Mak21p of *Saccharomyces cerevisiae*, a Homolog of Human CAATT-binding Protein, Is Essential for 60 S Ribosomal Subunit Biogenesis*

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Mak21-1 mutants are unable to propagate M₁ double-stranded RNA virus, a satellite of the L-A double-stranded RNA, encoding a secreted protein toxin lethal to yeast strains that do not carry M₁. We cloned MAK21 using its map location and found that Mak21p is homologous to a human and mouse CAATT-binding protein and open reading frames in Schizosaccharomyces pombe and Caenorhabditis elegans. Although the human protein regulates Hsp70 production, Mak21p is essential for growth and necessary for 60 S ribosomal subunit biogenesis. mak21-1 mutants have decreased levels of L-A coat protein and L-A double-stranded RNA. Electroporation with reporter mRNAs shows that mak21-1 cells cannot optimally express mRNAs which, like L-A viral mRNA, lack 3'-poly(A) or 5'-cap structures but can normally express mRNA with both cap and poly(A). The virus propagation phenotype of mak21-1 is suppressed by ski2 or ski6 mutations, each of which derepresses translation of non-poly(A) mRNA.

The L-A dsRNA virus has a single segment of 4.6 kilobases which replicates inside yeast cells, where it is maintained at a copy number of 1,000 or more without a substantial adverse effect on cell growth (1, 2). The icosahedral L-A virus particles are composed of 120 copies of the major coat protein (Gag) and about 2 copies of a Gag-Pol fusion protein (3, 4). The L-A (+) strand serves as the mRNA, encoding Gag and Gag-Pol, the latter formed by a −1 ribosomal frameshift event (5, 6). These proteins also support the propagation of a satellite RNA, called M₁ dsRNA, which encodes the secreted polypeptide “killer toxin” and immunity to this toxin (7). Propagation of M₁ is particularly sensitive to the efficiency of expression of the L-A mRNA, suggesting that only excess Gag and Gag-Pol, above the needs of L-A itself, are available to support M₁.

Because L-A mRNA lacks both a 5'-cap and 3'-poly(A) and induces a −1 ribosomal frameshift in expressing Gag-Pol, mutations affecting L-A virus propagation have included mutations in many components of the translation apparatus. Mutations in *SKI2, SKI3, SKI6, SKI7*, and *SKI8* result in elevated virus copy number (8, 9) because of enhanced translation of mRNAs lacking a 3'-poly(A) structure (10, 11). Mutation of *SKI11* enhances viral expression (8) because of a defect in a 5' → 3' exoribonuclease that degrades un capped RNAs (such as viral mRNA) (12–15). The efficiency of L-A’s programmed ribosomal frameshifting is affected by mutations in 5 S rRNA (16) as well as by several other genes and drugs influencing translation (17–20). Most of these agents and mutations accordingly influence viral propagation.

Mutations in genes resulting in an inability to propagate M₁ dsRNA are called mak mutations. 20 mak genes have been found to affect or be deficient in 60 S ribosomal subunits (21–23), but no mak mutants deficient in 40 S subunits were identified (22). Based on the proposal that the 3'-poly(A) structure may facilitate the 60 S subunit joining reaction (24), we suggested that 60 S-deficient mutants may be preferentially defective in translation of non-poly(A) mRNAs, such as viral mRNA (10, 22). However, there has been no direct evidence for this suggestion.

Here we clone the MAK21 gene and analyze the effects of mak21 mutations on translation and ribosome subunits.

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MATERIALS AND METHODS

Cloning of MAK21

*Saccharomyces cerevisiae* strain D2-3C (a mak21-1 ura3 his5 ade2) was provided by Daniel Masison. Strains 3164 (a arg1 lur-1 L-A-HN M₁) and 3165 (a arg1 thr1-1 x kar1-1 L-A-HN M₁) were used for cytoduction of L-A and M₁. Yeast genomic λ clones (25), ATCC71164, 70347, 70812, 70443, 71181, and 71075 were obtained from the American Type Culture Collection. Pfu polymerase (Stratagene) was used for PCR and a Bio-Rad kit for site-directed mutagenesis.

*λ* clones covering the region on chromosome IV where mak21-1 is located (26) were tested for complementation of mak21-1 using in vivo recombination with vector pBM2240 (27). Strain D2-3C was transformed by the spheroplast method with a mixture of 1 μg of λ clone DNA and 0.1 μg of linear plasmid pBM2240 digested with EcoRI and *XhoI*. These transformants were used as cytoduction recipients from strain 3165, and the cytoductants were tested by the killer assay (9). Only clone 71164 complemented mak21-1. Media have been defined previously (9).

Subcloning of MAK21

Subclones of λ ATCC71164 in the centromere plasmid pRS316 were tested for complementation of mak21-1 in strain D2-3C cytoduction as above. Only the 6.8-kilobase *XhoI* fragment shown in Fig. 1 complemented mak21-1. Subclones of this fragment inserted in pRS316 (Fig. 1) included the 4.7-kilobase *XhoI*-SacI fragment (p782) which complemented mak21-1.

The *XhoI*-Ndel fragment from p782 encompassing MAK21 was ligated into the *XhoI* site of pRS315 forming pH102 (=pRS315::MAK21). MAK21 bordered by *XhoI* and *SacI* sites was then transferred to the *XhoI*-SacI window of pRS313 and pRS316 (28), resulting in pH110 and pH107, respectively.

L. Benard, D. C. Masison, and R. B. Wickner, submitted for publication.
m(21): HIS3 Disruption

The XhoI-SalI fragment from p782 including mK21 was ligated into XhoI-SalI-digested pBS KS+. After ligation of a BglII linker (NEB1001) into the StuI site at base 3447 of mK21, the HIS3 gene of pJ215 (29), bordered by BamHI sites, was cloned into the BglII-digested mK21 plasmid, resulting in pH75 (Fig. 1 shows the region deleted). To confirm the disruption, genomic DNA of putative disruptant strain YHE353 was digested with SnaBI and PstI and probed with the SalI-PstI fragment from p782. This confirmed the integrity of the 3'-integration border. Probing YHE353 genomic DNA digested with SnaBI and NsiI with the BamHI-NsiI fragment from pH215 confirmed the integrity of the 5'-integration site. pH336-SK12 (30) and pH316-SK16 (11) have been described.

ADHI-luciferase and SSA3-luciferase Plasmids

The expression plasmids pH116 and pH118 were made by ligating the ADHI cassette from pVT103 (31), obtained after PCR amplification using primers HE66 (5’-ACAGCGGACCAGTATTCACTGCCAGGTTCT-3’) and HE67 (5’-ACAGAACATTTAAGTACGCGGGTGAACG-3’), into PvuII-digested pH331 (28), replacing the latter’s multiple cloning region. In pH116 the ADHI cassette is oriented opposite that in pH118, being directed toward the HIS3 gene. The luc− ORF, bordered by BamHI and XhoI sites, was transferred from pH96 (see below) into the BamHI-XhoI windows of pH116 and pH118, creating pH116 and pH117. Thus pH110 and pH117 have the ADHI-luciferase cassette in two orientations relative to HIS3. A 516-base pair SSA3 promoter fragment was obtained by PCR amplification from the genomic DNA of strain 21278b using primers HE35 (5’-GTCGTCGTCATCCTCGAGTTAATCTGGTCTCGATTT-3’) and HE36 (5’-GGGCGAGGAGCGGGGATGAG-3’). The product was cloned into the HindIII site of pH96 (pH91). The EcoRV-BamHI fragment from pH91 was replaced with the EcoRV-BamHI fragment from pTIL05, producing pH94.

An Smal site was introduced at the L-A AGT codon of pH94 by site-directed mutagenesis using oligonucleotide HE54 (5’-ACAAATCTTAGGCGGGGATGAG-3’), resulting in pH148. The Sp6 site of pH94 was changed into an Smal site by linker insertion using oligonucleotide HE52 (5’-ACCCGGGTACTG-3’), resulting in pH160. To place luc−/luc+ between StuI sites, the luc+ ORF was amplified by PCR from pTIR05 (5) using oligonucleotides HR71 (5’-CGACGGGCAGTGATTCACTGCCAGCAGTATAG-3’) and HE4 (5’-TTTGATGCGTTTATCGACCATGCTGTAATGTTG-3’). The product was cloned into the HindIII site of pH96 (pH91). The EcoRV-BamHI fragment from pH91 was replaced with the EcoRV-BamHI fragment from pH94.

A human CCAAT box-binding factor (hCBF) clone (pMT2-CBF) was kindly provided by Daniel Linzer (Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University). The hCBF ORF is tightly bordered by NcoI sites. The NcoI fragment of pMT2-CBF was cloned into pH92, producing pH93, in which hCBF expression is under the control of the ADHI promoter present on this LEU2-2 µm plasmid.

GAL-promoted mK21 Plasmid Construction

An XhoI site was introduced by site-directed mutagenesis (Bio-Rad) immediately downstream of the mK21 stop codon in pH115 (see below) using oligonucleotide HE81 (5’-GTTTGGTATTGAAGTGTATC-3’). The XhoI fragment of pH66 was cloned into pLS101, producing pHS23, in which hCBF expression is under the control of the ADHI promoter present on this LEU2-2 µm plasmid.
Subcloning this Xba fragment shown at the top of Fig. 1 complemented some IV, and the Xba and SalI sites are all inside YDR060W. Because this fragment is located at the genetic map position of mak21-1, YDR060W is the MAK21 gene and not a suppressor.

Because the rrp1 mutation maps close to mak21-1 and, like mak21-1, rrp1 results in loss of M₁ dsRNA, it was thought possible that RRP1 was identical to MAK21 (35). However, the restriction map of the clones carrying MAK21 are different from that of MAK21, and recently RRP1 has been sequenced, showing that it is identical to YDR087C and thus distinct from MAK21.

**MAK21 Is Essential**—One of the MAK21 genes of the diploid strain YHE305 (MATa/MATα ura3-1/ura3 his3-1/his3 trp1/- leu2/-) was replaced with the HIS3 gene using plasmid pH75 (see “Materials and Methods” and Fig. 1), resulting in strain YHE353, and the disruption was confirmed by Southern blotting. Of 32 meiotic tetrads from strain YHE353, 31 tetrads had only two viable spores, and the remaining tetrad contained only one viable spore. Of these 32 tetrads 11 were germinated at 30 °C, 10 at 25 °C, and 11 at 20 °C. Thus the germination temperature did not seem to influence spore viability. MAK21 is thus needed for spore germination or for growth.

To investigate if MAK21 is needed for spore germination or for cell growth, tetrads from strain YHE353 carrying pH107 (URA3 CEN MAK21, see “Materials and Methods”) were germinated on medium lacking uracil. Cells from eight tetrads containing four germinating ascospores were transferred to media containing 5-fluoroorotic acid to eliminate the URA3 plasmid. All eight tetrads showed 2:2 segregation for growth. Thus MAK21 is not only needed for spore germination, it is absolutely required for viability.

**Mak21p Homology with hCBF**—The 1,025-residue Mak21p shares substantial sequence similarity throughout its length with the hCBF (36). Other ORFs of unknown function from mouse, Caenorhabditis elegans, and Schizosaccharomyces pombe also show significant amino acid similarity with Mak21p (see Fig. 2). The hCBF stimulates expression from the hsp70 CCAAT box promoter in tissue culture cells. It was identified by its specific binding to this promoter. As shown diagrammatically in Fig. 2, 77 residues are conserved in all five homologs, and 90 are conserved in four of five homologs, and these are distributed through most of the length of the five proteins.

**Mak21p Is Not Required for Yeast Hsp70 (SSA3) Expression**—The control of human hsp70 expression by hCBF suggested that the homologous Mak21p might control the expression of yeast hsp70. The most homologous yeast hsp70 to the human hsp70 controlled by hCBP is Ssa3p (37, 38). Of its 649 residues, 445 are identical to the human hsp70. The promoter of the SSA3 gene contains an inverted CCAAT box.

To estimate expression from the SSA3 promoter, we constructed two plasmids (pH172 and pH173) in which transcription of luciferase was driven by the SSA3 promoter, differing only in the orientation of the SSA3-luciferase cassette relative to the HIS3 gene. Two similar plasmids, in which the ADH1 promoter drives luciferase transcription, were used as controls (see “Materials and Methods”). The four plasmids were transformed into strain YHE414 (MATα ura3 his3 mak21-1) containing either pRS316 (URA3 CEN plasmid) or pH107 (pRS316-MAK21). Transformsants were grown in 50 ml of SD medium to A₆₀₀ = 0.6. After resuspension of the cells in phosphate-buffered saline, protein extracts were prepared by disruption with glass beads. Luciferase activity was measured using the Promega luciferase assay kit and a luminometer (10, 36).
11) and was corrected for the protein concentration of the extract, measured using the Bradford assay.

For luciferase expression from the ADH1 promoter, the ratio of activity in a mak21-1 mutant to that in the wild type was 1.57 ± 0.46 (10 independent assays), probably not a significant increase. For luciferase from the SSA3 promoter, the same ratio was 4.7 ± 3.4. The increase in SSA3-promoted activity in mak21 cells is the opposite of what would be expected if Mak21p were functioning the same in yeast as does hCBF in human cells.

**hCBF Does Not Complement a mak21 Deletion**—From transfectants of a clone of hCBF under the ADH1 promoter (pH393-LEU2 2 μ) in YHE393 (MATa leu2 trp1 ura3 mak21::HIS3 pH107 [URA3 CEN MAK21] K+) were selected 5-fluoroorotic acid-resistant colonies. However, the appearance of resistant cells was below 1 in 10,000, suggesting that loss of the MAK21 plasmid was lethal. One resistant isolate was analyzed for the presence of plasmids by Southern blotting. This showed that recombination had taken place, resulting in loss of the original plasmids. Thus, hCBF cannot substitute for Mak21p in yeast.

**L-A Levels Are Reduced in mak21-1 Strains**—The same eight strains used to examine SSA3 expression above were used to compare L-A levels in mak21-1 and MAK21 strains. L-A Gag levels are at least 2-fold less in the mak21-1 strains compared with the MAK21-complemented strains (Fig. 3). Examination of phenol-extracted nucleic acids or RNase-resistant dsRNA showed likewise that there was twice as much L-A dsRNA present in the extract of MAK21 cells as in that of mak21-1 cells (Fig. 3).

**Mak21p Is Involved in 60 S Ribosomal Subunit Biogenesis**—Polysome profiles (10, 11) were examined from wild-type strain YHE413, the mak21-1 strain YHE414, YHE432 (YHE414 containing plasmid pRS313 {HIS3 CEN}) and YHE433 (YHE414 containing plasmid pH110 {HIS3 CEN MAK21}). Strains YHE413 and YHE414 were grown in YPAD, and strains YHE432 and YHE433 were grown in -His medium. The poly-
som profiles of the mak21-1 strains YHE414 and YHE432 show a marked increase in the ratio of free 40 S to 60 S ribosomal subunits compared with the wild-type strain YHE413 and also contain pronounced halfmer polysomes peaks (Fig. 4A). The 40 S:60 S ratio was restored to wild-type levels by the MAK21 expression plasmid (strain YHE433), with a concurrent disappearance of halfmer polysomes.

Because the ratio of free 60 S subunits to free 40 S subunits was decreased in mak21-1 strains, we examined the ratio of total 40 S to 60 S subunits. The mak21-1 strain YHE432 containing the vector pRS313 was compared with the same strain carrying the MAK21 expression plasmid pH110 grown in -His medium to an A_{600} between 0.4 and 0.5. Cells were broken in buffer lacking cyclohexamide and MgCl2, and the cell extract was loaded onto a sucrose gradient lacking MgCl2. The sucrose gradients were centrifuged for 14 h at 27,000 rpm. In the mak21-1 strain the 40 S:60 S ratio (as A_{260}) was 0.83, whereas this ratio was 0.64 in the isogenic MAK21 strain (Fig. 4B). Thus the mak21-1 strain has an imbalance in the 40 S:60 S ratio probably because of a decrease in 60 S ribosomal subunits since the 40 S peaks are nearly identical in the two strains whereas the 60 S peaks differ.

Mak21p Depletion and Overexpression—Because MAK21 is essential for cell viability we analyzed the effect of depleting Mak21p on ribosomal subunit biogenesis by putting Mak21p expression under the control of the GAL1 promoter. After transforming pH287 (GAL1::MAK21) into strain YHE393 (MATa leu2 trp1 ura3 mak21::HIS3 K+) pH107), the ADH1::MAK21 expression plasmid pH107 was eliminated by 5-fluoro-orotic acid selection, making Mak21p expression dependent on the activity of the GAL1 promoter of pH287 (strain YHE565).

Strain YHE565 was grown in galactose/raffinose medium to an A_{600} = 0.640. Cells were collected and resuspended in YPAD, to shut off production of Mak21p, or in YPAgal/raf. The doubling time in galactose/raffinose medium was 3 h and 20 min, but the initial doubling time after transfer to glucose medium was decreased to 2 h and 15 min. After 10 h of growth in dextrose the doubling time started to increase and was 10 h and 45 min after approximately 40 h of growth.

Polysomes were prepared from strain YHE565 grown for 20 h in glucose medium with a final A_{600} of 0.450 and final doubling time of 7.5 h. The polysome profile showed an extremely large 40 S ribosomal subunit peak (Fig. 5).

Potential MAK21 overexpression constructs included high copy (2 μ) plasmids in which expression of the intact MAK21 ORF, the NH2 terminus (Lys-507 = stop), or the COOH terminus (Asn-509 = start) is driven by the ADH1 promoter.
YHE414 transformed with these constructs in each case maintained the satellite virus, and had normal polysome profiles.

**mak21-1 Selectively Reduces Expression of mRNAs Lacking 5′-Cap or 3′-Poly(A)—**Luciferase expression of mRNAs carrying a 5′-cap, a 3′-poly(A) tail, or both or neither was examined by electroporation into isogenic mak21-1 and wild-type strains (Table I). The absence of either 5′-cap or 3′-poly(A) resulted in a modest, but consistent, decrease in expression of luc in this region. It has been postulated that hCBF contains the lysine-rich region postulated to be involved in nuclear localization. However, all of the Mak21/hCBF-like proteins have an abundance of lysine residues in this region. hCBF, which contains a nuclear localization signal in this part of the protein, is an important protein that is involved in nuclear processes.

**As shown in Table II, for luciferase inserted at the initiator AUG of the L-A sequence and at base 1834, the mak21-1 mutation results in a consistent decrease of about 2-fold in expression of these mRNAs. In other experiments, the same site of luciferase was at positions 197, 464, or 971 of L-A sequence (data not shown). Thus there is a clear decrease in translation in mak21-1 cells, compared with MAK21 cells, when the mRNAs are less competitive, i.e. if they lack a cap and a poly(A) tail.

**Mak21 5′- and 3′-Deletion Mutants and Their Polysomes—**Although the NH2-terminal region of Mak21p is not homologous to that of hCBF and is absent from the S. pombe homolog, the NH2-terminal region of hCBF has the ability to interact with p53 and the adenovirus E1A transcriptional activator (39). There is likewise little amino acid similarity among the COOH-terminal regions of Mak21/hCBF-like proteins, even between the human and mouse proteins. However, all of the Mak21/hCBF-like proteins have an abundance of lysine residues in this region. hCBF contains a nuclear localization signal in this part of the protein. Several 5′ and 3′ MAK21 deletions were constructed and transformed into strain YHE393 (MATa, URA3, his3, mak21::HIS3) (pH107) (URA3 CEN MAK21 K+). Complementation of MAK21 function was analyzed by spotting 10,000 cells onto a 5-fluoroorotic acid plate (thereby selecting for loss of the plasmid carrying the normal MAK21 gene) and scoring for growth.

**The first 207 amino acids of Mak21p are completely dispensable for its function (Fig. 5B). Up to 30 amino acids can be deleted from the NH2 terminus before the Mak21p function is partially debilitated (Fig. 5C). However, 127 amino acids can be deleted from the COOH terminus before Mak21p function is seriously debilitated (Fig. 5D).** Thus in contrast to hCBF, there does not seem to be an important role for the Mak21p NH2 terminus. This is in agreement with the fact that this whole region is absent in the S. pombe homolog (Figs. 1 and 2). Deleting 64 amino acids from the COOH terminus already causes some loss of function (Fig. 5E). However, 127 amino acids can be deleted from the COOH terminus before Mak21p function is seriously debilitated (Fig. 5F). This substantial part of the lysine-rich region postulated to be involved in nuclear localization can be deleted.

**Localization of Gfp-Mak21p and Gfp-Mak21p 3′-Deletions—**Because hCBFp and Mak21p are involved in nuclear processes, transcriptional activation of HSP70 genes and 60 S ribosomal subunit biosynthesis, respectively, we investigated the cellular localization of Mak21p. GFP was fused to the NH2 terminus of
Mak21p (pH209). This fusion protein was capable of complementing a mak21Δ mutation (YHE393), both for growth and ability to maintain M1, indicating that it had fully retained Mak21p activity. The GFP-Mak21 fusion protein localized to a discrete spot in the yeast cells (Fig. 6). However, formaldehyde fixation and/or digitonin permeabilization of these cells (for the purpose of staining the nuclei with DAPI) resulted in a diffuse distribution of the fusion protein, precluding the ability to relate GFP-Mak21 localization to specific cellular domains. A fusion protein from which the COOH-terminal amino acids were deleted up to Met-827 (pH283) was no longer able to complement a mak21Δ mutation. This fusion protein also lost the discrete cellular localization of the full-length fusion protein (Fig. 6).

ski6-2 and ski2Δ Suppress mak21-1 Effects on M1 Maintenance but Not on 60 S Levels—In general the effect on M1 satellite RNA propagation of mak mutations is suppressed by ski mutations. This is probably also true for mak21-1 because this mutation is suppressed by ski4 (40). However, the effect of mak mutations, ski mutations, or the presence of both mak and ski mutations on L-A propagation in the absence of M has not been analyzed in detail. To do so, mak21-1 ski6-2 and mak21-1 ski2Δ double mutants were constructed. These strains maintained M1, showing that indeed the mak21-1 mutation can be suppressed by ski mutations. Introduction of SKI6 or SKI2 expression plasmids into the corresponding mak21-1 ski6-2 or mak21-1 ski2Δ strains resulted in the loss of M1. Allowing the loss of these plasmids produced mak ski double mutants in which L-A propagation was not influenced by M1. After subsequently introducing MAK21, SKI6, or SKI2 expression plasmids, or just the expression vectors into these strains, the effect of mak21-1 and ski6-2, or ski2Δ mutations on L-A propagation was studied. L-A Gag levels were modestly decreased in mak21-1 mutants compared with the wild-type cells, and L-A levels were clearly increased in ski6-2 or ski2Δ mutants. In mak21-1 ski6-2 or mak21-1 ski2Δ double mutants L-A levels at least equaled those found in cells having only the ski mutation (data not shown).

The effect of a mak21-1 mutation combined with either a ski6-2 or a ski2Δ mutation on polysome profiles was analyzed. We showed above that mak21-1 causes a decrease in 60 S ribosomal subunits resulting in the appearance of halfmer polysomes. Although ski6-2 strains accumulate an abnormal 60 S subunit sedimenting at 38 S (11), this was not prominent on our gradients done on cells grown at a permissive temperature (30 °C). The ski2Δ strain also had a normal polysome profile. When a mak21-1 mutation is combined with either a ski6-2 or a ski2Δ mutation the polysome profiles of the resulting strains are similar to those of strains containing only the mak21-1 mutation (Fig. 7).

DISCUSSION

The mak21-1 mutation was isolated because it lost the M1 dsRNA satellite of the L-A virus. We show here that MAK21 is identical to YDR060W encoding a homolog of the hCBF. The hCBF was isolated based on its ability to bind specifically to the CCAAT site of the hsp70 promoter, and hCBF apparently regulates the expression of hsp70 in tissue culture cells. S. cerevisiae has a very close homolog of human hsp70, Ssa3p. In a mak21-1 strain, we find that expression from the SSA3 promoter is not decreased as expected but is actually increased. The observed result may be due to some “stress” resulting from the slow growth of mak21-1 cells. SSA3 transcription is increased in early stationary phase (38), and the slow growth of mak21-1 cells may mimic this condition. In addition, the NH2-terminal part of hCBF which interacts with p53 and E1A is not essential for the yeast gene. A substantial part of the COOH-terminal lysine-rich region believed to be involved in nuclear localization is likewise dispensable.

We find that mak21-1 results in a decrease in the level of free 60 S ribosomal subunits and that cells starved of Mak21p have very low levels of free 60 S particles and a large amount of free 40 S subunits. Because MAK21 is an essential gene, it is likely that its essential role is related to its role in ribosome biogenesis. Perhaps Mak21p is necessary for the transcription of some component necessary for 60 S ribosome biogenesis, but such a putative target gene remains to be identified. A fusion of GFP with Mak21p was found to localize in a single small central region of unfixed cells but was more diffuse in fixed cells,
precluding confirmation of our suspicion that this was the nucleolus. Similar behavior has been reported for Nip7p, another protein involved in 60 S ribosome biogenesis (41).

We have found previously that mutations in many genes resulting in deficiency in free 60 S ribosomal subunits result in loss of M₁ dsRNA (21–23). Here we have used electroporation of mRNAs to investigate the connection between deficiency of 60 S subunits and loss of M₁ dsRNA. We find that mRNAs lacking 5’-cap or 3’-poly(A) or both are translated about 2-fold less well in mak21-1 strains than in isogenic wild-type cells. Because the L-A mRNA lacks both 5’-cap and 3’-poly(A) (42, 43), it is expected that translation of L-A mRNA should be impaired in mak21-1 cells. In agreement with this result, the copy number of L-A virus, as judged by the levels of Gag protein or of L-A dsRNA, is about half the normal level in mak21-1 strains.

Why does a 2-fold decrease in L-A expression result in complete loss of M₁? We have suggested previously that L-A has limited cis-packaging, meaning that the L-A genome is preferentially encapsidated in the coat proteins that it encodes (17, 44). This implies that satellite dsRNAs, such as M₁, can only use the excess coat proteins produced from the L-A mRNA for their propagation. One particularly striking example is X dsRNA, a deletion mutant of L-A which lacks most of the Gag and Pol coding sequences but retains the sites on the RNA necessary for packaging, replication, and transcription of L-A (44–46). Although the parent molecule, L-A, is not lost in mak mutants that are 60 S subunit-deficient, X dsRNA is lost in these strains (44). A decrease in the efficiency with which L-A is translated would preferentially affect X or M₁. Here we show that in a mak21-1 strain, translation of any of several L-A-like mRNAs is indeed impaired if, as for the L-A mRNA itself, the 5’-cap and/or 3’-poly(A) is missing, but not if, as for cellular mRNAs, both cap and poly(A) are present.

These results explain why mak mutants deficient in translation of L-A lose M₁ dsRNA, but do not lose L-A dsRNA. It is likely that the deficiency of free 60 S subunits seen in mak21-1 strains is the cause of its inefficient translation of mRNAs lacking 5’-cap or 3’-poly(A) or both. However, the exact mechanism by which 60 S deficiency leads to this poor translation remains controversial. It has been suggested that the 3’-poly(A) structure is involved in joining of 60 S subunits to the 40 S complex waiting at the initiator AUG (47). In this model, a deficiency of 60 S subunits would be expected to favor poly(A)⁻ mRNAs over poly(A)⁺ mRNAs, as we observe. However, another model suggests that poly(A) is involved in attracting 40 S initiation complexes to the mRNA (48). This model would predict that some of the mak mutants would be deficient in 40 S subunits, a result we have not found (49). Only one of several 40 S-deficient strains loses M₁ dsRNA, suggesting that 40 S deficiency per se is not preferentially disadvantageous to non-poly(A) mRNAs in vivo. It is not clear by either model why deficiency of 60 S subunits would put cap-deficient mRNAs at more of a disadvantage than they are already. Further work will be necessary to resolve this problem.

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