

STRUCTURE, FUNCTION AND EVOLUTION OF SOLUTE TRANSPORTERS IN PROKARYOTES AND EUKARYOTES

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Summary

In both prokaryotes and eukaryotes, transport systems of organic solutes can be classified as passive transporters, such as channels and facilitated transporters, and active transporters, which utilize diverse energy-coupling mechanisms. In the past decade, our understanding of the biochemistry and molecular biology of transporters from *Escherichia coli* has progressed significantly, whereas the analysis of mammalian transporters has initially been limited by the ability to purify membrane proteins. The recent development of methods to detect the activity of recombinant proteins in individual cells, however, has led to the cloning of several novel mammalian transporter cDNAs. One of the most useful expression cloning systems is *Xenopus* oocytes in conjunction with uptake studies and electrophysiological experiments. Overall, the sequence information and the functional data derived from many transporters has revealed unifying designs, similar energy-coupling mechanisms and common evolutionary origins. Here, I will provide a general survey of the known transport systems in bacteria, yeast, plants, insects and vertebrates and illustrate the different types of transport systems in mammals by discussing transporters recently studied in our laboratory.

Introduction

Transport of organic solutes such as sugars, amino acids, peptides, neurotransmitters and drugs across cell membranes proceeds *via* specialized active and passive transporters. Active transporters couple solute transport to the input of energy and can be divided into two classes: ion-coupled and ATP-dependent transporters. Ion-coupled transporters link uphill solute transport to downhill electrochemical ion gradients. In general, these transporters are coupled to the cotransport of H⁺, Na⁺, Cl⁻ and/or to the countertransport of K⁺, although there are exceptions as discussed below. The electrochemical ion gradients are maintained by H⁺- or Na⁺/K⁺-ATPases located in cell membranes. ATP-dependent transporters are directly energized by the hydrolysis of ATP and translocate a heterogeneous set of substrates. Passive transporters include facilitated transporters and channels, which allow the diffusion of solutes across membranes. The goal of this review

Key words: membrane transport, glucose, amino acids, peptides, antibiotics, glutamate, neurotransmitter, urea, kidney, intestine, brain, lepidopteran insects, synaptic transmission, urinary concentrating mechanism, cystinuria, porin, ABC transporter, P-glycoprotein, periplasmic permease.

is to present selected examples of different transporters found in bacteria, yeast, plants, insects and higher vertebrates and to highlight unique properties, functional roles and common themes used in their design. Examples from mammalian transporters recently studied in our laboratory are also presented. These include the intestinal and renal Na⁺/glucose cotransporters SGLT1 (Hediger *et al.* 1987; Hediger and Rhoads, 1994; Lee *et al.* 1994) and SGLT2 (Kanai *et al.* 1994), the Na⁺- and K⁺-dependent neuronal and epithelial high-affinity glutamate transporter EAAC1 (Kanai and Hediger, 1992; Kanai *et al.* 1993*a,b*), the intestinal H⁺-coupled oligopeptide transporter PepT1 (Fei *et al.* 1994), the renal dibasic and neutral amino acid transport protein D2 (Wells and Hediger, 1992; Lee *et al.* 1993) and the renal facilitated urea transporter UT2 (You *et al.* 1993).

Transport proteins in bacteria

The major permeability barrier in bacteria is the cytoplasmic membrane. Gram-negative bacteria, such as *Escherichia coli*, also protect themselves by an additional structure called the outer membrane (Fig. 1A). The outer leaflet of the outer membrane is composed of an unusual lipid, a lipopolysaccharide, rather than the usual glycerophospholipid found in most biological membranes. This asymmetric bilayer provides an efficient barrier against rapid penetration by lipophilic agents such as antibiotics (Nikaido, 1994*a*). The periplasmic space between the two membranes contains proteins released by the cell, such as receptors which trap solutes.

Transport across the outer membrane

A special class of proteins, called porins (Cowan *et al.* 1992; Nikaido, 1994*b*; Nikaido and Saier, 1992), forms non-specific aqueous diffusion channels across the outer membrane to permit capture of nutrients from the environment in the periplasm (Fig. 1A). These are relatively hydrophilic proteins of approximately 36–38 kDa, which lack the long stretches of hydrophobic segments typically found in integral membrane transport proteins such as mammalian Na⁺/glucose cotransporters or glutamate transporters (see hydropathy plot, Fig. 12). Crystal structures of *E. coli* porins such as OmpF (Cowan *et al.* 1992; Eisele and Rosenbusch, 1989) revealed trimers of identical subunits, each subunit containing relatively short membrane-spanning segments that traverse the lipid bilayer 16 times as antiparallel β strands to form a β -barrel structure which surrounds a large channel. The β -barrel design requires minimal hydrophobicity and probably facilitates the export of the porin proteins across the cytoplasmic membrane into the periplasm, where they insert into the outer membrane to fold into a stable β -barrel configuration through interaction with the lipopolysaccharides (Sen and Nikaido, 1991).

The lambda receptor (LamB) of *E. coli* represents a special case within the porin family. It is a porin-like protein which allows diffusion of disaccharides, such as maltose, across the outer membrane. Bacteriophage λ has evolved the capacity to exploit the LamB protein as a receptor. The structural similarity of the LamB protein to the classical porins suggests that maltose diffusion occurs through a similar mechanism (Luckey and Nikaido, 1980). However, there is a specific binding site within the channel that interacts

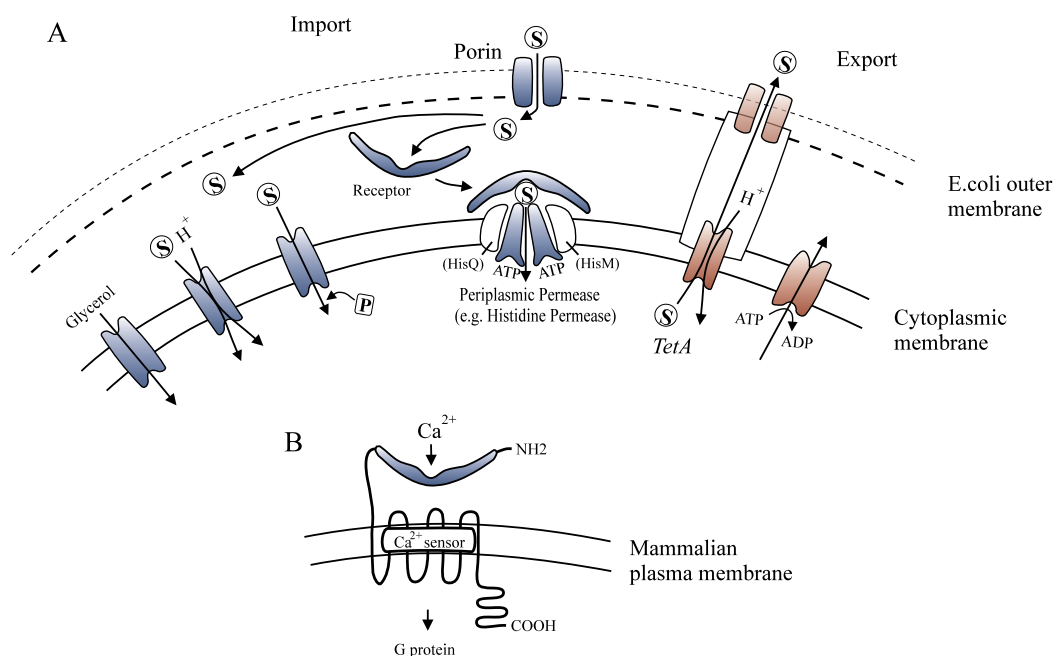


Fig. 1. (A) Schematic representation of the major classes of transport systems in the cytoplasmic and outer membranes of Gram-negative bacteria. Transports involved in import (left and center) or export (right) of substrates (S) is shown. P indicates phosphorylation of sugars by the phosphoenolpyruvate-dependent phosphotransferase system. (B) The extracellular domain of the mammalian Ca²⁺ sensor is proposed to be structurally similar to receptors of periplasmic permeases.

loosely with maltose. Kinetic studies revealed that this transport is a saturable process and that its properties are characteristic of facilitated diffusion (Freundlieb *et al.* 1988). The lambda receptor may therefore have evolved from a porin channel by the addition of a ligand-binding site to form a transporter. This illustrates an evolutionary route for the development of transporters for specific substrates.

The outer mitochondrial membrane of eukaryotic cells also possesses pore-forming proteins (see Fig. 4) whose structure and function are similar to those in the outer membrane of Gram-negative bacteria (Benz, 1990). No significant sequence homology, however, has been shown between the eukaryotic and bacterial porins.

Whether porin-like proteins also exist in mammalian plasma membranes is not clear (Fischbarg *et al.* 1993; Ha *et al.* 1993). The presence of large pores in plasma membranes would undoubtedly cause osmotic lysis, by analogy with the pore-forming membrane attack complex of the complement system (Davies and Lachmann, 1993). However, there may be porin-like plasma membrane proteins which include a ligand-binding site to form a transporter. Such proteins would be expected to have a hydrophobic α -helical leader sequence to direct their insertion into the plasma membrane (Dalbey, 1990) unless insertion occurs *via* an unconventional route.

Cytoplasmic uptake systems

These can be divided into the following classes: (1) facilitated transporters; (2) ion-coupled transporters; (3) transport systems that are energized by ATP hydrolysis; and (4) the phosphotransferase system that phosphorylates sugars subsequent to their translocation *via* a sugar-specific facilitated transporter (Fig. 1A). What follows is a discussion of the important aspects of the facilitated, ion-coupled and ATP-driven transporters. For information on the phosphotransferase system, the reader is referred to Meadow *et al.* (1990) and Stolz *et al.* (1993).

Facilitated transporters

The only known facilitated system in *E. coli* is the glycerol facilitator GlpF (Heller *et al.* 1980). GlpF is a 281-residue protein containing six putative transmembrane α -helices. It is a member of the major intrinsic protein (MIP) family of channel proteins and of the mammalian aquaporin family, which includes the mammalian water channel CHIP28 (Agre *et al.* 1993; Knepper, 1994).

Ion-coupled transporters

In bacteria, H^+ and Na^+ are the most common coupling ions used by ion-coupled transporters. Na^+ electrochemical gradients are formed in many bacteria, such as *E. coli*, by Na^+/H^+ antiport at the expense of the energy stored in the electrochemical ($\Delta\bar{\mu}_H$) gradient. Bacteria use Na^+ bioenergetics for special purposes, whereas the main energy-converting pathways, such as ATP synthesis, H^+ -coupled transport and flagellar motion, are energized directly by the H^+ electrochemical gradient $\Delta\bar{\mu}_H$ produced by oxidation *via* the respiratory chain. The glutamate transporter *gltS* (but not *gltP*), the proline transporter *putP* and the melibiose carrier *mel* are examples of Na^+ /cotransport (symport) systems. Because of the increased H^+ permeability of cell membranes at elevated temperatures, thermophilic bacteria have adapted to life at extreme temperatures by using Na^+ rather than H^+ as the coupling ion in solute transport (Konings *et al.* 1992).

How is uphill solute transport coupled to the downhill electrochemical ion gradients of H^+ and Na^+ ? Site-directed mutagenesis studies of the *E. coli* H^+ -coupled lactose permease (*lacY*) suggested that Arg-302, His-322 and Glu-325 play important roles in H^+ coupling (Roepe *et al.* 1990). Since these residues are predicted to be located close to each other in helices 9 and 10, it has been proposed that they act as an H^+ translocating network. A particularly interesting protein for studying the molecular mechanism of ion coupling is the melibiose transporter of *E. coli* (Botfield *et al.* 1990; Leblanc *et al.* 1993). This transporter can use either H^+ or Na^+ as the coupling ion, depending on which sugar is being transported. This versatility suggests that the ion-coupling mechanism for the melibiose transporter must be different from that proposed for lactose permease.

Many bacterial H^+ -coupled transporters are part of a large protein family that also shares significant homology to the mammalian facilitated glucose transporters (Maiden *et al.* 1987). For example the *E. coli* H^+ -coupled transporters of arabinose and xylose

show approximately 30% sequence identity to the facilitated glucose transporter GLUT1 from human erythrocytes. These proteins are all predicted to have 12 membrane-spanning regions and probably have a similar three-dimensional structure. In addition, there is internal homology between the first six and the second six putative membrane-spanning helices, indicating that these proteins evolved by duplication of an ancestral gene encoding six helices. Since transporters of the GLUT family are not H⁺-coupled (see Fig. 4), this homology indicates that interconversion of facilitated transporters and cotransporters did not necessarily require complete redesigning of the protein backbone during evolution.

The periplasmic permease

Periplasmic permeases of Gram-negative bacteria transport sugars, amino acids and ions with high affinity (10 μ M to 1 mM) (Fig. 1A, center). These permeases are typically composed of a soluble receptor located in the periplasm and a membrane-associated complex (Ames, 1993). As shown in Fig. 1A, the latter is composed of two hydrophobic proteins that form a diffusion channel and two copies of a hydrophilic ATPase component located on the cytoplasmic side. The ATPase component shows strong sequence homology to the ATPase component of members of the ABC (ATP-binding cassette) family (see below). A unique feature of periplasmic permeases is that they bind substrates to receptors prior to the translocation step. The high concentration of the receptors present in the periplasm permits trapping of substrates and results in accumulation before the actual translocation step. Gram-positive bacteria, such as *Streptococci* involved in dental plaque formation, have similar receptors that bind multiple sugars (Russell *et al.* 1992). Since these bacteria lack an outer membrane, the receptors would seem likely to be lost to the surroundings once secreted. It was proposed that they are anchored to the cytoplasmic membrane by lipids covalently linked to an N-terminal cysteine residue after removal of the signal peptide.

The best-studied periplasmic permeases of Gram-negative bacteria are the maltose system of *E. coli* and the histidine system of *Salmonella typhimurium* (see Ames, 1993, for a review). All five subunits of the complex – the receptor, the two hydrophobic proteins and the two ATPase components (see Fig. 1A) – are synthesized separately. An attractive hypothesis is that the ABC family (see below) evolved from an ancestral periplasmic permease by fusion of the receptor, the transmembrane subunits and two ATPase components to form a single protein. However, the transmembrane subunits HisQ and HisM of the histidine permease (see Fig. 1A) show no significant homology to members of the ABC family, indicating that this evolutionary relationship may be more complex.

Crystal structures of periplasmic receptors (Oh *et al.* 1993) confirmed the hypothesis of Quioco and his collaborators (Mao *et al.* 1982) that these receptors resemble a flytrap with two lobes that move like a pincer to envelop the ligand. Interestingly, the receptors appear to have a low level of structural similarity to the extracellular ligand domain of glutamatergic receptors (O'Hara *et al.* 1993) and to the recently cloned mammalian Ca²⁺ sensor (Brown *et al.* 1993) (Fig. 1B). This similarity led to the speculation that glutamate

receptors and sensors of Ca^{2+} , and possibly other ions, evolved by fusion of genes encoding a periplasmic receptor (the flytrap) and an integral membrane protein (the 'serpentine receptor'), which activates G-proteins.

Efflux systems

Active efflux systems of the *E. coli* inner and outer membranes play an important role in drug resistance (Nikaido, 1994a). Two classes of efflux systems have been described: (1) H^+ /drug antiporters that extrude drugs by using the proton-motive force and (2) ABC transporters (Fig. 1A, right). Efflux transporters are located in the cytoplasmic membrane. An important efflux system of Gram-negative bacteria is the TetA H^+ /tetracycline antiporter (Hinrichs *et al.* 1994). Binding of tetracycline to the *TetR* repressor causes dissociation of the repressor-operator DNA complex and triggers expression of the *TetA* gene. Since substrates would be pumped into the periplasm in Gram-negative bacteria, it has been proposed that accessory proteins in the periplasm bridge cytoplasmic H^+ /drug antiporters to outer membrane channels so that drugs are extruded directly into the surrounding medium (see Fig. 1A, right).

Understanding the key proteins involved in antibiotic resistance could be of great aid in developing new drugs. Efforts to produce more effective antibiotics, such as penicillins and cephalosporins, have involved modification of specific groups on antibiotic molecules to make them inert against antibiotic-inactivating enzymes. Such drugs could also be easily tested for efficient intestinal absorption using *Xenopus* oocytes expressing the recently cloned transporters PepT1 of oligopeptides and peptide-derived drugs (see below).

ABC transporters and multidrug resistance

These transporters are integral membrane proteins that include an ATP-binding cassette (ABC) (Gros and Buschman, 1993). In general, they are active transporters that carry out ATP-dependent extrusion of substrates from the cytoplasm. Transport is thought to be directly energized by ATP hydrolysis. The ABC transporter family includes the mammalian P-glycoprotein of multidrug resistance (MDR1–3) (Gros *et al.* 1988) (see Fig. 4, top right), the cystic fibrosis membrane conductance regulator (CFTR) (Riordan *et al.* 1994; Welsh and Anderson, 1993), the MHC (major histocompatibility complex)-encoded peptide transporters TAP1 and TAP2 (Momburg *et al.* 1994), the STE6 protein from *Saccharomyces cerevisiae* (Berkower and Michaelis, 1993) and bacterial export mechanisms (see above). The peptide transporters TAP1 and TAP2 are associated with antigen processing. These proteins transport larger peptides (approximately 20-mers) in an ATP-dependent manner into the lumen of the endoplasmic reticulum. There is no sequence homology between TAP1 and TAP2 and the oligopeptide transporter PepT1 (see below). The yeast STE6 transporter mediates export of *a*-factor, a 12-residue peptide derivative required to initiate the yeast mating program.

Sequence comparison of ABC family members revealed that P-glycoproteins CFTR and STE6 are composed of two homologous halves, each encoding six predicted

membrane-spanning regions and an ATP nucleotide binding fold. As indicated above, this fold is homologous to the hydrophilic ATPase component of bacterial periplasmic permease. Certain ABC transporters, such as TAP1 and TAP2, are half-molecules with only a single set of membrane-spanning regions and an ATP binding fold. A unique characteristic of this protein family is their heterogeneous spectrum of substrates. However, although evolutionary pressure must have acted vigorously to diversify their substrate specificities, the common transport mechanism among most members of this family was remarkably preserved.

Function of P-glycoproteins

Expression of P-glycoprotein MDR1 in the plasma membranes of mammalian cells in culture was shown to cause resistance to the toxic effects of a wide variety of chemically unrelated drugs (Gros and Buschman, 1993). This phenomenon constitutes a major mechanism of multidrug resistance in tumors. A major limitation of chemotherapeutic treatment of many types of human tumors is the formation and outgrowth of subpopulations of drug-resistant cells. These cells often display resistance to other drugs that share little similarity to the drug that induced the resistance. The only similarities among these compounds are that they are small, biplanar and hydrophobic molecules and contain a basic nitrogen atom. Their hydrophobicity permits entry into cells by simple diffusion across the plasma membrane.

By analogy with certain forms of antibiotic resistance in bacteria (see above), P-glycoprotein-mediated multidrug resistance is thought to be the result of active extrusion of cytotoxic drugs. However, the transport properties of P-glycoproteins are not yet completely established. Recent insights by Reuss and colleagues have revealed that the human P-glycoprotein MDR1 is both an efflux pump of drugs and a Cl⁻ channel activated by cell swelling (Altenberg *et al.* 1994*a,b*). The purpose of this dual function of P-glycoproteins has not yet been defined.

Membrane transport in plants

In general, the driving force for solute transport across plant cell membranes is the proton-motive force generated by plasma membrane H⁺-ATPases. The cellular uptake of sucrose, glucose, amino acids and nitrate is mediated by H⁺-coupled transporters (Bush, 1993; Caspari *et al.* 1994). cDNAs encoding an H⁺-coupled sucrose transporter (SUT1, see below), a sucrose-binding protein (SBP) (see below), an H⁺-coupled hexose transporter (Sauer and Tanner, 1989) similar to the mammalian facilitated glucose transporters (GLUTs), a neutral amino acid transporter (Hsu *et al.* 1993) and a nitrate transporter (CHL1, see below) have recently been isolated and characterized.

Distribution of photoassimilates

Sucrose is the main carbon metabolite in plants and the principal end product of photosynthetic carbon assimilation. H⁺-coupled sucrose transport across cell

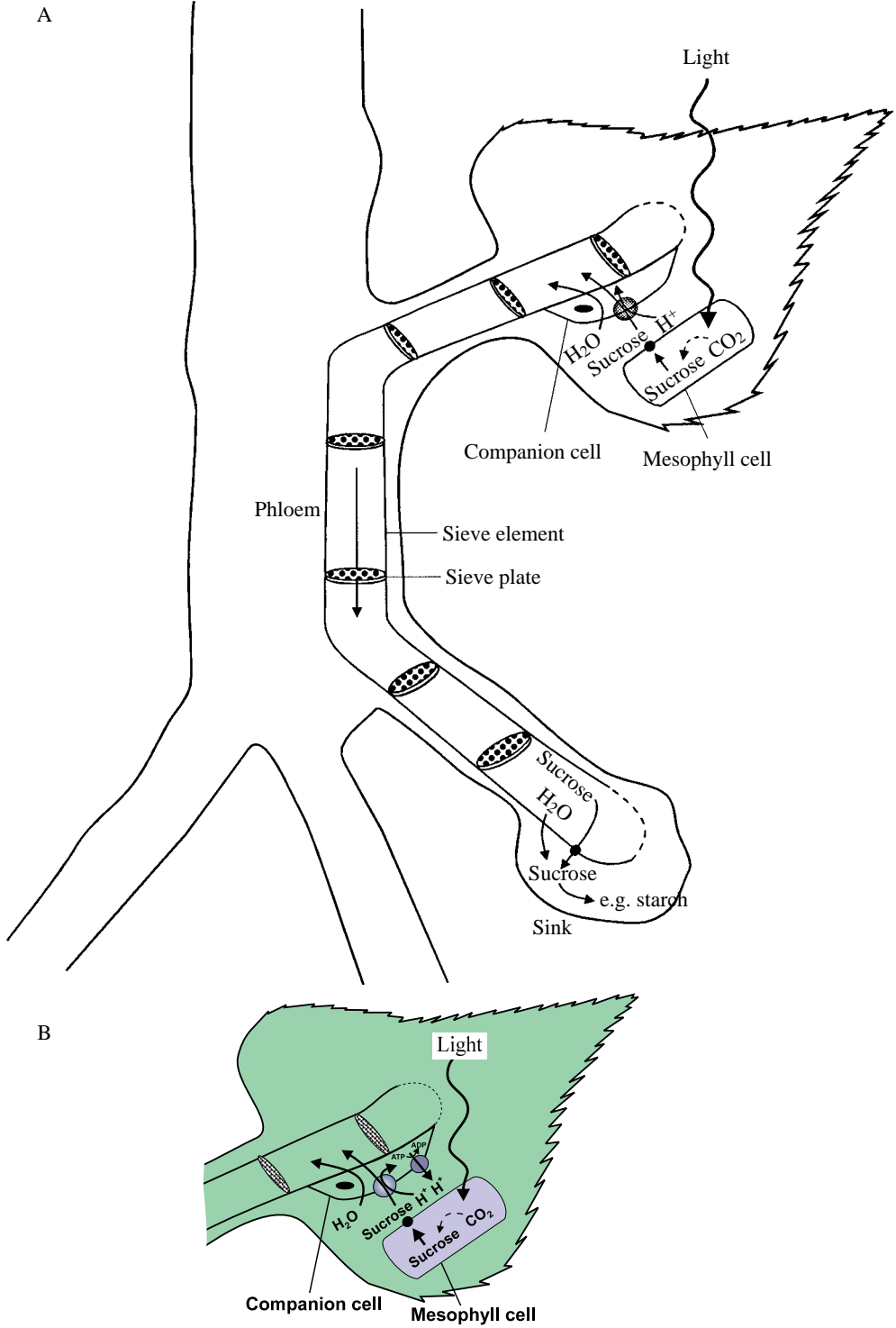


Fig. 2.

membranes of plants plays an important role in the distribution of assimilates (Bush, 1993) (Fig. 2A,B). The phloem is the main pathway through which sucrose and other carbohydrates formed during photosynthesis in the leaves are transported to all other parts of the plant. The conducting cells of the phloem elements are made up of long cellular aggregations of elongated cells, called sieve elements (Fig. 2A). Sieve elements are usually without nuclei and contain perforated end plates (sieve areas), which are specialized regions on the walls of overlapping cell tips where cytoplasmic connections occur. Closely associated with the sieve areas are the companion cells, which contain distinct nuclei (Fig. 2). In the sieve element/companion cell complex of the leaves, the companion cells are thought to accumulate sucrose and to transfer it to the sieve tubes.

The primary products of photosynthesis formed in chloroplasts of photosynthetically active mesophyll cells of the leaves are the triose phosphates. These are exported from the chloroplasts into the cytosol and metabolized into sucrose for export to non-photosynthetic cells. Sucrose is thought first to exit the mesophyll *via* a sucrose efflux system (Fig. 2). It is then loaded into the sieve element/companion cell complex of the small veins of the leaf in a process called phloem loading (Van Bel, 1993). This results in the entry of water into the sieve tubes by osmosis. Pressure builds up, forcing the solution to move towards the sinks. This movement permits allocation of assimilates to support the growth of heterotrophic tissues, such as developing leaves, apices, roots and reproductive organs (Fig. 2A). At the sinks, the sucrose is actively removed and water follows osmotically. Sugar transport to sink tissues may involve mechanisms similar to those involved in loading sucrose into the sieve element/companion cell complex.

Sucrose transporter SUT1

In many plants the phloem loading process depends on the function of an active H^+ -coupled sucrose transporter. Frommer and colleagues recently isolated a cDNA encoding the H^+ /sucrose transporter SUT1 responsible for phloem loading in spinach (Riesmeier *et al.* 1992a). The cloning was accomplished by complementing an engineered yeast mutant with a plant cDNA library. When SUT1 was expressed in yeast, the apparent K_m

Fig. 2. (A) Apoplastic transport of sugars in the phloem of plants. A close-up of the sugar transport systems in the leaf is shown in color in B. Sucrose and other photosynthesis products formed in the leaf (top right) are transported to a storage organ (sink, bottom right) *via* the phloem. Sucrose exiting the mesophyll is actively loaded into the sieve element/companion cell complex of the phloem. This is most likely achieved by the H^+ -coupled sucrose transporter SUT1. The H^+ -ATPase, which energizes the H^+ -cotransport process, was recently localized in transfer cells in the area of plasmalemma infoldings where active nutrient uptake is assumed to take place (Bouché-Pillon *et al.* 1994) (see Fig. 2B). Sucrose then moves across plasmodesma connections into sieve elements. For simplicity, the figure shows the phloem as a single sieve element complex only. However, sucrose is first loaded from the mesophyll into the phloem of minor veins and major veins are then involved in long-distance transport of sucrose. Some species are known to transport sucrose symplastically (Van Bel, 1993); this pathway is not considered in the figure.

for sucrose was 1.5 mM. Previous studies have revealed that sucrose transport is an electrogenic process with an H⁺:sugar stoichiometry of 1:1 (Bush, 1990). The SUT1 cDNA encodes a 525-residue protein with 12 putative membrane-spanning regions. No extensive sequence homology to other proteins was reported. Expression of SUT1 mRNA is high in mature exporting leaves and low in young leaves. Transgenic potato plants expressing SUT1 antisense constructs exhibited reduced levels of sucrose transporter mRNA (Riesmeier *et al.* 1992b). The dramatic reduction in root development and tuber yield of transgenic plants compared with control plants supported the hypothesis that this transporter plays an important role in phloem loading and in allocating assimilates to heterotrophic tissues. The plant H⁺-ATPase which provides the proton-motive force for sucrose transport has recently been localized to the plasma membrane of transfer cells (Bouché-Pillon *et al.* 1994).

The sucrose binding protein (SBP)

Grimes *et al.* (1992) identified a cDNA which encodes a 62 kDa sucrose-binding protein, referred to as SBP, which has several features that implicate a critical role in sucrose transport. Although SBP is an extrinsic hydrophilic protein, immunocytochemical evidence recently indicated that it is tightly associated with the extracellular leaflet of the plasma membrane of several cell types actively transporting sucrose, including the sieve element/companion cell complexes of mature phloem, the mesophyll cells of young leaves and the cotyledon sink cells (Overvoorde and Grimes, 1994). The precise functional role of this protein in sucrose transport, however, has not yet been determined.

Other sucrose transport systems

Despite these advances in molecular plant physiology, there are still many gaps in our understanding of sucrose transport. For example, the sucrose efflux system in the mesophyll has not yet been identified, although it is likely that it provides another important regulator for controlling carbon flux from the leaves. Likewise, the sugar and amino acid transporters in the sink tissues and in storage organs have not yet been described in detail (see Fig. 2A). Moreover, the carbon storage achieved by sucrose accumulation in vacuoles, such as in specialized storage cells of sugar cane, is thought to be coupled to the countertransport of H⁺. However, this protein has not yet been identified.

The nitrate transporter CHL1

In higher plants, amino acids are the primary source of nitrogen available to heterotrophic tissues. Inorganic nitrogen is typically absorbed from the soil as nitrate. The gene in *Arabidopsis* which encodes the nitrate transporter (CHL1) responsible for removal of nitrate from the soil was recently identified (Tsay *et al.* 1993). This transporter mediates H⁺-coupled nitrate uptake in the roots, has 12 putative membrane-spanning segments and shows weak homology to the intestinal H⁺-coupled peptide transporter PepT1 (see below). Since transport was found to be electrogenic, at least two H⁺ must be

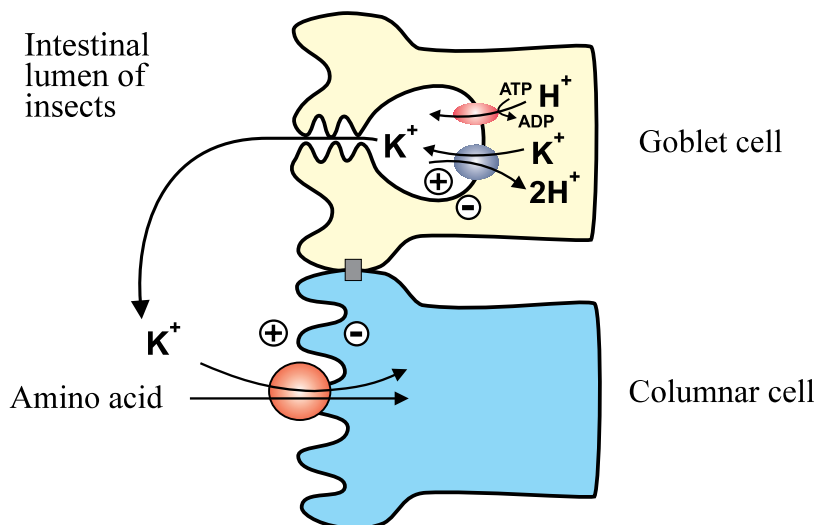


Fig. 3. K^+ -coupled amino acid transporters in brush-border membranes of insect midgut columnar cells. The plasma membrane V-ATPase of goblet cells is an electrogenic proton pump which energizes electrogenic K^+ secretion via $K^+/2H^+$ antiport.

cotransported with each NO_3^- . Nitrate absorbed by this protein is then transported to the leaves, where it is reductively assimilated. Subsequently, amino acids are exported to heterotrophic cells, where they are taken up by cells via H^+ -coupled transporters. The CHL1 nitrate transporter shows weak but significant homology to the peptide transporter PepT1 from rabbit small intestine (see below).

The K^+ -coupled amino acid transporters of insect intestine

In insect epithelial tissues, active electrogenic secretion of K^+ is an important mechanism of salt and fluid secretion. In the midgut of larval lepidopteran insects, the inwardly directed electrochemical K^+ gradient provides the driving force for uphill solute transport into enterocytes. The midgut is a straight tube consisting of two types of epithelial cells, columnar cells and goblet cells (Fig. 3). The goblet cells have a unique large apical cavity that is guarded at its apical end by a valve-like structure. The goblet cells actively secrete K^+ into this cavity, thereby providing the driving force for uphill K^+ -coupled amino acid transport from the lumen into columnar cells.

How is K^+ secreted into the goblet cavity? K^+ moves passively from the blood into goblet cells via K^+ channels. It is then secreted into the cavity via an electrogenic $K^+/2H^+$ antiporter (Lepier *et al.* 1994) (Fig. 3). This antiporter is driven by the proton-motive force provided by the electrogenic plasma membrane H^+ V-ATPase (Wieczorek, 1992). Thus, K^+ is actively pumped into the goblet cavity. This is accompanied by secretion of carbonate ions, which render the lumen highly alkaline. The lepidopteran larval midgut maintains a pH in the range 8–12. Secreted K^+ then moves from the goblet cavity into the

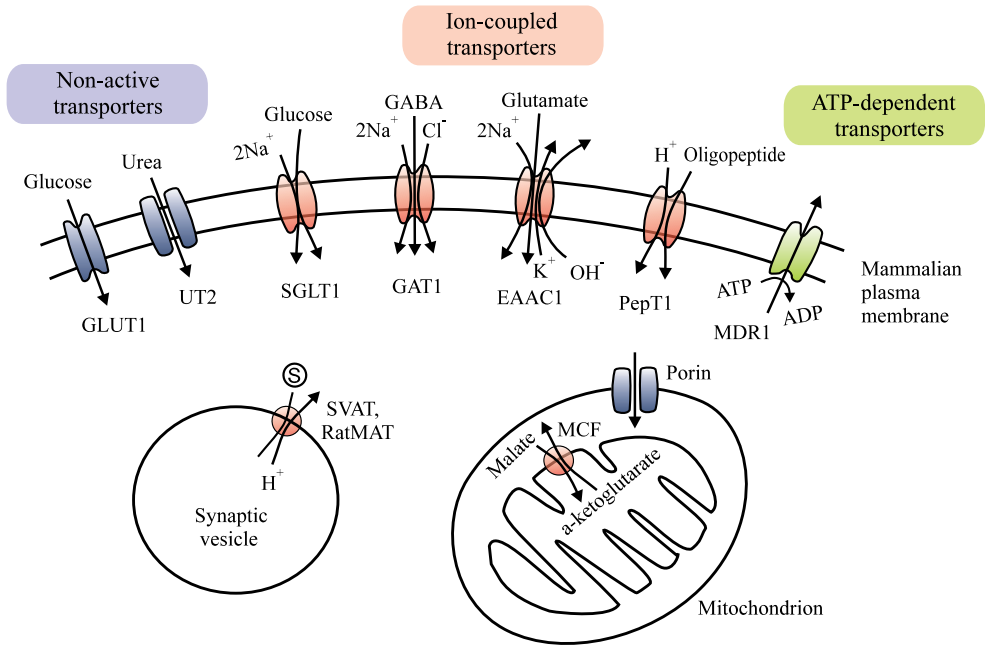


Fig. 4. Top: active and passive transport systems across plasma membranes of higher vertebrates. Bottom left: the synaptic vesicle amine transporter SVAT/RatMAT, which is coupled to the countertransport of H⁺. Bottom right: transport systems of the mitochondrial inner and outer membranes. The mitochondrial porins have β -barrel structures similar to that of the porins of the outer membrane of Gram-negative bacteria. MCF, mitochondrial carrier family (e.g. the malate/ α -ketoglutarate exchanger or the ATP/ADP carrier).

gut lumen. The electrical potential difference in excess of +150 mV (lumen positive) drives K⁺ along with amino acids across the brush-border membranes back into the columnar cells by cotransport systems (symport).

Experiments with intestinal brush-border membrane vesicles of lepidopteran larvae revealed the presence of six distinct K⁺-coupled amino acid transport systems (Giordana *et al.* 1989). These are transporters of (1) neutral amino acids, (2) proline, (3) glycine (with a higher affinity for Na⁺ than for K⁺), (4) lysine, (5) glutamate (but not aspartate) and (6) alanine.

Recent studies by Harvey and colleagues (Hennigan *et al.* 1993) of the larval *Manduca sexta* midgut have revealed that the rate of K⁺-coupled uptake of neutral amino acids reaches a maximum at approximately pH 10, consistent with transport being more effective at the high physiological pH characteristic of the anterior and middle gut lumen. Interestingly, the cation selectivity of phenylalanine and alanine uptake changed with pH. At pH 10 the selectivity was greater for K⁺ than for Na⁺, whereas at pH 8.0 the selectivity was equal for K⁺ and Na⁺. The cDNAs encoding K⁺-coupled amino acid transporters have not yet been isolated.

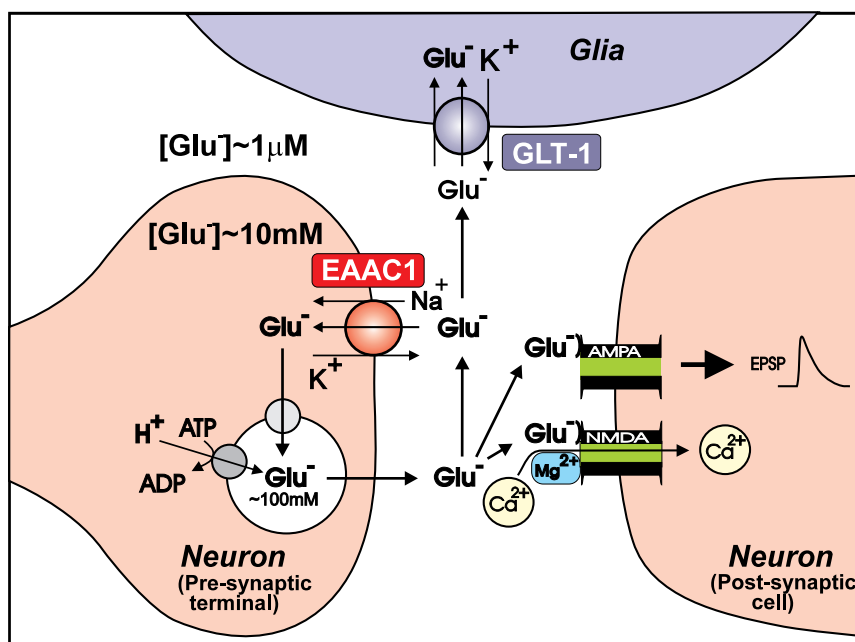


Fig. 5. Mobilization of glutamate at glutamatergic synapses. Glutamate (Glu⁻) stored in synaptic vesicle is released by fusion of vesicles to the presynaptic membrane. Subsequent stimulation of postsynaptic AMPA receptors by glutamate opens associated ion channels and induces an excitatory postsynaptic potential (EPSP). Glutamate is removed from the synaptic cleft through two processes: uptake into pre- or postsynaptic terminals and diffusion out of the synaptic cleft down the glutamate concentration gradient. According to the model, the high-affinity glutamate transporter EAAC1 is present in the presynaptic membrane. Diffusion of glutamate out of the synaptic cleft is driven by the high-affinity glutamate transporter GLT-1, located in glial plasma membranes. The glutamate anion is ‘packed’ into the synaptic vesicle by a Na⁺-independent vesicle transporter, driven by the inside-positive membrane potential generated by the vesicular ATP-dependent H⁺ transport. Its structure and functional properties have not yet been determined. Approximate glutamate concentrations are 10 mM in the presynaptic terminal, 100 mM in the synaptic vesicles and 1 μ M extracellularly. NMDA, *N*-methyl-D-aspartate transporter.

Mammalian ion-coupled organic solute transporters

In higher vertebrates, active absorption of organic solutes across plasma membranes proceeds *via* several classes of transporters that show distinct patterns of coupling to inorganic ions (Fig. 4). Because of the presence of Na⁺/K⁺-ATPases in plasma membranes, active transporters are frequently coupled to the inwardly directed Na⁺ electrochemical gradient (see Fig. 7). A number of transporters, however, are also coupled to the cotransport of Cl⁻ or to the countertransport of K⁺. In addition, some are coupled solely to the cotransport of H⁺.

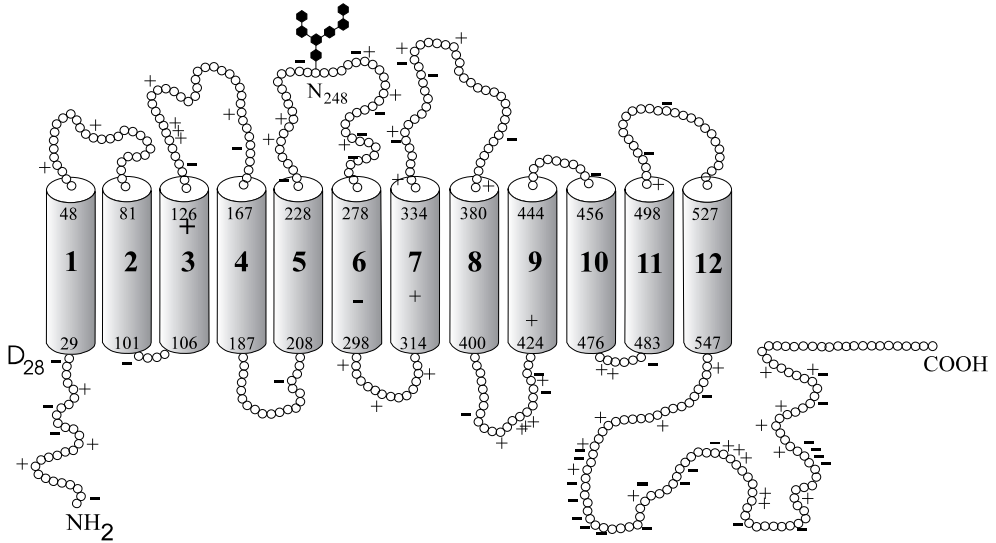


Fig. 6. A structural model of the intestinal Na^+ /glucose cotransporter SGLT1, showing 12 membrane-spanning domains. This model was predicted on the basis of the algorithm of Eisenberg *et al.* (1984) and hydropathy analysis (see Fig. 12). The transporter has a single N-glycosylation sites at Asn-248, as indicated by the hexagons. A mutation of the SGLT1 gene changing Asp-28 to Asn has been shown to account for the transport defect in a Syrian family affected with familial glucose-galactose malabsorption, a rare autosomal recessive disorder characterized by defective intestinal absorption of glucose and galactose (Desjeux, 1989; Turk *et al.* 1991).

In the past few years, we have isolated a number of cDNAs encoding these transporters using an expression cloning approach based on functional expression of cDNA libraries in *Xenopus laevis* oocytes. This strategy was originally developed for the intestinal Na^+ -coupled glucose cotransporter SGLT1 (see Hediger and Rhoads, 1994, for a review). Each of the transporter cDNAs we isolated in this manner encoded a member of a new family of membrane transport proteins.

The Na^+ /glucose cotransporters

Two types of glucose transporters have been identified in higher organisms: Na^+ -coupled glucose transporters (Na^+ /glucose cotransporters, SGLTs), which couple glucose uptake to the inwardly directed electrochemical Na^+ gradient (Crane *et al.* 1961), and facilitated glucose cotransporters (GLUTs), which permit passive movement of glucose across the plasma membranes down its concentration gradient (Fig. 4). Whereas facilitated glucose transporters are found in all cells examined, Na^+ /glucose cotransporters are classically found in tissues responsible for glucose recovery, such as epithelial cells of the small intestine and the proximal tubule of the kidney. Mueckler *et al.* (1985) reported the first primary structure of a facilitated glucose transporter, the human erythrocyte glucose transporter GLUT1. Subsequently,

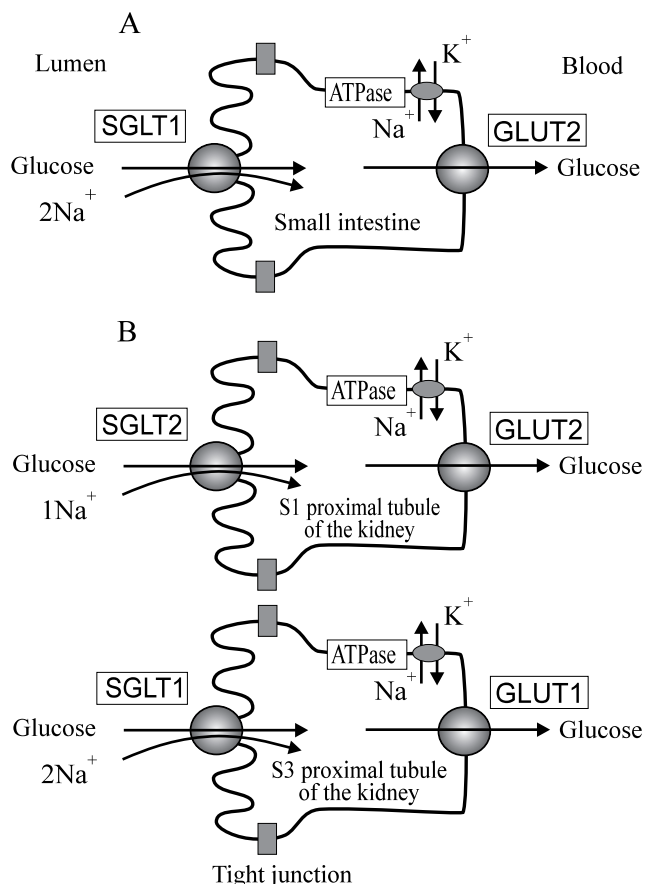


Fig. 7. Schematic model illustrating the mechanisms of transepithelial glucose transport in intestine (A) and proximal tubule of the kidney (B).

Hediger *et al.* (1987) reported the first primary structure of a Na^+ -dependent glucose transporter, SGLT1. An important observation was that these proteins share no sequence homology and, therefore, are members of two distinct families of mammalian transport proteins.

On the basis of hydropathy analysis (see Fig. 12), SGLT1 is a hydrophobic integral membrane protein with approximately 12 putative membrane-spanning domains (Fig. 6). Recently, the primary structure and functional properties of a second low-affinity Na^+ /glucose cotransporter isoform (SGLT2) were determined (Wells *et al.* 1992b; Kanai *et al.* 1994). The SGLT2 amino acid sequence is approximately 60% identical to that of SGLT1.

The SGLT family is a rapidly growing protein family

So far, seven distinctly different members have been identified: (1) the high-affinity

Na⁺/glucose cotransporter SGLT1 (Hediger *et al.* 1987); (2) the low-affinity Na⁺/glucose cotransporter SGLT2 (Wells *et al.* 1992*b*; Kanai *et al.* 1994) and its close homologue SNST1 (Pajor and Wright, 1992); (3) the Na⁺-dependent neutral amino acid transporter (SAAT) (Kong *et al.* 1993) related to system A; (4) the Na⁺-dependent myo-inositol transporter (SMIT) (Kwon *et al.* 1992); (5) the kidney SGLT-related transporter rkST1, whose transport substrate has not yet been identified (Hitomi and Tsukagoshi, 1994); (6) the proline transporter from *E. coli* (PutP) (Nakao *et al.* 1987); and (7) the pantothenic acid transporter from *E. coli* (PanF) (Jackowski and Alix, 1990). All these transporters have 12 predicted transmembrane domains and their sequences yield strikingly similar hydropathy plots.

Role of SGLT1 and SGLT2 in transepithelial glucose transport

Absorption of D-glucose across epithelial cells occurs against a concentration gradient by SGLTs in brush-border membranes and GLUTs in basolateral membranes (Fig. 7). Once inside the cell, glucose diffuses out of the cell into the blood *via* GLUT2 or GLUT1. The Na⁺/K⁺-ATPase (Skou *et al.* 1992) located in the basolateral membrane pumps Na⁺ out of the cell and maintains the inwardly directed Na⁺ electrochemical gradient required to drive uphill glucose transport across the brush-border membrane.

In the intestine (duodenum, jejunum and ileum), the digestion products of dietary carbohydrates, D-glucose and D-galactose, are absorbed by mature enterocytes in the upper third of intestinal villi *via* SGLT1 and GLUT2 (Fig. 7A). In the kidney, D-glucose is freely filtered at the glomerulus and then almost completely extracted from the urine by the proximal tubule and returned to the blood *via* SGLT2 and GLUT2 (Kanai *et al.* 1994) (Fig. 7B, upper part). Approximately 90% of the filtered glucose is reabsorbed by the early S1 segment of the proximal tubule and only a small fraction reaches the proximal straight tubule (S3 segments) (Fig. 7B, lower part). Studies of rat kidneys revealed that SGLT1 is also expressed in the proximal tubule S3 segments (Lee *et al.* 1994). However, SGLT1 has not yet been detected in human kidney.

Different functional roles of SGLT1 and SGLT2

SGLT1 is a high-affinity low-capacity transporter coupled to the cotransport of two Na⁺. In contrast, SGLT2 is a low-affinity high-capacity Na⁺/glucose cotransporter coupled to the cotransport of only one Na⁺. The stoichiometry of ion-coupled organic solute transporters is of fundamental importance both from a physiological and from a structural point of view. At equilibrium, the ratio of intracellular to extracellular glucose concentration is predicted by the equation:

$$[\text{glucose}]_i/[\text{glucose}]_o = \{([\text{Na}^+]_o/[\text{Na}^+]_i)e^{-FV_m/RT}\}^n,$$

where the subscripts i and o denote the intra- and extracellular concentrations, V_m is the membrane potential and n is the Na⁺ to glucose coupling ratio. A Na⁺ to glucose coupling ratio of 1 to 1 is reasonable to drive the absorption of the bulk of D-glucose in proximal tubule S1 segments with minimal energy consumption. In contrast, if we assume that in the proximal tubule cells (S3 segments) $[\text{Na}^+]_i$ is 10 mM, $[\text{Na}^+]_o$ is 140 mM and the

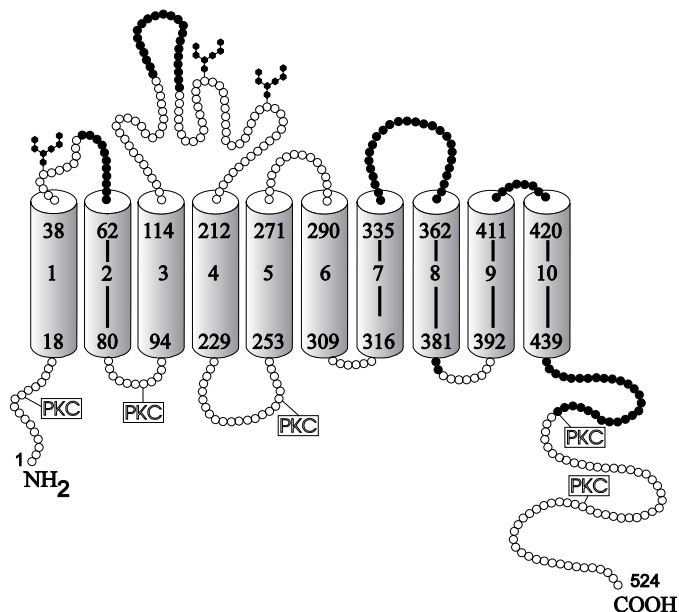


Fig. 8. Structural model of EAAC1. Domains corresponding to regions of extended sequence identity with the high-affinity glutamate transporter isoforms GLT-1 and GLAST are highlighted by filled circles or, if located inside potential membrane-spanning domains, by heavy lines. Potential protein kinase C phosphorylation sites are marked (PKC) and potential N-glycosylation sites are indicated by the hexagons.

membrane potential is -60 mV, the concentrating capacity of SGLT1, which has a Na^+ to glucose ratio of 2 to 1, is 140 times higher than that of SGLT2. This additional accumulative power of SGLT1 in proximal tubule S3 segments of rat kidney is necessary to pump the last traces of D-glucose from the lumen into tubular epithelial cells in this part of the kidney.

The high-affinity glutamate transporter EAAC1: identification of a new family of neurotransmitter transporters

Glutamate is the predominant excitatory neurotransmitter in the mammalian central nervous system. An important component of the glutamatergic transmission process is the rapid removal of released glutamate from the synaptic cleft. As illustrated in Fig. 5, high-affinity glutamate transporters are known to play an important role in terminating the postsynaptic action of glutamate (Kanai *et al.* 1993*a,b*). Transporters located in the pre- or postsynaptic membranes directly remove glutamate from the synaptic cleft. Transporters located in the plasma membrane of glial cells maintain the extracellular glutamate concentration at approximately $1 \mu\text{M}$ and establish a diffusion gradient which favors the movement of glutamate out of the synaptic cleft.

Expression cloning of a high-affinity glutamate transporter (EAAC1)

To isolate a cDNA encoding a high-affinity glutamate transporter, Kanai and Hediger (1992) screened a rabbit intestinal cDNA library for the ability to induce [¹⁴C]glutamate uptake in *Xenopus* oocytes. A cDNA was isolated which encodes a 524-residue protein, referred to as EAAC1 (Kanai *et al.* 1992) (Fig. 8). This protein has approximately 10 putative membrane-spanning regions. However, owing to the presence of a large hydrophobic stretch near the C terminus (residues 362–439; see Figs 8 and 12), alternative models with a different number of membrane-spanning regions can be constructed. EAAC1 from human kidney was subsequently isolated (Kanai *et al.* 1994) and its gene localized to human chromosome gp24 (Smith *et al.* 1994).

Tissue distribution and localization in brain

Northern blot analysis and *in situ* hybridization revealed that, in addition to small intestine and kidney, EAAC1 is abundantly expressed in neuronal tissues, including cerebral cortex, cerebellum, hippocampus, brainstem and retina. On the basis of these studies it was concluded that EAAC1 is of both neuronal and epithelial origin. In brain, EAAC1 probably provides a presynaptic glutamate uptake mechanism to terminate the action of released glutamate at glutamatergic synapses (Fig. 5) (Kanai and Hediger, 1992; Kanai *et al.* 1993*a,b*). Recent studies of rat brain revealed that EAAC1 is also expressed in γ -aminobutyric acid (GABA)-ergic cerebellar Purkinje cells. In these neurons, EAAC1 may serve to provide glutamate as a precursor for GABA synthesis (Y. Kanai and M. A. Hediger, in preparation). EAAC1 signals were also observed in kidney, liver and heart.

The EAAC family

Two additional cDNAs from rat brain that encode related high-affinity glutamate transporters have been reported. These are GLT-1 (Pines *et al.* 1992; Kanner, 1994) and GLAST (Storck *et al.* 1992), which show 51 and 55 % amino acid sequence identity, respectively, to rabbit EAAC1 (Kanai *et al.* 1993*b*). In contrast to EAAC1, these proteins are brain-specific and of glial origin. GLT-1, which is expressed in astrocytes, is ideally suited to maintain the low extracellular glutamate concentration of approximately 1 μ M below neurotoxic levels. The function of the GLAST protein remains unknown. Regions of extended sequence homology among these transporters are highlighted in Fig. 8 by filled circles or heavy lines. Recently, cDNAs encoding two other mammalian members of the EAAC1 family were reported: the neutral amino acid transporter ASCT1, with properties of system ASC (Arriza *et al.* 1993), and a second system-A-like neutral amino acid transporter, SATT1 (Shafqat *et al.* 1993). EAAC1 also has significant homology to the H⁺-coupled gltP glutamate transporters of *E. coli* (Tolner *et al.* 1992), *B. stearothermophilus* and *B. caldotenax* and to the dctA dicarboxylate transporter of *Rhizobium meliloti* (Engelke *et al.* 1989) with sequence identities ranging between 27 and 32 %. There is no homology between members of the EAAC1 family and those of the Na⁺- and Cl⁻-dependent GABA/neurotransmitter

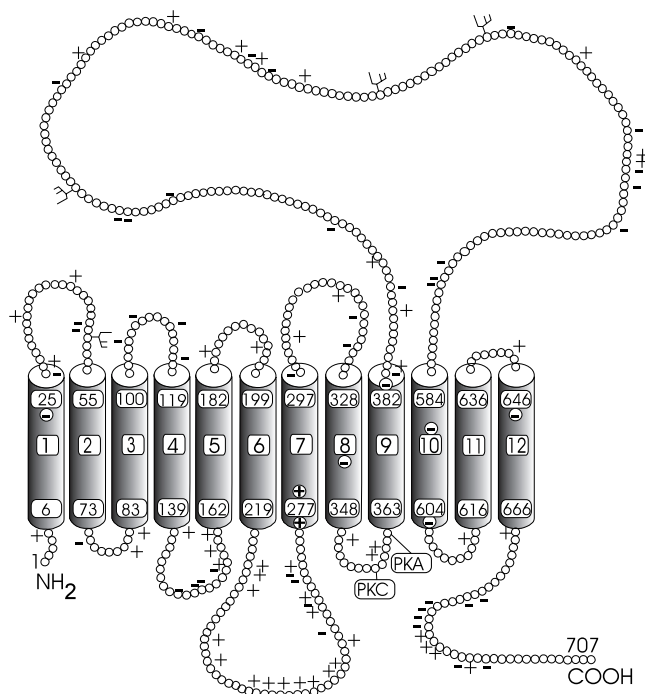


Fig. 9. Membrane model of the H^+ -coupled oligopeptide transporter PepT1. The amino acid sequence predicts a membrane protein with 12 membrane-spanning domains and a large hydrophilic loop containing three putative N-glycosylation sites (branches). PKC, putative protein kinase C phosphorylation site; PKA, putative protein kinase A phosphorylation site.

transporter family (Fig. 4), which includes transporters of GABA (Guastella *et al.* 1990), noradrenaline (Pacholczyk *et al.* 1991), dopamine (Kilty *et al.* 1991; Shimada *et al.* 1991), serotonin (Blakely *et al.* 1991), L-glycine (Smith *et al.* 1992*b*), L-proline (Freneau *et al.* 1992), choline (Mayser *et al.* 1992), taurine (Liu *et al.* 1992*a*; Smith *et al.* 1992*b*; Uchida *et al.* 1992) and betaine (Yamauchi *et al.* 1992). Likewise, there is no homology to the *E. coli* Na^+ /glutamate transporter *gltS* (Deguchi *et al.* 1989, 1990).

Coupling stoichiometry

EAAC1-mediated transport is electrogenic and dependent on extracellular Na^+ but not Cl^- . Studies of glutamate transport in salamander retinal glial cells and of EAAC1 expressed in oocytes revealed that high-affinity glutamate transport is coupled to the cotransport of two Na^+ and the countertransport of one K^+ and one OH^- (Bouvier *et al.* 1992; Y. Kanai and M. A. Hediger, in preparation). This ion coupling is very different from that of previously characterized Na^+ -dependent transporters such as the Na^+ /glucose cotransporters and Na^+ - and Cl^- -dependent neurotransmitter transporters. In brain, this coupling pattern provides the accumulative power required to terminate the neurotransmitter action of glutamate and to keep its extracellular concentration below neurotoxic levels. The lower limit of the extracellular glutamate concentration attainable

at equilibrium is set by this stoichiometry, and the predicted ion gradients and can be calculated from the equation:

$$E_{\text{Glu}} = 2E_{\text{Na}} + E_{\text{OH}} - E_{\text{K}} - V_{\text{m}},$$

where V_{m} is the membrane potential and E_{X} the Nernst equilibrium potential of ion X . E_{X} is given by:

$$E_{\text{X}} = \frac{RT}{zF} \ln \frac{[X]_{\text{o}}}{[X]_{\text{i}}},$$

where subscripts i and o denote the intra- and extracellular concentrations. Under physiological conditions, when the membrane potential is -90 mV, $\text{pH}_{\text{i}}=7.0$, $\text{pH}_{\text{o}}=7.4$, $[\text{K}^+]_{\text{i}}=[\text{Na}^+]_{\text{o}}=145$ mM, $[\text{K}^+]_{\text{o}}=2.5$ mM, $[\text{Na}^+]_{\text{i}}=10$ mM and $[\text{glutamate}]_{\text{i}}=10$ mM, this equation predicts a minimum extracellular glutamate concentration of approximately 0.6 μM . This value is reasonable since it is similar to the prevailing glutamate concentration in the cerebrospinal fluid of approximately 1 μM . The analysis clearly demonstrates the high accumulative power of EAAC1, which can concentrate glutamate more than 10 000-fold across plasma membranes.

The mammalian H^+ -coupled transporter of oligopeptides and peptide-derived antibiotics (PepT1)

Although active solute transport across plasma membranes of higher vertebrates has been thought to be driven primarily by the Na^+ gradient, it is increasingly recognized that there are also H^+ -coupled transporters. H^+ electrochemical gradients are formed in epithelial cells by Na^+/H^+ antiport at the expense of the energy stored in the Na^+ electrochemical gradient. Using expression cloning with *Xenopus laevis* oocytes to screen a rabbit intestinal cDNA library for the uptake of the biologically inert ^{14}C -labeled dipeptide glycyl-sarcosine (Gly-Sar, *N*-methyl glycine), we isolated a cDNA coding for a novel 707-residue peptide transporter called PepT1 (Fei *et al.* 1994).

The amino acid sequence of PepT1 predicts a membrane protein with 12 membrane-spanning domains (Fig. 9). The presence of an unusually large hydrophilic loop with several N-glycosylation sites makes this protein structure distinct from those of other organic solute transporters previously reported. PepT1 shows weak, but significant, similarity to the H^+ -dependent nitrate transporter CHL1 from plants (Tsay *et al.* 1993) (see above) and the yeast peptide permease (approximately 25 % identities). There is no homology of PepT1 to any other reported sequence, including the well-characterized prokaryotic H^+ -coupled transporter lactose permease.

When expressed in oocytes, this protein strongly increased the uptake of ^{14}C -labeled Gly-Sar (100 μM). Two-electrode voltage-clamp analysis of oocytes injected with PepT1 cRNA demonstrated that PepT1-mediated transport is electrogenic. Large inward currents were obtained when substrates such as dipeptides, tripeptides and β -lactam antibiotics (1 mM) were applied to the bath. Because transport is electrogenic, it was possible to screen a large number of compounds quickly to establish the substrate range

of PepT1 (Fei *et al.* 1994). Oligopeptides were transported, regardless of whether they contained acidic, basic or hydrophobic amino acids. It seems likely that any di-, tri- and tetrapeptide can serve as a substrate of PepT1. The affinities among dipeptides, however, varied substantially. PepT1 appears to have a preference for peptides containing bulky aliphatic side-chains. As referred to above (see 'Transport in bacteria', 'Efflux systems'), knowledge of the structural and functional properties of this protein may prove useful in designing orally administered therapeutic drugs.

H⁺ cotransport of PepT1 was directly demonstrated by measuring intracellular pH (pHi) of oocytes using a pH-sensitive microelectrode filled with a hydrogen-selective ionophore (Fei *et al.* 1994). Application of 10 mM Gly-Sar to PepT1 cRNA-injected oocytes caused pHi to decrease from 7.22 to 7.0 and the oocyte membrane to depolarize. Stoichiometric studies revealed that Gly-Sar is cotransported by PepT1 with one proton. On the basis of thermodynamic analysis and assuming that the extracellular pH (pHo) of the unstirred water layer in the intestinal lumen is 5.5, pHi is 7.0 and V_m is -60 mV, a 1:1 stoichiometry predicts that PepT1 can concentrate oligopeptides up to 316-fold across the brush-border membrane.

Classifying ion-coupled mammalian organic solute transporters

On the basis of the coupling patterns to inorganic ions and sequence comparison, it now appears reasonable to group ion-coupled solute transporters as follows (Fig. 4): (1) the predominantly Na⁺-coupled SGLT-family (Hediger *et al.* 1987); (2) the GABA/neurotransmitter transporter family, which is characterized by coupling to the cotransport of Na⁺ and Cl⁻ (Guastella *et al.* 1990; Kanner, 1994); (3) the glutamate/neurotransmitter family, which is characterized by coupling to the cotransport of Na⁺ and the countertransport of K⁺ and OH⁻ (Kanai *et al.* 1992; Pines *et al.* 1992; Storck *et al.* 1992); and (4) the oligopeptide transporter family, which is characterized by coupling to the cotransport of H⁺ (Fei *et al.* 1994).

This list does not include the plasma membrane Na⁺-coupled bile acid transporters from liver and intestine (Hagenbuch *et al.* 1991; Wong *et al.* 1994), the synaptic vesicle amine transporter (SVAT, RatMAT) (Fig. 4), which is coupled to the countertransport of H⁺ and is homologous to the *E. coli* TetA tetracycline resistance transporter (Edwards, 1992; Erickson *et al.* 1992; Liu *et al.* 1992b) (see above), and the mitochondrial carrier family (MCF) (Fig. 4) involved in energy transduction (Kuan and Saier, 1993). All these transporters are members of additional protein families.

D2H is a member of a novel transport protein family, defects in which cause cystinuria

Cystinuria is an autosomal recessive disorder of amino acid transport in kidney and is a common hereditary cause of kidney stones (Bergeron and Scriver, 1992; Segal and Thier, 1989). A defect of the transport system responsible for reabsorption of filtered cystine in the kidney results in excretion of cystine in the urine. The low solubility of cystine favors the formation of cystine-containing kidney stones. The screening of a rat renal cDNA

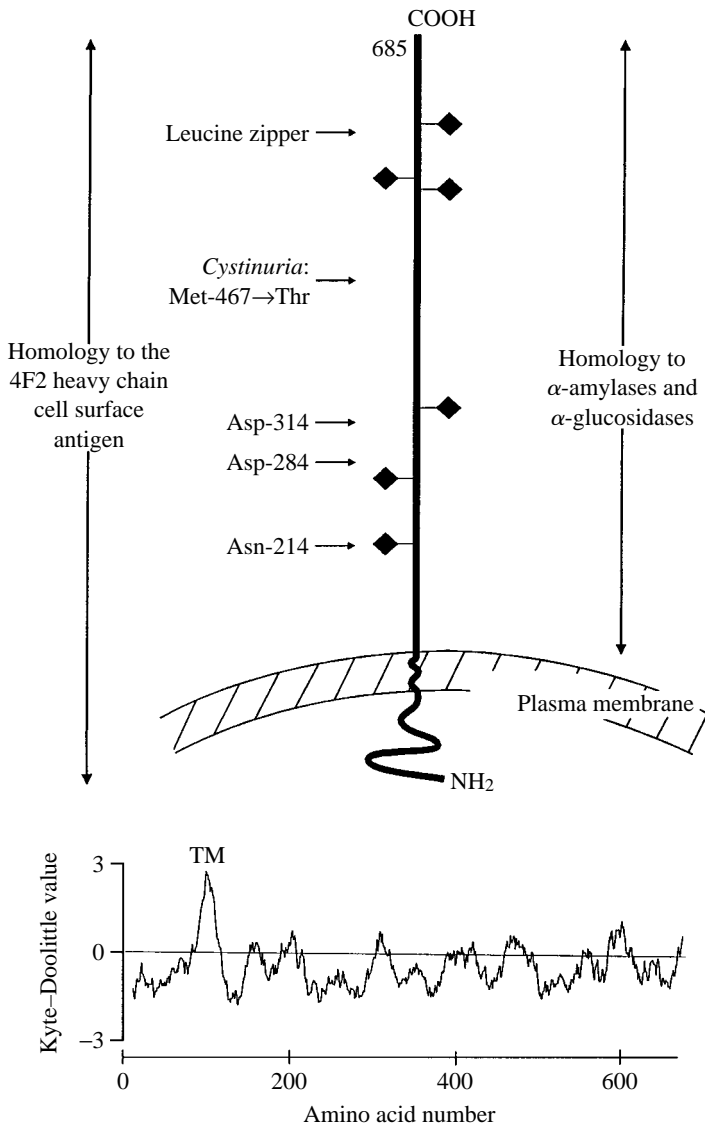


Fig. 10. Top: predicted structure of the kidney- and intestine-specific protein D2H/rBAT and its homologous 4F2 cell-surface antigen heavy chain. The D2H/rBAT and 4F2 heavy chain amino acid sequences are approximately 29% identical and constitute members of a new family of proteins involved in amino acid transport. They are type II membrane glycoproteins with a hydrophilic cytoplasmic NH₂ terminus, a single membrane-spanning domain and an extracellular COOH terminus. Mutation of Met-467 to Thr in D2H/rBAT causes cystinuria. The COOH-terminal domain is homologous to a family of carbohydrate-metabolizing enzymes. D2H/rBAT shares conserved residues with members of the D2H family: Asn-214 and Asp-284 are predicted to correspond to the proposed Ca²⁺-binding residues of porcine pancreatic amylase (Asn-100 and Asp-167) and Asp-314 is predicted to correspond to a catalytic aspartic acid residue of the amylase (Wells *et al.* 1992a). ♦, putative N-glycosylation sites. Bottom: Kyte and Doolittle (1982) hydropathy analysis of D2H using a window of 21. TM, transmembrane domain.

library for clones capable of inducing [^{14}C]cystine uptake in oocytes resulted in the isolation of the kidney- and intestine-specific clone, which encodes a 683-residue protein (D2) (Wells and Hediger, 1992). Two other groups isolated cDNAs encoding this protein from rabbit (rBAT) (Bertran *et al.* 1992b) and rat (NAA-Tr) (Tate *et al.* 1992), and clones encoding the human protein (D2H, rBAT) were also isolated (Bertran *et al.* 1993; Lee *et al.* 1993). On the basis of *Xenopus* oocytes expression studies, D2 and D2H induced the Na^+ -independent high-affinity transport of cystine, dibasic amino acids and, surprisingly, neutral amino acids. *In situ* hybridization of rat kidney sections revealed that D2 mRNA is abundant in the kidney proximal tubule S3 segments (Kanai *et al.* 1992), and immunocytochemistry localized the D2/NAA-Tr protein to renal and intestinal brush-border membranes (Mosckovitz *et al.* 1993). The human D2H gene, referred to as *SLC3A1*, was assigned to chromosome 2p21 by fluorescence *in situ* hybridization (Lee *et al.* 1993; X.-X. Zhang, R. Rosen, M. A. Hediger, P. Goodyer and P. Eydoux, in preparation).

The D2H protein family

Of great interest was the finding that the D2 amino acid sequence shows significant sequence identity (29%) to that of the heavy chain of the 4F2 cell-surface antigen. This antigen is a 125 kDa disulfide-linked heterodimer composed of an 85 kDa glycosylated heavy chain and a 41 kDa non-glycosylated light chain. It is associated with T-cell activation and tumor cell growth and is widely expressed in mammalian tissues. Expression of human 4F2 heavy chain in oocytes also stimulated the uptake of dibasic and neutral amino acids but, notably, not cystine (Bertran *et al.* 1992a; Wells *et al.* 1992a). In contrast to D2, 4F2-induced uptake of neutral, but not dibasic, amino acids was Na^+ -dependent (Wells *et al.* 1992a). Thus, D2- and 4F2-induced amino acid transport activities in oocytes have significantly different substrate specificities and Na^+ -dependencies.

Unique structures of D2 and 4F2

The D2 and 4F2 heavy chain amino acid sequences surprisingly predict proteins with a single transmembrane domain (Fig. 10), an atypical structure for membrane transport proteins. There is no homology between this protein family and the families of the classic transporters with 12 membrane-spanning domains. However, the COOH-terminal extracellular domains of D2 and 4F2 are significantly homologous to a family of carbohydrate-metabolizing enzymes that includes α -amylases and α -glucosidases (Wells and Hediger, 1992) (Fig. 10). The D2 protein and the 4F2 heavy chain therefore represent a new family of proteins involved in amino acid transport, possibly functioning as transport activators or regulators (i.e. as a subunit of system $\text{b}^{\text{0,+}}$). Alternatively, the proteins may self-aggregate to form homo-oligomeric pores that translocate amino acids or fold into a β -barrel structure to form porin-like proteins (see above, 'Transport in bacteria', 'Transport across the outer membrane'). The extracellular domains of D2 and 4F2 may include specific amino acid binding sites, by analogy with glucosidases that bind to specific oligosaccharides. It is likely that the discovery of this novel protein family will

lead to the identification of other structurally related proteins involved in the transport of amino acids or other solutes.

Cystinuria

The transporter implicated in patients with cystinuria was generally thought to transport only cystine and dibasic amino acids (Bergeron and Scriver, 1992; Segal and Thier, 1989). Except for transport of neutral amino acids, D2 and D2H/rBAT exhibited the substrate range and tissue distribution of the transporter proposed to be defective in cystinuria. Hybrid-depletion experiments demonstrated that D2 was responsible for most, if not all, of the cystine transport induced by kidney mRNA in oocytes and supported the role of D2H/rBAT in cystinuria (Wells and Hediger, 1992). There are several renal amino acid transporters that can compensate for abnormal neutral amino acid reabsorption.

Recent studies provided convincing evidence that cystinuria is caused by a defect in D2H/rBAT. Linkage of cystinuria in Libyan Jewish families to chromosome 2 markers (Pras *et al.* 1994) is consistent with our chromosomal localization of the D2H/rBAT gene on chromosome 2p21. Furthermore, D2H/rBAT mutations in cystinuria patients of Spanish families (Calonge *et al.* 1994; Palacín, 1994) were shown to segregate with the cystinuria phenotype, with the most common mutation changing Met-467 of the D2H/rBat extracellular region to Thr (Fig. 10). Expression of the Met467Thr mutant protein in *Xenopus* oocytes yielded only 20% of the wild-type L-Cys, L-Arg and L-Leu transport activity. It was therefore concluded that a defect of D2H/rBAT causes the transport defect in cystinuria. This demonstrates that D2H constitutes an essential part of the transporter that is defective in cystinuria. However, whether D2H/rBAT and 4F2 represent transporters or transporter activators remains to be determined.

The mammalian facilitated urea transporter: role in the urinary concentrating mechanism and in fluid balance

In mammals, urea constitutes the major end product of nitrogen metabolism (Marsh and Knepper, 1992). Mammals synthesize urea to eliminate ammonia, which is toxic to the central nervous system. Urea is formed in the liver *via* the urea cycle and synthesis from ammonium requires four ATP molecules. Urea is then filtered by the kidney and excreted in the urine. Movement of urea across epithelial cells lining the terminal part of kidney collecting ducts is thought to represent a major control site for the reabsorption of filtered urea.

How does urea move across cell membranes?

Classically, urea movement was thought to occur by lipid-phase permeation, but recent studies indicated the existence of specialized transporters (Chou *et al.* 1990a). Moreover, it is now becoming recognized that these transporters play a central role in the urinary concentrating mechanism and in nitrogen balance (Knepper *et al.* 1975; Knepper and Rector, 1991). The urea permeability of renal tubule segments was studied extensively

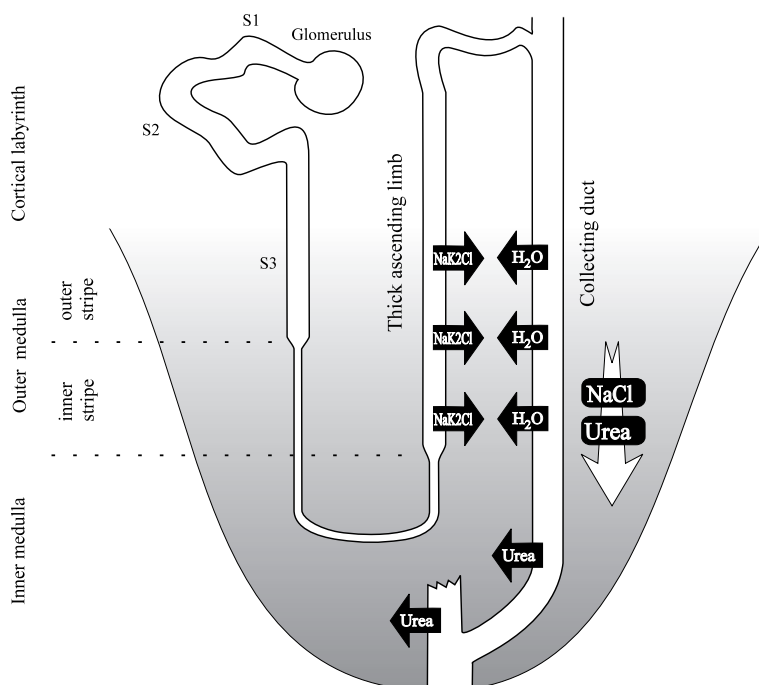


Fig. 11. Schematic representation of the mammalian urinary concentrating mechanism. Arrows indicate the activity of water channels, urea transporters and $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporters. During antidiuresis, vasopressin triggers the reabsorption of water driven by the osmotic gradient of NaCl and urea which exists in the kidney medulla. This gradient is established by $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporters in the thick ascending limbs and urea transporters in terminal part of inner medullary collecting ducts.

using *in vitro* perfused tubules (Knepper and Rector, 1991). The studies revealed that the highest permeability exists in the terminal part of the inner medullary collecting duct (terminal IMCD) and that there are specialized facilitated urea transporters, which are probably present in both apical and basolateral plasma membranes.

Urea transporters are characterized by a low affinity for urea. Saturation of transport with increasing substrate concentration is a characteristic feature of facilitated transport systems. Although urea and thiourea exhibit saturation, the kinetics of saturation are not completely understood. Studies by Mark Knepper (Chou *et al.* 1990b) using *in vitro* perfused tubules from inner medullary collecting ducts demonstrated that there is no saturation in zero-*trans* experiments when bath urea concentration was varied between 0 and 800 mM. However, when urea concentration was varied in both lumen and bath, the lumen-to-bath urea flux achieved a maximum value at 400–500 mM urea. The low affinities of urea transporters, together with this perplexing kinetic behavior, suggest that these proteins represent a transition between transporters and channels. Channels typically have low-affinity binding sites and substrate access to the binding sites occurs

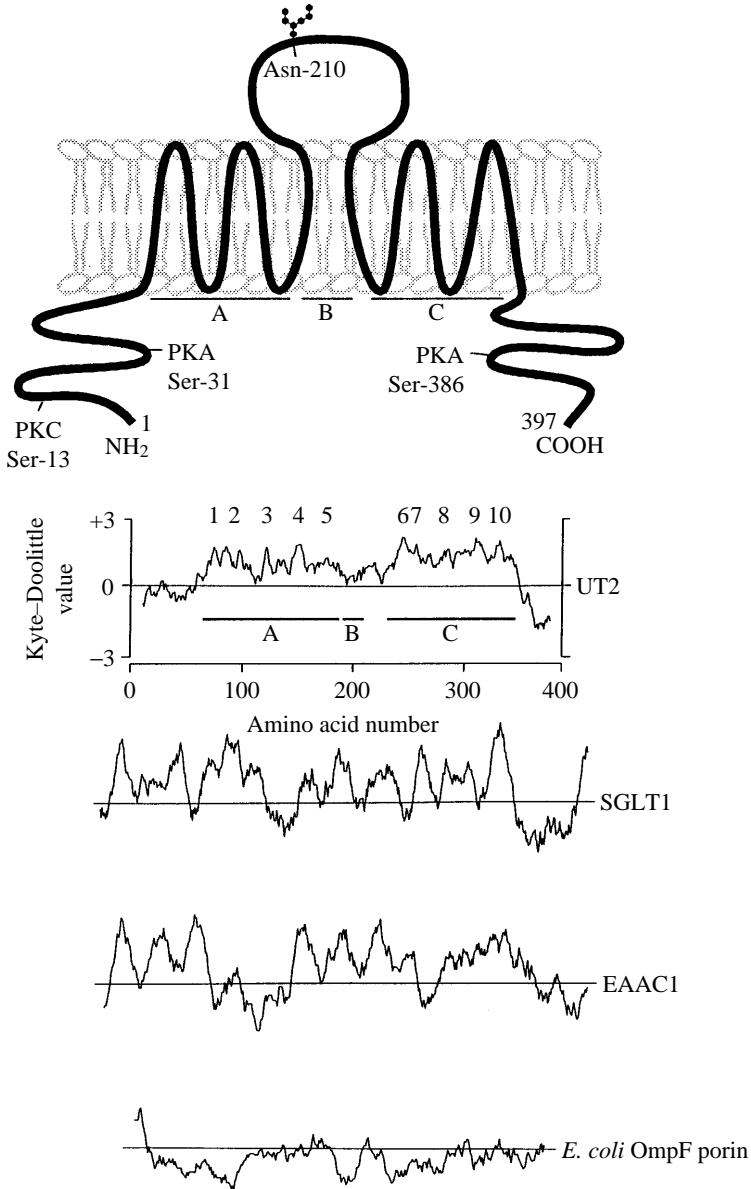


Fig. 12. Top: a membrane model of the urea transporter UT2 from rabbit kidney inner medulla. Potential protein kinase A (PKA) and protein kinase C (PKC) sites and a single N-glycosylation site (hexagons) are indicated. Bottom: comparison of the Kyte and Doolittle (1982) hydrophobicity plot of UT2 using a window of 21 with those of the Na⁺/glucose cotransporter SGLT1, the high-affinity glutamate transporter EAAC1 and the OmpF porin from *Escherichia coli* outer membrane. The figure illustrates the unique high degree of hydrophobicity of UT2.

simultaneously from both sides of the membrane. These properties may be shared by urea transporters.

Role of urea transport in the urinary concentrating mechanism

During antidiuresis, vasopressin activates water channels located in the plasma membranes of the initial part of the collecting duct epithelia through recruitment of channel proteins (WCH-CD) from intracellular vesicle pools (Fig. 11). Water is osmotically reabsorbed, a process that is driven by the potential energy of the corticopapillary osmotic gradient which exists in the kidney medulla (see arrows in Fig. 11). An important aspect of the water reabsorption mechanism is the presence of urea transporters in the plasma membranes of terminal IMCD cells, which open in response to vasopressin and allow urea to move into the medullary interstitium (Knepper and Star, 1990; You *et al.* 1993) (Fig. 11). This process, together with the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransport in the thick ascending limbs of the kidney nephron (Gamba *et al.* 1994) (see arrows in Fig. 11), establishes and maintains the corticopapillary osmotic gradient that provides the driving force for water reabsorption (Knepper and Rector, 1991).

Water channels (aquaporin and MIP family)

Agre and colleagues were the first to isolate a cDNA encoding a mammalian water channel (CHIP28) (Knepper, 1994; Preston *et al.* 1992). The channel is a 28 kDa integral membrane protein expressed abundantly in red blood cells. It is made up of six transmembrane domains that apparently arose by intragenic duplication of a three-spanner encoding sequence, a feature characteristic of members of the MIP family (see 'Transport in bacteria', 'Cytoplasmic uptake systems'). The predominant apical, vasopressin-regulated water channel of collecting ducts, referred to as WCH-CD (Fushimi *et al.* 1993), was subsequently isolated and is 42% identical to CHIP28.

Expression cloning of the urea transporter UT2

A transporter cDNA was isolated by screening a rabbit kidney cDNA library for the uptake of [^{14}C]urea. The cDNA encodes a 397-residue protein (UT2) (You *et al.* 1993). UT2 expressed in *Xenopus* oocytes mediated passive movement of urea across the oocyte plasma membrane and increased its permeability to urea 23-fold. A search of translated sequence databases revealed that UT2 is not homologous to any known sequence. Hydropathy analysis of UT2 revealed an unusual pattern of hydrophobicity, which was different from that found in previously studied transporters (Fig. 12). In contrast to the characteristic pattern of several distinct hydrophobic transmembrane segments interspersed with hydrophilic regions, as observed in most plasma membrane transport proteins, or the characteristic β -barrel structure of the relatively hydrophilic porins of the bacterial outer membrane, UT2 has two large extended hydrophobic domains. It is conceivable, therefore, that a major portion of the protein is entirely embedded in the membrane (Fig. 12).

In situ hybridization using UT2 antisense cRNA as a probe gave a strong signal in

epithelial cells lining the IMCD. This localization is consistent with the predicted distribution of the vasopressin-regulated urea transporter. Because of the central role of UT2 in fluid and nitrogen balance, studies of this protein are expected to provide important insights into the urinary concentrating mechanism and its regulation in response to different physiological states, such as overhydration, dehydration and changes in nitrogen balance.

Concluding remarks and future perspectives

Recent progress in the molecular physiology of solute transport has provided invaluable information on how transporters fulfill their functions in their specific environment. Since information from different research fields is often overlooked, this review serves to bring together important information on transporters from different organisms including bacteria, yeast, plants, insects and mammals.

The molecular cloning of cDNAs for solute transporters and their biochemical analysis in oocytes has made it possible to group transporters into distinct protein families on the basis of their primary sequences and functional characteristics, such as their coupling to electrochemical ion gradients or ATP hydrolysis. The findings that most transporters are composed of 6 or 12 membrane-spanning domains and that highly homologous ATP-binding domains are found in both bacterial periplasmic permeases and eukaryotic P-glycoproteins strongly suggest that these transporter families are variations of common themes that arose in response to evolutionary pressure to satisfy specific requirements, while basic transport mechanisms remained remarkably preserved.

Comparison of prokaryotic and eukaryotic transporters revealed possible evolutionary origins for these protein families and suggests that they are composed of precursors containing two, three or six membrane-spanning domains. The internal homologies seen in those containing 12 membrane-spanning domains, such as P-glycoproteins and facilitated glucose transporters, suggest that they evolved by duplication of genes encoding precursors with six membrane-spanning domains. Furthermore, the six-membrane spanners of the MIP and the MCF families (see Fig. 4) have internal homologies and may have arisen by gene duplications of three-spanner (MIP) or two-spanner (MCF) encoding sequences (Kuan and Saier, 1993; Maloney and Wilson, 1993). Proteins such as SGLT1, EAAC1, PepT1 or UT2, where there is no internal homology, may have evolved by fusion of different six-spanner precursors, although more complex evolutionary routes can be envisioned.

Important future goals will be (1) to identify additional putative transporters, such as brain high-affinity glutamate transporters or plant sucrose transporters, in order to enable a complete understanding of the physiological processes that hinge upon the functional state of these proteins, (2) to advance our understanding of how transporters are regulated, e.g. in response to changes in the physiological environment, (3) to delineate the different mechanisms of transport, (4) to clarify whether D2H and 4F2 function as transporters or transport activators, (5) to determine how UT2 is embedded in the plasma membrane and, most importantly, (6) to elucidate and compare the tertiary structures of these transporters.

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