The integration of YidC into the cytoplasmic membrane of Escherichia coli requires the signal recognition particle, SecA and SecYEG*

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The integration of the polytopic membrane protein YidC into the inner membrane of Escherichia coli was analyzed employing an in vitro system. Upon integration of in vitro synthesized YidC, a 42-kDa membrane protected fragment was detected, which could be immuno-precipitated with polyclonal anti-YidC antibodies. The occurrence of this fragment is in agreement with the predicted topology of YidC and probably encompasses the first two transmembrane domains and the connecting 320-amino acid-long periplasmic loop. The integration of YidC was strictly dependent on the signal recognition particle and SecA. YidC could not be integrated into the membrane in the absence of SecY, SecE, or SecG, suggesting that YidC, in contrast to its mitochondrial orthologue Oxa1p, cannot engage a SecYEG-independent protein-conducting channel.

Protein traffic in any living cell requires the specific recognition of the proteins to be transported and their selective transport across the lipid bilayer through an aqueous channel providing the polar environment for translocation. It is now evident that the core components of this protein-conducting channel, termed SecYEG in bacteria and Sec61 in the endoplasmic reticulum of eukaryotes, are conserved in all three kingdoms of life (1). The targeting of proteins to the SecYEG complex in Escherichia coli is mediated by two pathways exhibiting different substrate specificity. Largely hydrophobic membrane proteins are selectively recognized by the bacterial SRP (signal recognition particle), consisting of the protein Ffh and the 4.5 S RNA (2–4). Binding of SRP to the nascent chains of membrane proteins initiates a cotranslational targeting to FtsY, the bacterial homologue of the SRP-receptor, and subsequently to the SecYEG translocon (5, 6, 7). The translocation of secretory proteins, on the other hand, involves the postranslational binding of the preprotein by the chaperone SecB and its subsequent transfer to SecA, which then translocates the preprotein across the SecYEG channel in an ATP-dependent manner (8). While there is no general overlap between the SRP and SecA/AB pathways, the integration of a subset of membrane proteins, i.e. membrane proteins with large periplasmic domains, requires the cooperative activity of both SRP and SecA (7, 9). This has been demonstrated for a single spanning membrane protein carrying a 320-amino acid-long periplasmic loop, which is cotranslationally targeted to SecY by SRP but remains untranslocated until SecA is present (7).

Recent evidence indicates that the translocase activity and the integrase activity of the SecYEG complex depend on different domains of SecY and different components of the translocon (10, 11). In particular, the activity of SecG seems to be dispensable for the integration of SecA-independent membrane proteins (11). The engagement of different components of the translocon during SRP-dependent integration of membrane proteins and SecA-dependent translocation of secretory proteins is further corroborated by recent results indicating that the integration of membrane proteins requires an additional component, the 60-kDa membrane protein YidC (12, 13). YidC seems to be functionally and structurally closely associated with the SecYEG translocon (14, 15) and is presumably involved in the lateral transfer of transmembrane domains (TMs) from the Sec complex into the lipid bilayer (13, 16). Even small phase-derived membrane proteins, previously thought to spontaneously insert into the E. coli membrane, depend on YidC for their correct insertion (17). Since the integration of these phase proteins occurs most likely in a SecYEG-independent manner, it is assumed that for some proteins YidC can mediate membrane insertion independently of the SecYEG translocon (18).

Like YidC, its orthologues, Oxa1p in the inner mitochondrial membrane and Alb3 in the thylakoid membrane, are specifically involved in membrane protein assembly (18). Because mitochondria do not contain homologues to the bacterial SecYEG translocon, it has been suggested that Oxa1p represents a component of an individual integration machinery in the inner mitochondrial membrane through which membrane proteins, like the nuclear-encoded Oxa1p itself, are integrated from the matrix side into the inner membrane (19, 20).

Oxa1p consists of five transmembrane domains and a hydrophilic N-tail of about 100 amino acids, which is translocated into the intermembrane space of mitochondria (19). This topology is reminiscent of the bacterial protein ProW, which contains seven transmembrane domains and a periplasmic N-tail of about 100 amino acids (21). It has been demonstrated that the N-tail of ProW is translocated independently of the SecA/SecY system (21). The major topological difference between the E. coli YidC and Oxa1p/ProW is that it contains an additional N-terminal signal anchor sequence, which is connected by a 320-amino acid-long loop to the second TM (Ref. 22; Fig. 1B). In view of this special topology of YidC we wanted to analyze the mechanism of its integration. In particular, we asked whether the requirements for integration were comparable with those of single spanning membrane proteins, carrying large periplas-
mic domains. We demonstrate here that the integration of YidC into the inner membrane of *E. coli* requires a functional SecYE translocon and the coordinated activity of both the signal recognition particle and SecA.

### EXPERIMENTAL PROCEDURES

**Strains and Plasmids**—The following *E. coli* strains were used: MRE 600 (23), XL1-Blue (Stratagene), TY1 (ompT:kan, secY205) (24), CU164 (secY39) (25), CM124 (secE19-111, pCM22) (26), and KN553 (DsecB-C::Tn10 DsecG:kan) (27). For *in vitro* protein synthesis the following plasmids were used: pDMB (OmpA) (28), p717MttA-B (mannitol permease) (29), and the YidC gene cloned in pROEX-HTB (Invitrogen), kindly provided by Dr. Ross Dalbey.

**In Vivo Reactions**—The composition of the reconstituted transcription/translation system of *E. coli* and the purification of its components, the preparation of INV, urea extraction of INV, and the protease protection assay employed in this study have been described previously (11, 28, 29).

**YidC Purification and Production of Polyclonal Antibodies—**Overexpression and purification of YidC-His was performed in a similar manner as described (15). His-tagged YidC was expressed from pROEX-HTB-yidC in *E. coli* XL1-Blue, grown to mid-logarithmic phase in LB medium supplemented with ampicillin (100 μg/ml), and 0.2% glucose. Expression was induced by adding isopropyl-β-D-thiogalactopyranoside (1 mM) and growth was continued for another 2 h. Cells were disrupted by several passages through a French pressure cell (8000 p.s.i.), and unbroken cells were removed by centrifugation for 30 min at 30,000 × g (S30). Membranes were collected by ultracentrifugation (2.5 h, 45,000 rpm, Beckmann T50.2 rotor) of the S30 and solubilized in buffer A (10 mM Tris, pH 8.0, 20% glycerol, 100 mM KC1, 10 mM imidazole) containing 2% dodecylmaltoside. After removal of insoluble material (1 h, 100,000 × g) the solubilized proteins were applied to nickel-nitriilotriacetic acid-agarose (Quiagen, Hilden, Germany). The matrix was washed with buffer A containing 0.1% dodecylmaltoside, 40 mM imidazole, and bound material was eluted with buffer A containing 0.1% dodecylmaltoside and 400 mM imidazole. Polyclonal antibodies directed against purified and SDS-denatured YidC-His were raised in rabbits.

**Sample Analysis and Quantification—**All samples were analyzed on 13% SDS-polyacrylamide gels. Radiolabeled proteins were visualized by phosphorimaging using a Molecular Dynamics PhosphorImager and quantified using Imagequant software from Molecular Dynamics. The percentage of integration was calculated after correcting for the expected loss of methionine residues occurring during cleavage by proteinase K.

### RESULTS AND DISCUSSION

**In Vitro Synthesis and Integration of YidC—**For analyzing its integration, a His-tagged version of YidC was *in vitro* synthesized in the cell-free translation system in the presence of INV. 35S-Labeled translation products were subjected to a protease protection assay (0.5 mg/ml proteinase K (PK), 20 min, 25 °C) or directly precipitated with trichloroacetic acid, separated by SDS-PAGE and visualized by phosphorimaging. Arrows indicate the position of full-length YidC and the membrane-protected fragment of YidC (YidC-MPF) resistant toward proteinase K. Immunoprecipitation using anti-YidC antiserum confirmed the identity of the membrane protected fragment of YidC. The predicted topology model for YidC (21). The YidC protein consists of an N-terminal transmembrane domain, a large periplasmic loop, and five C-terminal transmembrane helices. The N and C termini are located on the cytoplasmic side (22).

**FIG. 1. Integration of YidC into inside-out inner membrane vesicles of *E. coli*. A, YidC was *in vitro* synthesized in a cell-free translation system in the presence of INV. **

- **B.** YidC-MPF → 44 kDa

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**A.**

| INV | - | + | + | + |
|-----|---|---|---|---|

- **anti-YidC**

| PK | - | + | + | + |
|----|---|---|---|---|

- **YidC**

| 69 kDa |

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**B.**

| N | 548 |
|----|----|

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been proposed that YidC can also function independently of SecYEG and as such might be sufficient for its own integration.

To address this, we analyzed the integration of YidC into INV prepared from different *E. coli* strains, carrying mutations within *secY*, *secE*, or *secG*. As controls we also tested the integration of the polytopic membrane protein mannitol permease (MtlA) and the translocation of the secretory protein OmpA. We have previously shown (11) that INV prepared from the *secY* mutants secY39 and secY205 were completely blocked in the transport of OmpA. The integration of MtlA on the other hand was not significantly influenced (Fig. 2). Similar to OmpA, both mutations also severely reduced the integration of YidC, which strongly argues for an involvement of SecY in YidC integration. The integration defect observed with the secY205 mutant vesicles furthermore points to a SecA participation in the integration of SecY, since the secY205 mutant is specifically impaired in the SecA-SecY interaction (24). This is why the integration of the SecA-independent membrane protein Mla is not impaired by this mutation.

In SecE-depleted membranes, SecY is rapidly degraded by the PhoH protease (30), causing pronounced transport defect for secretory proteins and membrane proteins (11). As for Mla and OmpA (Fig. 2), YidC integration was completely blocked in SecE-depleted INV, which confirms that YidC is integrated into the membrane through the SecYE translocon.

We have previously shown that SecG is dispensable for the integration of those membrane proteins, which do not require
SecA for proper integration (11). Thus, the function of SecG is probably restricted to its role in assisting the insertion of the SecA-preprotein complex into the translocation channel (27). INV prepared from a secG deletion mutant did therefore not affect the integration of MtlA, but were completely blocked in the translocation of OmpA (Fig. 2). These INV were also unable to support the integration of YidC, which underlines the involvement of SecA in the integration of YidC. In summary, these data suggest a SecYEG- and SecA-dependent integration activity of these vesicles could be fully restored. Full restoration was also possible in this in vitro system by adding just SecA, Ffh, and FtsY, suggesting that these components are essential for the integration of YidC.

The simultaneous dependence on SRP and SecA is reminiscent of single spanning membrane proteins with large periplasmic domains, like FtsQ or the fusion protein Momp2 (7, 11). A detailed analysis of Momp2 integration has provided a model on how SRP and SecA cooperate in the assembly of these membrane proteins: SRP mediates the cotranslational targeting of ribosome-associated nascent chains to the SecYEG translocon, but is unable to translocate the large hydrophilic domain, i.e. the protein is stably bound to the translocon but remains protease-sensitive until SecA is added (7). In vitro, Momp2 was found to be integrated even by SecA/SecB alone if SRP and FtsY were omitted. In this setup, however, SecA/SecB achieve both targeting and translocation in a posttranslational manner similar to the mechanism by which secretory proteins are translocated. The polytopic membrane protein YidC behaves clearly different from the single spanning Momp2, since it cannot be integrated by SecA/SecB alone in the absence of SRP. Presumably, if translocation would occur only after all TMs have been synthesized, a posttranslational binding of SecA would not lead to membrane assembly because of the tendency of the polytopic YidC to aggregate in solution. Thus, the occurrence of TMs following the large periplasmic loop of YidC seems to require a renewed binding of SRP probably to the third signal-anchor type TM.

Most secretory proteins depend not only on SecA for their translocation but also on the chaperone SecB, which binds to the preprotein and stabilizes it in a transport competent conformation (8). In addition to its chaperone activity, SecB has also a targeting function, which is mediated by its ability to interact with SecA. Whether SecB is involved in the topogenesis of membrane proteins requiring a concerted action of SRP and SecA, such as Momp2, has not been addressed so far.
Under our experimental conditions, i.e. in the presence of SRP and SecA, the integration of YidC does not seem to require SecB (Fig. 3). In addition, we show that in the absence of SRP integration of YidC cannot be accomplished by SecA and SecB alone. Thus, the chaperone activity of SecB seems to be unable to maintain YidC in an integration competent conformation, suitable for a posttranslational transport by SecA.

Under our conditions, i.e. saturating concentrations of SecA, the integration of YidC occurs in the absence of the membrane potential, since F1-ATPase is obviously not needed for this process (Fig. 3). The underlying mechanisms on how the proton motive force (pmf) effects protein translocation are mostly unclear (31). The pmf obviously influences the SecA reaction cycle, because high concentrations of SecA render the translocation process pmf-independent (32). The pmf presumably also affects the translocase and its pore size directly, and it might impose directionality to the translocation process (31). During the cotranslational targeting of SRP dependent proteins, a close contact between the translating ribosome and the SecYEG translocon is formed, and this seal should prevent any reverse translocation, therefore imposing directionality in the absence of the pmf.

In this paper we have analyzed the integration of YidC into the inner membrane of E. coli. Like single spanning membrane proteins with large periplasmic loops, its integration requires the coordinated activity of both SRP and SecA. Thus, the integration is probably initiated by a cotranslational binding of SRP to the signal anchor sequence of YidC and a subsequent targeting to the SecYEG complex. The activity of SecA is then required for the translocation of the large periplasmic loop. The SecA dependence of YidC is intriguing with respect to the so-called N-tail phenomenon (33), which describes the Sec-independent translocation of periplasmic N-tails in bacterial membrane proteins. The major structural difference between YidC and N-tail membrane proteins such as Oxa1p or ProW is that YidC, like its mitochondrial orthologue Oxa1p, catalyzes its own integration as part of a SecYEG-independent transport machinery.

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REFERENCES

1. Muller, M., Koch, H. G., Beck, K., and Schaefer, U. (2001) Prog. Nucleic Acids Res. Mol. Biol. 66, 107–157
2. MacFarlane, J., and Muller, M. (1995) Eur. J. Biochem. 233, 766–771
3. de Gier, J. W. L., Mansouri, P., Valenti, Q. A., Phillips, G. J., Lurink, J., and von Heijne, G. (1996) FEBS Lett. 399, 307–309
4. Ulbricht, N. D., Newitt, J. A., and Bernstein, H. D. (1997) Cell 88, 187–196
5. Valent, Q. A., Scotti, P. A., High, S., de Gier, J. W. L., von Heijne, G., Lentzen, G., Wintermeyer, W., Oudega, B., and Lurink, J. (1998) EMBO J. 17, 2504–2512
6. Beck, K., Wu, L. F., Brunner, J., and Muller, M. (2000) EMBO J. 19, 134–143
7. Neumann-Haefelin, C., Schafer, U., Muller, M., and Koch, H. G. (2000) EMBO J. 19, 6419–6426
8. Driessen, A. J. M., Manting, E. H., and van der Does, C. (2001) Nat. Struct. Biol. 8, 492–498
9. Scotti, P. A., Valenti, Q. A., Manting, E. H., Urbanus, M. L., Driessen, A. J. M., Oudega, B., and Lurink, J. (1999) J. Biol. Chem. 274, 29883–29888
10. Newitt, J. A., and Bernstein, H. D. (1998) J. Biol. Chem. 273, 12451–12454
11. Koch, H. G., and Muller, M. (2000) J. Cell Biol. 150, 689–694
12. Houben, E. N. G., Scotti, P. A., Valent, Q. A., Brunner, J., de Gier, J. W. L., Oudega, B., and Lurink, J. (2000) FEBS Lett. 476, 225–233
13. Urbanus, M. L., Scotti, P. A., Froderberg, L., Siaaf, A., de Gier, J. W. L., Brunner, J., Samuelsen, J. C., Dalbey, R. E., Oudega, B., and Lurink, J. (2001) EMBO Rep. 2, 524–529
14. Scotti, P. A., Urbanus, M. L., Brunner, J., de Gier, J. W. L., von Heijne, G., van der Does, C., Driessen, A. J. M., Oudega, B., and Lurink, J. (2000) EMBO J. 19, 542–549
15. van der Laan, M., Houben, E. N. G., Nouwen, N., Lurink, J., and Driessen, A. J. M. (2001) EMBO Rep. 2, 519–523
16. Beck, K., Eisner, G., Trescher, D., Dalbey, R. E., Brunner, J., and Muller, M. (2001) EMBO Rep. 2, 709–714
17. Samuelson, J. C., Chen, M., Jiang, F., Muller, I., Wiedmann, M., Kuhn, A., Phillips, G. J., and Dalbey, R. E. (2000) Nature 406, 637–641
18. Lurink, J., Samuelsen, T., and de Gier, J. W. L. (2001) FEBS Lett. 501, 1–5
19. Herrmann, J. M., Neupert, W., and Staute, D. A. (1997) EMBO J. 16, 2217–2226
20. Hell, K., Neupert, W., and Staute, R. A. (2001) EMBO J. 20, 1281–1288
21. Whitley, P., Zander, T., Ehrmann, M., Haardt, M., Bremer, E., and von Heijne, G. (1994) EMBO J. 13, 4653–4661
22. Saaf, A., Monne, M., de Gier, J. W. L., and von Heijne, G. (1998) J. Biol. Chem. 273, 30415–30418
23. Muller, M., and Blobel, G. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 7421–7425
24. Matsumoto, G., Yoshihisa, T., and Ito, K. (1997) EMBO J. 16, 6384–6393
25. Baba, T., Jacq, A., Beckmann, E., Beckwith, J., Taura, T., Ueguchi, C., Akiyama, Y., and Ito, K. (1990) J. Bacteriol. 170, 705–701
26. Traxler, B., and Murphy C. (1996) J. Biol. Chem. 271, 12384–12400
27. Nishiya, K., Suzuki, T., and Tokuda, H. (1996) Cell 85, 71–81
28. Behrmann, M., Koch, H. G., Hengelage, T., Wieseler, B., Hoffschulte, H. K., and Muller, M. (1998) J. Biol. Chem. 273, 13888–13904
29. Koch, H. G., Hengelage, T., Neumann-Haefelin, C., MacFarlane, J., Hoffschulte, H. K., Schimz, K.-L., Mechler, B., and Muller, M. (1999) Mol. Biol. Cell 10, 2163–2173
30. Akiyama, Y., Kihara, A., Tokuda, H., and Ito, K. (1996) J. Biol. Chem. 271, 31196–31201
31. Manting, E. H., and Driessen, A. J. M. (2000) Mol. Microbiol. 37, 226–238
32. Yamada, H., Matsuyama, S., Tokuda, H., and Mizushima, S. (1989) J. Biol. Chem. 264, 18577–18581
33. Dalbey, R. E., Kuhn, A., and von Heijne, G. (1995) Trends Cell Biol. 5, 380–383