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The DEAD box helicase, Dhh1p, functions in mRNA decapping and interacts with both the decapping and deadenylase complexes

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ABSTRACT

A major pathway of mRNA turnover in eukaryotic cells initiates with deadenylation, leading to mRNA decapping and subsequent 5′ to 3′ exonuclease digestion. We show that a highly conserved member of the DEAD box family of helicases, Dhh1p, stimulates mRNA decapping in yeast. In *dhh1*Δ mutants, mRNAs accumulate as deadenylated, capped species. Dhh1p's effects on decapping only occur on normal messages as nonsense-mediated decay still occurs in *dhh1*Δ mutants. The role of Dhh1p in decapping appears to be direct, as Dhh1p physically interacts with several proteins involved in mRNA decapping including the decapping enzyme Dcp1p, as well as Lsm1p and Pat1p/Mrt1p, which function to enhance the decapping rate. Additional observations suggest Dhh1p functions to coordinate distinct steps in mRNA function and decay. Dhh1p also associates with Pop2p, a subunit of the mRNA deadenylase. In addition, genetic phenotypes suggest that Dhh1p also has a second biological function. Interestingly, Dhh1p homologs in others species function in maternal mRNA storage. This provides a novel link between the mechanisms of decapping and maternal mRNA translational repression.

Keywords: deadenylase; decapping; helicase; mRNA turnover

INTRODUCTION

The process and regulation of mRNA turnover is a fundamental aspect of gene expression. A major pathway of mRNA turnover for both stable and unstable transcripts has been identified in *Saccharomyces cerevisiae*. In this pathway, degradation is initiated by deadenylation of the 3′ poly(A) tail (Muhlrad & Parker, 1992; Decker & Parker, 1993). In yeast, deadenylation is followed by removal of the 5′ ^{m7}GpppN cap structure, and exonucleolytic digestion in a 5′-3′ direction (Decker & Parker, 1993; Hsu & Stevens, 1993; Muhlrad et al., 1994; Beelman et al., 1996).

Poly(A) shortening requires two proteins, Ccr4p and Pop2p, which have recently been shown to be components of the yeast deadenylase (Tucker et al., 2001). Following deadenylation, the Dcp1p/Dcp2p decapping complex decaps mRNAs at their 5′ end (Beelman et al., 1996; Dunckley & Parker 1999). Although Dcp1p and Dcp2p are the only proteins known to be required for decapping (Dunckley & Parker, 1999, 2001), the rate of decapping is affected by several *trans*-acting factors. These accessory factors include Pat1p/Mrt1p, the Lsm complex (consisting of Lsm1p-Lsm7p), Vps16p, Edc1p, and Edc2p (Hatfield et al., 1996; Zhang et al., 1999; Bonnerot et al., 2000; Tharun et al., 2000; Wyers et al., 2000; Dunckley et al., 2001). Pat1p/Mrt1p was originally identified as a genetic lesion that slows mRNA decapping, and was later shown to also be required for efficient translational initiation (Hatfield et al., 1996; Wyers et al., 2000). There are nine Lsm proteins in yeast, which form two distinct seven-member ring structures of differing function (Salgado-Garrido et al., 1999). Lsm2p–Lsm8p associate in the nucleus and are involved in U6 snRNA metabolism, whereas Lsm1p–Lsm7p associate in the cytoplasm and affect decapping of mRNAs (Mayes et al., 1999; Bouveret et al., 2000; Tharun et al., 2000). Vps16p was identified as a mutant that affected decapping rates in vitro, and Edc1p and Edc2p have been shown to be in a complex with Dcp1p and Dcp2p (Zhang et al., 1999; Dunckley et al., 2001). Although the mechanism of how these proteins en-

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hance decapping is unclear, it is possible a class of decapping enhancers may do so by directly affecting the translatability of the mRNA. Indeed, translational repression may be a first step required for efficient decapping, as recent studies have shown the cap-binding protein, eIF-4E, can inhibit decapping activity both in vivo and in vitro (Schwartz & Parker, 1999, 2000). Given this, a critical issue to resolve in understanding mRNA decay is to determine the nature of transitions between translating and nontranslating states of mRNAs.

In yeast, the complex interplay of factors promoting deadenylation-dependent mRNA turnover with each other and the translating mRNP is not clearly understood. Clues as to how these processes may impinge upon one another and relate to translational control in metazoans may come from studies of the highly conserved DEAD box helicase Dhh1p.

Dhh1p belongs to a highly conserved subfamily of DEAD box helicase termed RCK/p54 helicases after the human protein (Akao et al., 1992; Lu & Yunis, 1992). Family members include Clam p47 (Minshall et al., 2001), mouse p54 (Seto et al., 1995), *Xenopus* Xp54 (Ladomery et al., 1997), *Drosophila* Me31B (de Valoir et al., 1991), *Caenorhabditis elegans* cgh-1 (Navarro et al., 2001), and *Schizosaccharomyces pombe* Ste13 (Maekawa et al., 1994). Several lines of evidence suggest that Dhh1p and other members of this family may play an important role in promoting mRNP remodeling during mRNA metabolism and decay. Dhh1p was isolated as a high copy suppressor of both *ccr4*Δ and *pop2*Δ cells (Hata et al., 1998). Although originally identified as transcription factors (Liu et al., 1998), both Ccr4p and Pop2p have recently been shown to be components of the cytoplasmic deadenylase in yeast (Tucker et al., 2001). Additionally, the yeast genomic two-hybrid analysis has identified possible interactions between the decapping enzyme, Dcp1p, and Dhh1p (Uetz et al., 2000). Lastly, the *Xenopus* homolog of Dhh1p, Xp54, is a major component of stored maternal mRNA particles, suggesting this protein may be involved in translational control following deadenylation (Ladomery et al., 1997). Taken together, these observations suggest Dhh1p may be important in mediating events following deadenylation, thereby promoting mRNA decapping and/or translational repression.

In this study, we present evidence that the highly conserved DEAD box helicase Dhh1p is required for efficient decapping of mRNAs following deadenylation. In addition, Dhh1p is unique in that it interacts with both the decapping complex and components of the deadenylase. Last, we provide evidence that Dhh1p has a second biological role that may relate to some other aspect of mRNA metabolism. These functions and the homology between other RCK/p54 helicases suggest that mRNA decay in yeast and maternal mRNA storage in higher eukaryotes may have mechanistic similarities.

RESULTS

*dhh1*Δ mutants fail to degrade mRNA efficiently

To determine if Dhh1p is involved in mRNA turnover, the half-life of both reporters and endogenous mRNAs were assayed in *dhh1*Δ mutant strains. The reporters used were the MFA2pG and PGK1pG (Decker & Parker, 1993). These two constructs are derivatives of the endogenous genes for *MFA2* and *PGK1* that contain a poly-guanosine tract (poly(G)) in their 3′ UTR and are under the transcriptional control of the *GAL1* UAS. These modifications allow mRNA turnover to be assessed by two means. First, the decay rate of full-length mRNA can be quantitated following repression of transcription by the addition of glucose to the growth medium. Second, the poly(G) tract inhibits exonucleolytic digestion thereby trapping decay intermediates (Vreken & Raue, 1992; Decker & Parker, 1993). The ratio of full-length mRNA to the mRNA decay intermediate is indicative of the efficiency of turnover (Hatfield et al., 1996; Cao & Parker, 2001).

Analysis of the MFA2pG and PGK1pG transcripts in *dhh1*Δ strains provided two observations that mRNA decay was deficient in the *dhh1*Δ cells. First, both reporters were stabilized in *dhh1*Δ mutants as compared to wild-type cells. The half-life of the unstable MFA2pG reporter was 8 min in the *dhh1*Δ mutant background compared to 3 min in wild-type (Fig. 1A). For the stable PGK1pG transcript the half-life was 33 min in the mutant as compared to 25 min in wild type, which is consistent with the stable *PGK1* mRNA being less responsive to subtle changes in decapping rate (see Cao & Parker, 2001). A change in mRNA decay rate in *dhh1*Δ cells was also supported by the observation that the level of mRNA decay intermediate was approximately fourfold less in *dhh1*Δ cells versus. wild type (Fig. 1A,B). We also observed that the endogenous *GAL1* mRNA (7 min wild type vs. 18 min *dhh1*Δ), *GAL7* mRNA (12 min wild type vs. 26 min *dhh1*Δ), and *GAL10* mRNA (7 min wild type vs. 18 min *dhh1*Δ) were stabilized in *dhh1*Δ cells. These results suggest that Dhh1p is required for efficient mRNA turnover in yeast.

Dhh1p affects decapping of mRNAs

Mutations that affect mRNA decay rate in yeast can affect deadenylation, decapping, or 5′-to-3′ exonucleolytic degradation. To determine the step of mRNA turnover affected in *dhh1*Δ mutants, three experiments were performed. First, steady state mRNA was analyzed on high-resolution polyacrylamide gels. Lesions in the deadenylase accumulate transcripts with longer poly(A) tails on average (Tucker et al., 2001). In contrast, lesions that affect decapping or 5′-to-3′ exonuclease digestion

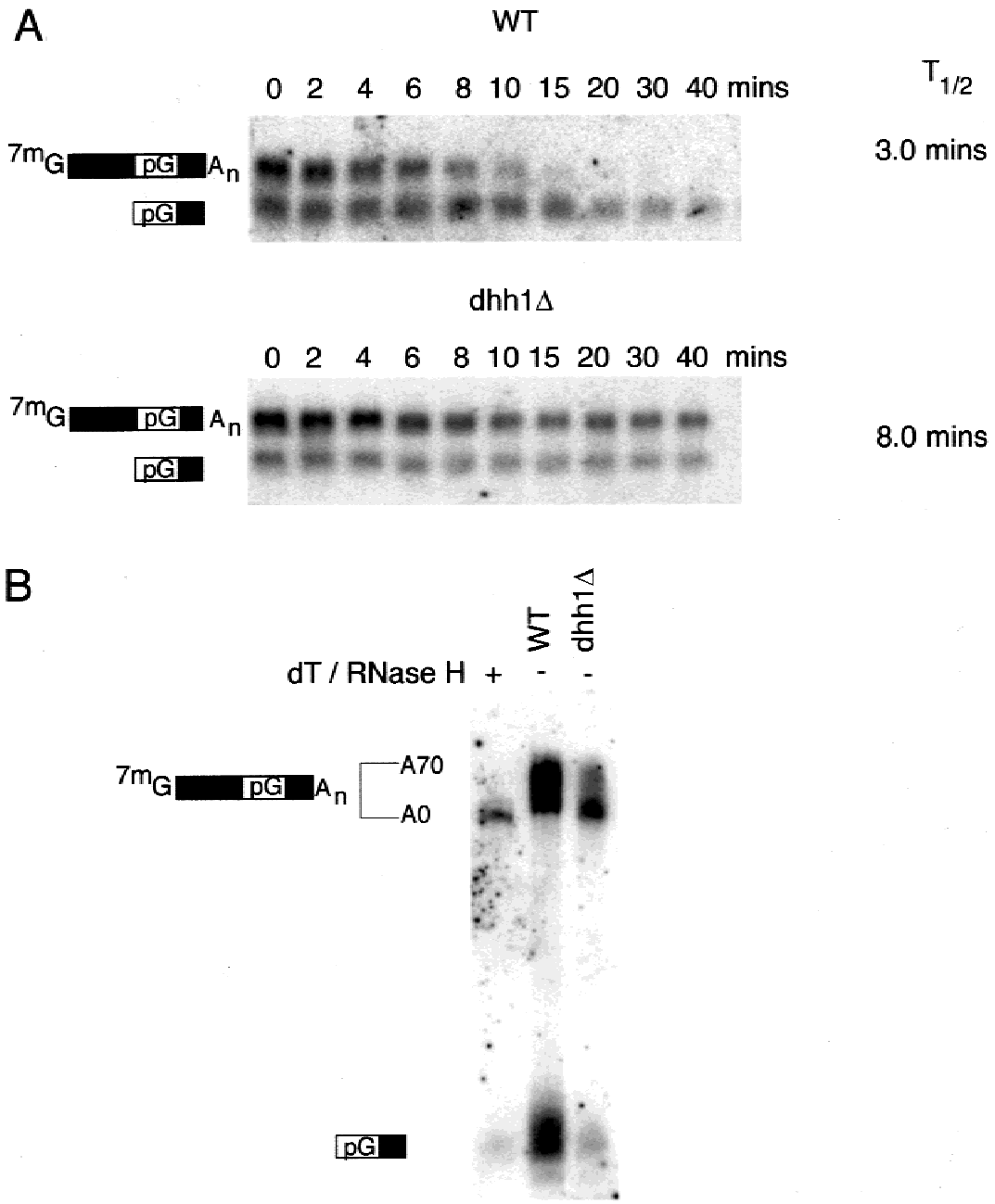

FIGURE 1. Dhh1p is required for efficient mRNA turnover. **A**: Half-life determination. Northern blot of the MFA2pG reporter following transcriptional repression in wild-type (WT) and *dhh1*Δ mutant strains. Time points are indicated above each panel in minutes. Half-lives shown were normalized to the amount of 18s rRNA in each lane. Schematic indicates the position of both the full-length species and the 5′ truncated decay intermediate. **B**: Steady-state analysis. Total mRNA from either WT or *dhh1*Δ mutants were analyzed on a polyacrylamide gel and then visualized by northern blot. Lane 1 indicates WT sample subjected to an oligo dT/RNase H cleavage to indicated fully deadenylated species. The relative positions of the full-length polyadenylated species, nonadenylated species, and decay intermediate are indicated at left.

accumulate deadenylated mRNAs (Hsu & Stevens, 1993; Muhlrad et al., 1994; Beelman et al., 1996). Northerns directed against the MFA2pG reporter revealed that this transcript accumulated as deadenylated species in *dhh1*Δ mutants as compared to the distribution in wild-type cells (Fig. 1B). This observation suggests that Dhh1p promotes either decapping or 5′-to-3′ exonucleolytic degradation.

In a second experiment, we examined the steps in mRNA turnover affected in a *dhh1*Δ using a transcriptional pulse-chase experiment. This assay utilizes the carbon source regulation of the *GAL1* UAS to rapidly induce and then repress transcription of the MFA2pG and PGK1pG genes, thereby producing a pulse of newly synthesized mRNA that can be analyzed over time. In this way, the rates of discrete stages of mRNA turnover

can be directly monitored. Although Dhh1p physically associates with the mRNA deadenylase complex (see below), there was no apparent effect on deadenylation rate for either MFA2pG or PGK1pG (Fig. 2 and data not shown). However, *dhh1*Δ mutants accumulated deadenylated species (Fig. 2). In addition, *dhh1*Δ mutants did not accumulate decay fragment as rapidly as wild-type cells. These observations are consistent with Dhh1p promoting decapping or exonucleolytic digestion.

A defect in mRNA decapping can be discriminated from a block in exonucleolytic digestion by a primer extension assay because the 5′ ends of decapped PGK1mRNAs are known to be 2 nt shorter than that of capped transcripts (Muhlrad et al., 1995; Hatfield et al., 1996; Zuk et al., 1999). To determine if Dhh1p is required for decapping or nuclease digestion, the nature of the 5′ end of the *PGK1* mRNA was determined in *dhh1*Δ mutants as compared to strains lacking the de-

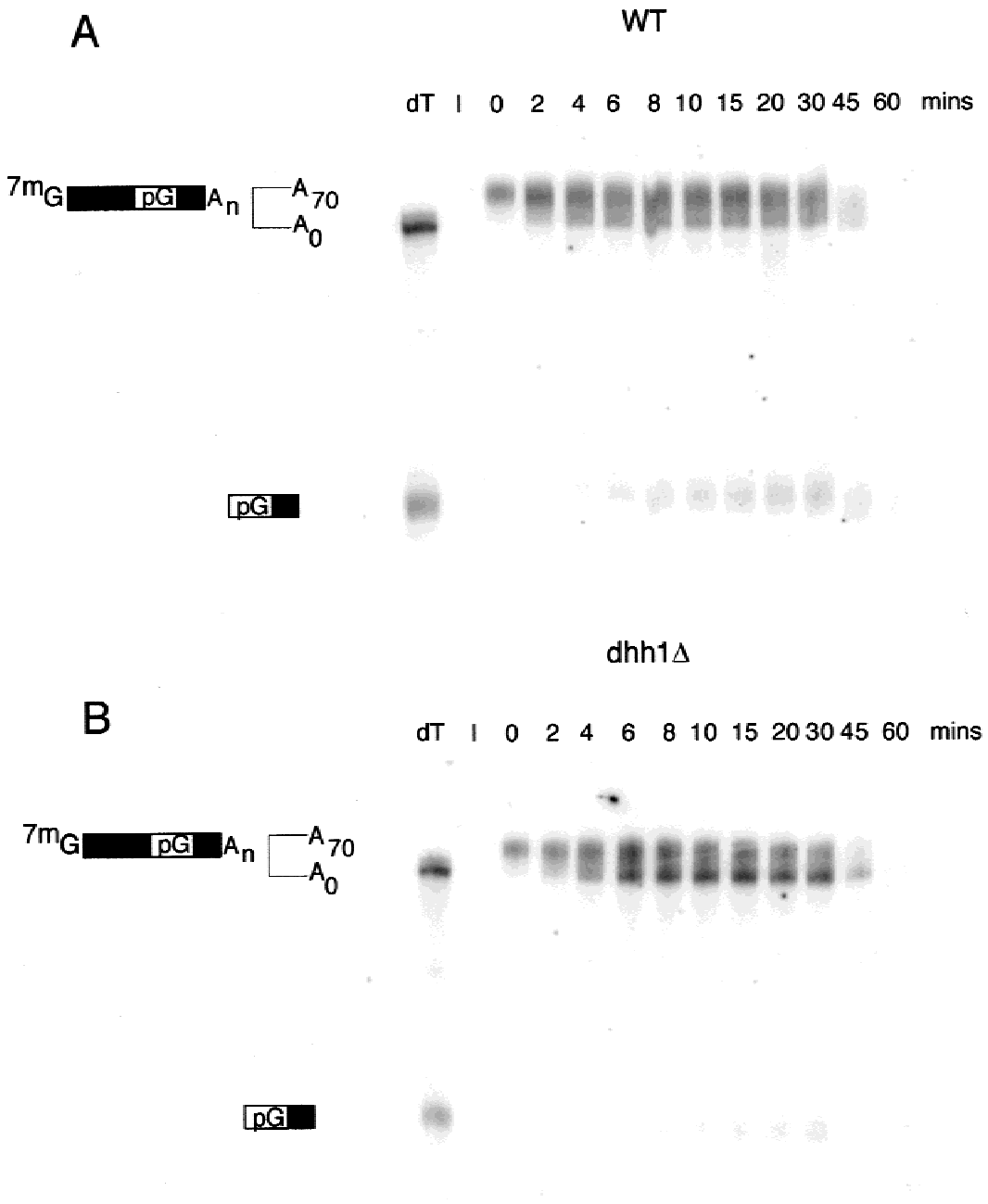

FIGURE 2. mRNA accumulated as non-adenylated species in *dhh1*Δ mutants. Shown is a transcriptional pulse-chase analysis of the MFA2pG reporter in either wild-type (WT) cells (**A**) or *dhh1*Δ mutants (**B**). dT indicates a sample of steady-state mRNA subjected to an oligo dT/RNaseH cleavage to indicated fully deadenylated species. I indicates a aliquot taken prior to induction of reporter transcription. Time points are indicated above each lane in minutes. The relative positions of the full-length polyadenylated species, nonadenylated species, and decay intermediate are indicated at left.

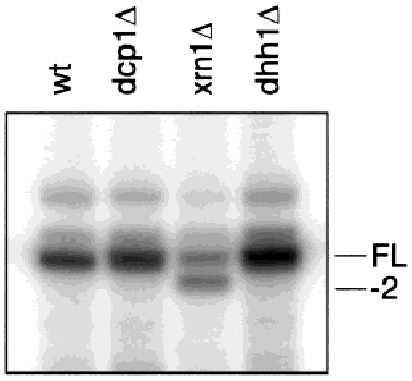

FIGURE 3. *dhh1*Δ mutants accumulate capped species. Primer extension analysis of the PGK1pG reporter in WT, *dcp1*Δ, *xrn1*Δ, and *dhh1*Δ mutant cells. Following extension of a radiolabeled probe (oRP131) by reverse transcriptase, samples were analyzed on a polyacrylamide gel and visualized by autoradiography. The positions of the full-length (FL) and 5′ truncated (−2) species are indicated at right.

capping enzyme (*dcp1*Δ) or mutant for the major 5′-3′ exonuclease (*xrn1*Δ). As expected, *PGK1* in wild type and *dcp1*Δ mutants only gave a full-length product, whereas in the *xrn1*Δ strain, a −2 species is also observed (Fig. 3). In the *dhh1*Δ mutant, only a full-length product was observed (Fig. 3). We interpret these results to indicate that Dhh1p is required for efficient decapping of mRNAs following deadenylation.

Dhh1p is not required for nonsense-mediated decay

mRNAs that contain premature translation termination codons are rapidly degraded in a process referred to as nonsense-mediated decay (NMD; for review, see Hilleren & Parker, 1999). In NMD, the aberrant mRNAs are decapped prior to deadenylation (Muhlrad & Parker, 1994). NMD requires the same decapping enzyme consisting of the Dcp1p and Dcp2p proteins (Beelman et al., 1996; Dunckley & Parker, 1999), but does not require the Lsm1-7 complex nor Pat1p, which promote decapping of normal mRNAs following deadenylation (Hatfield et al., 1996; Boeck et al., 1998; Bouveret et al., 2000). To determine if Dhh1p functions in NMD, we analyzed the decay of a *PGK1* mRNA with a nonsense allele at position 103 (referred to as PGK1n103; Muhlrad & Parker, 1994) in wild type and *dhh1*Δ mutants. In both wild-type and *dhh1*Δ cells, the PGK1n103 mRNA was unstable with a half-life of approximately 3 min (Fig. 4). These results indicate that Dhh1p is not required for NMD.

Turnover enzymes physically associate with Dhh1p

In principle, the role of Dhh1p in promoting decapping could be either direct or indirect. Interestingly, genomic

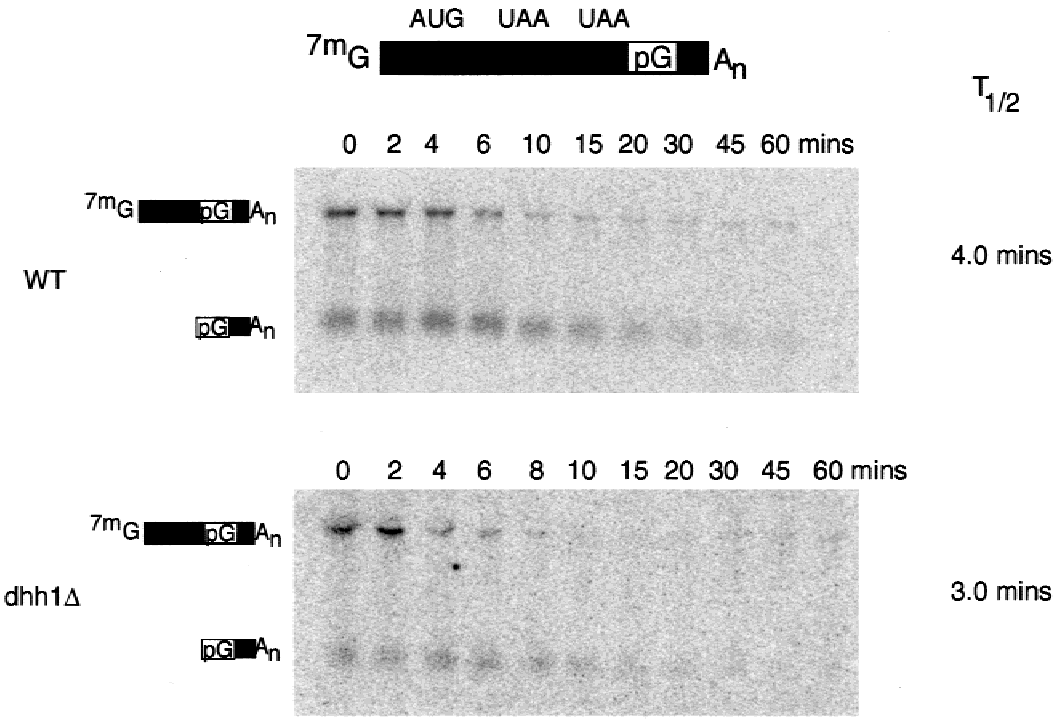

FIGURE 4. Dhh1p is not required for non-sense mediate decay. Half-life determination. Northern of a PGK1 reporter bearing a nonsense mutation in either wild-type (WT) cells or *dhh1*Δ mutants following transcriptional repression. Time points are indicated above each lane in minutes.

scale two-hybrid screens have identified several two-hybrid interactions between Dhh1p and proteins involved in mRNA decapping. Specifically, Dhh1p has shown two hybrid interactions with the decapping enzyme Dcp1p (Uetz et al., 2000), Pat1p and Lsm2p (Uetz et al., 2000; Ito et al., 2001). To determine the significance of these two hybrid interactions, we immunoprecipitated FLAG-tagged Dhh1p and examined if other proteins coimmunoprecipitated by western analysis. As predicted from the two-hybrid analysis, Dcp1p coimmunoprecipitated with Dhh1p, verifying this interaction (Fig. 5A, panel 1). Dhh1p also coimmunoprecipitated the decapping activators Lsm1p and Pat1p (Fig. 5A, panels 3 and 4). None of these interactions were RNase A sensitive, suggesting they associate as a complex rather than co-occupy an RNA molecule (Fig. 5A). These observations indicate that the Dhh1p can assemble, either directly or with additional components, with proteins directly involved in the decapping reaction.

Dhh1p associates with components of the major deadenylase

Dhh1p has also been shown to interact with Pop2p (Hata et al., 1998), a component of the yeast deadenylase (Tucker et al., 2001). Given this observation, we determined if the Dhh1p interaction with Pop2p was dependent on RNA. In this case, the immunoprecipitation of the FLAG–Dhh1p fusion was performed in a strain wherein Pop2p was tagged with multiple Myc epitopes (Tucker et al., 2001). The eluted complexes were then probed using an anti-myc antibody. Like Dcp1p, Pop2p also coimmunoprecipitated with Dhh1p (Fig. 5A, panel 2). Furthermore, this interaction was also not RNase A sensitive, suggesting directness. These results indicate that Dhh1p can physically interact with both the decapping enzyme and the deadenylase (see Discussion).

The decapping enzyme and deadenylase interact in vivo

The communication between Dhh1p and both the decapping and deadenylase complex implies there are either two distinct complexes with which Dhh1p associates, or there is an interaction between these two enzymes. To determine if the deadenylase and decapping enzyme physically interact, we immunoprecipitated the myc-tagged Pop2p and asked if Dcp1p coimmunoprecipitated as assessed by western analysis probing for the presence of Dcp1p. As shown in Figure 5B, Pop2p coimmunoprecipitated Dcp1p in an RNase A insensitive fashion. These results suggest that the decapping enzyme and the deadenylase complex can interact, at least transiently, in vivo (see Discussion).

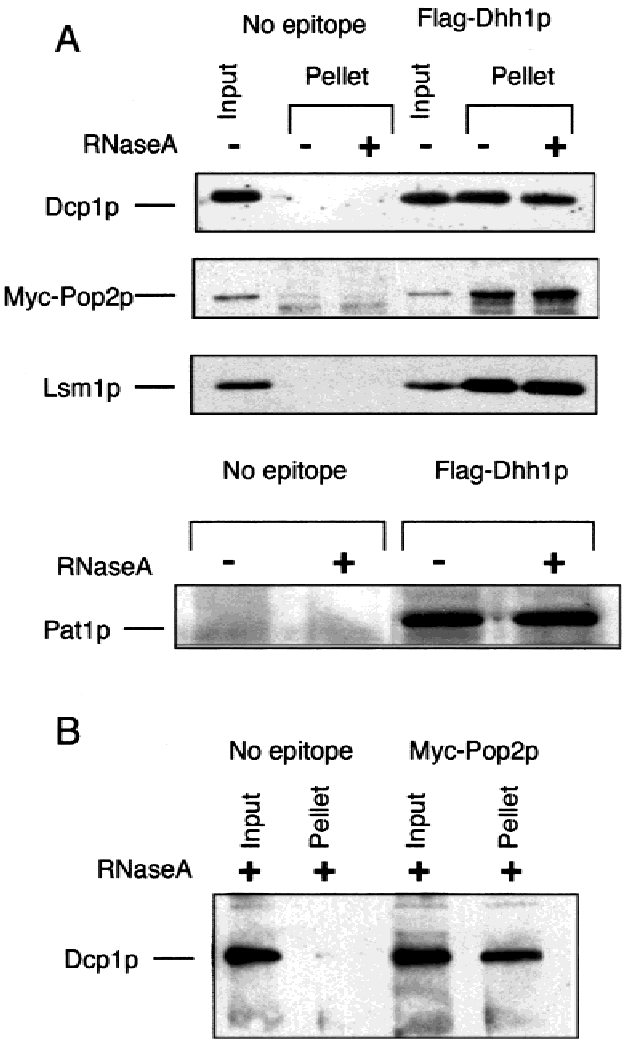

FIGURE 5. Dhh1p interacts with various turnover factors. Dhh1p interacts specifically with Dcp1p, Pop2p, Lsm1p, and Pat1p (**A**). Western analysis of immunoprecipitation reactions of yeast lysates made from either a wild-type strain (no epitope) or a strain expression a Flag–Dhh1p fusion. Blot was probed with either anti-Dcp1p antibody (panel 1) anti-myc (Pop2p) antibody (panel 2), anti-Lsm1p antibody (panel 3), or anti-Pat1p antibody (panel 4). The position of each protein is indicated to left. Input represents protein present in lysate prior to precipitation. Pellet indicates eluate from anti-Flag matrix following precipitation. Extracts were either treated (+) or not treated (−) with RNase A prior to incubation with affinity matrix. **B**: Dcp1p coimmunoprecipitates with Pop2p. Western analysis of immunoprecipitation reactions of yeast lysates made from either a wild-type strain (no epitope) or a strain expression a myc-Pop2p fusion. Blot was probed with anti-Dcp1p antibody. Immunoprecipitation was performed in presence of RNase A.

Links to Dhh1p and mRNA translation

Several lines of evidence suggest that Dhh1p has a role in mRNA metabolism outside its effects on mRNA turnover (see Discussion). To determine if this second function may be related to translation, the genetic relationships between *dhh1*Δ and alterations in the cap-binding protein were examined. A double mutant between *dhh1*Δ and a temperature-sensitive allele of

the yeast homolog of eIF-4E (*cdc33-1*) were alive but grew much more poorly at 30 °C than either single mutant (data not shown). In addition, the cold sensitivity of *dhh1*Δ cells, but not the sensitivity to higher temperatures, was suppressed by *caf20*Δ (Fig. 6A). Caf20p has previously been demonstrated to interact with the cap-binding complex and negatively modulates translation by competing with eIF-4G for interaction with eIF-4E (Altmann et al., 1997; Uetz et al., 2000). *caf20*Δ suppression of *dhh1*Δ is not due to a restoration of efficient decapping in these strains because analysis of steady-state mRNA at both 30 °C and 18 °C shows that *dhh1*Δ *caf20*Δ strains show the same defect in turnover as the *dhh1*Δ mutant alone (data not shown).

To obtain additional evidence for genetic interactions between the Caf20p and Dhh1p, we determined the effects of high copy expression of Caf20p on various strains. Wild type, *dhh1*Δ, *pat1*Δ, *lsm1*Δ, *ccr4*Δ, and *dcp2*Δ mutants were transformed with a high-copy plasmid expressing Caf20p under control of the *GAL1* UAS (Gal-CAF20; de la Cruz et al., 1997). As a control, cells were also transformed with the parental vector of this construct. After obtaining transformants, cells were plated onto minimal media containing either glucose or galactose, and incubated at 24 °C (Fig. 6). Both the *dhh1*Δ and *pat1*Δ mutants containing the Caf20p expression vector failed to grown on galactose, whereas the wild type, *ccr4*Δ, *dcp2*Δ, and *lsm1*Δ mutants grew as well as the vector control (Fig. 6B–D). On glucose, all cells grew as well as their vector control, indicating the lethality seen on galactose is due to overexpression of Caf20p. In combination, these results suggest that *dhh1*Δ and *pat1*Δ cells are hypersensitive to decreases in translation and imply a possible role for Dhh1p in translation (see Discussion).

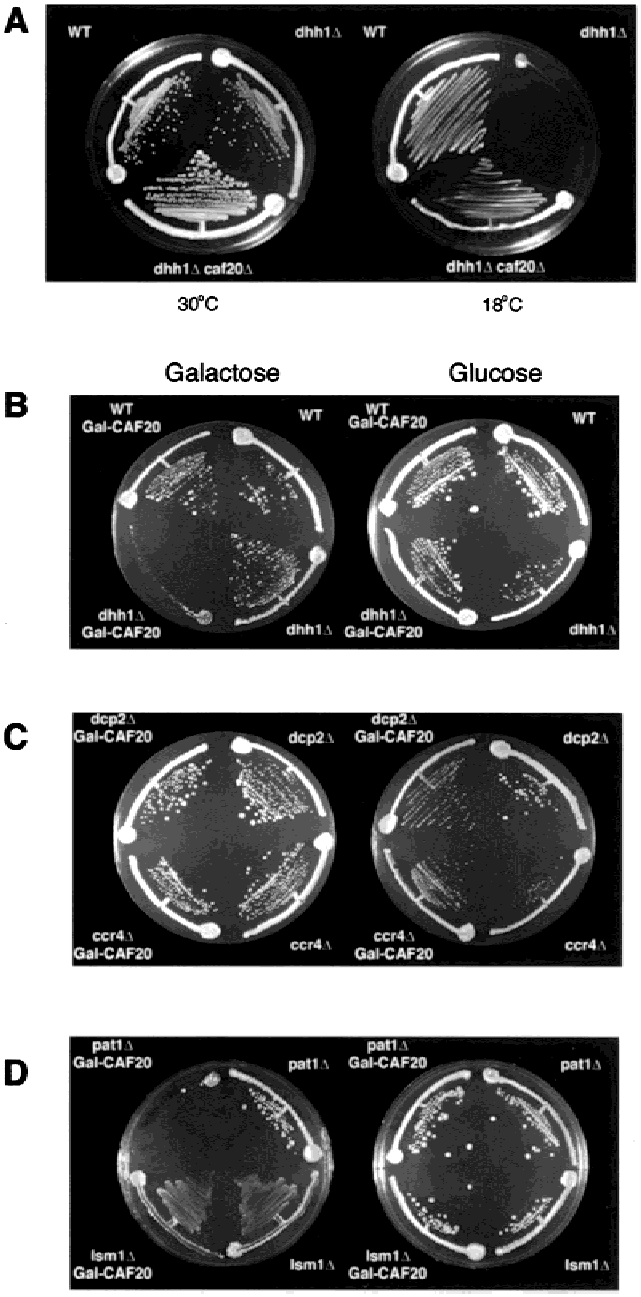

FIGURE 6. Genetic interactions between Dhh1p and Caf20p. **A**: *caf20*Δ suppresses the cold-sensitive phenotype of *dhh1*Δ mutants. Wild-type (WT), *dhh1*Δ mutants, and a *dhh1*Δ*:caf20*Δ double mutant were streaked onto plates and incubated at either 30 °C (left plate) or 18 °C (right plate) for several days. **B–D**: Overexpression of Caf20p is lethal in *dhh1*Δ and *pat1*Δ strains. Wild type, *dhh1*Δ (**B**); *ccr4*Δ, *dcp2*Δ (**C**); *and lsm1*Δ and *pat1*Δ (**D**) mutants were transformed with either empty vector or a high-copy 2-μ plasmid expressing Caf20p under control of the GAL1 UAS (Gal-CAF20). Cells were plated on either galactose (left plate) or glucose (right plate) and grown for several days at 24 °C

DISCUSSION

Dhh1p is required for efficient decapping of mRNAs

Several lines of evidence indicate that Dhh1p functions to enhance the rate of mRNA decapping. First *dhh1*Δ mutants show prolonged half-lives of several yeast mRNAs (Fig. 1). Second, *dhh1*Δ strains accumulate deadenylated transcripts (Fig. 2). Third, these deadenylated transcripts appear to be capped at their 5′ ends as indicated by primer extension analysis (Fig. 3). Fourth, as assessed by both two-hybrid and coimmunoprecipitation analysis, Dhh1p physically associates with the decapping enzyme, Dcp1p, and the decapping activators, Pat1p and Lsm1p (Fig. 5; Tharun et al., 2000; Uetz et al., 2000; Ito et al., 2001). Because *dhh1*Δ strains show a decrease in the rate of decapping, but not an absolute block, we conclude that the Dhh1p interacts with the decapping machinery and increases the efficiency of mRNA decapping.

Dhh1p may form complexes with decay factors

In this report, we show that Dhh1p interacts with several proteins involved in mRNA turnover. These factors

include a component of the deadenylase, Pop2p, the major decapping enzyme, Dcp1p, and the enhancers of decapping, Pat1p and Lsm1p.

The association of Dhh1p with both Dcp1p and Pop2p implies that there is a physical interaction between the deadenylase and the decapping enzyme in vivo. This hypothesis is further supported by the observation that a component of the deadenylase, Pop2p, coimmunoprecipitates with Dcp1p (Fig. 5). This interaction may be direct or possibly mediated through Dhh1p or other factors. These data raise the possibility that shortening of the poly(A) tail serves two functions, to promote the loss of Pab1p, thereby breaking the interactions between the 5′ and 3′ ends of the mRNA, and to alter the activity of a decapping enzyme associated with the deadenylase.

Dhh1p has a second biological role

Several observations indicate that Dhh1p has one or more additional roles in mRNA metabolism outside of mRNA turnover. First, although the defect in mRNA decapping is the same at all temperatures (data not shown), *dhh1*Δ strains are unable to grow at 18 °C and 36 °C. This is in contrast to mutants completely blocked for decapping such as *dcp1*Δ or *dcp2*Δ, which in our strain background are viable but slow growing at 18 °C and 36 °C (Beelman et al., 1996; Dunckley & Parker, 1999). Given this, the thermosensitivity of *dhh1*Δ strains suggests Dhh1p has a second biological role. Similarly, disruption of *caf20*Δ in a *dhh1*Δ background suppresses the cold-sensitive phenotype of *dhh1*Δ cells without suppressing the mRNA decay defect. In addition, we have observed that point mutations in the helicase motifs in Dhh1p allow growth at all temperatures even though mRNA decay is still defective (data not shown).

One possibility is that this second function relates to some aspect of mRNA translation. We also show that overexpression of Caf20p, which inhibits cap-dependent translation, is lethal to *dhh1*Δ and *pat1*Δ cells (Fig. 6). Importantly, this effect is specific for the *dhh1*Δ and *pat1*Δ mutants and overexpression of Caf20p does not exaggerate the slow growth of other strains with defects in mRNA turnover such as *lsm1*Δ, *ccr4*Δ, and *dcp2*Δ (Fig. 6). This phenotype is suggestive of some role in translation for Dhh1p and Pat1p because overexpression of Caf20p is also lethal to cells defective in the translational initiation factors eIF-4E, eIF-4A, eIF-4B, and eIF-4G (de la Cruz et al., 1997). Consistent with this interpretation, Pat1p has been suggested to be involved in translation initiation based on in vitro effects and alteration in polysomes profiles in vivo in *pat1*Δ mutants (Wyers et al., 2000). However, in our strain background, polysome profiles are largely unaffected in both *dhh1*Δ and *pat1*Δ strains as compared to wild type (data not shown). This implies that any roles of these proteins in translation are limited or transcript specific. This is consistent with the fact that neither Dhh1p nor Pat1p is essential for viability.

Alternatively, Dhh1p's second biological role may be related to some aspect of mRNA transport. Minshall et al. (2001) have shown that clam RCK/p54 helicase p47 translocates from cytoplasm to the nucleus during early embryogenesis. Consistent with this observation, Pat1p physically binds both Dhh1p and Crm1p/Xpo1p, an exportin (Ito et al., 2001). In this light, the genetic relationship seen between Dhh1p and Caf20p could be explained if *dhh1*Δ mutants are defective in some aspect of mRNA transport, thus reducing translational efficiency.

How does Dhh1p promote efficient mRNA decapping?

Insight into the role of Dhh1p in decapping may come from a consideration of the inverse relationship between the translational efficiency of a mRNA and its rate of decapping. This is based on the observations that a wide variety of alterations that lead to decreased translation initiation rate increase the rate of decapping (e.g., Muhlrad et al., 1995; LaGrandeur & Parker, 1999; Schwartz & Parker, 1999). In addition, purified cytoplasmic cap-binding protein, eIF-4E, inhibits Dcp1p in vitro (Schwartz & Parker, 2000). These results suggest that a critical step in mRNA decapping is the loss of eIF-4E from the mRNA, which allows the mRNA to become a substrate for decapping (Schwartz & Parker, 2000). Thus, one intriguing possibility is that Dhh1p may enhance mRNA decapping in two manners: (1) by facilitating the assembly of decay factors, and (2) by destabilizing the translational initiation complex following deadenylation, thereby increasing the access of the cap structure to Dcp1p. This could be envisioned as establishing a complex that is repressed for translation and posed for decapping. This possibility could provide a role of the putative helicase activity of Dhh1p and may also explain the role for RCK/p54 helicases in maternal mRNA storage (see below).

A related mechanism for mRNA decapping and maternal mRNA storage?

The homology between Dhh1p and other RCK/p54 helicases suggests an ancestral relationship between yeast mRNA decay and metazoan maternal mRNA storage. The *Xenopus* homolog, Xp54, is a component of stored maternal mRNAs whereas the *C. elegans* homolog, cgh-1, is a component of P granules and other possible mRNA–protein complexes (Ladomery et al., 1997; Navarro et al., 2001). Although no direct role for cgh-1 in translational control has been demonstrated, the recent work of Minshall et al. (2001) has shown that both the clam RCK/p54 helicase (p46) and the *Xenopus*

homolog (Xp54) interact with the cytoplasmic polyadenylation element binding protein (CPEB), which is known to participate in maternal mRNA storage (Minshall et al., 2001). In addition, tethering of Xp54 to the 3′ UTR of a reporter results in a three- to fivefold decrease in expression (Minshall et al., 2001). Similarly, Nakamura et al. (2001) have also shown that loss of the *Drosophila* homolog, Me31B, stimulates the unmasking of oskar and bicoid mRNAs during early development.

In essence, mRNA decapping and maternal mRNA storage may be manifestations of the same event. In both cases, RCK/p54 helicases may have a conserved role in promoting a transition from translational active to a repressed state (see below). In yeast, translational repression following deadenylation would lead to the decapping and destruction of the mRNA. In early metazoan development, however, deadenylation would lead to the storage of mRNAs as metabolically inert species until activated by cytoplasmic polyadenylation (Gray & Wickens, 1998). There are two striking similarities between the formation of an untranslated mRNP prior to decapping and the storage of maternal mRNAs. First, in both cases the mRNA is deadenylated and then enters a translational repressed state (Fig. 7). Second, in both cases, similar proteins are involved. For example, whereas Dhh1p is required for efficient decapping, the *Xenopus* homolog, Xp54, is a component of stored maternal mRNPs and stimulates repression when tethered (Ladomery et al., 1997; Minshall et al., 2001). Similarly, the yeast Pat1p is a distant homolog of the *Xenopus* protein P100, a putative component of stored mRNPs (Rother et al., 1992).

The decision to enter into a translational repressed state may be a consequence of the poly(A) status of the mRNA and the helicase activity of the RCK/p54 helicase. Interestingly, tethering Xp54 repress translation, whereas tethering mutants lacking putative helicase function stimulate translation on poly(A) minus substrates (Minshall et al., 2001). Moreover, tethering both wild-type and mutant Xp54 to poly(A) plus substrates has no effect on the translational activity of the RNA in oocytes (Minshall et al., 2001). One interesting possibility is that the poly(A) tail modulates RCK/p54 helicase activity and thus keeps the mRNA active, whereas deadenylation serves to activate helicase function and stimulate decapping/repression.

Given the similarities between mRNA decapping and maternal mRNA repression, the metazoan counterparts to decay factors such as Ccr4p, Pop2p, the Lsm1-7p complex, and Pat1p may also required for storage events. These mechanistic similarities also raise the possibility that an mRNA “storage-like” event may occur in yeast, perhaps on a subset of mRNAs whose decapping rate is slow, or during various stages of the life cycle.

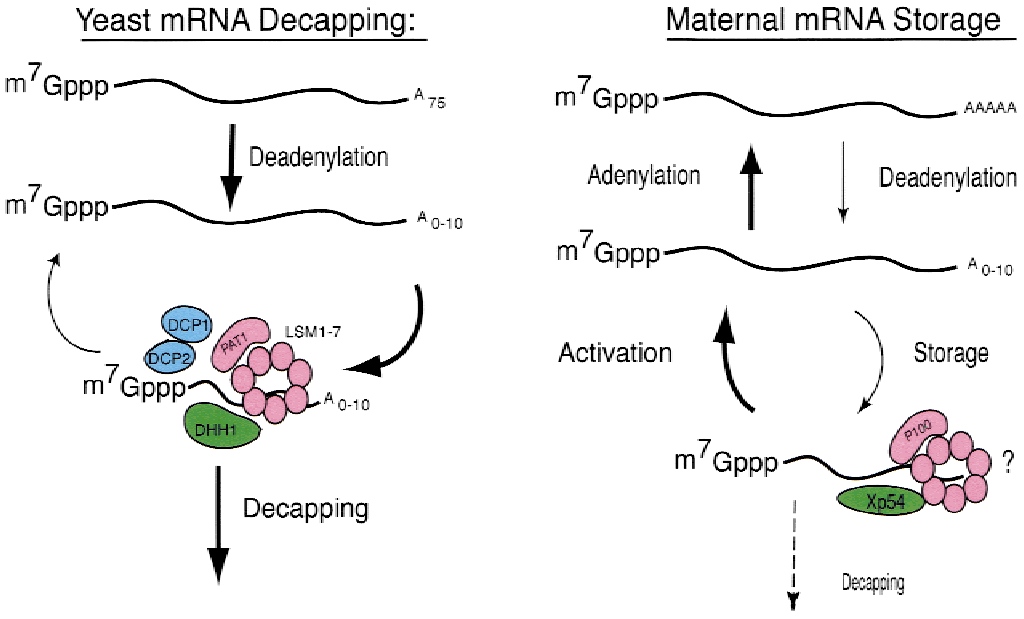

FIGURE 7. Possible relationship between mRNA decay and maternal mRNA storage. Both mRNA decay and maternal mRNA storage are initiated by deadenylation. Deadenylation presumably reduces the translational efficiency of the transcript and allows for the assembly of a “decay/storage” complex. The assembly of this complex traps the mRNA into a translational quiescent state by preventing recycling back into a translating pool. Whereas translational inhibition stimulates decapping in yeast, lack of decapping activity facilitates storage in oocytes. In principle, the components of the particle are conserved between yeast and *Xenopus* as indicated by the presence of Dhh1p/Xp54. Given this, other factors involved in yeast mRNA decapping may also be part of maternal mRNA storage particles.

MATERIALS AND METHODS

Plasmids

The FLAG-DHH1 plasmid (pRP1053) was created by PCR amplification of the DHH1 gene from yRP840, placing the FLAG epitope 5′ of DHH1 gene, and then ligating into the yeast expression vector pG-1 (Schena et al., 1991). The Gal-CAF20 vector used in Figure 6 was kindly provided by Patrick Linder (University of Geneva). The nonsense reporter used in Figure 4 (PGK1n103) was previously described in Muhlrad and Parker (1994).

Yeast

All yeast strains used in this study were derived from yRP841 (MAT∝, *leu2, lys2, trp1, ura3, cup1*::LEU2pm) with the following difference; yRP1561 (MAT∝, *his4, dhh1*::URA3), yRP1622 (MAT∝, Pop2p-MYC::NEO) and yRP1662 (MAT∝, *dhh1*::NEO), yRP1372 (MATa, *his4, pat1*::LEU2), yRP1346 (MATa, *his4, dcp2*::TRP1), yRP1616 (MATa, *his4*, *ccr4*::NEO), yRP1365 (MAT∝, *lsm1*::TRP1), yRP1318 (MAT∝, *caf20*Δ::URA3), yRP1663 (MATa, *dhh1*Δ::URA3, *caf20*Δ::URA3).

RNA analyses

All RNA analyses were performed as described in Muhlrad et al. (1994). For half-life determination in Figure 1, cells were grown to mid-log phase in media containing 2% galactose. Cells were harvested and briefly washed. Transcription was then repressed by the addition of media containing 4% glucose. Aliquots were taken over a brief time course and quickly frozen. For the transcriptional pulse-chase experiments described in Figure 2, cells were first grown on media containing 2% raffinose until mid-log phase. Transcription was then induced by the addition of galactose (final concentration = 2%). After a brief incubation of 8 min, cells were washed and then resuspended in media containing 4% glucose. Aliquots were then taken over time. Steady-state analyses as shown in Figure 1 were performed by extracting mRNA from cultures grown to mid-log phase in 2% galactose containing media.

Yeast total mRNA extractions were preformed as described in Muhlrad et al. (1994). RNAs were analyzed by running 10 μg of total mRNA on either a 1.5% formaldehyde agarose gel as in Figure 1A or a 6% polyacrylamide/7.5 M urea gel as in Figures 1B, 2, and 3. All northerns were performed using radiolabeled oligo probes directed against either the MFA2pG reporter (oRP140) or the PGK1pG reporter (oRP141).

Primer extension analysis was performed as previously described in Hatfield et al. (1996). Oligo oRP131, which is complementary to the 5′ end of the PGK1 transcript, was radiolabeled with 32P γ-ATP and polynucleotide kinase. Approximately, 2.0×10^6 cpm of radiolabeled oligo were added to 10 μg of total yeast mRNA. Extension was performed using Superscribe reverse transcriptase (Gibco BRL).

Immunopreciptations and western analysis

Immunoprecipitation experiments were performed as described in Tharun et al. (2000). Briefly, 200 mL yeast cultures were grown to mid-log phase. Extract was made by vortexing with glass beads in IP buffer (50 mM Tris, pH 7.5, 50 mM NaCl, 2 mM $MgCl_2$, 1 mM β-mercaptoethanol, 1× protease inhibitor, 0.1% NP40). Lysate was then spun at 15,000 rpm and the supernatant was incubated with anti-flag matrix (Sigma) for Figure 5A and anti-myc matrix (Covance) for Figure 5B for 3 h. Where indicated, samples were treated with 10 mg/mL RNase A prior to incubation with matrix. Following incubation, samples were pelleted and washed three times in IP wash (50 mM Tris, pH 7.5, 150 mM NaCl, 2 mM $MgCl_2$, 0.1% NP40). Bound complexes were eluted by boiling samples in SDS-loading buffer.

For western analysis, all samples were run on 10% SDS-polyacrylamide gels. Proteins were then transferred to nitrocellulose and probed with anti-Dcp1p, anti-Pat1p, anti-myc, or anti-Lsm1p. (Lms1p antibodies were a gift from A. Sachs, University of California, Berkeley).

Genetic interaction studies

Growth suppression between *DHH1* and *CAF20* was determined by crossing the *dhh1*Δ strain (yRP1562) with a *caf20*Δ mutant (yRP1318). The double mutant was then grown alongside the *dhh1*Δ mutant and wild-type strains at both 30 °C and 18 °C for several days. Caf20p high-copy lethality was determined by transforming a wild type (yRP841), *dhh1*Δ (yRP1561), *dcp2*Δ (yRP1346), *ccr4*Δ (yRP1616), *pat1*Δ (yRP1372), or a *lsm1*Δ (yRP1365) mutant with either pGAL-CAF20 (de la Cruz et al., 1997) or empty vector and plating cells on either glucose- or galactose-containing media. Cells were grown for several days at 24 °C and then scored for growth.

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