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Structure of the DNA Damage-Inducible Gene *DDR48* and Evidence for Its Role in Mutagenesis in *Sacharomyces cerevisiae*

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The *DDR48* gene of *Saccharomyces cerevisiae* is a member of a set of genes that displays increased transcription in response to treatments that produce DNA lesions or to heat-shock stress. Other members of this group include the *DDRA2* and *UBI4* genes. DNA sequence analysis of the *DDR48* gene demonstrates the presence of two overlapping open reading frames, each of which has the capacity to encode a protein with a molecular mass of approximately 45 kilodaltons. Fusions of the *DDR48* coding sequences to *lacZ* demonstrates that only one of these frames is expressed in yeast cells. The protein predicted from this sequence is extremely hydrophilic and contains multiple repeats of the peptide sequence Ser-Asn-Asn-X-Asp-Ser-Tyr-Gly where X is either Asn or Asp. Additionally, closely related sequences are found throughout the primary sequence. Primer extension data indicate that, after 4-nitroquinoline-1-oxide and heat-shock treatments, there are three major and two minor transcriptional start sites which are utilized. The function of the *DDR48* gene was investigated by disrupting this gene in diploid cells. Viable haploid cells containing the *DDR48* gene disruption were isolated after tetrad analysis. Although the *ddr48* mutant showed a slightly altered sensitivity to killing by 4-nitroquinoline-1-oxide and to heat shock compared with the *DDR48* haploid, the spontaneous mutation rate of reversion of a *his4* mutation was reduced 6- to 14-fold in the *ddr48* strain. These results implicate the *DDR48* gene in the production or recovery of mutations in *S. cerevisiae*.

Biological stress responses appear to be conserved throughout evolution. For example, a transient thermal stress or heat shock elicits an increase in the expression of specific sets of genes in bacteria as well as in human cells (17, 28). Moreover, it has been shown that the DnaK protein of *Escherichia coli*, a major heat shock protein, is approximately 48% identical in primary structure to that of the hsp70 heat-shock protein of *Drosophila melanogaster* (2). Although the biological roles of heat shock proteins have not been fully established, it appears that their importance for cells has dictated their continued presence throughout evolution.

The stress response that accompanies DNA damage to cells has been less well studied. In bacteria, disruption of DNA structure or replication stimulates a stress response designated the SOS response. A fundamental feature of this stress response is the increased expression of functions needed for DNA repair, mutagenesis, and inhibition of cell division. In several instances, the biochemical roles of the SOS genes are known (33, 34).

In eucaryotic cells, several DNA damage-inducible genes have been isolated by hybridization (20, 21), *lacZ* fusion (26), or immunological (1, 9, 15) approaches. The biological roles of only a few of these genes are known. For example, the major ubiquitin gene of yeast cells, *UBI4*, was shown to be induced by DNA-damaging treatments (32). Elledge and Davis (9) and Hurd et al. (15) have demonstrated that the small subunit of the nucleotide biosynthetic enzyme, ribonucleoside diphosphate reductase (*RNR2*), is induced by DNA damage. Moreover, we have recently determined that another DNA damage-inducible gene, *DIN1*, encodes a large subunit of the reductase enzyme (K. Yagle and K. McEntee, unpublished data). The roles or functions of several other DNA damage-inducible genes are unknown.

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In the present study, we describe the structure of the *DDR48* gene which was isolated in a differential hybridization screening for DNA damage-responsive genes. The levels of *DDR48* transcripts increase approximately 7- to 10-fold after treatment of yeast cells with 4 nitroquinoline-1-oxide (4NQO), UV light, or alkylating agents. Additionally, the *DDR48* gene is transcriptionally regulated by heat shock stress (21). DNA sequence analysis of the *DDR48* gene predicts an unusually hydrophilic protein containing multiple repeats of an octapeptide sequence. The role of the *DDR48* gene was investigated by disrupting the single copy of this gene in a haploid cell. Although the *ddr48* mutant was slightly less sensitive to killing by 4NQO than the nondisrupted strain and was only slightly more sensitive to thermal killing, the spontaneous mutation rate was reduced 3- to 14-fold in the disrupted strain, depending on the locus examined. These results suggest that the *DDR48* gene participates in one or more pathways for mutagenesis in *S. cerevisiae*.

MATERIALS AND METHODS

Strains. The strains of *Saccharomyces cerevisiae* used in this study are described in Table 1.

Media. Cells were routinely grown in YPD (1% yeast extract, 2% peptone, 2% glucose). SD minimal medium contained 0.67% yeast nitrogen base (without amino acids) and 2% glucose. Complete minimal medium (SDC) was SD medium supplemented with 20 mg each of uracil, tryptophan, histidine, and adenine per ml, and 30 mg each of lysine and leucine per ml. SD(Leu$^-$), SD(Ade$^-$), and SD(His$^-$) minimal media were the same as SDC except that for each medium the indicated amino acid was omitted. SD(Ura$^+$) was SD medium supplemented with 20 mg of uracil per ml only. YPD and minimal medium plates contained 2% agar. SDCC plates were SDC plates supplemented with canavanine (75 μg/ml). SD(X-Gal) plates consisted of

TABLE 1. *S. cerevisiae* strains, genotypes, and sources

Strain	Genotype	Source
M12B	α *trp1-289 ura3-52 gal2*	R. Davis (Stanford University)
T87 (X46)	a/α Δ*trp1*/Δ*trp1 ura3-52*/*ura3-52 leu2-3,112*/*leu2-3,112 lys2*/*lys2 ade2-101*/+ +/*his4-713*	E. Craig (University of Wisconsin)
JTD-100	Same as T87, except *ddr48*:*LEU2*/+	This study
JTD-101	Same as T87, except *ddr48*:*LEU2*/+	This study
JTD-110	Same as T87, except *ddr48*:*LEU2*/+	This study
JT-22A	α Δ*trp1 ura3-52 leu2-3,112 lys2 his4-713 ade2-101*	This study
JT-26A	a Δ*trp1 ura3-52 leu2-3,112 lys2 his4-713*	This study
JT-26B	a Δ*trp1 ura3-52 leu2-3,112 lys2 his4-713 ade2-101 ddr48*::*LEU2*	This study

SD minimal medium buffered with M63 salts (22) and containing 20 mg of uracil and 60 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside per ml (X-Gal; Boehringer Mannheim Biochemicals, Indianapolis, Ind.) dissolved in N',N'-dimethylformamide and 2% agar.

Growth of yeast cells and 4NQO and heat shock treatments. Strain M12B was grown in YPD medium at 30°C to a density of 0.5×10^7 to 1.0×10^7 cells/ml. The culture was divided into two aliquots and one was treated with 4NQO (Sigma Chemical Co., St. Louis, Mo.) or heat shock stressed as previously described (21).

RNA isolation and Northern analysis. RNA was extracted by glass bead disruption as described (21). Total RNA (100 μg per sample) was denatured in formamide, separated by electrophoresis in 1% agarose, and hybridized and washed as described previously (21).

Determination of direction of transcription. The 1.7-kilobase (kb) *Hin*dIII-*Cla*I fragment was subcloned into the *Hin*dIII and *Acc*I sites of M13mp18 and M13mp19 (36). The 17-base probe primer (BRL, Gaithersburg, Md.) was used to synthesize labeled probes that contained single-stranded unlabeled *DDR48* sequence by using the protocol of Hu and Messing (14). The probes were hybridized to total RNA (100 μg) from control and 4NQO-treated cells.

DNA sequencing. Restriction fragments were subcloned into phage vectors M13mp18 and M13mp19 (36). The single-stranded subcloned fragments were sequenced by the dideoxynucleotide method of Sanger et al. (27) by using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio) [α-^{35}S]dATP, and the protocol provided by the manufacturer. Both the universal primer (New England BioLabs, Inc., Beverly, Mass.) and synthetic oligonucleotides derived from the *DDR48* sequence were used to prime the sequencing reactions. The sequencing reaction products were separated on 6% acrylamide-8 M urea gels. Autoradiography of the dried gels was performed by overnight exposure of Kodak XAR film at room temperature.

Primer extension. A 35-base synthetic oligonucleotide (200 ng), complementary to nucleotides 187 to 221 of the *DDR48* sequence (see Fig. 2), was labeled with ^{32}P by using T4 polynucleotide kinase and [γ-^{32}P]ATP as described previously (19). The primer extension reactions were performed as described previously (10) with the following modifications. The labeled oligonucleotide (4 ng) was hybridized with 25 μg of total RNA at 45°C for 60 min in 10 mM Tris (pH 8.7), 1 mM EDTA, 270 mM KCl (final volume, 12 μl). To the annealed RNA templates, 24 μl of reverse transcription reaction buffer (20 mM Tris [pH 8.7], 10 mM $MgCl_2$, 10 mM dithiothreitol, 380 μM each dATP, dGTP, dCTP, and dTTP [final concentration]), 40 U of RNAsin (Promega, Madison, Wis), and 52 U of avian myeloblastosis virus reverse transcriptase (Life Sciences, Inc., St. Petersburg, Fla.) were added. The reaction mixtures were incubated at 45°C for 60 min, followed by the addition of 5 μl of 0.5 M EDTA, 200 μl of TE (10 mM Tris hydrochloride [pH 7.5], 1 mM EDTA), and 3 μl of glycogen (1 mg/ml) (Boehringer Mannheim) to stop the reaction. The samples were precipitated with ethanol and collected by centrifugation. The pellet was air dried and suspended in 5 μl of TE and 5 μl of loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol FF). One-half of the sample was loaded onto a 6% acrylamide-8 M urea sequencing gel. Sequencing reactions primed with the same oligonucleotide were used as markers.

Construction of *DDR48-lacZ* fusions. A 1.8-kb *Eco*RI-*Sst*I fragment containing approximately 1.3 kb of the upstream region and both potential translation start sites was subcloned into the polylinker region of pUC19 (36) to create plasmid pUC48USES. The 1.8-kb fragment was excised by digesting with *Eco*RI and *Bam*HI, which cuts in the polylinker, and was subcloned into the *Eco*RI and *Bam*HI sites of the expression plasmid pMC1790 (6). This construction created a fusion between codon 130 of open reading frame 2 (ORF2) (translation starts at nucleotide +1 of sequence; see Fig. 2) of *DDR48* and codon 8 of β-galactosidase. To create a fusion between *DDR48* ORF3 (translation starts at nucleotide +209 of *DDR48* sequence; see Fig. 2) and *lacZ*, pUC48BUSES was digested with *Bam*HI, and end-filled by using T4 DNA polymerase. This DNA was digested with *Eco*RI, and the resulting 1.8-kb fragment was subcloned into the *Eco*RI and *Sma*I sites of plasmid pMC1790.

Determination of correct reading frame. The two clones pMC1.8USEB (ORF2) and pMC1.8USEBF (ORF3), as well as pMC1790, were transformed into strain M12B by electroporation (30). To select TRP$^+$ transformants, cells were plated on SD(Ura$^+$) minimal plates and incubated at 30°C for 5 days. The TRP$^+$ colonies were restreaked onto SD(X-Gal) plates to test for β-galactosidase activity as determined by colony color.

Construction of a *ddr48*:LEU2-disrupted allele and replacement of the chromosomal gene. The 3.0-kb *Bgl*II fragment from YEP13 containing the *LEU2* gene was subcloned into the *Bam*HI site of the pUC19 polylinker region, thus creating plasmid pUCLEU2. The 3.8-kb *Hin*dIII-*Bam*HI fragment

of the *DDR48* gene was subcloned into pBR322 to create plasmid pBR48L3-2. The pBR48L3-2 plasmid was digested with *Sst*I and *Xba*I to remove approximately two-thirds of the coding region and up to 1.2 kb of the 3′ flanking region. The flanking *Sst*I and *Xba*I sites in the pUC19 polylinker were used to excise the *LEU2* gene from plasmid pUCLEU2, and this fragment was ligated into digested pBR48L3-2 plasmid to create plasmid pBR48L3-2/LEU2 (see Fig. 5). To introduce this disrupted copy of *DDR48* into a yeast diploid strain, the method of Rothstein was used (25). A portion (10 μg) of pBR48L3-2/LEU2 was digested with *Hin*dIII and *Bam*HI and used to transform T87 to LEU^+ by using the spheroplasting method of Hinnen et al. (12).

Isolation of genomic DNA and Southern hybridization analysis. Single colonies were innoculated into 5 ml of YPD and grown overnight at 30°C. Genomic DNA was isolated by the protocol of Holm et al. (13). Samples of DNA were digested with *Hin*dIII and *Bam*HI and separated by electrophoresis on 0.8% agarose gels. The gels were stained and transferred to nitrocellulose. A probe containing the 0.62-kb *Hin*dIII-*Sst*I fragment subcloned into M13mp18 was used for hybridization as previously described (21).

Sporulation and tetrad analysis. Single yeast colonies were inoculated into 5 ml of YPD, grown to saturation at 30°C, washed three times with equal volumes of distilled water, and suspended in 5 ml of distilled water. Aliquots of the cell suspension (0.2 ml) were added to 1.8 ml of potassium acetate solution (1.5%) supplemented with the appropriate nutrients at one-fourth the normal concentration. Suspensions were incubated at 30°C for 3 to 4 days, and the percentage of asci was monitored by microscopy. When the fraction of sporulating cells was greater than 0.10, the cells were washed three times with an equal volume of H_2O and suspended in 0.4 ml of H_2O. To analyze the tetrads, cells were treated with glusulase and asci were dissected on YPD plates by using a micromanipulator. The plates were incubated for 2 to 3 days at 30°C. To test for the LEU^+ phenotype of the colonies arising from the spores, cells were grown on SD(Leu^-) minimal plates for 2 to 3 days at 30°C.

Survival and induced mutagenesis measurements. Cells were grown in SDC medium at 30°C to a density of 0.5×10^7 to 1.0×10^7 cells/ml and divided into aliquots. Cultures were incubated at 30°C for 1 h with the indicated concentration of 4NQO or *N*′-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), after which an equal volume of 10% sodium thiosulfate was added. Cells were collected by centrifugation and suspended in 0.1 volume of 0.9% NaCl. A portion was diluted and spread on SDC plates to measure cell survival. The remaining cells were spread onto SD(His^-) or SD(Ade^-) minimal plates to determine the number of HIS^+ revertants or ADE^+ revertants, respectively.

Fluctuation test. For the fluctuation test, the procedure of Luria and Delbrück (18) was essentially followed. Single colonies of JT-22A and JT-26B were used to inoculate the SDC medium, and the cultures were incubated at 30°C to a cell density of about 10^7 cells/ml. An estimate of cell density was made by using the measured optical density at 595 nm, and samples of the overnight cultures were diluted to approximately 200 to 600 cells/ml in fresh SDC medium. A portion of these diluted cultures was spread on an SDC plate to measure actual cell density of the starting cultures. Twenty parallel samples for each strain were inoculated into test tubes (5 ml for experiment 1, 1 ml for experiment 2) and incubated at 30°C for approximately 40 h (20 doublings). Portions of the cultures were diluted in 0.9% NaCl and spread on SDC plates to measure cell number, and either one 100-μl portion (experiment 1) or two 200-μl portions (experiment 2) of each culture were spread onto SD(His^-) and SDCC plates to measure the number of HIS^+ or Can^r mutants, respectively. SDC plates were incubated at 30°C for 3 days, and SD(His^-) and SDCC selective plates were incubated at 30°C for 5 days before determining the number of mutants. The mutation rate was calculated by using the method of means.

RESULTS

DNA sequence analysis of the *DDR48* gene. By using a differential plaque filter hybridization screen (20), the *DDR48* gene was isolated as a 4.6-kb *Hin*dIII restriction fragment from a bacteriophage λ590 yeast genomic library. The *DDR48* gene hybridizes to an approximately 1.3-kb transcript (Fig. 1B) and is regulated at the level of transcription by both DNA damage and heat shock stresses (21). Transcript levels increase 7- to 10-fold within 30 min of exposure. Moreover, after these treatments, transcripts accumulate in the presence of cycloheximide (200 μg/ml), indicating that the *DDR48* gene is directly regulated by these environmental stresses (data not shown).

Restriction mapping of this region, subcloning, and Northern hybridization analysis were used to localize the transcribed region to a 2.2-kb *Hin*dIII-*Kpn*I restriction fragment (Fig. 1A). To determine the direction of transcription, the *Hin*dIII-*Cla*I fragment containing the *DDR48* gene was cloned into both M13mp18 and M13mp19, and the resulting single-stranded clones were used as probes for Northern hybridization analysis. The results (Fig. 1B) indicate that transcription is from left to right relative to the restriction map (Fig. 1A). Moreover, additional hybridization analysis indicated that transcription initiated approximately 0.2 to 0.3 kb downstream of the *Hin*dIII restriction site (data not shown).

The DNA sequence of the *DDR48* gene was obtained by subcloning restriction fragments into both M13mp18 and M13mp19 and by using the Sanger dideoxy chain termination method (27) and Sequenase. The sequencing strategy is shown in Fig. 1A. More than 92% of the *Hin*dIII-*Kpn*I fragment was sequenced on both strands, including all of the coding region. An overlapping *Sst*I fragment (approximately 11 kb) was isolated from a phage λgt1 genomic library by using the 0.62-kb *Hin*dIII-*Sst*I fragment. Approximately 80% of the contiguous upstream 0.6-kb *Hin*dIII fragment was sequenced (Fig. 1A).

The 2,710 base pairs (bp) of the DNA sequence is shown in Fig. 2. An unexpected feature of this sequence was the presence of two long, overlapping ORFs (beginning at nucleotides +1 and +209), each capable of encoding a protein with a molecular mass of approximately 45 kilodaltons. To determine the translational frame(s) expressed in yeast cells, protein fusions were constructed between the *DDR48* gene of *S. cerevisiae* and the *lacZ* gene of *E. coli*. Two plasmids were constructed by using the *lacZ* fusion vector pMC1790. Plasmid pMC1.8USEB resulted from a fusion of the *DDR48* ORF2 at predicted codon 130 with codon 8 of β-galactosidase. Fusing predicted codon 59 of *DDR48* ORF3 to the same *lacZ* codon created plasmid pMC1.8USEBF. The structures of the fusion junctions were confirmed by DNA sequencing (data not shown). Each of the constructs was introduced into *S. cerevisiae* M12B by electroporation (30), and Lac expression was determined on SD plates containing X-Gal. The results demonstrated that fusions to *DDR48* in ORF2 produced active enzyme as determined by an intense

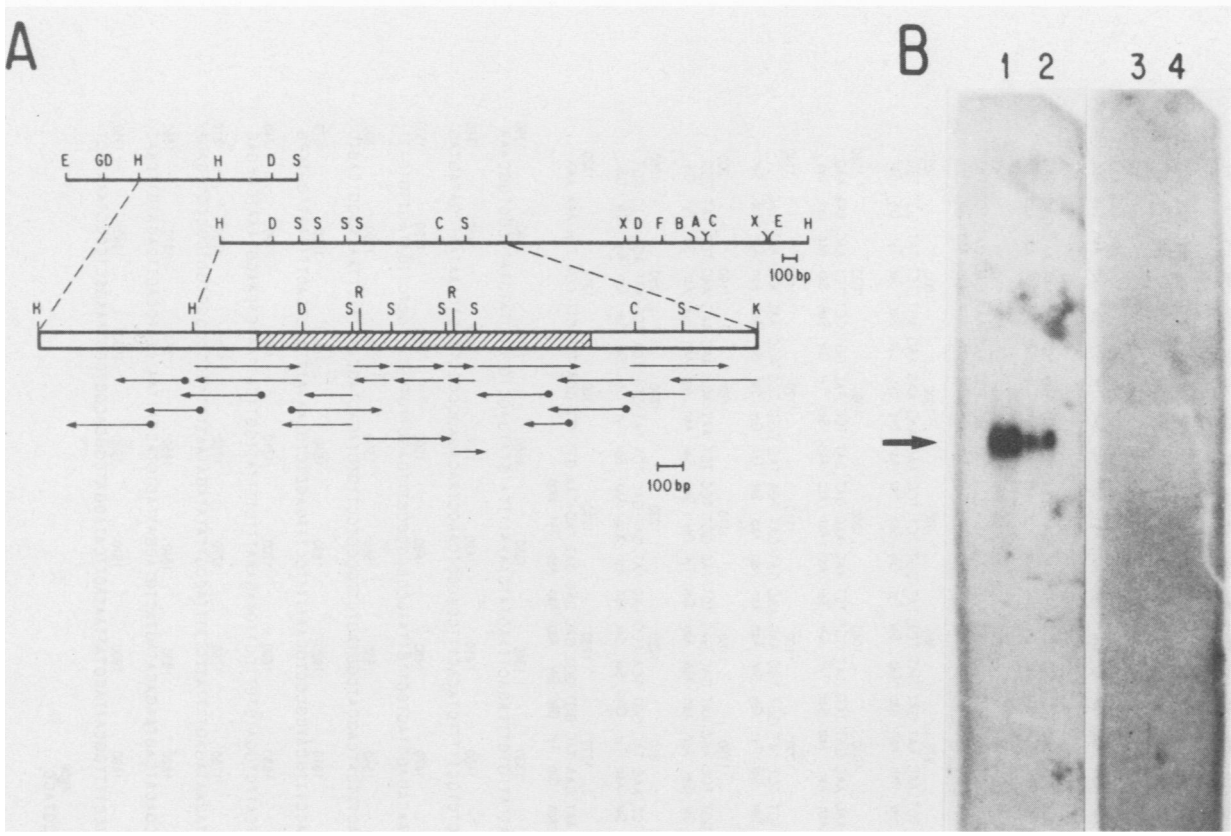


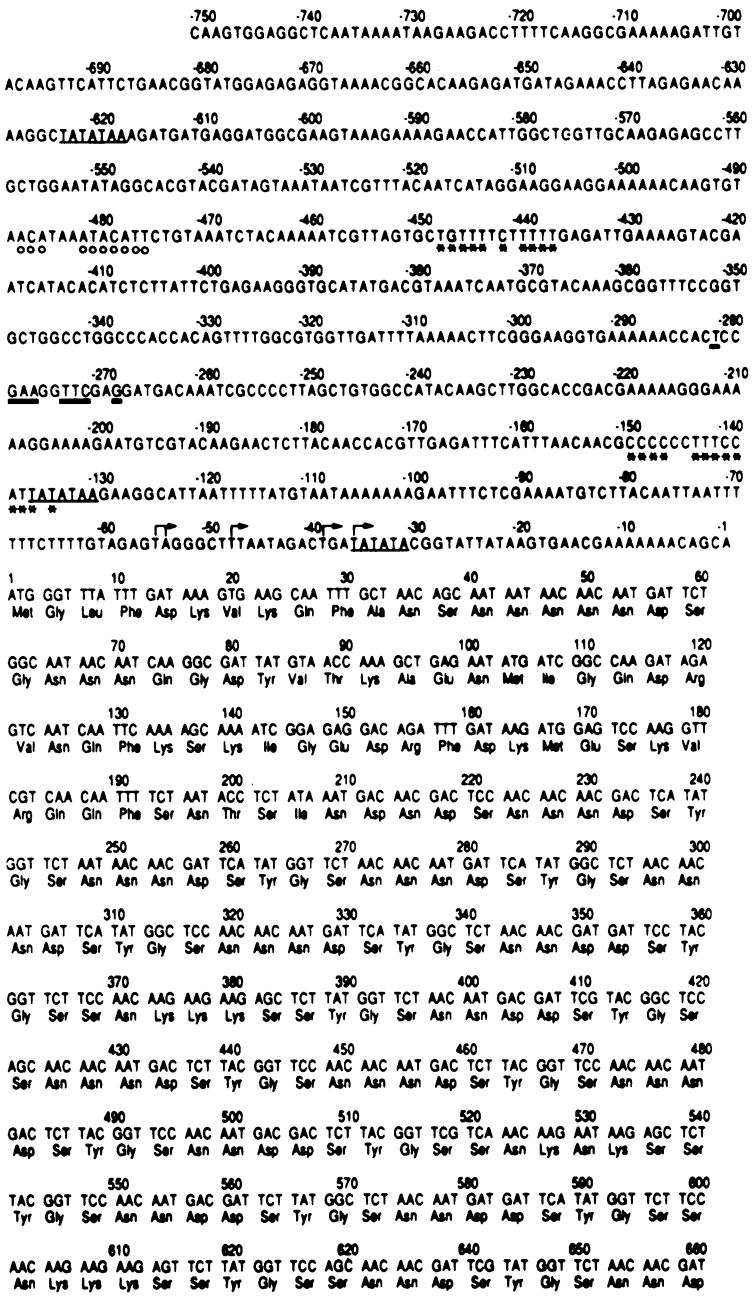
FIG. 1. Structure, sequencing strategy, and direction of transcription of the *DDR48* gene. (A) Restriction map of the *DDR48* gene and flanking DNA. The rightmost *Hin*dIII-*Kpn*I region was derived from a 4.6-kb *Hin*dIII fragment isolated from a yeast genomic λ590 library (20). The leftmost *Hin*dIII fragment was isolated from an overlapping *Sst*I genomic fragment cloned into λgt1. The position of the coding region is given by the cross-hatched area. The strategy used to sequence the *DDR48* gene and flanking DNA is shown below. Arrows indicate the direction of sequencing using subcloned fragments (→) and oligonucleotide primers (↔). Fragments were cloned into M13mp18 and M13mp19 for dideoxy sequencing of both strands. (B) Determination of direction of transcription of *DDR48*. Single-stranded DNA probes derived from M13mp18 and M13mp19 containing the 1.7-kb *Hin*dIII-*Cla*I fragment were used to hybridize to Northern filters containing total RNA from control or 4NQO-treated cells. RNA was hybridized with single-stranded probe M13mp18 H/C4 (lanes 1 and 2) or single-stranded probe M13mp19 H/C5 (lanes 3 and 4). The preparation of labeled single-stranded probes as described previously (13). Lanes: 1 and 3, RNA from 4NQO-treated cells; 2 and 4, RNA from untreated (control) cells. These results indicated that the direction of transcription of the region shown in panel A was from left to right as drawn. Restriction enzyme designations: A, *Ava*I; B, *Bam*HI; C, *Cla*I; D, *Hin*cII; E, *Eco*RI; F, *Sst*II; G, *Sal*I; H, *Hin*dIII; K, *Kpn*I; R, *Rsa*I; S, *Sst*I; X, *Xba*I.

blue colony color, whereas fusions to ORF3 produced no activity or very little activity as judged by the plate assay (data not shown). We concluded that translation frame 2 was the only reading frame expressed in vivo.

The predicted amino acid sequence (Fig. 2) was derived from ORF2. The coding region was 1,290 nucleotides in length and encoded a protein with a molecular mass of 46,187. The National Biomedical Research Foundation protein sequence data base was searched, and no significant homologies were detected between the predicted protein sequence of *DDR48* and any previously determined protein sequence.

Analysis of this putative protein sequence indicated that the *DDR48* gene product was extremely hydrophilic and was composed of multiply repeated peptide sequences of the form Ser-Asn-Asn-X-Asp-Ser-Tyr-Gly, where X is either Asn or Asp. Moreover, several related sequences were also found in the predicted primary sequence (Fig. 3). By using this structure, the *DDR48* protein was calculated to contain 27% Ser, 23% Asn, and 13% Asp, and to lack Cys, His, Pro, and Trp residues. Since the sequence Asn-X-Ser is a eucaryotic glycosylation sequence, it is possible that the *DDR48* protein is subject to posttranslational modification in yeast cells. A codon bias index of 0.29 was calculated (4) on the basis of the predicted amino acid sequence. This low value is indicative of genes which are not highly expressed. However, the results may not be truly indicative of the relative abundance of the transcript because 75% of the protein was highly repetitive.

In the 751 bp located upstream of the coding region of *DDR48*, there were three possible TATA boxes. While the distance between the mRNA initiation site(s) and the TATA box varies considerably among yeast genes, from 40 to 150 bp (31), only the TATA box at −137 to −131 in the *DDR48* gene sequence falls within this range. This TATA box is 75 bp upstream of the most 5′ transcription start site (Fig. 2). The 5′ flanking region contained a single heat shock consensus sequence at position −282 to −269 (Fig. 2). Also observed at positions −448 to −437 and at −150 to −135 were two sequences which showed considerable homology (10 of 12 and 13 of 16 matches, respectively) with a sequence found upstream of another DNA damage-responsive gene, *DDRA2* (N. Kobayashi, unpublished data). In addition, there was a possible RAP1 protein binding site at position


-750 -740 -730 -720 -710 -700
CAAGTGGAGGCTCAATAAAATAAGAAGACCTTTTCAAGGCGAAAAAGATTGT
-690 -680 -670 -660 -650 -640 -630
ACAAGTTCATTCTGAACGGTATGGAGAGAGGTAAAACGGCACAAGAGATGATAGAAACCTTAGAGAACAA
-620 -610 -600 -590 -580 -570 -560
AAGGCTATATAAAGATGATGAGGATGGCGAAGTAAAGAAAAGAACCATTGGCTGGTTGCAAGAGAGCCTT
-550 -540 -530 -520 -510 -500 -490
GCTGGAATATAGGCACGTACGATAGTAAATAATCGTTTACAATCATAGGAAGGAAGGAAAAAACAAGTGT
-480 -470 -460 -450 -440 -430 -420
AACATAAATACATTCTGTAAATCTACAAAAATCGTTAGTGCTGTTTTCTTTTTGAGATTGAAAAGTACGA
-410 -400 -390 -380 -370 -360 -350
ATCATACACATCTCTTATTCTGAGAAGGGTGCATATGACGTAAATCAATGCGTACAAAGCGGTTTCCGGT
-340 -330 -320 -310 -300 -290 -280
GCTGGCCTGGCCCACCACAGTTTTGGCGTGGTTGATTTTAAAAACTTCGGGAAGGTGAAAAAACCACTCC
-270 -260 -250 -240 -230 -220 -210
GAAGGTTCGAGGATGACAAATCGCCCCTTAGCTGTGGCCATACAAGCTTGGCACCGACGAAAAAGGGAAA
-200 -190 -180 -170 -160 -150 -140
AAGGAAAAGAATGTCGTACAAGAACTCTTACAACCACGTTGAGATTTCATTTAACAACGCCCCCCTTTCC
-130 -120 -110 -100 -90 -80 -70
ATTATATAAGAAGGCATTAATTTTTATGTAATAAAAAAAGAATTTCTCGAAAATGTCTTACAATTAATTT
-60 -50 -40 -30 -20 -10 -1
TTTCTTTTGTAGAGTAGGGCTTTAATAGACTGATATATACGGTATTATAAGTGAACGAAAAAAACAGCA
1 10 20 30 40 50 60
ATG GGT TTA TTT GAT AAA GTG AAG CAA TTT GCT AAC AGC AAT AAT AAC AAC AAT GAT TCT
Met Gly Leu Phe Asp Lys Val Lys Gln Phe Ala Asn Ser Asn Asn Asn Asn Asn Asp Ser
70 80 90 100 110 120
GGC AAT AAC AAT CAA GGC GAT TAT GTA ACC AAA GCT GAG AAT ATG ATC GGC CAA GAT AGA
Gly Asn Asn Asn Gln Gly Asp Tyr Val Thr Lys Ala Glu Asn Met Ile Gly Gln Asp Arg
130 140 150 160 170 180
GTC AAT CAA TTC AAA AGC AAA ATC GGA GAG GAC AGA TTT GAT AAG ATG GAG TCC AAG GTT
Val Asn Gln Phe Lys Ser Lys Ile Gly Glu Asp Arg Phe Asp Lys Met Glu Ser Lys Val
190 200 210 220 230 240
CGT CAA CAA TTT TCT AAT ACC TCT ATA AAT GAC AAC GAC TCC AAC AAC AAC GAC TCA TAT
Arg Gln Gln Phe Ser Asn Thr Ser Ile Asn Asp Asn Asp Ser Asn Asn Asn Asp Ser Tyr
250 260 270 280 290 300
GGT TCT AAT AAC AAC GAT TCA TAT GGT TCT AAC AAC AAT GAT TCA TAT GGC TCT AAC AAC
Gly Ser Asn Asn Asn Asp Ser Tyr Gly Ser Asn Asn Asn Asp Ser Tyr Gly Ser Asn Asn
310 320 330 340 350 360
AAT GAT TCA TAT GGC TCC AAC AAC AAT GAT TCA TAT GGC TCT AAC AAC GAT GAT TCC TAC
Asn Asp Ser Tyr Gly Ser Asn Asn Asn Asp Ser Tyr Gly Ser Asn Asn Asp Asp Ser Tyr
370 380 390 400 410 420
GGT TCT TCC AAC AAG AAG AAG AGC TCT TAT GGT TCT AAC AAT GAC GAT TCG TAC GGC TCC
Gly Ser Ser Asn Lys Lys Lys Ser Ser Tyr Gly Ser Asn Asn Asp Asp Ser Tyr Gly Ser
430 440 450 460 470 480
AGC AAC AAC AAT GAC TCT TAC GGT TCC AAC AAC AAT GAC TCT TAC GGT TCC AAC AAC AAT
Ser Asn Asn Asn Asp Ser Tyr Gly Ser Asn Asn Asn Asp Ser Tyr Gly Ser Asn Asn Asn
490 500 510 520 530 540
GAC TCT TAC GGT TCC AAC AAT GAC GAC TCT TAC GGT TCG TCA AAC AAG AAT AAG AGC TCT
Asp Ser Tyr Gly Ser Asn Asn Asp Asp Ser Tyr Gly Ser Ser Asn Lys Asn Lys Ser Ser
550 560 570 580 590 600
TAC GGT TCC AAC AAT GAC GAT TCT TAT GGC TCT AAC AAT GAT GAT TCA TAT GGT TCT TCC
Tyr Gly Ser Asn Asn Asp Asp Ser Tyr Gly Ser Asn Asn Asp Asp Ser Tyr Gly Ser Ser
610 620 630 640 650 660
AAC AAG AAG AAG AGT TCT TAT GGT TCC AGC AAC AAC GAT TCG TAT GGT TCT AAC AAC GAT
Asn Lys Lys Lys Ser Ser Tyr Gly Ser Ser Asn Asn Asp Ser Tyr Gly Ser Asn Asn Asp
670 680 690 700 710 720
GAT TCA TAT GGT TCT AAC AAC AAT GAT TCA TAT GGC TCT AAC AAC GAT GAT TCC TAC GGT
Asp Ser Tyr Gly Ser Asn Asn Asn Asp Ser Tyr Gly Ser Asn Asn Asp Asp Ser Tyr Gly
730 740 750 760 770 780
TCT TCC AAC AAG AAG AAG AGC TCT TAT GGT TCT AAC AAT GAC GAT TCG TAC GGC TCC AGC
Ser Ser Asn Lys Lys Lys Ser Ser Tyr Gly Ser Asn Asn Asp Asp Ser Tyr Gly Ser Ser
790 800 810 820 830 840
AAC AAC AAT GAC TCT TAC GGT TCC AAC AAT GAC GAC TCT TAC GGT TCG TCA AAC AAG AAT
Asn Asn Asn Asp Ser Tyr Gly Ser Asn Asn Asp Asp Ser Tyr Gly Ser Ser Asn Lys Asn
850 860 870 880 890 900
AAG AGC TCT TAC GGT TCT TCT AGC AAC GAT GAT TCT TAC GGA TCT TCC AAT AAC GAC GAC
Lys Ser Ser Tyr Gly Ser Ser Ser Asn Asp Asp Ser Tyr Gly Ser Ser Asn Asn Asp Asp
910 920 930 940 950 960
TCT TAC GGT TCT TCC AAC AAG AAG AAG AGT TCT TAT GGT TCC AAC AAT GAC GAT TCT TAT
Ser Tyr Gly Ser Ser Asn Lys Lys Lys Ser Ser Tyr Gly Ser Asn Asn Asp Asp Ser Tyr
970 980 990 1000 1000 1020
GGC TCT AAC AAT GAT GAT TCA TAT GGT TCT TCC AAC AAG AAG AAG AGT TCT TAT GGT TCC
Gly Ser Asn Asn Asp Asp Ser Tyr Gly Ser Ser Asn Lys Lys Lys Ser Ser Tyr Gly Ser
1030 1040 1050 1060 1070 1080
AGC AAC AAC GAT TCG TAT GGT TCT AAC AAC GAT GAT TCC TAC GGT TCT TCT AAC AAA AAG
Ser Asn Asn Asp Ser Tyr Gly Ser Asn Asn Asp Asp Ser Tyr Gly Ser Ser Asn Lys Lys
1090 1100 1110 1120 1130 1140
AAG AGT TCT TAT GGT TCC AAC AAC GAT GAT TCA TAC GGC TCC AGC AAC AAC AAT GAC TCT
Lys Ser Ser Tyr Gly Ser Asn Asn Asp Asp Ser Tyr Gly Ser Ser Asn Asn Asp Ser
1150 1160 1170 1180 1190 1200
TAC GGT TCC AAC AAT GAC GAC TCT TAC GGT TCC TCT AAT AGA AAC AAG AAC TCC TAT GGG
Tyr Gly Ser Asn Asn Asp Asp Ser Tyr Gly Ser Ser Asn Arg Asn Lys Asn Ser Tyr Gly
1210 1220 1230 1240 1250 1260
TCT TCC AAC TAC GGT TCA TCC AAC AAT GAT GAC TCT TAT GGT TCA TCT AAT AGA GGC GGT
Ser Ser Asn Tyr Gly Ser Ser Asn Asn Asp Asp Ser Tyr Gly Ser Ser Asn Arg Gly Gly
1270 1280 1290 1300 1310 1320
CGT AAT CAA TAC GGT GGT GAC GAC GAT TAC TAA GTT TTT GGA TGT CTT CGA TAA AAA AAA
Arg Asn Gln Tyr Gly Gly Asp Asp Asp Tyr stop
1330 1340 1350 1360 1370 1380 1390
TATATTATTGTGTTTAGACTTAAGTATGAAAATTTTATGTATGAGCTGTGGCTATGTATCCGCTGGCAAA
1400 1410 1420 1430 1440 1450 1460
TAGGTCTGCTTTTTCTACACTTTCCACCCTCAACCTAACAGAGCCCGCTGGCACAAATAATCGATAGTAG
1470 1480 1490 1500 1510 1520 1530
GACAACAGAGCTACTCCTTCTTATGCCCCGCCCCTTTGAGCTTGTTGTATTGCTCTTGATAGTTGTGTTT
1540 1550 1560 1570 1580 1590 1600
TTCACTTTCATCAGCATCGGTAGTCTTGCCGTCCTTGTCTTGACTAGCCATTTTCTTAAAAGCGTCACTC
1610 1620 1630 1640 1650 1660 1670
ACTACCTTTGCATCGCCCTGCAATTTTTCCTTTGAGCTCTGAAATATTTCGCTCACTTTTTGTTGGTCTG
1680 1690 1700 1710 1720 1730 1740
TATTCATTCTGGATGTCTTGGTTGTAGAAATTTCTTTTATTGGTTCATTAAAGTCAAGGTAAATGGCGAG
1750 1760 1770 1780 1790 1800 1810
AACTAGAATAGAGTTTTATTCTTTTTACCGTTATATAGATAATTCTAGCCGGGGGCGGTCGCCCCTGAGA
1820 1830 1840 1850 1860 1870 1880
TTCCGACATCAGTAAGACATAGTACTGTACGATTACTGTACGATTAATCTATCCACTTCAGATGTTCAAC
1890 1900 1910 1920 1930 1940 1950
AATTCCTTTTGGCATTACGTATTAATACTTCATAGGATCGGCACCCTCCCTTAAGCCTCCCCTAAATGCT
1958
TTCGGTACC

Amino Acid Sequence	No. of Repeats
SER ASN ASN ASN ASP SER TYR GLY	9
SER SER ASN ASN ASN ASP SER TYR GLY	2
SER ASN ASN ASP ASP SER TYR GLY	12
SER SER ASN ASN ASP ASP SER TYR GLY	3
SER SER ASN LYS LYS LYS SER SER TYR GLY	6
SER SER ASN LYS ASN LYS SER SER TYR GLY	2
SER SER ASN ASN ASP SER TYR GLY	1
SER SER SER ASN ASP ASP SER TYR GLY	1
SER SER ASN ARG ASN LYS ASN SER TYR GLY	1

FIG. 3. Structure and frequence of repeated motifs within the predicted *DDR48* protein. The families of repeated sequences and their frequencies are indicated as determined from the DNA sequence analysis of the *DDR48* coding region.

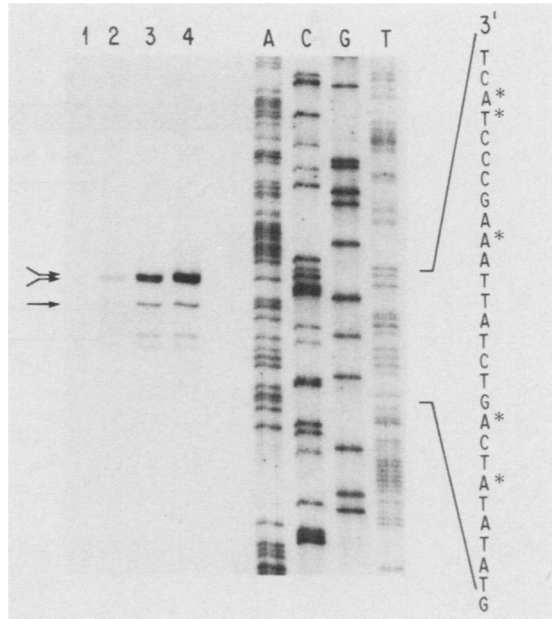


FIG. 4. Primer extension analysis of the start sites of *DDR48* transcription. Total RNA (25 μg) was isolated from control, 4NQO-treated, or heat shock-treated cells. The oligonucleotide primer (5'-GAGTCGTTGTCATTTATAGAGGTATTAGAAAATTG-3') was labeled at the 5' end with [γ-^{32}P]ATP and T4 polynucleotide kinase, extended with avian myeloblastosis virus reverse transcriptase, and the products were analyzed by electrophoresis in 6% polyacrylamide-8 M urea sequencing gels as described in Materials and Methods. The oligonucleotide primer was complementary to nucleotides 187 to 221 in the coding sequence (see Fig. 2). Lanes: 1, no RNA; 2, control RNA (25 μg); 3, RNA from 4NQO-treated cells (25 μg); 4, RNA from heat stock-treated cells (25 μg). The DNA sequencing lanes are on the right. The DNA sequence read from the sequencing gel is complementary to the sequence displayed in Fig. 2. The asterisks indicate the positions of the major extension products.

−488 to −476 (10 of 13 nucleotide matches). RAP1 protein has been shown to be a transcriptional activator for the MATα locus as well as for several genes involved in translation and glycolysis (5, 29). The role of these sequences in the regulation of *DDR48* transcription is unknown at the present time.

A considerable amount of 3' flanking region was also sequenced (667 bp). The *S. cerevisiae* sequence TAAAT AAA/G, which has been observed approximately 28 to 33 bp upstream of many yeast mRNA 3' termini (3), is not found in the *DDR48* sequence. However, there are several possible occurrences of the tripartate sequence TAG...TAGT or TATGT...(AT rich)...TTT which Zaret and Sherman (37) suggest has served as a transcription termination and polyadenylation signal.

Determination of the transcriptional start sites of *DDR48*. The transcriptional start site was identified by using primer extension by avian myelobastosis virus reverse transcriptase and RNA isolated from control, 4NQO-treated, and heat shock-treated cells (Fig. 4). Three major and two minor start sites were identified at positions −55, −54, −48, −39, and −36 relative to the ATG initiation codon for ORF2. The primer extension data suggested that the same start sites were used in damaged and undamaged cells and with the same relative frequency. Moreover, the same extension products were obtained by using RNA from yeast cells that had been subjected to brief heat shock to stimulate *DDR48* transcription. The possibility that one or more of these start sites resulted from premature termination by reverse transcriptase because of the secondary structure in the RNA was investigated by performing the extension reaction at two different temperatures (42 and 45°C). The pattern of extension products and their relative abundance were unchanged, suggesting that these products represent authentic transcription start sites (data not shown).

Disruption of the *DDR48* gene. To determine the biological role of the *DDR48* gene, a disrupted copy of this gene was prepared by inserting the selectable *LEU2* gene into the *DDR48* coding sequence (Fig. 5A). The genomic DNAs from two of the diploid LEU$^+$ transformants were analyzed by Southern hybridization (Fig. 5B). In parental strain T87, digestion of genomic DNA with *Hin*dIII and *Bam*HI produced a unique 3.8-kb fragment that hybridized with a 623-bp *DDR48* probe derived from the 5' end of the gene (Fig. 5B, lane 3). However, in digests of two LEU$^+$ diploid transformants, JTD-100 and JTD-101, two restriction fragments hybridized to the *DDR48* probe corresponding in size to 3.8 and 4.2 kb (lanes 1 and 2), the latter being the expected size of the disrupted *DDR48* allele.

The LEU2$^+$ diploid transformant JTD-100 was sporulated in potassium acetate medium, and the asci produced were subjected to tetrad analysis. In two experiments, 39 tetrads were dissected but only 28% of the spores were viable. However, upon further analysis, it was determined that both *LEU*$^+$ and *leu* mutant haploid strains were recovered at approximately equal frequency (57% *LEU*$^+$, 43% *leu* mu-

FIG. 2. DNA sequence analysis of the *DDR48* gene. The DNA sequence of the *DDR48* gene was determined by using the sequencing strategy shown in Fig. 1A. The two initiation codons which define the two long ORFs are located at +1 and +209 in the sequence. The transcriptional start sites are indicated by arrows above the sequence. Possible TATA boxes are underlined, and a single sequence resembling a heat shock element (17) is indicated with thick line. Sequences found upstream of *DDR48* and *DDRA2* are indicated with asterisks (N. Kobayashi, unpublished results). A possible RAP1 binding site is indicated (○).

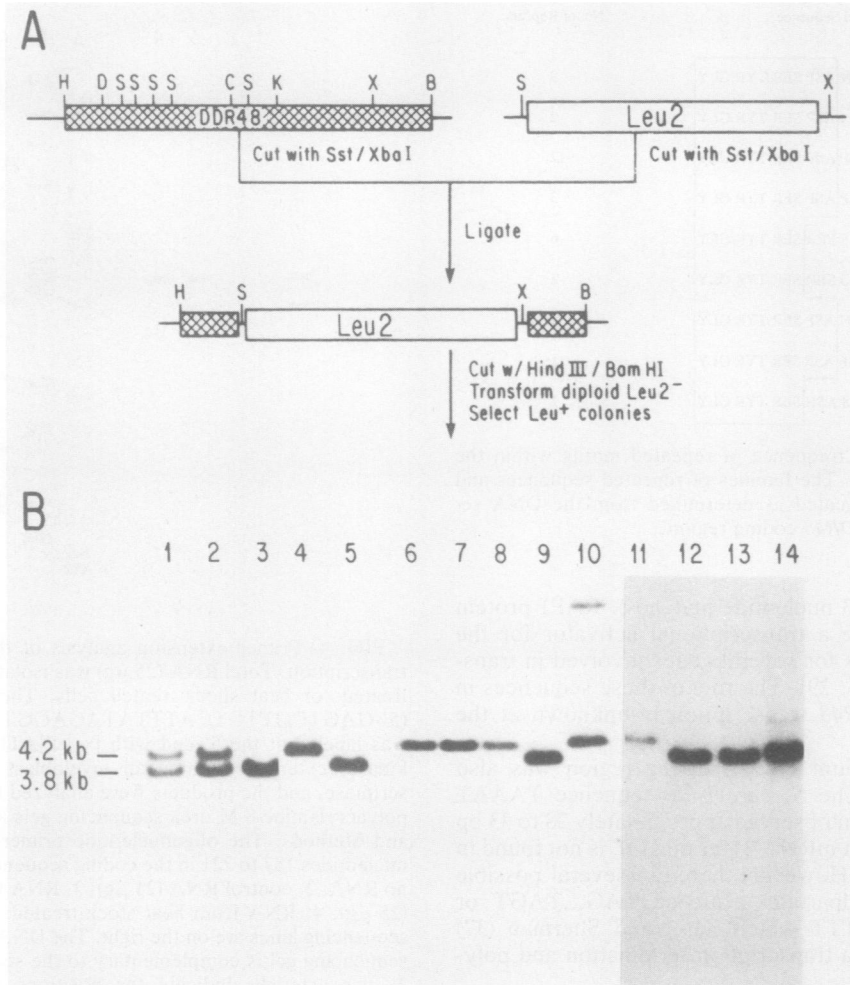


FIG. 5. Disruption of the *DDR48* gene of *S. cerevisiae*. (A) The *LEU2* gene, cloned on a *Bgl*II fragment and inserted at the *Bam*HI restriction site within the polylinker region of plasmid pUC19, was excised on an *Sst*I-*Xba*I fragment and ligated into *Sst*I-*Xba*I-cut plasmid pBR48L3-2 containing the *DDR48* gene. The resulting *ddr48*:*LEU2* region was excised by digestion with *Hin*dIII and *Bam*HI, and the DNA was used to transform diploid strain T87 to *LEU2*$^+$. (B) Southern hybridization analysis of *Hin*dIII- and *Bam*HI-digested genomic DNAs from heterozygous diploid transformants, *leu2* and *LEU2*$^+$ haploid cells. DNA was analyzed from two *LEU2*$^+$ diploid transformants (JTD-100 and JTD-101, lanes 1 and 2, respectively), parental *leu2* diploid T87 (lane 3), *LEU*$^+$ haploid strains JT-26B, JT-212A, and JT-214A (lanes 6, 7, and 8, respectively); and *leu2* haploid strains JT-26A, JT-221A, and JT-223A (lanes 12, 13, and 14, respectively). Lanes 4, 10, 11, Marker DNA contained the *ddr48*:*LEU2* disruption (lanes 4, 10, and 11) or the wild-type *DDR48* region (lanes 5 and 9). The disrupted allele migrated as a 4.2-kb *Hin*dIII-*Bam*HI fragment, whereas the wild-type allele migrated as a 3.8-kb fragment.

tant). Because of the low spore viability in this experiment, a *DDR48* heterozygote was constructed by mating two of the haploid progeny derived from the initial tetrad analysis. This diploid, JTD-110, was sporulated, and tetrads were analyzed. In this experiment, 26 tetrads were dissected and viability was 89%, which was equivalent to the observed spore viability of 93% obtained when the parental strain, T87, was dissected and analyzed. Approximately half (52%) of the spores were *LEU*$^+$ when tested.

Genomic DNA was isolated from three *LEU*$^+$ and three *leu* mutant haploids, digested with *Hin*dIII and *Bam*HI, and analyzed by Southern hybridization by using the 0.62-kb *Hin*dIII-*Sst*I *DDR48* fragment as probe (Fig. 5B). As expected, all three *LEU*$^+$ transformants contained a 4.2-kb restriction fragment which migrated at the same position as the *ddr48*:*LEU2* disruption marker. All three *leu* mutant haploids contained a 3.8-kb fragment that was identical to that found in the parental T87 diploid. These results demonstrated that the *DDR48* gene was not essential for the viability of *S. cerevisiae*. Moreover, the low viability measured in the first tetrad dissection experiment was apparently not due to the presence of the *ddr48*:*LEU2*-disrupted allele since a heterozygote made by mating *LEU*$^+$ and *leu*$^-$ haploid progeny showed high spore viability in a subsequent sporulation.

Survival of *DDR* gene disruption strains after DNA damage and heat shock stress. To determine whether *DDR48* functions during the cellular responses to different stresses, we examined the survival of haploid strains carrying the *DDR48* disruption after exposure to the DNA-damaging agent 4NQO and after heat shock.

Survival of haploid strains JT-26A (*DDR48*) and JT-26B (*ddr48*) after exposure to a DNA-damaging agent was measured by using different concentrations of 4NQO. The results of this experiment (Fig. 6) demonstrated that, at low 4NQO concentrations, there was little difference in survival between the wild-type and mutant strains and, at the highest concentration of 4NQO used (2 μg/ml), the strain containing

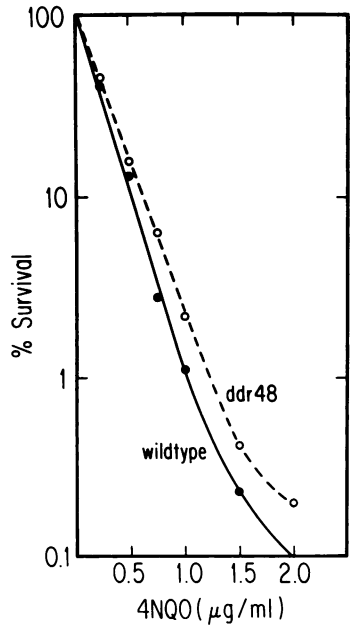


FIG. 6. Survival of a haploid yeast strain containing a disrupted *DDR48* gene. Strain JT-26B (*ddr48:LEU2*) (○) and isogenic *DDR48* strain JT-26A (●) were exposed to the indicated concentration of 4NQO for 60 min, and cell survival was measured by spreading dilutions of cells onto SDC plates. Surviving cells were counted after 3 days of incubation at 30°C: The data shown are the averages of three experiments. Identical results were obtained when cells were spread onto YPD plates (data not shown).

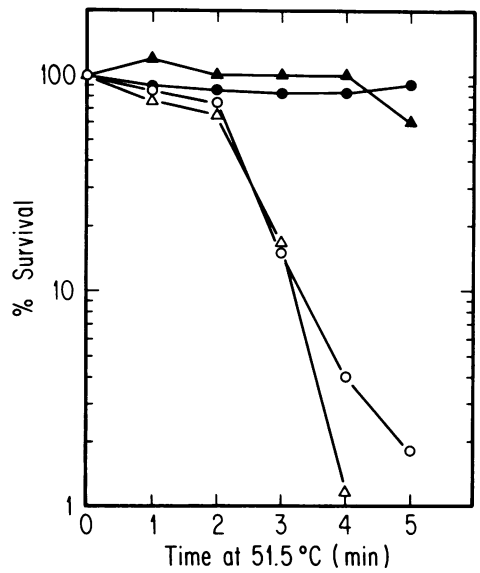


FIG. 7. Effects of the *DDR48* gene on heat shock killing and thermotolerance. Strains JT-26A (*DDR48*) (circles) and JT-26B (*ddr48:LEU2*) (triangles) were grown to early exponential phase in YPD medium at 27°C, and each culture was divided into two portions. One portion of each strain was exposed to 37°C for 2 h and incubated at 51.5°C for the indicated times (●, ▲). The second portion was incubated for an additional 2 h at 27°C and then shifted to 51.5°C (○, △). At the times indicated, samples were removed and diluted into 0.9% NaCl, and dilutions were spread on YPD plates to measure surviving cells.

the *ddr48* mutant allele showed a slightly greater survival than the nondisrupted strain. This increased survival of the *ddr48* mutant strain was observed in more than three independent experiments and when using two different pairs of strains (data not shown). Moreover, the *ddr48* mutant strain was also slightly more resistant to killing by UV irradiation and exposure to methyl methanesulfonate (data not shown).

The responses of the same haploid strains, JT-26A and JT-26B, to thermal stress was examined (i) after an acute heat shock, i.e., growth at 27°C followed by incubation at 51.5°C, and (ii) after conditions that produce thermotolerance in yeast, i.e., growth at 27°C, incubation at 37°C for 2 h, and then exposure to 51.5°C. The results of these survival and thermotolerance experiments are shown in Fig. 7. An acute heat shock of either strain JT-26A or JT-26B resulted in a relatively rapid exponential decrease in survival within the first 5 min of exposure to 51.5°C. We have routinely observed slightly greater killing of strain JT-26B (*ddr48:LEU2*) than strain JT-26A, but the magnitude of the effect was variable and only evident at relatively long exposure. Importantly, the *ddr48* disruption did not prevent the development of thermotolerance in strain JT-26B. Preincubation at 37°C resulted in a dramatic increase in cell survival at 51.5°C for both the JT-26A and JT-26B haploid strains. These results suggest that the *DDR48* gene is dispensable for acquisition of thermotolerance in *S. cerevisiae* and offer evidence that this gene does not play a significant role in recovery or protection of cells from acute heat shock.

Involvement of *DDR48* in spontaneous mutagenesis. To investigate the role of the *DDR48* gene in mutagenesis, we examined induced mutation frequencies at three different loci, *HIS4*, *ADE2*, and *CAN1* (35). Both forward mutations to canavanine resistance (Can^r) and reversion to histidine or adenine prototrophy were examined in wild-type (JT-22A and JT-26A) and *ddr48* mutant (JT-26B) strains. Cultures were exposed to increasing concentrations of the mutagens 4NQO or MNNG, and the mutation frequencies were determined as described in Materials and Methods (Table 2). Generally, there was a slight reduction in the induced mutation frequency in the *ddr48* mutant strains at all three loci examined after treatment with either 4NQO or MNNG.

We consistently observed more substantial effects of the *ddr48* mutation on the spontaneous mutation frequencies of these loci. The *ddr48* mutation resulted in an approximately 10-fold reduction in the spontaneous mutation frequency at the *HIS4* locus and a somewhat lower but reproducible 2- to 3-fold decrease at the *CAN1* and *ADE2* loci (Table 3). To investigate the effects of the *ddr48* mutation on spontaneous mutagenesis, a Luria-Delbrück fluctuation analysis was carried out to measure the actual mutation rates of the *HIS4* and *CAN1* loci in both *DDR48* and *ddr48* disruption strains. The mutation rates, calculated by using the method of the mean (18), are shown in Table 4. There was a 6- to 14-fold reduction in the mutation rate of the *his4-713* allele and a 3- to 4-fold reduction in the mutation rate of Can^r mutations in strains containing the *ddr48* gene disruption. Together these results implicate the *DDR48* gene in one or possibly more pathways of mutagenesis in *S. cerevisiae*.

DISCUSSION

The results presented in this paper define structural features of the *DDR48* gene, its regulatory region, and the protein it encodes and provide evidence for the function of this gene in mutagenesis. The predicted amino acid sequence of the protein encoded by this stress-responsive gene was quite unusual and consisted of multiple copies of a family of simple repeated sequences (Fig. 3). As a result, the protein is

TABLE 2. Induced mutation frequency in *DDR48* and *ddr48* strains

Treatment (concn)	His$^+$ revertants/10^6 survivors[a]			ADE$^+$ revertants/10^7 survivors[b]		
	DDR48	*ddr48*	Ratio	*DDR48*	*ddr48*	Ratio
1) 4NQO (μg/ml)						
0.25	34	21	1.6			
0.50	74	70	1.1			
1.0	303	84	3.6			
2.0	697	202	3.4			
2) MNNG (μg/ml)						
0.50				2.3	1.5	1.5
1.0				5.0	2.9	1.7
2.0				5.4	3.4	1.6
4.0				7.8	5.3	1.5
8.0				9.6	7.7	1.2

[a] The values represent the average of three experiments with strains JT-26A and JT-26B.
[b] The values represent the average of two experiments with strains JT-22A and JT-26B.

unusually rich in Ser, Asn, Asp, Tyr, and Gly residues. It is interesting to note that the sequence Asn-X-Ser, which is present more than 14 times in the *DDR48* protein sequence, is frequently used as a signal for glycosylation in eucaryotic proteins (16). There is no evidence at the present time that would indicate whether the *DDR48* protein is glycosylated in yeast cells.

The 750-bp sequence upstream of the start of translation contained several potentially important regulatory elements. A sequence resembling a binding site for the RAP1 (GRFI) transcription factor was found at approximately −480. A single consensus heat shock element was also located near −270. A T-rich region near position −440 is similar to a sequence found in the upstream region of another dually regulated gene, *DDRA2*. Deletion of this element in *DDRA2* abolishes expression of this gene (Kobayashi et al., unpublished data). A second sequence element shared by *DDR48* and *DDRA2* is located near −150 in the *DDR48* upstream region. This element, relatively GC-rich compared to surrounding sequences, has also been shown to be necessary for proper regulation of the *DDRA2* gene (Kobayashi et al., unpublished data).

An intact *DDR48* gene was shown to be nonessential on the basis of the removal of more than 70% of the protein coding region in haploid cells without loss of viability. We recently mated two haploid strains, each containing the *ddr48:LEU2* allele but of opposite mating type, in order to produce the homozygous *ddr48* diploid. Upon incubation in sporulation medium, this strain gave rise to four-spored asci at high efficiency. These results demonstrated that the *DDR48* gene was not required for mating or sporulation of yeast (J. Treger, unpublished data).

During the investigation of the phenotype of a haploid strain containing a *ddr48:LEU2* disruption gene, the responses to both DNA damage and thermal stress provided evidence that this gene did not contribute to the protection and recovery of cells from either of these environmental insults. However, we observed that the spontaneous mutation rate was greatly reduced in the *ddr48:LEU2* gene disruption strain compared with that of the wild-type strain. This antimutator effect of the *ddr48* mutant allele ranged from a 6- to 14 fold for the *his4-713* allele to approximately 3- to 4-fold for Can^r mutations. Interestingly, the antimutator effect of the mutant *ddr48* allele on either 4NQO- or MNNG-induced reversion of the *his4-713* and *ade2-101* alleles or on forward mutations at the *CAN1* locus were notably lower, ranging from 1.2- to 3.6-fold.

Although an allele-specific reduction in spontaneous mutation rate has been observed for other previously isolated antimutator mutations such as *ant1* (24), *rev3* (24) and *pso1* (7), which reduce the spontaneous mutation rate 2- to 12-fold depending on the allele examined, the molecular basis for this variation is not known. However, the variation may relate in some way to the type of base alteration needed for restoration of function or for production of drug resistance.

The *his4-713* mutation is a +1C addition to a proline codon of *HIS4* that was induced by the frameshift mutagen ICR-170 (8). Although the molecular nature of the HIS$^+$ revertants is not known, these mutations may be deletions of the extra G · C base pair rather than extragenic suppressor mutations. The reversion of the *his4-713* mutation appeared to be specific for 4NQO in that UV irradiation, methyl methanesulfonate, and MNNG did not increase the reversion frequency above spontaneous levels (data not shown). This result is consistent with the action of 4NQO at G · C base pairs (23).

In contrast, a greater variety of base alterations likely results in the inactivation of the arginine permease which is encoded by the *CAN1* locus (35). Indeed, the 4NQO-induced mutation frequency at the *CAN1* locus was significantly higher than the observed induced mutation frequency at the *HIS4* locus. It may be that the *DDR48* gene affects only a specific pathway, leading to spontaneous mutations in yeast

TABLE 3. Spontaneous mutation frequency in *DDR48* and *ddr48* strains

Strain	His$^+$/10^6 survivors	Can^r/10^6 survivors	ADE$^+$/10^7 survivors
JT-22A (*DDR48*)	2.2	2.8	0.92
JT-26A (*DDR48*)	2.7	ND[a]	ND
JT-26B (*ddr48*)	0.24	1.3	0.26

[a] ND, Not done.

TABLE 4. Mutation rate[a] in *DDR48* and *ddr48* strains

Expt	Mutation rate of *his4-713* → His$^+$ of strains *DDR48* and *ddr48* (ratio)	Mutation rate of Can^s → Can^r of strains *DDR48* and *ddr48* (ratio)
1	$2.7 \times 10^{-7}/1.9 \times 10^{-8}$ (14)	$3.4 \times 10^{-7}/1.0 \times 10^{-7}$ (3.4)
2	$4.4 \times 10^{-7}/7.4 \times 10^{-8}$ (6)	$4.1 \times 10^{-7}/1.4 \times 10^{-7}$ (2.9)

[a] The values indicated are given as HIS$^+$ mutations per cell per generation or Can^r mutations per cell generation, and the mutation rates were determined in two independent fluctuation tests with strains JT-22A and JT-26B.

cells, and that the reversion of the *his4-713* allele makes use of this pathway. Conversely, only a small subset of *CAN1* forward mutations to Can^r may utilize this particular pathway, thereby accounting for the smaller effect of the *ddr48* mutation on mutagenesis at the *CAN1* locus. Although the mutation rate at the *ADE2* locus was not measured, the spontaneous mutation frequency is reduced only about 3.5-fold in the *ddr48* mutant strain. This result also argues that the *DDR48* gene product participates in only a subset of all the mutation fixing or recovery pathways present in wild-type yeast cells.

Although mutations in a number of genes result in increased spontaneous mutation rates or reduced mutation frequencies after UV irradiation or chemical treatments (11), only a few mutations have been isolated which specifically reduce spontaneous mutation rates. The phenotype of the *ddr48* mutation is distinct from any of these previously described antimutator loci. The *ant1*, *rev3*, and *pso1* mutations confer slight to moderate sensitivity to killing by UV irradiation or chemical agents. The *ddr48* mutation has no associated sensitivity to killing by 4NQO. Furthermore, the *ddr48* mutation did not render the cells sensitive to UV irradiation, MNNG, or methyl methanesulfonate treatments (J. Treger, unpublished results). The *pso1* and *rev3* mutations also significantly reduce induced mutation frequencies. Although some locus-dependent variation was observed, the *ddr48* mutation appeared to have only small effects on 4NQO- and MNNG-induced mutagenesis at the loci examined.

There are at least two possible explanations for an antimutator phenotype with no associated DNA repair defect as seen with the *ddr48* disruption mutant. Possibly, the disrupted *DDR48* allele produces a partly functional polypeptide which participates in DNA repair but is unable to function in mutagenesis. The insertion of the *LEU2* fragment in *DDR48* after codon 130 would leave the amino-terminal coding portion of the gene intact. Alternatively, the *DDR48* protein might function in an error-prone repair pathway that competes with an efficient error-free process. The loss of the error-prone pathway as a result of *DDR48* inactivation would reduce mutagenesis without an accompanying DNA repair defect. Experiments are in progress to more precisely define the function of the *DDR48* gene and to investigate its role in other responses of yeast cells to DNA damage stress.

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