Regulated expression of the *Saccharomyces cerevisiae* DNA repair gene *RAD7* in response to DNA damage and during sporulation

Jeffery S.Jones+, Louise Prakash and Satya Prakash[1,*]
Department of Biophysics, University of Rochester School of Medicine, Rochester, NY 14642 and
[1]Department of Biology, University of Rochester, Rochester, NY 14627, USA

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ABSTRACT

The *RAD7* gene of *Saccharomyces cerevisiae* affects the proficiency of excision repair of DNA damaged by UV light. Here, we report our studies on the regulation of the *RAD7* gene in response to UV irradiation and during sporulation. *RAD7* transcript levels increased 6-fold within 40 min of exposure of cells to 37 J/m² of UV light. Higher UV doses also elicited rapid increases in the level of *RAD7* mRNA. *RAD7* mRNA levels increased in sporulating *MATa/MATα* diploid cells, but not in the asporogenous *MATa/MATa* strain exposed to sporulation conditions. The increase in *RAD7* mRNA level in *MATa/MATα* cells was 15-fold after 6 h and 9-fold after 7 h in sporulation medium; thereafter, *RAD7* mRNA levels declined. Periodic transcription of *RAD7* during sporulation suggests a role for *RAD7* in this process.

INTRODUCTION

Treatment of *Escherichia coli* with ultraviolet (UV) light induces transcription of over 20 genes, whose expression is coordinately regulated by the LexA repressor and the RecA activator proteins (1, 2). The inducible genes include the *UvrA, UvrB, UvrC,* and *UvrD* genes involved in excision repair, the *RecA* gene required for recombinational repair and UV mutagenesis, and the *UmuC* and *UmuD* genes required for UV mutagenesis (1, 2). It is not known whether a coordinated regulatory response to DNA damage also occurs in eukaryotes.

In the yeast *Saccharomyces cerevisiae*, over 30 genes are known to function in DNA repair (3). These genes have been classified into three epistasis groups (3, 4). The *RAD3* epistasis group consists of at least 10 genes that are required for excision repair of DNA damaged by UV light and by other agents that distort the DNA helix. Five of these genes, *RAD1, RAD2, RAD3, RAD4,* and *RAD10*, are required for incision of UV damaged DNA and mutations in these genes confer a high degree of sensitivity to UV light (5–7). The other five genes, *RAD7, RAD14, RAD16, RAD23,* and *MMS19*, affect the proficiency of excision repair and mutations in these genes render cells moderately UV sensitive (5, 7,8). The *RAD6* epistasis group consists of the genes required for postreplication repair and for UV mutagenesis, and includes the *RAD6, RAD18, REV1, REV2,* and *REV3* genes (3). Genes in the *RAD52* epistasis group function in DNA double strand break repair and in recombination, and include the *RAD50* through *RAD57* genes (4).

Of the known DNA repair genes of *S. cerevisiae*, only the *RAD2* and *RAD54* genes have been reported to be inducible by DNA damage (9–11). Transcription of the *RAD52* and *RAD54* genes also increases during meiosis (12). We have now examined whether the *RAD7* gene, involved in excision repair, is regulated in response to DNA damage and during meiosis. Previously, we showed that the *RAD7* gene transcribes an approximately 1.8 kb mRNA which encodes a 63.7-kD protein (13). In this paper, we show that *RAD7* transcript levels increase markedly in cells exposed to UV irradiation, and also during sporulation.

MATERIALS AND METHODS

Strains and media

The *S. cerevisiae* haploid strains used were: DBY747 *(MATa* his3-Δ1 leu2-3 leu2-112 trp1-289 ura3-52), g833-1B *(MATa* leu2 can1 his1-1 trp2), and g833-2D *(MATα* hom3-10 his1-7 ade2). The diploid strain g857 was obtained by crossing the strains g833-1B and g833-2D. The strain g721-2 *(MATa/MATa leu2-1/LEU2 can1^r/CAN1^S hom3-10/HOM3 his1-1/his1-7 trp2/TRP2 ade2/ADE2)* is closely related to the strain g857. YPD medium contained 2% Bacto-peptone, 2% glucose, and 1% yeast extract. Presporulation medium contained 6.7 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 presporulation medium. The presporulation medium was supplemented with 20 mg/l L-histidine (Sigma). Sporulation medium used for these experiments was 1% potassium acetate supplemented with 4 mg/l L-histidine.

* To whom correspondence should be addressed

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

UV Irradiation of yeast cells

Strain DBY747 was grown in YPD medium at 30°C to a density of 2×10^7 cells/ml, when cells were in mid logarithmic phase of growth. Cells were collected by filtration and resuspended in sterile glass distilled H_2O. 75 ml of cell suspension were placed in a sterile 9″×13″ pyrex dish and exposed to UV irradiation with constant stirring. Cells were then filtered and 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 various intervals thereafter. Cells from the 0 time points were diluted and spread on YPD plates to determine the fraction of survivors. The plates were incubated at 30°C in the dark to avoid photoreactivation. The survival was 82%, 76% and 71% after 37, 50 and 70 J/m^2 respectively.

Sporulation

The *MATa*/MATα diploid strain g857 was grown in YPD medium. Cells were then inoculated in presporulation medium and grown at 30°C to a density of approximately 2×10^7 cells/ml. The culture was filtered and cells resuspended in sporulation medium at a density of 2×10^7 cells/ml. The culture was incubated at 30°C, agitated vigorously and samples taken at various intervals. Each sample was examined microscopically and cell density and morphology recorded. To determine the period of commitment to meiotic recombination, dilutions of the samples were plated onto synthetic complete medium for viability determinations and onto synthetic complete medium lacking histidine to assay for *HIS1*$^+$ recombinants.

Isolation of total RNA, gel electrophoresis, and Northern hybridization

These methods were as described previously (9, 14).

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 1.8 kb *Xba*I/*Nru*I *RAD7* DNA fragment (13) was used as a probe for *RAD7* mRNA. The 1.1 kb *Hin*dIII fragment of pTRT2 (kindly provided by Dr. David Norris) which carries the 3′ two-thirds of the histone H2B-2 coding region (15) was used as a probe for histone H2B mRNA. The 1.1 kb *URA3*-containing *Hin*dIII fragment was used to detect the *ura3-52* transcript.

Radiolabeled probes were made using the Multiprime Kit (Amersham) as described by the manufacturer using α-[^{32}P]dATP (6000 Curies per mmole, Amersham) and incubated for 3−16 h at room temperature or 1−2 h at 37°C. Reactions were terminated by adding an equal volume (50 μl) 40 mM EDTA (pH 8.0), containing bromophenol blue dye. Spin columns for purification of large molecular weight probes from unincorporated radiolabeled nucleotides were made as described by Maniatis et al. (16) using sephadex G-50-80 (Sigma) and sterile 1 ml tuberculin syringe barrels as columns. Approximately 1 μl of the flowthrough was monitored for Cerenkov emission in a Beckman LS5000TD liquid-scintillation counter to ensure high efficiency labeling of the probe fragments. The probes generated using this method routinely had a specific activity of $1-3\times10^9$ cpm/μg.

Densitometry

Quantitation of the bands in various lanes on autoradiograms was carried out using an LKB Bromma ULTROSCAN XL automated scanning laser densitometer. Several exposures of each blot were analyzed to ensure linearity of response.

RESULTS

RAD7 mRNA levels increase in UV irradiated cells

Since *rad7* mutants are sensitive to UV light and show a deficiency in the removal of UV induced pyrimidine dimers from DNA, we examined whether UV light increases the expression of *RAD7*. Total RNA was isolated from the strain DBY747 irradiated with 37, 50, or 70 J/m^2 of UV light, and *RAD7* transcript levels determined by Northern blot hybridization. As is shown in Fig. 1A, B, and C, at all UV doses, *RAD7* mRNA levels became elevated rapidly after irradiation. Fig. 1D shows the quantitation of *RAD7* mRNA at different periods after UV irradiation. Since the ura3 mRNA levels do not change in UV irradiated cells, the RAD7 mRNA levels were normalized with the ura3 mRNA in each lane. *RAD7* mRNA levels fell immediately after UV irradiation. Thereafter, *RAD7* mRNA levels increased rapidly with maximal accumulation occurring at 40 min after 37/m^2 and 50 J/m^2 of UV light, and at 60 min following 70 J/m^2 of UV irradiation. Compared to the level in unirradiated cells from the logarithmic culture, the increase in *RAD7* mRNA was 6-fold after 37 and 70 J/m^2 and 4.5-fold after 50 J/m^2. Without normalization to ura3 mRNA, the level of increase in RAD7 mRNA is over 5 fold at all UV doses. These observations of UV induction of *RAD7* transcription were confirmed in two additional experiments. Control experiments in which *RAD7* mRNA levels were examined from cells that were not UV irradiated but were otherwise treated identically to the UV irradiated samples showed no change in the level of *RAD7* mRNA.

Since some *S. cerevisiae* genes show an increased expression both in response to DNA damage and to heat shock (17−19), we examined whether *RAD7* transcription was also inducible by heat shock. *RAD7* mRNA levels were examined by Northern blot analysis of total RNA isolated from *S. cerevisiae* cells held at 39°C for up to 2 h. However, we observed no increase in *RAD7* mRNA, whereas in these conditions, we observed a very rapid and dramatic increase in the heat shock inducible HSP26 mRNA (results not shown). Thus, *RAD7* transcription is induced specifically in response to UV damage rather than as a general stress response.

Regulated expression of *RAD7* during sporulation

RAD7 mRNA levels were examined during sporulation in the *MATa*/*MATα* diploid strain g857, a derivative of the strain SK1 (20). Strain g857 undergoes rapid, very efficient, and synchronous meiosis in sporulation medium. We observed that in this strain, in sporulation medium, commitment to recombination began at about 2 h and maximum recovery of *HIS1*$^+$ recombinants (~1%) occurred by 6 h, whereas sporulation began at approximately 7 h and was complete by 10 h when over 90% of cells had formed asci. Total RNA was isolated from cells grown in presporulation medium and at various times following transfer of cells to sporulation medium, and Northern blots were hybridized to *RAD7* and histone H2B-specific DNA probes (Fig. 2A). H2B mRNA levels increased betweeen

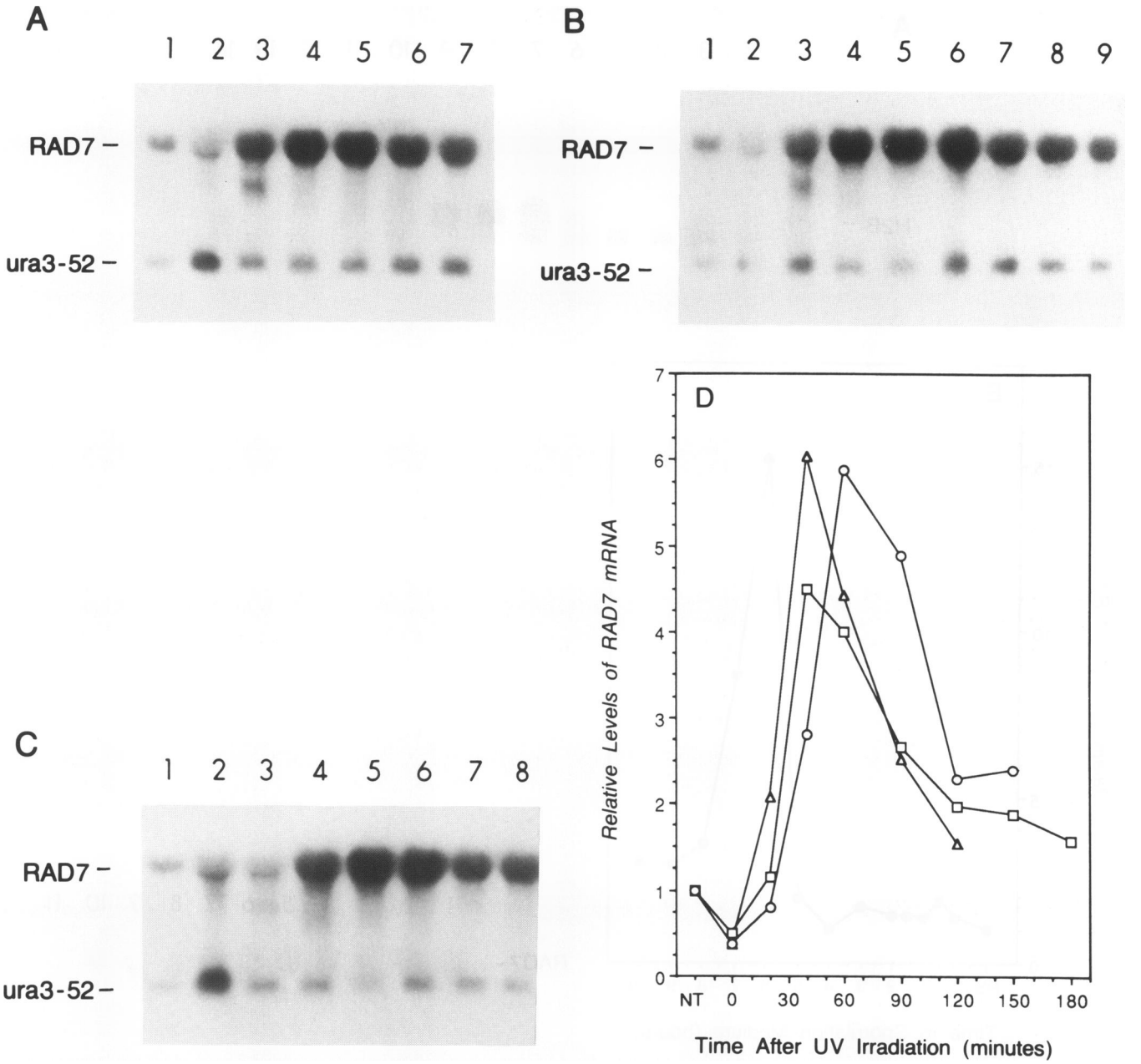

Fig. 1: *RAD7* mRNA levels in UV irradiated cells. Yeast strain DBY747 was UV irradiated and total RNA isolated. 50 μg of total RNA were resolved in each lane. The Northern blots were simultaneously probed with multiprime (Amersham) labeled *RAD7* and *URA3* specific probes. Lane 1 in (A), (B), and (C), RNA from unirradiated cells. (A) Lanes 2–7, RNA from cells at 0, 20, 40, 60, 90 and 120 min respectively after exposure to 37 J/m^2 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/m^2 UV radiation. (C) Lanes 2–8, RNA from cells at 0, 20, 40, 60, 90, 120 and 150 min after exposure to 70 J/m^2 UV radiation. (D) Quantitation of *RAD7* mRNA in autoradiograms shown in (A), (B) and (C). Since the *ura3* mRNA level is not affected by UV treatment, it can be used as an internal loading control. The ratio of *RAD7* mRNA to *ura3–52* mRNA was calculated for each lane and values determined for each time point were plotted relative to the value for unirradiated cells (NT). △, (37 J/m^2); □, (50 J/m^2); and ○, (70 J/m^2).

3 to 6 h, which is after the period of premeiotic DNA synthesis. The pattern of fluctuation in H2B mRNA observed by us resembles that reported by others (21). *RAD7* mRNA levels remained nearly constant during the first 5 h in the sporulation medium (Fig. 2A, lanes 2–9), and then increased sharply at 6 h (Fig. 2A, lane 10). Quantitation of *RAD7* mRNA levels during sporulation (Fig. 2B) indicates that *RAD7* mRNA levels were 15-fold higher at 6 h compared with the level present in cells from presporulation medium or in cells from sporulation medium during earlier periods. The level of *RAD7* mRNA was approximately 9-fold higher at 7 h and about 3-fold higher between 8 to 10 h. Two additional experiments verified our observation of increased *RAD7* expression during sporulation. To establish that the increase in *RAD7* mRNA levels was due to sporulation *per se* and not due to the starvation conditions of the sporulation medium, we examined the level of *RAD7* mRNA in the asporogenous *MATa/MATa* strain g721–2, which is closely related to the *MATa/MATα* strain g857. Strain g721–2

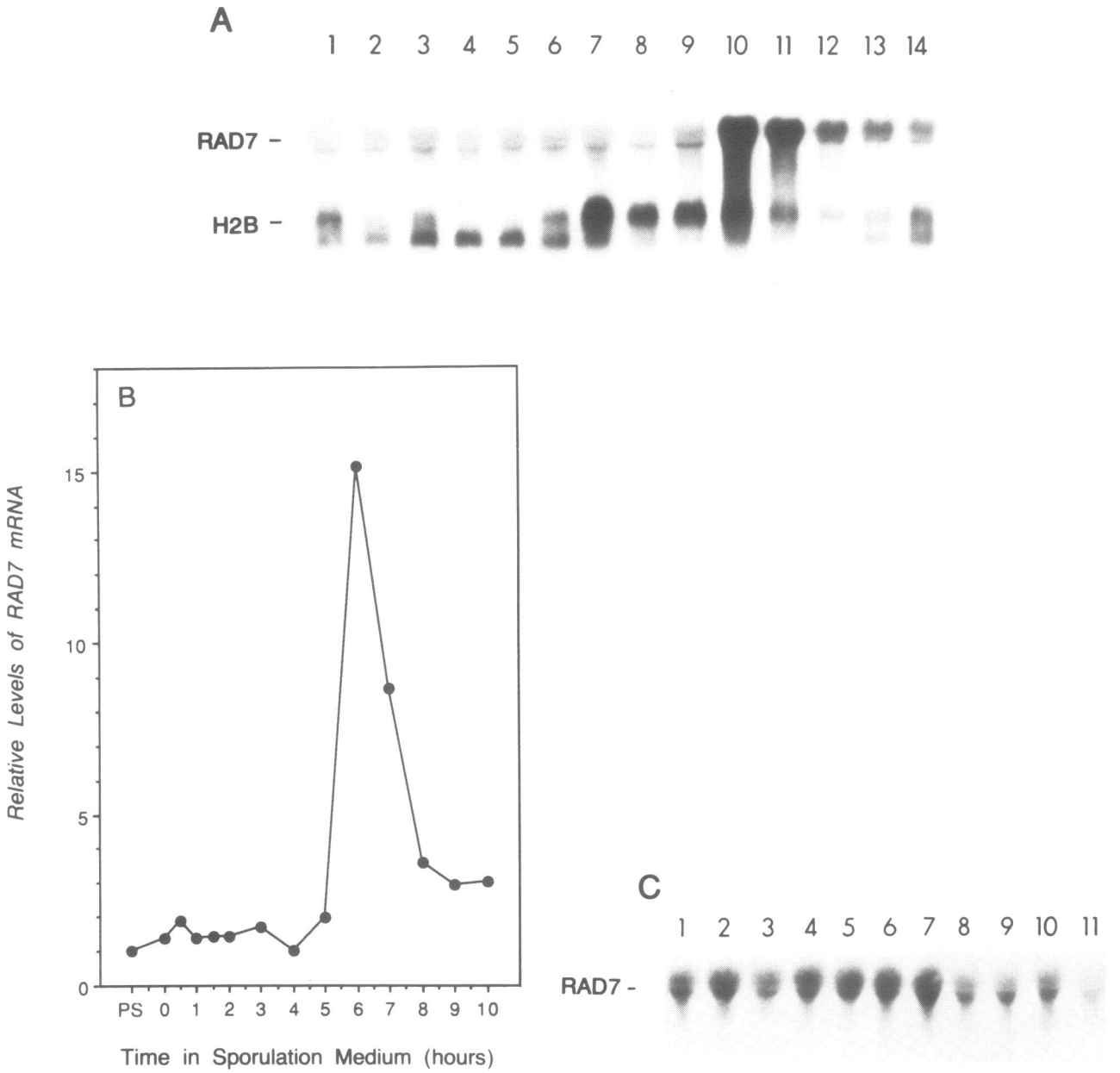

Fig. 2: *RAD7* mRNA levels during sporulation. The *MATa/MATα* yeast strain g857 was grown in presporulation medium, and transferred to sporulation medium. 50μg of total RNA were resolved per lane. After transfer to GeneScreen membrane, the RNA was visualized by shortwave UV light to ensure equality of RNA levels in each lane. Northern blots were probed with multiprime (Amersham) labeled *RAD7* and histone H2B (*TRT2*) specific probes. (A) *RAD7* and H2B mRNA levels during sporulation. Lane 1, RNA from cells grown in presporulation medium. Lanes 2–14, RNA from cells at 0, 0.5, 1.0, 1.5, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h respectively after transfer to sporulation medium. (B) Quantitation of *RAD7* mRNA levels in the Northern blot shown in (A). The *RAD7* mRNA levels for various time points were normalized to the level in cells from presporulation medium (PS). (C) RAD7 mRNA levels in MATa/MATa strain during incubation in sporulation medium. Yeast strain g721-2 was grown in presporulation medium, and then transferred to sporulation medium 50μg of total RNA were 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 *RAD7* specific probe. Lane 1, RNA from cells grown in presporulation medium. Lanes 2–11, RNA from cells at 0, 0.5, 1.0, 2, 3, 4, 5, 6, 7, and 8 h respectively after transfer to sporulation medium.

was grown in presporulation medium, transferred to sporulation medium, and the levels of *RAD7* mRNA examined by Northern blot hybridization. In contrast to the increased expression of *RAD7* in the *MATa/MATα* strain, *RAD7* mRNA levels declined somewhat in the *MATa/MATa* strain during the later periods of incubation in sporulation medium (Fig. 2C). Thus, our results show that *RAD7* mRNA levels increase specifically in response to sporulation of *MATa/MATα* diploid cells.

DISCUSSION

We have shown that the expression of the *RAD7* gene of *S. cerevisiae* increases in response to UV irradiation. At the UV doses examined, *RAD7* mRNA levels increased rapidly, reaching a peak between 40 to 60 min. The increase in the amount of *RAD7* mRNA was between 4.5 to 6 fold relative to the levels in cells from unirradiated logarithmically growing culture. Using a

different *S. cerevisiae* strain, G. Perozzi in our laboratory had observed a 5-fold increase in the level of *RAD7* mRNA at 30 min after 25 J/m^2 of UV light (22). Unlike the *DDR* (18) and *UBI4* (17, 19) genes whose expression is induced both by DNA damage and heat shock, *RAD7* mRNA levels did not rise in response to heat shock. Thus, *RAD7* transcription is elevated specifically in response to UV damage rather than as a stress response.

In addition to the *RAD2* gene (9, 10), the *RAD7* gene reported here, and the *RAD54* gene (11), several other genes also exhibit enhanced expression in response to DNA damage. These include the DNA replication genes *CDC9* (23), and *CDC17* (24), and the *RNR2* gene that encodes the small subunit of ribonucleotide reductase (25, 26). Expression of the *DIN* (27), *DDR* (28), and *UBI4* (19) genes is also induced upon exposure of cells to DNA damaging agents. Whether the expression of all of these genes is coordinately regulated in response to DNA damage is not known. However, since the upstream regulatory sequences that have been identified in the *RAD54* and *RNR2* genes (29–31) show no obvious similarity to each other and to upstream sequences in other DNA damage inducible genes, there may be multiple regulatory mechanisms that control the expression of various genes in response to DNA damage.

Expression of the *RAD7* gene is regulated in meiosis. *RAD7* mRNA levels rose 15 fold following incubation of *MATa/MATα* diploid cells in sporulation medium for 6 h. The increase in the level of *RAD7* mRNA is specific to cells undergoing sporulation and is not due to the starvation conditions of the sporulation medium, since the closely related *MATa/MATa* strain showed no enhancement in *RAD7* transcription upon incubation in sporulation medium. The period of maximal accumulation of *RAD7* mRNA during sporulation is later than is observed for the DNA replication genes *CDC9* and *CDC17*, that encode DNA ligase and DNA polymerase I, respectively. Peak accumulation of *CDC9* and *CDC17* mRNAs occurs at the time of premeiotic DNA synthesis (24, 32). The period of increase in the level of mRNAs encoded by the DNA double strand break repair and recombination genes *RAD52* and *RAD54* coincides with the time of commitment to meiotic recombination (12). The rise in the level of *RAD7* mRNA occurs after the period of commitment to recombination and may reflect a need for increased amounts of *RAD7* product during the later stages of sporulation. The fact that *rad7* mutations do not affect sporulation or spore viability adversely could be explained if there are other genes or alternate pathways which carry out functions similar to that of *RAD7*. In that case, it may be difficult to assess the biological role of *RAD7* in sporulation, unless double mutant combinations of *RAD7* with alternate genes are studied. In fact, a mutation in any of the excision repair genes *RAD1*, *RAD2*, and *RAD3* or in the *RAD18* gene, which functions in the postreplication DNA repair pathway (33), has no significant effect on sporulation, spore viability, or meiotic recombination. However, strains harboring mutations in two genes affecting alternate DNA repair pathways, *rad1 rad18*, *rad2 rad18*, and *rad3 rad18*, exhibit poor spore viability (34). Our observation of increased expression of *RAD7* during sporulation suggests a role for *RAD7* in this process.

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