

ERj1p uses a universal ribosomal adaptor site to coordinate the 80S ribosome at the membrane

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Ribosomes translating secretory and membrane proteins are targeted to the endoplasmic reticulum membrane and attach to the protein-conducting channel and ribosome-associated membrane proteins (RAMPs). Recently, a new RAMP, ERj1p, has been identified that recruits BiP to ribosomes and regulates translational activity. Here we present the cryo-EM structure of a ribosome–ERj1p complex, revealing how ERj1p coordinates the ribosome at the membrane and how allosteric effects may mediate ERj1p's regulatory activity.

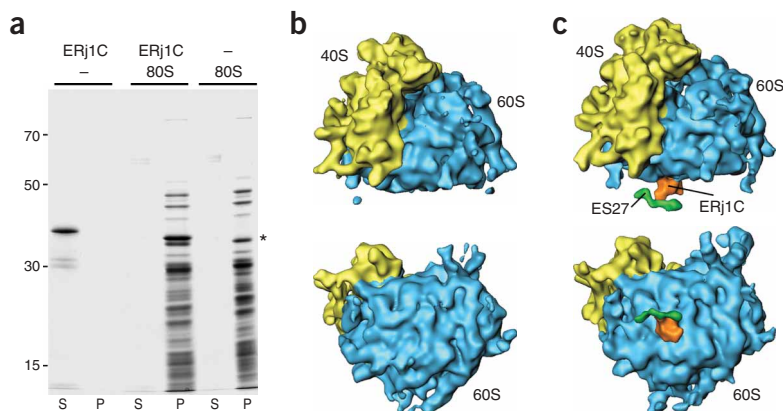
ERj1p was first discovered in a murine lung carcinoma cell line (where it is called Mtj1p)¹, but is also present in human cells (where it is called Htj1)² and dog pancreatic cells³. It is a 64-kDa type I membrane protein of the endoplasmic reticulum (ER) with a single transmembrane helix. In the lumen of the ER, the J-domain of ERj1p interacts as an Hsp40-type cochaperone with the luminal Hsp70 protein BiP, stimulating its ATPase activity^{3,4}. The cytosolic C-terminal domain is composed of two repeats resembling so-called myb domains, which are structurally related to the DNA-binding domains of Myb proteins and are also present in other transcription factors and regulators such as Swi3 and Ada2.

It has been shown previously that a truncated C-terminal fragment of ERj1p that lacks the N-terminal luminal domain, the transmembrane domain and part of the cytosolic domain (ERj1C- Δ C85, residues 173–467) can bind ribosomes³. This process involves a highly charged basic region (residues 177–193) that is similar to a peptide found in the protein SRP14 (residues 91–105) of the elongation-arresting Alu domain of SRP⁵. Notably, similar to SRP, ERj1p can also inhibit the translational activity of the ribosome. This effect has been attributed to allosteric interference by ERj1p, most likely with initiation (see accompanying paper by Dudek *et al.*⁶). It is believed that ERj1p's main role is in coordinating the luminal Hsp70 chaperone BiP at the site of nascent peptide translocation³, resulting in release of translational arrest⁶. However, it has been suggested that ERj1p has an additional function as a membrane-bound proteolysis-activated transcription factor⁷. Here, we used cryo-EM and single-particle reconstruction to analyze a ribosome–ERj1p complex.

Purified 80S ribosomes from dog pancreas and recombinant mouse ERj1C- Δ C85 (ERj1C for short) were used for complex formation. In agreement with our previous findings³, the ERj1C construct can be stably bound to the 80S ribosomes (Fig. 1a). The amount of ERj1C comigrating with ribosomes seems to be at least stoichiometric, and, therefore, we conclude that under our conditions ERj1C binds the ribosomes with a low off-rate and high occupancy.

The complex and a control sample consisting of identically treated unbound 80S ribosomes were visualized using cryo-EM, resulting in three-dimensional reconstructions with resolutions of 20 Å and 23 Å, respectively. Notably, the amount of particles necessary to achieve this resolution was relatively small (8,697 and 4,719), owing to the application of newly developed matched filters for amplitude correction during refinement (Supplementary Methods online).

Figure 1 Binding assay and cryo-EM map of the mammalian 80S ribosome–ERj1C complex. (a) Binding of ERj1C to 80S ribosomes. 20 pmol ribosomes and 50 pmol recombinant ERj1C were incubated and analyzed by centrifugation through a sucrose cushion followed by SDS-PAGE. The Coomassie-stained gel shows the supernatant (S) and pellet (P) fractions. Asterisk indicates ERj1C. Numbers indicate molecular weight (kDa). (b) Cryo-EM map of the dog pancreas 80S ribosome at a resolution of 23 Å. Yellow, small (40S) ribosomal subunit; blue, large (60S) subunit. Top, side view; bottom, rotated 90° to expose the side of the ribosome that attaches to the membrane. (c) Cryo-EM map of the 80S ribosome–ERj1C complex at a resolution of 20 Å. Orange, ERj1C; green, ES27.



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The overall appearance of both reconstructions resembles the typical mammalian 80S ribosome, as described previously⁸ (Fig. 1). However, a notable difference in the complex is the additional density located at the lower region of the large (60S) ribosomal subunit. This additional mass consists of a globular and an elongated region, and it is connected to the ribosome via two contacts in the immediate vicinity of the peptide tunnel exit (Fig. 1b,c). The elongated extra density has a slight twist, which is typical of an RNA helix at this resolution. The flexible ribosomal RNA expansion segment 27 (ES27) has been shown in yeast to adopt a conformation positioning its flexible region in exactly the same place⁹. Therefore, it is most likely that the elongated mass represents ES27 and the globular extra density represents the cytosolic domain of ERj1p.

The contacts of ERj1C with the ribosome are located on opposite sides of the peptide tunnel exit so that ERj1C forms a bridge (Fig. 2a). The large contact on one side involves the universal docking proteins L23p and L29p in *Escherichia coli*, which can also interact with SRP⁵, the Sec61 complex⁹ and, in prokaryotes, the chaperone trigger factor¹⁰. The second contact involves the tip of rRNA helix 24, which also functions as docking site for SRP⁵ and the Sec61 complex⁹. The space left between the tunnel exit and ERj1C is large enough for the nascent chain to emerge from the ribosomal peptide tunnel. However, exiting nascent peptides could be in contact with ERj1C in this position, as shown at the biochemical level in the accompanying paper⁶.

Binding of ERj1C to ribosomes most likely inhibits protein synthesis during initiation. However, in its observed position (Fig. 2b), ERj1C cannot interfere directly with the binding of factors such as eEF2. Hence, ERj1C's translation arrest activity must follow a different mechanism. Notably, comparison of the ribosome–ERj1p complex with the unbound ribosome reveals conformational differences in addition to the extra mass at the exit site. The difference map (Fig. 2c) shows that the flexible stalk region, consisting of the acidic P-proteins, adopts a more rigid conformation in the ribosome–ERj1C complex than in the unbound ribosome, where it is invisible. Moreover, the L1 protuberance is in the closed position in the unbound structure and in the open (outer) position in the complex. Thus, the binding of ERj1C may induce a conformational change in the ribosome either directly or through the coordination of ES27, which originates at the catalytically essential ribosomal subunit interface in a position to provide a link between ERj1p binding and factor binding.

The proximity between the tunnel exit and the binding site of ERj1C has several notable implications with respect to its function. First, the coordination of a ribosome on the ER membrane by binding to ERj1p would position the peptide tunnel exit in close proximity to the membrane. Hence, the ribosome would be ready to interact productively with other ER membrane components such as the translocon⁹. Second, our data are in agreement with the idea that ERj1p coordinates the luminal Hsp70 protein BiP at the site of nascent peptide translocation³. Because of partially overlapping binding sites, however, simultaneous binding of ERj1p and the translocon (the Sec61 complex) would require a minor rearrangement of ERj1p (Fig. 2b). Third, it is possible that a direct interaction between the emerging nascent chain and ERj1p takes place that could also

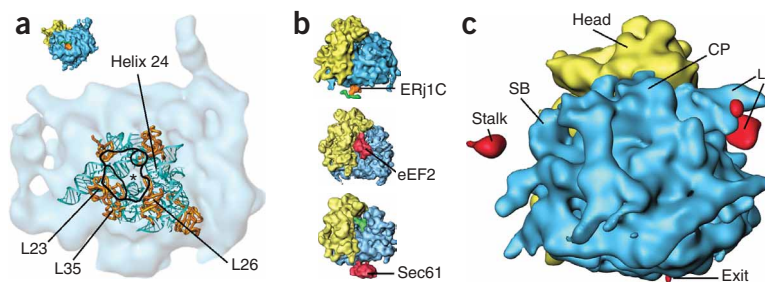


Figure 2 ERj1p's ribosomal contacts, overall position and conformational changes. (a) A molecular model of the peptide tunnel exit region, shown with a translucent ribosome and the contour of ERj1C (black lines). The two contacts of ERj1C involve L23, L35 and the tip of rRNA helix 24. Blue helices, rRNA; orange backbones, ribosomal proteins; asterisk, tunnel exit. Inset shows the orientation of the complex. (b) Comparison of the overall positions of ERj1C (top), eEF2 (middle) and Sec61 (bottom) on the 80S ribosome. (c) The 80S ribosome, shown with the difference map (red) resulting from the comparison with the ribosome–ERj1C complex. Red indicates substantial density differences in the complex. Notably, in addition to the difference at the exit site, there are different conformations of the stalk region and the L1 protuberance. Head, head of the 40S subunit; SB, stalk base; CP, central protuberance; exit, tunnel exit.

contribute to the modulation of translational activity, similar to the mechanisms observed for SecM¹¹ or for exit tunnel–blocking antibiotics. Finally, regarding the conformational changes of the ERj1C-bound ribosome, we suggest that these allosteric changes, induced directly or via ES27, are responsible for ERj1p-mediated translational arrest. The question remains how the binding of BiP to the luminal J-domain of ERj1p is communicated to the cytosolic side to release translational arrest.

Accession codes. The EM density maps have been deposited at the European Molecular Biology Laboratory 3D EM database (<http://www.ebi.ac.uk/msd-srv/emsearch/index.html>) with accession codes EMD-1168 and EMD-1169.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

ACKNOWLEDGMENTS

This work was supported by grants from the VolkswagenStiftung (R.B.), Deutsche Forschungsgemeinschaft (R.Z., J.D. and grant Sfb449 to M.B. and R.B.) and from the US National Institutes of Health (R01 NS 43258 and R01 GM 60635 to P.A.P.). The technical assistance of A. Müller is gratefully acknowledged. The cryo-EM data were collected, in part, on the JEOL 3000SFF electron microscope at the National Center for Macromolecular Imaging (Houston, USA), which is supported by the US National Institutes of Health through the National Center for Research Resources's P41 program (grant RR02250). We thank A. Paredes for his microscopy work.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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