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Minireview

ABC transporter architecture and mechanism: implications from the crystal structures of BtuCD and BtuF

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Abstract ABC transporters are ubiquitous membrane proteins that facilitate unidirectional substrate translocation across the lipid bilayer. Over the past five years, new crystal structures have advanced our understanding of how ABC transporters couple adenosine triphosphate (ATP) hydrolysis to substrate transport. In the following, we will briefly review the results of these structural investigations and outline their mechanistic implications.

Key words: ABC transporter; Crystal structure; Transport mechanism

ABC transporters use the energy of adenosine triphosphate (ATP) hydrolysis to move substrates across the lipid membranes surrounding cells or cell organelles, often against a concentration gradient. ABC transporters are strikingly diverse with respect to their physiological function, their substrate, and the direction of transport (import vs. export) [1]. This diversity is reflected in the finding that their membrane-spanning domains (MSDs) exhibit detectable sequence similarity only if they move related substrates in the same direction, i.e. either into or out of the cytoplasm [2]. However, ABC are unified by the presence of two cytoplasmic ATP binding cassettes (ABC) that contain critical conserved sequence motifs for binding and hydrolysis of ATP [3]. Thus, a common engine is attached to a specialized translocation pathway in all ABC transporters, and therefore a similar mechanism is likely used to couple ATP hydrolysis to unidirectional substrate translocation [4].

While most eukaryotic ABC transporters export hydrophobic molecules from the cytoplasm [5], the majority of bacterial ABC transporters import essential nutrients that are delivered to them by specific binding proteins [6]. These proteins bind their substrates selectively and with high affinity, which is thought to ensure the specificity of the transport reaction. There are also examples of bacterial ABC transporters serving as exporters, particularly to mediate the extrusion of toxic and hydrophobic molecules, thus contributing to drug and antibiotic resistance of infectious pathogens [7].

Because of their relevance to bacterial growth and human health, ABC transporters have attracted much attention over the past decade, and numerous studies are being conducted aiming at understanding the mechanism of these proteins [8]. High-resolution structures of close to a dozen isolated ABC domains have been determined by X-ray crystallography [9–19]. Moreover, in the past two years, three crystal structures of full-length ABC transporters have been reported, those of the lipid A-flippase MsbA from *Escherichia coli* and *Vibrio cholerae* at 4.5 and 3.8 Å resolution [20,21], and that of the *E. coli* vitamin B_{12} transporter BtuCD at 3.2 Å resolution [22]. Lessons learned from the structure of BtuCD, an ABC transporter mediating vitamin B_{12} uptake, and of BtuF, the cognate vitamin B_{12} binding protein, are discussed in the following.

1. Crystal structure of BtuCD

The physiological unit of the B_{12} transporter is a heterotetramer ($BtuC_2D_2$), and the four subunits assemble such that the two ABC domains as well as the transport MSDs are in close contact with each other, giving the transporter a compact appearance (Fig. 1). At the center of the heterotetramer, surrounded by all four subunits, a water-filled channel is present that connects with the cytoplasm. Three elements are particularly relevant to ABC transporter function:

1. Translocation pathway. Each of the two BtuC subunits traverses the membrane 10 times for a total of 20 transmembrane helices in the transporter. The interface between the two BtuC subunits is formed by the antiparallel packing of two pairs of helices, creating a large cavity that opens to the periplasmic space. This cavity is of sufficient size to accommodate the corrin ring of vitamin B_{12} and therefore likely represents the translocation pathway. The cavity has no structural resemblance to the binding pockets of B_{12}-dependent enzymes, consistent with its role as a relatively inert transport channel. There is no continuous pathway across the membrane because the cavity is closed to the cytoplasm by residues in two loops that appear to function as a gate. The single translocation pathway at the interface of the membrane-spanning subunits is likely a common feature of ABC transporters that move water-soluble substrates, although the size and the chemical nature of the internal surface may vary considerably.
2. Power source. The overall fold of the BtuD subunit closely resembles that of other ABC domains. Although it has been appreciated for some time that ABC transporters con-

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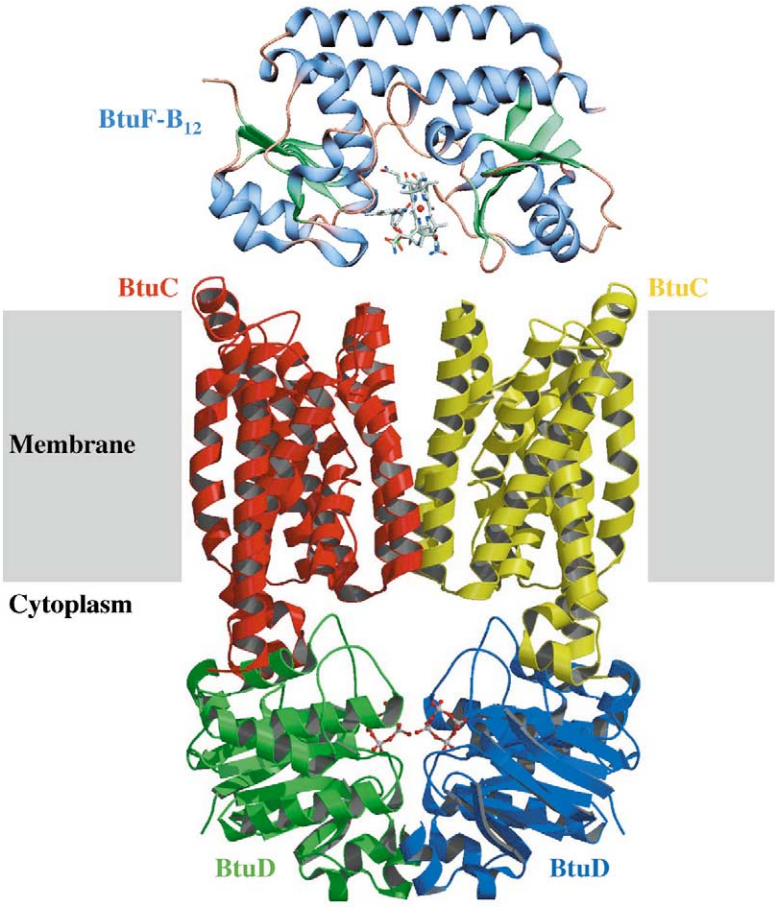

Fig. 1. Ribbon diagram of BtuCD and B_{12}-bound BtuF. The B_{12} transporter consists of two subunits each of BtuC (membrane-embedded) and BtuD (cytoplasmic ATP binding cassette). The large intersubunit gap evident at the center of BtuCD likely serves as the exit path for transported B_{12}. Two cyclotetravanadate molecules are bound at the ATP binding sites of the BtuD subunits. BtuF has been manually placed such that conserved surface glutamates align with conserved arginine residues of BtuCD, which places the bound B_{12} molecule over the entrance of the translocation pathway.

tained two ABC domains, their arrangement within the assembled transporter was, until recently, debated. This uncertainty was due to the observation of distinct dimeric arrangements in three crystal structures of isolated ABC domains that could not at the same time be general to the transporter superfamily [9–11]. Significantly, the BtuD subunits of the B_{12} transporter are aligned such that the nucleotide is bound at the dimer interface between the ABC signature motif of one subunit and the P-loop of the other. Hence, nucleotide binding and hydrolysis is a property of dimeric ATP binding cassettes, and not of individual domains. This arrangement had previously been observed in the crystal structure of the ABC protein Rad50 involved in DNA repair [10], and it is now thought to be the physiological arrangement of ABC domains in all ABC transporters. In the absence of nucleotide, the shared interface of the two ABC domains is small, which may account for the finding that the majority of structures of isolated ABC domains have been determined in the monomeric state. Physiologically relevant dimers of isolated ABC domains could be trapped either using non-hydrolyzing conditions (ATP/ethylenediamine tetraacetic acid (EDTA) [10,18]) or a hydrolysis-deficient mutant protein with a mutation in the second acidic residue of the Walker B motif (glutamate to glutamine [15]). In BtuCD, it appears that the MSDs keep the ABC domains properly aligned through extensive MSD–MSD and MSD–ABC interfaces. This observation suggests mutual conformational control of the MSD and ABC domains, which may be a common feature of full-length, physiologically assembled ABC transporters.

3. Transmission. The interface of the MSD and ABC domains transmits the conformational changes generated by ATP binding and hydrolysis from the BtuD subunits to the membrane-embedded BtuC subunits. Although the detailed contacts vary, this transmission interface appears to be architecturally conserved in ABC transporters (Fig. 2). Amino acid residues of BtuD involved in the interface with BtuC are predominantly clustered around the Q-loop [3], an element that has been proposed to act as a γ-phosphate sensor that may change its conformation upon nucleotide binding and hydrolysis [14]. Mutants at the transmission interface are expected to either disturb the assembly of the transporter or to affect coupling of ATP hydrolysis to transport. Indeed, mutation of Arg^{659} in TAP1 (transporter of antigenic peptides) results in severely affected coupling [23,24], while the deletion of Phe^{508} in CFTR (cystic fibrosis transmembrane conductance regulator) reduces the amount of correctly assembled protein in the plasma membrane to below 1%, indicating that folding/

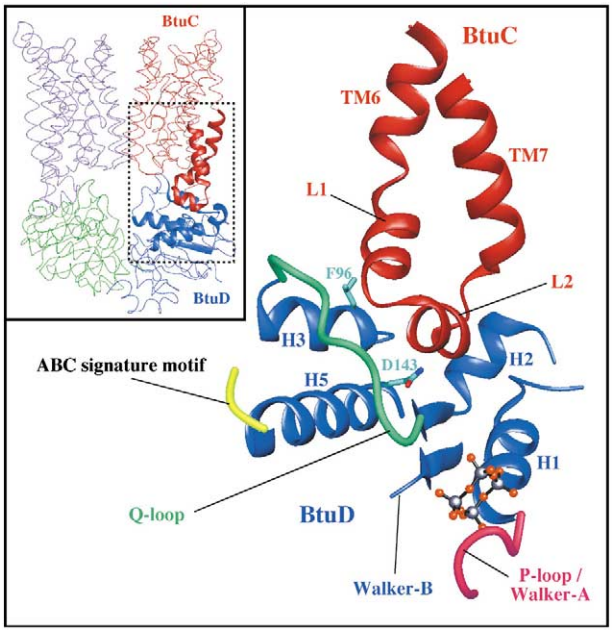

Fig. 2. Transmission interface. The main panel shows a close up of the BtuC–BtuD interface region, while the insert shows the interface region in the context of the entire BtuCD transporter. The surface of the ABC domain BtuD in contact with the helices L1 and L2 of BtuC comprises residues mostly around the Q-loop. Side chains are highlighted for residues Gln^{143}, which corresponds to Arg^{659} of Tap1, and Leu^{96}, which corresponds to Phe^{508} of CFTR. Mutations of these residues in Tap1 or CFTR lead to loss of coupling or assembly (see text).

assembly of the critical transmission interface has failed [25]. In BtuCD, the corresponding residues are found on the surface of BtuD in contact with BtuC (see Fig. 2). Strikingly, sequence alignments indicate that there is an architectural conservation of the L-loop of BtuC, in particular of helix L2 (Fig. 2). This may represent the only architectural feature of MSDs structurally conserved in all ABC transporters.

2. Crystal structure of BtuF

BtuF is the periplasmic binding protein that captures vitamin B_{12} after it has been transported across the outer membrane of *E. coli* by the TonB-dependent transporter BtuB, the structure of which was recently determined [26]. With B_{12} bound, BtuF docks to BtuCD and delivers the vitamin for uptake into the cytoplasm [27]. The substrate binding site is located in a deep cleft formed between the two lobes of BtuF, and B_{12} is bound in the 'base-on' conformation [28,29]. Although the details of the interaction between BtuCD and BtuF remain to be experimentally established, the structures of the individual proteins suggest that conserved glutamate residues on the surface of BtuF adjacent to the exposed ligand surface may be important for interaction with a set of conserved arginine residues in BtuCD [28]. Biochemical studies with purified components have demonstrated the formation of a stable complex between BtuF and BtuCD with the stoichiometry $BtuC_2D_2F$. A model of this complex, generated from the individual crystal structures through alignment of the conserved charged residues, positions the bound B_{12} over the entrance to the translocation pathway of BtuC [28].

3. Implications for ABC transporter mechanism

It is thought that a set of two or more gates is required in ABC transporters to alternatively block access to one side of the membrane or the other; the role of the energy-dependent steps being to control the proper sequence of gate opening and closing. Indeed, the BtuCD structure demonstrates that no continuous channel through the membrane is present. In the nucleotide-free structure of BtuCD, one gate in BtuC is definitely closed near the cytoplasmic side of the membrane. An attached binding protein may serve as an additional gate to prevent the escape of substrate on the wrong side of the membrane.

Long-range conformational changes, originating at the ABC domain interface, may operate the BtuC gate in an ATP-dependent manner. Alternatively, gate opening and substrate transport may be ATP-independent, with docking of the binding protein providing the necessary energy for these steps. In view of the tight complexes of binding protein and transporter observed for several importers [28,30], it is likely that one role of ATP hydrolysis is to dissociate the ternary complex and return the system to the resting state. The transporter thus appears to function as an airlock, with nucleotide binding and hydrolysis orchestrating a sequence of conformational changes that open and close the appropriate gates so that unidirectional translocation of a ligand is achieved without ever creating a continuous channel across the membrane.

What conformational changes are to be expected? The amplitude of protein motion concomitant with transport is likely substantial, as has been visualized by electron microscopy for multidrug resistance ABC transporters [31,32]. Although isolated ABC domains exhibit conformational flexibility between the helical and the ATP binding subdomains as well as induced local changes upon nucleotide binding [13,14,17], it is not clear if these same motions are occurring in full-length transporters, where the flexibility of the ABC domains may be controlled by the MSDs. This interpretation is supported by the finding that in full-length transporters, substrate binding to the MSDs stimulates ATP hydrolysis by the ABC domains, suggesting that the substrate binding state is signaled to the ATP binding sites. This level of control is lost in isolated ABC domain.

Based on the MsbA structure and on biochemical observations obtained from isolated ABC domains, it has been proposed that the ABC domains within an ABC transporter undergo cycles of association and dissociation during the transport cycle, pulling and pushing the partner membrane domains apart and together again as they move [20,33,34]. However, the BtuCD structure suggests that the ABC domains may remain in contact during the transport cycle and engage more tightly as ATP gets bound and hydrolyzed, with the sandwiched ATP molecules contributing substantially to the shared interface between ABCs. In particular, the signature motif of one ABC domain appears to move by several Ångstroms, thereby pinning the nucleotide against the P-loop of the opposing ABC domain [22]. How this change translates into movements of the MSDs is unknown.

In view of the existence of a common engine, it appears unlikely that ABC importers and exporters operate by a fundamentally different mechanism. We speculate that both ABC importers and exporters directly couple ATP hydrolysis to release of the substrate on the outside. In the case of import-

ers, the 'substrate' would correspond to the tightly attached binding protein, while the true substrate (the translocated molecule) would slip through the translocation channel in an earlier stage of the transport cycle. Exporters of hydrophilic molecules (such as antigenic peptides) may expose a substrate binding pocket where the water-filled, cytoplasmic channel between the four subunits is present in BtuCD. Future structural studies both of importers and exporters may clarify these questions.

4. Conclusions and outlook

Structural investigations of ABC transporter proteins have, in the past few years, provided a framework for addressing more detailed aspects of the transport mechanism. Crystallographic studies of transporters trapped at intermediate states will undoubtedly be a priority for the coming years, and may reveal the conformational changes concomitant with substrate transport and ATP binding and hydrolysis. Eventually, direct visualization of human ABC transporters at high resolution will be achieved and will provide us with a direct look at the medically most relevant members of this protein superfamily.

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