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Organization and stability of a polytopic membrane protein: Deletion analysis of the lactose permease of *Escherichia coli*

(membrane insertion/topology/*lacY–phoA* fusions/lactose transport)

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ABSTRACT **The overall topology of polytopic membrane proteins is thought to result from either the oriented insertion of the N-terminal α-helical domain followed by passive insertion of subsequent helices or from the function of independent topogenic determinants dispersed throughout the molecules. By using the lactose permease of *Escherichia coli*, a well-characterized membrane protein with 12 transmembrane domains and the N and C termini on the cytoplasmic surface of the membrane, we have studied the insertion and stability of in-frame deletion mutants. So long as the first N-terminal and the last four C-terminal putative α-helical domains are retained, stable polypeptides are inserted into the membrane, even when an odd number of helical domains is deleted. Moreover, even when an odd number of helices is deleted, the C terminus remains on the cytoplasmic surface of the membrane, as judged by *lacY–phoA* fusion analysis. In addition, permease molecules devoid of even or odd numbers of putative transmembrane helices retain a specific pathway for downhill lactose translocation. The findings imply that relatively short C-terminal domains of the permease contain topological information sufficient for insertion in the native orientation regardless of the orientation of the N terminus.**

Secondary structure models for most polytopic membrane proteins exhibit multiple hydrophobic domains in a linear array that traverse the membrane in zig-zag fashion as α-helices connected by hydrophilic segments ("loops"). The putative transmembrane domains are generally identified as hydrophobic stretches in the primary sequence that are ≈20 amino acid residues long, since a polypeptide of this length in α-helical conformation is sufficiently long to cross the hydrophobic core of the membrane and the position of the loops is dictated by the orientation of the putative transmembrane helices (1, 2). Regarding the orientation of the proteins with respect to the plane of the membrane if the disposition of any portion of the protein on the surfaces of the membrane can be ascertained experimentally, the topology of the remainder is presumed to follow directly from the secondary-structure model. Alternatively, in certain instances, topology is inferred by predicting the orientation of the most N-terminal hydrophobic domain (N1) (3).

The orientation of N1 varies in different polytopic membrane proteins (4, 5). Thus, it has been proposed for eukaryotic systems that there are two mechanisms for insertion of N1 and that topogenic information contained within the N terminus and N1 determines the mechanism utilized (4–6). By this means, the insertional orientation of N1 is thought to be solely responsible for the orientation of the remainder of the protein. In contrast, it has been suggested (7–9) that each hydrophobic transmembrane domain behaves as an independent entity. Accordingly, each putative transmembrane helical domain is predicted to contain topogenic information that specifies the orientation of that domain in the plane of the membrane. In other words, one proposed mechanism (3–6) implies that the primary information for the orientation of polytopic membrane proteins is contained within the N terminus of the protein, whereas the other hypothesis (7–9) suggests that topogenic information is spread throughout the protein.

To test these alternatives, we have used the lactose permease of *Escherichia coli*, a well characterized prokaryotic polytopic membrane protein that catalyzes the coupled translocation of β-galactosides and H^+ with a stoichiometry of unity (i.e., β-galactoside/H^+ symport or cotransport; for a current review, see ref. 10). The *lacY* gene, which encodes the permease, has been cloned and sequenced, and the amino acid sequence of the permease has been deduced from the DNA sequence (11). Based on circular dichroism and hydropathy analysis of the primary sequence, a secondary structure was proposed (12) in which the polypeptide has 12 hydrophobic domains in α-helical conformation that traverse the membrane in zig-zag fashion connected by hydrophilic loops (Fig. 1*a*). Evidence for the general features of the model and the demonstration that both the N and C termini are on the cytoplasmic surface of the membrane has been obtained from other spectroscopic measurements (13),† chemical modification (14), limited proteolysis (15, 16), and immunological studies (17–23), and strong exclusive support for the 12-helix motif has been obtained from analysis of an extensive series of *lacY–phoA* (*lac* permease–alkaline phosphatase) fusions (24).

Stochaj and coworkers (25, 26) demonstrated that sequences within the first 170 amino acid residues of the *lac* permease may be important for insertion. Moreover, a truncated permease containing only the N-terminal 50 amino acid residues is inserted into the membrane, and it was proposed that this region contains an internal "start transfer" sequence that results in the insertion of the N terminus as a "helical hairpin" (27, 28). With respect to the C terminus of *lac* permease, although the 17-amino acid C-terminal hydrophilic tail is not involved in insertion of the protein into the membrane, its stability, or its ability to catalyze transport, a 3-amino acid sequence at the end of the last putative transmembrane helix is critical for stability and hence activity once the protein is inserted into the membrane (29, 30).

Taking these considerations into account and the functional importance of residues within helices IX and X (for a review, see ref. 31), we have now constructed mutant *lac*

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Abbreviations: TDG, D-galactopyranosyl β-D-thiogalactopyranoside; CCCP, carbonylcyanide *m*-chlorophenylhydrazone; IPTG, isopropyl β-D-thiogalactopyranoside; mAb, monoclonal antibody.

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†In addition to circular dichroic and laser Raman spectroscopy, Fourier-transform infrared studies also show that purified lac permease is largely helical (P. D. Roepe, H.R.K., and K. J. Rothschild, unpublished work).

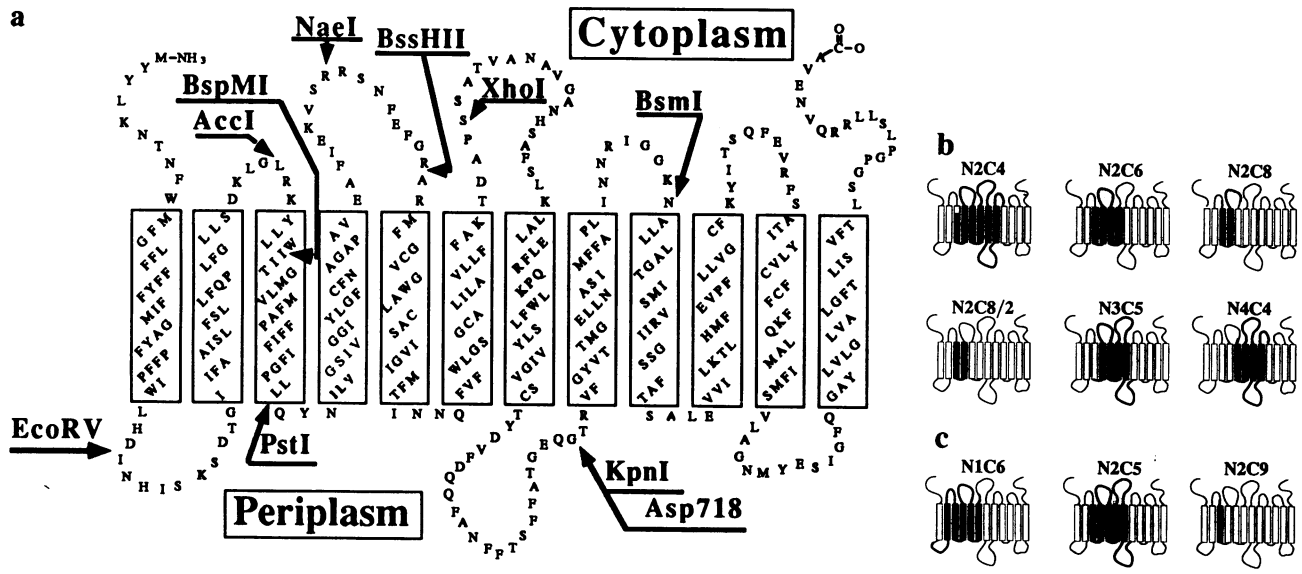

FIG. 1. Secondary structure of *lac* permease (*a*) showing polypeptides encoded by the *lacY* deletion constructs described in *b* and *c*. The single-letter amino acid code is used, hydrophobic transmembrane helices are shown in boxes, and deleted regions are solid boxes and thick lines. (*a*) Wild-type *lac* permease with restriction sites in the cassette *lacY* DNA indicated. (*b*) Constructs in which even numbers of putative transmembrane helices were deleted. (*c*) Constructs in which odd numbers of putative transmembrane helices were deleted.

permease molecules in which relatively extensive stretches of amino acid residues were deleted, but the N terminus and the first and last four putative transmembrane helices were left intact. The strategy behind the experiments was to delete even or odd numbers of transmembrane domains and to determine the effect of the deletions on insertion, stability, and the orientation of the C terminus with respect to the plane of the membrane and the activity of the constructs.

MATERIALS AND METHODS

Materials. [1-^{14}C]Lactose and L-[^{35}S]methionine were purchased from the Amersham; D-galactopyranosyl β,D-thiogalactopyranoside (TDG) was from Sigma and carbonylcyanide *m*-chlorophenylhydrazone (CCCP) was from Calbiochem. Antibodies against *E. coli* alkaline phosphatase were obtained from 5 Prime → 3 Prime, Inc. All other materials were reagent grade and obtained from commercial sources.

Bacterial Strains and Plasmids. *E. coli* HB101 [*hsdS20* (r_B^-, m_B^-),*recA13*,*ara-14*,*proA2*,*lacY1*,*galK2*,*rpsL20*(Sm^r),*xyl-5*,*mtl-1*,*supE44*,λ$^-$/F$^-$] (32) was used as carrier for the plasmids described and for detection of *lac* permease activity on MacConkey plates (Difco) containing 25 mM lactose. *E. coli* LJ915 is essentially HB101 mutated in the gene encoding enzyme I of the phospho*enol*pyruvate phosphotransferase system (generously provided by Milton Saier; University of California, San Diego). Both strains and also *E. coli* AZ-1 (*lacZ*$^+$Δ*lacY*) were used in downhill transport assays. *E. coli* T184 [*lacI*$^+$*O*$^+$*Z*$^-$*Y*$^-$(*A*),*rpsL*,*met*$^-$,*thr*$^-$,*recA*,*hsdM*,*hsdR*/*F'*,*lacI*^{q}*O*$^+$*Z*^{D118}(*Y*$^+$*A*$^+$)] (33) was used for overexpression of *lac* permease, [^{35}S]methionine-labeling, and lactose transport. Proteins were specifically labeled by using the T7 RNA polymerase system (34). A cassette *lacY* gene (EMBL-X56095) containing the *lac* promoter/operator was cloned into plasmid pT7-5 and used for all *lacY* gene manipulations. The cassette gene contains unique restriction sites in each segment of the gene encoding a putative loop (i.e., approximately every 100 base pairs). In most of the constructs, the plasmid was digested with appropriate restriction enzymes to remove the desired segment of the gene, treated with DNA polymerase I (Klenow fragment), and ligated to itself. In two constructs (N2C6 and N2C8/2), linkers were synthesized and inserted, and the cohesive ends were ligated. Oligodeoxynucleotides were synthesized on an Applied Biosystems model 391 DNA synthesizer. The fusion junctions of each construct were sequenced using the dideoxyoligonucleotide method (35, 36).

Growth of Cells, Overexpression, and Labeling of *lac* Permease. *E. coli* T184 harboring pGP1-2 and pT7-5(cassette *lacY*) or a derivative was grown at 30°C in LB supplemented with streptomycin (20 μg/ml), ampicillin (100 μg/ml), and kanamycin (50 μg/ml). Overnight cultures were diluted 1:10 with fresh medium at 30°C, and growth was continued for 3 h. The cultures were washed and resuspended in M9 salts, starved at 30°C for 30 min, and heat-shocked at 42°C for 20 min. Rifampicin (0.4 mM) and isopropyl β-D-thiogalactopyranoside (IPTG; 0.5 mM) were added, and the cultures were maintained at 42°C for 20 min and then transferred to 30°C. [^{35}S]Methionine labeling was carried out for 60 min with [^{35}S]methionine (1000 Ci/mmol; 1 Ci = 37 GBq) at a final concentration of 25 pM and membranes were prepared as described (29, 30).

Immunoprecipitation. Immunoprecipitation was carried out with monoclonal antibody (mAb) 4A10R, which is directed primarily against the C terminus of *lac* permease (37), as follows. Membranes were dissolved in 5% (wt/vol) NaDodSO$_4$/1.0 M Tris base (1 h at 37°C) and diluted to 1% NaDodSO$_4$ in a final volume of 0.1 ml. Then 0.9 ml of 50 mM Tris·HCl, pH 7.5/1.0% Triton X100/0.5% NaDodSO$_4$/100 mM NaCl/1.0 mM EDTA (immunoprecipitation buffer; IB) was added, and the solution was incubated with mAb 4A10R (40 μl of ascites fluid) for 3 h at room temperature. Protein A Sepharose conjugate equilibrated with IB containing 0.2% NaDodSO$_4$ (0.1 ml) was added, and the mixture was incubated for 45 min at room temperature. The Sepharose was then collected by low-speed centrifugation, washed three times with 1.0 ml of ice-cold IB containing 0.2% NaDodSO$_4$, and resuspended in 5% NaDodSO$_4$/20% (vol/vol) glycerol. Bound protein was dissociated at 70°C (10 min), incubated for 1 h at 37°C in sample buffer, and subjected to NaDodSO$_4$/polyacrylamide gel electrophoresis and autoradiography as described (29, 30).

Alkaline Phosphatase Assays. *E. coli* CC181 (*phoA*$^-$) was transformed with plasmid pT7-5 encoding the *lacY–phoA* fusion constructs and grown on LB plates. Replica plating on minimal agar containing 0.4 mM IPTG and 5-bromo-4-chloro-

3-indolyl phosphate (XP) (40 μg/ml) was used to assay the activity of the fusion proteins qualitatively. Quantitative assays were performed spectrophotometrically (38).

Downhill Lactose Transport. Overnight cultures of cells harboring various *lacY* genes were diluted 1:20 to 1:100 in fresh medium at 30°C, grown to an OD_{660} of 0.5, and induced with IPTG for 1.5 h. Cells were harvested by centrifugation, washed once in ice-cold 50 mM potassium phosphate, pH 7.3/10 mM magnesium sulfate, resuspended in the same buffer to an OD_{420} of 10 (≈1 mg of protein per ml), and CCCP was added to a final concentration of 20 μM. Where indicated, TDG or sucrose was added to final concentrations of 30 mM, and the cell suspensions were incubated at room temperature for 5 min prior to addition of 0.6 mM [1-^{14}C]lactose (10 mCi/mmol). Samples were taken during the first 30 min after addition of [1-^{14}C]lactose and rapidly filtered, and the filters were assayed for entrapped radioactivity by liquid scintillation spectrometry (39).

RESULTS

Deletion Mutagenesis of *lacY*. Construction of the deletions was facilitated by using a cassette *lacY* gene containing a unique restriction site at approximately every 100 base pairs within the coding sequence cloned into plasmid pT7-5. The nomenclature of the constructs (N*x*C*y*) describes the number of putative helices in the N-terminal (N*x*) and the C-terminal portions (C*y*) of the protein before and after the deletion (Fig. 1). In group I (N2C4, N2C6, N2C8, N2C8/2, N3C5, and N4C4; Fig. 1*b*), even numbers of putative transmembrane helices were deleted so that the orientation of the C terminus relative to the N terminus should remain the same as that of wild-type *lac* permease. In group II (N1C6, N2C5, and N2C9; Fig. 1*c*), odd numbers of putative transmembrane helices were deleted so that the orientation of the C terminus relative to the N terminus might be expected to be opposite to that of the wild-type permease.

Expression and Stability of Deletion Constructs. Expression of the deletion constructs was tested by studying [^{35}S]methionine incorporation in the presence of rifampicin using the T7 RNA polymerase system (34), followed by immunoprecipitation with mAb 4A10R, which reacts primarily with the C-terminal tail (37), and $NaDodSO_4$/polyacrylamide gel electrophoresis. Stability was tested by pulse–chase experiments with labeled and unlabeled methionine. Each deletion construct is expressed to an extent that is grossly comparable to wild-type permease (Fig. 2), and pulse–chase experiments demonstrate that the constructs are stable in the membrane for at least 12 h (Fig. 3).

Orientation of C-Terminal Domains. The orientation of the C terminus in each construct was determined by measuring the alkaline phosphatase activity of C-terminal *lacY–phoA* fusions. Thus, the full-length *lacY–phoA* fusion gene (24) was cloned into plasmid pT7-5, and unique restriction sites in the 5′ (*Bam*HI) and 3′ (*Spe* I) ends of the *lacY* cassette gene were used to replace the wild-type *lacY* gene with each of *lacY* deletions. As shown by Calamia and Manoil (24), alkaline phosphatase fusions to the C terminus of *lac* permease are markedly inactive relative to fusions in externally disposed domains of the protein, an observation consistent with other findings (14, 17–23) demonstrating that the C terminus is on the cytoplasmic surface of the membrane. Remarkably, when alkaline phosphatase is fused to the C terminus of each deletion construct, particularly those devoid of an odd num-

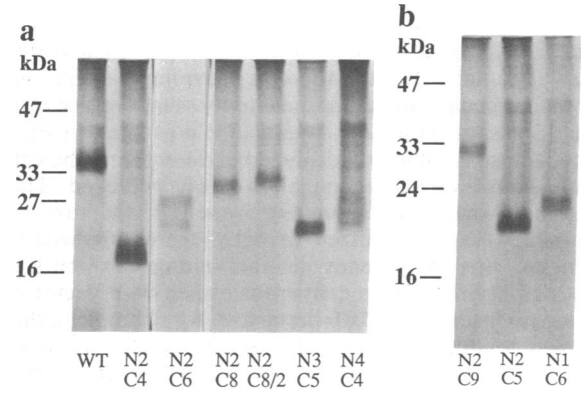

FIG. 2. Membrane insertion of *lac* permease in *E. coli* T184 harboring pGP1-2 and pT7-5(lacY) or pT7-5(NxCy). Solubilized membrane fractions from *E. coli* T184 harboring given *lacY* gene constructs in plasmid pT7-5 were labeled with [^{35}S]methionine, immunoprecipitated with mAb 4A10R, and subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis and autoradiography. Dried gels were exposed to film for 12 h. (*a*) Wild-type permease and constructs deleted of an even number of putative transmembrane helices. (*b*) Constructs deleted of an odd number of helices. Positions of prestained molecular mass markers (Bio-Rad) are indicated.

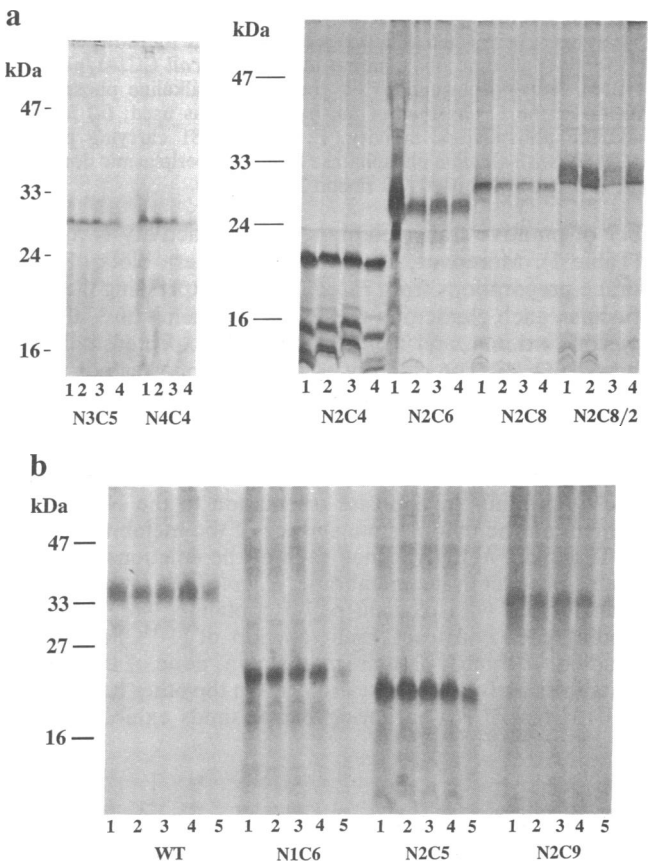

FIG. 3. Stability of *lac* permease in *E. coli* T184 harboring pGP1-2 and pT7-5(lacY) or pT7-5(NxCy). *E. coli* T184 harboring given *lacY* gene constructs were labeled with [^{35}S]methionine and chased with unlabeled methionine (40 mM). At 10 min, a sample of the cell suspension was rapidly frozen (0 time, lanes 1), and unlabeled methionine (40 mM, final concentration) was added to the remainder. At given times thereafter, samples were removed and rapidly frozen. Subsequently, the samples were thawed, membranes were prepared, solubilized in $NaDodSO_4$, and subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis, and the dried gels were exposed to film for 12 h. (*a*) Constructs deleted of an even number of putative transmembrane helices. Lanes: 1, no chase period; 2, 5-min chase; 3, 30-min chase; 4, 12- to 14-h chase. (*b*) Wild-type (WT) permease and constructs deleted of an odd number of putative transmembrane helices. Lanes: 1, no chase; 2, 10-min chase; 3, 30-min chase; 4, 60-min chase; 5, 12- to 14-h chase. Positions of prestained molecular mass markers (Bio-Rad) are indicated.

Table 1. Alkaline phosphatase activity of C-terminal fusion constructs

Construct	Alkaline phosphatase activity, units
pCM402	104.1
pT7-5	0.8
lacY–phoA	2.1
N2C4–*phoA*	1.1
N2C6–*phoA*	0.9
N2C8–*phoA*	1.5
N2C8/2–*phoA*	4.9
N3C5–*phoA*	0.9
N4C4–*phoA*	3.3
N1C6–*phoA*	0.8
N2C5–*phoA*	1.9
N2C9–*phoA*	0.9

E. coli CC181 (*phoA*$^-$) harboring given *phoA* fusion constructs were grown overnight in LB medium supplemented with spectinomycin (20 μg/ml) and ampicillin (100 μg/ml). The cultures were diluted 1:100 in fresh medium and induced at midlogrithmic phase with 0.5 mM IPTG for 1 h. Cells were washed once in 1.0 M Tris·HCl (pH 8.0) and resuspended in the same buffer. Alkaline phosphatase assays were performed as described (38) with the following controls: (*i*) for low alkaline phosphatase activity, *E. coli* CC181/pT7-5 or CC181 carrying plasmid pT7-5 encoding an alkaline phosphatase fusion at the C terminus of *lac* permease was used; (*ii*) for high alkaline phosphatase activity, *E. coli* CC181 carrying plasmid pCM402 with alkaline phosphatase fused to a periplasmic domain of *lac* permease was used (cf. fusion 2 in ref. 24).

ber of putative transmembrane helices, activity is minimal (Table 1). Moreover, as shown by Western blots of membrane preparations from *E. coli* CC181 expressing the given fusions, each chimera is present in the membrane, albeit to various extents, and migrates with the expected molecular mass (Fig. 4). The stability of the chimeras was not tested, however, because previous results (24) show that there is no correlation between the activity and the stability of LacY–PhoA fusion proteins. The results thus indicate that the C termini of the constructs, including those designed to have the C terminus in opposite orientation to the N terminus, remain on the cytoplasmic surface of the membrane.

Transport Activity. Since each of the deletions leads to a stable product in the membrane, *E. coli* T184 (*lacZ*$^-$*Y*$^-$) was transformed with plasmid pT7-5 containing *lacY* genes encoding each construct, and transport of [1-^{14}C]lactose was studied. Although data are not shown, none of the mutants catalyzes active lactose transport. On the other hand, *E. coli* HB101 (*lacZ*$^+$*Y*$^-$) harboring the plasmids exhibits variable

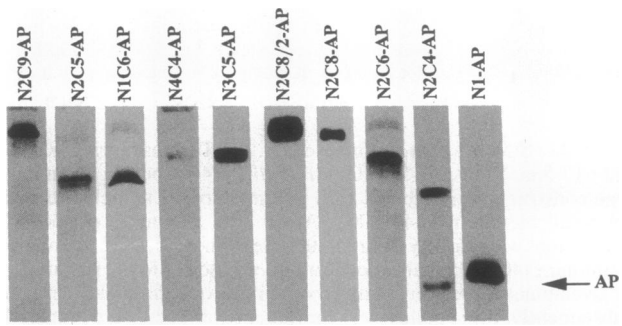

FIG. 4. Western blots of *lacY–phoA* fusion constructs expressed by *E. coli* CC181 harboring pT7-5(NxCy-phoA) plasmids. Membranes prepared from induced cultures were subjected to NaDodSO$_4$/polyacrylamide gel electrophoresis and electroblotted, and the nitrocellulose blot was incubated with anti-alkaline phosphatase antibodies. After incubation with horseradish peroxidase-protein A and a short incubation with the fluorescent substrate, the nitrocellulose blot was exposed to film for 30 min. AP, alkaline phosphatase.

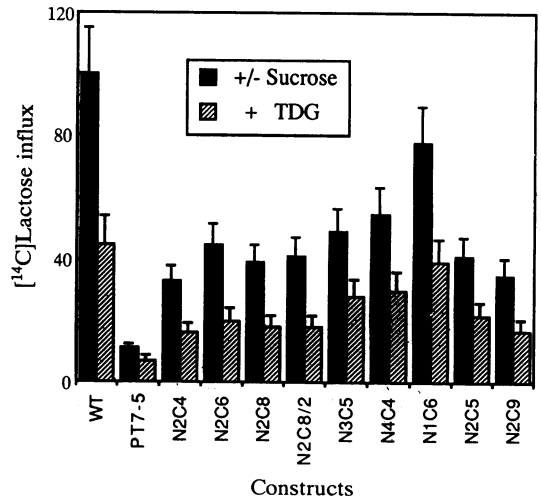

FIG. 5. Downhill lactose translocation by *E. coli* HB101, LJ915, or AZ-1 harboring pT7-5(lacY) or pT7-5(NxCy). The results represent the average of 16 measurements with *E. coli* HB101, LJ915, or AZ-1 transformed with each construct and are expressed as percentage of influx in wild-type cells. No significant differences were observed in the presence or absence of sucrose or between the various host strains.

intermediate phenotypes (from pink to red colonies) when grown on MacConkey agar containing 25 mM lactose, suggesting that the deletions may retain partial activity. To examine this possibility, "downhill" transport assays were performed with *E. coli* HB101, LJ915 (*lacZ*$^+$*Y*$^-$,*ptsEI*$^-$), or AZ-1 (*lacZ*$^+$Δ*Y*) with the following considerations taken into account. (*i*) Since *E. coli* HB101 harboring pT7-5(cassette *lacY*) catalyzes active lactose transport, the cells were "uncoupled" by addition of 20 μM CCCP. (*ii*) Cells overexpressing hydrophobic membrane proteins such as *lac* permease are fragile and might release cytosolic β-galactosidase into the medium during the transport assays. Since the enzyme has a high turnover number, release of even small amounts of enzyme would result in significant hydrolysis of [1-^{14}C]lactose in the external medium, generating [^{14}C]glucose, which could then be taken up by the phospho*enol*pyruvate phosphotransferase system. Thus, *E. coli* LJ915 (*lacZ*$^+$*Y*$^-$, *ptsEI*$^-$) was also used, as this strain is defective in enzyme I of the phosphoenolpyruvate phosphotransferase system and unable to transport glucose. In any event, since β-galactosidase is expressed in these strains, any [1-^{14}C]lactose that enters is rapidly cleaved, thereby providing a "sink" that drives the accumulation of radioactivity. The average results from 16 assays with each mutant are presented in Fig. 5. Since lactose is rapidly hydrolyzed upon entering the cell, a steady-state level of accumulation is not achieved. Therefore, initial rates of ^{14}C uptake from [1-^{14}C]lactose were determined and normalized to cells expressing wild-type permease. Strikingly, many deletion mutants exhibit significant activity relative to cells transformed with vector containing no insert and N1C6 permease, for example, exhibits ≈80% of wild-type activity. Importantly, moreover, in each instance, uptake is unaffected by the presence of excess sucrose, a poor substrate for the permease, but inhibited ≈50% by TDG, a high-affinity substrate.

DISCUSSION

The results presented in this communication are consistent with the conclusion that although the N-terminal hydrophobic domain of *lac* permease may be required for insertion into the membrane (25, 26) and is oriented toward the inner surface, it does not determine the topology of the C-terminal

domain. Thus, permease mutants containing intact N and C termini but deleted of even or odd numbers of intervening putative transmembrane helices are inserted into the membrane in a stable fashion with their C termini in the same orientation as native *lac* permease, as judged by the alkaline phosphatase activity of C-terminal LacY–PhoA fusions. Importantly, similar findings have been reported recently with *malF* (M. Ehrman, K. McGovern, and J. Beckwith, personal communication) and *secE* (P. J. Schatz, K. L. Bieker, K. M. Ottemann, T. J. Silhavy, and J. Beckwith, personal communication). Moreover, it has been shown that there are multiple sequences in *lac* permease that are independently able to support translocation of alkaline phosphatase (C. Manoil, personal communication). As a whole, the results are inconsistent with the hypothesis that the overall topology of polytopic membrane proteins is determined by the orientation of the first transmembrane helical domain followed by passive serpentine insertion of the subsequent helices.

Although the constructs with an even number of residual putative transmembrane domains do not present a problem with respect to overall topology, it is apparent that a relatively gross alteration in the topology of the odd-numbered deletion constructs must occur for the C termini to remain on the cytoplasmic surface of the membrane. Two possibilities, among others, are (*i*) one of the transmembrane domains is not inserted into the membrane and remains on either the cytoplasmic or periplasmic surface of the membrane or (*ii*) two fused hydrophilic loops of sufficient length traverse the membrane. In any case, it may be possible to resolve this aspect of the problem by constructing *phoA* fusions with *lacY* genes encoding the odd-numbered deletion constructs.

Finally, the observation that the deletion mutants translocate lactose downhill to various degrees suggests that many transmembrane domains can be removed without drastically altering facilitated influx. More specifically, from the data obtained with N1C6 (cf. Fig. 5), it appears that the pathway is contained largely within the last six putative transmembrane domains.

We thank Colin Manoil for providing *E. coli* CC181, plasmids encoding a *lacY–phoA* fusions, and for advice and discussion regarding construction of the *lac* permease–alkaline phosphatase fusions described. We are also grateful to Jonathan Beckwith for providing manuscripts prior to publication and to Milton Saier for providing *E. coli* LJ915.

1. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157**, 105–132.
2. Eisenberg, D., Schwarz, E., Komaromy, M. & Wall, R. (1984) *J. Mol. Biol.* **179**, 125–142.
3. Hartmann, E., Rapoport, T. A. & Lodish, H. F. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5786–5790.
4. Wickner, W. T. & Lodish, H. F. (1985) *Science* **230**, 400–407.
5. Rapoport, T. A. (1986) *CRC Crit. Rev. Biochem.* **20**, 73–137.
6. Singer, S. J., Maher, P. A. & Yaffe, M. P. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1960–1964.
7. Blobel, G. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1496–1500.
8. Popot, J. L. & Engelman, D. M. (1990) in *Protein Form and Function*, eds. Bradshaw, R. A. & Purton, M. (Elsevier, Cambridge, U.K.), p. 147.
9. Popot, J. L. & de Vitry, C. (1990) *Annu. Rev. Biophys. Biophys. Chem.* **19**, 369–403.
10. Kaback, H. R. (1990) in *The Bacteriai, Vol. XII*, ed. Krulwich, T. A. (Academic, New York), p. 151.
11. Büchel, D. E., Groneborn, B. & Müller-Hill, B. (1980) *Nature (London)* **283**, 541–545.
12. Foster, D. L., Boublik, M. & Kaback, H. R. (1983) *J. Biol. Chem.* **258**, 31–34.
13. Vogel, H., Wright, J. K. & Jähnig, F. (1985) *EMBO J.* **4**, 3625–3631.
14. Page, M. G. P. & Rosenbusch, J. P. (1988) *J. Biol. Chem.* **263**, 15906–15914.
15. Goldkorn, T., Rimon, G. & Kaback, H. R. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3322–3326.
16. Stochaj, V., Bieseler, B. & Ehring, R. (1986) *Eur. J. Biochem.* **158**, 423–428.
17. Carrasco, N., Tahara, S. M., Patel, L., Goldkorn, T. & Kaback, H. R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6894–6898.
18. Seckler, R., Wright, J. K. & Overath, P. (1983) *J. Biol. Chem.* **258**, 10817–10820.
19. Seckler, R. & Wright, J. K. (1984) *Eur. J. Biochem.* **142**, 269–279.
20. Carrasco, N., Viitanen, P., Herzlinger, D. & Kaback, H. R. (1984) *Biochemistry* **23**, 3681–3687.
21. Herzlinger, D., Viitanen, P., Carrasco, N. & Kaback, H. R. (1984) *Biochemistry* **23**, 3688–3693.
22. Carrasco, N., Herzlinger, D., Mitchell, R., DeChiara, S., Danho, W., Gabriel, T. F. & Kaback, H. R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4672–4676.
23. Seckler, R., Möröy, T., Wright, J. K. & Overath, P. (1986) *Biochemistry* **25**, 2403–2409.
24. Calamia, J. & Manoil, C. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4937–4941.
25. Stochaj, U. & Ehring, R. (1987) *Eur. J. Biochem.* **163**, 653–658.
26. Stochaj, U., Fritz, H.-J., Heibach, C., Markgraf, M., Schaewen, A. V., Sonnewald, U. & Ehring, R. (1988) *J. Bacteriol.* **170**, 2639–2645.
27. Von Heijne, G. & Blomberg, C. (1979) *Eur. J. Biochem.* **97**, 175–181.
28. Engleman, D. M. & Steitz, T. A. (1981) *Cell* **23**, 411–422.
29. Roepe, P. D., Zbar, R., Sarkar, H. K. & Kaback, H. R. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3992–3996.
30. McKenna, E., Hardy, D., Pastore, J. C. & Kaback, H. R. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 2969–2973.
31. Kaback, H. R., Bibi, E. & Roepe, P. D. (1990) *Trends Biochem. Sci.* **15**, 309–314.
32. Boyer, H. W. & Roulland-Dussoix, D. (1969) *J. Mol. Biol.* **41**, 459–472.
33. Teather, R. M., Bramhall, J., Riede, I., Wright, J. K., Fürst, M., Aichele, G., Wilhelm, V. & Overath, P. (1980) *Eur. J. Biochem.* **108**, 223–231.
34. Tabor, S. & Richardson, C. C. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1074–1078.
35. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5468.
36. Sanger, F. & Coulson, A. R. (1978) *FEBS Lett.* **87**, 107–110.
37. Herzlinger, D., Carrasco, N. & Kaback, H. R. (1985) *Biochemistry* **24**, 221–229.
38. Long, S., McCune, S. & Walker, G. C. (1988) *J. Bacteriol.* **170**, 4257–4265.
39. Trumble, W. R., Viitanen, P. V., Sarkar, H. K., Poonian, M. S. & Kaback, H. R. (1984) *Biochem. Biophys. Res. Commun.* **119**, 860–867.