Membrane association of FtsY, the *E. coli* SRP receptor

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Abstract FtsY, the *Escherichia coli* homologue of the eukaryotic SRP receptor (SRα), is located both in the cytoplasm and in the inner membrane of *E. coli*. Similar to SRα, FtsY consists of two major domains: a strongly acidic N-terminal domain (A) and a C-terminal GTP binding domain (NG) of which the crystal structure has recently been determined. The domains were expressed both in vivo and in vitro to examine their subcellular localization. The results suggest that both domains associate with the membrane but that the nature of the association differs.

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Key words: *Escherichia coli*; FtsY; Protein targeting; Signal recognition particle

1. Introduction

In eukaryotic cells, cotranslational targeting and insertion of proteins into the membrane of the endoplasmic reticulum (ER) is mediated by the signal recognition particle (SRP) and its receptor (SR) (for a recent review see [1]). The SRP consists of six polypeptides arranged on a 7S RNA scaffold. It binds via its 54 kDa subunit (SRP54) to hydrophobic targeting signals in short nascent polypeptides and inhibits further elongation. Interaction of the complex with the α-subunit of the SR in the membrane relieves the arrest of elongation and allows insertion of the nascent protein into the translocation pore of the ER membrane. SRβ functions as a membrane anchor for SRα. SRP54, SRα and SRβ are GTPases and GTP binding and hydrolysis are thought to induce conformational changes that regulate the targeting cycle.

In *Escherichia coli*, a related but less complex SRP machinery has been discovered. An SRP consisting of P48 (SRP54 homologue) and 4.5S RNA (7S RNA homologue) was shown to interact with nascent secretory and membrane proteins [2–4]. Recent genetic and biochemical evidence indicates that inner membrane proteins are particularly dependent on a functional SRP for proper targeting and membrane assembly [5–8].

Based on sequence similarity [9,10], affinity for SRP in vitro [11] and defective secretion upon depletion in a conditional strain [12], an *E. coli* homologue of SRα has been identified (FtsY). FtsY and SRα contain two distinct domains: a highly charged N-terminal domain (the A domain) which in SRα is involved in membrane association and a C-terminal domain (the NG domain) of which the crystal structure has recently been determined [13]. The structure reveals the existence of three subdomains: the α-helical N domain (197–280), the G domain that is related to the Ras GTPases (291–495) and the surface exposed I box (333–377), an α-β-α insertion in the G domain that is postulated to play a role in interaction with regulatory proteins.

FtsY is located partly in the cytosol and partly in the cytoplasmic membrane [12]. The mechanism of the association with the membrane is unclear. FtsY is a highly charged protein that does not contain any predicted membrane spanning segments. In addition, no obvious SRβ homologues have been identified in the *E. coli* genome sequence. In this study the structural domains of FtsY have been expressed separately and their ability to associate with the cytoplasmic membrane has been examined both in vitro and in vivo.

2. Materials and methods

2.1. General methods

Recombinant DNA techniques were carried out as described [14]. Protein concentration was determined according to Bradford [15]. Radiolabeled protein bands on dried polyacrylamide gels were visualized by PhosphorImaging using a Molecular Dynamics Phosphor Imager 473 and quantified using the Imagequant quantification software from Molecular Dynamics.

2.2. Strains and medium

E. coli BL21 F⁻ *hsdS gal* (DE3) harboring pLysE or pLysS was used for expression of *ftsY* (domains) cloned in pET vectors [16]. *E. coli* TOP10F (Stratagene, La Jolla, CA) was used for expression of *ftsY* (domains) cloned in pCL1920. *E. coli* MC4100 (F⁻ Δ*lacU*169 *araD* 136 *rpsL thi relA*) was used for the preparation of lysates and vesicles for in vitro targeting reactions. N4156::pAra14-FtsY [12] was used in complementation experiments. This strain was grown in LB supplemented with 0.4% fructose and 0.2% L-arabinose. The other strains were routinely grown in LB supplemented with 0.4% glucose and the appropriate antibiotics.

2.3. Cloning of FtsY (domains)

The constructions of pET9-FtsY and pET9-FtsY-NG have been described previously [12,17]. They encode full length FtsY and the NG fragment of FtsY containing the residues 197–497 plus six histidine residues at the C-terminus respectively. pET16b-FtsY-A encoding residues 1–197 was created by subcloning the PCR amplified 5′ end of the FtsY gene in pET16b. pET9-FtsY-G encoding residues 282–498 plus six histidine residues at the C-terminus was created by PCR amplification of the coding sequence. pET9-FtsY was used as a template in all PCRs. The expression vectors pET9 and pET16b were used for cloning of *ftsY* (segments) and have been described previously [16]. The *ftsY* (domains) mentioned above were also subcloned from their respective pET vectors into the low copy number expression vector pCL1920 [18] using *Xba*I and *Bam*HI.

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Abbreviations: ER, endoplasmic reticulum; IPTG, isopropyl-1-thio-β-D-galactopyranoside; GTP, guanosine-5′-phosphate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SR, SRP receptor; SRP, signal recognition particle; WT, wild-type

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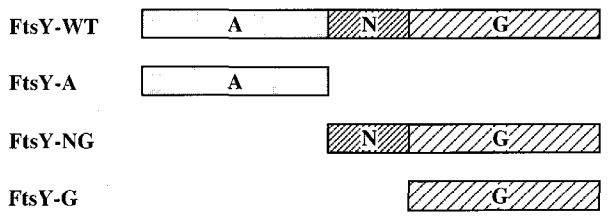

Fig. 1. Domain structure of FtsY and domains cloned in expression vectors. A, acidic domain; N, α-helical domain; G, GTP binding domain.

2.4. In vitro transcription, translation and targeting

The in vitro transcription, translation and targeting reactions were carried out basically as described using S-135 extracts and inverted cytoplasmic membrane vesicles (IMVs) [19]. After the targeting reaction, the membranes were collected by centrifugation through a sucrose cushion as described [20]. Association of the FtsY derivatives with the pelleted membranes was analyzed by flotation centrifugation (Valent and Luirink, in preparation). For flotation, the pellets were resuspended in 15 μl buffer I (50 mM HEPES-KOH pH7.6; 500 mM KOAc; 5 mM $Mg(OAc)_2$) and mixed with 105 μl buffer III (50 mM HEPES-KOH pH7.6; 500 mM KOAc; 5 mM $Mg(OAc)_2$; 250 mM sucrose; 50% OptiPrep (Nycomed Pharma AS, Oslo, Norway)). The samples were transferred to 1 ml tubes, overlaid with 580 μl of buffer II (50 mM HEPES-KOH pH7.6; 500 mM KOAc; 5 mM $Mg(OAc)_2$; 125 mM sucrose; 30% OptiPrep) and 300 μl buffer I and then centrifuged (166 000×*g*, 3 h, 4°C, TLS55 rotor, Beckmann). Four fractions (350 μl, 200 μl, 200 μl and 250 μl) were collected from the top and subjected to TCA precipitation. After precipitation, the samples were analyzed by SDS-PAGE and PhosphorImaging.

2.5. Subcellular localization of FtsY

Subcellular fractions were prepared essentially as described [21]. Cells were lysed by freezing and thawing combined with short ultrasonic treatment. The cell debris was removed from the lysate by sedimentation (7000×*g* for 15 min). Cell envelopes were separated from the soluble fraction (containing cytoplasmic and periplasmic proteins) by ultracentrifugation (356 000×*g* for 45 min). Peripheral cytoplasmic membrane proteins were extracted from cytoplasmic membrane vesicles with 1 M NaCl, 4 M urea and 0.2 M Na_2CO_3 as described [22].

3. Results and discussion

3.1. Cloning and expression of ftsY domains

FtsY consists of two domains, the highly charged N-terminal A domain and the C-terminal NG domain. Based on the recently solved crystal structure, the NG domain can be subdivided into the N domain (N-terminal) and the Ras-like G domain (C-terminal) which are connected by a linker peptide

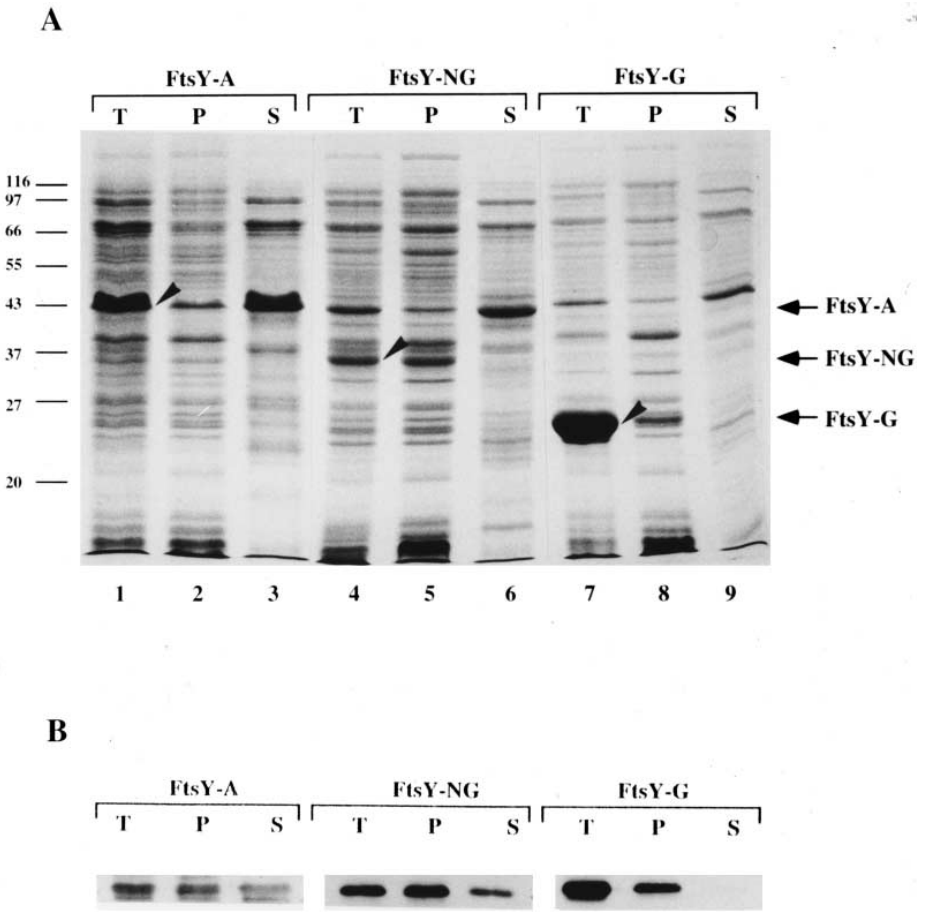

Fig. 2. Expression and subcellular distribution of FtsY domains. BL21(DE3) harboring pET16b-FtsY-A and pLysS or pET9-FtsY-NG and pLysE or pET9-FtsY-G and pLysE were grown to a culture turbidity at 660 nm of 0.3 and induced with 0.4 mM IPTG (A). Alternatively, the cells were induced with 50 μM IPTG (FtsY-A), 5 μM (FtsY-G) or left uninduced (FtsY-NG) (B). The cells were collected 2 h after induction, lysed and subjected to differential centrifugation. The protein concentration in the soluble fraction (S) was determined and 5 μg was applied per lane. Matched amounts of membrane pellet fraction (P) and total cell lysate (T) were applied as indicated. The fractions were analyzed by SDS-PAGE and Coomassie R-250 staining (A) or immunoblotting (B). The positions of molecular weight markers and FtsY domains are indicated.

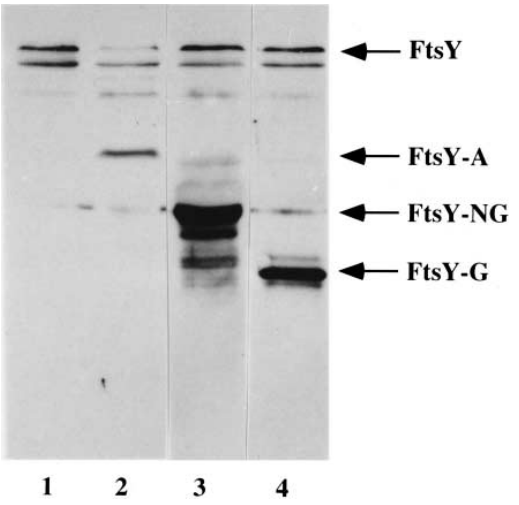

Fig. 3. Immunoblot analysis of IMVs purified from cells that express FtsY domains. IMVs were purified from cells that express FtsY-A (lane 2), FtsY-NG (lane 3), FtsY-G (lane 4) and control BL21(DE3) cells (lane 1). Expression was induced as indicated in the legend of Fig. 2 except that the induction time was 1 h. 0.05 OD_{280} units of IMVs were applied per lane and analyzed by immunoblotting using a polyclonal antiserum raised against purified FtsY.

(residues 282–292) [13]. The interdomain interface is mainly hydrophobic and involves a number of conserved residues. To facilitate structure-function studies, we cloned the A domain, the NG domain and the G domain separately in pET expression vectors (Fig. 1).

All domains were expressed albeit with different efficiencies (Fig. 2, cf. lanes 1, 4 and 7). FtsY-A migrated as a characteristically 'bulged' band at 43 kDa, whereas its predicted molecular mass is 22 kDa (Fig. 2, lane 1). In contrast, FtsY-NG and FtsY-G migrated at their expected molecular weights in SDS-PAGE (Fig. 2, lanes 4 and 7, respectively). This suggests that the aberrant slow and 'bulged' migration of full length FtsY in SDS-PAGE [12] is due to the extremely charged N-terminal part of the protein [23]. The bands were positively identified as derived from FtsY by means of immunoblotting using a polyclonal antiserum raised against purified FtsY (not shown).

Expression of FtsY-A and FtsY-NG induced some cell filamentation (not shown) similar to the effect of FtsY-WT expression [12]. Expression of FtsY-G induced the formation of large polar inclusion bodies (not shown), which might be caused by exposed hydrophobic side chains that are buried in the N/G interface of WT FtsY.

3.2. Complementation of FtsY conditional strain

FtsY is essential for cell growth. Thus, a conditional strain that carries *ftsY* under control of the arabinose promoter is only able to sustain growth in the presence of arabinose [12]. To test the ability of the domains to functionally complement FtsY depletion, they were introduced into the *ftsY* conditional strain. For this purpose the domains were recloned under *lac* promoter control in pCL1920 that carries a pSC101 origin and is able to replicate in the *ftsY* conditional strain which is *polA*. None of the mutants was able to grow in the absence of arabinose even when expression of the domains was induced with up to 1 mM IPTG and could be visualized by immunoblotting (data not shown). In contrast, WT FtsY cloned in pCL1920 supported growth even in the absence of both arabinose and IPTG probably due to leakiness of the *lac* promoter. From these data we conclude that the structurally distinct domains of FtsY are not functional when expressed as a separate entity. It is conceivable that truncated FtsY lacks important information for activity (like recognition of the SRP) or targeting of the protein to its cellular location.

3.3. Subcellular localization of FtsY domains in vivo

FtsY is an unusual protein in that it is located both in the cytosol and in the inner membrane whereas it is highly charged and does not contain any obvious membrane spanning sequences. To investigate if the domains harbor any targeting information, the subcellular location of the domains was determined. Cells fully induced to express the separate domains were disrupted and subjected to differential centrifugation to separate unlysed cells and possible inclusion bodies (in low speed pellet, not shown) from membrane associated material (in high speed pellet) and soluble proteins (in supernatant). FtsY-A was located primarily in the soluble fraction though a fraction was clearly in the membrane pellet (Fig. 2A, lanes 2 and 3) resembling the distribution of (overproduced) FtsY-WT [12]. FstY-NG was almost exclusively found in the membrane fraction (Fig. 2A, lanes 5 and 6). As expected, FtsY-G was mainly lost in the low speed pellet as a result of extensive inclusion body formation but there was also a notable fraction of FtsY-G that came down with the membranes (Fig. 2A, lane 8). To minimize localization artefacts due to the high expression, the domains were also expressed at a low level that requires immunoblotting for detection (Fig. 2B). The distribution of FtsY-NG and FtsY-G appeared unaltered (Fig. 2B, lanes 4–9) although aggregation of FtsY-G was less apparent. FtsY-A localization was somewhat shifted towards the membrane possibly due to a relatively higher number of available membrane attachment sites (see below).

It cannot be excluded that separately expressed FtsY domains at any expression level form small aggregates that cofractionate with the membranes. In order to examine this possibility, IMVs were isolated from cells expressing FtsY domains by sucrose density centrifugation and subjected to extraction with different chemical agents. In Fig. 3 an immunoblot is shown of the purified IMVs. Apparently, all domains cofractionated with the IMVs (Fig. 3, lanes 2, 3 and 4). It is notable that, although the amount of FtsY-A in the

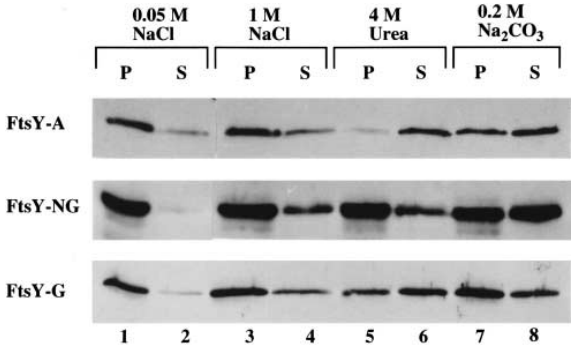

Fig. 4. Extraction of IMVs purified from cells that express FtsY domains. IMVs (0.1 OD_{280} units) were treated with the indicated agents. Pellet (P) and soluble (S) fractions were analyzed by immunoblotting using a polyclonal antiserum raised against purified FtsY.

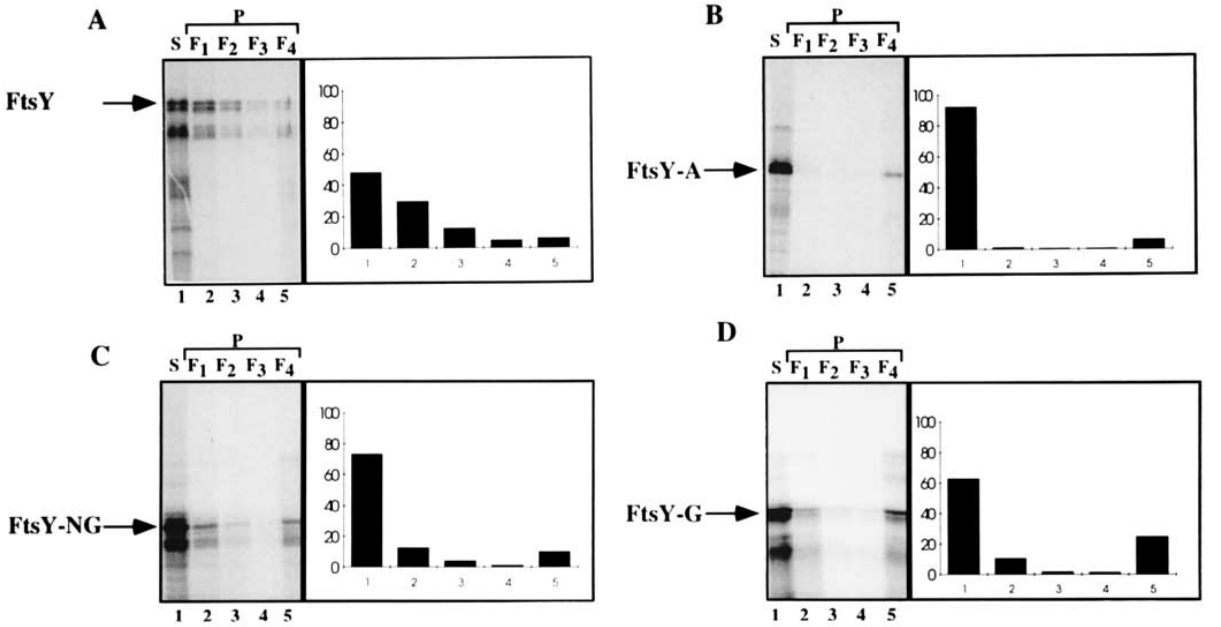

Fig. 5. Association of in vitro synthesized FtsY domains with IMVs. FtsY (domains) were synthesized in the presence of wild-type IMVs. After synthesis, the samples were sedimented through a sucrose cushion. The supernatant (S) was withdrawn and the pellet resuspended and subjected to flotation gradient analysis. Four fractions were taken from top (F1) to bottom (F4). All samples were TCA precipitated and analyzed by SDS-PAGE and PhosphorImaging. A quantitation of the PhosphorImage data is presented at the right hand side of each image. The total amount of FtsY (domains) present in supernatant and combined flotation fractions was set at 100%. Extra bands present below the expected molecular weight of FtsY-WT, FtsY-NG and FtsY-G probably represent degradation products as has been observed before for FtsY-WT [12]. The experiment was done twice with almost identical outcomes. The results of one experiment are shown.

IMVs is relatively low, it has a drastic effect on the amount of the endogenous FtsY in the membrane (Fig. 3, lane 2). Possibly, FtsY-A competes with endogenous FtsY for a limited number of membrane binding sites.

To examine the nature of the membrane association in more detail, the IMVs were treated with 1 M NaCl, 4 M urea or with 0.2 M Na_2CO_3 to remove peripherally associated proteins and with 0.05 M NaCl as a control incubation (Fig. 4). The association of FtsY-A domains was relatively resistant to extraction with 1 M NaCl, almost completely susceptible to extraction with 4 M urea, whereas a substantial fraction was also extracted upon treatment with 0.2 M Na_2CO_3. This suggests that most but not all of FtsY-A is peripherally associated with the membrane similar to FtsY-WT [12]. The association of FtsY-G and especially FtsY-NG was more resistant to extraction with urea and Na_2CO_3 suggesting that the nature of their association with the membrane differs from that of FtsY-A.

3.4. Targeting of FtsY domains in vitro

To examine the targeting of FtsY in more detail, we determined the in vitro association of FtsY and of the FtsY domains expressed in an S-135 extract in the presence of IMVs. After protein synthesis, the IMVs were purified by sedimentation through a high salt sucrose cushion and then subjected to flotation gradient analysis (Fig. 5).

A substantial fraction of FtsY-WT (33%) was found in the top two fractions of the flotation gradient (Fig. 5A, lanes 2 and 3) which is comparable to the fraction of integral membrane proteins like Lep and FtsQ that float with the membranes under these conditions (Valent and Luirink, manuscript in preparation). This suggests that FtsY-WT is able to associate efficiently with the inner membrane in vitro. In marked contrast, virtually no FtsY-A (1%) moved with the membranes (Fig. 5B, lanes 2 and 3). The residual sedimented material remained in the bottom fraction suggesting that part of the originally sedimented FtsY-A was not associated with the membrane (Fig. 5B, lane 5). This is supported by the presence of a similar percentage of sedimented material in this fraction when IMVs were lysed with Triton X-100 prior to sedimentation (not shown). The reason for the apparent discrepancy between the in vitro and in vivo localization of FtsY-A remains unclear but may be related to differences in folding and a tendency to aggregate in vitro.

A significant fraction of FtsY-NG (16%) and FtsY-G (12%) was found in the top fractions (Fig. 5C,D, lanes 2 and 3) indicative of association with the membrane. Again, a fraction of the originally sedimented material remained in the bottom fraction (Fig. 5C,D, lanes 5), also when IMVs were lysed with Triton X-100 prior to sedimentation (not shown). This fraction is especially significant for FtsY-G which may reflect its tendency to aggregate in vivo.

4. Concluding remarks

This study underlines the unusual mechanism by which FtsY associates with the membrane. The structurally distinct A and NG domains both seem to have affinity for the inner membrane but do not contain sequences sufficiently hydrophobic to anchor the protein in the membrane.

The nature of the interaction of FtsY-A with the membrane in vivo resembles that of WT FtsY [12]. The A domain is extremely negatively charged (p*I* 3.9) and unlikely to interact directly with phospholipids. Rather, it seems plausible that a receptor exists that plays a role in the membrane assembly of this domain possibly by a direct (electrostatic?) interac-

tion similar to membrane assembly of SRα by SRβ in the mammalian system [24]. The dynamics of the interaction might be regulated by conformational changes in the NG domain.

The NG domain is located partly at the inner membrane but the nature of the association seems different from that of WT FtsY given the stronger resistance to alkali and urea treatment. Also, in contrast to FtsY-A, FtsY-NG expression does not compete with WT FtsY for membrane association. Possibly, FtsY-NG is in direct contact with the lipid bilayer. This contact is probably mediated by the G domain since FtsY-G behaves like FtsY-NG in our fractionation studies. In addition, the electrostatic surface potential of FtsY-NG shows that the surface of the N domain is also negatively charged (Montoya and Sinning, unpublished), which makes a direct interaction with the phospholipids unlikely. Based on the structure it has been proposed that the G domain interacts with P48 [13]. Apparently, the association of the NG domain with the membrane is not sufficient to function as receptor for the SRP in *E. coli*. Interestingly, several bacterial FtsY homologues consist of only the NG domain (T. Samuelsson, personal communication).

After we had finished this study, Zelazny and coworkers [25] reported that FtsY lacking the A domain cannot complement FtsY depletion, which is consistent with our findings. Replacement of the A domain by integral membrane spanning segments from another inner membrane protein restored growth to a certain extent indicating that the A domain bears essential targeting information. Another study that demonstrates the importance of the A domain in FtsY localization and functioning was recently reported by Powers and coworkers [26]. *E. coli* SRP and FtsY were shown to support cotranslational targeting to mammalian microsomes. Removal of the first 46 residues of FtsY impaired but did not completely abolish membrane association of FtsY and reduced the efficiency of protein targeting suggesting that the two events are related. Increasing the concentration of this truncated FtsY could partly restore the targeting efficiency indicating that the residual part of FtsY also has some affinity for the microsomal membranes and retains activity.

Taken together these studies suggest that the A domain assists in targeting of FtsY to the membrane but is not absolutely indispensable. Functioning of the NG domain alone appears to depend on the organism or in vitro system used for reasons that remain unclear at present.

The subcellular distribution of FtsY is reminiscent of SecA, which is also a nucleotide binding protein and does not contain hydrophobic transmembrane sequences [22]. SecA is a translocon component that has affinity both for membrane lipids and the integral translocon component SecY (reviewed in [27]). Recent evidence indicates that SecA shuttles between a membrane inserted and de-inserted state, a process that is regulated by ATP binding and hydrolysis [28]. It is tempting to speculate that membrane assembly of FtsY is of similar complexity.

We are currently investigating the nature of membrane assembly of FtsY in more detail with special emphasis on a possible membrane receptor for FtsY. The in vitro assay should allow the identification of such a receptor.

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References

[1] Rapoport, T.A., Jungnickel, B. and Kutay, U. (1996) Annu. Rev. Biochem. 65, 271–303.
[2] Luirink, J., High, S., Wood, H., Giner, A., Tollervey, D. and Dobberstein, B. (1992) Nature 359, 741–743.
[3] Valent, Q.A., Kendall, D.A., High, S., Kusters, R., Oudega, B. and Luirink, J. (1995) EMBO J. 14, 5494–5505.
[4] Valent, Q.A., de Gier, J.-W.L., van Heijne, G., Kendall, D.A., ten Hagen-Jongman, C.M., Oudega, B. and Luirink, J. (1997) Mol. Microbiol. 25, 53–64.
[5] MacFarlane, J. and Muller, M. (1995) Eur. J. Biochem. 233, 766–771.
[6] Ulbrandt, N.D., Newitt, J.A. and Bernstein, H.D. (1997) Cell 88, 187–196.
[7] Seluanov, A. and Bibi, E. (1997) J. Biol. Chem. 272, 2053–2055.
[8] de Gier, J.W.L., Mansournia, P., Valent, Q.A., Phillips, G.J., Luirink, J. and von Heijne, G. (1996) FEBS Lett. 399, 307–309.
[9] Bernstein, H.D., Poritz, M.A., Strub, K., Hoben, P.J., Brenner, S. and Walter, P. (1989) Nature 340, 482–486.
[10] Römisch, K., Webb, J., Herz, J., Prehn, S., Frank, R., Vingron, M. and Dobberstein, B. (1989) Nature 340, 478–482.
[11] Miller, J.D., Bernstein, H.D. and Walter, P. (1994) Nature 367, 657–659.
[12] Luirink, J., Ten Hagen-Jongman, C.M., Van der Weijden, C.C., Oudega, B., High, S., Dobberstein, B. and Kusters, R. (1994) EMBO J. 13, 2289–2296.
[13] Montoya, G., Svensson, C., Luirink, J. and Sinning, I. (1997) Nature 385, 365–368.
[14] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[15] Bradford, M. (1976) Anal. Biochem. 72, 248–254.
[16] Studier, F.W., Rosenberg, A.H., Dunn, J.J. and Dubendorff, J.W. (1990) Methods Enzymol. 185, 60–89.
[17] Montoya, G., Svensson, C., Luirink, J. and Sinning, I. (1997) Proteins Struct. Funct. Genet. 28, 285–288.
[18] Lerner, C.G. and Inouye, M. (1990) Nucleic Acids Res. 18, 4631.
[19] De Vrije, T., Tommassen, J. and De Kruijff, B. (1987) Biochim. Biophys. Acta 900, 63–72.
[20] High, S., Flint, N. and Dobberstein, B. (1991) J. Cell Biol. 113, 25–34.
[21] Lugtenberg, B., Meyers, J., Peters, R., Van der Hoek, P. and Van Alphen, L. (1975) FEBS Lett. 58, 254–258.
[22] Cabelli, R.J., Dolan, K.M., Qian, L. and Oliver, D.B. (1991) J. Biol. Chem. 266, 24420–24427.
[23] Gill, D.R., Hatfull, G.F. and Salmond, G.P.C. (1986) Mol. Gen. Genet. 205, 134–145.
[24] Young, J.C., Ursini, J., Legate, K.R., Miller, J.D., Walter, P. and Andrews, D.W. (1995) J. Biol. Chem. 270, 15650–15657.
[25] Zelazny, A., Seluanov, A., Cooper, A. and Bibi, E. (1997) Proc. Natl. Acad. Sci. USA 94, 6025–6029.
[26] Powers, T. and Walter, P. (1997) EMBO J. 16, 4880–4886.
[27] Driessen, A.J.M., De Wit, J.G., Kuiper, W., Van der Wolk, J.P.W., Fekkes, P., Van der Does, C., Van Wely, K., Manting, E. and Den Blaauwen, T. (1995) Biochem. Soc. Trans. 23, 981–985.
[28] Economou, A., Pogliano, J.A., Beckwith, J., Oliver, D.B. and Wickner, W. (1995) Cell 83, 1171–1181.