JOURNAL OF BACTERIOLOGY, May 1995, p. 2315–2320
0021-9193/95/$04.00+0

Vol. 177, No. 9

Sequences Determining the Cytoplasmic Localization of a Chemoreceptor Domain

LENNY SELIGMAN,† JEANNIE BAILEY, AND COLIN MANOIL*

Department of Genetics, University of Washington, Seattle, Washington 98195

Received 14 November 1994/Accepted 24 February 1995

The *Escherichia coli* serine chemoreceptor (Tsr) is a protein with a simple topology consisting of two membrane-spanning sequences (TM1 and TM2) separating a large periplasmic domain from N-terminal and C-terminal cytoplasmic regions. We analyzed the contributions of several sequence elements to the cytoplasmic localization of the C-terminal domain by using chemoreceptor-alkaline phosphatase gene fusions. The principal findings were as follows. (i) The cytoplasmic localization of the C-terminal domain depended on TM2 but was quite tolerant of mutations partially deleting or introducing charged residues into the sequence. (ii) The basal level of C-terminal domain export was significantly higher in proteins with the wild-type periplasmic domain than in derivatives with a shortened periplasmic domain, suggesting that the large size of the wild-type domain promotes partial membrane misinsertion. (iii) The membrane insertion of deletion derivatives with a single spanning segment (TM1 or TM2) could be controlled by either an adjacent positively charged sequence or an adjacent amphipathic sequence. The results provide evidence that the generation of the Tsr membrane topology is an overdetermined process directed by an interplay of sequences promoting and opposing establishment of the normal structure.

The assembly of an integral membrane protein requires the insertion of the protein into the lipid bilayer in the appropriate topology and the folding of the structure into an active globular form (2, 3, 33). The detailed characterization of sequences directing membrane insertion is an essential step in understanding this process. The best-characterized determinants of membrane protein topology are apolar sequences that span the membrane and short positively charged sequences which orient adjacent spanning sequences (5, 6, 36). The orientation of a spanning sequence can also be determined by the position of the sequence in a polypeptide chain relative to those of other spanning sequences (25, 37).

We have analyzed the process of membrane protein assembly by using the *Escherichia coli* serine chemoreceptor (Tsr) as a model. Tsr inserts into the membrane in a structure that spans the bilayer twice, generating a simple topology (Fig. 1, left) (4, 10, 12, 15, 23). Tsr is presumed to function as a dimer (28) and localizes to the poles of cells (21). A model for the three-dimensional structure of the Tsr periplasmic domain has been formulated on the basis of that of the *Salmonella typhimurium* aspartate receptor (13). Genetic studies have provided detailed models for the structural organization of chemoreceptor transmembrane segments (20, 31) and have identified numerous mutations altering chemoreceptor function (1, 11, 29, 38).

We have sought to identify and characterize the major sequence determinants of Tsr topology. Our analysis has relied on the properties of alkaline phosphatase (AP) gene fusions (24). The AP activity of a hybrid protein coded for by such a fusion is a sensitive indicator of the cellular disposition of the fusion site in a membrane protein. For example, fusions to the periplasmic domain of Tsr have high AP activity, whereas fusions to the C-terminal cytoplasmic domain have low activity (Fig. 1, right) (12, 23). In earlier studies, we determined the minimum hydrophobicity requirements for TM1 to promote export (18), showed that the positive charge of the Tsr N-terminal cytoplasmic segment is important for periplasmic domain localization (14), and showed that TM2 and an amphipathic sequence (AS) adjacent to TM2 were necessary for efficient C-terminal domain localization to the cytoplasm (34). In this report, we present an analysis of the detailed sequence requirements for TM2 function, of the role of the large periplasmic domain in Tsr insertion, and of the ability of the AS to control the membrane orientation of an adjacent spanning sequence.

MATERIALS AND METHODS

Bacteria and plasmids. The following *E. coli* K-12 strains were used in this study: LS14 [Δ(*ara leu*)*7697* Δ(*lac*)X74 Δ*phoA Pvu*II Δ(*phoB phoR*) *galE galK thi rpsE rpoB argE*(Am) *recA1*], LS16 (LS14 *phoB*$^+$ *phoR*$^+$), DSM3 [*mutS215*::Tn*10* Δ(*ara leu*)*7697* Δ(*lac*)X74 *phoA20 galE galK thi rpsE rpoB argE*(Am) Δ(*recA*)::*cat*], DB6438 (Δ*lac rpoB argE metB mutT198*), CC701 [Δ(*ara leu*)*7697* Δ(*lac*)X74 Δ*phoA Pvu*II Δ*malF3 phoR galE galK thi rpsL pcnB zad*::Tn*10*/F′ *lacI lacZ*Δm 15], CC703 (CC701 *phoA*$^+$), and SM529 [Δ(*ara leu*)*7697* Δ(*lac*)X74 *phoR galE galK thi rpsL*].

The following plasmids have been described previously: pLS100a, pLS200, pLS210, pLS211, and pCM203 (23, 34). Plasmids pLS230-pLS236, pLS270-pCM273, and pCM615-pCM624 are derivatives of pLS200 which were created by oligonucleotide-directed mutagenesis. Plasmid pLS237a is a derivative of pLS234 that shows reduced toxicity. pLS237a was isolated after mutagenesis of pLS234 by growth in DB6438, followed by transformation of LS16 and screening for large, blue transformant colonies on L agar containing kanamycin and BCIP (5-bromo-4-chloro-3-indolylphosphate toluidinium salt).

Media and cell growth. Growth media have been described (27). Medium supplements were used at the following concentrations: glycerol (0.2%), amino acids (0.04%), thiamine (1 μg/ml), kanamycin (30 μg/ml in rich media and 15 μg/ml in minimal media), and BCIP (40 μg/ml).

DNA manipulations. Standard DNA preparations and manipulations were used (22). DNA was sequenced by the dideoxynucleotide termination method with a modified phage T7 DNA polymerase with double-stranded plasmid DNA templates and appropriate oligonucleotide primers (35).

Oligonucleotide-directed mutagenesis. Mutations were generated by annealing single-stranded template DNA with phosphorylated oligonucleotides at a molar ratio of 1:25 and then incubating them for 90 min at 37°C in the presence of deoxyribonucleotides, ATP, T4 DNA polymerase, T4 DNA ligase, and in some cases, T4 gene 32 protein (17, 34). The resulting mixtures were transformed into DSM3 with selection for growth on tryptone-yeast extract agar containing kanamycin and BCIP. Plasmid DNA was isolated from transformant colonies showing a range of blue color intensities, and the DNA was analyzed by restric-

* Corresponding author.
† Present address: Department of Pathology, University of Washington, Seattle, WA 98195.

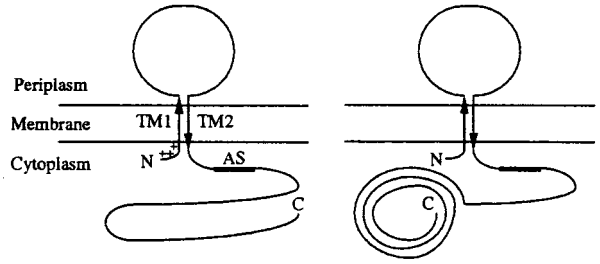

FIG. 1. The serine chemoreceptor and a serine chemoreceptor-AP hybrid protein. (Left) The serine chemoreceptor (Tsr). Membrane-spanning segments are indicated by arrows, with each arrowhead pointing toward the C terminus of the protein. The first membrane-spanning segment (TM1) consists of amino acid residues 7 through 30, the second membrane-spanning segment (TM2) consists of residues 192 through 214, and the AS consists of residues 222 through 234. (Right) A C-terminal Tsr-PhoA hybrid protein. AP is represented as a spiral. Cells producing the hybrid protein express low AP activity because of the cytoplasmic location of the AP moiety.

tion mapping and sequencing to identify appropriate mutants. The following oligonucleotides were used in this study: TM2Δ13 (AGGCGATGTGGATTCTG GTCTGGTTCGGTATTAAAG), TM2Δ9 (CGATGTGGATTCTGGTGGGCT TCGCCGTCTGGTTCGGTA), TM2Δ5 (GGATTCTGGTGGGCGTGATGG TCATCTTCGCCGTCTGGT), TM2 substitution (GGCGTGATG[C/G][G/A] C[C/G][G/A]CGTACTGGC), Δperiplasm (GGCGGTCTGTTCTTTAATGCC TTGAATTCCTACAGCCAGGCGATGTGG), Δ(TM1-periplasm) (GAAACC ATGTTAAAACGTATCTCCTACAGCCAGGCGATG), Δ(periplasm-TM2) (CC TTTTACAACTGACATCAGGCGCCTCGCTGGTAGCGCCA), NN (GAAA CCATGTTAAACAATATTTCCTACAGCCAG), and NNN (AGAAACCATG TTAAACAATATTAATATTGTGACCAGC).

AP assays. The AP activity of permeabilized cells was determined as described previously (26), except that iodoacetamide (1 mM) was present in wash and assay buffers (9). Prior to the assay, cultures were grown to early exponential phase (optical densities at 600 nm of 0.15 to 0.25) at 30°C in M63 minimal medium supplemented with 0.2% glycerol, 40 μg of amino acids per ml (all of the naturally occurring amino acids except methionine), and 15 μg of kanamycin per ml. TM2 mutants (Table 1) were assayed in a *pcnB* strain (CC701); other mutants (Table 2) were assayed in a *pcnB*$^+$ strain (LS14).

Hybrid protein synthesis. Rates of hybrid protein synthesis were measured after a 2-min exposure of the cells to [^{35}S]methionine by precipitation of the antibody to AP (18) or after whole-cell protein electrophoresis (34). Plasmids encoding toxic proteins (pCM203, pLS270, pLS230, pLS234, pLS236, and pLS237a) were assayed at low copy number in CC703, whereas remaining mutants were assayed at high copy number in SM529 or LS14.

TABLE 1. Tsr TM2 mutant derivatives

Plasmid	Mutation(s)[a]	AP activity[b] (U/OD$_{600}$)	Relative protein level[c]	Maximal activity (%)[d]
pLS100a	TM2Δ17	147 ± 20	(1.0)	(100)
pLS200	None	0.9 ± 0.2	1.6	0.4
pCM203[e]	None	135 ± 12	1.1	81
pLS270	TM2Δ13	65.0 ± 4.9	1.0	44
pLS271	TM2Δ9	4.6 ± 0.3	1.7	1.9
pLS272	TM2Δ5	1.5 ± 0.1	1.4	0.7
pLS230	I-201→D and V-202→D	5.3 ± 0.3	1.2	3.0
pLS231	I-201→D and V-202→G	1.9 ± 0.2	1.9	0.7
pLS232	I-201→G and V-202→G	1.8 ± 0.5	2.0	0.6
pLS233	I-201→H and V-202→G	2.6 ± 1.4	2.4	0.7
pLS234	I-201→R and V-202→G	1.3 ± 0.1	0.8	1.0
pLS235	I-201→G and V-202→D	3.2 ± 0.3	1.8	1.2
pLS236	I-201→R and V-202→D	6.9 ± 0.7	1.5	3.1
pLS237a	I-201→R, V-202→G, and K-215→T	15.5 ± 0.9	1.7	6.1

[a] AP is fused after residue 402 of the Tsr C-terminal cytoplasmic domain in all cases except in the protein coded for by pCM203, in which AP is fused after residue 164 of the periplasmic domain. Abbreviations: ΔTM2Δ17, deletion of second transmembrane segment (Δ[A-181–F-208]); TM2Δ13, Δ(V-197–A-209); TM2Δ9, Δ(V-199–I-207); TM2Δ5, Δ(I-201–A-205).

[b] Means ± standard deviations (3 to 7 assay values per strain). Assays were performed with plasmids at low copy number in CC701.

[c] Hybrid protein recovery relative to that of cells expressing pLS100a (see Materials and Methods).

[d] AP activity divided by that of pLS100a and the relative protein level.

[e] Periplasmic domain fusion junction.

RESULTS

Mutations altering TM2. In a previous study of Tsr mutants which exhibited C-terminal domain export, we identified mutations deleting most or all of TM2 (34). The smallest deletion shortened TM2 from 23 residues to 7 apolar residues (6 from TM2 and 1 from the periplasmic domain) and caused essentially complete C-terminal domain export in Tsr-PhoA hybrid proteins (Table 1; compare pLS100a and pCM203) (34). To examine the effect of less extreme changes, a series of mutant derivatives with progressively shorter TM2 sequences was constructed by site-directed mutagenesis of a C-terminal domain Tsr-PhoA gene fusion (Fig. 2) (see Materials and Methods). The AP activities of the derivatives were assayed as a measure of C-terminal domain export (34). Since several of the mutants were toxic when expressed from plasmids at high copy number (see below), AP activities were assayed with plasmids at low copy number in *pcnB* strains (see Materials and Methods). The AP activities were normalized for the rates of hybrid protein synthesis (see Materials and Methods). We found that although mutations shortening the 23-residue sequence to 14 or 18 residues caused little increase in AP activity (Table 1; pLS271 and pLS272), a mutation leaving 10 residues caused relatively efficient export of the AP moiety (Table 1; pLS270). These results imply that only about half of the TM2 sequence is required for stable cytoplasmic localization of the C-terminal domain.

As an additional test of the ability of TM2 to tolerate dramatic mutational change, we introduced charged residues into the center of TM2. Since previous genetic screens for mutants showing export of the C-terminal domain had failed to yield single-amino-acid substitutions within TM2 (34), we used a mutagenic strategy that could lead to the introduction of one or two (adjacent) charged residues. A degenerate oligonucleotide mixture capable of changing TM2 residues I-201 and V-202 (marked with asterisks in Fig. 2) to an arginine, aspartate, histidine, or glycine residue was used for site-directed mutagenesis (see Materials and Methods). Out of a possible 16 double-substitution mutations, 7 were identified. None of the seven mutations resulted in a maximal AP activity of greater than 3.1% (Table 1; pLS230 through pLS236), including changes introducing pairs of charged residues (I-201→D–V-202→D and I-201→R–V-202→D). Thus, as for the TM2 deletion mutants, the behavior of substitution mutants implies that the topology-determining function of TM2 is tolerant of changes dramatically decreasing its hydrophobic character.

Toxicity of TM2 mutations. Several of the mutant TM2 hybrid proteins (encoded by pLS270, pLS230, pLS234, and pLS236) were toxic to cells when expressed from a plasmid at high copy number. This toxicity was evident in the small colony size (compare Fig. 3A and B) and the presence of white papillae in colonies grown for extended periods on BCIP indicator plates. In all cases, such toxicity was alleviated when plasmid copy number was decreased in a *pcnB* host (not shown) (19).

To investigate the nature of the mutant TM2 toxicity, plasmids carrying second mutations reducing the toxicity of the I-201→R–V-202→G mutant (encoded by pLS234) were isolated (Materials and Methods). Two mutations were identified (Fig. 3C and D): one deleted TM2 (with the same change, Δ[A-181–F-208], found in pLS100a) and gave strong suppres-

TABLE 2. Tsr deletion derivatives

Plasmid	Mutation(s)[a]	AP activity[b] (U/OD_{600})	Relative protein level[c]	Relative activity[d]	Maximal activity (%)[e]
pLS210	None	23.5 ± 6.7	(1.0)	(1.0)	1.0
pLS211	ΔAS	395 ± 13	1.0	17	17
pCM615	ΔP	5.9 ± 1.6	1.2	0.2	0.2
pCM616	ΔP and ΔAS	44 ± 15	1.1	1.7	1.7
pCM617	ΔP and ΔTM1	475 ± 46	0.2	119	117
pCM618	ΔP and ΔTM2	3,755 ± 520	1.7	94	92
pCM619	ΔP, ΔTM1, and ΔAS	570 ± 22	0.3	81	79
pCM620	ΔP, ΔTM2, and ΔAS	3,110 ± 474	2.4	55	54
pCM621	ΔP, ΔTM1, and NN	116 ± 11	0.3	17	17
pCM622	ΔP, ΔTM2, and NNN	637 ± 68	1.4	19	19
pCM623	ΔP, ΔTM1, ΔAS, and NN	425 ± 36	0.3	53	52
pCM624	ΔP, ΔTM2, ΔAS, and NNN	2,327 ± 109	2.1	47	46

[a] AP is fused after residue 240 of the Tsr C-terminal cytoplasmic domain. Abbreviations: ΔP, deletion of periplasmic region (Δ[N-35–A-187]); ΔAS, deletion of amphipathic segment (Δ[M-222–G-234]); ΔTM1, deletion of first transmembrane segment (Δ[K-6–A-187]); ΔTM2, deletion of second transmembrane segment (Δ[G-27–K-215]); NNN, substitutions K-3→N, R-4→N, and K-6→N; NN, substitutions K-3→N and R-4→N (in a derivative deleted of K-6).
[b] Means ± standard deviations (4 to 16 assay values per strain). Assays were performed with plasmids at high copy number in LS14.
[c] Hybrid protein recovery relative to that of cells expressing pLS210. Values represent an average of at least two determinations per strain.
[d] AP activity divided by relative protein level and the AP activity of the parent (pLS210).
[e] Relative activity divided by the relative activity of pLS100a, determined to be 102 units relative to that of pLS210 under the same assay conditions (34).

sion, and another (encoded by pLS237a) neutralized lysine 215 (K-215→T, marked with an asterisk in Fig. 2) and gave partial suppression. The K-215→T mutation lengthened the uncharged sequence containing the C-terminal end of TM2 from 13 to 22 residues. Both suppressor mutations increased AP activity (Table 1; compare pLS234 with pLS100a and pLS237a).

Periplasmic domain deletion derivatives. Tsr contains a large periplasmic domain between TM1 and TM2 (Fig. 1, left). Different models for membrane protein insertion predict differences in the influence of such large domains on membrane protein topology (see Discussion). To determine whether the large size of the periplasmic domain influences C-terminal domain localization, a Tsr-PhoA derivative with a deletion shortening the domain from 161 to 8 residues (Δ[N-35–A-187]) was constructed (Fig. 4A). The mutation decreased AP activity about fivefold (Table 2; compare pCM615 with pLS210). A mutant with the shortened periplasmic domain that also lacked the AS showed a 10-fold-lower AP activity than the mutant lacking the AS alone (Table 2; compare pCM616 with pLS211). These results indicate that shortening the periplasmic domain increases the fidelity of C-terminal domain localization to the cytoplasm.

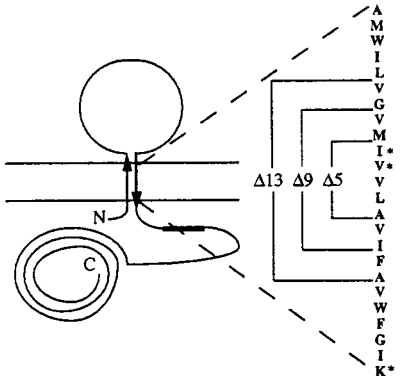

FIG. 2. Mutations altering Tsr TM2. Residues constituting wild-type TM2 (A-192–I-214) and the lysine residue immediately C terminal to TM2 (K-215) are shown. The endpoints of TM2 deletions are indicated, and residues altered by substitutions are marked with asterisks.

Competition between topology determinants. The positive N-terminal segment and the AS of Tsr each favor cytoplasmic disposition of the end of the spanning sequence (TM1 or TM2) adjacent to it (14, 34). We generated deletion mutants containing a single transmembrane sequence (TM1 or TM2) with the positive segment at its N-terminal end and the AS at its C-terminal end. The deletions create a competition between the two sequences for influence over the orientation of the spanning sequence. For both deletion derivatives, we observed essentially complete export of the C-terminal domain (Table 2; pCM617 and pCM618), and the additional deletion of the AS

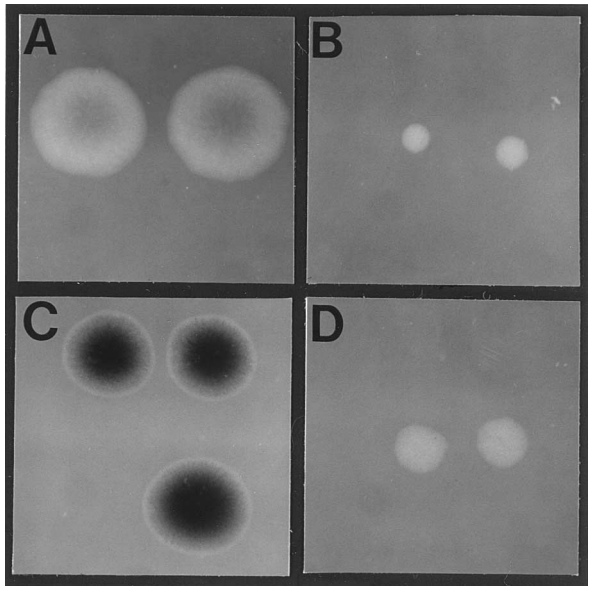

FIG. 3. Toxicity of TM2 mutants. LS14 colonies expressing mutant Tsr-PhoA hybrids with a cytoplasmic domain fusion junction (residue 402) are shown. (A) Wild-type TM2 (pLS200). (B) TM2 I-201→R–V-202→G (pLS234). (C) TM2 Δ(A-181–F-208) (pLS100a). (D) TM2 I-201→R–V-202→G–K-215→T (pLS237a). The photograph shows colonies grown on the same agar petri dish. Colonies were grown at 30°C for 75 h on morpholine/propane/sulfonic acid (MOPS)-glycerol medium (30) containing 1 mM P_i, all the common amino acids except methionine, thiamine, kanamycin, and BCIP.

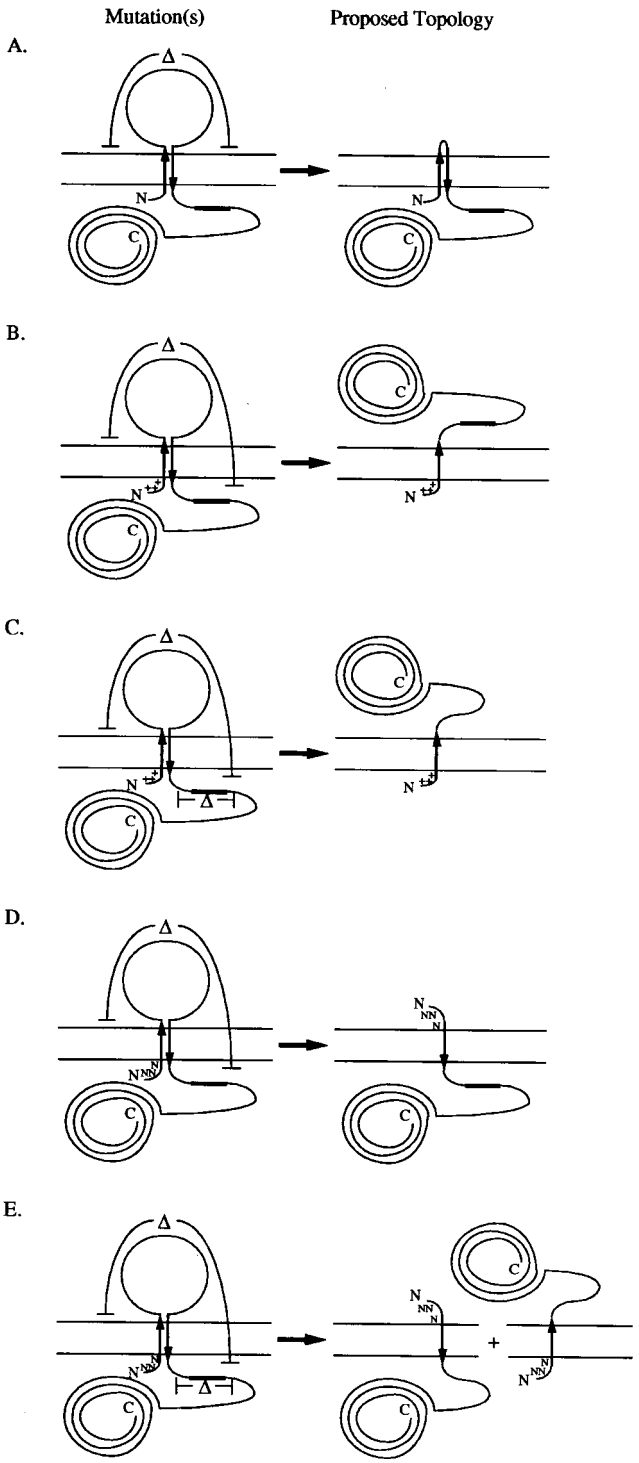

FIG. 4. Topology of the Tsr-PhoA deletion derivatives. (Left) Tsr-PhoA hybrids indicating the changes in Tsr. (Right) The proposed topologies of the resulting derivatives. (A) ΔP. (B) ΔP ΔTM2. (C) ΔP ΔTM2 ΔAS. (D) ΔP ΔTM2 NNN. (E) ΔP ΔTM2 ΔAS NNN.

from both constructs had no significant effect (Fig. 4B and C) (Table 2; pCM619 and pCM620). These results indicate that the positive N-terminal segment was dominant over the AS in forcing the intervening spanning sequence (TM1 or TM2) to orient with its N terminus facing the cytoplasm (Fig. 4B and C, right).

If the positive character of the N-terminal segment forces an N-cytoplasmic orientation on the spanning sequence in these derivatives, then mutations neutralizing the positive residues should reduce C-terminal domain export. To test this prediction, we converted the positive residues of the N-terminal segment into asparagine residues ("NN" or "NNN" in Table 2). In both cases, AP activity was reduced about fivefold (Table 2; pCM621 and pCM622), showing that the positive character of the N-terminal sequence was indeed essential for AP export (Fig. 4D). When the AS was additionally deleted from these derivatives, AP activity increased to about 50% maximal (Table 2; pCM623 and pCM624), suggesting a topologically mixed population (Fig. 4E). These results indicate that the AS forced an N_{out}-C_{in} orientation on the adjacent transmembrane sequence when the insertion function of the N-terminal region was weakened.

DISCUSSION

We have sought to analyze the major sequence elements directing the membrane insertion of a topologically simple protein, the serine chemoreceptor (Fig. 1, left). Earlier studies provided a detailed analysis of the roles of TM1 and the positive N-terminal region in periplasmic domain translocation (14, 18) and showed that TM2 and a novel AS were required for efficient C-terminal domain cytoplasmic localization (34). This report describes further studies of the sequences determining the cytoplasmic localization of the C-terminal domain. These studies assayed C-terminal domain localization by using C-terminal domain Tsr-PhoA hybrid proteins. The AP activity of such hybrid proteins is a sensitive measure of C-terminal domain export (34).

The earlier studies implied that the export function of TM1 is remarkably tolerant of mutation. For example, none of a variety of single charged residue substitutions in TM1 significantly disrupted its ability to promote periplasmic domain translocation (18). Is the function of TM2 in determining C-terminal domain cytoplasmic localization similarly tolerant of mutation? To address this question, we analyzed a number of mutations altering TM2. We found that although deletions removing 13 or more apolar residues of the 23-residue TM2 caused efficient C-terminal domain export in Tsr-PhoA hybrids, the removal of 5 or 9 residues caused little C-terminal domain export. The introduction of one or two charged residues near the center of TM2 was also without major effect. These findings imply that as few as 11 to 14 TM2 residues allow Tsr to insert into the membrane in its normal topology. These results are in accord with studies of the single-span membrane protein coded for by phage f1 gene III showing that newly synthesized deletion derivatives with shortened hydrophobic sequences (as few as 11 residues) associated correctly with the membrane (7, 8). The extra spanning sequence in Tsr by comparison with that in the gene III protein evidently does not greatly influence the hydrophobicity requirements for TM2 membrane association.

The earlier analysis of Tsr TM1 showed that only about half of the sequence was required for periplasmic domain export (18). Thus, even though TM1 and TM2 are oriented oppositely in the membrane, the hydrophobicity requirements for their topological functions are quite similar. This similarity suggests the existence of a common recognition step with an export machinery or the lipid bilayer in membrane insertion.

In the course of this analysis, we observed that several mutations reducing TM2 hydrophobicity caused the overproduced

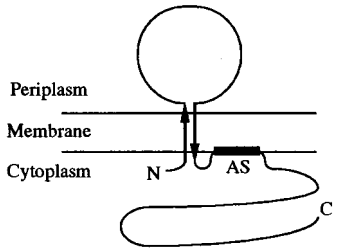

FIG. 5. Model for Tsr topology. The diagram shows the interaction of the AS with the membrane.

Tsr-PhoA hybrid to be quite toxic to cells. We have also observed that mutants with charged residues in TM1 sometimes display slow growth (18a). Second mutations reducing the toxicity of a mutation introducing an arginine residue into TM2 either deleted most of TM2 or neutralized a lysine residue immediately C terminal to TM2. These observations suggest that TM2 mutant toxicity may be due to the introduction of polar or charged residues into the bilayer, so that changes eliminating membrane interaction or lengthening the apolar sequence of TM2 alleviate the toxicity.

Does the large size of the periplasmic domain influence membrane insertion? One can imagine two ways in which the large size of the domain could affect insertion. If there existed a competition between TM1 and TM2 for insertion into the membrane in the outgoing (N_{in}-C_{out}) orientation, the longer it took for TM2 to be translated after TM1 had been made, the greater the opportunity for TM1 to interact with the membrane first and force the opposite orientation (N_{out}-C_{in}) on TM2. This model predicts that a shortening of the periplasmic domain would increase the probability of TM2 insertion prior to that of TM1, leading to increased C-terminal domain export. Alternatively, if premature folding of the normally periplasmic domain in the cytoplasm sometimes blocked export and forced an outgoing orientation on TM2, the large size of the periplasmic domain might favor TM2 misinsertion. This alternative predicts that a protein with a shortened periplasmic domain would exhibit decreased C-terminal domain export.

A deletion shortening the periplasmic domain from 161 to 8 residues caused about a 10-fold decrease in the basal level of export of the C-terminal AP of Tsr-PhoA hybrids. A deletion derivative lacking the AS showed a similar reduction in export relative to that of its parent. These results indicate that the large size of the Tsr periplasmic domain tends to promote membrane misinsertion and that the AS does not function specifically by counteracting this effect.

An earlier study had indicated that the Tsr AS might function by forcing the appropriate membrane orientation on TM2 (34). We tested this possibility by using a variety of Tsr deletion derivatives. The results indicate that the AS can influence the orientation of either TM1 or TM2. Since these and earlier results (34) imply that AS function does not specifically require TM1, TM2, or sequences C terminal to TM2, the AS may interact directly with the membrane (Fig. 5). Amphipathic helix interactions with lipid bilayers have been implied by several high-resolution membrane protein structures (16, 32).

The results of this and earlier studies of Tsr insertion (14, 18, 34) suggest that the generation of the protein's membrane topology is a process determined by a number of relatively autonomous sequence elements. The process is highly tolerant of mutational change, both because the transmembrane sequences are considerably more hydrophobic than is required for membrane insertion and because of the partial redundancy in the functions of the sequences controlling the orientations of the spanning sequences (14, 34). This picture of membrane protein insertion as an overdetermined process predicts that naturally occurring missense mutations that inactivate membrane proteins may do so only rarely by grossly altering the transmembrane topology.

ACKNOWLEDGMENTS

We thank Nick Kosuk for the isolation and sequencing of pLS237a and M. Lee for comments on the manuscript.

This work was supported by grants from the Public Health Service (GM46493) and the National Science Foundation (DMB-9205572). L.S. was a predoctoral fellow of the National Science Foundation.

REFERENCES

1. **Ames, P., and J. S. Parkinson.** 1988. Transmembrane signalling by bacterial chemoreceptors: *E. coli* transducers with locked signal output. Cell **55:**817–826.
2. **Blobel, G.** 1980. Intracellular protein topogenesis. Proc. Natl. Acad. Sci. USA **77:**1496–1500.
3. **Bormann, B., and D. Engelman.** 1992. Intramembrane helix-helix association in oligomerization and transmembrane signaling. Annu. Rev. Biophys. Biomol. Struct. **21:**223–242.
4. **Boyd, A., K. Kendall, and M. I. Simon.** 1983. Structure of the serine chemoreceptor in *Escherichia coli*. Nature (London) **301:**623–626.
5. **Boyd, D., and J. Beckwith.** 1990. The role of charged amino acids in the localization of secreted and membrane proteins. Cell **62:**1031–1033.
6. **Dalbey, R.** 1990. Positively charged residues are important determinants of membrane protein topology. Trends Biochem. Sci. **15:**253–257.
7. **Davis, N., and P. Model.** 1985. An artificial anchor domain: hydrophobicity suffices to stop transfer. Cell **41:**607–614.
8. **Davis, N. G., J. D. Boeke, and P. Model.** 1985. Fine structure of a membrane anchor domain. J. Mol. Biol. **181:**111–121.
9. **Derman, A. I., J. Puziss, P. Bassford, and J. Beckwith.** 1993. A signal sequence is not required for protein export in *prlA* mutants of *Escherichia coli*. EMBO J. **12:**879–888.
10. **Falke, J. J., A. Dernburg, D. Sternberg, N. Zalkin, D. Milligan, and D. E. Koshland.** 1988. Structure of a bacterial sensory receptor. J. Biol. Chem. **263:**14850–14858.
11. **Gardina, P., C. Conway, M. Kossman, and M. Manson.** 1992. Aspartate and maltose-binding protein interact with adjacent sites in the Tar chemotactic signal transducer of *Escherichia coli*. J. Bacteriol. **174:**1528–1536.
12. **Gebert, J., B. Overhoff, M. Manson, and W. Boos.** 1988. The Tsr chemosensory transducer of *Escherichia coli* assembles into the cytoplasmic membrane via a SecA-dependent process. J. Biol. Chem. **263:**16652–16660.
13. **Jeffery, C. J., and D. E. Koshland.** 1993. Three-dimensional structural model of the serine receptor ligand-binding domain. Protein Sci. **2:**559–566.
14. **Kimbrough, T. G., and C. Manoil.** 1994. Role of a small cytoplasmic domain in the establishment of serine chemoreceptor membrane topology. J. Bacteriol. **176:**7118–7120.
15. **Krikos, A., A. Krikos, M. Conley, A. Boyd, H. C. Berg, and M. I. Simon.** 1985. Chimeric chemosensory transducers of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82:**1326–1330.
16. **Kuhlbrandt, W., D. Wang, and Y. Fujiyoshi.** 1994. Atomic model of plant light-harvesting complex by electron crystallography. Nature (London) **367:**614–621.
17. **Kunkel, T. A., J. D. Roberts, and R. A. Zakour.** 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. Methods Enzymol. **154:**367–382.
18. **Lee, E., and C. Manoil.** 1994. Mutations eliminating the protein export function of a membrane-spanning sequence. J. Biol. Chem. **269:**28822–28828.

18a. **Lee, E., and C. Manoil.** Unpublished data.

19. **Lopilato, J., S. Bortner, and J. Beckwith.** 1986. Mutations in a new chromosomal gene of *Escherichia coli* K-12, *pcnB*, reduce plasmid copy number of pBR322 and its derivatives. Mol. Gen. Genet. **205:**285–290.
20. **Lynch, B. A., and D. E. Koshland.** 1991. Disulfide cross-linking studies of the transmembrane regions of the aspartate sensory receptor of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **88:**10402–10406.
21. **Maddock, J. R., and L. Shapiro.** 1993. Polar localization of the chemoreceptor complex in the *Escherichia coli* cell. Science **259:**1717–1723.
22. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Manoil, C., and J. Beckwith.** 1986. A genetic approach to analyzing membrane protein topology. Science **233:**1403–1408.
24. **Manoil, C., J. J. Mekalanos, and J. Beckwith.** 1990. Alkaline phosphatase fusions: sensors of subcellular location. J. Bacteriol. **172:**515–518.

25. **McGovern, K., M. Ehrmann, and J. Beckwith.** 1991. Decoding signals for membrane protein assembly using alkaline phosphatase fusions. EMBO J. **10:**2773–2782.
26. **Michaelis, S., H. Inouye, D. Oliver, and J. Beckwith.** 1983. Mutations that alter the signal sequence of alkaline phosphatase in *Escherichia coli*. J. Bacteriol. **154:**366–374.
27. **Miller, J. H.** 1992. A short course in bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
28. **Milligan, D., and D. E. Koshland.** 1988. Site-directed cross-linking: establishing the dimeric structure of the aspartate receptor of bacterial chemotaxis. J. Biol. Chem. **263:**6268–6275.
29. **Mutoh, N., K. Oosawa, and M. I. Simon.** 1986. Characterization of *Escherichia coli* chemotaxis receptor mutants with null phenotypes. J. Bacteriol. **167:**992–998.
30. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119:**736–747.
31. **Pakula, A. A., and M. I. Simon.** 1992. Determination of transmembrane protein structure by disulfide cross-linking: the *Escherichia coli* Tar receptor. Proc. Natl. Acad. Sci. USA **89:**4144–4148.
32. **Picot, D., P. Loll, and R. Garavito.** 1994. The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. Nature (London) **367:**243–249.
33. **Rees, D. C., H. Komiya, T. O. Yeates, J. P. Allen, and G. Feher.** 1989. The bacterial photosynthetic reaction center as a model for membrane proteins. Annu. Rev. Biochem. **58:**607–633.
34. **Seligman, L., and C. Manoil.** 1994. An amphipathic sequence determinant of membrane protein topology. J. Biol. Chem. **269:**19888–19896.
35. **Tabor, S., and C. Richardson.** 1987. DNA sequencing with a modified bacteriophage T7 DNA polymerase. Proc. Natl. Acad. Sci. USA **84:**4767–4771.
36. **von Heijne, G.** 1994. Membrane proteins: from sequence to structure. Annu. Rev. Biophys. Biomol. Struct. **23:**167–192.
37. **Wessels, H. P., and M. Speiss.** 1988. Insertion of a multispanning membrane protein occurs sequentially and requires only one signal sequence. Cell **55:**61–70.
38. **Yaghmai, R., and G. L. Hazelbauer.** 1992. Ligand occupancy mimicked by single residue substitutions in a receptor: transmembrane signaling induced by mutation. Proc. Natl. Acad. Sci. USA **89:**7890–7894.