Radiosensitive and Mitotic Recombination Phenotypes of the *Saccharomyces cerevisiae dun1* Mutant Defective in DNA Damage-Inducible Gene Expression

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ABSTRACT

The biological significance of DNA damage-induced gene expression in conferring resistance to DNA-damaging agents is unclear. We investigated the role of *DUN1*-mediated, DNA damage-inducible gene expression in conferring radiation resistance in *Saccharomyces cerevisiae*. The *DUN1* gene was assigned to the *RAD3* epistasis group by quantitating the radiation sensitivities of *dun1*, *rad52*, *rad1*, *rad9*, *rad18* single and double mutants, and of the *dun1 rad9 rad52* triple mutant. The *dun1* and *rad52* single mutants were similar in terms of UV sensitivities; however, the *dun1 rad52* double mutant exhibited a synergistic decrease in UV resistance. Both spontaneous intrachromosomal and heteroallelic gene conversion events between two *ade2* alleles were enhanced in *dun1* mutants, compared to *DUN1* strains, and elevated recombination was dependent on *RAD52* but not *RAD1* gene function. Spontaneous sister chromatid exchange (SCE), as monitored between truncated *his3* fragments, was not enhanced in *dun1* mutants, but UV-induced SCE and heteroallelic recombination were enhanced. Ionizing radiation and methyl methanesulfonate (MMS)-induced DNA damage did not exhibit greater recombinogenicity in the *dun1* mutant compared to the *DUN1* strain. We suggest that one function of *DUN1*-mediated DNA damage-induced gene expression is to channel the repair of UV damage into a nonrecombinogenic repair pathway.

THE expression of radiation-inducible genes is enhanced when cells are exposed to either UV or ionizing radiation. In prokaryotes, radiation induces the SOS response and triggers the expression of genes directly involved in DNA recombination, DNA repair, and cell cycle arrest (for review, see Friedberg *et al.* 1995). Inducible recombination in prokaryotes functions in postreplication repair of UV-induced DNA lesions (Rupp *et al.* 1971). The identification in *Saccharomyces cerevisiae* of both radiation-inducible genes (Ruby and Szostak 1985) and genes that arrest the cell cycle at specific cell cycle checkpoints (Weinert *et al.* 1994) underscores the importance of understanding whether radiation resistance in eukaryotes is conferred by elevating the expression of specific repair genes.

In *S. cerevisiae*, ~1% of the yeast genome is estimated to encode DNA damage-inducible genes (Ruby and Szostak 1985). These genes are involved in DNA metabolism, such as *RNR3* (large subunit of ribonucleotide reductase; Elledge and Davis 1990), *RNR2* (small subunit of ribonucleotide reductase; Elledge and Davis 1987), *CDC9* (DNA ligase; Barker *et al.* 1985), and *CDC17* (DNA polymerase α; Johnston *et al.* 1987), and in the recombinational repair of double-strand breaks, such as *RAD54* (Cole *et al.* 1987) and the yeast *recA* homolog, *RAD51* (Aboussekhra *et al.* 1992; Basile *et al.* 1992). Although it would seem logical that radiation-induced gene expression of DNA repair genes would enhance DNA repair, its biological significance in conferring radiation resistance is unclear. First, *RAD1* (Friedberg *et al.* 1995) and *RAD52* (Cole *et al.* 1989), genes that participate directly in the repair of UV or ionizing radiation-induced damage, respectively, are not induced after the exposure of radiation in mitotic cells but are constitutively expressed. Second, deletion of the damage response element (DRE) sequences upstream of either *RAD2* (Siede *et al.* 1989) or *RAD54* (Cole *et al.* 1989) does not confer enhanced radiation sensitivity in growing cultures after exposure to UV or ionizing radiation, respectively. Thus, the basal level of expression of DNA repair genes appears sufficient to maintain wild-type levels of DNA damage resistance in mitotic yeast.

The identification of protein kinase mutants defective in the transcriptional induction of *RNR3* and hypersensitive to DNA-damaging agents, however, has established a biological function for the DNA damage-inducible response in DNA repair. These protein kinases include the serine-threonine kinases Rad53/Sad1 (Allen *et al.* 1994) and Dun1 (Zhou and Elledge 1993), the ataxia-telangiectasia mutated (ATM)-like kinase, Mec1 (Weinert *et al.* 1994), and the casein kinase I isoform Hrr25 (Ho *et al.* 1997). The Dun1 kinase is hypostatic

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TABLE 1

Yeast strains

Lab name (synonym)	Genotype	Source
YA103 (W303-1A)	*MAT***a** [*ade2-1 can1-100 his3-11,15 leu2-3,112 trpl-1 ura3-1*]	Thomas and Rothstein (1989)
YA104 (W303-1B)	*MAT*α [*ade2-1 can1-100 his3-11,15 leu2-3,112 trpl-1 ura3-1*]	Thomas and Rothstein (1989)
	Strains derived from W303[a]	
YA145 (Y286)	*MAT*α *dun1-Δ100::HIS3*	Zhou and Elledge (1993)
YA133 (W839-52)	*MAT***a** *rad52::TRP1*	R. Rothstein
YA131 (W839-5D)	*MAT***a** *rad1::LEU2*	R. Rothstein
YA132 (W889)	*MAT*α *rad9::URA3*	R. Rothstein
YA154 (W841-11D)	*MAT*α *rad18::LEU2*	R. Rothstein
YA156 (YK12a)	*MAT***a** *ade2-n::URA3::ade2-a*	Huang and Symington (1994)
YA157 (YK12α)	*MAT*α *ade2-n::URA3::ade2-a*	Huang and Symington (1994)
YD100	*MAT***a** *dun1-Δ100::HIS3 ade2-n::URA3::ade2-a*	This study
YD101	*MAT***a** *dun1-Δ100::HIS3 ADE2*	This study
YD102	*MAT***a** *rad52::TRP1 ade2-n::URA3::ade2-a*	This study
YD103	*MAT***a** *dun1-Δ100::HIS3 rad52::TRP1*	This study
YD104	*MAT***a** *dun1-Δ100::HIS3 rad9::URA3*	This study
YD105	*MAT***a** *dun1-Δ100::HIS3 rad1::LEU2*	This study
YD106	*MAT***a** *dun1-Δ100::HIS3 rad9::URA3 rad52::TRP1*	This study
YD107	*MAT***a** *dun1-Δ100::HIS3 rad18::LEU2*	This study
YD108	*MAT***a** *rad1::LEU2 ade2-n::URA3::ade2-a*	This study
YD110	*MAT***a** *dun1-Δ100::HIS3 rad52::TRP1 ade2-n::URA3::ade2-a*	This study
YD111	*MAT***a** *dun1-Δ100::HIS3 rad1::LEU2 ade2-n::URA3::ade2-a*	This study
YD112	*MAT***a** *dun1-Δ100::HIS3 rad9::URA3 ade2-n::URA3::ade2-a*	This study
YD114	*MAT***a** *ade2-a*	This study
YD115	*MAT*α *ade2-n*	This study
YD116	*MAT***a**/*MAT*α *ade2-a/ade2-n*	This study
YD117	*MAT***a**/*MAT*α *ade2-a/ade2-n dun1-Δ100::HIS3/dun1-Δ100::HIS3*	This study
YD118	*MAT***a**/*MAT*α *ade2-a/ade2-n rad52::TRP1/rd52::TRP1*	This study
YD119	*MAT***a**/*MAT*α *ade2-a/ade2 dun1-Δ100::HIS3/dun1-Δ100::HIS3 rad52::TRP1/rad52::TRP1*	This study
YD120	*MAT***a**/*MAT*α *ade2-a/ade2-n rad1::LEU2/rad1::LEU2*	This study
YD121	*MAT***a**/*MAT*α *ade2-a/ade2-n dun1-Δ100::HIS3/dun1-Δ100::HIS3 rad1::LEU2/rad1::LEU2*	This study
YD122	*MAT***a** *trp1::his3-Δ3′his3-Δ5′ URA3*	This study
YD123	*MAT***a** *dun1-Δ100::his3::URA3*	This study
YD124	*MAT***a** *dun1-Δ100::his3::URA3 trp1::his3-Δ3′his3-Δ5′URA3*	This study

[a] Strains listed below are identical to W303-1A or W303-1B genotype except for the markers that are noted.

to Rad53/Sad1 and Mec1 protein kinases in the pathway for DNA damage induction (Allen *et al.* 1994), but its epistatic relationship with Hrr25 is unknown (Ho *et al.* 1997). A subset of DNA-damage inducible genes can still be induced in *dun1* mutants; for example, induction of *RAD51*, a gene involved in recombinational repair, is *DUN1* independent but *RAD9* dependent (Aboussekhra *et al.* 1996). Further studies have indicated that the pathways for *DUN2 (POLε)*-dependent transcriptional induction and *RAD9*-mediated cell cycle arrest are independent pathways that respond to DNA damage (Navas *et al.* 1996).

After observing that *rad9* mutants, which fail to arrest the cell cycle at the G_2 checkpoint (Weinert and Hartwell 1988), exhibit higher frequencies of chromosomal rearrangements (Fasullo *et al.* 1998), we investigated both the recombination and radiosensitive phenotypes of *dun1* mutants. We demonstrate that the *dun1 rad52* and *dun1 rad9* mutants exhibit a synergistic increase in UV sensitivity and that *dun1* mutants exhibit enhanced frequencies of spontaneous and UV-induced recombination. We suggest that the *DUN1* pathway for DNA damage-induced gene expression participates in the *RAD3* pathway, and that DNA damage tolerance in *dun1* mutants is partially conferred by *RAD52*-dependent recombinational mechanisms.

MATERIALS AND METHODS

Methods: Yeast extract, peptone, dextrose (YPD), synthetic dextrose (SD), synthetic complete (SC)-ADE, and SC-HIS are described by Sherman *et al.* (1982). YP(A)D contains YPD supplemented with 80 mg/liter of adenine. Ura^- isolates were selected on fluoroorotic acid (FOA) medium (Boeke *et al.* 1984). Yeast transformations were done according to Chen *et*

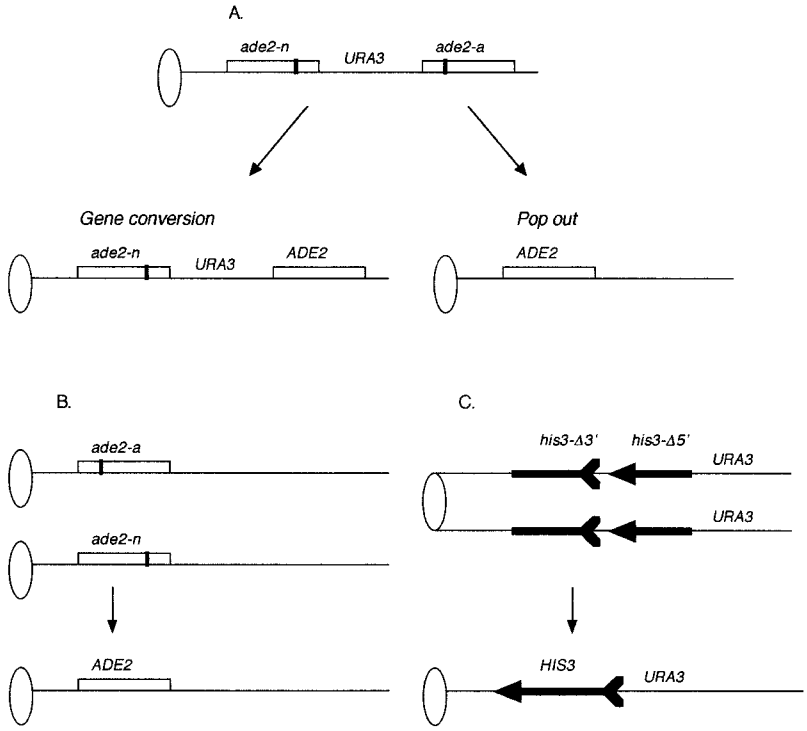

Figure 1.—Recombination assays used in this study. Ovals represent centromeres and lines represent chromosomes. For simplicity, the left arms of chromosomes are not included. (A) Intrachromatid recombination between two heteroalleles of *ade2*, *ade2-a*, and *ade2-n*. The *ade2* gene is represented by boxes and a heavy line marks the approximate position of the mutations. The genes are not drawn to scale. Mitotic recombination may result in either Ura^+, Ade^+ gene conversion events or Ade^+, Ura^- pop-out events, as described in Huang and Symington (1994). (B) Heteroallelic recombination between *ade2-a* and *ade2-n*. Mitotic recombination resulting in Ade^+ recombinants. (C) Sister chromatid exchange (SCE) between two fragments of *his3*. The *HIS3* gene is denoted by an arrow, the 3′ deletion lacking the arrowhead and the 5′ deletion lacking the feather tail. The fragments are not drawn to scale. Recombination between the fragments generates *HIS3*, as described previously (Fasullo and Davis 1987).

al. (1992). Petite colonies were scored on YP plates supplemented with 2% lactic acid (pH 5.5).

Yeast strains and plasmids: Yeast strains are described in Table 1. All yeast strains are derived from W303 (Thomas and Rothstein 1989). The *rad52*, *rad9*, *rad18*, and *rad1* mutations contain insertions of *TRP1*, *URA3*, *LEU2*, and *LEU2*, respectively. The *dun1*Δ mutant (YA145), kindly provided by S. Elledge, contains *dun1-Δ100::HIS3*. In the plasmid pZZ66, the *dun1-Δ100::HIS3* allele is present on a *Bam*HI restriction fragment (Zhou and Elledge 1993). The *dun1-Δ100::his3::URA3* mutation was constructed by inserting the 1.1-kb *URA3* fragment in the internal *Hin*dIII sites of *HIS3*. After digestion with *Bam*HI, the *dun1-Δ100::his3::URA3* fragment was then introduced into YA145 by one-step gene disruption (Rothstein 1983) to generate YD123. The UV sensitivity of the Ura^+ His^- transformants after 120 J/m^2 exposure was then confirmed. Double mutants were made by crossing the appropriate single mutants, dissecting tetrads of the resulting diploids, and identifying meiotic segregants that had the desired auxotrophic phenotype. The *rad9 dun1 rad52* triple mutant (YD106) was made by crossing the *rad9* single mutant (YA132) with the *dun1 rad52* double mutant (YD103), and dissecting tetrads from the sporulated diploid.

Construction of strains to monitor mitotic intrachromosomal and heteroallelic recombination: YA156 (YKH12a) and YA157 (YKH12α), kindly provided by L. Symington, contain a nontandem duplication of *ade2* that was generated upon integration of the pKH9 (*URA3*) plasmid (Figure 1). One copy of *ade2* contains the nonrevertible allele *ade2-a* and the other copy of *ade2* contains the nonrevertible allele *ade2-n* (Huang and Symington 1994). Haploid strains containing the integrated pKH9, including the *dun1* mutant (YD100), the *rad52* (YD102), and *rad1* (YD108) mutants, and the double *dun1 rad52* double mutant (YD110) are meiotic segregants that were obtained after genetic crosses and tetrad dissections. An Ade^+ Ura^- recombinant of YD100, YD101, was used in genetic crosses for making strains that monitor heteroallelic recombination.

Heteroallelic recombination was monitored in diploid strains containing *ade2-a* and *ade2-n* on different homologs. To identify Ura^- Ade^- haploids that contained either *ade2-a* or *ade2-n*, we obtained FOA^R isolates of YA156 and YA157. Primers 5′ CGCTATCCTCGGTTCTGCAT 3′ and 5′ TAACGC CGTATCGTGATTAA 3′ were used to amplify the *ade2* allele. Digestion of the PCR-amplified product with either *Aat*II or *Nde*I restriction endonucleases indicated whether the *ade2* allele contained one or both of the restriction sites. YD114 and YD115 (Table 1) containing *ade2-a* and *ade2-n* alleles, respectively, were mated to generate the diploid YD116; a strain with the identical genotype was made by Bai and Symington (1996). The *ade2-a* and *ade2-n* haploids containing *dun1*, *rad52*, and *rad1* mutations were generated by genetic crosses of YD114 and YD115 with YD101, YD108, or YD102 and sporulation.

The plasmid pNN287, containing tandem *his3* fragments (Fasullo and Davis 1987), was introduced into W303 by selecting for Ura^+ transformants (YD122). YD122 was then crossed with the *dun1-Δ100::his3::URA3* mutant, and a meiotic segregant containing both *dun1* and the integrated pNN287 was identified (YD123). Verification that His^+ recombinants of YD122 resulted from SCE was demonstrated by the linkage of *HIS3* and *URA3* contained on pNN287. FOA^R isolates of these His^+ recombinants should be both Ura^- and His^-. Of 105 His^+ recombinants, ~95% of the FOA^R were His^-, confirming that SCE occurred to generate the original recombinants.

Determining rates of spontaneous recombination and mutagenesis: Rates of spontaneous, mitotic recombination were determined by the method of the median as described by Lea and Coulson (1949) and executed by Esposito *et al.* (1982). Eleven independent colonies were used for each calculation. Three independent experiments were performed for each strain, and the significance of the differences was determined by the nonparametric statistical Whitney-Mann U-test (Zar 1996). Recombination rates were determined using colonies arising on YP(A)D medium, on which Ade^+ cells have no selective advantage, and both *ADE2* and *ade2* colonies appeared white.

Quantitating radiation sensitivity and radiation stimulation of recombination: To quantitate UV or γ-ray sensitivities, strains were grown in YPD to saturation, plated directly on YPD medium after appropriate serial dilution, and irradiated. A 254-nm germicidal lamp (2 J/m^2/sec) was used for UV irradiation. A nordion 1.8-kCi ^{137}Cs irradiator was used as a γ-ray source at 7.8 krads/hr. Colony-forming units (CFU) were counted after 3 days of growth and again after 1 wk. At least three independent experiments were performed for each indicated dose. Statistical differences were determined by the two-tailed *t*-test (Zar 1996).

To quantitate recombination after radiation or chemical exposure, yeast cultures were either grown to an A_{600} of 0.5–1 for log phase cells or to an A_{600} of 4 for stationary phase cells. To arrest cells at the G_2 phase of the cell cycle, cells were grown to an A_{600} of 0.5–1 in YPD, nocodazole [methyl-5-(2-thienylcarbonyl)-H-benzimidazole-2-yl-carbamate] (25) was added to a final concentration of 15 μg/ml, cells were incubated at 30° for 3 hr, and cell cycle arrest was confirmed as previously described (Fasullo *et al.* 1998). Irradiation protocols are described in Fasullo and Dave (1994). After irradiation, cells were plated for viability and for the generation of Ade$^+$ or His$^+$ recombinants. Colonies were counted after 3 days of incubation at 30° and again 1 wk later. For all experiments, measurements of the DNA damage-associated recombination were calculated by subtracting the spontaneous frequency from the frequency obtained after exposure to the agent, as in previous studies (Fasullo *et al.* 1994, 1998). Statistical differences were determined by the two-tailed *t*-test (Zar 1996).

RESULTS

UV sensitivity of *dun1* and assignment of *DUN1* to the *RAD3* epistasis group: The UV sensitivity of the *dun1* haploid mutant (YA145) was compared to the UV sensitivities of the *rad52* (YA133), *rad9* (YA132), *rad1* (YA131), and *rad18* (YA154) haploid mutants (Figure 2). The *dun1* mutant exhibited a level of UV sensitivity greater than wild type ($P < 0.01$) but not significantly different ($P > 0.2$) from the *rad52* mutant defective in recombinational repair. All other single mutants exhibited greater levels of UV sensitivity than the *dun1* mutant. To exclude the possibility that the *dun1* mutant contained an extragenic suppressor that increased UV resistance, we backcrossed the *dun1* mutant with the parental Rad$^+$ W303 strain and determined the UV sensitivity at 30 mJ/m^2 of 10 independent haploid segregants containing the *dun1::HIS3* disruption. All 10 *dun1* haploid segregants exhibited similar UV sensitivity (44% average survival), and all 10 *DUN1* haploid segregants exhibited similar UV sensitivity (80% average survival), indicating that the original *dun1* mutant did not contain an unlinked extragenic suppressor. Thus, the *dun1* mutant is UV sensitive but is significantly more resistant than the mutants defective in the *RAD3* or *RAD6* pathways.

To determine whether the *DUN1* gene participates in either the *RAD3*, *RAD52*, or *RAD9* pathways for UV resistance, the UV sensitivity of haploid mutants containing combinations of the *dun1* null mutation and the *rad1*, *rad9*, *rad52*, and *rad18* mutations was quantitated (Figure 2). If *DUN1* was important in one particular resistance pathway (epistasis group), then double mutants should exhibit no greater sensitivity than the most sensitive single mutant (Haynes and Kunz 1981). If *DUN1* participated in a different UV repair pathway, then the double mutant should exhibit a synergistic

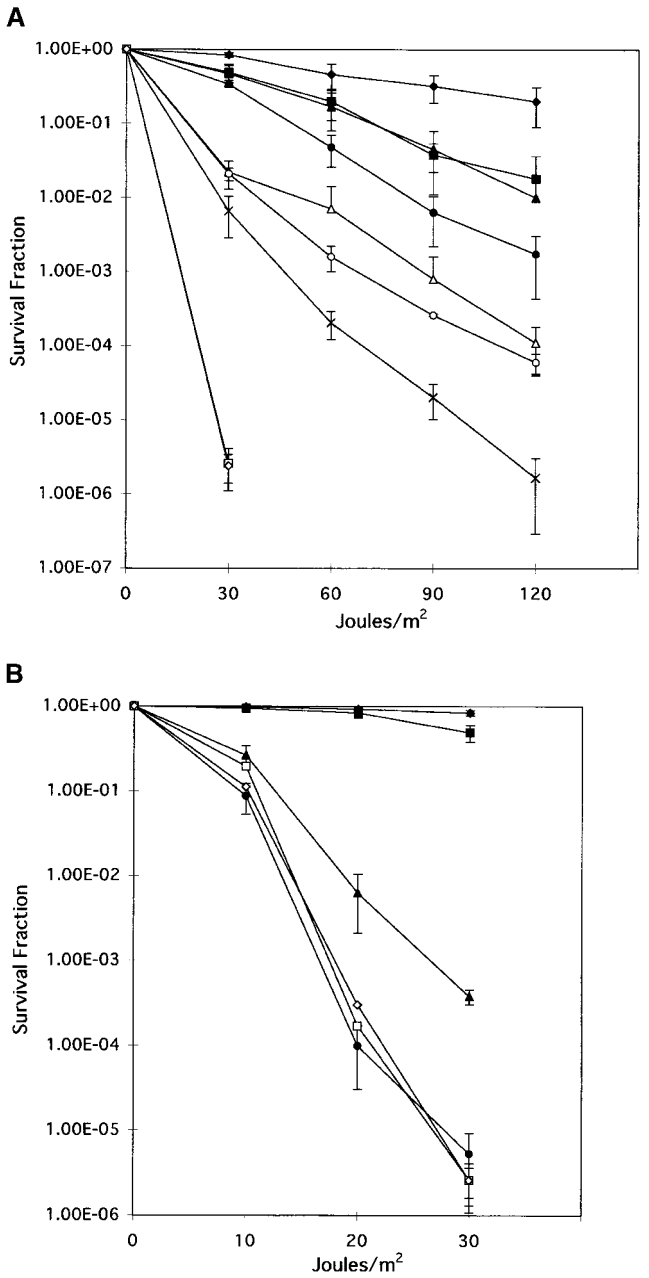

Figure 2.—Sensitivity to UV of *dun1*, *rad1*, *rad52*, *rad18*, and *rad9* single, double, and triple mutants. Survival curves are the mean of three independent experiments performed on independent segregants. Strains used are *DUN1* (YA103, ◆), *dun1::HIS3* (YA145, ■), *rad52::TRP1* (YA133, ▲), *rad1:LEU2* (YA131, ◇), *rad9::URA3* (YA132, ●), *rad18::LEU2* (YA154, ▲), *dun1::HIS3 rad52::TRP1* (YD103, X), *dun1::HIS3 rad9::URA3* (YD104, ○), *dun1::HIS3 rad1::LEU2* (YD105, □), *dun1::HIS3 rad9::URA3 rad52::TRP1* (YD106, △), *dun1::HIS3 rad18::LEU2.* (YD107, ●) (A) Survival fractions at 30–120 J/m^2 exposure. (B) Survival fractions at 10–30 J/m^2.

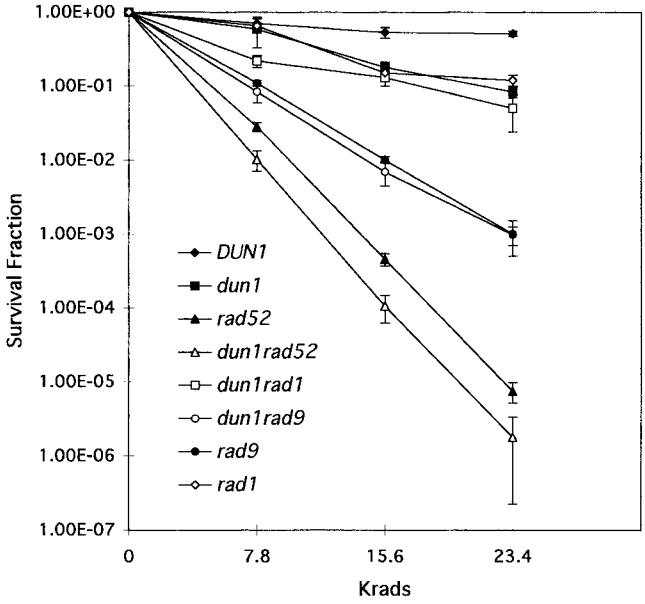

Figure 3.—Sensitivity to ionizing radiation of *dun1*, *rad1*, *rad52*, and *rad9* single and double mutants. Survival fraction is plotted against γ-ray dose in kiloradians (10 Gy = 1 krad). Survival curves are the mean of three independent experiments performed on independent segregants, and symbols are as described in legend to Figure 2.

(greater than additive) increase in UV sensitivity. The *dun1 rad52* (YD103) *dun1 rad9* (YD104), and *dun1 rad18* (YD107) double mutants exhibited synergistic increases in UV sensitivities compared to the single mutants. However, there was no significant difference ($P > 0.4$) between the UV sensitivity of the *dun1 rad1* double and the *rad1* single mutant, even at relatively low levels of radiation exposure (10 and 20 J/m^2). In comparison to the *dun1 rad52* and *dun1 rad9* double mutants, the *dun1 rad52 rad9* triple mutant (YD106) exhibited a further synergistic increase in UV sensitivity, although the UV sensitivity was not as great as the *rad1* mutant. This indicates that *DUN1*, *RAD52*, and *RAD9* participate in three independent pathways that confer resistance to UV-induced DNA damage and that *DUN1* is a member of the *RAD3* epistasis group.

We asked whether the synergistic decrease of UV resistance in the *dun1 rad52* mutants, compared to the single mutants, depended on haploidy or growth of cells in stationary phase. As observed for the *rad52 dun1* haploid cells, the same synergistic decrease in UV survival, compared to *rad52* (YD118) and *dun1* (YD117) diploid mutants, was observed in *rad52 dun1* diploid (YD119) cells (data not shown). This occurred when cells were grown to either log phase or stationary phase. This suggests that *RAD52*-dependent recombination pathways participate in the tolerance of UV lesions in both haploid or diploid *dun1* mutants.

Sensitivity of the *dun1* mutant to ionizing radiation: We asked whether a synergistic increase in ionizing radiation sensitivity, in comparison to the single mutants, also occurred in the *dun1 rad52*, *dun1 rad9*, and *dun1 rad1* double haploid (Figure 3). In comparison to wild type, all single mutants exhibit γ-ray sensitivity, and *rad1* and *dun1* mutants exhibit the same low level of γ-ray sensitivity ($P > 0.2$). The γ-ray sensitivities of the *dun1 rad9* and the *dun1 rad1* double mutants are not different from the sensitivity of the single *rad1* or *rad9* mutants ($P > 0.3$). The γ-ray sensitivity of the *dun1 rad52* double mutant was enhanced compared to the *rad52* single mutant ($P < 0.05$) but the increase in sensitivity was not synergistic. These results suggest that unrepaired DNA lesions induced by ionizing radiation in the *dun1* mutants are not channeled into either the *RAD52* or the *RAD6* pathways.

***dun1* exhibits higher rates of spontaneous, mitotic heteroallelic recombination:** The UV sensitivities of *dun1* mutants suggest that either the *RAD52* recombination pathway or the *RAD6* error-prone pathway participate in UV resistance in the *dun1* mutant. We therefore determined whether *dun1* strains exhibit a *RAD52*-dependent, hyper-recombination phenotype. Recombination assays used included heteroallelic recombination, intrachromosomal recombination, and SCE (Figure 1), since UV exposure stimulates these mitotic recombination events (Davies *et al.* 1975; Kadyk and Hartwell 1993; Galli and Schiestl 1995; Fasullo *et al.* 1998).

The spontaneous hyper-recombination phenotype of the *dun1* mutant was demonstrated by determining the rates of spontaneous Ade$^+$ prototrophs that result from mitotic, homolog recombination between two *ade2* alleles, *ade2-n* and *ade2-a*. These Ade$^+$ prototrophs can be distinguished from *ade2* strains by colony pigment; on YPD medium, Ade$^+$ colonies are white while *ade2* strains are red. Since YPD contains insufficient adenine to ensure equal growth rates of Ade$^+$ and Ade$^-$ colonies, Ade$^+$ white colony sectors outgrow the perimeter of the red colony, while off-white sectors that do not outgrow the red colonies are Ade$^-$ and petite. *Dun1* (YD117) colonies accumulated white Ade$^+$ sectors more readily than wild type, but *dun1 rad52* (YD119) colonies did not (Figure 4). When colonies are grown on YP(A)D, in which there is no growth advantage for Ade$^+$ sectors, the rate of generating Ade$^+$ prototrophs was ~5-fold above ($P < 0.05$) wild type (Table 2). Northern blots demonstrated that the level of *ade2* RNA from either *DUN1* or *dun1* strains was equivalent (data not shown), indicating that the increase in recombination is not due to elevated *ade2* transcription in the *dun1* mutant. The higher rate of recombination in the *dun1* mutant is *RAD52* dependent; the rate of heteroallelic recombination in the *dun1 rad52* double mutant is indistinguishable ($P > 0.05$) from that of the *rad52* mutant and is reduced ~15-fold compared to wild type (Table 2).

Since *DUN1* participates in the *RAD3* pathway, we asked whether the spontaneous hyper-recombination phenotype was dependent upon *RAD1* (Table 2). Rates

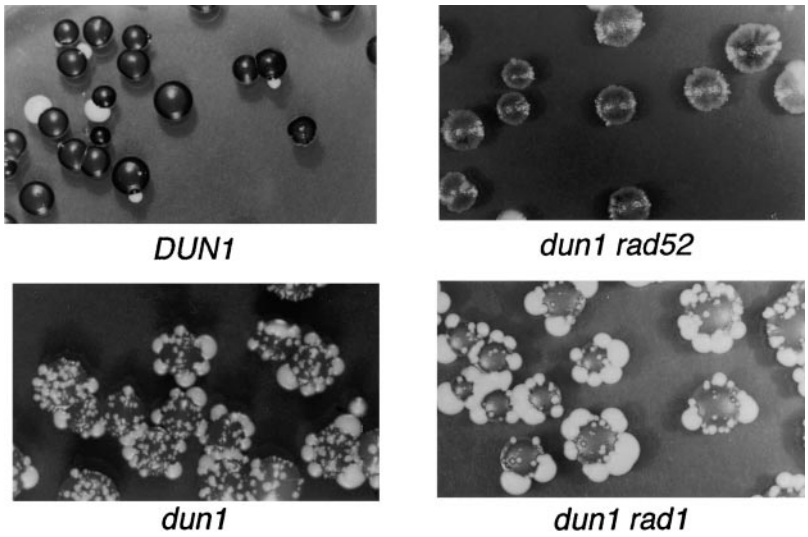

Figure 4.—Spontaneous *ade2* heteroallelic recombination is exhibited by changes in colony pigment from red (Ade$^-$) to white (Ade$^+$). Colony phenotypes are shown for *DUN1* wild-type (YD116), *dun1* (YD117), *dun1 rad1*(YD121), and *dun1rad52* (YD119) mutants. Cells were plated on YPD medium and colonies were photographed after a 2-wk incubation.

of mitotic heteroallelic recombination in the *rad1* strain (YD120) were similar to that of wild type, as expected from previous studies (Montelone *et al.* 1988). The hyper-recombination phenotype of the *dun1* mutant was also observed in the *rad1 dun1* double mutant (YD121), which is significantly different from wild type ($P < 0.05$) but not significantly different than *dun1* ($P > 0.05$). *Dun1 rad1* colonies also accumulate more white Ade$^+$ sectors than wild type on YPD medium (Figure 4). Thus, the hyper-recombination phenotype of *dun1* mutants is not dependent upon *RAD1*.

Aguilera and Klein (1988) identified two classes of mitotic hyper-recombination (*hpr*) mutants that exhibit elevated intrachromosomal recombination: those that result in increased gene conversion events, and those that result in reciprocal exchange events. We therefore determined whether Ade$^+$ recombinants, occurring by heteroallelic recombination, resulted from gene conversion or reciprocal exchange events. Ten independent, spontaneous Ade$^+$ recombinants isolated from wild type (YD116) and from *dun1* (YD117) diploids were sporulated, and tetrads dissected. For all 20 diploids, Ade$^+$::Ade$^-$ exhibited 2:2 segregation (data not shown). Ade$^-$ meiotic segregants from each Ade$^+$ diploid were backcrossed to *ade2-a* and *ade2-n* haploids, YD114 and YD115. If the Ade$^+$ diploid recombinant resulted from reciprocal exchange between the two alleles, then the *ade2* meiotic segregants would contain both *ade2-a* and *ade2-n* alleles (*ade2-a,n*), and would not yield spontaneous Ade$^+$ recombinants when backcrossed to YD114 and YD115. From each of the 20 *ade2* meiotic segregants from the 10 *dun1* recombinants and 10 wild-type recombinants, we obtained Ade$^-$ diploids from a single backcross that yielded Ade$^+$ recombinants. Conversion of *ade2-a* or *ade2-n* to *ADE2* occurred in approximately the same ratio, 8:12. Thus, we conclude that in all 20 Ade$^+$ recombinants from both wild-type and *dun1* mutants, *ADE2* resulted from gene conversion and not from reciprocal exchange of the two *ade2* alleles. We did not address whether such gene conversion events were associated with flanking marker exchange. Our data are consistent with observations that mitotic, heteroallelic recombination predominately results from gene conversion events (reviewed in Petes *et al.* 1991).

***dun1* mutants exhibit more intrachromosomal gene conversion events but fewer pop-out events:** Since the recombination assay that measures heteroallelic recombination generally monitors gene conversion events, we

TABLE 2

Effect of *dun1*, *rad1*, and *rad52* mutations on heteroallelic recombination

Strain	Genotype[a]	Rate of recombination[b]	Fold difference[c]
YD116	*DUN1*	$(1.1 \pm 0.1) \times 10^{-6}$	1
YD117	*dun1*	$(5.6 \pm 0.7) \times 10^{-6}$	5.2
YD118	*rad52*	$(7.8 \pm 2.8) \times 10^{-8}$	0.07
YD119	*dun1 rad52*	$(8 \pm 1.6) \times 10^{-8}$	0.07
YD120	*rad1*	$(2 \pm 0.4) \times 10^{-6}$	1.8
YD121	*dun1 rad1*	$(4.7 \pm 0.3) \times 10^{-6}$	4.3

[a] For full genotype, see Table 1.
[b] Rate ± SD; $N = 3$; rate, number of events/cell division.
[c] Rate of recombination in mutant/rate of recombination in wild type.

TABLE 3

Effect of *dun1*, *rad52*, and *rad1* mutations on intrachromosomal recombination

Strain	Genotype[a]	Rate[b]	% Pop-outs (total)[c]	Ratio[d]
YA156	*DUN1*	5.3×10^{-4}	57 (533 Ura^-/966 Ade^+)	1
YD100	*dun1::HIS3*	6.2×10^{-4}	3.7 (32 Ura^-/867 Ade^+)	0.06
YD102	*rad52::TRP1*	4.0×10^{-5}	71 (898 Ura^-/1271 Ade^+)	1.25
YD110	*rad52::TRP1 dun1::HIS3*	5.4×10^{-5}	22 (274 Ura^-/1267 Ade^+)	0.4
YD108	*rad1::LEU2*	9.1×10^{-5}	3.9 (99 Ura^-/2594 Ade^+)	0.07
YD111	*rad1::LEU2 dun1::HIS3*	2.5×10^{-4}	5.5 (66 Ura^-/1189 Ade^+)	0.1

[a] For full genotype, see Table 1.
[b] Rate, number of events/cell division.
[c] Data from four independent experiments.
[d] Percentage pop-outs in mutant/percentage pop-outs in wild type.

also determined whether *dun1* mutants exhibit a bias toward gene conversion events. We used an intrachromosomal recombination assay where pop-outs (loss of an integrated plasmid by either crossovers or gene conversion) could be readily detected (Figure 1). The assay consisted of direct repeats of *ade2* that flank *URA3* (Huang and Symington 1994). The rate of generating Ade^+ recombinants was approximately the same in wild type (YA156) as in the *dun1* mutant (YD100), but reduced 10-fold in both the *rad52* and the *rad52 dun1* mutants (Table 3). In wild type, 57% of Ade^+ recombinants are Ura^- and result from pop-outs; in *dun1*, *rad1*, and *dun1 rad1* mutants, ~5% were Ura^-. Consistent with this observation, the rate at which the integrated pKH9 plasmid is lost in *dun1* (YD100), as measured by the rate of FOA^R colonies, was 6.1×10^{-6}, ~10-fold less than the rate of 5.9×10^{-5} observed in the wild-type strain YA156. This indicates that spontaneous, mitotic recombination in the *dun1* mutant results in a higher proportion of gene conversion events and fewer pop-outs. The *rad52* and the *rad52 dun1* mutants exhibit higher percentages of pop outs, consistent with previous findings that gene conversion events are *RAD52*-dependent (Jackson and Fink 1981). Thus, spontaneous intrachromosomal recombination between direct repeats in the *dun1* mutant is biased toward gene conversion events that are not associated with crossover events.

DNA damage-induced mitotic recombination in the *dun1* mutant: If UV resistance in *dun1* mutants is partially conferred by *RAD52*-dependent recombinational repair, then UV-induced mitotic recombination should be enhanced in *dun1* mutants. Since the synergistic effect of *dun1* and *rad52* is observed in haploids, where heteroallelic recombination does not occur, as well as diploids, we investigated whether *dun1* mutants exhibit an enhanced level of UV-induced unequal SCE (Table 4). We found no differences in the rate of spontaneous SCE in the *dun1* mutant $(1.1 \pm 0.2) \times 10^{-6}$, compared to the rate in wild type $(1.4 \pm 0.3) \times 10^{-6}$. After UV irradiation, we observed significant ($P < 0.05$) two- to threefold increases in recombination, compared to wild type. However, if cells were arrested in G_2 with the microtubule inhibitor nocodazole prior to irradiation, there was no difference between the stimulation of SCE events in the *dun1* mutant compared to wild type. We speculate that *dun1*-enhanced UV-stimulated SCE depends on replication of the UV dimer.

Since spontaneous heteroallelic recombination is greater in *dun1* diploids than wild type, we exposed both wild-type and *dun1* diploid mutants to UV, γ-rays, and MMS to quantitate whether the stimulation of heteroallelic recombination was greater in *dun1* cells for any particular DNA damaging agent (Table 5). Although the *dun1*-enhanced recombination is modest (approximately twofold), it is significantly different ($P < 0.01$) from wild-type levels of UV stimulation. However, there was no increase in the levels of γ-ray and MMS-stimulated heteroallelic recombination in the *dun1* mutant (data not shown). Thus, the recombinogenicity of only a subset of DNA-damaging agents is enhanced in *dun1*.

Higher percentages of petites are generated in *dun1* mutants: We observed a 10-fold higher percentage of petites generated in the *dun1* (YA145) strain ($7.1 \pm 0.9\%$ or 57/804 total) compared to the wild-type (YA103) strain ($0.7 \pm 0.4\%$ or 13/1697 total). However, rates of spontaneous reversion to Trp^+ and Ade^+ prototrophy due to spontaneous mutagenesis are not higher in the *dun1* mutant (data not shown). Since mitochondrial DNA repair is less efficient than genomic DNA repair (Yakes and van Houten 1997), higher spontaneous numbers of petites is consistent with the DNA repair defect.

DISCUSSION

Three independent pathways participate in the repair of UV-induced DNA damage in *S. cerevisiae*. These include pathways for excision repair, mutagenic repair, and recombinational repair. Which pathway is chosen to repair UV-induced DNA damage is not completely understood. *DUN1* encodes a serine-threonine protein kinase that is activated by signal transduction pathways

TABLE 4

Stimulation of SCE by UV in *DUN1* and the *dun1* mutant

	Stimulation in *DUN1* (YD122)[a]		Stimulation in *dun1* (YD124)[a]		
UV Dose	Survival %	His^+/CFU $\times 10^{7}$[b]	Survival %	His^+/CFU $\times 10^{7}$[b]	Ratio[c]
60 J/m^2	97	98 ± 36	71	216 ± 36	2.2
90 J/m^2	87	142 ± 23	53	335 ± 89	2.4
120 J/m^2	77	166 ± 56	45	343 ± 98	2.1
After Nocodazole					
60 J/m^2	98	85 ± 50	86	85 ± 7	1.0
90 J/m^2	97	130 ± 113	94	125 ± 63	1.0

[a] For complete genotype, see Table 1.
[b] His^+/CFU $\times 10^7$ = (His^+ frequency w/agent − His^+ spontaneous frequency) $\times 10^7$; $N = 6$. Spontaneous frequency of YD122 = 1.6×10^{-5}, spontaneous frequency for YD124 = 2.2×10^{-5}.
[c] (His^+ per 10^7 CFU for *dun1*)/(His^+ per 10^7 CFU for *DUN1*).

for sensing DNA damage. *Dun1* mutants, which exhibit both UV and MMS sensitivity, are defective in DNA damage-inducible expression of ribonucleotide reductase (Zhou and Elledge 1993) and *MAG1* (Liu 1997) and are partially defective in DNA damage-induced G_2 checkpoint (Pati *et al.* 1997). In this study, we demonstrate that *DUN1* participates in the *RAD3* pathway for excision repair and that *dun1* mutants exhibit higher rates of spontaneous gene conversion and enhanced UV-induced mitotic recombination. We suggest that the *DUN1* pathway for DNA damage-induced gene expression channels the repair of UV damage into a nonrecombinogenic pathway.

The participation of *DUN1* in the *RAD3* epistasis group elucidates previous observations that *DUN2* (*POL*ε)-mediated transcriptional induction and *RAD9*-mediated cell cycle arrest are independent DNA damage-inducible pathways (Navas *et al.* 1996). The independence of these pathways was supported by the enhanced UV sensitivity of the *dun2 rad9* mutant, compared to the single mutants. Based on the participation of *DUN1* in the excision repair pathway, we speculate that the *DUN2* pathway may activate other genes that participate in UV excision repair.

Differences in genetic interactions between *rad9* and *dun1* regarding sensitivity to UV and ionizing radiation: Since several models of DNA double-strand break repair involve DNA synthesis (Szostak *et al.* 1983; Malkova *et al.* 1996), we expected that *dun1* mutants would also be sensitive to ionizing radiation. However, we observed no synergism between *rad9* and *dun1* with respect to γ-ray sensitivity. Nonetheless, the *dun1* γ-ray sensitivity is still consistent with *DUN1* belonging to the *RAD3* epistasis group, since *dun1* mutants exhibit the same level of γ-ray sensitivity as *rad1* mutants. Essentially identical γ-ray survival curves are obtained for single mutants *dun1* and *rad1* and for *rad1 dun1* double mutants (Figure 3), for *rad9* and *dun1 rad9* (Figure 3), and for *rad9* and *rad9 rad1* (Fasullo *et al.* 1998). Further experiments are in progress to determine whether *DUN1*, similar to *RAD1*, participates in the single-strand annealing (SSA) mechanism for double-strand break repair (Fishman-Lobell and Haber 1992).

Recombination phenotypes of the *dun1* mutants: Since tolerance to DNA damage in *dun1* mutants is partially conferred by *RAD52*-dependent mechanisms, it is interesting that only a subset of spontaneous *RAD52*-dependent recombination events is enhanced in the

TABLE 5

Stimulation of heteroallelic recombination by DNA-damaging agents in *DUN1* and the *dun1::HIS3* diploids

	Stimulation in *DUN1* (YD116)[a]		Stimulation in *dun1* (YD117)[a]		
UV Dose	Survival %	Ade^+/CFU $\times 10^{6}$[b]	Survival %	Ade^+/CFU $\times 10^{6}$[b]	Ratio[c]
30 J/m^2	94	76 ± 11	88	158 ± 16	2.1
90 J/m^2	85	475 ± 44	44	673 ± 64	1.4
120 J/m^2	72	455 ± 43	24	841 ± 74	1.8

[a] For complete genotype, see Table 1.
[b] Ade^+/CFU $\times 10^6$ = (Ade^+ frequency w/agent − Ade^+ spontaneous frequency) $\times 10^6$; $N = 4$. Spontaneous frequency of YD116 = 5.8×10^{-6}; spontaneous frequency for HD117 = 6.7×10^{-6}.
[c] (Ade^+ per 10^7 CFU for *dun1*$^-$)/(Ade^+ per 10^7 CFU for *DUN1*).

dun1 mutant, compared to wild-type strains. While spontaneous heteroallelic and intrachromosomal gene conversion events are enhanced, intrachromosomal pop-outs are decreased, and spontaneous unequal SCE is unchanged in *dun1* mutants. Although we do not understand why the proportion of intrachromosomal recombination events that result from exchange (pop-outs) is decreased in the *dun1* mutant, a similar phenomena (Table 3) was observed for *rad1* mutants, suggesting that *DUN1* also participates in the SSA mechanism for spontaneous recombination. We speculate that the unchanged rate of spontaneous SCE in *dun1* mutants, compared to the rate in wild type, results from a lower level of crossovers but a higher level of sister-chromatid gene conversions. However, due to the low frequency of spontaneous SCE, the percentages of spontaneous SCE that result from reciprocal exchanges and gene conversion events are unknown (Fasullo and Davis 1987; Kadyk and Hartwell 1992). Thus, detection of enhanced spontaneous recombination in *dun1* mutants may depend on the recombination assays used to monitor gene conversion.

Which spontaneous DNA lesions are more recombinogenic in the *dun1* mutant is unclear. Spontaneous DNA lesions include those that result from oxidation and alkylation damage (depurination). Since neither ionizing radiation nor MMS were more recombinogenic in *dun1* mutants, *dun1*-enhanced spontaneous recombination may result from spontaneous DNA lesions similar to those induced by UV.

The synergistic increase in UV sensitivity in the *rad52 dun1* double mutant, compared to the single mutants, for both haploids and diploids, suggests that one recombination mechanism by which UV damage is tolerated in *dun1* mutants is SCE. Although in comparison to wild type, the frequency of UV-stimulated recombinants that result from unequal SCE increased a modest two- to threefold in the *dun1* strain, a more direct physical measurement of SCE may demonstrate a greater enhancement. Less enhanced UV stimulation of heteroallelic recombination in the *dun1* mutant is consistent with observations that sister chromatids are preferred substrates for DNA repair (Kadyk and Hartwell 1992). Thus, we suggest that higher levels of SCE and heteroallelic recombination constitute a subset of *RAD52*-dependent mechanisms that lead to DNA damage tolerance in the *dun1* mutant.

Possible mechanisms for *dun1*-stimulated recombination: The *RAD1* independence of the spontaneous hyper-recombination phenotype of the *dun1* mutants suggests possible mechanisms for how *dun1*-enhanced recombination occurs. Similar to *dun1* recombination phenotypes, spontaneous *RAD1*-independent intrachromosomal recombination (Rattray and Symington 1995) and UV-induced *RAD1*-independent unequal SCE (Paulovich *et al.* 1998) mostly occurs by *RAD52*-dependent gene conversion. Kadyk and Hartwell (1993) suggest that such SCE events may be initiated when UV lesions are bypassed by the replication machinery; the resulting recombinogenic single-strand gaps may then be processed into either daughter-strand gap, double-strand break, or replication slippage pathways. The cell cycle dependence of the *dun1*-enhanced UV stimulation also suggests that in *dun1* mutants, some UV lesions are bypassed by the replication enzymatic machinery and the resulting recombinogenic single-strand gaps may trigger *RAD52*-dependent recombination.

The *RAD1*-independence of the *dun1* hyper-recombination phenotype differs from the *RAD1*-dependence of the hyper-recombination phenotypes of other DNA repair mutants. These include *rad9* mutants (Fasullo *et al.* 1998) and *rem* mutants (Malone and Hoekstra 1984) defective in the Rad3 helicase (Montelone *et al.* 1988). Rad1, as part of the Rad1-Rad10 endonuclease (Bardwell *et al.* 1994), may participate in mitotic recombination by excising nonhomologous DNA from recombinogenic double-strand breaks to form stable recombination intermediates (Fishman-Lobell and Haber 1992), or by processing DNA lesions into recombinogenic single-stranded DNA during excision repair (Montelone *et al.* 1988). The first role of *RAD1* may be important in hyper-recombination mutants where recombination between repeated sequences on nonhomologous chromosomes (ectopic recombination) is enhanced (Bailis *et al.* 1992; Fasullo *et al.* 1998). Since the *dun1*-enhanced recombination occurs between homologs or chromatids that are essentially identical, and the initiating lesion may be generated by DNA replication, *RAD1* may not be needed to either initiate or process the recombination intermediates.

DNA damage-inducible gene expression and *dun1* phenotypes: The *DUN1* pathway for DNA damage-inducible gene expression controls the expression of several genes involved in DNA repair, rendering it difficult to ascribe all the *dun1* phenotypes to a defect in the DNA damage inducibility of the *RNR* genes. However, the UV sensitivity of *dun1* mutants can be suppressed by the overexpression of *RNR1* (Zhao *et al.* 1998), and cells exposed to hydroxyurea, an inhibitor of ribonucleotide reductase, also exhibit more mitotic recombination (Galli and Schiestl 1996). Both *dun1* and *cdc8* (deoxythymidylate kinase; Jong *et al.* 1984) mutants exhibit higher levels of mitotic recombination and spontaneous petites (Sclafani and Fangman 1984). Thus, by analogy with *cdc8* phenotypes, nulceotide imbalance may result in some of the *dun1* phenotypes.

Since the substrates for the Dun1 protein kinase have not been identified, we cannot exclude the possibility that some *dun1* phenotypes directly result from failure to phosphorylate DNA repair proteins. For example, the Srs2/RadH/Hpr5 helicase, which exhibits amino acid similarity to UvrD, contains several potential phosphorylation sites for serine/threonine protein kinases

(genetics computer group, protein motif comparison). *Hpr5* mutants exhibit variable, elevated rates of mitotic gene conversion (Palladino and Klein 1992). Thus, a full explanation for the *dun1* recombination phenotypes awaits the identification of the substrates of Dun1 kinase.

Mutants defective in other yeast protein kinases, including Cdc5 (Aguilera and Klein 1988) and Pkc1 (Huang and Symington 1994), exhibit spontaneous hyper-recombination phenotypes. Currently the interactions between these protein kinases and Dun1 is unknown. *PKC1* is involved in stress responses that include heat and osmotic shock, and *pkc1* mutants exhibit an extended S phase (Levin and Bartlett-Heubusch 1992). Heat shock may induce recombination by causing oxidative damage (Brennan *et al.* 1994; Davidson *et al.* 1996). Thus, it would be interesting if *pkc1 rad52* double mutants exhibited a synergistic sensitivity to environmental stress.

Summary: We have shown that DNA damage tolerance in *dun1* mutants is partially conferred by *RAD52*-dependent recombination. This is the first demonstration that a defect in a protein kinase directly involved in the transcriptional induction of DNA damage-inducible genes also increases spontaneous, mitotic recombination in yeast. It will be important to determine whether other protein kinases involved in the DNA damage-inducible responses have similar phenotypes.

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