

ABC Transporter Architecture and Mechanism

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Transport proteins, embedded in lipid membranes, facilitate the import of nutrients into cells or the release of toxic products into the surrounding medium. The largest and arguably the most important family of membrane transport proteins are the ABC transporters. They are ubiquitous in biology and power the translocation of substrates across the membrane, often against a concentration gradient, by hydrolyzing ATP (Higgins, 1992).

Several human ABC transporters are medically relevant. For example, mutations in the CFTR (cystic fibrosis transmembrane conductance regulator) protein cause cystic fibrosis. A separate subclass of ABC transporters are associated with multidrug resistance in tumors, i.e. the ability of certain cancer cells to extrude cytotoxic agents used in chemotherapy. Yet another ABC transporter, the TAP protein, is critical for the proper functioning of the cellular immune response, as it pumps antigenic polypeptides from the cytoplasm into the endoplasmic reticulum, where they are being loaded onto MHC class I molecules for subsequent presentation on the cell surface. In bacteria, ABC transporters are predominantly involved in nutrient uptake, although they also participate in the export of bacterial toxins and harmful substances, contributing to bacterial multidrug resistance.

Despite the immense amount of biochemical studies, and recent advances in the visualization of ABC transporters, answers to critical questions about their translocation mechanisms have remained elusive. The structure of a bacterial ABC transporter facilitating vitamin B₁₂ import into *E. coli*, the BtuCD protein, was recently solved from data collected at SSRL. It is the first complete ABC transporter to have its high resolution (3.2 Å) structure determined in the physiological assembly, and it has yielded valuable insight into how ABC transporters work (Locher *et al.* 2002).

All ABC transporters contain two membrane-spanning domains that harbor a translocation pathway for a specific substrate. Attached are two cytoplasmic adenosine triphosphate-binding cassettes (hence ABC). As the ABC cassettes bind and hydrolyze ATP, conformational changes occur that are transmitted to the membrane-spanning domains, where they induce rearrangements that translocate the substrate from one side of the membrane to the other. The initial motion of the ABC cassettes has been dubbed the *power stroke*, and it is generally assumed that this rearrangement is similar in all ABC

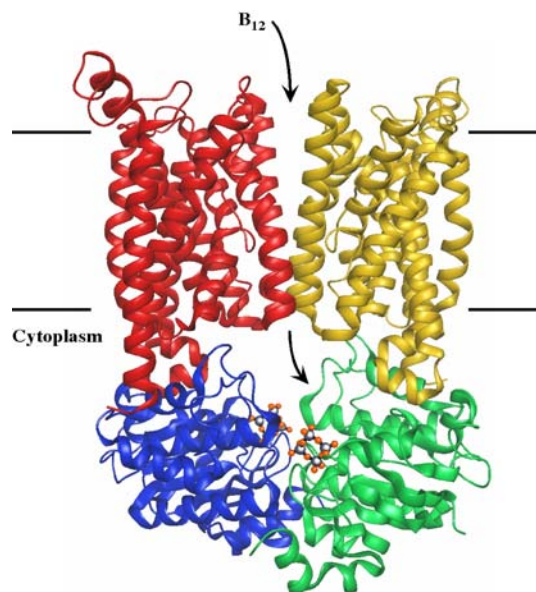


Figure 1. Ribbon diagram of the BtuCD protein structure. The transporter is assembled from two membrane-spanning BtuC subunits (red and yellow) and two ABC cassettes BtuD (green and blue). At the ATP binding sites, cyclotetranadate molecules are bound to the transporter (ball and stick models at the BtuD interface). Vitamin B₁₂ is delivered to the periplasmic side of the transporter by a binding protein (BtuF, not shown), then translocated through a pathway provided at the interface of the two membrane-spanning BtuC subunits. It finally exits into the cytoplasm at the large gap between the four subunits (arrows). This transport cycle is powered by the hydrolysis of ATP by the ABC cassettes BtuD.

transporters, irrespective of the size of the substrate to be transported or the directionality of the translocation (import or export).

The structure of the BtuCD protein provides insight at just how ABC transporters may carry out their tasks. In particular, three critical elements were visualized for the first time in an intact transporter:

1. Transport pathway: Accessible from the outside (periplasm), but sealed from the cytoplasm of the cell, a large cavity is buried at the interface of the two membrane-spanning BtuC subunits. The cavity is big enough to accommodate the corrin ring of the substrate, vitamin B₁₂, and therefore likely represents the transport pathway through the membrane.
2. Power source: The arrangement of the two BtuD subunits places the binding sites for two ATP molecules at the subunit interface, with each ABC cassette contributing to the binding site. Well-conserved motifs form the contact interface of the two ABC cassettes, suggesting that this arrangement is universal among ABC transporters. The placement of the ATP binding sites wedged between two opposing ABC cassettes provides an explanation for the cooperativity in ATP binding and hydrolysis observed for ABC transporters.
3. Relay station: The interface between the ABC cassette BtuD and the membrane-spanning subunit BtuC is where the mechanical energy produced by the hydrolysis of ATP is transmitted to the membrane-spanning domain. A prominent cytoplasmic loop of BtuC, forming two short helices, makes extensive contact with BtuD. Alignment with the protein sequences of other ABC transporters reveals that a comparable interface to that observed in BtuCD may exist in other transporters. The functionality and assembly of transporters with mutations in these critical interface residues is severely affected. As a striking example, 70% of cystic fibrosis patients have a single residue deleted that corresponds to a BtuD residue at the interface of the ABC cassette and the membrane-spanning domain.

While much remains to be learned about substrate binding as well as the detailed conformational changes concomitant with substrate translocation through ABC transporters, the BtuCD structure provides a framework for addressing these central mechanistic issues.

References:

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2. Locher, K. P., Lee, A. T., Rees, D. C. (2002) The *E. coli* BtuCD structure: a framework for ABC transporter architecture and mechanism. *Science* **296** 1091-1098.

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