MOLECULAR AND CELLULAR BIOLOGY, Jan. 1986, p. 90–96
0270-7306/86/010090-07$02.00/0

Vol. 6, No. 1

DNA Damage and Heat Shock Dually Regulate Genes in *Saccharomyces cerevisiae*

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Received 19 April 1985/Accepted 23 September 1985

Two *Saccharomyces cerevisiae* genes isolated in a differential hybridization screening for DNA damage regulation (*DDR* genes) were also transcriptionally regulated by heat shock treatment. A 0.45-kilobase transcript homologous to the *DDRA2* gene and a 1.25-kilobase transcript homologous to the *DDR48* gene accumulated after exposure of cells to 4-nitroquinoline-1-oxide (NQO; 1 to 1.5 μg/ml) or brief heat shock (20 min at 37°C). The *DDRA2* transcript, which was undetectable in untreated cells, was induced to high levels by these treatments, and the *DDR48* transcript increased more than 10-fold as demonstrated by Northern hybridization analysis. Two findings argue that dual regulation of stress-responsive genes is not common in *S. cerevisiae*. First, two members of the heat shock-inducible *hsp70* family of *S. cerevisiae*, *YG100* and *YG102*, were not induced by exposure to NQO. Second, at least one other DNA-damage-inducible gene, *DIN1*, was not regulated by heat shock treatment. We examined the structure of the induced RNA homologous to *DDRA2* after heat shock and NQO treatments by S1 nuclease protection experiments. Our results demonstrated that the *DDRA2* transcript initiates equally frequently at two sites separated by 5 base pairs. Both transcriptional start sites were utilized when cells were exposed to either NQO or heat shock treatment. These results indicate that *DDRA2* and *DDR48* are members of a unique dually regulated stress-responsive family of genes in *S. cerevisiae*.

Procaryotic and eucaryotic organisms possess stress responses to a variety of environmental stimuli. These responses generally involve rapid and often transient alteration in gene expression. Two of the best-studied stress responses are the heat shock response and the SOS response. The heat shock response has been observed in nearly all organisms from bacteria to human cells. In *Escherichia coli* (5, 45), *Saccharomyces cerevisiae* (12, 33), *Drosophila melanogaster* (1, 44), and mammalian cells in culture (26), regulation of the heat shock response occurs (at least in part) at the level of transcription. The synthesis of at least 13 heat shock proteins in *E. coli* requires the *htpR* gene which encodes a sigma factor that selectively directs recognition and binding of RNA polymerase to promoters of heat shock genes (14). In *D. melanogaster*, there is evidence that specific binding proteins are required for regulated expression of heat shock genes in vivo and in vitro (34, 44). Immunological and hybridization analyses reveal considerable protein and nucleotide sequence homology among heat shock genes and their products from very divergent organisms (5, 22). Although the biochemical functions of most heat shock proteins are not known, their sequence conservation suggests a vital role for these proteins in the cell.

The SOS response has been most fully characterized in *E. coli* in which, after treatment with DNA-damaging agents or arrest of DNA replication, more than 17 genes are transcriptionally activated. The molecular mechanism of this coordinately regulated response involves the proteolytic inactivation of a common repressor (*lexA* protein) by an activated form of the *recA* protein (for details, see reference 42). The functions of some of the SOS proteins are known and include DNA damage excision (*uvr*) (23), mutagenesis (*umuCD*) (3), recombinational repair (*recA*) (30), site-specific recombination (*himA*) (32), and inhibition of cell septation (*sfi*) (20).

Eucaryotic cells also manifest features of a stress response to DNA damage, although much of the evidence for this is indirect (9). We have recently reported the isolation of several DNA-damage-responsive (*DDR*) sequences from *S. cerevisiae* that are transcribed at elevated levels after treatment of cells with the mutagen-carcinogen 4-nitroquinoline-1-oxide (NQO) (29). In addition, Ruby et al. (37) have isolated a set of DNA-damage-inducible (*DIN*) sequences from among fusions of yeast genomic segments with the *lacZ* gene of *E. coli*. These latter results demonstrate that *S. cerevisiae* possesses a set of genes that are controlled by and respond to chemical and photochemical lesions in DNA. Moreover, Ruby et al. (37) have shown that at least one inhibitor of DNA synthesis induces certain *DIN* transcripts, indicating a coupling of replication and *DIN* gene expression analogous to the induction of the SOS response in *E. coli* after disruption of its chromosomal replication.

Recently, it has been demonstrated in *E. coli* that certain genes may respond to more than a single stress stimulus. Krueger and Walker (24) have reported that levels of two heat shock proteins, *groEL* and *dnaK*, increased markedly after exposure of cells to UV light or nalidixate treatments which are known to elicit the SOS response. Baluch et al. (4) observed induction of a protein (later identified as the *groEL* product) after similar treatments. The *groEL* protein is needed for phage λ morphogenesis (40), and *dnaK* protein is required for both *E. coli* and phage λ DNA replication (13). Moreover, the *dnaK* product has been shown to regulate the heat shock response (41). The induction of the *groEL* and *dnaK* proteins by these agents requires *htpR* but not *recA* or *lexA* function, indicating that their induction is not mediated by the normal SOS regulatory mechanism. Thus, the response of *E. coli* to DNA-damaging treatments or replication arrest is more complex than originally envisioned and involves the activation of more than one independent regulatory system. It is not known whether all the heat shock proteins are induced by DNA damage and replication block-

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age in *E. coli* or whether only a subset of these proteins is synthesized.

In this report we demonstrate that DNA damage and heat shock regulate two *DDR* genes in *S. cerevisiae*. The levels of transcripts homologous to the *DDRA2* and *DDR48* genes increase rapidly after a brief heat pulse or exposure to NQO. We used S1 nuclease protection experiments to locate the 5′ terminus of the *DDRA2* transcripts synthesized after heat shock and NQO treatments. Our results indicate that transcription initiates at two sites separated by 5 base pairs (bp) and that the sites of transcription initiation are the same after exposure to carcinogen or to elevated temperature. Our results also argue that dual regulation by heat shock and DNA damage is not a common regulatory motif for *DIN* and *DDR* sequences or for heat shock genes in *S. cerevisiae*.

MATERIALS AND METHODS

Growth of *S. cerevisiae*, exposure to DNA-damaging agents, and heat shock procedure. *S. cerevisiae* M12B (α *trp1-289 ura3-52 gal2*) was grown in YPD broth (2% Bacto-Peptone, 2% glucose, 1% yeast extract [Difco Laboratories, Detroit, Mich.]) with continuous shaking. For DNA-damaging treatment, the cells were grown at 30°C and treated in the early log phase (10^7 cells per ml) with NQO as described previously (29). The doses and duration of exposure were as described in the figure legends. For heat shock treatment, cells were grown at 23°C to the early log phase (10^7 cells per ml) and placed at 45°C for 2 min to rapidly raise the temperature of the culture to 37°C. The culture was incubated with shaking for 20 min at 37°C before the cells were harvested.

RNA isolation and poly(A)$^+$ RNA selection. RNA was extracted by glass bead disruption as previously described (29). Poly(A)$^+$ RNA was selected by chromatography on oligo(dT)-cellulose (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) as described previously (2).

Northern hybridization analysis. Total RNA (100 μg per lane) was denatured in 50% formamide at 65°C and electrophoresed in 1% agarose gels containing formaldehyde (25). The RNA in the gels was transferred to nitrocellulose by the method of Thomas (39), hybridized, and washed as previously described (29).

Source of plasmids, isolation, and radiolabeling. Plasmids pBRA2 and pBR48 containing the yeast genes *DDRA2* and *DDR48* were isolated as DNA-damage-responsive sequences in a differential plaque filter hybridization screen (29). The pYG100 and pYG102 plasmids were obtained from E. Craig; the pSZ214 plasmid was obtained from S. Ruby and J. Szostak. Small-scale plasmid DNA isolation and radioactive labeling of DNA by nick translation were carried out by standard procedures (19, 35).

DNA fragment isolation and subcloning. Restriction enzymes were obtained from Bethesda Research Laboratories, and digestions were carried out under conditions recommended by the manufacturer. DNA fragments for subcloning and for use as probes in S1 mapping experiments were obtained by isolation of the relevant restriction fragments from low-melting-temperature agarose gels (1%) (FMC Corp., Marine Colloids Div., Rockland, ME) by phenol extractions of molten gel slices, essentially as described previously (28). Vector M13 mp8 or M13 mp9 replicative-form DNAs (31) were digested with the indicated restriction enzymes, treated with bacterial alkaline phosphatase, (Bethesda Research Laboratories), and phenol extracted. A 2:1 molar excess of fragment and bacterial alkaline phosphatase-treated M13 mp8-M13 mp9 vector were ligated at 10 to 12°C for 16 to 36 h and used to transform competent *E. coli* JM103 cells [Δ(*lac-pro*) *thi rpsL supE endA sbcB15 hsdR4* F′ *traD36 proAB lacI^{q}ZΔM15*]. Transformants were spread on plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside and isopropyl-β-D-thiogalactopyranoside. Single colorless plaques were picked and grown in 2-ml cultures of JM103 cells overnight at 37°C, and samples (1 ml) of the clarified supernatant (titer approximately 10^{12} PFU/ml) were used to infect 200-ml cultures of log-phase JM103 cells for 5 h. Cells were collected by centrifugation, and replicative-form DNA was prepared from the cell pellet by a standard plasmid DNA isolation method (28), followed by equilibrium buoyant density centrifugation in CsCl gradients containing ethidium bromide. These subclones were radiolabeled by nick translation and used as probes for Northern hybridizations.

S1 nuclease mapping of 5′ ends of the *DDRA2* transcript. DNA fragments isolated as described above were end labeled as follows. The 5′ ends of restriction fragments were dephosphorylated with bacterial allkaline phosphatase, phenol extracted, and radioactively labeled with [γ-^{32}P]ATP (ICN Pharmaceuticals Inc., Irvine, Calif.) and T4 polynucleotide kinase (Bethesda Research Laboratories) as described previously (28). Labeled fragments were phenol extracted, separated from unincorporated nucleotides, and precipitated together with RNA for S1 mapping. The protocol employed was essentially that of Berk and Sharp (7), as modified for end-labeled probes (43). Conditions for DNA-RNA hybridization and S1 nuclease digestion were determined empirically. In a typical reaction, 5 to 20 μg of poly(A)$^+$ RNA from control, NQO-treated, or heat-shocked cells (isolated as described above) was hybridized with a fivefold molar excess of 5′-end-labeled restriction fragment (approximately 1×10^6 to 5×10^6 cpm/μg) at 45 to 47°C overnight in 80% deionized formamide–0.4 M NaCl–0.04 M PIPES (piperazine-*N*,*N*′-bis[2-ethanesulfonic acid]) (pH 6.4)–1 mM EDTA. For control hybridizations with no *S. cerevisiae* RNA added, *E. coli* tRNA (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was substituted. Digestions were performed at 20°C with approximately 370 U of S1 nuclease (P-L Biochemicals Inc., Millwaukee, Wis.) per ml in 200-μl reaction mixtures (0.25 M NaCl, 0.03 M potassium acetate [pH 4.5], 1 mM $ZnSO_4$, 20 μg of calf thymus DNA per ml). *E. coli* tRNA (Boehringer Mannheim) was added as a carrier for ethanol precipitation. Protected DNA fragments were analyzed on 0.3-mm, 5% acrylamide–8 M urea sequencing gels. Plasmid pBR322 DNA digested with *Hpa*II and end filled with [α-^{32}P]dCTP and the Klenow fragment of DNA polymerase I was used for size markers.

RESULTS

DNA damage induction of *DDRA2* and *DDR48* transcripts. We used a differential hybridization screen to identify and isolate genes in *S. cerevisiae* that display altered regulation in response to chemical or photochemical insult to cellular DNA. During an examination of more than 9,000 yeast genomic sequences inserted into bacteriophage λ, we identified approximately 30 clones displaying an increased hybridization response to cDNAs prepared from transcripts of UV- or NQO-damaged *S. cerevisiae* relative to untreated cells (29). The *DDRA2* gene was isolated in a screening with UV-irradiated cells as a source of poly(A)$^+$ RNA. As shown previously (29) and in Fig. 1A, *DDRA2* hybridized to a 0.45-kilobase (kb) transcript that is present in the RNA preparations from NQO-damaged cells but is absent in

control preparations. An increase in the steady-state level of the *DDRA2* transcript can be detected within 60 min after exposure of cells to 1.5 μg of NQO per ml (29; unpublished data).

A second clone, *DDR48*, was isolated in the same screening, using RNA from UV-irradiated cells to prepare cDNA probes. The *DDR48* gene is contained within a 4.6-kb *Hin*dIII fragment that is present once in the yeast genome (data not shown). Unlike *DDRA2*, low levels of a transcript homologous to *DDR48* could be detected in untreated cells (Fig. 1B). The amount of this transcript increased more than 10-fold within 30 min of NQO treatment (1 μg/ml) of yeast strain M12B (Fig. 1B). After 60 min of NQO treatment, the level of transcript decreased to a level which was three to five times the control value as determined by densitometry (Fig. 1B). A similar induction ratio could be seen 120 min after addition of NQO to a culture of yeast cells. Using the large and small rRNA subunits as molecular weight markers of 2.5 and 1.3 kb, respectively, we estimate the size of the *DDR48* transcript to be approximately 1.25 kb.

We examined the level of the *DDR48* transcript in cellular RNA prepared from yeasts that had been exposed to 0.1 or 1.0 μg of NQO per ml for 60 min. The results (Fig. 1C) indicate that the level of this transcript increases significantly only after exposure to the higher NQO concentration. A similar result has been obtained with the *DDRA2*-encoded transcript (29).

Heat shock induces *DDRA2* and *DDR48* transcripts. A culture of *S. cerevisiae* M12B growing exponentially at 23°C was exposed to a brief heat pulse (37°C, 20 min), and cellular RNA was isolated and analyzed by Northern analysis as described in Materials and Methods. A transcript homologous to the nick-translated pBRA2 probe could be detected in RNA from heat-treated cells (Fig. 1A). This transcript migrated at the same position as the 0.45-kb NQO-induced transcript encoded by the *DDRA2* gene. Although the level of this transcript appeared higher in the heat shock cells compared with the NQO-treated cells (Fig. 1A), in other experiments we observed comparable induction of the *DDRA2* transcript with NQO (data not shown). RNA prepared from heat-pulsed cells also contained high levels of a transcript homologous to the *DDR48* probe, pBR48, which migrated at approximately the same position as the *DDR48* transcript induced in NQO-treated cells (Fig. 1C). We routinely observed that the levels of the *DDR48* transcript synthesized in response to heat shock were comparable to those made in response to 30 min of NQO treatment.

DNA damage does not increase transcription of all heat shock genes. The *hsp70* genes are a major heat shock-regulated family in *S. cerevisiae* (5, 21). We used Northern hybridization analysis to investigate the effects of NQO treatment on the levels of two transcripts coded by two members of the *hsp70* family in yeast strain M12B. Plasmids pYG100 and pYG102 (21) were nick translated and used to probe nitrocellulose filters containing total RNA from control cells and cells treated with either 1.5 μg of NQO per ml for 1 h or heat shock treated at 37°C for 20 min. The results of these experiments are shown in Fig. 2A and B. As expected, brief heat treatment increased the level of transcripts homologous to the *YG100* and *YG102* probes. The level of transcript that hybridized to the *YG100* DNA increased approximately 4.3-fold, whereas the transcript homologous to the *YG102* probe showed a 2.7-fold increase in its steady-state level.

Exposure to NQO, however, failed to increase the level of either transcript in these cells (Fig. 2A and B). On the basis

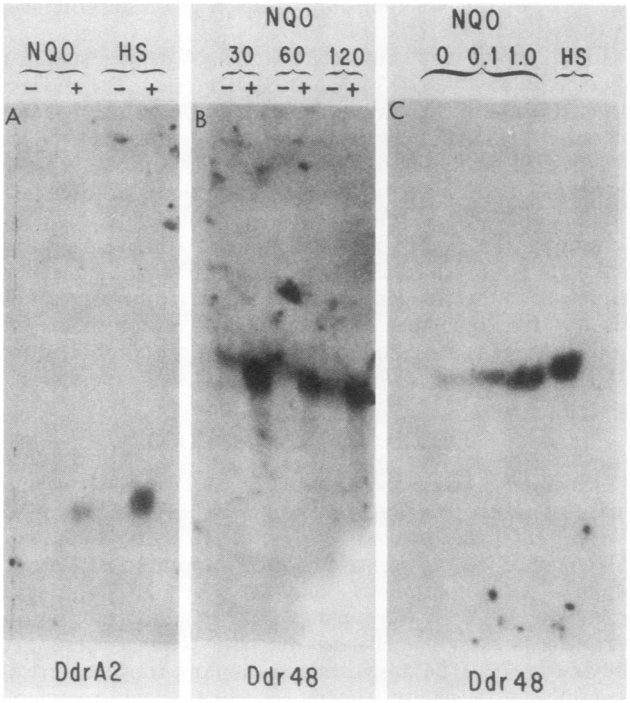

FIG. 1. DNA damage and heat shock (HS) treatments elevate the levels of *DDRA2* and *DDR48* transcripts in *S. cerevisiae*. Total cellular RNA (100 μg per lane) isolated from NQO-treated and heat-shocked M12B cells was separated by electrophoresis in formaldehyde-agarose gels (1%), transferred to nitrocellulose, and hybridized as described in Materials and Methods. (A) Probe, pBRA2; NQO −, control M12B cells, 30°C; NQO +, M12B cells treated with 1.5 μg of NQO per ml for 1 h; HS −, untreated M12B cells, 23°C; HS +, M12B cells, 37°C for 20 min. (B) Probe, pBR48; 30 −, 60 −, and 120 −, untreated cells at 30, 60, and 120 min, respectively; 30 +, 60 +, and 120 +, M12B cells treated with 1 μg of NQO per ml for 30, 60, and 120 min, respectively. (C) Probe, pBR48; left to right, M12B cells exposed to 0, 0.1, and 1.0 μg of NQO per ml for 1 h; HS, heat shock treated as above.

of densitometric measurements of these autoradiographs, we estimate that the levels increased less than 1.4-fold for the *YG100* and *YG102* transcripts. This result is consistent with the recent report of Brazzell and Ingolia (8) who found no increase in β-galactosidase levels after UV irradiation of yeast cells containing a fusion of the *YG100* heat shock gene with the *lacZ* gene of *E. coli*. We conclude that DNA damage does not increase transcription of all heat shock genes in *S. cerevisiae*.

Heat-shock does not increase transcription of all DNA-damage-responsive genes. Although our results indicated that the *hsp70* genes of *S. cerevisiae* do not respond to DNA-damaging treatments, we wished to determine whether heat shock treatment would increase transcript levels for other yeast genes that had been isolated as DNA-damage-responsive or -inducible sequences. For this experiment, we chose to examine the *DIN1* gene of *S. cerevisiae*. The *DIN1* gene was isolated as a gene fusion to the *E. coli lacZ* gene which displayed induction of β-galactosidase activity in yeast cells after UV irradiation or treatment with NQO (36, 37). The level of the transcript homologous to the *DIN1* gene was measured by Northern hybridization analysis with nick-translated plasmids pSZ214 as probe. This plasmid DNA hybridizes to two RNAs in yeast strain M12B with molecular weights of approximately 1.3 and 2.7 kb (27). Only the 2.7-kb

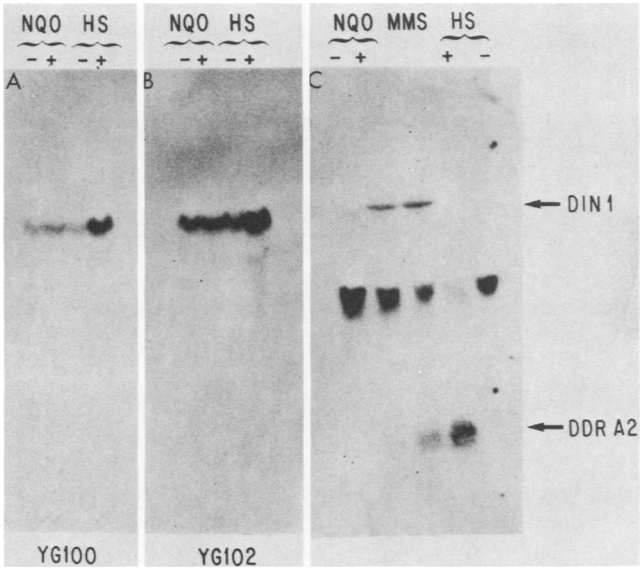

FIG. 2. Effects of DNA-damaging treatment and heat shock (HS) exposure on the levels of *YG100*, *YG102*, and *DIN1* transcripts in *S. cerevisiae*. Total RNA (100 μg per lane) was isolated from control, NQO-treated (1.5 μg/ml for panels A and B, 1 μg/ml for panel C, for 1 h), methyl methanesulfonate (MMS)-treated (0.05%, 1 h), or heat shock-treated (20 min, 37°C) M12B cells, electrophoresed in 1% formaldehyde–agarose gels and used to prepare Northern filters. Control cells for the NQO and methyl methanesulfonate experiments were grown at 30°C, whereas control cells for the heat shock experiment were grown at 23°C. The nick-translated probes used in the hybridization were as follows: (A) pYG100; (B) pYG102; and (C) pSZ214 and pBRA2. The concentration of NQO used in the experiment shown in panel C does not efficiently induce the *DDRA2* transcript after 1 h although the *DIN1* transcript is optimally induced.

transcript is inducible by DNA damage, whereas the smaller RNA is made constitutively (27, 37). The *DIN1* transcript was synthesized in response to treatment of M12B cells with either NQO or methyl methanesulfonate (Fig. 2C). The *DDRA2* transcript was also synthesized after treatment with these DNA-damaging agents, although the conditions used for the experiment shown in Fig. 2C were not optional for *DDRA2* transcript induction. The *DIN1* transcript is induced at lower concentrations of DNA-damaging agents than needed to induce *DDRA2* (29; unpublished data). However, we did not detect the *DIN1* transcript in RNA prepared from cells that had been heat shock treated, although we easily detected high levels of the *DDRA2* transcript in these RNA preparations (Fig. 2C). Moreover, the level of the 1.3-kb transcript decreased significantly after heat shock treatment. From these results we conclude that heat shock does not stimulate transcription of all DNA-damage-inducible genes in *S. cerevisiae*.

Localizing the *DDRA2* transcription unit in pBRA2. Although the results shown in Fig. 1 demonstrate significant increases in the level of transcripts homologous to the *DDRA2* and *DDR48* genes after either NQO treatment or heat shock, these data do not reveal whether the structures of the transcripts synthesized after these different treatments are identical. To investigate this point, we decided to localize the *DDRA2* transcription unit within the pBRA2 plasmid and to characterize the 5′ ends of the RNAs made in response to NQO and heat shock treatment.

To precisely locate the *DDRA2* transcription unit, we digested the 1.45-kb *Hin*dIII fragment contained within pBRA2 with *Hin*cII and *Hin*dIII and inserted the left 0.85-kb *Hin*dIII-*Hin*cII (*DDRA2*-A) and right 0.6-kb *Hin*cII-*Hin*dIII (*DDRA2*-B) fragments into the sequencing vectors M13 mp8 and M13 mp9. Replicative forms of these recombinants were nick translated and used to probe RNAs prepared from control and NQO-treated cells (Fig. 3). Both the *DDRA2*-A and *DDRA2*-B fragments hybridized to the 0.45-kb transcript, indicating that this *DDRA2* transcription unit spans the *Hin*cII site. When RNA was probed with the *Hin*dIII-*Pst*I fragment from the left end of the pBRA2 insert or with the *Cla*I-*Hin*dIII fragment from the right end of the insert, no

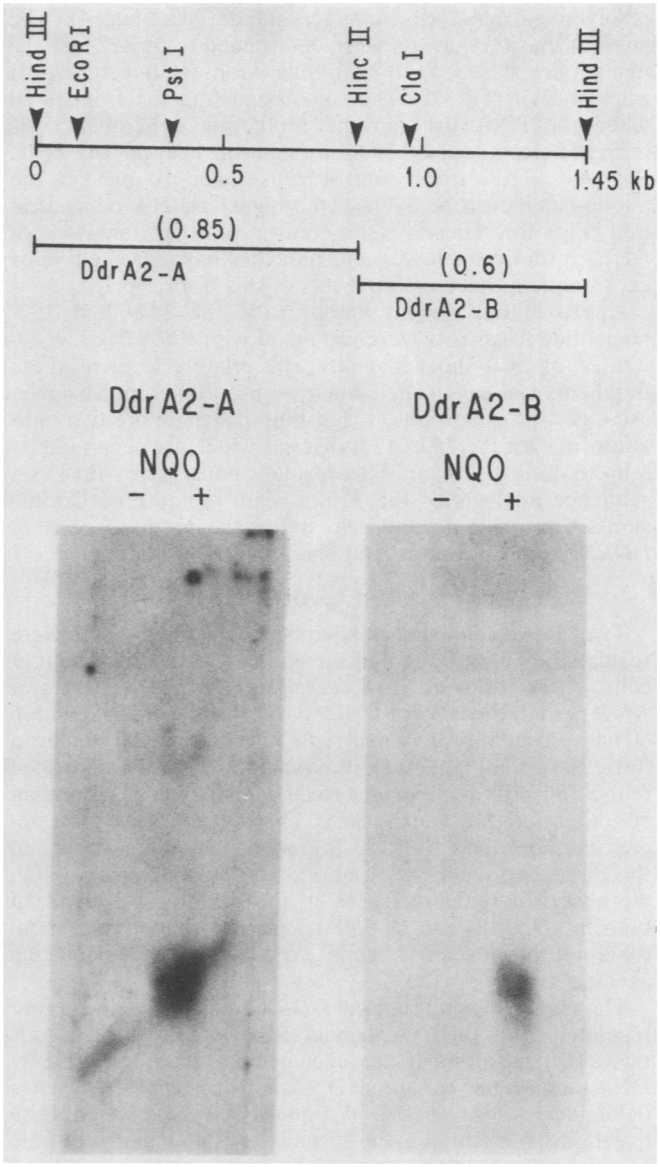

FIG. 3. Localization of the *DDRA2* transcription unit. The restriction map of the 1.45-kb insert in pBRA2 is shown in the upper portion of the figure. The indicated 0.85-kb *Hin*dIII-*Hin*cII restriction fragment (*DDRA2*-A) and 0.6-kb *Hin*cII-*Hin*dIII restriction fragment (*DDRA2*-B) were subcloned into both M13 mp8 and M13 mp9 vectors. Double-stranded replicative-form DNA from the indicated subclone was nick translated and used to probe nitrocellulose filters containing RNA (100 μg per lane) from control cells (−) and NQO-treated cells (1.5 μg/ml, 1 h) (+).

hybridization to the 0.45-kb transcript could be detected (data not shown). These results indicated that the *DDRA2* transcription unit was likely to be located between the *Pst*I and *Cla*I restriction sites in this region.

Mapping the 5′ end of the *DDRA2* transcript in NQO-treated and heat-shocked *S. cerevisiae*. The 5′ end of the *DDRA2* transcript was mapped by an S1 nuclease protection assay. A 760-bp *Hin*cII-*Eco*RV fragment was isolated from digests of pBRA2, and the 5′ ends of the fragment were dephosphorylated with bacterial alkaline phosphatase and end labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (see Materials and Methods). After hybridization with poly(A)$^+$ RNA from NQO-treated or heat-shocked cells and S1 nuclease digestion, two protected fragments could be resolved on denaturing polyacrylamide gels (Fig. 4). The sizes of these fragments were determined to be 157 and 152 nucleotides based upon their migration relative to *Hpa*II fragments of pBR322. The same-sized fragments appeared when the RNA used in the protection experiment was prepared from either NQO-treated or heat-pulsed cells. Poly(A)$^+$ RNA from control cells failed to protect the 5′-end-labeled probe even at the higher mRNA concentration (Fig. 4b). These results confirm Northern analyses of *DDRA2* transcript levels and indicate that untreated yeast cells contain undetectable levels of this transcript.

Approximately equal amounts of the 157- and 152-nucleotide fragments were protected with RNA from NQO-treated or heat-shocked cells. The relative level of these fragments was unchanged when we used different S1 nuclease digestion conditions, suggesting that there are two initiation sites for the *DDRA2* transcript which are separated by 5 bp (data not shown). This result is consistent with DNA sequence analysis of the 5′ upstream region (see Discussion). Thus, we detected no difference in the 5′ end of *DDRA2* transcript after heat shock or NQO treatment.

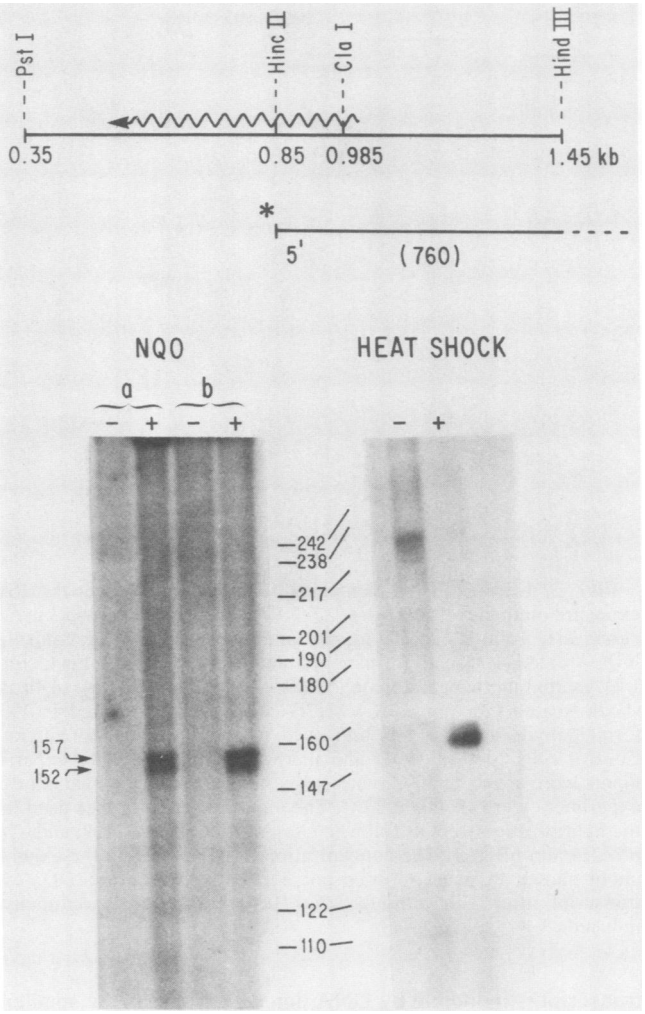

FIG. 4. S1 nuclease mapping of the 5′ ends of the *DDRA2* transcript from NQO- and heat shock-treated *S. cerevisiae*. A 760-bp *Hin*cII-*Eco*RV duplex fragment was labeled at its 5′ ends with ^{32}P as described in Materials and Methods. (The *Eco*RV site is in pBR322 DNA which does not hybridize with yeast RNA.) This end-labeled probe was hybridized with 15 μg (a) or 23 μg (b) of poly(A)$^+$ RNA from control (−) or NQO-treated (1.5 μg/ml, 1 h) (+) cells and with 5 μg of poly(A)$^+$ RNA from control (−) and heat-shocked (+) cells. The sizes of the protected fragments were determined by using end-filled *Hpa*II restriction fragments of pBR322 as markers. The location of the *Hin*cII site in the 1.1-kb *Pst-Hin*dIII fragment and the direction of transcription of the *DDRA2* gene are shown in the upper portion of the figure. The intensity of the upper protected fragment appears reduced in the heat shock lane. However, in other experiments we detected no difference in the relative intensity of the two bands.

DISCUSSION

Two *S. cerevisiae* genes have been described which were regulated by both DNA damage and heat shock treatment of cells. After exposure of *S. cerevisiae* M12B to 1.5 μg of NQO per ml, the level of *DDRA2* transcript increased within 60 min. Based on densitometry of autoradiographs similar to those shown in Fig. 1, we estimate that the steady-state level of the transcript homologous to *DDRA2* increased more than 50-fold after NQO exposure. The *DDR48* transcript increased more than 10- to 15-fold after a 30-min exposure to this damaging agent. After a brief heat pulse (20 min, 37°C), we observed rapid increases in the steady-state levels of both the *DDRA2* and *DDR48* transcripts that were comparable to those seen in cells exposed to DNA-damaging agents.

The demonstration that the *DDRA2* and *DDR48* genes are regulated by both DNA damage and heat shock responses raises two questions: (i) are all heat shock genes inducible by DNA damage and (ii) are all DNA-damage-responsive genes inducible by heat shock? We addressed the first of these questions by examining the response of two members of the *hsp70* family to NQO exposure. These yeast genes have been shown to be homologous to the *E. coli dnaK* gene which is inducible by both heat shock and DNA-damaging treatments (24). We reasoned that these conserved genes may be regulated in *S. cerevisiae* as the *dnaK* gene is regulated in *E. coli*. Using the pYG100 and pYG102 probes, we did not detect significant increases in homologous transcript levels after NQO treatment. However, we could readily detect increases in the steady-state levels of the transcripts after a brief heat pulse. Although we have not examined the response of other heat shock genes to DNA damage, we conclude that the *hsp70* family of yeast heat shock genes is not regulated by DNA-damaging treatments.

We found that the transcript coded by the *DIN1* gene of yeasts which is induced by NQO, methyl methanesulfonate, and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (27, 36) was not synthesized after heat shock treatment. This result argues that not all DNA-damage-responsive genes are regulated by heat stress. Another *DDR* gene isolated in this lab, *DDR3016B*, also is not induced by heat shock treatment (K.

McEntee, unpublished data). Thus, the *DDRA2* and *DDR48* genes represent a unique set of stress-responsive sequences that respond to both DNA damage and heat shock. It is perhaps worth noting that *DDRA2* and *DDR48*, although isolated based on their response to carcinogen treatment, are two new heat shock genes of *S. cerevisiae*.

We examined in detail the 5′ end of the *DDRA2* transcript after induction with both NQO and heat shock treatments to determine whether the same transcriptional start sites were used after these different conditions. Using S1 nuclease protection, we localized the 5′ ends of the *DDRA2* transcript approximately 152 and 157 bp upstream from a unique *Hin*cII site within the *DDRA2* coding sequence. We observed that these two protected fragments were equal in intensity in both NQO- and heat shock-treated cell RNA preparations. We believe that the appearance of these two fragments in denaturing gels is a consequence of there being two sites of transcription initiation in the *DDRA2* gene rather than an artifact of the S1 nuclease digestion because (i) the fragments were present in equimolar amounts when the S1 digestion conditions were varied with respect to RNA concentration, temperature, and amount of S1 nuclease (data not shown) and (ii) DNA sequence analysis indicated the presence of two TATATAAA sequences located approximately 90 and 100 bp upstream from the proposed sites of transcription initiation (T. McClanahan and K. McEntee, manuscript in preparation). The relative location of these putative TATA boxes to the transcriptional start sites we mapped is consistent with what is found for the *hsp90* heat shock gene (11) and several other highly expressed genes in *S. cerevisiae* (6, 17, 18). Our results argue that after heat shock or NQO exposure, both transcriptional start sites are utilized with equal efficiency.

The S1 nuclease protection experiments and DNA sequence analysis indicate that the *Cla*I restriction site is located approximately 25 bp downstream from the transcriptional start sites (McClanahan and McEntee, in preparation). This result explains our inability to detect the *DDRA2* transcript by Northern analysis with the *Cla*I-*Hin*dIII fragment from the pBRA2 clone since this fragment overlaps the RNA by only 25 bp.

The 3′ end of the *DDRA2* transcript is located approximately 450 nucleotides downstream from the unique *Cla*I restriction site on the basis of the location of the 5′ end and the size of the transcript. Our data do not allow us to conclude that there is a single termination site or several closely spaced sites, and we cannot say whether transcripts synthesized after NQO treatment or heat shock terminate at the same site(s). Additional experiments will be required to answer this question and to precisely locate the 3′ end of the message. In this regard, we have identified a single sequence which resembles a yeast poly(A) addition site approximately 435 bp downstream from the *Cla*I site (McClanahan and McEntee, in preparation), a result which is consistent with the Northern hybridization data.

In contrast to the *groEL* and *dnaK* genes of *E. coli*, which require higher doses of DNA-damaging agent and longer times for induction, the *DDRA2* and *DDR48* genes respond rapidly to relatively low levels of mutagen-carcinogen exposure as well as to brief heat pulse treatment. At present we know little about the molecular features of the regulation of these two stress-responsive genes. It is likely that these genes are regulated in *cis* by upstream activator sequences that are required for regulated transcription of many yeast genes (10, 15, 16, 38, 46). The dual regulation of these genes by DNA-damaging treatment and heat shock exposure might suggest that these stimuli exert their effects on each of these genes via separate upstream activator sequence elements. A prediction of this model is that it should be possible to modify or delete sequences upstream of the *DDRA2* and *DDR48* genes and differentially alter their response to either heat shock or DNA damage. Alternatively, both heat shock and DNA damage may regulate transcription of these genes through common upstream activator sequence elements. For example, heat treatment and exposure to DNA-damaging agents might similarly alter chromatin structure in the control regions of the *DDR* genes and allow binding of regulatory factors at or near the upstream activator sequence element. Experiments are in progress to distinguish between these possibilities.

ACKNOWLEDGMENTS

We wish to thank S. Ruby and J. Szostak for providing the *DIN1* clone, pSZ214, and E. Craig for supplying the YG100 and YG102 plasmids. We are indebted to D. Brown, L. Tabata, and V. Windsor for their help in preparing the manuscript.

T.M. was supported by funds from a Public Health Service Cellular and Molecular Biology training grant from the National Institutes of Health. This investigation was aided by grant NP-445 from the American Cancer Society (to K.M.).

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