

Physical interaction between components of DNA mismatch repair and nucleotide excision repair

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ABSTRACT Nucleotide excision repair (NER) and DNA mismatch repair are required for some common processes although the biochemical basis for this requirement is unknown. *Saccharomyces cerevisiae* RAD14 was identified in a two-hybrid screen using MSH2 as “bait,” and pairwise interactions between MSH2 and RAD1, RAD2, RAD3, RAD10, RAD14, and RAD25 subsequently were demonstrated by two-hybrid analysis. MSH2 coimmunoprecipitated specifically with epitope-tagged versions of RAD2, RAD10, RAD14, and RAD25. MSH2 and RAD10 were found to interact in *msh3 msh6* and *mlh1 pms1* double mutants, suggesting a direct interaction with MSH2. Mutations in *MSH2* increased the UV sensitivity of NER-deficient yeast strains, and *msh2* mutations were epistatic to the mutator phenotype observed in NER-deficient strains. These data suggest that MSH2 and possibly other components of DNA mismatch repair exist in a complex with NER proteins, providing a biochemical and genetical basis for these proteins to function in common processes.

Eukaryotes contain a DNA mismatch repair (MMR) system involving proteins related to the bacterial MutS and MutL proteins (for a review see ref. 1). The eukaryotic MMR system is more complex than the bacterial system. Instead of involving a single MutS-related protein, eukaryotic MMR involves two different heterodimeric complexes of MutS-related proteins, MSH2-MSH3 and MSH2-MSH6, that each have different mispair recognition specificity (1–7). Similarly, instead of a single MutL-related protein, eukaryotic MMR also involves a heterodimeric complex of two MutL-related proteins, MLH1-PMS1 (PMS2 in humans) (8, 9). Initial characterization of these pathways concentrated on their function in correcting mispaired bases resulting from DNA replication errors and the formation of heteroduplex recombination intermediates. Subsequent studies have suggested that MMR proteins may play more diverse roles in DNA metabolism.

MMR plays roles in genetic recombination beyond the repair of mispaired bases in recombination intermediates. MMR appears to regulate the extent of formation of heteroduplex tracts during recombination (10–12), possibly by regulating the resolution of Holliday junctions (11). MMR also suppresses recombination between divergent sequences (13–16), a process that may be similar to the proposed regulation of heteroduplex tract formation. The MSH2 and MSH3 proteins also act in recombination between duplicated DNA sequences (17, 18) and have been implicated in the removal of nonhomologous DNA strands greater than 30 bases long at the ends of recombining segments (19). This reaction involves the nucleotide excision repair (NER) complex RAD1-RAD10 (20, 21). It is unclear how MMR proteins function in these reactions; however, the ability of MSH2 and the MSH2-MSH6 complex to bind to Holliday junctions and

branched DNA structures (ref. 22 and G. T. Marsischky, S. Lee, J. Griffith, and R.D.K., unpublished results) suggest they could bind to branched DNA structures and either target resolution enzymes and endonucleases to these structures or alter their structure making them more susceptible to cleavage.

MMR proteins have been implicated in DNA repair processes requiring NER proteins. Transcription-coupled repair in *Escherichia coli* and human cells is defective in MMR-defective mutants (23–25) and MMR-defective mutations decrease transcription-coupled repair of thymine glycol adducts but not UV damage in *Saccharomyces cerevisiae* (26, 27). Mutations in *MSH2*, *MSH3*, *RAD1*, and *RAD10* cause a defect in gene conversion of a 26-base insertion mutation, suggesting they could be involved in the repair of large insertion/deletion mispairs (28), and *in vitro* studies in *Drosophila* have demonstrated that MEI9, a homologue of *S. cerevisiae* RAD1, is required for MMR (29). Similarly, it has been observed that NER can repair base-base mispairs *in vitro* (30), although involvement of MMR proteins in this *in vitro* reaction was not demonstrated. MSH2 and MSH2-MSH6 complexes bind to DNA damage adducts normally repaired by NER (31–34) although it is unclear whether this binding reflects a role of MMR proteins in the repair of such adducts. In two studies of human MMR *in vitro*, MMR proteins did not appear to play a role in repair of these types of adducts, whereas in another study MMR proteins did play a role in such repair (35–37). In *E. coli*, there is evidence that MMR proteins recognize UV and alkylation damage *in vivo*, although it is not clear whether MMR normally repairs such lesions in *E. coli* (38–41).

There is a considerable, but sometimes conflicting, body of data suggesting an interaction between MMR and NER. These pathways could have overlapping specificity for DNA damage or alternately some components from each pathway could function together in a hybrid repair pathway. At present, there is little biochemical data concerning how these pathways could interact. In the present study we demonstrate a physical interaction between MMR and NER proteins and provide genetic data supporting an overlapping repair specificity of these pathways.

MATERIALS AND METHODS

General Genetic Methods. Yeast extract/peptone/dextrose, synthetic drop-out, 5-fluoroorotic acid, canavanine, and 5-bromo-4-chloro-indolyl β -D-galactopyranoside media were as described (11, 42, 43). Transformations were performed by the lithium

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acetate method (44). Genotyping of mutants was performed both by replica plating onto appropriate minimal media and by PCR analysis using primer pairs that allowed amplification of either the wild-type or mutant alleles (10). The PCR primers used were as follows: *RAD10*, *rad10::HIS3*, 26408 (5'-GGACATGGCTTGA-TTTTTACAGTGCTC) and 26409 (5'-GCCTCGCAGTATT-TGAAGTTGGATGG); *RAD14*, *rad14::HIS3*, 26412 (5'-GTT-TGACGTTTGCTAAGTTGTAGGGAG) and PASC5 (5'-CC-GCTCGAGTTCAGTTTTCCGAGATAGTTAATTATGTA-CGAGTGACA); *RAD2*, *rad2::HIS3*, 26443 (5'-GATGCCGCC-ACATATAGAGACCTTAAAG) and 26444 (5'-CGTTGCGC-GTGTGGGTGGGTGCC); *MLH1*, *mlh1::hisG*, 25591 (5'-ACTTTTGGAGACCGCTTGCTGTT) and 25592 (5'-GTCTTTGGTACCGTTGAATAA) and *PMS1*, *pms1::hisG*, 25589 (5'-GCTGCTGCGGTTTGTGG) and 25590 (5'-ATCC-GTCCCTTTGGTCTGTATCT).

Strains. The *S. cerevisiae* strains used in this study are listed in Table 1. The *rad10::HIS3*, *rad14::HIS3*, and *rad2::HIS3* deletion strains were constructed by using an adaptation of a published method (45). The *rad10* disruption construct was generated by amplifying the *HIS3* gene present in pRS423 by PCR using primers (plasmid sequences are in lowercase) 24862 (5'-ACGT-AACACAAAAAGGGCATAAACAAAGTTGGGTATC-CTAGAAGggcctctctagtactact) and 24863 (5'-GGTAATAA-GCATGGAACAGATTTATTAAGAAAATAGGAATT-GTgcccgcctctctcagaatg). The *rad14* disruption construct was generated by using primers 24860 (5'-GAAAAAGAGTTTGG-ATCTTCGTAGTGAAGGTATCGAACGTAACGCTggcctc-ctctagtactact) and 24861 (5'-CTTATTATGACTTTCTTGTTA-TATTCTTATATACATAACCAACATgcccgcctctcagaatg). The *rad2* disruption construct was generated by using primers 26441 (5'-GTTCTACACGTCATCCATGAAGAAAAGCAT-TTTCGGGAGAACGCCAACTTCAGACgagcagattgtact-gagagtgcacc) and 26442 (5'-CTTTGTAAACATGCAGAAAC-AAAGGTAATGTTTATAAATAGTAAATCATACATAA-GTATATGTTActcctctacgatctgtgcccgtatttc).

The resulting PCR products were used to transform RKY2672, resulting in strains RKY2343, RKY2350, and RKY2352 in which the entire *RAD14*, *RAD10*, and *RAD2* ORFs, respectively, were replaced with the *HIS3* gene. Each disruption construct also was used to transform RKY2706 (a *msh2::hisG* strain (42)) to construct double mutant strains RKY2344, RKY2351, and RKY2353. The correct integration of each *rad::HIS3* mutant allele was verified by PCR analysis and by testing for its characteristic UV sensitivity. The *msh3 msh6* strain, RKY2567, and the corresponding isogenic wild-type strain, RKY2575, were described (5).

A *mlh1 pms1* double mutant strain RKY2752 was constructed by A. Datta in this laboratory by using the one-step disruption method (46). RKY2672 was transformed with *KpnI*- and *SphI*-digested pEAI105 (*mlh1::hisG-URA3-hisG*). Excision of the *hisG-URA3-hisG* was selected on 5-fluoroorotic acid media, followed by transformation with *SalI*-digested

pEAI100 (*pms1::hisG-URA3-hisG*) and a second round of selection for excision of *hisG-URA3-hisG*. The presence of the *mlh1* and *pms1* mutations was confirmed by mutator patch assays and PCR.

Plasmids. The MSH2 bait (pRDK371) was constructed as described elsewhere by cloning the entire *MSH2* ORF into pEG202 (47). The "prey" vector, pJG4-5 (48), was modified by introducing *AvrII* and *BssHII* sites between the *EcoRI* and *XhoI* sites present in the vector. To do this, oligonucleotides 22909 (5'-AATTTCGCGCTAGGCGAGCGCGCGAC) and 22910 (5'-TCGAGTCGCGCGCTCGCCTAGGCCG) were annealed and inserted into *EcoRI*- and *XhoI*-digested pJG4-5 to yield pRDK483.

The different *ScRAD* ORFs were inserted into pRDK483 digested with *EcoRI/XhoI* or *AvrII/XhoI*. *RAD1* and *RAD2* ORFs were PCR amplified following a previously described method (5) from pG12-*RAD1* and pG12-*RAD2*, respectively (kindly provided by Errol Friedberg, University of Texas Southwestern Medical Center, Dallas), using KlenTaq/Pfu polymerases (Ab Peptides, St Louis/Stratagene). The *RAD3*, *RAD10*, *RAD14*, and *RAD25* ORFs were similarly PCR-amplified from genomic DNA. The names of the prey plasmids and the primers used for amplification of each gene are listed in Table 2.

LexA- and 12CA5-fusion constructs were tested for expression of full-length fusion protein by Western blot analysis using an anti-LexA antibody (49) kindly supplied by Roger Brent and his laboratory (Massachusetts General Hospital, Boston) or an anti-12CA5 antibody (Babco, Richmond, CA). All *ScRAD* and MSH2 fusions constructed were verified by sequencing.

The bait plasmids containing the ORFs *hCDC2*, *hCDK2*, and *DmCDC2* were supplied by R. Brent and his laboratory, and bait plasmid containing *ScPRP20* and the prey plasmid containing *ScNPL3* were from Pam Silver (Dana-Farber Cancer Institute).

Mutator and UV-Sensitivity Assays. Mutation rate assays and patch tests to determine mutator phenotypes were performed exactly as described (5). To study UV sensitivity of the different mutant strains, liquid cultures were grown overnight to saturation in yeast extract/peptone/dextrose (YPD), serial 10-fold dilutions were prepared, and 10 μ l of each dilution was spotted onto YPD plates. The plates then were irradiated at different UV doses by using a 254-nm germicidal lamp as indicated in individual experiments. The colonies were counted after 3 days of incubation in the dark.

Two-Hybrid Techniques. A two-hybrid screen (48, 50) for MSH2 interactors was performed as described (47). To test for interaction between MSH2 and different ScNER proteins, the *S. cerevisiae* strain EGY48 was cotransformed with the different *HIS3* bait and *TRP1* prey plasmids described along with the *URA3 lacZ* reporter, pSH18-34 (48). Transformants were isolated and patched onto Ura⁻His⁻Trp⁻ plates, then replicated onto Ura⁻His⁻Trp⁻ 5-bromo-4-chloro-indolyl β -D-galactopyranoside plates containing either glucose or galactose to monitor β -galactosidase expression. Positive interactions also were verified by

Table 1. *S. cerevisiae* strains used in this study

Strain	Genotype
RKY2926	<i>his3, trp1, ura3-52, lex(leu2)3a</i> = EGY48 containing pSH18-34 (<i>URA3</i>) plasmid
RKY2575	<i>MATa, ade2, ura3-52, his3Δ1, trp1-289, leu2-3,112, lys2-bgl, hom3-10</i>
RKY2567	<i>MATa, ade2, ura3-52, his3Δ1, trp1-289, leu2-3,112, lys2-bgl, hom3-10 msh3::hisG-URA3-hisG, msh6::URA3</i>
RKY2672	<i>MATa, ade2Δ1, ade8, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2-bgl, hom3-10</i>
RKY2706	<i>MATa, ade2Δ1, ade8, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2-bgl, hom3-10, msh2::hisG</i>
RKY2752	<i>MATa, ade2Δ1, ade8, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2-bgl, hom3-10, pms1::hisG, mlh1::hisG</i>
RKY2350	<i>MATa, ade2Δ1, ade8, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2-bgl, hom3-10, rad10::HIS3</i>
RKY2351	<i>MATa, ade2Δ1, ade8, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2-bgl, hom3-10, msh2::hisG, rad10::HIS3</i>
RKY2343	<i>MATa, ade2Δ1, ade8, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2-bgl, hom3-10, rad14::HIS3</i>
RKY2344	<i>MATa, ade2Δ1, ade8, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2-bgl, hom3-10, msh2::hisG, rad14::HIS3</i>
RKY2352	<i>MATa, ade2Δ1, ade8, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2-bgl, hom3-10, rad2::HIS3</i>
RKY2353	<i>MATa, ade2Δ1, ade8, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2-bgl, hom3-10, msh2::hisG, rad2::HIS3</i>

Table 2. PCR primer pairs used to construct prey vectors

Plasmid	Primer	Primer sequence
pRDK589 (RAD1)	23793	5'-GTTCTAGGTTATTGCACTATCCTGTTGAAAATATCT
	PASC2	5'-AGGTCCGCTCGAGTTAAACAATATTTTACACAGGTGC
pRDK590 (RAD2)	23197	5'-AGACCTAGGAACTTCAGACATGGGTGTG
	23191	5'-CCGCTCGAGACATGCAGAAACAAAGGTAATG
pRDK591 (RAD3)	PASC54	5'-TCTCCTAGGTGACTACACTTTAAGAAGATTGGAAACAATGAAG
	PASC32	5'-CCGCTCGAGAGTTTATAGCAAAAAGCGTATCATTGC
pRDK592 (RAD10)	PASC3	5'-TGGAATTC AAGATGAACAATACTGATCCTACTTCA
	PASC4	5'-CCGCTCGAGAGGATGGTAATAAGCATGGAACAG
pRDK593 (RAD14)	PASC8	5'-GGAATTCATGACTCCCGAACAAAAGGCCAACTAGAGGCTAACAGGAAATTAGCAATAGAACGG
	PASC5	5'-CCGCTCGAGTTCAGTTTTTCCGAGATAGTTAATTATGTACGAGTGACA
pRDK594 (RAD25)	23195	5'-GGGAATTCATCATGACGGACGTTGAAGGC
	23196	5'-CCGCTCGAGGGTGACAATGAAACCAAGCCTATTC

galactose-dependent growth on Ura⁻His⁻Trp⁻Leu⁻ glucose and galactose plates.

Immunoprecipitation Experiments. Whole-cell extracts were prepared from EGY48 strains containing bait and prey plasmids essentially as described (51). Fifty milliliters of yeast cultures were grown to an A₆₀₀ of 1.5–2 in Ura⁻His⁻Trp⁻ glucose medium, induced for 4 hr with galactose, pelleted, quickly frozen, and stored at –80°C. Glass bead extracts were prepared as described (51) except that vanadate, chymostatin, and pepstatin were omitted from the modified H buffer and 2 mM benzamidine was included. 12CA5-tagged preys were immunoprecipitated essentially as described (47, 51) in 500- μ l reactions containing extracts in modified H buffer and 150 mM NaCl. All immunoprecipitation (IP)-related manipulations were performed at 4°C. Two hundred micrograms of cell extract were precleared by a 50-min rotation with 25 μ l of protein G-Sepharose (Pharmacia) followed by a 20-sec spin at low speed in a microcentrifuge. One microliter (\approx 1 μ g) of 12CA5 ascites then was added to each supernatant, the reaction rotated for 2.5 hr, 25 μ l of protein G-Sepharose added, the reaction rotated for 50 min, and the protein G beads pelleted. The beads from the IP and from the preclear were washed six times in H buffer containing 150 mM NaCl, resuspended in 25 μ l of NaDodSO₄ sample buffer (Bio-Rad) and frozen at –80°C. Subsequently the samples were boiled for 5 min and fractionated by 10% NaDodSO₄-PAGE.

Proteins were transferred and membranes were saturated as described (47). The membranes were incubated 2.5 hr in a 1:5,000 dilution of primary anti-LexA antibody in TBST buffer (10 mM Tris, pH 8/150 mM NaCl/0.05% Tween-20). The membranes then were washed three times in TBST and incubated for 50 min in a 1:10,000 dilution of secondary antibody [horseradish peroxidase (HRP)-conjugated anti-rabbit Ig, Amersham], washed six times, and visualized by ECL using an ECL kit (Amersham). To ensure appropriate expression and immunoprecipitation of 12CA5-tagged preys, the immunoblot was stripped by a 30-min incubation at 50°C in 62.5 mM Tris (pH 6.7), 2% NaDodSO₄, and 100 mM 2-mercaptoethanol, then blocked and probed again using 12CA5 as primary antibody and HRP-conjugated anti-mouse Ig.

RESULTS

Interaction Between MMR and NER Proteins. A two-hybrid screen was performed in *S. cerevisiae* using the LexA DNA-binding domain fused to the entire ORF of *MSH2* as bait and an activation-tagged library of *S. cerevisiae* genomic DNA as prey (47). One protein identified was ScEXO1, an exonuclease displaying properties consistent with a direct role in MMR. Another clone was identified that encoded a portion of *S. cerevisiae* RAD14 (52). To confirm that RAD14 interacts with MSH2, we constructed a prey vector that expressed full-length RAD14 protein to test its interaction with full-length MSH2 and found that

full-length RAD14 and MSH2 did interact by two-hybrid criteria (see Fig. 2).

To further analyze whether NER proteins could interact with MSH2, we constructed prey vectors containing ORFs of five additional NER proteins (RAD1, RAD2, RAD3, RAD10, and RAD25) and tested their ability to interact with a LexA-MSH2 bait and activate a *lacZ* reporter gene. We first confirmed that the LexA-MSH2 and RAD10 fusion proteins were functional and could complement *msh2* and *rad10* mutations, respectively (Fig. 1). Similar functional complementation was observed for the RAD14 and RAD2 prey constructs (data not shown); RAD1, RAD3, and RAD25 were not tested. In a typical experiment (Fig. 2), RAD10 was found to interact with MSH2. The observed interaction depended on MSH2 and induction of expression of RAD10 by the addition of galactose (compare galactose induction vs. glucose in Fig. 2). MSH2 did not interact with ScNPL3 control prey protein, and RAD10 did not interact with hCDC2, hCDK2, or DmCDC2 control bait proteins, suggesting the activation of the reporter gene was caused by a specific interaction between MSH2 and RAD10. Pairwise interactions between MSH2 bait and RAD1, RAD2, RAD3, RAD10, RAD14, and RAD25 preys were examined in a separate experiment; significant interaction was observed for all of these pairs of proteins under conditions where none of the preys interacted with a nonspecific control bait PRP20 (Fig. 2).

To verify the interaction between MSH2 and different NER proteins, various combinations of LexA-MSH2 and LexA-

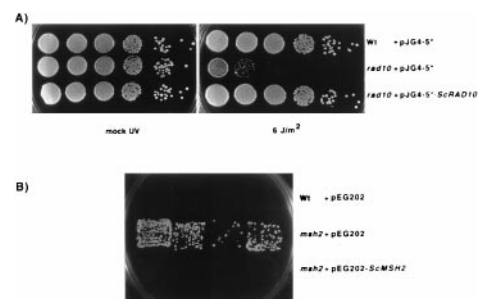


Fig. 1. Functional properties of RAD10 prey and LexA-MSH2 bait. (A) Complementation of the UV sensitivity of a *rad10* strain by expression of the RAD10 prey protein. The wild-type and *rad10* strains were transformed with indicated plasmids and expression of 12CA5-RAD10 protein in the strains was induced by transferring cells into synthetic drop-out (SD) Trp⁻ medium containing galactose followed by shaking for 4 hr. Then 10-fold serial dilutions were prepared and 10 μ l of different dilutions were spotted onto SD Trp⁻ plates containing galactose, and UV sensitivity was determined. (B) Complementation of the mutator phenotype of a *msh2* strain by expression of the LexA-MSH2 protein. Patches of the indicated strains on a yeast extract/peptone/dextrose plate were replica-plated to a canavanine plate to allow visualization of Can^r papilliae after incubation of the plates at 30° for 2 days.

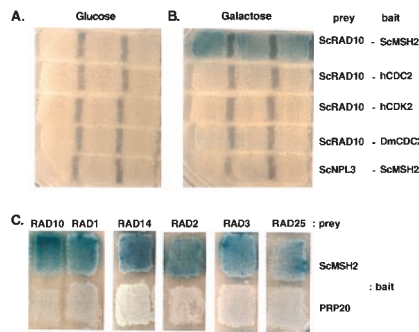


FIG. 2. Specific interaction of MSH2 with RAD10. The indicated baits and preys were cotransformed into EGY48 along with the *lacZ* reporter, pSH18-34. The preys are expressed only in the presence of galactose. (A and B) Three different transformants were patched on selective plates and then replica-plated onto 5-bromo-4-chloro-indolyl β -D-galactopyranoside (X-Gal) plates with either glucose or galactose. (C) Patches of one transformant containing each indicated pair of baits and preys replica plated onto X-Gal plates containing galactose. Interaction of a bait and a prey induces β -galactosidase activity producing blue coloration.

DmHAIRY were coexpressed with 12CA5-tagged RAD2, RAD10, RAD14, RAD25, and NPL3. Expression of proteins was monitored by Western blotting using anti-LexA and anti-12CA5 antibodies (data not shown). Prey proteins were immunoprecipitated with 12CA5 antibody and detected by Western blotting with LexA antibody. The results showed that LexA-MSH2 coimmunoprecipitated with RAD10 (Fig. 3). In contrast, RAD10 did not interact with the LexA-DmHAIRY control protein and the NPL3 control protein did not interact with LexA-MSH2 (Fig. 3). LexA-MSH2 also coimmunoprecipitated with RAD2, RAD14, and RAD25 proteins (Fig. 4). An EXO1 prey was used as positive control for coimmunoprecipitation (47); the level of precipitation of RAD10 and RAD14 was similar to that of EXO1, whereas the levels of precipitation of RAD2 and RAD25 were somewhat lower (Figs. 3 and 4). We ensured appropriate immunoprecipitation of all preys by stripping the membrane and Western blotting with anti-12CA5 antibodies (data not shown). RAD proteins did not coimmunoprecipitate nonspecifically with a LexA-DmHAIRY control, and 12CA5-NPL3 did not coimmunoprecipitate with LexA-MSH2. This finding indicates that neither LexA-MSH2 nor 12CA5-tagged NER proteins are nonspecifically "sticky" proteins in coimmunoprecipitation experiments.

The interactions observed could involve a direct interaction between MSH2 and one or more of the NER proteins tested or they could involve an indirect interaction, presumably requiring

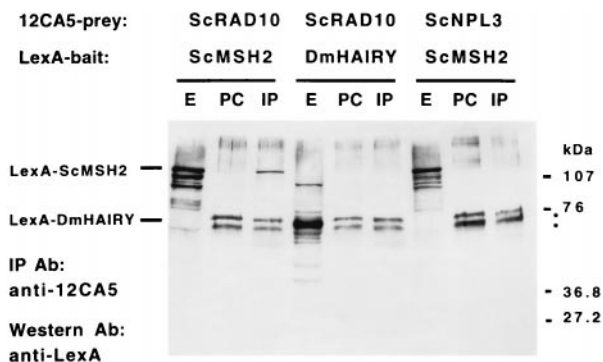


FIG. 3. Coimmunoprecipitation of MSH2 and RAD10. Whole-cell extracts (200 μ g) were prepared from EGY48 strains expressing LexA-tagged baits (MSH2, or DmHAIRY as a negative control) and 12CA5-tagged preys (RAD10, or ScNPL3 as a negative control). The extracts (E) then were analyzed by immunoprecipitation (IP) and Western blotting. PC, proteins eluted from protein G-Sepharose used to pre-clear; Ab, antibody; *, proteins nonspecifically precipitated by protein-G Sepharose.

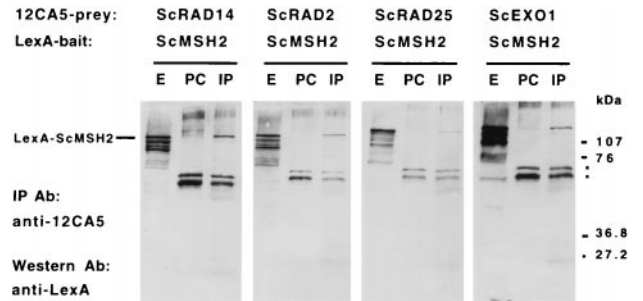


FIG. 4. Coimmunoprecipitation of MSH2 and different NER proteins. Whole-cell extracts (200 μ g) were prepared from EGY48 expressing LexA-tagged bait (MSH2) and 12CA5-tagged preys (RAD10, RAD14, RAD2, RAD25, or ScEXO1 as positive control). The extracts (E) then were analyzed by immunoprecipitation (IP) and Western blotting. PC, proteins eluted from protein G-Sepharose used to pre-clear; Ab, antibody; *, proteins nonspecifically precipitated by protein-G Sepharose.

either MSH3, MSH6, MLH1, or PMS1. To investigate this possibility, coimmunoprecipitation of LexA-MSH2 and RAD10 was studied in *msh3 msh6* and *mlh1 pms1* double mutants. The results show that LexA-MSH2 and RAD10 interacted in the absence of MSH3, MSH6, MLH1, or PMS1 (Fig. 5), consistent with the view that the observed interactions directly involve MSH2.

Overlapping Roles of MMR and NER Proteins in DNA Repair.

The observed interactions between MMR proteins and NER proteins suggest that these proteins could function in the same pathway(s) to some extent or that each of these repair pathways may recognize and repair some of the same types of DNA damage. To test these possibilities, we investigated the effects of mutations in NER and MMR genes on the rate of accumulation of mutations in target genes and on the sensitivity to UV irradiation. Mutations in *RAD2*, *RAD10*, and *RAD14* caused small increases in the rate of accumulation of Can^r mutations (2- to 3-fold) and reversion of the *hom3-10* (up to 2-fold) and *lys2-Bgl* alleles (up to 2-fold) (Table 3), consistent with previously published results on the mutator phenotypes caused by mutations in other NER genes like *RAD1* (53) and *RAD3* (54). When mutations in these genes were combined with a *msh2* mutation, the resulting mutation rates were the same as observed with the *msh2* single mutant. It is unlikely that additivity could have been observed because *msh2* mutations cause a much greater mutator phenotype than mutations in *RAD2*, *RAD10*, or *RAD14* but clearly a multiplicative effect was not seen. These results suggest that either *RAD2*, *RAD10*, and *RAD14* function in a MSH2-dependent repair pathway or they function in a different pathway that can suppress the accumulation of some types of mutations,

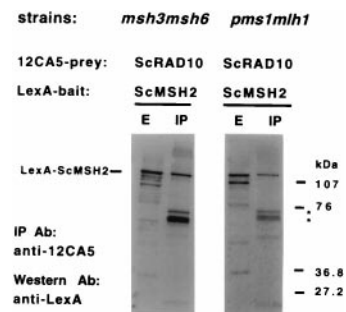


FIG. 5. Interaction of MSH2 and RAD10 in the absence of MSH3, MSH6, MLH1, and PMS1. Whole-cell extracts (200 μ g) were prepared from RKY2567 and RKY2752 expressing LexA-tagged bait (MSH2) and 12CA5-tagged prey (RAD10). The extracts (E) then were analyzed by immunoprecipitation (IP) and Western blotting. PC, proteins eluted from protein G-Sepharose used to pre-clear; Ab, antibody; *, proteins nonspecifically precipitated by protein-G Sepharose.

Table 3. Mutation rates in MMR- and NER-defective strains

Relevant genotype	Can ^r	Hom ⁺	Lys ⁺
Wild type	2.4×10^{-7} (1)	8.7×10^{-9} (1)	1.0×10^{-8} (1)
<i>rad10</i>	4.7×10^{-7} (1.9)	11.2×10^{-9} (1.3)	1.4×10^{-8} (1.4)
<i>rad2</i>	8.0×10^{-7} (3.2)	7.7×10^{-9} (0.9)	1.6×10^{-8} (1.6)
<i>rad14</i>	5.6×10^{-7} (2.2)	10.5×10^{-9} (1.2)	2.0×10^{-8} (2)
<i>msh2</i>	2.9×10^{-6} (12)	4.5×10^{-6} (517)	7.3×10^{-7} (73)
<i>msh2 rad10</i>	3.7×10^{-6} (15)	5.7×10^{-6} (655)	ND
<i>msh2 rad2</i>	3.3×10^{-6} (13)	4.5×10^{-6} (528)	6.4×10^{-7} (64)
<i>msh2 rad14</i>	2.8×10^{-6} (11.4)	5.0×10^{-6} (580)	5.9×10^{-7} (59)

Mutation rates (inactivation of *CAN1* and reversion of *hom3-10* or *lys2-Bgl*) were determined and the mean of 3-5 independent experiments is presented. The numbers in parentheses are the increase in mutation rate observed relative to wild type. The strains used are the strains listed in the legend to Fig. 6. Also see Table 2. ND, not determined.

most likely base substitution mutations (ref. 53 and C. Chen and R.D.K., unpublished results). The observed results are inconsistent with the idea that mutations in *RAD2*, *RAD10*, or *RAD14* result in the accumulation of DNA damage that is repaired by an MSH2-dependent pathway.

Previous studies of UV-induced recombination in NER-defective *E. coli* strains suggested that MMR can recognize or interact with UV damage, resulting in increased recombination (40, 41). To determine whether similar effects could be seen in *S. cerevisiae*, we tested the effect of a mutation in MSH2 on the UV sensitivity of strains containing *rad14*, *rad10*, and *rad2* deletion mutations. As expected, the *rad14*, *rad10*, and *rad2* single mutants were highly UV sensitive (Fig. 6). A *msh2* mutation did not cause any detectable UV sensitivity alone but did cause an increase in the UV sensitivity of *rad10* and *rad14* mutants (a 5- to 10-fold range of increased UV sensitivity was seen in different experiments). These data suggest that MSH2 can function in a minor pathway for the repair of UV damage distinct from *RAD10*, *RAD14*-dependent NER. The *msh2* mutation caused little increase in the UV sensitivity caused by the *rad2* mutation, suggesting that *RAD2* may function in both pathways.

DISCUSSION

The experiments presented here document *in vivo* interaction between *S. cerevisiae* MSH2 and six different NER proteins,

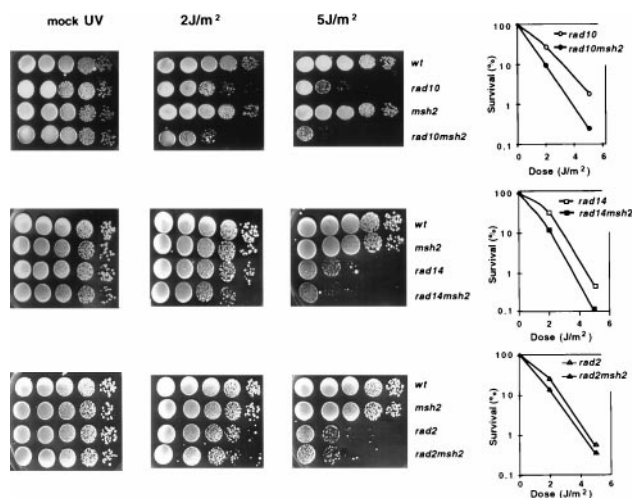


FIG. 6. Effect of an *msh2* mutation on UV sensitivity of NER mutants. The indicated mutant strains were analyzed for UV sensitivity. The graphs shown indicate the % survival observed relative to no UV irradiation. No significant killing of either the wild type or the *msh2* mutant strain was observed at the indicated UV dose. Three to 10 independent experiments were performed with each set of strains and one representative example is shown. The strains used were RKY2672, RKY2706 *msh2*, RKY2350 *rad10*, RKY2351 *msh2 rad10*, RKY2343 *rad14*, RKY2344 *msh2 rad14*, RKY2352 *rad2*, and RKY2353 *msh2 rad2*.

RAD1, *RAD2*, *RAD3*, *RAD10*, *RAD14*, and *RAD25*, which are components of an NER complex (55). We have not yet determined which NER protein(s) interacts directly with MSH2 or which other MMR proteins such as MSH3, MSH6, MLH1, and PMS1 are present in the observed MMR-NER complex(es). These interactions are consistent with genetic experiments implicating MMR and NER proteins in the same repair and recombination reactions.

There is a growing body of evidence that MMR and NER may functionally overlap and that MMR and NER proteins may act together in some types of repair. Previous studies have shown the *in vivo* requirement of MMR in transcription-coupled repair in *E. coli*, human, and for some types of damage, *S. cerevisiae* cells (23–27). NER can repair base-base mispairs *in vitro* (30), and MSH2 and MSH2-MSH6 complex are known to recognize UV damage, damage by platinum compounds and other adducts in DNA that are thought to be repaired by NER (31–34, 37). However, no clear picture has emerged as to whether the ability of one pathway to recognize damage normally repaired by the other pathway reflects significant repair reactions *in vivo* (35–37). The results presented here provide insight into these previous observations. For instance, the interaction of MSH2 with essentially all of the NER components required for transcription-coupled repair suggests it is the disruption of this complex in MMR mutants that causes a defect in transcribed strand repair. Second, our observation of increased UV sensitivity of MMR-NER double mutants compared with NER single mutants suggests that an MSH2-dependent process can serve as an alternate, albeit minor, pathway for the repair of UV damage that is independent of *RAD1*-*RAD10* and *RAD14* function. Our data showing that *rad2* mutants and *rad2 msh2* double mutants have similar UV sensitivity suggest that MSH2, and possibly other MMR proteins, recognize UV damage and target the *RAD2* endonuclease to this substrate. It should be noted that it has been suggested that XPG, the human *RAD2* homolog, plays additional roles in repair of oxidative damage to DNA besides its role in NER (56). Previous studies on the role of MMR in UV-stimulated recombination in *E. coli* NER-defective mutants and on the role of MMR in lethality of *E. coli dam* mutants caused by treatment with alkylating agents (38–41) are consistent with the view that MMR proteins can functionally recognize damage that usually is repaired by NER. Finally, our analysis of the mutator phenotype of NER mutants along with the results of previous studies of the mutator phenotype of *rad1* mutants and certain *rad3* mutants alleles (53, 54) suggests NER proteins could play a direct role in the repair of replication errors. Studies on gene conversion in MMR and NER mutants in *S. cerevisiae* and *in vitro* studies indicating the *Drosophila* MEI9 plays a role in MMR support the view that such repair could involve both NER and MMR proteins in some cases (28, 29), a result that is consistent with the results presented here.

The biological relevance of physical interaction between MMR proteins and NER proteins is even more clear for recombination. *RAD1*, *RAD10*, *MSH2*, and *MSH3* are required for recombina-

tion between duplicated sequences and double-strand-break-induced single-strand annealing recombination, and *RAD1* and *RAD10* are required to remove nonhomologous DNA from the 3' ends of recombining DNA during these processes (20, 57). *RAD1*, *RAD10*, and *MSH2*, but not *RAD2* or *RAD14*, are required for gene conversion of large insertion/deletion mutations that would form large loop mispairs in heteroduplex recombination intermediates (28). Consistent with this requirement, the *RAD1-RAD10* complex is a weak endonuclease *in vitro* that can cleave branched DNA structures and presumably large loop mispairs (57). The *RAD1-RAD10* endonuclease does not recognize base damage such as pyrimidine dimers in duplex DNA (58). It has been suggested that the specificity and reactivity of the *RAD1-RAD10* endonuclease is modulated by further protein-protein interactions with proteins such as *RAD14* that may target the *RAD1-RAD10* complex and other NER proteins to the site of DNA damage (for reviews see refs. 55 and 59). Thus, factor(s) likely exist to target this complex to other substrates (i.e., non-homologous tails and loop mispairs), during recombination events. The genetic data documenting the involvement of *MSH2* and *MSH3* in the *RAD1 RAD10* recombination pathway (17, 18, 28) and the physical interaction we observed suggest that this factor could be *MSH2* or the *MSH2-MSH3* complex. Indeed, *MSH2* by itself and in conjunction with *MSH3* has been shown to recognize loop mispairs (2, 5, 6, 60, 61) and *MSH2* and *MSH2-MSH6* complex recognize branched DNA structures such as Holliday junctions and Y structures in addition to different mispairs (refs. 22 and 60; G. T. Marsischky, S. Lee, J. Griffith, and R.D.K., unpublished results). It is interesting to note that in mammalian cells, the ERCC1 gene product (*RAD10* homologue) has been suggested to be involved in the processing of heteroduplex intermediates during recombination (62). Thus, it is possible that *MSH2*, *MSH2-MSH3*, and/or *MSH2-MSH6* complexes bind to such structures as branched DNAs and loop mispairs and then recruit the *RAD1-RAD10* endonuclease by the direct interaction demonstrated here. This interaction could explain the requirement of *RAD1*, *RAD10*, *MSH2*, and *MSH3* for recombination reactions and repair of large loops. Similarly, *MSH2* and *MSH2* containing complexes could target Holliday junction resolution enzymes to Holliday junctions.

The results presented here provide a physical basis for results suggesting functional interactions and overlap between MMR and NER. There are several possibilities for how this overlap could occur. One possibility is that there is a MMR-NER complex containing both the NER proteins described here and *MSH2* along with other components of MMR like *MSH3*, *MSH6*, *MLH1*, and *PMS1*. This complex would contain two different types of DNA damage recognition components, each of which has overlapping damage recognition specificity. Damage recognition by either component then could target other components of both MMR and NER to the site of damage where either could act in repair. Alternately, there could be different complexes containing subsets of MMR and NER proteins, each of which would likely have different DNA damage/structure recognition specificity and this differential specificity would target different combinations NER and/or MMR proteins to the repair of specific DNA structures. The available data suggest that there may exist at least two different types of complexes or reactions, a *MSH2*-dependent reaction that targets *RAD1-RAD10* endonuclease to branched structures and large loop mispairs and a *MSH2*-dependent reaction that targets *RAD2* endonuclease to UV damage. Additional biochemical and genetic studies will be required to distinguish among these possibilities.

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