Overview: ABC Transporters and Human Disease

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ABC transporters are found in all known organisms, and approximately 1,100 different transporters belonging to this family have been described in the literature. The family is defined by homology within the ATP-binding cassette (ABC) region, which extends outside of the more typical Walker motifs found in all ATP-binding proteins. Most family members also contain transmembrane domains involved in recognition of substrates, which are transported across, into, and out of cell membranes, but some members utilize ABCs as engines to regulate ion channels. There are approximately 50 known ABC transporters in the human, and there are currently 13 genetic diseases associated with defects in 14 of these transporters. The most common genetic disease conditions include cystic fibrosis, Stargardt disease, age-related macular degeneration, adrenoleukodystrophy, Tangier disease, Dubin–Johnson syndrome and progressive familial intrahepatic cholestasis. At least 8 members of this family are involved in the transport of a variety of amphipathic compounds, including anticancer drugs, and some appear to contribute to the resistance of cancer cells to chemotherapy.

KEY WORDS: ATP hydrolysis; diseases; multidrug resistance; nucleotide-binding domains; transmembrane domains; transport proteins; substrates; structure.

This introduction will give a brief overview of the current status of knowledge about ATP-binding cassette (ABC) transporters. The individual papers in this volume will provide more detailed information about the mechanism of action, subcellular distribution, and function in health and disease of many of these transporters.

ABC transporters can be recognized by a consensus ATP-binding region of approximately 90–110 amino acids, which includes two Walker motifs (A and B regions); a linker or dodecapeptide region, which lies between these Walker motifs (also known as the C region); and some additional regions of homology upstream and downstream from the Walker A and B motifs (Higgins, 1992) (Fig. 1). While ABCs are well conserved across all known organisms, they are usually associated with transmembrane (TM) domains consisting of six transmembrane helices, which confer substrate specificity, and are not so well-conserved. For the ABC transporters, there are two ABCs associated with two TMs, either as four single subunits as in most prokaryotes, or various combinations of fused subunits. In some of the transporters, the functional unit is formed by homodimerization of the basic components, one ABC domain plus one TM domain (such as HlyB (Zhang *et al.*, 1998) and most likely MXR), while in others it is composed of heterodimers (such as Tap1/Tap2). Two ABCs and two TMs are fused into a single polypeptide in most eukaryotic transporters (for reviews see Ambudkar and Gottesman, 1998). In addition, for some members of the MRP (multidrug-resistance–associated proteins) family (ABCC subfamily), there is an additional TM domain consisting of 5 TM helices at the amino-terminal end of the molecule. Many biochemical and molecular genetic studies indicate that the functional unit of an ABC transporter is composed of two TMs (twelve membrane-spanning helices) and two ABCs. This combination is achieved in a variety of ways, and some of these structural forms of ABC transporters are shown in Fig. 2.

Because of the importance of ABC transporters in many biological processes, and the mystery that surrounds their mechanism of action, much attention has been devoted to the elucidation of the crystal structure of ABC

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ATP-BINDING CASSETTE

Fig. 1. Schematic structure of a typical ATP-binding cassette (ABC). The highly conserved Walker A and B domains are separated by 90–110 amino acid residues. The "ABC signature" dodecapeptide (C region) is just upstream from the Walker B domain. The strongest amino acid consensus sequences among ATP sites of mammalian and avian P-glycoproteins are shown at the bottom of the figure in the standard single letter code.

transporters. To date, structures of nucleotide-binding regions of the ABC transporters, such as histidine permease (HisP), MutS, MalK, Rad50, MJ 1267 and the soluble ATP subunit, ArsA of the bacterial arsenite efflux pump, which is analogous to ABC transporters, have been reported (Diederichs *et al.*, 2000; Hopfner *et al.*, 2000; Hung *et al.*, 1998; Karpowich *et al.*, 2001; Lamers *et al.*, 2000; Obmolova *et al.*, 2000; Zhou *et al.*, 2000). However, there has been no successful crystallization reported for a complete ABC transporter that includes two ABCs and associated TMs, so mechanistic information derived from the existing structures is somewhat limited. The resolution of structures of HisP, MalK, Rad50, and ArsA indicates that the two functional ATP sites are each composed of residues from both the ABCs. Such a tertiary structural organization seems likely for mammalian transporters such as P-glycoprotein (Pgp) where both the ATP sites are essential for ATP hydrolysis (see Ambudkar *et al.*, 1999). In the case of Rad50, both the ABCs undergo dimerization in the presence of ATP because of extensive intersubunit contacts to the nucleotides, which are located in the interface of the two subunits (Hopfner *et al.*, 2000). It remains to be seen whether similar nucleotide-dependent dimerization of ABCs is a common feature shared by all members of this superfamily.

ABC transporters are ubiquitous with many family members in all known organisms. For example, there are 25 such transporters in *E. coli*, where they subserve functions ranging from substrate transport into the bacterium to extrusion of proteins and polysaccharides, and to antibiotic resistance; there are 30 in the yeast *Saccharomyes cerevisiae*, 56 in *Drosophila* and *C. elegans*, and approximately 50 (at present the exact number appears to be 48) in the human genome. There are seven known distinct subfamilies of ABC transporters, designated A-G, based on the homology in their ABCs (see article in this volume by Dean and Allikmets for summary). Most of the ABC transporters are involved directly in the transport of a variety of substrates, including sugars, amino acids, glycans, cholesterol, phospholipids, peptides, proteins, toxins, antibiotics, and xenobiotics. In general, substrate specificity is determined by the TM regions, and the energy for transport is provided by the ABCs. Substrates are moved into and out of cells, into and out of subcellular organelles, and, in the case of hydrophobic natural product drugs recognized by the multidrug transporter, substrates are detected and ejected directly from the plasma membrane.

Some ABC transporters are not directly involved in moving substrates, but appear to be part of regulated ion

Structural Organization of an ABC Transporter

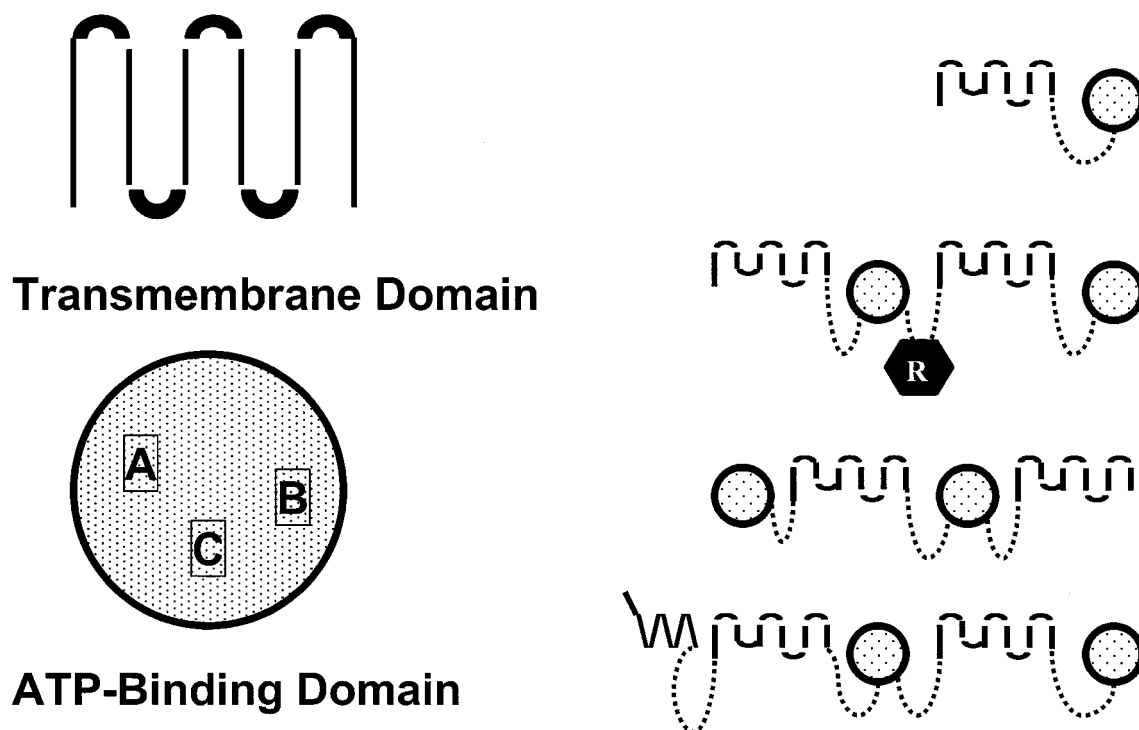**Transmembrane Domain**

ATP-Binding Domain

Fig. 2. Structural organization of a typical ABC transporter. On the left, the ATP-binding cassette is shown as a circle, with the letters A, B, C signifying regions of high homology (the Walker A and B domains and the dodecapeptide "ABC signature," also called the linker or C domain) in this family, and the transmembrane domain (TM) is shown as a series of six membrane-spanning helices. On the right, some of the various ways in which the ABCs (circles) and the TMs (each domain containing six helices) can be assembled in ABC transporters are illustrated. The hexagon with the R represents the regulatory R domain of CFTR (Riordan *et al.*, 1989), which is not found in any other ABC transporter. In some members such as MRP1, there is an additional membrane-spanning domain, with five transmembrane helices present at the amino terminal end as shown at the bottom right.

channels (such as the cystic fibrosis transmembrane regulator, or CFTR) or the sulfonylurea receptor (SUR). In these cases, it appears that the hydrolysis of ATP is linked to movement of the protein itself (for CFTR) or to other proteins (for SUR) to regulate opening and closing of ion channels. Finally, there are distantly related proteins, which utilize an ABC-like engine to drive processes as diverse as DNA repair (e.g., mutS; Obmolova *et al.*, 2000) and protein synthesis (e.g., elongation factor–3; Belfield *et al.*, 1995). For purposes of this discussion, such proteins are not considered "ABC transporters," but there may be features of their structure and mechanisms of action that are shared with ABC transporters.

Given the diversity of functions of ABC transporters, it is reasonable to ask what can be gained by studying them as a group. At the meeting on "ABC Transporters

and Human Disease" it became clear that there were many common features to their structures and mechanisms of action. For example, the best data suggest that two molecules of ATP are hydrolyzed during the transport of substrates, consistent with the finding of two ABCs in all known ABC transporters. Study of the human multidrug transporter, Pgp, indicates that each of these hydrolytic events subserves a different function; one is involved directly in a conformational change in the TMs that results in translocation of drugs out of the cell, while the second seems to be necessary to restore the transporter to its original high affinity state for substrates (see article by Sauna *et al.* in this volume; also Sauna and Ambudkar, 2000, 2001). It is reasonable to believe that the known requirement for the two ABCs for ABC transporters in which this stoichiometry has been studied may also reflect a requirement of

one hydrolytic event for transport and a second event to reset the transporter to its original state. It also became clear that despite vast evolutionary differences among bacteria, yeast, and man, the function of many of these transporters is entirely homologous in the three organisms. Thus, lessons learned from simpler systems, which are easier to manipulate, are relevant to human physiology and pathophysiology.

Since the original finding that resistance to anticancer drugs in human cancers is due to the expression of an ABC transporter (Chen *et al.*, 1986) (*MDR*1, *ABC* B1, or Pgp), it has become clear that many inherited human diseases result from defects in ABC transporters. In many cases in which positional cloning technology was used to zero in on the defective gene, such as the discovery of a defective *CFTR* gene in cystic fibrosis (Riordan *et al.*, 1989), a defective *ALDP* gene in adrenoleukodystrophy (Shani and Valle, 1998), the defective ABC1 cholesterol transporter in Tangier disease (Brooks-Wilson *et al.*, 1999; Rust *et al.*, 1999), or the defect in the *MRP6* gene in pseudoxanthoma elasticum (Ringpfeil *et al.*, 2000), the finding of an ABC transporter as the cause of disease was a complete surprise since the pathophysiology of the disease did not point to a transport problem. In others where candidate gene approaches were taken, such as the finding of a defective *SPGP* (sister of Pgp) gene in progressive familial intrahepatic cholestasis (Strauniek *et al.*, 1998) or the Dubin–Johnson hyperbilirubinemia syndrome in which there is a defect in MRP2 (cMOAT), the liver canalicular multiorganic anion transporter (Paulusma *et al.*, 1996; Wada *et al.*, 1998), the finding of an ABC transporter as the root cause of the disease was not a surprise. Table I summarizes human diseases known to date in which there are alterations in ABC transporters.

A large number of ABC transporters appear to be associated with drug resistance in prokaryotes and eukaryotes. This reflects the need for these systems to

Table I. Human Diseases Associated With an ABC Transporter

Disease	Transporter	References[a]
Cancer	ABCB1 (MDR1), ABCC1 (MRP1), ABCG2 (MXR)	(1–3)
Cystic fibrosis	ABCC7 (CFTR)	(4)
Stargardt disease and age-related macular degeneration	ABCA4 (ABCR)	(5)
Tangier disease and familial HDL deficiency	ABCA1 (ABC1)	(6)
Progressive familial intrahepatic cholestasis	ABCB11 (SPGP), ABCB4 (MDR2)	(7, 8)
Dubin–Johnson syndrome	ABCC2 (MRP2)	(9)
Pseudoxanthoma elasticum	ABCC6 (MRP6)	(10)
Persistent hypoglycemia of infancy	ABCC8 (SUR1), ABCC9 (SUR2)	(11, 12)
Sideroblastic anemia and ataxia	ABCB7 (ABC7)	(13)
Adrenoleukodystrophy	ABCD1 (ALD)	(14)
Sitosterolemia	ABCG5, ABCG8	(15)
Immune deficiency	ABCB2 (Tap1), ABCB3 (Tap2)	(16, 17)

[a] (1) Chen *et al.*, 1986; Gottesman and Pastan, 1993; (2) Cole *et al.*, 1992; (3) Doyle *et al.*, 1998; Miyake *et al.*, 1998; Allikmets *et al.*, 1998; (4) Riordan *et al.*, 1989; (5) Allikmets *et al.*, 1997; Sun *et al.*, 1999; (6) Brooks-Wilson *et al.*, 1999; Rust *et al.*, 1999; (7) Strautniesks *et al.*, 1998; (8) de Vree *et al.*, 1998; Dixon *et al.*, 2000; (9) Paulusma *et al.*, 1996; Wada *et al.*, 1998; (10) Ringpfeil *et al.*, 2000; (11) Thomas *et al.*, 1995; (12) Bryan and Aguilar-Bryan, 1999; (13) Allikmets *et al.*, 1999; (14) Shani and Valle, 1998; (15) Berge *et al.*, 2000; Lee *et al.*, 2001; (16) Powis *et al.*, 1991; (17) Momburg *et al.*, 1994.

protect organisms from the sea of toxic compounds in which they evolved. In humans, at least eight ABC transporters are known to confer resistance to cytotoxic compounds, and others are likely to be found. These are summarized in Table II. In addition to conferring resistance to cytotoxic compounds, these transport systems

Table II. Human ABC Transporters Which are Known to Transport Drugs[a]

Common names	Official name	Substrates	Normal location
Pgp, MDR1	ABC B1	Neutral and cationic, organic compounds	Intestine, liver, kidney, blood–brain barrier
MRP1	ABC C1	GS-X[b] and other conjugates, organic anions	Widespread
MRP2, cMOAT	ABC C2	GS-X and other conjugates, organic anions	Liver, kidney, intestine
MRP3, MOAT-D	ABC C3	GS-X conjugates, antifolates, bile acids, etoposide	Pancreas, kidney, intestine, liver, adrenal
MRP4, MOAT-B	ABC C4	Nucleoside analogs, methotrexate	Prostate, testis, ovary, intestine, pancreas, lung
MRP5, MOAT-C	ABC C5	Nucleoside analogs, cyclic nucleotides, organic anions	Widespread
MRP6, MOAT-E	ABC C6	Anionic cyclic, pentapeptide	Liver, kidney
MXR, BCRP, ABCP	ABC G2	Anthracyclines, mitoxantrone	Placenta, intestine, breast, liver

[a] Modified from Gottesman (in press).
[b] GS-X represents glutathione conjugates.

are extremely important in determining absorption, distribution, and excretion of many different pharmacologic compounds by the body. This has been demonstrated using knockout mice for *mdr*1a and b, and *mrp*1 (Johnson *et al.*, 2001; Schinkel *et al.*, 1997). In addition, studies with these triple knockout mice [*mdr*1a/1b $(-/-)$, *mrp*1 $(-/-)$] demonstrate that Pgp and MRP1 transporters contribute significantly to the development of resistance to anthracyclines, paclitaxel, and *Vinca* alkaloids (Allen *et al.*, 2000). In humans, a single-nucleotide polymorphism within the *MDR*1 gene (C3435T in exon 26) is linked to reduced levels of expression of this transporter in the gastrointestinal tract, which in turn affects absorption of commonly used drugs such as digoxin (Hoffmeyer *et al.*, 2000). In another study on the expression of Pgp in 100 placentas obtained from Japanese women, it was observed that 94% (61 of 65) samples, which had a C3435T allele, also had a mutant G2677A/T allele in exon 21, indicating a close association between these two polymorphisms (Tanabe *et al.*, 2001).

The study of ABC transporters is still in its infancy, but already work in this area has led to the elucidation of the cause of several significant inherited human diseases, and to new insights about treatment of cancer and drug pharmacokinetics.

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