Chaperonin-promoted
Post-translational Membrane Insertion of a Multispanning
Membrane Protein Lactose Permease*

(Received for publication, March 28, 1996, and in revised form, July 8, 1996)

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Using an in vitro membrane-free translation system from Escherichia coli, it is shown that chaperonin GroEL added cotranslationally interacts with newly synthesized lactose permease (LacY), a multispanning membrane protein, thereby preventing aggregation. Subsequently, when the isolated GroEL-LacY complex is incubated with inverted membrane vesicles, the permease is inserted into the membrane in a MgATP-dependent manner. Post-translational membrane insertion is also observed when aggregation of newly synthesized LacY is prevented by addition of the nonionic detergent n-dodecyl-β-D-maltoside during translation in place of GroEL. No membrane integration occurs with right-side-out vesicles, indicating that LacY interacts specifically only with the cytosolic face of the membrane. Ligand thiodigalactoside protection against alkylation of the Cys-148 residue in the permease shows proper post-translational insertion. Moreover, limited proteolysis of soluble LacY either complexed with GroEL or in detergent indicates that the newly synthesized protein assumes a conformation that is comparable to that of native, membrane-embedded permease prior to insertion into the membrane.

The Eschericia coli lactose permease (LacY) catalyzes the coupled translocation of β-galactosides and H+ across the cytoplasmic membrane. Within the framework of current interest in the biosynthesis and assembly of membrane proteins, LacY provides a particularly attractive model, since this hydrophobic polypeptide is membrane protein well characterized (1, 2). The functional unit of LacY is a monomer of 46.5-kDa containing 12 membrane-spanning hydrophobic α-helices (1) that traverse the membrane in a zig-zag fashion packed into a particle of about 50 × 40 Å (3). Although LacY represents one of the most extensively characterized transport proteins, it is not known how this protein or other integral membrane proteins are inserted into membranes. As shown previously, LacY can be synthesized in vitro (4, 5) and inserted cotranslationally into INV (5). Unlike secretory proteins, there is no evidence for post-translational insertion of LacY or other multispanning membrane proteins in E. coli, and, generally, little is known about biogenesis of multispanning membrane proteins. Membrane proteins are very hydrophobic and tend to aggregate in membrane-free translation systems (5). In this respect, molecular chaperones may act to prevent aggregation. It has been demonstrated by Bochkareva et al. (6) that chaperonin GroEL maintains the translocation-competent unfolded state of newly synthesized β-lactamase and that translocation across the membrane requires MgATP-promoted discharge of the protein from GroEL. The physiological role of molecular chaperones, including GroEL, is to interact with transiently exposed hydrophobic patches and to promote folding, assembly, or secretion of various proteins mainly by preventing aggregation (7–9). However, the possible role of GroEL or other molecular chaperones in the targeting and insertion of multispanning membrane proteins into the membrane is an open question.

EXPERIMENTAL PROCEDURES

Materials—Buffers: A, 1 mM DTT, 25 mM TEA acetate, pH 7.5; B, 50 mM KCl, 0.1 mM EDTA, 1 mM TEA acetate, pH 7.5; C, 50 mM KCl, 0.1 mM MgCl₂, 0.1 mM EDTA, 50 mM TEA acetate, pH 7.5. All chemicals (NEM, NEM-biotin, TDG, DM etc.) were purchased from Sigma. [35S]Met (code S 204 and specific activity ~1,000 Ci/mmol) was obtained from Amersham International. GroEL and GroES were purified as described (10). mRNA encoding LacY was prepared in a cell-free transcription mixture (11) containing T7 polymerase and plasmid pT7-5(LacY) (12) linearized by restriction endonuclease Scal. mRNA encoding LacY(Cys148) mutant was prepared similarly from the S148C version of C-less plasmid pC7S/C154V (13).

S-30 crude extract from the E. coli MRE600 was prepared as described (14) and purified from membranes by centrifugation. For this, 0.3 ml of the extract were loaded over 4.7 ml of 22% sucrose in buffer A and centrifuged at 18,000 rpm for 110 min at 45,000 rpm (SW50.1 rotor, Beckman L5-65 centrifuge). The top 3.5 ml were collected and diluted 3 times with the same buffer. The samples were then concentrated to 10.5 mg of protein per ml using Centricon 10 (Amicon) and stored at -80 °C. INV were prepared from E. coli MC1410 cells by a low pressure French press (14) in buffer containing 1 mM EDTA, 1 mM DTT, 50 mM TEA-HCl, pH 7.5. The final concentration of sucrose gradient-purified INV was 6 mg of protein per ml as determined according to Ref. 15. Using BSA as a standard. [35S]Labelled cells (10 A₀,₀,₀ per ml in the same buffer) were sonicated on ice by Microson (Heat Systems Inc.) for 4 × 15 s, and membranes were purified by two cycles of centrifugation at 4 °C for 45 min at 75,000 rpm (TLA100.1 rotor, Beckman TL100 centrifuge) through the same buffer containing 20% sucrose. SDS-Thrace PAGE was carried out according to Ref. 16 using 12.5% acrylamide and 0.42% bisacrylamide.

Cell-free Synthesis of [35S]LacY—Synthesis of LacY was carried out in a 30-µl translation mixture containing LacY mRNA, 45 µg of protein of membrane-free cell extract, 30 µCi of [35S]Met and other components as described (11) with 40 µl instead of 200 µl potassium acetate. Where indicated, 4.5–6.0 µg of GroEL or 0.1% DM was included. After a 15-min incubation at 37 °C, cold Met was added (final concentration 0.4 mM), and the translation was stopped by 1 mM puromycin and...
isolated in 45°C ice, the mixtures were centrifuged at 4°C for 35 min at 75,000 rpm. 

30 Na-CAPS (pH 12.4) so that the final pH in the samples was further incubation for 10 min at the same temperature.

Complex into INV—

3 times with the same buffer (without MgCl2). The washed INV were removed by centrifugation (40 min at 75,000 rpm in a Beckman TL100 centrifuge). 100-

mM NEM-biotin for 10 min at 25°C, and the reaction was quenched by extensive washing with DM buffer, the agarose beads were assayed by

l (1.5 mg/ml) of trypsin inhibitor from beef pancreas (type I-P, Sigma) in the case of trypsin, or with 3

m of total protein in the samples. 8-

m of total protein, 0.5–2.0

m of LacY, thereby preventing aggregation. In order to determine

Synthesis of [35S]LacY (Cys-148) mutant and

Functional Assay—Synthesis of [35S]LacY (Cys-148) mutant and post-translational insertion into INV were carried out as described above for wild type LacY but in 120–180 μl of translation mixture. Aliquots of isolated INV (30 μl) were treated, according to Frillingos and Kaback (17), by 0.4 mM NEM for 15 min at 25°C with or without 10 mM TDG in 40 μl of buffer C. Where indicated, TDG was replaced by 5 mM potassium succinate, 10 mM MgCl2, 35 mM potassium acetate, 2 mM DTT, 50 mM TEA acetate, pH 7.5) containing 10 μl of GroEL-[35S]LacY complex (1.5 × 10^6 cpm/1.7 μg of GroEL), 48 μg of INV, 3 μg of creatine kinase, 0.5 μM of creatine phosphate and, also, where indicated, 1 μg of GroES and/or 3.5 mM ATP. After a 30-min incubation at 37°C, ETD (5 μl of 0.2 M) was added, and the mixture was incubated for 10 min at 25°C and cooled on ice. The following isolation of INV carrying [35S]LacY is described in the legend to Fig. 2.

Analysis of the LacY’ Insertion from GroEL into INV—Typically, 15-μl aliquots of isolated INV (15 μg of total protein, ~1–2 × 10^6 cpm of [35S]LacY associated) and 5 μg of BSA in buffer A were mixed with 30 μl of the membrane-free biosynthesis of [35S]Met-labeled LacY leads to

and sedimenting the vesicles to the bottom at 4°C (40 min at 75,000 rpm in a TLA100.1 rotor, Beckman TL100 centrifuge). 100-μl aliquots were collected from the top, and fractions 7–9 containing GroEL were diluted 3 times with the same buffer (i), 30 μl of 0.15 M sodium carbonate, 0.15 M NaCl, pH 12.4 so that the final pH in the samples was ~12.2 (ii), 30 μl of 9 M urea in buffer A (iii), or 30 μl of 30 μM (1.5%) DM in buffer A (iv). After incubation for 1 h on ice, the mixtures were centrifuged at 4°C for 35 min at 75,000 rpm (TLA100.1 rotor, Beckman TL100 centrifuge). The pellets were solubilized in 45 μl of SDS-sample buffer (30 min at 37°C). 15-μl aliquots from the supernatants and solubilized pellets of each sample were counted in order to estimate the distribution of labeled material. An- other 15-μl aliquots were incubated with 5 μl of 5% SDS-sample buffer (30 min at 37°C) and analyzed by SDS-Tricine-PAGE. As a control, an analogous treatment in alkaline pH or with 6 μl of the GroEL-[35S]LacY complex did not reveal precipitation of radioactivity (data not shown). Functional Assay—Synthesis of [35S]LacY (Cys-148) mutant and post-translational insertion into INV were carried out as described above for wild type LacY but in 120–180 μl of translation mixture. Aliquots of isolated INV (~30 μg) were treated, according to Frillingos and Kaback (17), by 0.4 mM NEM for 15 min at 25°C with or without 10 mM TDG in 40 μl of buffer C. Where indicated, TDG was replaced by lactose (see Experiment 5 in Table 1). The reaction was quenched by addition of 2 mM DTT. The samples were diluted 4 times with buffer C, and TDG was removed by centrifugation (40 min at 75,000 rpm in a TLA100.1 rotor, Beckman TL100 centrifuge). The vesicles were washed 3 times with the same buffer (without MgCl2). The washed INV were dissolved in 40 μl of buffer C containing 0.05% DM, incubated with 0.4 mg of NEM-biotin for 10 min at 25°C, and the reaction was quenched by the addition of 10 mM DTT. Membranes were then incubated in alkaline pH as described above, and the excess of NEM-biotin was removed by centrifugation and 3 cycles of washing with buffer B. Pellets were dissolved in 100 μl of DM buffer (1% DM, 150 mM NaCl, 0.2 mM EDTA, 50 mM TEA acetate, pH 7.5), and the DM extracts (3–5 × 10^6 cpm) were incubated with 50 μl of 50% suspension of avidin-agarose beads for 1 h at room temperature with continuous rotation. After extensive washing with DM buffer, the agarose beads were assayed by liquid scintillation spectrometry.

GroEL-promoted Interaction of LacY with Right-side-out Membrane Vesicles Prior to and after Sonication of Them—Right-side-out membrane vesicles were prepared as described by Kaback (18) and converted into INV by sonication as described above for the whole cells in a buffer containing 0.1 mM DTT, 50 mM TEA acetate, pH 7.5. Sonicated vesicles were utilized without additional purification. Transfer of LacY from the GroEL-[35S]LacY complex into the right-side-out vesicles prior to or after sonication was carried out under identical conditions as described above. Then, the vesicles were isolated by centrifugation at 4°C for 45 min either at 14,000 rpm in an Eppendorf centrifuge 5415 C (for the right-side-out vesicles) or at 75,000 rpm through 20% sucrose layer in buffer B in a TLA100.1 rotor, Beckman TL100 centrifuge (for the sonicated vesicles). The membrane pellets were suspended in buffer A and treated in alkaline pH as described above.

DM-promoted Insertion of LacY into INV—The top fractions 1–3 (Fig. 3A) containing the soluble [35S]LacY were concentrated 10 times (final volume 30 μl) using Microcon 10 (Amicon). 4-μl aliquots were then added to 46 μl of INV, and insertion of LacY was carried out as described above. The residual concentration of DM was estimated to be 0.03–0.06 mM. Interaction of [35S]LacY with INV was analyzed by loading of 50 μl of sample over 600 μl of 20% sucrose layer in buffer B

RESULTS

In this study, we examine the effect of GroEL on the in vitro, membrane-free translation of LacY. As shown in Fig. 1, the membrane-free biosynthesis of [35S]Met-labeled LacY leads to almost complete aggregation. In contrast, if exogenous GroEL is added to the translation mixture, about 30% of the LacY synthesized remains soluble, co-sedimenting with GroEL. The finding indicates that GroEL interacts with newly synthesized LacY, thereby preventing aggregation. In order to determine whether or not the LacY’ bound to GroEL is able to interact with membranes, sucrose gradient fractions containing the GroEL-LacY’ complex were incubated with E. coli INV at 37°C. The membranes were then separated from the GroEL-LacY’ complex (Fig. 2A, fractions 8 and 2, respectively) by centrifu-
Fig. 2. Post-translational transfer of \([^{35}S]\text{LacY}\) from GroEL into INV. A, sucrose gradient centrifugation of samples containing GroEL-\([^{35}S]\text{LacY}\) complex and INV after incubation in the absence (open circles) or presence of Mg\(^{2+}\), ATP, and GroES (closed circles). Note: centrifugation of GroEL-\([^{35}S]\text{LacY}\) complex incubated with Mg\(^{2+}\), ATP, and GroES in the absence of INV does not reveal radioactivity in fractions 8 and 9 (data not shown). B, the effect of Mg\(^{2+}\) (10 mM), ATP (3.5 mM), or GroES (20 \(\mu\)gm) on \([^{35}S]\text{LacY}\) transfer from GroEL into INV. Incubation of INV with GroEL-\([^{35}S]\text{LacY}\) complex was carried out at 37 °C for 30 min. The incubation mixtures were then separated by centrifugation at 4 °C through 1.04 ml of a 7.5–20% sucrose gradient in the same buffer. After a 50-min centrifugation at 54,000 rpm (TLS 55 rotor, Beckman TL100 centrifuge), 130-\(\mu\)l fractions were collected from the top and analyzed as described above (Fig. 1). According to their density and as confirmed by Comassie staining profile (data not shown), INV were located on a boundary between 20 and 40% sucrose (fraction 8, A). C, extraction of \([^{35}S]\text{LacY}\) from the membrane. INV associated with \([^{35}S]\text{LacY}\) were treated with control buffer (pH 7.5), high pH buffer (pH 12), or buffer containing 6 mM urea or 1% DM and sedimented as described above ("Experimental Procedures."). Distribution of \([^{35}S]\text{LacY}\) between the supernatant (s) and the pellet (p) was estimated by the radioactivity counting (left panel) and by SDS-Tricine-PAGE (right panel). D, LacY inserts through the cytosolic and not through the periplasmic surface of membrane vesicles. The GroEL-\([^{35}S]\text{LacY}\) complex was incubated, in the presence of MgATP and GroES, for 30 min at 37 °C with the right-side-out membrane vesicles prior to (R) or after conversion of them into INV (I) by sonication. The following isolation of the membranes by centrifugation and treatment with high pH buffer (pH 12) were carried out as described under "Experimental Procedures." Molecular mass standards (kDa) are indicated at the right. 1, LacY bound to GroEL (half of input); 2 and 3, LacY transferred from GroEL into the membrane vesicles; 4 and 5 (supernatants) or 6 and 7 (membrane pellets, solubilized with 2% SDS), distribution of LacY after treatment of the vesicles with pH 12 buffer followed by centrifugation; 8 and 9, LacY extracted from the membrane pellets with 1% DM after the alkaline treatment.

Fig. 3. Post-translational insertion into INV of \([^{35}S]\text{LacY}\) maintained in a soluble state by DM. A, sucrose gradient centrifugation of translation mixtures containing \([^{35}S]\text{LacY}\) synthesized in the absence or presence of 0.1% DM. Synthesis of \text{LacY} and centrifugation were carried out as described in Fig. 1 (DM was added in place of GroEL). B, effect of EDTA (20 mM), Mg\(^{2+}\) (10 mM), or ATP (3.5 mM) on DM-promoted post-translational insertion of \([^{35}S]\text{LacY}\) into INV. Fractions 1–3 (A) were concentrated and incubated with INV as described in Fig. 2B. INV were then isolated by centrifugation. C, extraction of \([^{35}S]\text{LacY}\) from the membrane. Treatments of isolated INV containing LacY with control buffer (pH 7.5), alkaline pH (pH 12), 6 mM urea, or 1% DM were carried out as described in Fig. 2C. The membrane pellets were dissolved in SDS-sample buffer and analyzed by radioactivity counting (left panel) and by SDS-Tricine-PAGE (right panel).

The co-chaperonin GroES (a GroEL helper (7–9)) further increases the transfer of LacY from GroEL into the INV, and, in the presence of both MgATP and GroES, about 30% of the LacY bound to GroEL becomes associated with the membranes. Since MgATP and GroES are required for dissociation of GroEL complexes with various proteins (7–9), the observations suggest that discharge of LacY from GroEL is a prerequisite for the association of LacY with membranes. Integral membrane proteins are solubilized only under conditions that dissolve the membrane (i.e. in the presence of detergents) and remain membrane-bound under conditions that do not destroy the integrity of membrane (e.g. treatment with EDTA, alkaline pH, or high concentrations of chaotropes). As shown in Fig. 2C, treatment of INV-LacY under the conditions described reveals that LacY is solubilized only by the detergent DM which solubilizes the membrane. Extraction at high pH or with 6 mM urea, which is known to release peripheral membrane proteins (20–22), is not effective. The results support the conclusion that soluble, GroEL-bound LacY can insert into the plasma membrane.

Since interaction with GroEL maintains newly synthesized LacY in a soluble state, we reasoned that a mild detergent such as DM might mimic the chaperonin. In control experiments (not shown), it was demonstrated that the in vitro synthesis of \([^{35}S]\text{LacY}\) is inhibited by only 15–20% in the presence of 0.1% DM. Moreover, sucrose gradient separation of LacY synthesized in the presence of DM demonstrates that about one-third of the permease remains soluble (Fig. 3A). Fractions 1–3 from the sucrose gradient which contain DM-solubilized \([^{35}S]\text{LacY}\) were incubated with INV under conditions where the final concentration of DM in the incubation mixture (0.03–0.06 mM)
is dependent on the presence of Mg2+

LacY becomes associated with the membrane in a manner that labeling by avidin-Sepharose chromatography. In the present

alkali or 6M urea (Fig. 3

tracted only with high concentrations of DM (1%) and not with

MgATP. Furthermore, the membrane-associated LacY is ex-

posed, the same experiment was carried out with right-side-out

membrane vesicles prior to or after conversion of these mem-

brane fragments into INV by sonication. As shown in Fig. 2

lanes 2

and

3

in such a manner as to promote post-translational insertion.

To study the specificity of the transfer of LacY from GroEL-

LacY complex into membranes, we tested whether this produc-
tive interaction mimics the in vivo situation where insertion occurs from the cytosolic face of the membrane. For this pur-

pose, the same experiment was carried out with right-side-out

membrane vesicles prior to or after conversion of these mem-

brane fragments into INV by sonication. As shown in Fig. 2D, the inter-

action of LacY with the membranes is enhanced by sonication (lanes 2 and 3) that is a priori not surprising since after soni-
cation the surface area of the vesicles increases. However,
treatment under alkaline pH removes comparable quantities of LacY from the membrane in both cases (lanes 4 and 5). At the same time, the sonicated membranes contain a significantly higher quantity of LacY that is resistant to alkaline treatment and extractable only by detergents such as SDS (lanes 6 and 7) or DM (lanes 8 and 9). Thus, conversion of the right-side-out vesicles into INV clearly promotes insertion of LacY. This ob-

servation shows that, after release from ribosomes, LacY is able to recognize and target specifically to the cytosolic face of the membranes.

Does proper targeting of LacY lead to its proper insertion into the membranes? The most unequivocal means of answering the question is by functional assays. However, cell-free translation systems are known to be relatively inefficient, and, in this case, the amount of LacY inserted into the INV is so low that lactose transport assays are prohibited. Therefore, in order to test the function of post-translationally inserted permease, a more convenient assay was used which is based on the ability of the ligand TDG to protect LacY against alkylation by thiol reagents (1). As shown earlier (25), Cys-148 is the critical residue alkylation of which by NEM inactivates LacY, and this effect is inhibited by TDG (apparent K\text{inact} of about 1.0 mM in INV (17)). In the original protocol described by Friltingos and Kaback (17), membranes from cells expressing LacY(Cys148) mutant with a biotin acceptor domain in the middle cytoplasmic loop were incubated with radioactive NEM in the absence or presence of 10 mM TDG followed by analysis of the permease labeling by avidin-Sepharose chromatography. In the present study, we used a LacY(Cys148) mutant without a biotin acceptor domain, and the alkylation was carried out in two steps. Note: The behavior of the mutant was similar compared with the wild type regarding the in vitro biosynthesis, binding to GroEL, and membrane insertion. First, the INV containing [35S]LacY(Cys148) inserted post-translationally were treated with NEM in the absence or presence of TDG. Then, after removal of TDG (and DTT-inactivated reagent) by centrifugation, the formerly ligand-protected thiol groups were treated with NEM-biotinylation by more than 30% is observed.

TABLE I

Two-step treatments with thiol reagent: (i) the samples of [35S]LacY inserted post-translationally into INV were treated with 0.4 mM NEM (15 min at 25°C) in the absence or presence of 10 mM TDG, and (ii) after removal of the ligand (and DTT-inactivated reagent), the formerly TDG-protected thiol groups were treated with 0.4 mM NEM-biotin (10 min at 25°C) followed by isolation of the biotinylated [35S]LacY on avidin-agarose.

| Experiment | % of [35S]LacY input on avidin-agarose | Binding to avidin-agarose |
|------------|--------------------------------------|--------------------------|
|            | Background | −TDG | +TDG |
| 1          | 350       | 9    | 121 (−35%) |
| 2          | 250       | 7    | 108 (−31%) |
| 3          | 480       | 13   | 183 (−39%) |
| 4\*        | 480       | 12   | 165 (−35%) |
| 5\*        | 480       | 13   | 138 (−2%)  |

\* Nonspecific binding to avidin-agarose of samples untreated with NEM-biotin.

\* Numbers in parentheses represent the protective effect of TDG calculated from the ratio of the radioactivities \([+(+TDG)}−(−TDG)](+(−TDG)−background]\).

\* NEM-biotinylation was carried out for 30 min on ice.

\* Treatment with NEM was carried out in the presence of 10 mM lactose instead of TDG.
Current understanding of targeting and insertion of integral membrane proteins into lipid bilayer stems mainly from a generally accepted notion that the process is similar to the translocation of secretory proteins across the membrane (31–33). Accordingly, one possible mode of biogenesis of polytopic membrane proteins in E. coli is cotranslational, resembling the SRP system of eukaryotes. Alternatively, targeting and insertion may be post-translational, similar to the pathway for protein translocation across the cytoplasmic membrane in E. coli. Although both modes of biogenesis may operate simultaneously, this study deals specifically with questions related to post-translational membrane protein targeting and insertion. It seems evident that post-translational biogenesis of hydrophobic membrane proteins like LacY must require a specific mechanism to prevent newly synthesized molecules from aggregating. It has been suggested that folding and stability of membrane- and water-soluble proteins follow similar principles leading to comparable polarities on their interiors but different polarities on their surfaces to enable solubilization in the appropriate environments (34, 35). Specifically, membrane proteins possess an increased concentration of apolar residues on their surfaces and tend to aggregate, therefore, in an aqueous environment. Based on the finding presented here, it is proposed that GroEL or DM prevent aggregation by interaction with exposed hydrophobic patches on newly synthesized LacY. Moreover, by shielding the exposed hydrophobic residues, GroEL or DM may stabilize the transiently formed α-helices and helical hairpins and also their association to generate the proper folding. Stabilization of newly synthesized LacY in a near-native state in the presence of DM resembles the ability of permease, synthesized in the cell, to maintain close to native conformation in DM after extraction from membranes (36, 37). It could be suggested that the LacY surface is surrounded by the uniform belt of DM molecules which presumably stabilizes the hydrophobic, transmembrane region of the permease and mimics its interactions with lipids in the membrane (38, 39). Correspondingly, the apical hydrophobic surface in the central channel of GroEL, responsible for protein binding (40), could mimic the detergent belt and interact with the newly synthesized LacY molecule, thus stabilizing its native-like structure. It should be noted here that, unlike cytoplasmic proteins which must be unfolded in order to interact with GroEL (i.e. exposing their apolar interior (7–9)), the highly hydrophobic surface of membrane proteins like LacY may allow them to interact with the chaperonin also in the folded state.

The second step in biogenesis of membrane proteins is insertion into the membrane. This process has been postulated to occur by two alternative models. According to one hypothesis (41–43), insertion is directed by interactions between discrete topogenic regions in a newly synthesized membrane protein and specific receptor proteins in the membrane which initiates insertion through a water-filled proteinaceous channel. In the alternative proposal (44–46), thermodynamic considerations of lipid-protein interactions lead to a suggestion that protein integration into the membrane is a spontaneous process. In this case, the polypeptide chains move directly through a hydrophobic core of the lipid bilayer during insertion. Both concepts presume that the nascent polypeptide chain should be partially (or completely) unfolded in order to integrate into the membrane, and stabilization of hydrophobic α-helices or helical hairpins and their assembly into a native three-dimensional structure occur only within the membrane.

The data presented here suggest that: (i) under conditions where aggregation is prevented, LacY can fold into a state corresponding roughly to its tertiary structure within the membrane, and (ii) in the folded state, LacY can specifically recognize and interact with the cytosolic surface of the membrane followed by proper insertion (i.e. the folded state of LacY seems not a barrier for membrane insertion).

Finally, not much is known at present about the possible participation of GroEL in membrane protein biogenesis in intact cells, and to the best of our knowledge only one published preliminary work of Sato et al. (47) deals with this question. These authors observed that overproduced GroEL/ES protects an unstable, truncated form of the tetracycline H^+ antiporter in E. coli against degradation. Our results certainly predict that GroEL may play a physiological role in membrane protein
biogenesis, especially under stress conditions such as heat shock when it is overexpressed.

Acknowledgments—We are grateful to Drs. Ronald Kaback, Steve Karlsh, and Jack Kyte for helpful suggestions and stimulating discussions. We are also indebted to Dr. Ronald Kaback for generously providing the plasmid encoding LacY(Cys148).

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J. Biol. Chem. 1996, 271:22256-22261.
doi: 10.1074/jbc.271.36.22256

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