Members of the p56 family of mammalian proteins are strongly induced in virus-infected cells and in cells treated with interferons or double-stranded RNA. Previously, we have reported that human p56 inhibits initiation of translation by binding to the "e" subunit of eukaryotic initiation factor 3 (eIF3) and subsequently interfering with the eIF3/eIF2-GTP-Met-tRNA<sub>i</sub> (ternary complex) interaction. Here we report that mouse p56 also interferes with eIF3 functions and inhibits translation. However, the murine protein binds to the "c" subunit, not the "e" subunit, of eIF3. Consequently, it has only a marginal effect on eIF3-ternary complex interaction. Instead, the major inhibitory effect of mouse p56 is manifested at a different step of translation initiation, namely the binding of eIF4F to the 40 S ribosomal subunit-eIF3-ternary complex. Thus, mouse and human p56 proteins block different functions of eIF3 by binding to its different subunits.

One of the key features of the innate immune response is the induction of numerous cellular genes in response to viral stress. Viral stress conditions in cells are triggered by mechanisms commonly associated with a cell undergoing viral infection, such as the production of double-stranded RNA, the production of interferons, as well as other virus-mediated pathways that have yet to be elucidated. Previous studies from our laboratory have characterized the human viral stress-inducible protein p56, a 56-kDa protein (1). Human p56 (Hup56) has been shown to act as an inhibitor of protein synthesis through its association with the "e" subunit of eukaryotic initiation factor 3 (eIF3)\(^1\); the e subunit is also known as p48 or Int6) (2, 3). We have shown recently (4) that the inhibitory activity of human p56 occurs at the step of ternary complex stabilization by eIF3, a key step in the initiation pathway for protein synthesis in eukaryotes.

Eukaryotic initiation factor 3 is the largest of the 11 or more factors required for the initiation of protein synthesis in eukaryotes. eIF3 is composed of 12 subunits named eIF3a to eIF3l, although the exact stoichiometry and arrangement of the subunits are poorly understood (5). eIF3 has many functions in translation initiation, one of which is to serve as a ribosome dissociation factor by binding to the 40 S ribosomal subunit and preventing its re-association with 60 S subunits (6, 7). eIF3 also plays a role in stabilizing interactions with other components of the initiation pathway such as the ternary complex that consists of eIF2-GTP-Met-tRNA<sub>i</sub>, as well as stabilizing the formation of the 43 S complex that is formed when the ternary complex joins the 40 S ribosome (8, 9). Finally, eIF3 is also involved in binding to eIF4G of the heterotrimeric eIF4F complex, stabilizing its association with the 43 S complex (10, 11).

The p56 family of proteins includes several similar sized proteins in humans (p54, p56, p58, and p60) (12–15) as well as other species including hamster (16), mouse (17), and fish (18). The p56 family members share a structural homology consisting of a series of loosely conserved, 34-amino acid tetratricopeptide (TPR) tandem repeats (19). TPR motifs are known to mediate protein-protein interactions (20), and these motifs have been shown to be required for the interaction between human p56 and eIF3e (21). Conversely, the region of eIF3e that Hup56 interacts with is another loosely conserved structural motif known as the PCI motif. Named for Proteasome, COP9 signalosome and Initiation Factor, the three families of multi-subunit complexes that feature this motif, PCI motif-containing proteins, are potential target proteins for interaction with p56 family members based on structural homology (22). Three subunits of eIF3, "a," "c," and "e," contain PCI motifs (23, 24).

By extending the <i>in vitro</i> protein synthesis studies that showed that Hup56 can inhibit initiation of translation by interfering with eIF3 function, we followed a systematic investigation to reveal that all functions of eIF3 are not blocked by Hup56; it blocks only one specific step, namely the stabilization of the ternary complex of eIF2-GTP-Met-tRNA<sub>i</sub>, Hup56 does not interfere with the interactions of eIF3 with 40 S ribosomal subunits or eIF4F.

Because we are interested in studying the functions of the p56 family of proteins in interferon-infected animal models, we have extended our investigation to mouse (Mu)p56. Here we report that, like Hup56, Mup56 inhibited translation by binding to eIF3. However, unlike Hup56, it bound to the eIF3c (p110) subunit and not eIF3e. Consequently, Mup56 inhibited a function of eIF3 that is different from the one inhibited by Hup56.

**MATERIALS AND METHODS**

**Constructs**—The plasmid encoding full-length Hup56 was constructed by excising the full-length p56 cDNA from pBluescript KSII and inserting into pcDNA3 (Invitrogen). A full-length Mup56 plasmid for expression in mammalian cells was generated by PCR using an existing clone and then subcloning the cDNA sequence into...
Myc-pcDNA3. Its authenticity was confirmed by sequencing. This vector was generated by inserting six 30-nucleotide repeats of the c-Myc peptide in the N-terminal domain of the pcDNA3 expression vector. The construct encoding full-length Mup56 for expression in bacteria was generated by inserting the full-length cDNA for Mup56 into pET15b vector encoding a hexahistidine tag (Novagen). The full-length eIF3c construct was generated by reverse transcription-PCR and then inserting the cDNA sequence into pFLAG-CMV-2 (Kodak Scientific Imaging System). All constructs were confirmed by having the ligated junctions between vector and insert sequenced. Construction of a plasmid encoding full-length eIF3c was described previously (2).

**Antibodies**—The commercially available rabbit antibody (His Probe G-18) toward the His tag was used at a dilution of 1:666 for detection of the purified Mup56 in these studies (Santa Cruz Biotechnology). Transfected Mup56 was detected by the c-Myc 9E10 antibody at a 1:1000 dilution (Santa Cruz Biotechnology). A polyclonal antibody raised in guinea pigs against purified rabbit eIF3 was also used in these studies. At a 1:1000 dilution, this antibody recognized primarily the p110 (eIF3c) subunit of eIF3 and other eIF3 subunits to a lesser extent (4). A rabbit polyclonal antibody against Hup56 was used at a dilution of 1:2000 as described previously (2).

**Purification of Recombinant Mice p56 from Escherichia coli**—Mice p56 was purified by following the same procedure described previously for Hup56 (2), with a few modifications. A culture of E. coli was transformed with the pET15b/Mup56 construct, and the expression was induced with 1 mM isopropyl β-D-thiogalactoside for 12 h at room temperature. Mup56 was purified via nickel-affinity chromatography. Protein was then dialyzed against a high glycerol buffer (20 mM Tris, pH 7.9, 150 mM KCl, 0.5 mM dithiothreitol (DTT), 0.5 mM EDTA, 50% glycerol) and stored at −20 °C.

**Generation of Radiolabeled eIF4F**—Radiolabeled eIF4F was generated in vitro via reductive methylation using [14C]formaldehyde (PerkinElmer Life Sciences) as described previously (27).

**Purification of Ribosomal Subunits**—Free 40 S and 60 S ribosomal subunits were purified by using high salt sucrose gradients as described previously (7, 25).

**Generation of [35S]Met-tRNAi**— [35S]Met-tRNAi was prepared by using Brewer’s yeast tRNA (Ambion), E. coli aminoaeryl-tRNA synthetase, and [14C]methionine (56 mCi/mmol, PerkinElmer Life Sciences) as described previously (25).

**In Vitro Translation Inhibition Assay**—In vitro translations in rabbit reticulocyte lysate (Promega) were performed with luciferase mRNA in the presence of [35S]methionine. In vitro translations were performed with nuclease-treated rabbit reticulocyte lysate under conditions recommended by the manufacturer. A typical 25-μl reaction contained 17.5 μl of lysate, 0.02 mM amino acid mixture (minus methionine), 10 μl of [35S]methionine (1200 Ci/mmol), 20 units of RNase inhibitor (Roche Applied Science), and 0.5 μg of translation template. Mouse p56 was added post-translation amounts as indicated in the figure legends. Translations were allowed to proceed at 30 °C for 2 h. Following translation, a 5-μl aliquot of the reaction was resolved by 10% SDS-PAGE. Gels were dried and exposed to a PhosphorImager screen and incorporated radioactivity quantitated using the ImageQuant software (Amersham Biosciences).

**Cell Culture and Transfection**—HT1080 human fibrosarcoma cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (Invitrogen). Cells were transfected using FuGENE 6 (Roche Applied Science) according to the manufacturer’s protocol. Four micrograms of each plasmid was transfected, and cells were harvested after 18 h. Cell lysates were prepared for either Western blots or immunoprecipitations.

**Immunoprecipitation and Western Blot**—Immunoprecipitation of FLAG-tagged protein was performed in low salt buffer (20 mM Tris, pH 7.5, 50 mM KCl, 200 mM NaCl, 1 mM EDTA, 20% glycerol, 0.05% Triton X-100, 0.2 mM phenylmethylsulfonyl fluoride). M2 anti-FLAG-Sepharose beads (Sigma) were pre-soaked with 3 μg of bovine serum albumin for 15 min. Cell lysates were prepared as described (28), and 300 μg of whole-cell extracts were mixed with 500 μl of low salt buffer and 20 μl of preincubated anti-FLAG-Sepharose beads at 4 °C overnight. The immunocomplexes were washed with the low salt buffer and subjected to denaturing gel electrophoresis through a 10% polyacrylamide gel.

**Gel Filtration Chromatography**—The gel filtration binding assay was performed as described previously (2). Briefly, an XK 16/20 column (16 mm diameter, 70 cm height) was used in column volume ~100 ml, V0 = 35 ml. Western blots were performed with a 1:2000 dilution of a polyclonal p56 antibody (2). The Western blot for FLAG or Myc was performed using a 1:2000 dilution of anti-FLAG M2 antibody (Kodak Scientific Imaging System) and a 1:1000 dilution of anti-Myc 9E10 (Santa Cruz Biotechnology), respectively. Alternatively, 300 μg of cell lysate was incubated with 6 μl of antibody against Myc (c-Myc 9E10 monoclonal antibody; Santa Cruz Biotechnology) in 500 μl of RIPA buffer (150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholic acid, 0.1% SDS, 50 mM Tris, pH 8.0, 0.4 mM phenylmethylsulfonyl fluoride, and protease inhibitors) at 4 °C overnight. 20 μl of protein A-agarose (Roche Applied Science) was then added, followed by a 4-h incubation at 4 °C. Samples were washed four times with RIPA buffer and then subjected to SDS-PAGE followed by Western blotting with an antibody against FLAG (M2-monoclonal antibody, Sigma).

**In Vitro Pull Down**—Eighteen picomoles of purified His-Mup56 and 9E10 antibody were incubated 1:1000 dilution of c-Myc 9E10 (Santa Cruz Biotechnology), 1:2000 dilution of anti-FLAG M2 antibody (Kodak Scientific Imaging System) and 1:1000 dilution of anti-MyC 9E10 (Santa Cruz Biotechnology) in 500 μl of RIPA buffer (150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholic acid, 0.1% SDS, 50 mM Tris, pH 8.0, 0.4 mM phenylmethylsulfonyl fluoride, and protease inhibitors) at 4 °C overnight. 20 μl of protein A-agarose (Roche Applied Science) was then added, followed by a 4-h incubation at 4 °C. Samples were washed four times with RIPA buffer and then subjected to SDS-PAGE, transferred to nitrocellulose, and then Western-blotted using the His antibody.

**Ribosome Dissociation Assay**—The ribosome dissociation assay was performed as described in Ref. 7. Purified 40 S ribosomal subunits (0.7 A260 units) were incubated with 1.4 A260 units of purified 60 S ribosomal subunits to form 80 S ribosomes in a 100-μl reaction volume containing 100 mM KCl, 10 mM Tris-Cl, pH 7.5, 3 mM MgCl2, and 2 mM DTT. To dissociate 80 S ribosomes, 60 μl of eIF3 (3.75 μg, 600 nmol) was added to the reaction, incubated for 10 min at 37 °C, then layered on a 12-ml 10-25% sucrose gradient (100 mM KCl, 20 mM Hepes-KOH, pH 7.5, 5 mM MgCl2, 2 mM DTT), and centrifuged for 16 h at 20,000 rpm at 4 °C (Beckman SW28TI rotor). To test the effect of Mup56, 60 μl of purified p56 (3.2 μg, 600 nmol) was preincubated with 60 μl of eIF3 for 10 min at 30 °C prior to the addition of ribosomes. Gradients were unloaded via upward displacement using 60% sucrose, and UV absorbance was measured at 254 nm with an ISCO flow cell. For Western blot analysis of sucrose gradient fractions, 500 μl of each fraction was precipitated with a final concentration of 10% trichloroacetic acid, subjected to 10% SDS-PAGE, electroblotted onto a nitrocellulose membrane (Millipore), and then probed with either eIF3 antibody (1:1000) or His antibody (1:660) as described previously (2).

**eIF3/eIF4F Interaction Assay**—To observe the interaction of eIF3 and eIF4F, sucrose density centrifugation was performed as described in Ref. 27. Radiolabeled [3C]eIF4F (150 pmol, 30 μg) was incubated alone or with equimolar (150 pmol, 100 μg) amounts of unlabeled eIF3 in a 100-μl volume containing 20 μM Hepes-KOH, pH 7.5, 100 mM KCl, 1 mM MgCl2, 1 mM DTT, and 0.1 mM EDTA. Reactions were then layered on a 5–18% sucrose gradient (20 mM Tris-HCl, pH 7.5, 100 mM KCl, 1 mM MgCl2, 1 mM DTT) and centrifuged at 32,000 rpm for 18.5 h at 4 °C (Beckman SW60 rotor). To test the effect of Mup56 on this interaction, purified Mup56 (150 pmol, 8.4 μg) was preincubated with eIF3 for 10 min at 30 °C prior to addition of [3C]labeled eIF4F. Gradients were unloaded via needle syringe and 1-ml fractions collected. The radioactivity in 200 μl of each fraction was determined by liquid scintillation spectrometry.
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**RESULTS**

**Mouse p56 Inhibits Translation**—Mup56 and Hup56 are similar sized proteins, although they have only 50% sequence identity. However, both proteins contain six TPR motifs that are similarly spaced (Fig. 1). These motifs are defined by typical patterns of small and large hydrophobic residues, although no residues are completely invariant. The sequences within the same TPR motifs of the two proteins are highly conserved. For example, of the 34 residues of TPR2, 23 residues are identical and 6 more are similar (Fig. 1). To examine the functions of Mup56, we expressed a hexahistidine-tagged recombinant Mup56 in *E. coli*, and we purified it by affinity chromatography on a nickel-agarose column. The purified protein was then tested for its ability to inhibit translation in *vitro*. For this purpose, increasing quantities of Mup56 or Hup56 were added to a rabbit reticulocyte lysate system programmed with luciferase mRNA. Quantitation of the radiolabeled products revealed that both proteins inhibited translation in a dose-dependent fashion. Moreover, their potencies were similar: 50% inhibition was observed with about 50 nM concentration of each protein (Fig. 2). By having established that Mup56 is a potent inhibitor of translation, the underlying mechanism was further investigated.

**Mup56 Binds to eIF3**—We first examined whether Mup56, like Hup56, binds to eIF3. Size fractionation analysis by gel filtration chromatography showed that Mup56 existed as dimers and monomers (Fig. 3A). In the presence of purified eIF3, a portion of Mup56 appeared in fractions that contained eIF3, indicating the binding of the two proteins (Fig. 3B). Similar to what was observed with Hup56 (2) some Mup56 also appeared in the intermediate fractions (Fig. 3B, fractions 24–32), probably due to partial dissociation of the eIF3/Mup56 complex during gel filtration. Binding of Mup56 to eIF3 was confirmed by pull-down and immunoprecipitation assays (Fig. 4). When purified Mup56 and eIF3 were mixed *in vitro*, pulling down Mup56 by using its hexahistidine tag (Fig. 4A) or immunoprecipitation of eIF3 by its cognate antibody (Fig. 4B) resulted in co-purification of the associated partners (Fig. 4, A and B, lanes 2). Appropriate controls (Fig. 4, A and B, lanes 1 and 3) showed that the binding was efficient and specific. Moreover, the Western blot with the whole eIF3 antibody showed that Mup56 appeared to bind specifically to the c subunit of the eIF3 complex (Fig. 4A).

Because Hup56 binds to the e subunit of eIF3, the above result was unexpected. Hence we carried out a series of experiments to examine the specificities of *in vitro* interactions between p56 proteins and eIF3/eIF3e subunits (Fig. 5). For this purpose, Myc-tagged p56 proteins were expressed in human cells along with FLAG-tagged eIF3c or eIF3e. Each partner was immunoprecipitated or affinity-purified from the cell lysates, and the presence of the other partner in the precipitate was detected by Western blotting. The levels of expression of the transfected proteins and endogenous actin were measured by straight Western blotting of cell lysates with appropriate antibodies. When Mup56 was pulled down, eIF3c co-purified with it (Fig. 5A, lane 2). Similarly, when eIF3e was immuno-
precipitated, Mup56 was bound to it (Fig. 5B, lane 2) but Hup56 was not (Fig. 5B, lane 3). When eIF3e and p56 were co-expressed and eIF3e was immunoprecipitated, Hup56 interacted with it (Fig. 5C, lane 2) but Mup56 did not (Fig. 5C, lane 4). These results clearly showed that in vivo Mup56 and Hup56 specifically bound to the c and e subunits of eIF3, respectively. Both these proteins contain the PCI domain and predicted nuclear localization signals (Fig. 5D).

Mup56 Does Not Inhibit Several Functions of eIF3—We have shown previously that the inhibitory effect of Hup56 on translation initiation is primarily mediated by its ability to block the role of eIF3 in the stabilization of the eIF2-GTP-Met-tRNA,

ternary complex. The assay used to demonstrate this effect was a filter binding assay in which radiolabeled Met-tRNA was retained on nitrocellulose only as part of a fully formed complex with purified eIF2 and GTP. eIF3 is known to enhance this effect, stimulating the amount of ternary complex formed and subsequently retained on the filter (9). Thus, we were interested whether Mup56 had the same property as Hup56 despite the fact that it binds to a different subunit of eIF3. As can be seen in Fig. 6, Mup56 had only marginal effects on ternary complex stabilization, as compared with the effect of Hup56. These results indicated that Mup56 not only binds to a different subunit of eIF3 but also affects a function different from the one inhibited by Hup56.

The above observation led us to examine systematically the effects of Mup56 on other functions of eIF3. eIF3 promotes ribosomal dissociation, and Mup56 had no inhibitory effect on this property of eIF3, as shown by sucrose density gradient size separation analysis (Fig. 7). Sucrose gradient analyses were also used to examine the effects of Mup56 on binding of eIF3 to 40 S ribosomal subunits. Western blotting of sucrose gradient
C, the same as left 10–25% sucrose gradient. Gradients are shown as increasing density 80 S ribosomes as shown in a UV profile following centrifugation on a ribosomal subunits form dimers and comigrate with 60 S. eIF3 prior to addition to ribosomes. In this analysis, most of the 40 S indicated by A, 0.7 incubated with 1.4 column D, the same as column B with the addition of 300 nm Mup56. Column D, the same as column B with the addition of 300 nm eIF3. Column E, the same as column D except eIF3 was preincubated with equimolar Hup56 prior to addition. Column F, the same as column E except that Mup56 replaced Hup56. The experiment was performed in triplicate.

![Fig. 6](image1.png)

**Fig. 6.** Mup56 has a marginal effect on ternary complex stabilization by eIF3. Column A, retention of radiolabeled [14C]Met-tRNAi, alone (100 nM) on nitrocellulose filters was negligible and used to normalize results. Column B, ternary complex formed in the presence of 80 nm purified eIF2, 100 nm [14C]Met-tRNAi, and 100 μM GTP. Column C, the same as column B with the addition of 300 nm Mup56. Column D, the same as column B with the addition of 300 nm eIF3. Column E, the same as column D except eIF3 was preincubated with equimolar Hup56 prior to addition. Column F, the same as column E except that Mup56 replaced Hup56. The experiment was performed in triplicate.

fractions revealed that eIF3 was bound to 40 S subunits even in the presence of Mup56 (Fig. 8C). The same complex also contained Mup56 (Fig. 8B). However, binding of Mup56 to 40 S subunits required the presence of eIF3 (Fig. 8A). These results suggest that the eIF3-Mup56 complex can efficiently bind to 40 S ribosomal subunits. Similarly, a size separation analysis combining components of the previous assays, ternary complex and ribosomes, showed that eIF3-dependent binding of ternary complex to ribosomes was also not affected by Mup56 (Fig. 9).

**Fig. 7.** Mup56 does not affect ribosomal subunit dissociation. A, 0.7 A\text{260} units (14 pmol) of purified 40 S ribosomal subunits were incubated with 1.4 A\text{260} units (28 pmol) of purified 60 S subunits to form 80 S ribosomes as shown in a UV profile following centrifugation on a 10–25% sucrose gradient. Gradients are shown as increasing density left to right. Relative positions of 40 S, 60 S, and 80 S ribosomes are indicated by arrows. B, 60 pmol of purified eIF3 was added to dissociate 80 S ribosomes. C, equimolar (60 pmol) Mup56 was preincubated with eIF3 prior to addition to ribosomes. In this analysis, most of the 40 S ribosomal subunits form dimers and comigrate with 60 S.

**Fig. 8.** Mup56 binds to 40 S ribosomal subunits only if eIF3 is present. A, 600 nm (60 pmol, 3.2 μg) purified Mup56 was incubated with 0.7 A\text{260} (14 pmol) 40 S ribosomal subunits only and then layered on a 10–25% sucrose gradient. Gradient is shown as increasing density from left to right. Following centrifugation, fractions were collected, trichloroacetic acid-precipitated, and subjected to SDS-PAGE. The gel was transferred to nitrocellulose, and Western blot was then performed with anti-His antibody. Relative position of 40 S subunits in the gradient is shown on the top as determined by UV absorbance. The reaction in B was the same as A, except equimolar (600 nm, 60 pmol) purified eIF3 is added to the reaction. The reaction in C was the same as in B, except the Western was performed with an anti-eIF3 antibody.

complex, and 40 S subunit by a sucrose gradient analysis (a similar observation on the binding of eIF4F to 40 S complexes containing eIF3 and the ternary complex has been made). Radiolabeled eIF4F is able to bind to eIF3, which migrates as a 20 S complex (Fig. 11A). Confirming the results shown in Fig. 10, Mup56 did not affect the formation of this complex (Fig. 11B). In the presence of 40 S ribosomal units, eIF4F binds to 40 S-ternary complex-eIF3, as shown by the shift in the radioactivity from a 20 S peak to a 43 S peak (Fig. 11C). This association was completely blocked by Mup56 (Fig. 11D). Thus,

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Fig. 10. Mouse p56 does not interfere with the eIF3/eIF4F interaction. 

The p56 family of viral stress-inducible proteins is characterized by the presence of multiple TPR motifs. Because they are all of similar molecular weights and share partial sequence homology, it is not easy to identify the murine ortholog of a human member, such as human p56. Phylogenetic relationship suggests that among the three murine family members Mup56 (calculated Mr, 53.7) is the closest relative of the Hup56 (calculated Mr, 55.4), although the two proteins have only 50% sequence identity. Following the recent and more stringent definition of a TPR motif (31), both proteins contain six TPR motifs that are similarly spaced (Fig. 1). These motifs are known to mediate interactions with other proteins that often are subunits of multiprotein complexes. Consistent with this property, a protein interaction survey revealed that Hup56 interacts with eIF3e, and the TPR motif is required for this interaction (2). Moreover, the p56-interacting domain of eIF3e contains a long α-helical motif called the PCI motif. This led us to hypothesize that the p56 family members are designed to interact with proteins containing PCI motifs. The current study supports this hypothesis because Mup56 interacted with another PCI motif-containing subunit of eIF3, eIF3c. This observation demonstrated that all TPR motifs are not functionally equivalent. Similarly, all PCI motifs are also not equivalent. The structural basis of specific recognition between cognate TPR and PCI domains remains to be elucidated. However, we suspect that other members of the human and the murine p56 family will have similar specificity toward their cognate binding partners.

Although Mup56 and Hup56 bound to different subunits of eIF3, the global consequences were the same, inhibition of translation inhibition. However, our detailed investigation of the mechanism of the observed inhibition was quite revealing. Neither p56 protein inhibited the ability of eIF3 to interact with 40 S ribosomal subunits or eIF4F. But other functions were selectively inhibited by the two p56 proteins (Fig. 12). Hup56 blocks the interaction of eIF3 with the ternary complex (4), whereas Mup56 blocks the association of eIF3 and eIF4F on the surface of the 40 S ribosomal subunit. Although this may potentially reflect inherent properties of the p56 proteins, we anticipate that these effects reflect which of the eIF3 subunits has been bound. Thus, binding of a p56 protein to eIF3e has different consequences than binding to eIF3c, although either binding reduces the rate of translation initiation. The p56 family is emerging as an interesting group of proteins based on the ability of Hup56 and Mup56 to accentuate normal cellular mechanisms of translation regulation. The two best-studied mechanisms of general translational regulation are restriction of ternary complex formation or restriction of mRNA activation and binding to 40 S subunits (5, 32). By blocking the eIF3-ternary complex interaction, Hup56 achieves the same outcome as phosphorylation of eIF2α (4). The ability of Mup56 to block the eIF3/eIF4F interaction on the 40 S subunit leads to effects similar to those observed with reduced eIF4F activity as a reflection of 4E-BP dephosphorylation. Given that eIF3a also contains a PCI domain (5), it is possible that another of the p56 family members may bind this subunit causing an unpredictable pattern of translation initiation. Such findings are likely to result from our continued efforts to understand the mechanism of action of all the p56 proteins.

Given the mechanistic similarities of the p56 proteins to eIF2α phosphorylation or 4E-BP dephosphorylation, it should be noted that the addition of either exogenous eIF2 or eIF4F does not overcome the inhibition observed with Hup56 or Mup56 (see Ref. 4 and this report). Thus, these inhibitors behave as “dominant negative” inhibitors by poisoning 40 S-eIF3 complexes so they fail to bind eIF2 (as the ternary complex) or eIF4F (as the eIF4F-mRNA complex).

An interesting aspect revealed in these studies is the observation of a new step in the biochemical pathway of protein synthesis. By deconstructing the initiation pathway into simplified separate steps, we not only identified a specific target for Mup56, but we also were able to show the necessity for the ternary complex in order for eIF4F to associate with 40 S ribosomal subunits (Fig. 11E). Such a requirement has not been reported previously, although a similar observation has been made by Majumdar and Maitra. This observation is consistent with the many model 80 S initiation pathways that depict joining of the ternary complex to the 40 S subunit as an event required prior to binding of the mRNA. Biologically, this sequence makes sense as the required early binding of the
The initiator tRNA ensures that there will be the capability to recognize an initiating AUG codon as the mRNA is scanned by the 40 S complex (33). Binding of the mRNA first would possibly allow the scanning 40 S to bypass an initiating AUG (as noted in the regulated expression of GCN4) (34).

There are also a few questions raised by these experiments. eIF3 has been shown to form complexes with eIF2 (as ternary complex) (9) and eIF4F in the absence of the 40 S subunit (27). This raises the question as to whether it is these complexes (eIF3 ternary complex or eIF3 eIF4F) that bind to the 40 S subunit. Currently, there are insufficient data to answer this question, although the data from yeast (35, 36) and the data in Fig. 11 might suggest this (note the apparent conversion of eIF3 eIF4F complexes, as 20 S, in Fig. 11 A to 43 S complexes in Fig. 11 C). However, the requirement for the ternary complex to bind radiolabeled eIF4F to 40 S subunits is not consistent with the direct addition of the eIF3 eIF4F complex to the 40 S subunit. We anticipate that the weak interactions observed in the absence of the 40 S subunit (eIF3 eIF4F or eIF3 ternary complex) reflect much tighter associations that occur on the surface of the 40 S subunit. Given that eIF4F is not found associated with 40 S subunits when only eIF3 is present, one assumes that eIF3 must undergo a conformational change upon binding to the 40 S subunit that prevents stable interaction with eIF4F until the conformation of eIF3 is again altered by the binding of the ternary complex to eIF3-40 S.

As noted above, the human and mouse p56 proteins add new angles to the already known points of regulation in the eukaryotic pathway. It will be of interest to see if other viral stress-induced proteins mimic other regulated features of translation, which include start site selection and elongation rate. If so, these recognitions would be expected to be fundamentally different as the translation factors responsible for these effects lack the characteristic PCI domain common to the protein ligands of Hu- and Mup56.
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