Ski6p Is a Homolog of RNA-Processing Enzymes That Affects Translation of Non-Poly(A) mRNAs and 60S Ribosomal Subunit Biogenesis

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We mapped and cloned SKI6 of Saccharomyces cerevisiae, a gene that represses the copy number of the L-A double-stranded RNA virus, RNase PH. The ski6-2 mutant expressed electroporated non-poly(A) luciferase mRNAs 8- to 10-fold better than did the isogenic wild type. No effect of ski6-2 on expression of uncapped or normal mRNAs was found. Kinetics of luciferase synthesis and direct measurement of radiolabeled electroporated mRNA indicate that the primary effect of Ski6p was on efficiency of translation rather than on mRNA stability. Both ski6 and ski2 mutants show hypersensitivity to hygromycin, suggesting functional alteration of the translation apparatus. The ski6-2 mutant has normal amounts of 40S and 60S ribosomal subunits but accumulates a 38S particle containing 5'-truncated 25S RNA, apparently an incomplete or degraded 60S subunit. This suggests an abnormality in 60S subunit assembly. The ski6-2 mutation suppresses the poor expression of the poly(A)− viral mRNA in a strain deficient in the 60S ribosomal protein L4. Thus, a ski6 mutation bypasses the requirement of the poly(A) tail for translation, allowing better translation of non-poly(A) mRNA, including the L-A virus mRNA which lacks poly(A). We speculate that the derepressed translation of non-poly(A) mRNAs is due to abnormal (but full-size) 60S subunits.

The 5′ end of eucaryotic mRNA has a cap structure of the form m7G5′ppp5′Xp, while the 3′ end is polyadenylated. Both of these structures are essential for efficient translation and mRNA stability. The requirement for cap and poly(A) structure for messenger expression constitutes a handicap for RNA viruses whose mRNA is not made by the cellular machinery. L-A virus mRNA lacks both 5′ cap and 3′ poly(A) structures (4, 52), and its cytoplasmic location makes it unlikely that it can use cellular enzymes to modify its mRNA. Indeed, translation plays a large role in the interactions of the L-A double-stranded RNA (dsRNA) virus with its host, Saccharomyces cerevisiae (reviewed in references 11, 22, 32, and 58). L-A has two overlapping open reading frames, gag, encoding the major coat protein, and pol, encoding the RNA-dependent RNA polymerase and packaging function. L-A uses a −1 ribosomal frameshift to make a Gag-Pol fusion protein, and the efficiency of this frameshift is critical for viral propagation (12–14).

Many viruses adopt a variety of tricks to acquire a cap or poly(A) structure. Influenza viruses and Bunyaviruses steal caps from cellular mRNAs; reoviruses, rhabdoviruses, and togaviruses encode their own capping enzymes; and Picornaviridae carry out cap-independent translation (15, 32). Picornaviruses, togaviruses, influenza viruses, and many other RNA viruses have an encoded sequence that is copied to produce mRNA with a 3′ poly(A) structure, while rhabdoviruses have no encoded poly(A) but add it enzymatically to their transcripts. However, reoviruses and many plant virus mRNAs lack a 3′ poly(A).

The apparent lack of 5′ cap and 3′ poly(A) does not prevent L-A from being highly expressed. However, this lack of both structures makes L-A mRNA expression sensitive to chromosomal mutations affecting functions related to cap or poly(A). Accordingly, studies of L-A have secondarily brought insights into the roles of cap and poly(A).

For instance, the SKI genes (named SKI for superkiller) were identified by the superkiller phenotype of mutants (45, 53). The L-A particles can separately encapsidate a satellite dsRNA, called M dsRNA, encoding a secreted protein toxin (the killer toxin). ski mutants have higher copy numbers of M1 and L-A dsRNAs and so make more killer toxin (1). SKI2 later proved to be XRNI, encoding the 5′→3′ exoribonuclease specific for uncapped RNA and responsible for the major pathway of mRNA decay (21, 23, 37, 50, 51). It is evident how the uncapped L-A mRNA would be affected by this protein. To subvert the Ski1p/Xrn1p nuclease, the L-A major coat protein has a decapping activity which decapitates some cellular mRNAs to partially distract the nuclease from working on the capless L-A mRNA (2, 3, 31).

While SKI1 concerns RNA stability, SKI2, SKI3, and SKI8 encode a system that blocks the translation of non-poly(A) [poly(A)−] mRNA (31). ski2, ski3, or ski6 mutants translated electroporated C+ A− [Cap+ poly(A)−] mRNA nearly as well as C− A− mRNA. Both physical and functional stabilities of the electroporated mRNA showed little effect from these mutations. Ski3p is a 163-kDa nuclear protein (44), while Ski2p is an RNA helicase with a glycine-arginine-rich domain typical of nucleolar proteins and is homologous to a human nucleolar protein (7, 28, 61). Ski8p has two copies of the β-transducin (WD) repeat sequence but otherwise has no close homologs (33). This suggested to us that the effects of ski2 and ski3 (and

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ski6) on expression of poly(A)− mRNAs might best be explained by a function taking place in the nucleus. Strains with mutations in any of more than 20 genes resulting in deficiency of 60S ribosomal subunits are defective in viral propagation (5, 41). Mutations in ski2, ski3, or ski8 suppress the effect of 60S subunit deficiency on viral propagation (31, 54). We adopted a model in which 60S interaction with poly(A) is a prerequisite for joining 40S subunits waiting at the initiator AUG (reviewed in reference 22), and these SKI products mediate this effect by a role in 60S subunit biogenesis (31, 41). This model explains why a deficiency of 60S subunits impairs viral propagation more than cell growth, why ski mutants show increased virus copy number and expression, and why ski mutations suppress mutations producing deficiency of 60S subunit.

**Materials and Methods**

Genetic mapping of SKI6. M2 dsRNA is a killer toxin-encoding satellite of the L-A virus, meaning that its coat protein and replication proteins are encoded by L-A. At temperatures of 32°C or higher, M2 propagation depends on two genes, Afl ski6-2 (Fig. 1). The resulting pSK106 was digested with replacing a 369-bp segment of pSK1 ([poly(A)−] and T7 LUC [poly(A)− tail) have been described previously (19). For RNA synthesis, T7 LUC was linearized with DpnI. Transcripts were synthesized with the Ambion MEGAscript transcription kit in the presence or absence of the cap analog m7GppG in accordance with the manufacturer’s instructions. After DNase I treatment followed by precipitation with LiCl, RNAs were passed over G-50 columns (5 Prime-3 Prime Inc. SELECT-B). RNA was quantitated both by measuring the optical density at 260 nm (OD260) and by a comparison on agarose gels with known concentration standards.

For electroporation was done as described previously (17) with minor modifications. Cells for spheroplast preparations were grown in selective medium (H-Ura). After lyticase treatment, cells were incubated for 2 h in YP-adenosine medium to make them metabolically active. Two micrograms of RNA was used for electroporation, and cells were assayed for luciferase activity after 2 h for electroporation, and cells were assayed for luciferase activity after 2 h or 4 h.

**Expression of luciferase mRNAs.** The luciferase mRNA expression plasmids T7 LUC ([poly(A)−] and T7 LUC50 [50-mer poly(A)− tail) have been described previously (19). For RNA synthesis, T7 LUC was linearized with DpnI. Transcripts were synthesized with the Ambion MEGAscript transcription kit in the presence or absence of the cap analog m7GppG in accordance with the manufacturer’s instructions. After DNase I treatment followed by precipitation with LiCl, RNAs were passed over G-50 columns (5 Prime-3 Prime Inc. SELECT-B). RNA was quantitated both by measuring the optical density at 260 nm (OD260) and by a comparison on agarose gels with known concentration standards.

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isogenic ski2 disruptant (ski2::HIS3) were tested in the same manner onYPAD plates with or without drug.

RESULTS

SKI6 is an essential gene, and Ski6p is homologous to tRNA-processing enzymes and to proteins of Caenorhabditis elegans and Schizosaccharomyces pombe. We genetically mapped SKI6, finding it tightly linked to ADE3 on chromosome VII. We obtained 46 clones including this small area and cloned the gene from one of them (see Materials and Methods) (Fig. 1). Since the gene complementing the ski6 mutation was obtained from DNA mapping close to ADE3, we have cloned SKI6 and not a suppressor. Sequence analysis shows that the 246-residue Ski6 protein is closely related to proteins encoded by uncharacterized ORFs found in S. pombe and C. elegans (Fig. 2). These proteins are similar through most of their sequences except for the C. elegans SKI6 homolog, which has an N-terminal extension beyond the region of homology. Ski6p is also more distantly related to Mtr3p, a nucleolar protein required for mRNA export from the nucleus (25).

Ski6p also has homology to several tRNA-processing enzymes from bacteria, the RNase PH group. These are phosphate-dependent enzymes involved in the removal of the last few 3' nucleotides from tRNA precursors (10, 26, 27). They are highly specific in their action and work together with other nucleases in trimming the 3' end of the pre-tRNA molecule. They resemble polynucleotide phosphorylase in producing nucleoside diphosphates from RNA and phosphate and in their ability to synthesize RNA from nucleoside diphosphates (26).

Ski6p has weaker similarity to Bacillus subtilis polynucleotide phosphorylase. We have not tested whether ski6 mutations affect tRNA processing, but we present evidence below that they do affect ribosome biogenesis.

FIG. 1. Cloning of SKI6. The location of SKI6 was determined on the linkage map, and genomic clones in this area were screened for complementation (see text). Complementation tests of subclones localized SKI6 to YGR195w. The region deleted in the ski6::HIS3 disruption and the probe used are shown.

FIG. 2. Ski6 protein is homologous to tRNA-processing enzymes of bacteria (9, 27) and proteins encoded by uncharacterized ORFs of Schizosaccharomyces pombe (pombe; GenBank accession no. D89141) (64) and Caenorhabditis elegans (C. eleg; or C. e.; EMBL accession no. Z49909) (62). S.c. or S. cerev., S. cerevisiae; col or E. coli, Escherichia coli; H. flu, Haemophilus influenzae; Ps. aer., Pseudomonas aeruginosa.
TABLE 1. Translation of electroporated luciferase mRNAs

<table>
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<th>Strain</th>
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<td></td>
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ski6/SK1+ ratio

0.76 (1.0) 7.9 (10.4) 1.19 (1.56) 5.9 (7.8)

* Strain RV493 (K−) carrying either pSK16 (SK1+) or the vector pRS316 (ski6) was grown at 30°C in H-Ura and electroporated with 2 μg of luciferase mRNAs prepared with (C+) and without (C−) 5′ cap and with (A+) or without (A−) 3′ poly(A) as described in Materials and Methods.

† Luciferase activity is expressed in light units per microgram of protein. The blank is <0.01 light unit.

* This row of data shows the ski6/SK1+ ratio normalized on the assumption that this ratio is 1.0 for C+ A− mRNA.

A deletion-substitution mutation was produced by replacing the NdeI-EcoRV fragment of SK16 extending from just upstream of the AUG to codon 122 with the HIS3 gene. The meiotic tetrads produced two viable His− spores: two inviable spores (15 of 16 tetrads examined), indicating that SK16 is an essential gene. The ski6-2 mutant strains are temperature sensitive for growth at 39°C, and the temperature sensitivity is complemented by our clone of SK16. In liquid culture, ski6-2 cells gradually stop growth after about three doublings, with no unique morphology of the arrested cells. Revertants do not appear on plating a ski6 mutant at 39°C on rich medium.

Thus, SK16, whose mutation, like ski1, ski2, ski3, and ski8, increases expression of the uncapped, nonpolyadenylated viral mRNAs, is an essential gene with similarities to sequences corresponding to known 3′→5′ exonucleases. Ski6p might act on stability of mRNAs (on the Ski1p model) or on translation (like Ski2p, Ski3p, and Ski8p).

Ski6p blocks translation of non-poly(A) mRNA. We used electroporation of luciferase mRNAs to study the effect of a ski6-2 mutation (Table 1). In a wild-type strain, cap+ poly(A)+ mRNA was translated three times better than cap− poly(A)− mRNA (Table 1). In the isogenic ski6-2 strain, the poly(A)+ mRNA was translated only three times better than poly(A)− mRNA. Thus, the ski6-2 mutation increases the efficiency of translation of C+ A− mRNA 10-fold. A similar effect on C− A− mRNA translation was also seen, with an eightfold increase in translation in the ski6-2 strain (Table 1). In contrast, there is no effect of the ski6-2 mutation on translation of C+ A+ mRNA (Table 1).

Since the poly(A) structure is important in both translation and stability (reviewed in reference 22), we examined luciferase mRNA stability by direct assay of both structural integrity (Fig. 3) and functional integrity (Fig. 4). Radiolabeled mRNAs were electroporated into cells and incubated as they were for translation, samples were taken periodically, the cells were washed, and RNA was extracted. The amount of intact mRNA remaining was determined by electrophoresis and autoradiography (Fig. 3). Quantitation of the autoradiograms showed that each form of the luciferase mRNA was, if anything, more stable in the wild type than in the ski6-2 mutant, suggesting that mRNA turnover was not the basis of better expression in the mutant. However, since we could not determine into what compartments the electroporated mRNAs had been delivered, we examined the functional integrity of the luciferase mRNAs by measuring the kinetics of luciferase synthesis with the same samples (Fig. 4). This tests the stability of those mRNA molecules with access to the translation apparatus. For the poly(A)+ mRNAs, the kinetics of synthesis by mutant and wild type were similar. For the mRNAs lacking poly(A), synthesis was greater in the ski6 strain from the earliest time points, indicating a difference in translation rather than in mRNA stability. Plotted as percent maximal activity, the wild-type strain showed the same pattern as did the ski6 strain. If mRNA instability in the wild type were the cause of the reduced expression of the non-poly(A) mRNAs, it would quickly reach 100% maximal expression and then stop, while the ski6 mutant would continue expression (19). This was not observed (Fig. 4).

Antibiotic sensitivity of ski6 mutants. Many mutations affecting components of the translation apparatus produce hypersensitivity to hygromycin B (6, 49) and other drugs that increase translational errors (30). We found that ski6-2 strains are hypersensitive to hygromycin B (Fig. 5), supporting the notion that they affect translation. We also found that ski2 strains are hypersensitive to hygromycin B and slightly hyper-

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FIG. 3. SKI6 does not affect stability of electroporated luciferase mRNA. Labeled mRNA was electroporated into wild-type and ski6-2 cells. The kinetics of luciferase synthesis (Fig. 4) and mRNA degradation were determined on portions of the same samples taken at 0, 10, 20, and 30 min. Extracted RNA was analyzed by agarose gel electrophoresis and autoradiography as described previously (31).
ski6 mutants have a novel 25S rRNA-containing particle. At 30°C, growth rates of the isogenic ski6 and wild-type strains are almost identical, although the ski6 mutant shows a greater delay in leaving stationary phase than does the wild type. The amount of polysomes in ski6 strains is consistently lower than that in the wild-type (Fig. 6). The normal growth rates imply that translation is proceeding at an essentially normal rate, although, as shown above, non-poly(A) mRNAs are more translatable in ski6 cells under these conditions. In cells grown at 30°C, the polysome gradients reveal the existence of an extra peak in the ski6 strain, sedimenting slightly slower than the 40S subunits (Fig. 6 and 7). This 38S extra peak is most clearly distinguished from the 40S ribosomal subunit in longer centrifugation runs (Fig. 7). The amount of this extra peak is increased by a shift of temperature to 39°C, the nonpermissive temperature, and the mutant stops growing after three more generations. The ratio of polysomes in ski6-2 cells to those in SKI+ cells is similar at 39°C to the ratio at 30°C (Fig. 6).

We examined the distribution of rRNAs by Northern blot analysis of gradient fractions (Fig. 6). Fractions containing the extra peak in the mutant, close to the 40S peak, give the expected signal with an 18S rRNA probe for both strains. A probe specific for 25S rRNA detects a species in the novel peak that is smaller than 25S rRNA and does not appear in the wild type (Fig. 6). A probe specific for 3.8S rRNA shows that this species is absent from this part of the gradient in both the mutant and wild type (Fig. 6). Longer centrifugation shows that this separation of the extra peak (about 38S) from the 40S subunit peak and reduces contamination of free 60S subunits (Fig. 7A). Northern blot analysis confirms again in these fractions the presence of an RNA of about 20S, whose origin is the 25S rRNA. This 20S RNA might be a product of degradation of the 25S rRNA that is poorly protected in an incomplete 60S subunit. The ratio of 18S rRNA in the ski6 mutant to 18S rRNA in the wild type is 1.04, while the ratio of full-length 25S rRNA in the ski6 mutant to 25S rRNA in the wild type is 1.05. Thus, the ratios of free ribosomal subunits are similar.

Probing the RNA in the 38S peak with probes specific for the 5′ end or the 3′ end of 25S rRNA shows that the 25S-related species lacks the normal 5′ end but has sequences close to the 3′ end (Fig. 7B). The 25S-related sequence in the 38S peak can be distinguished both by size and by hybridization specificity from breakdown products of 25S rRNA found in the 60S peak of both mutant and wild-type strains (Fig. 7B). The latter hybridizes with both 5′ and 3′ probes, suggesting that they are a mixture of randomly broken molecules, while the former hybridizes with the 3′ probe but not with the 5′ probe.

Comparison of total 5.8S rRNA in isogenic mutant and wild-type strains shows a 1.9-fold decrease in the ski6-2 mutant cells, with 18S rRNA as the control (Fig. 8).

ski6-2 suppresses the effect of 60S subunit deficiency on L-A mRNA translation. The SKI genes were discovered based on their derepression of virus expression. Mutations needed for 60S ribosome biogenesis, including 60S subunit protein genes, show reduced copy number of L-A dsRNA and loss of the killer toxin-encoding satellite M1 dsRNA (4). The mak-7-1 mutation is deficient in ribosomal protein L4, has decreased free 60S ribosomal subunits, and shows halfmers, due to polysomes with a 40S subunit which is awaiting 60S joining (41). These mutants lose M1 dsRNA, but we find that ski6-2 mak-7-1 double mutants propagate M1 normally. The mak-7-1 ski6-2 strain 4566-2C (=MATa trpl1 leu2 ura3 ski6-2 mak-7-1 his− ade3) was transformed with either pSK6 or pYRC50 (41) or both to make isogenic strains defective in one or both genes.

FIG. 4. Kinetics of luciferase accumulation in ski6-2 and SKI+ cells indicates an effect on translation rather than mRNA degradation. (A) Kinetics of luciferase activity accumulation are plotted in the upper panels (luciferase activity in light units per microgram of protein), and percent maximal luciferase activity (% Max Luc Activity) is plotted in the lower panels. (B) Comparison of ski6-2 and SKI+ cells in translation of C+ A− mRNA over a 90-min time course. If accumulation were low in wild-type cells for C+ A− or C− A+ mRNAs because of mRNA degradation, then activity would accumulate rapidly and stop at later time points. The plateau (100%) would be expected early. In fact, the kinetics as percent maximal activity are similar for SKI+ and ski6 strains for all types of mRNA. The differences in rates of accumulation must be due to differences in translation rate. The percentage was calculated as follows: 100 [(value at time t) − (value at time t = 0)]/[30-min value]).

Polysome profiles were obtained as described in Materials and Methods, and the ability of the double mutant to propagate M1 was examined. The mak-7-1 strain lost M1, but the isogenic wild type and the ski6-2 mak-7-1 double mutant propagate M1 nor-
mally. This indicates that translation of the L-A mRNA [which lacks poly(A)] is improved as a result of the ski6-2 mutation. Moreover, the halfmer peak is absent in polysome gradients of the double mutants (Fig. 9), suggesting an alteration in the 60S joining reaction.

**DISCUSSION**

**Ski6p is involved in 60S ribosome assembly.** Mutations in ribosomal protein genes result in decreased rates and extents of ribosome assembly as expected from their being essential components of the final structure (36, 63). Other components that are not part of the finished product but are necessary for its construction have also been identified (29, 47, 55, 57).

Ski6p is clearly not one of the ribosomal proteins, but we show that a ski6 mutant, even at the permissive temperature, accumulates a novel particle that contains a fragment of 25S rRNA.
lacking the 5' end of the normal 25S rRNA. This is likely to be either a misassembled 60S subunit or a misassembled and then partially degraded 60S particle. This particle lacks 5.8S rRNA, and the cells show some deficiency of total 5.8S rRNA in comparison to an isogenic wild-type strain. There is no change in the relative levels of free 60S or 40S particles. It is unlikely that the 38S particle carries out any of the reactions of protein synthesis since it lacks both 5.8S rRNA and part of 25S rRNA.

However, the ski6 mutants plainly do have alterations of the translation apparatus itself. They are hypersensitive to hygromycin B, a phenotype typical of mutants in components of the translation apparatus. ski2 mutants show the same phenotype. This suggests that, in addition to the 38S defective 60S ribosomal subunits that accumulate in ski6 strains, the normal-sized 60S subunits are probably also functionally abnormal, perhaps by containing improperly processed rRNA.

Mitchell et al. have found that Ski6p is in a complex with other RNA-processing exoribonucleases (34), suggesting that it has a similar function. One of these exoribonucleases, Rrp4p, is known to be involved in 5.8S rRNA processing (35), and ski6 (renamed rrp41) mutants are likewise defective in 5.8S rRNA processing (34).

Does Ski6p derepress translation of non-poly(A) mRNAs via alterations of full-sized 60S subunits? Ski6p, like Ski2p, Ski3p, and Ski8p, is necessary for blocking the translation of non-poly(A) mRNAs, such as the viral mRNAs whose overexpression formed the basis of the original mutant isolations. The evidence points to translation, rather than mRNA turnover, as the basis of the derepressed expression of non-poly(A) mRNAs. The kinetics of luciferase accumulation and direct measurements of mRNA turnover show the pattern expected for a translation effect.

The ski2, ski3, ski6, and ski8 mutants translate non-poly(A) mRNAs nearly as well as they do poly(A)+ mRNAs (31; also this work), showing that the translation apparatus is inherently able to use non-poly(A) mRNAs. Indeed, it had already been shown that poly(A)-deficient mRNAs are found on polysomes in strains lacking the SKI1/XRN1 exoribonuclease that degrades uncapped mRNAs (21) and in a poly(A)+ polymerase temperature-sensitive mutant shifted to the nonpermissive temperature (43).

Elucidating the means by which Ski proteins block translation of non-poly(A) mRNA requires consideration of the role in translation of the 3' poly(A) structure of eukaryotic mRNA, an area which remains controversial (reviewed in references 22 and 48). Translation of electroporated mRNAs is stimulated by the 5' cap and 3' poly(A) structures by 12- and 200-fold, re-
The 3′ poly(A) structure is known to affect mRNA turnover (for an example, see reference 8), but the kinetics of reporter synthesis show that there is a substantial effect on synthesis, independent of mRNA stability differences (19).

One role suggested for poly(A) is to promote the joining of the 60S subunit to the 40S subunit waiting with associated initiation factors at the initiator AUG (38; for reviews, see references 22 and 39). This was proposed to occur by an interaction between the poly(A) and the 60S subunit, forming a circular mRNA, at least at the time of initiation. Our results support this model (31, 41; also this work). A deficiency of free 60S ribosomal subunits (in strains with mutations in any of 20 mak genes) results in poor translation of the viral mRNAs because they lack poly(A) and so compete poorly with the poly(A)− cellular mRNAs for limiting free 60S subunits (41). Indeed, limitation of 40S or 60S subunits in a strain temperature sensitive for poly(A) polymerase showed greater discrimination against non-poly(A) mRNA when 60S subunits were limiting (42). Mutations in the ski2, ski3, ski6, and ski8 genes improve viral mRNA translation [since they improve the translation of all non-poly(A) mRNAs] and suppress the effect of 60S subunit deficiency (without restoring the levels of 60S subunits) (31; also this work). Another model for the role of poly(A) in translation involves the association of the poly(A) binding protein with eIF-4G (48). However, this model does not suggest an explanation of our data.

We suggested that the Ski2, Ski3, and Ski8 proteins prepare 60S subunits so that they have the requirement for interaction with the 3′ poly(A) structure before they will join with the 40S subunit waiting at the AUG (31, 41, 58). We had no direct evidence that these proteins have these effects by altering 60S biogenesis besides the fact that Ski3p is nuclear (44) and that Ski2p is highly homologous to the mammalian Ski2p (28, 61) which has been localized to the nucleolus (28). In the case of ski6, we have shown directly that 60S subunits are altered.

**Conclusions.** We find that Ski6 encodes a homolog of bacterial RNA-processing enzymes and is necessary for a system that specifically blocks translation of non-poly(A) mRNA. We see a novel species in ski6 mutants that includes a fragment of 25S rRNA but lacks 5.8S rRNA, suggesting that in these strains 60S subunits are not properly formed. Hygromycin B hypersensitivity and suppression of halfmers in a mutant deficient in the ribosomal protein L4 also support the idea that a subtle modification exists in the functional ribosomes and suggests that this modification bypasses the requirement of a 3′ poly(A) for translation.

The results presented here and previously (31) suggest that the default for translation is efficient translation of mRNA independent of poly(A) and that, in a wild-type cell, the specificity for poly(A) mRNA is accomplished (by the Ski2, Ski3, Ski6, and Ski8 proteins) by repressing translation of poly(A)− mRNA.

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