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MINI-REVIEW

Genetic Basis of Multidrug Resistance of Tumor Cells

Susan E. Kane[1,2], Ira Pastan[3], and Michael M. Gottesman[1,4]

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Abstract

Multidrug resistance in animal cells is defined as the simultaneous resistance to a variety of compounds which appear to be structurally and mechanistically unrelated. One type of multidrug resistance is characterized by the decreased accumulation of hydrophobic natural product drugs, a phenotype which is mediated by an ATP-dependent integral membrane multidrug transporter termed P-glycoprotein or P170. The gene coding for P170 is called *MDR*. The nucleotide-binding domain of P-glycoprotein shares sequence homology with a family of bacterial permease ATP-binding components. In addition, P170 as a whole is structurally very similar to a number of prokaryotic and eukaryotic proteins believed to be involved in transport activities. This review summarizes our current knowledge of the molecular biology and clinical significance of *MDR* expression and P-glycoprotein transport activity, as well as some theories about the function of this protein in normal cells.

Key Words: Chemotherapy; ATP; drug transport; colchicine; actinomycin D; doxorubicin; vinblastine; vincristine; introns; evolution; P-glycoprotein; transmembrane domains; *MDR*1 gene.

Introduction

Many animal cells exhibit resistance to a variety of structurally unrelated cytotoxic agents. This phenomenon, known as multidrug resistance, is an inherent property of certain cancers which are never sensitive to chemotherapy. Other cancers are initially responsive to a given chemotherapeutic

[1] Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.
[2] Present address: Division of Surgery, City of Hope Medical Center, Duarte, California 91010.
[3] Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.
[4] To whom correspondence should be addressed.

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regimen but eventually acquire resistance to the drugs being used in the treatment and to other drugs as well. The acquisition of cross-resistance to one class of structurally unrelated drugs has been modeled in tissue culture (Biedler and Riehm, 1970; Akiyama *et al.*, 1985; Howell *et al.*, 1984; Croop *et al.*, 1988; Endicott and Ling, 1989; Gottesman and Pastan, 1988). In this review we shall summarize current knowledge of the molecular basis and clinical significance of resistance to these hydrophobic natural product drugs, e.g., the anthracyclines (doxorubicin, daunorubicin), *Vinca* alkaloids (vinblastine, vincristine), epipodophyllotoxins (etoposide, teniposide), actinomycin D, and taxol.

There are probably many systems involved in resistance to multiple drugs which are structurally dissimiliar, but the best understood mechanism of multidrug resistance is manifested by cells which accumulate much less drug than their parent cells which are drug sensitive. The resistant cells express an *mdr* gene, which encodes a 170-kDa membrane glycoprotein, termed P-glycoprotein or P170. The evidence for an association of P170 expression with multidrug resistance and the procedures used to clone the *mdr* genes have been reviewed elsewhere (Croop *et al.*, 1988; Endicott and Ling, 1989; Gottesman and Pastan, 1988). Expression of the mouse and human *mdr* *c*DNAs which code for P-glycoprotein in drug-sensitive cells is sufficient to confer the drug resistance phenotype (Gros *et al.*, 1986b; Ueda *et al.*, 1987a).

Though P170 is believed to be a pump (the multidrug transporter) which actively excludes drugs from resistant cells (Dano, 1973; Cornwell *et al.*, 1986, 1987; Horio *et al.*, 1988; Safa *et al.*, 1986), the actual mechanism for this activity is not understood and it is not known how a variety of structurally unrelated drugs might be handled by a single pump system. The involvement of P-glycoprotein in drug resistance of tissue culture cells is clear, but the role of this protein in resistance to cancer chemotherapy has been more difficult to assess. And since it is expressed in the plasma membrane of many normal tissues (see below), it seems likely that P170 performs a general function as a pump in normal cells as well. We do not know what the substrate(s) might be for P170 in such cells. This review will focus on recent data concerning the structure and function of a family of *mdr* genes, with particular attention to these issues of drug transport and normal pump activity.

Structure of Multidrug-Resistance Genes and Proteins

Gene Structure and Organization

Drug resistance in tissue culture cells is the result of overexpression and amplification of multidrug resistance genes, designated *MDR* (or PGY) in the human and *mdr* (*pgy* or *pgp*) in nonhuman species (Croop *et al.*, 1988; Endicott and Ling, 1989; Gottesman and Pastan, 1988). In human, mouse,

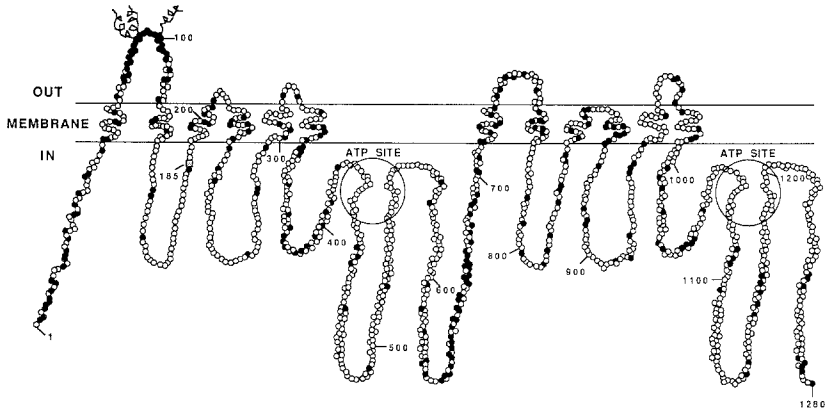

Fig. 1. Model of the human multidrug transporter. The ATP-binding sites, based on sequence homology with bacterial transport systems, are circled and putative N-linked carbohydrates are shown as curly lines. The glycine-to-valine mutation at amino acid residue 185, which is associated with preferred colchicine resistance, is shown as a cross-hatched square. The filled circles represent amino acids which differ between human *MDR*1 and mouse *mdr*1, the most divergent human and mouse sequences which are still functional in conferring multidrug resistance.

and hamster cells, these genes make up a small gene family, with two human (Roninson *et al.*, 1986; Van der Bliek *et al.*, 1987) and three rodent genes (Croop *et al.*, 1989; de Bruijn *et al.*, 1986; Gros *et al.*, 1988; Ng *et al.*, 1989). All code for a protein of approximately 1280 amino acids consisting of 12 transmembrane domains and two nucleotide binding sites (Fig. 1 and see Protein Structure) (Chen *et al.*, 1986; Gros *et al.*, 1986a). A homolog of *mdr*, *pfmdr*, has recently been cloned and sequenced from the malaria parasite *Plasmodium falciparum* (Wilson *et al.*, 1989; Foote *et al.*, 1989). The *pfmdr* gene is amplified and transcript levels are increased in some strains which are resistant to the antimalarial agent chloroquine.

The two human genes, *MDR*1 and *MDR*2, are linked within 330 kb of each other on chromosome 7, band q21.1 (Chin *et al.*, 1989; Callen *et al.*, 1987). The two genes encode proteins which are about 80% homologous at the amino acid level (Van der Bliek *et al.*, 1987; Chen *et al.*, 1986), suggesting that the genes arose by a duplication event, yet only the *MDR*1 gene has been shown to confer drug resistance in gene transfer experiments (Gros *et al.*, 1986b; Ueda *et al.*, 1987a). A function for the *MDR*2 gene has not been determined (see below).

The three rodent genes fall into two general classes. Mouse *mdr*1a and *mdr*1b (also referred to in the literature as *mdr*3 and *mdr*1, respectively) and their hamster homologs, *pgp*1 and *pgp*2, code for proteins which are most similar in amino acid sequence to P-glycoprotein encoded by human *MDR*1 (Chen *et al.*, 1986; Gros *et al.*, 1986a; Hsu *et al.*, 1989; Endicott *et al.*, 1987).

These genes all apparently code for P-glycoproteins which are functional in conferring drug resistance. Mouse *mdr*2 and hamster *pgp*3 are more related to human *MDR*2, and these genes are significantly divergent from the *MDR*1 class (Van der Bliek *et al.*, 1987; Gros *et al.*, 1988; Van der Bliek *et al.*, 1988). None of the *MDR*2-related genes have been shown to mediate multidrug resistance (Gros *et al.*, 1988; Van der Bliek *et al.*, 1988).

As for human *MDR*1 and *MDR*2, the rodent genes all lie on the same chromosome (Van der Bliek *et al.*, 1986; Raymond *et al.*, 1990). Sequence analysis of *c*DNA clones for the three rodent genes show that *mdr*1a and *mdr*1b (and *pgp*1 and *pgp*2 in the hamster) share certain amino acids which are different from those of *MDR*1, suggesting that these genes resulted from a duplication event sometime after primates and rodents diverged. And many of the amino acid residues which are the same in *mdr*1a and *mdr*1b are also different from *mdr*2, indicating that *MDR*1-class and *MDR*2-class genes arose before the divergence of primates and rodents (Gros *et al.*, 1986a, 1988; Hsu *et al.*, 1989).

The rodent *mdr*1 genes are more related to human *MDR*1 than to *MDR*2, and *mdr*1a (*pgp*1) appears to be more similar to *MDR*1 than is *mdr*1b (*pgp*2) (Hsu *et al.*, 1989; Endicott *et al.*, 1987). Support for this conclusion comes from species-to-species comparisons of sequences in the least conserved region of P170. P-glycoprotein is made up of two homologous halves which are joined by a linker region (see below). Whereas there is about 43% identity between the two halves of a given molecule, and up to 85% homology between the deduced protein sequences of different molecules, the linker regions of the various proteins are much more divergent. This region is, however, more highly conserved within a homologous set of *mdr* genes across species than between classes within a given species. In terms of exact amino acid matches, mouse *mdr*1a and human *MDR*1 are 68% identical in the linker, while *mdr*1a and *mdr*1b are only 47% identical. Mouse *mdr*1b shares only 48% identity with *MDR*1, but as much as 67% identity with hamster *pgp*2. The mouse and human *MDR*2-class sequences are 60% similar in the linker region, but mouse *mdr*2 vs. *mdr*1a or *mdr*1b are only 25 and 20% homologous, respectively (Hsu *et al.*, 1989).

The fact that P-glycoprotein, and therefore *MDR* genes, are composed of two homologous halves has suggested that the gene itself was generated by a gene duplication event sometime before *MDR*1 and *MDR*2 divergence. Yet there appears to be greater relatedness between entire *MDR*1-class molecules of different species (i.e., *MDR*1 vs. *mdr*1a or *mdr*1b) than between the two halves of any single gene. Furthermore, a very surprising finding has come from recent studies of the intron/exon structure of human *MDR*1 and mouse *mdr*1b genes (Chen *et al.*, 1990; Raymond and Gros, 1989). For both of these genes, although each half of the gene contains about the same number of

exons and introns, most of the introns occur at different places in the two halves of the gene. That is, rather than seeing analogous introns at the same positions in their respective halves, as expected from gene duplication, most pairs of introns interrupt the coding sequence at different amino acids in each half of the gene. Two intron pairs which *are* located at conserved positions in the two halves are within the highly conserved nucleotide binding domains of the protein (see below). The human and mouse genes analyzed in this way are remarkably similar in their exon/intron organization, such that the intron positions relative to coding sequence are strongly conserved between species but not between halves of the gene. It is possible that this gene structure is a result of gene duplication of a primordial gene containing few introns, and subsequent insertion of additional introns in the resulting duplicated gene (Raymond and Gros, 1989). However, it seems more likely that the gene for P170 arose by fusion of two structurally similar genes which had evolved separately, not by gene duplication (Chen *et al.*, 1990). Interestingly, the recent report on the cloning of the cystic fibrosis gene indicates a similar phenomenon—a gene with two structurally homologous halves which are nonetheless organized differently with respect to intron/exon boundaries, (Riordan *et al.*, 1989). Some implications of this type of evolution, relative to function, will be discussed later.

Protein Structure

As indicated above, a number of sequences have been determined for mouse, hamster, and human *c*DNA clones of *MDR* genes and they indicate that P-glycoprotein consists of two homologous halves plus a variable linker region (Chen *et al.*, 1986; Gros *et al.*, 1986a). Amino acid sequence analysis shows six hydrophobic transmembrane segments and a hydrophilic nucleotide binding domain in each half of the protein. According to the current model of P-glycoprotein orientation in the membrane, the highly charged amino acid terminis are both intracellular, the nucleotide binding domains are cytoplasmic, and there are three potential glycosylation sites in the first extracellular loop (Fig. 1). Support for this model can be summarized as follows: (1) Antibodies to specific regions of P-glycoprotein recognize extracellular or intracellular determinants as predicted (Kartner *et al.*, 1985; Yoshimura *et al.*, 1989); (2) ATP sites in the cytoplasm would be expected if the protein functions to pump drugs out of cells; and (3) at least one of the putative extracellular glycosylation sites contains carbohydrate residues since treatment with endoglycosidase F allows an antipeptide antibody to bind at this site (Bruggemann *et al.*, 1989).

There are striking similarities in sequence and apparent structure between P170 and a variety of polypeptides involved in cellular export or

import activities in prokaryotes (Chen *et al.*, 1986; Gros *et al.*, 1986a; Higgins *et al.*, 1985, 1986; Higgins, 1989). The presumed nucleotide binding domains were originally identified on the basis of their similarity with a variety of ATP-binding proteins, many of which are subunits of multicomponent transport systems in bacteria. Within this class of proteins the strongest homologies are with *hlyB*, the ATP-binding hemolysin export protein in *E. coli* (Felmlee *et al.*, 1985); *ndvA*, a protein possibly involved in β-(1 $\rightarrow$ 2)glucan export in *Rhizobium meliloti* (Stanfield *et al.*, 1988); *lktB*, a leukotoxin secretion determinant in *Pasteurella haemolytica*, analogous to *hly*B (Strathdee and Lo, 1989); and *cya*B, a factor in *Bordetella pertussis* involved in secretion of adenylate cyclase (Glaser *et al.*, 1988). Although these proteins are most similar to P170 in their ATP-binding domains, the homologies do extend outside of these regions. Other ATP-binding proteins with P-glycoprotein homology, primarily in the nucleotide-binding domain of P170, include bacterial transport components *hisP*, *malK*. *pstB*, *rbsA*, *oppD*, and *oppF*, as well as proteins involved in cell division (*ftsE* in *E. coli*; Gill *et al.*, 1986) and nodulation (*nodI* in *Rhizobium*; Evans and Downie, 1986) (reviewed in Higgins *et al.*, 1986). In addition to their ATP-binding subunits, the bacterial transport complexes contain a substrate-binding subunit and two hydrophobic integral membrane components (Ames, 1986), though the sequences of these proteins are not known to be homologous to the corresponding portions of P170. Other gene products associated with the FtsE and NodI proteins in mediating cell division and nodulation do not resemble known transport or membrane-associated structures (Higgins *et al.*, 1986). FtsE and NodI might therefore be related to the transport proteins only by virtue of a common ATP-binding function which couples ATP hydrolysis with a variety of biological activities.

Recent reports indicate that P-glycoprotein is structurally very similar to a number of eukaryotic proteins also thought to be involved in cellular transport mechanisms. As already mentioned, there is at least one homolog of the *mdr* gene in malaria parasites (Wilson *et al.*, 1989; Foote *et al.*, 1989). The brown protein and the white protein in *Drosophila* are involved in eye pigmentation probably at the level of pigment transport into pigment cells (Sullivan and Sullivan, 1975; Sullivan *et al.*, 1979). Both proteins contain sequence and structural features in common with P170 and the bacterial transport complexes, each with an amino-terminal nucleotide-binding site and extensive hydrophobic stretches in the carboxy-terminal one-third of the molecule (Dreesen *et al.*, 1988; O'Hare *et al.*, 1984; Mount, 1987). Finally, a yeast protein has just been described which probably is involved in the secretion of mating type factor **a**. The product of the STE6 gene shows almost 57% amino acid sequence similarity with P-glycoprotein, allowing for substitution of conserved amino acids. The predicted structure of STE6 is virtually identical to that of P-glycoprotein with two homologous halves,

each containing six transmembrane regions and an ATP-binding site (McGrath and Varshavsky, 1989; Kuchler *et al.*, 1989).

The identification of two domains within P-glycoprotein bearing strong similarities with bacterial ATP-binding proteins clearly supports observations of ATP-dependent activity for P-glycoprotein. The association of many of these ATP-binding subunits with transport systems, the more extensive homologies with efflux pumps such as HlyB, and the isolation of eukaryotic transport proteins with similar structures, including STE6 and *Drosophila* brown and white, all strengthen the notion that P170 is also involved in pumping cytotoxic drugs (and possibly other as yet unidentified substrates) out of cells (see below). Because the best homologies have been found for pumps involved in protein efflux (*hly*B and *STE6*), it has been suggested that one function of the *mdr* system may involve efflux of proteins or peptides (Gerlach *et al.*, 1986; McGrath and Varshavsky, 1989; Kuchler *et al*, 1989). However, none of the substrate binding sites for these transporters have been identified, and until such information is available it is not possible to predict substrate specificity from sequence data.

Evolution of Structure

It is interesting to speculate on the evolution of P170 protein structure in light of similarities with other known and proposed transport systems. Considering the surprising findings on intron/exon organization of *MDR*1, it seems likely that each half of the gene evolved independently. Each half could have at one time encoded a protein whose structure was strikingly similar to that of the HlyB-class proteins and some eukaryotic "transport" proteins. Each would contain a nucleotide binding domain, which in turn probably had evolved separately and joined with a number of genes encoding substrate binding and transport functions (this concept is illustrated in Fig. 2). In the case of the bacterial complexes, the various activities involved in transport are encoded on separate polypeptide subunits, whereas in eukaryotic systems and some prokaryotic proteins, all of the functional domains apparently reside in a single molecule.

Thus, perhaps the P170 half-molecules once were independent proteins involved in different aspects of ATP-mediated cellular activity. Alternatively, there is some evidence that other transport systems require multiple hydrophobic domains and/or ATP domains for activity. Within many of the bacterial complexes (discussed elsewhere in this issue), two ATP-binding subunits are needed for transport. For example, the *E. coli* ribose transport ATP-binding subunit (RbsA) is apparently a two-domain protein, with each domain having a putative ATP binding site (Higgins *et al.*, 1986; Bell *et al.*, 1986). For the multisubunit bacterial transporters, such as *mal*K (Gilson *et al.*,

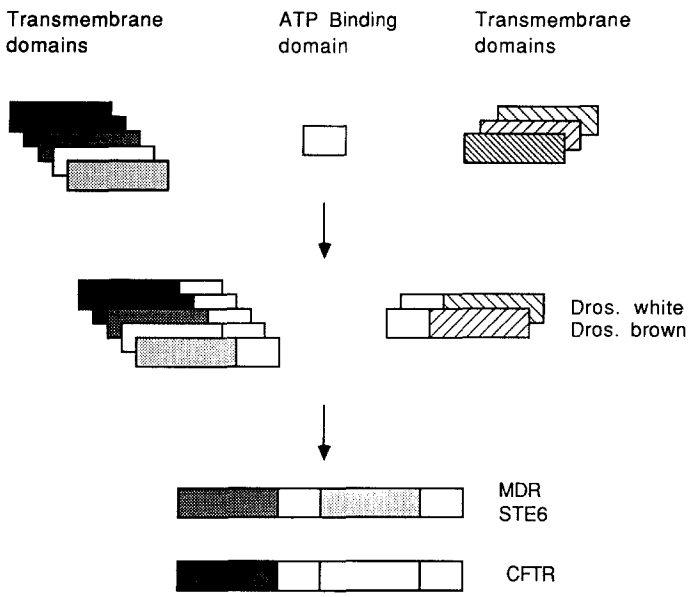

Fig. 2. Schematic representation of a model for evolution of *MDR*-related genes. Shaded rectangles are genetic elements encoding transmembrane (TM) domains of six membrane-spanning regions each. The gene for ATP-binding sites is depicted as an open box. In the model, the ATP-binding site and transmembrane domains might once have functioned as separate entities as parts of a complex analogous to bacterial transport systems (first line). The genes for these elements would then have fused, either in TM–ATP or ATP–TM configurations, to give a fully functional gene (the *Drosophila* brown and white genes, for example) or half-genes (line 2). These would have subsequently fused again (line 3) to form genes with two structurally redundant but possibly functionally unique halves. The resulting gene fusions sometime have extensive internal amino acid homology, as in the case of *MDR*, or very little similarity between halves, for the cystic fibrosis gene (*CFTR*). The representation of TM domains and TM–ATP fusions is not meant to be exhaustive, nor do we mean to exclude the possibility of an evolutionary relationship between the mature genes sometime after the "gene assembly" process.

1982) and *his*P (Higgins *et al.*, 1982), it is not known how many ATP-binding subunits are involved in the transport complex. It is posssible, perhaps likely on the basis of analogy to *rbs*A and *mdr*, that two such subunits are needed (Higgins *et al.*, 1986). In addition, some of these complexes, such as the bacterial permease systems for oligonucleotides, maltose, histidine, and phosphate, seem to require two integral membrane components, each with several transmembrane segments (Gilson *et al.*, 1982; Higgins *et al.*, 1982, 1985; Hiles *et al.*, 1987; Surin *et al.*, 1985). Likewise, there is some genetic evidence that the *Drosophila* white and brown proteins, both homologous in structure to one-half of P170, interact with each other in the pigment cell membrane to mediate uptake of a pigment precursor (Dreesen *et al.*, 1988).

So perhaps the two halves of P170, once separate proteins as in the bacterial systems, actually were two subunits in a complex, working together to perform their cellular function. A gene fusion event would have favored coordinate regulation of the two polypeptides and brought together two hydrophobic domains and two ATP-binding domains. If this were the case, we might expect the left and right halves of P170 to make distinct contributions to the activity of the protein, such that a gene fusion event was needed rather than a gene duplication to maintain a functional transport unit. There is some circumstantial evidence consistent with this reasoning. Constructions which contain major deletions in either half of the transporter do not confer drug resistance (Currier *et al.*, 1989). Also, based on photoaffinity labeling studies, both halves of the protein seem to interact with the substrate (Bruggemann *et al.*, 1989, see below). We know very little about the contributions of different regions of P-glycoprotein to drug transport, but it is possible that the two halves work in concert to mediate specificity in substrate binding, ATP binding, and transport. The fusion of two different membrane transporters might well have allowed interaction with the very broad range of compounds recognized by the multidrug transporter. Preliminary freeze-fracture experiments suggest that P-glycoprotein forms multimeric complexes in the plasma membrane, indicating an even higher order of structure (Sehested *et al.*, 1989).

We have much to learn by analyzing the overall family of ATP-dependent transport proteins. Even though sequence homologies are sometimes weak, the similarities in structure appear to be overwhelming. Many clues about the mechanism of action of P170 and potential methods for inactivating transport function might be obtained from comparisons with other systems. It would also be interesting to determine whether domains of bacterial, yeast, or *Drosophila* proteins can be interchanged with those of P-glycoprotein, in hopes of understanding more about substrate binding specificity and transport. The relationship between structure and function will be discussed further below.

When considering the general structure of transmembrane proteins, a still higher order of structural similarity to the *mdr* family of proteins appears (Unwin, 1986). The general motif of 6, 12, or 24 transmembrane segments is found commonly in other transmembrane proteins, even some lacking ATP-interaction sites. For example, ion channels, such as the sodium channel and calcium channels, contain 24 transmembrane segments, arranged in groups of six (Noda *et al.*, 1984; Tanabe *et al.*, 1987; Catterall, 1988). The cystic fibrosis protein, also possibly involved in ion movement across membranes, has limited sequence homology with P170 but very similar structural motifs —12 transmembrane regions plus two nucleotide-binding domains (Riordan *et al.*, 1989). Mammalian adenylate cyclase, a transmembrane enzyme, has a

similar structure, suggesting the possibility that it may have derived from a transport system and may still have a transport function in addition to its catalytic function (Krupinski *et al.*, 1989). The glucose transporter has two stretches of transmembrane domains consisting of six transmembrane regions each (Maiden *et al.*, 1987; Mueckler *et al.*, 1985). Gap junctions are formed from six transmembrane segments (Kumar and Gilula, 1986; Paul, 1986). Perhaps these data are telling us that packing of hydrophobic protein halves within a membrane to form a central pore commonly occurs in a hexameric array; perhaps interaction of these hexamers is essential to form higher-order structures (12, 24, etc.). Answers to some of these questions must await successful crystallization and X-ray diffraction analysis of this family of transmembrane proteins in a lipid bilayer, or the application of other physical techniques with high enough resolution to delineate structure.

Function of P170

That P170 is involved in drug resistance in tissue culture cells is well established (and has been reviewed extensively elsewhere, Croop *et al.*, 1988; Endicott and Ling, 1989; Gottesman and Pastan, 1988). The similarities with transport proteins discussed above, and data from *in vitro* and *in vivo* models detailed below, suggest that P-glycoprotein is a membrane-associated, ATP-dependent drug efflux pump. It has been difficult to determine the contribution of such drug transport to clinical multidrug resistance, however. And the possible role of *MDR*1- or *MDR*2-class P-glycoproteins in normal cellular transport activity is not known. These issues will be discussed in terms of the expression and activity of P170 *in vitro* and *in vivo*.

Interaction of Drugs with the Multidrug Transporter

There are a number of tissue culture systems available for studying the multidrug resistance phenotype. From these it was originally observed that multidrug resistant cells expressing P-glycoprotein take up drugs just as well as sensitive cells but they fail to accumulate these drugs (Dano, 1973; Fojo *et al.*, 1985a; Juliano and Ling, 1976; Willingham *et al.*, 1986). Drug efflux by these cells is energy-dependent. Thus, P170 appears to be functioning in a system responsible for the ATP-driven efflux of drugs from multidrug resistant cells. Indirect evidence that P170 is the multidrug transporter itself comes from drug-binding studies. [^{3}H]-Vinblastine binds to plasma membrane vesicles prepared from multidrug resistant cells (Cornwell *et al.*, 1987). In addition, radiolabeled photoaffinity analogs of vinblastine specifically label P-glycoprotein in these vesicles (Cornwell *et al.*, 1986; Safa *et al.*, 1986).

Many compounds of low cytotoxicity inhibit drug binding to P170 (Cornwell *et al.*, 1986) and appear to reverse multidrug resistance in culture, by acting as competitive inhibitors of the pump which swamp out its ability to transport more highly toxic agents. Such reversing agents include verapamil (Fojo *et al.*, 1985a), quinidine (Tsuruo *et al.*, 1981), and reserpine (Beck *et al.*, 1988), all of which are presumed to be substrates for the P-glycoprotein pump system (Cano-Gauci and Riordan, 1987). Affinity analogs of verapamil (Safa, 1988; Yusa and Tsuruo, 1989) and azidopine (Safa *et al.*, 1987), another reversing agent, also label P-glycoprotein, suggesting that both cytotoxic drugs and reversing agents have a single site or a small number of sites for binding on P170.

Careful analysis of the drugs which interact with P170 reveals a few common features including hydrophobicity, positive charge at neutral pH, and a planar aromatic ring structure with a relatively fixed van der Waals radius (Zamora *et al.*, 1988). However, other apparent substrates for the transport system include gramicidin D (Ling, 1985) and valinomycin (M. Horio, I. Pastan, and M. M. Gottesman, unpublished observations), both hydrophobic peptides which intercalate in the plasma membrane and do not have the same structure features as most other drug substrates. Thus, it is still necessary to be cautious in defining the substrates for P-glycoprotein.

Efforts to identify a specific chemical structure which is recognized by the pump have been generally unsuccessful, but recently Pearce *et al.*, reported studies with reserpine analogs in which a potential "pharmacophore" structure has been defined (Pearce *et al.*, 1989). Although many of the substrates for P-glycoprotein clearly do not include this "pharmacophore," such an approach might eventually be helpful in defining the nature of the substrate-pump interaction site(s) (see Structure and Function).

One hint as to how P-glycoprotein recognizes substrates for transport comes from recent studies utilizing a photoactivatable compound, [^{125}I]-INA (iodinated naphthaleneazide) (Raviv *et al.*, 1990). This extremely hydrophobic reagent labels transmembrane regions of proteins in lipid domains nonspecifically, including P-glycoprotein in multidrug-resistant cells. If cells are incubated with doxorubicin, [^{125}I]-INA can be activated by energy transfer from doxorubicin so as to define the location of the drug within lipid domains of cells. As shown in Fig. 3, in drug-sensitive cells activation of [^{125}I]-INA via doxorubicin irradiation results in a nonspecific protein-labeling pattern indistinguishable from that produced by direct activation of [^{125}I]-INA. This result demonstrates that doxorubicin is normally widely distributed in cell membranes. However, in multidrug-resistant cells, P-glycoprotein is the predominant protein labeled when the doxorubicin activation technique is used, indicating that essentially all the doxorubicin in these cells is associated with P-glycoprotein. Thus, the multidrug transporter might act as a

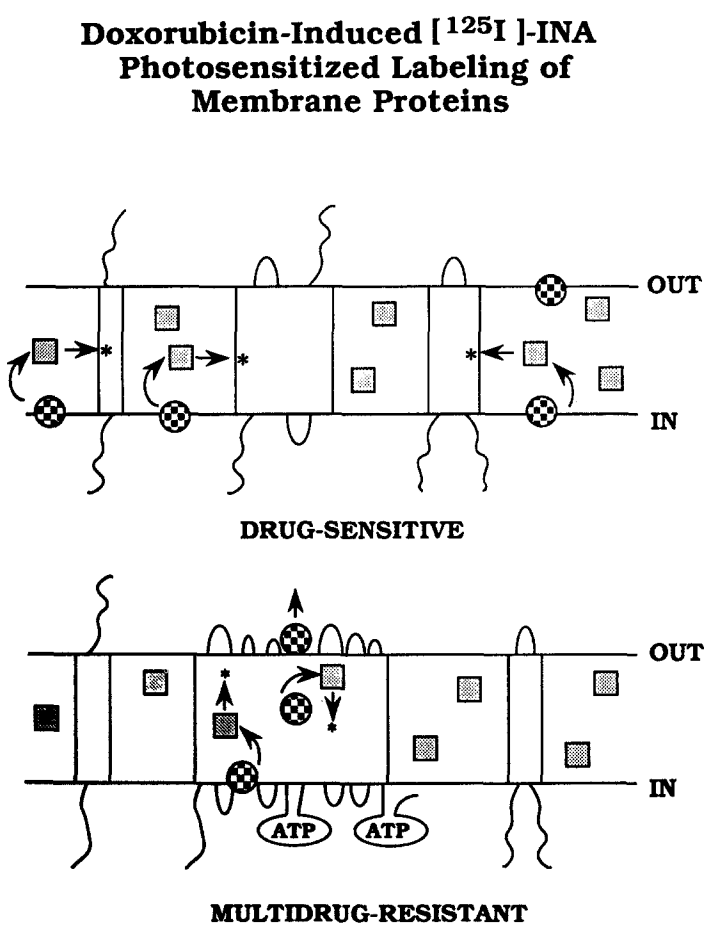

Fig. 3. Cartoon of [^{125}I]-INA labeling in drug-sensitive and drug-resistant cells. Top panel shows the random distribution of doxorubicin (checkered balls) and nonspecific labeling of membrane proteins by [^{125}I]-INA (shaded square) in drug-sensitive cells. Bottom panel depicts the concentration of doxorubicin in the vicinity of P170 and the resulting specific labeling of P170 by doxorubicin-activated [^{125}I]-INA in drug-resistant cells.

hydrophobic "vacuum cleaner" to remove drugs, and perhaps hydrophobic peptides, dissolved in the plasma membrane.

Direct Evidence for Transport of Drugs

Direct evidence for the transport function of P-glycoprotein has come from studies using partially purified membrane vesicles (Horio *et al.*, 1988). Vesicles made from drug-resistant cells but not from drug-sensitive cells will bind radiolabeled photoaffinity drug analogs and will transport

[^{3}H]vinblastine in an ATP-dependent fashion. A nonhydrolyzable analog of ATP acts as a competitive inhibitor of drug transport, and vanadate, an ATPase inhibitor, inhibits transport noncompetitively, indicating that active transport of vinblastine across a membrane requires ATP hydrolysis. Other drugs which contribute to the multidrug resistance phenotype also inhibit transport, as do agents which reverse multidrug resistance (Horio *et al.*, 1988).

Vesicle-transport experiments have also been performed with bile canalicular vesicles containing P170. In this system, using inside-out membrane vesicles prepared from apical membranes of hepatocytes, ATP-dependent transport of daunomycin has been demonstrated (Kamimoto *et al.*, 1989). Vesicle systems such as these provide good models for characterizing the biochemistry of P-glycoprotein and determining the effects of various mutations in P170 on transport activity. To date, functional reconstitution of purified P-glycoprotein into a lipid bilayer has not been achieved. However, the recent report of successful reconstitution of a multisubunit bacterial transporter (hisP) with homology to P-glycoprotein suggests that reconstitution of the ATP-dependent multidrug transporter is feasible (Bishop *et al.*, 1989).

Additional evidence for an ATPase activity associated with P-glycoprotein has come from the laboratory of Tsuruo. P-glycoprotein purified over an MRK16 monoclonal antibody affinity column has residual ATPase activity. Unfortunately, this activity is not drug-dependent (Hamada and Tsuruo, 1988a, b).

A transport function for P170 is also suggested from experiments with epithelia formed by Madin–Darby canine kidney (MDCK) cells (Horio *et al.*, 1989). Both wild-type MDCK cells and MDCK cells infected with a retrovirus carrying a human MDR1 *c*DNA form epithelia in which P170 is expressed in a polar fashion, only on the apical surface. This is consistent with the expression of P170 on the lumenal surface of kidney proximal tubule in normal tissue (see below), and suggests that the protein itself contains the signals needed for proper membrane localization in these cells. The virus-infected cells (MDR-MDCK) are 100-fold more resistant to colchicine than wild-type cells and express correspondingly higher amounts of P170. Epithelia formed by either cell type exhibit net basal to apical flow of vinblastine, vincristine, daunomycin, actinomycin D, and the reversing agent verapamil, with significantly higher rates of transport by the MDR-MDCK cells over wild-type cells. Verapamil, a reversing agent, inhibits basal to apical transport of drugs. These results indicate transepithelial transport of drugs, most likely mediated by P-glycoprotein (Horio *et al.*, 1989).

Our recent report of a transgenic mouse system demonstrates that P-glycoprotein can function *in vivo* to confer drug resistance (Galski *et al.*,

1989). Mouse embryos were injected with a plasmid carrying a human *MDR*1 *c*DNA under the control of a chicken β-actin promoter. The resulting transgenic mice expressed *MDR*1 mRNA mainly in bone marrow and spleen. P-glycoprotein was detected by immunofluorescence on the surface of bone marrow cells, and transgenic mice treated with daunomycin were resistant to leukopenia which normally results from treatment with cytotoxic drugs (Galski *et al.*, 1989). Bone marrow suppression due to such cytotoxicity is often a major obstacle in cancer chemotherapy. By circumventing the bone marrow suppression, this transgenic mouse system potentially represents a model for determining whether high doses of drugs can be used to treat previously unresponsive cancers. This model also provides the first evidence for the minimal level of *MDR*1 gene expression needed to confer drug-resistance *in vivo*. This result is important for interpreting levels of expression of the *MDR*1 gene in human cancers (see below).

Function in Normal Cells

Expression of the *MDR*1 gene has been detected in a number of normal human tissues, both as mRNA and protein. By immunohistochemical staining of normal human tissues, P170 is found in liver, adrenal, pancreas, kidney, colon, and jejunum (Fojo *et al.*, 1987b). Localization within these tissues appears to be very specific. It is on the apical surface only of epithelial cells in liver, kidney, colon, and jejunum; on the biliary canalicular front of hepatocytes; on small biliary and pancreatic ductules; and on cells of the adrenal cortex (Thiebaut *et al.*, 1987; Sugawara *et al.*, 1988a, b). P-glycoprotein has also been detected in human placenta (Sugawara *et al.*, 1988a) and is expressed on specialized endothelial cells in the brain and testis (Cordon-Cardo *et al.*, 1989; Thiebaut *et al.*, 1989). Preliminary results suggest that in the brain it is on the lumenal surface of endothelial cells of some post-capillary venules. Gene-specific RNA analysis indicates that most of the P170 in normal tissues results from MDR1 expression. Expression of the human MDR2 gene is at lower levels, with highest expression in the liver (Van der Bliek *et al.*, 1987; Chin *et al.*, 1989).

In rodents, the three *mdr* genes appear to be expressed in different tissues. Mouse *mdr*1a mRNA is at very high levels in the intestine, with more moderate amounts in brain, heart, and lung, and lower levels in kidney and muscle. mRNA specific to *mdr*1b is very high in adrenal and the uterus of pregnant mice, the latter being localized to the lumenal surface of the endometrium. Moderate amounts of *mdr*1b are expressed by heart and kidney and very low *mdr*1b mRNA is detected in the nongravid uterus. Finally, mouse *mdr*2 is expressed to varying degrees in liver, muscle, spleen, and heart (Croop *et al.*, 1989; Arceci *et al.*, 1988). Preliminary data for the hamster indicate a similar distribution of expression of the three hamster genes (Baas and Borst, 1988; Mukhopadhyay *et al.*, 1988).

These expression data and the similarities in structure between P-glycoprotein and other transport proteins strongly suggest that P170 has a function in normal cells as well as in multidrug-resistant cells. The localization of P170 to specialized cells in human tissues indicates that it might be involved in transepithelial secretion of toxic substances or unknown cell metabolites into bile, urine, or the lumen of the GI tract. In the mouse, *mdr*1a and *mdr*1b would presumably divide this function, since either gene or both are expressed in liver, kidney, and intestine. The high-level expression of *MDR*1 and *mdr*1b in the adrenal suggests a function for secretion of steroids or other adrenocortical products by these genes. *MDR*1 *c*DNA cloned from RNA isolated from the human adrenal has essentially the same sequence as *MDR*1 *c*DNA from multidrug-resistant cells, indicating that the P-glycoprotein in the adrenal should also be fully functional as a multidrug transporter (Kioka *et al.*, 1989). Since *mdr*1b is also expressed in secretory glands of the gravid mouse endometrium, it will be interesting to learn whether the *MDR*1 gene is expressed in the pregnant human uterus as well, but this has not yet been determined. It is possible that the evolution of the rodent *mdr*1b gene may reflect its special function in the uterus during pregnancy in the rodent, and it may not have a human counterpart.

The discovery of P-glycoprotein in the endometrium of mouse gravid uterus also suggests that it might be involved in secretion of steriod hormones, or be regulated by hormones. In a multidrug-resistant mouse cell line, binding of [^{3}H]azidopine (a reversing agent) or [^{3}H]vinblastine to P-glycoprotein is inhibited by the steroid progesterone (Yang *et al.*, 1989). Progesterone also increases [^{3}H]vinblastine accumulation in this system and increases the sensitivity of the multidrug-resistant cells to vinblastine. Thus an endogenous steroid is at least capable of interacting with P-glycoprotein and might represent one class of natural substrates for the pump activity.

Another possibility, in terms of function, is that P-glycoprotein-dependent efflux represents an alternative pathway for secretion of proteins, peptides, or other substances which have no formal signal sequence for secretion (McGrath and Varshavsky, 1989; Kuchler *et al.*, 1989; Gerlach *et al.*, 1986). This idea was originally suggested to explain the broad range of drugs transported by P-glycoprotein, since they could be bound to different proteins, which in turn were transported by the pump (Gerlach *et al.*, 1986). Although it has been shown that host substrates interact directly with the transporter itself, obviating the need for carrier proteins, other data suggest that certain proteins may be substrates for P-glycoprotein. It is believed that one requirement for P170 substrates is hydrophobicity, which helps target potential substrates to the plasma membrane to be recognized by the P170 efflux system. Indeed, there is a direct relationship between hydrophobicity and ability to serve as a drug substrate of P170, such that the ability of

steroids to inhibit P170 in mouse cells increases with increasing hydrophobicity (Yang *et al.*, 1989). In addition, multidrug-resistant cells are resistant to the hydrophobic cytotoxic peptides gramicidin D and valinomycin. And yeast mating factor **a**, the natural substrate for the STE6 gene product, is a hydrophobic dodecapeptide containing a lipophilic farnesyl group at its carboxyl terminus (Anderegg *et al.*, 1988). One possible substrate for P170 according to this mechanism of protein secretion is interleukin-1 (IL-1) (McGrath and Varshavsky, 1989; Kuchler *et al.*, 1989), a secreted protein which apparently is not handled by the classical secretory pathway. Like yeast mating factor **a**, IL-1 precursor has no signal sequence (March *et al.*, 1985) and the mature peptide is modified by the lipid myristic acid (Bursten *et al.*, 1988). If *MDR*1 is not responsible for protein efflux, it is possible that *MDR*2, or a more distantly related transporter, will be found to play such a role.

Another hint as to the possible function of the *mdr* genes comes from their mode of regulation. In the rat, damage to the liver, either by partial hepatectomy (Thorgeirsson *et al.*, 1987) or treatment of the animals with cytotoxic drugs (reviewed in Gottesman, 1988), results in large increases in *mdr* RNA levels. The mechanism of this regulation is not known, but it may involve RNA stabilization, since new transcription as measured by nuclear run-off assays is not seen (Marino *et al.*, 1990). Studies of gene regulation will be facilitated by the development of tissue culture model systems in which expression of the *MDR*1 gene can be modulated. Current examples include the induction by heat shock of the *MDR*1 gene in human renal adenocarcinoma cells (Chin *et al.*, 1990) and the increased expression seen in human neuroblastoma cells after retinoic acid treatment (Bates *et al.*, 1989). Regulation of the *MDR*1 gene also appears to involve differential promoter utilization. In human multidrug-resistant KB cells, transcription initiates at one of two major promoters (Ueda *et al.*, 1987b), whereas in most normal tissues only the downstream promoter is used (Ueda *et al.*, 1987b). The significance of activation of the upstream promoter is unclear, but it may be related to abnormal expression of the *MDR*1 gene after drug selection since human childhood acute lymphocytic leukemias can show activation of this promoter (Rothenberg *et al.*, 1989).

Structure and Function

If the structural homologies between P-glycoprotein and many other transport proteins are significant, it seems that a motif of six transmembrane regions plus a nucleotide binding site is important for function. There might, for conformation reasons, be a need for at least two such units, as in P170,

which in turn might exist as dimeric or tetrameric complexes in the plasma membrane. One possibility is that a large structure is required to form a membrane channel or pore big enough to accommodate bulky or high-molecular-weight substrates. Alternatively, each unit of transmembrane region plus ATP-binding domain might contribute uniquely to substrate binding specificity or function in some other way, such that the P-glycoprotein molecule must be thought of as a whole rather than as two homologous halves. This is consistent with the observation that neither half of P170 can function alone (Currier *et al.*, 1989).

However, we know very little at this point about the contributions of different structural domains to the function of P-glycoprotein. P170 appears to be very sensitive to perturbations in its amino acid sequence, in assays measuring ability of mutant P170s to confer drug resistance. Even though *MDR*1 and *MDR*2 are 85% homologous, the *MDR*2 gene product has not been known to mediate multidrug resistance (Gros *et al.*, 1988; Van der Bliek *et al.*, 1988). Furthermore, a deletion of as few as 53 residues at the carboxyl terminus of the *MDR*1 coding sequence completely abolishes the multidrug resistance phenotype when the mutated gene is transfected into drug-sensitive NIH 3T3 cells (Currier *et al.*, 1989). The same is true for substantial internal deletions of 267 bp in the amino half of the protein (removing the first cytoplasmic loop through the fourth transmembrane domain) or 906 bp in the carboxyl half (removing the sixth through twelfth transmembrane domains). Deleting 23 C-terminal residues reduces the ability of P170 to confer resistance but does not eliminate activity altogether (Currier *et al.*, 1989).

Independent evidence indicates that the nucleotide binding sites in each half of P-glycoprotein are important in drug resistance, but the two sites apparently are not identical. Inactivation of both binding sites abolishes all activity, while inactivation of either site alone leaves either no activity or only a small amount of residual resistance to colchicine, vinblastine, and actinomycin D (Azzaria *et al.*, 1989; I. Roninson, personal communication). The patterns of residual resistance are different, however, depending on which nucleotide binding site is mutated. This suggests that both halves of P170, and possibly the nucleotide binding sites themselves, are somehow involved in specificity of drug recognition or binding. Indeed, the two halves might be closely associated with each other in the folded protein, such that the integrity of P170 structure or conformation is important.

This idea is supported by studies on the interaction between P-glycoprotein and [^{3}H]azidopine, a photoaffinity ligand which probably binds P170 in a manner similar to cytotoxic drug substrates and specifically labels the mutidrug transporter. In these experiments, P170 is allowed to react with [^{3}H]azidopine, and proteolytic fragments of the labeled protein are analyzed. Two distinct labeled regions, one from each half of the protein, are detected,

suggesting that either there are two drug binding sites or there is one site which is composed of regions from both halves of the molecule (Bruggemann *et al.*, 1989). It will be interesting to know the results of similar labeling experiments performed with transporter proteins which are mutated either in putative drug binding domains or in ATP-binding sites.

The issue of drug binding and specificity is also intriguing in light of very early observations that drug-resistant cell lines are usually most resistant to the drug in which resistance was originally established (Croop *et al.*, 1988; Moscow and Cowan, 1988; Shen *et al.*, 1986). Although these cells are cross-resistant to the whole battery of drugs within the multidrug resistance phenotype, the pattern of cross-resistance can vary from cell line to cell line. Some information on drug specificity comes from the discovery that P170 in colchicine-selected human cells differs from P170 in vinblastine-selected or in wild-type, drug-sensitive cells at only a single amino acid residue (Choi *et al.*, 1988; Kioka *et al.*, 1989). The "mutant" protein encoding a preference for colchicine resistance has a valine at codon 185 in the P170 sequence, while the "wild-type" sequence is glycine at that residue. When the mutant gene is transfected into drug-sensitive cells, the cells become relatively more resistant to colchicine than vinblastine or adriamycin, the drugs used in these studies. Cells containing the wild-type gene are uniformly resistant to these agents. Thus residue 185 seems to be responsible for preferential resistance to colchine, and might be involved in the specificity of drug interaction with P170. Amino acid 185 is contained within one of the regions labeled by [^{3}H]azidopine in the experiments described above (Bruggemann *et al.*, 1989).

There also appears to be a difference in resistance conferred by the three rodent *mdr* gene products. Within a single mouse cell line, J774.2, as drug resistance increases during stepwise selection with increasing amounts of vinblastine, *mdr* gene expression also changes. At low concentrations of vinblastine, *mdr*1b mRNA and protein are expressed, while at higher concentrations of drug, cells expressing the *mdr*1a gene predominate (Hsu *et al.*, 1989). These results suggest that there might be a selective advantage at high vinblastine levels for those cells which express *mdr*1a, as opposed to *mdr*1b, indicating probable functional differences between the two protein products. These observations might also be explained by a regulated switch for *mdr*1b to *mdr*1a synthesis with higher drug concentrations. These results are consistent with the different tissue-specific expression of *mdr*1a and *mdr*1b, and reinforce the idea that these genes, although both capable of conferring multidrug resistance, have different functions in the mouse.

Further comparisons between *mdr*1a and *mdr*1b sequences and analysis of their respective functions in multidrug resistance or other substrate transport could be very informative. This will be true for all three of the rodent genes as well as for *MDR*1 and *MDR*2 in humans, in light of tissue-specific

differences in expression and perhaps function. It appears that the determinants of substrate interaction and transport function will be complex and probably encompass the entire structure of these proteins. Intermolecular and interspecies chimeras might be interesting in terms of dissecting the relationship between structure and function.

Clinical Relevance of Expression of the *MDR*1 Gene

Expression of the MDR1 Gene in Human Cancers

If expression of the *MDR*1 gene contributes to the phenomenon of multidrug resistance in human cancer, it should be possible to detect this expression in human tumors and expression should correlate with chemotherapy responsiveness. Two general approaches have been used for analyzing *MDR* expression in human cancer—those which determine levels of *MDR*1 mRNA (RNA slot blots or Northern analysis, *in situ* hybridizations, and PCR amplification of RNA samples) and those which look at amounts of P170 protein (Western blots and immunohistochemistry). Each method must be considered in terms of sensitivity, specificity for *MDR*1 expression, use as a quantitative assay, and ability to detect *MDR*1 expression within a heterogeneous background of nonexpressing cells.

Findings so far indicate that *MDR*1 expression depends very much on tumor type. Expression is high in those tumors which derive from tissues that normally express a lot of P170 (see *Function in Normal Cells*), including colon, kidney, adrenal, liver, and pancreas (Fojo *et al.*, 1987b; Goldstein *et al.*, 1989). The level of expression in these tumors is comparable to the expression in bone marrow of transgenic mice carrying the *MDR*1 gene, and the marrow of these mice is known to be daunomycin-resistant *in vivo* (Galski *et al.*, 1989). Human tumors of this type are most often intrinsically resistant to standard chemotherapy, that is, they respond poorly to drug treatment. Adenocarcinomas of the kidney, which are derived from the proximal tubule cells which normally express P-glycoprotein, have been studied in most detail. The most differentiated tumors express the most P-glycoprotein (Kanamaru *et al.*, 1989), and there is a correlation between levels of *MDR*1 RNA and resistance to vinblastine in tissue culture explants from these tumors (Kakehi *et al.*, 1988). Cell lines derived from adenocarcinomas of the kidney express *MDR*1 RNA and are multidrug resistant, and this resistance is reversible by drugs such as quinidine and verapamil, which are known inhibitors of multidrug transport by P-glycoprotein (Fojo *et al.*, 1987a). Taken together, these data suggest that expression of the *MDR*1 gene contributes to the clinical multidrug resistance of renal adenocarcinomas.

Many other cancers, including neuroblastomas, leukemias, lymphomas, sarcomas, and ovarian and breast cancers, tend to show low *MDR*1 gene expression prior to chemotherapy and often have increased *MDR*1 RNA levels after treatment (Goldstein *et al.*, 1989; Salmon *et al.*, 1989). In some cases, however, such as most lung cancers (Lai *et al.*, 1989), intrinsically resistant tumors do not have detectable levels of P170 and *MDR*1 expression does not always correlate with response to chemotherapy, suggesting that other mechanisms of drug resistance must exist (see Moscow and Cowan, 1988, for a review of other mechanisms of multidrug resistance).

It remains to be seen, after much more extensive sampling and appropriate longitudinal studies pre- and post-treatment, whether *MDR*1 gene expression varies with stage of treatment for certain tumors and whether expression of the multidrug transporter is diagnostic for nonresponse to chemotherapy. A preliminary study of adult patients with acute nonlymphocytic leukemia suggests that the level of expression of the *MDR*1 gene is one of the best prognostic indicators available for response to a chemotherapy regimen including daunomycin (Sato *et al.*, 1990a, b). Similar studies will be needed for other cancers which express the *MDR*1 gene to assess the relative contribution of the multidrug transporter to the drug-resistance phenotype.

Future Directions for Clinical Studies

Because of the association of *MDR*1 gene expression with drug resistance in many human cancers, a number of clinical trials have been initiated with agents which are known to inhibit the pump activity of the multidrug transporter *in vitro*. These agents might also sensitize cancers to chemotherapy with natural product cytotoxic drugs. A clinically ideal reversing agent will be one which enhances the cytotoxic effects of chemotherapeutic drugs on tumor cells without increasing nonspecific cytoxicity for normal tissues. Many classes of reversing agents are known, including verapamil and other calcium channel blockers, amiodarone, quinidine, reserpine, phenothiazines, cyclosporins, and many other hydrophobic natural products (Gottesman and Pastan, 1989). Yet, most of these compounds already have significant pharmacologic effects. Nevertheless, trials have been reported which use verapamil in low doses for treatment of drug-resistant multiple myeloma, and the cancers show a renewed response to chemotherapy (Dalton *et al.*, 1989). Trials are ongoing at the National Cancer Institute to evaluate the usefulness of amiodarone or quinidine for the treatment of colon and kidney cancer with adriamycin and etoposide (A. Fojo, I. Pastan, and M. M. Gottesman, unpublished data). Results from these and other studies should be available within the next year or so. However, it is clear that less toxic reversing agents will need to be developed before more extensive clinical trials can be

considered. To do this, nontoxic analogs of many natural products are being screened in tissue culture for their reversing ability, and these will need to be tested in an appropriate animal model for activity, and undergo Phase I (toxicity) trials in humans. We are still many months from the development of a safe and effective inhibitor of the multidrug transporter (if such exists) which could be used to test the role of this transporter in the drug resistance of human cancer.

Other Applications of the MDR Genetic System

The MDR1 Gene as a Selectable Marker for Eukaryotic Expression Systems

In transfection experiments with tissue culture cells, a *c*DNA of the human *MDR*1 gene is able to confer multidrug resistance on previously drug-sensitive cells (Ueda *et al.*, 1987a). Furthermore, in cell lines selected for multidrug resistance which overexpress the endogenous *MDR*1 gene, the gene is frequently amplified and surrounding genomic sequences are coamplified (Fojo *et al.*, 1985b; Shen *et al.*, 1986). These observations led us to develop a system which uses the *MDR*1 *c*DNA as a selectable marker for expressing heterologous genes in eukaryotic cells. Cells stably transfected with the *MDR*1 vector plus a second plasmid can be selected for drug resistance by virtue of *MDR*1 expression, and these cells also express the cotransfected sequences (Kane *et al.*, 1988). Expression of the *MDR*1 gene and of the cotransfected gene can be amplified by growing the cells in increasing concentrations of a selective agent. The *MDR*1 expression system has recently been modified so that *MDR*1 and the heterologous sequences are present on a single plasmid (Kane *et al.*, 1989). Transfected cells selected for drug resistance also stably express and amplify the nonselected gene with extremely high efficiency. These methods are useful for synthesizing very high levels of a variety of proteins in tissue culture cells.

Gene Therapy Using the MDR1 Gene as a Selectable Marker

Human gene therapy involves the introduction of genes which are frequently unselectable into a stably expressing cell type or tissue to correct an inborn error of metabolism or provide a therapeutic gene product. Several strategies have been devised to link selectable genes to the nonselectable genes of interest so as to guarantee stable expression of the nonselectable gene. The *MDR*1 gene, because it confers resistance to a wide variety of cytotoxic drugs, is a potential selectable marker for such gene transfer experiments. We have successfully introduced a human *MDR*1 *c*DNA into a retroviral

expression vector and infected mouse bone marrow cells so as to obtain vinblastine- and colchicine-resistant granulocyte macrophage colony-forming units with good efficiency (McLachlin *et al.*, 1990).

Once conditions have been established for selecting drug-resistant marrow (or some other tissue) *in vivo*, it will be desirable to introduce a nonselectable gene along with the *MDR*1 *c*DNA. To guarantee that *MDR*1 expression is tightly linked to expression of the nonselected gene, we have demonstrated the feasibility of forming functional chimeric proteins by fusions at the carboxy-terminus of P-glycoprotein. One such fusion between the multidrug transporter and an adenosine deaminase (ADA) *c*DNA has been described (Germann *et al.*, 1989). After selection for colchicine resistance, the fusion protein is localized to the plasma membrane of transfected cells. The protein retains both multidrug transport function and ADA enzyme activity. The fusion construction is cloned in a retroviral expression vector such that MDR-ADA virus can be isolated after transfection of appropriate packaging cell lines. The MDR-ADA virus can be used to stably introduce the ADA gene into cultured cells and, potentially, into bone marrow.

Conclusions

The pursuit of the molecular basis for resistance to chemotherapeutic drugs has unearthed a novel ATP-dependent transport system with analogs in many organisms. Further analysis of this system should reap benefits in terms of our understanding of plasma membrane transport systems, and in the treatment of human disease.

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