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MAMMALIAN ABC TRANSPORTERS IN HEALTH AND DISEASE

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■ **Abstract** The ATP-binding cassette (ABC) transporters are a family of large proteins in membranes and are able to transport a variety of compounds through membranes against steep concentration gradients at the cost of ATP hydrolysis. The available outline of the human genome contains 48 ABC genes; 16 of these have a known function and 14 are associated with a defined human disease. Major physiological functions of ABC transporters include the transport of lipids, bile salts, toxic compounds, and peptides for antigen presentation or other purposes. We review the functions of mammalian ABC transporters, emphasizing biochemical mechanisms and genetic defects. Our overview illustrates the importance of ABC transporters in human physiology, toxicology, pharmacology, and disease. We focus on three topics: (a) ABC transporters transporting drugs (xenotoxins) and drug conjugates. (b) Mammalian secretory epithelia using ABC transporters to excrete a large number of substances, sometimes against a steep concentration gradient. Several inborn errors in liver metabolism are due to mutations in one of the genes for these pumps; these are discussed. (c) A rapidly increasing number of ABC transporters are found to play a role in lipid transport. Defects in each of these transporters are involved in human inborn or acquired diseases.

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INTRODUCTION

The name ABC transporters was introduced in 1992 by Chris Higgins in a memorable review (1). The designation ABC was based on the highly conserved ATP-binding cassette, the most characteristic feature of the superfamily. Traffic ATPases and P-glycoproteins (Pgps) are other names used for this family. An inventory of all 48 known and putative human ABC transporters (2) can be found on the Web site of M. Müller, University of Wageningen, The Netherlands (2a). Table 1 lists the 24 ABC transporters with known function or involvement in disease, which are discussed in this review. The cutoff is somewhat arbitrary. We include transporters like ABCB6, for which the function is surmised rather than known, and we have omitted transporters for which a function will probably be found before this chapter is published.

We have omitted three well-characterized ABC transporters, ABCC7–9, because they are not known to mediate any uphill transport. ABCC7 (CFTR) is a chloride channel, and channels are not transporters. Valverde et al. (3) reported evidence in 1992 that P-glycoprotein can exist in two states, as drug transporter and as chloride channel. On this basis, Higgins (1, 4) raised the question of whether CFTR “might also be bifunctional, with a transport function yet to be identified.” Subsequent work has shown, however, that P-glycoprotein is not a chloride channel (5, 6) and has failed to identify a transport function for CFTR. Hence, CFTR is not discussed here. ABCC8 and 9 (SUR1 and 2), the sulfonyl-urea receptors, are the ATP-sensing subunits of a complex potassium channel and are not known to transport anything.

The subunit structure of ABC transporters varies, as shown in Figure 1. The basic structure, as found in P-glycoprotein (ABCB1), is thought to consist of 12

transmembrane segments and two ATP-binding sites in a protein of about 1300 amino acids. This basic structure may be assembled from two equal (ABCG2) or unequal (ABCG5 and 8) halves. Some ABC transporters (e.g., MRP1) have additional domains and are even larger than P-glycoprotein. How ABC transporters work is still not known in detail. Models for the transport mechanism are briefly reviewed here.

This chapter focuses on four overlapping topics, emphasizing biochemical mechanisms, genetic defects, and regulatory mechanisms:

1. ABC transporters play a role in the transport of drugs (xenotoxins) and drug conjugates. Classically this role is exemplified by MDR1 P-glycoprotein, MRP1 (the multidrug resistance protein 1, ABCC1), or BCRP1 (MXR, ABCP, ABCG2), which can cause multidrug resistance (MDR) in cancer cells. It has become clear that these transporters can play an important role in preventing the uptake of toxic compounds, including many drugs and food components, from the gut into the body, and in protecting vital structures in the body, such as the brain, the cerebrospinal fluid, the testis, and the fetus, against toxins that enter the body. Many drugs are detoxified by conjugation with glutathione, glucuronate, or sulfate, which results in acidic charged conjugates that cannot diffuse through cell membranes. Members of the MRP family mediate the export of these conjugates. Two members of the family, MRP4 (ABCC4) and MRP5 (ABCC5), can transport cyclic nucleotides and nucleotide analogs; these transporters might contribute to resistance against base and nucleoside analogs used in the chemotherapy of cancer and viral disease.

2. Mammalian secretory epithelia use ABC transporters to excrete endogenous metabolites, sometimes against a steep concentration gradient. In the liver these compounds include bile salts (transported by BSEP, the bile salt export pump, ABCB11), phosphatidylcholine (MDR3 P-glycoprotein, ABCB4), bilirubin glucuronides (MRP2, ABCC2), and drugs (MDR1 P-glycoprotein, ABCB1). The physiological functions of these pumps are discussed.

3. An increasing number of human inborn diseases are found to be caused by defects in ABC transporter genes; we discuss these. Table 1 provides a summary.

4. Many mammalian ABC transporters are under tight transcriptional regulation by nuclear receptors. This is especially the case for the lipid transporters but also for MDR1 and MRP2. The mechanisms of regulation and their importance for physiology are discussed.

Space limitations preclude the discussion of other important functions of ABC transporters, such as peptide transport. The drug-transporting P-glycoproteins (ABCB1) are excellent transporters of hydrophobic peptides, such as gramicidin D or cyclosporin A (7, 8). A heterodimeric ABC transporter, the transporter associated with antigen processing (TAP) transports peptides for antigen presentation (9), and an ABC transporter related to TAP have been found to export peptides from mitochondria (10).

Important information on the function of ABC transporters has been deduced from genetic defects, either in humans or in knockout (KO) mice, and we make

TAP2	ABCB3	653 (7?)	mTap2 rTap2	Ubiquitous	As TAP1	As TAP1	As TAP1	As TAP1	Forms dimer with TAP1
PSF2									
RING11									
ABC18									
PGY3	ABCB4	1279 (12)	mMdr2 rMdr2	Liver hepato- cytes	Plasma mem- brane (apical)	Long-chain phosphatidyl- choline	Some amphi- pathic drugs	PFIC-3, cho- lestasis of pregnancy	Also defense function against xeno- toxins?
MDR3(2)									
PFIC-3									
ABCB6	ABCB6	842 (6?)	Rat Mouse	Ubiquitous	Mitochondria	Iron?	—	—	—
ABC14									
UMAT									
MTABC3									
EST45597									
ABC7	ABCB7	752 (6?)	mAbc7	Ubiquitous	Mitochondria	Iron?	—	Anemia with ataxia	—
ATMIP									
ASAT									
(EST140535)									
ABC10	ABCB10	738 (6?)	mAbc10? ?	?	Mitochondria	Intermediates in heme/biosyn- thesis?	—	—	—
M-ABC2									
EST20237									
ABC-me?									
BSEP	ABCB11	1321 (12?)	mBsep rBsep	Liver hepato- cytes	Plasma mem- brane (apical)	Bile salts	Paclitaxel	PFIC-2	Liver disease Drug resistance is low
sPGP									
PFIC-2									
PGY4									

TABLE 1 Continued

Name	Symbol	Size ^b (tms)	Rodent gene	Main locations			Physiological substrates		Other substrates		Disease ^c		Miscellaneous
				Tissue	Subcellular		substrates	substrates	Human	Rodent			
MRP1 MRP	ABCC1	1531 (17)	mMrp1 rMrp1	Ubiquitous	Plasma membrane (basolateral) and endosomes	LTC ₄	Anionic drug conjugates, GSSG, GSH	—	—	Drug hypersensitivity	Also cotransports drugs with GSH		
MRP2 cMOAT	ABCC2	1545 (17)	rMrp2	Liver, intestine, kidney	Plasma membrane (apical)	Bilirubin-glucuronides, GSSG and GSH; acidic bile salts	Anionic drug conjugates	Dubin-Johnson syndrome	Altered drug handling ^d	Also cotransports drugs with GSH			
MRP3	ABCC3	1527 (17?)	rMrp3 mMrp3	Liver, bile ducts, gut, adrenal cortex	Plasma membrane (basolateral)	Bile salts	Anionic drug conjugates ^e	—	?	Strongly upregulated in cholestasis			
MRP4	ABCC4	1325 (12?)	rat mMrp4	Many tissues	Plasma membrane (apical?)	Cyclic nucleotides	Nucleotide analogs, organic anions	—	—	—			
MRP5	ABCC5	1437 (12?)	rMrp5 mMrp5	Ubiquitous	Plasma membrane (basolateral)	Cyclic nucleotides	Nucleotide analogs, organic anions	—	—	—			
MRP6	ABCC6	1503 (17?)	rMrp6 mMrp6	Kidney, liver	Plasma membrane (basolateral)	?	BQ-123 (an acidic peptide)	Pseudo-xanthoma	—	—			
ALD ALDP	ABCD1	745 (6?)	mAld	Many	Peroxisomal membrane	Very long chain saturated fatty acyl-CoA	—	Adrenoleukodystrophy	As humans	Probably heterodimer with ABCD2, ABCD3, ABCD4			

ALDL1 ALDR	ABCD2	740 (6?)	rAbcd2	Many	Peroxisomal membrane	Very long chain saturated fatty acyl-CoA?	—	—	ABCD1, ABCD3, ABCD4?
PMP70 PXMP1	ABCD3	659 (6?)	mPmp70 rPmp70	Many	Peroxisomal membrane	Very long chain saturated fatty acyl-CoA?	—	—	ABCD1, ABCD2, ABCD4?
PMP69 P70R PXMP1L	ABCD4	606 (6?)	mP69r	Many	Peroxisomal membrane	Very long chain saturated fatty acyl-CoA?	—	—	ABCD1, ABCD2, ABCD3?
BCRP1 MXR1 ABCP	ABCG2	655 (6)	mBcrp1	Placenta, breast, liver, endothelium	Plasma membrane (apical)	None known	Amphipathic drugs	—	Drug hypersensitivity Major defense against xenotoxins
ABCG5	ABCG5	651 (6?)	—	Liver, intestine	Plasma membrane? (apical?)	Plant sterols	Cholesterol?	Sitosterolemia?	Probably heterodimer with ABCG8
ABCG8	ABCG8	673 (6?)	—	Liver, intestine	Plasma membrane? (apical?)	Plant sterols	Cholesterol?	Sitosterolemia	Probably heterodimer with ABCG5

^aSee also <http://www.nutri.gene.4t.com/humanabc.htm>, which gives the complete list of 51 putative ABC transporters in human cells. Abbreviations: CoA, coenzyme A; GSH, glutathione; GSSG, oxidized GSM; LTC₄, leukotriene.

^bSize in number of amino acids first, and topology as the most probable number of transmembrane segments (tms) in parentheses.

^c—: No homozygous null alleles have been observed (humans, rats) or constructed (knockout mice). ?; No phenotype has yet been found.

^dDecreased biliary drug clearance and increased oral drug availability; decreased biliary excretion of bilirubin glucuronides.

^ePreference for glucuronosyl derivatives of drugs and steroids; does not transport GSH.

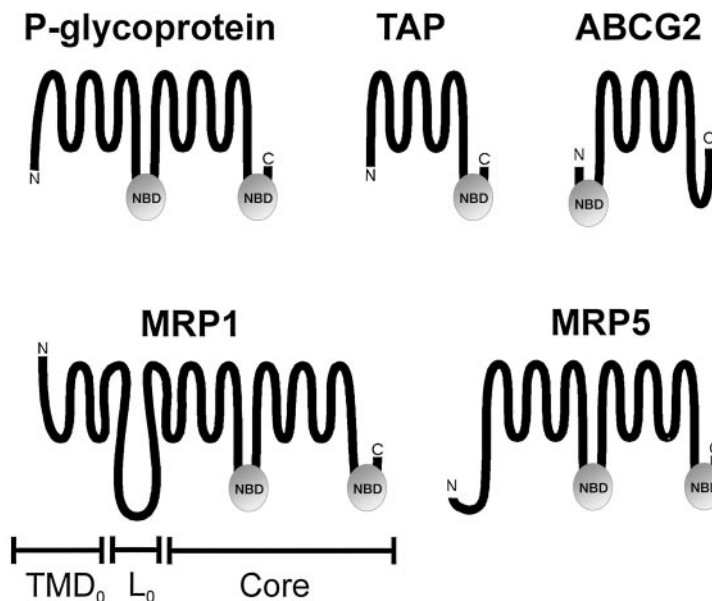


Figure 1 Predicted topology of the major classes of mammalian ABC transporters. This simplified scheme shows the intracellular nucleotide-binding domains (NBDs) and the transmembrane segments and indicates the N and C termini of the protein. Note that the predicted topology is often based on minimal data, as in the case of ABCG2 (BCRP1/MXR/ABCP) and MRP5 (ABCC5). TAP, the transporter associated with antigen presentation, probably has more than six transmembrane segments (9, 41). The half-size transporter TAP functions as a heterodimer of TAP1 and TAP2, and ABCG2 functions as a homodimer. See text for further details.

liberal use of this information in this review. When the defect results in disease the picture is usually clear, although one should keep in mind that genetic defects can result in abnormal protein products with nonphysiological effects, or can have long-range *cis* effects on neighboring genes. Absence of phenotype is more difficult to interpret. Gene function may not be required under the experimental conditions tested—usually a rather dull existence in a mouse cage—or may be masked by transporters with overlapping substrate specificity that might even be upregulated.

ABC transporters are favorite targets of reviewers. A treatise on all known ABC transporters is in the making (11); mammalian ABC transporters have been reviewed as a group (2); and there are numerous recent reviews on subfamilies of transporters (12–23) or individual transporters (24–28, 28a). To avoid unproductive repetition, we focus here on some generalizations that could be useful to people outside this field of research, and on recent developments and controversies.

STRUCTURE OF ABC TRANSPORTERS

ABC transporters are large membrane proteins and it has been technically difficult to get crystals suitable for X-ray crystallographic analysis. Recently this problem was solved by Chang & Roth (29) for MsbA, a bacterial homolog of a multidrug resistance transporter from *Escherichia coli*. MsbA is a lipid transporter but is closely related to LmrA from *Lactococcus lactis*, which functions as a multidrug transporter. The substrate specificities of LmrA and Pgp (ABCB1) are similar (30), and if LmrA is introduced into animal cells it confers multidrug resistance, like Pgp (31). The high levels of sequence, topological, and functional similarity between LmrA and Pgp make it likely that there is also structural similarity between the bacterial transporters and Pgp (32), even though MsbA and LmrA are both half-size ABC transporters functioning as homodimers.

Chang & Roth (29) succeeded in crystallizing the MsbA homodimer in the detergent dodecyl- α -D-maltoside in the presence of OsCl₃. Their structure has a conelike transmembrane domain with the top of the cone pointing out of the cell. This domain contains 12 transmembrane α -helices, confirming the commonly accepted structure for Pgp (33) and refuting a recently proposed alternative (34, 35). The structure looks very different from the structure of Pgp deduced from electron micrographs by Rosenberg et al. (36), however, which looks like a 10-nm flower floating in the membrane and facing out. The Rosenberg structure has a large central pore that is closed at the cytoplasmic face of the membrane and forms an aqueous chamber within the membrane. In contrast, the Chang-Roth structure is open at the cytoplasmic side of the membrane and not at the exoplasmic side. The structure also has a central chamber, easily accessible by substrate from the inner leaflet of the membrane, while excluding substrate from the outer leaflet. This is in full agreement with biochemical studies on Pgp and LmrA (see below).

The nucleotide-binding domains (NBDs) of MsbA are remarkably far from the membrane and connected to the membrane domain by a third domain, the intracellular domain. The NBD structure is not fully visible in the crystal structure, but in the modeled structure the two NBDs are rather far apart. This is difficult to reconcile with the kinetic and spectroscopic studies that indicate that the ATPase domains of ABC transporters are closely interacting and adjacent in space (see below). Interacting ATP-binding sites are clearly found in the structures of two distant relatives of ABC transporters, the Rad50 ATPase involved in double-strand break repair (37) and the ArsAB arsenite/antimonite pump of *E. coli* (38). In the 3-D structure of the ATPase part (ArsA) of the pump the two ATP-binding sites are very close together. In fact, each ATP-binding site is made up of residues from both domains of ArsA.

How the transmembrane segments of ABC transporters form the postulated transmembrane pore is controversial. Looking at P-glycoprotein one would expect a head-tail, head-tail structure with transmembrane segment (TMS) 1 close to 12, and 6 close to 7. This simple arrangement may not be correct,

however. Loo & Clarke (39) constructed an active cysteineless version of the human MDR1 Pgp and made a systematic study of the structure and function of this transporter by introducing cysteines at defined positions in the molecule [reviewed in (33)]. In cross-linking studies they found interaction of TMS 6 with TMS 10, 11, and 12, and interaction of 12 with 4, 5, and 6 (40). On this basis, they propose a structure in which the two halves of Pgp are displaced to allow 4, 5, 6 to lie opposite 10, 11, 12. An even more radical displacement has been deduced from a different set of experiments for TAP by Vos et al. (41), who think that the two subunits of TAP interact in a head-head, tail-tail fashion [see also (42)]. Such a configuration in Pgp would also be compatible with the results of Loo & Clarke (40).

In contrast, the MbsA structure (29) shows a head-tail, head-tail arrangement for the two monomers but an unusual one, as in each monomer TMS 1 is juxtaposed to 6. It should be stressed, however, that the crystal form is only one conformation of a structure that must undergo considerable rearrangements during the catalytic cycle. Reits et al. (43) have shown that the lateral mobility of TAP in the membrane increases substantially when the protein becomes inactive and decreases again when it pumps peptides. This implies major structural rearrangements. Higgins & Linton (32) mention unpublished results indicating a substantial lateral repacking of the transmembrane helices during the catalytic cycle. Obviously, the landmark structure of Chang & Roth (29) was obtained under rather unusual conditions, without membrane lipids, adenine nucleotides, or substrate. Undoubtedly, an avalanche of additional structural information will follow, which will provide a test for some of the models for transporter function now available (29, 32, 38).

HOW DO ABC TRANSPORTERS WORK?

ABC transporters are versatile. They are used in nature to transport a large range of compounds out of cells. Kinetic experiments and directed mutagenesis have generated a large volume of data that delineate key features of the modus operandi of ABC transporters. A review of these data is beyond the scope of this chapter. Here we summarize some general principles. The most extensive work has been done on Pgp, but the recent experiments on LmrA (see above) are also of interest. As LmrA is a half-size ABC transporter functioning as a homodimer, this implies that the two halves of Pgp must also have an equivalent function.

Inactivation of one of the two nucleotide-binding domains (NBDs) of Pgp, by amino acid substitution or by nucleotide trapping in the presence of vanadate, blocks drug transport and even ATP hydrolysis by the unaffected NBD. This shows that the two interact strongly and cannot hydrolyze Mg-ATP independently. A model in which the two NBDs alternate in ATP hydrolysis during the catalytic cycle of Pgp was proposed by Senior et al. (44). This can be generalized to CFTR (45), to the TAP1/2 heterodimer (46), and to the bacterial LmrA

homodimer (47), and has been refined by recent work in several labs (33, 48–54). Nothing is simple in the world of ABC transporters, however, as rather different results were obtained with another drug transporter, MRP1 (55, 56). The two NBDs in MRP1 are clearly nonequivalent. The most striking difference with Pgp is that inactivation of NBD1 does not completely eliminate drug transport, whereas inactivation of NBD2 does (55). This is obviously incompatible with a model in which drug transport is dependent on alternating ATP hydrolysis by NBD1 and 2, as favored for Pgp.

There is still doubt about the position and number of substrate-binding sites in ABC transporters, such as Pgp. Older studies had shown that mutations scattered through the entire protein may affect substrate specificity. Substrates cross-linked onto Pgp mainly label TMS 5, 6, 11, and 12, which suggests that these segments are part of the drug-binding site(s) [reviewed in (57, 58)]. Using a verapamil analog that can be coupled to single cysteines introduced into a cysteineless version of Pgp, recent studies show targeting of TMS 4, 6, 10, 11, and 12 (59). The NBDs are not required for drug binding (33, 60).

Van Veen et al. (47) find two vinblastine-binding sites on the LmrA homodimer, a high-affinity site on the cytoplasmic side and a low-affinity site on the extracellular side. They present an alternating two-site (two-cylinder engine) mechanism in which each subunit alternates between the high-affinity and the low-affinity conformation. This elegant model is a simplification of the situation in Pgp, where there is compelling evidence for at least two substrate-binding sites, which differ in substrate specificity. These can interact in a positively cooperative manner (61–63). There is even evidence for a third drug-binding site (64) and for drug-binding sites with allosteric effects on drug transport (31, 65).

Hydrophobic substrates are probably taken from the inner leaflet of the plasma membrane by Pgp (66, 67) and LmrA (68). Results with LmrA indicate, however, that the transporter does not flip the substrate from the inner to the outer leaflet of the plasma membrane, as envisaged by Higgins & Gottesman (69), but transfers substrate directly into the extracellular medium (68, 70).

THE DRUG-TRANSPORTING P-GLYCOPROTEINS

Drug-transporting ABC transporters, like P-glycoprotein (Pgp), MRP1, and BCRP, were discovered as proteins associated with acquired multidrug resistance in cancer cells. Resistance is a result of the ability of these transporters to extrude several classes of anticancer drugs from the cell, lowering the effective drug concentration inside the cell (25). Pgp is able to cause the highest resistance to bulky amphipathic drugs, such as paclitaxel (taxol), anthracyclines, and *Vinca* alkaloids. The hydrophobic parts of these drugs allow their rapid insertion in the membrane. The hydrophilic residues prevent rapid flipping of the drug to the inner leaflet of the membrane, slowing down entry into the cell; in fact, for an anthracycline like doxorubicin (Adriamycin) this takes about a minute, giving the

Pgp pump ample opportunity to deal with the influx (71, 72). The rate of spontaneous flip-flop is relevant, because estimates of the turnover number of Pgp substrates range from 1 to 10 s^{-1} , which is low (73, 74).

The substrate specificity of drug-transporting Pgps is wide, but still not completely defined for technical reasons. In most studies, cells are transfected with a Pgp gene construct and the drug resistance of the transfected cell relative to its parent is determined. If several independent transfected cell lines are compared, this assay system is direct and reliable, but relatively insensitive. Substrates that enter cells faster than Pgp can pump them out are missed. A case in point is methotrexate (MTX), a hydrophilic negatively charged drug, long thought to be the prototype of a drug not touched by Pgp. However, cells in which MTX enters at a very low rate because its main import route is lacking could readily be made MTX resistant by introducing Pgp (75). The sensitivity of the transfected cell system is also compromised by the presence of endogenous levels of Pgp and other drug transporters in nontransfected cells. This is illustrated by the fact that mouse fibroblasts from KO mice lacking drug-transporting Pgps and MRP1 were 10- to 20-fold more sensitive to paclitaxel, *Vinca* alkaloids, and epipodophyllotoxins than their wild-type counterparts (76).

A more sensitive assay system for drugs transported by Pgp is provided by stable kidney cell lines that can be grown as monolayers on a filter, separating a basal and an apical compartment (77). Transporters introduced in these cells by transfection properly route to the apical or the basolateral membrane of the cell, which allows quantitative measurements of rates of drug transport (78–84). This system was used, for instance, to demonstrate that valspodar (PSC833), an efficient inhibitor of Pgp, is also transported by Pgp at a low rate, confirming that good inhibitors of Pgp are usually also substrates (85). The best inhibitors may have such a high affinity for Pgp that their off rate is too low to detect transport. The kidney monolayer system is also marred by endogenous transporters (which may show some clonal variation in expression, further complicating transport analysis) and by the fact that for some substrates, such as vincristine, entry into the cell may be rate-limiting.

Drug transport has also been studied in membrane vesicles isolated from disrupted cells. Depending on the cell type and conditions of the isolation procedure, different ratios of right-side-out and inside-out vesicles can be obtained. Inside-out vesicles can be used to study ATP-dependent transport directly, which allows the determination of kinetic parameters. With Pgp this system has not been so useful, as most substrates are rather hydrophobic and diffuse back out. In addition, these hydrophobic substrates display extensive nonspecific binding to the membranes, thereby dramatically decreasing the signal-to-noise ratio. In contrast, vesicular transport has been an important tool for studying substrate specificity of the MRPs, ABC transporters that transport anionic hydrophilic substrates. In addition to these direct transport assays, Pgp and MRPs have also been studied extensively by various indirect methods, such as measuring substrate binding, nucleotide trapping, and ATPase activity. The

advantages and pitfalls of these indirect methods have been reviewed (63) and are not discussed here.

The picture emerging from of all these studies is that Pgp is an omnivore, transporting a vast range of drugs. The preference is for large amphipathic molecules that are neutral or weakly basic, but if pushed, Pgp can also inefficiently handle an anionic highly charged compound, such as MTX. It is therefore hardly surprising that it has proven difficult to define the elements common to all Pgp substrates. Hydrophobicity, planar aromatic rings, and the presence of tertiary amino groups favor interaction with Pgp, but no highly conserved elements of recognition have been found [reviewed in (25, 86–88)]. How a single protein can accommodate so many different structures has long been a mystery. Some of the principles involved are illustrated by the structural analysis of the bacterial transcription regulator BmrR, a soluble protein that can tightly bind very different cationic drugs. Key elements in binding are a central flexible cavity within the protein; the cavity contains a negatively charged residue in a hydrophobic environment. The flexibility of the cavity allows multiple Van der Waals contacts and stacking interactions that do not require precise positioning of the ligand, as is the case for hydrogen bonding (89, 90). If the ligand enters from the inner leaflet of the membrane, steric requirements can be imposed by the protein to prevent entry of natural membrane lipids. Although this model is not directly applicable to Pgp, it has helped to explain some of the principles of promiscuous substrate binding.

P-Glycoprotein in the Defense Against Drugs/Xenotoxins

There is little doubt that Pgp is a key player in the defense of the body against amphipathic xenotoxins. In the gut mucosa it prevents entry of toxins into the body; in the blood-brain barrier, placental trophoblasts, testis, and bone marrow it provides protection of vital body parts; and in the gut, liver, and kidney Pgp helps to eliminate toxins from the body. The wide range of toxic compounds transported by Pgp makes it a guardian for all seasons. The downside is that Pgp can also interfere with the delivery of drugs to target tissues, but the development of potent Pgp inhibitors with low toxicity has opened up new ways to overcome this undesirable interference of Pgp with medical treatment.

Although there are many examples in the older literature of Pgp defense functions, the detailed analysis of these functions was greatly simplified by the generation of Pgp KO mice (91–93). Mice have two Abcb1-type drug transporters, known as the products of genes *Mdr1a* (*Mdr3*) and *Mdr1b* (*Mdr1*), that appear to do the job of a single Pgp, the MDR1 Pgp (ABCB1) in humans. The *Mdr1a* Pgp is the only Pgp present in the gut and in the blood-brain barrier, and the *Mdr1a* ($-/-$) mice have been used extensively to study the effect of Pgp on oral availability and brain penetration of drugs. The *Mdr1b* ($-/-$) mice have hardly been used for pharmacological studies, since the *Mdr1a/1b* ($-/-$) mice and the triple KO mice (94, 95), additionally defective in Mrp1, were available. The KO mice have no abnormalities as long as they are not exposed to drugs.

This may depend somewhat on the environment, however, as an inflammatory bowel disease has been reported for the *Mdr1a* ($-/-$) strain outside Amsterdam (96). Environmental effects on the cytochrome P450 system in the KO mice have also been described (97). There is no evidence for the compensatory upregulation of other transporter genes in the KO mice, but only a few genes have been checked. The ordered arrays of all mouse genes that will soon be available should settle the issue.

Recent experiments on the defense function of Pgps have concentrated on three drug barriers: the gut mucosa, the blood-brain barrier, and the maternal-fetal barrier.

BLOOD-BRAIN BARRIER This barrier consists of the endothelial cells of the brain capillaries, which are linked together by tight junctions and lack the holes found in capillaries elsewhere in the body. Hence, drugs getting into the brain have to pass the endothelial cells and get by the Pgp present at high level in the apical membrane of these cells (98). Many observations in the older literature suggest a contribution of Pgp to the blood-brain barrier [reviewed in (99–102)], but the importance of Pgp did not become clear until the *Mdr1a* ($-/-$) mice were analyzed. These mice were 100-fold more sensitive to the neurotoxic pesticide ivermectin, a good Pgp substrate (78), and accumulated 100-fold more ivermectin in their brains than wild-type mice (92). Subsequent work has demonstrated that the brain accumulation of a large range of drugs is strongly decreased by P-glycoprotein. These include anticancer drugs, such as doxorubicin, paclitaxel, and vincristine; cardiac drugs, such as digoxin and quinidine; antiarrhythmic drugs such as loperamide; antiemetics, such as ondansetron and domperidone; and the immunosuppressant cyclosporin A (98, 101, 103, 104). In fact, any good Pgp substrate is severely affected, unless it diffuses rather rapidly through membranes, like dexamethasone, which is strongly affected when it is at low concentrations (105), but much less affected at high concentrations (78). The interest in Pgp in the blood-brain barrier was further stimulated by the discovery that the HIV protease inhibitors indinavir, nelfinavir, and saquinavir are Pgp substrates and that Pgp limits the penetration of these drugs into the brain (106, 107). Pgp might also affect the treatment of primary brain tumors with an intact brain-type vasculature (101, 108, 109). A problem is that the available Pgp inhibitors seem unable to fully inhibit the high concentrations of Pgp in the blood-brain barrier (14, 85, 110–113). We expect this problem to be solved by the development of more effective Pgp inhibitors.

The blood capillaries of the inner ear are similar in structure and barrier function to the capillaries in the brain and also contain Pgp. Zhang et al. (114) have shown that the absence of Pgp in the *Mdr1a* ($-/-$) mice results in a dysfunction of the blood-inner ear barrier, and they conclude that the Pgp in this barrier prevents the ototoxicity that could be caused by drugs that are Pgp substrates.

P-GLYCOPROTEIN AND THE MATERNAL-FETAL BARRIER In 1998, Lankas et al. (115) discovered that the naturally occurring *Mdr1a* ($-/-$) mutants of the CF-1 mouse stock display an enhanced sensitivity to the teratogenic effects of the pesticide avermectin. They found that the *Mdr1a* Pgp (Abcb1, Mdr3) is present in the fetus-derived epithelial cells that make up the exchange border between the fetal and maternal circulation. Pgp in the apical border of these cells faces the maternal circulation and is optimally placed to protect the fetus against incoming amphipathic toxins.

This work was extended by Smit et al. (116) in the *Mdr1a/1b* ($-/-$) mice. They found that digoxin, saquinavir, or paclitaxel given intravenously to pregnant mother mice penetrated the ($-/-$) fetuses more effectively than ($+/+$) fetuses. A similar increase in drug penetration was obtained by inhibiting placental Pgp with the Pgp inhibitors PSC833 or GG918. These striking results show that "Pgp makes a major contribution to yet another biological important protective barrier" (116). It is now clear that in mice the *Mdr1a* isoform is the major one in placenta (93, 115). The high *Mdr1b* RNA levels found in placenta by Croop et al. (117) must have been due to a misinterpretation.

P-GLYCOPROTEIN AND ORAL AVAILABILITY OF DRUGS Pgp was known to be present in the apical membranes of the mucosal cells of the gut from the early studies using immunohistochemistry, but until 1996 the idea that Pgp might actually do something in that location and interfere with the uptake of amphipathic drugs from the gut was mainly based on studies with Pgp inhibitors (118–120). Mayer et al. (121) then found that the predominantly fecal excretion of digoxin in wild-type mice shifted toward urinary excretion in the *Mdr1a* ($-/-$) mice. They also found that intravenously injected digoxin is excreted directly into the gut in wild-type mice (16% of administered dose in 90 minutes), but hardly at all (2%) in the KO mice.

This result showed that Pgp can affect the oral availability of Pgp substrate drugs, and it suggested that paclitaxel (taxol), an excellent Pgp substrate that has to be given intravenously to patients because of its very poor oral availability, might be prevented from entering the body from the gut by Pgp. This was verified by Sparreboom et al. (122). They showed that the oral availability of paclitaxel went from 11% in wild-type mice to 35% in *Mdr1a* ($-/-$) mice. Even more striking was the reduction in fecal excretion of an oral dose of paclitaxel from 87% in wild-type mice to <3% in the KO mice, which probably represents the combined intestinal and biliary excretion. Inhibition of Pgp in wild-type mice with PSC833 resulted in a dramatic ten-fold increase in the availability of paclitaxel (123), presumably because the PSC833 affected not only the uptake of paclitaxel from the gut but also metabolism of it, as paclitaxel and cyclosporins are both substrates for the P450 3A4 isoenzymes. The translation of these results from mice to new treatment modalities for human patients is in full swing (124). The principle has been proven to work in human patients, both for paclitaxel

(125, 126) and docetaxel (127). Most other anticancer drugs are less affected by intestinal Pgp (128).

There are other candidate drugs and other transporters for which this principle should work, as MRP2 and BCRP are also present in the apical membrane of the enterocyte. It should also be realized that the oral availability of a range of drugs could be affected by variations in Pgp activity, either because of Pgp polymorphisms (see below) or because of variations in dietary components that can inhibit Pgp, such as unidentified components of grapefruit juice. Furthermore, the expression of *MDRI* is modulated by many drugs and toxins via the nuclear receptor *SXR* and possibly others. An important example is paclitaxel, which induces expression of both *CYP3A* and *MDRI*, thereby inducing its own metabolism and excretion (see below).

The Role of P-Glycoprotein in Clinical Anticancer Drug Resistance

It is now 25 years since P-glycoprotein was discovered. Reliable methods are available to detect the presence of P-glycoprotein in tumor samples by immunohistochemistry or functional tests (129). Potent, relatively nontoxic inhibitors of P-glycoprotein have been developed; these can virtually eliminate the effects of P-glycoprotein on drug accumulation in cells in model systems. Still there is no agreement on the usefulness of P-glycoprotein blockers in the treatment of human tumors containing Pgp. Four factors contribute to this unsatisfactory state of affairs:

1. It has been difficult to determine what amounts of Pgp are significant and in which cells they are localized. This is necessary in order to focus trials with reversal agents on tumors in which Pgp makes a real contribution to resistance. The problem is illustrated by a recent reinvestigation of the variable results published for Pgp in breast cancer. Faneyte et al. (130) showed by immunohistochemistry that the Pgp in these tumors is present only in the variable number of infiltrating macrophages and T cells and not in the tumor cells.

2. The early clinical trials with MDR reversal agents were done with drugs, such as verapamil, that inhibit Pgp rather poorly at clinically achievable dose. With hindsight, this makes the negative early trials with reversal agents uninterpretable. Even PSC833 (valsopodar), the reversal agent used most in recent clinical trials, may not be able to completely block Pgp in all nooks and corners of the body (85), as pointed out before.

3. Reversal agents also affect systemic drug disposition, either by decreasing drug elimination via Pgp, or by interfering with drug metabolism via the P450 system. Hence, if effective Pgp blockers are combined with drugs like doxorubicin or paclitaxel, the patient is exposed to more anticancer drug than without the blocker, making the interpretation of the clinical results difficult. To eliminate this confounder, it is necessary to adjust the dose of the anticancer drug downward, and this has been done in only some of the most recent clinical trials (131–133).

4. Tumors use whatever drug resistance mechanisms are available, and it is now abundantly clear that Pgp is not the only defense available, nor even the only drug transporter in tumors. It is not easy to determine to what extent resistance of a given tumor is due to Pgp, or due to other resistance mechanisms. With increasing insight into drug resistance mechanisms and the rapidly increasing ability to analyze genetic alterations in tumors in detail, this problem should eventually be solved.

The potential contribution of Pgp to resistance in human cancer patients cannot yet be assessed (134–138). A priori, it is unlikely that some tumors would not use such a wonderful mechanism to defend themselves against drugs, and there is ample anecdotal evidence for a role of P-glycoprotein in drug resistance (134, 139, 140). Compelling evidence for a contribution of Pgp to survival of myeloma tumor cells comes from the work of Mickley et al. (141). They analyzed the nature of the overexpressed *MDR1* allele(s) in myeloma cells from patients in which the two alleles present could be distinguished by a polymorphism. In each case where *MDR1* was overexpressed, only a single allele was found to be overexpressed. This shows that MDR1 Pgp does play a role in clinical resistance in myeloma. No other reasonable mechanism would consistently select for overexpression of a single *MDR1* allele. A similar analysis in patients with acute myeloid leukemia was negative (142). This suggests that differential expression of the two Pgp alleles might be a good criterion for patient selection in future trials with Pgp blockers.

Physiological Substrates of P-Glycoprotein

Mice lacking the major drug-transporting Pgps, Mdr1a and b, are normal and fertile in a protected environment (93). This shows that these transporters have no essential function in physiology other than defending the body against xenotoxins. Pgps are good at transporting lipid analogs (81, 143, 144), however, and the ABCB4-type Pgps are dedicated phosphatidylcholine (PC) translocators (91). This has raised the question of whether the drug-transporting Pgps might not do small lipid jobs on the side in normal lipid trafficking [an issue reviewed in more detail in (15, 145)].

The fact that Mdr1 Pgp (ABCB1) does not functionally replace Mdr2 (ABCB4) in the Mdr2 knockout model predicts that natural PC is not a substrate for MDR1 Pgp (91), but a direct test must still be done to prove this. There is indirect evidence for transport by MDR Pgp of two other long-chain membrane lipids, sphingomyelin (146) and glucosylceramide (147), and a short-chain lipid, platelet-activating factor (148, 149).

Dexamethasone, an artificial steroid, is effectively transported by Pgp, which has raised the question of whether Pgp might also transport endogenous steroids. MDR1 Pgp is present at high concentration in the human adrenal cortex, which has prompted speculations about an essential role of drug-transporting Pgps in cortical hormone transport (150, 151). It should be noted, however, that rat and hamster adrenals do not have this high adrenal level of Pgp (152–155) and that

the *Mdr1a/1b* ($-/-$) mouse has no signs of adrenal or genital insufficiency (93). The Pgp in the human adrenals may therefore either be accidental or reflect the exposure of primates to toxins especially deleterious to the adrenal cortex. Whether cholesterol, the major sterol in mammalian membrane, is translocated by Pgp (156, 157) is doubtful in our opinion and requires confirmation by direct transport experiments, as pointed out by Johnstone et al. (151). This also holds for the postulated role of Pgp in cholesterol esterification (158, 159).

Five other physiological functions have been attributed to the drug-transporting Pgps (151): transport of cytokines, particularly interleukin 2 (IL-2), IL-4, and gamma interferon (IFN- γ) (160, 161); increased self-renewal and decreased differentiation of hematopoietic stem cells (162); migration of antigen-presenting dendritic cells and T lymphocytes (163); inhibition of alloantigen-dependent, but not mitogen-dependent, T-cell activation (164); and inhibition of apoptosis [reviewed in (151)], but not cytolysis by pore-forming proteins (165, 166). The evidence for these physiological functions rests mainly on the effects of Pgp inhibitors, gross overexpression of a Pgp gene, or the effects of anti-Pgp antibodies, and remains the subject of lively discussions (167).

Mutations in Human P-Glycoprotein and Their Effects on Drug Disposition

As the important role of Pgp in protecting our body against drugs and xenotoxins was being delineated in recent years, two questions arose: Is this role significantly affected by natural polymorphisms, and what would be the fate of humans homozygous for a null allele of the *MDR1* Pgp gene? After Mickley et al. (141) identified the first two natural polymorphisms in *MDR1* (*ABCB1*), more extensive surveys turned up 16 polymorphisms in Germany (168, 169) and 9 in Japan (170, 171). The most interesting mutation is a silent exonic C3435T mutation that correlates with the intestinal level of Pgp (168). On average, the TT homozygotes have a more than twofold lower level of intestinal Pgp. This difference remains after rifampin induction and is associated with a significantly increased uptake of digoxin from the gut (168).

No null allele of the *MDR1* Pgp gene has been found thus far, but only about 500 people have been screened. It is surprising, however, given the liberal use of amphipathic drugs in human society and the high levels of amphipathic toxins in the human environment, that no known inborn hypersensitivity to drugs in humans is attributable to Pgp absence. Null alleles have turned up in mice (172, 173) and in collie dogs. The mice have a retroviral insertion in their *Mdr1a* gene. The dogs have a 4-base-pair deletion in the relevant Pgp gene, resulting in a frameshift and a stop around amino acid 90 (A. Roulet, personal communication). Both the collie dogs and the mice are hypersensitive to ivermectin, a neurotoxin kept out of the brain by Pgp (92, 174). In contrast, more than seven million people in Africa have been treated with ivermectin for river blindness (onchocerciasis) without reported untoward effects [see (92, 174)]. Perhaps natural selection tends to weed out *MDR1* null alleles in humans. A possible

mechanism is suggested by the remarkable results of Lankas et al. (115) in mice. They treated *Mdr1a* (+/-) heterozygous mice with an ivermectin analog and found that all the (-/-) progeny had a cleft palate, but none of the (+/+) mice did. The most remarkable result, however, was that about 30% of the (+/-) heterozygous fetuses had a cleft palate as well. This shows that a full dose of Pgp is important to protect the fetus against teratogenic effects of amphipathic toxins. Humans with their single Pgp gene may be even more vulnerable than mice with their two genes, *Mdr1a* and *1b*. Another explanation, equally speculative, is that Pgp is essential in the early development of humans but not mice. More work is required, however, to determine whether null alleles of *MDR1* really are rare or nonexistent in humans.

BCRP (ABCG2), A NEW TRANSPORTER IMPORTANT IN MAMMALIAN DEFENSE

The intensive study of cell lines selected for MDR in the past three decades led to the discovery of Pgp and MRP1. Although the increased level of either transporter could account for the drug resistance of most of these cell lines, the resistance of a few cell lines remained unexplained. These lines were characterized by high mitoxantrone resistance and lower resistance to anthracyclines and camptothecins. Resistance was a result of decreased drug accumulation, which suggested the presence of a new drug transporter. This transporter was finally identified by Doyle et al. (175) as the breast cancer resistance protein (BCRP), a half-size ABC transporter overproduced in MCF7 breast cancer cells, and by Allikmets et al. (176) as ABCP, a transporter present at high concentration in placenta. A third name, MXR for mitoxantrone resistance protein, was adopted by Miyake et al. (177). BCRP/ABCP/MXR belongs to the ABCG subfamily and has been renamed ABCG2. Presumably, ABCG2 functions as a homodimer (178); there are no indications for other intracellular partners. The range of drugs to which ABCG2 can confer resistance is less broad than found for Pgp. In addition to mitoxantrone, topotecan derivatives, and anthracyclines, these drugs include bisantrene, etoposide, prazosin, and flavopiridol (179–183). Other typical Pgp substrates, such as *Vinca* alkaloids and taxanes, are not included in the ABCG2 resistance spectrum. Like Pgp, ABCG2 does not require glutathione (GSH) for the transport of electroneutral amphipathic drugs (183a).

It now appears that Pgp, MRP1, and ABCG2 can explain MDR in all cell lines analyzed thus far. This is underlined by a study by Allen et al. (180), who used fibroblast lines from triple KO mice, which lack both murine drug-transporting Pgps and Mrp1, to select new lines resistant to topotecan, mitoxantrone, or doxorubicin. Each of the MDR lines isolated overexpressed *Abcg2*. Interestingly, two types of phenotypes were observed. The doxorubicin-selected lines had much higher relative resistance to doxorubicin, etoposide, and bisantrene than did the lines selected with mitoxantrone or topotecan. In parallel, Robey et al.

(181) noted that some cells overexpressing ABCG2 transport rhodamine 123, whereas others do not. The two phenotypes were recently explained by the discovery of polymorphisms in ABCG2 (179). The wild-type version of the protein has an Arg at position 482, which is associated with the inability to transport rhodamine 123, low resistance to anthracyclines, and high resistance to mitoxantrone and topotecan. The broad ABCG2 phenotype, including rhodamine transport and doxorubicin resistance, was associated with replacement of Arg482 in ABCG2 by Gly or Thr.

ABCG2 is present in the plasma membrane of cultured cells (184, 185), and in polarized cells the murine *Abcg2* routes to the apical membrane (186). ABCG2 RNA is detectable in many tissues, with the highest levels in the placenta (175, 176). The recent finding that ABCG2 is present in venous and capillary endothelial cells of almost all tissues (183) necessitated a more precise analysis of the tissue distribution of ABCG2 by immunohistochemistry. ABCG2 was found in the apical membranes of placental syncytiotrophoblasts, hepatocytes, the epithelial lining of the small intestine and colon, and the ducts and lobules of the mammary gland (183). Hematopoietic progenitor cells, but not mature blood cells, contain ABCG2 as well. The localization of ABCG2 suggests that this protein could have an important defense function, i.e., by limiting uptake of drug from the gut and preventing entry of drug into the fetus. These predictions have been verified in *Mdr1a/1b* ($-/-$) mice by using GF120918, a good inhibitor of Pgp and also highly active against ABCG2 (180, 187). Whereas the *Mdr1a/1b* ($-/-$) mice have lost the intestinal barrier function toward taxanes, as a result of the presence of Pgp, they still take up topotecan rather sluggishly. This barrier can be eliminated by the drug GF120918, which also promotes penetration of topotecan through the maternal-fetal barrier in the KO mice (186). GF120918 also decreases hepatobiliary excretion of topotecan in the *Mdr1a/1b* ($-/-$) mice.

From these results it is clear that ABCG2 represents a second transporter, besides Pgp, that can limit the oral availability of certain classes of amphipathic drug. As pointed out by Allen et al. (180), GF120918 provides a low-toxicity agent with dual reversal and sensitizing action, which could be useful for increasing oral availability of drugs (186) and their accumulation in tumors that overexpress Pgp and/or ABCG2. For specific inhibition of ABCG2, fumitremorgin C (188, 189) and its analogs (190) may be used.

No physiological function for ABCG2 is known, beyond its role in defense. An *Abcg2* ($-/-$) mouse is under investigation (A. H. Schinkel, personal communication). Zhou et al. (191) have recently found, however, that *Abcg2* is highly expressed in primitive hematopoietic stem cells of mice, especially in the so-called side population (SP) that does not stain well with Hoechst 33342 dye. Zhou et al. (191) show that *Abcg2* extrudes the Hoechst dye. The SP population is enriched for cells that can repopulate bone marrow, and SP cells may also be responsible for the bone-marrow-reconstituting capacity of stem cells isolated from muscle and neural tissue. Enforced overexpression of *Abcg2* in murine

bone-marrow cells expands the SP population and partially inhibits hematopoietic development. The authors speculate that *Abcg2* exports an endogenous metabolite capable of inducing differentiation in stem cells. Clearly it will be important to carefully check the SP population in *Abcg2* ($-/-$) mice to see whether there is any decrease in this population. This is an important control to exclude the possibility that the association between *Abcg2* expression and SP population is accidental and that the enforced overexpression of ABCG2 results in an abnormal block in differentiation.

THE MRPs

Toxic compounds that enter the body are often modified by oxidation (phase I metabolism) and/or made more water soluble by conjugation to glutathione (GSH), sulfate, or glucuronate (phase II metabolism). The resulting conjugates are too hydrophilic to diffuse out of the cell and require dedicated transporters to help their exit, as first pointed out by Ishikawa (192). As Pgps prefer non-anionic substrates, Ishikawa concluded that there must be other GS-X pumps (glutathione-X conjugate pumps) to do the job. In fact, such a pump was long known: cMOAT, the canalicular multispecific organic anion transporter, which transports bilirubin-glucuronides from the liver into the bile. The limited tissue distribution of this transporter was not compatible with a role as a universal GS-X pump, however.

The first GS-X pump identified with a wide tissue distribution was the multidrug resistance (associated) protein, MRP (now MRP1), discovered by Cole et al. (193) in cells selected for MDR. Initially, the substrate specificity of MRP1 looked similar to that of Pgp, but vesicular transport experiments established that MRP1 is in fact a GS-X pump. In 1996 the gene *cMOAT* (now *MRP2*) was cloned (194, 195). MRP3–5 soon followed (196) when Allikmets et al. (197) identified 21 potential human ABC transporters. Recent work has added four more members to this MRP family: MRP6 (see below), MRP7 (198), and MRP8 and 9 (199). This probably completes the family, as there are no other putative MRP genes among the 52 human ABC transporter genes. The MRPs, CFTR, and the SURs are thought to have evolved from a common ancestor (200), and these proteins are now grouped together in the C branch of the ABC transporter family (Table 1).

The MRPs studied thus far, MRP1–5, are all organic anion pumps, but they differ in substrate specificity, tissue distribution, and intracellular location, as should be clear from Table 1. MRPs come in two structural types (Figure 1), one with 17 transmembrane segments (MRP1, 2, 3, 6), and one with 12 (MRP4, 5, 7, 8). Experiments by Bakos et al. (201) have shown that TMD₀ (Figure 1) is not essential for catalytic function or intracellular routing; the function of this domain is unclear. Long MRPs share an L₀ segment (Figure 1) with a highly conserved sequence near its N terminus. This sequence is also present near the

N terminus of the short MRPs. It is essential for function and appears to associate with the membrane. Structural studies on MRPs have just begun (202).

Here we discuss the substrate specificity, the physiological functions, and the potential role in drug resistance of the six members of the MRP family that have been biochemically characterized, MRP1–6.

MRP1

MRP1 is a prototype GS-X pump and a remarkably versatile one. It transports a variety of drugs conjugated to GSH, to sulfate or to glucuronate, as well as anionic drugs and dyes, but also neutral/basic amphipathic drugs and even oxyanions. The oxyanions arsenite and antimonite and the neutral/basic drugs are cotransported with GSH. Notwithstanding this enormous range of substrates transported, MRP1 is not indiscriminate. Whereas estradiol-17 β -glucuronide is a good substrate, the 3-isomer is not (203). The older work on MRP1 has been reviewed (26). Here we deal with a number of recent developments and open questions.

SUBSTRATE SPECIFICITY Initial transport studies on intact cells and isolated membrane vesicles suggested that MRP1 can transport organic anions, such as glutathione conjugates, without the help of free GSH. Recent experiments paint a more complex picture, however, since the transport of at least three authentic organic anions, glucuronosyl-etoposide (204), estrone-3-sulfate (205), and the glucuronosyl derivative of a nicotine-derived carcinogen, NNAL-O-glucuronide (206), was strongly stimulated by GSH. The stimulation was relatively specific: No stimulation was seen with estradiol-3 β -glucuronide (a poor MRP1 substrate), or estradiol-17 β -glucuronide (a good substrate). No cotransport of GSH and the estrone-3-sulfate could be demonstrated—in contrast to the clear cotransport of GSH and vincristine (207–209) or daunorubicin (210)—but Leslie et al. (206) note that GSH uptake is hard to measure for technical reasons and that low rates of cotransport might have been missed. The stimulating GSH could be replaced by *S*-methyl GSH and even by ophthalmic acid, a GSH analog in which the central cysteine is replaced by a non-sulfur-containing unusual amino acid, α -aminobutyrate (206).

Remarkably, MRP2, which has an overall substrate specificity similar to that of MRP1 (211, 212), transports NNAL-O-glucuronide in the absence of GSH and transport is even inhibited by GSH (206). Nevertheless, MRP2 also needs GSH to transport a neutral drug, such as vinblastine, out of the cell (213). The experiments of Evers et al. (213) with MRP2 have also shown that GSH acts stoichiometrically rather than catalytically, as there is a constant ratio of GSH and vinblastine molecules exported over a range of vinblastine concentrations.

Several investigators have tried to rationalize these and other experimental results in a model for MRP1/2 containing a bipartite substrate-binding site (207,

213, 214). Evers et al. have proposed a simple model containing a site with high affinity for GSH (G-site) and low affinity for hydrophobic ligands (drugs) and a D-site with the inverse properties. At low drug concentrations the D-site is occupied by drug and the G-site by GSH, resulting in cotransport. At higher drug concentrations, drugs such as sulfipyrazone may also occupy the G-site, resulting in inhibition of GSH transport (213), whereas other drugs such as vinblastine cannot. This model is undoubtedly an oversimplification. As MRP1 can bind and transport a large number of compounds, the binding pockets must be complex and flexible, and various types of competition or cooperativity between substrates can be envisaged.

Like Pgp and BCRP, the substrate specificity of MRP1 and 2 is sensitive to amino acid substitutions in the protein, as shown by Cole and coworkers who find that substitutions of a single amino acid in two different parts of MRP1 can dramatically alter substrate specificity (206, 215, 216). Their results indicate that MRP1 has a complex binding pocket. The basic mode of binding may be to the flexible, hydrophobic core of the protein (possibly constituting the D-site) by Van der Waals and stacking interactions. Acidic residues may help to bind basic amphipathic drugs. Basic residues (possibly in the G-site) may help to bind acidic drugs, as reported for MRP2 (217). Presumably, these basic residues must be shielded by substrate, or GSH, to get drug transport at all. The overall picture differs from that sketched for Pgp, where there is considerable evidence for two independent, nonoverlapping, but allosterically interacting sites for substrate binding. A more detailed discussion of this complex issue is provided (206, 215, 216).

PHYSIOLOGICAL FUNCTIONS OF MRP1 Even though MRP1 is the major high-affinity transporter of LTC₄, *Mrp1* (-/-) mice are fine in a protected environment (218, 219). These mice do show a diminished response to a nonspecific inflammatory stimulus (218), as expected, but they are nevertheless more resistant to an experimental *Streptococcus pneumoniae* infection than wild-type mice, presumably because the inability of macrophages, mast cells, and granulocytes to secrete LTC₄ secondarily leads to increased LTB₄ excretion and more effective recruitment of phagocytic cells (220). *Mrp1* (-/-) mice are also defective in the mobilization of antigen-bearing dendritic cells from the epidermis and in directing these cells to lymph nodes (221). No pathology has been reported for the *Mrp1* (-/-) mice that can be attributed to this effect on dendritic cell trafficking.

In theory, MRP1 should be able to affect the redox balance in cells through its ability to extrude reduced and oxidized glutathione. GSSG is transported at high rate with a K_m of 93 μ M (222). Transport of GSH is sluggish and the K_m must be very high, around 10 mM. Nevertheless, export of GSH by MRP1 is probably significant, as intracellular GSH concentrations are around 10 mM. Indeed, *Mrp1* (-/-) mice have raised levels of GSH in their tissues (223), and there is considerable evidence that MRP2 is the transporter responsible for excretion of

GSH from the liver into bile (224, 225). Although it is difficult to exclude the possibility that the GSH is cotransported with an unknown endogenous cellular ligand, the unambiguous demonstration of vesicular transport of GSH into MRP1-containing vesicles (226) shows that MRP1 can transport GSH. As GSSG is transported more efficiently by MRP1 than GSH, one may expect MRP1 to counteract the rising GSSG/GSH ratio in cells exposed to oxidative stress. This has been verified in cultured rat astrocytes (227).

The effect of the loss of Mrp1 on anticancer drug resistance has been studied both in *Mrp1* ($-/-$) mice and in triple KO (TKO) mice in which the disrupted Mrp1 alleles are combined with disruptions of the two drug-transporting Pgp (ABCB1) genes, *Mdr1a* and *Mdr1b* (94, 95). MRP1 and murine Mrp1 are normally located in intracellular vesicles of undefined nature and in the basolateral membrane of epithelial membranes (80, 228–231). Hence, MRP1 secretes drugs into the body, rather than moving them out of the body as Pgp or MRP2 do. This makes MRP1 a system of cellular defense rather than one of total organism defense like Mdr1 Pgp and MRP2, which eliminate drugs from the body. The importance of this cellular function is highlighted by the fact that mice lacking Mrp1 are hypersensitive to etoposide (94, 95, 218, 219, 230), whereas an increased sensitivity to vincristine is uncovered in the TKO mice (95). This results from three effects, reviewed by Borst et al. (253):

1. Increased sensitivity of bone marrow precursor cells, normally protected by Mrp1 (219, 230).
2. Increased sensitivity of some epithelia containing high levels of Mrp1. This is especially significant in the collecting tubules of the kidney (resulting in drug-induced polyuria) and in the basal layer of the oropharyngeal mucosa (resulting in a severe oral mucositis) (230). More widespread drug-induced damage in other organs is found in the TKO mice (94, 95).
3. Protection of vital body cavities, such as the contents of the testicular tubules (230) and the cerebrospinal fluid (94). The epithelium of the testicular tubules is made up of Sertoli cells, which contain high amounts of Mrp1 in their basolateral membrane. Absence of Mrp1 leads to increased drug-induced destruction of sperm cell (precursors). Mrp1 is therefore an integral part of the blood-testis barrier and it may play a similar function in the blood-inner ear barrier (232).

In the same fashion, the epithelial cells of the choroid plexus in the brain have an important function in the “cleansing” of toxic compounds from cerebrospinal fluid (CSF) (233, 234). These cells normally contain high levels of Mrp1/MRP1 in their basolateral membrane (94, 235); an absence of Mrp1 causes etoposide levels to increase ten-fold in the CSF after intravenous administration of the drug (94). Obviously, the body needs a basolateral transporter to protect sperm or CSF. Pgp in the apical membrane would pump drug into the cavity rather than protect its content.

In mice, loss of Mrp1 is associated only with increased sensitivity to epipodophyllotoxins (etoposide) and *Vinca* alkaloids (vincristine), the drugs also

most affected by the absence of Mrp1 in *Mrp1* ($-/-$) embryonic stem cells (236) and in fibroblast cell lines from TKO mice (76, 95). Other drugs modestly affected by the absence of Mrp1 in cell culture—anthracyclines, taxanes, camptothecins—have not been systematically analyzed in vivo.

INHIBITORS OF MRP1 AND MRP2 No good inhibitors of MRP1 and 2 are available that are suitable for use in intact cells and animals/patients (237). The prototype Pgp inhibitor, verapamil, has only limited and variable effects on cells rendered multidrug resistant by MRP1 (238). The newer agents that block Pgp and/or BCRP efficiently at low nontoxic concentrations—cyclosporin A, PSC833, GF120918, Pluronic L61, fumitremorgin—inhibit MRP1 only at high concentrations or not at all (239–241). The only inhibitors widely used thus far are relatively nonspecific inhibitors of organic anion transport, used in the old days for driving out urate by blocking its reabsorption in the kidney. These include sulfinpyrazone, probenecid, and benzbromarone. Draper et al. (242) noted that indomethacin, a nonsteroidal antiinflammatory drug (NSAID), was able to reverse resistance of MRP1 cells. This was followed up by Duffy et al. (243), who showed that some other NSAIDs have a similar effect, although it is unrelated to the action of these drugs on cyclooxygenase. Since all the active drugs are (relatively weak) organic anions, this may be the common denominator of these MRP inhibitors. Sulfinpyrazone is a substrate of MRP1 and 2. Indomethacin is rapidly converted into a glucuronide conjugate in cells, and it is possible that this conjugate is transported by MRP1 rather than the free drug.

Flavonoids are another group of compounds studied as inhibitors of MRPs. Hooijberg et al. (244) were able to reverse decreased daunorubicin accumulation in MRP1 cells with several flavonoids. The cytotoxicity of flavonoids is relatively high, however, and Leslie et al. (245) obtained only partial reversal of vincristine resistance with quercetin and no reversal at all with other flavonoids at concentrations that were not cytotoxic by themselves. Given the high cytotoxicity of flavonoids and their diverse biochemical targets in the cell (see 245), it seems unlikely that flavonoids can be modified to yield MRP inhibitors with sufficient specificity and therapeutic width to be useful in clinical practice.

Because the NSAIDs and uricosuric inhibitors of MRPs were or are in clinical use, it has been suggested that these drugs might be tried in patients to reverse MRP-mediated drug resistance. Another clinically tested compound, the leukotriene D₄ receptor antagonist MK571, is an excellent MRP1 inhibitor in vesicular transport experiments, but has only a limited effect on MRP1 in intact cells even at subtoxic dose (237). This suggests that good MRP inhibitors will have to be made as prodrugs in which the acidic groups are shielded (241). Norman and coworkers have found, however, that certain tricyclic isoxazoles do inhibit MRP1 in intact cells at micromolar concentration. They are much less active against Pgp, BCRP (ABCG2), MRP2, and MRP3 (237, 246, 247). How these

compounds work has not been reported, but they might become a useful tool for research on MRP1.

INVOLVEMENT OF MRP1 IN DRUG RESISTANCE IN CANCER PATIENTS MRP1 is present in virtually all human tissues and in most human tumor cell lines and tumor samples. It is therefore likely that MRP1 contributes to the resistance of tumors against etoposide, vincristine, and anthracyclines, the drugs most affected by the presence of Mrp1 in fibroblast cell lines (76). Whether MRP contributes in practice to tumor cell resistance in cancer patients is not known yet. The correlation between MRP1 levels in tumors and tumor resistance is not striking (26).

The ability of MRP1 to cause arsenite resistance in transfected or selected cells (248, 249) and the overexpression of MRP1 in cells selected for arsenite (249) has raised the question of whether MRP1 might be responsible for the arsenite resistance of patients treated with arsenite for acute promyelocytic leukemia (250). This remains to be tested. It is noteworthy, however, that the *Mrp1* (-/-) mouse is not hypersensitive to arsenite (219), which suggests that MRP1 is not a critical factor in the cellular defense against arsenite. This could be due to the rapid excretion of the complexes of arsenite and methylarsenite with glutathione into bile (224, 251, 252).

MRP2

MRP2 and MRP1 have about the same size and putative membrane topology (Figure 1), and they both transport a similar large range of organic anions [reviewed in (211, 212)]. However, the tissue distribution of MRP2 is much more restricted than that of MRP1, and MRP2 is located in the apical membrane of epithelial cells, whereas MRP1 is basolateral. MRP2 has an important function in the biliary excretion of endogenous metabolites, such as glucuronosyl-bilirubin, as well as many exogenous compounds (96, 253). MRP2 is expressed not only in the liver but also in the kidney (254) and the intestine of rats (255, 256) and humans (257). There is considerable species difference in the level of expression in these two organs compared to the liver. The most extreme example is in rabbits, in which expression in kidney and intestine is higher than in the liver (258). Intestinal MRP2 excretes organic anions into the gut (255) and plays a role in reducing the oral availability of PhIP, a food-derived carcinogen (259, 260). Mutations in the *MRP2* gene cause the Dubin-Johnson syndrome [see (96)]. These patients suffer from an inherited conjugated hyperbilirubinemia, which indicates that bilirubin can enter the hepatocytes and is conjugated with glucuronate, but is not secreted into bile. The syndrome is rare and generally described as benign; in view of the function of MRP2, however, it is possible that the patients could be hypersensitive to certain drugs.

Because of the similarity in the substrates transported by MRP1 and 2, one would expect cells transfected with the *MRP2* gene constructs to become

resistant to the same range of anticancer drugs as *MRP1* transfectants. It has been technically difficult to verify this prediction because *MRP2* is not routed efficiently to the plasma membrane in most transfected cells (261). It is now clear, however, that *MRP2* can cause resistance to vincristine (262, 263), vinblastine (213, 263), anthracyclines (262, 263), camptothecin derivatives (262), mitoxantrone (253), methotrexate (MTX) (244), and cisplatin (263). Indirect evidence for an association between *MRP2* and cisplatin resistance had already been obtained with cancer cell lines (196, 264). Probably the cisplatin is transported as a complex with two GSH moieties (265), but this remains to be verified.

Like *MRP1* and 3, *MRP2* is able to confer resistance to brief (4-h) exposures to very high concentrations of MTX (83, 244), but not to chronic MTX exposure. This has been explained by a competition between transport of MTX by MRPs and the conversion of MTX into MTX_{glu} by folylpoly- γ -glutamate synthetase (FPGS), which adds additional glutamates to MTX (83, 244, 244a). At low MTX concentrations the FPGS efficiently converts MTX into MTX_{glu} , which is not transported by MRPs. As the MRPs have a low affinity for MTX, they cannot compete with FPGS for the low concentrations of MTX present. In contrast, short exposures to high concentrations of MTX lead to high intracellular concentrations of MTX, which allow *MRP* to pump out a substantial fraction of the MTX before it is polyglutamylated. The low affinity of MRPs for MTX makes sense, as otherwise cells might be depleted of their normal stock of folates. Low-affinity transport of some folates by *MRP1* has been observed by Keppler et al. (266) and G. Jansen (personal communication). Expression of transfected *MRP1*, 2, or 3 genes in ovarian carcinoma cell lines leads to a 30% reduction in folate pools (267).

Whether *MRP2* contributes to anticancer drug resistance in patients remains to be seen. *MRP2* has been detected in renal, lung, gastric, colorectal, and hepatocellular carcinomas (211, 268), but no correlation between *MRP2* and clinical resistance has emerged.

MRP3

MRP3 is an organic anion transporter, basolateral like *MRP1* (83, 269), and prominently present in liver, gut, and kidney (196, 270) like *MRP2*. The strong upregulation of *MRP3* in the liver under some cholestatic conditions (271–274) and the ability of *MRP3* to transport some bile salts (198, 275) have led to speculations that *MRP3* might play a role in the enterohepatic recycling of bile salts and in the removal of toxic organic anions from the hepatocyte under cholestatic conditions (83, 272). This is discussed further below.

MRP3 differs from *MRP1* and 2 in that it appears unable to transport GSH (83, 226). This may explain why cells transfected with *MRP3* gene constructs are not resistant to most of the anticancer drugs that are probably cotransported with

GSH by MRP1/2 (83, 269, 276). The only exceptions are the epipodophyllotoxins etoposide and teniposide (83, 226, 269, 276). MRP3-mediated resistance against these drugs does not require intracellular GSH, and etoposide appears to be transported by MRP3 in unmodified form (226).

MRP3 is present in cancer cell lines from many tissues (196, 277), but initial studies on MRP3 in a panel of drug-resistant cancer cell lines did not turn up any association between MRP3 levels and resistance (196). A more recent survey of MRP3 in lung cancer lines (278, 279) found a strong correlation between MRP3 and doxorubicin resistance. As MRP3 does not cause anthracycline resistance in transfected cell lines, the clinical significance of this correlation is doubtful.

MRP3 levels are high in the adrenal cortex (270). Given the high affinity of MRP3 for estradiol-17 β -glucuronide (275, 280, 281), it is therefore possible that MRP3 contributes to the transport of endogenous steroid conjugates. As glucuronosyl-drug conjugates are good MRP3 substrates (226, 275, 280, 281), MRP may also contribute to the disposal of toxic/carcinogenic agents detoxified by glucuronidation. The *Mrp3* ($-/-$) mice generated in our laboratories are healthy and fertile (unpublished data), but have not been tested yet for drug disposition.

MRP4 and MRP5, Transporters of Cyclic Nucleotides and Nucleotide Analogs

MRP4 and MRP5 are “short” MRPs that lack the TMD₀ present in MRP1 (Figure 1). They are both organic anion pumps, but they have the interesting ability to transport cyclic nucleotides and nucleotide analogs, a class of organic anions apparently not transported by MRP1–3 or 6. The transport of nucleotide analogs by MRP4 and 5 can result in resistance to clinically used base, nucleoside, and nucleotide analogs, at least in transfected cells that highly overproduce MRP4 or 5. The rate of cyclic nucleotide transport by these transporters is low and the physiological role of this transport remains to be defined.

Initial studies on MRP5 by McAleer et al. (282) and Wijnholds et al. (84, 283) showed that this protein is an organic anion pump, able to transport acidic organic dyes, *S*-(2,4-dinitrophenyl)glutathione, GS-DNP, and GSH, and inhibited by sulfinpyrazone. However, substantial drug resistance in *MRP5*-transfected cells was found only for 6-mercaptopurine (6MP) and thioguanine (TG), two purine bases that are definitely not acidic (84, 283). This paradoxical result was rationalized when Schuetz et al. (284) discovered that a cell line selected for resistance against PMEA, an adenine nucleotide analog, highly overexpressed the *MRP4* gene. This suggested that MRP4 and 5 are nucleotide transporters and that the resistance of MRP5 cells to thiopurines was a result of conversion of the bases into the corresponding nucleotides and extrusion of the nucleotides from the cell by MRP5. This has been confirmed in subsequent work (84; P.R. Wielinga, G. Reid, J. Schuetz, J. Beijnen & P. Borst, unpublished results). Whereas MRP4 appears to prefer methylated thioIMP, MRP5 prefers the unmethylated thionucleotides. There is no indication that MRP5 can transport nucleoside di- or triphosphate analogs (84).

Cells with high concentrations of MRP4 are highly resistant to PMEA and AZT and much less resistant to other nucleoside analogs used in antiviral therapy, such as lamivudine, ddC, and d4T (284). MRP4 (285), but not MRP5 (84), confers resistance to short-term incubation with high concentrations of MTX. Like MRP4, MRP5 can transport nucleotide analogs with a normal pyrimidine ring (286).

The list of substrates transported by MRP4 and 5 was substantially broadened by vesicular transport studies. Jedlitschky et al. (287) discovered that MRP5 can transport cyclic GMP and AMP (cGMP and cAMP), and Hopper et al. (198) recently found this for MRP4 as well [see also (290)]. The affinity for cGMP is higher than for cAMP. There is no indication that MRP4 or 5 can use standard 5'- or 3'-mononucleotides as substrate. Estradiol-17 β -glucuronide is transported relatively well by MRP4 (288). Interestingly, the cGMP transport by MRP5 is efficiently inhibited by the inhibitors of cGMP phosphodiesterase, sildenafil (Viagra), trequinsin, and zaprinast (287).

The tissue distribution of MRP4 and MRP5 is still not well known. Recent studies suggest that *MRP4* is more widely expressed than initially thought (196, 197, 286, 289), with the highest levels in kidney and prostate. Lee et al. (285) found MRP4 in the basolateral membrane of the acinar cells in the prostate. In contrast, Van Aubel et al. (290) reported that MRP4 is in the apical membrane, not the basolateral membrane, of rat and human kidney cells. Whether MRP4 is indeed targeted to different membranes in different epithelial tissues needs verification with antibodies that allow more conclusive immunohistochemistry.

Analysis of tissue RNA suggests that *MRP5* is ubiquitously expressed. The highest levels are found in skeletal muscle and brain (196, 282, 291, 292). All attempts to generate antibodies that allow the localization of MRP5 in tissues with anti-MRP5 antibodies have failed thus far. This is presumably because the expression levels are too low, as these antibodies readily detect MRP5 in transfected cells (84). On Western blots MRP5 can be detected in human and murine erythrocytes (287), which might explain the observed cGMP transport in these cells (293), although this needs verification using red cells from *Mrp5* (-/-) mice.

The physiological functions and possible role in drug resistance of MRP4 and 5 remain to be defined. Obviously, the discovery that these pumps can transport cyclic nucleotides, notably cGMP, has raised the question of whether MRP4/5 can affect the signal transduction role of cGMP by removing it from the cell, which would supplement the degradation by phosphodiesterases. There is also evidence for an extracellular signaling role for cGMP in kidney and several other tissues, and MRP4/5 might be involved.

Any role that MRP4/5 may have in drug resistance is also under investigation. As nucleobase and nucleoside analogs are used extensively in anticancer and antiviral therapies, there is potential for MRP4/5 to mediate resistance to these compounds. As pointed out by Chen et al. (288), 6MP and methotrexate are both

used in the treatment of childhood leukemias and MRP4 is the only drug transporter known thus far that can transport both drugs.

No human disease has been associated with alterations in MRP5, and the Mrp5 KO mouse, generated by Wijnholds et al. (84), has no obvious phenotype. It is possible, however, that the overlapping substrate specificities of MRP5 and MRP4 (and possibly MRP8 and 9) may hide the physiological function of Mrp5, e.g., in cyclic nucleotide transport, and that the breeding of mice lacking all these transporters may lead to an understanding of the physiological function of each of them.

MRP6

Human *MRP6* is mainly expressed in liver and kidney (196, 294, 295), like *Mrp6* (*MLP-1*), its rat homolog (271, 280, 296), but low RNA levels have also been detected in other tissues. In initial immunofluorescence studies, Madon et al. (296) localized rat Mrp6 in the basolateral and apical membranes of hepatocytes, but more recent work by the same group (personal communication) strongly indicates that MRP6 is only in the basolateral membrane of polarized cells. In contrast to some other MRPs, expression of *Mrp6* is stable, whatever damage is inflicted on the liver (296).

The substrate specificity of MRP6 is still a mystery. Madon et al. (296) tested a series of typical MRP substrates in vesicular transport studies and found transport only of BQ-123, an anionic cyclopentapeptide and an antagonist for the endothelin A receptor. Endothelin-1 itself was transported by Mrp2, but not by Mrp6. These results suggest that MRP6 could be a highly selective pump for organic anions. It should be noted, however, that Madon et al. (296) tested radioactive substrates only at relatively low concentrations. No competition experiments were done with high competitor concentrations, substrates such as methotrexate were not tested, and standard inhibitors of MRPs were not tested either.

Amplification of the 3' part of the *MRP6* gene was found in leukemia cells selected for anthracycline (epirubicin) resistance (297–299). The anthracycline resistance was initially thought to be due to a new resistance determinant, called the anthracycline resistance gene, *ARA*. Subsequent work has shown, however, that the epirubicin resistance of cell lines with *ARA* gene amplification can be explained by coamplification of the *MRP1* gene together with the 3' half of the adjacent *MRP6* gene (294, 295). There is no indication that the *MRP6* gene is ever associated with anticancer drug resistance.

How defects in MRP6 cause pseudoxanthoma elasticum, a heritable disorder characterized by calcification of elastic fibers in skin, arteries, and retina, is unclear. Why the loss of a highly specialized pump located in the basolateral membrane of liver and kidney cells would lead to such a generalized connective tissue disease is hard to explain. Speculations include indirect effects on Ca^{2+}

metabolism or elastic fiber assembly through excretion of cytokine-like organic anionic peptides (300–302).

ABC TRANSPORTERS INVOLVED IN LIPID TRANSPORT

The first mammalian transporters that were discovered transported mainly amphipathic drugs, as cell lines overexpressing these genes were easily isolated by drug selection. More recently an even larger contingent of transporters was identified that are dedicated to the translocation of lipids. These were found either on the basis of homology with known transporters or as causative genes in disease loci. Within the ABCB family, MDR3 translocates the phospholipid PC, and BSEP transports bile salts, derivatives of cholesterol. In the ABCA subfamily, ABCA1 is involved in phospholipid and cholesterol efflux from peripheral cells; ABCR (ABCA4) translocates the lipid *N*-retinylidene-phosphatidylethanolamine; and four half-size transporters in the ABCD subfamily are thought to be involved in the transport of very long chain fatty acyl coenzyme A (CoA) esters. Finally, two members of the ABCG family, ABCG5 and ABCG8, were recently identified as pumps for plant sterols and possibly also cholesterol.

That protein-mediated lipid translocation is a crucial biochemical mechanism in the complex mammalian organism is underscored by the fact that mutations in nearly all these lipid transporter genes are associated with clear-cut disease phenotypes. In this section we discuss the various lipid translocation processes mediated by these ABC transporters and the diseases associated with their absence. We do not discuss the transport of lipid analogs by other ABC transporters, such as the ABCB1 type P-glycoproteins. In our view, lipid analogs are handled as drugs by these transporters and there is no conclusive evidence that they transport natural membrane lipids, as discussed in the Pgp section.

MDR3/Mdr2 P-Glycoprotein (ABCB4)

Mdr2 Pgp in the mouse was the first ABC transporter to be identified as a phospholipid flippase; actually, it is the first flippase characterized at the genetic, molecular, and functional levels. Earlier work has shown that the translocation of aminophospholipids across the plasma membrane is protein mediated, but the protein responsible for this function has remained elusive. It may be the P-type ATPase encoded by the ATP8A1 gene, but this is still under debate. Disruption of the *Mdr2* gene in the mouse led to a total abrogation of biliary phospholipid secretion into bile (91). Secretion of cholesterol was also abolished, but this is secondary to the absence of phospholipids. Transgenic expression of the human *MDR3* gene in *Mdr2* ($-/-$) mice completely rescued the phenotype, proving that the two genes are orthologs (303). Translocation of phospholipids and phospholipid analogs by the Mdr2/MDR3 Pgp is specific for the phosphorylcholine (PC)

head group [see (15)], as expected from the fact that PC is the only phospholipid in bile. Nevertheless, this Pgp can also transport some amphipathic drugs and it is inhibited by inhibitors of the MDR1-type (ABCB1) Pgps (304).

Biliary lipid secretion directly depends on bile salt secretion and is generally assumed to involve bile salt-induced solubilization of phospholipids from the apical membrane of the hepatocyte. Simple micelles of bile salt are hardly capable of solubilizing cholesterol, whereas mixed micelles of bile salts and phospholipids have a much higher affinity for cholesterol. This explains why cholesterol secretion secondarily depends on phospholipid secretion (91, 305). Little is known about the actual mechanism by which bile salts extract phospholipids from the canalicular membrane after their Mdr2-mediated translocation. It appears to involve vesiculation from the outer leaflet of the membrane (306). Vesiculation is not stopped by cholestasis, since the formation of the abnormal cholestatic lipoprotein X, which is observed in many different states of cholestasis, completely depends on the activity of Mdr2 Pgp and is absent in *Mdr2* ($-/-$) mice (307). Thus, biliary lipid vesicles continue to be formed during cholestasis, but they are discharged into the blood instead of the bile.

Phospholipid translocation in the canalicular membrane is specific for PC with relatively short fatty acyl chains (16 and 18 carbon atoms) compared to the overall composition of membrane PC. The simplest explanation for this specificity would be if only Pgp translocates these PC subtypes, because otherwise a surplus of phospholipid with long fatty acyl chains would build up in the canalicular membrane, which would require back-flipping of nonextracted phospholipids. Although an ATP-independent flippase function has been described in canalicular membrane fractions, its specificity for nonextracted phospholipids was not investigated (308). The preference for translocation of short-chain PC might be explained by assuming that this PC subtype more easily detaches laterally from the lipid leaflet to enter into the binding cavity of Pgp in the membrane. This assumption could also explain why sphingomyelin (SM), which also has a phosphorylcholine head group, is not translocated; SM has longer and saturated fatty acyl chains, which require more energy for lateral detachment from the lipid leaflet. The canalicular membrane contains relatively high levels of SM and cholesterol, which probably explains why the canalicular membrane does not dissolve in the high concentration of bile salt present in the canalicular lumen. In the presence of cholesterol the long and saturated acyl chains of SM form domains in the liquid ordered state; these rigid domains are highly resistant to solubilization mediated by c.q. bile salts (309).

In spite of the resistance of the canalicular membrane to the acute solubilization by bile salts, the absence of biliary lipid secretion does harm to the liver. *Mdr2* ($-/-$) mice suffer from progressive liver inflammation and fibrosis (310). As expected, an analogous inherited human disease exists (311, 312). A subgroup of patients with progressive familial intrahepatic cholestasis (PFIC) was found to have mutations in the *MDR3* gene. These are now called type 3 patients. They suffer from liver inflammation and fibrosis, which progresses to cirrhosis and

liver failure. The severity of the liver pathology depends on the cytotoxicity of the bile salts in the enterohepatic circulation. The more hydrophobic bile salts cause more damage, as shown by feeding different types of bile salts to *Mdr2* (-/-) mice (313). PFIC type 3 can be distinguished from other types of PFIC (see below) by high serum levels of gamma-glutamyltransferase (GGT) activity in the patient (312). GGT is an ectoenzyme on the canalicular membrane. The prevailing idea is that the bile salt micelles solubilize GGT from the membrane and that the enzyme reaches the circulation either by transcytosis or by the opening of the tight junctions between hepatocytes.

Interestingly, defects in the *MDR3* gene do not give rise only to pediatric liver disease. Jacquemin et al. (314) reported that the mother of a patient with PFIC type 3 and several other women from this family suffered from intrahepatic cholestasis of pregnancy. These women are heterozygotes for the mutation in the *MDR3* gene. Moreover, Rosmorduc et al. (315) reported on six patients with gallstones who had mutations in the *MDR3* gene. These two publications suggest that the severe phenotype associated with complete or nearly complete absence of *MDR3* function is complemented by milder phenotypes associated with a reduction but not a complete absence of *MDR3* Pgp function.

Neither in the mice nor in patients with a complete lack of *MDR3* Pgp was any extrahepatic defect observed. Although low levels of *MDR3/Mdr2* mRNA are present in adrenal glands, muscle, tonsil, and spleen, it is not certain whether the corresponding protein is present as well.

BSEP (ABCB11)

Childs et al. (316) isolated a partial cDNA from pig tissue on the basis of its homology with *MDR1* and called it sister of P-glycoprotein. Gerloff et al. (317) used this sequence to obtain a homologous full-size cDNA from the rat. Plasma membrane vesicles from Sf9 cells transfected with this rat *Bsep* cDNA took up taurocholate in an ATP-dependent fashion with a K_m of 5.3 μM (317). These vesicles also transported the taurine conjugates of chenodeoxycholate and ursodeoxycholate, but not glycocholate and cholate (317). Murine *Bsep* was shown to transport taurocholate, taurochenodeoxycholate, and glycocholate (318–320). With transfected cell lines, Lecureur et al. (319) demonstrated that murine *Bsep* transports taurocholate, vinblastine, and calcein-AM, but not taxol and several other *MDR1* Pgp substrates. This contrasts with the observation by Childs et al. (321) that rat *Bsep* confers (slight) resistance to taxol. Our impression is that *Bsep* contributes little to *MDR*, if it transports drugs at all.

In parallel with the identification of *Bsep* as an export pump for bile salt, Strautnieks et al. (322) found a locus on chromosome 2q24 in a subgroup of PFIC patients. They subsequently located *BSEP* in this locus and showed that the disease is associated with mutations in this gene (323). This subtype of PFIC is now referred to as type 2. These patients have extremely low biliary concentra-

tions of bile salt, which along with the transfection studies with rat and murine Bsep has led to the conclusion that BSEP is the main transporter of bile salt in the canalicular membrane. Although the etiology of PFIC type 3 (damage by canalicular, lipid-free micelles of bile salt) and that of PFIC type 2 (absence of bile salt secretion) are very different, the clinical progression is so similar that the two were originally grouped together. An important diagnostic difference is that patients with PFIC type 3 (MDR3 Pgp deficiency) have a high serum level of gamma-glutamyltransferase (GGT) whereas type 2 patients have a low level. This fits the idea that GGT release is mediated by canalicular bile salts, which are absent in type 2 patients.

Very recently, Wang et al. (324) reported on a knockout mouse for the *Bsep* gene. *Bsep* ($-/-$) mice are cholestatic in the sense that taurocholate accumulates in their liver because its secretion into bile is strongly impaired. However, in contrast to the human patients, the mice excrete substantial amounts of tauro-muricholate into bile as well as a hitherto undefined tetrahydroxy bile salt. Apparently, Bsep is not the only transporting system for bile salt in the murine canalicular membrane; another system is capable of excreting these hydrophilic bile salts. This escape route prevents severe and progressive cholestasis in *Bsep* ($-/-$) mice and as a consequence the animals have hardly any histopathological signs of liver injury. Because humans cannot convert bile salts into muricholate or tetrahydroxy bile salts, this escape route is not present in humans. Interestingly, *Bsep* ($-/-$) mice were found to have an increased rate of phospholipid and cholesterol secretion into bile. The reason for this is unclear, but the accumulation of taurocholate in the liver might lead to an induction of *Mdr2* expression, which would increase phospholipid and cholesterol secretion because *Mdr2* Pgp activity is the rate-controlling step in biliary lipid secretion (303, 325). It was shown in rats that enlarging the bile salt pool increases the expression of *Mdr2* and phospholipid secretion into bile (325).

ABCR (ABCA4)

In 1997 purification of the Rim protein led to the identification of *ABCR* (*ABCA4*) (326, 327). This large, 210-kilodalton glycoprotein was already known to be localized in the rims of outer segment discs of rods in the retina. Rod outer segments are a stack of membranous structures under the plasma membrane in which the chemical reactions of visual signal transduction take place. As with many other ABC transporters, the importance of ABCR was discovered by the consequences of its deficiency. Mutations in *ABCR* lead to Stargardt disease (328), a progressive disease starting with delayed dark adaptation, followed by deposition of lipofuscin in the retinal epithelium. The lipofuscin accumulation may be the cause of the subsequent atrophy of the epithelium. Weng et al. (329) produced knockout mice for the *Abcr* gene and observed a phenotype similar to Stargardt disease. In experiments in which the retina of these animals was

exposed to light, Weng et al. (329) found a decreased clearance of all-*trans*-retinaldehyde (atRAL), formed by photobleach, during the subsequent dark phase. Concomitantly, a decreased formation of all-*trans*-retinol (atROL) from atRAL was observed. Thus, ABCR had to mediate a step in the clearance of atRAL after photobleach. Sun et al. (330) had shown that the ATPase activity in membranes containing reconstituted ABCR was stimulated by atRAL (and several other retinoids), but this activation was dependent on the presence of phosphatidylethanolamine (PE). From these data Weng et al. (329) composed the following model: Upon light exposure, atRAL bound to metarhodopsin II is released in the disc interior and reacts with the primary amine of PE via a Schiff's base. The condensation product, *N*-retinylidene-phosphatidylethanolamine (N-RPE), is then translocated by ABCR to the cytoplasmic leaflet of the disc membrane, where the complex dissociates and atRAL is reduced to atROL. The lack of N-RPE translocation probably also explains the lipofuscin accumulation, because a major component of lipofuscin is A2-E, a positively charged Schiff's base condensation product of two retinaldehydes with ethanolamine. Insufficient clearance of this product leads to its accumulation in the lysosomes of the retinal epithelium and subsequent death of these cells. Importantly, Mata et al. (331) showed more recently that mice heterozygous for *Abcr* gene disruption [*Abcr* (+/-)] display the same phenotype as *Abcr* (-/-) mice, but with later onset. This suggests that there is not a large overcapacity of *Abcr* activity and that heterozygous *ABCR* (+/-) mutations may also predispose humans to retinal disease.

Screening for mutations in the *ABCR* gene has revealed a host of mutant alleles, some of which occur quite frequently (332). Several cases were reported in which *ABCR* mutations segregated with retinal dystrophies of substantially different phenotype, although all are associated with delayed dark adaptation of rods. An obvious question is whether age-related macular degeneration (AMD) is also associated with mutations in *ABCR*. This is difficult to prove because it is a frequent age-related disease (mild or early forms occur in nearly 30% of people older than 75 years). Allikmets (333) has demonstrated, however, a weak but highly significant association between heterozygosity for two mutant *ABCR* alleles and development of AMD.

ABCA1

The *ABCA1* gene was cloned in 1994 by the Chimini group (334) on the basis of its homology with other ABC transporters. *ABCA1* expression during embryonic development was found to correlate spatially and temporally with areas of apoptosis (335), which suggested that it played a role in programmed cell death. Indeed, ABCA1 was found to contribute to the engulfment of apoptotic bodies, very much like a homolog in *Caenorhabditis elegans*, *ced-7* (336). Additional evidence for this putative function came from the observation that ABCA1 drives

phosphatidylserine (PS) translocation across the plasma membrane (335), since it is known that exposure of PS on the outer leaflet of the membrane is necessary for the recognition of apoptotic cells. However, it was recently found that ABCA1 has an essential role in cellular lipid efflux, as mutations in the *ABCA1* gene cause Tangier disease (337–340), a rare inherited disorder characterized by the virtual absence of high-density lipoprotein (HDL). These patients are not known to suffer from any defect in the clearance of apoptotic bodies. Hence, the function of ABCA1 in lipoprotein metabolism is apparently the more important one in humans.

The defect in Tangier disease involves the absence of cholesterol efflux from peripheral tissues. Fibroblasts from these patients are incapable of donating cholesterol and phospholipid to lipid-poor apolipoprotein A-I (apoA-I), a step that is essential for maturation of the lipoprotein. When the association between *ABCA1* defects and Tangier disease was discovered, the initial suggestion was that ABCA1 transports cholesterol, but recent work demonstrates that this is not the case. In an elegant study Wang et al. (341) investigated the molecular mechanism of ABCA1-mediated cholesterol efflux from transfected cells. In line with earlier findings by Chambenoit et al. (342), they demonstrated that apoA-I binding to the cell membrane involves binding to ABCA1 and that binding depends on activity of the pump. Inhibition of the pump with glibenclamide not only prevented cholesterol and phospholipid transfer but also eliminated binding of apoA-I to the cells. Depletion of cholesterol from the cells by extraction with the cholesterol acceptor cyclodextrin resulted in a strong decrease of ABCA1-mediated cholesterol efflux, while phospholipid transfer continued. Conversely, cholesterol efflux to mature HDL, which contains phospholipid, or to cyclodextrin, which does not need phospholipid for cholesterol extraction, is ABCA1 independent. This led Wang et al. (341) to the conclusion that ABCA1 functions primarily as an outward phospholipid translocase rather than a cholesterol transporter. The authors propose the following model: ATP binding/hydrolysis on ABCA1 probably induces a conformational change of the transporter, which leads to the binding of apoA-I and phospholipid translocation. The binding site may consist of phospholipids together with ABCA1 itself. Once apoA-I has recruited the phospholipids translocated by ABCA1, the nascent complexes promote cholesterol efflux. Apparently, phospholipid-free apoA-I has little affinity for cholesterol, and addition of phospholipid to the complex enhances the affinity for the sterol.

Transgenic mice that overexpress *ABCA1* have increased plasma levels of HDL, as expected, but also an increased concentration of cholesterol in bile (343). This could mean that ABCA1 also fulfills a function in lipid secretion in the canalicular membrane. To investigate this in a more unambiguous model, Groen et al. (344) measured cholesterol secretion rates into bile in *Abca1* (–/–) mice during bile diversion. They observed no difference between *Abca1* (–/–) mice and controls. This rules out the possibility that *Abca1* contributes to biliary

cholesterol efflux, unless this function would be completely taken over by another transporter in *Abca1* ($-/-$) mice, which is rather unlikely.

The cell-specific expression and subcellular distribution of ABCA1 are still not settled. The Mangelsdorf group (345) has suggested that the protein is present in the apical membrane of enterocytes, but their indirect evidence is open to other interpretations (see below). Neufeld et al. (346) have provided evidence to suggest that ABCA1 cycles between plasma membrane and endosomes. Immunohistochemistry with good antibodies on normal tissues will be required to determine the cell-type-specific expression as well as the subcellular localization of ABCA1.

ABCG5 and ABCG8

Like other members of the ABCG family, ABCG5 and ABCG8 (also called sterolin 1 and 2, respectively) are half-transporters. Mutations in the *ABCG5* and *8* genes were recently linked to sitosterolemia (347). The hallmark biochemical feature of this rare inherited disease is the elevated concentration of plant sterols in plasma (348), most importantly sitosterol (24-ethyl cholesterol). Both *ABCG5* and *8* are expressed mainly in liver and intestine (347, 349). The fact that mutations in either *ABCG5* or *ABCG8* cause sitosterolemia suggests that these two half-transporters form a functional (and obligatory) heterodimer. No transport studies have been reported yet with transfected cell lines. Also, the localization of the proteins in normal human tissues has not been described yet.

Besides sitosterol, such other sterols as campesterol, stigmasterol, and brassicasterol are present in plants and other components of the diet like shellfish. The absorption of these plant sterols in the intestine is strongly increased in sitosterolemic patients. Control subjects absorb about 50–70% of dietary cholesterol, but less than 5% of the ingested plant sterols. Sitosterolemic patients absorb plant sterols to a much higher extent (20–40%). As a consequence, there is a marked accumulation of these sterols in plasma and tissues. In normal subjects, the low amounts of absorbed sitosterol are quickly secreted into bile so that only trace amounts of it can be found in blood (348). In sitosterolemic patients, the biliary secretion of sitosterol and other phytosterols is impaired (350–352), which results in a bile/plasma ratio of total plant sterols that is reduced more than 30-fold compared to controls (352). This shows that phytosterol handling in these patients is impaired at two levels: Absorption in the intestine is increased and secretion into bile decreased. This suggests that the ABCG5/ABCG8 heterodimer is an outward pump for plant sterols. In the intestine this pump reduces absorption and in the liver it mediates canalicular secretion of these unwanted sterols.

Sitosterolemic patients also suffer from hypercholesterolemia and decreased biliary cholesterol secretion. In contrast to other forms of hypercholesterolemia,

the form in these patients is markedly sensitive to cholesterol levels of the diet (348), which suggests that the intestinal absorption of cholesterol might also be disturbed. This could mean that cholesterol is also a substrate for the transporter, but this hypothesis remains to be tested.

ABCD1–4

The ABCD subfamily represents another group of half-transporters. ABCD1 (ALDP) was the first mammalian half-transporter to be discovered. In 1993, Mosser et al. (353) showed that mutations in the *ALD* gene cause X-linked adrenoleukodystrophy (X-ALD). This is a severe neurodegenerative disorder mainly characterized by adrenal insufficiency and progressive demyelination of the central nervous system. The biochemical hallmark is an accumulation of very long chain fatty acids (VLCFA) that results from a defect in their β -oxidation, a process confined to the peroxisomes. Since the activation of very long chain fatty acids occurs outside the peroxisome, whereas the β -oxidation itself takes place inside, it was suggested that ALD might be involved in the translocation of the acyl-CoA esters across the peroxisomal membrane (354). No direct evidence exists for this hypothesis. The main problem is a technical one: it is not yet possible to isolate intact peroxisomes from mammalian tissues or cells. Upon isolation of these organelles they become leaky, making it impossible to perform transport experiments. Hettema et al. (355) showed that the yeast homologs of ALD, Pat1 and Pat2, are necessary for proper peroxisomal β -oxidation of long-chain fatty acids using yeast knockouts for these genes. Only the oxidation of long-chain fatty acids, esterified in the cytosol, was impaired and not that of medium-chain fatty acids, which are activated in the peroxisomes. This is compatible with the idea that Pat1/2 are required to import the long-chain fatty acyl-CoA esters into peroxisomes.

Whereas yeast Pat1 and Pat2 form an obligatory heterodimer, the situation in mammals seems to be more complex. Mammals have four ABCD transporters, ABCD1–4, which are also called ALDP, ALDR, PMP70, and PMP69, respectively. Interaction between these proteins was demonstrated by coimmunoprecipitation and yeast two-hybrid assays (355, 356). Only mutations in the *ALD* gene have been detected, which suggests that the others are redundant with respect to VLCFA oxidation. Indeed, overexpression or induction of *ABCD2* or *ABCD3* restores VLCFA oxidation in fibroblasts from X-ALD patients or *Ald* ($-/-$) mice (357, 358). Clearly, the elucidation of the substrate specificity and molecular mechanism of the ABCD transporter subfamily awaits a proper experimental system for direct transport experiments.

ABC Transporters and Bile Formation

Liver research has probably benefited most from the discovery of new ABC transporters. Almost all excretory functions in the apical membrane of the hepatocyte are mediated by ABC transporters: bile salts via BSEP, phospholipid via MDR3 Pgp, and bilirubin and other organic anions via MRP2. Whereas MDR3 Pgp and BSEP seem to be liver-specific transporters, MRP2 also functions in kidney and intestine. In this section we describe some specific functions of ABC transporters in the liver that have not been dealt with above. The importance of biliary secretion is evidenced by the diseases that are caused by mutations in genes for liver-specific transporters, notably *MDR3*, *BSEP*, and *MRP2*. These diseases are discussed above in the sections on these transporters.

The combination of the pump function of ABC transporters with the anatomy of the canaliculi, which are tiny 1- μm tubules between adjacent hepatocytes, causes huge concentration gradients between hepatocytes and bile. Estimations for the gradient of bile salts and organic anions range from 100 to 1000. This makes the biliary excretion system efficient but it also has inherent dangers. Many of the excreted compounds are amphipathic, which means that they have the potency to diffuse back into cells. This is especially relevant for the epithelial cells of the bile duct, through which the bile flows. As mentioned in the section on MDR3 Pgp, high concentrations of bile salt are harmful to both hepatocytes and epithelial cells of the bile duct, and the main mechanism of protection is the secretion of phospholipids and cholesterol. The lipids in bile may also represent a mechanism of defense against amphipathic drugs, because lipid vesicles and mixed micelles constitute a lipid sink in which amphipathic compounds are taken up, thereby preventing their diffusion into cells [(359) and references therein].

The presence of lipids in the canalicular lumen is also important for the net excretion of drugs. For instance, the excretion of indocyanine green into bile is reduced by 90% in *Mdr2* ($-/-$) mice (360) even though this dye is at least partly transported via MRP2. Indocyanine green is a hydrophobic organic anion, and the dependence of its biliary excretion on lipid secretion could be explained in two ways: On the one hand, such hydrophobic drugs may be cosecreted with biliary lipid vesicles without actually being translocated by Mdr2 Pgp. On the other hand, these hydrophobic drugs may extensively diffuse back into the cell if lipid vesicles are not present in the canalicular lumen. A similar strong dependence of secretion on lipid has been observed for protoporphyrin, an endogenous, hydrophobic, anionic intermediate in the synthesis of heme, and for α -tocopherol (361). Biliary tocopherol secretion is significantly impaired in the *Mdr2* ($-/-$) mouse and this compound undergoes back diffusion in cells of the biliary tree. When injected into the bile duct in a retrograde fashion, 60% of the α -tocopherol was absorbed.

The extreme concentrations in the canaliculus also pose problems for the liver. The Vore group (362) demonstrated that the well-known cholestatic

effect of estradiol 17- β -glucuronide (E17G) depends on its secretion into bile via Mrp2. This anionic endogenous estrogen metabolite is partly secreted via Mrp2, as evidenced by its impaired secretion in Mrp2-deficient TR⁻ rats. It may also be transported to some extent via Mdr1 Pgp (363). It is known that E17G leads to cholestasis when it is administered to the perfused liver. Huang et al. (362) found that this cholestatic effect was strongly reduced in the TR⁻ rat, which suggests that cholestasis was induced after secretion of E17G from the canalicular side of the membrane. In transport studies with membrane vesicles from Sf9 cells that were transduced with Bsep and Mrp2 or with Bsep alone, Stieger et al. (364) showed that inhibition of Bsep, which is the cause of cholestasis, occurs from the *trans* side of the membrane. Most likely, E17G is pumped into the vesicle lumen to such high concentrations that it *trans*-inhibits Bsep, even though its affinity for Bsep is low.

Similarly, it was recently shown by Dietrich et al. (365) that cholestasis induced by α -naphthylisothiocyanate (ANIT) depends on its secretion via Mrp2 and that this is glutathione dependent. It had been shown before that ANIT forms an extremely labile covalent complex with GSH (366). Dietrich et al. observed that Mrp2-deficient TR⁻ rats are protected against cholestasis by ANIT, which causes extensive damage to epithelial cells of the bile duct [for review see (367)]. It is therefore plausible that the ANIT-GSH molecule is secreted in high concentrations into bile, where it dissociates. The hydrophobic ANIT molecule then diffuses into epithelial cells of the bile duct, where it causes extensive damage and cell death because these cells are not equipped with Mrp2 to protect themselves.

An interesting feature of ABC transporters involved in bile formation is that transport is subject to short-term regulation by shuttling of the proteins between intracellular vesicles and the canalicular membrane. Kipp et al. (368–370) showed that several ABC transporters (such as Bsep and Mrp2) are transported directly to the canalicular membrane after their synthesis, rather than via the basolateral membrane as reported for canalicular ectoenzymes (371). Bsep is not simply delivered to the canalicular membrane, however; a substantial fraction is stored in an intracellular compartment, presumably late endosomes. From this compartment Bsep is mobilized to the canalicular membrane in a regulated fashion. Indeed, in the perfused liver, bile salt secretion can be induced by a mechanism dependent on phosphatidylinositol 3 (PI3)-kinase (a wortmannin-sensitive mechanism), and this is accompanied by transfer of the protein from intracellular membranes to the canalicular membrane (372, 373). These experiments suggest that at least 50% but maybe as much as 80% of the Bsep protein is present within the cell and not in the canalicular membrane. This mechanism provides an attractive form of short-term regulation that prevents a high and potentially toxic concentration of bile salts either in the cell or in the canalicular lumen.

The MRPs in the liver are also subject to long-term regulation as a mechanism to protect against the toxic consequences of cholestasis (i.e., loss of bile flow).

Under normal conditions, apical MRP2 expression in the liver is high, whereas the level of MRP3 is low and confined to epithelial cells of the bile duct. However, during cholestasis in rats, the levels of MRP2 rapidly drop, whereas there is a very strong induction of MRP3 in hepatocytes (272, 273, 374, 375). As a consequence, secretion of many organic anions (including bile salts) shifts from the apical to the basolateral direction, which prevents intrahepatic accumulation to toxic concentrations.

REGULATION OF ABC TRANSPORTER EXPRESSION; A HOME FOR ORPHAN NUCLEAR RECEPTORS

The expression of several ABC transporters is under tight transcriptional regulation. The group of orphan nuclear receptors especially appears to play an important role in this regulation. Nuclear receptors constitute a family of transcription factors that act as heterodimers, which bind to promoter elements and induce gene expression. The common theme is that RXR, the retinoid receptor, is an obligatory partner in the heterodimer; the other partner can be any of the other members of the family. It is this other partner that determines the specificity for the activating ligand and for the target gene (376). Only relatively recently were ligands for these receptors recognized, as well as an increasing number of target genes. Nuclear receptors relevant for the expression of ABC transporters are LXR, the "liver X" receptor; FXR, the farnesoid receptor for which bile salts are important endogenous ligands; and PXR and SXR, the pregnane- and the steroid-activated receptors, which are expressed in rodents and humans, respectively. These two receptors turn out to be important xenobiotic sensing receptors. Finally, PPAR α and PPAR γ , already known as the receptors involved in peroxisome proliferation, are actually key regulators in lipid and carbohydrate metabolism.

Repa et al. (345) reported that *ABCA1* expression can be strongly induced (up to 20-fold) by the ligand-bound receptor dimer RXR/LXR. They found in mice that this induction was obtained only with specific ligands for LXR and not with ligands for the PPARs, FXR, and PXR. In addition, they observed that this induction took place in the intestine, where basal expression is very low; no induction was detected in liver, where basal expression is high. In line with this observation, induction was absent in LXR α/β double knockout mice. The endogenous ligands for LXR are oxysterols, metabolites of cholesterol. Hence LXR is activated in states of cholesterol surplus. In rodents, the receptor also induces expression of *CYP7A1*, the key enzyme in bile salt synthesis, and thereby stimulates shunting of cholesterol into the bile salt pathway. Intriguingly, the massive induction of *Abca1* expression by LXR was accompanied by a dramatic reduction in cholesterol absorption in the intestine, underlining the role of LXR in preventing cholesterol overload. This led the authors to suggest that ABCA1 is involved in cholesterol efflux from the apical membrane of the

enterocyte, but this hypothesis has lost its luster with the increasing evidence that ABCA1 is a phospholipid transporter rather than a cholesterol transporter. Two alternatives provide more plausible explanations for the LXR-mediated decrease in cholesterol absorption: First, the expression of the *ABCG5* and *ABCG8* half-transporters is also induced by LXR activation (376). The ABCG5/8 heterodimer is now thought to diminish plant sterol absorption in the gut and it may also affect cholesterol absorption (see above). Second, yet another protein (possibly an ABC transporter) might be induced by LXR and affect cholesterol absorption. In fact, the expression of several other ABC transporters of unknown function, such as ABCA2, ABCA6, ABCA7, and ABCG1, is also induced by cholesterol (377–380). Clearly, this is an important pharmacological issue, because a reduction in cholesterol absorption could reduce the incidence of cardiovascular disease.

The regulation of *ABCA1* is complex, as agonists for PPAR α , PPAR γ , and PPAR δ induce *ABCA1* expression (381, 382). Most likely, this PPAR-mediated regulation occurs via LXR α , because the authors found that the PPAR agonists enhanced expression of LXR α (but not LXR β). Simultaneous treatment with a PPAR agonist and LXR agonist (oxysterol) had an additive effect on *ABCA1* expression (381).

Recent work has shown that FXR plays a central role in the regulation of bile salt metabolism. Activation of FXR by bile salts, such as chenodeoxycholate, is stronger than by farnesol, the ligand originally linked to this receptor (383–385). Moreover, expression of both the binding protein for ileal bile acid and CYP7A1, key proteins in bile salt metabolism, is induced by activation of FXR. A FXR-binding element is present in the 5' sequence of the gene for the human bile salt export pump (BSEP) and expression of this gene is induced via FXR as well (386). In line with these observations, the expression of *Bsep* is dramatically decreased in *Fxr* (–/–) mice (387). Hence, in a state of bile salt excess, *BSEP* expression normally goes up, which stimulates the hepatocanicular clearance of bile salts.

Members of the ABCD subfamily are also regulated by nuclear receptors. Expression of *ABCD2* and *ABCD3* is induced by fibrates, ligands for PPARs, and this induction may be mediated via PPAR α , because fibrate induction of *ABCD2* expression was absent in *PPAR* α knockout mice (388). As described above, LXR α expression is also induced by PPAR activation. Hence, the observed induction of *ABCD2* expression might also be mediated via LXR α , because a potential LXR response element appears to be present in the *ABCD2* promoter region. Induction of *ABCD2* and *ABCD3* expression is relevant because increased expression may compensate for the loss of ABCD1 in patients with X-linked adrenoleukodystrophy (see above).

It has long been known that the expression of the drug transporter MDR1 can be induced by various drugs, such as rifampin. A potential mechanism for this induction was provided recently, through the identification of the transcription factor SXR (the homolog in rodents is called PXR) as a regulator of *MDR1*

expression (389). SXR is also a member of the family of nuclear receptors (390). It has a flexible binding cavity that can accommodate many structurally different ligands that include rifampin, phenobarbital, pregnenolone-16 α -carbonitrile, paclitaxel (taxol), clotrimazole, hyperforin, lithocholate, and also the HIV protease inhibitor ritonavir and ingredients of St. John's wort (391). Indeed, SXR is best described as a receptor for xenobiotics. There is considerable species difference in the affinity of the receptor for various ligands as evidenced by the very different response in rodents and humans (390). Dussault et al. (392) showed very recently that ritonavir, a peptide mimetic that inhibits HIV protease, strongly induces the expression of *MDR1* in intestinal LS180 cells together with *CYP3A4*, *CYP2C8*, and *MRP2*. This induction most likely occurs via SXR and, indeed, a consensus sequence for PXR/SXR has recently been identified in the promoter sequence of rat *Mrp 2* (F. Simon, personal communication). The role of SXR in *MDR1* expression now explains why *MDR1* is induced by compounds like rifampin, paclitaxel, or St. John's wort, a commonly used herbal medicine (168, 344, 393). Variations in intestinal Pgp can affect uptake of digoxin (393) and cyclosporin A (394, 395) from the gut.

SXR may be considered a master regulator of xenobiotic metabolism, given the fact that it induces proteins of phase I metabolism and transporters simultaneously. An important example of this dual action is paclitaxel, which is metabolized by cytochrome P450 and excreted via Pgp. Since paclitaxel is a ligand for SXR, it induces its own metabolism as well as its secretion from the body. Hence, SXR is an interesting target for the modulation of oral availability and pharmacokinetics of drugs. Antagonism of this receptor may not only increase the oral bioavailability of drugs that are substrates for *MRP2* and *MDR1* Pgp, but at the same time decrease their metabolism by cytochrome P450. Synold et al. (389) showed that ecteinascidin-743 is a strong antagonist of SXR (nearly full inhibition at 50 nM) and found that this compound indeed represses SXR-induced expression of *MDR1*. This illustrates the potential pharmacological importance of this approach, even though experiments in rodents cannot be simply translated to man because of the difference in ligand specificity of SXR and PXR.

CONCLUDING REMARKS

This chapter provides a snapshot of fast-moving targets, the mammalian ABC transporters. Some of these transporters, such as Pgp or *MRP2*, were discovered long ago and their function is now fairly well defined, although much remains to be done. Others have recently arrived on the scene and their outline still lacks focus. By far the largest group is still no more than a sequence in a database, a promise for new insights to be gained. Given the enormous investments in the biochemistry of metabolism and drug disposition in the past 50 years, it is remarkable that so many of these transporters, now known to be vital, remained

out of sight for so long. The coming years will see a concerted effort to determine the function of each of the 48 human ABC transporters. We expect that genetic defects in humans and gene disruption in mice will play an important role in unraveling function, as they have in the recent past. Experiments with highly purified transporters reconstituted in artificial lipid membranes together with the 3-D structure of some transporters should establish how ABC transporters work.

The medical implications of this new knowledge should rapidly increase. As detailed in this chapter, ABC transporters such as Pgp and BCRP affect the oral availability of drugs, the accumulation of drugs in brain, fetus, testis, etc., and the disposition and elimination of drugs in the body. This knowledge will be exploited in drug design or by combining drugs with effective inhibitors for these transporters. A new area will be the exploration of natural polymorphisms in transporters that affect drug disposition or response to drugs, a topic hardly touched thus far. Finally, genetic defects in each of the 48 human ABC transporters will be identified, serious ones that might justify prenatal diagnosis and abortion, minor ones that may be neutralized by treatment or lifestyle adjustments. Given this potential, this chapter is not more than a first-quarter report of a rich and fruitful biochemical year lying ahead.

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LITERATURE CITED

- Higgins CF. 1992. *Annu. Rev. Cell Biol.* 8:67–113
- Dean M, Rzhetsky A, Allikmets R. 2001. *Genome Res.* 11:1156–66
- Müller M. 2001. *48 Human ATP-Binding Cassette Transporters*. <http://www.nutrigene.4t.com/humanabc.htm>
- Valverde MA, Diaz M, Sepúlveda FV, Gill DR, Hyde SC, Higgins CF. 1992. *Nature* 355:830–33
- Gill DR, Hyde SC, Higgins CF, Valverde MA, Mintenig GM, Sepúlveda FV. 1992. *Cell* 71:23–32
- Valverde MA, Bond TD, Hardy SP, Taylor JC, Higgins CF, et al. 1996. *EMBO J.* 15:4460–68
- Bond TD, Higgins CF, Valverde MA. 1998. *Methods Enzymol.* 292:359–70
- Sarkadi B, Müller M, Homolya L, Holló Z, Sepródi J, et al.. 1994. *FASEB J.* 8:766–71
- Borgnia MJ, Eytan GD, Assaraf YG. 1996. *J. Biol. Chem.* 271:3163–71
- Schmitt L, Tampé R. 2000. *ChemBioChem* 1:16–35
- Young L, Leonhard K, Tatsuta T,

- Trowsdale L, Langer T. 2001. *Science* 291:2135–38
11. Holland IB, Kuchler K, Higgins C, Cole S, eds. 2002. *ABC Proteins: From Bacteria to Man*. London: Academic
12. Hrycyna CA, Gottesman MM. 1998. *Drug Resist. Updates* 1:81–83
13. Van Veen HW, Konings WN. 1997. *Semin. Cancer Biol.* 8:183–91
14. Sugiyama Y, Kusuhara H, Suzuki H. 1999. *J. Control.* 62:179–86
15. Borst P, Zelcer N, van Helvoort A. 2000. *Biochim. Biophys. Acta* 1486:128–44
16. Suzuki H, Sugiyama Y. 2000. *Eur. J. Pharm. Sci.* 12:3–12
17. Hooiveld GJEJ, Van Montfoort JE, Meijer DKF, Müller M. 2001. *Eur. J. Pharm. Sci.* 12:525–43
18. Borst P. 1997. *Semin. Cancer Biol.* 8:131–34
19. Arnberg AC, Van Bruggen EF, Clegg RA, Upholt WB, Borst P. 1974. *Biochim. Biophys. Acta* 361:266–76
20. Sanders JP, Flavell RA, Borst P, Mol JN. 1973. *Biochim. Biophys. Acta* 312:441–57
21. Suzuki H, Sugiyama Y. 1999. In *Membrane Transporters as Drug Targets*, ed. GL Amidon, W Sadée, pp. 387–439. New York: Kluwer Academic/Plenum
22. Oude Elferink R, Groen AK. 2001. *Biochim. Biophys. Acta*. Preprint
23. Jansen PLM, Müller M, Sturm E. 2001. *Hepatology* 34:1067–74
24. Gottesman MM, Pastan I, Ambudkar SV. 1996. *Curr. Opin. Genet. Dev.* 6:610–17
25. Ambudkar SV, Dey S, Hrycyna CA, Pastan IRM, Gottesman MM. 1999. *Annu. Rev. Pharmacol. Toxicol.* 39:361–98
26. Hipfner DR, Deeley RG, Cole SPC. 1999. *Biochim. Biophys. Acta* 1461:359–76
27. Leslie EM, Deeley RG, Cole SPC. 2001. *Toxicology* 167:3–23
28. Hipfner DR, Mao Q, Qiu W, Leslie EM, Gao M, et al. 1999. *J. Biol. Chem.* 274:15420–26
- 28a. Schmitz G, Langmann T, Heimerl S. 2001. *J. Lipid Res.* 42:1–8
29. Chang G, Roth CB. 2001. *Science* 293:1793–800
30. Van Veen HW, Venema K, Bolhuis H, Oussenko I, Kok J, et al. 1996. *Proc. Natl. Acad. Sci. USA* 93:10668–72
31. Van Veen HW, Callaghan R, Socenecantu L, Sardini A, Konings WN, Higgins CF. 1998. *Nature* 391:291–95
32. Higgins CF, Linton KJ. 2001. *Science* 293:1782–84
33. Loo TW, Clarke DM. 1999. *Biochem. Cell Biol.* 77:11–23
34. Jones PM, George AM. 1999. *J. Membr. Biol.* 166:133–47
35. Jones PM, George AM. 1999. *FEMS Microbiol. Lett.* 179:187–202
36. Rosenberg MF, Callaghan R, Ford RC, Higgins CF. 1997. *J. Biol. Chem.* 272:10685–94
37. Hopfner K-P, Karcher A, Shin DS, Craig L, Arthur LM, et al. 2000. *Cell* 101:789–800
38. Zhou T, Radaev S, Rosen BP, Gatti DL. 2000. *EMBO J.* 19:4838–45
39. Loo TW, Clarke DM. 1995. *J. Biol. Chem.* 270:843–48
40. Loo TW, Clarke DM. 2000. *J. Biol. Chem.* 275:5253–56
41. Vos JC, Reits EAJ, Wojcik-Jacobs E, Neeffjes J. 2000. *Curr. Biol.* 10:1–7
42. Reits EAJ, Griekspoor AC, Neeffjes J. 2000. *Immunol. Today* 21:598–600
43. Reits EAJ, Vos JC, Grommé M, Neeffjes J. 2000. *Nature* 404:774–78
44. Senior AE, Al-Shawi MK, Urbatsch IL. 1995. *FEBS Lett.* 377:285–89
45. Senior AE, Gadsby DC. 1997. *Semin. Cancer Biol.* 8:143–50
46. Arora S, Lapinski PE, Raghavan M. 2001. *Proc. Natl. Acad. Sci. USA* 98:7241–46
47. Van Veen HW, Margolles A, Müller M, Higgins CF, Konings WN. 2000. *EMBO J.* 19:2503–14

48. Senior AE, Bhagat S. 1998. *Biochemistry* 37:831–36
49. Sauna ZE, Ambudkar SV. 2001. *J. Biol. Chem.* 276:11653–61
50. Druley TE, Stein WD, Ruth A, Roninson IB. 2001. *Biochemistry* 40:4323–31
51. Sauna ZE, Ambudkar SV. 2000. *Proc. Natl. Acad. Sci. USA* 97:2515–20
52. Sauna ZE, Smith MM, Müller M, Ambudkar SV. 2001. *J. Biol. Chem.* 276:33301–4
53. Szakács G, Ozvegy C, Bakos E, Sarkadi B, Váradi A. 2000. *Biochem. Biophys. Res. Commun.* 276:1314–19
54. Szakács G, Ozvegy C, Bakos E, Sarkadi B, Váradi A. 2001. *Biochem. J.* 356:71–75
55. Gao M, Cui H-R, Loe DW, Grant CE, Almquist KC, et al. 2000. *J. Biol. Chem.* 275:13098–108
56. Nagata K, Nishitani M, Matsuo M, Kioka N, Amachi T, Ueda K. 2000. *J. Biol. Chem.* 275:17626–30
57. Germann UA. 1996. *Eur. J. Cancer* 32A:927–44
58. Ueda K, Taguchi Y, Morishima M. 1997. *Semin. Cancer Biol.* 8:151–59
59. Loo TW, Clarke DM. 2001. *J. Biol. Chem.* 276:14972–79
60. Loo TW, Clarke DM. 1999. *J. Biol. Chem.* 274:24759–65
61. Shapiro AB, Ling V. 1997. *Eur. J. Biochem.* 250:130–37
62. Dey S, Ramachandra M, Pastan I, Gottesman M, Ambudkar SV. 1997. *Proc. Natl. Acad. Sci. USA* 94:10594–99
63. Sharom FJ, Liu R, Qu Q, Romsicki Y. 2001. *Cell Dev. Biol.* 12:257–65
64. Shapiro AB, Fox K, Lam P, Ling V. 1999. *Eur. J. Biochem.* 259:841–50
65. Martin C, Berridge G, Higgins CF, Callaghan R. 1997. *Br. J. Pharmacol.* 122:765–71
66. Shapiro AB, Corder AB, Ling V. 1997. *Eur. J. Biochem.* 250:115–21
67. Shapiro AB, Ling V. 1997. *Eur. J. Biochem.* 250:122–29
68. Bolhuis H, Van Veen HW, Molenaar D, Poolman B, Driessen AJM, Konings WN. 1996. *EMBO J.* 15:4239–45
69. Higgins CF, Gottesman MM. 1992. *Trends Biochem. Sci.* 17:18–21
70. Margolles A, Putman M, Van Veen HW, Konings WN. 1999. *Biochemistry* 38:16298–306
71. Eytan GD, Regev R, Oren G, Assaraf YG. 1996. *J. Biol. Chem.* 271:12897–902
72. Regev R, Eytan GD. 1997. *Biochem. Pharmacol.* 54:1151–58
73. Urbatsch IL, Al-Shawi MK, Senior AE. 2001. *Biochemistry* 33:7069–76
74. Ambudkar SV, Cardarelli CO, Pashinsky I, Stein WD. 1997. *J. Biol. Chem.* 272:21160–66
75. De Graaf D, Sharma RC, Mechetner EB, Schimke RT, Roninson IB. 1996. *Proc. Natl. Acad. Sci. USA* 93:1238–42
76. Allen JD, Brinkhuis RF, Van Deemter L, Wijnholds J, Schinkel AH. 2000. *Cancer Res.* 60:5761–66
77. Tanigawara Y, Okamura N, Hirai M, Yasuhara M, Ueda K, et al. 1992. *J. Pharmacol. Exp. Ther.* 263:840–45
78. Schinkel AH, Wagenaar E, Van Deemter L, Mol CAAM, Borst P. 1995. *J. Clin. Invest.* 96:1698–705
79. Schinkel AH, Wagenaar E, Mol CAAM, Van Deemter L. 1996. *J. Clin. Invest.* 97:2517–24
80. Evers R, Zaman GJR, Van Deemter L, Jansen H, Calafat J, et al. 1996. *J. Clin. Invest.* 97:1211–18
81. van Helvoort A, Smith AJ, Sprong H, Fritzsche I, Schinkel AH, et al. 1996. *Cell* 87:507–17
82. Evers R, Kool M, Van Deemter L, Jansen H, Calafat J, et al. 1998. *J. Clin. Invest.* 101:1310–19
83. Kool M, Van der Linden M, De Haas M, Scheffer GL, De Vree JML, et al. 1999. *Proc. Natl. Acad. Sci. USA* 96:6914–19
84. Wijnholds J, Mol CAAM, Van Deemter L, De Haas M, Scheffer GL, et

- al. 2000. *Proc. Natl. Acad. Sci. USA* 97:7476–81
85. Smith AJ, Mayer U, Schinkel AH, Borst P. 1998. *J. Natl. Cancer Inst.* 90:1161–66
 86. Bain LJ, McLaclan JB, LeBlanc GA. 1997. *Environ. Health Perspect.* 105: 812–18
 87. Seelig A. 1998. *Eur. J. Biochem.* 251: 251–61
 88. Seelig A, Landwojtowicz E. 2001. *Eur. J. Pharm. Sci.* 12:31–40
 89. Zheleznova EE, Markham P, Edgar R, Bibi E, Neyfakh AA, Brennan RG. 2000. *Trends Biochem. Sci.* 25:39–42
 90. Vazquez-Laslop N, Zheleznova EE, Markham PN, Brennan RG, Neyfakh AA. 2000. *Biochem. Soc. Trans.* 28:517–20
 91. Smit JJM, Schinkel AH, Oude Elferink RPJ, Groen AK, Wagenaar E, et al. 1993. *Cell* 75:451–62
 92. Schinkel AH, Smit JJM, Van Tellingen O, Beijnen JH, Wagenaar E, et al. 1994. *Cell* 77:491–502
 93. Schinkel AH, Mayer U, Wagenaar E, Mol CAAM, Van Deemter L, et al. 1997. *Proc. Natl. Acad. Sci. USA* 94:4028–33
 94. Wijnholds J, De Lange ECM, Scheffer GL, Van den Berg DJ, Mol CAAM, et al. 2000. *J. Clin. Invest.* 105:279–85
 95. Johnson DR, Finch RA, Lin ZP, Zeiss CJ, Sartorelli AC. 2001. *Cancer Res.* 61:1469–76
 96. Panwala CM, Jones JC, Viney JL. 1998. *J. Immunol.* 161:5733–44
 97. Schuetz EG, Umbenhauer DR, Yasuda K, Brimer C, Nguyen L, et al. 2000. *Mol. Pharmacol.* 57:188–87
 98. Schinkel AH. 1999. *Adv. Drug Deliv. Rev.* 36:179–94
 99. Naito M, Tsuruo T. 1996. In *Multidrug Resistance in Cancer Cells*, ed. S Gupta, T Tsuruo, pp. 321–33. Chichester, UK: Wiley
 100. Tsuji A, Tamai I. 1998. In *Introduction to the Blood-Brain Barrier*, ed. WM Pardridge, pp. 238–47. Cambridge: Cambridge Univ. Press
 101. Borst P, Schinkel AH. 1998. See Ref. 100, pp. 198–206
 102. Van Asperen J, Mayer U, Van Tellingen O, Beijnen JH. 1997. *J. Pharm. Sci.* 86:881–84
 103. Tamai I, Tsuji A. 2000. *J. Pharm. Sci.* 89:1371–88
 104. Fromm MF. 2000. *Int. J. Clin. Pharmacol. Ther.* 38:69–74
 105. Meijer OC, De Lange ECM, Breimer DD, de Boer AG, Workel JO, De Kloet ER. 1998. *Endocrinology* 139:1789–93
 106. Kim RB, Fromm MF, Wandel C, Leake B, Wood AJJ, et al. 1998. *J. Clin. Invest.* 101:289–94
 107. Van der Sandt ICJ, Vos CMP, Nabulsi L, Blom-Roosemalen MCM, Voorwinden HH, et al. 2001. *AIDS* 15:483–91
 108. Tews DS, Nissen A, Külgén C, Gammann AKA. 2000. *J. Neurooncol.* 50:227–37
 109. Bredel M. 2001. *Brain Res. Rev.* 35:161–204
 110. Drion N, Lemaire M, Lefauconnier J-M, Scherrmann J-M. 1996. *J. Neurochem.* 67:1688–93
 111. Desrayaud S, Guntz P, Scherrmann J-M, Lemaire M. 1997. *Life Sci.* 61:153–63
 112. Kusuhara H, Suzuki H, Terasaki T, Kakee A, Lemaire M, Sugiyama Y. 1997. *J. Pharmacol. Exp. Ther.* 283: 574–80
 113. Mayer U, Wagenaar E, Dorobek B, Beijnen JH, Borst P, Schinkel AH. 1997. *J. Clin. Invest.* 100:2430–36
 114. Zhang Z-J, Saito T, Kimura Y, Sugimoto C, Ohtsubo T, Saito H. 2000. *Brain Res.* 852:116–26
 115. Lankas GR, Wise LD, Cartwright ME, Pippert T, Umbenhauer DR. 1998. *Reprod. Toxicol.* 12:457–63
 116. Smit JW, Huisman T, Van Tellingen O, Wiltshire HR, Schinkel AH. 1999. *J. Clin. Invest.* 104:1441–47
 117. Croop JM, Raymond M, Haber D,

- Devault A, Arceci RJ, et al. 1989. *Mol. Cell. Biol.* 9:1346–50
118. Keller RP, Altermatt HJ, Donatsch P, Zihlmann H, Laissue JA, Hiestand PC. 1992. *Int. J. Cancer* 51:433–38
119. Su S-F, Huang J-D. 1996. *Drug Metab. Dispos.* 24:142–47
120. Terao T, Hisanaga E, Sai Y, Tamai I, Tsuji A. 1996. *J. Pharm. Pharmacol.* 48:1083–89
121. Mayer U, Wagenaar E, Beijnen JH, Smit JW, Meijer DKF, et al. 1996. *Br. J. Pharmacol.* 119:1038–44
122. Sparreboom A, Van Asperen J, Mayer U, Schinkel AH, Smit JW, et al. 1997. *Proc. Natl. Acad. Sci. USA* 94:2031–35
123. Van Asperen J, Van Tellingen O, Sparreboom A, Schinkel AH, Borst P, et al. 1997. *Br. J. Cancer* 76:1181–85
124. Schellens JHM, Malingré MM, Kruijtzter CMF, Bardelmeijer HA, Van Tellingen O, et al. 2000. *Eur. J. Pharm. Sci.* 12:103–10
125. Terwogt JMM, Beijnen JH, Huinink WWT, Rosing H, Schellens JHM. 1998. *Lancet* 352:285
126. Terwogt JMM, Malingré MM, Beijnen JH, Huinink WWT, Rosing H, Koopman FJ, et al. 1999. *Clin. Cancer Res.* 5:3379–84
127. Malingré MM, Richel DJ, Beijnen JH, Rosing H, Koopman FJ, et al. 2001. *J. Clin. Oncol.* 19:1160–66
128. Van Asperen J, Van Tellingen O, Beijnen JH. 2000. *Drug Metab. Dispos.* 28:264–67
129. Broxterman HJ, Schuurhuis GJ, Lankelma J, Oberink JW, Eekman CA, et al. 1997. *Br. J. Cancer* 76:1029–34
130. Faneyte IF, Kristel PM, van de Vijver MJ. 2001. *Int. J. Cancer* 93:114–22
131. Fracasso PM, Brady MF, Moore DH, Walker JL, Rose PG, et al. 2001. *J. Clin. Oncol.* 19:2975–82
132. Baekelandt M, Lehne G, Tropé CG, Szántó I, Pfeiffer P, et al. 2001. *J. Clin. Oncol.* 19:2983–93
133. Advani R, Fisher GA, Lum BL, Hausdorff J, Halsey J, et al. 2001. *Clin. Cancer Res.* 7:1221–29
134. Sonneveld P. 1996. *Bailliere's Best Pract. Clin. Haematol.* 9:185–203
135. Arceci RJ. 2000. *Br. J. Haematol.* 110:285–91
136. Sikic BI, Fisher GA, Lum BL, Halsey J, Beketic-Oreskovic L, Chen G. 1997. *Cancer Chemother. Pharmacol.* 40(Suppl.):S13–19
137. Sandor V, Fojo T, Bates SE. 1998. *Drug Resist. Updates* 1:190–200
138. Tan B, Piwnica-Worms D, Ratner L. 2000. *Curr. Opin. Oncol.* 12:450–58
139. Chan HSL, De Boer G, Haddad G, Gallie BL, Ling V. 1995. *Hematol. Oncol. Clin. North Am.* 9:275–318
140. Goldstein L. 1995. *Curr. Probl. Cancer* 19:67–124
141. Mickley LA, Lee J-S, Weng Z, Zhan Z, Alvarez M, et al. 1998. *Blood* 91:1749–56
142. Van den Heuvel-Eibrink MM, Wiemer EAC, De Boevere MJ, Van der Holt B, Vossebeld PJM, et al. 2001. *Blood* 97:3605–11
143. Bosch I, Dunussi-Joannopoulos K, Wu R-L, Furlong ST, Croop J. 1997. *Biochemistry* 36:5685–94
144. Romsicki Y, Sharom FJ. 2001. *Biochemistry* 40:6937–47
145. Borst P, van Meer G, Oude Elferink R. 2002. See Ref. 11, pp. In Press
146. Bezombes C, Maestre N, Laurent G, Levade T, Bettaïeb A, Jaffrézou J-P. 1998. *FASEB J.* 12:101–9
147. Riggers RJ. 2001. *Lipid Translocation by Multidrug Transporters*, pp. 7–128. AMC/Univ. Amsterdam
148. Ernest S, Bello-Reuss E. 1999. *J. Am. Soc. Nephrol.* 10:2306–13
149. Riggers RJ, Vogels I, van Meer G. 2001. *Biochem. J.* 357:859–65
150. Gruol DJ, Vo QD, Zee MC. 1999. *Biochem. Pharmacol.* 58:1191–99
151. Johnstone RW, Ruefli AA, Smyth MJ. 2000. *Trends Biochem. Sci.* 25:1–6
152. Fojo AT, Ueda K, Slamon DJ, Poplack

- DG, Gottesman MM, Pastan I. 1987. *Proc. Natl. Acad. Sci. USA* 84:265–69
153. Bradley G, Georges E, Ling V. 1990. *J. Cell. Physiol.* 145:398–408
154. Trezise AEO, Romano PR, Gill DR, Hyde SC, Sepúlveda FV, et al. 1992. *EMBO J.* 11:4291–303
155. Brown PC, Thorgeirsson SS, Silverman JA. 1993. *Nucleic Acids Res.* 21:3885–91
156. Barnes KM, Dickstein B, Cutler JGB, Fojo T, Bates SE. 1996. *Biochemistry* 35:4820–27
157. Wang E, Casciano CN, Clement RP, Johnson WW. 2000. *Biochem. Biophys. Res. Commun.* 276:909–16
158. Debry P, Nash EA, Neklason DW, Metherall JE. 1997. *J. Biol. Chem.* 272:1026–31
159. Luker GD, Nilsson KR, Covey DF, Piwnica-Worms D. 1999. *J. Biol. Chem.* 274:6979–91
160. Raghu G, Park SW, Roninson IB, Mechetner EB. 1996. *Exp. Hematol.* 24:1258–64
161. Drach J, Gsur A, Hamilton G, Zhao S, Angerler J, et al. 1996. *Blood* 88:1747–54
162. Bunting KD, Galipeau J, Topham D, Benaim E, Sorrentino BP. 1999. *Ann. NY Acad. Sci.* 872:125–41
163. Randolph GJ, Beaulieu S, Pope M, Sugawara I, Hoffman L, et al. 1998. *Proc. Natl. Acad. Sci. USA* 95:6924–29
164. Frank MH, Denton MD, Alexander SI, Khoury SJ, Sayegh MH, Briscoe DM. 2001. *J. Immunol.* 166:2451–59
165. Johnstone RW, Cretney E, Smyth MJ. 1999. *Blood* 93:1075–85
166. Johnstone RW, Tainton KM, Ruefli AA, Froelich CJ, Cerruti L, et al. 2001. *J. Biol. Chem.* 276:16667–73
167. Borst P, Van Blitterswijk WJ, Borst J, Tepper AD, Schinkel AH. 1998. *Drug Resist. Updates* 1:337–39
168. Hoffmeyer S, Burk O, Von Richter O, Arnold HP, Brockmöller J, et al. 2000. *Proc. Natl. Acad. Sci. USA* 97:3473–78
169. Cascorbi I, Gerloff T, John A, Meisel C, Hoffmeyer S, et al. 2001. *Clin. Pharmacol. Ther.* 69:169–74
170. Ito S, Ieiri I, Tanabe M, Suzuki A, Higuchi S, Otsubo K. 2001. *Pharmacogenetics* 11:175–84
171. Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, et al. 2001. *J. Pharmacol. Exp. Ther.* 297:1137–43
172. Umbenhauer DR, Lankas GR, Pippert TR, Wise LD, Cartwright ME, et al. 1997. *Toxicol. Appl. Pharmacol.* 146:88–94
173. Bleicher KB, Pippert TR, Glaab WE, Skopek TR, Sina JF, Umbenhauer DR. 2001. *J. Biochem. Mol. Toxicol.* 15:133–42
174. Lankas GR, Cartwright ME, Umbenhauer D. 1997. *Toxicol. Appl. Pharmacol.* 143:357–65
175. Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, et al. 1998. *Proc. Natl. Acad. Sci. USA* 95:15665–70
176. Allikmets R, Schriml LM, Hutchinson A, Romano-Spica V, Dean M. 1998. *Cancer Res.* 58:5337–39
177. Miyake K, Mickley L, Litman T, Zhan Z, Robey R, et al. 1999. *Cancer Res.* 59:8–13
178. Ozvegy C, Litman T, Szakács G, Nagy Z, Bates S, et al. 2001. *Biochem. Biophys. Res. Commun.* 285:111–17
179. Brangi M, Litman T, Ciotti M, Nishiyama K, Kohlhagen G, et al. 1999. *Cancer Res.* 59:5938–46
180. Allen JD, Brinkhuis RF, Wijnholds J, Schinkel AH. 1999. *Cancer Res.* 59:4237–41
181. Robey RW, Medina-Pérez WY, Nishiyama K, Lohusen T, Miyake K, et al. 2001. *Clin. Cancer Res.* 7:145–52
182. Maliepaard M, van Gastelen MA, De Jong LA, Pluim D, Van Waardenburg RCAM, Ruevekamp-Helmers MC. 1999. *Cancer Res.* 59:4559–63
183. Maliepaard M, Scheffer GL, Faneyte IF, van Gastelen MA, Pijnenborg

- ACLM, et al. 2001. *Cancer Res.* 61:3458–64
- 183a. Maliepaard M, van Gastelen MA, Tohgo A, Hausheer FH, van Waardenburg RCAM, et al. 2001. *Clin. Cancer Res.* 7:935–41
184. Rocchi E, Khodjakov A, Volk EL, Yang CH, Litman T, et al. 2000. *Biochem. Biophys. Res. Commun.* 271:42–46
185. Scheffer GL, Kool M, Heijn M, De Haas M, Pijnenborg ACLM, et al. 2000. *Cancer Res.* 60:5269–77
186. Jonker JW, Smit JW, Brinkhuis RF, Maliepaard M, Beijnen JH, et al. 2000. *J. Natl. Cancer Inst.* 92:1651–56
187. de Bruin M, Miyake K, Litman T, Robey R, Bates SE. 1999. *Cancer Lett.* 146:117–26
188. Rabindran SK, He HY, Singh M, Brown E, Collins KI, et al. 1998. *Cancer Res.* 58:5850–58
189. Rabindran SK, Ross DD, Doyle LA, Yang WD, Greenberger LM. 2000. *Cancer Res.* 60:47–50
190. Van Loevezijn A, Allen JD, Schinkel AH, Koomen G-J. 2001. *Bioorg. Med. Chem. Lett.* 11:29–32
191. Zhou S, Schuetz JD, Bunting KD, Colapietro A-M, Sampath J, et al. 2001. *Nat. Med.* 7:1028–34
192. Ishikawa T. 1992. *Trends Biochem. Sci.* 17:463–68
193. Cole SPC, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, et al. 1992. *Science* 258:1650–54
194. Paulusma CC, Bosma PJ, Zaman GJR, Bakker CTM, Otter M, et al. 1996. *Science* 271:1126–28
195. König P, Giraldo R, Chapman L, Rhodes D. 1996. *Cell* 85:125–36
196. Kool M, De Haas M, Scheffer GL, Scheper RJ, Van Eijk MJT, et al. 1997. *Cancer Res.* 57:3537–47
197. Allikmets R, Gerrard B, Hutchinson A, Dean M. 1996. *Hum. Mol. Genet.* 5:1649–55
198. Hopper E, Belinsky MG, Zeng H, Tosolini A, Testa JR, Kruh GD. 2001. *Cancer Lett.* 162:181–91
199. Tammur J, Prades C, Arnould I, Rzhetsky A, Hutchinson A, et al. 2001. *Gene* 273:89–96
200. Varádi A, Tusnády GE, Sarkadi B. 2002. See Ref. 11, In press
201. Bakos E, Evers R, Szakács G, Tusnády GE, Welker E, et al. 1998. *J. Biol. Chem.* 273:32167–75
202. Rosenberg MF, Mao Q, Holzenburg A, Ford RC, Deeley RG, Cole SPC. 2001. *J. Biol. Chem.* 276:16076–82
203. Loe DW, Almquist KC, Cole SP, Deeley RG. 1996. *J. Biol. Chem.* 271:9683–89
204. Sakamoto H, Hara H, Hirano K, Adachi T. 1999. *Cancer Lett.* 135:113–19
205. Qian Y-M, Song W-C, Cui H, Cole SPC, Deeley RG. 2001. *J. Biol. Chem.* 276:6404–11
206. Leslie EM, Ito K, Upadhyaya P, Hecht SS, Deeley RG, Cole SPC. 2001. *J. Biol. Chem.* 276:27,846–54
207. Loe DW, Almquist KC, Deeley RG, Cole SPC. 1996. *J. Biol. Chem.* 271:9675–82
208. Loe DW, Deeley RG, Cole SPC. 1998. *Cancer Res.* 58:5130–36
209. Renes J, De Vries EGE, Nienhuis EF, Jansen PLM, Müller M. 1999. *J. Pharmacol.* 126:681–88
210. Salerno M, Garnier-Suillerot A. 2001. *Eur. J. Pharmacol.* 421:1–9
211. König J, Nies AT, Cui Y, Leier I, Keppler D. 1999. *Biochim. Biophys. Acta* 1461:377–94
212. Renes J, De Vries EGE, Jansen PLM, Müller M. 2000. *Drug Resist. Updates* 3:289–302
213. Evers R, De Haas M, Sparidans R, Beijnen J, Wielinga PR, et al. 2000. *Br. J. Cancer* 83:375–83
214. Heijn M, Hooijberg JH, Scheffer GL, Szabó G, Westerhoff HV, Lankelma J. 1997. *Biochim. Biophys. Acta* 1326:12–22

215. Zhang D-W, Cole SPC, Deeley RG. 2001. *J. Biol. Chem.* 276:13231–39
216. Ito K, Olsen SL, Qiu W, Deeley RG, Cole SPC. 2001. *J. Biol. Chem.* 276:15616–24
217. Ryu S, Kawabe T, Nada S, Yamaguchi A. 2000. *J. Biol. Chem.* 275:39617–24
218. Wijnholds J, Evers R, van Leusden MR, Mol CAAM, Zaman GJR, et al. 1997. *Nat. Med.* 11:1275–79
219. Lorico A, Rappa G, Finch RA, Yang D, Flavell RA, Sartorelli AC. 1997. *Cancer Res.* 57:5238–42
220. Schultz MJ, Wijnholds J, Peppelenbosch MP, Vervoordeldonk MJB, Speelman P, et al. 2001. *J. Immunol.* 166:4059–64
221. Robbiani DF, Finch RA, Jager D, Muller WA, Sartorelli AC, Randolph GJ. 2000. *Cell* 103:757–68
222. Leier I, Jedlitschky G, Buchholz U, Center M, Cole SPC, et al. 1996. *Biochem. J.* 314:433–37
223. Rappa G, Lorico AL, Flavell RA, Sartorelli AC. 1997. *Cancer Res.* 57:5232–37
224. Paulusma CC, Van Geer MA, Evers R, Heijn M, Ottenhoff R, et al. 1999. *Biochem. J.* 338:393–401
225. Ballatori N, Rebbeor JF. 1999. *Semin. Liver Dis.* 18:377–87
226. Zelcer N, Saeki T, Reid G, Beijnen JH, Borst P. 2001. *J. Biol. Chem.* 276:46400–7
227. Hirrlinger J, König J, Keppler D, Lindenau J, Schulz JB, Dringen R. 2001. *J. Neurochem.* 76:627–36
228. Flens MJ, Zaman GJR, Van der Valk P, Izquierdo MA, Schroeijers AB, et al. 1996. *Am. J. Pathol.* 148:1237–47
229. Kartenbeck J, Leuschner U, Mayer R, Keppler D. 1996. *Hepatology* 23:1061–66
230. Wijnholds J, Scheffer GL, Van der Valk M, Beijnen JH, Scheper RJ, Borst P. 1998. *J. Exp. Med.* 188:797–808
231. Raggars RJ, van Helvoort A, Evers R, van Meer G. 1999. *J. Cell Sci.* 112:415–22
232. Saito T, Zhang Z-J, Tokuriki M, Ohtsubo T, Noda I, et al. 2001. *Brain Res.* 895:253–57
233. Gao B, Meier PJ. 2001. *Microsc. Res. Tech.* 52:60–64
234. Ghersi-Egea J-F, Strazielle N. 2001. *Microsc. Res. Tech.* 52:83–88
235. Rao VV, Dehlheimer JL, Bardgett ME, Snyder AZ, Finch RA, et al. 1999. *Proc. Natl. Acad. Sci. USA* 96:3900–5
236. Lorico A, Rappa G, Flavell RA, Sartorelli AC. 1996. *Cancer Res.* 56:5351–55
237. Norman BH. 1998. *Drugs Future* 23:1001–13
238. Loe DW, Deeley RG, Cole SPC. 2000. *J. Pharmacol. Exp. Ther.* 293:530–38
239. Böhme M, Jedlitschky G, Leier I, Büchler M, Keppler D. 1994. *Adv. Enzyme Regul.* 34:371–80
240. Kornberg A, Baker TA. 1992. *DNA Replication*, pp. 637–87. San Francisco: Freeman
241. Evers R, Kool M, Smith AJ, Van Deemter L, De Haas M, Borst P. 2000. *Br. J. Cancer* 83:366–74
242. Draper MP, Martell RL, Levy SB. 1997. *Br. J. Cancer* 75:810–15
243. Duffy CP, Elliott CJ, O'Connor RA, Heenan MM, Coyle S, et al. 1998. *Eur. J. Cancer* 34:1250–59
244. Hooijberg JH, Broxterman HJ, Kool M, Assaraf YG, Peters GJ, et al. 1999. *Cancer Res.* 59:2532–35
- 244a. Zeng H, Chen Z-S, Belinsky MG, Rea PA, Kruh GD. 2001. *Cancer Res.* 61:7225–32
245. Leslie EM, Mao Q, Oleschuk CJ, Deeley RG, Cole SPC. 2001. *Mol. Pharmacol.* 59:1171–80
246. Tabas L, Shepard RL, Pratt S, Gruber JM, Norman BH, et al. 2001. *Proc. Am. Assoc. Cancer Res.* 42:949 (Abstr.)
247. Norman BH, Gruber JM, Kroin JS, Lander PA, Lohman MCP, et al. 2001.

- Proc. Am. Assoc. Cancer Res.* 42:949 (Abstr.)
248. Cole SPC, Sparks KE, Fraser K, Loe DW, Grant CE, et al. 1994. *Cancer Res.* 54:5902–10
249. Vernhet L, Allain N, Payen L, Anger J-P, Guillouzo A, Fardel O. 2001. *Biochem. Pharmacol.* 61:1387–91
250. Shen Z-X, Chen G-Q, Ni J-H, Li X-S, Xion S-M, et al. 1997. *Blood* 9:3354–60
251. Kala SV, Neely MW, Kala G, Prater CI, Atwood DW, et al. 2000. *J. Biol. Chem.* 275:33404–8
252. Vernhet L, Séité MP, Allain N, Guillouzo A, Fardel O. 2001. *J. Pharmacol. Exp. Ther.* 298:234–39
253. Borst P, Evers R, Kool M, Wijnholds J. 2000. *J. Natl. Cancer Inst.* 92:1295–302
254. Schaub TP, Kartenbeck J, König J, Vogel O, Witzgall R, et al. 1997. *J. Am. Soc. Nephrol.* 8:1213–21
255. Gotoh Y, Suzuki H, Kinoshita S, Hirohashi T, Kato Y, Sugiyama Y. 2000. *J. Pharmacol. Exp. Ther.* 292:433–39
256. Mottino AD, Hoffman T, Jennes L, Vore M. 2000. *J. Pharmacol. Exp. Ther.* 293:717–23
257. Fromm MF, Kauffmann HM, Fritz P, Burk O, Kroemer HK, et al. 2000. *Am. J. Pathol.* 157:1575–80
258. Van Aubel RAMH, Hartog A, Bindels RJ, Van Os CH, Russel FGM. 2001. *Eur. J. Pharmacol.* 400:195–98
259. Dietrich CG, De Waart DR, Ottenhoff R, Schoots IG, Oude Elferink RP. 2001. *Mol. Pharmacol.* 59:974–80
260. Dietrich CG, De Waart DR, Ottenhoff R, Bootsma AH, Van Gennip AH, Oude Elferink RP. 2001. *Carcinogenesis* 22:805–11
261. Borst P, Evers R, Kool M, Wijnholds J. 1999. *Biochim. Biophys. Acta* 1461:347–57
262. Koike K, Kawabe T, Tanaka T, Toh S, Uchiumi T, et al. 1997. *Cancer Res.* 57:5475–79
263. Cui Y, König J, Bucholz U, Spring H, Leier I, Keppler D. 1999. *Mol. Pharmacol.* 55:929–37
264. Ohga T, Koike K, Ono M, Makino Y, Itagaki Y, et al. 1996. *Cancer Res.* 56:4224–28
265. Ishikawa T, Wright CD, Ishizuka H. 1994. *J. Biol. Chem.* 269:29085–93
266. Keppler D, Leier I, Jedlitschky G, König J. 1998. *Chem. Biol. Interact.* 111–12:153–61
267. Jansen G, Assaraf YG, Priest DG, Bunni MA, Kool M, et al. 2002. In *Chemistry and Biology of Folates and Pteridines*, ed. S Milstien, Norwell, MA: Kluwer Acad. pp. 643–48
268. Nies AT, König J, Pfannschmidt M, Klar E, Hofmann WJ, Keppler D. 2001. *Int. J. Cancer* 94:492–99
269. König J, Rost D, Cui Y, Keppler D. 1999. *Hepatology* 29:1156–63
270. Scheffer GL, Kool M, De Haas M, De Vree JML, Pijnenborg ACLM, et al. 2002. *Lab. Invest.* 82:193–201
271. Hirohashi T, Suzuki H, Ito K, Ogawa K, Kume K, et al. 1998. *Mol. Pharmacol.* 53:1068–75
272. Ogawa K, Suzuki H, Hirohashi T, Ishikawa T, Meier PJ, et al. 2000. *Am. J. Physiol. Gastrointest. Liver Physiol.* 278:G438–46
273. Soroka CJ, Lee JM, Azzaroli F, Boyer JL. 2001. *Hepatology* 33:783–91
274. Donner MG, Keppler D. 2001. *Hepatology* 34:351–59
275. Hirohashi T, Suzuki H, Takikawa H, Sugiyama Y. 2000. *J. Biol. Chem.* 275:2905–10
276. Zeng H, Bain LJ, Belinsky MG, Kruh GD. 1999. *Cancer Res.* 59:5964–67
277. Yamada A, Kawano K, Koga M, Matsumoto T, Itoh K. 2001. *Cancer Res.* 61:6459–66
278. Young LC, Campling BG, Voskoglou-Nomikos T, Cole SPC, Deeley RG, Gerlach JH. 1999. *Clin. Cancer Res.* 5:673–80
279. Young LC, Campling BG, Cole SPC,

- Deeley RG, Gerlach JH. 2001. *Clin. Cancer Res.* 7:1798–804
280. Hirohashi T, Suzuki H, Sugiyama Y. 1999. *J. Biol. Chem.* 274:15181–85
281. Zeng H, Liu G, Rea PA, Kruh GD. 2000. *Cancer Res.* 60:4779–84
282. McAleer MA, Breen MA, White NL, Matthews N. 1999. *J. Biol. Chem.* 274:23541–48
283. Wijnholds J, Mol CAAM, Scheffer GL, Scheper RJ, Borst P. 1999. *Proc. Am. Assoc. Cancer Res.* 40:315 (Abstr.)
284. Schuetz JD, Connelly MC, Sun D, Paibir SG, Flynn PM, et al. 1999. *Nat. Med.* 5:1048–51
285. Lee K, Klein-Szanto AJP, Kruh GD. 2000. *J. Natl. Cancer Inst.* 92:1934–40
286. Borst P, Reid G, Saeki T, Wielinga P, Zelcer N. 2002. See Ref. 11. In press
287. Jedlitschky G, Burchell B, Keppler D. 2000. *J. Biol. Chem.* 275:30069–74
288. Chen Z-S, Lee K, Kruh GD. 2001. *J. Biol. Chem.* 276:33747–54
289. Lee K, Belinsky MG, Bell DW, Testa JR, Kruh GD. 1998. *Cancer Res.* 58:2741–47
290. Van Aubel RAMH, Smeets PHE, Peters JGP, Bindls RJM, Russel FGM. 2002. *J. Am. Soc. Nephrol.* In press
291. Belinsky MG, Bain LJ, Balsara BB, Testa JR, Kruh GD. 1998. *J. Natl. Cancer Inst.* 90:1735–41
292. Zhang Y, Han HY, Elmquist WF, Miller DW. 2000. *Brain Res.* 876:148–53
293. Schultz C, Vaskinn S, Kildalsen H, Sager G. 1998. *Biochemistry* 37:1161–66
294. Kool M, Van der Linden M, De Haas M, Baas F, Borst P. 1999. *Cancer Res.* 59:175–82
295. Belinsky MG, Kruh GD. 1999. *Br. J. Cancer* 80:1342–49
296. Madon J, Hagenbuch B, Landmann L, Meier PJ, Stieger B. 2000. *Mol. Pharmacol.* 57:634–41
297. Longhurst TJ, O'Neill GM, Harvie RM, Davey RA. 1996. *Br. J. Cancer* 74:1331–5
298. O'Neill GM, Peters GB, Harvie RM, MacKenzie HB, Henness S, Davey RA. 1998. *Br. J. Cancer* 77:2076–80
299. Kuss BJ, O'Neill GM, Eyre H, Doggett NA, Callen DF, Davey RA. 1998. *Genomics* 51:455–58
300. Bergen AA, Plomp AS, Schuurman EJ, Terry S, Breuning M, et al. 2000. *Nat. Genet.* 25:228–31
301. Le Saux O, Urban Z, Tschuch C, Csiszar K, Bacchelli B, et al. 2000. *Nat. Genet.* 25:223–27
302. Ringpfeil F, Lebowohl MG, Christiano AM, Uitto J. 2000. *Proc. Natl. Acad. Sci. USA* 97:6001–6
303. Smith AJ, De Vree JML, Ottenhoff R, Oude Elferink RPJ, Schinkel AH, Borst P. 1998. *Hepatology* 28:530–36
304. Smith AJ, van Helvoort A, van Meer G, Borst P, Szabó K, et al. 2000. *J. Biol. Chem.* 275:23530–39
305. Oude Elferink RPJ, Ottenhoff R, Van Wijland M, Smit JJM, Schinkel AH, Groen AK. 1995. *J. Clin. Invest.* 95:31–38
306. Crawford AR, Smith AJ, Hatch VC, Oude Elferink RPJ, Borst P, Crawford JM. 1997. *J. Clin. Invest.* 100:2562–67
307. Oude Elferink RPJ, Ottenhoff R, Van Marle J, Frijters CMG, Smith AJ, Groen AK. 1998. *J. Clin. Invest.* 102:1749–57
308. Cornacchia L, Domdey H, Mössner J, Berr F. 1997. *Biochem. Biophys. Res. Commun.* 231:277–82
309. London E, Brown DA. 2000. *Biochim. Biophys. Acta* 1508:182–95
310. Mauad TH, Van Nieuwkerk CMJ, Dingemans KP, Smit JJM, Schinkel AH, et al. 1994. *Am. J. Pathol.* 145:1237–45
311. De Vree JML, Jacquemin E, Sturm E, Cresteil D, Bosma PJ, et al. 1998. *Proc. Natl. Acad. Sci. USA* 95:282–87
312. Jacquemin E, De Vree JML, Cresteil D,

- Sokal EM, Sturm E, et al. 2001. *Gastroenterology* 120:1448–58
313. Van Nieuwkerk CMJ, Oude Elferink RPJ, Groen AK, Ottenhoff R, Tytgat GNJ, et al. 1996. *Gastroenterology* 111:165–71
314. Jacquemin E, Cresteil D, Manouvrier S, Boute O, Hadchouel M. 1999. *Lancet* 353:210–11
315. Rosmorduc O, Hermelin B, Poupon R. 2001. *Gastroenterology* 120:1459–67
316. Childs S, Yeh RL, Georges E, Ling V. 1995. *Cancer Res.* 55:2029–34
317. Gerloff T, Stieger B, Hagenbuch B, Madon J, Landmann L, et al. 1998. *J. Biol. Chem.* 273:10046–50
318. Noe J, Hagenbuch B, Meier PJ, St-Pierre MV. 2001. *Hepatology* 33:1223–31
319. Lecureur V, Sun D, Hargrove P, Schuetz EG, Kim RB, et al. 2000. *Mol. Pharmacol.* 57:24–35
320. Green RM, Hoda F, Ward KL. 2000. *Gene* 241:117–23
321. Childs S, Yeh RL, Hui D, Ling V. 1998. *Cancer Res.* 58:4160–67
322. Strautnieks SS, Kagalwalla AF, Tanner MS, Knisely AS, Bull L, et al. 1997. *Am. J. Hum. Genet.* 61:630–33
323. Strautnieks SS, Bull LN, Knisely AS, Kocoshis SA, Dahl N, et al. 1998. *Nat. Genet.* 20:233–38
324. Wang R, Salem M, Yousef IM, Tuchweber B, Lam P, et al. 2001. *Proc. Natl. Acad. Sci. USA* 98:2011–16
325. Frijters CMG, Ottenhoff R, Van Wijland MJA, Van Nieuwkerk CMJ, Groen AK, Oude Elferink RPJ. 1997. *Biochem. J.* 321:389–95
326. Azarian SM, Travis GH. 1997. *FEBS Lett.* 409:247–52
327. Illing M, Molday LL, Molday RS. 1997. *J. Biol. Chem.* 272:10303–10
328. Allikmets R, Singh N, Sun H, Shroyer NF, Hutchinson A, et al. 1997. *Nat. Genet.* 15:236–45
329. Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH. 1999. *Cell* 98:13–23
330. Sun H, Molday RS, Nathans J. 1999. *J. Biol. Chem.* 274:8269–81
331. Mata NL, Tzekov RT, Liu X, Weng J, Birch DG, Travis GH. 2001. *Invest. Ophthalmol. Vis. Sci.* 42:1685–90
332. Allikmets R. 2000. *Am. J. Hum. Genet.* 67:793–99
333. Allikmets R. 2000. *Am. J. Hum. Genet.* 67:487–91
334. Luciani MF, Denizot F, Savary S, Mattei MG, Chimini G. 1994. *Genomics* 21:150–59
335. Luciani MF, Chimini G. 1996. *EMBO J.* 15:226–35
336. Moynault A, Luciani MF, Chimini G. 1998. *Biochem. Soc. Trans.* 26:629–35
337. Rust S, Rosier M, Funke H, Real J, Amoura Z, et al. 1999. *Nat. Genet.* 22:352–55
338. Bodzioch M, Orsó E, Klucken J, Langmann T, Böttcher A, et al. 1999. *Nat. Genet.* 22:347–51
339. Drobniak W, Liebisch G, Biederer C, Trumbach B, Rogier G, et al. 1999. *Arterioscl. Thromb. Vasc. Biol.* 19:28–38
340. Brooks-Wilson A, Marcil M, Clee SM, Zhang L-H, Roomp K, et al. 1999. *Nat. Genet.* 22:336–45
341. Wang N, Silver DL, Thiele C, Tall AR. 2001. *J. Biol. Chem.* 276:23742–47
342. Chambenoit O, Hamon Y, Marguet D, Rigneault H, Rosseneu M, Chimini G. 2001. *J. Biol. Chem.* 276:9955–60
343. Vaisman BL, Lambert G, Amar M, Joyce C, Ito T, et al. 2001. *J. Clin. Invest.* 108:303–9
344. Groen AK, Brandsma RH, Ottenhoff R, Chimini G, Kuipers F. 2001. *J. Clin. Invest.* 108:843–50
345. Repa JJ, Turley SD, Lobaccaro JA, Medina J, Li L, et al. 2000. *Science* 289:1524–29
346. Neufeld EB, Remaley AT, Demosky SJ, Stonik JA, Cooney AM, et al. 2001. *J. Biol. Chem.* 276:27584–90

347. Berge KE, Tian H, Graf GA, Yu L, Grishin NV, et al. 2000. *Science* 290: 1771–75
348. Salen G, Shefer S, Nguyen L, Ness GC, Tint GS, Shore V. 1992. *J. Lipid Res.* 33:945–55
349. Lee MH, Lu KL, Hazard S, Yu H, Shulenin S, et al. 2001. *Nat. Genet.* 27:79–83
350. Lutjohann D, Bjorkhem I, Beil UF, Von Bergmann K. 1995. *J. Lipid Res.* 36:1763–73
351. Miettinen TA. 1980. *Eur. J. Clin. Invest.* 10:27–35
352. Gregg RE, Connor WE, Lin DS, Brewer HBJ. 1986. *J. Clin. Invest.* 77:1864–72
353. Mosser J, Douar AM, Sarde CO, Kioschis P, Feil R, et al. 1998. *Nature* 361: 726–30
354. Hetteima EH, Tabak HF. 2000. *Biochim. Biophys. Acta* 1486:18–27
355. Hetteima EH, Van Roermund CW, Distel B, Van den Berg M, Vilela C, et al. 1996. *EMBO J.* 15:3813–22
356. Shani N, Valle D. 1996. *Proc. Natl. Acad. Sci. USA* 93:11901–6
357. Braiterman LT, Zheng S, Watkins PA, Geraghty MT, Johnson G, et al. 1998. *Hum. Mol. Genet.* 7:239–47
358. Kemp S, Wei HM, Lu JF, Braiterman LT, McGuinness MC, et al. 1998. *Nat. Med.* 4:1261–68
359. Verkade HJ, Wolbers MJ, Havinga R, Uges DR, Vonk RJ, Kuipers F. 1990. *Gastroenterology* 99:1485–92
360. Huang L, Vore M. 2001. *Drug Metab. Dispos.* 29:634–37
361. Mustacich DJ, Shields J, Horton RA, Brown MK, Reed DJ. 1998. *Arch. Biochem. Biophys.* 350:183–92
362. Huang L, Smit JW, Meijer DK, Vore M. 2001. *Hepatology* 32:66–72
363. Huang L, Hoffman T, Vore M. 1998. *Hepatology* 28:1371–77
364. Stieger B, Fattinger K, Madon J, Kullak-Ublick GA, Meier PJ. 2001. *Gastroenterology* 118:422–30
365. Dietrich CG, Ottenhoff R, De Waart DR, Oude Elferink RPJ. 2001. *Toxicology* 167:73–81
366. Carpenterdeyo L, Marchand DH, Jean PA, Roth RA, Reed DJ. 1991. *Biochem. Pharmacol.* 42:2171–81
367. Klaassen CD, Watkins JB III. 1984. *Pharmacol. Rev.* 36:1–67
368. Kipp H, Pichetshote N, Arias IM. 2001. *J. Biol. Chem.* 276:7218–24
369. Kipp H, Arias IM. 2000. *Semin. Liver Dis.* 20:339–51
370. Kipp H, Arias IM. 2000. *J. Biol. Chem.* 275:15917–25
371. Maurice M, Schell MJ, Lardeux B, Hubbard AL. 1994. *Hepatology* 19:648–55
372. Misra S, Ujhazy P, Varticovski L, Arias IM. 1999. *Proc. Natl. Acad. Sci. USA* 96:5814–19
373. Misra S, Ujhazy P, Gatmaitan Z, Varticovski L, Arias IM. 1998. *J. Biol. Chem.* 273:26638–44
374. Kiuchi Y, Suzuki H, Hirohashi T, Tyson CA, Sugiyama Y. 1998. *FEBS Lett.* 433:149–52
375. Stockel B, Konig J, Nies AT, Cui Y, Brom M, Keppler D. 2000. *Eur. J. Biochem.* 267:1347–58
376. Di Croce L, Okret S, Kersten S, Gustafsson JA, Parker M, et al. 1999. *EMBO J.* 18:6201–10
377. Kaminski WE, Wenzel JJ, Piehler A, Langmann T, Schmitz G. 2001. *Biochem. Biophys. Res. Commun.* 285: 1295–301
378. Kaminski WE, Piehler A, Pullmann K, Porsch-Ozcurumez M, Duong C, et al. 2001. *Biochem. Biophys. Res. Commun.* 281:249–58
379. Langmann T, Porsch-Ozcurumez M, Unkelbach U, Klucken J, Schmitz G. 2000. *Biochim. Biophys. Acta* 1494: 175–80
380. Kaminski WE, Orso E, Diederich W, Klucken J, Drobnik W, Schmitz G. 2000. *Biochem. Biophys. Res. Commun.* 273:532–38
381. Chinetti G, Lestavel S, Bocher V,

- Remaley AT, Neve B, et al. 2001. *Nat. Med.* 7:53–58
382. Oliver WRJ, Shenk JL, Snaith MR, Russell CS, Plunket KD, et al. 2001. *Proc. Natl. Acad. Sci. USA* 98:5306–11
383. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, et al. 1999. *Science* 284:1362–65
384. Wang H, Chen J, Hollister K, Sowers LC, Forman BM. 1999. *Mol. Cell* 3:543–53
385. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, et al. 1999. *Science* 284:1365–68
386. Ananthanarayanan M, Balasubramanian N, Makishima M, Mangelsdorf DJ, Suchy FJ. 2001. *J. Biol. Chem.* 276:28857–65
387. Schuetz EG, Strom S, Yasuda K, Lecureur V, Assem M, et al. 2001. *J. Biol. Chem.* 276:39411–18
388. Fourcade S, Savary S, Albert D, Gauthier D, Gondcaille C, et al. 2001. *Eur. J. Biochem.* 268:3490–500
389. Synold TW, Dussault I, Forman BM. 2001. *Nat. Med.* 7:584–90
390. Xie W, Barwick JL, Downes M, Blumberg B, Simon CM, et al. 2000. *Nature* 406:435–39
391. Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, et al. 2001. *Proc. Natl. Acad. Sci. USA* 98:3369–74
392. Dussault I, Lin M, Hollister K, Wang EH, Synold TW, Forman BM. 2001. *J. Biol. Chem.* 276:33309–12
393. Dürr D, Stieger B, Kullak-Ublick GA, Rentsch KM, Steinert HC, et al. 2000. *Clin. Pharmacol. Ther.* 68:598–604
394. Lown KS, Mayo RR, Leichtman AB, Hsiao HL, Turgeon DK, et al. 1997. *Clin. Pharmacol. Ther.* 62:248–60
395. Kaplan B, Lown K, Craig R, Abecassis M, Kaufmann D, et al. 1999. *Transplantation* 67:333–35



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