

## Review

# About a switch: how P-glycoprotein (ABCB1) harnesses the energy of ATP binding and hydrolysis to do mechanical work

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### Abstract

The efflux of drugs by the multidrug transporter P-glycoprotein (Pgp; ABCB1) is one of the principal means by which cancer cells evade chemotherapy and exhibit multidrug resistance. Mechanistic studies of Pgp-mediated transport, however, transcend the importance of this protein per se as they help us understand the transport pathway of the ATP-binding cassette proteins in general. The ATP-binding cassette proteins comprise one of the largest protein families, are central to cellular physiology, and constitute important drug targets. The functional unit of Pgp consists of two nucleotide-binding domains (NBD) and two transmembrane domains that are involved in the transport of drug substrates. Early studies postulated that conformational changes as a result of ATP hydrolysis were transmitted to the transmembrane domains bringing about drug transport. More recent structural and biochemical studies on the other hand suggested that ATP binds at the interface of the two NBDs and induces the formation of a closed dimer, and it has been hypothesized that this dimerization and subsequent ATP hydrolysis powers transport. Based on the mutational and biochemical work on Pgp and structural studies with isolated NBDs, we review proposed schemes for the catalytic cycle of ATP hydrolysis and the transport pathway. [Mol Cancer Ther 2007; 6(1):13–23]

### Introduction

The movement of solutes, nutrients, and ions across cell membranes is a critical biological process. Transport proteins constitute 15% to 30% of membrane proteins in a cell, and as much as 60% of the energy consumption in mammalian cells is devoted to transport processes. The ATP-binding cassette (ABC) family represents one of the largest and most diverse family of transport proteins (1–3). The importance of membrane transport is indicated not only by the large number of genes, their ubiquitous occurrence, and primordial origin but also by the fact that, of the 48 human ABC proteins, 17 are implicated in human diseases (2). P-glycoprotein (Pgp; ABCB1) was the first eukaryotic ABC transporter identified because of its role in conferring multidrug resistance to cancer cells (4). The overexpression of Pgp in human cancer cells can make the cells simultaneously resistant to a variety of chemically unrelated cytotoxic drugs, many of which are anticancer agents, rendering chemotherapy ineffective. It has been estimated that ~40% of all human cancers develop multidrug resistance, making it a major obstacle to the effective treatment of cancer. In addition to the enormous clinical importance of Pgp, this protein is one of the most extensively studied ABC transporters and can contribute to understanding how this important class of proteins works.

Pgp has the molecular architecture of an ABC protein (5), a core structure of four domains [i.e., two nucleotide-binding domains (NBD) and two transmembrane domains (TMD)]. The cytosolic NBDs bind and hydrolyze ATP, whereas each TMD consists of six transmembrane helices, which together form the drug-binding site(s). Although the TMDs show little sequence homology, the NBDs of all ABC proteins show extensive amino acid sequence identity and conserved motifs. These include the Walker A and Walker B, common to most proteins that bind nucleotide, and the signature motif (LSGGQ motif, linker peptide, or C motif), which is diagnostic of ABC proteins. In addition, the NBDs of ABC transporters contain the D-loop, H-loop, and Q-loop and we have recently proposed that a highly conserved A-loop (an aromatic residue 25 amino acids upstream of the Walker A) be considered an integral part of the ABC (6, 7). Thus, Pgp has discrete NBDs and drug-binding sites and the protein does two independent, albeit coupled, functions: ATP hydrolysis and substrate transport.

The basic elements of the mechanism of the transport pathway of Pgp have been in place for over a decade (8).

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There is now considerable evidence that transport mediated by Pgp is energy dependent (see refs. 8–12 for reviews). Structural and biochemical studies also suggest that conformational changes at the NBDs are transmitted to the TMDs. Although the precise causal events at the NBDs that drive conformational changes at the drug-binding site remain unresolved, it would be reasonable to postulate that a transport event involves, at a minimum, the following steps: (a) initiation of the transport pathway by binding ATP and/or drug, (b) conformational changes at the NBDs as a consequence of ATP binding and/or hydrolysis, (c) transmission of the conformational changes at the NBDs to the TMDs to cause a high-affinity to low-affinity switch at the drug-binding site, and (d) resetting the pump. The crystal structures of NBDs of several bacterial ABC proteins in conjugation with mutational and biochemical studies have expanded our understanding of the ATP-binding pocket. A major theme that has emerged from studies with isolated NBDs is the ATP-driven dimerization, where the ATP alters the electrostatic charge balance at the ATPase active site and permits the association of the two halves of the ATP site. This has resulted in the reexamination of the mechanisms of transport proposed for Pgp. Recent studies on Pgp based on these reformulations have in turn contributed significantly to our understanding of the mechanisms of transport in ABC proteins. Due to space limitations, the scope of this review is limited to the catalytic cycle of ATP hydrolysis and transport pathway of Pgp. There are several excellent reviews of both bacterial and eukaryotic ABC transporters (13–16) not discussed here.

### Initiation of the Transport Pathway of Pgp

Recent work suggests that the drug-binding sites of Pgp lie in a large funnel-shaped “binding pocket” with overlapping sites formed by multiple helices from both TMDs (17–21). The transport pathway of Pgp is initiated by the binding of drug and ATP. The drug binds to the protein at an inward (cytoplasmic) facing high-affinity site and is expelled via a conformational change that transforms it to a low-affinity outward (extracellular) facing site. The biochemistry of the ATPase reaction of human, mouse, and hamster Pgps has been studied since the early 1990s, and these studies have been exhaustively reviewed (8, 10, 22, 23). A high-resolution structure of HisP, the ATPase subunit of the bacterial histidine permease, was obtained in 1998 (24) and was the first for an ABC domain. Several structures of ABC domains and a few of full-length ABC proteins have since been elucidated, and it is now possible to understand ATP binding, one of the steps that initiate the transport pathway, in greater detail.

Each NBD of Pgp consists of conserved subdomains: Walker A; Walker B; signature motif; and A-loop, D-loop, H-loop, and Q-loop. The structures of ABC proteins show that the Walker A (also called the P-loop) binds the nucleotide through electrostatic interactions with the triphosphate moiety (13). Residues of the Walker B on

the other hand show hydrogen bonding with the coordinating divalent cations (generally  $Mg^{2+}$ ). Interactions between the Walker A and Walker B are broadly consistent with those found in most ATPases, and the specific interactions between conserved Walker A and Walker B residues and the nucleotide have been recently reviewed (6, 21). The Walker A and Walker B together constitute one of three structurally and functionally distinct subdomains that characterize ABC proteins (25) and are often referred to as the ABC core subdomain. The emerging contour of a NBD of Pgp is that the ATP is flanked by the Walker A and Walker B and H-loop and Q-loop of one NBD on one side and the cavity is closed by the signature sequence and D-loop of the other NBD. Thus, a head-to-tail configuration of two apposing elements becomes apparent and these are referred to as either lobe I and II or arm I and II. Although a high-resolution structure of Pgp is not available, the biochemical evidence is consistent with such a view. For example, fluorescence resonance energy transfer analysis shows that the two ATPase sites are closely associated (26) and cross-linking studies show that the LSGGQ motif in each NBD is adjacent to the opposing Walker A sequence (27). Such an organization seems to be unique to ABC transporters, and it has been argued that the formation of the ATP sandwich may have a central role in harnessing the free energy of ATP to produce conformational changes that result in drug substrate translocation. The role of the NBD as a switch during the transport mechanism of Pgp is both complex and mostly unresolved at present and is discussed in a separate section. However, there is evidence that binding of ATP mediates NBD:NBD dimer formation (28, 29). It is also important to emphasize that the NBD residues make contacts with the opposite monomer via the nucleotide and not directly; thus, the bound ATP at the interphase is sometimes referred to as the ‘glue’ that binds the monomers together (13).

The mechanical movements that accompany ATP binding have been documented at various levels. These include fluorescence resonance energy transfer and cross-linking studies that show subdomain movement and two-dimensional and three-dimensional structural and biochemical studies that show reduced affinity for drugs in the transition state. Another important question *vis-à-vis* the initiation of the transport cycle is how do drugs modulate ATP binding and/or hydrolysis? Transport/drug substrates affect the basal activity of Pgp differently. One class of compounds stimulates ATPase activity at low concentrations but inhibits the activity at high concentrations, a second class of compounds enhances ATPase activity in a dose-dependent manner without any inhibition, whereas a third class of compounds inhibits both basal-stimulated and verapamil-stimulated ATPase activity (23). Moreover, the mechanism by which transport/drug substrates stimulate ATP hydrolysis is unclear. Early studies that characterized the ATPase activity of Pgp clearly showed that drug substrates did not effect the  $K_m$  (ATP) but only the  $V_{max}$  (30–33). Thus, it seems unlikely that drug substrates directly affect the affinity of ATP for Pgp.

Similarly, the use of photoaffinity probes showed that drugs do not directly affect 8-azido[ $\alpha$ - $^{32}\text{P}$ ]ATP binding and nucleotides do not affect binding of the azido-labeled transport substrate [ $^{125}\text{I}$ ]iodoarylazidoprazosin ([ $^{125}\text{I}$ ]IAAP; refs. 34, 35). In addition, some workers have suggested that drugs may lower the activation energy for ATP hydrolysis (12). There have been a few studies that have estimated the activation energy of Pgp-mediated ATP hydrolysis (36–38), and these results seem to suggest that drugs do not have a significant effect on the activation energy of ATP hydrolysis. The most detailed thermodynamic analyses were conducted by Al-Shawi et al. (38) who measured the activation energy for the Pgp ATPase reaction in the presence of several drug substrates. This study reported that the activation energy for basal ATPase is  $104.2 \pm 2.9$  kJ/mol, whereas in the presence of drugs it varies from 94.9 to 116.2 kJ/mol. The work of Clarke et al. (39) offers some clues about the mechanistic details of how drug substrates affect ATP hydrolysis. Using a cysteine-less mutant of Pgp and chemical cross-linking, they show that compounds that stimulate or inhibit ATP hydrolysis do so by increasing and decreasing respectively the distance between the Walker A and the LSGGQ sequences. This would imply that the former favors the formation of the sandwich dimer (see below), a view that is consistent with that of Tomblin et al. (40).

### Formation of the NBD Sandwich Dimer

Biochemical studies with Pgp and the crystal structures of NBDs of ABC proteins support the concept of an ATP-driven dimerization of NBDs. Both biochemical and genetic studies show that a functional mammalian ABC transporter is either a homodimer or a heterodimer (see refs. 12, 23, 41, 42 for reviews) and that each monomer consists of a NBD and a TMD. The general architecture of the NBD:NBD dimer was originally predicted by the modeling studies of Jones and George (43) based on the first high-resolution structure of an ABC subunit, the HisP (24). The essential feature of the nucleotide-binding pocket is that the ATP is sandwiched between the Walker A, Walker B, Q-loop, and H-loop of one NBD and the D-loop and signature sequence of the apposing NBD, hence the term “ATP sandwich.” Such a dimer has since been shown to occur in the structures of Rad50 (28), MutS (44, 45), BtuD (46), MJ0796 (29) HlyB (47), and Sav1866 (48). Although the structures of several ABCs show the formation of the NBD dimer, reservations about the physiologic significance of this state have been expressed (13). The main concern is that no high-resolution structure of a dimer has been obtained to date of an intact ABC protein in its native state using physiologic substrates. Structures that show the nucleotide sandwich have been obtained with mutant or incomplete NBDs, wild-type NBDs in the absence of  $\text{Mg}^{2+}$ , or wild-type NBDs in the presence of transition state analogues. Of course, the flip side to this argument is that preventing ATP hydrolysis either by disabling the ABC by mutagenesis or by using nonhydrolyzable analogues of ATP is the only way to

capture a stable dimer (29), and several lines of evidence now converge to suggest that ATP-induced dimerization may play an important role in the catalytic cycle of ABC proteins.

Solution-phase experiments also support the idea of a NBD dimer during the transport pathway of ABC proteins. The proximity of the Walker A and the apposing signature sequence has been shown in the orthovanadate (Vi)-trapped “transition state” of MalK (49). Similarly, it has been shown that ATP-dependent dimerization occurs in the mitochondrial ABC transporter from *Saccharomyces cerevisiae*, Mdl1p (50, 51). Recently, Gadsby et al. (52) have taken a unique approach to showing the ATP sandwich in the cystic fibrosis transmembrane regulator protein. They identified two amino acid residues on opposite faces of the NBD:NBD dimer most likely to be coupled with each other based on a statistical analysis of 10,000 NBDs (53). These residues, R555 and T1246, were then mutated to generate a set of two single mutants (R555K and T1246N) and a double mutant (R555K/T1246N). Using these mutants, they were able to construct thermodynamic double-mutant cycles pioneered by Fersht et al. (54) to show energetic coupling between the two faces of the NBD during the ATP-driven tight dimerization of the two NBDs.

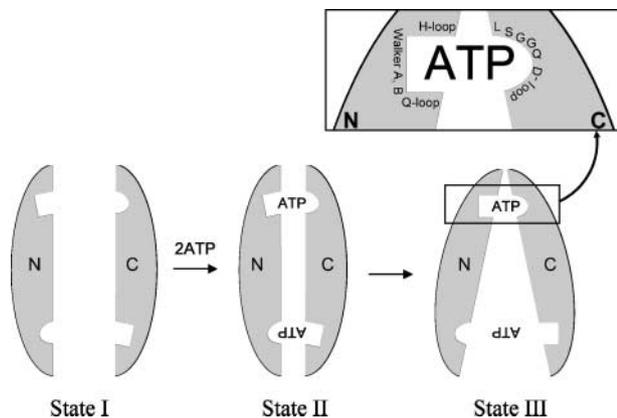
### The Reaction Intermediate of Pgp with Occluded ATP

The studies described above suggest that dimerization of the two NBDs driven by ATP binding is an important step during the ATP hydrolysis. The NBDs of ABC proteins have a highly conserved glutamate in the Walker B region, which is sometimes referred to as the “catalytic carboxylate” (29), a characterization that has been disputed by some investigators (16). In human Pgp, the double-mutant E556Q/E1201Q does not show steady-state hydrolysis but can occlude the nucleoside triphosphate in a reaction intermediate (21, 55). Similar results were obtained as a result of equivalent mutations in mouse Pgp (56). The mutant Pgp with ATP has been described as the “occluded nucleotide conformation” (57). In the occluded nucleotide conformation, the nucleotide is predominantly the nucleoside triphosphate and is tightly bound in a nonexchangeable form in the absence of Vi or beryllium fluoride and the formation of this conformation is strongly temperature dependent (56, 58). These studies suggest that the “ATP-bound” state and the “occluded nucleotide” state may not be the same (57, 58).

The distinction between a nucleotide bound and occluded state is an important one because, although the crystal structures of several isolated domains show 2 mol of nucleotide per dimer (28, 29, 47, 59), for Pgp, several studies have shown that the “occluded” state contains only 1 mol ATP/mol Pgp. Tomblin and Senior (57) have speculated that there are three distinct states of Pgp leading up to the occluded conformation. The first is an open dimer, the second is a closed dimer that is symmetrical with ATPs bound at both NBDs, and the third is the

asymmetrical occluded state where one of the two ATPs is tightly bound in a nonexchangeable form. A molecular dynamics simulation of the ATP-binding process in BtuCD (an ABC protein that is the vitamin B<sub>12</sub> importer in *Escherichia coli*) favors the idea of an asymmetrical occluded state (60). This study found that, if both nucleotides were docked in identical positions relative to the Walker A and Walker B of each NBD, MgATP binding occurred across the dimer interface of one NBD. At the other NBD, the binding pocket actually opened up, preventing the formation of hydrogen bonds between ATP and the signature motif of the apposing cassette. More recently, the same group has done a simulation of the ATP-driven closure of MalK, the ATP subunit of the maltose transporter, but the results were inconclusive (61). In two simulations, the separation distance between the Walker A and the signature motifs equilibrated (after 15 ns) to the same value at both ATP sites, whereas in three simulations they did not exhibit an asymmetry reminiscent of the BtuCD study. The authors suggest that the presence of TMDs may be required for asymmetrical nucleotide binding, an assertion that is consistent with the finding that a structure of MsbA (such as the TMDs) in the ADP-Vi-trapped state showed only one bound nucleotide (62). Another view is that the isolated NBDs may not “use the same catalytic pathway and transition state structure” as the intact protein (57).

Figure 1 shows likely transition states during the formation of the occluded conformation, which occurs in mutants of Pgp where both “catalytic carboxylates” have been altered (E556Q/E1201Q for human Pgp). The state I shows that, in the absence of nucleotide, the two apposing domains of the ATP sites are in the “open” conformation.



**Figure 1.** Schematic comparison of the sandwich dimer and the occluded state of Pgp. State I depicts an open conformation of the NBDs of Pgp in the absence of nucleotide. ATP binding results in the apposing faces of the nucleotide-binding site coming together (state II) and two ATP molecules are probably bound at this stage. Formation of state II is temperature independent and the nucleotides are exchangeable. State III is a transient intermediate, and one of the two ATPs is occluded in a nonexchangeable form equivalent to the E-S state. Occlusion of ATP is temperature dependent. See text for additional details. *Inset*, contour of the functional ATP-binding site based on the biochemical data with Pgp and structural information on isolated NBDs. *N*, NH<sub>2</sub>-terminal NBD; *C*, COOH-terminal NBD.

The binding of two molecules of ATP results in the formation of the nucleotide sandwich (state II) that draws together the apposing faces of the ATP sites. This is followed by a conformational change in which one of the two ATPs is “occluded” in a nonexchangeable form (state III). We have recently shown that, unlike the “ATP-bound” state, the formation of the “ATP-occluded” state of Pgp is strongly temperature dependent (58). Similarly, Tampe et al. (50) followed the formation of the NBD dimer of the mitochondrial ABC protein Mdl1. By using the E599Q mutant (equivalent to the human Pgp mutant E556Q/E1201Q described above) and site-specific labeling with a fluorophore, they were able to monitor the kinetics of the occlusion in real time and obtain initial rates. Arrhenius plots of the association of the two NBDs show that formation of the occluded state has relatively high activation energy of ~70 kJ/mol. This suggests that relatively large conformational changes are associated with the formation of NBD dimers. It must, however, be noted that this study used isolated NBDs and the stoichiometry of ATP:protein was not 1 as observed for Pgp.

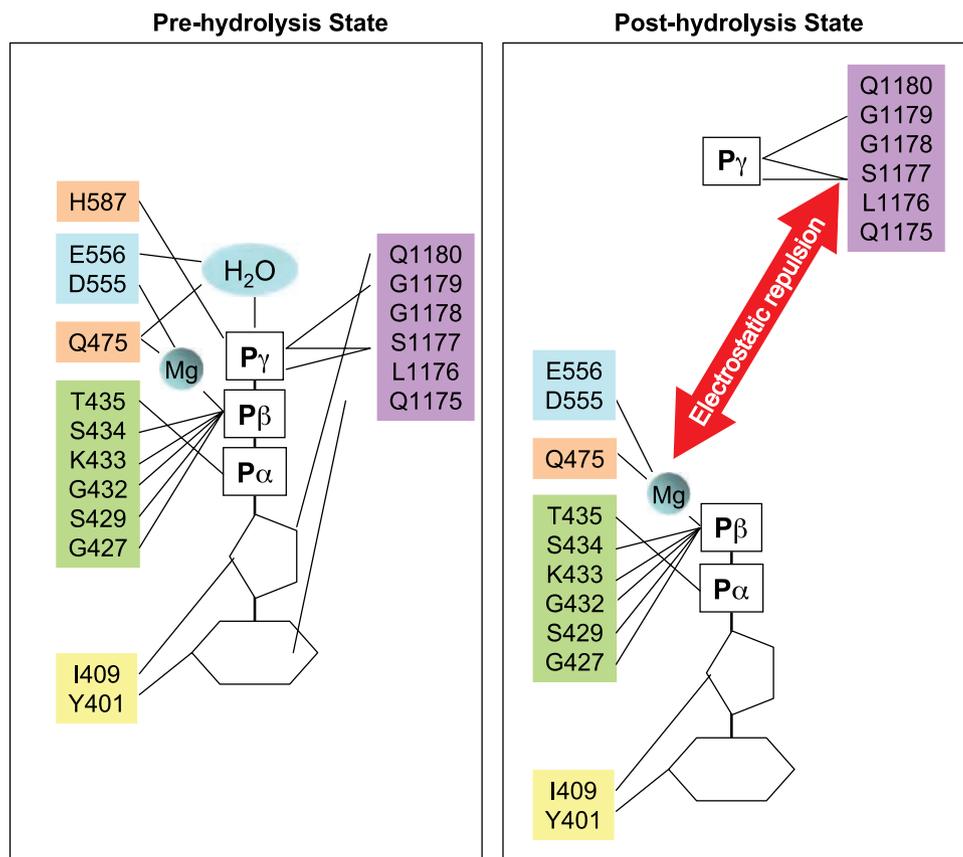
### The Occluded Nucleotide Conformation and Alternating Catalysis

There is extensive biochemical literature characterizing the Vi-trapped state of Pgp (see refs. 10, 63 for reviews). These studies show that the trapped nucleotide is the nucleoside diphosphate, and only one of the two catalytic sites is occupied by ADP. These findings formed the basis of the “alternating catalysis model” (8). The Vi-trapped reaction intermediate of Pgp represents a posthydrolysis reaction intermediate. It is thus plausible that the ATP occluded in the Walker B glutamate mutant Pgps described above is the one subsequently hydrolyzed and trapped in the presence of Vi. The molecular basis for alternating catalysis is poorly understood, but recent structural studies and real-time molecular dynamics simulations with bacterial ABC transporters and isolated NBD domains provide some important insights.

It has been shown that, in MalK, the hydrolysis of ATP (i.e., presence of ADP) at the active site leads to the dissociation of the dimer (64). The “catalytic carboxylate” mutants of MJ0796 and MJ1267 (ABC transporters from *Methanococcus jannaschii*) form dimers with ATP but not with ADP (65). Molecular dynamics simulations of the ATP-induced dimerization MalK show that interactions between the  $\gamma$ -phosphate and the signature motif are the first points of contact during the closure of the ATP site (61). These findings are consistent with the proposal of Hunt et al. (29) that ATP acts as a molecular glue neutralizing the electrostatic charge imbalance. Formation of ADP results in the disassociation of the dimer (see illustration in Fig. 2).

The findings detailed above and in preceding sections may provide a mechanism for alternating catalysis (Fig. 3). We have seen above that, in Pgp, ATP-driven dimerization of the NBDs is rapidly followed by the occlusion of ATP at one of the two catalytic sites (Fig. 3A, steps I and II).

**Figure 2.** Mechanochemistry of the prehydrolysis and posthydrolysis reactions. *Left*, major interactions between the residues in NBDs of Pgp and ATP at one nucleotide-binding site based on the X-ray crystallographic structure of MJ0796 and HlyB (29, 47). Essentially most of the interactions between the  $\gamma$ -phosphate are with the LSGGQ, signature sequence, whereas a conserved aromatic residue and the Walker A and Walker B residues interact with the adenine ring and the  $\alpha$ -phosphate and  $\beta$ -phosphate. The posthydrolysis electrostatic repulsion (*right*) would therefore result in the ADP moving with lobe 1 and the  $\gamma$ -phosphate with lobe II (see text for description of lobes I and II).



Occlusion of the ATP is followed by hydrolysis, resulting in the active site being occupied by ADP (Fig. 3A, step III). Evidence from bacterial systems suggests that a NBD occupied by ADP is unstable and leads to the disassembly of the occluded site (29, 64, 65), and this would result in the release of ADP (Fig. 3A, step IV). The ADP/ATP exchange at site 1 may favor the occlusion of ATP at site 2, thus bringing about alternate catalysis. A significant caveat is in order here. Tampe et al. (51) have recently proposed the “processive clamp model of ATP hydrolysis” in lieu of the alternative catalysis model. In such a model on ATP binding, both NBDs associate to a dimer. There is no distinction between the symmetrical “dimerization” and asymmetrical “occlusion.” The ATP-driven dimerization is followed by hydrolysis of the two ATPs in succession and the disassembly of the ATP sandwich. The evidence for an occluded nucleotide conformation of Pgp with a stoichiometry of one ATP molecule per transporter molecule (56, 58), the molecular dynamic simulations (60), and suggestions that the presence of TMDs leads to asymmetrical binding of ATP at one NBD (61) suggest that, in intact transporters, the alternating catalytic site model may be operational but additional evidence is clearly needed. Additional support for this view comes from the recently solved structure of Sav1866, a multidrug transporter from *Staphylococcus aureus* (48). This structure deviates from all previous structures in that the transmembrane helices are

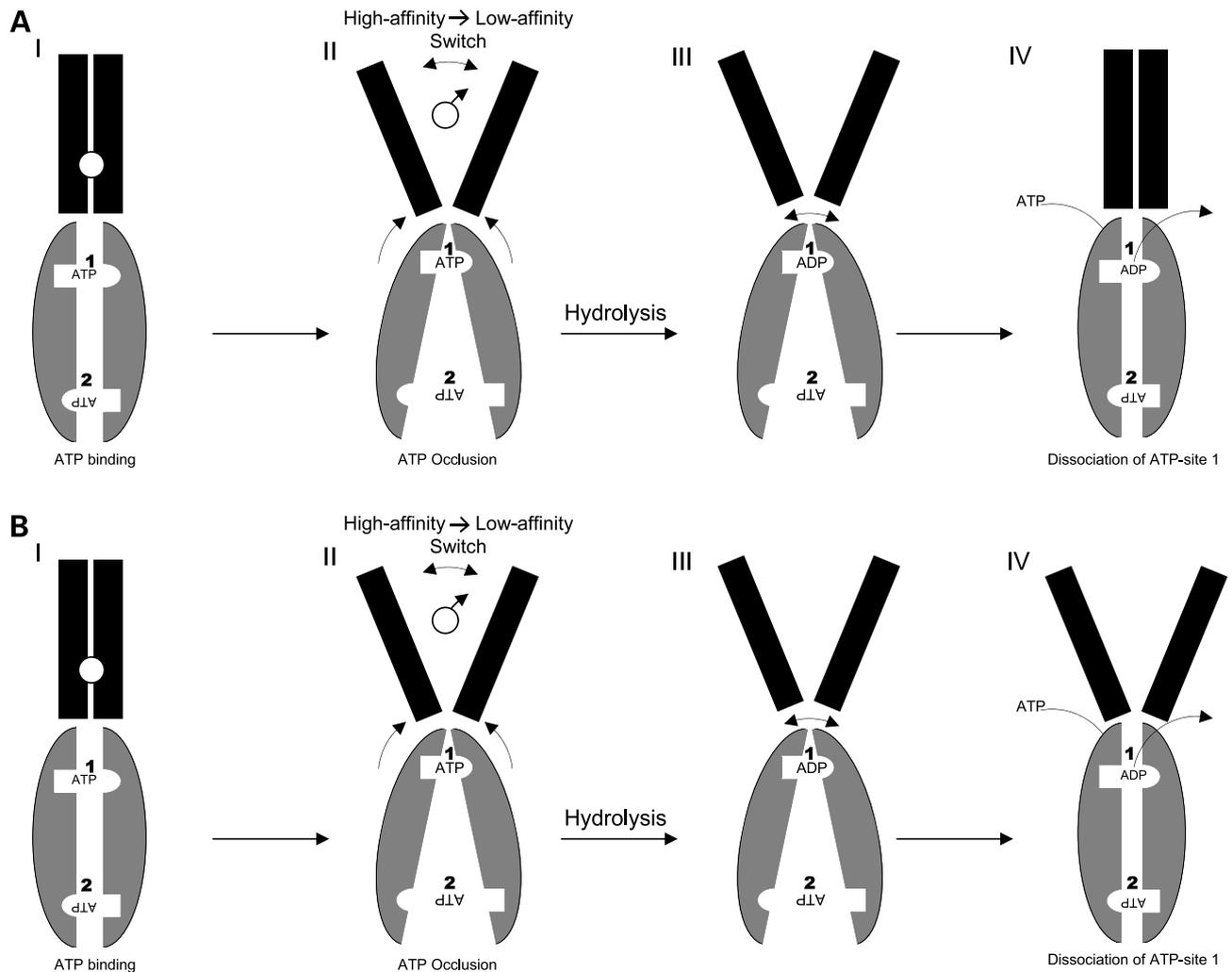
not aligned side by side but are intricately interleaved and their maximal separation during the catalytic cycle is likely to be limited. This has led Schuldiner (66) in the accompanying commentary to state that “this view contradicts mechanistic models that suggest that the NBDs join together upon binding ATP and then dissociate upon completing a transport cycle.” Nonetheless, the mechanochemical principles suggested in the processive clamp model (i.e., ATP-induced dimerization and ADP-induced dissociation) seem to be consistent with some structural and biochemical data (28, 29, 50, 59, 65).

### The Occluded Nucleotide Conformation of Pgp Exhibits the High-Affinity to Low-Affinity Switch

The formation of an ATP-driven NBD:NBD dimer per se does not necessarily imply that the resultant conformational changes are coupled to the movements at the drug-binding site that bring about transport. Hunt et al. (29) in describing the crystal structure of the MJ0796 dimer provided the first postulate of how such a coupling may occur and suggested two alternative scenarios. (a) The formation of the nucleotide sandwich may be directly harnessed to displacements of the transmembrane helices that provide the power stroke for the pump (i.e., the conformational changes that bring about the high-affinity

to low-affinity switch at the drug-binding site). The free energy gain on ATP hydrolysis, on the other hand, is used to drive the thermodynamic destabilization of the nucleotide sandwich dimer, which in turn allows the pump to

reset. (b) ATP hydrolysis results in electrostatic repulsion between the ADP product bound to the Walker A motif and the inorganic phosphate ( $P_i$ ) product bound to the LSGGQ signature motif in the apposing subunit. The



**Figure 3.** Ocluded nucleotide conformation in the transport pathway. *Gray ovals*, NBDs; *black rectangles*, TMDs that interact with drug substrate. The two ATP sites are labeled 1 and 2, respectively. The domains lining each ATP site are as shown in the *inset* to Fig. 1. For clarity, only ADP is shown as the hydrolysis product and not  $P_i$ . This is because several studies have shown that  $P_i$  has an extremely low affinity for ABC transporters and is released before ADP. *White circle*, drug substrate molecule. **A**, ATP-driven occlusion drives transport and ADP-driven disassembly of ATP site resets the TMDs: step I represents the sandwich dimer with a molecule of ATP bound at each ATP site (state II in Fig. 1). This is rapidly followed by the asymmetrical occlusion of ATP at site 1 depicted as state III in Fig. 1. Conformational changes that occur as a consequence of ATP occlusion are transmitted to the TMD and bring about the high-affinity to low-affinity switch [i.e., the drug-binding site is exposed extracellularly and affinity for drug substrate is reduced (step II)]. The occluded nucleotide is hydrolyzed to ADP at site 1 (step III). Presence of ADP at site 1 results in dissociation of the two faces of this ATP site as illustrated in Fig. 2, returning the NBDs to the “nucleotide bound” conformation (step IV). ATP hydrolysis and disassembly of the ATP site 1 result in the drug-binding sites at the TMDs being reset. **B**, ATP-driven occlusion drives high-affinity to low-affinity switch but ADP-driven disassembly of ATP site does not reset the TMDs. The model is identical to that depicted in **A**, except that ADP hydrolysis, dissociation, and release do not reset the TMDs. In subsequent steps, it is plausible that site 2, which is already occupied by ATP, has an advantage vis-à-vis occlusion over site 1 where the ADP/ATP exchange is occurring. This may bring about alternate catalysis. Data from our laboratory suggest that hydrolysis and ADP release at site 1 does not reset the TMDs (see text). These data favor the model depicted in **B**; however, this issue has not been resolved and thus the scenario outlined in **A** is also plausible. Structures of intact ABC transporters, particularly that of Sav1866 (48), suggest that intramolecular movements may be relatively constrained and complete association and disassociation of NBDs during the reaction pathway are unlikely (66). Other models have, however, been proposed, including the processive clamp model (51). This model does not distinguish between ATP-driven dimerization and the asymmetrical occlusion of ATP at one of the two sites. In this model, ATP binding to two monomeric NBDs leads to formation of a NBD dimer that, after hydrolysis of both ATPs, dissociates and releases ADP. The ATP switch model (12) proposes that the conformational changes described at the ATP sites in processive clamp model are transmitted to the TMDs during the transport pathway. Thus, the assembly of the nucleotide sandwich is accompanied by the high-affinity to low-affinity switch and disassembly of the nucleotide sandwich resets the drug substrate site. It is important to note that, in both **A** and **B**, simplified versions are presented and it is quite possible that there are additional steps involved, which have not yet been identified.

conformational changes resulting from this separation of the ABCs could be the ones coupled to the movement of drugs. The ATP switch model, proposed recently, endeavors to make the case that the former scenario is operational in ABC proteins and hypothesizes that ATP binding, not hydrolysis, provides the power stroke for the pump (12). This hypothesis is supported by the following. (a) A 10 Å resolution structure of Pgp was determined by electron cryomicroscopy of two-dimensional crystals, grown in the absence of nucleotide and in the presence of either AMPPNP (a nonhydrolyzable ATP analogue) or ADP and Vi. Although this level of resolution does not provide much structural detail at the atomic level, it is clear that the projection maps of the extracellular surface of the proteins were very different from each other. These workers contend that the differences could not be accounted for by a change in the oligomeric state of Pgp and must correspond to conformational changes in the monomer (67). (b) The affinity of a Pgp transport substrate, vinblastine, was drastically reduced when the Pgp was trapped with AMPPNP, ATP- $\gamma$ -S, or ADP + Vi (68, 69).

The discussion in section 4 suggests the “nucleotide binding” involves the ATP-driven dimerization of the NBDs (Fig. 1, state II) followed by a rapid, temperature-dependent occlusion of ATP at one of the two ATP sites (Fig. 1, state III). It would thus be important to determine which of these steps (if any) brings about the high-affinity to low-affinity switch at the drug-binding site. We have summarized above the evidence that shows that a “prehydrolysis” state of Pgp obtained with AMP-PNP or ATP- $\gamma$ -S exhibits conformational changes at the drug-binding site. However, it is not clear from these data whether the nonhydrolyzable analogues of ATP represent the “nucleotide bound” state or the “occluded nucleotide” state of Pgp. Recent work from our laboratory (58) suggests that the formation of the ATP-occluded conformation of the mutant human Pgp E556Q/E1201Q is energy dependent with an activation energy of  $\sim 70$  kJ/mol, a value that is comparable with that obtained for ATP-driven dimerization of an equivalent mutant of Mdl1 (50). These results suggest that relatively large conformational changes accompany the occlusion of ATP (38). We have furthermore shown that occlusion of ATP in the mutant human Pgp E556Q/E1201Q is accompanied by a decreased binding of transport substrate. We also showed that the activation energies for nucleotide trapping and decrease in drug binding are equivalent (58). This suggests that the high-affinity to low-affinity switch at the drug-binding site and occlusion of ATP are also coupled. This is illustrated in Fig. 3 (step II in Fig. 3A and B). More important, our studies indicate that, in the absence of occlusion of nucleotide, we do not observe a high-affinity to low-affinity switch at the drug-binding site.

It is, however, important to note that the Vi-trapped state of Pgp, which is clearly a posthydrolysis state, also shows features comparable with the prehydrolysis state, occluded nucleotide conformation. Thus, for example, projection maps calculated from two-dimensional crystals of Pgp show that the crystals grown in the presence of AMP-PNP

are very different from those grown in the absence of nucleotide. The lattice dimensions of the crystals grown in AMP-PNP or ADP + Vi are roughly comparable ( $a = 64 \pm 0.5$  Å;  $b = 65 \pm 0.4$  Å;  $c = 119 \pm 0.5$  Å versus  $a = 64 \pm 0.4$  Å;  $b = 68 \pm 0.2$  Å;  $c = 119 \pm 0.5$  Å). Similarly, both AMP-PNP-bound and ADP-Vi-trapped states of Pgp show reduced affinity for the drug (34, 35, 68–70). As both occluded ATP and trapped ADP do not show reduced binding of drug substrates, it would suggest that, although ATP occlusion may be sufficient to bring about the high-affinity to low-affinity switch, the subsequent hydrolysis of the occluded nucleotide is not sufficient to reset the TMDs (Fig. 3B, steps II–IV). An interesting observation by Callaghan et al. was that, although Pgp in the presence of ATP- $\gamma$ -S or ATP and Vi shows reduced affinity for [ $^3$ H]vinblastine, the nucleotides do not affect the binding of [ $^3$ H]XR9576. The authors speculate that this reflects either the different affinities of the two radioligands or the fact that vinblastine is a transport substrate of Pgp whereas XR9576 is a non-transported modulator (68). Dey et al. (71) on the other hand showed that, although binding of [ $^{125}$ I]IAAP was reduced in the Vi-trapped conformation of Pgp, there were alternative pathways to resetting the Pgp molecule. Thus, in the absence of a modulator, *cis*(Z)-flupentixol, a second round of ATP hydrolysis was necessary to recover [ $^{125}$ I]IAAP binding, whereas the second ATP hydrolysis event was unnecessary when recovery of [ $^{125}$ I]IAAP was effected in the presence of *cis*(Z)-flupentixol. These findings suggest that transport substrates, inhibitors, and modulators may influence the transport pathway of Pgp in different ways and should be investigated further. The reaction intermediates of the ATPase reaction that have been studied and the effect of each on the drug-binding site are summarized in Table 1. Although different laboratories have used diverse probes and techniques to monitor conformational changes at the drug-binding site, they share the common strategy of exploiting reaction intermediates of the ATPase reaction to trap the transporter at different steps of the ATPase reaction. One objection to such an analysis, however, is that stable trapped states need not display the binding properties of the true transition states (see ref. 72). Such a view, moreover, is consistent with the observation of Qu et al. (73) that the “transition state” Pgp binds drugs and modulators with unchanged affinity. This study contradicts the findings from several laboratories (see above) for which there could be several explanations. The experiments are conducted with purified soluble protein in CHAPS, detergent solution in the presence or absence of added lipid. It remains to be determined whether the conformation of the protein in this system is the same as that of native protein or protein reconstituted in a lipid bilayer free of detergent. Quenching of tryptophan fluorescence is used to determine the affinity of drug substrates. This is a measure of a ‘global’ conformational change as the human Pgp contains 11 tryptophans randomly distributed across the molecule. For this approach to be useful, a tryptophan-less Pgp should be constructed and tryptophans should be reintroduced into

**Table 1. Conformational changes in the drug-binding sites during the different reaction intermediates of the ATPase reaction mediated by Pgp**

| Reaction intermediate | Trapped intermediate                 | Effect on drug-binding site  | References  |
|-----------------------|--------------------------------------|--|---|
| E + S                 | Pgp + ATP                            | No effect on IAAP binding  | (34, 70)  |
| E•S                   | Pgp (E556Q/E1201Q)•ATP<br>Pgp•AMPPNP | Reduced affinity for IAAP<br>Reduced affinity for vinblastine<br>Structural changes in transmembrane domains observed by electron microscopy   | (55, 58)<br>(67, 69)<br>(67, 78)                                  |
| E•P                   | Pgp•ATP- $\gamma$ -S<br>Pgp•ADP•Vi   | Conformational changes using the antibody UIC2<br>Reduced affinity for vinblastine<br>Reduced affinity for IAAP<br>Reduced affinity for vinblastine<br>Structural changes in transmembrane domains observed by electron microscopy | (67)<br>(68) and *<br>(34, 35, 37, 55, 70)<br>(67–69)<br>(67, 78) |
| E + P                 | Pgp + ADP + Vi ( $P_i$ )             | Conformational changes using the antibody UIC2<br>Rotation of helices TM6 and TM12<br>Reduced affinity for IAAP  | (67, 79, 80)<br>(76)<br>(34, 35)                                  |

\*Sauna and Ambudkar, unpublished data.

specific TMDs. Similarly, an alternative method used by this group involves the labeling of the ATP sites of Pgp with MIANS and obtaining affinity measurements of transport substrates based on the inhibition of MIANS fluorescence. The transport substrate sites are located in the TMDs, and the NBDs are in the cytoplasmic loops. These are spatially separated molecular targets, and thus,  $K_d$  values are obtained by a “long-range” interaction where the ligand does not directly interact with the fluorescent probe. Thus, as these are all indirect methods, these approaches need to be validated by using point mutations either in the transport substrate site or in NBDs that affect ATP hydrolysis and drug specificity.

### Resetting the Transport Pathway

The final step in the transport pathway involves resetting the molecule to its ground state (i.e., a conformation that can bind another molecule of the drug with high affinity to begin the next cycle). This is probably the least understood step in the transport pathway. Higgins and Linton (12) have proposed a generalized model for the transport pathway of ABC proteins. The essential feature of this model is the “ATP switch,” where ATP binding and ATP hydrolysis, respectively, bring about formation and dissociation of the NBD dimer. Formation of the closed dimer (ATP binding) brings about conformational changes in the TMDs that reduce the affinity for the drug and expose it to the extracellular milieu. ATP hydrolysis resets the protein. Such a scenario is depicted in Fig. 3A, and the high-affinity to low-affinity switch would occur at step II and the TMDs would be reset at step IV. As is evident from sections 4 to 6, this is an oversimplification (at least for Pgp). ATP-driven dimerization and an asymmetrical occlusion of nucleotide at one of the two catalytic sites seem to be two distinct steps (Fig. 1). Moreover, it is the second of these two steps that is

energetically driven, involves relatively large conformational changes, and seems to drive the high-affinity to low-affinity switch at the drug-binding site (58). The occluded ATP has also been described as “committed” to ATP hydrolysis (74), and it is plausible that this represents an early step, the E•S state, in the ATP hydrolysis reaction (represented in a simplified form as  $E + S \rightarrow E \cdot S \rightarrow E \cdot P \rightarrow E + P$ , where E is Pgp and S and P are ATP and ADP, respectively). Published studies with Pgp show that the nucleoside diphosphate can be trapped at the catalytic site in the presence of Vi (10). Formation of the Pgp•ADP•Vi reaction intermediate follows release of  $P_i$  and thus represents a posthydrolysis (E•P) state of the ATPase reaction. There is also evidence, using several different techniques, that the Vi-trapped state of Pgp shows reduced affinity for drugs (34, 35, 67–69). Similarly, cross-linking studies using a cysteine-less mutant of Pgp show rotations in the transmembrane helices TM6 and TM12 (implicated in drug substrate binding) in the Vi-trapped state (75, 76). One explanation could be that the high-affinity to low-affinity switch occurs during the formation of the E•S intermediate of the ATPase reaction. What is not evident is the effect (if any) of the subsequent steps, release of  $P_i$  and ADP in the ATPase reaction on the TMDs. Thus, the biochemical data seem to indicate that conformational changes associated with  $P_i$  and ADP release are not sufficient to reset the Pgp (Fig. 3B, step IV). Only we have carried out studies that experimentally followed events beyond the formation of the E•P intermediate and found that, after formation of the Pgp•ADP•Vi reaction intermediate, release of both  $P_i$  and ADP was not sufficient to restore binding of the transport substrate [ $^{125}$ I]IAAP (34, 35). These studies indicated that an additional ATP hydrolysis event was necessary to advance the transport pathway to completion. However, the studies relied on the use of azido-labeled transport substrate and nucleotide and

UV cross-linking, which have inherent drawbacks in the interpretation of kinetic data (72). Similar studies with other ABC transporters using alternative techniques are thus necessary to unequivocally resolve the issue “what resets the transporter?” Is it  $P_i$  release, ADP release, or an additional ATP binding and/or hydrolysis event? A reliable estimation of the stoichiometry of moles ATP hydrolyzed per mole of substrate transported would also help in understanding the transport pathway. However, the values reported in the literature range from 1 to 50 mol of ATP per mol of substrate transported (see ref. 77 for an overview and for a discussion of the limitations of the methods used).

## Conclusions

Our current understanding of the coupling between the steps in the ATPase reaction and mechanical movements at the drug-binding site is summarized in Table 1. Although several studies have determined the conformational changes at the TMDs during the formation of the E-S and E-P intermediates of the ATPase reaction, very few studies have experimentally monitored the effect of the subsequent steps of the ATPase catalytic cycle on the drug-binding site(s). This is an important area of investigation that needs greater attention. Additionally, the formation of the ATP sandwich dimer in ABC proteins has in the last few years emerged as a core theme in elucidating the transport pathway. Studies with mutants of the “catalytic carboxylate” of Pgp suggest that the asymmetrical occlusion of ATP follows dimerization of the NBDs. The latter seems to drive conformational changes that facilitate transport. Much of the structural evidence that allows us to understand the ATP sandwich has come from isolated NBDs. However, there are suggestions based on molecular dynamic simulations, structures of intact ABC transporters, and biochemical data that the presence of TMDs may be necessary for asymmetrical nucleotide occlusion compared with the symmetrical binding observed in isolated domains. High-resolution structures of intact Pgp and other ABC proteins that include both the NBDs and the TMDs harboring the drug-binding sites captured in the ground state and various intermediate steps during the catalytic cycle are thus necessary for us to understand the coupling between ATP hydrolysis and drug transport.

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## References

- Dean M, Hamon Y, Chimini G. The human ATP-binding cassette (ABC) transporter superfamily. *J Lipid Res* 2001;42:1007–17.
- Gottesman MM, Ambudkar SV. Overview: ABC transporters and human disease. *J Bioenerg Biomembr* 2001;33:453–8.
- Dean M, Annilo T. Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. *Annu Rev Genomics Hum Genet* 2005;6:123–42.
- Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat Rev Cancer* 2002;2:48–58.
- Higgins CF, Hiles ID, Salmond GPC, et al. A family of related ATP-binding subunits coupled to many distinct biological processes in bacteria. *Nature* 1986;323:448–50.
- Ambudkar SV, Kim IW, Xia D, Sauna ZE. The A-loop, a novel conserved aromatic acid subdomain upstream of the Walker A motif in ABC transporters, is critical for ATP binding. *FEBS Lett* 2006;580:1049–55.
- Kim IW, Peng XH, Sauna ZE, et al. The conserved tyrosine residues 401 and 1044 in ATP sites of human P-glycoprotein are critical for ATP binding and hydrolysis: evidence for a conserved subdomain, the A-loop in the ATP-binding cassette. *Biochemistry* 2006;45:7605–16.
- Senior AE, Al-Shawi MK, Urbatsch IL. The catalytic cycle of P-glycoprotein. *FEBS Lett* 1995;377:285–9.
- Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem* 1993;62:385–427.
- Sauna ZE, Smith MM, Muller M, Kerr KM, Ambudkar SV. The mechanism of action of multidrug-resistance-linked P-glycoprotein. *J Bioenerg Biomembr* 2001;33:481–91.
- Davidson AL. Mechanism of coupling of transport to hydrolysis in bacterial ATP-binding cassette transporters. *J Bacteriol* 2002;184:1225–33.
- Higgins CF, Linton KJ. The ATP switch model for ABC transporters. *Nat Struct Mol Biol* 2004;11:918–26.
- Jones PM, George AM. The ABC transporter structure and mechanism: perspectives on recent research. *Cell Mol Life Sci* 2004;61:682–99.
- Reyes CL, Ward A, Yu J, Chang G. The structures of MsbA: insight into ABC transporter-mediated multidrug efflux. *FEBS Lett* 2006;580:1042–8.
- van der Does C, Tampe R. How do ABC transporters drive transport? *Biol Chem* 2004;385:927–33.
- Hanekop N, Zaitseva J, Jenewein S, Holland IB, Schmitt L. Molecular insights into the mechanism of ATP-hydrolysis by the NBD of the ABC-transporter HlyB. *FEBS Lett* 2006;580:1036–41.
- Loo TW, Clarke DM. Do drug substrates enter the common drug-binding pocket of P-glycoprotein through “gates”? *Biochem Biophys Res Commun* 2005;329:419–22.
- Loo TW, Bartlett MC, Clarke DM. The drug-binding pocket of the human multidrug resistance P-glycoprotein is accessible to the aqueous medium. *Biochemistry* 2004;43:12081–9.
- Sauna ZE, Andrus MB, Turner TM, Ambudkar SV. Biochemical basis of polyvalency as a strategy for enhancing the efficacy of P-glycoprotein (ABCB1) modulators: stipiamide homodimers separated with defined-length spacers reverse drug efflux with greater efficacy. *Biochemistry* 2004;43:2262–71.
- Loo TW, Clarke DM. Determining the dimensions of the drug-binding domain of human P-glycoprotein using thiol cross-linking compounds as molecular rulers. *J Biol Chem* 2001;276:36877–80.
- Ambudkar SV, Kim IW, Sauna ZE. The power of the pump: mechanisms of action of P-glycoprotein (ABCB1). *Eur J Pharm Sci* 2006;27:392–400.
- Sharom FJ. The P-glycoprotein efflux pump: how does it transport drugs? *J Membr Biol* 1997;160:161–75.
- Ambudkar SV, Dey S, Hrycyna CA, et al. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Annu Rev Pharmacol Toxicol* 1999;39:361–98.
- Hung LW, Wang IXY, Nikaido K, et al. Crystal structure of the ATP-binding subunit of an ABC transporter. *Nature* 1998;396:703–7.
- Karpowich N, Martsinkevich O, Millen L, et al. Crystal structures of the MJ1267 ATP binding cassette reveal an induced-fit effect at the ATPase active site of an ABC transporter. *Structure* 2001;9:571–86.
- Chen J, Sharma S, Quioco FA, Davidson AL. Trapping the transition state of an ATP-binding cassette transporter: evidence for a concerted mechanism of maltose transport. *Proc Natl Acad Sci U S A* 2001;98:1525–30.
- Loo TW, Bartlett MC, Clarke DM. The “LSGGQ” motif in each nucleotide-binding domain of human P-glycoprotein is adjacent to the opposing Walker A sequence. *J Biol Chem* 2002;277:41303–6.

28. Hopfner KP, Karcher A, Shin DS, et al. Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily. *Cell* 2000;101:789–800.
29. Smith PC, Karpowich N, Millen L, et al. ATP binding to the motor domain from an ABC transporter drives formation of a nucleotide sandwich dimer. *Mol Cell* 2002;10:139–49.
30. Ambudkar SV, Lelong IH, Zhang J, et al. Partial purification and reconstitution of the human multidrug-resistance pump: characterization of the drug-stimulatable ATP hydrolysis. *Proc Natl Acad Sci U S A* 1992;89:8472–6.
31. Al-Shawi MK, Senior AE. Characterization of the adenosine triphosphatase activity of Chinese hamster P-glycoprotein. *J Biol Chem* 1993;268:4197–206.
32. Sarkadi B, Price EM, Boucher RC, Germann UA, Scarborough GA. Expression of the human multidrug resistance cDNA in insect cells generates a high activity drug-stimulated membrane ATPase. *J Biol Chem* 1992;267:4854–8.
33. Sharom FJ, Yu X, Chu JW, Doige CA. Characterization of the ATPase activity of P-glycoprotein from multidrug-resistant Chinese hamster ovary cells. *Biochem J* 1995;308:381–90.
34. Sauna ZE, Ambudkar SV. Evidence for a requirement for ATP hydrolysis at two distinct steps during a single turnover of the catalytic cycle of human P-glycoprotein. *Proc Natl Acad Sci U S A* 2000;97:2515–20.
35. Sauna ZE, Ambudkar SV. Characterization of the catalytic cycle of ATP hydrolysis by human P-glycoprotein: the two ATP hydrolysis events in a single catalytic cycle are kinetically similar but affect different functional outcomes. *J Biol Chem* 2001;276:11653–61.
36. Romsicki Y, Sharom FJ. The ATPase and ATP-binding functions of P-glycoprotein—modulation by interaction with defined phospholipids. *Eur J Biochem* 1998;256:170–8.
37. Sauna ZE, Smith MM, Muller M, Ambudkar SV. Functionally similar vanadate-induced 8-azidoadenosine 5'-[ $\alpha$ -(32)P]diphosphate-trapped transition state intermediates of human P-glycoprotein are generated in the absence and presence of ATP hydrolysis. *J Biol Chem* 2001;276:21199–208.
38. Al-Shawi MK, Polar MK, Omote H, Figler RA. Transition state analysis of the coupling of drug transport to ATP hydrolysis by P-glycoprotein. *J Biol Chem* 2003;278:52629–40.
39. Loo TW, Bartlett MC, Clarke DM. Drug binding in human P-glycoprotein causes conformational changes in both nucleotide-binding domains. *J Biol Chem* 2003;278:1575–8.
40. Tomblin G, Donnelly DJ, Holt JJ, et al. Stimulation of P-glycoprotein ATPase by analogues of tetramethylrosamine: coupling of drug binding at the "R" site to the ATP hydrolysis transition state. *Biochemistry* 2006;45:8034–47.
41. Borst P, Elferink RO. Mammalian ABC transporters in health and disease. *Annu Rev Biochem* 2002;71:537–92.
42. Haimeur A, Conseil G, Deeley RG, Cole SPC. The MRP-related and BCRP/ABCG2 multidrug resistance proteins: biology, substrate specificity, and regulation. *Curr Drug Metab* 2004;5:21–53.
43. Jones PM, George AM. Subunit interactions in ABC transporters: towards a functional architecture. *FEMS Microbiol Lett* 1999;179:187–202.
44. Lamers MH, Perrakis A, Enzlin JH, et al. The crystal structure of DNA mismatch repair protein MutS binding to a G  $\times$  T mismatch. *Nature* 2000;407:711–7.
45. Obmolova G, Ban C, Hsieh P, Yang W. Crystal structures of mismatch repair protein MutS and its complex with a substrate DNA. *Nature* 2000;407:703–10.
46. Locher KP, Lee AT, Rees DC. The *E. coli* BtuCD structure: a framework for ABC transporter architecture and mechanism. *Science* 2002;296:1091–8.
47. Zaitseva J, Jenewein S, Jumpertz T, Holland IB, Schmitt L. H662 is the linchpin of ATP hydrolysis in the nucleotide-binding domain of the ABC transporter HlyB. *EMBO J* 2005;24:1901–10.
48. Dawson RJP, Locher KP. Structure of a bacterial multidrug ABC transporter. *Nature* 2006;443:180–5.
49. Litman T, Brangi M, Hudson E, et al. The multidrug-resistant phenotype associated with overexpression of the new ABC half-transporter, MXR (ABCG2). *J Cell Sci* 2000;113:2011–21.
50. van der Does C, Presenti C, Schulze K, Dinkelaker S, Tampe R. Kinetics of the ATP hydrolysis cycle of the nucleotide-binding domain of Mdl1 studied by a novel site-specific labeling technique. *J Biol Chem* 2006;281:5694–701.
51. Janas E, Hofacker M, Chen M, et al. The ATP hydrolysis cycle of the nucleotide-binding domain of the mitochondrial ATP-binding cassette transporter Mdl1p. *J Biol Chem* 2003;278:26862–9.
52. Vergani P, Lockless SW, Nairn AC, Gadsby DC. CFTR channel opening by ATP-driven tight dimerization of its nucleotide-binding domains. *Nature* 2005;433:876–80.
53. Lockless SW, Ranganathan R. Evolutionarily conserved pathways of energetic connectivity in protein families. *Science* 1999;286:295–9.
54. Serrano L, Horovitz A, Avron B, Bycroft M, Fersht AR. Estimating the contribution of engineered surface electrostatic interactions to protein stability by using double-mutant cycles. *Biochemistry* 1990;29:9343–52.
55. Sauna ZE, Muller M, Peng XH, Ambudkar SV. Importance of the conserved Walker B glutamate residues, 556 and 1201, for the completion of the catalytic cycle of ATP hydrolysis by human P-glycoprotein (ABCB1). *Biochemistry* 2002;41:13989–4000.
56. Tomblin G, Bartholomew LA, Urbatsch IL, Senior AE. Combined mutation of catalytic glutamate residues in the two nucleotide binding domains of P-glycoprotein generates a conformation that binds ATP and ADP tightly. *J Biol Chem* 2004;279:31212–20.
57. Tomblin G, Senior AE. The occluded nucleotide conformation of P-glycoprotein. *J Bioenerg Biomembr* 2005;37:497–500.
58. Sauna ZE, Nandigama K, Ambudkar SV. Exploiting reaction intermediates of the ATPase reaction to elucidate the mechanism of transport by P-glycoprotein (ABCB1). *J Biol Chem* 2006;281:26501–11.
59. Chen J, Lu G, Lin J, Davidson AL, Quijcho FA. A tweezers-like motion of the ATP-binding cassette dimer in an ABC transport cycle. *Mol Cell* 2003;12:651–61.
60. Oloo EO, Tieleman DP. Conformational transitions induced by the binding of MgATP to the vitamin B-12 ATP-binding cassette (ABC) transporter BtuCD. *J Biol Chem* 2004;279:45013–9.
61. Oloo EO, Fung EY, Tieleman DP. The dynamics of the MgATP-driven closure of MalK, the energy-transducing subunit of the maltose ABC transporter. *J Biol Chem* 2006;281:28397–407.
62. Reyes CL, Chang G. Structure of the ABC transporter MsbA in complex with ADP-vanadate and lipopolysaccharide. *Science* 2005;308:1028–31.
63. Ambudkar SV, Kimchi-Sarfaty C, Sauna ZE, Gottesman MM. P-glycoprotein: from genomics to mechanism. *Oncogene* 2003;22:7468–85.
64. Lu G, Westbrooks JM, Davidson AL, Chen J. ATP hydrolysis is required to reset the ATP-binding cassette dimer into the resting-state conformation. *Proc Natl Acad Sci U S A* 2005;102:17969–74.
65. Moody JE, Millen L, Binns D, Hunt JF, Thomas PJ. Cooperative, ATP-dependent association of the nucleotide binding cassettes during the catalytic cycle of ATP-binding cassette transporters. *J Biol Chem* 2002;277:21111–4.
66. Schuldiner S. Structural biology: the ins and outs of drug transport. *Nature* 2006;443:156–7.
67. Rosenberg MF, Velarde G, Ford RC, et al. Repacking of the transmembrane domains of P-glycoprotein during the transport ATPase cycle. *EMBO J* 2001;20:5615–25.
68. Martin C, Berridge G, Mistry P, et al. Drug binding sites on P-glycoprotein are altered by ATP binding prior to nucleotide hydrolysis. *Biochemistry* 2000;39:11901–6.
69. Martin C, Higgins CF, Callaghan R. The vinblastine binding site adopts high- and low-affinity conformations during a transport cycle of P-glycoprotein. *Biochemistry* 2001;40:15733–42.
70. Ramachandra M, Ambudkar SV, Chen D, et al. Human P-glycoprotein exhibits reduced affinity for substrates during a catalytic transition state. *Biochemistry* 1998;37:5010–9.
71. Maki N, Moitra K, Ghosh P, Dey S. Allosteric modulation bypasses the requirement for ATP hydrolysis in regenerating low affinity transition state conformation of human P-glycoprotein. *J Biol Chem* 2006;281:10769–77.
72. Russell PL, Sharom FJ. Conformational and functional characterization of trapped complexes of the P-glycoprotein multidrug transporter. *Biochem J* 2006;399:315–23.
73. Qu Q, Chu JWK, Sharom FJ. Transition state P-glycoprotein binds

drugs and modulators with unchanged affinity, suggesting a concerted transport mechanism. *Biochemistry* 2003;42:1345–53.

74. Tomblin G, Muharemagic A, White LB, Senior AE. Involvement of the "occluded nucleotide conformation" of P-glycoprotein in the catalytic pathway. *Biochemistry* 2005;44:12879–86.

75. Loo TW, Clarke DM. Cross-linking of human multidrug resistance P-glycoprotein by the substrate, tris-(2-maleimidoethyl)amine, is altered by ATP hydrolysis. *J Biol Chem* 2001;276:31800–5.

76. Loo TW, Clarke DM. Vanadate trapping of nucleotide at the ATP-binding sites of human multidrug resistance P-glycoprotein exposes different residues to the drug-binding site. *Proc Natl Acad Sci U S A* 2002;99:3511–6.

77. Shapiro AB, Ling V. Stoichiometry of coupling of rhodamine 123

transport to ATP hydrolysis by P-glycoprotein. *Eur J Biochem* 1998;254:189–93.

78. Rosenberg MF, Callaghan R, Modok S, Higgins CF, Ford RC. Three-dimensional structure of P-glycoprotein: the transmembrane regions adopt an asymmetric configuration in the nucleotide-bound state. *J Biol Chem* 2005;280:2857–62.

79. Druley TE, Stein WD, Roninson IB. Analysis of MDR1 P-glycoprotein conformational changes in permeabilized cells using differential immunoreactivity. *Biochemistry* 2001;40:4312–22.

80. Druley TE, Stein WD, Ruth A, Roninson IB. P-glycoprotein-mediated colchicine resistance in different cell lines correlates with the effects of colchicine on P-glycoprotein conformation. *Biochemistry* 2001;40:4323–31.

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## About a switch: how P-glycoprotein (ABCB1) harnesses the energy of ATP binding and hydrolysis to do mechanical work

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