Mechanism of eIF6-mediated Inhibition of Ribosomal Subunit Joining*

Received for publication, December 17, 2009, and in revised form, February 9, 2010. Published, JBC Papers in Press, March 31, 2010. DOI 10.1074/jbc.C109.096057

Thorsten Mielke, Michael Blau, Jean-Paul Armache, Thorsten Mielke¶, Maya Topf¶, and Roland Beckmann

The abbreviations used are: eIF6, eukaryotic initiation factor 6; eEF2, eukaryotic translation elongation factor 2; cryo-EM, cryo electron microscopy; eIF6 binding site involves mainly rpL23 (L14p in Saccharomyces cerevisiae). The structure reveals that the eIF6 binding site involves mainly rpl23 (L14p in Escherichia coli). Based on our structural data, we propose that the mechanism of the anti-association activity of eIF6 is based on steric hindrance of intersubunit bridge formation around the dynamic bridge B6.

The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. 1S and 2S and Table 1S.

To whom correspondence should be addressed. Tel.: 49-89-2180-76900; Fax: 49-89-2180-76945; E-mail: beckmann@lmb.uni-muenchen.de.

Complex Reconstitution—The full-length protein eIF6 from Saccharomyces cerevisiae was overexpressed in Escherichia coli cells and purified via a His<sub>6</sub> tag as described previously (6, 12). An anti-association assay with yeast 80 S ribosomes was performed to check for the activity of the purified eIF6 (12). For preparation of the sample, 2 pmol of gradient-purified 60 S subunits were mixed with 10 pmol of eIF6 to reconstitute the 60 S-eIF6 complex in buffer A (20 mm Hepes, 100 mm KCl, 2.5 mm Mg(OAc)<sub>2</sub>, 1 mm dithiothreitol). The cryo-EM reconstruction of the 60 S-eIF6 complex appeared to be limited by preferential orientation of the particles on the grid. Densities for the large ribosomal subunit and for eIF6 were isolated using binary masks. The cryo-EM crystal structure of S. cerevisiae (Protein Data Bank (PDB) entry 1G62) was taken as a basis for flexible fitting. First, rigid fitting of the eIF6 structure into the density was performed using a local exhaustive search with the software Mod-EM guided by a cross-correla-
and allows the identification of the binding site of eIF6 on the 60 S subunit (Fig. 1B, supplemental Fig. 2S). The main interaction partner of eIF6 is the ribosomal protein rpL23 (L14p in *E. coli*), which is located at the intersubunit surface of the 60 S subunit (Fig. 1, C and D). This binding site appears to be conserved between kingdoms because it is in agreement with a similar binding site that has recently been suggested for the archaeal aIF6 on the basis of chemical cross-linking and RNA protection experiments (20). The crystal structure of eIF6 from *S. cerevisiae* (PDB entry 1G62) was taken as a basis for flexible fitting into the assigned EM density for eIF6 using the software Flex-EM (21). The eIF6 binding interface with the 60 S subunit is in good agreement with previously published genetic data. Suppressor mutations in the TIF6 gene which rescue sdo1Δ cells locate to this particular region that has also been suggested to mediate ribosome binding (8). To fit the density, the C-terminal part of rpL23 was adjusted, leading to a small inward movement of the helical C terminus of rpL23 relative to the conformation observed in the 80 S ribosome. The hydrophobic C-terminal interaction surface of rpL23 interacts with the helical binding region of eIF6, both of which show a high degree of conservation. In addition to rpL23, the neighboring ribosomal protein rpL24 (L24e in *Haloarcula marismortui*) and the highly conserved sarcin-ricin loop (SRL) also contribute to the interaction with eIF6 (Fig. 1D).

**Anti-association Activity of eIF6**—The anti-association activity of eIF6 prevents binding of the 40 S ribosomal subunit to the 60 S subunit. When the 40 S subunit in its 80 S-bound conformation is superimposed with the 60 S-eIF6 complex, it is evident that both eIF6 and the 40 S subunit share a common binding region on the 60 S subunit and cannot be present simultaneously (Fig. 2A). A large part of the eIF6 density would clash with the rRNA of the 40 S subunit, explaining the mutually exclusive binding by simple steric hindrance (Fig. 2B). The position of eIF6 at the 60 S subunit surface coincides exactly with the dynamic intersubunit bridge B6 formed by the C-terminal region of the ribosomal protein rpL23 (22). Therefore, in the presence of eIF6, the bridge B6 and the nearby located bridge B5a, both formed by rpL23, are directly affected. In addition, the surrounding intersubunit contacts B7, B3, and B5b also can no longer be formed, explaining the observed highly efficient prevention of subunit joining (Fig. 2B). The phosphorylation sites of eIF6, Ser-174 and Ser-175, are located at the accessible

RESULTS AND DISCUSSION

**Structure of the Ribosomal 60 S-eIF6 Complex**—We reconstituted a ribosomal 60 S-eIF6 complex from *S. cerevisiae in vitro* and used cryo-EM in combination with single particle analysis for a three-dimensional reconstruction. The structure of the 60 S-eIF6 complex reveals an extra density representing eIF6 in the intersubunit space (Fig. 1A). The characteristic five-fold pseudosymmetric structure (12) is resolved in the cryo-EM map (11.8 Å resolution at 0.5 FSC cutoff, supplemental Fig. 1S) and the density information.

This search was taking into account both sides and the five possible in-plane rotations of the structure. Next, the best fits (with the highest CCC) were further validated by comparison with the best local fits achieved using the Chimera fit-in-map module (18). Finally, the best eIF6 fit (CCC 0.886) was refined by using the software Flex-EM (19) together with its ribosomal interaction partner rpL23, taking into account the binding interface when eIF6 is bound to rpL23 (supplemental Table 1S). The last C-terminal 20 amino acids of eIF6 were not used for the final fit because they did not influence the fitting result. They are highly likely to be non-structured and, in agreement with that, they could not be localized in the map as density information.

**FIGURE 1. Localization of eIF6 in a cryo-EM reconstruction of the 60 S-eIF6 complex.** A, the map is shown in the crown view. B, close up of the fitted eIF6 structure in EM-density (mesh). C and D, molecular environment of the eIF6 binding site on the 60 S subunit. Thumbnails show the respective orientation. Colors are: orange, eIF6; magenta, sarcin-ricin loop (SRL); blue, rpL23; green, rpL24; gray, 60 S ribosomal subunit. Abbreviations are: CP, central protuberance; L1, L1 stalk; SB, stalk base; N/C, N and C terminus of eIF6, respectively.  

**FIGURE 1.** Localization of eIF6 in a cryo-EM reconstruction of the 60 S-eIF6 complex. A, the map is shown in the crown view. B, close up of the fitted eIF6 structure in EM-density (mesh). C and D, molecular environment of the eIF6 binding site on the 60 S subunit. Thumbnails show the respective orientation. Colors are: orange, eIF6; magenta, sarcin-ricin loop (SRL); blue, rpL23; green, rpL24; gray, 60 S ribosomal subunit. Abbreviations are: CP, central protuberance; L1, L1 stalk; SB, stalk base; N/C, N and C terminus of eIF6, respectively.
surface of the bound eIF6, which is not involved in the interaction with the 60 S subunit (Fig. 1B). Also, the C-terminal nuclear localization sequence (and Ser-235), which was not visualized in the crystal structure, would be located at the accessible outer surface of eIF6.

**Dissociation of eIF6 from the 60 S Ribosomal Subunit**—The elongation factor-like GTPase Efl1p, which is required for the release of eIF6 in yeast, is highly homologous to eEF2. It has been shown that Efl1p can compete with eEF2 for ribosome binding, resulting in inhibition of the ribosome-associated GTPase activity of eEF2 (23). Therefore, it is safe to assume that they share a similar binding site, which is known for eEF2 to be the canonical translation factor binding site (24). We modeled Efl1p using HHpred (25) and MODELLER (26) with the ribosome-bound eEF2 (PDB entry 1S1H) as a template, resulting in a model for the 60 S ribosomal subunit-bound Efl1p. Strikingly, in this position, Efl1p could bind concomitantly with eIF6 and would perfectly embrace eIF6 to facilitate an interaction with its GTPase domain (Fig. 2C). A small conformational change of Efl1p in this position would be sufficient to trigger GTP-dependent release of eIF6 from the 60 S subunit (Fig. 2D).

Our structural information provides the basis for a molecular understanding of the conserved activity of eIF6 as a ribosomal anti-association factor. It remains to be seen to what extent the ribosomal binding mode of eIF6 is also functionally involved in its additional activities in higher eukaryotes.

**REFERENCES**
