Failure To Induce a DNA Repair Gene, *RAD54*, in *Saccharomyces cerevisiae* Does Not Affect DNA Repair or Recombination Phenotypes

GARY M. COLE^{1†*} AND ROBERT K. MORTIMER²

Genetics Department¹ and Department of Biophysics and Medical Physics,² University of California, Berkeley, Berkeley, California 94720

Received 2 February 1989/Accepted 11 May 1989

The *Saccharomyces cerevisiae* *RAD54* gene is transcriptionally regulated by a broad spectrum of DNA-damaging agents. Induction of *RAD54* by DNA-damaging agents is under positive control. Sequences responsible for DNA damage induction (the DRS element) lie within a 29-base-pair region from –99 to –70 from the most proximal transcription start site. This inducible promoter element is functionally separable from a poly(dA-dT) region immediately downstream which is required for constitutive expression. Deletions which eliminate induction of *RAD54* transcription by DNA damage but do not affect constitutive expression have no effect on growth or survival of noninducible strains relative to wild-type strains in the presence of DNA-damaging agents. The DRS element is also not required for homothallic mating type switching, transcriptional induction of *RAD54* during meiosis, meiotic recombination, or spontaneous or X-ray-induced mitotic recombination. We find no phenotype for a lack of induction of *RAD54* message via the damage-inducible DRS, which raises significant questions about the physiology of DNA damage induction in *S. cerevisiae*.

The yeast *Saccharomyces cerevisiae* offers an attractive model for the study of DNA repair in eucaryotic organisms. Extensive genetic analyses have established that mutations in many genes which lead to increased sensitivity to DNA-damaging agents can be ordered into three epistasis groups, the *RAD3*, *RAD6*, and *RAD52* groups (3, 5, 9, 21, 24). The three groups are thought to represent separate repair systems, with the *RAD3* group responsible for excision repair, the *RAD6* group for an error-prone pathway, and the *RAD52* group for a repair activity involving recombination (reviewed in references 7, 8, and 12). Phenotypes of representative mutants from each group support such a conclusion. In particular, several *RAD52* group mutants, including *rad54* mutants, show reduced sporulation and spore viability (10; E. Dowling, Ph.D. Thesis, University of California, Berkeley, 1982) and are unable to carry out homothallic mating type switching, an event which is presumed to be lethal in such mutants since *rad52 HO* and *rad54 HO* spores die (27; J. Game, personal communication). Additionally, *RAD52* group mutants are very sensitive to ionizing radiation, which results in double-strand breaks that are thought to require genetic recombination for repair (10, 22).

In *Escherichia coli*, many DNA repair activities are coordinately regulated by the *lexA/recA* regulatory network, in which the *lexA* protein represses transcription of many DNA repair genes by binding to their operators and *recA*, activated by DNA damage (or other stress), causes proteolysis of *lexA* and enhanced transcription of these genes, a process known as the SOS response (reviewed in reference 32). In *S. cerevisiae*, by contrast, no such coordinated program of gene expression in response to DNA-damaging agents has been demonstrated, although a number of individual genes

have been shown to be damage inducible, including members of two epistasis groups (4, 25). At least two of these genes, *RAD2* and *RAD54*, are induced by several different agents, despite the fact that *rad2* and *rad54* mutants are only slightly sensitive to some of these agents. However, many other genes in these epistasis groups do not appear to be transcriptionally regulated by DNA damage. Also, analyses of the promoter regions of inducible genes have failed to discern homologous upstream regulatory sequences (G. M. Cole, unpublished results).

We report here the characterization of a damage-inducible promoter in *S. cerevisiae*, that of the *RAD54* gene. *RAD54* is induced 3 to 12-fold by a diverse array of DNA-damaging agents (4), including X-rays, UV, and methyl methanesulfonate (MMS). We show that induction under all these conditions is achieved through the same 29-base-pair (bp) region, 70 and 102 bp upstream of the two *RAD54* transcription start sites, and is positively regulated. Deletion of this inducible promoter and a consequent inability to induce *RAD54* had no effect on the growth rate of cells exposed to low levels of MMS. Also, cells lacking the inducible promoter showed survival identical to that of the wild type in response to various doses of X-rays. Deletion strains were indistinguishable from wild-type strains for the ability to switch mating type. In wild-type cells, *RAD54* mRNA is induced ~15-fold during meiosis (4a). This induction and the meiotic recombination coincident with it were unaffected in homozygous deletion strains. Although homozygous *rad54* diploids have dramatically reduced rates of spontaneous and X-ray-induced mitotic recombination (8, 27), we were unable to detect any difference between inductionless and isogenic wild-type strains. In short, yeast cells lacking the damage-inducible promoter element show no phenotype associated with *rad54* mutants. This raises questions about the physiological significance of *RAD54* transcriptional regulation by DNA-damaging agents.

* Corresponding author.

† Present address: Research Institute of Scripps Clinic, Molecular Biology Department MB-7, 10666 North Torrey Pines Road, La Jolla, CA 92037.

TABLE 1. Yeast strains used in these experiments

Strain	Genotype
4c.....	<i>MATa leu2-3 leu2-112 his 1-1 his3Δ hom3-10</i>
YNN281.....	<i>MATa trp1Δ his3-Δ200 ura3-52 lys2-801 ade2-1</i>
XS560-1C.....	<i>MATa trp1Δ his3-Δ200 ura3-52 leu2-3 leu2-112</i>
XSM28.....	Isogenic to XS560-1C with the <i>RAD54</i> promoter deletion M28 integrated at <i>RAD54</i>
GCHOM28.....	<i>MATa/MATα HO/ho leu2/leu2</i> heterozygous for the <i>RAD54</i> promoter deletion M28 integrated at <i>RAD54</i>
g999.....	<i>MATa/MATα leu2/leu2 can1/can1 HOM3/hom3-10 ADE2/ade2 TRP2/trp2 his1-1/his1-7</i>
g999M28.....	Isogenic to g999 except homozygous for the <i>RAD54</i> promoter deletion M28 integrated at <i>RAD54</i>
g538-26a.....	<i>MATa rad54-3 can1 ura3 his1 trp2 leu2 ade4</i>

MATERIALS AND METHODS

Strains and growth conditions. All yeast strains used in these experiments are shown in Table 1. All subcloning and deletion experiments were performed in *E. coli* HB101. Presporulation medium, YEPD, and selective glucose medium were as described by Sherman et al. (28). Sporulation medium was 1% potassium acetate. Standard yeast genetic techniques were also as described by Sherman et al. (28). Cell densities were determined by hemacytometer.

***RAD54* promoter deletions.** Deletions of the *RAD54* promoter in plasmid pSZ211-54 (4) were made by cutting the target sequence with the relevant enzyme, either *Hind*III, *Mlu*I, or *Bgl*II, and then digesting with BAL 31 exonuclease for various times. The deleted plasmids were then recircularized, with an 8-bp linker (New England Biolabs), either *Hind*III, *Mlu*I, or *Bam*HI, added to the ligation mix prior to transforming *E. coli*. The unidirectional deletions shown in Fig. 1 could therefore be generated by subcloning deleted fragments back into the original vector. The four 5'-to-3' *Mlu*I deletions (M28, M45, M54, and M57) also included a deletion of the *Hind*III-*Nsp*CI restriction fragment. Sequences of all deletions were determined by subcloning into M13. The subclones were then sequenced using a modification of the Sanger dideoxy technique (2). Deletions between restriction sites were created by cutting with the relevant enzymes, filling in with Klenow fragment (Pharmacia), and religating the plasmid by the methods of Maniatis et al. (20). Deleted pSZ211-54 plasmids were then integrated into the *ARS1* locus in strain 4c by cutting at a unique *Nhe*I site (23). The noninducible promoter deletion M28 was integrated into the *RAD54* locus of strain XS560-1C by cutting at an *Xba*I site in the *RAD54* promoter. Yeast transformation was by the lithium acetate method of Ito et al. (16). Single-copy integration at *ARS1* was verified by Southern blot hybridization.

DNA damage induction assays. Conditions and procedures for assaying *lacZ* activity in response to X-rays, UV, and MMS were as described previously (4). The X-ray tube used in these experiments was a Machlett OEG-60 with a beryllium window. MMS was purchased from Kodak. Assays of total protein were as described by Lowry et al. (18). All values represent an average of three separate trials.

RNA preparation and analysis. Total RNA was prepared as follows: 50 ml of mid-log-phase cells (MMS induction assays) or 50 ml of cells at 2.5×10^7 /ml in sporulation medium (meiotic induction assays) was pelleted and washed in ice-cold 2.5% β -mercaptoethanol. Cells were repelleted and suspended in 1 ml of LETS buffer (0.1M Tris hydrochloride [pH 7.5], 1 mM EDTA, 0.1 M LiCl) with 0.5% sodium dodecyl sulfate. Silanized glass beads (0.45-mm diameter; purchased from Sigma) were added just below the meniscus, and tubes were vortexed at top speed four times for 30 s each, with ice cooling between bursts. Breakage of cells was

monitored by light microscopy and was routinely >95%. Another milliliter of LETS plus 0.5% sodium dodecyl sulfate, containing 400 μ g of proteinase K (Sigma), was added to the tube, and the tubes were incubated at 37°C for 30 min. The mixture was extracted once with an equal volume of 25:24:1 phenol-chloroform-isoamyl alcohol and reextracted with 1 volume of 24:1 chloroform-isoamyl alcohol. To 2 ml of the aqueous phase was added 37 μ l of 5 M NaCl and 4 ml of absolute ethanol. Tubes were then chilled at -70°C for 1 h. Total nucleic acids were precipitated by centrifugation at 10,000 rpm in a Beckman JA-20 rotor at 4°C for 30 min. The pellet was dried in a vacuum desiccator and suspended in sterile TE buffer at a concentration of ~10 mg/ml. Total nucleic acid was quantitated by A_{260} and A_{280} . Immediately before samples were loaded on the gels, each aliquot of 15 μ g was treated with 13 μ g of RNase-free DNase (Bethesda Research Laboratories) for 10 min at room temperature in a total volume of 10 μ l. RNA samples were run in 1.0% agarose-formaldehyde denaturing gels as described by Maniatis et al. (20).

A 15- μ g sample of total RNA was loaded per lane for all time points. To verify quantitation of total RNA, duplicate samples were loaded onto a separate gel and stained with ethidium bromide (0.5 μ g/ml) after electrophoresis. Gels were blotted to GeneScreen Plus nylon membranes (DuPont) according to the manufacturer's instructions. Hybridizations were carried out for 16 h at 42°C in 50% formamide-1 M NaCl-10% dextran sulfate-1% sodium dodecyl sulfate. Probes were prepared by nick translation using a premixed DNase I-DNA polymerase I solution (Bethesda Research Laboratories) according to the manufacturer's instructions. Nick translations were carried out in the presence of all four α - ^{32} P-labeled nucleotides (800 Ci/mmol; Amersham).

The *RAD54* probe was made from a 1.9-kilobase *Bam*HI fragment internal to the *RAD54* coding region (H. Emery, N. Kellogg, D. Schild, and R. Mortimer, manuscript in preparation). Autoradiography was on Kodak XAR-5 X-ray film at -70°C with two DuPont Cronex intensifier screens. Densitometer analysis of autoradiographs was on an RTF scanning densitometer manufactured by the Transodyne Corporation.

Survival curve assays. Mid-log-phase cells were plated on YEPD at various densities and irradiated with various doses of X-rays. After several days, survival was scored as the fraction of colonies on experimental plates relative to unirradiated control plates. Values represent an average of three separate trials.

Meiosis experiments. g999 and g999M28 strains were grown to a density of 2.5×10^7 /ml in presporulation medium, pelleted, washed once in sporulation medium, resuspended in sporulation medium at 2.5×10^7 /ml, and shaken with vigorous aeration at 30°C. At $t = 0$ and at 3-h time intervals, samples were withdrawn and diluted. Various densities of these cells were plated on histidine selective plates. After

several days of incubation at 30°C, colonies were counted and the frequency of histidine prototroph formation was calculated relative to a dilution of cells at $t = 0$ on synthetic complete medium. Additionally, samples of total RNA were prepared from g999M28 at 2-h time intervals as described above. Sporulation was monitored by light microscopy.

RESULTS

The *RAD54* promoter consists of a DNA damage-inducible and a constitutive element. We have previously demonstrated that a *lacZ* fusion construct containing 406 bp of the region upstream of the *RAD54* structural gene is sufficient to account completely for the response of the wild-type gene to both constitutive and inducing conditions (4). Here we present a detailed analysis of this region which identifies two elements, a 29-bp damage regulatory sequence (DRS) and a 56-bp A+T-rich sequence which is responsible for constitutive *RAD54* expression. Using the *RAD54-lacZ* fusion plasmid pSZ211-54 (4), we constructed a number of deletions in the *RAD54* promoter from several sites (Fig. 1). For precise quantitation, these deletions and the undeleted control were integrated in single copy at the *ARS1* site in *S. cerevisiae* 4c (Table 1). As an additional control, an undeleted copy of pSZ211-54 was integrated at the chromosomal *RAD54* locus. Constitutive and induced levels of β -galactosidase in this construct were identical to those of a strain having a single copy of pSZ211-54 at *ARS1*, demonstrating that integration at *ARS1* does not affect expression of the plasmid (data not shown).

5' Deletions which remove all sequences up to the *MluI* site at -258 from the ATG start codon (H66, H96, H110, H117, H126, H140, and H148) have no significant effect on either X-ray induction or constitutive expression of *RAD54*. Similarly, 3' deletions of sequences upstream of the *MluI* site (M-26, M-28, M-34, and M-46) do not alter expression of the gene. However, deletions of the region immediately downstream of the *MluI* site (M28, M45, M54, and M57) abolish X-ray induction of *RAD54* without affecting its constitutive expression. The smallest of these, M28, removes 28 bp of this region and is phenotypically indistinguishable from the longer deletions M45, M54, and M57. This result suggests that this region contains an essential component of an X-ray-inducible element that does not affect the constitutive expression of *RAD54*. However, if one extends the deletion from the *MluI* site to the *BglII* site (deletion MB), a distance of 86 bp, very little constitutive expression of *RAD54* can be measured. Consequently, sequences required for basal levels of *RAD54* expression, downstream from the damage response element, also lie in the *MluI-BglII* region. 3' Deletions from the *BglII* site confirm this conclusion, with B-26 and B-40 showing approximately equal basal levels of expression as wild-type, while B-57 significantly reduces constitutive *lacZ* activity. Interestingly, the 3' deletion B-40 overlaps the 5' deletions M54 and M57, yet each shows roughly the same level of basal transcription. This implies that the basal control element is redundant, since either the 5' segment or the 3' segment is sufficient to direct basal expression of the gene. Analysis of the sequence in this region shows it to be very A+T rich, with 44 out of 56 bases adenine on the coding strand. There are two long stretches of uninterrupted dA-dT in the region, one of 11 bp and one of 8 bp. Poly(dA-dT) regions have been demonstrated to regulate the constitutive expression of a number of yeast genes (26, 30), and the redundancy of these sequences in the *MluI-BglII* region makes it likely that they

TABLE 2. Expression of *RAD54-CYC1* promoter fusions in response to X-rays^a

Plasmid	Description	U of β -galactosidase/mg of total protein at:		Relative induction
		0 h	4 h	
PLGΔ312S	Wild-type <i>CYC1</i> derivative	203	287	1.4
ΔSMA-SSP	PLGΔ312S deleted for entire <i>CYC1</i> promoter	<0.004	<0.004	1.0
PLG54-HB	<i>RAD54 HindIII-BglII</i> fragment replacing <i>CYC1 SmaI-SspI</i> fragment	0.19	0.93	4.8
PLG54-MB	<i>RAD54 MluI-BglII</i> fragment replacing <i>CYC1 SmaI-SspI</i> fragment	0.58	2.3	4.0

^a Each strain was irradiated with a dose of 24 krad of X-rays and returned to growth in selective medium prior to the β -galactosidase assays.

perform a similar function in *RAD54*. The longer deletion from the *BglII* site, B-57, also demonstrates the independence of the DRS from the constitutive regulator. Even though cells containing this deletion have significantly reduced *lacZ* expression, they remain capable of inducing this expression about fivefold in response to X-rays. This result argues that the DRS is entirely contained within the first 29 bp of the sequence immediately downstream of the *MluI* site, with a maximum 5' boundary defined by the *MluI* site and a maximum 3' boundary defined by B-57.

Comparison of the DRS with the promoter regions of other yeast genes, in particular DNA damage-inducible genes, did not reveal significant homologies. However, we did observe a perfect match within the DRS to the canonical CCAAT pentanucleotide sequence element. Various CCAAT elements have been shown to selectively bind transcription factors in several different eucaryotic systems (reviewed by P. Mitchell and R. Tjian, Science, in press). It remains to be seen whether these sequences are actually involved in regulating expression of damage-inducible genes.

The *MluI-BglII* fragment of *RAD54* is sufficient to direct basal and damage-inducible expression. The foregoing deletion analysis demonstrates that sequences contained within the 86-bp *MluI-BglII* fragment of the *RAD54* promoter are necessary for the regulation of *RAD54* transcription, both by DNA-damaging agents and at constitutive levels. However, these deletions do not prove that this region is sufficient to regulate *RAD54*. To determine whether this was the case, we constructed fusions of the *RAD54* upstream sequences to a *CYC1* promoter element fused to *lacZ* (in plasmid PLGΔ312S; 11). We then assayed these constructs in strain YNN281 for basal and inducible levels (Table 2). The parent plasmid PLGΔ312S (a multicopy YEP24 derivative), which contains the *CYC1* UAS1 and UAS2 upstream regulatory elements as well as functional TATA sequences, was not inducible by a dose of 24 krad of X-rays (Table 2). This plasmid had quite high basal levels of expression (203 U/mg). Deletion of all known *CYC1* regulatory sequences, from the *SmaI* site at -312 to the *SspI* site at -20 from the most upstream transcription start site, yielded a derivative which had very low levels of β -galactosidase activity, less than 0.01% of the undeleted vector, or <0.004 U/mg. This activity was not inducible by X-rays. However, if the *CYC1* regula-

FIG. 1. Deletion analysis of the *RAD54* promoter. Plasmid pSZ211-54 (4), which contains 406 bp of the region upstream of the *RAD54* ATG fused to the *E. coli lacZ* structural gene, was used as the substrate for the deletion analysis. All constructs shown were integrated in single copy at the *ARS1* locus in strain 4c. Deletions were created using BAL-31 exonuclease as described in the text. Assays for β -galactosidase activity were also as described in the text. Deleted sequences are indicated by a solid bar. Deletions are designated by endpoint, direction, and extent in base pairs (e.g., H96 refers to a deletion of 96 bp from 5' to 3' starting from the *HindIII* restriction site, while M-34 deletes 34 bp from the *MluI* site 3' to 5'). C, Constitutive *lacZ* activity prior to X-irradiation. I, Induced *lacZ* activity 4 h after irradiation with 24 krad of X-rays. IR, Ratio of induced to constitutive values. For clarity of presentation, we have drawn a dashed line in the IR column where we did not observe induction. The striped box represents the region responsible for X-ray induction. Arrows indicate the two primary transcription start sites (Emery et al., in preparation). The unfilled boxes represent A+T-rich regions responsible for constitutive expression of *RAD54*. The sequence of the *RAD54* promoter is presented below the deletions. Restriction sites are marked above the sequence. The X-ray-inducible promoter element is underlined.

tory sequences were replaced by an intact *RAD54* promoter, a significant basal level was restored (0.19 to 0.58 U/mg), and such constructs were then fully inducible by X-rays, four- to fivefold. Replacement of the *CYC1* *SmaI-SspI* fragment by either the *HindIII-BglII* fragment (240 bp) or the *MluI-BglII* fragment (86 bp) of *RAD54* resulted in this pattern of

expression. From these experiments, we conclude that the 86-bp *MluI-BglII* sequence contains both the DRS and a constitutive element that regulates *RAD54*.

The *RAD54* DRS element regulates responses to several forms of DNA damage. *RAD54* is induced by several DNA-damaging agents, including MMS, UV, and X-rays (4). To

TABLE 3. Elimination of UV and MMS induction in deletions lacking X-ray induction

Deletion strain	Treatment (amt)	U of β -galactosidase/ mg of total protein	Induction ratio
H110	UV (0)	0.14	2.7
	UV (100 J/m ²)	0.38	
M28	UV (0)	0.12	1.0
	UV (100 J/m ²)	0.12	
M57	UV (0)	0.15	1.2
	UV (100 J/m ²)	0.18	
H110	MMS (0)	0.15	4.1
	MMS (0.01%)	0.62	
M28	MMS (0)	0.19	1.0
	MMS (0.01%)	0.16	
M57	MMS (0)	0.12	1.0
	MMS (0.01%)	0.12	

determine whether these different responses were initiated at the same regulatory site or were in fact the result of multiple control at different sites, we assayed deletions of the *RAD54* DRS for induction by UV and MMS (Table 3). Because of the possibility that control regions for responses to various DNA-damaging agents could be overlapping subsets of a larger region, we analyzed both the smallest (M28) and the largest (M57) deletions which eliminated X-ray induction of *RAD54* without affecting basal expression. The fluence of UV (100 J/m²) and the concentration of MMS (0.01%) were chosen because these levels induced maximal or near-maximal expression in previous experiments with undeleted *RAD54-lacZ* fusions (4). As a positive control, deletion strain H110 was also assayed. We observed that both the smaller and the larger DRS deletion was not inducible by either UV or MMS. Control strain H110, on the other hand, had about a threefold increase in β -galactosidase activity in response to UV and about a fourfold induction in response to MMS. Therefore, the same 29-bp region responsible for X-ray induction of *RAD54* is also required for UV and MMS induction.

Failure to induce *RAD54* mRNA has no effect on growth rate in the presence of MMS. Since we now possessed a deleted *RAD54* promoter which eliminated DNA damage induction but left constitutive expression unaltered, we were in a position to determine the physiological significance of *RAD54* induction. By integrating the *RAD54-lacZ* fusion plasmid at the *RAD54* chromosomal locus, we replaced the wild-type *RAD54* promoter with the noninducible deletion derivative from M28 (Fig. 2). Using Southern blot analysis, we verified that the deleted plasmid had recombined properly into the *RAD54* chromosomal locus in a single copy (not shown). This construction puts the *lacZ* fusion gene under the control of the wild-type *RAD54* promoter and the wild-type *RAD54* gene under the control of the DRS-deleted promoter. When exposed to DNA damage, cells containing this construct (XSM28) should induce *lacZ* but remain at constitutive levels for *RAD54*. To verify that such was in fact the case, we took RNA samples from transformed and untransformed isogenic XS560-1C cells which had been exposed to various doses of MMS. Fifteen micrograms of total RNA from each sample was run on denaturing formaldehyde gels, blotted, and probed for *RAD54* message (Fig. 2). Both the transformed and wild-type cells had approximately equal levels of *RAD54* mRNA at 0 h. MMS was then added directly to the YEPD medium at a concentration of either 0.002 or 0.01%. A second RNA sample was taken 90

FIG. 2. Replacement of the chromosomal *RAD54* promoter with a noninducible deletion and demonstration that this construct is noninducible by MMS. Strain XS560-1C was transformed with deletion plasmid M28 (Fig. 1), which eliminates DNA damage induction. Prior to transformation, this plasmid was cut with *Xba*I in the *RAD54* promoter, which directs integration to the chromosomal locus of *RAD54* (23). As shown in panel A, this construct results in a tandem duplication of the *RAD54* promoter. The *RAD54-lacZ* fusion comes under the control of the wild-type promoter, while the wild-type *RAD54* gene has the deleted promoter which possesses only a constitutive poly(dA-dT) element. As shown in the Northern (RNA) blot in panel B, this construct renders the chromosomal *RAD54* gene uninducible by MMS. RNA was prepared from either the transformed strain XS560M28 or the untransformed isogenic strain XS560-1C 90 min after the addition of various concentrations of MMS directly to the medium. All lanes were loaded with 15 μ g of total RNA and probed with a sequence specific to wild-type *RAD54* message, which does not recognize the *RAD54-lacZ* fusion message. Lanes 1 and 2, Control XS560-1C cells with (1) no MMS and (2) 0.01% MMS. Lanes 3, 4, and 5, Promoter-deleted XS560M28 cells with (3) no MMS, (4) 0.002% MMS, and (5) 0.01% MMS.

min after addition of the MMS. Levels of *RAD54* mRNA in the wild-type strain increased dramatically after addition of 0.01% MMS (Fig. 2; ~15-fold as measured by densitometric analysis of underexposed blots). In the deletion strain, however, *RAD54* expression remained relatively constant at both concentrations tested, 0.002 and 0.01% MMS. There was a slight decrease in message at 0.002% MMS (about 30%) and a slight increase at 0.01% (about 70%). These variations in *RAD54* mRNA levels probably represent non-specific physiological responses to various concentrations of MMS and not specific alterations in *RAD54* transcription.

If noninducing cells are at a selective disadvantage relative to wild-type cells in the presence of MMS, we should be able to detect even slight differences by means of competitive growth experiments. As a necessary control for such experiments, we first had to show that the *RAD54-lacZ* fusion

FIG. 3. Competitive growth of wild-type and isogenic noninducible *RAD54* cells in medium containing MMS. Equal samples of 10^6 cells of wild-type XS560-1C and noninducible XS560M28 strains (which had been grown to mid-log phase in YEPD medium) were placed in fresh YEPD medium supplemented with 30 μ g of L-leucine per ml and containing either 0.01% MMS ($\square$) or no MMS ($\blacklozenge$). Cells were shaken at 30°C in identical flasks and maintained in mid-log phase by repeated subculturing of 10^6 cells into identical fresh medium. Samples of $\sim 1,000$ cells were plated every 12 h on YEPD. After colonies had grown, plates were replicated to synthetic complete-minus-leucine plates. Because XS560M28 contains the *LEU2* selectable marker, XS560M28 colonies could be scored by their leucine prototrophy. The fraction of noninducible cells could then be calculated.

would not complement *rad54*. We transformed a *rad54-3* mutant strain, g538-26a, with the undeleted plasmid pSZ211-54 and assayed for survival in response to X-rays. We did not detect any complementation by the plasmid (data not shown). We therefore added equal numbers of mid-log-phase XS560-1C and XSM28 cells to rich liquid medium containing either no MMS or 0.01% MMS. Samples were maintained in mid-log growth phase by subculturing at 12-h intervals. At $t = 0$ and every 12 h thereafter, samples were diluted and plated on YEPD. Colonies from these plates were replica plated to leucine selective plates. Cells containing the noninducible *RAD54* gene were scored by their *LEU*⁺ phenotype, since the integrated plasmid contained the *LEU2* marker. The number of *LEU*⁺ cells as a fraction of the total was then determined (Fig. 3). A comparison of the relative numbers of *LEU*⁺ cells in the no-MMS and 0.01%-MMS samples showed no appreciable difference in growth rates between the two populations over a period of 72 h. If noninducing cells grew more slowly than wild type, we would expect a steadily diminishing fraction of *LEU*⁺ cells over time, which did not occur. We repeated this experiment twice with 0.01% MMS and twice with 0.002% MMS, obtaining similar results (data not shown).

Induction of *RAD54* is not required for survival in response to X-rays. In addition to the competitive growth experiments with MMS, we also measured the ability of noninducible cells to survive ionizing radiation. Assays of this type measure the fraction of irradiated cells that survive increasing doses of X-rays relative to unirradiated controls. When survival is plotted as a function of dose, a characteristic survival curve emerges. Mutants which completely lack a *RAD54* function show a dramatic decrease in survival, as high as 1,000-fold at moderate doses (10). We analyzed the survival of both haploid and diploid cells unable to induce *RAD54* in response to X-irradiation and compared them with

TABLE 4. X-ray survival of cells lacking the *RAD54* damage-inducible element relative to wild-type and mutant *rad54* strains

Strain	% Survival at X-ray dose (krad):						
	0	3	6	12	24	48	60
Haploids							
XS560-1C (wild type)	100	65	44	36	28	14	15
XSM28 (noninducible)	100	65	39	36	24	22	9
g538-26a (<i>rad54-3</i>)	100	29	10	0.2	<0.03	<0.03	<0.03
Diploids							
g999 (wild type)	100	100	92	90	58	35	26
g999M28 (noninducible)	100	100	94	88	61	31	25

wild-type strains (Table 4). Since survival rates were very similar for these strains, we have not drawn survival curves but present instead the survival data. For both haploid and diploid yeasts, survival of the noninducible *RAD54* strains was virtually indistinguishable from that of the control strains. For the sake of comparison, we also analyzed survival in a haploid *rad54-3* mutant, g538-26a. This strain is very sensitive to X-rays; survival was 3 orders of magnitude less than that of either the wild type or the noninducible strain at doses of ≥ 24 krad. Homozygous *rad54-3* diploid strains are as sensitive as or more sensitive than *rad54-3* haploid strains (Game, personal communication). Clearly, a failure to induce *RAD54* mRNA by X-rays does not lead to a phenotype resembling that of the mutant.

The inducible DRS promoter is not required for homothallic mating type switching. In homothallic yeast strains, the action of the HO endonuclease produces a double-strand break at the mating type locus prior to the conversion event that results in mating type switching (17). In the absence of *RAD54*, this process is aborted and results in lethality (Game, personal communication). To determine whether the *RAD54* DRS is required for switching, we constructed a diploid strain heterozygous for both the *HO* gene and the *RAD54* promoter, strain GCHOM28 (Table 1). This strain was sporulated, and 20 tetrads were dissected. We did not observe any inviability or slow growth that would be expected to cosegregate with a noninducible *RAD54* *HO*⁺ phenotype if switching were affected in these cells. Of 20 tetrads, 13 produced four viable spores, with 21 *RAD54*⁺ *HO*⁺ spores and 10 noninducible *RAD54* *HO*⁺ spores. These results are not significantly different from the 1:1 segregation expected for these two unlinked genes ($\chi^2 \sim 0.15$). All of these spores successfully switched mating type and mated with daughter cells to produce normal *MATa/MAT α* diploids capable of sporulation. Of six three-viable-spored tetrads, the inviable spore in every case was *HO*⁺. Three of these were wild type for *RAD54*, and three were noninducible. The remaining ascus produced two inviable spores, both of which were *HO*⁺ and lacked the *RAD54* DRS element. These data make it clear that a functional damage-inducible *RAD54* promoter is not required for the successful completion of mating type switching.

Meiotic induction and DNA damage induction of *RAD54* utilize different regulatory mechanisms. We have shown elsewhere that *RAD54* is transcriptionally regulated during meiosis, increasing its steady-state mRNA level some 15-fold (4a). Additionally, *rad54* homozygous diploids show reduced rates of sporulation and reduced spore viability

FIG. 4. Expression of *RAD54* during meiosis in strains homozygous for the noninducible *RAD54* promoter. g999M28 was grown to late log phase in presporulation medium and transferred to sporulation medium at a density of 2.5×10^7 cells per ml. RNA was prepared, electrophoresed, blotted, and hybridized as described in the text. RNA preparations were made every 2 h (numbers above lanes) after transfer of cells to sporulation medium. Fifteen micrograms of total RNA was loaded in each lane.

relative to the wild type (10; Dowling, thesis). To see whether this induction is under the control of the DRS element, and if so, whether a failure to induce *RAD54* in meiosis has any effect on sporulation, we constructed a diploid strain homozygous for the M28 deletion, g999M28. This strain is derived from an SK-1 background and undergoes a rapid and synchronous meiosis in response to sporulation conditions. Diploids were made by transforming two haploid strains of opposite mating type with the M28 plasmid integrated at the chromosomal *RAD54* promoter. Insertion in single copy was verified by Southern blot analysis (not shown), and these strains were mated to produce the homozygous diploid g999M28. As a control, the same untransformed haploids were mated to make diploid strain g999. These strains were grown in presporulation medium and then suspended in sporulation medium. For the noninducible *RAD54* strain, preparations of total RNA were made at 2-h intervals. Fifteen-microgram samples of these were run on formaldehyde denaturing gels, which were blotted and probed for *RAD54* mRNA (Fig. 4). *RAD54* was definitely induced in this strain, with message levels at 12 h approximately ninefold greater than at $t = 0$ (as determined by densitometric scan). Asci were first visible at 10 h, and sporulation was >70% complete at 12 h. The profile of induction in this DRS-less strain was very similar to that observed in wild-type strains (4a). To determine whether this strain possesses any defects in recombination, we undertook meiotic pullback experiments. g999M28 and g999 are heteroallelic at the *his1* locus. During meiosis, recombination at this locus increases approximately 1,000-fold relative to vegetative growth to yield histidine prototrophs at a frequency approaching 1%. Because the commitment to recombination precedes and is temporally separable from spore formation, it is possible to "rescue" such recombinants as viable diploids before they have sporulated (6, 29). The frequency of histidine prototroph formation, as measured by colony formation on histidine selective plates, provides a reliable index of the commitment to recombination during the course of meiosis. Using this technique, we assayed both g999 and g999M28 for recombination at the *his1* locus (Fig. 5). For both strains, recombinants were first detectable at 6 h, increasing to a frequency of about 0.25% at 12 h. Asci

FIG. 5. Meiotic recombination at the *HIS1* locus in wild-type and noninducible *RAD54* strains. g999 (□) and g999M28 (◆) were grown to late log phase and suspended in sporulation medium at a density of 2.5×10^7 cells per ml. g999M28 is isogenic to g999 except for a homozygous deletion of the damage-inducible promoter. Both strains contain the *his1-1* and *his1-7* heteroalleles. At the indicated intervals after transfer to sporulation medium, various concentrations of cells were plated on either synthetic complete medium or synthetic complete minus histidine. Recombination at the *HIS1* locus was assayed as the frequency of histidine prototrophic colonies relative to total colonies on synthetic complete medium.

were first visible at 9 h, and by 17 h sporulation was essentially complete. By this time, recombinant colonies were almost entirely germinated spores. There were no significant differences in the time course or relative level of histidine prototroph formation in the two strains. Additionally, spore formation and spore viability appeared unaffected in g999M28 relative to g999 (data not shown). We conclude that the *RAD54* DRS element has no effect on *RAD54* transcription during sporulation and is completely dispensable for meiosis.

Spontaneous and X-ray-induced mitotic recombination is unaffected in inductionless *RAD54* strains. Mutations in the *rad54* gene lead to significantly reduced rates of both spontaneous and X-ray-induced mitotic recombination (8, 27). To assay this phenomenon in cells lacking the DRS element, we once more took advantage of the *his1* heteroalleles in strain g999M28 and the isogenic g999. Recombination at the *his1* locus occurs at a low but detectable frequency ($\sim 10^{-5}$) during vegetative growth. This frequency is increased 10- to 20-fold by X-irradiation. To assay for spontaneous recombination, we performed a variation of the Luria-Delbruck fluctuation test (19). Ten histidine auxotrophic colonies each of g999 and g999M28 were grown in separate flasks of synthetic complete medium and plated on histidine selective plates at a density of 10^7 per plate. The values presented in the no-X-ray column of Table 5 represent the median value for 10 trials. The noninducible *RAD54* and wild-type strains generated histidine recombinants at nearly identical frequencies, approximately 10^{-5} . Separate histidine selective plates were also spread with 10^6 cells per plate from three of these cultures for each strain and X-rayed at either 6 or 36 krad (Table 5). Irradiated cells demonstrated about an order of magnitude increase in recombination, but there was no appreciable difference between the inducible and noninducible *RAD54* strains. Hence, we were unable to detect any alteration in either spontaneous or induced mitotic recombination in cells lacking the noninducible *RAD54* promoter.

TABLE 5. Spontaneous and X-ray-induced mitotic recombination in wild-type and noninducible *RAD54* strains

Strain	X-ray dose (krad)	Frequency of histidine prototroph formation (10^{-5})
g999 (wild type)	0	1.3
	6	16
	36	11
g999M28 (homozygous noninducible <i>RAD54</i>)	0	1.2
	6	26
	36	13

DISCUSSION

The study of inducible DNA repair in eucaryotes lags significantly behind that in *E. coli*. Even so, a number of fundamental differences are readily apparent. In *S. cerevisiae*, for example, there does not appear to be a correlate of the SOS response seen in bacteria. Many yeast genes for which damage-sensitive mutations have been isolated do not appear to be damage inducible. The genetics of DNA damage sensitivity in *S. cerevisiae* indicate the existence of multiple pathways for DNA repair (reviewed in references 7, 8, and 12), with mutations in different pathways significantly more sensitive to some DNA-damaging agents than to others. Nonetheless, at least two genes in two very different systems, *RAD2* and *RAD54*, are inducible by many different agents, including those to which *rad2* and *rad54* mutants are not sensitive (4, 25), leading to the possibility that a common signal may serve as an inducer for these separate pathways.

As an initial step in exploring this possibility, we carried out an analysis of the *RAD54* promoter. We demonstrate here the isolation of a *cis*-acting DNA damage-responsive sequence, or DRS, in the *RAD54* promoter. A 29-bp region containing this element is necessary and sufficient to induce *RAD54* expression 3- to 10-fold in the presence of several diverse DNA-damaging agents, including X-rays, UV, and MMS. The mechanism of action for the DRS is positive, since deletions of this element do not affect constitutive expression of *RAD54*, but eliminate DNA damage induction. Constitutive expression of *RAD54* is under the control of an adjacent poly(dA-dT) region, which operates independently of the DRS. Interestingly, *RAD54* lacks a functional TATA element, which is required for the expression of many if not most yeast genes (reviewed in reference 31).

The biological significance of *RAD54* induction is not clear. Given that we could separate constitutive and inducible promoter elements, we sought to determine whether inductionless strains of *S. cerevisiae* would exhibit any of the phenotypes associated with *rad54* mutations. This proved not to be the case. Cells of a noninducible strain were able to survive a wide range of X-ray doses at frequencies indistinguishable from an isogenic inducible strain. In the presence of low concentrations of MMS, these strains exhibited identical growth rates. Induction of *RAD54* was not required for mating type switching in homothallic strains. Also, the DRS element was not required for the transcriptional induction of *RAD54* seen in meiosis, nor did absence of the DRS affect either meiotic, or spontaneous or induced mitotic, recombination frequencies.

How, then, can these data be explained? Several possibilities suggest themselves, as follows.

(i) The function of *RAD54* is duplicated, and another gene (or genes) is capable of substituting for *RAD54* in the repair of DNA damage. A model of this sort seems very unlikely,

however, since, if the repair function of *RAD54* were duplicated, mutations in the *RAD54* gene should not lead to the severe radiation-sensitive phenotypes observed.

(ii) The primary biochemical function of *RAD54* is not in DNA repair, although it may be required for some types of repair. Basal levels of *RAD54* are capable of effecting this secondary repair function, whereas induced levels are required for the primary, as yet uncharacterized function.

(iii) *RAD54* induction may be required only for a specific type of repair not yet assayed. Since a wide range of agents which produce very different DNA lesions are all capable of inducing *RAD54*, the DRS may be a general "damage-induction box" which nonspecifically responds to many types of DNA damage. A parallel for this type of regulation exists in *S. cerevisiae* in the general amino acid regulation mediated by *GCN4*. In this system, genes for many amino acid biosynthetic pathways are coordinately regulated via a hexanucleotide sequence in their promoters which binds *GCN4* protein (14, 15). Starvation for any one of a group of amino acids leads to induction of all genes under *GCN4* control, regardless of the levels of the end product for any given pathway (13).

We should also point out that general amino acid regulation provides another instance of an apparently "inessential" induction. Deletion of the *GCN4*-responsive sequences of the *HIS4* promoter, leaving only sequences which allow basal levels of transcription, does not lead to histidine auxotrophy (1). Such cells are fully capable of synthesizing sufficient histidine for growth using constitutive levels of the *HIS4* product. The relative growth rates of these cells versus wild-type cells has not been determined, however, and they may be at a competitive disadvantage in histidine-limited environments.

The presence of a DNA damage-inducible element in the *RAD54* promoter which does not appear to have a physiological role in certain aspects of DNA repair or recombination, despite the fact that *RAD54* is required for these functions, seems paradoxical. Transcriptional regulation of DNA repair in *S. cerevisiae* seems more complex than in *E. coli*. It is likely that a complete understanding of DNA damage induction will require a more thorough elucidation of the biochemistry of DNA repair in eucaryotes.

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