Reconstitution of ATP-dependent Leukotriene C₄ Transport by Co-expression of Both Half-molecules of Human Multidrug Resistance Protein in Insect Cells*

(Received for publication, July 12, 1996, and in revised form, August 13, 1996)

Mian Gao‡§¶, Douglas W. Loe‡, Caroline E. Grant‡, Susan P. C. Cole‡§¶, and Roger G. Deeley‡§¶**
From the Cancer Research Laboratories‡¶ and Department of Pathology§¶, Queen’s University, Kingston, Ontario K7L 3N6, Canada.

Multidrug resistance protein (MRP) confers a multidrug resistance phenotype similar to that associated with overexpression of P-glycoprotein. Unlike P-glycoprotein, MRP has also been shown to be a primary active ATP-dependent transporter of conjugated organic anions. The mechanism(s) by which MRP transports these compounds and increases resistance to natural product drugs is unknown. To facilitate studies on the structure and function of MRP, we have determined whether a baculovirus expression system can be used to produce active protein. Full-length MRP as well as molecules corresponding to either the NH₂- or COOH-proximal halves of the protein were expressed individually and in combination in Spodoptera frugiperda Sf21 cells. High levels of intact and half-length proteins were detected in membrane vesicles from infected cells. Although underglycosylated, the full-length protein transported leukotriene C₄ (LTC₄) with kinetic parameters very similar to those of MRP produced in transfected HeLa cells. Neither half-molecule was able to transport LTC₄. However, a functional transporter with characteristics similar to those of intact protein could be reconstituted when both half-molecules were co-expressed. Transport of LTC₄ by Sf21 membrane vesicles containing either intact or reconstituted MRP was competitively inhibited by both S-decylglutathione and 17β-estradiol 17-β-d-glucuronide, with Ki values similar to those reported previously for MRP expressed in HeLa cells (Loo, D. W., Almquist, K. C., Deeley, R. G., and Cole, S. P. C. (1996) J. Biol. Chem. 271, 9675–9682; Loo, D. W., Almquist, K. C., Cole, S. P. C., and Deeley, R. G. (1996) J. Biol. Chem. 271, 9683–9689). These studies demonstrate that human MRP produced in insect cells can function as an active transporter of LTC₄ and that the NH₂- and COOH-proximal halves of the protein can assemble efficiently to form a transporter with functional characteristics similar to those of the intact protein.

Resistance to multiple drugs is encountered frequently during treatment of many types of cancer. Both intrinsic and acquired clinical multidrug resistance often involve a spectrum of drugs that includes many natural products and their derivatives. The most extensively characterized mechanism known to cause resistance to natural product drugs in vitro is overexpression of the ATP-dependent transmembrane transporter P-glycoprotein (P-gp)¹ (1). However, recently, a second member of the ATP binding cassette (ABC) superfamily, multidrug resistant protein (MRP), has been shown to confer a multidrug resistance phenotype similar to that associated with P-gp (2–4). Since its discovery in the human small cell lung cancer cell line H69AR, MRP has been identified in non-P-gp multidrug-resistant cell lines from a variety of tumor types (2, 5). Drug-resistant cell lines that overexpress both MRP and P-gp have also been described (6–8).

MRP was predicted from its cDNA sequence to be 1531 amino acids long with a polypeptide molecular weight of 171,000. MRP contains multiple potential transmembrane helices, two nucleotide binding domains (NBDs), and 14 potential N-linked glycosylation sites (2). Biochemical analyses confirmed that MRP is synthesized as a M₉ 170,000 precursor that is processed to a mature N-glycosylated form with an apparent molecular weight of 190,000 (9, 10).

Most drug-selected cell lines and transfected cells overexpressing MRP display reductions in drug accumulation and increases in the rate of ATP-dependent drug efflux (4, 11–16). However, there is no direct evidence that MRP can bind unmodified forms of the drugs to which it confers resistance, and there are conflicting reports of its ability to transport these compounds (17–19). MRP can function as a primary active ATP-dependent transporter of cysteiny1 leukotrienes, other organic glutathione conjugates including oxidized glutathione, as well as etoposide glucuronide, certain steroid glucuronides, and bile salt derivatives (18–24). In addition, the protein has been shown to transport the Vinca alkaloid vincristine, but only in the presence of GSH (19). The mechanism by which this occurs is not known.

Elucidation of the molecular mechanism by which MRP transports conjugated organic anions will require identification of the specific protein domains and amino acid residues that participate in substrate binding and transport. As an initial step, we have expressed intact human MRP as well as the NH₂- and COOH-proximal halves of the protein, both independently and in combination, using a baculovirus expression system. The ability of these molecules to transport leukotriene C₄ (LTC₄) into inside-out membrane vesicles has been determined. We found that intact MRP expressed in Spodoptera

---

1 The abbreviations used are: P-gp, P-glycoprotein; MRP, multidrug resistance protein; LTC₄, leukotriene C₄; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; mAb, monoclonal antibody; NBD, nucleotide binding domain; CFTR, cystic fibrosis transmembrane conductance regulator; ABC, ATP binding cassette; PNGase F, peptide N-glycosidase F; wt, wild type.
frugiperda Sf21 cells, although underglycosylated, exhibited a high level of ATP-dependent LTC4 transport with kinetic parameters similar to those obtained with vesicles from MRPltransfected human cells. We also found that neither half-molecule alone would support LTC4 transport, but a high level of ATP-dependent transport was obtained when both half-molecules were co-expressed. These results indicate that the two half-molecules of MRP can assemble to form an active transporter despite the lack of covalent linkage, and that transport of LTC4 requires interaction of both halves of MRP.

**EXPERIMENTAL PROCEDURES**

**Generation of Constructs**—The MRP expression cassette (MRP1) in pBlueScript II KS+ (Strategene) used previously to generate a vector for transfection of mammalian cell lines included 86 nucleotides of the 5'-untranslated region of MRP mRNA (3). To eliminate the potential of this very GC-rich region to decrease the translational efficiency in insect cells, a second MRP construct lacking this sequence was constructed. The 5'-end of the MRP coding sequence was amplified by polymerase chain reaction (PCR) using primers 5'-TCCCCGGGCGGCCATGGCTCGGGGGTTC-3' (forward primer), which includes a Smal site (underline) and consensual Kozak sequence (double underline), and 5'-GAAGATAGCCTGCAACGT (reverse primer). A PCR product of approximately 1.1 kilobases was generated and subsequently digested at the Smal site in the primer and at a BamHI site in the MRP coding sequence located at nucleotide 1840 (8). This 2.9-kilobase fragment was inserted in the MRPl expression cassette subsequent to removal of the existing Smal-BamHI fragment to produce the new expression cassette pBSMRP-6-ATG. A 4.7-kilobase SacI-KpnI fragment containing the full-length coding region flanked by an untranslated sequence of 31 and 77 nucleotides at the 5' and 3'-ends, respectively, was isolated from pBSMRP-6-ATG and inserted into the donor plasmid pFASTBAC1 (Life Technologies, Inc.) downstream from the polyhedrin promoter.

To generate the constructs in which only one-half of the MRP was expressed, two fragments corresponding to nucleotides 2355–2808 and 2782–3279 were amplified by PCR. The primers used for the 2355–2808 fragment were primer 10.2F4, 5'-CGGCTGAGCTCGGAACGTGGG-3' (forward primer), and primer 10.1'G-CCGCTGCCATCTGAGCTGGGTC-5' (reverse primer). The primer 10.13 contained an in-frame stop codon TGA (double underline) and a KpnI site (underline). The primers used for the 2782–3279 fragment were primer 1014, 5'-CACGAAGCTATGGCAGAACTGCAGAAAGCTG-3' (forward primer), and primer 1022R3, 5'-GAAGATAGCCTGCAACGT (reverse primer). The primer 1013 contained an in-frame stop codon TGA (double underline) and a KpnI site (underline). The primers used for the 2782–3279 fragment were primer 1014, 5'-CACGAAGCTATGGCAGAACTGCAGAAAGCTG-3' (forward primer), and primer 1022R3, 5'-CGCCATGGCGCTCCGGGGCTTC-3' (reverse primer). A PCR product of approximately 1.1 kilobases was generated and subsequently digested at the Smal site in the primer and at a BamHI site in the MRP coding sequence located at nucleotide 1840 (8). This 2.9-kilobase fragment was inserted in the MRPl expression cassette subsequent to removal of the existing Smal-BamHI fragment to produce the new expression cassette pBSMRP-6-ATG. A 4.7-kilobase SacI-KpnI fragment containing the full-length coding region flanked by an untranslated sequence of 31 and 77 nucleotides at the 5' and 3'-ends, respectively, was isolated from pBSMRP-6-ATG and inserted into the donor plasmid pFASTBAC1 (Life Technologies, Inc.) downstream from the polyhedrin promoter.

**Immunoblotting and Glycosylation Studies**—Membrane proteins were solubilized in Laemmli buffer (25) and subjected to SDS-polyacrylamide gel electrophoresis (PAGE), electrotransferred to a polyvinylidene difluoride membrane (Millipore), and blotted as described (26). Blots were incubated with MRP-specific mAb QCR-L1 (purified, diluted 1:10,000; Centocor, Malvern, PA) (26, 27), or mAb MRP6 (ascites, diluted 1:250; kindly provided by Dr. R. Scheper, Free University Hospital, Amsterdam, The Netherlands) (28) followed by horseradish peroxidase-conjugated goat anti-mouse IgG (and IgM + H+L) and F(ab')2 (Fierce) (10, 26). Antibody binding was determined by enhanced chemiluminescence detection (Amersham Corp.). Membrane proteins were de-glycosylated by incubation with peptide N-glycosidase F (PNGase F; New England Biolabs, Mississauga, Ontario, Canada) as described (10).

**Quantification of MRP Protein by Immunoblotting**—Since the epitopes for MRP-specific mAbs QCR-L1 and MR6 remained unaltered in the various MRP constructs used to infect Sf21 cells, immunoblotting with one or the other of these mAbs was used to estimate the amount of MRP in different membrane preparations. To reduce size-dependent differences in transfer efficiency, membrane proteins were resolved on a 5–15% gradient gel, and aliquots of membranes from MRP-transfected HeLa T14 cells (14) were loaded on both sides of the gel to serve as internal standards. The relative amount of MRP or MRP fragments in various samples was quantified by laser densitometry of appropriately exposed films following enhanced chemiluminescence detection using a Molecular Dynamics (Sunnyvale, CA) computing densitometer.

**Vesicle Transport of LTC4**—Membrane vesicles were prepared from Sf21 cells infected with virus encoding intact MRP or MRP fragments, as well as from control cells transfected with virus encoding β-glucuronidase, as described previously for the preparation of vesicles from MRP-transfected HeLa cells (19). Uptake of [3H]LTC4 (50 nM, 132 Ci/mmol), [3H]NBD2, [3H]β-glucuronide, and [3H]estradiol 17β- (D-glucuronide) on LTC4 uptake by Sf21 vesicles was determined (29) with modifications (19). Initial rates of LTC4 uptake were determined at various concentrations of ATP and the leukotriene itself, as described (29) with modifications (19). Initial rates of LTC4 uptake were determined at various concentrations of ATP and the leukotriene itself, and double reciprocal plots of the data were used to determine Km values for both LTC4 and ATP (19). The effects of S-decylglutathione and 17β-estradiol on LTC4 uptake by Sf21 vesicles prepared from cells infected with vectors expressing either intact MRP or both half-molecules were determined at various LTC4 concentrations in the presence of a fixed concentration of inhibitor. Double reciprocal plots of the data were used to determine Ks values for both compounds, as described (19).

**RESULTS**

**Generation of Recombinant Baculoviruses**—Fig. 1 illustrates a possible topology of MRP, showing the maximal number of transmembrane regions predicted from hydrophathy analyses (5, 30). The figure also indicates regions of MRP expressed by vectors encoding the NH2- and COOH-proximal half-molecules used in this study. The NH2-proximal half-molecule, desig-
and titers were determined, which usually ranged between 5–15% gradient SDS-PAGE, electroblotted, and incubated with MRP-specific mAb QCRL-1. The sizes of protein standards are indicated in kilodaltons.

Membrane proteins from Sf21 cells infected with control vector, with or without 

FIG. 2. Immunoblot analyses, glycosylation, and quantification of wild-type MRP expressed in Sf21 cells. A, membranes were prepared from Sf21 cells infected with the recombinant baculovirus coding for the full-length MRP (Sf21/wt) and with the recombinant baculovirus coding for the β-glucuronidase (Sf21/c). Total membrane proteins (1 μg) were resolved on 5–15% gradient SDS-PAGE, electroblotted, and incubated with MRP-specific mAb QCRL-1. The sizes of protein standards are indicated in kilodaltons. B, membrane proteins (20 μg) from Sf21/wt and T14 cells were denatured at 65 °C for 10 min and treated with 1 International Union of Biochemistry milliunit of PNGase F at 37 °C overnight in a total volume of 30 μl. Membrane proteins from PNGase F-treated or untreated samples of T14 and Sf21/wt cells were then resolved on 7.5% SDS-PAGE and analyzed by immunoblotting with mAb QCRL-1. C, serial dilutions of membrane proteins from Sf21/wt and T14 cells were resolved on 5–15% gradient SDS-PAGE, and the relative amount of MRP was analyzed as described under "Experimental Procedures." D, ATP-dependent uptake of [3H]LTC4 was measured at various LTC4 concentrations (20–800 nM) for 25 and 30 s, respectively. Kinetic parameters (Vmax and Km) were determined from regression analysis of the Lineweaver-Burke transformation of the data. D, ATP-dependent uptake of [3H]LTC4 was measured at various concentrations of ATP (4 μM–4 mM) in the presence of 50 nM [3H]LTC4. Kinetic parameters were determined from regression analysis of the Lineweaver-Burke data transformation. Data points in each panel represent means of triplicate determinations in a single experiment. Bars, S.E.

Synthesis of Recombinant MRP in Sf21 Cells—Immunoblotting using the MRP-specific mAb QCRL-1 revealed an abundant protein of approximately 170 kDa in membranes from Sf21 cells infected with the full-length MRP vector (Sf21/wt) that was not detectable in membranes from Sf21 cells infected with virus coding for β-glucuronidase (Sf21/c; Fig. 2A). The apparent molecular weight of MRP produced in Sf21/wt cells was approximately 20,000 less than that of the mature protein produced in transfected HeLa cells (data not shown) and was similar to that of its unglycosylated precursor (10). To determine whether the difference in molecular weight was attributable to underglycosylation of the baculovirus-encoded MRP, membrane proteins from T14 and Sf21/wt cells were treated with PNGase F, resolved by SDS-PAGE, and immunoblotted with mAb QCRL-1 (Fig. 2B). PNGase F treatment reduced the apparent molecular weight of MRP in T14 membranes from 190,000 to 170,000, as observed previously (10). Deglycosylation reduced the apparent molecular weight of MRP produced in insect cells to a much lesser extent, so that following PNGase F treatment the proteins produced in Sf21 and HeLa cells co-migrated. The relative levels of MRP expressed in Sf21/wt and HeLa T14 transfectants were compared by immunoblotting of a serial dilution of total membrane protein isolated from each cell type (Fig. 2C). Densitometry of the immunoblot indicated that the levels of baculovirus-encoded MRP were 50–75% of those present in T14 membranes. Thus full-length MRP can be efficiently expressed and integrated into the membranes of Sf21 cells, but the protein is clearly underglycosylated.

MRP Expressed in Sf21 Cells Functions as an ATP-dependent LTC4 Transporter—The cysteinyl leukotriene LTC4 has been shown to be a high affinity substrate for human MRP. Its transport characteristics have been established in several laboratories using membrane vesicles from both drug-selected MRP-overexpressing cells and MRP transfectants such as the T14 HeLa cell population (19–22). We used this substrate to determine whether MRP produced by Sf21 cells was functional and, if so, whether its transport characteristics were similar to those of the protein produced in human cells. Fig. 3, A and B, shows the time course and ATP dependence of [3H]LTC4 accumulation by vesicles prepared under identical conditions from T14 and Sf21/wt cells, respectively. Accumulation was measured at room temperature at an initial concentration of 50 nM [3H]LTC4 in the presence of 4 mM ATP or AMP, and comparable amounts of total vesicle protein were used. With vesicles from both cell types, ATP-dependent uptake of [3H]LTC4 was rapid and linear up to 20–25 s. In both cases, steady state was approached after 120 s. During the linear phase, the rate of ATP-dependent uptake was approximately 250 and 150 pmol mg−1 min−1 for vesicles from T14 and Sf21/wt cells, respectively. The very low levels of LTC4 uptake by Sf21/wt vesicles in the presence of AMP were similar to those observed for vesicles from Sf21 cells infected with control vector, with or without...
ATP. Initial rates of uptake were determined at several LTC4 concentrations. Double-reciprocal plots of the data yielded an apparent $K_m$ of 60 nM and a $V_{max}$ of 495 pmol mg$^{-1}$ min$^{-1}$ for MRP from T14 cells and an apparent $K_m$ of 67 nM and a $V_{max}$ of 450 pmol mg$^{-1}$ min$^{-1}$ for MRP from Sf21/wt cells (Fig. 3C).

Similar analyses were also used to determine $K_m$ values for ATP, examples of which are shown in Fig. 3D. With different preparations of T14 vesicles, we have obtained $K_m$ values for ATP ranging from 50 to 70 µM. A $K_m$ of 100 µM was obtained in two independent analyses of vesicles from Sf21/wt cells.

**Expression of Half-molecules of MRP in Sf21 Cells**—MRP half-molecules were expressed either individually or together in Sf21 cells, and the relative levels of the NH2- and COOH-proximal halves of the molecule were determined by immunoblotting with the MRP-specific mAbs QCRL-1 and MRPm6, respectively (Fig. 4). Both the NH2- and COOH-proximal half-molecules were efficiently expressed and recovered in the membrane preparations as polypeptides of the anticipated molecular weights of 104,000 and 67,000, respectively. Minor amounts of higher molecular weight species were also detected. Based on their apparent molecular weights and the observation that their levels increased when protein samples were subjected to less rigorous denaturation prior to electrophoresis (data not shown), the minor species appear to be homodimers and possibly trimers of the half-molecules. Using the same multiplicities of infection of baculoviruses, the expression levels of the half-molecules were similar to each other and approximately the same as those obtained with full-length MRP. In addition, when co-expressed, the levels of both half-molecules were comparable with those obtained when they were expressed individually.

**LTC4 Transport by Individual and Co-expressed MRP Half-molecules**—Membrane vesicles were prepared from cells infected with vectors coding for the NH2-proximal (Sf21/ΔC) and the COOH-proximal (Sf21/ΔN) half-molecules of MRP either individually or together (Sf21/ΔC+ΔN). Membranes from Sf21/wt cells were analyzed for comparison. Membrane proteins (1 µg) of each sample were subjected to 5–15% gradient SDS-PAGE and transferred to a polyvinylidene fluoride membrane. Left, expression of ΔC was detected with mAb QCRL-1. Right, expression of ΔN was detected with mAb MRPm6. The sizes of protein standards are indicated in kilodaltons.

**DISCUSSION**

Intact MRP has been expressed efficiently in several types of transfected mammalian cells (3, 4, 16). However, we have found that a number of MRP mutant proteins fail to accumulate in the plasma membrane despite high levels of expression of the cognate mRNAs. Similar studies of other members of the ABC superfamily have demonstrated that trafficking of

---

3 C. E. Grant, unpublished observations.
various LTC4 concentrations (12.5–1000 nM) in the presence of an apparent

erator for each inhibitor and vesicle preparation and used to determine

gلجنة at each LTC4 and inhibitor concentration.

some mutated forms of these proteins to the plasma membrane is very inefficient (31–33), probably as the consequence of ab-

dominal integrity of MRP produced in Sf21 cells, we have taken

determined whether this system may be a useful alternative
to transfected mammalian cells for structure-function studies of MRP.

Our results demonstrate that full-length MRP can be

equated naturally occurring mutation of CFTR, ΔF508, fails to fold correctly in the endoplasmic reticulum when expressed in mammalian cells growing at

37 °C (32, 37), in insect cells grown at 27 °C (38), CFTR-ΔF508 is

efficiently synthesized and is functional. Truncated forms of both CFTR and P-gp have also been synthesized efficiently in baculovirus-infected insect cells (39, 40). Consequently, we have determined whether this system may be a useful alternative to transfected mammalian cells for structure-function studies of MRP.

CFTR produced in insect cells forms a Cl− channel with characteristics similar to those found in mammalian cells (43). In addition, baculovirus-encoded P-gp is functional with respect to both its drug-binding and ATPase activities (44, 45). However, there has been no demonstration of direct ATP-dependent drug transport by membrane vesicles from P-gp-infected insect cells, and since the infected cells do not replicate, drug sensitivity assays are not possible. To assess the functional integrity of MRP produced in Sf21 cells, we have taken advantage of the fact that its ability to transport LTC4 and the

inhibition of this process by various organic anions have been charac-
terized in several mammalian cell transfec
tants (19, 20, 22, 23). No ATP-dependent LTC4 uptake could be detected by vesicles from cells infected with the control virus, and ATP-independent uptake was considerably lower than observed with membrane vesicles from HeLa cells. In contrast, ATP-de-
pendent LTC4 transport was readily demonstrable using vesi-
cles from cells infected with virus encoding intact MRP. We confirmed previously that the rates of LTC4 uptake by vesicles from T14 HeLa transfec
tants and drug-selected H69AR cells were approximately proportional to their relative levels of MRP expression (14, 19). Based on immunoblotting data, the levels of MRP in Sf21 membranes were 50–75% of those in T14 cells, and the Vmax values for LTC4 transport were 80–90%. The Km values for LTC4 and ATP and the Ki values for the competitive inhibi
tors S-decylglutathione and 17-estradiol 17-(β-D-glucu-

ronide) were also similar to those obtained previously with T14 vesicles, indicating that there are no major differences between the transport activity and substrate binding characteristics of MRP expressed in either HeLa or Sf21 cells.

Many ABC transporters contain four structural domains that include two polytopic membrane-spanning regions and two cytoplasmic NBDs (46). However, the membrane-spanning regions and NBDs of some prokaryotic ABC transporters are separate polypeptides (47, 48). In addition, some ABC proteins (49, 50), appear to be “half-molecules” with only a single set of six transmembrane segments and one NBD that may function as homodimers. These observations have been interpreted to suggest that many members of the ABC superfamily have evolved by duplication and/or fusion of previously autonomous “half-molecules” or individual domains (46). The fusion or separation of functional domains has, in some cases, been mimicked experimentally (46, 51). Notably, co-expression of both halves of P-gp in SY9 cells was required for drug stimulation of ATPase activity, indicating the ability of the two fragments in association to bind substrate (40). However, direct drug transport by the co-expressed halves of P-gp has not been demonstrated, and when co-expressed in HEK 293 cells, no drug-resistant clones were obtained. Consequently, it remains to be established that the two halves of P-gp can associate to form an active drug pump (40). In contrast, the NH2-proximal 836 amino acid residues of CFTR, encompassing only the first six transmembrane helices, the first NBD, and the regulatory do-

main, can form Cl− channels with conductive properties iden
tical to those of full-length CFTR (43).

The predicted topologies of MRP and, more recently identi
cified, related ABC transporters such as the sulfonlurea recep
tor, the canalicular multispecific organic action transporter, yeast-cadmium factor I, and epithelial basolateral conductance regulator, are not consistent with two similarly organized halves, largely because of the extremely hydrophobic NH2-terminal extension present in these proteins (30, 52–54). Consequen
tly, the NH2- and COOH-truncated MRP fragments we have generated differ considerably from each other. The NH2-

proximal “half-molecule” may contain as many as 12 transmembrane helices, in addition to the first NBD and most of the conector region, whereas the COOH-terminal fragment con
tains only 4–6 membrane-spanning helices and the second NBD (Fig. 1). Despite their structural differences, each of the two MRP half-molecules was able to integrate into the mem-

branes of infected cells equally well when expressed either alone or together. Levels of expression were also comparable to that of the intact protein, suggesting that interaction between the two halves of the protein has little effect on the efficiency of integration into the membrane or on protein trafficking. Al

though no ATP-dependent LTC4 transport was detected with

\[\text{LTC}_{4} \text{Transport by Co-expressed MRP Half-molecules}\]

![Graph](Image)
LTC4 Transport by Co-expressed MRP Half-molecules

Schneider E., Horton, J. K., Yang, C.-H., Nakagawa, M., and Cowan, K. H. (1994) Cancer Res. 54, 152–158

Cole, S. P. C., Sparks, K. E., Fraser, K., Lee, D. W., Grant, C. E., Wilson, G. M., and Deeley, R. G. (1994) Cancer Res. 54, 1521–1524

Binashi, M., Supino, R., Gambetta, R. A., Giancone, G., Prosperi, E., Caprancico, G., Cataldo, I., and Zunino, F. (1995) Int. J. Cancer 62, 84–89

Breunling, L. M., Paul, G., Ghosh, T., & Chakravarti, A. (1993) Cell 75, 5719–5725

Jedlitschky, G., Leier, I., Buchholz, U., Barnouin, K., Kurz, G., and Keppler, D. (1996) Cancer Res. 56, 988–994

Lee, D. W., Almquist, K. C., Deeley, R. G., and Cole, S. P. C. (1996) J. Biol. Chem. 271, 9675–9682

Lee, I., Jedlitschky, G., Buchholz, U., Cole, S. P. C., Deeley, R. G., and Keppler, D. (1994) J. Biol. Chem. 269, 27807–27810

Lee, I., Jedlitschky, G., Buchholz, U., Center, M., and Keppler, D. (1994) Cancer Res. 54, 4833–4836

Muller, M., Meijer, C., Zaman, G. J. B., Borst, P., Schepers, R. J., Mulder, N. H., de Vries, K. E. G., and Jansen, P. L. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 13053–13057

Leier, I., Jedlitschky, G., Buchholz, U., Center, M., Cole, S. P. C., Deeley, R. G., and Keppler, D. (1996) Biochem. J. 314, 433–437

Lee, D. W., Almquist, K. C., Cole, S. P. C., and Deeley, R. G. (1996) J. Biol. Chem. 271, 9683–9689

Laemmli, U. K. (1970) Nature 227, 680–685

Hipher, D. R., Gaudie, S. D., Deeley, R. G., and Cole, S. P. C. (1994) Cancer Res. 54, 5788–5792

Hipher, D. R., Almquist, K. C., Stride, B. D., Deeley, R. G., and Cole, S. P. C. (1996) Cancer Res. 56, 3307–3314

Flens, M. J., Izierdito, M. A., Scheffler, G. L., Fritz, J., Meijer, C. J. L. M., Schepers, R. J. E., and Zaman, G. J. B. (1994) Cancer Res. 54, 4557–4563

Leier, I., Jedlitschky, G., Buchholz, U., and Keppler, D. (1994) Eur. J. Biochem. 220, 599–606

Stride, B. D., Valdimarsson, G., Gerlach, J. H., Wilson, G. W., Cole, S. P. C., and Deeley, R. G. (1996) Mol. Pharmacol. 49, 962–971

Cheng, S. H., Gregory, R. J., Marshall, J. P., Paul, S., Souza, D. W., White, G. A., O'Farrell, C. G., and Smith, A. E. (1990) Cell 63, 827–834

Lukas, G. L., Mohamed, A., Kartner, N., Chang, X.-B., Riordan, J. R., and Grinstein, S. (1994) EMBO J. 13, 6076–6086

Ward, C. L., and Kopito, R. R. (1994) J. Biol. Chem. 269, 25710–25718

Fried, S., Riordan, J. R., and Williams, D. B. (1994) J. Biol. Chem. 269, 12784–12788

Yang, Y., Janich, S., Cohn, J. A., and Wilson, J. M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 599–604

Lee, T. W., and Clarke, D. M. (1995) J. Biol. Chem. 270, 21839–21844

Denning, G. M., Anderson, M. P., Amaral, J. F., Marshall, J., Smith, A. E., and Welsh, M. J. (1992) Nature 358, 761–764

Li, C., Ramjieszing, M., Reyes, E., Jensen, T., Chang, X.-B., Rommens, J. M., and Bear, C. E. (1993) Nat. Genet. 3, 311–316

Sheppard, D. N., Osetgaard, L. S., Rich, D. P., and Welsh, M. J. (1994) Cell 76, 1091–1098

Lee, T. W., and Clarke, D. M. (1994) J. Biol. Chem. 269, 7750–7755

Schinkel, A. H., Kemp, S., Dalle, M., Rudenko, G., and Wagenaar, E. (1993) J. Biol. Chem. 268, 7471–7478

Bakos, E., Hagedus, T., Halle, Z., Welker, E., Tsuanyda, G. E., Zaman, G. J. B., Flens, M. J., Varadi, A., and Sarkadi, B. (1996) J. Biol. Chem. 271, 12322–12326

Kartner, N., Hanrahan, J. W., Jensen, T. J., Naishtat, A., Sun, S., Ackerley, C. A., Reyes, E. F., Tsui, L.-C., Rommens, J. M., Bear, C. E., and Riordan, J. R. (1991) Cell 64, 681–691

Sarkadi, B., Price, E. M., Boucher, R. C., Germann, U. A., and Scarborough, G. A. (1992) J. Biol. Chem. 267, 4854–4858

Ahmad, S., Safa, A. R., and Glazer, R. I. (1994) Biochemistry 33, 10313–10318

Higgins, C. F. (1992) Annu. Rev. Cell Biol. 8, 67–113

Ames, B. G. (1987) Annu. Rev. Biochem. 55, 397–425

Hyde, S. C., Emsley, P., Hartshorn, M. J., Mimmack, M. M., Gileadi, U., Pearce, S. R., Gallagher, M. P., Gill, D. R., Hubbard, R. E., and Higgins, C. F. (1999) Nature 381, 362–365

Koronakis, V., and Hughes, C. (1993) Semin. Cell Biol. 4, 7–15

Korovnich, J. G. and DeGorte, D. (1993) Cancer Res. 53, 5842–5847

Paulusma, C. C., Bosma, P. J., Zaman, G. J. B., Bakker, C. T., Otter, M., Scheffler, G. L., Schepers, R. J., Borst, P., and Oude Elferink, R. P. J. (1996) J. Biol. Chem. 271, 2853–2857

van Kuijck, M. A., van Aubel, R. A. M. H., Busch, A. E., Lang, F., Russel, F. G., and van den Boogaard, R. J. M. T. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5401–5406