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Opposing Effects of Ubiquitin Conjugation and SUMO Modification of PCNA on Replicational Bypass of DNA Lesions in *Saccharomyces cerevisiae*

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The Rad6-Rad18 ubiquitin-conjugating enzyme complex of *Saccharomyces cerevisiae* promotes replication through DNA lesions via three separate pathways that include translesion synthesis (TLS) by DNA polymerases ζ (Polζ) and Polη and postreplicational repair mediated by the Mms2-Ubc13 ubiquitin-conjugating enzyme and Rad5. Here we report our studies with a proliferating cell nuclear antigen (PCNA) mutation, *pol30-119*, which results from a change of the lysine 164 residue to arginine. It has been shown recently that following treatment of yeast cells with DNA-damaging agents, the lysine 164 residue of PCNA becomes monoubiquitinated in a Rad6-Rad18-dependent manner and that subsequently this PCNA residue is polyubiquitinated via a lysine 63-linked ubiquitin chain in an Mms2-Ubc13-, Rad5-dependent manner. PCNA is also modified by SUMO conjugation at the lysine 164 residue. Our genetic studies with the *pol30-119* mutation show that in addition to conferring a defect in Polζ-dependent UV mutagenesis and in Polη-dependent TLS, this PCNA mutation inhibits postreplicational repair of discontinuities that form in the newly synthesized strand across from UV lesions. In addition, we provide evidence for the activation of the *RAD52* recombinational pathway in the *pol30-119* mutant and we infer that SUMO conjugation at the lysine 164 residue of PCNA has a role in suppressing the Rad52-dependent postreplicational repair pathway.

Proliferating cell nuclear antigen (PCNA) is the eukaryotic sliding clamp required for processive DNA synthesis. The *Saccharomyces cerevisiae POL30* gene encoding PCNA is essential for cell viability (7), and conditional lethal *pol30* mutations confer defects in DNA replication (1, 3). PCNA is loaded onto the template-primer junctions of DNA by replication factor C in an ATP-dependent reaction. DNA polymerase δ (Polδ) then binds PCNA and carries out processive DNA synthesis (6, 23). In reconstituted systems containing viral origin sequences, PCNA and Polδ carry out replication of both the leading and lagging strands (26, 38). In addition to its essential role in DNA replication, PCNA has been shown to be required for various DNA repair processes, including nucleotide excision repair (NER), base excision repair, and mismatch repair (23); more recently, PCNA has been shown to interact physically and functionally with the various translesion synthesis (TLS) polymerases of yeasts and humans (9–11, 13).

Genetic studies of *S. cerevisiae* have indicated that PCNA is involved in *RAD6*-dependent error-free postreplicative bypass of UV-damaged DNA (35). Postreplicative bypass processes come into play when the DNA replicational machinery encounters a DNA lesion in the template strand and is unable to replicate past the lesion. Replication of damaged DNA templates can occur by error-free damage avoidance processes in which the undamaged complementary sequence is used to accomplish replication through the damaged site (14) or it may involve TLS by a specialized DNA polymerase across from the lesion. The *S. cerevisiae RAD6* and *RAD18* genes are required for the error-free as well as mutagenic modes of damage bypass (25, 30). Rad6, a ubiquitin-conjugating enzyme, exists in vivo in a tight complex with Rad18, a DNA binding protein (4, 5). Mutations in *RAD6* and *RAD18* confer a high degree of sensitivity to UV light, and they engender a defect in the replication of UV-damaged DNA (28). Also, UV-induced mutagenesis does not occur in either *rad6* or *rad18* mutants (2, 8, 25).

The Rad6-Rad18-mediated ubiquitin conjugation promotes replication through DNA lesions via three different pathways: the Polζ- and Polη-dependent TLS pathways and the Rad5-, Mms2-Ubc13-dependent postreplicational repair pathway (34). The Rev3 and Rev7 proteins together comprise DNA Polζ (27), which promotes TLS by extending from the nucleotides inserted opposite the lesion site by another DNA polymerase (29). For certain DNA lesions (such as, for example, UV lesions and abasic sites), Polζ promotes mutagenic TLS through the lesion (12, 17, 20), whereas for a lesion such as thymine glycol, it promotes error-free replication through the lesion (22).

The *RAD30* gene of yeast encodes Polη, which promotes error-free replication through cyclobutane pyrimidine dimers. Polη is unique among eukaryotic DNA polymerases in its proficient ability to replicate through a *cis-syn* thymine-thymine dimer (19, 21, 39, 40), and genetic studies of yeasts as well as of humans have indicated a major role for Polη in promoting

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error-free replication through the cyclobutane pyrimidine dimers formed at TC and CC sites (32, 41).

The *RAD5*, *MMS2*, and *UBC13* genes function in a third Rad6-Rad18-dependent lesion bypass pathway. Deletion of any of these genes inactivates the postreplicational repair of discontinuities that form in the DNA synthesized from UV-damaged DNA templates (34). This process might involve a copy-choice type of DNA synthesis, wherein the newly synthesized daughter strand of the undamaged complementary sequence is used as the template for bypassing the lesion. Rad5 is a member of the SWI/SNF family of ATPases, and it has a C3HC4 motif characteristic of ubiquitin ligase (E3) proteins (18). Mms2 associates with Ubc13, a ubiquitin-conjugating (E2) enzyme, and the Mms2-Ubc13 complex assembles polyubiquitin chains linked through lysine 63 of ubiquitin (16). Rad5 physically interacts with Rad18 and Ubc13 (36).

More recently, it has been shown that in yeast cells treated with DNA-damaging agents, PCNA becomes monoubiquitinated at its lysine 164 residue via the action of Rad6-Rad18; subsequently, this PCNA residue is polyubiquitinated via a lysine 63-linked ubiquitin chain in an Mms2-, Ubc13-, and Rad5-dependent reaction (15). Epistasis analyses with a *pol30* mutation in which the lysine 164 residue has been changed to arginine and which disables the ubiquitin conjugation reaction have indicated a role for PCNA ubiquitination in Rad6-Rad18-dependent repair processes (15, 33).

In studies that were carried out in our laboratory with *pol30-119*, a mutant of yeast PCNA that was initially isolated in Connie Holms laboratory (1) and which is due to a change of lysine 164 to arginine, we had observed results similar to those that have been reported by Jentsch and colleagues (15, 33). In view of the finding that the *pol30* K164R mutation inactivates PCNA ubiquitination, we have reexamined our data and we report these results here. Our studies confirm the previously reported observations with this *pol30* mutation, and they extend them in several ways. In particular, we find that in addition to conferring a defect in Polζ-dependent UV mutagenesis and in Polη-dependent TLS, the *pol30-119* mutation impairs the postreplicational repair of discontinuities that form in the newly synthesized DNA across from UV lesions. Even though the *pol30-119* mutation apparently impairs all three Rad6-Rad18-dependent lesion bypass processes, intriguingly, its level of UV sensitivity is much less than that of the *rad6*Δ and *rad18*Δ mutants. Our genetic data support the inference that the Rad52-dependent postreplicational repair pathway is activated in the *pol30-119* mutant and that that accounts for the higher level of UV resistance of this PCNA mutant compared to that of the *rad6*Δ or *rad18*Δ mutants. PCNA is additionally modified by SUMO conjugation at the lysine 164 and lysine 127 residues (15); our studies point to an inhibitory role of SUMO conjugation at the lysine 164 residue of PCNA for Rad52-dependent postreplicational repair.

MATERIALS AND METHODS

Strains and plasmids. Mutations in the *POL30* gene which encodes PCNA were obtained by PCR mutagenesis and plasmid shuffle and screened for cold sensitivity (14°C) and for sensitivity to methyl methanesulfonate (MMS) as described previously (1). One of the mutations, *pol30-119*, had no effect on growth at any of the temperatures, but it conferred MMS and UV sensitivity. All yeast strains used for epistasis analysis and for alkaline sucrose gradient sedimentation are isogenic to EMY74.7 (*MAT***a** *his3-*Δ*1 leu2-3-112 trp1*Δ *ura3-52*). The one-step gene replacement method was used to generate genomic deletions of the *RAD1*, *RAD5*, *RAD6*, *RAD52*, *REV3*, and *RAD30* genes as described previously (34, 35). An SacI, PvuI digest of plasmid pCH1654 (containing the *pol30-119* mutant gene in which arginine replaces the lysine 164 residue) was used to generate an integrant of the *pol30-119* mutation at the *POL30* site in the genome. The various deletion mutations were subsequently generated in the *pol30-119* strain. The wild-type EMY74.7 strain and its *pol30-119* derivative were used to measure UV-induced $CAN1^S$-to-$can1^r$ forward mutation frequencies. Ethidium bromide mutagenesis was used to obtain [rho^0] derivatives which lack mitochondrial DNA, yielding isogenic strains YR1-65 (*rad1*Δ) and YR1-131 (*rad1*Δ *pol30-119*) used for alkaline sucrose gradient sedimentation.

UV survival and UV mutagenesis. UV irradiation and determination of UV-survival and UV-induced mutation frequencies were done as previously described (35).

Sedimentation in alkaline sucrose gradients of nuclear DNA from UV-irradiated cells. Strains grown overnight at 30°C in synthetic complete medium lacking uracil but supplemented with uridine were UV irradiated at a confluence rate of 0.1 J/m^2/s and pulse labeled for 15 min with [^{3}H]uracil. Following UV irradiation, cells were washed and resuspended in high uracil medium and incubated for an additional 30 min or 6 h. Conversion of cells to spheroplasts, alkaline sucrose sedimentation, and processing of samples were done as described previously (34, 35).

RESULTS

***The pol30-119* mutation.** Mutations of the *POL30* gene were obtained using PCR mutagenesis and plasmid shuffle (1). A number of mutations were identified by their sensitivity to MMS and by a cold-sensitive growth phenotype at 14°C. One of the mutants, *pol30-119*, exhibits normal growth, cell viability, and cell cycle progression phenotypes at 14, 30, and 37°C. The *pol30-119* mutant also grows normally in 0.1 M hydroxyurea-containing medium, indicating a normal synthetic-phase (S-phase) checkpoint. The *pol30-119* mutation, however, confers sensitivity to UV light and MMS. The *pol30-119* mutation is due to the change of lysine 164 to arginine. This residue is located at the monomer-monomer interface of PCNA, and it is conserved in PCNA from diverse sources, including yeasts, *Drosophila* spp., frogs, mice, and humans.

Epistasis of *pol30-119* with mutations in genes belonging to the *RAD6* group. In yeast, genes belonging to three epistasis groups function in the repair of UV-damaged DNA (30). Genes in the *RAD3* group are required for the removal of damage by NER, those in the *RAD6* group promote error-free and mutagenic bypass of DNA lesions during replication, and those in the *RAD52* group mediate double-strand break repair and genetic recombination. To determine the epistasis relationships of *pol30-119* with mutations in genes belonging to these groups, we combined the *pol30-119* mutation with the *rad1*Δ mutation defective in NER, the *rad6*Δ mutation defective in replicational bypass of DNA lesions, or the *rad52*Δ mutation defective in double-strand break repair and genetic recombination and compared the UV sensitivity of these double mutants with that of the respective single mutants. The *pol30-119* mutation exhibits a synergistic increase in UV sensitivity in combination with the *rad1*Δ mutation (Fig. 1A) and the *rad52*Δ mutation (Fig. 1B), suggesting that *pol30-119* impairs a repair pathway distinct from the NER and genetic recombination pathways. The UV sensitivity of the *pol30-119* *rad6*Δ mutant, however, was no greater than that of the *rad6*Δ strain (Fig. 1C). The epistasis of the *rad6*Δ mutation over *pol30-119* suggested that this PCNA mutation inactivates *RAD6*-dependent lesion bypass processes.

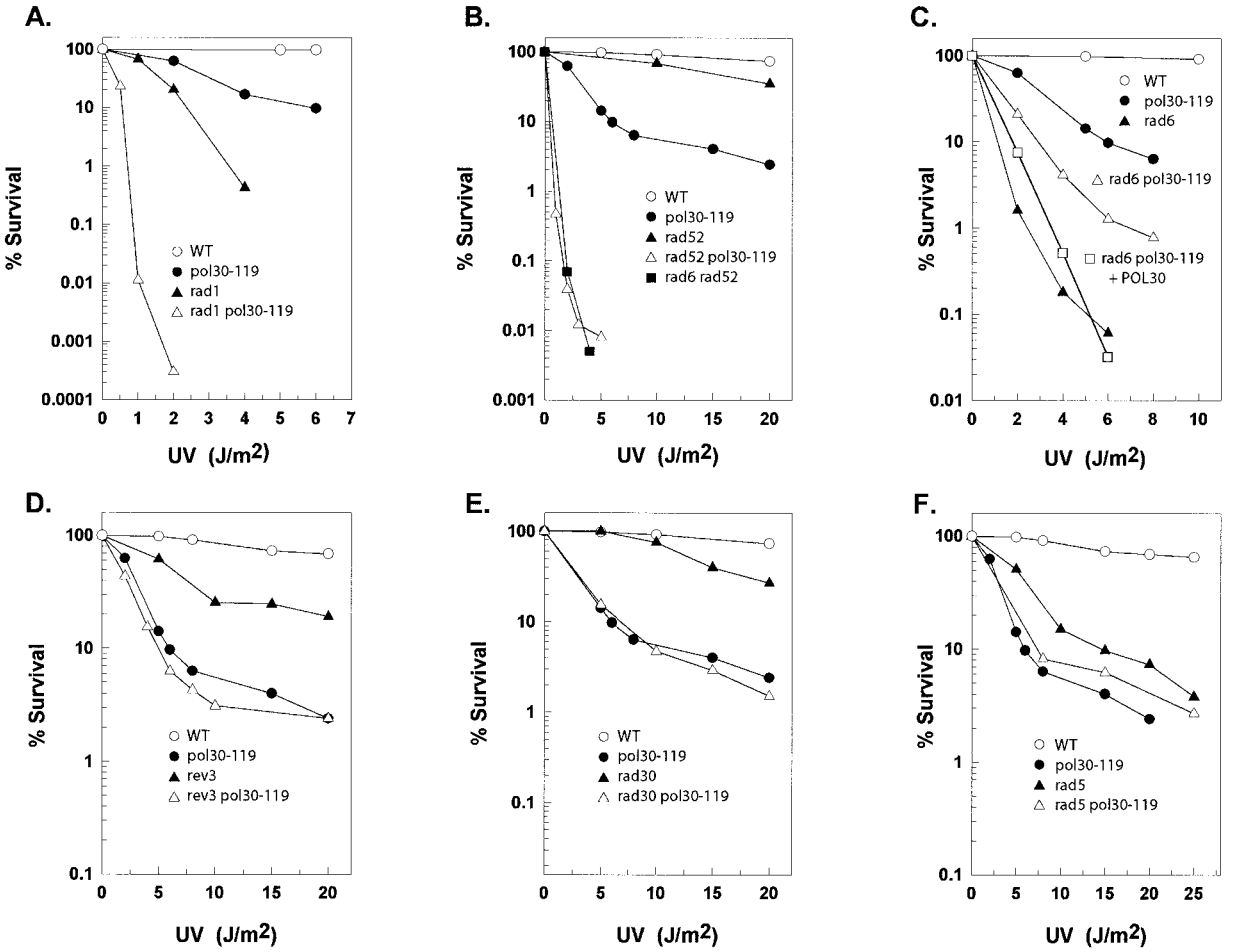

FIG. 1. Epistasis analysis of the *pol30-119* mutation. Survival curves after UV irradiation of wild-type (WT) strain EMY74.7, its isogenic *pol30-119* derivative, and isogenic derivatives carrying deletion mutations of different *RAD* genes and of the *REV3* gene are shown. Survival curve results represent averages of at least two different experiments for each strain. □ (C), *rad6*Δ *pol30-119* strain carrying the wild-type *POL30* gene on the *CEN ARS TRP1* plasmid pBL230.

To identify which of the Rad6-Rad18-dependent pathways is specifically inactivated by the *pol30-119* mutation, we examined the epistasis relationships of the *pol30-119* mutation with the *rev3*Δ, *rad30*Δ, and *rad5*Δ mutations. Surprisingly the *pol30-119* mutation exhibited an epistatic relationship with all three deletion mutations, for in each case the UV sensitivity of the double mutant was no greater than that of the *pol30-119* mutation (Fig. 1D to F). These epistatic relationships suggested that the *pol30-119* mutation interferes with all three Rad6-Rad18-dependent lesion bypass processes.

Defective UV mutagenesis in the *pol30-119* mutant. The epistasis of *pol30-119* with the *rev3*Δ mutation suggested a role of PCNA in *REV3*-dependent mutagenic bypass of UV lesions. To verify this, we compared the frequency of UV-induced mutations in *pol30-119* with that in the wild-type strain. As shown in Fig. 2, the frequency of *CAN1*^{S}-to-*can1*^{r} forward mutations fell precipitously in the *pol30-119* strain, indicating a defect in UV mutagenesis.

Impaired postreplicational repair in the *pol30-119* mutant. Even though postreplicational repair of UV-damaged DNA is inhibited in both the *rad5*Δ and *mms2*Δ mutants, the *rad5*Δ mutation confers a much higher level of UV sensitivity than the *mms2*Δ mutation (34). This observation has suggested an additional role for Rad5 in a process other than postreplicational repair, raising thereby the possibility that the epistasis of *pol30-119* over *rad5*Δ was due to the inactivation of this other Rad5-dependent repair process. To ascertain whether PCNA ubiquitination was in fact necessary for postreplicational repair, we carried out experiments to directly check for this.

To examine the effect of the *pol30-119* mutation on postreplicational repair, we determined the size of newly synthesized DNA in the *rad1*Δ and *rad1*Δ *pol30-119* strains following UV irradiation. Because of the lack of NER in the *rad1*Δ strain, UV damage persists in DNA and replication of such DNA requires the various lesion bypass processes. The *rad1*Δ and *rad1*Δ *pol30-119* strains were UV irradiated at 2.5 J/m^2, and the size of newly synthesized DNAs was examined by alkaline sucrose gradient sedimentation following pulse labeling of DNA with [^{3}H]uracil for 15 min and an additional 30-min chase in medium containing a high concentration of unlabeled uracil. In both the *rad1*Δ and *rad1*Δ *pol30-119* strains, DNA sediments towards the top in alkaline sucrose gradients (indicative of discontinuities in the newly synthesized strand) (Fig. 3). In the unirradiated *rad1*Δ and *rad1*Δ *pol30-119* cells, how-

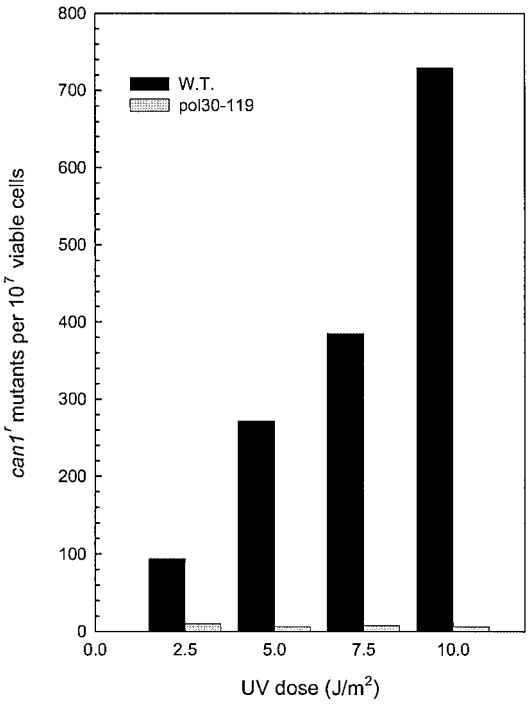

FIG. 2. Effect of *pol30-119* on UV-induced *CAN1*^{S}-to-*can1*^{r} mutations. The results represent an average of five or more experiments for the wild-type (W.T.) and *pol30-119* mutant strains.

ever, the size of DNA synthesized following the 15-min pulse and 30-min chase periods was the same as in uniformly labeled cells (data not shown), indicating that this period is sufficient to synthesize normally sized DNA in unirradiated cells. Previously, we had shown that the size of newly synthesized DNA in the *rad1* mutant decreases with increasing UV dose and correlates with the average distance between photoproducts present in the parental DNA (28). These discontinuities presumably reflect gaps that form in the newly synthesized strand across from the damage site in the template strand. In *rad1*Δ cells incubated for 6 h following UV irradiation, daughter strands attain the same size as in unirradiated cells, indicating that postreplicative gap-filling processes have restored normal size to daughter strands (Fig. 3A). By contrast, the *rad1*Δ *pol30-119* strain is unable to restore normally sized DNA in UV-irradiated cells following a 6-h incubation period (Fig. 3B). Thus, the *pol30-119* mutation impairs the efficiency of postreplicational repair of UV-damaged DNA.

Requirement of *RAD52* for the suppression of UV sensitivity of *rad6*Δ by *pol30-119*. Although the various genetic observations strongly suggest that *pol30-119* impairs all three Rad6-Rad18-dependent pathways of lesion bypass, intriguingly, this mutation confers a much lower degree of UV sensitivity than the *rad6*Δ or *rad18*Δ mutations. This observation suggested either that the *pol30-119* mutation imparts only a partial deficiency in Rad6-Rad18-dependent bypass processes or that an alternate lesion bypass pathway becomes activated in this PCNA mutant. To examine this point further, we first determined whether the *pol30-119* mutation suppresses the UV sensitivity of the *rad6*Δ or *rad18*Δ mutants (since that would imply the activation of an alternate Rad6-Rad18-independent pathway of lesion bypass). As shown in Fig. 1C, the *rad6*Δ *pol30-119* mutant is not as UV sensitive as the *rad6*Δ mutation, which implies that the *pol30-119* mutation suppresses the repair defectiveness of the *rad6*Δ mutation. Similar results were obtained with the *rad18*Δ mutation (data not shown). Introduction of the wild-type *POL30* gene on an *ARS CEN TRP1* plasmid increased the UV sensitivity of the *rad6*Δ *pol30-119*

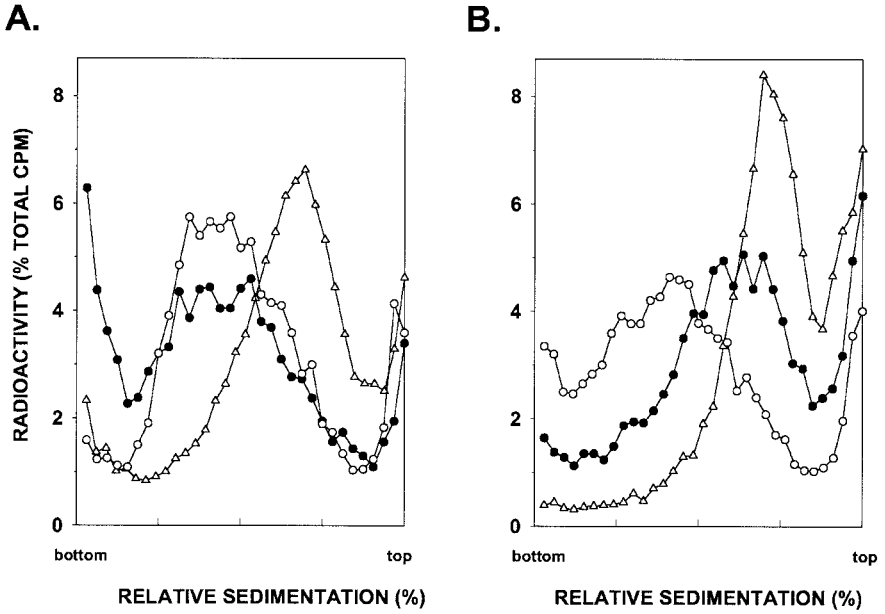

FIG. 3. Sedimentation in alkaline sucrose gradients of nuclear DNA from cells incubated for different periods following UV irradiation with 2.5 J/m^2. The *rad1*Δ (A) and *rad1*Δ *pol30-119* (B) strains (YR1-65 and YR1-131, respectively) were UV irradiated and then pulse labeled with [^{3}H]uracil for 15 min followed by a 30-min (△) or 6-h (●) chase in high-uracil medium before conversion to spheroplasts and sedimentation of DNA in alkaline sucrose gradients. Unirradiated cells pulse labeled for 15 min and chased for 6 h in high-uracil medium are indicated (○).

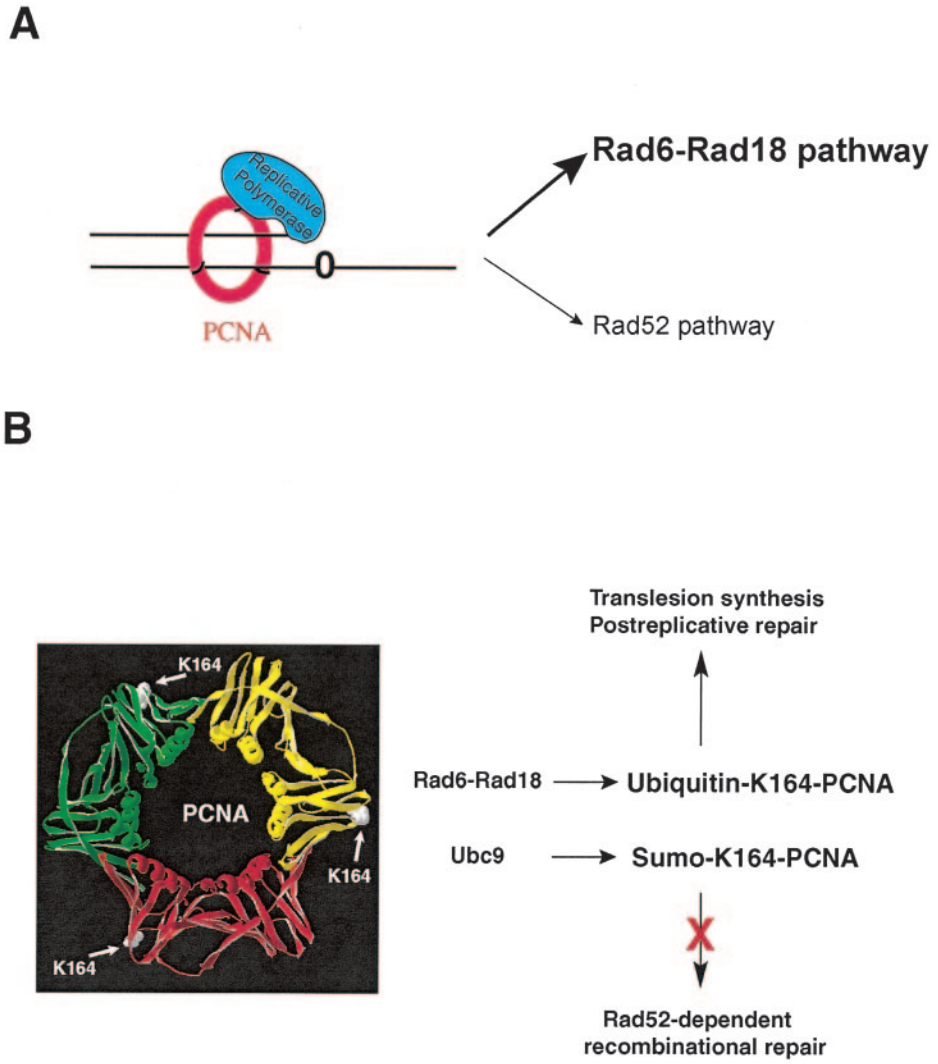

FIG. 4. Roles of Rad6-Rad18-mediated ubiquitin conjugation and Ubc9-mediated SUMO attachment to the lysine 164 residue of PCNA. (A) In wild-type yeast cells, Rad6-Rad18-dependent processes play a predominant role in lesion bypass whereas Rad52-dependent recombinational bypass plays a relatively minor role. (B) Rad6-Rad18-dependent ubiquitin conjugation at the lysine 164 residue of PCNA promotes TLS by DNA Polζ and Polη and postreplicational repair of discontinuities that form in the newly synthesized DNA across from UV lesions. Ubc9-mediated SUMO modification of lysine 164 of PCNA is proposed to inhibit Rad52-dependent lesion bypass.

mutant to the *rad6*Δ level. We conclude from these observations that in the *pol30 K164R* mutant an alternate repair pathway becomes activated and that in the presence of wild-type PCNA this alternate pathway remains relatively dormant.

In addition to Rad6-Rad18-dependent postreplicational repair, the Rad52-dependent recombinational pathway contributes to the postreplicational repair of UV-damaged DNA (28). If suppression in the *pol30-119* mutant were due to the activation of Rad52-dependent postreplicational repair, we would expect the UV sensitivity of the *pol30-119 rad52*Δ double mutant to be the same as that of the *rad6*Δ *rad52*Δ mutant (since both the Rad6- and Rad52-dependent lesion bypass pathways would then be inactivated in the *pol30-119 rad52*Δ mutant). From the very similar UV sensitivities of the *pol30-119 rad52*Δ and *rad6*Δ *rad52*Δ double mutants (Fig. 1B), we conclude that activation of the Rad52 pathway underlies the reduced UV sensitivity of the *pol30-119* mutant and the suppression of the UV sensitivity that occurs in the *rad6*Δ or *rad18*Δ mutants when they are combined with the *pol30-119* mutation. The implications of these results for the effects of SUMO modification of the lysine 164 residue of PCNA on Rad52-dependent postreplicational repair are discussed below.

DISCUSSION

It has been shown previously that in vivo, the Rad6 and Rad18 proteins mediate the monoubiquitination of PCNA at the lysine 164 residue and that Mms2-Ubc13 in conjunction with Rad5 (15) modulates the polyubiquitination of this residue through lysine 63-linked chains of ubiquitin. Here we show that the *pol30-119* mutation, which results from a change of the lysine 164 residue of PCNA to arginine, confers a defect in the Rad6-Rad18-dependent lesion bypass processes. In addition to exhibiting epistasis with the *rad6*Δ and *rad18*Δ mutations, the

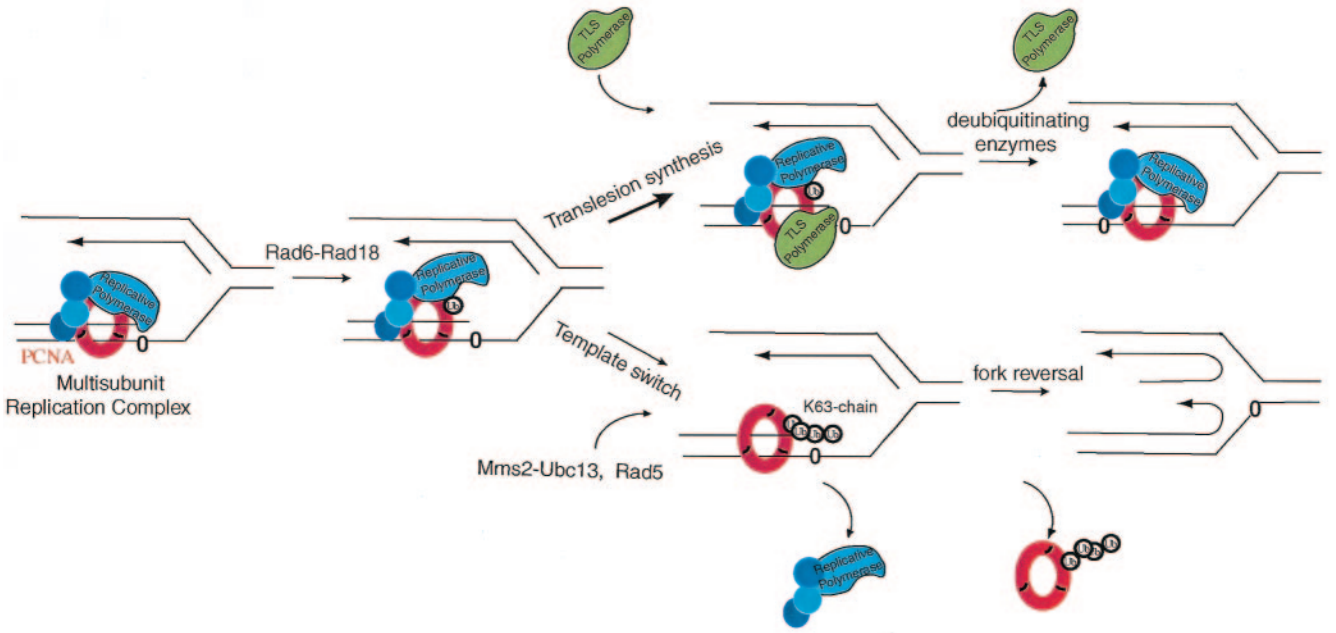

FIG. 5. A model for the role of Rad6-Rad18-dependent monoubiquitination (Ub) and Mms2-Ubc13-, Rad5-dependent polyubiquitination at the lysine 164 residue of PCNA in TLS and in template switching, respectively. For simplicity, the possible SUMO modification of lysine 164 in one of the PCNA monomers is not shown. See Discussion for further details. 0, DNA lesion.

pol30-119 mutation displays epistatic interactions with the *rev3*Δ, *rad30*Δ, and *rad5*Δ mutations, each of which inactivates an alternate Rad6-Rad18-dependent lesion bypass processes. In addition, we show an impairment of UV mutagenesis and inhibition of postreplication repair in the *pol30-119* mutant. The results of epistasis analyses and UV mutagenesis confirm the observations that have been reported recently with regard to this *pol30* mutant (15, 33). Our finding that there is a very considerable inhibition of postreplicational repair in the *pol30-119* mutant could not have been predicted from the epistasis results with *rad5*Δ, however, since in addition to its involvement in postreplicational repair, Rad5 is likely involved in another repair process as well (34). Without a direct demonstration of a postreplicational repair defect, therefore, one could not be certain which of the Rad5 functions was inactivated by *pol30-119*. Thus, in addition to confirming the observations that have been reported previously with the K164R mutation of PCNA, our studies extend them and provide further support to the conclusion that PCNA ubiquitination is a prerequisite for all three Rad6-Rad18-dependent lesion bypass processes.

If all three Rad6-Rad18-dependent lesion bypass pathways are inactivated by the *pol30-119* mutation, how do we then account for the much higher level of UV resistance of the *pol30-119* mutant compared to that of the *rad6*Δ and *rad18*Δ mutants? Although Rad6-Rad18-dependent lesion bypass is the predominant way by which replication through DNA lesions is accomplished in yeast cells, the Rad52 system does contribute to lesion bypass (28), albeit in a subsidiary way (Fig. 4A). Under certain circumstances, however (as in the absence of the Srs2 DNA helicase, for example, which actively disrupts the Rad51-nucleoprotein filament and whose presence therefore would inhibit the Rad52-dependent recombinational bypass of DNA lesions), the Rad52 pathway becomes activated (24, 37). Consequently, the *srs2*Δ mutation suppresses the DNA damage sensitivity of the *rad6*Δ and *rad18*Δ mutants (31). Here we provide genetic evidence that the *pol30-119* mutation suppresses the UV sensitivity of the *rad6*Δ and *rad18*Δ mutants and that this suppression is mediated via the Rad52 pathway.

SUMO is conjugated to PCNA at the lysine 164 residue and also at lysine 127; lysine 164, however, is the primary site of this modification (15). On the basis of the observation that the *pol30* K127R K164R mutation (in which both the lysine residues subject to SUMO modification have been changed to arginine) confers a somewhat higher level of UV resistance than the *pol30* K164R mutation (in which SUMO conjugation can still occur at the K127 site), it has been suggested that SUMO conjugation at lysine 127 is inhibitory for DNA repair (33). Since the difference in the UV survival rates of the K127R K164R and K164R mutants is very slight, however, the inhibitory effect of SUMO conjugation at K127 on DNA repair is likely to be minimal. From our observation indicating that the Rad52 pathway is activated in the *pol30* K164R mutant, we infer a role for SUMO modification of lysine 164 in the inhibition of Rad52-dependent lesion bypass. Thus, we suggest that in wild-type yeast cells, whereas ubiquitin conjugation at the lysine 164 residue of PCNA activates all three Rad6-Rad18-dependent lesion bypass processes, SUMO modification of this PCNA residue inactivates Rad52-dependent lesion bypass (Fig. 4B). SUMO modification of PCNA, which is prevalent during the S phase (15), might be a device used for keeping the Rad52 recombinational pathway in check during the S phase because of the attendant risk of chromosome rearrangements. Our results, taken together with previously published observations (15, 33), support the conclusion that attachment of a single ubiquitin molecule at lysine 164 of PCNA

activates TLS by Polζ and Polη whereas polyubiquitination of this PCNA residue through a lysine 63-linked ubiquitin chain activates a template switch mechanism (Fig. 5). It is conceivable that in one PCNA trimer, ubiquitin and SUMO modifications can occur on the K164 residues of two different monomers and that that accounts for the simultaneous activation and inhibition of Rad6-Rad18- and Rad52-dependent lesion bypass processes, respectively.

In Fig. 5, we present a model to explain the possible role of ubiquitin modification of PCNA in TLS and in template switching. During normal replication, the PCNA-bound replicative polymerase is at the primer template junction. Monoubiquitination of the lysine 164 residue of PCNA disrupts the replication ensemble so that the polymerase can no longer access the primer-template junction, and this PCNA modification enables the entry of a TLS polymerase into the replication ensemble. Since the TLS polymerases are able to bind PCNA in the absence of any ubiquitin modification, this modification may have no effect on PCNA binding by the TLS polymerases; instead, it could be important for disrupting the association of some PCNA-bound protein that is a part of the replication ensemble and which is otherwise inhibitory to the binding of PCNA by the TLS polymerases. After the lesion has been bypassed, deubiquitination of the monoubiquitinated lysine 164 residue of PCNA could lead to the repositioning of the replicative polymerase at the primer-template junction and to the exit of the TLS polymerase from the replicational complex. For template switching to occur, we envision that PCNA polyubiquitination destabilizes the replication ensemble to such an extent as to cause its dissociation from the replication fork, thereby providing access of the primer end to the various proteins required for template switching and synthesis.

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