Replacement of Alanine 58 by Asparagine Enables the Melibiose Carrier of Klebsiella pneumoniae to Couple Sugar Transport to Na**

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The melibiose carrier of Klebsiella pneumoniae couples sugar transport to H⁺ and Li⁺, while that of Escherichia coli uses Na⁺ besides the other two cation species (Hama and Wilson, 1992). We have shown that the K. pneumoniae melibiose carrier is capable of recognizing Na⁺ when the amino-terminal 81 residues are replaced by the corresponding region of the E. coli melibiose carrier (Hama and Wilson, 1993). In this amino-terminal region there are 5 residues that are not conserved between the two carriers. In this study, we changed each of the 5 residues of the K. pneumoniae carrier to the one in the E. coli carrier. The substitutions are Ile-36 → Val, Val-43 → Leu, Leu-54 → Trp, Ala-58 → Asn, and Cys-68 → Ala.

With four of the five mutants, Ile-36 → Val, Val-43 → Leu, Leu-54 → Trp, and Cys-68 → Ala, sugar accumulation was not affected by Na⁺. In striking contrast, melibiose and methyl-1-thio-β-D-galactopyranoside accumulation was greatly stimulated by Na⁺ with the Ala-58 → Asn mutant. Furthermore, Na⁺ uptake coupled to downhill melibiose transport was observed with the Ala-58 → Asn mutant. These results indicate that the Ala-58 → Asn substitution enables the K. pneumoniae melibiose carrier to couple sugar transport to Na⁺. It is clear that the Asn-58 residue (Asn-54 in the E. coli carrier) is involved in Na⁺ recognition.

All living cells take up various solutes from the environment. One common mechanism for solute uptake is cation-substrate cotransport, which can be found in cells ranging from bacteria to mammals. In general, the coupling cation for cotransport systems is correlated to the ion species that is primarily involved in the energy transduction at the membrane in which they are located. For example, many of the cotransporters in the mammalian plasma membrane, where Na⁺ is the primary cation for energy transduction, use this cation for cotransport. On the other hand, in bacterial membranes where H⁺ is the primary cation for energy transduction, H⁺-solute cotransporters are most common. There are interesting exceptions, such as the Escherichia coli melibiose carrier, which is a cytoplasmic membrane protein responsible for cotransport of α- and β-galactosides with monovalent cations. Although H⁺ is the primary ion that is involved in energy transduction at the cytoplasmic membrane of E. coli, this carrier uses Na⁺, Li⁺, or H⁺. Furthermore, the preferable coupling cation is variable depending on sugar substrates (see Tsuchiya et al. (1985) for a review). This unique feature makes the carrier a useful system to study the mechanism of cation-sugar coupling.

The melibiose carrier of E. coli is encoded by the melB gene. The melibiose carrier consists of 469 amino acid residues (Yazyu et al., 1984) and is considered to have 12 transmembrane α-helices (Botfield et al., 1992). Several groups have been studying cation and sugar recognition sites of this carrier. Approximately 30 different mutants have been isolated, which shows changes in cation or sugar recognition (Yazyu et al., 1985; Kawakami et al., 1988; Botfield and Wilson, 1988). Pourcher et al. (1991, 1993) made site-specific mutants at aspartate 31, 51, 55, and 120, which are located in the putative transmembrane regions, and showed that these residues are in or near the Na⁺ binding site. Wilson and Wilson (1992) also showed that aspartate 51 and 120 are important for Na⁺-stimulated melibiose accumulation.

Recently we undertook another approach to learn more about the Na⁺ recognition site in the melibiose carrier. We cloned and sequenced the melB gene of Klebsiella pneumoniae and showed that this carrier uses H⁺ and Li⁺ but not Na⁺ (Hama and Wilson, 1992). Although its primary structure was 78% identical to the E. coli melibiose carrier, we were not able to identify region(s) involved in Na⁺ recognition by comparing the amino acid sequences, because the differences were seen throughout the molecule. Therefore, we made chimeric carriers to narrow the choice of the amino acid residues on which we should focus. When the amino-terminal 81 residues of the K. pneumoniae carrier were replaced by the corresponding 77 residues of the E. coli carrier, the resulting chimeric carrier, E2K10, was able to couple sugar transport to Na⁺ (Hama and Wilson, 1993). There are only 5 amino acid residues that are not conserved between the two carriers in this amino-terminal region, indicating that one (or more) of the 5 residues must be directly involved in Na⁺ recognition.

In this study we changed each one of the 5 residues of the K. pneumoniae carrier to the one found in the E. coli carrier. With four of the five mutants, Ile-36 → Val, Val-43 → Leu, Leu-54 → Trp, and Cys-68 → Ala, Na⁺ had no effect on sugar transport. In striking contrast, the Ala-58 → Asn mutation had a remarkable effect. This mutant had the ability to couple sugar transport to Na⁺. These results show that Asn-58 (Asn-54 in the E. coli carrier) is involved in Na⁺ recognition.

EXPERIMENTAL PROCEDURES

Materials—[α-32P]dATP and [14C]lactose were purchased from Amersham Corp. [14C]TMG was purchased from DuPont-NEN. [3H]Melibiose was a generous gift from Dr. Gérard Leblanc of Département de Biologie du Commissariat à l'Energie Atomique, Villefranche-sur-Mer, France. The nonradioactive sugars were obtained from Sigma. Restriction endonucleases and T4 DNA ligase were used.

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The abbreviation used is: TMG, methyl-1-thio-β-D-galactopyranoside.
pSUL54W; with 0.1 mM [3H]melibiose (0.2 pCi/ml) at 23 °C for 10 min in the
purchased from Stratagene Cloning Systems. The Sequenase kit was
pSUC68A.
pSUI36V; absence
LiCl (gray
DWl(pcn) cells expressing each carrier were washed and incubated
with 0.1 mM [3HTMG (0.2 pCi/ml) at 23 °C for 10 min in the
absence (white bar) or presence (black bar) of 10 mM NaCl or 10 mM
LiCl (gray bar). Wild type, DW1(pcn)/pSUBS25; I36V, DW1(pcn)/
pSUJ54V; V43L, DW1(pcn)/pSU43V; L54W, DW1(pcn)/
pSUL54W; A58N, DW1(pcn)/pSU58N; C68A, DW1(pcn)/
pSU68A.

FIG. 1. Effect of Na+ and Li+ on melibiose accumulation. DW1(pcn) cells expressing each carrier were washed and incubated with 0.1 mM [3H]melibiose (0.2 pCi/ml) at 23 °C for 10 min in the absence (white bar) or presence (black bar) of 10 mM NaCl or 10 mM LiCl (gray bar). Wild type, DW1(pcn)/pSUBS25; I36V, DW1(pcn)/pSUJ54V; V43L, DW1(pcn)/pSU43V; L54W, DW1(pcn)/pSUL54W; A58N, DW1(pcn)/pSU58N; C68A, DW1(pcn)/pSU68A.

Table I
Oligodeoxynucleotides used for site-directed mutagenesis

| Oligonucleotide* | Sequence | Location* |
|------------------|----------|-----------|
| KpmelAC          | TCGGTACCCGGGGATCTACGC | -163 to -155' |
| KpC              | GACCTGGAATTCATCGATTTTCAATG | -12 to 15 |
| Kp151A           | GAAAACCCTGGCTAAGG    | 465 to 453 |
| KpT36VC          | CTACCCAGATGGTGTTGCGTATA | 96 to 117 |
| KpI36V           | TAACGCAACAACATCGGTTAG | 96 to 117 |
| KpV43LC          | ATCCGTCGGGGCCTGGTGCCACC | 117 to 137 |
| KpV43LA          | GGTGCAACACCCGGCAAGGCAGT  | 117 to 137 |
| KpL54WA          | GGGTATCGCATCCCGAGGCTTGCAC | 148 to 174 |
| KpL54WA          | CTGGCAAAAGTCCGGATGATCC | 148 to 174 |
| KpC68AA          | CGACCGGTAGCCGTTAAGCATC | 192 to 213 |

*Oligonucleotides with the suffix "C" and "A" represent coding and anticoding sequences, respectively.
The sequence of KpmelAC corresponds to the junction of the vector and the insert of pSUBS25. The 12-base sequence preceding the base -163, which contains the Smal site, is derived from the multiple cloning site of pSU2718.

Table II
Stimulation of melibiose accumulation by cations

| Carrier  | Stimulation* | NaCl | LiCl |
|----------|--------------|------|------|
| Wild type| 1.0          | 0.6  |      |
| Ile-36→Val| 1.1          | 0.7  |      |
| Val-43→Leu| 1.1          | 0.7  |      |
| Leu-54→Trp| 1.0          | 1.4  |      |
| Ala-58→Asn| 3.2          | 2.2  |      |
| Cys-68→Ala| 1.0          | 0.9  |      |

*Stimulation was calculated from the values shown in Fig. 1.

FIG. 2. Effect of Na+ and Li+ on TMG accumulation. DW1(pcn) cells expressing each carrier were washed and incubated with 0.1 mM [3HTMG (0.2 pCi/ml) at 23 °C for 10 min in the absence (white bar) or presence (black bar) of 10 mM NaCl or 10 mM LiCl (gray bar). Wild type, DW1(pcn)/pSUBS25; I36V, DW1(pcn)/pSUJ54V; V43L, DW1(pcn)/pSU43V; L54W, DW1(pcn)/pSUL54W; A58N, DW1(pcn)/pSU58N; C68A, DW1(pcn)/pSU68A.

Table III
Effect of NaCl and LiCl on lactose accumulation

| Carrier  | Lactose accumulation* | NaCl | LiCl |
|----------|-----------------------|------|------|
| Wild type| 0.3                   | 0.3  | 1.3  |
| Ile-36→Val| 0.3                   | 0.3  | 1.3  |
| Val-43→Leu| 0.3                   | 0.4  | 1.7  |
| Leu-54→Trp| 0.3                   | 0.3  | 0.6  |
| Ala-58→Asn| 0.1                   | 0.6  | 3.8  |
| Cys-68→Ala| 0.3                   | 0.3  | 0.9  |

*DW1(pcn) cells expressing each carrier were incubated with 0.2 mM [3H]lactose for 60 min.
These were added to give a final concentration of 10 mM.

Site-directed Mutagenesis—Site-directed mutagenesis was carried out by the overlap extension method (Ho et al., 1989). Oligonucleotides used as primers are listed in Table I. DNA amplification was carried out with Pfu DNA polymerase as follows: a cycle of 5 min at 95 °C, 1 min at 40 °C, and 30 s at 75 °C; 29 cycles of 1 min at 95 °C, 1 min at 40 °C, and 30 s at 75 °C; 5 min at 75 °C. One hundred pmoles of each primer was added to a total of 100 μl of reaction mixture. pSUBS25 (3 ng) was used as a template for the first set of amplification. The combination of primers for the first amplification were: KpmelAC-KpV43LA and KpV43LC-Kp151A for the Val-36→Val mutation; KpmelAC-KpL54WA and KpL54WC-Kp151A for the Leu-54→Trp mutation; KpI36VA-KpC68AA and KpC68AC-Kp151A for the Ala-58→Asn mutation; Kp151A-KpL54WA and KpL54WC-Kp151A for the Cys-68→Ala mutation. After the first amplification, the reaction mixtures were diluted 100-fold, and 5 μl of each was used as a template for the second amplification. The second amplification was

purchased from New England Biolabs. Pfu DNA polymerase was purchased from Stratagene Cloning Systems. The Sequenase kit was purchased from United States Biochemical. All other chemicals were reagent grade.

Bacterial Strains and Plasmids—Plasmid copy number mutation (pcn:2Tn10) (Lopilato et al., 1986) was transferred into E. coli DW1 (lacZΔ[ZY] melAΔ[AB]) (Wilson and Wilson, 1987) and DW2 (lacZΔ[ZY] melAΔ[Δβ]) (Bothfield and Wilson, 1988) by P1 transduction. The resulting strains, DW1(pcn) and DW2(pcn), were used as host strains for transport assay and sugar fermentation assay, respectively. E. coli TG1 (Δlac-pro super thi hsdS5 F' traD36 proAB lacF' lacZAM15) (Amersham International) was used as a host strain for cloning and plasmid isolation.

pSUBS25 (Hama and Wilson, 1992), which has the BglII-Smal 2.5-kilobase fragment containing the K. pneumoniae melB gene at the BamHI-HincII sites of pSU2718 (Martinez et al., 1988), was used as a source of the wild type gene.
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FIG. 3. The uptake of Na⁺ and H⁺ driven by downhill melibiose entry into cells. Na⁺ and H⁺ uptake measurements were carried out as described under “Experimental Procedures.” After a base line value was recorded for 2–3 min, an anaerobic 1 M melibiose solution was added at the point indicated by arrows to give a final concentration of 10 mM. Wild type, DW1(pcN)/pSUBS25; AsnN, DW1(pcN)/pSUAS8N.

carried out with primers KpmelAC-Kp151A for Ile-36–Val, Val-43–Leu, and Leu-54–Trp mutations and KpIC-Kp151A for Ala-58–Asn and Cys-68–Ala mutations. To clone DNA fragments containing Ile-36–Val, Val-43–Leu, or Leu-54–Trp mutation, the amplified products were digested with SmaI and BamHI of which recognition sites were in the KpmelAC primer and in the melB gene (positions 156 and 236), respectively. DNA fragments containing each mutation were used to replace the corresponding region of pSUBS25. With the Ile-36–Val and the Val-43–Leu mutants, the 80-base pair BamHI-BamHI fragment (156–236), which was lost during this cloning procedure, was inserted later. This was not necessary for the Leu-54–Trp mutant because the first BamHI site had been disrupted to create the mutation. The resulting plasmids, pSU386V, pSU439L, and pSU548W, were sequenced. DNA fragments containing the Ala-58–Asn or the Cys-68–Ala mutation were cloned into the Smal site of pUC18 and sequenced. The BamHI-BamHI 80-base pair fragment (156–236) containing each mutation was subsequently cut out and used to replace the corresponding segment of pSUBS25. The resulting plasmids were named pSUAS8N and pSUC68A.

Sequencing was carried out with double strand plasmid DNA by the chain termination method (Sanger et al., 1977) using T7 DNA polymerase (Sequenase, United States Biochemical). In all cases the melB genes were expressed constitutively in DW1(pcN).

Sugar Transport Assay—Cells were grown in LB medium containing 10 μg/ml tetracycline and 30 μg/ml chloramphenicol. Transport assay was carried out as described previously (Hama and Wilson, 1993).

Measurement of Cation Movement—Cells were grown in LB medium containing 10 μg/ml tetracycline and 30 μg/ml chloramphenicol. Proton and Na⁺ uptake coupled to sugar transport was measured as described previously (Hama and Wilson, 1983). Melibiose was added to give a final concentration of 10 mM.

RESULTS

Five amino acid residues of the K. pneumoniae melibiose carrier, Ile-36, Val-43, Leu-54, Ala-58, and Cys-68, were changed to Val, Leu, Trp, Asn, and Ala, respectively, by site-directed mutagenesis. At first, we intended to overproduce the mutated carriers using the pKK223–3 (Pharmacia KLB Biotechnology Inc.) expression vector. However, the growth of the cells overexpressing these carriers was very slow and deletions of a part of the melB gene occurred frequently. We therefore used pSUBS25, which has been successfully used for the physiological study of the K. pneumoniae melibiose carrier to clone the mutated genes. A part of the melB gene containing each mutation was used to replace the corresponding region of the wild type melB gene of pSUBS25 as described under “Experimental Procedures.” The plasmids pSU386V, pSU439L, pSU548W, pSUAS8N, and pSUC68A express melibiose carriers with Ile-36–Val, Val-43–Leu, Leu-54–Trp, Ala-58–Asn, and Cys-68–Ala mutations, respectively. During the construction of these plasmids, we used pmt strains as hosts. This mutation has been shown to lower the copy number of multicopy plasmids (Lopilato et al., 1986). pmt strains carrying each of the plasmids grew much faster than the corresponding strain without the pmt mutation (data not shown). When transformed with each of the plasmids, colonies of DW2(pcN), which has the 3-galactosidase but lacks its own melibiose carrier, turned red on melibiose-MacConkey agar plates indicating that all of the mutant carriers had melibiose transport activity (data not shown).

Effect of Cation on Sugar Transport—Each plasmid containing either one of the mutated genes or the normal melB gene was placed in DW1(pcN), which lacks both the melibiose carrier and the 3-galactosidase, and used for physiological study. When exposed to radioactive melibiose in the absence of NaCl or LiCl, DW1(pcN)/pSUBS25, which expressed the wild type carrier, accumulated this sugar to a concentration 90 times higher than that in the external medium (Fig. 1). The accumulation was not affected by adding NaCl and was slightly lowered by LiCl. This result is consistent with what we obtained with DW1(pcN)/pSUBS25 (Hama and Wilson, 1992). With DW1(pcN)/pSU386V, pSU439L, or pSUC68A, melibiose accumulation was about 70% of that with the wild type carrier. The effect of NaCl and LiCl on melibiose accumulation with the three mutants was the same as with the wild type carrier. Melibiose accumulation in the cells carrying pSU548W was slightly lower than in the cells with the wild type carrier in the absence of cations (about 60% of the wild type), and NaCl had no effect. LiCl stimulated the accumulation of this sugar 1.4-fold (Fig. 1 and Table II), suggesting the possibility that the Leu-54–Trp mutant had acquired the ability to couple melibiose transport to Li⁺. In striking contrast, melibiose accumulation in DW1(pcN)/pSUAS8N was only 22-fold (or 23% of normal) in the absence of cations and greatly stimulated by adding NaCl or LiCl. The Na⁺ and Li⁺ stimulation were 3.2- and 2.2-fold, respectively. This result strongly suggests that the Ala-58–Asn mutation enables the carrier to couple melibiose transport to Na⁺ or Li⁺.

TMG transport is coupled to H⁺ and Li⁺ in the K. pneumoniae melibiose carrier (Hama and Wilson, 1992). TMG accumulation in DW1(pcN)/pSUBS25 was 9.3-fold without added cations (Fig. 2). Li⁺ stimulated the accumulation 6-fold while Na⁺ had no effect with the wild type carrier. With Ile-36–Val, Val-43–Leu, and Cys-68–Ala mutants, TMG accumulation in the absence or presence of cations was about the same as with the wild type carrier. In the cells expressing the carrier with Leu-54–Trp or Ala-58–Asn mutations the accumulation was very poor in the absence of cations (1.3 and 1.0-fold, respectively), while it was comparable with the others in the presence of Li⁺, indicating the possibility of impaired H⁺-TMG coupling. A striking result is that Na⁺ stimulated TMG accumulation in DW1(pcN)/pSUAS8N 18-fold, while there was no effect with the Leu-54–Trp mutant. This result is consistent with the idea that the Ala-58–Asn mutant can use Na⁺ for sugar transport.

Lactose is a relatively poor substrate for the K. pneumoniae melibiose carrier. The cells with the K. pneumoniae carrier accumulated this sugar only in the presence of Li⁺ (Hama and Wilson, 1992). The accumulation of lactose was very low when DW1(pcN) was used as a host strain, and Li⁺ stimulation was barely seen (Table III). With Ile-36–Val, Val-43–Leu, Leu-54–Trp, and Cys-68–Ala mutants, lactose accumulation was as low as in the case with the wild type carrier.

2 The nucleotide numbering for the melB gene of K. pneumoniae is based on Hama and Wilson (1992).
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**Fig. 4. The location of the substitutions in a model of the K. pneumoniae melibiose carrier.** The model is based on the one for the E. coli melibiose carrier (Botfield et al., 1992). Amino acid numbering is different from the one for E. coli, because the K. pneumoniae carrier has 4 additional residues at the amino terminus. The 5 residues mutated in this study are indicated by filled circles. The aspartic acid residues in the putative transmembrane regions (Asp-55, -59, -69, and -124) are marked with ©. These Asp residues correspond to positions 31, 51, 55, and 120 in the E. coli carrier.

However, in the presence of Li⁺, cells expressing the Ala-58→Asn mutant carrier accumulated lactose 3 times as much as the cells with the wild type carrier. It was not clear if Na⁺ had a stimulatory effect.

**Cation Movement Induced by Downhill Melibiose Transport—**When a high concentration of substrate of a cotransporter is added to cell suspensions, rapid downhill entry of the substrate occurs through the carrier and is associated with entry of cations into the cells. The decrease of the extracellular concentration of the cation caused by such uptake can be measured by an ion-selective electrode. H⁺ and Na⁺ uptake coupled to melibiose transport has been demonstrated with the E. coli melibiose carrier (Tschiya and Wilson, 1978) and chimeric carriers derived from the E. coli and the K. pneumoniae melibiose carriers (Hama and Wilson, 1993). We tested melibiose-induced Na⁺ uptake by DW1(pcn)/pSUAS25. As we reported previously, no Na⁺ uptake was observed when 10 mM melibiose was added to cells expressing the K. pneumoniae melibiose carrier (Fig. 3). On the other hand, rapid Na⁺ uptake was observed with DW1(pcn)/pSUAS58N. This result unambiguously indicates that melibiose transport is coupled to Na⁺ in the Ala-58→Asn mutant.

H⁺ uptake coupled to downhill melibiose transport was observed with both DW1(pcn)/pSUAS25 and DW1(pcn)/pSUAS58N (Fig. 3), indicating that melibiose transport is coupled with H⁺ in both the wild type and the mutant carrier. The initial rate was somewhat lower with the Ala-58→Asn mutant. Because the accumulation of melibiose and TMG was also lower in the cells with the Ala-58→Asn mutant than with the wild type carrier in the absence of cation (Figs. 1 and 2), it appears that H⁺-coupled pathway is less efficient in the mutant.

**DISCUSSION**

The most important observations with the mutant Ala-58→Asn were: 1) melibiose and TMG accumulation was stimulated by Na⁺, and 2) Na⁺ uptake was coupled to downhill melibiose transport. It is concluded that this mutant couples sugar transport to Na⁺. This study provides strong evidence for the idea that the region surrounding the position 58 of the K. pneumoniae melibiose carrier (54 in the E. coli carrier) forms a critical part of the Na⁺ recognition site.

The Leu-54→Trp mutant seems to have Li⁺-coupled melibiose transport activity. With the E2K10 chimeric carrier, which contains all of the five substitutions made in this study, Na⁺ and Li⁺ stimulated melibiose accumulation to the same extent. Because Li⁺, but not Na⁺, was effective on melibiose accumulation with the Leu-54→Trp mutant and Na⁺ was more effective than Li⁺ with the Ala-58→Asn mutant, the effect of the cations on melibiose transport with the E2K10 carrier appears to be caused by the combination of these two substitutions.

The locations of the 5 residues targeted in this study are shown in Fig. 4. The Ala-58 is very close to the Asp-59 and the Asp-58 residues, which have been shown to be important for Na⁺ recognition in the E. coli carrier (Pourcher et al., 1991; Wilson and Wilson, 1992; Pourcher et al., 1993). In addition, the Ala-58 may also be close to the Asp-35 and the Asp-124, which are also implicated in Na⁺ recognition by studies in E. coli. Thus, the 4 Asp residues and the Asn-58 may form a Na⁺ binding "pocket" in the mutant melibiose carrier. The way the Asn residue interacts with Na⁺ could be either direct or indirect. The replacement of Ala-58 by Asn might enable the carrier to form an additional hydrogen bond required to bind Na⁺. Alternatively, Asn-58 (Asn-54 in E. coli) might be necessary for the correct conformation of one (or more) of the Asp side chains, which may form hydrogen bonds with Na⁺. Further mutagenesis study at the position 58 (54 in E. coli) would provide valuable information to answer these questions.

It is of interest to note that Ala-58→Asn mutant has higher lactose transport activity than the wild type K. pneumoniae carrier (Table III). In other words, the mutation caused improved recognition for both Na⁺ and lactose. Botfield and Wilson (1988) identified 22 single mutations that altered both Li⁺ and TMG recognition. These observations suggest that the cation and the sugar recognition sites may overlap.

The fact that H⁺-sugar coupling is slightly lowered in the Ala-58→Asn mutant (Figs. 1–3) suggests that the mutant has acquired functional Na⁺ recognition site at the expense of a slight distortion of the H⁺ recognition site. Thus, Na⁺ and H⁺ recognition sites in the melibiose carrier appear to overlap. According to a systematic analysis of primary structure of transporters carried out by Marger and Saier (1993), Na⁺-solute cotransporters make distinct groups from H⁺-solute cotransporters and facilitators. We believe the melibiose carriers could be considered as intermediate type, because they have the capacity to bind either H⁺ or Na⁺. Another example
is the alanine carrier of thermophilic bacteria, which is one of the few transporters with sequence similarity to the melibiose carrier and also uses both H+ and Na+ (Kamata et al., 1992).

We speculate that spontaneous Na+-coupled mutants of the K. pneumonia melibiose carrier would arise under certain selective pressure such as alkaline pH, high external Na+ concentration, or high temperature, because Na+-coupled cotransporters are found in bacteria living in these environments (Dimroth, 1987; de Vrij et al., 1989). It would be an interesting system in which a transporter undergoes diversification in response to the environment.

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REFERENCES
Botfield, M. C., and Wilson, T. H. (1988) J. Biol. Chem. 263, 12909-12915
Botfield, M. C., Noguchi, K., Tsuchiya, T., and Wilson, T. H. (1992) J. Biol. Chem. 267, 1818-1822

de Vrij, W., Bulthuis, R. A., van Iwaarden, P. R., and Koning, W. N. (1988) J. Bacteriol. 171, 1118-1125
Dimroth, P. (1987) Microbiol. Rev. 51, 329-340
Hama, H., and Wilson, T. H. (1992) J. Biol. Chem. 267, 18371-18376
Hama, H., and Wilson, T. H. (1993) J. Biol. Chem. 268, 10060-10065
Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K., and Pease, L. R. (1989) Gene (Amst.) 77, 51-59
Kamata, H., Akiyama, S., Morosawa, H., Ohta, T., Hamamoto, T., Kambe, T., Kagawa, Y., and Hira, H. (1992) J. Biol. Chem. 267, 21650-21655
Kawakami, T., Akizawa, Y., Ishikawa, T., Shimamoto, T., Tsuda, M., and Tsuchiya, T. (1986) J. Biol. Chem. 261, 14376-14380
Lopatin, J., Bortner, S., and Beckwith, J. (1986) Mol. & Gen. Genet. 205, 285-290
Margr, M. D., and Saier, M. H., Jr. (1988) Trends Biochem. Sci. 13, 13-20
Martínez, E., Bartolome, S., and de la Cruz, F. (1988) Gene (Amst.) 68, 159-162
Pourcher, T., Deckert, M., Baseliana, M., and Leblanc, G. (1991) Biochem. Biophys. Res. Commun. 178, 1179-1185
Pourcher, T., Zani, M.-L., and Leblanc, G. (1993) J. Biol. Chem. 268, 3209-3215
Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463-5467
Tsuchiya, T., and Wilson, T. H. (1978) Membr. Biochem. 2, 63-79
Tsuchiya, T., Wilson, D. M., and Wilson, T. H. (1985) Ann. N. Y. Acad. Sci. 456, 542-549
Wilson, D. M., and Wilson, T. H. (1987) Biochim. Biophys. Acta 904, 191-200
Wilson, D. M., and Wilson, T. H. (1992) J. Bacteriol. 174, 3083-3086
Yazyu, H., Shiota-Niya, S., Shimamoto, T., Kanazawa, H., Futai, M., and Tsuchiya, T. (1984) J. Biol. Chem. 259, 4320-4326
Yazyu, H., Shiota, S., Futai, M., and Tsuchiya, T. (1985) J. Bacteriol. 162, 853-857