Transcript levels of the *Saccharomyces cerevisiae* DNA repair gene *RAD18* increase in UV irradiated cells and during meiosis but not during the mitotic cell cycle

Jeffery S. Jones⁺ and Louise Prakash^{*}

Department of Biophysics, University of Rochester School of Medicine, Rochester, NY 14642, USA

Received October 3, 1990; Revised and Accepted January 4, 1991

ABSTRACT

We have examined the transcript levels of the *Saccharomyces cerevisiae* DNA repair gene *RAD18* in UV irradiated cells, in the mitotic cell cycle, and during meiosis. Levels of *RAD18* mRNA increased upon UV irradiation, but remained constant during the mitotic cell cycle. During meiosis, *RAD18* mRNA levels rose about 4 fold at a stage coincident with the period when high levels of genetic recombination occur. *RAD18* resembles the other DNA repair genes, *RAD2*, *RAD6*, *RAD7*, *RAD23*, and *RAD54*, all of which also exhibit increased transcription in response to DNA damage and during meiosis. Comparisons of sequences in 5' flanking regions of *RAD* genes suggest that different upstream sequences are involved in regulating the expression of DNA repair genes belonging to different epistasis groups.

INTRODUCTION

Organisms respond to environmental stresses by increasing the production of specific classes of proteins. The *Escherichia coli* SOS response is a well-studied example of transcriptional induction in response to DNA damage, where the *LexA* and *RecA* genes control the coordinate induction of about 20 DNA repair genes (1,2). A large number of genes, over 30, are known to be involved in DNA repair in the yeast *Saccharomyces cerevisiae*. Of these genes, the *RAD2*, *RAD6*, *RAD7*, *RAD23*, and *RAD54* have been shown to be inducible by DNA damage (3–8). The *RAD2*, *RAD7*, and *RAD23* genes function in excision repair, the *RAD6* gene is required for postreplication repair and for DNA damage induced mutagenesis and sporulation, and the *RAD54* gene is required for the repair of DNA double strand breaks and for recombination. Transcription of these genes also increases during meiosis (5,6,8–11). Several DNA damage inducible genes, *DIN* (12) and *DDR* (13) have been identified in *S. cerevisiae* by direct screening procedures, and transcription of the DNA replication genes *CDC9* and *CDC17*, that encode DNA ligase and DNA polymerase I, respectively, increases in response

to DNA damage (14,15). Other DNA damage inducible genes in yeast include the *UBI4* (16) and *RNR2* (17,18) genes.

In contrast to *Escherichia coli*, in yeast, the evidence for a common regulatory mechanism controlling the induced transcription of genes in response to DNA damage has been lacking. However, since the various DNA damage inducible genes of yeast, such as *UBI4*, *RNR2*, and possibly *DIN* and *DDR*, are involved in aspects of cellular metabolism not directly related to DNA repair, they could be responding to an inducing signal other than DNA damage. Likewise, the DNA damage induced expression of DNA replication genes might be occurring in response to other factors such as inhibition of DNA replication. In contrast, genes involved specifically in repairing DNA damage could all be responding to the same signal and might be controlled by a common regulatory mechanism. Alternatively, genes belonging to only a particular epistasis group, *i.e.*, excision repair, postreplication repair, or the double strand break repair and recombination group, might share a common regulatory mechanism. Thus, with the aim of determining the mechanism(s) of regulation of DNA repair genes, we have studied the transcription of DNA repair genes which belong to different epistasis groups in response to DNA damage, and also during the mitotic and meiotic cell cycles.

In this paper, we report our studies on the regulation of the *S. cerevisiae* *RAD18* gene. *rad18* mutants are highly sensitive to a variety of DNA damaging agents, including ultraviolet light (UV), ionizing radiation, and alkylating agents. The *RAD18* gene belongs to the *RAD6* epistasis group and like the *RAD6* gene, it is required for postreplication repair of UV damaged DNA (19). However, unlike *RAD6*, *RAD18* is not involved in UV mutagenesis (20). Previously, we showed that *RAD18* encodes a 55.5 kD protein containing putative ATP binding sequences and zinc finger domains for DNA binding (21). Here, we show that *RAD18* transcript levels increase in cells exposed to UV light and also during meiosis, but remain constant during the mitotic cell cycle.

Comparisons of 5' flanking sequences in various DNA damage inducible *RAD* genes suggest the involvement of different upstream sequences in transcriptional induction of genes

* To whom correspondence should be addressed

⁺ Present address: McArdle Laboratory for Cancer Research, University of Wisconsin, 1400 University Avenue, Madison, WI 53706, USA

belonging to different epistasis groups. The common upstream sequences present in the *RAD18* and *RAD6* genes differ from the upstream regulatory sequences that have been identified in genes belonging to the excision repair group or in the double strand break repair and recombination group gene *RAD54*.

MATERIALS AND METHODS

Strains and Media

S. cerevisiae haploid strains used were DBY747: *MATa his3-Δ1 leu2-3 leu2-112 trp1-289 ura3-52*; J19: strain DBY747 with *rad18-Δ:LEU2*; 4910-3-3a (from J. Huberman): *MATa bar1-1 cdc7-4 his7 ura1*; g833-1B (from J. Game): *MATa leu2 can1 his1-1 trp2 ho gal*; g833-2D (from J. Game): *MATα hom3-10 his1-7 ade2 ho gal*. The diploid strain g857 was obtained by crossing strains g833-1B and g833-2D followed by selection of diploids on synthetic minimal medium supplemented with histidine. Strains were propagated using media prepared as described (22) except that prespore and sporulation media used to test transcript levels during meiosis were similar to media described (23) with modifications suggested by John Game. Prespore medium contained 6.7 grams per liter (gm/l) Difco Yeast Nitrogen Base without amino acids, 10 gm/l Difco Yeast Extract, 20 gm/l Difco Bacto Peptone, and 10 gm/l potassium acetate (Sigma). Potassium phthalate (Sigma) was prepared as a 0.25 M stock, the pH adjusted to 5.0 with KOH and diluted 1:5 to a final concentration of 0.05 M in the media. The medium was supplemented with 20 milligrams per liter (mg/l) L-histidine (Sigma). Sporulation medium used for these experiments was 1% potassium acetate supplemented with 4 mg/l L-histidine.

UV Irradiation of Liquid Cultures

Strain DBY747 was grown in YPD medium at 30°C to a density of 2×10^7 cells/ml. Cells were collected by filtration and resuspended in sterile glass distilled H₂O. 75 ml of cell suspension were placed in sterile pyrex dishes (9" × 13" baking dishes) and exposed to UV radiation and cell suspensions were continuously mixed with sterile glass rods to ensure uniform UV exposure. Following UV irradiation, cells were filtered, resuspended in fresh YPD medium prewarmed to 30°C, and incubated at 30°C in yellow light to avoid photoreactivation. The time of resuspension was considered to be 0 min after irradiation. Samples were collected at time 0 and at 20 min intervals for the first hour, and 30 min intervals thereafter. Cells from the 0 time points were diluted and plated to determine the fraction of survivors, which was 82%, 76% and 71% after 37, 50 and 70 J/m² respectively.

Heat Shock

A 500 ml culture of strain DBY747 was grown in YPD medium at room temperature (23°C) with constant agitation, to a density of approximately 4×10^6 cells/ml. The culture was heat shocked by transferring the culture to 39°C. Samples of cells were collected immediately and at 15, 30, 45, and 60 min after the temperature shift.

Mitotic Cell Cycle

Strain 4910-3-3a (24) was grown at 23°C in YPD medium to a density of 5×10^6 cells per ml. Yeast mating factor α (Sigma) was added to the culture to a final concentration of 20 ng/ml. Samples were collected at 1 hour intervals and aliquots inspected microscopically to determine cell morphology. Three hours after

addition of α factor, cells were filtered, washed once in sterile glass distilled H₂O, once in fresh YPD medium and resuspended in a volume of fresh YPD medium equal to the pre-filtration volume. Cells were incubated, samples taken at 30 min intervals, and scored for morphology.

Meiosis

A preculture of the *MATa/MATα* diploid strain g857 was grown in 10 ml liquid presporulation medium at 30°C with constant agitation. A large volume prespore medium culture was inoculated from the preculture and grown overnight to a density of approximately 2×10^7 cells per ml. The culture was filtered and cells resuspended in sporulation medium prewarmed to 30°C. The culture was incubated at 30°C, agitated vigorously and samples were taken at 30 min intervals for the first two hours and at one hour intervals thereafter. Each sample was examined microscopically and cell density and morphology recorded. Dilutions of the samples were plated on synthetic complete and synthetic complete medium lacking histidine to assay for *HIS1*⁺ recombinants formed during meiosis.

RNA Isolation, Gel Electrophoresis, and Northern Hybridization

These methods were as described previously (3,25).

Radiolabeling and Purification of Double Strand Hybridization Probes

DNA fragments were obtained by digestion with restriction endonucleases, gel purified and electroeluted or isolated with Gene Clean (Bio 101) and diluted to a concentration of 2.5–5 ng/μl. The 745 base pair *Sall/StuI* fragment of pJJ140 (21) was used as a probe for *RAD18*. The 1.1 kb *HindIII* fragment of pTRT2 (kindly provided by Dr. David Norris) which carries the 3' two-thirds of the histone H2B coding region (26) was used as a probe for histone H2B mRNA. A 1.2 kb *BglII/PstI* fragment (provided by Dr. S. Lindquist, University of Chicago) containing the coding region of HSP26 (27) was used to detect the heat shock inducible *HSP26* mRNA. The 1.1 kb *URA3*-containing *HindIII* fragment of YEp24 was used to detect *URA3* and *ura3-52* transcripts. An approximately 1.9 kb fragment of pSB4 (provided by Dr. S. Baker, University of Rochester) was used to detect *HIS4* mRNA.

Radiolabeled probes were made as described (6).

Densitometry

Quantitation of mRNA levels was carried out using an LKB Bromma ULTROSAN XL automated scanning laser densitometer as described earlier (6).

RESULTS

RAD18 mRNA levels increase in UV irradiated cells

To determine the effect of UV irradiation on *RAD18* expression, total RNA was isolated from strain DBY747 exposed to 37, 50, or 70 J/m² of UV light, and *RAD18* mRNA levels examined by Northern blot hybridizations (Fig. 1A–C). Fig. 1D shows the quantitation of *RAD18* mRNA in UV irradiated cells. Since the level of *ura3* mRNA does not change in UV irradiated cells, *RAD18* mRNA levels were normalized with the *ura3* mRNA to correct for variation in loading. At all UV doses, *RAD18* mRNA levels declined immediately after UV irradiation. Thereafter, *RAD18* mRNA levels increased rapidly, with maximal

Fig. 1: *RAD18* mRNA levels are elevated after UV irradiation. Yeast strain DBY747 was exposed to UV radiation and total RNA isolated. 50 μ g of total RNA were resolved per lane. The Northern blots were simultaneously probed with multiprime (Amersham) labeled *RAD18* and *URA3* specific probes. Composites of different exposures of the blots were combined to show optimal exposures of the *RAD18* and *ura3-52* bands. (A) Lane 1 in (A), (B), and (C), RNA from untreated cells. Lanes 2–7, RNA from cells at 0, 20, 40, 60, 90 and 120 min, respectively after exposure to 37 J/m² UV radiation. (B) Lanes 2–9, RNA from cells at 0, 20, 40, 60, 90, 120, 150 and 180 min, respectively after exposure to 50 J/m² UV radiation. (C) Lanes 2–8, RNA from cells at 0, 20, 40, 60, 90, 120 and 150 min, respectively after exposure to 70 J/m² UV radiation. (D) Quantitation of the autoradiograms shown in (A), (B) and (C) indicates the extent to which the levels of *RAD18* mRNA change after exposure of cells to UV radiation. Multiple exposures of the blots were scanned on a laser densitometer. The ratio of the level of *RAD18* mRNA to that of the *ura3-52* mRNA (*ura3* mRNA level is not affected by UV treatment) was calculated for each lane to correct for unequal loading. The values determined for each timepoint were plotted relative to the value for untreated cells (NT). Symbols: □, (37 J/m²); ○, (50 J/m²); and △, (70 J/m²).

accumulation occurring at 40 min after 37 and 50 J/m² and at 60 min after 70 J/m². *RAD18* mRNA levels then fell to levels which were lower than those observed in cells from the vegetatively growing culture (Fig. 1A–C, lane 1), suggesting that under these experimental conditions, lower basal levels of *RAD18* mRNA are attained. At all UV doses, peak levels of *RAD18* mRNA are about 4 fold higher than the levels reached under these experimental conditions at later periods. We have confirmed the observation of UV induction of *RAD18* mRNA levels in several different experiments. No increase in *RAD18* mRNA levels occurred in cells that were not UV irradiated but were otherwise treated in an identical manner to the UV irradiated sample.

Heat shock does not induce *RAD18* transcription

Since some of the DNA damage inducible genes of *S. cerevisiae* are also induced by heat shock (16,28,29), we examined whether *RAD18* transcription was inducible by heat shock. Upon transfer of yeast cells to 39°C, *RAD18* mRNA levels fell in the 15 and 30 min samples and then recovered to basal levels by 60 min (Fig. 2A). *HIS4* mRNA levels also showed a similar response to heat shock, a pattern characteristic of non heat shock inducible

Fig. 2: The level of *RAD18* mRNA is not elevated following heat shock. Yeast strain DBY747 was heat shocked by shifting the culture to 39°C. Aliquots were taken immediately after the shift, time 0 (lane 1) and at 15, 30, 45 and 60 min after the shift (lanes 2–5 respectively). (A) 50 μ g of total RNA were resolved per lane, and probed for the *HIS4* and *RAD18* mRNAs. (B) 2 μ g of total RNA were resolved per lane and probed for the *HSP26* mRNA.

Fig. 3: *RAD18* mRNA levels in the mitotic cell cycle. (A) Yeast strain 4910-3-3a was grown in YPD medium to a density of 4.5×10^6 cells/ml, and α factor added to synchronize cells in G1. Samples were collected prior to the addition of α factor (NT, not treated with α factor), after 1, 2 and 3 hours of α factor arrest (indicated by numbers with minus signs), immediately after release from α factor arrest (designated 0) and at 30 min intervals for 5 hours. Aliquots of the samples were examined microscopically and the cell number (■) and percent budded cells (□) for each time point determined. (B) Total RNA from the various timepoints in (A) was isolated and 50 μ g total RNA resolved per lane. The Northern blots were simultaneously probed for *RAD18*, and histone *H2B* mRNA, and later washed and reprobbed with a *URA3* specific probe. This figure is a composite to show optimal exposures of the different transcripts. Lane 1, RNA from the *rad18*- Δ strain JJ9. The *ura3*-52 transcript present in this strain comigrates with the *H2B* transcript. Lane 2, RNA from cells prior to α factor treatment (NT in panel A). Lanes 3–5, RNA from cells exposed to α factor for 1, 2, and 3 h. Lanes 6–16, RNA from cells at time 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 h respectively after release from the α factor arrest. The light *URA3* bands in lanes 5 and 6 are due to a transfer artifact. (C) Quantitation of *RAD18* and *H2B* mRNA levels in the mitotic cell cycle. The autoradiograms corresponding to lanes 2–16 in (B) were scanned with a laser densitometer. To control for unequal loading, the values determined for the *URA3* mRNA level (which is not regulated in the mitotic cell cycle) were used to normalize the *RAD18* (□) and *H2B* (○) mRNA levels and the values plotted. The *URA3* mRNA level in lane 4 was used for quantitating the relative *RAD18* and *H2B* mRNA levels in lanes 5 and 6. The time of release from α factor arrest is indicated by 0. Numbers with minus signs represent time during the α factor treatment.

genes. However, in these experimental conditions, we observed a rapid and dramatic increase in the amount of heat shock inducible *HSP26* mRNA (Fig. 2B).

***RAD18* mRNA levels remain constant during the mitotic cell cycle**

Since *RAD18* functions in postreplication repair of UV damaged DNA (19), and *rad18* mutants show an increased incidence of

spontaneous mitotic recombination and mutation (30,31), *RAD18* could be a component of the DNA replication machinery, and like the DNA replication genes *CDC8*, *CDC21* (32), *CDC9* (14), and *CDC17* (15), may show increased transcription at the G1/S phase boundary in the cell cycle. To examine the levels of *RAD18* mRNA in the mitotic cell cycle, we used the strain 4910-3-3a because it can be easily synchronized by low α factor concentrations, and synchronous cell divisions are maintained

Fig. 4: The level of *RAD18* mRNA is elevated during sporulation. Yeast strain g857 was grown in presporulation medium, and transferred to sporulation medium at a density of 2×10^7 cells/ml. Aliquots were taken at various times after the transfer to sporulation medium, total RNA isolated and 50 μ g of total RNA resolved per lane. After transfer to GeneScreen membrane, the RNA was visualized by shortwave UV light to ensure equality of RNA levels in each lane. Northern blots were probed with multiprimer (Amersham) labeled *RAD18* and histone *H2B* (*TRT2*) specific probes. (A) *RAD18* and *H2B* mRNA levels during sporulation. This figure is a composite of different exposures of the two transcripts. Lanes 1–11, total RNA from cells at 0, 0.5, 1.0, 1.5, 2, 3, 4, 5, 6, 7 and 8 h respectively after transfer to sporulation media. (B) Quantitation of *RAD18* mRNA levels during sporulation. The *RAD18* transcript levels for the various timepoints were normalized to *RAD18* transcript level at time 0.

for at least two cell cycles after release from α -factor arrest (24). Fig. 3A shows the level of synchrony achieved by α factor treatment. Following incubation with α factor for 3 hours, almost all of the cells arrested as single, large, and unbudded G1-stage cells. After release from α factor arrest, three synchronous cell divisions occurred, as attested by the periodic increase in the frequency of budded cells which was followed by a doubling in cell density. Fig. 3B shows the *RAD18*, histone *H2B*, and *URA3* mRNA levels during different stages of the cell cycle and Fig. 3C shows the quantitation of *RAD18* and *H2B* mRNA normalized to *URA3* mRNA, since *URA3* mRNA remains constant during the cell cycle. *H2B* mRNA levels declined dramatically in α factor treated cells (compare lanes 3–5 to lane 2, Fig. 3B). After release from α factor arrest, *H2B* mRNA levels showed three cycles of periodic accumulation (Fig. 3C), and the peak level of *H2B* mRNA at 0.5, 2.5, and 4 h (lanes 7, 11, and 14, Fig. 3B; Fig. 3C) immediately precedes the time when the maximal frequency of budding cells is observed (Fig. 3A). The pattern of cyclic fluctuation in *H2B* mRNA is consistent with previous studies which demonstrated that *H2B* mRNA levels reach a maximum

A. Excision repair genes

RAD2 -172 C T C C G T G G A G G C A T T A A A A
RAD23 -300 C T C G G T G G C G A A A T T G A A A

B. Double strand break repair and recombination gene

RAD54 -256 C G C G T T A C C C A A T A T A G C A A A G T T T C G C G

C. Postreplication repair genes

RAD6 -228 T G A C T A C A T T T T C C C G
RAD18 -335 T A A C T T C T T T T T C C C G

Fig. 5: Comparisons of upstream sequences in DNA damage inducible *RAD* genes. The numbers refer to the distance between the first nucleotide in the sequence and the translation initiating ATG codon. Sequences are from References 3 and 10 for *RAD2*; H. Qiu and S. Prakash (personal communication) for *RAD23*; 39 for *RAD54*; 40 for *RAD6*; and 21 for *RAD18*.

early in S phase (33, 34). In sharp contrast to the periodic expression of *H2B* mRNA, *RAD18* mRNA levels remained constant throughout the cell cycle (Fig. 3C).

RAD18 mRNA levels increase during meiosis

Since the DNA repair genes *RAD2*, *RAD6*, *RAD7*, *RAD23*, and *RAD54* show induced expression in response to DNA damage as well as during meiosis, we determined whether *RAD18* also shows regulated expression during meiosis. We examined *RAD18* mRNA levels during sporulation in the *MATa/MAT α* diploid strain g857. This strain undergoes very efficient and synchronous meiosis. As judged by the appearance of *HIS1*⁺ prototrophs, meiotic recombination in this strain began at about 2 h and plateaued by about 5 h. No spore formation occurred until about 6 h. The frequency of sporulating cells increased dramatically between 7 and 8 h, and over 90% of cells had formed asci by 10 h. At various times after transfer of strain g857 to sporulation medium, total RNA was isolated and Northern blots hybridized with *RAD18* and histone *H2B* probes. Fig. 4A shows the levels of *RAD18* and *H2B* mRNA during sporulation, and Fig. 4B presents the quantitation of *RAD18* mRNA. *RAD18* mRNA levels fell shortly after transfer to sporulation medium (Fig. 4A, lane 2), then the level of *RAD18* mRNA rose gradually, increasing 4 fold at peak levels between 4 and 5 h (Fig. 4A, lanes 7 and 8). *RAD18* mRNA levels then declined to near basal levels in 6–8 h samples (Fig. 4A, lanes 9–11). Other independent experiments have verified the increased expression of *RAD18* during meiosis. The pattern of *H2B* mRNA accumulation during meiosis is similar to that observed previously by us and others (5,6,35).

To confirm that the increase in *RAD18* mRNA occurred as a specific response of cells undergoing meiosis and was not due to starvation conditions of the sporulation medium, we examined the level of *RAD18* mRNA in the asporogenous *MATa/MAT α* strain g721–2, which is closely related to the *MATa/MAT α* strain g857. Strain g721–2 was grown in presporulation medium, transferred to sporulation medium, and *RAD18* mRNA levels examined at various periods up to 8 h after transfer to sporulation medium. In this strain, *RAD18* mRNA levels remained constant during incubation in sporulation medium (results not shown). Thus, these observations demonstrate that *RAD18* mRNA levels increase during meiosis.

DISCUSSION

Our observations indicate that *RAD18* mRNA levels increase in UV irradiated cells. Compared to the level in vegetatively growing cells, *RAD18* mRNA levels were elevated about 2.5 fold at 1 hour following 70 J/m² of UV irradiation. However, relative to the basal *RAD18* mRNA levels attained under these experimental conditions, the increase in *RAD18* mRNA in UV irradiated cells was about 4 fold. The magnitude of increase in the level of *RAD18* mRNA in response to UV irradiation is lower than that observed by us and our coworkers for the other UV inducible genes: *RAD2*, *RAD6*, *RAD7*, and *RAD23* (3,5,6,8). In contrast to the DNA damage inducible genes *DDR* (29) and *UBI4* (16,28), which are also induced by heat shock, *RAD18* transcription did not increase upon heat shock. Thus, the increase in the level of *RAD18* mRNA in UV irradiated cells reflects a specific response of *RAD18* to DNA damage rather than a general stress response.

RAD18 resembles the damage inducible DNA repair genes *RAD2*, *RAD6*, and *RAD23* (5,8,9) in exhibiting constant transcript levels during the mitotic cell cycle. This indicates that the increase in *RAD18* mRNA levels in UV irradiated cells does not arise from the cell cycle arrest phenomenon that occurs in response to DNA damage (36,37).

RAD18 mRNA levels increased about 4 fold during meiosis between 4 and 5 h. Using the same strain and identical experimental conditions, we and our coworkers previously reported increased transcription of *RAD2*, *RAD6*, *RAD7*, and *RAD23* genes in meiosis (5,6,8,9). For all these genes, maximal mRNA accumulation occurs between 3 to 7 h, which is coincident with the period when high levels of meiotic recombination occur. A function for *RAD18* in meiosis is indicated from studies in which a *rad18* mutation was coupled with mutations in the excision repair genes *RAD1*, *RAD2*, or *RAD3* (38). Even though a single mutation in any of the excision repair genes or in the *RAD18* gene has no obvious effect on sporulation, spore viability, or meiotic recombination, *rad1 rad18*, *rad2 rad18*, and *rad3 rad18* double mutants suffer from a drastic reduction in spore viability (38), suggesting that as for DNA repair in mitotic cells, the excision repair genes and the *RAD18* gene constitute alternate DNA repair pathways during meiotic development.

A comparison of sequences between the upstream regions of several DNA damage inducible *RAD* genes is shown in Fig. 5. The excision repair genes *RAD2* and *RAD23* share 14 identical residues in a 19 nucleotide long sequence (Fig. 5A). Deletion analysis of the 5' flanking region in *RAD2* suggests the involvement of this sequence in DNA damage inducible expression (10). This upstream sequence has also been implicated in the UV induction of *RAD23* transcription (H. Qiu and S. Prakash, personal communication). The upstream regulatory sequence in the *RAD2* and *RAD23* genes is different from the upstream sequence that has been shown to regulate the DNA damage induction of *RAD54* (39), a double strand break repair and recombination gene (Fig. 5B). The *RAD6* and *RAD18* genes do not possess any upstream sequence that shows a high degree of homology to either the *RAD2* or *RAD23* sequence, or the *RAD54* sequence. Instead, *RAD6* and *RAD18* share a different sequence in which 12 of the 15 nucleotides match. These observations suggest that regulatory mechanisms may differ among DNA repair genes belonging to different epistasis groups.

ACKNOWLEDGEMENT

This work was supported by Public Health service Grant GM19261 from the National Institutes of Health.

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