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Genetics

Isolation and characterization of the *RAD3* gene of *Saccharomyces cerevisiae* and inviability of *rad3* deletion mutants

(DNA repair/excision/pyrimidine dimers/DNA crosslinks/cloning)

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ABSTRACT **The *RAD3* gene of *Saccharomyces cerevisiae* is required for nicking of DNA containing pyrimidine dimers or interstrand crosslinks. We have cloned the *RAD3* gene and physically mapped it to 2.6 kilobase of DNA. A DNA segment of the cloned *RAD3* insert was ligated into plasmid YIp5, which transforms yeast by homologous integration, and shown to integrate at the *RAD3* site in chromosome V, thus verifying the cloned DNA segment to be the *RAD3* gene and not a suppressor. The *RAD3* gene encodes a 2.5-kilobase mRNA, extending between the *Kpn* I site and the *Sau*3A1/*Bam*HI fusion junction in plasmid pSP10, and the direction of transcription has been determined. The 2.5-kilobase transcript could encode a protein of about 90,000 daltons. We also show the deletions of the *RAD3* gene to be recessive lethals, indicating that the *RAD3* gene plays an important role in other cellular processes in addition to incision of damaged DNA.**

Excision of UV-induced pyrimidine dimers in the yeast *Saccharomyces cerevisiae* is a complex process involving at least 10 genes: *RAD1*, *RAD2*, *RAD3*, *RAD4*, *RAD7*, *RAD10*, *RAD14*, *RAD16*, *RAD23*, and *MMS19*. The excision defectiveness of *rad1* mutants was determined by chromatographic measurements of pyrimidine dimers in DNA (1, 2) as well as by using a more sensitive assay depending on the specificity of the phage T4 UV endonuclease for dimer-containing DNA (3). The excision defectiveness of *rad2* mutants was demonstrated by an indirect method in which crude extracts of UV-irradiated yeast cells were used to compete with UV-irradiated *Haemophilus influenzae* transforming DNA for photoreactivating enzyme (4), and the reduced ability of *rad3*, *rad4*, *rad7*, *rad10*, *rad14*, *rad16*, *rad23*, and *mms19* mutants to excise UV-induced pyrimidine dimers was demonstrated by determining the susceptibility to nicking of nuclear DNA from UV-irradiated cells by T4 or *Micrococcus luteus* UV endonuclease (5–9). The *rad1*, *rad2*, *rad3*, *rad4*, and *rad10* mutants were found to be defective in incision of UV-irradiated DNA and incision of DNA crosslinked by treatment with psoralen plus 360-nm light (10–12). These observations suggest that the structural genes for the yeast incising activity acting at major distortions in DNA may be found in this group. However, it is also likely that some of these genes code for regulatory proteins rather than the incising activity.

Attempts to identify a pyrimidine dimer-incising activity in cell-free extracts of yeast have been unsuccessful (13). If the yeast pyrimidine dimer-incising activity exists as a complex of several proteins, this complex might be disrupted during the routine procedures used for cell fractionation; another possible source of difficulty in identifying such an activity may be the low levels of enzyme present in unirradiated cells.

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Our goal is to identify and characterize the protein products of the *S. cerevisiae RAD* genes involved in incision and to examine their structure and regulation. As a first step toward reaching this aim, we have isolated the *RAD3* gene and characterized its mRNA product. We have also created deletion mutants of the *RAD3* gene and show these mutants to be inviable.

MATERIALS AND METHODS

Strains. The following yeast strains were constructed in our laboratory: LP2649-1A, *MATα leu2-3 leu2-112 can1 ura3-52 rad3-2*; LP2649-1D, *MATa aro7-1 can1 RAD*$^+$; LP2649-1B, *MATα aro7-1 ura3-52 RAD*$^+$; LP2693-4B, *MATα his3-Δ1 leu2-3 leu2-112 trp1-289 ura3-52 rad4-4*; B-635, *MATa cyc1-115 his1 lys2 trp2 RAD*$^+$. Strains 7799-4B, *MATa his4-17 ura3-52 RAD*$^+$, and 7799-5C, *MATα his4-17 ura3-52 RAD*$^+$, were obtained from G. Fink. We constructed the *RAD*$^+$/*rad3-2* diploid DH-62 by mating GP19-6B, *MATa ade2-1 ura3-52 rad3-2*, with 7799-5C. Yeast strains containing plasmids are designated by the letter "p" preceding the strain number, followed in parentheses by the plasmid harbored by that particular strain.

Plasmids. We used a recombinant plasmid library obtained from D. Botstein, which contains yeast genomic DNA that had been subjected to partial digestion with *Sau*3A1 followed by ligation into the *Bam*HI site of the 7.6-kilobase (kb) hybrid plasmid YEp24 (14, 15). YEp24 contains plasmid pBR322, a 2.2-kb *Eco*RI fragment of yeast 2-μm circle DNA, form B (allowing for autonomous replication in yeast), and a 1.1-kb *Hin*dIII fragment containing the yeast *URA3* gene (for selection in yeast).

The plasmid YIp5 was used for cloning DNA fragments and for integration into the yeast genome. YIp5 is a 5.5-kb plasmid also derived from pBR322; it contains the yeast *URA3* gene but does not replicate autonomously in yeast because it contains no yeast origin of replication sequence and transforms only by homologous integration in the yeast genome (16) and has unique *Eco*RI, *Hin*dIII, *Bam*HI, and *Sal* I sites.

pAB108, obtained from S. Baim, a 6.1-kb derivative of pBR322, replicates autonomously in yeast because of the *ARS2* and contains the yeast *URA3* gene.

Transformation Procedures and UV Irradiation. Transformation of yeast was carried out either by the spheroplast method of Hinnen *et al.* (17) or by the treatment of intact yeast cells with lithium acetate to promote DNA uptake (18). Response to UV irradiation of various yeast strains was determined by the spot test as described by Prakash and Prakash (19) or from survival curves after UV irradiation.

Abbreviations: kb, kilobase(s); Ura$^+$, uracil-independent; cM, centimorgans.

Nucleic Acid Purifications. Plasmid DNA from yeast was purified by a modified method of Hirt (20). Yeast genomic DNA was purified by a mini-prep method obtained from G. S. Roeder. Growth of *Escherichia coli* containing plasmids was carried out as described by Norgard *et al.* (21) but with nucleosides omitted. Plasmid DNA was purified by the cleared lysate method of Clewell and Helinski (22) but with 0.1% Triton X-100 instead of Brij 58. For screening of large numbers of *E. coli* containing plasmids, the procedure of Ish-Horowicz and Burke (23) for DNA extraction from plasmid or cosmid cultures was used. DNA fragments were purified either by electroelution from 40 mM Tris/20 mM sodium acetate/2 mM EDTA, pH 7.8 (24), agarose gels followed by passage through DEAE-Sephacel as described by Maniatis *et al.* (25) or by electroelution from Tris/acetate/EDTA agarose gels containing electrophoretically pure agarose. Total RNA was purified from yeast by a modification of the method described by Broach *et al.* (26).

Gel Electrophoresis, Transfer to Nitrocellulose Filters, Preparation of Probes, and Hybridizations. Restriction endonucleases and other enzymes were purchased from New England BioLabs and used as recommended. Mung bean nuclease was purchased from P-L Biochemicals. Electrophoresis in agarose slab gels to separate restriction fragments was carried out in 89 mM Tris·HCl/23 mM phosphoric acid/2.5 mM EDTA buffer, pH 8.3. Agarose gels also contained 0.5 μg of ethidium bromide per ml.

Nick-translation of DNA fragments for radioactive labeling was carried out by using the nick-translation kit purchased from Amersham. The specific activities of various probes ranged from 6.4×10^6 to 3.1×10^7 cpm/μg of DNA and a total of about 2×10^6 cpm was used for each Southern blot. The method of Southern (27) was used to transfer restriction fragments to nitrocellulose paper and hybridizations were carried out as described by Maniatis *et al.* (25).

RNA was fractionated by electrophoresis in formaldehyde agarose gels, transferred to nitrocellulose paper, and hybridized with ^{32}P-labeled DNA as described (25). Single-stranded probes were prepared by cloning of specific restriction fragments in M13mp8 and M13mp9 phage (28) and the DNA was labeled as described by Hu and Messing (29). Specific activities of the probes used for blot hybridizations were between 3.1×10^7 and 4.95×10^7 cpm/μg of DNA and about 4×10^6 cpm was used per blot. All autoradiographs were exposed on Kodak XL-1 (X-Omat) film at −70°C with DuPont Lightning Plus intensifying screens.

RESULTS

Isolation of the *RAD3* Gene. The pool of yeast genomic DNA in YEp24 was used to transform the yeast strain LP2649-1A, *MATα leu2-3 leu2-112 can1 ura3-52 rad3-2* to uracil independence (Ura$^+$). About 10,000 Ura$^+$ transformants were screened for UV resistance by the spot test and four UV-resistant colonies were obtained. These colonies were subcloned and retested for UV resistance as well as for retention of the genetic markers present in the original untransformed strain. Plasmid DNA from each of the four UV-resistant transformants was used to transform *E. coli* strain HB101 to ampicillin resistance. Plasmid DNA from these *E. coli* strains was used to transform the *ura3-52 rad3-2* strain LP2649-1A to Ura$^+$. In all four cases, all Ura$^+$ transformants were also UV resistant, indicating that the factor conferring UV resistance to *rad3-2* strains resided on the plasmid and probably was the *RAD3* gene of yeast. The four plasmids were designated pSP1 to pSP4. Plasmids pSP1, pSP3, and pSP4, each containing a 14-kb insert of yeast DNA, gave identical patterns of digestion with *Hin*dIII and other restriction enzymes indicating that all three plasmids were the same. Plasmid pSP2 contained a 20-kb insert of yeast DNA and differed from the other three plasmids due to the additional 6 kb of DNA. Because the *rad3* complementing plasmids contained large yeast DNA inserts, subcloning of the DNA insert was achieved by subjecting the pSP3 DNA to partial *Eco*RI digestion followed by electrophoresis of digestion products in a Tris/acetate/EDTA 0.7% agarose gel. DNA fragments ranging in size between about 8 and 20 kb were isolated by electroelution and then ligated with T4 DNA ligase. The ligation mixture was used to transform *E. coli* strain HB101 to ampicillin resistance. Plasmid DNAs from the transformants were digested with *Eco*RI to distinguish the various classes of insert deletions and used to transform the *rad3-2* strain LP2649-1A to Ura$^+$. Of the *rad3-2* complementing plasmids obtained, the one with the smallest remaining insert was pSP6. A partial restriction map of this plasmid, which contains a 3.7-kb insert, is given in Fig. 1.

Genetic Mapping. To verify that the cloned DNA segment in the pSP6 plasmid contained the yeast *RAD3* gene and not a suppressor of *rad3-2*, or some other gene, which, when present in a multicopy plasmid, could fortuitously complement *rad3-2*, we genetically mapped the 2.2-kb *Eco*RI/*Bgl* II fragment of pSP6 to the *RAD3* site in the yeast genome. The 2.2-kb *Eco*RI/*Bgl* II fragment of pSP6 (Fig. 1) was isolated by electroelution and ligated into *Eco*RI/*Bam*HI-digested YIp5, a plasmid that contains the yeast *URA3* gene but no yeast origin of replication and therefore transforms yeast by homologous integration into the yeast chromosome. The resulting plasmid, pSP8, was used to transform yeast strains 7799-4B (*ura3-52 RAD*$^+$) and LP2693-4B (*ura3-52 rad4-4*) to Ura$^+$. Two Ura$^+$ integrants of 7799-4B were crossed to LP2693-4B to generate the diploids DH-74 and DH-75 and one Ura$^+$ integrant of LP2693-4B was crossed to 7799-4B to generate the diploid DH-77, as indicated in Table 1. The *ura3* gene is located on the left arm of chromosome V, about 8.0 cM from the centromere; the *rad4* gene is located on the right arm of chromosome V, about 135 cM from the centromere; and the *rad3* gene is located about 16 cM from *rad4*, distal to the centromere (30). If we had cloned the *RAD3* gene, then the plasmid pSP8 should integrate at the *RAD3* locus on chromosome V by homologous recombination, leading to tight linkage of *URA3* on the plasmid pSP8 to the *RAD3* locus in the chromosome. The *URA3* marker can then be mapped in relation to the *rad4* locus. However, because the plasmid pSP8 also carried the *URA3* gene, integration might also occur at the *ura3* locus in the genome (31, 32). Tetrad analysis of the three diploids examined gave a map distance of 11–13 cM between *ura3* and *rad4* (Table 1). The linkage of *ura3* to *rad4* indicates that *URA3* had integrated at the *rad3* locus and that the *RAD3* gene was present on plasmid pSP6.

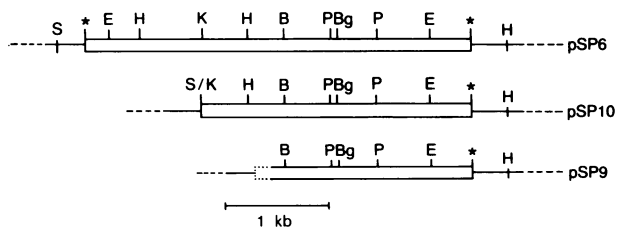

FIG. 1. Partial restriction maps of recombinant plasmids containing the *RAD3* gene and various subclones. Insert of the yeast DNA segment in pSP6, given by the open bar, is in the *Bam*HI site of the *tet* gene of YEp24; the thin line represents YEp24 DNA. The asterisk denotes the *Sau*3A1/*Bam*HI junction of the fusion of insert with plasmid. Symbols for restriction enzymes are as follows: B, *Bam*HI; Bg, *Bgl* II; E, *Eco*RI; H, *Hin*dIII; K, *Kpn* I; P, *Pst* I; and S, *Sal* I. Plasmids pSP6 and pSP10 have *RAD3* function; plasmid pSP9 does not. See text for details.

Table 1. Genetic mapping of pSP8 containing the 2.2-kb *Eco*RI/*Bgl* II segment of pSP6 inserted into the yeast integrating plasmid YIp5

Diploid	*MAT*a parent	*MAT*α parent	No. of tetrads PD	NPD	T	*ura3–rad4* distance, cM
DH-74	p7799-4B (pSP8 integrant no. 1) (*URA*$^+$ *RAD*$^+$)	LP2693-4B (*ura3-52 rad4-4*)	20	—	7	13
DH-75	p7799-4B (pSP8 integrant no. 2) (*URA*$^+$ *RAD*$^+$)	LP2693-4B (*ura3-52 rad4-4*)	18	—	5	11
DH-77	7799-4B (*ura3-52 RAD*$^+$)	pLP2693-4B (pSP8 integrant no. 1) (*URA*$^+$ *rad4-4*)	19	—	6	12

PD, parental ditype; NPD, nonparental ditype; T, tetratype; cM, centimorgans.

Location of the *RAD3* Gene. In order to localize the *RAD3* gene within the 3.7-kb insert of pSP6 (Fig. 1), the 3.1-kb *Eco*RI fragment of pSP6 was ligated into the single *Eco*RI site of pAB108. The plasmid generated, pSP11, failed to restore UV resistance to the *rad3-2* strain LP2649-1A, indicating that one or both *Eco*RI sites are within the *RAD3* gene. Determination of which *Eco*RI site cut within the *RAD3* gene was achieved by subjecting plasmid pSP6 to complete digestion with *Kpn* I and *Sal* I, both of which have unique sites in pSP6. The resulting 10-kb *Kpn* I/*Sal* I fragment, consisting of most of the vector and the *RAD3* insert, was separated by electroelution from the 1.4-kb *Kpn* I/*Sal* I fragment containing 0.275 kb of vector and 1.125 kb of insert. The *Kpn* I protruding 3′ extension and the *Sal* I protruding 5′ extension of 10-kb fragment were recessed by treatment with mung bean nuclease. The resulting blunt-ended fragment was ligated and the DNA was amplified by transformation and growth of *E. coli* strain HB101. This plasmid, designated pSP10 (Fig. 1), pSP6 with the 1.4-kb *Kpn* I/*Sal* I fragment deleted, was found to still complement *rad3* for UV resistance (Fig. 2). The complementation of this subcloned insert was complete, as was that of the original pSP3 plasmid or the pSP6 plasmid (Fig. 2). Another deletion plasmid was obtained in which the *Hin*dIII site that is 0.45 kb to the right of the *Kpn* I site of pSP10 had been deleted but the *Bam*HI site was retained; this plasmid, pSP9 (Fig. 1), failed to complement *rad3* (Fig. 2). Because the 3.1-kb *Eco*RI fragment does not complement *rad3* mutants, whereas a fragment with a deletion of the leftward *Eco*RI site (Figs. 1 and 2) does complement *rad3*, the rightward *Eco*RI site and part or all of the remaining 0.4 kb of insert DNA must be required for *RAD3* function. These results taken together suggest that the *RAD3* gene extends between the *Kpn* I and the *Sau*3A1/*Bam*HI fusion junction of the insert and vector in plasmid pSP10 (Fig. 1).

Size of the *RAD3* Transcript and Transcription Direction. The 3.1-kb *Eco*RI DNA fragment of pSP6, when used as a probe for hybridization to total yeast RNA from the *RAD*$^+$ strain B-635, shows two transcripts, of 2.5 kb and 1.2 kb (Fig. 3, lane A). In order to determine the *RAD3* transcript and the direction of transcription, segments of DNA were inserted into M13 phages for the generation of single-stranded probes. The rightward 0.9-kb *Bgl* II/*Eco*RI fragment of pSP6 (see Fig. 1), which is an internal fragment of the *RAD3* gene, was inserted into *Bam*HI/*Eco*RI-digested M13mp8 and M13mp9 phages described by Messing and Vieira (28). The recombinant phages containing the appropriate inserts were used as templates for DNA synthesis to generate radioactively labeled single-stranded probes (29), which were then used in hybridizations to total yeast RNA from the *RAD*$^+$ strain B-635. The 0.9-kb *Bgl* II/*Eco*RI fragment cloned in M13mp8 hybridized to the 2.5-kb transcript (Fig. 3, lane B), whereas the same fragment inserted in M13mp9 did not (Fig. 3, lane C). Because the restriction sites of M13mp8 are arranged in the order 5′–*Eco*RI–*Sma* I, *Xma*I–*Bam*HI–*Sal* I, *Acc* I, *Hin*cII–*Pst* I–*Hin*dIII–3′ and those of M13mp9 are 5′–*Hin*dIII–*Pst* I–*Hin*cII, *Acc* I, *Sal* I–*Bam*HI–*Sma* I, *Xma* I–*Eco*RI–3′, these results indicate that the direction of transcription is from *Bgl* II to *Eco*RI and that the *RAD3* gene specifies a single transcript of 2.5 kb. In addition, the leftward 2.2-kb *Eco*RI/*Bgl*

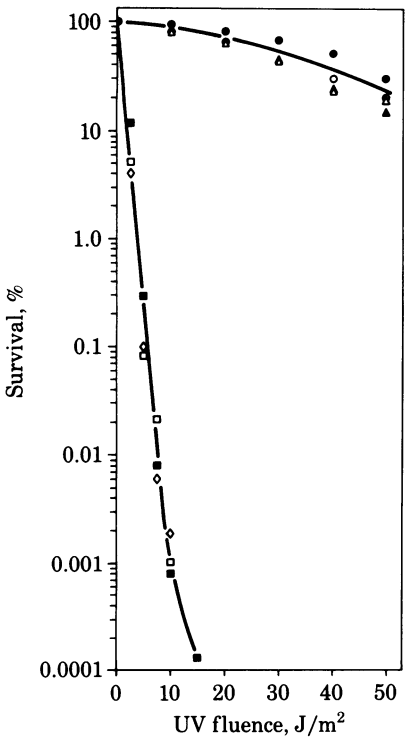

FIG. 2. Survival after UV irradiation of yeast cells harboring various *RAD3* insert-containing plasmids. Cells were grown in minimal medium supplemented with the necessary nutrients except that plasmid-bearing strains were grown in medium lacking uracil. Irradiation was as described by Prakash and Prakash (19). ●, Strain LP2649-1B, *MAT*α *aro7-1 ura3-52 RAD*$^+$; ■, LP2649-1A, *MAT*α *leu2-3 leu2-112 can1 ura3-52 rad3-2*; ◇, pLP2649-1A(YEp24); □, pLP2649-1A(pSP9); ○, pLP2649-1A(pSP3); ▲, pLP2649-1A(pSP6); △, pLP2649-1A(pSP10).

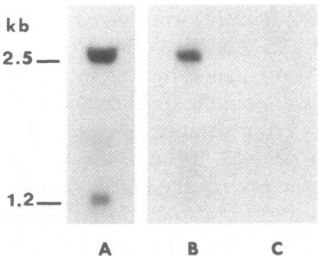

FIG. 3. Transcripts of the *RAD3* region. Total RNA (20 μg per lane) from yeast cells was fractionated on formaldehyde/agarose gels, transferred to nitrocellulose, and hybridized to various double- and single-stranded DNA fragments of the *RAD3* region. Lane A, RNA from the *RAD*$^+$ strain B-635 hybridized to the 3.1-kb *Eco*RI fragment of pSP6, labeled by nick-translation. Lanes B and C, as in lane A, but RNA was hybridized to the rightward 0.9-kb *Bgl* II/*Eco*RI fragment of pSP6 (see Fig. 1), cloned in M13mp8 (lane B) or M13mp9 (lane C).

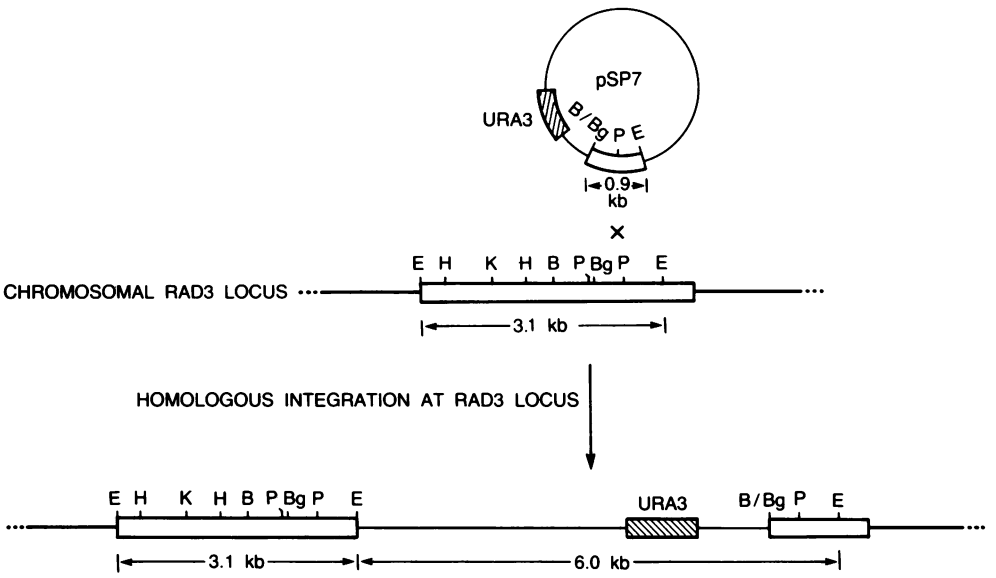

FIG. 4. Generation of a duplicated deletion of the *RAD3* gene by homologous integration at the *RAD3* locus of an internal segment of the *RAD3* gene. The 0.9-kb *Bgl* II/*Eco*RI fragment of pSP6 was ligated into *Bam*HI/*Eco*RI-digested YIp5 to generate the plasmid pSP7. As indicated diagrammatically, integration by homologous recombination at the *RAD3* locus will generate a duplicated deletion of *RAD3* flanking the YIp5 sequence. Thin line, pBR322 sequences; open bar, *RAD3* region DNA; hatched bar, yeast *URA3* gene; thick line, yeast chromosomal DNA; symbols for restriction enzymes are as given for Fig. 1.

II fragment, when cloned in M13mp9, hybridizes with the same two transcripts as observed with the 3.1-kb *Eco*RI probe, whereas the 2.2-kb *Eco*RI/*Bgl* II fragment, when cloned in M13mp8, shows no hybridization to yeast RNA. These results indicate the direction of the 1.2-kb transcript to be the same as that of the *RAD3* transcript.

Inviability of *rad3* Deletion Mutants. A deletion mutation of the *RAD3* gene was generated by the method of Shortle *et al.* (32). The 0.9-kb *Bgl* II/*Eco*RI fragment, which is an internal fragment of the *RAD3* gene (Fig. 1), was inserted into the *Bam*HI/*Eco*RI-digested integrating vector YIp5, generating the plasmid pSP7. This plasmid can transform *ura3* auxotrophs to prototrophy by homologous integration at either the *rad3* or *ura3* loci. If integration occurs at the *rad3* locus, then a duplication of the 0.9-kb *Bgl* II/*Eco*RI fragment flanking the integrated pBR322 sequences is generated. However, neither *RAD3* sequence contains an intact gene (Fig. 4), both sequences having undergone deletions of a portion of the *RAD3* gene. When the haploid yeast strain 7799-5C (*ura3-52 RAD*$^+$) was transformed to Ura$^+$ with pSP7, all of the over 100 Ura$^+$ transformants were *RAD*$^+$, suggesting that, in all these prototrophs, integration had occurred at the *ura3* site, because integration at the *RAD3* locus should yield radiation-sensitive transformants due to deletion of the *RAD3* gene. The absence of radiation-sensitive transformants could arise if integrations at the *RAD3* locus generating deletions are lethal.

In order to test the possibility that *rad3* deletions are lethal, the *RAD3/rad3-2 ura3-52/ura3-52* diploid DH-62 was transformed to Ura$^+$ with pSP7. Four of the UV-sensitive Ura$^+$ diploids, which must have resulted from integration in the *RAD3* gene to generate the UV-sensitive *rad3-Δ/rad3-2* diploid, were sporulated and subjected to tetrad analysis. As expected for inviability of *rad3* deletions, all four diploids yielded no tetrads with 3 or 4 viable spores, and the viable spores were always *ura3*$^-$ (Table 2). Three diploids in which integration of pSP7 had occurred at the *ura3* locus gave both *URA3*$^+$ and *ura3*$^-$ spores (Table 2).

Physical evidence that integration had occurred at the *RAD3* locus in the UV-sensitive diploids was provided by purifying genomic DNA from the parent nontransformed strain and three UV-sensitive diploid integrants. After restriction with *Eco*RI and separation of DNA fragments by gel electrophoresis, DNA was transferred to nitrocellulose by the method of Southern (27) and hybridized to pBR322 or a DNA fragment from the *RAD3* gene labeled by nick-translation. The 1.4-kb *Bam*HI/*Eco*RI fragment homologous to the 0.9-kb *Bgl* II/*Eco*RI fragment of pSP7, but also containing an additional 0.5 kb of DNA from the *RAD3* region, from the left of the *Bgl* II site (Fig. 1), was used as the probe for *RAD3*. In such gel-transfer hybridization experiments with genomic DNA from diploid yeast, a single band of 3.1 kb hybridizing to the yeast DNA probe is expected in the nontransformed diploid, while an additional 6.0-kb band is expected from the transformed diploids (Fig. 4). On the other hand, if pBR322 DNA is used as the probe, no hybridization band should occur for the nonintegrant, while integrants should contain a single 6.0-kb hybridization fragment (Fig. 4). Results of such a gel-transfer experiment are given in Fig. 5. As predicted for integration at the *RAD3* locus, the expected results are obtained with either the pBR322 or yeast DNA probes. The appearance of the 6.0-kb hybridization band in the integrants is diagnostic of integration at *RAD3*. Our results indicate that a deletion of the *RAD3* gene causes inviability of haploid cells.

DISCUSSION

We have isolated the *S. cerevisiae RAD3* gene and determined that it encodes an mRNA of 2.5 kb, whereas the length of the DNA insert in plasmid pSP10, which fully complements the UV sensitivity of *rad3* mutants, is 2.6 kb, suggesting that the *RAD3* gene spans most of the insert DNA in pSP10 (Fig. 1). The *RAD3* gene has also been cloned by Naumovski and Friedberg (33).

Table 2. Tetrad analysis of the *RAD3/rad3-2 ura3-52/ura3-52* diploid (DH-62) transformed with plasmid pSP7

Transformant no.	Locus of integration	No. of tetrads with given no. of viable spores per tetrad				*URA3*$^+$:*ura3*$^-$ spores
		4	3	2	1	
1	*RAD3*	0	0	24	36	0:84
2	*RAD3*	0	0	8	31	0:47
6	*RAD3*	0	0	14	30	0:58
7	*RAD3*	0	0	4	23	0:31
3	*ura3*	5	15	0	0	37:28
4	*ura3*	3	8	0	0	20:16
5	*ura3*	4	11	3	0	29:26

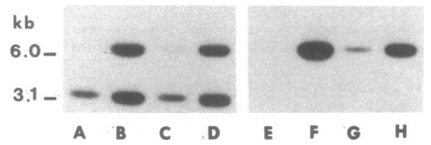

FIG. 5. Physical evidence for integration of plasmid pSP7 containing the 0.9-kb *Bgl* II/*Eco*RI internal fragment of the *RAD3* gene at the *RAD3* locus in the yeast genome. Plasmid pSP7 was used to transform the *RAD*$^+$/*rad3-2 ura3-52/ura3-52* diploid DH-62 to Ura$^+$. Genomic DNA from the untransformed strain (lanes A and E) and three UV-sensitive integrants (lanes B–D and F–H) was purified and digested with *Eco*RI. DNA fragments separated by electrophoresis in agarose gels were transferred to nitrocellulose and hybridized to a ^{32}P-labeled 1.4-kb *Bam*HI/*Eco*RI fragment of pSP6 (lanes A–D) or pBR322 DNA (lanes E–H).

The direction of *RAD3* transcription, determined by using the single-stranded phages M13mp8 and M13mp9 containing the internal 0.9-kb *Bgl* II/*Eco*RI fragment of the *RAD3* gene, was found to be rightward (Fig. 1). The *RAD3* promoter probably lies in the *Kpn* I/*Hin*dIII segment and the *RAD3* transcript must extend through most of the insert DNA in pSP10 close to the *Sau*3A1/*Bam*HI fusion junction (Fig. 1). The 2.5-kb *RAD3* transcript could code for a polypeptide of molecular weight about 90,000. The molecular weights of the polypeptides encoded by the *E. coli uvrA*, *uvrB*, and *uvrC* genes, which also function in incision of DNA damaged by UV and other DNA damaging agents, are 114,000, 84,000 and 68,000 respectively (34–36). Thus, the *RAD3* gene appears to resemble the *uvrB* gene in the size of its protein product.

Our results show that deletions of the *RAD3* gene created by integration of an internal 0.9-kb *Bgl* II/*Eco*RI fragment in the *RAD3* gene are recessive lethals. The UV resistance of *rad3* mutants harboring multicopy plasmids such as pSP3, pSP6, or pSP10 is similar to that of the *RAD*$^+$ cells, and we have also not observed any effect on the viability of these cells. These results suggest either that *RAD3* product levels are regulated in multicopy plasmid-containing strains or that a large amount of the *RAD3* gene product has little or no effect. The inviability of *rad3* deletion mutants indicates that the *RAD3* gene performs other important functions in addition to incision of damaged DNA and suggests that the *RAD3* gene does not code for an activity that functions only in incision. In this respect, the *RAD3* gene seems to differ from the *uvrA* and *uvrB* genes of *E. coli* because strains with insertions of the Mud(Ap,*lac*) operon fusion vector into the *uvrA* or *uvrB* genes, which inactivate these genes, are fully viable (37, 38).

Because the *RAD3* gene functions in excision repair and other cellular processes affecting viability, it is possible that the *RAD3* gene codes for a regulatory protein that controls various enzyme activities related to DNA repair and replication and chromosome segregation. Alternatively, the *RAD3* gene may code for an enzyme required for these various functions. Isolation and characterization of *rad3* mutants that are temperature sensitive for growth should provide some insight into the *RAD3* function. Determining the nucleotide sequence of the *RAD3* gene should allow us to construct well-defined *in vitro* mutations of the gene and should facilitate structure–function studies.

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