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Roles of elusive translational GTPases come to light and inform on the process of ribosome biogenesis in bacteria

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Summary

Protein synthesis relies on several translational GTPases (trGTPases), related proteins that couple the hydrolysis of GTP to specific molecular events on the ribosome. Most bacterial trGTPases, including IF2, EF-Tu, EF-G, and RF3, play well-known roles in translation. The cellular functions of LepA (also termed EF4) and BipA (also termed TypA), on the other hand, have remained enigmatic. Recent studies provide compelling *in vivo* evidence that LepA and BipA function in biogenesis of the 30S and 50S subunit, respectively. These findings have important implications for ribosome biogenesis in bacteria. Because the GTPase activity of each of these proteins depends on interactions with both ribosomal subunits, some portion of 30S and 50S assembly must occur in the context of the 70S ribosome. In this review, we introduce the trGTPases of bacteria, describe the new functional data on LepA and BipA, and discuss the how these findings shape our current view of ribosome biogenesis in bacteria.

Abbreviated summary (for Graphical Abstract)

LepA and BipA are paralogs of EF-G highly conserved among bacteria. Recent studies show that these GTPases act in biogenesis of the 30S and 50S subunit, respectively. An important implication of these findings is that, for each subunit, some portion of the assembly process occurs in the context of the 70S ribosome.

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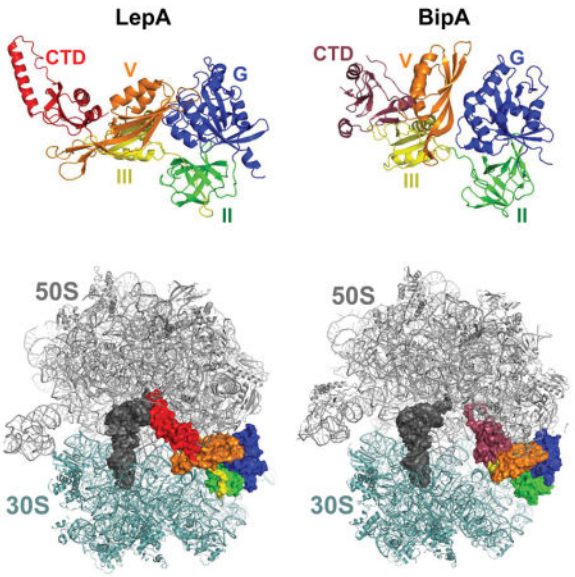

The P-loop GTPases represent a large family of proteins involved in various cellular processes including translation, signal transduction, cell motility, intracellular transport, protein trafficking, and chromosome partitioning (Leipe *et al.*, 2002). There is an important subfamily of P-loop GTPases termed the translational GTPases (trGTPases). Characteristic of the trGTPases is a domain architecture in which the highly-conserved GTPase (G) domain is followed by a β barrel domain (domain II). In bacteria, there are nine trGTPases including initiation factor IF2; elongation factor EF-Tu and similar proteins SelB and CysN; and elongation factor EF-G and similar proteins RF3, TetM, BipA, and LepA (Margus *et al.*, 2007). With the sole exception of CysN, these trGTPases bind the 70S ribosome and exhibit ribosome-dependent GTP hydrolysis activity (Burdett, 1996; Carlson *et al.*, 2017; De Laurentiis and Wieden, 2015; deLivron and Robinson, 2008). CysN is a component of the enzyme ATP sulfurylase, which forms activated sulfate (adenosine 5′-phosphosulfate; APS) from ATP and sulfate, an obligate step in the metabolic assimilation of sulfur (Leyh and Suo, 1992). GTP hydrolysis by the CysN subunit regulates APS formation in many bacteria (Liu *et al.*, 1998; Margus *et al.*, 2007). Structures of IF2, EF-Tu, SelB, EF-G, RF3, TetM, BipA, and LepA on the ribosome have been determined by cryo-electron microscopy and/or X-ray crystallography (Fischer *et al.*, 2016; Gagnon *et al.*, 2014; Gao *et al.*, 2009; Kumar *et al.*, 2015; Li *et al.*, 2013; Schmeing *et al.*, 2009; Sprink *et al.*, 2016; Zhou *et al.*, 2012). These factors bind the ribosome in a similar manner with domains G and II making specific contacts to the 50S and 30S subunit, respectively, on the A-site side of the ribosome (Fig. 1). The G domain interacts with a conserved region of the 50S subunit including the sarcin-ricin loop (SRL) of the 23S rRNA, contacts critical for GTPase activation.

Most ribosome-associated trGTPases play well-established roles in the cell. IF2 promotes the binding of initiator tRNA (fMet-tRNA^{fMet}) to the small (30S) subunit of the ribosome and the subsequent docking of the large (50S) subunit during initiation (Antoun *et al.*, 2003; Antoun *et al.*, 2006; Goyal *et al.*, 2015; Grigoriadou *et al.*, 2007; Marshall *et al.*, 2009; Pavlov *et al.*, 2011). The GTP-bound form of IF2 is important for these functions, and the

hydrolysis of GTP (which occurs upon subunit joining) facilitates subsequent IF2 release. EF-Tu catalyzes the binding of aminoacyl-tRNA (aa-tRNA) to the A site of the ribosome during elongation (Geggier *et al.*, 2010; Gromadski and Rodnina, 2004; Johansson *et al.*, 2012; Loveland *et al.*, 2017; Ogle *et al.*, 2002; Satpati *et al.*, 2014; Voorhees *et al.*, 2010). EF-Tu•GTP•aa-tRNA samples ribosomes carrying peptidyl-tRNA in the P site, and pairing between the anticodon of aa-tRNA and the codon in the 30S A site triggers GTP hydrolysis by EF-Tu. This causes release of the acceptor end of aa-tRNA from EF-Tu, allowing the aa-tRNA to move fully into the ribosome (A/A site) and participate in peptide bond formation. The GTP hydrolysis event is functionally irreversible and hence provides two independent opportunities for rejection of incorrect aa-tRNA substrates, increasing the overall accuracy of aa-tRNA selection. SelB resembles EF-Tu in structure and function but specifically facilitates incorporation of selenocysteinyl-tRNA at appropriate UGA codons (Fischer *et al.*, 2016; Kotini *et al.*, 2015; Zinoni *et al.*, 1990). Such UGA codons are marked by a stem-loop structure (SECIS element), which lies just downstream in the mRNA. SelB binds selenocysteinyl-tRNA^{Sec} exclusively and has a unique domain that interacts with the SECIS element, thereby directing site-specific incorporation of selenocysteine. EF-G catalyzes translocation, the movement of tRNAs (and paired codons) to their adjacent sites in the ribosome (Belardinelli *et al.*, 2016; Chen *et al.*, 2016; Cunha *et al.*, 2013; Savelsbergh *et al.*, 2003; Wasserman *et al.*, 2016; Zhang *et al.*, 2016; Zhou *et al.*, 2014). Binding of EF-G•GTP to the pretranslocation complex (carrying deacylated tRNA in the P site and peptidyl-tRNA in the A site) results in rapid GTP hydrolysis, which is followed by a conformational change that “unlocks” the ribosome and promotes tRNA-mRNA movement. Hydrolysis of GTP speeds tRNA-mRNA movement and is critical for subsequent release of EF-G from the posttranslocation complex. TetM is structurally similar to EF-G, and confers resistance to the antibiotic tetracycline (Burdett, 1991; Burdett, 1996; Li *et al.*, 2013). TetM•GTP binds the ribosome and catalyzes release of tetracycline from the 30S A site. This presumably occurs via conformational changes induced by TetM, although the detailed mechanism has yet to be elucidated. RF3 catalyzes the dissociation of release factor RF1 or RF2 (RF1/2) during termination (Freistroffer *et al.*, 1997; Jin *et al.*, 2011; Koutmou *et al.*, 2014; Peske *et al.*, 2014; Shi and Joseph, 2016; Zhou *et al.*, 2012). RF3•GTP binds the posttermination ribosome (containing deacylated tRNA in the P site and RF1/2 in the A site) and promotes intersubunit rotation, which destabilizes RF1/2. Hydrolysis of GTP by RF3 allows dissociation of both termination factors from the ribosome. LepA and BipA are structurally similar to EF-G, with the three proteins sharing four homologous domains (Fig. 1) (Evans *et al.*, 2008; Fan *et al.*, 2015). The biological roles of LepA and BipA have been difficult to pin down.

A phylogenetic analysis of trGTPases in >200 representative bacterial genomes revealed that IF2, EF-Tu, EF-G, and LepA are the most ubiquitous (Margus *et al.*, 2007). Genes encoding IF2, EF-Tu, and EF-G were found in all bacteria, while the *lepA* gene was found in all but one particular strain of *Streptococcus pyogenes*. BipA was found in most (86%) of the bacteria analyzed, often being absent in those lineages with minimal genomes. By comparison, RF3, a well-known participant in translation termination, was found in 62% of the bacteria analyzed. These observations imply that LepA and BipA play important roles in the bacterial cell.

LepA functions in 30S subunit biogenesis

In 1985, the *lepA* gene was originally identified as the gene upstream from the leader peptidase (*lep*) gene in *Escherichia coli* (March and Inouye, 1985). Dibb and Wolfe (1986) disrupted the *lepA* gene, and the null mutant conferred no obvious defects in growth or protein secretion (Dibb and Wolfe, 1986). Since then, other investigators have further analyzed the *ΔlepA* mutant and found only subtle phenotypes, such as increased sensitivity to tellurite (Shoji *et al.*, 2010) and compromised fitness under conditions of low pH, low temperature, and high Mg^{2+} (the latter phenotypes being revealed only in growth competition assays) (Pech *et al.*, 2011). Loss of LepA confers more obvious phenotypes in other organisms. In *Helicobacter pylori,* LepA was found to be essential under acidic conditions (Bijlsma *et al.*, 2000). In *Streptomyces coelicolor*, loss of *lepA* leads to overproduction of an antibiotic (Badu-Nkansah and Sello, 2010). Eukaryotes also have LepA in the form of mitochondrial and chloroplast homologs. In *Saccharomyces cerevisae*, loss of LepA (Guf1) causes heat and cold sensitivity and reduced levels of cytochrome oxidase (Bauerschmitt *et al.*, 2008). In mice, a mitochondrial *lepA* knockout leads to decreased spermatogenesis and male stertility (Gao *et al.*, 2016). In *Arabidopsis thaliana*, deletion of *cpLEPA* impairs chloroplast development and increases sensitivity to light (Ji *et al.*, 2012). These disparate phenotypes across various lineages have been puzzling and provided little clarity regarding the physiological role of LepA.

In 2006, it was proposed that LepA acts as a “back-translocase,” catalyzing reverse tRNA-mRNA movement in the ribosome (Qin *et al.*, 2006). This idea was attractive, due to the structural similarity between LepA and EF-G (Fig. 1). However, multiple laboratories have since been unable to confirm this biochemical activity (Balakrishnan *et al.*, 2014; Ermolenko; Liu *et al.*, 2010; Rodnina). Using toeprinting, which monitors the position of mRNA in the ribosome, Balakrishnan *et al.* (2014) found no evidence that LepA could catalyze reverse translocation in various ribosomal complexes, although the protein exhibited robust ribosome-dependent GTPase activity (Balakrishnan *et al.*, 2014). Cooperman and co-workers used puromycin and fluorescent probes on mRNA and tRNA to interrogate the effects of LepA on translocation (Liu *et al.*, 2010). Addition of LepA to ribosomes in the posttranslocation state promoted some movement of tRNA with respect to the 50S subunit, reducing puromycin reactivity of bound peptidyl-tRNA to an intermediate level. Codon-anticodon movement followed at a very slow rate (0.0009 s^{-1}), virtually identical to that seen in the absence of LepA (Liu *et al.*, 2010). Thus, while LepA can interact with the ribosome and compete with elongation factors (Liu *et al.*, 2010; Liu *et al.*, 2011), LepA does not substantially accelerate reverse codon-anticodon movement (i.e., back translocation) as initially proposed (Qin *et al.*, 2006).

Important clues about the role of LepA came from Balakrishnan *et al.* (2014). Using ribosome profiling, they found that loss of LepA alters the average ribosome density (ARD) on hundreds of mRNAs in *E. coli* (Balakrishnan *et al.*, 2014). The effects on ARD depend on the sequence of the translation initiation region, with a tendency for mRNAs with “strong” Shine-Dalgarno sequences to exhibit reduced translation efficiency in the absence of LepA. While clearly influencing ARD, LepA had virtually no effect on ribosome distribution along mRNA, arguing against a role in elongation. Indeed, direct measurements

of elongation rates showed no difference in wild-type versus *ΔlepA* cells (Balakrishnan *et al.*, 2014). Furthermore, Shoji *et al.* (2010) showed that *ΔlepA* does not alter the frequency of miscoding or frameshifting (-1 or +1, spontaneous or programmed) (Shoji *et al.*, 2010). Together these data suggested that LepA primarily influences initiation, with little to no impact on elongation (Balakrishnan *et al.*, 2014).

Balakrishnan *et al.* (2014) also screened for synthetic phenotypes by moving *ΔlepA* mutation into every strain of the Keio collection, a set of strains in which each non-essential gene is deleted (Baba *et al.*, 2006). The results showed that *ΔlepA* confers a synthetic growth defect in strains compromised for gene regulation (*dksA*, *molR*), transport (*tatB*, *tonB*, *tolR*), respiration (*ubiF*, *ubiG*, *ubiH*) and ribosome assembly (*rsgA*) (Balakrishnan *et al.*, 2014). Of these genes identified, *rsgA* is only one clearly linked to the ribosome and hence potentially most pertinent to LepA function. RsgA (also termed YjeQ) is a GTPase known to be important in 30S subunit biogenesis (Campbell and Brown, 2008; Goto *et al.*, 2011; Leong *et al.*, 2013), raising the possibility that LepA also contributes to this process. Balakrishnan *et al.* (2014) hypothesized that loss of LepA causes a defect in ribosome assembly that indirectly alters translation initiation in the cell (Balakrishnan *et al.*, 2014).

To directly test the role of LepA in ribosome assembly, Gibbs *et al.* (2017) used SILAC (stable isotope labeling of amino acids in culture) (Ong *et al.*, 2002) and mass spectrometry to determine r-protein composition of ribosomal particles in the presence and absence of LepA (Gibbs *et al.*, 2017). They found that four r proteins (S3, S10, S14, and S21) are disproportionately underrepresented in 30S particles in the *ΔlepA* strain. These proteins assemble at a late stage of 30S subunit biogenesis and are involved in the folding of the 3′ major and minor domains of the 16S rRNA. In addition, 30S particles in the *ΔlepA* mutant contain elevated levels of precursor (17S) rRNA. This defect in 30S assembly did not appear to be an indirect consequence of altered protein production rates. Based on ribo-seq data, loss of LepA caused no appreciable decrease in small subunit protein or assembly factor production rates. Collectively, these data indicate that LepA functions in 30S subunit biogenesis. The genetic link between *lepA* and *rsgA* was further investigated by performing a similar SILAC analysis in *ΔrsgA* cells. Ribosomal protein composition of fractions from *ΔrsgA* cells showed accumulation of 30S particles lacking S2, S3, S10, and/or S21 (Gibbs *et al.*, 2017). These particles, which presumably represent stalled pre-30S intermediates, resemble those seen in the absence of LepA. Thus, LepA and RsgA appear to play partially redundant roles in 30S subunit biogenesis (Gibbs *et al.*, 2017).

BipA functions in 50S subunit biogenesis

BipA (BPI-inducible protein A) was first identified in *Salmonella typhimurium* as a protein produced when cells are treated with bactericidal/permeability increasing protein (BPI), an antimicrobial secreted by human neutrophils (Qi *et al.*, 1995). BipA has since been implicated in the virulence of several human pathogens. In enteropathogenic *E. coli* (EPEC), loss of the factor leads to decreased cytoskeletal rearrangements in host cells, sensitivity to host defense peptides, and hypermotility (Farris *et al.*, 1998; Grant *et al.*, 2003). BipA influences thermoregulation of the *E. coli* capsule, leading to increased transcription of K-antigens at 37°C and reduced transcription at 20°C (Rowe *et al.*, 2000). In *E. coli* K12,

ΔbipA leads to reduced growth rate at 20°C (Pfennig and Flower, 2001), suggesting that BipA activity depends on temperature. The *ΔbipA* mutant also exhibits hypersensitivity to antibiotics that target the ribosome, such as chloramphenicol and tobramycin (Duo *et al.*, 2008). In *Pseudomonas aeruginosa,* BipA is important for virulence, antimicrobial resistance, and biofilm formation (Neidig *et al.*, 2013).

Biochemical studies showed that BipA, like other trGTPases, exhibits 70S-dependent GTP hydrolysis activity (deLivron and Robinson, 2008; Kumar *et al.*, 2015). The structure of BipA is similar to LepA and EF-G except for a unique C-terminal domain (CTD, Fig. 1), which is crucial but not sufficient for ribosome binding (deLivron *et al.*, 2009). The truncated factor BipAΔCTD exhibits elevated and ribosome-independent GTP hydrolysis activity, suggesting the CTD is necessary for GTPase control (deLivron *et al.*, 2009). BipA can also bind ppGpp, the bacterial stress alarmone, and BipA•ppGpp appears to preferentially interact with the 30S subunit (deLivron and Robinson, 2008; Fan *et al.*, 2015). Structural studies have revealed that, off the ribosome, BipA adopts virtually the same structure regardless of the nucleotide bound (GDPCP, GDP, ppGpp, or no nucleotide) (Fan *et al.*, 2015; Kumar *et al.*, 2015). On the ribosome, BipA•GDPCP exhibits a different, more compact structure (Kumar *et al.*, 2015). Although structures of ribosome-bound BipA•ppGpp have yet to be reported, modeling of ppGpp in place of GDPCP predicts a steric clash between the 3′ diphosphate and the SRL of the 50S subunit (Kumar *et al.*, 2015). This may explain why BipA•ppGpp associates with 30S rather than 70S particles. It was postulated that BipA acts as a stress factor, with ppGpp allosterically controlling ribosome binding specificity to regulate synthesis of virulence and stress factors (deLivron and Robinson, 2008). However, to our knowledge, no additional evidence has since materialized to bolster this hypothesis.

Compelling *in vivo* evidence suggests instead that BipA functions in biogenesis of the 50S subunit of the ribosome. Flower and coworkers screened for Tn5 insertion mutations that suppress the cold sensitive phenotype of *ΔbipA* in *E. coli* (Krishnan and Flower, 2008). They found several independent insertions, all of which mapped to *rluC*, which encodes a pseudouridine synthase that modifies U955, U2504, and U2580 of the 23S rRNA. It was further shown that loss of RluC or a triple substitution of its three target nucleotides (U955, U2504, U2580) could suppress the cold-sensitive and capsule synthesis phenotypes of the *ΔbipA* mutant (Krishnan and Flower, 2008). As ribosome modification and assembly are coordinated processes, this raised the possibility that BipA plays a role in 50S biogenesis. Indeed, further work by Flower and coworkers showed that loss of *bipA* leads to accumulation of pre-50S particles and elevated levels of precursor 23S rRNA in cells grown at 20°C (Choudhury and Flower, 2015). Additionally, they found that deletion of *deaD*, encoding a DEAD-box helicase that acts on the 23S rRNA, exacerbates the cold-sensitivity and 50S assembly phenotypes of the *ΔbipA* mutant (Choudhury and Flower, 2015). Together, these data provide strong evidence that BipA participates in ribosome assembly. A defect in 50S biogenesis could be the basis of the pleiotropic phenotypes of *ΔbipA* reported previously (Duo *et al.*, 2008; Farris *et al.*, 1998; Grant *et al.*, 2003; Neidig *et al.*, 2013; Pfennig and Flower, 2001; Qi *et al.*, 1995; Rowe *et al.*, 2000).

Implications for ribosome assembly

The bacterial 70S ribosome is a ~2.5 MDa enzyme composed of three large rRNAs (16S, 23S, 5S) and ~50 r proteins. Biogenesis of the ribosome is complicated and begins with transcription of the three rRNA molecules, which are usually co-transcribed in a single operon. The primary transcript is cleaved by endonucleases into precursor rRNAs, which are later trimmed to form mature 16S, 23S, and 5S rRNA. Assembly of each subunit involves the folding of rRNA and binding of r proteins in a generally hierarchical manner (Fig. 2). Transcription and cleavage, rRNA modification, r protein binding, and rRNA folding are coordinated, although there are many random-order events in the assembly process, resulting in multiple parallel pathways (Mulder *et al.*, 2010; Talkington *et al.*, 2005). Early studies showed that each ribosomal subunit can self-assemble *in vitro* using purified components (Nierhaus and Dohme, 1974; Traub and Nomura, 1968). However, self-assembly is slow and requires non-physiological conditions such as elevated temperature and Mg^{2+} concentration. In the cell, ribosome assembly is considerably faster and more efficient due to dozens of assembly factors (AFs), including ribonucleoprotein-binding (RNP-binding) proteins, modification enzymes, helicases, chaperones, and GTPases (Shajani *et al.*, 2011; Wilson and Nierhaus, 2007) (Table 1).

Assembly of the small and large subunits have generally been considered separately, as though they are independent processes. However, factors such as LepA and BipA, which are important for 30S and 50S biogenesis, respectively, each require the 70S ribosome for GTP hydrolysis. This implies that at least a portion of the assembly process occurs in the context of the 70S ribosome. It has been proposed for example, that LepA acts late in the assembly process by binding a precursor 70S particle and catalyzing a conformational change in the 30S subunit head domain that provides another opportunity for correct folding of the 3′ domain of 16S rRNA (Gibbs *et al.*, 2017). This activity is in line with other trGTPases, such as EF-G, TetM, and RF3, which promote conformational changes in the 70S ribosome as part of their biological function.

A growing body of evidence indicates that, in all organisms, ribosome assembly is checked through quality control mechanisms (Karbstein, 2013). In eukaryotes, a number of AFs mimic r proteins or translation factors and bind specific regions of the pre-40S, occluding the functional sites of the subunit until maturation is complete (Strunk *et al.*, 2011). For example, Rio2, Tsr1, and Dim1 occupy binding sites for eIF1, eIF1A, and initiator tRNA, thereby preventing premature translation initiation (Strunk *et al.*, 2011). Intriguingly, final assembly of the 40S subunit includes a functional “test drive” in which 80S complexes lacking mRNA and tRNA are formed and subsequently dissociated prior to canonical translation initiation (Strunk *et al.*, 2012). The test drive begins with a pre-40S particle bound by several AFs (Enp1, Pno1, Dim1, Rio2, Tsr1, and Nob1). Initiation factor eIF5B promotes 60S subunit docking in a GTP-dependent manner, resulting in an 80S complex and release of Rio2. The ATPase Fap1 then promotes intersubunit rotation, causing dissociation of Dim1 and Tsr1 (Ghalei *et al.*, 2017). Release factor homolog Dom34 and ATPase Rli1 then function to break apart the 80S complex, using the energy of ATP hydrolysis. The endonuclease Nob1 cleaves the ends of the precursor 18S rRNA either before or after subunit dissociation (Strunk *et al.*, 2012). The mature 40S subunit is then ready to bind

mRNA and start translation initiation. This test drive effectively checks the functional centers of the ribosome and licenses the mature 40S to enter the translation pool.

Similar quality control mechanisms are likely at play in bacteria, with late-stage assembly events occurring in the context of the 70S ribosome. Recent work from Varshney and colleagues suggests that ribosome assembly coincides with translation initiation in *E. coli* (Shetty and Varshney, 2016). Depletion of initiator $tRNA^{fMet}$ inhibited ribosome maturation, as indicated by cold sensitivity and elevated levels of unprocessed rRNA. Expression of mutant $tRNA^{fMet}$ with basepair substitutions in the anticodon stem also conferred defects in ribosome assembly, and 70S particles with precursor 17S rRNA accumulated in such strains. Based on these and additional data, the authors proposed that the final stages of ribosome assembly take place during the ribosome's virgin round of initiation (Shetty and Varshney, 2016). This model can rationalize several earlier observations: (i) the presence of precursor rRNAs in polysomes (Mangiarotti *et al.*, 1974; Srivastava and Schlessinger, 1988), (ii) specific inhibition of ribosome assembly by lamotrigine, which targets IF2 (Stokes *et al.*, 2014), and (iii) genetic interactions between IF2 and assembly factors (Campbell and Brown, 2008).

An alternative hypothesis is that assembly involves formation of a 70S complex that contains AFs in the binding sites of tRNA and initiation factors, more akin to the "test drive" described in eukaryotes. Recent cryo-EM studies show that RsgA binds the interface surface of the 30S subunit, with domains occupying the sites for IF1, IF3, and initiator tRNA (Lopez-Alonso *et al.*, 2017) (Fig. 3), similar to how the eukaryotic AFs occlude the same sites of pre-40S subunits. A pre-30S particle bound by AFs including RsgA may participate in a translation-like cycle in which the 50S subunit docks and is subsequently released, events coordinated with late-stage 30S maturation. IF2 could mediate 50S docking in the absence of initiator tRNA, as eIF5B does in the eukaryotic case, rationalizing the functional links between IF2 and ribosome assembly (Stokes et al., 2014; Campbell and Brown, 2008). In this scenario, LepA would presumably act in later events, in the context of the 70S ribosome. Clearly, future work will be necessary to test these two hypotheses and gain further insight into ribosome assembly in bacteria.

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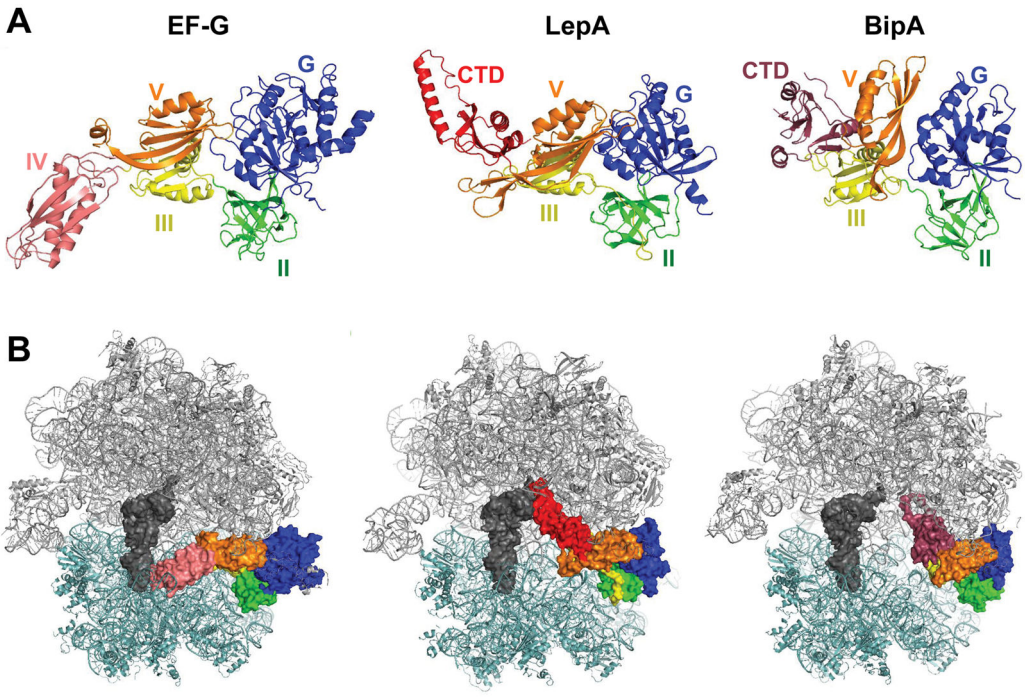

Figure 1. Homologous proteins EF-G, LepA, and BipA bind the ribosome similarly

(A) Structures of EF-G, LepA, and BipA. Domains G (blue), II (green), III (yellow), and V (orange) are homologous among the factors. Unique domains include EF-G domain IV (pink), LepA C-terminal domain (red), and BipA C-terminal domain (raspberry). Images are based on PDB ID files 4V5F, 5J8B, and 4ZCI. (B) Structures of EF-G, LepA, and BipA bound to the 70S ribosome. 50S subunit, gray; 30S subunit, light teal; P-site tRNA; dark gray. Color coding of GTPase domains as in panel A. Images are based on PDB ID files 4V5F, 4W2E, and 5AA0.

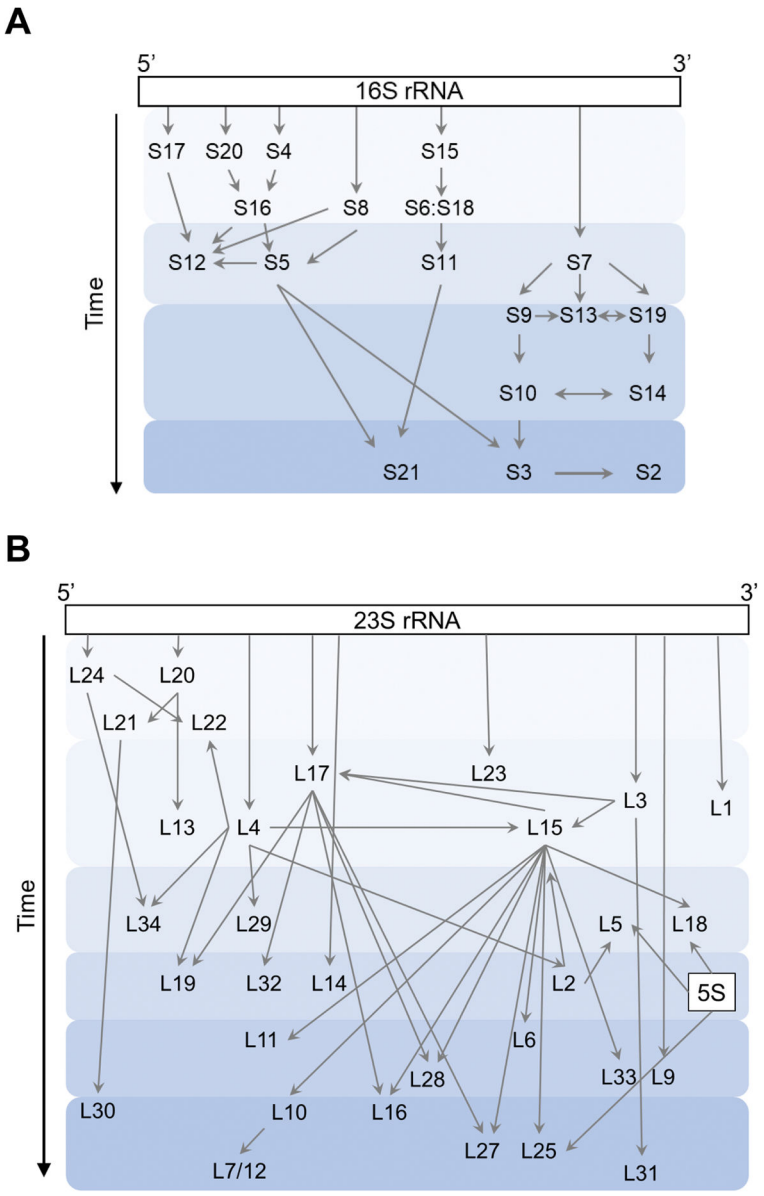

Figure 2. Assembly maps

Protein binding during assembly of the small (A) or large (B) subunit is depicted schematically. Arrows indicate hierarchical dependencies and shaded regions indicate temporal stages. Schemes are based on (Chen and Williamson, 2013; Davis *et al.*, 2016).

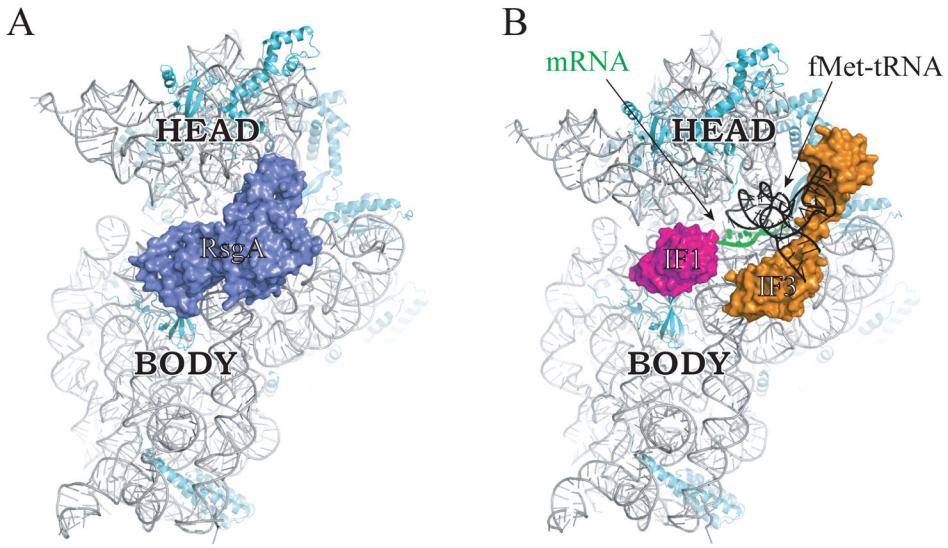

Figure 3. RsgA binds the 30S subunit in a way that occludes IF1, IF3, and initiator tRNA

Interface view of the 30S subunit bound by RsgA (A) or by IF1, IF3, fMet-tRNA, and mRNA (B), as determined by cryo-EM studies (Hussain *et al.*, 2016; Lopez-Alonso *et al.*, 2017). 16S rRNA, gray; r proteins, cyan; RsgA, blue; IF1, magenta; IF3, orange; mRNA, green; fMet-tRNA, black. Head and body regions of the subunit are labeled. Images are based on PDB ID files 5NO3 and 5LMV.

Table 1

A non-comprehensive list of *E. coli* proteins implicated in ribosome assembly

Assembly factor[1]	Type	Ribosomal subunit
RbfA	RNP binding	30S
RimJ	RNP binding	30S
RimM	RNP binding	30S
RimP	RNP binding	30S
YhbY	RNP binding	50S
KsgA (RsmA)	Modification enzyme	30S
RsmC	Modification enzyme	30S
RlmA (RrmA)	Modification enzyme	50S
RlmE (RrmJ)	Modification enzyme	50S
RluB	Modification enzyme	50S
RluC	Modification enzyme	50S
RluD	Modification enzyme	50S
DeaD (RhlD)	Helicase	50S
DbpA (RhlC)	Helicase	50S
SrmB (RhlA)	Helicase	50S
RhlE	Helicase	50S
DnaK/DnaJ/GrpE	Chaperone	30S, 50S
GroES/GroEL	Chaperone	50S
Era	GTPase	30S
RsgA (YjeQ)	GTPase	30S
LepA (EF4)	GTPase	30S
Der (EngA)	GTPase	50S
YihA (EngB)	GTPase	50S
BipA (TypA)	GTPase	50S

[1] Assignments based on (Keseler *et al.*, 2017; Shajani *et al.*, 2011; Wilson and Nierhaus, 2007) and references therein.