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Genetics

Functional substitution of mouse ribosomal protein L27′ for yeast ribosomal protein L29 in yeast ribosomes

(ribosome assembly/cycloheximide resistance/interspecific complementation)

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ABSTRACT **A cDNA clone of mouse ribosomal protein L27′ was shown previously to be 62% identical in amino acid residues to yeast ribosomal protein L29. The L27′ cDNA was expressed in yeast to determine the ability of the mouse protein to substitute for yeast L29 in assembling a functional ribosome. In a yeast strain resistant to cycloheximide by virtue of a recessive mutation in the L29 protein, the murine cDNA did not produce a sensitive phenotype, indicating failure of the mouse L27′ protein to assemble into yeast ribosomes. However, when the mouse L27′ gene was expressed in cells devoid of L29 and otherwise inviable, the murine protein supported normal growth, demonstrating that mouse ribosomal protein L27′ indeed was interchangeable with yeast L29. We conclude that mouse ribosomal protein L27′ is assembled into ribosomes in yeast, but yeast L29 is assembled preferentially when both L29 and L27′ are present in the same cell.**

The ribosome is an organelle whose constituent building blocks have been extensively catalogued. With identification of ribosomal components completed, attention has turned toward deducing the arrangement of the various protein and RNA moieties in the overall structure as well as assigning functions to individual or groups of ribosomal components. A particularly profitable means of determining the function of individual ribosomal components has been through genetic and biochemical analysis of mutants resistant or hypersensitive to inhibitors of protein synthesis (1, 2). Another approach has been *in vitro* reconstitution of ribosomes (3); omission of specific ribosomal proteins from reconstitution mixtures often results in absence of a specific function in the completed particles, thereby implicating the missing protein in that function (4–6). Although eukaryotic ribosomes have been reconstructed with groups of ribosomal proteins (7–9) or subunits (10), reconstitution experiments have been successful primarily with ribosomes of prokaryotic origin, the only type of ribosomes reassembled from totally separated components (3). Other strategies for determining the function or location of ribosomal components have relied on cross-species comparisons. Similarities between eukaryotic ribosomal proteins and *Escherichia coli* ribosomal proteins (e.g., size, charge, or immunological crossreactivity) have been extrapolated to functional similarities (11–13). Relationships between ribosomal proteins of different organisms can also be inferred from evolutionarily conserved rRNA domains (14); proteins from different organisms that each bind the same rRNA sequence or structure can be assumed to share a similar function and/or location in the ribosome (15–18). Finally, deductions from DNA sequences have revealed amino acid homologies among ribosomal proteins from different organisms, homologies that suggest equivalent function (19–25).

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Cycloheximide is an inhibitor of the peptidyltransferase reaction in eukaryotic ribosomes. In yeast, resistance to cycloheximide results from mutations in the gene *CYH2* (26, 27), which encodes ribosomal protein L29 (28). Therefore, L29 is believed to be a part of the ribosome's peptidyltransferase center. Recently, Belhumeur *et al.* (29) isolated a cDNA clone encoding mouse ribosomal protein L27′. The protein sequence deduced from this cDNA showed 62% identity to yeast L29 and 74% homology when conservative amino acid changes were considered. Based on this sequence relationship, Belhumeur *et al.* proposed that the two proteins perform the same function in ribosomes. In this report we describe experiments to test the hypothesis that yeast ribosomal protein L29 and mouse ribosomal protein L27′ are equivalent proteins. We show that L27′ substituted for L29 *in vivo*, confirming a functional homology previously indicated by the sequence homology. However, analysis of the cycloheximide-resistance phenotype of cells containing L27′ suggested that yeast L29 is preferentially, perhaps exclusively, assembled into ribosomes when both L29 and L27′ are produced in yeast cells.

MATERIALS AND METHODS

Strains. Plasmids used in this study were propagated in *E. coli* strain MC1061 [*araD139* Δ(*ara–leu*)*7697* Δ*lacX74 GalU GalK hsr hsm strA*], provided by M. Casadaban (University of Chicago, Chicago). The following strains of *Saccharomyces cerevisiae* were used: DB745 (*MATα ura3-52 ade1-101 leu2-3,112*) from D. Botstein (Massachusetts Institute of Technology, Boston); HF160X1 (*MATa his3-Δ1 ade5 trp⁻ cyh2*); HF203X1 (*MATa his3-Δ1 leu2-3,112 ura3-52 ade⁻ cyh2*); HF194X1 (*MATa ura3-52 his3-Δ1 ade⁻ cyh2*); HF194GCYHX23 (*MATα leu2-3,112 his3-Δ1 trp1-289 ade⁻ UAS_G::cyh2*). The designation *cyh2* refers to the recessive cycloheximide-resistance allele of *CYH2*, the gene encoding ribosomal protein L29. HF194GCYHX23 contained a chromosomal *cyh2* allele into the promoter of which had been inserted UAS_G, the upstream activation sequence from the *GAL10* gene (30); consequently UAS_G::*cyh2* can be expressed only when cells are grown in the presence of galactose.

Yeast Transformation. Plasmids were introduced into yeast by the spheroplast method (31). Strains pre-grown on galactose usually transformed poorly or not at all; transformation efficiency was greatly enhanced by substituting in the selective medium a mixture of 0.75 M sorbitol and 0.75 M mannitol in place of the usual 1.0 M sorbitol.

Plasmid Constructions. Mouse ribosomal protein L27′ was produced in yeast by inserting cDNA clone cCL3 (29) into two types of expression vector.

Single-copy, conditional expression vector. Plasmid pBM150 (32), which contained the yeast *GAL10–GAL1*

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bidirectional promoter and a centromere causing its copy number to remain at 1 when introduced into yeast cells, was modified to accept the L27′ cDNA. After the *Kpn* I site in the centromeric sequence was eliminated with phage T4 DNA polymerase, the *Sal* I site 275 base pairs (bp) distal to the *GAL1* promoter was converted to a *Kpn* I site by filling-in with the Klenow fragment of *E. coli* DNA polymerase and ligation to a *Kpn* I oligonucleotide linker. The *Hin*dIII site 3′ of the L27′ cDNA sequence was similarly converted to a *Kpn* I site; the L27′ cDNA was then cleaved at a *Bam*HI site 15 bp 5′ of the L27′ translation start codon and at the newly created *Kpn* I site 3′ of the gene, and the resulting fragment was inserted into pBM150 between the *Bam*HI site 54 bp 3′ of the *GAL1* transcription start and the *Kpn* I site further downstream. Transcription of L27′ was thereby made conditional, since the *GAL1* promoter is functional only when cells are grown on galactose. A yeast transcription termination sequence was also inserted at the *Kpn* I site 3′ of the L27′ cDNA; the terminator consisted of a *Kpn* I–*Nru* I (converted to *Kpn* I) fragment derived from the distal portion of the yeast *CYC1* gene contained in plasma pAB16 (kindly provided by F. Sherman, University of Rochester, Rochester, NY). The final construct was designated pBML27′.

High-copy, constitutive expression vector. The *Bam*HI–*Hin*dIII fragment containing the L27′ cDNA was inserted between the *Bam*HI and *Hin*dIII sites in the polylinker of pVT102L (33), placing the L27′ gene adjacent to the yeast alcohol dehydrogenase promoter in this high-copy-number plasmid. The promoter is active constitutively.

Analysis of mRNA. Nitrocellulose blot hybridization of yeast RNA was performed as described (30). Analysis of yeast polyribosomes by sucrose density gradient centrifugation was carried out as described by Warner *et al.* (34).

RESULTS

Diploid yeast cells heterozygous for resistance to cycloheximide, designated *cyh2/CYH2*, where *cyh2* represents the resistance allele, display a sensitive phenotype at inhibitor concentrations above 1.0–2.0 μg/ml (28). This fact was used to test the function of mouse ribosomal protein L27′ in yeast. The mouse cDNA encoded a wild-type (sensitive) L27′ protein; thus, if after introduction of the L27′ gene into a *cyh2* yeast strain the mouse ribosomal protein was incorporated into yeast ribosomes, transformants were expected to acquire a cycloheximide-sensitive phenotype.

For expression of the mouse L27′ coding sequence in yeast, the cDNA was placed adjacent to the *GAL1* promoter. The *GAL1* promoter is active only when cells are propagated on galactose (32). The *GAL1*–L27′ gene was introduced on a single-copy plasmid into the *cyh2* strain HF194X1. Transformants were replica-plated to either glucose medium or galactose medium, both containing cycloheximide at 5 μg/ml, and all transformants grew on both media. This failure to convert the cycloheximide-resistant phenotype of the recipient cells to a sensitive phenotype on galactose demonstrated that mouse ribosomal protein L27′ probably did not become incorporated into yeast ribosomes. To be certain that continued growth in the presence of cycloheximide was not due to the mouse ribosomal protein also conferring resistance, we introduced the same plasmid into a *CYH2* yeast strain; in this case cells remained sensitive.

We also performed RNA hybridization analysis to be certain that the *GAL1*–L27′ hybrid gene was actually transcribed. Total cellular RNA was isolated from transformants grown either in expressing (galactose) or nonexpressing (glucose) conditions, separated by size in denaturing gels, and transferred to nitrocellulose. Fig. 1 shows an autoradiograph of the transferred RNA that was hybridized simultaneously with a ^{32}P-labeled L27′ DNA probe and a probe

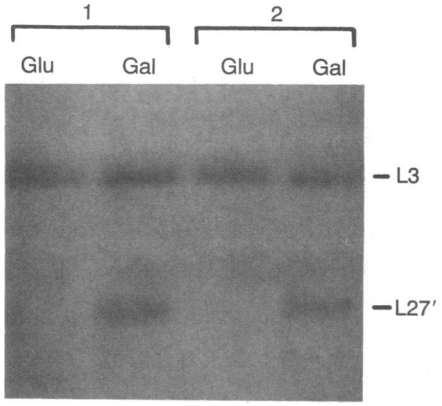

FIG. 1. RNA blot hybridization analysis of L27′ mRNA. Yeast strain HF194X1 was transformed with pBML27′, a plasmid that carried the murine L27′ ribosomal protein gene transcribed under control of the *GAL1* promoter. Two independent transformants were grown on glucose (*GAL1* promoter off) or galactose (*GAL1* promoter on) before isolation of total cell RNA. The RNA was size-fractionated by electrophoresis under denaturing conditions and transferred to nitrocellulose. The transferred RNA was hybridized simultaneously to two ^{32}P-labeled DNA probes specific for mouse ribosomal protein L27′ and yeast ribosomal protein L3, respectively.

complementary to yeast ribosomal protein L3 mRNA, which served as a control. RNA extracted from cells grown on nonexpression medium hybridized only to the L3 probe, whereas a second RNA species of a size expected for the L27′ mRNA was revealed in RNA prepared from cells grown on medium enabling expression of the *GAL1*–L27′ gene. These results proved that the L27′ gene was transcribed.

It remained possible that, despite the L27′ gene being transcribed, the L27′ mRNA was translated inefficiently or not at all. Poor translation was a possibility since, with respect to the AUG initiator codon, the L27′ mRNA contained a guanine at the −3 position. The −3 residue in mRNA is believed to influence the efficiency of translational initiation (35), and in yeast guanine occurs at this position in only 13% of all sequenced mRNAs (75% have adenine; see ref. 36). To measure its translational efficiency, the distribution of L27′ mRNA on and off polysomes was analyzed. Strains containing the *GAL1*–L27′ plasmid were grown on galactose to allow expression of the L27′ gene and the total cell contents were separated by sucrose density centrifugation into fractions containing large polysomes, smaller polysomes, monomers, and nonribosomal material (Fig. 2*A*). RNA was isolated from each fraction and hybridized as described above (Fig. 2*B*). The majority of mRNA for yeast ribosomal protein L3 was found in the fraction containing large polysomes. The L27′ mRNA was also found in the polysome fractions, indicating that it associated with ribosomes and presumably had been translated into protein. However, in contrast to L3 mRNA, the L27′ mRNA also was found in all fractions of the gradient, suggesting that much of the mRNA was not being efficiently translated, perhaps due to inefficient initiation as predicted from the sequence of its 5′ untranslated region.

To this point our results indicated that mouse ribosomal protein L27′ was not incorporated into yeast ribosomes, although it probably was synthesized in the yeast cells. Polysome profiles alone, however, were insufficient to determine just how much L27′ protein was produced. And in any case, regardless of the level of L27′ synthesis, the mouse ribosomal protein may have been unable to compete successfully with endogenous yeast ribosomal proteins (presumably L29) for assembly into ribosomal subunits. To address both of these questions, we introduced the L27′ gene into a yeast strain in which synthesis of endogenous L29 could be

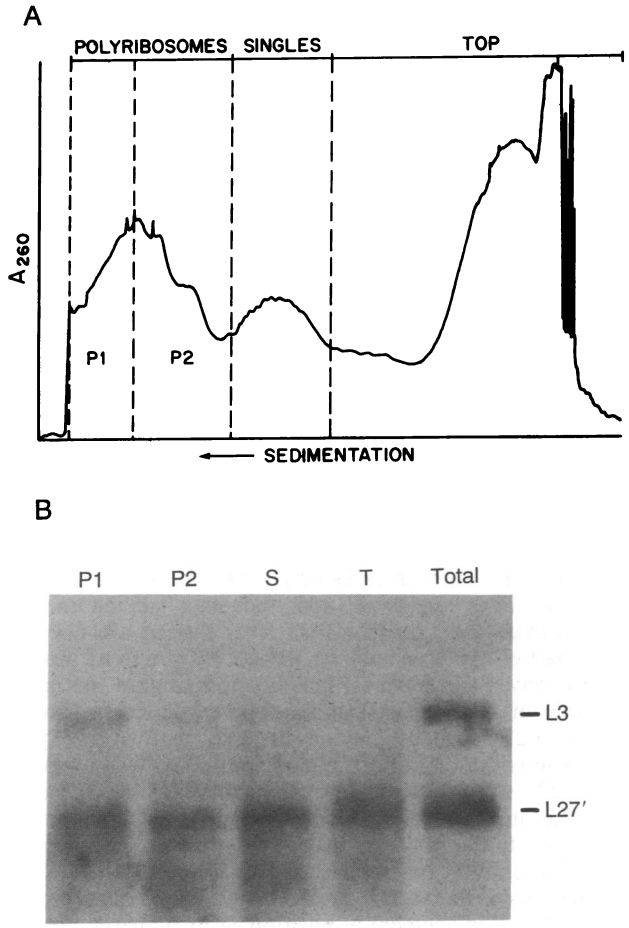

FIG. 2. Distribution of L27′ mRNA in ribosomes. Yeast strain HF194X1 transformed with pBML27′ was grown on galactose to allow expression of the mouse L27′ gene. Cell extract was sedimented through a sucrose density gradient and fractions of the gradient were pooled as indicated (*A*) for RNA hybridization analysis (*B*). P1, larger polysomes; P2, smaller polysomes; S, monomeric ribosomes; T, top of the gradient; Total, cell extract before sedimentation through the sucrose gradient.

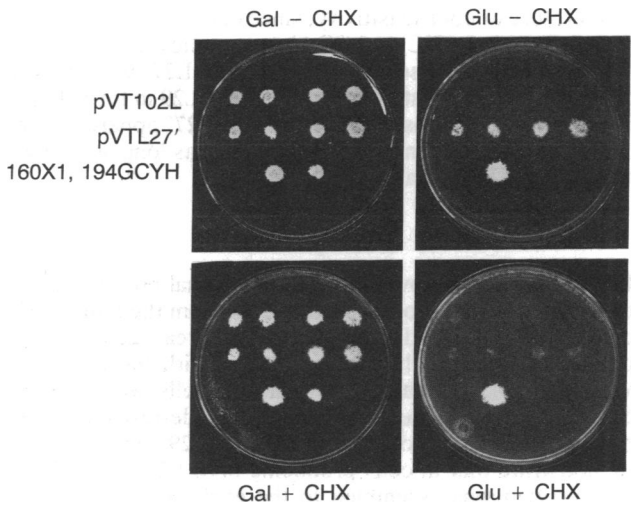

FIG. 3. Complementation of L29-deficient cells by L27′. Yeast strain HF194GCYHX23 transformed with plasmid vector pVT102L (top row) or pVTL27′ containing the murine L27′ gene (middle row) were replica-plated to glucose and galactose media with and without cycloheximide (CHX) at 5 μg/ml. Prior to transformation, HF194GCYHX23 contained a single copy of the yeast L29 gene that conferred resistance to cycloheximide and that required galactose for its expression. The L27′ gene carried by pVTL27′ was expressed regardless of carbon source. When transformed with pVT102L, HF194GCYHX23 was inviable on glucose; the same strain transformed with pVTL27′ was viable, demonstrating that the mouse ribosomal protein was a functional substitute for yeast L29. Further proof for the expression of murine L27′ was revealed by the fact that HF194GCYHX23 cells also expressing (on galactose) the yeast L29 ribosomal protein gene were resistant to cycloheximide, whereas cells expressing only the L27′ gene were sensitive to the drug as indicated by lack of growth on glucose medium containing cycloheximide. Other strains (bottom row): 160X1, HF160X1, a cycloheximide-resistant strain with an L29 gene expressed on all carbon sources; 194GCYH, HF194GCYHX23.

totally eliminated (30). Ordinarily, absence of L29 results in inability of cells to grow. If L27′ was produced at a low level but nonetheless able to assemble a functional ribosomal subunit, we expected that the protein would rescue the growth defect of cells lacking L29, albeit perhaps poorly. Alternatively, if L27′ was sufficiently abundant but unable to compete for assembly, absence of endogenous yeast L29 would eliminate the competitive disadvantage and also permit L27′ to rescue L29-deficient cells.

The L27′ cDNA was inserted adjacent to the constitutive alcohol dehydrogenase promoter in plasmid pVT102L (33), yielding pVTL27′. Both pVTL27′ and pVT102L were introduced into HF194GCYHX23; this yeast strain had a chromosomal copy of *cyh2* under control of the *GAL10* promoter (30) and consequently was unable to produce ribosomal protein L29 unless grown on galactose. Fig. 3 shows that X23 cells transformed with pVT102L (lacking the L27′ cDNA) grew on galactose but were unable to grow on glucose. However, the same strain transformed with pVTL27′ grew on both carbon sources. This result demonstrated that, indeed, mouse ribosomal protein L27′ was able to substitute for L29 and lead to formation of functional ribosomes. To demonstrate that L27′ had actually become incorporated into the ribosomes, we replica-plated the transformants onto both glucose and galactose media containing cycloheximide at 5 μg/ml. Recall that the recipient strain possessed the *cyh2* allele expressed from the *GAL10* promoter. On galactose, HF194GCYHX23 carrying the L27′ gene grew in the presence of cycloheximide, as expected since *cyh2* was expressed and L27′ was shown not to affect cycloheximide resistance when L29 was also produced. However, on glucose plus cycloheximide, a condition in which L29 was not produced, the same cells now failed to grow, implying that mouse ribosomal protein L27′ had replaced yeast ribosomal protein L29, the only polypeptide in the host yeast strain capable of conferring cycloheximide resistance. To obtain still further confirmation of functional substitution of L29 by murine L27′, transformants were grown in the presence of leucine, a nonselective condition that permitted loss of the *LEU2*-containing pVTL27′ plasmid. All cells that had lost the plasmid, indicated by reversion to leucine auxotrophy, also gave rise to colonies unable to grow on glucose (data not shown). This simultaneous loss of leucine prototrophy and the ability to grow without galactose demonstrated that the plasmid-borne L27′ gene had complemented the L29 deficiency.

To assess whether yeast ribosomes assembled with L27′ were as efficient as L29-containing ribosomes, we compared the growth rate of HF194GCYHX23 transformed with pVTL27′ on galactose and glucose to the growth rate of the same strain transformed with a multicopy plasmid carrying the L29 gene. The growth rates of these strains were the same regardless of plasmid or carbon source (data not shown). Thus, at least in the presence of abundant mouse L27′, the functioning of the ribosome was sufficient to maintain normal growth. Ribosomes assembled with L27′ also did not exhibit

temperature or cold sensitivity, since cells were unaffected in growth at either 37°C or 14°C (data not shown).

In summary, mouse ribosomal protein L27′ was found to be equivalent to yeast ribosomal protein L29 in formation of functional yeast ribosomes. However, L27′ apparently was unable to assemble into yeast ribosomes as long as L29 was also present in the same cell.

DISCUSSION

In this report we show that yeast ribosomal protein L29 can be replaced by ribosomal protein L27′ from the mouse. This conclusion was based on the finding that yeast cells deprived of L29 grew normally when provided with the L27′ gene; without L27′, the same L29-deficient cells were inviable. Second, we made use of the cycloheximide-resistant phenotype conferred by one allele of the L29 gene (*cyh2*) to demonstrate that in cells producing both L29 and L27′, the L29 protein was assembled to the exclusion of L27′. This conclusion originated from the fact that the mouse gene, encoding wild-type L27′, failed to alter the cycloheximide-resistant phenotype of a *cyh2* strain.

Murine L27′ was found to share 62% strict identity to yeast L29 and 74% homology when conservative amino acid changes were considered (29). It is therefore not overly surprising that the two proteins should be interchangeable. Nearly 10 years ago, Wejksnora and Warner (37) demonstrated that hybrid cells formed by fusion of mouse 3T3 cells and Chinese hamster ovary (CHO) cells assembled hybrid ribosomes built of a mixture of mouse and hamster components. Recently, Rhoads and Roufa (38) showed that human ribosomal protein S14 could assemble into ribosomes of CHO cells. In CHO cells a mutant allele of S14 confers resistance to emetine. Rhoads and Roufa found that introduction of the (wild-type) human S14 gene into emetine-resistant cells converted those cells to emetine sensitivity, indicating that the human counterpart of S14 had assembled into some fraction of the CHO ribosomes. Unlike the assembly of mouse L27′ into yeast ribosomes, the human S14 protein was not excluded from assembly in the presence of the indigenous CHO S14 protein.

Since exclusion of murine L27′ from the yeast ribosome was inferred from maintenance of the cycloheximide-resistant phenotype of *cyh2* cells transformed with the L27′ gene, it could be argued that presence of the drug inadvertently selected for exclusive assembly of L29 so as to remain cycloheximide-resistant. This possibility is unlikely since *cyh2/CYH2* diploid cells (analogous to L29/L27′ cells) are sensitive to very low concentrations of cycloheximide (1.0–2.0 μg/ml; see ref. 28). Furthermore, introduction of additional copies of *cyh2* genes into a *CYH2* strain increases resistance only proportionately to *cyh2* gene dosage, and resistance is never as great as that of a strain having only *cyh2* alleles (>10.0 μg/ml; see ref. 28). Thus, presence of a *cyh2* allele in a cell also synthesizing the wild-type version of L29 is not sufficient for cycloheximide to select for a population of fully resistant cells when both versions of the protein are equally proficient in assembly. We therefore concluded that L27′ must have been unable to assemble in the presence of L29 in order for cells to remain as resistant as untransformed *cyh2* cells. Of course, the presence of cycloheximide may have promoted greater exclusion of an already assembly-impaired L27′ protein than would have occurred otherwise in the absence of the inhibitor.

Exclusion of L27′ from yeast ribosomes in the presence of L29 may have occurred either during transport of the protein into the nucleus or during formation of the large ribosomal subunit. Two 7 amino acid segments responsible for the nuclear localization of L29 have been deduced (M. Underwood and H.M.F., unpublished results). Sequence comparison reveals almost perfect identity between L29 and L27′ within the two nuclear localization sequences, diverging only in a single residue in which leucine replaces histidine within one of the two sequences. A number of silent amino acid substitutions have been produced in the yeast L29 nuclear localization segments (M. Underwood and H.M.F., unpublished results), but it is not known whether a His → Leu change is deleterious as far as function of the nuclear localization segment is concerned. Studies with simian virus 40 and polyoma virus tumor (T)-antigen proteins have shown that the rate of nuclear uptake is dependent upon the number of nuclear localization sequences contained within a protein (39–42); multiple localization sequences facilitate receptor recognition at the nuclear pore (42). Possibly the murine L27′ protein has but one functional yeast nuclear localization sequence (the other, with His → Leu, being nonfunctional) and is therefore at a competitive disadvantage during nuclear uptake.

Finally, it should be borne in mind that we did not show the mouse L27′ protein actually to have been in the finished yeast ribosome, only that L27′ supported growth of an L29-deficient cell. Ribosomes lacking L29 may indeed be functional, as is the case for *E. coli* ribosomes lacking one of a number of different proteins (43). Even though L29 does end up as part of the large subunit, inviability due to its absence simply may result from an inability to maintain the proper pathway of ribosome assembly. The evidence in favor of murine L27′ actually having become part of the yeast ribosome is the fact that L29-deficient cells, when supplied with L27′, were sensitive to cycloheximide. L29 is assumed to be the site of cycloheximide binding to the ribosome, since resistance to the inhibitor results from amino acid substitutions that convert the uncharged Gln-37 of L29 to a charged residue (lysine in one case and glutamic acid in another; refs. 44 and 45). Replacement at position 37 by a charged residue presumably prevents inhibitor binding. Wild-type L27′ possesses an uncharged amino acid at position 37, namely methionine, and it seems reasonable to conclude that sensitivity to cycloheximide was due to the presence of L27′ in the ribosome itself.

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1. Moldave, K. (1985) *Annu. Rev. Biochem.* **54,** 1109–1149.
2. Vazquez, D. & Jimenez, A. (1980) in *Ribosomes: Structure, Function, and Genetics*, eds. Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L. & Nomura, M. (University Park Press, Baltimore), pp. 847–869.
3. Nomura, M., Mizushima, S., Ozaki, M., Traub, P. & Lowry, C. V. (1969) *Cold Spring Harbor Symp. Quant. Biol.* **34,** 49–61.
4. Nierhaus, K. H. (1980) in *Ribosomes: Structure, Function, and Genetics*, eds. Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L. & Nomura, M. (University Park Press, Baltimore), pp. 267–294.
5. Spirin, A. (1986) *Ribosome Structure and Protein Biosynthesis* (Benjamin/Cummings, Menlo Park, CA), pp. 187–215.
6. Bielka, H. (1982) *The Eukaryotic Ribosome* (Springer, New York), pp. 172–176.
7. Cox, R. A. & Greenwall, P. (1980) *Biochem. J.* **186,** 861–872.
8. Vioque, A. & Palacian, E. (1985) *Mol. Cell. Biochem.* **66,** 55–60.
9. Lee, J. C. & Anderson, R. (1986) *Arch. Biochem. Biophys.* **245,** 248–253.
10. Altamura, S., Cammarano, P. & Londei, P. (1986) *FEBS Lett.* **204,** 129–133.
11. Lake, J. A. (1983) in *Progress in Nucleic Acids Research and Molecular Biology*, eds. Cohn, W. E. & Moldave, K. (Academic, New York), Vol. 30, pp. 163–194.
12. Lake, J. A. (1985) *Annu. Rev. Biochem.* **54,** 507–530.
13. Matheson, A. T., Moller, W., Amons, R. & Yaguchi, M. (1980) in *Ribosomes: Structure, Function, and Genetics*, eds. Cham-

bliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L. & Nomura, M. (University Park Press, Baltimore), pp. 297–332.
14. Noller, H. F. (1984) *Annu. Rev. Biochem.* **53,** 119–162.
15. Svoboda, A. J. & McConkey, E. H. (1981) *Eur. J. Biochem.* **116,** 397–402.
16. Gourse, R. L., Thurlow, D. L., Gerbi, S. A. & Zimmermann, R. A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2722–2726.
17. El-Baradi, T. T. A. L., Raue, H. A., de Regt, V. C. H. F., Verbree, E. C. & Planta, R. J. (1985) *EMBO J.* **4,** 2101–2107.
18. El-Baradi, T. T. A. L., de Regt, V. C., Einerhand, S. W., Teixido, J., Planta, R. J., Ballestra, J. P. & Raue, H. A. (1987) *J. Mol. Biol.* **195,** 909–917.
19. Lin, A., McNally, J. & Wool, I. G. (1983) *J. Biol. Chem.* **258,** 10664–10671.
20. Sor, F. & Nomura, M. (1987) *Mol. Gen. Genet.* **210,** 52–59.
21. Larkin, J. C., Thompson, J. R. & Woolford, J. L. (1987) *Mol. Cell. Biol.* **7,** 1764–1775.
22. Leer, R. J., van Raamsdonk-Duin, M. M. C., Molenaar, C. M. T., Cohen, L. H., Mager, W. H. & Planta, R. J. (1982) *Nucleic Acids Res.* **10,** 5869–5878.
23. Nakanishi, O., Oyanagi, M., Kuwano, Y., Tanaka, T., Nakayama, T., Mitsui, H., Nabeshima, Y. & Okata, K. (1985) *Gene* **35,** 289–296.
24. Itoh, T., Otaka, E. & Matsui, K. A. (1985) *Biochemistry* **24,** 7418–7423.
25. Nielsen, H., Andreasen, P. H., Dreisig, H., Kristiansen, K. & Engberg, J. (1986) *EMBO J.* **5,** 2711–2717.
26. Mortimer, R. K. & Hawthorne, D. C. (1973) *Genetics* **74,** 33–54.
27. McLaughlin, C. S. (1974) in *Ribosomes*, eds. Nomura, M., Tissieres, A. & Lengyel, P. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 815–827.
28. Fried, H. M. & Warner, J. R. (1982) *Nucleic Acids Res.* **10,** 3133–3148.
29. Belhumeur, P., Paterno, G. D., Boileau, G., Claverie, J.-M. & Skup, D. (1987) *Nucleic Acids Res.* **15,** 1019–1029.
30. Fried, H. M., Nam, H. G., Loechel, S. & Teem, J. (1985) *Mol. Cell. Biol.* **5,** 99–108.
31. Boguslawski, G. (1985) in *Gene Manipulations in Fungi*, eds. Bennett, J. W. & Lasure, L. L. (Academic, New York), pp. 161–195.
32. Johnston, M. & Davis, R. W. (1984) *Mol. Cell. Biol.* **4,** 1440–1448.
33. Vernet, T., Dignard, D. & Thomas, D. Y. (1987) *Gene* **52,** 225–233.
34. Warner, J. R., Mitra, G., Schwindinger, W. F., Studeny, M. & Fried, H. M. (1985) *Mol. Cell. Biol.* **5,** 1512–1521.
35. Kozak, M. (1984) *Nature (London)* **308,** 241–246.
36. Cigan, M. A. & Donohue, T. F. (1987) *Gene* **59,** 1–18.
37. Wejksnora, P. J. & Warner, J. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5554–5558.
38. Rhoads, D. D. & Roufa, D. J. (1987) *Mol. Cell. Biol.* **7,** 3767–3774.
39. Dingwall, C., Sharnick, S. V. & Laskey, R. A. (1982) *Cell* **30,** 449–458.
40. Landford, R. E., Kanda, P. & Kennedy, R. C. (1986) *Cell* **46,** 575–582.
41. Roberts, B. L., Richardson, W. D. & Smith, A. E. (1987) *Cell* **50,** 465–475.
42. Newmeyer, D. D. & Forbes, D. J. (1988) *Cell* **52,** 641–653.
43. Dabbs, E. R. (1985) in *Structure, Function, and Genetics of Ribosomes*, eds. Hardesty, B. & Kramer, G. (Springer, New York), pp. 733–748.
44. Stocklein, W. & Piepersberg, W. (1980) *Curr. Genet.* **1,** 177–183.
45. Kaufer, N. F., Fried, H. M., Schwindinger, W. F., Jasin, M. & Warner, J. R. (1983) *Nucleic Acids Res.* **11,** 3123–3135.