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RAD7 Gene of *Saccharomyces cerevisiae*: Transcripts, Nucleotide Sequence Analysis, and Functional Relationship between the RAD7 and RAD23 Gene Products

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The *RAD7* gene of *Saccharomyces cerevisiae* was cloned on a 4.0-kilobase (kb) DNA fragment and shown to provide full complementation of a *rad7*-Δ mutant strain. The nucleotide sequence of a 2.2-kb DNA fragment which contains the complete *RAD7* gene was determined. Transcription of the *RAD7* gene initiates at multiple sites in a region spanning positions −61 to −8 of the DNA sequence. The 1.8-kb *RAD7* mRNA encodes a protein of 565 amino acids with a predicted size of 63.7 kilodaltons. The hydropathy profile of the RAD7 protein indicates a highly hydrophilic amino terminus and a very hydrophobic region toward the carboxyl terminus. A *RAD7* subclone deleted for the first 99 codons complements the *rad7*-Δ mutation, but not the *rad7*-Δ *rad23*-Δ double mutation, indicating that the RAD23 protein can compensate for the function that is missing in the amino-terminally deleted RAD7 protein. The *RAD7* and *RAD23* genes in multicopy plasmids do not complement the *rad23*-Δ and *rad7*-Δ mutations, respectively. These observations could mean that although the two proteins might share a common functional domain, they must also perform distinct functions. Alternatively, an interaction between the RAD7 and RAD23 proteins could also account for these observations.

In *Saccharomyces cerevisiae*, incision of DNA containing pyrimidine dimers or interstrand cross-links is a complex process requiring as many as 10 genes. Mutants with mutations in six of these genes, *RAD1*, *RAD2*, *RAD3*, *RAD4*, *RAD10*, and *MMS19*, are highly defective in incision of DNA containing pyrimidine dimers (46, 58) or interstrand cross-links (30), whereas mutants with mutations in the *RAD7*, *RAD14*, *RAD16*, and *RAD23* genes show partial incision defectiveness (30, 31, 58). Our goal is to characterize the structure, regulation, and protein products of the genes involved in incision, and to reconstitute the incision activity in vitro. We and others have previously reported on the characterization of the *RAD1* (16, 59), *RAD2* (14, 36), *RAD3* (15, 34, 35, 44), and *RAD10* (39, 45) genes. In this paper we present the transcript analysis, mapping of the 5′ and 3′ ends of the mRNA, and the nucleotide sequence of the *RAD7* gene. We have also examined the complementation ability of a partial *RAD7* gene, deleted for the 99 amino-terminal codons, in the presence or absence of a wild-type genomic *RAD23* gene. The *RAD7* and *RAD23* genes are related in several ways: mutants with mutations in either gene are sensitive to UV light and only partially defective in excision repair, whereas the *rad7 rad23* double mutant shows greater UV sensitivity and excision defectiveness than either single mutant (31). The *RAD7* and *RAD23* genes occur in two different gene clusters, one located on chromosome X, which includes the three genes *CYC1-OSM1-RAD7* (COR cluster), and the other located on chromosome V, which includes the *ANP1-RAD23-CYC7* genes (ARC cluster), and it has been proposed that the COR and ARC clusters are related by duplication and transposition (27). In this paper, we show that the *RAD23* gene can provide complementation ability to the *RAD7* gene deleted for the first 99 codons.

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MATERIALS AND METHODS

Strains and culture media. The following strains of *S. cerevisiae* were used in this study: LP2741-3B (*MATα ade1 his3-Δ1 leu2-3 leu2-112 trp1-289 ura3-52 rad7-Δ*); LP2727-14A (*MATa ade1 his3-Δ1 leu2-3 leu2-112 trp1-289 ura3-52 RAD*$^+$); S211-1D (*MATa cyc1-11* (or *cyc1-363*) *his1 ura3-52 rad23-Δ*) (from F. Sherman); GP32-1B (*MATα arg4-17 ura3-52 rad7-Δ rad23-Δ*); 7799-4B (*MATa his4-17 ura3-52 RAD*$^+$) (from G. Fink); and DBY746 (*MATα his3-Δ1 leu2-3 leu2-112 trp1-289 ura3-52 RAD*$^+$) (from D. Botstein). The *rad7*-Δ mutation, originally isolated as *cyc1-1* (55), is a deletion encompassing the *CYC1*, *OSM1*, and *RAD7* genes on chromosome X (52). The *rad23*-Δ mutation was originally isolated as *CYC7-H3* (27), and is a deletion of the entire *ANP1* and *RAD23* coding regions, as well as the 5′ region of the *CYC7* gene on chromosome V. The *rad7*-Δ *rad23*-Δ double mutant (GP32-1B) was constructed by genetic manipulation of the two single *rad* mutants. Strains S211-1D and LP2727-4C (*MATα ade1 trp1-289 ura3-52 rad7-Δ*), carrying the previously described deletions, were mated, the resulting diploid (GP32) was induced to sporulate, and the tetrads were dissected. The resulting haploid strains were screened for UV sensitivity and backcrossed to the parent strains to determine the *rad* genotype.

Escherichia coli HB101 was used to amplify all plasmids. *E. coli* JM101 was used to propagate M13 recombinant bacteriophages. Strain MC1066 (from M. Casadaban) was used to screen *lacZ* fusion constructions.

YPD and minimal media for growth of *S. cerevisiae* strains were prepared as described by Prakash and Prakash (40). *E. coli* strains were cultured in LB, YT, or M9 medium as described by Maniatis et al. (24), with the addition of ampicillin (100 μg/ml) as required.

Vectors and plasmids. The following yeast-*E. coli* shuttle vectors were used in this study. Plasmid pTB62 contains the

ARS2 origin of replication from yeast cells (57) cloned in pBR322 and the *URA3* gene for selection in yeasts and is maintained in *S. cerevisiae* as an autonomously replicating plasmid. Plasmid YIp5 results from insertion of the yeast *URA3* gene into the *E. coli* plasmid pBR322 (50). This plasmid lacks a yeast origin of replication and is maintained in yeast cells following integration into the genome by homologous recombination. Plasmid pTB199 was constructed in our laboratory by insertion of the 2.1-kilobase (kb) *Eco*RI fragment that contains the origin of replication of the yeast 2μm circle (8) into the *Bal*I site of YIp5. This plasmid is present in high copy number in yeast cells (20 to 30 copies per cell [7]). Plasmids pGP4 and pGP8 are described below. Plasmid pGP15 was constructed by inserting the *Xho*I-*Eco*RI *RAD7* fragment from plasmid pGP4 into the *Sal*I-*Eco*RI sites of the single-copy vector YCp50 containing the yeast *CEN4* sequences (22). Plasmid pPP18 contains the entire *RAD23* gene in the 5.6-kb *Sal*I-*Xho*I fragment from plasmid pAB109 (obtained from F. Sherman; described by McKnight et al. [27]), inserted into the *Sal*I site of pTB199.

Transformation procedures and UV irradiation. Yeast cells were transformed by the method of Ito et al. (19). Transformation of *E. coli* cells was as described by Maniatis et al. (24). UV irradiation and measurements of survival were as described by Prakash and Prakash (40).

Preparation of nucleic acids. Plasmid DNA was purified by equilibrium centrifugation in CsCl gradients containing ethidium bromide to separate the supercoiled and the nicked DNA circles. Rapid plasmid minipreparations for restriction analysis were as described by Maniatis et al. (24). Single-stranded DNA from M13-infected cells was prepared as described by Heidecker et al. (13). Total RNA was extracted from exponentially growing cells as described by Reed et al. (43). These RNA preparations were enriched in poly(A)$^+$ RNA over an oligo(dT)-cellulose column, with 0.5 M KCl–0.01 M Tris hydrochloride (pH 7.5) as binding buffer and 0.01 M Tris hydrochloride (pH 7.5) as elution buffer, as indicated by the manufacturer (P-L Biochemicals, Inc.).

Northern hybridizations and preparation of radiolabeled probes. Total RNA (20 μg) or poly(A)$^+$ RNA (4 μg) was fractionated on 1.5% agarose gels containing formaldehyde by the method of Maniatis et al. (24) and transferred to GeneScreen membrane as described by the manufacturer (New England Nuclear Corp.). The RNA blots were hybridized to single-stranded DNA probes that had been radioactively labeled with [α-^{32}P]dATP (3,000 Ci/mmol; Amersham Corp. or New England Nuclear) as described by Hu and Messing (17). The specific activity of the probes was about 5 × 10^7 cpm/μg of DNA. Approximately 5 × 10^6 to 10 × 10^6 cpm was used in each hybridization. Hybridizations were performed at 42°C for 12 h in a solution containing 50% deionized formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 1% sodium dodecyl sulfate, 0.02% each of bovine serum albumin, Ficoll, and polyvinylpyrrolidone, and 100 μg of denatured, sonicated salmon sperm DNA per ml.

Mapping of the 5′ and 3′ mRNA termini by S1 nuclease digestion. Mapping of the S1-protected DNA fragments was performed by a modification of the method of Favaloro et al. (9). The appropriate restriction fragments (1 to 2 μg) were treated with alkaline phosphatase from calf intestine (molecular biology grade; Boehringer Mannheim Biochemicals) in a buffer containing 0.01 M Tris hydrochloride (pH 8.0), 1 mM $MgCl_2$, and 10 nM $ZnCl_2$ at 37°C for 30 min to remove the 5′ phosphates. After phenol-chloroform extractions and ethanol precipitation, the DNA fragments were 5′ end labeled in a buffer containing 50 mM Tris hydrochloride (pH 7.5), 10mM $MgCl_2$, 5 mM dithiothreitol, 0.1 mM spermidine, and 0.1 mM EDTA, by using T4 polynucleotide kinase (Bethesda Research Laboratories) in the presence of 150 to 200 μCi or [γ-^{32}P]ATP (3,000 Ci/mmol; New England Nuclear or Amersham) at 37°C for 30 min; 3′ end labeling was carried out by extension of 3′ recessed termini generated by restriction enzyme digestion, with the Klenow fragment of DNA polymerase I (Bethesda Research Laboratories), in the presence of the appropriate α-^{32}P-labeled deoxynucleotides (3,000 Ci/mmol; New England Nuclear or Amersham) and unlabeled deoxynucleotides. The end-labeled fragments were strand separated by the method of Maxam and Gilbert (26) by electrophoresis in 4% acrylamide gels (50:1 ratio of acrylamide to bisacrylamide), with 0.5× TBE buffer (1× TBE buffer is 89 mM Tris hydrochloride, 89 mM boric acid, and 2 mM EDTA) as running buffer. The gels were run at 4°C for about 16 h at 600 V. The band-containing strips of acrylamide, identified by autoradiography, were cut out of the gel, crushed into small pieces, and incubated in 0.6 ml of 50 mM Tris hydrochloride (pH 7.5) at 37°C overnight. The aqueous phase, containing the eluted fragments, was recovered by centrifugation through glass wool and concentrated by ethanol precipitation after the addition of 20 μg of tRNA as carrier. The precipitated probes were suspended in TE buffer (pH 7.5), and ca. 10^5 cpm was coprecipitated with 30 to 100 μg of poly(A)$^+$ RNA. The hybridization was performed at 45°C for at least 5 h in 80% formamide. The DNA-RNA hybrids were digested with 10, 150, and 300 U of S1 nuclease (Bethesda Research Laboratories) in 300 μl of S1 buffer (24) at 15°C for 1 to 2 h and gave similar results.

DNA sequencing. Restriction fragments were cloned in the M13 phage derivatives mp8, mp9, mp18, and mp19 (28). The nucleotide sequence was determined by the dideoxy chain termination method of Sanger et al. (49), in the presence of either [α-^{32}P]dATP or [α-^{35}S]thio-dATP (600 to 800 Ci/mmol; New England Nuclear or Amersham), as described by Biggin et al. (3). The sequencing reactions were fractionated on 5 to 8% acrylamide gels (19:1 ratio of acrylamide to bisacrylamide) containing 8 M urea.

Assays of β-galactosidase activity. β-Galactosidase assays were performed by a modification of the method of Ruby and Szostak (47). The units of β-galactosidase activity were calculated as follows: 1,000 (OD_{420} per milliliter)/micrograms of total protein per milliliter, where OD_{420} is the optical density at 420 nm.

RESULTS

Cloning of the *RAD7* gene and complementation analysis. The *RAD7* gene is one of at least seven tightly linked genes which are clustered in a region that spans about 1.5 centimorgans on the right arm of chromosome X. The entire region was cloned by chromosome walking, and the resulting 38 kb of DNA were cut into 13 fragments with the restriction endonucleases *Eco*RI and *Hin*dIII and separately cloned into the *E. coli* vector pBR322 (52). We obtained the DNA fragments IV through X (52), transferred them to the yeast autonomously replicating multicopy vectors pTB62 or pTB199, and tested their ability to complement a *rad7* deletion (*rad7*-Δ) mutation. Neither the 0.8-kb DNA fragment VII in plasmid pGP1 nor the 6.0-kb segment, including fragments IV-V-VI in plasmid pTB148, displayed any complementation ability (Fig. 1 and 2). When fragments IV, V, VI, and VII were ligated together in the original order in plasmid pGP3, a *rad7*-complementing function was reconstructed (Fig. 1). Further subcloning of this 6.8-kb fragment

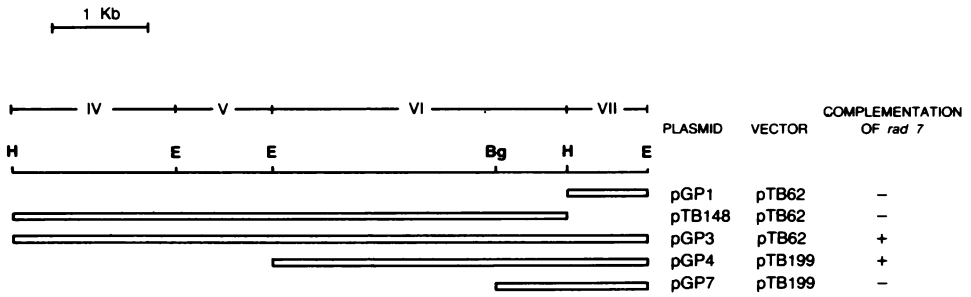

FIG. 1. Construction and localization of a functional *RAD7* gene. The ability of various DNA fragments to complement the UV sensitivity of a *rad7* deletion mutation is indicated. Fragment numbers in the top line are from Shalit et al. (52). The open bars indicate the DNA fragments from the *RAD7* region present in the vector named at the right. Symbols: Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII.

restricted the complementation ability to the 4.0-kb *Eco*RI-*Eco*RI fragment in plasmid pGP4, which encompasses the original fragments VI and VII (Fig. 1 and 2). Construction of plasmid pGP7 by deletion of the DNA between the *Eco*RI and the *Bgl*II sites in fragment VI in plasmid pGP4 destroyed the complementation ability of this clone (Fig. 1 and 2).

The *Bgl*II-*Hin*dIII fragment is internal to the *RAD7* gene. The subcloning results indicate that sequences to the left of the *Bgl*II site in fragment VI and to the right of the *Hin*dIII site between fragments VI and VII (Fig. 1) are necessary for the *RAD7* function. Therefore, the *Bgl*II-*Hin*dIII fragment should be internal to the gene. To test whether this was the case, the *Bgl*II-*Hin*dIII fragment was cloned into plasmid YIp5 (50), which does not contain a yeast origin of replication and is maintained in yeast cells only following integration into the genome by homologous genetic recombination. Integration of a plasmid containing an internal fragment of a gene by recombination with its chromosomal copy results in the disruption of the gene on the chromosome, giving rise to two truncated copies that are separated from each other by vector sequences (54). Wild-type yeast cells were transformed with plasmid pGP13, containing the *RAD7* *Bgl*II-*Hin*dIII fragment in YIp5, which had been linearized by digestion with the restriction endonuclease *Hin*dIII to create a recombinogenic free end in the region of homology with *RAD7* (37). The resulting transformants displayed the same levels of UV sensitivity as did the original *rad7*-Δ mutant (Fig. 2). Several independent transformants were crossed to the original *rad7*-Δ mutant, and the resulting diploids were found to retain the UV sensitivity, indicating that the disruption was in the *RAD7* gene. These results verify that the *Bgl*II-*Hin*dIII fragment is internal to the *RAD7* gene.

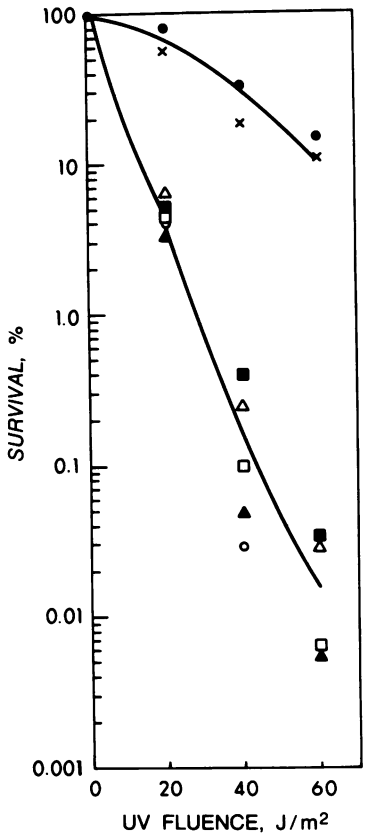

FIG. 2. Survival after UV irradiation of the *rad7*-Δ mutant with or without plasmids carrying different fragments from the *RAD7* chromosomal region. Symbols: ●, LP2727-14B, *RAD*⁺; □, LP2741-3B, *rad7*-Δ; ×, LP2741-3B(pGP4); △, LP2741-3B(pGP7); ○, LP2741-3B(pTB148); ▲, LP2741-3B(pTB199); ■, DBY746(pGP13); this strain contains the disrupted *RAD7* gene.

Size and direction of the *RAD7* transcript. To determine the size and direction of the *RAD7* transcript, the *Bgl*II-*Hin*dIII *RAD7* internal fragment was cloned in the two phage M13 derivatives mp8 and mp9 (29). Radioactively labeled single-stranded DNA probes from both recombinant phages were used in two separate hybridizations to poly(A)⁺ RNA from a *RAD*⁺ strain. Hybridization to an approximately 1.8-kb transcript was observed only when the M13 mp9 recombinant phage DNA was used. Since the order of the cloning sites in M13mp9 is 5′-*Hin*dIII-*Bam*HI-3′, the direction of transcription of the *RAD7* gene is from *Bgl*II toward *Hin*dIII (Fig. 1). In the chromosomal context, the gene is transcribed in the direction away from the centromere and toward the telomere of the right arm of chromosome X.

5′ and 3′ end mapping of the *RAD7* transcript. The 5′ and 3′ ends of the *RAD7* transcript were mapped by determining the size of the protected DNA fragments resulting from S1 nuclease digestion of RNA-DNA hybrids obtained by using the 5′-end-labeled *Pvu*II-*Xho*I and the 3′-end-labeled *Hin*dIII-*Eco*RI fragments as probes (Fig. 3). The results are shown in Fig. 4. Identical results were obtained when poly(A)⁺ RNA from a yeast strain bearing the *RAD7* gene in the multicopy plasmid pGP4 was used instead of poly(A)⁺ RNA from an untransformed wild-type strain. The *RAD7* transcript seems to initiate at a number of sites, spanning the region between positions −61 and −8 in the *RAD7* DNA sequence shown in Fig. 5. The three major S1-protected DNA fragments, 221, 225, and 229 nucleotides long (Fig. 4),

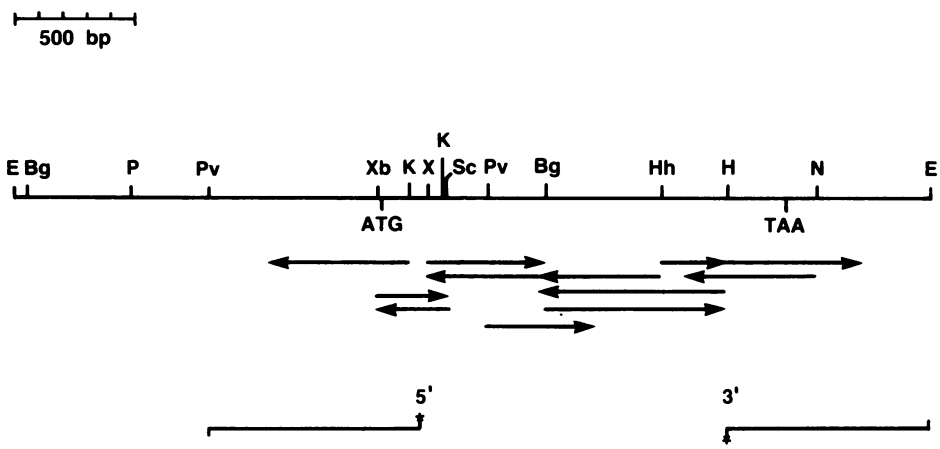

FIG. 3. Sequencing strategy. Restriction fragments from the *RAD7* region were cloned in the M13 vector pairs mp8 and mp9 or mp18 and mp19, so that the sequence of both strands of the DNA could be determined independently. The initiation and termination codons of the open reading frame are shown on the restriction map. The arrows indicate the direction and length of the sequence obtained from each cloned fragment. The end-labeled, strand-separated restriction fragments used as probes in the S1 mapping experiments are shown in the lower part of the figure. Symbols: Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; Hh, *Hha*I; K, *Kpn*I; N, *Nru*I; P, *Pst*I; Pv, *Pvu*II; Sc, *Sac*I; X, *Xho*I; Xb, *Xba*I.

correspond to the 5′ mRNA ends at positions −8, −12, and −16, respectively, and the S1-protected DNA fragments of 240, 265, 266, and 274 nucleotides correspond to the 5′ mRNA ends at −27, −52, −53, and −61, respectively (Fig. 5). The smallest S1-protected DNA fragment, of 211 nucleotides, corresponds to the 5′ mRNA end within the first codon of the open reading frame, at position +3; the significance of this 5′ end within the open reading frame is unclear. The 3′ end of the *RAD7* mRNA maps at position +1761, 63 nucleotides downstream of the translation termination codon TAA (Fig. 5).

Nucleotide sequence analysis of the *RAD7* gene. The nucleotide sequence of the *RAD7* gene was determined by the dideoxy chain termination method (49) on *RAD7* DNA fragments cloned in both orientations in the appropriate M13 phage vectors (28). The sequencing strategy in Fig. 3 shows that the sequence of both DNA strands was independently determined. The sequence of 2,231 base pairs (bp), corresponding to the strand identical to the mRNA, is shown in Fig. 5. A consensus sequence for termination of transcription by RNA polymerase III, TTTTCTTTTTTT, is found around position −150 downstream of a previously unidentified tRNA gene present in the region immediately upstream of *RAD7*. This sequence is part of an inverted repeat of 28 bp, between positions −158 and −129, that could potentially base pair, resulting in a hairpin structure presumed to be involved in RNA polymerase III transcription termination (51). The overall base composition of the *RAD7* gene reflects the G+C content of bulk yeast genomic DNA, with a value of 39% G+C in the coding region, while both the 5′ and 3′ nontranscribed regions display a lower G+C content (31 to 32%).

The *RAD7* nucleotide sequence contains an open reading frame 1,695 bp long, which encodes a protein of 565 amino acids with a predicted molecular weight of 63,705. Translation of most eucaryotic mRNAs begins at the 5′ proximal AUG (21; S. B. Baim, C. T. Goodhue, D. F. Pietras, D. C. Eustice, M. Labhard, L. R. Friedman, D. M. Hampsey, J. I. Stiles, and F. Sherman, UCLA Symp. Mol. Cell. Biol., in press), since 40S ribosomal subunits most probably scan the 5′ end of the mRNA in a linear fashion (20). The first in-frame AUG on the *RAD7* transcript is found between 8 and 61 nucleotides downstream of the mapped 5′ ends of the message. This AUG is preceded by a G at position −3, rather than the A found at this position in the majority of eucaryotic mRNAs (21).

To determine whether the *RAD7* open reading frame was translated in yeast cells, we constructed an in-frame *lacZ* fusion in plasmid pGP4, in the *Bgl*II site at position +702 in the *RAD7* DNA sequence, corresponding to codon 234 (Fig. 5). This fusion produces β-galactosidase activity in yeast cells, indicating that the *RAD7* open reading frame is translated. In *RAD7*, 60 of the 61 possible codons are used, indicating the absence of the codon bias toward the major isoacceptor tRNA species that characterizes some highly expressed yeast genes (2). The *RAD7* protein contains 37.3% nonpolar, 34.0% polar, 14.9% acidic, and 13.8% basic amino acids. The major clusters of both acidic and basic amino acids are located at the amino-terminal end of the protein, where 49% of the total charged residues are found within the first 200 amino acids. A cluster of five basic residues is contained within the first 10 amino acids, followed by a predominantly acidic region that spans about 120 residues. A stretch of seven contiguous arginine and lysine residues occurs between amino acids 147 and 153. The central portion of the protein sequence, between residues 200 and 350, contains about the same numbers of acidic and basic amino acids, while in the last 200 residues there are about twice as many acidic amino acids as basic ones, although no clustering of charged residues is observed at the carboxyl terminus. A Kyte and Doolittle hydropathy profile (23) of the *RAD7* protein indicates a highly hydrophilic region that includes the first 200 amino acids, followed by a mostly nonpolar region that extends throughout the rest of the protein and becomes quite hydrophobic toward the carboxyl terminus (Fig. 6).

5′ and 3′ flanking sequences of the *RAD7* gene. Yeast genes transcribed by RNA polymerase II, like RNA pol II genes in higher eucaryotes, are often preceded by one or more TATA boxes in the region upstream of the mRNA start site. In yeast cells, the spatial requirement between this putative RNA polymerase entry site and the site of transcription initiation does not seem to be as strict as in higher eucaryotes (12). The sequences TATTAT at position −30 to −25, TATAA at position −37 to −33, and TATTTAT at position −128 to −122 resemble the canonical sequence

TATAT(A)AT(A) usually found 26 through 34 nucleotides upstream of the site of transcription initiation in higher eucaryotes (6, 11). A computer search in the *RAD7* noncoding region did not reveal any direct or inverted repeats, with the exception of the previously described RNA pol III terminator. The consensus sequence AATAAA, which is believed to represent the signal for poly(A)$^+$ addition in higher eucaryotes (42), was observed in the 3′ noncoding region of *RAD7* between positions +1702 and +1707. The region between positions +1699 and +1707, overlapping the AATAAA sequence, also resembles the sequence TAAATAAA(G), which is found in many yeast genes approximately 28 to 33 nucleotides upstream of the 3′ end of the mRNA (1). Between positions +1730 and +1757, the sequence TAG. . .TAATGTTTT appears to be similar to the consensus sequence TAG. . .TAGT or TATGT. . .(AT rich). . .TTT, proposed to act in yeast cells as a transcription termination signal (60).

Expression of a truncated *RAD7* gene lacking the amino-terminal region of the protein. During subcloning analysis we observed that plasmid pGP8, derived from plasmid pGP4 by the deletion of about 2.0 kb of DNA upstream of the *Xho*I site at position +209 in the *RAD7* sequence (Fig. 5 and 7), complements the *rad7*-Δ mutation. Fusion of the *Xho*I site within the *RAD7* coding region with the *Sal*I site present in the pBR322 sequence of the vector results in a clone that lacks the entire 5′ upstream region as well as the first 209 bp of the open reading frame of *RAD7*. Transcriptional analysis and *RAD7-lacZ* fusion studies suggest that the 5′-deleted *RAD7* gene in plasmid pGP8 and the complete *RAD7* gene in plasmid pGP4 are expressed about equally. Northern hybridizations to a *RAD7*-specific probe of poly(A)$^+$ RNA from yeast cells containing plasmid pGP8 or plasmid pGP4 show a difference of about 200 nucleotides in the size of the two transcipts (1.6 and 1.8 kb, respectively), suggesting that the *RAD7* transcriptional start in plasmid pGP8 is located close to the *Sal*I/*Xho*I junction. The abundance of *RAD7* transcript from the two plasmids is approximately the same. In-frame *RAD7-lacZ* fusions in plasmids pGP8 and pGP4 that place the *E. coli lacZ* gene in the *Bgl*II site of the *RAD7* gene at position +702, corresponding to codon 234 (Fig. 5), show that the hybrid β-galactosidase activity from the two *RAD7-lacZ* fusion plasmids in yeast cells is about the same, 40 and 24 U/μg of total protein in plasmids pGP9 (derived from pGP8) and pGP11 (derived from pGP4), respectively.

We identified the 5′ end(s) of the *RAD7* mRNA transcribed in yeast cells from plasmid pGP8 by the S1 nuclease mapping procedure, using the 5′-end-labeled *Nru*I-*Bgl*II single-stranded DNA fragment of pGP8 as a probe (results not shown). This 813-nucleotide fragment includes 493 nucleotides of the *Xho*I-*Bgl*II region in *RAD7* and 320 nucleotides of the *Nru*I-*Sal*I region of pBR322 that lies upstream of the *Sal*I/*Xho*I junction in pGP8 (Fig. 7). Of the three S1-protected DNA fragments observed, 417, 490, and 525 nucleotides long, the two smaller ones map approximately at positions +289 and +216, respectively, within the *RAD7* sequence (Fig. 5), while the largest one maps within the pBR322 sequence, about 30 bp upstream of the *Sal*I/*Xho*I junction in plasmid pGP8. As was observed previously (56), these results with the truncated *RAD7* gene in plasmid pGP8 suggest that a cryptic promoter in pBR322 is being used. In the transcripts initiating in pBR322 and at +216 in *RAD7*, the presence of three out-of-frame AUGs in the *RAD7* sequence upstream of the in-frame AUG at +298 (Fig. 5), could make initiation of translation at the *RAD7* AUG codon at +298 rather unlikely (21; Baim et al., in press). Translation of the

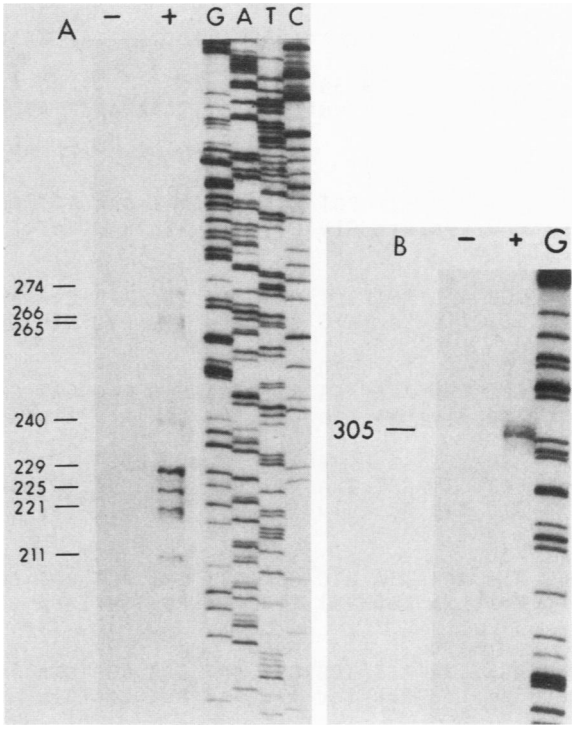

FIG. 4. Mapping of the 5′ and 3′ termini of the *RAD7* transcript by S1 nuclease digestion of RNA-DNA hybrids. (A) Heterogeneity of S1-protected DNA fragments at the 5′ end of the transcript. The probe is the 900-bp *Pvu*II-*Xho*I fragment shown in Fig. 3, 5′ end labeled and strand separated prior to hybridization to poly(A)$^+$ RNA from the *RAD*$^+$ strain 7799-4B. The length of the protected fragments was determined from the sequence of the *Hha*I-*Hin*dIII fragment shown on the right. (B) S1-protected fragment at the 3′ end of the *RAD7* transcript. The probe is the 800-bp *Hin*dIII-*Eco*RI fragment shown in Fig. 3, 3′ end labeled, strand separated, and hybridized as in panel A. The G lane of the sequence of the *Pvu*II-*Bgl*II fragment, used for size determination, is shown on the right.

mRNA initiating at +289 in the *RAD7* region could start at the in-frame AUG at position +298. The out-of-frame AUG at position +290 is too close to the 5′ end of this mRNA for initiating translation. Since in pGP8 the *RAD7-lacZ* fusion constructed in the *Bgl*II site at position +702 expresses β-galactosidase activity in yeast cells and since no in-frame AUG occurs upstream of the *Bgl*II site other than the one at position +298 (Fig. 5), the RAD7 protein encoded by this plasmid is expected to lack the first 99 amino acids at the amino terminus, which corresponds to the highly hydrophilic region of the RAD7 protein (Fig. 6).

The amino terminal region of the RAD7 protein is required for complementation of the *rad7*-Δ mutation in the absence of a functional *RAD23* gene. When the *rad7*-Δ mutant strain is transformed with plasmid pGP8, the UV sensitivity of the mutant is restored to wild-type levels (Fig. 8). This could result from the presence of an excess of a partially active RAD7 protein, owing to the high-copy-number vector, or to complementation by the RAD23 protein, which, because of its functional relationship with RAD7, might provide some function that is missing in the partially deleted RAD7 protein, or both. For determining the effect of plasmid copy number, the *RAD7 Xho*I-*Eco*RI fragment of plasmid pGP8 (Fig. 7) was cloned in the yeast centromere-containing vector YCp50 (22), which is maintained in yeast cells as an

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      -150       -140       -130       -120       -110       -100        -90        -80        -70
TTTTCTTTTTTTCTGGAAAATAAGAAAACTTATTTATGGAAGCAAAAATGGAATAAAGGATTGGGACGAAGTTAGTGAAAAAAAACTGAAA

      -60        -50        -40        -30   XbaI -20        -10         1          10
TAGTCCTAGAGTAACTCCGAAGTGTCTTTGTATAAGCTATTATCTAGAAATCACGAGAGGGGAAGAA ATG TAT CGC AGT AGA AAC
                                                                    Met Tyr Arg Ser Arg Asn

 20         30         40         50         60         70         80
CGA CCA AAA AGA GGT GGA GAA AAT GAA GTT AAG GGA CCA AAT TCT GCC TTG ACT CAA TTT TTA AGA GAA
Arg Pro Lys Arg Gly Gly Glu Asn Glu Val Lys Gly Pro Asn Ser Ala Leu Thr Gln Phe Leu Arg Glu

 90        100        110        120        130        140        150
GAA GGG ATC AGT GCT GAA AAT ATC AAA CAA AAA TGG TAC CAG CGA CAG TCG AAG AAG CAA GAA GAT GCA
Glu Gly Ile Ser Ala Glu Asn Ile Lys Gln Lys Trp Tyr Gln Arg Gln Ser Lys Lys Gln Glu Asp Ala

160        170        180        190        200        210 XhoI   220
ACA GAC GAA AAA AAA GGT AAA GCG GAG GAT GAT AGC TTT ACT GCC GAG ATA TCT CGA GTA GTT GAA GAT
Thr Asp Glu Lys Lys Gly Lys Ala Glu Asp Asp Ser Phe Thr Ala Glu Ile Ser Arg Val Val Glu Asp

230        240        250        260        270        280        290
GAA GAA ATT GAT GAA ATT GGA ACA GGT AGT GGT ACC GAG ACA GAA AGA GCT CAG GTT TCC TAC GAT GCC
Glu Glu Ile Asp Glu Ile Gly Thr Gly Ser Gly Thr Glu Thr Glu Arg Ala Gln Val Ser Tyr Asp Ala

300        310        320        330        340        350        360
AGG ATG AAA TTA GTC CCT GCT GAT AGT GAT GAA GAA GAA TAT GAA ACT AGC CAC ATT TCT GAC ACG CCA
Arg Met Lys Leu Val Pro Ala Asp Ser Asp Glu Glu Glu Tyr Glu Thr Ser His Ile Ser Asp Thr Pro

370        380        390        400        410        420        430
GTC AGT TTA AGT TCG GCT AAT GAC CGG GAA TCA TTG ACT AAA AAA AGG CAA AAT ACT GCA AAA ATT ATC
Val Ser Leu Ser Ser Ala Asn Asp Arg Glu Ser Leu Thr Lys Lys Arg Gln Asn Thr Ala Lys Ile Ile

440        450        460        470        480        490        500
CAA AAT CGT CGC AGA AAG CGT AAA AGA GCA GCT GAC CTA TTG GAT AGA CGC GTC AAC AAA GTA TCC AGC
Gln Asn Arg Arg Arg Lys Arg Lys Arg Ala Ala Asp Leu Leu Asp Arg Arg Val Asn Lys Val Ser Ser

510        520        530        540        550        560        570
TTA CAA AGT CTT TGT ATT ACG AAA ATT AGT GAA AAT ATA TCC AAG TGG CAA AAA GAG GCT GAT GAA TCA
Leu Gln Ser Leu Cys Ile Thr Lys Ile Ser Glu Asn Ile Ser Lys Trp Gln Lys Glu Ala Asp Glu Ser

580        590        600        610        620        630
TCA AAG TTG GTA TTT AAC AAA TTG AGA GAT GTC CTT GGT GGC GTA TCA ACC GCT AAT TTG AAT AAT TTG
Ser Lys Leu Val Phe Asn Lys Leu Arg Asp Val Leu Gly Gly Val Ser Thr Ala Asn Leu Asn Asn Leu

640        650        660        670        680        690        700 BglII
GCA AAA GCA CTA TCG AAG AAT AGG GCC CTG AAT GAT CAT ACT TTG CAA CTT TTC TTG AAG ACA GAT CTA
Ala Lys Ala Leu Ser Lys Asn Arg Ala Leu Asn Asp His Thr Leu Gln Leu Phe Leu Lys Thr Asp Leu

710        720        730        740        750        760        770
AAA AGG TTA ACT TTC AGC GAT TGT TCT AAA ATT TCA TTT GAT GGT TAC AAA ACG CTA GCC ATT TTT TCG
Lys Arg Leu Thr Phe Ser Asp Cys Ser Lys Ile Ser Phe Asp Gly Tyr Lys Thr Leu Ala Ile Phe Ser

780        790        800        810        820        830        840
CCA CAC CTA ACC GAA TTA TCC CTA CAA ATG TGT GGG CAG TTG AAC CAT GAA TCA TTG CTT TAC ATT GCT
Pro His Leu Thr Glu Leu Ser Leu Gln Met Cys Gly Gln Leu Asn His Glu Ser Leu Leu Tyr Ile Ala

850        860        870        880        890        900        910
GAA AAG CTA CCG AAC TTG AAA TCG CTG AAT TTA GAT GGA CCA TTT CTG ATC AAC GAG GAC ACA TGG GAG
Glu Lys Leu Pro Asn Leu Lys Ser Leu Asn Leu Asp Gly Pro Phe Leu Ile Asn Glu Asp Thr Trp Glu

920        930        940        950        960        970        980
AAG TTC TTT GTA ATA ATG AAA GGT AGA TTA GAA GAG TTC CAC ATT TCT AAT ACG CAC CGC TTC ACC GAC
Lys Phe Phe Val Ile Met Lys Gly Arg Leu Glu Glu Phe His Ile Ser Asn Thr His Arg Phe Thr Asp

990       1000       1010       1020       1030       1040       1050
AAA TCA TTA TCT AAT TTA TTG ATC AAC TGC GGT TCT ACC TTG GTA TCC TTA GGG TTA TCC AGA CTA GAT
Lys Ser Leu Ser Asn Leu Leu Ile Asn Cys Gly Ser Thr Leu Val Ser Leu Gly Leu Ser Arg Leu Asp

1060       1070       1080       1090       1100       1110       1120
TCT ATA TCA AAT TAC GCT TTA TTA CCG CAG TAC CTA GTC AAC GAC GAA TTT CAC AGT CTC TGT ATT GAA
Ser Ile Ser Asn Tyr Ala Leu Leu Pro Gln Tyr Leu Val Asn Asp Glu Phe His Ser Leu Cys Ile Glu

1130       1140       1150       1160       1170       1180       1190
TAT CCA TTC AAT GAA GAG GAT GTT AAC GAC GAG ATC ATC ATA AAT CTG CTA GGT CAA ATC GGG CGC ACG
Tyr Pro Phe Asn Glu Glu Asp Val Asn Asp Glu Ile Ile Ile Asn Leu Leu Gly Gln Ile Gly Arg Thr
```

FIG. 5. Nucleotide sequence of the *RAD7* gene and predicted amino acid sequence of the protein. The second digit from the right denotes the numbered base. The positions of the 5′ and 3′ ends of the transcript are indicated by arrows. The thickness of the arrows at the 5′ end reflects the intensity of the protected DNA fragment bands in the gel shown in Fig. 4. Pertinent restriction sites are indicated.

autonomously replicating single-copy plasmid. The *RAD7*-flanking pBR322 sequences in the resulting plasmid pGP15 are the same as in plasmid pGP8. Plasmid pGP15 was introduced into a *rad7*-Δ mutant, and the UV survival of the plasmid-containing strain was intermediate between those of the *rad7*-Δ mutant and wild-type strains (Fig. 8), indicating that copy number is only partially responsible for the complementation ability of the 5′ terminally deleted *RAD7* gene. To test whether the *RAD23* function is involved in the partial complementation ability of the truncated *RAD7* gene, a *rad7*-Δ *rad23*-Δ mutant strain was constructed and transformed with either plasmid pGP4 or pGP8. The UV survival of the mutant strains and transformants is shown in Fig. 8. The *rad7*-Δ *rad23*-Δ double mutant is more UV sensitive than either one of the two single mutants. Plasmid pGP4, which contains the entire *RAD7* gene, restores the UV suvival of the double-mutant strain to that of a single *rad23*-Δ mutant. However, plasmid pGP8, which is missing the amino terminus of the RAD7 protein, does not show any complementation ability when introduced into a strain with the *rad7*-Δ *rad23*-Δ double-mutation background. These observations suggest that the RAD23 protein can compensate for the function of the missing amino-terminal region of RAD7.

Lack of cross-complementation between *RAD7* and *RAD23*. One interpretation of the results of the complementation analysis of the amino-terminally truncated *RAD7* subclone is that the RAD7 and RAD23 proteins share a common function. On the basis of the proposed common evolutionary origin of the two genes (27), it could be postulated that the two gene products perform similar functions in the excision repair of UV-damaged DNA in *S. cerevisiae* cells, although they might be present in different amounts in the cell, as is the case for the highly conserved iso-1- and iso-2-cytochrome *c*, encoded by the *CYC1* and *CYC7* genes, respectively. Iso-2-cytochrome *c* makes up only about 5% of the total cytochrome *c* in yeast cells (53). However, overproduction of iso-2-cytochrome *c* can compensate for the deficiency of iso-1-cytochrome *c* in *cyc1* mutants. To determine the extent of the functional relationship between the *RAD7* and *RAD23* gene products, we tested whether the absence of one of the two gene products could be complemented even partially by overproduction of the other. For this purpose, the *RAD7* gene on a multicopy vector (plasmid pGP4), was introduced into a *rad23*-Δ mutant yeast strain and the UV survival of transformants was determined. The reciprocal experiment was also performed by testing the UV survival of a *rad7*-Δ strain bearing the *RAD23* gene on a

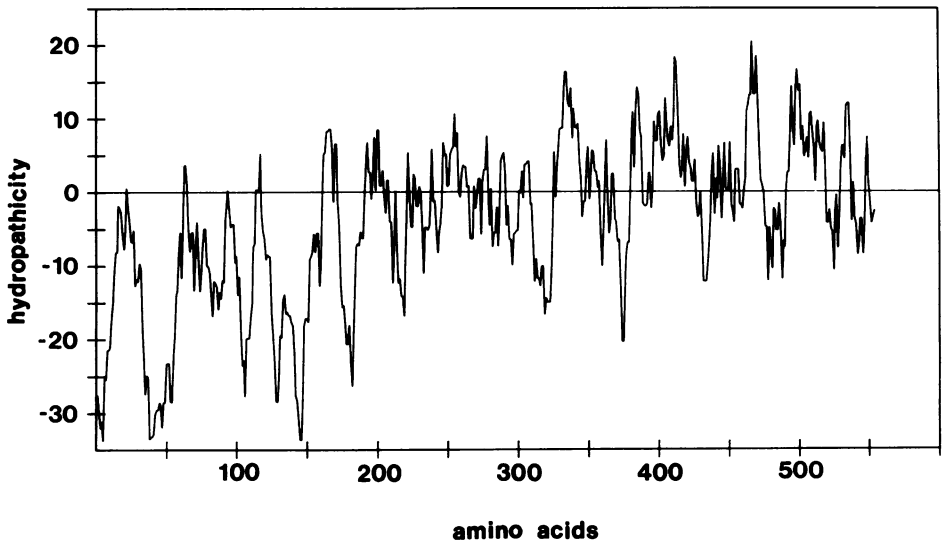

FIG. 6. Kyte and Doolittle (23) hydropathy profile of the RAD7 protein. The computer program continuously determines the average hydropathy of a moving segment of given length. Each value in the figure is calculated over a range of 11 amino acids and represents the sum of the individual values for these residues. The first plotted value corresponds to the amino acid residue number 5. Negative values indicate hydrophilic regions in the protein.

multicopy vector (plasmid pPP18, constructed as described in Materials and Methods). Neither of the two genes, when present at high copy number in the reciprocal single-mutant background, was able to complement even partially the UV-sensitive mutant phenotype. This result indicates that the RAD7 and RAD23 proteins must differ from each other in some domain(s) of activity, which renders them functionally distinct.

DISCUSSION

The *RAD7* gene transcribes a 1.8-kb mRNA with several 5′ ends, which, except for one, map between 61 and 8 nucleotides upstream of the first ATG at position +1 (Fig 4A and 5). The *RAD7*-encoded protein contains 565 amino acids with a predicted size of 63.7 kilodaltons. The RAD7 protein does not show any significant homology with the *E. coli* uvrA (A. Sancar, personal communication), uvrC (48), and uvrD (10) proteins. Also, no homology is observed with the protein sequences of RAD1 (P. Reynolds, L. Prakash, and S. Prakash, unpublished observation), RAD2 (K. Madura and S. Prakash, submitted for publication), RAD3 (34, 44), and RAD10 (45) of *S. cerevisiae*.

The RAD7 protein is highly structured, with 50% of the charged residues clustered in the amino-terminal region. The hydropathy profile (23) indicates the presence of a highly hydrophilic amino-terminal region and a hydrophobic region toward the carboxyl terminus (Fig. 6). Kyte and Doolittle (23) showed a high correlation between protein regions, with average hydropathy values of a given 19-amino acid segment greater than 1.6 and membrane-spanning regions of membrane-bound proteins. The hydropathy analysis of the RAD7 protein indicates two possible membrane-binding domains,

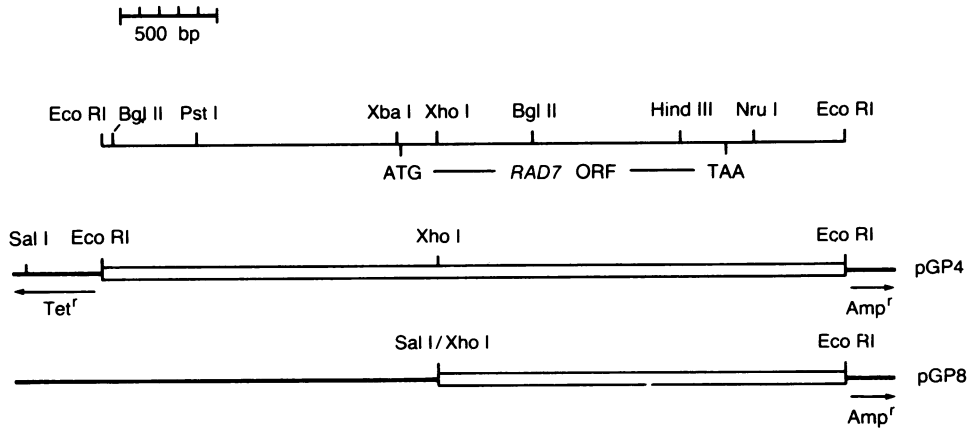

FIG. 7. *RAD7* subclone lacking the entire 5′ noncoding region and the amino-terminal region of the RAD7 protein. The restriction map of the *RAD7* gene with the relevant restriction sites is given in the upper part of the figure, and the *RAD7* open reading frame is indicated. Plasmid pGP8 was derived from plasmid pGP4 by deletion of the region of DNA between the *Sal*I site in the pBR322 sequence and the *Xho*I site at position +209 in the *RAD7* sequence. The distance between the *Sal*I site and the closest *Eco*RI site is not to scale. The direction of transcription of the genes that confer ampicillin and tetracycline resistance is indicated by the arrows.

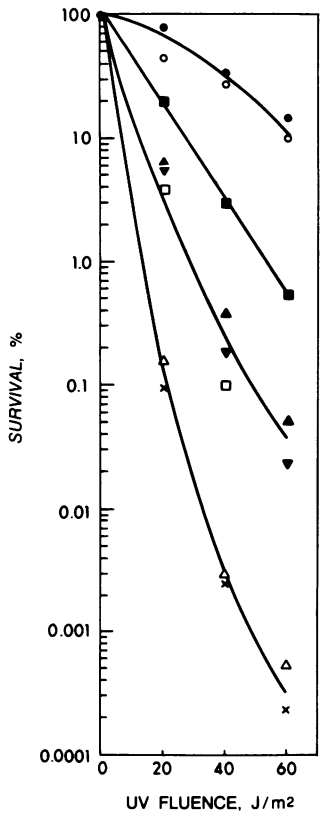

FIG. 8. Survival after UV irradiation of the *rad7*-Δ and *rad23*-Δ single-mutation strains and of the *rad7*-Δ *rad23*-Δ double-mutation strain, with or without plasmids. Plasmid pGP4 contains the entire *RAD7* gene. The 5′ deleted *RAD7* gene described in the text is inserted in a multicopy vector in plasmid pGP8 and in a single-copy vector in plasmid pGP15. Symbols: ●, LP2727-14B, *RAD*$^+$; □, LP2741-3B, *rad7*-Δ; ▼, S211-1D, *rad23*-Δ; △, GP32-1B, *rad7*-Δ *rad23*-Δ; ○, LP2741-3B(pGP8); ■, LP2741-3B(pGP15); ▲, GP32-1B(pGP4); ×, GP32-1B(pGP8).

both of which are located in the hydrophobic region toward the carboxyl terminus, around the amino acid residues 425 and 475. A comparison of the RAD7 protein sequence with those of the outer membrane proteins OmpC (32), OmpF (18), and PhoE (38) of *E. coli* did not reveal any significant homology.

A deletion in the *RAD7* gene of the first 99 codons, which represent a major portion of the hydrophilic region, retains partial complementation ability of a *rad7*-Δ mutant strain in a single-copy plasmid, while it provides full complementation when present in a multicopy plasmid (Fig. 8). The complementation ability of the multicopy plasmid containing the amino-terminally deleted *RAD7* gene is abolished in a strain that is deleted for both the *RAD7* and the *RAD23* genes. One interpretation for these results is that the *RAD7* and *RAD23* genes share a region that codes for a similar activity in the two proteins. In the absence of this region in the RAD7 protein, the RAD23 protein could supply the missing function. However, since the *rad7*-Δ and the *rad23*-Δ mutants cannot be cross-complemented even by the multicopy plasmids containing the *RAD23* and *RAD7* genes, respectively, the two proteins must also contain distinct functions. Alternatively, an interaction between the RAD7 and RAD23 proteins could account for the activity or the stability, or both, of the amino-terminally deleted RAD7 protein.

rad7 or *rad23* deletion mutants, as well as *rad14* mutants, are only partially defective in the excision repair of UV-damaged DNA, as well as of psoralen-plus light-induced DNA interstrand cross-links (31, 41). In contrast, mutants with mutations in the *RAD1, RAD2, RAD3, RAD4, RAD10,* and *MMS19* genes are highly defective in excision repair of these lesions (see reference 30 and references therein). The difference between these two groups of mutants resides in the lack of incision in the *rad1, rad2, rad3, rad4, rad10,* and *mms19* mutants (30, 46, 58), while the *rad7, rad14,* and *rad23* mutants are only partially incision defective (30, 31, 58). The partial incision deficiency of the *rad7*-Δ mutant suggests that the RAD7 protein might play an accessory role in the incision process; for example, its activity might be required to render a subset of the lesions in chromatin accessible to the incision complex. DNA lesions in different regions of chromatin might be repaired with different efficiency depending upon the chromatin structure, location of chromatin in the nucleus, or association of chromatin with the nuclear matrix or with other nuclear structural components (4, 5, 25, 33). The membrane-binding domains indicated by the hydropathic structure might suggest that RAD7 is a nuclear membrane protein and affects the repair of chromosomal regions associated with the nuclear membrane. Purification, characterization, and cellular localization of the RAD7 protein should provide some insight into its role in excision repair.

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LITERATURE CITED

1. **Bennetzen, J. L., and B. D. Hall.** 1982. The primary structure of the *Saccharomyces cerevisiae* gene for alcohol dehydrogenase I. J. Biol. Chem. **257**:3018–3025.
2. **Bennetzen, J. L., and B. D. Hall.** 1982. Codon selection in yeast. J. Biol. Chem. **257**:3026–3031.
3. **Biggin, M. D., T. J. Gibson, and G. F. Hong.** 1983. Buffer gradient gels and ^{35}S label as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA **80**:3963–3965.
4. **Bohr, V., and P. C. Hanawalt.** 1984. Factors that affect the initiation of excision-repair in chromatin, p. 109–125. *In* A. Collins, R. T. Johnson, and C. S. Downes (ed.), DNA repair and its inhibition. IRL Press, Oxford.
5. **Bohr, V. A., C. A. Smith, D. S. Okumoto, and P. C. Hanawalt.** 1985. DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. Cell **40**:359–369.
6. **Breathnach, R., and P. Chambon.** 1981. Organization and expression of eucaryotic split genes coding for proteins. Annu. Rev. Biochem. **50**:349–383.
7. **Broach, J. R.** 1982. The yeast plasmid 2μ circle. Cell **28**: 203–204.
8. **Broach, J. R., and J. B. Hicks.** 1980 Replication and recombination functions associated with the yeast plasmid 2μ circle. Cell **21**:501–508.
9. **Favaloro, J., R. Treisman, and R. Kamen.** 1980. Transcription maps of polyoma virus-specific RNA: analysis by two-dimensional nuclease S1-gel mapping. Methods Enzymol. **65**:718–749.

10. **Finch, P. W., and P. T. Emmerson.** 1984. The nucleotide sequence of the *uvrD* gene of *E. coli*. Nucleic Acids Res. **12**:5789–5799.
11. **Gannon, F., K. O'Hare, F. Perrin, J. P. LePennec, C. Benoist, M. Cochet, R. Breathnach, A. Royal, A. Garapin, B. Cami, and P. Chambon.** 1979. Organization and sequences at the 5' end of a cloned complete ovalbumin gene. Nature (London) **278**:428–434.
12. **Guarente, L.** 1984. Yeast promoters: positive and negative elements. Cell **36**:799–800.
13. **Heidecker, G., J. Messing, and B. Gronenborn.** 1980. A versatile primer for DNA sequencing in the M13mp2 cloning system. Gene **10**:69–73.
14. **Higgins, D. R., L. Prakash, P. Reynolds, and S. Prakash.** 1984. Isolation and characterization of the *RAD2* gene of *Saccharomyces cerevisiae*. Gene **30**:121–128.
15. **Higgins, D. R., S. Prakash, P. Reynolds, R. Polakowska, S. Weber, and L. Prakash.** 1983. Isolation and characterization of the *RAD3* gene of *Saccharomyces cerevisiae* and inviability of *rad3* deletion mutants. Proc. Natl. Acad. Sci. USA **80**:5680–5684.
16. **Higgins, D. R., S. Prakash, P. Reynolds, and L. Prakash.** 1983. Molecular cloning and characterization of the *RAD1* gene of *Saccharomyces cerevisiae*. Gene **26**:119–126.
17. **Hu, N. T., and J. Messing.** 1982. The making of strand-specific M13 probes. Gene **17**:271–277.
18. **Inokuchi, K., N. Mutoh, S.-I. Matsuyama, and S. Mizushima.** 1982. Primary structure of the *ompF* gene that codes for a major outer membrane protein of *Escherichia coli* K-12. Nucleic Acids Res. **10**:6957–6968.
19. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
20. **Kozak, M.** 1983. Comparison of initiation of protein synthesis in procaryotes, eucaryotes, and organelles. Microbiol. Rev. **47**:1–45.
21. **Kozak, M.** 1984. Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNA. Nucleic Acids Res. **12**:857–872.
22. **Kuo, C. L., and J. L. Campbell.** 1983. Cloning of *Saccharomyces cerevisiae* DNA replication genes: isolation of the *CDC8* gene and two genes that compensate for the *cdc8-1* mutation. Mol. Cell. Biol. **3**:1730–1737.
23. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
24. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Mansbridge, J. N., and P. C. Hanawalt.** 1983. Domain-limited repair of DNA in ultraviolet irradiated fibroblasts from xeroderma pigmentosum complementation group C, p. 195–207. *In* E. C. Friedberg and B. N. Bridges (ed.), Cellular responses to DNA damage. Alan R. Liss, Inc., New York.
26. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
27. **McKnight, G. L., T. S. Cardillo, and F. Sherman.** 1981. An extensive deletion causing overproduction of yeast iso-2-cytochrome c. Cell **25**:409–419.
28. **Messing, J., R. Crea, and P. H. Seeburg.** 1981. A system for shotgun DNA sequencing. Nucleic Acids Res. **9**:309–321.
29. **Messing, J., and J. Vieira.** 1982. A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. Gene **19**:269–276.
30. **Miller, R., L. Prakash, and S. Prakash.** 1982. Genetic control of excision of *Saccharomyces cerevisiae* interstrand cross-links induced by psoralen plus near-UV light. Mol. Cell. Biol. **2**:939–948.
31. **Miller, R., L. Prakash, and S. Prakash.** 1982. Defective excision of pyrimidine dimers and interstrand DNA crosslinks in *rad7* and *rad23* mutants of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **188**:235–239.
32. **Mizuno, T., M.-Y. Chou, and M. Inouye.** 1983. A comparative study on the genes for three porins of the *Escherichia coli* outer membrane: DNA sequence of the osmoregulated *ompC* gene. J. Biol. Chem. **258**:6932–6940.
33. **Muellenders, L. H. F., A. C. Van Kesteren, C. J. M. Bussmann, A. A. Van Zeeland, and A. T. Natrajan.** 1984. Preferential repair of nuclear matrix associated DNA in xeroderma pigmentosum complementation group C. Mutat. Res. **141**:75–82.
34. **Naumovski, L., G. Chu, P. Berg, and E. C. Friedberg.** 1985. *RAD3* gene of *Saccharomyces cerevisiae*: nucleotide sequence of wild-type and mutant alleles, transcript mapping, and aspects of gene regulation. Mol. Cell. Biol. **5**:17–26.
35. **Naumovski, L., and E. C. Friedberg.** 1983. A DNA repair gene required for the incision of damaged DNA is essential for viability in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **80**:4818–4821.
36. **Naumovski, L., and E. C. Friedberg.** 1984. *Saccharomyces cerevisiae RAD2* gene: isolation, subcloning, and partial characterization. Mol. Cell. Biol. **4**:290–295.
37. **Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein.** 1983. Genetic applications of yeast transformation with linear and gapped plasmids. Methods Enzymol. **101**:228–245.
38. **Overbeeke, N., H. Bergmans, F. Van Mansfeld, and B. Lugtenberg.** 1983. Complete nucleotide sequence of *phoE*, the structural gene for the phosphate limitation inducible outer membrane pore protein of *E. coli* K12. J. Mol. Biol. **163**:513–532.
39. **Prakash, L., D. Dumais, R. Polakowska, G. Perozzi, and S. Prakash.** 1985. Molecular cloning of the *RAD10* gene of *Saccharomyces cerevisiae*. Gene **34**:55–61.
40. **Prakash, L., and S. Prakash.** 1977. Isolation and characterization of MMS-sensitive mutants of *Saccharomyces cerevisiae*. Genetics **86**:33–55.
41. **Prakash, L., and S. Prakash.** 1979. Three additional genes involved in pyrimidine dimer removal in *Saccharomyces cerevisiae: RAD7, RAD14,* and *MMS19*. Mol. Gen. Genet. **176**:351–359.
42. **Proudfoot, N. J., and G. G. Brownlee.** 1976. 3' non-coding region sequences in eukaryotic messenger RNA. Nature (London) **263**:211–214.
43. **Reed, S. I., J. Ferguson, and J. C. Groppe.** 1982. Preliminary characterization of the transcriptional and translational products of the *Saccharomyces cerevisiae* cell cycle gene *CDC28*. Mol. Cell. Biol. **2**:412–425.
44. **Reynolds, P., D. R. Higgins, L. Prakash, and S. Prakash.** 1985. The nucleotide sequence of the *RAD3* gene of *Saccharomyces cerevisiae*: a potential adenine nucleotide binding amino acid sequence and a nonessential acidic carboxyl terminal region. Nucleic Acids Res. **13**:2357–2372.
45. **Reynolds, P., L. Prakash, D. Dumais, G. Perozzi, and S. Prakash.** 1985. Nucleotide sequence of the *RAD10* gene of *Saccharomyces cerevisiae*. EMBO J. **4**:3549–3552.
46. **Reynolds, R. J., and E. C. Friedberg.** 1981. Molecular mechanisms of pyrimidine dimer excision in *Saccharomyces cerevisiae*: incision of ultraviolet-irradiated deoxyribonucleic acid in vivo. J. Bacteriol. **146**:692–704.
47. **Ruby, S. W., and J. W. Szostak.** 1985. Specific *Saccharomyces cerevisiae* genes are expressed in response to DNA-damaging agents. Mol. Cell. Biol. **5**:75–84.
48. **Sancar, G. B., A. Sancar, and W. D. Rupp.** 1984. Sequences of the *E. coli uvrC* gene and protein. Nucleic Acids Res. **12**:4593–4608.
49. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
50. **Scherer, S., and R. W. Davis.** 1979. Replacement of chromosome segments with altered DNA sequences constructed in vitro. Proc. Natl. Acad. Sci. USA **76**:4951–4955.
51. **Sentenac, A., and B. Hall.** 1982. Yeast nuclear RNA polymerases and their role in transcription, p. 561–606. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Har-

bor, N.Y.
52. **Shalit, P., K. Loughney, M. Olson, and B. Hall.** 1981. Physical analysis of the *CYC1-sup4* interval in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **1**:228–236.
53. **Sherman, F., and J. W. Stewart.** 1971. Genetics and biosynthesis of cytochrome c. Annu. Rev. Genet. **5**:257–296.
54. **Shortle, D., J. E. Haber, and D. Botstein.** 1982. Lethal disruption of the yeast actin gene by integrative DNA transformation. Science **217**:371–373.
55. **Singh, A., and F. Sherman.** 1978. Deletions of the iso-1-cytochrome c and adjacent genes of yeast: discovery of the *osm1* gene controlling osmotic sensitivity. Genetics **89**:653–665.
56. **Struhl, K.** 1981. Position effects in *Saccharomyces cerevisiae*. J. Mol. Biol. **152**:569–575.
57. **Tschumper, G., and J. Carbon.** 1982. Delta sequences and double symmetry in a yeast chromosomal replicator region. J. Mol. Biol. **156**:293–307.
58. **Wilcox, D. R., and L. Prakash.** 1981. Incision and post-incision steps of pyrimidine dimer removal in excision defective mutants of *Saccharomyces cerevisiae*. J. Bacteriol. **148**:618–623.
59. **Yang, E., and E. C. Friedberg.** 1984. Molecular cloning and sequence analysis of the *Saccharomyces cerevisiae RAD1* gene. Mol. Cell. Biol. **4**:2161–2169.
60. **Zaret, K. S., and F. Sherman.** 1982. DNA sequence required for efficient transcription termination in yeast. Cell **28**:563–573.