Considerable uncertainty surrounds the stoichiometry of coupling of ATP hydrolysis to drug pumping by P-glycoprotein, the multidrug transporter. To estimate relative turnovers for pumping of the drug vinblastine and ATP hydrolysis, we began by measuring the number of P-glycoprotein molecules on the surface of murine NIH3T3 cells expressing the human MDR1 gene. Fluorescence of cells treated with monoclonal antibody UIC2 was determined as a function of (i) amount of antibody at a fixed number of cells and (ii) increasing cell number at constant antibody. The two together gives $1.95 \times 10^6$ P-glycoprotein molecules/cell. Initial uptake rates of vinblastine ± verapamil measure the ability of P-glycoprotein to extract vinblastine from the plasma membrane before it enters the cell. As a function of [vinblastine] at $37^\circ C$, they give the maximum rate of this component of outward pumping as $2.1 \times 10^6$ molecules s$^{-1}$ cell$^{-1}$ or a turnover number of $1.1$ s$^{-1}$. Initial rates of one-way efflux as a function of [vinblastine] at $25^\circ C \pm$ glucose give the maximum rate of this component of pumping as $0.59 \times 10^6$ molecules s$^{-1}$ cell$^{-1}$. The ratio of ATPase activity of P-glycoprotein at $37$ and $25^\circ C$ is $4.6$. Appropriating this ratio for pumping, maximum one-way efflux at $37^\circ C$ is $4.6 \times 0.59 = 2.7 \times 10^6$ molecules s$^{-1}$ cell$^{-1}$, a turnover number of $1.4$ s$^{-1}$. The vinblastine-stimulated ATPase activity of P-glycoprotein has a turnover number of $3.5$ s$^{-1}$ at $37^\circ C$, giving $2.8$ molecules of ATP hydrolyzed for every vinblastine molecule transported in a particular direction. These calculations involve several approximations, but turnover numbers for pumping of vinblastine and for vinblastine-stimulated ATP hydrolysis are comparable. Thus, ATP hydrolysis is probably directly linked to drug transport by P-glycoprotein.

Most people who die from cancer do so because their tumors have metastasized and become resistant to chemotherapy. Until we find ways to prevent cancer entirely, overcoming drug resistance is the main hope to save lives. P-glycoprotein (P-gp), the product of the MDR1 gene, contributes to multidrug resistance in many cell types (1, 2) and is expressed in many tumors (3–8). P-gp pumps out its drug substrate from the tumor cell, reducing the effectiveness of administered chemotherapeutic agents (9). It has ATPase (10–14) activity enhanced by numerous substrates and substrate analogs. A very wide range of substrates are pumped out of cells by P-gp (15). Major efforts have been made toward finding clinically useful reversers of P-gp that can block its action, leading to renewed accumulation of drugs within erstwhile resistant cells (16–17). Secure knowledge of the mechanism of action of P-gp is the basis for designing new and more effective reversers.

Four lines of evidence indicate that P-gp can expel its substrates directly out of the cell membrane (reviewed in Ref. 18). First, the substrates of P-gp are lipophilic and reside, most of the time, within cell membranes. Thus, it is within the membrane that the pump will find it most easy to locate its substrate. Second, kinetic analyses show that drug accumulation is reduced by the action of P-gp from the earliest times that measurements can be made, i.e. before significant amounts of the drug can enter the cell yet when it is already present within the membrane (19). Hence it seems to be pumped out from the membrane itself before it crosses it. Third, Raviv et al. (20), making use of the photosensitive probe 5-iodonaphthalene-1-azide to label membrane proteins, showed that doxorubicin was expelled from cell membranes of P-gp-containing but not drug-sensitive cells. In addition, fluorescent dyes such as Hoechst 33342 (21) and calcine-AM (22) have been used to demonstrate removal of substrate from the lipid bilayer by P-gp. These considerations favor a “vacuum cleaner” model (20) for P-gp in which this protein is an ATP-driven pump that pumps its substrates directly out of the plasma membrane. A major criticism of this model is, however, the lack of reliable information on the stoichiometry of ATP hydrolysis to drug pumping. Attempts to measure this stoichiometry in phospholipid vesicles containing pure P-gp suggested a minimum of 50 ATP molecules hydrolyzed per drug molecule transported, however, such a figure is difficult to reconcile with the metabolic potential of multidrug resistant cells (12). Recently, Eytan et al. (23) attempted to derive the stoichiometry by measuring the effect of P-gp on valinomycin-facilitated transport of $^{86}$Rb$^+$ into proteoliposomes containing P-gp, valinomycin being a P-gp substrate. They reported 0.5–0.8 molecules of the complex valinomycin-Rb$^+$ transported for every ATP molecule hydrolyzed. They assumed, however, that only Rb$^+$-complexed valinomycin was transported.

The abbreviations used are: P-gp, P-glycoprotein; Ab, antibody; FACS, fluorescence-activated cell sorter; VBL, vinblastine; PBS, phosphate-buffered saline; BSA, bovine serum albumin.
molecules were transported by P-gp, whereas we observed that vinblastinemediated ATP hydrolysis by P-gp is unaffected by its charged state. Transport of charged vinblastinemolecules in the experiments by Eytan et al. (23) was carried out in the presence of a substantial excess of the uncharged form, which would not contribute to the measured flux but would contribute to ATP hydrolysis. In addition, their assay was carried out in solutions that were not free of potassium, which would compete with rubidium, complicating their calculations of the stoichiometry. We deemed it important, therefore, to determine this stoichiometry using an independent approach.

Here we determine the number of P-gp molecules present on the surface of P-gp-expressing cells using an antibody (Ab) titration procedure and FACS analysis. In the same cell type, we measure the maximum capacity of P-gp to reduce the entry of its substrate vinblastine (VBL) by determining the difference between initial rates of VBL uptake in the presence and absence of the P-gp blocker verapamil. These two numbers, taken together, can determine the catalytic constant (molecules pumped per second) for the effect of P-gp on reducing VBL accumulation. Also, we measure the maximum capacity of such cells to accelerate efflux of loaded VBL in the presence of an energy source, giving another measure of the transport capacity and of the catalytic constant for the effect of P-gp on VBL transport. Comparing these catalytic constants with that obtained for the effect of VBL on accelerating ATP hydrolysis by P-gp, we compute the ratio of the maximum rate of VBL transport to the maximum rate of VBL-stimulated ATP hydrolysis. This ratio is not far from unity. Thus, only a small number of ATP molecules seem hydrolyzed for each VBL molecule transported by P-gp.

EXPERIMENTAL PROCEDURES

Cells and Cell Culture—We used the following previously characterized murine NIH3T3 fibroblasts: sensitive cells (NIH3T3 cell line), wild type human P-gp-expressing MDR1-Gly-185, and mutant MDR1-Val-185 (in which glycine at position 185 is substituted by valine) as described in Stein et al. (19). Human MDR1-transfected cell lines N3–30, N3–600, and N3–2400 (resistant to vincristine at 30, 600, and 2400 ng/ml, respectively) were characterized by Germann et al. (24). Cells were grown as monolayer cultures as described earlier (19, 24).

FACS Analysis—0.125 to 8 million cells (from a 10-cm dish harvested by trypsinization into PBS, washed twice with PBS containing 0.1% bovine serum albumin (PBS-BSA), and resuspended in PBS-BSA) were incubated for 30 min on ice with 0.125–6.4 μg/ml of human P-gp-specific IgG2a Ab (PharMingen), washed twice with PBS-BSA, and reacted with fluorescence-conjugated (fluorescein isothiocyanate-labeled) goat anti-mouse IgG2a Ab (1–10 μg; Jackson Immunoresearch Laboratories, West Grove, PA) for 30 min on ice. The cells were again washed twice in PBS-BSA and the levels of FL1 fluorescence were analyzed using a FACSsort flow cytometer with Cell Quest software (Becton Dickinson FACS System, San Jose, CA).

Transport Experiments—Cells were seeded onto 5-cm dishes at 400,000 cells/2 ml of complete medium (in the absence of cytotoxic drugs) and used 2 days later at 105 cells/dish after washing in ice-cold PBS. Uptake was measured from 5 s to 120 min at 37 °C from PBS containing 5 mM glucose and 15–25 Ci/mmol H-VBL (Amersham) at concentrations from 1.4 nm to 50 μM and either 0 or 50 μM verapamil (to block P-gp), and stopped by washing rapidly, twice, in ice-cold PBS. Zero time uptakes were determined by adding ice-cold solutions of the H-VBL to the cells. Cells were removed from dishes by trypsinization, and dish contents were transferred to vials for liquid scintillation counting. Cells from dishes (PBS-washed as for the transport experiments) were trypsinized for counting in the hemocytometer. For efflux, cells were first loaded with VBL by suspending in appropriate concentrations of H-VBL, for 40 min at 25 °C from PBS containing 10 mM sodium azide and 10 mM 2-deoxyglucose to deprive the cells of metabolic energy (and block the action of P-gp). Efflux was initiated after washing cells rapidly in PBS followed by resuspension in PBS containing either sodium azide and 2-deoxyglucose as before or 5 mM glucose. Efflux was terminated by washing the dish rapidly with ice-cold PBS, and cells were taken for scintillation counting as before. Data are reported in terms of the volume of external solution taken up by, or still remaining in, 1 × 10⁶ cells at the reported time (19). In all cases aliquots of the H-VBL solutions used were taken for scintillation counting to calculate this quantity.

ATP Hydrolysis Assays—ATP hydrolysis by crude cell membranes was determined by measuring the release of inorganic phosphate from 5 mM ATP in the presence of 10 mM MgCl₂, 5 mM sodium azide, 1 mM ouabain, and 0.5 mM EGTA, and in the presence and absence of 0.3 mM ouabain (26, 35).

Data Analysis—Equations 1–3 were used to obtain the best fit curves by using the program SIGMA PLOT (Jandel, Inc.), which uses the Marquard-Levenburg algorithm.

RESULTS

Using FACS, we first studied binding of P-gp-specific monoclonal Ab UIC2 to cells of various cell lines engineered to express human P-gp. Fig. 1 depicts the results of experiments where we used control mouse IgG2a Ab (A) and UIC2 Ab (B) binding to NIH3T3 cells transfected with the human MDR1 gene (Gly-185 cells) at four concentrations of Ab, 0, 0.2, 1.6, and 4.8 μg/tube containing 200 μl of medium. In panel C, at these same concentrations with binding being to Val-185 cells, NIH3T3 cells transfected with a mutant strain of MDR1 where valine replaces glycine in position 185 of the polypeptide chain of P-gp; in panel D, binding was done with a single amount (1 μg) of antibody to five different cell strains, using the non-transfected NIH3T3 cells, cells transfected with wild type MDR1 gene and selected at low dose (30 ng/ml vincristine; N3–30), with a higher dose (600 ng/ml; N3–600), and with a still higher dose (2400 ng/ml; N3–2400), as well as the MDR1 Gly-185 cell strain grown in the presence of 60 ng/ml colchicine (see panel B). In each case, the data are plotted as the number of cells on the ordinate that are labeled with the fluorescence intensity denoted on the abscissa. Cells tested with the control IgG Ab showed very little fluorescence with no increase as the concentration of Ab is raised, whereas the Gly-185 and Val-185 strains, tested with monoclonal Ab UIC2, demonstrate a clear and similar fluorescence signal that increases in intensity as the concentration of Ab increases. The various cell strains depicted in panel D show a marked difference in signal intensity, consistent with differences in the amount of P-gp present on the surface of these different cells (24).

To determine the number of available P-gp molecules on the cell surface, we used the same monoclonal Ab UIC2 and a depletion assay. We first quantitate how the degree of reaction depends on the amount of Ab. Fig. 2 (panels A and C) depicts the median fluorescence of a sample of Gly-185 and Val-185 3T3 cells, respectively, when treated with increasing Ab concentration (0–8 μg of Ab/sample mixture of 200 μl) using a fixed number of cells, 5 × 10⁴ reaction at 4 °C. We fitted the data by the simple binding equation.

\[
\text{BOUND} = \frac{\text{BOUND}_{\text{MAX}} \cdot [\text{Ab}]}{K_m + [\text{Ab}]} \quad (\text{Eq. 1})
\]

where [Ab] is the concentration of the Ab present in the reaction sample, BOUND is the measured median fluorescence due to binding of Ab to the cells, BOUND_{MAX} is the maximum fluorescence determined by the maximum Ab bound to the amount of cells present, while \( K_m \) is the concentration of Ab that gives one-half maximal binding (i.e., 0.5 × 10⁴ reaction at 4 °C, etc.) of the affinity of Ab for ligand, here P-gp). Next, we titrated the Ab, present at an amount less than the values of \( K_m \) found in the previous titrations, against an increasing number of added cells, using up to 8 million cells/reaction sample. As the number of cells per sample increases, less and less Ab is available for each cell. The

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2 S. V. Ambudkar, unpublished data.
median fluorescence per cell decreases as Ab is depleted from the reaction mixture by being bound to the cells themselves. We fitted the data by the depletion equation.

\[
\text{BOUND} = \frac{\text{BOUND}_{\text{init}} \cdot K_n}{K_n + [\text{Cells}]}
\]  

(Eq. 2)

where [Cells] is the number of cells present per reaction sample, BOUND is the measured median fluorescence due to binding of Ab to cells, BOUND_{init} is the initial fluorescence determined by the maximum Ab bound per cell at the weight of Ab present, while \(K_n\) is that number of cells that depletes by one-half the maximal amount of binding (a measure of the number of sites that bind the Ab, i.e. of P-gp molecules per cell).

For the Gly-185 cells, we found \(K_n\) to be 1.87 ± 0.24 μg of Ab, while \(K_n\) was 2.97 ± 0.77 million cells/reaction sample. We calculate the number of P-gp molecules exposed to Ab as follows. In Fig. 2B, 1 μg of Ab is present per reaction sample, and \(K_n\) is 2.97. Therefore 29.7 million cells are sufficient to deplete one-half or 0.5 μg of Ab from the reaction mixture. Now 0.5 μg of Ab is 2.02 × 10^{13} molecules (assuming molecular mass of the Ab to be 150 kDa). Hence each cell binds 2.02/2.97 or 0.68 million molecules of Ab. But from Fig. 2A we find the \(K_m\) of Ab for P-gp as 1.87 μg. Thus at the 1 μg concentration used in Fig. 2B, Ab-binding sites are only saturated to a fraction of \(1/(1 + 1.87)\) or 0.345. Therefore, the true number of Ab-binding sites is 0.68/0.345 = 1.95 ± 0.53 million P-gp sites/Gly-185 3T3 cell on computing for the combination of errors. Performing the Ab binding procedures at 25 and 37 °C gave essentially the same values (data not shown).

For the Val-185 3T3 cells, similarly, the number of P-gp sites per cell is 2.63 ± 0.87 million, not significantly different from the number present per Gly-185 3T3 cell. We performed the same experiments also for the N3–2400 strain of human MDR1-transfected 3T3 cells (see Ref. 24 and Fig. 1D). For these cells (data not shown), the value of \(K_m\) was found to be 2.9 ± 0.4 μg of Ab while \(K_n\) was 0.56 ± 0.14 × 10^{6} 3T3 control cells. Calculating, as described above, gives 14.2 ± 3.8 × 10^{6} molecules of P-gp/cell for this highly resistant strain.

In addition, with the MDR1 Gly-185-transfected 3T3 cells, we used another human P-gp-specific monoclonal Ab with an external epitope, MRK-16 (27), and also the Fab and F(ab')_2 fragments prepared from this Ab. The depletion titration gave a \(K_n\) value of 1.90 ± 0.37 × 10^{6} cells for the MRK-16 Ab and 2.76 ± 0.62 × 10^{6} for the F(ab')_2 fragment, not significantly different. Similar results were obtained with Fab (monovalent) fragment (data not shown). Considering these data it is reasonable to assume that 1 molecule of Ab binds to 1 P-gp molecule.

P-gp can act on its substrates in two ways; it can pump drug from the cytoplasm of the cell and also pump it out from the membrane before it reaches the cytoplasm (19). We determined the maximum rate of VBL pumping by P-gp by these two
pathways. First, we studied the extraction of VBL from the membrane before it accumulated inside the cell. Fig. 3 shows the time course of uptake of VBL into Gly-185 3T3 cells at 37 °C in PBS. (In Figs. 3 and 4, the ordinate is the amount of VBL taken up and expressed as the volume of external medium cleared of VBL (in \( \mu l \)) per million cells at the time stated (19). Multiplying this measure by the concentration of VBL in the external medium would give the amount of VBL that enters the cell in the given time period). In Fig. 3A, the time course was measured over an extended range. The final level of uptake is dramatically increased by adding 50 \( \mu M \) of the reverser verapamil to the cells (filled circles). In Fig. 3B, however, uptake is measured for 12 s at 37 °C. Uptake is linear from zero time during this interval. In succeeding experiments, uptakes were performed at 10 s in the initial rate range. Fig. 4A depicts the uptake of VBL during 10 s in the presence (filled circles) and absence (open squares) of 50 \( \mu M \) verapamil. In the presence of verapamil there is little change in the VBL uptake as the concentration of the drug is increased. In its absence, uptake increases with the concentration of VBL. Two curves, with and without verapamil, begin to approach one another, which is consistent with increasing concentrations of VBL saturating the pumping ability of P-gp so that it cannot cope with the inflow of the drug. Thus, the difference between uptake of VBL in presence and absence of verapamil gives the component of pumping that takes place as the drug crosses the membrane but before it enters the cytoplasm (18, 19). In Fig. 4B we plot this difference, i.e. pumping of VBL by P-gp, as a function of [VBL] by combining data from different experiments. The filled circles are from Fig. 4A, open circles are from a similar experiment at other concentrations of VBL, whereas the open square is from the 10-s data points of Fig. 3B of Fig. 3 (open square) as a function of [VBL]. The fitted line is for Equation 3 in the text.

\[
v = \frac{V_{\text{max}} \cdot S}{K_p + S},
\]

where \( V_{\text{max}} \) is the maximum velocity of pumping (in moles/cell/second), whereas \( K_p \) is that [VBL] giving one-half maximum pumping velocity. We fitted the data of Fig. 4B to Equation 3 where \( v/S \) is the uptake of VBL (volume of external medium cleared per 10 s) and obtained an estimate of \( K_p \) and \( V_{\text{max}} \). We
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Fig. 5. Time course of efflux of vinblastine at 25 °C from Gly-185-transfected NIH3T3 cells loaded with VBL during 40 min at 25 °C in PBS containing 10 mM sodium azide and 10 mM 2-deoxyglucose at VBL concentrations of 1.4 μM (A) or 100 μM (B). Efflux of 3H-VBL was followed into this same medium (filled circles) or into PBS containing 5 mM glucose (filled squares). The solid lines are the best-fitted exponential decay curves. Panel C plots the energy-dependent efflux, calculated as the difference between the VBL efflux during 2 min at 25 °C into glucose and azide/deoxyglucose at various VBL concentrations as indicated on the abscissa. The filled circles and triangles are from different experiments as in panels A and B, whereas the open squares are the data from panels A and B themselves. The fitted line is for Equation 3 in the text.

We next studied zero trans-efflux (28) from cells loaded with VBL. In Fig. 5A, Gly-185 3T3 cells were loaded with 1.4 mM VBL at 25 °C for 40 min in the presence of 10 mM sodium azide and 10 mM 2-deoxyglucose to deplete the energy (19). (These experiments were performed at 25 rather than 37 °C since we found that during loading of the cells at 37 °C, in the absence of an energy source, they became less adherent to the dishes, preventing an accurate determination of efflux rates.) Cells were washed rapidly with ice-cold PBS and then exposed to a washout medium at 25 °C, free of VBL but containing either PBS + 10 mM sodium azide + 10 mM 2-deoxyglucose (filled circles) or PBS + 5 mM glucose to restore energy (filled squares). The data were fitted by an exponential efflux equation, giving values for τ_{1/2} of 37 and 4.4 min into azide/2-deoxyglucose or glucose medium, respectively. Efflux into glucose medium is thus some 8.5 times that into azide/2-deoxyglucose. A parallel experiment performed with [VBL] at 100 μM is depicted in Fig. 5B. Rates into azide/2-deoxyglucose and glucose are now similar to τ_{1/2} values of 13 and 8.4 min, respectively. In addition, results of similar experiments with different concentrations of VBL are depicted in Fig. 5C. Here we plot efflux during 2 min, reported as the difference between efflux into glucose and into azide/2-deoxyglucose and expressed as a fraction of the zero time value, as a difference between efflux into glucose and into azide/2-deoxyglucose at various VBL concentrations as indicated on the abscissa. The filled circles and triangles are from different experiments as in panels A and B, whereas the open squares are the data from panels A and B themselves. The fitted line is for Equation 3 in the text.

Our data provide an estimate of the number of P-gp molecules present at the surface of the cell by using an antibody depletion method. The number we find for both Gly-185 MDR1 and Val-185 MDR1-transfected cell lines appears reasonable in light of the previous determinations. Thus, Pastan et al. (29), using MRK-16 Ab and ferritin bridge labeling, determined that MDR1 virus-infected colchicine-resistant MDCK cells express about 5.5 × 10^6 P-gp molecules/cell. Sehested et al. (30), using freeze-fracture electron microscopy found 500 P-gp molecules/μm^2 of plasma membrane, corresponding to 8 × 10^6 molecules/cell. Shapiro and Ling (12) estimated that multidrug-resistant Chinese hamster ovary cells contain 3 to 4 × 10^6 P-gp molecules/cell based on the yield of purified P-gp from these cells. Although, we compare different cell lines with different degrees of drug resistance, our estimates of 2 × 10^6 molecules of P-gp for the Gly-185 3T3 cells, 2.6 × 10^6 for the Val-185 cells, and 14 × 10^6 for the highly resistant N3–2400 cells are compatible with previous data. The Ab depletion method has the advantage of giving an in situ determination of the number of Ab-binding molecules per cell and requires no assumptions as to the yield of protein extracted from the plasma membrane or the yield of membranes that can be prepared from the whole cells.

Our data on the maximum velocity of drug efflux can be compared with two values in the literature. Ghauharali et al. (31) studied daunorubicin efflux from multidrug resistant human KB–8–5 epidermoid carcinoma cells using a fluorescence assay and reported a maximum efflux of 180 pmol of daunorubicin/10^6 cells/min at 37 °C to be compared with 58 pmol/min/10^6 cells at 25 °C in our study or perhaps 4.63 × this value (269 pmol/min/10^6 cells/min) if we take the temperature dependence of the ATPase activity as appropriate for pumping. A far higher value of 2,000 pmol/min/10^6 cells was calculated by Stein (18) from data reported by Nielsen et al. (32) for daunorubicin efflux from the multidrug resistant Ehrlich ascites tumor cells. The reason for this discrepancy is not clear, but these experiments were not designed to determine the maximum value for daunorubicin pumping. The higher value for daunorubicin efflux (2 nmol/min/10^6 cells) gives rise to a higher drug/ATP stoichiometry in the calculations of Stein (18).

We can compare the turnover numbers that we obtain on combining our transport data and the estimates of the number of P-gp molecules per cell with the turnover numbers that are available for the purified protein (12), these being in the range 1–10/s. Urbatsch et al. (13), using purified P-gp from drug resistant Chinese hamster ovary cells report a turnover number of 4.9 s^-1 for the basal ATPase activity and 9.2 s^-1 for the activity in the presence of verapamil. The verapamil-stimulated activity would thus have a turnover number of 4.3 s^-1. Our data (see also Table I) using purified and reconstituted...
P-gp (isolated from KB-V1 cells) gives turnover numbers of the basal activity of 2.9 s\(^{-1}\), 12.8 s\(^{-1}\) of the maximal verapamil-stimulated activity and 6.4 s\(^{-1}\) of the maximal VBL-stimulated activity. This would give values for the verapamil component alone of 9.9 s\(^{-1}\) and for the VBL component of 3.5 s\(^{-1}\). It is difficult to know which of these turnover numbers are appropriate to compare with the turnover numbers for VBL pumping (1.1–1.4 s\(^{-1}\)) that we have determined.

In addition, our data for VBL efflux necessarily had to be determined at 25 °C whereas the values for the turnover number of the ATPase activity have been determined at 37 °C. We did, however, also measure ATPase activities at 25 °C for all the P-gp species that we studied for three different drugs that stimulate the activity of P-gp (Table I). In particular, the ATPase activity in the presence of verapamil and VBL was reduced 3.5- and 4.6-fold, respectively, as temperature was reduced from 37 to 25 °C, comparable with the reduction of 3.6-fold found in the two determinations of the VBL pumping rates in Gly-185 cells.

Our turnover numbers for VBL pumping may be underestimated. In particular, we measured pumping rates in the living cell at prevailing ATP concentrations. The \(K_m\) for ATP for P-gp ATPase is reported as being in the range 0.28–1.4 mM (10, 12). This concentration, vinblastine inhibits activity in this mutant.

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### REFERENCES
Coupling of P-gp-mediated ATP Hydrolysis and Drug Transport

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