

Interplay of signal recognition particle and trigger factor at L23 near the nascent chain exit site on the *Escherichia coli* ribosome

Ronald S. Ullers,¹ Edith N.G. Houben,¹ Amanda Raine,² Corinne M. ten Hagen-Jongman,¹ Måns Ehrenberg,³ Joseph Brunner,⁴ Bauke Oudega,¹ Nellie Harms,¹ and Joen Luirink¹

¹Department of Molecular Microbiology, Vrije Universiteit, 1081 HV Amsterdam, Netherlands

²Department of Pharmaceutical Biosciences and ³Department of Cell and Molecular Biology, Uppsala University, S-7514 Uppsala, Sweden

⁴Institute of Biochemistry, Eidgenössische Technische Hochschule Zürich, CH-8093 Zürich, Switzerland

As newly synthesized polypeptides emerge from the ribosome, they interact with chaperones and targeting factors that assist in folding and targeting to the proper location in the cell. In *Escherichia coli*, the chaperone trigger factor (TF) binds to nascent polypeptides early in biosynthesis facilitated by its affinity for the ribosomal proteins L23 and L29 that are situated around the nascent chain exit site on the ribosome. The targeting factor signal recognition particle (SRP) interacts specifically with the signal anchor (SA) sequence in nascent inner membrane proteins

(IMPs). Here, we have used photocross-linking to map interactions of the SA sequence in a short, in vitro-synthesized, nascent IMP. Both TF and SRP were found to interact with the SA with partially overlapping binding specificity. In addition, extensive contacts with L23 and L29 were detected. Both purified TF and SRP could be cross-linked to L23 on non-translating ribosomes with a competitive advantage for SRP. The results suggest a role for L23 in the targeting of IMPs as an attachment site for TF and SRP that is close to the emerging nascent chain.

Introduction

The crystal structure of the large ribosomal subunit reveals an extended cavity, with a length of ~ 100 Å and a diameter of ~ 15 Å, that runs from the peptidyl transferase center to the ribosomal surface (Nissen et al., 2000). This water-filled space is thought to constitute the major polypeptide exit tunnel. The limited width of the tunnel precludes folding and interactions with cellular components. Only when the nascent polypeptide emerges from the exit tunnel may it interact cotranslationally with molecular chaperones and targeting factors that assist folding, prevent aggregation, and facilitate localization to the correct subcellular destination.

Cross-linking studies have shown that trigger factor (TF)* is the first chaperone in line to interact generically with

nascent polypeptides in *Escherichia coli* (Valent et al., 1995, 1997; Hestekamp and Bukau, 1996). It was recently demonstrated that this early role of TF is made possible by its specific interaction with L23 and L29, two ribosomal proteins that are strategically positioned at the nascent chain exit site (Kramer et al., 2002). TF binds to unfolded proteins with moderate affinity (Maier et al., 2001) and has a preference for short hydrophobic peptides (Patzelt et al., 2001). In vitro, TF has prolyl isomerase activity (Stoller et al., 1995; Hestekamp and Bukau, 1996), but the in vivo relevance of this property is enigmatic because the interaction with substrate proteins is independent of the presence of proline residues (Stoller et al., 1995; Patzelt et al., 2001).

Similar cross-link studies have identified signal recognition particle (SRP) as the first targeting factor to specifically interact with nascent inner membrane proteins (IMPs) (Valent et al., 1997). The SRP consists of a 48-kD GTPase designated Ffh (for fifty-four homologue) and 4.5S RNA, which are homologous to the eukaryotic SRP54 and the 7S RNA. The latter two form part of a larger SRP that functions in the cotranslational targeting of proteins to the endoplasmic reticulum membrane (for review see Herskovits et al., 2000). Strikingly,

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Address correspondence to Joen Luirink, Dept. Molecular Microbiology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, Netherlands. Tel.: 31-20-4447175. Fax: 31-20-4446979. E-mail: joen.luirink@falw.vu.nl

*Abbreviations used in this paper: EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; Ffh, fifty-four homologue; IMP, inner membrane protein; SA, signal anchor; SRP, signal recognition particle; TF, trigger factor.

Key words: signal recognition particle; trigger factor; ribosome; protein targeting; membrane protein

it has been shown recently that SRP54 is juxtaposed to the eukaryotic homologues of L23 and L29 (Pool et al., 2002).

The cellular function of TF in targeting and folding is unclear. Recent evidence points to a cooperation with DnaK in cotranslational folding, although mechanistic details have remained elusive (Deuerling et al., 1999; Teter et al., 1999). It has been suggested that TF also plays a role in targeting by interacting specifically with the early mature region of pre-secretory proteins, thus preventing interaction of the SRP with the (mildly hydrophobic) signal peptide and funneling this class of proteins into the SecB/SecA targeting pathway (Beck et al., 2000). In contrast, other studies pointed to default nascent chain binding by TF and a decisive role for the SRP in conferring targeting specificity by high affinity interaction with a particularly hydrophobic signal anchor (SA) sequence in a nascent IMP (Valent et al., 1997; Lee and Bernstein, 2001).

In this study, we have investigated the initial recognition of a nascent IMP by chaperones and targeting factors in the *E. coli* cytosol. Interactions of a short targeting intermediate, with most of its SA sequence exposed just outside the ribosome, were analyzed in an unbiased site-specific photocross-linking approach. In addition to contacts with L23 and L29, both TF and SRP were found to interact with the SA with partially overlapping binding specificity and a competitive advantage for SRP. Interestingly, both purified TF and SRP could be cross-linked to L23 on purified ribosomes. The results suggest an important role for L23 at the nascent chain exit site, where decisions related to translation, folding, and targeting are being made based on specific sequence elements in the emerging nascent chain.

Results and discussion

Model IMP and experimental approach

We have analyzed the molecular environment of a short nascent IMP in the *E. coli* cytosol using a scanning in vitro photocross-linking approach. FtsQ, a bitopic type II IMP, was synthesized from truncated mRNA to a length of 77 amino acids in a cell- and membrane-free *E. coli* extract. At this nascent chain length, the majority of the SA sequence is expected to be exposed outside the ribosome (Fig. 1 A). Previous studies have indicated that 77FtsQ represents a short targeting intermediate of FtsQ (Urbanus et al., 2001). It is efficiently recognized by the Sec-translocon in the inner membrane, whereas a truncate that is seven residues shorter is defective in targeting. Consequently, it is expected that 77FtsQ interacts in the cytosol with factors that force the decision for cotranslational targeting to the membrane. A single stop codon (TAG) was introduced at positions 25–43 and 49 in the SA sequence and at positions 10 and 24 in the flanking hydrophilic region of 77FtsQ (Fig. 1 A). The TAG codons were suppressed during in vitro synthesis by the addition of (Tmd)Phe-tRNA^{Sup}, a suppressor tRNA that carries a photoreactive probe (Brunner, 1996). The translation mixture also contained [³⁵S]methionine to label the nascent chains. After translation, the samples were irradiated with UV light to induce cross-linking.

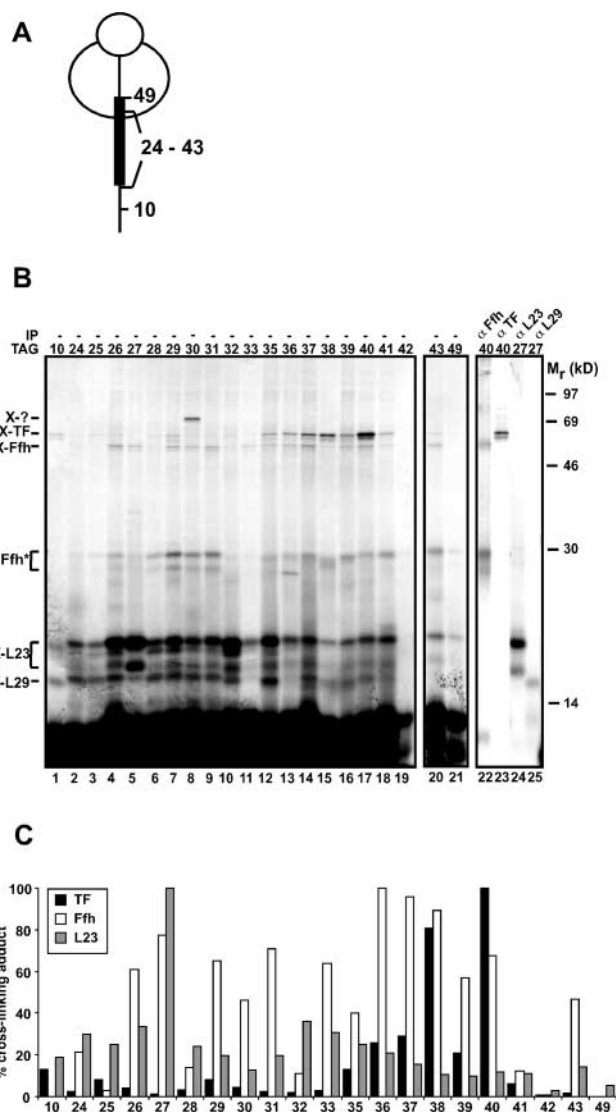


Figure 1. Scanning photocross-linking of nascent 77FtsQ. (A) Schematic representation of the position of (Tmd)Phe in nascent 77FtsQ. (B) In vitro translation of nascent 77FtsQTAG mutants was performed in the presence of (Tmd)Phe-tRNA^{Sup}. After translation, samples were irradiated with UV light to induce cross-linking, and the ribosome–nascent chain complexes were purified and analyzed by SDS-PAGE. UV-irradiated ribosome–nascent chain complexes of 77FtsQTAG27 and 77FtsQTAG40 were immunoprecipitated as indicated. (C) Quantification of Ffh, TF, and L23 cross-linking adducts. The highest value for cross-linking efficiency was taken as 100%.

The SA sequence of nascent 77FtsQ is close to SRP, TF, and the ribosomal proteins L23 and L29

Except for the TAG mutation at position 34, all mutations were efficiently suppressed by the modified suppressor tRNA, resulting in nascent FtsQ of the expected molecular weight and with a photoreactive probe at the desired position (Fig. 1 B). Compared with nonirradiated samples (not depicted), UV irradiation resulted in cross-linking to the chaperone TF and to the SRP subunit Ffh, as demonstrated by immunoprecipitation (Fig. 1 B, lanes 22 and 23).

Cross-linking to Ffh appeared rather efficient, considering the low cellular abundance of *E. coli* SRP (Jensen and Peder-

sen, 1994). This is consistent with the proposed role of the SRP in recognizing particularly hydrophobic SA sequences in IMPs for subsequent delivery at the Sec-translocon (Valent et al., 1998; Beck et al., 2000). The contact of Ffh with the SA sequence is extensive, ranging at least from position 24 to 43 (34–53 residues from the peptidyltransferase center), but does not appear uniform in intensity (Fig. 1 C). It seems plausible that already at this stage, the SA region has adopted an ordered structure that is accommodated in the hydrophobic binding pocket formed by part of the Ffh M domain and 4.5S RNA (Batey et al., 2000).

Cross-linking to TF was relatively modest, considering its cellular abundance (Lill et al., 1988), and appeared more focused to the COOH-terminal region of the SA sequence (positions 35–40) that is expected to be close to the nascent chain exit site on the ribosome (Fig. 1 A). This region does not comply with the proposed peptide binding motif of TF (Patzelt et al., 2001). Rather, the observed specificity seems related to the recently demonstrated affinity of TF for L23 and L29, which are located around the putative exit site, thus positioning TF in the vicinity of any emerging polypeptide (Kramer et al., 2002).

Interestingly, at all positions, cross-linking products of ~16–20 kD were observed (Fig. 1 B, lanes 1–21) corresponding to cross-linking partners of ~8–12 kD. Based on the small weight of the cross-linking partners and taking into account that the SA is not fully exposed outside the ribosome, we hypothesized that these adducts represented ribosomal proteins. Indeed, we could positively identify the ribosomal proteins L23 and L29 as cross-linking partners by immunoprecipitation (Fig. 1 B, lanes 24 and 25). Consistently, artificial release of the nascent chain from the ribosome by puromycin treatment diminished subsequent cross-linking to L23 and L29 (unpublished data). Cross-linking to L23 was observed along the exposed part of the nascent chain but appeared especially strong at position 27, suggesting a looped structure of the emerging SA sequence (Fig. 1 C). To our knowledge, this is the first demonstration that L23 and L29 are in close proximity to an emerging nascent polypeptide, consistent with their proposed location at the nascent chain exit site (Nissen et al., 2000). Other small cross-linking partners could not be identified. In addition, we found that residue 30 was specifically cross-linked to a yet unidentified ~70-kD component (Fig. 1 B, lane 8).

Cross-linking of nascent 77FtsQ to Ffh is preferred over cross-linking to TF and L23

In the *in vitro* translation approach described above, we have used a crude *E. coli* S-135 cell extract. In the final reaction mixture, the cellular components are diluted ~10-fold compared with the intracellular *E. coli* milieu. To investigate the influence of higher concentrations of interacting components, we switched to a reconstituted *in vitro* translation system consisting of purified components necessary for the translation reaction instead of using a crude S-135 extract. This approach offers the advantage of introducing TF and SRP into the system in defined concentrations in the absence of other cytosolic factors that might influence interactions at the nascent chain exit site. To ensure that endoge-

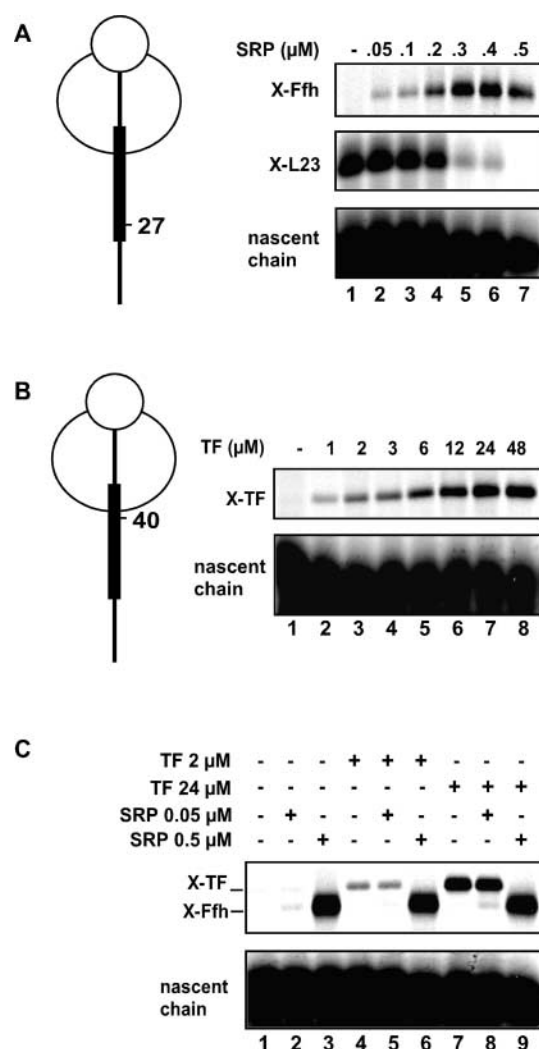


Figure 2. Cross-linking of 77FtsQ upon translation in a reconstituted translation system. (A–C) 77FtsQTAG27 (A) and 77FtsQTAG40 (B and C) were synthesized in a reconstituted translation system in the presence of (Tmd)Phe-tRNA^{Sup} and various concentrations of purified SRP (A), purified TF (B), or combinations of SRP and TF (C), as indicated. After translation, the ribosome–nascent chain complexes were purified and analyzed by SDS-PAGE.

nous TF was absent from the system, ribosomes were prepared from a TF⁻ strain. Two constructs were used, 77FtsQTAG27 and 77FtsQTAG40, that exhibit strong cross-linking to Ffh and to TF, respectively (Fig. 1). Both nascent chains were synthesized in the presence of varying concentrations of SRP and/or TF and subjected to cross-linking to analyze effects on nascent chain interactions.

Cross-linking of 77FtsQTAG27 to Ffh was readily detectable at 0.05 μM SRP (Fig. 2 A). Cross-linking of SRP was concentration dependent and saturated at 0.3 μM. Strikingly, the high cross-linking efficiency to the ribosomal protein L23 decreased at elevated levels of SRP and was completely eliminated at a concentration of 0.5 μM of SRP. Apparently, Ffh is able to compete with L23 for the proximity to nascent 77FtsQTAG27.

Cross-linking of 77FtsQTAG40 to TF was detected at 1 μM of TF (Fig. 2 B). Increasing amounts of TF resulted in

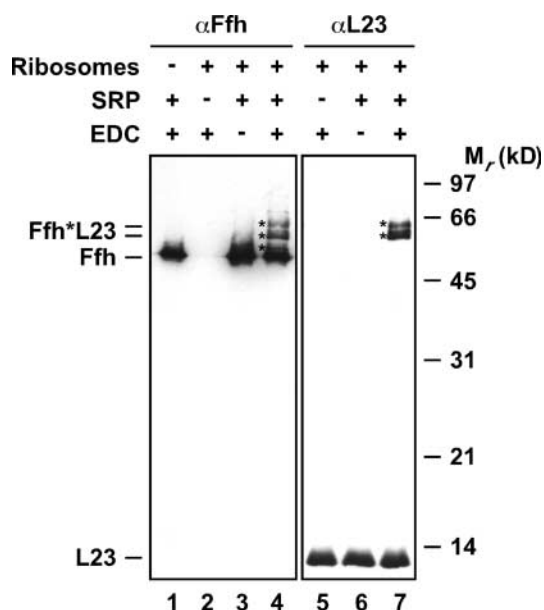


Figure 3. L23 is a ribosome attachment site for Ffh. Purified ribosomes (1 μ M) were incubated with SRP (300 nM) for 5 min at 26°C. Cross-linking was induced by the addition of 10 mM EDC, and samples were incubated for another 10 min at 26°C. Cross-linking was quenched, and the samples were TCA precipitated and analyzed by SDS-PAGE and immunoblotting using antisera against Ffh and L23, as indicated.

higher cross-linking efficiency. The minimal saturating concentration for TF cross-linking was \sim 24 μ M (Fig. 2 B).

Next, we examined the effect of simultaneously adding SRP and TF at low and saturating concentrations (Fig. 2 C). Notably, 0.5 μ M of the SRP completely prevented cross-linking of TF at position 40, even when TF was present at high concentrations. Vice versa, 24 μ M of TF had no effect on Ffh cross-linking at this position, even when it was present at the lowest detectable concentration. At position 27, the addition of TF up to a concentration of 48 μ M did not result in detectable TF cross-linking nor did it compete with cross-linking of Ffh and L23 (unpublished data). Together, the data indicate specific contacts of the 77FtsQ SA sequence with TF and L23/L29, but especially a dominating and high affinity interaction with the SRP component Ffh. This supports the notion that the SRP plays a decisive role in conferring targeting specificity (Valent et al., 1997; Lee and Bernstein, 2001) by effectively competing with the interaction of TF and L23 with the hydrophobic SA sequence of nascent IMPs. To address the issue of targeting specificity in more detail, it will be of interest to map the contacts of a nascent secreted protein with a less hydrophobic signal sequence in a similar experimental approach.

SRP interacts with the ribosomal protein L23

As SRP and L23 appear to compete for the same binding site on the nascent FtsQ, we considered the possibility that the SRP associates with L23 near the exit site. Purified nontranslating ribosomes were mixed with purified SRP and subsequently incubated with the carboxyl-amine cross-linker 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), pre-

cipitated with TCA, and analyzed by SDS-PAGE and immunoblotting using antisera against Ffh and L23 (Fig. 3).

Ffh and L23 migrate in SDS-PAGE at an apparent molecular mass of 49 kD and 12 kD, respectively. Only when both ribosomes and SRP were present were cross-linking adducts of \sim 50, \sim 56, and \sim 60 kD detected using antiserum against Ffh (Fig. 3, lane 4). The \sim 56- and \sim 60-kD adducts also cross-reacted with antibodies against L23 (Fig. 3, lane 7), indicating that they represent cross-linked Ffh–L23 complexes. The presence of two bands might be caused by cross-linking at different positions in the proteins. Alternatively, another small ribosomal protein might participate in complex formation. This putative protein might also contact Ffh directly, giving rise to the \sim 50-kD cross-linking adduct (Fig. 3, lane 4).

The observed cross-linking of Ffh to L23 on nontranslating ribosomes suggests that L23 serves as the docking site for Ffh on the ribosome to strategically position the SRP at the nascent chain exit site. This position is compatible with the recently reported Ffh-dependent cross-link of the 4.5S RNA with a particular region in the 23S RNA structure (Rinke-Appel et al., 2002). These contacts are likely to be dynamic in nature, given the substoichiometric ratio of SRP to ribosomes in the cell (Jensen and Pedersen, 1994).

Extrapolation of structural data from ribosome–translocon complexes of other species suggests that L23 forms part of one of the major connections between the ribosome and the translocon during cotranslational IMP insertion (Beckmann et al., 2001; Morgan et al., 2002). To allow docking of the ribosome at the SecYEG translocon, Ffh would have to evacuate L23. Interestingly, it has been shown recently by Pool et al. (2002) that the mammalian SRP54 interacts with L23a and L35, homologues of *E. coli* L23 and L29, respectively. When SRP54 contacts the SRP receptor, it moves away from L23a. It is tempting to speculate that the *E. coli* SRP receptor FtsY plays a similar role in the repositioning of Ffh on the ribosome during or after targeting. So far, we have not been able to detect cross-linking between Ffh and L29, which may be due to an unfavorable positioning of potentially cross-linking residues. In any case, L29 was extensively cross-linked to 77FtsQ, suggesting proximity between L29 and Ffh as well.

TF and SRP compete for binding to L23 on the ribosome

Because we detected cross-linking of Ffh to L23 and because an interaction between TF and L23 has been demonstrated previously (Kramer et al., 2002), we examined whether TF and the SRP compete for interaction with L23 using the EDC cross-linking procedure described above.

Cross-linking adducts that contain Ffh and L23 could again be observed when only SRP was incubated with the ribosomes (Fig. 4, lane 3). When only TF and ribosomes were present, a \sim 65-kD cross-linking product appeared (Fig. 4, lane 1) that was also detected using antiserum against TF (not depicted), indicating a TF–L23 interaction that is consistent with published data (Kramer et al., 2002). When SRP and TF were present in equimolar amounts, both TF–L23 and Ffh–L23 cross-linking products appeared (Fig. 4,

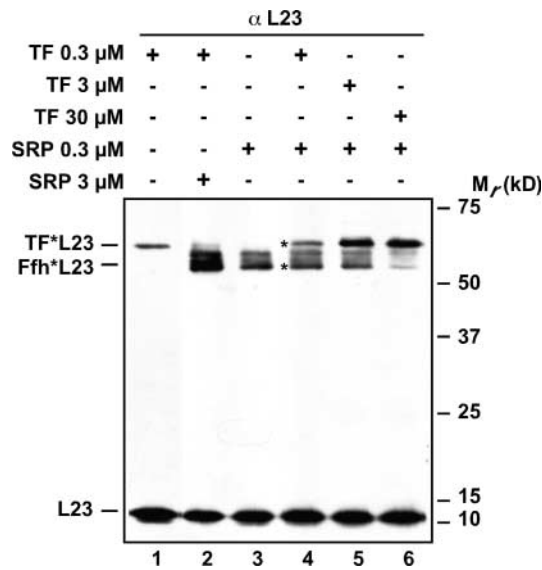


Figure 4. **TF and SRP compete for cross-linking to L23.** Purified ribosomes (1 μM) were incubated with various concentrations of SRP and TF for 5 min at 26°C as indicated. The samples were cross-linked and analyzed as described under Fig. 3.

lane 4), possibly because ribosomes were present in an approximately threefold molar excess in the reaction mixture. An ~10-fold molar excess of the SRP over TF resulted in higher cross-linking efficiency of Ffh to L23 whereas cross-linking of TF to L23 was almost completely eliminated (Fig. 4, lane 2). Increasing amounts of TF resulted in a higher cross-linking efficiency of TF to L23 (Fig. 4, lanes 5 and 6). Interestingly, an ~100-fold molar excess of TF over the SRP was necessary to almost completely prevent the cross-linking of Ffh to L23 (Fig. 4, lane 6).

The simplest explanation of these results is that SRP and TF share L23 as a common attachment site on the ribosome. This adds another layer of complexity to the regulation of the earliest stages of protein targeting. It appears unlikely that both SRP and TF interact simultaneously with L23, given the small region of L23 that is exposed at the surface of the ribosome (Harms et al., 2001). Consistent with a mutual exclusive interaction with L23, we could not detect any TF–Ffh cross-links in our experiments (Fig. 4). Rather, our data hint at a competition between SRP and TF for interaction with L23, with a competitive advantage for SRP, although this needs to be substantiated in proper binding studies. At present, it cannot be excluded that SRP or TF alters the conformation of L23, thus indirectly influencing the affinity of L23 for the other cytosolic factor. Finally, the conformation of L23 and its affinity for SRP and TF may be affected by the nascent polypeptide. Interestingly, it has recently been shown that mammalian SRP binds more tightly to translating ribosomes even when the nascent chain is very short and still buried in the ribosome (Flanagan et al., 2003).

In summary, L23 is likely to play an important role in the targeting of IMPs because it interacts with, or is in proximity to, all key players in this fundamental process: the nascent IMP itself, TF, SRP, and the translocon in the mem-

brane. Future studies will focus on the resolution of the intricate interactions at the nascent chain exit site and their influence on folding and targeting in a physiological context.

Materials and methods

Reagents, enzymes, and sera

Restriction enzymes and the Expand long template PCR kit were purchased from Roche Molecular Biochemicals GmbH. T4 DNA ligase was from Epicenter Technologies. Megashort T7 transcription kit was from Ambion. [³⁵S]methionine, protein A–Sepharose, protein G–Sepharose, and RNA guard (RNase inhibitor) were from Amersham Biosciences. EDC was from Pierce Chemical Co. Sigma-Aldrich supplied all other chemicals. Antisera against L29 and L23 were gifts of R. Brimacombe (Max Planck Institute for Molecular Genetics, Berlin, Germany). Anti-TF was provided by W. Wickner (Dartmouth Medical School, Hannover, NH). Purified TF and SRP were gifts of I. Sinning (University of Heidelberg, Heidelberg, Germany).

Strains and plasmid constructs

Strain Top10F' was used for routine maintenance of plasmid constructs (Stratagene). Strain MRE600 was used to obtain translation lysate for suppression of TAG stop codons in the presence of (Tmd)Phe-tRNA^{SUP} (Ellman et al., 1991). Strain MC4100 Δ*tig* [*tig*:Cm] was a gift from P. Genevaux (University of Geneva, Geneva, Switzerland) and was used to prepare purified TF⁻ ribosomes for translation in the reconstituted system.

To introduce single TAG codons in nascent 77FtsQ, plasmid pC4Meth77FtsQ (Urbanus et al., 2001) was mutated in a two-step PCR procedure as previously described (Scotti et al., 2000). The nucleotide sequences of the mutant genes were confirmed by DNA sequencing.

In vitro transcription, translation, and cross-linking in S-135 extract

Truncated mRNA was prepared as previously described (Scotti et al., 2000) from HindIII-linearized pC4Meth77FtsQ derivative plasmids. In vitro translation was performed in either an *E. coli* cell- and membrane-free S-135 extract described previously (Urbanus et al., 2001) or in a reconstituted in vitro translation system based on the purified components (see below). Photocross-linking was performed as previously described (Scotti et al., 2000). Ribosome–nascent chain complexes were collected by centrifugation and analyzed either directly by SDS-PAGE or after immunoprecipitation as previously described (Luirink et al., 1992) using fourfold the amount used for direct analysis. The labeled bands were quantified using the ImageQuant software (Molecular Dynamics) and corrected for translation efficiency of the nascent chains by dividing the amount of cross-linked material by the amount of total labeled material.

Translation in reconstituted in vitro translation system

The components of the reconstituted in vitro translation system are described in detail in the online supplemental material (available at <http://www.jcb.org/cgi/content/full/jcb.200302130/DC1>). (Tmd)Phe-tRNA^{SUP} was added to the factor mix. Purified SRP and TF were added to the factor mix in concentrations ranging from 0 to 2 μM and 0 to 48 μM, respectively.

After preincubating the ribosome mix and factor mix for 5 min at 37°C, the translation was started by adding the factor mix to the ribosome mix, and incubation was continued for a further 5 min at 37°C. Subsequently, the reaction mixture was chilled on ice for 5 min before photocross-linking. The samples were further processed as described above.

Cross-linking of SRP and TF to ribosomes

Ribosomes (for preparation see the online supplemental material, available at <http://www.jcb.org/cgi/content/full/jcb.200302130/DC1>) were mock treated or mixed with purified SRP and/or TF at the indicated final concentrations between 0.3 and 30 μM. The samples were incubated for 10 min at 25°C. Cross-linking was induced by the addition of 10 mM EDC, and the samples were incubated for another 10 min at 25°C. Cross-linking was quenched with 4 mM β-mercaptoethanol for 10 min on ice. Samples were TCA precipitated, analyzed by SDS-PAGE, and immunoblotted using antisera against Ffh, TF, and L23.

Online supplemental material

Additional Materials and methods on the reconstituted translation system and the purification of ribosomes are available online at <http://www.jcb.org/cgi/content/full/jcb.200302130/DC1>.

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References

- Batey, R.T., R.P. Rambo, L. Lucast, B. Rha, and J.A. Doudna. 2000. Crystal structure of the ribonucleoprotein core of the signal recognition particle. *Science*. 287:1232–1239.
- Beck, K., L.F. Wu, J. Brunner, and M. Mueller. 2000. Discrimination between SRP- and SecA/SecB-dependent substrates involves selective recognition of nascent chains by SRP and trigger factor. *EMBO J.* 19:134–143.
- Beckmann, R., C.M. Spahn, N. Eswar, J. Helmers, P.A. Penczek, A. Sali, J. Frank, and G. Blobel. 2001. Architecture of the protein-conducting channel associated with the translating 80S ribosome. *Cell*. 107:361–372.
- Brunner, J. 1996. Use of photocrosslinkers in cell biology. *Trends Cell Biol.* 6:154–157.
- Deuerling, E., A. Schulze-Specking, T. Tomoyasu, A. Mogk, and B. Bukau. 1999. Trigger factor and DnaK cooperate in folding of newly synthesized proteins. *Nature*. 400:693–696.
- Ellman, J., D. Mendel, S. Anthony-Cahill, C.J. Noren, and P.G. Schultz. 1991. Biosynthetic method for introducing unnatural amino acids site-specifically into proteins. *Methods Enzymol.* 202:301–336.
- Flanagan, J.J., J.-C. Chen, Y. Miao, Y. Shao, J. Lin, P.E. Bock, and A.E. Johnson. 2003. SRP binds to ribosome-bound signal sequences with fluorescence-detected subnanomolar affinity that does not diminish as the nascent chain lengthens. *J. Biol. Chem.* In press.
- Harms, J., F. Schluenzen, R. Zarivach, A. Bashan, S. Gat, I. Agmon, H. Bartels, F. Franceschi, and A. Yonath. 2001. High resolution structure of the large ribosomal subunit from a mesophilic eubacterium. *Cell*. 107:679–688.
- Herskovits, A.A., E.S. Bochkareva, and E. Bibi. 2000. New prospects in studying the bacterial signal recognition particle pathway. *Mol. Microbiol.* 38:927–939.
- Hestekamp, T., and B. Bukau. 1996. Identification of the prolyl isomerase domain of *Escherichia coli* trigger factor. *FEBS Lett.* 385:67–71.
- Jensen, C.G., and S. Pedersen. 1994. Concentrations of 4.5S RNA and Ffh protein in *Escherichia coli*: the stability of Ffh protein is dependent on the concentration of 4.5S RNA. *J. Bacteriol.* 176:7148–7154.
- Kramer, G., T. Rauch, W. Rist, S. Vorderwulbecke, H. Patzelt, A. Schulze-Specking, N. Ban, E. Deuerling, and B. Bukau. 2002. L23 protein functions as a chaperone docking site on the ribosome. *Nature*. 419:171–174.
- Lee, H.C., and H.D. Bernstein. 2001. The targeting pathway of *Escherichia coli* presecretory and integral membrane proteins is specified by the hydrophobicity of the targeting signal. *Proc. Natl. Acad. Sci. USA*. 98:3471–3476.
- Lill, R., E. Crooke, B. Guthrie, and W. Wickner. 1988. The 'trigger factor cycle' includes ribosomes, presecretory proteins and the plasma membrane. *Cell*. 54:1013–1018.
- Luirink, J., S. High, H. Wood, A. Giner, D. Tollervy, and B. Dobberstein. 1992. Signal sequence recognition by an *Escherichia coli* ribonucleoprotein complex. *Nature*. 359:741–743.
- Maier, R., C. Scholz, and F.X. Schmid. 2001. Dynamic association of trigger factor with protein substrates. *J. Mol. Biol.* 314:1181–1190.
- Morgan, D.G., J.F. Menetret, A. Neuhof, T.A. Rapoport, and C.W. Akey. 2002. Structure of the mammalian ribosome-channel complex at 17Å resolution. *J. Mol. Biol.* 324:871–886.
- Nissen, P., J. Hansen, N. Ban, P.B. Moore, and T.A. Steitz. 2000. The structural basis of ribosome activity in peptide bond synthesis. *Science*. 289:920–930.
- Patzelt, H., S. Rudiger, D. Brehmer, G. Kramer, S. Vorderwulbecke, E. Schaffitzel, A. Waitz, T. Hestekamp, L. Dong, J. Schneider-Mergener, et al. 2001. Binding specificity of *Escherichia coli* trigger factor. *Proc. Natl. Acad. Sci. USA*. 98:14244–14249.
- Pool, M.R., J. Stumm, T.A. Fulga, I. Sinning, and B. Dobberstein. 2002. Distinct modes of signal recognition particle interaction with the ribosome. *Science*. 297:1345–1348.
- Rinke-Appel, J., M. Osswald, K. von Knoblauch, F. Mueller, R. Brimacombe, P. Sergiev, O. Avdeeva, A. Bogdanov, and O. Dontsova. 2002. Crosslinking of 4.5S RNA to the *Escherichia coli* ribosome in the presence or absence of the protein Ffh. *RNA*. 8:612–625.
- Scotti, P.A., M.L. Urbanus, J. Brunner, J.W. de Gier, G. von Heijne, C. van der Does, A.J. Driessen, B. Oudega, and J. Luirink. 2000. YidC, the *Escherichia coli* homologue of mitochondrial Oxa1p, is a component of the Sec translocase. *EMBO J.* 19:542–549.
- Stoller, G., K.P. Rucknagel, K.H. Nierhaus, F.X. Schmid, G. Fischer, and J.U. Rahfeld. 1995. A ribosome-associated peptidyl-prolyl cis/trans isomerase identified as the trigger factor. *EMBO J.* 14:4939–4948.
- Teter, S.A., W.A. Houry, D. Ang, T. Tradler, D. Rockabrand, G. Fischer, P. Blum, C. Georgopoulos, and F.U. Hartl. 1999. Polypeptide flux through bacterial Hsp70: DnaK cooperates with trigger factor in chaperoning nascent chains. *Cell*. 97:755–765.
- Urbanus, M.L., P.A. Scotti, L. Froderberg, A. Saaf, J.W. de Gier, J. Brunner, J.C. Samuelson, R.E. Dalbey, B. Oudega, and J. Luirink. 2001. Sec-dependent membrane protein insertion: sequential interaction of nascent FtsQ with SecY and YidC. *EMBO Rep.* 2:524–529.
- Valent, Q.A., D.A. Kendall, S. High, R. Kusters, B. Oudega, and J. Luirink. 1995. Early events in preprotein recognition in *E. coli*: interactions of SRP and trigger factor with nascent polypeptides. *EMBO J.* 14:5494–5505.
- Valent, Q.A., J.W. de Gier, G. von Heijne, D.A. Kendall, C.M. ten Hagen-Jongman, B. Oudega, and J. Luirink. 1997. Nascent membrane and presecretory proteins synthesized in *Escherichia coli* associate with signal recognition particle and trigger factor. *Mol. Microbiol.* 25:53–64.
- Valent, Q.A., P.A. Scotti, S. High, J.W. de Gier, G. von Heijne, G. Lentzen, W. Wintermeyer, B. Oudega, and J. Luirink. 1998. The *E. coli* SRP and SecB targeting pathways converge at the translocon. *EMBO J.* 17:2504–2512.