Assembly of a cytoplasmic membrane protein in *Escherichia coli* is dependent on the signal recognition particle

Jan-Willem L. de Gier[a], Parvaneh Mansournia[a], Quido A. Valent[b], Gregory J. Phillips[c], Joen Luirink[b], Gunnar von Heijne[a,*]

[a]*Department of Biochemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden*
[b]*Department of Microbiology, BioCentrum Amsterdam, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands*
[c]*Department of Microbiology, Iowa State University, Ames, IA 50011, USA*

Received 31 October 1996

Abstract Targeting of the cytoplasmic membrane protein leader peptidase (Lep) and a Lep mutant (Lep-inv) that inserts with an inverted topology compared to the wild-type protein was studied in *Escherichia coli* strains that are conditional for the expression of either Ffh or 4.5S RNA, the two components of the *E. coli* SRP. Depletion of either component strongly affected the insertion of both Lep and Lep-inv into the cytoplasmic membrane. This indicates that SRP is required for the assembly of cytoplasmic membrane proteins in *E. coli*.

Key words: *Escherichia coli*; Signal recognition particle; Membrane protein; Leader peptidase; Protein targeting

1. Introduction

Targeting of secretory proteins to the cytoplasmic membrane of *E. coli* can follow different pathways that probably converge at the membrane embedded translocation machinery, the translocon [1–3]. The so-called SRP pathway involves the signal recognition particle (SRP), a complex of a 4.5S RNA and the Ffh protein which are homologous to the 7S RNA and 54 kDa constituents of the mammalian SRP, respectively [2,3]. FtsY, an *E. coli* homologue of the mammalian SRP receptor, has been identified on the basis of sequence similarity [4] and has been found to have affinity for the *E. coli* SRP in vitro [5]. The SRP subunits 4.5S RNA and Ffh as well as their receptor FtsY are essential for viability and their depletion results in defective protein secretion [6–9].

In *E. coli* there seems to be a correlation between the affinity of a signal sequence for the SRP-targeting pathway and the hydrophobicity of the signal sequence core region [10]. This seems to hold also for the SRP-targeting pathways in *Saccharomyces cerevisiae* [11] and chloroplasts (S. High, personal communication). Whether the SRP is also involved in the targeting and assembly of cytoplasmic membrane proteins in *E. coli* is not known, however. Based on the relatively strong hydrophobicity of signal anchor domains and their ability to be cross-linked to SRP in vitro, it has been proposed that, like in mammalian cells, membrane proteins are targeted via the SRP pathway [10]. A role for the SRP in the targeting of the cytoplasmic membrane protein lactose permease (LacY) has been suggested using an indirect approach [12]. In addition, overproduction of another cytoplasmic membrane protein, leader peptidase (Lep), results in reduced levels of free SRP and a SRP depletion secretion phenotype (our unpublished data).

In this study, the membrane assembly of wild-type Lep and a Lep mutant (Lep-inv) [13] that inserts with an inverted topology compared to the wild-type protein has been studied in *E. coli* strains that are conditional for the expression of either Ffh or 4.5S RNA. We find that depletion of either component strongly reduces the level of properly inserted Lep and Lep-inv. While Lep is known to depend on the SecA and SecY components of the translocon for its membrane assembly [14], SRP is the first component with a demonstrable effect on the insertion of the Sec-independent Lep-inv protein.

2. Materials and methods

2.1. Strains, plasmids and growth conditions

The 4.5S RNA conditional strain FF283 was cultured as described [8]. To deplete cells for 4.5S RNA, culturing was carried out in the absence of IPTG. The Ffh conditional strain WAM121, which is similar to WAM113 [6] except that it harbors a kanamycin instead of an ampicillin resistance marker, was cultured in LB medium supplemented with 0.4% glucose and 0.1% arabinose. O/N cultures were washed once with LB and backdiluted 1:20. To deplete cells for Ffh, cells were cultured in the absence of arabinose. 30 min prior to labeling the WAM121 cells were transferred to M9 minimal medium. When appropriate, antibiotics were added to the medium: ampicillin (final concentration 100 μg/ml) and kanamycin (final concentration 50 μg/ml). Lep and Lep-inv were expressed by arabinose induction from the pING1 vector [15] in strain FF283 and by IPTG induction from the pKK388-1 vector (Clonetech) in strain WAM121.

2.2. Construction of WAM121

The background stain for the ffh depletion experiments was WAM100, an ara$^-$ derivative of MC4100 (F$^-$, Δ[argF$^-$lac]U169, araD139, rpsL, relA1, deoC1, ptsF25, rbsR, flb5301, l$^-$). The ffh depletion strain WAM121 (WAM100; ffh1::kan; attB::R6Kori, ParaBAD$^-$ffh$^+$, cat [CmR]), although similar in phenotype to the previously reported stain WAM113 [7], was constructed to permit introduction of ampicillin-based recombinant plasmids. WAM121 was constructed by placing the ffh$^+$ under regulation of the araBAD promoter (ParaBAD) on pBAD18 [16] creating pAraFfh18. A DNA fragment containing the ParaBAD$^-$ ffh$^+$ construct was then inserted into pCD11PKS and introduced to the chromosome of MC4100 by site-specific recombination. The development of the site-specific integration system will be reported elsewhere (Phillips, Platt and Drescher, manuscript in preparation).

2.3. Assay for membrane targeting

For all experiments cells were grown to mid-log phase. Expression of Lep and Lep-inv was induced for 5 min with either IPTG (final

*Corresponding author. Fax: (46) (8) 153679.
E-mail: gunnar@biokemi.su.se

Abbreviations: Ffh, Fifty-four homologue; SRP, signal recognition particle; Lep, leader peptidase; LacY, lactose permease; IPTG, isopropyl-1-thio-β-D-galactopyranoside; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

PII S0014-5793(96)01354-3

concentration 1 mM) or arabinose (final concentration 0.2%). Cells were labeled with [^{35}S]methionine (150 μCi/ml) for 15 s whereupon non-radioactive methionine was added (final concentration 500 μg/ml). Cells were either directly converted to spheroplasts or were first subjected to a pulse-chase protocol prior to spheroplasting. For spheroplasting, cells were collected at 14000 rpm for 2 min in a microfuge, resuspended in ice-cold buffer (40% w/v sucrose, 33 mM Tris pH 8.0), and incubated with lysozyme (final concentration 5 μg/ml) and 1 mM EDTA for 15 min on ice. Aliquots of the spheroplast suspension were incubated on ice for 1 h in the presence or absence of proteinase K (final concentration 0.3 mg/ml). Subsequently, phenylmethylsulfonyl fluoride (PMSF) was added to the spheroplast suspensions (final concentration 0.33 mg/ml). After addition of PMSF, samples were precipitated with trichloroacetic acid (final concentration 10%), resuspended in 10 mM Tris/2% SDS, immunoprecipitated with antisera to Lep, OmpA and AraB, washed, and analyzed by SDS-PAGE. Gels were scanned in a Fuji BAS1000 phosphoimager.

3. Results

3.1. Lep and Lep-inv depend on SRP for efficient insertion into the cytoplasmic membrane

To study the role of SRP in the targeting and membrane insertion of Lep and Lep-inv, the proteins were expressed in strains that were depleted for either Ffh or 4.5S RNA. Cells were labeled with [^{35}S]methionine and Lep was detected by immunoprecipitation and SDS-PAGE. Membrane insertion was monitored using a protease protection assay (Fig. 1).

Depletion of Ffh in WAM121 cells significantly decreased the efficiency of translocation of the C-terminal P2 domain in Lep (Fig. 2A). Depletion of 4.5S RNA in FF283 cells had a similar effect (data not shown). Similarly, a significant fraction of Lep-inv failed to insert into the cytoplasmic membrane in FF283 cells depleted for 4.5S RNA as evidenced by their resistance to proteinase K (Fig. 2B). Depletion of Ffh in WAM121 had a similar effect on Lep-inv (data not shown).

As shown by the proteinase K sensitivity of OmpA (an outer membrane protein with a large periplasmic domain that is protease-resistant in intact cells but not in spheroplasts) and band X (a cytoplasmic control) (Fig. 2B), the spheroplasts were efficiently produced and were largely intact. Consistent with the observation that the secretion of OmpA is SRP-independent [6–8], no effect of Ffh or 4.5S RNA depletion on the formation of mature OmpA is seen.

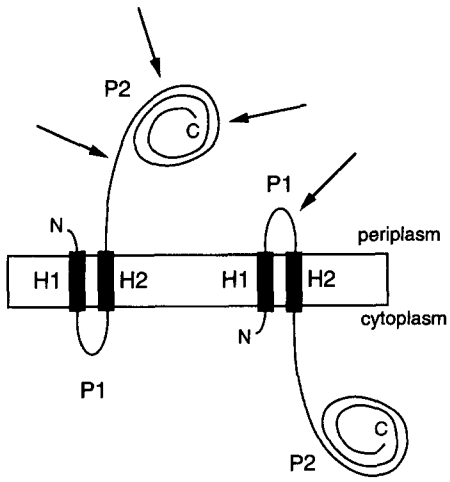

Fig. 1. Orientation of Lep and Lep-inv in the cytoplasmic membrane. The Lep-inv mutant was derived from Lep by adding 3 lysine codons between codons 4 and 5, inserting 10 codons encoding the sequence Gly-Gln-Ser-Leu-Asn-Ala-Pro-Thr-Ser-Gly between codons 22 and 24, deleting residues 30–52, and changing Lys_{56} to Asn and Glu_{61} to Val (cf. [13,18]). In spheroplasts, proteinase K degrades the P2 domain of Lep and the P1 loop of Lep-inv (arrows). For Lep-inv, this treatment gives rise to a protease-resistant H2-P2 fragment that can be immunoprecipitated with a Lep antiserum, whereas no immunoprecipitable material remains when the P2 domain in Lep has been digested.

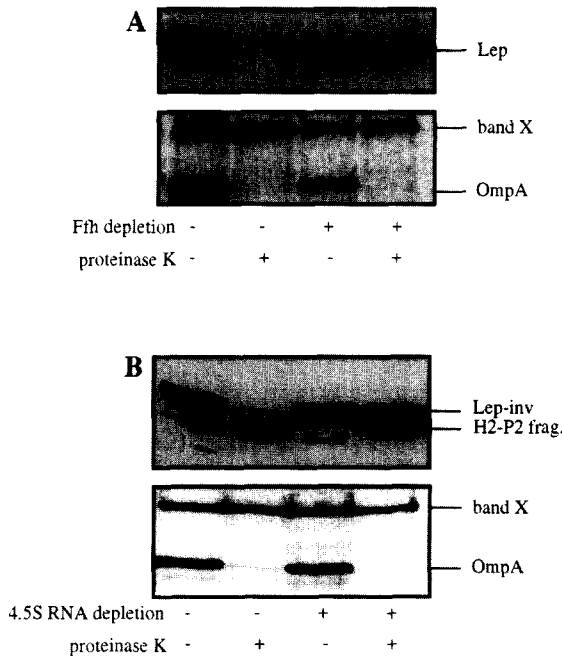

Fig. 2. SRP is necessary for efficient membrane assembly of Lep and Lep-inv. A: Proteinase K accessibility of Lep (top) and OmpA/band X (bottom) in WAM121 spheroplasts not depleted and depleted for Ffh. OmpA is only degraded by proteinase K in cells with a disrupted outer membrane. Band X is an unknown protein that is fortuitously precipitated by an antiserum raised against an AraB fusion protein of the same molecular weight as band X. Since the band X protein is protease-resistant in spheroplasts and becomes protease-sensitive only after disruption of the cytoplasmic membrane with the detergent Triton X-100 (data not shown), it serves as a convenient control for the intactness of the spheroplasts. B: Proteinase K accessibility of Lep-inv (top) and OmpA/band X (bottom) in FF283 spheroplasts not depleted and depleted for 4.5S RNA. The H2-P2 product is a protease-resistant fragment resulting from cleavage in the P1 loop (cf. Fig. 1).

3.2. Mode of insertion of leader peptidase

To test whether the non-assembled Lep and Lep-inv that accumulates under Ffh or 4.5S RNA depletion can insert post-translationally into the membrane, pulse-chase experiments were performed. As seen in Fig. 3, little or no Lep-inv became protease-sensitive during a 15 min chase in cells depleted for 4.5S RNA. Similar results were obtained under Ffh depletion in strain WAM121 and for Lep under the same conditions (data not shown).

4. Discussion

We have studied the role of Ffh and 4.5S RNA (the two components of *E. coli* SRP) in the targeting and assembly of the cytoplasmic membrane protein Lep. It has previously been found that membrane assembly of Lep is SecB independent (R. Dalbey, personal communication) but is dependent on the translocon components SecA and SecY [14,17]. In contrast,

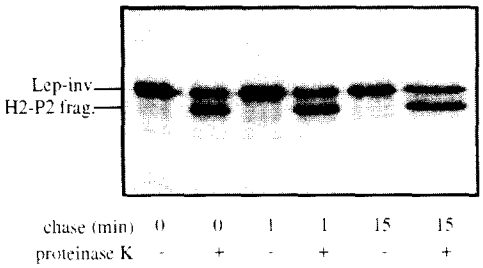

Fig. 3. Lep-inv does not assemble post-translationally in the absence of SRP. Lep-inv was expressed in strain FF283 after depletion of 4.5S RNA. Cells were pulse-labeled for 15 s, and excess non-radioactive methionine was added. Cells were converted to spheroplasts after 1 min and 15 min and were further processed as in Fig. 2.

membrane insertion of Lep-inv is SecA and SecY independent [13].

The membrane insertion of both Lep and Lep-inv was significantly reduced upon depletion of either Ffh or 4.5S RNA, strongly suggesting that the membrane assembly of both forms of Lep requires SRP. SRP is thus the first component of the secretory machinery in *E. coli* that has been found to play a role in the membrane assembly of a SecA/Y-independent protein such as Lep-inv. The requirement for SRP in the targeting of Lep is further supported by our recent findings that SRP can be crosslinked in vitro to the first transmembrane segment of Lep [10], and that overproduction of Lep results in reduced levels of free SRP and a SRP depletion secretion phenotype (our unpublished data).

In summary, we have shown that two forms of the inner membrane protein Lep, one that depends on the SecA/Y translocase for membrane insertion and one that does not, both depend on the *E. coli* SRP for membrane assembly. As shown by pulse chase experiments, molecules that fail to interact properly with SRP quickly become incompetent for membrane assembly. This is in keeping with the suggestion that SRP functions as a chaperone to prevent aggregation of hydrophobic proteins [10].

Acknowledgements: This work was supported by a TMR fellowship from the European Community to J.W.d.G., and by grants from the Swedish Natural Sciences Research Council, the Swedish Cancer Foundation, and the Göran Gustafsson Foundation to G.v.H. Q.V. was supported by the Netherlands Organization for Scientific research and G.J.P. was supported by the National Institutes of Health.

References

[1] Pugsley, A.P. (1993) Microbiol. Rev. 57, 50–108.
[2] Wolin, S.L. (1994) Cell 77, 787–790.
[3] Luirink, J. and Dobberstein, B. (1994) Mol. Microbiol. 11, 9–13.
[4] Römisch, K., Webb, J., Herz, J., Prehn, S., Frank, R., Vingron, M. and Dobberstein, B. (1989) Nature 340, 478–482.
[5] Miller, J.D., Bernstein, H.D. and Walter, P. (1994) Nature 367, 657–659.
[6] Phillips, G.J. and Silhavy, T.J. (1992) Nature 359, 744–746.
[7] 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.
[8] Ribes, V., Römisch, K., Giner, A., Dobberstein, B. and Tollervey, D. (1990) Cell 63, 591–600.
[9] Poritz, M.A., Bernstein, H.D., Strub, K., Zopf, D., Wilhelm, H. and Walter, P. (1990) Science 250, 1111–1117.
[10] Valent, Q.A., Kendall, D.A., High, S., Kusters, R., Oudega, B. and Luirink, J. (1995) EMBO J. 14, 5494–5505.
[11] Ng, D.T.W., Brown, J.D. and Walter, P. (1996) J. Cell Biol. 134, 269–278.
[12] MacFarlane, J. and Müller, M. (1995) Eur. J. Biochem. 233, 766–771.
[13] von Heijne, G. (1989) Nature 341, 456–458.
[14] Wolfe, P.B. and Wickner, W. (1984) Cell 36, 1067–1072.
[15] Johnston, S., Lee, J.H. and Ray, D.S. (1985) Gene 34, 137–145.
[16] Guzman, L.M., Belin, D., Carson, M.J. and Beckwith, J. (1995) J. Bacteriol. 177, 4121–4130.
[17] Wolfe, P.B., Rice, M. and Wickner, W. (1985) J. Biol. Chem. 260, 1836–1841.
[18] Nilsson, I.M. and von Heijne, G. (1990) Cell 62, 1135–1141.