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PCNA Modifications for Regulation of Post-Replication Repair Pathways

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Abstract

Stalled DNA replication forks activate specific DNA repair mechanism called post-replication repair (PRR) pathways that simply bypass DNA damage. The bypassing of DNA damage by PRR prevents prolonged stalling of DNA replication that could result in double strand breaks (DSBs). Proliferating cell nuclear antigen (PCNA) functions to initiate and choose different bypassing pathways of PRR. In yeast, DNA replication forks stalled by DNA damage induces monoubiquitination of PCNA at K164, which is catalyzed by Rad6/Rad18 complex. PCNA monoubiquitination triggers the replacement of replicative polymerase with special translesion synthesis (TLS) polymerases that are able to replicate past DNA lesions. The PCNA interaction motif and/or the ubiquitin binding motif in most TLS polymerases seem to be important for the regulation of TLS. The TLS pathway is usually error-prone because TLS polymerases have low fidelity and no proofreading activity. PCNA can also be further polyubiquitinated by Ubc13/Mms2/Rad5 complex, which adds an ubiquitin chain onto monoubiquitinated K164 of PCNA. PCNA polyubiquitination directs a different PRR pathway known as error-free damage avoidance, which uses the newly synthesized sister chro matid as a template to bypass DNA damage presumably through template switching mechanism. Mammalian homologues of all of the yeast PRR proteins have been identified, thus PRR is well conserved throughout evolution. Mutations of some PRR genes are associated with a higher risk for cancers in mice and human patients, strongly supporting the importance of PRR as a tumor suppressor pathway.

Keywords

damage bypass; PCNA ubiquitination; post-replication repair; RAD6/RAD18 complex; template switching; translesion synthesis; UBC13/MMS2/RAD5 complex

INTRODUCTION

The most important task for dividing cells is preserving its genetic information during DNA replication. However, cells constantly encounter many types of genotoxic challenges that can result in mutations that can alter genetic information. Such challenges include exogenous genotoxic insults, such as radiation and toxic chemicals, and endogenous cellular byproducts, such as cellular metabolites and reactive oxygen species. Spontaneous errors during DNA replication are another threat to the maintenance of genomic integrity. DNA lesions that cannot be accommodated into the active sites of replicative DNA polymerases in S phase are extremely dangerous because such DNA lesions block the progression of DNA replication forks. Prolonged stalling of DNA replication can result in collapse of DNA

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replication forks and the concurrent production of double strand breaks (DSBs), and/or gross chromosomal rearrangements, which can lead to cell death.

Post-replication repair (PRR) suppresses prolonged stalling of DNA replication by bypassing DNA lesions (Barbour and Xiao, 2003), thereby allowing DNA replication to continue. DNA lesions remaining after bypass are repaired later, in G2 phase, by various DNA repair mechanism such as base excision repair, nucleotide excision repair, or homologous recombination.

There are two known DNA damage bypassing PRR pathways in eukaryotes. The first pathway known as translesion synthesis (TLS) uses specialized DNA polymerases for bypassing DNA lesions. In response to DNA damage, the replicative DNA polymerase (Pol δ or Pol ε) are replaced by translesion polymerases. These specialized polymerases can add nucleotides opposite DNA lesions because they have a more open active site that can accommodate the bulky lesions. Due to their accommodating active site, these polymerases are very low in fidelity and incorporate both correct and incorrect nucleotides opposite the lesion. Thus, even though TLS is an efficient way to bypass DNA damage, it is intrinsically error-prone and can cause mutations. In contrast to TLS, the second pathway known as damage avoidance pathway is error-free and probably uses the replicated, undamaged sister chromatid for template by template switching mechanism. However, the exact mechanism or downstream proteins participating in this pathway is largely unknown. The proposed nature of error-free bypassing mechanism and the requirement of Rad52 (Gangavarapu et al., 2007) suggest it might share some features of homologous recombination.

The activation mechanism of bypassing pathway was a long-lived mystery. Recently, however, many studies in yeast and human cells have revealed the direct links between the initiation of both bypassing pathways and the covalent modifications of proliferating cell nuclear antigen (PCNA) by ubiquitin.

PCNA modification in DNA repair pathway

PCNA forms a doughnut-shaped homotrimeric clamp structure that encircles DNA. PCNA serves as a processivity factor for DNA polymerases and a platform for binding of many proteins involved in DNA replication and repair. In yeast, PCNA activity can be regulated by covalent linkage to the small protein modifiers, ubiquitin (Ub) and small Ub-like modifier (SUMO) (Fig. 1). PCNA modification not only promotes the replication fork to bypass DNA lesions but also determines whether bypass modes operate in an error-prone or an error-free manner.

In response to DNA damage, PCNA is monoubiquitinated at the Lysine (K) 164 residue by the E2 Ub conjugating enzyme Rad6 and the E3 Ub ligase Rad18 (Rad6/Rad18 complex) (Fig. 1) (Hoege et al., 2002). Rad18 not only binds to Rad6 and PCNA, but also to DNA (Bailly et al., 1997). Thus, Rad18 recruits the ubiquitination machinery to the chromatin-bound target, PCNA. Biochemical experiments indicate that PCNA ubiq uitination is limited to PCNA that has been loaded onto DNA by replication factor C (Garg and Burgers, 2005). Since PCNA is ubiquitinated in response to DNA damage, it is believed that PCNA monoubiquitination occurs at DNA lesions that stall DNA replication forks. This PCNA monoubiquitination promotes error-prone TLS pathway.

Monoubiquitinated PCNA can be further polyubiquitinated by Ubc13 and Mms2 (E2 and E2 variant, respectively) and E3 Ub ligase Rad5 by extending ubiquitin chain from the monoubiquitinated K164 residue (Fig. 1). The polyubiquitin chain in PCNA is linked through the K63 residue of Ub (Hoege et al., 2002). Unlike the canonical polyubiquitin chain linked through K48 of Ub, the non-canonical K63 polyubiquitin chain does not

promote proteosomal degradation (Spence et al., 1995). Rad5 can bind DNA and has a SWI/SNF ATPase/helciase domain and a RING domain for E3 Ub ligase activity (Johnson et al., 1992). Ubc13 and Mms2 form a heterodimeric E2 enzyme that catalyzes the building of the K63-linked polyubiquitin chain. This PCNA polyubiquitination promotes error-free damage avoidance pathway. The ubiquitination complexes, Rad6/Rad18 and Ubc13/Mms2/Rad5 sequentially ubiquitinate PCNA, however, the regulation of their distinct pathways is not clearly understood.

PCNA can also be sumoylated at K164 and K127 residue. This process requires another E2/E3 complex Ubc9/Siz1 (Hoege et al., 2002; Stelter and Ulrich, 2003). Sumoylated PCNA is known to recruit the helicase Srs2, which prevents inappropriate homologous recombination by disrupting Rad51-ssDNA filaments (Haracska et al., 2004; Papouli et al., 2005; Pfander et al., 2005).

Translesion synthesis polymerases

In eukaryotes, five special polymerases have been identified that perform translesion DNA synthesis (Prakash et al., 2005). Four of them Pol η, Pol ι, Pol κ, and REV1 belong to the Y-family of DNA polymerases, which have the characteristics of low fidelity and processivity and lack proofreading exonuclease activity. They all have relatively non-restrictive active sites compared to replicative DNA polymerases, which allow them to accommodate DNA mispairs and bulky lesions. (Lehmann et al., 2007; Prakash et al., 2005). Pol ζ consists of a catalytic subunit REV3 that belongs to the B-family of DNA polymerase and an accessory factor REV7 (Nelson et al., 1996).

Pol η, which is found in all eukaryotes, incorporates nucleotides opposite UV-induced cyclobutane pyrimidine dimers (CPD) in a relatively error-free manner, despite the distortion caused by the cyclobutane ring (Masutani et al., 2000; McCulloch et al., 2004b). However, Pol η misincorporates a guanine (G) opposite a thymine-thymine (T-T) CPD or undamaged T-T bipyrimidine, up to once in every 25 TLS events *in vitro* (McCulloch et al., 2004a; Washington et al., 2001). Mutations of Pol η cause the variant form of Xeroderma Pigmentosum (XP-V), which has a very high risk of sunlight-induced skin cancer. XP-V cells have a greatly increased frequency of UV-induced mutations (Cordonnier and Fuchs, 1999; Johnson et al., 1999; Masutani et al., 1999). Pol η is the first DNA polymerase demonstrated to act as a tumor suppressor in humans.

REV1, found in all eukaryotes, is a dCMP transferase that can insert cytosine (C) opposite either Gs or abasic sites *in vitro* and *in vivo* (Jansen et al., 2006; Lawrence et al., 2000). REV1 is essential for many types of damage-induced mutagenesis (Jansen et al., 2006; Lawrence et al., 2000; Wood et al., 2007), but interestingly its catalytic activity is not required for mutagenesis (Otsuka et al., 2005). Since REV1 interacts with the other three Y-family polymerases as well as the REV7 subunit of Pol ζ (Guo et al., 2003; Murakumo et al., 2001; Ohashi et al., 2004; Tissier et al., 2004), REV1 is thought to function as a scaffold for other TLS polymerases during mutagenic TLS.

Pol ι is a TLS polymerase found only in vertebrates and has very low processivity, which is reflected by its inability to extend after the incorporation of a single base opposite DNA lesion (Johnson et al., 2000). It has a very high error rate, particularly at the incorporation opposite T, at which it inserts G in preference instead of the correct adenine (A) (Tissier et al., 2000). Pol ι can also incorporate bases opposite minor-groove adducts via Hoogsteen base pairing, despite low efficiency (Choi and Guengerich, 2006). Although Pol ι physically interacts with Pol η (Kannouche et al., 2003), the effect of Pol ι on the UV-induced mutagenesis showed controversial outcomes (Choi et al., 2006; Dumstorf et al., 2006); one group showed that Pol ι deficiency reduced the UV-induced mutagenesis and the other

group showed that knockdown of Pol ι did not affect the mutant frequency by UV irradiation.

Pol κ, present in *Schizosaccharomyces pombe* (*S. pombe*) and vertebrates but absent in *Saccharomyces cerevisiae* (*S. cerevisiae*), is able to bypass polycyclic aromatic hydrocarbons like benzo[a]pyrene-guanine. Therefore, Pol κ deficiency makes cells sensitive to these types of chemicals (Ogi et al., 2001). Although Pol κ could not bypass UV photoproducts in *in vitro* systems, Pol κ-deficient mouse cells are sensitive to UV light, possibly due to reduced nucleotide excision repair (NER) activity (Ogi and Lehmann, 2006). Pol κ is located at DNA replication foci only in a small proportion of S-phase cells, unlike all other Y-family polymerases for TLS (Ogi et al., 2005).

Pol ζ, found in all eukaryotes, lacks the proofreading exonuclease activity which is found in other B-family of DNA polymerases like Pol δ and Pol ε. Interestingly, Pol ζ has higher fidelity compared to Y-family polymerases (Haracska et al., 2001; Johnson et al., 2000), but is indispensable for UV-induced mutagenesis (Lawrence et al., 1984). In yeast, Pol ζ is not essential for cell viability (Johnson et al., 1998); However, the disruption of murine Rev3 caused embryonic lethality, suggesting that Pol ζ may have more complex functions in higher organisms (Bemark et al., 2000; Esposito et al., 2000; Wittschieben et al., 2000).

PCNA monoubiquitination and TLS

PCNA monoubiquitination has been proven to promote a TLS pathway by most Y family polymerases. The relationship between PCNA modification and TLS pathway was first demonstrated by the activation of the TLS pathway by the RAD6-mediated PCNA monoubiquitination in yeast (Stelter and Ulrich, 2003). The PCNA ubiquitination dependent activation of two TLS polymerases Pol η and REV1 was further demonstrated in an *in vitro* purified yeast system (Garg and Burgers, 2005). The importance of PCNA ubiquitination for TLS pathway in mammals can be appreciated from observations that human PCNA become monoubiquitinated following UV irradiation in a RAD18 dependent manner and Pol η specifically interacts with monoubiquitinated PCNA (Kannouche et al., 2004). In addition, Pol η localizes to sites of DNA damage and interacts with monoubiquitinated PCNA in a RAD18 dependent manner in mice (Watanabe et al., 2004).

The regulation of TLS by PCNA monoubiquitination might be mediated by specific ubiquitin binding motifs (UBMs) in TLS polymerases. All Y family TLS polymerases have these motifs, which can bind to the Ub moiety on PCNA. For example, Pol η and Pol κ contain a UBZ (Ubiquitin Binding Zinc finger) motif, whereas Pol ι and REV1 contain one and two UBM motifs, respectively (Bienko et al., 2005). It has been suggested that these UBM or UBZ motifs are important for function of TLS polymerase in damaged cells (Bienko et al., 2005; Guo et al., 2006; Parker et al., 2007; Plosky et al., 2006; Wood et al., 2007). Specifically, UBMs were required for the localization of Pol η, Pol ι, and REV1 to replication foci and for their association with PCNA. At least in the case of Pol η and REV1, TLS activities of both TLS polymerases are dependent on the presence of UBMs.

Various TLS polymerases such as Pol η, ι, and κ interact with PCNA via their consensus PCNA interacting peptide (PIP) motif, which binds the inter-domain connector loop of PCNA. Many genetic and biochemical studies demonstrated that these domains required for PCNA binding are also important for TLS functions of these polymerases (Prakash et al., 2005). Based on the two PCNA interaction motifs (PIP and UBM/UBZ) in TLS polymerases, it has been postulated that there are two types of interacting modes for the regulation of TLS by PCNA modification. The first interaction is the one between the PIP motif in TLS polymerases and the inter-domain connector loop motif in PCNA. The second interaction between the UBM/UBZ motif in TLS polymerase and the K164-attached Ub

moiety in PCNA could further stabilize the interaction. However, this idea has recently been challenged by several reports indicating that the activation of TLS polymerases by PCNA ubiquitination is not direct process. PCNA ubiquitination does not affect the interaction of Pol δ with PCNA or the binding affinity of the TLS polymerases like Pol η, Pol ζ, and REV1 to PCNA (Haracska et al., 2006). In addition, PCNA ubiquitination does not stimulate TLS activities of Pol η *in vitro.* The mutations in the UBZ motif also do not affect the stimulation of DNA synthesis by Pol η with PCNA or Ub-conjugated PCNA (Acharya et al., 2007). Lastly, PCNA ubiquitination at K164 does not affect the accessibility of Pol η to DNA replication forks stalled by T-T CPD or the *in vitro* catalytic activity of Pol η across this DNA lesion (Nikolaishvili-Feinberg et al., 2008). Therefore, the major role of PCNA monoubiquitination may be to alter the interaction affinity of the replicative polymerase or accessory proteins with PCNA, thereby allowing Pol η or other TLS polymerases to interact PCNA via their PIP motifs.

TLS polymerases provide an important function by allowing cells to bypass DNA lesions that would otherwise lead to DSBs. However, the error-prone nature of TLS polymerases demands cells to tightly regulate the usage of TLS polymerases. The molecular mechanism of exchange between replicative DNA polymerase and TLS polymerases at DNA damage sites is beginning to be uncovered. The exchange of Pol δ with Pol η requires both the stalling of DNA replication holoenzyme and PCNA monoubiquitination in a reconstituted yeast system (Zhuang et al., 2008). In addition, the reverse exchange step to bring back Pol δ is inhibited when the K164 of PCNA is occupied by Ub, suggesting that PCNA deubiquitination is likely required for the reverse exchange step after lesion bypass by Pol η (Zhuang et al., 2008). Recently, ubiquitin specific protease 1 (USP1) was identified in a siRNA screening as a putative deubiquitination (DUB) enzyme for PCNA (Huang et al., 2006). Thus USP1 may catalyze the reverse exchange step by deubiquitinating PCNA, and allow access by the replicative polymerase.

Signaling for PCNA monoubiquitination

Both the E3 Ub ligase RAD18 and the DUB enzyme USP1 regulate the level of PCNA ubiquitination. When a DNA replication fork stalls at DNA lesions, uncoupling between the stalled DNA replicative polymerase and the MCM helicases occurs. This results in the accumulation of single-stranded DNA (ssDNA), which will be subsequently coated by the hetero-trimeric ssDNA-binding protein, replication protein A (RPA). RAD18 can bind to ssDNA coated with RPA (Bailly et al., 1997; Hoege et al., 2002; Watanabe et al., 2004). Therefore, it is thought that ssDNA at the stalled DNA replication forks would activate RAD6/RAD18 pathway and promote PCNA ubiquitination. Recently, it was reported that RPA is important for damage-induced PCNA ubiquitination (Davies et al., 2008). RPA directly interacted with Rad18 both in yeast and mammalian cells and recruited Rad18 to ssDNA *in vitro*. However, the direct DNA binding by Rad18 seems also important for PCNA ubiquitination, because a Rad18 mutant defective in DNA binding could not ubiquitinate PCNA (Davies et al., 2008).

The level of PCNA ubiquitination in cells is also regulated by the DUB enzyme USP1, which removes the ubiquitin from monoubiquitinated PCNA (Huang et al., 2006). In response to DNA damage, USP1 is degraded by an autocleavage mechanism, which in turn promotes the enhancement of ubiquitinated PCNA in cells. In contrast, the ectopic expression of a non-degradable USP1 decreased the level of damage-induced PCNA monoubiquitination (Huang et al., 2006). Therefore, the role of USP1 is to keep PCNA ubiquitination at a low level in undamaged cells to prevent the unwanted employment of TLS polymerases that could result in mutagenesis.

RPA-coated ssDNA is a signal for the activation of checkpoint kinase ataxia telangiectasia RAD3-related (ATR) as well as RAD6/RAD18 pathway (Byun et al., 2005). The ATR activation is mediated by the interaction between RPA and ATRIP, an interacting protein for ATR. The alternative sliding clamp, RAD9/HUS1/RAD1 (termed 9-1-1 complex; Ddc1/Mec3/Rad17 in *S. cerevisiae,* respectively) is also recruited to stalled replication forks where RPA-coated ssDNA is accumulated (Parrilla-Castellar et al., 2004). Many attempts to find putative regulatory mechanism between ATR or 9-1-1 signaling and PCNA ubiquitination led to a conclusion that both pathways seem to exist in a mutually independent manner. The loss of ATR or 9-1-1 signaling by the depletion of ATRIP or RAD1 expression respectively did not impair PCNA ubiquitination in Xenopus egg extract (Chang et al., 2006). In *S. pombe* and *S. cerevisiae,* the activations of PCNA modification and cell cycle checkpoints are independent each other responding to DNA damage (Davies et al., 2008; Frampton et al., 2006). However, a recent report demonstrated a possible regulation mechanism of PCNA ubiquitination by CHK1, an effecter kinase of ATR (Yang et al., 2008). This study demonstrated that CHK1 was important for the DNA damage-induced PCNA ubiquitination, independent of ATR and ataxia telangiectasia mutated (ATM). It was not the kinase activity of CHK1 that is important for PCNA ubiquitination but the interaction between CHK1 and DNA replication protein Claspin, which was stabilized by CHK1 and promoted the binding of RAD18 to chromatin.

PCNA ubiquitination might be regulated by tumor suppressor protein p53 and downstream cyclin-dependent kinase inhibitor p21. It was suggested by the observation of the increased efficiency and the decreased fidelity of TLS in p53 or p21-deficient mouse fibroblast cells (Avkin et al., 2006). In addition, the knockdown of p53 or p21 expression reduced PCNA ubiquitination. In contrast, the overexpression of a non-degradable mutant p21 suppressed PCNA ubiquitination (Soria et al., 2006). This suppression was not mediated by direct interaction with PCNA, because a mutant p21 defective in binding to PCNA also gave a similar inhibition of PCNA ubiquitination. Despite the contradictory results between these two reports, it appears that damage induced PCNA ubiquitination is affected by p21.

PCNA polyubiquitination and error-free PRR pathway

In yeast, the stalling of DNA replication fork promotes PCNA polyubiquitination via a non-canonical K63 linkage to monoubiquitinated K164 residue in PCNA (Frampton et al., 2006; Hofmann and Pickart, 1999; Ulrich and Jentsch, 2000). The heterodimeric E2 enzyme, Ubc13/Mms2 and an E3 ligase Rad5 catalyze PCNA polyubiquitination (Fig. 1) (Frampton et al., 2006; Hofmann and Pickart, 1999; Ulrich and Jentsch, 2000).

Recently, PCNA polyubiquitination has been observed in mammalian cells. PCNA is polyubiquitinated in UV-irradiated human cells, which is dependent on UBC13 (Chiu et al., 2006). Furthermore, the inhibition of K63 polyubiquitin chain formation increases TLS-mediated mutations, suggesting that PCNA polyubiquitination can suppress the TLS pathway mediated by PCNA monoubiquitination (Chiu et al., 2006). Although the yeast and human PRR pathways are well conserved, there seems to be an important difference with regards to protein requirements. While yeast Mms2 is absolutely required for PCNA polyubiquitination, knockdown of MMS2 in human cells still shows PCNA polyubiquitination (Brun et al., 2008). This is in contrast to strong suppression of PCNA polyubiquitination by knockdown of RAD18 or UBC13 (Brun et al., 2008). In addition, PCNA polyubiquitination is observed in mms2-null mouse embryonic stem cells (Brun et al., 2008). Therefore, it appears that there might be redundancies in the damage avoidance pathway in mammalian cells compared to yeast.

SHPRH was recently identified as a putative mammalian homologue of yeast RAD5 (Motegi et al., 2006; Unk et al., 2006). Human SHPRH belongs to the SWI/SNF family of

AT-Pases and contains a C_3HC_4-type RING-finger motif that is located between the conserved SWI/SNF helicase domains as in yeast Rad5 (Sood et al., 2003). A number of mutations of *SHPRH* gene are found in many types of tumor cell lines, indicating that SHPRH may be a tumor suppressor gene (Motegi et al., 2006; Unk et al., 2006). Like yeast Rad5, SHPRH physically interacts with the RAD6/RAD18 and UBC13/MMS2 complexes (Motegi et al., 2006) and promoted PCNA polyubiquitination at its K164 in RAD18-dependent manner *in vivo* (Motegi et al., 2006) and *in vitro* (Unk et al., 2006). In addition, the reduced expression of SHPRH by shRNA knockdown enhances DNA damage sensitivity and genomic instability.

Recently, another putative mammalian homologue of yeast Rad5 was identified (Motegi et al., 2008; Unk et al., 2008). HLTF has slightly higher sequence homology with yeast Rad5 than SHPRH. Similar to SHPRH, HLTF contains a RING domain and the SWI/SNF helicase domain. HLTF also has a HIRAN domain that has been predicted to function as a sensor for DNA damage and/or an initiator for DNA repair activities (Iyer et al., 2006). Interestingly, this domain is present in yeast Rad5, but not in SHPRH. The expression of HLTF is frequently silenced in many colorectal tumors by the methylation of its promoter suggesting HLTF could be a tumor suppressor gene (Bird, 2002). HLTF physically interacts with PCNA, RAD6/RAD18 and UBC13/MMS2 complexes and promotes the K63-linked polyubiquitination of PCNA at its K164 residue, similar to SHPRH and yeast Rad5 (Motegi et al., 2008). Furthermore, the de-repression of HLTF expression in colorectal cancer cells enhances PCNA polyubiquitination (Motegi et al., 2008). Intriguingly, HLTF and SHPRH interacts with one another suggesting possible interplay between the two mammalian Rad5 homologs in the regulation of PCNA polyubiquitination (Motegi et al., 2008). The reduced expression of HLTF in human or mouse cells increased sensitivity to DNA damage and genomic instability.

The evidence from both yeast and human cells supports a distinct role of the UBC13/MMS2/RAD5 complex in promoting an error-free damage avoidance pathway that is distinct from the TLS pathway. In yeast, the separation of the error-free PRR pathway from the TLS pathway is supported by synergistic UV sensitivity of *ubc13Δ* or *rad5Δ* strain by an additional *rev3Δ* mutation (Brusky et al., 2000). In addition, the *rad5Δ, ubc13Δ,* or *mms2Δ* mutation increased spontaneous or damage-induced mutagenesis in a REV3 dependent manner (Broomfield et al., 1998; Brusky et al., 2000; Johnson et al., 1992). Pol η also contributes to error-free bypass for UV damage in addition to the RAD5 pathway. Consequently, the frequency of UV induced mutations rises dramatically when both the RAD5-dependent PRR and Pol η-dependent TLS pathways were simultaneously inactivated (McDonald et al., 1997). In human, the reduced expression of human MMS2 increases the frequency of UV-induced mutagenesis about 2-fold without increasing UV-induced cell death (Li et al., 2002). Consistent with this result, the inhibition of K63 polyubiquitination also increases the frequency of UV-induced mutagenesis (Chiu et al., 2006). Two to three folds induction of mutagenesis by the reduced expression of SHPRH or HLTF in human cells (Motegi et al., 2008) supports that SHPRH/HLTF also function similarly in promoting UBC13/MMS2-dependent PCNA polyubiquitination for an error-free damage avoidance PRR in a similar manner to that of Rad5 in yeast.

Mechanistically the error-free damage avoidance PRR pathway is suggested to use the newly synthesized sister chromatid as a template for bypass and a template switching or copy-choice mechanism involving homologous sister chromatid invasion, DNA synthesis, and the resolution of the Holliday junction (Lawrence, 1994). Unfortunately, the exact mechanism of the error-free damage avoidance PRR pathway is not clearly understood because some genes directly involved in it have not been identified. In addition, the transient nature of intermediates formed during the damage avoidance PRR adds more difficulty to

understand the mechanism because conventional genetic methods cannot easily detect such an event. So far, genetic studies in yeast looking at the template-switch copy choice mode of recombination have demonstrated its requirement for Rad5 (Zhang and Lawrence, 2005).

It is still under debate whether DNA replication fork reversal could be the first step of template switching. The DNA replication fork reversal model, also known as a chicken foot model presumably requires the helicase activity to drive the regression of the DNA replication fork and allow annealing between the replicated sister strand and the stalled strand. In support of this model, the DNA helicase activity of yeast Rad5 can promote *in vitro* DNA replication fork regression by the unwinding and annealing of the nascent and the parental strands (Blastyak et al., 2007). However, several other groups suggested the harmful effect of the regression of stalled DNA replication forks, thereby being usually prevented by the DNA damage checkpoint (Sogo et al., 2002; Tercero and Diffley, 2001). Clearly the mechanism of error-free PRR is complex and much more work is needed to understand this process.

CONCLUDING REMARKS

Studies in yeast and mammals have clearly demonstrated the significance of the PRR pathway in maintaining genomic stability. There are two bypassing mechanism of PRR that allow cells to recover from prolonged stalled DNA replication forks, thereby avoiding dangerous DSBs. The complex interplay between these two pathways is not well understood, but together they inhibit genomic instability that can lead to cell death. In support of this, the high incidence of sunlight-induced skin cancer by the mutation of human Pol η in all XP-V patients and recently identified mammalian homologous of yeast Rad5, SHPRH and HLTF that are putative tumor suppressors strongly suggest the importance of PRR pathways as tumor suppressors.

How do cells balance error-prone mutagenic TLS and error-free damage avoidance PRR and what determines the choices of each pathway? Even though it is not well understood, this paradigm of balance seems to be well conserved from yeast to mammals. The last decade has been an exciting period for the PRR field because of the identification of many PRR proteins and their human homologs and the key observation that PCNA modifications are essential for the PPR mechanism. In next decade, studies about the regulation and function of these proteins and modifications will reveal a more detailed molecular mechanism of this important DNA repair pathway.

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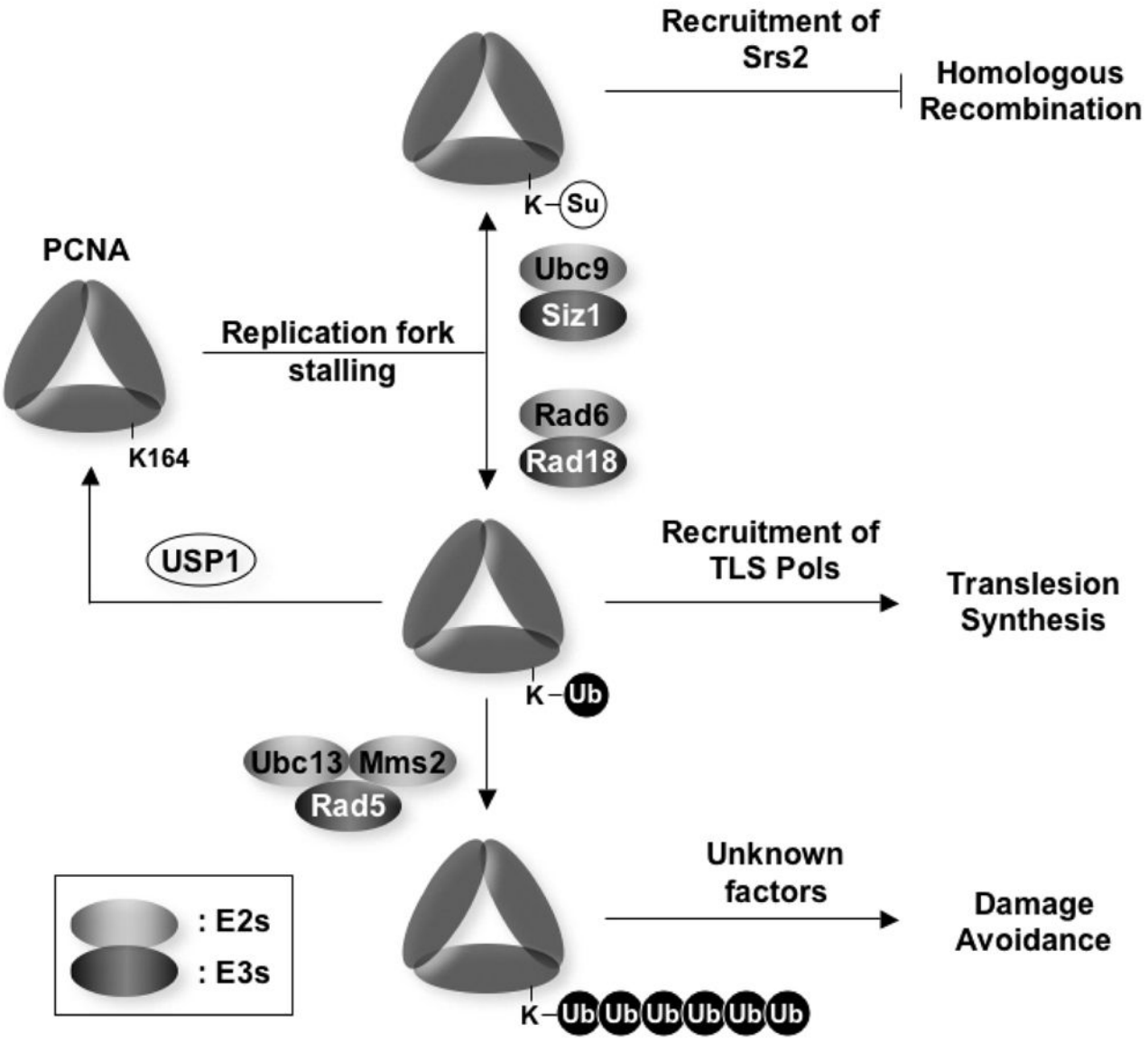

Fig. 1.

Different PCNA modifications determine DNA repair pathways. PCNA can be modified by either sumoylation (Su) or ubiquitination (Ub) at the same K164 residue in response to the stalling of DNA replication fork. The E2/E3 complex Ubc9/Siz1 or Rad6/Rad18 are responsible for each modification, respectively. Monoubiquitinated PCNA can be further polyubiquitinated by the E2/E3 complex Ubc13/Mms2/Rad5 to form a non-canonical K63-linked polyubiquitin chain. The DUB enzyme USP1 removes the ubiquitin from monoubiquitinated PCNA, thereby regulates the level of PCNA ubiquitination. Sumoylated PCNA recruits the Srs2 helicase to block the formation of Rad51-ssDNA filament to prevent inappropriate homologous recombination. PCNA monoubiquitination promotes the recruitment of TLS polymerases (Pols) to facilitate DNA damage bypass, whereas PCNA polyubiquitination is thought to promote the error-free damage avoidance through template switching, although the molecular mechanism is not clearly understood. The model presented in this figure regarding PCNA modification and its function is based on studies

mainly in *S. cerevisiae,* except the role of USP1 in PCNA deubiquitination, which was recently identified in human cells. In mammals, PCNA sumoylation has not been reported and two Rad5 homologs (SHPRH and HLTF) that can promote PCNA polyubiquitination were identified.