Copyright © 2009 by the Genetics Society of America
DOI: 10.1534/genetics.108.092601

Stm1 Modulates mRNA Decay and Dhh1 Function in *Saccharomyces cerevisiae*

Vidya Balagopal and Roy Parker[1]

Department of Molecular and Cellular Biology and Howard Hughes Medical Institute, University of Arizona, Tucson, Arizona 85721-0206

Manuscript received June 12, 2008
Accepted for publication November 10, 2008

ABSTRACT

The control of mRNA degradation and translation are important for the regulation of gene expression. mRNA degradation is often initiated by deadenylation, which leads to decapping and 5′–3′ decay. In the budding yeast *Saccharomyces cerevisae*, decapping is promoted by the Dhh1 and Pat1 proteins, which appear to both inhibit translation initiation and promote decapping. To understand the function of these factors, we identified the ribosome binding protein Stm1 as a multicopy suppressor of the temperature sensitivity of the *pat1*Δ strain. Stm1 loss-of-function alleles and overexpression strains show several genetic interactions with Pat1 and Dhh1 alleles in a manner consistent with Stm1 working upstream of Dhh1 to promote Dhh1 function. Consistent with Stm1 affecting Dhh1 function, *stm1*Δ strains are defective in the degradation of the *EDC1* and *COX17* mRNAs, whose decay is strongly affected by the loss of Dhh1. These results identify Stm1 as an additional component of the mRNA degradation machinery and suggest a possible connection of mRNA decapping to ribosome function.

CONTROL of mRNA translation and degradation are important points of regulation of eukaryotic gene expression. In eukaryotic cells there are two general mechanisms for the degradation of mRNAs, both of which initiate with deadenylation, leading either to 3′–5′ exonucleolytic degradation or to decapping followed by 5′–3′ exonucleolytic destruction of the mRNA (reviewed in Parker and Song 2004; Garneau *et al.* 2007; Shyu *et al.* 2008). In *Saccharomyces cerevisiae*, the major pathway of mRNA decay involves decapping followed by 5′–3′ decay (Coller and Parker 2004), with removal of the poly(A) tail predominantly promoted by the Ccr4/Pop2/Not deadenylase complex (Decker and Parker 1993; Muhlrad *et al.* 1994; Tucker *et al.* 2001). The 5′ m^7G cap is then removed by the Dcp1/2 decapping enzyme and 5′–3′ decay is performed by the exonuclease Xrn1 (Hsu and Stevens 1993; Beelman *et al.* 1996; Dunckley and Parker 1999).

Decapping is a critical step in this decay pathway as it permits destruction of the mRNA and is a site of numerous control inputs. Moreover, many observations indicate that decapping and translation in yeast cells are intertwined processes that are often in competition. For example, when mRNAs are maintained in association with ribosomes by the inhibition of translation elongation using cycloheximide, the rate of decapping is reduced (Beelman and Parker 1994). Conversely, mRNAs poorly translated because of *cis* elements, such as secondary structures in the 5′ untranslated region or a poor AUG context, are decapped faster than their well-translated counterparts (Muhlrad *et al.* 1995; Lagrandeur and Parker 1999). Moreover, mutation of initiation factors such as eIF-4E, the cap binding protein, or Prt1 (part of the eIF3 complex) lead to faster degradation of mRNAs (Schwartz and Parker 2000). Consistent with this competition, eIF-4E has been shown to inhibit the decapping enzyme *in vitro* (Schwartz and Parker 1999). Thus, a key step in mRNA decapping is exchanging translation initiation factors for the mRNA decapping machinery.

The balance between translation and decay also correlates with the type of mRNP formed and its subcellular localization. When mRNAs exit translation, they form nontranslating mRNPs, which can undergo decapping and degradation and/or accumulate in cytoplasmic foci referred to as P-bodies (Sheth and Parker 2003). P-bodies are cytoplasmic foci that accumulate translationally repressed mRNA along with the decay machinery and translational repressors (reviewed in Eulalio *et al.* 2007; Parker and Sheth 2007). Analyses of P-bodies provide additional evidence for an inverse relationship between translation and formation of mRNPs capable of mRNA decapping. For example, blocking translation initiation using mutations in initiation factors leads to an increase in the P-body size and number along with accelerated decay rates. Conversely, inhibition of translation elongation and trapping of the mRNAs in polysomes lead to the loss of P-bodies (Sheth and Parker 2003; Teixeira *et al.* 2005).

An important question is the mechanism by which mRNAs cease translation initiation and form nontranslating mRNPs capable of decapping and accumulation

[1] *Corresponding author:* Department of Molecular and Cellular Biology and Howard Hughes Medical Institute, University of Arizona, 1007 E. Lowell St., Tucson, AZ 85721-0206. E-mail: rrparker@u.arizona.edu

TABLE 1

Yeast strains used in this study

Name	Genotype	Reference
yRP2065	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Coller and Parker (2005)
yRP2066	*MAT***a**, *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 dhh1::NEO*	Coller and Parker (2005)
yRP2323	*MATα, his3Δ1 leu2Δ0 ura3Δ0 dhh1::NEO, pat1::HIS3*	Gift from J. Coller
yRP1437	*MAT***a**, *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 stm1::NEO*	Winzeler *et al.* (1999)[a]
yRP840	*MAT***a** *leu2–3,112 trp1 ura3-52 his4-539 cup1::LEU2/PGK1pG/MFA2pG*	Hatfield *et al.* (1996)
yRP1372	*MAT***a** *leu2–3,112 trp1 ura3-52 his4-539 cup1:LEU2/PGK1pG/MFA2pG pat1::LEU2*	Tharun *et al.* (2000)

[a] Purchased from Open Biosystems.

in P-bodies. In yeast, the Dhh1 and Pat1 proteins appear to be involved in this transition from translation to the nontranslating mRNP (Coller and Parker 2005). Dhh1 and Pat1 appear to act, at least partially, independently of each other. Strains lacking either Dhh1 or Pat1 show reductions in decapping rates, while strains lacking both proteins are severely blocked for decapping (Coller and Parker 2005). Moreover, overexpression of either Dhh1 or Pat1 causes global translational repression, as seen by a decrease in polysomes and an increase in size and number of P-bodies in a manner independent of each other (Coller and Parker 2005). Finally, Dhh1 has been shown to directly repress translation *in vitro* (Coller and Parker 2005).

Although these general translation repressors have been identified, much remains to be understood about their mode of action. One major unresolved issue is understanding how Dhh1 and Pat1 interact with the translation machinery to promote translation repression and/or target mRNAs for decapping. We have approached this issue by using genetic methods to try to find proteins that could link Dhh1 and/or Pat1 to the translation machinery. In this work we identified Stm1 as a high-copy suppressor of the temperature-sensitive growth defect of the *pat1Δ* strain. Stm1 has been shown to associate with ribosomes (Van Dyke *et al.* 2004, 2006) and was initially identified as a suppressor of Tom1, which has a role in the export of messenger RNAs from the nucleus (Utsugi *et al.* 1995). In this study we show that Stm1 has genetic interactions with Pat1 and Dhh1, affects the accumulation of Dhh1 in P-bodies, and can affect the decay of a subclass of yeast mRNAs. Taken together, this identifies Stm1 as a component of the decapping machinery that also interacts with the translation machinery.

MATERIALS AND METHODS

Yeast strains, growth conditions, and plasmids: The genotypes of all strains used in this study are listed in Table1. Strains were grown in either standard yeast extract/peptone medium (YP) or synthetic medium (SC) supplemented with appropriate amino acids and 2% dextrose. Strains were grown at 30° unless otherwise stated. For overexpression studies, strains were grown in YP or SC media supplemented with appropriate amino acids, 2% galactose, and 0.5% sucrose. All plasmids and oligonucleotides used in the study are listed in Table 2 and Table 3, respectively.

RNA analysis: All RNA analyses were performed as described in Muhlrad and Parker (1992). For half-life measurements, cells were grown to mid-log phase containing 2%

TABLE 2

Plasmids used in this study

Name	Description	Reference
pRP948	Stm1 under the control of its endogenous promoter on a 2μ *URA3* vector	This study
pRP1360	Stm1 overexpression using *GAL10* promoter on a 2μ *URA3* vector	Gelperin *et al.* (2005)[a]
pRP1728	Stm1 overexpression using *GAL10* promoter on a 2μ *LEU2* vector	This study
pRP1361	Overexpression of Dhh1 using *GAL10* promoter on a 2μ *URA3* vector	Gelperin *et al.* (2005)[a]
pRP485	*MFA2* mRNA with a poly(G) tract under *GAL10* promoter on a CEN *URA3* vector	Muhlrad and Parker (1992)
pRP469	*PGK1* mRNA with a poly(G) tract under *GAL10* promoter on a CEN *URA3* vector	Decker and Parker (1993)
pRP1189	*EDC1* mRNA with a poly(G) tract under *GAL10* promoter on a CEN *TRP1 URA3* vector	Muhlrad and Parker (2005)
pRP1151	GFP-tagged Dhh1 on a CEN *LEU2* vector	Coller *et al.* (2001)
pRP1574	mCherry-tagged Edc3 on CEN *URA3* vector	Buchan *et al.* (2008)
pRP1007	*COX17* mRNA under *GAL10* promoter on a 2μ *LEU2* vector (pG74/ST30)	Olivas and Parker (2000)

[a] Purchased from Open Biosystems.

TABLE 3

Oligonucleotides used in this study

Name	Sequence
oRP140	ATATTGATTAGATCAGGAATTCC
oRP141	AATTGATCTATCGAGGAATTCC
oRP100	GTCTAGCCGCGAGGAAGG
oRP1211	AATTGCTTTGGATGACCAGATCC
oRP1427	GGTTGTCGGCAGACTGTCAG

galactose. Transcription was repressed by the addition of media containing 4% dextrose. Aliquots were collected over a brief course of time and frozen. Total RNA was extracted as described in Caponigro *et al.* (1993) and analyzed by running 20 μg of total RNA on 1.25% formaldehyde agarose gels. All Northern analyses were performed using radiolabeled oligonucleotide probes directed against *MFA2pG* (oRP140), *PGK1pG* (oRP141), *COX17* (oRP1427), and *EDC1pG* (oRP1121). Loading corrections were done using oRP100, an oligonucleotide probe directed against *SCR1* RNA, a stable RNA polymerase III transcript. Half-lives were determined by quantitation of blots using a Molecular Dynamics phosphorimager (Sunnyvale, CA).

Microscopy: For the analysis of P-bodies under logarithmic growth conditions, cultures were grown to an OD_{600} of 0.3–0.4 in the appropriate media. Cells grown in YP media were harvested and washed with SC supplemented with appropriate amino acids and 2% dextrose and observed under the microscope. Cells grown in SC media were directly harvested, resuspended in a smaller volume of the same media, and observed under the microscope.

For the analysis of P-bodies following glucose depletion, cells grown to mid log phase were washed with SC supplemented with appropriate amino acids (no sugar), resuspended in the same media, and incubated in a flask in a shaking water bath for 15 min. The cells were then harvested and observed under the microscope.

For the analysis of P-bodies at high cell densities, the cultures were allowed to grow overnight to an OD_{600} of 1.0 or for 2 days to an OD_{600} of >3.0. The cells were harvested and observed under the microscope. All microscopy was done on a deconvolution microscope (Deltavision RT, Applied Precision) using an objective (UPlan Sapo ×100 1.4 NA; Olympus). Images were collected using software (softWoRx) as 512 × 512-pixel files with a camera (CoolSNAP HQ; Photometrics) using 1 × 1 binning. Images are Z series that have been adjusted to the same contrast range with ImageJ software.

Western analysis: Western analysis of proteins was conducted by preparing whole-cell extracts from the appropriate strains. Protein concentration was determined by Bio-Rad protein assay and equal amounts of total protein were loaded on the gel. Protein-A-tagged Dhh1 and Stm1 proteins were detected using peroxidase antiperoxidase antibody (DAKO). GFP-tagged Dhh1 and Stm1 proteins were detected using anti-GFP antibody (Covance).

RESULTS

Stm1 is a high-copy suppressor of *pat1*Δ temperature sensitivity: To identify additional proteins involved in translation repression and/or mRNA decay, we screened for high-copy suppressors of the temperature-

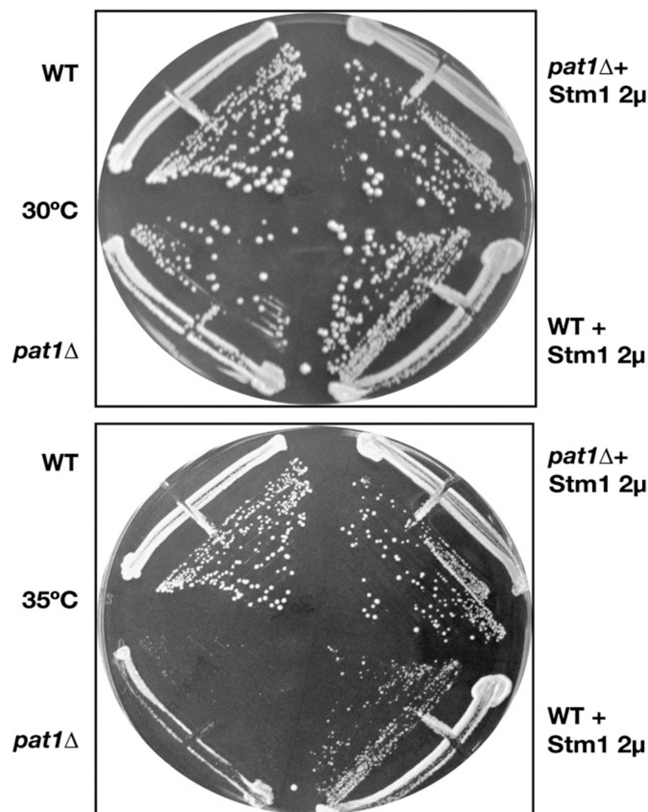


Figure 1.—Stm1 is a high-copy suppressor of *pat1*Δ^{ts}. Stm1 was overexpressed using a 2μ overexpression plasmid in wild-type (yRP840) and *pat1*Δ (yRP1372) strain backgrounds and the growth was monitored under the conditions mentioned. Overexpression of Stm1 allowed the *pat1*Δ strains to survive at nonpermissive temperatures.

sensitive phenotype of *PAT1* loss-of-function alleles using a *PAT1* nonsense allele termed *mrt1-3* (Hatfield *et al.* 1996; Tharun *et al.* 2000). A *mrt1-3* strain was transformed with a 2μ *URA3* high-copy yeast genomic library, and >10,000 transformants were screened for colonies able to grow at the nonpermissive temperature (35°). The plasmids from yeast capable of growth at nonpermissive temperature were rescued, and the yeast genomic sequences within the plasmids were identified by DNA sequence analysis. The analysis of overlapping plasmid inserts suggested that *STM1* was a high-copy suppressor of the *mrt1-3* temperature-sensitive phenotype, which was confirmed by subcloning only *STM1* on a high-copy plasmid and showing that it could suppress the temperature sensitivity of both the *mrt1-3* allele and a complete *pat1*Δ (data not shown and Figure 1). We also observed the *RCN2* gene as a weak high-copy suppressor of the *mrt1-3* and *pat1*Δ temperature sensitivity (data not shown). However, since the *RCN2* suppression phenotype was weak, it was not pursued. These results identified *STM1* as a high-copy suppressor of the temperature sensitivity of loss-of-function alleles in *PAT1*.

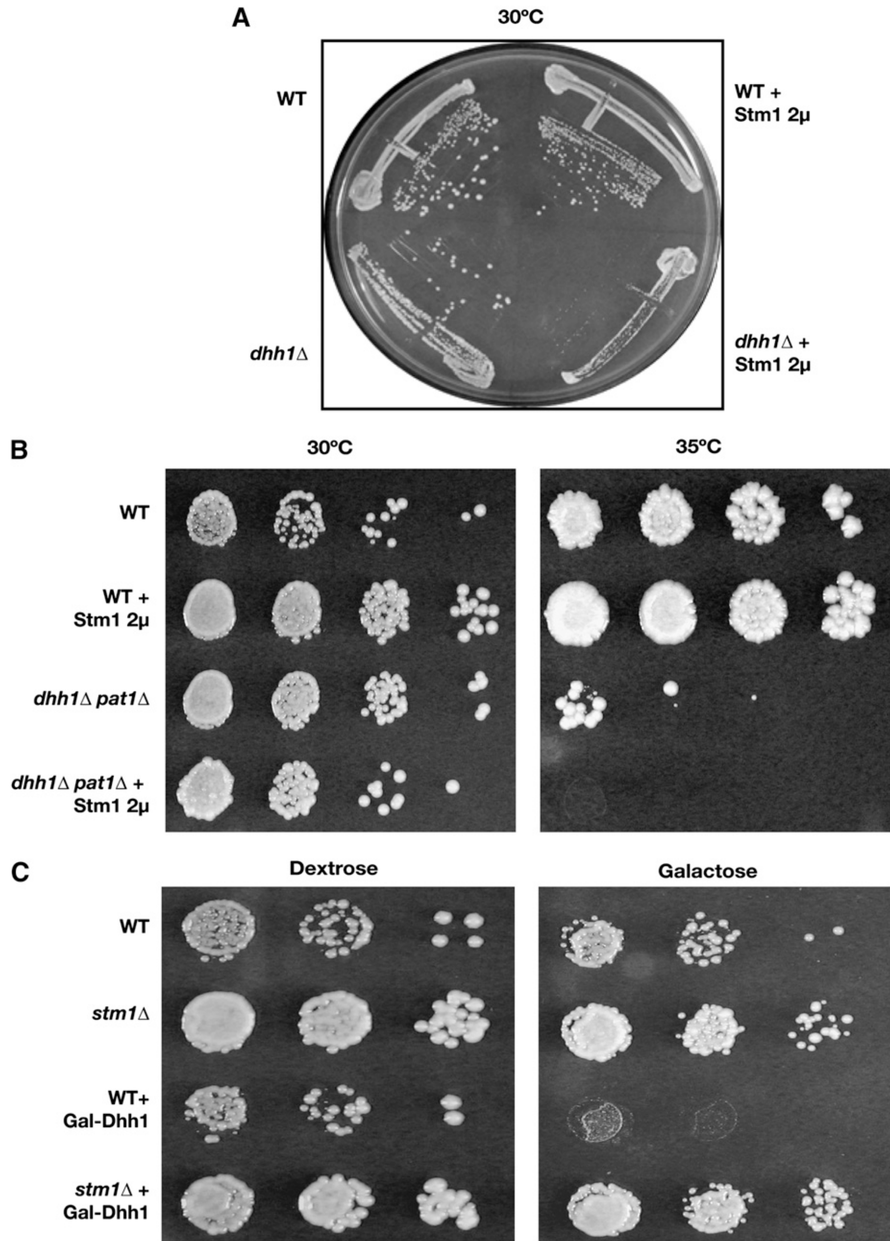


FIGURE 2.—Stm1 has genetic interactions with the general translation repressors Dhh1 and Pat1. Stm1 was overexpressed using a 2μ plasmid in wild type (yRP2065) and *dhh1*Δ (yRP2066) (A) and *dhh1*Δ *pat1*Δ (yRP2323) (B) double-deletion strain backgrounds. Growth was monitored over a period of 3 days. Overexpression of Stm1 has a deleterious effect on growth in *dhh1*Δ and *dhh1*Δ *pat1*Δ strain backgrounds. (C) Dhh1 was overexpressed using *GAL10* promoter in wild-type (yRP2065) and *stm1*Δ (yRP1437) strains. Growth was monitored over a period of 3 days. Deletion of *STM1* prevented the overexpression lethality of Dhh1.

Stm1 has genetic interactions with Dhh1 and Pat1: To examine possible mechanisms by which Stm1 suppressed *pat1*Δ, we examined how overexpression of Stm1 from a 2μ plasmid affected the growth of a *dhh1*Δ strain, which is also temperature sensitive. Surprisingly, we observed that overexpression of Stm1 impaired the growth of the *dhh1*Δ strain as compared to a *dhh1*Δ strain carrying the empty vector (Figure 2A). In contrast, 2μ overexpression of Stm1 in a wild-type strain had little or no effect on growth rate (Figure 2A). This observation demonstrated that Stm1 affects the cells' requirement for Dhh1 function and suggested that Stm1 and Dhh1 might functionally interact.

The different effects of Stm1 overexpression on the *pat1*Δ and *dhh1*Δ strains could be explained in two ways. First, overexpression of Stm1 might suppress the *pat1*Δ by promoting the function of Dhh1 in translation repression and mRNA decay. In this model, the overexpression of Stm1 in a *dhh1*Δ might be detrimental by committing mRNAs to a Dhh1-mediated event that would be lacking in a *dhh1*Δ strain. An alternative possibility is that Stm1 functions independently of Dhh1 and Pat1 in a third pathway that compensates for the loss of Pat1. To test these two possibilities, we overexpressed Stm1 (using a 2μ plasmid) in a *dhh1*Δ *pat1*Δ double deletion. If Stm1 functions independently of both Pat1 and Dhh1, overexpression of Stm1 in the double mutant should improve its growth at high temperatures. Alternatively, if Stm1 suppresses the growth defects of the *pat1*Δ by increasing the function of Dhh1, one predicts that overexpression of Stm1 should be detrimental to the growth of *dhh1*Δ *pat1*Δ.

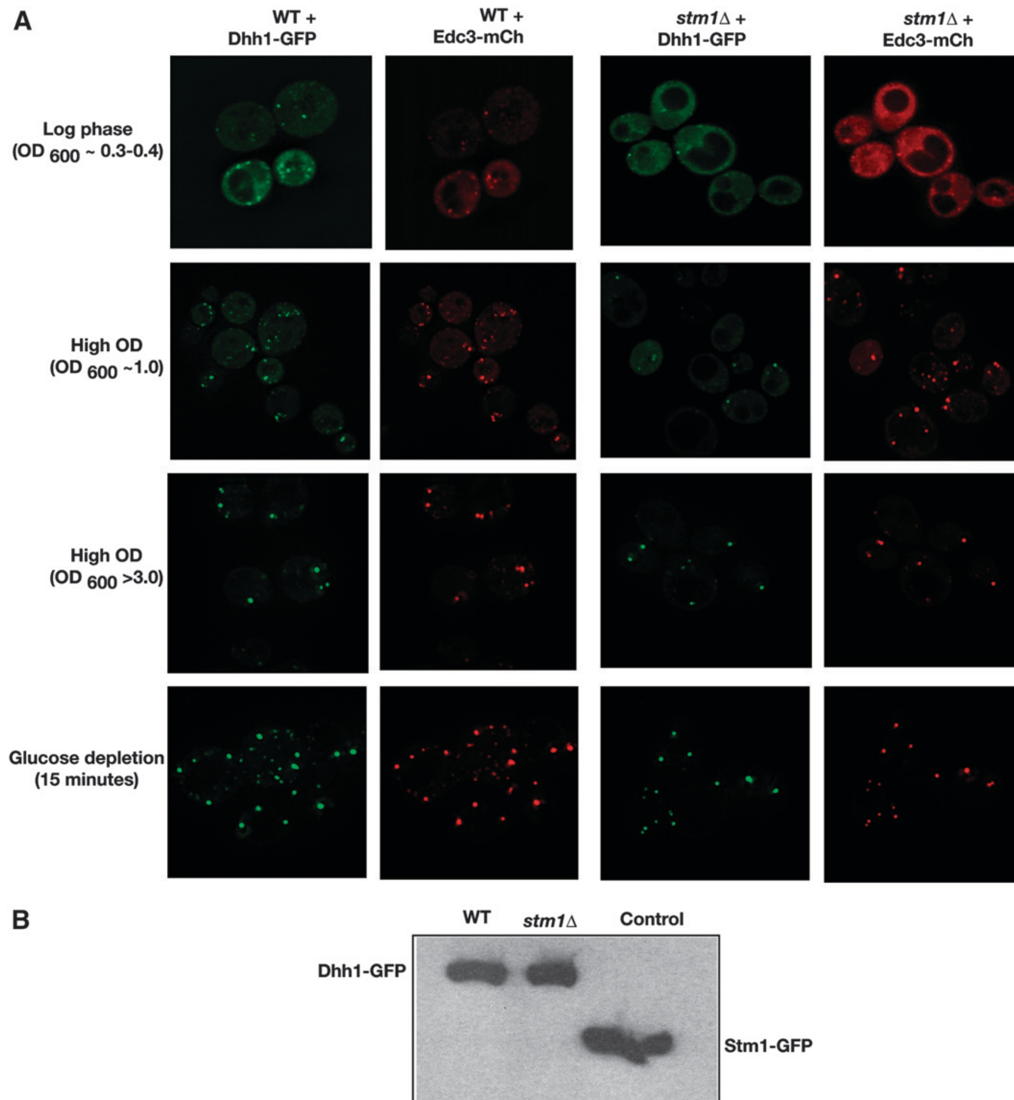


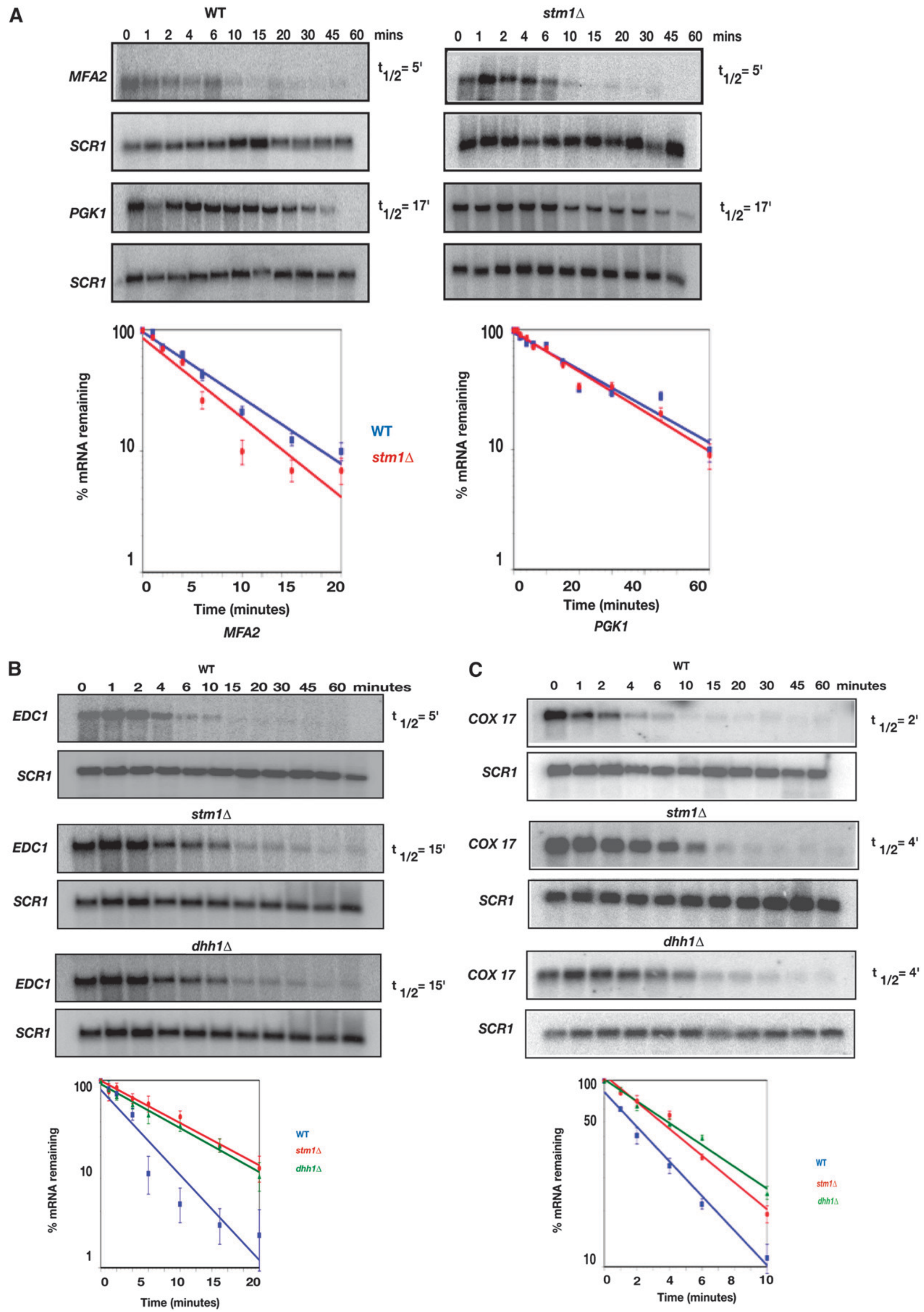
Figure 3.—Stm1 promotes the accumulation of Dhh1 in P-bodies. (A) Wild-type (yRP2065) and *stm1*Δ (yRP1437) strains were cotransformed with GFP-tagged plasmid of Dhh1 and mCherry-tagged plasmid of Edc3, and the localization of these proteins was observed by microscopy under glucose depletion or high cell densities (see materials and methods). Deletion of *STM1* affects the accumulation of Dhh1 in P-bodies under high cell densities. (B) Whole-cell extracts were prepared from wild-type (yRP2065) and *stm1*Δ (yRP1437) strains carrying Dhh1-GFP plasmid and Western analysis was performed using anti-GFP antibody (Covance). Equal amounts of total protein were loaded on the gel.

We observed that the *dhh1*Δ *pat1*Δ strain grows poorly at the restrictive temperature, but overexpressing *STM1* made it considerably worse (Figure 2B). We interpret this result to suggest that Stm1 suppresses the *pat1*Δ growth defect by promoting the function of Dhh1.

The above results suggested that Stm1 overexpression enhances the function of Dhh1. If Stm1 functions to increase Dhh1 activity in some manner, this would predict that a deletion of *STM1* reduces the function of Dhh1. To test this possibility, we took advantage of previous work showing that overexpression of Dhh1 under control of the *GAL10* promoter inhibits cell growth (Coller and Parker 2005). Given this, we asked if deletion of *STM1* had an effect on the overexpression lethality of the Dhh1.

We observed that deletion of *STM1* prevents the growth inhibition by overexpression of Dhh1 (Figure 2C). Moreover, as judged by Western analysis the *stm1*Δ strain showed similar levels of GAL-Dhh1 expression as compared to the wild-type strain (data not shown). Taken together, these observations argue that Stm1 promotes Dhh1 function.

Stm1 affects, but is not required for, the accumulation of Dhh1 in P-bodies: The above results suggested that Stm1 acts to enhance the function of Dhh1. Dhh1 is thought to function in translation repression and P-body formation by first decreasing translation initiation and then by accumulating with the translationally repressed mRNA in a P-body (Coller and Parker 2005). A prediction of Stm1 enhancing Dhh1 function is that strains lacking Stm1 might show a deficit in Dhh1 accumulation in P-bodies. To test this possibility, we examined the formation of P-bodies in *stm1*Δ strains and their ability to accumulate a GFP-tagged version of Dhh1. For this experiment, we cotransformed a GFP-tagged plasmid of Dhh1 and a mCherry-tagged plasmid of another P-body marker, Edc3, into wild-type and *stm1*Δ strains. The strains were examined for P-body formation during glucose depletion, which is a stress condition that leads to rapid assembly of P-bodies


A
WT
stm1Δ
0 1 2 4 6 10 15 20 30 45 60 mins
MFA2
SCR1
PGK1
SCR1
t 1/2= 5'
t 1/2= 17'
% mRNA remaining
Time (minutes)
MFA2
PGK1
WT
stm1Δ
B
WT
0 1 2 4 6 10 15 20 30 45 60 minutes
EDC1
SCR1
stm1Δ
dhh1Δ
t 1/2= 5'
t 1/2= 15'
WT
stm1Δ
dhh1Δ
% mRNA remaining
Time (minutes)
C
WT
0 1 2 4 6 10 15 20 30 45 60 minutes
COX 17
SCR1
stm1Δ
dhh1Δ
t 1/2= 2'
t 1/2= 4'
WT
stm1Δ
dhh1Δ
% mRNA remaining
Time (minutes)

(Teixeira *et al.* 2005), and at higher cell density where P-bodies are enlarged (Teixeira *et al.* 2005).

We first examined P-body formation at higher cell densities where P-bodies enlarge over time from the small P-bodies observed in mid-log phase cultures (Teixeira *et al.* 2005). We observed that in wild-type strains, P-bodies as judged by the accumulation of Dhh1 and Edc3 were clearly visible when the cells reached an OD_{600} of 1.0 (Figure 3A). In *stm1*Δ strains, P-bodies formed at similar cell densities as judged by the accumulation of Edc3 in these foci (Figure 3A), but these P-bodies contained reduced amounts of Dhh1 as compared to wild-type cells (Figure 3A). To be sure that these differences in P-body formation were not due to changes in the levels of Dhh1, we showed by Western analysis that the protein levels of Dhh1-GFP were comparable between the wild-type and the *stm1*Δ strains (Figure 3B). This indicates that Stm1 can affect the accumulation of Dhh1 in P-bodies.

Two observations indicate that, although Stm1 affects Dhh1 accumulation in P-bodies, Stm1 is not absolutely required for Dhh1 accumulation in P-bodies. First, we observed that when cells reached higher cell densities (OD_{600} > 3.0 after 2 days of growth), Dhh1-GFP accumulated in P-bodies, even in the *stm1*Δ strain (Figure 3A). Moreover, during acute glucose deprivation, where translation decreases to <5% of normal in a few minutes and mRNA accumulates in P-bodies (Ashe *et al.* 2000; Teixeira *et al.* 2005), Dhh1-GFP accumulated in P-bodies in both wild-type and *stm1*Δ strains (Figure 3A). Taken together, these observations indicate that Stm1 promotes, but is not required for, the accumulation of Dhh1 in P-bodies and is consistent with Stm1 acting upstream of Dhh1 to promote its function.

Stm1 enhances the degradation of the *EDC1* and *COX17* mRNAs: The above results document genetic and cell biological interactions between Stm1 and Dhh1, suggesting that Stm1 enhances the function of Dhh1. Since Dhh1 functions in both translation repression and mRNA decay, it suggested that Stm1 might affect the decay of some mRNAs. To determine if Stm1 affects mRNA degradation, we examined the effect of *stm1*Δ on the decay of specific mRNA reporters. The specific reporters used were the *PGK1pG*, *MFA2pG*, *COX17*, and *EDC1pG* mRNAs under the control of the *GAL10* promoter, which allows transcriptional shutoff by the addition of glucose (Decker and Parker 1993). The *PGK1pG* and *MFA2pG* transcripts are canonical reporters that represent general mRNA decay, while *COX17* and *EDC1pG* mRNAs are transcripts that are more sensitive to the loss of *DHH1* (Cheng *et al.* 2005; Muhlrad and Parker 2005). These experiments resulted in the following observations.

First, we observed that the *PGK1pG* and *MFA2pG* mRNAs showed similar decay rates in the wild-type and *stm1*Δ strains (Figure 4A). In contrast, the *dhh1*Δ strain or *pat1*Δ strains show significant changes in the decay rates of these mRNAs by affecting their rates of decay (Tharun *et al.* 2000; Coller *et al.* 2001). These observations indicate that Stm1 is not normally required for decay at least of the *MFA2* and *PGK1* transcripts.

A second, and important, observation was that the half-life of the *EDC1* transcript in a *stm1*Δ strain was prolonged ($t_{1/2} = 15$ min) as compared to a wild-type strain ($t_{1/2} = 5$ min) (Figure 4B) and was similar to the observed decay rate seen in a *dhh1*Δ strain ($t_{1/2} = 15$ min). Overexpression of Stm1 did not alter the half-life of the *EDC1* transcript (data not shown). Similarly, the *COX17* mRNA was approximately twofold more stable in the *stm1*Δ and *dhh1*Δ strains as compared to wild-type cells (Figure 4C). The three- to fourfold stabilization of *EDC1* mRNA and twofold stabilization of *COX17* mRNA observed in a *stm1*Δ strain provide direct evidence that Stm1 has a role in mRNA decay, although its function may primarily be limited to specific mRNAs.

Stm1 overexpression does not restore mRNA decay in a *pat1*Δ strain: The above results indicated that Stm1 is a high-copy suppressor of the temperature sensitivity of *pat1*Δ and appeared to act by promoting Dhh1 function. Given this, one possibility is that Stm1 overexpression would suppress the decay defect in the *pat1*Δ strain by enhancing Dhh1 function. To examine this possibility, we compared the decay rates of *MFA2pG*, *PGK1pG*, and *EDC1pG* mRNAs between *pat1*Δ strains and *pat1*Δ strains overexpressing Stm1.

We observed that overexpression of Stm1 did not restore the defect in mRNA degradation seen in the *pat1*Δ strain for some mRNAs. Specifically, strains lacking *PAT1* show increased stability of the *MFA2pG* and *PGK1pG* transcripts, while the *EDC1* mRNA is not affected by *pat1*Δ, which is consistent with this mRNA being predominantly dependent on Dhh1 for its degradation (Figure 5). When Stm1 was overexpressed in a *pat1*Δ strain, the half-lives of *MFA2pG* ($t_{1/2} = 9$ min),

Figure 4.—Stm1 deletion stabilizes *EDC1* and *COX17* mRNAs. (A) Decay of the reporters *MFA2pG* and *PGK1pG* following transcriptional repression in wild-type (yRP2065) and *stm1*Δ (yRP1437) strains. *MFA2pG* and *PGK1pG* were detected using oligonucleotide probes oRP140 and oRP141, respectively. *SCR1* was detected using oRP100. (B) The decay of the *EDC1pG* reporter following transcriptional repression in wild-type (yRP2065), *stm1*Δ (yRP1437), and *dhh1*Δ (yRP2066) strains. Oligonucleotide probes oRP1211 and oRP100 were used to detect *EDC1* and *SCR1* transcripts, respectively. (C) The decay of the *COX17* mRNA following transcriptional repression in wild-type (yRP2065), *stm1*Δ (yRP1437), and *dhh1*Δ (yRP2066) strains. Oligonucleotide probes oRP1427 and oRP100 were used to detect *COX17* and *SCR1* transcripts, respectively. (A–C) Time points represent minutes after transcriptional repression. *SCR1* was used as a loading control. Each decay experiment was done in triplicate. A graphical representation of the data is shown by plotting the percentage of mRNA remaining as a function of time. Best-fit lines were determined by exponential curve fitting using the graphing software Deltagraph. Error bars denote the calculated standard deviation.

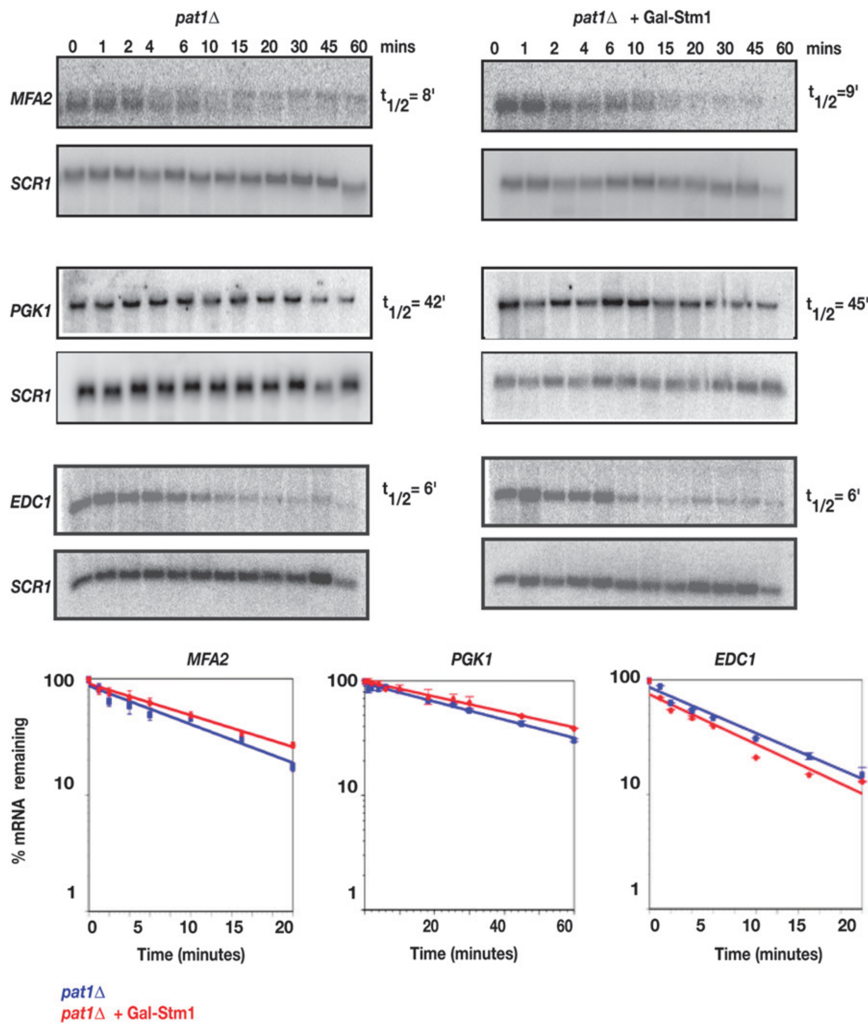


FIGURE 5.—Overexpression of Stm1 does not affect the half-life of mRNAs in a *pat1*Δ strain. The decay of *MFA2pG*, *PGK1pG*, and *EDC1pG* reporters following transcriptional repression in the *pat1*Δ (yRP1372) strain overexpressing Stm1 or empty vector under a *GAL10* promoter is shown. Time points indicated represent minutes after transcriptional repression. *SCR1* was used as a loading control. *MFA2pG*, *PGK1pG*, and *EDC1pG* transcripts were detected using oligonucleotide probes oRP140, oRP141, and oRP1211, respectively, while *SCR1* was detected using oRP100. A graphical representation of the data is shown by plotting the percentage of mRNA remaining as a function of time. Best-fit lines were determined by exponential curve fitting using the graphing software Deltagraph. Experiments were done in triplicate and error bars denote the standard deviation.

PGK1pG ($t_{1/2} = 45$ min), and *EDC1pG* ($t_{1/2} = 6$ min) were not significantly altered. This argues that the suppression of the temperature sensitivity of *pat1*Δ, brought about by Stm1, is not due to a general stimulation of mRNA decay.

Strains lacking Pat1 also show a defect in translation (WYERS *et al.* 2000), and this may account for the temperature sensitivity of *pat1*Δ. To examine whether Stm1 overexpression was suppressing the defect in translation in the *pat1*Δ strains, we measured the incorporation of ^{35}S-labeled amino acids into protein with and without Stm1 overexpression. We observed that *pat1*Δ strains showed a defect in amino acid incorporation (65% of wild type at 37°), which was partially suppressed by Stm1 overexpression (up to 90% of wild type). In contrast, overexpression of Stm1 in wild-type strains had no effect on amino acid incorporation. This suggests that at least part of the suppression of *pat1*Δ temperature sensitivity is due to Stm1 overexpression correcting a defect in translation rates. However, we cannot rule out that Stm1 overexpression may also suppress a defect in mRNA degradation for a subset of mRNAs.

Stm1 overexpression inhibits mRNA degradation in a *dhh1*Δ strain: One surprising genetic interaction was that the overexpression of Stm1 in a *dhh1*Δ affected its growth adversely (Figure 2A). This suggested that Stm1 performs a function that both enhances the activity of Dhh1 and increases the cellular need for Dhh1 function. A prediction of this model is that overexpression of Stm1 might also increase the role of Dhh1 in the degradation of mRNAs. To examine this possibility, we compared the decay rates of the *MFA2pG*, *PGK1pG*, and *EDC1pG* mRNAs between *dhh1*Δ strains and a *dhh1*Δ overexpressing Stm1.

We observed that overexpression of Stm1 strongly inhibited the degradation of the *EDC1* mRNA in the *dhh1*Δ strain where the half-life went from ~15 min to ≥50 min (Figure 6). In contrast, overexpression of Stm1 in the *dhh1*Δ strain did not affect the decay rates of the *MFA2pG* or *PGK1pG* mRNA. We interpret these observations to suggest that overexpression of Stm1 commits an mRNA to a Dhh1-specific pathway of decay. Moreover, the different effects on *MFA2pG* and *EDC1pG* suggest that Stm1 might affect only a subset of mRNAs that includes *EDC1*.

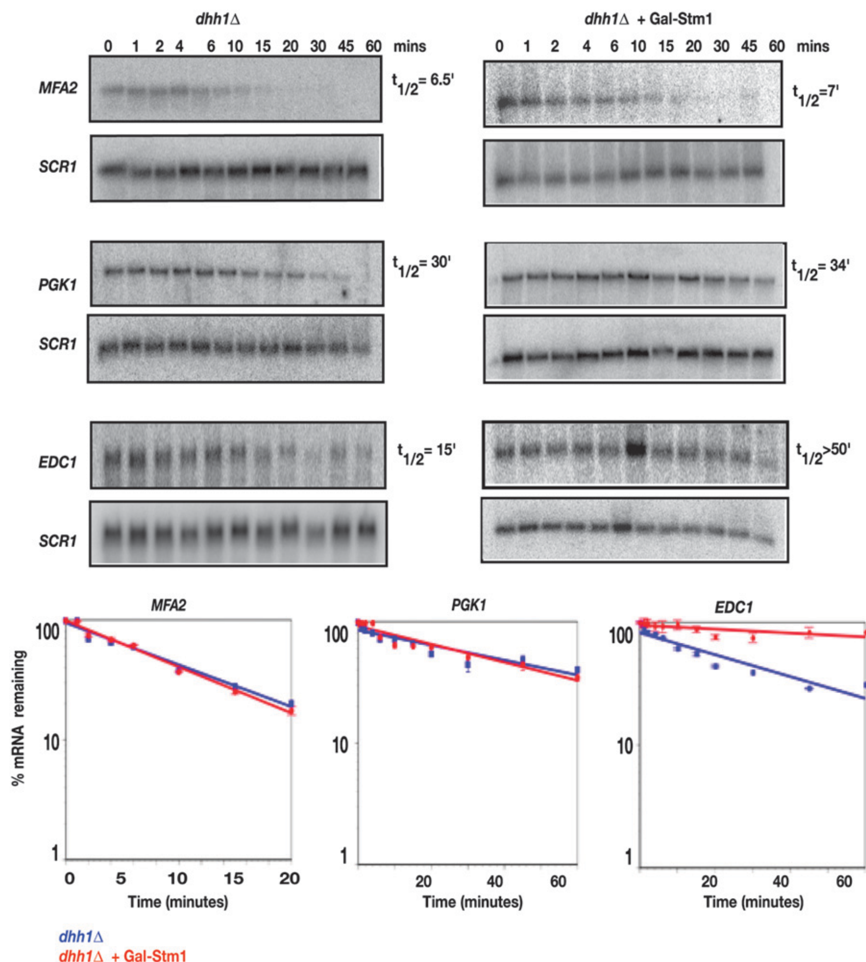


Figure 6.—Overexpression of Stm1 prolongs the half-life of *EDC1* mRNA in a *dhh1*Δ strain. The decay of *MFA2pG*, *PGK1pG*, and *EDC1pG* reporters following transcriptional repression in *dhh1*Δ (yRP2066) strain overexpressing Stm1 or empty vector under a galactose promoter is shown. Time points indicated represent minutes after transcriptional repression. *SCR1* was used as a loading control. *MFA2pG*, *PGK1pG*, and *EDC1pG* transcripts were detected using oligonucleotide probes oRP140, oRP141, and oRP1211, respectively, while *SCR1* was detected using oRP100. A graphical representation of the data is shown by plotting the percentage of mRNA remaining as a function of time. Best-fit lines were determined by exponential curve fitting using the graphing software Deltagraph. Experiments were done in triplicate and error bars denote the standard deviation.

DISCUSSION

Stm1 is a modulator of translation repression and/or mRNA decay: Several lines of evidence argue that Stm1 affects the process of targeting an mRNA for degradation. First, we show that Stm1 is a high-copy suppressor of the temperature-sensitive phenotype of the *pat1*Δ strain (Figure 1). Second, we observe that *stm1*Δ strains show defects in the decay of the *EDC1* and *COX17* mRNAs, but not in *MFA2* or *PGK1* mRNAs (Figure 4). This demonstrates that Stm1 can affect decay of at least a subset of mRNAs. In addition, previous work has shown that overexpression of Stm1 partially suppresses a *pop2*Δ strain (Hata *et al.* 1998) and suppresses the synthetic lethality of a *pop2*Δ *pab1–rrm2*Δ combination (Ohn *et al.* 2007). Taken together, we suggest that Stm1 can influence the process of targeting an mRNA for degradation. Moreover, since translation repression and targeting an mRNA for degradation are often coupled, these results imply that Stm1 may also function in the process of translation repression.

Stm1 promotes the function of Dhh1 in translation repression and mRNA decay: Several observations argue that Stm1 acts to enhance the function of Dhh1 in promoting translation repression and mRNA decay. First, the high-copy suppression by Stm1 of the temperature-sensitive phenotype of *pat1*Δ strains requires Dhh1 (Figure 1 and Figure 2B). Second, overexpression of Stm1 in a *dhh1*Δ strain affects growth adversely (Figure 2A); suggesting that Stm1 function creates a condition that requires Dhh1 for resolution. Consistent with this proposal, we observed that overexpression of Stm1 in *dhh1*Δ strains further inhibited the rates of decay of *EDC1* mRNA in these conditions (Figure 6). Third, we observed that in a *stm1*Δ strain, Dhh1-GFP is less efficiently recruited to P-bodies as yeast cells reach high cell densities (Figure 3A). Finally, *stm1*Δ strains are resistant to inhibition of cell growth due to Dhh1 overexpression (Figure 2C). Taken together, the simplest interpretation of these observations is that Stm1 function stimulates Dhh1 function.

Possible mechanisms of Stm1 function: An unresolved issue is the specific mechanism by which Stm1 promotes Dhh1 function. Stm1 has previously been shown to interact with yeast ribosomes (Van Dyke *et al.* 2004, 2006). This suggests two general models for how Stm1 could function (Figure 7). In one model, Stm1 recruits Dhh1 through physical interactions to translating ribosomes and by doing so leads to Dhh1 triggering

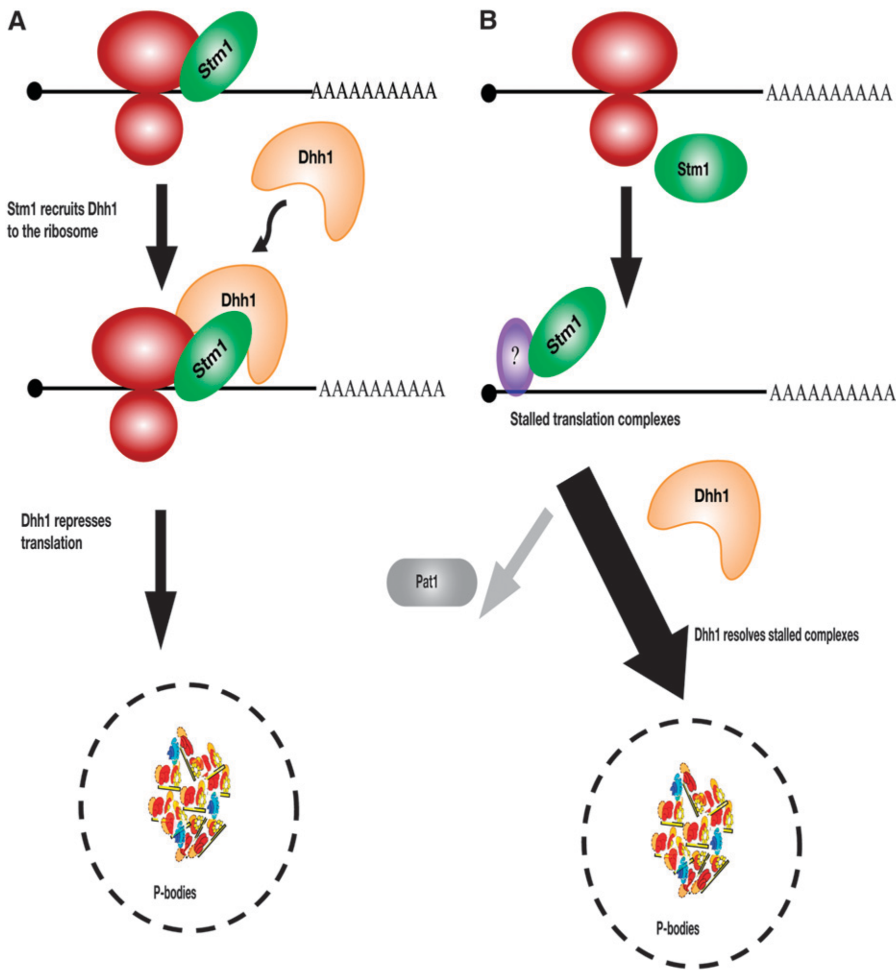


Figure 7.—Possible models for the function of Stm1. (A) Stm1 physically recruits Dhh1 to the translating ribosomes, and this allows Dhh1 to repress translation. (B) Stm1 stalls ribosomes at some stage of translation and represses translation, and this triggers the function of Dhh1.

translation repression. One limitation of this model is that it would not provide an explanation for why overexpression of Stm1 in the absence of Dhh1 is growth inhibitory. An alternate model is that Stm1 binds to the ribosome and inhibits a specific step of its function. This would create a stalled translation complex that would then be disassembled/resolved by Dhh1 promoting the mRNAs repression. The latter model is appealing since it would explain why Stm1 overexpression in the absence of Dhh1 is detrimental. Translation complexes would be predicted to be stalled in unproductive states that are poorly resolved without Dhh1 function. Consistent with this model, it is known that Dhh1 function in targeting an mRNA for decapping requires that ribosomes are able to load on the mRNA (Coller and Parker 2005). In either case, on the basis of its reported interactions with ribosomes, Stm1 could provide a necessary link between the translation machinery and Dhh1, serving as a link to repression and mRNA degradation. Unraveling this process will be important in understanding the regulation of translation and decay of mRNA.

We thank all members of the Parker laboratory, especially Wehai He, Guy Pilkington, Tracy Nissan, Denise Muhlrad, and Carolyn Decker for helpful discussions, technical assistance, and critical review of the manuscript. Thanks are also due to Jeff Coller for the gift of the yeast strain YRP2323. This work was supported by funds from the National Institutes of Health (grant R37 GM45443) and the Howard Hughes Medical Institute.

LITERATURE CITED

Ashe, M. P., S. K. De Long and A. B. Sachs, 2000 Glucose depletion rapidly inhibits translation initiation in yeast. Mol. Biol. Cell **11:** 833–848.

Beelman, C. A., and R. Parker, 1994 Differential effects of translational inhibition in cis and in trans on the decay of the unstable yeast MFA2 mRNA. J. Biol. Chem. **269:** 9687–9692.

Beelman, C. A., A. Stevens, G. Caponigro, T. E. LaGrandeur, L. Hatfield *et al.*, 1996 An essential component of the decapping enzyme required for normal rates of mRNA turnover. Nature **382:** 642–646.

Buchan, J. R., D. Muhlrad and R. Parker, 2008 P bodies promote stress granule assembly in Saccharomyces cerevisiae. J. Cell Biol. **183:** 441–455.

Caponigro, G., D. Muhlrad and R. Parker, 1993 A small segment of the MAT alpha 1 transcript promotes mRNA decay in Saccharomyces cerevisiae: a stimulatory role for rare codons. Mol. Cell. Biol. **13:** 5141–5148.

Cheng, Z., J. Coller, R. Parker and H. Song, 2005 Crystal structure and functional analysis of DEAD-box protein Dhh1p. RNA **11:** 1258–1270.

Coller, J., and R. Parker, 2004 Eukaryotic mRNA decapping. Annu. Rev. Biochem. **73:** 861–890.

Coller, J., and R. Parker, 2005 General translational repression by activators of mRNA decapping. Cell **122:** 875–886.

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.

Decker, C. J., and R. Parker, 1993 A turnover pathway for both stable and unstable mRNAs in yeast: evidence for a requirement for deadenylation. Genes Dev. **7:** 1632–1643.

Dunckley, T., and R. Parker, 1999 The DCP2 protein is required for mRNA decapping in Saccharomyces cerevisiae and contains a functional MutT motif. EMBO J. **18:** 5411–5422.

Eulalio, A., I. Behm-Ansmant and E. Izaurralde, 2007 P bodies: at the crossroads of post-transcriptional pathways. Nat. Rev. Mol. Cell Biol. **8:** 9–22.

Garneau, N. L., J. Wilusz and C. J. Wilusz, 2007 The highways and byways of mRNA decay. Nat. Rev. Mol. Cell Biol. **8:** 113–126.

Gelperin, D. M., M. A. White, M. L. Wilkinson, Y. Kon, L. A. Kung *et al.*, 2005 Biochemical and genetic analysis of the yeast proteome with a movable ORF collection. Genes Dev. **19:** 2816–2826.

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.

Hatfield, L., C. A. Beelman, A. Stevens and R. Parker, 1996 Mutations in trans-acting factors affecting mRNA decapping in Saccharomyces cerevisiae. Mol. Cell. Biol. **16:** 5830–5838.

Hsu, C. L., and A. Stevens, 1993 Yeast cells lacking $5' \rightarrow 3'$ exoribonuclease 1 contain mRNA species that are poly(A) deficient and partially lack the $5'$ cap structure. Mol. Cell. Biol. **13:** 4826–4835.

LaGrandeur, T., and R. Parker, 1999 The cis acting sequences responsible for the differential decay of the unstable MFA2 and stable PGK1 transcripts in yeast include the context of the translational start codon. RNA **5:** 420–433.

Muhlrad, D., and R. Parker, 1992 Mutations affecting stability and deadenylation of the yeast MFA2 transcript. Genes Dev. **6:** 2100–2111.

Muhlrad, D., and R. Parker, 2005 The yeast EDC1 mRNA undergoes deadenylation-independent decapping stimulated by Not2p, Not4p, and Not5p. EMBO J. **24:** 1033–1045.

Muhlrad, D., C. J. Decker and R. Parker, 1994 Deadenylation of the unstable mRNA encoded by the yeast MFA2 gene leads to decapping followed by $5' \rightarrow 3'$ digestion of the transcript. Genes Dev. **8:** 855–866.

Muhlrad, D., C. J. Decker and R. Parker, 1995 Turnover mechanisms of the stable yeast PGK1 mRNA. Mol. Cell. Biol. **15:** 2145–2156.

Ohn, T., Y. C. Chiang, D. J. Lee, G. Yao, C. Zhang *et al.*, 2007 CAF1 plays an important role in mRNA deadenylation separate from its contact to CCR4. Nucleic Acids Res. **35:** 3002–3015.

Olivas, W., and R. Parker, 2000 The Puf3 protein is a transcript-specific regulator of mRNA degradation in yeast. EMBO J. **19:** 6602–6611.

Parker, R., and U. Sheth, 2007 P bodies and the control of mRNA translation and degradation. Mol. Cell **25:** 635–646.

Parker, R., and H. Song, 2004 The enzymes and control of eukaryotic mRNA turnover. Nat. Struct. Mol. Biol. **11:** 121–127.

Schwartz, D. C., and R. Parker, 1999 Mutations in translation initiation factors lead to increased rates of deadenylation and decapping of mRNAs in Saccharomyces cerevisiae. Mol. Cell. Biol. **19:** 5247–5256.

Schwartz, D. C., and R. Parker, 2000 mRNA decapping in yeast requires dissociation of the cap binding protein, eukaryotic translation initiation factor 4E. Mol. Cell. Biol. **20:** 7933–7942.

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

Shyu, A. B., M. F. Wilkinson and A. van Hoof, 2008 Messenger RNA regulation: to translate or to degrade. EMBO J. **27:** 471–481.

Teixeira, D., U. Sheth, M. A. Valencia-Sanchez, M. Brengues and R. Parker, 2005 Processing bodies require RNA for assembly and contain nontranslating mRNAs. RNA **11:** 371–382.

Tharun, S., W. He, A. E. Mayes, P. Lennertz, J. D. Beggs *et al.*, 2000 Yeast Sm-like proteins function in mRNA decapping and decay. Nature **404:** 515–518.

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.

Utsugi, T., A. Toh-e and Y. Kikuchi, 1995 A high dose of the STM1 gene suppresses the temperature sensitivity of the tom1 and htr1 mutants in Saccharomyces cerevisiae. Biochim. Biophys. Acta **1263:** 285–288.

Van Dyke, M. W., L. D. Nelson, R. G. Weilbaecher and D. V. Mehta, 2004 Stm1p, a G4 quadruplex and purine motif triplex nucleic acid-binding protein, interacts with ribosomes and subtelomeric Y′ DNA in Saccharomyces cerevisiae. J. Biol. Chem. **279:** 24323–24333.

Van Dyke, N., J. Baby and M. W. Van Dyke, 2006 Stm1p, a ribosome-associated protein, is important for protein synthesis in Saccharomyces cerevisiae under nutritional stress conditions. J. Mol. Biol. **358:** 1023–1031.

Winzeler, E. A., D. D. Shoemaker, A. Astromoff, H. Liang, K. Anderson *et al.*, 1999 Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science **285:** 901–906.

Wyers, F., M. Minet, M. E. Dufour, L. T. A. Vo and F. Lacroute, 2000 Deletion of the *PAT1* gene affects translation initiation and suppresses a *PAB1* gene deletion in yeast. Mol. Cell. Biol. **20:** 3538–3549.

Communicating editor: F. Winston