JOURNAL OF BACTERIOLOGY, Jan. 1989, p. 230–237
0021-9193/89/010230-08$02.00/0

Vol. 171, No. 1

The *REV1* Gene of *Saccharomyces cerevisiae*: Isolation, Sequence, and Functional Analysis

FRANK W. LARIMER,[1]* JACK R. PERRY,[2]† AND ALICE A. HARDIGREE[1]

Biology Division, Oak Ridge National Laboratory,[1] and The University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences,[2] Oak Ridge, Tennessee 37831

Received 29 March 1988/Accepted 27 September 1988

The *REV1* gene of *Saccharomyces cerevisiae* is required for normal induction of mutations by physical and chemical agents. We have determined the sequence of a 3,485-base-pair segment of DNA that complements the *rev1-1* mutant. Gene disruption was used to confirm that this DNA contained the *REV1* gene. The sequenced segment contains a single long open reading frame, which can encode a polypeptide of 985 amino acid residues. The *REV1* transcript is 3.1 kilobase pairs in length. Frameshift mutations introduced into the open reading frame yielded a Rev$^-$ phenotype. A base substitution, encoding Gly-193 to Arg-193, was found in this open reading frame in *rev1-1*. Deletion mutants, lacking segments of the 5′ region of *REV1*, had intermediate mutability relative to *REV1* and *rev1-1*; a complete deletion exhibited lower mutability than *rev1-1*. *REV1* is not an essential gene. An in-frame fusion of the 5′ end of the *REV1* open reading frame to the *lacZ* gene produced β-galactosidase activity constitutively. The predicted REV1 protein is hydrophilic, with a predicted pI of 9.82. No homologies to RAD1, RAD2, RAD3, RAD7, or RAD10 proteins were noted. A 152-residue internal segment displayed 25% identity with UMUC protein.

The *Saccharomyces cerevisiae REV1* gene product functions in a cellular process required for mutagenesis caused by UV radiation and many chemical mutagens (14, 20, 25). The *REV1* gene is one of a small group of yeast genes, including *REV3*, *REV7*, *CDC7*, and *NGM2*, whose mutant alleles have a severe and general effect on mutability (21–24, 34, 35). Mutants of *REV1* exhibit little or no mutability either by UV light or by many chemicals and are only slightly sensitive to the lethal effects of these agents (23, 24).

The *REV1* gene product is thought to constitute part of a DNA repair process that is "error prone" or "mutagenic" in its function (14, 20, 25). Just as SOS processing by *umuDC* is distinguished from other Rec functions and from *uvrABC*-controlled excision repair in *Escherichia coli* (56), mutagenic repair is distinct from the other major repair pathways in yeasts (14, 20, 25). Excision repair is controlled by genes of the *RAD3* epistasis group, and recombinational strand break repair is controlled by genes of the *RAD52* epistasis group. The *REV1* gene is part of the *RAD6* epistasis group; the *RAD6* gene product has recently been identified as a ubiquitin transferase that may be required to process histones on chromatin to initiate DNA repair (18).

As an initial step toward understanding the regulation and function of mutagenic repair, we sought to clone and characterize the *REV1* gene. We hope to use such a clone to overproduce the *REV1* gene product to facilitate purification, characterization, and in vitro reconstruction of the mutagenic repair complex. In this regard, the cloning of the *REV3* gene has been reported (J. F. Lemontt, S. V. Lair, A. K. Beck, and E. G. Bernsteine, abstr., p. 33, 11th Int. Conf. Yeast Genet. Mol. Biol., Montpellier, France, 1982), a fragment which complements *rev2* has been identified (53), and *CDC7*, which potentiates mutability when present at high copy number (51), has been shown to encode a protein homologous to protein kinases (38).

In this paper we describe the isolation of the *REV1* gene and the mutant allele *rev1-1* and report their nucleotide and deduced amino acid sequences. Deletion analysis was used to determine the functional limits of the *REV1* coding sequence and its 5′- and 3′-flanking sequences. A preliminary characterization of the response of *REV1*::*lacZ* fusions to heat shock and DNA-damaging agents is presented. Similarity analysis suggests that a segment of the REV1 protein is homologous to UMUC protein.

MATERIALS AND METHODS

Media. LB medium (29) (with antibiotic supplements as appropriate) was used to propagate *E. coli* strains and plasmid-bearing derivatives. YEPD and synthetic complete (SC) yeast media have been described before (24). SC-URA is SC without uracil, SC-LYS is SC without lysine, and SC-LEU is SC without leucine.

Yeast strains. *S. cerevisiae* S288C α *gal2 mel mal SUC2 CUP1* was used as the source of wild-type DNA for library construction. Strains XL68-3D α *rev1-1 lys1-1 leu2-3 his4-38 trp1-289 ura3-52*, XL69-5A α *REV1 lys1-1 leu2-3 trp1-289 his3Δ1*, and XL70-1B α *REV1 lys1-1 leu2-3 trp1-289 ura3-52* were constructed in this laboratory, using standard techniques for mating, sporulation, and tetrad analysis (52).

Bacterial strains. *E. coli* BNN45 (F$^-$ *metB thi hsdR lacY supE44 supF*), derived from ED8654 (31), was used routinely as a cloning host. *E. coli* DH1 *recA1* (F$^-$ Δ*lac-pro*, *endA1 gyrA96 thi-1 hsdR17 supE44 relA1*) (13) was used as a cosmid host. *E. coli* JM107 (Δ*lac-pro endA1 gyrA96 thi-1 hsdR17 supE44 relA1* [F′ *traD36 proAB*$^+$ *lacI*^{q}*ZΔM15*]) (57) was used as the host for M13 vectors. *E. coli* GM215 (F$^-$ *dam-3 endA1 rna-1 thi-1 supE44*) (30) was used to produce Dam$^-$ plasmid DNA for *BclI* subcloning.

Plasmids, enzymes, and biochemicals. Plasmids YCp50, YIp5, YEp13, and pHC79 have been described previously (6, 15, 19, 50). Restriction endonucleases, T4 DNA ligase, and Klenow fragment of DNA polymerase I were purchased from New England BioLabs or Bethesda Research Laboratories and used according to the specifications of the manu-

* Corresponding author.
† Present address: Department of Microbiology, University of Tennessee, Knoxville, TN 37919.

facturer. Plasmid and cosmid DNAs were prepared from *E. coli* by the alkaline lysis technique (29). High-molecular-weight yeast DNA was prepared as described previously (36). Cosmid and plasmid DNA was recovered from yeast cells by alkaline lysis (10). Oligonucleotides used as adapters, linkers, or sequencing primers were synthesized on a Systec 1410 synthesizer, using phosphoramidite chemistry. Restriction fragments for DNA sequencing were cloned into M13-mp18 or M13-mp19. Radioisotopes for sequencing and labeling were obtained from Dupont, NEN Research Products. Nick translation kits were purchased from Amersham Corp. *o*-Nitrophenyl-β-D-galactopyranoside and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) were obtained from Sigma Chemical Co.

Cosmid library. The shuttle vector YCp50 (*URA3 ARS1 CENIV* Amp^r Tet^r) was modified by inserting a 1.65-kilobase pair (kbp) *Bgl*II fragment bearing the lambda *cos* site (from pHC79) into the *Bam*HI site in the *tet* gene (pFL17). A new *Bam*HI site was introduced by inserting a *Bam*HI oligonucleotide linker into a unique *Pvu*II site in the *cos* segment (pFL18). Total yeast DNA from S288C (>100 kbp) was partially digested with *Sau*3A and separated by neutral sucrose density gradient centrifugation. DNA fragments of 35 to 40 kbp in length were ligated to *Bam*HI-digested cosmid pFL18 and packaged into phage particles (29). Approximately 12,000 clones were derived from this packaging, or 32 genome copies. An amplified DNA library was made from 2,500 pooled clones in DH1.

Transformation. Bacterial transformations were performed by the method of Hanahan (13), while yeast transformations were by the lithium acetate method of Ito et al. (17).

Rev activity screen. Rev activity in cosmid-transformed isolates was monitored by using a replica plate papillation assay. Master plates were prepared by transferring each isolate to an SC-URA plate. Twenty independent isolates per plate were applied in "patches" (1 by 1 cm) to form a master plate. Master plates were incubated at 30°C for 24 h. Two replicas of each master plate were made on SC-LYS. One of the SC-LYS replicas was exposed to 40 J of 254-nm UV light per m^2. The replicas were incubated at 30°C for 3 to 5 days, and the patches were scored for Lys^+ papillae. Rev^- patches normally exhibit 0 to 2 papillae, while Rev^+ patches have 10 to 30 papillae. Quantitative mutagenesis assays of Lys^+ reversion were performed as described by Lemontt (24).

Gene disruptions and integrations. Integrating plasmids were targeted to *REV1* by restriction endonuclease cleavage within the *REV1* segment (37). One-step gene disruption was performed by the method of Rothstein (47). The structures of all integrations and disruptions were confirmed by Southern transfer hybridization analysis (29).

***REV1* transcript analysis.** Total RNA was extracted from XL70-1B transformed with the multicopy YEp13-*REV1* plasmid pFL190. Cells were grown in SC-LEU to a titer of 10^7, and RNA was extracted by vortexing with phenol and glass beads, followed by extraction with phenol-chloroform-isoamyl alcohol and precipitation with LiCl (52). Dot hybridizations were applied to Schleicher & Schuell BA 85 nitrocellulose and performed according to their specifications. Glyoxylated total RNA was subjected to agarose gel electrophoresis, transferred to GeneScreen (Dupont, NEN Research Products), and hybridized by using the protocol of the manufacturer. RNA ladder from Bethesda Research Laboratories was used to provide size standards. Mapping of the start points of the transcript was performed by the S1 nuclease protection assay described previously (12).

DNA sequence analysis. The dideoxy chain termination method of Sanger et al. (49) was used to determine the nucleotide sequence of both strands of the *REV1* gene. Computer-assisted analyses of nucleic acid sequence data and prediction of protein hydropathy and secondary structure were performed by using a set of programs developed by Stephens (54), the IBI-Pustell Sequence Analysis Programs (International Biotechnologies, Inc.), and the National Biomedical Research Foundation Protein Identification Resource Data Base software.

β-Galactosidase assays. Plate assays were done by replica plating to X-Gal plates. For quantitative β-galactosidase assays (48), cells were grown to an optical density at 600 nm of approximately 1.0 in YEPD medium. For induction experiments, cultures were split and the desired treatment was applied to a sample. At various time points, 5 ml of sample was removed and assayed for β-galactosidase activity. A 1-ml portion was used to measure optical density at 600 nm, while the remaining cells were pelleted by centrifugation and suspended in 1 ml of Z buffer (48). The assay of *o*-nitrophenyl-β-D-galactopyranoside-hydrolyzing activity has been described before (48). One unit = $OD_{420} \times 1{,}000/[t(\text{h}) \times V(\text{ml}) \times OD_{600}]$, where V is the volume of the sample assayed and OD is optical density.

RESULTS

Isolation of *REV1* by complementation. Cosmid library DNA was used to transform XL68-3D to uracil independence. Some 440 Ura^+ transformants were screened for complementation of *rev1-1*. Two Rev^+ transformants were identified. Cosmids were recovered from each transformant, and preliminary restriction maps were obtained (Fig. 1). The two cosmid inserts shared a 15.5-kbp overlapping segment. Subcloning of *Hin*dIII and *Bgl*II fragments showed that the Rev^+ segment was contained within an 8.0-kbp *Bgl*II fragment (pFL41; Fig. 1), whereas all of the *Hin*dIII subclones were Rev^-. Both the cosmid and the *Bgl*II clones fully complemented *rev1-1* (Fig. 2). Deletions of either the *Pvu*II or *Cla*I fragments of pFL41 were Rev^- (not shown), extending the limits of the Rev^+ segment of 1,100 bp.

Disruption of the *REV1* gene. The subcloning results indicated that a 736-bp *Hin*dIII-*Cla*I fragment was wholly contained within the complementing region. Therefore, this fragment should be internal to the gene. To test this prediction, the *Hin*dIII-*Cla*I fragment was cloned into the integrating plasmid YIp5 (50). Integration of a plasmid containing an internal fragment of a gene into the homologous chromosomal copy results in the disruption of the chromosomal sequence, yielding a gene that is interrupted by the vector sequences. Rev^+ strain XL70-1B was transformed with plasmid pFL44 (Fig. 1), which had been linearized by partial digestion with the restriction endonuclease *Eco*RI, creating recombinogenic free ends to direct integration into the region of homology. The transformants obtained displayed the Rev^- phenotype (Table 1). A disruption strain was crossed to the *rev1-1* mutant, and the resulting diploid was shown to be Rev^-. After sporulating this diploid, all spores were Rev^- (11 tetrads). The disruption was crossed to a Rev^+ strain, and after the resulting diploid was sporulated and tetrads were dissected, the disruption (Rev^- Ura^+) segregated 2:2 (nine tetrads). These results indicate that the *Hin*dIII-*Cla*I fragment is internal to the *REV1* gene.

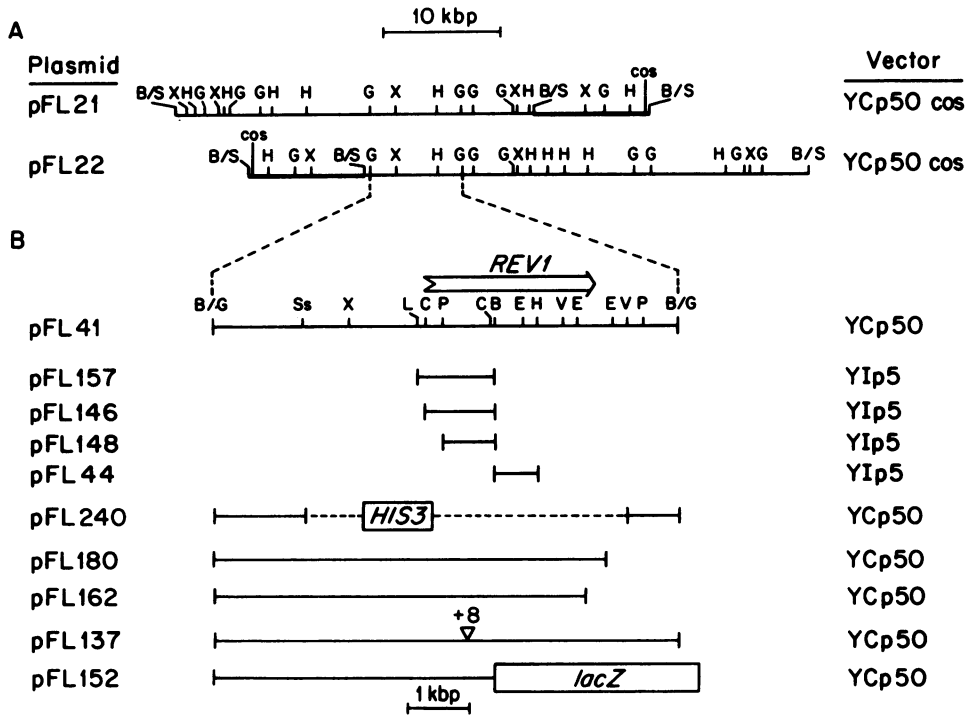

FIG. 1. Plasmid structures. Restriction sites are indicated by letters. B, *Bam*HI; B/G, *Bam*HI-*Bgl*II junction; B/S, *Bam*HI-*Sau*3A junction; C, *Cla*I; E, *Eco*RI; G, *Bgl*II; H, *Hin*dIII; L, *Bcl*I, P, *Pst*I; Ss, *Bss*HII; V, *Pvu*II; X, *Xho*I; cos, lambda cohesive end site. (A) Cosmids complementing *rev1-1*. Heavy line indicates cosmid CEN vector pFL18 sequences; light line indicates yeast DNA insert. (B) Subclones of *REV1* fragments and derivative plasmid constructions. The *lacZ* fragment of pFL152 is from pMC1871 (7). Not shown: pFL190, being the 8-kbp *Bgl*II *REV1* fragment in vector YEp13.

A deletion of *REV1* was constructed by one-step gene disruption (47). A segment of the *REV1* fragment was deleted and replaced by a segment bearing the *HIS3* gene (pFL240; Fig. 1). The Rev insert (plus *HIS3*) was excised from pFL240 and used to transform the haploid XL69-4A. The resulting His^+ strains were Rev^- (Table 1), as a consequence of replacement of the chromosomal sequences with the *rev1*Δ::*HIS3* segment. Because the deletion is viable in a haploid, *REV1* is not an essential gene.

Direction of transcription and size of transcript. The *Cla*I-*Eco*RI *REV1* internal fragment from pFL44 was cloned into M13 phage mp19. Both orientations of the fragment with respect to the phage were obtained. Radioactively labeled single-stranded DNA probe (16) from each phage was used in two separate dot hybridizations to total RNA. Hybridization was obtained only with the phage priming the Rev fragment 5′-*Eco*RI-*Cla*I-3′, indicating that the direction of transcription of *REV1* is from *Cla*I toward *Eco*RI. Northern transfer hybridization analysis of total RNA, using the *REV1* probe, revealed a 3.1-kilobase transcript (Fig. 3A).

Functional limits of the *REV1* gene. To map the 5′ end of the minimum complementing segment of *REV1*, deletion subclones (pFL157, pFL146, and pFL148) were constructed in the integrating vector YIp5 (Fig. 1). Integration was targeted into *REV1* by linearizing the plasmids at an *Hpa*I site within the *REV1* segment. These integrations produce a sequential series of interruptions of the 5′ end of *REV1*. The mutability phenotypes of these interruptions are shown in Fig. 4. The interruption terminating at the *Bcl*I site (pFL157) shows full Rev^+ mutability, whereas the *Cla*I (pFL146) and *Pst*I (pFL148) interruptions are intermediate in mutability with respect to *REV1* and *rev1-1*.

BAL31 deletions were used to localize the 3′ end of *REV1*. Plasmid pFL41 was linearized at the *Sal*I site of the *tet* gene, and timed BAL31 digestions were carried out to degrade the 3′ end of the *REV1* subclone. Both Rev^- and Rev^+ deletions (pFL162 and pFL180, Fig. 1, Table 1) were found whose endpoints fell within the 640-bp *Eco*RI fragment.

Nucleotide sequence of the *REV1* gene. The dideoxynucleotide chain termination method (49) was used to determine the nucleotide sequence of *REV1* DNA fragments cloned in both

TABLE 1. Mutability of Rev constructions and reference alleles

Allele [free plasmid]	Lys^+ revertants per 10^8 survivors (% survival)		
	No UV	40 J of UV per m^2	80 J of UV per m^2
Haploids			
REV1[a]	8.3	6,014 (42)	17,321 (8)
rev1::pFL44[a]	13	161 (17)	153 (3)
*rev1*Δ::*HIS3*[b]	4.7	11 (17)	21 (6)
rev1-1[c]	16	83 (14)	234 (2)
*REV1*Δ*3355*[c] [pFL180]	8.9	3,529 (59)	8,547 (14)
*rev1*Δ*2933*[c] [pFL162]	4.0	83 (12)	123 (6)
rev1::1104(+8)[c] [pFL137]	5.0	208 (14)	214 (2)
Diploids			
REV1/REV1	6.5	790 (63)	2,398 (28)
rev1::pFL44/*rev1*::pFL44	3.3	24 (22)	38 (12)
rev1-1/rev1-1	2.0	7.5 (26)	8.7 (11)
rev1::pFL44/*rev1-1*	4.1	42 (22)	14 (11)

[a] XL70-1B.
[b] XL69-5A.
[c] XL68-3D.

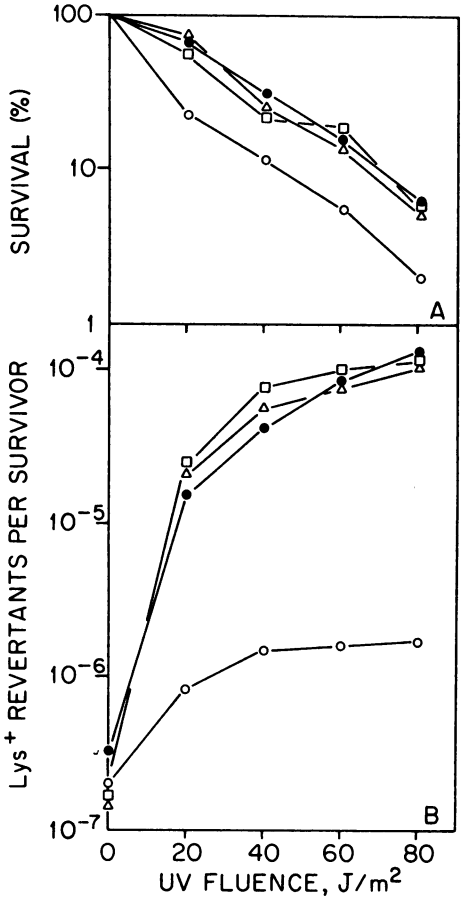

FIG. 2. Survival and mutability of Rev strains and plasmid-transformed strains following UV irradiation. (A) UV survival; (B) UV mutability. Symbols: ●, XL70-1A *REV1*; ○, XL68-3D *rev1-1*; □, XL68-3D *rev1-1* (pFL22 *REV1*); △, XL68-3D *rev1-1* (pFL41 *REV1*).

orientations in M13 phage vectors. The sequencing strategy is shown in Fig. 5. The sequence of each DNA strand was independently determined. Figure 6 shows the sequence of 3,485 bp corresponding to the mRNA and regions flanking the gene. The overall base composition of the *REV1* fragment is slightly AT rich, being 64% A+T, with respect to the bulk yeast genomic DNA. The *REV1* coding sequence is somewhat purine rich at 55% A+G in the strand corresponding to mRNA.

The 5′ end of the *REV1* transcript was mapped by S1 nuclease protection. Two transcription start points were observed (Fig. 3B), one at nucleotide (nt) 318 and one at nt 342. Each start point is in a short T run. The first downstream ATG (nt 361 to 363) defines an open reading frame 2,955 bp in length, which can encode a protein of 985 amino acids with a predicted molecular weight of 112,239. A *lacZ* translational fusion (pFL152; Fig. 1) into the *Bam*HI site at nt 1518 in this reading frame produces β-galactosidase activity, demonstrating that this open reading frame is transcribed and translated. Furthermore, a dGGTCGACC oligonucleotide linker inserted into the *Hpa*I site at nt 1104 (pFL137; Fig. 1) yielded a Rev⁻ frameshift mutant (Table 1). All other reading frames are extensively blocked by termination codons. In the *REV1* gene all 61 possible codons are used, unlike the codon bias characteristic of highly expressed yeast genes (3).

Isolation and sequence of *rev1-1*. Targeted integration of plasmid pFL157 into the Rev⁻ strain XL68-3D yielded Rev⁺ transformants (not shown), mapping the *rev1-1* mutation between the *Bcl*I site and the *Hpa*I site. To recover *rev1-1*, plasmid pFL41 was digested with *Pvu*II and *Bss*HII, and the vector fragment with sequences flanking *REV1* was used to transform XL68-3D. The plasmid was reconstituted in vivo by gapped-plasmid repair, which transfers the missing chromosomal sequence (*rev1-1*) into the plasmid by gene conversion (37). The repaired *rev1-1* plasmid (pFL73; not shown) was recovered from yeast cells and the *rev1-1* insert was sequenced. The *rev1-1* allele consists of a G-to-A substitution at nt 937, encoding a Gly-193 to Arg-193 amino acid substitution (Fig. 6).

5′- and 3′-flanking sequences of the *REV1* gene. The 5′-flanking region of *REV1* is 67% AT and is rich in poly(dA-dT) sequences. There are four striking dA-dT regions: 10 T's from nt 74 to 83; 18 T's, 3 A's, and 1 C from nt 41 to 62; 25 A's, 5 T's, 6 G's, and 3 C's from nt 100 to 138; and 15 T's, 14 A's, 3 C's, and 1 G from nt 254 to 286. The two T-rich regions resemble elements required for constitutive transcription of the *HIS3*, *PET56*, and *DED1* genes (55). The sequence TATATA occurs at nt 185 to 190 upstream of the coding sequence. This sequence resembles the canonical TATA(T/A)A(T/A) usually found 26 through 34 nt upstream of the point of initiation of transcription in higher eucaryotes

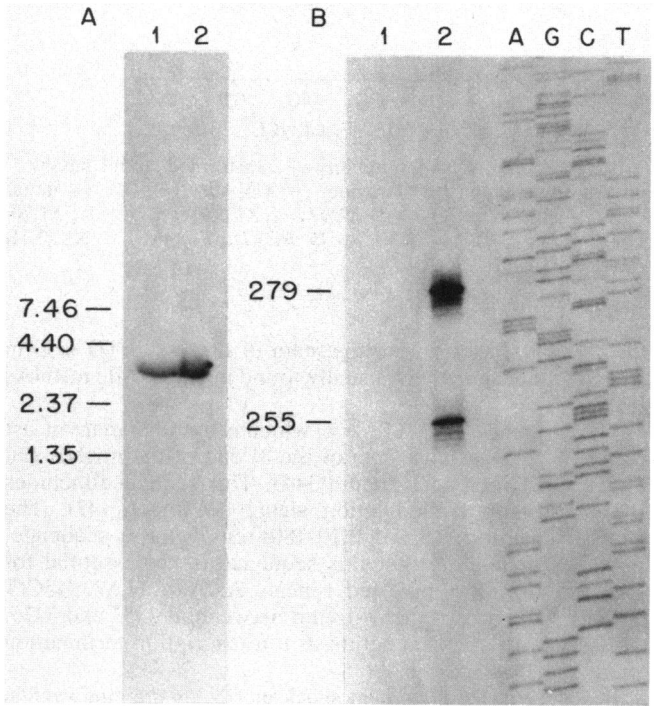

FIG. 3. Northern (RNA) hybridization analysis (A) and S1 nuclease mapping of the 5′ termini (B) of the *REV1* transcript. (A) Lane 1, 10 μg of total yeast RNA; lane 2, 20 μg of RNA. Indicated in the margin are the locations and sizes (kilobases) of marker RNAs. Complementary *Eco*RI-*Cla*I probe was labeled with ^{32}P by primer extension of the appropriate M13 clone. (B) Lane 1, 5 ng of *REV1* probe alone; lane 2, 5 ng of *REV1* probe plus 200 μg of total yeast RNA. Indicated in the margin are the sizes (nt) of the protected fragments. Complementary *Bcl*I-*Pst*I probe was labeled with ^{32}P at the *Pst*I end, and the fragment was hybridized with or without RNA in 80% formamide at 45°C for 12 h. The hybrids were digested with S1 nuclease (500 U/ml) for 30 min at 37°C. The sequencing ladder shown is M13mp18, using −52 primer.

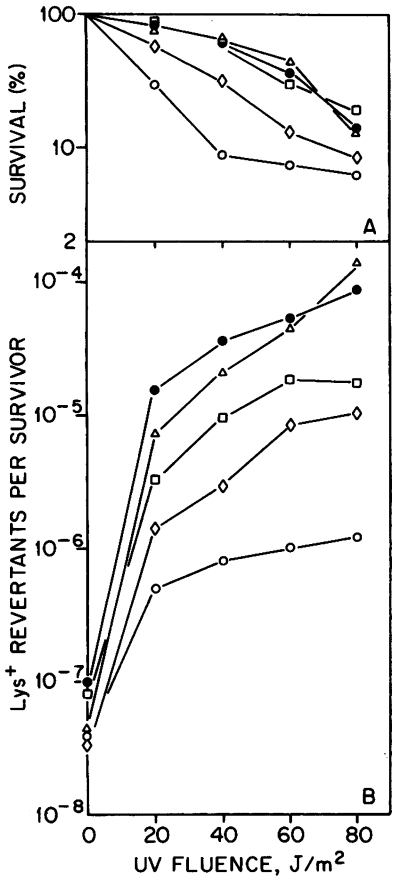

FIG. 4. Survival and mutability of transformed strains having 5′ interruptions of the *REV1* region. (A) UV survival; (B) UV mutability. Symbols: ●, XL70-1B *REV1*; ○, XL68-3D *rev1-1*; △, XL70-1B *REV1*::pFL157; □, XL70-1B *REV1*::pFL146; ◇, XL70-1B *REV1*::pFL148.

(5, 11). The base at 3 nt upstream of the first ATG is G in *REV1*, rather than the A usually found in eucaryotic mRNAs (8).

The sequence TAAATAAA, which is found in many yeast genes 28 to 33 nt upstream of the 3′ end of the mRNA (2), appears between nt 3436 and 3443. This sequence includes the possible polyadenylation signal AATAAA (42). The Rev$^+$ 3′ deletion Del3355 (pFL180) removes this sequence, however, indicating that this sequence is not essential for Rev function. An inverted repeat, AAAAAaTAAgGaCtT TAaTTTTT, occurs downstream between nt 3453 and 3475. This structure might function as a transcription termination signal.

Response of *REV1* to heat shock or DNA-damaging agents. The Rev$^+$ strain XL70-1B carrying the *REV1*::*lacZ* fusion CEN plasmid pFL152 showed no β-galactosidase activity on X-gal plates. Low basal activity was observed by direct assay (Table 2). As some DNA repair genes from yeasts are known to be inducible by DNA-damaging agents such as UV (1, 9, 27, 28, 41, 46, 48) or 4-nitroquinolone-*N*-oxide (27, 48), we monitored β-galactosidase activity following treatment with these agents. In addition, the response to heat shock was examined. The *REV1*::*lacZ* fusion did not respond to such treatments (Table 2). Similar results were obtained for treatments at 30°C, as well as with an integrated *REV1*::*lacZ* fusion (data not shown).

TABLE 2. β-Galactosidase activity of *REV1*::*lacZ* fusion in *S. cerevisiae*

Treatment	Assay time (min)	β-Galactosidase activity (U)
None (22°C)	0	2.7
	60	2.7
	120	2.4
Heat shock (37°C)	60	1.3
	120	1.3
UV, 80 J/m^2 (22°C)	60	2.2
	120	2.4
4-Nitroquinoline-*N*-oxide, 0.5 μg/ml (22°C)	60	2.5
	120	2.3

DISCUSSION

Cosmid cloning. Since the Rev phenotype did not easily lend itself to a positive selection scheme, we chose to construct a cosmid library to minimize the number of transformed clones to be individually screened. This choice was quickly justified by obtaining two independent clones of *REV1*, among slightly less than a genome equivalent of clones. Our library also derives two advantages from the choice of a *CEN* vector: large plasmids are stabilized, and genes that might be lethal at high copy number are maintained in single copy, although the *REV1* gene did not prove deleterious when present at high copy number.

REV1 protein. The translation of the *REV1* open reading frame indicates a 112,239-dalton protein containing 37.2% nonpolar, 35.0% polar, 11.8% acidic, and 16.0% basic amino acids. The REV1 protein is substantially basic, with a predicted pI of 9.82. The translation of the *REV1* open reading frame does not exhibit any significant sequence similarity with the *S. cerevisiae* RAD1 (45), RAD2 (28), RAD3 (43), RAD7 (39), or RAD10 (44) proteins. A protein similarity search (26) in the National Biomedical Research Foundation Protein Identification Resource and the GenBank data banks did not reveal any strong similarities. The highest score returned indicated a weak similarity (*z* score of 4.65) with an alignment of the *E. coli* UMUC protein (40).

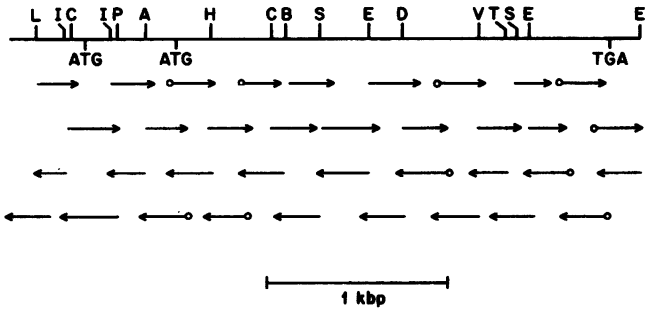

FIG. 5. DNA sequencing strategy. Plain arrows denote subcloned segments primed with M13 sequencing primer; arrows with open circles denote segments primed internally with oligonucleotides synthesized by using deduced sequence. The positions of the translational initiator (ATG) and terminator (TGA) codon are indicated below the sequenced fragment; restriction endonuclease cleavage sites used to generate subclones are shown above. A, *Acc*I; B, *Bam*HI; C, *Cla*I; D, *Hin*dIII, E, *Eco*RI, H, *Hpa*I; I, *Hin*cII; L, *Bcl*I; P, *Pst*I; S, *Sca*I; T, *Sst*I; V, *Pvu*II.

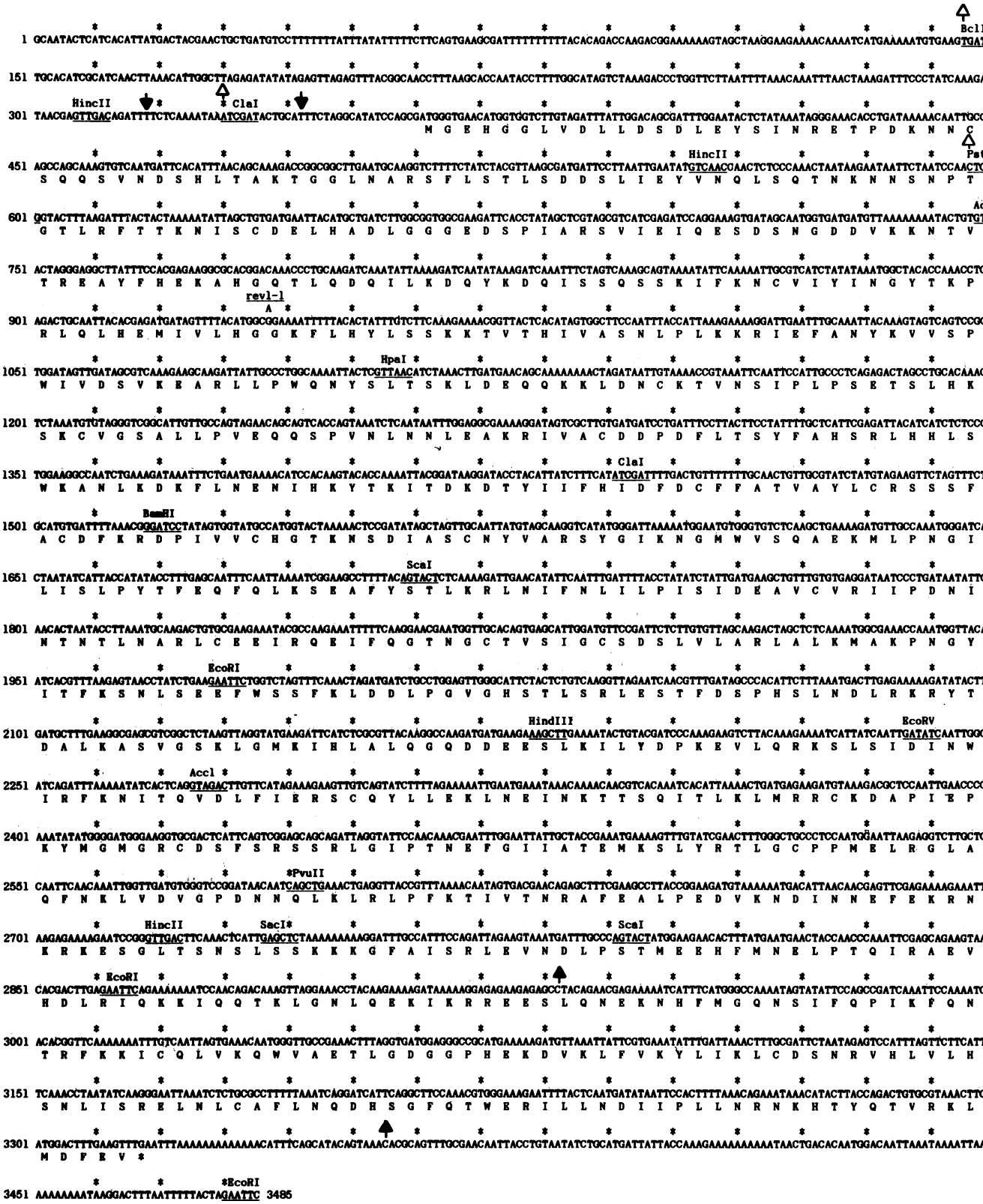

FIG. 6. DNA sequence of the *REV1* gene and its flanking regions. Restriction endonuclease cleavage sites are underlined. Nucleotide numbers are shown in the margin. The deduced amino acid sequence (in single-letter code) is given under the nucleotide sequence. Symbols: ▼, transcript start points; △, endpoints of 5′ deletions; ▲, endpoints of 3′ deletions.

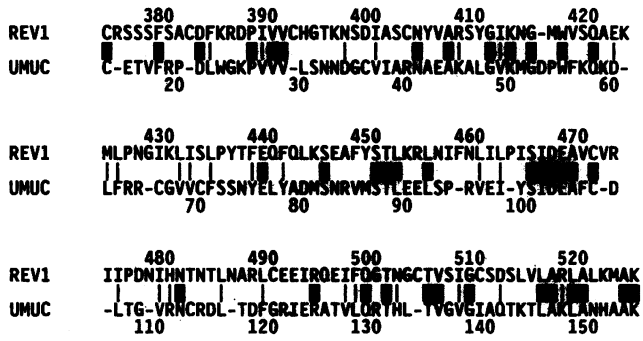

FIG. 7. Similarity between REV1 protein and UMUC protein. Identities are indicated by solid blocks; similarities scoring >+1 (using the Mutation Data Matrix) are indicated by vertical lines. A FASTP similarity search (26) was performed (*k*-tuple = 2), using the translation of *REV1* against translations of 16,749 sequences in GenBank. The average score was 24.4, with a standard deviation of 22.26. The optimized score for REV1 versus UMUC was 128. The *z* score for this alignment was 4.65.

Figure 7 gives the alignment of 152 residues of the REV1 protein with a segment of the UMUC protein. There are 38 matches (25% identity) in this alignment. When residues with similarity scores of >+1 are added to the identities, the two sequences show 42% similarity. The most notable block of identities, SIDEA-C, is also partly conserved (SIDE) in the *umuC* homolog *mucB* (40). Both *REV1* and *umuC* are required for error-prone repair, functionally supporting the suggestion that these peptide segments are homologous. This alignment should prove useful as a basis for targeting mutations to identify functionally important segments of the REV1 protein.

Expression of *REV1*. The *REV1*::*lacZ* fusion results suggest that *REV1* is constitutively expressed, as are a number of other yeast repair genes (32, 33). The occurrence of poly(dA-dT) tracts is consistent with constitutivity (55), although the *Bcl*I deletion (pFL157) indicates that the most prominent T-rich segment is not required for full mutability.

Both *Cla*I (pFL146) and *Pst*I (pFL148) deletions lack upstream sequences presumed necessary for transcription, yet both interruptions exhibit significant residual mutability. Both of these constructs are immediately downstream from a vector sequence known to have weak promoter activity in *S. cerevisiae* (4). The *Cla*I deletion could produce a hybrid transcript or initiate from the downstream start point and be translated normally. The *Pst*I deletion, however, has removed part of the N-terminal coding sequence. The sequence across the *Pst*I site into the vector shows an in-frame ATG codon 34 bp upstream. A transcript of this truncated gene could initiate translation at this ATG, or elsewhere, and yield a truncated REV1 protein. This suggests that an N-terminal segment of the REV1 protein is dispensable.

Residual mutability of *rev1-1*. The extreme Rev$^-$ phenotype of the *rev1*Δ::*HIS3* gene disruption, compared with the reference allele *rev1-1* (Table 1), implies that the Arg-193 missense protein encoded by *rev1-1*, and perhaps even the truncated gene product of *rev1*::pFL44, retains residual Rev activity. Consequently, the precise description of *rev1-1* mutability (14, 20, 25) may need to be reinterpreted in light of *rev1-1* leakiness. The location of the *rev1-1* substitution within the protein defines this segment as critical to some functional domain. With the *REV1* clone now in hand, the generation of new missense alleles, by either site-localized or site-directed mutagenesis, will facilitate the identification of additional domains and perhaps generate novel mutability phenotypes.

ACKNOWLEDGMENTS

We are pleased to acknowledge Bruce Suttle for technical assistance in DNA sequencing. We are indebted to Richard Mural and Thomas Soper for discussion of the manuscript.

This research was sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

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. **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.
3. **Bennetzen, J. L., and B. D. Hall.** 1982. Codon selection in yeast. J. Biol. Chem. **257**:3026–3031.
4. **Braus, G., G. Paravivini, and R. Hütter.** 1988. A consensus transcription termination sequence in the promoter region is necessary for efficient gene expression of the *TRP1* gene of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **212**:495–504.
5. **Breathnach, R., and P. Chambon.** 1981. Organization and expression of eucaryotic split genes coding for proteins. Annu. Rev. Biochem. **50**:349–383.
6. **Broach, J. R., J. N. Strathern, and J. B. Hicks.** 1979. Transformation in yeast: development of a hybrid cloning vector and isolation of the *CAN1* gene. Gene **8**:121–133.
7. **Casadaban, M. J., A. Martinez-Arias, S. K. Shapira, and J. Chou.** 1983. β-Galactosidase gene fusions for analyzing gene expression in *Escherichia coli* and yeast. Methods Enzymol. **100**:293–308.
8. **Cigan, A. M., and T. F. Donahue.** 1987. Sequence and structural features associated with translational initiator regions in yeast—a review. Gene **59**:1–18.
9. **Cole, G. M., D. Schild, S. T. Lovett, and R. K. Mortimer.** 1987. Regulation of *RAD54*- and *RAD52-lacZ* gene fusions in *Saccharomyces cerevisiae* in response to DNA damage. Mol. Cell. Biol. **7**:1078–1084.
10. **Filetici, P., N. Junakovic, and P. Ballario.** 1985. Rapid alkaline preparation for yeast circular covalently closed DNA molecules. Curr. Genet. **9**:123–126.
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. **Hames, B. D., and S. J. Higgins.** 1985. Nucleic acid hybridization. IRL Press, Oxford.
13. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **155**:557–580.
14. **Haynes, R. H., and B. A. Kunz.** 1981. DNA repair and mutagenesis in yeast, p. 371–414. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*. Life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Hohn, B., and J. Collins.** 1980. A small cosmid for efficient cloning of large DNA fragments. Gene **11**:291–298.
16. **Hu, N. T., and J. Messing.** 1982. The making of strand-specific M13 probes. Gene **17**:271–277.
17. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.
18. **Jentsch, S., J. P. McGrath, and A. Varshavsky.** 1987. The yeast DNA repair gene *RAD6* encodes a ubiquitin-conjugating enzyme. Nature (London) **329**:131–134.
19. **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.
20. **Lawrence, C. W.** 1982. Mutagenesis in *Saccharomyces cerevisiae*. Adv. Genet. **21**:173–274.
21. **Lawrence, C. W., G. Das, and R. B. Christensen.** 1985. *REV7*, a new gene concerned with UV mutagenesis in yeast. Mol. Gen. Genet. **200**:80–85.
22. **Lawrence, C. W., P. E. Nisson, and R. B. Christensen.** 1985. UV and chemical mutagenesis in *rev7* mutants of yeast. Mol. Gen. Genet. **200**:86–91.
23. **Lawrence, C. W., T. O'Brien, and J. Bond.** 1984. UV-induced reversion of *his4* frameshift mutations in *rad6*, *rev1*, and *rev3* mutants of yeast. Mol. Gen. Genet. **195**:487–490.
24. **Lemontt, J. F.** 1971. Mutants of yeast defective in mutation induced by ultraviolet light. Genetics **68**:21–23.
25. **Lemontt, J. F.** 1980. Genetic and physiological factors affecting repair and mutagenesis in yeast, p. 85–120. *In* W. M. Generoso, M. D. Shelby, and F. J. deSerres (ed.), DNA repair and mutagenesis in eukaryotes. Plenum Publishing Corp., New York.
26. **Lipman, D. J., and W. R. Pearson.** 1985. Rapid and sensitive protein similarity searches. Science **227**:1435–1441.
27. **McClanahan, T., and K. McEntee.** 1984. Specific transcripts are elevated in *Saccharomyces cerevisiae* in response to DNA damage. Mol. Cell. Biol. **4**:2356–2363.
28. **Madura, K., and S. Prakash.** 1986. Nucleotide sequence, transcript mapping, and regulation of the *RAD2* gene of *Saccharomyces cerevisiae*. J. Bacteriol. **166**:914–923.
29. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Marinus, M. G.** 1973. Location of DNA methylation genes on the *Escherichia coli* K-12 genetic map. Mol. Gen. Genet. **127**: 47–55.
31. **Murray, N. E., W. J. Brammer, and K. Murray.** 1977. Lambdoid phages that simplify the recovery of in vitro recombinants. Mol. Gen. Genet. **150**:53–61.
32. **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.
33. **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.
34. **Nisson, P. E., and C. W. Lawrence.** 1986. The isolation and characterization of *ngm2*, a mutation that affects nitrosoguanidine mutagenesis in yeast. Mol. Gen. Genet. **204**:90–97.
35. **Njagi, G. D. E., and B. J. Kilbey.** 1982. *cdc7-1*, a temperature sensitive cell-cycle mutant which interferes with induced mutation in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **186**:478–481.
36. **Olson, M. V., K. Loughney, and B. D. Hall.** 1979. Identification of the yeast DNA sequences that correspond to specific tyrosine-inserting nonsense suppressor loci. J. Mol. Biol. **132**:387–410.
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. **Patterson, M., R. A. Sclafani, W. L. Fangman, and J. Rosamond.** 1986. Molecular characterization of cell cycle gene *CDC7* from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6**:1590–1598.
39. **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.
40. **Perry, K. L., S. J. Elledge, B. B. Mitchell, L. Marsh, and G. C. Walker.** 1985. *umuDC* and *mucAB* operons whose products are required for UV light- and chemical-induced mutagenesis: UmuD, MucA, and LexA proteins share homology. Proc. Natl. Acad. Sci. USA **82**:4331–4335.
41. **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.
42. **Proudfoot, N. J., and G. G. Brownlee.** 1976. 3′ non-coding region sequences in eukaryotic messenger RNA. Nature (London) **263**:211–214.
43. **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.
44. **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.
45. **Reynolds, P., L. Prakash, and S. Prakash.** 1987. Nucleotide sequence and functional analysis of the *RAD1* gene of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7**:1012–1020.
46. **Robinson, G., C. Nicolet, D. Kalainov, and E. Friedberg.** 1986. A yeast excision-repair gene is inducible by DNA damaging agents. Proc. Natl. Acad. Sci. USA **83**:1842–1846.
47. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
48. **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.
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 **74**:5463–5467.
51. **Sclafani, R. A., M. Patterson, J. Rosamond, and W. L. Fangman.** 1988. Differential regulation of the yeast *CDC7* gene during mitosis and meiosis. Mol. Cell. Biol. **8**:293–300.
52. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
53. **Siede, W., and F. Eckardt-Schupp.** 1986. DNA repair genes of *Saccharomyces cerevisiae*: complementing *rad4* and *rev2* mutations by plasmids which cannot be propagated in *Escherichia coli*. Curr. Genet. **11**:205–210.
54. **Stephens, R. M.** 1985. A sequencers' sequence analysis package for the IBM PC. Gene Anal. Technol. **2**:67–75.
55. **Struhl, K.** 1985. Naturally occurring poly (dA-dT) sequences are upstream promoter elements for constitutive transcription in yeast. Proc. Natl. Acad. Sci. USA **82**:8419–8423.
56. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48**:60–93.
57. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.