REVIEW

nature structural & molecular biology

The ATP switch model for ABC transporters

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ABC transporters mediate active translocation of a diverse range of molecules across all cell membranes. They comprise two nucleotide-binding domains (NBDs) and two transmembrane domains (TMDs). Recent biochemical, structural and genetic studies have led to the ATP-switch model in which ATP binding and ATP hydrolysis, respectively, induce formation and dissociation of an NBD dimer. This provides an exquisitely regulated switch that induces conformational changes in the TMDs to mediate membrane transport.

The vectorial transport of molecules across membrane lipid bilayers is central to cell physiology, including the uptake of nutrients, elimination of waste products, protein secretion, energy generation and movement of signaling molecules. ABC (ATP-binding cassette) transporters, which mediate much of this traffic, form a large protein family found in all prokaryotic and eukaryotic cells. In *Escherichia coli*, genes encoding ABC transporters occupy almost 5% of the genome. In humans, mutations in ABC transporter genes underlie many genetic disorders, including Tangier disease (cardiovascular), persistent hyperinsulinemic hypoglycemia of infancy (pancreas), Stargardt disease (eye), Wegener's granulomatosis (immune system), cystic fibrosis (lung and gut) and Dubin-Johnson syndrome (liver). Other ABC transporters protect cells from cytotoxins and can confer resistance to antibiotics, antifungals, herbicides and, in man, anticancer drugs. Indeed, it is difficult to characterize any physiological system in depth without identifying a role for an ABC transporter[1].

The vast majority of ABC proteins are active transporters, requiring energy in the form of ATP to translocate specific transport substrates across cell membranes (see **Table 1** for a list of ABC proteins referred to in this review). Most eukaryotic ABC transporters that have been characterized thus far mediate export of substrate from the cytoplasm, out of the cell or into organelles. The exceptions are ABC transporters that transport substrate from the mitochondrial matrix, which is equivalent to the cytoplasm of the original symbiont. In contrast, some bacterial ABC transporters import into the cytoplasm and, for this, require an additional extracellular protein, the periplasmic binding protein (PBP). Other ABC proteins have evolved to couple the conformational changes induced by ATP binding and hydrolysis to activities other than transport, including K^+ channel regulation (SUR1, also known as ABCC8), chromosome organization (SMC proteins), DNA repair (MutS and Rad50), and mRNA export from the nucleus (Elf1p).

The first ABC transporter gene was sequenced >20 years ago[2]. Since then, many elegant biochemical and genetic studies of both prokaryotic and eukaryotic ABC transporters have been published, yet only with the recent availability of structural data has it become possible to combine these data into a coherent model for the mechanism of transport—the ATP switch.

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Published online 27 September 2004; doi:10.1038/nsmb836

Structure of ABC transporters

ABC transporters have four core domains (**Fig. 1**). Two TMDs each consist of multiple membrane-spanning α-helices, which together form the pathway through which the transported substrate crosses the lipid bilayer, and a specific binding site (or sites) for the transport substrate. Two NBDs couple conformational changes induced by ATP binding, ATP hydrolysis and ADP release to the transport process. These four domains are sometimes encoded as separate polypeptides and sometimes fused into multidomain proteins[3]. For any given ABC transporter the two TMDs are similar if not identical, as are the two NBDs, generating an internal two-fold (or pseudo two-fold) symmetry (**Fig. 1**). Together these four domains form the minimal functional unit that is both necessary and sufficient for transport.

The NBDs of all ABC transporters, whether of prokaryotic or eukaryotic origin and irrespective of the transport substrate, share extensive amino acid sequence identity and several characteristic motifs[3]. Studies of bacterial ABC transporters first showed that the NBDs bind and hydrolyze ATP, and that ATP hydrolysis is required for transport[4–7]. High-resolution X-ray structures of 11 NBDs from ABC transporters and 3 NBDs associated with nontransport functions show a very similar tertiary fold in which the conserved amino acid motifs contribute to the nucleotide-binding pocket (**Fig. 2**). The Walker A motif (or P loop) and the Walker B motif are common to many nucleotide-binding proteins and hydrogen-bond extensively with the bound nucleotide. In addition, the Walker B contributes the catalytic base. The ABC signature motif (sometimes incorrectly referred to as Walker C), the H loop, Q loop and stacking aromatic are peculiar to ABC NBDs, and although they coordinate bound nucleotide or nucleophilic water, their precise role in the molecular mechanism is less clear.

Several dimer interfaces have now been observed in crystals of isolated NBDs. The interface first observed in the Rad50 (ref. 8), LolD[9], and MutS[10,11] structures, and subsequently in the complete BtuCD transporter[12], is the only interface observed more than once by

Table 1 ABC proteins referred to in this review

Protein	Transport substrate (disorder)	Species
Transporters		
ABCA1	Cholesterol (Tangier disease/HDL deficiency)	Human
ABCA4	Retinal (Stargardt disease)	Human
TAP (ABCB2/3)	Peptides (antigen presentation/Wegener's granulomatosis)	Human
MRP2 (ABCC2)	GSH conjugates (Dubin-Johnson syndrome)	Human
P-gp (ABCB1)	Hydrophobic drugs (multidrug resistance)	Mammals
MRP1 (ABCC1)	Leukotrienes (multidrug resistance)	Human
LolD	Amino acids	*M. jannaschii*
BtuCD	Vitamin B12	*E. coli*
$MalEFGK_2$	Maltose	*E. coli*
$HisJQMP_2$	Histidine	*S. typhimurium*
OppABCDF	Oligopeptides	*S. typhimurium*
MsbA	Lipid A	*E. coli, V. cholera*
LmrA	Hydrophobic drugs	*L. lactis*
Mdlp1	Peptides (from mitochondria)	*S. cerevisiae*
GlcV	Glucose	*S. solfataricus*
Nontransporter ABC proteins		
SUR1 (ABCC8)	K^+ channel regulation (PHHI)	Human
CFTR (ABCC7)	Chloride channel (cystic fibrosis)	Human
SMC	Chromosome organization	*T. maritima*
MutS	DNA mismatch repair	*E. coli, T. aquaticus*
MSH2/6	DNA mismatch repair	Human
Rad50	Double-strand break DNA repair	*P. furiosus*
Elf1p	mRNA export from nucleus	*S. pombe*

crystallography, is consistent with genetic and biochemical data, and is now generally considered to reflect the physiological NBD dimer. In this dimer, the two bound nucleotides are sandwiched at the protein-protein interface and each is coordinated by amino acids from both NBDs (**Fig. 2**). Thus, rather than having two distinct ATP-binding sites, one on each NBD, it is more appropriate to think in terms of two ATP-binding pockets with both NBDs contributing to each pocket. The concept that ABC NBD dimerization might be central to the mechanism by which ABC proteins work first arose from studies of the DNA repair enzyme Rad50 (ref. 8). In the ATP-switch model, formation and dissociation of the NBD dimer upon ATP binding and hydrolysis, respectively, provide a regulatable switch that induces conformational changes in the TMDs to mediate transport.

In contrast to the NBDs, the TMDs have been relatively refractory to structural analysis and the only structural data available are in the context of the intact transporter. Each TMD is predicted to consist of 5 membrane-spanning α-helices. The overall shape of an ABC transporter was first characterized at low-to-medium resolution for the mammalian multidrug-resistance P-glycoprotein (P-gp; ABCB1) by single-particle analysis[13] and cryo-EM of two-dimensional crystals[14,15], and elegantly described at high-resolution for the *E. coli* vitamin B12 transporter BtuCD[12] (**Fig. 1**). Both structures indicate that an aqueous chamber is formed within the membrane at the interface of the TMD dimer, and that this chamber is open at the extracellular face but closed intracellularly. The two NBDs are exposed as cytoplasmic lobes, one interacting with each TMD. Although a structure for the lipid A transporter of *E. coli*, MsbA, suggested an inverted V-like organization with the NBDs well separated[16], it is now considered unlikely that this structure reflects the physiological domain organization[17]. The architecture of ABC transporters is very different from that of other ATP-dependent transporters such as the P-type ATPases (Ca^{2+} pump) or F_1-F_0 ATPase, in which the membrane-spanning α-helices are more closely packed and the nucleotide-binding sites more widely distanced from the membrane.

Although the TMDs of different subfamilies of ABC transporters share the same gross architecture, they can have different numbers of membrane-spanning α-helices and share little sequence identity. The two high-resolution TMD structures available (BtuD[12] and MsbA[16]) have folds that cannot be modelled on one another. Although all NBDs share a common evolutionary origin and mechanism, it seems that they are coupled to different subfamilies of TMDs. Given the diversity of substrates handled by different ABC transporters, it is not unreasonable to anticipate mechanistic differences between these TMD subfamilies.

Mechanisms of transport

Elegant studies of the mammalian multidrug resistance P-glycoprotein (P-gp) by Alan Senior defined the biochemistry of the ATPase cycle and proposed the alternating catalytic sites model[18]. Subsequently, several related models for transport appeared, based principally on data obtained for the bacterial histidine and maltose transporters and

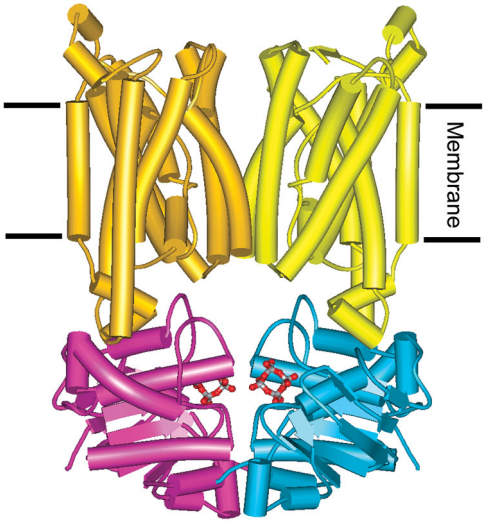

Figure 1 Schematic representation of the vitamin B12 transporter BtuCD of *E. coli* (PDB entry 1L7V)[12]. In this transporter, the two TMDs (BtuC) are identical to each other, as are the two NBDs (BtuD), giving a clear two-fold symmetry. The TMD dimer is gold and yellow and the NBD dimer is magenta and blue. Each TMD is composed of ten membrane-spanning α-helices (most ABC transporters have fewer than ten α-helices per domain) and together they form an aqueous chamber in the membrane that is open to the extracellular surface. The NBDs form a dimer with the two ATP-binding pockets sandwiched at the dimer interface (see **Fig. 2**). Two molecules of cyclic tetravanadate (shown in ball-and-stick and colored elementally) mimic nucleotide bound in the two pockets formed at the protein-protein interface of the NBD dimer.

P-gp[19–23]. These models generally assumed that ATP hydrolysis provides the principal energy input or power stroke for transport, and that the two NBDs operate alternately and may therefore be coupled to distinct steps in the transport cycle.

Three recent data sets, however, have caused the underlying tenets of these models to be revisited: first, biochemical data suggesting that ATP binding, rather than ATP hydrolysis, provides the power stroke for transport; second, the finding that for some ABC transporters (such as the ABCC family), the two NBDs are not equivalent in their abilities to bind and hydrolyze ATP; and third, structural data (see above) showing that the two ATP-binding pockets are located at the interface of an NBD dimer, implying that the two NBDs act in concert at a single step rather than influencing distinct steps in the transport cycle.

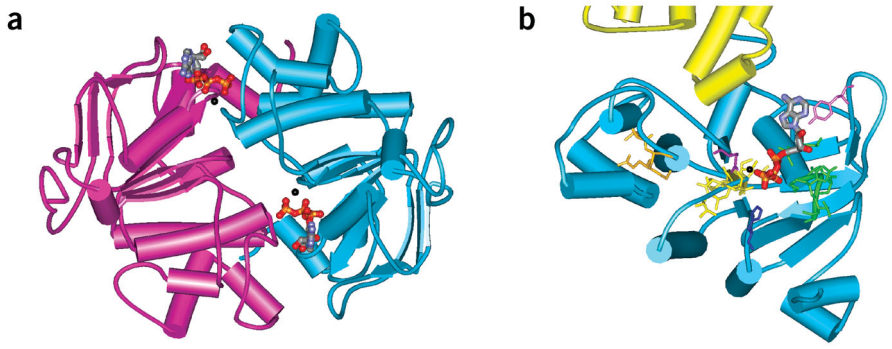

Figure 2 Structure of the NBDs of ABC transporters. (**a**) The NBD closed dimer viewed from above, as if looking down through the membrane and TMDs (which are hidden for clarity). As no complete transporter with bound ATP has been crystallized, a consensus structure based on structures of several NBDs and modeled for P-gp[17] is shown. The two ATP molecules and two magnesium ions, shown in ball-and-stick and colored by atom types, occupy the two nucleotide-binding pockets at the interface between the two NBDs and are coordinated by residues from both NBDs (see also **b**). (**b**) NBD1 (blue) viewed from NBD2 (which is hidden for clarity). The intracellular loops of TMD1 that form an interface with NBD1 are yellow (toward top). The residues and motifs coordinating the Mg-ATP are shown as stick models and colored as follows: stacking aromatic (Tyr401, light purple), Walker A motif (427-GSGCGKST-435, green), Walker B motif (551-ILLLDEAT-558, yellow), Q-loop glutamine (Gln475, dark purple), H-loop histidine (His587, dark blue). The ABC signature motif of this NBD (531-LSGGQ-535, orange) is not involved in coordinating the Mg-ATP in **b**, but does contact the Mg-ATP (not shown) bound to the second nucleotide-binding pocket.

The ATP-switch model

The ATP-switch model involves repeated communication, in both directions, between the NBDs and TMDs (**Fig. 3**). Unlike in P-type transport ATPases, there are no phosphorylated protein intermediates[24] and communication involves only noncovalent conformational changes. At the heart of the model is a switch between two principal conformations of the NBDs: formation of a closed dimer upon binding two ATP molecules at the dimer interface; and dissociation to an open dimer facilitated by ATP hydrolysis and release of P_i and ADP. The kinetics of this switch are enhanced by cooperativity between the two nucleotide-binding pockets, and can be tightly regulated by signals from the TMDs. Switching between the open and closed configurations of the dimer induces conformational changes in the TMDs necessary for vectorial transport of substrate across the membrane. The general description below is for an exporter; adaptations required to mediate uptake of substrate into the cell are discussed toward the end of this review.

Step I. Binding of substrate to the TMDs initiates the transport cycle by facilitating ATP-dependent closed dimer formation. During a transport cycle, the binding site for the transport substrate must be reoriented across the membrane and its substrate-binding affinity must be reduced. For active transport, this requires energy input and can be defined as the power stroke. Substrate binding to the transporter must initiate the transport cycle and precede this power stroke, otherwise the transport cycle will be futile. Assuming that the power stroke is due to NBD closed dimer formation (step II), binding of substrate to its high-affinity site on the TMDs is predicted to lower the activation energy for ATP-dependent dimerization.

The clearest indication that substrate binding initiates the transport cycle comes from characterization of substrate-independent mutants of the bacterial histidine and maltose transporters. In these mutants, amino acid changes lead to loss of control by the transport substrate over the NBDs, and consequently ATP is hydrolyzed continuously in a futile cycle[25,26]. More recently, it has been demonstrated that binding of the transport substrate to the TMDs induces a conformational change in the NBDs. For example, spectroscopic and protease or chemical accessibility studies show substrate-induced conformational changes in the NBDs of P-gp[27,28], TAP (ABCB2/3)[29], MRP1 (ABCC1)[30], and the bacterial histidine[31] and maltose[32] permeases. After initial demonstrations for the bacterial histidine and oligopeptide permeases[6,7] and mammalian P-gp[33], transport substrates have been shown to stimulate ATP hydrolysis by many ABC transporters. Similarly, ATPase activity of the DNA repair ABC protein MutS is stimulated by its substrate, mismatch DNA[34].

Thus far, few studies have attempted to distinguish whether substrate-induced ATPase activity is due to enhancement of ATP binding or to stimulation of the hydrolytic step itself. The observation that the ATP-binding properties of isolated NBDs differ from those of the intact transport complex[35] implies that the TMDs can influence ATP binding itself. Direct evidence that binding of transport substrate *per se* enhances ATP binding has recently been obtained for four ABC proteins. (i) A mutant *E. coli* maltose transporter has been characterized in which the requirement for maltose to stimulate ATP hydrolysis has been lost[26]. The NBDs of this mutant have a 30-fold increase in affinity for ATP[32]. (ii) The transported substrate LTC4 increases ATP binding by MRP1, specifically at NBD1 (ref. 36). (iii) Vinblastine, a transport substrate for P-gp, increases the apparent affinity of the NBDs for ATP by ~60-fold (K.J.L., C. Wooding and C.F.H., unpublished data). Similarly, use of a fluorescence probe in the nucleotide-binding pocket showed that several drug substrates increase the affinity of the NBDs for ATP, albeit modestly (about four-fold)[37]. Although some studies of P-gp have concluded that drug substrate may not alter the affinity for ATP[18,33], this affinity was estimated by measuring ATPase activity: in these studies basal ATPase activity in the absence of added substrate must be attributable either to an unknown endogenous substrate (in which case it does not actually report activity in the absence of substrate), or to uncoupling of the ATPase cycle from substrate binding (which would then reflect the anticipated futile cycle). (iv) For the MutS mismatch repair enzyme, binding of mismatch DNA to its DNA-binding domain (analogous to the TMDs of an ABC transporter) stimulates ATP binding by the NBDs[38,39]. Thus, the available

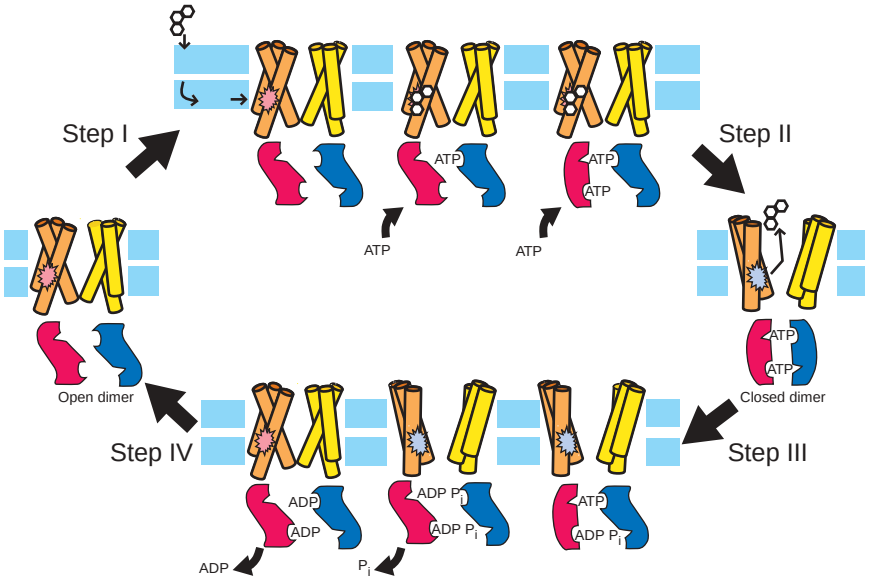

Figure 3 The ATP switch model for the transport cycle of an ABC transporter. The schematic is for a drug exporter; variations required for an importer are discussed in the text. The TMDs, shown as cylinders spanning the membrane, are viewed in the plane of the membrane. The NBDs are shown as shapes at the cytoplasmic face of the membrane and, for clarity, as if viewed from above the membrane as in **Figure 2a**. The transporter in its basal state has the NBDs in an open dimer configuration, with low affinity for ATP. The drug-binding site (red) is high-affinity and faces the inner leaflet of the membrane. Step I: the transport cycle is initiated by binding of substrate to its high-affinity site on the TMDs from the inner leaflet of the membrane. The affinity of the NBDs for ATP is increased, effectively lowering the activation energy for closed dimer formation. Two molecules of ATP bind, cooperatively, to generate the closed dimer. The detail here varies for different transporters. For example, in some transporters one nucleotide-binding pocket has a higher affinity for ATP than the other, whereas in other transporters, which pocket is occupied first by ATP may be stochastic. Step II: the closed NBD dimer induces a conformational change in the TMDs such that the drug-binding site is exposed extracellularly and its affinity is reduced, releasing the bound drug. Step III: ATP is hydrolyzed to form a transition-state intermediate. Hydrolysis of the two ATP molecules is normally sequential, although for some ABC transporters only one ATP may be hydrolyzed. Step IV: sequential release of P_i, and then ADP, restores the transporter to its basal configuration.

data suggest that binding of the transport substrate to the TMDs initiates the transport cycle by transmitting a conformational change to the NBDs, enhancing the binding of ATP and lowering the activation energy for closed dimer formation.

The molecular events involved in signaling from the TMDs to enhance ATP binding and initiate closed dimer formation remain speculative. Interdomain conformational changes seem likely to be transmitted through interaction of the L loops (EAA sequence) of the TMDs[12,40], and the Q loops[9] and 'structurally diverse' regions[41] of the NBDs, which are in close proximity to each other. Comparison of the structures of the GlcV NBD in its nucleotide-free and ATP-bound forms[42] suggests that displacement of the Walker A loop in response to a signal transmitted from the TMDs may allow ATP to gain access to its binding sites. Alternatively, ATP may access the nucleotide-binding pockets at all times, with low affinity, and the transport substrate–induced conformational change aligns the ABC signature motifs with respect to the rest of the nucleotide-binding pockets to facilitate high-affinity ATP-binding and closed dimer formation. ABC transporters may differ in this respect, reflecting different relative basal affinities for ATP in the absence of an initiating signal from the transport substrate.

Step II. ATP binding induces closed dimer formation, the power stroke for transport. Active export across a cell membrane requires that a high-affinity binding site for the transport substrate is accessible from the cytoplasmic face of the membrane, and that this site is converted during the transport cycle to a low-affinity site accessible from the extracellular face of the membrane[43]. Three lines of evidence suggest that ATP binding, rather than hydrolysis, provides the energy input required for this power stroke.

First, ATP binding induces changes in the substrate-binding properties of the TMDs. The affinity of transport substrates for ABC transporters has been difficult to measure directly, and indirect measurements (through stimulation of ATPase activity) often reflect other rate-limiting steps. Recently, direct measurement of substrate (vinblastine) binding to P-gp in the presence of nonhydrolyzable ATP analogs showed that ATP binding, in the absence of hydrolysis, is sufficient to reduce substrate-binding affinity[14,44,45]. Both AMP-PNP and ATP-γ-S reduced drug-binding affinity and had little effect on binding capacity, or affinity for a modulator that binds to an allosteric site but is not transported, arguing against nonspecific disruption of protein structure. Similarly, for MRP1, ATP-γ-S converts the high-affinity leukotriene-binding site to a low-affinity site[46]. Whether these transport substrate–binding sites are reoriented has not yet been addressed. However, for the related bacterial drug transporter LmrA, the high-affinity site has been shown to be accessible from the inner leaflet of the membrane, whereas the low-affinity site is exposed to the extracellular medium[21].

Studies of two other ABC proteins that are, however, not active transporters, also suggest that ATP binding and not hydrolysis provides the power stroke. For the cystic fibrosis protein, an ABC ion channel, initial studies led to the hypothesis that ATP hydrolysis drives channel opening[23]. However, a more detailed analysis has recently revealed that channel opening can be mediated by ATP binding to the NBDs in the absence of hydrolysis[47,48]. Similarly, for the DNA repair proteins MutS and hMSH2/6, ATP binding in the absence of hydrolysis induces the key conformational change that enables the DNA repair complex to assemble[34,38,39].

Second, ATP-binding induces substantial conformational changes in the TMDs. Spectroscopic, protease accessibility and crosslinking studies have shown that ATP binding to the NBDs induces conformational changes in the TMDs of MRP1 (ref. 30), HisPMQ[31], LmrA[49] and P-gp[28]. These conformational changes have been visualized directly for P-gp by two-dimensional crystallography[14,15]. Two-dimensional crystal structures of AMP-PNP-bound P-gp showed that the major conformational change during the transport cycle occurs upon ATP binding, and that subsequent ATP hydrolysis introduces more limited changes[14]. Spectroscopic studies of LmrA have led to a similar conclusion[49].

The nature of the conformational changes induced in the TMDs remains to be elucidated. Although the substrate-binding site is

exposed to the opposite face of the membrane, thermodynamics argue against shuttling of the site across the plane of the membrane, and experimental evidence that suggested threading of α-helices into and out of the lipid bilayer has been shown to reflect an artifact[50]. Cryo-EM of two-dimensional crystals of P-gp suggests repacking of the membrane-spanning α-helices, possibly involving rigid-body rotation of the two TMDs with respect to each other[14,15]. Rotation and tilting of transmembrane α-helices may both contribute to these conformational changes. As the TMDs of different subfamilies of ABC transporters differ (see above), it may be that different types of conformational change are involved in the transport of different types of substrates.

Third, ATP binding induces NBD closed dimer formation. The observation that amino acids from both NBDs coordinate with each ATP, and that amino acids required for hydrolysis of the γ-phosphate bond are only positioned appropriately in the closed dimer configuration[8,9,51], allows few alternatives to the hypothesis that ATP binding drives closed dimer formation. In addition, around half the area buried at the dimer interface is contributed by the two ATP molecules, suggesting that ATP is essential for formation of the closed dimer[9,51].

Biochemical studies of intact transport complexes suggest that the conformational changes in the NBDs are relatively small[52,53], and therefore do not involve a major reorientation of the NBDs with respect to other domains. The NBDs are relatively flexible in the absence of ATP[42,51] and ATP binding induces a rigid body rotation of subdomains I and II with respect to each other[8,9,51]. This aligns residues in the ABC signature motif of one NBD with the Walker A and B motifs of the other NBD to form two complete ATP-binding pockets. At least two sets of interactions are likely to provide energy for this conformational change: interactions of the γ-phosphate of ATP with the conserved glutamine in the ABC signature motif of the other NBD, and the interaction of the conserved glutamine in the Q loop with bound nucleotide and the hydrolytic water[9]. There is strong biochemical evidence that binding of the two ATP molecules can be cooperative, for example for the histidine permease[35,54], the maltose transporter[55], MRP1 (refs. 46,56) and P-gp[57,58]. This provides for kinetic control of closed dimer formation.

As the transport substrate–binding site has low affinity in the ATP-bound (closed dimer) configuration, it is assumed, but has not been demonstrated, that the substrate leaves the transporter at this stage of the cycle.

Step III. ATP is hydrolyzed. ABC transporters normally hydrolyze ATP as part of the transport cycle. ATP hydrolysis seems to initiate resetting of the transporter to its basal, open dimer configuration by destabilizing the NBD closed dimer.

Many studies of the role of ATP hydrolysis have involved trapping a posthydrolytic transition state with vanadate, originally characterized for P-gp[59], in which the γ-phosphate bond has been hydrolyzed and P_i has been replaced by vanadate. Biochemical evidence suggests that this is still an activated state, suggesting that the NBDs have not yet been restored to the open dimer configuration[60]. Consistent with this, studies of P-gp and MRP1 show that the vanadate-trapped intermediate still has low affinity for the transport substrate[14,46,61], that (for LmrA) the substrate-binding site remains exposed to the extracellular face of the membrane[21], and that the conformation of the TMDs remains distinct from that of the nucleotide-free state[14]. Similar conclusions have been reached for bacterial PBP-dependent transporters[62]. Although a recent report suggests that transport substrate–binding affinity is the same in the trapped transition state as for native P-gp[63], an indirect measure of drug binding was used that did not detect the high-affinity site. Thus, hydrolysis of the γ-phosphate bond in itself does not seem to restore the transporter to its basal state.

The trigger that initiates ATP hydrolysis is unknown. Release of transport substrate from the TMDs may induce a conformational change that is transmitted to the NBDs. Alternatively, ATP hydrolysis might be autocatalytic—a direct consequence of the geometry of ATP with respect to specific hydrolytic residues introduced by closed dimer formation. This latter model is supported by the observation that stable closed dimers of isolated NBDs are difficult to obtain in the presence of ATP[35,42]. Indeed, crystals of the isolated LolD dimer with bound ATP could be obtained only when a specific mutation (E171Q) preventing ATP hydrolysis was introduced[9].

Step IV. Release of P_i and ADP. P_i and ADP are released sequentially, restoring the protein to its basal state, so it is ready to initiate another transport cycle. After ATP hydrolysis, the closed dimer is destabilized by electrostatic repulsion between ADP coordinated with the Walker A motif of one NBD and P_i coordinated to the signature motif of the other NBD[9]. This leads to rigid body rotation of the subdomains of each NBD, P_i and ADP release, and restoration of the open dimer configuration. The observation that vanadate traps a transition-state intermediate, replacing P_i before ADP can be released, suggests that P_i is normally released before ADP. This has been shown experimentally for P-gp[18], the maltose transporter[64], MRP1 (ref. 46) and Mdl1p[65]. It also indicates that the NBDs are not restored to the open dimer configuration upon P_i release. In apparent contrast, evidence has been presented suggesting that Pi release (while ADP remains bound) restores the high-affinity substrate-binding site, indicating that the ADP-bound configuration differs from that of the transition-state intermediate (with both ADP and P_i or vanadate bound)[45]. These steps remain to be clarified.

Notably, failure to release ADP from NBD2 of MRP1 prevents continuation of the transport cycle[46]. Similarly, it has been suggested that ADP release by P-gp can be rate-limiting for the ATPase cycle[18,66,67]. It is difficult to see how a step that is not dependent on transport substrate can be rate-limiting given that transport substrates can stimulate the ATPase cycle. Furthermore, under saturating concentrations of transport substrate, ADP release is apparently not rate-limiting[68]. This apparent discrepancy may be resolved by the observation that, for MutS, ADP release is rate-limiting[38] because it remains bound to one NBD until substrate binding induces a conformational change in the NBDs such that ADP is displaced by ATP[39]. It may be that for some transporters, binding of the transport substrate to its high-affinity site on the TMDs (step I) stimulates both ADP release at the end of one transport cycle and ATP binding at the beginning of the next.

Energetics of transport

Although many eukaryotic ABC transporters normally operate down a concentration gradient, they are undoubtedly true active transporters and can pump substrate against a concentration gradient[69]. This requires net energy input. Nevertheless, it has been demonstrated that the LmrA transporter can operate as a passive facilitator in the absence of the NBDs, and that drug transport by intact LmrA can be reversed with concomitant ATP synthesis when a reverse substrate concentration gradient is imposed[70,71]. Thus, ATP binding and hydrolysis are not required for substrate transport in itself, but confer directionality, kinetic advantage and provide the energy required to drive substrate transport against a concentration gradient. Although it now seems that ATP binding, rather than ATP hydrolysis, provides the power stroke for transport, this does not necessarily indicate that this is the only energy input into the system. Substantial conformational

changes, each of which has an activation energy, are induced by binding of transport substrate, ATP binding, ATP hydrolysis, P_i release and ADP release. A detailed analysis of the free energy input at each step of the transport cycle still seems some way off. It is also possible to envisage an adaptation in which the conformational changes induced in the TMDs are reversed so that formation of the closed dimer sets the substrate-binding site intracellularly, and restoration to the open dimer reorients the site so it is exposed extracellularly. Indeed, the bacterial PBP transporters described below seem to reflect an adaptation that achieves exactly this, using an additional protein rather than relying solely on variation in the TMDs.

Stoichiometry of ATP binding and hydrolysis

In the ATP-switch model presented above it is assumed that in the closed dimer both nucleotide-binding pockets have bound ATP. Two bound nucleotides have been observed in crystallographic dimers of LolD E171Q[9], MalK[51] and Rad50 (ref. 8), and have been detected biochemically in the Mdl1p dimer[65] and intact P-gp[37,60]. For CFTR there is indirect evidence that ATP must bind to both sites to open the channel[47,48]. It is not impossible, however, to envisage a variation on this model in which one ATP is sufficient to stabilize the closed dimer, depending on the nature of the protein-protein interface.

The situation with respect to hydrolysis is a little less clear. For most ABC transporters, both ATP-binding pockets have the capacity to hydrolyze ATP; this has been most clearly demonstrated for P-gp[72,73], TAP[74] and the histidine permease[20], suggesting that two ATPs are hydrolyzed per transport cycle. However, ATP hydrolysis at only a single pocket might be sufficient to destabilize the closed dimer, albeit at a slower rate. Consistent with this, it has been shown by experimental manipulation that ATP hydrolysis by both NBDs is not essential for transport. Thus, when one pocket of the histidine transporter is mutated to abolish ATP hydrolysis without inhibiting ATP hydrolysis by the other pocket, histidine is still transported, though at a reduced rate[20]. Similarly for P-gp, recent studies show that inactivation of one of the nucleotide-binding pockets by a mutation that does not prevent ATP hydrolysis by the other pocket does not prevent transport[75] (K.J.L. and C.F.H., unpublished data). Earlier studies showing that mutations in either NBD inactivate the protein can be explained by their inactivation of the second nucleotide-binding pocket through allosteric coupling. For CFTR it has also been shown that although ATP is normally hydrolyzed, this is not absolutely required by either NBD: hydrolysis simply seems to enhance the rate of nucleotide release and formation of an open dimer[47]. Notably, only one nucleotide-binding pocket of CFTR seems to hydrolyze ATP during a normal cycle of channel opening and closing[76]. Although this might be peculiar to CFTR, for which rates of channel opening and closing do not need to be rapid, some ABC transporters lack key residues in the ABC signature motif of one NBD, suggesting that this pocket may not normally hydrolyze ATP.

The interpretation of the roles of the two NBDs has been complicated by data showing that the two nucleotide-binding pockets of some ABC transporters hydrolyze ATP in an alternating fashion, including P-gp[18,55,57,59,60], MutS[39], and the bacterial histidine[35,54] and maltose[77] transporters. This has often been interpreted in terms of the two hydrolysis events affecting different steps of the transport cycle, a concept initially reinforced by studies showing that inactivation of either one of the pockets prevents transport. However, these data may now be interpreted better in terms of cooperative ATP binding and hydrolysis, such that binding of ATP by one pocket enhances ATP binding by the other, likewise for ATP hydrolysis. What determines which pocket binds ATP first? For transporters such as P-gp, each of the two sites binds or hydrolyzes ATP first in roughly equivalent proportions[58]. This can be interpreted in one of two ways: either alternating hydrolysis occurs within a single transport cycle and the choice of which pocket hydrolyzes first is stochastic, or alternating hydrolysis continues between cycles such that one pocket hydrolyzes first in one cycle and the other pocket hydrolyzes first in the subsequent cycle. This would require a 'memory' from the previous transport cycle, possibly reflecting lack of ADP release from one nucleotide-binding pocket (see above). Even for transporters in which the two NBDs are identical in sequence (HisP), there is some asymmetry between the two NBDs in the transport complex[31,58,78,79], consistent with a memory between cycles. In contrast, for members of the ABCC family, including MRP1 (ref. 80) and SUR1 (ABCC8)[81], the NBDs are clearly asymmetric, with the pocket predominantly formed by NBD1 providing the initial ATP-binding site. An acidic residue in the Walker B motif contributes to the difference between the two pockets[46]. For these transporters the other pocket (that is, the one in which the Walker A and B motifs are derived from NBD2) seems to hydrolyze ATP preferentially[36,82–84], and in MdlP1 hydrolysis of one ATP is much slower than that of the other[65]. Whichever is the case, the net result is the same: ATP hydrolysis, nucleotide release and destabilization of the closed dimer.

It seems likely that cooperative ATP binding and ATP hydrolysis enhance the kinetics of closed dimer formation and dissociation to the open dimer. Support for this interpretation comes from biochemical studies of the DNA repair ABC protein MutS. A single-residue mutation, R697A, appropriately positioned at the NBD dimer interface to interact with the P loop of the opposing NBD, abolishes alternating hydrolysis and reduces the efficiency of DNA repair[39]. Although Arg697 is not conserved in most ABC transporters, an equivalent residue could potentially serve a similar role. Cooperativity and variations among transporters provide differences in kinetic control of these transporters and illustrate the versatility of the ATP switch in regulating cellular events.

Binding sites for transport substrates

The transport substrate specificity of ABC transporters, with the exception of the PBP-dependent transporters discussed below, is determined by one or more substrate-binding sites located on the TMDs. Some ABC transporters transport only one molecule of substrate per transport cycle. For example, TAP seems to have a single peptide-binding site with the N terminus of the translocated peptide binding to one TMD and the C terminus to the other TMD[85]. In contrast, other ABC transporters have multiple substrate-binding sites. For example, some MRPs seem to transport both cytotoxic drugs and glutathione (GSH), which bind to distinct binding sites[86,87]. As they can also transport drug-GSH conjugates, the number of sites may be a semantic issue: one site for a drug-GSH conjugate becomes two sites when the ligands are not conjugated. For P-gp, detailed pharmacological studies have identified three interacting substrate-binding sites from which transport can occur, and a further site at which nontransported modulators bind[88,89]. Where there are multiple sites, one substrate frequently stimulates transport of the other[21,90,91], either through cooperative binding[88] and/or because occupancy of two sites more effectively stimulates ATP binding and initiates NBD closed dimer formation (step I). In support of this latter hypothesis, there is strong evidence that both GSH and drug must bind to MRP1 to efficiently induce activating conformational changes in the NBDs[30].

The location of the substrate-binding site(s) on the TMDs is unknown despite many mutagenesis and photoaffinity labeling studies, and no ABC transporter has yet been crystallized together with

substrate. For transporters that handle hydrophilic substrates, it is likely that the sites are within an aqueous transmembrane chamber. However, for ABC transporters with hydrophobic substrates that interact from the inner leaflet of the membrane (such as P-gp and LmrA), the initial interactions may involve α-helices exposed to the acyl chains of the lipid bilayer. These apparent differences suggest that the TMDs of different subfamilies of ABC transporters may be substantially different.

Finally, several groups have tried to estimate the stoichiometry of transport, the number of substrate molecules transported per ATP hydrolyzed. This has proved technically difficult, although it generally gives estimates between 1 and 2 (refs. 7,92,93). It now seems possible that for some ABC transporters, two ATP molecules are hydrolyzed per transport cycle, whereas for others it is only one (see above). Similarly, as some transporters have a single substrate-binding site, whereas others have multiple sites that may not all be always occupied, a variable stoichiometry may be anticipated depending on the particular transporter and substrate(s) studied.

Bacterial ABC transporters that mediate solute uptake

The first ABC transporters identified and characterized were the PBP-dependent transporters of Gram-negative bacteria. In contrast to eukaryotic ABC transporters, the PBP-dependent transporters mediate uptake of substrate into the cell. This requires an additional protein, the PBP, which binds substrate extracellularly (in the periplasm) and delivers it to the membrane-bound ABC transporter complex. The structures of many PBPs have been determined and, despite lack of sequence similarity, all adopt a similar tertiary fold. A single molecule of substrate is bound in a cleft between the two subdomains of the PBP, trapping the substrate and sequestering it from the surrounding aqueous solvent (the Venus flytrap model)[94]. This conformational change also generates a site that allows the PBP–ligand complex to interact with the TMDs of the ABC transporter at the extracellular face of the membrane[95,96]. The two lobes of the closed form of the PBP interact with the two TMDs of the ABC transporter, orienting the cleft so that bound substrate faces the chamber of the transporter. Structural evidence for the vitamin B12 transporter implies that only a single PBP can normally interact productively with the TMDs at any one time, delivering a single substrate molecule to the transporter[97].

A transport cycle is then initiated that fundamentally seems similar to that of other ABC transporters. Docking of the PBP–substrate complex onto the TMDs of the ABC transporter initiates the transport cycle, in an analogous fashion to the interaction of transport substrate with the TMDs of exporters. Even though all aspects have not yet been confirmed experimentally, subsequent steps seem similar to those of exporters involving ATP-dependent transitions between the open and closed configurations of the NBD dimer coupled to conformational changes in the TMDs. One key difference, however, is that the conformational changes induced in the TMDs must have different consequences to achieve import. Thus, the power stroke must expose a low-affinity site to the cytoplasm. This does not require a fundamental difference in coupling ATP binding and hydrolysis to transport, but a difference between the TMDs of exporters and importers. There is a further potential complexity. Genetic evidence indicates that the TMDs have determinants that impart substrate specificity in addition to that conferred by the PBP[98,99]. These data can be interpreted in one of two ways: either transport substrate released from the PBP binds to a site on the TMDs that is reoriented during a subsequent step in the cycle so it is exposed to the cytoplasm (in an analogous fashion to exporters), or, perhaps more probably, the specificity imposed by the TMDs is analogous to that of the pore of an ion channel, restricting passage but not requiring reorientation of a site. In the latter case, it is predicted that ATP binding and closed dimer formation transmit a signal to the PBP, inducing it to release the ligand into the chamber of the transporter from which it passes to the cytoplasm. It has been shown that maltose is released to the cytoplasm in the vanadate-trapped transition state[62].

Perspectives

Biochemical, structural and genetic data from many laboratories, obtained for several different ABC transporters, have led to the ATP-switch model. The ATP-dependent dimerization of the ABC domains provides a versatile and controllable switch. The model describes the basic principles by which an ABC transporter operates and can reconcile apparent differences among ABC transporters. These differences lie in two major areas: first, whether the two ATP-binding pockets are equivalent in their ability to bind and hydrolyze ATP, and second, the nature of the substrates transported and the direction of transport. Although we now understand the general principles by which these transporters work, many details remain to be elucidated, particularly how the switch is adapted to ensure differential control of different transporters, how the TMDs of different ABC transporters are adapted to handle different substrates, and how conformational changes are transduced between domains. Answers will only emerge from a combination of further genetic, biochemical and structural data.

ACKNOWLEDGMENTS

We are grateful for the contributions of many present and former members of the laboratory and collaborators, particularly R. Callaghan, R. van Veen, C. Martin, R. Ford and M. Rosenberg. We also thank many colleagues throughout the world for sharing information and stimulating discussions. The authors' research is funded by the UK Medical Research Council.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Received 27 April; accepted 8 September 2004
Published online at http://www.nature.com/nsmb/

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