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# Regulation of the *Saccharomyces cerevisiae* DNA repair gene *RAD16*

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## ABSTRACT

**The *RAD16* gene product has been shown to be essential for the repair of the silenced mating type loci [Bang *et al.* (1992) *Nucleic Acids Res.* 20, 3925–3931]. More recently we demonstrated that the *RAD16* and *RAD7* proteins are also required for repair of non-transcribed strands of active genes in *Saccharomyces cerevisiae* [Waters *et al.* (1993) *Mol. Gen. Genet.* 239, 28–32]. We have studied the regulation of the *RAD16* gene and found that the *RAD16* transcript levels increased up to 7-fold upon UV irradiation. Heat shock at 42°C also results in elevated levels of *RAD16* mRNA. In sporulating *MATα/MATa* diploid cells *RAD16* mRNA is also induced. The basal level of the *RAD16* transcript is constant during the mitotic cell cycle. G1-arrested cells show normal induction of *RAD16* mRNA upon UV irradiation demonstrating that the induction is not a secondary consequence of G2 cell cycle arrest following UV irradiation. However, in cells arrested in G1 the induction of *RAD16* mRNA after UV irradiation is not followed by a rapid decline as occurs in normal growing cells suggesting that the down regulation of *RAD16* transcription is dependent on progression into the cell cycle.**

## INTRODUCTION

DNA repair mechanisms have important roles in the maintenance of the integrity of DNA. In the yeast *Saccharomyces cerevisiae* >30 genes called *RAD* genes are known to be involved in DNA repair (11,12). Amongst the *RAD* genes the *RAD2*, *RAD6*, *RAD7*, *RAD18*, *RAD23* and *RAD54* genes have been reported to be inducible upon DNA damage and during meiosis (4,5, 19,20,23–26). The *RAD2*, *RAD7*, *RAD23* gene products are involved in nucleotide excision repair, *RAD6* and *RAD18* are required for induced mutagenesis and post-replication repair and *RAD54* functions in the repair of double-strand breaks and recombination (12). Furthermore, several other DNA damage-inducible genes have been identified, such as *PHR1* (30), *DIN* (35), *DDR* (28), *UB14* (38), *RNR2* (8,16) and the DNA replication genes *CDC9* (29) and *CDC17* (21) that encode DNA ligase and DNA polymerase I, respectively.

In *Escherichia coli* the response to DNA damage is regulated by a well-studied mechanism called the SOS-response (15,17,44). LexA protein represses transcription of the genes that are part of the SOS regulon by binding to the LexA box in the promoter regions, and RecA which is activated by DNA damage, causes proteolysis of LexA thereby eliminating the repression of these genes (17,41,44). In yeast, no evidence for such a common regulatory mechanism in controlling the response to DNA damage has been observed so far. A comparison of the promoter regions from various DNA damage inducible *RAD* genes did not result in the recognition of motives that are common to all these genes suggesting that the mechanism of regulation may differ among DNA repair genes (18).

In this paper we study the regulation of the *S.cerevisiae RAD16* gene. The *RAD16* gene belongs to the *RAD3* epistasis group and is partially deficient in excision repair as is reflected by the moderate UV sensitivity of *rad16* mutants (1,11–13). In a *rad16* mutant the transcriptional silenced *HMLα* locus is not repaired while the expressed *MATα* is repaired although at a slower rate than in repair proficient cells (1). Recently, studies on repair of the *RPB2* gene have shown that the non-transcribed strand is not repaired at all in both *rad16* and *rad7* mutants, whereas the transcribed strand is repaired at a rate similar to that in repair-proficient cells (40). These results indicate that *RAD16* and *RAD7* proteins are not only essential for repair of transcriptional silent regions but also function in repair of non-transcribed strands of active genes (40). The sequence of the *RAD16* gene shows that this gene encodes a 91.3 kD protein containing two putative zinc finger domains and several helicase domains that are also present in several other proteins like the *SNF2*, *RAD54*, *BRM* and *ERCC6* gene products from different eukaryotic origins (1). Here we report that the *RAD16* gene is inducible by UV irradiation, to some extent by heat shock and during meiosis.

## MATERIALS AND METHODS

### Strains and growth conditions

The diploid strain DDB.C7 was obtained by crossing strains *MATa* DDB7c and *MATα* DDB7b. All yeast strains were grown in complete medium (YEPD; 1% yeast extract, 2% Bacto peptone, 2% glucose) as previously described (1) except that presporulation and sporulation media used to measure transcript levels during

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meiosis were similar to the described media (31) with some modifications. Presporulation medium contains 0.3% yeast extract, 0.3% Bacto peptone, 10% glucose. The sporulation medium contains 2% sodium acetate, 0.05% yeast extract, 0.8% glucose.

### UV irradiation and sampling procedures

For UV irradiation, the yeast cells were grown in YEPD medium at 28°C to $OD_{700}$ = 1.2. Cells were collected, suspended in the same volume of sterile water and irradiated with 254 nm UV light (Phillips T UV 30 W) at a rate of 3.5 J/m$^2$/s in 50 ml portions using a 20 cm diameter glass dish. For survival experiments appropriate dilutions of the cells were plated on YEPD agar and the survival was scored after 3–4 days of incubation at 28°C. For induction experiments, the UV-irradiated cells were collected and resuspended in fresh YEPD medium (prewarmed to 28°C) and incubated at 28°C in a red flask to avoid photoreactivation. The time of resuspension was considered to be $t = 0$ after irradiation. Samples were collected at different time intervals. The samples were immediately cooled and stored on ice for RNA isolation.

### RNA isolation

Total RNA was isolated as follows (33): cells from 10 ml suspension were centrifuged and resuspended in 400 µl AE buffer (50 mM sodium acetate pH 5.3, 10 mM EDTA). Forty µl 10% SDS was added and the mixture was intensively shaken on a vortex. An equal volume of phenol (phenol/AE saturated, pH 5.3) was added and the mixture was incubated at 65°C for 4 min. The samples were frozen on dry-ice/ethanol and centrifuged for 5 min in a microfuge (14 kr.p.m.). The lysate was extracted with phenol/chloroform. RNA was precipitated with ethanol 96%, washed once with prechilled ethanol 70% and dried at room temperature for 10 min. RNA was resuspended in 100 µl sterile water and stored at –20°C. The RNA concentration was quantified by $A_{260}$ measurement. The samples were diluted to a concentration of 1 µg/µl. To verify quantification and quality of RNA preparations of 5 µg RNA from each sample was loaded on 1% agarose TAE gel with ethidium bromide (2 µg/ml).

### RNA denaturing gel and Northern blotting

Fifty to eighty µg of total RNA was dried in a speedvac and resuspended in 50% formamide (deionized), 6% formaldehyde, 12 mM Tris, 6 mM sodium acetate, 0.3 mM EDTA pH 7.5 and denatured at 65°C for 15 min. The samples were kept on ice for 5 min. Two µl tracking dye was added and the samples were loaded on a RNA denaturing agarose gel (1% agarose, 12 mM Tris, 6 mM NaAc, 0.3 mM EDTA pH 7.5). The RNA was transferred to Genescreen plus (NEN) by capillary blotting as described in the manual of the supplier. Following transfer the nylon membrane was dried for 5 min at room temperature and baked at 80°C for 20 min. The nylon membrane was prewetted in sterile water and sealed in a hybridization bag with 10 ml of hybridization solution ($NaHPO_4$ 0.5 M pH 7, SDS 7%) and incubated at 65°C for 18 h. These Northern blots were first hybridized with a *RAD16* specific probe. Then the *RAD16* probe was removed and the blots were reprobed with others probes (*HSP86* or *URA3*) as controls. The transcript levels were quantified with a Betascope 603 blot analyzer (Betagen).

### Preparation of radiolabeled probes

DNA fragments were obtained by digestion with restriction endonucleases, gel purified and diluted to a concentration of 5 ng/µl. The 0.2 kb *Hin*dIII/*Cla*I and the 0.9 kb *Pst*I/*Sph*I *RAD16* DNA fragments were used as probes for the *RAD16* mRNA. The 1.1 kb *Hin*dIII fragment of *URA3* was used to detect *URA3* transcripts. The 0.5 kb *Eco*RI fragment of the heat shock *HSP86* gene cloned in pUC19 (kind gift from Dr Jaap Venema) was used as probe to detect *HSP86* transcripts. DNA probes were labeled using random hexanucleotides and [α-$^{32}$P]dCTP (Amersham).

### Sporulation

The diploid strain *MATa/MAT*α DDB.C7 was grown in YEPD medium. The cells were inoculated in presporulation medium and grown overnight at 28°C to a density of $OD_{700}$ = 0.8. Cells were collected by centrifugation at 7 kr.p.m. for 5 min and resuspended in the same volume of sporulation medium prewarmed to 28°C. The culture was incubated at 28°C, shaken vigorously and samples were taken at intervals of 1 h. Each sample was checked by microscope and the cell morphology was recorded.

### Heat shock

For heat shock experiments, cells of the yeast strain K107 were grown in YEPD medium at room temperature to a density of 5 × 10$^7$ cells/ml. Ten ml of culture was taken as the $t = 0$ sample and stored on ice. The rest of cells was collected by centrifugation at 7 kr.p.m. for 5 min, resuspended in 100 ml YEPD prewarmed to 42°C and incubated in a water bath-agitator at 42°C. Samples were collected at 10, 20, 40, 60, 80, 100 and 120 min after the temperature shift.

### Mitotic cell cycle

The haploid yeast *MATa DDB7c* strain was grown at 28°C in YEPD medium to a density of 2 × 10$^7$ cells/ml. Yeast mating type α factor (Sigma) was added to a final concentration of 15 ng/ml. Samples were collected at 1 h intervals and examined microscopically to determine cell morphology. Three h after adding α factor cells were collected by centrifugation and washed twice with sterile water and once with YEPD medium and then resuspended in an equal volume of fresh YEPD medium. Cells were incubated at 28°C in a shaking waterbath. Samples were taken at various intervals, examined microscopically for cell morphology and the RAD16 mRNA levels were determined.

## RESULTS

### Expression of the *RAD16* gene in response to UV irradiation

To determine the effect of UV irradiation on *RAD16* expression total RNA was isolated from cells of strain K107 (*RAD*$^+$) at different times after these cells were exposed to 40 or 70 J/m$^2$ of UV light. Equal amounts of RNA were loaded on denaturing agarose gels and *RAD16* mRNA levels were examined by Northern blot analyses using a labeled *RAD16* specific probe. The blots were then washed and rehybridized with a *URA3* probe as an internal control since it has previously been reported that the *URA3* mRNA level does not change after UV-irradiation of the cells (19,21,24). Figure 1A shows the results of such an analysis. Hybridization with *RAD16* specific probes, either N-terminal or C-terminal, always resulted in two signals on the Northern blots

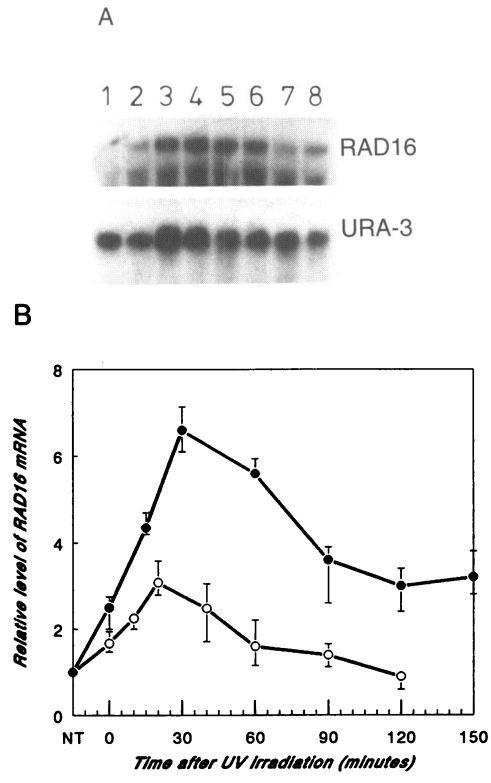


**Figure 1.** *RAD16* mRNA levels in UV-irradiated cells. Yeast strain K107 was UV-irradiated and total RNA was isolated. Eighty μg of total RNA was loaded per lane. The Northern blots were hybridized with labeled *RAD16* and *URA3* probes, respectively. (**A**) Autoradiograms of the blot were combined to show the *RAD16* and *URA3* specific bands. Lane 1: RNA from unirradiated cells that were collected 15 min before UV irradiation. Lanes 2–8: RNA from cells at 0, 10, 20, 40, 60, 90 and 120 min after exposure to 40 J/m$^2$ UV radiation. The autoradiograms were scanned using a β-scope analyzer. (**B**) Quantification of *RAD16* mRNA levels following 40 J/m$^2$ (○) and 70 J/m$^2$ (●). For each time-point the *RAD16* mRNA signal was corrected for the *URA3* mRNA signal that was used as control. The values for each time-point were calculated and plotted relative to the value for unirradiated cells (NT). The curve of 40 J/m$^2$ shown in B is of the experiment shown in A. The error bars are based on three different experiments.

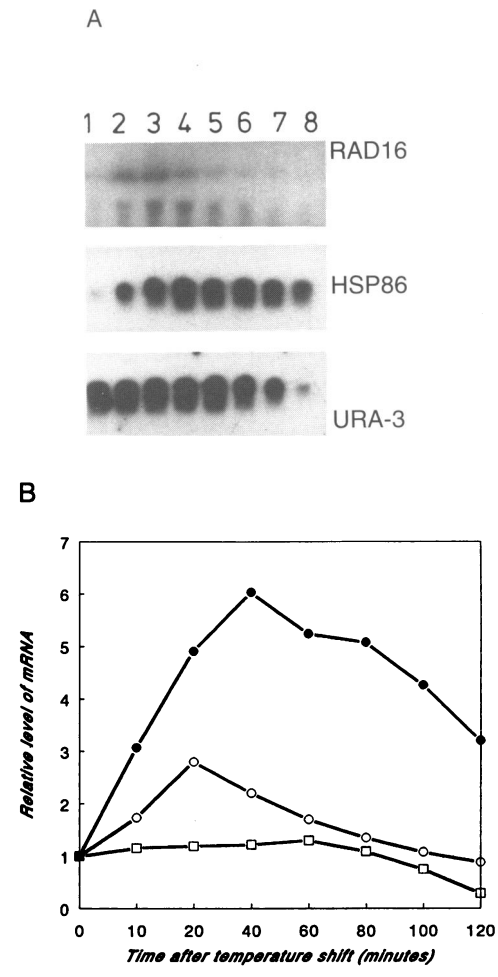


**Figure 2.** *RAD16* mRNA levels following heat shock treatment. The yeast strain K107 was grown at 25°C and shifted to 42°C. The samples were collected before and after the temperature shift at various times and total RNA was isolated. Eighty μg of RNA was applied in each lane. The Northern blots were subsequently probed with *RAD16*, *HSP86* and *URA3* labeled probes. The autoradiograms were scanned by a β-scope analyzer. (**A**) Autoradiograms showing the *RAD16*, *HSP86* and *URA3* bands. Lane 1: RNA from yeast cells maintained at 25°C. Lanes 2–8: RNA from cells incubated for 10, 20, 40, 60, 80, 100 and 120 min at 42°C. (**B**) Quantification of *RAD16* (○), *URA3* (□) and *HSP86* (●) mRNA levels during heat shock. The values determined for each time-point were plotted relative to the value for cells at 25°C ($t = 0$, lane 1). The experiment was repeated twice with similar results.

corresponding to mRNAs of ~2.3 and 1.9 kb. Quantification of the signals using the β-scope showed that they had the same kinetics of induction and both signals disappeared when RNA isolated from a *rad16* disruption mutant was used. Therefore the two signals were taken together in the quantitative analysis. The amount of *RAD16* mRNA in every lane was corrected for the *URA3* signal in the same lane. Under our experimental conditions we always observed a slight increase of the *URA3* signals upon UV irradiation. Figure 1B shows the relative amounts of *RAD16* mRNA plotted against the time after UV irradiation. Clearly, *RAD16* mRNA is induced upon UV irradiation. After 40 J/m$^2$ the induction is ~3-fold and after 70 J/m$^2$ 7-fold. In a control experiment in which unirradiated cells were treated in a manner identical to the UV-irradiated cells we observed no increase in *RAD16* mRNA level (results not shown).

## Heat shock induced *RAD16* transcription

Some yeast genes that are induced by DNA damage are also inducible by heat shock (10,27,38). We tested whether this is also the case for the *RAD16* gene. Cells were grown at 25°C then shifted to 42°C and maintained at this temperature for 2 h. Total RNA at different times after the temperature shift was isolated and analyzed by Northern blotting. Figure 2A shows that *RAD16* mRNA levels increase immediately after the temperature shift and reach a maximum after 20 min. The maximal induction is ~2.8-fold. As a control we used *HSP86*, a genuine heat shock gene. In this case we observe a 6.5-fold increase in mRNA levels (Fig. 2B) whereas hardly an increase in *URA3* mRNA is observed (Fig. 2B).

## Expression of the *RAD16* gene during meiosis

To examine whether the *RAD16* gene is induced during meiosis as was observed for other UV-inducible *RAD* genes, we measured *RAD16* mRNA levels during sporulation in the *MAT***a**/*MAT*α diploid strain DDB.C7. This strain is characterized by rapid and efficient sporulation. In our experiments we found that spore

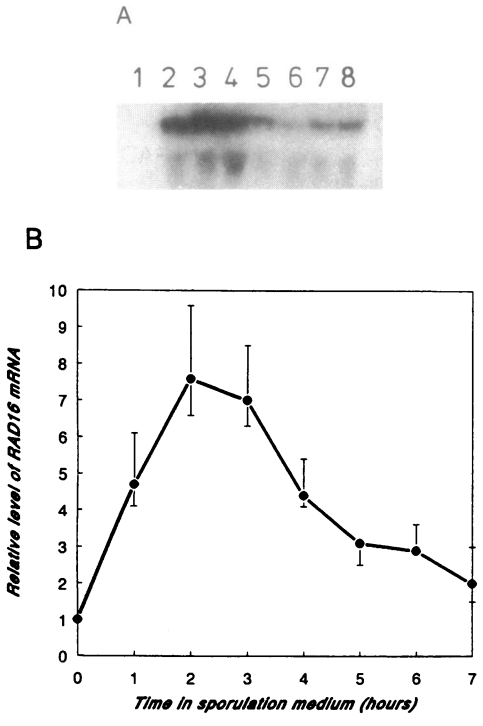


**Figure 3.** *RAD16* mRNA during sporulation. Yeast strain *MATα/MATa* DDB.C7 was transferred from presporulation medium to sporulation medium and incubated at 28°C with vigorous aeration. Samples were collected at various intervals after medium shift and RNA was isolated. Eighty μg RNA was loaded/lane. Northern blots were probed with labeled *RAD16* specific probes. (**A**) *RAD16* mRNA levels during sporulation. Lane 1: RNA from cells grown in presporulation medium ($t = 0$). Lanes 2–8: RNA from cells at 1, 2, 3, 4, 5, 6 and 7 h after transfer from presporulation to sporulation medium. (**B**) Graphic representation of the quantification of the Northern blot. The values of *RAD16* mRNA levels at various times are plotted relative to the value of *RAD16* mRNA level from cells grown in presporulation medium ($t = 0$). The error bars are derived from two other experiments.

formation becomes visible after 5 h of incubation in sporulation medium and after 11 h >90% of the cells have sporulated. Samples were collected before and at various times after transfer of the cells to sporulation medium and total RNA was isolated and analyzed on Northern blots. Figure 3A shows the *RAD16* mRNA levels during meiosis. *RAD16* mRNA levels increase rapidly and reach a maximum at 2 h after transfer to sporulation medium. Quantification of the *RAD16* mRNA levels during sporulation (Fig. 3B) indicates that the maximal induction is 7.5-fold after 2 h of incubation in sporulation medium.

## Expression of *RAD16* during the mitotic cell cycle

The regulation of *RAD16* during the mitotic cell cycle was investigated by sampling cells from synchronised cultures at different stages of the cell cycle and analyzing the *RAD16* transcript levels. We used the haploid *MATa* DDB7c strain because this strain can be synchronized by a low amount of α factor (15 ng/ml). Synchronous cell division was observed for at least two cell cycles after release from α factor. The level of synchronisation achieved using α factor is shown in Figure 4. Quantification of the *RAD16* mRNA signals shows that the level of *RAD16* expression is constant throughout the mitotic cell cycle.

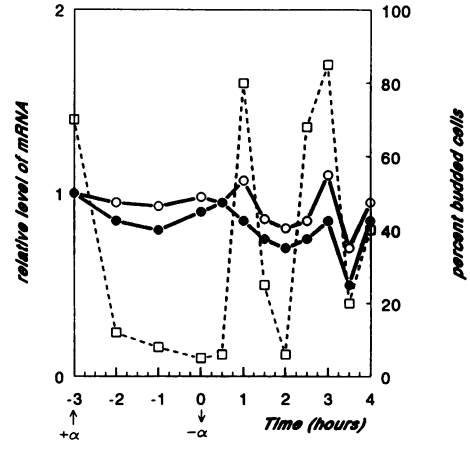


**Figure 4.** *RAD16* mRNA in the mitotic cell cycle. The yeast haploid strain DDB7c *MATa* was grown in YEPD medium to a density of $2 \times 10^7$ cells/ml and arrested in the G1 stage by α factor treatment (15 ng/ml). Samples were collected just before to the addition of α factor, after 1 and 2 h of incubation with α factor (indicated: –1, –2), immediately after release from α factor ($t = 0$) and subsequently at 30 min intervals. Samples were examined microscopically and the percent of budded cells (□) were recorded. Total RNA were isolated from each sample and 80 μg RNA was loaded per lane. The Northern blots were hybridized with labeled *RAD16* probe then washed and reprobed with *URA3* probe. The autoradiograms were scanned by a β-scope analyzer. Quantification of *RAD16* (●) and *URA3* (○) mRNA levels. The values of *RAD16* mRNA levels at various times are plotted relative to the value of *RAD16* mRNA level from cells not treated with α factor (–3).

## *RAD16* mRNA levels after UV irradiation of G1 cells

The observed induction of *RAD16* mRNA after UV irradiation could be a result from a direct effect of UV irradiation or alternatively from an indirect effect due to UV-induced arrest in the G2 phase of the cell cycle. To discriminate between these two possibilities we irradiated (70 J/m$^2$) a culture of haploid *MATa* DDB5b cells arrested in G1 with α factor and measured *RAD16* mRNA levels at different times after irradiation (Fig. 5A). When non-irradiated G1 cells are released from α factor we observe bud formation after 30 min (Fig. 4). UV irradiation of the G1 cells lead to a budding delay of 30 min (Fig. 5B) as described earlier (45). The induction of the *RAD16* gene in UV-irradiated G1 cells is not significantly different from that in non-synchronized cells. The *RAD16* mRNA level increases 4–5-fold at 30 min (Fig. 5A, lane 5) then decreases rapidly. Such a rapid induction and decline is always observed in *RAD16* induction experiments. However, in UV-irradiated G1 cells that are not released from α factor, the expression of the *RAD16* gene remains at the high induced level. This finding suggests that down-regulation of RAD16 expression is dependent on progression into the cell cycle. To test this the following experiments were performed. Cells were arrested in G1 by α factor, then UV-irradiated with 70 J/m$^2$ and further incubated in medium with α factor to ensure that the cells stayed arrested in G1. In one experiment α factor was removed at 120 min after UV irradiation and in the other α factor was not removed. In the first experiment the *RAD16* mRNA level increases rapidly reaching a peak after 30 min and remains at this high level until 120 min after UV irradiation when the α factor is released (Fig. 6). At that moment RAD16 mRNA level drops almost immediately. In the other experiment with cells that were continuously maintained in

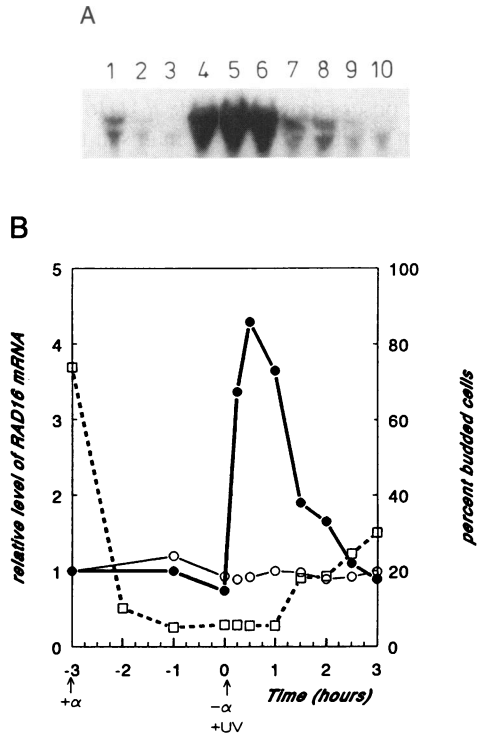


**Figure 5.** *RAD16* mRNA levels in UV-irradiated G1 cells. Cells from the haploid DDB7c *MATa* strain were grown in YEPD medium to a density of $2 \times 10^7$ cells/ml and α factor was added to synchronize cells in G1. Samples were collected just before the addition of α factor and after 3 h of incubation with α factor and checked microscopically for the percentage of budded cells. The cells were washed, resuspended in water and UV-irradiated with a dose of 70 J/m$^2$. Following UV radiation the cells were collected and resuspended in YEPD medium. Total RNA from cells at different time-points was isolated and 80 µg RNA was loaded per lane. The blot was hybridized with labeled *RAD16* specific probe. (**A**) Lane 1: RNA from untreated cells. Lane 2: RNA from unirradiated cells exposed to α factor for 3 h. Lanes 3–10: RNA from cells at time $t = 0, 15, 30, 60, 90, 120, 150$ and 180 min after UV irradiation. (**B**) Graphic representation of the quantification of the Northern blots. The amount of *RAD16* mRNA (●) at each time-point was normalized to the *RAD16* mRNA level in not treated cells (indicated: –3). Also shown are the relative levels of *URA3* mRNA (○) and the percent of budded cells (□).

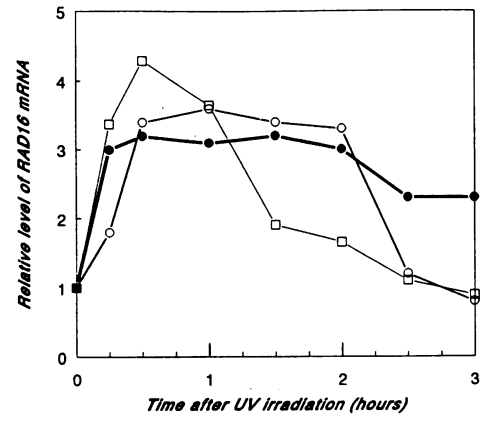


**Figure 6.** *RAD16* mRNA levels in UV irradiated G1 cells maintained in the presence and absence of α factor. The haploid DDB7c *MATa* strain was grown in YEPD medium to a density of $2 \times 10^7$ cells/ml. The cells were arrested in G1 by α factor for 3 h, washed and resuspended in water and UV-irradiated at 70 J/m$^2$ and then kept in the medium without or with α factor to ensure that the cells remained in G1. 120 min after UV irradiation α factor was removed. Total RNA was isolated and 80 µg of total RNA was loaded per lane. The blot was hybridized with labeled *RAD16* probe then washed and reprobed with *URA3* probe and the bands on the blots were quantified using a β-scope analyzer. The graph represents the relative amount of *RAD16* mRNA in cells released from α factor immediately after UV irradiation (□), in cells released from α factor 2 h after UV irradiation (○) or in cells maintained in the continuously presence of α factor (●). The value for each time-point was normalized to *RAD16* transcript level in the sample $t = 0$.

the presence of α factor, the *RAD16* mRNA level remains at the induced level until 180 min (Fig. 6). At this point the mRNA levels of both the *RAD16* and the *URA3* genes drop dramatically. Probably the cells die as a result of the 3 h of incubation in medium containing α factor after UV radiation. Indeed these cells are not able to divide any more after release from α factor. These results suggest that indeed the *RAD16* gene can only be down regulated when the cells progress from the G1 phase further into the cell cycle.

## DISCUSSION

We have studied the inducibility of the *RAD16* gene upon UV irradiation by Northern blot analysis. Hybridization with probes derived from different regions of the *RAD16* gene always resulted in two *RAD16* signals on the blots. One possible explanation for the two signals could be that one signal is derived from *RAD16* gene and the other is derived from one of the genes that are homologous to the *RAD16* gene, since the *RAD16* gene has been shown to be a member of the *SNF2* helicase gene family. The *RAD16* gene is strongly homologous in its putative helicase domains to at least three other yeast genes: *SNF2*, *RAD5* and *RAD54* (1,9,18,22). However, both signals disappeared when mRNA was isolated from a *rad16* disruption mutant. Within the coding sequence of the *RAD16* gene a consensus for a branch point for splicing of yeast polymerase II transcripts is present, which means that the *RAD16* mRNA could be alternatively spliced and therefore give two signals on the blots. However, PCR on cDNA made on polyA$^+$ mRNA or on chromosomal DNA using different combinations of *RAD16*-specific primers always resulted in the same specific PCR product (our unpublished data). This would suggest that the *RAD16* mRNA is not spliced. Furthermore, multiple transcripts have been observed for several members of the *SNF2* helicase gene family like *S.cerevisiae RAD54* (4), *S.pombe rad8* (7) *rhp51*$^+$ (46), *Drosophila* Lodestar (14), mouse *CHD1* (6) and human *ERCC6* (39). Possibly this is a common feature of the members of this family. In most cases the phenomenon has been attributed to alternative polyadenylation (7). The two signals showed the same kinetics for UV induction, therefore in all our calculations the two signals were taken together. At all UV doses examined *RAD16* mRNA levels rapidly increased after irradiation reaching a peak at ~30 min. Compared to the level of *RAD16* mRNA in cells from unirradiated, exponentially growing culture, the increase of *RAD16* mRNA levels was 3–7-fold following UV doses of 40 and 70 J/m$^2$, respectively. The induction factors obtained under our experimental conditions are comparable to those observed for other UV inducible genes (5,19,20,23,25).

We have shown that repair of the transcriptional silenced *HMLα* locus is absent in both *rad16* and *rad7* mutants (36). The *RAD7* gene also has been shown to be an UV-inducible gene (20). The kinetics of UV induction of the *RAD16* gene are similar to

that of the *RAD7* gene. The maximum induction of both *RAD16* and *RAD7* mRNA occurs ~30–40 min after UV irradiation. We have shown that, upon UV irradiation, the difference between repair of *MATα* and *HLMα* seems to be due to a time lag of 30–40 min in the repair of *HLMα* (37). The time needed for maximal induction of both *RAD16* and *RAD7* seems to coincide with the time lag of repair of *HMLα*. Therefore, one could speculate that this time lag is due to the necessity to induce *RAD16* and *RAD7* for the repair of *HMLα*. The *RAD16* and *RAD7* genes were shown to be epistatic among the genes involved in nucleotide excision repair since no differences in phenotype were found between the two single mutants and the double mutant (40). Analysis of repair of the *RPB2* gene has shown that the repair of the non-transcribed strand is absent in both *rad16* and *rad7* mutants (40). Probably the *RAD16* and *RAD7* gene products act in consecutive steps in the same pathway or function in a complex. One could argue that *RAD16* might be necessary for induction of *RAD7* (42) or vice versa. However, this is not the case since both *RAD16* in a *rad7* mutant and *RAD7* in a *rad16* mutant are normally induced by UV (unpublished results).

In contrast to many UV-inducible genes, *RAD16* resembles the damage inducible genes *DDR* (27) and *UB14* (38) which are both induced by UV damage and heat stress. A short heat stress resulted in a 2.8-fold *RAD16* transcript accumulation within 20 min of heat treatment. However *RAD16* transcripts levels obtained after heat shock were much lower than those reached upon UV irradiation and furthermore the *RAD16* induction upon heat stress is not comparable to that of a genuine heat shock protein like *HSP86* (6.5-fold). In contrast to their similar behaviour on UV induction, *RAD16* and *RAD7* differ in their response to heat shock since heat stress has no effect on *RAD7* expression (20). The increase of *RAD16* mRNA level in both UV-irradiated cells and in cells under heat stress suggests that *RAD16* could play a role in the response not only to UV damage but also to other stress conditions.

Expression of the *RAD16* gene is enhanced during meiosis. *RAD16* mRNA levels accumulated up to 7.8-fold after incubation of *MATα/MAT***a** diploid cells in sporulation medium for 2 h. The accumulation of *RAD16* mRNA levels during meiosis is specific to cells undergoing sporulation and is not due to the stress caused by starvation in sporulation conditions, since both a haploid *MATα* strain and an asporogenous *MAT***a***/MAT***a** strain showed no enhancement of *RAD16* transcription following 6–10 h of incubation in sporulation medium (data not shown). Without an internal control for mitotic events it is hard to make comparisons with other sporulation induced genes, but it seems that the early induction of *RAD16* might resemble that of the DNA replication genes: DNA ligase *CDC9* and DNA polymerase I *CDC17* that occurs at the premitotic DNA synthesis stage 3 h after transfer to sporulation medium (19). In contrast to the early induction of the *RAD16*, the *RAD7* gene is induced late at 7–8 h after transfer to sporulation medium (20). The high level of *RAD16* mRNA in the early stage of sporulation might reflect the need of the *RAD16* product in meiosis. However, a *rad16* mutation does neither affect sporulation nor spore viability. So far, it seems that all the *RAD* genes that were shown to be inducible by UV are also induced during meiosis. This could suggest a possible overlap in the regulatory mechanisms involved in UV induction and induction during meiosis.

The fact that *RAD16* expression is constant during the mitotic cell cycle, and that the induction of *RAD16* gene in G1-arrested cells is identical to that of vegetatively growing cells, shows that UV induction is not an indirect effect of cell cycle arrest that occurs in response to DNA damage (33,43).

An interesting observation is that the *RAD16* expression after UV irradiation of G1-arrested cells remains at the high-induced level when the cells stay arrested in the G1 phase in the constant presence of α factor. There are several possible explanations for this phenomenon. It could be the result of remaining dimers in the DNA as a consequence of a low level of repair in G1 cells. However, using dimer-specific antibodies to measure dimer removal from DNA, which was isolated from the same UV-irradiated G1 cells, we found that the overall repair rate was comparable to that of normal UV-irradiated cells (unpublished data). Another possibility is that in fact transcription does go down, but does not result in a lower signal for *RAD16* mRNA in G1 cells because *RAD16* mRNA is more stable in G1 cells than in other phases of the cell cycle. Last, but not least, the down regulation of *RAD16* expression in UV-irradiated cells might be dependent on an unknown factor that is absent in G1 cells. This could be a protein that is only present in other stages of the mitotic cell cycle or, alternatively, the replication process might be involved in down regulation of the *RAD16* gene. Such an involvement of replication in gene regulation has been demonstrated in the establishment of silencing of the mating-type loci in yeast.

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