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 Raue, 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 heteropentapeptide complex, termed the exosome, has recently been described (Mitchell et al., 1997). Three of the proteins from this complex (Rrp4p, Ski6p/Rrp41p and Rrp43p) have been shown to have 3’ to 5’ exonucleolytic activity in vitro, and the remaining two (Rrp42p and Rrp43p) have sequence similarity to bacterial 3’ to 5’ exonuclease (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/Rrp4p 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 (Muhrad and Parker, 1994; Muhrad 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 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; Muhrad et al., 1994, 1995)
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 MF A2pG 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 MF A2pG 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...
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 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 XRNI 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 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 MF2pG 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 3’ to 5’ decay of the poly(G)→3’ end fragment as a percentage of total mRNA (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 experiments.

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<th>Fragment half-life (min)</th>
<th>Percentage of fragment</th>
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<td>PGK1pG</td>
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<td>&gt;60</td>
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<td>19</td>
<td>&gt;60</td>
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The table gives the phenotypes of wild-type (yRP840), ski2Δ (yRP1195), ski3Δ (yRP1196), rrp41/ski6-100 (yRP1204), rrp41/ski6-2 (yRP1203) and ski8Δ (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, 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 experiments.

ND = not determined.
glucose and cycloheximide. The full-length and poly(G) fragments after a shift to the restrictive temperature, (Figure 5B, the MF A2pG activity for the 3′→5′ mRNP degradation. In order to explore this possibility we examined the processing of the 5.8S RNA in the ski2Δ, ski3Δ, ski6/rrp41, ski8 and rrp4 gene products function to promote 3′→5′ mRNA degradation. The rRNA processing role of the exosome components Sk16/Rpp41p and Rrp4p raised the possibility that a defect in 5.8S processing might indirectly affect 3′→5′ mRNA degradation. If decreased 3′→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 addition to exploring this possibility we examined the processing of the 5.8S RNA in the ski2Δ, ski3Δ, ski6/rrp41-2, ski6/rrp41-100, ski8A 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 ski2Δ, ski3Δ and ski8A strains showed no such defect (Figure 6). This observation argued that the effect of the ski2Δ, ski3Δ and ski8A mutations was specific for 3′→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′→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 stable and unstable transcripts can be degraded by the 3′→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′→3′ and 3′→5′ mRNA decay. In this model, it is predicted that any mutation that inactivates the 5′→3′ pathway, such as the dcp1Δ or the xrn1Δ, would be synthetically lethal with any mutation that inactivates the 3′→5′ decay mechanism. We tested this prediction in a series of genetic crosses and found that the following double mutants were inviable: dcp1Δ and ski2Δ, dcp1Δ and ski3Δ, dcp1Δ and ski8A, xrn1Δ and ski2Δ, xrn1Δ and ski3Δ, and xrn1Δ and ski8Δ (see Materials and methods). Thus, any double mutant that contained a block to the 5′ decay pathway (xrn1Δ or dcp1Δ) with a block to the 3′ decay pathway (ski2Δ, ski3Δ or ski8Δ) 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 ski8Δ strain was crossed to a dcp1Δ strain carrying a plasmid with the temperature-sensitive dcp1-2 allele. When dissected spores were germinated at the restrictive

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′→5′ mRNA degradation. The rRNA processing role of the exosome components Sk16/Rpp41p and Rrp4p was specific for the 3′→5′ 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 ski2Δ, ski3Δ and ski8Δ strains showed no such defect (Figure 6). This observation argued that the effect of the ski2Δ, ski3Δ and ski8Δ mutations was specific for 3′→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′→5′ degradation of mRNA in a more direct manner (see Discussion).

RRP4 mutations also disrupt 3′ to 5′ mRNA degradation

Sk16p/Rtp41p is a component of a multiprotein complex called the exosome, which is required for correct 3′→5′ processing of the 5.8S rRNA (see Introduction; Mitchell et al., 1997). Since ski6/rrp41 mutants were found to be defective in 3′→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′→5′ mRNA degradation. Additionally, these observations implied that the exosome may be the functional unit of nucleolytic activity for the 3′→5′ mRNA degradation process.

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) ski2Δ (yRP1195) strains in which dextrose (to inhibit transcription) and cycloheximide (to inhibit decapping) were added at zero time. Similar results were obtained for ski3Δ, ski8Δ 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) fragment species are indicated with cartoons on the left. The probe for this experiment was oRP141 (see Figure 2 legend).
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 ski8Δ 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, ski8Δ, dcp1-2 and dcp1-2 ski8Δ 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 ski8Δ double mutant (Figure 8). For example, in wild-type and ski8Δ 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 dcp1Δ 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 ski8Δ 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.
5.8S rRNA (probe b, Mitchell ski8 dcp1-2 YEPD plates grown at 24°C (permissive for Several observations now indicate that 3

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 degrading both wild-type (yRP840), ski8Δ (yRP1197), dcp1-2 (yRP1205) and dcp1-2 ski8Δ (yRP1202) stains are 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.

![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), ski2Δ (yRP1195), ski3Δ (yRP1196), ski6/rrp41-2 (yRP1203), ski8Δ (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).](Image 62x333 to 293x448)

![Fig. 7. Conditional synthetic lethality of the dcp1-2 and ski8Δ 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), ski8Δ (yRP1197), dcp1-2 (yRP1205) and dcp1-2 ski8Δ (yRP1202)](Image 327x569 to 541x741)

![Table II. mRNA half-lives in strains mutant for mRNA decay](Image 106x554)

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The half-lives (in min) of various mRNAs in the wild-type (yRP840), ski8Δ (yRP1197), dcp1-2 (yRP1205) and dcp1-2 ski8Δ (yRP1202) are shown. Transcriptional repression experiments were performed 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.

isms 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.

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 cerevisiae* 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 ski8Δ* 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 exonuclease-degrading activity. 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' exonuclease 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 possibility 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 *ski2Δ* and *ski8Δ* strains as compared with wild-type (Masison et al., 1995). Interestingly, the *ski2Δ* and *ski8Δ* 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 *ski2Δ, ski3Δ* and *ski8Δ* 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 mRNPs, 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, ski3Δ* and *ski8Δ* 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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>yRP684</td>
<td>MATa his4-539 leu2-3,112 lys2-201 trpl-Δ1 ura3-52</td>
<td>Hatfield et al., 1996</td>
</tr>
<tr>
<td>yRP685</td>
<td>MATa his4-539 leu2-3,112 lys2-201 trpl-Δ1 ura3-52</td>
<td>Hatfield et al., 1996</td>
</tr>
<tr>
<td>yRP840</td>
<td>MATa his4-539 leu2-3,112 trpl-Δ1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG</td>
<td>Hatfield et al., 1996</td>
</tr>
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<td>yRP1192</td>
<td>MATa his4-539 leu2-3,112 lys2-201 trpl-Δ1 ura3-52 skil:::URA2</td>
<td>this study</td>
</tr>
<tr>
<td>yRP1193</td>
<td>MATa his4-539 leu2-3,112 lys2-201 trpl-Δ1 ura3-52 skil:::TRP1</td>
<td>this study</td>
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<tr>
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<td>yRP1196</td>
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<td>this study</td>
</tr>
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<td>yRP1198</td>
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<td>this study</td>
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<tr>
<td>yRP1200</td>
<td>MATa his4-539 leu2-3,112 trpl-Δ1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG skik:::URA3</td>
<td>this study</td>
</tr>
<tr>
<td>yRP1201</td>
<td>MATa his4-539 leu2-3,112 trpl-Δ1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG dcp1-2/URA3</td>
<td>this study</td>
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<tr>
<td>yRP1202</td>
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<td>this study</td>
</tr>
<tr>
<td>yRP1203</td>
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<td>this study</td>
</tr>
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<td>yRP1204</td>
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<td>this study</td>
</tr>
<tr>
<td>yRP1205</td>
<td>MATa leu2-3,112 lys2-201 trpl-Δ1 ura3-52 cup1::LEU2/PGK1pG/MFA2pG dcp1-2/URA3 [dcp1-2/TRA1]</td>
<td>this study</td>
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<td>yRP1223</td>
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<td>this study</td>
</tr>
<tr>
<td>P54</td>
<td>MATa ade1-100 his4-519 leu2-3,112 ura3-52</td>
<td>Mitchell et al., 1996</td>
</tr>
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<td>MATa ade3 his(5,6) ura3 skil-2 K1+</td>
<td>Ridley et al., 1984</td>
</tr>
</tbody>
</table>

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 sk confirming the synthetic lethality of sk2 and xrn1 (Johnson and Kolodner, 1995) could be observed in our strains, yRP1198 (sk2Δ) was crossed to yRP1199 (xrn1Δ). Dissection of the diploid gave 66.25% viability, and no sk2::LEU2 xrn1::URA3 segregants in >20 tetrads. To confirm the synthetic lethality of sk2 and xrn1, xrn1 was disrupted by insertion of the diploid generated by the cross of yRP1193 (sk2Δ::URA3) and yRP840. The resulting double heterozygote was dissected, which gave a viability of 61.4% and no sk2::TRP1 xrn1::URA3 segregants in >10 tetrads. In both crosses, control dissections had viabilities of >95%.

### Synthetic lethal crosses

**sk2 xrn1 and sk3 xrn1 synthetic lethality.** In order to confirm that the synthetic lethality of sk2 and xrn1 was observed, the results were consistent with the synthetic lethality of sk2::URA3 xrn1::URA3 haploids.

**dcpl sk2 synthetic lethality.** In order to determine if sk2 and dcpl were synthetically lethal, yRP1199 (xrn1Δ::URA3) was crossed to yRP1197 (sk2Δ::URA3), and the resulting diploid was sporulated and dissected. Viability was 81.25% (control >90%), and all 2 Ura+ tetrads were recovered, and all 4 Ura- tetrads were also 4 Ura-. We concluded that these results were consistent with the synthetic lethality of sk2::URA3 xrn1::URA3 haploids.

### 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 quantification 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**

**sk2 xrn1 and sk3 xrn1 synthetic lethality.** In order to confirm that the synthetic lethality of sk2 and xrn1 (Johnson and Kolodner, 1995) could be observed in our strains, yRP1198 (sk2Δ) was crossed to yRP1199 (xrn1Δ). Dissection of the diploid gave 66.25% viability, and no sk2::LEU2 xrn1::URA3 segregants in >20 tetrads. To confirm the synthetic lethality of sk2 and xrn1, xrn1 was disrupted by insertion of the diploid generated by the cross of yRP1193 (sk2Δ::URA3) and yRP840. The resulting double heterozygote was dissected, which gave a viability of 61.4% and no sk2::TRP1 xrn1::URA3 segregants in >10 tetrads. In both crosses, control dissections had viabilities of >95%.

**sk2 xrn1 synthetic lethality.** In order to determine if sk2 and xrn1 were synthetically lethal, yRP1199 (xrn1Δ::URA3) was crossed to yRP1197 (sk2Δ::URA3), and the resulting diploid was sporulated and 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%), and all 2 Ura+ tetrads were recovered, and all 4 Ura- tetrads were also 4 Ura-. We concluded that these results were consistent with the synthetic lethality of sk2::URA3 xrn1::URA3 haploids.

**dcpl sk2 synthetic lethality.** In order to determine if sk2 and dcpl were synthetically lethal, yRP1192 (sk2Δ::LEU2) was crossed to yRP1200 (dcpl::URA3) and the resulting diploid was sporulated and dissected. Viability was 72.2% (control 97.6%), and no sk2::LEU2 dcpl::URA3 haploids were recovered in >45 tetrads. We conclude that sk2 is synthetically lethal with dcpl.

**dcpl sk3 synthetic lethality.** To determine if dcpl and sk3 were synthetically lethal, dcpl was disrupted with the URA3 gene in the diploid generated by crossing yRP1193 (sk2Δ::TRA1) and yRP840. The resulting double heterozygote was sporulated and dissected with a viability of 72.1% (control 100%). The failure to recover dcpl::URA3 haploids.
skin1/3:TRP1 spores in >120 tetrads led to the conclusion that dcp1 is synthetically lethal with skin3
dcp1 skin8 synthetic lethality. In order to determine if skin8 and dcp1 were synthetically lethal, yRP1201 (dcp1Δ) was crossed to yRP1194 (skin8Δ), and the resulting diploid was dissected. Since both disruptions were marked with the UR43 gene, it was not possible to determine directly if double mutants were recovered. However, viability was 64.5% (control >90%), no 2.2 Ura::yRP1201 tetrads were recovered, and all 4.0 live dead tetrads were also 4.0 Ura::yRP1201. Additionally, we determined that skin8::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 dcp1Δ was synthetically lethal with skin8Δ.

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