The epidermal permeability barrier is composed of intercellular lipids in the stratum corneum and protects skin from dessication. After acute permeability barrier disruption, induced experimentally by either tape stripping or topical acetone treatment, the barrier is restored by a coordinate process involving increased lipid synthesis, cytokine and growth factor production, and DNA replication. Whereas many of these repair responses are linked directly to an increase in transepidermal water loss (a measure of barrier integrity), others, such as increased pro-inflammatory cytokine production, may be the result of the attendant epidermal cell injury that accompanies barrier disruption. Utilizing differential display, we have identified a protein whose mRNA was up-regulated after acute permeability barrier disruption. The corresponding cDNA was cloned and sequenced. Here we report that based on its high degree of predicted amino acid sequence identity (>97%), the cDNA is the murine ortholog of the recently cloned human translation initiation factor 6 (eIF6) for which a yeast homolog has been identified.

The role of eIF6 in translation is not firmly established. 80 S ribosomal subunits, which are released from polysomes after translation termination, are in equilibrium with 40 S and 60 S subunits. Purified eIF6 from rabbit reticulocyte lysates binds to the 60 S ribosomal subunit and exhibits ribosome anti-association activity in an in vitro assay (11). eIF6 and another translation initiation factor, eIF3 (a multiprotein complex that also exhibits anti-association activity via its interaction with the 40 S ribosomal subunit) are thought to regulate the supply of ribosomal subunits necessary for translation initiation (10, 12).

Interestingly, in the budding yeast Saccharomyces cerevisiae, temperature-sensitive mutants of other translation initiation factors (eIF4E (mRNA cap-binding protein) and eIF3h (a component of eIF3)) arrest in G1 and are classified as cell division cycle (cdc) mutants cdc33 and cdc63, respectively (13, 14). In addition to isolating the full-length cDNA sequence of murine eIF6 and reporting the up-regulation of murine eIF6 mRNA levels in response to epidermal injury, we initiated studies using S. cerevisiae as a model to begin to understand the role(s) of eIF6 in the cell. We show here that complete deletion of the gene for the yeast eIF6 homolog YPR016c from S. cerevisiae is lethal, indicating that YPR016c is essential. Furthermore, expression of either YPR016c-GFP or murine eIF6-GFP fusion proteins restored viability, suggesting that the YPR016c gene product is indeed yeast eIF6. A nuclear/perinuclear localization of the eIF6-GFP fusion proteins in yeast is also reported. Finally, we show that depletion of eIF6 results in reduced polysome size and dramatically reduces free 60 S ribosomal subunit content. Taken together, these results suggest that eIF6 plays a role in 60 S ribosomal subunit assembly, stability, or nucleo-cytoplasmic transport.

**EXPERIMENTAL PROCEDURES**

**Materials**—Mouse total RNA from assorted tissues was purchased from Ambion. Oligonucleotides were purchased from Operon. RX Fuji media and chemicals were purchased from Sigma or Fisher. RX Fuji

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EBI Data Bank with accession number(s) AP047046.

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Polymerase Chain Reaction—PCR reactions were performed using the Advantage cDNA Polymerase Mix (CLONTECH Labs, Inc) according to the manufacturer’s instructions. All reactions were performed in a model TC480 DNA thermal cycler (Perkin Elmer Corp.).

Deletion of the Yeast eIF6 Homolog, YPR016c—Standard procedures were employed for all studies in yeast (16). Saccharomyces cerevisiae strain CBS8/13 (see Table I), a derivative of S288c (kindly provided by C. Beh, University of CA, Berkeley), was used for one-step gene replacement (17) of the yeast eIF6 homolog, YPR016c. Briefly, the TRP1 gene was PCR-amplified from linearized plasmid pRS404 (18) using primers 5'-GAGCATTTGGGAAACAAGACGATACTTCACATCTGGAACAAACAAATACGTCCTACGATGCGCATC-3' and 5'-AAACAGACTTGAGGGAAGGAGGATACCTGGATCGTGTTATCTTCACACCG-3'. The 1.0kb PCR product, designed for homologous recombination at the YPR016c locus, was transformed into yeast by the lithium acetate procedure (19). Tryptophan prototrophs were isolated from minimal plates (SD). The YPR016c deletion was confirmed by PCR analysis using primers which flank the integration site: 5'-CCCATATGTCCTTTGTGCAAG-3' and 5'-GTCACTATAATATACACACACACACGT-3'. A TRP+ heterozygous (diploid) colony, which contained the expected 1065-bp wild type YPR016c PCR product and the 1330-bp PCR product (where the TRP1 gene had replaced the YPR016c ORF) was sporulated and subjected to tetrad analysis.

Plasmid and Strain Constructions—pLW-1, a yeast expression plasmid that contains the GAL1 promoter in front of an amino-terminal fusion of yeast YPR016c and GFP (20), was constructed as follows. The YPR016c ORF was amplified by PCR from S288c yeast genomic DNA using 5'-CGCGCCGCCGGCAGCTAAATTTGAAACTCAATGAAA-3' and 5'-CGCGCCGCCGGCAGTGGTTTTTGCTAAAGATCATGACG-3'. The resulting PCR product, with Fse1 sites engineered on both ends, was cut with Fse1 and ligated into the Fse1 site of pACA1580 to create a GAL1-regulated YPR016c-GFP fusion.

pLW-2 contains 990 bp upstream of the initiating ATG with the YPR016c ORF to create an amino-terminal fusion of yeast YPR016c and GFP (20), was constructed as follows. The YPR016c ORF was amplified by PCR from S288c yeast genomic DNA using 5'-CGCGCCGCCGGCAGCTAAATTTGAAACTCAATGAAA-3' and 5'-CGCGCCGCCGGCAGTGGTTTTTGCTAAAGATCATGACG-3'. The resulting PCR product, with Fse1 sites engineered on both ends, was digested with these restriction enzymes and ligated into the pSpI and Fse1 sites of plasmid pPJ1.

pCWL1 was constructed for GAL1-regulated expression of murine eIF6 as an amino-terminal fusion to GFP in yeast and was constructed as follows. eIF6 was PCR-amplified from mouse liver Marathon-Ready cDNA (CLONTECH) using primers 5'-CGCGCCGCCGGCAGCTAAATTTGAAACTCAATGAAA-3' and 5'-CGCGCCGCCGGCAGTGGTTTTTGCTAAAGATCATGACG-3'. The resulting PCR product, with Fse1 sites engineered on both ends, was ligated into the Fse1 site of pACA1580.

pLW-5 contains a genomic DNA fragment including the YPR016c gene. This plasmid was isolated from a genomic library (2 μ, LEU2) by its ability to suppress the lethality of a yrpr016cΔ:TRP1 deletion in a multicopy suppression screen.

To assess the functional interchangeability of murine eIF6 with the presumptive yeast counterpart Ypr016c, we carried out a plasmid shuffling experiment. LWX3, which contains the yrpr016cΔ::TRP1 deletion and is viable by virtue of the presence of pLW-5 (YPR016cΔ, LEU2), was transformed with either pLW-1 (GAL1-YPR016c-GFP, URA3) or pCWL1 (GAL1-murine eIF6-GFP, URA3). The two transformants, LWX5 and LWY7 (also described in Table I), were grown under non-selective conditions in the presence of leucine and galactose, and the yeast uracil prototrophs LWX4 and LWY6, which maintained pLW-1 and

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### Table 1: Yeast Strains

| Name      | Genotype                  |
|-----------|---------------------------|
| CBS8/13   | MATa ura3-52 ura3-52 trp1Δ-63 leu2Δ-1 his3Δ-200 mal/mal hap2/hap2 |
Fluorescence-activated Cell Sorting—Yeast were grown in 5Gal + amino acid supplements and shook overnight at 30 °C. The next morning, cells were centrifuged and then resuspended in 5SD + amino acid supplements to A_{600} = 0.05 and incubated at 30 °C for as long as 24 h. Cells were harvested in log phase (A_{600} < 1.0) by centrifugation and washed once with water. Cells were then fixed in 500 μl of 7% EtOH for either 1 h at room temperature or overnight at 4 °C. They were then washed twice in 20× TE (10 mM Tris, 1 mM EDTA, pH 8.0) and resuspended in 100 μl of 20× TE + RNase A (1 mg/ml), and incubation was continued for 4 h at 37 °C. Finally, cells were washed two times with phosphate-buffered saline, resuspended in 50 μl of phosphate-buffered saline and 100 μg/ml propidium iodide, and incubated overnight at 4 °C in the dark. Before FACS analysis, cells were diluted 10–20-fold in phosphate-buffered saline. A Becton-Dickinson FACScan benchtop cytometer was utilized at the Laboratory for Cell Analysis (University of California, San Francisco Mount Zion Cancer Center, San Francisco, CA).

Polysome/Ribosomal Subunit Profile Analysis—Analyses of polysome/ribosomal subunit profiles in strain LWY4 grown either in 5Gal (eIF6- induced) or for 16 h in 5SD (eIF6-depleted) were performed exactly as described by Zanchin et al. (21), with the following exceptions. Cell extracts were isolated from 200-ml cultures grown to mid-exponential phase. Cycloheximide was added (10 mg/ml), and cells were centrifuged, washed once, and resuspended in breaking buffer (as described previously) that also contained phenylmethylsulfonyl fluoride (17 μg/ml). After cell lysis and centrifugation (as described previously), identical amounts of cell extracts (20 A_{600} units) were loaded onto 12-mL linear sucrose gradients (10–50%). After a 3-h centrifugation, the gradients were fractionated manually into 0.22-mL fractions starting from the bottom of the tubes. A spectrophotometer was used to monitor absorbance at 254 nm.

Fluorescence Microscopy—GFP and 4,6-diamidino-2-phenylindole-dihydrochloride fluorescence was evaluated in living yeast using a Nikon Microphot fluorescence microscope. Yeast were incubated in the presence of 1 μg/ml 4,6-diamidino-2-phenylindole-dihydrochloride while being grown in liquid culture for 1 h before microscopic analysis. The control yeast strain used in the fluorescence microscopic studies was strain CBS13 (see Table I) harboring pACA1580.

RESULTS
Isolation of Murine eIF6 cDNA—RNA fingerprinting was used to identify epidermal mRNAs that are differentially expressed 2 h after acute permeability barrier disruption by tape stripping. We chose this time point because previous work showed that mRNAs encoding proteins involved in permeability barrier repair are up-regulated within 0.5–4 h after acute barrier disruption (2–5). Fig. 1 displays the RNA fingerprinting results (for details, see “Experimental Procedures”). The cDNA band labeled clw1 was more abundant in the tape-stripped samples; therefore, it was isolated from the gel and PCR-amplified, and the purified clw1 subclone (isolation was described under “Experimental Procedures”) was used to probe an epidermal RNA blot.

A RNA blot was prepared from the epidermis of outbred hairless mice that were subjected to acute barrier disruption by tape stripping. A single 1.3-kb mRNA hybridized with the 150-bp partial clw1 cDNA probe (Fig. 2). Furthermore, the levels of this epidermal mRNA (clw1) were increased after tape stripping, confirming the differential expression of clw1 mRNA in response to barrier disruption. Subsequent Northern analysis using the partial clw1 cDNA as a probe revealed that a single cross hybridizing 1.3-kb mRNA was present in a wide variety of mouse tissues.2

We cloned the full-length clw1 cDNA from mouse liver by 5' and 3' RACE. Based on the high degree (>97%) of predicted amino acid sequence identity, it is likely that clw1 cDNA encodes the murine ortholog of human eIF6 (9). The mouse liver eIF6 cDNA sequence has been submitted to GenBank (accession number AF047046). Analysis of the amino acid sequence of murine eIF6 does not reveal any obvious clues as to its function in translation. However, murine eIF6 and the human, yeast, and Drosophila presumptive homologs contain a potential nuclear export signal, LSSLIQVPLVA, which conforms to the consensus nuclear export signal described as a short, leucine-rich, hydrophobic sequence (22). Whether this consensus sequence is functional is not known at the present time.

Functional Studies of a eIF6 Homologous Sequence in S. cerevisiae—We initiated studies in S. cerevisiae to delineate the cellular function(s) of eIF6. As a first step, the entire YPR016c ORF was replaced with the Δ:TRP1 gene to assess the phenotype of Ypr016c (eIF6) knockout yeast. Tetrad dissection of 18 ascis from the heterozygous diploid LWY1 (ypr016c Δ::TRP1/ YPR016c) resulted in a segregation pattern of two live spores and two dead spores. None of the viable segregants were TRP+, indicating that YPR016c is required for viability. Microscopic examination of the meiotic segregants revealed that the ypr016c Δ::TRP1 cells had arrested between the second and fourth cell divisions.2

The diploid strain LWY1 (ypr016c Δ::TRP1/YPR016c) was transformed with pLW-2 and sporulated. pLW-2 expresses a

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2 L. C. Wood, C. Grunfeld, and K. R. Feingold, unpublished results.
YPR016c-GFP fusion protein under the control of the endogenous YPR016c promoter. Tetrad analysis of LWY1 harboring pLW-2 produced four viable segregants (two TRP+ segregants and two TRP− segregants), indicating that the YPR016c-GFP fusion protein was functional and could substitute for the endogenous YPR016c protein product.

To determine whether murine eIF6 could functionally replace YPR016c in yeast, ypr016c::TRP1 strains that contained either YPR016c (LWY4) or murine eIF6 (LWY6) expressed as GAL1-regulated GFP fusion proteins were constructed. The growth characteristics of LWY4 and LWY6 on SGal, conditions that up-regulate GAL1 promoter activity, were compared with a control strain (CBS13 harboring GAL1-GFP) in Fig. 3. As shown in Fig. 3 (left side), the growth rates of LWY4 (harboring GAL1-YPR016c-GFP) and LWY6 (harboring GAL1-murine eIF6-GFP) versus control appeared somewhat slower on SGal, indicating that overexpression of the eIF6-GFP fusion proteins had no major effects on cell growth. This result also indicated that the murine eIF6-GFP fusion protein functionally replaced YPR016c gene function. When the growth rates of these strains were monitored in liquid SGal for three to four generations, both LWY4 and control yeast exhibited a doubling time of 4 h, whereas the doubling time for LWY6 was 6 h. Importantly, when LWY4 and LWY6 were grown on SD (Fig. 3, right side), conditions that repress GAL1 promoter activity, cell growth was arrested. This result demonstrated that the cells were dependent upon GAL1-regulated expression of either the YPR016c-GFP (in LWY4) or the murine eIF6-GFP (in LWY6) fusion proteins for viability.

Localization of murine eIF6-GFP and YPR016c-GFP Fusion Proteins in Yeast—We examined live cells by fluorescence microscopy to localize the eIF6-GFP fusion proteins in strains LWY2, LWY4, and LWY6. In LWY2, we did not detect any GFP fluorescence above the background (data not shown), in agreement with previous findings showing that eIF6 is in very low abundance intracellularly (11). However, upon examination of nonsynchronous LWY4 and LWY6 cells during log-phase growth in liquid SGal, a major portion of the GFP fluorescence was concentrated in a nuclear/perinuclear location that colocalized with nuclear 4',6-diamidine-2-phenylindole-dihydrochloride staining (Fig. 4 C, D, G, and H). In contrast, GFP fluorescence in control yeast (grown under the same conditions) that harbor the parent plasmid was localized uniformly throughout the yeast cell (Fig. 4A).

Because we only observed GFP fluorescence when the eIF6-GFP fusion proteins were overexpressed, we next determined whether the repression of YPR016c-GFP expression by incubating LWY4 cells in decreasing amounts of galactose would alter the GFP fluorescence pattern in living yeast. For these determinations, LWY4 and control cells were incubated during log phase (A600 < 1.0) for 4 h in either SGal or SGal:SRaf (1:2); SRaf contains 6.7 g/liter yeast nitrogen base without amino acids, 20 g/liter raffinose), conditions that did not alter growth rates (data not shown), and then examined by fluorescence microscopy. Again, a distinct nuclear/perinuclear localization of YPR016c-GFP was detected in LWY4 cells grown in SGal:SRaf (Fig. 4E). The localization of GFP fluorescence in control yeast (harboring pACA1580) grown in SGal:SRaf (1:2) was nearly identical to that shown in Fig. 4A and was therefore omitted.

Because temperature-sensitive mutants of two other translation initiation factors (cdc33 and cdc63) arrest in G1, we performed FACS analysis of the conditional mutant strain LWY4. For these determinations, LWY4 was grown for up to 24 h in SD, conditions that strongly repress YPR016c-GFP expression. For comparison, the control LWYS strain was analyzed at the same A600 reading after only 5–7 h in SD. These different time points were chosen because 3–4 h after the switch from SGal to SD, the growth of LWY4 begins to decline in comparison to that of LWY5. No differences in FACS
profiles were detected when LWY4 cells were grown for 1, 4, or 8 h in SD as compared with control LWY5 cells. However, FACS profiles showed that the eIF6 conditionally mutant strain LWY4 arrested in G1 after several generations in SD (after either 16 h or 24 h; Fig. 5). Thus, depletion of eIF6 from *S. cerevisiae* resulted in the same cell division cycle phenotype as reported for two other translation initiation factors, *cdc33* and *cdc63*. Based on this result, we have designated the genetic locus YPR016c as *cdc95*. In addition, by forward angle scattering, we also determined that the relative size of LWY4 cells grown for several generations in SD is the same as that of LWY5 (control) cells grown to the same *A*$_{600}$ (data not shown).

The effect of eIF6 depletion on translation initiation was determined by examining the polysome/ribosomal subunit profiles obtained from strain LWY4 grown in either SGal (eIF6-induced) or SD (eIF6-depleted). For these studies, LWY4 was grown to mid-exponential phase for 16 h (three to four generations) in either SGal or SD medium, cells were harvested in the presence of cycloheximide, and sucrose density gradient analyses were performed using previously published procedures (21). Fig. 6 displays one set of representative results obtained from three separate experiments. Peaks representing polysomes, monosomes, and ribosomal subunits are labeled as follows: *s*, small ribosomal subunit (40 S); *l*, large ribosomal subunit (60 S); *m*, monoribosomes; *p*, polysomes.

Although more difficult to quantitate, the pool of free 60 S subunits was greatly reduced as well. The pool of free 40 S subunits varied between experiments (increasing in some and decreasing in others) but remained, on average, roughly constant. The decrease in polysome size could reflect either a decrease in the cellular content of 60 S subunits (which would appear as an initiation defect) or a decrease in ribosomes (40 S and 60 S subunits) if the mRNA content remained the same. The latter seems to best account for the observed profiles. These results, when taken together with the nuclear/perinuclear localization of eIF6-GFP fusion proteins, are consistent with a role for eIF6 in 60 S ribosomal subunit assembly, stability, or nucleo-cytoplasmic transport.

**DISCUSSION**

During initiation, which is generally the rate-limiting step in translation, a number of soluble protein factors interact tran-
siently with macromolecular complexes consisting of ribosomes, aminoacyl-tRNAs, and messenger ribonucleoproteins and form translation initiation complexes (10). These initiation factors are known to exert control over total protein synthesis; however, these factors may also play key roles in the nucleus and/or in the regulation of the cell cycle. The current model is largely based on reconstitution experiments using purified factors in conjunction with macromolecular complexes. Whereas the role of some of these factors is firmly established, many aspects of the translation initiation model require in vivo confirmation that can be obtained using \textit{S. cerevisiae} and its attendant genetic and molecular biological tools.

In this study, we report the cloning of murine eIF6, a translation initiation factor that was previously identified in rabbit reticulocyte lysates by its activity in preventing ribosomal subunit association (11). The predicted amino acid sequence of murine eIF6 is >97\% identical to eIF6 from human skeletal muscle (9). Several presumptive eIF6 homologs were identified by BLAST search, and an examination of their amino acid sequences indicated that this protein is highly conserved throughout evolution (9). Interestingly, the archaeabacterial \textit{S. acidocaldarius} eIF6 homolog is transcribed as part of a polycistronic mRNA that also encodes two large ribosomal subunit proteins, RL46 and RL31 (24).

Our functional studies of eIF6 in \textit{S. cerevisiae} revealed new insights regarding the cellular role(s) of this highly conserved protein. Deletion of the yeast eIF6 homolog \textit{YPR016c} was lethal, indicating that \textit{YPR016c} is an essential gene. Furthermore, \textit{YPR016c} was functionally replaced by murine eIF6 when expressed as an amino-terminal GFP fusion protein, indicating that the \textit{YPR016c} locus encodes the yeast eIF6. We found that a substantial fraction of the murine and yeast eIF6-GFP fusion proteins was localized in a nuclear/perinuclear compartment. This localization is consistent with a putative role for eIF6 in the coordination of nuclear and cytoplasmic events. A nuclear localization has been reported for two other eIFs, namely, eIF5A (25) and eIF4E-4E (26).

Depletion of yeast eIF6-GFP in the conditionally mutant strain LWY4 resulted in decreased polysome content, a dramatic reduction in 60 S ribosomal subunit content, and diminished 80 S monoribosome content. When taken together with the nuclear/perinuclear localization of eIF6-GFP fusion proteins reported herein, these results suggest that eIF6 plays a key role in 60 S subunit assembly, stability, or nucleo-cytoplasmic transport. Other yeast mutants in nonribosomal protein genes that are defective in 60 S subunit assembly have been reported previously (21, 27, 28). Whether eIF6 plays a direct role in the initiation phase of translation or functions primarily to provide physiologic quantities of stable 60 S ribosomal subunits remains to be determined.

Depletion of eIF6-GFP also caused G\textsubscript{1} arrest, as has been reported for temperature-sensitive alleles of two other yeast eIFs, \textit{cdc63} (which encodes the \eta subunit of eIF3; Refs. 14 and 23) and \textit{cdc33} (which encodes eIF4E or mRNA cap-binding protein; Ref. 13). Furthermore, temperature-sensitive mutants in two other protein components of the eIF3 complex also arrest in G\textsubscript{1} (29, 30). Because depletion of yeast eIF6 caused a cell division cycle type of arrest, we designated the \textit{YPR016c} locus as \textit{cdc65}.

In yeast, as in mammalian cells, critical growth requirements in G\textsubscript{1} must be met for the cells to enter S phase and replicate their DNA (31). However, experiments that used protein synthesis inhibitors such as cycloheximide demonstrated that protein synthesis is required for progression through all stages of the yeast cell cycle (32, 33). Why then, would depletion of three different eIFs in the cell cause a specific arrest in G\textsubscript{1}?

Each of the three eIFs in question is thought to function by providing critical components of translation initiation complexes. One role ascribed to eIF3 is the dissociation of 80 S ribosomes into 40 S and 60 S subunits (12, 34). Our results in yeast show that eIF6 plays a key role in determining free 60 S ribosomal subunit content, whereas eIF4E plays a role in the recruitment of capped mRNAs to the translation initiation complex (10). It has been shown that when alterations such as mutations in eIF3 in \textit{cdc63} cells (35) cause translation initiation complexes to become rate limiting, inefficiently translated mRNAs are affected preferentially (36). One such mRNA encodes the \text{G}_{s}-specific cyclin Cln3p, which is necessary for the \text{G}_{s}-\text{S}-phase transition.

Polyenesis and Schmidt (35) recently demonstrated that the translational regulation of \textit{CLN3} provides a mechanism to link cell growth and cell division. They showed that a translational control element (an upstream ORF) in the 5’ leader of \textit{CLN3} mRNA functions to diminish \textit{CLN3} expression in either \textit{cdc63} cells or poor growth media, conditions in which translation initiation components are low or rate-limiting. Because the results from our gradient analyses indicate that eIF6 functions to provide free 60 S ribosomal subunits for translation, we predict that \textit{CLN3} mRNA would also be inefficiently translated in cells in which \textit{YPR016c} expression is conditionally repressed. Translational regulation of another cell cycle-regulatory molecule, the cyclin-dependent kinase inhibitor p27\(^{kip1}\), has also been reported (37, 38).

The mechanism(s) responsible for the relatively rapid increase of eIF6 mRNA levels in murine epidermis after tissue injury is(are) yet to be determined. It is tempting to speculate that cytokines may be the mediators, because these molecules are known to be produced rapidly in response to cutaneous barrier disruption (3, 4, 39, 40). It will be of interest to determine whether other types of genotoxic stress, such as UV irradiation and DNA-damaging agents, also affect eIF6 expression in yeast and mammalian tissues. It is also important to determine the downstream consequences of increased cellular eIF6 levels in relation to protein synthesis, in particular.

Recent studies have shown additional roles for known eIFs. For example, eIF5A is believed to mediate the nucleo-cytoplasmic transport of specific cellular RNAs and ribonucleoproteins through the nuclear pores (25, 41, 42). It will therefore be of interest to identify proteins that interact with eIF6 to clarify the role(s) of this essential protein. The yeast two-hybrid assay may be a useful approach.

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Noted Added in Proof—During the preparation of this manuscript we became aware of the publication of the eDNA sequence for murine mast cell eIF6, also referred to as \textit{imc-415} (Cho, S. H., Cho, J.-J., Kim, I. S., Vilagofis, H., Metealf, D. D., and Oh, C. K. (1998) Biochem. Biophys. Res. Commun. 252, 123–127).

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