JOURNAL OF BACTERIOLOGY, June 1986, p. 914–923
0021-9193/86/060914-10$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 166, No. 3

Nucleotide Sequence, Transcript Mapping, and Regulation of the *RAD2* Gene of *Saccharomyces cerevisiae*

KIRAN MADURA AND SATYA PRAKASH*
Department of Biology, University of Rochester, Rochester, New York 14627

Received 3 January 1986/Accepted 20 March 1986

We determined the nucleotide sequence, mapped the 5′ and 3′ mRNA termini, and examined the regulation of the *RAD2* gene of *Saccharomyces cerevisiae*. A long open reading frame within the *RAD2* transcribed region encodes a protein of 1,031 amino acids with a calculated molecular weight of 117,847. A disruption of the *RAD2* gene that deletes the 78 carboxyl terminal codons results in loss of RAD2 function. The 5′ ends of *RAD2* mRNA show considerable heterogeneity, mapping 5 to 62 nucleotides upstream of the first ATG codon of the long *RAD2* open reading frame. The longest *RAD2* transcripts also contain a short open reading frame of 37 codons that precedes and overlaps the 5′ end of the long *RAD2* open reading frame. The *RAD2* 3′ mRNA end maps 171 nucleotides downstream of the TAA termination codon and 20 nucleotides downstream from a 12-base-pair inverted repeat that might function in transcript termination. Northern blot analysis showed a ninefold increase in steady-state levels of *RAD2* mRNA after treatment of yeast cells with UV light. The 5′ flanking region of the *RAD2* gene contains several direct and inverted repeats and a 44-nucleotide-long purine-rich tract. The sequence T G G A G G C A T T A A found at position −167 to −156 in the *RAD2* gene is similar to a sequence present in the 5′ flanking regions of the *RAD7* and *RAD10* genes.

The *RAD2* gene of *Saccharomyces cerevisiae* is one of 10 genes, *RAD1*, *RAD2*, *RAD3*, *RAD4*, *RAD7*, *RAD10*, *RAD14*, *RAD16*, *RAD23*, and *MMS19*, involved in excision repair of DNA containing pyrimidine dimers or cross-links (27, 28, 44, 55). Mutants in the *RAD1*, *RAD2*, *RAD3*, *RAD4*, *RAD10*, and *MMS19* genes are highly defective in incision activity (27, 44, 55), while mutants in the other four genes show various degrees of incision defects (27, 28, 55). To study the structure, regulation, and function of these genes, we and others have cloned and characterized the *RAD1* (15, 56), *RAD2* (13, 33), *RAD3* (14, 31, 32, 41), *RAD7* (35), and *RAD10* (37, 42, 54) genes. We had previously located the *RAD2* gene on a cloned DNA fragment, shown that it encodes a 3.3-kilobase (kb) transcript, and determined its direction of transcription (13). Disruptions of the *RAD2* gene, made by integrating a plasmid containing an internal *RAD2* DNA fragment in the yeast chromosomal *RAD2* site, are viable but highly sensitive to UV light (13). Similar results are observed for disruptions or deletions of the *RAD1* and *RAD10* genes (15, 37, 42). In contrast, disruptions or deletions of the *RAD3* gene are recessive lethal mutations (14, 32, 41). In this paper we report the complete nucleotide sequence of the *RAD2* gene, map its 5′ and 3′ mRNA termini, and show that steady-state levels of *RAD2* mRNA increase significantly after UV irradiation of yeast cells.

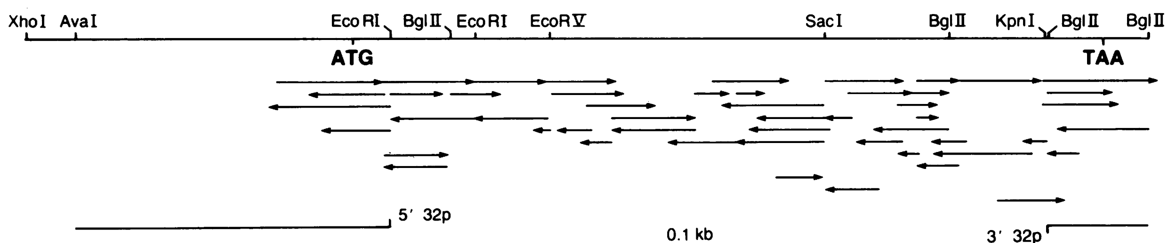

FIG. 1. Sequencing strategy for the *RAD2* gene. The arrows indicate the DNA strand and the distance that was sequenced. Six-base-recognizing restriction enzyme sites that were used for cloning into various M13 vectors are shown. Four-base-recognizing restriction enzyme sites are not indicated, although they were used extensively. The ATG initiation codon lies 152 nucleotides upstream of the 5′ EcoRI site, and the TAA termination codon lies 235 nucleotides 3′ to the *KpnI* site. The restriction fragments for mapping the 5′ and 3′ ends of the *RAD2* transcript are also shown.

MATERIALS AND METHODS

Yeast and bacterial strains. *S. cerevisiae* 7799-4B *MAT***a** *his4-17 ura3-52 RAD*$^+$ was used for transcript analyses, and strain DBY746 *MAT*α *his3-Δ1 leu2-3 leu2-112 trp1-289 ura3-52 RAD*$^+$ was used for *RAD2-lacZ* fusion analyses.

The *Escherichia coli* strains used were HB101 for maintenance and propagation of plasmids, JM103 for propagation of M13 phage derivatives, and MC1066 for maintenance and propagation of *lacZ* fusion plasmids.

DNA sequencing. Restriction fragments were cloned into the M13 derivatives M13mp8, M13mp9, M13mp18, and M13mp19 wherever possible. Frequently we used shotgun cloning of fragments released by restriction enzymes recognizing four base sequences. The nucleotide sequences were determined by the dideoxy chain termination method of

* Corresponding author.

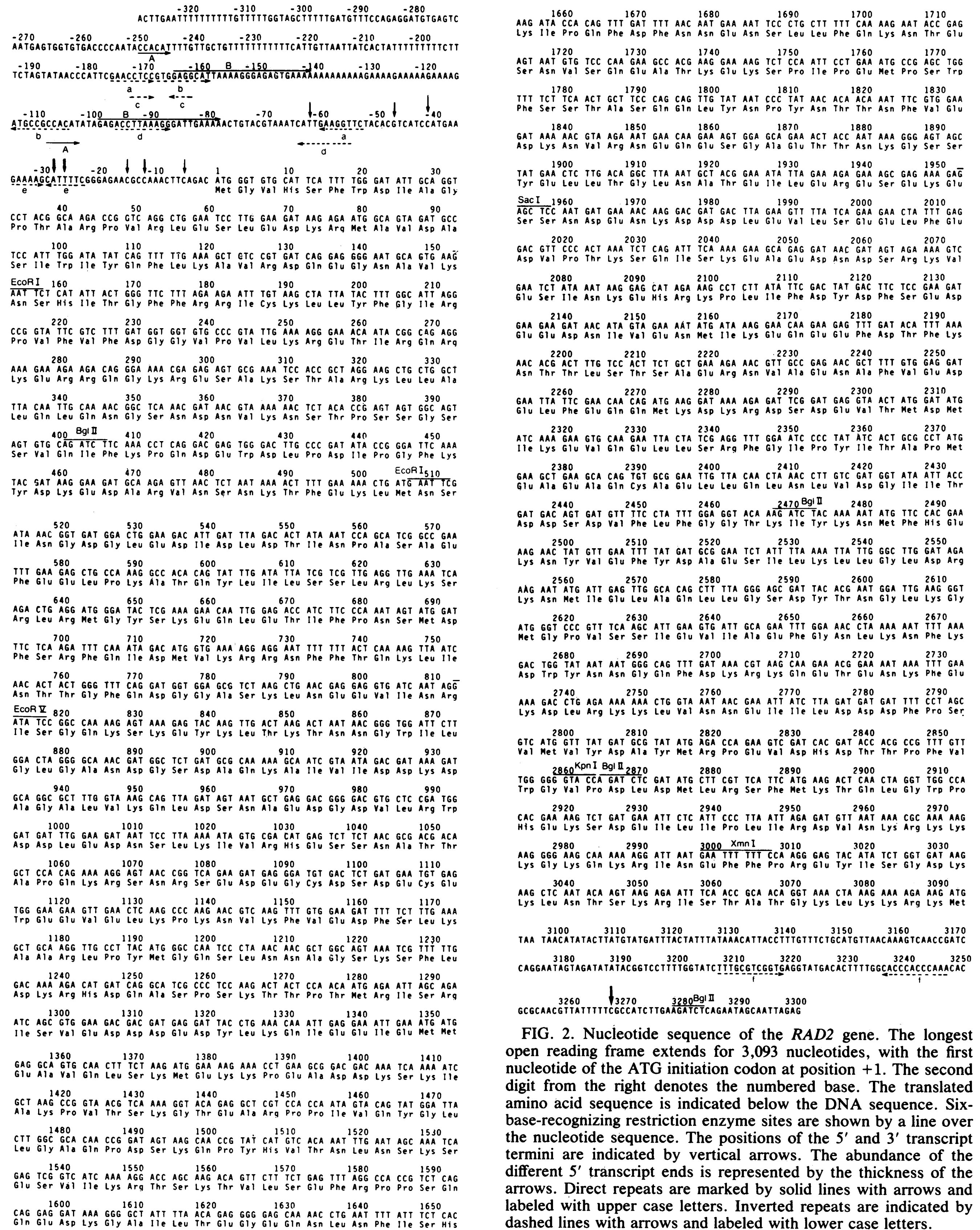

FIG. 2. Nucleotide sequence of the *RAD2* gene. The longest open reading frame extends for 3,093 nucleotides, with the first nucleotide of the ATG initiation codon at position +1. The second digit from the right denotes the numbered base. The translated amino acid sequence is indicated below the DNA sequence. Six-base-recognizing restriction enzyme sites are shown by a line over the nucleotide sequence. The positions of the 5′ and 3′ transcript termini are indicated by vertical arrows. The abundance of the different 5′ transcript ends is represented by the thickness of the arrows. Direct repeats are marked by solid lines with arrows and labeled with upper case letters. Inverted repeats are indicated by dashed lines with arrows and labeled with lower case letters.

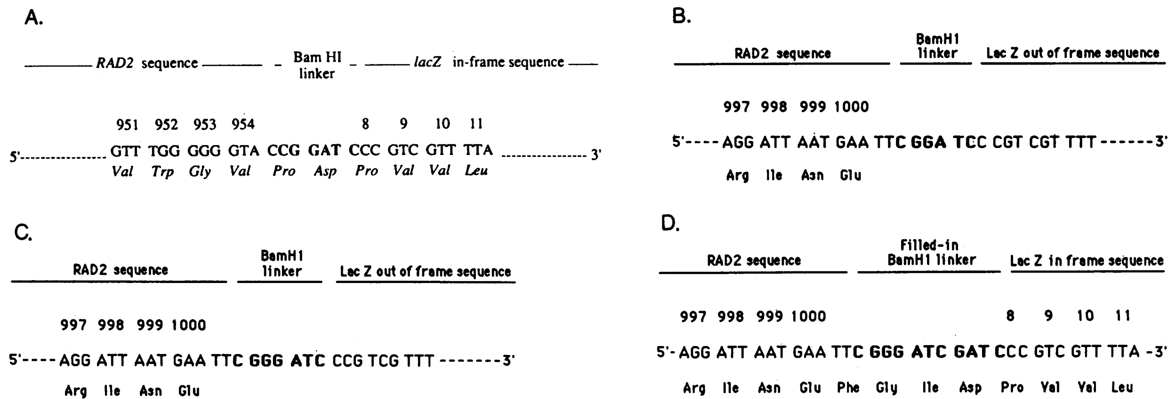

FIG. 3. In-frame and out-of-frame *RAD2-lacZ* fusions. The nucleotide sequence of the *RAD2* gene is followed by the nucleotide sequence of the *BamHI* linker, shown in bold letters, and the *lacZ* gene. The codon numbers are indicated above the *RAD2* and *lacZ* nucleotide sequences. (A) Codon 8 of the *lacZ* gene is fused in-frame with codon 954 of the *RAD2* gene in plasmid pKM12. (B and C) At position +3002, the *RAD2* gene is fused to the *BamHI* linker followed by the *lacZ* gene. An octamer or decamer *BamHI* linker was used in fusions shown in B and C, respectively, and the plasmids with these fusions are designated pKM16 and pKM17, respectively. In both cases, the *lacZ* coding region is out-of-frame with the *RAD2* coding region. (D) The fusion plasmid pKM17 (C) was restricted at the *BamHI* site, and the 3′ recessed ends were filled in as described in the text. These modified ends were ligated back together to yield plasmid pKM15. In the resulting construction, the *RAD2* coding region is in-frame with that of *lacZ*, consistent with the open reading frame shown in Fig. 2.

Sanger et al. (49) with deoxyadenosine 5′-(α[^{35}S]thio)triphosphate (6). The reactions were fractionated on 5% polyacrylamide gels (acrylamide-bis,19:1) containing 8 M urea (Schwarz/Mann, Orangeburg, N.Y.). After electrophoresis, the gels were treated with 5% acetic acid and methanol for 20 min. Gels were then transferred to Whatman filter paper and dried under vacuum. Autoradiography generally required less than 48 h with Kodak XAR-5 or XRP-1 film. The nucleotide sequence has been determined on both strands and includes overlaps and multiple analyses of various regions (Fig. 1).

S1 nuclease mapping. All the RNA procedures were with poly(A)$^+$ RNA, obtained by chromatography of Rad$^+$ total RNA on 1-ml columns of oligo(dT) cellulose, under conditions recommended by the manufacturer (Pharmacia, Inc., Piscataway, N.J.). The 1.3-kb *AvaI-EcoRI* DNA fragment for 5′ end labeling (Fig. 1) was dephosphorylated with calf intestinal phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). The 5′ end labeling was carried out as described by Maxam and Gilbert (25) with 125 μCi of [γ-^{32}P]ATP (3,000 Ci/mmol; New England Nuclear Corp., Boston, Mass.), and T4 polynucleotide kinase (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). For 3′ end labeling, the 3′ recessed ends of the 413-base pair *BglII* DNA fragment (Fig. 1) were filled with the large fragment of DNA polymerase I (Bethesda Research Laboratories) in the presence of [α-^{32}P]dATP and unlabeled deoxynucleoside triphosphates (24). Samples of 100 μg of poly(A)$^+$ RNA were used for all mapping experiments. The 5′ and 3′ *RAD2* mRNA termini were mapped with S1 nuclease by a modification (53) of the Berk and Sharp method (5). The conditions for the S1 protection experiments were as previously described (41). The protected DNA fragments were electrophoresed on DNA sequencing gels.

***RAD2* transcript analysis.** Yeast Rad$^+$ cells of strain 7799-4B were grown to a density of 1×10^7 to 3×10^7/ml in yeast extract-peptone-dextrose (YEPD) medium. The cells were pelleted by centrifugation or filtration, washed once with water, and suspended in water at a density of 10^7/ml. The cells were UV irradiated at a dose rate of 1 J/m^2 per s for 25 or 50 s with constant stirring in petri dishes (150 by 20 mm). The cells were pelleted again, resuspended in YEPD medium, and incubated at 30°C in the dark. Aliquots were withdrawn at various intervals for RNA isolation. Total RNA was isolated as described by Reed et al. (40). A 100-μg amount of total RNA was dissolved in 1 M glyoxal and electrophoresed through a 1% agarose gel in 10 mM $NaPO_4$ (pH 6.5). The RNA was transferred to a Gene Screen membrane, hybridized as described by the manufacturer (New England Nuclear), and probed with an internal *RAD2* fragment (*EcoRI-BglII*) cloned into M13mp9. The probes were nick translated to a specific activity of 2×10^8 to 3×10^8/μg of DNA with [α-^{32}P]dATP, [α-^{32}P]dCTP (3,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.), dGTP, and dTTP. We generally added 10^7 cpm of probe for each hybridization. As an internal control, *URA3* mRNA coded by the *ura3-52* gene in strain 7799-4B was also probed with the plasmid YIp5, which contains the *URA3* gene on a 1.1-kb *HindIII* fragment. Because of the greater abundance of this transcript, we added three to five times fewer counts per minute.

Construction of *RAD2-lacZ* fusions. The *RAD2* gene in plasmid pLP1 (13) was restricted at the unique *KpnI* site at position +2859 (Fig. 2) with the isoschizomer Asp718 (Boehringer Mannheim). The 3′ recessed ends were filled in with the large fragment of DNA polymerase I in the presence of deoxynucleoside triphosphates (Bethesda Research Laboratories). *BamHI* linkers d(pCGGATCCG) were ligated to the blunt ends, and a 4.5-kb *BamHI-XhoI* DNA fragment of *RAD2* was isolated and ligated to *BamHI-SalI*-treated plasmid pRR35, a modification of the *E. coli*-yeast *lacZ* vector pMC2010 (8) where *EcoRI* was replaced with *SalI*. This plasmid contains the *E. coli lacZ* gene, in addition to the yeast 2μm origin of replication, and the yeast *TRP1* gene. This construction results in an in-frame fusion of codon 954 of the *RAD2* gene with codon 8 of the *lacZ* gene (Fig. 3A).

Three different *RAD2-lacZ* fusions were made at the *XmnI* site of *RAD2* at position +3002 as follows. The 6.5-kb *XhoI-HindIII* fragment from plasmid pLP1 (13) was cloned into plasmid pUC8 to create the plasmid pKM1. A *BamHI* linker (New England BioLabs, Inc., Beverly, Mass.) was attached to *XmnI*-cleaved pKM1 DNA. After cleavage with *BamHI*, a 4.6-kb DNA fragment containing all *RAD2* sequences 5′ to the *XmnI* site at +3002 (Fig. 2), including 1.3

Phe	UUU	35 (3.4)	Ser	UCU	21 (2.0)	Tyr	UAU	14 (1.4)	Cys	UGU	4 (0.4)
Phe	UUC	13 (1.3)	Ser	UCC	13 (1.3)	Tyr	UAC	9 (0.9)	Cys	UGC	0 (0.0)
Leu	UUA	21 (2.0)	Ser	UCA	14 (1.4)	---	UAA	1 ---	---	UGA	0 ---
Leu	UUG	24 (2.3)	Ser	UCG	10 (1.0)	---	UAG	0 ---	Trp	UGA	10 (1.0)
Leu	CUU	9 (0.9)	Pro	CCU	8 (0.8)	His	CAU	6 (0.6)	Arg	CGU	4 (0.4)
Leu	CUC	6 (0.6)	Pro	CCC	9 (0.9)	His	CAC	4 (0.4)	Arg	CGC	1 (0.1)
Leu	CUA	10 (1.0)	Pro	CCA	14 (1.4)	Gln	CAA	23 (2.2)	Arg	CGA	3 (0.3)
Leu	CUG	13 (1.3)	Pro	CCG	10 (1.0)	Gln	CAG	22 (2.1)	Arg	CGG	2 (0.2)
Ile	AUU	25 (2.4)	Thr	ACU	18 (1.7)	Asn	AAU	37 (3.6)	Ser	AGU	17 (1.6)
Ile	AUC	13 (1.3)	Thr	ACC	8 (0.8)	Asn	AAC	29 (2.8)	Ser	AGC	11 (1.1)
Ile	AUA	19 (1.8)	Thr	ACA	16 (1.6)	Lys	AAA	52 (5.0)	Arg	AGA	24 (2.3)
Met	AUG	25 (2.4)	Thr	ACG	9 (0.9)	Lys	AAG	45 (4.4)	Arg	AGG	17 (1.6)
Val	GUU	12 (1.2)	Ala	GCU	16 (1.6)	Asp	GAU	61 (5.9)	Gly	GGU	14 (1.4)
Val	GUC	10 (1.0)	Ala	GCC	5 (0.5)	Asp	GAC	22 (2.1)	Gly	GGC	11 (1.1)
Val	GUA	13 (1.3)	Ala	GCA	19 (1.8)	Glu	GAA	67 (6.5)	Gly	GGA	13 (1.3)
Val	GTG	16 (1.6)	Ala	GCG	9 (0.9)	Glu	GAG	33 (3.2)	Gly	GGG	13 (1.3)

Ala	49	(4.8)	Leu	83	(8.0)
Arg	51	(4.9)	Lys	97	(9.4)
Asn	66	(6.4)	Met	25	(2.4)
Asp	83	(8.0)	Phe	48	(4.7)
Cys	4	(0.4)	Pro	41	(4.0)
Gln	45	(4.4)	Ser	86	(8.3)
Glu	100	(9.7)	Thr	51	(4.9)
Gly	51	(4.9)	Trp	10	(1.0)
His	10	(1.0)	Tyr	23	(2.2)
Ile	57	(5.5)	Val	51	(4.9)

FIG. 4. Codon usage and amino acid composition in *RAD2*. The *RAD2* open reading frame encodes a protein of 1,031 amino acids with a predicted molecular weight of 117,847. In the *RAD2* gene, 60 of the possible 61 codons are used. The percent occurrence of codons and amino acids is given in parentheses.

kb of the 5′ flanking region, was cloned into the *lacZ* vector pRR35. The authenticity of the fusion junction was determined by restriction with *Eco*RI, whose recognition sequence GAATTC is created by accurate ligation. The constructions were made with both an octamer, d(pCGGATCCG), and a decamer, d(CGGGATCCCG), *Bam*HI linker. In both constructions, the *RAD2* gene was out-of-frame with *lacZ* (Fig. 3B and C). The *RAD2* sequences were fused in frame with the *lacZ* sequences by filling in the *Bam*HI site of the decamer linker, using the large fragment of DNA polymerase I in the presence of deoxynucleoside triphosphates (Fig. 3D).

β-Galactosidase activities were measured by the method of Ruby and Szostak (46). Units of β-galactosidase specific activity were calculated as $OD_{420} \times 1{,}000/t(h) \times V(ml) \times OD_{600}$, where V is the volume of the sample assayed and OD_{600} is the optical density of the culture at 600 nm.

RESULTS

Nucleotide sequence of the *RAD2* gene. Within the *rad2*-complementing DNA segment, the nucleotide sequence shows a long open reading frame which begins with the ATG codon at position +1 and ends with the TAA codon at position +3094 (Fig. 2). This open reading frame is consistent with the size (3.3 kb) and direction of the *RAD2* transcript (13). To determine if the *RAD2* open reading frame is translated and expressed in yeast, we constructed an in-frame fusion of *RAD2* with the *E. coli lacZ* gene. Codon 8 of the *lacZ* gene was fused with the *Bam*HI-linkered *RAD2* gene at codon 954 (Fig. 3A). The yeast 2μm multicopy plasmid pKM12 containing this *RAD2-lacZ* fusion produced 300 U of β-galactosidase activity in *S. cerevisiae*, indicating that the *RAD2* open reading frame is translated. The *RAD2* open reading frame encodes a protein of 1,031 amino acids with a predicted molecular weight of 117,847. In the *RAD2* gene, 60 of the possible 61 codons are used (Fig. 4). The *RAD2* gene does not show the codon bias characteristic of the highly expressed yeast genes (4).

Recently, Nicolet et al. (34) reported a sequence of the *RAD2* gene containing an open reading frame of 2,925 bp that encodes a protein of 975 amino acids. The most significant difference between the two sequences occurs downstream of the position +2871 (Fig. 2). Following the CTC codon, located at position +2869 to +2871, the sequence we studied shows GAT ATG CTT . . . . . codons (Fig. 2 and 5); at this position, the sequence of Nicolet et al. shows the codons GGA TAT GCT . . . . . Thus, the sequence of Nicolet et al. differs from the one in this study by the presence of the additional G underlined in the GGA codon. This additional G in the former sequence accounts for the observed difference in the carboxyl terminal codons between the two sequences. The sequence in our study contains 74 additional codons beyond the position +2871, whereas the sequence of Nicolet et al. shows only 18 codons, and these differ from the codons in the sequence in our study.

Since in the *RAD2-lacZ* fusions shown (Fig. 3A), the *lacZ* gene was fused in-frame to the *RAD2* gene upstream of the position where the two sequences differ, we constructed other *lacZ* fusions at position +3002 in the *RAD2* gene to verify the sequence in our study (Fig. 3B to D). Two *RAD2-lacZ* fusions shown (Fig. 3B and C) put the *lacZ* gene out of frame with the *RAD2* gene. These fusions in the 2μm multicopy plasmids pKM16 and pKM17 did not show any β-galactosidase activity in *S. cerevisiae*. The fusion shown (Fig. 3D) connects the *RAD2* reading frame with the *lacZ* reading frame, and the 2μm multicopy plasmid pKM15 containing this fusion produced 300 U of β-galactosidase in *S. cerevisiae*. These results demonstrate that the *RAD2* open reading frame continued through position +3002 (Fig. 2).

Other differences between the sequence we studied and that of Nicolet et al. (34) lie in the 5′ and 3′ flanking regions. In the 5′ region, the former sequence between −323 to −306 is A $(T)_{10}$G T T T T T G, whereas the latter sequence in this region is A $(T)_{13}$C T T T T T T T G; these sequences differ in the underlined bases. The sequence in this study from −224 to −234 contains a run of 11 Ts rather than the run of 12 Ts

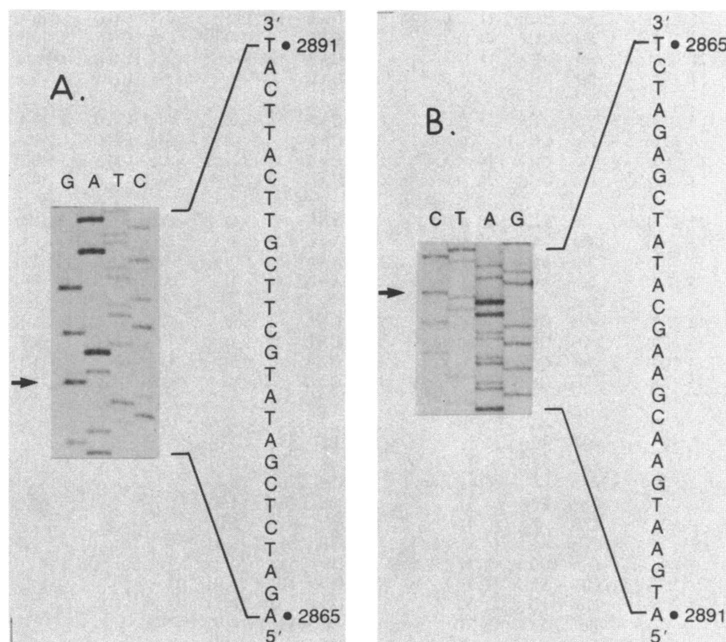

FIG. 5. Complementary nucleotide sequences of a region of the *RAD2* coding sequence extending from +2865 to +2891 in the sense (A) and the antisense (B) DNA strand. The arrow indicates the presence of a single G residue at position +2872 (A) and the single C in the complementary DNA strand (B).

which was present in the other sequence. In the 3′ region, the sequence in this study from +3176 to +3178 is AAT, whereas the other sequence is AAAT.

Mapping of the 5′ and 3′ ends of *RAD2* mRNA. For mapping the 5′ end of *RAD2* mRNA, the 1.3-kb *Eco*RI-*Ava*I DNA fragment was 5′ end labeled (Fig. 1) and hybridized to poly(A)$^+$ RNA from an unirradiated Rad$^+$ yeast strain, and the size of S1-nuclease-protected DNA fragments was determined on DNA sequencing gels. We observed three major clusters of S1-protected DNA fragments of 170-173, 184-186, and 199-205 nucleotides and relatively minor protected DNA fragments of 163 and 220 nucleotides (Fig. 6A). The 5′ mRNA ends correspond to nucleotides at positions −5, −12, −15, −26, −28, −41, −47, and −62 (Fig. 2).

For mapping the 3′ end of *RAD2* mRNA, the 3′-end-labeled 413-bp *Bgl*II DNA fragment (Fig. 1) located at position +2865 to +3278 (Fig. 2) was hybridized to poly(A)$^+$ RNA from a Rad$^+$ yeast strain, and the size of the S1-nuclease-protected DNA fragment was determined on DNA sequencing gels. A single protected DNA fragment of 402 nucleotides was observed (Fig. 6B), indicating that the 3′ mRNA end of *RAD2* is located at position +3267 (Fig. 2).

5′ and 3′ flanking sequences of the *RAD2* gene. The *RAD2* 5′ and 3′ flanking regions contain 65.5% and 62.2% A + T, respectively, and the coding region contains 59.9% A + T. In the 5′ flanking region, several long tracts of Ts are located upstream of the position −190; three of these runs of Ts are 9, 10, and 11 nucleotides long. The most striking feature in the 5′ flanking region is the existence of a 44-nucleotide-long purine-rich tract located from position −157 to −114, which contains 33 As, 10 Gs, and a single T residue. This purine tract is flanked at both ends by a 10-nucleotide inverted repeat (designated b in Fig. 2). The 5′ flanking region also contains several direct and inverted repeats (Fig. 2). The 26-nucleotide sequence from −165 to −140 (designated B in Fig. 2), when aligned with the 23-nucleotide sequence from −99 to −77, shows 20 identical nucleotides.

The T A T A A/T A A/T sequence usually found 26 to 34 bp upstream of the mRNA start site in higher eucaryotes and proposed to be required for proper transcription initiation (7, 11) is not observed in the *RAD2* 5′ region. However, a T A T A sequence is present at position −103 to −100, and a T A T A A sequence occurs at position −188 to −184 (Fig. 2). Eucaryotic mRNAs usually contain an A at position −3 and a G at position +4, relative to the translation initiation codon (18, 19). The *RAD2* gene contains a G at positions −3 and +4 (Fig. 2).

The sequence A A T A A A, found about 20 nucleotides upstream of the 3′ end of mRNAs of higher eucaryotes (38), or the sequences T A A A T A A A/G (3), T T T T T A T A (12), or T A G. . .T A G T or T A T G T . . . .(A+T rich) . . . . T T T . . . . (57), found in the 3′ ends of various *S. cerevisiae* genes, and postulated to be required for transcription termination, are absent from the *RAD2* 3′ flanking region. However, a 12-nucleotide inverted repeat (designated f in Fig. 2) occurs 20 nucleotides upstream of the 3′ mRNA terminus at +3267. In this inverted repeat, 10 of the 12 nucleotides could potentially form base pairs and form a stem and loop configuration in the mRNA or the DNA strand, which might affect the termination or the stability of mRNA, or both.

Increase in *RAD2* levels after UV irradiation. *RAD2* mRNA levels were examined in the Rad$^+$ yeast strain 7799-4B after UV radiation (25 and 50 J/m^2). The UV dose of 25 J/m^2 did not affect survival or growth of cells appreciably; 75% of cells were still viable after this treatment. However, after 50 J/m^2, only 10% of cells were viable. At various times before and after UV irradiation, RNA samples were prepared from yeast cells. Equal amounts of total RNA samples were electrophoresed through agarose gels, blotted onto a Gene Screen membrane, and probed with the radiolabeled 1.96-kb *Eco*RI-*Bgl*II *RAD2* internal DNA fragment, located at position +507 to +2468 (Fig. 2), and with the 1.1-kb *Hin*dIII DNA fragment of the *URA3* gene. Since the level of *URA3*

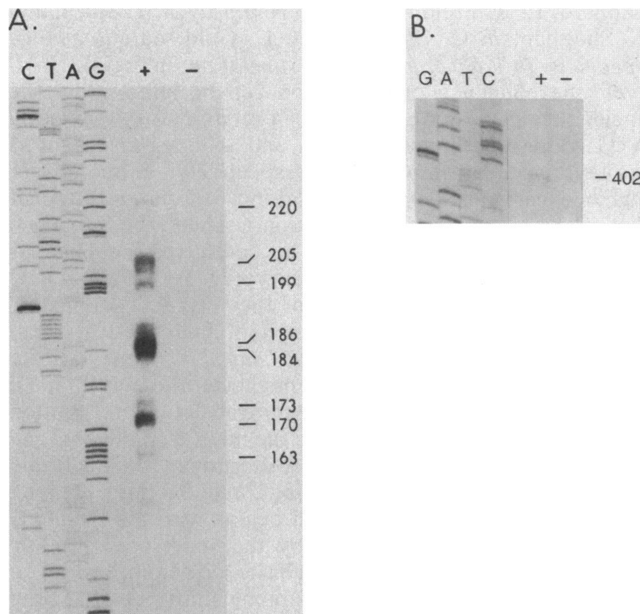

FIG. 6. S1 nuclease mapping of 5′ and 3′ termini of *RAD2* mRNA. The presence (+) or absence (−) of 100 μg of poly(A)$^+$ RNA from Rad$^+$ cells in the hybridization reaction is indicated. (A) The 5′ S1-protected fragments originate from the 1.3-kb *Ava*I-*Eco*RI DNA fragment, which was ^{32}P-end-labeled at the *Eco*RI site (Fig. 1). The sequence ladder shown on the left is of the *RAD2 Bgl*II fragment from position +2865 to +3278 (Fig. 2). The sizes of the protected fragments were the same when determined against the sequence ladder of the 1.3-kb *Ava*I-*Eco*RI fragment used in these mapping experiments. (B) 3′ S1-protected fragment. The protected 402-nucleotide fragment originates from the 413-bp *Bgl*II DNA fragment (Fig. 1) whose 3′ ends were labeled with [^{32}P]dATP and [^{32}P]dGTP. The sequence ladder is from the *RAD2 Bgl*II fragment from position +2865 to +3278 used in the mapping experiment. Faint bands in sequence lanes are sequencing artifacts.

mRNA is not affected by UV irradiation, *URA3* mRNA was used as an internal control for equality of RNA amounts loaded in each lane.

The *RAD2* probe hybridizes to a transcript of about 3.3 kb (13). The *RAD2* transcript levels increased significantly at 30 and 60 min after a 25-J/m^2 exposure of UV light (Fig. 7A). The *RAD2* mRNA levels as quantitated by densitometric scanning were 1.0, 9.0, 6.5, 3.0, 2.6, 2.8, 2.6, and 2.5 at 0, 30, 60, 90, 120, 150, 180, and 240 min after UV irradiation, respectively, relative to *RAD2* mRNA amounts in the unirradiated control culture. *URA3* mRNA levels remained constant in all of these lanes. Four different experiments were performed at 25 J/m^2 and gave similar results. Control experiments in which cells were treated similarly but not exposed to UV light showed no change in *RAD2* mRNA amounts. Thus, exposure of cells to 25 J/m^2 elicited a ninefold increase in the intracellular levels of *RAD2* mRNA. Treatment of cells with 50 J/m^2 showed a fivefold increase in *RAD2* transcripts (Fig. 7B). After 50 J/m^2, the levels of *RAD2* transcripts relative to the unirradiated control were 1.0, 2.0, 4.4, 5.6, 3.2, 2.3, 2.3, and 1.5 at 0, 20, 40, 60, 80, 120, 180, and 240 min, respectively, whereas upon normalization of *RAD2* mRNA with the *URA3* mRNA, the corresponding *RAD2* mRNA levels were 1.0, 1.5, 3.2, 4.7, 2.9, 2.0, 2.0, and 1.7.

DISCUSSION

RAD2 protein. The S1 nuclease mapping results indicate several 5′ mRNA termini located at positions −5, −12, −15, −26, −28, −41, −47, and −62; the most abundant cluster of 5′ mRNA termini occurs at position −26 and −28. Transcripts starting at position −28 or downstream from it contain a single open reading frame which initiates from ATG at +1 and terminates with the TAA codon at +3094. This open reading frame encodes a protein of 1,031 amino acids with a calculated molecular weight of 117,847, and contains 17.8% acidic, 15.3% basic, 31.6% hydrophilic, and 35.3% hydrophobic residues. The RAD2 protein does not show any significant sequence homology with the *E. coli* UVRA (A. Sancar, personal communication), UVRB (C. Backendorf, personal communication), UVRC (48), or UVRD (10) proteins, or with the *S. cerevisiae* RAD1 (P. Reynolds, L. Prakash, and S. Prakash, unpublished observations), RAD3 (31, 41), RAD6 (43), RAD7 (35), or RAD10 (42) proteins. A protein homology search in the National Biomedical Research Foundation Library data bank was carried out by the method of Lipman and Pearson (22) and showed no significant similarities.

The distribution of the acidic and basic amino acids is nonuniform along the length of the RAD2 protein. The amino acids 361 to 376 contain 10 acidic and no basic residues, and the 6 residues from amino acids 435 to 440 are acidic. The 205 amino acids from residues 611 to 815 are also highly acidic containing 59 acidic and 22 basic residues. Both the amino and carboxyl terminal regions of the RAD2 protein are basic. In the 100 amino acids at the amino terminus, 22 basic and 11 acidic residues are present, while the last 44 amino acids at the carboxyl terminus contain 18 basic and 3 acidic residues. These basic regions of the RAD2 protein

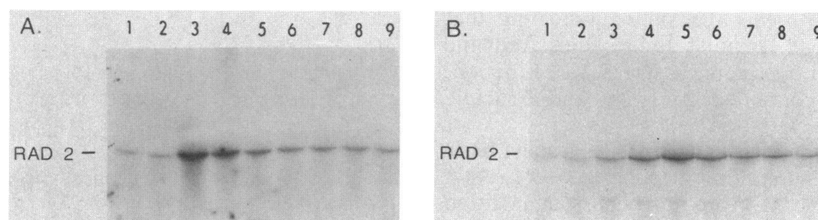

FIG. 7. Northern analysis showing elevated levels of *RAD2* transcripts after UV irradiation. Northern blots of RNA from UV irradiated and unirradiated control cells were hybridized with the nick translated *RAD2* probe. (A) RNA levels after 25 J/m^2. Lane 1, RNA (100 μg) from control cells; lanes 2 through 9, RNAs (100 μg per lane) prepared at 0, 30, 60, 90, 120, 150, 180, and 240 min, respectively, after UV irradiation. (B) RNA levels after 50 J/m^2. Lane 1, RNA (100 μg) from control cells; lanes 2 through 9, RNAs (100 μg per lane) prepared at 0, 20, 40, 60, 80, 120, 180, and 240 min, respectively, after UV irradiation.

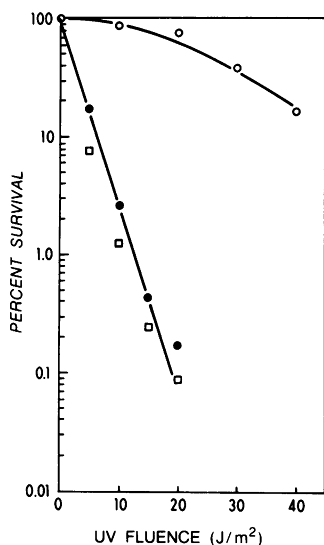

FIG. 8. Survival after UV irradiation of a yeast *rad2-2* mutant strain, LP2804-5B, *MAT***a** *his1 his3-Δ1 leu2-3 leu2-112 trp1-289 ura3-52 rad2-2*, carrying the multicopy plasmid pLP1 or pLP7 (13). Plasmid pLP1 contains the entire functional *RAD2* gene. In plasmid pLP7, the *Kpn*I site of the *RAD2* gene in plasmid pLP1 is disrupted as described in the text. Plasmid containing strains were grown in selective medium lacking uracil. Symbols: ○, LP2804-5B + pLP1; ●, LP2804-5B + pLP7; □, LP2804-5B + YEp24.

could be involved in binding to DNA or to acidic portions of the other proteins in the incision complex or both.

Loss of *RAD2* function in a carboxyl terminal deletion. A disruption of the *RAD2* gene at the *Kpn*I site present at position +2859 to +2864 was made by removing the 3′ extensions resulting from *Kpn*I digestion by using the 3′-5′ exonuclease activity of T4 DNA polymerase. Ligation of blunt ends thus formed resulted in the loss of the four nucleotides G T A C from position +2860 to +2863. After the GGG codon at position +2857 to +2859, the nucleotide sequence of the *Kpn*I disrupted *RAD2* gene is:

CAG ATC TCG ATA TGC TTC GTT CAT TCA TGA
Gln Ile Ser Ile Cys Phe Val His Ser Stop

Thus, the *RAD2* gene disrupted at the *Kpn*I site contained only nine codons beyond the GGG codon, instead of the 78 codons present in the wild-type *RAD2* gene. We had previously shown that the multicopy plasmid pLP7, which contains the entire *RAD2* gene but has the *Kpn*I site disrupted as described above, does not complement the *rad2* mutation as determined by spot tests (13). UV survival curves (Fig. 8) showed that the UV sensitivity of the *rad2-2* mutant with or without the plasmid pLP7 was the same, indicating that deletion of the 78 carboxyl terminal amino acids from the RAD2 protein resulted in complete loss of RAD2 function. These 78 amino acids contained 23 basic and 9 acidic residues.

Overlapping open reading frame that encodes a short basic peptide. In the transcripts initiating at positions −41, −47, and −62, the 5′ proximal AUG codon occurs at position −40. In 95% of higher eucaryotic mRNAs, translation begins at the first AUG codon (18, 19). Extensive studies with the *CYC1* gene of yeast also suggest that protein synthesis begins at the 5′ proximal AUG codon in the mRNA (S. B. Baim, C. T. Goodhue, D. F. Pietras, D. C. Eustice, M. Labhard, L. R. Freidman, D. M. Hampsey, J. I. Stiles, and F. Sherman, *in* C. Calendar and L. Gold, ed., *Sequence Specificity in Transcription and Translation*, in press). However, the efficiency of translation can be affected by the nucleotides at positions −3 and +4 relative to the initiating ATG codon. The nucleotides A and to a lesser extent G are favored at these positions, respectively, in mRNAs of higher eucaryotes (18, 19); furthermore, C is preferred at the −1, −2, −4, and −5 positions. Thus, the sequence C C A/G CC A T G(G) appears to be a preferred sequence for translation initiation in higher eucaryotes (19). The C A T C C A T G A sequence context of the −40 ATG of *RAD2* differs from the consensus version of higher eucaryotes. However, studies with the *S. cerevisiae CYC1* gene indicate that while translation efficiency might be affected in small measure by the sequence context, translation most often initiates at the first AUG codon in the mRNA (Baim et al., in press). The ATG at −40 initiates a short open reading frame of 37 codons, and this open reading frame overlaps the long *RAD2* open reading frame which begins with the +1 ATG (Fig. 9). The peptide encoded by the short open reading frame is unusual; it contains eight basic, no acidic, and seven glycine residues. Since the overlapping short peptide frame terminates at the TAA codon at position +72, translation of the RAD2 protein might reinitiate from the ATG codon at position +79 (Fig. 2) (23), encoding a RAD2 protein missing the 26-amino terminal residues.

The *URA3* gene of *S. cerevisiae* also contains a short overlapping open reading frame and multiple transcription initiation sites as described above for the *RAD2* gene. In the *URA3* gene, the short open reading frame precedes and overlaps the *URA3* coding sequence and encodes a peptide

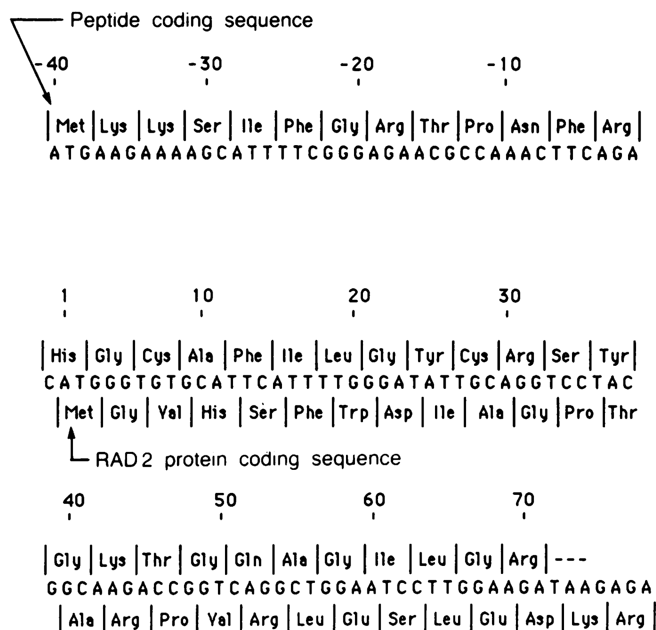

FIG. 9. Amino acid sequence encoded by a 37-codon open reading frame which overlaps the 5′ end of the *RAD2* open reading frame. The upstream ATG at −40 is out of frame with the +1 ATG initiating the long *RAD2* open reading frame. The short reading frame extends into the long *RAD2* open reading frame and terminates at a TAA termination codon at +72. The short peptide sequence is above the DNA sequence and the long RAD2 protein sequence is below it.

RAD2	-167	T G G A G G C A T T A A
RAD7	-122	T G G A A G C A A A A A
RAD10	-213	C G G T G G C A A C A A

FIG. 10. Comparison of the DNA sequences in the 5′ flanking regions of the *RAD2*, *RAD7*, and *RAD10* genes of *S. cerevisiae*. The position of the sequences is relative to the first ATG codon at position +1 in the open reading frame. The common base pairs in all three sequences are boxed, while the sequences shared between any two genes are underlined.

of 28 amino acids. Rose and Botstein (45) have shown that fusions of the *E. coli* β-galactosidase gene in the overlapping peptide frame are productive, indicating that this reading frame is transcribed and translated. The 5′ ends of minor *URA3* mRNAs map upstream of the overlapping peptide frame, whereas the 5′ ends of the major *URA3* transcripts lie upstream of the *URA3* coding sequence, and these could encode only the URA3 protein. Interestingly, the minor and the major *URA3* transcripts are regulated differentially (45). We are now determining whether the RAD2 overlapping peptide frame is expressed in *S. cerevisiae*, and if it has any regulatory or functional significance.

The sequences 5′ and 3′ to the *RAD2* coding region. The *RAD2* 5′ mRNA ends are highly heterogeneous, mapping over an approximately 60-bp region. The transcripts of the *CYC1* and *HIS1* genes of *S. cerevisiae* also display similar 5′ end heterogeneity (9, 16). The *CYC1* and *HIS1* genes contain several T A T A A/T A A/T-like sequences, and each of these can be associated with a separate 5′ mRNA end. The *RAD2* gene contains the sequence T A T A at position −103 to −100 and T A T A A at position −188 to −184; however, it has been shown that the T A T A and the T A T A A sequences do not promote transcription initiation in the *HIS4* gene of *S. cerevisiae* (29). Probably the most interesting feature in the 5′ flanking region of the *RAD2* gene is the purine tract beginning at position −157, and containing 33 As, 10 Gs, and only 1 T residue. Studies with poly(dA) · poly(dT) sequence segments in recombinant DNA molecules indicate that nucleosomes cannot form over an 80-bp poly(dA) · poly(dT) segment, and that poly(dA) · poly(dT) segments as small as 20 bp are disfavored during nucleosome formation (21, 39). Promoter-up constitutive mutants of the *ADR2* gene of *S. cerevisiae*, which codes for the glucose-repressed ADHII enzyme, have an addition of 33 to 34 adenines to a block of 20 consecutive adenine nucleotides which occurs 222 bp upstream of the gene (47). The purine tract in the *RAD2* gene could promote the entry of RNA polymerase, thereby affecting the expression of the gene. We are examining the role of this sequence in initiation of transcription and on mRNA levels of *RAD2*.

The *RAD2* 3′ flanking region does not contain any of the sequences that have been associated with transcription termination in yeasts or in higher eucaryotes. However, a 12-bp inverted repeat occurs 20 nucleotides upstream of the 3′ end of *RAD2* mRNA. It would be of interest to determine if this inverted repeat functions in transcription termination.

Regulation of the *RAD2* gene. We show that steady-state *RAD2* transcript levels were elevated ninefold after UV radiation of 25 J/m^2 and about fivefold after 50 J/m^2. This increase occurred within 30 min at 25 J/m^2 and within 1 h at 50 J/m^2, after which mRNA levels began to decline. Using *RAD2-lacZ* fusions, Robinson et al. (44a) have found an increase in β-galactosidase levels after DNA-damaging treatments, suggesting that the enhanced *RAD2* transcript levels result in higher amounts of RAD2 protein. In *E. coli*, various DNA repair genes, including the excision genes *uvrA*, *uvrB*, *uvrC*, and *uvrD*, are coordinately induced in a *recA-lexA*-dependent manner after treatments with DNA damaging agents (52). The *uvrA*, *uvrB*, *uvrC*, and *uvrD* genes show about a fivefold induction similar to that observed for the *RAD2* gene (17, 20, 50, 51). However, the *lacZ* fusions of the *RAD1* and *RAD3* genes of *S. cerevisiae* do not show any evidence of increased β-galactosidase levels after DNA damaging treatments (30, 31, 44a).

Several DNA-damage-inducible genes have been identified in *S. cerevisiae*, and these include the DNA ligase gene *CDC9* (36), and several genes of unknown function: *din* genes (46), *ddr* genes (26), and the *RecAsc* gene (1). It is not yet clear if DNA damage in yeasts and higher eucaryotes induces a set of coordinately regulated genes as DNA damage does in *E. coli*.

A comparison of 5′ flanking sequences of *RAD2* with *RAD1* (P. Reynolds, L. Prakash, and S. Prakash, unpublished observations), *RAD3* (31, 41), *RAD6* (43), *RAD7* (35), *RAD10* (42), and the *CDC9* genes (2) reveals a short common sequence in the *RAD2*, *RAD7*, and *RAD10* genes (Fig. 10). G. Perozzi in our laboratory has observed higher levels of *RAD7* mRNA after UV irradiation than in unirradiated cells (G. Perozzi, Ph.D. thesis, University of Rochester, Rochester, N.Y., 1985). However, we do not have any information on the regulation of the *RAD10* gene. For defining the promoter and regulatory elements in the *RAD2* gene, we are examining transcription and regulation in various deletions of its 5′ flanking sequence, including deletions of the sequence mentioned above (Fig. 10).

ACKNOWLEDGMENTS

We are grateful to Paul Reynolds for help and advice during various phases of this work, Louise Prakash for her interest and support, and Alan Morrison for a critical reading of the manuscript. We thank Aziz Sancar, Claude Backendorf, David G. Barker, and Lee H. Johnston for providing DNA sequences before publication and Errol Friedberg for sending the paper (44a) before publication.

This work was supported by grants CA-35035 from the National Cancer Institute and DE-FG02-85ER60281 from the U.S. Department of Energy.

LITERATURE CITED

1. **Angulo, J. F., J. Schwenke, P. L. Moreau, E. Moustacchi, and R. Devoret.** 1985. A yeast protein analogous to *Escherichia coli* RecA protein whose cellular level is enhanced after UV irradiation. Mol. Gen. Genet. **201**:20–24.
2. **Barker, D. G., J. H. M. White, and L. H. Johnston.** 1985. The nucleotide sequence of the DNA ligase gene (CDC9) from *Saccharomyces cerevisiae*: a gene which is cell cycle regulated and induced in response to DNA damage. Nucleic Acids Res. **13**:8323–8337.
3. **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.
4. **Bennetzen, J. L., and B. D. Hall.** 1982. Codon selection in yeast. J. Biol. Chem. **257**:3026–3031.
5. **Berk, A. J., and P. A. Sharp.** 1978. Spliced early mRNAs of simian virus 40. Proc. Natl. Acad. Sci. USA **75**:1274–1278.
6. **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.
7. **Breathnach, R., and P. Chambon.** 1981. Organization and expression of eucaryotic split genes coding for proteins. Annu. Rev. Biochem. **50**:349–383.

8. **Casadaban, M. J., A. Martinez-Arias, S. K. Shapira, and J. Chou.** 1983. β-galactosidae gene fusions for analyzing gene expression in *Escherichia coli* and yeast. Methods Enzymol. **100**:293–308.
9. **Faye, G., D. W. Leung, K. Tatchell, B. D. Hall, and M. Smith.** 1981. Deletion mapping of sequences essential for *in vivo* transcription of the iso-1-cytochrome *c* gene. Proc. Natl. Acad. Sci. USA. **78**:2258–2262.
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. **Henikoff, S., J. D. Kelly, and E. H. Cohen.** 1983. Transcription terminates in yeast distal to a control sequence. Cell **33**:607–614.
13. **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.
14. **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.
15. **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.
16. **Hinnebusch, A. G., and G. R. Fink.** 1983. Repeated DNA sequences upstream from *HIS1* also occur at several other co-regulated genes in *Saccharomyces cerevisiae*. J. Biol. Chem. **258**:5238–5247.
17. **Kenyon, C. J., and G. C. Walker.** 1981. Expression of the *E. coli uvrA* gene is inducible. Nature (London) **289**:808–810.
18. **Kozak, M.** 1983. Comparison of initiation of protein synthesis in procaryotes, eucaryotes, and organelles. Microbiol. Rev. **47**: 1–45.
19. **Kozak, M.** 1984. Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNA. Nucleic Acids Res. **12**:857–872.
20. **Kumura, K., and M. Sekiguchi.** 1984. Identification of the *uvrD* gene product of *Escherichia coli* as DNA helicase II and its induction by DNA-damaging agents. J. Biol. Chem. **259**: 1560–1565.
21. **Kunkel, G. R., and H. G. Martinson.** 1981. Nucleosomes will not form on double-stranded RNA or over poly (dA) · poly (dT) tracts in recombinant DNA. Nucleic Acids Res. **9**:6869–6888.
22. **Lipman, D. J., and W. R. Pearson.** 1985. Rapid and sensitive protein similarity searches. Science **227**:1435–1441.
23. **Liu, C.-C., C. C. Simonsen, and A. D. Levinson.** 1984. Initiation of translation at internal AUG codons in mammalian cells. Nature (London) **309**:82–85.
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. **Maxam, A. M., and W. Gilbert.** 1977. A new method for sequencing DNA. Proc. Natl. Acad. Sci. USA **74**:560–564.
26. **McClanahan, T., and K. McEntee.** 1984. Specific transcripts are elevated in *Saccharomyces cerevisiae* in response to DNA damage. Mol. Cell. Biol. **4**:2356–2363.
27. **Miller, R., L. Prakash, and S. Prakash.** 1982. Genetic control of excision of *Saccharomyces cerevisiae* interstrand DNA crosslinks induced by psoralen plus near-UV light. Mol. Cell. Biol. **2**:939–948.
28. **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.
29. **Nagawa, F., and G. R. Fink.** 1985. The relationship between the "TATA" sequence and transcription initiation sites at the *HIS4* gene of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **82**:8557–8561.
30. **Nagpal, M. L., D. R. Higgins, and S. Prakash.** 1985. Expression of the *RAD1* and *RAD3* genes of *Saccharomyces cerevisiae* is not affected by DNA damage or during the cell division cycle. Mol. Gen. Genet. **199**:59–63.
31. **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.
32. **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.
33. **Naumovski, L., and E. C. Friedberg.** 1984. *Saccharomyces cerevisiae RAD2* gene; isolation, subcloning, and partial characterization. Mol. Cell. Biol. **4**:290–295.
34. **Nicolet, C. M., J. M. Chenevert, and E. C. Freidberg.** 1985. The *RAD2* gene of *Saccharomyces cerevisiae*: nucleotide sequence and transcript mapping. Gene **36**:225–234.
35. **Perozzi, G., and S. Prakash.** 1986. *RAD7* gene of *Saccharomyces cerevisiae*: transcripts, nucleotide sequence analysis, and functional relationship between the RAD7 and RAD23 gene products. Mol. Cell. Biol. **6**:1497–1507.
36. **Peterson, T. A., L. Prakash, S. Prakash, M. A. Osley, and S. I. Reed.** 1985. Regulation of *CDC9*, the *Saccharomyces cerevisiae* gene that encodes DNA ligase. Mol. Cell. Biol. **5**:226–235.
37. **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.
38. **Proudfoot, N. J., and G. G. Brownlee.** 1976. 3' non-coding region sequences in eukaryotic messenger RNA. Nature (London) **263**:211–214.
39. **Prunell, A.** 1982. Nucleosome reconstitution on plasmid-inserted poly (dA) · poly(dT). EMBO J. **1**:173–179.
40. **Reed, S. I., J. Ferguson, and J. C. Groppe.** 1982. Preliminary characterization of the transcriptional and translational products of the *Saccharomyces cerevisiae* cell division cycle gene *CDC28*. Mol. Cell. Biol. **2**:412–425.
41. **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.
42. **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.
43. **Reynolds, P., S. Weber, and L. Prakash.** 1985. *RAD6* gene of *Saccharomyces cerevisiae* encodes a protein containing a tract of 13 consecutive aspartates. Proc. Natl. Acad. Sci. USA **82**: 168–172.
44. **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.

44a. **Robinson, G. W., C. M. Nicolet, D. Kalainov, and E. C. Friedberg.** A yeast excision-repair gene is inducible by DNA damaging agents. Proc. Natl. Acad. Sci. USA **83**:1842–1846.

45. **Rose, M., and D. Botstein.** 1983. Structure and function of the yeast *URA3* gene. Differentially regulated expression of hybrid β-galactosidase from overlapping coding sequences in yeast. J. Mol. Biol. **170**:883–904.
46. **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.
47. **Russell, D. W., M. Smith, D. Cox, V. M. Williamson, and E. T. Young.** 1983. DNA sequences of two yeast promoter-up mutants. Nature (London) **304**:652–654.
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. **Schendel, P. F., M. Fogliano, and L. D. Strausbaugh.** 1982.

Regulation of the *Escherichia coli* K-12 *uvrB* operon. J. Bacteriol. **150**:676–685.

51. **van Sluis, C. A., G. F. Moolenaar, and C. Backendorf.** 1983. Regulation of the *uvrC* gene of *Escherichia coli* K12: localization and characterization of a damage-inducible promoter. EMBO J. **2**:2313–2318.
52. **Walker, G. C.** 1985. Inducible DNA repair systems. Annu. Rev. of Biochem. **54**:425–457.
53. **Weaver, R. F., and C. Weissman.** 1979. Mapping of RNA by a modification of the Berk-Sharp procedure: the 5′ termini of 15 S β-globin mRNA precursor and mature 10 S β-globin mRNA have identical map coordinates. Nucleic Acids Res. **7**:1175–1193.
54. **Weiss, W. A., and E. C. Friedberg.** 1985. Molecular cloning and characterization of the yeast *RAD10* gene and expression of the RAD10 protein in *E. coli*. EMBO J. **4**:1575–1582.
55. **Wilcox, D. R., and L. Prakash.** 1981. Incision and postincision steps of pyrimidine dimer removal in excision-defective mutants of *Saccharomyces cerevisiae*. J. Bacteriol. **148**:618–623.
56. **Yang, E., and E. C. Friedberg.** 1984. Molecular cloning and nucleotide sequence analysis of the *Saccharomyces cerevisiae RAD1* gene. Mol. Cell. Biol. **4**:2161–2169.
57. **Zaret, K. S., and F. Sherman.** 1982. DNA sequence required for efficient transcription termination in yeast. Cell **28**:563–573.