Pharmaceutical Research, Vol. 12, No. 12, 1995

Review

Biology of Membrane Transport Proteins

Wolfgang Sadée,[1,3] Volkmar Drübbisch,[1] and Gordon L. Amidon[2]

Received June 20, 1995; accepted August 6, 1995

Membrane transporter proteins are encoded by numerous genes that can be classified into several superfamilies, on the basis of sequence identity and biological function. Prominent examples include facilitative transporters, the secondary active symporters and antiporters driven by ion gradients, and active ABC (ATP binding cassette) transporters involved in multiple-drug resistance and targeting of antigenic peptides to MHC Class I molecules. Transported substrates range from nutrients and ions to a broad variety of drugs, peptides and proteins. Deleterious mutations of transporter genes may lead to genetic diseases or loss of cell viability. Transporter structure, function and regulation, genetic factors, and pharmaceutical implications are summarized in this review.

KEY WORDS: membrane transporters; glucose; peptides; multidrug resistance; transporter regulation; transporter gene families; genetic transporter defects.

INTRODUCTION

The viability of cellular life forms critically depends on the integrity of lipid bilayer membranes protecting the cell's interior. However, as the diffusion of polar substrates across lipid bilayers is extremely limited, essential transporter proteins have evolved to permit the utilization of vital substances from the environment, such as anions and cations, vitamins, sugars, nucleosides, amino acids, peptides, bile acids, and porphyrins. The biological significance of transporters is highlighted by their universal phylogenetic distributions among prokaryotes and eukaryotes (1–6). In the mammalian organism, major transporter functions include nutrient absorption in the gut, secretion and reabsorption in the kidney, glucose uptake into all tissues, bile acid transfer in the gut and liver, and penetration of the blood-brain-barrier. Regulation of neurotransmitter concentration in the synaptic cleft by presynaptic reuptake transporters is of particular biomedical relevance. Further, transporters direct the access of substrates across intracellular membrane barriers, for example to facilitate binding of antigenic peptide fragments to MHC class I molecules in an endosomal compartment, and neurotransmitter uptake into synaptic vesicles.

Medical implications of transporter malfunction are illustrated by genetic defects, such as glucose malabsorption and insulin-resistant glucose transport. Multiple drug resistance (MDR) genes encoding transporter molecules are implicated in native and acquired resistance to antineoplastic agents. Mutations of the related cystic fibrosis transmembrane conductance regulator (CFTR) have now been recognized as a cause of cystic fibrosis. Lastly, transport proteins in the plasma membrane may surreptitiously serve as receptors for viral attachment and cellular infection (7).

Over the past years, numerous genes encoding membrane transporters have been cloned (Table I) (8–35). On the basis of the deduced primary amino acid sequences, one can classify transporters into families with similar primary structure and function. Further, hydropathy analysis suggests common structural features underlying overall topology and function of several transporter families even if their primary structures are dissimilar. The cloned genes serve as probes to discover additional members of each gene family, providing insights into substrate specificities observed in different organs *in vivo*. Lastly, transfer of transporter genes into target tissues could serve to overcome genetic defects, e.g., delivery of the CFTR gene to lung tissues, or to permit high dose chemotherapy by transfection of MDR genes into normal hematopoietic stem cells to prevent dose-limiting hematotoxicity.

From a pharmaceutical view-point, transporter proteins either serve as drug receptors, such as the dopamine transporter as a cocaine receptor, or they are crucial to drug delivery, determining intestinal absorption, hepatic and renal disposition, penetration of the blood-brain-barrier, and access to tumor cells. A spectrum of peptide-like drugs, such as cephalosporins and ACE inhibitors, depends on intestinal dipeptide transporters for efficient absorption. Nucleosides and their analogs, among them antivirals and antineoplastics, also depend on specific transporters to reach their target sites. For CNS activity, drugs must cross the lipophilic blood-brain-barrier to be active. Numerous studies deal with transport of drugs and peptides in and out of the CNS, either through endothelial cells of the blood-brain-barrier or the choroid plexus (36,37). The discovery of numerous neuropeptides presents opportunities for novel therapies, but CNS access is limited unless specific transport routes exist

[1] School of Pharmacy, University of California, San Francisco, California 94143-0446.

[2] College of Pharmacy, The University of Michigan, Ann Arbor, Michigan 48109-1065.

[3] To whom correspondence should be addressed.

0724-8741/95/1200-1823$07.50/0

Table I. Selected Mammalian Transporter Proteins Encoded by Cloned Genes

Substrate	Transport mode	Function, tissue	Reference
A. Symporters/antiporters, facilitative transporters			
Glucose	a. Na^+-glucose cotransp. (11 TMDs)	Small intestines, kidney nutrient absorption ubiquitous	8
	b. Facilitative transporter GLUT 1-5 (12-TMDs) GLUT-1	dehydroascorbic acid transport	9
Neutral amino acid	Na^+-independent system L transporter (4 TMDs)	ubiquitous transport of amino acids	10
Cationic amino acid	Na^+-independent, similar to the Y+ cationic L-amino acid transporter (14 TMDs)	ubiquitous, mouse ecotropic retrovirus receptor	7
Dipeptides	H^+ contransport (12 TMD)	intestines, kidney isoforms transports peptoid drugs	11–13
Nucleosides	Na^+-dependent and facilitative	ubiquitous, isoforms may transport nucleoside drugs	14,15
Taurine	Na^+ and Cl^- dependent; similar to neurotransmitter transp. (12 TMDs)	kidney (regulated by hypertonicity)	16
		brain (inhibited by GABA)	17
Bile acids	Na^+/bile acid cotransporter (7 TMDs), Na^+-independent, ABC transporters	liver, intestines various isoforms	
Prostaglandins	facilitative (12 TMDs)	epithelial tissues, isoforms Na^+-dependent	18
Na^+/H^+ exchanger	Antiporter (10 TMDs)	various isoforms, some hormone sensitive (growth hormone, adrenergic)	19
		ileal villus cells	20
Phosphate	Na^+/Pi cotransporter (6–8 TMDs)	kidney, reabsorption of phosphate	21
Sulfate	Na^+-cotransport (12 TMDs)	kidney, liver, intestines different isoforms	22,23
Neurotransmitters		synaptic reuptake	
Norepinephrine	Na^+/Cl^- cotransporter (12 TMDs)	cocaine, antidepressant sensitive	24
Dopamine	Na^+/Cl^- cotransporter (12 TMDs)	cocaine-sensitive	25,26
Serotonine	(12 TMDs)	antidepressant sensitive	27
GABA	GAT-1, Na^+/Cl^- dependent (12 TMDs)	GABA reuptake	28
Glycine	Na^+/Cl^- dependent (12 TMDs)	colocalized with NMDA receptors, splice variants	29
Glutamate	Na^+ cotransport, K^+/OH^- counter-transport (6–10 TMDs proposed)	glial, neuronal reuptake limiting excitotoxic cell damage	30
Biogenic amines	(12 TMD)	neuronal vesicular uptake MPP^+ resistance	31
B. ABC transporters			
Lipophilic drugs	MDR genes, MRP (12 TMDs, 2 ABC domains)	multi-drug resistance	32
Cl^-	Cl^- channel (CFTR) homology to ABC transport.	cystic fibrosis transmembrane conductance regulator	33
Antigenic peptides	TAP1 and TAP2 heterodimer (one ABC and one TMD region on each half-transporter).	active transport to Class I MHC molecules	34,35

or the peptides are chemically modified to exploit a preexisting transport system (36).

The advancing insights into the biology of transporters are of particular pharmaceutical relevance because:

1. Molecular cloning of transporter genes invariably reveals the presence of multiple genes encoding subtypes with similar function, but distinct tissue distribution, regulation, and specificity towards drugs. An obvious goal is to clone all subtypes to unravel the complexities of structure-activity relationships for drug transport. Availability of the cloned genes will enable one to dissect complex kinetic systems that cannot be resolved by any other approach.

2. Upon cloning and expression of the respective gene, one can study its function and regulation. mRNA analysis permits the study of cellular expression by Northern blot or in situ hybridization. Moreover, the cloned gene facilitates the production of monoclonal antibodies to determine cellular distribution of the transporter protein, and its trafficking to and from its target membrane. Functional insertion of glucose transporter subtypes into the plasma membrane is hormonally regulated and plays a crucial role in glucose utilization. Hence, modulation of transporter activity could be exploited in drug targeting.

3. Transporters are likely to exist in the form of multiple alleles floating through the population, some of which encoding proteins with functional defects. Nonlethal deleterious defects manifest themselves in genetic disorders; however, some mutant alleles may permit normal physiological function of the encoded transporter but are defective with respect to drug transport or binding. Drug malabsorption

could thus be the result of a defective transporter allele, a very likely scenario if one considers that drugs, such as the cephalosporins, are given to millions of patients.

4. Using site-directed mutagenesis, one can map the molecular sites of ligand binding and transport, offering the hope that structural transporter models can be developed for the design of drugs that serve as transport substrates or inhibit transport. However, whereas transport studies have yielded a rather detailed understanding of the transport kinetics, advances in determining transporter structure and topology are still limited by the lack of direct measurements, because of difficulties in crystallizing these integral membrane proteins. First glimpses of the overall topology have been obtained from electron density maps of two-dimensional arrays of certain P-type ATPases (e.g., Na^+/K^+-ATPase, primary ion pump) (see 6).

This review broadly summarizes current knowledge of the molecular biology and structure of transporter proteins in general, and it discusses selected carriers of particular relevance to pharmaceutical and biomedical applications. No attempt was made to cover all transporters and related ion channels, but rather, the selected transporters serve as examples for the emerging understanding of this complex superfamily of proteins.

FUNCTIONAL CLASSIFICATION OF TRANSPORTERS: UNIPORTERS, SYMPORTERS, ANTIPORTERS, PRIMARY ACTIVE TRANSPORTERS

The translocation of many solute molecules across biological membranes is mediated by transporters or channels (pores). Other mechanisms such as receptor mediated endocytosis (LDL and transferrin receptors) will not be considered here. Pores and channels may have binding sites accessible to solutes from either side of the membrane. The porins, abundant proteins in the outer membrane of gram negative bacteria, have the shape of a β-barrel, consisting of multiple transmembrane β-strands, as determined by x-ray crystallography (3). They permit passive penetration of ions and small nutrient molecules. Further, porin-like channels serve as facilitators, having specific binding sites for selected solutes (3). The often more selective channels may undergo conformational changes, thereby, regulating whether they are open or closed to solute traffic. In contrast, transporters require a conformational change during the process of solute translocation across the membrane, and the solute binding site of a transporter is accessible to only one side of the membrane at any time. Whereas passage of solutes through channels is extremely fast, solute flux through transporters is orders of magnitude slower. Moreover, the secondary structure of transporters is thought to consist of multiple α-helices serving as transmembrane domains (TMDs), rather than β-barrels (3).

Transporters can be further classified on the basis of their energy requirements (Table I). Passive transporters, allowing the passage of a single kind of solute across the membrane (uniporters) can only facilitate net solute flux down an electrochemical concentration gradient (facilitative diffusion), with the glucose facilitator family providing a prime example. In contrast, active transporters can move solutes against an electrochemical gradient across the membrane. Hence, the solute transport must be coupled to a free energy yielding process to drive solute uptake. A number of ion pumps are primary active transporters, coupled to an energy yielding chemical or photochemical reaction. Examples include bacteriorhodopsin, which utilizes the energy derived from the absorption of a photon by retinal to translocate protons across the membrane, and the ATP binding cassette (ABC) transporters which exploit the chemical energy released upon ATP hydrolysis (e.g., Na^+/K^+-ATPase). In most cases, the ion pumps are electrogenic (a net movement of charge resulting from the primary pump operation), thereby, generating a charge separation and voltage difference across the membrane.

Numerous substrates of pharmaceutical interest are transported by the secondary active transporters which utilize voltage and ion gradients generated by primary active transporters (Table I). Co-transporters (symporters) translocate two or more different solutes in the same direction, whereas antiporters couple the transport of solutes in opposite directions, exploiting a chemical gradient for one of the solutes. In prokaryotes, H^+ symporters represent the most common paradigm of energy utilization, whereas in eukaryotic cells, Na^+ symporters are more frequently encountered (6,38).

CLASSIFICATION OF TRANSPORTERS BY SEQUENCE SIMILARITIES

With a rapidly increasing number of cloned transporter genes available, the deduced primary structure (amino acid sequence) can serve to generate families of closely related genes. We define sequence identity by the percent identical amino acid residues in the two sequences under comparison, while sequence similarity takes into account the frequency of mutational substitutions with related amino acids during evolution, using a PAM score (point accepted mutation score). Given a sufficiently high score and additional statistical criteria, we assume two sequences to be homologous, i.e., derived from the same ancestral precursor gene by divergence. Hence, there is no gliding scale of homology; a gene and its encoded protein are either homologous to another gene, or they are not. Closely related homologs represent a gene family, which can be part of a superfamily of several groups of less similar homologous genes. These definitions are needed to understand the classification of transporter genes in the literature.

Several large gene families encode channels and transporters with little or no apparent sequence similarity among them; yet, most transporters share common structural features as deduced from hydropathy analysis of their amino acid sequences. A similar number of putative transmembrane domains (TMDs) suggest that topology and translocation mechanism are similar across several families despite seemingly unrelated primary structures. On the other hand, within the same family of transporters with similar sequence, we can find transporters with greatly differing substrate specificity and energy coupling mechanisms. Therefore, classification of transporters by substrate or energy coupling mechanism may not do justice to homology established by sequence comparisons. Different energy coupling mechanisms may have evolved and added to the principal structure

at a later point in evolution. The fact that the LacY H^+ symporter can be converted by a single amino acid substitution to a facilitative uniporter (see 3) corroborates the hypothesis that specific energy coupling is an additional feature superimposed on the principal transporter function.

A selection of cloned transporter genes is provided in Table I. Whereas there is high sequence identity among individual genes of a family of closely related transporters, very little if any sequence identity occurs between different transporter gene families, such as neurotransmitter reuptake, glucose, and amino acid transporters. The question remains open whether all of these families have evolved by convergence from different ancestral genes, or by divergence from one or only a few parent genes. Similarity of function (transport) and membrane topology (often 12 predicted TMDs) is striking even when no significant sequence identity is observed. Moreover, short sequence motifs may be repeated in transporters with otherwise unrelated primary structure. Lastly, the putative 12-TMD structure appears to have arisen from an internal gene duplication of a 6-TMD ancestral gene fragment (6 + 6 structure) (2). The two halves of the 12-TMD transporters show varying degree of sequence conservation, i.e., the N-terminal half is more highly conserved when comparing distantly related families, whereas the C-terminal is often better conserved within a subfamily of related genes. Different degrees of conservation imply different functions for the two halves, such as general functions supporting the translocation mechanism (N-terminal) and substrate recognition (C-terminal). These combined results suggest possible homology even among dissimilar sequences.

On the basis of primary structure analysis, Marger and Saier (1) identified a major facilitator superfamily (MFS) having the 6 + 6 topology, with five gene clusters: 1. bacterial drug resistance proteins (e.g., tetracyclins); 2. sugar facilitators; 3. facilitators of Krebs cycle intermediates (e.g., citrate); 4. phosphate ester-phosphate antiporters; 5. oligosaccharide symporters. These related transporters (>50 genes) display distinct energy requirements. On the other hand, the distinct solute/Na^+ symporters comprise seven groups with little or no sequence similarity among each other or any other family (neurotransmitters, bile acids, sugars-amino acids, vitamins-nucleosides-galactoside, citrate, glutamate, phosphate) (1,4). Not all of these share the 12-TMD topology, e.g., the glutamate transporters, important for the reuptake of excitatory amino acids, have 6–8 putative TMDs (74). Yet another group of facilitative amino acid transporters arose from a common ancestor, as did the P-type ATPases serving as primary transporters (ABC transporters) and ion exchangers (1,3).

The anion exchanger Cl^-/HCO_3^- (band 3, AE1), a member of a distinct family of symporters and antiporters, represents a major erythrocyte membrane protein. Additional family members include the Na^+/Ca^{++} exchanger of cardiac sarcolemma, the amiloride sensitive Na^+/H^+ exchanger, and the $Na^+/K^+/2Cl^-$ cotransporter which is inhibited by the highly selective diuretic bumetanide, all of which have the 12-TMD topology (6). The latter cotransporter belongs to a new family of electrochemically neutral transporters (also Na^+/Cl^- cotransporters) regulating cell volume (RBC) and epithelial salt transport, and they are targets of the diuretic thiazides and sulfonylbenzoates (bumetanide) (39).

A different type of transporter family is represented by the major intrinsic protein (MIP) of bovine lens, forming pores with diverse functions, such as water transport and osmotic regulation (5). With 6 putative TMDs, the functional MIP holo-protein is thought to consist of a homotetramer. Furthermore, each subunits appears to have arisen from intragenic duplication of a 3-TMD fragment (3 + 3 structure). The bacterial glycerol facilitator is considered a homolog of MIP (5), and various mitochondrial exchangers also have a 6-TMD topology, including the ADP/ATP exchanger and the H^+/OH^- exchanger (uncoupling protein (5). Furthermore, the primary structure of the aquaporins is similar to that of MIP. Urinary water reabsorption is regulated by vasopressin acting at the V2 receptor. The hormone enhances the insertion of the water channel, aquaporin-2, into the apical membrane of cells in the collecting ducts (40), whereas the constitutively high water permeability of the proximal tubule and the descending part of the loop of Henle is attributed to the presence of aquaporin-1. A mutation of the aquaporin-2 gene was identified in a patient with nephrogenic diabetes insipidus (40).

Recently, a facilitative urea transporter was cloned from rabbit renal medulla. The unique sequence with two large hydrophobic domains contains approximately 10 TMDs, and the transporter is regulated by vasopressin (41). Thus renal function is tightly regulated by hormonal control of transporter functions.

A newly emerging family of proteins involved in neutral and dibasic amino acid transport contains only one obvious TMD (6). These proteins may fold into more helices within the membrane than predicted from hydropathy or they may aggregate to form homopolymers or heteropolymers with other yet unknown subunits. Defective epithelial transport of cystine results in cystinurea, a classical inherited disorder leading to the deposition of calculi in the kidney, because of the insolubility of cystine. Recently, Carlone et al. (42) identified several single point mutations in the neutral/dibasic amino acid transporter gene, *rBAT*, the most common of which (M467T) abolished amino acid transport in transfected cells. These results provide an example of the physiological consequences of genetic transporter defects.

In summary, numerous transporters exist with diverse primary structures, topology, and function. Yet, the underlying principle of the transport mechanism may be identical for a vast majority of the identified transporter gene products, with the 12-TMD structure providing a central structural motif.

ION PUMPS AND ION EXCHANGERS

The ubiquitous Na^+/K^+-ATPase is a prominent primary active ion transporter (ABC family) which forces strong opposite gradients of Na^+ and K^+ across the cell membrane and maintains cell viability. By generating a Na^+ gradient for solute transport across the membrane, this exchanger also provides the driving force for the secondary active, Na^+-dependent transporters. A neuronal Na^+/K^+ pump is regulated by NGF, and thereby, NGF may play a role in neuronal survival by maintaining a Na^+ gradient for nutrient transport

(43). Furthermore, cloning of the gene encoding the ATP-dependent K^+ channel which regulates insulin secretion of pancreatic beta-cells, revealed a structure typical of the AT-Pase superfamily (2 × 6 TMDs, 2 ABCs). This ABC protein has been identified as the high affinity receptor of the sulfonylureas, widely used to promote insulin secretion in the treatment of non-insulin dependent diabetes mellitus. Mutations of this gene resulting in aberrant processing of the encoded mRNA have been associated with familial persistent hyperinsulinemia of infancy (44). These finding provide examples of transporters serving as drug receptors and being implicated in a growing number of hereditary diseases.

Driven by the Na^+ gradient, the Na^+/inorganic phosphate (Pi) symporters concentrate phosphate into the cell (see Table I for further examples of secondary ion transporters). Recent cloning of Na^+/Pi cotransporters revealed a membrane topology with only 8 putative TMDs (21). The activity of this transporter, which affects Pi homeostasis by mediating renal tubular Pi reabsorption, is tightly regulated by a number of hormones, including insulin, vitamin D3, PTH, and glucocorticosteroids. Sulfate homeostasis also depends on specialized transporters (22,23). Urinary sulfate is largely reabsorbed by a Na^+/sulfate cotransporter in the tubular brush border membrane which shares the 8-TMD topology but little sequence identity with the Na^+/Pi symporter. Because of limited sulfate pools in the body, sulfate reabsorption maintains metabolic reactions requiring sulfate, including drug metabolism. Transepithelial reabsorption is further facilitated by a basolateral Na^+-independent sulfate transporter (23). Such cooperative transport between two classes of transporters with different energy requirements emerges as a major paradigm of transcellular transport (see also bile acid and nucleoside transporters).

Functionally coupled to the Na^+/K^+ ATPase is the family of Na^+/H^+ exchangers which have similar membrane topology (10–12 putative TMDs) but little sequence identity to other transporters. Identified functions for Na^+/H^+ exchangers include regulation of intracellular pH, recovery from an acid load, and maintenance of intestinal lumenal pH (20). Differential inhibition by the diuretic amiloride suggested the presence of isoforms, now termed NHE 1–4 (19). Using transfected fibroblasts, Counillon *et al.* (45) identified point mutations (e.g. F167L) that confer resistance of NHE1 to amiloride because of impaired drug binding to the transporter. This study provides an example of how drugs can affect transporter activity directly by binding to a target site on the transporter molecule. In addition, the activity of Na^+/H^+ exchangers may also be affected *via* growth hormone receptors or G protein-coupled receptors, such as the β_2 adrenergic receptor (19,20). Hence, transporter activities often are under multiple physiological controls, which in the case of the Na^+/H^+ exchanger could affect the activity of other secondary transporters dependent on H^+ cotransport. Sequential coupling to the Na^+/H^+ exchanger involves a series of H^+ symporters such as the intestinal dipeptide transporters, which utilize the generated H^+ gradient to concentrate substrates into the cell and play an important role in intestinal drug absorption (see below).

Further Na^+-coupled ion transporters include a family of electrically silent Na^+/Cl^- and $Na^+/K^+/2Cl^-$ cotransporters, all with 12 predicted TMDs (6). These proteins are responsible for net epithelial salt transport, and represent the targets of the diuretic thiazides and sulfonylbenzoates (bumetanide) which inhibit transport activity (39). Thus, multiple ion transporters not only serve essential physiological functions, but they are also important drug receptors. We will now address selected transporters in greater detail.

Glucose Transporters. Among the best studied transporters are the Na^+-glucose cotransporters and facilitative glucose transporters. Whereas the former mainly serve in dietary glucose absorption, the latter are found throughout the body with differential distribution for each isoform (GLUT 1–4) (46). Proposed to have 11 TMDs (cotransporter) and 12 TMDs (facilitative), the primary structures of these two types are seemingly unrelated to each other (47); however, they may be functionally linked. Renal glucose reabsorption requires several transporters, including the brush-border Na^+/glucose symporter and the basolateral facilitative transporters, with different isoforms being expressed in different segments (48).

The facilitative glucose transporters GLUT1–4 represent major membrane proteins and play important physiological roles throughout the body (8). GLUT4 is the main insulin regulatable isoform expressed in muscle tissue, while insulin and thyroid hormone regulates GLUT1 and GLUT4 in opposite directions (49). In the absence of insulin, GLUT4 resides mainly within the cell associated with endocytic clathrin coated vesicles (CCV), while undergoing constitutive recycling between the plasma membrane and CCVs (50). However, within minutes of insulin exposure, GLUT4 transfer to the cell surface increases dramatically to satisfy the cell's metabolic needs (51). Young and Holman (51) demonstrated that insulin acts by enhancing the rate of GLUT4 exocytosis, rather than blocking endocytosis, thereby, altering its subcellular distribution.

Such regulation of transporter function sets a prime example for the study of other transporters with relevance to drug transport. Exploiting the differential localization of GLUT4 and GLUT1 in vesicles and the plasma membrane, respectively, Marshall *et al.* (52) constructed GLUT1/GLUT4 chimeras to identify transporter domains that confer intracellular localization to GLUT4. By studying the cellular trafficking of the chimeras, residues 24–132 were shown to play a role in intracellular sequestration. However, the question which cellular factors mediate transporter trafficking remains to be resolved; possible candidates include a ras/GTPase or a 165 kD protein associated with GLUT4 vesicles in adipocytes (53). This protein translocates to the plasma membrane together with GLUT4 upon insulin stimulation, and it may therefore play a role in the translocation process. Conversely, transporter and receptor internalization from the plasma membrane may be regulated by an MHC class I molecule (54). A peptide derived from the class I molecule was shown to bind to the class I molecule at the cell surface, but not in the antigenic peptide groove, thereby, inhibiting internalization of several membrane proteins. As a result, this peptide caused an increase in GLUT4 activity in the plasma membrane (54). The ability to regulate the activity of certain transporters is potentially valuable in drug delivery.

Several genetic diseases are linked to mutations of glucose transporter genes. Glucose/galactose malabsorption is a genetic disorder resulting in severe diarrhea, caused by a

defect in the Na^+/glucose cotransporter of the brush border of intestinal epithelium. A single point mutation was identified in the coding frame of the gene resulting in a D28A conversion at the head of the first TMD (55). This mutation may have affected Na^+ cotransport, thereby, abolishing the driving force of the transporter. Genetic alterations in facilitative glucose transport have been linked to the pathophysiology of diabetes. Defective alleles of the facilitative glucose transporters (e.g. GLUT4) can result in impaired insulin action and insulin resistance (8), accounting for 1% of type II diabetes with normal insulin levels (non-insulin-dependent diabetes mellitus, NIDDM) (56). Furthermore, the translocation of GLUT4 to the cell surface may be impaired, possibly involving a ras/GTPase-like factor, and GLUT2 may be downregulated in β cells of the pancreas (56). Genetic differences in activity and subcellular distribution of GLUT4 and other isoforms may therefore account in part for insulin resistance.

The use of transgenic mice provides a powerful tool to study transporter functions. Liu et al. (57) generated transgenic mice expressing the 5′ flanking region of the human GLUT4 gene linked to a CAT reporter gene and determined that the reporter gene was indeed specifically expressed in tissues normally expressing GLUT4 mRNA. Moreover, changes in CAT mRNA levels induced by fasting and refeeding paralleled those observed for the endogenous GLUT4 mRNA (57), indicating that GLUT4 gene expression is determined by the 5′ flanking region. These studies provide a model for drug transport. Indeed, facilitative hexose transporters may also determine the cellular uptake of pharmaceutically relevant substrates. By injecting the GLUT1 mRNA into frog oocytes, Vera et al. (9) showed that ascorbic acid is transported by GLUT1 into the cell in its oxidized form, dehydroascorbic acid, to be subsequently reduced back to ascorbic acid for use inside the cell. These results suggest a mechanism for the concentrative uptake of vitamin C into mammalian cells.

The Dipeptide-H^+ Symporter. Our laboratories focus on the intestinal dipeptide transporters because of their essential role in the absorption of several important drugs, including ACE inhibitors, renin inhibitors, beta-lactam antibiotics, and cephalosporins (58). The acidic pH in the intestines generated by the brush-border Na^+/H^+ exchanger serves as the driving force for intestinal absorption of dipeptides, tripeptides, and peptoid drugs. However, transcellular penetration appears to depend on at least two distinct oligopeptide transporters, an apical H^+/dipeptide transporter and a basolateral H^+-independent transporter. Different translocation mechanisms and subcellular transporter locations were determined in the human colon carcinoma cell line, Caco-2, which represents a useful cellular model of intestinal absorption (59). Very little is known about the regulation of these transporters, but protein kinase C activation was shown to suppress transport activity in Caco-2 cells (60).

Photoaffinity labeling with a cephalexin derivative revealed a major protein band of 127,000 D (61); further, the transporter can be readily expressed in *Xenopus laevis* oocytes by injection of total mRNA isolated from rabbit intestines or human colon carcinoma cell lines such as Caco-2 (62). We have used ^{3}H-Gly-Sar under acidic conditions to measure mRNA induced uptake in frog oocytes (63). Size selection of rabbit intestinal mRNA revealed a single fraction capable of inducing ^{3}H-Gly-Sar uptake in oocyte, suggesting that the dipeptide transporter consists of only one subunit (63). To develop a cloning strategy, we then determined the ability of several cell lines to concentrate ^{3}H-Gly-Sar in the presence of an H^+ gradient. As reported previously, the human colon carcinoma cell lines HT-29 and Caco-2 displayed saturable uptake, whereas COS-7 cells did not, thus, serving as a possible recipient of a cloned dipeptide transporter gene. Nonsaturable uptake in HT-29 cells was several fold less than in Caco-2 cells; nevertheless, mRNA isolated from HT-29 cells yielded functional expression of ^{3}H-Gly-Sar transport in oocyte even stronger than that achieved with mRNA from rabbit intestines. Therefore, we constructed an expression cloning library in pCDN8 (Invitrogen). From this library, using pools of 3,000 cDNA clones, cRNA was produced and injected again into oocytes (Fig. 1). Expression of ^{3}H-Gly-Sar uptake with cRNA derived from a pool of clones (pool Ab) was significantly above the control, suggesting that the library contains a functional dipeptide transporter gene.

Meanwhile, several groups independently cloned two distinct genes encoding putative H^+/dipeptide transporters (11–13,64). Fei et al. (11) used rabbit intestinal mRNA to construct a library for expression cloning in frog oocytes. The cloned rabbit H^+/oligopeptide transporter gene (rPEPT1) showed little homology to other transporters. The human PEPT1 homolog is 92% similar to the rabbit transporter (12), whereas a recently cloned kidney isoform shows considerably less sequence identity, and its substrate specificity remains to be investigated (13). These transporters contain the common 12 TMD topology, deduced from the hydropathy plot (Fig. 2); however, the number of predicted TMDs strongly depends on the algorithm used to determine the hydropathy profile. Direct measurements will be required to verify the TMD topology, e.g, the insertion of reporter epitopes into the proposed extra- and intracellular loops, to

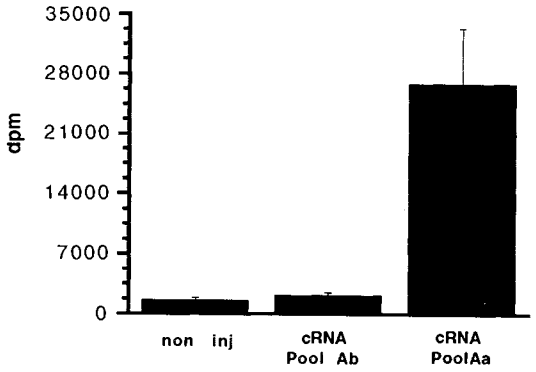

Fig. 1. ^{3}H-Gly-Sar uptake in *xenopus laevis* oocytes injected with cRNA from HT-29 cells. A cDNA library was prepared from HT-29 cells, in pCDN8 (Invitrogen) (complexity 3×10^6 clones, >1.6 kb size selected). Pools of 4,000 clones were tested for dipeptide transport activity, by preparing cRNA with SP-6 RNA polymerase and injecting the capped cRNA into the oocytes at pH 5.5 to maintain a H^+ gradient. ^{3}H-Gly-Sar uptake was tested after 48 hrs by microinjection into the oocytes. The pool shown gave positive results in several experiments.

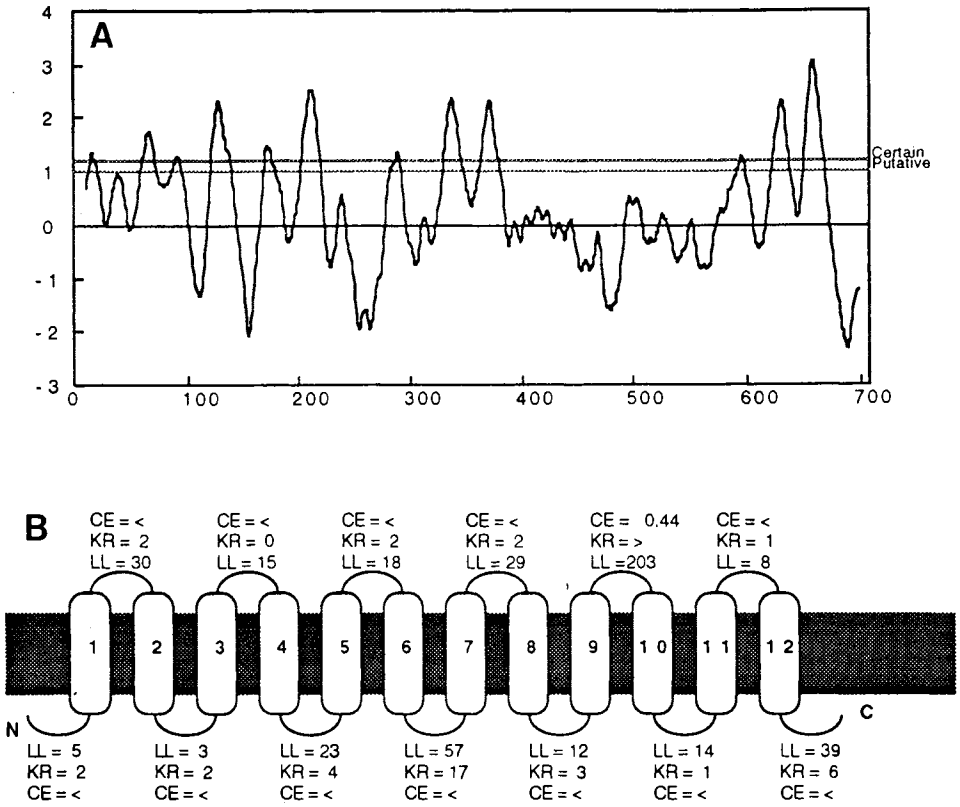

Fig. 2. Hydropathy plot and putative transmembrane topology of the hPEPT1 transporter protein (101). The Kyte-Doolittle scale and a 11 residue moving window were used for calculating hydropathy. H > 1.2 was taken to indicate certain TMD, and H > 1.0 was putative. Under these criteria, all 12 TMDs were assumed to be certain. LL = loop length, KR = number of Lys and Arg.

permit direct assay of their orientation by immunofluorescence microscopy.

In order to determine whether any other peptide transporter genes are expressed in rabbit intestinal mRNA, Fei et al. (11) used a hybrid depletion procedure to eliminate the mRNA corresponding to the cloned transporter gene. Upon injection of the depleted intestinal mRNA, dipeptide transport was no longer induced in frog oocytes, suggesting that the cloned rPEPT1 gene alone can account for the activity detected in oocytes. Using hPEPT1 as a probe, Liang et al. (12) detected mRNA expression in Caco-2 cells, but not HT-29 cells. This result suggests the possibility that other transporter isoforms may exist in HT-29 cells, because of the ability of mRNA derived from HT-29 cells to induce dipeptide transport in frog oocytes.

Dantzig. et al. (64) cloned a distinct gene encoding a putative intestinal H^+/dipeptide transporter (HPT1) from Caco-2 cells. This cadherin-like protein with only a single TMD enhanced H^+-dependent cephalexin transport when transfected into CHO cells (64). The cloning strategy involved the use of a monoclonal antibody that blocked uptake of cephalexin in Caco-2 cells, and transfection into mammalian cells, rather than oocytes. Putative transporters with only a single TMD have been reported previously to be involved in the transport of neutral and dibasic amino acids (6); however, it is unclear whether these proteins indeed represent the transporter per se. HPT1 mRNA activity was detectable in HT-29 cells (46), but in our studies, HPT1 cDNA could not be confirmed by PCR to be present in the clonal pools with positive dipeptide transporter expression in oocytes (unpublished). Therefore, it is possible that additional dipeptide transporter isoforms remain to be discovered.

Organic Cation Transport. Epithelial cells of the renal proximal tubules and hepatocytes are involved in the secretion of cationic drugs of various types, including sedatives, antihistamines, antiarrhythmics, and antibiotics. Using frog oocyte expression, Gründemann et al. (65) cloned a cDNA from rat kidney that encodes an organic cation transporter, OCT1, with 11 putative TMDs. Its characteristics are similar to organic cation uptake into cells of the renal proximal tubules (basolateral membrane) and hepatocytes. This polyspecific transporter is distinct from the multidrug resistance genes and the biogenic amine transporters. Since cationic drugs of different structures with widely different hydrophobicities are substrates of the transporter, it may play a general role in drug disposition in vivo. It will be of interest to see whether additional isoforms can be identified.

Bile Acid Transporters. The Na^+/bile acid transporters in the liver and in the intestines represent two isoforms of a homologous gene (66). A cloned hamster ileal N^+/taurocholate transporter with 7 putative TMDs is not expressed in the liver, while it is 35% identical to the equivalent rat liver transporter protein (66). In the liver, transcellular transport of bile acids is required from the basolateral (sinusoidal) to the canalicular side. Several transporters with different

translocation mechanisms are working cooperatively or in tandem (67). These include Na^+-dependent electrogenic transporters, ATP-dependent active transporters (largely at the canalicular side), and Na^+-independent transporters, in addition to intracellular specific binding proteins (67).

To facilitate drug absorption in the intestines, one could exploit ileal bile acid transporters by designing drug-substrate conjugates that translocate an otherwise impermeable agent across the cell membrane. Such a strategy proved successful for several bile acid conjugates when tested with ileal and hepatic bile acid transporter (68,69). However, it appears to be difficult to preserve both recognition and transport of the drug conjugate, and there may be size limitations for any drug attached to the bile acid carrier molecule.

Nucleoside Transporters. These transporters have been implicated in sensitivity or resistance to nucleoside antimetabolites in the treatment of cancer and viral infections. The heterogeneity of the ubiquitous nucleoside transporters suggests that a complex gene family exists (70). The need to complement *de novo* nucleotide biosynthesis by salvaging nucleosides and nucleobases (especially in the CNS) requires efficient conservation and transport into and across cells. Thus, expression of nucleoside transporter mRNA is readily detectable in epithelioid tissues (71) and the blood-brain-barrier. Transport studies and molecular cloning revealed the presence of two major classes of nucleoside transporters, facilitative and concentrative (70). The facilitative transporters are distinguished by their sensitivity to the inhibitor, nitrobenzylthioinosine, while the secondary active transporters are coupled to Na^+ contransport. Nucleoside distribution is affected by transporters throughout the body, such as in the blood-brain-barrier, placenta, intestines, and kidney. The question of nucleoside transport across the blood barriers into and out of the CNS is relevant to antiviral therapy, including the treatment of AIDS with CNS complications.

Recently, two types of Na^+-dependent nucleoside transporters have been cloned, SNST1 from rabbit kidney with homology to the Na^+/glucose transporter (14), and cNT1 from rat intestines having a distinct primary structure (15). The latter transports adenosine and pyrimidine nucleosides, including antivirals such as azidothymidine and dideoxycytidine. The therapeutic efficacy and targeting of nucleosides can be expected to depend on the spectrum of transporters involved in their cellular uptake.

Prostaglandin Transporters. The efficient removal of prostaglandins from the blood during passage through lung, liver and kidney critically depends on selective transporters, that may be either facilitative of Na^+-dependent. Thereby, the prostaglandins are effectively limited to an autacoid role. In contrast, prostacyclin (PGI2) is not recognized by the prostaglandin transporter in lung tissue and largely escapes during lung passage into the general circulation (18 and references therein). Organic anion transport inhibitors, such as indocyanine green, block PGE transport and enhance its passage through the lung. Recently, the previously cloned rat metrin F/G gene was identified as a facilitative prostaglandin transporter gene, showing 37% amino acid identity to the rat liver bile salt transporter (18). The transporter recognizes PGE_1, PGE_2, and PGF_{2a} with similar affinity, but it has low capacity for the 6-keto PGF1a metabolite and for iloprost (18). It appears that anions transporters consist of a multigene family with relevance to drug disposition.

Neurotransmitter Transporters. Secretion of neurotransmitters into the synaptic cleft is usually followed by rapid presynaptic reuptake. Following the cloning of the GABA reuptake transporter (28), many sequences of similar neurotransmitter transporters are now available (Table I). The human glycine transporter exists in multiple subtypes with several splice isoforms (29), but the significance of these isoforms remains elusive because selective inhibitors are lacking. Meanwhile, genes encoding transporters for proline, taurine, betaine, and choline have been cloned (72), and the presence of a selective transporter has become a criterion for acceptance of a neuronal substrate as a bona fide neurotransmitter. Most genes are rather closely related to each other (e.g., norepinephrine, dopamine, serotonin uptake systems), whereas the glutamate transporter shares little or no sequence identity with the other transporters (30). In general, the neuronal reuptake proteins are secondary active symporters, dependent upon both a Na^+ and Cl^- gradient, and they have 12 putative TMDs. Because there is no signal peptide, one assumes that the N and C terminal domains of the 12-TMD structure are both localized in the cytoplasm (Fig. 3) (72). Leucine zipper motifs (4 Leu residues spaced by 6 residues, i.e., two helical turns) are a general feature of 12-TMD transporters (e.g., in TMD2,9 of the dopamine transporter) which may serve to stabilize interhelical interactions (72). However, for the glutamate transporters, between 6 and 10 TMDs have been proposed (30), and these transporters function as Na^+-K^+-OH^- countertransporters.

The neurotransmitter reuptake systems are targets for numerous drugs, in particular antidepressants and neurostimulants. For many substrates and drugs, selectivity among the most closely related reuptake systems of norepinephrine, dopamine and serotonin is limited, but more selective drugs are being discovered at a fast pace. Curiously, the racemic amphetamine displays different stereoselectivity in inhibiting different transporters; whereas D-amphetamine is 10-fold more potent than L-amphetamine at the dopamine transporter, both enantiomers display similar potencies at the norepinephrine transporter (72). The dopamine reuptake system is considered a "cocaine receptor", and occupancy of the binding sites on this transporter and its inhibition correlate with induced locomotor activity in rodents (25,26). Mazindol is a potent, reasonably selective inhibitor of this subtype (25). The dopamine transporter may also mediate the targeted effect of various neurotoxins such as MPTP and 6-hydroxydopamine. By expressing the dopamine transporter in COS-7 cells, the toxic metabolite MPP^+ was shown to be selectively concentrated into the transfected cells (73).

The distribution of polar amino acids in the TMDs displays some analogy of the dopamine transporter to the G protein coupled receptors selective for biogenic amines. Residue Asp79 in TMD1, crucial to transporter function, is analogous to critical Asp residues in TMD2,3 in the biogenic amine receptors (74). Point mutations of Asp79 greatly reduce transport and binding for all substrates. Moreover, two serine residues in TMD7 of the dopamine transporter are analogous to a serine pair in TMD5 of catechol receptors. Mutation of Ser355 and Ser359 in TMD7 to Ala or Gly re-

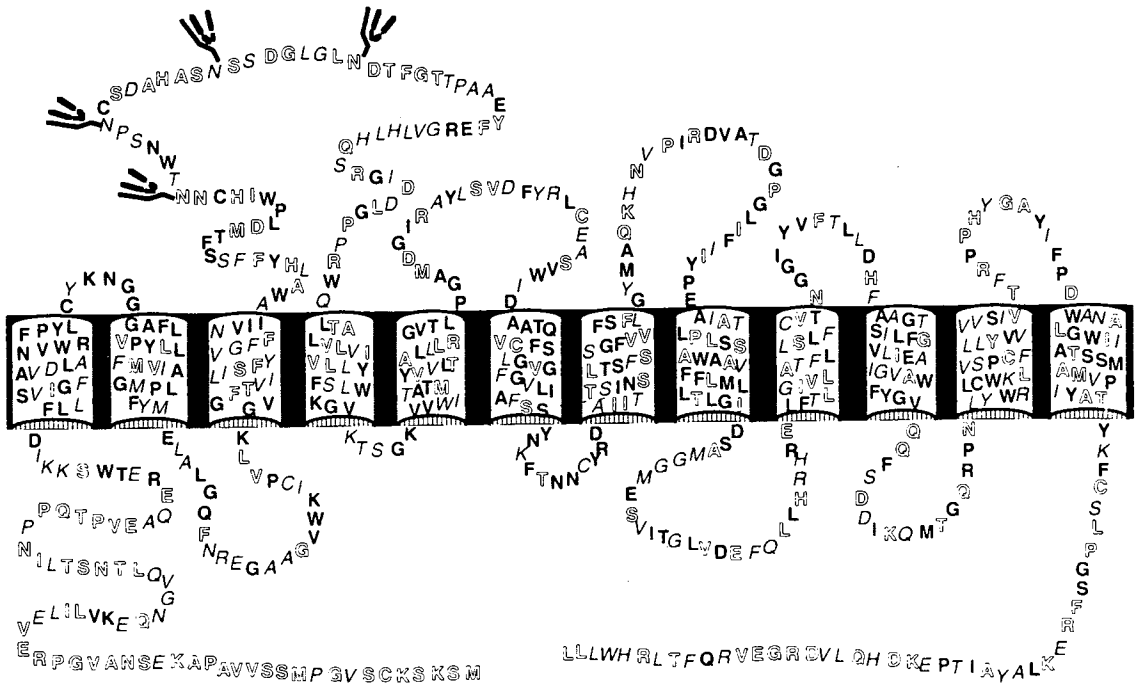

Fig. 3. Deduced amino acid sequence and putative TMD topology of the dopamine transporter. Amino acids conserved in GABA, dopamine (DA) and norepinephrine (NE) transporters are in boldface, amino acids conserved in DA and NE transporters in italics, and residues present only in the DA transporter are in open letters. (Reproduced from (25))

duced dopamine transport, but it had little effect on the binding of CFT, a cocaine analog (74). This result establishes that the substrate binding site overlaps, but is distinct from the binding site of the inhibitor. Thus, one can now search for ligands that may inhibit the binding of cocaine (cocaine antagonist) without affecting the normal function of the dopamine transporter. Such agents could prove valuable in the treatment of cocaine abuse.

By constructing chimeric dopamine-norepinephrine genes, Buck and Amara (75) delineated domains important to the transport of catechols and MPP^+. At least three regions appeared to contribute to substrate selectivity (TMD1–3, TMD4–8, TMD10–11), with TMD4–8 playing a role in MPP^+ transport. Chimeric human and rat serotonin transporters were used to delineate domains responsible for species differences in imipramine and D-amphetamine binding (76). Regions distal to residue 532 were identified in these cross-species chimeras to affect substrate affinity.

Neurotransmitter transporters can be exploited for diagnostic study of neuronal degeneration *in vivo* by imaging with suitable tracers and PET, or they may serve to visualize dopaminergic or serotonergic neurons by concentrating fluorigenic substrates (77). Furthermore, drugs can be targeted to tissues expressing these transporters. In disseminated neuroblastoma, a tumor of early childhood frequently expressing abundant norepinephrine transporters, clinical trials have been pursued with ^{131}I-metaiodobenzylguanidine (78), which is a substrate of the norepinephrine uptake system, and hence, selectively accumulates in the tumor for diagnosis and localized irradiation therapy.

This discussion has so far been restricted to neurotransmitter transporters expressed at the cell surface. However, a distinct set of genes has been identified for transporters responsible for concentrating neurotransmitters into presynaptic vesicles. Therefore, the storage of neurotransmitters also depends on specialized transporters. In the nematode, an acetyl choline neuronal vesicular transporter of similar molecular architectures (12 TMDS) was shown to be vital; nonfunctional mutations proved to be lethal (79). This result underscores the biological importance of such transporter gene products. Detailed kinetic studies have been performed with the vesicular acetyl choline transporter of mammalian cells, also referred to as the receptor for vesamicol which inhibits acetylcholine uptake (80). A vesicular biogenic amine transporter with the 12-TMD topology has been recently cloned (31), by exploiting its ability to confer resistance to the neurotoxin MPP^+, a metabolite of MPTP. The primary structure of the vesicular transporter is different from the neurotransmitter transporters expressed at the cell surface, but it shares some sequence identity with a bacterial tetracyclin transporter.

Given the extraordinary role that neurotransmitter transporter play in neuronal functions, one might expect genetic mutations to contribute to mental disorders. The dopamine transporter is implicated in the mechanisms underlying psychostimulant abuse, Parkinsonism, and Tourette's syndrome. Cloning of the human dopamine transporter gene has led to the discovery of dimorphic RFLPs (81). However, it remains to be seen whether genetic defects of neurotransmitter transporters underly mental diseases and drug addiction.

ATP BINDING CASSETTE (ABC) SUPERFAMILY OF TRANSPORTERS

The ABC transporter gene family has rapidly grown to over 30 cloned members with extensive sequence homology

(82). The ATP binding domains consisting of a cassette of some 200 amino acids include ATP binding motifs (Walker motifs) present in the nucleotide binding pocket of a large variety of proteins (ABC proteins), which include the transporters discussed here. Taking advantage of the known crystal structure of another ABC protein, adenylyl kinase, a structural model of the ABC domain of transporters was developed, on the basis of homology between their ATP domains (82). ATP hydrolysis provides the energy for substrate translocation by these active transporters of prokaryotes and eukaryotes; however the mechanism of energy coupling remains obscure. A change in ABC conformation upon ATP binding is thought to confer conformational changes on the TMD structure, and loops 2 and 3 appear to play a role in this process (82).

Prominent examples of ABC transporters include the P-glycoproteins associated with multiple drug resistance in tumors (MDR genes), the cystic fibrosis transmembrane regulator (CFTR), the MHC linked antigenic peptide transporters (HAM1,2 and TAP1,2) (Table I), the STE6 gene product responsible for the secretion of peptide mating factors in yeast, and various drug resistance genes in bacteria and parasites (6,82–84). The functional transporter complex usually consists of four domains, i.e., two hydrophobic domains of 6 TMDs each and two ABC domains. Each domain may be expressed as a separate gene product, as found for the oligopeptide permease of *Salmonella typhimurium*, or two domains each are combined into one gene requiring simultaneous expression of two genes to yield the functional heterodimeric transporter (TAP1,2). Alternatively, all four domains are fused into one single gene encoding a contiguous polypeptide chain as found in several eukaryotic systems (MDR, CFTR).

CFTR Channel. The CFTR gene product appeared to display chameleon-like qualities of either a transporter or an ion channel. Despite the rather high sequence identity to MDR, CFTR does not represent an ABC transporter in the strict sense, but rather functions as a Cl^- channel which is merely regulated by ATP binding to the ABC portions (no active transport) (85). Thus, its rapid turnover number is more akin to that of channels rather than to the slower turnover of active transporters (85). The cAMP stimulated CFTR protein consists of five domains, i.e., the ususal 2 + 2 topology plus a fifth regulatory domain which serves as a gatekeeper. Since there is a consensus building that CFTR is truly an ion channel that only looks like a channel, translocating anions such as Cl^-, and possibly water and small solutes such as urea, it will not be discussed here in much detail. Nevertheless, numerous CFTR mutations found to be associated with cystic fibrosis provide insights into the structure and function of an integral tansmembrane protein having the ABC transporter topology. The single point mutation dF508 (deletion of Phe508), found in 70% of CFTR patients, occurs in one of the two ABC portions and may alter ATP binding (33). More importantly, it fails to reach the apical membrane surface and is retained in the ER even though it is fully functional as a Cl^- channel (86). This finding raises the question why the defective protein fails to move past the ER, and whether drug treatment could induce normal cellular trafficking. Because of the high prevalence of CFTR, much effort will be spent on drug development, and the results will be relevant to the general transporter field. In fact, 5% of Caucasians are heterozygous with respect to the dF508 mutation, leading to speculation that heterozygous carriers may have selective advantages, such as a possible protection against enterotoxin elicited diarrhea and severe dehydration (87). Bacterial transporters of neutral and branched amino acids show homology to CFTR and have provided a CFTR mutation model to localize the domains critical to function (88). Gene therapy of CFTR appears to be promising. In CF transgenic mice, application of a CFTR encoding plasmid in liposomes was capable of correcting defective ion transport (89).

Multiple Drug Resistance Genes (MDR). In a landmark paper, Keld Danø demonstrated in 1973 active outward transport of daunomycin (doxorubicin) in resistant Ehrlich ascites tumor cells, and he already then suggested that resistance may be overcome by the coadministration of agents that inhibit this drug exporter (90). Subsequently, Juliano and Ling (91) isolated a surface glycoprotein modulating drug permeability in CHO cell mutants, introducing the term P-glycoprotein (P = permeability). The P-glycoprotein product of the cloned MDR-1 human gene turned out to be a protein of Mr 170 kD (32), and a member of the growing family of ABC transporters. MDR gene products transport a variety of largely lipophilic drugs out of the cell, thereby, conferring resistance against certain antineoplastic agents (32). Exposure of transformed cells to a cytotoxic agent, such as doxorubicin, can lead to MDR overexpression, and thus, to acquired drug resistance. Since the MDR transporters display a low degree of specificity and tend to recognize several antineoplastic agents, multiple drug resistance ensues (92). Fortuitously, some Ca^{++} channel blockers, such as verapamil and nifedipine, were also found to bind to MDR transporters. Therefore, blocking the export of cytotoxic drugs out of the cell by coadministration of inhibitors represents a viable strategy to overcome multidrug resistance caused by MDR overexpression. However, the clinical relevance of antineoplastic drug resistance in treatment failure is still under investigation (93,94).

Two-dimensional amino acid sequence analysis of the human MDR1 gene product demonstrates that the 2 + 2 topology of the protein (2 × 6 TMDs and 2 × ABC) is comprised of internal repeats generated by tandem intragenic duplication (Fig. 4). The substrate binding pockets of MDR are under intense study, using labeling and mutational analysis. For example, photoaffinity labeling involving prazocine and forskolin demonstrated that each of these drugs binds to identical sites in both halves of the protein (95). Furthermore, a glycine to valine mutation was shown to change the preferred substrate selectivity in favor of colchicine resistance (96), indicating that drug selectivity can differ substantially. In the absence of an accurate 3D model, however, it will be difficult to characterize fully the binding sites.

Therapy with many antineoplastic agents is dose-limited by their bone marrow toxicity. Thus, MDR gene transfer into a cancer patient's healthy bone marrow cells represents an indirect approach which could allow one to increase substantially the doses of antineoplastic agents. When the human MDR-1 gene was transferred with a viral vector into murine bone marrow cells, a substantial enrichment of protected bone marrow cells was achieved by selection in vitro with

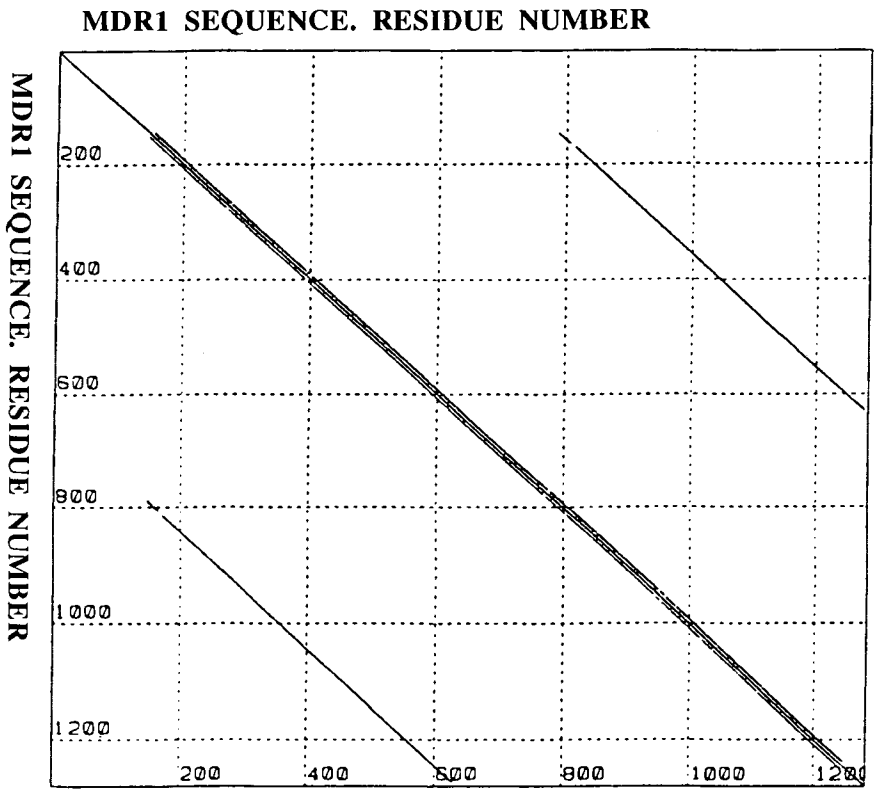

Fig. 4. Lawrence plot comparing the amino acid sequence of the human MDR1 gene product against itself. At the stringency selected (ndev > 4, window 21), one can see two shorter lines parallel to the central line of identity, representing the first and second portion of the protein. This pattern suggests that the protein consists of two homologous repeat units, each containing one ABC and one 6-TMD domain. The longer parallel line immediately adjacent to the central line of identity may have been generated by α-helical periodicities and are commonly observed with other integral membrane proteins having multiple TMDs.

the cytotoxic MDR substrate taxol (97). The ability to amplify clones of transfected hematopoietic cells *in vitro* for reinjection into the same host could generate a drug resistant bone marrow allowing high-dose chemotherapy. Alternatively, cotransfection of the MDR-1 gene with nonselectable genes would allow one to amplify transfected cells carrying both the MDR-1 selection marker and the gene of interest. This approach could facilitate gene therapy of inherited disorders, such as deficiencies of β-globin or adenosine deaminase (severe combined immunodeficiency) (97).

Recently, MDR gene products were found in intestinal tissues where they transport absorbed drug molecules out of the cells back into the gut lumen (98). The presence of P glycoprotein indeed reduced drug absorption in the Caco-2 cell culture model (99). This process may significantly limit the bioavailability of selected drugs that are P-glycoprotein substrates (100), and the relevance of intestinal MDR expression to drug bioavailability is under intense study.

MHC Linked Transporter of Peptides Assembled in Class I Molecules. Antigenic peptides generated by degradation of the intact antigen by proteasomes, bind to MHC class I molecules in the endoplasmic reticulum as a required step in antigen presentation at the cell's surface (50,53). Genetic studies pointed to genes within the MHC Class II region as playing a role in this process. Two genes in this region, each encoding a "half ABC transporter", are now recognized to transport antigenic peptides into the ER, thereby facilitating association with MHC class I molecules (34,35,101). These two genes share sequence homology with the MDR genes, but they need to form heterodimers for functional peptide transport (e.g. HAM1 + HAM2 (mouse) and TAP1 + TAP2 (human)). Evidence for the essential role of both gene product stems from studies with mutant cell lines unable to present endogenous antigens because of inability to assemble antigen peptides and MHC Class I molecules (101). The defect was traced to a defective half-transporter gene, and transfection with the corresponding wild-type gene to replace the defective monomer rescued antigen-MHC class I assembly. These results also provided direct evidence for the role of ATP in driving peptide transport (101). Subsequently, the TAP heterodimer was shown to associate directly with MHC/β2-microglobulin complexes, thereby facilitating antigenic peptide-MHC complexation (102). These results provide another example of physical interaction of a transporter with HLA molecules which could affect cellular transporter trafficking.

Extensive polymorphism of the peptide transporter genes suggested that the structural features of the MHC

class I molecules are not the only determinant of specific antigen presentation. Rather, polymorphic transporter genes were shown to alter the spectrum of bound peptides (103), and the antigenic peptide sequence affected recognition and transport by TAP1 and TAP2 (101). Furthermore, human HLA class I molecules largely depend for their expression on the supply of peptides by the TAP protein. A homozygous human TAP peptide transporter mutation was found to cause HLA class I deficiency and suppression of the immune response (104). These new insights into antigen presentation may result in effective measures to influence the immune system.

Drug Resistance in Bacteria and Parasites. Bacterial drug resistance can result from ABC transporters extruding toxic substances. For example, resistance to antimonials and arsenicals is conveyed by a plasmid encoded ATP driven anion pump (83). Two separate genes (Ars A and Ars B), containing each one TMD region and one ABC domain, are required for cellular export. Similarly, the active transporter pfMDR has been implicated in chloroquin resistance of malarial parasites (82 and references therein).

In contrast, arsenical resistance of trypanosomes, which cause African sleeping sickness, was associated with defects in genes encoding two high affinity adenosine transport systems (P1 and P2) which are secondary active transporters without ABC domains (84). Specifically, the melaminophenyl arsenical, melasoprol, was found to be transported by the P2 type, genetic defects of which lead to drug resistance. On the other hand, structural similarities between adenine and melamine suggests that melamine conjugates could serve to target drugs to trypanosomes via the intact P2 system (84).

CONCLUSIONS

The cloning of numerous transporter genes enables one to view their structure and functions on a molecular-genetic basis. Even though structural models at atomic resolution are not available, structure-function analysis using sequence comparison and mutational effects is beginning to reveal the binding sites, possible channel location, transduction mechanisms, and energy coupling of active transport. Transporters are essential to the cell's survival, and they provide required pathways for drug access to the target, or they represent the drug receptors *per se*. Not surprisingly, transporter activity is often tightly regulated by hormones and other factors. Understanding the mechanism of regulation, including post-translational modifications such as phosphorylation or cellular trafficking, will prove valuable in developing strategies in drug therapy. Indeed, hormonal regulation of transporter movement to the plasma membrane critically determines the activity of many transporters. Furthermore, an increasing number of transporter genes is implicated in genetic diseases, but few of these are understood in molecular detail. Dozens of inherited defects of membrane transport are known to contribute to human diseases (105), including defects of transporters of amino acids, hexoses, LDL, urate, anions and cations, water, and vitamins. In the pharmaceutical field, such transporter defects need to be considered for drug delivery, drug resistance, and drug design. If a transporter is required to facilitate drug absorption, as is the case for the intestinal absorption of cephalosporins and ACE inhibitors *via* H^+/dipeptide transporters, one must study the prevalence of any defective alleles in the patient population. For non-essential gene products, the prevalence of defective alleles could be rather high, resulting in ineffective oral therapy in affected patients. These considerations make a compelling case for cloning the transporter genes and their subtypes, studying the substrate specificity for each, and initiating genetic studies on the allelic complexity and distribution of transporter genes.

In conclusion, the vast literature on membrane transporters spans multiple scientific disciplines, including genetics, molecular biology, physiology, drug design and targeting, and pharmacokinetics/phamacodynamics. We intended to illustrate how these diverse disciplines overlap and merge, requiring a multidisciplinary approach to exploit transporters for therapeutic applications.

ACKNOWLEDGMENTS

This study was supported by a US Public Health research grant from the NIH, General Medical Sciences, GM37188.

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