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Homologous Sugar Transport Proteins in Escherichia coli and Their Relatives in Both Prokaryotes and Eukaryotes

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Homologous sugar transport proteins in *Escherichia coli* and their relatives in both prokaryotes and eukaryotes

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Separate proteins for proton-linked transport of D-xylose, L-arabinose, D-galactose, L-rhamnose and L-fucose into *Escherichia coli* are being studied. By cloning and sequencing the appropriate genes, the amino acid sequences of proteins for D-xylose/H^+ symport (XylE), L-arabinose/H^+ symport (AraE), and part of the protein for D-galactose/H^+ symport (GalP) have been determined. These are homologous, with at least 28 % identical amino acid residues conserved in the aligned sequences, although their primary sequences are not similar to those of other *E. coli* transport proteins for lactose, melibiose, or D-glucose. However, they are equally homologous to the passive D-glucose transport proteins from yeast, rat brain, rat adipocytes, human erythrocytes, human liver, and a human hepatoma cell line. The substrate specificity of GalP from *E. coli* is similar to that of the mammalian glucose transporters. Furthermore, the activities of GalP, AraE and the mammalian glucose transporters are all inhibited by cytochalasin B and *N*-ethylmaleimide. Conserved residues in the aligned sequences of the bacterial and mammalian transporters are identified, and the possible roles of some in sugar binding, cation binding, cytochalasin binding, and reaction with *N*-ethylmaleimide are discussed. Each protein is independently predicted to form 12 hydrophobic, membrane-spanning α-helices with a central hydrophilic segment, also comprised of α-helix. This unifying structural model of the sugar transporters shares features with other ion-linked transport proteins for citrate or tetracycline.

Introduction

In 1963 Peter Mitchell suggested that transport of nutrients into the cells of microorganisms might employ a trans-membrane gradient of protons to energize the uptake process (Mitchell 1963). The idea required that an individual transport system catalyse the obligatory translocation of protons with a nutrient molecule, 'symport', or the experimentally indistinguishable 'antiport' of hydroxyl ions, so that energy released by respiration or ATP hydrolysis and 'stored' as the electrochemical gradient of protons could drive accumulation of the nutrient (Mitchell 1961, 1963, 1973). The principle is illustrated in figure 1. The mechanism was originally conceived to account for the respiration-dependence and uncoupler-sensitivity of the lactose transport system of *Escherichia coli* (LacY; Kennedy 1970). Direct evidence for the coupling of lactose and proton movement was obtained when Ian West devised experimental conditions that revealed a sugar-promoted alkaline pH change in appropriately-induced strains of *E. coli* (West 1970; West and Mitchell 1972, 1973). The involvement of the respiration-generated electrochemical gradient of protons in lactose transport was further confirmed by exploiting the sensitivity of subcellular vesicles to ionophores and uncoupling

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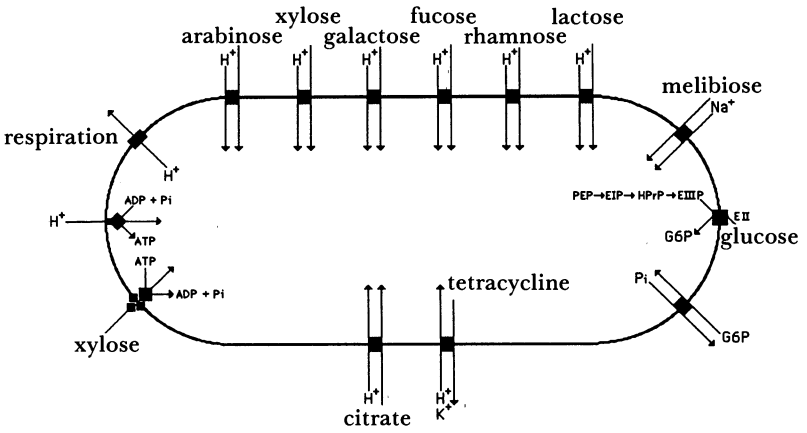

FIGURE 1. An illustration of selected transport systems in *Escherichia coli*. The large oval represents the cytoplasmic membrane of the microorganism. A trans-membrane electrochemical gradient of protons is generated by respiration, depicted on the top left. This gradient is used for the energisation of ATP synthesis (left) or uptake of sugars (top) and citrate (bottom) via a series of substrate/H^+ symporters; it can also energize tetracycline/H^+ efflux via an antiporter (bottom). Other transport mechanisms are sugar/Na^+ symport (top right), PEP-sugar phosphotransferase (right), hexose-P/Pi exchange (bottom right) and binding protein dependent transport, probably energized by ATP (bottom left).

agents (reviewed by Kaback (1972, 1987)). We have combined these approaches with the use of appropriate mutants to characterize at least five other proton-linked sugar transport systems in *Escherichia coli*. They catalyse transport of L-arabinose, D-xylose, D-galactose, L-rhamnose, or L-fucose (figure 1; table 1) (Henderson & Macpherson 1986; Bradley *et al.* 1987). There are additional mechanisms for transport of sugars and related compounds into *E. coli*, such as the phosphotransferase systems, ATP-dependent binding-protein systems, sugar/Na^+ symport and hexosephosphate/Pi antiport (figure 1); these are the subjects of other articles in this volume

TABLE 1. HOMOLOGOUS SUGAR TRANSPORT PROTEINS IN DIFFERENT ORGANISMS

sugar	organism	evidence	reference
D-xylose	*Escherichia coli*	primary sequence	Maiden *et al.* (1987)
L-arabinose	*Escherichia coli*	primary sequence	Maiden *et al.* (1987)
D-galactose	*Escherichia coli*	cytochalasin binding	Cairns *et al.* (1989)
lactose	*Kluyveromyces lactis*	primary sequence	Chang & Dickson (1988)
D-glucose	*Leishmania donovani*	cytochalasin binding	Zilberstein *et al.* (1986)
D-glucose 1	*Saccharomyces cerevisiae*	primary sequence	Celenza *et al.* (1988)
D-glucose 2	rat brain	primary sequence	Birnbaum *et al.* (1986)
	human hepatoma	primary sequence	Mueckler *et al.* (1985)
	human erythrocyte	primary sequence	Mueckler *et al.* (1985)
	human and rat adipocyte	DNA probe	Flier *et al.* (1988)
	human and rat heart	DNA probe	Flier *et al.* (1988)
D-glucose 3	rat liver	primary sequence	Thorens *et al.* (1988)
	rat intestine	DNA probe	Thorens *et al.* (1988)
	rat kidney	DNA probe	Thorens *et al.* (1988)
	rat β-islet pancreas	DNA probe	Thorens *et al.* (1988)
D-glucose 4	rat adipocyte	primary sequence	James *et al.* (1989)
	rat heart	primary sequence	James *et al.* (1989)
	rat muscle	DNA probe	James *et al.* (1989)

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and will not be considered further here. (See papers by Lengeler *et al.*, Kornberg, Quiocho, Higgins *et al.*, Pourcher *et al.*, Maloney, this symposium).

By cloning and sequencing the *araE* and *xylE* genes of *E. coli* (Davis & Henderson 1987; Maiden *et al.* 1988) it was found that the predicted amino acid sequences of the D-xylose/H^+ and L-arabinose/H^+ transport proteins (AraE and XylE) were substantially homologous (Maiden *et al.* 1987). Surprisingly, each one exhibited a similar degree of homology to the D-glucose transport proteins from a human hepatoma cell line and from rat brain (Mueckler *et al.* 1985; Birnbaum *et al.* 1987; Maiden *et al.* 1987; Baldwin & Henderson, 1989). Another surprise was their *lack* of similarity, at least at the primary sequence level, with the proteins for transport of lactose/H^+ or melibiose/Na^+ (LacY and MelB) into the *same* organism, *E. coli* (Muller-Hill *et al.* 1980; Yazyu *et al.* 1984). Furthermore, the glucose/Na^+ transport protein from mammalian intestine was unlike any of the sugar transporters from either mammals or other organisms (Hediger *et al.* 1988).

Recent evidence shows that the family of homologous sugar transport proteins listed in table 1 occurs in organisms as diverse as bacteria, yeasts, rat and man. In this article we examine selected properties of the members of that family that occur in *E. coli*; their substrate specificities, the effects of certain inhibitors, and their aligned sequences, and compare them with their eukaryote relatives. The recurrence of certain motifs in the primary sequences are highlighted, together with the presence or absence of residues that might be expected to interact with the sugars, the proton, or the inhibitors. We consider their possible relation with transport proteins for the apparently unrelated substrates, citrate and tetracycline, and present a unifying two-dimensional model for the structure of all the homologous proteins in the membrane.

Recognition of substrate molecules by the sugar transport systems

Proton-linked transport systems can be assayed by measuring substrate-promoted pH changes in de-energized suspensions of appropriately-induced intact bacterial cells (Henderson & Macpherson 1986) or by measuring uptake of radioisotope-labelled substrate into right-side out subcellular vesicles (Kaback 1972; Henderson 1986). By utilizing different sugars for these assays the substrate specificity of an individual transporter can be extensively characterized. If a desired sugar is not available in a radioisotope-labelled form, then its interaction with a transport system may be determined by measuring its competition with an established substrate; it is then important to distinguish between actual transport of the substrate and binding without transport ('dead-end' inhibition) (Cleland 1963; Deves & Krupka 1978). Substrate–inhibitor specificity can also be determined by measuring their ability to displace reagents such as *N*-ethylmaleimide or cytochalasin B (see below).

The results of these studies are summarized in table 2. As far as possible, the substrates are ranked in the order of their affinities for each individual transport system. This is not completely reliable, because different assay methods had to be used for different transporters; for example, the mammalian D-glucose transporter does not translocate protons. The structures of the sugars are shown in figure 2 to facilitate comparison between substrates. Such comparison will aid the elucidation of the molecular mechanism of substrate recognition by the structurally and functionally related transport proteins.

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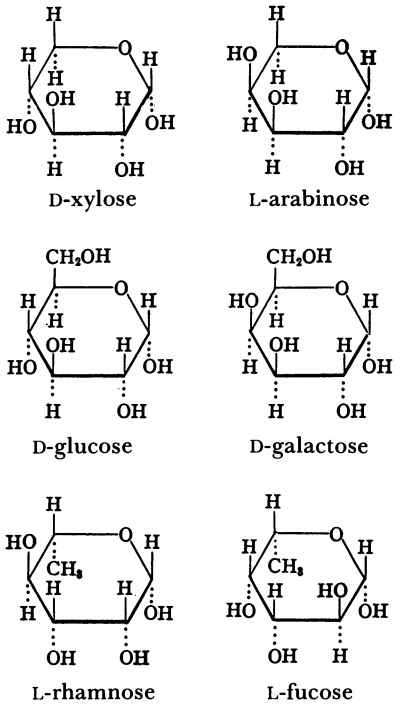

FIGURE 2. Substrates for sugar transport systems.

TABLE 2. MOLECULAR RECOGNITION BY SUGAR TRANSPORTERS

xylose/H^+ symporter (*E. coli*)	arabinose/H^+ symporter (*E. coli*)	galactose/H^+ symporter (*E. coli*)	glucose transporter (erythrocyte)	glucose transporter (adipocyte)
D-xylose	L-arabinose	D-glucose	2-deoxy-D-glucose	6-F-D-galactose
	5-CH_3-L-arabinose (D-fucose)	2-deoxy-D-glucose	D-glucose	D-glucose
		D-galactose	6-deoxy-D-glucose	6-deoxy-D-glucose
	5-FCH_2-L-arabinose (6-F-D-galactose)	6-F-D-glucose	D-mannose	2-deoxy-D-galactose
	D-xylose	6-F-D-galactose	D-galactose	D-galactose
		6-deoxy-D-glucose	2-deoxy-D-galactose	6-deoxy-D-galactose (D-fucose)
		D-talose	D-xylose	D-talose
		2-deoxy-D-galactose	L-arabinose	3-deoxy-D-glucose
		D-mannose	6-deoxy-D-galactose (D-fucose)	D-xylose
		6-deoxy-D-galactose (D-fucose)		L-arabinose
		D-xylose		

The L-arabinose and D-xylose transporters of E. coli

The D-xylose/H^+ transporter (XylE) of *E. coli* is very fastidious; only D-xylose is a substrate. In particular, it does not transport the corresponding 5-methylpentose or D-hexose (6-deoxy-D-glucose or D-glucose). Furthermore, pentoses with a different orientation of an —OH residue in either 2-, 3- or 4-position in the pyranose ring are not substrates. Thus the D-xylose/H^+ transporter can discriminate against L-arabinose (figure 2).

The L-arabinose/H^+ transporter (AraE) of *E. coli* is more tolerant of variations in sugar

structure. The corresponding 5-methylpentose (6-deoxy-D-galactose (D-fucose)) is an even better substrate and so is 6-deoxy-6-fluoro-D-galactose. However, it does not bind D-galactose, revealing a finely-tuned discrimination against the 6-hydroxyl group. AraE does bind the C-4-epimer, and so transports D-xylose (figure 2), albeit with a reduced affinity, but it does not accept epimers with a different orientation of —OH residues in either the C-3 or C-2 position in the pyranose ring. As the primary sequences of their proteins are homologous (see below) such discrimination must result from quite subtle differences in the three-dimensional shape of the proteins.

Neither methyl-α-D-xylose nor methyl-β-D-xylose interact with XylE, nor does methyl-β-L-arabinose interact with AraE, as judged by their failures to elicit sugar/H^+ symport in appropriately-induced strains of *E. coli* (P. J. F. Henderson, unpublished data). Therefore, substitutions at the 1-position of the sugar would seem to exclude its transport by these proteins.

The D-galactose transporter of E. coli

The D-galactose/H^+ transporter (GalP) of *E. coli* does not accept either of the pentoses, L-arabinose or D-xylose, but the corresponding methylpentoses are substrates (table 2). Thus the presence of the 6-methylene group is a prerequisite for binding, which contrasts with the situation for XylE (above). The presence of the C-6 hydroxyl residue enhanced sugar binding (table 2), rather than preventing it as in the case of AraE (above). The preferred position of the C-4 hydroxyl is the *gluco* configuration, so that the best substrate for GalP is D-glucose, though this is not of physiological relevance because D-glucose does not induce biosynthesis of the D-galactose transporter. (D-Glucose is transported into *E. coli* by a phosphotransferase mechanism; see Lengeler *et al.* and Kornberg, this symposium). As D-gulose is not a substrate (data not shown), GalP does not accommodate changes in the configuration of the C-3 hydroxyl residue. However, the absence or inversion of the C-2 hydroxyl residue is unimportant because other substrates include 2-deoxy-D-glucose, 2-deoxy-D-galactose, D-mannose, and D-talose (table 2).

Substitution of a methyl residue in the α- or β-positions of D-galactose or D-glucose abolished the ability to elicit sugar/H^+ symport activity of GalP. Curiously, however, methyl-β-D-galactoside effectively protected the GalP protein against labelling with *N*-ethylmaleimide (Henderson *et al.* 1983). This compound may be a dead-end inhibitor of GalP activity but more investigation is needed to establish the point.

The D-glucose transporters of mammalian cells

Substrate recognition by the human-erythrocyte D-glucose transporter was catalogued some time ago (LeFevre 1961; Barnett *et al.* 1973). Its substrate specificity is very similar to that of GalP (table 2), with perhaps a greater preference for D-glucose over D-galactose. Rees & Holman (1981) examined the specificity of the rat adipocyte transporter for sugars (table 2). They concluded the following.

> 'The important hydrogen bonding positions on the sugar appear to be the ring-oxygen, the β-position at C-1, a hydroxyl in the *gluco* configuration at C-3 and to a lesser extent at C-6. The C-2 and possibly the C-4 hydroxyls seem to be relatively unimportant. In many respects, therefore, the adipocyte sugar transport system resembles that of the human erythrocyte (Barnett *et al.* 1973). The most obvious differences, however, appear to indicate an apparent lack of specificity of the adipocytes system for the C-4 and C-6 positions.... Also, the loss of the C-6 hydrogen bond is less important when compared with the human erythrocyte systems.'

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It appears that the differences in the sugar specificities of the mammalian D-glucose transporters and the *E. coli* GalP protein are relatively minor. As the rat brain D-glucose transporter is reported to be virtually identical in sequence to the human erythrocyte transporter (Birnbaum *et al.* 1987; Mueckler *et al.* 1985), then (some of) the differences between its sequence and that of the rat adipocyte transporter (65% identity; James *et al.* (1989)) should account for their differences in substrate recognition identified by Rees & Holman (1981) (see above). Intriguingly, the sequence of the GalP protein is likely to be much more similar to AraE than to the D-glucose transporters.

The L-rhamnose and L-fucose transporters of E. coli

L-Rhamnose is 6-deoxy-L-mannose and L-fucose is 6-deoxy-L-galactose; they can be regarded as either 6-deoxy-hexoses or 5-methyl-pentoses (figure 2). The L-rhamnose/H^+ or L-fucose/H^+ transporters of *E. coli* accept the corresponding hexoses or pentoses, albeit with reduced affinity, so loss of the normal substrate's methyl group or substitution of an —OH group in it does not prevent binding (Bradley *et al.* 1987; Muiry 1989). However, each transport protein recognizes the correct orientation of the —OH at the C-2, C-3 or C-4 position in the pyranose ring and so a sugar differing in only one of these configurations is not a substrate (Muiry 1989). Thus the L-rhamnose transporter accepts L-rhamnose, L-mannose and L-lyxose, but not L-fucose, L-galactose and D-arabinose, which are substrates of the L-fucose transporter. It is not yet known whether these transport proteins are similar to each other at the amino acid sequence level, but it seems that the L-rhamnose/H^+ transporter of *Salmonella typhimurium*, at least, is not homologous to the transporters listed in table 1 (Muiry 1989).

Inhibitors of sugar H^+ symport

N-ethylmaleimide

When incubated with a sulphydryl reagent, such as *N*-ethylmaleimide, the *E. coli* transport proteins for L-arabinose/H^+, D-xylose/H^+ and D-galactose/H^+ lose 70–90% of their activity (Macpherson *et al.* 1981, 1983; Henderson & Macpherson 1986; Davis 1986). The appropriate sugar substrates protect these proteins against *N*-ethylmaleimide. In this respect they resemble the lactose/H^+ and melibiose/Na^+ symporters (Fox & Kennedy 1965; Carter *et al.* 1968; G. Leblanc, personal communication), despite the differences in their amino acid sequences. However, the transport of L-fucose/H^+ or L-rhamnose/H^+ is insensitive to *N*-ethylmaleimide under the same conditions (P. J. F. Henderson, T. P. McDonald & S. A. Bradley, unpublished data).

The protection by substrates was exploited to label the susceptible transport proteins, AraE, XylE and GalP, with radioactive *N*-ethylmaleimide; they were identified as proteins that migrated with an apparent relative molecular mass (M_r) of 35000–40000 in SDS-polyacrylamide gels (Macpherson *et al.* 1981, 1983; Henderson *et al.* 1983; Henderson & Macpherson 1986; Davis 1986). By similar procedures the lactose/H^+ transport protein was the first to be identified, with an apparent M_r of about 30000 (Jones & Kennedy 1969). Such labelling experiments are very important for the identification of hydrophobic transport proteins of low abundance (0.3–1.0% of the membrane proteins), which are refractory towards the usual methods of solubilisation and purification.

The D-glucose transporter of human erythrocytes is also inhibited by sulphydryl reagents (Bloch 1974). However, in this case the presence of a transport substrate *accelerates* the rate of

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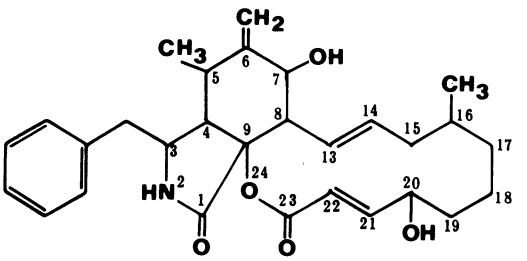
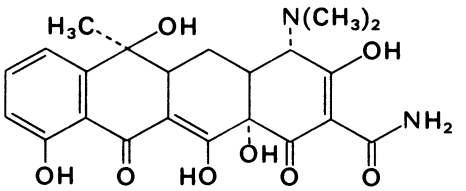
inactivation by, e.g. *N*-ethylmaleimide (Rampal & Jung 1987). These results imply that the environment of a sulphydryl group is affected by the binding of a sugar to the transport proteins. However, it is not easy to identify the residue(s) involved (see below).

Cytochalasin B

Cytochalasin B (figure 3*a*) is a fungal product that inhibited mammalian D-glucose transport proteins (Jung & Rampel 1977), and covalently labelled them when irradiated with U.V. light (Carter-Su *et al.* 1982; Shanahan 1982; Baly & Horuk 1988).We found that it inhibited the transport of L-arabinose via AraE or D-galactose via GalP (but not D-xylose via XylE) into vesicles made from appropriately-induced strains of *E. coli* (T. P. McDonald & P. J. F. Henderson, unpublished data). When activated by UV light the L-arabinose and D-galactose transport proteins (AraE and GalP) reacted covalently with radioactive cytochalasin B, and they could be identified on SDS-polyacrylamide gel electrophoresis as labelled proteins of apparent M_r 36000–38000; appropriate sugar substrates in the incubation protected the transporters against this labelling (Smith *et al.* 1989; Cairns *et al.* 1989).



cytochalasin B



tetracycline

FIGURE 3. The structures of cytochalasin B and tetracycline.

These results reinforced the conclusion that the prokaryote and eukaryote sugar transport proteins are structurally similar and the identification of the AraE and GalP transport proteins by using *N*-ethylmaleimide was confirmed. The corollary is that all transport proteins that interact with cytochalasin B are likely to be related. The D-glucose transport protein of *Leishmania donovani* (Zilberstein *et al.* 1986; Baly & Horuk 1988) is included in the family of transporters in table 1 for this reason. Because the L-fucose/H^+, L-rhamnose/H^+, and lactose/H^+ transporters are not susceptible to cytochalasin B it might be concluded that they are not structurally related to this family. However, this suggestion must be treated with caution, because attempts to inhibit or photolabel the XylE transporter with cytochalasin B have failed, despite its homology to those in table 1. Cytochalasin B will be an invaluable tool in the elucidation of the subtle differences between these sugar transport proteins, and in the determination of their structures and molecular mechanisms.

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6-*Deoxy*-D-*glucose*

This sugar analogue was not a substrate for XylE in the sugar/H^+ symport assay, although it did inhibit the transport of D-xylose, the natural substrate (E. O. Davis 1986; B. J. McKeown, unpublished data). In addition, 6-deoxy-D-glucose was more effective in protecting XylE against reaction with *N*-ethylmaleimide than D-xylose (E. O. Davis & P. J. F. Henderson, unpublished data). Although it was not a substrate for AraE in the sugar/H^+ symport assay, 6-deoxy-D-glucose protected AraE against cytochalasin B. By contrast, 6-deoxy-D-glucose was a good substrate for GalP (table 2).

These preliminary observations implied that 6-deoxy-D-glucose either bound to the normal (possibly external) sugar binding site of XylE and AraE, but failed to trigger the events leading to sugar/H^+ symport, or perhaps that it bound to a second (possibly internal) site that may also interact with cytochalasin B (Walmsley 1988). More investigations are required to confirm and expand these initial observations; however, the availability of dead-end inhibitors, which 6-deoxy-D-glucose and cytochalasin B appear to be, will be important to exploit kinetic measurements of the bacterial transporter activities to the level that has already been valuable in mechanistic studies of the mammalian D-glucose transporter (Deves & Krupka 1978; Lowe & Walmsley 1986; Walmsley 1988).

Amino acid sequence homologies between sugar transport proteins from prokaryotes and eukaryotes

Escherichia coli

The sequences of the amino acids in the arabinose/H^+ and xylose/H^+ transport proteins of *E. coli* were established from the DNA sequences of their genes (Davis & Henderson 1987; Maiden *et al.* 1987, 1988). The AraE and XylE proteins were homologous, with 141 identical residues out of 472 and 491, respectively (figure 4) (Maiden *et al.* 1987). There were additional conservative substitutions throughout the proteins, so that nearly 40% of the aligned sequences could be regarded as homologous (Maiden *et al.* 1987; Baldwin & Henderson 1989). Only the 96 N-terminal residues of the *E. coli* D-galactose/H^+ transporter (GalP) have been sequenced so far, but 72 were identical with those in the aligned L-arabinose/H^+ transporter (D. C. M. Moore & P. J. F. Henderson, unpublished data). If this very high degree of identity persists throughout the AraE and GalP sequences, the differences should reveal features that enable discrimination between the pentose and the hexose, as defined in the studies described above. Because GalP and AraE appear to be more closely related to each other than they are to XylE, they presumably resulted from a more recent gene duplication and subsequent divergence (Doolittle 1981; von Heijne 1987).

The sequences of the lactose/H^+ and melibiose/Na^+ transport proteins of *E. coli* were

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Figure 4. The aligned amino acid sequences of transport proteins. The single letter code for amino acids is employed for the following transport proteins: Xyl, xylose/H^+ symporter of *E. coli* (Davis & Henderson 1987); Gl1, glucose transporter of *Saccharomyces cerevisiae* (Celenza *et al.* 1988); Gl2, the glucose transporter of a human hepatoma cell line (Mueckler *et al.* 1985); Ara, arabinose/H^+ symporter of *E. coli* (Maiden *et al.* 1988); Cit, plasmid-encoded citrate/H^+ symporter (Sasatsu *et al.* 1985; Ishiguro *et al.* 1985); Tet, plasmid pBR322-encoded tetracycline/H^+ antiporter (Bertrand 1983). The dotted rectangles enclose hydrophobic regions predicted to form membrane-spanning α-helices.

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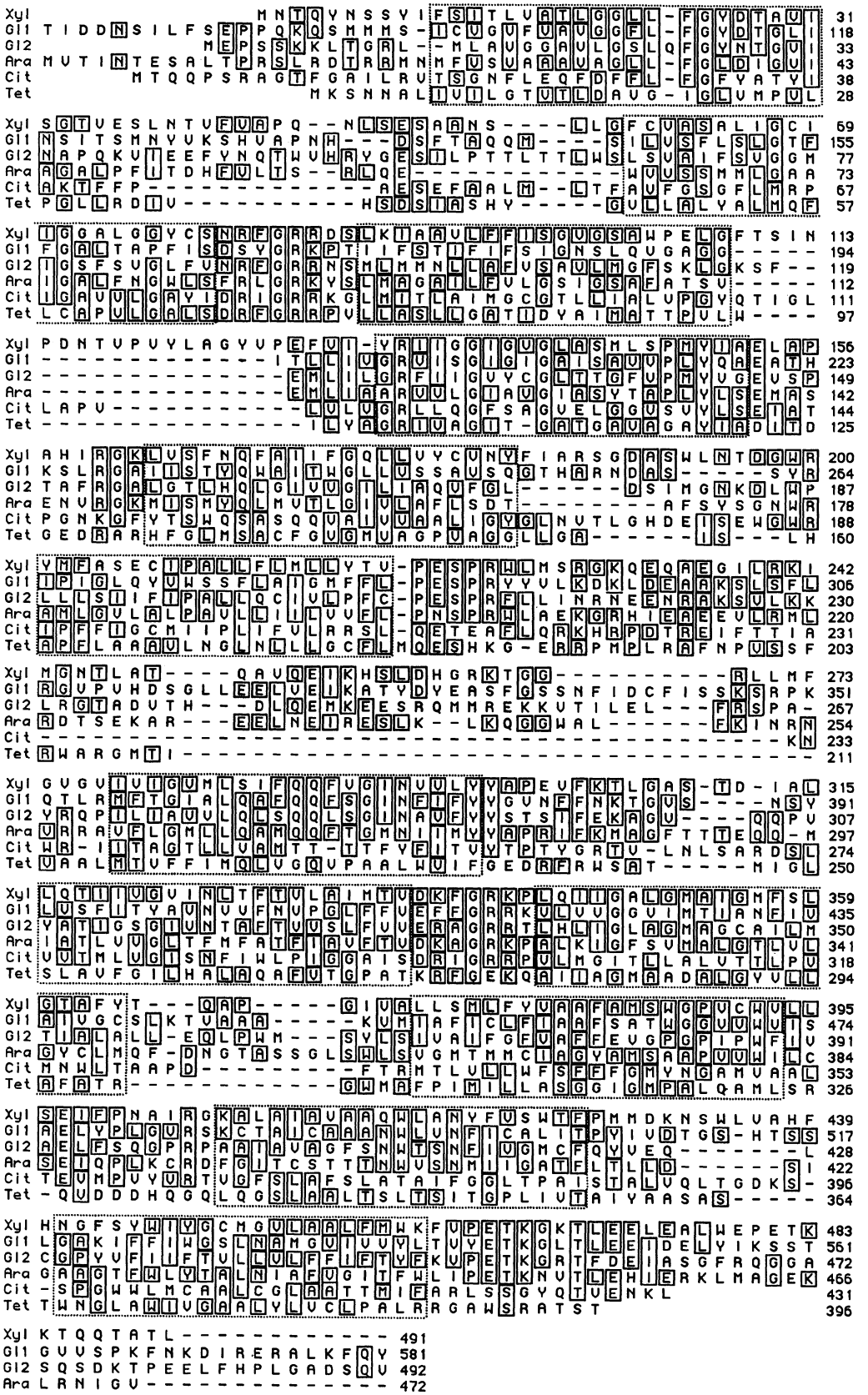

FIGURE 4. For description see opposite.

deduced from their gene DNA sequences by Buchel *et al.* (1980) and by Yazyu *et al.* (1984), respectively. Curiously, considering the similarity of their function (sugar/cation symport) they were not similar at the primary sequence level either to each other or to the monosaccharide transport proteins (table 1). Furthermore, the primary sequence of the L-rhamnose/H^+ transport protein of *Salmonella typhimurium* is completely different from any of these (Muiry 1989); however, the three-dimensional structures of all these proteins may be much more similar than their sequences indicate.

Yeasts

The existence of proton-linked and facilitated diffusion transport systems for sugars in various species of yeasts is well established (Eddy 1982; Kotyk 1983). The primary sequence of a D-glucose transport protein from *Saccharomyces cerevisiae* was recently deduced from the DNA sequence of the *SNF*3 gene (Celenza *et al.* 1988). Out of 884 residues in the amino acid sequence those from 86 to 581 were 28–31 % identical with the bacterial transporters AraE and XylE and with the mammalian D-glucose transporters discussed below; the C-terminal 87 residues were not necessary for transport function (Celenza *et al.* 1988) (figure 4). The primary sequence of a lactose transport protein from *Kluyveromyces lactis* was deduced from the DNA sequence of the *LAC12* gene (Chang & Dickson 1988). Out of 587 residues those from 117 to 530 revealed homology to residues 60–480 of the sequences of AraE, XylE and the mammalian D-glucose transporters, even though the number of identities between the monosaccharide transporters was greater than between any one of them and the yeast disaccharide transporter.

The yeast lactose transport protein was not similar to the LacY protein of *E. coli* (Chang & Dixon 1988). Thus two proteins of very different primary sequence have evolved to transport the same sugar (it is not yet clear whether the yeast protein catalyses sugar/H^+ symport or facilitated diffusion). It remains to be determined whether the two proteins have similar three-dimensional structures.

Mammalian D-glucose transport proteins

Mueckler *et al.* (1985) cloned and sequenced cDNA encoding a facilitated diffusion D-glucose transport protein from the human hepatoma HepG2 cell line. Of its 492 residues 28–31 % were identical to those in the aligned AraE and XylE pentose/H^+ transporters from *E. coli* (Maiden *et al.* 1987) (figure 4) and, taking into account conservative substitutions, each bacterial protein was as similar to the human protein as they were to each other. Immunological and peptide sequence data indicated that the hepatoma protein was very similar, if not identical, to the intensively-studied D-glucose transporter of human erythrocytes (Wheeler & Hinkle 1986; Baly & Horuk 1988; Baldwin & Henderson 1989). The D-glucose transporter of rat brain was virtually identical, with only seven different residues out of 492 (Birnbaum *et al.* 1986). There is probably another related D-glucose transporter in brain (Kayano *et al.* 1988).

A rat-liver D-glucose-transport protein, cloned sequenced and expressed in *E. coli*, was substantially homologous to the hepatoma–brain–erythrocyte transporter, but with about 55 % identical residues in the aligned sequences (Fukumoto *et al.* 1988; Thorens *et al.* 1988). These structural differences must account for the different kinetic properties of the liver protein (Ciaraldi *et al.* 1986; Baly & Horuk 1988), which are presumably allied to the special physiological role of D-glucose transport in liver tissue (Nordlie 1985; Baly & Horuk 1988). Hybridization experiments showed that it also occurred in intestine, kidney, and β-pancreatic islet cells (Thorens *et al.* 1988).

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James *et al.* (1989) sequenced cDNA of the insulin-regulatable D-glucose transporter from rat adipose tissue. Its predicted amino acid sequence was 65% identical to that of the HepG2–brain transporter. The differences between the various types of D-glucose transporters (table 1) appeared to be most significant in the third predicted membrane-spanning segment (James *et al.* 1989), see below, and presumably reflected different properties necessary for the special physiological role of D-glucose transport in each tissue (Baly & Horuk 1988). DNA–RNA hybridization and immunological experiments indicated that the insulin-regulatable D-glucose transporter was present in brown and white adipose tissue, heart and red and white skeletal muscle, but not in brain, liver, or HepG2 cells (James *et al.* 1989).

Therefore, mammals have a series of different D-glucose transport proteins expressed to different levels in each tissue, each with a particular role in the body's mechanisms for capturing, distributing, or utilizing D-glucose (Kayano *et al.* 1988; Baly & Horuk 1988).

D-Glucose transport proteins in other organisms

D-Glucose/H^+ symport activities have been reported in species of the alga, *Chlorella* (Komor & Tanner 1974), in a few of the cyanobacteria, e.g. *Synechocystis*, Nostoc Mac and *Plectonema* (Raboy & Padan 1978; Beauclerk & Smith 1978; Flores & Schmetterer 1986) and in the protozoan parasite *Leishmania donovani* (Zilberstein & Dwyer 1985). It seems likely that these, and the D-glucose transport proteins in other organisms from the primitive bacteria to the highest eukaryotes, will prove to be related when their sequences become available. A sucrose/H^+ transporter in plants (Giaquinta 1983; Reinhold & Kaplan 1984) may also be a member of this family.

Features of the aligned sequences

There are probably 12 *membrane-spanning* α*-helices*

When the hydropathic profile of each of the homologous sugar-transport proteins is analysed independently there appear to be 12 hydrophobic regions, predicted to be membrane-spanning α-helices (Mueckler *et al.* 1985; Maiden *et al.* 1987; Celenza *et al.* 1988; Chang & Dickson 1988). In some cases, uncertainty over the predicted position of these regions is overcome when the sequences are aligned according to the amino acid identities. One example of this is loop 11 of the AraE protein (Baldwin & Henderson 1989) (figures 4, 6). Faint evidence of duplication in the two halves of each protein (see below) suggests that the membrane-spanning regions should be regarded as two groups of six.

The predicted occurrence of 12 membrane-spanning α-helices is not restricted to the homologous sugar-transport proteins listed in table 1. It also occurs for the láctose/H^+ (Foster & Kaback 1983), glucose-6-phosphate/Pi (Friedrich & Kadner 1987), glycerol-3-phosphate/Pi (Eiglmeier *et al.* 1987), proline/Na^+ (Nakao *et al.* 1987) and possibly the melibiose/Na^+ (Pourcher *et al.*, this symposium), transporters of *E. coli*, the plasmid- or transposon- encoded citrate and tetracycline transporters (see below), and the uracil transporter of yeast (Jund *et al.* 1988). Perhaps this is a general property of many transport proteins, even when their amino acid sequences are not similar. However, there are exceptions, for example the rhamnose/H^+ transporter of *E. coli* (Muiry 1989) and the D-glucose/Na^+ transporter of intestine (Hediger *et al.* 1987).

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There is a central hydrophilic region

In all the sugar transport proteins there is a central hydrophilic region of 55–70 residues strongly predicted to contain a high proportion of α-helix. It contains several conserved regions, and may be identified with a protease-sensitive region of the erythrocyte D-glucose transporter that is cleaved only by trypsin applied to the cytoplasmic face of the membrane (Baldwin *et al.* 1980) (fig. 2 in Baldwin & Henderson 1989).

Significance of the conserved amino acid residues

When six different sugar transporters for L-arabinose (*E. coli*) or D-xylose (*E. coli*) or D-glucose (*S. cerevisiae*, human hepatoma, human liver, rat adipocyte) are aligned there are 45 absolutely conserved residues and about 150 conservative substitutions. These residues may be important features of the common structure and mechanism of these proteins. An analysis (Maiden 1986) of four transporters for L-arabinose, D-xylose, D-glucose and citrate (figure 4) (see below) showed that the most commonly conserved residues were glycine, proline, arginine and glutamate, the first two probably reflecting the unique structural properties of their side chains.

Aspartate, asparagine, glutamate and glutamine residues are involved in the H-bonded interaction of soluble proteins with sugar molecules in their binding sites (Quiocho 1986, this symposium). It is therefore of interest that these residues are highly conserved in some locations predicted to be within membrane-spanning helices (loops 1, 6, 7, 8, 10) (see figures 4, 6). Site-directed mutagenesis experiments could evaluate their possible roles in sugar binding.

The bacterial transporters, unlike the mammalian transporters, are energized by the co-transport of a proton with the sugar; hence, the proton-translocating residues might be conserved only in the bacterial proteins. Likely candidates for such residues are histidine, glutamate and aspartate. However, despite the proposed involvement of histidine in proton translocation by the functionally similar lactose/H^+ transporter of *E. coli* (Kaback 1987; Kaback *et al.*, this symposium), no histidine residues are conserved in the bacterial proteins (figure 4) (Baldwin & Henderson 1989). Some acidic residues are conserved in the bacterial but not the mammalian transporters, and are candidates for site-directed mutagenesis experiments to evaluate their role in cation translocation. It is uncertain whether the more recently characterized D-glucose transporters from *S. cerevisiae* or liver or adipose tissue (Celenza *et al.* 1988; Thorens *et al.* 1988; James *et al.* 1989) translocate cations.

In view of their inhibition by sulphydryl reagents (above), it is interesting that no cysteine residues are conserved in the aligned transporters (Maiden *et al.* 1987); such inhibition may stem from stearic hindrance of substrate binding or translocation, rather than from an essential mechanistic role of —SH groups. There are no cysteine residues in the N-terminal half of the AraE protein, so the substrate-protected residue(s) must be in the C-terminal part of AraE and, by extension, of the other proteins. Walmsley (1988) has suggested that a reagent-sensitive cysteine residue in the glucose transporter may be at position 347, or possibly 417, in the C-terminal half of the protein. Directed mutagenesis could confirm the location of such residues, as in the case of the lactose/H^+ transporter, where both peptide labelling–mapping and site-directed mutagenesis experiments identified Cys148 as the residue susceptible to *N*-ethylmaleimide (Beyreuther *et al.* 1980; Menick *et al.* 1987). However, this is predicted to occur in the N-terminal half of the LacY protein (Kaback 1987; Kaback *et al.*, this symposium).

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The RXGRR motif

Between the putative membrane-spanning helices 2 and 3 and helices 8 and 9 is a sequence motif RXGRR (figures 4, 6). The first and last R may be replaced by K; X is usually an amino acid with a large hydrophobic side chain, and it is often preceded by D or N. It is predicted to form a β-turn, linking the adjacent helices. The positively-charged side chains may interact with the head-group of lipids. The finding of this motif and the occurrence of twelve hydrophobic regions in a novel sequence are initial clues that the protein belongs to this homologous group (see below).

The PESPR and PETK motifs

These occur after the ends of the putative membrane-spanning helices numbers 6 and 12, respectively, in all the monosaccharide transporters (figures 4, 6). They are also found in the lactose transporter of *K. lactis* (Chang & Dickson 1988), but not in the citrate or tetracycline transporters (see below).

A diffused motif through helices 4 *and* 5

The following pattern is conserved in the region including putative helices 4 and 5 of the aligned monosaccharide transporters (figure 4):

R---G---G------P-Y--E------RG------Q-----G.

No gaps are inserted to achieve this matching. This motif presumably has structural or functional significance, or both, and it is conserved, albeit imperfectly, in the transport proteins for lactose (Chang & Dickson 1988), citrate, and tetracycline (figures 4, 6).

The tryptophan residue in helix 11

Photolabelling of a sugar transporter with cytochalasin B probably results from photoactivation of an aromatic amino acid residue in the protein rather than from activation of the inhibitor (Deziel *et al.* 1984) (see above). Peptide mapping experiments indicated that the covalently-bound cytochalasin B was located between Phe389 and Trp412 in the mammalian D-glucose transporter (Cairns *et al.* 1984; Holman & Rees 1987). The only aromatic residue conserved in this region of the bacterial transporters is a tryptophan in helix 11 (figures 4, 6). Experiments are being done to determine whether this is the actual site of labelling, and to discover why the XylE protein does not bind cytochalasin B.

Similarities between the citrate, tetracycline and sugar transporters

The citrate/H^+ *transporter*

A plasmid-encoded citrate transport activity is thought to be proton-linked (Reynolds & Silver 1983). The transport protein was sequenced via its gene by two groups independently (Sasatsu *et al.* 1985; Ishiguro & Sato 1985) and appeared to have only a slight similarity to the sugar transporters at the primary sequence level (Maiden *et al.* 1987). Nevertheless, when analysed with the algorithm of Eisenberg *et al.* (1984), the citrate transporter was also predicted to have two groups of six membrane-spanning α-helices separated by a central hydrophilic domain (Maiden 1986). Furthermore, between the predicted helices 2–3 and 8–9 were the DRXGRR motifs and through the predicted helices 4 and 5 was the diffused motif

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described above (figure 4). There were other similarities between the citrate transporter and individual sugar transport proteins (figure 4), but only a few more identities that occurred in every protein (figure 4). The most noticeable differences were the relative shortness of the central (24 residues) and the C-terminal (14 residues) hydrophilic regions in the citrate transporter, which largely account for its shorter length of 431 residues compared to 472–522 residues in the sugar transporters.

The tetracycline/H^+, K^+ transporter

One class of tetracycline genes encodes proteins that effect the active efflux of the antibiotic from the cell (McMurry *et al.* 1980; Levy 1984). The mechanism may be an obligatory coupling to the movement of protons (presumably antiport) or potassium ions (Dosch *et al.* 1984). The sequences of several such tetracycline transporters have been determined (Peden 1983; Waters *et al.* 1983; Hillen & Schollmeier 1983). They are extensively homologous to each other, but not obviously so to the sugar transporters (Levy 1987). One example is shown in figure 4. Nevertheless, each one is predicted to have twelve membrane-spanning α-helices by the same algorithm used to analyse the sugar transporters (figures 4, 5); between the putative helices 2 and 3 there is a perfect RXGRR motif (figures 4, 6); through the putative helices 4 and 5 is the diffused motif (figures 4, 5, 6); and between the putative helices 8 and

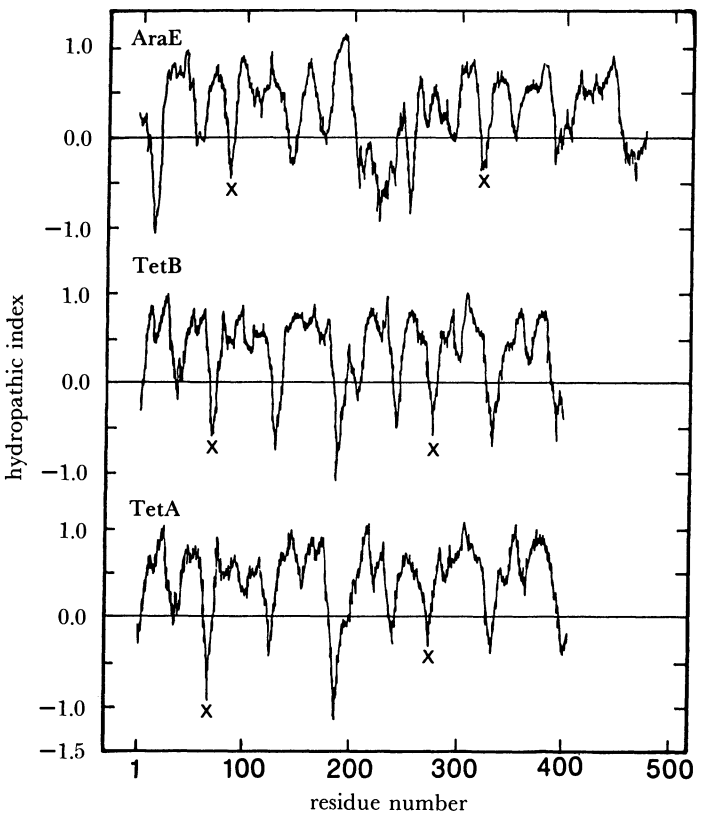

FIGURE 5. Comparison of the hydropathic profile of the arabinose/H^+ symporter (AraE) with the hydropathic profiles of the tetracycline/H^+ antiporters of *E. coli* (TetB is shown in figure 4). The profiles were calculated by the program CETH2 with a nine-residue span by using the hydrophobicity scale of Eisenberg *et al.* (1984). X indicates the positions of the RXGRR motifs discussed in the text.

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9 is RXGEK, similar to RXGRR (figures 4 and 6). There are other similarities in primary sequence between the tetracycline, citrate and individual sugar transporters (figure 4), but few of them are conserved in all the transporters. Superficially, the structures of tetracycline and cytochalasin B appear similar to each other (figure 3), or at least they resemble each other more than a monosaccharide does (but see Griffin *et al.* (1982)). Perhaps the cytochalasin binding site on a sugar transport protein resembles a tetracycline binding site?

These similarities permit the speculation that the tetracycline transporters, which are probably representatives of a wider class of antibiotic resistance factors, have three-dimensional structures in the membrane that are fundamentally similar to those of the series of homologous sugar transporters.

A MODEL FOR THE FOLDING OF THE TRANSPORT PROTEINS IN THE MEMBRANE

Many membrane proteins are believed to be folded so that hydrophobic α-helices are inserted through the membrane, joined by hydrophilic regions located in the aqueous environment (Kyte & Doolittle 1982; von Heijne 1988; Lodish 1988). There are very few proteins, bacteriorhodopsin from *Halobacterium halobium* and components of the photosynthetic reaction centre of *Rhodopseudomonas viridis*, where this model is supported by detailed structural

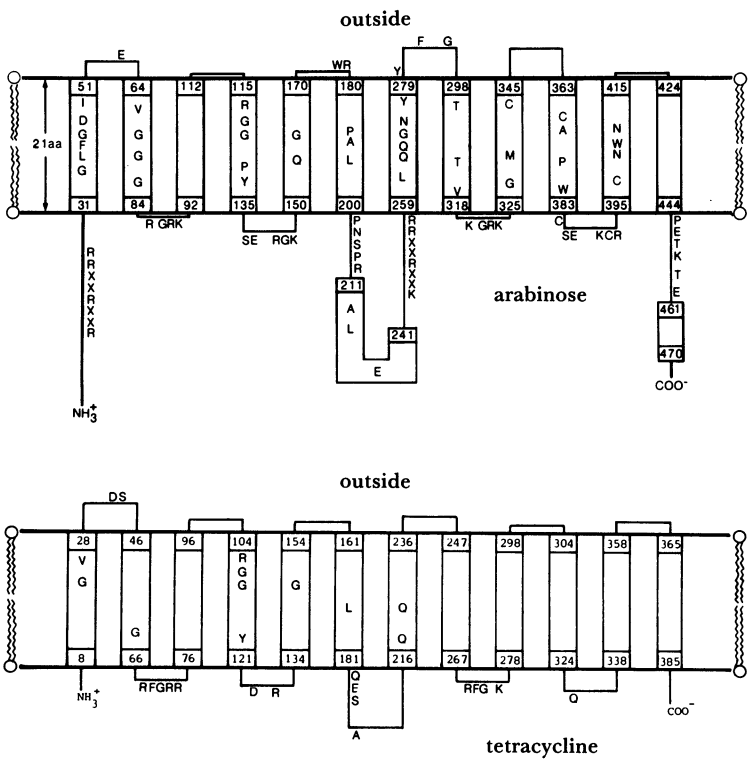

FIGURE 6. Proposed orientations of the arabinose/H^+ symporter and the tetracycline/H^+ antiporter in the membrane. The models are based on predictions of membrane-spanning regions and α-helix content and on the alignment with the glucose transporter (Eisenberg *et al.* 1984; Mueckler *et al.* 1985; von Heijne 1987. The 12 membrane-spanning segments are depicted as rectangles with their end residues numbered from the N-terminus. The rectangles outside the membrane represent predicted α-helices. The single residues marked are usually conserved in all five aligned sequences (figure 4) and some recur at equivalent positions in each half of the molecule.

information determined by electron diffraction or X-ray crystallographic measurements (Henderson & Unwin 1975; Henderson & Schertler, this symposium; Diesenhofer *et al.* 1985). Nevertheless, the burgeoning mass of primary sequence information shows that numerous other membrane proteins, including those involved in transport, also contain a series of hydrophobic regions, each sufficiently long to span the membrane as an α-helix, separated by hydrophilic regions. Models of membrane protein structure based on such sequence data alone should be viewed with caution (Lodish 1988), but they do generate testable hypotheses of structural features, for example that the N-terminus and the C-terminus are on the same side of the membrane, and they set criteria for comparisons between different proteins, as illustrated above.

Two examples of such models are given in figure 6, the L-arabinose/H^+ transporter of *E. coli* and the tetracycline/H^+K^+ transporter encoded on a plasmid. Importantly, they include the positions of residues conserved in all the monosaccharide transporters, four of which are shown in figure 4, and some sequence motifs apparently repeated in similar positions in each half of the molecule.

Concluding remarks

The family of sugar transport proteins described in this article has been found in such diverse organisms so far that it seems likely to occur in most forms of life. This implies that investigations of the molecular mechanism of their transport activities in microorganisms, with the attendant conveniences of experimental manipulation, will uncover features equally valid for their operation in higher organisms, including man. It seems that insight into the mechanism of transport of substrates other than sugars will also accrue, if the apparent similarities to the citrate and tetracycline (and possibly other) transporters described here are real. Also, the homologies described imply a fundamental similarity between proteins that catalyse facilitated diffusion ('passive transport') and substrate/H^+ symport ('secondary active transport'); a perceived difference in mechanism that seems not to require a profound difference in structure. The purpose of cataloguing their substrate and inhibitor specificities is to gain insight into the nature of the ligand binding sites and to define the relation between the transport proteins; the availability of a variety of sugars and their structural analogues and the known sequence homologies of the L-arabinose, D-xylose, D-galactose and D-glucose transporters, make this a particularly promising series of proteins in which to establish the characteristics of molecular recognition and their evolution (Fersht 1985; Quiocho 1986, this symposium). If the relatively unspecific D-glucose–D-galactose hexose transporters arose first, one can speculate that they then evolved to discriminate against the 6-OH residue and recognize the precise orientation of the 4-OH residue to become more specific for either of the pentoses, L-arabinose or D-xylose. When the substrate specificity of the homologous yeast lactose transporter (Chang & Dickson 1988) is known in more detail, then it may be possible to learn about molecular discrimination between mono- and di-saccharides. There also exist transport proteins with the same function of sugar–cation symport, but without any detectable similarity in primary sequence, i.e. the lactose/H^+, L-rhamnose/H^+ and melibiose/Na^+ transporters of Enterobacteria and the D-glucose/Na^+ transporter of mammalian intestine; perhaps these arose by convergent evolution.

A real understanding of all these phenomena is critically dependent upon determination of

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the three-dimensional structure of transport proteins at the level of atomic resolution. Tentative models of transport protein structure such as those in figure 6 are the basis of present and future experiments in which 'topological' protein reagents and antibodies, and gene fusions (Manoil & Beckwith 1986), and other techniques, are used to explore the actual folding of the protein in the membrane. The structural and functional roles of individual amino acids may also be evaluated by *in vivo* and *in vitro* mutagenesis (see Botfield & Wilson (1988); Kaback (1987); Kaback *et al.*, this symposium). Such approaches are the most practicable at the present time, but they are unlikely to yield a molecular model of sufficient detail to understand the different substrate specificities, the process of cation recognition, the mechanism of inhibitor action, the translocation mechanism, and the evolution of the related transport proteins described in this article. The determination of a more detailed three-dimensional structure probably requires crystallisation and X-ray diffraction analysis. The first step is the purification of sufficient stable, undenatured protein. This may be difficult to achieve for membrane proteins, but perhaps no more so than the apparent impossibility of establishing their primary sequences that we faced a decade ago.

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