MOLECULAR AND CELLULAR BIOLOGY, Mar. 1988, p. 1132–1136
0270-7306/88/031132-05$02.00/0

Vol. 8, No. 3

Expression of the Yeast *UBI4* Gene Increases in Response to DNA-Damaging Agents and in Meiosis

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Received 4 September 1987/Accepted 11 December 1987

The polyubiquitin gene, *UBI4*, of *Saccharomyces cerevisiae* is regulated by a variety of environmental stresses and physiological conditions. After exposure of rapidly growing yeast cells to DNA-damaging agents (4-nitroquinoline-1-oxide and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine), intracellular levels of *UBI4* transcript increased rapidly. Induction of *UBI4* transcripts occurred within 30 to 60 min of exposure to 4-nitroquinoline-1-oxide in *RAD*$^+$, *rad52*, and *rad6* repair-deficient yeast strains. In high-density *RAD*$^+$ cultures, the effect of alkylating agents on *UBI4* transcript levels is attenuated, in part because of significant increases in the basal level of this message in untreated cells. We also observed that the levels of *UBI4* transcripts increased significantly when diploid cells were exposed to sporulation conditions. Maximal levels of *UBI4* transcripts were reached after 6 to 8 h in sporulation medium. Accumulation of *UBI4* transcripts occurred in a/α diploids that undergo meiosis but not in asporogenous α/α diploids exposed to the same nutritional conditions.

Ubiquitin is a 76-residue polypeptide that shows extraordinary sequence conservation among eucaryotes and is found both free and conjugated with other cellular proteins (6). There is considerable biochemical and genetic evidence indicating that coupling of ubiquitin to certain intracellular proteins results in their degradation (2, 3, 6). Moreover, ubiquitin has also been found conjugated with platelet-derived growth factor receptor and other cellular receptors (14, 15).

Varshavsky and co-workers have isolated and characterized a ubiquitin gene family in *Saccharomyces cerevisiae*. The four ubiquitin genes exist as fusion genes: *UBI1*, *UBI2*, and *UBI3* genes encode ubiquitins with carboxyl-terminal tails of unrelated amino acid sequences. The *UBI4* gene consists of five head-to-tail repeats of the ubiquitin-coding sequence (10, 11). Recent experiments by Finley et al. (5) demonstrated that the *UBI4* gene is responsive to heat stress and is required for cell survival under a variety of stress conditions, including chronic heat treatment, exposure to amino acid analogs, and nutrient starvation. Moreover, *ubi4/ubi4* diploids fail to sporulate, and haploid spores containing a *ubi4* mutation display an unusual time-dependent loss of germination ability (5). These and other observations demonstrate that the *UBI4* gene is important for growth of yeast cells and is regulated in response to different environmental stresses.

We have identified a family of genes in *S. cerevisiae* which displays increased transcription after treatment of cells with a variety of DNA-damaging agents (8). Recently, we demonstrated that two of these DNA damage-responsive (DDR) genes are transcriptionally responsive to heat stress as well as to treatments that damage DNA (9). During the course of this work, we investigated the response of the *UBI4* gene to DNA damage stress. We show in this paper that the levels of the *UBI4* transcript were markedly increased after exposure of cells to two different chemical mutagens and carcinogens. Transcript accumulation was rapid in both *RAD*$^+$ and *rad* mutant strains of *S. cerevisiae* during early exponential growth. Interestingly, this DNA damage response of the *UBI4* gene was greatly attenuated in dense cell cultures. We also demonstrate that the *UBI4* gene transcripts accumulated during sporulation in a/α diploids but not in isogenic but asporogenous α/α diploids. These results provide additional insight into the roles of ubiquitin in *S. cerevisiae* and demonstrate further regulatory complexity of the *UBI4* gene.

MATERIALS AND METHODS

Yeast strains and conditions for growth. *S. cerevisiae* M12B (α *trp1-289 ura3-52 gal2*) is wild type for DNA repair functions (*RAD*$^+$); strain SX46A-rad6Δ (*rad6 ura3*) contains a deletion of the *rad6* gene and was kindly provided by L. Prakash, University of Rochester; strain YNN209 (*trp1 ade2-1 his3-Δ1 ura3-52 rad52-1 rad50*) was obtained from Ron Davis, Stanford University. The isogenic diploid strains AP1 a/α (*ade2*) and AP1 α/α (*ade2*) were obtained from P. Magee, Michigan State University (4).

Cells were grown in YPD broth (2% Bacto-Peptone, 2% glucose, 1% yeast extract [Difco Laboratories, Detroit, Mich.]) with continuous shaking. Unless otherwise stated, cells were treated with 4-nitroquinoline-1-oxide (4NQO) at 30°C, when cells reached a density of 10^7/ml (early log phase). The doses of 4NQO (Sigma Chemical Co., St. Louis, Mo.) and the duration of exposure were as given in the figure legends. Heat shock treatment of strain M12B was performed as described previously (9). The conditions for exposure of cells to the alkylating agent *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG; Sigma) have been previously described (7).

Sporulation conditions. Cultures of strains AP1 a/α and AP1 α/α were grown overnight at 30°C in YPD medium until early stationary phase. Cells (150 ml) were washed twice with an equal volume of distilled water and suspended in sporulation medium (200 ml; 1.5% potassium acetate supplemented with one-fourth the normal concentration of required nutrients). At t = 0, 2, 4, 6, 8, 9.5, and 23 h after suspension in sporulation medium, a 25-ml sample of cells was removed. A small sample (200 μl) was examined by light microscopy to determine the percentage of mature asci. Asci were observed only at t = 23 h in cultures of strain AP1 a/α (24% of total cells). RNA was extracted from the rest of the 25-ml sample as indicated below.

RNA isolation and Northern (RNA) hybridization analysis.

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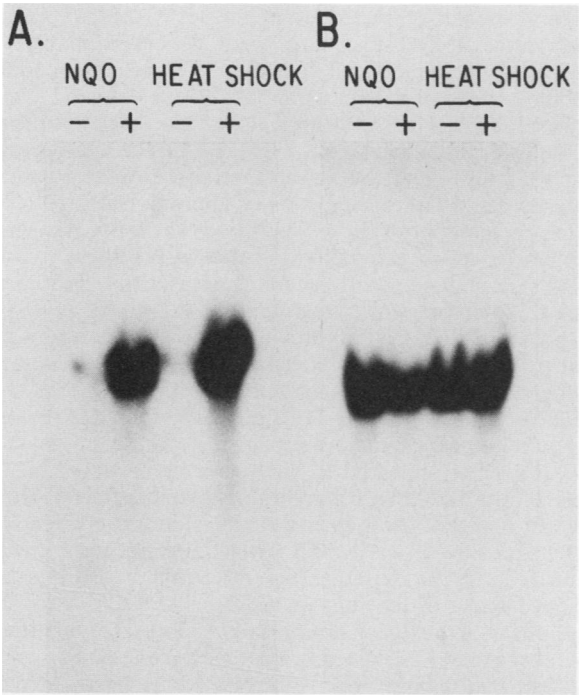

FIG. 1. DNA damage and heat shock induction of *UBI4* transcripts. Total RNA was isolated from exponentially growing M12B control cells and from cells that had been stressed with 4NQO or heat treatment. Samples (100 μg each) were separated by electrophoresis in formaldehyde-agarose gels (1%), transferred to nitrocellulose, and hybridized with radiolabeled *UBI4* probe (A) or *ADC1* probe (B) as described in Materials and Methods. Lanes: NQO −, cells grown at 30°C, no treatment; NQO +, 1 μg of 4NQO per ml, 60 min; heat shock −, cells grown at 23°C, no treatment; heat shock +, cells treated at 37°C for 20 min.

RNA was extracted from yeast cells by glass bead disruption as previously described (8). Total RNA was denatured and separated by electrophoresis in agarose gels (1%) containing formaldehyde, as described previously (9).

Radiolabeling of plasmid DNA. Plasmid pUB200, containing the *UBI4* polygene (11), was radiolabeled with a multiprime kit (Amersham Corp., Arlington Heights, Ill.) and [α-^{32}P]dCTP (ICN Pharmaceuticals Inc., Irvine, Calif.; 3,000 Ci/mmol) according to manufacturer directions.

RESULTS

***UBI4* gene transcripts accumulate in *S. cerevisiae* after exposure to DNA damage and heat shock stresses.** Exposure of *S. cerevisiae* M12B to 4NQO (1 μg/ml) for 60 min resulted in markedly increased levels of *UBI4* RNA, as judged by Northern hybridization analysis (Fig. 1). In early exponentially growing cultures, the level of *UBI4* transcript was relatively low and increased more than 20-fold after 4NQO treatment. A brief heat shock stress also resulted in *UBI4* transcript accumulation within 20 min of treatment. The levels of *UBI4* transcripts attained after 4NQO treatment were comparable to those reached after heat shock. In the same experiment, we measured the levels of transcripts homologous to the *ADC1* locus, the constitutively expressed alcohol dehydrogenase structural gene (1). The results (Fig. 1B) demonstrated that *ADC1* transcript levels were unaffected by either 4NQO or heat shock treatment, which dramatically affected *UBI4* expression.

We examined the dose response and time course of *UBI4* transcript accumulation after exposure of cells to another DNA-damaging agent, MNNG. *UBI4* transcript levels increased after exposure to 4 μg/ml, up to approximately 8 μg of MNNG per ml (Fig. 2A). At higher concentrations of this damaging agent, *UBI4* transcript levels decreased slightly, a response that has also been observed for other DDR genes. We also observed a dose-dependent increase in *UBI4* transcript levels after exposure to 4NQO (see Fig. 3A).

The kinetics of *UBI4* transcript accumulation were examined after exposure of cells to 8 μg of MNNG per ml or 1.5 μg of 4NQO per ml (Fig. 2B). Significant increases in the levels of *UBI4* transcript were evident after exposure of cells to 4NQO for 30 min. Transcripts continued to accumulate for up to 60 min in cells treated with either MNNG or 4NQO and were still elevated 120 min after addition of MNNG or 4NQO.

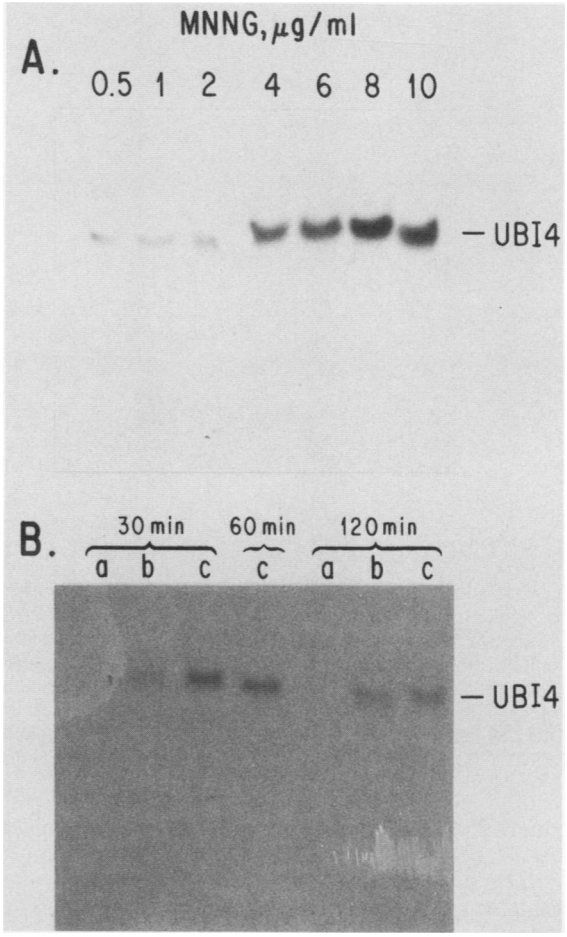

FIG. 2. Dose response and kinetics of *UBI4* transcript accumulation after DNA damage. (A) Total RNA (50 μg per lane) was isolated from cultures of strain M12B that had been exposed to the indicated concentrations of MNNG for 60 min. The gel was blotted to nitrocellulose and analyzed by Northern hybridization with radiolabeled pUB200 probe. (B) Total RNA (50 μg per lane) was isolated from untreated M12B cells (lanes a), from cells treated with 8 μg of MNNG per ml (lanes b), and from M12B cells exposed to 1.5 μg of 4NQO per ml (lanes c) for the indicated times. RNA was analyzed by Northern hybridization with ^{32}P-labeled pUB200 probe as described in Materials and Methods. Control hybridizations indicated that comparable amounts of RNA were present in all of the lanes.

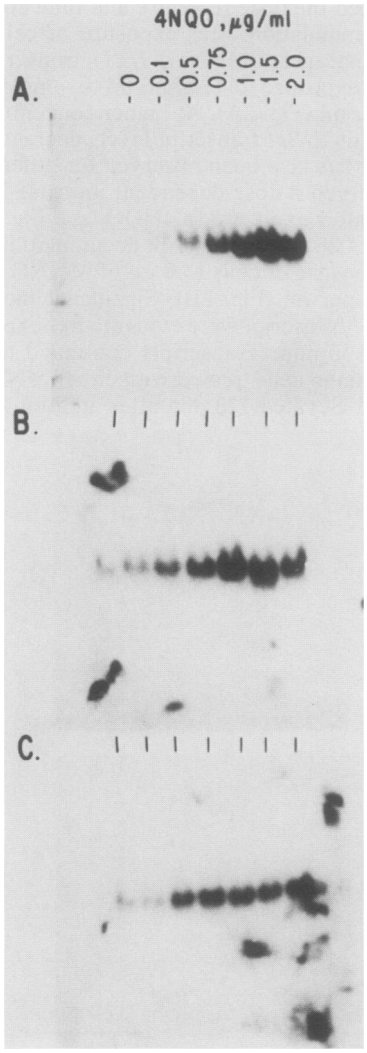

FIG. 3. Induction of *UBI4* transcripts in *rad6*, *rad50 rad52*, and *RAD*$^+$ yeast strains by 4NQO. Yeast strains growing in YPD were exposed to the indicated concentrations of 4NQO for 60 min. RNA was isolated and analyzed by Northern hybridization as described in Materials and Methods, with a radiolabeled *UBI4* probe. Panels: A, total RNA (50 μg per lane) isolated from strain M12B; B, total RNA (100 μg per lane) isolated from strain YNN209 (*rad50 rad52*); C, total RNA (100 μg per lane) isolated from strain SX46A-rad6Δ.

Induction of *UBI4* transcripts in different *rad* mutant yeast strains. To determine the genetic requirements for regulating the *UBI4* gene in response to mutagen-carcinogen treatment, we examined the levels of *UBI4* transcripts in different *rad* mutant yeast strains. Treatment of a *rad52 rad50* strain (YNN209) or a *rad6* strain (SX46A-rad6Δ) with 4NQO resulted in a dose-dependent increase in the levels of *UBI4* transcripts (Fig. 3). The *RAD52* gene is required for efficient homologous recombination and double-strand break repair, whereas the *RAD6* gene is needed for most chemical and radiation mutagenesis in *S. cerevisiae* (12, 13). A comparison of these dose-response data suggests that the *UBI4* transcript was induced at the same concentrations of 4NQO in the *rad6* mutant as in the *rad52* or *RAD*$^+$ strain. Thus, neither the *RAD52* nor the *RAD6* gene is required for induction of *UBI4* transcripts after 4NQO exposure.

Cell density effects on *UBI4* transcript induction by 4NQO. The largest relative increases in the levels of *UBI4* transcripts after 4NQO exposure were observed in rapidly growing cultures at low cell densities. In these exponentially growing cultures of haploid strain M12B, we detected a very low basal level of *UBI4* transcripts by Northern hybridization analysis (control lanes, Fig. 1). As the cell density increased, however, the basal level of *UBI4* transcripts increased such that, at densities of approximately 10^8 cells per ml, the amount of the 1.5-kilobase (kb) *UBI4* transcript in untreated cells was readily detected by Northern hybridization (Fig. 4, lane 13). A second transcript that hybridized to the *UBI4* probe, which migrated as a 0.7- to 0.8-kb RNA, was present in control cultures and was unaffected by cell density. This transcript is likely derived from one or more members of the ubiquitin gene family, with which *UBI4* has extensive homology (10). Treatment of strain M12B with 4NQO (1.5 μg/ml, 60 min) produced a significant increase in *UBI4* RNA but appeared to have little or no effect on the levels of the 0.7-kb cross-hybridizing transcripts (Fig. 4, lanes 10 and 12). At higher cell densities, the effects of 4NQO exposure on the 1.5-kb *UBI4* transcript were greatly attenuated as the basal levels of *UBI4* transcripts approached those of the fully induced cells. Thus, the *UBI4* gene appears to be under complex physiological regulation in haploid strain M12B. We also examined the response of the *UBI4* gene in diploid yeast strain AP1 a/α. In low-density cultures (Fig. 4, lanes 1 through 4) of this strain, the *UBI4* transcript rapidly accumulated after 4NQO exposure, whereas in higher-density cultures (Fig. 4, lanes 5 through 8) the effects of 4NQO were much less pronounced, partly

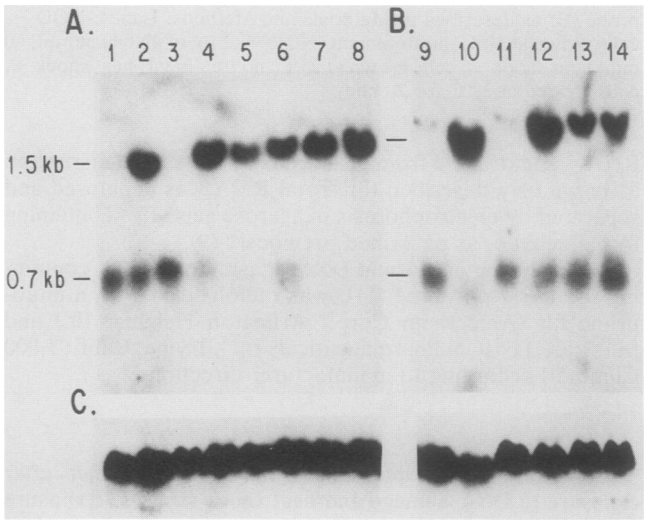

FIG. 4. Cell density effects on basal and 4NQO-induced levels of *UBI4* transcripts in diploid and haploid *S. cerevisiae*. Cultures of yeast strains AP1 a/α and M12B were grown to different cell densities, and half of each culture was treated with 4NQO (1.5 μg/ml, 60 min). Control and 4NQO-treated cells were collected, and total RNA was prepared by glass bead disruption. Samples of total RNA (100 μg per lane) were separated by electrophoresis in formaldehyde-agarose gels (1%), transferred to nitrocellulose, and hybridized with radiolabeled *UBI4* probe. Lanes: 1 to 8, strain AP1; 9 to 14, strain M12B. Cell density was determined by turbidity at 600 nm (lanes): 1 and 2, 0.17; 3 and 4, 0.48; 5 and 6, 0.78; 7 and 8, 1.7; 9 and 10, 0.18; 11 and 12, 0.42; 13 and 14, 1.8. The even-numbered lanes contained RNAs from 4NQO-treated cell cultures isolated at the indicated cell densities. For panel C, the filter was hybridized with a control probe containing a constitutively expressed member of the *HSP70* gene family (YG102 [9]).

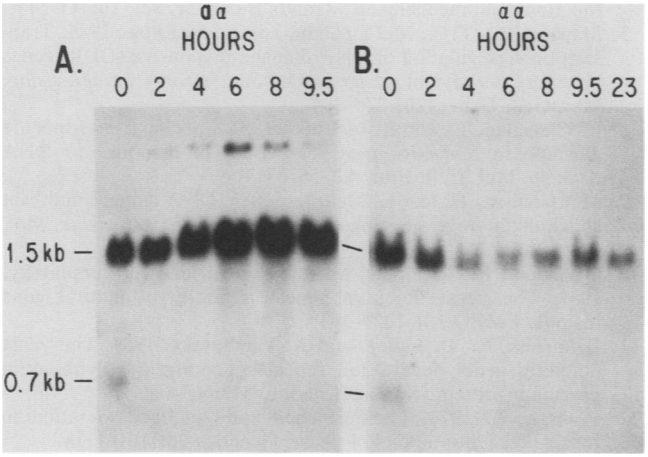

FIG. 5. Induction of *UBI4* transcripts during meiosis. Strains AP1 **a**/α and AP1 α/α were incubated in sporulation medium as described in Materials and Methods. At the indicated times after transfer to potassium acetate, samples of cells were collected and total RNA was prepared. RNA (25 μg per lane) was fractionated by electrophoresis in formaldehyde-agarose gels (1%) and analyzed by Northern hybridization with a *UBI4* probe. Panels: A, strain AP1 **a**/α; B, strain AP1 α/α. The origin of the large transcript that hybridized to the *UBI4* probe after 6 to 8 h of sporulation of strain AP1 **a**/α was not determined.

because of the significant increase in the basal level of this message. Interestingly, the amount of the 0.7-kb transcript appeared to decrease at higher densities of the diploid cell culture (compare lanes 1 and 7).

Regulation of the *UBI4* gene in meiosis. Finley and coworkers (5) have demonstrated a requirement for the *UBI4* gene in meiosis: *ubi4*/*ubi4* homozygous diploids fail to sporulate. This result is complicated by the fact that *ubi* mutant yeast strains are sensitive to nutrient starvation, and starvation for nitrogen is a requisite condition for inducing sporulation in wild-type diploid *S. cerevisiae*. To investigate a possible role for *UBI4* in meiosis, we examined the levels of its transcript by Northern hybridization. These results are shown in Fig. 5A. In diploid yeast strain AP1 **a**/α, the levels of *UBI4* transcript were initially high because of the high density of cells necessary for efficient sporulation. By 6 h of incubation in sporulation medium, there was a substantial increase in the amount of *UBI4* transcript. The transcript level remained high after 8 h of incubation in sporulation medium and started to decrease slightly by 9.5 h. That this increase in *UBI4* transcript levels was due to sporulation per se and not to nonspecific starvation effects was demonstrated by measurement of the levels of *UBI4* transcripts in cultures of the isogenic AP1 α/α strain incubated under the same conditions in sporulation medium. This α/α strain is incapable of undergoing meiosis and served as a control for starvation effects. Levels of *UBI4* transcripts actually decreased after exposure of this asporogenous diploid to sporulation conditions for up to 23 h (Fig. 5B). We conclude that the *UBI4* transcripts accumulate in cells during the first 8 h of sporulation as part of the meiotic response of **a**/α diploids. Upon longer exposure of the autoradiogram, we detected the 0.7-kb transcripts in RNA samples of both strains AP1 **a**/α and AP1 α/α. However, by 2 h of incubation in sporulation medium, the levels of these transcripts were substantially reduced, suggesting that one or more of the other *UBI* genes is sensitive to starvation (or more precisely, sporulation conditions) and this effect is independent of cell type.

DISCUSSION

The *UBI4* gene of *S. cerevisiae* is regulated by a variety of environmental and physiological stimuli. Finley et al. (5) have demonstrated that this gene is expressed at elevated levels during heat shock stress or starvation. Furthermore, these researchers demonstrated increased levels of *UBI4* transcripts in stationary cultures of *S. cerevisiae* compared to levels in exponentially growing cells. In this report, we demonstrate additional regulatory features of the *UBI4* gene. We found that treatment of cells with DNA-damaging agents such as 4NQO and MNNG caused a rapid increase in the levels of *UBI4* transcripts in exponentially growing cells. The dose response and kinetics of transcript accumulation were similar to those reported for the *DDRA2* and *DDR48* genes, which, like *UBI4*, also respond to heat shock stress (9). We also have observed induction of *UBI4* transcripts after brief exposure to methyl methane sulfonate (K. McEntee, unpublished data). After exposure of cells to the mutagen-carcinogen 4NQO, *UBI4* transcripts accumulate in *RAD*$^+$, *rad52*, and *rad6* mutant yeast strains.

We found that the relative increase in the amount of *UBI4* transcripts upon exposure to 4NQO was dependent on the density of the culture. Optimal induction was found with rapidly growing, low-cell-density exponential cultures, whereas with higher-cell-density cultures the effects of 4NQO treatment were significantly reduced. This result is due, at least in part, to the substantial increase in the basal level of *UBI4* transcripts in untreated, dense cultures. This basal level of transcripts approaches that seen after 4NQO exposure of exponential cultures. We presume that altered physiological regulation of *UBI4* at high cell densities accounts for the inability of 4NQO to elicit elevated expression of this gene.

The *UBI4* gene is also regulated in meiosis such that, within the first 8 h of incubation in sporulation medium, transcript levels increase three- to fivefold. This accumulation of *UBI4* transcripts was observed in **a**/α diploid yeast strains and not in α/α strains, which are incapable of sporulation. Increased production of *UBI4* transcripts and, presumably, ubiquitin protein in sporulating cells may be necessary for increased degradation of intracellular proteins during this developmental switch. The fact that *ubi*/*ubi* diploids are incapable of sporulation is consistent with this notion. The kinetics of transcript accumulation are also compatible with this idea, since the increase in *UBI4* message peaks relatively early (between 6 and 8 h) in the meiotic program, and this would be the expected period in which degradation of preexisting proteins is likely to occur if removal of these proteins is necessary for successful completion of meiosis.

There are at least two explanations to account for increased expression of *UBI4* after exposure of yeast cells to different chemical mutagens-carcinogens. According to the first explanation, the *UBI4* gene, like the *DDRA2* and *DDR48* genes, responds to lesions in DNA, such as nicks, gaps, or double-strand breaks, that result from exposure to different damaging agents. (It is also possible that replication blockage by these lesions is the primary signal.) Alternatively, the *UBI4* gene might respond to increased protein alkylation produced by these chemical agents. Several chemical agents which alkylate DNA or produce strand breaks are capable of alkylating proteins and free amino acids. This second possibility is more easily reconciled with the proposal that structurally abnormal proteins are substrates for and regulators of the ubiquitination system in *S.*

cerevisiae (5, 6). Experiments are in progress to test these possibilities.

ACKNOWLEDGMENTS

We thank A. Varshavsky and D. Finley for the pUB200 plasmid, L. Prakash for the *rad6* deletion mutant SX46A-rad6Δ, and P. Magee for the isogenic diploid strains. We are indebted to L. Tabata and J. Ransom for help in preparing the manuscript.

J.M.T. was supported by funds from a Public Health Service tumor cell biology training grant (CA09056) from the National Institutes of Health. This investigation was aided by a grant (NP445) from the American Cancer Society (to K.M.).

LITERATURE CITED

1. **Bennetzen, J. L., and B. D. Hall.** 1982. The primary structure of the *Saccharomyces cerevisiae* gene for alcohol dehydrogenase I. J. Biol. Chem. **257**:3018–3025.
2. **Busch, H., and I. L. Goldknopf.** 1981. Ubiquitin-protein conjugates. Biochemistry **40**:173–187.
3. **Ciechanover, A., D. Finley, and A. Varshavsky.** 1984. Ubiquitin dependence of selective protein degradation demonstrated in the mammalian cell cycle mutant ts85. Cell **37**:57–66.
4. **Clancy, M. J., B. Buten-Magee, D. J. Straight, A. L. Kennedy, R. M. Partridge, and P. T. Magee.** 1983. Isolation of genes expressed preferentially during sporulation in the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **80**:3000–3004.
5. **Finley, D., E. Ozkaynak, and A. Varshavsky.** 1987. The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation, and other stresses. Cell **48**:1035–1046.
6. **Finley, D., and A. Varshavsky.** 1985. The ubiquitin system: functions and mechanisms. Trends Biochem. Sci. **10**:343–346.
7. **Maga, J. A., T. A. McClanahan, and K. McEntee.** 1986. Transcriptional regulation of DNA damage responsive (DDR) genes in different *rad* mutant strains of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **205**:276–284.
8. **McClanahan, T., and K. McEntee.** 1984. Specific transcripts are elevated in *Saccharomyces cerevisiae* in response to DNA damage. Mol. Cell. Biol. **4**:2356–2363.
9. **McClanahan, T., and K. McEntee.** 1986. DNA damage and heat shock dually regulate genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6**:90–96.
10. **Ozkaynak, E., D. Finley, M. J. Soloman, and A. Varshavsky.** 1987. The yeast ubiquitin genes: a family of natural gene fusions. EMBO J. **6**:1429–1439.
11. **Ozkaynak, E., D. Finley, and A. Varshavsky.** 1984. The yeast ubiquitin gene: head-to-tail repeats encoding a polyubiquitin precursor protein. Nature (London) **312**:663–666.
12. **Prakash, L.** 1974. Lack of chemically induced mutation in repair-deficient mutants of yeast. Genetics **78**:1101–1118.
13. **Resnick, M. A., and P. Martin.** 1976. The repair of double-stranded breaks in nuclear DNA of *Saccharomyces cerevisiae* and its genetic control. Mol. Gen. Genet. **143**:119–129.
14. **Siegelman, M., M. W. Bond, W. M. Gallatin, T. St. John, H. T. Smith, V. A. Fried, and I. L. Weissman.** 1986. Cell surface molecule associated with lymphocyte homing is a ubiquinated branched-chain glycoprotein. Science **231**:823–829.
15. **Yarden, T., J. A. Escobedo, W.-J. Kuang, T. L. Yang-Feng, T. O. Daniel, P. M. Tremble, E. Y. Chen, M. E. Ando, R. N. Harkins, U. Francke, V. A. Fried, A. Ulrich, and L. T. Williams.** 1986. Structure of the receptor for platelet derived growth factor helps define a family of closely related growth factor receptors. Nature (London) **323**:226–232.