The *Escherichia coli* lactose carrier is an energy-transducing H⁺/galactoside cotransport protein which strictly couples sugar and proton transport in 1:1 stoichiometry. Here we describe five lactose carrier mutants which catalyze "uncoupled" sugar-independent H⁺ transport. Symptoms similar to uncoupling by a proton ionophore have been observed in cells expressing these mutant carriers. The mutations occur at two separate loci, encoding substitutions either for alanine 177 (valine) or tyrosine 236 (histidine, asparagine, phenylalanine, or serine).

Compared to the parent, cells expressing the valine 177 carrier grew slowly on minimal media with glucose as carbon source. When washed cells were incubated in the absence of added sugars the mutant showed a reduced protonotive force compared with the parent. Addition of either thiodigalactoside or α-D-nitrophosphorylglactoside reduced the defect in protonotive force.

Sugar-independent H⁺ entry rate into cells expressing either the normal carrier or the Val-177 mutant were measured directly using the pH electrode. Following sudden acidification of the external medium (by either oxygen-pulse or acid-pulse) protons entered more rapidly into cells expressing the Val-177 carrier. This novel sugar-independent mode of H⁺ transport probably depends on an acquired capacity of the Val-177 carrier to bind the transported proton with higher than normal affinity in a transition state involving the binary carrier/H⁺ complex.

Among the many cation/substrate cotransporters which exist in nature, the *Escherichia coli* lactose carrier is the most thoroughly studied example. The structural gene (lacY) encoding the lactose carrier has been sequenced (1) and cloned on multicopy plasmids (2). Such plasmids have been used to express the carrier in vitro (3), and to purify and reconstitute it in active form (4, 5). There is strong evidence to support Mitchell's hypothesis (6) that the energy for active lactose transport in *E. coli* is derived from the proton electrochemical gradient via a mechanism involving H⁺-lactose symport.

Although the existence of symport was directly demonstrated by West (7), and is now generally accepted as the mechanism of active lactose accumulation (8), the molecular mechanism is not yet understood. Implicit in the notion that galactoside transport occurs "exclusively" by cotransport with H⁺ is that specific mechanisms (structural attributes of the carrier) exist to forbid the conformational changes which would cause either cosubstrate to be transported in the form of a binary complex with the carrier. In other words, there must be some concrete mechanism to enforce Jencks' rule that activation energy for isomerizations involving binary complexes between the carrier and either cosubstrate ought to be maximized, while activation energy for isomerization of the ternary complex involving the carrier and both cosubstrates ought to be minimized (9). Despite this conceptual understanding, there has been little research aimed at probing those structural features of the lactose carrier which conspire to prevent its functioning as a unipporter.

Since prevention of unipport reactions is fundamental to the molecular mechanism of energy transduction via symport, our interest has begun to focus on mutations which lower the activation energy for isomerization of binary complexes between the lactose carrier and its cosubstrates. Lactose carrier mutations are known in which sugar is transported in the absence of cation movement. For example, a mutation of His-322 (10, 11), Glu-325 (12), Arg-302 (13), or Gly-262 (14) can result in lactose transport which is not stoichiometrically coupled to proton translocation. Net sugar transport under these conditions must occur via carrier-substrate isomerization plus the return of the empty carrier. The deleterious effect of these mutations on energy coupling may be related to their capacity to affect the relative magnitude of the energy barrier to "uncoupled" versus "coupled" transport via the binary carrier-sugar (CS) and the ternary carrier-sugar-H⁺ complexes. Recent studies indicate that balance between the symport and unipport pathways is influenced both by the nature of the position 322 substituent and by the structure of the sugar substrate. Whereas the Tyr-322 mutant transports melibiose with H⁺ but lactose without H⁺, the Phe-322 mutant transports both sugars with H⁺ (10, 11). Thus, the main attraction of this "energy-barrier" hypothesis is that the characteristics of the various uncoupled mutants (10–16) may be accommodated without invoking any fatal structural defect in the detailed pathway of H⁺ conduction through the protein.

Previous work with uncoupled mutants has been restricted to mutants which transport sugar without an accompanying proton (10–16). Here we describe a complementary family of lactose carrier mutations which decrease the energy barrier to H⁺ transport via the binary carrier-H⁺ (CH) complex.
Uncoupled Proton Transport by the Lactose Carrier

Proton transport by these mutants is shown to be galactoside-independent and to require expession of the lactose carrier. Certain substrates (TDG and α-pNPG) of the lactose carrier were found to decrease the "proton leak," presumably by binding to the lactose carrier and decreasing the mole fraction of carrier present as the "leaky" CH complex. This represents the first description of mutations which confer upon a cotransporter the ability to catalyze cation uniport. The properties of such mutants should be somewhat unusual given the deleterious effect of this phenotype on the wide array of processes which derive energy from the transmembrane ion-motive force.

**EXPERIMENTAL PROCEDURES**

Materials—TDG and α-pNPG were obtained from Sigma. [3H]Proline and reagents for oligonucleotide-directed mutagenesis were from Amersham Corp. [3H]Taurine, [3C]Taurine, [14C]benzoic acid, [methyl-3H]TMG, and [3H]H2O were from Du Pont-New England Nuclear. Silicone oil (specific gravity 1.016) was from the Contour Chemical Company, Woburn, MA. Cellulose nitrate filters (0.6-μm pores) used in transport experiments were obtained from Sartorius, Chemicals, obtained from usual sources, were of the highest quality commercially available.

Bacterial Strains—The E. coli K-12 strains used in these experiments were DW1, lacIa (Z) mel (ΔD) strA (17); MAA23, Aflac (ΔD) RecA::TnlO. The F'-factor from E. coli AE60 (18) has the genotype lacO'P'A(Z) Y+A(A) Amp's Tets (2). The mutant lacY was cloned on a small high copy plasmid, pB15, by selecting for mutants which have the genotype lacO'P'A(Z) Y+A(A) Amp's Tets (2). Both plasmids express a lactose carrier with enhanced specificity for sucrose and malate (40).

Sugar Transport—Exponentially growing cells were harvested by centrifugation and washed twice with 100 mM potassium phosphate buffer (pH 7.0). Cells were resuspended in this buffer (to give ~3 X 10^7/ml) and were treated as indicated in the figure legends. Cells were separated from radio-labeled substrates by filtration and washed with 5 ml of 100 mM potassium phosphate buffer (pH 7.0) containing 5 mM HgCl2. The filters were dissolved in 4 ml of Liqisint (National Diagnostics) and radioactivity was quantitated in a scintillation spectrometer.

Measurement of Intracellular Water—Cytoplasmic water (measured for each strain) was taken as the difference between the total aqueous space, measured with [3H]H2O (1.4 μCi/ml), and the [14C]taurine (0.3 μCi/ml) space (24). Cells were separated from the extracellular medium by centrifugation through sucrose oil as described previously (25) except that cell pellets were placed directly into 4 ml of Liqisint and incubated for about 1 h, with intermittent vortex mixing to dislodge the pellet, before quantitating radioactivity by two-channel scintillation spectrophotometry. Counting efficiency for 3H was 21% in channel A and 0.06% in channel B. Counting efficiency for 14C was 8.9% in channel A and 71% in channel B.

Measurement of Proton Gradient—Exponentially growing cells were harvested by centrifugation and washed once (or twice if lacY was induced by growth in the presence of 1 mM IPTG) with 120 mM potassium phosphate buffer (pH 5.9) in which protomotive force is manifest substantially as ΔpH (26). Cells were separated from the extracellular medium by centrifugation through sucrose oil and processed as described above. Other conditions were as described in the figure legends.

Measurement of Protomotive Force—The total protomotive force was estimated from the distributions of [14C]benzoic acid (0.1 μCi/ml) and/or [3H]tetrathenylphosphonium (0.5 μCi/ml) across the plasma membrane. Cells used in these experiments were treated with IPTG as described previously (27). [3H]Taurine (0.6 μCi/ml) or [14C]taurine (0.3 μCi/ml) were used as markers for the extracellular space (25–27). Cells were separated from the extracellular medium by centrifugation through sucrose oil and processed as described above.

RESULTS AND DISCUSSION

We recently isolated a mutant based on its ability to catalyze sucrose transport via the lactose carrier which was expressed from an F'-factor. The mutant lacY was cloned on a plasmid, pB15, and DNA sequencing revealed that the sucrose-positive phenotype was conferred upon the carrier by a point mutation which changed alanine 177 to valine (40). Brooker and Wilson (20) had independently isolated this mutation on a small high copy plasmid, pB15, by selecting for mutants able to recognize maltose. Cells expressing the lactose carrier grown in minimal medium (25-27) grew poorly in minimal media with glucose as carbon source (Fig. 1). The same cells uninduced for the lactose carrier grew normally. Cells possessing a plasmid with the normal lacY grew well with or without induction of IPTG as described in the legend to Fig. 1.

### Table 1

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Unusual sugar specificities</th>
<th>Amino acid substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTE18 (parent)</td>
<td>--</td>
<td>Wild-type</td>
</tr>
<tr>
<td>pB15</td>
<td>+</td>
<td>Ala-177 to Val</td>
</tr>
<tr>
<td>pTKY-A177V</td>
<td>+</td>
<td>Ala-177 to Val</td>
</tr>
<tr>
<td>pB5</td>
<td>+</td>
<td>Tyr-236 to His</td>
</tr>
<tr>
<td>pL13</td>
<td>+</td>
<td>Tyr-236 to Asn</td>
</tr>
<tr>
<td>pP7</td>
<td>+</td>
<td>Tyr-236 to Ser</td>
</tr>
<tr>
<td>pF1</td>
<td>+</td>
<td>Tyr-236 to Phe</td>
</tr>
</tbody>
</table>

---

*The abbreviations used are: TDG, β-D-galactopyranosyl-1-thio-β-D-galactopyranoside; pNPG, 4-nitrophenoxy-α-D-galactopyranoside; TMG, methyl-1-thio-β-D-galactopyranoside; β-ONPG, 2-nitrophenoxy-β-D-galactopyranoside; IPTG, isopropyl-1-thio-β-D-galactopyranoside.*
of the lactose carrier. We believe that the expression of the mutant carrier seriously compromises growth and this provides strong selection for spontaneous revertants to the normal carrier. Indeed, Collins et al. (28) found that certain double mutants containing the Val-177 substitution together with substitutions at position 322 were prone to reverse mutation at position 322. Similarly, we have encountered cases in which wild-type lacY DNA sequence was unexpectedly obtained from cells ostensibly carrying only pB15 (40), suggesting reversion from the mutant Val-177 to the wild-type Ala-177.

Among the several hypothetical causes for the slower growth rate of the mutant, and hence the cause of selective pressure for reverse mutation, was the possibility that the Val-177 carrier catalyzed proton transport in the absence of sugar. This uncoupled proton transport would lead to a reduction in proton motive force (with results similar to addition of a proton ionophore). Proton transport could occur via the binary CH complex shown in Fig. 2A. If true, sugar substrate might be expected to prevent the proton leak by a mass action effect which drives the carrier into the ternary “CSH” complex (Fig. 2B). Several experiments were carried out to test these hypotheses.

Proton motive Force—Measurement of transmembrane ΔpH and Δψ indicate that the mutant has a distinct defect (Table II). Expression of the Val-177 carrier significantly reduced the transmembrane ΔpH, but had little effect on Δψ. Thus, when the external pH was 5.5 the proton motive force (ΔµH+) was 71 mV more negative in cells expressing the normal carrier from pTE18 than in cells expressing the mutant carrier from pTKY-A177V and 38 mV more negative when the external pH was increased to 5.9. The precence of 500 µM TDG restored ΔµH+ to near normal levels in cells expressing the Val-177 carrier. This protective effect of TDG was due exclusively to improvement of ΔpH. Other data (Fig. 3) show that the effect of TDG and α-pNP on the proton gradient are concentration dependent, reflecting apparent sugar affinities similar to values expected for the wild-type carrier (33, 34). Independent evidence, obtained from inhibition of β-ONPG transport, indicates that the affinity of the Val-177 carrier for TDG and α-pNP is indeed identical to values for the normal carrier (40).

That these effects on ΔpH were due to expression of lacY was affirmed by experiments showing that the phenomenon can be brought under control of the lac repressor (Fig. 4). Whereas induction (1 mM IPTG) of the lactose carrier in cells harboring pb15 (Val-177 carrier) decreased the proton gradient by 60% (18- to 7-fold), the analogous experiment performed with cells harboring pTE18 (normal carrier) showed a modest 11% increase (18- to 20-fold) in the magnitude of the proton gradient. These observations are consistent with the idea that the Val-177 carrier, but not the wild-type carrier, can transport protons by a sugar-independent mechanism (Fig. 2A).

Inasmuch as the Val-177 carrier is one example from a larger family of mutants which share the common property of enhanced maltose specificity (20), we tested whether other
Uncoupled Proton Transport by the Lactose Carrier

mutants from this family might also share in common the propensity to leak protons. Induction of the lac operon from pR5 (His-236), pL13 (Asn-236), pF1 (Phe-236), and pP7 (Ser-236) did indeed decrease the proton gradient (Fig. 4). Mutant pR5 appeared to have the greatest impact on the proton gradient and mutant pP7 the least impact. Thus, to varying degrees all of the "maltose" recognition mutants appear to catalyze sugar-independent H⁺ transport (Fig. 4A).

Measurement of the Sugar-independent Proton Transport ("Internal Leak") by the Mutant Carrier—Direct evidence that the Val-177 carrier leaks H⁺ was obtained by using the pH electrode to monitor the rate of proton entry into the cell following a sharp acidification of the extracellular medium. The method has previously been used to measure the proton leak in E. coli mutants which fail to couple ATP synthesis to proton transport via the F₁Fₒ ATPase (31, 32). In the present experiments, cells were made anaerobic to block proton pump-

Fig. 5. Comparison of H⁺ entry rate following acid-pulse delivered to anaerobic cells expressing either the normal lactose carrier or the mutant Val-177 carrier. E. coli DW1 (3 × 10⁹/ml) harboring plasmid pTE18 (Parent) or pTKY-A177V (Mutant) was made anaerobic by bubbling nitrogen into the medium (120 mM KCl, 30 mM KSCN) for 30-60 min as described under "Experimental Procedures." The extracellular pH was sharply acidified (at arrow) by adding 50 μl of anaerobic 5 mM HCl to a 2.5-ml sample of the anaerobic cells.

Fig. 6. Comparison of H⁺ entry rate following oxygen-pulse delivered to anaerobic cells expressing either the normal lactose carrier or the mutant Val-177 carrier. E. coli DW1 (3 × 10⁹/ml) harboring plasmid pTE18 (Parent) or pTKY-A177V (Mutant) were made anaerobic by bubbling nitrogen into the medium (120 mM KCl, 30 mM KSCN) for 30-60 min as described under "Experimental Procedures." The extracellular pH was sharply acidified (at arrow) by adding 25 μl of aerobic medium (120 mM KCl, 30 mM KSCN) to a 2.5-ml sample of the anaerobic cells.
of the medium is the cytoplasm itself; this creates a large driving force for proton reentry. For this reason, larger acidifications were necessary in the "acid-pulse" experiment than in oxygen-pulse experiment (compare scale bars in Figs. 5 and 6).

**TMG Transport**—One might expect that the proton leak found in Val-177 would affect various physiological processes that utilize a proton motive force as a source of energy. One of the processes is sugar transport by the lactose carrier. In one set of experiments cells were exposed to $[^{14}C]$TMG and at timed intervals samples were filtered, washed, and counted. Because of the possibility of rapid exit of the sugar during filtration, a second set of experiments was carried out in which HgCl$_2$ was added to cells before filtration to rapidly inactivate the carrier (Fig. 7). The washing buffer also contained HgCl$_2$ to prevent TMG efflux during the wash. Under these conditions rapid uptake was observed in both mutant and parent, followed by a fall in sugar content to a constant level, equal to that observed in the parallel experiments in which reactions were not quenched with HgCl$_2$. During filtration there was apparently a very rapid exit of sugar from both parental and mutant strains. This exit was prevented by using HgCl$_2$ to block the carrier before filtration.

The dramatic fall in intracellular sugar concentration after reaching the peak ($IN/OUT = 25$) is probably due to a fall in the proton motive force. Such a fall in proton motive force has been shown to occur when there is a sudden rapid entry of protons plus sugar via the lactose carrier. Presumably, sugar influx, stoichiometrically coupled to $H^+$, exceeds the capacity of the electron transport chain to pump protons out of the cell. The result is that proton motive force is reduced and consequently both ATP synthesis and cell growth are compromised (29, 30). This "overshoot" phenomenon is not observed in either normal or Val-177 mutant when the lacY gene is expressed in a plasmid construction with 50% of the lactose carrier expression of pH15.

This diminution of proton motive force, although consistent with the declining phase of TMG concentration (Fig. 7), does not explain why the cell expressing the Val-177 carrier should settle to a lower steady-state level than the cell expressing a normal carrier. The parallel $H^+$ leak (Fig. 2A) sufficient to produce a permanent lowering of the proton motive force (Table II) could produce such a difference in the steady-state sugar accumulation.

**Effect of pTKY-A177V on Proline Transport**—Any effect of the Val-177 carrier on the proton motive force ought to have a measurable effect on other transport systems that depend on the proton electrochemical gradient for energy. We studied proline transport to test this prediction. $[^{3}H]$Proline transport in E. coli DW1 was unaffected by the presence of wild-type lactose carrier expressed constitutively from pTE18 (Fig. 8A). In contrast, proline transport, particularly the initial rate, was substantially reduced in the strain expressing the Val-177 carrier from pTKY-A177V. In order to establish more clearly that the observed transport defect was indirect and related to uncoupled turnover of the Val-177 carrier (Fig. 2A),

![Fig. 7](image_url)

**Fig. 7** Comparison of TMG transport with or without rapid quenching by mercuric chloride. E. coli DW1 (4 x 10^9/ml) harboring plasmid pTE18 (●, ○) or pB15 (△, △) were exposed to 0.1 mM $[^{14}C]$TMG (0.1 µCi/ml) and washed with 5 ml buffer containing 5 mM HgCl$_2$. Initial rates of TMG uptake were obtained by a modified procedure (B) either in the presence (open symbols) or absence (solid symbols) of 1 mM TDO. Twenty µl of a 5-fold concentrated solution (containing 5 mM TDO and 5 µM $[^{3}H]$Proline at 0.5 µCi/ml) was rapidly diluted with 500 µl of cell suspension (4 x 10^9/ml) expressing lactose carrier from pTE18 (●, ○) or pTKY-A177V (△, △). The transport reactions were quenched at the indicated intervals by adding 1 ml of buffer containing 20 mM HgCl$_2$. The quenched sample was then filtered through a filter which was then washed as described for A.

![Fig. 8](image_url)

**Fig. 8.** Inhibition of proline transport by activity of the Val-177 lactose carrier mutant. E. coli DW1 (●) or DW1 expressing lactose carrier from pTE18 (○) or pTKY-A177V (△) were exposed to 1 µM $[^{3}H]$proline (0.1 µCi/ml) in buffer (100 mM potassium phosphate, pH 7.0). Aliquots (0.1 ml) were filtered at intervals indicated (A) and washed with 5 ml buffer containing 5 mM HgCl$_2$. Initial rates of proline transport were obtained by a modified procedure (B) either in the presence (open symbols) or absence (solid symbols) of 1 mM TDO. Twenty µl of a 5-fold concentrated solution (containing 5 mM TDO and 5 µM $[^{3}H]$Proline at 0.5 µCi/ml) was rapidly diluted with 500 µl of cell suspension (4 x 10^9/ml) expressing lactose carrier from pTE18 (●, ○) or pTKY-A177V (△, △). The transport reactions were quenched at the indicated intervals by adding 1 ml of buffer containing 20 mM HgCl$_2$. The quenched sample was then filtered through a filter which was then washed as described for A.
we studied the effect of TDG on the initial rate of proline transport (Fig. 5B). Whereas TDG had no discernible effect on the rate of proline transport in the cell expressing the normal lactose carrier, it increased the rate nearly 2-fold in cells expressing the Val-177 carrier.

These data are consistent with the view that H⁺ in the extracellular medium can associate with the Val-177 carrier and cross the membrane via the binary CH complex (Fig. 2A). The "internal H⁺ leak" is ameliorated by TDG which occupies the sugar binding site, thereby reducing the mole fraction of carrier present as the leaky CH intermediate (Fig. 2B). Presumably, the improvement in proline transport is secondary to an improvement in the magnitude of the protonmotive force. Thus, implicit in this scheme is that any proton cycling involved in maintaining the steady-state TDG gradient is much less "wasteful" of energy than proton cycling in the futile cycle involving the electron transport chain and the leaky CH intermediate of the Val-177 mutant.

Mechanism of H⁺ Uniport—Although the mechanism by which the "H⁺-leaky" mutants catalyze proton transport in the absence of sugar is not known, two general possibilities may be considered: (i) reduced proton binding affinity for the carrier (to form the CH complex) and (ii) increased proton binding affinity for the carrier in the transition state (35, 36). We favor the second possibility as a working hypothesis. One recent observation is consistent with this view. We have found that the Val-177 carrier has a \( V_{\text{max}} \) for \( \beta \)-ONPG which exceeds that of the parent by about 3-fold (40). There was no effect on the \( k_{\text{cat}} \). The \( V_{\text{max}} \) for cotransport is probably jointly a function of the binding energies for both cosubstrates in the transition state. In other words, return of the unloaded carrier should not be uniquely rate-limiting, since different substrates have different values of \( k_{\text{cat}} \). One simple mechanism consistent with the data is that the component of proton binding energy which accounts for the increased H⁺ uniport rate is similarly realized in the transition state involving \( \beta \)-ONPG and thereby increases the \( V_{\text{max}} \) for cotransport. Stated differently, \( \beta \)-ONPG binding is unaffected by the Val-177 mutation and the effect on \( \beta \)-ONPG transport depends fully on the change in interaction of the carrier with H⁺. More complicated effects on both sugar and H⁺ binding have been observed with other sugars (40).

Irrespective of the manner in which proton binding energy is perturbed during the H⁺ uniport cycle, a detailed description of the process must explain how nonpolar amino acid side chains (alanine and valine) in the 177 position can modify the interaction between H⁺ and the carrier. The mechanism seems unlikely to be direct. To the extent that the relevant H⁺ binding sites involve charged groups several indirect mechanisms might affect affinity of the carrier for protons: (i) perturbations affecting the dielectric constant at the binding site; (ii) steric effects on the proximity of neighboring charges to the proton binding site; (iii) steric effects on the depth of proton binding sites within the membrane (since the effective binding energy is the free energy intrinsic to the pKᵢ of the binding site plus the free energy obtained from moving the proton down its electrochemical gradient to the position of that site (37, 38)); and (iv) steric effects on internal energy (9, 35) of the carrier (i.e. the Gibbs energy difference between the unloaded carrier and the "activated" conformation of the unloaded carrier which binds H⁺ in the transition state).

Thermodynamic Considerations in Mutational Analysis of Transport—In cyclic processes such as cotransport, the sum of the Gibbs free energy changes (\( \Delta G \)) associated with each step of the complete cycle must be equal to zero. Thus a change in the chemical potential of one intermediate must be accompanied by a change in the \( \Delta G \) value associated with at least two steps in the cycle (in order to maintain the zero sum). Experimentally it may sometimes be difficult to identify the second alteration. However, in the case of the "proton-leaky" mutations two alterations are clearly demonstrable: one related to interaction with the cation (H⁺) and the second related to interaction with the sugar. A distinctive feature of the proton-leaky mutants is that the defect in H⁺ binding can be studied in the absence of sugar, leaving no question that the mutation exerts its effect on proton recognition without any free energy contribution from the cosubstrate. Nevertheless, the Val-177 mutant has pleiotropic effects on the kinetics of sugar transport, altering specificity for many sugars (sucrose, cellobiose, palatinose, melibiose, TMG, and \( \beta \)-ONPG (40) including maltose (20), the sugar originally used to select the Val-177 mutant. Stated differently, multiple effects of a point mutation are obligatory and thus need not be ascribed a priori to gross structural perturbations devoid of mechanistic significance.

The generality of this "mutational double-effect" principle may be illustrated by example. Other mutants with two distinct phenotypes include the group of "sugar-leaky" mutants (Fig. 2C) of the putative "charge-relay" domain (10, 11, 15, 16) and the sugar-leaky Cys-262 mutant (14). These mutants show distinct abnormalities in both cation transport and sugar recognition. In the E. coli melibiose carrier, many mutants show alterations in both sugar recognition and cation recognition (39). As these multiple effects are likely to be the result of obligate thermodynamic constraints imposed by the cyclic nature of transport processes, it is unclear whether such loci should be assigned to regions in or near the cation site on the one hand or the sugar binding domain on the other (10, 11).

There seems now to be a growing family of mutants known to affect interaction of the symport protein with both cosubstrates (10–16, 28, 39). These mutations should provide helpful information regarding structural attributes of the carrier which conspire to satisfy the obligatory thermodynamic linkages influencing the kinetics and/or efficiency of energy-conserving reactions in the transport cycle. In particular, further study of the uncoupled mutants such as the proton-leaky carriers described here should help to identify critical domains in the lactose carrier which, because of their effect on energy barriers to uncoupled transport, play at least a permissive role in energy transduction.

REFERENCES

Uncoupled Proton Transport by the Lactose Carrier

Characterization of Escherichia coli lactose carrier mutants that transport protons without a cosubstrate. Probes for the energy barrier to uncoupled transport.

S C King and T H Wilson


Access the most updated version of this article at http://www.jbc.org/content/265/17/9645

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/17/9645.full.html#ref-list-1