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Specific Transcripts Are Elevated in *Saccharomyces cerevisiae* in Response to DNA Damage

TERRILL McCLANAHAN AND KEVIN McENTEE*

Department of Biological Chemistry, University of California School of Medicine, Los Angeles, California 90024

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Differential hybridization has been used to identify genes in *Saccharomyces cerevisiae* displaying increased transcript levels after treatment of cells with UV irradiation or with the mutagen/carcinogen 4-nitroquinoline-1-oxide (NQO). We describe the isolation and characterization of four DNA damage responsive genes obtained from screening ca. 9,000 yeast genomic clones. Two of these clones, λ78A and pBR178C, contain repetitive elements in the yeast genome as shown by Southern hybridization analysis. Although the genomic hybridization pattern is distinct for each of these two clones, both of these sequences hybridize to large polyadenylated transcripts ca. 5 kilobases in length. Two other DNA damage responsive sequences, pBRA2 and pBR3016B, are single-copy genes and hybridize to 0.5- and 3.2-kilobase transcripts, respectively. Kinetic analysis of the 0.5-kilobase transcript homologous to pBRA2 indicates that the level of this RNA increases more than 15-fold within 20 min after exposure to 4-nitroquinoline-1-oxide. Moreover, the level of this transcript is significantly elevated in cells containing the *rad52-1* mutation which are deficient in DNA strand break repair and gene conversion. These results provide some of the first evidence that DNA damage stimulates transcription of specific genes in eucaryotic cells.

Treatment of the bacterium *Escherichia coli* with agents that directly damage DNA (UV light, mitomycin C, 4-nitroquinoline-1-oxide [NQO]) or arrest DNA replication (nalidixate) has been shown to induce a complex cellular stress response called the SOS response (18, 30). Among the first events occurring in cells after their exposure to these agents is the transcriptional activation of more than 12 genes that participate in efficient DNA repair (10, 14), mutagenesis (8), and inhibition of cell division (13). In addition, cells lysogenic for phage λ respond to DNA damage by inducing expression of the repressed prophage (19). Recently, it has been shown that the mechanism of activation of these unlinked genes is through the proteolytic destruction of a common cellular repressor (*lexA* protein) by an activated form of the *recA* protein (17). The repressor of phage λ, the *cI* protein, is also a substrate for *recA* protein-directed cleavage (4, 23). The SOS response in procaryotic cells provides a molecular model for coordinate gene regulation in response to specific stress stimuli.

Despite extensive genetic and biochemical evidence for a DNA damage stress response in *E. coli*, there is little evidence that DNA damage stimulates new gene expression or increased gene expression in eucaryotic cells. Much of the evidence for an "SOS-like" response to chemical or photochemical damage in mammalian cells is indirect. Reactivation and induced mutagenesis of herpes simplex virus (6) appear similar to Weigle reactivation of bacteriophage λ in *E. coli*, an SOS response (29, 30). In addition, one group has reported that UV irradiation of monkey cells before infection with simian virus stimulates reversion of a temperature-sensitive mutation in the viral gene A (26). Biochemical evidence suggests that pretreatment of cells with carcinogens, before or shortly after infection with simian virus 40, enhances the replication of virus containing pyrimidine dimers. (K. Dixon and J. Maga, unpublished data).

Since a fundamental feature of the SOS response in procaryotes is transcriptional activation of several genes, we reasoned that a differential hybridization screen could be used to identify DNA damage responsive (Ddr) genes in the simple eucaryote *Saccharomyces cerevisiae*. Similar approaches have been useful in identifying genes that are transcriptionally regulated by galactose (27), by inorganic phosphate (15), by heat shock (9), and during sporulation in yeast (3). Using cDNA hybridization probes prepared from cells exposed either to UV irradiation or to the mutagen/carcinogen NQO, we have identified unique and repetitive DNA segments that are transcriptionally regulated by DNA damage. In this report, we describe the isolation and characterization of four Ddr genes.

MATERIALS AND METHODS

The yeast strains used in this study are described in Table 1.

Growth of *S. cerevisiae* and exposure to DNA-damaging agents. *S. cerevisiae* was grown in YPD broth (2% Bacto-Peptone, 2% glucose, 1% yeast extract [Difco]) at 30°C with continuous shaking. For irradiation, cells from half of the original culture were collected at a density of 2×10^7 cells per ml, washed with 1 volume of sterile saline (0.9% NaCl), and suspended in 1 volume of saline. Irradiation was performed at 23°C in sterile Corning Pyrex dishes (12 by 7.5 in.) (30.48 by 19.05 cm) with a 15-W germicidal UV lamp to a final dose of 150 J/m^2. After UV irradiation, cells were collected by centrifugation, suspended in fresh YPD medium, and allowed to recover at 30°C for 1 h. Control (non-irradiated) cells were processed identically, except that they did not receive UV treatment. RNA was isolated from these cells as described below.

Treatment of *S. cerevisiae* with NQO (Sigma Chemical Co.) was performed in YPD medium either at 1.5 μg/ml for 6 h or as described in the text. The initial cell density was between 1×10^7 and 2×10^7 cells per ml. After NQO treatment, sodium azide was added to a final concentration of 0.02% and cycloheximide was added to 200 μg/ml. Cells were collected by centrifugation and disrupted for RNA isolation.

* Corresponding author.

TABLE 1. *S. cerevisiae* strains and genotypes

Strain	Genotype	Source
M12B	α *trp1-289 ura3-52 gal2*	R. Davis
X5122 49C	α *rad52 leu2*	YGSC[a]
X5122 57D	a *rad52 ura3*	YGSC
XS214-1B	a *rad52-1 leu2 trp5 arg4 his6 ile3 ura1 lys9 ade2 met2*	YGSC
YNN209	α *trp1-289 ade2-1 his3-Δ1 ura3-52 rad52-1*	R. Davis
X10-1C	a *rad6-1 ade2-1*	YGSC
SPX143-6C	a *rad56-1 leu1 ura3 aro7 ade1 his2 his6 trp1 arg4*	YGSC

[a] YGSC, Yeast Genetic Stock Center, Department of Biophysics and Medical Physics, University of California, Berkeley.

RNA extraction and oligodeoxythymidylate-cellulose column chromatography. RNA was extracted by either of two methods. In method 1, cells were suspended in 1/20 volume of cold extraction buffer (0.1 M Tris, 0.1 M LiCl, 0.1 mM EDTA [pH 7.4]). An equal volume of cold sterile glass beads was added and used to disrupt the cell wall by repeated vortexing. After the addition of sodium dodecyl sulfate to 0.5%, RNA was purified by repeated phenol-chloroform (1:1) extraction and then by ethanol precipitation. In method 2, cells were suspended in 1/40 volume of 50 mM sodium phosphate [pH 7.5]–10 mM β-mercaptoethanol–1 M sorbitol. Zymolyase 60,000 (Miles Laboratories, Inc.) was added in sufficient amounts to allow cell lysis in 5 min with the addition of 1% sodium dodecyl sulfate. This mixture was made to 0.5 M NaCl–0.2 M Tris (pH 7.4)–0.01 M EDTA and was phenol-chloroform (1:1) extracted and ethanol precipitated. Polyadenylated [poly(A)$^+$] RNA was selected by oligodeoxythymidylate-cellulose chromatography (Bethesda Research Laboratories) as described previously (1).

Reverse transcription and labeled cDNA purification. Reverse transcription of the *S. cerevisiae* poly(A)$^+$ RNA was performed as described previously (27), with avian myeloblastosis virus reverse transcriptase (Life Sciences, Inc.) and [α-^{32}P]dCTP (Amersham Corp.). Reactions were incubated at 40°C for 60 to 90 min and stopped by the addition of sodium dodecyl sulfate (1%) and 100 mM NaOH. RNA was hydrolyzed at 37°C for 30 min, neutralized by the addition of HCl and 100 mM Tris (pH 7.5), and separated from unincorporated [α-^{32}P]dCTP by gel filtration through Sephadex G-50 (1 by 6 cm). The void fractions containing radioactivity were used for hybridization.

Hybridization to a λ-yeast genomic library. ^{32}P-labeled cDNAs were used to probe a λ590 *Hin*dIII genome library of *S. cerevisiae* S288C. Originally, nitrocellulose replicas of petri plates containing between 300 and 800 plaques were prepared by the method of Benton and Davis (2) and used for hybridization. However, this procedure proved unsatisfactory because of the variable plaque size and lack of reproducibility of the hybridization signal. Instead, we chose to hybridize to ordered plaque arrays containing between 90 and 100 plaques per plate. The increased plaque size assured a stronger hybridization signal and greater reproducibility during the screening. Hybridizations were performed at 42°C in 50% formamide–5× SSPE (1× SSPE is 0.15 M NaCl, 0.01 M NaH_2PO_4, 1 mM EDTA [pH 7.4]), 0.3% sodium dodecyl sulfate, and 500 μg of heat-denatured calf thymus DNA per ml for ca. 48 to 64 h. After hybridization, filters were washed with 2× SSPE at 37°C for 2 h and autoradiography was done at −70°C with Cronex intensifying screens.

DNA isolation, restriction digestion, and separation on agarose gels. DNA was isolated from stationary-phase *S. cerevisiae* by the method of Cryer et al. (5). Restriction digestions were performed under conditions recommended by the manufacturer and with a three- to fivefold excess of enzyme. DNA fragments were separated by electrophoresis in 0.7% agarose gels as described previously (20).

Southern hybridization analysis. DNA in agarose gels was transferred to nitrocellulose (Schleicher & Schuell, Inc.) essentially as described by Maniatis et al. (20). Nick translations of purified plasmids or λ clones were done by the procedure of Rigby et al. (22). Filters were hybridized, washed, and subjected to autoradiography as described above.

Northern hybridization analysis. Total (100 μg) or poly(A)$^+$ (4 μg) RNA was denatured in 50% formamide at 65°C and electrophoresed in 1% agarose gels containing formaldehyde (16). The RNA in the gels was transferred to nitrocellulose by the method of Thomas (28). Filters were hybridized with nick-translated DNA probes as described for Southern hybridization, except that 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) was used in the hybridization and washing steps.

Subcloning *S. cerevisiae* fragments into vector pBR322. Plate stocks of the λ590-yeast clones of interest were prepared, phage were purified by banding in a cesium chloride block gradient, and the phage DNA was isolated by phenol extraction. Purified DNA was digested with *Hin*dIII and mixed with the appropriate amount of pBR322 that had been digested with *Hin*dIII, treated with bacterial alkaline phosphatase for 60 min at 65°C, phenol extracted, and ethanol precipitated. T4 DNA ligase (Bethesda Research Laboratories) and ATP were added, and the ligation was incubated overnight at 12°C. Ligated DNA mixtures were mixed with HB101 cells made competent by treatment with $CaCl_2$, and ampicillin-resistant (Amp^r) transformants were selected. Transformants that were Amp^r and tetracycline-sensitive were recloned, and their plasmid DNA was isolated by a rapid boiling procedure (12). Large quantities of the desired plasmids were prepared by chloramphenicol amplification (20) followed by equilibrium buoyant-density centrifugation in CsCl gradients containing ethidium bromide.

Restriction mapping of the Ddr genes. Purified plasmids containing Ddr sequences were digested with the indicated restriction enzymes alone or in combination, under conditions recommended by the manufacturer, and were analyzed by electrophoresis in agarose gels.

General techniques. *E. coli* K-12 strains MC1061 [F$^-$ *hsdR araD* Δ(*ara-leu*) Δ*lacX74*] and HB101 (F$^-$ *hsdS recA13 proA lacY galK*) were grown in LB medium (21) before phage plating or transformation. When necessary, ampicillin was added to a final concentration of 50 μg/ml.

RESULTS

Isolation of Ddr genes. The conditions selected for treating cells with UV irradiation or NQO were based on two considerations. First, we wanted to use a dose of UV light or carcinogen that would introduce damage into most of the cells in the culture without reducing cell viability more than 50%. Second, we wanted to be able to identify genes that are activated immediately after DNA damage as well as those that are activated at later times. Based on preliminary experiments, we chose to use a UV dose of 150 J/m^2 (greater than 80% survival) and to collect cells within 1 h of treatment. The second condition we chose was treatment of cells for 6 h with 1.5 μg of NQO per ml (55% survival). In two

separate experiments, RNA was prepared from either UV-irradiated or NQO-treated cells as well as control cells, and labeled cDNAs were prepared for hybridization probes.

More than 9,000 λ clones containing genomic *Hin*dIII fragments of *S. cerevisiae* S288C were transferred in duplicate to nitrocellulose and hybridized with these labeled cDNA probes as described above. The conditions for hybridization and length of autoradiographic exposure were chosen so that 20 to 70% of the plaques typically gave detectable hybridization signals after 3 to 5 days of exposure (see Fig. 1).

From these two plaque filter screenings, more than 30 plaques were identified that gave stronger hybridization signals when probed with cDNA prepared from damaged cells than from control cells. An example of one such λ clone is shown in Fig. 1. Several of these λ clones were plaque purified, and their DNA was isolated and digested with *Hin*dIII. The sizes of the yeast inserts were determined by comparing their mobilities in agarose gels with those of *Bam*HI restriction fragments of phage λ.

Routinely, these digested DNAs were transferred to nitrocellulose by "sandwich blotting" (20) and were hybridized separately with labeled cDNA probes prepared from control and DNA-damaged cells. These clone blots were used to confirm the results of the plaque filter hybridization and to identify the yeast fragment to be subcloned (data not shown).

During our screening of the λ-yeast library, we detected four clones that showed an unexpected differential response: they hybridized more strongly to cDNA prepared from control cells than to cDNA prepared from the NQO-treated cells (data not shown). These clones likely represent "turn-off" or "turn-down" sequences which appear to have no counterpart in the bacterial SOS response.

Restriction and hybridization analysis of Ddr genes. Four λ clones showing a stronger hybridization signal to cDNA from damaged cells were chosen for further characterization. These isolates were designated λ178C, λ78A, λ3016B, and λA2. The λA2 clone was among the first to be identified in screenings with cDNA probes prepared from UV-irradiated cells. All of the other clones were obtained with cDNA preparations from NQO-treated cells that were used to screen the λ-yeast library.

The sizes of the *Hin*dIII yeast inserts in the λ178C, λ3016B, and λA2 clones were determined to be 1.95, 1.70, and 1.45 kilobases (kb), respectively. These unique *Hin*dIII restriction fragments were isolated from agarose gels and ligated into the *Hin*dIII site of pBR322. The resulting plasmids were designated pBR178C, pBR3016B, and pBRA2. When λ78A was restricted with *Hin*dIII, three small restriction fragments were resolved on a 1% agarose gel with sizes of 1.74, 1.53, and 0.45 kb (data not shown). The presence of these three *Hin*dIII fragments in the purified λ78A clone suggests that an incomplete *Hin*dIII digest was used in constructing the λ-yeast library. The pBR178C, pBR3016B, and pBRA2 recombinant plasmids were further digested with several different restriction enzymes for construction of restriction maps of the genomic inserts. These results are shown in Fig. 2. A preliminary restriction map of the λ78A clone indicated that this fragment is distinct from the others (data not shown). This result was also confirmed by hybridization analysis, which failed to show any homology among the clones.

The purified pBR178C, λ78A, pBR3016B, and pBRA2 DNAs were nick translated with [α-^{32}P]dCTP and were used to probe *Hin*dIII digests of genomic yeast DNA bound to nitrocellulose filters. Both the pBR178C and λ78A clones hybridized to several genomic fragments, among which were

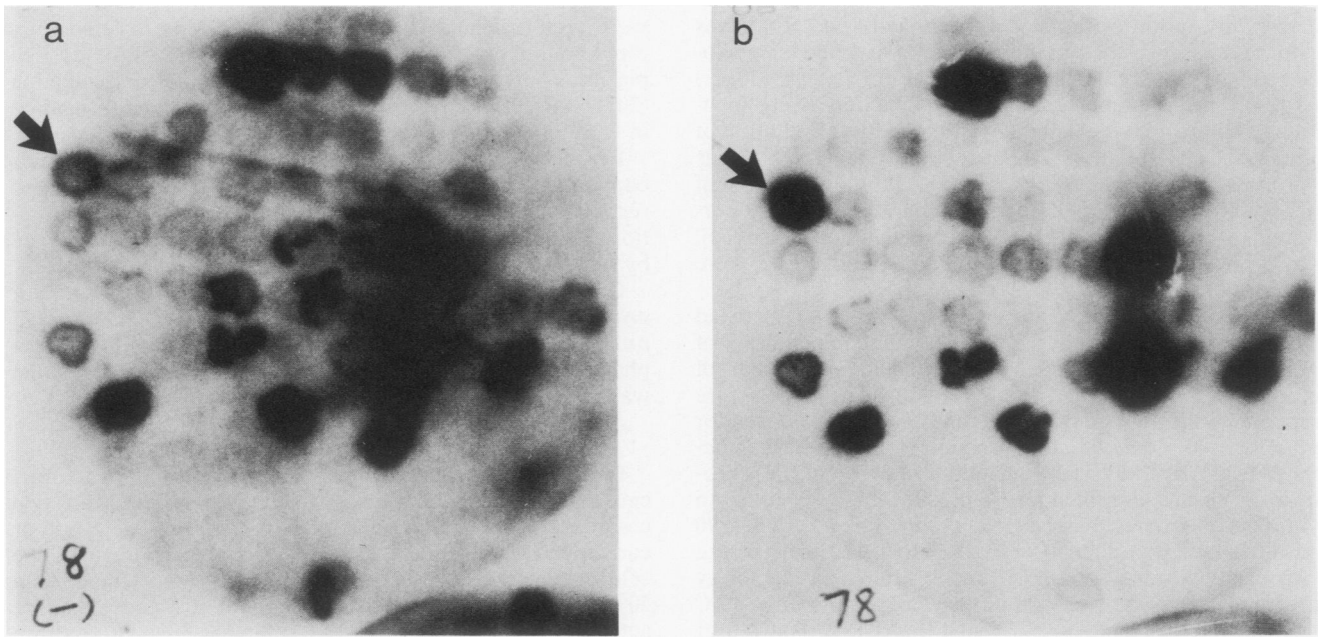

FIG. 1. Differential plaque filter hybridization with cDNA probes from control and DNA-damaged cells. *S. cerevisiae* M12B was grown in YPD medium to a cell density of 2×10^7 cells per ml, and the culture was split into control (no drug) and 1.5 μg of NQO per ml. Cells were incubated for 6 h at 30°C. RNA was isolated from each culture, and labeled cDNA probes were prepared as described in the text. Duplicate nitrocellulose plaque filters were hybridized with (a) cDNA from untreated cells and (b) cDNA from NQO-treated cells. The arrow indicates the position of the λ78A clone.

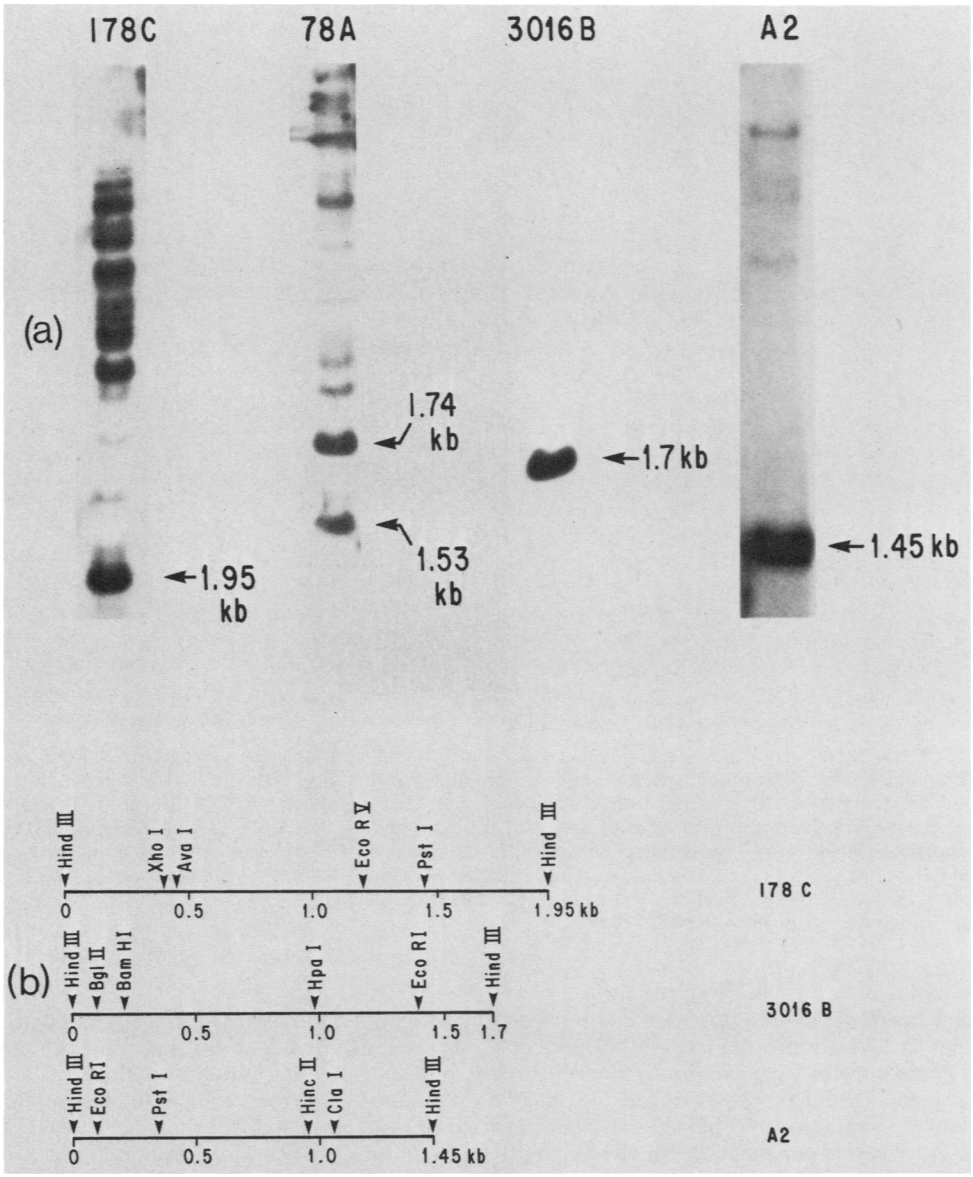

FIG. 2. Genomic hybridization and restriction enzyme mapping of Ddr clones. (a) Genomic DNA from strain M12B was digested to completion with *Hin*dIII, electrophoresed, transferred to nitrocellulose, and hybridized with the indicated nick-translated Ddr probe. The fragments identified by arrows are identical in size to the cloned Ddr sequences. The 0.45-kb fragment contained in λ78A migrated off this gel. Restriction fragments of phage λ were used as molecular weight markers. (b) Restriction enzyme maps were determined by a combination of single and double digests on intact clones. The following enzymes were tested for their ability to cleave each of the four clones: *Bam*HI, *Bcl*I, *Bgl*II, *Bst*EII, *Cla*I, *Eco*RI, *Hin*dIII, *Hpa*I, *Kpn*I, *Pst*I, *Pvu*II, *Sal*I, *Sst*I, *Sst*II, and *Xho*I. In addition, both *Eco*RV and *Ava*I sites were found in clone 178C, and a *Hin*cII site was found in clone A2.

fragments identical in size to the cloned inserts (Fig. 2). The pattern of fragments homologous to the pBR178C insert differed significantly from the hybridization pattern obtained with the λ78A probe. We conclude that the pBR178C and λ78 clones contain repetitive sequences that are present 10 to 15 times in the yeast genome.

Both pBR3016B and pBRA2 hybridized to unique *Hin*dIII restriction fragments in the yeast genome (Fig. 2). Furthermore, the sizes of these fragments, 1.7 and 1.45 kb, corresponded to the sizes of the inserts in pBR3016B and pBRA2, respectively.

Northern analysis of the Ddr sequences. We examined the sizes of the transcripts homologous to the four Ddr clones by Northern hybridization. Poly(A)$^+$ RNA (4 μg) prepared from control and UV- or NQO-treated cells was electrophoresed in agarose gels and transferred to nitrocellulose. RNA filters were hybridized with the indicated nick-translated Ddr sequence probe, washed, and autoradiographed. The results are shown in Fig. 3a through d. Both pBR178C and λ78A hybridized to single large transcripts. Based upon the mobilities of the 25S and 18S ribosomal subunits, we estimate the sizes of these transcripts to be ca. 5 kb (Fig. 3a and b).

Densitometry of these autoradiographs indicated that the level of the transcript homologous to pBR178C was ca. eightfold higher in NQO-treated cells than in control cul-

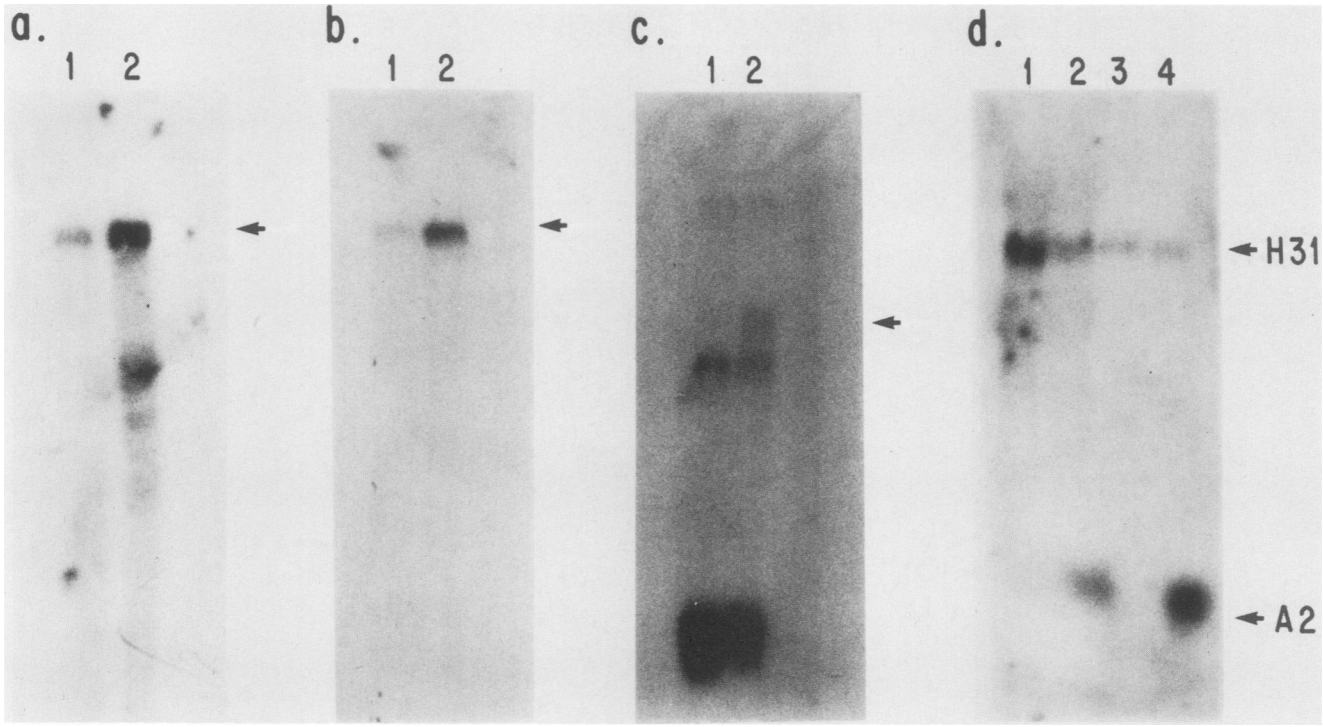

FIG. 3. Northern analysis of Ddr sequences displaying increased transcript levels after DNA damage. RNA from NQO-treated and UV-irradiated cells was prepared as described in the text, and the Northern filters were hybridized with the indicated nick-translated Ddr probe. (a) Probe: pBR178C. Lane 1, poly(A)$^+$ RNA (4 μg) from control cells, 6 h; lane 2, poly(A)$^+$ RNA (4 μg) from NQO-treated cells, 6 h. (b) Probe: λ78A. Lane 1, poly(A)$^+$ RNA (4 μg) from control cells, 6 h; lane 2, poly(A)$^+$ RNA (4 μg) from NQO-treated cells, 6 h. (c) Probe: pBR3016B. Lane 1, poly(A)$^+$ RNA (4 μg) from control cells, 6 h; lane 2, poly(A)$^+$) RNA (4 μg) from NQO-treated cells, 6 h. (d) Probe: pBRA2. Lane 1, total RNA (100 μg) from control cells, 1 h; lane 2, total RNA (100 μg) from UV-irradiated cells, 1 h; lane 3, poly(A)$^+$ RNA (4 μg) from control cells, 1 h; lane 4, poly(A)$^+$) RNA (4 μg) from UV-irradiated cells, 1 h. Gel d was also hybridized with λH31, which is homologous to a constitutive transcript from the *Trp5* region.

tures. The transcript homologous to λ78A was also present in NQO-treated cells at levels more than sevenfold greater than that found in control mRNA preparations (Fig. 3b). In both cases, there were significant basal levels of these transcripts in control *S. cerevisiae* cultures (Fig. 3a, lane 1, and Fig. 3b, lane 1), a result consistent with the original plaque filter screening (Fig. 1).

The pBR3016B probe hybridized to two poly(A)$^+$ RNAs from control cells and to an additional transcript in RNA preparations from cells treated with NQO (Fig. 3c, lanes 1 and 2). We estimated the sizes of the former transcripts to be 2.5 and 0.6 kb. The size of the transcript unique to the NQO-treated cell preparation was ca. 3.2 kb. This transcript was not detectable in control RNA preparations even after several days of autoradiography. However, because of the relatively low abundance of this RNA in damaged cells, it was difficult to accurately determine an induction ratio. Because the hybridization signal was ca. fivefold greater than background on the autoradiogram, a minimum estimate is a fivefold increase of this 3.2-kb transcript in damaged cells.

The pBRA2 probe hybridized to a single 0.5-kb transcript that was 8 to 20 times more abundant in RNA isolated from UV-irradiated cells (Fig. 3d, lanes 1 to 4) or cells treated with NQO (see below). The transcript could be detected in total RNA from UV-irradiated cells (Fig. 3, lane 2) and was substantially enriched in the poly(A)$^+$ RNA fraction (Fig. 3, lane 4).

Kinetics and dose response of induction of the Ddr 0.5-kb transcript. The 0.5-kb transcript homologous to pBRA2 was induced by UV irradiation within 1 h after exposure. We examined the kinetics of induction of this transcript after treatment with the mutagen/carcinogen NQO (1.5 μg/ml). This transcript appeared within 20 min after addition of the DNA-damaging agent and increased up to 60 min after treatment (Fig. 4a).

We next examined the dose response of this transcript after exposure of cells to different concentrations of NQO. After 60 min, the 0.5-kb transcript was present in cells treated with greater than 1 μg of NQO per ml (Fig. 4b). We have not examined the production of this transcript at later times with the lower NQO concentrations. In addition to UV irradiation and NQO, other DNA-damaging agents, both alkylating and cross-linking, were tested by Northern analysis of total RNA for their ability to stimulate production of the 0.5-kb transcript. Methylmethane sulfonate (0.1%) but not mitomycin C (5 μg/ml) or nitrofurantoin (1.0 μg/ml) significantly increased the level of this transcript after exposure for 60 min (data not shown). We are currently examining the kinetics of transcript accumulation and dose response to different mutagens/carcinogens for the other Ddr sequences.

Expression of the Ddr 0.5-kb transcript in *rad52 S. cerevisiae* strains. We next examined the production of the 0.5-kb transcript homologous to pBRA2 in *S. cerevisiae* strains that were altered in their ability to repair DNA damage. These

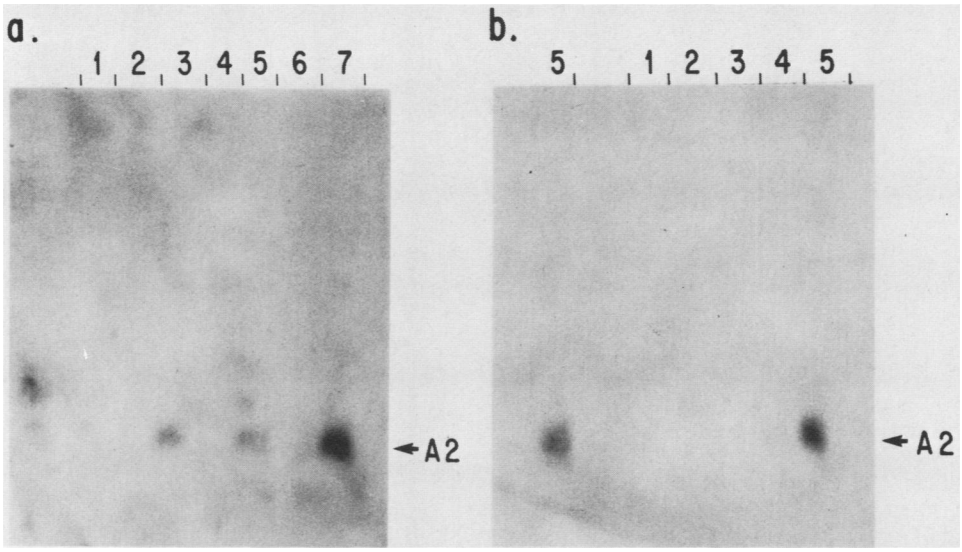

FIG. 4. Kinetics and dose response of A2 transcript accumulation after DNA damage. (a) Kinetics of transcript production. NQO (1.5 μg/ml) was added to early log phase cells. At the indicated times, cells were collected from both control and drug-treated cultures and total RNA was prepared as described in the text. Lane 1, control cells, $t = 0$; lane 2, control cells, $t = 20$ min; lane 3, NQO-treated cells, $t = 20$ min; lane 4, control cells, $t = 40$ min; lane 5, NQO-treated cells, $t = 40$ min; lane 6, control cells, $t = 60$ min; lane 7, NQO-treated cells at $t = 60$ min. (b) Dose response of transcript production. NQO was added to individual early log phase cultures at the indicated doses and incubated for 60 min. Cells were collected and total RNA was isolated. Lane 1, control cells; lane 2, NQO-treated cells, 0.1 μg/ml; lane 3, NQO-treated cells, 0.5 μg/ml; lane 4, NQO-treated cells, 1.0 μg/ml; lane 5, NQO-treated cells, 1.5 μg/ml.

strains included the *rad6-1* mutant X10-1C, *rad56* mutant SPX143-6C, and *rad52* mutant strain XS12249C. The levels of the 0.5-kb transcript increased in all of the strains after treatment with NQO (1.5 μg/ml) (data not shown). However, in the *rad52* mutant strains that were defective in double-strand break repair, significantly higher levels of the Ddr 0.5-kb transcript were detected in the absence of any DNA damage compared with RAD$^+$ strain M12B (Fig. 5). To determine whether this increase was due to the *rad52* mutation or to differences in the genetic backgrounds of the strains, we examined the levels of the 0.5-kb transcript in three additional *rad52* mutants. All of the *rad52* mutants contained high levels of the 0.5-kb A2 transcript without exposure of the cells to DNA-damaging agents (Fig. 5).

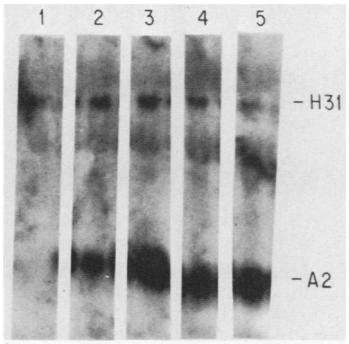

FIG. 5. Levels of transcript homologous to pBRA2 in *rad52* strains of *S. cerevisiae*. Cells in early to mid-log phase were collected, and the RNA was extracted as described in the text. Total RNA (100 μg per lane) was electrophoresed through formaldehyde-agarose gels, transferred to nitrocellulose, and hybridized with nick-translated pBRA2. Lane 1, strain M12B (wild type); lane 2, strain X512249C *rad52*; lane 3, strain X512257D *rad52*; lane 4, strain SX5-1B *rad52*; lane 5, strain YNN209 *rad52*.

DISCUSSION

DNA damage initiates a complex cellular response in *E. coli* that includes the derepression of several unlinked genes through the inactivation of a common repressor. Many of the genes that are transcriptionally activated by the damaging event have been identified and include functions involved in excision repair (*uvrA* and *uvrB*) (10, 14), in blocking cell septation (*sfi*) (13), and in mutagenesis (*umuC*) (8). The SOS response in bacteria is an example of a stress response much like the heat shock response. In both cases, an environmental stress condition activates transcription of a small group of genes. In the case of heat shock, this stress response has been found in both procaryotic and eucaryotic organisms. However, there has been little or no direct evidence for an SOS-like stress response in eucaryotic cells.

The results reported here are among the first to demonstrate that UV or chemical insult to DNA in *S. cerevisiae* can increase the level of specific transcripts. We have characterized four clones that were isolated by a differential plaque filter hybridization screen of yeast genomic DNA fragments. Two of these Ddr segments are repetitive elements in the yeast genome, as demonstrated by Southern hybridization analysis. Although these two clones, 78A and 178C, do not cross-hybridize (data not shown), and share homology with different sets of genomic *Hin*dIII fragments, each hybridizes to a single transcript that is ca. 5 kb and is eightfold more abundant in cells exposed to the DNA-damaging agent NQO.

At the present time, the precise relationship between the 178C and 78A sequences is not known, nor do we know whether each hybridizes to the same or different transcripts. A transcript size of greater than 5 kb is uncommon in *Saccharomyces*, with most transcripts ranging in size from 1 to 3.5 kb. However, two transcripts homologous to the transposable element Ty1 have been identified and characterized (7). The major transcript, which appears to be

homologous to the entire transposable element, is 5.7 kb, and a minor transcript is ca. 5 kb. We have recently found that several of the Ddr sequences isolated in our differential screening share some homology with the Ty1 element (T. McClanahan and V. Bradshaw, unpublished data). These results suggest that one or more members of a Ty1 series of transposons is regulated transcriptionally by DNA damage. Experiments are in progress to determine the relationship among these sequences and the optimal conditions for their expression after DNA damage.

Two unique sequences have also been identified which code for transcripts that accumulate more than fivefold after treatment with NQO. The pBR3016B clone hybridizes to a 3.2-kb transcript that can be detected 6 h after NQO treatment. The pBRA2 clone codes for a 0.5-kb transcript that increases more than 15-fold after 20 min of NQO treatment. Since this 0.5-kb transcript appears almost immediately after exposure to NQO, it is likely to represent a DNA sequence more directly regulated by DNA damage. For this reason, we have examined the expression of this gene in more detail. In addition to its rapid kinetics of expression, we have found that *rad52* mutant yeast strains, deficient in DNA double-strand break repair and in gene conversion (11), synthesize high levels of this 0.5-kb transcript without DNA-damaging treatment. Although we do not understand how RAD52 controls the level of the 0.5-kb transcript, we believe that it is likely to be indirect and may result from persistent unrepaired lesions in the chromosomes of this repair-deficient cell. In *E. coli*, the level of *recA* protein (and transcript) increases after UV irradiation or exposure to chemical damaging agents. In certain strains lacking DNA repair functions (e.g., *recF* mutants), the level of *recA* protein is significantly elevated, even in the absence of damaging treatments (25).

An elevation or increase in steady-state levels of transcripts may result from an increased rate of transcription initiation, a decreased rate of processing or degradation, or both. The mechanism by which DNA damage alters the levels of the Ddr transcripts is not known in all cases. Pulse-labeling experiments indicate that in the case of the 0.5-kb transcript homologous to pBRA2, the increase in transcript levels results from an increased rate of synthesis (V. Bradshaw and K. McEntee, unpublished data). We are performing similar kinetic analyses with the other Ddr sequences we have isolated.

These results, together with those of Ruby et al. (24), are among the first to demonstrate an SOS-like response in eucaryotic cells treated with DNA-damaging agents. We do not know, nor can we accurately estimate, the number of DNA damage regulated genes in *Saccharomyces*. Each of the techniques that has been used to isolate such sequences, differential hybridization and *lacZ* gene fusions, has limitations. For example, *Ddr* sequences contained on *Hin*dIII fragments together with highly transcribed genes will go undetected. For detection of damage-inducible sequences by fusion with the *E. coli lacZ* gene, the fusion must align the yeast sequence and the *lacZ* gene in the correct translational reading frame. These limitations notwithstanding, it should be possible to isolate additional *Ddr* (*din* [damage-inducible]) sequences. It is worth noting that the *din1* clone isolated by gene fusion displays a transient response to low levels of NQO (0.05 μg/ml) (24). This response has not been observed for the clones we have described. These data suggest that eucaryotes may possess complex regulatory mechanisms for controlling gene expression in response to treatment with mutagens/carcinogens.

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