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INVITED REVIEW

Portrait of multifaceted transporter, the multidrug resistance-associated protein 1 (MRP1/ABCC1)

Éva Bakos · László Homolya

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Abstract MRP1 (ABCC1) is a peculiar member of the ABC transporter superfamily for several aspects. This protein has an unusually broad substrate specificity and is capable of transporting not only a wide variety of neutral hydrophobic compounds, like the MDR1/P-glycoprotein, but also facilitating the extrusion of numerous glutathione, glucuronate, and sulfate conjugates. The transport mechanism of MRP1 is also complex; a composite substrate-binding site permits both cooperativity and competition between various substrates. This versatility and the ubiquitous tissue distribution make this transporter suitable for contributing to various physiological functions, including defense against xenobiotics and endogenous toxic metabolites, leukotriene-mediated inflammatory responses, as well as protection from the toxic effect of oxidative stress. In this paper, we give an overview of the considerable amount of knowledge which has accumulated since the discovery of MRP1 in 1992. We place special emphasis on the structural features essential for function, our recent understanding of the transport mechanism, and the numerous assignments of this transporter.

É. Bakos
Institute of Enzymology, Hungarian Academy of Sciences,
Karolina út 29-31,
1113 Budapest, Hungary

L. Homolya (✉)
Membrane Research Group for Membrane
Biology and Immunopathology,
Hungarian Academy of Sciences, National Medical Center,
Diószegi u. 64,
1113 Budapest, Hungary
e-mail: homolya@biomembrane.hu

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Abbreviations

ABC	ATP-binding cassette transporters
MDR	multidrug resistance
MRP1	multidrug resistance-associated protein 1 (ABCC1), ABCC-type protein
ABCC	C subfamily of ATP-binding cassette transporters
MDR1	multidrug resistance protein 1 (P-glycoprotein, ABCB1), ABCB-type protein
MDR3	multidrug resistance protein 3 (ABCB4), ABCB-type protein
MRP2	multidrug resistance-associated protein 2 (cMOAT, ABCC2), ABCC-type protein
sPgp	sister-P-glycoprotein (BSEP, ABCB11), ABCB-type protein
CFTR	cystic fibrosis transmembrane conductance regulator (ABCC7), ABCC-type protein
SUR	sulfonylurea receptor (ABCC8 and ABCC9), ABCC-type protein
ABCG2	multidrug transporter (breast cancer resistance protein, MXR/BCRP/ABCP)
NBD	nucleotide-binding domain, ABC domain
TMD	transmembrane domain
TMH	transmembrane helix
MOAT	multispecific organic anion transporter
LTA_4-D_4	leukotriene A_4-D_4
GSH	reduced glutathione
GSSG	glutathione disulfide
GS-X	glutathione conjugate

$E_2$17βG	estradiol-17-β-D-glucuronide
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
ALLN	*N*-acetyl-leucinyl-leucinyl-norleucinal
VP-16	etoposide
AML	acute myeloblastic leukemia
ALL	acute lymphoblastic leukemia
CLL	chronic lymphoblastic leukemia
SCLC	small-cell lung cancer
NSCLC	nonsmall-cell lung cancer
LRP/MVP	lung resistance-related protein, major vault protein
CYP	cytochrome P450
AM	acetoxy methyl ester
MK571	inhibitor of MRP1, MRP2, MRP4, and MRP7; LTD_4 receptor antagonist
Nrf2	nuclear factor-E2 p45-related factor
Ycf1	yeast cadmium factor-1
Kir6.2	inwardly rectifying K^+ channel
MJ0796	*Methanococcus jannaschii* hypothetical ABC transporter
HlyB	*Escherichia coli* hemolysin transporter
MalK	ATP-hydrolyzing subunit of the *Escherichia coli* maltose transporter
Rad50	*Pyrococcus furiosus* DNA repair ABC ATPase
BtuCD	*Escherichia coli* vitamin B_{12} importer
MsbA	*Salmonella typhimurium* lipid A transporter
PKC	protein kinase C
ROS	reactive oxygen species
γ-GCSh	γ-glutamylcysteine synthetase heavy subunit

Introduction

Multidrug resistance (MDR) is a major impediment to successful chemotherapy of cancer. The MDR phenotype is frequently associated with the overexpression of the MDR1/P-glycoprotein, which was identified as an active drug-extruding transporter with a broad substrate specificity. This distinctive feature of this drug pump explained the cross-resistance to a series of structurally and functionally unrelated anticancer agents. However, numerous unexplained cases of MDR phenotype implicated the existence of other mechanisms for MDR. Identification of the multidrug resistance protein 1 (MRP1, also denoted as ABCC1) in 1992 [24], and its initial characterization, elucidated that this protein indeed represents an alternative drug pump. The *MRP1* gene maps to chromosome 16p13.1 and encodes for a protein of 1,531 amino acids. Sequence analysis identified MRP1 as a member of the ATP-binding cassette (ABC) superfamily, to which the MDR1/P-glycoprotein also belongs. MRP1 is an integral membrane glycophosphoprotein with an apparent molecular weight of 190 kDa [3, 41] and functions as a primary active transporter utilizing the energy of ATP binding/hydrolysis [25, 181]. The transport activity of MRP1, similar to that of other ABC transporters, is characterized by vanadate sensitivity and lack of a phosphorylated intermediate. Like MDR1/P-glycoprotein, MRP1 also possesses a broad substrate specificity and can confer resistance to a wide variety of anticancer agents in tumor cell lines [25, 45, 181].

Subsequent analysis of MRP1 revealed that, in contrast to MDR1/P-glycoprotein, MRP1 also transports organic anions such as compounds conjugated to glutathione, glucuronate, or sulfate [60, 80, 102]. Following this perception, MRP1 has been identified as one of the elusive multispecific organic anion transporters (MOATs) or glutathione-conjugate (GS-X) pumps, which were described well before the discovery of MRP1. Since the cellular release of the inflammatory cytokine LTC_4 was attributed to the activity of MOATs, the MRP1 transporter has been proposed to play a role in leukotriene-mediated inflammatory responses [80]. Similarly, efflux of oxidized glutathione (GSSG) from cells during oxidative stress was reported years before the cloning of MRP1. The ability of MRP1 to transport GSSG [79] made this transporter the number one candidate for the GSSG overflow system.

Collectively, with the detailed investigation of MRP1, several previously discovered phenomena, such as non-P-glycoprotein-mediated drug resistance, transport of conjugates, and cellular release of LTC_4 and GSSG, converged and became explainable by the function of a single transporter. Due to its extraordinary transport properties, MRP1 contributes to several physiological functions and pathophysiological incidents.

Structure of MRP1 and the role of the amino-terminal regions

The cDNA encoding MRP1 was first cloned from a doxorubicin-selected, multidrug resistant human lung cancer cell line (H69R) by differential cDNA screening [24]. As mentioned above, on the basis of its primary sequence, MRP1 has been classified into the superfamily of ABC transporters. Thus, it contains characteristic short ATP-binding motifs (Walker A and Walker B) and in-between an additional conserved sequence (ABC signature motif). In addition to sequence homology, ABC proteins share a common molecular architecture: they are composed of the combinations of nucleotide-binding domains (NBDs), which harbor conserved peptide motifs, and transmembrane domains (TMDs), which generally consist of six transmembrane helices (TMHs).

Subsequent to the cloning of MRP1, several MRP-related proteins have been identified and classified into a subfamily. By the systematic classification of the human ABC transporters, the nine human MRPs, together with the cystic fibrosis transmembrane conductance regulator (CFTR) and two sulfonylurea receptors (SURs), form the ABCC subgroup. Accordingly, a systematic name, ABCC1, has been assigned to MRP1. In contrast to other ABC transporters, which exhibit a high similarity between the two NBDs, members of the ABCC subfamily share the peculiarity of "NBD asymmetry", that is, the two NBDs are fairly dissimilar in these proteins. The main difference comes from a small 13-amino-acid-long sequence between the Walker A and the ABC signature motifs, which is present only in the C-terminal NBDs (NBD2) of ABCC proteins. Other ABC proteins contain this "insert" in both NBDs. Other dissimilarities between the two NBDs of the ABCC subfamily members are found in sequences within the ABC signature regions and around the Walker B motifs.

The first sequence analysis of MRP1 indicated that this protein is not a canonical ABC transporter because the predicted membrane topology of the TMDs was peculiar. Although a common TMD1–NBD1–TMD2–NBD2 domain arrangement has been proposed, the N- and C-terminal membrane-bound regions have been described as domains consisting of eight and four TMHs, respectively [24]. This topology model was challenged by limited proteolysis experiments and hydrophobicity analysis; consequently, a revised secondary structure for MRP1 has been proposed independently by two research groups [7, 149]. According to this new topology model, MRP1 has a domain arrangement of TMD0–L0–TMD1–NBD1–TMD2–NBD2, i.e., the ABC transporter "core region" is extended with an N-terminal transmembrane domain (TMD0), which is connected to the core region with a characteristic intracellular linker region (L0). The three membrane-bound regions, TMD0, TMD1, and TMD2, consist of five, six, and another six TMHs, respectively (Fig. 1). Subsequent investigations introducing mutations to the glycosylation sites [49] and inserting hemagglutinin epitopes [67, 68] fully supported the latter membrane topology model. Furthermore, studies using an antibody against the N-terminal 1–18 amino acids also confirmed the extracellular localization of the amino terminus of MRP1 [14].

The first but very limited set of structural data for MRP1 was obtained at 22 Å resolution by electron microscopy of single particles of the purified protein [137]. The MRP1 monomer showed a pentagonal ring around a large pore. This ring exhibits a twofold pseudosymmetry, which may correspond to the core structure consisting of two TMDs and two NBDs. One of the two small dense regions, seen on the external side of the ring, might represent the TMD0 domain.

Although the TMD0 region is characteristic of several members of ABCC family, this domain was found to be dispensable for the function of MRP1. A truncated mutant, which lacks this domain, was functional with respect to transport activity, and similarly to the wild-type protein, localized to the basolateral membrane in polarized cells [6]. On the other hand, certain mutations in TMD0 resulted in significant conformational changes and reduced transport activity [59, 180]. Based on these observations, it has been suggested that certain residues in TMD0 contribute to the maintenance of the correct structure of the protein. Recently, a detailed study investigating the influence of TMD0 on the subcellular localization of MRP1 revealed that, although TMD0 is not essential for basolateral trafficking of MRP1, deletion of the TMD0 region affects the subcellular distribution of the protein [169]. About 50% of truncated protein was found in the recycling endosomes, whereas this was only 20% in the case of the intact MRP1. This finding suggests that TMD0 domain is important for the retention or the recycling of MRP1 to the plasma membrane. It has also been shown that both TMD0 and the remainder of the protein contain routing signals, and TMD0 becomes essential in trafficking when the COOH-terminal region of MRP1 is mutated or truncated [169].

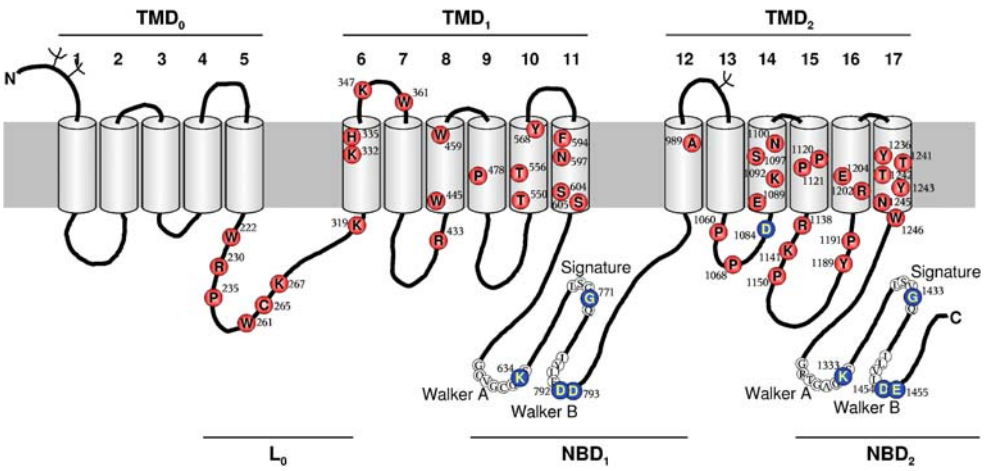

Fig. 1 Membrane topology model of MRP1. The depicted model was constructed on the basis of sequence analysis and the available experimental data (see text for details). *TMD* Transmembrane domain, *NBD* nucleotide-binding domain, *L0* linker region between TMD0 and TMD1. Mutations affecting substrate specificity and catalytic activity are indicated with *red and blue marks*, respectively

It is important to note that the TMD0 domains in other MRP1-related proteins, including the human MRP2 and yeast cadmium factor-1 (Ycf1), are required for correct apical targeting and for proper vacuolar localization, respectively [37, 98]. The TMD0 region of the human SUR1 anchors the protein to Kir6.2 potassium channel subunit. This interaction enhances their trafficking to the cell surface and supports the nucleotide-dependent gating of the channel [22].

In MRP1, the deletion of TMD0, together with the L0 linker region, abrogates the activity of the pump and causes its accumulation in intracellular membranes, indicating that the L0 domain is essential for function and proper trafficking [6]. Coexpression of the L0 peptide with the inactive core region results in restored activity and basolateral targeting of the transporter, suggesting that L0 forms a distinct domain that works in specific interaction with the core region of MRP1 [4]. The region in L0 responsible for the proper basolateral trafficking was mapped between amino acids 208 and 270, whereas the region of L0 required for transport activity falls between amino acids 208 and 260 [170]. Photoaffinity labeling and mutational studies implicated contribution of the L0 region to the formation of substrate-binding site because the L0 region was required for the binding of photoreactive analogs of certain characteristic substrates (see below) [126, 130, 170].

Surprisingly, the isolated L0 peptide, which was predicted to localize in the cytoplasm, was found to be attached to membranes [4]. Despite the relatively low sequence similarity, the proposed secondary structure of the L0 regions seems to be conserved in all members of the ABCC family. In accordance with the secondary structure predictions, the L0 region contains two helices, one of which is a characteristic amphipathic helix. This helix in MRP1 (amino acids 221–233) is responsible for the interaction with cell membranes because elimination of this helix abolishes membrane attachment of L0 [4]. In CFTR, an acidic cluster in the second helix of L0 was shown to interact with the R (regulatory) domain, which controls the channel activity [106]. In the case of MRP1, the nature of the interaction between the L0 linker region and the core structure is yet to be clarified.

Handling of substrates

The substrates of MRP1, identified indirectly by cytotoxicity assays and substrate-stimulated ATPase measurements, as well as directly by cellular and vesicular transport studies, comprise a vast variety of hydrophobic compounds, organic anion conjugates, and anionic nonconjugated substances. Table 1 summarizes the most characteristic substrates of MRP1. The typical conjugate substrates include glutathione, glucuronate, and sulfate conjugates, such as the cysteinyl leukotriene LTC_4 [80, 102], estradiol-17-β-D-glucuronide ($E_2$17βG) [60, 85], estrone 3-sulfate [127], and sulfated bile acids [60]. The ability of MRP1 to transport glutathione conjugates and the expression of this protein in numerous tissues make MRP1 a ubiquitous glutathione-conjugate pump.

MRP1 can confer resistance not only to many commonly used neutral natural product chemotherapeutic agents [25, 45], like MDR1/P-glycoprotein, but also to various anionic compounds, such as the folic acid antimetabolite, methotrexate [52], certain heavy metal oxyanions (arsenical,

Table 1 Selected substrates of human MRP1

Substrates	References
Conjugates	
Endobiotic conjugates	
GSH conjugates	
Leukotriene C_4, leukotriene D_4, leukotriene E_4	[60, 80, 102]
Prostaglandin A_2-SG	[35]
Hydroxynonenal-SG	[132]
Glucuronide conjugates	
Estradiol-17-β-D-glucuronide	[60, 85]
Glucuronosylbilirubin	[61]
Sulfate conjugates	
Estrone 3-sulfate,	[127]
Dehydroepiandrosterone 3-sulfate	[60]
Sulfatolithocholyl taurine	[60]
Xenobiotic conjugates	
GSH conjugates	
Aflatoxin B_1-epoxide-SG	[89]
Chlorambucil-SG, melphalan-SG	[9]
Glucuronide conjugates	
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)-*O*-glucuronide	[84]
Therapeutic agents	
Anticancer drugs	
Anthracyclines	
Daunorubicin, doxorubicin	[25, 45, 133]
Plant alkaloids	
Vinblastine, vincristine, SN-38, etoposide	[25, 45, 87]
Others	
Methotrexate	[52]
Antiandrogens (flutamide)	[46]
HIV protease inhibitors	
Saquinavir, ritonavir	[115]
Other compounds	
Metalloids	
Sodium arsenite, potassium antimonite	[25, 58]
Peptides	
GSH, GSSG	[79, 87]
N-acetyl-Leu-Leu-norleucinal (ALLN)	[31]
Fluorescent probes	
Calcein-AM, calcein	[51]
BCECF	[33]

antimonial) [25, 58], and antiandrogens (flutamide) [46]. Contrary to the MDR1/P-glycoprotein, the MRP1-mediated active transport of unmodified chemotherapeutic drugs, as well as that of certain anionic conjugates, requires the presence of glutathione (GSH). The mechanism by which GSH participates in MRP1-mediated efflux is rather complex. The transport of some hydrophobic agents, such as vincristine, is stimulated by GSH, and conversely, these compounds stimulate GSH transport [87]. This cross-stimulation was originally explained by the cotransport of the two compounds, although a recent review by Borst et al. [15] suggests a mechanism of mutual heterotropic cooperativity of substrate-binding sites. On the other hand, glutathione stimulates the transport of certain substrates, but these compounds have no effect on GSH transport. These are exemplified by estrone 3-sulfate, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol-*O*-glucuronide (NNAL-*O*-glucuronide), or daunorubicin [84, 127, 133]. In contrast to these substrates, certain compounds, such as verapamil and bioflavonoids, are not transported by MRP1 but they enhance the MRP1-mediated transport of GSH [82, 88].

To identify the regions of MRP1, which are directly involved in substrate binding, photoaffinity labeling studies and mutational analyses have been performed. The GSH-dependent azidoagosterol A labeling of the protein was detected only in TMD2 [130], whereas the azido analogs of GSH and unconjugated drugs, such as rhodamine123, or quinoline, labeled both halves of MRP1, reacting with TMHs 10–11 in TMD1 and TMHs 16–17 in TMD2 [29, 65, 126]. The iodoarylazido analog of LTC_4 labeled the above two regions as well as TMH 12 [66], while the labeling with unmodified LTC_4 showed that the TMHs 6, 7, 10, and 17 are parts of the LTC_4 binding site [176].

As mentioned earlier, the L0 region has also been shown to participate in substrate binding. Removal of the L0 region, together with TMD0, abolished the binding of LTC_4 or a photoaffinity analog of glutathione (azidophenacyl-GSH). The GSH-dependent binding of azidoagosterol A was also eliminated, although direct labeling of the L0 peptide with these compounds could not be demonstrated [126, 130, 170]. In contrast, the binding of azidoaryl derivative of glutathione to the L0 linker region has been detected [65]. Typical substrates, which are transported as glutathione or glucuronide conjugates, e.g., LTC_4 or $E_2$17βG, are not transported by an MRP1 variant mutated in the L0 region (W222L, W223L, R231A, W261A, K267M). In contrast, the transport of an unconjugated substrate (SN38) was not affected in this variant [108]. These observations suggest that L0 participates in the formation of the site interacting with glutathione or glucuronide moieties. A photoaffinity analog of LTC_4 also labeled the TMD0 domain; however, considering the fact that MRP1 lacking TMD0 is fully functional, it is more likely that TMD0 region does not directly contribute to the binding site of LTC_4, and this region was labeled due to its close proximity to the photoreactive group [66].

A molecular model of MRP1 indicated that five residues of TMHs 10, 11, 16, and 17 form an "aromatic basket", lining the putative substrate translocation pathway [20]. Mutational analyses demonstrated that amino acid changes affecting substrate specificity are found in almost all TMHs of TMD1, TMD2, as well as in the L0 region (for a review, see the work of Haimeur et al. [47]). However, these mutations are located predominantly in TMHs 11 and 17 and in the cytoplasmic loop connecting TMH 15 and TMH 16 (see Fig. 1).

Many substrates cross-inhibit the transport of one another, whereas several mutations inhibiting the transport of certain substrates have no effect on the transport of other substrates. These data indicate that these substrates bind to a common substrate-binding pocket and make contacts with overlapping but nonidentical sets of residues. In addition to the GSH-dependent transport, in some other cases, substrate-stimulated transports have also been observed. These include the cross-stimulation of the transport of LTC_4 and estrone 3-sulfate at low concentrations [127], the acceleration of LTC_4 transport by NNAL-*O*-glucuronide [84], and the stimulation of *N*-ethylmaleimide glutathione transport by indomethacin at low concentrations [5]. These observations suggest the existence of more than one allosterically cooperative, nonoverlapping substrate-binding sites within the large substrate-binding pocket. At low concentration, various substrates can bind to distinct substrate-binding sites, facilitating the binding and transport of one another, whereas at higher concentrations, they saturate all the available binding sites and, thus, cross-inhibit the transport of one another.

Catalytic cycle of MRP1

As mentioned above, the transport of MRP1, similar to that of most ABC transporters, is driven by ATP binding/hydrolysis. Unlike P-type ATPases, ABC transporters do not form phosphorylated intermediates during their transport cycle. To characterize the individual steps of the catalytic cycle, such as ATP binding, formation of the transition state, cleavage of the terminal phosphate bond (ATP hydrolysis), and release of ADP and phosphate, various experimental approaches are used. ATP binding is often examined by photoaffinity labeling under nonhydrolytic conditions by using azido-ATP. The transition-state complex formation can be followed by the so-called "nucleotide trapping" measurement. This also means photoaffinity labeling with azido-ATP but under hydrolytic conditions in the presence of a phosphate-mimicking anion,

e.g., vanadate [43, 53, 105]. This anion stabilizes the transition-state complex (MRP1·Mg·ADP·P_i) by replacing the gamma phosphate and arrests the transporter in this state. Another approach to characterize the individual steps of the ATPase cycle is the vanadate-catalyzed photooxidative cleavage reaction [69]. This method is based on the observation that the cleavage of the polypeptide chain upon UV irradiation in the presence of vanadate depends on the conformation of the protein. Thus, the ATP binding and the transition-state complex formation can be assessed by the accumulation of various cleavage products generated under nonhydrolytic and hydrolytic conditions, respectively. The full catalytic cycle, which also includes ATP hydrolysis and ADP/phosphate release, can be followed by ATPase measurements, determining the rate of phosphate liberation [5].

The scheme of the catalytic cycle of the ABC transporters is further complicated by the fact that these proteins contain two cooperating NBDs. In a number of bacterial ABC proteins, the NBDs and TMDs are expressed as separate proteins, which associate to form a functional unit. The analysis of the solved structures of dimeric NBDs of MJ0796, HlyB, MalK, and Rad50, as well as the studies on the structures of "complete" ABC transporters, which also contain the TMDs (BtuCD and MsbA), demonstrated that the two NDBs dimerize in a head-to-tail orientation and form two composite ATP-binding sites/catalytic sites [118]. In these dimeric arrangements, the Walker A of one NBD and the ABC signature motif of the other NBD are involved in the formation of an ATP-binding site. For simplicity, we subsequently refer to an active site in NDB1 or NBD2, which includes Walker A and Walker B of the respective NBD.

Both vanadate-induced cleavage reactions and azido-ATP labeling experiments resulted in the conclusion that both NBDs bind ATP, although NBD1 has higher affinity for ATP than NBD2 [43, 53, 69, 178]. Nucleotide trapping has also been shown in both catalytic sites, but the rate of the transition-state complex formation was greater in NBD2 than in NBD1 [43, 53, 105]. These observations imply that both sites are catalytically active, but the hydrolytic activity of NBD2 is higher than that of NBD1.

The two NBDs of MDR1/P-glycoprotein are functionally equivalent, and the integrity of both NBDs is needed for transport. Inactivation of any one of the two ATP-binding sites results in the complete loss of trapping activity of the protein [101]. In contrast, chemical modifications or mutations of the consensus motifs in the two NBDs of MRP1 have different effects on the transport [43, 53, 121, 178]. Mutations of key residues in the Walker A or Walker B of NBD1 decreased LTC_4 transport activity to 30–50% of the wild-type protein, whereas the corresponding mutations in NBD2 essentially inactivated the protein [43, 53, 121]. On the other hand, substitution of the conserved Gly in the ABC signature sequence of NBD1 for Ala completely inactivates the protein, while this mutation in NDB2 causes only a partial inactivation [121].

Unambiguous evidence for allosteric interactions between NBD1 and NBD2 has been provided by azido-ATP labeling experiments, demonstrating that binding of ATP at NBD1 increases the affinity for ATP and ADP trapping at NBD2 [54]. Conversely, the trapping of ADP at NBD2 has also been shown to enhance the ATP binding at NBD1 in one report [53], while other studies indicated that mutations affecting nucleotide binding and trapping at NDB2 had no effect on ATP binding at NBD1 [43, 121].

Collectively, the different properties of nucleotide binding and transition-state complex formation of the individual NBDs, the diverse consequences of mutations in these domains, as well as their different allosteric regulation implicate distinguishable activities and functions for the two NBDs of MRP1. The data detailed above suggest that ATP binding to both NBDs and the hydrolysis at the NBD2 are the key steps for MRP1-mediated transport. Although ATP is also hydrolyzed at NBD1, the significance of this hydrolysis in the transport is unknown. It is interesting to note that Yang et al. [179] and Zhao and Chang [184] found that certain mutations in the NBD1 decreased the affinity for ATP but increased the transport activity. They interpreted their data in a way that the dissociation rate of nucleotide from the mutated variants of NBD1 is higher than that of wild type, and they concluded that the transport is promoted by the release of the bound nucleotide from NBD1 regardless of ATP hydrolysis. Similar functional asymmetry of NBDs has been shown in CFTR, where ATP binding to both NBDs is required for channel opening, while hydrolysis at NBD2 is necessary for the channel closing [12].

From among the several sequence differences between the NBDs of MRP1, the only one which was established to contribute to the observed functional differences is the putative catalytic base following the Walker B motifs [122]. This residue is Asp in NBD1 and Glu in NBD2. An Asp to Glu mutation in NBD1 enhanced the hydrolytic activity of NBD1, whereas the corresponding mutation of Glu to Asp decreased the rate of hydrolysis at NBD2 and the rate of ADP release. These observations suggest that the presence of Asp in this position is partially responsible for the low hydrolytic activity of NBD1.

It has been documented that purified MRP1 reconstituted in proteoliposomes as well as MRP1 in isolated insect cell membranes shows ATPase activity, which is stimulated by certain transported substrates of MRP1 [5, 97]. However, the mechanism by which transported substrates promote ATP hydrolysis is controversial. Gao et al. [43] found that LTC_4 increased the binding of azido-ATP to NBD1 several

folds, whereas it had little effect on nucleotide binding to NBD2. In contrast, others have found that LTC_4 enhanced ATP binding to NBD2 but not to NBD1 [131]. These data differ from those obtained previously from nucleotide trapping studies on MDR1/P-glycoprotein [154] and recently from vanadate cleavage experiments with MRP1 [69]. These studies demonstrated that the transported substrates have no effect on nucleotide binding but accelerate the formation of the transition-state complex in both catalytic sites, suggesting that substrates stimulate a reaction step of the hydrolytic cycle after ATP binding but precede the formation of the transition-state complex. In accordance with this observation, the trapping of azido-ADP was found to be enhanced by LTC_4 at both NBDs [43]. The discrepancy considering the effect of substrates on ATP binding may be due to the fact that the experiments were performed in two different systems: azido-ATP labeling was done with coexpressed N-half and C-half of MRP1, while vanadate cleavage was done with the full-length wild-type protein.

It has been commonly accepted that during the transport cycle of ABC transporters, the substrate binds to a high-affinity binding site, which is subsequently reoriented, and its substrate binding affinity becomes reduced, facilitating the release of the substrate. High- and low-affinity drug binding states of MRP1 were also distinguished. Either ATP with or without vanadate, or the poorly hydrolyzable ATP analog, ATPγS, can result in the transition from a high- to a low-affinity state [121]. Prolonged binding of either ATP or ADP in NBD2 effectively locked the protein in a low-affinity substrate binding state, while increased ADP trapping in NBD1 prevented this transition [122]. Based on these observations, it has been proposed that occupancy of both NBDs with ATP results in the formation of a low-affinity drug binding state, which persists until NDB2 is occupied with ADP. Manciu et al. [96] have characterized the structural changes of MRP1 during transport by using protease accessibility and infrared spectroscopy. They have demonstrated that ATP binding induces conformational changes in TMDs and increases the accessibility of the protein toward the aqueous medium, while no additional change in the conformational state of TMHs takes place upon ATP hydrolysis. These findings indicate that ATP binding rather than the hydrolysis drives the major structural reorganization in MRP1. However, in the case of a bacterial ABC transporter, the MsbA, a recent electron paramagnetic resonance spectroscopic study has shown that the accessibility of the protein further increases at the external side upon the formation of the high-energy ADP-bound intermediate [32].

Taking together the structural and biochemical data discussed above, as well as the observed conformational changes during the catalytic cycle, the following transport model can be proposed. First, the substrate binds to its high-affinity binding site and MgATP binds to NBD1. Binding of ATP at NBD1 promotes the ATP binding to NBD2. ATP binding results in the formation of a closed dimer NBD conformation, and the subsequent conformational changes cause reconfiguration of the TMDs, resulting in lowering the affinity of the substrate binding site. A closed dimerization of NBDs allows the transition-state complex formation at NDB2. This causes a further conformational change, which results in getting the low-affinity substrate-binding site even more exposed to the extracellular medium, and the substrate becomes released. The step which follows the ATP binding and leads to the transition-state complex formation is accelerated by the presence of substrates. After ATP hydrolysis, the terminal phosphate and subsequently ADP are released from NBD2. There are three options at this point to restart the transport cycle: (1) the protein returns to the high-affinity substrate-binding state after ATP hydrolysis at NBD1 and ADP release, (2) the recovery takes place subsequent to the release of nonhydrolyzed ATP from NBD1, or (3) the protein is capable of binding of another substrate molecule without releasing the ATP from NBD1.

Substrate binding and ATP hydrolysis are promoted by different segments of the protein. However, the substrate-stimulated ATP hydrolysis and the ATP-energized substrate translocation require intramolecular interactions between the two halves of the protein. Studies on the structure of the MsbA transporter [134] revealed that the intracellular loop between TMH 2 and TMH 3 of this protein (ICD1), which consists of three helices in a U-like configuration, establishes contacts between the TMD and the NBD. The position of ICD1 makes feasible that this region is part of the pathway transmitting the conformational changes between the NBDs and the substrate-binding pocket. The corresponding first cytoplasmic loops in TMD1 and TMD2 of MRP1 have the same predicted architecture as the ICD1 in MsbA. Mutation of Asp 1084 in one of these loops in MRP1 drastically decreased nucleotide trapping at NBD2 and prevented the transition from high- to low-affinity state of protein, whereas ATP binding was unaffected [182]. These findings suggest that this intracellular region contributes to the transduction of conformational changes in MRP1, triggered by nucleotide binding and hydrolysis. However, the structural changes introduced into this loop by replacement of two prolines with alanines had no effect on the overall transport activity of the protein [71].

A recent study from our laboratory suggests that the ABC signature motif is a candidate for intramolecular communication [157]. The structures of ATP-bound NBD dimers show that the conserved second Gly residue in the ABC signature motif interacts with the oxygen of the gamma-phosphate of the ATP, bound to the Walker motif of

the opposite subunit [118]. Substitution of these glycines for aspartic acids in either NBDs of MRP1 eliminates transport activity, but the mutants are still capable of forming the transition-state complex [157]. It is interesting to note that in these mutants, the formation of the intermediate complex is inhibited by substrates, whereas it is accelerated in the wild-type protein. Based on these results, we suggested that substrate binding facilitates the signature region to turn toward the ATP molecule bound to the opposing NDB. This movement allows the formation of the proper dimer interface for ATP hydrolysis. When a critical residue is mutated in this region, the characteristic substrate-induced conformational change is altered, implicating that the movement of the signature region is at the endpoint of the substrate-induced allosteric changes, and this region is a key component of the coupling of substrate binding and ATP hydrolysis. Similar observations have been made with the MDR1/P-glycoprotein [156].

Regulation of MRP1 expression and function

MRP1 expression is a subject of multiple regulatory mechanisms. In some multidrug resistant cell lines that overexpress MRP1, the *MRP1* gene is amplified, while in others, an increased transcription occurs without gene amplification. The 5′-end region of *MRP1* gene was cloned, and the analysis of this region showed that, similar to other human multidrug transporters, *MRP1* has a TATA-less promoter [186]. The basal promoter activity was localized to nucleotides −91 to +103 in a GC-rich region of the *MRP1* gene. There are putative Sp1 binding sites in the GC elements, and it was demonstrated that Sp1 in fact interacts with these binding sites, which were proven to be essential for optimal MRP1 transcriptional activity [187].

High-level MRP1 expression was observed even in tumors originating from tissues, which originally exhibit little MRP1 expression. This elevation in MRP1 expression is likely due to the upregulation by components that are involved in malignant transformation, such as oncogenes and tumor suppressor proteins. The wild-type p53 tumor suppressor protein has been shown to repress the transcription of *MRP1* partially by deactivation of promoter-bound Sp1, whereas mutant p53 abrogates the repression of MRP1 [168]. Therefore, a loss of p53 function and/or an increase in Sp1 activity in tumor cells could contribute to an upregulation of the *MRP1* gene, as demonstrated in various tumor types including prostate [153] and nonsmall-cell lung cancer (NSCLC) [117]. The *MRP1* promoter contains a putative AP-1 site (−498 through −492), which interacts with a complex, containing c-*jun* and *jun*D oncogenes [75]. Accordingly, downregulation of MRP1 expression has been found in cells transfected with mutant c-*jun* [27].

Numerous chemotherapeutic agents, including doxorubicin [144] and vinblastine [141], as well as heavy metals such as arsenite, cadmium, and mercury [58, 70], have been reported to induce MRP1 expression. In addition, reactive oxygen species (ROS) have been found to regulate the expression of MRP1. High intracellular ROS levels induce MRP1 expression, whereas the elevation in GSH concentration lowers the ROS levels and consequently results in downregulation of MRP1 [177]. Prooxidants, such as menadione, *tert*-butylhydroquinone, 2,3-dimethoxy-1,4-naphthoquinone [177], and sulindac [161], which produce ROS and generate oxidative stress, have been shown to induce the expression of MRP1. It has been clearly demonstrated that the nuclear factor-E2 p45-related factor 2 (Nrf_2), the key transcriptional factor of antioxidant responsive element-driven genes [48], is involved in the regulation of MRP1 expression.

Similar to MRP1, another oxidative stress-sensitive gene product, the heavy subunit of γ-glutamylcysteine synthetase (γ-GCSh), which is the rate-limiting enzyme in glutathione biosynthesis, frequently shows elevated expression in many drug-resistant cell lines. Expressions of MRP1 and γ-GCSh are coinduced by many cytotoxic and redox active agents [58, 161]. Coordinated elevation in GSH production and in MRP1 pump activity ensures the effective extrusion of these toxic compounds by an MRP1-mediated, GSH-dependent transport mechanism. In addition, the expression of *MRP1* and *γ-GCSh* genes can also be upregulated by interleukin-1β through generation of nitric oxide, which is easily converted to reactive nitrogen species, reminiscent of ROS [56].

The mechanism underlying the concerted regulation of the *MRP1* and *γ-GCSh* genes is unknown. Several oxidative stress-responsive elements, located upstream from the promoter of the *MRP1* and the *γ-GCSh* genes, have been identified [186]. One possibility is that these putative *cis*-acting elements mediate the oxidative stress-induced expression by interacting with redox-sensitive transcription factors. On the other hand, it has recently been demonstrated that doxorubicin upregulates the transcription of *MRP1* gene via doxorubicin-activated c-*jun* N-terminal kinase (JNK), which enhances the association of the activated form of c-*jun* with the AP-1 site in the *MRP1* promoter [144]. Moreover, stimulation of JNK activity has been observed upon depletion of intracellular GSH. These results, together with the finding that *γ-GCSh* gene also contains an AP-1 binding site, implicate that c-*jun*/AP-1 may play a role in the coregulated expression of MRP1 and γ-GCSh.

Very little is known about the posttranslational regulations of MRP1. This transporter is extensively glycosylated, but either the transport function or the localization is not significantly modulated by protein glycosylation

[7, 103]. MRP1 has been shown to be phosphorylated primarily on serine residues. Initially, it has been proposed that phosphorylation by protein kinase C (PKC) plays a role in the regulation of transport function of MRP1 because PKC inhibitors blocked MRP1-mediated transport [93]. However, this result might reflect a direct interaction of PKC inhibitors with MRP1 instead of a regulation through protein phosphorylation.

Tissue distribution and subcellular localization

In contrast to MRP2 and MRP3, which exhibit a rather constrained tissue distribution, MRP1 is ubiquitously expressed in the body. The tissues showing the highest level of MRP1 expression include the lung, testis, kidney, heart, and placenta, whereas a moderate MRP1 expression was found in the small intestine, colon, brain, and peripheral blood mononuclear cells [24, 42, 116, 123]. Because most of these tissues belong to the defense lines of the body, it is somewhat unexpected that MRP1 is expressed at a very low level in the liver, the major organ of xenobiotic metabolism and detoxification. It is interesting to note that MRP1 expression is elevated in the regenerating regions of the liver after tissue damage [136]. Because animal models are often used for pharmacokinetic studies, it is noteworthy that certain differences in tissue distribution of MRP1 can be seen among various species, e.g., the canine liver shows a relatively high expression of MRP1, whereas, as mentioned earlier, in the normal human liver, MRP1 expression is hardly detectable [26, 42]. Gender differences in MRP1 expression in the kidney and liver have also been reported, i.e., higher MRP1 expressions were found in female mice [94].

In tissues where MRP1 is expressed at a relatively high level, the transporter is not distributed uniformly but primarily expressed in special cell types. Table 2 summarizes the cell-type-specific expression pattern of MRP1 in various tissues. In general, cells with a specialized barrier function or cells at a high proliferative status exhibit higher expression of MRP1 than the surrounding cell types. The former is exemplified by the choroid cells in the blood cerebrospinal fluid barrier [129], or the syncytiotrophoblasts and fetal blood-vessel endothelial cells in the placenta [147], whereas the latter notion is demonstrated by the reactive type II pneumocytes in hyperplastic alveoli of the lung [17], or the crypt Paneth cells in the small intestine [123]. The increased level of MRP1 expression in the reactive ductules of the damaged liver is explained by the activation of hepatic progenitor cells [136], which also represent a rapidly multiplying cell type. Similarly, the increase in MRP1 expression during culturing of brain microvessel isolates can be a consequence of the presence of a cell subpopulation with higher proliferative potential [143].

With respect to subcellular localization, MRP1 is primarily expressed in the plasma membrane [41, 181]. Immunocytochemical studies demonstrated basolateral lo-

Table 2 Cell type-specific distributions of MRP1

Tissues	High MRP1 expression	No/low MRP1 expression	References
Lung	Bronchial epithelial cells, bronchiolar epithelial cells, seromucinous glands, reactive type II pneumocytes, alveolar macrophages	Normal type I and II alveolar pneumocytes	[17, 42, 140, 175]
Testis	Sertoli cells in the seminiferous tubules, Leydig cells, testosterone-producing interstitial cells outside the seminiferous tubules		[42, 163, 173]
Kidney	Glomeruli, ascending limb cells, epithelial cells of the loop of Henle, distal and collecting duct cells	Proximal tubule cells	[123, 173]
Placenta	Fetal blood vessels of the terminal and intermediate villi, syncytiotrophoblasts, epithelial cells of the endoplacental yolk sac		[104, 146, 147]
Small intestine	Crypt cells (Paneth cells)	Enterocytes	[123]
Colon	Entire crypt–villous axis		[123]
Brain	Brain capillary endothelial cell		[107, 143, 151, 183]
	Choroidal and ependymal cells, tanycytes in the choroid plexus		[129, 171]
	Glial cells, parenchyma astrocytes		[28, 44]
Blood	Erythrocytes, T-cells, mast cells		[10, 111, 116, 125, 172]

calization for MRP1 in various polarized cell types, including drug-selected and transfected cells as well as epithelial and endothelial cells of normal tissues [17, 36, 83, 123, 140, 175]. In contrast, other ABC transporters which are thought to be crucial in tissue defense, such as MDR1/P-glycoprotein, MRP2, and ABCG2, reside in the apical membrane of polarized cells [19, 95, 162]. The relative position of MRP1 and these transporters in selected tissues is depicted in Fig. 2. Surprisingly, MRP1 was found to localize to the apical surface of the brain capillary endothelial cells [107, 151, 183]. Thus, the ipsilateral distribution of MRP1 with MDR1/P-glycoprotein, MRP2, and ABCG2 in the brain vessels represents an exception. Contradicting data have been published on the subcellular localization of MRP1 in the placental trophoblasts. Initial reports suggested apical localization [146]; however, more recent studies revealed that this transporter localizes to the basolateral membrane of syncytiotrophoblasts [104, 147].

In addition to the plasma membrane localization, intracellular expression of MRP1 has been found in various cell lines [3, 24, 44, 128, 167] and normal tissue samples [42, 174]. The subcellular organelles where MRP1 accumulation was seen include endocytic vesicles [3], perinuclearly located lysosomes [128], and trans-Golgi vesicles [44, 167]. The role of intracellular MRP1 is yet to be clarified. Because MRP1-dependent accumulation of fluorescent drugs into these organelles was also demonstrated [128, 167], it has been proposed that, in addition to extruding drugs through the plasma membrane, MRP1 can confer drug-resistant phenotype to cells by sequestering the drugs into the intracellular compartments. This suggestion would resolve the contradiction how a drug-resistant cell line expressing MRP1 may exhibit the same level of intracellular drug accumulation as its drug-sensitive counterpart [24]. Because these studies were performed with cells overexpressing the transporter and/or with MRP1 tagged with fluorescent proteins, for the evaluation of the physiological relevance of these observations, it should be considered that either overexpression or tagging may greatly influence the subcellular localization pattern. However, the predominant cytoplasmic expression of MRP1 seen in several normal tissues [42, 174] indicates that the intracellular localization of the transporter might have a physiological role. The interesting finding that MRP1 rapidly translocates from the Golgi to the plasma membrane in response to unconjugated bilirubin exposure [44] suggests that intracellularly expressed MRP1 serves as a cellular reservoir, which can be easily mobilized and targeted to the site of action. The particular trafficking signals directing MRP1 to the plasma membrane, as well as the retention and recycling mechanisms determining the further cellular routing of the transporter, are not known yet. However, certain regions such as the L0 domain [4, 6, 170], and the C-terminus in cooperation with the TMD0 [169], were found to be crucial for proper targeting.

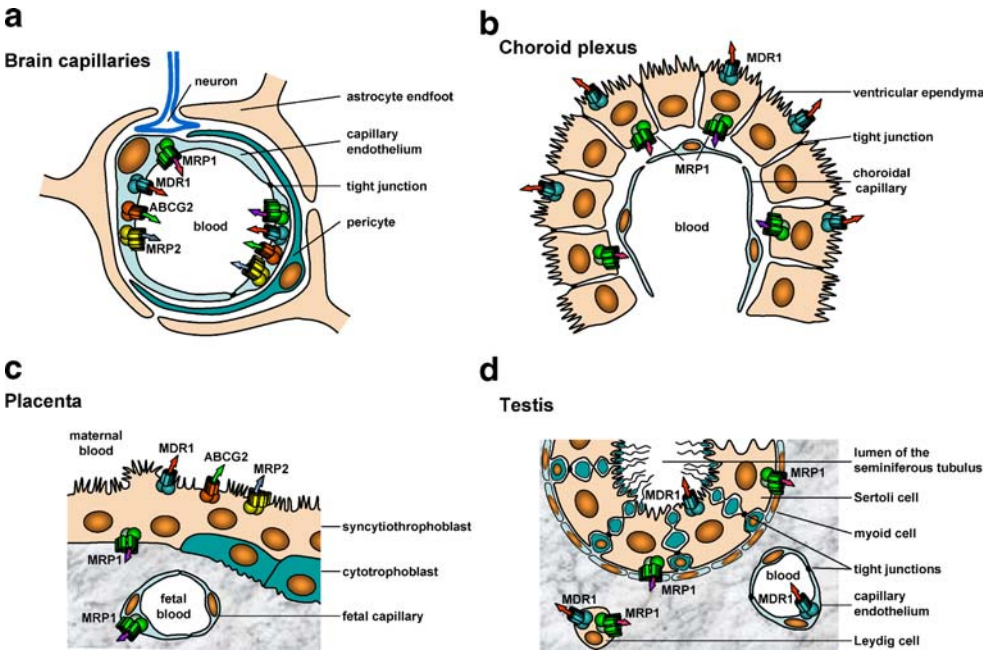

Fig. 2 Cell-type-specific expression and subcellular localization of MRP1 in various physiological barriers. The figure illustrates the relative position of MRP1 and other selected ABC transporters (MDR1/P-glycoprotein, ABCG2, and MRP2) at the blood–brain barrier (**a**), the blood–cerebrospinal fluid barrier (**b**), the placenta (**c**), and the blood–testis barrier (**d**). MRP1 is expressed in the basolateral membrane of the ventricular epithelial cells in the choroid plexus, the syncytiotrophoblasts in the placenta, and the Sertoli cells in the seminiferous tubules. In contrast, MRP1 is localized to the apical membrane of the brain capillary endothelial cells, ipsilaterally to MDR1/P-glycoprotein, ABCG2, and MRP2. MRP1 is also expressed in the fetal blood vessels in the placenta and in the testosterone-producing Leydig cells in the testis

Dual role of MRP1 in the physiological barriers

The substrate specificity of MRP1, together with its distribution in tissues which are considered as the major defense lines of the body, implies a physiological role for MRP1 in protection against xenobiotics and endogenous toxic metabolites (endobiotics). The cellular detoxification process comprises four stages: the uptake of the toxic compounds (phase 0), followed by oxidation (phase I) and conjugation with an anionic group (phase II), and finally, the conjugates are extruded from the cells in an ATP-dependent manner (phase III). MRP1 and related proteins were suggested to play a role in this terminal step of detoxification [57].

Unambiguous verification for this function of MRP1 has emerged from studies using knockout animals. Mice deficient in mrp1 (mrp1 (−/−)) were independently generated by two groups and found to be viable, healthy, and fertile with normal histological and hematological parameters [92, 172]. Challenging the knockout animals with etoposide (VP-16), however, revealed that the mrp1 (−/−) mice are hypersensitive to this cytotoxic drug. The affected tissues include bone marrow, oropharyngeal mucosa, Sertoli cells in the seminiferous tubules of the testis, and urinary collecting tubules in the kidney [92, 173]. Similarly, developing spermatocytes and spermatids were damaged in the testis of mrp1 (−/−) mice after a treatment with the pesticide methoxychlor [163].

Although numerous in vitro studies clearly demonstrated the MRP1-mediated transport of various xeno- and endobiotics, several attempts failed to prove this protective role of MRP1 in vivo. Constitutive expression of MRP1 failed to protect mice from the toxic effect of arsenical and antimonial compounds [90]. The carcinogenicity of aflatoxin B1 in the mrp1 (−/−) mice was not different from that seen in wild-type animals [91]. Similarly, the permeation of several drugs and conjugates across the blood–brain barrier and the blood–cerebrospinal fluid barrier was unaffected in the mrp1-deficient mice [23, 76]. These data were explained by the presence of other transporters which also contribute to these defense mechanisms and can compensate for the lack of MRP1. This explanation is rather plausible because numerous transmembrane export pumps with partially overlapping substrate specificities are present and highly expressed in the physiological barriers. The ABC transporters which are thought to play a crucial role in detoxification and tissue defense include MDR1/P-glycoprotein, MRP1, MRP2, and ABCG2 (for recent review, see the paper of Leslie et al. [83]).

More compelling results were obtained from studies combining different single knockouts and/or using multiple knockout animals. In mice deficient in mdr1a, mdr1b, and mrp1, vincristine and etoposide were found to be toxic to the bone marrow and the gastrointestinal mucosa [62], indicating their compensatory role in protection against these drugs. By using different knockout animals, the role of mrp1 in the tissue distribution and excretion of grepafloxacin, a new quinolone antibiotic, has also been demonstrated [139]. Contribution of MRP1 to blood–cerebrospinal fluid barrier was unambiguously shown by comparing the etoposide levels in the blood–cerebrospinal fluid in mdr1a/mdr1b double and mdr1a/mdr1b/mrp1 triple knockout mice after intravenous administration of this compound [171]. Similarly, the protective function of mrp1 in the blood–brain barrier was demonstrated by the decreased elimination rate of estradiol-17-β-D-glucuronide from the brain of the triple knockout mice [151].

With the exception of brain capillary endothelial cells, in most physiological barriers, MRP1 is expressed in the basolateral membrane of polarized cells, contralaterally to MDR1/P-glycoprotein, MRP2, and ABCG2 (see Fig. 2). This subcellular distribution confers a function for MRP1 distinct from that of the other efflux pumps. In the first line defense fronts of the body, in the lung and the gut, the primary task of the protective efflux system is the removal of toxic compounds back to the lumen. In the airway and intestinal epithelial cells, however, the transport of MRP1 is contrary to the luminal extrusion [123, 140]. Similarly, in the kidney and the liver, which are the major sites of elimination of xeno- and endobiotics, MRP1, when expressed, is facing the serosal side, opposite to the direction of excretion [123, 136]. Thus, it has been proposed that the role of MRP1 in these tissues is protecting the special, sensitive cell types from toxic effects of xeno- and endobiotics rather than the elimination of these compounds from the body [83]. This concept is supported by the note that a high level of MRP1 expression was observed in cells at a high proliferative status, such as hyperplastic reactive type II pneumocytes in the lung [17], Paneth cells in the small intestine [123], and hepatic progenitor cells in the damaged liver [136] (see above).

On the other hand, the role of MRP1 in the blood–tissue barriers seems to be different. As verified by the studies using mrp1-deficient mice, this transporter greatly contributes to the barrier function, protecting the various sanctuary sites of the body. Both in the blood–brain barrier and the choroid plexus, MRP1 is expressed at the serosal side, i.e., the apical surface of brain capillary [107, 151, 183], and the basolateral membrane of the ventricular epithelial (choroidal and ependymal) cells [129, 171], respectively. Thus, transport activity of MRP1 prevents the accumulation of toxins in the brain and cerebrospinal fluid by extruding these compounds into the blood. In the placenta, MRP1 is primarily expressed in the abluminal (basolateral) side of fetal vessel endothelial cells [104, 146] and in the epithelial cells of the endoplacental yolk sac [147]. This localization

brings about the protection of the fetus by limiting the entry and "quarantining" the toxic compounds. In contrast, the low-level expression of MRP1 found in the basolateral membrane of syncytiotrophoblasts rather serves as a defense system for the trophoblast itself or possibly mediates the transport of important metabolites across the maternal–fetal interface [83, 104]. In accordance with this notion, an increase in MRP1 expression can be observed with placental maturation [120, 147]. In the testis, MRP1 most probably plays a role in the local defense, protecting the special cell types such as the Sertoli cells and the developing spermatocytes in the seminiferous tubules. This suggestion has emerged from the observation that etoposide or methoxychlor exposure damaged primarily these cells in the testes of mrp1 −/− mice [163, 173]. A special role for MRP1 has been proposed in the testosterone-producing Leydig cells, where the transporter is expressed at a high level [42, 173]. Because this cell type is the major site of estrogen conjugation in the testis, and estrogen conjugates are known as high-affinity MRP1 substrates, it is not implausible to assume that MRP1 plays a role in protecting the testis from the feminizing effect of estrogens [127].

The role of MRP1 in leukotriene metabolism and oxidative stress defense

In addition to exogenous and endogenous toxic compounds, the inflammatory cytokine LTC_4 and its metabolites LTD_4 and LTE_4 were also identified as MRP1 substrates [80, 86]. These cysteinyl leukotrienes are known to increase vascular permeability and smooth-muscle contraction at the site of inflammation. LTC_4 is synthesized from LTA_4 by conjugation with GSH in leukotriene-generating cells such as mast cells, macrophages, basophilic and eosinophilic granulocytes, dendritic cells, and platelets. LTC_4 is released from these cells in an ATP-dependent manner and rapidly converted extracellularly to LTD_4 and LTE_4 by γ-glutamyltranspeptidase and dipeptidase, respectively. Because numerous photoaffinity labeling and vesicular transport studies have demonstrated that LTC_4 is one of the highest affinity MRP1 substrates [60, 80, 86, 102], it has been postulated that this transporter is responsible for the cellular release of LTC_4 from the leukotriene-producing cells. This hypothesis attributes an additional physiological function to MRP1, which greatly differs from its role in protecting special, sensitive cell types and sanctuary sites of the body.

Unambiguous evidence for the role of MRP1 in the leukotriene-mediated inflammatory responses was provided by studies with the mrp1 (−/−) mice. Bone marrow-derived mast cells isolated from the knockout animals exhibited only a minor residual LTC_4 release, which was accompanied by an intracellular accumulation of this cytokine [172]. This observation clearly demonstrated MRP1 as the major efflux pump for LTC_4 in mast cells. Even more compelling are the observations showing impaired immune reactions in the mrp1 (−/−) mice. The response to arachidonic acid, a leukotriene-inducing inflammatory stimulus, was greatly reduced in the mrp1-deficient mice as measured by decreased ear edema and vascular permeability, whereas the responses to topical application of phorbol ester (prostaglandin-induced inflammatory stimulus) or to intradermal injection of LTC_4 were unaffected [172].

The mobilization of dendritic cells from the skin to the lymph nodes was also found to be greatly reduced in the mrp1 knockout animals, whereas this defect was overcome by the exogenous administration of cysteinyl leukotrienes (LTC_4 and LTD_4), demonstrating the crucial role of MRP1 in the LTC_4 release [135]. Surprisingly, mrp1 (−/−) mice were found to be more resistant to *Streptococcus pneumoniae*-induced pneumonia than wild-type animals [142]. This observation also connects MRP1 with leukotriene metabolism. As a consequence of the lack of mrp1, intracellular LTC_4 is accumulated in the leukotriene-producing cells, which results in the accumulation of LTA_4, a common precursor for LTC_4 and LTB_4. Thus, LTA_4 becomes more available for conversion to LTB_4. This suggestion is supported by the observations that (1) LTB_4 concentration was elevated in the bronchoalveolar lavage fluid of the mrp1 (−/−) mice; (2) LTB_4-dimethyl amide, an LTB_4 antagonist, abolished the survival advantage of knockout animals; (3) peritoneal macrophages of mrp1 (−/−) mice produce more LTB_4 than macrophages from wild-type mice in response to arachidonic acid or heat-killed *S. pneumoniae*. Collectively, these studies with mrp1 (−/−) mice firmly established the intimate involvement of MRP1 in the leukotriene metabolism.

Several elements of the transcriptional regulation of *MRP1* gene, such as oxidative stress-responsive elements of its promoter region [186] and regulation of expression by Nrf2 [48] and by ROS [161, 177], implicate a connection between this transporter and the redox state of the cell. At a high oxidative state, the cell abolishes the toxic effect of peroxides by their reduction using glutathione (GSH) as an electron donor, producing glutathione disulfide (GSSG). GSH is normally recycled by GSSG reductase enzyme, but during oxidative stress, GSSG is also released from several cell types via an overflow system. Implicating an involvement of MRP1 in the GSH/GSSG metabolism, in the mrp1 (−/−) mice, increased accumulation of GSH was found in tissues which normally express MRP1, whereas GSH concentration was unchanged in tissues known to express little MRP1 [92].

Reduced glutathione was proven to be a poor substrate of MRP1 [79, 87], but its transport is stimulated by

bioflavonoids and verapamil by increasing the apparent affinity of the transporter for GSH [82, 88]. In contrast, GSSG by itself was found to be a substrate with an apparent K_m value of around 100 μM [79]. This affinity makes MRP1 capable of extruding GSSG from cell during oxidative stress when the activity of GSSG reductase becomes rate limiting. Although overexpression of MRP1 failed to increase resistance against oxidative stress in transfected cell lines [8], endogenously expressed MRP1 was shown to mediate cellular GSSG release in response to oxidative stress in astrocytes and endothelial cells [50, 100]. The specificity in these studies was demonstrated by blocking the GSSG efflux by the MRP1 inhibitor MK571 or by MRP1 siRNA. Further support was provided by the findings that the elevated endothelial GSSG efflux observed in the aorta of hypertensive mice was blocked by MK571 and was absent in hypertensive mrp1 (−/−) mice [100]. In addition to its role in the GSSG efflux, MRP1 also contributes to defense against oxidative stress by removal of GS conjugates formed in the cells at a high oxidative state. This is most well exemplified by 4-hydroxynonenal, the predominant toxic lipid peroxidation product, which was found to be bound to and transported by MRP1 [132]. In summary, MRP1 seems to be a multifunctional efflux pump, which not only functions as a part of a defense system against xenobiotics and toxic metabolites but also contributes to immunological responses and to the elimination of toxic effects caused by oxidative stress.

The pathophysiological aspect of MRP1

In addition to its diverse physiological functions, there is a significant body of evidence that MRP1 also contributes to the clinical MDR of several hematological and solid tumors. The MDR phenotype was described in drug-selected tumor cell lines, which exhibited cross-resistance to a wide range of structurally and functionally unrelated anticancer agents. With regard to the mechanism, it has been generally accepted that MDR is a consequence of the presence and activity of export pumps with broad substrate specificity. These transporters extrude cytotoxic agents from the cells, maintaining the drug level below a cell-killing threshold. Several ABC transporters, including MDR1/P-glycoprotein, MRP1-6, ABCG2, MRP7-8, MDR3 (ABCB4), sPgp (ABCB11), and ABCA2, have been shown to confer drug resistance for cell lines in vitro; however, their involvement in the clinical MDR has been established only for MDR1/P-glycoprotein, MRP1, and ABCG2.

As mentioned earlier, MRP1 was originally cloned from a doxorubicin-selected lung cancer cell line, which exhibited resistance to a range of cytotoxic agents without increased expression of MDR1/P-glycoprotein [24]. The substrate specificity of MRP1, as assessed in initial transport measurements with inside-out vesicles, did not correlate with the drug resistance profile found in cell lines. Most of these discrepancies were resolved by the findings that several drugs are transported by MRP1 as glutathione or glucuronide conjugates or require GSH for their transport. The former is exemplified by chlorambucil-GS, melphalan-GS, or etoposide-glucuronide [9, 60], while the latter is represented by vincristine and daunorubicin [86, 87, 133]. Overexpression of MRP1 has been found in numerous drug-selected cell lines derived from various tumors, including lung cancers, breast cancer, gastric and colon carcinomas, prostate cancer, melanoma, neuroblastoma and glioma, fibrosarcoma and epidermoid carcinoma, as well as diverse types of leukemias. Unlike MRP1-enriched inside-out vesicles, these cellular model systems most likely possess all the features, such as glutathione or the drug-conjugating apparatus, which are conceivably needed for effective transport. However, the MRP1-conferred drug resistance profiles found in these cell lines were still dissimilar. This variability was observed not only in cell lines with different origin but also in cells derived from the same parental cell line [30, 145], implicating that the MDR phenotype is multifactorial and possibly involves other resistance factors such as DNA-topoisomerases, cytochrome P450 enzymes, and other ABC transporters capable of conferring drug resistance. Consistent with this idea, several reports indicated that overexpression of MDR1/P-glycoprotein followed MRP1 overexpression in sequential selection procedures [18, 145, 185]. Thus, for a better evaluation of drug resistance in cell lines, a more complex approach is required. Recently, a novel pharmacogenomic method was introduced by Szakacs et al. [155], determining expression profiles for the entire human ABC transporter panel in 60 various cancer cell lines (NCI-60) and correlating them with the growth inhibitory profile of about 1,500 potential anticancer drugs.

To circumvent the problems deriving from multifactorial cellular responses to selective pressure of drug exposure, cell lines transfected with MRP1 cDNA have been generated to investigate MRP1-conferred MDR [3, 25, 45, 181]. These studies revealed similar drug resistance profiles in transfected cells of various origins, although minor differences still existed. In general, the transfectants exhibited increased resistance to doxorubicin, daunorubicin, epirubicine, vincristine, and etoposide (VP-16) but not to cisplatin, taxol, and mitoxantrone. Colchicine and vinblastine resistance conferred by MRP1 slightly varied from cell line to cell line. In addition to these "classical" anticancer agents, resistance to methotrexate [52], ethacrynic acid [99], arsenical and antimonial oxyanions [25], as well as to cytotoxic peptides such as ALLN and 4A6

[31] was observed in MRP1-transfected cells. Not only the drug resistance of these cells but also reduced intracellular accumulation and increased efflux of cytotoxic agents have been shown [25, 36, 52, 181]. After cloning the murine and rat orthologs of human MRP1, some remarkable species differences have been revealed. Cells transfected with mouse or rat mrp1 did not exhibit resistance to anthracyclines; furthermore, rat mrp1-transfected cells also remained sensitive to vinca alkaloids [148, 159]. These species differences call our attention to a careful interpretation of pharmacological studies using these animal models.

Although the cellular model systems are powerful tools to investigate the MDR phenotype conferred by MRP1, the clinical relevance of MRP1 in human malignancies was established by studies investigating the expression and/or function of MRP1 in clinical samples. Increased level of MRP1 expression has been found in a wide range of hematological and solid tumors (for references, see Table 3). Some of them, such as nonsmall-cell lung cancer (NSCLC) [13, 109, 150, 175] or chronic lymphoblastic leukemia [63, 111], generally exhibit high level of MRP1 expression and these tumors are intrinsically multidrug resistant, whereas others such as small-cell lung cancer (SCLC) [175], gastric carcinoma [2, 34], neuroblastoma [114], and retinoblastoma [21] exhibit high MRP1 expression with a lower frequency. A correlation between the MRP1 expression and the stage of the tumor has been reported in certain tumor types, such as acute myeloblastic leukemia (AML) [165], myelodysplastic syndromes (MDS) [124], and prostate cancer [153]. On the other hand, in lung adenocarcinomas, a reverse correlation was seen with tumor grading [13]. High MRP1 expression was also found to be associated with higher grade of tumor differentiation in digestive tract carcinomas [160], endometrial carcinomas [72], and various subtypes of NSCLC, such as lung adenocarcinoma and squamous cell carcinoma [109, 150].

In numerous cases, elevated MRP1 expression was found to be associated with transcriptional regulation events. Because p53 is a suppressor of MRP1 transcription [168], it is not surprising that a strong correlation between mutant p53 status and MRP1 expression was found in prostate cancer [153] and NSCLC [117]. Similarly, an association between the expression of MRP1 and the N-myc oncogen has been firmly established in neuroblastoma [114, 119]. Increased MRP1 expression was observed after chemotherapy in relapsed SCLC [73], bladder carcinomas [158], and acute lymphoblastic leukemia (ALL) [11]. Whether this is a consequence of repopulation of drug-resistant cells (selection) or upregulation of expression in response to drug exposure (induction) remains to be established.

Various clinical parameters, e.g., overall survival, event-free survival, responsiveness to chemotherapy, etc., were correlated with MRP1 expression to explore its contribution to clinical drug resistance. Many of these studies failed to establish a causative role for MRP1 in the negative disease outcome, whose failure is mainly attributable to the impediments hindering this kind of investigations. One of these difficulties is the fact that MRP1 is expressed in almost all normal tissues, and contamination of tumor samples with variable amounts of normal tissues can lead to ambiguous results. Another factor, which can also complicate the interpretation of the results of such studies, is the possible presence of other drug resistance-associated proteins, e.g., LRP/MVP or MDR1/P-glycoprotein. Moreover, the drug extrusion activity of the transporter is not necessarily proportional to the amount of mRNA or protein, especially at high levels of expression. Therefore, definitive conclusions can be obtained only from carefully designed and performed multiparametric studies, which include the identification of the tumor cells, detection of alternative resistance-associated proteins, and use of a specific functional assay, whenever possible.

The significance of this issue is clearly demonstrated by the example of acute myeloblastic leukemia (AML). Several independent studies indicated that MRP1 expression is not predictive of negative therapy outcome in AML [39, 81, 124]. However, when MDR1/P-glycoprotein and MRP1 were assessed together by an appropriate functional assay, the activity of the multidrug transporters was proven to be a negative prognostic factor for achievement of complete remission [64, 77, 78, 166]. Despite all these difficulties discussed above, MRP1 expression has been found to be predictive of poor response to chemotherapy in NSCLC [13, 117] and SCLC

Table 3 Tumor types with elevated MRP1 expression

Tumor types	References
Acute myeloblastic leukemia (AML)	[77, 78, 124, 165]
Acute lymphoblastic leukemia (ALL)	[11]
Chronic lymphoblastic leukemia (CLL)	[63, 111]
Nonsmall-cell lung cancer (NSCLC)	[13, 73, 109, 150, 175]
Small-cell lung cancers (SCLC)	[13, 73, 175]
Breast cancer	[38, 110, 138]
Prostate cancer	[152, 164]
Gastric carcinoma	[2, 34]
Esophageal squamous cell carcinoma (ESCC)	[112, 160]
Colorectal cancer	[40, 160]
Endometrial carcinoma	[72]
Glioma	[1]
Neuroblastoma	[113, 114]
Retinoblastoma	[21]

[55, 74]. Similarly, MRP1 positivity was strongly associated with reduction in both overall survival and event-free survival in breast cancer [38, 110] and neuroblastoma [113, 114, 119]. In addition, a strong correlation between MRP1 expression and shorter times to relapse has been found in breast cancer [38, 110, 138].

Because MDR represents a major obstacle to cancer treatment, the observations discussed above emphasize the significance of MRP1 with respect to clinical practice. Enormous efforts have been made to develop chemotherapeutics and modulators (reversing agents) to prevent MDR (for recent review, see the work of Boumendjel et al. [16]). However, application of modulators is a double-edged sword because these agents may abolish the physiological barriers or result in inhibition of other essential physiological functions of these transporters. Consequently, a more complex approach for better therapy achievements should include a proper, multiparametric diagnosis comprising the selective detection of all possible drug-resistance proteins, as well as a personalized therapeutic intervention that takes into consideration of the results of such diagnosis.

Conclusions

Since the identification of MRP1, considerable amount of experimental data accumulated on the structure, transport function, substrates, cosubstrates, and inhibitors of this transporter. Still, "we see in a mirror dimly" regarding the transport mechanism due to the complexity and the puzzling details of the transport cycle. Although MRP1 has been originally identified as an alternative multidrug transporter in drug-selected cell lines, it turned out to be a protein with multiple physiological functions. Generation and detailed investigation of knockout animals brought a breakthrough in understanding of the diverse physiological roles of MRP1, although these studies were also hampered by the compensatory effects of other ABC transporters with a substrate specificity partially overlapping that of MRP1. Collectively, according to our current understanding, this transporter (1) protects special cell types from the toxic effects of xeno- and endobiotics; (2) contributes to the barrier function of the defense lines of certain sanctuary sites of the body; (3) participates in leukotriene-mediated inflammatory responses through LTC_4 transport; and (4) contributes to defense against oxidative stress by providing an overflow system for GSSG. In addition to these numerous beneficial functions, there is also a vicious aspect of this transporter. Namely, MRP1 has been proven to be responsible for clinical MDR in several human malignancies such as lung, breast, prostate cancer, pediatric tumors, and various forms of leukemia.

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