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The Basis of Multidrug Resistance in Mammalian Cells: Homology With Bacterial Transport

Minireview

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It is always gratifying to uncover a unifying relationship among vastly different organisms. A new example of such universality is described by two research groups in this issue of *Cell* (Gros et al., pp. 371–380; Chen et al., pp. 381–389). These investigators have found that a class of mammalian membrane glycoproteins implicated in the phenomenon of multidrug resistance (Mdr) in tumor cells bears strong homology to a class of well-studied bacterial transport proteins.

The development of resistance to cytotoxic agents in tumor cells is one of the causes of failure in cancer chemotherapy. The problem is compounded by the fact that tumors become resistant simultaneously to many of the commonly used antineoplastic agents, several of which are structurally unrelated. Several groups have isolated drug-resistant rodent and human cell lines by exposing cells to sequentially increasing concentrations of a single drug (such as vinblastine, colchicine, or adriamycin). In all cases, the final cell line displayed resistance to additional drugs to which it had not been exposed. These studies reproduced the pattern observed in vivo during treatment of tumors, and opened the way for standard genetic and biochemical approaches in uniform cell populations.

Physiological studies of these Mdr cell lines have provided strong indications that this resistance has its basis in an altered ability to accumulate the drugs intracellularly. In the parent cells, drug uptake occurs by passive diffusion; inhibitors of energy metabolism raise the intracellular level of the drug, suggesting that the cell actively pumps out the drug against its concentration gradient (Skovsgaard, Cancer Res. *38*, 1785–1791, 1978). Resistant cells contain lower levels of several drugs (regardless of which drug was used initially to isolate the resistant line), apparently by expelling the internalized drug at a rate higher than that of the sensitive parent cells (Fojo et al., Cancer Res. *45*, 3002–3007, 1985; Riordan and Ling, Pharmac. Therapeut. *28*, 51–75, 1985). Surprisingly, however, in Mdr cells there is no correspondence between the level of resistance to a drug and its ability to compete in transport and binding of other drugs; a possible interpretation of this puzzling result is presented below.

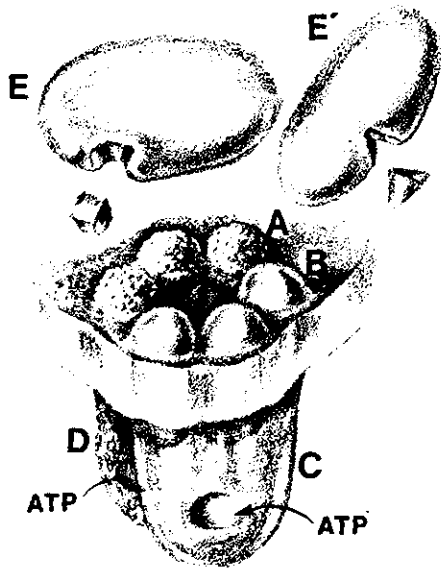
Considerable progress has been made toward understanding the biochemical basis of the Mdr phenomenon. Mdr cell lines have been shown to contain a ~140 kb region of amplified DNA whose level of amplification correlates with the level of drug resistance (Gros et al., PNAS *83*, 337–341, 1986; Fojo et al., PNAS *82*, 7661–7665, 1985; Roninson et al., PNAS *83*, 4538–4542, 1986). When drug-sensitive cells are transfected with these genomic DNA sequences (from both rodent and human cells), they acquire the same Mdr pattern as donor cells (Shen et al. and Gros et al., MCB *6*, in press). A ~5 kb mRNA is transcribed from the amplified DNA and is overproduced in Mdr cells to extents that correlate well with the level of resistance. Hybridization studies suggest that the amplified DNA contains a small family of related genes (*mdr* genes); however, a single member of this family is sufficient to confer multiple drug resistance.

Several alterations in the protein composition of Mdr cells have been detected. One of the most striking and consistent changes is an increase in the amount of a 170 kd membrane glycoprotein, named gp170 (Riordan et al., Nature *316*, 817–819, 1985), which has been shown to bind vinblastine and vincristine (Cornwell et al., PNAS *83*, 3847–3850, 1986; Cornwell et al., JBC, in press) and is phosphorylated (Carlsen et al., BBA *467*, 238–250, 1977). The *mdr* sequences code for gp170, and *mdr* transcripts have been found in a variety of normal tissues; adrenal tissues contain a particularly high level (Fojo et al., *PNAS*, in press).

In this issue of *Cell*, two groups report the sequence of *mdr* cDNAs from human (Chen et al.) and mouse (Gros et al.). The deduced amino acid sequences show a high level of homology and correspond to proteins of ~1280 amino acids. Both proteins are composed of two homologous halves, each half including a very hydrophobic region and a hydrophilic region. Surprisingly, the hydrophilic regions share strong homology with bacterial transport proteins.

Gram-negative bacteria transport a variety of substrates (sugars, amino acids, ions, and peptides) through periplasmic transport systems (permeases), so-called because one of their components is a soluble protein located within the periplasm, a space between the inner and outer cell membranes (Ames, Ann. Rev. Biochem. *55*, 397–425, 1986). Periplasmic permeases, which typically have high affinity for their substrates, have a complex organization, consisting of at least four proteins: a periplasmic protein that tightly binds the solute being transported; two hydrophobic membrane proteins that might also bind the solute and that probably originated by duplication since they display various levels of homology, and possibly form a pseudodimer; and one peripheral membrane protein that has a hydrophilic sequence, and binds ATP or a related nucleotide.

The ATP-binding proteins of all of these permeases share extensive homology and are highly homologous to the hydrophilic portions of the Mdr proteins. The homology includes, and extends far beyond, the ATP-binding site proper, indicating a strongly conserved functional similarity. It is interesting that the genes coding for the homologous ATP-binding proteins are often duplicated, and either maintained as such or as a fusion gene that codes for a single protein made up of two homologous halves, each having an ATP-binding site (Buckel et al., JBC *261*, 7659–7662, 1986). On the other hand, there is very poor or no homology between the various periplasmic proteins (except between related permeases), or between various



pairs of the hydrophobic membrane proteins. It is important to note that the membrane-bound complex in several of these permeases is used by more than one periplasmic component, thus channeling the transport of different (though somewhat related) solutes through the same membrane complex (e.g., the single membrane-bound complex of the histidine permease transports substrates as different as histidine, arginine, and O-diazo-acetylserine). It is likely that the membrane complex forms a pore with only partial specificity for a particular substrate. In each permease, the specificity would be greatly influenced by the periplasmic component.

How do the structure and function of bacterial permeases relate to the Mdr phenomenon? Given the known facts concerning periplasmic permeases and the involvement of Mdr in transport, it is possible to put together the following hypothetical model and analogies. Protein gp170 has incorporated into a single molecule the features of the entire membrane-bound complex of the bacterial counterpart—two homologous hydrophobic domains and two homologous hydrophilic domains, the latter involved in binding ATP. Each set of domains might be involved in the formation of pseudodimers. Substrates can bind to this molecule and the ATP-binding domain is somehow involved in energizing its transport function. To complete the analogy with bacterial systems, specific substrate-binding proteins might be present in excess; these would function in "trapping" the drug with high affinity and "delivering" it to gp170. Several substrate-binding proteins with different specificities could use the same (or a class of related) gp170, thus raising (and/or altering) the specificity and efficiency of transport. This might explain the puzzling lack of reciprocity in drug competition studies referred to earlier. If gp170 normally is the limiting component, increasing its level in Mdr cells would result in increased transport of several drugs.

The figure depicts this model in schematic form: structures A and B represent a pseudodimer of the two hydrophobic proteins (or of the two hydrophobic domains of a gp170 molecule); structures C and D represent a homodimer or pseudodimer of the ATP-binding protein (or of domains of a single molecule); and structures E and E' represent two different specific substrate-binding proteins.

An interesting aspect is that of directionality. The outward activity of the Mdr system might be due to its being positioned in the opposite orientation relative to the cell interior, as compared to bacterial systems. The postulated specific substrate-binding protein would be in the cytoplasm, while the ATP-binding domain of gp170 would be located outside the cell (it is questionable whether sufficient ATP would be available there, however). Alternatively, gp170 might be organized so that both the ATP-binding domain and the substrate-binding protein are on the cytoplasmic side of the membrane.

Clues to the mechanism of action of these systems (bacterial and eukaryotic) may come from an understanding of the function of other bacterial proteins that also share extensive homology with the ATP-binding transport components: UvrA (DNA repair), HlyB (export of hemolysin), FtsE (cell division), and nodI (nodulation in Rhizobium) (Doolittle et al., Nature *323*, 451–453, 1986; Higgins et al., Nature *323*, 448–450, 1986). Although most of these systems involve membrane-associated functions, it seems unlikely that they use a periplasmic-type transport mechanism. Thus, this group of homologous proteins, including gp170, may perform a basic function related to energy coupling but not necessarily to transport. It should be noted that several systems actively expelling noxious drugs exist in bacteria, such as the amplifiable tetracycline resistance pumping activity encoded by several transposons (Levy, in *Antimicrobial Drug Resistance*, ed. L. E. Bryan, Academic Press, Inc., 1984, pp. 191–240), and pumps for ions such as arsenate and calcium (Rosen et al., Ann. NY Acad. Sci. *456*, 235–244, 1985).

Among the most important questions to be answered is one concerning the normal function of the Mdr system that may be used "illicitly" by these drugs. Considering the high level of expression of the *mdr* gene(s) in adrenal tissues, it is likely that this system has an important physiological role in this tissue, possibly related to steroid and/or catecholamine production, packaging, or secretion. This knowledge may be put to use towards designing drugs that are not recognized by this system or that are actively pumped into the cell through an unrelated system. In this respect, the concept of "illicit transport" and its practical implications (Ames et al., PNAS, *70*, 456–458, 1973) may be useful towards developing alternative chemotherapeutic drugs.