Multidrug ABC transporters from bacteria to man: an emerging hypothesis for the universality of molecular mechanism and function

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The ATP-binding cassette (ABC) superfamily of membrane-associated transporters encompasses a diverse group of proteins in a wide variety of organisms and cell types, many of which have been implicated in human diseases. In addition to highly homologous ATP-binding domains, most of these proteins have multiple transmembrane (TM) spanning domains and function as ATP-dependent transporters.[1,2] In eukaryotic cells, members of this family include the multidrug transporter, P-glycoprotein (P-gp), the related multidrug resistance-associated protein (mrp1) encoded by the *MRP1* gene,[3] the cystic fibrosis transmembrane regulator (CFTR),[4] the STE6 a-mating factor transporter in the yeast *Saccharomyces cerevisiae*,[5,6] and the ABCR protein in retina implicated in macular degeneration.[7,8] Additional members of this family are the peroxisomal membrane pumps, PMP70[9-11] and ALDP[12] and the related ALDRP[13] whose mutated versions have been implicated in the cerebro-hepato-renal autosomal recessive disorder Zellweger syndrome and adrenoleukodystrophy, repectively. Additionally, other membrane-associated transporters in the endoplasmic reticulum have been shown to be involved in the transport of peptides for major histocompatibility Class I antigen presentation (TAP1 and TAP2).[14-16] ABC proteins have also been identified in a number of prokaryotes including *Escherichia coli* and *Bacillus subtilis*. Interestingly, the complete genome sequence of *B. subtilis* revealed the existence of 77 putative ABC proteins suggesting an extremely important role for these types of proteins in this Gram-positive bacterium.[17] P-gp, the protein closely associated with multidrug resistance in human cancers encoded by the *MDR1* gene, has served as a paradigm for studying the mechanism of action of ABC transporters. Study of these related transporters should offer further insight into how human P-gp functions and will hopefully lead to the development of more effective means to combat the multidrug resistance phenomenon in cancer.

In the 15 January, 1998 issue of *Nature*,[18] van Veen et al reported functional complementation of the human multidrug-resistance P-gp gene (*MDR1*) in human lung fibroblast cells using the bacterial antibiotic-resistance gene, *lmrA*, from *Lactococcus lactis*. The *lmrA* gene was cloned and sequenced in 1996 by the same group.[19] The LmrA protein differs strikingly from human P-gp in that it is only half the size, being composed of six transmembrane domains and one ATP binding/utilization domain (Fig. 1). It is 34% identical to each half of human P-gp, and, although most of the identity between the molecules lies in the ATP binding/utilization domain, the transmembrane domain does show a remarkable 23% and 27% identity to the N- and C-half transmembraane domains of P-gp. Interestingly, this transmembrne domain homology includes the putative transmembrane domains implicated as determinants of substrate specificity in human P-gp.[1] The *lmrA* gene was then functionally expressed in *E. coli*, a feat difficult to duplicate with human P-gp,[20] and in the human lung

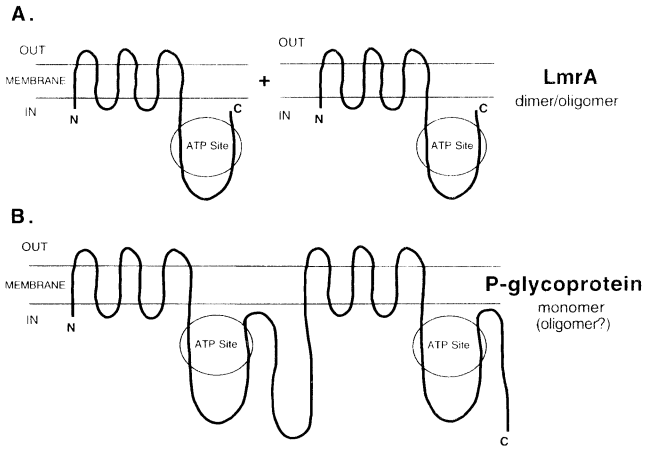

Fig. 1. Schematic diagrams of the hypothetical models of bacterial LmrA (A) and human P-glycoprotein (B). LmrA contains six TMs and one ATP site per subunit and is thought to function as a dimer or higher order oligomer. P-glycoprotein has 12 TMs and can function as a monomer.

fibroblast cell line, GM0637, demonstrating its potential for use not only as a prokaryotic model but also in mammalian cells as well. Strikingly, even with significant differences in codon usage, when expressed transiently in human cells, the protein is targeted to the plasma membrane and confers drug resistance to a variety of typical MDR agents on these cells. However, it is unclear from these studies whether this resistance is comparable in degree to that conferred by human P-gp expressed under similar conditions, or whether it is possible to generate stable drug resistant cell lines using *lmrA*. In in vitro studies using LmrA and P-gp expressed in insect cells, both proteins demonstrate similar characteristics with respect to vanadate-sensitive drug-stimulated ATPase activity, substrate and modulator specificities and allosteric binding site properties for substrates and non-competitive inhibitors as well as in ability to extract drugs from the lipid bilayer,[22] strongly suggesting that these two multidrug-resistance proteins share a common mechanism of substrate recognition and transport.

The 'half-molecule' quandary has been the subject of intense interest recently with the discovery of a number of related mammalian and prokaryotic ABC transporters having this structure. A gene encoding a P-gp-like transporter from *Trichomonas vaginalis*, an early divergent protozoan, has also been cloned and sequenced; the gene, Tvpgp1, encodes a 589-amino acid polypeptide containing 6 putative TM domains and one putative ATP-binding cassette.[23] Sequence analysis of the deduced primary structure of Tvpgp1, currently being investigated in our laboratory, also reveals greatest overall similarity to the amino and carboxyl-terminal halves of human P-gp. The study of these half-molecules should be especially important in studying the function of human P-gp and, in fact all ABC transporters, by defining the minimal functional unit of human P-gp and addressing the issue of whether or not a single half transporter is capable of full function. These studies will also offer the opportunity to learn more about the structure and function of ABC transporters themselves. The 'half-molecules' are thought to function as dimers (see Figure 1); in the case of the TAP transporter, the two proteins are known to form a heterodimer. Similarly, the *Saccharomyces cerevisiae* homologs of the mammalian peroxisomal PMP70 and ALDP proteins have also been shown to be heterodimers.[24] LmrA and the white and brown pigment ABC proteins in *Drosophila melanogaster*[25,26] are unique, however, in that they are heterodimers targeted to the plasma membrane whereas all other half-molecules described to date are found in intracellular membranes.[27]

The next major breakthrough in understanding the mechanism of action of human P-gp and all ABC transporters will occur with the generation of high resolution structures. Attempts at obtaining such information using electron microscopy and single particle image analysis have met with some preliminary success,[27] but as for many membrane proteins, the difficulty in making large quantities of P-gp for structural studies and the hydrophobicity of the protein conspire against obtaining higher resolution images of 2-dimensional and 3-dimensional crystals. It is clear that for human P-gp, both halves are necessary for proper functioning and most data suggests that the 12 TM-2 ATP site P-gp can function as a monomer.[21] It will therefore be of interest to determine the subunit structure of LmrA since a half molecule functioning independently as a monomer would be an ideal candidate for pursuing 2-dimensional and 3-dimensional structural studies. However, higher order structure (dimer or greater) seems likely (Fig. 1).

An additional prospect for LmrA, and in fact other 'half-molecules' capable of conferring drug resistance, is in the applications to gene therapy. The goal of gene therapy is to modify cells genetically so that they can supply a necessary or useful function to the host. Vectors employing drug resistance genes as selectable markers in vivo have useful applications not only in the treatment of cancer by conferring drug resistance on drug sensitive normal tissues such as bone marrow, but also in the treatment of hereditary metabolic disorders presently curable by bone marrow transplantation. In this approach, the MDR-based vector expressing either the *MDR1* gene alone or coupled to a non-selectable gene of interest is transfected or transduced into drug-sensitive bone marrow cells.[3] Following treatment with MDR drugs, the untransduced/untransfected cell will be killed and the cells expressing the genes of interest will necessarily expand. One of the problems presently facing the field of gene therapy lies in the efficient packaging and delivery of the genes of interest. This problem is, in significant part, due to the large size of the vectors presently being used given that the *MDR1* gene is approximately 4 kB in length. If LmrA, or another half molecule capable of conferring significant drug resistance to cells, could replace *MDR1* in this system, greater gene delivery efficiency could be obtained making the therapy more feasible and effective.

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