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Biochimica et Biophysica Acta 1461 (1999) 347–357

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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Review

The multidrug resistance protein family

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Received 15 September 1999; accepted 15 September 1999

Abstract

The human multidrug resistance protein (MRP) family contains at least six members: MRP1, the godfather of the family and well known as the multidrug resistance protein, and five homologs, called MRP2–6. In this review, we summarize what is known about the protein structure, the expression in tissues, the routing in cells, the physiological functions, the substrate specificity, and the role in multidrug resistance of the individual members of the MRP family.

Keywords: Multidrug resistance protein; Multidrug resistance; Protein routing; Glutathione–drug conjugate pump; GSH; Cisplatin

Contents

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0005-2736/99/$ – see front matter
PII: S0005-2736(99)00167-4

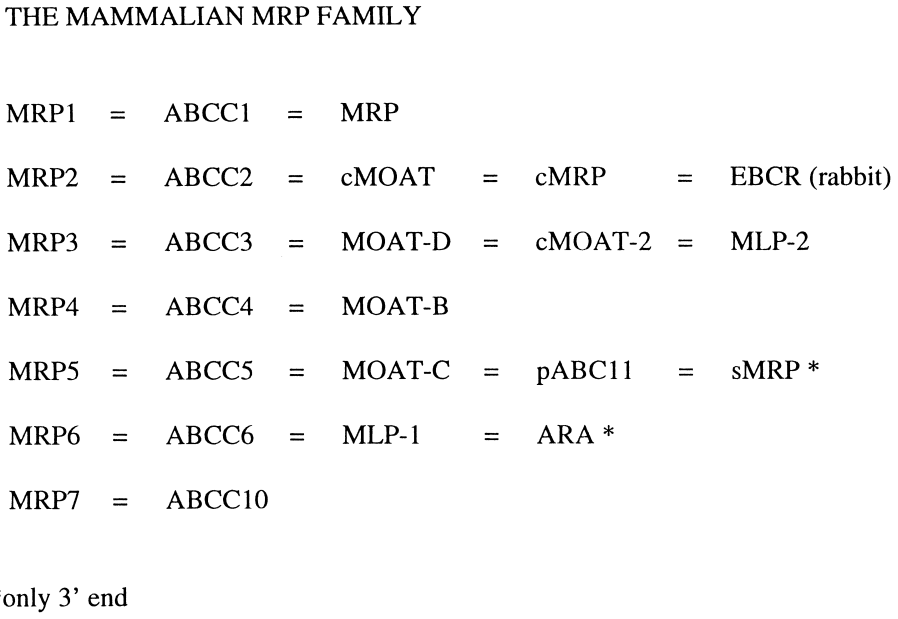

Fig. 1. Overview of the MRP family and the alternative names used in the literature for the individual members.

1. Introduction

The MRP family entered the drug resistance scene in 1992 when Susan Cole and Roger Deeley cloned the multidrug resistance-associated protein gene, now known as MRP1 [1]. MRP2 followed in 1996 [2–4] and the notion of an MRP family with five members was introduced at the Gosau meeting on ABC transporters in 1997 by Marcel Kool [5,6]. MRP6 was added in 1998 [7,8] and the existence of a 7th family member, MRP7, has only been inferred from a database search. It will not be discussed any further here.

Sequences corresponding to each of these members of the MRP family were already identified in the ground-breaking paper by Allikmets et al. [9], which identified 21 new ABC transporters on the basis of conserved sequences. Some of these MRPs are also known by other names, as summarized in Fig. 1. MRP2 was characterized as the canalicular multispecific organic anion transporter (cMOAT) long before its cloning [10], because this transporter is missing in rats with an inborn error in the biliary secretion of organic anions, including the conjugated bilirubins (see [3,11] and König et al., this volume). Hence, the term MOAT has also been used for other members of the MRP family [11–14]. The sMRP [15] is a cloning artifact and ARA [16,17] represents the 3′-end of the MRP6 gene that is incidentally co-amplified with MRP1 in cells selected for doxorubicin resistance [8].

Within the MRP family, homology is highest be-

Table 1
Percentage amino acid identity between the human MRP proteins for which the complete sequence is known

	MRP1 1531 aa chr 16	MRP2 1545 aa chr 10	MRP3 1527 aa chr 17	MRP4 1325 aa chr 13	MRP5 1437 aa chr 3	MRP6 1503 aa chr 16
MRP1	100					
MRP2	49	100				
MRP3	58	48	100			
MRP4	39	37	36	100		
MRP5	34	35	33	36	100	
MRP6	45	38	43	34	31	100

For the accession numbers of the MRP genes, see legend of Fig. 3.

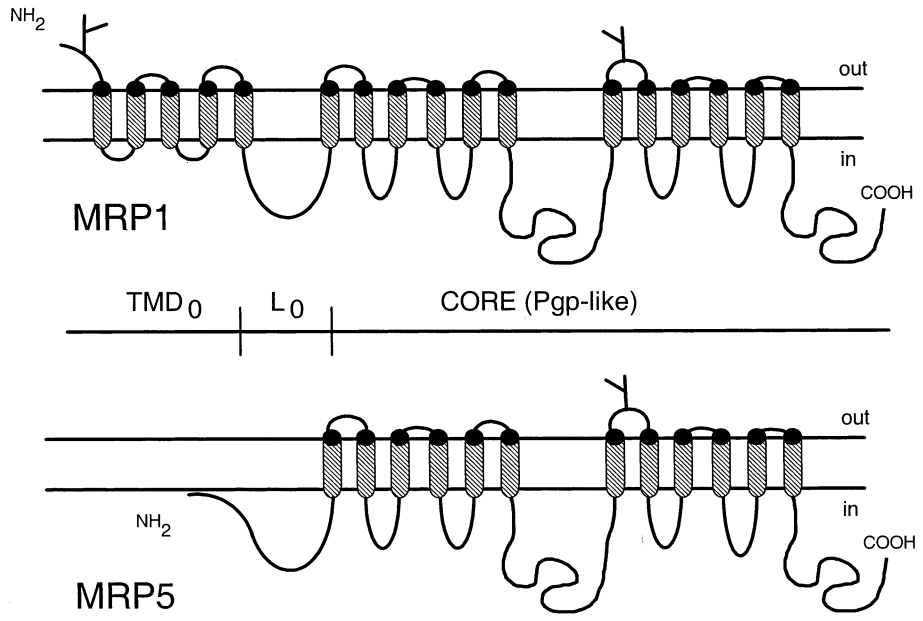

Fig. 2. Membrane topology models for MRP1 and MRP5 according to [20]. MRP2, MRP3 and MRP6 have a similar structure as MRP1 and are characterized by the presence of the extra N-terminal extension with five transmembrane regions (TMD_0) connected to a Pgp-like core by a cytoplasmic linker (L_0). MRP4 has a similar structure as MRP5. They both lack the TMD_0 domain, but contain the intracellular L_0 domain in addition to the Pgp-like core.

tween MRP1, -2, -3 and -6 (Table 1). These MRPs also share the characteristic TMD_0L_0 segment [18,19], also seen in glutathione–drug conjugate (GS-X) pumps from simple eukaryotes, such as yeast and Leishmania (Fig. 2). Although this segment is absent in MRP4 and -5, it should be noted that these still have the basic structure that seems to be required for GS-X pump activity in MRP1 [20], i.e. the P-glycoprotein-like core structure and the L_0 loop (Fig. 2).

Within the group of mammalian ABC transporters the MRPs form a cluster that is clearly demarcated from the other known groups, such as the P-glycoproteins, CFTR, and the sulfonylurea receptors (Fig. 3). Although the functions of the family members may turn out to be diverse, for the moment it therefore seems reasonable to speak of an MRP family. We focus here on the ability of MRPs to transport anti-cancer drugs and on the physiological functions of MRPs.

Table 2
General features of MRPs

	GS-X pump[a]	Transport of MTX[b]	Transport of (some) MDR drugs[a]	Main location in the body[c]	KO mice (rats) available[d]
MRP1	+	+	+	Ubiquitous (low in liver)	+
MRP2	+	+	+	Liver, kidney, gut	+
MRP3	+	+	+	Liver, adrenals, pancreas, kidney,gut	−
MRP4	?	?	?	Prostate, lung, muscle, pancreas, testis, ovary,bladder, gallbladder,	−
MRP5	+	−?	?	Ubiquitous	+
MRP6	?	?	?	Liver, kidney	−

[a]See other sections of this paper.
[b]See [21–24].
[c]Based on RNA expression data [6,8,12,13,25,26,59].
[d]See Section 6.

2. General features of MRPs

Some of the general features of MRPs are summarized in Table 2 and Fig. 4. MRP1, -2, -3 and -5 are able to transport glutathione conjugates, such as dinitrophenyl glutathione (DNP-GS). For MRP4 and -6, this remains to be tested. MRP1–3 can transport methotrexate (MTX) [21–24], experiments with transfected MRP5 cells have thus far been negative. The same holds for clinical MDR drugs, as will be discussed in more detail below. MRP1, MRP4 and MRP5 RNAs are widely distributed in the body, whereas MRP2, MRP3 and MRP6 appear mainly to function in liver, kidney and gut [6,8,12,13,25,26]. In the case of MRP1–3, the RNA data summarized in Table 2 have been substantiated by protein localization. For the other MRPs no protein data are available yet.

In polarized monolayers of kidney cells, MRP2 is routed to the apical membrane and MRP1, -3 and -5 to the basolateral membrane (Fig. 4). MRP4 and -6 have not been analyzed. With specific (monoclonal) antibodies MRP2 has also been located in apical, and MRP1 and MRP3 in basolateral membranes of tissues [2,23,26–28]. A substantial fraction of MRP1 of normal tissues is not in the plasma membrane, but located in undefined intracellular vesicles [2]. The MRP5-specific antibodies available thus far do not detect MRP5 in tissue sections and the localization of this protein in vivo remains to be sorted out.

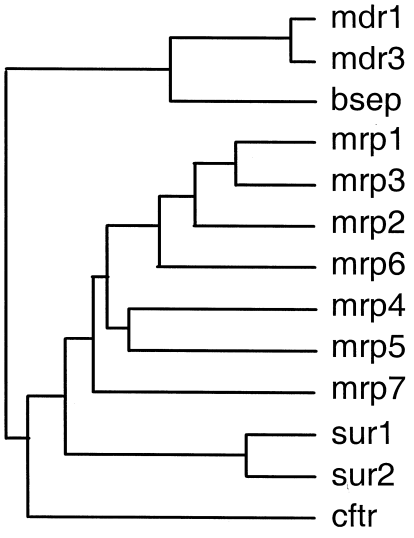

Fig. 3. Phylogenetic rooted tree showing the relationship between the human MRP homologs, human SUR1 and SUR2, human CFTR, and the three Pgp homologs, MDR1, MDR3 and BSEP. To generate this tree the C-terminal end of each protein was used that aligns with amino acids 955–1531 of MRP1. This part of the protein includes the C-terminal six transmembrane domains as well as the second nucleotide binding domain. For the multiple sequence alignment the PILEUP program from the University of Wisconsin Genetics Group (GCG) package (version 9.1) was used. The following accession numbers were used: MRP1, L05628; MRP2, U49248; MRP3, AF009670; MRP4, AF071202; MRP5, AF104942; MRP6, AF076622; MRP7, U66684; SUR1, L78207; SUR2, AF061289; CFTR, M28668; MDR1, M14758; MDR3, M23234; BSEP, AF091582.

3. Five problems in the study of MRPs and some solutions

(1) The MRPs characterized thus far are organic anion transporters (Table 2). Hence, many good substrates for these transporters are highly charged and do not penetrate the cell membrane. This complicates the study of MRPs in intact cells. There are hydrophobic pro-drugs, however, that are converted in the cell into anions. Examples are 1-chloro-2,4-dinitrobenzene (CDNB) that is converted in the cell into DNP-GS by a glutathione-*S*-transferase; and calcein-AM that is rapidly hydrolyzed to calcein in the cell. A disadvantage of these prodrugs is that their conversion into MRP substrate may be limiting. For the characterization of substrate specificity, it is therefore necessary to study vesicular transport by MRPs in inside-out vesicles. Insect Sf9 cells transfected with baculovirus MRP constructs have proven the most convenient source of such vesicles.

(2) Antibodies against one MRP often cross-react with another member of the MRP family. This problem has been solved by generating monoclonal antibodies against the non-conserved parts of MRPs and by rigorously checking specificity against a panel of different MRPs.

(3) Cells selected for drug resistance often overexpress multiple transporter genes. Attempts to relate the resistance profile of such cells to a single transporter are therefore risky. The use of specific inhibitors or transfected cells can solve this problem. Unfortunately, there are no drugs yet that specifically inhibit a single MRP species without effect on other transporters. In fact, most inhibitors of MRP1 and MRP2 studied thus far are organic anions that act as

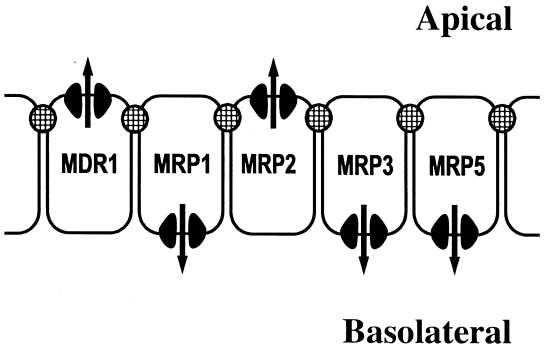

Fig. 4. Schematic representation of the localization of MDR1 Pgp, MRP1, MRP2, MRP3 and MRP5 in transfected polarized epithelial cells. The physical barrier between the apical and basolateral plasma membrane is formed by tight junctions.

substrates for MRPs. Attempts have been made to specifically block only a single MRP by inhibiting its synthesis by anti-sense oligonucleotides. Although partial inhibition in short-term experiments has been observed with selected cell lines ([29] and M. Kool, unpublished data), this approach is unsuitable for systematic studies.

The characterization of substrate specificity of MRPs therefore requires cells transfected with MRP expression constructs. By comparing the transfected cell with its untransfected parent, the activity of the MRP introduced can (in principle) be deduced.

(4) It has been difficult to get MRP transfectants giving high expression and product routed to the cell membrane. Some of the initial transfection constructs produced low levels of transporter, but this could be remedied by shortening the construct segment corresponding to the 5′-untranslated region of the mRNA and improving the translational start. Transfected cells may also lose expression over time and it is therefore necessary to use early passages, reclone if necessary, and regularly check expression levels by antibody. Two serious problems remain, however. (a) Most cells contain endogenous (organic anion) transporters resulting in background transport activity. This activity may vary somewhat by clonal variations in expression of endogenous transporters. As the endogenous transporters are often uncharacterized and, hence, undetectable by antibody, e.g. in the pig and dog polarized kidney cells often used for transport studies, there is no simple solution for this problem. However, endogenous P-glycoprotein may be suppressed by adding specific inhibitors, such as PSC833 [30]. We have generated cell lines from KO mice lacking both murine P-gps and Mrp1. These should eventually provide parental cells with much lower background resistance/transporter activities than the human cells now used for transfection experiments. (b) Transfected cells produce a transporter that is trapped in an endosomal compartment and does not reach the plasma membrane. The problem is illustrated by the (largely, unpublished) data for MRP2 summarized in Table 3. Some cell lines transfected with a retroviral MRP2 construct do not express the protein at all, or in a patchy fashion with only 10% of the cells positive. This 10% is maintained after cloning and may reflect a deleterious effect of the transporter on cell viability. Intra-cellular routing of MRP2 is also sensitive to growth conditions and in kidney cells or hepatocytes

Table 3
Overview of MRP2-transfected cell lines

Transfected cell	Tissue of origin	Expression level	Fraction of positive cells	MRP2 in the plasma membrane
HCT8	Colon carcinoma	−	−	−
PXN94	Ovarian carcinoma	−	−	−
SW 1573	Lung carcinoma	+	10%	−
T24	Bladder carcinoma	++	10%	−
2008	Ovarian carcinoma	+++	>75%	Some
A2780	Ovarian carcinoma	+++	>75%	Some
LLC-PK1	Pig kidney	++	>90%	Most[a]
MDCKII	Canine kidney	+++	>90%	Most[a]

[a]MRP2 is only routed to the plasma membrane when cells are growing as a monolayer.

MRP2 only goes to the plasma membrane if cells touch [30,31].

(5) Cells transfected with MRP1, MRP2 or MRP5 cDNAs secrete GSH into the medium and this might be one of the reasons why it has been more difficult to get cells expressing high levels of MRP than of P-gp. If cells transfected with an MRP1 cDNA construct are transferred from rich growth medium to buffered saline, they are rapidly depleted of GSH, suggesting that they must be producing GSH at high rate in growth medium to counteract this drain. We have found no simple way to stop this efflux or to compensate it with glutathione esters. We return to the mechanism of the efflux in the next section.

4. GSH excretion by MRP transfected cells

It was found at an early stage that the organic anion transporter MRP1 was also able to transport non-anionic organic drugs, such as anthracyclines and Vinca alkaloids. Attempts to detect derivatives of these drugs conjugated to an anionic ligand (GSH, glucuronic acid, sulfate) have remained unsuccessful and the consensus is now that these drugs are transported as such. Early experiments also established that cellular resistance to these drugs mediated by MRP1 requires intracellular GSH, suggesting that GSH is either required as an activator or as a co-transported substrate.

More recent experiments have shown that GSH is required for vesicular transport of vincristine by MRP1 and for inhibition by vincristine of the transport of organic anions by MRP1 [32]. Vanadate-mediated nucleotide trapping experiments directly showed a low-affinity binding of GSH to MRP1 [33]. These experiments did not answer the question whether GSH is an activator or a co-transported substrate. Recent experiments with kidney cells transfected with a MRP2 cDNA construct have shown, however, that vinblastine transport mediated by MRP2 induces a proportional GSH transport. The stoichiometry is odd, as the ratio of vinblastine/GSH transported is 2–3 [34] in this system. Nevertheless, the results strongly support a co-transport model.

Another question that has remained unanswered concerns the GSH secreted by cells transfected with MRP1, MRP2 and MRP5 cDNA constructs. The importance of MRP1-mediated GSH export in vivo is obvious from the observation that Mrp1 (−/−) mice have raised levels of GSH in their tissues [35]. Is this GSH co-transported with an endogenous ligand or is GSH transported by itself? MRP1 can bind GSH with low affinity, but can it also transport GSH? The low affinity of GSH for MRPs has made it difficult to study transport of radioactive GSH in vesicles. It has been shown, however, that GSH competitively inhibits uptake of other MRP1 substrates in vesicles [36]. This is compatible with GSH transport by MRP1. Important supporting evidence has come from experiments with yeast. Rebbeor et al. [37] have shown that yeast cadmium resistance factor 1 (Ycf1), the yeast ortholog of MRP1/2 [38], transports GSH with low affinity ($K_m = 15$ mM).

All these results and other experiments summarized by Paulusma et al. [36] and Ballatori and Rebbeor [39] support the idea that MRP1/2 can transport GSH. As no endogenous ligand co-transported with GSH has been found ([36,39] and our unpublished data with cultured cells), we favor a minimal working model [34] for MRP1/2 with two drug binding sites: one with a relatively high affinity for GSH (G-site) and a low affinity for drug, and one with a relatively high affinity for drug and a low affinity for GSH (D-site). In the absence of drugs, both binding sites are occupied by GSH resulting in a slow export of GSH. At low drug concentrations, the G-site remains occupied by GSH and the D-site becomes occupied by drug, resulting in co-transport of both compounds. We infer that the two sites show positive cooperativity as we observe stimulation of GSH transport by MDCKII-MRP2 cells in the presence of the organic anions sulfinpyrazone and indomethacin [34]. This may also explain why sulfinpyrazone is able to stimulate DNP-GS export in MDCKII-MRP2 cells [30]. At high drug concentrations some (negatively charged) drugs appear to be able to occupy both the G- and D-site resulting in transport of drug alone. MRP1 and MRP2 do not require free GSH for the transport of compounds that are conjugated to glutathione, glucuronide or sulfate [30,40–42]. We suggest that such substrates have a relatively high affinity for both the G- and D-site and are therefore transported efficiently without requiring GSH or stimulating GSH export. As cells with

high levels of MRP3 do not secrete GSH [23], the G-site in this transporter may have a very low affinity for GSH.

5. What do MRPs contribute to drug resistance of cancer cells?

Table 2 provides a brief summary of current knowledge. MRP1 can cause resistance to a wide range of MDR drugs, as discussed in detail by Hipfner et al. in this volume. Hooijberg et al. [22] have recently shown that MRP1 can also cause high level resistance to short-term methotrexate (MTX) exposure. The same was found for MRP2 and MRP3 [22,23]. MTX is transported, but the polyglutamylated forms of MTX, accumulating during long exposure, are not transported (our unpublished data) and hence resistance nearly disappears during long-term incubations. The clinical significance of the high levels of MRP1 found in some human tumors, remains to be established (see Hipfner et al., this volume).

The substrate specificity of MRP2 was defined in detail using natural rat mutants (TR^-/GY or EHBR) lacking MRP2, and by vesicular transport studies (see König et al., this volume). However, only some MDR drugs were included in these studies. More recently, we observed that the variable expression of MRP2 in lung cancer cells selected for doxorubicin resistance, correlated very well with resistance to cisplatin [6]. This was of interest as Ishikawa and coworkers had already shown that cisplatin and GSH can form a 1:2 complex that might be removed from cells by a GS-X pump [43]. The complex was shown to inhibit LTC_4 transport by MRP1 [44], but all attempts to demonstrate cisplatin resistance in MRP1-transfected cells were unsuccessful (see Hipfner et al., this volume).

Recent results have shown, however, that MRP2 can confer resistance to cisplatin, in addition to a range of MDR drugs. Koike et al. used a stable anti-sense cDNA construct of MRP2 to transfect the human HepG2 hepatoma cell line [45]. This resulted in sensitization to a remarkable series of drugs: cisplatin, CPT-11, the camptothecin derivative SN-38, vincristine and doxorubicin. It also led to a substantial decrease in MRP2 and to a 4-fold increase in cellular GSH [45]. Since the anti-sense construct was rather long (805 bp) it is not completely excluded that these remarkably strong effects on resistance could have been due to inhibition of the synthesis of several different MRPs.

Recent transfection experiments have substantiated the ability of MRP2 to transport the Vinca alkaloid vinblastine [30]. The study of drug resistance in transfected cells was complicated, however, by low levels of MRP2 expression and poor routing to the plasma membrane (Table 3). In the best transfectants, 2008 ovarium carcinoma cells, we only observed marginal resistance to vincristine and mitoxantrone (M. Kool, unpublished data). However, Cui et al. [46] succeeded in getting sufficient expression of MRP2 in human embryonic kidney (HEK-293) and MDCK cells to demonstrate clear, i.e. 5- to 10-fold, resistance to etoposide, vincristine, doxorubicin and cisplatin (see further discussion by König et al., this volume). These results establish the ability of MRP2 to confer resistance to cisplatin and a range of MDR drugs. It remains to be seen whether this ever occurs in patients.

Early studies on MRP3 did not find any correlation between expression of this transporter and drug resistance [6]. A more recent survey of a panel of lung cancer cell lines showed, however, a strong correlation between MRP3 and doxorubicin resistance and a weaker, but still highly significant correlation with resistance to vincristine, etoposide and cisplatin [47]. In an important study using MRP3-containing vesicles from MRP3-transfected animal cells, Hirohashi et al. [24] showed that MRP3 is a typical organic anion transporter, but, unlike MRP1 and -2, it prefers glucuronides as substrates over glutathione conjugates. Hirohashi et al. [24] also found that MRP3 transports MTX and this was independently shown by Kool et al. [23]. In addition, Kool et al. [23] found low etoposide and teniposide resistance in MRP3-transfected cells. As it is difficult to generate transfected cells with high concentrations of MRP3 in the plasma membrane, it remains possible that higher levels of MRP3 will also result in resistance to doxorubicin and possibly other drugs, as suggested by the correlation study of Young et al. [47].

Little is known as yet about MRP5. The intriguing possibility that this transporter causes resistance to

thio-purines [48] remains to be substantiated by drug accumulation and vesicular transport studies.

6. Physiological functions of MRPs

Defense against toxic compounds is a major concern of all living organisms and ABC transporters play an essential role in this defense. The PgpA of *Leishmania*, an MRP avant la lettre, because it was discovered before MRP1, but not recognized as a novel class of ABC transporter, contributes to the defence of this unicellular parasite against arsenite and antimonite [49]. In *Caenorhabditis elegans*, MRPs contribute to the defence against heavy metal ions and against some of the toxins that bacteria produce to escape being eaten by this bacterial predator [50]. It is, therefore, likely that mammalian MRPs also help to protect their host against toxic compounds and studies on Mrp1 KO mice have shown that this is indeed the case. The Mrp1 (−/−) mice are hypersensitive to etoposide [51,52], and this is especially manifest in bone marrow, testis, kidney, the oropharyngeal mucosa, i.e. in cells which normally have a substantial amount of Mrp1 [53].

As Mrp1 is located at the basolateral side of epithelium cells, it tends to pump drugs into the body, rather than into bile, urine or gut for disposal, as P-glycoprotein does. Nevertheless, Mrp1 can protect vital cell layers from destruction, for instance, the basal stem cell layer in the oral mucosa [53]. There are also important organs in the body that require a basolateral transporter for protection. A good example are the testicular tubules. The male germ cells are protected by a ring of Sertoli cells with their apical surface towards the lumen. The high concentration of Mrp1 in the basal membrane of the Sertoli cells helps to protect the germ cells by pumping out drug from the testicular tubule [53]. Another position in the body where a basolateral transporter is useful for protecting a vital organ is the choroid plexus. This structure protrudes into the cerebrospinal fluid (CSF) and is essential for exchange of metabolites between blood and CSF. The epithelial cell layer of the plexus contains a high amount of Mrp1 ([54] and our unpublished data) and we have recently shown, that the absence or Mrp1 results in considerable accumulation of intravenously administered etoposide in the CSF (J. Wijnholds, unpublished data).

Whether MRP2–6 also play a role in protection against exogenous toxic compounds remains to be determined. There is no Mrp2 KO mouse, but there are natural rat mutants lacking Mrp2. These mutant rats are compromised in the excretion of organic anions from the liver (see König et al., this volume). The Mrp5 (−/−) mouse was only recently generated and pharmacological studies with this mutant remain to be done (J. Wijnholds, unpublished data).

The presence of a range of ABC transporters available for defence against toxic compounds, has allowed the recruitment during evolution of some of these transporters for metabolic purposes. An example is the MDR3 P-glycoprotein, which evolved from a duplicate copy of the gene for a drug-transporting P-glycoprotein into a dedicated transporter for phosphatidylcholine [55]. There is abundant evidence that the MRPs also have important physiological functions. MRP2 provides a major route for the secretion of organic anions from the liver, and rats and humans lacking this transporter develop a mild liver disease, mainly due to the inability of the liver to excrete bilirubin-glucuronides (see König et al., this volume).

MRP1 is the major high-affinity transporter of leukotriene C_4 (LTC_4) as shown by biochemical experiments and the analysis of KO mice [51]. Remarkably, the absence of the major LTC_4 excretion pathway does not have any detectable deleterious effect on mice in the congenial surroundings of a quality animal house. Actually, the mice are more resistant than wild-type mice against an inflammatory stimulus and even against a bacterial lung infection [51,56]. This does not mean that MRP1 is more important for defense than for normal metabolism. It remains possible that there is overlap in function between MRPs and that double or triple KO mice lacking several Mrps may reveal a more substantial metabolic role for MRP1 than is now apparent.

The tissue distribution of MRP3 shown in Table 2 [6,13,23,25,26] and its basolateral position in polarized cells [23,26] suggest that this protein has an important metabolic role in the intestinal uptake of organic anions and in the removal of organic acids from bile and liver cells under conditions of cholestasis. Mrp3 is highly upregulated in the liver of rats

made cholestatic by bile duct ligation [57,58] and in cholestatic human liver [23]. MRP3 is also present at high level in the adrenal cortex ([6] and M. Kool, unpublished immunohistochemistry data). This raises the possibility that MRPs are responsible for secretion of some hydrophilic steroid hormone derivatives, such as dehydroepiandrosterone sulfate. The birth of the Mrp3 (−/−) mouse that should help to clarify the physiological role of Mrp3 is eagerly awaited.

The Mrp5 KO mouse, recently generated in our laboratory [48], is healthy and fertile and has no obvious macroscopic defects. This shows that Mrp5 does not have an indispensable function, but whether it is mainly a defense protein or fulfils functions not yet revealed, remains to be established.

7. Outlook

The discovery of the MRP family has considerably broadened the study of MDR in tumor cells and has led to widespread interest in the possible function(s) of the members of this family in normal metabolism. It is still impossible to assess the potential contribution of MRP1, the best studied MRP, to clinical drug resistance and we still have a long way to go before we know for each family member whether it helps tumors to escape from drug treatment. Likewise, our understanding of the role of each MRP in normal metabolism varies from being sketchy to non-existent. Transfected cells and KO mice are providing insight into the physiological functions of MRPs, but it should be clear from this review that a vast amount of work remains to be done. Eventually, KO mice combining defects in several transporters will have to be generated to dissect overlapping functions and get a complete picture. Interesting years lie ahead. Mother Cole and father Deeley can be pleased with their thriving family.

Acknowledgements

Our experimental work on MRPs is supported by grants from the Dutch Cancer Society to P.B.

References

[1] S.P.C. Cole, G. Bhardwaj, J.H. Gerlach, J.E. Mackie, C.E. Grant, K.C. Almquist, A.J. Stewart, E.U. Kurz, A.M.V. Duncan, G.R. Deeley, Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line, Science 258 (1992) 1650–1654.

[2] M.J. Flens, G.J.R. Zaman, P. Van der Valk, M.A. Izquierdo, A.B. Schroeijers, G.L. Scheffer, M. De Haas, C.J.L.M. Meijer, R.J. Scheper, Tissue distribution of the multidrug resistance-associated protein, Am. J. Pathol. 148 (1996) 1237–1247.

[3] M. Büchler, J. König, M. Brom, J. Kartenbeck, H. Spring, T. Horie, D. Keppler, cDNA cloning of the hepatocyte canalicular isoform of the multidrug resistance protein, cMrp, reveals a novel conjugate export pump deficient in hyperbilirubinemic mutant rats, J. Biol. Chem. 271 (1996) 15091–15098.

[4] K. Taniguchi, M. Wada, K. Kohno, T. Nakamura, T. Kawabe, M. Kawakami, K. Kagotani, K. Okumura, S. Akiyama, M. Kuwano, A human canalicular multispecific organic anion transporter (cMOAT) gene is overexpressed in cisplatin-resistant human cancer cell lines with decreased drug accumulation, Cancer Res. 56 (1996) 4124–4129.

[5] M. Kool, M. de Haas, C. Paulusma, G. Scheffer, R. Scheper, R. Oude Elferink, F. Baas, P. Borst, (1997) FEBS Advanced Lecture Course 105 (Abstract).

[6] M. Kool, M. De Haas, G.L. Scheffer, R.J. Scheper, M.J.T. Van Eijk, J.A. Juijn, F. Baas, P. Borst, Analysis of expression of *cMOAT (MRP2), MRP3, MRP4*, and *MRP5*, homologs of the multidrug resistance-associated protein gene *(MRP1)*, in human cancer cell lines, Cancer Res. 57 (1997) 3537–3547.

[7] M. Kool, J. Wijnholds, R. Evers, Z. Holló, G.L. Scheffer, M. de Haas, M. van der Linden, C.A.A.M. Mol, L. van Deemter, R.J. Scheper, F. Baas, P. Borst, Proc. AACR 39 (1998) 167 (Abstract).

[8] M. Kool, M. Van der Linden, M. De Haas, F. Baas, P. Borst, Expression of human *MRP6*, a homologue of the multidrug resistance protein gene *MRP1*, in tissues and cancer cells, Cancer Res. 59 (1999) 175–182.

[9] R. Allikmets, B. Gerrard, A. Hutchinson, M. Dean, Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the expressed sequence tags database, Hum. Mol. Genet. 5 (1996) 1649–1655.

[10] P.L.M. Jansen, W.H. Peters, W.H. Lamers, Hereditary chronic conjugated hyperbilirubinemia in mutant rats caused by defective hepatic anion transport, Hepatology 5 (1985) 573–579.

[11] C.C. Paulusma, P.J. Bosma, G.J.R. Zaman, C.T.M. Bakker, M. Otter, G.L. Scheffer, R.J. Scheper, P. Borst, R.P.J. Oude Elferink, Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene, Science 271 (1996) 1126–1128.

[12] K. Lee, M.G. Belinsky, D.W. Bell, J.R. Testa, G.D. Kruh, Isolation of MOAT-B, a widely expressed multidrug resis-

tance-associated protein canalicular multispecific organic anion transporter-related transporter, Cancer Res. 58 (1998) 2741–2747.
[13] M.G. Belinsky, L.J. Bain, B.B. Balsara, J.R. Testa, G.D. Kruh, Characterization of MOAT-C and MOAT-D, new members of the MRP/cMOAT subfamily of transporter proteins, J. Natl. Cancer Inst. 90 (1998) 1735–1741.
[14] S. Cole, Correspondence. Re: Characterization of MOAT-C and MOAT-D, new members of the MRP/cMOAT subfamily of transporter proteins, J. Natl. Cancer Inst. 91 (1999) 888–889.
[15] T. Suzuki, K. Nishio, H. Sasaki, H. Kurokawa, F. Saito-Ohara, T. Ikeuchi, S. Tanabe, M. Terada, N. Saijo, cDNA cloning of a short type of multidrug resistance protein homologue, SMRP, from a human lung cancer cell line, Biochem. Biophys. Res. Commun. 238 (1997) 790–794.
[16] T.J. Longhurst, G.M. O'Neill, R.M. Harvie, R.A. Davey, The anthracycline resistance-associated (*ara*) gene, a novel gene associated with multidrug resistance in a human leukaemia cell line, Br. J. Cancer 74 (1996) 1331–1335.
[17] B.J. Kuss, G.M. O'Neill, H. Eyre, N.A. Doggett, D.F. Callen, R.A. Davey, *ARA*, a novel ABC transporter, is located at 16p13.1, is deleted in inv(16) leukemias, and is shown to be expressed in primitive hematopoietic precursors, Genomics 51 (1998) 455–458.
[18] E. Bakos, T. Hegedüs, Z. Holló, E. Welker, G.E. Tusnády, G.J.R. Zaman, M.J. Flens, A. Váradi, B. Sarkadi, Membrane topology and glycosylation of the human multidrug resistance-associated protein, J. Biol. Chem. 271 (1996) 12322–12326.
[19] G.E. Tusnády, E. Bakos, A. Váradi, B. Sarkadi, Membrane topology distinguishes a subfamily of the ATP-binding cassette (ABC) transporters, FEBS Lett. 402 (1997) 1–3.
[20] E. Bakos, R. Evers, G. Szakács, G.E. Tusnády, E. Welker, K. Szabó, M. De Haas, L. Van Deemter, P. Borst, A. Váradi, B. Sarkadi, Functional multidrug resistance protein (MRP) lacking the N-terminal transmembrane domain, J. Biol. Chem. 273 (1998) 32167–32175.
[21] M. Masuda, Y. I'izuka, M. Yamazaki, R. Nishigaki, Y. Kato, K. Ni'inuma, H. Suzuki, Y. Sugiyama, Methotrexate is excreted into the bile by canalicular multispecific organic anion transporter in rats, Cancer Res. 57 (1997) 3506–3510.
[22] J.H. Hooijberg, H.J. Broxterman, M. Kool, Y.G. Assaraf, G.J. Peters, P. Noordhuis, R.J. Scheper, P. Borst, H.M. Pinedo, G. Jansen, Antifolate resistance mediated by the multidrug resistance proteins MRP1 and MRP2, Cancer Res. 59 (1999) 2532–2535.
[23] M. Kool, M. Van der Linden, M. De Haas, G.L. Scheffer, J.M.L. De Vree, A.J. Smith, G. Jansen, G.J. Peters, N. Ponne, R.J. Scheper, R.P.J. Oude Elferink, F. Baas, P. Borst, MRP3, an organic anion transporter able to transport anti-cancer drugs, Proc. Natl. Acad. Sci. USA 96 (1999) 6914–6919.
[24] T. Hirohashi, H. Suzuki, Y. Sugiyama, Characterization of the transport properties of cloned rat multidrug resistance-associated protein 3 (MRP3), J. Biol. Chem. 274 (1999) 15181–15185.
[25] Y. Kiuchi, H. Suzuki, T. Hirohashi, C.A. Tyson, Y. Sugiyama, cDNA cloning and inducible expression of human multidrug resistance associated protein 3 (MRP3), FEBS Lett. 433 (1998) 149–152.
[26] J. König, D. Rost, Y. Cui, D. Keppler, Characterization of the human multidrug resistance protein isoform MRP3 localized to the basolateral hepatocyte membrane, Hepatology 29 (1999) 1156–1163.
[27] C.C. Paulusma, M. Kool, P.J. Bosma, G.L. Scheffer, F. Ter Borg, R.J. Scheper, G.N.J. Tytgat, P. Borst, F. Baas, R.P.J. Oude Elferink, A mutation in the human *cMOAT* gene causes the Dubin–Johnson syndrome, Hepatology 25 (1997) 1539–1542.
[28] T.P. Schaub, J. Kartenbeck, J. Koenig, O. Vogel, R. Witzgall, W. Kriz, D. Keppler, Expression of the conjugate export pump encoded by the *mrp2* gene in the apical membrane of kidney proximal tubules, J. Am. Soc. Nephrol. 8 (1997) 1213–1221.
[29] A.J. Stewart, Y. Canitrot, E. Baracchini, N.M. Dean, S.P.C. Cole, Reduction of expression of the multidrug resistance protein (MRP) in human tumor cells by antisense phosphorothioate oligonucleotides, Biochem. Pharmacol. 51 (1996) 461–469.
[30] R. Evers, M. Kool, L. Van Deemter, H. Jansen, J. Calafat, L.C.J.M. Oomen, C.C. Paulusma, R.P.J. Oude Elferink, F. Baas, A.H. Schinkel, P. Borst, Drug export activity of the human canalicular multispecific organic anion transporter in polarized kidney MDCK cells expressing *cMOAT (MRP2)* cDNA, J. Clin. Invest. 101 (1998) 1310–1319.
[31] H. Roelofsen, C.T.M. Bakker, B. Schoenmaker, M. Heijn, P.L.M. Jansen, R.P.J. Oude Elferink, Redistribution of canalicular organic anion transport activity in isolated and cultured rat hepatocytes, Hepatology 21 (1995) 1649–1657.
[32] D.W. Loe, R.G. Deeley, S.P.C. Cole, Characterization of vincristine transport by the M_r 190,000 multidrug resistance protein (MRP): Evidence for cotransport with reduced glutathione, Cancer Res. 58 (1998) 5130–5136.
[33] Y. Taguchi, A. Yoshida, Y. Takada, T. Komano, K. Ueda, Anti-cancer drugs and glutathione stimulate vanadate-induced trapping of nucleotide in multidrug resistance-associated protein (MRP), FEBS Lett. 401 (1997) 11–14.
[34] R. Evers, M. De Haas, R. Sparidans, J. Beijnen, P.R. Wielinga, J. Lankelma, P. Borst, Drug export mediated by the multidrug resistance protein MRP2 (cMOAT) is associated with glutathione export, submitted for publication.
[35] G. Rappa, A.L. Lorico, R.A. Flavell, A.C. Sartorelli, Evidence that the multidrug resistance protein (MRP) functions as a co-transporter of glutathione and natural product toxins, Cancer Res. 57 (1997) 5232–5237.
[36] C.C. Paulusma, M.A. Van Geer, R. Evers, M. Heijn, R. Ottenhof, P. Borst, R.P.J. Oude Elferink, The canalicular multispecific organic anion transporter (cMOAT/MRP2) mediates low-affinity transport of reduced glutathione, Biochem. J. 338 (1999) 393–401.

[37] J.F. Rebbeor, G.C. Connolly, M.E. Dumont, N. Ballatori, ATP-dependent transport of reduced glutathione on YCF1, the yeast orthologue of mammalian multidrug resistance associated proteins, J. Biol. Chem. 273 (1998) 33449–33454.
[38] R. Tommasini, R. Evers, E. Vogt, C. Mornet, G.J.R. Zaman, A.H. Schinkel, P. Borst, E. Martinoia, The human multidrug resistance-associated protein functionally complements the yeast cadmium resistance factor 1, Proc. Natl. Acad. Sci. USA 93 (1996) 6743–6748.
[39] N. Ballatori, J.F. Rebbeor, Roles of MRP2 and oatp1 in hepatocellular export of reduced glutathione, Sem. Liver Dis. 18 (1999) 377–387.
[40] G. Jedlitschky, I. Leier, U. Buchholz, K. Barnouin, G. Kurz, D. Keppler, Transport of glutathione, glucuronate, and sulfate conjugates by the *MRP* gene-encoded conjugate export pump, Cancer Res. 56 (1996) 988–994.
[41] G. Jedlitschky, I. Leier, U. Buchholz, J. Hummel-Eisenbeiss, B. Burchell, D. Keppler, ATP-dependent transport of bilirubin glucuronides by the multidrug resistance protein MRP1 and its hepatocyte canalicular isoform MRP2, Biochem. J. 327 (1997) 305–310.
[42] K. Ito, H. Suzuki, T. Hirohashi, K. Kume, T. Shimizu, Y. Sugiyama, Functional analysis of a canalicular multispecific organic anion transport cloned from rat liver, J. Biol. Chem. 273 (1998) 1684–1688.
[43] T. Ishikawa, F. Ali-Osman, Glutathione-associated cis-diamminedichloroplatinum (II) metabolism and ATP-dependent efflux from leukemia cells, J. Biol. Chem. 268 (1993) 20116–20125.
[44] T. Ishikawa, J. Bao, Y. Yamane, K. Akimaru, K. Frindrich, C.D. Wright, M.T. Kuo, Coordinated induction of *MRP*/*GS-X* pump and τ-glutamylcysteine synthetase by heavy metals in human leukemia cells, J. Biol. Chem. 271 (1996) 14981–14988.
[45] K. Koike, T. Kawabe, T. Tanaka, S. Toh, T. Uchiumi, M. Wada, S.-i. Akiyama, M. Ono, M. Kuwano, A canalicular multispecific organic anion transporter (*cMOAT*) antisense cDNA enhances drug sensitivity in human hepatic cancer cells, Cancer Res. 57 (1997) 5475–5479.
[46] Y. Cui, J. König, U. Bucholz, H. Spring, I. Leier, D. Keppler, Drug resistance and ATP-dependent conjugate transport mediated by the apical multidrug resistance protein, MRP2, permanently expressed in human and canine cells, Mol. Pharmacol. 55 (1999) 929–937.
[47] L.C. Young, B.G. Campling, T. Voskoglou-Nomikos, S.P.C. Cole, R.G. Deeley, J.H. Gerlach, Expression of multidrug resistance protein-related genes in lung cancer: correlation with drug response, Clin. Cancer Res. 5 (1999) 673–680.
[48] J. Wijnholds, C.A.A.M. Mol, G.L. Scheffer, R.J. Scheper, P. Borst, Proc. AACR 40 (1999) 315 (Abstract).
[49] M. Ouellette, D. Légaré, B. Papadopoulou, Microbial multidrug resistance ABC transporters, Trends Microbiol. 2 (1994) 407–411.
[50] A. Broeks, B. Gerrard, R. Allikmets, M. Dean, R.H.A. Plasterk, Homologs of the human multidrug resistance genes *MRP* and *MDR* contribute to heavy metal resistance in the soil nematode *Caenorhabditis elegans*, EMBO J. 15 (1996) 6132–6143.
[51] J. Wijnholds, R. Evers, M.R. van Leusden, C.A.A.M. Mol, G.J.R. Zaman, U. Mayer, J. Beijnen, M. Van der Valk, P. Krimpenfort, P. Borst, Increased sensitivity to anticancer drugs and decreased inflammatory response in mice lacking MRP, Nat. Med. 11 (1997) 1275–1279.
[52] A. Lorico, G. Rappa, R.A. Finch, D. Yang, R.A. Flavell, A.C. Sartorelli, Disruption of the murine *MRP* (Multidrug Resistance Protein) gene leads to increased sensitivity to etoposide (VP-16) and increased levels of glutathione, Cancer Res. 57 (1997) 5238–5242.
[53] J. Wijnholds, G.L. Scheffer, M. Van der Valk, J.H. Beijnen, R.J. Scheper, P. Borst, Multidrug resistance protein 1 protects the oropharyngeal mucosal layer and the testicular tubules against drug-induced damage, J. Exp. Med. 188 (1998) 797–808.
[54] V.V. Rao, J.L. Dehlheimer, M.E. Bardgett, A.Z. Snyder, R.A. Finch, A.C. Sartorelli, D. Piwnica-Worms, Choroid plexus epithelial expression of *MDR1* P glycoprotein and multidrug resistance-associated protein contribute to the blood–cerebrospinal-fluid drug-permeability barrier, Proc. Natl. Acad. Sci. USA 96 (1999) 3900–3905.
[55] J.J.M. Smit, A.H. Schinkel, R.P.J. Oude Elferink, A.K. Groen, E. Wagenaar, L. Van Deemter, C.A.A.M. Mol, R. Ottenhof, N.M.T. Van der Lugt, M. Van Roon, M.A. Van der Valk, G.J.A. Offerhaus, A.J.M. Berns, P. Borst, Homozygous disruption of the murine *mdr*2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease, Cell 75 (1993) 451–462.
[56] M.J. Schultz, J. Wijnholds, M.P. Peppelenbosch, M.J.B.M. Vervoordeldonk, P. Speelman, S.J.H. Van Deventer, P. Borst, T. Van der Poll, Mice lacking the multidrug resistance protein 1 are resistant to *streptococcus pneumoniae*-induced pneumonia, J. Exp. Med., submitted for publication.
[57] T. Hirohashi, H. Suzuki, K. Ito, K. Ogawa, K. Kume, T. Shimizu, Y. Sugiyama, Hepatic expression of multidrug resistance-associated protein-like proteins maintained in eisai hyperbilirubinemic rats, Mol. Pharmacol. 53 (1998) 1068–1075.
[58] D.F. Ortiz, S. Li, R. Iyer, X. Zhang, P. Novikoff, I.M. Arias, MRP3, a new ATP-binding cassette protein localized to the canalicular domain of the hepatocyte, Am. J. Physiol. 276 (1999) G1493–G1500.
[59] G.J.R. Zaman, C.H.M. Versantvoort, J.J.M. Smit, E.W.H.M. Eijdems, M. De Haas, A.J. Smith, H.J. Broxterman, N.H. Mulder, E.G.E. De Vries, F. Baas, P. Borst, Analysis of the expression of *MRP*, the gene for a new putative transmembrane drug transporter, in human multidrug resistant lung cancer cell lines, Cancer Res. 53 (1993) 1747–1750.