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The 3′ to 5′ degradation of yeast mRNAs is a general mechanism for mRNA turnover that requires the SKI2 DEVH box protein and 3′ to 5′ exonucleases of the exosome complex

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One major pathway of mRNA decay in yeast occurs by deadenylation-dependent decapping, which exposes the transcript to 5′ to 3′ exonucleolytic degradation. We show that a second general pathway of mRNA decay in yeast occurs by 3′ to 5′ degradation of the transcript. We also show that the *SKI2, SKI3, SKI6/RRP41, SKI8* and *RRP4* gene products are required for 3′ to 5′ decay of mRNA. The Ski6p/Rrp41p protein has homology to the *Escherichia coli* 3′ to 5′ exoribonuclease RNase PH, and both the Ski6p/Rrp41p and Rrp4p proteins are components of a multiprotein complex, termed the exosome, that contains at least three polypeptides with 3′ to 5′ exoribonuclease activities. These observations suggest that the exosome may be the nucleolytic activity that degrades the body of the mRNA in a 3′ to 5′ direction, and the exosome's activity on mRNAs may be modulated by Ski2p, Ski3p and Ski8p. Blocking both 3′ to 5′ and 5′ to 3′ decay leads to inviability, and conditional double mutants show extremely long mRNA half-lives. These observations argue that efficient mRNA turnover is required for viability and that we have identified the two major pathways of mRNA decay in yeast.

Keywords: 3′ to 5′/DEVH box/exosome/mRNA degradation/yeast

Introduction

An important control point in the modulation of gene expression is the stability of the mRNA, which can vary significantly and be regulated in response to a variety of physiological cues (for reviews, see Beelman and Parker, 1995; Ross, 1995; Caponigro and Parker, 1996a; Jacobson and Peltz, 1996). A critical step in understanding mRNA turnover is to determine the mechanisms by which mRNAs are degraded and their generality. One pathway of mRNA degradation in yeast occurs by shortening of the poly(A) tail, followed by a decapping reaction, thereby exposing the mRNA to 5′ to 3′ degradation (Decker and Parker, 1993; Hsu and Stevens, 1993; Muhlrad *et al.*, 1994, 1995). Two lines of evidence have suggested that this deadenylation-dependent decapping and 5′ to 3′ exonucleolytic digestion is a general mechanism of mRNA decay able to act on many transcripts. First, each of the three yeast mRNAs whose pathways of decay have been analyzed extensively are degraded in this manner (Muhlrad *et al.*, 1994, 1995; Caponigro and Parker, 1996b). In addition, mutation of either the decapping enzyme, encoded by the *DCP1* gene, or the 5′ to 3′ exonuclease, encoded by the *XRN1* gene, leads to a stabilization of many different mRNAs (Hsu and Stevens, 1993; Muhlrad *et al.*, 1994, 1995; Beelman *et al.*, 1996).

In addition to the general 5′ to 3′ mechanism of mRNA degradation, there must be other pathways of mRNA turnover able to act on many transcripts. This conclusion is based on the observations that strains blocked in 5′ to 3′ decay due to mutation are viable and all mRNAs examined continue to degrade, albeit at slower rates. Other general mRNA decay pathways could include both 3′ to 5′ mechanisms of decay and endoribonuclease cleavage-mediated decay mechanisms (for a review, see Beelman and Parker, 1995). A 3′ to 5′ pathway of mRNA degradation is a good candidate for a second general mRNA turnover pathway, for three reasons. First, the known endonuclease cleavage sites in mRNAs require specific sequences, which are not likely to be found in every mRNA (Bernstein *et al.*, 1992; Nielsen and Christiansen, 1992; Brown *et al.*, 1993; Binder *et al.*, 1994; Dompenciel *et al.*, 1995). Second, one yeast mRNA, encoded by the *PGK1* gene, had been shown to be degraded in a 3′ to 5′ direction when the 5′ to 3′ pathway was blocked (Muhlrad *et al.*, 1995). This conclusion was based on the use of poly(G) insertions in the transcript, which can block 5′ to 3′ and 3′ to 5′ exonucleases, and thereby trap intermediates in decay (Vreken and Raué, 1992; Decker and Parker, 1993). By determining the structure of the intermediates, the directionality of degradation can be determined. For example, when *PGK1* transcripts with a poly(G) tract in the 3′-untranslated region (3′ UTR) are degraded in the absence of the 5′ to 3′ decay mechanism, an mRNA fragment trimmed to the 3′ side of the poly(G) tract accumulates (Muhlrad *et al.*, 1995). Finally, a heteropentaprotein complex, termed the exosome, has recently been described (Mitchell *et al.*, 1997). Three of the proteins from this complex (Rrp4p, Ski6p/Rrp41p and Rrp44p) have been shown to have 3′ to 5′ exoribonuclease activity *in vitro*, and the remaining two (Rrp42p and Rrp43p) have sequence similarity to bacterial 3′ to 5′ exonucleases (Mitchell *et al.*, 1997). While the complex is known to be required for proper 5.8S rRNA processing in the nucleus in yeast, a homologous complex in HeLa cells is also found in the cytoplasm (Mitchell *et al.*, 1997), suggesting a potential role in 3′ to 5′ mRNA degradation.

An important issue is whether the 3′ to 5′ mechanism of mRNA turnover is a general pathway acting on many mRNAs, and what gene products perform and modulate the nucleolytic events. In this work, we present evidence that several different mRNAs can be degraded by a 3′ to 5′ mechanism. Given the broad range of substrates identified, we hypothesized that the 3′ to 5′ mechanism would be a general pathway of mRNA decay able to perform

mRNA turnover at rates sufficient for viability. This view predicted that mutations that inactivate the 5′ to 3′ pathway might be synthetically lethal with mutations that inactivate the 3′ to 5′ mechanism. This logic led us to examine the process of mRNA degradation in a set of Ski⁻ mutants, as well as strains mutated for two components of the exosome. Mutations in the *SKI* genes originally were isolated as allowing overexpression of gene products from a yeast double-stranded RNA virus (Ridley *et al.*, 1984), which could be explained by changes in mRNA decay. Strikingly, mutations of the *SKI2* or *SKI3* genes are synthetically lethal with deletions of the *XRN1* gene, which encodes the 5′ to 3′ exonuclease required for the 5′ to 3′ decay pathway (Johnson and Kolodner, 1995). This observation suggested to us that *SKI2* and *SKI3*, as well as the phenotypically similar genes *SKI6* and *SKI8*, might be required for 3′ to 5′ degradation of mRNAs. Since Ski6p/Rrp41p is a component of the exosome complex (Mitchell *et al.*, 1997), we also asked if another component, Rrp4p, was required for 3′ to 5′ decay. Examination of the 3′ to 5′ decay mechanism in *ski2*, *ski3*, *ski6/rrp41*, *ski8* and *rrp4* mutants demonstrated that these gene products were indeed required for normal 3′ to 5′ mRNA turnover. These observations suggest that the exosome is the nucleolytic activity that can degrade the body of the mRNA in a 3′ to 5′ direction, and the exosome's activity on mRNAs is modulated by Ski2p, Ski3p and Ski8p. Cells in which both 3′ to 5′ and 5′ to 3′ decay are blocked by separate mutations are not viable, and conditional double mutants show extremely long mRNA half-lives. These observations argue that efficient mRNA turnover is required for viability and that we have identified the two major pathways of mRNA decay in yeast.

Results

Stable and unstable yeast mRNAs can be degraded by a 3′ to 5′ mechanism

As discussed above, prior work had demonstrated that in the absence of 5′ to 3′ degradation the body of the *PGK1* transcript was degraded in a 3′ to 5′ direction to produce 3′-trimmed mRNA fragments (Muhlrad and Parker, 1994; Muhlrad *et al.*, 1995). These intermediates are easily observed when 5′ to 3′ decay is inhibited either in *cis* or in *trans*, and the mRNA under examination contains a poly(G) tract, which can block the 3′ to 5′ exonuclease. In order to determine if other yeast mRNAs could be degraded 3′ to 5′, we asked if the *MFA2* mRNA was also a substrate for 3′ to 5′ degradation when the 5′ to 3′ pathway was blocked. We utilized the *MFA2pG* transcript for this experiment since this mRNA contains a poly(G) tract to facilitate the detection of decay intermediates. In addition, the *MFA2pG* mRNA is known normally to undergo deadenylation-dependent decapping, yet this transcript still turns over when decapping is blocked (Beelman *et al.*, 1996).

In this experiment, we utilized a strain with a temperature-sensitive allele (*dcp1-2*) of the *DCP1* gene, which encodes the decapping enzyme, and examined the decay of the *MFA2pG* transcript at the restrictive temperature as compared with a wild-type strain. Strains carrying the *dcp1-2* allele are viable at all temperatures, and have a wild-type mRNA decay phenotype at the permissive

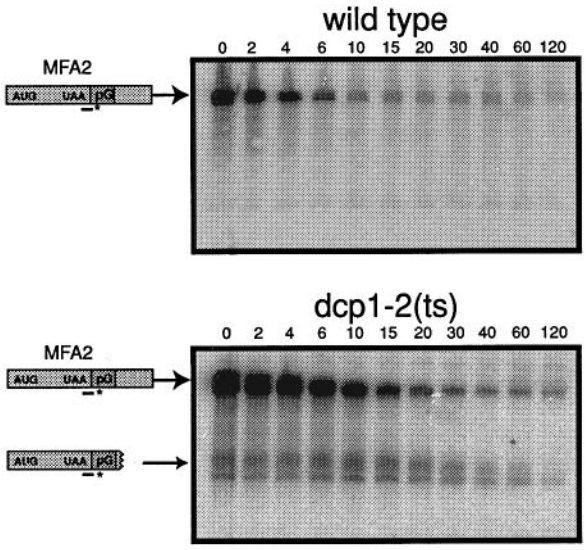

Fig. 1. 3′ to 5′ decay of the *MFA2pG* mRNA in a *dcp1-2* mutant. Polyacrylamide gel Northern blots of transcriptional repression experiments from wild-type (yRP840) and *dcp1-2* (yRP1205) strains after a shift to 36°C for 1 h. The identity of the various RNA species is indicated with cartoons at the left. These blots were probed with an oligonucleotide of sequence 5′-CCAAATTCCTAGATCTCTTGG-3′, which hybridizes to the poly(G) insertion and flanking 5′ sequence in the *MFA2pG* mRNA. The position of hybridization is indicated by the black bar on the cartoon RNAs on the left.

temperature, but show essentially a complete block to decapping at the restrictive temperature (S.Tharun and R.Parker, in preparation). As shown in Figure 1, we observed that at the restrictive temperature in the *dcp1-2* strain, the *MFA2pG* transcript was more stable than in wild-type cells. Furthermore, the ~190 nucleotide mRNA fragment produced by 5′ to 3′ exonucleolytic degradation to the 5′ side of the poly(G) tract [referred to as the poly(G)→3′ end fragment, see Figure 2A] was not detected using an oligonucleotide probe known to detect this species (data not shown, oRP140, Caponigro and Parker, 1995). A critical observation was that a new intermediate of ~210 nucleotides was observed using an oligonucleotide probe spanning the poly(G) tract and 5′-flanking sequences. Based on probing with different oligonucleotides and RNase protection experiments (data not shown), it was demonstrated that this mRNA fragment [referred to as the 5′ end→poly(G) fragment, Figure 2A] was missing the sequences from the 3′ side of the poly(G) tract to the 3′ end of the mRNA. Thus, like the stable *PGK1pG* mRNA, the unstable *MFA2pG* mRNA can be degraded in a 3′ to 5′ direction, although in wild-type cells the rate of this process for the *MFA2* transcript is normally slower than the process of decapping and 5′ to 3′ degradation. Interestingly, the rate at which the *MFA2pG* mRNA was degraded by the 3′ to 5′ mechanism was faster than the rate at which the *PGK1pG* mRNA was degraded 3′ to 5′. This indicated that the 3′ to 5′ pathway of mRNA decay can maintain the relative rates of degradation observed in wild-type cells.

The poly(G)→3′ end mRNA fragments are also substrates for the 3′ to 5′ decay machinery

In wild-type yeast, mRNAs are degraded primarily 5′ to 3′ (Decker and Parker, 1993; Muhlrad *et al.*, 1994, 1995)

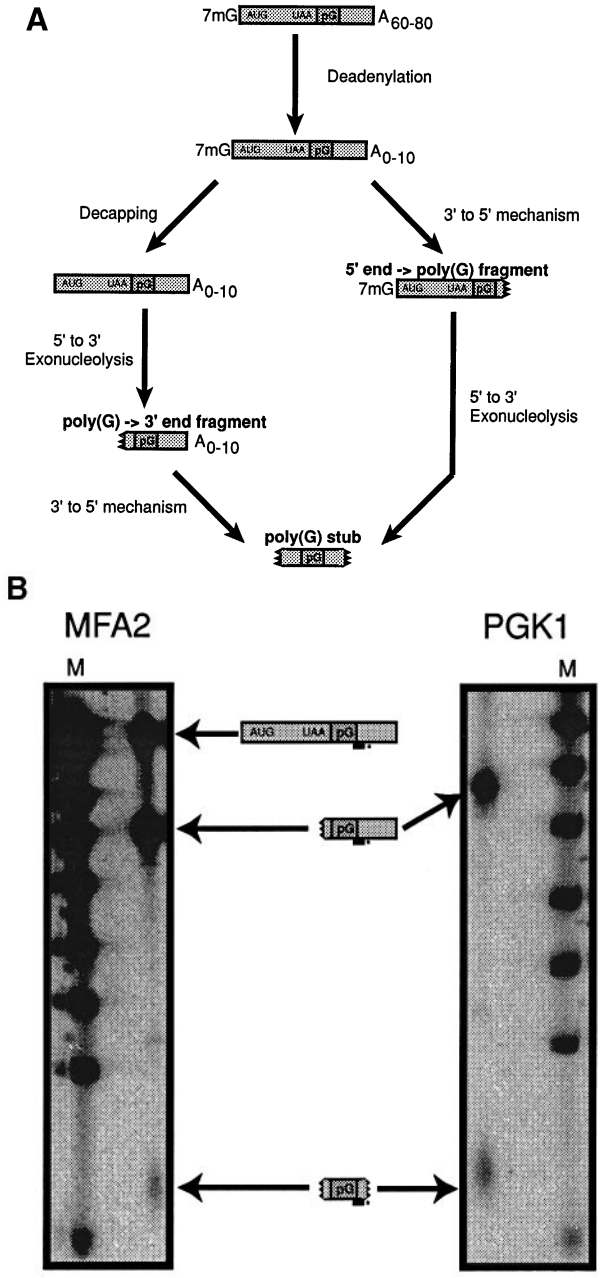

Fig. 2. The poly(G)→3′ end fragments from *MFA2pG* and *PGK1pG* decay 3′ to 5′. **(A)** A diagram of the interrelationship of the major pathways of mRNA decay in yeast. (**B**) Polyacrylamide gel Northern blots of steady-state RNA from a wild-type strain (yRP840) probed with oligonucleotide probes that specifically hybridize to the poly(G) insertions and flanking 3′ sequences in *MFA2pG* (left panel) and *PGK1pG* (right panel) [oRP140 (*MFA2pG*) and oRP141 (*PGK1pG*) in Caponigro and Parker, 1995]. The general structure of the various RNA species is indicated with cartoons in the middle. The full-length 1.5 kb *PGK1pG* band is not visible because it does not transfer out of the polyacrylamide gel. The lanes labeled M are molecular weight markers. Marker sizes are 489, 404, 353, 242, 190, 147, 110, 89, 67 and 34 nucleotides in the left panel, and 242, 190, 147, 110, 89, 67 and 34 nucleotides in the right panel.

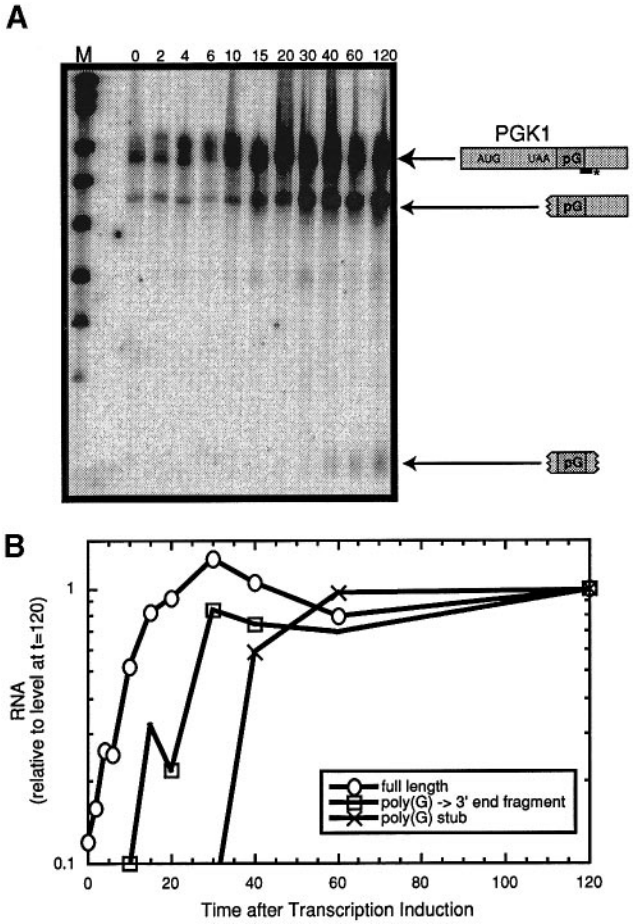

Fig. 3. The poly(G) stub is a product of poly(G)→3′ end fragment degradation. The induction of the *PGK1pG* mRNA and its decay intermediates in a wild-type strain (yRP840) at 30°C following the induction of transcription by the addition of galactose at time = 0 is shown. The numbers above the lanes in (A) indicate minutes after transcriptional induction. The lane labeled M contains molecular weight markers, of 489, 404, 353, 242, 190, 147, 110, 89 and 67 nucleotides. The probe for this experiment was oRP141 (see Figure 2 legend). (**A**) Northern gel; (**B**) quantitation of the relevant species.

and, when a transcript has an inserted poly(G) tract, poly(G)→3′ end fragments of mRNAs are easily detected. Since the poly(G)→3′ end fragment is resistant to 5′ to 3′ decay, a simple hypothesis is that these mRNA fragments are also substrates for the 3′ to 5′ mRNA degradation machinery. This model predicts that another decay intermediate, trimmed on both the 5′ and 3′ sides of the poly(G) tract, should be produced (see Figure 2A). In order to determine if this product was in fact present, RNA from cells expressing the *MFA2pG* and the *PGK1pG* transcripts was examined on polyacrylamide Northern blots. As seen in Figure 2, a band of the appropriate size was detected with probes specific for either the *MFA2pG* transcript or the *PGK1pG* transcript (Figure 2B).

The detection of an mRNA decay product trimmed on both the 5′ and 3′ sides [referred to as the poly(G) ‘stub’] suggests that the fragment generated by 5′ to 3′ decay is ultimately degraded by a 3′ to 5′ nucleolytic pathway. This hypothesis predicts that in a time course of induction, the poly(G) ‘stub’ would not be produced until after the poly(G)→3′ end fragment was produced and was itself being degraded. In order to test this prediction, we induced the transcription of the *PGK1pG* mRNA, which was under control of the GAL1 upstream activating sequence (UAS), and followed the levels of the various mRNA fragments as a function of time. As seen in Figure 3, the poly(G)→3′ end fragment (produced by 5′ to 3′ decay) increased in abundance after the full-length mRNA, consistent with

Table I. mRNA decay phenotypes of *ski2*, *ski3*, *ski6/rrp41* and *ski8* mutants

Strain	Condition	Full-length half-life (min)		Fragment half-life (min)		Percentage of fragment	
		MFA2pG	*PGK1pG*	*MFA2pG*	*PGK1pG*	*MFA2pG*	*PGK1pG*
Wild-type	30°C	5	25	15	15	47%	21%
*ski2*Δ	30°C	5	25	>60	>60	81%	72%
*ski3*Δ	30°C	5	25	>60	>60	81%	72%
ski6-2	30°C	ND	ND	33	33	60%	35%
*ski8*Δ	30°C	5	25	>60	>60	81%	72%
Wild-type	37°C	3	19	10	10	49%	24%
ski6-10	37°C	3	19	>60	>60	56%	35%

The table gives the phenotypes of wild-type (yRP840), *ski2*Δ (yRP1195), *ski3*Δ (yRP1196), *rrp41/ski6-100* (yRP1204), *rrp41/ski6-2* (yRP1203) and *ski8*Δ (yRP1197) strains for six characteristics: the half-lives of full-length *MFA2pG* and *PGK1pG*, the half-lives of the poly(G)→3′ end fragment for *MFA2pG* and *PGK1pG* transcripts, and the amount of poly(G)→3′ end fragment present at steady-state as a percentage of full-length + poly(G)→3′ end fragment. Fragment half-lives were determined by transcriptional repression in the presence of cycloheximide (see Materials and methods). Half-lives of >60 min indicate that at the end of the 60 min time course, mRNA levels had not reached 50% of the initial value. All values are averages of at least two independent time courses.
ND = not determined.

the precursor–product relationship previously demonstrated for these two species in transcriptional pulse–chase experiments (Decker and Parker, 1993; Muhlrad *et al.*, 1995). In addition, the levels of the poly(G) 'stub' only rose after the levels of the poly(G)→3′ end fragment reached a steady-state (Figure 3). We interpreted these results to indicate the poly(G)→3′ end mRNA fragment was also a substrate for the 3′ to 5′ degradation machinery (cartooned in Figure 2A).

The SKI2, SKI3, SKI6/RRP41 and SKI8 gene products are required for normal 3′ to 5′ mRNA decay

The above results indicated that a variety of different mRNA species and mRNA fragments could be degraded in a 3′ to 5′ direction. An important goal was to identify the gene products involved in 3′ to 5′ degradation of mRNAs and to determine how their activities were modulated. Based on the synthetic lethality of the *ski2* or *ski3* mutations with deletions of the *XRN1* gene (see Introduction), we examined the process of mRNA degradation in strains mutated in either the *SKI2*, *SKI3*, *SKI6/RRP41* or *SKI8* genes. The Ski6p/Rrp41p protein was of particular interest since it is a component of the 3′ to 5′ exonuclease complex called the exosome (Mitchell *et al.*, 1997). Since the *SKI2*, *SKI3* and *SKI8* genes were non-essential, we utilized deletion mutations of these genes in our analysis. However, deletions of the *SKI6/RRP41* gene were inviable (see Materials and methods; L.Bernard, K.Carroll, R.C.P.Valle and R.B.Wickner, manuscript submitted), so we examined the process of mRNA decay in *SKI6/RRP41* mutants by utilizing both a strain carrying an original viable allele of *ski6/rrp41* (Ridley *et al.*, 1984) and a strain carrying a temperature-sensitive allele which we isolated (termed *ski6/rrp41-100*; see Materials and methods).

Our first analysis of the *ski2*, *ski3*, *ski6/rrp41* and *ski8* mutations was to examine their effect on mRNA turnover. As shown in Table I, mutations in any of these genes do not change the turnover rates of the full-length *MFA2pG* or *PGK1pG* transcripts. Since these transcripts are known to be degraded by deadenylation-dependent decapping and subsequent 5′ to 3′ decay, this observation indicated that these gene products were not required for the 5′ to 3′ pathway of degradation. However, the amount of the poly(G)→3′ end fragment was increased in the *ski2*, *ski3*, *ski6/rrp41* and *ski8* mutants relative to wild-type. This effect can be seen in an increased amount of the trapped poly(G)→3′ end fragment, as a percentage of total mRNA species present (Table I). This observation suggested that the 3′ to 5′ decay of the poly(G)→3′ end fragment might be defective in these mutants, and that the increased levels of the poly(G)→3′ end fragment were due to a failure to degrade it efficiently.

In order to determine if the Ski$^-$ mutations were inhibiting the 3′ to 5′ degradation of the poly(G)→3′ end fragment, we directly measured the decay rate of this species in wild-type and *ski2*, *ski3*, *ski6/rrp41* and *ski8* mutants. The experimental approach was to block the production of new poly(G)→3′ end fragments by the addition of cycloheximide, which rapidly inhibits the decapping of mRNAs (Beelman and Parker, 1994). Subsequently, the decay rate of the pre-existing poly(G)→3′ end fragment could be observed over time.

In wild-type cells, the poly(G)→3′ end fragment of either the *MFA2pG* or *PGK1pG* transcripts decayed with a half-life of ~15 min at 30°C (Figure 4 and Table I). In contrast, in the *ski2*Δ, *ski3*Δ and *ski8*Δ strains, the same intermediates decayed with half-lives of >60 min. Moreover, the phenotype of a *ski2*Δ *ski3*Δ *ski8*Δ triple mutant was similar, consistent with these mutations all affecting the same decay mechanism (data not shown). The *rrp41/ski6-2* mutation also stabilized the poly(G) fragment, albeit to a lesser extent (Table I), presumably because this allele is a partial loss of function. Similar results are seen with the temperature-sensitive *rrp41/ski6-100* allele, although the rates of degradation were slightly faster due to the higher temperatures (Table I). These observations argued that the *ski2*Δ, *ski3*Δ, *ski6/rrp41* and *ski8*Δ mutants are defective in the 3′ to 5′ mechanism of mRNA decay.

Additional evidence that these mutations were inhibiting 3′ to 5′ degradation was provided by analysis of the fragments from the *MFA2pG* (Figure 5) and *PGK1pG* (similar results not shown) mRNAs accumulating in the *ski2*, *ski3*, *ski6/rrp41* and *ski8* mutant strains on polyacrylamide Northern gels. All of these mutant strains accumu-

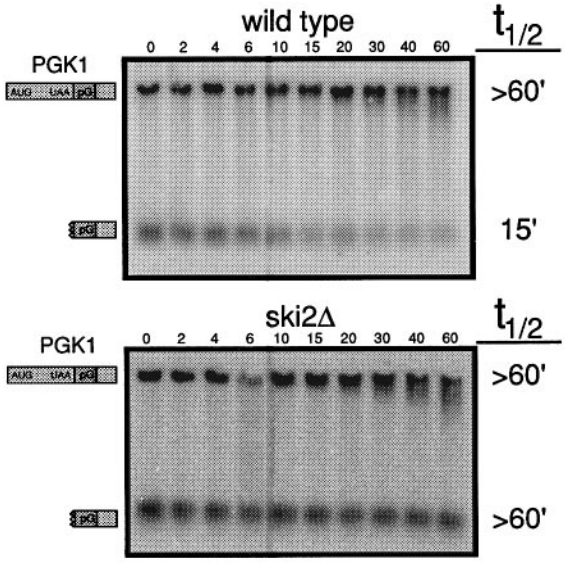

Fig. 4. Decay of the poly(G)→3′ end fragment in wild-type and *ski* mutant strains. A representative transcriptional repression experiment is shown in (**A**) wild-type (yRP840) and (**B**) *ski2*Δ (yRP1195) strains in which dextrose (to inhibit transcription) and cycloheximide (to inhibit decapping) were added at zero time. Similar results were obtained for *ski3*Δ, *ski8*Δ and *ski6/rrp41-100* strains (see Table II). The numbers above each lane indicate minutes after addition of glucose and cycloheximide. The full-length and poly(G)→3′ end fragment species are indicated with cartoons on the left. The probe for this experiment was oRP141 (see Figure 2 legend).

lated higher levels of the poly(G)→3′ end fragment from the *MFA2pG* transcript (Figure 5; standardized to levels of the 7S RNA) and failed to produce the poly(G) 'stub' seen in wild-type (based on longer exposures, not shown). In addition, a number of smaller RNA species also accumulated (Figure 5). Utilizing different probes, we determined that these new mRNA fragments were shortened by different amounts from the 3′ end of the mRNA. For example, when the blots were probed with an oligonucleotide that hybridizes near the 3′ end of the transcript, the smaller species were no longer detectable (data not shown). Therefore, some residual 3′ to 5′ degradation was occurring in the *ski2*, *ski3*, *ski6/rrp41* and *ski8* mutants, although at a significantly reduced rate. These results argued that the proteins encoded by the *SKI2*, *SKI3*, *SKI6/RRP41* and *SKI8* genes were required for efficient 3′ to 5′ degradation of mRNAs.

RRP4 mutations also disrupt 3′ to 5′ mRNA degradation

Ski6p/Rrp41p is a component of a multiprotein complex called the exosome, which is required for correct 3′ to 5′ processing of the 5.8S rRNA (see Introduction; Mitchell *et al.*, 1997). Since *ski6/rrp41* mutants were found to be defective in 3′ to 5′ mRNA decay, we were interested in determining if other exosome components were also required for this process. To investigate this, we examined the accumulation of 3′-trimmed decay intermediates of the poly(G)→3′ end fragment in a temperature-sensitive *rrp4-1* strain (Mitchell *et al.*, 1996). As shown in Figure 5B, the *rrp4-1* mutant also accumulated 3′-trimmed fragments after a shift to the restrictive temperature, similarly to the *rrp41/ski6-100* mutant. [No such fragments were observed at the permissive temperature (data not shown).] The poly(G)→3′ end fragment was also more stable in a *rrp4-1* strain (relative to a wild-type control) (data not shown). These observations argued that Rrp4p is also required for normal rates of 3′ to 5′ mRNA degradation. Additionally, these observations implied that the exosome may be the functional unit of nucleolytic activity for the 3′ to 5′ mRNA degradation process.

The ski2Δ, ski3Δ and ski8Δ lesions do not affect processing of the 5.8 S rRNA

An important goal is determining how the *SKI2*, *SKI3*, *SKI6/RRP41*, *SKI8* and *RRP4* gene products function to promote 3′ to 5′ mRNA degradation. The rRNA processing role of the exosome components Ski6/Rrp41p and Rrp4p raised the possibility that a defect in 5.8S processing might indirectly affect 3′ to 5′ mRNA degradation. If decreased 3′ to 5′ degradation of mRNA was an indirect consequence of a defect in rRNA processing, then all of these mutants would be expected to affect 5.8S RNA processing. In order to explore this possibility we examined the processing of the 5.8S RNA in the *ski2*Δ, *ski3*Δ, *ski6/rrp41-2*, *ski6/rrp41-100*, *ski8*Δ and *rrp4-1* strains on polyacrylamide Northern blots using a probe hybridizing to sequences just 3′ of the mature 5.8S RNA and therefore specific for the 3′-processed sequences (probe b, Mitchell *et al.*, 1996). Consistent with the work of Mitchell *et al.*, both *ski6/rrp41* and *rrp4* show a defect in 5.8S processing (Figure 6). However, the *ski2*Δ, *ski3*Δ and *ski8*Δ strains showed no such defect (Figure 6). This observation argued that the effect of the *ski2*Δ, *ski3*Δ and *ski8*Δ mutations was specific for 3′ to 5′ decay of mRNA and was not due to a defect in 5.8S rRNA processing. Given this result, it is likely that Ski2p, Ski3p, Ski6/Rrp41p, Ski8p and Rrp4p affect 3′ to 5′ degradation of mRNA in a more direct manner (see Discussion).

Cells lacking 5′ to 3′ and 3′ to 5′ mRNA decay pathways are inviable

Since both stable and unstable transcripts can be degraded by the 3′ to 5′ pathway, we hypothesized that this was a general mechanism of mRNA turnover able to work on many transcripts. This view explains the observation that mutations in the *SKI2* and *SKI3* genes are synthetically lethal with deletions of *XRN1* (Johnson and Kolodner, 1995), because of the disruption of both 5′ to 3′ and 3′ to 5′ mRNA decay. In this model, it is predicted that any mutation that inactivates the 5′ to 3′ pathway, such as the *dcp1*Δ or the *xrn1*Δ, would be synthetically lethal with any mutation that inactivates the 3′ to 5′ decay mechanism. We tested this prediction in a series of genetic crosses and found that the following double mutants were inviable: *dcp1*Δ and *ski2*Δ, *dcp1*Δ and *ski3*Δ, *dcp1*Δ and *ski8*Δ, *xrn1*Δ and *ski2*Δ, *xrn1*Δ and *ski3*Δ, and *xrn1*Δ and *ski8*Δ (see Materials and methods). Thus, any double mutant that contained a block to the 5′ decay pathway (*xrn1*Δ or *dcp1*Δ) with a block to the 3′ decay pathway (*ski2*Δ, *ski3*Δ or *ski8*Δ) was synthetically lethal.

In order to confirm that the failure to recover double mutants in these crosses was due to synthetic lethality, and not to failure of the double mutants to germinate, a *ski8*Δ strain was crossed to a *dcp1*Δ strain carrying a plasmid with the temperature-sensitive *dcp1-2* allele. When dissected spores were germinated at the restrictive

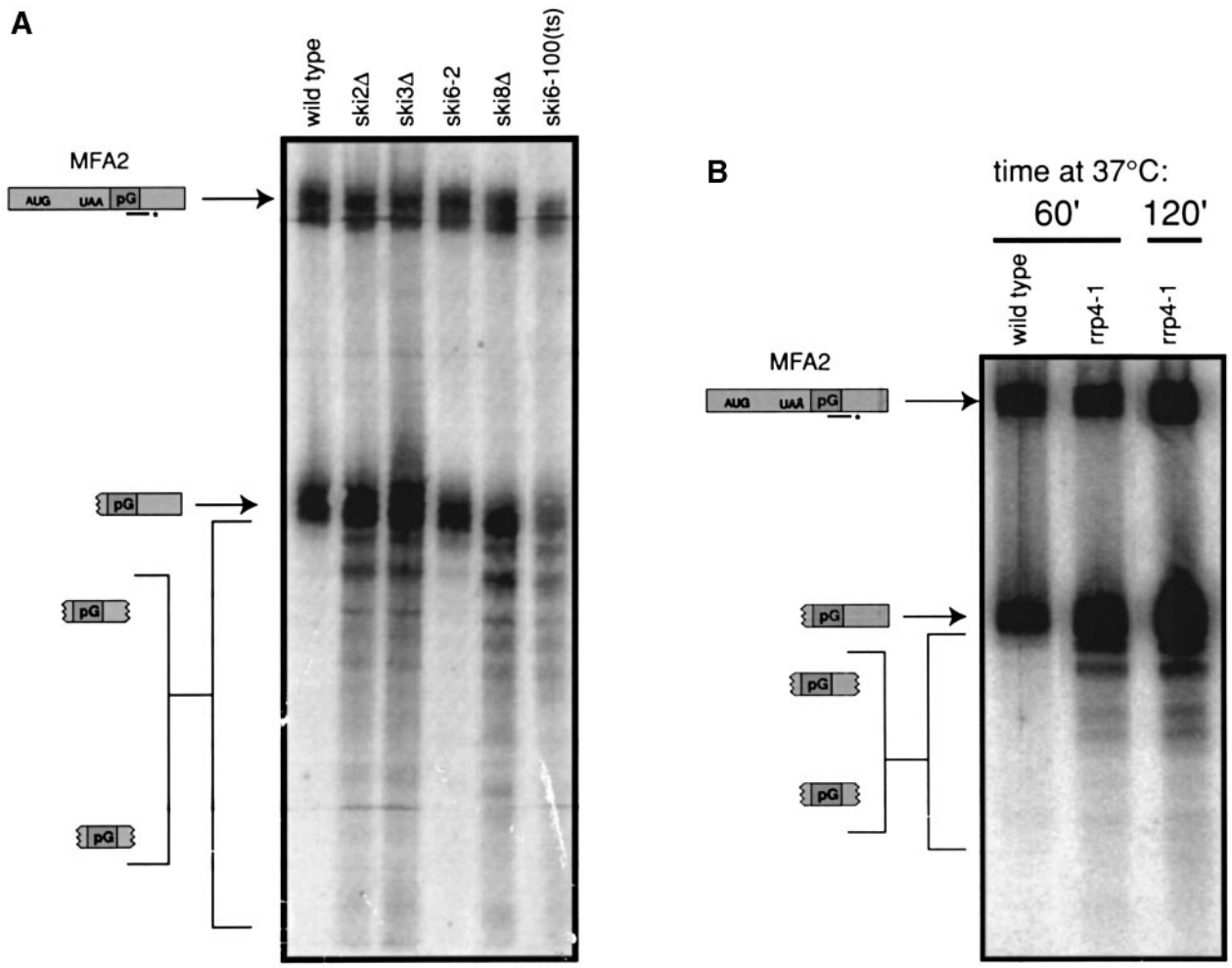

Fig. 5. Accumulation of 3′-trimmed RNA fragments in *ski2*, *ski3*, *ski6/rrp41*, *ski8* and *rrp4* mutants. Polyacrylamide Northerns of the *MFA2pG* transcripts using the oligonucleotide probe that hybridizes just 3′ of the poly(G) tract in the *MFA2pG* sequence (oRP140, Caponigro and Parker, 1995). All strains in (**A**) were grown and harvested at 30°C, with the exception of *rrp41/ski6-100*, which was grown at 30°C and shifted to 37°C for 2 h prior to harvest. All strains in (**B**) were grown at 24°C and shifted to 37°C for the time indicated above each lane prior to harvest. Shifts of 1 and 2 h of wild-type to 37°C were identical (data not shown). The identity of the various RNA species is indicated with cartoons on the left. The strains are: wild-type (yRP840), *ski2*Δ (yRP1195), *ski3*Δ (yRP1196), *ski6/rrp41-2* (yRP1203), *ski8*Δ (yRP1197), *ski6/rrp41-100* (yRP1204) and *rrp4-1* (yRP1223).

temperature, the double mutant was not recovered. However, when the spores were germinated at the permissive temperature, it was possible to recover the *dcp1-2 ski8*Δ double mutant, which subsequently was shown to be unable to grow at higher temperatures (Figure 7).

dcp1-2 ski8Δ double mutants have a severe defect in mRNA decay

The hypothesis that mutations that disrupt both decay mechanisms will be synthetically lethal due to a lack of mRNA decay strongly predicts that double mutants will be more defective for mRNA decay than either single mutant. In order to test this hypothesis, we examined the degradation of several mRNAs in wild-type, *ski8*Δ, *dcp1-2* and *dcp1-2 ski8*Δ strains. The strains were grown at 24°C, where the double mutant is viable, and mRNA decay was assayed at 37°C, where the temperature-sensitive *dcp1-2* allele behaves like a strong loss-of-function allele.

A key result was that the decay of the full-length *MFA2pG* mRNA was greatly slowed in the *dcp1-2 ski8*Δ double mutant (Figure 8). For example, in wild-type and *ski8*Δ strains, the *MFA2pG* mRNA had a half-life of 3 min, which was increased to 8 min in the *dcp1-2* strain. [This was a faster rate of decay than is seen for the *MFA2pG* mRNA in *dcp1*Δ mutants at 30°C and was presumably due to the higher temperature, where decay is generally faster (Herrick *et al.*, 1990).] In contrast, in the *dcp1-2 ski8*Δ double mutant, the *MFA2pG* half-life was at least 60 min and the mRNA became heterogeneous in size at late time points. Similar results were seen with the *PGK1pG*, *GAL10*, *GAL1* and *GAL7* mRNAs, which also showed extremely slow rates of mRNA degradation in the double mutant (Table II). The very slow mRNA decay rates seen in the double mutant argued that we have identified the two major pathways of mRNA decay in yeast, and suggested that efficient mRNA turnover by one of these mechanisms was required for viability (see Discussion). It should be noted that the heterogeneous size distribution of the mRNA at late time points suggested that there was still some residual mRNA degradation occurring in the double mutant, although at a greatly reduced rate and therefore not likely to be of substantial significance.

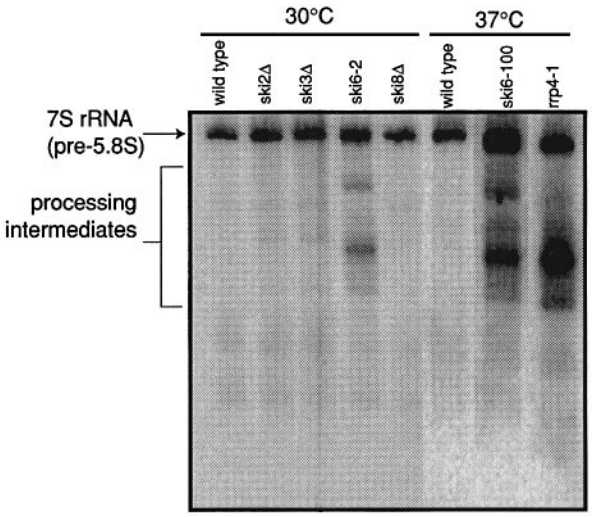

Fig. 6. Processing of the 5.8S rRNA in *ski2*, *ski3*, *ski6/rrp41*, *ski8* and *rrp4* mutants. Polyacrylamide Northern blots of total RNA from wild-type (yRP840), *ski2*Δ (yRP1195), *ski3*Δ (yRP1196), *ski6/rrp41-2* (yRP1203), *ski8*Δ (yRP1197), *ski6/rrp41-100* (yRP1204) and *rrp4-1* (yRP1223). Strains were assayed at the temperature indicated above each lane; strains assayed at 37°C were grown at 24°C and shifted to 37°C for 1 h prior to harvest. The blot was probed with an oligonucleotide specific to sequences 3′ of the 3′ end of the mature 5.8S rRNA (probe b, Mitchell *et al.* 1996).

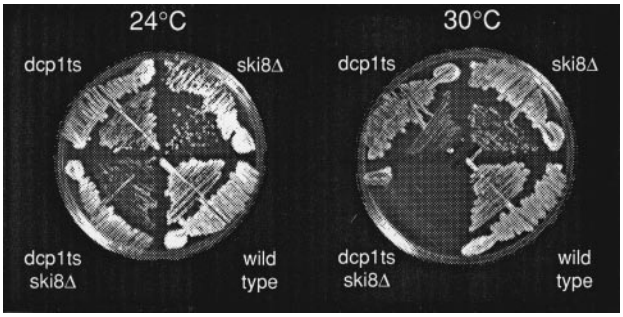

Fig. 7. Conditional synthetic lethality of the *dcp1-2* and *ski8*Δ alleles. YEPD plates grown at 24°C (permissive for *dcp1-2*) and 30°C (restrictive for *dcp1-2*) are shown. Strains are wild-type (yRP840), *ski8*Δ (yRP1197), *dcp1-2* (yRP1205) and *dcp1-2 ski8*Δ (yRP1202)

Discussion

3′ to 5′ degradation of the mRNA body is a general pathway of mRNA decay in yeast

Several observations now indicate that 3′ to 5′ nucleolytic degradation of the transcript body is a general mechanism of mRNA turnover in yeast, capable of acting on many mRNAs. This evidence includes the analysis of mRNA decay when decapping and 5′ to 3′ degradation are blocked, which indicated that both the stable *PGK1* mRNA (Muhlrad *et al.*, 1995) and the unstable *MFA2* mRNA were degraded 3′ to 5′ (Figure 1). Similarly, mRNA fragments, which were protected from 5′ to 3′ decay by strong RNA secondary structures, were also ultimately degraded 3′ to 5′ (Figures 2 and 3). In addition, the extreme stability of the *MFA2pG*, *PGK1pG*, *GAL1*, *GAL7* and *GAL10* mRNAs observed in strains defective for both 5′ to 3′ and 3′ to 5′ decay mechanisms indicated that these mRNAs were all substrates for 3′ to 5′ degradation (Table II). These observations suggest that there are two general mechanisms for degrading the mRNA body in yeast, decapping leading to 5′ to 3′ degradation, or 3′ to 5′ degradation. Moreover, since transcripts were extremely long-lived in the absence of these two mechanisms of degradation (Figure 8 and Table II), there are unlikely to be other major nucleolytic activities that can act to degrade mRNAs at a reasonable rate.

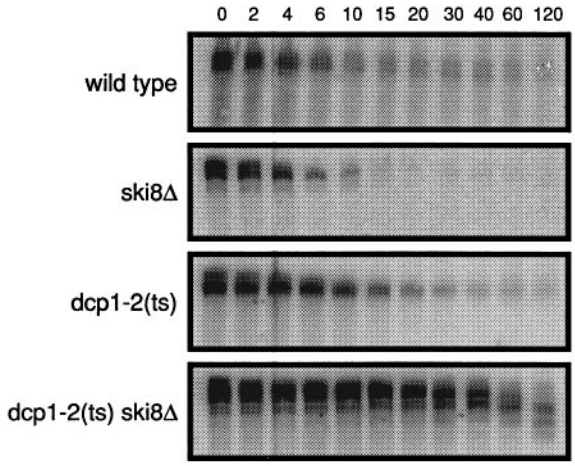

Fig. 8. The *MFA2pG* mRNA is extremely stable in *dcp1-2 ski8*Δ double mutants. Polyacrylamide Northerns analyzing the decay of the *MFA2pG* transcript following transcriptional repression for wild-type (yRP840), *ski8*Δ (yRP1197), *dcp1-2* (yRP1205) and *dcp1-2 ski8*Δ (yRP1202) are shown. Strains were grown in YEP medium with 2% galactose at 24°C. At mid-log phase, cultures were shifted to 37°C. After 1 h, transcription of the reporter mRNAs was repressed by the addition of dextrose to a final concentration of 4%. RNA was prepared from samples taken at various times after this repression. The numbers above each lane indicate minutes after repression of transcription. These blots were probed as in Figure 2A.

Table II. mRNA half-lives in strains mutant for mRNA decay

Strain	*MFA2pG*	*PGK1pG*	*GAL1*	*GAL7*	*GAL10*
Wild-type	3	15	6	2	6
*ski8*Δ	3	15	4	5	6.5
dcp1-2	8	20	8	7	9
*dcp1-2 ski8*Δ	60	110	50	50	65

The half-lives (in min) of various mRNAs in the wild-type (yRP840), *ski8*Δ (yRP1197), *dcp1-2* (yRP1205) and *dcp1-2 ski8*Δ (yRP1202) strains are given. Transcriptional repression experiments were perfomed as in Figure 8. Some half-lives are shorter than previously described due to being assayed at 37°C. mRNAs were detected on Northern blots by probing with oligonucleotides specific for each mRNA.

mRNA decay is essential

The generality of the 3′ to 5′ decay mechanism is strengthened by the synthetic lethal interactions between mutations that inactivate the 5′ to 3′ decay pathway and mutations that inactivate the 3′ to 5′ pathway (see Results). This observation argues that efficient mRNA degradation, by either one of these pathways, is essential for viability. Thus, mRNA turnover is a redundant and essential process in yeast. Moreover, several of the proteins required for these mRNA decay mechanisms, including the products of *XRN1*, *SKI2*, *SKI6/RRP41* and *RRP4*, have homologs in other eukaryotic cells, including mammals (Dangel *et al.*, 1995; Lee *et al.*, 1995; Bashkirov *et al.*, 1997; Mitchell *et al.*, 1997). The existence of these homologs

argues that these pathways of mRNA turnover occur in all eukaryotic cells and are likely to be the two general mechanisms of mRNA decay.

Relative roles of the 3′ to 5′ and 5′ to 3′ decay pathways

An interesting issue is the relationship between 5′ to 3′ degradation and 3′ to 5′ degradation of mRNA and their respective roles in eukaryotic cells. Currently, the available evidence suggests that the major mechanism of mRNA decay in *Saccharomyces cerevisae* is by decapping and 5′ to 3′ degradation (Decker and Parker, 1993; Hsu and Stevens, 1993; Muhlrad *et al.*, 1994, 1995; Beelman *et al.*, 1996). However, the 3′ to 5′ mechanism of degradation is likely to have unique functions. For example, it is likely that particular mRNAs are degraded preferentially by the 3′ to 5′ mechanism even in wild-type cells. This possibility is suggested by the observation that the *GAL7* mRNA shows a small but reproducible increase in half-life in *ski8* mutants (Table II). Similarly, since the pathways of mRNA degradation have only been examined under an extremely limited set of growth conditions, there may be specific conditions where the 3′ to 5′ mechanism is primary. In addition, the 3′ to 5′ mechanism of degradation may play an antiviral role by reducing expression from viral poly(A)$^-$ transcripts (Masison *et al.*, 1995). Finally, it should be considered that in other eukaryotes the relative importance of these two mechanisms may be different. For example, in oat seedlings, the phytochrome A mRNA appears to be degraded by both 5′ to 3′ and 3′ to 5′ decay mechanisms at similar rates (Higgs and Colbert, 1994).

The SKI2, SKI3, SKI6/RRP41, SKI8 and RRP4 gene products are required for 3′ to 5′ degradation of the transcript body

Two observations demonstrated that the *SKI2*, *SKI3*, *SKI6/RRP41*, *SKI8* and *RRP4* gene products were required for the 3′ to 5′ decay pathway. First, an mRNA fragment known to be degraded 3′ to 5′ was no longer degraded efficiently in these mutants, and additional fragments trimmed at the 3′ end accumulated (Figure 5). Second, the 3′ to 5′ decay of full-length mRNAs observed in the *dcp1-2* mutant is blocked in the *dcp1-2 ski8*Δ double mutant (Figure 8). An important question is how these proteins function to promote 3′ to 5′ mRNA degradation.

Several observations lead to a model wherein the exosome could be the complex performing the exonucleolytic degradation. First, mutations in both *SKI6/RRP41* and *RRP4* have similar phenotypes with regard to 3′ to 5′ mRNA degradation. The products of both of these genes have 3′ to 5′ exoribonuclease activity when recombinant protein is isolated from *E.coli* (Mitchell *et al.*, 1997). Additionally, in mammalian cells, a homologous complex has been localized to the cytoplasm as well as the nucleus (Mitchell *et al.*, 1997). However, we cannot rule out the formal possiblity that the exosome has an indirect effect on mRNA degradation.

What are the roles of Ski2p, Ski3p and Ski8p? Mutations in these genes have other phenotypes that are consistent with a defect in 3′ to 5′ degradation of poly(A)$^-$ mRNAs. For example, the overexpression of the mRNAs from the double-stranded RNA killer virus could be due to a stabilization of the poly(A)$^-$ viral mRNAs (Masison *et al.*, 1995). In addition, poly(A)$^-$ mRNAs introduced into yeast by electroporation showed a longer functional stability in *ski2*Δ and *ski8*Δ strains as compared with wild-type (Masison *et al.*, 1995). Interestingly, the *ski2*Δ and *ski8*Δ strains also showed increased initial rates of protein production from electroporated poly(A)$^-$ transcripts (Masison *et al.*, 1995). This observation was interpreted to indicate that these proteins function to repress translation of poly(A)$^-$ mRNAs due to an alteration in the biogenesis of the 60S ribosomal subunit and that the longer functional mRNA stability was a consequence of differences in translation rates (Masison *et al.*, 1995). However, several observations now suggest that Ski2p, Ski3p and Ski8p affect 3′ to 5′ mRNA degradation more directly. First, polysome profiles in *ski2*Δ, *ski3*Δ and *ski8*Δ mutants are identical to wild-type (Masison *et al.*, 1995), and our examination of 5.8S processing indicated that at least this aspect of rRNA processing was normal in these mutants (Figure 6). Second, since the *ski2*, *ski3*, *ski6/rrp41*, *ski8* and *rrp4* mutants affected the 3′ to 5′ degradation of the poly(G)→3′ end fragments (Figure 5 and Table I), which are mRNA 3′ UTR fragments that are not being translated, it is unlikely that an increase in translation rate in the mutant strains could be protecting the RNA indirectly from 3′ to 5′ degradation. Given this, there are two possible explanations for the results with electroporated mRNAs. First, if there is a competition between 3′ to 5′ degradation and translation initiation for electroporated mRNAs, when the RNAs are first introduced into cells, more transcripts would be getting translated, but at the same initiation rate, in the mutant strains. Alternatively, Ski2p, Ski3p and Ski8p might function in remodeling mRNP structure, perhaps by promoting the disassociation of proteins from the 3′ UTR, which might decrease the translation rate and also make the 3′ end more accessible to the exosome.

A simpler model is that Ski2p, Ski3p and Ski8p function to adapt, or recruit, the exosome to mRNA substrates. This is a particularly appealing model for Ski2p, which is a member of the DEVH box family of proteins and thus a putative RNA helicase, because some 3′ to 5′ exonuclease complexes have been shown to have associated RNA helicases of this type (Margossian *et al.*, 1996; Py *et al.*, 1996). In this view, other proteins would serve as ‘adaptors’ for other exosome substrates, such as the 5.8S pre-rRNA. This hypothesis would explain why the *ski2*Δ, *ski3*Δ and *ski8*Δ mutations do not affect 5.8S processing (Figure 6). Strikingly, mutations in another DEVH protein closely related to Ski2p, Dob1p, show a defect in processing of the 5.8S pre-rRNA (de la Cruz *et al.*, 1998). This observation suggests that the Dob1p might serve as the exosome ‘adaptor’ for 5.8S pre-rRNA. This view makes the testable predictions that Ski2p, and perhaps Ski3p and Ski8p, will show interactions with the exosome and will directly affect its ability to degrade mRNA substrates.

Materials and methods

Plasmids and strains

The genotypes of the strains used in this study can be found in Table III. All strains with yRP numbers are isogenic, with the exception of yRP1223, which is a spore from a cross between yRP841 and P54 (Mitchell *et al.*, 1996).

Table III. Strains used in this study

Strain	Genotype	Source
yRP684	MATα *his4-539 leu2-3,112 lys2-201 trp1-Δ1 ura3-52*	Hatfield *et al*., 1996
yRP685	MATa *his4-539 leu2-3,112 lys2-201 trp1-Δ1 ura3-52*	Hatfield *et al*., 1996
yRP840	MATa *his4-539 leu2-3,112 trp1-Δ1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG*	Hatfield *et al*., 1996
yRP841	MATα *leu2-3,112 lys2-201 trp1-Δ1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG*	Hatfield *et al*., 1996
yRP1192	MATα *his4-539 leu2-3,112 lys2-201 trp1-Δ1 ura3-52 ski2::LEU2*	this study
yRP1193	MATα *his4-539 leu2-3,112 lys2-201 trp1-Δ1 ura3-52 ski3::TRP1*	this study
yRP1194	MATα *his4-539 leu2-3,112 lys2-201 trp1-Δ1 ura3-52 ski8::URA3*	this study
yRP1195	MATa *his4-539 leu2-3,112 trp1-Δ1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG ski2::LEU2*	this study
yRP1196	MATa *his4-539 leu2-3,112 trp1-Δ1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG ski3::TRP1*	this study
yRP1197	MATa *his4-539 leu2-3,112 lys2-201 trp1-Δ1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG ski8::URA3*	this study
yRP1198	MATa *his4-539 leu2-3,112 trp1-Δ1 ura3-52 rpb1-1 ski2::LEU2*	this study
yRP1199	MATα *his4-539 leu2-3,112 trp1-Δ1 ura3-52 xrn1::URA3*	this study
yRP1200	MATa *his4-539 leu2-3,112 trp1-Δ1 ura3-52 dcp1::URA3*	this study
yRP1201	MATa *his4-539 leu2-3,112 lys2-201 trp1-Δ1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG dcp1::URA3*	this study
yRP1202	MATa *his4-539 leu2-3,112 lys2-201 trp1-Δ1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG ski8::URA3 dcp1::URA3* [*dcp1-2/TRP1*]	this study
yRP1203	MATα *his4-539 leu2-3,112 lys2-201 trp1-Δ1 ura3-52 ski6::URA3* [*ski6-2/LYS2*]	this study
yRP1204	MATα *his4-539 leu2-3,112 lys2-201 trp1-Δ1 ura3-52 ski6::URA3* [*ski6-100/LYS2*]	this study
yRP1205	MATa *leu2-3,112 lys2-201 trp1-Δ1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG dcp1::URA3* [*dcp1-2/TRP1*]	this study
yRP1223	MATα *ade1-100 leu2-3,112 trp1-Δ1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG rrp4-1*	this study
P54	MATa *ade1-100 his4-519 leu2-3,112 ura3-52*	Mitchell *et al*., 1996
2898:4156-4a	MATa *ade3 his(5,6) ura3 ski6-2 K1+*	Ridley *et al*., 1984

All strains with yRP numbers are isogenic, with the exception of yRP1223 (see Materials and methods). The construction of the *cup1::LEU2/PGK1pG/MFA2pG* disruption was described previously (Hatfield *et al*., 1996).

The *ski6/rrp41::URA3* disruption strain was constructed by PCR amplification of the regions flanking the *SKI6/RRP41* gene from genomic DNA. The PCR products were subcloned (using sites introduced in the PCR primers) on either side of the *URA3* gene in a pBluescript vector, and the resulting *ski6/rrp41::URA3* construct was gel purified and used to transform a wild-type diploid (yRP840 crossed with yRP841). Heterozygous transformants were identified using genomic Southern blots, and the lethality of the *ski6/rrp41::URA3* disruption was determined by dissection.

To isolate conditional *ski6/rrp41* alleles, the coding region of the *SKI6/RRP41* gene was PCR amplified and subcloned downstream of the GAL1 UAS (using sites introduced in the PCR primers), on a *TRP1* cloning vector (Elledge and Davis, 1988). This GAL–SKI6/RRP41(TRP1) construct was transformed into the *ski6/rrp41::URA3/SKI6/RRP41* heterozygote, and a *ski6/rrp41::URA3* [GAL–SKI6/RRP41(TRP1)] strain was recovered by dissection. The *ski6/rrp41* conditional allele was generated by PCR mutagenesis of the wild-type *SKI6/RRP41* open reading frame (Muhlrad *et al.*, 1992). The mutagenized PCR product was co-transformed with a gapped *LYS2* cloning vector (Sikorski and Boeke, 1991) into the *ski6/rrp41::URA3* [GAL–SKI6/RRP41(TRP1)] strain, and transformants were selected on Lys^- plates at 24°C. The transformants were then screened at 37°C in order to identify transformants carrying temperature-sensitive alleles and lacking the GAL–SKI6/RRP41 plasmid. Recovering the SKI6/RRP41 plasmid in *E.coli* and re-transformation into the *ski6/rrp41::URA3* [GAL–SKI6/RRP41] strain demonstrated that the temperature-sensitivity was due to a mutated *SKI6/RRP41* gene on the plasmid. Subsequent experiments demonstrated that this allele gave a wild-type phenotype at the permissive temperature (data not shown). The *ski6-2* allele was isolated by PCR amplification of genomic DNA from the original isolate (Ridley *et al.*, 1984). The PCR product was then co-transformed into yeast with a gapped *LYS2* vector, as described above.

RNA procedures

RNA preparation, blotting and quantitation. RNA samples were prepared and isolated as previously described (Caponigro *et al.*, 1993). Half-lives were determined by quantitation of blots using a Molecular Dynamics phosphorimager. Loading corrections for quantitation were determined by stripping blots and re-hybridizing with an oligonucleotide probe to the 7S RNA, an RNA polymerase III transcript that is part of the signal recognition particle (Caponigro *et al.*, 1993).

Determining precursor–product relationships. Steady-state transcriptional induction experiments were performed by growing strains in YEP with 2% raffinose. Transcription was induced by addition of galactose to a final concentration of 2%, and samples taken at various times after the addition of galactose allowed a determination of precursor–product relationships among the mRNA decay products.

Measurement of poly(G)→3′ end fragment decay rates. These experiments were performed by blocking transcription by addition of dextrose to a final concentration of 4%, and inhibiting decapping by addition of cycloheximide to a final concentration of 0.1 mg/ml (Beelman and Parker, 1994). Analysis of RNA prepared from samples taken after the addition of dextrose and cycloheximide allowed a determination of the decay kinetics of the poly(G)→3′ end fragments.

Synthetic lethal crosses

ski2 xrn1 and ski3 xrn1 synthetic lethality. In order to confirm that the synthetic lethality of *ski2* and *xrn1* (Johnson and Kolodner, 1995) could be observed in our strains, yRP1198 (*ski2*Δ) was crossed to yRP1199 (*xrn1*Δ). Dissection of the diploid gave 66.25% viability, and no *ski2::LEU2 xrn1::URA3* segregants in >20 tetrads. To confirm the synthetic lethality of *ski3* and *xrn1*, *xrn1* was disrupted by *URA3* insertion in the diploid generated by the cross of yRP1193 (*ski3*Δ*::TRP1*) and yRP840. The resulting double heterozygote was dissected, which gave a viability of 61.4% and no *ski3::TRP1 xrn1::URA3* segregants in >10 tetrads. In both crosses, control dissections had viabilities of >95%.

ski8 xrn1 synthetic lethality. In order to determine if *ski8* and *xrn1* were synthetically lethal, yRP1199 (*xrn1*Δ*::URA3*) was crossed to yRP1197 (*ski8*Δ*::URA3*), and the resulting diploid was dissected. Since both disruptions were marked with the *URA3* gene, it was not possible to determine directly if double mutants were recovered. However, viability was 81.25% (control >90%), no 2:2 Ura^+:Ura^- tetrads were recovered, and all 4:0 live:dead tetrads were also 4:0 Ura^+:Ura^-. We concluded that these results were consistent with the synthetic lethality of *ski8::URA3 xrn1::URA3* haploids.

dcp1 ski2 synthetic lethality. In order to determine if *ski2* and *dcp1* were synthetically lethal, yRP1192 (*ski2*Δ*::LEU2*) was crossed to yRP1200 (*dcp1*Δ*::URA3*) and the resulting diploid was sporulated and dissected. Viability was 72.2% (control 97.6%), and no *ski2::LEU2 dcp1::URA3* haploids were recovered in >45 tetrads. We conclude that *ski2* is synthetically lethal with *dcp1*.

dcp1 ski3 synthetic lethality. To determine if *dcp1* and *ski3* were synthetically lethal, *dcp1* was disrupted with the *URA3* gene in the diploid generated by crossing yRP1193 (*ski3*Δ*::TRP1*) and yRP840. The resulting double heterozygote was sporulated and dissected with a viability of 72.1% (control 100%). The failure to recover *dcp1::URA3*

ski3::TRP1 spores in >120 tetrads led to the conclusion that *dcp1* is synthetically lethal with *ski3*

dcp1 ski8 synthetic lethality. In order to determine if *ski8* and *dcp1* were synthetically lethal, yRP1201 (*dcp1*Δ) was crossed to yRP1194 (*ski8*Δ), and the resulting diploid was dissected. Since both disruptions were marked with the *URA3* gene, it was not possible to determine directly if double mutants were recovered. However, viability was 64.5% (control >90%), no 2:2 Ura^+:Ura^- tetrads were recovered, and all 4:0 live:dead tetrads were also 4:0 Ura^+:Ura^-. Additionally, we determined that *ski8::URA3 dcp1::URA3* strains could be recovered, but only in the presence of a plasmid carrying a copy of *DCP1*. When the *dcp1* allele on the plasmid was temperature sensitive (*dcp1-2*), the double mutant strain was unable to grow at temperatures restrictive for Dcp1p activity (see Figure 7). Based on these observations, we concluded that *dcp1*Δ was synthetically lethal with *ski8*Δ.

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