The Engine of ABC Proteins

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Proteins that belong to the ATP-binding cassette superfamily span from bacteria to humans and comprise one of the largest protein families. These proteins are characterized by the presence of two nucleotide-binding domains, and recent studies suggest that association and dissociation of these domains is a common basic molecular mechanism of operation that couples ATP binding/hydrolysis to substrate transport across membranes.

Although the overall sequence identity among ABC proteins is low, identity among the NBDs is considerably higher. The presence of conserved NBDs is consistent with the view that the various functions of ABC proteins (e.g., ion channels, lipid transporters, peptide transporters) depend mostly on the divergent transmembrane domains (TMDs), whereas nucleotide binding and hydrolysis, common to all ABC proteins, require the conserved NBDs. Because of the large number of ABC proteins and the fact that their sequences differ significantly, it is not surprising that the substrates of ABC proteins include small inorganic ions, amino acids, large polypeptides, lipids, small sugars, and complex polysaccharides (4). Most ABC proteins are highly specific, but others, such as the multidrug resistance proteins P-glycoprotein and MRP1, have broad substrate specificity (1, 6). It is generally believed that ABC proteins are structurally related, but since our knowledge of the structure/function relationship of ABC proteins is very limited, it is unknown how structurally similar ABC proteins can accommodate such different substrates.

Domain organization of ABC proteins

Figure 1A illustrates the domain organization of ABC proteins. The core structure (shown in yellow) consists of two multispanning TMDs (TMD1 and TMD2) and two NBDs (NBD1 and NBD2). The NBDs of ABC proteins are probably positioned near the plasma membrane but are not integrated into it. Some ABC proteins contain additional domains or associated proteins. Most members of the MRP (ABCC) subfamily have an additional multispanning TMD (TMD0 in Fig. 1A) of unknown function (6). A NBD1-TMD2 linker polypeptide of variable length is present in many ABC proteins. In some cases, the linker is important for regulation of the protein function by phosphorylation (17, 18). In CFP, this linker domain is known as regulatory domain (R domain), and its phosphorylation by protein kinase A is a critical event for channel activation (18). Finally, many bacterial permeases associate with substrate-binding periplasmic proteins that deliver the substrate to the transport complex (4).

The number of polypeptides that form the basic core structure varies (see Fig. 1Bb, where separate polypeptides are...
Role of NBDs in different ABC proteins

Many ABC proteins from lower organisms contain two identical NBDs, and the NBD1 and NBD2 of many other ABC proteins, including P-glycoprotein, have very similar sequences and functional properties (1, 4, 16). In contrast, the two NBDs of other ABC proteins have very different functional roles. Proteins of this kind include those that belong to the multidrug resistance protein subfamily, such as CFTR, SUR, and MRP1 (6, 8, 18). Both NBDs are required for proper function of mammalian ABC proteins, and a mandatory positive catalytic cooperativity between the NBDs occurs in P-glycoprotein (1, 18). In this protein, NBD1 and NBD2 are equivalent and ATP binds randomly to either NBD. Hydrolysis occurs at only one NBD per hydrolysis cycle, and NBD1 and NBD2 alternate in the hydrolysis of ATP. Although there are some asymmetries between the NBDs, both NBDs of P-glycoprotein behave fairly similarly from a functional point of view (1, 18). The current working hypothesis for CFTR channel gating also proposes cooperativity between NBD1 and NBD2 (18). The simplest interpretation of single-channel analysis studies using a number of nucleotide analogs and mutations at NBD1 and/or NBD2 is that the main effects of ATP hydrolysis by NBD1 and NBD2 are to “open” and “close” the channel, respectively. However, recent data point toward a more complex picture that involves a more stable nucleotide interaction and a slower rate of hydrolysis at NBD1 compared with NBD2. The NBDs of SUR and MRP1 also have distinct functions (2, 6, 8). It has been proposed that their ATPase activity resides at NBD2, whereas NBD1 binds but does not significantly hydrolyze ATP.

NBD structure

There is general agreement that the structure of the NBDs of different ABC proteins is very similar, independently of the level of conservation in primary sequence (3, 9–12). A representative NBD (HisP, the ATP-binding subunit of a histidine permease; see Ref. 9) is depicted in Fig. 2A. The ribbon model shows that each NBD is L-shaped with two arms or lobes. Arm I contains the basic domain homologous to the F1-ATPase (red α-helices and β-sheets), with the motifs A and B (conserved motifs are underlined in Fig. 2B) as well as the antiparallel β-sheet subdomain (ABCβ, shown in yellow) that interacts with the ribose and base of the nucleotide. Motifs A and B are far apart in the sequence (>90 amino acids) but close in the tertiary structure. The structural aspects of the binding of α- and β-nucleotide phosphates and Mg2+ are similar for ABC proteins and the F1-ATPase. ABC proteins and the F1-ATPase share motifs that, together with those of the hydrogen bonds are formed between the main-chain
acidic residue that interacts with Mg$^{2+}$. In contrast to the ribose and adenine are buried in the F1-ATPase, whereas in monomer NBDs of ABC proteins (see Refs. 10 and 11). The loop).

 Association between NBDs

It is well established that there is functional interaction between the two NBDs, and each NBD has to be “normal” for the ABC proteins to display proper function. Nucleotide binding to the NBD monomer produces conformational changes via an induced-fit mechanism (10). The structural changes induced by ATP on each NBD, although important, are not likely responsible for the power stroke that couples the use of ATP energy to substrate transport. It has been proposed that changes in the interactions between the two NBDs during the transport cycle can provide this power stroke (12, 15).

Details on how the NBDs interact physically have only recently started to emerge. The comparison of the high-resolution crystal structures of isolated NBDs of a number of ABC proteins (bacterial and human TAP1), the two available full-length bacterial ABC proteins, and a few ABC-related proteins have created controversy (see Ref. 11). Dimers have been identified or proposed for all of the NBDs crystallized, but the proposed structural relationships between the NBDs vary significantly (see Fig. 3). For instance, HisP dimers are proposed to be arranged “back-to-back” in such a way that the two arm II structures form the common surface that interacts with the TMDs and the motifs A and C of each monomer face away from those of the other monomer. As a result, the two ATP molecules bound would be ~40 Å apart, and the motif C would not participate in nucleotide binding. The proposed dimeric structure of MalK, the ATP-binding subunit of a bacterial maltose transporter, shows a slightly asymmetric interlocking association with the motifs A of each monomer facing each other but still far away (~40 Å apart), whereas the motifs C are closer to each other (~18 Å).

A different picture has emerged from the analysis of the...
structure of the NBDs of Rad50 and the full-length ABC protein BtuCD (7, 12). Rad50 is a DNA repair enzyme that does not belong to the ABC superfamily, but its catalytic domains are related to the NBDs of ABC proteins. The NH₂- and COOH-terminal NBDs of Rad50 coexpressed and copurified [Rad50 catalytic domain (Rad50CD)] dimerize in the presence of ATP (7). In the dimeric crystal structure of Rad50CD obtained in the presence of a nonhydrolyzable ATP analog, residues in motifs A and B of one monomer interact with the pyrophosphate moiety of ATP, whereas motif C of the other monomer contributes additional interactions with the phosphate, adenine, and ribose.

The structure of BtuCD obtained in the absence of ATP resembles that of Rad50CD and suggests that motif A of one NBD and motif C of the other NBD move away from each other in the absence of nucleotide (7, 12). Recently, structural studies of NBDs from Archaeabacteria support a Rad50-like mechanism (15). According to these data, ATP binding induces dimerization of the NBDs, forming a nucleotide sandwich dimer. Mutagenesis studies of HisP, MalK, and Rad50CD support or are compatible with each of the proposed dimer structures (see Ref. 11). Recently, however, most attention has been focused on the relevance of the Rad50CD model because of important supporting data that include: 1) the structure of BtuCD (12), 2) vanadate photocleavage experiments on the maltose transporter complex that suggest proximity of motif A of one NBD and motif C of the other NBD during ATP hydrolysis (5), and 3) cross-linking experiments on P-glycoprotein that suggest that motif A of NBD1 and motif C of NBD2 of P-glycoprotein are very close to each other at the same stage during the ATP binding/hydrolysis cycle (13). The fact that in the Rad50CD model ATP binding and hydrolysis require two NBDs helps explain the cooperativity of ATP hydrolysis in ABC proteins. It is possible that isolated ABC NBDs and MsbA crystallized as monomers and that the proposed dimeric structures are artifactual (i.e., intermolecular forces present during crystal formation produce contacts between monomers that do not represent biologically relevant association interfaces between monomers).

In summary, although the balance of the data may have recently shifted toward a Rad50CD-like NBD dimer structure, it is presently unclear how NBDs interact in functional ABC proteins with the complete core domain structure. Crystallization artifacts can explain some of the apparently conflicting results, but different NBD arrangements in different ABC proteins and/or the dynamics of the NBD associations during the transport cycle can be important. In the ABC protein SUR (MRP subfamily), recent mutagenesis data suggest that motif C is not directly involved in ATP binding (14), as proposed for Rad50CD and ABC proteins with identical NBDs or NBDs of similar function (e.g., P-glycoprotein).

Toward a unified mechanism of transport by ABC proteins

If dimerization of the NBDs does indeed occur in ABC proteins, it could not only explain the cooperativity between the NBDs but could also provide a unified molecular mechanism of coupling between ATP hydrolysis and substrate transport. A scheme of possible interactions between the NBDs during the ATP binding/hydrolysis cycle is depicted in Fig. 4. Since each ATP binding site is conformed by residues in the two NBDs, ATP binding promotes dimerization of the NBDs. After ATP hydrolysis, the electrostatic repulsion between the inorganic phosphate bound to motif C and the ADP bound to motif A could lead to the dissociation of the NBD dimer. Phosphate is rapidly released, and on release of ADP the cycle can restart. The power stroke for substrate transport can be the formation of the dimer or the dissociation of the dimers. Because of the tight association between the NBDs and the TMDs, the pulling out of the NBDs after ATP hydrolysis may open the “gate” of the “channel” formed by intracellular loops in BtuCD (12). Whether the tightening of the packing of some helices on the cytoplasmic side is a key step for ABC proteins that mediate substrate efflux (e.g., P-glycoprotein), as opposed to influx (BtuCD), is unknown. In this context, it is interesting that low-resolution images of P-glycoprotein suggest that the major conformational changes are associated with ATP binding and not hydrolysis (16). Since cross-linking experiments suggest that the drug-binding pocket of P-glycoprotein has a funnel-like shape, with the larger opening facing the extracellular side, similar to that of the vitamin B₁₂ importer BtuCD (12), it cannot be ruled out that the power stroke is ATP binding for the efflux pumps (closing access of substrates to the cytoplasmic side or inner leaflet of the plasma membrane) and ATP hydrolysis (opening access of substrates to the cytoplasm) for the influx pumps.

![FIGURE 4. Model of association/dissociation of NBDs during the ATP binding/hydrolysis cycle. The first step (A) involves binding of ATP to a monomer NBD by interaction with the core and antiparallel subdomains (red), which produces a rotation in the α-helical subdomain (green) that is coupled to the interaction of the γ-phosphate of ATP with the conserved Gly in the γ-phosphate linker (see Fig. 2B). This linker connects the core F₁-like ATP binding subdomain to the α-helical subdomain. ATP binding to the monomer NBDs is followed by dimerization driven by the conformational changes that favor the interaction of the bound ATP with the signature sequence (motif C) of the other monomer (B). ATP is then hydrolyzed, and the conformational changes are reversed (C). The electrostatic repulsion between the ADP bound to motif A of one monomer and the phosphate bound to motif C of the other monomer could destabilize the dimer (D). Based on the model proposed by J. F. Hunt and colleagues (15).](http://physiologyonline.physiology.org/)
Undoubtedly, more high-resolution structures of ABC proteins will soon be available and will provide essential missing information, but more detailed and sophisticated biochemical and biophysical experiments will be needed to understand how ABC proteins work and to identify basic common steps of the molecular mechanism of transport by such diverse proteins.

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References


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