PART I

ABC PROTEINS: AN OVERVIEW AND DESCRIPTION OF THE STRUCTURE, GENOME, NORMAL TISSUE EXPRESSION, PHYSIOLOGICAL ASPECT, AND MECHANISM OF ACTION

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THE P-GLYCOPROTEIN 170: JUST A MULTIDRUG RESISTANCE PROTEIN OR A PROTEAN MOLECULE?

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1.1. INTRODUCTION

The ATP-binding cassette (ABC) proteins represent a highly diversified superfamily in all living kingdoms, with 49 human proteins, 14 of which are associated with various diseases (1, 2). They are found in all animal and plant species from prokaryotes to eukaryotes, and their functional characteristics are extended from ion transport to macromolecule efflux (3, 4). Although differences are observed in their functions, substrate specificities, molecular mechanisms, and *in vivo* localizations, they share a high degree of sequence and structural homology (5). The best known and best characterized of them is P-glycoprotein (P-gp; subfamily B, member 1: ABCB1), which is encoded by the *MDR1* (now *ABCB1*) gene, located on chromosome 7 in humans. It is the first eukaryotic ABC member identified and was discovered by Juliano and Ling (6) because of its implication in multidrug resistance (MDR) of cancer cells to chemotherapy (7). It consists of two halves that share a high degree of similarity. Each homologous half contains six hydrophobic transmembrane domains (TMDs) and a relatively hydrophilic intracellular loop encoding an adenosine triphosphate (ATP) binding site (nucleotide-binding domain [NBD]). By extruding cytotoxic drugs out of the cells before they reach their cellular target, P-gp expression leads to failure of AIDS and cancer chemotherapy (8). It is now recognized that several causes can explain its overexpression, such as gene amplification and gene polymorphisms. The studies on *ABCB1* polymorphism and its functional consequences have become a major topic of research (9, 10). In addition, many studies have shown that P-gp is expressed in several normal tissues (e.g., intestinal epithelial cells, blood–brain barrier [BBB], and placenta.) and that its primary function is to prevent the uptake of toxic compounds from the gut into the body, to expel them in the bile or urine, and to protect some very sensitive organs, such as the brain, from them (11). P-gp is also involved in other physiologic processes, such as control and regulation of apoptosis, stress, hypoxia, stem-cell differentiation, cellular immune response, or plasma membrane dynamic (12–14).

1.2. P-gp 170: FROM GENE TO PROTEIN

1.2.1. ABCB1 Gene: Structure, Regulation

MDR or *ABCB* genes constitute a small family in which two genes are closely related in humans (*MDR1* and *MDR2*, now *ABCB1* and *ABCB4*) and in rodents (*mdr1, mdr2*, and *mdr3*) (15). Full-length cDNAs for human *ABCB1* and rodent *mdr1* and *mdr3* genes were shown to confer an MDR phenotype to drug-sensitive cells after DNA-mediated transfer. The proteins encoded by the human *MDR2* gene and by its mouse counterpart are specifically involved in phosphatidylcholine translocation between plasma membrane leaflets (16). The human *MDR* genes are adjacent to each other on the long arm of chro-

mosome 7, distant by 330 kpb. *ABCB1* and *ABCB4* coding sequences are 76% identical. *ABCB1* gene has been shown to contain 29 exons and 28 introns (one of them longer than 40 kbp) with a total span greater than 120 kbp (17). The degree of *ABCB1* gene amplification tightly parallels the expression of the MDR phenotype in cell lines selected for resistance. Chromosomal rearrangements have been observed in several cell lines and clinical samples. For instance, translocation has been observed between chromosomes 4 and 7; the resulting somatic cell hybrids showed an overexpression of *ABCB1*, and this translocation provides a model for activation of *ABCB1* (18, 19). Nevertheless, amplified genomic regions are not observed in all resistant cell lines. In the human *ABCB1* promoter (Fig. 1.1), analysis of sequences upstream from *ABCB1*-coding regions has revealed that two distinct transcription start sites can be used, respectively located 136 and 140 bp upstream from the first ATG codon. The proximal site is used in most MDR cell lines and normal tissues (20–22). Two other minor transcription start sites are located about 100 bp downstream from these promoters. The proximal promoter (P1) spans the region from –198 to +43. It is TATA-less and contains two Y-box consensus sequences (–113 to –118), at least two GC boxes, and other GC-rich regions which may bind Sp1 factors. In this downstream promoter, sequences from –6 to +11 (relative to the P1 transcription start site) are sufficient for proper transcriptional initiation. This transcription start site has a strong homology with the initiator (*Inr*) sequence of the murine terminal deoxynucleotidyl

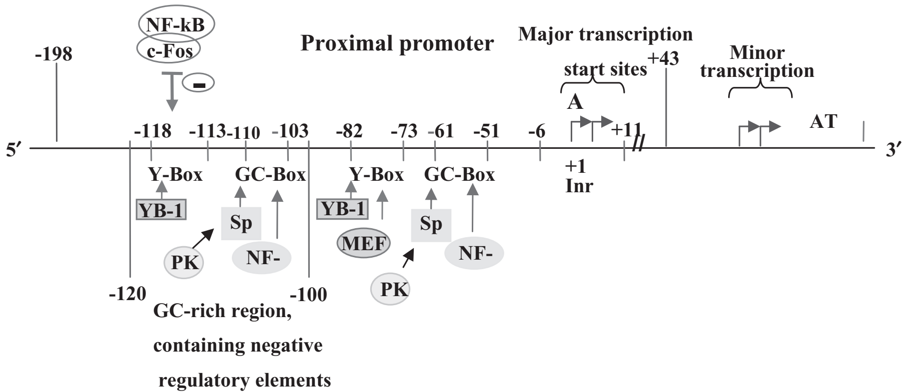

FIGURE 1.1. Schematic illustration of the human *MDR1* gene promoter used in MDR cells. The positive transcription factors YB-1 (Y-box protein 1) and MEF1 (MDR1 promoter-enhancing factor) bind to Y-box elements (or CCAAT-box like). The complex formed with transcription factors NF-kB/c-Fos also negatively regulates these regions. This complex is only detected in sensitive cells. GC boxes are recognized by transcription activators NF-Y and Sp1, activated by signal transduction pathways involving AMPc-dependent kinases (PKC). The Inr sequence (–6 at +11) is sufficient to promote transcription in the absence of TATA box promoters.

transferase gene (20). The *Inr* sequences are located at the transcription start site and can direct transcription from a RNA polymerase II promoter in the absence of a TATA box. Recently, some authors have shown the role of the highly structured 5′ end region of *ABCB1* mRNA in P-gp overexpression (23).

The transcriptional regulation of *ABCB1* gene expression is highly regulated by complex events and several signaling pathways. For example, *ABCB1* gene transcription requires transcriptional factors and coregulators such as p53, c-myc, c-jun, HIF-1, and CtBP1. Altered methylation of the human *ABCB1* promoter is sometimes associated with acquired MDR (24–26). Moreover, *ABCB1* gene expression can also be regulated by different physiological processes, including differentiation factors (retinoic acid, sodium butyrate), steroid hormones (estradiol), and environmental stress (thermic and osmotic shock, low external pH). Antitumoral agents can also induce *ABCB1* gene expression in human and rodent cell lines by transcriptional regulation. Overexpression can also result from spontaneous selection of mutants overexpressing P-gp rather than a direct induction of its expression (27).

Basal transcription of the human *ABCB1* gene is controlled by a negative regulation involving a GC-rich region, located from –56 to –45 and from –110 to –103. Moreover, the region containing Y-box and GC elements seems essential for activation of *ABCB1* after UV irradiation, suggesting a cooperative interaction between these boxes (28). A CAAT element binds two transcriptional factors, NF-κB and c-Fos (bases –116 to –113) in cells such as MCF7 cells (29). This protein complex is absent in MCF7 doxorubicin-resistant cells; and consequently, it has been suggested that it inhibits *ABCB1* gene expression in sensitive parental cells. Conversely, the proximal promoter also contains different sites recognized by transcriptional activators, such as Sp1-activated by AMPc-dependent kinases (29). The transcriptional factor Y-box-binding protein 1 (YB-1) accumulates in the nucleus of MDR cells, where it binds to Y boxes and might also activate *ABCB1* transcription. In sensitive counterparts, this factor is only detected in cytoplasm (30, 31). These Y boxes are also involved in the overexpression of *ABCB1* gene in HL60 vincristine-resistant cells (32), by the mean of MEF-1 transcriptional factor (*MDR1* promoter-enhancing factor); the interaction is also absent in sensitive cells (33). *In vivo* studies have shown that *RAS* and *RAF* oncogenes can regulate human P-gp expression.

The transcription rate of the *ABCB1* gene can also be modulated by p53 itself or by p53 family members in response to a large subset of stimuli. For example, in the *ABCB1* gene promoter, an Sp1 binding site is present and binds the promoting transcription heteroduplex Sp1-p53, modulating the expression of *ABCB1* gene, when cells are treated with pro-apoptotic agents. Several studies suggested that p53 could be a potent repressor of *ABCB1* gene transcription when activated by cytotoxic agents. Nevertheless, the repression is dependent on the interaction of p53 with other transcription factors; whereas

the interaction of p53 with an *ABCB1* promoter, via a novel p53 DNA binding site (the HT site), leads to a direct repression of transcription (34). Another study showed that the reintroduction of wild-type p53 in doxorubicin resistant cells confers a sensitive phenotype that is correlated with a decrease in their tumorigenicity (35). On the other hand, p53 can inhibit P-gp function by mediating the inhibition of protein kinase C-alpha (PKC-α) promoter activity, because PKC-α can phosphorylate and activate P-gp (36). Other members of the p53 family (namely p63 and p73) can regulate the transcription of the *ABCB1* gene, but a differential regulation can be observed. In fact, p63 and p73 regulate the majority of p53 target genes, but transient transfection assays demonstrated that p63 and p73 activated rather than repressed *ABCB1* transcription. This upregulation is DNA binding-dependent but not through the HT site; p63 and p73 interact with the *ABCB1* promoter via the alternate p63/p73 element, APE (37).

The human *ABCB1* gene promoter presents many regulating sequences that are bound by several different kinds of transcription factors. Analyses point out specific sequences upstream from the *ABCB1* gene such as, the inverted CCAAT sequence, also called the Y box (–82 to –73), which binds the NF-YA transcription factor to regulate *ABCB1* expression in a positive way (38). This sequence is also involved in the binding of another transcription factor, CCAAT/enhancer binding protein beta (C/EBPβ). Cotransfection assays by either C/EBPβ or C/EBPβ-LIP (a dominant-negative form of C/EBPβ) in the breast cancer cell line MCF-7 and its doxorubicin resistant variant MCF-7/ADR have shown that mutations inside the Y box abolished *ABCB1* expression by C/EBPβ. The binding of C/EBPβ to another sequence, AP-1 box (–123 to –111), negatively regulates the expression of the *ABCB1* gene (39). The mechanisms of *ABCB1* activation by C/EBPβ also involve interactions with Y-box-associated proteins and differential sequences binding in a certain cellular biochemical context. Some Y-box-associated proteins, such as the YB-1, also regulate the transcription of genes involved in cell growth, DNA replication, and DNA repair. Finally, a study has identified a cis-regulating element for *ABCB1* gene transcription (40). These authors characterized the invMED1 sequence in the 5′-flanking region of the human *ABCB1* gene; this one interacts with a nuclear protein, LRP130, and stimulates the transcription of *ABCB1* in CEM leukemia cells. Interestingly, the level of LRP130 did not vary with the resistance level, but its binding intensity is variable with the *ABCB1* gene expression. Furthermore, as this invMED1 sequence is also located in promoter regions of other *MDR*-related genes, the invMED1/LRP130 couple could be a potential central regulator of the transcription of these genes. Another protein frequently mutated in cancers, the transcription factor c-myc, is also a strong activator of *ABCB1* transcription. It acts by binding the E-box motif (namely, CACGTG), which is localized within the proximal promoter of the *ABCB1* gene (–272, –444). In neuroblastoma, a childhood cancer, the overexpression of the neuronal variant N-myc (*MYCN*) enhances *ABCB1* gene expression and constitutes a marker for poor prognosis

(41). Indeed, N-myc overexpression is frequently associated with the MDR phenotype and high expression of *ABCB1* in neuroblastoma metastasic tumors. Epigenetic changes in histone H3 methylation induced by cytotoxic drug treatments have been shown to be responsible for the *ABCB1* gene overexpression in cancer-resistant cells (42).

1.2.2. ABCB1 Gene Polymorphisms

The expression level and function of *ABCB1* gene also depends on some gene polymorphisms. During the last decade, several single-nucleotide polymorphisms (SNPs) have been identified in the coding region of the gene (15, 43). The first studies carried out in normal human patients showed significant correlations between polymorphisms in exon 26 (C3435T) of *ABCB1* and expression levels and functions of *ABCB1* (44). Some other polymorphisms may be associated with altered *ABCB1* expressions and/or P-gp functions; they can be associated with altered drug metabolisms and/or pharmacokinetics and have an impact on drug efficiency and toxicity. In the context of rheumatoid arthritis, a study showed that the *ABCB1* genotypes 3435CC and 3435TC result in lower probabilities of remission after treatment with methotrexate and glucocorticosteroids, compared with patients with the 3435TT genotype (45). Other authors have shown that the *ABCB1* polymorphisms could be a risk factor for several other diseases such as renal epithelial tumor, bowel diseases, and Parkinson's disease (46–48). On the contrary, Morita et al. (49) did not observe differences in transcellular transport and intracellular accumulation between cells with polymorphic variants (G2277T/A and C3435T) and cells expressing the wild-type genotype.

Furthermore, the C3435T polymorphism in exon 26 may affect the function of P-gp by influencing its expression level, thus modifying cancer prognosis in breast cancer (due to chemotherapy resistance) (50, 51). It may be one of the risk factors for susceptibility in upper aerodigestive tract cancers, which are associated with tobacco use and alcohol consumption (52). Furthermore, another variation, G1199A, appears to alter the transepithelial permeability and efflux of fluorescent substrates *in vitro*. It confers more resistance to cells selected by cytotoxic agents such as vinblastine and vincristine (53). This feature could be an explanation for the relative interindividual difference in sensitivity to antineoplastic agents and drug resistance. In addition to the numerous SNPs identified, insertions, duplications, or deletions of sequences in the *ABCB1* gene could also play a role in altered P-gp functions (54). For example, an increase of *ABCB1* DNA copy numbers leads to an enhanced P-gp expression, which is characteristic of drug-resistant cell lines in comparison with the drug-sensitive parental cell lines. A study based on the comparison of the SNPs occurring in the entire 200 kb of the *ABCB1* gene in five different populations (Chinese, Malays, Indians, Caucasians, and African-Americans) has shown that a recent positive selection has occurred at the

human *ABCB1* gene locus. This positive and population-dependent selection confers a typical haplotype of the *ABCB1* locus in a given population and, consequently, a potential population-dependent susceptibility to MDR (55). Numerous correlations were observed between ethnicity-related polymorphisms and haplotypes in the human *ABCB1* gene. For example, Kimchi-Sarfaty et al. (56) identified that the 3435C > T occurred in 24.2% of the U.S. population and in 69.3% of the Ashkenazi-Jewish population.

It appeared that genotype analysis of *ABCB1* SNPs is becoming increasingly important in identifying genetic variants underlying susceptibility to human disease. Recent results suggested that *ABCB1* polymorphisms might influence the intracellular concentration of cyclosporine, a P-gp substrate preventing graft rejection after solid organ transplantation. The *ABCB1* 1199A carriers presented a 1.8-fold decreased cyclosporine intracellular concentration, whereas the 3435T carriers showed a 1.7-fold increase. In contrast 61A > G, 1236C > T, and 2677G > T polymorphisms did not modify cyclosporine intracellular and blood concentrations (57). Nevertheless, opposite results appeared to be likely due to differences in cancer types (58, 59). Future research on *ABCB1* polymorphism will allow to better understand the factors that contribute to interindividual variability in drug exposure, response, and toxicity (10, 43).

1.2.3. P-gp Structure

The human *ABCB1* gene encodes P-gp, a protein of 170 kDa containing ~1280 amino acids (approximate mass of 170–180 kDa). It is organized in two homologous halves, corresponding to duplication of an ancestral gene and/or fusion of two ancestral molecules. A structural model for the glycoprotein was proposed by Jones and George (60). It was obtained from hydropathy plots and computer prediction algorithms (Fig. 1.2).

During the year 2001, the bacterial P-gp homologue (MsbA) of *Escherichia coli* was the first ABC transporter to be crystallized. Nevertheless, the described structure was controversial, and new structures of bacterial multidrug ABC transporter at high resolutions (3Å) were proposed (61). Among these is SAV1866, the bacterial P-gp homologue (62). On the other hand, since 1997, Rosenberg et al. (63) studied the structural organization of the P-gp. They have obtained low- to high-resolution three-dimensional (3D) structures for P-gp using cryo-electron microscopy of two-dimensional (2D) crystals. During the year 2005, they obtained the first 3D structure for an intact eukaryotic ABC transporter (64). It contains a wide hydrophilic pore (5 nm for internal diameter and 10 nm for external diameter), closed on the internal cytosolic side, forming an aqueous compartment inside the hydrophobic membrane bilayer. This cup-shaped chamber has been proposed to include an opening allowing a lateral entry of drug substrates to be excluded. The accepted model for

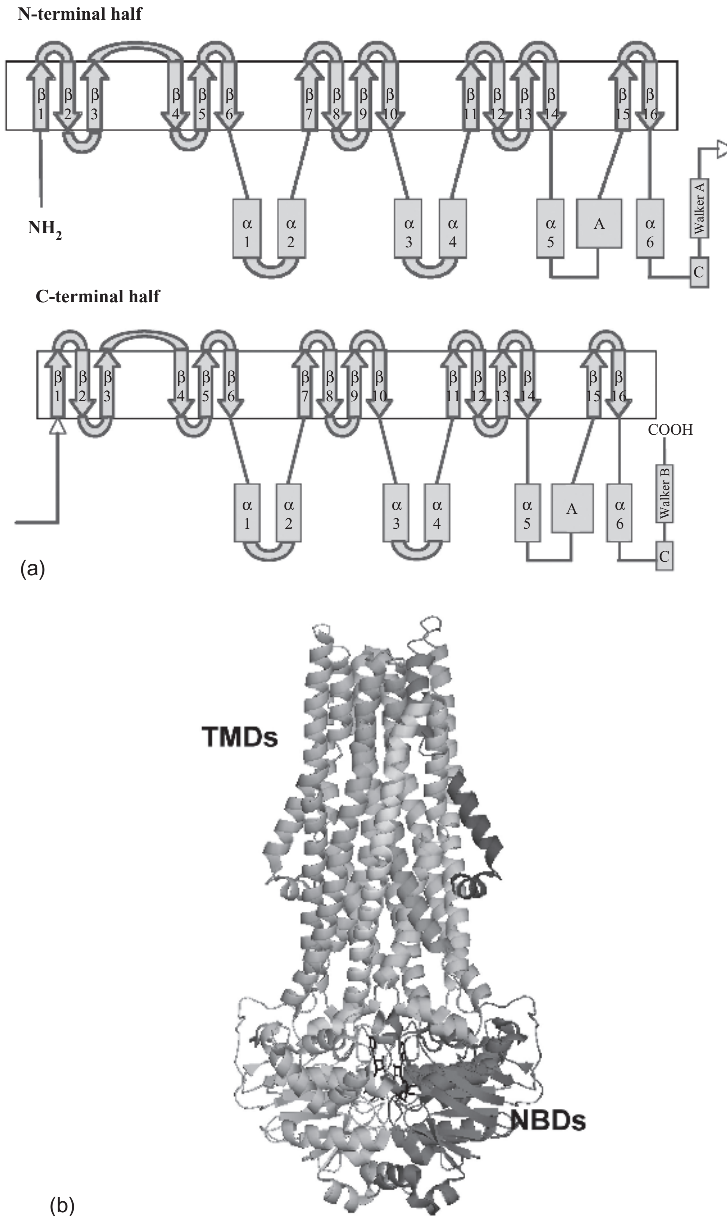

FIGURE 1.2. Predicted membrane topology and three-dimensional structure of P-gp. (a) Each of both N- and C-terminal halves are composed of 16 transmembrane anti-parallel β-sheets and 6 cytoplasmic α-helices. The A, B, and C rectangles correspond to the ATP-binding domains. Adapted from Jones and George (60). (b) The single polypeptide chain is folded in two halves, each containing six transmembrane α-helices. The transmembrane α-helices are connected by extracellular or cytosolic loops, followed, in cytosol, by large domains containing for each half a Nucleotide Binding Domain (NBD). Drawing with PyMOL.

human P-gp suggests that the single polypeptide chain is folded in two transmembrane domains (TMDs), each half containing six transmembrane α-helices. There is evidence that the two TMDs together constitute the drug transport pore. The transmembrane α-helices are connected by extracellular or cytosolic loops, followed by a large cytosolic domain containing an ATP-binding site (65) or NBD (NBD1 and NBD2 for the first and the second half, respectively). Each NBD contains nucleotide-binding motifs, including Walker A (P-loop) and Walker B sequences, and the ABC signature motif (LSGGQ). A central sequence connects the two homologous halves of the protein and is called the "linker" region. The two halves share 43% sequence identity and 78% similarity, and TMDs of these proteins display β-sheets rather than α-helices. The linker region also plays an important role in P-gp function. Its flexible secondary structure is sufficient for the coordinate functioning of both halves of P-gp, which are likely required for the proper interaction of the two ATP-binding sites. Both NBDs of P-gp can bind and hydrolyse ATP.

There is great evidence that for efficient ATP hydrolysis, the two NBDs have to interact by forming a sandwich dimer so that the LSGGQ motif of one NBD comes into contact with the loop of the other NBD to form the nucleotide-binding pocket (66). Moreover, it is evident that the other transmembrane segments, such as segment 1 (67) and segment 7 (68), play roles in the drug-binding pocket, whereas a mutation in segment 6 (residue G346) affected drug transport in cells by a reduction in basal ATP hydrolysis, but had no effect on drug binding (69).

1.2.4. Posttranslational Modification

Phosphorylation. The linker region (75 amino acids long: 633–709) contains phosphorylable serine residues (661, 667, 671, 675, and 683) recognized by different kinases, such as protein kinases C (PKC) and protein kinases A (PKA) (70). PKA inhibition does not influence P-gp expression and function, but P-gp phosphorylation by PKC modulates the activity of the pump (71). The first studies have indeed reported that enhancement of PKC activity by phorbol esters increased the resistance level of cells and reduced drug accumulation (72).

Since phorbol ester treatment increases P-gp phosphorylation, these results suggest that phosphorylation may enhance drug efflux. Ratsaninghe et al. have observed (73) differential expressions and activities of PKC and tyrosine phosphatase in MCF7 MDR cells in comparison to sensitive counterparts. This relationship of P-gp efflux activity with decreased and with increased phosphorylation suggests that its activity may be modulated not only by kinases but also by phosphatases (74). Moreover, PKC inhibitors may directly interact with P-gp. On the contrary, other authors (75) concluded that phosphorylation did not play a significant role in regulating P-gp activity in MCF-7/ADR cells. As a conclusion, the mechanisms of P-gp inhibition by PKC inhibitors and the role of its phosphorylation remain unclear. PKC blockers may affect drug

transport both by (i) direct competition with transported drugs for binding to P-gp and (ii) indirect inhibition through a pathway involving PKC inhibition, but independent of P-gp phosphorylation (76).

Specifically, Ser-661, Ser-667, and Ser-671 are, both *in vitro* and *in vivo*, the major sites of phosphorylation, and they all occur within classical PKC consensus motifs. The number and identity of the kinases that phosphorylate P-gp in MDR cells remained uncertain for a long time (77). It seems most likely that P-gp is phosphorylated by one or more PKC isoenzymes (78). In the PKC family, PKC-α phosphorylates and activates P-gp, whereas its inhibition by p53 leads to decreased P-gp phosphorylation (36). Previously, a critical role for the linker region Ser-661 in the positive regulation of P-gp ATPase activity by PKC-α was suggested by the demonstration that mutation of this serine to asparagine abolished the enhancement of drug-stimulated P-gp ATPase activity by PKC-α in a baculovirus expression system (79). Moreover, results on proteoliposomes containing P-gp suggest that differential phosphorylation patterns of the transporter could to be linked to environmental molecular composition (lipids, presence of detergents) and structure (80).

Glycosylation The P-gp apparent molecular weight is reduced from 170 kDa to 140 kDa after enzymatic treatment with different glycosidases such as peptide-N-glycosidase F or endo-β-N-acetylglucosaminidase (81). The primary sequence of P-gp suggests that 10 putative N-glycosylation sites are present. Nevertheless, only three potential sites of extracellular N-glycosylation (residues Asn 91, 94, and 99) exist in the first extracellular loop. In fact, glycosylation may contribute to a precise folding and a correct trafficking of P-gp to the plasma membrane. It is first synthesized in the endoplasmic reticulum (ER) as a core-glycosylated intermediate with a molecular mass of about 150 kDa. The carbohydrates are subsequently modified in the Golgi apparatus to yield a protein of about 170 kDa that is consequently delivered to the cell membrane. Using the mutational studies, Loo and Clarke found that 10% of the point mutations affected the processing of P-gp. These mutants are retained in the ER as core-glycosylated intermediates associated with the molecular chaperones calnexin (82) and Hsc70 (83). However, tunicamycin treatment inhibiting glycosylation of P-gp in MDR cells does not affect drug sensitivity, although the efficiency in obtaining drug-resistant clones is drastically reduced (84). Thus, glycosylation seems to be involved in P-gp processing and/or stability. Transfection of MDR cells with wild-type ubiquitin or treatment with an N-glycosylation inhibitor increased the ubiquitination of P-gp and increased its degradation in the proteasome (85). On the other hand, Gribar et al. (86), using a vaccinia virus-based transient expression system, obtained HeLa cells expressing several types of P-gp mutants. First, HeLa expressing "P-gp-N/Q" (91, 94, 99N→Q) showed a 40%–50% lower cell surface compared to HeLa cells expressing the wild-type protein, although the substrate specificity of the pump was not affected. The reduced expression was not due to glutamine substitution but to sugar moiety deprivation; indeed, in

HeLa cells expressing a P-gp with the substitution 99N→D or with the 99N deletion, the level of cell-surface P-gp remained unchanged. In the same way, mutagenesis of the three sites in the human protein (Asn to Gln, Ala, or Asp) reduced the apparent molecular size to around 140 kDa, but did not modify the ATPase activity of the mutated P-gp, which remained able to confer drug resistance (87). Moreover, the nature and sequence of glycosylated chains are very complex. Recently, Greer and Ivey (88) have described several possible N-glycanic structures of overexpressed human P-gp. One of them contains a high-mannose complex oligosaccharide, while two other structures present terminal sialic acids. The α6 sialyl terminal groups and β1–6 branching glycans are highly expressed in cancers due to the regulation of acetylglucosaminyl-transferase V, which could include the glycosylation of P-gp (89).

1.3. TISSULAR, CELLULAR, AND ORGANELLE EXPRESSION OF P-gp 170

1.3.1. Expression in Normal Tissues and Tumors

Several normal tissues express high levels of the *ABCB1* gene, such as apical membranes of epithelial cells from kidney proximal tubule, intestine, and lung. *ABCB1* gene is also found in brain microvascular endothelia, placenta, adrenal cortex, testis, uterus, lymphocytes, and hematopoietic cells (90–92). In such tissues, P-gp localization and its highly conserved structure during evolution suggest an important role for this protein in protecting mammalian cells against various toxins and/or in transporting endogenous substrates (93, 94).

As a result of this tissue localization, P-gp functions in three main areas (95): (i) P-gp limits drug entry into the body after oral drug or toxin administration as a result of its expression in the luminal (apical) membrane of enterocytes; (ii) once the xenobiotic has reached the blood circulation, P-gp promotes drug elimination into bile and urine as a result of its expression in the canalicular membrane of hepatocytes and in the luminal membrane of proximal tubule cells in the kidneys, respectively; (iii) in addition, once a xenobiotic has reached the systemic blood circulation, P-gp limits drug penetration into sensitive tissues. In particular, in the blood brain barrier (BBB), P-gp is localized in both luminal and abluminal membranes of capillary endothelial cells, pericytes, and astrocytes (96). This localization strongly suggests an important efflux role of P-gp, restricting the penetration of drugs and toxic agents in the central nervous system, thus playing the role of a gatekeeper (97). Studies on knockout mice lacking P-gp have confirmed these ideas since these animals show a disrupted BBB and can be up to 100-fold more sensitive to several neurotoxic drugs (98, 99). Furthermore, the knockout mice studies have clarified that MDR plays a more important role in preventing drug absorption and uptake in gut and brain than in drug excretion in the bile and urine (100).

ABC transporters were often detected in a wide variety of stem cells, including melanoma and hematopoietic stem cells (101, 102). P-gp especially is expressed in primitive stem cells, including human CD34+ cells, which can be identified by their ability to transport fluorescent dyes that are P-gp substrates, such as rhodamine 123 (103). Maturation of these cells was accompanied by a decrease in P-gp expression and functional activity. It was suggested that ABC transporters in human stem cells could act as protectors from genetic damage by naturally occurring xenobiotics (104). However, as initially described by Gottesman and Pastan (105), this constitutes a "double-edged sword" because the conserved expression of P-gp after the stem cells' malignant transformation in acute myeloid leukemia could decrease sensitivity of leukemia cells to chemotherapy (102). Today, several therapeutic assays have been conducted using retroviral *ABCB1* gene transfer to convert drug-sensitive hematopoietic cells into drug-resistant cells, in order to protect normal cells from intensive cancer chemotherapy (106). The aim of this approach is to combine high-dose chemotherapy with transplantation of *ABCB1*-transduced hematopoietic stem cells; clinical benefits are under investigation.

A recent study (107) also reported expression and function of P-gp in human fetal neural stem/progenitor cells, hNSPCs. Data suggested that P-gp was functionally expressed in cultured hNSPCs and was downregulated during differentiation, indicating that *ABCB1* expression might be important in maintaining hNSPCs in an undifferentiated state. Those data are corroborated by a recent review from Mizutani et al. (108) who reported that high expression of P-gp prevents stem-cell differentiation, leading to the proliferation and amplification of this cell repertoire. Links between *ABCB1* expression and the differentiation stage were also investigated in neoplastic cells treated with all-trans retinoic acid (ATRA), which is used against certain forms of leukemia. Data appeared controversial, as reported by Stromskaya et al. (109). The authors showed that increasing differentiation of leukemic cells (induced by RARα overexpression) induced an increase in *ABCB1* gene expression in cells from solid tumors. Nevertheless, it did not result in elevation of constitutive P-gp functional activity, but it could participate in the control of P-gp induction. Sulová et al. (110) recently reported that combined treatment of P-gp positive cells with verapamil and ATRA induced a depression of P-gp expression and/or transport function whereas ATRA alone did not. Taken together, these data show that interconnections between retinoic acid-mediated differentiation and MDR regulation remain complex and dependent on the cell context.

P-gp is also expressed in the cancer cells that have developed drug resistance (111). It corresponds to the first known function of this protein, described in 1976 by Juliano and Ling (6). Certain tumors originating from tissues with naturally high levels of P-gp expression may be intrinsically drug resistant (e.g., colon, kidney, pancreas, and liver carcinoma) (112, 113). On the other hand, tumors with low basic levels of P-gp expression (such as hematological malig-

nancies) sometimes display a marked increases after chemotherapy (114, 115); this phenomenon is associated with acquired resistance. There is a poor understanding of events leading to overexpression of *ABCB1* in response to chemotherapy. An induction of P-gp by chemotherapeutic agents has been suggested, although the mechanism of this induction remains unclear (12). Upon exposure to both endogenous and exogenous stresses (metabolic modifications, hypoxia, chemotherapy), cancer cells are committed to adaptation. Enhancement of *ABCB1* expression constitutes one part of the response.

1.3.2. Cellular Localization of P-gp

Numerous studies have suggested a different intracellular localization of cytotoxic drugs between sensitive and MDR cells (116–120). Most of the drug accumulates in the nucleus of sensitive cells. In MDR cells overexpressing P-gp, the protein is mainly located in the plasma membranes of the cells, and altered drug distribution has been observed in resistant cells. The drug is largely excluded from the nuclei and is sequestered in perinuclear vesicles that move toward the cell periphery to create punctate cytoplasmic distribution patterns (121). The number of these drug-accumulating vesicles per cell seems to correlate with the level of drug resistance, as observed in an MDR Chinese hamster ovary cell line (116). Vesicle formation displays biphasic kinetics, with an initial rapid increase followed by a plateau where no further increase is observed. It has been suggested that a pH shift in various cytoplasmic organelles might contribute to this intracellular redistribution of anticancer drugs (122). Owing to their positive electric charge at physiologic pH, most anticancer drugs (vinca alkaloids, anthracyclines) are accumulated under their protonated form on the side of a membrane at which the pH is lower. This suggests that cationic molecules become "acid-trapped" in acidic cytoplasmic vesicles (123).

Several studies have tried to identify the drug sequestration compartments associated with P-gp function (124). Ferrao et al. (125) demonstrated the involvement of P-gp in drug compartmentalization in leukemic cell lines and patient samples, suggesting that cytoplasmic localization could be involved in the sequestration of doxorubicin in organelles, preventing it from reaching its nuclear targets. Moreover, it has also been detected in the nuclear membrane, in the cytosol (126), and in several cytoplasmic compartments of different cell lines, such as the Golgi apparatus (127) and the ER. A study suggested that P-gp was first present in ER before moving to the Golgi and finally reaching the plasma membrane. Moreover, drug accumulation was raised when P-gp was localized in ER or in the Golgi rather than on plasma membrane (128). On the other hand, Bennis et al. (129) observed a preferential accumulation of doxorubicin in subcellular components distinct from nuclei in doxorubicin-resistant K562 cells. In cells transfected with the *ABCB1* gene, P-gp was detected in vesicles located around the periphery of the nuclei (130), suggesting a mitochondrial pattern, while Gong et al. (131) have shown that

accumulation of daunorubicin occurred in mitochondria-like organelles in K562-resistant cells. In addition, Munteanu et al. (132), then Solazzo et al. (133), independently demonstrated a mitochondrial P-gp localization by several methods and different specific monoclonal antibodies in K562 cells' MDR variants, in MDR1 P1(0.5) hepatocarcinoma cells, and in *ABCB1*-transfected (PNA1)NIH/3T3 cells. The two groups have studied P-gp expression in whole cells by confocal microscopy and in purified isolated mitochondria by western blot. They used functional assays on isolated whole mitochondria by flow cytometry (assays requiring different washing and centrifugations to eliminate debris and contaminations by other membranes such as plasma membranes) to verify that the mitochondrial P-gp was functional. In contrast, Paterson and Gottesman (134) did not observe P-gp in mitochondria of MCF-7 ADR and KB-V1 cells. Thus, the presence of P-gp in mitochondria is dependent on the MDR cell origin.

1.4. P-gp 170: A PROTEIN IMPLICATED IN NUMEROUS FUNCTIONS

1.4.1. Multi-Molecule Transporter

As previously mentioned, P-gp was originally identified in resistant tumor cells as part of the mechanism of MDR; but over the last decade, it has been demonstrated that P-gp is also expressed throughout the body to confer intrinsic resistance to the tissues by exporting unnecessary or toxic exogenous substances or metabolites (135). It is thought that MDR substrates enter the cell through the lipid bilayer by passive diffusion and bind reversibly to P-gp in the bilayer or on the cytoplasmic side of the cell membrane. Subsequently, P-gp utilizes energy from the ATP hydrolysis to transport MDR drugs out of the cell against a concentration gradient (136, 137). P-gp can interact mainly with two classes of compounds: The first one, classically considered as substrates, are generally hydrophobic, positively charged or neutral, and include natural products, chemotherapeutic drugs, or steroids. The second group is constituted by modulators that are able to reverse MDR by blocking P-gp drug efflux without being transported by the pump (see Part IV, Chapter 8).

Drug transport involves two steps. First, there is a catalytic cycle of ATP hydrolysis, which drives transport. This involves low-affinity binding of ATP to both NBDs, which induces the formation of a putative nucleotide sandwich dimer (138). Second, the drug is moved from the cytoplasmic side to the extracellular side of the membrane. The P-gp drug-binding site is constituted by the transmembrane helices and is located within the cytoplasmic membrane leaflet. Three models of P-gp mechanisms of action (Fig. 1.3), not rigorously exclusive of each other, are currently reported: classical pump, vacuum cleaner, and flippase (2).

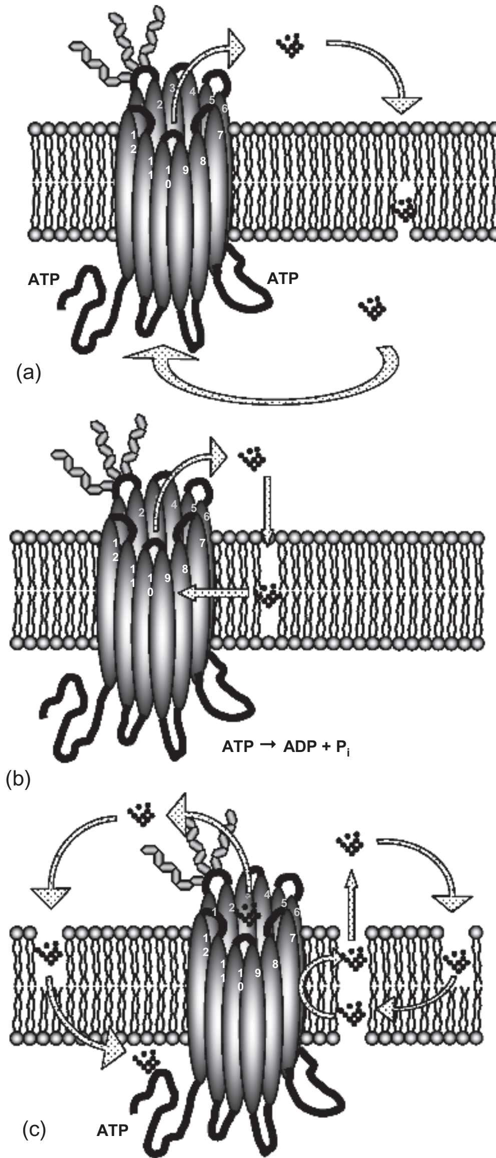

FIGURE 1.3. Different functional models of P-gp. (a) The pump model according to which P-gp may form a transmembrane pore, through which drugs expelled from MDR cells pass, thanks to the ATP hydrolysis energy. (b) In the vacuum cleaner model, drugs interact with the membrane lipids, then with P-gp, which turns inside the membrane and may also release drugs in the extracellular medium.
(c) "Flippase" model: The drug inserted in the inner leaflet of the lipidic bilayer may be translocated ("flipped") on the external leaflet from which it may slowly diffuse in the extracellular medium.

In the classical-pump model, P-gp forms a pore composed of the clustering of the 12 hydrophobic segments, and actively translocates (in an ATP-dependent manner) polar compounds out of the cell as the ion-translocating pumps (105). Evidence for the direct interaction of many of the substrates or reverting agents with the transporter has been obtained, such as drug-binding studies and photoaffinity labeling experiments. The majority of experimental data strongly supports this drug-pump model (137). Drugs interact in cytoplasm with the transmembrane region of the two halves of P-gp (transmembrane segments 5–6 and 11–12), coming together to form a single, large, and flexible drug-binding pocket, possibly containing several binding sites for the substrate (8). It seems that at least two molecules can simultaneously bind different overlapping regions. Then, P-gp expels the drugs directly into the extracellular medium due to the energy from ATP hydrolysis. Authors have demonstrated that the drug-stimulated ATPase activity was directly correlated to the ability of P-gp to transport these drugs (139, 140). Even if data concerning the stoichiometry of the exchange has remained controversial for a long time, probably because of the high basal ATPase activity (141, 142), it seems that one nucleotide is cleaved per P-gp molecule (143). Thus, the function of P-gp is associated mainly with a reduced accumulation of intracellular drugs by way of an active efflux and/or by an intracellular redistribution of these drugs. No substrate-transporter binding that is able to transfer it to P-gp has been described.

According to the “hydrophobic vacuum cleaner” model, P-gp may detect and eliminate hydrophobic substrates directly from the lipid bilayer (144, 145). As most substrates are hydrophobic, it has been proposed that they first equilibrate between the aqueous internal compartment and the inner membrane leaflet before P-gp meets the substrate. In a second step, nucleotide binding and/or ATP hydrolysis causes conformational changes of the transporter, which subsequently can extract substrates from the inner leaf and pump them directly to the external aqueous medium (146, 147). This model is strengthened by data demonstrating unidirectional transport of fluorescent P-gp substrates from the cytoplasmic leaflet of the plasma membrane to the external aqueous environment (148).

In the “flippase” model, P-gp encounters drugs in the inner leaflet of the plasma membrane and flips them to the outer leaflet from which they diffuse into the extracellular medium (149). This model is based on the analogy between amphipathic drugs and the normal phospholipids of membranes. Whereas the lateral mobility of phospholipids within the membrane is high, the spontaneous rate of flipping between the two leaflets of the membrane is very low because the polar-heads groups of the phospholipids cannot be easily transferred across the hydrophobic internal part of the membrane, which is constituted by the acyl chains of the phospholipids. Although this model was initially only based on theoretical considerations, it received a considerable boost when Smit et al. (150) found that the murine *mdr2* P-gp is essential for the normal transport of phosphatidylcholine from the hepatocytes into bile.

According to this model, P-gp may flip drugs from the inner to the outer leaflet of the bilayer where they can partition with the aqueous phase. Recognition and binding of diverse sets of substrates must be associated with a preferred membrane location, determined by molecular properties and lipid interactions (2, 151, 152). It remains that it would be difficult to distinguish experimentally between the hydrophobic vacuum cleaner model and the flippase model.

1.4.2. Lipid Transport

In agreement with this flippase function, a growing number of publications have reported a role of P-gp in phospholipid translocation. P-gp has been reported to regulate the translocation of phosphatidylcholine and phosphatidylethanolamine, as well as sphingomyelin and several other short-chain phospholipid analogs (108, 153). This could explain in part the wide range of substrates recognized by P-gp, due to the different hydrophobic interactions inside lipid bilayers. More specifically, the simple glycosphingolipid (GlcCer) is a P-gp substrate candidate. It is synthesized from ceramide on the cytosolic surface of the Golgi apparatus and enters the outer leaflet of the plasma membrane. Interestingly, GlcCer levels are much lower in cells lacking MDR transporters (154). Nevertheless, it remains unclear whether P-gp translocates natural long chain lipids since *ABCB1* knockout transgenic mice have no detectable abnormality in lipid metabolism (155, 156). P-gp could also be involved in trafficking cholesterol from the plasma membrane to the ER, even if it remains unclear whether the P-gp-facilitated cholesterol trafficking is associated with its conventional drug transport activity (154, 157). Another study conducted by Garrigues et al. (158) suggested a coupling between the basal ATPase activity of P-gp and its intramembrane cholesterol-redistribution function. Data were fully consistent with the possibility that P-gp may actively translocate cholesterol in the membrane. Finally, P-gp-mediated cholesterol redistribution in the cell membrane makes it likely that the protein contributes to stabilizing the cholesterol-rich microdomains, especially rafts, and that it is involved in the regulation of cholesterol trafficking in cells. Thus, P-gp activity is particularly sensitive to its lipid environment. In some cases, P-gp appears to be within specialized raft-like membrane microdomains, where its ATPase activity is five times higher than in crude membranes (159, 160). These observations remain controversial (161). More generally, P-gp retains its function in liquid-ordered cholesterol and sphingolipid model membranes, and P-gp activity requires a microenvironment of raft microdomains or intermediate-density domains (162, 163).

1.4.3. Control and Regulation of Apoptosis

A growing number of publications debate about the role of P-gp in apoptosis (164). Of course, due to its drug efflux function, P-gp exerts a strong down-regulatory effect on drug-induced cell death, but it seems that this prevention

is not limited to this mechanism. Several works reported that P-gp might play a role in regulation of cell death against different stimuli. Robinson et al. (165) showed that P-gp overexpression was associated with resistance to serum starvation-induced apoptosis in Chinese hamster ovary fibroblasts and that the resistance was reversed by verapamil, indicating that P-gp was required for this resistance. Other groups demonstrated that functional P-gp can confer resistance to a wide range of caspase-dependent apoptotic stimuli (death receptor ligation, UV radiation, etc.). Different mechanisms could underlie this function. It has been demonstrated that functional P-gp could inhibit activation of the caspase cascade (especially caspases 8 and 3), downstream FAS ligation without disturbance of death-inducing signaling complex (DISC) formation. The inhibition seemed to be dependent on ATP hydrolysis (166). By contrast, the caspase-independent apoptosis pathway was not affected by P-gp expression, suggesting a caspase-specific role for P-gp. Caspase inhibition could also be explained by an increase in intracellular pH due to expression of functional P-gp, while apoptotic events such as caspase activation need acidic pH (164). In addition, cellular stresses (tumor necrosis factor [TNF], FAS ligation, radiation) are often associated with ceramide generation, which can directly induce mitochondrial cytochrome *c* release. It has been demonstrated that P-gp might both decrease ceramide production by reducing the availability of sphingomyelin and augmented ceramide glycosylation by translocating glucosylceramide across the Golgi membrane, thus detoxifying and inhibiting their apoptotic functions (167). Recently, it was suggested that downregulation of P-gp consecutive to CIAPIN1 inhibition, a new apoptosis inhibitor, could sensitize leukemia cells to chemotherapeutic drugs by upregulating the pro-apoptotic BAX protein (168). Similar results were obtained with hepatocellular carcinoma cells expressing the MDR phenotype. In this model, apoptosis could be restored by downregulation of P-gp expression (169). These data were corroborated by studies of the association between phosphatidylinositol 3-kinase/AKT pathway and MDR of gastric cancer cells (170). It was shown that inhibition of P-AKT expression significantly upregulates p53 expression, and downregulates P-gp expression and *ABCB1* transcription.

1.4.4. P-gp Importance in Immune Response

Variable levels of P-gp expression have been reported in lymphocytes, ranging from 20% to 80% in B cells and from 30% to 100% in T cells. Thus, the link between P-gp expression and the function of lymphocytes remains controversial (171). It has been shown that inhibition of P-gp efflux by monoclonal antibodies or pharmacological inhibitors resulted in the reduction of NK and CD8+ cytotoxic activity. P-gp expression was also reported in skin dendritic cells. These cells are key players in the immune system with the capacity to support innate and specific immunity and to initiate primary immune responses. P-gp seems to be involved in dendritic-cell migration toward lymph nodes through afferent lymphatic vessels (172). One can hypothesize that P-gp could

modulate both NK and CD8+ activity and dendritic-cell migration by regulating cytokine transport, since it has been shown that IL-1β, IL-2, IFNγ, and TNF could be transported across the cellular membrane out of activated lymphocytes (164). Nevertheless, the real place and biological relevance of P-gp implication in physiological immune system functions remains to be demonstrated.

Overexpression of P-gp was also found in lymphocytes from various autoimmune diseases such as rheumatoid arthritis or systemic lupus erythematosus. It may be due to a long-term use of drugs inducing P-gp expression (173). Persistence of activated cell compartments characterizing these diseases could induce P-gp expression. Another role for P-gp in autoimmune diseases was suspected with observations performed on *ABCB1* knockout mice. The animals have been reported to be more susceptible to inflammatory bowel diseases. It has been suggested that P-gp, in regard to its gut localization, could prevent accumulation of inflammation-inducing bacteria (94).

1.5. CONCLUSION

Finally, since the discovery of P-gp (product of the *ABCB1* gene) in 1976 in cancer tissue, several thousands of articles have been published, showing the interest of the knowledge of its gene, its structure, and its role. From this amount of data, a more rational approach to P-gp inhibition should emerge. Recent studies have focused on *ABCB1* pharmacogenetics, which is involved in both drug pharmacokinetics and cancer MDR (2, 10). Nevertheless, numerous data about P-gp remain partial and/or unclear. For instance, the mechanism of P-gp-mediated drug transport is not yet completely elucidated, especially the coupling between ATP cleavage and transport; the molecular phenomena leading to *ABCB1* overexpression in response to chemotherapy is poorly understood; and the effects of various genotypes and haplotypes on P-gp function remain controversial. Today, we know that P-pg is present in normal cells and in tumor cells, where it plays a role to efflux hydrophobic endogenous and exogenous compounds. P-gp is involved in numerous physiological and pathologic pathways, in normal and cancer tissues; and the implication of P-gp in so many processes has opened several important new topics of investigation.

REFERENCES

1. Kimura Y, Morita SY, Matsuo M, and Ueda K. 2007. Mechanism of multidrug recognition by MDR1/ABCB1. *Cancer Sci* 98: 1303–1310.
2. Sharom FJ. 2008. ABC multidrug transporters: Structure, function and role in chemoresistance. *Pharmacogenomics* 9: 105–127.
3. Dassa E and Bouige P. 2001. The ABC of ABCS: A phylogenetic and functional classification of ABC systems in living organisms. *Res Microbiol* 152: 211–229.

4. Szakács G, Paterson JK, Ludwig JA, Booth-Genthe C, and Gottesman MM. 2006. Targeting multidrug resistance in cancer. *Nat Rev Drug Discov* 5: 219–234.
5. Higgins CF. 2007. Multiple molecular mechanisms for multidrug resistance transporters. *Nature* 446: 749–757.
6. Juliano RL and Ling V. 1976. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta* 455: 152–162.
7. Gottesman MM and Ling V. 2006. The molecular basis of multidrug resistance in cancer: The early years of P-glycoprotein research. *FEBS Lett* 580: 998–1009.
8. Loo TW and Clarke DM. 2005. Recent progress in understanding the mechanism of P-glycoprotein-mediated drug efflux. *J Membr Biol* 206: 173–185.
9. Vaalburg W, Hendrikse NH, Elsinga PH, Bart J, and van Waarde A. 2005. P-glycoprotein activity and biological response. *Toxicol Appl Pharmacol* 207: 257–260.
10. Chinn LW and Kroetz DL. 2007. ABCB1 pharmacogenetics: Progress, pitfalls, and promise. *Clin Pharmacol Ther* 81: 265–269.
11. Hennessy M and Spiers JP. 2007. A primer on the mechanics of P-glycoprotein the multidrug transporter. *Pharmacol Res* 55: 1–15.
12. Callaghan R, Crowley E, Potter S, and Kerr ID. 2008. P-glycoprotein: So many ways to turn it on. *J Clin Pharmacol* 48: 365–378.
13. Lin T, Islam O, and Heese K. 2006. ABC transporters, neural stem cells and neurogenesis—A different perspective. *Cell Res* 16: 857–871.
14. Sarkadi B, Homolya L, Szakács G, and Váradi A. 2006. Human multidrug resistance ABCB and ABCG transporters: Participation in a chemoimmunity defense system. *Physiol Rev* 86: 1179–1236.
15. Huang Y and Sadée W. 2006. Membrane transporters and channels in chemoresistance and -sensitivity of tumor cells. *Cancer Lett* 239: 168–182.
16. Van Helvoort A, Smith AJ, Sprong H, Fritzsche I, Schinkel AH, Borst P et al. 1996. MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. *Cell* 87: 507–517.
17. Chen CJ, Clark D, Ueda K, Pastan I, Gottesman MM, and Roninson IB. 1990. Genomic organization of the human multidrug resistance (MDR1) gene and origin of P-glycoproteins. *J Biol Chem* 265: 506–514.
18. Mickley LA, Spengler BA, Knutsen TA, Biedler JL, and Fojo T. 1997. Gene rearrangement: A novel mechanism for *MDR-1* gene activation. *J Clin Invest* 99: 1947–1957.
19. Huff LM, Lee J-S, Robey RW, and Fojo T. 2006. Characterization of gene rearrangement leading to activation of *MDR-1*. *J Biol Chem* 281: 36501–36509.
20. Van Groenigen M, Valentijn LJ, and Baas F. 1993. Identification of a functional initiator sequence in the human MDR1 promoter. *Biochim Biophys Acta* 1172: 138–146.
21. Huff LM, Wang Z, Iglesias A, Fojo T, and Lee JS. 2005. Aberrant transcription from an unrelated promoter can result in MDR-1 expression following drug selection in vitro and in relapsed lymphoma samples. *Cancer Res* 65: 11694–11703.
22. Lutterbach B, Sun D, Schuetz J, and Hiebert SW. 1998. The MYND motif is required for repression of basal transcription from the multidrug resistance 1 promoter by the t(8;21) fusion protein. *Mol Cell Biol* 18: 3604–3611.

23. Randle RA, Raguz S, Higgins CF, and Yagüe E. 2007. Role of the highly structured 5′-end region of MDR1 mRNA in P-glycoprotein expression. *Biochem J* 406: 445–455.
24. Chen G, Jaffrezou JP, Fleming WH, Duran GE, and Sikic BI. 1994. Prevalence of multidrug resistance related to activation of the mdr1 gene in human sarcoma mutants derived by single-step doxorubicin selection. *Cancer Res* 54: 4980–4987.
25. Desiderato L, Davey MW, and Piper AA. 1997. Demethylation of the human MDR1 5′ region accompanies activation of P-glycoprotein expression in a HL60 multidrug resistant subline. *Somat Cell Mol Genet* 23: 391–400.
26. Kusaba H, Nakayama M, Harada T, Nomoto M, Kohno K, Kuwano M et al. 1999. Association of 5′ CpG demethylation and altered chromatin structure in the promoter region with transcriptional activation of the multidrug resistance 1 gene in human cancer cells. *Eur J Biochem* 262: 924–932.
27. Chaudhary PM and Roninson IB. 1993. Induction of multidrug resistance in human cells by transient exposure to different chemotherapeutic drugs. *J Natl Cancer Inst* 85: 632–639.
28. Labialle S, Gayet L, Marthinet E, Rigal D, and Baggetto LG. 2002. Transcriptional regulators of the human multidrug resistance 1 gene: Recent views. *Biochem Pharmacol* 64: 943–948.
29. Ogretmen B and Safa AR. 1999. Negative regulation of MDR1 promoter activity in MCF-7, but not in multidrug resistant MCF-7/Adr, cells by cross-coupled NF-kappa B/p65 and c-Fos transcription factors and their interaction with the CAAT region. *Biochemistry* 38: 2189–2199.
30. Rohlff C and Glazer RI. 1998. Regulation of the MDR1 promoter by cyclic AMP-dependent protein kinase and transcription factor Sp1. *Int J Oncol* 12: 383–386.
31. Bargou RC, Jurchott K, Wagener C, Bergmann S, Metzner S, Bommert K et al. 1997. Nuclear localization and increased levels of transcription factor YB-1 in primary human breast cancers are associated with intrinsic MDR1 gene expression. *Nat Med* 3: 447–450.
32. Ogretmen B and Safa AR. 2000. Identification and characterization of the MDR1 promoter-enhancing factor 1 (MEF1) in the multidrug resistant HL60/VCR human acute myeloid leukemia cell line. *Biochemistry* 39: 194–204.
33. Ohga T, Uchiumi T, Makino Y, Koike K, Wada M, Kuwano M et al. 1998. Direct involvement of the Y-box binding protein YB-1 in genotoxic stress-induced activation of the human multidrug resistance 1 gene. *J Biol Chem* 273: 5997–6000.
34. Johnson RA, Ince TA, and Scotto KW. 2001. Transcriptional repression by p53 through direct binding to a novel DNA element. *J Biol Chem* 276: 27716–27720.
35. Zhan M, Yu D, Lang A, Li L, and Pollock RE. 2001. Wild type p53 sensitizes soft tissue sarcoma cells to doxorubicin by down-regulating multidrug resistance-1 expression. *Cancer* 92: 1556–1566.
36. Zhan M, Yu D, Liu J, Glazer RI, Hannay J, and Pollock RE. 2005. Transcriptional repression of protein kinase C alpha via Sp1 by wild type p53 is involved in inhibition of multidrug resistance 1 P-glycoprotein phosphorylation. *J Biol Chem* 280: 4825–4833.

37. Johnson RA, Shepard EM, and Scotto KW. 2005. Differential regulation of MDR1 transcription by the p53 family members. Role of the DNA binding domain. *J Biol Chem* 280: 13213–13219.
38. Okamura H, Yoshida K, Sasaki E, Morimoto H, and Haneji T. 2004. Transcription factor NF-Y regulates mdr1 expression through binding to inverted CCAAT sequence in drug-resistant human squamous carcinoma cells. *Int J Oncol* 25: 1031–1037.
39. Chen GK, Sale S, Tan T, Ermoian RP, and Sikic BI. 2004. CCAAT/enhancer-binding protein beta (nuclear factor for interleukin 6) transactivates the human MDR1 gene by interaction with an inverted CCAAT box in human cancer cells. *Mol Pharmacol* 65: 906–916.
40. Labialle S, Dayan G, Gayet L, Rigal D, Gambrelle J, and Baggetto LG. 2004. New invMED1 element cis-activates human multidrug-related MDR1 and MVP genes, involving the LRP130 protein. *Nucleic Acids Res* 32: 3864–3876.
41. Blanc E, Goldschneider D, Ferrandis E, Barrois M, Le Roux G, Leonce S et al. 2003. MYCN enhances P-gp/MDR1 gene expression in the human metastatic neuroblastoma IGR-N-91 model. *Am J Pathol* 163: 321–331.
42. Baker EK, Johnstone RW, Zalcberg JR, and El-Osta A. 2005. Epigenetic changes to the MDR1 locus in response to chemotherapeutic drugs. *Oncogene* 24: 8061–8075.
43. Shilling RA, Venter H, Velamakanni S, Bapna A, Woebking B, Shahi S et al. 2006. New light on multidrug binding by an ATP-binding-cassette transporter. *Trends Pharmacol Sci* 27: 195–203.
44. Cascorbi I, Gerloff T, Johne A, Meisel C, Hoffmeyer S, Schwab M et al. 2001. Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. *Clin Pharmacol Ther* 69: 169–174.
45. Pawlik A, Baśkiewicz-Masiuk M, Machaliński B, Safranow K, and Gawrońska-Szklarz B. 2005. Involvement of P-glycoprotein in the release of cytokines from peripheral blood mononuclear cells treated with methotrexate and dexamethasone. *J Pharm Pharmacol* 57: 1421–1425.
46. Siegsmund M, Brinkmann U, Scháffeler E, Weirich G, Schwab M, Eichelbaum M et al. 2002. Association of the P-glycoprotein transporter MDR1(C3435T) polymorphism with the susceptibility to renal epithelial tumors. *J Am Soc Nephrol* 13: 1847–1854.
47. Taniguchi S, Mochida Y, Uchiumi T, Tahira T, Hayashi K, Takagi K et al. 2003. Genetic polymorphism at the 5′ regulatory region of multidrug resistance 1 (MDR1) and its association with interindividual variation of expression level in the colon. *Mol Cancer Ther* 2: 1351–1359.
48. Drozdźik M, Białecka M, Myśliwiec K, Honczarenko K, Stankiewicz J, and Sych Z. 2003. Polymorphism in the P-glycoprotein drug transporter MDR1 gene: A possible link between environmental and genetic factors in Parkinson's disease. *Pharmacogenetics* 13: 259–263.
49. Morita N, Yasumori T, and Nakayama K. 2003. Human MDR1 polymorphism: G2677T/A and C3435T have no effect on MDR1 transport activities. *Biochem Pharmacol* 65: 1843–1852.

50. Kafka A, Sauer G, Jaeger C, Grundmann R, Kreienberg R, Zeillinger R et al. 2003. Polymorphism C3435T of the MDR-1 gene predicts response to preoperative chemotherapy in locally advanced breast cancer. *Int J Oncol* 22: 1117–1121.
51. Vaclavikova R, Nordgard SH, Alnaes GI, Hubackova M, Kubala E, Kodet R et al. 2008. Single nucleotide polymorphisms in the multidrug resistance gene 1 (ABCB1): Effects on its expression and clinicopathological characteristics in breast cancer patients. *Pharmacogenet Genomics* 18: 263–273.
52. Sam SS, Thomas V, Sivagnanam K, Reddy KS, Surianarayanan G, and Chandrasekaran A. 2007. ABCB1 genetic polymorphism and risk of upper aerodigestive tract cancers among smokers, tobacco chewers and alcoholics in an Indian population. *Pharmacogenet Genomics* 17: 861–866.
53. Woodahl EL, Yang Z, Bui T, Shen DD, and Ho RJ. 2004. Multidrug resistance gene G1199A polymorphism alters efflux transport activity of P-glycoprotein. *J Pharmacol Exp Ther* 310: 1199–1207.
54. Yasui K, Mihara S, Zhao C, Okamoto H, Saito-Ohara F, Tomida A et al. 2004. Alteration in copy numbers of genes as a mechanism for acquired drug resistance. *Cancer Res* 64: 1403–1410.
55. Li D, Zhang GL, Lou YQ, Li Q, Wang X, and Bu XY. 2007. Genetic polymorphisms in MDR1 and CYP3A5 and MDR1 haplotype in mainland Chinese Han, Uygur and Kazakh ethnic groups. *J Clin Pharm Ther* 32: 89–95.
56. Kimchi-Sarfaty C, Marple AH, Shinar S, Kimchi AM, Scavo D, Roma MI et al. 2007. Ethnicity-related polymorphisms and haplotypes in the human ABCB1 gene. *Pharmacogenomics* 8: 29–39.
57. Crettol S, Venetz JP, Fontana M, Aubert JD, Ansermot N, Fathi M et al. 2008. Influence of ABCB1 genetic polymorphisms on cyclosporine intracellular concentration in transplant recipients. *Pharmacogenet Genomics* 18: 307–315.
58. Elens L, Capron A, Kerckhove VV, Lerut J, Mourad M, Lison D et al. 2007. 1199G > A and 2677G > T/A polymorphisms of ABCB1 independently affect tacrolimus concentration in hepatic tissue after liver transplantation. *Pharmacogenet Genomic* 17: 873–883.
59. Green H. 2008. Pharmacogenomics of importance for paclitaxel chemotherapy. *Pharmacogenomics* 9: 671–674.
60. Jones PM and George AM. 1998. A new structural model for P-glycoprotein. *J Membr Biol* 166: 133–147.
61. Dawson RJ and Locher KP. 2006. Structure of a bacterial multidrug ABC transporter. *Nature* 443: 180–185.
62. Zolnerciks JK, Wooding C, and Linton KJ. 2007. Evidence for a Sav1866-like architecture for the human multidrug transporter P-glycoprotein. *FASEB J* 21: 3937–3948.
63. Rosenberg MF, Kamis AB, Callaghan R, Higgins CF, and Ford RC. 2003. Three-dimensional structures of the mammalian multidrug resistance P-glycoprotein demonstrate major conformational changes in the transmembrane domains upon nucleotide binding. *J Biol Chem* 278: 8294–8299.
64. Rosenberg MF, Callaghan R, Modok S, Higgins CF, and Ford RC. 2005. Three-dimensional structure of P-glycoprotein: The transmembrane regions adopt an

asymmetric configuration in the nucleotide-bound state. *J Biol Chem* 280: 2857–2862.

65. Vandevuer S, Van Bambeke F, Tulkens PM, and Prévost M. 2006. Predicting the three-dimensional structure of human P-glycoprotein in absence of ATP by computational techniques embodying crosslinking data: Insight into the mechanism of ligand migration and binding sites. *Proteins* 63: 466–478.
66. Lee JY, Urbatsch IL, Senior AE, and Wilkens S. 2008. Nucleotide-induced structural changes in P-glycoprotein observed by electron microscopy. *J Biol Chem* 283: 5769–5779.
67. Loo TW, Bartlett MC, and Clarke DM. 2006. Transmembrane segment 1 of human P-glycoprotein contributes to the drug-binding pocket. *Biochem J* 396: 537–545.
68. Loo TW, Bartlett MC, and Clarke DM. 2006. Transmembrane segment 7 of human P-glycoprotein forms part of the drug-binding pocket. *Biochem J* 399: 351–359.
69. Storm J, O'Mara ML, Crowley EH, Peall J, Tieleman DP, Kerr ID et al. 2007. Residue G346 in transmembrane segment six is involved in inter-domain communication in P-glycoprotein. *Biochemistry* 46: 9899–9910.
70. Staats J, Marquardt D, and Center MS. 1990. Characterization of a membrane-associated protein kinase of multidrug-resistant HL60 cells which phosphorylates P-glycoprotein. *J Biol Chem* 265: 4084–4090.
71. Parissenti AM, Gannon BR, Villeneuve DJ, Kirwan-rhude AF, Chadderton A, and Gluck S. 1999. Lack of modulation of MDR1 gene expression by dominant inhibition of cAMP-dependent protein kinase in doxorubicin-resistant MCF-7 breast cancer cells. *Int J Cancer* 82:893–900.
72. Gupta KP, Ward NE, Gravitt KR, Bergman PJ, and O'Brian CA. 1996. Partial reversal of multidrug resistance in human breast cancer cells by an N-myristoylated protein kinase C-alpha pseudosubstrate peptide. *J Biol Chem* 271: 2102–2111.
73. Ratsaninghe D, Phang JM, and Yeh GC. 1998. Differential expression and activity of phosphatases and protein kinases in adriamycin sensitive and resistant human breast cancer MCF-7 cells. *Int J Oncol* 13: 79–84.
74. Kannan S. 2006. Therapeutic significance of ectophosphatase inhibitors in reversal of multi-drug resistance. *Med Hypotheses* 66: 1041–1042.
75. Smith CD and Zilfou JT. 1995. Circumvention of P-glycoprotein-mediated multiple drug resistance by phosphorylation modulators is independent of protein kinases. *J Biol Chem* 270: 28145–28152.
76. Castro AF, Horton JK, Vanoye CG, and Altenberg GA. 1999. Mechanism of inhibition of P-glycoprotein-mediated drug transport by protein kinase C blockers. *Biochem Pharmacol* 58: 1723–1733.
77. Sachs CW, Chambers TC, and Fine RL. 1999. Differential phosphorylation of sites in the linker region of P-glycoprotein by protein kinase C isozymes alpha, betaI, betaII, gamma, delta, epsilon, eta, and zeta. *Biochem Pharmacol* 58: 1587–1592.
78. Szabó K, Bakos E, Welker E, Müller M, Goodfellow HR, Higgins CF et al. 1997. Phosphorylation site mutations in the human multidrug transporter modulate its drug-stimulated ATPase activity. *J Biol Chem* 272: 23165–23171.
79. Ahmad S, Safa AR, and Glazer RI. 1994. Modulation of P-glycoprotein by protein kinase C alpha in a baculovirus expression system. *Biochemistry* 33: 10313–10318.

80. Lelong-Rebel IH and Cardarelli CO. 2005. Differential phosphorylation patterns of P-glycoprotein reconstituted into a proteoliposome system: Insight into additional unconventional phosphorylation sites. *Anticancer Res* 25: 3925–3935.
81. Loo TW, Bartlett MC, and Clarke DM. 2004. Processing mutations located throughout the human multidrug resistance P-glycoprotein disrupt interactions between the nucleotide binding domains. *J Biol Chem* 279: 38395–38401.
82. Loo TW and Clarke DM. 1994. Prolonged association of temperature-sensitive mutants of human P-glycoprotein with calnexin during biogenesis. *J Biol Chem* 269: 28683–28689.
83. Loo TW and Clarke DM. 1995. P-glycoprotein. Associations between domains and between domains and molecular chaperones. *J Biol Chem* 270: 21839–21844.
84. Kramer R, Weber TK, Arceci R, Ramchurren N, Kastrinakis WV, Steele G, Jr. et al. 1995. Inhibition of N-linked glycosylation of P-glycoprotein by tunicamycin results in a reduced multidrug resistance phenotype. *Br J Cancer* 71: 670–675.
85. Zhang Z, Wu JY, Hait WN, and Yang JM. 2004. Regulation of the stability of P-glycoprotein by ubiquitination. *Mol Pharmacol* 66: 395–403.
86. Gribar JJ, Ramachandra M, Hrycyna CA, Dey S, and Ambudkar SV. 2000. Functional characterization of glycosylation-deficient human P-glycoprotein using a vaccinia virus expression system. *J Membr Biol* 173: 203–214.
87. Urbatsch IL, Wilke-Mounts S, Gimi K, and Senior AE. 2001. Purification and characterization of N-glycosylation mutant mouse and human P-glycoproteins expressed in *Pichia pastoris* cells. *Arch Biochem Biophys* 388: 171–177.
88. Greer DA and Ivey S. 2007. Distinct N-glycan glycosylation of P-glycoprotein isolated from the human uterine sarcoma cell line MES-SA/Dx5. *Biochim Biophys Acta* 1770: 1275–1282.
89. Krueger KE and Srivastava S. 2006. Posttranslational protein modifications: Current implications for cancer detection, prevention, and therapeutics. *Mol Cell Proteomics* 5: 1799–1810.
90. Fojo AT, Ueda K, Slamon DJ, Poplack DG, Gottesman MM, and Pastan I. 1987. Expression of a multidrug-resistance gene in human tumors and tissues. *Proc Natl Acad Sci U S A* 84: 265–269.
91. Fromm MF. 2003. Importance of P-glycoprotein for drug disposition in humans. *Eur J Clin Invest* 33: 6–9.
92. Thuerauf N and Fromm MF. 2006. The role of the transporter P-glycoprotein for disposition and effects of centrally acting drugs and for the pathogenesis of CNS diseases. *Eur Arch Psychiatry Clin Neurosci* 256: 281–286.
93. Konishi T, Satsu H, Hatsugai Y, Aizawa K, Inakuma T, Nagata S et al. 2004. A bitter melon extract inhibits the P-glycoprotein activity in intestinal Caco-2 cells: Monoglyceride as an active compound. *Biofactors* 22: 71–74.
94. Leslie EM, Deeley RG, and Cole SP. 2005. Multidrug resistance proteins: Role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. *Toxicol Appl Pharmacol* 204: 216–237.
95. Fromm MF. 2004. Importance of P-glycoprotein at blood-tissue barriers. *Trends Pharmacol Sci* 25: 423–429.

96. Bendayan R, Ronaldson PT, Gingras D, and Bendayan M. 2006. In situ localization of P-glycoprotein (ABCB1) in human and rat brain. *J Histochem Cytochem* 54: 1159–1167.
97. Ha SN, Hochman J, and Sheridan RP. 2007. Mini review on molecular modeling of P-glycoprotein (Pgp). *Curr Top Med Chem* 7: 1525–1529.
98. Borst P and Elferink RO. 2002. Mammalian ABC transporters in health and disease. *Annu Rev Biochem* 71: 537–592.
99. Doran A, Obach RS, Smith BJ, Hosea NA, Becker S, Callegari E et al. 2005. The impact of P-glycoprotein on the disposition of drugs targeted for indications of the central nervous system: Evaluation using the MDR1A/1B knockout mouse model. *Drug Metab Dispos* 33: 165–174.
100. Lin JH and Yamazaki M. 2003. Clinical relevance of P-glycoprotein in drug therapy. *Drug Metab Rev* 35: 417–454.
101. Keshet GI, Goldstein I, Itzhaki O, Cesarkas K, Shenhav L, Yakirevitch A et al. 2008. MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun* 368: 930–936.
102. de Grouw EP, Raaijmakers MH, Boezeman JB, van der Reijden BA, Van de Locht LT, and de Witte TJ. 2006. Preferential expression of a high number of ATP binding cassette transporters in both normal and leukemic CD34 + CD38- cells. *Leukemia* 20: 750–754.
103. Chaudhary PM and Roninson IB. 1991. Expression and activity of P-glycoprotein, a multidrug efflux pump, in human hematopoietic stem cells. *Cell* 66: 85–94.
104. Dean M, Fojo T, and Bates S. 2005. Tumour stem cells and drug resistance. *Nat Rev Cancer* 5: 275–284.
105. Gottesman MM and Pastan I. 1988. The multidrug transporter, a double-edged sword. *J Biol Chem* 263: 12163–12166.
106. Mitsuhashi J, Tsukahara S, Suzuki R, Oh-hara Y, Nishi S, Hosoyama H et al. 2007. Retroviral integration site analysis and the fate of transduced clones in an MDR1 gene therapy protocol targeting metastatic breast cancer. *Hum Gene Ther* 18: 895–906.
107. Islam MO, Kanemura Y, Tajria J, Mori H, Kobayashi S, Shofuda T et al. 2005. Characterization of ABC transporter ABCB1 expressed in human neural stem/progenitor cells. *FEBS Lett* 579: 3473–3480.
108. Mizutani T, Masuda M, Nakai E, Furumiya K, Togawa H, Nakamura Y et al. 2008. Genuine functions of P-glycoprotein (ABCB1). *Curr Drug Metab* 9: 167–174.
109. Stromskaya TP, Rybalkina EY, Zabotina TN, Shishkin AA, and Stavrovskaya AA. 2005. Influence of RARalpha gene on MDR1 expression and P-glycoprotein function in human leukemic cells. *Cancer Cell Int* 5: 15.
110. Sulová Z, Macejová D, Seres M, Sedlák J, Brtko J, and Breier A. 2008. Combined treatment of P-gp-positive L1210/VCR cells by verapamil and all-trans retinoic acid induces down-regulation of P-glycoprotein expression and transport activity. *Toxicol In Vitro* 22: 96–105.
111. Gottesman MM, Fojo T, and Bates SE. 2002. Multidrug resistance in cancer: Role of ATP-dependent transporters. *Nat Rev Cancer* 2: 48–58.

112. Huang CC, Wu MC, Xu GW, Li DZ, Cheng H, Tu ZX et al. 1992. Overexpression of the MDR1 gene and P-glycoprotein in human hepatocellular carcinoma. *J Natl Cancer Inst* 84: 262–264.
113. Kramer R, Weber TK, Morse B, Arceci R, Staniunas R, Steele G, Jr. et al. 1993. Constitutive expression of multidrug resistance in human colorectal tumours and cell lines. *Br J Cancer* 67: 959–968.
114. Petrini M, Di Simone D, Favati A, Mattii L, Valentini P, and Grassi B. 1995. GST-pi and P-170 co-expression in multiple myeloma. *Br J Haematol* 90: 393–397.
115. Soto-Vega E, Arroyo C, Richaud-Patin Y, García-Carrasco M, Vázquez-Lavista LG, and Llorente L. 2008. P-glycoprotein activity in renal clear cell carcinoma. *Urol Oncol* in press.
116. Sognier MA, Zhang Y, Eberle RL, Sweet KM, Altenberg GA, and Belli JA. 1994. Sequestration of doxorubicin in vesicles in a multidrug-resistant cell line (LZ-100). *Biochem Pharmacol* 48: 391–401.
117. Belhoussine R, Morjani H, Millot JM, Sharonov S, and Manfait M. 1998. Confocal scanning microspectrofluorometry reveals specific anthracyline accumulation in cytoplasmic organelles of multidrug-resistant cancer cells. *J Histochem Cytochem* 46: 1369–1376.
118. Larsen AK, Escargueil AE, and Skladanowski A. 2000. Resistance mechanisms associated with altered intracellular distribution of anticancer agents. *Pharmacol Ther* 85: 217–229.
119. Bour-Dill C, Gramain MP, Merlin JL, Marchal S, and Guillemin F. 2000. Determination of intracellular organelles implicated in daunorubicin cytoplasmic sequestration in multidrug-resistant MCF-7 cells using fluorescence microscopy image analysis. *Cytometry* 39: 16–25.
120. Benderra Z, Trussardi A, Morjani H, Villa AM, Doglia SM, and Manfait M. 2000. Regulation of cellular glutathione modulates nuclear accumulation of daunorubicin in human MCF7 cells overexpressing multidrug resistance associated protein. *Eur J Cancer* 36: 428–434.
121. Seidel A, Hasmann M, Loser R, Bunge A, Schaefer B, Herzig I et al. 1995. Intracellular localization, vesicular accumulation and kinetics of daunorubicin in sensitive and multidrug-resistant gastric carcinoma EPG85-257 cells. *Virchows Arch* 426: 249–256.
122. Schindler M, Grabski S, Hoff E, and Simon SM. 1996. Defective pH regulation of acidic compartments in human breast cancer cells (MCF-7) is normalized in adriamycin-resistant cells (MCF-7adr). *Biochemistry* 35: 2811–2817.
123. Warren L, Jardillier JC, and Ordentlich P. 1991. Secretion of lysosomal enzymes by drug-sensitive and multiple drug-resistant cells. *Cancer Res* 51: 1996–2001.
124. Shapiro AB, Fox K, Lee P, Yang YD, and Ling V. 1998. Functional intracellular P-glycoprotein. *Int J Cancer* 76: 857–864.
125. Ferrao P, Sincock P, Cole S, and Ashman L. 2001. Intracellular P-gp contributes to functional drug efflux and resistance in acute myeloid leukemia. *Leuk Res* 25: 395–405.
126. Maraldi NM, Zini N, Santi S, Scotlandi K, Serra M, and Baldini N. 1999. P-glycoprotein subcellular localization and cell morphotype in MDR1 gene-transfected human osteosarcoma cells. *Biol Cell* 91: 17–28.

127. Molinari A, Cianfriglia M, Meschini S, Calcabrini A, and Arancia G. 1994. P-glycoprotein expression in the Golgi apparatus of multidrug resistant cells. *Int J Cancer* 59: 789–795.
128. Fu D, Bebawy M, Kable EPW, and Roufogalis BD. 2004. Dynamic and intracellular trafficking of P-glycoprotein-EGFP fusion protein: Implications in multidrug resistance in cancer. *Int J Cancer* 109: 174–181.
129. Bennis S, Ichas F, and Robert J. 1995. Differential effects of verapamil and quinine on the reversal of doxorubicin resistance in a human leukemia cell line. *Int J Cancer* 62: 283–290.
130. Rajagopal A and Simon SM. 2003. Subcellular localization and activity of multidrug resistance proteins. *Mol Biol Cell* 14: 3389–3399.
131. Gong Y, Wang Y, Chen F, Han J, Miao J et al. 2000. Identification of the subcellular localization of daunorubicin in multidrug-resistant K562 cell line. *Leuk Res* 24: 769–774.
132. Munteanu E, Verdier M, Grandjean-Forestier F, Stenger C, Jayat-Vignoles C, Huet S et al. 2006. Mitochondrial localization and activity of P-glycoprotein in doxorubicin-resistant K562 cells. *Biochem Pharmacol* 71: 1162–1174.
133. Solazzo M, Fantappiè O, Lasagna N, Sassoli C, Nosi D, and Mazzanti R. 2006. P-gp localization in mitochondria and its functional characterization in multiple drug-resistant cell lines. *Exp Cell Res* 312: 4070–4078.
134. Paterson JK and Gottesman MM. 2007. P-Glycoprotein is not present in mitochondrial membranes. *Exp Cell Res* 313: 3100–3105.
135. Sakaeda T, Nakamura T, and Okumura K. 2002. MDR1 genotype-related pharmacokinetics and pharmacodynamics. *Biol Pharm Bull* 25: 1391–1400.
136. Hendrikse NH, Franssen EJ, van der Graaf WT, Vaalburg W, and de Vries EG. 1999. Visualization of multidrug resistance in vivo. *Eur J Nucl Med* 26: 283–293.
137. Sharom FJ. 2006. Shedding light on drug transport: Structure and function of the P-glycoprotein multidrug transporter (ABCB1). *Biochem Cell Biol* 84: 979–992.
138. Sauna ZE and Ambudkar SV. 2007. About a switch: How P-glycoprotein (ABCB1) harnesses the energy of ATP binding and hydrolysis to do mechanical work. *Mol Cancer Ther* 6: 13–23.
139. Callaghan R, Berridge G, Ferry DR, and Higgins CF. 1997. The functional purification of P-glycoprotein is dependent on maintenance of a lipid-protein interface. *Biochim Biophys Acta* 1328: 109–124.
140. Ambudkar SV, Kim IW, and Sauna ZE. 2006. The power of the pump: Mechanisms of action of P-glycoprotein (ABCB1). *Eur J Pharm Sci* 27: 392–400.
141. Ambudkar SV, Cardarelli CO, Pashinsky I, and Stein WD. 1997. Relation between the turnover number for vinblastine transport and for vinblastine-stimulated ATP hydrolysis by human P-glycoprotein. *J Biol Chem* 272: 21160–21166.
142. Shapiro AB and Ling V. 1998. Stoichiometry of coupling of rhodamine 123 transport to ATP hydrolysis by P-glycoprotein. *Eur J Biochem* 254: 189–193.
143. Tombline G, Muharemagic A, White LB, and Senior AE. 2005. Involvement of the "occluded nucleotide conformation" of P-glycoprotein in the catalytic pathway. *Biochemistry* 44: 12879–12886.

144. Stein WD, Cardarelli C, Pastan I, and Gottesman MM. 1994. Kinetic evidence suggesting that the multidrug transporter differentially handles influx and efflux of its substrates. *Mol Pharmacol* 45: 763–772.
145. Romsicki Y and Sharom FJ. 1999. The membrane lipid environment modulates drug interactions with the P-glycoprotein multidrug transporter. *Biochemistry* 38: 6887–6896.
146. Rosenberg MF, Kamis AB, Callaghan R, Higgins CF, and Ford RC. 2003. Three-dimensional structures of the mammalian multidrug resistance P-glycoprotein demonstrate major conformational changes in the transmembrane domains upon nucleotide binding. *J Biol Chem* 278: 8294–8299.
147. Hennessy M and Spiers JP. 2007. A primer on the mechanics of P-glycoprotein the multidrug transporter. *Pharmacol Res* 55: 1–15.
148. Shapiro AB and Ling V. 1998. Transport of LDS-751 from the cytoplasmic leaflet of the plasma membrane by the rhodamine-123-selective site of P-glycoprotein. *Eur J Biochem* 254: 181–188.
149. Higgins CF and Gottesman MM. 1992. Is the multidrug transporter a flippase? *Trends Biochem Sci* 17: 18–21.
150. Smit JJM, Schinkel AH, Oude Elferink RPJ, Groen AK, Wagenaar E, van Deemter L et al. 1993. Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. *Cell* 75: 451–462.
151. Romsicki Y and Sharom FJ. 2001. Phospholipid flippase activity of the reconstituted P-glycoprotein multidrug transporter. *Biochemistry* 40: 6937–6947.
152. Siarheyeva A, Lopez JJ, and Glaubitz C. 2006. Localization of multidrug transporter substrates within model membranes. *Biochemistry* 45: 6203–6211.
153. Bosch I, Dunussi-Joannopoulos K, Wu RL, Furlong ST, and Croop J. 1997. Phosphatidylcholine and phosphatidylethanolamine behave as substrates of the human MDR1 P-glycoprotein. *Biochemistry* 36: 5685–5694.
154. Raggers RJ, Pomorski T, Holthuis JCM, Kälin N, and Van Meer G. 2000. Lipid traffic: The ABC of transbilayer movement. *Traffic* 1: 226–234.
155. Van Meer G. 2005. Cellular lipidomics. *EMBO J* 24: 3159–3165.
156. Borst P, Zelcer N, and Van Helvoort A. 2000. ABC transporters in lipid transport. *Biochem Biophys Acta* 1486: 128–144.
157. Luker GD, Nilsson KR, Covey DF, and Piwnica-Worms D. 1999. Multidrug resistance (MDR1) P-glycoprotein enhances esterification of plasma membrane cholesterol. *J Biol Chem* 274: 6979–6991.
158. Garrigues A, Escargueil AE, and Orlowski S. 2002. The multidrug transporter, P-glycoprotein, actively mediates cholesterol redistribution in the cell membrane *Proc Natl Acad Sci U S A* 99: 10347–10352.
159. Bucher K, Besse CA, Kamau SW, Wunderli-Allenspach H, and Krämer SD. 2005. Isolated rafts from adriamycin-resistant P388 cells contain functional ATPases and provide an easy test system for P-glycoprotein-related activities. *Pharm Res* 22: 449–457.
160. Basco Z, Nagy H, Goda K, Bene L, Fenyvesi F, Matkó J et al. 2004. Raft and cytoskeleton associations of an ABC transporter: P-glycoprotein. *Cytometry A* 61: 105–116.

161. Reungpatthanaphong P, Marbeuf-Gueye C, Le Moyec L, Salerno M, and Garnier-Suillerot A. 2004. Decrease of P-glycoprotein activity in K562/Adr cells by MbCD and filipin and lack of effect induced by cholesterol oxidase indicate that this transporter is not located in rafts. *J Bioenerg Biomembr* 36: 533–543.
162. Modok S, Heyward C, and Callaghan R. 2004. P-glycoprotein retains function when reconstituted into a sphingolipid- and cholesterol-rich environment. *J Lipid Res* 45: 1910–1918.
163. Radeva G, Perabo J, and Sharom FJ. 2005. P-Glycoprotein is localized in intermediate-density membrane microdomains distinct from classical lipid rafts and caveolar domains. *FEBS J* 272: 4924–4937.
164. Johnstone RW, Ruefli AA, and Smyth MJ. 2000. Multiple physiological functions for multidrug transporter P-glycoprotein? *Trends Biochem Sci* 25: 1–6.
165. Robinson LJ, Roberts WK, Ling TT, Lamming D, Sternberg SS, and Roepe PD. 1997. Human MDR 1 protein overexpression delays the apoptotic cascade in Chinese hamster ovary fibroblasts. *Biochemistry* 36: 11169–11178.
166. Ruefli AA, Tainton KM, Darcy PK, Smyth MJ, and Johnstone RW. 2002. P-glycoprotein inhibits caspase-8 activation but not formation of the death inducing signal complex (disc) following Fas ligation. *Cell Death Differ* 9: 1266–1272.
167. Turzanski J, Grundy M, Shang S, Russell N, and Pallis M. 2005. P-glycoprotein is implicated in the inhibition of ceramide-induced apoptosis in TF-1 acute myeloid leukemia cells by modulation of the glucosylceramide synthase pathway. *Exp Hematol* 33: 62–72.
168. Li X, Hong L, Zhao Y, Jin H, Fan R, Du R, Xia L, Luo G, and Fan D. 2007. A new apoptosis inhibitor, CIAPIN1 (cytokine-induced apoptosis inhibitor 1), mediates multidrug resistance in leukemia cells by regulating MDR-1, Bcl-2, and Bax. *Biochem Cell Biol* 85: 741–750.
169. Fantappiè O, Solazzo M, Lasagna N, Platini F, Tessitore L, and Mazzanti R. 2007. P-glycoprotein mediates celecoxib-induced apoptosis in multiple drug-resistant cell lines. *Cancer Res* 67: 4915–4923.
170. Han Z, Hong L, Han Y, Wu K, Han S, Shen H et al. 2007. Phospho Akt mediates multidrug resistance of gastric cancer cells through regulation of P-gp, Bcl-2 and Bax. *J Exp Clin Cancer Res* 26: 261–268.
171. Köck K, Grube M, Jedlitschky G, Oevermann L, Siegmund W, Ritter CA et al. 2007. Expression of adenosine triphosphate-binding cassette (ABC) drug transporters in peripheral blood cells: Relevance for physiology and pharmacotherapy. *Clin Pharmacokine* 46: 449–470.
172. Randolph GJ, Beaulieu S, Pope M, Sugawara I, Hoffman L, Steinman RM et al. 1998. A physiologic function for p-glycoprotein (MDR-1) during the migration of dendritic cells from skin via afferent lymphatic vessels. *Proc Natl Acad Sci U S A* 95: 6924–6929.
173. Richaud-Patin Y, Soto-Vega E, Jakez-Ocampo J, and Llorente L. 2004. P-glycoprotein in autoimmune diseases. *Autoimmun Rev* 3: 188–119.