An Essential Role for the *Saccharomyces cerevisiae* DEAD-Box Helicase DHH1 in G1/S DNA-Damage Checkpoint Recovery

Megan Bergkessel[1] and Joseph C. Reese[2]

Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, Pennsylvania 16802

Manuscript received October 31, 2003
Accepted for publication January 9, 2004

ABSTRACT

The eukaryotic cell cycle displays a degree of plasticity in its regulation; cell cycle progression can be transiently arrested in response to environmental stresses. While the signaling pathways leading to cell cycle arrest are beginning to be well understood, the regulation of the release from arrest has not been well characterized. Here we show that *DHH1*, encoding a DEAD-box RNA helicase orthologous to the human putative proto-oncogene p54/RCK, is important in release from DNA-damage-induced cell cycle arrest at the G1/S checkpoint. *DHH1* mutants are not defective for DNA repair and recover normally from the G2/M and replication checkpoints, suggesting a specific function for Dhh1p in recovery from G1/S checkpoint arrest. Dhh1p has been suggested to play a role in partitioning mRNAs between translatable and nontranslatable pools, and our results implicate this modulation of mRNA metabolism in the recovery from G1/S cell cycle arrest following DNA damage. Furthermore, the high degree of conservation between *DHH1* and its human ortholog suggests that this mechanism is conserved among all eukaryotes and potentially important in human disease.

THE G1-to-S phase transition, termed START in yeast, represents an important and thus highly regulated decision point in the cell cycle, as it signifies a commitment to completion of cell division (Levine *et al.* 1995; Reed 1997). Eukaryotic cells are capable of undergoing a transient arrest at the G1/S transition if conditions that would be unfavorable for cell division, such as nutrient limitation (Gallego *et al.* 1997), environmental toxins (Philpott *et al.* 1998), or damaged DNA are encountered. This capacity for transient arrest allows the cell to respond to environmental stresses in such a way that viability is maximized. Disruption of either the ability to initiate the arrest or the ability to subsequently recover from the arrest and resume cell division appears to be detrimental (Hartwell *et al.* 1994; Lydall and Weinert 1995; Shaulian *et al.* 2000).

In the case of DNA damage, much more is known about the signaling cascade leading to the initiation of the transient arrest, known as the checkpoint response, than about the mechanisms regulating the subsequent release from checkpoint arrest. The DNA-damage signaling cascade appears to be highly conserved throughout eukaryotes (Lydall and Weinert 1996). Damage activates a series of phosphorylation events leading to phosphorylation of the *ATM* homologs, *MEC1* and *TEL1* (Morrow *et al.* 1995; Siede *et al.* 1996). These lipid kinase family members are believed to be partially redundant in the DNA-damage signaling cascade and cause phosphorylation of a kinase encoded by *RAD53* (Sanchez *et al.* 1996; Sun *et al.* 1996). Rad53p then phosphorylates the transcription factor component Swi6p, which causes a delay in the accumulation of mRNA for G1 cyclins and thus a transient cell cycle arrest (Sidorova and Breeden 1997). While much is understood about the initiation of the transient G1/S checkpoint arrest, little is known about the downstream events, regulating release from the arrest. Our evidence suggests that the yeast gene *DHH1* plays a role in this process.

DHH1 encodes a highly conserved putative DEAD-box RNA helicase that has been shown to associate with factors that are reported components of mRNA decapping, deadenylation, and transcription complexes in yeast (Coller *et al.* 2001; Fischer and Weis 2002; Maillet and Collart 2002). Dhh1p stimulates mRNA decapping by the decapping enzyme Dcp1p, and it has been shown to localize, along with other proteins involved in decapping and mRNA degradation, to discrete cytoplasmic foci known as P-bodies. P-bodies are believed to be involved in sequestering mRNAs in a nontranslating pool, from which they are subsequently degraded or possibly reactivated for translation (Sheth and Parker 2003). Highly conserved orthologs of Dhh1p have been shown to play a role in repressing translation of specific messages as part of the intricate program of translational control that operates in early development of Xenopus (Smillie and Sommerville 2002), Drosophila (Nakamura *et al.* 2001), and *Spisula solidissima*

[1]*Present address:* Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94107.

[2]*Corresponding author:* Department of Biochemistry and Molecular Biology, Penn State University, 203 Althouse Lab, University Park, PA 16802. E-mail: jcr8@psu.edu

(clam; Minshall *et al.* 2001). The Xenopus ortholog of Dhh1p, Xp54, is known to localize to stored maternal mRNPs in oocytes (Ladomery *et al.* 1997), where its role in sequestering mRNAs to a nontranslating pool in discrete cytoplasmic foci appears to be analogous to the role of Dhh1p in yeast P-bodies. Indeed, overexpression of Xp54 in a *dhh1*Δ yeast strain can functionally compensate for the lack of Dhh1p (Tseng-Rogenski *et al.* 2003).

The significance of the interactions between Dhh1p and components of deadenylation and transcription complexes is less clear. Physical and genetic interactions between Dhh1p and Ccr4p, Pop2p, and Not1p have been reported (Hata *et al.* 1998; Coller *et al.* 2001; Maillet and Collart 2002). Ccr4p is the catalytic subunit in the yeast deadenylation machinery, and Pop2p and at least some of the Not proteins also appear to be components of the deadenylation complex (Tucker *et al.* 2001, 2002). The Ccr4-Not complex is also believed to act in the regulation of transcription (Collart and Struhl 1994; Hata *et al.* 1998; Deluen *et al.* 2002). It has been suggested that the Ccr4-Pop2-Not complex may act to coordinate the behavior of a transcript from its transcription through its deadenylation (Tucker *et al.* 2001; Maillet and Collart 2002), and the reported interactions of Dhh1p with components that appear to be involved in both regulating transcription (the Not proteins) and decapping (Dcp1p and Dcp2p) suggests Dhh1p may contribute to or even extend this coordinated regulation.

Interestingly, the human homolog of *DHH1*, p54/RCK (DDX6), is a target gene of a chromosomal translocation breakpoint (11q23.3) fusion from a B-cell lymphoma and is overexpressed in several malignant cell types (Lu and Yunis 1992; Akao *et al.* 1995; Nakagawa *et al.* 1999); thus, it is a candidate proto-oncogene.

Here we show that *dhh1*Δ mutant cells are hypersensitive to DNA damage and deficient in cell cycle reentry following activation of the G1/S checkpoint by DNA damage. These defects are specific to DNA damage-induced G1/S arrest, as *DHH1* is not required for recovery from α-factor-induced G1 arrest or for recovery from the replication (S) or G2/M DNA-damage checkpoints. Partially inactivating the G1/S checkpoint by deletion of *MEC1* can alleviate the requirement for *DHH1* in passing through START following DNA damage. However, overriding cell cycle checkpoints completely or constitutively overexpressing the G1 cyclin *CLN3* is lethal in *dhh1*Δ cells, even in the absence of exogenous DNA damage. Deleting the gene encoding the mRNA decapping enzyme Dcp1 likewise caused increased sensitivity to DNA damage. In conjunction with the recent studies that strongly implicate Dhh1p and its orthologs in regulation of mRNA stability and translation, these results suggest that crucial aspects of G1/S checkpoint regulation are carried out at the level of mRNA metabolism. The conservation of sequence and function between *DHH1* and its human ortholog implies that the disruption of normal checkpoint functions is a likely mechanism for p54/RCK-associated oncogenesis.

MATERIALS AND METHODS

Strains and growth conditions: All strains are isogenic with PH499 (*MAT***a**, *ade2-101 ochre his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 amber*), unless otherwise noted. Strains used throughout these studies are listed in Table 1. Strains YRP840 (*DCP1*) and YRP1071 (*dcp1*Δ) were described previously (Tharun and Parker 2001). The coding sequence of p54/RCK (DDX6) was amplified by PCR from a HeLa cell cDNA expression library and cloned into a derivative of a yeast expression vector (pG1; Schena *et al.* 1991) containing two HA epitope sequences inserted at the *Bam*HI site. *CLN3* derivatives were expressed from the *GAL1* promoter using the vector pYES2 (Clontech, Palo Alto, CA). The destruction box mutant (CLN3ΔDB) was constructed by introducing a stop codon at residue 398. Cultures for fluorescence-activated cell sorting (FACS) analysis were grown in minimal media (yeast nitrogen base, dextrose, and complete amino acids). Other cultures were grown in rich media (1% yeast extract, 2% Bacto-Peptone, and 2% dextrose). Doses of ultraviolet (UV) radiation or methyl methanesulfonate (MMS) and growth conditions following treatment with DNA-damaging agents are described in the figure legends.

FACS and checkpoint analysis: Synchronization in G1 was achieved by treatment of a midlog phase culture with α-factor (Sigma, St. Louis) at a concentration of 0.2 μg/ml (or 5 μg/ml for strains with an intact *BAR1* gene) for 3–3.5 hr. Synchronization in S phase was achieved by treatment of a midlog phase culture with hydroxyurea at a concentration of 150 mM for 3–3.5 hr, and G2/M synchronizaton was achieved by treatment of a midlog phase culture with nocodazole at a concentration of 10 μg/ml for 3–3.5 hr. To induce DNA damage by ultraviolet radiation, cultures were centrifuged and resuspended in a small volume of media, spread onto 150-mm solid media plates at a density of ~13–14 OD_{600nm} units per plate, and then exposed to either 50 or 60 J/m^2 of UV light. Cells were then scraped off the plates, further washed to remove α-factor, and resuspended in fresh media at OD_{600nm} of 0.5. To induce DNA damage by the alkylating agent MMS, cultures were treated with MMS at a concentration of 0.2% for 30 min. Cells were then collected by centrifugation, washed once with media containing 10% sodium thiosulfate to inactivate the MMS, and washed twice more with fresh media to remove α-factor, before resuspension in fresh media at OD_{600nm} of 0.5. Cells were collected and RNA was isolated for Northern blotting or processed for FACS analysis and budding indices were measured as described in previous publications (Weinert *et al.* 1994; Sidorova and Breeden 1997).

DNA repair assay: DNA-damage repair assays were conducted as described in a previous publication with modifications (Gillette *et al.* 2001).

DHH1 (YJR218), *dhh1*Δ (YJR219), and *rad23*Δ (Research Genetics, Birmingham, AL) cells were grown in YPAD at 30° to an OD_{600} of 0.6–0.7. A 50-ml aliquot of cells was collected by centrifugation, washed in sterile water, and resuspended into 40 ml ice-cold PBS. From this point on all manipulations were performed under low-light conditions and using amber centrifuge tubes. Twenty milliliters of the cell suspension was transferred to each of two 150-mm petri dishes and exposed to 40–60 J/m^2 UV irradiation using a Stratalinker 2400 (Stratagene, La Jolla, CA). The cells in the experiment shown in Figure 4 were exposed to 50 J/m^2, but *dhh1*Δ cells repaired DNA damage as well as the wild type when exposed to 40, 50, or 60 J/m^2 of UV irradiation (not shown). The cells were

TABLE 1

Strains constructed in this study

PH499	*MAT***a**, *ade2-101 ochre his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 amber*
PH500	As PH499; *MAT*α
PH501	As PH499; *MAT***a**/α diploid
YJR218	As PH499; *bar1Δ::hisg*
YJR219	As PH499; *dhh1Δ::HIS3 bar1Δ::hisg*
YJR530	As PH501; *dhh1Δ::HIS3/DHH1 mec1Δ::LEU2/MEC1 sml1Δ::URA3/SML1*
YJR531	As PH499; *dhh1Δ::HIS3 mec1Δ::LEU2 sml1Δ::URA3*
YJR532	As PH500; *dhh1Δ::HIS3 mec1Δ::LEU2 sml1Δ::URA3*
YJR533	As PH499; *mec1Δ::LEU2 sml1Δ::URA3*
YJR534	As PH499; *dhh1Δ::HIS3 sml1Δ::URA3*
YJR535	As PH499; *sml1Δ::URA3*
YJR536	As PH501; *dhh1Δ::HIS3/DHH1 rad53Δ::TRP1/RAD53 sml1Δ::URA3/SML1*
YJR537	As PH499; *rad53Δ::TRP1 sml1Δ::URA3*
YJR538	As PH500; *rad53Δ::TRP1 sml1Δ::URA3*
YJR721	As PH501; *dhh1Δ::HIS3/DHH1 rad53Δ::TRP1/RAD53 sml1Δ::LEU2/SML1*
YJR722	As PH501; *dhh1Δ::HIS3/DHH1 rad53Δ::TRP1/RAD53 sml1Δ::URA3/SML1[pRS416-DHH1]*
YJR723	As PH499; *dhh1Δ::HIS3 rad53Δ::TRP1 sml1::LEU2[pRS416-DHH1]*
YJR724	As PH499; *dhh1Δ::HIS3 mec1Δ::LEU2 sml1Δ::TRP1*
YJR725	As PH499; *dhh1Δ::HIS3 mec1Δ::LEU2 sml1Δ::TRP1[pRS416-DHH1]*
YJR726	As PH499; *dhh1Δ::HIS3 mec1Δ::LEU2 sml1Δ::TRP1 tel1Δ::KanMx[pRS416-DHH1]*
YJR727	As PH499; *rad53Δ::TRP1 sml1::LEU2*
YJR745	As PH499; *sml1Δ::LEU2*
YJR746	As PH499; *mec1Δ::LEU2 sml1Δ::TRP1*
YJR748	As PH499; *mec1Δ::LEU2 sml1Δ::URA3 tel1Δ::KanMx*
YJR749	As PH499; *mec1Δ::LEU2 sml1Δ::TRP1 tel1Δ::KanMx*
YJR750	As PH501; *dhh1Δ::HIS3/DHH1 mec1Δ::LEU2/MEC1 sml1Δ::TRP1/SML1 tel1Δ::KanMx/TEL1*
YJR751	As PH500; *dhh1Δ::HIS3 sml1Δ::TRP1*
YJR752	As PH500; *sml1Δ::TRP1*
YJR753	As PH500; *dhh1Δ::HIS3 mec1Δ::LEU2 sml1Δ::TRP1*
YJR754	As PH499; *tel1Δ::KanMx*
YJR755	As PH500; *tel1Δ::KanMx*
YJR756	As PH499; *sml1Δ::TRP1 tel1Δ::KanMx*
YJR757	As PH500; *sml1Δ::TRP1 tel1Δ::KanMx*
YJR758	As PH499; *mec1Δ::LEU2 sml1Δ::TRP1 tel1Δ::KanMx*
YJR759	As PH499; *dhh1Δ::HIS3 tel1Δ::KanMx*
YJR760	As PH500; *dhh1Δ::HIS3 tel1Δ::KanMx*
YJR761	As PH499; *dhh1Δ::HIS3 sml1Δ::TRP1 tel1Δ::KanMx*
YJR762	As PH499; *dhh1Δ::HIS3 sml1Δ::TRP1 tel1Δ::KanMx*

transferred to a 50-ml tube and an aliquot was immediately removed and placed on ice for the $t = 0$ sample. The cells were collected by centrifugation, returned to prewarmed YPAD media, and allowed to recover in the dark. Experiments were also repeated where cells were maintained in PBS during the recovery phase to prevent the dilution of the adducts by DNA replication (Gillette *et al.* 2001), and under these conditions the removal of adducts was similar in the wild type and mutant (not shown). At the specified time point cells were collected, washed in ice-cold STE (10 mm Tris-HCl, pH 7.5, 100 mm NaCl, 1 mm EDTA), and frozen at −80°. Genomic DNA was isolated using standard techniques and quantified by agarose gel electrophoresis. Approximately 100 ng of DNA was denatured in 0.3 ml of 0.4 m NaOH, 1 mm EDTA for 10 min at 65° and was applied to HybondN+ (Amersham-Pharmacia) using a slot blot manifold. The membrane was blocked in 2% nonfat milk (NFM) in TBST (50 mm Tris-HCl, ph 7.4, 150 mm NaCl, 0.05% Tween 20) for 1 hr and incubated for 1 hr at 37° with anti-thymidine dimer monoclonal antibody KTM53 (Kamiya Biomedical, Seattle, WA) prepared in 0.5% NFM in TBST at a 1:300 dilution. After washing, the membrane was incubated at room temperature with a secondary antibody/horseradish peroxidase conjugate in 0.5% NFM in TBST. The secondary antibody was detected by chemiluminescence. Western blot signals were corrected for the amount of DNA bound to the membrane, which was determined by probing the membrane with radiolabeled total genomic DNA.

RESULTS

***DHH1* is highly conserved and *dhh1Δ* cells are sensitive to DNA damage:** A *DHH1* null mutant is viable, but grows more slowly than the isogenic wild-type strain and displays temperature-sensitive growth (Strahl-Bolsinger and Tanner 1993; Hata *et al.* 1998; Supplemental Figure 1 at http://www.genetics.org/supplemental/). We found that it is also hypersensitive to various DNA-damaging agents, including UV irradiation and the DNA alkylating agent MMS (Figure 1A).

Dhh1p displays a remarkably high identity (68%) and similarity (82%) to its human ortholog p54/RCK over the central 400 amino acids of the yeast protein (Figure 1B). This unusually high degree of conservation argues that the functions of these genes have been highly con-

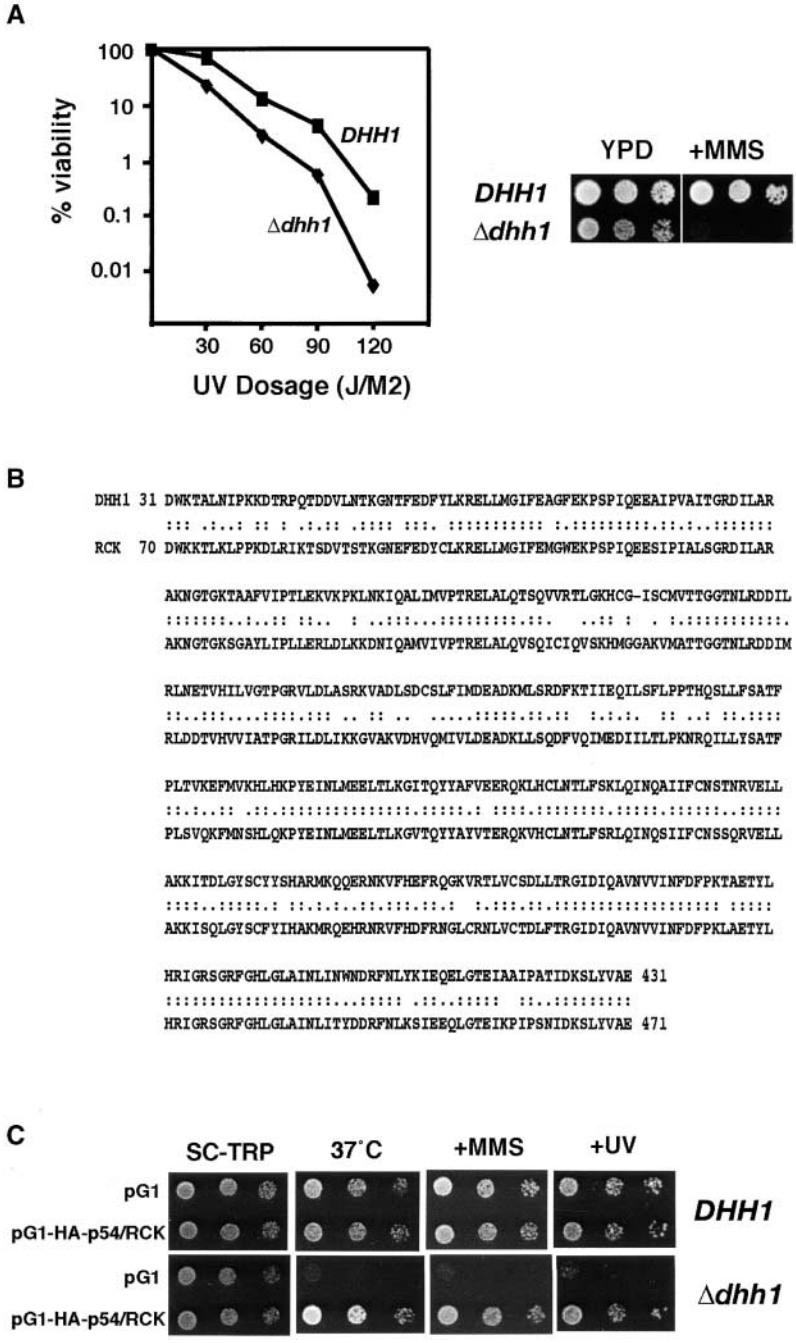


FIGURE 1.—*DHH1* is the ortholog of p54/RCK and is required for resistance to DNA damage. (A) Viability after exposure to UV irradiation and growth on YPD plates containing 0.015% MMS were measured in YJR218 (*DHH1*) and YJR219 (*dhh1*Δ). Plates lacking and containing MMS were grown for 2 or 3 days at 30°, respectively. (B) Sequence conservation between Dhh1p and p54/RCK. The lengths of Dhh1p and p54/RCK are 506 and 482 amino acids, respectively. (C) Complementation of the temperature-sensitive and DNA-damage-sensitive phenotypes of the *dhh1*Δ mutant by p54/RCK expression. Fivefold serial dilutions of cultures of YJR218 and YJR219 transformed with pG1 or pG1-$(HA)_2$-p54/RCK were spotted onto SC-tryptophan plates or the same medium containing 0.01% MMS, and one was exposed to 60 J/m^2 UV irradiation after plating. Plates were placed at 30° for 2 or 3 days (DNA damage exposure and 37° conditions).

served throughout evolution. To address this, the coding sequence of p54/RCK was amplified and inserted into a yeast expression vector (pG1), and the resulting plasmid was introduced into cells carrying a *DHH1* null allele. The results shown in Figure 1C reveal that expression of p54/RCK can complement the temperature-sensitive growth and DNA-damage-sensitive phenotypes of the *dhh1*Δ cells, suggesting that the two proteins perform many of the same functions in their respective organisms. These results provide strong evidence that *DHH1* is indeed the ortholog of the putative oncogene p54/RCK.

***dhh1*Δ cells are defective in G1/S DNA-damage checkpoint recovery:** Because DNA-damage sensitivity is often associated with defects in checkpoint-induced cell cycle arrest and because *DHH1* is genetically linked to cell cycle control (MORIYA and ISONO 1999; REESE and GREEN 2001), we investigated the cell cycle characteristics of the *dhh1*Δ strain using FACS analysis. The FACS profile of an asynchronous population of *dhh1*Δ cells is indistinguishable from that of the wild type, indicating that they are not delayed at any particular point in a normal, uninterrupted cell cycle and that the slow-growth phenotype is due to generally slowed progres-

sion throughout the cell cycle (Supplemental Figure 1). We next investigated the checkpoint response of *dhh1*Δ cells after inducing DNA damage. Populations of wild-type and *dhh1*Δ cells were synchronized in the G1 phase of the cell cycle, using the mating pheromone α-factor. Half of each population was exposed to the DNA-damaging agent MMS, and then the α-factor and MMS were removed by washing with fresh media.

In the absence of DNA damage, *dhh1*Δ cells required an additional 15–20 min to emerge from α-factor-induced arrest relative to the wild-type cells (Figure 2A, top), consistent with the overall reduced growth rate of the strain. Wild-type cells displayed a delay in G1/S progression after exposure to DNA damage due to activation of the G1/S checkpoint, but these cells resumed cell cycle progression by 90–120 min after release, and approximately half the cells entered G2 by 180 min (Figure 2A, bottom left). Like the wild type, the *dhh1*Δ mutant activated its checkpoint and arrested, indicating no defects in checkpoint activation. However, the *dhh1*Δ cells showed a severely protracted G1 arrest and a somewhat slowed S-phase compared to that of the wild-type strain (Figure 2A, bottom right). *dhh1*Δ cells began to resume progression into S-phase only at 4 hr after release and required 6 hr for a small population of cells to enter G2. Similar results were observed in UV-treated cells (Figure 2B). This phenotype is specific for DNA-damage-induced checkpoints because *dhh1*Δ cells did not show an extensive delay relative to wild-type cells in emerging from G1/S arrest in the absence of DNA damage (Figure 2A, top right). Thus, *dhh1*Δ cells are competent for cell cycle arrest, but appear to be unable to subsequently recover from the arrest. This behavior is in contrast to that of known DNA-damage cell cycle checkpoint mutants, which fail to arrest after DNA damage (Hartwell and Kastan 1994; Weinert *et al.* 1994; Lydall and Weinert 1995; Zhou and Elledge 2000).

The FACS analysis presented above indicates that *dhh1*Δ cells delayed DNA replication under conditions of DNA damage. However, this assay cannot distinguish cells that failed to progress through the G1/S boundary from those that passed through the checkpoint and arrested prior to DNA replication. To further characterize the precise position of the protracted cell cycle arrest in *dhh1*Δ cells following DNA damage, we followed the accumulation of the mRNA for the cyclin *CLN2* over the same arrest-and-release time course. A strong but transient burst of expression of the G1 cyclins *CLN1* and *CLN2* is part of the cascade of gene expression that defines passage through the G1/S transition (START; Levine *et al.* 1995; Nasmyth 1996). It has been shown that the activation of the G1/S checkpoint affects its cell cycle delay at least in part by causing a delay in the reaccumulation of *CLN2* mRNA (Sidorova and Breeden 1997) and, therefore, measuring CLN2 expression is a direct way of monitoring release from the G1/S DNA-damage checkpoint. Thus, if the failure in cell cycle progression observed for *dhh1*Δ cells is truly a failure in reemergence from checkpoint arrest, then it should be characterized by a protracted delay in *CLN2* reaccumulation. This is what was observed upon measuring *CLN2* reaccumulation by Northern blotting. In the absence of DNA damage, the peak of *CLN2* expression is delayed ~20–30 min in the *dhh1*Δ strain compared to the wild type, as might be expected from the slight delay observed in the FACS profiles shown in Figure 2A (Figure 2C). However, following UV treatment, the peak of *CLN2* mRNA is delayed ~120–180 min in the mutant cells compared to the wild-type cells, again consistent with the FACS data. These data indicate that the *dhh1*Δ cells are delayed at the G1/S boundary prior to START, at the cell cycle position of the G1/S checkpoint arrest.

The protracted G1/S arrest of *dhh1*Δ cells is checkpoint dependent and not due to repair defects: If indeed the protracted arrest of the *dhh1*Δ cells is due specifically to an inability to recover from a checkpoint arrest, then one prediction is that an intact checkpoint response should be required in order to observe the protracted cell cycle delay phenotype. To test this prediction, we began by isolating a double mutant in which *MEC1* was deleted in a *dhh1*Δ background. Viability of the *dhh1*Δ *mec1*Δ strain was preserved by also deleting *SML1* (***S**uppression of **M**ec1 **L**ethality*), an inhibitor of ribonucleotide reductase, the deletion of which preserves viability of deletion mutants for the essential checkpoint genes *MEC1* and *RAD53* (Zhao *et al.* 1998). The double mutant showed no significant synthetic effect with regard to DNA-damage sensitivity, with viability after exposure to UV irradiation approximately the same as the viability of the *mec1*Δ strain (Figure 3A). This places *DHH1* in an epistasis group with *MEC1* with regard to the checkpoint response and supports the notion that the role of *DHH1* in the checkpoint response may be in recovery from the checkpoint arrest. To further characterize the checkpoint behavior of the *dhh1*Δ *mec1*Δ strain, the arrest and release time course described in Figure 2 was repeated with the double mutant, using UV irradiation as the DNA-damaging agent. The protracted delay observed in cells lacking *DHH1* is indeed dependent upon an intact checkpoint, as might be expected if the role of *DHH1* is specific to checkpoint recovery. The *dhh1*Δ *mec1*Δ strain was greatly accelerated through the checkpoint, as measured by FACS analysis, compared to the *dhh1*Δ strain. The fact that deletion of *MEC1* failed to completely reverse the *dhh1*Δ-induced delay (Figure 3B, compare bottom right to top right) is likely due to residual checkpoint activity in *MEC1* deletion mutants (Morrow *et al.* 1995; Sanchez *et al.* 1996). Progression through START was also measured by *CLN2* mRNA accumulation in the double mutant, as described in Figure 2 (Figure 3C). Again, the Northern results support the suggestion by FACS that inactivation of the checkpoint response suppresses the requirement for *DHH1* in passage through START following DNA dam-

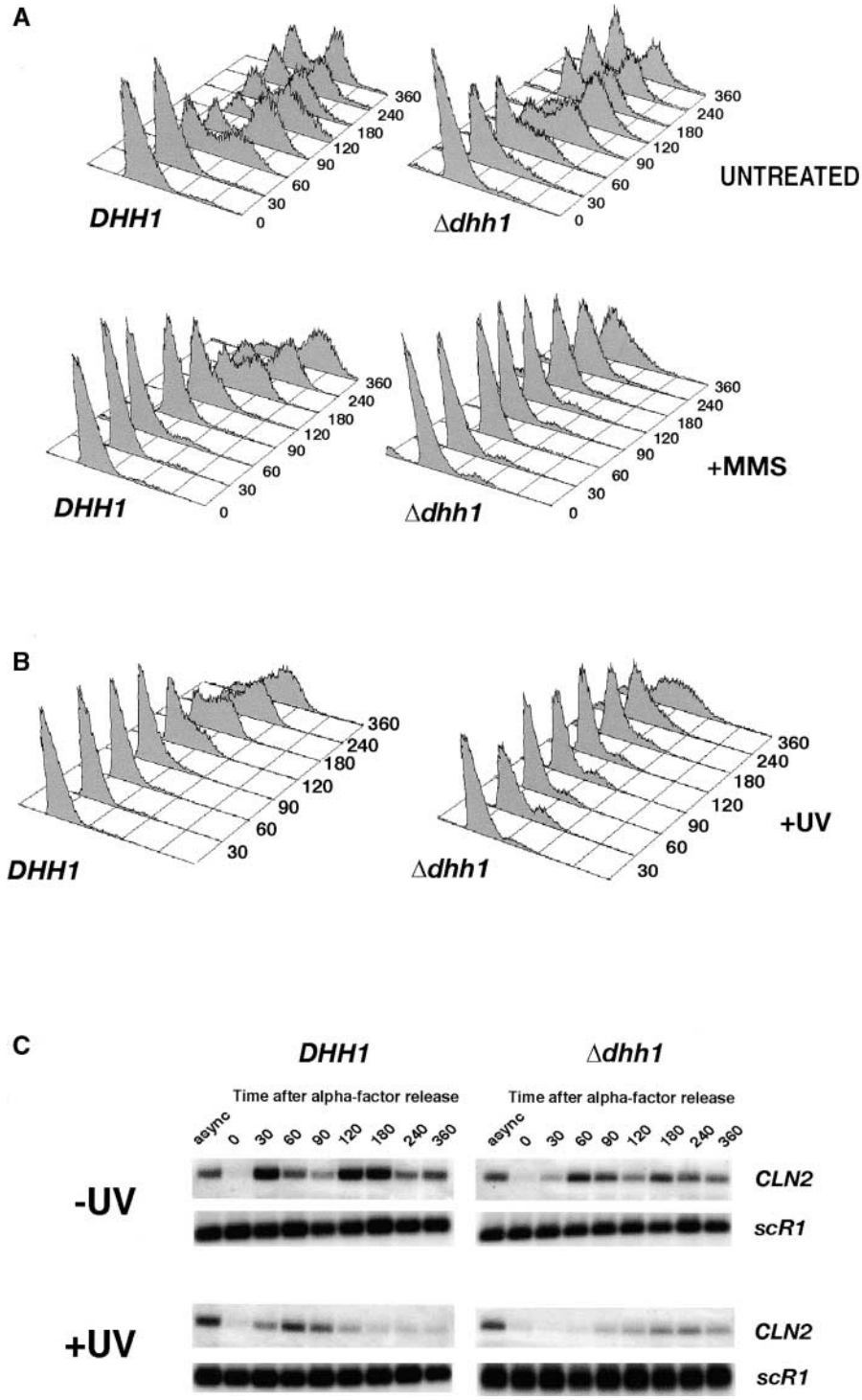


FIGURE 2.—*dhh1*Δ mutant is severely delayed in emergence from G1/S checkpoint arrest. (A, top) Wild-type (YJR218) and *dhh1*Δ (YJR219) cells were synchronized in G1 with α-factor, then the α-factor was removed, and aliquots were taken for FACS analysis over a 6-hr time period. (A, bottom) Wild-type and *dhh1*Δ cells were exposed to the DNA-alkylating agent methyl methanesulfonate (MMS) while arrested in G1 with α-factor, and cell cycle progression after removal of α-factor and MMS was followed by FACS. (B) Wild-type (YJR218) and *dhh1*Δ (YJR219) cells were subjected to the same time course as described in A, but they were treated with 60 J/m^2 UV irradiation rather than MMS. (C) Reaccumulation of *CLN2* mRNA is severely delayed in *dhh1*Δ cells following activation of the G1/S checkpoint by UV-induced DNA damage. Aliquots were taken for quantifying G1 cyclin mRNA by Northern blotting over the same time course as in A and B. *ScR1* is a loading control.

age. These results further support the model that *DHH1* plays a specific role in checkpoint recovery, following checkpoint activation and cell cycle arrest.

One possibility suggested by the results described above is that *DHH1* plays a role in repairing DNA damage, such that deletion of *DHH1* impairs repair, resulting in persistence of the damage-signaling cascade that mediates G1/S checkpoint arrest. To investigate this possibility, we followed repair of UV photoproducts in wild-type and *dhh1*Δ cells, using an antibody against thymidine dimers. As a control, repair was also followed in *rad23*Δ cells, which are known to be defective for the nucleotide excision repair pathway (GILLETTE *et al.* 2001; Figure 4). The *dhh1*Δ strain shows no defect compared to the wild type in the repair of UV-induced damage, while the control *rad23*Δ strain is obviously defective in repair as measured by this assay. These results suggest that the role of *DHH1* in checkpoint

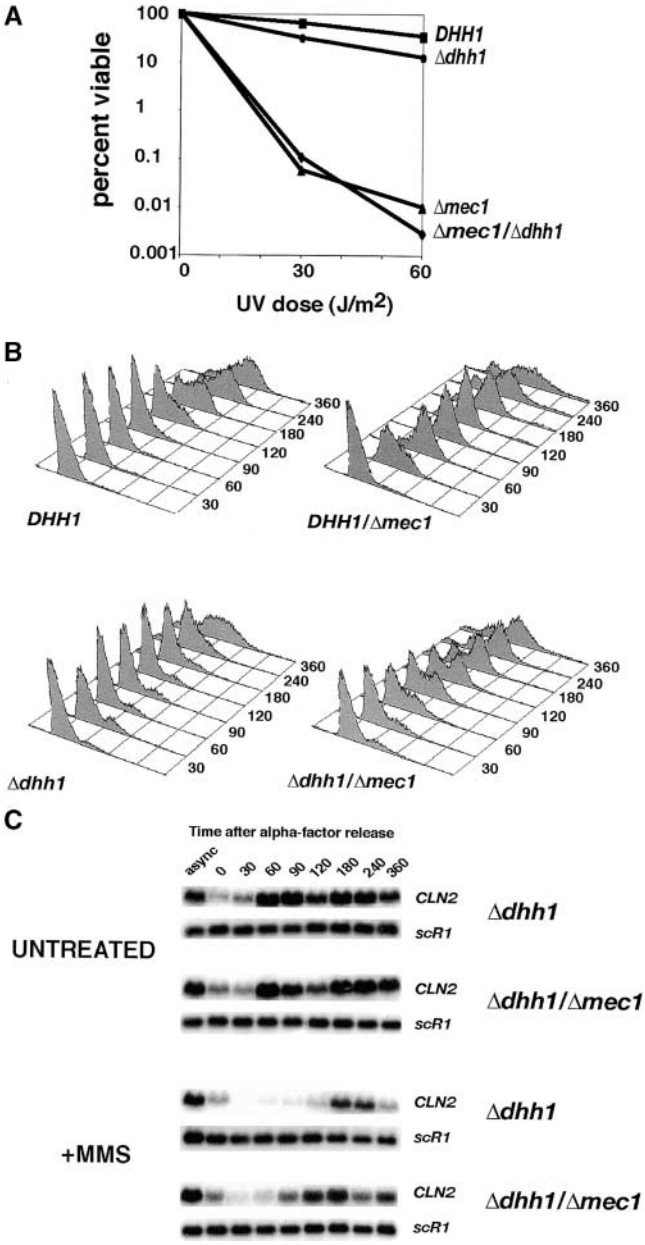


FIGURE 3.—The protracted G1 arrest of *dhh1*Δ cells is dependent upon an intact checkpoint response. (A) Viability after exposure to UV irradiation was measured in strains carrying deletions of *DHH1* and *MEC1*. Wild-type (*sml1*Δ*::URA3,* YJR535), *dhh1*Δ *sml1*Δ (YJR534), *mec1*Δ *sml1*Δ (YJR533), and *dhh1*Δ *mec1*Δ *sml1*Δ (YJR531) cells are shown. All strains used contain a deletion of *SML1* to preserve the viability of the *mec1*Δ and *rad53*Δ strains (ZHAO *et al.* 1998), but only the relevant, distinguishing genotypes are listed here and in the text. (B) Cells were synchronized in G1 with α-factor and exposed to UV irradiation and then released from α-factor and followed by FACS as in Figure 2A. (C) Samples of *dhh1*Δ and *dhh1*Δ *mec1*Δ cells were collected over the same time course, after induction of DNA damage by MMS, for analysis of *CLN2* mRNA expression by Northern blotting. *ScR1* is a loading control.

recovery is at the level of regulation of cell cycle progression, rather than at the level of actually repairing DNA damage.

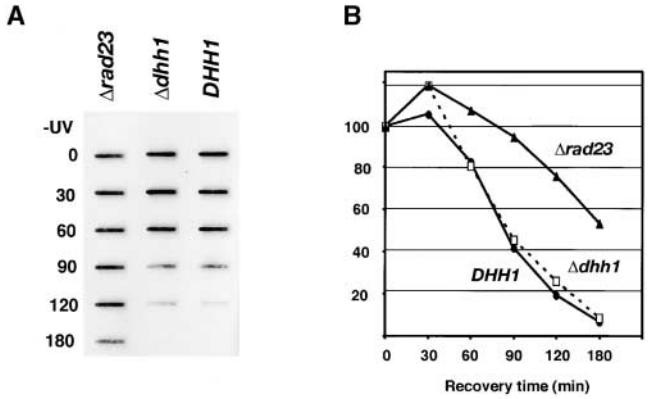


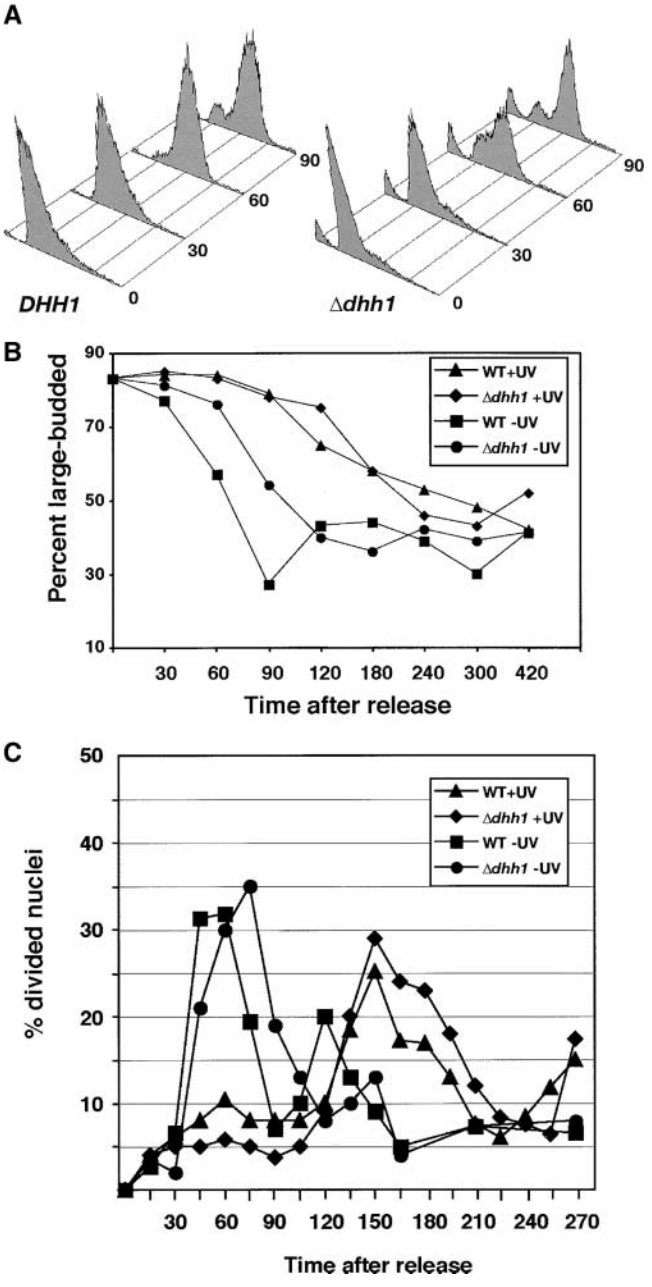
FIGURE 4.—*DHH1* is not required for DNA damage repair. *DHH1*, *dhh1*Δ, and *rad23*Δ cells were exposed to 50 J/m^2 and allowed to recover for 30–180 min. Genomic DNA was prepared, denatured, and applied to a membrane. (A) Western blot using anti-thymidine dimer monoclonal antibody KTM53. (B) Quantification of the results. Values represent percentage of signal at $t = 0$ and are normalized to total DNA as described in MATERIALS AND METHODS.

The checkpoint recovery defect of *dhh1*Δ cells is specific to the G1/S checkpoint: Many DNA-damage checkpoint genes characterized to date in yeast regulate the G1/S, S, and G2/M DNA-damage checkpoints (HARTWELL and KASTAN 1994; WEINERT *et al.* 1994; LYDALL and WEINERT 1995; ZHOU and ELLEDGE 2000). To determine whether the severe delay in emergence from G1/S checkpoint arrest observed for the *dhh1*Δ strain is specific to the regulation of START or a general defect in checkpoint recovery, we examined the recovery of *dhh1*Δ cells from the replication (S) and G2/M checkpoints.

To examine the activation of and release from the S-phase replication checkpoint, cells were arrested in S-phase with hydroxyurea (HU), and cell cycle progression was followed by FACS analysis after its removal. *dhh1*Δ cells were capable of arresting in response to HU treatment similarly to wild-type cells and upon release reentered the cell cycle at nearly the same rate as wild-type cells (Figure 5A). The mutant required ~15–20 additional minutes to enter G2/M compared to the wild-type strain, but clearly the delay was not nearly as severe as what was observed at the G1/S DNA-damage checkpoint. Instead, the extended period of time required by the *dhh1*Δ cells to enter G2/M after release from HU block was similar to that observed for its emergence from α-factor arrest in the absence of DNA damage. This slight delay is attributable to the overall slowed growth of this strain and likely does not indicate defects in emerging from this checkpoint specifically (see also below).

To examine the activation of and release from the G2/M checkpoint, cells were synchronized in G2/M with nocodazole, exposed to UV irradiation, and released into the cell cycle by removal of the drug. Progres-

sion out of the G2 checkpoint arrest and through M phase was monitored by calculating budding indices on the basis of counting large-budded cells (Figure 5B) and also by calculating the percentage of cells that were binucleate as visualized following 4′,6-diamidino-2-phenylindole staining (Figure 5C). As previously observed for α-factor and HU arrests, the *dhh1*Δ cells required an additional 20–30 min to emerge from nocodazole block in the absence of DNA damage compared to wild-type cells. However, and more importantly, they emerged from the G2/M DNA-damage checkpoint at a rate indistinguishable from that of the wild type. Thus, *DHH1* is specifically required for the recovery from the G1/S DNA-damage checkpoint. Furthermore, these data provide additional support to the model that the role played by Dhh1p at the G1/S checkpoint impinges on the cell cycle regulatory machinery and not directly on damage repair. If the protracted G1/S arrest seen in *dhh1*Δ cells was due solely to defects in actually repairing DNA damage, these defects in damage repair would be likely to cause a protracted checkpoint arrest at the S-phase and G2/M checkpoints as well.



***dhh1*Δ cells are hypersensitive to additional cell cycle perturbations:** Since the role of *DHH1* in recovery from G1 cell cycle arrest following DNA damage appeared to be at the level of cell cycle regulation, we wondered whether it would be possible to fully suppress the requirement for *DHH1* in cell cycle reentry simply by further reducing checkpoint activity or by increasing expression of positive cell cycle progression factors such as G1 cyclins. Since the deletion of *MEC1* had caused a partial suppression of the G1 delay seen in the *dhh1*Δ strain following DNA damage, we started by attempting to fully inactivate the G1 checkpoint to see if this could lead to a full suppression of the delay. Starting with a strain background lacking *SML1* to preserve viability of *RAD53* and *MEC1* mutants (Zhao *et al.* 1998), we attempted to isolate a triple mutant lacking *DHH1*, *MEC1*, and its partially redundant homolog *TEL1* or a double mutant for *DHH1* and *RAD53*, the kinase of which is believed to act downstream of *MEC1* and *TEL1* in the DNA-damage signaling pathway at G1/S (Sun *et al.* 1996). These attempts were unsuccessful unless *DHH1* was supplied on a *URA3* plasmid during strain construction (data not shown). After successfully isolating the *dhh1*Δ *mec1*Δ *tel1*Δ and *dhh1*Δ *rad53*Δ mutants carrying the wild-type *DHH1* on a *URA3* plasmid, cultures of these strains were spotted on plates containing 5-fluoroorotic acid (5-FOA) to test for viability of the mutants after the loss of *DHH1* (Figure 6A). Surprisingly, fully disabling the known G1/S checkpoint activation pathway proved to be synthetically lethal with deletion of *DHH1*, even in the absence of exogenous DNA damage. *MEC1* and *RAD53* do play additional roles in cell cycle regulation besides activation of the G1/S checkpoint response, as evidenced by the fact that they

Figure 5.—*dhh1*Δ cells are not deficient in recovery from S phase or G2/M checkpoints. (A) The S-phase checkpoint was activated in wild-type and *dhh1*Δ cells using the replication inhibitor hydroxyurea (HU) and cell cycle progression after removal of HU was followed by FACS. (B) Wild-type and *dhh1*Δ cells were synchronized in the G2 phase of the cell cycle using the microtubule polymerization inhibitor nocodazole and exposed to UV irradiation to activate the G2/M checkpoint. Cell cycle progression following removal of nocodazole was assessed by observing bud morphology. The symbols are as follows: squares, *DHH1*, −UV irradiation; circles, *dhh1*Δ, −UV irradiation; triangles, *DHH1*, +UV irradiation; diamonds, *dhh1*Δ, +UV irradiation. (C) As in B except the percentage of binucleate cells was counted as a measure of progression through the G2/M checkpoint at 15-min intervals following release.

are essential for cell viability regardless of damage, if the negative regulator of dNTP pools, *SML1*, is functional (Zhao *et al.* 1998). It has been suggested that the essential roles of these proteins are related to dealing with minor "damage" that occurs as part of each cell cycle in the G1, S, and G2 phases of the cell cycle (Zhao *et al.* 2001). Our observation of synthetic lethality of *MEC1 TEL1* or *RAD53* deletion with *DHH1* deletion does not address the mechanism or elucidate the relevant cell cycle phase at which these proteins may interact. However, this observation does support the model that *DHH1* contributes to the cell cycle regulatory machinery that allows the cell to deal with the cell cycle perturbations that are regularly encountered.

We next decided to attempt to alter expression of cell cycle regulatory factors known to act specifically at the G1/S transition, to suppress the requirement for *DHH1* following DNA damage. Since at least one target of the checkpoint machinery leading to cell cycle delay at G1 is *CLN2* transcription (Sidorova and Breeden 1997), we wondered whether the checkpoint recovery defect of *dhh1*Δ cells could be suppressed by expressing G1 cyclins from an exogenous promoter. *CLN2* was placed under the control of the *ADH1* promoter contained on a low-copy-number plasmid, which gives constitutive, moderate levels of expression. Expressing *CLN2* from the *ADH1* promoter failed to accelerate the progression of the *dhh1*Δ strain through the DNA-damage checkpoint (Figure 6B). *CLN2* mRNA accumulated in the *dhh1*Δ cells to levels equal to that of the wild-type strain, indicating that the failure of exogenous *CLN2* expression to accelerate checkpoint progression was not due to trivial expression defects in this mutant (Supplemental Figure 2 at http://www.genetics.org/supplemental/). These results indicate that *DHH1* plays a role in regulating G1/S progression that is broader than triggering G1 cyclin transcription. This notion is supported by some of the phenotypes observed in *dhh1*Δ mutants. For example, deletion of *DHH1* causes phenotypes consistent with cell wall defects (Hata *et al.* 1998), and genes required for cell wall formation are, along with the G1 cyclins, activated at START (Levine *et al.* 1995; Nasmyth 1996).

Another part of the cascade of gene expression activated at START, which is upstream of *CLN2* expression, involves upregulation of *CLN3*. *CLN3* is a G1 cyclin that is expressed at low levels throughout the cell cycle, and its post-transcriptional upregulation during G1 drives the expression of *CLN2* and many other genes required for progression through G1/S. Overexpression of *CLN3*

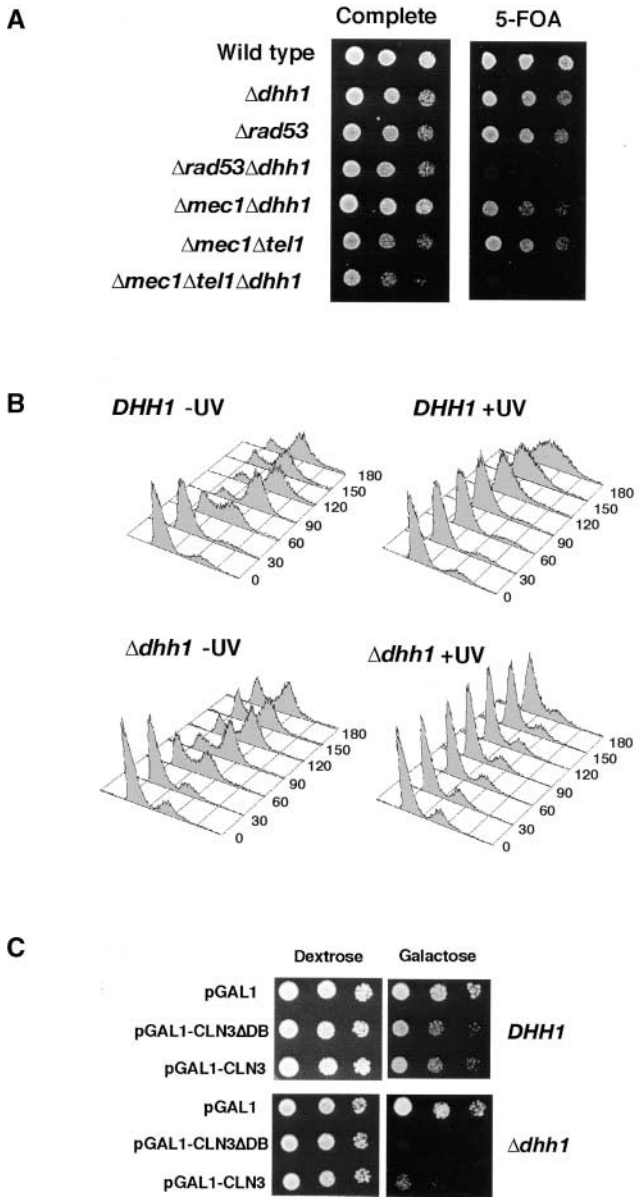


Figure 6.—*dhh1*Δ cells are hypersensitive to cell cycle perturbations. (A) Complete inactivation of G1/S checkpoint function is lethal in a *dhh1*Δ background. Strains analyzed in this figure are *sml1*Δ (YJR745), *dhh1*Δ *sml1*Δ (YJR747), *rad53*Δ *sml1*Δ (YJR727), *dhh1*Δ *rad53*Δ *sml1*Δ (YJR723), *dhh1*Δ *mec1*Δ *sml1*Δ (YJR725), *mec1*Δ *tel1*Δ *sml1*Δ (YJR749), and *dhh1*Δ *mec1*Δ *tel1*Δ *sml1*Δ (YJR726). The viability of YJR723 and YJR726 strains was isolated in the presence of *DHH1* supplied on the *URA3* plasmid pRS416-*DHH1*. These strains were then grown in the presence of 5-fluoroorotic acid (5-FOA) to evaluate their viabilities in the absence of *DHH1* expression. Note that all strains contained the *sml1*Δ mutation, but this information is not indicated within the figure to highlight the relevant phenotypes. Overexpression of G1 cyclins in *dhh1*Δ cells is shown. (B) Constitutive expression of *CLN2* does not rescue the *dhh1*Δ cell cycle delay phenotype. Cells were transformed with a plasmid carrying the *CLN2* gene under control of the *ADH1* promoter on a low-copy plasmid that allows for moderate, constitutive expression. Cells were subjected to a similar synchronization, damage, and release time course as described in Figure 2, except that UV irradiation was used as a mutagen. A small fraction of cells escape α-factor block due to the constitutive expression of *CLN2*, but this does not obscure the analysis. (C) Overexpression of *CLN3* or a derivative lacking its destruction box (CLN3ΔDB) from the inducible *GAL1* promoter contained on a high-copy-number vector is lethal in a *dhh1*Δ background, but not in a wild-type background.

precociously drives cells through start, circumventing normal cell cycle control and checkpoint mechanisms (Levine *et al.* 1995; Nasmyth 1996). Furthermore, the post-transcriptional regulation of *CLN3* mRNA under conditions of cell stress influences cell cycle progression (Gallego *et al.* 1997; Polymenis and Schmidt 1997; Philpott *et al.* 1998). Therefore, we wondered whether *CLN3* might be a target of regulation by *DHH1* and whether overexpressing *CLN3* could suppress the cell cycle delay phenotype seen in *dhh1*Δ cells. However, inducing overexpression of *CLN3* from the *GAL1* promoter severely reduced the growth and viability of *dhh1*Δ cells, and expression of a version lacking its destruction box (ΔDB) proved lethal in this background (Figure 6C). Together with the observed synthetic lethalities in the *dhh1*Δ *mec1*Δ *tel1*Δ strain and the *dhh1*Δ *rad53*Δ strain, this result supports the model that *DHH1* contributes to the balance of positive and negative signals that modulate cell cycle progression following DNA damage. While the inherent plasticity of the cell cycle may allow the cell to tolerate a strong perturbation or the loss of some part of its regulatory machinery, combinations of these insults lead eventually to cell death. The fact that loss of *DHH1* shows synthetic lethality with strong cell cycle perturbations in the form of DNA damage and with loss of regulation via checkpoint deletion or cyclin overexpression implicates it as part of the regulatory network that maximizes viability by contributing to control of cell cycle progression.

***dcp1*Δ mutants are also sensitive to DNA damage:** Previous studies have suggested associations between Dhh1p and several proteins with roles in mRNA degradation in yeast, including the decapping enzyme Dcp1p (Coller *et al.* 2001; Fischer and Weis 2002). We wondered whether the DNA-damage phenotypes observed in the *dhh1*Δ strain would also be associated with disruption of the decapping machinery.

DCP1 encodes the major yeast mRNA-decapping enzyme, and *DHH1* is reported to stimulate its decapping activity (Fischer and Weis 2002). We next assayed the DNA-damage sensitivity of a *dcp1*Δ mutant and found it to be nearly as sensitive to UV irradiation and MMS as the *dhh1*Δ strain (Figure 7). Unfortunately we were unsuccessful in performing the block, damage, and release studies described in Figure 2 to assess the integrity of the G1/S checkpoint in this mutant because it arrests poorly in response to α-factor (not shown). The *dcp1*Δ mutant also showed more severe growth defects than the *dhh1*Δ mutant and is inviable in some genetic backgrounds. This is consistent with *DHH1* playing a stimulatory and/or regulatory role in decapping, whereas *DCP1* plays an essential role. Nonetheless, these data support the notion that the DNA-damage sensitivity phenotypes observed in *dhh1*Δ cells are closely linked to the function of Dhh1p in the decapping complex.

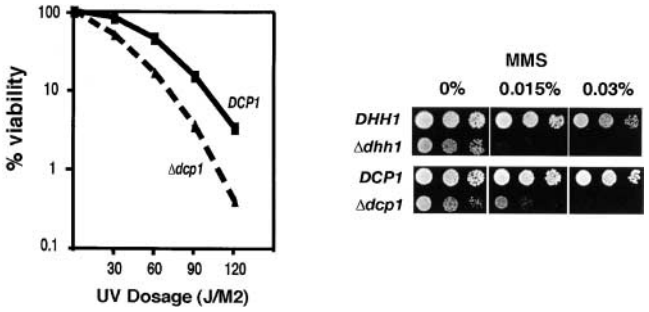


Figure 7.—DNA damage sensitivity phenotype of the *dhh1*Δ strain is shared by *dcp1*Δ cells. UV and MMS sensitivity were assayed as described in Figure 1 for *dcp1*Δ (YRP1071) and its isogenic wild-type strain (YRP840). *dhh1*Δ and its isogenic wild-type strain were included in the MMS sensitivity assay for direct comparison. The *dcp1*Δ strain is nearly as sensitive as the *dhh1*Δ strain.

DISCUSSION

DNA-damage sensitivity phenotypes are linked to mRNA metabolism: That Dhh1p is known to colocalize with and stimulate the decapping machinery in cytoplasmic foci (Fischer and Weis 2002; Sheth and Parker 2003) and also appears to interact with the mRNA deadenylation complex (Hata *et al.* 1998; Coller *et al.* 2001; Tucker *et al.* 2002) strongly suggests that the role of Dhh1p in G1/S checkpoint recovery is at the level of regulation of mRNA metabolism. Recently, deletion of *DHH1* has also been shown to be synthetically lethal with mutations in *DBP5* and *DED1*, DEAD-box helicases with roles in mRNA export and translation initiation, respectively (Tseng-Rogenski *et al.* 2003). This result also supports a model in which the major role of *DHH1* is post-transcriptional. Further, that deletions of other components of the decapping and decay machinery also cause DNA-damage sensitivity phenotypes similar to that observed in the *dhh1*Δ strain suggests that the role of Dhh1p in checkpoint recovery is closely linked to its function in mRNA decapping. We have shown here that deletion of the decapping protein *DCP1* causes increased sensitivity to UV irradiation. In addition, a *lsm1*Δ strain was shown to be moderately hypersensitive to UV irradiation in a genome-wide screen for deletions conferring DNA-damage hypersensitivity (Birrell *et al.* 2001), and *pat1*Δ cells have also been shown to be mildly hypersensitive to UV irradiation (Wang *et al.* 1999).

While our results cannot rule out a role for Dhh1p in regulating transcription in response to DNA damage, it seems unlikely that this is the case. Although physical and genetic interactions exist between *DHH1* and components of the Ccr4-Not complex that has been implicated in transcriptional regulation, it seems that Dhh1p function is more closely linked to the Ccr4 deadenylase and the Dcp1 decapping complexes. Ccr4p, Pop2p, and

Dhh1p all associate with the N-terminal domain of Not1p, the only essential component of the Ccr4-Not complex, while the other Not proteins, mutations of which cause the strongest transcription phenotypes, all associate with the C-terminal domain of Not1p (Bai *et al.* 1999; Deluen *et al.* 2002; Maillet and Collart 2002). It has been proposed that the apparent functions of the Ccr4-Not complex in both transcriptional regulation and deadenylation suggest a high degree of coordinated regulation of mRNA metabolism throughout the lifetime of an mRNA, from its transcription through its destruction (Maillet and Collart 2002; Tucker *et al.* 2002). If Dhh1p is a modulator of mRNA metabolism, it is reasonable to expect that it would associate with a complex or complexes that affect an mRNA throughout its lifetime.

There are several possible roles that a DEAD-box RNA helicase might be imagined to play in association with the deadenylation and decapping machinery, which could affect regulation of mRNA stability, regulation of the translational state of the mRNA, or both. DEAD-box helicases involved in other processes such as splicing and translation are known to be required for taking apart protein-RNA complexes so that they can be remodeled to allow for the next step in these processes (Schwer 2001; Tanner and Linder 2001). It might be that Dhh1p has a similar role in helping to remodel the interactions between mRNAs and their associated mRNP proteins as the status of the mRNP changes over the course of its lifetime. Indeed, several lines of recent evidence suggest that the DDX6-like DEAD-box helicases, which include *DHH1*, associate with mRNAs throughout the lifetime of the mRNA. In addition to the associations between Dhh1p and transcription, deadenylation, and decapping complexes in yeast, the Xenopus *DHH1* ortholog Xp54 has been shown to interact with nascent transcripts in the nuclei of transcriptionally active oocytes, but to localize to the cytoplasm in transcriptionally quiescent oocytes. Furthermore, its shuttling between the nucleus and cytoplasm is developmentally regulated (Smillie and Sommerville 2002).

An attractive model is that Dhh1p and its orthologs associate with a subset of mRNAs and regulate their stability and/or translation. Such a model would suggest that efficient recovery from G1/S checkpoint arrest requires Dhh1p either to stimulate the decay or to alter the translational status of a subset of mRNAs or possibly even to perform both functions. However, the specific mRNAs that may be affected and the ways in which they are affected have yet to be determined. Studies in Drosophila (Nakamura *et al.* 2001) and clam (Minshall *et al.* 2001) have shown that the *DHH1* orthologs in these organisms act to repress translation of maternal mRNAs to which they bind during early development. In Drosophila, two mRNAs, *osk* and *BicD*, that are normally silenced until they are transported into the oocyte, are prematurely translated in nurse cells when the Dhh1p ortholog is inactivated (Nakamura *et al.* 2001). In other organisms, no specific mRNA targets of Dhh1p have been identified. While the very specific G1/S checkpoint recovery defect associated with deletion of *DHH1* may seem to be suggestive of specific mRNA targets of Dhh1p, it is also possible that G1/S-specific transcripts are simply the most sensitive among a very large number of mRNA targets of Dhh1p to this type of regulation of mRNA metabolism.

Post-transcriptional control at the G1/S boundary: Recent evidence strongly suggests that post-transcriptional or translational control mechanisms play an important role in regulating cell cycle progression through G1/S in higher eukaryotes. Both inhibitors of G1/S progression, such as p53 and the cyclin-dependent kinase inhibitor p21 (Peter 1997; Wang *et al.* 2000) and proto-oncogene stimulators of cell cycle progression (Landers *et al.* 1997) have been shown to be regulated at the level of mRNA stability and translation. Many of these G1/S regulatory messages have naturally short half-lives and thus are particularly sensitive to regulation of mRNA stability (Chen and Shyu 1995). In yeast, G1/S progression is known to be sensitive to regulation at the level of mRNA stability and translational efficiency. Mutation of the cap-binding protein, eIF4E, which destabilizes some mRNAs, leads to cell cycle arrest in G1. Interestingly, this arrest can be overcome by overexpression of *CLN3* (Danaie *et al.* 1999). The exceptionally high sequence conservation with *DHH1* displayed by the human protein p54/RCK, and its ability to substitute for *DHH1*, indicates that a putative regulatory mechanism in which it plays a role, affected by modulation of mRNA stability, is highly conserved among all eukaryotes.

Checkpoint function and neoplastic transformation: Appropriate response to DNA damage involves balancing checkpoint signaling leading to cell cycle arrest with mitogenic signaling leading to cell cycle reentry such that genomic damage is minimized and viability is maximized. It has long been clear that inactivating checkpoints, disrupting the balance toward mitogenic signaling, is catastrophic to cells (Hartwell *et al.* 1994; Weinert 1997). However, it is becoming clear that mitogenic signaling to allow cell cycle reentry is also essential (Shaulian *et al.* 2000). In higher eukaryotes, disruption of this balance can lead either to unregulated growth and neoplastic transformation or to apoptosis. Others have recently reported that deletion of *DHH1* suppresses the deleterious effects of heterologously expressing the human tumor suppressor gene *BRCA1* and suggest that this is due to a normal role for Dhh1p at the G1/S transition, where *BRCA1* may serve in a checkpoint role in human cells (Westmoreland *et al.* 2003). Our findings indicate that *DHH1* plays an important role in the cell cycle reentry process at the G1/S DNA-

damage checkpoint, potentially helping to strike the balance between terminal arrest and inappropriate cell cycle progression. Loss of *DHH1* function renders cells hypersensitive to DNA damage, apparently due to an inability of *dhh1*Δ cells to recover from checkpoint arrest. However, it also renders cells incapable of dealing with perturbations that accelerate cell cycle progression, such as full inactivation of the G1/S checkpoint or overexpression of *CLN3*. Interestingly, deletion of *DHH1* or other genes involved in decapping in yeast has been reported to cause a range of apoptotic phenotypes (Mazzoni *et al.* 2003). These results provide new insight into the potential cell cycle regulatory mechanisms acting at the level of mRNA stability and translatability in yeast. Furthermore, they suggest that these regulatory mechanisms are highly conserved and that their disruption in human cells, via overexpression of the *DHH1* ortholog, can contribute to neoplastic transformation.

The authors thank Dr. Roy Parker for advice and providing information on *DHH1* prior to publication. We also are grateful to Todd Cohen, Drs. Parker, Steve Elledge, Rodney Rothstein, and Linda Breeden for strains used in early stages of this work and colleagues for advice and discussion regarding this article. Financial support for this project was provided by the National Institutes of Health (GM58672), the National Leukemia Research Association, a Penn State University Innovation Grant to J.C.R., and an American Society of Microbiologists Undergraduate Research Fellowship to M.B.

LITERATURE CITED

Akao, Y., O. Marukawa, H. Morikawa, K. Nakao, M. Kamei *et al.*, 1995 The rck/p54 candidate proto-oncogene product is a 54-kilodalton D-E-A-D box protein differentially expressed in human and mouse tissues. Cancer Res. **55:** 3444–3449.

Bai, Y., C. Salvadore, Y. C. Chiang, M. A. Collart, H. Y. Liu *et al.*, 1999 The CCR4 and CAF1 proteins of the CCR4-NOT complex are physically and functionally separated from NOT2, NOT4, and NOT5. Mol. Cell. Biol. **19:** 6642–6651.

Birrell, G. W., G. Giaever, A. M. Chu, R. W. Davis and J. M. Brown, 2001 A genome-wide screen in Saccharomyces cerevisiae for genes affecting UV radiation sensitivity. Proc. Natl. Acad. Sci. USA **98:** 12608–12613.

Chen, C. Y., and A. B. Shyu, 1995 AU-rich elements: characterization and importance in mRNA degradation. Trends Biochem. Sci. **20:** 465–470.

Collart, M. A., and K. Struhl, 1994 NOT1(CDC39), NOT2 (CDC36), NOT3, and NOT4 encode a global-negative regulator of transcription that differentially affects TATA-element utilization. Genes Dev. **8:** 525–537.

Coller, J. M., M. Tucker, U. Sheth, M. A. Valencia-Sanchez and R. Parker, 2001 The DEAD box helicase, Dhh1p, functions in mRNA decapping and interacts with both the decapping and deadenylase complexes. RNA **7:** 1717–1727.

Danaie, P., M. Altmann, M. N. Hall, H. Trachsel and S. B. Helliwell, 1999 CLN3 expression is sufficient to restore G1-to-S-phase progression in Saccharomyces cerevisiae mutants defective in translation initiation factor eIF4E. Biochem. J. **340** (1): 135–141.

Deluen, C., N. James, L. Maillet, M. Molinete, G. Theiler *et al.*, 2002 The Ccr4-not complex and yTAF1 (yTaf(II)130p/yTaf (II)145p) show physical and functional interactions. Mol. Cell. Biol. **22:** 6735–6749.

Fischer, N., and K. Weis, 2002 The DEAD box protein Dhh1 stimulates the decapping enzyme Dcp1. EMBO J. **21:** 2788–2797.

Gallego, C., E. Gari, N. Colomina, E. Herrero and M. Aldea, 1997 The Cln3 cyclin is down-regulated by translational repression and degradation during the G1 arrest caused by nitrogen deprivation in budding yeast. EMBO J. **16:** 7196–7206.

Gillette, T. G., W. Huang, S. J. Russell, S. H. Reed, S. A. Johnston *et al.*, 2001 The 19S complex of the proteasome regulates nucleotide excision repair in yeast. Genes Dev. **15:** 1528–1539.

Hartwell, L., T. Weinert, L. Kadyk and B. Garvik, 1994 Cell cycle checkpoints, genomic integrity, and cancer. Cold Spring Harbor Symp. Quant. Biol. **59:** 259–263.

Hartwell, L. H., and M. B. Kastan, 1994 Cell cycle control and cancer. Science **266:** 1821–1828.

Hata, H., H. Mitsui, H. Liu, Y. Bai, C. L. Denis *et al.*, 1998 Dhh1p, a putative RNA helicase, associates with the general transcription factors Pop2p and Ccr4p from *Saccharomyces cerevisiae*. Genetics **148:** 571–579.

Ladomery, M., E. Wade and J. Sommerville, 1997 Xp54, the Xenopus homologue of human RNA helicase p54, is an integral component of stored mRNP particles in oocytes. Nucleic Acids Res. **25:** 965–973.

Landers, J. E., S. L. Cassel and D. L. George, 1997 Translational enhancement of mdm2 oncogene expression in human tumor cells containing a stabilized wild-type p53 protein. Cancer Res. **57:** 3562–3568.

Levine, K., A. H. Tinkelenberg and F. Cross, 1995 The CLN gene family: central regulators of cell cycle Start in budding yeast. Prog. Cell Cycle Res. **1:** 101–114.

Lu, D., and J. J. Yunis, 1992 Cloning, expression and localization of an RNA helicase gene from a human lymphoid cell line with chromosomal breakpoint 11q23.3. Nucleic Acids Res. **20:** 1967–1972.

Lydall, D., and T. Weinert, 1995 Yeast checkpoint genes in DNA damage processing: implications for repair and arrest. Science **270:** 1488–1491.

Lydall, D., and T. Weinert, 1996 From DNA damage to cell cycle arrest and suicide: a budding yeast perspective. Curr. Opin. Genet. Dev. **6:** 4–11.

Maillet, L., and M. A. Collart, 2002 Interaction between Not1p, a component of the Ccr4-not complex, a global regulator of transcription, and Dhh1p, a putative RNA helicase. J. Biol. Chem. **277:** 2835–2842.

Mazzoni, C., P. Mancini, L. Verdone, F. Madeo, A. Serafini *et al.*, 2003 A truncated form of KlLsm4p and the absence of factors involved in mRNA decapping trigger apoptosis in yeast. Mol. Biol. Cell **14:** 721–729.

Minshall, N., G. Thom and N. Standart, 2001 A conserved role of a DEAD box helicase in mRNA masking. RNA **7:** 1728–1742.

Moriya, H., and K. Isono, 1999 Analysis of genetic interactions between DHH1, SSD1 and ELM1 indicates their involvement in cellular morphology determination in Saccharomyces cerevisiae. Yeast **15:** 481–496.

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.

Nakagawa, Y., H. Morikawa, I. Hirata, M. Shiozaki, A. Matsumoto *et al.*, 1999 Overexpression of rck/p54, a DEAD box protein, in human colorectal tumours. Br. J. Cancer **80:** 914–917.

Nakamura, A., R. Amikura, K. Hanyu and S. Kobayashi, 2001 Me31B silences translation of oocyte-localizing RNAs through the formation of cytoplasmic RNP complex during Drosophila oogenesis. Development **128:** 3233–3242.

Nasmyth, K., 1996 At the heart of the budding yeast cell cycle. Trends Genet. **12:** 405–412.

Peter, M., 1997 The regulation of cyclin-dependent kinase inhibitors (CKIs). Prog. Cell Cycle Res. **3:** 99–108.

Philpott, C. C., J. Rashford, Y. Yamaguchi-Iwai, T. A. Rouault, A. Dancis *et al.*, 1998 Cell-cycle arrest and inhibition of G1 cyclin translation by iron in AFT1-1(up) yeast. EMBO J. **17:** 5026–5036.

Polymenis, M., and E. V. Schmidt, 1997 Coupling of cell division to cell growth by translational control of the G1 cyclin CLN3 in yeast. Genes Dev. **11:** 2522–2531.

Reed, S. I., 1997 Control of the G1/S transition. Cancer Surv. **29:** 7–23.

Reese, J. C., and M. R. Green, 2001 Genetic analysis of TAF68/61 reveals links to cell cycle regulators. Yeast **18:** 1197–1205.

Sanchez, Y., B. A. Desany, W. J. Jones, Q. Liu, B. Wang *et al.*, 1996

Regulation of RAD53 by the ATM-like kinases MEC1 and TEL1 in yeast cell cycle checkpoint pathways. Science **271:** 357–360.

Schena, M., D. Picard and K. R. Yamamoto, 1991 Vectors for constitutive and inducible gene expression in yeast. Methods Enzymol. **194:** 389–398.

Schwer, B., 2001 A new twist on RNA helicases: DExH/D box proteins as RNPases. Nat. Struct. Biol. **8:** 113–116.

Shaulian, E., M. Schreiber, F. Piu, M. Beeche, E. F. Wagner *et al.*, 2000 The mammalian UV response: c-Jun induction is required for exit from p53-imposed growth arrest. Cell **103:** 897–907.

Sheth, U., and R. Parker, 2003 Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. Science **300:** 805–808.

Sidorova, J. M., and L. L. Breeden, 1997 Rad53-dependent phosphorylation of Swi6 and down-regulation of CLN1 and CLN2 transcription occur in response to DNA damage in Saccharomyces cerevisiae. Genes Dev. **11:** 3032–3045.

Siede, W., J. B. Allen, S. J. Elledge and E. C. Friedberg, 1996 The Saccharomyces cerevisiae MEC1 gene, which encodes a homolog of the human ATM gene product, is required for G1 arrest following radiation treatment. J. Bacteriol. **178:** 5841–5843.

Smillie, D. A., and J. Sommerville, 2002 RNA helicase p54 (DDX6) is a shuttling protein involved in nuclear assembly of stored mRNP particles. J. Cell Sci. **115:** 395–407.

Strahl-Bolsinger, S., and W. Tanner, 1993 A yeast gene encoding a putative RNA helicase of the "DEAD"-box family. Yeast **9:** 429–432.

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.

Tanner, N. K., and P. Linder, 2001 DExD/H box RNA helicases: from generic motors to specific dissociation functions. Mol. Cell **8:** 251–262.

Tharun, S., and R. Parker, 2001 Targeting an mRNA for decapping: displacement of translation factors and association of the Lsm1p-7p complex on deadenylated yeast mRNAs. Mol. Cell **8:** 1075–1083.

Tseng-Rogenski, S. S., J. L. Chong, C. B. Thomas, S. Enomoto, J. Berman *et al.*, 2003 Functional conservation of Dhh1p, a cytoplasmic DExD/H-box protein present in large complexes. Nucleic Acids Res. **31:** 4995–5002.

Tucker, M., M. A. Valencia-Sanchez, R. R. Staples, J. Chen, C. L. Denis *et al.*, 2001 The transcription factor associated Ccr4 and Caf1 proteins are components of the major cytoplasmic mRNA deadenylase in Saccharomyces cerevisiae. Cell **104:** 377–386.

Tucker, M., R. R. Staples, M. A. Valencia-Sanchez, D. Muhlrad and R. Parker, 2002 Ccr4p is the catalytic subunit of a Ccr4p/Pop2p/Notp mRNA deadenylase complex in Saccharomyces cerevisiae. EMBO J. **21:** 1427–1436.

Wang, W., H. Furneaux, H. Cheng, M. C. Caldwell, D. Hutter *et al.*, 2000 HuR regulates p21 mRNA stabilization by UV light. Mol. Cell. Biol. **20:** 760–769.

Wang, X., P. M. Watt, R. H. Borts, E. J. Louis and I. D. Hickson, 1999 The topoisomerase II-associated protein, Pat1p, is required for maintenance of rDNA locus stability in Saccharomyces cerevisiae. Mol. Gen. Genet. **261:** 831–840.

Weinert, T., 1997 Yeast checkpoint controls and relevance to cancer. Cancer Surv. **29:** 109–132.

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.

Westmoreland, T. J., J. A. Olson, W. Y. Saito, G. Huper, J. R. Marks *et al.*, 2003 Dhh1 regulates the G1/S-checkpoint following DNA damage or BRCA1 expression in yeast. J. Surg. Res. **113:** 62–73.

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.

Zhao, X., A. Chabes, V. Domkin, L. Thelander and R. Rothstein, 2001 The ribonucleotide reductase inhibitor Sml1 is a new target of the Mec1/Rad53 kinase cascade during growth and in response to DNA damage. EMBO J. **20:** 3544–3553.

Zhou, B. B., and S. J. Elledge, 2000 The DNA damage response: putting checkpoints in perspective. Nature **408:** 433–439.

Communicating editor: F. Winston