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

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**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 *RRN2* (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 repair 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...
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: MATα his3Δ1 leu2-3 leu2-112 trp1-289 ura3-52; 199: strain DBY747 with rad18Δ-ΔLEU2; 4910-3-3a (from J. Huberman): MATα barl-1 cdc7-4 his1 ura1; g833-1B (from J.Game): MATα 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 (23) 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 medium. 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 x 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" x 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/m^2 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 x 10^9 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 x 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.

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/m^2 of UV light, and RAD18 mRNA levels examined by Northern blot hybridizations (Fig. 1A–C). Fig. 1D shows the quantification 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. Therefore, 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 μg 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, peaks 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 genes.

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 μg of total RNA were resolved per lane, and probed for the HIS4 and RAD18 mRNAs. (B) 2 μg 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 $4.5 \times 10^6$ cells/ml, and $\alpha$ factor added to synchronize cells in G1. Samples were collected prior to the addition of $\alpha$ factor (NT, not treated with $\alpha$ factor), after 1, 2 and 3 hours of $\alpha$ factor arrest (indicated by numbers with minus signs), immediately after release from $\alpha$ 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 µg total RNA resolved per lane. The Northern blots were simultaneously probed for RAD18, and histone H2B mRNA, and later washed and reprobed with a URA3 specific probe. This figure is a composite to show optimal exposures of the different transcripts. Lane 1, RNA from the rad18-A strain JJ9. The ura3-52 transcript present in this strain comigrates with the H2B transcript. Lane 2, RNA from cells prior to $\alpha$ factor treatment (NT in panel A). Lanes 3-5, RNA from cells exposed to $\alpha$ 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 $\alpha$ 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 $\alpha$ factor arrest is indicated by 0. Numbers with minus signs represent time during the $\alpha$ factor treatment.

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 $\alpha$ factor concentrations, and synchronous cell divisions are maintained.
mRNA is consistent with previous studies fluctuation in of budding cells is observed (Fig. 3A). The pattern of cyclic treated cells (compare lanes 3-5 to lane 2, Fig. 3B). After release mRNA levels declined dramatically in a factor H2B mRNA remains constant during mRNA, since URA3 to cell density. Fig. 3B shows the quantitation of RAD18 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 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).

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 x 10^7 cells/ml. Aliquots were taken at various times after the transfer to sporulation medium, total RNA isolated and 50ug 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 multiprime (Amersham) labeled RAD18 and histone H2B (TRT2) specific probes. (A) RAD18 and H2B mRNA levels during sporulation. (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.

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/MATα 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 asc 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/MATa strain g721-2, which is closely related to the MATa/MATα 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.

REFERENCES