REPORT

Dynamic association of human Ebp1 with the ribosome

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ABSTRACT

Ribosomes are the macromolecular machines at the heart of protein synthesis; however, their function can be modulated by a variety of additional protein factors that directly interact with them. Here, we report the cryo-EM structure of human Ebp1 (p48 isoform) bound to the human 80S ribosome at 3.3 Å resolution. Ebp1 binds in the vicinity of the peptide exit tunnel on the 80S ribosome, and this binding is enhanced upon puromycin-mediated translational inhibition. The association of Ebp1 with the 80S ribosome centers around its interaction with ribosomal proteins eL19 and uL23 and the 28S rRNA. Further analysis of the Ebp1-ribosome complex suggests that Ebp1 can rotate around its insert domain, which may enable it to assume a wide range of conformations while maintaining its interaction with the ribosome. Structurally, Ebp1 shares homology with the methionine aminopeptidase 2 family of enzymes; therefore, this inherent flexibility may also be conserved.

Keywords: ribosome; Ebp1; single-particle cryo-EM

INTRODUCTION

While peptide bond formation is performed by ribosomes, they interact with a variety of translation factors to facilitate initiation, elongation, and termination thereby greatly enhancing the efficiency of translation (Rodnina et al. 1997; Savelsbergh et al. 2003; Spahn et al. 2004; Schmeing et al. 2009; Shao et al. 2016). In addition to the canonical translation factors, the eukaryotic ribosome has evolved expansion segments within their rRNAs that serve as scaffolds for interactions with a variety of additional factors (Melnikov et al. 2012; Anger et al. 2013; Simsek et al. 2017). These associations may enable regulation of translation of specific transcripts and thereby facilitate ribosomes to perform specialized roles (Voorhees et al. 2014; Simsek et al. 2017). While the function of many of these factors remains to be completely understood, the interaction sites have been found to cluster in hotspots on the ribosomal surface.

One such key hotspot on the ribosomal surface is in the vicinity of the peptide exit tunnel. Some of the factors that are known to bind near the peptide exit tunnel and interact with the nascent chain include methionine aminopeptidases (MAPs/MetAPs), amino-terminal acetyltransferases

(NATs), nascent polypeptide-associated complex (NAC), and signal recognition particle (SRP)/Sec61 translocon (Ball and Kaesberg 1973; Walter and Blobel 1980; Wiedmann et al. 1994; Lauring et al. 1995; Gautschi et al. 2003; Raue et al. 2007; Voorhees et al. 2014; Magin et al. 2017; Braunger et al. 2018; Knorr et al. 2019; Lin et al. 2020). Apart from factors that act cotranslationally, in yeast, the ribosome biogenesis factor Arx1 (yeast homolog of Ebp1), which shares the same fold as methionine aminopeptidase 2 (MetAP2), also binds close to the peptide exit tunnel in pre-60S particles in the nucleolus (Bradatsch et al. 2007, 2012; Hung et al. 2008; Greber et al. 2012, 2016). Arx1 along with Alb1 and Rei1 binds to the pre-60S particle as a complex in the nucleolus and facilitates its export to the cytoplasm by increasing its interaction with the nuclear pore (Bradatsch et al. 2007, 2012; Hung et al. 2008; Greber et al. 2016). Intriguingly, out of the many factors that bind near the peptide exit tunnel, yeast Arx1, MAPs, and NAT also recruit the expansion segment 27L (ES27L) of the 28S rRNA (Greber et al. 2016; Knorr et al. 2019). Furthermore, deletion of ES27L leads to a reduction in the association of MAPs and NAT with the ribosome and is lethal in yeast (Fujii et al. 2018; Shankar et al. 2020).

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In this study, we report the structure of human Ebp1 (p48 isoform) bound to human 80S ribosomes. We find that Ebp1 binds in close proximity to the peptide exit tunnel in mature 80S ribosomes. The association of Ebp1 with 80S ribosomes is predominantly mediated by ribosomal proteins eL19 and uL23 and the 28S rRNA with the insert domain (residues 245–306) of Ebp1. Furthermore, we find that the Ebp1 can undergo a rotation around this insert domain, which suggests a dynamic mode of association with the ribosome.

RESULTS AND DISCUSSION

Architecture of human Ebp1-ribosome complex

We purified ribosomes from HEK expi293F cells using two different protocols and subjected them to single-particle cryo-EM analysis (Supplemental Fig. S1). As reported previously, ribosomes isolated from cells treated with puromycin were in the rotated conformation and contained additional densities corresponding to eEF2 and an E-site tRNA (Supplemental Fig. S2A,C; Anger et al. 2013). In the second protocol, when ribosomes were initially isolated by ultracentrifugation in a 50% sucrose cushion prior to puromycin treatment, the ribosomes were predominantly found in the nonrotated POST state (Supplemental Fig. S2B,C; Khatter et al. 2015). Successive 3D classification of both data sets resulted in identification of a subset of particles that contained an unknown density close to the peptide exit tunnel (Supplemental Figs. S1, S2). In order to increase the total number of particles containing this additional density, the signal corresponding to either the 40S subunit or the 40S subunit bound to eEF2 and an E-site tRNA was subtracted from the particles in the respective data sets (Supplemental Fig. S1). The density subtracted particles from both data sets were merged and used for subsequent rounds of 3D classification and refinement. For the final reconstruction, 20,500 particles were used to obtain a map of the 60S subunit with the additional density at 3.3 Å resolution and was subsequently used for atomic model building (Fig. 1A; Supplemental Fig. S3; Supplemental Table S1).

FIGURE 1. Interaction interfaces of the Ebp1-ribosome complex. (A) Ebp1 (green)-ribosome (60S subunit [rRNA in light blue and ribosomal proteins in gray]) complex is represented as surface in two orientations. (B) Overview of Ebp1 binding site on human ribosome. Ebp1 (green), eL19 (yellow), uL23 (magenta), uL24 (orange), uL29 (wheat) proteins, fragment of 5.8S rRNA (purple), and different regions (H53, H59, and ES27L) of the 28S rRNA (light blue) are shown as cartoons. (C–E) Closer view of interaction interface of Ebp1 (green) with eL19 (yellow) and H59 (light blue), uL23 (magenta), and H53 (light blue), and uL29 (wheat) and 5.8S rRNA (purple). Interacting residues are shown as sticks, and polar interactions are marked by dotted lines.

In order to identify the protein that accounted for the additional density located near the peptide exit tunnel, the structures of known proteins that bound in this region were docked on to the cryo-EM map and visually inspected. Based on the orientation of the secondary structures in the density and sequence assignment, the additional density could be unambiguously identified as Ebp1 (Supplemental Figs. S4–S6). Furthermore, the presence of Ebp1 and absence of MetAP2 in the purified samples was confirmed by western blot and mass spectrometry (Supplemental Fig. S5C; Supplemental Table S2). As binding of Ebp1 is proximal to the peptide exit tunnel, we reasoned that the association of Ebp1 to ribosomes could be enhanced upon translational inhibition. To this end, control and puromycin treated HEK expi293F cells were subjected to polysome profiling experiments using sucrose density gradient

centrifugation. The fractions of the sucrose gradient were probed for Ebp1 and showed an increase in association of Ebp1 to the 80S monosome upon puromycin-mediated translational inhibition (Supplemental Fig. S7).

Ebp1 binding to the ribosome is mediated by its interactions with both ribosomal proteins (uL23, uL29, and eL19) and rRNA (5.8S and 28S rRNA) (Fig. 1B). The interaction with eL19 is predominantly mediated by polar contacts between Asn34 and Asn36 of eL19 and Glu254 and Tyr255 of Ebp1 (Fig. 1C; Supplemental Fig. S8A left panel). Compared to the apo 80S structure, H59 of 28S undergoes remodeling upon Ebp1 binding. The bases of C2708 and G2711 stack against Phe266 and Arg263 of Ebp1, respectively (Fig. 1C; Supplemental Fig. S8A middle panel). These stacking interactions are further bolstered by hydrophobic interactions involving Leu257 and Leu301 (Fig. 1C). These interactions are surrounded by multiple polar contacts involving residues Lys260, Ser267, and Arg271 of Ebp1 and U2709 and G2712 of the 28S rRNA (Fig. 1C; Supplemental Fig. S8A right panel). The Ebp1 interface with uL23 is centered around a hydrophobic core formed by Met87, Ile90, Glu91 and Leu147 of uL23 and Met259, Lys288, Met291, and Val294 of Ebp1 (Fig. 1D; Supplemental Fig. S8B left panel). These hydrophobic interactions are buttressed on one side by the polar interaction between Arg290 of Ebp1 and Asn151 of uL23 and on the other side by polar interactions between Lys258 of Ebp1 and Glu84 of uL23 (Fig. 1D; Supplemental Fig. S8B middle panel). Lys258 of Ebp1 also interacts with the backbone phosphate group of C2526 of H53 of 28S (Fig. 1D; Supplemental Fig. S8B right panel). In addition, Ala40 of uL29 and G78 of the 5.8 rRNA contribute to Ebp1 binding by interacting with Lys238, Asp239, and Ala240 (Fig. 1E; Supplemental Fig. S8C). The binding of Ebp1 to the 80S ribosome recruits ES27L of 28S rRNA and this interaction is predominantly mediated by regions in two amino-terminal helices of Ebp1 (Supplemental Fig. S8D). Due to the limited resolution of ES27L in our cryo-EM map, we could not assign the bases and this density is modeled as a poly (G)-poly(C) A-form helix.

Comparison of Ebp1 and Arx1 bound ribosome structures

While homology with Arx1 allowed us to initially identify Ebp1, their structures are not identical. Several features are conserved between these two proteins and the insert domains of yeast Arx1 and human Ebp1 are placed in similar positions and both interact with uL23 and eL19 and helices 53 and 59 of the 28S (yeast 25S rRNA) (Fig. 2A; Greber et al. 2016). Furthermore, many of the amino acids that form these interactions are also conserved between Arx1 and Ebp1. Interestingly, we observed a striking difference in the orientation of ES27L in the two structures. Specifically, ES27L undergoes a rotation of $\sim 35^\circ$ in the human

FIGURE 2. Comparison of the human Ebp1-80S and yeast Arx1-60S complexes (Greber et al. 2016). (A) Superposition of yeast Arx1-ribosome complex (PDB: 5APN [Greber et al. 2016]) on Ebp1-ribosome complex. Ebp1 (green), Arx1 (dark beige), eL19 (yellow), uL23 (magenta), uL24 (orange), uL29 (wheat) proteins, fragment of 5.8S rRNA (purple), and different regions (H53, H59, and ES27L) of the 28S/25S rRNA (light blue and blue) are shown as cartoon. Insert domains of Ebp1 and Arx1 are highlighted with a box. (B) Rotation of ES27L in human Ebp1 (green)-ribosome complex in comparison with yeast Arx1 (dark beige)-60S complex. Only 28S/25S rRNA of human and yeast ribosome are displayed (light blue and blue) for clarity. (C) Additional insertion regions of yeast Arx1 (dark beige) that mediate interaction with uL24 (orange), uL29 (wheat), 5.8S rRNA (purple), and H24 of 28S/25S rRNA are indicated with boxes. Other proteins and rRNA fragments are colored as in A.

Ebp1-ribosome complex compared to the Arx1-ribosome complex (Fig. 2B; Greber et al. 2016). Moreover, in contrast to Arx1, the lack of additional insertion regions in Ebp1 reduces its interaction surface with the ribosome (2209 Å² for Arx1-60S complex vs. 1191 Å² for Ebp1-80S complex without considering ES27L) (Fig. 2C; [Supplemental Fig. S9A](#)). Particularly, residues 112–121 and 342–350 of Arx1 mediate additional interactions with uL29 (Fig. 2C; Greber et al. 2016). Moreover, in Arx1, two helices formed by residues 445–450 and 482–490 wedge between 5.8S rRNA and uL24 thereby providing additional interactions (Fig. 2C; [Supplemental Fig. S9A](#)). It is interesting to note that while a region corresponding to helix 5 (region 206–212) of Ebp1 is not a helix in Arx1 (Greber et al. 2016), a loop region (residues 287–294) mediates the corresponding interaction with the 25S rRNA ([Supplemental Fig. S9A,B](#)). It is also noteworthy that the regions in Arx1 (residues 271–303, 326–333, and 426–496) that mediate several binding interactions with Rei1 and Alb1 during 60S assembly in yeast (Greber et al. 2016) are also absent in Ebp1 ([Supplemental Fig. S9C](#)). While Alb1 is a known modulator of the Arx1 interaction with pre-60S particle, Rei1 has a dual function wherein it senses the peptide exit tunnel architecture and is necessary for the release of Arx1 from the pre-60S particle (Lebreton et al. 2006; Meyer et al. 2007; Greber et al. 2012, 2016). Thus, the lack of Alb1 and Rei1 interaction regions in Ebp1 suggests that human Ebp1 might not have a role in ribosome biogenesis, which is consistent with RNAi knockdown of Ebp1 in human cells having been shown to have a minimal effect on 60S biogenesis (Wild et al. 2010).

Ebp1-ES27L can rotate around the hinge domain

While this manuscript was in preparation, other studies also reported the structure of Ebp1 in complex with ribosomes (Kraushar et al. 2020; Liang et al. 2020; Thoms et al. 2020; Wells et al. 2020; Wild et al. 2020). The structure of the Ebp1-ribosome complex reported in our study recapitulates most of the primary interactions with the 28S rRNA, uL23, and eL19 proteins that are observed in other recent structures ([Supplemental Table S3](#); Kraushar et al. 2020; Liang et al. 2020; Thoms et al. 2020; Wells et al. 2020; Wild et al. 2020). Superposition of the Ebp1-ribosome structure obtained in this study with those previously determined showed different orientations of Ebp1 with respect to the ribosome. The structure determined by Wild et al., however, showed the largest conformational change in the orientation of Ebp1 with respect to the core of the 60S subunit (Fig. 3; [Supplemental Movie S1](#)). Further analysis of this conformational change revealed that Ebp1-ES27L in the structure determined by Wild et al. is rotated by ~8–10° in comparison to all the other structures (Fig. 3C; [Supplemental Table S4](#)). Interestingly, the rotation axis passes through the insert domain of Ebp1, which serves

as its major interaction surface for association with the ribosome (Fig. 1). Consequently, the residues in this domain that contribute to the interaction with the ribosome undergo minimal change in their position owing to their proximity to the rotational axis. On the other hand, residues in the MetAP2 core domain of the Ebp1 undergo a larger change in their position as they are placed away from the rotational axis (Fig. 3C). Interestingly, the structure of the Ebp1-ribosome complex determined by Liang et al. shows another conformational change wherein the interaction of Ebp1 with uL29 and 5.8S rRNA is absent ([Supplemental Fig. S10](#)). The structural comparison of these different Ebp1-ribosome complexes indicates that Ebp1's interactions with uL23, eL19 and H53 and H59 of the 28S are consistent in all the determined structures while those with uL24, uL29, 5.8S rRNA, and other regions of 28S rRNA (except H53, H59, and ES27L) are variable ([Supplemental Table S3](#)).

We then analyzed the potential consequence of the observed rotation of Ebp1 along this axis. First, we find that the rotation of Ebp1 results in either loss or weakening of the interaction of the MetAP core domain of Ebp1 with uL24 and 28S rRNA (region 389–391) (Fig. 4). In the Wild et al. structure, the interaction of Ebp1 and uL24 is mediated by Lys210 (Ebp1) and Asn91 (uL24) (Wild et al. 2020). Furthermore, Lys210 of Ebp1 is involved in a hydrogen bond interaction with A389 of the 28S rRNA (Wild et al. 2020). Lys211 and Lys215 of Ebp1 also contribute toward its interaction with uL24 and 28S rRNA (region 389–391) (Wild et al. 2020). These interactions, however, are not seen in the structure of the Ebp1-ribosome complex reported here and the side chains of Lys211 and Lys215 of Ebp1 are not ordered in our structure. Similarly, the structure by Thoms et al. and Wells et al. also show fewer interactions in this region as compared to the structure reported by Wild et al. ([Supplemental Table S3](#); Thoms et al. 2020; Wells et al. 2020). Rotation of Ebp1 translates helix 5 (region 206–212) by ~5 Å leading to an increase in the distance to the 28S rRNA (region 389–391) (Fig. 4). Interestingly, the presence of an equivalent interaction corresponding to Ebp1 helix 5 and the 28S rRNA interaction in the Arx1-60S ribosomal subunit structure and the presence of additional Arx1 interaction partners such as Rei1 and Alb1 could restrict a similar rotation of Arx1 (Fig. 2; [Supplemental Fig. 9A–C](#); Greber et al. 2016). Helix 5 of Ebp1 is not present in human MetAP2 ([Supplemental Fig. 9D](#); Liu et al. 1998), which suggests a potentially even more dynamic interaction between MetAP2 and the ribosome.

In the Wild et al. (2020) structure, Ebp1 binds in the proximity of the peptide exit tunnel and almost blocks it completely with little lateral gap. The rotation of Ebp1 around the insert domain and the loss of the Ebp1-uL24/28S rRNA (region 389–391) interaction increases this lateral gap by ~2 Å. To test the possible range of rotation of Ebp1 that would be allowed, we generated a series of Ebp1 conformations that are rotated along the observed

FIGURE 3. Ribosome bound Ebp1 can undergo a rotation around the insert domain. (A) Overview of Ebp1 binding site on human ribosome obtained in this study is shown in two orientations. Ebp1 (green), eL19 (yellow), uL23 (magenta), uL24 (orange), uL29 (wheat) proteins, fragment of 5.8S rRNA (purple), and different regions (H53, H59, and ES27L) of the 28S rRNA (light blue) are shown as cartoon. (B) Overview of Ebp1 binding site on human ribosome from a previous study is shown in two orientations (PDB: 6SXO [Wild et al. 2020]). Ebp1 (light pink), eL19 (yellow), uL23 (magenta), uL24 (orange), uL29 (wheat) proteins, fragment of 5.8S rRNA (purple), and different regions (H53, H59, and ES27L) of the 28S rRNA (light blue) are shown as cartoon (as in A). (C) Relative orientation of Ebp1 (green and light pink) with respect to 60S subunit is shown based on superposition of ribosomal proteins in two orientations. The axis and degree of rotation of Ebp1 are indicated. Arrows (gray) indicate a few regions of Ebp1 that undergo conformational change.

rotational axis in both directions while keeping the 60S subunit fixed (Supplemental Fig. S11A). These models were then used to calculate clash scores using MolProbity and the binding interface was analyzed using “Protein interfaces, surfaces, and assemblies” (PISA) (Krissinel and Henrick 2007; Williams et al. 2018). The clash score and predicted decrease in energy of solvation due to complex formation were used to evaluate the feasibility of each conformation. This analysis pointed out that there is a window of $\sim 20\text{--}30^\circ$ (represented by the region between the vertical lines in the Supplemental Fig. S11B) where there is not a drastic increase in clashes or predicted free energy. This suggests that Ebp1–ES27L could rotate around this axis without dissociating from the ribosome (Supplemental Fig. S11B).

In this study, we report the structure of human Ebp1 bound to the mature 80S ribosome in proximity of the peptide exit tunnel. While the precise role of Ebp1 in regulating translation remains to be elucidated, comparison of the Ebp1-ribosome complex obtained in this study with the recently reported structures suggests that the Ebp1 can adopt a range of conformation while remaining bound to the ribosome even when ES27L wobbles on the ribosome (Kraushar et al. 2020; Liang et al. 2020; Thoms et al. 2020; Wells et al. 2020; Wild et al. 2020). While Ebp1 preferentially binds the empty 80S ribosome, this inherent flexibility may enable Ebp1 to remain bound to a translating ribosome while inhibiting other factors that also recognize this surface. Consequently, the levels of Ebp1 in the cell could shape the overall translational output of the cell. This is consistent with the change in overall and newly synthesized protein levels observed upon Ebp1 depletion (Kraushar et al. 2020). It is interesting to note that Ebp1 is highly expressed in some tumors and during embryonic development (Liu et al. 2006; Neilson et al. 2017; Yu et al. 2017; Nguyen et al. 2019; Hwang et al. 2020). Since Ebp1 is known to have a role in transcription and it also interacts with ribosomes, it is tempting to speculate a role for Ebp1 in coupling transcription and translation in the cell.

MATERIALS AND METHODS

Sample preparation for cryo-EM

Mature 80S ribosomes were isolated using two different protocols from the HEK Expi293F cells as described elsewhere with modifications (Khatter et al. 2014, 2015; Bhaskar et al. 2020). The ribosomal pellet obtained in the final step was then resuspended in resuspension buffer (50 mM HEPES 7.6, 80 mM Potassium acetate, 5 mM Magnesium chloride, and 1 mM DTT) to obtain a final concentration of ribosomes at 0.5–0.7 mg/mL. A total of 4 μL of the sample was applied on the Quantifoil R2/2 Cu 300 mesh grids (Quantifoil, Micro Tools GmbH). The grids were blotted for 3 sec at 4°C and 80% humidity and subsequently plunge frozen in liquid ethane using Leica EM GP plunge freezer (Leica Microsystems). Alternatively, 4 μL of the sample was applied on

FIGURE 4. Rotation of Ebp1 leads to remodeling of its interactions with the ribosome. Two conformations of Ebp1 (green and light pink [PDB: 6SXO; Wild et al. 2020]) bound to the 80S ribosome are shown. Fragments of 28S rRNA are displayed in light blue. Loss of interaction with uL24 (orange) is indicated by an arrow.

the Quantifoil R2/2 Cu 300 mesh grids coated with 3 nm continuous carbon (Quantifoil, Micro Tools GmbH). The grids were blotted for 3 sec at 4°C and 80% humidity and subsequently plunge frozen in liquid ethane using Vitrobot (Thermo Fisher Scientific).

Image acquisition and data processing

A C_s -corrected (CEOS GmbH) FEI Titan Krios microscope (Thermo Fisher Scientific) operated at 300 kV equipped with a Gatan-K2 Summit direct detector (operated in counting mode) (Gatan Inc.) and Quantum-LS Gatan Image Filter (GIF, slit width of 20 eV) was used for data acquisition. A total of 7438 micrographs were recorded as movies (40 frames/micrograph) in two different data sets (calibrated magnification of 58140 $\times$ corresponding to 0.86 Å/pixel). Defocus range of 0.5 to 2.5 μ m was used for both data sets. Motion correction, dose-weighting were done using full-frame motion correction and the CTF estimation was performed using CTFFIND4 in cryoSPARCs (Rohou and Grigorieff 2015; Punjani et al. 2017). Subsequently, an initial subset of particles was picked manually and was used as template for automatic picking in cryoSPARCs (Punjani et al. 2017). Picked particles were subjected to multiple rounds of initial 2D classification. Subsequently the selected particles were exported, and the following steps of processing were carried out in RELION-3.0 (Scheres 2012; Zivanov et al. 2018). The exported particles from both data sets were subjected to initial 3D refinement in RELION-3.0 separately (Scheres 2012; Zivanov et al. 2018). Then, the map obtained from this refinement was used for generating a mask for the density corresponding to the Ebp1–ES27L region and was used for initial 3D classification. Subsequently, the particles containing Ebp1 were sorted, refined and the density corresponding to 40S in data set 1 or 40S along with eEF2 and E-site tRNA in data set 2 were subtracted from the particles using a refined map. The density subtracted particles from both data sets were merged and used for multiple rounds of focused 3D classification (using Ebp1–ES27L mask) and refinement (Supplemental Fig. S1). After the final round of refinement, the final maps were corrected for the modulation transfer function (MTF) of the K2 camera, multiple user defined B-factors (20, 40, 60) were applied and low-pass filtered with the resolution cut-off corresponding to the Fourier shell correlation (FSC) value of

0.143 (calculated using two half data sets) in RELION-3.0 (Zivanov et al. 2018). These maps were used for model building and model refinement. Variation in local resolution of the map was estimated in RELION (Scheres 2012; Zivanov et al. 2018).

Refinement, model building, and structure analysis

Previously deposited atomic models of the 60S ribosomal subunit (PDB: 6Y0G) and Ebp1 (PDB: 2V6C and 2Q8K) were chosen as the initial atomic model for the refinement (Kowalinski et al. 2007; Monie et al. 2007; Bhaskar et al. 2020). These models were docked into the map using UCSF Chimera and were subjected to an initial round of rigid body refinement, and real-space refinement with global minimization, local grid search, simulated annealing, and ADP (B-factor) refinement using Phenix-real space refine (Pettersen et al. 2004; Adams et al. 2010; Afonine et al. 2018a,b). This refined model was used for multiple iterative rounds of model building in Coot and subjected to real space refinement with global minimization, local grid search, and ADP (B-factor) refinement in Phenix (Adams et al. 2010; Emsley et al. 2010; Afonine et al. 2018b). As the resolution of ES27L was limited, a double stranded RNA helix composed of poly(G) and poly(C) was placed in the corresponding density in Coot. This model was subjected to a final round of Phenix-real space refinement with global minimization, local grid search, and ADP (B-factor) refinement (Adams et al. 2010; Afonine et al. 2018b). Figures were prepared using the Pymol (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC) and UCSF Chimera softwares (Pettersen et al. 2004). The rotation axis of Ebp1 was calculated using the `draw_rotation_v2.py` (https://raw.githubusercontent.com/Pymol-Scripts/Pymol-script-repo/master/draw_rotation_axis.py) script available from github in PyMol and was subsequently used for generating multiple rotated conformation of Ebp1. Analysis of the interface and calculation of the solvation free energy gain were done using PISA server (http://www.ebi.ac.uk/pdbe/prot_int/pistart.html) (Krissinel and Henrick 2007). Clash scores for models containing different conformations of Ebp1 with respect to the ribosome were estimated using Phenix validation tool (Williams et al. 2018; Afonine et al. 2018a).

Sucrose density gradient and western blots

HEK Expi293F cells were maintained in FreeStyle media (Thermo Fisher Scientific) at 37°C and 5% CO₂. Control and puromycin treated (100 μ g/mL) HEK Expi293F cells were lysed in ice cold lysis buffer composed of 20 mM HEPES pH 7.4, 150 mM KCl, 15 mM MgCl₂, 1 mM DTT, 2% NP40, RNasin (Promega), 1 $\times$ protease inhibitor (Bimake). Lysate was incubated on ice for 30 min, then centrifuged (13,000 rpm for 10 min at 4°C) to pellet the insoluble fraction. A 15% to 60% (w/v) sucrose gradient was prepared (20 mM HEPES pH 7.4, 150 mM KCl, 15 mM MgCl₂, 1 mM DTT, 15% or 60% sucrose) with Biocomp Gradient

Master. A total of 500 μ L of supernatant were layered on top of gradients, and the samples were centrifuged in an SW40 rotor at 39,000 rpm for 3 h at 4°C. Gradients were fractionated (0.60 mL each) and subsequently pooled together based on the OD₂₆₀. The polysome profile fractions were analyzed by western blot using Anti-Ebp1 amino-terminal (Merck) and Anti-RPS15 antibodies (Abcam).

DATA DEPOSITION

The cryo-EM maps and the corresponding atomic model reported in this study are available from the Electron Microscopy Data Bank and the Protein Data Bank with accession numbers EMD-12189 and 7BHP, respectively.

SUPPLEMENTAL MATERIAL

Supplemental material is available for this article.

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Author contributions: V.B., J.D., and J.A.C. designed the experiments. V.B. and A.G.M. contributed toward preparation and freezing of the sample. V.B., S.C., and A.D.S. carried out image acquisition and data processing. Map interpretation, modeling, and bioinformatic analysis was carried out jointly by V.B. and J.D. Biochemical experiments were designed and performed by J.D. The manuscript was written by V.B, J.D., and J.A.C. with input from all other authors.

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