Transcript levels of the *Saccharomyces cerevisiae* DNA repair gene *RAD23* increase in response to UV light and in meiosis but remain constant in the mitotic cell cycle

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ABSTRACT

The *RAD23* gene of *Saccharomyces cerevisiae* is required for excision-repair of UV damaged DNA. In this paper, we determine the location of the *RAD23* gene in a cloned DNA fragment, identify the 1.6 kb *RAD23* transcript, and examine *RAD23* transcript levels in UV damaged cells, during the mitotic cell cycle, and in meiosis. The *RAD23* mRNA levels are elevated 5-fold between 30 to 60 min after 37 J/m^2 of UV light. *RAD23* mRNA levels rise over 6-fold during meiosis at a stage coincident with high levels of genetic recombination. This response is specific to sporulation competent *MATa/MATα* diploid cells, and is not observed in asporogenous *MATa/MATa* diploids. *RAD23* mRNA levels, however, remain constant during the mitotic cell cycle.

INTRODUCTION

Procaryotic and eucaryotic cells have evolved mechanisms that regulate the expression of genes in response to a variety of environmental stresses. Treatment of *Escherichia coli* with ultraviolet (UV) light or with agents that introduce bulky adducts in DNA causes the coordinate induction of about 20 genes that are members of the SOS regulatory network (1, 2). The SOS system is regulated by the LexA and the RecA proteins. In the absence of DNA damage, the LexA repressor binds to the operator sequences of the SOS genes and turns off their expression. Upon treatment of cells with DNA damaging agents, the RecA protein becomes an active protease and cleaves the LexA repressor, resulting in increased transcription of SOS genes. Activation of RecA apparently occurs by its binding to the single-stranded DNA left during replication of noncoding lesions. The SOS inducible genes include the *UvrA, UvrB, UvrC,* and *UvrD* genes, required for excision repair, the *RecA* gene required for recombinational repair, the *UmuC* and *UmuD* genes required for mutagenesis, and the *SfiA* gene required for inhibition of cell division in response to DNA damage (1, 2).

In contrast to *E. coli,* in eucaryotes the regulatory response of DNA repair genes to DNA damage is not well defined. In the yeast *Saccharomyces cerevisiae,* over 30 genes are involved in the repair of DNA damage (3). At least ten of these genes are required for excision repair of DNA damaged by UV light and by other agents that deform the DNA helix (4–7). Mutations in five of these genes, *RAD1, RAD2, RAD3, RAD4,* and *RAD10,* render the cell highly susceptible to UV light and these mutants lack the ability to incise damaged DNA (4, 6, 7). The other five genes, *RAD7, RAD14, RAD16, RAD23,* and *MMS19,* are likely to affect the efficiency of excision repair since strains carrying mutations in these genes are not as sensitive to UV and retain some degree of excision ability (4, 5, 7). Of the excision repair genes of *S. cerevisiae,* only the *RAD2* and *RAD7* genes have thus far been shown to be inducible by DNA damage (8–10). The other genes shown to be inducible in response to DNA damage are the *RAD6* gene (11), required for postreplication repair, UV mutagenesis, and sporulation, and the *RAD54* gene (12), required for the repair of DNA double strand breaks and for recombination. All of these genes also show induced expression during meiosis (10, 11, 13–15). Several other genes, *DIN* (16) and *DDR* (17), have been identified in *S. cerevisiae* by directly screening for genes whose expression is induced in response to DNA damaging treatments. However, the role of these genes in DNA repair remains unknown. The other DNA damage inducible genes in *S. cerevisiae* include the DNA replication genes *CDC9* (18) and *CDC17* (19) that encode DNA ligase and DNA polymerase I, respectively. Transcription of the *S. cerevisiae RNR2* gene encoding the small subunit of ribonucleotide reductase is also induced upon DNA damage (20, 21). It is not known whether DNA damage induction of all of these genes is coordinately regulated.

We have begun a systematic analysis of regulation of DNA repair genes in response to DNA damage, during the mitotic cell cycle, and in meiosis. In this paper, we report our studies on the regulation of the *RAD23* gene. The *RAD23* gene is tightly linked to the *ANP1* and *CYC7* genes on chromosome V (22). We identified the location of the *RAD23* gene in a *CYC7* region DNA fragment obtained from F. Sherman. The size and direction of the *RAD23* transcript were determined and transcription of *RAD23* was examined in UV irradiated cells, during the cell division cycle, and in meiosis. We find that *RAD23* transcript levels increase in response to UV irradiation and show a periodic fluctuation during meiosis, but remain constant during the mitotic cell cycle.

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MATERIALS AND METHODS

Strains, media, plasmids, and general recombinant methods

The *S. cerevisiae* strains used for the preparation of total RNA were; DBY746 (*MATα* his3-Δ1 leu2-3 trp1-289 ura3-52 RAD+) KM135 (*MATα* his3-Δ1 leu2-3 leu2-112 trp1-289 ura3-52 rad23Δ), 4910-3-3a (*MAT*a *his7 ura1 cdc7-4 bar1-1*), g833-1B (*MATa* leu2 can1 his1-1 trp2), g833-2D (*MATα* hom3-10 his1-7 ade2), and g721-2 (*MAT*a/MATa leu2-1/LEU2 can1r/CAN1S hom3-10/HOM3 his1-1/his1-7 trp2/TRP2 ade2/ADE2). Strain S211-10B (*MAT*a *cyc1-11 or cyc1-362 ura3-52 cyc7-4 rad23*-Δ1) carries the *CYC7-H3* mutation which causes overproduction of iso-2-cytochrome c. The *CYC7-H3* mutation is a 5 kb deletion which encompasses the *RAD23* gene (22). We have previously named this deletion as *rad23*Δ-1 (5). Strain 4910-3-3a was provided by J. Huberman, and strains g833-1B, g833-2D, and g721-2 by J. Game. The diploid strain g857 was constructed by crossing the haploid strains g833-1B and g833-2D, followed by selection of diploids on minimal synthetic medium supplemented with histidine. Strain g51-6d (*MATa* bar1-1 cry1-3 ade6 his6 leu1 lys2) was obtained from I. Herskowitz.

Yeast strains were grown in YPD medium (2% Bacto-peptone, 2% glucose, 1% yeast extract [Difco Laboratories, Detroit, Mich.]) at 30°C unless indicated otherwise. Yeast strain 4910-3-3a was grown at 23°C. Pre-sporulation medium contained 0.67% yeast nitrogen base without amino acids, 1% yeast extract, 2% Bacto-peptone, 1% potassium acetate, 50mM potassium phthalate (pH 5.0), 20 mg L-histidine/l . Sporulation medium contained 1% potassium acetate and 20 mg L-histidine/l.

Isolation of DNA restriction fragments and agarose gel electrophoresis were performed as described (23). M13 RF and plasmid DNAs were purified by cesium chloride isopycnic centrifugation from *E. coli* JM101 and HB101 strains, respectively.

Deletion of the *RAD23* gene

In plasmid pDG28, the 1.1kb *Bgl*II DNA fragment present within the *RAD23* open reading frame (ORF) was replaced by a 3.8 kb *Bgl*II-*Bam*H1 DNA fragment containing the yeast *URA3* gene, flanked by Salmonella *hisG* DNA sequences (24). The 4.8 kb EcoRI DNA fragment containing the *U*RA3 gene with flanking *RAD23* sequences was purified and introduced into the yeast strain DBY746 (25). The resulting *rad23*Δ Ura$^+$ strain KM134 was crossed to known *rad23* mutant strains and shown to be allelic as determined by UV sensitivity of diploids, and by tetrad analysis of segregants. Ura$^-$ derivatives of yeast strain KM134 were isolated by selection on 5-FOA containing medium, yielding the strain KM135.

UV-light treatment of yeast cells

Yeast strain DBY746 was grown in YPD medium to a density of 1 to 3×10^7cells/ml. Cells were collected by filtration and resuspended in sterile distilled water at a density of 1×10^7cells/ml. A 75ml cell suspension in a 30×20 cms pyrex dish was exposed to UV-light at a flux of 1J/m^2/sec with constant stirring. Irradiated cells were collected by filtration, resuspended in pre-warmed YPD medium at the original density, and incubated at 30°C in yellow light to avoid photoreactivation. Samples from the culture were withdrawn at intervals after irradiation, washed with an equal volume of distilled water, and the cell pellet frozen at −70°C. Total RNA was isolated when all the samples had been collected. Survival of cells after 37 J/m^2 and 50 J/m^2 was 82% and 60%, respectively.

Cell-cycle synchronization

Yeast strain 4910-3-3a (26), which carries a *bar1-1* mutation, was grown at 23°C in YPD medium to a density of ~ 1.5×10^7 cells/ml. Cell-cycle arrest was achieved by the addition of 10ng/ml α-factor (Sigma Chemicals Co). After 3 h of α-factor treatment, over 97% of the cells were arrested as single, large and unbudded G1 'schmoos'. Following α-factor removal by filtration, cells were washed with an equal volume of YPD medium (equilibrated to 23°C), resuspended in fresh YPD medium, and incubated at 23°C. Cell morphology and density were examined at 30 min intervals. The doubling time of this strain grown under these conditions is 2 h. Total RNA was purified from samples withdrawn from the culture before, during, and after α-factor arrest.

Sporulation conditions

The *MAT*a/MATα diploid strain g857 was obtained from freshly mated haploid strains g833-1B and g833-2D. Following growth of strain g857 for approximately 15 h in YPD medium, cells were inoculated at low density in pre-sporulation medium and grown for 18 h to a density of 4×10^7cells/ml. Cells were then collected by filtration, washed with sporulation medium, and resuspended at 2×10^7cells/ml in sporulation medium. The culture was incubated at 30°C with vigorous aeration. Aliquots of the culture were withdrawn at various times during sporulation, and total RNA was purified. Each sample was also examined microscopically for morphology and cell density. The period of commitment to recombination was determined by plating dilutions of samples onto synthetic complete medium for viability determinantions and onto synthetic complete medium lacking histindine to determine the frequency of *HIS1*$^+$ recombinants.

Isolation of total RNA, gel electrophoresis, Northern hybridization, and densitometry

These methods were as described previously (8). Transcript levels were quantitated by densitometry with an LKB laser-densitometer. To ensure linearity of response, X-ray films with varying exposures were quantitated.

Preparation of radiolabeled probes

RAD23 mRNA was detected by hybridization to nick-translated plasmid pPP1 which contains the internal 1.1kb *Bgl*II fragment of *RAD23* in the yeast integrating plasmid, YIp5. *RAD23* mRNA was also detected by hybridization to nick-translated probe 1923-04, in which the 0.5 kb *Pst*I-*Hin*dIII *RAD23* DNA fragment, which is internal to the 1.1 kb *Bgl*II fragment in the *RAD23* ORF (Fig. 1), was cloned into M13mp19. The 1.1 kb *Hin*dIII fragment of *URA3* was used to probe for *URA3* mRNA. Histone H2B mRNA was detected by hybridization to nick-translated plasmid pTRT2 (obtained from L. Hereford), in which an 1.1 kb *Hin*dIII fragment carrying the H2B gene is cloned into pBR322. Nick-translation of plasmid DNA was carried out by using materials from Amersham, Inc. Plasmid DNA (100ng) was nick-translated in a reaction containing 50μCi ^{32}P-dATP (3000Ci/mMole), 50μCi ^{32}P-dCTP (3000Ci/mMole), dGTP and dTTP. The reaction mixture was incubated at 15°C for 2 h, and reaction terminated by the addition of an equal volume of 0.1M Na_2-EDTA. Unincorporated isotope was removed by passage through a 0.8ml Sephadex G-50 column (Boehringer Manneheim Co.). The specific activity of radiolabeled probes was routinely between 1 to 3×10^8cpm/μg.

RESULTS

Location of the *RAD23* gene

Plasmid pAB109, obtained from F. Sherman, contains a 7.2 kb *SalI-Hin*dIII *S. cerevisiae* DNA fragment cloned in YIp5. Fig. 1 shows the positions of the *ANP1, RAD23,* and *CYC7* genes in this DNA fragment. The position of the *CYC7* gene was determined previously (22) and the position of *ANP1* was obtained from L. Melnick and F. Sherman (personal communication). The 7.2 kb *SalI-Hin*dIII DNA fragment was cloned into the yeast low copy plasmid YCp50, yielding plasmid pJI15. The 2.1 kb *Eco*RI fragment in the middle of the *SalI-Hin*dIII fragment was cloned into the yeast multi-copy plasmid pTB220, yielding the plasmid pPP12. Transformation of the *rad23*Δ-1 strain S211-10B with the plasmid pJI15 or pPP12 resulted in Rad$^+$ levels of UV resistance. The 2.1 kb *Eco*RI fragment in low copy plasmid YCp50 also restored Rad$^+$ levels of UV resistance to the *rad23*Δ-1 strain. The 1.1 kb *Bgl*II fragment present within the 2.1 kb *Eco*RI fragment was cloned into the yeast integrating plasmid YIp5, yielding the plasmid pPP1. Transformation of the Rad$^+$ yeast strain DBY746 with pPP1 resulted in a UV sensitive strain due to the disruption of the *RAD23* gene. Allelism tests with the *rad23*Δ-1 mutation confirmed that the *RAD23* gene had been disrupted. Since integration of plasmid pPP1 into the yeast chromosome disrupts the *RAD23* gene, the 1.1 kb *Bgl*II fragment is internal to the *RAD23* gene (Fig. 1). We also constructed a deletion of the *RAD23* gene by the method of Alani, *et al.* (24) by replacing the *RAD23* 1.1 kb *Bgl*II fragment with the yeast *URA3* gene flanked by *HisG* sequences. The *RAD23* region *Eco*RI DNA fragment in which the 1.1 kb *Bgl*II fragment had been replaced by *URA3* was purified and transformed into the Rad$^+$ yeast strain DBY746 to generate a *rad23*Δ mutation. The *RAD23* gene on plasmids pJI15 and pPP12 complemented this *rad23*Δ mutation in strain KM135. Nucleotide sequence analysis of the 2.1 kb *Eco*RI fragment, carried out in the laboratory of F. Sherman (L. Melnick and F. Sherman, personal communication), shows an ORF of 398 codons that encodes a polypeptide of ~42.5 kD. This ORF is expressed in yeast, since we found that in-frame fusions of *E. coli lacZ* gene at the *Bgl*II sites located at codon 16 or 368 produce β-galactosidase in *S. cerevisiae*.

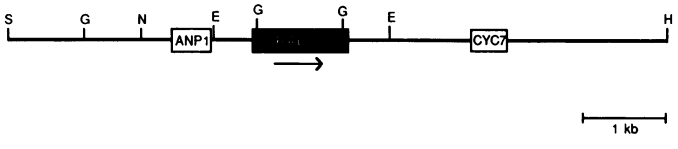


Fig. 1: Location of the *RAD23* gene and direction of transcription. The location of the *RAD23* gene on a 7.2 kb *SalI-Hin*dIII yeast DNA fragment cloned into plasmid pAB109 is shown. The positions of pertinent restriction sites (which are not necessarily unique) are indicated. The solid box represents the *RAD23* open reading frame. The direction of *RAD23* transcription is indicated by the arrow. The positions of the *ANP1* and *CYC7* genes are indicated. Symbols: ᴱ, *Eco*RI; G, *Bgl*II; H, *Hin*dIII; N, *Nco*I; S, *Sal*I.

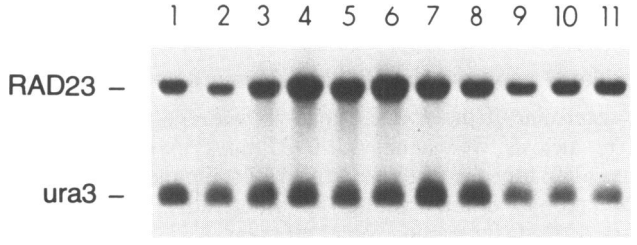
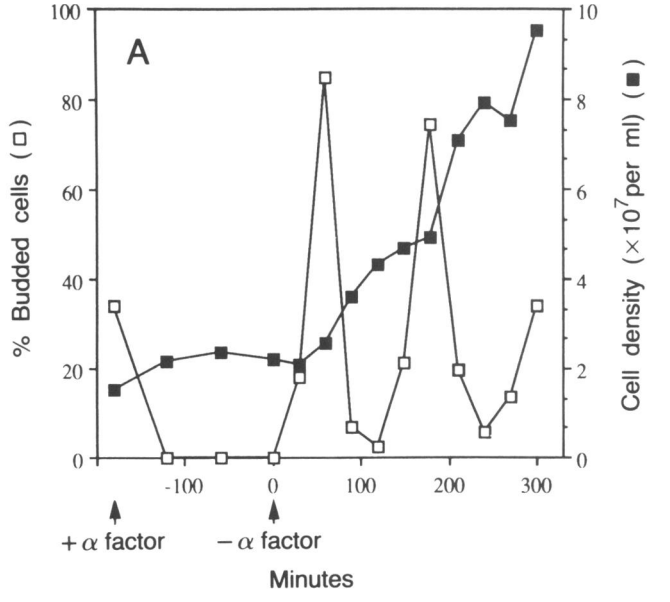


Fig. 2: Levels of *RAD23* mRNA following 37 J/m^2 of UV irradiation. Total RNA was isolated from yeast strain DBY746 before and after UV irradiation. 25 μg of RNA per lane were electrophoresed and *RAD23* and *ura3* mRNA detected by Northern hybridizations. Since the level of *ura3* mRNA does not change in UV irradiated cells, it can be used as an internal control for mRNA loading. Lane 1, RNA from unirradiated cells. Lanes 2 to 11, RNA from cells at 0, 15, 30, 45, 60, 90, 120, 150, 180, and 240 min following 37 J/m^2 of UV light.

Size and direction of the *RAD23* transcript

To determine the size and direction of the *RAD23* transcript, total and polyA$^+$ RNA were purified from three different Rad$^+$ and isogenic *rad23*Δ strains, and Northern blots hybridized to nick-translated plasmid pPP1. In this plasmid, the internal 1.1 kb *Bgl*II fragment of *RAD23* is cloned in YIp5. A 1.6 kb transcript was present in RNA isolated from Rad$^+$ strains and this transcript was absent in *rad23*Δ strains. (Data not shown).

To determine the direction of *RAD23* transcription, strand-specific DNA probes were prepared. M13 clones 1823-04 and 1923-04 contain the 0.5 kb *Pst*I-*Hin*dIII *RAD23* DNA fragment, which is internal to the 1.1 kb *Bgl*II fragment, cloned into M13mp18 and M13mp19, respectively. Single-stranded



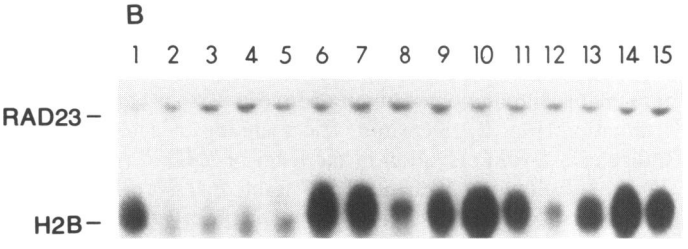


Fig. 3: *RAD23* mRNA levels during the mitotic cell cycle. A. Strain 4910-3-3a was arrested at the G1 stage by treatment with α-factor for 180 min, after which cells were collected by filtration, resuspended in fresh YPD medium, and incubated at 23°C. The graph shows the fraction of budded cells and cell density of the culture at various time intervals. **B.** 50 μg of total RNA per lane were electrophoresed and hybridized to *RAD23* and histone H2B specific probes. The equality of RNA amounts was verified by also probing these blots with a *URA3* probe, since *URA3* mRNA levels remain constant during the cell cycle. Lane 1, RNA from exponentially growing cells. Lanes 2 to 4, RNA from cells treated with 10ng/ml α-factor for 2, 2.5, and 3 h, respectively. Lanes 5 to 15, RNA isolated at 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300 min, respectively, following release from α-factor arrest.

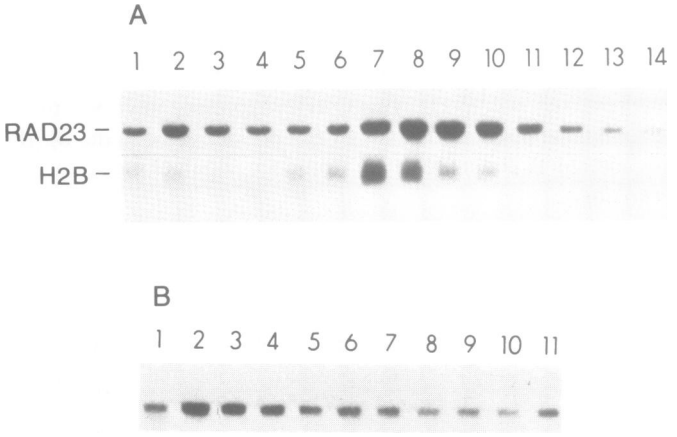


Fig. 4: Elevated levels of *RAD23* mRNA during meiosis. **A.** *MAT***a**/MATα diploid strain g857 was grown in YPD medium and then transferred to pre-sporulation medium and grown for 18 h. Cells were collected by filtration and transferred to sporulation medium. 25 μg of total RNA were loaded per lane. Northern blots were hybridized to the *RAD23* and histone H2B specific probes. The blots were also hybridized to a *URA3* probe. As reported previously (11), *URA3* mRNA levels remained constant for 6 h in sporulation medium and then declined. Lane 1, RNA from cells grown in pre-sporulation medium. Lanes 2 to 14, RNA isolated at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, and 12 h following transfer to sporulation medium. **B.** *RAD23* mRNA levels in the *MAT***a**/MAT**a** strain during incubation in sporulation medium. Yeast strain g721-2 was grown in presporulation medium, and then transferred to sporulation medium. 25 μg of total RNA were loaded in each lane. Lanes 1 to 11, total RNA from cells incubated for 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, and 8 h in sporulation medium.

radiolabeled DNA probes were hybridized to Northern blots of RNA purified from Rad$^+$ and *rad23*Δ strains. A single 1.6 kb transcript was detected only with the probe 1923-04, indicating that the direction of transcription is as indicated in Fig. 1, and is consistent with the size and direction of the *RAD23* ORF. (Data not shown).

Regulated expression of the *RAD23* gene in response to UV irradiation

Total RNA was isolated from a logarithmic phase culture of the Rad$^+$ strain DBY746 exposed to UV light and levels of *RAD23* mRNA were determined by Northern hybridizations. We also examined *RAD23* mRNA levels in cells that were not UV irradiated but were otherwise treated identically to the UV irradiated samples. *RAD23* mRNA levels remained constant in these control experiments. Exposure of cells to 37 J/m^2 of UV light elicited a rapid increase in *RAD23* mRNA levels, with maximal accumulation occurring between 30 to 60 min after UV irradiation (Fig. 2). Densitometric scanning of autoradiograms indicates a 5-fold elevation in the level of *RAD23* mRNA after 37 J/m^2. Following 50 J/m^2 of UV light, *RAD23* mRNA levels increased 4-fold (results not shown). The observation of elevated *RAD23* mRNA levels upon UV irradiation has been verified by us in many different experiments.

Heat shock does not induce *RAD23* transcription

The dual response of a number of *S. cerevisiae* genes to DNA damage and heat-shock has been reported (27–29). To determine if the *RAD23* gene was also inducible by heat shock, we examined the levels of *RAD23* mRNA during heat stress. Northern blot analysis showed that following transfer of yeast cells to 38°C, as is typical of non heat shock inducible genes, *RAD23* mRNA levels declined in the 10 and 20 min samples, and then increased to basal levels by 60 min (results not shown). In contrast, under these conditions we observed a very rapid and dramatic increase in the heat shock inducible *HSP26* mRNA. Thus, the elevation in *RAD23* mRNA levels occurs specifically in response to DNA damage, rather than as a general stress response.

RAD23 mRNA levels remain constant during the mitotic cell cycle

In addition to its role in DNA repair, *RAD23* product may function in DNA replication. Since many DNA replication genes, such as *CDC9* (18), *CDC8, CDC21* (30), and *CDC17* (19), are periodically regulated during the cell cycle, we determined whether the *RAD23* gene is also regulated in a similar manner. We used the strain 4910-3-3a for these studies because it can be easily synchronized in the cell cycle by very low concentrations of dsa-factor and synchronous divisions are maintained for at least two generations after release from α-factor arrest (26). Fig. 3A shows the percent budded cells and cell density before, during, and following α-factor treatment. Approximately 35% of the cells in the exponentially growing unsynhcronized culture were actively budding. Three hours of treatment with α-factor resulted in greater than 97% unbudded, single, and large G1 stage cells. Following release from α-factor arrest, a rapid increase in the frequency of budded cells was observed at 60 min, which was followed by a doubling in cell density. At approximately 2 h after the first budding peak, a second peak of budding cells was detected which was once again followed by a doubling in cell density. Clearly, at least two cycles of synchronous cell division occur following release of cells from α-factor arrest. Fig. 3B shows *RAD23* and histone H2B mRNA levels at different times during the cell cycle. Histone H2B mRNA levels change dramatically during the cell cycle, reaching a maximum early in S phase (31, 32). H2B mRNA levels declined precipitously in α-factor treated cells (compare lanes 2–4 to lane 1, Fig. 3B) while *RAD23* mRNA levels remained constant. Following release from α-factor arrest, H2B mRNA levels showed three periodic cycles of peak accumulation. The peak level of H2B mRNA at 30, 150, and 270 min (lanes 6, 10, and 14, Fig. 3B) immediately preceded the peak levels of budding (Fig. 3A). Also consistent with the results presented in Fig. 3A is the observation that H2B mRNA levels peaked at 2 h intervals, similar to the observed doubling time of this strain. In sharp contrast to the regulated expression of H2B mRNA, *RAD23* mRNA levels remained constant at all times throughout the cell cycle. To test the possibility that the *cdc7-4* mutation present in strain 4910-3-3a might have influenced the results observed, a similar α-factor arrest experiment was performed in the *CDC7*$^+$ strain g51-6d. In this strain also, *RAD23* mRNA levels remained constant throughout the cell cycle (results not shown).

Regulated expression of *RAD23* during meiosis

To determine if the *RAD23* gene shows regulated expression during meiosis, we examined *RAD23* transcript levels at various times after transfer of the *MAT***a**/MATα diploid strain g857 to sporulation medium. Strain g857, a derivative of SK1 (33), is characterized by rapid, very efficient (> 95% asci) and synchronous sporulation. We found that in this strain, commitment to recombination began at about 2 h and maximum recovery of recombinants was achieved by 5 to 6 h. Spore formation began after 6 h and was complete by 10 h. Total RNA was isolated from cells grown in pre-sporulation and sporulation medium and Northern blots were hybridized with *RAD23* and

histone H2B-specific probes. Fig. 4A shows the results of this analysis. During the first 2 h in sporulation medium (lanes 3–6, Fig. 4A), *RAD23* mRNA levels remained nearly constant. A significant elevation in *RAD23* mRNA was observed between 3 to 6 h (lanes 7–10, Fig. 4A) which coincides with the period during which recombination occurs. Between 7 to 12 h (lanes 11–14, Fig. 4A), *RAD23* mRNA levels gradually declined. Histone H2B mRNA levels became substantially elevated (lanes 7 and 8, Fig. 4A) subsequent to premeiotic DNA synthesis, which occurs from 1 to 3 h. Afterwards, H2B mRNA levels declined (lanes 9–14, Fig. 4A). The pattern of H2B mRNA accumulation is similar to that observed by others (34). *RAD23* mRNA levels were quantitated by densitometry and the results indicate a 6-fold increase in the amount of *RAD23* mRNA between 4 and 5 h during sporulation. Several additional experiments have confirmed our observation of increased expression of the *RAD23* gene during sporulation. To verify that the changes in *RAD23* mRNA levels were meiosis specific, we examined *RAD23* mRNA levels in an asporogenous *MAT***a**/MAT**a** strain g721-2, which is closely related to the *MAT***a**/MATα strain g857. In this strain, *RAD23* mRNA levels declined during the course of incubation in sporulation medium (Fig. 4B). Thus, our observations show that the expression of *RAD23* is regulated during meiosis.

DISCUSSION

We have identified the location of the *RAD23* gene in a cloned DNA fragment and determined the size and direction of its transcript. *RAD23* encodes a 1.6 kb transcript whose orientation is consistent with the ORF characterized by L. Melnick and F. Sherman (personal communication). In our studies of *RAD23* gene regulation, we examined the levels of *RAD23* mRNA by Northern blot hybridizations. *RAD23* mRNA levels were elevated 5-fold after 37 J/m^2 and 4-fold after 50 J/m^2 of UV light. Some of the DNA damage inducible genes, *DDR* (28) and *UBI4* (27, 29) are also inducible by heat shock. However, *RAD23* transcript levels did not rise in response to heat shock, indicating a specific response of *RAD23* transcription to DNA damage rather than a general stress response.

Stage-specific expression during the mitotic cell cycle has been shown for a number of yeast genes required for DNA replication; these include the DNA ligase *CDC9* gene (18), DNA polymerase I *CDC17* gene (19), thymidylate synthase *CDC21* gene and thymidylate kinase *CDC8* gene (30). The mRNA levels of the *CDC9* and *CDC17* genes have also been shown to rise upon DNA damage (18, 19). However, we observed no periodic fluctuation in *RAD23* mRNA levels during the cell cycle. This observation is consistent with the requirement of the *RAD23* product throughout the cell cycle rather than at a particular stage. The fact that *RAD23* mRNA levels remain constant during the cell cycle indicates that UV induction of *RAD23* transcription does not arise from the cell cycle arrest phenomenon that occurs in response to DNA damage (35, 36).

The level of *RAD23* mRNA increased during meiosis between 3 to 6 h. This period is coincident with meiotic recombination, suggesting that increased *RAD23* transcription may be required for achieving high levels of genetic recombination, mismatch repair, or for both processes. Even though *rad23* mutants undergo sporulation and produce viable spores, it is not known whether meiotic recombination or mismatch repair is affected. Moreover, the existence of multiple pathways may preclude determination of the role of single genes unless double mutant combinations of genes in alternate pathways are examined. Thus, even though the single mutations in excision repair genes *RAD1*, *RAD2*, and *RAD3* or in the postreplication repair gene *RAD18* display no significant effects on sporulation, spore viability, or meiotic recombination, *rad1 rad18*, *rad2 rad18*, and *rad3 rad18* double mutants exhibit a dramatic reduction in spore viability (37).

Several DNA damage inducible genes of *S. cerevisiae* also show increased expression during meiosis. They include the DNA repair genes *RAD2* (13, 14), *RAD6* (11), *RAD7* (10), and *RAD54* (15), DNA replication genes *CDC9* and *CDC17* (19, 38), and the polyubiquitin gene *UBI4* (29). However, these genes differ in the period when they become induced during meiosis. The period of maximal accumulation of *CDC9* and *CDC17* mRNAs in meiosis is coincident with the period of premeiotic DNA synthesis (19, 38), whereas peak mRNA levels of DNA repair genes occur later and coincide with the period of genetic recombination (10, 11, 13, 15). It is not known whether common or different regulatory mechanisms control the expression of these genes in response to DNA damage and during meiosis.

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