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Minireview

Substrate recognition and transport by multidrug resistance protein 1 (ABCC1)

Roger G. Deeley*, Susan P.C. Cole

Division of Cancer Biology and Genetics, Cancer Research Institute, Queen's University, Kingston, Ont., Canada K7L 3N6

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Abstract **Multidrug resistance protein (MRP) 1 belongs to the 'C' branch of the ABC transporter superfamily. MRP1 is a high-affinity transporter of the cysteinyl leukotriene C_4 and is responsible for the systemic release of this cytokine in response to an inflammatory stimulus. However, the substrate specificity of MRP1 is extremely broad and includes many organic anion conjugates of structurally unrelated endo- and xenobiotics. In addition, MRP1 transports unmodified hydrophobic compounds, such as natural product type chemotherapeutic agents and mutagens, such as aflatoxin B_1. Transport of several of these compounds has been shown to be dependent on the presence of reduced glutathione (GSH). More recently, GSH has also been shown to stimulate the transport of some conjugated compounds, including sulfates and glucuronides. Here, we summarize current knowledge of the substrate specificity and modes of transport of MRP1 and discuss how the protein may recognize its structurally diverse substrates.**

Keywords: Multidrug resistance protein 1; Leukotriene C_4; Glutathione; Atomic homology model; Site-directed mutagenesis; Organic anion transport; ABC transporter; Multidrug resistance

1. Introduction

Multidrug resistance protein 1 (MRP1/ABCC1) was identified in 1992 as the cause of multidrug resistance in an anthracycline selected human small cell lung carcinoma cell line, H69AR [1]. The drug resistance profile of H69AR cells includes several classes of natural product cytotoxic agents, as well as certain heavy metal oxyanions [2–4]. An intriguing feature of the H69AR cells was their collateral sensitivity to the glutathione (GSH) synthesis inhibitor, buthionine sulfoximine (BSO) as a consequence of an unexplained 6-fold decrease in cellular GSH levels relative to parental H69 cells [5]. The cross-resistance of H69AR cells to natural products such as *Vinca* alkaloids and epipodophyllotoxins, as well as anthracyclines, suggested the possible involvement of P-glycoprotein (P-gp/ABCB1), which at the time was the only known human multidrug transporter [6]. However, resistance was not modulated by agents that reversed P-gp-mediated resistance, and no increase in P-gp levels could be detected [2,7,8]. In contrast, the MRP1 gene was extensively amplified in H69AR cells and MRP1 mRNA levels were elevated approximately 100-fold [1]. The marked increase in levels of MRP1 mRNA relative to the parental H69 cells facilitated the cloning of MRP1 cDNA by differential hybridization. Sequencing of MRP1 revealed it to be an ABC transporter, but only very distantly related to P-gp. Its closest known human relative at the time was the cystic fibrosis transmembrane conductance regulator (CFTR/ABCC7). Despite the lack of amino acid sequence identity with P-gp (approximately 19% overall), gene transfection experiments confirmed the ability of MRP1 to confer resistance to a number of natural product drugs, as well as arsenical and antimonial oxyanions, consistent with the established phenotype of H69AR cells [9,10]. It is now known that MRP1 is a member of a family of nine, possibly ten, proteins in humans, the members of which have been characterized functionally to varying extents, as described elsewhere in this issue. The MRPs (ABCC1–6, 10–12), together with the ATP-gated chloride channel, CFTR (ABCC7), and the ATP-dependent sulfonylurea receptors (SURs) (ABCC8 and ABCC9), which are potassium channel regulators, comprise the 'C' branch of the ABC superfamily [11].

Clinical studies have documented the expression of MRP1 in a range of solid and haematological cancers and, in some cases, have correlated MRP1 expression with negative response to treatment and disease outcome. These studies have been reviewed extensively elsewhere [12–15]. In this minireview, we summarize current knowledge of the substrate specificity and modes of transport of MRP1 and discuss how the protein may recognize its diverse substrates (see Table 1).

2. Substrate specificity of MRP1

The convergence of several unrelated lines of investigation contributed to the early identification of a large number of

*Corresponding author. Fax: +1 613 533 6830.
E-mail address: deeleyr@post.queensu.ca (R.G. Deeley).

Abbreviations: BSO, buthionine sulfoximine; LTC_4, leukotriene C_4; MRP1, multidrug resistance protein 1; P-gp, P-glycoprotein; CFTR, cystic fibrosis transmembrane conductance regulator; $E_2$17βG, estradiol-17β-D-glucuronide; GSH, glutathione; NBD, nucleotide-binding domain; MSD, membrane spanning domain; SUR, sulfonylurea receptor; MALDI TOF, matrix-assisted laser desorption/ionization time of flight; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; TM, transmembrane

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Table 1
Substances transported by MRP1 (ABCC1)

Compound Class	Examples
Drugs/Xenobiotics	
Antineoplastics	Folate-based antimetabolites (methotrexate, edatrexate, ZD1694), anthracyclines (doxorubicin, daunorubicin, epirubicin, idarubicin), plant alkaloids (etoposide, vincristine, vinblastine, paclitaxel, irinotecan, SN-38), antiandrogens (flutamide, hydroxyflutamide)
Antivirals	Saquinavir, ritonavir
Antibiotics	Difloxacin, grepafloxacin
Metalloids	Sodium arsenite, sodium arsenate, potassium antimonite, potassium antimony tartrate
Fluorescent probes	Calcein, Fluo-3, BCECF, SNARF
Toxicants	Aflatoxin B_1, methoxychlor, fenitrothion, chlorpropham
Drug/Xenobiotic conjugates	
GSH conjugates	2,4-Dinitrophenyl-SG, bimane-SG, *N*-ethylmaleimide-SG, doxorubicin-SG, thiotepa-SG, cyclophosphamide-SG, melphalan-SG, chlorambucil-SG, ethacrynic acid-SG, metolachlor-SG, atrazine-SG, sulforaphane-SG, aflatoxin B_1-epoxide-SG, 4-nitroquinoline 1-oxide-SG, $As(SG)_3$
Glucuronide conjugates	Etoposide-Gluc, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)-3β-*O*-Gluc, SN-38-gluc, 4-methylumbelliferyl-β-D-gluc, 6-hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridylmethyl) benzothiazole sulfate (E3040S)-Gluc
Naturally occurring metabolites	
GSH conjugates	Leukotriene C_4, prostaglandin A_2-SG, 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2-SG, hydroxynonenal-SG
Glucuronide conjugates	17β-Estradiol-17-β-D-gluc, glucuronosylbilirubin, bis-lucuronosylbilirubin, hyodeoxycholate-6-α-Gluc
Sulfate conjugates	Estrone 3-sulfate, dehydroepiandrosterone sulfate, sulfatolithocholate
Folates	Folic acid, L-leucovorin
Peptides	GSH, GSSG
Other	Bilirubin

likely MRP1 substrates. The ability of MRP1 to transport many of these putative substrates has now been confirmed by in vitro transport studies using membrane vesicles from MRP1 transfected cells. One contributing factor was the prior characterization of the drug resistance profile of H69AR cells from which MRP1 was cloned [2–4,7]. However, another factor stemmed from the suspicion that MRP1 was the protein defective in naturally occurring strains of mutant (TR^- and Esai) rats [16,17]. Both mutant strains are characterized by a biliary transport deficit, similar to that seen in the rare human inherited disorder, Dubin–Johnson Syndrome [18]. By analyzing the bile composition of the mutant rats and their ability/inability to secrete a wide variety of compounds into bile, a great deal of information had been acquired concerning the substrate specificity of the transporter(s) involved. Biochemical studies had also identified a conjugated organic anion transporter in the plasma membrane of mast cells and eosinophils with functional characteristics similar to that of the hepatocanalicular transporter defective in the TR^- and Esai rats [19]. In fact, the transport defect in these rats and in Dubin–Johnson Syndrome involves MRP2 (ABCC2) whereas the transporter in mast cells and eosinophils is MRP1. Nevertheless, because the substrate specificities of the two proteins overlap extensively, the information from both lines of investigation was extremely useful in the early characterization of the substrate profiles of MRP1 and subsequently, MRP2.

The first MRP1 substrate identified by vesicle transport studies was the conjugated cysteinyl leukotriene C_4 (LTC_4), which was a known substrate of the conjugated organic anion transporter in mast cells [20–23] (Fig. 1). Mouse knock-out studies have confirmed that this inflammatory cytokine is indeed a physiological substrate of MRP1 [24]. The protein has also been shown to be capable of ATP-dependent transport of anionic glutathione, glucuronide and sulfate conjugates of an extremely diverse array of endo- and xenobiotics (reviewed in [13,25,26]). The structural diversity of some examples of the better characterized MRP1 substrates is illustrated in Fig. 1. The affinity of MRP1 for some of its substrates is relatively high for such a promiscuous transporter. For example, the K_m values for LTC_4 and the GS-conjugate of aflatoxin B_1 epoxide are 100–200 nM [22,27]. The protein can also display surprising structural specificity. For example, 17β and 16α glucuronides of estriol differ by more than 20-fold in their ability to competitively inhibit LTC_4 transport [28]. One of the general observations to emerge from vesicular transport studies of MRP1 is that structurally unrelated compounds with different anionic substituents, which may be substrates or simply able to bind to the protein without being transported, often act as competitive inhibitors of transport of well-characterized substrates such as LTC_4 and estradiol-17β-D-glucuronide ($E_2$17βG) [22,28]. The studies also show that the rank order of potency of inhibition is substrate dependent, prompting the suggestion that the substrates and 'inhibitors' establish distinct but overlapping sets of contacts with a common binding pocket on the protein [22,29].

3. Modes of MRP1-mediated transport

Although gene transfer experiments confirmed that MRP1 could enhance the efflux of several natural product drugs, initial attempts with membrane vesicle preparations to demonstrate that the protein was a direct, ATP-dependent drug transporter were unsuccessful [22]. Several observations using drug resistant cell lines overexpressing MRP1 suggested that cellular GSH levels influenced the ability of the transporter to efflux at least some of the drugs to which it conferred resistance. GSH depletion with BSO diminished efflux of certain drugs, while the relatively non-specific organic anion transport inhibitor, probenecid, both decreased GSH release and increased drug accumulation [30–32]. Overall, these observations suggested that MRP1 may mediate the efflux of both GSH and drugs, and that these processes might in some way be coupled. Fig. 1 illustrates the structures of some GSH-dependent MRP1 substrates. Vesicular transport studies confirmed that transport of certain unmodified drugs, such as vincristine, and xenobiotics such as aflatoxin B_1, is GSH-dependent [22,27,33]. Both inhibition studies and determination of kinetic parameters of transport indicated that GSH markedly increased the affinity

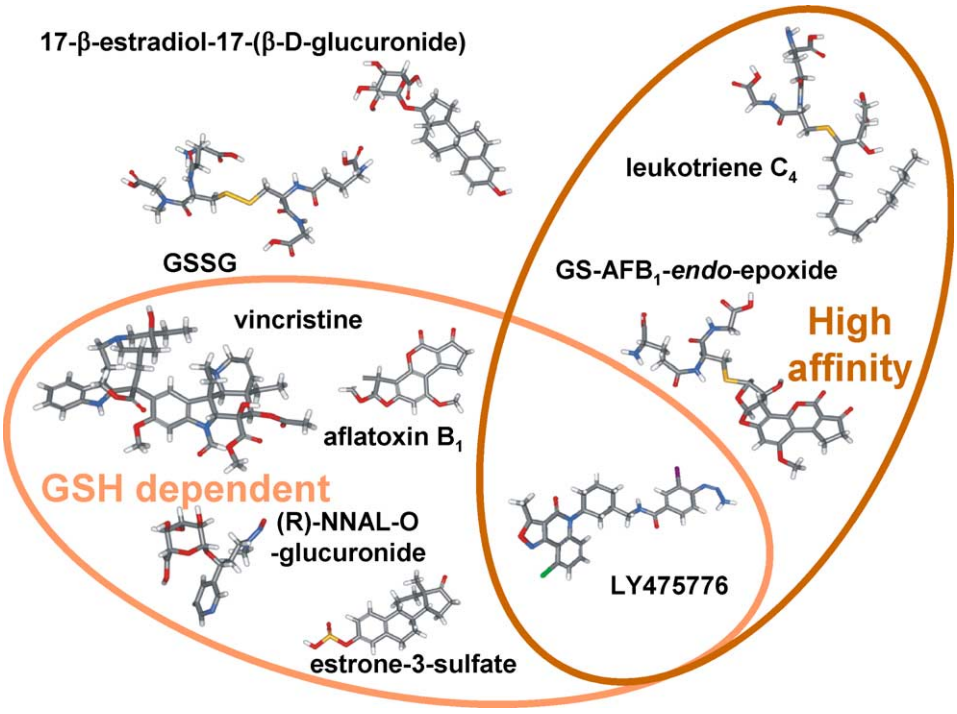

Fig. 1. Structures of some compounds that bind and/or are transported by MRP1 in a GSH-dependent or independent manner. The figure illustrates the structures of a number of MRP1 substrates, including molecules (leukotriene C_4 and GS-aflatoxin B_1-endo-epoxide) that are transported with relatively low K_m values (~100–200 nM) [20,22,27], as well as examples of conjugated and unconjugated substrates that are transported in a GSH-dependent manner. LY475776 is a tricyclic isoxazole that is a GSH-dependent inhibitor of MRP1 that inhibits LTC_4 transport with an IC_{50} of 60–70 nM [67]. AFB_1, aflatoxin B_1; GSSG, oxidized glutathione.

of MRP1 for the second substrate. With vincristine, it is also clear that the presence of drug increases the affinity of MRP1 for GSH [33]. Initially, it was thought that the positive cooperativity observed between transport of GSH and a second substrate might be a mechanism that was restricted to unconjugated compounds. However, there are now several examples of sulfate, glucuronide and glutathione conjugates that display GSH-dependent or stimulated transport [34–38]. It is also apparent that at least in some cases, such as estrone sulfate and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)-glucuronide, GSH transport is not stimulated, despite the fact that the presence of GSH both decreases the K_m and increases the V_{max} for the second substrate [35,36]. Conversely, a number of compounds have been identified, notably certain bioflavonoids and verapamil derivatives, that markedly stimulate GSH transport by both decreasing its K_m and increasing its V_{max}, apparently without being transported themselves [39–41]. The stimulatory effect of GSH can also be obtained with a variety of GSH analogs including non-sulfur containing tripeptides, such as ophthalmic acid [26,41] or short chain alkyl derivatives, such as *S*-methyl GSH, which is actually more effective than GSH in enhancing transport of estrone sulfate but less effective than GSH in stimulating vincristine transport [33,35,36]. The effect of GSH and its derivatives on the transport of a second substrate can be very large. However, it may be an extreme example of a more general mechanism. Several examples of 'cooperativity' between two substrates or a substrate and another ligand, other than GSH, have been documented for MRP1 and other related proteins such as MRP2 [42,43].

A conceptual framework that may be applicable to the interaction of ABC drug transporters with more than one substrate or ligand is provided by studies of soluble multidrug binding proteins, such as QacR from *Staphylococcus aureus*. QacR is a drug-dependent transcription factor rather than a drug transporter. However, like a multidrug resistance protein such as P-gp, QacR binds structurally diverse hydrophobic cations [44]. The protein has also been crystallized in the presence of various ligands [44]. QacR has a large and flexible binding pocket that is exceptionally rich in aromatic and polar amino acids, as well as critical glutamate residues involved in ionic interactions with the protein's cationic substrates. The binding pocket can be viewed as containing multiple mini-pockets for individual drugs that overlap to various extents. The pocket can also undergo substantial changes in shape and volume upon interaction with a ligand. This flexibility allows the simultaneous accommodation of more than one compound, despite the fact that in some cases these compounds may form binary complexes with the protein in which their binding sites would be predicted to overlap [45]. Thus the binding of one compound effectively creates an alternative binding site for another. In principle, this site could have a higher or lower affinity for the second compound than occupied by that ligand in a binary complex. However, if it has a higher affinity then positive cooperativity would be expected. In this type of model, the binding could be ordered or random. In the former case, only one substrate may be affected, as observed with the effect of GSH on MRP1-mediated transport of estrone sulfate and NNAL-glucuronide [35,36], while in the latter the interaction with both substrates may be altered, as observed with the combination

of GSH and vincristine [33]. Although many studies of ABC drug transporters indicate that substrate binding results in conformational changes in the protein, the possibility cannot be excluded that the binding of one substrate may mask residues that disfavor binding of the second compound, or the compound itself may contribute directly to the creation of a second binding site, without an alteration in protein conformation. At least with respect to GSH binding to MRP1, however, the evidence that the tripeptide induces conformational changes in the transporter is quite strong [46,47]. To what extent this may influence the substrate specificity and transport activity of the protein under physiological conditions is not known.

4. Substrate recognition by MRP1

Unlike many ABC proteins, MRP1 contains three rather than two membrane spanning domains (MSDs) (Fig. 2) [1,48–50]. The additional MSD (MSD0) of MRP1 has been shown experimentally to contain five transmembrane (TM) helices, while the two core MSDs (MSD1 and MSD2) have a more typical ABC structure, each containing 6 TM helices and an intracellular COOH-terminus connected to a nucleotide-binding domain (NBD). Thus, this NH_2-terminus of MRP1 is glycosylated and extracellular [49]. Several other ABCC proteins contain a third NH_2-terminal MSD with similar predicted topologies. These include MRPs 2, 3, 6 and 7 and the two SURs. Partial deletions of MSD0 in MRP1 and mutation of certain amino acids in this domain interfere with protein processing and activity [51–53]. However, it appears likely that they do so by interfering with correct folding of the remainder of the protein, since MSD0 can be removed in its entirety (amino acids 1–203) and the truncated protein retains the ability to traffic to its normal, basolateral location in the plasma membrane. The MSD0-less protein can also transport a number of substrates [54–58]. Although not essential for trafficking to the plasma membrane or transport activity, MSD0 is required for efficient retention of MRP1 at the

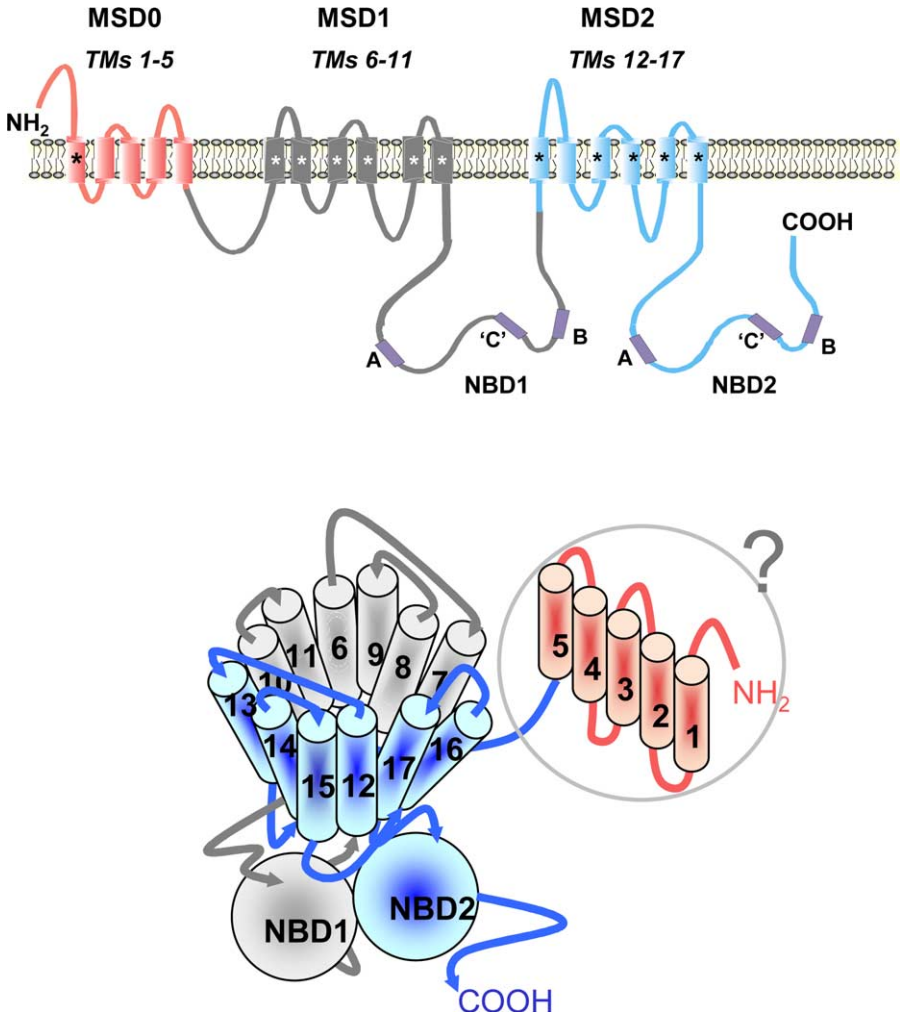

Fig. 2. Cartoons of the predicted topology and transmembrane helix packing of MRP1. The upper panel is a schematic of the predicted topology of the MSDs MRP1 based on experimental evidence, secondary structure prediction algorithms, such as HMMTOP, and molecular dynamics modeling [61]. Transmembrane helices (TMs) known to contain amino acid residues that influence substrate specificity are marked with an asterisk. Conserved motifs in the nucleotide binding domains (NBDs) are indicated by: A (Walker A), B (Walker B) and 'C' (the ABC signature). The coloring of various regions of the protein is consistent between upper and lower panels. The lower panel shows a cartoon of the predicted packing of the TM helices of MSD1 (grey) and MSD2 (blue) of MRP1 based on the crystal structure of the bacterial lipid transporter MsbA [100–102] (see text). The arrangement of TM helices in MSD0 (pink) is presently completely unknown, as is the manner in which it interacts with the remainder of the protein.

cell surface. MSD0 can also rescue the trafficking of NH_2-terminally truncated forms of MRP1 that are retained in the endoplasmic reticulum following elimination of trafficking signals located at the COOH-terminal end of the protein [59,60]. Thus MSD0 and the COOH-terminal region of MRP1 contain at least partially redundant elements that contribute to the processing and trafficking of the protein [60]. Nevertheless, the demonstration that MRP1 retains transport activity in the absence of MSD0 indicates that the major determinants of substrate binding and translocation are located in the core region of the protein. As described below, this region of the protein has been extensively mutated resulting in a topological 'map' of amino acids that are involved in determining substrate specificity, as well as the overall activity of the protein. More recently, energy minimized models of MSD1 and MSD2 of MRP1 have been developed [61]. The model provides the opportunity to integrate functional data from the mutational studies with a possible three-dimensional structure of the substrate translocation pathway. It has also provided a framework for further mutational and/or cross-linking studies designed to test its validity.

The mapping of regions and individual amino acids involved in substrate binding and transport by MRP1 has relied extensively on site-directed mutagenesis and UV-crosslinking studies with photoactivateable substrates and other ligands. Photolabeling studies have been carried out with unmodified LTC_4 [22,56] and an arylazido LTC_4 derivative [62], azido-GSH derivatives [63,64], compounds that bind to the protein in a GSH-dependent manner (e.g. azidoagosterol-A) [55,65] and the tricyclic isoxazole LY475776 [63,67,68] and others that bind independently but are less specific for MRP1 (e.g. iodoaryl azido derivatives of the quinoline, *N*-(hydrocinchonidin-8′-yl)-4-azido-2-hydroxybenzamide (IACI) and rhodamine 123 (IAARh123) [69,70]). Co-expression studies with various fragments of MRP1, partial proteolysis and, most recently, MALDI TOF mass spectrometry of complete proteolytic digests have been used to localize regions of the protein to which these various ligands are cross-linked [71]. In general, the major sites of cross-linking detected by co-expression and partial proteolysis studies appear to involve, at the very least, regions spanning TMs 10 and 11 in MSD1 and TMs 16 and 17 in MSD2. However, the distribution of labeling differs depending on the type of photoactivateable agent used.

LTC_4 and azidophenacyl-GSH predominantly label MSD1, while GSH-dependent compounds such as Agosterol A and LY475776 primarily label MSD2, and compounds such as IACI and IAARh123 label sites in both MSDs to more or less equivalent extents. Recent MALDI TOF mass spectrometry studies of MRP1 labeled with LTC_4 have identified TM6 and TM7, as well as TM10 and TM17 as sites of cross-linking [71]. These studies also detected labeling of the COOH-proximal region of the cytoplasmic linker connecting MSD0 and MSD1 which appears contrary to previous reports using co-expressed fragments of MRP1 labeled with either LTC_4 or azidophenacyl-GSH [57]. However, weak labeling of the MSD0–MSD1 linker was observed with ^{125}I-labeled aryl-azido GSH [64]. There has also been a report of the photolabeling of MSD0 itself by an azido-derivative of LTC_4 [62]. Regardless of the reason for differences in photolabeling results, the region of the MSD0–MSD1 linker between amino acids 204–280 has been shown to be essential for substrate binding, transport activity and trafficking of MRP1 [54,57,56,65,72]. The differences in labeling profile between GSH-dependent ligands, such as Agosterol A and LY475776 that predominantly label MSD2, and the preferential labeling of MSD1, as opposed to the MSD0–MSD1 linker, by LTC_4 and azidophenacyl-GSH, suggest that regions of MSD1 may be of critical importance for binding of the GS-moiety of these compounds. Site-directed mutagenesis studies summarized below support this suggestion.

Mutagenesis studies of MRP1 have confirmed the importance of residues in TMs 10, 11, 16 and 17 in determining substrate specificity and overall activity [29,61,66,73–80]. They have also revealed the contribution of residues in other TM helices (TMs 6, 7, 8, 9, 12, 14, and 15) [52,53,73–75,81–86] and cytoplasmic loops [75,87,88], such as that connecting TMs 15 and 16, in defining the substrate specificity of the protein. In some cases, mutagenesis has been guided by functional differences observed between highly conserved mammalian orthologs of MRP1 [79,84,89–94] and in others, by structural differences between MRP1 and its human homologs with overlapping substrate specificity, such as MRP2 and MRP3 [95–98]. In addition, amino acids with side chains expected to contribute to substrate recognition and interhelical interaction (e.g. polar, ionizable and aromatic residues), or to α-helical geometry (e.g. Pro) and positioning within the membrane (e.g. aromatic and basic residues) have been extensively mutated.

These studies have also revealed that major differences in substrate specificity between MRP1 orthologs can be attributable to changes in a single amino acid. For example, despite the fact that human MRP1 was cloned from an anthracycline selected cell line, several highly conserved non-primate mammalian orthologs do not confer resistance to this class of drugs [89,91–93]. Those that have been tested are also poor transporters of some steroid conjugates which are transported relatively well by human MRP1. In mouse and rat Mrp1, these differences can be largely eliminated simply by conversion of a Gln residue in TM14 and an Ala residue in TM17 to Glu and Thr, respectively, which are the corresponding amino acids in human MRP1 [79,84,92]. It is also apparent that the relationship between conservation of function and structure among homologs is complex and impossible to predict from amino acid sequence alone, as shown by mutational studies of TM17 of human MRP1, MRP2 and MRP3. TM17 is the most highly conserved TM among these homologs. Between MRP1 and MRP3 the helix differs in only two residues. However, it is clear from mutation of polar residues in TM17 of all three proteins that comparable conserved residues establish different interactions with common substrates [29,96–98]. For example, with respect to the transport of $E_2$17βG by MRP1 and MRP3 there is little overlap between the residues involved. In MRP1, all residues shown to affect interaction with $E_2$17βG are located in the predicted inner leaflet region of the helix. In contrast, in MRP3 several critical amino acids are in the predicted outer leaflet region of the helix and mutations of the corresponding amino acids in MRP1 have no effect on transport of this common substrate.

Mutations in TM17 of MRP1 affect drug resistance profiles and transport of a number of anionic and conjugated substrates, but none have been found that selectively alter LTC_4 transport. The lack of specific interaction of residues in TM17 with LTC_4 is supported by the observation that replacement

of the entire helix up to and including position 1248 at the predicted membrane-cytosol interface with Ala residues has little effect on LTC_4 transport [99]. However, mutation of Arg^{1249} does abrogate LTC_4 binding and overall activity of the transporter [80]. In TM16, the presence of a Glu residue at position 1204 is critical for efficient transport of GSH and LTC_4 but not several other substrates [80]. However, to date this is the only mutation in these two helices that has been shown to selectively affect transport of GSH and the GSH conjugated leukotriene. Overall, these results and those from recent mass spectrometry studies [71] are consistent with the suggestion raised by cross-linking studies that the major determinants of binding of these compounds are in MSD1.

Mutational analysis of TM11 has identified Asn^{590}, Arg^{593}, Phe^{594} and Pro^{595} (all located in predicted TM11) as being important for LTC_4 binding [61,74–76]. However, alanine substitution of Asn^{590} affects transport of a variety of substrates, apparently by reducing the binding of ATP by NBD1 [76], although other amino acid substitutions have no effect. On the other hand, while alanine substitution of Phe^{594} eliminates LTC_4 binding and overall transport activity, conservative mutations have differential effects on substrate specificity suggesting that this residue may interact directly with substrate [61]. Although photolabeling studies have implicated the region encompassing TMs10 and 11 as the major site of cross-linking of GSH-derivatives and conjugates, no additional polar, aromatic or ionizable residues in these TMs have been identified that selectively affect the transport of LTC_4. Thus it appears highly likely that other regions are critical for the specific recognition of GSH, LTC_4 and possibly other GS-conjugates. TM6 is a probable candidate [71,74,81]. It is relatively rich in charged residues, mutation of which affects either overall activity (as exemplified by Asp^{336}), or selectively decreases transport of LTC_4 and GSH (as exemplified by Lys^{332} and His^{335}) [74,81]. In addition, Bao et al. [99] replaced amino acids 320-337 (corresponding to TM6) with alanine and demonstrated that unlike TM17, TM6 is critical for the binding and transport of LTC_4. Finally, mass spectrometry analyses also indicate that TM6 is part of the LTC_4 binding site of MRP1 [71].

Another of the more intriguing observations to emerge from mutational studies of MRP1 concerns the role of aromatic amino acids in substrate recognition. A number of studies indicate that aromatic amino acids in the inner leaflet regions of helices in MSD1 and MSD2 are involved in recognition of specific substrates [29,73]. However, recent models derived using molecular dynamics simulations of MSD1 and MDS2 of MRP1 indicate that they may also play a more general role in defining a 'gateway' to the translocation pathway [61]. The models are based on the crystal structures of the bacterial lipid transporter MsbA from *V. cholera* and *E. coli*, and an *E. coli* MsbA based homology model of P-gp that incorporates data from cysteine cross-linking studies [100–102]. The models indicate that Trp^{553} (TM10), Trp^{1198} (TM16), Tyr^{1243} and Trp^{1246} (TM17) that are known to affect substrate specificity and/or overall activity [29,73,77] could form an aromatic 'basket' located close to the cytosolic interface of a possible translocation pathway that would be completed by Phe^{594} in TM11 (Fig. 3). The results of mutational studies are consistent with this suggestion [61].

Aromatic amino acids are extremely important in the initial interaction of substrates with soluble multidrug binding proteins. For example, in QacR, initial interaction with a substrate such as rhodamine 123 displaces two Tyr residues, which in the absence of drug are buried in the drug-binding pocket. This displacement then results in conformational changes that shift the protein from its DNA- to drug-bound form [44]. If this predicted feature of MRP1 is correct, it raises the interesting possibility that interactions between these residues and substrate play a pivotal role in transducing conformational changes resulting from initial substrate binding to other regions of the protein.

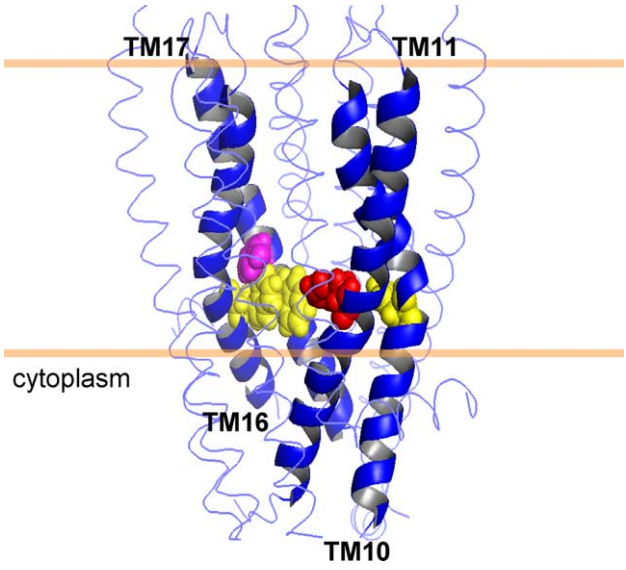

Fig. 3. Putative aromatic 'basket' close to the cytoplasmic end of the translocation pathway of MRP1 predicted by atomic homology models of the second and third MSDs of the transporter. The figure was generated using PyMol and is based on the molecular dynamics models of MSD1 and MSD2 of MRP1 [61]. A lateral view in the plane of the membrane is shown with TM helices 10, 11, 16 and 17 shown as ribbons. Trp^{553} TM10, Trp^{1198} TM16, and Trp^{1246} TM17 are shown as yellow spheres. Tyr^{1243} TM17 and Phe^{594} TM11 are shown in pink and red, respectively. The predicted positions of membrane interfaces are indicated by the orange lines.

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