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Topical Review

Phosphate Transport Processes in Eukaryotic Cells

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Introduction

Inorganic phosphate (P_i) plays a central role in cellular energy metabolism, in the synthesis and hydrolysis of ATP and in many other reactions. Because P_i is a constituent of DNA and membrane lipids, net uptake of P_i is essential for cell growth and replication. Eukaryotic cells require several P_i transport systems, in the plasma membrane and in the membranes of various organelles, and larger organisms require specialized, physiological mechanisms for accumulating from the environment the P_i needed by their cells. Some of these transport systems resemble those used by prokaryotes, while others are unique to eukaryotes. Excellent and detailed reviews exist describing certain individual P_i transport systems. It is the goal of this review to provide an overview of all of the P_i transport processes, to illustrate the relationships among the different systems, and to compare the mechanisms which have evolved. Given the scope of this review, we cannot describe all the important work that has been conducted. In particular, the important topic of physiological regulation of P_i transport is not discussed. We have chosen to emphasize the molecular mechanisms of P_i transport, the coupling of energy to drive transport, and, wherever possible, the isolation and sequencing of P_i transport proteins and genes.

Inorganic phosphate is an anion and a weak acid. These factors complicate both the nature of P_i transport mechanisms and their study. The concentration gradient of P_i, the transmembrane electrical potential, and the transmembrane pH gradient are all involved in determining the energy cost of net P_i translocation. Most cells maintain a plasma membrane electrical potential which is negative inside, and respiring mitochondria maintain an electrical potential as high as −180 mV. These potentials produce an automatic energy cost for anion uptake, especially for dianions. Unfortunately, reliable values for the concentration of free P_i and the pH are not always available for the compartments of interest; thus, separating energy cost-related from mechanism-based effects of P_i, Na^+, and H^+ concentrations has been difficult.

On an evolutionary time scale the oldest systems for active transport of P_i appear to be proton gradient-driven mechanisms. The energy resulting from primary proton translocation by ATPases or oxidative enzymes in the plasma membrane is used by prokaryotes to drive uptake of P_i and other metabolites from the environment. Mechanisms of this type are found in the mitrochondria of eukaryotes, as well as in the plasma membranes of higher plants. Animals, with their well-developed Na^+/K^+ ATPase, have developed mechanisms to use the electrochemical gradient of sodium to drive transport of P_i (and other compounds) across the plasma membrane. Lower eukaryotes, such as yeast and fungi, may use both proton- and sodium-driven mechanisms.

In addition to secondary active transport systems, there are systems that catalyze equilibrium transport of P_i by anion/anion exchange. These include the P_i/dicarboxylate exchange system of mitochondria, the P_i/3-phosphoglycerate exchange system in chloroplasts, and exchange of P_i via the band 3 Cl^-/HCO_3^- exchanger of erythrocytes. Several of the exchange-type mechanisms also resemble prokaryotic systems. Although actual evolutionary relationships have not yet been established among the various P_i transporters, Fig. 1 illustrates the distribution of the major P_i transport mechanisms throughout nature. Similarities in substrate preference, kinetic and thermodynamic characteristics and inhibitor sensitivities, rather than genetic infor-

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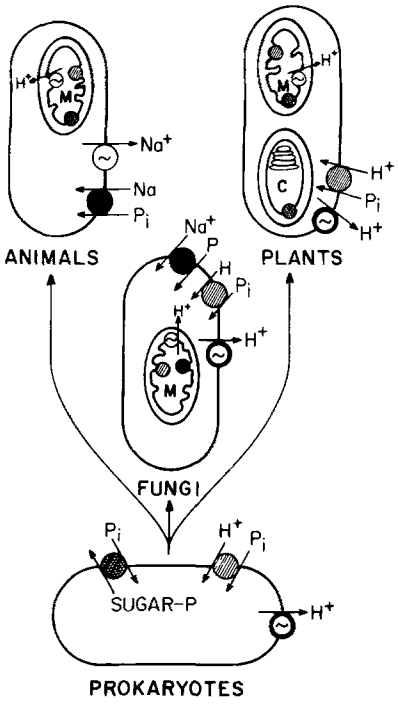

Fig. 1. Distribution of different P_i transport processes throughout nature. Processes with similar mechanisms, indicated here by identical symbols, may not actually be evolutionary related. Striped circle: H^+/P_i cotransporters. Cross-hatched circles: P_i/anion exchangers. Filled circles: Na^+/P_i cotransporters. Squiggle circles: primary active transporters

mation, form the basis for this hypothetical "family tree."

Phosphate Transport Driven by the H^+ Gradient

The transporters in this category have in common the translocation of P_i against its electrochemical gradient at the expense of a proton electrochemical gradient generated independently. Although we have chosen to describe these as H^+/P_i symporters, an obligatory cotransport of protons with P_i is macroscopically identical to obligatory exchange of P_i for hydroxyl ion, and no study has yet differentiated between these two mechanisms.

Mitochondrial Phosphate Uptake is a Major Energy Cost of ATP Synthesis

The most well-studied eukaryotic H^+/P_i cotransport system is that found in the inner membrane of mitochondria. (For a review *see* Wohlrab, 1986.) This system translocates P_i from the cytoplasm into the mitochondrial matrix, where it is used to synthesize ATP and to drive the accumulation of respiratory substrates (via P_i/dicarboxylate exchange; *see below*). In several respects it functions like the Pit transport system found in *Escherichia coli*, which catalyzes uptake of P_i from the environment at the expense of a proton gradient generated by electron transport and ATPase enzymes (Rosenberg, Gerdes & Harold, 1979). It is possible that the mitochondrial H^+/P_i cotransporter, like the electron transport enzymes and membrane lipid composition of these organelles, may have a prokaryotic origin. However, an important difference between the mitochondrial and the *E. coli* transporter is that the latter is insensitive to N-ethylmaleimide (Rosenberg et al., 1982).

The energy cost for P_i uptake by respiring mitochondria is difficult to calculate. Concentrations of cytoplasmic free P_i measured using in vivo NMR (nuclear magnetic resonance) spectroscopic techniques range from 0.5 to 2.0 mM depending on the tissue, similar to or somewhat lower than P_i values measured by traditional extraction methods. A value of 1.7 mM has been observed in dog brain in vivo (Nioka et al., 1987) and concentrations of 1.5 mM in glucose-perfused and 0.6 mM in pyruvate-perfused isolated guinea pig heart have been reported by Zweier and Jacobus (1987). Measurement of intramitochondrial free P_i is considerably more difficult. Matrix P_i may be bound to proteins, or complexed with Ca^{2+}, so that chemical determinations of total mitochondrial P_i will, in general, overestimate the free ion concentration. In a ^{31}P NMR study of the isolated, perfused rat heart Garlick et al. (1983) observed two resonances from P_i, the more alkaline of which exhibited behavior consistent with an assignment to intramitochondrial P_i. Making some assumptions about the NMR relaxation behavior of the intramitochondrial P_i, they estimated an intramitochondrial P_i concentration of 2.6 times the cytosolic concentration.

These observations suggest that both concentration and electrical potential gradients oppose uptake of P_i into the matrix of respiring mitochondria. The needed energy is provided by coupling transport of P_i to the energy of the electrochemical gradient of protons, which is created by respiration. As many as 25% of the protons extruded by the respiratory chain may be used to support uptake of P_i (Lehninger & Reynafarje, 1982). Transport of P_i into the mitochondrial matrix appears to be an electroneutral event (Guerin, Guerin & Klingenberg, 1970; Ligeti et al., 1985). Although this process is usually referred to as $H^+/H_2PO_4^-$ cotransport, it is possible that the actual substrate of the P_i transporter is HPO_4^{2-}. In a study using fluorophosphate analogs, Freitag and Kadenbach (1978) found that

the divalent fluorophosphate supported mitochondrial swelling, suggesting that it was transported, while monovalent phosphate analogs were inhibitors of P_i uptake. Studies of "inside-out" vesicles of mitochondrial inner membrane indicate that energy-dependent *efflux* of P_i from intact mitochondria may be catalyzed by the same transporter operating electrophoretically at the expense of the electrical component of the proton gradient (Wehrle, Cintron & Pedersen, 1978).

The mitochondrial H^+/P_i cotransporter has a relatively low substrate affinity, approximately 1.6 mM for total P_i (Coty & Pedersen, 1974), close to the cytoplasmic P_i concentration. Fructose (Erecińska et al., 1977), ethanol (Desmoulins, Cozzone & Canioni, 1987), and other substrates have been found to profoundly alter cytoplasmic P_i, precisely in the concentration range of the transporter K_m. However, the V_{max} for P_i transport in intact rat liver mitochondria is approximately 3000 nmol/min · mg of mitochondrial protein (at 22°C, Ligeti et al., 1985) and the maximal rate of ATP synthesis in rat liver mitochondria is approximately 400 nmol/min · mg of mitochondrial protein (Jeffries, LaNoue & Radda, 1986). Thus it is unlikely that P_i transport becomes rate limiting for ATP synthesis under physiological conditions.

Mitochondrial P_i Transport is Dependent on a Free Sulfhydryl Residue

The first characteristic of the H^+/P_i cotransporter to be identified was its sensitivity to reagents reactive with free sulfhydryl groups (Fonyo & Bessman, 1968). The free sulfhydryl residue (now identified as Cys 42 in the bovine heart transporter, *see below*) necessary for transport reacts with a wide variety of mercurial, dithiol, and alkylating reagents (Fonyo, 1976). The H^+/P_i cotransporter can be distinguished from other mitochondrial transporters on the basis of this nonselective sensitivity to –SH reagents. The P_i/dicarboxylate exchanger is sensitive to mercurials but not to N-ethylmaleimide (NEM) (Coty & Pedersen, 1976*b*). The (ADN) exchanger is sensitive to NEM under certain conditions, but not to mercurials (Aquila, Eiermann & Klingenberg, 1982*a*). Some sulfhydryl reagents such as 5,5′-dithiobis (2-nitrobenzoate), mersalyl, and diazobenzenesulfonate (Wehrle & Pedersen, 1981; Fonyo & Vignais, 1980) and eosin-5-maleimide (Houstek & Pedersen, 1985) inhibit P_i transport under conditions in which they have been rigorously shown not to enter the mitochondrion. Griffiths et al. (1982) examined a series of *N*-polymethylene carboxymaleimides and found that those with four to ten carbons between the carboxylate "anchor" and the reactive maleimide could inhibit P_i transport. Taken together these results suggest that the reactive sulfhydryl is somewhat below the surface, at the cytoplasmic side of the inner membrane.

Fonyo and co-workers have shown that, while binding of P_i does not protect the critical sulfhydryl group, acidification of the mitochondrial matrix reduces the inhibition of the transporter by sulfhydryl group reagents (Fonyo & Vignais, 1980; Ligeti & Fonyo, 1984) and alkalinization of the matrix increases inhibition. This is true even for nonpenetrant sulfhydryl reagents, suggesting that matrix pH affects the accessibility or reactivity of a cytoplasmic sulfhydryl. Studies of inverted vesicles from the inner membrane have suggested that the same essential sulfhydryl group may be accessible from the matrix surface under some conditions (Wehrle et al., 1978). In recent studies Bukusoglu and Wohlrab (1987) have inhibited the P_i transporter with the affinity label 4-azido-2-nitrophenylphosphate. Inhibition by this reagent from the cytoplasmic side is prevented by P_i, ADP, and succinate. This supports the suggestion of the sulfhydryl labeling studies that important changes take place in the carrier when it is functioning during State 3 respiration.

A Single 34-kD Protein is Responsible for Mitochondrial Phosphate Transport

In 1975 Coty and Pedersen (1975*a*) attempted to identify the H^+/P_i cotransporter using a protocol exploiting its characteristic inhibition pattern. A protein of 32 kD was preferentially labeled by radiolabeled NEM. In 1979 Banerjee and Racker solubilized and reconstituted mercurial-sensitive P_i transport in artificial phospholipid vesicles. After partial purification, the reconstitutively active fraction contained several proteins, but only the 30-kD region exhibited NEM-labeling strongly inhibited by P_i. By comparing mercurial-sensitive NEM labeling in blowfly flight muscle, beef heart, and rat liver mitochondria, Wohlrab (1979) was able to identify the H^+/P_i cotransport protein as a 34-kD polypeptide. P_i/P_i exchange activity or unidirectional P_i uptake have been reconstituted from the detergent-solubilized mitochondria of beef heart (Wohlrab, 1980; Wohlrab & Flowers, 1982), pig heart (Mende et al., 1982), rat liver (Wehrle & Pedersen, 1983), and blowfly flight muscle (Wohlrab, Collins & Costello, 1984*a*). Several phospholipid mixtures have proven successful for reconstitution, especially those containing the characteristic mitochondrial phospholipid cardiolipin (diphosphatidylglycerol)

(Wohlrab et al., 1984*a*). P_i transport in various reconstituted systems is appropriately inhibited by mercurials, NEM, and diazobenzenesulfonate but not by *n*-butylmalonate.

Procedures for purification of the H^+/P_i cotransporter exploit the fact that, like the ADN exchanger (Riccio, Aquila & Klingenberg, 1975) and the P_i/dicarboxylate exchanger (Kaplan & Pedersen, 1985) and H^+/P_i cotransporter is not adsorbed to hydroxylapatite from detergent solution. It has been suggested that these proteins all bind unusually high amounts of lipid and detergent, preventing their interaction with the hydroxylapatite column (Klingenberg, Riccio & Aquila, 1978). The presence during the extraction process of dithiothreitol and cardiolipin are important for maximal P_i transport activity of the reconstituted preparation. The transporter from blowfly flight muscle, which has a highly simplified mitochondrial inner membrane, can be completely purified in a reconstitutively active state by chromatography on celite using a gradient of SDS in Triton X-100 (Wohlrab et al., 1984*a*). The transporter from other sources, while highly active following a similar chromatographic treatment, is still substantially contaminated.

Separation of the H^+/P_i cotransporter from the other 30–35 kD proteins is more difficult and requires the use of sodium dodecylsulfate (SDS), possibly to remove bound lipid or to unfold the protein. Complete purification of the beef heart H^+/P_i cotransport protein has been accomplished by Kolbe et al. (1984). As a second chromatographic step, these authors used chromatography on hydroxylapatite in SDS, urea, and β-mercaptoethanol. The purified transport system from beef heart or blowfly flight muscle appear as two closely spaced bands on SDS-PAGE, designed "PTP α" "PTP β" by Kolbe et al. (1984). Bisaccia and Palmieri (1984) purified the H^+/P_i cotransporter from porcine heart in a reconstitutively active form as a single 34-kD species. Kaplan, Pratt and Pedersen (1986), using sequential chromatography on hydroxylapatite, DEAE-cellulose, and Affigel (Bio-Rad Laboratories), have purified the rat liver mitochondrial H^+/P_i cotransporter to near homogeneity. Depending upon the mercury content of the Affigel preparation, the rat liver transporter may appear as a single or two closely spaced bands in the 34-kD region on SDS-PAGE. Significantly, both preparations exhibit similar specific activities of transport, indicating that both α and β forms are catalytically active. Gibb, Reid and Lindsay (1986) report the purification of the rat liver transporter to a single 34-kD polypeptide using chromatography on hydroxylapatite and celite, combined with sequential Triton X-114 and Triton X-100 extraction. Although this preparation was not tested for transport activity, an antibody raised against the protein inhibits phosphate transport in mitoplasts (mitochondria with the outer membrane removed).

The Mitochondrial Phosphate Transport Gene is Homologous to Genes for the Adenine Nucleotide Exchanger and the Brown Adipose Tissue Uncoupler Protein

Differences between "PTP-α" and "PTP-β" appear minimal. One-dimensional peptide maps of the two SDS-PAGE bands from bovine heart mitochondria are nearly identical, and the N terminal sequences of the two forms are identical (Kolbe et al., 1984). Reaction with NEM, or carbamoylation of the protein causes the two bands to run as a single band. The amino acid composition of the beef heart transporter is 59% hydrophobic or nonpolar residues, similar to the ADN exchanger (Aquila et al., 1982*a*) and appropriate for a transmembrane protein. The first large peptide of the transporter to be sequenced was the 47-residue peptide at the N terminus, which is released by formic acid hydrolysis (Kolbe & Wohlrab, 1985). This peptide includes the unique site of alkylation by NEM, a cysteine (Cys 42 in the bovine heart protein) which is flanked by positive charges (Lys and Arg). Significantly, three other cysteines in this peptide (as well as four others elsewhere in the protein) do not react with NEM when the transporter is inhibited. Kolbe and Wohlrab (1985) suggest that the basic flanking residues, by stabilizing the reactive thiolate anion, account for the hyper-reactivity of this cysteine.

The mitochondrial H^+/P_i transporter is coded by the nuclear genome and carries a 49-residue import sequence, which is removed when the protein is embedded in the mitochondrial membrane (Runswick et al., 1987). From the structure of the 1–47 peptide, homology between the H^+/P_i transporter and the mitochondrial ADP/ATP exchanger was clearly apparent (Kolbe & Wohlrab, 1985). The complete sequence of the beef heart transporter was obtained by DNA sequencing techniques (Runswick et al., 1987). The P_i transporter peptide contains three internally homologous regions, as indicated in Fig. 2. This pattern of threefold repeat is also found in the ADP/ATP exchanger (Saraste & Walker, 1982) and in the uncoupling protein from brown fat mitochondria (Aquila, Link & Klingenberg, 1985) and the primary structures of all three proteins are significantly homologous. Runswick et al. (1987) have modeled each of the three proteins with six membrane-spanning hydrophobic segments

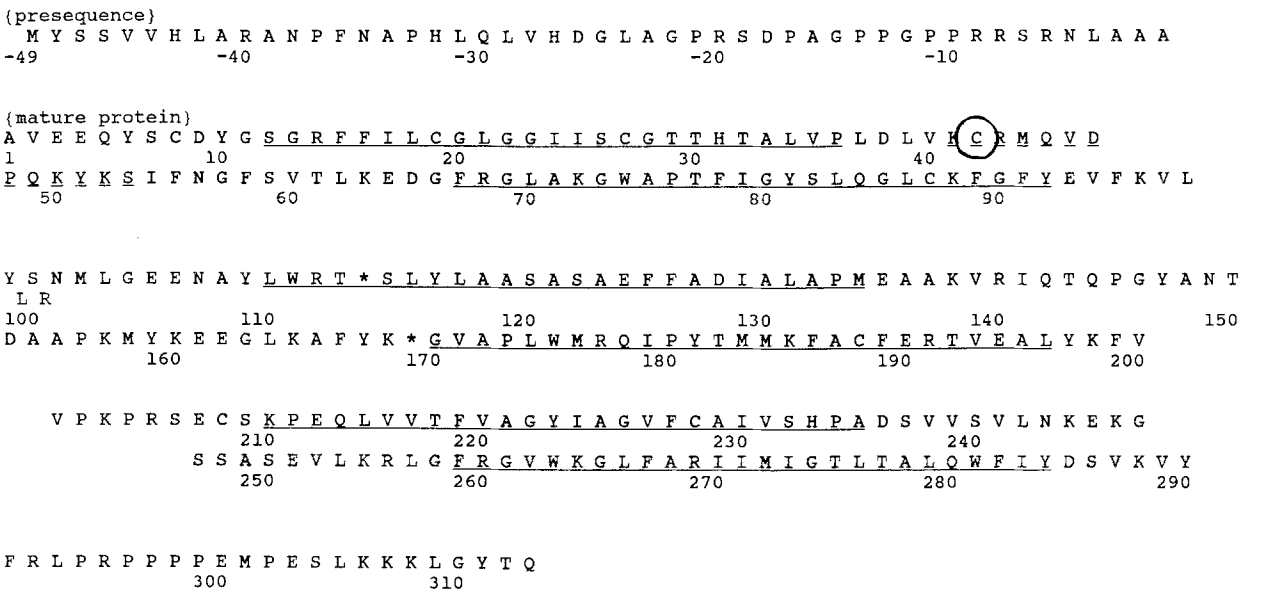

Fig. 2. Amino acid sequence of the bovine heart mitochondrial H^+/P_i cotransporter. Data are from Runswick et al., 1987. Three homologous regions are found within the mature protein. Residues have been aligned for maximal homology, with * indicating an added space. In each segment two sequences suitable to form transmembrane helices can be identified (underlined). The dashed sequence indicates the region suggested by Aquila, Link and Klingenberg (1987) to form a β-strand turning back across the membrane. This region contains the critical cysteine residue 42 (circled)

connected by hydrophilic loops. In one model the unique, NEM-binding cysteine of the H^+/P_i transporter falls in the first loop, on the cytoplasmic side of the membrane. An alternative model has been proposed by Aquila, Link and Klingenberg (1987), in which the peptide, after the first helical region, turns abruptly back through the membrane. This places the critical cysteine below the membrane surface, and implies an opposite direction for the remaining helices. Further study will be required to resolve this difference.

A full-length cDNA clone of the rat liver P_i transport gene has been obtained by Ferreira, Pratt and Pedersen (1989) which agrees with the amino acid composition of the purified rat liver transporter and with the sequence of the N-terminal amino acids. The DNA sequence and hydropathy profile of the rat liver system show a close correspondence to the bovine heart protein. Nonetheless, 32 out of 312 residues in the phosphate transport proteins from these two sources are not conserved. This shows considerably more variability than, for instance, the β subunit of the ATP synthase enzyme, in which only five residues out of 480 differ between the rat liver and the beef heart enzymes (Ysern, Amzel & Pedersen, 1988).

Antibodies raised against the P_i transporter (two) and the ADP/ATP transporter (one) from bovine heart fail to cross-react, although one of the anti-P_i transporter antibodies recognized the P_i transporter purified from pig heart, rat heart, rat liver, and blowfly flight muscle mitochondria (Rasmussen & Wohlrab, 1986). Another antibody against beef heart P_i transporter recognized other heart mitochondrial P_i transporters, but not those from liver or flight muscle, indicating some tissue-dependent differences in protein structure.

H^+/P_i Cotransport is also Found in the Plasma Membrane of Fungi

Yest and related fungi are able to grow under a variety of conditions and have a complex system of P_i uptake processes. *Saccharomyces cerevisiae* has three systems for P_i uptake. The constitutive transport system is similar to the mitochondrial H^+/P_i cotransporter, with a K_m for P_i of 1.7 mM (Goodman & Rothstein, 1957). When grown in low P_i medium, these and similar yeast and fungi activate a P_i transport system of relatively high affinity (5–20 μM) and simultaneously lose the low-affinity mechanism (Nieuenhuis & Borst-Pauwels, 1984). The high-affinity system, which is also a H^+/P_i cotransport (Roomans & Borst-Pauwels, 1979), is found in intact cells, but protoplasts prepared by osmotic shock have only the low-affinity activity. High-affinity transport is correlated with the appearance

of a binding protein for P_i on the plasma membrane (Jeanjean & Fournier, 1979). This 52-kD protein, which can be released from the yeast by osmotic shock, has been purified to homogeneity from *Candida tropicalis* (Jeanjean et al., 1984). Antibodies to the purified binding protein inhibit P_i translocation in intact cells but not in protoplasts. Jeanjean and co-workers suggest that the binding protein acts to modify the activity of the constitutive system rather than to replace it.

H^+/P_i Cotransport in Higher Plants Appears to Include a High-Affinity Component

Inorganic phosphate uptake across the plasma membrane of green plants appears to be driven by H^+ cotransport. It is not enhanced by Na^+ (Ullrich-Eberius & Yingchol, 1974), but it is enhanced by increasing ΔpH and by fusicoccin, a stimulator of the electrogenic H^+ pump (Lin, 1979). As in mitochondria, transport is inhibited by the uncoupler FCCP (*p*-trifluoromethoxy carbonylcyanide phenylhydrazone) and by mersalyl, but not by anion exchange inhibitors such as SITS (4-acetamido-4′-isothiocyanatostilbene-2,2′ sulfonic acid) (Lin, 1981). Uptake of P_i in *Lemna gibba* (duckweed) produces a transient depolarization of the plasma membrane, suggesting that positive charge transfer is involved. Ullrich-Eberius et al. (1981) have calculated that above pH 6 the H^+ concentration gradient alone would be insufficient to support P_i uptake into duckweed maintaining a high transmembrane potential. H^+/P_i cotransport in duckweed exhibits biphasic kinetics with respect to P_i concentration, with apparent K_m's of 6–8 μM and 65–75 μM (Ullrich-Eberius, Novacky & van Bel, 1984). This high affinity, as well as the translocation of net charge, represents a substantial change from the mitochondrial transporter, and resembles instead the yeast high-affinity transport process.

An interesting study of P_i transport in vivo has been performed by Brodelius and Vogel (1985) using perfused cultures of two plant cell lines. ^{31}P NMR spectroscopy was used to study *Catharanthus roseus* and *Daucus carota*, which differ in their responses to the addition of P_i to growth medium. *C. roseus* has the ability to take up large amounts of P_i very rapidly into an acidic intracellular storage compartment, presumably the vacuole. This study demonstrated that with time P_i accumulated in the vacuole is sequestered into an NMR-invisible compartment, as the total amount of P_i accumulated (measured by radiolabel) remained constant but the NMR signal due to vacuolar P_i decreased. In contrast to the pattern of rapid accumulation and slower utilization observed in *C. roseus*, *D. carota* took up P_i slowly, directly into the cytoplasm, only as required for growth. It was shown that neither plant stores P_i as polyphosphate or phytates.

Phosphate Transport Driven by the Sodium Gradient

Active transport of P_i into many types of animal cells appears to occur by a sodium-dependent process. Evolution of such a mechanism reflects the development of an active Na^+/H^+ ATPase, and probably a reduced desirability of large H^+ concentration gradients within complex multicellular organisms. This type of P_i transport has been studied most extensively in kidney and intestine, where it is responsible for the rate-limiting step in transepithelial P_i transport. Studies have now been initiated on P_i transport processes in heart and skeletal muscle (Nuutinen & Hassinen, 1981; Medina & Illingsworth, 1980), nerve fibers (Jirounek et al., 1982, 1984), across the placenta (Stulc & Stulcova, 1984; Brunette & Allard, 1985), and in a variety of cultured cell lines. In addition, uptake of P_i by cancer cells has been studied (Wehrle & Pedersen, 1982; Bowen & Levinson, 1983), partly to determine whether P_i uptake plays a role in rapid growth. A derepressible, sodium-dependent, high-affinity P_i transport system is also found in yeast (Roomans, Blasco & Borst-Pauwels, 1977). The interactions among Na^+, P_i, and H^+ as substrates, as effectors, and as driving forces are complex, and a consensus as to a precise mechanism for any of the Na^+/P_i cotransport systems has yet to be reached.

Na^+/P_i Cotransport across the Apical Membrane of Kidney Proximal Tubule Epithelial Cells Drives Whole Body P_i Resorption

The physiological aspects of renal phosphate transport and its regulation have been reviewed extensively elsewhere (Bonjour & Caverzasio, 1984; Mizgala & Quamme, 1985; Gmaj & Murer, 1986) and only a summary will be presented here to place the mechanistic and molecular studies in context. Renal P_i resorption plays a major role in whole body P_i homeostasis and is also important in maintenance of acid-base balance. Although species differences have been observed in regulation of transport, considerable similarity exists in the properties of the

renal Na^+/P_i cotransport system from a variety of sources. Kidneys of rats and rabbits have received the most study. Several types of preparations have been used to study this transport system, including in vivo micropuncture, isolated tubule perfusion, brush border membrane vesicles (BBMV), and both primary and established kidney cell lines in tissue culture.

Transepithelial P_i movement from urine to plasma in the kidney occurs across a single cell layer and is the net result of two transport processes: sodium-dependent active transport at the apical membrane and a second translocation step at the basolateral membrane. P_i transport at the basolateral membrane is not yet well characterized. An active transport system is unlikely to be necessary, as both the P_i concentration gradient and the transmembrane potential gradient probably favor anion efflux across the basolateral membrane. Both sodium-dependent (Schwab, Klahr & Hammerman, 1984*a*,*b*) and anion exchange-type mechanisms (Murer & Burckhardt, 1983) have been suggested for transport across the basolateral membrane.

Inorganic phosphate resorption from the glomerular filtrate appears to occur entirely through a sodium-dependent carrier in the apical membrane of the epithelial cell, with no measurable "leak" component and little back flux from plasma to lumen (Dennis & Brazy, 1982). This step requires an energy input, as the membrane potential (30–60 mV, negative inside) opposes anion accumulation. The concentration gradient for P_i across the apical membrane must vary somewhat along the nephron. Intracellular P_i in the kidney as a whole is approximately 2.4 mM, as measured by NMR spectroscopy in vivo (Freeman et al., 1986), but it is not possible to determine the intracellular concentration of the proximal segment epithelial cells specifically. The P_i concentration in the glomerular filtrate is approximately 90% of the plasma P_i concentration (depending on the protein and calcium concentrations of the plasma), typically 2.5 mM at the beginning of the proximal tubule under normal conditions. Although as much as 95% of the filtered P_i is resorbed in the proximal segment, the simultaneous resorption of water results in the concentration of P_i remaining above 1.5 mM in the tubule fluid under normal conditions (Harris et al., 1974). Some P_i uptake does occur in the distal convoluted tubule (Amiel, Kuntzinger, & Richet, 1970), but probably accounts for resorption of no more than 5–10% of the filtered load of P_i. Separate segments of the nephron as well as nephrons from different regions of the kidney appear to differ not only in basal transport capacity, but also in their response to diet and hormones (Burnette & Beliveau, 1984). These authors have observed biphasic kinetics for P_i transport at 37°C, in contrast to single phase kinetics observed by them and other laboratories at temperatures below 30°C. This has lead them to suggest the presence of two different P_i transporters, with differential distribution along the nephron (Brunette, Beliveau & Chan, 1984).

The transport system in rat and rabbit kidney BBMV has an apparent K_m for total P_i of approximately 0.08 mM under physiological conditions (Hoffmann, Thees & Kinne, 1976; Cheng & Sacktor, 1981). Because P_i transport rates increase with increasing luminal or extravesicular pH, it was originally suggested that HPO_4^{2-} was the transported species. Arsenate, a dianion throughout the pH range of interest, is a competitive inhibitor of P_i transport. Sacktor and Cheng (1981) observed an enhancement of P_i uptake into rabbit BBMV by an internally acidic ΔpH and suggested that this was due to preferential translocation of HPO_4^{2-}. The enhancement was greater when amiloride was added to block dissipation of ΔpH via the Na^+/H^+ exchanger. A smaller enhancement by ΔpH was observed by Amstutz et al. (1985) in rat kidney BBMV. Failure of others (Burckhardt, Stern & Murer, 1981) to measure such stimulation may be due to the use of SCN^-, which is permeant as an anion but also, to a limited extent, as an acid (Mitchell & Moyle, 1969) and might dissipate small pH gradients or compete with P_i transport for the pH gradient.

The effects of pH on transport kinetics are apparently not exclusively due to the titration of P_i, as they are still observed when the concentration of HPO_4^{2-} is held constant (Cheng & Sacktor, 1981). The pH dependence of P_i uptake can be substantially reduced by increasing sodium concentration in the assay (Burckhardt et al., 1981), but even at saturating levels of Na^+ an effect of pH is observed. Amstutz et al. (1985) reported a 50% increase in apparent K_m for total P_i and a 24% decrease in V_{max} at pH 6.4 relative to pH 7.4, even in the presence of 300 mM Na^+. Because the apparent affinity drops by only 40% while the concentration of HPO_4^{2-} drops by 90%, these results suggest that $H_2PO_4^-$ may also be a substrate. Alternatively, a direct pH-dependent increase in the affinity for HPO_4^{2-} might occur over this range. The stimulation of P_i uptake by high pH can be reversed by feeding a high phosphate diet (Cheng, Liang & Sacktor, 1983). P_i uptake in cultured pig kidney cells (LLC-PK1) is faster at pH 6.4 than at pH 7.4 (Brown et al., 1984). The level of P_i in culture medium may be high enough to mimic the effects of feeding a high P_i diet to animals. This observation is especially interesting in light of the observation (*see below*) that the intestinal Na^+/P_i

cotransporter appears to function more rapidly at low than at high pH.

Although P_i transport is strongly sodium dependent in intact renal tubules, isolated whole cells and BBMV, the degree of Na^+ specificity of P_i varies. In BBMV no other cation supports more than 10% of the uptake rate observed in the presence of Na^+ (Hoffman et al., 1976; Cheng & Sacktor, 1981), although Li^+ appears somewhat more effective than other nonsodium cations. In intact LLC-PK1 cells uptake in the presence of several other cations approaches 30% of the rate in the presence of Na^+ (Noronha-Blob, Filburn & Sacktor, 1984). The initial rate of P_i uptake into BBMV depends linearly on the Na^+ concentration gradient; however, Na^+ also causes a four to sixfold increase in the initial rate of equilibrium P_i transport (Hoffman et al., 1976; Cheng & Sacktor, 1981). Concentrative P_i uptake driven by a transmembrane proton gradient without a Na^+ gradient also requires Na^+ in the medium (Sacktor & Cheng, 1981).

During concentrative uptake of P_i in the presence of a sodium gradient Na^+ ions are transported for each P_i (Hoffman et al., 1976; Amstutz et al., 1985). This square concentration dependence on Na^+ has been invoked to explain the observation that P_i uptake is more sensitive to reductions in luminal Na^+ concentration than is transport of other metabolites such as glucose (Dennis & Brazy, 1982). Increasing pH increases the affinity of the carrier for Na^+ from a K_m of approximately 150 mM at pH 6.4 to 70 mM at 7.4. In rat renal BBMV, increasing Na^+ appears to decrease the K_m for P_i and to increase the V_{max} for transport (Amstutz et al., 1985). In BBMV from LLC-PK1 cells, only the affinity increase is observed (Brown et al., 1984). Kinetic analysis of sodium stimulation of P_i transport in the absence of a sodium gradient indicates a first-order dependence on Na^+ concentration (Cheng & Sacktor, 1981). These results suggest that binding of a single sodium is sufficient to allow transport, while binding of a second ion provides sufficient energy to drive net accumulation.

Above neutral pH the transport of P_i is predominately electroneutral, with changes in the vesicular transmembrane electrical potential altering P_i uptake by less than 15% (Hoffman et al., 1976; Cheng & Sacktor, 1981). Since transport in this pH range appears to require two Na^+ ions per P_i, HPO_4^{2-} must be the transported species above pH 7. This will result in the effective net translocation of H^+ if there is a pH difference between the two compartments. P_i transport is not enhanced by an internally negative electrical potential (Cheng & Sacktor, 1981; Burckhardt et al., 1981), but these latter authors, using potential-sensitive probes, did measure the generation of some internal positive charge during sodium-dependent P_i uptake into rat renal BBMV. In addition, a second-order dependence on Na^+ is observed throughout the entire pH range. It seems likely that the carrier binds two Na^+ ions both above and below neutrality and that protonation of a carrier protein residue or binding of protonated P_i results in the translocation of some positive charge. This may provide an additional energy source to drive P_i uptake at acid pH, where Na^+ binds poorly.

Inhibition of Na^+/P_i Cotransport in the Kidney Suggest that Arginine and Cysteine May Be Important

As in other systems, arsenate dianion is a competitive inhibitor of P_i transport in the kidney, but the K_i for arsenate (1 mM) is an order of magnitude higher than the K_m for P_i at pH 7.4 (Hoffman et al., 1976). Szczepanska-Konkel et al. (1986) have shown that small phosphonocarboxylates (formic and acetic) are competitive inhibitors of both concentrative and equilibrium Na^+/P_i cotransport in BBMV and in vivo. The K_i of the best inhibitor (phosphonoformic acid, 0.46 mM) is still quite high, but this may be due to the relatively high pK_a of the phosphonate (pH 7.24, Warren & Williams, 1971). The fractional inhibition of P_i transport by phosphonoformic acid is the same at each pH, but without a complete kinetic analysis it is impossible to distinguish substrate and inhibitor titrations from effects on binding constants and transport rates. Analogs with larger organic moieties do not inhibit, suggesting that the binding site for P_i is relatively small.

Strevey, Brunette and Beliveau (1984) found that rat kidney BBMV incubated with arginine-reactive reagents such as phenylglyoxal experience a 65% inhibition of concentrative P_i transport without inhibiting equilibrium transport (equal Na^+ inside and out). The Na^+ stoichiometry and affinity for P_i transport appeared unchanged. Extra vesicular P_i provides only partial protection against phenylglyoxal inhibition, but adding sodium increases protection to nearly 100% (Beliveau & Strevey, 1987). Debiec and Lorenc (1984) have examined the effect of several amino acid reagents on the Na^+/P_i cotransport in the absence of a sodium gradient. Certain sulfhydryl group reagents inhibit Na^+/P_i cotransport, provided they are present at the internal (cytoplasmic) surface of the vesicles. Disulfide-reducing reagents do not inhibit from either side of the membrane. The amino group reagent trinitrobenzenesulfonate (TNBS) inhibits P_i transport, but only when Na^+ is present during the inhibition,

again suggesting that a sodium-dependent conformational change occurs. This would also be consistent with the substantial alterations in kinetic parameters induced by Na^+.

Pratt and Pedersen (1989) have shown that membrane-permeant reagents specific for cysteine or tyrosine inhibit Na^+-dependent P_i uptake by rat renal BBMV, while impermeant analogs do not. Inhibition by NBD-Cl (7-chloro-4-nitrobenzo-2-oxa-1,3-diazole) is not prevented by Na^+ plus P_i, or by competitive inhibitors of P_i uptake. The reactivity of the critical sulfhydryl(s) is much less than that of the mitochondrial H^+/P_i cotransporter. Histidine- and carboxylate-specific reagents also cause substantial inhibition.

Identification of the Kidney Na^+/P_i Cotransporter Has Been Difficult

No protein has been unequivocally identified with Na^+/P_i cotransport activity in kidney. Hammerman, Hansen and Morrissey (1983) have attempted to identify the carrier in dog kidney BBMV by cAMP-dependent phosphorylation. A small decrease in P_i uptake is observed after in vitro cAMP-dependent phosphorylation of BBMV, accompanied by differential phosphorylation of two proteins with molecular masses of 96 and 62 kD. These effects are enhanced by prior treatment of the animals to lower circulating levels of PTH. The 62-kD protein is dephosphorylated with a time course corresponding to the recovery of transport activity. In the rat, fewer proteins are phosphorylated in a cAMP-dependent fashion, although a 62-kD protein with a dephosphorylation time course similar to recovery of P_i transport has been reported by Hruska, Kurnik and Tsutsumi (1984). This group has also observed the ADP-ribosylation of a 62-kD protein. A chloroform-methanol soluble phosphate-binding protein isolated from rabbit kidney has been characterized by Kessler, Vaughn and Fanestil (1982). While it appears to co-purify with P_i transport activity (*see below*), liposomes reconstituted with this protein alone do not carry out Na^+-dependent P_i accumulation (Schäli, Vaughn & Fanestil, 1986).

Lin, Schwarc and Stroh (1984) fractionated an octylglucoside extract of BBMV by chromatofocusing and reconstituted several sodium-dependent transport activities. Sodium-dependent P_i uptake was found in all fractions. Schäli and Fanestil (1985) solubilized rabbit kidney BBMV with octylglucoside and reconstituted the unresolved protein mixture into phosphatidylcholine plus cholesterol vesicles. Chromatofocussing of a similar extract prepared with the nonionic detergent NP-40 resulted in resolution of the BBMV proteins into three fractions, one of which contained Na^+-stimulated P_i transport activity which was inhibited by arsenate (Schäli et al., 1986). No evidence for reconstitution of concentrative uptake of P_i from kidney has yet been obtained.

Na^+/P_i Cotransport in the Intestine Resembles Transport in the Kidney

Inorganic phosphate transport in BBMV from rat small intestine has an apparent K_m for total P_i of 0.1–0.2 mM in the presence of 100 mM Na^+ (Berner, Kinne & Murer, 1976; Danisi, van Os & Straub, 1984) and a second order dependence on Na^+ concentration between pH 6 and 7.6. Similar results have been obtained in BBMV from rabbit duodenum (Danisi, Murer & Straub, 1984). The affinity for Na^+ is a strong function of pH, with more Na^+ required to saturate the carrier at lower pH, as is observed for the renal transport system. At saturating levels of Na^+, the rate of P_i transport in intestinal BBMV decreases with increasing pH, in contrast to observations for kidney BBMV. This behavior, coupled with a strong dependent of uptake rate on charge compensation have lead Shirazi-Beechey, Gorvel and Beechey (1988) to propose a model where two sodiums are taken up with one HPO_4^-. However, these kinetic differences between the kidney and intestine transporters may not reflect a complete difference in transport mechanism. At low Na^+ the pH dependence of the intestinal transporter is reduced (Danisi et al., 1984). Furthermore, the pH dependence of the renal transporter was found to be reversed in a number of situations (*see above*).

Shirazi-Beechey et al. (1988) have used the localization pattern of Na^+/P_i cotransport in the rabbit intestine to identify the transport protein. A mixture of antibodies raised against proteins from the duodenum of the rabbit inhibits P_i transport, while antibodies against segments which do not transport P_i do not inhibit. A different approach is that taken by Peerce (1988) who showed that, like the kidney transporter, the Na^+/P_i cotransporter from intestine is sensitive to the arginine reagents phenylglyoxyl. Peerce (1988) inhibited the intestinal BBMV P_i transporter with fluorescently labeled phenylglyoxyl. Inhibition is completely prevented by Na^+ plus P_i, suggesting that a residue near the active site is modified. Only two protein bands on SDS-PAGE showed a pattern of Na^+ plus P_i-protectable labeling with fluorescent phenylglyoxal, one of 145 kD and

another of 45 kD. When membrane proteins were solubilized with the zwitterionic detergent CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate) and separated by chromatofocussing, the sodium-dependent P_i transport activity was found in two fractions. SDS-PAGE analysis of the proteoliposomes which contained P_i transport activity revealed the presence of the 145-kD peptide, but not the 45-kD peptide. This evidence supports the assignment of the intestinal Na^+/P_i transport activity to this protein. This information should be useful for identifying the renal transporter.

Na^+/P_i Cotransport is a Mechanism Used by Many Animal Cells

Uptake of P_i by kidney and intestinal epithelial cells may be considered a special case because of the crucial role of this transport step in whole body P_i, Ca^{2+}, and pH balance. However, transport of P_i across the plasma membrane of other types of animal cells, while less well studied, appears very similar. For example, Brunette and Allard (1985) studied the P_i transport system in human placental membranes. In BBMV from placental membranes transport is sodium-dependent, with an apparent K_m at pH 7 of approximately 0.9 mM, which is reduced to 0.5 mM at pH 8.5. At low substrate concentrations the optimal uptake is obtained at pH 7.0. Sodium increases the V_{max} for transport, and not the affinity for P_i. This active transport through the placental brush border membrane is believed to be responsible for the maternal-fetal gradient of phosphate observed during pregnancy.

Na^+/P_i cotransport has been described in Simian virus-40 transformed 3T3 fibroblasts (Hamilton & Nilsen-Hamilton, 1978) and is also the mechanism of net P_i uptake by Ehrlich ascites tumor cells, a rapidly growing, highly malignant murine tumor line. The K_m for uptake into intact Ehrlich cells is approximately 0.3 mM for total P_i (Wehrle & Pedersen, 1982) and the V_{max} is quite similar to that of LLC-PK1 cells (Noronha-Blob et al., 1984). The rate of P_i transport varies little with pH unless cells are preincubated at the pH of interest before the transport assay. Using this protocol Bowen and Levinson (1983) observed that transport activity increases with increasing pH at higher P_i concentrations, although at lower P_i concentrations a maximum was observed at pH 7. The nature of the changes occurring after preincubation is unknown. One explanation is that the pH of the cytoplasmic compartment is changed, resulting in an alteration in activity. Uptake is a function of $H_2PO_4^-$ at constant HPO_4^{2-} and a function of HPO_4^{2-} at constant $H_2PO_4^-$ (Bowen & Levinson, 1983). Either monofluorophosphate (exclusively dianionic) or difluorophosphate (exclusively monoanionic) will substantially inhibit P_i uptake by Ehrlich cells, although monofluorophosphate is a significantly better inhibitor (Wehrle & Pedersen, 1982). Thus, for this Na^+/P_i cotransport system it appears that either mono- or divalent P_i can function as a substrate.

As reported for cultured pig kidney (LLC-PK1) cells, the specificity for Na^+ of the Ehrlich cell and SV-3T3 cell P_i transporters is much less than that observed in kidney BBMV. In particular, Li^+ appears to substitute well for a large portion of the Na^+. Transport appears to be nonelectrogenic at pH 7.3, and there is some evidence that H^+ translocation may accompany P_i transport. Significantly, P_i uptake is not inhibited by the H^+/P_i inhibitor mersalyl, nor by the anion exchanger inhibitor SITS. A substantial inhibition by the carboxylate-reactive chemical DCCD (N,N′-dicyclohexylcarbodiimide) suggests that an aspartic or glutamic acid residue may be necessary for activity, perhaps as a Na^+ or a H^+ binding site.

Figure 3 illustrates a composite of the characteristics of sodium-dependent P_i transport in eukaryotic plasma membrane. The order of binding of the substrates, the rate constants and potential dependence of the original steps, and other molecular mechanistic details remain to be elucidated.

Phosphate Exchange Transporters

Unlike the two modes of transport discussed so far, P_i/anion exchangers are a diverse group of translocators, with different mechanisms and unrelated structures. They have in common that they promote exchange of P_i for an anion other than the hydroxyl anion, and probably function as passive exchangers, moving anions down their electrochemical gradient.

P_i/Dicarboxylate Exchange Drives Substrate Uptake in Mitochondria

The dicarboxylate exchanger of the inner membrane catalyzes the transport of respiratory substrates such as malate and succinate into the mitochondrion. In addition, the exchanger may facilitate translocation of dicarboxylates into the cytoplasm for gluconeogenesis. This system can exchange certain dicarboxylic acids for others, but it also uses matrix P_i, which is accumulated in an energy-dependent reaction *via* the H^+/P_i cotransporter to promote and possibly regulate the distribution of sub-

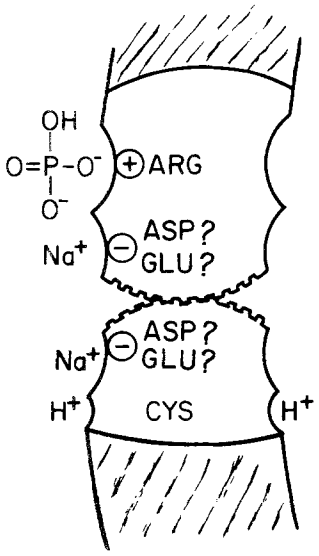

Fig. 3. An idealized Na^+/P_i cotransporter. Characteristics common to the various transporters have been synthesized. The binding site for P_i seems to at or near an arginine residue. Because FITC-conjugated phenylglyoxal inhibits, this residue may be near the outer surface of the transporter. Two sodium sites have been assigned to carboxylates, one necessary for both equilibrium and concentrative uptake and a second specifically utilized to drive concentrative uptake. One proton binding site (on the outside) would contribute to ΔpH-driven uptake. A second, on the inside of the membrane, would be used for control of transport rates by intracellular pH. Either sodium or proton binding sites might be generated by carboxylates, and so responsible for the inhibition of transport by DCCD or diethyl pyrocarbonate. The location of important cysteine residues is in question. Cytoplasmic, extracellular, and intramembrane sites have been suggested. Not indicated is the possibility of a tyrosine residue necessary for normal activity. For references and discussion, *see* the text

strate anions. The K_m values for dicarboxylate substrates (50–200 μM) are an order of magnitude less than the K_m for P_i (1.5 mM, Palmieri et al., 1971). The V_{max} for exchange in intact mitochondria (estimated at ≈70 nmol/min · mg of mitochondrial protein at 25°C in Wohlrab, 1986) is no greater than the rate of ATP synthesis, rather than greatly in excess, as is the H^+/P_i cotransport activity.

P_i transport via the exchanger can be physically distinguished from H^+/P_i cotransport using the competitive inhibitors *n*-butylmalonate (8) or phenylsuccinate (Chappell & Robinson, 1968), which have no effect on H^+/P_i cotransport. Although P_i/dicarboxylate exchange is completely inhibited by mercurial reagents and thus requires one or more free sulfhydryl residues for activity, it is not inhibited by the sulfhydryl alkylator NEM.

Dicarboxylate exchange activity has been reconstituted in artificial phospholipid vesicles from mitochondrial inner membrane solubilized with Triton X-100 (Saint-Macary, Laine & Foucher, 1979). More recently Kaplan and Pedersen (1985), using a highly purified preparation of dicarboxylate exchanger from rat liver mitochondria, reported successful reconstitution of malate self-exchange activity. In its purification behavior, the dicarboxylate exchanger closely resembles other mitochondrial transporters. The exchanger is solubilized by Triton X-114, fails to adsorb to hydroxylapatite from the detergent extract, and requires cardiolipin and liposome reconstitution for stability. A purification of 230-fold was achieved by hydroxylapatite chromatography, assuming equivalent efficiency of reconstitution. Malate exchange is completely inhibited by mercurials and *n*-butylmalonate, but is not inhibited by NEM. External unlabeled malate, malonate, or succinate inhibit malate uptake, but citrate and α-ketoglutarate do not (Kaplan & Pedersen, 1985), mimicking the behavior of the exchanger in intact mitochondria (Palmieri et al., 1971). P_i inhibits malate exchange, but only at high concentrations. Transport of P_i by this reconstituted system has not been reported. At least six distinct bands of the partially purified transporter are visible on silver-stained SDS-PAGE. Saint-Macary and Foucher (1985) reconstituted dicarboxylate exchange activity from mitochondria from several sources. All of the preparations contained a group of proteins in the vicinity of 30 kD, which raises the possibility that the P_i dicarboxylate exchanger may resemble the H^+/P_i cotransporter, as was suggested years ago (Coty & Pedersen, 1975*a*).

P_i/Triose Phosphate/Phosphoglycerate Exchange in Chloroplasts Releases Fixed Carbon to the Cytoplasm

During photosynthesis newly fixed carbon leaves the chloroplast in the form of triose phosphate (triose-P); consequently, P_i must be continually replaced to maintain a steady state within the organelle. Transport of P_i across the chloroplast inner envelop membrane has been shown to occur by an obligatory one-for-one exchange with triose-P (Heldt & Rapley, 1970). In addition to exchange of triose-P for P_i, the chloroplast exchanger can promote exchange of triose-P for 3-phosphoglycerate (3-PGA), which results in the delivery of NADH plus ATP or of NADPH to the cytoplasm via recycling reactions. The exchanger accepts P_i or various three-carbon molecules phosphorylated at either the 1 or 3 position but not at carbon 2 (Fleige et al., 1978). The preferred form of P_i as substrate appears to be HPO_4^{2-}. In the dark P_i and 3-PGA are accepted

with similar affinity for exchange against triose-P, but during photosynthesis exchange of 3-PGA is considerably reduced relative to exchange of P_i, resulting in net carbohydrate release to the cytoplasm. This exchanger resembles the P_i/hexaose-P exchanger found in many bacteria, including *Streptococcus lactis* and *Staphylococcus aureus* (Ambudkar & Maloney, 1984; Maloney et al., 1984; Sonna & Maloney, 1988).

Inorganic phosphate/triose-P exchange is inhibited by phenylglyoxal, which reacts primarily with arginine residues, and by pyridoxal 5'-phosphate and TNBS, which alter free amino groups (Flügge & Heldt, 1979). Inhibition by these reagents can be prevented by substrate phosphates, suggesting that fixed positive charges on these residues form the P_i binding site. Mercurials also inhibit transport, but the reactive sulfhydryl does not appear to be at the substrate site. By labeling with radioactive inhibitors, the exchanger has been identified as a 29-kD protein comprising a large fraction of the chloroplast inner membrane. The P_i/triose-P exchanger has been solubilized using Triton X-100 and transport has been reconstituted in phospholipid vesicles (Flügge, Gerber & Heldt, 1981), where it exhibits pH gradient-dependent alterations in substrate specificity identical to those observed in the intact chloroplast. The molecular mass of the exchanger in the Triton X-100 extract is estimated to be 61 kD (Flügge & Heldt, 1984), or double that of the monomer. Thus, the functional unit may be a dimer of identical subunits.

Recently the cytoplasmic gene coding for the P_i/triose-P exchanger has been cloned and sequenced (Flügge et al., 1989). The gene codes for a 44.2-kD precursor peptide, which can be inserted into the chloroplast inner envelop membrane in an ATP-dependent reaction. Upon insertion the protein is processed to a mature transporter of 34 kD. It is interesting to note that both the precursor and the mature transporter appear somewhat larger by sequence analysis than had been predicted by their mobility on SDS-PAGE. The sequence of the P_i/triose-P exchanger contains no significant internal homology and does not resemble the mitochondrial H^+/P_i cotransporter. It will be extremely interesting to see whether the mitochondrial P_i/dicarboxylate transporter is homologous with the chloroplast exchanger.

Inorganic Phosphate Exchange in Erythrocytes Occurs via the Cl^-/HCO_3^- Exchanger

The primary function of this glycoprotein, called "band 3" due to its mobility on SDS-PAGE gels, is transport of HCO_3^- for the control of respiration and acid balance. This system differs significantly from the other systems described, which are specifically designed for the transport of P_i. The band 3 protein is designed for the electroneutral exchange of monoanions, but it does transport SO_4^{2-}, though at a much reduced rate. This raises the question of which form of P_i is transported. The rate of chloride transport is reduced at lower pH, increases as the pH increases, then plateaus above pH 7 (Brahm, 1977). In contrast, translocation of sulfate (in exchange for internal Cl^-) occurs with a maximal velocity at approximately 6.3, followed by a decline at higher pH (Jennings, 1976). This suggests that the extra negative charge of SO_4^{2-} must be neutralized in order to maximize transport rates. The pH-rate profile for P_i transport had a sharp maximum at pH 6.3 and is lower both above and below this value, leading Runyon and Gunn (1984) to conclude that only $H_2PO_4^-$ is transported. Berghout et al. (1985) took advantage of their earlier observation (Legrum, Fasold & Passow, 1980) that modification of the erythrocyte membrane with dansyl chloride inhibits the transport of monovalent and enhances the transport of divalent anions. Following dansylation, transport of P_i is reduced at lower pH, but enhanced at pH values above 7.5. Thus, at low pH P_i behaves like Cl^-, but at high pH it behaves partially like SO_4^{2-}. These authors suggest that HPO_4^{2-} can be transported, but that the rate is very slow compared to that of $H_2PO_4^-$.

The classical competitive and noncompetitive inhibitors of band 3 anion exchange are stilbenesulfonates such as SITS and other sulfonates such as DIDS (4,4'-diisothiocyanato-1,2-diphenylethane-2,2'-disulfonic acid). In contrast to other P_i transport systems, the band 3 anion exchanger is not inhibited by sulfhydryl reagents. Inhibition has been observed by reagents that react with lysine (Jennings, 1982), arginine (Weith, Bjerrum & Borders, 1982), and tyrosine residues (Craik, Gounden & Reithmeier, 1986). The tyrosine modified by NBD-Cl is not protected by competitive inhibitors of anion transport. In contrast, inhibition of transport by the nonpenetrant carbodiimide, 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide can be prevented by the competitive inhibitors DNDS (4,4-dinitrostilbene-2,2-disulfonic acid). The reactivity of this carboxylate is altered by manipulations known to alter the carrier orientation (Craik & Reithmeier, 1985).

The band 3 transporter consists of a dimer or tetramer of identical 95-kD peptides, each of which appears to be independently capable of catalyzing anion exchange (Macara & Cantley, 1983). Each peptide has a 43-kD glycosylated, water-soluble cytoplasmic domain which binds to the cytoskeleton and a 52-kD hydrophobic domain which traverses the erythrocyte membrane. Jennings (1985) has pro-

posed a model for the hydrophobic domain which includes seven transmembrane sequences. The gene for the band 3 transporter has recently been expressed in *Xenopus* oocytes (Gunn & Kopito, 1989).

Conclusion

Much more work has been done on P_i transport processes, even in the last five years, than we have been able to mention in the space available. We have restricted our discussion to studies on mechanisms of transport or transport regulation, identification of transport proteins and their essential amino acids, and isolation, purification, and reconstitution of P_i transport systems. Many valuable studies on the physiology of P_i transport and its regulation and P_i transport in nonepithelial cells have also been conducted. Transport of P_i into and out of organelles other than the mitochondrion is gaining well-deserved attention, as are transport processes in fungi and plants. It is hoped that in another five years many P_i transport processes will be understood in true molecular terms and that this will increase our knowledge of cellular bioenergetics and metabolism.

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