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MINI-REVIEW

Anion Exchange Reactions in Bacteria

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Abstract

Bacterial anion exchange now includes both "carboxylate-linked" reactions, in which there is an antiport of mono- and dicarboxylic acids, and "Pi-linked" reactions that build on phosphate (Pi) and organic phosphates. To illustrate the general features of this expanding class, this article discussed the biochemistry, physiology, and molecular biology of Pi-linked antiporters that accept glucose 6-phosphate (G6P) as their primary substrate. Kinetic and biochemical analysis suggsts that Pi-linked exchangers have a bifunctional active site that accepts a pair of negative charges. For this reason, exchange stoichiometry moves between the limits of 2 : 1 and 2 : 2 to reflect the ratio of mono- and divalent substrates at either membrane surface. This results in a particularly interesting reaction sequence *in vivo*, where, because cytosolic pH is relatively alkaline, one can expect the asymmetric exchange of two monovalent G6P anions against a single divalent G6P. In this way, an otherwise futile self-exchange of G6P gives a net flux driven (indirectly) by the pH gradient. Despite this biochemical and physiological complexity, Pi-linked carriers resemble all other secondary carriers at a molecular level. Indeed, sequence analysis leads one to infer a common (albeit low resolution) structural theme in which each functional unit has two sets of six trans-membrane α helices separated by a central hydrophilic loop. Present examples show that this topology can derive from either a single protein, as is typical in bacteria, or from pairs of identical subunits, as found in mitochondria and chloroplasts. The finding of this common structure should make it possible to build detailed structural models that have implications for all membrane carrier proteins.

Key Words: Membrane transport; reconstitution phosphate transport; hydropathy; models; osmolytes.

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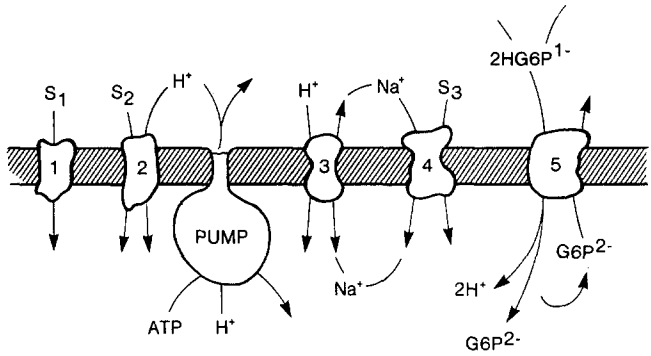

Fig. 1. Chemiosmotic circuits at the bacterial membrane. The functional organization of a membrane centers on its collection of pumps and carriers. The ones given here constitute a minimal set, as might be found in anaerobes or in facultative organisms growing under anaerobic conditions. The proton circulation is usually initiated at an F_0F_1 ATPase and completed by a number of secondary reactions. From Maloney (1987).

Chemiosmotic Circuits

Cell and organelle membranes are organized so as to construct one or more chemiosmotic circuits. In the bacterial world a simple circuit of this sort (Fig. 1) would specify a primary ion pump (almost always a proton pump) whose activity maintains both an electrical and a chemical (pH) gradient. These ion-motive gradients, in their turn, would drive solute transport by an accompanying set of secondary carriers. Because there are only a few different kinds of primary pumps (Maloney and Wilson, 1985), it is really the diversity among secondary carriers that confers flexibility as living systems interact with their environments. Indeed, as indicated at a later point, this diversity extends to include a secondary carrier that disguises itself as a primary pump.

Among secondary carriers, one recognizes three distinct reaction types. Thus, the diagram of Fig. 1 includes *uniport* (mediated by carrier No. 1), a reaction which seems to be of restricted value to bacteria; *symport* (Nos. 2 and 4), which is both widely distributed (Maloney, 1987) and well-studied (Kaback, 1986); and *antiport* (No. 3) or exchange. Cation antiporters are a frequent finding (Rosen, 1986), and these take on special significance if they support the simultaneous operation of ionic currents with different chemical specificity (e.g., for H^+ or Na^+, Fig. 1). The last example treated by Fig. 1 (No. 5) falls within the category of antiport and represents both the newest and most complex of secondary carriers found in bacteria—this *anion exchanger* mediates a bifunctional reaction that accepts either monovalent or divalent substrates in an overall neutral exchange. For example, during glucose 6-phosphate (6GP) transport, a pair of monovalent anions might be accepted at the external surface. Subsequently, in the relatively alkaline

cytoplasm, these monovalent substrates would be stripped of their accompanying protons to generate a pair of divalent anions, one of which would recycle to complete the neutral exchange.

In the present article there is a specific focus on these anion antiporters, which, as noted above, carry out the most elaborate reactions associated with secondary carriers. And because anion exchange during glucose 6-sugar phosphate transport is the best understood bacterial exchange mechanism, there will be an emphasis on such "Pi-linked" reactions. At the end, however, discussion will extend to include a new example, a "carboxylate-linked" exchange, whose properties are rather unusual.

Historical

Phosphate Transport and Exchange

The anion exchange illustrated in Fig. 1 is called Pi-linked to indicate that it can mediate exchanges among inorganic and organic phosphates. As a result, one can imagine three distinct reactions that might occur: (i) the self-exchange involving phosphate alone (Pi:Pi); (ii) the heterologous exchange of Pi and sugar phosphate (written as Pi : G6P, with unspecified stoichiometry); and (iii) an exchange based solely on movements of the organic substrates (G6P : G6P, as in Fig. 1). Understandably, the complexity of this behavioral phenotype might make it difficult to distinguish anion-exchange reactions from other transport mechanisms (Fig. 1), and for this reason, it is useful to precede a discussion of Pi-linked antiport with a short chronology of the study of phosphate transport in bacteria.

Multiple Pathways for Phosphate Transport

In the mid 1950's Mitchell reported that resting cells of *Micrococcus pyogenes* (now *Staphylococcus aureus*) showed a rapid Pi : Pi exchange based on a selective movement of the monovalent anion, $H_2PO_4^{-1}$ (Mitchell and Moyle, 1953; Mitchell, 1954). It was presumed that this reaction reflected a system whose normal function was to accumulate Pi during cell growth. A decade later, Harold *et al.* (1965) made quite different observations regarding Pi transport in another gram-positive cell, *Streptococcus faecalis*. In that case, no exchange reaction was found, and when the net flux was examined, it proved to have an absolute dependence on concurrent metabolism (ATP) and a selectivity favoring the divalent anion, HPO_4^{-2}.

These striking differences (*S. aureus* vs. *S. faecalis*) predicted a diversity appreciated only much later, when studies of *Escherichia coli* revealed four systems, each of which carried ^{32}Pi into the cell (Bennett and Malamy, 1970; Rosenberg *et al.*, 1977; Willsky and Malamy, 1980). Two of these (Pit and Pst) proved relatively specific for phosphate itself, while two others (UhpT

and GlpT) appeared to accept Pi as a low-affinity analog of the natural substrate—a hexose phosphate for UhpT (Deitz, 1976) or glycerol 3-phosphate for GlpT (Hayashi *et al.*, 1964).

We now know a good deal about the ways in which ^{32}Pi moves across *E. coli* membranes, Thus, Pst, which has an associated high-affinity Pi-binding protein, belongs to a family of "solute ATPases" that uses internal ATP to drive substrate transport (Berger, 1973; Bishop *et al.*, 1989). Pit, on the other hand is a simple chemiosmotic carrier mediating H^+/Pi symport (Rosenberg *et al.*, 1979). This paired use of a solute ATPase and a chemiosmotic carrier is often found in the organization of bacterial membranes, and the same is probably true of eukaryotes as well, but there the analysis is less complete.

Until recently, the other ^{32}Pi transporters in *E. coli* have also been considered, along with Pit, as H^+/anion symporters. But this classification is clearly incorrect. Instead, we now believe that systems taking ^{32}Pi come in two functional varieties—those designed for net Pi movement (Pst, Pit, etc.), and those that engage in anion exchange, using Pi or phosphorylated substrates (UhpT, GlpT, etc.). With hindsight, we would conclude that Harold *et al.*, (1965) described one in the former (dedicated) category, while Mitchell's (1954) initial discovery belongs to the second (exchange) class (Sonna and Maloney, 1988).

Experimental Evidence for Pi-Linked Anion Exchange

The idea that bacteria harbor Pi-linked anion exchange is based on three kinds of experiments, each at a different level of analysis. Work with intact cells allowed a thorough characterization of homologous Pi:Pi antiport and provided the first crucial clue as to physiologic function. On the other hand, use of membrane vesicles was essential to validation of exchange as a biochemical mechanism, since only this work has clearly ruled out other possible scenarios. Finally, studies of reconstituted protein in an artificial system have verified the initial conclusions and, unexpectedly, led to useful general tool for analysis of these and other membrane systems (see Maloney and Ambudkar, 1989).

Homologous ^{32}Pi:Pi Exchange in Streptococcus lactis

The fundamental properties of homologous Pi:Pi exchange have been best described in gram-positive cells. For example, the experiment given by Fig. 2 shows this reaction as it occurs in *S. lactis*. That work examined ^{32}Pi transport by washed cells suspended at pH 7. Such resting cells, which lack internal metabolizable reserves, have an internal Pi pool that is elevated to about 50 mM from its the usual level of 5 mM. Samples taken after addition of 20 μM ^{32}Pi showed that labeled Pi has a ready access to this pool, whether

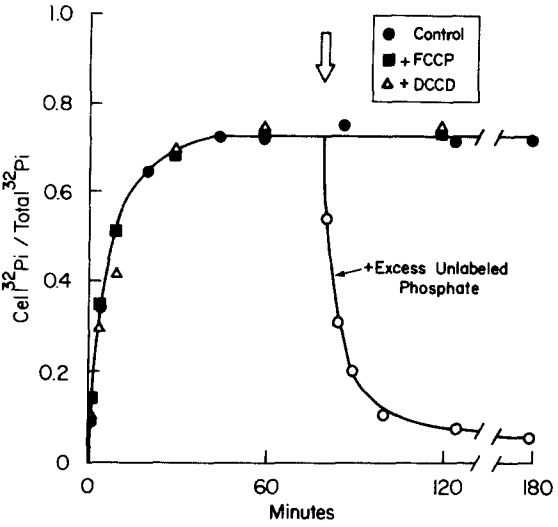

Fig. 2. Phosphate exchange in *Streptococcus lactis*. Incorporation of ^{32}Pi was monitored by centrifugation through silicone oil after addition of 20 μM ^{32}Pi to washed cells in 300 mM KCl and 20 mM MOPS/K (pH 7). At the arrow, part of the control suspension was given 4 mM KPi. As indicated, 10 μM FCCP was present or stock cells were treated for 30 min with 1 mM DCCD before assay. From Maloney *et al.* (1984).

or not assays were done in the presence of FCCP, a protonophore (*p*-trifluorocarbonylcyanidemethoxyphenylhydrazone), or DCCD (*N*, *N'*-dicyclohexylcarbodiimide), a covalent inhibitor of the F_0F_1 ATPase. These latter findings seemed paradoxical at first. The cell membrane was clearly "permeable" to ^{32}Pi, yet this same membrane sustained a Pi chemical gradient (2500-fold) even when sources of metabolic energy were interrupted. This conflict resolves in a simple way only if ^{32}Pi movement reflects the exchange of internal and external substrate. In *S. lactis* this suggestion was verified clearly by showing that the pool of external Pi (^{32}Pi *plus* ^{31}Pi) remained constant in size even though most of the added ^{32}Pi was taken up by cells (Maloney *et al.*, 1984).

Further work has revealed several distinctive features of Pi self-exchange. (1) The reaction does not distinguish between Pi and AsO_4. (2) The homologous exchange is electrically neutral. (3) There is an unusual selectivity that favors *mono*valent Pi (AsO_4) over the divalent anion; this conclusion derived initially from a kinetic analysis (Maloney *et al*, 1984; see also Mitchell, 1954), but this role for monovalent phosphate is also supported by studies of heterologous exchange (see below). (4) Finally, it seems clear that systems which display Pi : Pi exchange are really concerned with the transport of organic substrates. In *S. lactis*, for example, low levels of glucose, 2-deoxyglucose, or mannose 6-phosphate inhibit ^{32}Pi : Pi exchange (Maloney *et al.*

1984) and also act as high-affinity substrates during a neutral heterologous exchange (Ambudkar and Maloney, 1984). In *E. coli* the UhpT carrier shares this same substrates specificity (Deitz, 1976; Ambudkar and Maloney, unpublished), while GlpT accepts glycerol 3-phosphate as the primary substrate (Hayashi *et al.*, 1964; Ambudkar *et al.*, 1986a).

In its failure to discriminate between Pi and AsO_4 and in its use of the monovalent anion, ^{32}Pi:Pi antiport in *S. lactis* resembles that originally described for *S. aureus* (Mitchell, 1954). It was not surprising, then, to find that in *S. aureus* Pi-linked exchange also accepts sugar phosphates (Sonna and Maloney, 1988). In this case, the range of substrates resembles that found for *S. lactis* or *E. coli*, but also includes glycerol 3-phosphate (G3P), at low affinity. With clarification of the staphylococcal example, the finding of Pi exchange becomes diagnostic for this family of anion-exchange proteins.

UhpT Function in Membrane Vesicles of Escherichia coli

The work with intact cells (above) predicted that phosphate and sugar phosphate(s) should engage in a direct exchange. This antiport was demonstrated first by tests using Pi-loaded membrane vesicles of *S. lactis* (Ambudkar and Maloney, 1984), but the subsequent work with vesicles of *S. aureus* (Sonna and Maloney, 1988) and *E. coli* (Sonna *et al.*, 1988) has been the more significant. As well as verifying Pi:G6P antiport, these latter experiments have also ruled out the possibility that such exchange is a partial reaction by a proton-coupled symporter acting in the absence of the ion-motive gradient. Until ruled out, this explanation was a viable alternative to the model of antiport, since proton-coupled porters can show a prominent substrate exchange, often faster than the reaction of net flux (Wong and Wilson, 1970; Kaczorowski and Kaback, 1979). And, in fact, Essenberg and Kornberg (1975) had concluded that UhpT function in *E. coli* was mediated by a H^+/anion symport reaction. To address this issue directly, the method of Kaback (1971) was adapted to prepare right-side-out membrane vesicles *without* internal Pi. These vesicles were competent according to the usual criteria—they supported a cation-motive force near 140 mV and accumulated both Pi and proline by ion-coupled mechanisms during respiration. Yet these same vesicles could not take up glucose 6-phosphate (Fig. 3). Rather, sugar phosphate was found only when an internal pool of Pi had been set up by prior action of H^+/Pi symport. This experiment established UhpT function as having a dependence on substrate(s) *in trans*, a dependence that is understood in a simple way only when anion exchange forms the basis of sugar phosphate transport.

Reconstitution of Pi-Linked Exchange

Many of the general features of Pi-linked antiport have been confirmed in an artificial system by using methods of reconstitution to analyze the

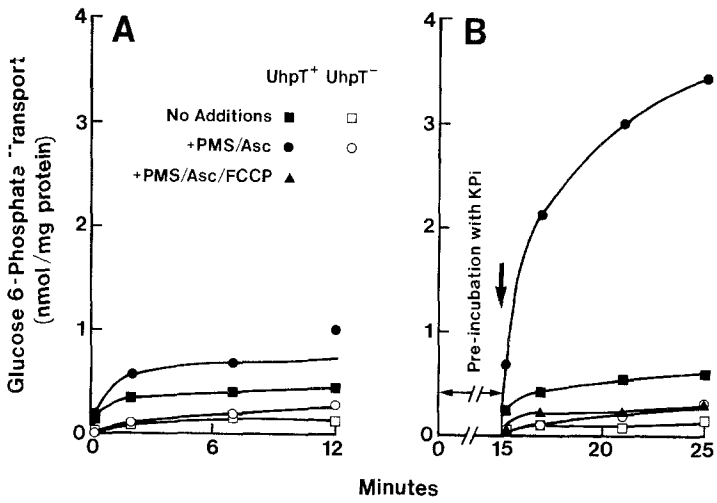

Fig. 3. G6P accumulation requires internal Pi. MOPS-loaded vesicles of a wild type (UhpT$^+$) or transport-negative (UhpT$^-$) strain were placed in a MES-based buffer at pH 6 in the absence or presence of PMS/ascorbate as the oxidizable substrate; 5 μM FCCP was used, as indicated. (A) Labeled G6P was added to 0.1 mM. (B) In parallel tubes, vesicles were preincubated for 15 min with 0.1 mM KPi before adding labeled G6P.

reaction in proteoliposomes. In this effort, our approach (Maloney and Ambudkar, 1989) has been to exploit a special class of protein stabilants —"compatible" compounds known as osmolytes—whose presence at high concentration during detergent solubilization strongly promotes full retention of activity. Among these osmolytes, which include betaine, glycine, proline, various sugars, glycerol, and higher polyols (Yancey *et al.*, 1982), we most often choose to use glycerol for its high membrane permeability.

Table I illustrates the striking response obtained when detergent solubilization is performed in the presence of osmolytes at high concentration (Ambudkar and Maloney, 1986; Maloney and Ambudkar, 1989). Thus, when ^{32}Pi:Pi exchange from *S. lactis* was reconstituted using traditional methods (Racker *et al.*, 1979; Newman and Wilson, 1980), recovered specific activity was so low as to suggest a massive inactivation—indeed, such behavior is typical of many membrane proteins. But when any of several osmolytes was present at the time of exposure to detergent, the final specific activity became 10 to 20-fold increased, indicating full retention of activity in proteoliposomes. This positive reaction to glycerol (and to other osmolytes) (Table I) is not confined to Pi-linked exchange, nor to bacterial proteins, but is found for a large number of prokaryote and eukaryote membrane proteins, including ion-motive and solute ATPases (see Bishop *et al.*, 1989), various secondary carriers, and several membrane-bound enzymes (summarized by Maloney

Table I. Osmolytes and Reconstitution of Pi-Linked Exchange

Test compound	^{32}Pi Transport[a] (n mol/mg protein)
Methanol (20%)	27 ± 5
None	36 ± 9
Ethylene glycol (15%)	42 ± 16
Proline (12%)	306
Glycine (8%)	410
Glucose (20%)	425
Glycerol (20%)	530 ± 98

[a]Steady-state values (± SD) for ^{32}Pi transport by proteoliposomes loaded with 100 mM KPi and exposed to 50 μM external substrate. From Ambudkar and Maloney (1986); see also Maloney and Ambudkar (1989).

Table II. Pi-Linked Anion Exchange[a]

Cell	Substrate(s)	Gene
E. coli	G6P	*uhpT*
	G3P	*glpT*
S. typhimurium[b]	PGA, PEP	*pgtP*
S. aureus	G6P, G3P	
S. lactis	G6P	
R. prowazekii[c]	ATP, ADP	*atpX*

[a]Systems also described by four-letter genetic symbols are of known sequence. Among those, *uhpT*, *glpT* and *pgtP* show close homology.
[b]As judged by sequence homology and by the presence of ^{32}Pi:Pi exchange in intact cells (unpublished).
[c]The role of Pi is uncertain in this case.

and Ambudkar, 1989). It is on this basis that we have concluded that such "osmolyte-mediated" reconstitution will benefit others working in this area.

A Family of Pi-Linked Antiporters

The kinds of experiments summarized above have led to positive identification of several examples of Pi-linked antiport, as described in Table II. Within this family, the best described members are those with preference for G6P—the *E. coli* UhpT protein and its counterparts in *S. lactis* and *S. aureus*. *E. coli* also houses GlpT (encoded by *glpT*), which functions as Pi-linked antiport on the basis of both direct tests (Ambudkar *et al.*, 1986a) and sequence homology with UhpT (Eiglmeier *et al.*, 1987). PgtP of *Salmonella typhimurium* also appears to operate by anion exchange, at least insofar as can be judged by the sequence homology to both UhpT and GlpT (Goldrick *et al.*, 1988) and the presence of a ^{32}Pi:Pi exchange in intact cells (unpublished). *Rickettsia prowazekii* harbours still another example of bacterial anion exchange (Winkler, 1976), and while this

protein has no obvious homology with other examples listed here (Krause *et al.*, 1985; H. Winkler, personal communication), its function clearly warrants its inclusion here. Since anion exchange is so prominent in eukaryote cells, one would be surprised if the family of Pi-linked antiport were limited to the examples of Table I.

Models of Pi-Linked Exchange

Exchange Stoichiometry

The way in which we think of anion exchange has been very much influenced by measurements of exchange stoichiometry in *S. lactis*. Two facts have emerged from those studies. (1) The heterologous exchange of phosphate and glucose 6-phosphate has a stoichiometry of 2 : 1 (Pi : G6P) for the normal conditions of assay at pH 7 (Ambudkar and Maloney, 1984). (2) As assay pH is lowered past the pK_2 of G6P ($pK_2 = 6.1$), this stoichiometry falls, reaching its lower limit of 1 : 1 at pH 5.2 (Ambudkar *et al.*, 1986b). These findings can be incorporated into a molecular model that has interesting consequences.

Biochemical Model for Anion Exchange

The data concerning exchange stoichiometry are viewed as entirely in keeping with the distinctive features of Pi :Pi exchange. Thus, the simplest interpretation is that a 2 : 1 exchange stoichiometry at pH 7 arises as two monovalent Pi anions move in neutral exchange against one divalent G6P, the predominant sugar phosphate anion at pH 7. An appropriate biochemical model (Fig. 4A) would therefore invoke a bifunctional carrier that effects neutral exchange by the binding and transport of either a pair of monoanions or of a single divalent substrate, much as the (cation) ionophore A23187 uses a pair of carboxylate oxygens to bind either two protons or a single calcium or magnesium ion.

With this working model in mind, the pH dependence of stoichiometry is understandable if the monoanion binding sites accept monovalent sugar phosphate as well as monovalent Pi. This is certainly a reasonable suggestion, and is supported strongly by the finding that the K_t for sugar phosphate transport is independent of pH between pH 5 and pH 7 (Ambudkar *et al.*, 1986b). As a result, at any particular pH one imagines that as the carrier selects its substrates at random it generates a macroscopic stoichiometry which titrates a mixture of molecular exchanges that are either 2 : 2 (mono : mono), 2 : 1 (mono : di), or 1 : 1 (di : di). It might also be emphasized that this analysis is made easy by a fortunate circumstance—of the anionic species which might serve as substrates (monovalent and divalent Pi and sugar

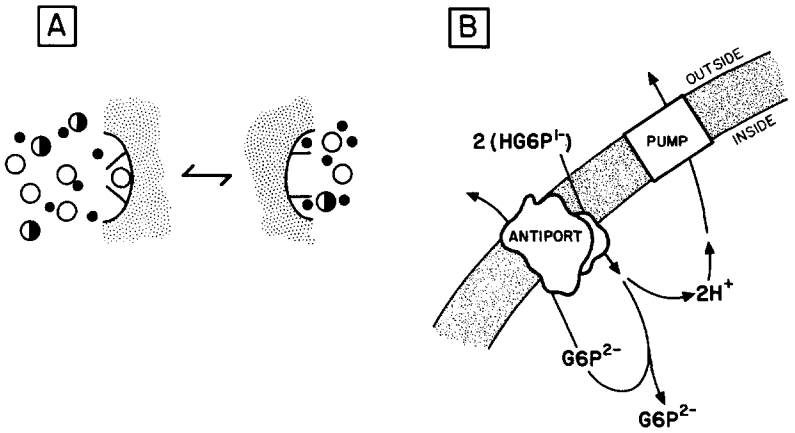

Fig. 4. Biochemical and cellular models of Pi-linked exchange. (A) A general model for neutral antiport involving three substrates—monovalent Pi (●) and mono- (◑) or divalent (○) G6P. Reorientation requires occupancy at both binding sites, by either a pair of monoanions or a single divalent substrate. As shown, the bifunctional protein binds either a single divalent substrate (left) or a pair of monovalent substrates (right). (B) The model of part A predicts that during G6P transport a pH gradient (alkaline inside) leads to an asymmetric exchange as two monovalent G6P anions move in exchange for a single divalent G6P. From Ambudkar *et al.* (1986b) and Maloney *et al.* (1986).

phosphates), the divalent Pi anion is *not* accepted. If monovalent and divalent Pi were equally effective substrates, pH effects on kinetics and exchange ratios would be more difficult to analyze.

Physiologic Consequences of the Biochemical Model for Anion Exchange

The biochemical model of Fig. 4A accounts for exchange stoichiometry as well as certain earlier findings. For example, Essenberg and Kornberg (1975) had shown that Pi-linked exchange in *E. coli* (UhpT) behaves as H^+/anion symport, at least phenotypically. We can now understand why. Note that both mono- and divalent sugar phosphates are substrates; and recall as well that the bacterial cytoplasm is alkaline with respect to the periplasm. For these reasons, the self-exchange of sugar phosphate could act to transport material in the net if efflux of a single divalent sugar phosphate is used to support entry of two sugar phosphate monoanions. This physiological model (Fig. 4B; see also Fig. 1) results in net entry of $2H^+$ and $1G6P^{2-}$, so that the anion exchange reaction masquerades effectively as a proton-linked symport reaction! This reaction mode is feasible, at least in principle, since the K_t for sugar phosphate transport is low (micromolar) compared to that for Pi transport (millimolar). Nevertheless, the *in vivo* balance among the various homologous and heterologous reactions is not entirely clear, and it is doubtful that any single reaction type is used exclusively.

We know that in *E. coli* the carbon-to-phosphorous ratio is about 40 : 1 (mol : mol) (Luria, 1960), so that, over the long term, sugar phosphates provide too little carbon and too much phosphorous. Thus, it is likely that during growth of G6P or G3P, a manageable C : P ratio is achieved by some mixture of exchange modes that ends up bringing in carbon and extruding excess Pi; this problem is discussed in more detail elsewhere (Maloney, 1990).

Molecular Model of Anion Exchange

Although the earlier discussion emphasized the biochemical and cellular aspects of Pi-linked exchange, these antiport systems serve equally well to frame general arguments about the molecular structure of membrane carriers. Hydropathy analysis using the amino acid sequence of either UhpT (Friedrich and Kadner, 1987) or GlpT (Eiglmeier *et al.*, 1987; Gott and Boos, 1988) suggests a secondary structure with hydrophobic or amphipathic stretches that might accommodate 12 α-helical transmembrane segments (Fig. 5). Indeed this global topology is the most striking general feature we associate with membrane transport proteins, and overall it is conserved in the nearly two dozen secondary carriers now sequenced, including both prokaryote and eukaryote examples (see Maloney 1990). Surely, this reflects some sort of structural paradigm, whether or not it is simple as shown here. It is also striking that in UhpT and GlpT (and most other carriers), these hydrophobic columns appear as two clusters separated by a large cytoplasmic loop. This seems to implicate an internal dimer (6 + 6) as the fundamental unit of structure and, remarkably, one can use recent studies of mitochondrial and

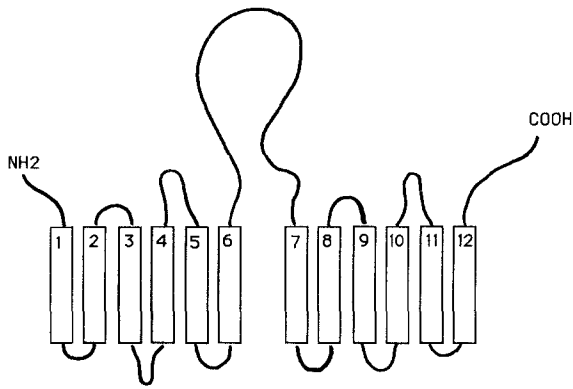

Fig. 5. Molecular model of membrane carriers. A low-resolution view of UhpT and GlpT secondary structures suggests a bipartite organization in which two clusters of membrane-embedded α helices are separated by a cytoplasmic loop. This is a common theme in membrane transport. See text for further comment. From Maloney (1990).

chloroplast antiporters to draw much the same conclusion. It has been argued that the organelle carriers function as homodimers (Klingenberg, *et al.* 1980; Aquila *et al.*, 1987; Flugge and Heldt, 1986). And as it turns out, the relevant amino acid sequences now show these proteins as having 6 (or 7) hydrophobic columns (Aquila *et al.*, 1987, Runswick *et al.*, 1987; Flugge *et al.*, 1989), so that the dimeric structure is also best represented by 6 + 6. One should not read too much into such correlations, of course, but it is of obvious interest that this 6 + 6 organizational plan provides a structural basis for the bifunctional reaction scheme characteristic of Pi-linked exchange. At any rate, such thinking offers a sensible way to organize the next generation of experiments.

Carboxylate-Linked Exchange

A review of bacterial anion exchange is not complete without mention of recent work (Anantharam *et al.*, 1989) identifying a new family of antiport —a carboxylate-linked reaction in *Oxalobacter formigenes*. This cell is an anaerobe that exploits oxalate transport and metabolism to sustain membrane energetics, and current evidence now points to a mechanism whereby a proton-motive force is established by operation of a secondary carrier (anion exchange) rather than a primary pump. The data suggest a 1 : 1 exchange of divalent oxalate and monovalent formate (the product of oxalate metabolism) as the electrogenic event underlying generation of membrane potential. A pH gradient would be formed secondarily, following consumption of a proton during the intracellular decarboxylation of oxalate to formate (which may be written: $^{-}OOC\text{–}COO^{-} + H^{+} \rightarrow HCOO^{-} + CO_2$). And taken together, these separate events—oxalate^{-2} influx, oxalate decarboxylation, formate^{-1} efflux—constitute an "indirect" H^{+} pump with a net stoichiometry of $1H^{+}$ extruded/turnover. This cycle is of interest for several reasons. It shows, for example, in simple biochemical terms, how and enzyme (a decarboxylase) might mediate the "transduction" of chemical into electrochemical energy. In more practical terms, the finding of oxalate : formate exchange raises the possibility that antiport contributes to energy conservation in other anion-degrading systems. If so, the scheme of anion^{-n} : $(n - 1)OH^{-}$ may be more generally relevant, since the use of OH^{-} as the counterion spares carbon for biosynthesis. In this setting, an extension to the case of $n = 1$ is of special interest, for that describes a monocarboxylate uniport or channel, of which there is one possible example—the anaerobic decarboxylation of acetate to yield CO_2 *plus* methane in several methanogenic bacteria (Zehnder and Brock, 1979). The presence of an archibacterial uniport or channel could be of real significance to the way in which we view the origins, not only of anion exchange, but of membrane transport as well. This exciting

possibility is made all the more enticing by the extraordinarily high velocity found for carboxylate-linked antiport in *O. formigines*. In the *crude* reconsitituted preparation, this anion exchange moves substrates more rapidly than any purified carrier yet studied (Anantharam *et al.*, 1989).

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