenesis, DNA damage signaling, genome breakage and cell survival. This work shows comprehensively the relative *in vivo* contributions of a number of mammalian TLS-associated polymerases to replication of damaged DNA and to DNA damage signaling responses when cells are exposed to common food-derived genotoxins and to UV light. Additionally, this thesis provides evidence that ssDNA gaps can be transmitted through mitosis into the subsequent cell cycle and converted into DSBs that underlie genome instability, in a DNA replication-dependent fashion. Lastly, I have shown that Rev1, a key TLS polymerase, is required for preventing premature aging in mice.

THE COMPLEXITIES OF TLS

Many *in vitro* studies of individual TLS polymerases have been conducted and these have yielded important insights into their biochemical properties (Waters *et al.*, 2009), rather than on their functions and contribution to TLS in a living cell. In Chapters 2 and 3, I described the roles of different mammalian TLS polymerases in response to DNA damages at nuclear chromatin. Even if, at the genome, some types of DNA lesions, such as CPDs, may be bypassed by only one TLS polymerase at the replication fork (Yoon *et al.*, 2009), the present idea of TLS is that persistent DNA damage is replicated by the concerted action of at least two TLS polymerases: inserter polymerases and extender polymerases. The inserter TLS polymerase is capable of inserting nucleotides across DNA lesions, while extension beyond the lesions is performed by extender TLS polymerases (Prakash *et al.*, 2002; Shachar *et al.*, 2009; Yoon *et al.*, 2009; Yoon *et al.*, 2010). So far, mammalian Polκ and Polζ have been implicated in the latter stage (Haracska *et al.*, 2002; Prakash *et al.*, 2002). Data in this thesis suggest that the extender function of Polκ might be limited to specific DNA lesions, since MEFs deficient for Polζ display persistent stalled replication forks at UV-induced (6-4)PPs and at BPDE lesions (Chapters 2 and 3). This suggests that, for these DNA lesions, (i) other TLS polymerases, including Polκ, are not capable of acting as an extender polymerase, or that (ii) Polκ-mediated extension might be Polζ-dependent.

REGULATION OF TLS

Because TLS is an inherently mutagenic process, its activity should be tightly controlled. This thesis and work by others provide evidence that Rev1 and Polζ are indispensable for bypassing strongly helix-distorting (6-4)PPs (Shachar *et al.*, 2009; Szuts *et al.*, 2008), whereas they have a minor role in the bypass across mildly helix-distorting CPDs. In

addition, CPDs and Benzo[*a*]pyrene diepoxyde (BPDE)-induced DNA adducts seem to be cognate DNA lesions for Polη and Polκ, respectively (Bi *et al.*, 2005; Yoon *et al.*, 2009; Zhang *et al.*, 2002). It is not known how the "optimal" TLS polymerase is recruited to a specific lesion. In yeast and in human cells, arrest of the replication fork by DNA adducts elicits TLS via the mono-ubiquitination of PCNA at K164 (PCNA-Ub), especially for TLS by Polη across mildly distorting CPDs (Bienko *et al.*, 2010). This may suggest a "tool belt" mechanism whereby all TLS polymerases are bound to PCNA which, upon fork stalling and ubiquitination, selects the best suited TLS polymerase to perform the bypass (Freudenthal *et al.*, 2010). However, data in chapter 3 and from Edmunds *et al* suggest that Rev1 and Rev3-mediated TLS is independent from PCNA-Ub (Edmunds *et al.*, 2008). Therefore, a reasonable possibility could be that the TLS polymerase selection is based on a trial and error basis. In such a model, when a particular TLS polymerase would not be able to appropriately bypass the lesion, another might be invoked, possibly only after repriming of replication downstream of the lesion (see below). Other processes and pathways that may regulate mutagenic TLS include (i) proofreading *in trans* by the replicative polymerases (Bebenek *et al.*, 2001a), (ii) DNA mismatch repair (Tsaalbi-Shtylik *et al.*, in preparation), and (iii) the Fanconi anemia (FA) pathway. The FA pathway has been implicated in the regulation of Rev1 and Rev3 via its FANCC component (Niedzwiedz *et al.*, 2004). In support, it has recently been shown that the FA-associated protein (FAAP20) interacts with Rev1 through its ubiquitin-binding domain (UBZ4) (Kim *et al.*, 2012). Depletion of FAAP20 leads to loss of recruitment of Rev1 to UV damages, indicating that the FA complex may stimulate TLS. Furthermore, the FA complex possesses ubiquitin ligase activity, and it is tempting to speculate that the FA complex may regulate TLS by ubiquitination of TLS-related proteins in response to strongly helix-distorting DNA lesions. In addition, the FA complex may also indirectly influence TLS, since recent data indicate that the FA complex stabilizes stressed replication forks. Accordingly, in the absence of the FA complex, destabilized forks may not be suitable for Rev1-dependent TLS (Lossaint *et al.*, 2013).

Recently, it has been shown that a subunit of the yeast replicative DNA polymerase Polδ, Pol32, interacts with Polζ and that this interaction is required for Polζ-mediated TLS (Johnson *et al.*, 2012). Likewise, in human cells, the human ortholog of Pol32, POLD3, interacts with Rev1 and Polζ as well as with the ubiquitin-binding protein Spartan. Depletion of Spartan leads to enhanced UV-induced mutagenesis, which is dependent on Rev1 and Polζ (Kim *et al.*, 2013). Together, these data suggest a link between the coordination and regulation of replicative and TLS DNA polymerases.

MECHANISTIC BASIS FOR TLS

To bypass DNA lesions, TLS may act in two modes: (i) direct bypass and (ii) post-replicative gap filling. Post-replicative gaps can be generated by re-priming of processive

replication beyond the lesions or by the convergence of an adjacent replicon. Possibly, cells employ both TLS modes to bypass DNA damages, depending on the architecture of DNA lesions. For instance, the mildly-helix distorting CPDs might be competently bypassed by Polη via direct bypass, while TLS across the strongly helix-distorting (6-4) PPs is thought to involve post-replicative gap filling (Jansen *et al.*, 2007). It was shown by electron microscopy that TLS-deficient yeast cells accumulate ssDNA gaps 30 min after UV exposure, providing evidence for TLS as a gap-filling mechanism (Lopes *et al.*, 2006). It would be intriguing to apply the same approach to human or mouse Xpc mutant cells that are deficient for global-genome nucleotide excision repair (GG-NER) of UV lesions, but proficient for TLS. Consistent with a role in post-replicative gap filling, Rev1 or Rev3 mutant mouse cells show the accumulation of (6-4)PPs-containing ssDNA patches in the G_2 phase (Jansen *et al.*, 2009a; Jansen *et al.*, 2009b). Although also in mammalian cells gap-filling TLS at an episomal substrate can be separated from replication (Diamant *et al.*, 2012), the physiological role of post-replicative gaps at the genome is not established, as it is difficult to exclude the possibility that, in Rev1 or Rev3-proficient cells, CPDs and (6-4)PPs are bypassed directly. ssDNA gaps activate the Atr-mediated DNA damage signaling pathway and the loading of the 9-1-1 complex to 5' primer-dimer termini (Majka *et al.*, 2006; Nam *et al.*, 2011). Furthermore, yeast Rev1, Polζ as well as Polκ might be recruited to damaged chromatin and interact with the 9-1-1 complex (Jansen *et al.*, 2007; Kai *et al.*, 2003; Sabbioneda *et al.*, 2005; Yu *et al.*, 2003). In yeast, it has been demonstrated that 9-1-1 mutant yeast cells display a hypomutable phenotype to DNA damaging agents (Murakami-Sekimata *et al.*, 2010; Paulovich *et al.*, 1998). This implies that the 9-1-1 complex is at the intersection between TLS and DNA damage signaling in yeast. Recently, it was found that Rev1, via its N-terminal BRCT domain, specifically binds 5' phosphorylated primer-template termini *in vitro*, a result consistent with recruitment of the protein to post-replicative gaps (de Groote *et al.*, 2011). Further study for the interaction between Rev1 or Rev3 and the 9-1-1 complex in mammalian cells might provide more insight into the linkage of TLS and DNA damage signaling.

ISOLATION AND CHARACTERIZATION OF TLS COMPLEXES

Unraveling the composition of TLS polymerase-containing complexes in response to a particular kind of DNA damage in the genome, and dissecting whether such complexes act directly at stalled replication forks or at post-replicative gaps will contribute in understanding TLS at the molecular level. This can be investigated by using different approaches. To study the proximity of photolesions in single-stranded DNA, different DNA polymerases, and the 9-1-1 complex, the proximity ligation assay can be applied. This assay is a highly sensitive, antibody-dependent, technique to investigate the proximity of biomolecules *in situ* (Gullberg *et al.*, 2003). Similarly, chromatin immunoprecipitation (ChIP), using an antibody specific for a protein or DNA adduct in concert with western blotting or mass spectrometry, may be employed

to analyze protein complexes at ssDNA containing CPDs or (6-4)PPs. Additionally, such ChIP analysis also allows the identification of histone modifications, which may provide evidence on the existence of preferred chromatin regions for TLS polymerases. To isolate protein complexes at replication forks, an assay called Isolation of proteins on nascent DNA (iPOND) can be used (Sirbu *et al.*, 2012; Sirbu *et al.*, 2013). This assay relies on the pull-down of DNA replication forks that have incorporated 5-ethynyl-2'-deoxyuridine (EdU) prior to stalling, with associated proteins, which can be followed by western blotting or mass spectrometry. To enhance the specificity, an antibody that specifically binds to single-stranded CPDs or (6-4)PPs may be used to pull down replication complexes at these lesions, prior to iPOND. In addition, epitope-tagged ubiquitin or other post-translational modifications will provide a map showing which proteins are modulated in response to stalled replication forks by specific DNA damaging agents.

GENERATION OF CELL LINES WITH MULTIPLE DEFICIENCIES IN TLS-RELATED GENES: NEXT GENERATION MUTAGENESIS STRATEGIES

After identifying TLS-associated complexes at particular lesions, cells deficient for specific TLS-associated proteins or TLS complexes could be generated in order to study TLS at the genome, using the toolbox developed here. With regard to the generation of cell lines deficient in certain factors, at present two techniques are widely used: siRNA (or shRNA)-mediated knockdown and gene-targeting. The disadvantages of siRNA or shRNA knockdown are efficiency, specificity and stability, while gene-targeting is costly, time-consuming and laborious. The generation of double-mutant cells is even more difficult. Recently, Wang *et al* have demonstrated a novel gene-targeting technology using the CRISPR/Cas system. This technique is highly efficient and can target multiple genes in cells and in mice simultaneously (Wang *et al.*, 2013). Applying this approach to TLS-associated genes will allow the generation of cell lines with various combinations of TLS mutants in a limited time span.

FROM SINGLE STRAND GAPS TO GENOME INSTABILITY

Previous work has shown that *Rev1*$^{-/-}$*Xpc*$^{-/-}$ MEFs that are defective in the bypass of (6-4)PPs replicate UV-damaged DNA almost completely, leaving unreplicated (6-4) PPs in ssDNA gaps (Jansen *et al.*, 2009a). In Chapter 5 of this thesis, the fate of these ssDNA gaps was studied. It was found that, at environmentally relevant levels of UV lesions, these gaps, despite activating DNA damage signaling, are transmitted through mitosis into the subsequent cell cycle leading to the formation of DSBs during the ensuing S phase. Yet, high levels of UV damage result in poor cell cycle progression and a strong mitotic arrest ((Jansen *et al.*, 2009a) and data not shown). These data suggest that high levels of ssDNA gaps activate a strong cell cycle checkpoint that precludes

cells from entering a new cell cycle. Apparently, relatively low levels of ssDNA gaps are tolerated in cultured mammalian cells. In the second S phase, ssDNA gaps may stall replisomes resulting in DSBs as reflected by the formation of phosphorylated Atm and the induction of micronuclei (MN). It should be noted, however, that phosphorylated ATM represents only a fraction of DSBs, mainly in heterochromatin (Riballo *et al.*, 2004). In the near future, it would be intriguing to determine whether chromosomal aberrations accompany the induction of MN in the second cell cycle. Furthermore, live cell imaging of *Rev1*$^{-/-}$*Xpc*$^{-/-}$ MEFs expressing GFP-tagged 53BP1, a DSB marker, allows the analysis of 53BP1 foci formation in the first and second cell cycle after UV exposure. Therefore, such an analysis may provide additional insights in the occurrence of UV-induced DSBs in *Rev1*$^{-/-}$*Xpc*$^{-/-}$ MEFs. Recently, this approach revealed that in aphidicolin-treated cells 53BP1 foci are formed, when the cells are in the subsequent cell cycle following treatment (Lukas *et al.*, 2011). These data suggest that incompletely replicated DNA in the first cell cycle is transmitted to the daughter cells, comparable with the findings presented in Chapter 5. Surprisingly, Rev1-deficient cells are likely defective in the repair of DSBs (Sharma *et al.*, 2012). This might aggravate the genome instability induced by UVC in *Rev1*$^{-/-}$*Xpc*$^{-/-}$ MEFs. However, also *Xpc*$^{-/-}$ cells accumulate DSBs during the second S phase upon high UVC exposure indicating that the delayed genome instability described in Chapter 5 is not restricted to Rev1-deficient cells.

The MN assay is an important standard assay in genotoxicity testing (Kirsch-Volders *et al.*, 2011). The advantages of the assay are its (i) simplicity of scoring, (ii) accuracy, (iii) sensitivity and (iv) rapidity. Nonetheless, this assay requires cycling cells and therefore appropriate doses of test agents are critical to prevent the induction of permanent cell cycle arrests. The Organisation for Economic Co-operation and Development (OECD) guidelines for standard testing for MN recommends the second cell division to be covered in MN analysis (Kirsch-Volders *et al.*, 2011). However, a mechanistic insight related to this advice was lacking. In line with this, the data in Chapter 5 demonstrates that the induction of MN following exposure to agents inducing replication-blocking DNA lesions may require two cell divisions to convert primary DNA damage (a ssDNA gap) into a DSB. Therefore, the testing guidelines released by OECD, including (i) the standard *in vivo* MN test guideline 474 and (ii) the *in vitro* MN test guideline 487, should be obligated.

TLS AND PREMATURE AGING

As already mentioned, stalled replication forks at DNA damage lead to Atr-mediated DNA damage signaling. The bypass of DNA adducts by TLS polymerases results in completion of replication and the quenching of DNA damage signaling. This suggests that there might be an equilibrium between these two mechanisms. It has been demonstrated that TLS, while mutagenic, paradoxically suppresses tumor promotion in mice by mitigating excessive proliferation that likely is caused by DNA damage

signaling associated with chronic replicative stress (Tsaalbi-Shtylik *et al.*, 2009). Perturbation of the balance between mutagenesis, replicative stress and proliferation might lead to pathologies, such as cancer and premature aging. Premature aging correlates with the accumulation of endogenous DNA damages, induced mainly by free radicals (de Boer *et al.*, 2002; Hasty *et al.*, 2003). Lipid peroxidation products, generated endogenously by cells or found in fatty diets, induce free radicals, DNA lesions and large amounts of protein damages. Chapter 2 of this thesis shows that Rev1 and Polι play a role in bypassing DNA lesions induced by hydroxynonenal (HNE), an end product of lipid peroxidation. As described in Chapter 6, *Rev1*$^{-/-}$*Xpc*$^{-/-}$ mice display phenotypes of accelerated aging that most likely originates in enhanced replication stress caused by defective TLS at endogenous DNA damages. These endogenous DNA damages might be related, among others, to lipid peroxidation-induced DNA adducts.

Polι has been implicated not only in bypassing HNE adducts (Chapter 2), but it might also act as 5'-deoxyribose phosphate lyase, which is essential for the removal of free radical-induced DNA damages by Base Excision Repair (BER) (Bebenek *et al.*, 2001b). Indeed, depletion of Polι in human cells enhanced their sensitivity to oxidative stress (Petta *et al.*, 2008). At present, it is unclear whether Polι prevents premature aging in mice. It would be interesting to study mice with a defined deletion in the Polι gene.

Although Rev3 performs only a minor role in bypassing HNE-induced lesions (Chapter 2), Rev3 mutant mice display embryonic lethality (Van Sloun *et al.*, 2002; Wittschieben *et al.*, 2000). This suggests that the endogenous DNA damages causing the embryonic lethality of Rev3-deficient mice may not be related to lipid peroxidation products. It has been found that Rev3 mutant chicken DT40 cells are hypersensitive to a nitric oxide inducer, suggesting a role of Rev3 in TLS across nitric oxide-induced DNA damages (Wu *et al.*, 2006). Nitric oxides are generated in cells and interact with oxygen, resulting in the reactive nitrogen species (RNS) that bind to both DNA and proteins. In order to shed light on the role of Rev3 in preventing replication stress induced by nitric oxide and aging, the addition of nitric oxide scavengers to Rev3-defective embryos may prevent embryonic lethality.

Premature aging may be provoked by mutations in DNA of mitochondria, (Lagouge *et al.*, 2013). Mitochondria have no Nucleotide Excision Repair (NER) (LeDoux *et al.*, 1992), thus, there is a possibility that endogenous lipid peroxidation products might induce mutations in mtDNA. In yeast cells, Rev1 and Rev3 are recruited to mitochondria to perform mutagenic TLS (Zhang *et al.*, 2006). At present, a role for mammalian TLS polymerases in mitochondria is not known. Therefore, mutation analysis on mtDNA of TLS-deficient mammalian cells may unveil this process in mammals. Moreover, investigation on the integrity of mitochondria in cells lacking a TLS polymerase might provide a molecular link between TLS, cellular metabolism and aging.

Telomere shortening is also related to premature aging of somatic cells (Lopez-Otin *et al.*, 2013). Telomeres can be classified as a DNA damage trap as they possess limited

DNA repair activity leading to persistent DNA damages, telomere exhaustion and attrition. It is not yet clear whether TLS polymerases play a role in preventing telomere shortening or in preventing DNA damage signaling at persistent ssDNA gaps at telomeres. Further study using mice described in Chapter 6 may provide insights into the role of TLS polymerases in protecting telomeres, and ultimately aging.

REFERENCES

Bebenek K, Matsuda T, Masutani C, Hanaoka F, Kunkel TA (2001a). Proofreading of DNA polymerase eta-dependent replication errors. *J Biol Chem*. **276:** *2317-2320*

Bebenek K, Tissier A, Frank EG, McDonald JP, Prasad R, Wilson SH, Woodgate R, Kunkel TA (2001b). 5'-Deoxyribose phosphate lyase activity of human DNA polymerase iota in vitro. *Science*. **291:** *2156-2159*

Bi X, Slater DM, Ohmori H, Vaziri C (2005). DNA polymerase kappa is specifically required for recovery from the benzo[a]pyrene-dihydrodiol epoxide (BPDE)-induced S-phase checkpoint. *J Biol Chem*. **280:** *22343-22355*

Bienko M, Green CM, Sabbioneda S, Crosetto N, Matic I, Hibbert RG, Begovic T, Niimi A, Mann M, Lehmann AR *et al.* (2010). Regulation of translesion synthesis DNA polymerase eta by monoubiquitination. *Mol Cell*. **37:** *396-407*

de Boer J, Andressoo JO, de Wit J, Huijmans J, Beems RB, van Steeg H, Weeda G, van der Horst GT, van Leeuwen W, Themmen AP *et al.* (2002). Premature aging in mice deficient in DNA repair and transcription. *Science*. **296:** *1276-1279*

de Groote FH, Jansen JG, Masuda Y, Shah DM, Kamiya K, de Wind N, Siegal G (2011). The Rev1 translesion synthesis polymerase has multiple distinct DNA binding modes. *DNA Repair (Amst)*. **10:** *915-925*

Diamant N, Hendel A, Vered I, Carell T, Reissner T, de Wind N, Geacinov N, Livneh Z (2012). DNA damage bypass operates in the S and G2 phases of the cell cycle and exhibits differential mutagenicity. *Nucleic Acids Res*. **40:** *170-180*

Edmunds CE, Simpson LJ, Sale JE (2008). PCNA ubiquitination and REV1 define temporally distinct mechanisms for controlling translesion synthesis in the avian cell line DT40. *Mol Cell*. **30:** *519-529*

Freudenthal BD, Gakhar L, Ramaswamy S, Washington MT (2010). Structure of monoubiquitinated PCNA and implications for translesion synthesis and DNA polymerase exchange. *Nat Struct Mol Biol*. **17:** *479-484*

Gullberg M, Fredriksson S, Taussig M, Jarvius J, Gustafsdottir S, Landegren U (2003). A sense of closeness: protein detection by proximity ligation. *Curr Opin Biotechnol*. **14:** *82-86*

Haracska L, Prakash L, Prakash S (2002). Role of human DNA polymerase kappa as an extender in translesion synthesis. *Proc Natl Acad Sci U S A*. **99:** *16000-16005*

Hasty P, Campisi J, Hoeijmakers J, van Steeg H, Vijg J (2003). Aging and genome maintenance: lessons from the mouse? *Science*. **299:** *1355-1359*

Jansen JG, Fousteri MI, de Wind N (2007). Send in the clamps: control of DNA translesion synthesis in eukaryotes. *Mol Cell*. **28:** *522-529*

Jansen JG, Tsaalbi-Shtylik A, Hendriks G, Gali H, Hendel A, Johansson F, Erixon K, Livneh Z, Mullenders LH, Haracska L *et al.* (2009a). Separate domains of Rev1 mediate two modes of DNA damage bypass in mammalian cells. *Mol Cell Biol*. **29:** *3113-3123*

Jansen JG, Tsaalbi-Shtylik A, Hendriks G, Verspuy J, Gali H, Haracska L, de Wind N (2009b). Mammalian polymerase zeta is essential for post-replication repair of UV-induced DNA lesions. *DNA Repair (Amst)*. **8:** *1444-1451*

Johnson RE, Prakash L, Prakash S (2012). Pol31 and Pol32 subunits of yeast DNA polymerase delta are also essential subunits of DNA polymerase zeta. *Proc Natl Acad Sci U S A*. **109:** *12455-12460*

Kai M, Wang TS (2003). Checkpoint activation regulates mutagenic translesion synthesis. *Genes Dev*. **17:** *64-76*

Kim H, Yang K, Dejsuphong D, D'Andrea AD (2012). Regulation of Rev1 by the Fanconi anemia core complex. *Nat Struct Mol Biol*. **19:** *164-170*

Kim MS, Machida Y, Vashisht AA, Wohlschlegel JA, Pang YP, Machida YJ (2013). Regulation of error-prone translesion synthesis by Spartan/C1orf124. *Nucleic Acids Res*. **41:** *1661-1668*

Kirsch-Volders M, Plas G, Elhajouji A, Lukamowicz M, Gonzalez L, Vande Loock K, Decordier I (2011). The in vitro MN assay in 2011: origin and fate,

biological significance, protocols, high throughput methodologies and toxicological relevance. *Arch Toxicol*. **85:** *873-899*

Lagouge M, Larsson NG (2013). The role of mitochondrial DNA mutations and free radicals in disease and ageing. *J Intern Med*. **273:** *529-543*

LeDoux SP, Wilson GL, Beecham EJ, Stevnsner T, Wassermann K, Bohr VA (1992). Repair of mitochondrial DNA after various types of DNA damage in Chinese hamster ovary cells. *Carcinogenesis*. **13:** *1967-1973*

Lopes M, Foiani M, Sogo JM (2006). Multiple mechanisms control chromosome integrity after replication fork uncoupling and restart at irreparable UV lesions. *Mol Cell*. **21:** *15-27*

Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013). The hallmarks of aging. *Cell*. **153:** *1194-1217*

Lossaint G, Larroque M, Ribeyre C, Bec N, Larroque C, Decaillet C, Gari K, Constantinou A (2013). FANCD2 Binds MCM Proteins and Controls Replisome Function upon Activation of S Phase Checkpoint Signaling. *Mol Cell*. **51:** *678-690*

Lukas C, Savic V, Bekker-Jensen S, Doil C, Neumann B, Pedersen RS, Grofte M, Chan KL, Hickson ID, Bartek J *et al.* (2011). 53BP1 nuclear bodies form around DNA lesions generated by mitotic transmission of chromosomes under replication stress. *Nat Cell Biol*. **13:** *243-253*

Majka J, Binz SK, Wold MS, Burgers PM (2006). Replication protein A directs loading of the DNA damage checkpoint clamp to 5'-DNA junctions. *J Biol Chem*. **281:** *27855-27861*

Murakami-Sekimata A, Huang D, Piening BD, Bangur C, Paulovich AG (2010). The Saccharomyces cerevisiae RAD9, RAD17 and RAD24 genes are required for suppression of mutagenic post-replicative repair during chronic DNA damage. *DNA Repair (Amst)*. **9:** *824-834*

Nam EA, Cortez D (2011). ATR signalling: more than meeting at the fork. *Biochem J*. **436:** *527-536*

Niedzwiedz W, Mosedale G, Johnson M, Ong CY, Pace P, Patel KJ (2004). The Fanconi anaemia gene FANCC promotes homologous recombination and error-prone DNA repair. *Mol Cell*. **15:** *607-620*

Paulovich AG, Armour CD, Hartwell LH (1998). The Saccharomyces cerevisiae RAD9, RAD17, RAD24 and MEC3 genes are required for tolerating irreparable, ultraviolet-induced DNA damage. *Genetics*. **150:** *75-93*

Petta TB, Nakajima S, Zlatanou A, Despras E, Couve-Privat S, Ishchenko A, Sarasin A, Yasui A, Kannouche P (2008). Human DNA polymerase iota protects cells against oxidative stress. *EMBO J*. **27:** *2883-2895*

Prakash S, Prakash L (2002). Translesion DNA synthesis in eukaryotes: a one- or two-polymerase affair. *Genes Dev*. **16:** *1872-1883*

Riballo E, Kuhne M, Rief N, Doherty A, Smith GC, Recio MJ, Reis C, Dahm K, Fricke A, Krempler A *et al.* (2004). A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to gamma-H2AX foci. *Mol Cell*. **16:** *715-724*

Sabbioneda S, Minesinger BK, Giannattasio M, Plevani P, Muzi-Falconi M, Jinks-Robertson S (2005). The 9-1-1 checkpoint clamp physically interacts with polzeta and is partially required for spontaneous polzeta-dependent mutagenesis in Saccharomyces cerevisiae. *J Biol Chem*. **280:** *38657-38665*

Shachar S, Ziv O, Avkin S, Adar S, Wittschieben J, Reissner T, Chaney S, Friedberg EC, Wang Z, Carell T *et al.* (2009). Two-polymerase mechanisms dictate error-free and error-prone translesion DNA synthesis in mammals. *EMBO J*. **28:** *383-393*

Sharma S, Hicks JK, Chute CL, Brennan JR, Ahn JY, Glover TW, Canman CE (2012). REV1 and polymerase zeta facilitate homologous recombination repair. *Nucleic Acids Res*. **40:** *682-691*

Sirbu BM, Couch FB, Cortez D (2012). Monitoring the spatiotemporal dynamics of proteins at replication forks and in assembled chromatin using isolation of proteins on nascent DNA. *Nat Protoc*. **7:** *594-605*

Sirbu BM, McDonald WH, Dungrawala H, Badu-Nkansah A, Kavanaugh GM, Chen Y, Tabb DL, Cortez D (2013). Identification Of Proteins At Active, Stalled, And Collapsed Replication Forks Using Isolation Of Proteins On Nascent DNA (iPOND) Coupled With Mass Spectrometry. *J Biol Chem*.

Szuts D, Marcus AP, Himoto M, Iwai S, Sale JE (2008). REV1 restrains DNA polymerase zeta to ensure frame fidelity during translesion synthesis of UV photoproducts in vivo. *Nucleic Acids Res*. **36:** *6767-6780*

Tsaalbi-Shtylik A, Verspuy JW, Jansen JG, Rebel H, Carlee LM, van der Valk MA, Jonkers J, de Gruijl FR, de Wind N (2009). Error-prone translesion replication of damaged DNA suppresses skin carcinogenesis by controlling inflammatory hyperplasia. *Proc Natl Acad Sci U S A*. **106:** *21836-21841*

Van Sloun PP, Varlet I, Sonneveld E, Boei JJ, Romeijn RJ, Eeken JC, De Wind N (2002). Involvement of mouse Rev3 in tolerance of endogenous and exogenous DNA damage. *Mol Cell Biol*. **22:** *2159-2169*

Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, Zhang F, Jaenisch R (2013). One-step generation of mice carrying mutations in multiple genes by

CRISPR/Cas-mediated genome engineering. *Cell*. **153:** *910-918*

Waters LS, Minesinger BK, Wiltrout ME, D'Souza S, Woodruff RV, Walker GC (2009). Eukaryotic translesion polymerases and their roles and regulation in DNA damage tolerance. *Microbiol Mol Biol Rev*. **73:** *134-154*

Wittschieben J, Shivji MK, Lalani E, Jacobs MA, Marini F, Gearhart PJ, Rosewell I, Stamp G, Wood RD (2000). Disruption of the developmentally regulated Rev3l gene causes embryonic lethality. *Curr Biol*. **10:** *1217-1220*

Wu X, Takenaka K, Sonoda E, Hochegger H, Kawanishi S, Kawamoto T, Takeda S, Yamazoe M (2006). Critical roles for polymerase zeta in cellular tolerance to nitric oxide-induced DNA damage. *Cancer Res*. **66:** *748-754*

Yoon JH, Prakash L, Prakash S (2009). Highly error-free role of DNA polymerase eta in the replicative bypass of UV-induced pyrimidine dimers in mouse and human cells. *Proc Natl Acad Sci U S A*. **106:** *18219-18224*

Yoon JH, Prakash L, Prakash S (2010). Error-free replicative bypass of (6-4) photoproducts by DNA polymerase zeta in mouse and human cells. *Genes Dev*. **24:** *123-128*

Yu X, Chini CC, He M, Mer G, Chen J (2003). The BRCT domain is a phospho-protein binding domain. *Science*. **302:** *639-642*

Zhang H, Chatterjee A, Singh KK (2006). Saccharomyces cerevisiae polymerase zeta functions in mitochondria. *Genetics*. **172:** *2683-2688*

Zhang Y, Wu X, Guo D, Rechkoblit O, Wang Z (2002). Activities of human DNA polymerase kappa in response to the major benzo[a]pyrene DNA adduct: error-free lesion bypass and extension synthesis from opposite the lesion. *DNA Repair (Amst)*. **1:** *559-569*