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NORF5/HUG1 Is a Component of the *MEC1*-Mediated Checkpoint Response to DNA Damage and Replication Arrest in *Saccharomyces cerevisiae*

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Analysis of global gene expression in *Saccharomyces cerevisiae* by the serial analysis of gene expression technique has permitted the identification of at least 302 previously unidentified transcripts from nonannotated open reading frames (NORFs). Transcription of one of these, *NORF5/HUG1* (hydroxyurea and UV and gamma radiation induced), is induced by DNA damage, and this induction requires *MEC1*, a homolog of the ataxia telangiectasia mutated (*ATM*) gene. DNA damage-specific induction of *HUG1*, which is independent of the cell cycle stage, is due to the alleviation of repression by the Crt1p-Ssn6p-Tup1p complex. Overexpression of *HUG1* is lethal in combination with a *mec1* mutation in the presence of DNA damage or replication arrest, whereas a deletion of *HUG1* rescues the lethality due to a *mec1* null allele. *HUG1* is the first example of a NORF with important biological functional properties and defines a novel component of the *MEC1* checkpoint pathway.

A major accomplishment of genome-era research was the complete elucidation of the genomic sequence of the eukaryote *Saccharomyces cerevisiae*. As a direct result of this effort, 6,275 open reading frames (ORFs) representing all ORFs larger than 100 contiguous amino acids were identified (10, 14). However, identification of genes encoded by small ORFs (<100 amino acids) based on sequence analysis alone has been severely limited by high false-positive rates, and traditional functional screens have been similarly hampered by the small target size for mutagenesis (4). Evidence from several microorganisms suggests that a significant fraction of genomes are encoded by small genes. For example, the *Escherichia coli* genome encodes 381 proteins of less than 100 amino acids in length from a total of 4,288 annotated ORFs (8.9% [37a]), and random protein sequencing in the fully sequenced cyanobacterium *Synechocystis* revealed that 11.8% of the total proteins were encoded by ORFs of <100 codons (8a). Extrapolation of such studies to yeast would suggest that there may be as many as 800 small ORFs in the entire yeast genome, of which only 177 have been identified (20a). The subset of small ORFs will likely encode important proteins in all organisms, including humans. In *S. cerevisiae*, these small proteins include mating pheromones, proteins involved in energy metabolism, proteolipids, chaperonins, stress proteins, transporters, transcriptional regulators, nucleases, ribosomal proteins, thioredoxins, and metal ion chelators. In multicellular organisms, there is a rich diversity of short peptides, including many hormones, antibacterial defensins, cecroporins, and magainins (3). There are also small ORFs encoding transporter proteins, homeobox proteins, transcription factors, and kinase regulatory subunits reported in the nematode *Caenorhabditis elegans* (29a).

Analysis of global gene expression in *S. cerevisiae* by the serial analysis of gene expression (SAGE) technique (39, 40) has permitted the identification of at least 302 previously unidentified transcripts from nonannotated ORFs (NORFs). Whether any of these NORFs are important for the growth and biology of yeast is unclear. We report herein the first systematic analysis of NORFs in the yeast genome and the characterization of *NORF5/HUG1*. Our analysis of the 30 most highly transcribed NORFs has shown that 12 of the 30 NORF genes are evolutionarily conserved with mammalian homologs (28a). *NORF5/HUG1* was chosen for further analysis because its dramatic expression in hydroxyurea (HU)-treated cells suggested a potential role in transcriptional response after replication arrest and DNA damage.

Several checkpoint genes in *S. cerevisiae* are required for transcriptional induction of a large regulon of genes that facilitate DNA repair, cause cell cycle arrest, and mediate recovery from DNA damage (12, 41). A central component of these checkpoints is *MEC1*, the budding yeast homolog of the hereditary ataxia telangiectasia *ATM* gene and a member of the phoshatidylinositol-3-kinase family (32, 45). Signals of DNA damage normally pass from sensor genes such as *RAD9*, *RAD17*, *RAD24*, *MEC3*, and *DDC1* to *MEC1*, leading to phosphorylation of Rad53p, replication protein A, and potentially other targets, causing cell cycle arrest and transcriptional response (2, 8, 12, 19, 29, 36). We found that genes in the *MEC1* checkpoint pathway are required for the transcriptional induction of *NORF5/HUG1* in response to replication arrest and DNA damage. Additional experiments have shown that *NORF5/HUG1* has distinct genetic interactions with *MEC1*. These findings highlight the importance of the development and application of new technologies in the total-genome sequence era to fully understand the genetic complexity of an organism.

MATERIALS AND METHODS

Analysis of NORF data. Yeast genome intergenic regions were defined as regions outside annotated ORFs or the 500-bp region downstream of annotated ORFs (yeast genome sequence and tables of annotated ORFs were obtained from the Stanford Genome Database (35a). Based on sequence analysis, a total of 9,524 putative ORFs of 25 to 99 amino acids were present in the intergenic

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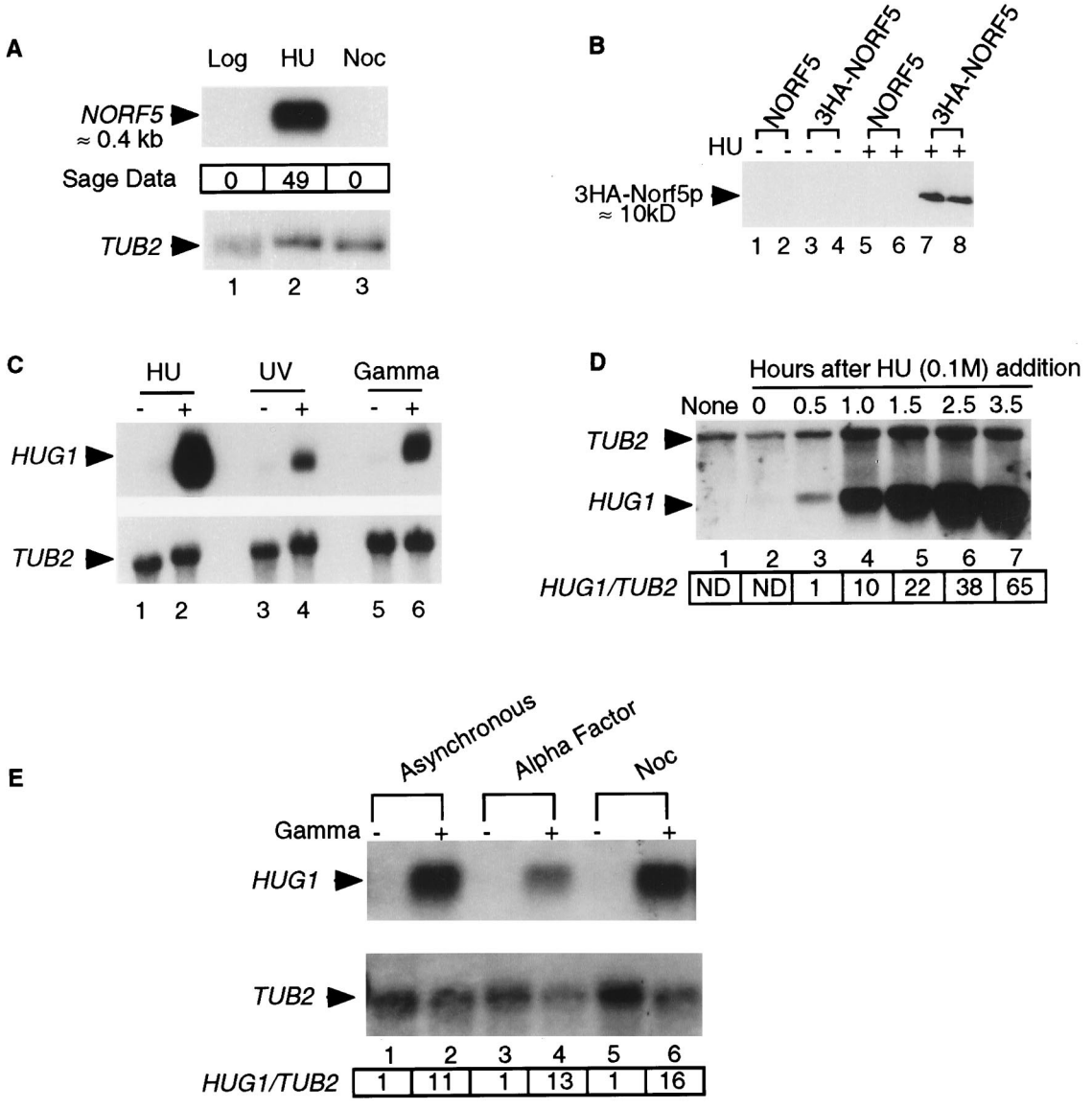

FIG. 1. Transcription of *NORF5*/*HUG1* is induced by replication arrest and DNA damage. (A) NORF5 transcription is upregulated in cells arrested with HU. Results are from Northern blot analysis with wild-type cells (YPH499) grown logarithmically (lane 1) and arrested with HU (lane 2) or nocodazole (Noc) (lane 3). The expression pattern observed by SAGE is indicated at the bottom (0:49:0) (40). (B) NORF5 is translated in cells arrested with HU. Western blot analysis was done with protein extracts from transformants (YMB711) containing pMB366 (*3HA-NORF5 LEU2 CEN*) or plasmid pMB363 (*NORF5 LEU2 CEN*) grown logarithmically (lanes 1 to 4) or arrested with HU (lanes 5 to 8) and probed with HA antibody as described previously. (C) Transcription of *NORF5*/*HUG1* is HU and UV and gamma radiation induced. Results are from Northern blot analysis with wild-type cells (YPH499) grown logarithmically (lanes 1, 3, and 5), arrested with HU (lane 2), exposed to UV radiation (lane 4), or exposed to gamma radiation (lane 6). (D) *HUG1* transcription is delayed upon replication arrest with HU. Results are from Northern blot analysis using logarithmically grown wild-type cells (YPH499) (lane 1) or after addition of HU (0.1 M) and incubation for 0 h (lane 2), 0.5 h (lane 3), 1.0 h (lane 4), 1.5 h (lane 5), 2.5 h (lane 6), or 3.5 h (lane 7) at 30°C. The levels of *HUG1* in lanes 1 and 2 were below the background level and hence are denoted as ND (not detected). *HUG1*/*TUB2* indicates the ratio of the intensity of the *HUG1* signal to the *TUB2* signal normalized to the *HUG1*/*TUB2* ratio in lane 3 (0.5 h) set to 1.0 as described in Materials and Methods. (E) *HUG1* transcription is independent of the cell cycle stage. Northern blot analysis was done with wild-type cells (YPH499) grown logarithmically (lanes 1 and 2), arrested in G_1 phase by treatment with alpha factor (lanes 3 and 4), and arrested in G_2/M with nocodazole (lanes 5 and 6), either before (lanes 1, 3, and 5) or after exposure to gamma radiation (lanes 2, 4, and 6). The arresting agents were present throughout the incubations. *HUG1*/*TUB2* for lanes 2, 4, and 6 indicates the ratio of the intensity of the *HUG1* signal to the *TUB2* signal normalized to the *HUG1*/*TUB2* ratio in control lanes 1, 3, and 5, respectively, as described in Materials and Methods.

regions. Of the 60,633 SAGE tags analyzed, there were 302 unique SAGE tags that matched the genome uniquely, were in the correct orientation, and were expressed at levels greater than 0.3 transcript copies per cell. The 302 unique SAGE tags were either within or adjacent to intergenic ORFs (100 bp upstream or 500 bp downstream of the ORF). Homology searches for 30 highly transcribed NORFs can be obtained from reference 28a.

Strains and plasmids used. The strains used included YPH499 (*MAT***a** *ura3-52 lys2-801 ade2-101 his3-Δ200 trp1-Δ63 leu2-Δ1*), YPH987 (*MAT***a**/α *ura3-52/ura3-52 lys2-801/lys2-801 ade2-101/ade2-101 trp1-Δ63/trp1-Δ63 leu2-Δ1/leu2-Δ1 his3-Δ200/his3-Δ200 CFIII CEN3L. YPH983TRP1SUP11*), YMB711 (*MAT*α *ura3-52 lys2-801 ade2-101 his3-Δ200 trp1Δ63 leu2-Δ1 hug1Δ1::HIS3*), and YMB847 (*MAT***a** *ura3-52 lys2-801 ade2-101 his3-Δ200 trp1-Δ63 leu2-Δ1 hug1Δ2::HIS3*) (our collection); Y203 (*MAT***a** *ade2-1 his3 leu2-3,112 lys2 trp1 ura3-Δ100 rnr3::RNR3-URA3-TRP1*), Y203-*dun1* (*dun1* in Y203), Y217(*MAT***a** *ade2-1 his3 leu2-3, 112 lys2 trp1 ura3-Δ100 rnr3::RNR3-URA3-TRP1 crt4-2/tup1*), Y231 (same as Y217, except with *crt8-91/ssn6* instead of *crt4-2/tup1*), Y300 (*MAT***a** *can1-100 ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1*), and Y577 (*crt1-Δ1::LEU2* in Y300) from S. Elledge (16); W1588-4A (*MAT***a** *leu2,3-112 ade2-1 can1-100 his3-11, 15 ura3-1 trp1 RAD5*), U952-3C (*sml1Δ::HIS3* in W1588-4A), U953-61D (*mec1Δ::TRP1 sml1Δ::HIS3* in W1688-4A), and U971

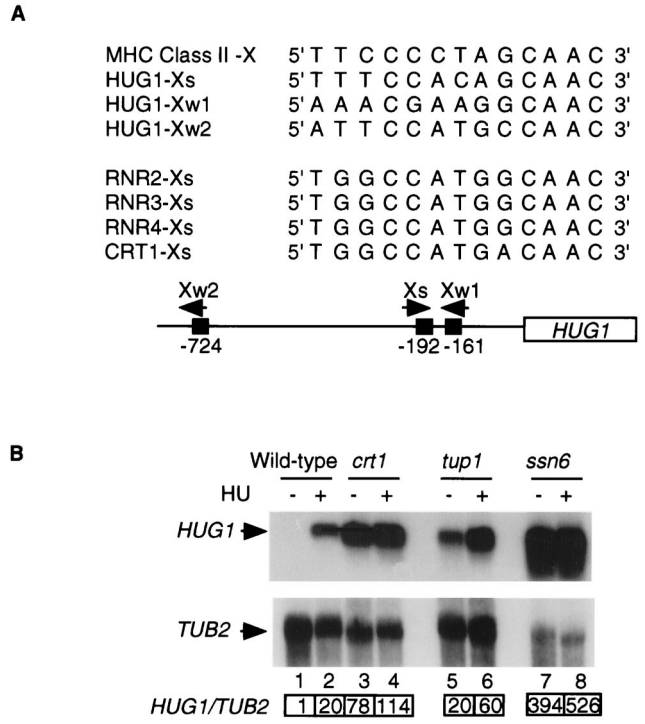

FIG. 2. Crt1p, Ssn6p, and Tup1p are negative regulators of *HUG1* transcription in the absence of DNA damage or replication arrest. (A) The promoter of *HUG1* contains X-box-related sequences Xs and Xw, with strong and weak homology, respectively, to the consensus sequence in mammalian MHC class II and *S. cerevisiae RNR* and *CRT1* genes (16, 26, 27). (B) Transcription of *HUG1* in the absence of DNA damage is repressed by the Crt1p-Ssn6p-Tup1p complex. Northern blot analysis was performed with the wild-type strain (Y300) and the *crt1-Δ1*::*LEU2* (Y577), *crt4-2*/*tup1* (Y217), and *crt8-91*/*ssn6* (Y231) strains, grown logarithmically (lanes 1, 3, 5, and 7) or arrested with HU (lanes 2, 4, 6, and 8). *HUG1*/*TUB2* for lanes 2 to 8 indicates the ratio of the intensity of the *HUG1* signal to the *TUB2* signal normalized to the *HUG1*/*TUB2* ratio in control lane 1 as described in Materials and Methods.

(*MAT*α *leu2,3-112 ade2-1 can1-100 his3-11, 15 ura3-1 trp1 RAD5 dun1Δ::URA3*) from R. Rothstein (46); TWY312 (*MAT***a** *ura3 trp1 his7 rad53*/*mec2-1*), TWY316 (*MAT***a** *ura3 trp1 his3 mec3-1*), TWY397 (*MAT***a** *ura3 his7 trp1 leu2*), DLY62 (*MAT***a** *ura3 leu2 his3 trp1 ade2*), and DLY258 (*MAT***a** *ura3 leu2 his3 trp1 ade2 mec1-1 sml1*) from T. Weinert (44); and YMP10381 (*MAT***a** *ade2 ade3-130 ura3 leu2 trp1 cyh2 SCR::URA3*), YMP10535 (*rad9Δ::LEU2* in YMP10381), YMP11108 (*rad17Δ::LEU2* in YMP10381), YMP10533 (*rad24Δ::TRP1* in YMP10381), YEF610 (*MAT***a** *ade2 ade3 leu2 ura3 trp1 mec1Δ::TRP1 sml1-1* [*pEF208=URA3 ADE3 MEC1 CEN*] lacking pEF208 by loss on 5-fluoroorotic acid (5-FOA) medium, YEF630 (*MAT***a** *leu2 ura3 his3 sml1-1*), and yPP8 (*MAT*α *ade2 ade3 leu2 ura3 trp1 mec1Δ::TRP1 his3* [*pEF208=URA3 ADE3 MEC1 CEN*] from the L. Hartwell laboratory. Plasmid pMB363 (*HUG1 LEU2 CEN*) contains the *HUG1* ORF and sequences 272 bp upstream of the start codon and 191 bp downstream of the stop codon of *HUG1* in pRS315 (35). Plasmid pMB366 (*3HA-HUG1 LEU2 CEN*) contains three copies of the hemagglutinin (HA) epitope after the second amino acid in Hug1p and was derived by ligation of 3HA from plasmid pSM937, a gift from S. Michaelis. Plasmid pMB379 (*GAL1-HUG1 URA3-2*μ) contains the *HUG1* ORF and sequences 36 bp upstream of the start codon and 66 bp downstream of the stop codon of *HUG1* in pRS426GAL1 (*GAL1-URA3-2*μ) (22). Plasmid pMB386 (*HUG1fsLEU2CEN*) contains a frameshift in the *HUG1* ORF at codon 14 of *HUG1*.

Cell cycle arrest and Northern and Western blot analyses. For cell cycle arrest and exposure to DNA damage, we used early-logarithmic-phase cultures. For replication arrest, cells were incubated in the presence of HU (0.1 M) for 3.5 h; for G_1 arrest, cells were incubated with alpha factor (Sigma; T-6901) (3×10^{-2} M) for 2 h; for G_2/M arrest, cells were incubated with nocodazole (Sigma; M-1404) (15 μg/ml) for 90 min at 30°C. For each arrest (>90%), we examined cell morphology and determined DNA content by flow cytometry (5). For exposure to UV radiation, cells were spread on the surface of yeast extract-peptone-dextrose (YPD) plates and irradiated (Stratagene; UV Stratalinker 2400) at 60 J/m^2. For exposure to gamma radiation, liquid cultures were irradiated with a dose of 2 Gy with a Shepherd Mark ^{137}I-Cs irradiator. After irradiation with UV and gamma radiation, cells were incubated at 30°C for 1 h. For thermal stress, cells were shifted to 37°C for 2 h. Cells from each treatment were washed, and cell pellets were frozen at −70°C for RNA preparation. Total RNA was made by the hot phenol method as described previously (3) from cell pellets (−70°C) of treated or untreated cultures, and Northern blot analysis was performed as described previously (11). Quantitation was done with a Fuji Phosphoimager, model BAS1500. We have previously determined that the SAGE tag abundance for *TUB2* is 10:7:8 and that of *ACT1* is 81:38:84 in log-phase–S phase–G_2/M-phase cells (40). Hence, we used *TUB2* as the loading control for RNA. For most of the blots, we detected very low levels of *HUG1* transcript in the no treatment (control) lane. For example, we determined that the ratio of the intensity of the *HUG1* signal to the *TUB2* signal (*HUG1*/*TUB2*) in the control lane ranges from 0.04 to a maximum of 1.2 in one case. The ratio of *HUG1*/*TUB2* was set to 1.0 for the control lane, and the value of the ratio of *HUG1*/*TUB2* in the treated lanes was divided by the value of the ratio in the control lane. The result of this ratio is presented at the bottom of each panel as *HUG1*/*TUB2*. Background values were subtracted from the values obtained for each observation. Exceptions are in Fig. 1D and 2D, lane 5 (see figure legends).

Sensitivity to HU, UV radiation, ionizing radiation, and methyl methanesulfonate was determined as described previously (21). Western blot analysis was done as described previously (18) by using whole-cell extracts from *hug1Δ1::HIS3* (YMB711) transformants containing plasmid pMB363 or pMB366. Filters were incubated with the primary HA antibody (1:5,000 dilution) followed by secondary antibody GAMHRP (goat anti-mouse horseradish peroxidase) (dilution of 1:10,000) and then with the enhanced chemiluminescence reagent (Amersham) and exposed to film.

Genetic analysis. The *HUG1* ORF was replaced by *HIS3* by a PCR-based method (6). *hug1Δ1::HIS3* (YMB711) replaces the *HUG1* ORF, including sequences 153 bp upstream of the start codon (ATG) and 65 bp downstream of the stop codon (TAA). *hug1Δ2::HIS3* (YMB847) replaces the *HUG1* ORF, including sequences 153 bp upstream of the start codon (ATG) and 53 bp upstream of the stop codon (TAA). Deletions were made in diploid strain YPH987. Deletion of *HUG1* was verified by PCR and Southern blot analysis, the diploid was sporulated, and tetrad analysis showed 2:2 segregation of the *hug1Δ::HIS3* in each of the tetrads. For genetic interactions with *MEC1*, tetrad analyses of two independent matings were done. In the first case, YMB711 was mated to YEF610 lacking pEF208. From a total of 14 tetrads dissected, we obtained 3, 8, and 3 tetrads containing 4, 3, and 2 viable spores, respectively. Among these were 14 *hug1Δ1::HIS3* and 15 *mec1Δ::TRP1* spores, and from these, 7 were *hug1Δ1::HIS3 mec1Δ::TRP1*. In a second experiment we analyzed tetrads from a mating between YMB847 and yPP8. The strain yPP8 is inviable without the pMEC1 plasmid (pEF208). From a total of 34 tetrads, we obtained 14, 9, and 11 tetrads containing 4, 3, and 2, viable spores, respectively. Among these were 40 *hug1Δ2::HIS3* and 32 *mec1Δ::TRP1* spores, and from these, 15 were *hug1Δ2::HIS3 mec1Δ::TRP*. The latter spores were viable without pMEC1, as evidenced by growth on 5-FOA (7). Genetic interactions between *DUN1* and *HUG1* were determined by tetrad analysis of a diploid derived by mating strains YMB847 and U971. From a total of 22 tetrads, we obtained 41 *hug1Δ2::HIS3* and 37 *dun1Δ::URA3* spores: 19 of these were *dun1Δ*/*hug1Δ2*, and all of the double mutants were resistant to HU.

RESULTS

SAGE analysis reveals transcription from NORFs that are evolutionarily conserved. As previously reported (40), SAGE has identified transcripts that correspond to NORFs in the intergenic regions of *S. cerevisiae*. We performed a systematic analysis of the SAGE tags that correspond to the NORFs (see Materials and Methods). Of the 60,633 SAGE tags analyzed, there were 302 unique SAGE tags that were either within or adjacent to intergenic ORFs of <100 amino acids. The 302 SAGE tags were expressed at levels ranging from 0.6 to 94 transcript copies per cell. The 30 most abundant of the transcripts detected by SAGE were observed at least nine times. We found that 12 of the 30 highly expressed NORF genes are evolutionarily conserved with mammalian homologs (28a). Northern blot analysis of four of the NORFs (NORF1, NORF5, NORF14, and NORF17) has confirmed their transcription in *S. cerevisiae* (data not shown). In addition, the SAGE data facilitated the addition of 27 new ORFs (<100 amino acids) to the *S. cerevisiae* genome database (35b).

Transcription of *NORF5*/*HUG1* is induced by replication arrest and DNA damage. NORF5, a putative 68-amino-acid protein, corresponds to a previously unidentified ORF transcribed in HU-arrested cells (40) HU, a potent inhibitor of ribonucleotide reductase (RNR), which is required for deoxynucleoside triphosphate (dNTP) synthesis, leads to replica-

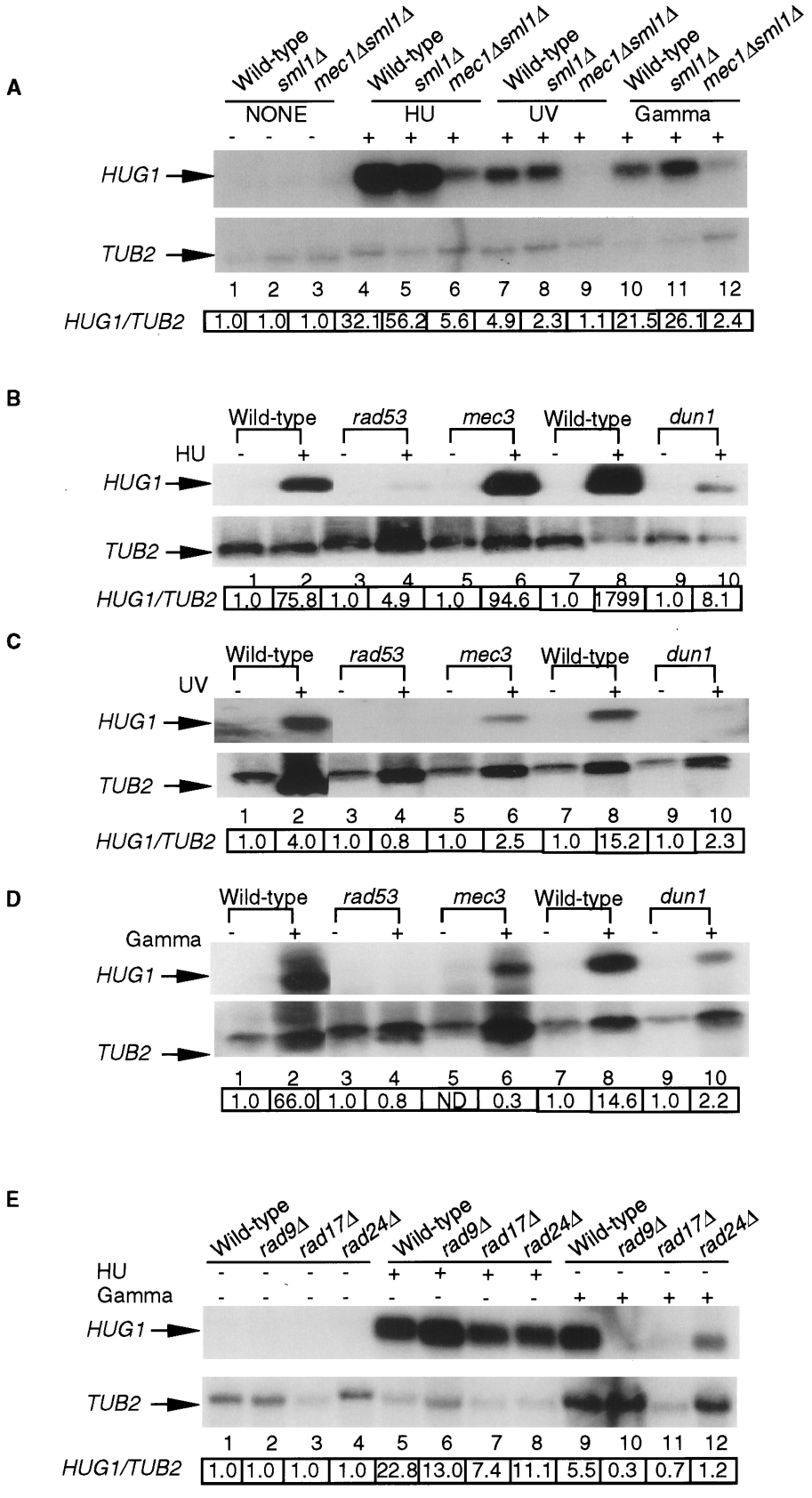

FIG. 3. Genes in the *MEC1* checkpoint pathway are required for the DNA damage- and replication arrest-induced transcription of *HUG1*. (A) Northern blot analysis was done with logarithmically grown, HU-arrested, UV or gamma radiation-exposed cells. The strains used were isogenic to the wild-type strain (W1588-3A) and the *sml1*Δ::*HIS3* (U952-3C) and *mec1*Δ::*TRP1 sml1*Δ::*HIS3* (U953-61D) strains. *HUG1/TUB2* indicates the ratio of the intensity of the *HUG1* signal to the *TUB2*

tion arrest in S phase (13, 15). The transcript abundance for NORF5 in logarithmically grown yeast cells was <1 copy/cell, whereas in HU-arrested cells, it was 37 copies/cell, exhibiting a higher level of differential gene expression in HU-arrested cells than any other *S. cerevisiae* gene (40). Northern blot analysis supported SAGE data, because a transcript of approximately 400 bp, corresponding to NORF5, is present in RNA prepared from HU-arrested cells (Fig. 1A). Consistent with these results, Western blot analysis of the candidate epitope-tagged 68-amino-acid ORF (chromosome 13, coordinates 158760 to 158966) confirmed a protein of about 10 kDa in HU-arrested cells (Fig. 1B). Transcription of NORF5 is also induced in cells exposed to UV or gamma radiation (Fig. 1C). The transcriptional induction of NORF5 appears to be specific to replication arrest and DNA damage, since there was no induction of NORF5 in cells subjected to heat shock (data not shown) or nocodazole-induced G_2/M arrest (Fig. 1A). On the basis of its transcription pattern, we named the NORF5 gene *HUG1*. We found that following addition of HU, low levels of *HUG1* transcription are detected at earlier time periods of 0.5 and 1.0 h, followed by an almost linear increase until 3.5 h post HU addition (Fig. 1D). The DNA damage-dependent transcription of *HUG1* is not restricted to any particular stage of the cell cycle. Cells arrested in G_1 with alpha factor or G_2/M with nocodazole show similar patterns of transcription of *HUG1* compared to asynchronous populations upon exposure to gamma radiation (Fig. 1E), and, therefore, DNA damage-induced transcription of *HUG1* can occur in the G_1 and G_2/M phases.

Crt1p, Ssn6p, and Tup1p are negative regulators of *HUG1* transcription in the absence of DNA damage or replication arrest. Promoters of DNA damage- or replication arrest-inducible genes, such as *RNR2*, *RNR3*, *RNR4*, and *CRT1*, often contain X-box sequences (16). In *S. cerevisiae*, the X box mediates Crt1p-dependent repression of the *RNR* genes by recruitment of the general repressors Ssn6p and Tup1p (37) to the promoters of damage-inducible genes (16). X-box sequences sharing a high degree of identity to those found in the promoters of mammalian major histocompatibility complex (MHC) class II genes (26) and *S. cerevisiae* genes were found in the promoter of *HUG1* (Fig. 2A), suggesting that *HUG1* may also be repressed by Crt1p. Accordingly, Northern blot analysis showed that *HUG1* is constitutively transcribed in *crt1*, *ssn6*, and *tup1* mutants that are deficient for Crt1p-mediated repression. In the absence of DNA damage, *HUG1* is transcribed at levels 78-, 394-, and 20-fold higher in the *crt1*, *ssn6*, and *tup1* mutants than wild-type cells (Fig. 2B). Thus, Crt1p, Ssn6p, and Tup1p are negative regulators of *HUG1* transcription in the absence of DNA damage or replication arrest.

Checkpoint genes in the *MEC1* pathway are required for the transcriptional induction of *HUG1*. Checkpoint genes in the *MEC1* pathway are required for the alleviation of DNA damage-dependent repression of *RNR* genes by the Crt1p-Ssn6p-Tup1p complex (16). The checkpoint genes mediate multiple responses following damage to DNA or the spindle apparatus including cell cycle arrest, transcriptional induction of damage-inducible genes, and repair of DNA damage (12). Unlike most other checkpoint genes, null alleles of *MEC1* (*mec1*Δ) are lethal (17, 47), but mutations in *SML1* (*sml1-1* or *sml1*Δ) (46), *CRT1* (16), or *CLN1* and *CLN2* (38) can suppress this lethality. Since the *sml1*Δ mutation does not affect the transcription of *HUG1* (Fig. 3A), we decided to use a *mec1*Δ *sml1*Δ strain for evaluation of the role of *MEC1* in the transcriptional induction of *HUG1*. Northern blot analysis showed that *MEC1* is required for the transcriptional induction of *HUG1* in response to replication arrest with HU and DNA damage from UV or gamma radiation (Fig. 3A). In contrast, *TEL1*, a functional homolog of *MEC1* (21), is not required for the HU-induced transcription of *HUG1* (data not shown). These results prompted us to determine if other genes in the *MEC1* pathway (see Fig. 7) were required for the transcriptional induction of *HUG1*. Our results showed that the HU (Fig. 3B)-, UV (Fig. 3C), and gamma (Fig. 3D) radiation-induced transcription of *HUG1* is dependent on *RAD53* and partially dependent on *DUN1*. Additionally, transcriptional induction of *HUG1* is dependent on *MEC3* (Fig. 3A, B, and C), *RAD9*, *RAD17*, and *RAD24* (Fig. 3E) upon exposure to UV and gamma radiation, but independent of these genes in the presence of HU. These effects do not appear to be simply due to delayed induction, since no *HUG1* induction is detected in the mutants after 3.5 h in 0.1 M HU, whereas marked induction of *HUG1* is observed as early as 1 h in wild-type cells (Fig. 1D). We conclude that the transcriptional induction of *HUG1* is dependent on *MEC1* and other genes in the checkpoint pathway (Fig. 3 and 7).

Deletion of *HUG1* suppresses *mec1* lethality, and overexpression of *HUG1* increases the sensitivity of the *mec1 sml1-1* strain to HU. To elucidate the role of Hug1p in DNA damage and replication arrest, we deleted the *HUG1* ORF and examined several phenotypes. Deletion of *HUG1* in a haploid strain does not affect growth, sensitivity to DNA-damaging agents, or HU (data not shown). Given the transcriptional dependence of *HUG1* on *MEC1*, we examined the effect of *hug1*Δ on the essential and checkpoint functions of *MEC1*. The *mec1*Δ *sml1-1* strain is viable due to the *sml1-1* mutation (46). We mated a *hug1*Δ *SML1* strain to a *mec1*Δ *sml1-1* strain, sporulated the heterozygous diploid, and analyzed the tetrads. Genetic analysis showed that *hug1*Δ suppresses the lethality due to *mec1*Δ, because we obtained *hug1*Δ *mec1*Δ spores at the expected frequency (see Materials and Methods). The *hug1*Δ *mec1*Δ strain is as sensitive to DNA damage and replication arrest as the parent *mec1*Δ *sml1-1* strain (data not shown). These results were confirmed by tetrad analysis of a mating between the *hug1*Δ and *mec1*Δ[*pMEC1 CEN URA3*] strains. We obtained *hug1*Δ *mec1*Δ[*pMEC1 CEN URA3*] spores that

signal normalized to the *HUG1*/*TUB2* ratio in control lanes 1, 2, and 3 (lanes 4, 7, and 10 normalized to lane 1, lanes 5, 8, and 11 to lane 2, and lanes 6, 9, and 12 to lane 3) as described in Materials and Methods (B, C and D) Northern blot analysis was done with strains grown logarithmically, arrested with HU (B), or exposed to UV (C) or gamma (D) radiation. The strains used were wild type (TWY397), *rad53*/*mec2-1* (TWY312), *mec3-1* (TWY316), wild type (Y203), and *dun1* (Y203-*dun1*). Two lanes between lanes 2 and 3 in panels B, C, and D were deleted because they represented data not relevant to the experiment. *HUG1*/*TUB2* indicates the ratio of the intensity of the *HUG1* signal to the *TUB2* signal in cells treated with HU or UV or gamma radiation and normalized to the *HUG1*/*TUB2* ratio in control lanes without treatment (lane 1 normalized to lane 2, lane 3 to lane 4, lane 5 to lane 6, lane 7 to lane 8, lane 9 to lane 10, and lane 11 to lane 12). Transcription of *TUB2* is not induced by UV or gamma radiation; the data reflect unequal loading of the lanes as evidenced by ethidium bromide staining of the gels (data not shown). (For Fig. 2D, lane 5, the level of *HUG1* was below the background level and hence was denoted as not detected [ND].) The wild-type strain isogenic to the *rad53* and *mec3* mutants is represented in lanes 1 and 2. The wild-type strain isogenic to the *dun1* mutant is represented in lanes 9 and 10. (E) Northern blot analysis using logarithmically grown cells, arrested with HU or exposed to gamma radiation. The strains used were isogenic to the wild-type strain (YMP10381), *rad9*Δ::*LEU2* (YMP10535), *rad17*Δ::*LEU2* (YMP11108), and *rad24*Δ::*TRP1* (YMP10533). *HUG1*/*TUB2* indicates the ratio of the intensity of the *HUG1* signal to the *TUB2* signal in cells treated with HU or gamma radiation and normalized to the *HUG1*/*TUB2* ratio in control lanes without treatment (lanes 5 and 9 normalized to lane 1, lanes 6 and 10 to lane 2, lanes 7 and 11 to lane 3, and lanes 8 and 12 to lane 4).

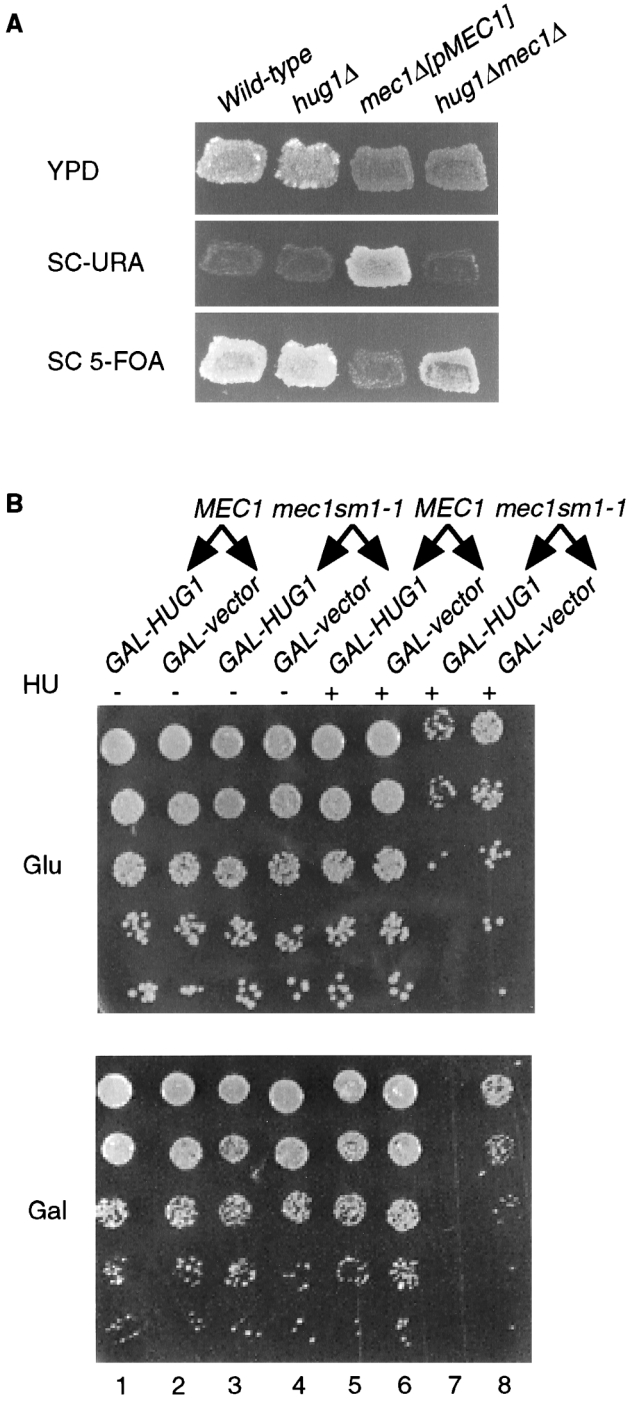

FIG. 4. Genetic interactions between *HUG1* and *MEC1*. (A) Deletion of *HUG1* suppresses the lethality of *mec1*Δ. Strains derived from a mating between the *hug1*Δ (YMB847) and *mec1*Δ*SML1* (pMEC1) (yPP8) strains were plated on control medium YPD and then replica plated to SC-Ura and SC with 5-FOA. The *mec1*Δ *SML1* strain is inviable without the pMEC1 plasmid (pEF208) (growth on SC-Ura, 5-FOA sensitive). The wild-type, *hug1*Δ and *hug1*Δ *mec1*Δ strains can lose the pMEC1 plasmid (no growth on SC-Ura, 5-FOA resistant). (B) Overexpression of *HUG1* (pMB379) increases the sensitivity of *mec1 sml1-1* (DLY258) mutants to replication arrest, with no effect in the wild-type strain (DLY62). Strains were grown logarithmically in either the absence or presence of HU (0.1 M) for 3.5 h, and 5 μl of a fivefold serial dilution series was plated on SC-Ura with glucose (Glu) or SC-Ura with raffinose plus galactose (Gal).

were viable without the pMEC1 plasmid, thus confirming the suppression of *mec1*Δ lethality by deletion of *HUG1* (Fig. 4A). Therefore, *hug1*Δ suppresses *mec1*Δ lethality, but not sensitivity to DNA damage or replication arrest. These results also suggest that *HUG1* may be transcribed either at low levels or in a small fraction of the cells in the absence of DNA damage or replication arrest.

Consistent with the ability of a *HUG1* deletion to suppress *mec1*Δ lethality, we found that overexpression of *HUG1* (*GAL1-HUG1*) increased the sensitivity of the *mec1 sml1-1* strain (DLY258) to HU (Fig. 4B) and UV radiation (data not shown) and had no phenotype in wild-type cells (DLY62) (Fig. 4B). Almost identical results were obtained with another *mec1*Δ *sml1-1* strain (YEF610 lacking pEF208), suggesting that the dosage lethality phenotype is not strain specific (data not shown). The phenotype was specifically due to the *HUG1* protein, because a frameshift mutation in the *HUG1* ORF abolished the dosage lethality phenotype (data not shown).

***HUG1* and *SML1* are adjacent to each other and are transcribed independently.** Similar to the phenotype of a *hug1*Δ, mutations in *SML1* (*sml1-1* or *sml1*Δ) also suppress *mec1*Δ lethality (46). The start codon of *SML1* is 417 bp downstream of the stop codon of *HUG1*, and both genes are transcribed from the same strand of DNA (Fig. 5). Hence, we examined whether *SML1* played a role in the phenotype of suppression of *mec1*Δ lethality by a deletion of *HUG1*. We determined that *HUG1* and *SML1* are transcribed and regulated independently (Fig. 5). For example, unlike *HUG1*, the transcription of *SML1* is not induced by replication arrest or DNA damage and is unaffected by mutations in checkpoint genes (data not shown and reference 46). Additionally, *HUG1* transcription is not affected by a deletion of *SML1* or vice versa (Fig. 5). We also determined that *SML1* is transcribed in a *hug1*Δ *mec1*Δ strain (data not shown). It is interesting to note that in contrast to *sml1*Δ, the *sml1-1* mutation present in most laboratory *mec1* strains (46) overlaps with the 3′ untranslated region of *HUG1* and abolishes the transcription of *HUG1* (Fig. 5).

Deletion of *HUG1* suppresses the HU sensitivity of the *dun1*Δ strain. The protein kinase *DUN1* gene acts downstream of *MEC1* (1, 12, 24, 48) and is required for the efficient induction of *HUG1* following replication arrest and DNA damage (Fig. 3 and 7). The *dun1* mutants exhibit an HU sensitivity that can be suppressed by overexpression of *RNR1* (46). Given the ability of *HUG1* overexpression to increase the sensitivity of the *mec1 sml1-1* strain to replication arrest and DNA damage, we determined whether *HUG1* expression might modulate the HU sensitivity of the *dun1*Δ strain. Genetic analysis demonstrated that a deletion of *HUG1* (*hug1*Δ) suppressed the HU sensitivity of a *dun1*Δ strain (Fig. 6A). As expected, this HU sensitivity was restored in the *dun1*Δ *hug1*Δ strain by a *HUG1*-containing plasmid (Fig. 6B). These findings further support the role of *HUG1* as a critical downstream mediator of the *MEC1* pathway.

DISCUSSION

Here, we show that the SAGE technique (39, 40) used to determine global gene expression can identify transcripts corresponding to NORFs. Systematic analysis of SAGE tags corresponding to intergenic regions suggests the presence of at least 302 NORFs. These NORFs may correspond to small ORFs (<99 amino acids) or large ORFs (>99 amino acids) that may have been overlooked due to possible sequencing errors. Homology searches have shown that 12 of the 30 most highly transcribed NORFs are evolutionarily conserved. One of the NORFs, *NORF5*/*HUG1*, encodes a novel DNA damage

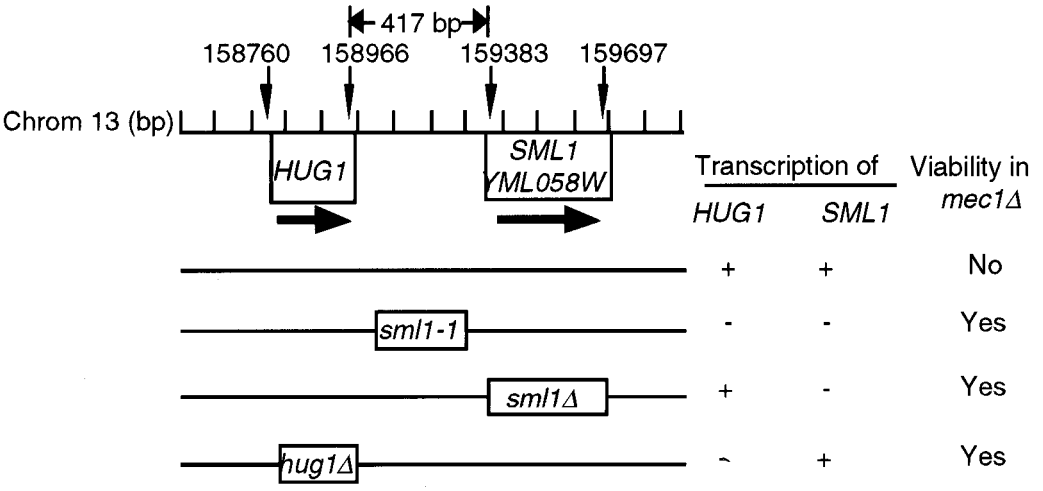

FIG. 5. *HUG1* and *SML1* are transcribed independently, and deletions of either gene suppress *mec1*Δ lethality. The strains used were the wild type (W1588-4A) and the *sml1-1* (YEF630), *sml1*Δ(U952-3C), and *hug1*Δ*2* (YMB847) mutants. Transcription of *SML1* was detected in logarithmically grown cells, whereas that of *HUG1* was only detected in cells arrested with HU. The *sml1-1* mutation (46) deletes a 290-bp region between two direct repeats of 11 bp; the first repeat is 7 bp downstream of the *HUG1* stop codon. The *sml1-1* mutation is present in most laboratory *mec1* strains (25, 46).

and replication arrest-induced gene that is transcriptionally regulated by the genes in the *MEC1* pathway. Our results validate the idea that the NORFs are biologically relevant and highlight the importance of global approaches such as SAGE to identify a significant number of genes in yeast and other organisms that may be missed by sequence analysis alone.

Further characterization of the transcriptional regulation of *HUG1* showed that the promoter of *HUG1* contains three X-box-related sequences (16, 26): one strongly conserved X box (Xs) and two weakly conserved X boxes (Xw). X-box sequences (13 bp in length) were originally identified in the promoters of all MHC class II genes (26) and subsequently

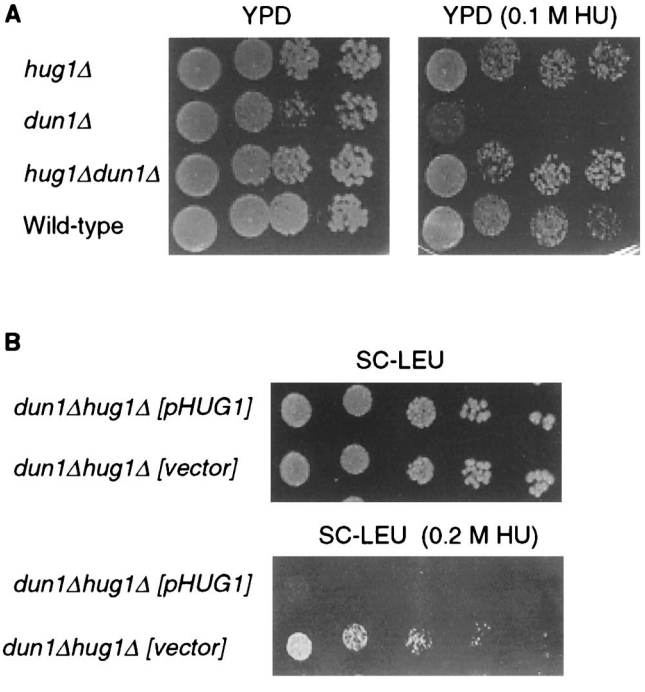

FIG. 6. Deletion of *HUG1* suppresses the HU sensitivity of the *dun1*Δ strain. (A) *hug1*Δ *dun1*Δ strains are resistant to HU. Spores from tetrad analysis of a mating between the *hug1*Δ*2* (YMB847) and *dun1*Δ (U971) strains were plated on YPD medium with or without HU (0.1 M). (B) *HUG1* restores HU sensitivity in a *dun1*Δ *hug1*Δ strain. The *hug1*Δ *dun1*Δ spores from panel A were transformed with pMB363 (*CEN HUG1 LEU2*) or vector alone (pRS315) and plated on SC-Leu with or without HU (0.2 M).

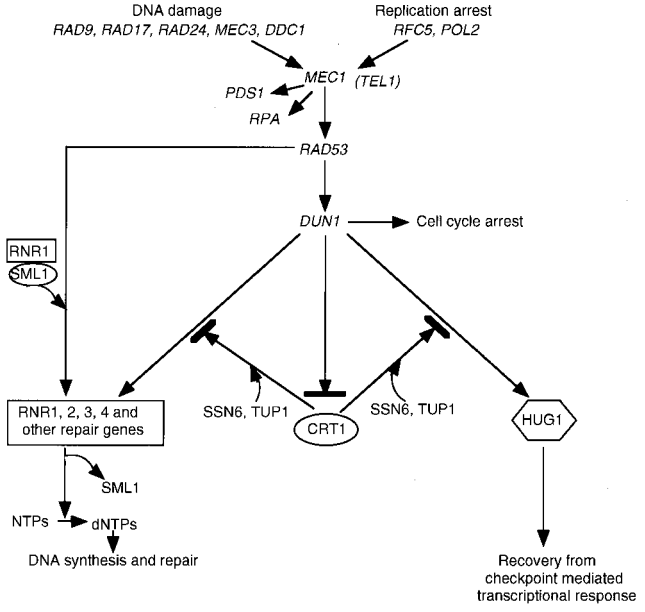

FIG. 7. *HUG1* is a critical component of the checkpoint response. Signals received from the sensors for DNA damage and replication arrest are transduced through the kinases *MEC1* and *TEL1*, leading to phosphorylation and activation of *RAD53* and *DUN1*, causing cell cycle arrest and transcriptional induction, which can be *DUN1* independent or dependent (12). *SML1* (46) and *CRT1* (16) function to negatively regulate the *MEC1* effectors *RNR1* and *RNR1* to 4, respectively. Transcription of *HUG1* is induced in response to replication arrest and DNA damage in a checkpoint-dependent manner. Deletion of *HUG1* rescues the lethality of *mec1*Δ and the HU sensitivity of *dun1*Δ strains; overexpression of *HUG1* is lethal in combination with a *mec1* mutation in the presence of replication arrest or DNA damage. These observations, along with the delayed induction of *HUG1* in response to HU, suggest that *HUG1* may function, in part, through the negative regulation of *MEC1* effectors, perhaps facilitating recovery from the transcriptional response after DNA damage and replication arrest.

found in the promoters of *RNR2*, *RNR3*, *RNR4*, and *CRT1* (16). There is a high degree of conservation between the X boxes; for example, 10 of the 13 bases of Xs in *HUG1* are identical to the Xs of the MHC class II X box (26). Also, the location and orientation of the X boxes in *HUG1* are similar to those of the other X-box-containing genes in *S. cerevisiae*; Xs and Xw in *HUG1* are in opposite orientations located 30 bp apart (16). It has been shown that Crt1p binds specifically to X-box sequences in the promoters of *RNR* genes and mediates repression of these genes by recruitment of the Tup1p-Ssn6p corepressor complex to the promoters of these genes. DNA damage leads to hyperphosphorylation of Crt1p with loss of DNA binding and loss of repression (16). Northern blot analysis showed that *HUG1* is constitutively transcribed in *crt1*, *ssn6*, and *tup1* mutants that are deficient for Crt1p-mediated repression. The degree of derepression of *HUG1* transcription was as follows: *ssn6*>*crt1*>*tup1* mutants. Similar results were reported for the derepression of the *RNR2* promoter in the *ssn6*, *crt1*, and *tup1* mutants (16).

In *S. cerevisiae*, there is a large regulon of genes that show increased transcription in response to DNA damage and replication arrest (1, 12, 19, 20, 24, 28). The checkpoints that are sensitive to DNA damage or replication arrest act in multiple phases of the cell cycle (G_1, S, or G_2 phases) (2, 12, 23, 34, 41–43). The checkpoint genes regulate transcription, facilitate the repair of DNA, and mediate cell cycle arrest and recovery from DNA damage-induced responses (12, 41). The results presented in this paper show that the DNA replication arrest and damage-induced transcription of *HUG1* are dependent on the signal transduction pathway involving the checkpoint genes *RAD9*, *RAD17*, *RAD24*, *MEC3*, *MEC1*, *RAD53*, and *DUN1*.

Despite the major advances in the delineation of the *MEC1* checkpoint pathway, the full complexity of this pathway is just beginning to be addressed (16, 30, 33, 38, 41, 46). The current findings suggest that the small protein Hug1p, the product of a NORF, is a critical mediator of the *MEC1* pathway. Induction of *HUG1* by DNA damage and replication arrest requires an intact *MEC1* pathway, and a deletion of *HUG1* can rescue phenotypes associated with defects in the *MEC1* pathway. Although the precise mechanism of action of *HUG1* remains unclear, several observations suggest that *HUG1* may function, in part, through the negative regulation of *MEC1* pathway effectors, perhaps facilitating the recovery from the transcriptional response after DNA damage and replication arrest. First, mutations in the other two genes (*SML1* and *CRT1*) besides *HUG1* that can rescue *mec1*Δ lethality function to negatively regulate effectors of the *MEC1* pathway (16, 46). Second, overexpression of *HUG1* is lethal in combination with a *mec1* mutation in the presence of DNA damage or replication arrest; this is in contrast to the *MEC1* effectors *RNR1* and *RNR3*, whose overexpression rescues *mec1*Δ lethality (9). Third, transcription of *HUG1* is delayed in response to replication arrest (Fig. 1D), unlike the rapid induction of *RNR3* (16). This delay in *HUG1* induction may allow time for DNA synthesis and repair before recovery. Taken together, these results suggest that *HUG1* is a critical component of the checkpoint response (Fig. 7).

Consistent with the importance of the coordinated response to DNA damage, several key features of these pathways are conserved in human, yeast, and other systems. The *S. cerevisiae MEC1* gene, for example, is homologous to the *Schizosaccharomyces pombe rad3*$^+$ gene, the *Drosophila melanogaster mei-41* gene, and the human *ATM* gene (31). By analogy, a *HUG1* homolog regulated by ATM or p53 may be present in humans. It is not surprising that database searches have failed to detect a homolog of *HUG1*, because it has only been detected in DNA-damaged or replication-arrested cells. Identification and characterization of homologs of *HUG1* from other organisms, including humans, may further our understanding of the role of *MEC1* in budding yeast and may allow greater insight into the ATM- and p53-mediated checkpoint pathway in humans.

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REFERENCES

1. **Aboussekhra, A., J. E. Vialard, D. E. Morrison, M. A. de la Torre-Ruiz, L. Cernakova, F. Fabre, and N. F. Lowndes.** 1996. A novel role for the budding yeast *RAD9* checkpoint gene in DNA damage-dependent transcription. EMBO J. **15:**3912–3922.
2. **Allen, J. B., Z. Zhou, W. Siede, E. C. Friedberg, and S. J. Elledge.** 1994. The *SAD1*/*RAD53* protein kinase controls multiple checkpoints and DNA damage-induced transcription in yeast. Genes Dev. **8:**2401–2415.
3. **Ausubel, F. M. (ed.).** 1997. Current protocols in molecular biology. John Wiley & Sons, Inc., New York, N.Y.
4. **Basrai, M. A., P. Hieter, and J. D. Boeke.** 1997. Small open reading frames: beautiful needles in the haystack. Genome Res. **7:**768–771.
5. **Basrai, M. A., J. Kingsbury, D. Koshland, F. Spencer, and P. Hieter.** 1996. Faithful chromosome transmission requires Spt4p, a putative regulator of chromatin structure in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:**2838–2847.
6. **Baudin, A., O. Ozier-Kalogeropoulos, A. Denouel, F. Lacroute, and C. Cullin.** 1993. A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. Nucleic Acids Res. **21:**3329–3330.
7. **Boeke, J. D., F. LaCroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine-5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197:**345–346.
8. **Brush, G. S., D. M. Morrow, P. Hieter, and T. J. Kelly.** 1996. The ATM homologue *MEC1* is required for phosphorylation of replication protein A in yeast. Proc. Natl. Acad. Sci. USA **93:**15075–15080.

8a. **Cyano2Dbase.** 14 January 1999, revision date. ORFs. [Online.] http://www.kazusa.or.jp/tech/sazuka/cyano/proteome.html. [23 August 1999, last date accessed.]

9. **Desany, B. A., A. A. Alcasabas, J. B. Bachant, and S. J. Elledge.** 1998. Recovery from DNA replicational stress is the essential function of the S-phase checkpoint pathway. Genes Dev. **12:**2956–2970.
10. **Dujon, B., D. Alexandraki, B. Andre, W. Ansorge, V. Baladron, J. P. Ballesta, A. Banrevi, P. A. Bolle, M. Bolotin-Fukuhara, P. Bossier, et al.** 1994. Complete DNA sequence of yeast chromosome XI. Nature **369:**371–378.

10a. **E. coli Genome Center.** 5 September 1997, revision date. ORFs. [Online.] E. coli Genome Center, University of Wisconsin, Madison. http://genetics/wisc.edu/. [23 August 1999, last date accessed.]

11. **el-Deiry, W. S., T. Tokino, V. E. Velculescu, D. B. Levy, R. Parsons, J. M. Trent, D. Lin, W. E. Mercer, K. W. Kinzler, and B. Vogelstein.** 1993. WAF1, a potential mediator of p53 tumor suppression. Cell **75:**817–825.
12. **Elledge, S. J.** 1996. Cell cycle checkpoints: preventing an identity crisis. Science **274:**1664–1672.
13. **Elledge, S. J., Z. Zhou, J. B. Allen, and T. A. Navas.** 1993. DNA damage and cell cycle regulation of ribonucleotide reductase. Bioessays **15:**333–339.
14. **Goffeau, A., B. G. Barrell, H. Bussey, R. W. Davis, B. Dujon, H. Feldmann, F. Galibert, J. D. Hoheisel, C. Jacq, M. Johnston, E. J. Louis, H. W. Mewes, Y. Murakami, P. Philippsen, H. Tettelin, and S. G. Oliver.** 1996. Life with 6000 genes. Science **274:**546, 563–567.
15. **Huang, M., and S. J. Elledge.** 1997. Identification of *RNR4*, encoding a second essential small subunit of ribonucleotide reductase in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **17:**6105–6113.
16. **Huang, M., Z. Zhou, and S. J. Elledge.** 1998. The DNA replication and damage checkpoint pathways induce transcription by inhibition of the Crt1 repressor. Cell **94:**595–605.

17. **Kato, R., and H. Ogawa.** 1994. An essential gene, *ESR1*, is required for mitotic cell growth, DNA repair and meiotic recombination in *Saccharomyces cerevisiae*. Nucleic Acids Res. **22:**3104–3112.
18. **Kenna, M. A., C. B. Brachmann, S. E. Devine, and J. D. Boeke.** 1998. Invading the yeast nucleus: a nuclear localization signal at the C terminus of Ty1 integrase is required for transposition in vivo. Mol. Cell. Biol. **18:**1115–1124.
19. **Kiser, G. L., and T. A. Weinert.** 1996. Distinct roles of yeast *MEC* and *RAD* checkpoint genes in transcriptional induction after DNA damage and implications for function. Mol. Biol. Cell **7:**703–718.
20. **McClanahan, T., and K. McEntee.** 1986. DNA damage and heat shock dually regulate genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6:**90–96.
20a. **MIPS.** 9 August 1999, revision date. ORFs. [Online.] Munich Information Centre for Protein Sequences, Martinsfried, Germany. http://www.mips.biochem.mpg.de/proj/yeast/tables/small_orfs.html. [23 August 1999, last date accessed.]
21. **Morrow, D. M., D. A. Tagle, Y. Shiloh, F. S. Collins, and P. Hieter.** 1995. *TEL1*, an *S. cerevisiae* homolog of the human gene mutated in ataxia telangiectasia, is functionally related to the yeast checkpoint gene *MEC1*. Cell **82:**831–840.
22. **Mumberg, D., R. Muller, and M. Funk.** 1994. Regulatable promoters of Saccharomyces cerevisiae: comparison of transcriptional activity and their use for heterologous expression. Nucleic Acids Res. **22:**5767–5768.
23. **Navas, T. A., Z. Zhou, and S. J. Elledge.** 1995. DNA polymerase epsilon links the DNA replication machinery to the S phase checkpoint. Cell **80:**29–39.
24. **Pati, D., C. Keller, M. Groudine, and S. E. Plon.** 1997. Reconstitution of a *MEC1*-independent checkpoint in yeast by expression of a novel human *fork head* cDNA. Mol. Cell. Biol. **17:**3037–3046.
25. **Paulovich, A. G., R. U. Margulies, B. M. Garvik, and L. H. Hartwell.** 1997. *RAD9*, *RAD17*, and *RAD24* are required for S phase regulation in Saccharomyces cerevisiae in response to DNA damage. Genetics **145:**45–62.
26. **Reith, W., C. Herrero-Sanchez, M. Kobr, P. Silacci, C. Berte, E. Barras, S. Fey, and B. Mach.** 1990. MHC class II regulatory factor RFX has a novel DNA-binding domain and a functionally independent dimerization domain. Genes Dev. **4:**1528–1540.
27. **Reith, W., C. Ucla, E. Barras, A. Gaud, B. Durand, C. Herrero-Sanchez, M. Kobr, and B. Mach.** 1994. RFX1, a transactivator of hepatitis B virus enhancer I, belongs to a novel family of homodimeric and heterodimeric DNA-binding proteins. Mol. Cell. Biol. **14:**1230–1244.
28. **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.
28a. **Sagenet.** 7 March 1999, revision date. ORFs. [Online.] http://www.sagenet.org/NORF/NORF.html. [23 August 1999, last date accessed.]
29. **Sanchez, Y., B. A. Desany, W. J. Jones, Q. Liu, B. Wang, and S. J. Elledge.** 1996. Regulation of *RAD53* by the ATM-like kinases *MEC1* and *TEL1* in yeast cell cycle checkpoint pathways. Science **271:**357–360.
29a. **Sanger Website.** 13 August 1999, revision date. ORFs. [Online.] http://www.songer.ac.uk/Projects/C_elegans. [23 August 1999, last date accessed.]
30. **Santocanale, C., and J. F. Diffley.** 1998. A Mec1- and Rad53-dependent checkpoint controls late-firing origins of DNA replication Nature **395:**615–618.
31. **Savitsky, K., S. Sfez, D. A. Tagle, Y. Ziv, A. Sartiel, F. S. Collins, Y. Shiloh, and G. Rotman.** 1995. The complete sequence of the coding region of the ATM gene reveals similarity to cell cycle regulators in different species. Hum. Mol. Genet. **4:**2025–2032.
32. **Shiloh, Y.** 1997. Ataxia-telangiectasia and the Nijmegen breakage syndrome: related disorders but genes apart. Annu. Rev. Genet. **31:**635–662.
33. **Shirahige, K., Y. Hori, K. Shiraishi, M. Yamashita, K. Takahashi, C. Obuse, T. Tsurimoto, and H. Yoshikawa.** 1998. Regulation of DNA-replication origins during cell-cycle progression. Nature **395:**618–621.
34. **Siede, W., A. S. Friedberg, and E. C. Friedberg.** 1993. *RAD9*-dependent G1 arrest defines a second checkpoint for damaged DNA in the cell cycle of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **90:**7985–7989.
35. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
35a. **Stanford Genome Database.** 17 July 1999, revision date. ORFs. [Online.] Department of Genetics, Stanford University School of Medicine, Stanford, Calif. http://genome-www.stanford.edu/Saccharomyces/. [23 August 1999, last date accessed.]
35b. **Stanford Genome Database.** 17 July 1999, revision date. ORFs. [Online.] http://genome-www.stanford.edu/Saccharomyces/newORF.html. [23 August 1999, last date accessed.]
36. **Sun, Z., D. S. Fay, F. Marini, M. Foiani, and D. F. Stern.** 1996. Spk1/Rad53 is regulated by Mec1-dependent protein phosphorylation in DNA replication and damage checkpoint pathways. Genes Dev. **10:**395–406.
37. **Tzamarias, D., and K. Struhl.** 1995. Distinct TPR motifs of Cyc8 are involved in recruiting the Cyc8-Tup1 corepressor complex to differentially regulated promoters. Genes Dev. **9:**821–831.
38. **Vallen, E. A., and F. R. Cross.** 1999. Interaction between the MEC1-dependent DNA synthesis checkpoint and G1 cyclin function in *Saccharomyces cerevisiae*. Genetics **151:**459–471.
39. **Velculescu, V. E., L. Zhang, B. Vogelstein, and K. W. Kinzler.** 1995. Serial analysis of gene expression. Science **270:**484–487.
40. **Velculescu, V. E., L. Zhang, W. Zhou, J. Vogelstein, M. A. Basrai, D. E. Bassett, Jr., P. Hieter, B. Vogelstein, and K. W. Kinzler.** 1997. Characterization of the yeast transcriptome. Cell **88:**243–251.
41. **Weinert, T.** 1998. DNA damage checkpoints update: getting molecular. Curr. Opin. Genet. Dev. **8:**185–193.
42. **Weinert, T. A., and L. H. Hartwell.** 1993. Cell cycle arrest of *cdc* mutants and specificity of the *RAD9* checkpoint. Genetics **134:**63–80.
43. **Weinert, T. A., and L. H. Hartwell.** 1988. The *RAD9* gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. Science **241:**317–322.
44. **Weinert, T. A., G. L. Kiser, and L. H. Hartwell.** 1994. Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair. Genes Dev. **8:**652–665.
45. **Zakian, V. A.** 1995. ATM-related genes: what do they tell us about functions of the human gene? Cell **82:**685–687.
46. **Zhao, X., E. G. Muller, and R. Rothstein.** 1998. A suppressor of two essential checkpoint genes identifies a novel protein that negatively affects dNTP pools. Mol. Cell **2:**329–340.
47. **Zheng, P., D. S. Fay, J. Burton, H. Xiao, J. L. Pinkham, and D. F. Stern.** 1993. *SPK1* is an essential S-phase-specific gene of *Saccharomyces cerevisiae* that encodes a nuclear serine/threonine/tyrosine kinase. Mol. Cell. Biol. **13:**5829–5842.
48. **Zhou, Z., and S. J. Elledge.** 1993. *DUN1* encodes a protein kinase that controls the DNA damage response in yeast. Cell **75:**1119–1127.