MOLECULAR AND CELLULAR BIOLOGY, Oct. 1990, p. 5553–5557
0270-7306/90/105553-05$02.00/0

Vol. 10, No. 10

The DNA Damage-Inducible Gene *DIN1* of *Saccharomyces cerevisiae* Encodes a Regulatory Subunit of Ribonucleotide Reductase and Is Identical to *RNR3*

KEVIN YAGLE AND KEVIN McENTEE*

Laboratory of Biomedical and Environmental Sciences and Department of Biological Chemistry, UCLA School of Medicine, University of California, 900 Veteran Avenue, Los Angeles, California 90024-1786

Received 23 April 1990/Accepted 2 July 1990

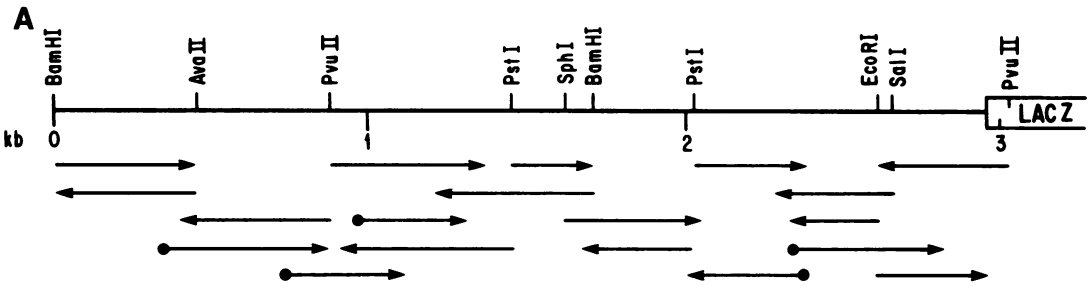
The sequence of the *DIN1* gene of *Saccharomyces cerevisiae* is identical to *RNR3*, a gene encoding a DNA damage-inducible regulatory subunit of ribonucleotide reductase. Two sequence elements located upstream of *DIN1*(*RNR3*) are homologous to putative DNA damage regulatory elements in the promoter of the reductase catalytic subunit gene, *RNR2*. The transcript start sites for *DIN1*(*RNR3*) have been localized, and induction by different agents has been compared with other DNA damage-regulated genes.

The *DIN1* gene of *Saccharomyces cerevisiae* was originally isolated from a pool of *lacZ* gene fusions and was identified on the basis of its increased expression of β-galactosidase in cells treated with different DNA-damaging agents, including UV irradiation, 4-nitroquinoline-1-oxide (4NQO), and methyl methanesulfonate (MMS) (10, 11). Unlike the DNA damage-responsive (*DDR*) genes *DDRA2*, *DDR48*, and *UBI4*, the *DIN1* gene is not induced by heat shock stress (9), a result which suggests that the stress regulation of *DIN1* might involve different control elements. A preliminary physical characterization of the yeast cell DNA contained on the *DIN1-lacZ* expression plasmid pSZ214 demonstrated that the regulatory and coding regions of the gene were localized to approximately 3 kilobase pairs of DNA upstream of the *lacZ* fusion junction (Fig. 1A). The complete nucleotide sequence of this region was determined for both strands by the dideoxynucleotide chain terminator method (12). Appropriate restriction fragments were subcloned into single-stranded vectors M13mp18 and M13mp19, and sequencing was performed by using both universal primers as well as additional synthetic oligonucleotides (Fig. 1B). A comparison of the *DIN1* sequence with those contained in the GenBank database indicated that this gene was homologous to the cDNA sequence of the large subunit of murine ribonucleotide reductase (M1) (1). A single, long open reading frame extended from nucleotide position 703 to the *lacZ* junction, and the predicted DIN1 protein sequence was 63% identical to or, allowing for conservative substitutions, 78% similar to the mouse reductase M1 subunit. The alignment of the murine M1 subunit sequence with the first 755 amino acids of the predicted DIN1 protein are shown in Fig. 2. Among the conserved residues are two cysteines that have been implicated in the catalytic reduction of ribonucleotides (1) and correspond to residues 408 and 426 in the DIN1 sequence. Although this sequence is not the complete coding region of *DIN1*, it is likely that it represents more than 94% of the full-length M1 protein on the basis of a comparison with the murine reductase.

While this work was in progress, Elledge and Davis reported the isolation and characterization of two yeast genes, *RNR1* and *RNR3*, encoding alternate large subunits of ribonucleotide reductase (6). These genes were isolated on the basis of their homology with a murine cDNA probe encoding the large regulatory subunit. We have compared the *DIN1* gene with both the *RNR1* and *RNR3* genes and concluded that *DIN1* is identical to *RNR3*. This conclusion is based on three pieces of evidence. (i) The induction of *DIN1* transcript after DNA-damaging treatment is quantitatively similar to that reported for *RNR3* (>100-fold) rather than that reported for *RNR1* (3- to 5-fold). (ii) The restriction map of *DIN1* closely resembled that of the *RNR3* gene (6). (iii) The predicted amino acid sequence of the first 100 residues of the DIN1 reductase subunit matched a corresponding region of RNR3 at 94 of these positions (Fig. 3). (The six differences may be due to sequencing errors or to strain variations.) These results demonstrate that the *DIN1* and *RNR3* genes of yeast cells are identical, and for the remainder of this paper the gene will be designated *DIN1*(*RNR3*).

The transcript start sites were determined for the chromosomal *DIN1*(*RNR3*) gene by primer extension with avian myeloblastosis virus reverse transcriptase (15). Initially, an oligonucleotide primer (primer A) corresponding to the sequence between positions 721 and 737 shown in Fig. 1B was used. This 17-mer corresponded to the amino terminus of the *DIN1*(*RNR3*) coding region. The results of these experiments (Fig. 4, primer A) indicate the presence of seven extension products with RNA from cells that had been treated with MMS or 4NQO. Interestingly, the two most abundant extension products were also present in experiments with RNA from untreated cells. This result was unexpected, since Northern (RNA) hybridization studies indicated that the *DIN1*(*RNR3*) gene was expressed at extremely low levels in untreated cells (see Fig. 5). A second experiment with an oligonucleotide (primer B) complementary to the sequence between positions 692 and 709 containing only seven nucleotides of coding sequence was performed. The products obtained with this 18-mer are shown in Fig. 4 (primer B), and, in this case, none of the seven major extension products was detected in RNA from unstressed cells, but these RNAs were readily detected in RNA pre-

* Corresponding author.



B

```
   1 GGATCCAGAAGGAAACACTCAAGGGTGTTGTCGTCGCTGGAGGCGTACTAGCCGGCGCTGTGGCCGTGGCTAGTTTCTTCTTAAGAAACAAGAGAAGGTAACAAGCACATAAAAAATCAG

 121 CACATACGTACATACATAAGAATGAATCGCACGCACGCGTAAACATTTATCATTTAATCTTCAGTTGTTAGATAAAAAAAAAAAGAAAAGAAAAGAAAGTGAAGGCTTGTTTCAGTTTGA

 241 ACTAGGTAGCAGAGCAAGCCCTCGTTCTTGGCTGCTAATTTTCCTAAAGTAGTAAAAAAAGCCAAGTTATCTGCCTACGGTTGTCACAGCAACATTGCGTGCCGTTGTTCTTTTGTTTTT

 361 TTTTTTTTTTTTTTTTTCGTGGTTGTCGCAGCAACGACACCTAGGCGCTGCTCAAAGGGGCAAAAACCCGGTTGCCATGGCGAGGACCAAACGACAAGATGGGAAAAAAACAATAGTCTA

 481 TTGTTAAATCGTAATACTGTATTGTGAGATGCTGACGCGTTTCGTTTTTCGTGTCAGACGTTCTTTATATTGTTTCGTGTTCTGCTGCAAAACGTATATAAACGCACTGCTATTTTGCCT

 601 TCTTTTGCCTTCTTCCTTGCCTTTCTCTCATCTCATATCCAAGTTGAAATAAATATGACAAGCAAGAATAGCAGCAGCAATAAATCAAATACTCCCACACAAATGTACGTTATTAAAAGA
                                                                                                                  M  Y  V  I  K  R

 721 GACGGCCGCAAAGAGCCCGTTCAATTCGATAAAATTACCTCCCGTATCACCCGTTTGTCATACGGTTTAGACCCAAACCGTATTGATGCTGTTAAGGTAACCCAACGTATTATTTCTGGT
       D  G  R  K  E  P  V  Q  F  D  K  I  T  S  R  I  T  R  L  S  Y  G  L  D  P  N  R  I  D  A  V  K  V  T  Q  R  I  I  S  G

 841 GTGTACTCCGGTGTTACTACCGTTGAGCTGGACAATCTTGCAGCTGAAACATGTGCATACATGACCACTGTGCACCCTGATTATGCCACTCTAGCCGCTAGAATCGCCATCTCTAACTTA
       V  Y  S  G  V  T  T  V  E  L  D  N  L  A  A  E  T  C  A  Y  M  T  T  V  H  P  D  Y  A  T  L  A  A  R  I  A  I  S  N  L

 961 CATAAGCAAACCACAAAGCAATTCTCCAAAGTTATTGAGGATTTACACGACTGGATTAACCCAGCTACTGGAAAGCATGCTCCTATGATTTCGGACGAAATTTACAACATTGTCATGGAA
       H  K  Q  T  T  K  Q  F  S  K  V  I  E  D  L  H  D  W  I  N  P  A  T  G  K  H  A  P  M  I  S  D  E  I  Y  N  I  V  M  E

1081 ACAAGATACTTGAACTCGGCCATCGTGTACGATAGGGATTTCCAGTATACGTATTTCGGATTCAAGACACTGGAGCGTTCGTACTTGCTAAGACTGAACGGTGAAGTGGCAGAACGTCCT
       T  R  Y  L  N  S  A  I  V  Y  D  R  D  F  Q  Y  T  Y  F  G  F  K  T  L  E  R  S  Y  L  L  R  L  N  G  E  V  A  E  R  P

1201 CAGCATTTGGTAATGCGTGTGGCGCTAGGTATCCATGGTAGCGATATCGAATCTGTGCTGAAGACTTATAATTTGATGTCGTTAAGATACTTCACTCACGCTTCCCCAACTTTATTCAAC
       Q  H  L  V  M  R  V  A  L  G  I  H  G  S  D  I  E  S  V  L  K  T  Y  N  L  M  S  L  R  Y  F  T  H  A  S  P  T  L  F  N

1321 GCTGGTACGCCACTTCCTCAAATGTCTTCATGTTTCTTAATTGCCATGAAGGATGACTCTATCGAAGGTATTTATGATACTTTGAAAGAATGTGCTATGATTTCCAAAACTGCAGGTGTG
       A  G  T  P  L  P  Q  M  S  S  C  F  L  I  A  M  K  D  D  S  I  E  G  I  Y  D  T  L  K  E  C  A  M  I  S  K  T  A  G  V

1441 TTGTCTCATATCAACAACATCCGTTCCACAGGTTCTTATATCGCTGGTACCAACGGTACTTCAAACGGGTTGATTCCTATGATTCGTGTTTTCAATAATACTGCCCGTTATGTGACCAGG
       L  S  H  I  N  N  I  R  S  T  G  S  Y  I  A  G  T  N  G  T  S  N  G  L  I  P  M  I  R  V  F  N  N  T  A  R  Y  V  T  R

1561 GTGGTAACAAGAGACCTGGTGCTTTCGCCCTTTTCTTGGAGCCATGGCATGCAGATATCTTCGACAAAGTTCGATATCAGAAAAACACATGGGAAGGAAGAAATTCGTGCAAGAGATTTG
       V  V  T  R  D  L  V  L  S  P  F  S  W  S  H  G  M  Q  I  S  S  T  K  F  D  I  R  K  T  H  G  K  E  E  I  R  A  R  D  L

1681 TTCCCTGCTCTATGGATCCCTGATCTTTTCATGAAACGTGTTCAAGAGGATGGGCCTTGGACTTTGTTTTCGCCCAGTGCTGCCCCAGGTTTAGATGATGTGTGGGGTGATGAATTTGAA
       F  P  A  L  W  I  P  D  L  F  M  K  R  V  Q  E  D  G  P  W  T  L  F  S  P  S  A  A  P  G  L  D  D  V  W  G  D  E  F  E

1801 GAACTATATACTCGTTACGAAAGAGAAGGTCGTGGTAAAACAATTAAAGCCCAAAAGTTGTGGTATGCCATTTTGCAAGCACAGACAGAAACAGGTACACCTTTCATGGTTTATAAGGAC
       E  L  Y  T  R  Y  E  R  E  G  R  G  K  T  I  K  A  Q  K  L  W  Y  A  I  L  Q  A  Q  T  E  T  G  T  P  F  M  V  Y  K  D

1921 GCATGTAACAGGAAGACAAACCAACAGAACTTAGGTACTATCAAATCATCTAATTTATGTTGTGAAATCGTCGAATATTCCTCCCCGGATGAAACTGCAGTTTGTAATTTAGCTTCTATT
       A  C  N  R  K  T  N  Q  Q  N  L  G  T  I  K  S  S  N  L  C  C  E  I  V  E  Y  S  S  P  D  E  T  A  V  C  N  L  A  S  I

2041 GCCCTACCAGCATTCGTTGAGGTTTCAGAAGATGGTAAAACTGCAAGCTATAATTTCGAGAGATTACACGAGATTGCTAAAGTCATTACTCACAACTTGAACAGAGTTATCGACCGTAAT
       A  L  P  A  F  V  E  V  S  E  D  G  K  T  A  S  Y  N  F  E  R  L  H  E  I  A  K  V  I  T  H  N  L  N  R  V  I  D  R  N

2161 TACTATCCAGTTCCCGAGGCTAGAAATTCAAATATGAAGCATAGACCTATTGCTCTTGGTGTCCAGGGTTTGGCCGATACTTATATGATGTTGCGTCTACCCTTTGAATCGGAAGAAGCT
       Y  Y  P  V  P  E  A  R  N  S  N  M  K  H  R  P  I  A  L  G  V  Q  G  L  A  D  T  Y  M  M  L  R  L  P  F  E  S  E  E  A

2281 CAAACTCTAAACAAACAAATCTTCGAAACTATTTACCATGCTACTCTTGAAGCCTCCTGTGAATTGGCCCAAAAAGAAGGTAAATATTCTACTTTTGAAGGTTCTCCAGCTTCTAAGGGT
       Q  T  L  N  K  Q  I  F  E  T  I  Y  H  A  T  L  E  A  S  C  E  L  A  Q  K  E  G  K  Y  S  T  F  E  G  S  P  A  S  K  G

2401 ATTTTACAATTCGATATGTGGAACGCTAAACCATTTGGCATGTGGGATTGGGAAACCTTAAGAAAGGACATTGTTAAACATGGGTTAAGAAACTCTTTGACTATGGCACCAATGCCAACC
       I  L  Q  F  D  M  W  N  A  K  P  F  G  M  W  D  W  E  T  L  R  K  D  I  V  K  H  G  L  R  N  S  L  T  M  A  P  M  P  T

2521 GCCTCAACTTCCCAAATTCTTGGTTATAATGAATGCTTCGAACCAGTGACCTCAAACATGTACTCTCGTCGTGTCCTGTCTGGTGAATTCCAAGTTGTTAATCCATATTTACTACGTGAT
       A  S  T  S  Q  I  L  G  Y  N  E  C  F  E  P  V  T  S  N  M  Y  S  R  R  V  L  S  G  E  F  Q  V  V  N  P  Y  L  L  R  D

2641 TTAGTCGACCTGGGTATTTGGGATGATAGTATGAAACAATATCTAATTACACAAAATGGTTCTATTCAAGGCTTACCAAATGTGCCACAAGAATTGAAGGAATTATACAAAACCGTCTGG
       L  V  D  L  G  I  W  D  D  S  M  K  Q  Y  L  I  T  Q  N  G  S  I  Q  G  L  P  N  V  P  Q  E  L  K  E  L  Y  K  T  V  W

2761 GAAATCTCTCAAAAGACCATTATCAATATGGCTGCTGATCGTGCCATCTACATCGATCAGTCTCATTCCTTGAATCTTTTCTTGCAAGCACCATCAATGGGTAAGATTACTAGTATGCAT
       E  I  S  Q  K  T  I  I  N  M  A  A  D  R  A  I  Y  I  D  Q  S  H  S  L  N  L  F  L  Q  A  P  S  M  G  K  I  T  S  M  H

2881 TTCTACGGTTGGAAGAAGGGTTTAAAAACTGGTATGTACTACTTAAGAACGCAAGCAGCCTCCGCTGCTATTCAATTTACCATTGATCC
       F  Y  G  W  K  K  G  L  K  T  G  M  Y  Y  L  R  T  Q  A  A  S  A  A  I  Q  F  T  I  D
```

pared from damaged (+4NQO) or replication-blocked (hydroxyurea [HU]-treated) cells. The constitutive (or weakly inducible) transcripts from undamaged cells that annealed to primer A (Fig. 4, lane 2) likely originated from the *RNR1* gene. This primer annealed to a region of the *DIN1*(*RNR3*) gene which is highly conserved between this gene and the murine sequence and is likely conserved between the *RNR1* and *DIN1*(*RNR3*) genes. By way of contrast, oligonucleotide primer B corresponds to a less-conserved region of the *DIN1*(*RNR3*) transcription unit and does not anneal to the *RNR1* transcripts.

Recently, Elledge and Davis (3) and Hurd et al. (7) reported the cloning and sequence of the *RNR2* gene of yeast cells encoding the small subunit of ribonucleoside diphosphate reductase. The *RNR2* gene, like *DIN1*(*RNR3*), is transcriptionally activated by DNA-damaging treatments, and transcript levels increase approximately 10- to 20-fold on induction (3, 4, 7). Furthermore, the protein sequence of RNR2 is 60% identical to the small reductase subunit of mouse.

The regulatory region of the *RNR2* gene has been examined by a series of deletion and promoter fusion studies and a 42-base-pair element has been identified which is necessary and sufficient for transcriptional regulation of this gene by HU and damaging agents (5, 8). This damage response element (DRE) contains two redundant positive regulatory elements and a negative control element (5). In order to determine whether the *DIN1*(*RNR3*) gene contained homologous control elements, we compared the nucleotide sequences upstream of *DIN1*(*RNR3*) with the DRE element described by Elledge and Davis (5). Previously, we had determined that the 700-base-pair region upstream of the *DIN1*(*RNR3*) coding region contained all the sequences necessary and sufficient for regulation by DNA-damaging agents. As shown in Table 1, two sequences were found which matched with a central region of the DRE element and these were located at positions 55 to 72 (matching at 13 of 18 positions) and 429 to 456 (matching at 14 of 18 positions) in the *DIN1*(*RNR3*) upstream region (Fig. 1). This comparison suggested that the sequence CnnTnGCCnTGGCnA may be important for mediating damage regulation of these genes. Additionally, a sequence containing a 9 of 11 nucleotide match to a RAP1 consensus binding site was identified between nucleotides 387 and 409. A RAP1 binding site is closely associated with the DRE of *RNR2* (5, 8) and may be important for its regulation.

The *RNR2* gene is strongly induced by HU, which directly inhibits the reductase by interacting with a tyrosyl-free radical in the catalytic site contained on the small subunit (13). We examined by Northern blots the transcript response of the chromosomal *DIN1*(*RNR3*) gene to HU as well as to 4NQO and MMS treatment. At the same time, these RNAs were hybridized with radiolabeled *DDRA2*, *DDR48*, and *UBI4* probes (Fig. 5). The results demonstrate that, for *DIN1*, HU treatment is more effective than either DNA-damaging treatment for inducing transcription. However, for *DDRA2* and *UBI4* (and, to a lesser extent, for *DDR48*), exposure of cells to 4NQO or MMS was more effective than HU treatment for inducing transcription from these genes. These results raise the possibility that the *DIN1*(*RNR3*) gene is responding to different cellular stress signals than are the *DDRA2* and *UBI4* genes. For example, *DIN1*(*RNR3*) gene transcription may be coupled to changes in nucleotide levels in cells which would be more significantly affected by HU than by 4NQO or MMS treatments.

Our results confirm and extend the recent studies of

```
  1 MHVIKRDGRQERVMFDKITSRIQKLCYGLNMDFVDPAQITMKVIQGLYSG 50
    : ::::::: : : :::::::: ::::::: : ::: :: ::: :::::
  1 MYVIKRDGRKEPVQFDKITSRITRLSYGLDPNRIDAVKVTQRIISGVYSG 50

 51 VTTVELDTLAAETAATLTTKHPDYAILAARIAVSNLHKETKKVFSDVMED 100
    ::::::: ::::: : ::: ::::: :::::::::::::: : :: ::::
 51 VTTVELDNLAAETCAYMTTVHPDYATLAARIAISNLHKQTTKQFSKVIED 100

101 LYNYINPHNGRHSPMVASSTLDIVMANKDRLNSAIIYDRDFSYNYFGFKT 150
    : ::::: ::: :::      :::: : :::::::::::: : ::::::
101 LHDWINPATGKHAPMISDEIYNIVMETRY.LNSAIVYDRDFQYTYFGFKT 149

151 LERSYLLKINGKVAERPQHMLMRVSVGIHKEDIDAAIETYNLLSEKWFTH 200
    :::::::::::: :::::::::::: :::: ::: : :::::: :::::
150 LERSYLLRLNGEVAERPQHLVMRVALGIHGSDIESVLKTYNLMSLRYFTH 199

201 APPTLFNAGTNRPQLSSCFLLSMKDDSIEGIYDTLKQCALISKSAGGIGV 250
    : :::::::  :::::::::::::::::::::::::::::: :: ::
200 ASPTLFNAGTPLPQMSSCFLIAMKDDSIEGIYDTLKECAMISKTAGVLS. 248

251 AVSCIRATGSYIAGTNGNSNGLVPMLRVYNNTARYVDQGGNKRPGAFAIY 300
      :  :: :::::::::: :::::::::::::::::::     :    ::
249 HINNIRSTGSYIAGTNGTSNGLIPMIRVFNNTARYVTRVVTRDLVLSPFS 298

301 LEPWHLDIFEFLDLKKNTGKEEQRARDLFFALWIPDLFMKRVETNQDWSL 350
    :           ::::: :::: :::::: :::::::::::::: : : :
299 WSHGMQISSTKFDIRKTHGKEEIRARDLFPALWIPDLFMKRVQEDGPWTL 348

351 MCPNECPGLDEVWGEEFEKLYESYEKQGRVRKVVKAQQLWYAIIESQTET 400
    :::   :::::::::::: ::  ::::::  : :::: ::::::: ::::
349 FSPSAAPGLDDVWGDEFEELYTRYEREGRG.KTIKAQKLWYAILQAQTET 397

401 GTPYMLYKDSCNRKSNQQNLGTIKCSNLCTEIVEYTSKDEVAVCNLASLA 450
    :::::::: :::: :::::::::::::: ::::: : :: :::::::::
398 GTPFMVYKDACNRKTNQQNLGTIKSSNLCCEIVEYSSPDETAVCNLASIA 447

451 LNMYVTPEH.....TYDFEKLAEVTKVIVRNLNKIIDINYYPIPEAHLSN 495
    :  ::           :::::: :: ::: :::::::: :::::::::
448 LPAFVEVSEDGKTASYNFERLHEIAKVITHNLNRVIDRNYYPVPEARNSN 497

496 KRHRPIGIGVQGLADAFILMRYPFESPEAQLLNKQIFETIYYGALEASCE 545
    :::::::::::: ::::: :::: ::: :::::::::: : ::::::
498 MKHRPIALGVQGLADTYMMLRLPFESEEAQTLNKQIFETIYHATLEASCE 547

546 LAKEYGPYETYEGSPVSKGILQYDMWNVAPTDLWDWKPLKEKIAKYGIRN 595
    ::   : : :::::: :::::::::   : :::::  ::  : : ::::
548 LAQKEGKYSTFEGSPASKGILQFDMWNAKPFGMWDWETLRKDIVKHGLRN 597

596 SLLIAPMPTASTAQILGNNESIEPYTSNIYTRRVLSGEFQIVNPHLLKDL 645
    :: ::::::::: :::: :::::: ::::: :::::::::::: :::::
598 SLTMAPMPTASTSQILGYNECFEPVTSNMYSRRVLSGEFQVVNPYLLRDL 647

646 TERGLWNEEMKNQIIACNGSIQSIPEIPDDLKQLYKTVWEISQKTVLKMA 695
    : ::::: ::  ::  :::::::::::::::::::::::::::::: ::
648 VDLGIWDDSMKQYLITQNGSIQGLPNVPQELKELYKTVWEISQKTIINMA 697

696 AERGAFIDQSQSLNIHIAEPNYGKLTSMHFYGWKQGLKTGMYYLRTRPAA 745
    :::: :::::::::: :  :  :::::::::::: :::::::::: ::
698 ADRAIYIDQSHSLNLFLQAPSMGKITSMHFYGWKKGLKTGMYYLRTQAAS 747

746 NPIQFTLN 753
    ::::::::
748 AAIQFTID 755
```

FIG. 2. DIN1 homology to the murine ribonucleotide reductase large subunit M1. The upper sequence is the murine M1 reductase subunit sequence determined by Caras et al. (1), and the lower sequence represents the predicted sequence of the DIN1 protein. Amino acid identities and conservative substitutions are indicated by the dashed lines. The GenBank search was performed by using the University of Wisconsin FAST N program on a VAX computer.

FIG. 1. Structure and sequence of the *DIN1* gene in plasmid pSZ214. (A) A 3-kilobase-pair region immediately upstream of the *lacZ* gene containing *DIN1* coding and regulatory sequences is shown together with pertinent restriction sites and DNA sequencing strategy. (B) The nucleotide sequence and predicted amino acid sequence for the first 755 residues of the DIN1 protein up to the LacZ fusion junction are shown. The DNA sequence was determined by the dideoxy-chain termination method with both universal and synthetic oligonucleotide primers and fragments subcloned into single-stranded phage vectors M13mp18 and M13mp19. Sequence translation was performed on an IBM PC by using the CYBORG program from IBI/PUSTELL.

```
          1                                                  50
RNR3  MYVIKRDGRKEPVQFDKITSVLTRLSYGLDPNRIDAFKVTQRIISGVYSG

DIN1  ------------------RI--------------V------------

          51                                                 100
RNR3  VTTVELDILAAETCAYMTHVHPDYATLAARIATSNLHKQTTKQFSKVIED

DIN1  -------N----------T-------------I----------------
```

FIG. 3. Sequence comparison of the predicted N-terminal residues of DIN1 and RNR3. The first 100 residues of the predicted DIN1 protein sequence were compared with the corresponding region reported by Elledge and Davis (6) for RNR3. A dashed line indicates amino acid identity between the sequences.

Elledge and Davis (6) demonstrating that *S. cerevisiae* possesses a DNA damage-inducible gene for a large subunit of ribonucleoside diphosphate reductase. Interestingly, yeast cells also contain a second gene which encodes a homologous protein and which is expressed at much higher levels than *DIN1(RNR3)* in undamaged cells. This gene, designated *RNR1*, is essential in yeast cells, whereas the *DIN1(RNR3)* gene is nonessential for normal vegetative growth (8; K. Yagle, unpublished data). However, overexpression of the *RNR3* gene allows growth of a strain carrying a disruption of the *RNR1* gene (6). Thus, *DIN1(RNR3)* appears functionally equivalent to *RNR1*.

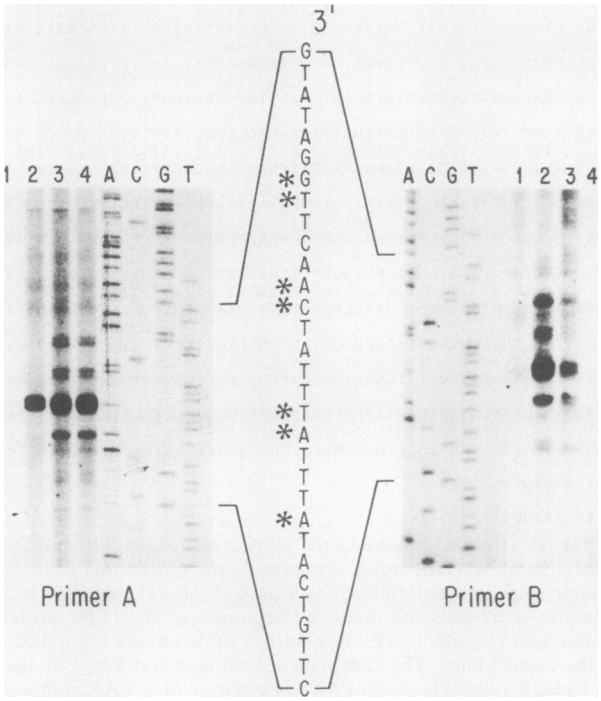


FIG. 4. Identification of the *DIN1(RNR3)* transcript start sites. Total cellular RNA was isolated from untreated, 4NQO, MMS, or HU-treated cells (strain M12B), and the 5' ends of the *DIN1(RNR3)* transcripts were determined by using avian myeloblastosis virus reverse transcriptase and oligonucleotide primer A (complementary to nucleotides 721 to 737 in Fig. 1) or primer B (complementary to nucleotides 692 to 709). The conditions for annealing and primer extension are those described by Treger and McEntee (15), except that 40 and 100 μg of total RNA were used for each extension reaction for experiments with primer A and primer B, respectively. The DNA sequence ladders obtained by using primers A and B are shown. The DNA sequence is complementary to that shown in Fig. 1, beginning at residue 634. Primer A: lane 1, no RNA; lane 2, RNA from untreated (control) cells; lane 3, RNA from cells treated with MMS (0.1%, 1 h); lane 4, RNA from cells treated with 4NQO (1 μg/ml, 1 h). Primer B: lane 1, no RNA; lane 2, RNA from untreated (control) cells; lane 3, RNA from cells treated with HU (100 mM, 90 min); lane 4, RNA from cells treated with 4NQO (1 μg/ml, 1 h). *, transcript start sites.

Taken together, these results suggest a model for the control of ribonucleotide reductase in yeast cells. Under normal growth conditions, yeast cells possess a reductase composed of the RNR2 gene product (M2 counterpart) and a large subunit (M1) encoded by a constitutively expressed *RNR1* gene. After a period of exposure to DNA-damaging agents or the antitumor drug HU, expression of the *RNR2* gene increases from 5- to 20-fold. This increase in the amount of small subunit is more than matched by induction of a second gene encoding a M1-like protein, *DIN1(RNR3)*. In damaged cells or cells arrested for DNA synthesis, the reductase therefore consists of multiple species because of the association of the small subunit with either the constitutively expressed (RNR1) or the inducible [DIN1(RNR3)] large subunits. This unusual regulation may be important to the physiology of damaged or stressed cells. The reductase enzymes from eucaryotic cells are subject to complex allosteric regulation by ribonucleotides and deoxyribonucleotides (2). In damaged yeast cells or yeast cells depleted of normal nucleotide pools, it may be important to modify the allosteric properties of the reductase in order to restore normal DNA synthesis or reestablish balanced nucleotide pool levels. In *S. cerevisiae*, this may be accomplished by synthesizing a distinct regulatory subunit protein which, in combination with the small subunit (*RNR2* gene product), could function more effectively under the conditions of cellular stress described here. Consistent with this proposal is the observation that the large subunit contains the binding sites for the allosteric effectors of reductase activity (1, 2). Enzymatic characterization of ribonucleotide reductase from damaged cells as well as molecular characterization of its regulatory region should provide further insight into this unusual DNA damage stress response in yeast cells.

TABLE 1. Homologies between the *DIN1(RNR3)* upstream regions and the DRE of *RNR2*

Region[a]	Sequence
DIN1(RNR3) nt 429 to 456	CggTtGCCATGGCgAgGA
DIN1(RNR3) nt 55 to 72	GCGCtgTgGCCgTGGCtA
RNR2 DRE	GCGCGATCGCCATGGCAACGA
Consensus	CnnTnGCCnTGGCnA

[a] nt, Nucleotide.

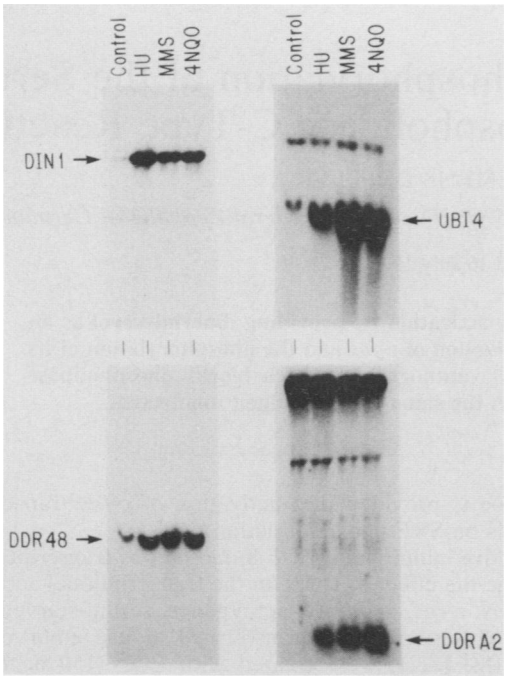


FIG. 5. HU and DNA damage induction of *DIN1*(*RNR3*) and *DDR* genes. Total cellular RNA was isolated from strain M12B by glass bead disruption and examined by Northern hybridization as previously described (14). Cells were treated with HU (200 mM, 90 min), 4NQO (1.5 μg/ml, 60 min), or MMS (0.1%, 60 min), and the RNA (100 μg/lane) was hybridized with multiprimed ([α-^{32}P]dCTP; Amersham) *DIN1*(*RNR3*), *UBI4*, *DDR48*, and *DDRA2* probes. The positions of the induced transcripts are indicated. A low-abundance, cross-hybridizing transcript is present in the autoradiograph of the *UBI4* Northern hybridization. The *DDRA2* probe contained a small amount of *TY1* DNA, which hybridized to a 5.6-kilobase transcript seen at the top of the gel.

Nucleotide sequence accession number. The GenBank accession number for the *DIN1*(*RNR3*) nucleotide sequence is M37684.

We thank S. Ruby and J. Szostak for the *DIN1*(*RNR3*) clone pSZ214 and S. Elledge for communicating his unpublished results to us. We are indebted to L. Tabata and J. Ransom for their help in preparing the manuscript and to Richard Ruettinger for assistance with the DNA sequence analysis.

This work was supported by Public Health Service grant GM38456 from the National Institutes of Health to K.M. and by Office of Energy Research, Office of Health and Environmental Research, Contract no. De-FC03-87-ER60615 (operated for the U.S. Department of Energy by the University of California). K.M. is a recipient of a faculty research award from the American Cancer Society.

LITERATURE CITED

1. **Caras, I. W., B. B. Levinson, M. Fabry, S. R. Williams, and D. W. Martin, Jr.** 1985. Cloned mouse ribonucleotide reductase subunit M1 cDNA reveals amino acid sequences homology with *Escherichia coli* and herpesvirus ribonucleotide reductases. J. Biol. Chem. **260**:7015–7022.
2. **Cory, J. G., D. A. Rey, G. L. Carter, and P. E. Bacon.** 1985. Nucleoside 5′-diphosphates as effectors of mammalian ribonucleotide reductase. J. Biol. Chem. **260**:12001–12007.
3. **Elledge, S. J., and R. W. Davis.** 1987. Identification and isolation of the gene encoding the small subunit of ribonucleotide reductase from *Saccharomyces cerevisiae*: DNA damage-inducible gene required for mitotic viability. Mol. Cell. Biol. **7**:2783–2793.
4. **Elledge, S. J., and R. W. Davis.** 1989. DNA damage induction of ribonucleotide reductase. Mol. Cell. Biol. **9**:4932–4940.
5. **Elledge, S. J., and R. W. Davis.** 1989. Identification of the DNA damage-responsive element of *RNR2* and evidence that four distinct cellular factors bind it. Mol. Cell. Biol. **9**:5373–5386.
6. **Elledge, S. J., and R. W. Davis.** 1990. Two genes differentially regulated in the cell cycle and by DNA damaging agents encode alternative regulatory subunits of ribonucleotide reductase. Genes Dev. **4**:740–751.
7. **Hurd, H. K., C. W. Roberts, and J. W. Roberts.** 1987. Identification of the gene for the yeast ribonucleotide reductase small subunit and its inducibility by methyl methanesulfonate. Mol. Cell. Biol. **7**:3673–3677.
8. **Hurd, H. K., and J. W. Roberts.** 1989. Upstream regulatory sequences of the yeast *RNR2* gene include a repression sequence and an activation site that binds the RAP1 protein. Mol. Cell. Biol. **9**:5359–5372.
9. **McClanahan, T., and K. McEntee.** 1986. DNA damage and heat shock dually regulate genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6**:90–96.
10. **Ruby, S. W., and J. W. Szostak.** 1985. Specific *Saccharomyces cerevisiae* genes are expressed in response to DNA-damaging agents. Mol. Cell. Biol. **5**:75–84.
11. **Ruby, S. W., J. W. Szostak, and A. W. Murray.** 1983. Cloning regulated yeast genes from a pool of lacZ fusions. Methods Enzymol. **101**:253–268.
12. **Sanger, F., S. Nicklen, and A. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
13. **Thelander, M., A. Gräslund, and L. Thelander.** 1985. Subunit M2 of mammalian ribonucleotide reductase. J. Biol. Chem. **260**:2737–2741.
14. **Treger, J. M., K. A. Heichman, and K. McEntee.** 1988. Expression of the yeast *UBI4* gene increases in response to DNA-damaging agents and in meiosis. Mol. Cell. Biol. **8**:1132–1136.
15. **Treger, J. M., and K. McEntee.** 1990. The structure of the DNA damage-inducible gene *DDR48* and evidence for its role in mutagenesis in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10**:3174–3184.