J. Membrane Biol. 143, 89–102 (1995)

The Journal of
Membrane
Biology

Topical Review

The Biology of the P-Glycoproteins

C.R. Leveille-Webster, I.M. Arias
Tufts University School of Medicine, Department of Physiology, 136 Harrison Ave, Boston, Massachusetts 02111

Received: 24 March 1994/Revised: 8 June 1994

Introduction

Resistance of neoplastic cells to the cytotoxic action of multiple chemotherapeutic agents is a major obstacle in clinical cancer treatment. Some tumors are intrinsically resistant to multiple drugs, whereas other neoplasms acquire multidrug resistance (MDR) following exposure to cytotoxic agents. The molecular basis for MDR is starting to be unraveled. The discovery of p-glycoprotein, a 170 kD plasma membrane protein that functions as an ATP-driven chemotherapeutic drug efflux pump in cancer cells, was a major advance in the field of drug resistance. Biochemical investigations revealed that p-glycoprotein-mediated drug efflux could be inhibited by a number of agents. Clinical scientists were quick to initiate clinical trials combining chemotherapeutic agents with reversing agents to treat drug refractory neoplasms. Basic scientists, intrigued by the finding that p-glycoproteins are present in normal tissues, have sought to define a role for these proteins in normal cellular physiology.

The p-glycoproteins are actually a family of proteins. One group mediates MDR while the function of another group, which has no role in MDR, is unknown. Recent evidence suggests these p-glycoproteins may be involved in membrane phospholipid transport.

Progress in p-glycoprotein research has been rapid and the subject of several reviews [68, 77, 81, 82, 145, 146]. This review will focus on the biology and biochemistry of the p-glycoproteins.

Key words: Drug resistance — ATPases — Chemotherapy — Cancer — Membrane Transport — Xenobiotics

Correspondence to: C.R. Leveille-Webster

The Multidrug Resistance Phenotype is Defined in Cell Culture

The development of MDR has been extensively studied in cancer cell lines. MDR clones of these cancer cell lines developed by sequential selection in increasing concentrations of lipophilic cytotoxic drugs were resistant, not only to the selecting agent, but also to a number of structurally and functionally unrelated drugs [17, 126]. Cross resistance was seen between the *Vinca* alkaloids, anthracyclines, epipodophyllotoxins, actinomycin D and taxol (Table 1). Drug resistance was associated with decreased intracellular drug accumulation which was maintained by energy-dependent, enhanced drug efflux. The MDR phenotype in these cells could be reversed by a structurally diverse group of drugs (Table 2) including verapamil, cyclosporine, progesterone and trifluoperazine [75, 103, 184]. Concurrent administration of these chemosensitizers with chemotherapeutic drugs restored drug sensitivity in MDR cells, but had no effect on drug sensitivity in parental cell lines. Chemosensitization was associated with enhanced intracellular drug accumulation and decreased drug efflux.

MDR cell lines were found to overexpress a 170 kD plasma membrane glycoprotein [98, 143]. The level of expression of this protein, called p-glycoprotein, correlated with the degree of drug resistance and the magnitude of drug accumulation defects in MDR cells [17, 126]. Photoactive analogues of chemotherapy drugs, chemosensitizing agents and ATP were shown to bind to p-glycoprotein in MDR cell plasma membranes [18, 52, 123]. Kinetic analysis suggested that chemotherapeutic agents and MDR chemosensitizers were bound to a common site on p-glycoprotein. ATP binding was not blocked by chemotherapeutic drugs or chemosensitizers

Table 1. Chemotherapeutic agents associated with the multidrug resistance phenotype

Vinca alkaloids	Epipodophyllotoxins
Vincristine	Etoposide
Vinblastine	Teniposide
Colchicine	Taxol
Anthracyclines	Actinomycin D
Doxorubicin	
Daunorubicin	
Mitoxantrone	

Table 2. Chemosensitizing agents which reverse the multidrug resistance phenotype

Calcium channel blockers	Antibiotics
Verapamil	Cephalosporins
Diltiazem	Bafilomycin
Azidopine	Erythromycin
Dihydroperidines	Calmodulin inhibitors
Hormones and steroids	Trifluoperazine
Progesterone	Chlorpromazine
Tamoxifen	Lysosomotrophic agents
Cortisol	Monensin
Hydrophobic peptides	Chloroquine
Cyclosporine	Miscellaneous
Valinomycin	Reserpine
Gramicidin	Yohimbine
Detergents	Quinidine
Tween 80	Staurosporine
Triton X-100	Dipyridamole
Nonidet P-40	FK 506

suggesting separate ATP and drug binding sites on p-glycoprotein.

P-Glycoproteins Are Members of the ABC Transporter Superfamily

P-glycoprotein genes have been cloned from mouse [61, 83, 85], human [73, 109, 142, 147, 189], hamster [127] and rat [60, 169] tissues. P-glycoprotein is encoded by a small multigene family. There are three gene products in rodents [60, 83, 85, 127] and two in man [145, 189]. The mouse genes are termed mdr1 (or mdr1b), mdr2 and mdr3 (or mdr1a) [83, 85]. The human genes are MDR1 and MDR2 (or MDR3) [145, 189]. Transfection studies with full-length cDNAs divide the p-glycoprotein gene family into two groups. When transfected into drug-sensitive cells the Group 1 gene products, MDR1, mdr1 and mdr3, confer the MDR phenotype [61, 83, 160, 185]. Transfection of Group II gene products, MDR2 or mdr2, however, does not confer drug resistance [61, 85, 157].

The full-length cDNA sequence for p-glycoprotein

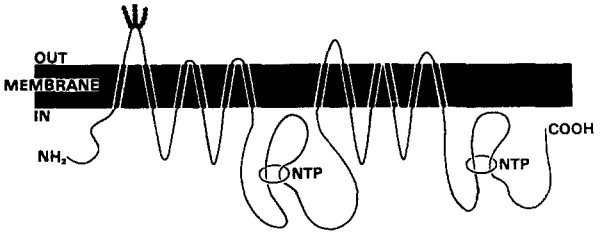

Fig. 1. Schematic diagram of p-glycoprotein structure. Reproduced, with permission, from *Advances in Pharmacology* **21,** c1990, by Academic Press.

encodes a 1280 amino acid protein with significant homology between C and N terminal halves [40, 80, 83]. Hydrophobicity plots are most consistent with a topology which includes six transmembrane domains within each half of the protein (Fig. 1). The hydrophilic cytoplasmic portion of each half contains an approximately 200 amino acid consensus sequence common to all members of the ATP binding cassette (ABC) superfamily of membrane transport proteins [90]. This consensus region contains the ATP binding motifs known as the Walker A and B domains.

The majority of ABC proteins are involved in active membrane transport [90]. Approximately 50 prokaryotic members of the ABC superfamily have been identified [71]. These bacterial cell membrane proteins are involved in the ATP-dependent transport of a variety of substrates including proteins, peptides, amino acids, sugars, inorganic anions and polysaccharides. Homology of these prokaryotic proteins with p-glycoprotein resides primarily in the region surrounding the nucleotide binding domains. Several eukaryotic ABC transporters have been identified in yeast. The STE 6 gene product in *Saccharomyces cerevisiae,* which shares homology to p-glycoprotein in both the nucleotide binding region and within the transmembrane domains [105, 117], is responsible for the secretion of "a" mating pheromone across the yeast plasma membrane. The PDR5 and SNQ2 genes in *S. cerevisiae* [108, 128] and the pmd 1 + gene in *Schizosaccharomyces pombe* [161], which share less homology with p-glycoprotein than STE 6, are associated with fungal drug resistance. In *S. pombe* a gene associated with cadmium resistance, HMT 1, is involved in ATP-dependent transport of phytochelatins, small peptides equivalent to mammalian glutathione, across the yeast vacuolar membrane [130; D. Ortiz, *personal communication*). The pfmdr1 gene in *Plasmodium falciparium* shares a high degree of homology to p-glycoprotein. The protein product of this gene has been localized to the trophozoite digestive vacuole where it may function in drug resistance [53, 54, 74, 102]. In Leishmania *sp,* several p-glycoprotein gene homologues have been identified in drug-resistant parasites [30, 49, 88, 131]. P-glycoprotein homologues have also been cloned from

Entameoba histolytica [152], *Caenorhabditis elegans* [156] *Drosophila* [195] and marine sponges [107]. The demonstration of p-glycoprotein gene homologues across phylogenic lines suggests that p-glycoprotein is an ancient protein associated with fundamental cellular functions.

Several mammalian homologues of p-glycoprotein have been identified. The TAP1 and TAP2 proteins present on the endoplasmic reticulum of T lymphocytes are involved in the ATP-dependent transport of cytosolic peptides derived from internalized antigens into the lumen of the endoplasmic reticulum [62, 165]. A hepatic peroxisomal membrane protein, PMP 70, has sequence homology to p-glycoprotein [100]. When this protein is mutated, a fatal cerebro-hepato-renal dysfunction known as Zellweger syndrome develops [76]. The cystic fibrosis transmembrane conductance regulator protein, (CFTR), which is an ATP-dependent, cAMP-regulated chloride channel, also shares homology with p-glycoprotein [144].

Although the 12 transmembrane domain model for p-glycoprotein topology is supported by antibody localization data [198], recent studies suggest an alternative topographical model. Evaluation of the murine mdr1 or human MDR1 gene products translated in a cell free system or expressed in *Xenopus* oocytes revealed a glycosylation site in the second half of p-glycoprotein, as well as the previously reported sites in the first extracellular loop [173, 202]. This second glycosylation site links transmembrane domains 8 and 9 in the traditional model of p-glycoprotein topology suggesting that they are located extracellularly. The authors propose a model for p-glycoprotein topology with 10 transmembrane domains.

The functional unit of p-glycoprotein in the plasma membrane has not been established. Recent studies have isolated oligomeric complexes of p-glycoprotein from detergent extracts of human and hamster MDR cells [135]. These oligomers were derived from the noncovalent association of p-glycoprotein monomers and were able to bind ATP.

P-Glycoproteins Are Present in Normal Tissue

Examination of mdr mRNA expression [55, 73, 157, 187] and immunomorphometric localization of p-glycoprotein [28, 157, 176, 181] revealed the presence of p-glycoprotein in normal tissues. P-glycoprotein is found on the apical surfaces of many secretory epithelial cells. The human MDR1 gene product is found on the brush borders of the intestinal epithelial cells and renal proximal tubule cells [181]. It has also been localized to cells of the adrenal cortex [181], trophoblastic cells of the placenta [176], capillary endothelial cells in the brain and testis [51] and on the surface of lymphocytes [38] and hematopoietic stem cells [39]. Small amounts of MDR1 are found on the hepatic biliary canalicular membrane and on the apical surface of the small biliary and pancreatic ductules [73, 181]. In mice, the mdr1 gene product has a distribution similar to MDR1 in the adrenal gland, kidney, pregnant uterus and placenta [10, 55].The murine mdr3 gene product is found primarily in the intestine with small amounts in the brain and on the hepatic biliary canalicular membrane [27, 55]. The murine mdr2 gene product is the predominant p-glycoprotein on the biliary canalicular membrane with small amounts found in the adrenals, heart, muscle and B lymphocytes [27, 55]. Human MDR2 mRNA has been localized to the liver and adrenals [43, 157].

P-Glycoproteins Are Membrane ATPases

P-glycoprotein ATPase activity was investigated in plasma membrane fractions and partially purified and reconstituted protein preparations from MDR cells. The initial purification of p-glycoprotein from MDR hamster cells did not preserve ATPase activity [143]. P-glycoprotein purified from MDR human leukemia cells had low ATPase activity (1.2 nmol/min/mg protein) [86, 87]. Four groups have subsequently reported p-glycoprotein ATPase activity in partially purified soluble plasma membrane fractions derived from human or hamster MDR cells. In these preparations, p-glycoprotein ATPase activity ranged from 0.37–3.4 μmol/min/mg protein [4, 9, 65, 162]. In the most highly purified (90% pure) preparation, hamster p-glycoprotein [162] had an ATPase activity of 0.321 μmol/min/mg protein. P-glycoprotein ATPase activity has also been associated with plasma membranes from Sf9 cells transfected with human MDR1 cDNA, (0.03 μmol/min/mg protein) [153] and with a beta-galactosidase p-glycoprotein fusion protein expressed in and purified from fibroblasts (0.18 μmol/min/mg protein) [166]. In all preparations, p-glycoprotein ATPase activity was magnesium dependent and substitution with calcium supported little, if any, activity. ATP was the preferred substrate although GTP and ITP were hydrolyzed to some extent. The K_m for ATP ranged from 0.5 to 1.4 mM. Inhibitors of other known membrane ATPases, such as sodium azide, oligomycin, ouabain and EGTA, had no effect on p-glycoprotein enzyme activity. Vanadate, which acts as a transitional state analogue for the phosphate release from the P-type ATPases, inhibited p-glycoprotein ATPase activity, but at concentrations [1–12 μM) greater than those required for inhibition of the P-type ATPases (50–500 nM). The mechanism of vanadate inhibition of p-glycoprotein is unknown. No phosphorylated intermediate has been demonstrated for p-glycoprotein and the aspartyl-phosphate site conserved in the P-type ATPases is not present in p-glycoprotein. A different site for covalent enzyme phosphate complex formation may be present.

Sulfhydryl reagents, such as N-ethylmaleimide and $HgCl_2$, inhibited p-glycoprotein ATPase activity [4, 65, 162, 166] implying the presence of an important cysteine residue in the p-glycoprotein catalytic and/or nucleotide binding domains. N-ethylmaleimide inhibition of p-glycoprotein ATPase activity in hamster MDR plasma membranes was protected by ATP and was not reversible with dithiothreitol [5]. The important sulfhydryls were shown to be localized in both the C and N terminal halves of the molecule.

Substrate-stimulated P-glycoprotein ATPase activity was demonstrated in plasma membrane fractions prepared from transfected Sf9 insect cells [153] or MDR hamster cells [4]. In partially solubilized membrane fractions from MDR human or hamster cells, drug-stimulated activity could not be demonstrated until the preparations were reconstituted into proteoliposomes [9, 162, 164]. Apparently, p-glycoprotein requires stabilization of its transmembrane domains in a lipid environment for optimum ATPase activity [66]. ATPase activity of partially purified reconstituted human p-glycoprotein was stimulated three- to fourfold by doxorubicin, daunorubicin, vinblastine, actinomycin D and verapamil [9]. None of these drugs changed the affinity of the ATPase for ATP. Drug-stimulated ATPase activity was inhibited by vanadate. In proteoliposomes containing partially purified hamster p-glycoprotein, ATPase activity was stimulated 50% by verapamil, trifluoperazine, progesterone and colchicine, whereas daunorubicin and vinblastine paradoxically inhibited ATPase activity [164]. ATPase activity in a highly purified hamster p-glycoprotein preparation was stimulated tenfold by verapamil and fourfold by vinblastine with lesser stimulation by other MDR active agents [162]. In this preparation, verapamil decreased the apparent affinity of ATP for the transporter as evidenced by an increase in the K_m for ATP from 0.5 to 2.76 mM. Differences in the degree of basal and drug-stimulated ATPase activity in these preparations could reflect differences in the lipid composition of the proteoliposomes used for reconstitution, effects of detergent solubilization, contamination with other membrane ATPases or differences in ATPase activity between human and hamster p-glycoprotein.

Group I P-Glycoproteins Mediate Drug Transport

Several groups have attempted to characterize p-glycoprotein-mediated drug transport in intact cells, plasma membrane vesicles or reconstituted proteoliposomes. A note of caution is advised in evaluating p-glycoprotein transport studies since a number of factors can complicate the generation of accurate kinetic data in these experiments. Since p-glycoprotein substrates are hydrophobic, they tend to partition into or bind to internal and external membranes. P-glycoprotein transport of hydrophobic substrates must be separated from membrane binding by demonstrating temperature dependence, inhibition by detergent permeation and/or sensitivity to osmotic collapse of the vesicular space. ATP-dependent transport of hydrophobic substrates must be differentiated from the diffusion of these compounds through membranes. Kinetic data must be calculated using initial rates of drug uptake and not steady-state levels of accumulation. At high concentrations, hydrophobic substrates tend to self-associate and complicate the generation of saturation curves necessary to make these calculations [58].

Plasma membrane vesicles from MDR human [95] and hamster [64] cells have been used to demonstrate ATP-dependent transport of chemotherapeutic drugs. In vesicles from human MDR cells, accumulation of vinblastine was osmotically sensitive and temperature and ATP dependent [95]. The K_m for ATP was 38 μM. Nonhydrolyzable ATP analogues did not support transport. Vanadate, verapamil and MDR active chemotherapeutic agents inhibited vinblastine accumulation. Colchicine and vinblastine accumulation in hamster MDR plasma membrane vesicles was saturable, osmotically sensitive, ATP dependent and disrupted by membrane detergent permeation [64]. Transport was inhibited by MDR active agents, vanadate and N-ethylmaleimide. Drug transport has been demonstrated in partially purified hamster p-glycoprotein reconstituted into proteoliposomes [164]. Uptake of colchicine into proteoliposomes was ATP and temperature dependent, osmotically sensitive and saturable. Transport was inhibited by daunorubicin, vinblastine and verapamil.

Apical membrane vesicles from normal rat tissues show p-glycoprotein-mediated drug transport. ATP-dependent daunorubicin and vinblastine transport was reported in inside-out rat biliary canalicular membranes vesicles (CMV) [101, 171]. Transport was osmotically sensitive and saturable with a K_m of 49 μM for daunorubicin and 26 μM for vinblastine. The K_m for ATP was 80 μM. GTP stimulated daunorubicin transport slightly, but nonhydrolyzable ATP analogues did not. Transport was sensitive to vanadate, but was unaffected by ouabain, sodium azide and N-ethylmaleimide. Other p-glycoprotein substrates inhibited daunorubicin transport. Rat intestinal brush border membrane vesicles also had ATP-dependent p-glycoprotein-mediated daunorubicin transport [167].

The kinetics of daunorubicin transport has been studied in intact MDR cells [175]. Saturable p-glycoprotein-mediated accumulation of daunorubicin with a K_m of approximately 1.5 μM was demonstrated. In these studies, substrate self-aggregation was monitored by fluorescent microscopy and values for the passive permeation of daunorubicin were determined so that diffusion, as well as p-glycoprotein-mediated pump activity, could be considered. In cell lines with low levels of p-glycoprotein,

the passive efflux rate of daunorubicin made a significant contribution to the total daunorubicin efflux rate. Only in cell lines with high levels of expression of p-glycoprotein did the contribution from passive efflux become negligible. Maximal transport velocities for daunorubicin correlated with the amount of p-glycoprotein in the cell lines.

P-glycoprotein-mediated drug transport has been studied in cell lines which form polarized epithelial surfaces when grown on porous supports. Net basal to apical transepithelial transport of vinblastine was demonstrated in the Madin Darby canine kidney cell line and in the human intestinal adenocarcinoma cell lines, CaCo-2, T_{84} and HCT-8 [94, 97]. P-glycoprotein was immunolocalized to the apical surface of these cells. Transepithelial flux of vinblastine in these cells was inhibited by other MDR active agents and by monoclonal antibodies to p-glycoprotein.

The murine mdr genes have been expressed in secretory vesicle membranes in *S. cerevisiae* sec 6-4 mutants [148]. In these mutants, a temperature-sensitive defect in the final step of the vesicular secretory pathway allows the accumulation of large amounts of secretory vesicles containing newly synthesized plasma membrane proteins when the yeast are grown at the permissive temperature. Yeast sec 6-4 mutants transfected with either the murine mdr1 or mdr3 gene exhibited verapamil-sensitive vinblastine and colchicine accumulation [148].

Is There an Endogenous Substrate for Group I P-Glycoproteins?

Group I p-glycoproteins can function as ATP-dependent drug transport pumps in cancer cell lines and in some normal tissues. Although it is unlikely that chemotherapy drug transport is the primary function for p-glycoprotein in normal tissue, the search for an endogenous substrate for the protein has been elusive. Its polarized distribution on secretory epithelia and role in drug efflux suggest that p-glycoprotein may function in excretion of naturally occurring toxins or commonly encountered xenobiotics.

Recently, mdr3 knockout mice were shown to have an increased sensitivity to the toxic effects of vinblastine [158]. When the same dose of vinblastine was given to normal mice and mdr3 knockout mice, the knockout mice had higher vinblastine concentrations in many tissues at several time points after drug administration. The knockout mice also had a striking sensitivity to the neurotoxic effects of the hydrophobic antiparasitic compound, ivermectin. Large increases in the brain concentration of this drug were documented in the knockout mice most likely due to lack of p-glycoprotein-mediated drug transport across the cerebral endothelial cells of the blood-brain barrier.

Known substrates for p-glycoprotein represent a structurally and functionally diverse group of compounds, many of which are natural products of fungal, bacterial or plant origin [75]. The structures of some typical MDR substrates are shown in Fig. 2. Three-dimensional analysis of over 120 compounds known to interact with p-glycoprotein suggests that a p-glycoprotein substrate is a cationic, hydrophobic molecule with at least two planar rings and a molecular weight of 400–1500 [12, 132]. A number of naturally occurring compounds that share these characteristics have been tested in rat CMV for their ability to inhibit daunorubicin transport. To date, neither sterols, reduced or oxidized glutathione, epinephrine, various amines, sphingosine or prostaglandins E1, B1 and E2 have proven to be substrates [12].

Several observations suggest an interaction between progesterone and p-glycoprotein. Progesterone photoaffinity labels p-glycoprotein, inhibits the binding of chemotherapy drugs to p-glycoprotein and reverses the MDR phenotype in MDR cell lines [136]. Progesterone is not a substrate for the transporter, since steady-state levels of progesterone accumulation were the same in resistant and sensitive cells and no enhanced efflux of progesterone was demonstrated in MDR cell lines [197]. Levels of the mdr1 gene product increase dramatically on the luminal surface of the secretory epithelium of the mouse uterus under the influence of progesterone [10, 11]. Progesterone has also been shown to regulate the activity of the murine mdr1 promoter [133].

Since high levels of p-glycoprotein are found in the adrenal cortex where extensive steroidogenesis occurs, the role of glucocorticoids as endogenous substrates for p-glycoprotein has been explored. MDR cell lines show reduced accumulation and increased ATP-dependent efflux of cortisol [190]. Exposure to dexamethasone increased mdr3 and mdr1 mRNA expression in murine hepatoma cells [203]. In mouse Y1 adrenocortical carcinoma cells, increased steroidogenesis induced by adrenocortical hormone was accompanied by increased p-glycoprotein mRNA levels [3]. P-glycoprotein reversing agents disrupted steroid secretion in Y1 cells [44]. A null mutation in one allele of the murine mdr1 gene in Y1 cells inhibited steroid secretion [3].

When transfected with a human adrenal MDR1 cDNA, a porcine renal proximal tubule cell line, LLC-PK1, which forms a polarized epithelial surface when grown on a porous support, had verapamil-sensitive transepithelial flux of vinblastine, cortisol, aldosterone and dexamethasone, but not progesterone [186]. In these cells, cortisol and dexamethasone did not inhibit azidopine photoaffinity labeling of p-glycoprotein whereas progesterone did. Slight differences in the structure among these steroids may determine whether a particular compound is transported by p-glycoprotein or simply competes for drug binding.

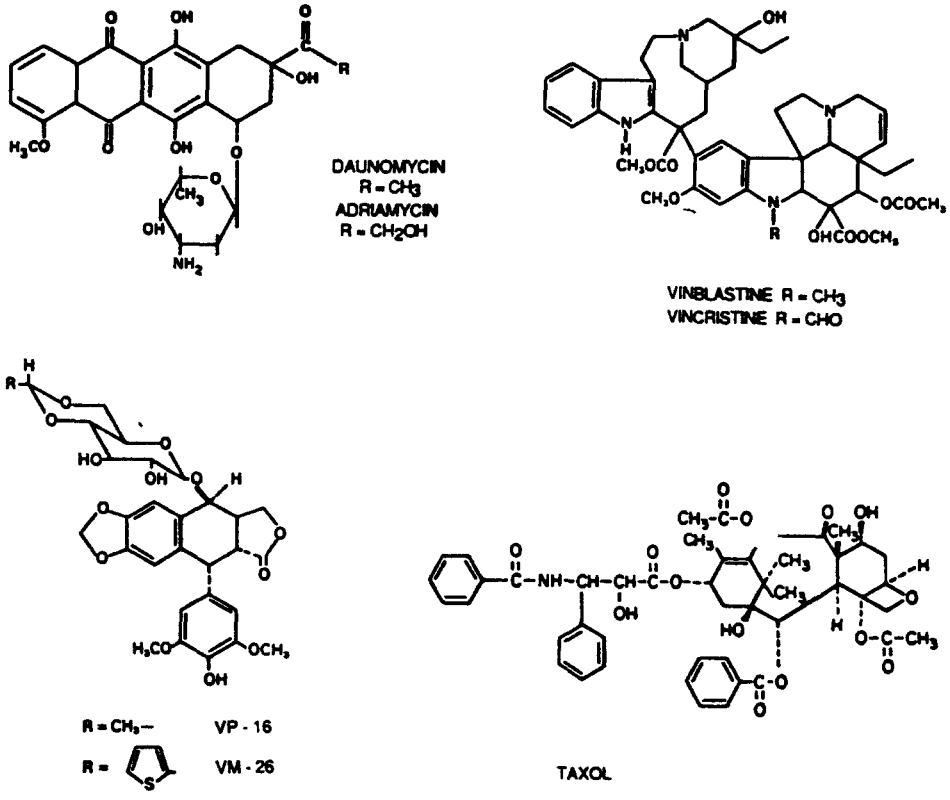

Fig. 2. Structure of some representative p-glycoprotein substrates. Reproduced, with permission, from the *Annual Review of Medicine* **42,** c1991, by Annual Review.

P-glycoprotein is present on the capillary endothelium of the brain [51] where it may play a role in maintaining the blood-brain barrier. Lipophilic chemotherapy agents, such as those involved in the MDR phenotype, cannot enter the brain. The mdr3 gene product is the predominant p-glycoprotein expressed in the murine brain [158]. When mdr3 knockout mice and normal mice are given comparable doses of vinblastine, the knockout mice have profound increases in the brain concentration of this cytotoxic agent when compared to the brain concentration in normal mice [158]. Mouse brain capillary endothelial cell lines that stably expressed p-glycoprotein were established to study the role of p-glycoprotein in the blood brain barrier [177]. When grown on a porous filter, these cells demonstrated polarized p-glycoprotein expression and had verapamil-sensitive, unidirectional transepithelial transport of vinblastine. No transepithelial flux of fluorouracil, a non-MDR chemotherapy agent that is also excluded from the brain, could be demonstrated.

MDR hamster cells manifest verapamil- and vinblastine-sensitive, energy-dependent accumulation of morphine and binding of morphine to plasma membrane fractions [29]. Synthetic opiates including meperidine, pentazocine and methadone, increased vinblastine accumulation in MDR cells and inhibited photoaffinity labeling of plasma membranes with a doxorubicin analogue.

Homology of p-glycoprotein with several eukaryotic ABC transporters suggests a role for p-glycoprotein in peptide transport. P-glycoprotein is highly homologous to the STE6 gene product in yeast which is responsible for the secretion of "a" mating factor, a hydrophobic dodecapeptide [105]. Expression of a cDNA for mouse mdr 1 [139] or human MDR1 [82] in yeast strains that lack the STE 6 gene product restored the ability to secrete the pheromone. P-glycoprotein shares a high degree of homology with the hyl B gene product in *Escherichia coli* that is responsible for the ATP-dependent secretion of the protein, hemolysin [71]. In *S. pombe,* a p-glycoprotein homologue transports phytochelatins, small gamma-glutamyl-cysteine-glycine tripeptides (D. Ortiz, *personal communication*). The TAP1 and TAP2 gene products, which transport cytosolic peptides into the endoplasmic reticulum, share homology with p-glycoprotein [62, 165]. P-glycoprotein expression and multidrug resistance were induced in tissue culture by exposure of hamster cells to the cytotoxic synthetic hydrophobic tripeptide, N-acetyl-leucyl-leucyl norleucine [163]. Cyclosporines, cyclic hydrophobic peptides, bind to and may be substrates for p-glycoprotein [150, 184]. Hamster MDR cells display verapamil-sensitive resistance to the toxic hydrophobic peptides, gramicidin D and valinomycin [113, 124, 149]. Since the liver normally functions to extract a variety of small peptides from the circulation, a number of synthetic and naturally occurring hydrophobic peptides, including insulin, cholecystokinin, neurokinin A and RNase-S-peptide, were tested for their ability to inhibit ATP-dependent

daunorubicin transport in rat CMV. Inhibition was not observed at peptide concentrations as high as 200 mM [12].

Group I P-Glycoproteins Interact with a Diverse Group of Lipophilic Compounds

How does p-glycoprotein interact with such a large variety of substrates? Substrate specificity has been addressed by examining the effect of sequence differences among cloned p-glycoprotein genes on drug resistance phenotypes and drug binding. Although the murine mdr3 and mdr1 gene are highly homologous (92% identity), when transfected into cells they resulted in different MDR phenotypes [61]. Cell clones transfected with the mdr1 gene were less resistant to doxorubicin, colchicine and mitoxantrone than were those transfected with the mdr3 gene. Cell clones with mdr3 were more resistant to actinomycin D than were mdr1 clones. Both the mdr1 and mdr3 genes are homologous to the mdr2 gene (74% identity), yet the mdr2 gene product is not associated with the MDR phenotype [85]. Examination of a series of chimeras, constructed throughout the whole length of mdr1 with segments of mdr2, revealed that only the highly homologous ATP binding sites and the highly divergent linker regions were interchangeable enough to produce a functional molecule [28]. Similar results have been demonstrated with human MDR1 and MDR2 [82]. Another MDR1/MDR2 chimera, which had an 89 amino acid region of MDR2 including the first cytoplasmic loop and parts of the third and fourth TM domains substituted into MDR1, did not confer MDR, but when four amino acids in the cytoplasmic loop were changed back to those found in MDR1, biological function was restored [56]. This suggests that the first cytoplasmic loop is important for p-glycoprotein function.

Investigation of several point mutations or deletions in p-glycoprotein suggests that the predicted transmembrane domains play an important role in determining substrate binding and transport activity. A naturally occurring mutation of glycine to valine at position 185 in transmembrane domain 3 of human MDR1 changed the specificity of the transporter so that colchicine and etoposide resistance were enhanced while resistance to vinblastine, vincristine and actinomycin D was decreased [48, 104, 150]. Despite this resistance pattern, the mutant protein showed increased binding of vinblastine and decreased binding of colchicine. This discrepancy between the observed phenotype and drug binding suggested that the glycine to valine substitution did not affect initial drug binding, but instead altered the efficiency of dissociation of drugs from p-glycoprotein. Mutation of glycine 338 to alanine and alanine 339 to proline in transmembrane domain 6 in hamster p-glycoprotein decreased resistance to several drugs while maintaining normal resistance to actinomycin D [63]. When serine 941 in transmembrane domain 11 of murine mdr1 was mutated to phenylalanine, cell clones transfected with the mutated gene acquired resistance to vinblastine while colchicine and doxorubicin resistance was decreased [84].

Progress has been made in identifying the drug binding site on p-glycoprotein by examining proteolytic fragments of azidopine or azidoprazosin photoaffinity-labeled human MDR1 and murine mdr1 [24, 82]. Both the amino terminal and carboxy terminal halves were labeled. Photoaffinity binding has been localized to two regions: (1) transmembrane domains 5 and 6 or the cytoplasmic domain immediately following TM6 and (2) a region within the transmembrane domain 12 or the cytoplasmic region immediately following TM 12 [82]. This labeling pattern is consistent with the presence of two different drug binding sites or the presence of one binding site which is formed by two homologous halves of the molecule.

Point mutations in either of the two conserved glycine and lysine residues residing within the A fold of the Walker consensus sequence in the mdr1 gene resulted in loss of biological function despite persistent binding of azido ATP [13]. This result agreed with vesicle studies in which ATP hydrolysis, and not simply binding, was necessary for p-glycoprotein function. Amino terminal human MDR1 b-galactosidase fusion proteins expressed in fibroblasts were capable of ATP hydrolysis [166]. Expression of cDNAs encoding the amino or carboxy terminal half molecules of human MDR1 in Sf9 cells revealed that each half molecule had basal ATPase activity [114]. Drug-stimulated ATPase activity, however, was not present until both half molecules were expressed together. In separate studies, deletions of either the carboxy- or amino terminal half of p-glycoprotein resulted in loss of transport [57]. These results suggest that coupling of ATPase activity to drug binding and transport requires cooperative interaction between the two halves of p-glycoprotein.

Post-Translational Modification May Regulate P-Glycoprotein

P-glycoprotein is post-translationally modified by phosphorylation and glycosylation. Glycosylation does not appear to be necessary for protein function although it may play a role in the trafficking or stability of the protein [16, 110, 156].

Phosphorylation may have a role in regulating p-glycoprotein function. P-glycoprotein is phosphorylated by protein kinase C (PKC) [33, 34], protein kinase P [172] and protein kinase A(PKA) [118]. PKC inhibitors reduced the phosphorylation of p-glycoprotein and reversed the drug accumulation defect in MDR cells [32,

72, 115]. Cotransfection of a PKC-deficient breast carcinoma cell line with cDNAs for p-glycoprotein and PKC resulted in increased resistance to vinblastine and doxorubicin and increased phosphorylation of p-glycoprotein when compared to cells transfected with p-glycoprotein cDNA alone [199]. Basal levels of cAMP-dependent PKA were necessary to maintain p-glycoprotein mRNA levels in MDR cells [1, 44]. In vivo, p-glycoprotein was rapidly phosphorylated and dephosphorylated in MDR cells [115]. PKC phosphorylation sites in the human MDR1 gene product were mapped to multiple serine residues clustered in the linker region of the molecule [35]. Analysis of phosphorylation in the murine mdr1 gene product mapped phosphorylation sites to the linker region, specifically on serine 669 for PKC and serine 681 for PKA [129]. The authors postulate that the mdr linker region is structurally equivalent to the R domain of the CFTR protein. Phosphorylation of the R domain by PKA regulates chloride permeability by CFTR. Although much smaller than the R domain (55 *vs.* 241 amino acids), the linker region in p-glycoprotein, like the R domain in CFTR, is encoded by a single exon, has several highly charged alternating basic and acidic amino acids and contains several PKA and PKC phosphorylation consensus sites [129].

Group I P-Glycoproteins Mediate Clinical Tumor Drug Resistance

There is strong evidence implicating p-glycoprotein as a clinically important mediator of multidrug resistance in human tumors. P-glycoprotein is frequently present in tumors derived from tissues normally expressing p-glycoprotein including hepatic, renal, intestinal and adrenal tumors [42, 73, 79, 96]. In general, these tumors tend to be intrinsically resistant to chemotherapy. Cancer cells derived from tissues that do not normally express p-glycoprotein acquire p-glycoprotein either as a result of malignant transformation [80] or after exposure to chemotherapeutic agents [31, 41, 59, 121]. In breast carcinoma [192], neuroblastoma [36], rhabdomyosarcoma [37], esophageal carcinoma [140], multiple myeloma [111], ovarian carcinoma [92], small cell lung cancer [92] and leukemia and lymphoma [134, 151, 154] the presence of p-glycoprotein in biopsy samples correlates wtih a poor response to chemotherapy.

Therapeutic strategies to circumvent p-glycoprotein action may influence the success of chemotherapeutic drug protocols. A large group of chemosensitizers interact with p-glycoprotein to reverse drug resistance in MDR cell lines [75, 103, 184]. This structurally diverse group of compounds (Table 2) includes calcium channel blockers, calmodulin antagonists, steroids and hormones, hydrophobic peptides, lysosomotropic agents and nonionic detergents. Some reversing agents including verapamil [200] cyclosporine [149, 184] and progesterone [136, 197] appear to be competitive inhibitors that interact with p-glycoprotein at a common drug binding site. A note of caution, however, since kinetic analysis of these interactions is complicated by the hydrophobic nature of these compounds. Verapamil and cyclosporine may also be substrates for the transporter [149, 200]. Other agents, such as bile acids [116], are noncompetitive inhibitors interacting with p-glycoprotein at a site other than the drug binding site.

The growth of murine tumor xenografts derived from MDR cells can be inhibited by the administration of chemosensitizers along with chemotherapeutic agents [75, 77, 103]. Human clinical trials using p-glycoprotein chemosensitizers have been conducted [19, 168]. Some drug-refractory leukemias and myelomas responded to chemotherapy regimes when either verapamil or cyclosporine was added to the protocol [59, 112, 121]. Clinical trials with solid tumors, such as ovarian and colonic carcinoma, have met with limited success [193, 196]. Most of these clinical trials have been complicated by systemic toxicity. The maintenance of serum verapamil concentrations in the range associated with MDR reversal in vitro predictably led to dose-limiting cardiotoxicity [59, 121]. Patients given cyclosporine as a chemosensitizing agent developed hyperbilirubinemia [192, 196], consistent with this peptide's ability to inhibit ATP-dependent bile acid and nonbile acid organic anion transport in hepatocytes [99]. Clinical trials with trifluoperazine were limited by extrapyramidal side effects [120]. Progesterone, quinidine and tamoxifen have also been evaluated as chemosensitizing agents in clinical trials [19, 50, 168].

Expression of P-Glycoproteins Is Modulated by Environmental Stimuli

The regulation of p-glycoprotein gene expression in normal and neoplastic tissues is an area of active investigation. The liver serves as a good model system to study p-glycoprotein regulation since normal hepatic p-glycoprotein levels can be influenced by a number of environmental stimuli and the liver normally expresses both the Group I and II p-glycoprotein gene products which enables concurrent investigation of the effect of various factors on the expression of both gene products.

The induction of p-glycoprotein expression has been studied in hepatocarcinogenesis. Increased p-glycoprotein expression has been detected in clinical biopsy samples from human hepatocellular carcinoma (HHC) [42, 96]. In mice, overexpression of mdr3 occurred in four models of HCC [106, 178], chemical carcinogenesis induced by dimethylhydrazine and diethylnitrosamine in C57BL/6N mice, spontaneous carcinogenesis in C3H/HeN mice and in tumors arising in transgenic mice carrying either the human hepatitis B virus large envelope polypeptide or the SV40 large T antigen. The mdr3 gene

was activated in the latter stages of carcinogenesis in these models. Increased mdr1 mRNA levels were occasionally seen, but mdr2 mRNA levels did not change. In experimental models of HCC in rats, increased p-glycoprotein mRNA levels were detected in neoplastic tissue [23, 69, 182]. P-glycoprotein expression appeared to be associated with tumor progression rather than with tumor initiation in these rodent models. Several experimental observations support such a hypothesis. The human MDR1 gene promoter is a target for the Ras oncogene and the p53 tumor suppressor gene [47], both of which are associated with tumor progression. P-glycoprotein is usually heterogeneously expressed in clinical tumor samples, but was found in high concentrations in the invasive edges and distant metastases in colonic carcinoma samples [194].

Rat liver cells transfected with v-H-Ras or v-Raf have increased p-glycoprotein expression and increased resistance to doxorubicin and vinblastine [25]. These results suggest that cellular transformation is sufficient to induce increased p-glycoprotein and that selective pressure from cytotoxic drugs is not necessary.

Hepatocyte p-glycoprotein expression can be influenced by other environmental factors. Hepatic regeneration induced by partial hepatectomy in rats was accompanied by increased expression of hepatic mdr1a and mdr1b RNA [125, 182]. These increases were noted within 24 hr and correlated with the previously described pattern of DNA replication seen in regenerating liver. In contrast, mdr2 RNA levels were increased at 48 hr post hepatectomy after the major wave of cell proliferation had already occurred [125]. These results suggest differential regulation of the two gene products. In mice, mdr1a and mdr2 mRNA levels were also increased in regenerating liver [179]. Cholestasis in rats, induced by bile duct ligation or exposure to alpha-naphthylisothiocyanate, increased mdr1a and mdr1b mRNA levels [159]. In monkeys, alpha-naphtylisothiocyanate cholestasis induced both MDR1 and MDR2 mRNA expression in the liver [159]. Rodents fed various cytotoxic xenobiotic agents, including aflatoxin, acetyl-aminofluorene, dioxin, phenothiazide and isosafrole, have increased mdr1a and mdr1b RNA expression [26]. Acetyl-aminofluorene induction of mdr1a and mdr1b in rats was associated with a verapamil-sensitive increase in biliary excretion of vinblastine [159]. In rat primary hepatocyte cultures, increased p-glycoprotein expression occurred upon exposure of cells to cytotoxic agents [45]. Induction of hepatic p-glycoprotein expression secondary to a variety of environmental insults may represent part of a coordinated hepatic cytoprotective response which has already been shown to include induction of metallothioneins, cytochrome p450 enzymes and phase II drug conjugating enzymes such as glutathione-S-transferases and UDP glucuronyl transferases.

P-glycoprotein gene activation has been correlated with cellular differentiation. Exposure of several human colonic carcinoma cell lines to the differentiating agents, sodium butyrate and dimethylsulfoxide, increased MDR1 mRNA levels [119]. These agents, however, did not alter p-glycoprotein gene expression in primary rat hepatocyte cultures [70]. Increased p-glycoprotein mRNA levels occurred in human colonic carcinoma cells exposed to verapamil and cyclosporine [89]. Concomitant with verapamil treatment were electron microscopic and biochemical changes consistent with increased differentiation. In human renal, colonic and gastric carcinomas, MDR1 expression has been histologically correlated with the degree of tumor differentiation [14, 15, 122].

Two heat-shock consensus elements have been identified in the major MDR1 promoter [47]. Exposure to heat shock or heavy metals increased MDR1 gene expression in human kidney cell lines, but not in liver, adrenal, cervical or colonic cell lines [46].

Little is known about the developmental regulation or expression of p-glycoprotein. MDR1 has been identified in human fetal tissues [191]. Recently, p-glycoprotein was detected in mouse oocytes and early cleavage embryos [67]. In embryo cultures, p-glycoprotein expression was associated with a verapamil reversible increase in daunorubicin accumulation. Treatment of pregnant female mice with verapamil increased the toxicity of doxorubicin toward the developing zygote. These results suggest p-glycoprotein may play a protective role in early embryogenesis.

Group II P-Glycoproteins May Function in Phospholipid Transport

The mdr2 gene product is not capable of chemotherapeutic drug binding or transport [27,85], although its nuclear binding domain is capable of ATP hydrolysis [28]. In mice, this gene product is primarily localized on the hepatic biliary canalicular membrane [27]. Homozygous disruption of the mdr2 gene in mice resulted in the absence of phospholipid in bile and the development of liver lesions [174]. By 6–12 weeks of age, mdr2 knockout mice had scattered hepatocyte necrosis, proliferation of bile ducts and mild portal inflammation. Bile analysis showed an absence of phospholipid. Heterozygous mice had bile phospholipid values 50% of those of normal mice and developed no hepatic pathology. Biliary phospholipid is normally incorporated into mixed micelles with bile acids, the major component of bile. It was hypothesized that in the absence of phospholipid solubilization, bile acids, by virtue of their detergent action, damage the biliary canalicular membrane and the apical membranes of the biliary ductules, giving rise to the pathology seen in the mdr2 knockout mice [174].

These studies suggest that the mdr2 gene product may be involved in phospholipid transfer from hepato-

cytes to the bile. The protein could function by facilitating the entry or exit of phospholipids from the canalicular membrane. Alternatively, it might be involved in the movement of phospholipids across the canalicular membrane. The latter function could be accomplished if the mdr2 gene product functioned as a flippase, translocating phospholipids from the inner to the outer leaflet of the canalicular membrane where they can then gain access to bile. The functional activity of a phosphatidylcholine flippase has been described in rat endoplasmic reticulum [21] and in CMV [20]. The relationship of the latter translocator to the mdr2 gene product has not been investigated.

A Number of Phenomena Associated with P-Glycoprotein Suggest Different Models of Action

Higgins and Gottesman hypothesized that the multidrug transporter might actually function as a "hydrophobic vacuum cleaner" which removes drugs from the plasma membrane before they reach the cytoplasm [82, 91]. This model of transport, they claim, would explain some puzzling aspects of the MDR phenotype. First, the broad substrate specificity of the transporter might be accounted for. In their model, the primary determinant of substrate specificity is the ability of a drug to interact with the lipid bilayer and secondarily to interact with the binding site of the transporter. This fits with the finding that all p-glycoprotein substrates are lipophilic compounds and that the major determinant of the ability of a given substance to be transported by p-glycoprotein is its relative hydrophobicity [201]. Second, the discrepancies in the kinetics of p-glycoprotein transport might be explained. These kinetic aberrations include an inability to correlate initial rate of drug efflux with p-glycoprotein concentration in MDR cells, differences between the level of drug resistance and the magnitude of drug accumulation defects seen in MDR cells and differences between the affinity constants determined in vesicle systems with the actual concentrations of cytotoxic agents that the transporter is able to protect cells from. In the Gottesman and Higgins model, drugs first partition into the lipid bilayer and then interact with p-glycoprotein. The actual drug concentration seen by the transporter would not correspond to the concentration of drug used in the experiment, but would depend on the ability of the drug to partition into the lipid bilayer, as well as on the lipid composition of the membrane. Changes in membrane fluidity have been shown to alter drug transport in rat CMV [171].

Several observations support the Gottesman and Higgins model. Doxorubicin photoactivates a highly lipophilic iodinated naphthalene azide membrane probe in MDR cells, but not in their sensitive counterparts [138]. In MDR cells, photosensitization was associated with exclusive localization of doxorubicin to the plasma membrane. MDR1-transfected fibroblasts extrude the hydrophobic acetoxy methyl ester derivatives of several fluorescent dyes [93]. The free acid forms of these dyes, rapidly formed by the action of cytoplasmic esterases, are not extruded by the transporter. The free acid forms fail to accumulate in MDR cells, suggesting that the intact dye may interact with p-glycoprotein before it ever enters the cytoplasm [93].

Chloride channel activity was demonstrated in MDR1-transfected cells [6, 7, 188]. Another ABC protein, CFTR, has already been shown to be a chloride channel. MDR1-associated chloride currents exhibited rectification and were activated by changes in cell volume [188], whereas CFTR is a linear, small conductance channel activated by PKA-dependent phosphorylation in response to elevated cAMP levels [144]. In situ hybridization experiments showed complementary patterns of MDR1 and CFTR gene expression in epithelial tissues [183] suggesting that the two genes may be coordinately regulated. Epithelial cells that expressed CFTR lacked MDR1 and vice versa. In some tissues, different cell subpopulations preferentially expressed one of the gene products. For example, intestinal crypt cells expressed CFTR, whereas the apical villus cells expressed MDR1.

The electrophysiological properties of MDR1-associated chloride channel activity are similar to the volume-regulated chloride channels present in many epithelial cells, suggesting that MDR1 chloride channel activity may have a physiologic role in cell volume control. Recent studies in MDR1-transfected human breast cancer cells, which have p-glycoprotein-like chloride channel activity, failed to show that these channels were involved in volume regulation in these cells [6].

Chloride channel activity and multidrug transport appeared to be separate functions of p-glycoprotein. Agents which inhibited chloride channel activity in MDR1-transfected fibroblasts had no effect on multidrug transport [78]. in fibroblasts transfected with a MDR1 gene containing mutations in the ATP binding site, drug resistance was abolished, but chloride channel activity was maintained [78]. Hyposmotic solutions activated chloride channels in MDR1-transfected human breast cancer cells, but did not disturb p-glycoprotein-mediated efflux of the fluorescent dye, rhodamine [7]. Recent studies have cast doubt on p-glycoprotein's role as a chloride channel [137]. These experiments failed to show a quantitative correlation between p-glycoprotein expression and the activity of volume-sensitive chloride channel activity in four epithelial cell lines.

ATP channel activity has been ascribed to p-glycoprotein [2]. Channel activity was spontaneous and not associated with changes in cell volume. The authors suggest that the driving force for drug extrusion via p-glycoprotein might be an electrochemical gradient of ATP rather than ATP hydrolysis.

Many MDR cell lines have elevated intracellular pH [22, 170, 180]. In a series of myeloma cell lines which were sequentially selected for different levels of MDR1 expression, a linear relationship between intracellular pH and the initial rate of chemotherapeutic drug efflux was demonstrated [141]. In contrast, the initial rate of drug efflux was not associated with the level of p-glycoprotein expression. The authors postulate that p-glycoprotein is involved in intracellular alkalinization and is only indirectly involved in drug transport possibly by mediating a pH-dependent alteration in either membrane drug partitioning in the membrane or intracellular/extracellular distribution of cationic drugs.

P-glycoprotein's role in altering intracellular pH in MDR cell lines has been challenged. Some studies have failed to document consistent changes in intracellular pH in MDR cells [8]. In hamster and human MDR cell lines, experimental manipulation of the intracellular pH failed to effect p-glycoprotein-mediated drug efflux [8]. In yeast secretory vesicles expressing mdr3, vinblastine accumulation was independent of proton movements and unaffected by the presence or absence of a steep proton gradient [148].

Conclusions

The initial discovery of p-glycoprotein in the plasma membrane of MDR cancer cell lines was followed quickly by the cloning of its gene. Sequence analysis of cloned cDNAs revealed that p-glycoprotein was a member of the ABC family of membrane transporters. Subsequent biochemical characterization demonstrated the binding of chemotherapeutic drugs and ATP to p-glycoprotein. P-glycoprotein-mediated drug transport and drug-stimulated ATPase activity were documented in plasma membrane vesicles and in proteoliposomes containing the partially purified protein. P-glycoprotein was shown to be phosphorylated and the effect of this modification on the protein's biological function was explored. P-glycoproteins were found in many normal tissues and their overexpression was documented in numerous cancers. An important role for p-glycoprotein in intrinsic and acquired drug resistance in clinical oncology was established. Despite all that has been learned about p-glycoprotein over the last few years, additional studies will be necessary to address the many questions that have been left unanswered. Determination of p-glycoprotein structure and membrane topology should help elucidate the nature of chemotherapeutic drug binding sites and the mechanism whereby drug movement is coupled to ATP hydrolysis. Complete purification and functional reconstitution of p-glycoprotein into defined lipid vesicles will permit further characterization of drug transport and ATPase activity and give us the means by which p-glycoprotein's apparent dual function as a transporter and a channel can be clarified. Structural and functional studies on p-glycoprotein will also provide information needed to develop specified inhibitors that can be used clinically to overcome MDR in cancer patients. Further study of the mechanisms whereby p-glycoprotein expression is induced and regulated during malignant transformation is indicated. The development of biliary phospholipid deficiency in mdr2 knockout mice and xenobiotic hypersensitivity in mdr3 knockout mice have given us the first clues into the normal physiologic roles for the p-glycoproteins. The search for endogenous substrates for the p-glycoproteins will continue to be an area of active investigation.

Continued investigation of p-glycoprotein's functions should result in better understanding of an important class of prokaryotic and eukaryotic membrane transporters. The potential of exploiting the knowledge garnered from these studies in the treatment of neoplastic, parasitic and inherited and acquired liver disease may be greater than we can now imagine.

References

1. Abraham, I., Hunter, R.J., Sampson, K.E., Smith, S., Gottesman, M.M., Mayo, J.K. 1987. *Mol. Cell. Biol.* **7**:3098–3106
2. Abraham, E.H., Prat, A.G., Gerweck, L., Seneveratne, T., Arceci, R.J., Kramer, R., Guidotti, G., Cantiello, H.F. 1993. *Proc. Natl. Acad. Sci. USA* **90**:312–316
3. Altuvia, S., Stein, W.D., Goldenberg, S., Kane, S.E., Pastan, I., Gottesman, M.M. 1993. *J. Biol. Chem.* **268**:27127–27132
4. Al-Shawi, M.K., Senior, A.E. 1993. *J. Biol. Chem.* **268**:4197–4206
5. Al-Shawi, M.K., Urbatsch, I.L., Senior, A.E. 1994. *J. Biol. Chem.* **269**:8986–8992
6. Altenberg, G.A., Dietmer, J.W., Glass, D.C., Reuss, L. 1994. *Cancer Res.* **54**:618–622
7. Altenberg, G.A., Vanoye, C.G., Han, E.S., Dietmer, J.W., Reuss, L. 1994. *J. Biol. Chem.* **269**:7145–7149
8. Altenberg, G.A., Young, G., Horton, J.K., Glass, D., Belli, J.A., Reuss, L. 1993. *Proc. Natl. Acad. Sci. USA* **90**:6735–6738
9. Ambudkar, S.V., Lelong, I.H., Zhang, J., Cardarelli, C.O., Gottesman, M.M., Pastan, I. 1992. *Proc. Natl. Acad. Sci. USA* **89**:8472–8476
10. Arceci, R.J., Baas, F., Raponi, R., Horwitz, S.B., Housman, D.R., Croop, J.M. 1990. *Mol. Rep. Dev.* **25**:101–109
11. Arceci, R.J., Croop, J.M., Horwitz, S.B., Housman, D. 1988. *Proc. Natl. Acad. Sci. USA* **85**:4350–4354
12. Arias, I.M., Gatmaitan, Z., Mazzanti, R., Kumamoto, Y. 1991. *In:* Xenobiotic and Cancer. L. Ernster et al., editors. pp 229–239. Japan Science Society, Tayko, Taylor and Francis, London
13. Azzaria, M., Schurr, E., Gros, P. 1989. *Mol. Cell. Biol.* **9**:5289–5297
14. Bates, S.E., Mickley, L.A., Chen, Y.N., Richert, N., Rudick, J., Biedler, J.L., Fojo, A.T. 1989. *Mol. Cell. Biol.* **9**:4337–4344
15. Bates, S.E., Shieh, C.Y., Tsokos, M. 1991. *Am. J. Pathol.* **139**:305–315
16. Beck, W.T., Cirtain, M. 1982. *Cancer Res.* **42**:184–189
17. Beck, W.T., Danks, M.K. 1991. *In:* I. Roninson, editor. pp. 3–46. Plenum, New York
18. Beck, W.T., Qian, X.D. 1992. *Biochem. Pharmacol.* **43**:89–93

19. Bellamy, W.T., Dalton, W.S., Dorr, R.T. 1990. *Cancer Invest.* **8:**545–560
20. Berr, F., Meier, P.J., Stieger, B. 1993. *J. Biol. Chem.* **268:**3976–3979
21. Bishop, W.R., Bell, R.M. 1985. *Cell* **42:**51–60
22. Boscoboinik, D., Gupta, R.S., Epand, R.M. 1990. *Br. J. Cancer* **61:**568–572
23. Bradley, G., Sharma, R., Rajalakshmi, S., Ling, V., 1992. *Cancer Res.* **52:**5154–5161
24. Bruggeman, E.P., Currier, S.J., Gottesman, M.M., Pastan, I. 1992. *J. Biol. Chem.* **267:**21020–21026
25. Burt, R.K., Garfield, S., Johnson, K., Thorgeirsson, S.S. 1988. *Carcinogenesis* **9:**2329–2332
26. Burt, R.K., Thorgeirsson, S.S. 1988. *J. Natl. Cancer Inst.* **80:**1383–1386
27. Buschman, E., Arceci, R.J., Croop, J.M, Arias, I.M., Che, M., Housman, D.E., Gros, P. 1992. *J. Biol. Chem.* **267:**18093–18099
28. Buschman, E., Gros, P. 1991. *Mol. Cell. Biol.* **11:**595–603
29. Callaghan, R., Riordan, J.R. 1993. *J. Biol. Chem.* **268:**16059–16064
30. Callahan, H.L., Beverly, S.M. 1991. *J. Biol. Chem.* **266:**18427–18430
31. Campos, L., Guyotat, D., Archimbaud, E. 1992. *Blood* **79:**473–476
32. Chambers, T.C., Chalikonda, I., Eilon, G. 1990. *Biochem. Biophys. Res. Commun.* **169:**253–259
33. Chambers, T.C., McAvoy, E.M., Jacobs, J.W., Eilon, G. 1990. *J. Biol. Chem.* **265:**7679–7686
34. Chambers, T.C., Zheng, B., Kuo, J.F. 1992. *Mol. Pharmacol.* **41:**1008–1015
35. Chambers, T.C., Pohl, J., Raynor, R.L., Kuo, J.F. 1993. *J. Biol. Chem.* **268:**4592–4595
36. Chan, H.S.L., Haddad, G., Thorner, P.S., DeBoer, G., Lin, Y.P., Ondrusek, N., Yeger, H., Ling, V. 1991. *N. Engl. J. Med.* **325:**1608–1614
37. Chan, H.S.L., Thorner, P.S., Hadda, G., Ling, V. 1990. *J. Clin. Oncol.* **8:**689–704
38. Chaudhary, P.M., Mechetner, E.B., Roninson, I. 1992. *Blood* **80:**1–5
39. Chaudhary, P.M., Roninson, I.B. 1991. *Cell* **66:**85–94.
40. Chen, C., Chin, J.E. Udea, K., Clark, D.P., Pastan, I., Gottesman, M.M., Roninson, I. 1986. *Cell* **47:**381–389
41. Cheng, A.-I., Su, I.-J., Chen, Y.-C., Chen, Y.-C., Lee, T.-C., Wang, C.-H. 1993. *J. Clin. Oncol.* **11:**109–115
42. Chenivesse, X., Franco, D.F., Brechot, C. 1993. *J. Hepatol.* **18:**168–172
43. Chin, J.E., Soffir, R., Noonan, K.E., Choi, K., Roninson, I. 1989. *Mol. Cell. Biol.* **9:**3808–3820
44. Chin, K.V., Chauhan, S.S., Abraham, I., Sampson, K., Krolczyk, A. 1992. *J. Cell. Physiol.* **152:**87–94
45. Chin, K.V., Chauhan, S., Pastan, I., Gottesman, M.M. 1990. *Cell Growth Diff.* **1:**361–365
46. Chin, K.V., Tanaka, S., Darlington, G., Pastan, I., Gottesman, M.M. 1990. *J. Biol. Chem.* **265:**221–226
47. Chin, K.V., Ueda, K., Pastan, I., Gottesman, M.M. 1992. *Science* **255:**459–462
48. Choi, K., Chen, C.J., Kriegler, M., Roninson, I. 1989. *Cell* **53:**519–529
49. Chow, L., Wong, A., Ullman, B., Wirth, D. 1993. *Mol. Biochem. Pharmacol.* **60:**195–208
50. Christen, R.D., McClay, E.F., Plaxe, S.C., Yen, S.S.C., Kim, S., Kirmani, S., Wilgus, L.L., Heath, D.D., Shalinsky, D.R., Freddo, J.L., Braly, P.S., O'Quigley, J., Howell, S.B. 1993. *J. Clin. Oncol.* **11:**2417–2426
51. Cordon-Cardo, C., O'Brien, J.P., Casal, D., Rittman-Grauer, L., Biedler, J.L., Melamed, M.R., Bertino, J.R. 1989. *Proc. Natl. Acad. Sci. USA* **86:**695–698
52. Cornwall, M.M., Tsuruo, T., Gottesman, M.M., Pastan, I. 1987. *FASEB J.* **1:**51–54
53. Cowman, A.F., Galatis, D., Thompson, J.K. 1994. *Proc. Natl. Acad. Sci. USA* **91:**1143–1147
54. Cowman, A.F., Karcz, S., Galatis, D., Culvenor, J.G. 1991. *J. Biol. Chem.* **113:**1033–1042
55. Croop, J.M., Raymond, M., Haber, D., DeVault, A., Arceci, J., Gros, P., Housman, D.E. 1989. *Mol. Cell. Biol.* **9:**1346–1350
56. Currier, S.J., Kane, S.E., Willinghan, M.C., Cardarelli, C.O., Pastan, I., Gottesman, M.M. 1992. *J. Biol. Chem.* **267:**25153–25159
57. Currier, S.J., Ueda, K., Willingham, M.C., Pastan, I., Gottesman, M.M. 1989. *J. Biol. Chem.* **264:**14376–14381
58. Dalmark, M., Storm, H. 1981. *J. Gen. Physiol.* **78:**349–364
59. Dalton W.S., Grogan, T.M., Meltzer, P.S., Scheper, R.J., Durle, B.G.M., Taylor, C.W., Miller, T.P., Salmon, S.E. 1989. *J. Clin. Oncol.* **7:**415–424
60. Deuchars, K.L., Duthie, M., Ling, V. 1992. *Biochim. Biophys. Acta* **1130:**157–165
61. DeVault, A., Gros, P. 1990. *Mol. Cell. Biol.* **10:**1652–1663
62. Deverson, E.V., Gow, J.R., Coadwell, J., Monaco, J.J., Butcher, G.W., Howard, J.C. 1990. *Nature* **348:**738–741
63. Devine, S.E., Ling, V., Melera, P.W. 1992. *Proc. Natl. Acad. Sci. USA* **89:**4564–4568
64. Doige, C.A., Sharom, F.J. 1992. *Biochim. Biophys. Acta* **1109:**161–171
65. Doige, C.A., Yu, X., Sharom, F.J. 1992. *Biochim. Biophys. Acta* **1109:**149–160
66. Doige, C.A., Yu, X., Sharom, F.J. 1993. *Biochim. Biophys. Acta* **1146:**65–72
67. Elbling, L., Berger, W., Rehberger, A., Waldhor, T., Micksche, M. 1993. *FASEB J.* **7:**1499–1506
68. Endicott, J.A., Ling, V. 1989. *Annu. Rev. Biochem.* **58:**137–171
69. Fairchild, C.R., Ivy, S.P., Rushmore, T., Lee, G., Koo, P., Goldsmith, M.E., Myers, C.E., Farber, E., Cowan, K. 1987. *Proc. Natl. Acad. Sci. USA* **84:**7701–7705
70. Fardel, O., Loyer, D., Morel, F., Ratanasavanh, D., Guillouzo, A. 1992. *Biochem. Pharmacol.* **44:**2259–2268
71. Fath, M.J., Kolter, R. 1993. *Microbiol. Rev.* **57:**995–1017
72. Fine, R.L., Patel, J., Chabner, B.A. 1988. *Proc. Natl Acad. Sci. USA* **85:**582–586
73. Fojo, A.T., Ueda, K., Salmon, D.J., Poplack, D.G., Gottesman, M.M., Pastan, I. 1987. *Proc. Natl. Acad. Sci. USA* **84:**265–269
74. Foote, S.J., Thompson, J.K., Cowman, A.F., Kemp, D.J. 1989. *Cell* **57:**921–930
75. Ford, J.M., Hait, W.N. 1990. *Pharmacol. Rev.* **42:**155–199
76. Gartner, J., Moser, H., Valle, D. 1992. *Nature Genet.* **1:**16–23
77. Georges, E., Sharom, F.J., Ling, V. 1990. *Adv. Pharmacol.* **21:**185–213
78. Gill, D., Hyde, S.C., Higgins, C.F., Valverde, M.A., Mintenig, G.M., Sepúlveda, F.V. 1992. *Cell* **71:**23–32
79. Goldstein, L.J., Galski, H., Fojo, A., Willingham, M., Lai, S., Gasdar, A., Pirker, R., Green, A., Crist, W., Brodeur, G.M., Lieber, M., Cossman, J., Gottesman, M.M., Pastan, I. 1989. *J. Natl. Cancer Inst.* **81:**116–124
80. Gottesman, M.M., Pastan, I. 1988. *J. Biol. Chem.* **263:**12163–12166
81. Gottesman, M.M., Pastan, I. 1991. *Annu. Rev. Med.* **42:**277–286
82. Gottesman, M.M., Pastan, I. 1993. *Annu. Rev. Biochem.* **62:**385–427
83. Gros, P., Croop, J., Housman, D. 1986. *Cell* **47:**371–380

84. Gros, P., Dhir, R., Croop, J., Talbot, F. 1991. *Proc. Natl. Acad. Sci. USA* **88:**4564–4568
85. Gros, P., Raymond, M., Bell, J. 1988. *Mol. Cell. Biol.* **8:**2770–2778
86. Hamada, H., Tsuruo, T. 1988. *Cancer Res.* **48:**4926–4932
87. Hamada, H., Tsuruo, T. 1988. *J. Biol. Chem.* **263:**1454–1458
88. Hendrickson, N., Sifri, C.D., Henderson, D.M., Allen, T., Wirth, D., Ullman, B. 1993. *Mol. Biochem. Pharmacol.* **60:**53–64
89. Herzog, C.E., Tsokos, M., Bates, S.E., Fojo, A.T. 1993. *J. Biol. Chem.* **268:**2946–2952
90. Higgins, C.F. 1992. *Annu. Rev. Cell Biol.* **8:**67–113
91. Higgins, C.F., Gottesman, M.M. 1992. *Trends Biochem. Sci.* **17:**18–21
92. Holzmayer, T.A., Hilsenbeck, S., Von Hoff, D.D. 1992. *J. Natl. Cancer Inst.* **84:**1486–1491
93. Homolya, L., Hollo, Z., Germann, U.A., Pastan, I., Gottesman, M.M., Sarkadi, B. 1993. *J. Biol. Chem.* **268:**21493–21496
94. Horio, M., Chin, K.-V., Currier, S.J., Goldenberg, S., Williams, C., Pastan, I., Gottesman, M.M., Handler, J. 1989. *J. Biol. Chem.* **264:**14880–14884
95. Horio, M., Gottesman, M.M., Pastan, I. 1988. *Proc. Natl. Aca. Sci. USA* **85:**3580–3584
96. Huang, C., Wu, M., Xu, G., Li, D., Cheng, H., Tu, Z., Jiang, H., Gu, J. 1992. *J. Natl. Cancer Inst.* **84:**262–263
97. Hunter, J., Jepson, M.A., Tsuruo, T., Simmons, N.L., Hirst, B.H. 1993. *J. Biol. Chem.* **268:**14991–14997
98. Juliano, R.L., Ling, V. 1976. *Biochim. Biophys. Acta* **455:**152–162
99. Kadmon, M., Klunemann, C., Bohme, M., Ishikawa, T., Gorgas, K., Otto, G., Herfarth, C., Keppler, D. 1993. *Gastroenterology* **104:**1507–1514
100. Kamijo, K., Taketan, S., Yokota, S., Osumi, T., Hashimoto, T. 1990. *J. Biol. Chem.* **265:**4534–4540
101. Kamimoto, Y., Gatmaitan, Z., Hsu, J., Arias, I. 1989. *J. Biol. Chem.* **264:**11693–11698
102. Karcz, S., Cowman, A.F. 1991. *Exp. Parasitol.* **73:**233–240
103. Kaye, S.B. 1990. *Cancer Treat. Rev.* **17:**37–43
104. Kioka, N., Tsubota, J., Kakehi, Y., Komano, T., Gottesman, M.M., Pastan, I. 1989. *Biochem. Biophys. Res. Commun.* **162:**244–231
105. Kuchler, K., Sterne, R., Thorner, J. 1989. *EMBO J.* **8:**3973–3984
106. Kuo, M.T., Khao, J.Y., Teeter, L.D., Ikeguchi, M., Chisari, F.V. 1992. *Cell Growth Diff.* **3:**5321–540
107. Kureluc, B., Krca, S., Pivcevic, B., Ugarkovic, D., Bachmann, M. 1992. *Carcinogenesis* **13:**69–76
108. Leppart, G., McDevitt, R., Falco, S.C., Van Dyk, T.K., Ficke, M.B., Golin, J. 1990. *Genetics* **125:**13–20
109. Lincke, C.R., van der Bliek, A.M., Schuurhuis, G.J., van der Velde-Koerts, T., Smit, J.M., Borst, P. 1990. *Cancer Res.* **50:**1779–1785
110. Ling, V., Kartner, N., Sudo, T., Siminovitch, L., Riordan, J. 1983. *Cancer Treat. Rep.* **67:**869–874
111. Linsenmeyer, M.E., Jefferson, S., Wolf, M., Matthews, J.P., Board, P.G., Woodcock, D.M. 1992. *Br. J. Cancer* **65:**471–475
112. List, A.F., Spier, C., Greer, J., Wolff, S., Hutter, J., Dorr, R., Salmon, S., Futscher, B., Baier, M., Dalton, W. 1993. *J. Clin. Oncol.* **11:**1652–1660
113. Loe, D.W., Sharom, F. 1994. **1190:**72–84
114. Loo, T.W., Clarke, D.M. 1994. *J. Biol. Chem.* **270:**7750–7755
115. Ma, L., Marquardt, D., Takemoto, L., Center, M.S. 1991. *J. Biol. Chem.* **266:**5593–5599
116. Mazzanti, R., Fatappie, O., Kamimoto, Y., Gentilini, P., Arias, I. 1994. *Hepatology* **20:**170–176
117. McGrath, T., Varshavsky, A. 1989. *Nature* **340:**400–404
118. Mellado, W., Horwitz, S.B. 1987. *Biochemistry* **26:**6900–6904
119. Mickley, L.A., Bates, S.E., Richert, N.D., Currier, S., Tanaka, S. 1989. *J. Biol. Chem.* **264:**18031–18040
120. Miller, R.L., Bukowski, R.M., Budd, T., Purvis, J., Weick, J.K., Shepard, K., Midha, K.K., Ganapathi, R. 1988. *J. Clin. Oncol.* **6:**880–888
121. Miller, T.P., Grogan, T.M., Dalton, W.S., Spier, C.M., Scheper, R.J., Salmon, S.E. 1991. *J. Clin. Oncol.* **9:**17–24
122. Mizoguchi, T., Yamada, K., Furukawa, T., Hidaka, K., Hisatsugu, T., Shimazu, H., Tsuruo, T., Sumizawa, S., Akimaya, S.I. 1990. *J. Natl. Cancer Inst.* **82:**1679–1683
123. Naito, M., Hamada, H., Tsuruo, Y. 1988. *J. Biol. Chem.* **263:**11887–11891
124. Naito, M., Hoshino, T., Matsushita, Y., Hirai, R., Tsuruo, T. 1991. *J. Cell. Pharmacol.* **2:**263–267
125. Nakatsukasa, H., Silverman, J.A., Gant, T.W., Evarts, R.P., Thorgeirsson, S.S. 1993. *Hepatology* **8:**1202–1207
126. Neilsen, D., Skovsgaard, T. 1992. *Biochim. Biophys. Acta* **1139:**169–183
127. Ng, W.F., Sarangi, F., Zastaway, R.L., Veinot-Drebot, L., Ling, V. 1989. *Mol. Cell. Biol.* **9:**1224–1228
128. Nishi, K., Yoshida, M., Nishikawa, M., Nishiyama, M., Horinouchi, S., Beppu, T. 1992. *Mol. Microbiol.* **6:**761–769
129. Orr, G.A., Han, E.K.H., Browne, P.C., Nieves, E., O'Conner, B.M., Yang, C.-P.H., Horwitz, S.B. 1993. *J. Biol. Chem.* **268:**25054–25062
130. Ortiz, D.F., Kreppel, L., Speiser, D.M., Scheel, G., McDonald, G., Ow, D.W. 1992. *EMBO J.* **11:**3491–3499
131. Papadopoulou, B., Roy, G., Dey, S., Rosen, B., Ouellette, M. 1994. *J. Biol. Chem.* **269:**11980–11986
132. Pearce, H.L., Safa, A.R., Bach, N.J., Winter, M.A., Cirtain, M.C., Beck, W.T. 1989. *Proc. Natl. Acad. Sci. USA* **86:**5128–5132
133. Piekarz, R.L., Cohen, D., Horwitz, S.B. 1993. *J. Biol. Chem.* **268:**7613–7616
134. Pirker, R., Wallner, J., Geizler, K., Linkesch, W., Haas, O.A., Bettelheim, P., Hopfner, M., Scherrer, R., Valent, P., Havelec, L., Ludwig, H., Lechner, K. 1991. *J. Natl. Cancer Inst.* **83:**708–712
135. Poruchynsky, M., Ling, V. 1994. *Biochemistry* **33:**4163–4174
136. Qian, X.-D., Beck, W.T. 1990. *J. Biol. Chem.* **265:**18753–18756
137. Rasola, A., Galietta, L.J.V., Gruenert, D.C., Romeo, G. 1994. *J. Biol. Chem.* **269:**1432–1436
138. Raviv, Y., Pollard, H.B., Bruggemann, E.P., Pastan, I., Gottesman, M.M. 1990. *J. Biol. Chem.* **265:**3975–3980
139. Raymond, M., Gros, P., Whiteway, M., Thomas, D.Y. 1992. *Science* **256:**232–233
140. Robey-Cafferty, S.S., Rutledge, M.L., Bruner, J.M. 1990. *Am. J. Clin. Pathol.* **93:**1–7
141. Roepe, P.D. 1992. *Biochemistry* **31:**12555–12564
142. Riordan, J.R., Deuchars, K., Kartner, N., Alon, N., Trent, J., Ling, V. 1985. *Nature* **316:**817–819
143. Riordan, J.R., Ling, V. 1979. *J. Biol. Chem.* **254:**12701–12705
144. Riordan, J.R., Rommens, J.M., Kerem, B.-S., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.-L., Drumm, M.L., Iannuzzi, M.C., Collins, F.S., Tsui, L.-C. 1989. *Science* **245:**1066–1073
145. Roninson, I.B., editor. 1991. Molecular and Cellular Biology of Multidrug Resistance in Tumor Cells. Plenum, New York
146. Roninson, I.B. 1992. *Biochem. Pharmacol.* **43:**95–102
147. Roninson, I.B., Chin, J.E., Choi, K., Gros, P., Housman, D.E., Fojo, A., Shen, D.W., Gottesman, M.M., Patsan, I. 1986. *Proc. Natl. Acad. Sci. USA* **83:**4538–4542
148. Ruetz, S., Gros, P. 1994. *J. Biol. Chem.* **269:**12277–12284
149. Saeki, T., Ueda, K., Tanigawara, Y., Mori, R., Komano, T. 1993. *J. Biol. Chem.* **268:**6077–6080

150. Safa, A.R., Stern, R.K., Choi, K., Agresti, M., Tamai, I., Mehta, N.D., Roninson, I.B. 1990. *Proc. Natl. Acad. Sci. USA* **87:**7225–7229
151. Salmon, S.E., Grogan, T.M., Miller, T. 1989. *J. Natl. Cancer Inst.* **81:**696–701
152. Samuelson, J., Ayala, E., Orozoo, C., Wirth, D. 1990. *Mol. Biochem. Parasitol.* **38:**281–290
153. Sarkadi, B., Price, E.M., Boucher, R.C., Germann, U.A., Scarborough, G.A. 1992. *J. Biol. Chem.* **267:**4854–4858
154. Sato, H., Preisler, H., Day, R. 1990. *Br. J. Haematol.* **75:**340–345
155. Schinkel, A.H., Borst, P. 1991. *Semin. Cancer Biol.* **2:**213–226
156. Schinkel, A.H., Kemp, S., Dolle, M., Rudenko, G., Wagenaar, E. 1993. *J. Biol. Chem.* **268:**7474–7481
157. Schinkel, A.H., Roelofs, M.E.M., Borst, P. 1991. *Cancer Res.* **51:**2628–2635
158. Schinkel, A.H., Smit, J.J.M., van Tellingen, O., Beijnen, J.H., Waganaar, E., van Deemter, L., Mol, C.A.A.M., van der Valk, M.A., Robanus-Maandag, E.C., te Riele, H.P., Berns, A.J.M., Borst, P. 1994. *Cell* **77:**491–502
159. Schrenk, D., Gant, T.W., Preisegger, K-H., Silverman, J.A., Marino, P.A., Thorgeirsson, S.S. 1993. *Hepatology* **17:**854–860
160. Schurr, E., Raymond, M., Bell, J.C., Gros, P. 1989. *Cancer Res.* **49:**2729–2734
161. Servos, J., Haase, E., Brendel, M. 1993. *Mol. Gen. Genet.* **236:**214–218
162. Shapiro, A.B., Ling, V. 1994. *J. Biol. Chem.* **269:**3745–3754
163. Sharma, R.C., Inoue, S., Roitelman, J., Schimke, R.T., Simoni, R.D. 1992. *J. Biol. Chem.* **267:**5731–5734
164. Sharom, F.J., Yu, X., Doige, C. 1993. *J. Biol. Chem.* **268:**24197–24202
165. Shepard, J.C., Schumacher, T.N.M., Ashton-Rickardt, P.G., Imaeda, S., Ploegh, H.L., Janeway, C.A., Tonegawa, S. 1993. *Cell* **74:**577–584
166. Shimabuka, A.M., Nishimoto, T., Ueda, K., Komano, T. 1992. *J. Biol. Chem.* **267:**4308–4311
167. Shu, H., Gatmaitan, Z., Arias, I.M. 1992. *Gastroenterology* **102:**879–885
168. Sikic, B.I. 1993. *J. Clin. Oncol.* **11:**1629–1635
169. Silverman, J.A., Raunio, H., Gant, T.W., Thorgeirsson, S.S. 1991. *Gene* **106:**229–236
170. Simon, S., Roy, D., Schindler, M. 1994. *Proc. Natl. Acad. Sci. USA* **91:**1128–1132
171. Sinicrope, F.A., Dudeja, P.K., Bissonnette, B.M., Safa, A.R., Brasitus, T.A. 1992. *J. Biol. Chem.* **267:**24995–25002
172. Skaats, J., Marquardt, D., Center, M.S. 1990. *J. Biol. Chem.* **265:**4084–4090
173. Skach, W.R., Calayag, C., Lingappa, V.R. 1993. *J. Biol. Chem.* **268:**6903–6908
174. Smit, J.M.M., Schinkel, A.H., Oude Elferink, R.P.J., Groen, A.K., Wagenaar, E. van Deemter, L., Mol, C.A.A.M., Ottenhoff, R., van der Lugt, N.M.T., van Roon, M.A., van der Valk, M.A., Berns, A.J.M., Borst, P. 1993. *Cell* **75:**451–462
175. Spoelstra, E., Westerhoff, H., Dekker, H., Lankelma, J. 1992. *Eur. J. Biochem.* **207:**567–579
176. Sugawara, I., Kataoka, I., Morishita, Y., Hamada, H., Tsuruo, T., Itoyama, S., Mori, S. 1988. *Cancer Res.* **48:**1926–1929
177. Tatsuta, T., Naito, M., Oh-hara, T., Sugawara, I., Tsuruo, T. 1993. *J. Biol. Chem.* **267:**20383–20391
178. Teeter, L.D., Becker, F.F., Chisari, F.V., Li, D., Kuo, M.T. 1990. *Mol. Cell. Biol.* **10:**5728–5735
179. Teeter, L.D., Chan, J.Y., Kuo, M.T. 1991. *Mol. Carcinogen.* **4:**358–361
180. Theibault, F., Currier, S.J., Whitaker, J., Haugland, R.P., Gottesman, M.M., Pastan, I. 1990. *J. Histochem. Cytochem.* **38:**685–690
181. Theibault, F., Tsuruo, T., Hamada, H., Gottesman, M.M., Pastan, I., Willingham, M.C. 1987. *Proc. Natl. Acad. Sci. USA* **84:**7735–7738
182. Thorgeirsson, S.S., Huber, B.E., Sorrell, S., Fojo, A., Pastan, I., Gottesman, M.M. 1987. *Science* **236:**1120–1122
183. Trezise, A.E., Romano, P.R., Gill, D.R., Hyde, S.C., Sepúlveda, F.V. 1992. *EMBO J.* **11:**4291–4303
184. Twentyman, P.R. 1992. *Biochem. Pharmacol.* **43:**109–117
185. Ueda, K., Cardarelli, C., Gottesman, M.M., Pastan, I. 1987. *Proc. Natl. Acad. Sci. USA* **84:**3004–3008
186. Ueda, K., Okamura, N., Hirai, M., Tanigawara, Y., Saeki, T., Kioka, N., Komano, T., Hori, R. 1992. *J. Biol. Chem.* **267:**24248–24252
187. Ueda, K., Yamano, Y., Kioka, N., Kaehi, Y., Yoshida, O. 1989. *Jpn. J. Cancer Res.* **80:**1127–1132
188. Valverde, M.A., Diaz, M., Sepúlveda, F.V., Gill, D.R., Hyde, S.C., Higgins, C.F. 1992. *Nature* **155:**830–833
189. van der Bliek, A.M., Kooiman, P.M., Schneider, C., Borst, P. 1988. *Gene* **71:**410–411
190. van Kalken, C.K., Broxterman, H.J., Pinedo, H.M., Feller, N., Dekker, H., Lankelma, J., Giaccone, G. 1993. *Br. J. Cancer* **67:**284–289
191. van Kalken, C.K., Giaccone, G., van der Valk, P., Kuiper, C.M., Hadisaputro, M.M.N., Bosma, S.A.A., Scheper, R.J., Meijer, C.J.L.M., Pinedo, H.M. 1992. *Am. J. Pathol.* **141:**1063–1072
192. Verrelle, P., Meissonnier, F., Fonck, Y., Feillel, V., Dionet, C., Kwiatkowski, F., Plagne, R., Chassagne, J. 1991. *J. Natl. Cancer Inst.* **83:**111–116
193. Verweij, J., Herweijer, R., Oosterom, R., van der Burg, M.E.L., Planting, A.S.Th., Seynaeve, C., Stoter, G., Nooter, K. 1991. *Br. J. Cancer* **64:**361–364
194. Weinstein, R.S., Jakate, S.M., Dominguez, J.M., Lebovitz, M.D., Koukoulis, G.K., Kuszak, J.R., Klusens, L.F., Grogan, T.M., Saclarides, T.J., Roninson, I.B., Coon, J.S. 1991. *Cancer Res.* **51:**2720–2726
195. Wu, C.T., Budding, M., Griffen, M.S., Croop, J.M. 1991. *Mol. Cell. Biol.* **11:**3940–3948
196. Yahanda, A.M., Adler, K.M., Fisher, G.A., Brophy, N.A., Halsey, J., Hardy, R.L., Gosland, M.P., Lum, B.L., Sikic, B.I. 1992. *J. Clin. Oncol.* **10:**1624–1634
197. Yang, C.H., Depino, S.G., Greenberger, L.M., Arceci, R.J., Horwitz, S.B. 1989. *J. Biol. Chem.* **264:**782–788
198. Yoshimura, A., Kumazuru, Y., Sumizama, T., Ichikawa, M., Ikeda, S. 1989. *J. Biol. Chem.* **264:**16281–16291
199. Yu, G., Ahmad, S., Aquino, A., Fairchild, C.R., Trepel, J.B. 1991. *Cancer Commun.* **3:**181–188
200. Yusa, K., Tsuruo, T., 1989. *Cancer Res.* **49:**5002–5006
201. Zamora, J.M., Pearce, H.L., Beck, W.T. 1988. *Mol. Pharmacol.* **33:**454–462
202. Zhang, J.T., Ling, V. 1991. *J. Biol. Chem.* **266:**18224–18232
203. Zhao, J.-Y., Ikeguchi, M., Eckersberg, T., Kuo, M.T. 1993. *Endocrinology* **133:**521–524

Note Added in Proof: A recent study (Ruetz, S., Gros, P. 1994. *Cell* **77:**1–20) utilizing yeast secretory vesicles which overexpress the mdr2 gene product has conclusively demonstrated that this protein functions as an ATP-dependent phosphatidylcholine translocase.