Drug-stimulated Nucleotide Trapping in the Human Multidrug Transporter MDR1

COOPERATION OF THE NUCLEOTIDE BINDING DOMAINS*

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The human multidrug transporter (MDR1 or P-glycoprotein) is an ATP-dependent cellular drug efflux pump, and its function involves a drug-stimulated, vanadate-inhibited ATPase activity. In the presence of vanadate and MgATP, a nucleotide (ADP) is trapped in MDR1, which alters the drug binding properties of the protein. Here, we demonstrate that the rate of vanadate-dependent nucleotide trapping by MDR1 is significantly stimulated by the transported drug substrates in a concentration-dependent manner closely resembling the drug stimulation of MDR1-ATPase. Non-MDR1 substrates do not modulate, whereas N-ethylmaleimide, a covalent inhibitor of the ATPase activity, eliminates vanadate-dependent nucleotide trapping. A deletion in MDR1 (Δ amino acids 78–97), which alters the substrate stimulation of its ATPase activity, similarly alters the drug dependence of nucleotide trapping. MDR1 variants with mutations of key lysine residues to methionines in the N-terminal or C-terminal nucleotide binding domains (K433M, K1076M, and K433M/K1076M), which bind but do not hydrolyze ATP, do not show nucleotide trapping either with or without the transported drug substrates. These data indicate that vanadate-dependent nucleotide trapping reflects a drug-stimulated partial reaction of ATP hydrolysis by MDR1, which involves the cooperation of the two nucleotide binding domains. The analysis of this drug-dependent partial reaction may significantly help to characterize the substrate recognition and the ATP-dependent transport mechanism of the MDR1 pump protein.

Overexpression of the human multidrug transporter (MDR1 or P-glycoprotein) is responsible for the phenomenon of multiple drug resistance in various cancer cell types. MDR1 is an integral plasma membrane protein that acts as an ATP-dependent efflux pump to reduce the intracellular concentration of diverse hydrophobic compounds (reviewed in Refs. 1–3). MDR1 belongs to the superfamily of the ATP-binding cassette (ABC) transporters and contains a tandem repeat of transmembrane domains and conserved nucleotide-binding motifs connected by a central “linker” region (1, 4, 5).

MDR1 exhibits an ATP hydrolytic activity closely related to its drug transport function. This ATPase activity is significantly stimulated by the transported substrate drugs but is blocked by low concentrations of vanadate (6–10). Senior and co-workers (11–14) demonstrated that in the presence of vanadate and MgATP, similar to the effect observed with myosin and other related ATPases (see Ref. 15), MDR1 forms a strong complex with a radioactively labeled nucleotide. Vanadate stops the full catalytic cycle of MDR1, most probably by replacing inorganic phosphate, and stabilizes a protein-trapped form of a nucleotide, which was found to be exclusively ADP (11). Experimentally, this nucleotide trapping can be followed by using [α-32P]ATP as an energy donor substrate, because the MDR1-trapped labeled nucleotide is not removable by washings even in the presence of high concentrations of MgATP and/or MgADP. A covalent MDR1 labeling occurs if the photoaffinity analog, 8-azido-[α-32P]ATP is used in the trapping reaction, followed by UV light treatment.

It has been documented (12) that the formation of enzyme-bound (trapped) ADP from MgATP occurs randomly at the two nucleotide binding sites of MDR1, and whereas one MDR1 molecule is capable for the binding of two MgATP molecules, the saturation stoichiometry for the trapped ADP/MDR1 is one to one. The ADP-associated form of MDR1 may represent a high energy intermediate of the protein, required for the drug-pump function (13).

In their experiments, Senior and colleagues (11–13) did not observe a modulation by the transported drug substrates either of the vanadate-dependent nucleotide trapping, or of the release of the trapped nucleotide in MDR1. In contrast, a substrate stimulation of the labeled nucleotide trapping has recently been reported in the case of MRP, another ABC transporter involved in drug resistance (16). Moreover, when studying the drug substrate interactions with MDR1, Urbatsch and Senior (17) and Dey et al. (18) found a significant inhibition of drug binding after vanadate-dependent nucleotide trapping, indicating a strong interaction between drug binding and ATP hydrolysis.

In the present paper, we demonstrate that the addition of transported drug substrates significantly increases the rate of vanadate-dependent nucleotide trapping in MDR1, when this process is studied under properly selected experimental conditions. To assay the reactions of the wild-type and mutant
MDR1, the transport protein and its variants were expressed using the baculovirus-Spodoptera frugiperda (Sf9) insect cell system and characterized by analyzing their nucleotide trapping in isolated membrane preparations. The experiments presented clearly show that the vanadate-dependent nucleotide trapping in MDR1 reflects a drug-dependent partial reaction of the transport cycle, which is significantly modulated by site-directed mutations in the pump protein. Our experiments also indicate a close cooperation of the two nucleotide binding sites in the drug-dependent trapping of nucleotides.

MATERIALS AND METHODS

Chemicals—8-azido-[α-32P]ATP (666 GBq/mmol) and [α-32P]ATP (111 TBq/nmol) were obtained from ICN Biomedicals.

MDR1 Expression and Membrane Preparation—Sf9 cells were cultured and infected with baculovirus vectors as described in Ref. 19. Baculovirus transfer vectors were constructed as described earlier (20, 21) by using the human MDR1 cDNA encoding a protein with the following mutants: Δaa 78–97, K433M, K1076M, and K433M/K1076M.

The virus-infected Sf9 cells were suspended in a low ionic strength medium (containing 50 mM Tris-HCl, pH 7.0, 50 mM mannitol, 2 mM EGTA, 10 μg/ml leupeptin, 8 μg/ml aprotinin, 0.5 mM phenylmethylsulfonyl fluoride, 2 mM dithiothreitol) and disrupted using a glass-Teflon homogenizer. Membrane fractions were isolated by repeated centrifugations and homogenizations, and the membrane protein concentrations were determined as described in Ref. 6.

Electrophoresis and Immunoblotting—Membranes were suspended in a disaggregation buffer (6). Samples (20 μl) were run on 6% Laemmli-type gels and electroblotted onto polyvinylidene difluoride membranes. Quantitative estimation of the expression of human MDR1 was performed using the polyclonal anti-MDR1 antibody 4077 (22), and a secondary antibody (anti-rabbit peroxidase-conjugated IgG; 20,000 × dilution, Jackson Immunoresearch), as described in Ref. 21. Horseradish peroxidase-dependent luminescence (ECL, Amersham Pharmacia Biotech) was determined by luminography and quantitated by the Bio-Rad phosphorimagery system.

Measurement of ATPase Activity—ATPase activity sensitive to vanadate was measured in isolated membranes as described in Ref. 6.

Vanadate-dependent Nucleotide Trapping—Isolated Sf9 cell membranes (100 μg protein) were incubated for 30 s to 10 min at 37 °C in a reaction buffer containing 50 mM Tris-KCl, pH 7.0, 100 mM mannitol, 2 mM EGTA, 10 μg/ml leupeptin, 5 μg/ml aprotinin, 0.5 mM phenylmethylsulfonyl fluoride, 2 mM dithiothreitol, and 1 mM NaF.

The incubation medium contained either 5 μM Mg-8-azido-[α-32P]ATP or 5 μM MgATP containing 0.1–0.2 MBq of [32P]ATP. The reaction was stopped by the addition of 500 μl of ice-cold Tris-EGTA-MgATP-vanadate buffer (50 mM Tris-KCl, 0.1 mM EGTA, pH 7.0, 10 mM MgATP, 200 μM sodium orthovanadate), the membranes were spun for 20 min at 4 °C at 15,000 × g, the supernatant was removed, 500 μl of Tris-EGTA-ATP-vanadate buffer were added, and the centrifugation was repeated. In the case of [α-32P]ATP labeling, the membranes were dissolved in electrophoresis buffer, and activity was measured in a scintillation...
counter, whereas in the case of 8-azido-[α-32P]ATP labeling, the washed pellet was resuspended in 20 μl of Tris-EGTA buffer, placed in a drop on a Parafilm-covered glass plate, cooled, and kept on ice. The samples were irradiated for 10 min with a UV lamp (λ<sub>max</sub> about 250 nm) at a distance of 3 cm. Thereafter the membranes were collected in 40 μl of the electrophoresis buffer, and the samples were run on 6% Laemmli-type gels. The proteins were electroblotted onto polyvinylidene difluoride membranes as described above and the blots were dried and subjected to autoradiography in a phosphorimager (Bio-Rad). The identity of the 32P-azido-nucleotide labeled bands was assured by immuno-staining with anti-MDR1 specific antibody (antibody 4077) on the same blot.

**RESULTS**

**Nucleotide Trapping in MDR1 from Mg-8-azido-ATP**—In this set of experiments, we examined the vanadate-dependent nucleotide trapping in isolated Sf9 cell membranes expressing the human MDR1 by using the photoaffinity ATP analog, 8-azido-[α-32P]ATP. 8-Azido-ATP was reported to be an efficient energy donor substrate for the MDR1-ATPase, although both the K<sub>m</sub> and the V<sub>max</sub> values were found to be lower for Mg-8-azido-ATP than for MgATP (23). Labeling was performed in the presence of sodium orthovanadate as described under “Materials and Methods” for the time intervals and nucleotide concentrations indicated in Figs. 1 and 2. Verapamil and 5-fluorouracil (5FU) were used as test drugs because verapamil is a well-known substrate and activator of the MDR1 ATPase, whereas 5FU is not transported by MDR1 and does not stimulate MDR1-ATPase activity (1, 6, 24). The addition of 1 mM MgATP to the reaction medium abolished 8-azido-nucleotide trapping in MDR1, indicating a competition of MgATP and Mg-8-azido-ATP at the specific nucleotide binding sites (data not shown).

Fig. 1A shows an autoradiogram, and Fig. 1B shows the corresponding immunoblot of the isolated Sf9 cell membranes labeled with 8-azido-[α-32P]ATP under the vanadate-dependent nucleotide trapping conditions. The major labeled protein band in the Sf9 cell membranes after 2 min of incubation at 37 °C with 5 μM Mg-8-azido-[α-32P]ATP is human MDR1. A weak labeling was also observed in the 70–80-kDa proteolytic fragments of MDR1, which were formed during the labeling, washing, and irradiation procedure and which were also visible as reactive bands on the immunoblots (Fig. 1B). The addition of verapamil greatly increased the labeling of MDR1, whereas this labeling was unaffected by 5FU.

The sulfhydrl reagent N-ethylmaleimide (NEM), by reacting with cysteine residues at the nucleotide binding sites, was shown to be a powerful covalent inhibitor of the MDR1 ATPase and drug transport function but did not affect primary nucleotide or drug binding (10). As demonstrated in Fig. 1A, nucleotide trapping was fully inhibited by 500 μM NEM. The addition of verapamil greatly increased the labeling of MDR1, whereas this labeling was unaffected by 5FU.

The sulfhydrl reagent N-ethylmaleimide (NEM), by reacting with cysteine residues at the nucleotide binding sites, was shown to be a powerful covalent inhibitor of the MDR1 ATPase and drug transport function but did not affect primary nucleotide or drug binding (10). As demonstrated in Fig. 1A, nucleotide trapping was fully inhibited by 500 μM N-ethylmaleimide. There was no measurable 32P incorporation into membranes expressing β-galactosidase. As estimated from measurements of 32P incorporation in the washed membranes and in the

![Fig. 3](image-url)
excised bands of MDR1 from the blots, UV treatment resulted in the cross-linking of 40–50% of the trapped $^{32}$P activity in each experiment.

Fig. 2A represents the 8-azido-ATP concentration dependence of nucleotide trapping by MDR1, measured in the presence or absence of 50 $\mu$M verapamil after 2 min of incubation at 37 °C. Verapamil stimulation of labeling was 5–15-fold at 5–50 $\mu$M 8-azido-ATP, and although the level of nucleotide trapping increased, the percentage of drug stimulation was much less at higher 8-azido-ATP concentrations. A saturation of the 8-azido-nucleotide trapping was observed at about 100 $\mu$M Mg-8-azido-ATP, both with and without the addition of verapamil. Fig. 2B shows the time course of $^{32}$P-nucleotide trapping when the isolated Sf9 cell membranes were incubated with 5 $\mu$M 8-azido-$[^{32}$P]ATP at 37 °C. As shown, the addition of 50 $\mu$M verapamil increased the labeling 4–7-fold at 1 min, whereas less stimulation was observed at increasing time periods (after 5 min, the degradation of ATP significantly reduced the rate of further labeling). In each set of experiments, both $\beta$-galactosidase-expressing and NEM-pretreated MDR1-expressing membranes served as controls. Altogether, these experiments strongly suggest that the rate of nucleotide trapping in MDR1 from 8-azido-ATP is significantly increased by the addition of a known drug substrate of this transporter, whereas N-ethylmaleimide pretreatment blocks the formation of a trapped nucleotide.

**Neurotoxic Trapping in MDR1 from MgATP**—The physiological energy donor for drug transport by MDR1 is MgATP; therefore, we performed experiments similar to those described above by using Mg[$\alpha$-$^{32}$P]ATP. Because nucleotide cross-linking could not be found in this case (see also Ref. 12), nucleotide trapping was measured by counting the membrane-bound radioactivity after repeated washings of the membranes in the presence of 200 $\mu$M sodium orthovanadate and 10 mM MgATP at 4 °C (see “Materials and Methods”).

Fig. 3A represents the MgATP concentration dependence of nucleotide trapping, measured in the MDR1-expressing Sf9 cell membranes after 1 min of incubation with Mg[$\alpha$-$^{32}$P]ATP at 37 °C. Under these conditions, verapamil increased membrane labeling 4–8-fold at 5–50 $\mu$M MgATP, whereas the relative drug stimulation of nucleotide trapping was lower at higher MgATP concentrations. The saturation of the vanadate-dependent nucleotide trapping reaction could not be exactly determined in these experiments, because at higher MgATP concentrations (above 300–500 $\mu$M) nonspecific membrane labeling became relatively high.

It is to be noted that in contrast to verapamil, 5-fluorouracil had no effect on vanadate-dependent nucleotide trapping at any MgATP concentration examined (not shown), and $^{32}$P incorporation was significantly lower in the control, $\beta$-galactosidase-expressing membranes. Nucleotide trapping was significantly reduced in the NEM-pretreated, MDR1-expressing membranes, and in the presence of this MDR1 transport and ATPase inhibitor, $^{32}$P incorporation was not influenced by verapamil or by other MDR1 substrate drugs (see below).

Fig. 3B shows the time course of $^{32}$P-nucleotide trapping when the MDR1-expressing isolated Sf9 cell membranes were incubated with 50 $\mu$M [$\alpha$-$^{32}$P]ATP at 37 °C. We found that 50 $\mu$M verapamil increased the labeling 4–6 times at 30 s to 1 min, whereas significantly less verapamil stimulation was observed after longer incubation periods. Again, a low level of $^{32}$P-nucleotide trapping was found in the NEM-pretreated membranes and in the control, $\beta$-galactosidase-expressing membranes; the level of trapping was not influenced by verapamil.

**Effects of Various Drugs on the Nucleotide Trapping in MDR1**—Fig. 4 shows the detailed examination of the effects of various drugs on the nucleotide trapping in MDR1-expressing membranes incubated at 37 °C for 2 min with 5 $\mu$M 8-azido-[\$\alpha$-$^{32}$P]ATP (Fig. 4A), or for 1 min with 50 $\mu$M Mg[$\alpha$-$^{32}$P]ATP (Fig.
4B). As shown in Fig. 4A, covalent labeling after nucleotide trapping from 8-azido-ATP was found to be significantly stimulated by the MDR1 substrate drugs verapamil, cyclosporine A (CsA), or calcein AM (CaAM), whereas free calcein (Ca free) and 5FU, which are not MDR1 substrates (see Refs. 1, 2, 6–8, and 24), had no effect on this phenomenon.

Fig. 4B demonstrates the drug concentration dependence of nucleotide trapping in MDR1 from Mg-[α-32P]ATP. Again, verapamil, CsA, vincristine, and rhodamine 123 significantly stimulated nucleotide trapping in a concentration-dependent manner, whereas free calcein and 5FU had no effect. The stimulation of nucleotide trapping was evoked by much lower concentrations of CsA, verapamil, or calcein AM than of, for example, rhodamine 123. This pattern closely corresponds to the drug concentration dependence of the MDR1-ATPase activity (see Refs. 6, 8, 10, and 25), although the stimulatory effect of cyclosporine A was much more pronounced than in the MDR1-ATPase activity measurements (see “Discussion”).

Nucleotide Trapping in a Substrate Affinity Mutant of MDR1—A mutant form of MDR1 with a 20-amino acid deletion in its first extracellular loop (Δaa 78–97 MDR1), has been shown to have a reduced drug transport capacity (27) and a low level of ATPase activity, stimulated only by extremely high concentrations (above 200 μM) of verapamil (20). Nucleotide trapping in this mutant MDR1, expressed in Sf9 cell membranes, was followed in the presence of 5 μM Mg-8-azido-[α-32P]ATP and 200 μM sodium orthovanadate.

As shown in Fig. 5, in the Δaa 78–97 MDR1 protein, nucleotide trapping was almost negligible without added drugs, and 50 μM verapamil or 20 μM calcein AM (which produced a maximum stimulation of nucleotide trapping in the wild-type MDR1) had little effect on this labeling. However, the addition of high concentrations of verapamil (300 μM) produced a significant labeling even in the mutant MDR1, approaching the level seen in the wild-type protein (Fig. 5C).

Nucleotide Trapping in Nucleotide Binding Site Mutants of MDR1—In the following experiments, we examined nucleotide trapping with 8-azido-[α-32P]ATP (followed by photo-cross-linking) in MDR1 variants in which essential lysine residues in the Walker A motifs were mutated. These lysines were replaced by methionines either in the N-terminal ABC domain (K433M), in the C-terminal ABC domain (K1076M), or in both ABC domains (K433M/K1076M). These mutant MDR1s, when expressed in Sf9 cells, were shown to demonstrate significant 8-azido-ATP binding but no drug transport or drug-stimulated ATPase activity (21). Because, in these mutant proteins, nucleotide binding may also be altered at low MgATP concentra-
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Affinity, with a $K_D$ of about 200–500 $\mu$M. The binding of MgATP occurs randomly at the two nucleotide binding sites, and the stoichiometry of MgATP/MDR1 at saturating MgATP concentrations is 2:1 (12, 13). Data in the literature indicate that this MgATP binding is not affected by the presence of drug substrates (1, 10, 28). Experimental data also suggest that primary drug binding by MDR1 (as measured at 4°C) is similarly unaffected by the presence or absence of MgATP (see Refs. 17 and 30); thus, initial MgATP and drug binding seem to be independent reactions.

In contrast to MgATP binding, ATP hydrolysis requires an interaction of the protein with the transported drug substrate. Human MDR1-ATPase activity, as measured in isolated Sf9 cell membranes, is about 5–6-fold higher in the presence than in the absence of drug substrates, e.g. verapamil (the “basic” MDR1-ATPase activity is probably supported by endogenous lipid-like or other hydrophobic molecules in the membrane preparation). As we demonstrate in this report, the partial reaction of MDR1-ATPase (reflected in the MDR1 nucleotide trapping), is also strongly accelerated by the transported substrates.

In previous studies (11–13), this drug stimulation of nucleotide trapping was not observed, probably because the experiments were carried out at relatively high ATP concentrations and/or for long measurement periods. Drug substrate stimulation of the nucleotide trapping in our experiments is also restricted to early time periods and unsaturating MgATP (or 8-azido-MgATP) concentrations, that is, under conditions where the drug-dependent acceleration still significantly affects the “titration” of the relevant nucleotide trapping site. It is interesting to note that the differences between the concentration dependence and the time course of nucleotide trapping observed here for Mg-8-azido-ATP and for MgATP can be well explained by the lower $K_m$ and $V_{max}$ values of Mg-8-azido-ATP than of MgATP (17, 29); although higher MgATP concentrations are required to saturate the nucleotide binding sites, both ATP splitting and nucleotide trapping are faster with MgATP than with Mg-8-azido-ATP. In our present experiments under near-saturating conditions, the molar ratio of nucleotide trapping by MDR1 was estimated to be about 0.4–0.6, supporting the conclusion of Urbatsch et al. (12) that only one nucleotide is promoted to an occluded state during this partial reaction.

The drug substrate concentration dependence of the MDR1-ATPase activity and that of the nucleotide trapping were found to be similar, that is, a significant stimulation of both reactions was obtained by similar concentrations of verapamil, vincristine, rhodamine 123, or calcine AM. The data obtained for the deletion mutant in the first extracellular loop of MDR1 (Δaa 78–97), namely that stimulation could be obtained only with extremely high concentrations of verapamil, also strongly suggest that substrate interactions modify the process of nucleotide trapping similarly to that of the MDR1-ATPase activity. Still, some substrates (such as CsA) that yield only very small stimulation of the ATPase but strongly inhibit its verapamil stimulation (31) produced a much greater stimulation of nucleotide trapping (the inhibition of this reaction at higher CsA concentrations was also apparent). These data indicate that the partial reaction of nucleotide trapping may be efficiently promoted by some substrates that can yield only a low turnover for the MDR1-ATPase activity, which reflects the full catalytic cycle.

Interaction of the multidrug transporter with SH group reagents, such as NEM, preferentially occurs at the two cysteine residues in the two nucleotide binding domains (29, 32). Pretreatment of MDR1 with SH group reagents blocks MDR1-ATPase activity (29) but at low concentrations does not inhibit the primary binding of MgATP or drug substrates, as shown by the MgATP- or drug-dependent quenching of an MDR1-bound fluorescent SH-reactive probe (32). A recent communication (33) suggests that NEM may in fact increase drug binding by MDR1 under certain conditions. Because, as reported here, NEM fully blocks drug-stimulated nucleotide trapping, MgATP and primary drug substrate binding most probably occur independently from the following reaction steps, leading to ATP hydrolysis.

The mutant MDR1 proteins, in which lysines in the first (K433M), second (K1076M), or both nucleotide binding domains are replaced by methionines, were demonstrated to bind MgATP less efficiently at low MgATP concentrations (2–5 $\mu$M) but similarly to the wild-type MDR1 at concentrations above 10 $\mu$M MgATP (21). None of these MDR1 variants possess measurable ATPase activity (21); thus, a mutation in one ATP binding domain is sufficient to eliminate the catalytic reaction in the whole pump protein (see also Refs. 26 and 34). This finding was interpreted to indicate a strong cooperative inter-
37 °C for 2 min with 5 mM vanadate-induced nucleotide trapping significantly reduced the drug binding site(s). As shown by Urbatsch and Senior (17), probably significantly alter the conformation and/or location of domains and the appropriate drug binding domains within based on the functional cooperation of the two ATP binding phosphate. All of the steps after MgATP and drug binding are cleavage and a concomitant occlusion of a MgADP molecule, the structure of the MDR1 protein induce a drug-dependent is initiated by the independent primary binding of MgATP and molecular mechanism of the vectorial drug transport by MDR1. The ABC domains already in this partial reaction.

nucleotide trapping entirely, showing a cooperation between other nucleotide binding domains, e.g. CFTR, TAP, or MRP, may help to understand the reaction mechanism and transport characteristics of these ABC transporters.

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Fig. 7. Nucleotide trapping in the nucleotide binding site mutants of MDR1. Labeling was performed in Sf9 cell membranes expressing either the wild-type human MDR1 or the nucleotide binding site mutants K433M (MK) and K1076M (KM). 100 μg of membranes were incubated in the presence of 200 μM sodium orthovanadate at 37 °C for 2 min with 5 μM Mg-8-azido-[α-32P]ATP or for 10 min with 50 μM Mg-8-azido-[α-32P]ATP (in each case the total amount of radioactivity was 0.2 MBq/sample). The incubation medium contained 50 μM verapamil. Nucleotide trapping and covalent photofinity labeling were performed as described under “Materials and Methods.” Panel A, autoradiogram of the isolated Sf9 cell membranes labeled with 8-azido-Mg-[α-32P]ATP or for 10 min with 50 μM sodium orthovanadate at 37 °C for 2 min with 5 μM [γ-32P]ATP (in each case the total amount of radioactivity was 0.2 MBq/sample). The incubation medium contained 50 μM verapamil. Nucleotide trapping and covalent photofinity labeling were performed as described under “Materials and Methods.” Panel B, immunoblot corresponding to panel A, developed by the MDR1-specific polyclonal antibody 4077.

action between the two nucleotide binding domains of MDR1. As we demonstrate here, independently of the presence or absence of drug substrates, none of these nucleotide binding site mutants perform the nucleotide trapping reaction. Thus, a mutation in one of the nucleotide binding domains eliminates nucleotide trapping entirely, showing a cooperation between the ABC domains already in this partial reaction.

Based on the above-described features, we propose that the molecular mechanism of the vectorial drug transport by MDR1 is initiated by the independent primary binding of MgATP and the drug substrate. The following conformational changes in the structure of the MDR1 protein induce a drug-dependent cleavage and a concomitant occlusion of a MgADP molecule, whereas the full catalytic cycle involves the transport of drug substrate to the external membrane surface and the full hydrolysis of ATP and the dissociation of ADP and inorganic phosphate. All of the steps after MgATP and drug binding are based on the functional cooperation of the two ATP binding domains and the appropriate drug binding domains within MDR1.

The drug-dependent ATP hydrolysis and occlusion most probably significantly alter the conformation and/or location of the drug binding site(s). As shown by Urbatsch and Senior (17), vanadate-induced nucleotide trapping significantly reduced azidogaline labeling in MDR1. Recent experiments of Dey et al. (18) demonstrated the presence of two nonidentical drug-interaction sites in the MDR1 protein. In this study the C-terminal drug-recognition (“on”) site was found to be significantly more sensitive to vanadate trapping of nucleotides than the N-terminal (“off”) site, and ATP hydrolysis was essential for the