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Mutations in *trans*-Acting Factors Affecting mRNA Decapping in *Saccharomyces cerevisiae*

LIANNA HATFIELD,[1]† CLARE A. BEELMAN,[1] AUDREY STEVENS,[2] AND ROY PARKER[1,3]*

Department of Molecular and Cellular Biology[1] and Howard Hughes Medical Institute,[3] University of Arizona, Tucson, Arizona 85721, and Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831[2]

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The decay of several yeast mRNAs occurs by a mechanism in which deadenylation precedes decapping and subsequent 5′-to-3′ exonucleolytic decay. In order to identify gene products required for this process of mRNA turnover, we screened a library of temperature-sensitive strains for mutants with altered mRNA degradation. We identified seven mutations in four genes that inhibited mRNA turnover. Two mutations were alleles of the *XRN1* 5′-to-3′ exoribonuclease known to degrade mRNAs following decapping. One mutation defined a new gene, termed *DCP1*, which in subsequent work was demonstrated to encode a decapping enzyme or a necessary component of a decapping complex. The other mutations defined two additional genes, termed *MRT1* and *MRT3* (for mRNA turnover). Mutations in the *MRT1* and *MRT3* genes slow the rate of deadenylation-dependent decapping, show transcript-specific effects on mRNA decay rates, and do not affect the rapid turnover of an mRNA containing an early nonsense codon, which is degraded by a deadenylation-independent decapping mechanism. Importantly, cell extracts from *mrt1* and *mrt3* strains contain normal levels of the decapping activity required for mRNA decay. These observations suggest that the products of the *MRT1* and *MRT3* genes function to modulate the rates of decapping that occur following deadenylation.

The process of mRNA turnover is a critical step in determining the regulation and levels of gene expression (for reviews, see references 3, 6, and 30). In eukaryotic cells, a major pathway of mRNA turnover is initiated by shortening of the poly(A) tail, which is followed by degradation of the transcript body (for reviews, see references 1 and 9). In *Saccharomyces cerevisiae*, mRNA deadenylation leads to removal of the 5′ cap structure of mRNAs (decapping) and then rapid 5′-to-3′ exonucleolytic digestion of the mRNA body (8, 16, 21, 22). Although there is no direct evidence for decapping being a step in mRNA decay in more complex eukaryotes, mRNAs lacking the cap structure are rapidly degraded in many eukaryotic cells (e.g., see reference 10) and enzymatic activities that could catalyze the removal of the cap structure and subsequent 5′-to-3′ degradation of the transcript have been identified in mammalian cells (e.g., see reference 7). Thus, deadenylation-dependent decapping followed by 5′-to-3′ exonucleolytic decay may be a conserved mechanism of eukaryotic mRNA degradation.

Since decapping is the final step that leads to degradation of the transcript body, decapping rates significantly affect mRNA decay rates. Thus, decapping rates are controlled by several inputs. For example, the unstable *MFA2* mRNA is rapidly decapped following deadenylation, while the stable *PGK1* transcript is decapped slowly after deadenylation (8, 21, 22). Differences in decapping rates are at least partially determined by specific sequences within these mRNAs (21–23). Similarly, the major effect of the poly(A) tail on mRNA stability appears to be an inhibition of decapping (5). Finally, early nonsense codons promote extremely rapid mRNA decay by triggering decapping independently of deadenylation (24). Given the importance of decapping, a knowledge of the mechanisms and control of mRNA decapping will be essential to understanding the process of mRNA decay.

To identify gene products required for mRNA decapping, a library of temperature-sensitive strains was screened for mutants with altered mRNA degradation. We identified seven mutations in four genes that inhibited mRNA turnover. Two mutations were alleles of the *XRN1* 5′-to-3′ exoribonuclease known to degrade mRNAs following decapping (16, 21, 22). One mutation defined a new gene, termed *DCP1*, which in subsequent work was demonstrated to encode a component of the decapping enzyme (2). The other mutations defined two additional genes, termed *MRT1* and *MRT3* (for mRNA turnover), whose gene products are required for mRNA decapping. Interestingly, the rapid turnover of an mRNA containing an early nonsense codon, which is degraded by a deadenylation-independent decapping mechanism (24), was not inhibited in the *mrt1* and *mrt3* strains. These observations suggest that the *MRT1* and *MRT3* gene products modulate the rates of decapping that occur following deadenylation.

MATERIALS AND METHODS

Plasmid construction. The plasmid pRP484 was used for integrating the MFA2pG and PGK1pG genes under the transcriptional control of the *GAL1* upstream activation sequence (UAS), along with the *LEU2* gene as a marker (collectively termed *LEU2* [PM]), into the *CUP1* locus. By standard cloning techniques, pRP484 was derived from pRP480, which contains the following sequences located in the polylinker region of the *LEU2* integration vector pRS405 (33). An ~450-bp *Sau*3A-*Eco*RI fragment containing sequences from the 5′ region of the *CUP1* locus was isolated from pUC8-cup1^s (12) after filling the *Eco*RI site with Klenow and was located between the *Bam*HI and *Pst*I-Klenow-filled polylinker site. An ~1,200-bp *Hin*dIII-*Xho*I fragment containing sequences from the 3′ region of the *CUP1* locus was isolated from pBS-Cup-Sma (20) after the *Sma*I site had been changed to an *Xho*I site by linker insertion and was located between the *Hin*dIII and *Xho*I sites of the polylinker. An *Sph*I-*Xho*I fragment containing the *GAL1* UAS and PGK1pG transcription unit was isolated from pRP469 (8) after the *Hin*dIII site was changed to *Xho*I by linker insertion and the *Sph*I site was filled with Klenow. This fragment was oriented within the *Xho*I site with the blunt-ended *Sph*I end at the insert-vector junction. The *Xho*I site at the insert-vector junction was lost in pRP480. To create the insertion plasmid pRP484, *Xho*I linkers were added to the *Pvu*II-*Hin*dIII fragment containing the *GAL1* UAS and the MFA2pG transcription unit from pRP485 (8) prior to insertion into the unique *Xho*I site at the PGK1pG frag-

* Corresponding author. Phone: (520) 621-9347. Fax: (520) 621-4524.
† Present address: Uintah Basin Campus, Utah State University, Vernal, UT 84078.

TABLE 1. Strains used in this study

Strain	Mutant	Genotype
yRP684	Wild type	*MAT*α *trp1 ura3-52 leu2-3,112 his4-539 lys2-201*
yRP685	Wild type	*MAT***a** *trp1 ura3-52 leu2-3,112 his4-539 lys2-201*
yRP693	*rpb1-1*	*MAT*α *ura3-52 leu2-3,112 rpb1-1*
yRP838	Wild type	*MAT***a** *trp1 ura3-52 leu2-3,112 his4-539 cup1*::*LEU2* (PM) *lys2-201*
yRP839	Wild type	*MAT*α *trp1 ura3-52 leu2-3,112 his4-539 cup1*::*LEU2* (PM) *lys2-201*
yRP840	Wild type	*MAT***a** *trp1 ura3-52 leu2-3,112 his4-539 cup1*::*LEU2* (PM)
yRP841	Wild type	*MAT*α *trp1 ura3-52 leu2-3,112 lys2-201 cup1*::*LEU2* (PM)
yRP850	*pab1*Δ	*MAT*α *trp1 ura3-52 leu2-3,112 pab1*::*URA3 cup1*::*LEU2* (PM) with pAS137
yRP881	*spb2*Δ *pab1*Δ	*MAT***a** *trp1 ura3-52 leu2-3,112 his4-539 spb2*::*URA3 pab1*::*URA3*
yRP884	*xrn1*Δ	*MAT***a** *trp1 ura3-52 leu2-3,112 lys2-201 cup1*::*LEU2* (PM) *xrn1*::*URA3*
yRP890	*dcp1-1 MDC1-1*	*MAT*α *trp1 ura3-52 leu2-3,112 lys2-201 cup1*::*LEU2* (PM) *dcp1-1 MDC1-1*
yRP891	*dcp1-1 MDC1-1*	*MAT***a** *trp1 ura3-52 leu2-3,112 his4-539 cup1*::*LEU2* (PM) *dcp1-1 MDC1-1*
yRP892	*dcp1-1*	*MAT***a** *trp1 ura3-52 leu2-3,112 lys2-201 cup1*::*LEU2* (PM) *dcp1-1*
yRP903	*xrn1*Δ *pab1*Δ	*MAT*α *lys2 trp1 ura3 leu2 cup1*::*LEU2* (PM) *xrn1*::*URA3 pab1*::*URA3*
yRP1063	*dcp1-1*	*MAT*α *trp1 ura3-52 leu2-3,112 his4-539 cup1*::*LEU2* (PM) *dcp1-1*
yRP1064	*xrn1-230*	*MAT*α *trp1 ura3-52 leu2-3,112 lys2-201 cup1*::*LEU2* (PM) *xrn1-230*
yRP1065	*xrn1-428*	*MAT*α *trp1 ura3-52 leu2-3,112 lys2-201 cup1*::*LEU2* (PM) *xrn1-428*
yRP1066	*mrt1-3*	*MAT***a** *trp1 ura3-52 leu2-3,112 his4-539 cup1*::*LEU2* (PM) *mrt1-3*
yRP1067	*mrt3-1*	*MAT***a** *trp1 ura3-52 leu2-3,112 his4-539 cup1*::*LEU2* (PM) *mrt3-1*
yRP1069	*dcp1*Δ	*MAT*α *trp1 ura3-52 leu2-3,112 lys2-201 cup1*::*LEU2* (PM) *dcp1*::*URA3*
yRP1102	*rpb1-1*	*MAT***a** *ura3-52 his4-539 rpb1-1*
yRP1103	*mrt1-3 rpb1-1*	*MAT***a** *ura3-52 mrt1-3 rpb1-1*
yRP1104	*dcp1-1 rpb1-1*	*MAT*α *ura3-52 dcp1-1 rpb1-1*
yRP1105	*mrt3-1 rpb1-1*	*MAT***a** *ura3-52 his4-539 mrt3-1 rpb1-1*
yRP1130	*mrt1-3 pab1*Δ	*MAT*α *trp1 ura3-52 leu2-3,112 his4-539 pab1*::*URA3 cup1*::*LEU2* (PM) *mrt1-3*
yRP1131	*mrt1-2 pab1*Δ	*MAT***a** *trp1 ura3-52 leu2-3,112 his4-539 pab1*::*URA3 cup1*::*LEU2* (PM) *mrt1-2*
yRP1132	*dcp1-1 pab1*Δ	*MAT*α *trp1 ura3-52 leu2-3,112 his4-539 pab1*::*URA3 cup1*::*LEU2* (PM) *dcp1-1 MDC1-1*
yRP1133	*mrt3-1 pab1*Δ	*MAT***a** *trp1 ura3-52 leu2-3,112 his4-539 pab1*::*URA3 cup1*::*LEU2* (PM) *mrt3-1*
yRP1134	*mrt1-2*	*MAT***a** *trp1 ura3-52 leu2-3,112 his4-539 cup1*::*LEU2* (PM) *mrt1-2*
yRP1135	*mrt1-1*	*MAT***a** *trp1 ura3-52 leu2-3,112 his4-539 cup1*::*LEU2* (PM) *mrt1-1*

ment-3′ *CUP1* junction of pRP480. The promoter-proximal end of this DNA insertion is adjacent to the 5′ *CUP1* sequence as determined by DNA sequencing (data not shown).

Yeast strains and screening of the temperature-sensitive mutant yeast collection. The *mrt* and *dcp1-1* and other strains used in this study are listed in Table 1. The *mrt* and *dcp1-1* strains were obtained from at least two backcrosses of the original mutants obtained from the screen.

The starting yeast strains for this project were constructed in the following manner. First, by utilizing the *HO* gene (15), two isogenic *MAT***a** and *MAT*α strains, yRP685 and yRP684, were constructed. Each of these strains was then transformed with *Hin*dIII-linearized pRP484, thereby integrating at the *CUP1* locus the PGK1pG and MFA2pG genes under the control of the *GAL1* UAS (termed PM). All integrations were confirmed by Southern analysis (data not shown). To facilitate selection of diploids during subsequent analysis, yRP839 (yRP685 with PM) was transformed with a 4.5-kb fragment of the *LYS2* gene, thereby creating yRP840. Similarly, yRP838 was transformed with a 3.3-kb fragment of the *HIS4* gene, thereby creating yRP841.

A temperature-sensitive library was constructed by standard ethyl methanesulfonate mutagenesis, yielding 640 and 262 temperature-sensitive strains from yRP840 and yRP841, respectively. This library was screened by Northern analysis with RNA prepared (4) after growing cells at 36°C for 1 h. In this screen, oligonucleotide probes specific for either the PGK1pG (oRP141) (5) or the MFA2pG (oRP140) (5) mRNA and their corresponding decay fragments were used to examine the effects of mutations on both transcripts. These probes are complementary to a portion of the poly(G) insertion (8) and to *MFA2* or *PGK1* sequences just 3′ of the insertion.

Genetic analysis of *mrt* and *dcp1-1* mutants and linkage of mRNA phenotype with temperature-sensitive growth. The original temperature-sensitive mutants were first backcrossed at least twice to the parental strains yRP840 and yRP841. In each case, spores from multiple tetrads were examined both for temperature-sensitive growth and, by Northern analysis, for alterations in mRNA decay. In each case, analysis of more than 20 tetrads indicated that the mutant mRNA phenotype segregated 2:2, indicating that it was due to a single lesion. For *mrt1* alleles, the mRNA phenotype was always linked to the temperature sensitivity (25 tetrads), indicating that the *mrt1* alleles cause temperature-sensitive growth.

Novelty of *MRT* genes with respect to other genes whose products are involved in mRNA stability in *S. cerevisiae*. The *DCP1* gene has been shown to encode a component of the decapping enzyme (2). To determine if the other isolated mutants represented alleles of either the *XRN1*, *CCA1*, or *DCP1* gene, we transformed each mutant with a centromere plasmid carrying these genes, expressed under their native promoters (pRP635, pRP636, and pRP717, respectively) and examined the growth and mRNA phenotypes. Two of the isolates were complemented by the *XRN1* plasmid, indicating that these were new alleles of *XRN1*. This was also confirmed by linkage analysis by a cross to an *xrn1*Δ strain (yRP884). None of the mutants were complemented by the *CCA1* gene. Neither *mrt1-3* nor *mrt3-1* was complemented by the *DCP1* gene, suggesting that the *mrt1-3* and *mrt3-1* lesions were not in the *DCP1* gene. This conclusion was confirmed by linkage analysis by crossing yRP1066 (*mrt1-3*) and yRP1067 (*mrt3-1*) to a *dcp1*Δ strain, yRP1069.

Complementation and linkage analysis of *mrt* mutants. By standard techniques, *mrt1-1*, *mrt1-2*, *mrt1-3*, *dcp1-1*, and *mrt3-1* were examined by both complementation analysis (examining both growth and mRNA phenotypes) and pairwise linkage analysis (examining mRNA phenotypes). This defined three unlinked complementation groups, which we defined as *MRT1*, *DCP1*, and *MRT3*.

Suppression of *pab1*Δ by *mrt* and *dcp1* mutations. To examine if the *mrt1-2*, *mrt1-3*, *dcp1-1*, and *mrt3-1* lesions would suppress *pab1*Δ, these mutants were crossed to yRP850 (*pab1*::*URA3*) bearing pAS137 (see reference 5). Diploids auxotrophic for tryptophan (diploids which lost the pAS137 *PAB1* plasmid) were sporulated, and their spores were dissected by standard procedures. The predominance of tetrads exhibiting a 3:1 growth ratio (three spores viable to one dead spore) and a 1:3 URA^+ ratio (only one viable URA^+ spore) was interpreted to suggest that the *mrt* and *dcp1-1* mutations and *pab1*Δ were unlinked and that the *mrt* and *dcp1-1* mutations suppress *pab1*Δ lethality. *dcp1-1* suppressed *pab1*Δ only if spores were grown at 24°C; therefore, spores from all crosses were grown at 24°C. The steady-state MFA2pG mRNA distributions from the *mrt1-3* (yRP1066)-and-*pab1*::*URA3* cross supported our interpretation of the tetrad analysis showing one wild-type spore, one *mrt1-3* spore, and a unique mRNA phenotype in the *mrt1-3 pab1*::*URA3* spore (an apparent combination of both the *mrt1-3* and *pab1*Δ [5] effects [data not shown] on MFA2pG mRNA).

RNA analysis. Analysis of mRNA was done by the following previously published procedures: RNA preparation (4), immunoprecipitations with antisera directed against the cap structure (21, 25), RNase H reactions (23), primer extension analysis (21), and measurements of mRNA half-lives by transcriptional repression of the *GAL1* UAS and by temperature shifts of *rpb1-1* (26) strains (27). Transcriptional pulse-chase experiments were done essentially as previously described (8), except that cells were grown in minimal medium with 2% raffinose and 2% sucrose as neutral carbon sources prior to a transcriptional induction period of 8 min, without a temperature shift at the end of the induction period.

Cell-free decapping activity assays. Decapping activity was assayed after partial purification as described by Beelman et al. (2). Wild-type (yRP840), *mrt1-3*

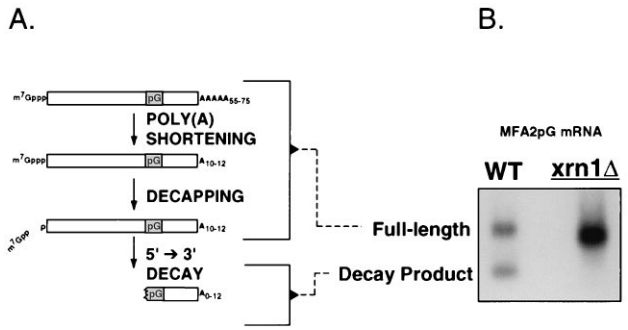

FIG. 1. Strategy to isolate mutations affecting mRNA decay. (A) A common pathway of mRNA degradation in *S. cerevisiae*. In the schematic, the shaded box labeled pG represents the site of insertion of the poly(G) tract, which serves as a barrier to exonucleases. (B) A Northern blot illustrating the different steady-state distributions of full-length MFA2pG mRNA and its corresponding decay product seen in wild-type (WT), yRP840, and *xrn1*Δ, yRP884 strains. The probe for this experiment was an oligonucleotide, oRP140 (5), which hybridizes to the junction of the poly(G) insertion and the MFA2 3′ UTR sequences and is therefore specific for the MFA2pG transcript and its decay product.

(yRP1066), and *mrt3-1* (yRP1067) cells were grown in yeast extract-peptone-dextrose (YEPD) medium, and the decapping enzymatic activity was eluted from the heparin-agarose column with buffer A containing 700 mM $(NH_4)_2SO_4$.

RESULTS

Strategy. The approach that we utilized to identify gene products involved in mRNA decay was to screen a collection of approximately 900 temperature-sensitive mutants for lesions that alter the normal pathway of mRNA decay (Fig. 1A). The logic behind this screen was that mutations that inhibit mRNA degradation were expected to increase the levels of full-length mRNA and reduce or abolish the levels of decay products. The major observed decay product corresponds to a fragment produced by 5′-to-3′ exonucleolytic digestion to an insertion of a poly(G) tract (pG) into the 3′ UTR (8) (depicted in Fig. 1A). This pG insertion forms a strong secondary structure that inhibits exonucleases, thereby stabilizing this decay fragment (e.g., see reference 8). An example of the assay used is shown in Fig. 1B, in which a strain where the *XRN1* gene was deleted, which encodes the 5′-to-3′ exoribonuclease responsible for mRNA degradation (16, 19, 21, 22), accumulated full-length *MFA2* transcripts and showed a decrease in the levels of decay product.

The isolation of *mrt* mutants. As the first step in this screen, we constructed a pair of strains in which the *PGK1* and *MFA2* genes containing the 3′ UTR pG insertion, termed PGK1pG and MFA2pG, were integrated into the genome. In addition, to allow subsequent analysis, the PGK1pG and MFA2pG genes were under the control of the *GAL1* UAS (see below). Following mutagenesis and purification, approximately 900 temperature-sensitive strains were screened by Northern analysis for accumulation of full-length PGK1pG and MFA2pG transcripts and a corresponding decrease in the levels of their decay products.

Seven different strains were identified that showed an increase in the levels of full-length mRNA and a decrease in the levels of decay product (similar to the *xrn1*Δ phenotype shown in Fig. 1B). Genetic analysis of these isolates demonstrated the following points (see Materials and Methods for details). (i) In each case, the alteration in the distribution between full-length mRNA and decay product was due to a single genetic locus. (ii) One of the mutations, originally termed *mrt2-1*, was in the *DCP1* gene, which appears to encode an essential component of the decapping enzyme (2). This point mutation in the *DCP1* gene was subsequently named *dcp1-1*. (iii) None of the mutations were in the *CCA1* gene, which is thought to affect mRNA decay indirectly by inhibiting translation elongation (28). (iv) Two of the mutants carried new alleles of *XRN1*, which we have termed *xrn1-230* and *xrn1-428*. (v) The remaining four mutations defined two different genes. On the basis of these observations, we concluded that we had identified mutations in two genes that affect mRNA turnover (see results below). We termed these genes *MRT1* (three alleles [*mrt1-1*, *mrt1-2*, and *mrt1-3*]) and *MRT3* (one allele [*mrt3-1*]).

The *mrt1* and *dcp1-1* mutations can affect growth at high temperature. Because of the library screened, each original isolate in this screen was temperature sensitive for growth at 36°C. To determine if this conditional growth was due to the *mrt* or *dcp1-1* alleles, we examined whether the alteration in mRNA distributions and temperature sensitivity cosegregated in backcrosses of the mutants to wild-type strains. For the *mrt3-1* mutation and the alleles of *XRN1*, the temperature sensitivity and the alteration in mRNA distribution segregated away from each other. These mutants did not prevent growth at any temperature tested. In contrast, in *mrt1-1*, *mrt1-2*, and *mrt1-3* strains, the mutation responsible for the mRNA phenotype cosegregated with conditional lethality in 25 tetrads, indicating that these lesions in the *MRT1* gene cause temperature-sensitive growth. It should be noted, however, that the alteration in mRNA distribution was seen at both the permissive and nonpermissive temperatures for all three *mrt1* alleles. It is, therefore, possible that effects on mRNA decay are lethal only when the cells are grown at 36°C or that the *mrt1* lesions cause lethality at 36°C because of loss of a second, essential function.

The linkage between the effects on mRNA distribution and growth at 36°C for the *dcp1-1* mutation was complex. Although in every tetrad two spores showed alteration in mRNA distributions (as judged by Northern analysis), only one-half of the spores with a mutant mRNA phenotype and none of the wild-type spores from the first backcross were temperature sensitive. These results suggested that a second, unlinked mutation, which we termed *MDC1-1* (modifier of dcp1), caused *dcp1-1* strains to be temperature sensitive.

To test this model, two crosses were performed (for a similar analysis discussed in more detail, see reference 18). First, a four-spore tetrad was identified in the *dcp1-1* backcross in which no spores were temperature sensitive, suggesting that the modifier had segregated away from the *dcp1-1* allele and was present in the spores that do not show an alteration in mRNA distribution. Such a spore was picked and then crossed to a temperature-sensitive *dcp1-1* strain, which would be expected to give a diploid homozygous for the modifier mutation. As predicted, dissection of this diploid gave 2:2 segregation for temperature sensitivity with each temperature-sensitive spore showing alterations in mRNA distributions. Additional evidence for such a modifier mutation came from taking a spore from a tetrad similar to that described above, which was predicted to have the *dcp1-1* allele but not the modifier lesion, and by backcrossing it to the wild type. As predicted, dissection of this diploid, which would be lacking the modifier, gave 2:2 segregation for alterations in the mRNA phenotype; however, no spores were temperature sensitive. The modifier mutation, termed *MDC1-1*, was later seen to be dominant, since a diploid homozygous for *dcp1-1* and heterozygous for the modifier mutation was temperature sensitive.

The *MDC1-1* mutation had no phenotype by itself with respect to cell growth or mRNA decay (data not shown) but was

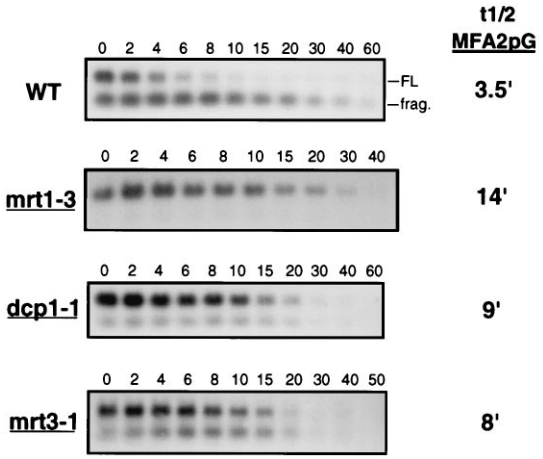

FIG. 2. MFA2pG mRNA is stabilized in *mrt* and *dcp1-1* mutants. The figure shows measurement of the decay rate of the MFA2pG mRNA in wild-type, *mrt*, and *dcp1-1* strains on a Northern blot following the inhibition of transcription by the addition of glucose to the growth media. The time points represent minutes after the addition of glucose to the media. The strains utilized were wild type (WT) (yRP840), *mrt1-3* (yRP1066), *dcp1-1* (yRP890), and *mrt3-1* (yRP1067). Other alleles of *MRT1*, *mrt1-1*, and *mrt1-2* exhibit a similar stabilization of MFA2pG mRNA as seen in the *mrt1-3* mutant (data not shown). The *xrn1-230* and *xrn1-428* mutants isolated in this screen both yield MFA2pG half-lives of approximately 9 min (data not shown). To specifically detect the MFA2pG transcript and its decay product, the oligonucleotide oRP140 (5) was used as the probe. FL, full length; frag., fragment.

synthetically temperature sensitive with the *dcp1-1* mutation. This indicated that the *dcp1-1* mutation itself does not limit growth at high temperature but can become required when combined with a second lesion. Although the basis for the temperature sensitivity of the *dcp1-1 MDC1-1* mutant is unknown, it can be suppressed by expression of the *DCP1* gene (2). This observation implies that the temperature-sensitive phenotype is due, at least in part, to a defect in mRNA decapping (see below).

mRNAs are stabilized in *mrt* and *dcp1-1* mutant strains. To determine if the changes in distribution between full-length mRNA and decay products were due to a change in the mRNA decay rate, we measured the half-lives of the MFA2pG and PGK1pG mRNAs in wild-type, *dcp1-1*, and *mrt* mutant strains. In these experiments, cells were grown in media containing galactose and transcription of the MFA2pG and PGK1pG mRNAs was repressed by the addition of glucose (27). Comparison of the decay rates of the indicated mutants showed rates of decay two- to fourfold lower than those seen in wild-type cells for the MFA2pG (Fig. 2) and PGK1pG transcripts (Table 2). It should be noted that the decay rate of the PGK1pG transcript in this wild-type strain is slightly higher than that previously reported (e.g., see reference 27) because of the different strain background. The observation that the *mrt1-3*, *dcp1-1*, and *mrt3-1* mutations affected mRNA decay rates demonstrated that the *MRT1*, *MRT3*, and *DCP1* gene products are required for normal rates of mRNA turnover.

The *mrt1* and *mrt3* mutations affect mRNA decapping. In principle, the *mrt1*, *mrt3*, and *dcp1-1* mutations could affect mRNA decay by slowing deadenylation, decapping, and/or 5′-to-3′ digestion. We have recently cloned the *DCP1* gene and shown that this gene encodes a component of the decapping enzyme (2). Accordingly, loss-of-function mutations in the *DCP1* gene lead to a block of decapping in vivo (2). To determine the step(s) in the decay pathway affected by lesions in the *MRT1* and *MRT3* genes, we analyzed the decay of the

TABLE 2. mRNA half-lives in wild-type and *mrt* strains

mRNA	Half-life (min)[a]		
	Wild type	*mrt1-3*	*mrt3-1*
PGK1pG	22.5	47.8	41.8
PAB1	20.8	20	14.5
Pre-CYH2	<2	<2	<2
CYH2	26	48.5	38
HIS3	7	17	16.5
GAL10	5	17	6.2
MFA2pG	3.5	16	7

[a] Half-lives are based on multiple determinations and typically vary by less than 20% between individual experiments.

MFA2pG transcript in these mutant strains by transcriptional pulse-chase analysis (8). In this type of experiment, carbon source regulation of the *GAL1* UAS is utilized to rapidly induce and then repress transcription, thereby producing a pool of newly made transcripts the turnover of which can be monitored over time to observe rates of deadenylation and subsequent decay of the MFA2pG transcript.

Decay of the MFA2pG transcript in wild-type, *mrt1-3*, and *mrt3-1* strains is shown in Fig. 3 and reveals two important observations. First, the deadenylation rate for the MFA2pG mRNA was not significantly different in the *mrt1-3* and *mrt3-1* mutants from that in wild-type cells (cf. bottom of bands in 0- through 8-min lanes in Fig. 3). Second, both mutant strains showed an accumulation of full-length deadenylated transcripts relative to the wild-type strain (cf. 30-min lanes). We interpret these observations to indicate that the primary effect of the *mrt* lesions on mRNA decay is to affect steps that occur after deadenylation, such as decapping and/or 5′-to-3′ exonuclease digestion.

To distinguish whether the *mrt1* and *mrt3* mutations blocked mRNA decapping or 5′-to-3′ exonuclease digestion, we uti-

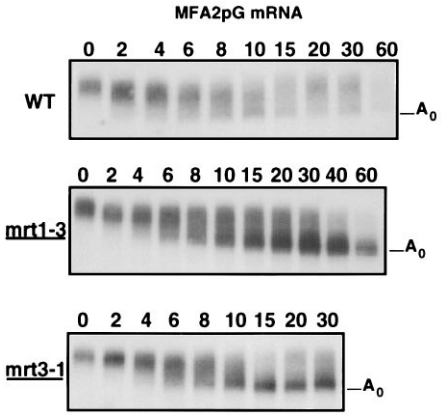

FIG. 3. *mrt* mutations inhibit mRNA decay after deadenylation; transcriptional pulse-chase analysis of the decay of the MFA2pG transcript in wild-type (yRP840 [WT]) and *mrt* strains. MFA2pG mRNA was analyzed on a 6% acrylamide–8 M urea Northern gel. Numbers above the lanes are minutes after transcriptional repression by the addition of glucose following an 8-min induction of transcription (see reference 8). The mutant strains utilized were *mrt1-3* (yRP1066) and *mrt3-1* (yRP1067), and the blot was probed with oRP140 (5). The positions of fully deadenylated MFA2pG transcripts are shown on the right, based on lanes in which the RNA was treated with RNase H and oligo(dT) prior to electrophoresis (not shown). In wild-type cells, the decay product arising from 5′-to-3′ exonucleolytic decay accumulates beginning at the 8-min time point (not shown). In the *mrt* strains, the appearance of this decay fragment is severely reduced or abolished in the transcriptional pulse-chase experiment (data not shown; see Fig. 2 for steady-state comparison).

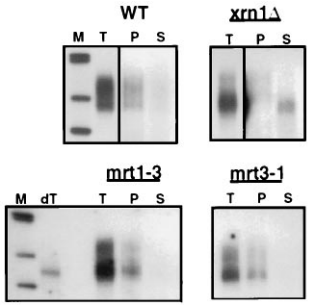

FIG. 4. *mrt* mutants accumulate deadenylated, capped transcripts; immunoprecipitation of MFA2pG mRNA with antisera directed against the cap structure. Following immunoprecipitation of total RNA under steady-state conditions (prepared from cells growing in media containing galactose), the RNA was analyzed on polyacrylamide Northern gels, and probe oRP140 (5) was used to visualize MFA2pG mRNA. Lanes: T, total RNA before immunoprecipitation; P, pellet (immunoprecipitable fraction); S, supernatant. Since some RNA is lost during the immunoprecipitation protocol (8), the T lanes of the wild type (yRP840), the *mrt* mutants *mrt1-3* (yRP1066) and *mrt3-1* (yRP1067), and the *xrn1*Δ control (yRP884) show a lighter exposure than those of the immunoprecipitated fractions, thereby allowing a comparison of the poly(A) distribution in each case. The small amount of the RNA that is not immunoprecipitable even in the wild-type cells represents RNA that is capped but is not precipitated because of the limited antisera utilized in these experiments. The use of increased amounts of antisera precipitates these species (data not shown). M lanes, DNA fragment size markers; dT lane, WT mRNA treated with oligo(dT) and RNase H to show the size of full-length MFA2pG mRNA lacking poly(A) tails.

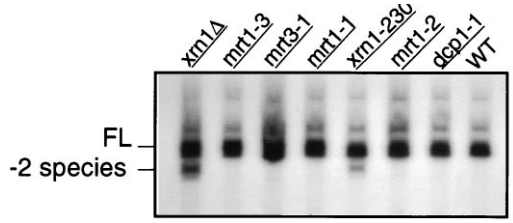

FIG. 5. Primer extension analysis of the *PGK1* transcript in *mrt* mutants; primer extension analysis of the *PGK1* transcripts in various mutants affecting mRNA decay. FL, the cDNA product corresponding to full-length *PGK1* transcripts; −2 species, the cDNA product previously shown to arise from a decapped *PGK1* RNA (22). The labels above each lane indicate the strains from which RNA was prepared (*xrn1*Δ [yRP884], *mrt1-3* [yRP1066], *mrt3-1* [yRP1067], *mrt1-1* [yRP1115], *xrn1-230* [yRP1064], and *mrt1-2* [yRP1114]). In this experiment, the oligonucleotide used to prime cDNA synthesis (oRP131 [24]) produces cDNA products from both the endogenous *PGK1* gene and the integrated PGK1pG construct.

lized antisera directed against the 5′ cap structure to separate steady-state mRNA from wild-type cells and *mrt* mutants into capped and decapped populations (21, 22). Mutations that block 5′-to-3′ exonucleolytic digestion, such as *xrn1*Δ, are known to accumulate deadenylated, decapped transcripts (16, 21, 22). In contrast, lesions that inhibit decapping were expected to accumulate deadenylated, capped transcripts. In keeping with the transcriptional pulse-chase analysis, examination of the MFA2pG mRNA present in the *mrt* mutants at steady-state indicated that these mutants accumulated full-length, deadenylated transcripts (Fig. 4 [T lanes]). As seen in the lanes containing immunoprecipitated RNA in Fig. 4 (P lanes), both of the *mrt* mutants accumulated a deadenylated species that was immunoprecipitable, indicating that these full-length deadenylated transcripts are capped. In contrast, *xrn1*Δ strains accumulated a deadenylated species that lacks the 5′ cap, as judged by their presence in the soluble fraction. These observations indicated that the *mrt1* and *mrt3* mutations inhibit decapping of the MFA2pG transcript.

To obtain additional evidence that the *mrt1* and *mrt3* mutations inhibit decapping, we took advantage of the observation that the decapped PGK1pG transcripts that accumulate in *xrn1*Δ strains are shorter at their 5′ ends by two nucleotides (21, 22). We determined if decapped PGK1pG transcripts accumulated in the *mrt* mutants by examining the 5′ end of PGK1pG mRNA in these strains by primer extension analysis. If decapping is blocked in an *mrt* mutant, then full-length (capped) PGK1pG transcripts would be expected to accumulate in these strains (labeled FL in Fig. 5). This can be seen in *dcp1-1* strains (Fig. 5 [*dcp1-1* lane]), which are deficient in decapping activity (2). Conversely, if 5′-3′ exonuclease digestion is blocked in the *mrt* strains, as in the *xrn1*Δ strains, then decapped transcripts should accumulate as cDNA extension products that are shorter by two nucleotides (labeled −2 species [Fig. 5, *xrn1*Δ lane]). As expected, in both the *xrn1*Δ and the *xrn1-230* strains, cDNA extension products shorter by 2 bases were observed. In contrast, only full-length cDNA products were observed in the *mrt1* and *mrt3* strains (Fig. 5). We interpreted the results of this primer extension assay, in combination with the results from the direct assay of cap immunoprecipitations on MFA2pG mRNA described above, to indicate that mutations in the *MRT1* and *MRT3* genes inhibit mRNA decapping.

Lesions in the *MRT1* and *MRT3* genes affect the decay rates of several endogenous transcripts. The degradation pathway of deadenylation-dependent decapping has been speculated to be a common pathway of yeast mRNA decay (1, 9). Since the *mrt1* and *mrt3* lesions affected decapping of MFA2pG and PGK1pG mRNAs, we determined if these lesions affected the turnover of additional yeast transcripts. To perform this experiment, the *mrt1-3* and *mrt3-1* mutations were introduced into a strain carrying a temperature-sensitive lesion in RNA polymerase II (*rpb1-1*) (26). Subsequently, transcription in these strains was inhibited by shifting the cells to 36°C, and the decay of a number of endogenous mRNAs over time was monitored (14, 27). The temperature shift is not expected to change the effects of the *mrt* mutations on mRNA decay, because the alterations in mRNA distribution between full-length mRNA and decay product due to the *mrt* mutations are the same at both 24 and 36°C (data not shown). As summarized in Table 2, both the *mrt1* and the *mrt3* mutations affected the degradation of a number of different cellular mRNAs, suggesting that the function of the *MRT* gene products in mRNA decay is relatively general (see discussion). The stabilization effect was greatest for unstable mRNAs, such as the *HIS3* and MFA2pG transcripts. This is presumably because the normal rate of decapping and 5′-to-3′ decay of these mRNAs is much faster than the rates of possible alternative mRNA decay mechanisms for these transcripts (such as 3′-to-5′ decay). Thus, a block in decapping will have a more dramatic effect on *HIS3* and MFA2pG mRNA stability compared with more stable transcripts, such as PGK1pG, that undergo both slow 5′-to-3′ decay and slow 3′-to-5′ decay (see reference 22 for discussion). Interestingly, although the *mrt1-3* mutation stabilized the *GAL10* transcript by three- to fourfold compared with the wild-type strain, the *mrt3-1* mutation did not significantly affect the decay rate of this transcript. This observation implied that mRNA-specific interactions with the *MRT* gene products may play a role in differential rates of mRNA decapping and decay (see discussion).

The stability of the *PAB1* transcript was not affected by *mrt1* or *mrt3* mutations. Moreover, the stability of this transcript was not affected in *dcp1-1* strains (data not shown). These observations show that mRNA stability is not increased by default in

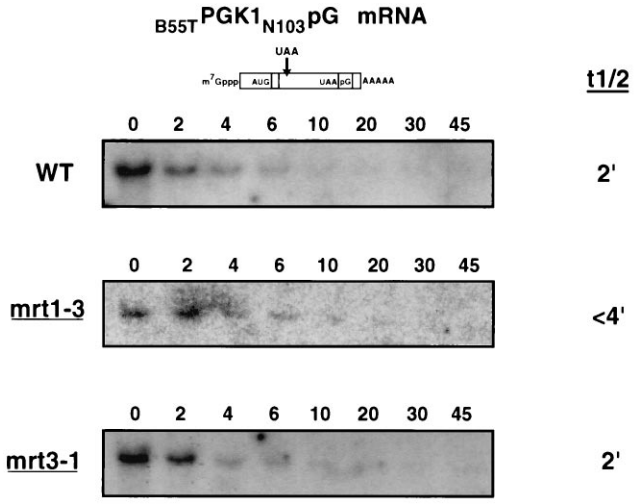

FIG. 6. *mrt* mutations do not inhibit the decay of PGK1pG mRNA containing an early nonsense codon; measurement of decay of a PGK1pG transcript containing an early nonsense codon ($_{B55T}$PGK1$_{N103}$pG) (24). In this experiment, a plasmid expressing the $_{B55T}$PGK1$_{N103}$pG transcript under the control of the *GAL1* UAS (pRP611 [24]) was introduced into a wild-type strain (WT) (yRP840) and *mrt1-3* (yRP1066) and *mrt3-1* (yRP1067) mutants. Transcription was repressed at zero time by the addition of glucose, and the levels of $_{B55T}$PGK1$_{N103}$pG present were determined by probing with an oligonucleotide probe specific for the $_{B55T}$PGK1$_{N103}$pG mRNA, oRP252 (5′-CTTGGACAGAGATCAATTCG-3′). This probe is complementary to an oligonucleotide inserted at position 55 of the *PGK1* sequence in the $_{B55T}$PGK1$_{N103}$pG transcript and *PGK1* mRNA sequence 3′ of the tag. $t_{1/2}$, half-life.

the *mrt* and *dcp1* mutants and suggest that *PAB1* mRNA may be primarily degraded by an alternative decay mechanism.

PGK1pG mRNAs containing an early nonsense codon are not stabilized in *mrt* mutant strains. In principle, the *mrt1* and *mrt3* mutations could inhibit decapping in two general ways. First, the *MRT1* and *MRT3* gene products could be direct regulators of Dcp1p activity by promoting the synthesis or increasing the activity of this decapping enzyme. Alternatively, the *MRT* gene products might influence messenger ribonucleoprotein (mRNP) structure after deadenylation, resulting in increased accessibility of the 5′ cap structure to the decapping enzyme. To distinguish between these possibilities, we first examined the degradation of transcripts containing early nonsense codons in the *mrt1* and *mrt3* mutants. This experiment is based on the observation that even though mRNAs containing premature nonsense codons are decapped prior to deadenylation (24), these transcripts are decapped by the same enzyme that decaps normal transcripts, Dcp1p (2). If the *mrt1* and *mrt3* lesions directly affect the activity of the decapping enzyme, then they would be expected to affect the decapping of a transcript with an early nonsense codon. Alternatively, if the *mrt1* and *mrt3* mutations are required only for modulating the rate of deadenylation-dependent decapping, then they would not be expected to affect the decay of a transcript with an early nonsense codon.

To perform this experiment, we transformed the *mrt1* and *mrt3* strains with a plasmid expressing a PGK1pG transcript containing an early nonsense codon, termed $_{B55T}$PGK1$_{N103}$pG (24). Since this mRNA is under the transcriptional control of the *GAL1* UAS, we measured its decay rate following inhibition of transcription by the addition of glucose. As shown in Fig. 6, the *mrt1-3* and *mrt3-1* mutations do not significantly affect the decay rate of the $_{B55T}$PGK1$_{N103}$pG transcript. In agreement with these results, the *mrt1-3* and *mrt3-1* mutations do not affect the rapid decay of the inefficiently spliced precursor to the *CYH2* mRNA (Table 2), which is thought to be degraded by the same mechanism that degrades transcripts containing early nonsense codons (13). These results demonstrated that the *MRT1* and *MRT3* gene products are not involved in deadenylation-independent decapping and suggested that they may specifically function to modulate rates of decapping following deadenylation (see discussion).

***mrt1* and *mrt3* mutants contain normal levels of the mRNA decapping activity.** The previous results suggested that the *mrt1-3* and *mrt3-1* mutants must contain at least some decapping activity. To directly test the levels of decapping activity in these mutants, we assayed cell extracts from wild-type, *mrt1*, and *mrt3* strains for the decapping activity recently shown to be required for mRNA decay (2). This experiment demonstrated that cell extracts from both *mrt* mutants contain essentially wild-type levels of in vitro decapping activity (relative decapping activities were 1.0 for wild type, 0.9 for *mrt1-3*, and 1.2 for *mrt3-1*). This observation implies that *mrt1* and *mrt3* lesions may affect the rates of mRNA decapping by altering the interaction of the decapping enzyme with its substrate (see Discussion).

Mutations in the *MRT1*, *MRT3*, and *DCP1* genes suppress the lethality of *pab1*Δ. The above results suggested that the *MRT1* and *MRT3* gene products promote mRNA decapping. However, decapping can also be negatively controlled. For example, since decapping generally occurs following deadenylation, the poly(A) tail can be considered an inhibitor of mRNA decapping. This negative affect on decapping requires the poly(A) binding protein (Pab1p) (31), since mRNAs are decapped prior to deadenylation in *pab1*Δ strains (5). It was hypothesized that such deadenylation-independent decapping in *pab1*Δ strains might result in the premature degradation of mRNAs, thereby contributing to the lethality of *pab1*Δ. This model was supported by the observation that inhibition of mRNA turnover downstream of decapping, by deletion of the *XRN1* gene, suppresses the lethality of *pab1*Δ (5). A prediction of this model is that mutations that inhibit mRNA decapping should suppress the lethality of *pab1*Δ. To test this possibility, we examined whether the *mrt1*, *mrt3*, and/or *dcp1-1* lesion could suppress the lethality of *pab1*Δ by a standard genetic cross (see Materials and Methods). As shown in Fig. 7, mutations in the *MRT1*, *MRT3*, and *DCP1* genes suppress the lethality of *pab1*Δ. These observations suggest that one class of suppressors of *pab1*Δ lethality will be those that alter mRNA degradation following deadenylation (see reference 5).

DISCUSSION

Two pathways of mRNA turnover converge on mRNA decapping. Two different but related pathways of mRNA degradation utilize mRNA decapping to expose transcripts to 5′-to-3′ exonucleolytic degradation (for a review, see reference 1). One of these pathways is a mechanism whereby mRNAs are deadenylated and then decapped. This process of mRNA decay appears to function on normal yeast mRNAs spanning a wide range of decay rates (8, 21, 22). A related pathway of degradation functions on mRNAs that contain early nonsense codons wherein transcripts are decapped without prior deadenylation (11, 24). Several lines of evidence suggest that these two pathways converge at a common step of mRNA decapping (Fig. 8). First, both pathways require the function of the same decapping enzyme, which is encoded by the *DCP1* gene (2). Similarly, following decapping, the same 5′-to-3′ exonuclease, Xrn1p, degrades the body of the transcript (11, 16, 21, 22, 24).

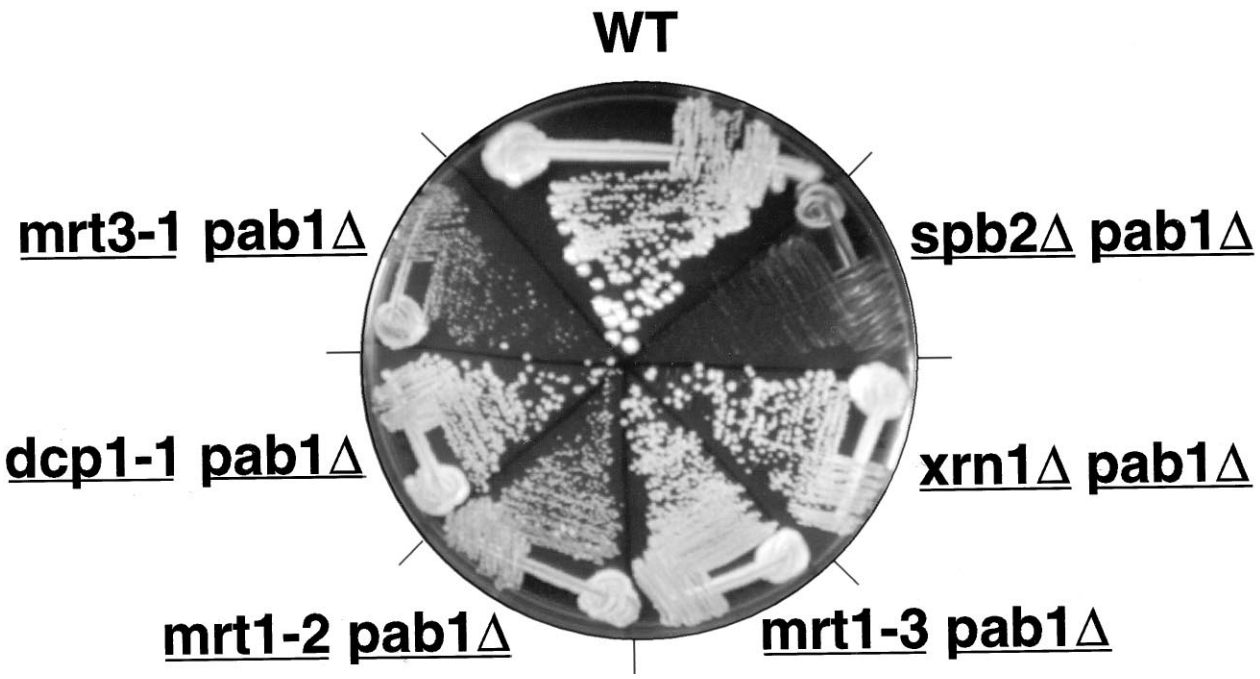

FIG. 7. Mutations that inhibit mRNA decay suppress *pab1*Δ. YEPD plate on which *pab1*Δ strains in combination with the mutations shown were streaked for single colonies at 24°C. Strains shown include yRP881 (*spb2*Δ *pab1*Δ), yRP903 (*xrn1*Δ *pab1*Δ), yRP1110 (*mrt1-3 pab1*Δ), yRP1111 (*mrt1-2 pab1*Δ), yRP1112 (*dcp1-1 pab1*Δ), and yRP1113 (*mrt3-1 pab1*Δ). The wild-type (WT) strain used for comparison was yRP840. The *spb2*Δ mutation, which suppresses *pab1*Δ lethality (32), was used as a positive control for *pab1*Δ growth suppression.

Finally, the convergence of these pathways is supported by the observation that the 5′ end of the decapped transcript is the same in deadenylation-independent and deadenylation-dependent decapping (cf. references 22 and 24). In both of these pathways, different features of transcripts including the poly(A) tail, the status of translation termination, and specific mRNA sequences need to be recognized and utilized to affect the rate of the decapping reaction.

Identification of mutations affecting mRNA decapping. In this work, we have identified mutations in two genes, *MRT1* and *MRT3*, that affect mRNA decay. Since these recessive mutations lead to the stabilization of mRNAs, the *MRT1* and *MRT3* gene products presumably function to promote mRNA degradation. Several lines of evidence indicated that mutations in these genes primarily lead to an inhibition of mRNA decapping. First, in transcriptional pulse-chase analysis of the *mrt1* and *mrt3* mutants, MFA2pG transcripts are deadenylated essentially normally but persist as full-length deadenylated species (Fig. 3). Second, immunoprecipitation of MFA2pG transcripts showed that the full-length deadenylated transcripts that accumulate in the *mrt* mutants have a cap structure (Fig. 4). Third, primer extension analysis of the PGK1pG transcript detects only a full-length species and not a species lacking 2 bases at the 5′ end that is detected in strains blocked at 5′-to-3′ exonucleolytic degradation (Fig. 5). We interpret these observations to indicate that the *MRT1* and *MRT3* gene products promote mRNA decapping. We have shown by complementation and linkage analysis that the *MRT1* and *MRT3* genes are distinct from the *DCP1* gene, which encodes the decapping enzyme or a necessary component of the decapping complex required for mRNA decapping in vivo (2). Thus, at the present time, the products of the *MRT1* and *MRT3* genes and Dcp1p represent the three gene products known to affect mRNA decapping.

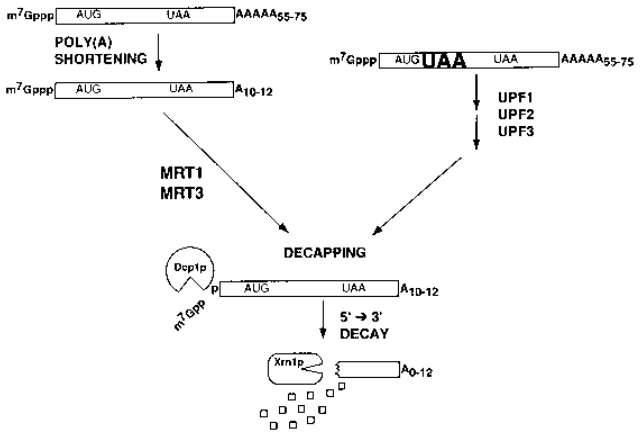

FIG. 8. Model of *MRT* gene product function; converging pathways of deadenylation-dependent decay of normal mRNAs and the deadenylation-independent decapping of transcripts containing premature stop codons. The positions at which various gene products are thought to function, including the *MRT* gene products, are shown.

Two classes of gene products affect mRNA decapping. The three gene products defined by the *MRT1*, *MRT3*, and *DCP1* loci fall into two classes with regard to their role in mRNA decapping. The first class consists of gene products required for the production of active decapping enzyme and thereby should affect both deadenylation-dependent and deadenylation-independent decapping. The only known member of this class is the *DCP1* gene (2). Strains from which the *DCP1* gene is deleted are defective in deadenylation-dependent and deadenylation-independent decapping and show no decapping activity when assayed in cell extracts. A point mutation in this gene, *dcp1-1*, which was isolated during this work, shows phenotypes similar to that of *dcp1*Δ (2). One might predict that

other gene products that fall into this class of decapping gene products will be isolated. These gene products could function to increase the level of expression of Dcp1p or to modulate its enzymatic activity, perhaps as another subunit of a decapping complex or by posttranslational modification of Dcp1p.

The second class of gene products affecting mRNA decapping consists of the products of the *MRT1* and *MRT3* genes. In contrast to the general effects of *dcp1*Δ, mutations in these genes do not affect deadenylation-independent decapping (Fig. 6; also see Table 2 [*CYH2* precursor mRNA]) and therefore may be functionally limited to transcripts that require deadenylation before decapping (depicted in Fig. 8). This model is supported by the demonstration that cell extracts from *mrt1-3* and *mrt3-1* strains show normal levels of decapping activity. In this light, it is striking to note that some mRNAs are affected differently by the *mrt1-3* and *mrt3-1* lesions. For example, the *GAL10* transcript is stabilized by a *mrt1-3* lesion but is not affected by an *mrt3-1* mutation. This raises the possibility that the *MRT1* and *MRT3* gene products are involved in determining mRNA-specific rates of decapping following deadenylation.

An important issue is how *mrt1* and *mrt3* mutations inhibit mRNA decapping. Previous observations have shown that inhibition of translational elongation, because of either cycloheximide (e.g., see reference 14) or the *cca1-1* mutation (28), can stabilize mRNAs. This raises the possibility that the *mrt1* and *mrt3* lesions inhibit mRNA decapping as a secondary consequence of an inhibition of translation in these mutants. Although we cannot formally rule out this possibility, the mRNA-specific effects observed in the *mrt1* and *mrt3* mutants are different from the general stabilization of all mRNAs examined because of the inhibition of translation resulting from cycloheximide or *cca1-1*.

Two general models may explain how the *MRT1* and *MRT3* gene products more directly affect decapping rates. In one view, these gene products are envisioned to interact with Dcp1p in a manner that would recruit Dcp1p to the mRNA after deadenylation and thus promote decapping. An appealing alternative view is suggested by the proposed role of the *UPF* gene products in the decay of mRNAs with early nonsense codons. In that case, the *UPF* gene products have been hypothesized to function in sensing premature termination and subsequently alter the mRNP structure at the 5′ end of the transcript to allow rapid deadenylation-independent decapping (6, 11, 17, 24, 29). By analogy to this model, the *MRT1* and *MRT3* gene products may promote the formation of or stabilize a particular mRNP structure, after loss of the poly(A) tail, that is susceptible to decapping by Dcp1p. In this model, modulation of decapping rate by specific mRNA features could affect the interaction of proteins such as translation initiation factors with the 5′ cap structure, thereby sterically competing with the decapping activity. Such a model is appealing in that it provides a mechanistic basis for the interrelationship between the translation and turnover of eukaryotic mRNAs.

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L.H. and C.A.B. contributed equally to this work.

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