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Requirement of *RAD52* Group Genes for Postreplication Repair of UV-Damaged DNA in *Saccharomyces cerevisiae*[▽]

Venkateswarlu Gangavarapu, Satya Prakash, and Louise Prakash*

Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, 301 University Blvd., Galveston, Texas 77555-1061

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In *Saccharomyces cerevisiae*, replication through DNA lesions is promoted by Rad6-Rad18-dependent processes that include translesion synthesis by DNA polymerases η and ζ and a Rad5-Mms2-Ubc13-controlled postreplicational repair (PRR) pathway which repairs the discontinuities in the newly synthesized DNA that form opposite from DNA lesions on the template strand. Here, we examine the contributions of the *RAD51*, *RAD52*, and *RAD54* genes and of the *RAD50* and *XRS2* genes to the PRR of UV-damaged DNA. We find that deletions of the *RAD51*, *RAD52*, and *RAD54* genes impair the efficiency of PRR and that almost all of the PRR is inhibited in the absence of both Rad5 and Rad52. We suggest a role for the Rad5 pathway when the lesion is located on the leading strand template and for the Rad52 pathway when the lesion is located on the lagging strand template. We surmise that both of these pathways operate in a nonrecombinational manner, Rad5 by mediating replication fork regression and template switching via its DNA helicase activity and Rad52 via a synthesis-dependent strand annealing mode. In addition, our results suggest a role for the Rad50 and Xrs2 proteins and thereby for the MRX complex in promoting PRR via both the Rad5 and Rad52 pathways.

DNA lesions in the template strand block the progression of the replication fork. Genetic studies of the yeast *Saccharomyces cerevisiae* have indicated a crucial role for the Rad6-Rad18 ubiquitin conjugating enzyme complex (3, 4) in promoting replication through DNA lesions via three different pathways that include DNA polymerase η (Polη)- and Polζ-dependent translesion synthesis (TLS) and an Mms2-Ubc13-Rad5-dependent postreplicational repair (PRR) pathway which promotes the repair of discontinuities that form in the DNA synthesized from damaged templates (20, 33, 39, 51).

UV light induces the formation of cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts in DNA. Both in yeast and in humans, Polη promotes error-free synthesis through the CPDs; consequently, inactivation of Polη confers an increase in the incidence of UV mutagenesis (21, 32, 43, 57) and causes a cancer-prone syndrome, the variant form of xeroderma pigmentosum, in humans (19, 30). Polζ promotes TLS through the UV lesions by extending from the nucleotides inserted opposite the CPD or the (6-4) photoproduct by another DNA Pol (17, 23). By contrast to Polη, which mediates error-free TLS through the CPDs, Polζ promotes error-prone TLS through the UV lesions (26–28).

Although the mechanism by which the Mms2-Ubc13-Rad5-dependent PRR pathway operates is not defined, it is likely to involve a transient template switching mechanism and a copy choice type of DNA synthesis wherein the newly synthesized daughter strand of the undamaged complementary sequence is used as a template for bypassing the lesion (5). In the Mms2-Ubc13-Rad5 complex, the Mms2-Ubc13 complex promotes the assembly of lysine 63-linked polyubiquitin chains (13) and Rad5 functions as an ubiquitin ligase (E3) for promoting Mms2-Ubc13-dependent ubiquitination (12, 54). In addition to the E3 activity, Rad5 has a DNA-dependent ATPase activity, characteristic of the SWI2/SNF2 family of ATPases to which it belongs (18, 22). Genetic studies of yeast have indicated that both the ATPase and E3 activities of Rad5 are indispensable for its role in PRR (9).

All the Rad6-Rad18 lesion bypass processes are dependent upon lysine 164 ubiquitination of PCNA. In DNA-damaged yeast cells, PCNA becomes monoubiquitinated at its lysine 164 residue via the action of Rad6-Rad18, and subsequently, this lysine residue is polyubiquitinated via a lysine 63-linked polyubiquitin chain by the Mms2-Ubc13-Rad5 enzyme complex (12). Genetic studies of yeast have indicated the requirement of PCNA monoubiquitination for Polη- and Polζ-dependent TLS and of PCNA polyubiquitination for Rad5-dependent PRR (10, 12, 44).

By contrast to inactivation of *RAD6* and *RAD18*, which confers a large increase in UV sensitivity because of the inhibition of different Rad6-Rad18-dependent lesion bypass processes, mutations in the *RAD52* epistasis group of genes confer only a modest increase in UV sensitivity (40). Instead, the genes of the *RAD52* group play a crucial role in homology-dependent recombinational repair of double-strand DNA breaks that are induced upon exposure to ionizing radiation, such as X rays and γ rays; hence, inactivation of these genes confers a large increase in sensitivity to ionizing radiation (25, 47, 50). Rad51, Rad52, and Rad54 are three key proteins indispensable for recombinational repair. Rad51 forms an extended helical filament on single-stranded (ss) DNA (34, 46, 48). This nucleoprotein filament then invades the homologous region in duplex DNA, resulting in the formation of a three-stranded D loop. Rad52 and Rad54 stimulate the DNA pairing function of Rad51; Rad52 enhances the ability of Rad51 to displace RPA from ss DNA (42, 45), and Rad54 functionally

* Corresponding author. Mailing address: University of Texas Medical Branch at Galveston, 301 University Blvd., Galveston, TX 77555-1061. Phone: (409) 747-8601. Fax: (409) 747-8608. E-mail: l.prakash@utmb.edu.

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cooperates with Rad51 at different steps of the pairing reaction. Rad54, a Swi2/Snf2 family DNA-dependent ATPase, physically interacts with Rad51; it stabilizes Rad51-ss DNA filaments and stimulates the DNA pairing reaction of Rad51 (31, 36, 41), and it also dissociates Rad51 from duplex DNA via its DNA translocase activity (1), thereby freeing up the primer end to initiate DNA synthesis.

Mutations in the *RAD50*, *MRE11*, and *XRS2* genes also render cells highly sensitive to ionizing radiation and defective in double-strand break (dsb) repair (25, 50). In addition to a role in dsb repair by homologous recombination, these genes are required for dsb repair by nonhomologous end joining (25, 50). Mre11, Rad50, and Xrs2 exist in a stable complex, the MRX complex, in yeast cells (52, 55). Rad50 belongs to the structural maintenance of chromosome family of proteins, and like the other members of this family, it has a bipartite ATPase domain located at its amino and carboxy termini, which are separated by a long, antiparallel coiled-coil domain. The protein folds onto itself, and the two termini form a globular ATPase domain. Two molecules of Mre11 bind to two molecules of Rad50 in the vicinity of the globular ATPase domain. The globular head, formed by two molecules of Rad50 and two molecules of Mre11, contains two DNA binding sites in it, which could promote the alignment of the two DNA ends for dsb repair by nonhomologous end joining, and which could also mediate the alignment of DNA ends with the sister chromatid in recombination (2, 8, 14–16). Xrs2 binds the Rad50-Mre11 complex, and it helps target the MRX complex to DNA ends (53).

Here, we have examined the roles of the *RAD51*, *RAD52*, and *RAD54* genes and of the *RAD50* and *XRS2* genes in PRR of DNA synthesized from UV-damaged templates. Although all these genes contribute to the PRR of UV damaged DNA in yeast cells, rather unexpectedly, they differ in their modus operandi. By contrast to Rad51, Rad52, and Rad54, which carry out PRR in a manner independent of the Rad5-mediated PRR, we find that Rad50 and Xrs2 contribute to PRR via both the Rad5- and Rad52-mediated PRR pathways.

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 yeast strains used in these studies are all derived from EMY74.7, *MAT***a** *his3-*Δ*1 leu2-3,112 trp1*Δ *ura3-52*: YR1-65, *rad1*Δ [*rho*^{0}]; YR1-118, *rad1*Δ *rad5*Δ [*rho*^{0}]; YR1-347, *The rad1*Δ *rad50*Δ [*rho*^{0}]; YR1-315, *rad1*Δ *rad51*Δ [*rho*^{0}]; YR1-231, *rad1*Δ *rad52*Δ [*rho*^{0}]; YR1-318, *rad1*Δ *rad54*Δ [*rho*^{0}]; YR1-350, *rad1*Δ *xrs2*Δ [*rho*^{0}]; YR1-241, *rad1*Δ *rad5*Δ *rad52*Δ [*rho*^{0}]; YR1-385, *rad1*Δ *rad50*Δ *rad5*Δ [*rho*^{0}]; YR1-400, *rad1*Δ *xrs2*Δ *rad5*Δ [*rho*^{0}]; YR1-356, *rad1*Δ *rad50*Δ *rad52*Δ [*rho*^{0}]; and YR1-408, *rad1*Δ *xrs2*Δ *rad52*Δ [*rho*^{0}].

UV irradiation and sedimentation in alkaline sucrose gradients. For postreplication repair experiments, alkaline sucrose gradient sedimentation is used to determine the size of nuclear DNA synthesized in cells following UV irradiation. To avoid confusion between UV-damaged nuclear DNA synthesized after UV irradiation that has not been repaired and undamaged mitochondrial DNA, which could be similar in size, these experiments are best performed with [*rho*^{0}] strains, which lack mitochondrial DNA. Although [*rho*^{0}] strains are respiratory deficient in medium containing glucose as a carbon source, the growth of [*rho*^{0}] cells and that of [*rho*$^+$] cells are very similar.

Yeast cells were grown to logarithmic phase in synthetic complete medium lacking uracil but containing 5 μg of uridine/ml. When the cells reached a density of 0.5×10^7 to 1.0×10^7 cells per ml, they were UV irradiated at room temperature in the same growth medium in 150- by 20-mm petri dishes with constant stirring at a dose rate of 0.1 J/m^2/s. To avoid photoreactivation, all operations after UV irradiation were performed in yellow light. Following UV irradiation, cells were labeled with radioisotope and incubated for various times, followed by conversion to spheroplasts. Briefly, after UV irradiation, cells collected by filtration were resuspended in fresh uridine medium at a density of 1×10^8 to 2×10^8 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, CA) 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, and incubated for an additional period of 30 min or 6 h. Cells were converted to spheroplasts as described previously (9), and a 0.3-ml aliquot of the spheroplast suspension was layered directly onto a 0.2-ml lysing layer (0.79 M sorbitol, 0.66 M EDTA, 2.5% sarkosyl, 0.3 M NaCl) on top of a 15 to 30% (wt/vol) linear alkaline sucrose gradient made in 0.3 M NaOH, 0.7 M NaCl, 40 mM EDTA, 1% Sarkosyl (pH 12.5). Centrifugation was carried out in an SW41 rotor (Beckman) at 21,000 rpm for 15 h and 30 min at 4°C as described previously (9). Processing of samples was done as described previously (9).

UV sensitivity. Cells grown to mid-logarithmic phase in yeast extract-peptone-dextrose medium were washed and sonicated to disperse clumps. Following sonication, they were pelleted by centrifugation and suspended in sterile distilled water to a density of 2×10^8 cells per ml. The cell suspensions were diluted, spread onto the surface of yeast extract-peptone-dextrose plates, and irradiated at a dose rate of 0.1 J/m^2/s for doses of 10 J/m^2 and below or at 1 J/m^2/s for doses above 10 J/m^2. Plates were incubated in the dark, and colonies were counted after 3 to 5 days.

RESULTS

Defective postreplication repair in the *rad51*Δ, *rad52*Δ, and *rad54*Δ mutants. To determine the effects of the *rad51*Δ, *rad52*Δ, and *rad54*Δ mutations on PRR, we examined the sizes of newly synthesized DNA in *rad1*Δ *rad51*Δ, *rad1*Δ *rad52*Δ, and *rad1*Δ *rad54*Δ mutant strains. Because of the absence of nucleotide excision repair in the *rad1*Δ strain, UV lesions are not removed from DNA and replication of such lesion-containing DNA templates becomes highly dependent upon the various lesion bypass processes. As we have shown previously (9), and the results are presented here for comparison (Fig. 1A), when *rad1*Δ cells are UV irradiated at 3.5 J/m^2 and the size of newly synthesized DNA is examined by pulse-labeling of DNA with [^{3}H]uracil for 15 min, followed by a chase for 30 min, the DNA sediments toward the top of the alkaline sucrose gradient, indicating the presence of discontinuities in the newly synthesized DNA (Fig. 1A). By contrast, in unirradiated *rad1*Δ cells, the size of newly synthesized DNA following the 15-min pulse and 30-min chase period becomes the same as that in uniformly labeled cells. Incubation of *rad1*Δ cells for 6 h following the 15-min pulse after UV irradiation results in the formation of normal-size DNA, indicating that postreplicative gap filling processes have restored normal size to DNA synthesized from the UV-damaged templates (Fig. 1A). In the *rad1*Δ *rad51*Δ, *rad1*Δ *rad52*Δ, and *rad1*Δ *rad54*Δ cells, however, we find that normal-size DNA is not reconstituted in cells UV irradiated at 3.5 J/m^2 and then given a 15-min pulse followed by a 6-h recovery period, and the efficiency of PRR is reduced to about the same extent in all three mutant strains (Fig. 1B, C, and D).

Rad5 and Rad52 control alternate pathways of PRR. Since an increase in UV sensitivity is observed when deletion mutations of the genes in the *RAD52* group are combined with the *rad6*Δ or *rad18*Δ mutations, *RAD52* and the other genes of this group are presumed to function in PRR via a pathway that operates independently of Rad6-Rad18-mediated lesion bypass pathways. Because Rad6-Rad18-dependent PRR is subsumed via the Rad5-Mms2-Ubc13 action (9, 51), we examined whether the introduction of the *rad5*Δ mutation enhances the PRR defect of the *rad52*Δ mutation. As shown in Fig. 2, the

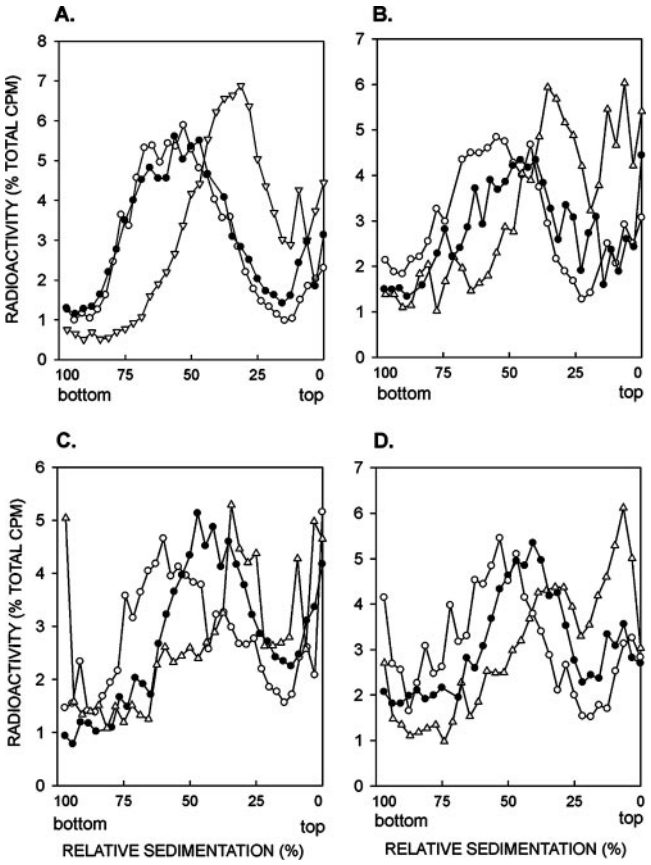

FIG. 1. Requirement of *RAD51*, *RAD52*, and *RAD54* genes for postreplication repair of UV-damaged DNA. Sedimentation in alkaline sucrose gradients of nuclear DNA from cells incubated for different periods following UV irradiation with 3.5 J/m^2. The *rad1*Δ (A), *rad1*Δ *rad51*Δ (B), *rad1*Δ *rad52*Δ (C), and *rad1*Δ *rad54*Δ (D) strains were UV irradiated at 3.5 J/m^2 and then pulse-labeled with [^{3}H]uracil for 15 min, followed by a 30-min (Δ) or 6-h (●) chase in high-uracil medium. Synthesis of normal-size DNA from unirradiated cells pulse-labeled with [^{3}H]uracil for 15 min was followed by a 6-h chase (○). The data for the *rad1*Δ strain in panel A shown here for comparison are taken from reference 9.

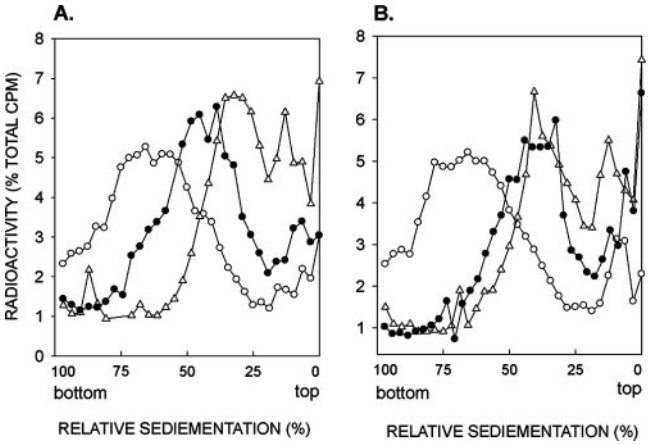

FIG. 2. *RAD5* and *RAD52* control alternate pathways for postreplication repair of UV-damaged DNA. Sedimentation in alkaline sucrose gradients of nuclear DNA from cells incubated for different periods following UV irradiation with 3.5 J/m^2. The *rad1*Δ *rad5*Δ (A) and *rad1*Δ *rad5*Δ *rad52*Δ (B) strains were UV irradiated at 3.5 J/m^2 and then pulse-labeled with [^{3}H]uracil for 15 min, followed by a 30-min (Δ) or 6-h (●) chase in high-uracil medium. Synthesis of normal-size DNA from unirradiated cells pulse-labeled with [^{3}H]uracil for 15 min was followed by a 6-h chase (○). The data for the *rad1*Δ *rad5*Δ strain in panel A shown here for comparison are taken from reference 9.

*rad1*Δ *rad5*Δ *rad52*Δ strain displays a much higher level of PRR defect than the *rad1*Δ *rad5*Δ strain or the *rad1*Δ *rad52*Δ strain (compare Fig. 1 and 2). In fact, compared to the intermediate level of repair capacity that remains in the *rad1*Δ *rad5*Δ or the *rad1*Δ *rad52*Δ strain, there is little, if any, evidence of repair in the *rad1*Δ *rad5*Δ *rad52*Δ strain. From these observations, we infer that PRR in yeast cells is effected via two pathways, a Rad6-Rad18 and Rad5-Mms2-Ubc13-dependent pathway and a Rad52-dependent pathway, and these two pathways function independently of one another for promoting replication through UV lesions.

Impaired PRR in *rad50*Δ and *xrs2*Δ mutants. Since the *RAD50*, *MRE11*, and *XRS2* genes function in the repair of dsb's by homologous recombination that is mediated by the proteins encoded by the *RAD52* group genes, we determined whether the MRX complex contributes also to efficient PRR and whether it carries out its role via the Rad52-dependent PRR pathway. To examine this, the *rad50*Δ and *xrs2*Δ mutations were combined with the *rad1*Δ mutation and the sizes of newly synthesized DNA examined in the double mutants following UV irradiation. As shown in Fig. 3A and B, normal-size DNA is not reconstituted in the *rad1*Δ *rad50*Δ and *rad1*Δ *xrs2*Δ strains that were UV irradiated at 3.5 J/m^2 and then given a 15-min pulse followed by a 6-h recovery period. Although the efficiency of PRR is affected in both of the mutant strains, in different experiments we have consistently observed a higher level of PRR defect in the *rad1*Δ *xrs2*Δ strain than in the *rad1*Δ *rad50*Δ strain (Fig. 3A and B).

To examine the possibility that Rad50 and Xrs2 function in the Rad52-dependent PRR pathway, we examined the efficiency of PRR in the *rad1*Δ *rad50*Δ *rad52*Δ and *rad1*Δ *xrs2*Δ *rad52*Δ strains. As expected, the PRR deficiency in these strains was no greater than that in the *rad1*Δ *rad52*Δ strain, thus suggesting a role for Rad50 and Xrs2 in the Rad52-dependent PRR pathway (compare Fig. 3E and F with Fig. 1C).

To verify that the role of Rad50 and Xrs2 was restricted to the Rad52-dependent pathway and that they did not contribute to Rad5-dependent PRR, we examined the PRR efficiency of the *rad1*Δ *rad50*Δ *rad5*Δ and *rad1*Δ *xrs2*Δ *rad5*Δ mutant strains. However, surprisingly, the PRR defect in these mutant strains was no greater than that observed in the *rad1*Δ *rad5*Δ strain (compare Fig. 3C and D with Fig. 2A). These observations lead us to conclude that the role of Rad50 and Xrs2 in PRR is not restricted just to the Rad52-dependent pathway but contributes also to Rad5-dependent PRR.

Epistasis relationship of the *rad50*Δ and *xrs2*Δ mutations with the *rad5*Δ and *rad52*Δ mutations. Although the *RAD50*, *MRE11*, and *XRS2* genes function in the repair of dsb's by homologous recombination in collaboration with the *RAD52* group of genes, and mutations in the genes encoding the MRX complex and in the genes belonging to the *RAD52* group display epistasis for γ-ray sensitivity, our observation that Rad50 and Xrs2 contribute to PRR mediated by both the Rad5- and

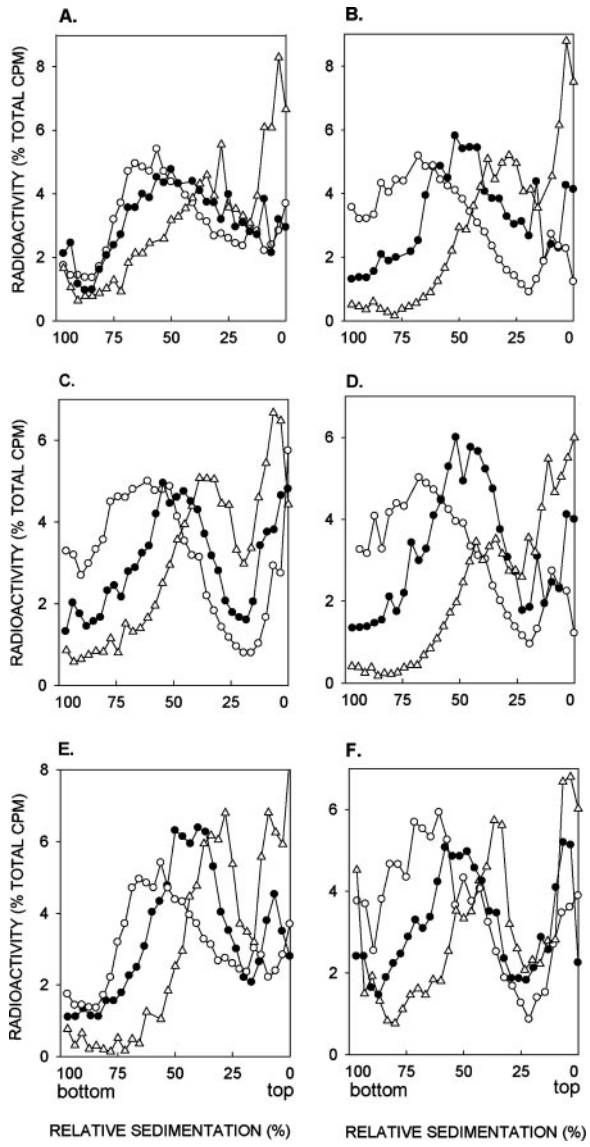

FIG. 3. Involvement of *RAD50* and *XRS2* in postreplication repair of UV-damaged DNA. Sedimentation in alkaline sucrose gradients of nuclear DNA from cells incubated for different periods following UV irradiation with 3.5 J/m^2. The *rad1*Δ *rad50*Δ (A), *rad1*Δ *xrs2*Δ (B), *rad1*Δ *rad50*Δ *rad5*Δ (C), *rad1*Δ *xrs2*Δ *rad5*Δ (D), *rad1*Δ *rad50*Δ *rad52*Δ (E), and *rad1*Δ *xrs2*Δ *rad52*Δ (F) strains were UV irradiated at 3.5 J/m2 and then pulse-labeled with [^{3}H]uracil for 15 min, followed by a 30-min (Δ) or 6-h (●) chase in high-uracil medium. Synthesis of normal-size DNA from unirradiated cells pulse-labeled with [^{3}H]uracil for 15 min was followed by a 6-h chase (○).

Rad52-dependent pathways has necessitated a reexamination of the epistasis relationships among these genes for repairing UV damage. As shown in Fig. 4A, the *rad5*Δ *rad50*Δ strain displays the same UV sensitivity as the *rad5*Δ strain and the UV sensitivity of the *mms2*Δ *rad50*Δ strain resembles the UV sensitivity profile of the *rad50*Δ strain. Thus, an epistatic relationship is indicated among the *rad5*, *mms2*, and *rad50* mutations. Since the UV sensitivity of the *rad50*Δ *rad52*Δ strain is about the same as that of the *rad50*Δ or *rad52*Δ strains (Fig. 4D), an epistatic relationship is also indicated between the *rad50* and *rad52* genes. For the *xrs2* mutation, however, we observe increases in the UV sensitivities of the *xrs2*Δ *rad5*Δ and *xrs2*Δ *mms2*Δ double mutants compared to the UV sensitivity of the more sensitive single-mutant strain (Fig. 4B), and the *xrs2*Δ *rad52*Δ double mutant also exhibits a higher level of UV sensitivity than either of the single mutants (Fig. 4D).

The epistatic relationship of the *rad50*Δ mutation with both the *rad5*Δ and *rad52*Δ mutations and the somewhat increased UV sensitivity of the *xrs2*Δ mutation with both the *rad5*Δ and *rad52*Δ mutations raised the possibility that as a structural element, the MRX complex not only affects the efficiency of PRR mediated by both the Rad5- and Rad52-dependent pathways but also contributes to TLS mediated by Pols η and ζ. For this reason, we examined the UV sensitivities of the *rad50*Δ and *xrs2*Δ mutations in combination with the *rad30*Δ or the *rev3*Δ mutation. Such double mutants, however, exhibit higher levels of UV sensitivity than the corresponding single mutants (Fig. 4C), which suggests that Rad50 and Xrs2 do not contribute to TLS by Pols η and ζ.

DISCUSSION

Here, we show that *RAD51*, *RAD52*, and *RAD54*, which are indispensable for mediating dsb repair by homologous recombination, contribute also to the repair of discontinuities that form in the newly synthesized DNA in UV-irradiated yeast cells. The results with the *rad52*Δ mutation validate the observations that we reported previously for the *rad52-1* point mutation (38). Our observation that the PRR of UV-damaged DNA is inhibited to a much greater extent in the *rad1*Δ *rad5*Δ *rad52*Δ mutant than in the *rad1*Δ *rad5*Δ or the *rad1*Δ *rad52*Δ strain indicates that Rad5 and Rad52 promote PRR via alternate pathways.

Replication through UV-induced DNA lesions in yeast cells thus is mediated by Rad6-Rad18-dependent processes and by a Rad52-dependent pathway which acts independently of Rad6 and Rad18 (Fig. 5). The existence of alternate PRR pathways raises a number of questions: how do the PRR pathways controlled by Rad6-Rad18 and by the Rad52 group of proteins differ? Do they compete for bypassing DNA lesions, and does the action of each pathway extend to repairing the gaps opposite lesions in both the leading and lagging strand templates, or are the two pathways confined to act specifically on one of the two DNA strands? Although our study yields no definitive answers to these questions, the available data allow us to make certain observations that are discussed below.

In the Mms2-Ubc13-Rad5-controlled PRR pathway, the Mms2-Ubc13 complex promotes the polyubiquitination of PCNA, and Rad5 acts as the ubiquitin ligase. In addition, Rad5 has a DNA helicase activity capable of performing replication fork regression (5), a reaction that was envisaged in a model for damage bypass by template switching proposed over 30 years ago (11). The requirement of Rad5, however, is not restricted just to PRR, as it contributes also to TLS (9). By contrast, all the available genetic evidence is consistent with the involvement of the Mms2-Ubc13 complex in PRR only (51). Whereas a combination of mutations in genes that function in competing pathways results in a synergistic enhancement in sensitivity to DNA-damaging agents and in repair deficiency, a combination of mutations in genes that affect different but noncom-

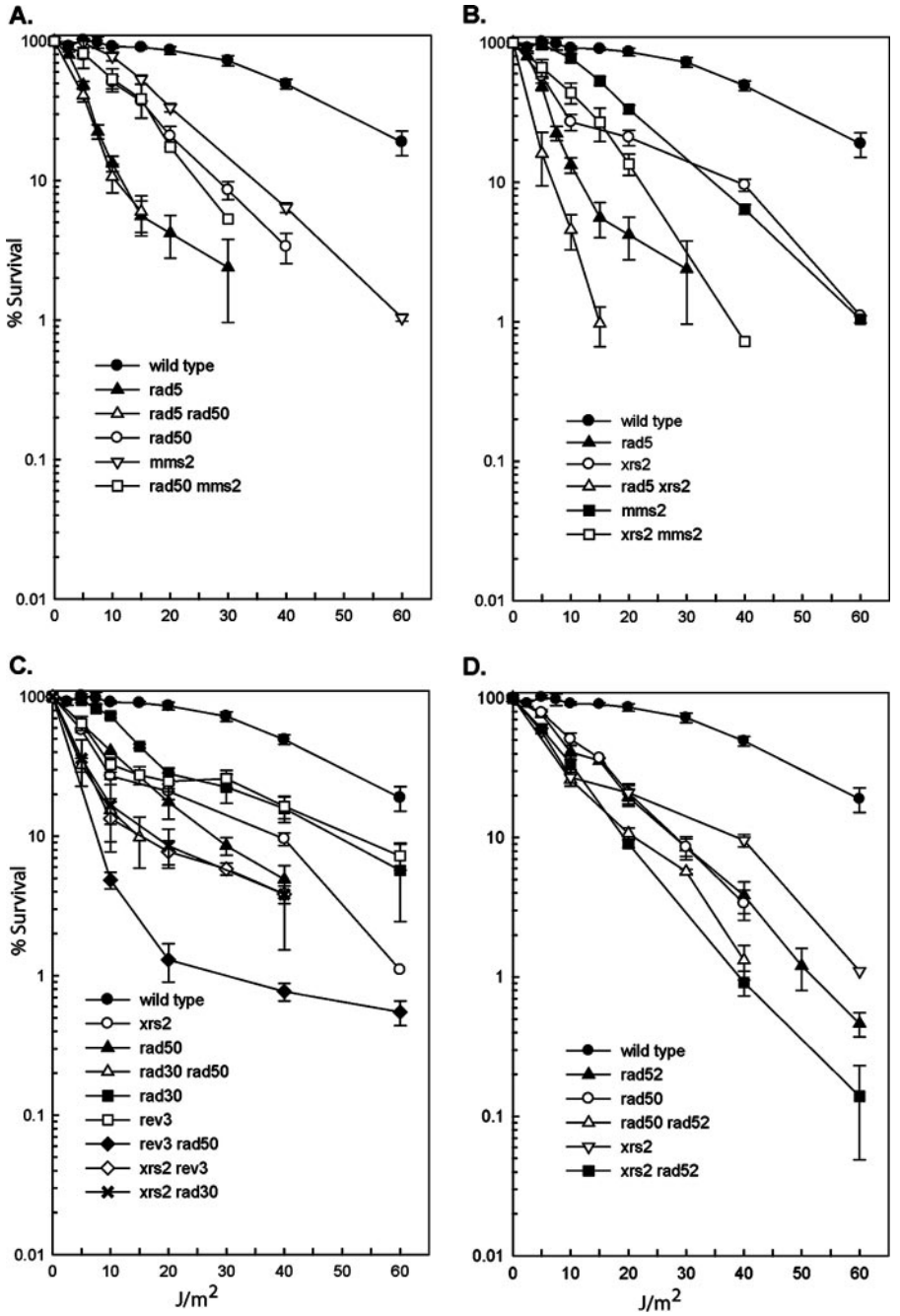

FIG. 4. Epistasis relationships of *rad50* and *xrs2* with *rad5* and *rad52.* Survival after UV irradiation of wild-type strain EMY74.7 and its isogenic derivatives. Survival curves represent an average for at least three different experiments for each strain. (A) Epistasis of *rad50*Δ with *mms2*Δ and with *rad5*Δ; (B) lack of epistasis of *xrs2*Δ with *rad5*Δ and with *mms2*Δ; (C) increased UV sensitivity of the *rad50*Δ and *xrs2*Δ mutations when combined with the *rev3*Δ or *rad30*Δ mutations; (D) epistasis of *rad50*Δ with the *rad52*Δ mutation and the lack of epistasis between the *xrs2*Δ and *rad52*Δ mutations. Error bars represent standard errors.

peting pathways results in an additive increase in sensitivity to DNA-damaging agents and in repair deficiency. Because the increased UV sensitivity of the *mms2*Δ *rad52*Δ double mutant over that of the corresponding single mutants approximates an additive rather than a synergistic relationship (51), and because the PRR defect of the *rad1*Δ *rad5*Δ *rad52*Δ strain compared to the PRR defect in the *rad1*Δ *rad5*Δ or *rad1*Δ *rad52*Δ strain is indicative of an additive relationship (compare Fig. 1 and 2), we suggest that the Rad5- and Rad52-dependent PRR pathways act predominantly in a noncompeting manner.

Genetic experiments in which *rad1*Δ yeast strains harboring the *rad18*Δ, *rad5*Δ, or *rad52*Δ mutation were transformed with a plasmid that carried a single (6-4) TT photoproduct in each DNA strand and that were 28 bp apart have indicated the requirement of Rad18, Rad5, and Rad52 for replication through this DNA lesion (58). Since a (6-4) TT photoproduct

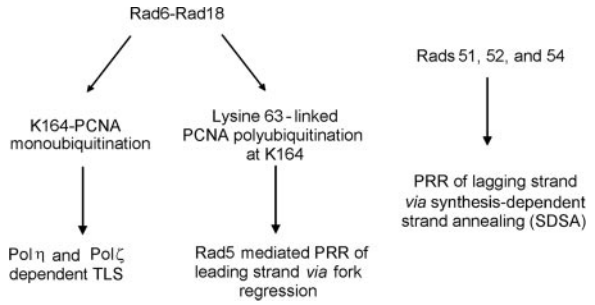

FIG. 5. Rad6-Rad18-dependent and Rad51-, Rad52-, and Rad54-dependent pathways for replication of UV-damaged DNA in yeast. It is proposed (see text for details) that Rad5-mediated PRR is restricted to the leading strand and that Rad proteins 51, 52, and 54, which are also likely to involve the other proteins that function with this group, such as Rad55 and Rad57, promote lesion bypass on the lagging strand. Further, it is suggested that both of the PRR pathways utilize nonrecombinational means that involve fork regression and template switching mediated by Rad5 (5) and the SDSA pathway, in which the Rad51-coated ss nucleoprotein filament formed on the strand with the 3′-OH terminus from the gapped region on the lagging strand invades the duplex on the leading strand side, and this is followed by D-loop formation, synthesis, and reannealing reactions (25, 47).

presents a strong block to synthesis by the TLS Pols, TLS makes only a small contribution (~4%) to replication through this lesion. Whereas Rad18 was responsible for the replication of ~70% of the plasmids and Rad5 was involved in ~60% of the replication events, Rad52 accounted for ~45% of the replication events (58). The nearly equivalent contribution of Rad5 and Rad52 for promoting replication through the (6-4) TT photoproduct suggested in this study is also in accord with the idea that Rad5 and Rad52 lesion bypass pathways do not compete. The inference that Rad5 and Rad52 modulate PRR via noncompeting pathways suggests that their action is confined to one of the two template strands. For reasons that we elaborate upon below, we suggest a role for the Rad5 pathway in promoting replication when the lesion is on the leading strand template and for the Rad52 pathway in promoting replication when the lesion is on the lagging strand template (Fig. 5).

Analyses of replication products from human cell extracts replicating simian virus 40-derived plasmids (49) have indicated that a site-specific T-T dimer when placed in the template of the leading strand of newly synthesized DNA blocks the synthesis on the leading strand, but synthesis on the lagging strand continues. When the T-T dimer is placed in the template for the lagging strand, however, there is little inhibition of replication fork progression and a small gap is left in the lagging strand, which presumably results from the inhibition of Okazaki fragment completion (49). An uncoupling of leading strand synthesis from lagging strand synthesis similar to that observed in human cells has also been suggested to occur in UV-irradiated yeast cells when the lesion is on the leading strand template (29). Overall, the various studies with mammalian and yeast cells (6, 7, 29, 49) have pointed to the existence of fundamental differences in the effects a lesion has on replication fork progression when it is located on the leading versus the lagging strand template.

The Rad5 and Rad52 PRR pathways differ in that the former pathway has the requirement for PCNA polyubiquitination whereas the latter pathway requires no such PCNA modification. This raises the possibility that when the lesion is on the leading strand template, PCNA polyubiquitination modulates the uncoupling of leading strand synthesis from lagging strand synthesis. In that case, the Rad5 pathway could act primarily when leading strand synthesis is blocked. The role of the Rad52 PRR pathway would then be relegated to the lagging strand.

The various genetic and biochemical observations with Rad5 have suggested a role for Rad5 in mediating error-free lesion bypass by transient template switching, wherein its DNA helicase activity, which is highly specialized for replication fork regression, could promote template switching and a copy choice type of DNA synthesis, in which the lesion on the leading strand is bypassed by template switching using the newly synthesized lagging strand as the template that would be formed upon fork regression (5). Although the proteins encoded by the *RAD52* group of genes are required for recombinational repair of dsb's, we consider it highly probable that the gap left opposite the DNA lesion on the lagging strand is filled in by the action of the Rad52 group proteins via a nonrecombinational pathway like that depicted in the synthesis-dependent strand annealing (SDSA) model, wherein the Rad51-coated ss nucleoprotein filament invades the DNA duplex on the leading strand side and the gap on the lagging strand is then filled in by DNA synthesis using the newly synthesized leading strand as the template (see references 25 and 47 for details of the SDSA model). The reason for suggesting a nonrecombinational mode of PRR for the Rad52-dependent pathway is that recombination is normally suppressed in yeast cells and it is only in the absence of Srs2 DNA helicase (24, 40, 56) or in the absence of Siz1-mediated PCNA sumoylation (10, 35, 37) that the recombinational repair pathway becomes activated.

The involvement of Rad50 and Xrs2 in PRR and our observation that the PRR defect of *rad1*Δ *rad50*Δ or *rad1*Δ *xrs2*Δ mutants is not increased in the presence of *rad5*Δ or *rad52*Δ mutations might suggest that the MRX complex contributes to both the Rad5- and Rad52-dependent PRR pathways, and it raises the possibility that the MRX complex plays a structural role in aligning the sister chromatids into close proximity, and thereby, this complex contributes to both of these pathways.

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