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# Requirement of *RAD5* and *MMS2* for Postreplication Repair of UV-Damaged DNA in *Saccharomyces cerevisiae*

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**UV lesions in the template strand block the DNA replication machinery. Genetic studies of the yeast *Saccharomyces cerevisiae* have indicated the requirement of the Rad6-Rad18 complex, which contains ubiquitin-conjugating and DNA-binding activities, in the error-free and mutagenic modes of damage bypass. Here, we examine the contributions of the *REV3*, *RAD30*, *RAD5*, and *MMS2* genes, all of which belong to the *RAD6* epistasis group, to the postreplication repair of UV-damaged DNA. Discontinuities, which are formed in DNA strands synthesized from UV-damaged templates, are not repaired in the *rad5*Δ and *mms2*Δ mutants, thus indicating the requirement of the Rad5 protein and the Mms2-Ubc13 ubiquitin-conjugating enzyme complex in this repair process. Some discontinuities accumulate in the absence of *RAD30*-encoded DNA polymerase η (Polη) but not in the absence of *REV3*-encoded DNA Polζ. We concluded that replication through UV lesions in yeast is mediated by at least three separate Rad6-Rad18-dependent pathways, which include mutagenic translesion synthesis by Polζ, error-free translesion synthesis by Polη, and postreplication repair of discontinuities by a Rad5-dependent pathway. We suggest that newly synthesized DNA possessing discontinuities is restored to full size by a "copy choice" type of DNA synthesis which requires Rad5, a DNA-dependent ATPase, and also PCNA and Polδ. The possible roles of the Rad6-Rad18 and the Mms2-Ubc13 enzyme complexes in Rad5-dependent damage bypass are discussed.**

Replication of damaged DNA templates can occur by translesion synthesis. This process is usually mutagenic but can be error free as well (see below). In *Escherichia coli,* the *umuC*-encoded DNA polymerase V (PolV) promotes mutagenic translesion synthesis (29, 35). Error-prone translesion synthesis, however, accounts for only a minor portion of the damage bypass in *E. coli*, whereas much of the damage bypass occurs by means of error-free mechanisms. Error-free bypass mechanisms come into play when the replication machinery terminates synthesis at the site of a DNA lesion and replication restarts downstream of the lesion. This results in the formation of a gap in the newly synthesized strand across from the DNA lesion (30). In *E. coli*, this gap is filled in by RecA-dependent recombination mechanisms by which the DNA strand from the undamaged sister duplex is transferred to the gapped strand in the damaged duplex (31). In mammalian cells, a copy choice type of DNA synthesis has been invoked as the major mechanism for the filling in of gaps formed in the newly synthesized strand opposite DNA lesions (11). In this mechanism, the newly synthesized daughter strand of the undamaged complementary sequence is used as the template to bypass the lesion. Once the lesion is bypassed, DNA polymerase switches back to copying the damaged template strand.

Genetic studies of the yeast *Saccharomyces cerevisiae* have indicated the requirement of the *RAD6* and *RAD18* genes in error-free, as well as mutagenic, damage bypass processes (22, 28). *rad6* and *rad18* mutants exhibit a high degree of sensitivity to UV light and to other DNA-damaging agents, and they are defective in the postreplication repair of discontinuities that form in DNA synthesized from UV-damaged DNA templates (27) and in UV-induced mutagenesis (1, 5, 22). Rad6, a ubiquitin-conjugating (UBC) enzyme, exists in vivo in a complex with Rad18, a DNA-binding protein, and it has been suggested previously that Rad18 targets the Rad6 UBC activity to single-stranded DNA that results from blockage of DNA replication by DNA lesions (2, 3). However, how Rad6-Rad18-dependent protein ubiquitination promotes postreplication repair is not known.

In addition to *RAD6* and *RAD18*, whose functions in damage bypass are expected to be regulatory, the products of the *REV1*, *REV3*, and *REV7* genes play a direct role in mutagenic translesion synthesis. The Rev3 and Rev7 proteins together form DNA Polζ (26), which functions in translesion synthesis by extending from the nucleotide inserted opposite a DNA lesion by another DNA polymerase (10, 14, 19). For example, Polζ is highly inefficient at inserting nucleotides opposite the 3′ T of a *cis-syn* thymine-thymine (TT) dimer or a (6-4) TT photoproduct, but it is very efficient at extending from nucleotides inserted opposite the 3′ T of either lesion by another DNA polymerase (14, 19). The Rev1 protein has a deoxycytidyl transferase activity which transfers a dCMP residue to the 3′ end of the DNA primer (25). This activity, however, appears to have little or no role in promoting replication through UV-induced DNA lesions.

*RAD30*, another member of the *RAD6* epistasis group, functions in the error-free replication of UV-damaged DNA (17, 24), and *RAD30*-encoded DNA Polη (16) replicates through a *cis-syn* TT dimer with the same efficiency and accuracy with which it replicates through undamaged T's (20, 39). In addition to inducing the formation of cyclobutane dimers at two adjacent thymines, UV light induces the formation of *cis-syn* cy-

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clobutane dimers and (6-4) photoproducts at 5′-TC-3′ and 5′-CC-3′ sequences. In both yeasts and humans, UV-induced mutations occur primarily by a 3′ C-to-T transition, and the incidence of UV-induced mutations at TC and CC sites is elevated severalfold in the *rad30*Δ strain over that in the wild-type strain (41). These observations have implicated Polη in the error-free bypass of UV lesions formed at TC and CC sites.

*RAD5*, *MMS2*, and *UBC13* also belong to the *RAD6* epistasis group (4, 12, 15). Rad5, a member of the SNF2/SWI2 family of ATPases, contains the seven consensus helicase motifs, and it has a $C_3HC_4$ RING finger motif, which is embedded in the middle of the helicase-like domain (15). Purified Rad5 displays a DNA-dependent ATPase activity, but it is devoid of any DNA helicase activity (18). Since UV-induced mutations still occur in the absence of *RAD5* and a synergistic increase in UV sensitivity occurs in the absence of *RAD5* and *REV3*, *RAD5* is expected to promote damage bypass in an error-free manner (15). The *mms2*Δ mutation also results in proficiency in UV-induced mutagenesis, and it leads to a synergistic increase in UV sensitivity when it is combined with the *rev3*Δ mutation, implicating the involvement of *MMS2* in error-free damage bypass as well (4). The Mms2 protein belongs to a family of noncanonical E2s, known as UBC enzyme variant proteins, that lack UBC (E2) activity because of the absence of an active-site cysteine residue (4). Mms2 forms a specific complex with the *UBC13*-encoded E2, and this complex assembles polyubiquitin chains linked through lysine 63 (12). A role for K63-linked ubiquitin chains has been previously suggested for *RAD6*-dependent damage bypass, since yeast cells carrying a mutated form of ubiquitin in which lysine 63 is replaced by arginine (ubiK63R) display a UV-sensitive phenotype and the *rad6*Δ mutation with the ubiK63R mutation results in epistasis (32). A *ubc13*Δ yeast strain also exhibits UV sensitivity, and *UBC13*, *MMS2*, and ubiquitin (*ubiK63R*) single, double, and triple mutants display comparable UV sensitivity phenotypes, suggesting that all these genes function in the same damage bypass pathway (12).

Here, we examine the roles of various *RAD6* group genes, *RAD5*, *MMS2*, *RAD30*, and *REV3*, in the postreplication repair of DNAs synthesized from UV-irradiated DNA templates. We show that *RAD5* and *MMS2* make a major contribution to postreplication repair, and we suggest that conjugation of ubiquitin to Rad5 and/or associated proteins by the sequential action of the Rad6-Rad18 and Mms2-Ubc13 enzyme complexes promotes the assembly of Rad5 and associated proteins into the stalled replication machinery. Additionally, we discuss a model in which Rad5, together with PCNA and DNA Polδ, promotes postreplication repair by a copy choice type of DNA synthesis.

## MATERIALS AND METHODS

**Strains.** For postreplication repair studies, yeast strains were treated with ethidium bromide to obtain [*rho*$^0$] derivatives lacking mitochondrial DNA. The following isogenic yeast strains, all derived from EMY74.7, *MAT***a** *his3-*Δ*1 leu2-3,112 trp1*Δ *ura3-52*, were used in these studies: YR1-65, *rad1*Δ [*rho*$^0$]; YR1-118, *rad1*Δ *rad5*Δ [*rho*$^0$]; YR1-77, *rad1*Δ *rev3*Δ [*rho*$^0$]; YR1-120, *rad1*Δ *rad5*Δ *rev3*Δ [*rho*$^0$]; YR1-206, *rad1*Δ *rad30*Δ [*rho*$^0$]; YR1-205, *rad1*Δ *rad5*Δ *rad30*Δ [*rho*$^0$]; YR1-218, *rad1*Δ *mms2*Δ [*rho*$^0$]; and YR1-225, *rad1*Δ *mms2*Δ *rad5*Δ [*rho*$^0$].

The following *rad*Δ single mutant strains, used for determining UV sensitivity, were derived from EMY74.7 and the *rad*Δ *mms2*Δ double mutant combinations were obtained from the EMY74.7 isogenic *mms2*Δ *ura3* derivative strain YMMS2.6 by the gene replacement method. The strains and their relevant genotypes are as follows: YR1-62, *rad1*Δ; YR6-100, *rad6*Δ; YR5-62, *rad52*Δ; YR30.2, *rad30*Δ; YR5-50, *rad5*Δ; YR1-203, *mms2*Δ *rad1*Δ; YR6-190, *mms2*Δ *rad6*Δ; YR52-55, *mms2*Δ *rad52*Δ; YR30-19, *mms2*Δ *rad30*Δ; and YR5-53, *mms2*Δ *rad5*Δ.

**Analysis of DNA synthesized from UV-irradiated templates by sedimentation in alkaline sucrose gradients.** Asynchronously growing yeast cells were UV irradiated in logarithmic phase at a density of $0.5 \times 10^7$ to $1.0 \times 10^7$ cells per ml of synthetic complete medium lacking uracil but containing 5 μg of uridine/ml at room temperature with constant stirring in 150- by 20-mm petri dishes at a dose rate of 0.1 J/m$^2$/s. All operations after irradiation were performed in yellow light to avoid photoreactivation. Cells were labeled with a radioisotope following UV irradiation and incubated for various times, followed by conversion to spheroplasts as described previously (27). Briefly, after UV irradiation, cells were collected by filtration and resuspended in fresh uridine medium at a density of $1 \times 10^8$ to $2 \times 10^8$ cells per ml. Pulse-labeling was achieved by the addition of 100 μCi of [$^3$H]6′-uracil (20 to 25 Ci/mmol, 1 mCi/ml; Moravek Biochemicals and Radiochemicals, Brea, Calif.) to 1 ml of cells, followed by vigorous shaking for 15 min at 30°C. Cells were then washed, resuspended in synthetic complete medium containing 1.67 mg of uracil (high-uracil medium)/ml as described previously (27), 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 (36, 37).

**UV sensitivity.** Cells were grown to midlogarithmic phase in yeast extract-peptone-dextrose medium, washed, sonicated to disperse cell clumps when necessary, and resuspended in sterile distilled water to a density of $2 \times 10^8$ cells per ml. Cell suspensions were diluted, spread onto yeast extract-peptone-dextrose medium, and irradiated at a dose rate of 1 J/m$^2$/s. Plates were incubated in the dark, and colonies were counted after 3 to 5 days.

## RESULTS

**Impaired postreplication repair in the *rad5*Δ mutant.** To determine if *RAD5* was required for postreplication repair of UV-damaged DNA, we examined the sizes of newly synthesized DNA in UV-irradiated *rad1*Δ and *rad1*Δ *rad5*Δ cells. Because of the defect in the removal of UV-induced DNA damage, survival of UV-irradiated *rad1*Δ cells depends upon lesion bypass processes. The *rad1*Δ cells were UV irradiated at 2.5 J/m$^2$, and the size of newly synthesized DNA formed from UV-damaged templates was examined by pulse-labeling of DNA with [$^3$H]uracil for 15 min, followed by a chase for 30 min. DNA from *rad1*Δ cells obtained following this treatment sediments toward the top of the alkaline sucrose gradient, indicating the presence of discontinuities in the newly synthesized DNA (Fig. 1A). In unirradiated *rad1*Δ cells, the size of newly synthesized DNA following the 15-min pulse and 30-min chase periods was the same as in overnight-labeled cells (data not shown), indicating that this time interval is sufficient to reconstitute normal-size DNA in unirradiated cells. A previous study has indicated that the size of newly synthesized DNA in UV-irradiated *rad1* cells correlates with the average distance between photoproducts present in the template strands (27), an expected result if replication is arrested at the site of UV lesions and a gap is formed in the newly synthesized strand across from the damage site.

In *rad1*Δ cells that were UV irradiated and allowed to have a 6-h repair period following the 15-min pulse, the size of daughter strands became the same as in unirradiated control cells, indicating proficient filling-in of daughter strand gaps (Fig. 1A). In the *rad1*Δ *rad5*Δ mutant strain, however, the efficiency of daughter strand gap filling was greatly reduced, as normal-size DNA was not reconstituted in this strain when cells were UV irradiated and allowed to have a 15-min pulse and a 6-h chase period (Fig. 1B). In the unirradiated *rad1*Δ

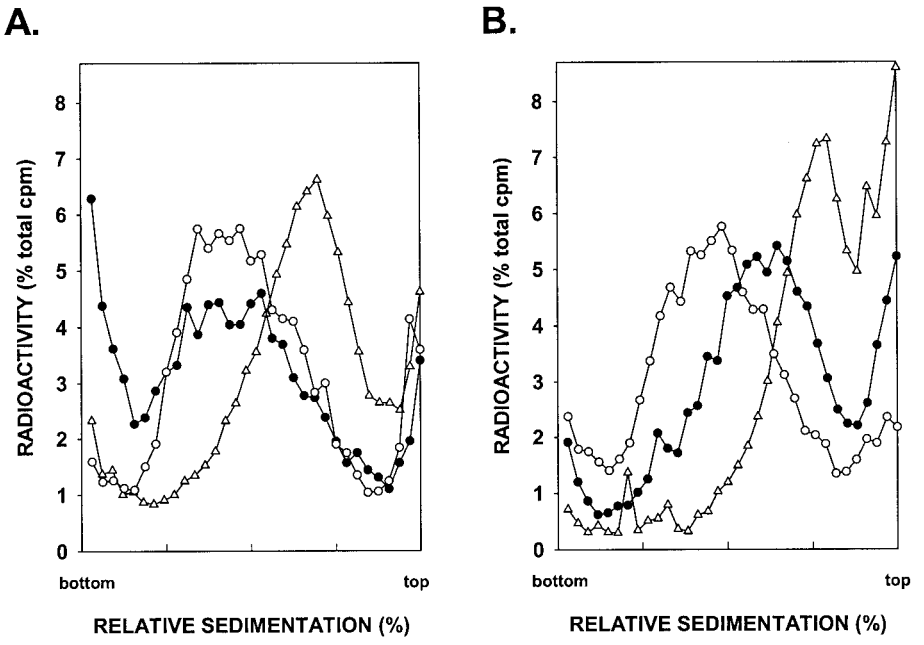


FIG. 1. Requirement of *RAD5* for postreplication repair of UV-damaged DNA. *rad1*Δ (A) and *rad1*Δ *rad5*Δ (B) cells were UV irradiated at 2.5 J/m$^2$ and then pulse-labeled with [$^3$H]uracil for 15 min, followed by a 30-min (△) or 6-h (●) chase in high-uracil medium, prior to conversion to spheroplasts and sedimentation of DNA in alkaline sucrose gradients. Synthesis of normal-sized DNA from unirradiated cells pulse-labeled with [$^3$H]uracil for 15 min was followed by a 6-h chase (○). In the unirradiated *rad1*Δ and *rad1*Δ *rad5*Δ cells, normal-sized DNA reconstituted after a 15-min pulse with [$^3$H]uracil and a 30-min chase. The total sedimentation counts was for cells pulse-labeled for 15 min followed by a 30-min or 6-h chase in high-uracil medium and for the unirradiated cells pulse-labeled for 15 min and then chased for 6 h were ~11,100, 14,500, and 23,850, respectively.

*rad5*Δ cells, normal-sized DNA was reconstituted following a 15-min pulse with [$^3$H]uracil and a 30-min chase (data not shown). These results indicate the requirement of *RAD5* in daughter strand gap filling of UV-damaged DNA.

**Effect of the *rev3*Δ and *rad30*Δ mutations on postreplication repair.** The *REV3*- and *RAD30*-encoded DNA polymerases function in mutagenic and error-free modes of translesion DNA synthesis, respectively. Here, we examine the contribution of these polymerases to the reconstitution of normal-sized DNA following the replication of UV-damaged DNA. A previous study had indicated that postreplicative gap filling of UV-damaged DNA was not affected in the *rad1 rev3* mutant (27). However, since the *rev3* mutation used in that study was a missense allele, the possibility that *REV3* function was not entirely inhibited by this mutation could not be excluded. As shown in Fig. 2A, however, the *rad1*Δ *rev3*Δ mutant strain was also fully proficient in postreplicative gap filling of UV-damaged DNA. Since the UV sensitivity of the *rev3*Δ *rad5*Δ mutant is much greater than that of the *rev3*Δ or *rad5*Δ single mutant (15), we examined the ability of the *rad1*Δ *rev3*Δ *rad5*Δ mutant strain to carry out gap filling. As shown in Fig. 2B, the sedimentation profile of DNA from *rad1*Δ *rev3*Δ *rad5*Δ cells that had been UV irradiated and allowed a 6-h repair period following a 15-min pulse period was the same as the size of DNA from *rad1*Δ *rad5*Δ cells (compare Fig. 1B and 2B). Thus, the *rev3*Δ mutation does not enhance the postreplication repair defect of the *rad1*Δ *rad5*Δ mutant strain. From these observations, we infer that Polζ makes no significant contribution to the filling in of postreplicative gaps formed opposite UV lesions.

The *rad30*Δ mutation impaired to some degree the restoration of normal-sized DNA in UV-irradiated cells. The *rad1*Δ *rad30*Δ mutant strain was not quite as proficient in the repair of discontinuities formed in the newly synthesized DNA strand following UV irradiation as the *rad1*Δ mutant (compare Fig. 1A and 2C), and the defectiveness in the repair of discontinuities was also somewhat greater in the *rad1*Δ *rad30*Δ *rad5*Δ triple mutant than that in the *rad1*Δ *rad5*Δ double mutant (compare Fig. 1B and 2D). Thus, in the absence of translesion synthesis by Polη, some discontinuities accumulate in the DNAs synthesized from UV-damaged templates.

**Epistasis of *RAD5* to *MMS2*.** Based on the UV survival curves of single and double mutants, *MMS2* has been assigned to the *RAD6* epistasis group. However, from these studies, it was not clear if *MMS2* also contributed to nucleotide excision repair (NER), since levels of survival after UV irradiation of the *rad4* mutant defective in NER and the *rad4 mms2* double mutant were not very different (4). Therefore, we have reexamined the epistasis relationships between a strain with the *mms2*Δ mutation and strains with the *rad1*Δ, *rad52*Δ, and *rad6*Δ mutations, which are defective in NER, recombinational repair, and damage bypass, respectively. We found a synergistic enhancement in UV sensitivity when the *mms2*Δ mutation was combined with the *rad1*Δ mutation (Fig. 3A) or with the *rad52*Δ mutation (Fig. 3B), whereas the UV sensitivity of the *mms2*Δ *rad6*Δ strain was no greater than that of the *rad6*Δ

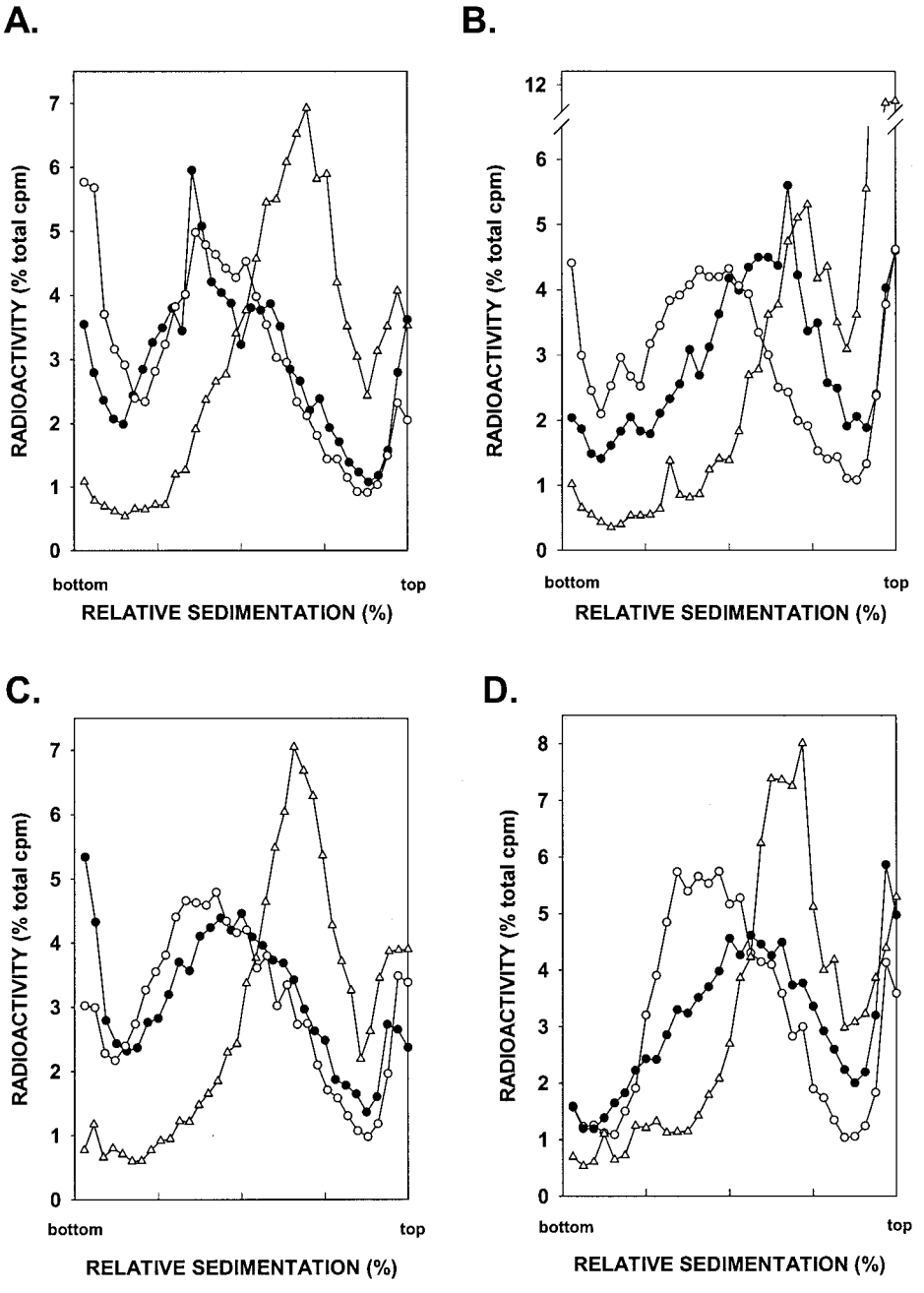


FIG. 2. Effect of the *rev3*Δ and *rad30*Δ mutations on postreplication repair of UV-damaged DNA. *rad1*Δ *rev3*Δ (A), *rad1*Δ *rad5*Δ *rev3*Δ (B), *rad1*Δ *rad30*Δ (C), and *rad1*Δ *rad5*Δ *rad30*Δ (D) cells were UV irradiated at 2.5 J/m$^2$ and pulse-labeled with [$^3$H]uracil for 15 min followed by a 30-min (△) or 6-h (●) chase in high-uracil medium. Cells were converted to spheroplasts, and the size of nuclear DNA was examined by sedimentation in alkaline sucrose gradients. Sedimentation patterns of DNA from unirradiated cells pulse-labeled with [$^3$H]uracil for 15 min followed by a 6-h chase (○) are also shown.

strain (Fig. 3C). These observations provide confirmatory evidence for the involvement of *MMS2* in *RAD6*-dependent damage bypass.

*MMS2* functions in the error-free pathway of *RAD6*-dependent damage bypass, since UV-induced mutagenesis was not affected in the *mms2*Δ mutant and a synergistic enhancement in UV sensitivity occurs when the *mms2*Δ mutation is combined with the *rev3*Δ mutation (4). In contrast to the requirement of *REV3*-encoded Polζ for error-prone damage bypass, error-free bypass of UV lesions is mediated by two alternate pathways that involve *RAD30* and *RAD5*, respectively. This conclusion stems from observations that the *rad5*Δ *rad30*Δ double mutant strain displays a synergistic increase in UV sensitivity compared to the levels of sensitivity of the *rad5*Δ and *rad30*Δ single mutants and that the incidence of UV-induced mutations is much higher in the double mutant than in the single mutants (17, 24). The UV sensitivities of strains with the *rad5*Δ and *rad30*Δ mutations in combination with the *mms2*Δ

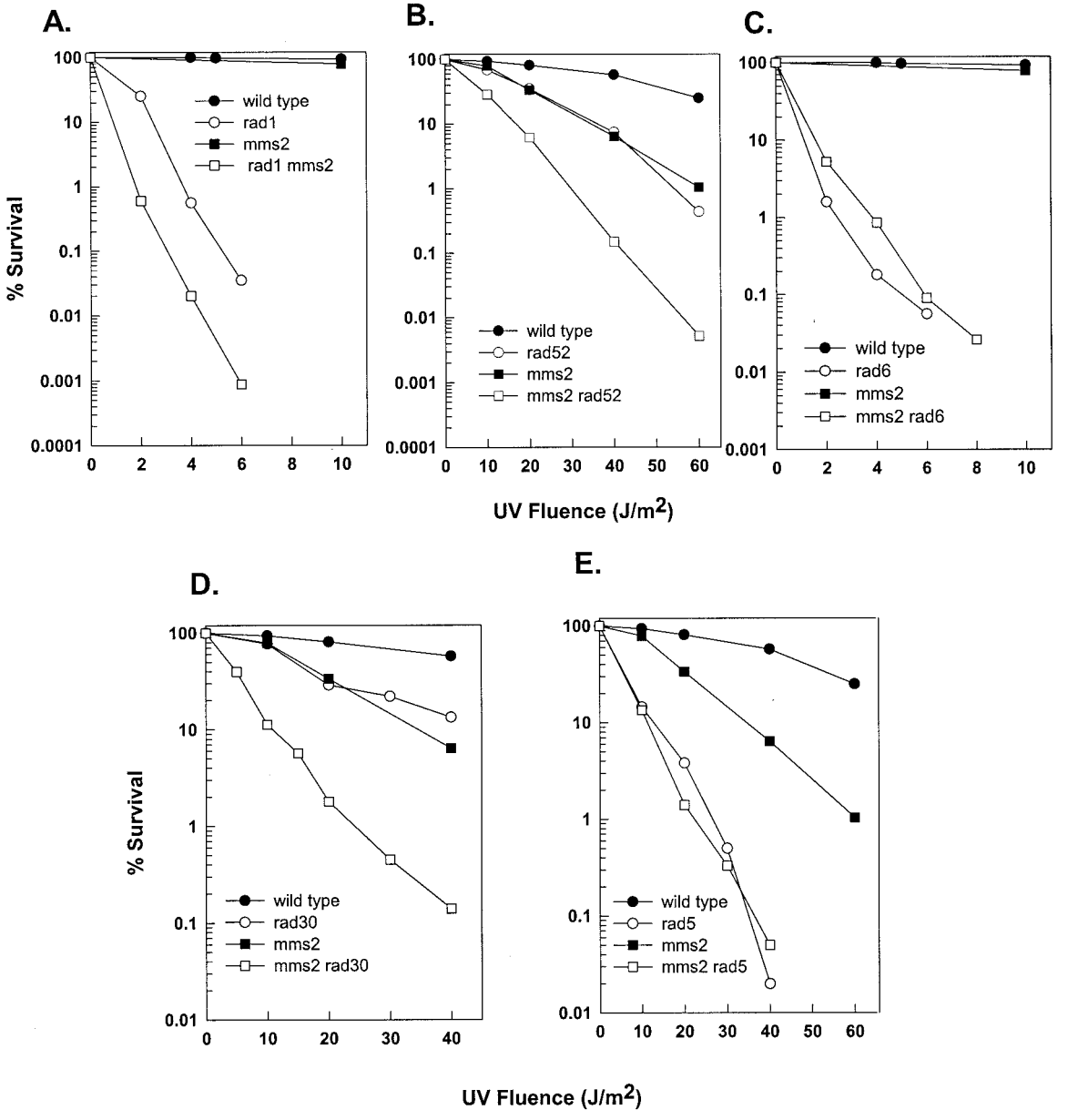


FIG. 3. Epistasis analysis of the *mms2*Δ mutation. Shown is survival after UV irradiation of wild-type strain EMY74.7, its isogenic *mms2*Δ derivative strain YMMS2.6, and EMY74.7 strains carrying deletion mutations of different *RAD* genes constructed by the gene replacement method. Survival curves present results from an average of approximately three experiments for each strain.

mutation have been examined previously by two independent groups. While in one study, *rad5* was shown to be epistatic to *mms2* and additive with *rad30* (38), in another study, *rad5* showed additivity with *mms2* (40). Because of these conflicting observations, we reexamined the epistasis relationships of *mms2* with *rad5* and *rad30.* We found that, compared to the UV sensitivities of the *mms2*Δ and *rad30*Δ single mutants, the UV sensitivity of the *mms2*Δ *rad30*Δ double mutant was synergistically enhanced (Fig. 3D) but that the UV sensitivity of the *mms2*Δ *rad5*Δ double mutant was the same as that of the *rad5*Δ mutant (Fig. 3E). These observations support a role for *MMS2* in *RAD5*-dependent error-free postreplication repair.

**Defective postreplication repair in the *mms2*Δ mutant.** Next, we examined the effect of the *mms2*Δ mutation on postreplication repair. The *mms2*Δ mutation was combined with the *rad1*Δ mutation, and the double mutant was UV irradiated at 2.5 J/m$^2$. The size of newly synthesized DNA was then examined by centrifugation in alkaline sucrose gradients. As shown in Fig. 4A, the efficiency of postreplication repair was greatly reduced by the *mms2*Δ mutation, since there was only a small increase in the size of newly synthesized DNA even after a 6-h repair period in the *rad1*Δ *mms2*Δ strain. Also, little, if any, postreplication repair occurred in the *rad1*Δ *mms2*Δ *rad5*Δ mutant strain (Fig. 4B). The requirement of *MMS2* for pos-

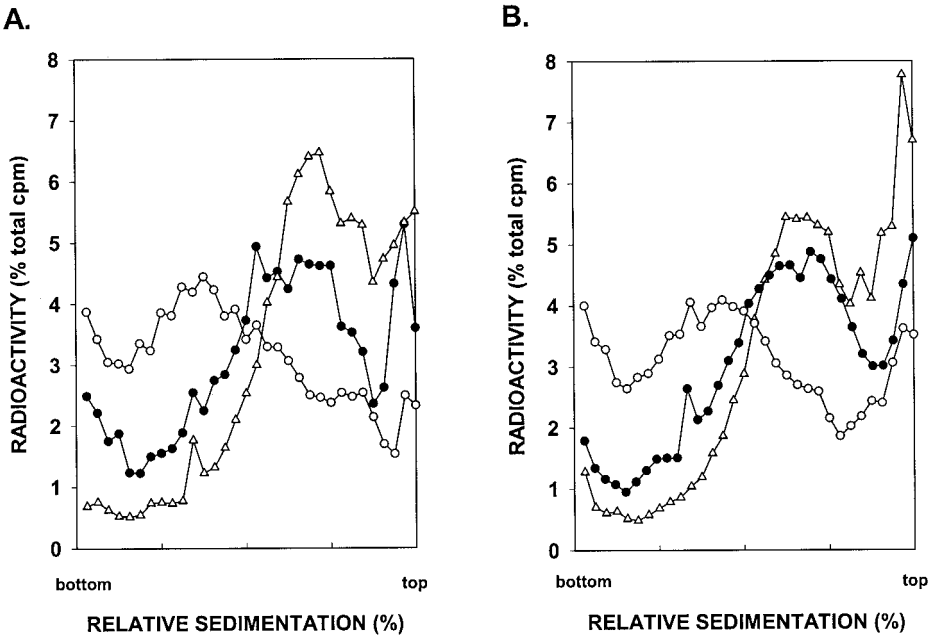


FIG. 4. Requirement of *MMS2* for postreplication repair of UV-damaged DNA. *rad1*Δ *mms2*Δ (A) and *rad1*Δ *mms2*Δ *rad5*Δ (B) cells were UV irradiated at 2.5 J/m$^2$ and then pulse-labeled with [$^3$H]uracil for 15 min, followed by a 30-min (△) or 6-h (●) chase in high-uracil medium, prior to conversion to spheroplasts and sedimentation of DNAs in alkaline sucrose gradients. Synthesis of normal-sized DNA from unirradiated cells pulse-labeled with [$^3$H]uracil for 15 min followed by a 6-h chase (○) is also shown.

treplication repair implies that ubiquitin conjugation by the Mms2-Ubc13 complex is indispensable for this repair process.

## DISCUSSION

**Roles of *REV3*, *RAD30*, and *RAD5* in the replication of UV-damaged DNA.** Here, we show that the discontinuities formed in the strands synthesized from UV-irradiated DNA templates are repaired with a much-reduced efficiency in the presence of the *rad5*Δ mutation but that the *rev3*Δ mutation has little effect on this repair process and that some discontinuities accumulate in the absence of translesion synthesis by Polη. These observations indicate a major role for *RAD5* in the postreplication repair of UV-damaged DNA, whereas Polζ and Polη contribute to damage bypass by translesion DNA synthesis.

On its own, Polζ bypasses UV lesions quite poorly. This is so because Polζ is highly inefficient at inserting nucleotides opposite a *cis-syn* TT dimer or a (6-4) TT photoproduct. However, Polζ efficiently extends from nucleotides inserted opposite the 3′ T of these lesions by another DNA polymerase. For both lesions, Polζ efficiently extends from a G opposite the 3′ T (14, 19), and that accounts for the T-to-C transitions that occur at these lesion sites.

Polη promotes highly efficient and error-free bypass of *cis-syn* TT dimers, and genetic studies have indicated a role for Polη in the error-free bypass of cyclobutane dimers formed at 5′-TC-3′ and 5′-CC-3′ sites. Thus, we expect Polη to play a prominent role in the bypass of cyclobutane dimers formed at different dipyrimidine sites. Polη may also promote replication through some of the (6-4) dipyrimidine photoproducts (also see below) since it is able to insert a G, from which Polζ subsequently extends, opposite the 3′ T of the (6-4) TT lesion (14).

In contrast to the involvement of Polζ and Polη in translesion DNA synthesis, the Rad5-dependent bypass pathway functions in the postreplication repair of discontinuities that form in the DNA synthesized from UV-damaged templates. These discontinuities may arise primarily opposite the (6-4) dipyrimidine lesions present in the template DNA. In contrast to a *cis-syn* cyclobutane pyrimidine dimer (CPD), which has only a modest effect on DNA structure, a (6-4) lesion induces a large structural distortion, leading to a 44° bend in the DNA helix, and the 3′ nucleotide of the lesion is held perpendicular to the 5′ nucleotide (21). Consequently, while Polη can efficiently replicate through a CPD, it is unable to bypass a (6-4) lesion, and although Polη can insert a G opposite the 3′ T of the (6-4) TT lesion, it does so with a reduced efficiency (14). Thus, because of the less efficient insertion of nucleotides opposite (6-4) lesions, we expect translesion synthesis to promote the bypass of only a fraction of these lesions. Consequently, the Rad5-dependent postreplication repair pathway may be the major means for bypassing the (6-4) dipyrimidine lesions.

Although Polη and Rad5 may primarily promote the bypass of CPDs and (6-4) photoproducts, respectively, we expect some overlap in their lesion bypass abilities. This overlap is suggested by the facts that the UV sensitivity of the *rad5*Δ *rad30*Δ double mutant is greater and its level of mutagenesis is higher than those of the *rad5*Δ and *rad30*Δ single mutants (17, 24). Thus, in the absence of *RAD30*, we expect the *RAD5*-dependent pathway to promote the bypass of CPDs as well.

In summary, the bypass of UV lesions in *S. cerevisiae* is mediated via three separate Rad6-Rad18-dependent path-

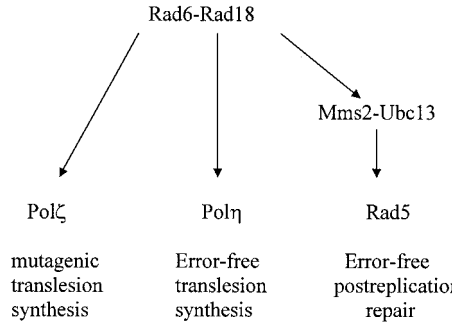


FIG. 5. Rad6-Rad18-dependent pathways for the replication of UV-damaged DNA in yeast. Whereas Polη primarily carries out error-free translesion synthesis through CPDs and the Rad5-dependent error-free postreplication repair pathway is proposed to primarily promote the bypass of (6-4) dipyrimidine lesions by a copy choice type of DNA synthesis, Polζ contributes to the mutagenic bypass of both these UV lesions. The Mms2-Ubc13 protein complex is proposed to function specifically in the Rad5-dependent postreplication repair pathway. Subsequent to the attachment of ubiquitin to Rad5 and/or associated proteins by the Rad6-Rad18 complex, the Mms2-Ubc13 complex may attach additional ubiquitin moieties to these proteins, and that may be important for the assembly of these repair proteins into the replication machinery.

ways: Polζ-dependent mutagenic translesion synthesis, Polη-dependent error-free translesion synthesis through CPDs, and Rad5-dependent repair of postreplicative gaps which presumably are formed in the newly synthesized strand opposite the highly distorting (6-4) dipyrimidine lesions (Fig. 5).

**Requirement of the Mms2-Ubc13 UBC enzyme complex for Rad5-dependent postreplication repair.** In agreement with previous observations (38), we find epistasis of the *mms2*Δ mutation with *rad5*Δ but that UV sensitivity is greatly enhanced when the *mms2*Δ mutation is combined with the *rad30*Δ mutation (Fig. 3) or the *rev3*Δ mutation (4). Consistent with the epistasis of *rad5*Δ and *mms2*Δ mutations, we find a drastic inhibition in postreplication repair of UV-damaged DNA in the absence of the Mms2 protein. From these observations, we infer the requirement of the Mms2-Ubc13 enzyme complex for Rad5-dependent postreplication repair. Curiously, even though the *mms2*Δ strain is not as UV sensitive as the *rad5*Δ strain (Fig. 3E), it is much more defective in postreplication repair than the *rad5*Δ strain (compare Fig. 1B and 4A). Thus, while the Mms2-Ubc13 complex is almost indispensable for postreplication repair, perhaps, in the absence of Rad5, another protein can partially substitute for it in this repair process. The higher UV sensitivity of the *rad5*Δ strain than that of the *mms2*Δ strain may suggest that, in addition to having a role in postreplication repair, Rad5 promotes the repair of UV lesions by some other means as well but that the Mms2-Ubc13 function is primarily dedicated to the filling in of postreplicative gaps.

**Possible roles of Rad6-Rad18 and Mms2-Ubc13 enzyme complexes in Rad5-dependent postreplication repair.** Rad5 physically interacts with both Rad18 and Ubc13 (38), suggesting either that Rad5 is a target of ubiquitin conjugation by the Rad6-Rad18 and Mms2-Ubc13 enzyme complexes or that Rad5 serves to recruit these UBC complexes for the ubiquitination of other postreplication repair proteins. Thus, the Rad6-Rad18 and Mms2-Ubc13 complexes may either act on Rad5 directly or coordinate the ubiquitination of some Rad5-associated protein(s) via their interactions with Rad5. Since Rad6 attaches ubiquitin directly to a protein substrate with or without the help of a ubiquitin ligase (7, 13, 33, 34) and the Mms2-Ubc13 complex assembles unanchored polyubiquitin chains linked through lysine 63 (12), following the Rad6-Rad18-mediated attachment of ubiquitin to Rad5 or to Rad5-associated proteins, the Mms2-Ubc13 complex may attach additional ubiquitin moieties linked through lysine 63. Since lysine 63-linked ubiquitin chains are not substrates for proteolytic degradation (6, 8), they may facilitate the assembly of Rad5 and/or its associated proteins into the replication machinery.

**Model for Rad5 action in postreplication repair.** Previously, it was shown that the *pol30-46* mutation in PCNA results in a defect in the error-free postreplication repair of UV-damaged DNA in yeast (37) and that postreplication repair is also severely inhibited by the yeast *pol3-3* mutation, which results in a temperature-sensitive defect in Polδ (36). The requirement of Rad5, PCNA, and Polδ for postreplication repair suggests that these proteins function together in this process.

The *RAD6*, *RAD18*, and *RAD5* genes do not function in genetic recombination, and mutational inactivation of these genes results in a hyper-recombination phenotype (23, 28). A plausible way by which the Rad5 pathway may promote replication of UV-damaged templates is through a copy choice type of DNA synthesis. Upon stalling of the replication fork at the damage site, ubiquitination of Rad5 and/or associated proteins by the Rad6-Rad18 and Mms2-Ubc13 complexes may promote the assembly of these proteins into the stalled replication machinery. Although Rad5 has no DNA helicase activity (18), such an activity may appear in Rad5 upon its ubiquitination or an as yet unidentified DNA helicase may unwind the 3′ end of the nascent strand, which would then anneal with the homologous strand of the undamaged sister duplex. An assembly of PCNA, Polδ, Rad5, and additional, as yet unidentified proteins may perform limited DNA synthesis through the undamaged sister duplex. Once the 3′ end of the invading strand has been copied past the site of DNA lesion, Polδ would switch back to copying the original template strand. A copy choice type of mechanism has been proposed to account for replication across from damage sites in mammalian cells (11), and a purified system consisting of DNA polymerase holoenzyme, gene 32 protein, dda DNA helicase, and uvsx protein supports a copy choice type of DNA synthesis in bacteriophage T4 (9).

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