Catalytic and transport cycles of ABC exporters

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Abstract

ABC (ATP-binding cassette) transporters are arguably the most important family of ATP-driven transporters in biology. Despite considerable effort and advances in determining the structures and physiology of these transporters, their fundamental molecular mechanisms remain elusive and highly controversial. How does ATP hydrolysis by ABC transporters drive their transport function? Part of the problem in answering this question appears to be a perceived need to formulate a universal mechanism. Although it has been generally hoped and assumed that the whole superfamily of ABC transporters would exhibit similar conserved mechanisms, this is proving not to be the case. Structural considerations alone suggest that there are three overall types of coupling mechanisms related to ABC exporters, small ABC importers and large ABC importers. Biochemical and biophysical characterization leads us to the conclusion that, even within these three classes, the catalytic and transport mechanisms are not fully conserved, but continue to evolve. ABC transporters also exhibit unusual characteristics not observed in other primary transporters, such as uncoupled basal ATPase activity, that severely complicate mechanistic studies by established methods. In this chapter, I review these issues as related to ABC exporters in particular. A consensus view has emerged that ABC exporters follow alternating-access switch transport mechanisms. However,

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some biochemical data suggest that alternating catalytic site transport mechanisms are more appropriate for fully symmetrical ABC exporters. Heterodimeric and asymmetrical ABC exporters appear to conform to simple alternating-access-type mechanisms.

**Introduction**

The ABC (ATP-binding cassette) transporters constitute one of the largest superfamilies of membrane proteins. They are ubiquitous in all phyla. Conserved motifs in NBDs (nucleotide-binding domains) of ABC transporters constitute the defining elements of this family and represent some of the most conserved motifs in biology. Consequently, many mechanistic models based on structural observations have been proposed to account for ABC transporter functionality. These proteins are found on plasma membranes and internal organelle membranes. ATP hydrolysis is the energy source for coupled substrate transport by the ABC transporters, thus substrate transport can be ‘uphill’ against a concentration gradient. These proteins act as importers and exporters from cells and organelles. The genetic split between importers and exporters occurred before the divergence of prokaryotes and eukaryotes [1]. Substrates or ‘allocrites’ for this family of transporters encompass all types of molecules that need to be transported across a membrane against a concentration gradient [2]. Accessory substrate-binding proteins are utilized by prokaryotic ABC importers that accumulate substrates into the cell. There are between 45 and 48 annotated ABC transporter genes in humans and many are associated with diseases or genetic disorders. To ease confusion in the field, the genes have been organized into seven subfamilies in the human genome: subfamily ABCA to subfamily ABCG [3]. This chapter is devoted to presenting current ideas about the coupling of ATP hydrolysis to the transport function of these ABC transporter pumps, with a focus on ABC exporters. Coupling models have been highly contentious and dependent on the type of experimental data considered and the perspective while building the model. As will be seen, ABC exporters present some unique challenges in analysing their coupling mechanisms as they are different from standard primary transporters in many interesting ways.

**Structural organization of ABC transporters and mechanistic implications**

ABC transporters contain TMDs (transmembrane domains) that form the translocation pathway and two NBDs that constitute the power units (Figure 1). The general structure, topology and conserved motifs have been thoroughly reviewed in the previous chapter by Zolnerciks et al. Each NBD contains two nucleotide-binding motifs, the Walker A and Walker B motifs. The Walker A motif is involved in binding of nucleotide phosphates and the Walker B motif is involved in Mg\(^{2+}\) and water co-ordination at the catalytic
Figure 1. Topological arrangements of ABC exporters

Topological models of three ABC exporters illustrating different arrangements of the TMDs and NBDs. Within the TMDs, the numbered cylinders represent transmembrane \( \alpha \)-helices. Relative locations of conserved motifs of the NBD are also shown.

The NBDs also contain other conserved motifs that define the ABC family of proteins. These include the Q-loop, involved in Mg\(^{2+}\) co-ordination and binding to a water molecule and the ABC signature motif (also referred to as the C motif, consensus sequence LSGGQXQR) that is also involved in ATP binding. Both are located between the Walker motifs. In ABC exporters alone, directly preceding the signature motifs, there are X-loops (consensus sequence TEVGERG) which
appear to be involved in interdomain communication and cross-talk between NBDs and TMDs. Upstream of the Walker A motif is the A-loop in which a conserved aromatic residue interacts with the adenine ring of ATP through π–π interactions. Downstream of the Walker B motif is the D-loop involved in indirect co-ordination of the γ-phosphate of ATP through a water molecule and also the H-loop involved in hydrogen-bonding the γ-phosphate of ATP.

Two NBDs come together to form two NBSs (nucleotide-binding sites) with two nucleotides sandwiched in at the interface of the two NBDs (Figure 2). Together, both NBDs (NBD1 and NBD2) are involved in forming the two functional NBSs (NBS1 and NBS2). In this ‘nucleotide sandwich’ ‘head-to-tail’ arrangement, each NBS comprises the D-loop and ABC signature motif of one NBD (e.g. NBD2) and the Walker A and B motifs, H-loop and Q-loop from the other NBD (e.g. NBD1 in this example) to form NBS1. Thus ATP molecules that occupy the two NBSs (NBS1 or NBS2) can be simultaneously hydrogen-bonded by residues from each of the NBDs (NBD1 and NBD2). This type of ‘head-to-tail’ ATP-binding site (NBS) formed at the interface of the opposite NBD subunits was first predicted by Jones and George [4] and subsequently verified in isolated nucleotide dimer crystal structures [5] and later in full transporter structures [6].

Some ABC transporters such as Pgp (P-glycoprotein; ABCB1) and the homodimeric ‘half-transporter’ BCRP (breast cancer-resistance protein; ABCG2) contain two consensus NBSs (NBS1 and NBS2), both being capable of full ATP hydrolytic function. In such transporters, the NBDs are interchangeable [7]. Other transporters, such as the MRP (multidrug-resistance protein) family members (ABCC family), contain only one fully functional

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**Figure 2. Structure of nucleotide-binding domains**

Ribbon diagrams of the two nucleotide-binding domains of the homodimeric ABC exporter Sav1866 binding two non-hydrolysable AMP-PNP (adenosine 5′-[β,γ-imido]triphosphate) molecules shown as stick figures (PDB code 2ONJ). Each of the two nucleotides is hydrogen-bonded to both NBDs and ‘glues’ them together. Two NBSs are formed in the nucleotide-binding sandwich conformation. (A) View from inside the membrane. (B) Side view.
and conserved consensus NBS (NBS1) and one less conserved, degenerate and less hydrolytically active non-consensus NBS (NBS2) [8,9]. Consequently, the mechanism of coupled ATP hydrolysis might not be fully conserved between all ABC transporter subfamilies.

The minimal functional ABC transporter unit capable of transport consists of a complex of two NBDs and two TMDs. Given that the two NBSs are formed at the interface of NBD1 and NBD2, cross-talk between all four functional domains is expected and observed [10]. These four domains can be separately encoded [11] or are encoded as one polypeptide, as in the case of Pgp (ABCB1) and MRP1 (ABCC1) (Figure 1). Proteins containing all four domains are called ‘full transporters’. Alternatively, transporters are encoded as multidomain proteins in various combinations, such as the two identical homologous halves of the homodimeric half-transporter BCRP (ABCG2) (Figure 1). Similar to BCRP, bacterial ABC exporters are half-transporters with an NBD linked to a TMD in a single polypeptide form. Several ABC transporters also have additional domains or subunits that aid in regulation of transport of their particular substrates, such as accessory substrate-binding proteins, regulatory domains, regulatory proteins and extra TMDs (e.g. TMD0 of MRP1 in Figure 1). In prokaryotic importers, the NBDs, TMDs, accessory regulatory domains and substrate-binding proteins are encoded as different polypeptides. For any given transporter, each of the two NBDs and two TMDs may be identical or similar, generating a two-fold symmetrical or two-fold pseudo-symmetrical unit [12]. Each TMD in ABC exporters is usually composed of six α-helices. However, in prokaryotic ABC importers, other topologies ranging from five to ten α-helices per TMD have been observed [13,14]. Thus the TMDs of importers and exporters cannot be easily interchanged in models of transport.

It is best to consider the coupling mechanisms of these proteins in three distinct groups on the basis of their structures [15,16]. (i) ABC exporters, which are found in all kingdoms of life, contain six α-helices per TMD (12 transmembrane helices in total in the core unit, see Figure 3). The two TMDs are not simply joined side by side, but have significant twists and each has two transmembrane helices ‘domain-swapped’ into the other domain [17]. In this group, the α-helices extend well beyond the membrane into the cytoplasm as ICLs (intracellular loops). Consequently, the NBDs are approximately 25 Å (1 Å=0.1 nm) from the membrane. Each half-transporter or half-molecule has two short α-helices, called coupling helix 1 (between transmembrane helices 2 and 3 and on ICL1 of the N-terminal half) and coupling helix 2 (between transmembrane helices 4 and 5 and on ICL2 of the N-terminal half). Thus, in a whole transporter, there are four coupling helices that provide most of the interactions between the TMDs and NBDs. Coupling helices 1 make contacts with both NBDs during the formation of the nucleotide sandwich, but are released from contacts with the opposing NBD in the apo forms (Figure 3). In contrast, coupling helices 2 are always
domain-swapped into the other half-molecule’s NBD. This is not seen in other ABC proteins. Furthermore, NBD X-loops interact with the opposite TMD, mainly through ICL1 [18], but also through ICL2 [19]. Consequently, switching of conformations and orientations of the TMDs coupled to ATPase activity at the NBDs/NBSs is expected to be completely different for ABC exporters and importers. (ii) Group 2 are ‘small’ prokaryotic ‘type I ABC importers’ that have five or six transmembrane helices per TMD (usually 12 in total). The core consists of two TMDs each containing five transmembrane helices that are positioned side by side. An additional N-terminal transmembrane helix is occasionally found that is domain swapped into the other TMD. In contrast with ABC exporters, coupling helices 2 are not domain-swapped into the other half molecule, but are associated with their own NBD. (iii) Group 3 are ‘large’ prokaryotic ‘type II ABC importers’ that have ten transmembrane helices per TMD (20 in total). Here there is no domain-swapping between TMDs or NBDs. For further details, see the previous chapter by Zolnerciks et al.

All of these widely divergent architectures and distant lineages suggest that the mechanism of coupled ATP hydrolysis might not be fully conserved between all ABC transporters. Excellent reviews are available.
describing the mechanisms of ABC importers which are generally thought to obey ‘alternating-access’-type schemes [15,16,20]. Many of the controversial issues discussed in this chapter regarding basal ATPase, power strokes, stoichiometries of transport and hydrolysis, symmetry and asymmetry have their counterparts in ABC importers, but are beyond the scope of this chapter.

**Mechanistic requirements for coupling of substrate transport to ATP hydrolysis**

In active transport, the uphill change in chemical potential of the transported allocrite, or work performed, is coupled to and driven by a spontaneous process that releases energy. ATP hydrolysis powers all primary ABC transporters in performing their varied pump functions. A pump needs at least two gates that must be alternately open, but never be open simultaneously [21]. Here a gate is defined as a protein conformation that stops movement of the allocrite across it in the closed conformation, but allows movement in the open conformation. Each of the two gates must open only to one side of the membrane and not to the same side as the other gate. When both gates are closed, there is an ‘occluded state’, and the transported entity is trapped within the protein. A change in chemical potential (from low to high) of the transported allocrite occurs during this process of occlusion/deocclusion. The step of the reaction cycle in which this change in chemical potential of the transported entity occurs is the ‘power stroke’. Chemical energy is transformed to vectorial energy (osmotic or electrochemical energy) in this step [22].

In principle, any two obligatorily coupled reactions cycles, such as chemical and vectorial transport cycles sharing common intermediates, can drive each other [23]. In ABC proteins, the chemical reaction cycle constitutes the intermediates and steps of ATP hydrolysis on the transporter. The vectorial transport cycle represents the sequential protein conformations and allocrite-bound states and steps that are involved in transporting the allocrite from one side of the membrane to the other side. However, in active transport, there is an additional requirement that any reaction steps that would lead to uncoupled partial reactions must be avoided [24]. This is achieved by interleaving the partial reactions of the chemical reaction and the vectorial transport cycle such that they are inextricably interwoven. Neither the vectorial reaction nor the chemical reaction can occur unless the other reaction occurs. Coupling and energy conversion thus require that the partial reactions of ATP hydrolysis control the coordinated opening and closing of gates and the occlusion of the transported entity. ABC proteins are very dynamic by nature and exhibit large conformational changes at most, if not all, of the steps of the hydrolytic reaction and transport cycles (Figure 3E). Thus, to define the molecular mechanism of transport and coupling, it is necessary to know the exact step (partial reaction) at
which the change in chemical potential of the transported entity occurs. This is a difficult endeavour, which has led to the proposal of many contentious alternative and sometimes mutually exclusive models of the molecular mechanisms for the coupling of transport to ATP hydrolysis by the different ABC transporters.

**Transport and coupling mechanism of ABC exporters**

To consider the mechanism of allocrite transport by ABC exporters, it is instructive to use the MRP Pgp (ABCB1) to establish ground rules from which exceptions observed in other ABC exporters can be interpreted and understood. Pgp is most useful in this role because it was the first ABC exporter recognized and, as a consequence, and due to its medical importance, it is the most thoroughly analysed ABC transporter physiologically, genetically, functionally, structurally, biochemically and biophysically [25,26]. It is a single polypeptide which appears to have arisen by tandem duplication of an ancestral half-transporter and now contains all four core functional domains with no complicating accessory subunits [27] (Figure 1). Consequently, it is nearly symmetrical in the two NBDs and NBSs, which are functionally equivalent and interchangeable [7]. Both NBSs are fully conserved consensus NBSs that are both catalytically and equivalently active [28,29]. Pgp is pseudo-symmetrical in the two TMDs (Figure 3). It can be purified to homogeneity and reconstituted with full functionality into proteoliposomes of defined composition [30]. Transport substrates of Pgp are well defined and characterized. Most importantly, changes in drug chemical potential on transport have been directly recorded in well-defined proteoliposomes containing only Pgp [31]. A moderate-resolution X-ray crystal structure of apo (nucleotide-free) Pgp is also available [32].

There has been a tremendous amount of information generated on the molecular features that allow Pgp to transport drugs or allocrites coupled to the hydrolysis of ATP. The various approaches used in these studies include mutational analyses and detailed biochemical, biophysical and pharmacological characterizations that have been reviewed thoroughly previously [25,26,33–35]. It has been shown conclusively that allocrites interact with Pgp directly. Drug transport by Pgp is thermodynamically coupled to ATP hydrolysis. Binding of ATP or drugs to Pgp leads to conformational changes. Pgp pumps drugs directly rather than altering drug distribution indirectly. The minimal functional unit of Pgp is a monomer. Drugs are extracted from the cytoplasmic leaflet of the plasma membrane and pumped to the external medium. Many purine NTPs can power the reaction cycle, implying a large degree of flexibility in the NBSs. Both catalytic sites (NBSs) were shown to be essential for turnover and inactivation of either inhibited turnover. But, at any given point in the reaction cycle, only one NBS is catalytically active and so catalysis alternates between the two sites (see below).
The conundrum of reaction pathway control: ABC transporters are both tightly coupled and fully uncoupled under different conditions

Allocrite transport by ABC transporters is tightly coupled to ATP hydrolysis, but ABC transporters also exhibit the very unusual phenomenon of basal ATPase activity in the absence of transport allocrites. At first glance, this phenomenon seems to violate the rule of coupling that states that reactions that lead to uncoupling must be avoided. With the rules of coupling relaxed, a ‘reverse water wheel’ (water pump)-type mechanism was initially proposed in which Pgp is continually hydrolysing ATP and alternating transport-site access (inside and outside) whether the transport-site is occupied or not [36]. However, this type of model was unsatisfactory in explaining the very tight coupling of allocrite transport to ATPase activity by Pgp under optimal conditions as described below. To maintain the rules of coupling, others had ascribed the basal ATPase activity to an ‘apparent uncoupled ATPase activity’ associated with the transport of an elusive undiscovered endogenous allocrite (reviewed in [25,26]).

The ATPase activity of Pgp shows three transport allocrite-dependent phases: basal (no allocrite), allocrite-activated (at moderate allocrite concentrations), and allocrite-inhibited (at high allocrite concentrations) [30,37]. At optimal concentrations of activating drug (allocrite), the maximal transport rate (and equivalent coupled ATPase activity) is a characteristic of the particular drug and is inversely proportional to the number of hydrogen bonds the drug can form with Pgp [38]. This indicates direct and tight control of the ATPase and transport activities of Pgp by the bound drug. Further thermodynamic analysis also revealed that, when drug concentrations were optimal, all ATPase activity proceeded through a very tightly coupled pathway. But, in the absence of any transport substrate, all ATPase activity proceeds through a different catalytic pathway [39] (Figure 4). At intermediate drug concentrations, the ATPase activity partitions between the two different catalytic pathways in proportion to the activating drug concentration bound to this proportion of the Pgp population of molecules. Thus there is an allocrite-dependent transport and coupled ATPase pathway and a different non-productive basal ATPase side reaction. Each of these two pathways has different chemical intermediates and partial reactions. The model in Figure 4, by having two separate distinct ATP hydrolytic pathways, reconciles how Pgp can be both tightly coupled when optimal concentrations of transport allocrites are available and also completely uncoupled when no transport allocrites are present.

The apparent affinity of Pgp for ATP increases when drugs bind to the ‘inward-facing’ conformation (Figures 3A and 3B) which facilitates the binding of a second ATP molecule (Figure 4, unshaded cycle). Consequently, ATP hydrolysis and drug transport proceed through the coupled catalytic pathway. If no drug binding occurs, the apparent affinity for the second ATP remains low and a second ATP molecule rarely binds to initiate the basal ATPase
pathway and so the level of basal ATPase activity is usually kept low (Figure 4, shaded cycle). Membrane lipid composition and particularly cholesterol modulates the relative partitioning level between the two pathways [39] by modulating the ATP-binding affinity. This may be related to the need to fill up the drug-binding cavity with cholesterol for efficient coupling between the ATPase reaction cycle and the drug-transport cycle [40] as described in Chapter 3 by Zolnerciks et al.

Another ABC MRP, PDR5 (pleiotropic drug resistance 5), found in yeast, has one canonical NBS and one degenerate NBS. In contrast with Pgp, PDR5 appears to proceed mostly through the uncoupled pathway by a ‘reverse water wheel’-type mechanism [41].
Thus it now appears that a very important feature of ABC exporters is to control the reaction pathway, through a large set of alternative dynamic intermediates. This allows for a much greater flexibility in transport and in selection of different substrates. As a consequence, the mechanistic and transport pathways followed by different ABC transporters are likely to be different and to be optimized for each one’s particular function.

**Alternating-access switch models represent the current consensus view of the mechanism of ABC exporters**

Switch-based models were formulated for Pgp by analogy to the putative mechanisms of ABC importers and from detailed structural analyses of full ABC transporters and isolated NBDs as described in the previous chapter by Zolnerciks et al. The alternating-access switch models are derived from the Jardetzky conformational model for transport [42]. All such models have three consistent elements [12]. (i) Binding of transport substrate to the TMDs of the apo form in the ‘high-affinity inward-facing orientation’ initiates the transport cycle by allowing the reaction cycle to proceed. (ii) ATP binding induces the formation of the closed nucleotide sandwich structure. ATP acts as molecular glue that holds the two NBDs together. The binding energy gained by the NBPs/NBSs is transmitted to the TMDs which then change their access to the ‘low-affinity outward-facing orientation’ on the other side of the membrane. Thus the gate to the inside is closed and the gate to the outside of the membrane is opened, and the affinity of the transported entity for the ABC transporter changes (switches) from high affinity (low chemical potential of substrate) to low affinity (high chemical potential). Consequently the ATP-binding step can be considered as the power stroke in which the chemical potential of the transported entity changed. (iii) ATP hydrolysis leads to the formation of extra negative charge, thus opening the closed nucleotide sandwich structure by charge repulsion [43]. Charge repulsion driving a conformational change is a common attribute of most, if not all, mechanisms envisaged. The opening of the nucleotide sandwich structure facilitates P_i release and ADP dissociation, which in turn allows the TMDs and access-gates to reset to the high-affinity orientation on the original side of the membrane.

The first premise of switch-type models is the establishment of stable dimeric nucleotide sandwich structures containing two ATP molecules symmetrically positioned in the two NBSs. However, these structures have never been observed in the presence of physiologically relevant ATP concentrations and have only been observed when the NBDs were made non-functional by mutation of a critical residue, by removing the essential Mg^{2+} cofactor or by use of non-hydrolysable ATP analogues [26]. Under physiological conditions, the ATP concentration is so high that it is unlikely that Pgp is ever found in a nucleotide-depleted apo form [44] as required by some models. EPR spectroscopy of the intact ABC exporter MsbA (a homodimeric half-transporter homologous with Pgp) shows that ATP binding in the absence of Mg^{2+} does
not close the NBDs and Mg\(^{2+}\) itself was without effect [45]. Another premise of switch-type models is that the affinity for transported drug should decrease on ATP binding before initiation of the catalytic transition state. This was indeed demonstrated in some experiments employing non-hydrolysable analogues of ATP with Pgp [46] or BCRP (ABCG2) [47]. However, in other experiments using physiological nucleotides, the affinities of drugs for Pgp measured in the resting state, before ATP binding, was essentially the same as their affinities during the catalytic transition state [48,49], complicating the application of this premise to Pgp. As discussed in the previous chapter, the evidence for switch type models from interpretations of static structural snapshots may be overwhelming, but these mechanisms do not satisfactorily explain all available biochemical and biophysical experimental observations for Pgp under physiological-like conditions.

**Alternating catalytic site mechanisms are appropriate for fully symmetrical ABC exporters**

Alternating catalytic site models, first proposed for Pgp [50], have several consistent elements. (i) ATPase activity coupled to drug transport alternates sequentially between the two kinetically equivalent NBSs, thus generating asymmetry at the NBSs. (ii) Each ATP hydrolytic event leads to generation of a distinct charge-repulsion event and hence a distinct power stroke capable of driving an allocrite across the membrane (Figure 5). (iii) The allocrite-transport cycle and the ATP-hydrolysis cycle are inextricably interleaved as required by coupling considerations. (iv) Such models have a theoretical transport stoichiometry of one ATP hydrolysed per allocrite moved that has been demonstrated experimentally for Pgp under optimal conditions [51]. In sharp contrast, a value of two ATP hydrolysed per substrate moved was determined in some tightly coupled ABC importers [52]. Again this would imply that different coupling mechanisms exist for ABC exporters and ABC importers.

A prediction of alternating catalysis was that one ATP should be occluded at a particular catalytic site (either NBS1 or NBS2) before being committed to hydrolysis in that NBS. The occlusion represents an interleaved partial step of the ATPase reaction with a limited transfer of ATP’s chemical potential to Pgp which is probably employed to close the inward-facing drug-access gate. This predicted single asymmetrical occluded ATP at either NBS was directly demonstrated in a catalytically inactivated Pgp mutant where the Walker B catalytic glutamate residues of the NBDs were changed to alanine [53]. The mutations eliminated two of the critical negative charges (Figure 5) required for initiation of the power stroke by charge repulsion on ATP hydrolysis. Later, the single asymmetrical occluded ATP was also observed in normal Pgp by the use of the non-hydrolysable ATP analogue adenosine 5’-[\(\gamma\)-thio]-triphosphate [54,55]. Binding of a second ATP triggers transient occlusion
of the low-affinity ATP bound in the previous cycle, followed by immediate hydrolysis of this occluded ATP. Thus, in fully intact Pgp, one can either trap one catalytic transition state or one occluded ATP [53,55], but never trap two ATPs at the same time as required by switch-type models. The occluded ATP leads to asymmetry in the NBDs, NBSs and TMDs [56] and this asymmetry constitutes the conformational memory required by the alternating catalytic mechanism [50]. Such alternating catalysis (Figure 5) is also supported by molecular dynamics computations [57,58] and by electron microscopy structural studies [59].

Variations on the themes and hybrid mechanisms

Sauna and Ambudkar [60] have proposed a hybrid ‘occluded nucleotide-binding switch’ model for Pgp. Here ATP binds to the NBDs and induces the closed nucleotide sandwich structure as above, but with no associated substrate affinity changes. Then the switch of high affinity to
low affinity by the drug-binding site is produced by occlusion of one ATP molecule (tight binding and going from high to low chemical potential) that occurs randomly at a given NBS. The occluded nucleotide is committed to hydrolysis and is hydrolysed. Next, dissociation of ADP or exchange for ATP resets the drug-binding site to a high-affinity form. This model does not overcome problems of intrinsic reaction pathway uncoupling generated by NBS asymmetry and TMD symmetry as discussed below.

Other hybrid mechanisms include the ‘processive clamp model’ [61]. ATPase activity in the homodimer of fragmented NBDs of Md11 (ABCB10), was not co-operative in both NBDs and therefore hydrolysis must be sequential between the NBDs (processive). It was concluded that ATP binding in separated NBDs leads to dimerization of the NBDs (clamp) to form the NBSs and that this clamp step provides the power stroke for the reorientation of the TMDs from inward-facing to outward-facing conformation as in the switch models. Then a random stochastic ATP hydrolysis at either NBS occurs, followed by the hydrolysis of the other ATP at the other NBS, which leads to separation of the NBDs and relaxation of the TMDs to the inward-facing conformation. The processive clamp model was based on results obtained using isolated NBDs (fragmented protein) and on extrapolation from structural observations obtained with prokaryotic ABC importers, which appear to function differently from ABC exporters. This hybrid processive clamp model is more similar to the switch models. On the other hand, the recently proposed hybrid ‘affinity-switch model’ [62] first proposed for the single consensus NBS-containing ABC Cl− channel CFTR (cystic fibrosis transmembrane conductance regulator; ABCC7) and extended to Pgp (ABCB1) is more similar to alternating catalytic site models. Here it was concluded that ATP binding to the consensus NBS2 (NBS1 is always ATP-occupied) leads to a closed dimer formation that drives a change of orientation of the TMDs from inward-facing to outward-facing, but without an affinity change for the transported substrate. Hydrolysis of ATP at the catalytically competent NBS2 is the power stroke that brings about the affinity change of the substrate by further subtle conformational changes in the TMDs. Opening of NBS2 follows, and dissociation of Pi and ADP allow the transporter and TMDs to reset to the initial inward-facing conformation.

**Symmetry and asymmetry considerations**

Early on it was recognized that the original formulation of the alternating catalytic site mechanism [50] had a problem in postulating one symmetrical allocrite-binding site with two gates, controlling access to the inside and outside, located between the symmetrical TMDs. Essentially, an alternating asymmetrical power stroke cannot drive a symmetrical alternating-access model without violating the principles of coupling and thus leading to intrinsic uncoupling. In other words, the two sets of coupled reactions (chemical ATP hydrolysis and vectorial allocrite transport) have to be either fully symmetrical (as in the case of switch-type models) or fully asymmetrical to obtain coupling.
Otherwise, uncoupling chemical and transport reaction shortcuts are feasible (bypass pathways) that are not strictly interleaved with the other coupled reaction pathway.

The alternating catalytic site (two-cylinder engine) transport model [63] was proposed to account for the requirements of asymmetry throughout the reaction/transport cycle of the homodimeric LmrA. (i) In this model there are two allocrite channels that alternate in transport and each allocrite channel is located on a given half-transporter (or homologous half of Pgp) or at the interface between the two transmembrane helices. (ii) It is implied that there are two gates in each drug channel (four gates in total) controlling the orientation of the drug-binding site within. Whereas the two-cylinder model gives an excellent fit to biochemical observations, it was abandoned due to presumed incompatibilities with the available structural data. In particular there was a lack of structurally observed independent channels with the added presence of domain swapping between the two half-transporters. However, given the dynamic nature of LmrA, these structural considerations might, in fact, be more of a misperception than true incompatibilities.

The original alternating catalytic site model for Pgp has been modified to include asymmetries of the NBSs and TMDs to avoid uncoupling and to be more consistent with recent structural studies. Figure 6 represents such a

![Figure 6. Alternating catalytic site and asymmetrical Y transport model of Pgp](image)

Circles, squares and hexagons represent different conformations of the N-terminal and C-terminal catalytic sites (NBS1 and NBS2). Both NBSs bind ATP with similar affinities and both hydrolyse ATP, but not in the same catalytic cycle. The two NBSs interact strongly and cannot hydrolyse ATP independently. Clockwise from top left: the transport and catalytic cycle is initiated on binding of a drug molecule (blue circle) to TMD2 through an open gate to the inner leaflet of the plasma membrane. ATP binding to NBS1 closes the gate, occludes the drug, and commits to hydrolysis the ATP bound previously at NBS2. Hydrolysis of ATP proceeds at NBS2 generating a high chemical potential form of the enzyme (catalytic transition state, hexagon). Relaxation of the high chemical potential protein conformation in NBS2 performs the transport work by changing the conformation of the asymmetrical TMDs such that TMD2 exposes the drug to the shared permeation pathway to the extracellular medium. ADP release from NBS2 resets the transporter to the alternative conformation. An inner leaflet gate is now opened on TMD1 and the shared permeation pathway gate to the outside is closed. Binding of the next drug molecule (red circle) to TMD1 allows the cycle to continue in the alternative conformation with ATP hydrolysis at NBS1. At any given point in the cycle, there is no symmetry in either the NBSs or TMDs.
model based on results obtained in our laboratory. This scheme, which we call the ‘alternating catalytic site and asymmetrical Y drug transport model’, has a minimum of three gates, two occluded drug states and two occluded nucleotide forms. It shares many elements with the alternating catalytic site (two-cylinder engine) transport model [63].

One may speculate that an ancestral, four-gate and two-channel, homodimeric ABC transporter was an original form of ABC exporters, but as the need for transport of large substrates emerged, the mechanism evolved. The alternating catalytic site and asymmetrical Y transport model has two asymmetrical gates controlling transported substrate entry in accordance with the asymmetry required by the two NBDs and two NBSs. Structural elements of the exit gate are shared, but in an asymmetrical form in the two half-cycles (Figure 6). In other words, the two NBSs and TMDs are always asymmetrical, as has been experimentally demonstrated. Transport of the drug through a given channel is facilitated by the observed asymmetrical transmembrane helix movements as described in the ‘solvation-exchange mechanism’ of drug transport by Pgp where a large change in affinity for the transported drug is not essential [38].

**Nature’s re-simplification of ABC exporters by inhibiting ATP hydrolysis at one nucleotide-binding site**

The alternating catalytic site and asymmetrical Y transport model describes a complicated scheme devised by Nature to allow for the transport of many large substrates by a transporter that is simultaneously symmetrical in structural elements, but completely asymmetrical in action. An easy simplification of the ‘asymmetrical Y transport channel’ would be to remove one of the arms of the Y such that the transporter is asymmetrical in all actions at all times. There is evidence that Nature has performed this simplification many times during the evolution of asymmetrical ABC exporters. Examples are found in the heterodimeric TAP (transporter associated with antigen processing) 1/TAP2 (ABCB2/ABCB3) [8], or the asymmetrical MRP family (ABCC family) (Figure 1) [64] or even the asymmetrical Cl⁻ channel CFTR (ABCC7) [62]. This simplification is achieved by changing one of the consensus NBSs to a non-consensus NBS that cannot or only poorly hydrolyses ATP. As was detailed for the TAP1/TAP2 system, ATP hydrolysis by the consensus NBS alone still allows peptide transport. This was demonstrated directly by studies that further mutated the degenerate NBS to prevent any possible ATP hydrolysis at this degenerate site (reviewed in [65]). As a direct consequence of asymmetry in the NBSs, one of the asymmetrical gates controlling entry of substrate into the transporter (one arm of the Y) is always closed due to the continual presence of ATP in the non-consensus NBS that cannot be hydrolysed to open this gate. In such an asymmetrical system, transport more or less conforms to the allosteric alternating-access model [42].
Conclusion

It has generally been presumed that all ABC transporters share similar types of transport and coupling mechanisms even though there is no compelling theoretical justification for this presumption. In the ABC transporter family, there are distant genetic lineages; very diverse and not fully conserved structural and topological elements; very diverse structural transmission and coupling pathways; different symmetries and asymmetries of nucleotide-binding domains and TMDs; different nucleotide-binding sites that are fully functional, partly functional or completely non-functional; differing levels of coupled and uncoupled ATPase activity; and differing coupling stoichiometries. All of this diversity argues against a single universal transport and coupling mechanism. Rather, the mechanistic and transport pathways followed by different ABC transporter classes and individual transporters are likely to be optimized for their particular function. Most structural studies, and especially crystallographic ones, are performed outside the realm of dynamics and time domains and cannot account for chemical potential changes or protein dynamics. On the other hand, kinetic analyses and some biochemical and biophysical studies can be performed under physiologically relevant conditions and within dynamic time domains. As ABC proteins are very dynamic, they do not like to be ‘coerced’ into ordered crystals. Furthermore, the detergents used do remove membrane lipids that might be required to maintain physiologically relevant structures. Thus many structural analyses are performed in extra-physiological domains where proteins do not operate. To move forward, more complementary and interlaced kinetic, biochemical, biophysical and structural studies are urgently needed. Additionally, a new thrust towards elucidating and understanding the nature and pathways of protein dynamics during the transport and catalytic cycles is needed to better define the transport mechanism of ABC exporters in molecular terms. Such a molecular-based understanding is an absolute prerequisite for any rational medical intervention in the myriad diseases mediated by the functioning or the lack of functioning of ABC exporters.

Summary

- ABC transporters are structurally heterogeneous and are likely to have three overall types of coupling mechanisms specific to ABC exporters, small ABC importers and large ABC importers.
- Even within these three ABC transporter classes, the catalytic and transport mechanisms are not fully conserved.
- ABC transporters perform dynamic reaction pathway control. Partitioning between highly coupled transport cycles and uncoupled basal ATPase is controlled to aid in selection of diverse transport substrates.
• Alternating-access switch transport models of ABC exporters are in good agreement with structural studies, but not in total agreement with biochemical studies on full and homodimeric ABC exporters.

• Alternating catalytic site transport models of ABC exporters are in general agreement with biochemical studies, symmetry and asymmetry considerations, and can accommodate many current structural observations, but are not in full agreement with all structural observations.

• Heterodimeric ABC exporters and full ABC exporters containing a non-functional nucleotide-binding site probably evolved to conform to simple alternating-access transport-type mechanisms.

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References


