tive cooperativity of vinblastine binding was observed. The presence of two vinblastine-binding sites is further supported by *i*) heterologous displacement experiments in which vinblastine bound by LmrA was displaced by the inhibitor CP 100-356 in a biphasic fashion, and *ii*) stoichiometric determinations which reveal the binding of two vinblastine molecules per LmrA transporter. Trapping of LmrA in the ADP/vanadate-bound transition-state conformation resulted in reduced efficiency of photo-affinity labelling with (3H)APDA, indicating that drug binding and ATP hydrolysis are coupled. Vinblastine-displacement experiments and vinblastine/transporter stoichiometry determinations suggest that the ADP/vanadate-trapped transporter contains a single low-affinity drug-binding site.

These observations have been incorporated in a sequential two-site mechanistic model of the LmrA transporter. It is obvious from the observations described above that LmrA is not only a structural but also a functional homologue of P-glycoprotein. To test that this is indeed the case, LmrA was functionally expressed in insect cells, oocytes, and human fibroblast cells. The analysis of the pharmacological properties of LmrA in these heterologous expression systems showed that LmrA and P-glycoprotein behave very similar.

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Modulation of Multidrug Resistance in Cancer Cells by Inhibitors of P-Glycoprotein

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Most metastatic cancers are either intrinsically resistant to chemotherapy or acquire drug resistance subsequent to chemotherapy. Therefore, a major problem in cancer chemotherapy is the development of resistance to chemotherapeutic agents in tumor cells.

In vitro selection of cancer cells for resistance to lipophilic cytotoxic agents usually results in the development of cross-resistance to many other drugs which share little structural similarity. These drugs include *Vinca* alkaloids, anthracyclins, epipodophyllotoxins, taxol, actinomycin D, colchicine, puromycin, gramicidin D_1, and others. The hallmark of this type of multidrug resistance is overexpression of the MDR1-gene product, a 170 kDa phosphoglycoprotein termed P-glycoprotein (P-gp), which is involved in the efflux of these agents. We and others have shown that many structurally distinct agents reverse multidrug resistance (MDR) by binding to P-gp and inhibiting the efflux of the MDR-related cytotoxic agents. However, the precise mechanism of how these agents interact with P-gp and reverse MDR is not well understood.

To investigate how the MDR-related cytotoxic agents bind to P-gp and inhibits effluxes, and whether there is a direct relationship between MDR reversal and modulator interaction at the level of P-gp inhibition, we have used five approaches including *1*) reversing MDR in drug-resistant cell lines by using potent MDR modulators, *2*) performing drug-accumulation studies in drug-sensitive and MDR variants, *3*) synthesizing photoaffinity analogs of the MDR-related cytotoxic agents and modulators, and identifying and characterizing their binding sites by photoaffinity labeling, proteolytic digestion, and using P-gp epitope-specific antibodies, *4*) inhibiting vinblastine, doxorubicin, verapamil, and azidopine by photolabeling of P-gp, and *5*) performing kinetic analysis and inhibition of binding of radioactive vinblastine and vincristine to plasma-membrane vesicles under equilibrium conditions. Using these approaches, we have found that potent modulators of P-gp interact either competitively or allosterically with the cytotoxic-drug-binding sites of P-gp and inhibit its function, thereby allowing the cytotoxic agent to accumulate in the MDR cells, inhibit growth, and cause cell death. Among diverse classes of modulators, we have found that cyclosporin A and its non-immunosuppressive analog PSC 833, FK-506, two diaminoquinazolines CP100356 and CP114476, the quinoline derivative CP117227, tamoxifen, several reserpine and yohimbine derivatives, megestrol acetate, and phenothiazines and related compounds, particularly *trans*-flupentixol and iodoazidophenethylspiperone (I-NAPS) are effective inhibitors of P-gp.

Our results using these modulators demonstrate a direct relationship between the reversal of MDR and their interaction with the drug-binding sites of P-gp. Moreover, our data revealed that vinblastine, verapamil, and azidopine bind to at least one common binding domain located within or immediately *C*-terminal to transmembrane domain 6 (TMD 6) of P-gp. However, kinetic analysis revealed that vinblastine and cyclosporin A competitively interact for the same binding site while they non-competitively interact with the azidopine-binding site. Therefore, these results suggest that vinblastine and azidopine bind to separate binding sites on a common domain. Interestingly, deletion of a phenylalanine at position 335 of P-gp (located in TMD 6) has been shown to cause resistance to cyclosporin A and PSC 833. Our data provide direct evidence that TMD 6 is an important domain of P-gp for recognition of some MDR-related drugs and potent P-gp inhibitors.