The *Saccharomyces cerevisiae* *RAD18* gene encodes a protein that contains potential zinc finger domains for nucleic acid binding and a putative nucleotide binding sequence

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Received April 7, 1988; Revised and Accepted June 21, 1988

ABSTRACT

The *RAD18* gene of *Saccharomyces cerevisiae* is required for postreplication repair of UV damaged DNA. We have isolated the *RAD18* gene, determined its nucleotide sequence and examined if deletion mutations of this gene show different or more pronounced phenotypic effects than the previously described point mutations. The *RAD18* gene open reading frame encodes a protein of 487 amino acids, with a calculated molecular weight of 55,512. The RAD18 protein contains three potential zinc finger domains for nucleic acid binding, and a putative nucleotide binding sequence that is present in many proteins that bind and hydrolyze ATP. The DNA binding and nucleotide binding activities could enable the RAD18 protein to bind damaged sites in the template DNA with high affinity. Alternatively, or in addition, RAD18 protein may be a transcriptional regulator. The *rad18* deletion mutation resembles the previously described point mutations in its effects on viability, DNA repair, UV mutagenesis, and sporulation.

INTRODUCTION

Mutations of the *RAD18* gene of the yeast *Saccharomyces cerevisiae* confer a high level of sensitivity to ultraviolet light (UV), ionizing radiation, alkylating agents, and a variety of other DNA damaging agents (1,2). *RAD18* belongs to the *RAD6* epistasis group, and like *rad6* mutants, *rad18* mutants are defective in postreplication repair of discontinuities that occur in newly synthesized DNA following UV irradiation (3,4). In contrast to the *RAD6* gene, which is absolutely required for UV mutagenesis, *rad18* mutations have no effect on UV mutagenesis (2). Sporulation, spore viability, and meiotic recombination occur normally in *rad18* homozygous diploids (1,5,6), whereas *rad6/rad6* diploids are defective in sporulation (1,2). In both *rad18* and *rad6* mutants, levels of spontaneous mitotic recombination and spontaneous mutations are elevated (7-9).

To understand the role of *RAD6* epistasis group genes in postreplication repair and in other cellular processes, we have begun to clone and characterize the important genes of this group. The cloned *RAD6* gene was used to overproduce the RAD6 protein in yeast, facilitating its purification and characterization (10, 11). In this paper, we report the isolation and nucleotide sequence of the *RAD18* gene. The *RAD18* encoded protein contains 487 amino acids with a calculated molecular weight of 55,512. The RAD18 protein contains three sequences that show homology with the zinc binding, nucleic acid binding sequence motif present in a variety of

transcriptional activators and in other nucleic acid binding proteins (12,13). The RAD18 protein also contains a sequence that is present in various proteins that bind and hydrolyze ATP (14). The possible roles of the putative DNA binding and nucleotide binding activities of the RAD18 protein in DNA repair are discussed.

MATERIALS AND METHODS

Strains

Escherichia coli strain HB101 was used for subcloning and propagation of plasmids, and XL1-Blue (15) for propagation of M13 phage derivatives and color selection of pUC derivatives. The *S. cerevisiae* haploid *RAD*⁺ strains used in this study were: 7799-4B (*MAT α his4-17 ura3-52*); DBY747 (*MAT α his3- Δ 1 leu2-3 leu2-112 trp1-289 ura3-52*); LP2729-3B (*MAT α arg4-17 leu2-3 leu2-112 trp1-289 ura3-52*); Y428 (*MAT α ade2-101 leu2- Δ 98 lys2-80 trp1- Δ ura3-52*); and Y433 (*MAT α ade2-101 his3- Δ 200 leu2- Δ 98 lys2-80 ura3-52*). Haploid *rad18* strains were: 966-3C (*MAT α leu1 rad18-1*); LP2729-4B (*MAT α his3- Δ 1 leu2-3 leu2-112 trp1-289 ura3-52 rad18-2*); LP2729-5B (*MAT α arg4-17 his3- Δ 1 ura3-52 rad18-2*); LP2814-12A (*MAT α trp1-289 ura3-52 rad18-1*); LP2815-5A (*MAT α his3- Δ 1 trp1-289 ura3-52 rad18-3*); LP2816-2C (*MAT α his3- Δ 1 trp1-289 ura3-52 rad18-4*); JJ9 (DBY747*rad18- Δ :LEU2*); JJ11 (DBY747 *rad18- Δ :HIS3*); JJ16 (LP2729-3B *rad18- Δ :LEU2*); JJ17 (Y428 *rad18- Δ :LEU2*) and JJ18 (Y433 *rad18- Δ :LEU2*). Diploid strains used for determining sporulation frequency and spore viability, JJ41 (*RAD*⁺/*RAD*⁺), JJ42 (*rad18- Δ /RAD*⁺), JJ43 (*RAD*⁺/*rad18- Δ*), and JJ40 (*rad18- Δ /rad18- Δ*), were obtained from crosses of Y428 X Y433, JJ17 X Y433, Y428 X JJ18, and JJ17 X JJ18, respectively.

Isolation of the *RAD18* Gene

The *rad18* complementing DNA insert (Fig. 1) was isolated from a cosmid library, kindly provided by G. Fink (MIT), by overlap hybridization with the *SUP61* gene, which is tightly linked to *RAD18* (16). A 4.3 kb *Eco*RI fragment from λ MG3 clone 5226 and a 6.0 kb *Bam*HI fragment from λ MG3 clone 5228 (17), containing DNA inserts known to flank the *SUP61* gene and kindly provided by M. Olson (Washington University), were each labeled with α -[³²P]dCTP by nick translation and used for colony hybridization as described (18). The two probes are separated by about 22 kb and thus span approximately 32 kb surrounding the *SUP61* locus. Cosmids from positive colonies were isolated and tested for complementation of the UV sensitivity in *rad18* mutant strains. Percent survival after UV (11) or gamma irradiation (19) was determined using logarithmically growing liquid cultures plated at various dilutions on rich or selective media.

Sequencing Strategy

DNA fragments were obtained by digestion with restriction enzymes (New England Biolabs and Boehringer Mannheim Biochemicals) with four and six nucleotide recognition sequences as shown in Fig. 3. These fragments were cloned into various sites in the polylinker

regions of M13mp18, M13mp19, M13um20 and M13um21(International Biotechnology Inc.). The nucleotide sequence of *RAD18* was determined by the chain termination method of Sanger *et al.* (20) as modified by Biggin *et al.* (21), and described in Madura and Prakash (22), using deoxyadenosine 5'-(α -[35 S]thio)triphosphate (Amersham). Unlabeled nucleotides, primers and polymerase I Klenow fragment were obtained from Bethesda Research Laboratories and New England Biolabs.

RAD18 Transcript Analysis

Isolation of total RNA from cells of the *RAD*⁺ yeast strain 7799-4B, glyoxylation, fractionation, transfer to Gene Screen membrane, and hybridization were carried out as described by Madura and Prakash (22). Oligo dT cellulose (PL Biochemicals) was used to prepare poly(A)⁺ RNA from total RNA as described by the manufacturer. To determine transcript direction, strand specific probes were constructed by insertion of the 227 base *SalI*-*XbaI* fragment (Fig. 3) into the *SalI* and *XbaI* sites of M13mp18 and M13mp19 phages. The probes were labeled by primer extension to a specific activity of 3.3×10^6 and 4.2×10^6 cpm/ μ g of DNA for M13mp18 and M13mp19, respectively, and both blots were probed with approximately 1.3×10^6 cpm.

Construction of *RAD18* Genomic Deletion Strains (*rad18-Δ*)

Strains containing deletions of the genomic *RAD18* gene, designated *rad18-Δ*, were constructed by the gene replacement method (23) by using plasmids pJJ209 and pJJ202, in which all but 44 of the 487 codons of the *RAD18* coding region had been replaced by either the yeast *HIS3* or *LEU2* gene. The 5.8 kb *RAD18* -containing *HindIII*-*BamHI* fragment (Fig. 1) was cloned into the *HindIII*-*BamHI* sites of pUC18, generating the plasmid pJJ192. A 1353 nucleotide fragment of *RAD18* extending from the *BaI* site at position +132 to the *StuI* site at position +1484 (20 bases beyond the TAA) in the *RAD18* open reading frame (ORF) (see Fig. 4) in pJJ192 was replaced with a *BglIII* linker (Pharmacia), generating the plasmid pJJ193, or the *LEU2* -containing *HpaI* fragment of plasmid YEp13, yielding the plasmid pJJ202. The *HIS3* -containing *BamHI* fragment of plasmid pSZ515 (24) was inserted into the unique *BglIII* site of pJJ193 to generate the *HIS3*-containing plasmid pJJ209. The 6.4 kb *HindIII*/*BamHI* fragment of plasmid pJJ202 was used to transform the *RAD*⁺ strains DBY747, LP2729-3B, Y428, and Y433 to *LEU2*⁺ to generate the *rad18-Δ* strains JJ9, JJ16, JJ17, and JJ18, respectively. The 4.2 kb *EcoRI* fragment of plasmid pJJ209 was used to transform the strain DBY747 to *HIS3*⁺ to generate the *rad18-Δ* strain JJ11. All deletion strains were tested for allelism to *rad18*.

Mutagenesis and Sporulation

Strains were grown to mid exponential phase in synthetic complete medium and plated on selective medium and exposed or not exposed to UV light to determine the frequency of spontaneous and induced reversion of the *arg4* -17 allele. Sporulation frequency and spore viability were determined as described by Morrison, *et al.*(11). Over 1000 cells were counted in each diploid to obtain the sporulation frequency.

Figure 1. Localization of *rad18* complementing ability and partial restriction map of the yeast DNA in the complementing cosmid. DNA segments from the approximately 34 kb yeast DNA insert in cosmid pSCW395, which contains *rad18* complementing ability, were inserted into either the 2 μ plasmid pTB220, or the *CEN4 ARS1* plasmid YCp50, and tested for their ability to restore UV resistance to *rad18* mutants. The open bars represent DNA fragments from the *RAD18* region that were cloned in the vector indicated on the right; the filled-in bar represents cosmid DNA. Symbols: A, *AhaII*; B, *BamHI*; E, *EcoRI*; H, *HindIII*; K, *KpnI*; S, *StuI*; Sa, *SalI*; X, *XbaI*; Xh, *XhoI*.

RESULTS

Cloning of the *RAD18* Gene

Since the *RAD18* gene is about 0.4 cM from the *SUP61* gene, we cloned the *RAD18* gene by using the *SUP61* gene as a probe to screen a cosmid library by colony hybridization. DNA from one of the positive clones, designated pSCW395, was found to complement the UV sensitivity of the *rad18-2* strain LP2729-4B. A partial restriction map of the approximately 34 kb yeast DNA insert in the cosmid pSCW395 is shown in Fig. 1. DNA fragments from pSCW395 were inserted into either the multicopy yeast plasmid pTB220 (10), which contains the yeast 2μ replication origin, or the centromere-containing plasmid YCp50 (25), which is maintained at about one copy per cell. Subcloning of the leftwards *XhoI-XhoI* fragment from pJJ127 yielded plasmid pJJ140, containing a 5.8 kb *BamHI-HindIII* fragment with *rad18* complementing activity (Fig. 1). This plasmid restored UV resistance to all the *rad18* mutants tested: *rad18-1*, *rad18-2*, *rad18-3*, and *rad18-4* (Fig. 2). The 3.2 kb *BamHI-StuI* segment in pJJ155 is the smallest DNA fragment tested which retains *rad18* complementation ability. A partial restriction map of a 2.5 kb *EcoRI-AhaII* fragment located within the 3.2 kb *BamHI-StuI* fragment is shown in Fig. 3.

Nucleotide Sequence of the *RAD18* Gene

The nucleotide sequence of the 2471 base *EcoRI-AhaII* fragment, was determined using the sequencing strategy given in Fig. 3. As shown in Fig. 4, there is only a single large open

Figure 2. Survival after UV irradiation (A) or ionizing radiation (B) in *rad18* and *RAD*⁺ strains with or without plasmids. Symbols: □, DBY747 + YCp50, *RAD*⁺; ■, LP2814-12A (*rad18-1*) + pJJ140; ○, LP2729-4B (*rad18-2*) + pJJ140; ●, LP2814-12A (*rad18-1*) + YCp50; △, LP2729-4B (*rad18-2*) + YCp50; ▲, DBY747 *rad18Δ::HIS3*. In this strain, the genomic *RAD18* gene is deleted. Similar results were obtained for complementation of *rad18-3* (strain LP2815-5A) and *rad18-4* (strain LP2816-2C) by plasmid pJJ140.

Figure 3. Restriction map of the 2.5 kb *EcoRI-AhaII* DNA fragment and strategy for determining its nucleotide sequence. The ATG initiating codon of the *RAD18* gene, to the left of the *BclI* site, and the TAA termination codon, to the right of the *StuI* site, are indicated. The horizontal lines with arrowheads represent the direction and extent of sequencing.

reading frame (ORF) in this region, beginning with the ATG codon at position +1 and extending to the TAA codon at position +1462.

5' and 3' Flanking Sequences of the *RAD18* Gene

The 5' flanking region of the *RAD18* gene is 66% AT and contains many poly(dA-dT) stretches. Deletion analysis of the upstream promoter elements in the *HIS3*, *PET56*, and *DED1* genes of yeast indicates that poly(dA-dT) stretches upstream of the TATA element are required for wild type constitutive transcription levels (26). The most striking poly(dA-dT) stretch in the *RAD18* gene is a 30 base region located at position -494 to -465; it contains 27 Ts, 19 of which are continuous, 2 Cs, and 1 A. This 30 base pair segment could be part of the constitutive promoter of the *RAD18* gene. An eleven base pair portion within this region, the sequence C A T T T T C T T T T, located at positions -475 to -465, is duplicated at positions -372 to -362. Although the 5' region is generally AT rich, no perfect matches occur for the proposed eukaryotic promoter element T A T A T/A A T/A which is usually found 26 to 34 nucleotides upstream of the mRNA start site in higher eukaryotes and is necessary for proper initiation of transcription (27,28). In mRNAs of higher eukaryotes, the nucleotides A, and to a lesser extent, G, are favored at positions -3 and +4 relative to the translation initiating ATG codon (29, 30). The *RAD18* gene has a C at position -3 and a G at position +4 (Fig. 4). The consensus sequence T A G ... T A G T or T A T G T ... (AT rich) ... T T T proposed to be a signal for transcription termination in yeast (31) occurs in the *RAD18* gene between positions +1565 and +1599.

The Coding Region of the *RAD18* Gene

The *RAD18* ORF encodes a protein of 487 amino acids, with a predicted molecular weight of 55,512. In the predicted RAD18 protein, 59 of the 61 codons are used, indicating no codon

Table 1. Amino acid composition of the RAD18 protein
(Percent amino acids is given in parentheses)

Ala	15	(3.1)	Leu	46	(9.4)
Arg	25	(5.1)	Lys	41	(8.4)
Asn	31	(6.4)	Met	16	(3.3)
Asp	31	(6.4)	Phe	17	(3.5)
Cys	12	(2.5)	Pro	26	(5.3)
Gln	22	(4.5)	Ser	61	(12.5)
Glu	34	(7.0)	Thr	29	(6.0)
Gly	18	(3.7)	Trp	4	(0.8)
His	11	(2.3)	Tyr	8	(1.6)
Ile	22	(4.5)	Val	18	(3.7)

Figure 5. Charge profile of the RAD18 protein. Vertical lines extending below the horizontal line represent the acidic residues, while those extending above the horizontal line represent the basic residues. Short lines that cross the horizontal represent proline residues.

bias as is observed for highly expressed genes of *S. cerevisiae*, such as alcohol dehydrogenase I and glyceraldehyde 3-phosphate dehydrogenase (32). This suggests that *RAD18* is not a well-expressed gene.

The amino acid composition of RAD18 protein is given in Table 1. The RAD18 protein contains 33.7% nonpolar, 37.2% polar, 13.3% acidic, and 15.8% basic residues, and thus is somewhat basic. The RAD18 protein also has a high serine content (12.5%). The distribution of acidic and basic residues within the RAD18 protein is striking (Fig. 5), since between residues 106 and 140, there are 11 acidic amino acids and no basic amino acids, and the carboxyl terminal 68 amino acids contain 20 acidic and only 6 basic residues. The region of 279 residues between these two "acidic regions" contains 58 basic and only 25 acidic amino acids.

The RAD18 protein has 12 cysteine residues, 10 of which are found in regions which could form zinc binding DNA binding domains (12,13, 33). The zinc binding, nucleic acid binding finger domains were first described in the transcription factor TFIIIA (33), which is involved in controlling 5S RNA transcription in *Xenopus*. Since then, these sequences have been found in other transcriptional factors, and also in proteins that bind nucleic acids, such as the DNA repair UvrA protein of *E. coli* and the single-strand nucleic acid binding gene 32 protein of phage T4 (13). As shown in Table 2, the RAD18 protein contains three sequences that match the consensus metal binding sequences present in nucleic acid binding proteins.

Table 2. Putative metal binding, nucleic acid binding, finger domains in the RAD18 protein.

Residues	Sequence
28 - 48	C - X ₂ - C - X ₁₁ - C - X ₄ - C
51 - 65	C - X ₃ - H - X ₆ - C - X ₂ - C
190 - 210	C - X ₂ - C - X ₁₂ - H - X ₃ - C
Consensus sequence:	C/H - X₂₋₄ - C/H - X₂₋₁₅ - C/H - X₂₋₄ - C/H

The consensus sequence is from (12,13).

The RAD18 protein also contains a conserved sequence that is present in a number of proteins that bind and hydrolyze ATP (14), such as the *E. coli* DNA repair and recombination proteins UvrA (34), UvrD (35), RecA (14), and RecB(36), and the *S. cerevisiae* RAD3 protein (37) (Table 3). In the 21 amino acid segment shown in Table 3, 8 residues are identical between the RAD18 and RAD3 proteins. The *RAD3* gene of *S. cerevisiae* is essential for cell viability (38,39) and is also required for excision repair of UV damaged DNA. The RAD3 protein is a single-standed DNA-dependent ATPase and possesses DNA helicase activity that can unwind long regions of double-stranded DNA (40,41).

Comparison of the RAD18 protein and protein sequences present in GenBank, EMBL, and NBRF databanks, analyzed using the University of Wisconsin programs (42) did not identify any protein which is homologous to the RAD18 protein.

Transcription of the *RAD18* Gene

The 227 nucleotide *SalI*-*XbaI* internal fragment of the *RAD18* gene, when cloned in M13mp18 but not M13mp19, hybridizes to a single transcript of about 1.7 kb (Fig. 6), indicating that the direction of transcription is from the *SalI* site toward the *XbaI* site (Fig. 3). The observed size of the *RAD18* transcript and the direction of transcription are consistent with the identification of the *RAD18* ORF (Fig. 4).

Characterization of a *rad18-Δ* Mutation

Since all the previously known mutations in the *RAD18* gene were point mutations and therefore could be leaky, we constructed a genomic deletion of the *RAD18* gene and examined its effect on viability, sporulation, UV sensitivity, and UV mutagenesis. The genomic *rad18* deletion extends from the *BalI* site at nucleotide +132 of the open reading frame to nucleotide +1484, 20 bases beyond the TAA termination codon (Fig. 4). As expected, the *rad18Δ* mutation was allelic to *rad18* point mutations, confirming that the cloned gene was indeed *RAD18*. We observed no effect of the *rad18Δ* mutation on viability, indicating that *RAD18* is not an essential

Table 3. Homology between putative nucleotide binding sequence of the RAD18 protein and other known yeast and *E. coli* ATPases.

Protein	Residues	Sequence															Reference						
RAD18	353-373	<u>G</u>	<u>G</u>	I	<u>S</u>	<u>K</u>	<u>L</u>	M	I	M	K	S	N	<u>G</u>	<u>K</u>	<u>S</u>	S	<u>S</u>	Y	R	K	<u>L</u>	this paper
RAD3	35-55	<u>G</u>	<u>G</u>	N	<u>S</u>	<u>I</u>	<u>L</u>	E	M	P	S	G	T	<u>G</u>	<u>K</u>	<u>T</u>	V	<u>S</u>	L	L	S	<u>L</u>	37
UvrA	633-653	G	L	F	T	C	I	T	G	V	S	G	S	<u>G</u>	<u>K</u>	<u>S</u>	T	L	I	N	D	T	34
UvrD	22-42	R	S	N	L	L	<u>V</u>	L	A	G	A	G	S	<u>G</u>	<u>K</u>	<u>T</u>	R	V	L	V	H	R	35
RecA	59-79	G	R	I	V	E	<u>I</u>	Y	G	P	E	S	S	<u>G</u>	<u>K</u>	<u>T</u>	T	L	T	L	Q	V	14
RecB	16-36	Q	G	E	R	L	<u>I</u>	E	A	S	A	G	T	<u>G</u>	<u>K</u>	<u>T</u>	F	T	I	A	A	L	36

Identical or similar residues shared by all proteins are boxed. Identical residues between RAD18 and RAD3 proteins are underlined.

Figure 6. Transcript of the *RAD18* gene. Poly(A)⁺ RNA (2 μ g) was dissolved in glyoxal, fractionated through a 1% agarose gel, transferred to gene screen paper, and hybridized to the 227 base *Sall-XbaI* internal fragment of the *RAD18* gene (see Fig. 3) cloned into M13mp18 phage. No hybridization was detected when the 227 base *Sall-XbaI* fragment was cloned into M13mp19 phage (results not shown). The size markers on the right are ³²P end labeled *HaeIII* digested ϕ X174 DNA.

gene. The *rad18*- Δ strains were as sensitive to UV and gamma irradiation as the strains carrying the most sensitive *rad18* point mutant alleles (Fig. 2). Percent sporulation and spore viability were similar for *rad18*- Δ /*rad18*- Δ and isogenic *RAD*⁺/*RAD*⁺ or *RAD*⁺/*rad18*- Δ strains. UV induced reversion at the *arg4-17* locus was similar in *RAD*⁺ and *rad18*- Δ strains.

DISCUSSION

We have cloned the *RAD18* gene and determined its nucleotide sequence. The protein predicted by the *RAD18* open reading frame contains 487 amino acids with a predicted molecular weight of 55,512. Since deletions of the *S. cerevisiae* *RAD3* gene are recessive lethals (38,39), we examined whether strains carrying a *rad18* deletion mutation show any additional phenotypes, and whether in *rad18* Δ mutants, cellular processes are affected to a greater extent than in the *rad18* point mutants. However, deletions of the *RAD18* gene do not affect viability, are no more UV sensitive than the most sensitive *rad18* point mutants, and show wild type levels of sporulation, spore viability, and UV induced reversion.

RAD18 is in the same epistasis group as *RAD6*, which has recently been shown to be an E2 ubiquitin-conjugating enzyme that attaches ubiquitin to histones H2A and H2B *in vitro* (43). *RAD6* shows considerable sequence homology with *CDC34*, a cell division cycle mutant which is another E2 ubiquitin-conjugating enzyme with the same substrate specificity as *RAD6* (44). The sequence of *RAD18* protein was compared to that of *RAD6* and *CDC34*, and also to the yeast ubiquitin proteins UBI1, UBI2, and UBI3 (45), but no significant homologies were detected.

The predicted sequence of the *RAD18* protein contains two structural features of special interest. Three regions in the *RAD18* protein could form zinc binding, nucleic acid binding finger domains (Table 2, Fig. 4). The first region, from residues 28 to 48, resembles the CC-CC type sequence that has been identified in various proteins, including the yeast regulatory proteins

GAL4, PRR1, and ARGII which bind the specific upstream promoter sequences (13). This sequence also occurs in the *E. coli* UvrA protein, which binds damaged sites in DNA and is required for excision repair of DNA (13). The second region, from residues 51 to 65, is similar to the CH-CC type sequence present in the phage T4 gene 32 protein (12,13), which binds single-stranded nucleic acids in a non-specific manner, and is required for T4 DNA replication, recombination, and repair. The third region, from residues 190-210, resembles the CC-HC type sequence that has been observed in the retrovirus nucleic acid binding protein(s) and in the yeast transcriptional activator SWI5 (13). Another feature of interest in the RAD18 protein is the presence of a sequence between residues 353 to 373 which shows homology with the amino acid sequence present in proteins that bind and hydrolyze ATP (Table 3). The structural features observed in the RAD18 encoded protein suggest that it binds nucleic acids via its zinc fingers, and binds and hydrolyzes a nucleotide(s).

rad18 mutants are defective in postreplication repair of UV damaged DNA (3,4). Discontinuities in the newly synthesized DNA from UV irradiated templates persist in *rad18* mutants, whereas in Rad⁺ strains these discontinuities are repaired. Since *rad18* mutations do not affect mutagenesis or recombination after UV irradiation, the RAD18 gene product seems to be involved in error free nonrecombinational repair of gaps that presumably occur in the newly synthesized DNA strand across from the damage in the template strand. One possibility for the action of RAD18 protein in DNA repair is that it binds the damaged site in the template DNA strand. This binding could interfere with mutagenic bypass of the lesion and allow DNA polymerases and other components of the repair complex to carry out error free repair of the gap, perhaps by using the non-damaged strand of the sister duplex as template. The RAD18 protein could bind a nucleotide such as ATP, and the ATP-bound form of RAD18 might have higher affinity for the damaged DNA, whereas ATP hydrolysis by the RAD18 protein might effect its turnover from the DNA. The RecA protein of *E. coli* binds preferentially to UV irradiated DNA and this activity has been suggested to effect bypass of lesions with reduced fidelity (46). Another possibility for the action of RAD18 protein is that it is a transcriptional activator of genes involved in DNA repair. The RAD18 protein may bind specific upstream regulatory sequences via its zinc fingers, and nucleotide binding by the RAD18 protein may modulate this activity. Like the RecA protein of *E. coli*, RAD18 may be involved in DNA repair both mechanistically and in a regulatory role. The RAD18 gene is being fused to a highly expressed yeast promoter to facilitate overproduction, purification, and characterization of the encoded protein. Such an approach has been used successfully in the purification and characterization of the RAD3 and RAD6 proteins (11,40,41).

ACKNOWLEDGEMENTS

We thank Maynard Olson for providing the *SUP61* probes used for isolating the RAD18 gene and Gerry Fink for providing the cosmid library. This work was supported by NIH grant GM19261.

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