The signal recognition particle binds to protein L23 at the peptide exit of the *Escherichia coli* ribosome

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ABSTRACT

The signal recognition particle (SRP) from *Escherichia coli*, composed of Ffh protein and 4.5S RNA, mediates membrane targeting of translating ribosomes displaying a signal or signal-anchor sequence. SRP binds at the peptide exit of the large ribosomal subunit. Structural details of the interaction are not known. Here, the position of Ffh or SRP on the ribosome was probed by using site-specific UV-induced crosslinking by *p*-azidophenacyl bromide (AzP) attached to a number of cysteine residues engineered into surface positions of Ffh. Efficient crosslinking to vacant ribosomes took place from two positions (AzP17 and AzP25) in the N domain of Ffh, both with Ffh and SRP. Both AzP17 and AzP25 were predominantly crosslinked to ribosomal protein L23 that is located at the peptide exit of the 50S subunit. The SRP receptor, FtsY, did not change the crosslink pattern, whereas the presence of a nascent signal peptide on the ribosome resulted in a second crosslink between Ffh(AzP17) and protein L23, indicating that binding to the nascent signal peptide induced a slightly different arrangement of SRP on the ribosome. These results indicate a model of the topographical arrangement of SRP at the peptide exit of the 50S ribosomal subunit.

Keywords: 4.5S RNA; membrane targeting; ribosome nascent chain complexes; signal peptide; crosslinking

INTRODUCTION

The signal recognition particle (SRP) coordinates the cotranslational targeting of secretory and membrane proteins to the membrane of the endoplasmic reticulum in eukaryotes or the plasma membrane in bacteria. The SRP in *Escherichia coli* is composed of a 48-kD protein, Ffh, and 4.5S RNA. The SRP receptor, FtsY, binds to the SRP-ribosome nascent-chain complex and mediates the transfer of the translating ribosome to the site of protein translocation (translocon) in the membrane. Ffh, 4.5S RNA, and FtsY share significant sequence homology and structural conservation with their respective eukaryotic functional counterparts, SRP54, 7S RNA, and SRα (Keenan et al. 2001).

Ffh is composed of three domains: the N-terminal N domain, the central GTPase (G) domain, and the C-terminal methionine-rich M domain (Freymann et al. 1997, 1999). The N domain is composed of four antiparallel α-helices; it appears to influence GTP binding and hydrolysis in the G domain. Furthermore, biochemical studies indicate a role for the N domain in integrating signal transduction events that occur during cotranslational protein targeting (Lu et al. 2001). The G domain has a β/α fold common to all GTP binding proteins. A unique feature of SRP-related GTPases is an insertion, I box, in the switch 1 region of the G domain (Freymann et al. 1997; Montoya et al. 1997). The M domain of Ffh contains the binding sites for the signal sequence and the 4.5S RNA (Batey et al. 2000). Binding of 4.5S RNA to Ffh stabilizes the M domain without significantly affecting binding of signal peptides (Zheng and Gierasch 1997). 4.5S RNA plays an important role in modulating the complex conformation of Ffh and its receptor, FtsY (Peluso et al. 2000, 2001; Jagath et al. 2001).

The binding site of SRP on the ribosome is not known in detail so far. The signal peptide is recognized cotranslationally by the SRP, indicating a location of SRP on the ribosome in the vicinity of the exit channel. Using UV-induced crosslinking to the ribosome from thio-U-substituted 4.5S RNA, two binding sites for 4.5S RNA were identified (Rinke-Appel et al. 2002). The crosslink from position 84 of 4.5S RNA to nucleotides 2828–2837 of 23S rRNA was dependent on the presence of both Ffh and a nascent peptide, indicating the significance of this 4.5S RNA binding site for

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protein targeting. Crosslinks from positions 29–50 of 4.5S RNA were found to the small ribosomal subunit and may be involved in functions of 4.5S RNA other than SRP-dependent targeting. The labeled N termini of signal peptides can be crosslinked to nt 91 of 23S rRNA in the neighborhood of L23 and L29 (Choi and Brimacombe 1998), indicating that SRP should also bind there. In fact, the mammalian homolog of Ffh, SRP54, was crosslinked to proteins L23a and L35 of eukaryotic ribosomes, the homologs of *E. coli* L23 and L29, respectively, indicating that SRP54 binds in the vicinity of the peptide exit of the large ribosomal subunit (Pool et al. 2002). Recently, the trigger factor (TF), a bacterial chaperone that binds to the nascent peptide chains emerging from the ribosome (Teter et al. 1999; Beck et al. 2000), was crosslinked to L23 at the peptide exit of the 50S ribosomal subunit (Kramer et al. 2002).

In this paper, we localize the position of the Ffh moiety of SRP on the *E. coli* ribosome by UV-induced crosslinking of a *p*-azidophenacyl (AzP) group attached to cysteine residues engineered into different surface positions of Ffh. The combined length of AzP and cysteine side chain is ~10Å. AzP-modified Ffh was used to probe the spatial proximity to ribosomal residues on both vacant ribosomes and translating ribosomes exposing a signal peptide. The results are discussed in terms of the topographical arrangement of SRP at the peptide exit of the 50S ribosomal subunit.

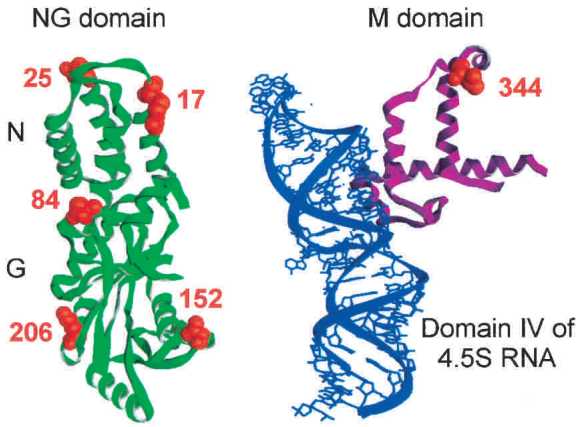

FIGURE 1. Positions in Ffh of AzP-modified cysteine residues. (*Left*) NG domain of Ffh from *Thermus aquaticus* (PDB entry 2FFH; Keenan et al. 1998). (*Right*) M domain of *Escherichia coli* Ffh complexed with a 49-nucleotide fragment of 4.5S RNA (1DUL; Batey et al. 2000). Amino acids replaced with cysteine are indicated in red and labeled by *E. coli* numbers; residue 344 corresponds to residue 335 of the *T. aquaticus* structure. The structure of the parts of the M domain missing in the structure of the RNA complex, including the finger loop comprising residue 344, was modeled on the basis of the crystal structure of full-length Ffh (2FFH).

RESULTS

Modification of Ffh with AzP

The crosslinker AzP was incorporated at single cysteine residues that were engineered into six different surface positions in the N, G, and M domains of Ffh (Fig. 1), replacing nonconserved amino acids. The native cysteine at position 406 of Ffh was replaced with serine, and the C terminus was extended with a tag of six histidines for affinity purification. Labeling positions were chosen so as to cover most of Ffh, that is, the tip of the N domain (positions 17 and 25), the interface between the N and G domains (position 84), the I box insertion in the G domain (position 152), the side of the G domain opposite to the I box (position 206), and the so-called finger loop in the M domain (position 344).

The functional activity of AzP-modified Ffh was tested in gel-shift assays with 4.5S RNA and FtsY (Jagath et al. 2001). According to these assays, all Ffh-AzP derivatives used for the crosslinking experiments were as active as native Ffh in binding 4.5S RNA and the SRP receptor, FtsY (data not shown).

Crosslinking of Ffh-AzP to the ribosome

First, crosslinking of Ffh or SRP to nontranslating vacant ribosomes was studied. SRP has a significant affinity to vacant ribosomes (~0.05 μM, unpubl.). Ribosome-Ffh/SRP complexes were prepared at 1 μM concentrations of both ribosomes and Ffh/SRP. The photoreactive azido group was activated for crosslinking by UV irradiation at 305 nm. Irradiated ribosome complexes were subjected to two rounds of sucrose gradient centrifugation to separate (1) 30S and 50S subunits and (2) rRNA from ribosomal proteins (Stade et al. 1989). The crosslinked products from the rRNA and ribosomal proteins pools were denatured by heating, and RNA was digested with RNase T1. Proteins were separated by SDS-PAGE, and crosslinked Ffh was visualized by immunoblotting by using an antibody against the His tag of Ffh.

Given the molecular weights of Ffh (48 kD) and most ribosomal proteins, molecular masses of crosslinked products are expected at ≥~60 kD. For AzP-modified Ffh at positions of 17 and 25, Ffh(AzP17) and Ffh(AzP25), crosslinks were found to both 23S rRNA and to proteins of the 50S subunit (Fig. 2A). The protein–protein crosslink product gave a band at ~60 kD, distinct from the position of Ffh. Interestingly, crosslinks of Ffh to ribosomal proteins were not affected by 4.5S RNA, indicating that binding of Ffh to the ribosome does not depend on 4.5S RNA and that the location on the ribosome of Ffh alone and Ffh in SRP is similar. A slight difference is indicated by the observation that the crosslink to 23S rRNA observed with Ffh alone disappeared on addition of 4.5S RNA. Because the crosslink to 23S RNA was rather weak, ~50× weaker than that to ribosomal proteins, it was not analyzed further. No crosslinks were found from other positions in Ffh (Fig. 2B).

Ribosome-nascent chain complexes (RNC) were formed by translating a 3′-truncated fragment of leader peptidase

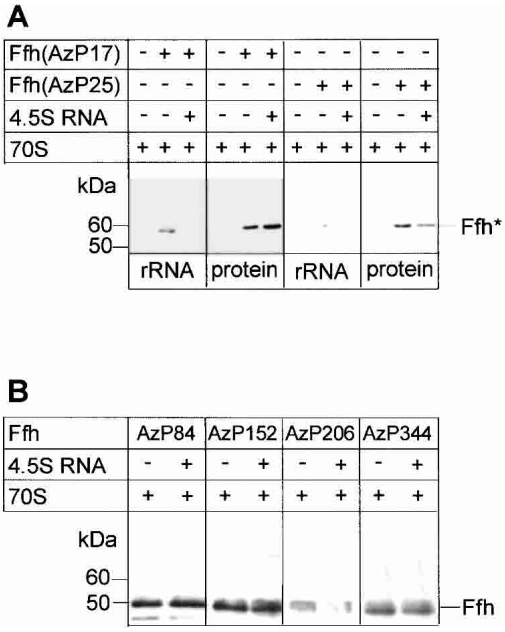

FIGURE 2. Crosslinking of AzP-modified Ffh and SRP to vacant 70S ribosomes. (*A*) Identification of crosslinks from Ffh positions 17 and 25. Crosslinks to 23S rRNA (rRNA) or 50S ribosomal proteins (protein) were analyzed after two rounds of sucrose gradient centrifugation, which removed noncrosslinked Ffh (for details, see Materials and Methods). Ffh* indicates position of Ffh crosslinked to rRNA or protein. (*B*) No crosslinks from positions 84, 152, 206, and 344 of Ffh. Irradiated complexes were analyzed without sucrose gradient centrifugation. In *A* and *B*, Ffh was identified by immunoblotting using antibodies against the His tag of Ffh. Without UV irradiation, no crosslinking products were found with either mutant (data not shown).

(Lep) mRNA coding for the first 94 amino acids of the protein. The fraction of the ribosomes that contained the nascent chains was ~50%, as quantified from the number of f[^{3}H]Met-labeled peptide chains per ribosome. The length of the nascent chains was as expected (>90 amino acids), as calculated from the number of ^{14}C-labeled leucine residues incorporated per f[^{3}H]Met and verified by SDS-PAGE and phosphoimaging (data not shown).

The affinity of SRP to the ribosome is increased >10-fold when the SRP-specific signal peptide of leader peptidase is exposed on the ribosome (unpubl.). Therefore, we used smaller concentrations of Lep-RNC and Ffh/SRP (0.1 μM each) in order to minimize Ffh/SRP binding to nontranslating ribosomes present in the preparation. Crosslinks from both Ffh(AzP17) and Ffh(AzP-25) to Lep-RNC were obtained, as identified with an antibody against the His tag of Ffh (data not shown). Other positions in Ffh gave no significant crosslink product.

Identification of ribosomal proteins crosslinked to Ffh

Ribosomal proteins crosslinked to Ffh(AzP17) or Ffh(AzP25) were analyzed using antibodies against about half of all ribosomal proteins (kindly provided by R. Brimacombe, Berlin, Germany; see Materials and Methods). Crosslinking to vacant ribosomes of Ffh(AzP17) alone or in SRP resulted in a single crosslink to protein L23 (Fig. 3A). The same protein was the major crosslinking product of Ffh(AzP25), both in the absence and presence of 4.5S RNA. The efficiency of crosslinking to L23 was high, >10% and ~30% with Ffh(AzP17) and Ffh(AzP25), respectively (Fig. 3B). Low-yield crosslinks were formed between Ffh(AzP25) and two other proteins, L21 and L27; Ffh(AzP17) did not form crosslinks other than to L23.

L23 was also the major crosslinking target on the Lep-RNC (Fig. 4). Unlike with vacant ribosomes, with RNC the crosslink products between Ffh(AzP17) and L23 appeared in two bands, indicating the formation of two L23-Ffh adducts with slightly different electrophoretic mobilities. Both products are probably owing to crosslinks to RNCs, as at the conditions used (equimolar amounts of RNC and Ffh/SRP at low concentration, Ffh/SRP binding to vacant ribosomes is expected to be very low. UV irradiation of the Lep-RNC with Ffh(AzP25) resulted in single crosslink product with L23, as with vacant ribosomes. Crosslinking products of Ffh(AzP25) with L21 and L27 were observed in only small amounts.

FtsY did not change the crosslink pattern on Lep-RNC

At the plasma membrane, the RNC-SRP complex binds to the SRP receptor, FtsY. In the presence of GTP, a stable complex between Ffh and FtsY is formed that is necessary to promote release of the signal peptide from SRP. The crosslinking of Ffh(AzP17) and Ffh(AzP25) to the Lep-RNC did not change on addition of FtsY and a nonhydrolyzable GTP analog, GDPNP (Fig. 5). Also in the presence of FtsY, two crosslinking products to L23 were observed with Ffh(AzP17), and the yield of both crosslinks was only slightly decreased. The major crosslink of Ffh(AzP25) to L23 was not affected by FtsY. This is in contrast to the eukaryotic system, in which binding of the Ffh homolog, SRP54, or SRP to the SRP receptor, SRα, results in the loss of a crosslink of SRP54 to the eukaryotic homolog of L23 (Pool et al. 2002), indicating that prokaryotic and eukaryotic complexes in that respect behave differently. Consistent with this notion, Pool et al. (2002) reported that the difference induced by SRα binding was less when *E. coli* SRP was present on the eukaryotic RNC.

DISCUSSION

The present results localize the binding site of SRP on the ribosome in the vicinity of the peptide exit of the large ribosomal subunit. In bacteria, the peptide exit is surrounded by proteins L23, L29, L22, and L24 (Harms et al. 2001). We show that the tip of the N domain of Ffh (positions 17 and 25) is located in the proximity of L23. Al-

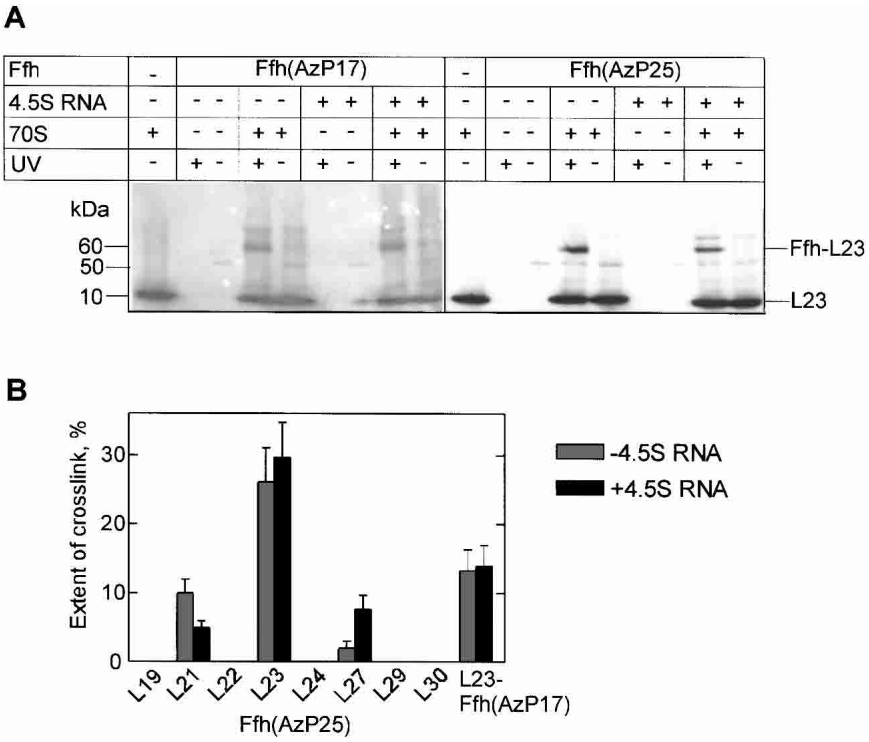

FIGURE 3. Identification of ribosomal proteins crosslinked from positions 17 and 25 of Ffh/SRP bound to vacant ribosomes. (*A*) Immunoblots using an antibody against the ribosomal protein L23. (*B*) Crosslinking efficiencies using antibodies against several large subunit proteins. Shaded bars indicate Ffh alone; solid bars, SRP.

though the yield of crosslinks to L23, particularly from position 25 of Ffh is very high, no crosslinks to the neighboring protein L29 were found, indicating that the N domain of Ffh binds to the side of L23 opposite from L29. Another constraint for positioning SRP on the ribosome comes from the crosslink formed between the position 84 of 4.5S RNA and nt 2828–2837 of 23S rRNA (Rinke-Appel et al. 2002). Thus, SRP must bind to the ribosome in such a manner that the N domain of Ffh is located at L23, whereas the 3′ end of 4.5S RNA is pointing away from the exit tunnel in the direction of nt 2828–2837 of 23S rRNA.

The structure of SRP is not known, and the domain arrangement in full-length Ffh is unclear. In the crystal structure of full-length Ffh (Keenan et al. 2001), the protein is found as a trimer, and the structure of the loop connecting the NG and M domains is not resolved, precluding the unambiguous assignment of the relative domain orientation in one protein. The M domain from the molecule designed A in the Protein Data Bank (PDB) entry (2FFH) may belong to the protein with the NG domain that is denoted as chain A or to the one denoted as chain B. In the following, we term these relative domain arrangements A/A (both NG and M domains belong to the same chain in 2FFH) or B/A (the NG domain belongs to chain B, whereas the M domain belongs to chain A), respectively. The two arrangements predict a different orientation of 4.5S RNA relative to the NG domain, as can be deduced by superimposing the structure of the M domain from Ffh (2FFH) with the M domain in the complex with domain IV of 4.5S RNA (1DUL; Batey et al. 2000). In the complex with 4.5S RNA, some adjustment of the NG domain is necessary to avoid an otherwise extensive steric clash with 4.5S RNA. Docking the two alternative SRP structures to the ribosome by using the crosslinks from positions 17 and 25 of Ffh to L23 and from position 84 of 4.5S RNA to nt 2828–2837 of 23S rRNA as constraints yielded two alternative SRP orientations, as described below. Both arrangements satisfy the crosslinking results, as positions 84, 152, and 206 of Ffh are located far from any ribosomal component, and position 344 in the M domain is oriented away from the ribosome, in agreement with the lack of crosslinks from these positions. Only an extended form of RNA, rather than a bent form, could span the distance between the crosslinks on L23 and nt 2828–2837 of 23S rRNA, indicating that the functionally active form of 4.5S RNA on the ribosome may be extended (Gorodkin et al. 2001).

Fitting the tip of the N domain in the A/A model of SRP toward L23 results in an arrangement in which the NG and M domains of Ffh enclose the peptide exit (Fig. 6A). The tetra-loop region of 4.5 RNA is oriented toward the tunnel, whereas the 3′ end can be placed very close to the site of the crosslink to 23S RNA (Rinke-Appel et al. 2002) by introducing a slight bent in 4.5S RNA. According to this model (A/A), the emerging signal peptide could easily contact both NG and M domains, explaining that the signal peptide could be crosslinked to both domains (Zopf et al. 1990;

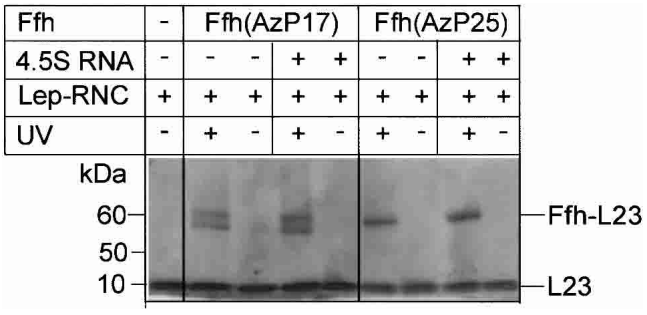

FIGURE 4. Crosslinking from positions 17 and 25 of Ffh bound to Lep-RNC. An immunoblot of the crosslinking products using an antibody against L23 is shown. Crosslinking efficiencies to L23, L21, and L27 were as in Figure 3.

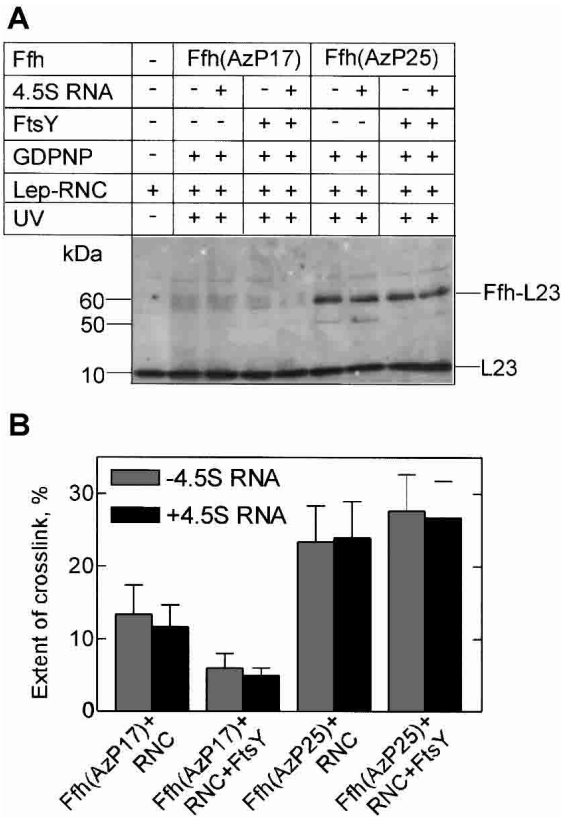

FIGURE 5. Crosslinking from positions 17 and 25 of Ffh bound to Lep-RNC in the presence of FtsY. (*A*) Immunoblot of the crosslinking products using antibody against L23. (*B*) Crosslinking efficiencies. Shaded bars indicate Ffh alone; solid bars, SRP.

High and Dobberstein 1991; Lütcke et al. 1992; Cleverley and Gierasch 2002). It has been suggested that FtsY binds at the I box of the NG domain of Ffh (Montoya et al. 1997). With this assumption, the arrangement of the NG domain of Ffh in model A/A would place FtsY in direct proximity to the ribosome, in keeping with the interaction between FtsY and the ribosome reported recently (Herskovits et al. 2002).

The alternative orientation of NG and M domains of Ffh, the B/A orientation, brings the tetra loop of 4.5S RNA close to the peptide exit and places the M domain on the top of L23 and L29, whereas the bulk of the NG domain protrudes away from the ribosome. According to this model, the emerging signal peptide is more likely to bind to the hydrophobic groove of the M domain (Keenan et al. 1998), whereas the G domain appears to be too distant to allow a simultaneous interaction of the N-terminal part of the signal sequence with the M domain (Zopf et al. 1990; High and Dobberstein 1991; Lütcke et al. 1992) and of the hydrophobic part of the signal sequence with the G domain of Ffh (Cleverley and Gierasch 2002). Also, the simultaneous interaction of FtsY with the ribosome and the I box of Ffh would be unlikely according to the B/A model. However, this does not exclude the B/A model, because the contacts mentioned above may be established sequentially, rather than simultaneously.

The present results show that bacterial SRP binds to a site on the ribosome which overlaps with that of TF, a chaperone involved in the cotranslational folding of cytosolic proteins, which also crosslinks to protein L23 (Kramer et al. 2002). TF binds to short nascent chains of any kind, with preference for peptides enriched in basic and aromatic residues (Patzelt et al. 2001), whereas SRP is specific for peptides containing a signal or signal-anchor sequence (Valent et al. 1995; Beck et al. 2000). A simple model assumes that SRP and TF alternate in transient binding to the ribosome until a nascent peptide emerges. On recognition of a nascent peptide, the binding of either SRP or TF is stabilized, and this event determines whether the RNC is targeted to the membrane for cotranslational protein translocation or membrane insertion (SRP) or whether the growing peptide is handed over to chaperones downstream of TF. There is evidence indicating that the ribosome may sense the nature of the nascent peptide while it is still in the exit tunnel (Liao et al. 1997; Nakatogawa and Ito 2002), raising the possibility that the ribosome actively recruits SRP or TF to the peptide exit.

MATERIALS AND METHODS

Reagents

Buffer A contains 50 mM Tris-HCl (pH 7.5), 70 mM NH_4Cl, 30 mM KCl, and 7 mM $MgCl_2$. AzP was from Sigma. GTP, GDPNP, pyruvate kinase, and phosphoenolpyruvate were from Roche Diagnostics. Ni-NTA agarose was from Qiagen. Nikkol (Octa-ethylene glycol mono-*n*-dodecyl ether) was from Nikko Chemicals, Japan. All other chemicals were obtained from Sigma or Merck.

E. coli strains and plasmids

BL21 (DE3) pLysS strain was used for expressing FtsY and Ffh from pET9-FtsY(Trp343) and pET24-Ffh, respectively (Jagath et al. 2000). The plasmid pT7–4.5S was used to prepare 4.5S RNA by transcription in vitro using T7 RNA polymerase (Lentzen et al. 1994).

Ffh, FtsY, and 4.5S RNA

Mutants of Ffh containing a single cysteine residue at positions 17, 25, 84, 152, 206, or 344 were generated by PCR mutagenesis by the QuickChange method using Pfu polymerase (Promega). The single cysteine residue present at position 406 of native Ffh was substituted with serine. Mutations were generated in plasmid pET24-Ffh coding for Ffh extended by six histidines at the C terminus. Mutations were confirmed by DNA sequencing.

Ffh mutants were expressed in *E. coli* BL21 (DE3) pLysS cells and purified on Ni-NTA agarose under non-denaturing conditions. Four grams of cell pellet was resuspended in 40 mL of buffer (20 mM Hepes at pH 7.5, 300 mM NaCl, 0.1 mM EDTA, 0.01%

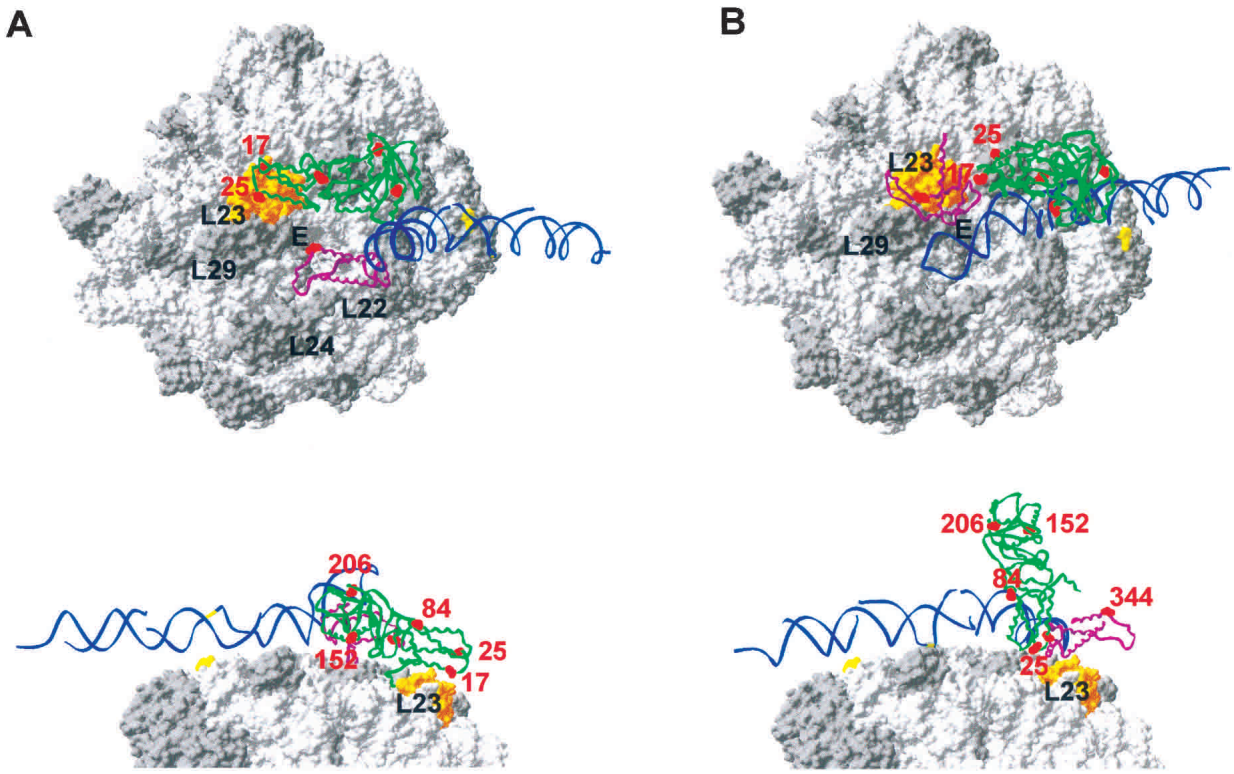

FIGURE 6. Model of the SRP-ribosome complex. (*A*) Docking of SRP with the A/A orientation (2FFH) of the NG and M domains. (*B*) Docking of SRP with the B/A orientation of the NG and M domains. In both models, the NG domain is shifted slightly to remove the steric clash with 4.5S RNA. For details of the models, see text. Green ribbon indicates, NG domain of Ffh; purple ribbon, M domain of Ffh; red ribbon, AzP-modified positions in Ffh; blue ribbon, 4.5S RNA. The crosslink between 4.5S RNA (position 84) and 23S rRNA (Rinke-Appel et al. 2002) is indicated in yellow. The ribosomal surface was calculated from the coordinates of the 50S subunit from *Haloarcula marismortui* (1JJ2; Ban et al. 2000) and is depicted in light gray (rRNA), with protein L23 in orange and other proteins in dark grey. E indicates peptide exit.

Nikkol, 0.1 mM Pefablock SC, and 10 mM 2-mercaptoethanol), and the suspension was sonicated 3× for 5 min each on ice (Branson Sonifier, duty cycle 50%, output 4). The extract was centrifuged at 20,000*g* for 30 min (Beckman, JA25.5). The supernatant was incubated with 1 mL of Ni-NTA agarose, equilibrated with buffer (20 mM Hepes at pH 7.5, 300 mM KCl, 10 mM 2-mercaptoethanol), for 60 min on ice under shaking. The resin was washed with buffer (20 mM Hepes at pH 7.5, 1 M KCl, 10 mM imidazole, 10 mM 2-mercaptoethanol) to remove unspecifically bound proteins. Ffh was labeled while bound to Ni-NTA agarose with 200 μM AzP in 20 mM Hepes (pH 7.5), 300 mM KCl, 10% methanol, and 10% glycerol in the dark for 2 h. After washing with the same buffer to remove excess crosslinking reagent, the protein was eluted by high-imidazole buffer (20 mM Hepes at pH 7.5, 300 mM KCl, 250 mM imidazole, 25% glycerol). Labeled Ffh was rebuffered into buffer A with 50% glycerol and concentrated on 30-kD Centricon filters at 4°C. The purity of proteins was >90% according to SDS-PAGE analysis. 4.5S RNA and FtsY(Trp343) were prepared as described (Jagath et al. 2000).

Ribosome-nascent chain complexes

70S ribosomes from *E. coli* MRE 600 and purified components of the translation system were prepared as described (Rodnina and Wintermeyer 1995; Matassova et al. 1999; Rodnina et al. 1999). Aminoacyl-tRNA was prepared by aminoacylation of total tRNA by the protein supernatant fraction after centrifugation at 100,000*g* (S100) from *E. coli* in the presence of 19 amino acids (0.3 mM each) except leucine, L-[^{14}C]leucine (30 μM), and ATP (3 mM). Aminoacyl-tRNA was phenolized and purified by ion exchange chromatography on MonoQ. RNCs were prepared as follows. 70S ribosomes (0.5 μM) were programmed with truncated Lep-mRNA (2 μM) coding for the first 94 amino acids of leader peptidase (de Gier et al. 1996) in the presence of purified initiation factors 1, 2, 3 (0.85 μM each), f[^{3}H]Met-tRNA^{fMet} (1 μM), and GTP (1 mM) in buffer A for 60 min at 37°C. Translation was started by mixing 70S initiation complexes (0.05 μM after mixing) with a preincubated mixture of EF-Tu (40 μM), EF-Ts (0.04 μM), EF-G (0.3 μM), purified aminoacyl-tRNA containing [^{14}C]Leu-tRNA (21 μM), GTP (1 mM), phosphoenolpyruvate (3 mM), and pyruvate kinase (0.08 mg/mL) in buffer A. After translation for 45 min at 37°C, RNCs were purified by ultracentrifugation through 400 μL 1.1 M sucrose in buffer A containing 20 mM $MgCl_2$ for 1.5 h at 259,000*g* in a 55S swing-out rotor in a Sorvall M120GX centrifuge. Pellets were dissolved in buffer A, shock-frozen, and stored at −80°C.

UV-induced crosslinking of Ffh(AzP) to the ribosome

SRP was formed by incubating AzP-modified Ffh and 4.5S RNA for 5 min at 25°C.

Crosslinking experiments were performed in buffer A containing, in addition, 0.02% Brij 35, 1 mM DTT, and 10% glycerol. Ffh/SRP was added to vacant ribosomes (1 μM each), to RNC (0.1 μM each), or to RNC (0.1 μM each) in the presence of FtsY (0.4 μM) and GDPNP (200 μM) to form the complex under subdued light. Mixtures were irradiated in a microtiter plate on ice for 10 min using four Philips Cleo 15 W lamps at a distance of 10 cm. To minimize photodegradation of protein or RNA, a 305 nm cut-off filter was placed between light source and sample.

Irradiated samples were denatured by boiling for 2 min, digested by RNase T1, and subjected to SDS-PAGE analysis (see below). In some experiments (Fig. 2A), irradiated samples were subjected to two rounds of sucrose gradient centrifugation prior to gel analysis: In the first run, 30S and 50S subunits were separated and, in the second, were performed in the presence of SDS; rRNA and proteins were separated (Stade et al. 1989). The material from the rRNA peak was digested with RNase T1 and subjected to SDS-PAGE analysis. The material from the protein peak was analyzed directly.

Crosslinked ribosomal proteins were identified by immunoblotting. Proteins were separated on a 7% SDS-PAGE and transferred to nitrocellulose membranes by electroblotting. The membrane was incubated either with anti-penta-His antibody (Qiagen) to identify His-tagged Ffh or with primary antibodies against a large number of ribosomal proteins of the small and large subunit (provided by R. Brimacombe). Initial screening indicated positive signals for L21, L23, and L27. Additional antibodies used for routine testing were against L19, L22, L24, L28, L29, and L30, which were always negative. Detection was by enhanced chemiluminescence using secondary antibodies conjugated to horseradish peroxidase (DAKO). For quantitative analysis, films were scanned, and the intensities of L23 crosslinked to Ffh relative to total L23 were determined.

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