An Analysis of Suppressor Mutations Suggests That the Two Halves of the Lactose Permease Function in a Symmetrical Manner*

(Received for publication, June 9, 1997, and in revised form, July 28, 1997)

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A conserved motif, GXXX(D/E)(R/K)XC[(X)(R/K)](R/K), is located in loop 2/3 and loop 8/9 in the lactose permease, and also in hundreds of evolutionarily related transporters. The importance of conserved residues in loop 8/9 was previously investigated (Pazdernik, N. J., Jessen-Marshall, A. E., and Brooker, R. J. (1997) J. Bacteriol. 179, 735–741). Although this loop was tolerant of many substitutions, a few mutations in the first position of the motif were shown to dramatically decrease lactose transport. In the current study, a mutant at the first position in the motif having very low lactose transport, Leu^280, was used as a parental strain to isolate second-site revertants that restore function. A total of 23 independent mutants that were sequenced and found to have a second amino acid substitution at several locations (G46C, G46S, F49L, A50T, L212Q, L216Q, S233P, C333G, F354C, G370C, G370S, and G370V). A kinetic analysis revealed that the first-site mutation, Leu^280, had a slightly better affinity for lactose compared with the wild-type strain, but its V_max for lactose transport was over 30-fold lower. The primary effect of the second-site mutations was to increase the V_max for lactose transport, in some cases, to levels that were near the wild-type value.

When comparing this study to second-site mutations obtained from loop 2/3 defective strains, a striking observation was made. Mutations in three regions of the protein, codons 45–50, 234–241, and 366–370, were able to restore functionality to both loop 2/3 and loop 8/9 defects. These results are discussed within the context of a C1/C2 alternating conformation model in which lactose translocation occurs by a conformational change at the interface between the two halves of the protein.

The lactose permease is a model protein for the study of sugar transport and cation/solute symport. The protein is encoded by the lacY gene of the lac operon of Escherichia coli. The gene has been cloned and sequenced revealing an open reading frame that encodes a protein of 417 amino acids (1, 2). The gene has been cloned and sequenced revealing an open reading frame that encodes a protein of 417 amino acids (1, 2). The lactose permease is a member of a very large superfamily of solute transporters (9–11). The superfamily members transport a variety of solutes including sugars, amino acids, Kreb's cycle intermediates, and antibiotics. In general, most members are predicted to contain 12 transmembrane segments with a large cytoplasmic loop between helices 6 and 7, although a few exceptions to this pattern have been noted (12). Among members of the superfamily, a general homology exists between the first and second halves of the proteins, consistent with the hypothesis that the superfamily arose by a gene duplication/fusion event of a primordial gene encoding a protein of 6 transmembrane segments (13). A model for the tertiary structure of the lactose permease has been proposed by examining the superfamily with regard to its pattern of structural features as well as specific data on particular transporters (12). The tertiary structure is predicted to have 8 transmembrane segments forming the channel lining regions and 4 segments that are embedded in the membrane to form scaffolding regions.

A striking feature of the superfamily is the identification of a conserved motif, GXXX(D/E)(R/K)XC[(X)(R/K)](R/K), located in hydrophilic loop 2/3 and repeated again at the analogous region in the second half of the protein in loop 8/9. Its conservation within such a large superfamily suggests that it may play a general role in protein structure or function. The importance of conserved residues in this motif has been extensively studied in the lactose permease and tetracycline antipporter (14–18). With regard to transport function, the residues at the first position in both loops, and the fifth position in loop 2/3 appear to be the most important. The other sites in the motif tend to be tolerant of amino acid substitutions to varying degrees. The basic residues may be important for structural stability, a feature that is consistent with the positive inside rule (19).

A useful approach to investigate the functional role of important amino acids within a protein is to isolate second-site revertants that restore activity to mutant strains having a first-site mutation that alters a critical amino acid. For example, previous suppressor mutations of the lactose permease identified potential salt-bridge interactions of TM-6/TM-7, TM-7/TM-11, TM-8/TM-10, and TM-9/TM-10 (20–23). More recently, this approach has been used by us, and by Yamaguchi and colleagues (24–26), to study the functional roles of mutations in the loop 2/3 motif. In the tetracycline antipporter, two second-site revertants have been characterized (26). One al-

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*This work was supported by Grant GM53259 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

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1 The abbreviation used is: TM, transmembrane.
**TABLE I**

**Bacterial strains and plasmids**

| Strain     | Relevant genotype (chromosome/F/plasmid) | Ref. |
|------------|------------------------------------------|-----|
| HS4006/FpI**^P** Y | Δ(lac-Pro)Δmal B 101/ | 36 |
|            | Lac^I^ lac O^- lac Z^- lac Y^- |     |
| T184       | lac I^- lac O^- lac Z^- lac Y^- lac Z^-lac Y^- | 37 |
| pTE18 (plasmid) | Δ(lac I^- lac O^- Δlac Z) | 37 |
| pLac184 (plasmid) | Δ(lac I^- lac O^- Δlac Z) | 38 |

**RESULTS**

Isolation of Second-site Suppressor Mutants—A conserved motif, GXXG/D/E(K/R)K(X/K/R)/K, is found within hydrophilic loop 2/3 and repeated again in loop 8/9 among members of the superfamily that includes the lactose permease (9–11). In loop 8/9, the first three or four residues within the motif are predicted to lie within the cytoplasmic end of transmembrane segment 8. A glycine is commonly found in the first position although the lactose permease and some other members of the superfamily have a proline at this position. In the lactose permease, this corresponds to Pro^280^.

Both glycine and proline are strong α-helix breakers, and this property has been previously suggested to be important at the first position in the motif (16). In a previous study with the lactose permease, bulky residues that are strong α-helical formers were inhibitory when substituted at this position. Less bulky residues such as cysteine, aspartate, glutamine, and asparagine were tolerated at this position as were bulky residues that are not strong α-helical formers such as tyrosine and phenylalanine. A Leu^280^ substitution showed downhill lactose transport that was less than 5% of the wild-type value even though the amount of permease protein in the membrane was normal (15). Due to its low level of lactose uptake, the uphill accumulation of the Leu^280^ strain was also lower than wild-type (i.e. 13%), but not completely defective. In the current study, this Leu^280^ strain was used as a parental strain to isolate suppressors (i.e. second-site revertants). These are termed suppressors because they suppress the inhibitory effects of the first-site mutation.

To identify suppressor mutations, the Leu^280^ strain was streaked on 1% melibiose MacConkey plates. Melibiose is an α-galactoside that is transported very well by the wild-type lactose permease but poorly by the Leu^280^ parental strain. Therefore, the Leu^280^ parent forms white colonies due to its low level of sugar transport. After a few days, however, suppressor mutations were identified as red flecks in the primary streak. These red flecks were restreaked to isolate individual red colonies and to confirm that the mutation suppressed the white phenotype of the parental strain. A total of 28 independent suppressors were saved, and then subjected to DNA sequencing across codon 280. Five of the 28 suppressors contained first-site reversions which altered the Leu^280^ to another residue. The remaining 23 suppressors retained a leucine residue at codon 280. These were saved for further study. It should be noted that melibiose MacConkey plates were used instead of lactose MacConkey plates because the Leu^280^ mutation had a pink phenotype on lactose MacConkey plates which made it more difficult to identify red revertants.

DNA Sequencing of the Second-site Suppressors—In addition to the Leu^280^ mutation, all of the 23 second-site suppressor strains had a single nucleotide substitution at various locations throughout the lacY gene. Table II describes the nature of these suppressor mutations.
mutations, and their predicted locations within the secondary topology of the lactose permease is shown in Fig. 1. Overall, the suppressors were loosely grouped into 3 regions: the periplasmic side of TM-2 and loop 1/2; TM-7 and loop 6/7; and the periplasmic side of TM-11 and loop 11/12 (Fig. 1, rectangles). Only one suppressor, pP280L/C333G, is not located in these three regions. It is found on the cytoplasmic side of TM-10.

Sugar Transport—To evaluate the effects of the second-site mutations, the wild-type, parent, and suppressor strains were initially screened for their ability to transport sugars based on their phenotype on MacConkey indicator plates. These plates contained lactose (α-β-galactoside), melibiose (α-galactoside), or maltose (α-glucoside) at three different concentrations: 0.2, 0.4, and 1.0%. A strain exhibiting good transport of these sugars will have a red phenotype. Strains having intermediate levels of transport will show a red center or pink phenotype, while a white phenotype indicates very low transport activity. As shown in Table III, the wild-type is red on lactose and melibiose plates while the Leu280 strain is white, except for a pink phenotype at high lactose concentrations. The suppressors have a red or red center phenotype on 1% melibiose MacConkey plates. This is expected since they were originally isolated on these types of plates. There were three suppressors with pink phenotypes at lower melibiose concentrations: pP280L/C333G, pP280L/L212Q, and pP280L/G46S. However, these three strains and all of the remaining suppressors had a red phenotype at all the lactose concentrations tested.

The phenotype of E. coli strains on maltose MacConkey plates can provide an indication of an alteration of sugar specificity since the lactose permease normally does not recognize this sugar very well. To carry out this analysis, the wild-type and mutant plasmids were transformed into a strain that is leu280 (i.e., the Leu280 strain) and substantially restore the ability to translocate lactose. The G370V suppressor, for example, raised the \( V_{\text{max}} \) to values that were similar to the Leu280 parent. However, as described later, the results can be explained by the differences in sugar concentrations between the two experiments. The lactose concentration in the plates is much higher (5.6 mM at 0.2% to 11.1 mM at 0.4%) than the transport assay (0.1 mM). Suppressors with higher \( K_m \) and higher \( V_{\text{max}} \) values may have relatively low rates of transport at low lactose concentrations but substantially higher rates of transport at saturating concentrations.

To obtain a better understanding of the effects of the suppressor mutations, a selected number of suppressor strains were subjected to a kinetic analysis. The apparent \( K_m \) and \( V_{\text{max}} \) values are described in Table IV. The parent mutation has a dramatically lower \( V_{\text{max}} \) and a slightly higher affinity compared with the wild-type (15). A general trend was observed among the suppressor strains. They tended to restore the kinetic parameters back to values that were similar to the wild-type strain; the more dramatic effect was an elevation of the \( V_{\text{max}} \) for lactose transport. The G370V suppressor, for example, raised the \( V_{\text{max}} \) to values that were similar to the wild-type strain. Several other suppressors (G46C, F49L, L216Q, S233P, and F354C) raised the \( V_{\text{max}} \) from 6- to 15-fold while the \( K_m \) values were only affected 3–5-fold. Two mutations that showed a low value for lactose transport in Fig. 2, pP280L/C333G and pP280L/S233P, have \( K_m \) values of 0.4 and 1.3, respectively. The decreased affinity for lactose is coupled with an enhanced \( V_{\text{max}} \) and explains why these strains showed low [14C]lactose transport at 0.1 mM but were red on MacConkey plates that have saturating lactose concentrations.

To gain insight into the kinetic effects of the suppressor mutations, two of them, A50T and G370V, were separated from the P280L parent mutation by restriction digestion. As shown at the bottom of Table IV, both of the single suppressor mutations had \( V_{\text{max}} \) values that were substantially lower than the corresponding double mutant strain with a P280L mutation (66.2 versus 15.2 and 199.9 versus 50.7). These results suggest the first-site mutation alone and the suppressor mutations alone tend to perturb the structure of the permease in a way that inhibits conformational changes associated with lactose transport. However, when they are coupled together, the two mutations compensate for each other’s structural perturbations and substantially restore the ability to translocate lactose.

**Table II: Codon changes and location of suppressor mutants**

| Strain   | Number of isolates | Second-site codon change | Location in secondary structure |
|----------|--------------------|--------------------------|---------------------------------|
| pLAC184  |                    |                          |                                 |
| pP280L   |                    |                          |                                 |
| pP280L/G46C | 3               | GGT to TGT               | Periplasmic loop 1/2            |
| pP280L/G46S | 5               | GGT to AGT               | Periplasmic loop 1/2            |
| pP280L/F49L | 3               | TTT to TTG               | Periplasmic side of TM-2        |
| pP280L/A50T | 1               | GCC to ACC               | Periplasmic side of TM-2        |
| pP280L/L212Q | 2              | CTG to CAG               | Cytoplasmic loop 6–7            |
| pP280L/L216Q | 1              | CTG to CAG               | Cytoplasmic loop 6–7            |
| pP280L/L233P | 2              | TCC to CCC               | Middle of TM-7                  |
| pP280L/L233Q | 1              | TGC to GCC               | Cytoplasmic side of TM-10       |
| pP280L/F354C | 1              | TGC to GTC               | Middle of TM-11                 |
| pP280L/G370C | 1              | GGC to TGC               | Periplasmic loop 11–12          |
| pP280L/G370S | 2              | GGC to AGC               | Periplasmic loop 11–12          |
| pP280L/G370V | 1              | GGC to GTG               | Periplasmic loop 11–12          |
Suppressor Analysis of the Lactose Permease

**DISCUSSION**

In the current study, we have isolated and characterized suppressor mutations that restore a significant amount of lactose permease function to a protein that has a defective mutation in the first position of the conserved motif in cytoplasmic loop 8/9. Since suppressors could potentially restore activity in a variety of ways, the locations and character of these second-site revertants offers insight into their mechanism of action. One possibility is that a suppressor mutation could be very close to the first-site mutation in the tertiary structure and thereby influence the structure of loop 8/9 directly. Based on our tertiary model in Fig. 3, this could be the case for the suppressors in loop 6/7 (i.e. L212Q and L216Q) and/or the second-site mutation could compensate for the first-site mutation. Based solely on the locations of the suppressor mutations described in Table II, it is not possible to distinguish between these two possibilities. However, a comparison of the locations of loop 2/3 suppressor mutations isolated in previous studies suggests that many of the suppressors identified in this study are acting to restore the ability of the lactose permease to make necessary conformational changes rather than restoring the local structure of loop 8/9 directly.

Table V summarizes the locations of suppressor mutations that were isolated by starting with parental mutations that caused a defect in loop 2/3 (24, 25) or loop 8/9 (this study). Certain suppressor locations seem to be loop specific. For example, suppressor mutations in TM-1 (i.e. codons 28 and 29) were only obtained in loop 2/3 suppressor screens while suppressor mutations in loop 6/7 (i.e. codons 212 and 216) were only obtained from a defective loop 8/9 parent. However, suppressors are located centrally or near the periplasmic side of transmembrane segments or within hydrophilic regions of periplasmic loops. An alternative way that a suppressor could act is by altering protein structure from a distance. To correct the defect in lactose transport, two types of events could occur. One possibility is that a suppressor could have a substantial effect on protein secondary structure that would affect the protein conformation in such a way as to restore the correct structure to loop 8/9. In this scenario, the suppressor mutation would "fix" the local structure of loop 8/9. A second possibility is that the suppressor mutations may not actually restore the correct structure to the loop 8/9 region. Instead, the second-site mutations may change the structure of the permease so it can make necessary conformational changes that were initially inhibited by the first-site mutation in the loop 8/9 motif. In other words, the second-site mutation could compensate for the first-site mutation.

### Table III

| Strain          | 0.2% Mel | 0.4% Mel | 1% Mel | 0.2% Lac | 0.4% Lac | 1% Lac | 0.2% Malt | 0.4% Malt | 1% Malt |
|-----------------|----------|----------|--------|----------|----------|--------|----------|----------|--------|
| pP280L/G46C     | Pink     | Red      | Red    | White    | Red      | White  | White    | White    | White  |
| pP280L/G46C     | Red      | Red      | Red    | Red      | Red      | White  | White    | White    | White  |
| pP280L/A50T     | Red      | Red      | Red    | Red      | Red      | Red    | Red      | Red      | Red    |
| pP280L/G49L     | Red      | Red      | Red    | Red      | Red      | Red    | Red      | Red      | Red    |
| pP280L/S354C    | Pink     | Red      | Red    | Red      | Red      | Red    | White    | White    | White  |
| pP280L/G370V    | Red      | Red      | Red    | Red      | Red      | Red    | White    | White    | White  |
| pP280L/G370C    | Red      | Red      | Red    | Red      | Red      | Red    | White    | White    | White  |
| pP280L/S233P    | R. C.    | Red      | Red    | Red      | Red      | Red    | White    | White    | White  |
| pP280L/L212Q    | Pink     | R. C.    | Red    | Red      | Red      | Red    | Red      | Red      | Red    |
| pP280L/L216Q    | Red      | Red      | Red    | Red      | Red      | Red    | White    | White    | White  |
| pP280L/C333G    | Pink     | Pink     | R. C.  | Red      | Red      | Red    | White    | White    | White  |

* R. C., red center.

### Table IV

| Mutation/revertant | K<sub>m</sub> ± S.E. | V<sub>max</sub> ± S.E. |
|--------------------|---------------------|----------------------|
| pLac184            | 0.5 ± 0.1           | 197.0 ± 37.6         |
| pP280L<sup>a</sup> | 0.1 ± 0.1           | 6.1 ± 3.1            |
| pP280L/G46C        | 0.3 ± 0.04          | 51.2 ± 5.3           |
| pP280L/F49L        | 0.4 ± 0.1           | 47.2 ± 9.7           |
| pP280L/A50T        | 0.9 ± 0.4           | 66.2 ± 28.6          |
| pP280L/L212Q       | 0.1 ± 0.1           | 8.2 ± 1.9            |
| pP280L/L216Q       | 0.4 ± 0.1           | 89.6 ± 24.1          |
| pP280L/S233P       | 1.3 ± 0.6           | 53.8 ± 25.9          |
| pP280L/C333G       | 0.4 ± 0.1           | 14.1 ± 4.1           |
| pP280L/F354C       | 0.5 ± 0.1           | 37.7 ± 5.0           |
| pP280L/G370V       | 1.1 ± 0.2           | 199.9 ± 34.7         |
| pA50T              | 1.1 ± 0.2           | 15.2 ± 4.9           |
| pG370V             | 2.4 ± 0.4           | 50.7 ± 14.7          |

<sup>a</sup> The single mutants having a low V<sub>max</sub> were tested for their protein levels in the membrane as described under "Materials and Methods." Relative to the wild-type strain, the protein levels in the three single mutants, P280L, A50T, and G370V were 92%, 70%, and 96%, respectively.
pressors in three regions of the lactose permease including codons 45–50, 234–241, and 366–370, were able to restore functionality to both loop 2/3 and loop 8/9 defects. One identical suppressor mutation, A50T, was able to restore functionality to a G64S mutation in loop 2/3 and to a P280L mutation in loop 8/9. From a structural point of view, it seems very unlikely that this A50T substitution could restore the correct local structure to both loop 2/3 and loop 8/9, particularly since it is located in a periplasmic loop and not likely to be near loop 8/9. Instead, we propose that many of the suppressors in these three regions (i.e. codons 45–50, 234–241, and 366–370) are restoring the ability of the lactose permease to make the necessary conformational changes that are associated with lactose transport.

Before we discuss how certain suppressors may exert their effects, it is helpful to consider the symmetry of our tertiary model for the lactose permease. In this model proposed previously, the two halves of the protein form a rotationally symmetrical structure at an interface involving TM-2/TM-11 and TM-5/TM-8 (Ref. 12, and also see Fig. 3A). Based on the observation that the two halves of the protein are evolutionarily related, this model has the α-helices including TM-1 to TM-6 and TM-7 to TM-12 arranged in the same manner. Another critical feature of our model is that the two halves of the protein (TM-1 to TM-6 and TM-7 to TM-12), form independent domains that meet at this interface. This idea is supported by other studies involving the lactose permease, tetracycline antiporter, and glucose transporter (32–34). When the two halves of these transporters were independently expressed from separate promoters, the segments encoding TM-1 to TM-6 and TM-7 to TM-12 were efficiently inserted into the membrane and associated with each other to form a functional protein. In our model of Fig. 3A, the interface between the two halves of the protein is the critical region that promotes conformational changes facilitating transport. Loop 2/3 and loop 8/9 occupy symmetrical locations at this interface. Mutations in these loops primarily affect the $V_{\text{max}}$ for transport, suggesting that the loops may play an important role in keeping the proper configuration so the permease can alternate between the C1 and C2 conformations.

To explain how second-site suppressor mutations in regions 45–50, 234–241, and 366–370, could restore functionality, we first need to consider how the first-site mutations in loop 2/3 and loop 8/9 may exert their effects. Based on their locations in our tertiary model, and also based on their detrimental effects on the $V_{\text{max}}$ for lactose transport, we propose that the first site mutations may perturb the optimal spatial arrangement of helices at the interface. In this regard, it is worth emphasizing that our model has TM-2 and TM-8 located at the interface, and

![Figure 3: Symmetrical effects of first-site mutations in loop 2/3 and loop 8/9.](image-url)
A summary of suppressor mutations that correct loop 2/3 or loop 8/9 defects

| Location of second-site suppressor | Location of first-site mutation Location of second-site mutation in secondary model |
|-----------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Pro^{28} → Ser, Leu, Thr          | X  | TM-1, near periplasm                                                                                                               |
| Phe^{29} → Ser                    | X  | TM-1, near periplasm                                                                                                               |
| Thr^{45} → Arg                    | X  | Periplasmic loop 1/2                                                                                                                |
| Gly^{46} → Cys, Ser               | X  | Periplasmic loop 1/2                                                                                                                |
| Phe^{49} → Leu                     | X  | X   | TM-2, very near periplasm                                                                                                           |
| Ala^{50} → Thr                     | X  | X   | TM-2, very near periplasm                                                                                                           |
| Cys^{154} → Gly                    | X  | X   | TM-5, central                                                                                                                        |
| Leu^{272} → Gln                    | X  | X   | Cytoplasmic loop 6/7                                                                                                                 |
| Leu^{276} → Gln                    | X  | X   | Cytoplasmic loop 6/7                                                                                                                 |
| Ser^{233} → Pro                    | X  | X   | TM-7, central                                                                                                                        |
| Cys^{234} → Phe, Trp               | X  | X   | TM-7, central                                                                                                                        |
| Gln^{241} → Leu                    | X  | X   | TM-7, central                                                                                                                        |
| Phe^{263} → Val                    | X  | X   | TM-8, near periplasm                                                                                                                 |
| Thr^{266} → Ile                     | X  | X   | TM-8, central                                                                                                                        |
| Cys^{333} → Gly                    | X  | X   | TM-10, near cytoplasm                                                                                                               |
| Phe^{354} → Cys                     | X  | X   | TM-11, central                                                                                                                        |
| Ser^{366} → Phe                     | X  | X   | TM-11, very near periplasm                                                                                                          |
| Val^{367} → Glu                     | X  | X   | Periplasmic loop 11/12                                                                                                               |
| Ala^{369} → Pro                    | X  | X   | Periplasmic loop 11/12                                                                                                               |
| Gly^{370} → Cys, Ser, Val           | X  | X   | Periplasmic loop 11/12                                                                                                               |

* Suppressor mutations of loop 2/3 defects were identified in previous studies (24, 25).

The mutations at the first position in the conserved motif (i.e., positions 64 and 280) are actually predicted to lie at the cytoplasmic edges of TM-2 and TM-8. Furthermore, the first-site mutations (Gly^{46} → Cys, Ser, Val; Pro^{28} → Leu) are bulky and less likely to promote a turn compared with the wild-type residues.

Provided that the two halves of the protein form a rotationally symmetrical structure, a mutation in loop 2/3 could have the same net effect as a mutation in loop 8/9. This could occur if a mutation affected the spatial arrangement of helices in a symmetrical way at the interface. Fig. 3, B and C, provides a conceptual example to illustrate this idea. It is important to emphasize that this figure schematically illustrates how a mutation in loop 2/3 or the analogous site in loop 8/9 could have the same effect based on a proposed symmetry in the permease. It is not meant to imply that we understand how the first-site mutations actually disrupt protein structure. Regardless of the precise nature of this disruption, this model shows how a mutation in loop 2/3 or loop 8/9 could alter protein structure in the same way. As shown in Fig. 3B, a loop 2/3 mutation could cause a “symmetrical twisting” at the interface. In this drawing, the first half of the protein (TM-1 to TM-6) is twisted toward the reader and the second half of the protein (TM-7 to TM-12) is twisted toward the plane of the paper. With regard to helices at the interface, TM-2 is tilted downward relative to TM-11, and TM-5 is tilted upward relative to TM-8. In Fig. 3C, an analogous mutation in loop 8/9 could have the same net effect. A first-site mutation would disrupt the interface so that the second half of the protein is twisted toward the reader and the first half is twisted toward the plane of the paper. The striking thing to note is that two structures shown in Fig. 3, B and C, are equivalent to each other. In both cases, TM-2 is tilted downward relative to TM-11, and TM-5 is tilted upward relative to TM-8. Therefore, due to the inherent symmetry in our model, mutations at analogous sites in the two halves of the protein (i.e., first position of the conserved motif in loop 2/3 and loop 8/9) could have the same net effect on permease structure.

To explain how suppressor mutations at codons 45–50, 234–241, and 366–370 could restore functionality to both loop 2/3 and loop 8/9 defects, we suggest that they also effect the interface between the two halves of the permease, but in a way that compensates for the defect imposed by the first site mutation in loop 2/3 or loop 8/9. According to our schematic illustration in Fig. 3, B and C, the suppressor mutations would twist the protein back to its original configuration as shown in Fig. 3A. Based on these locations and the character of the substitutions, all of these suppressor mutations could restore the correct interface boundary by altering the TM-2/TM-11 side of the protein. Amino acids 45–50 are located in the loop 1/2 and TM-2 region and could alter the topology of TM-2 or the interaction between TM-2 and TM-11. Amino acids 366–370 are located in TM-11 or in loop 11/12. These mutations could alter the topology of TM-11 or the interaction between TM-11 and TM-2. Lastly, mutations in the region including codons 233–241 are located on TM-7 which is adjacent to TM-11. These mutations are either highly disruptive Ser^{233} → Pro, or involve major changes in side chain volume. The amino acid substitutions could indirectly restore the TM-11/TM-2 interface by altering the topology of TM-11.

In summary, we have carried out several studies concerning the importance of the conserved motif in loop 2/3 and loop 8/9, as well as the isolation of second-site suppressor mutations. This work has provided important implications regarding the structure-function relationships within solute transporters of the superfamily. First-site mutations in loop 2/3 and loop 8/9 greatly inhibit the maximal velocity for solute transport, suggesting that the motifs play a key role in promoting conformational changes associated with transport. Suppressor mutations, which seem to be clustered at the interface, are able to restore the ability of the transporter to make the required conformational changes. These results are consistent with a structural model that has a symmetrical arrangement between the two halves of the protein and inconsistent with all previous models in which the 12 transmembrane segments do not form a symmetrical structure. Solute transport may occur by a conformational change at this interface. As proposed earlier, such a conformational change could be explained by small shear motions at the TM-2/TM-11 and the TM-8/TM-5 interface (24). In water-soluble proteins that have been crystallized in both opened and closed conformations, shear motions are commonly involved in conformational changes that alter accessibility to the solute binding site (35). During motion, interdigitating side chains of adjacent a-helices can alter their torsional angles so that they move among conformational states of nearly the same energy without crossing large energy barriers. This type of event could explain the interconversion between the C1 and C2 conformations that has been described in Fig. 3A.

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