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Solute-Binding Sites in ABC Transporters for Recognition, Occlusion and Trans-Inhibition

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Introduction

For all living cells, the ability to accumulate useful molecules and to extrude waste products or toxic materials is essential. In many cases, this involves specific transporters, which are able to pump their substrates across a cell membrane against a concentration or electrochemical gradient. In the case of ATP-binding cassette (ABC) transporters, ATP provides the energy required for this uphill movement. ABC transporters have been scrutinized for many years because of their ability to export drugs from mammalian cells, thus hindering treatment of bacterial infections and cancer. Although the true physiological substrates for the drug extruders is not clear, other ABC exporters with defined specificity for molecules, such as amino acids, peptides, and cholesterol, have been identified and characterized. Unlike eukaryotic cells, eubacteria and archaea also utilize ABC transporters to import a range of substances. Over the last few years, six full-length importers, including those recently described by Gerber et al. and Kabada et al.,[1,2] and two exporters have been crystallized and their high-resolution structures have been determined. This is in addition to numerous structures of soluble components of the ABC systems.[3–5] As each new structure is added, it becomes increasingly possible to understand the molecular function of this medically important family of proteins.

All ABC transporters contain a transmembrane domain (TMD), via which the solute moves across the membrane, and a cytosolic nucleotide-binding domain (NBD), which binds and hydrolyzes ATP to produce energy for the transport.[3–5] The NBDs are highly conserved motors that are also involved (without the TMD) in other essential cellular processes (DNA repair, protein translation or ribosome assembly). Conversely, the TMDs vary considerably as may be expected from the wide variety of transported solutes, from small hydrophobic drugs to large (poly) peptides.

Transmembrane domains tailored to their substrates

As mentioned above, the X-ray structures of six ABC importer proteins have been solved. The *Escherichia coli* methionine transporter (MetNI) was recently described by Kabada et al.,[2] and has the simplest TMD architecture, with just five membrane spanning helices per MetI subunit—the MetI component is the TMD, while the NBD is on a separate polypeptide chain (MetN); two copies of each comprise the full transporter. In the recently solved *Methanosarcina acetivorans* transporter structure (maModBC), the TMD subunits (ModB) have six transmembrane helices each.[1] As shown by Kabada et al., these TMDs may be superimposed on each other with high accuracy; the five helices of MetI corresponding to the five C-terminal helices in ModB. The same is also true for the previously solved *Archaeoglobus fulgidus* transporter afModBC.[6] The two TMD subunits from the *E. coli* maltose transporter structure (MalF and MalG), in which the TMD is a heterodimer coupled to a MalK NBD homodimer,[7] may also be superimposed (Figure 1b). All of these proteins transport relatively small substrates: methionine, molybdate and tungstate, and the disaccharide maltose, respectively. On the other hand, the importers that deal with larger substrates, the vitamin B12 transporter BtuCD[8] and the metal-chelate-type transporter HI1470/71,[9] possess a quite different TMD architecture compared with the aforementioned transport system, but are themselves very similar (Figure 1a). This suggests that the TMDs are adapted to optimally transport specific solutes. So far, only two high-resolution structures of ABC exporters are available,[10,11] but interestingly, their TMDs, though similar to each other, are different to either of the known importer configurations (Figure 1c).

A mechanism for substrate transport modulation

The two new structures are novel in that they contain a substrate bound to the extended domains, C-terminal to the NBDs, on the cytosolic side of the membrane.[1,2] Interestingly, there are two solute-binding sites located at a dimer interface, similar to that seen for the two ATP-binding sites in the NBDs. The apparent function of the solute binding in this region is to curtail transporter function by preventing the dimerization of the NBDs. This is shown by the inhibition of the ATPase activity of maModBC or MetNI by increasing concentrations of molybdate and tungstate, or methionine, respectively.[1,2] In the maModBC structure, the NBDs are further apart than in the afModBC protein, which does not have a regulatory domain. The benefits of this trans-inhibition are clear; the accumulation of potentially toxic substrates or use of energy for import of substrate beyond that required is clearly

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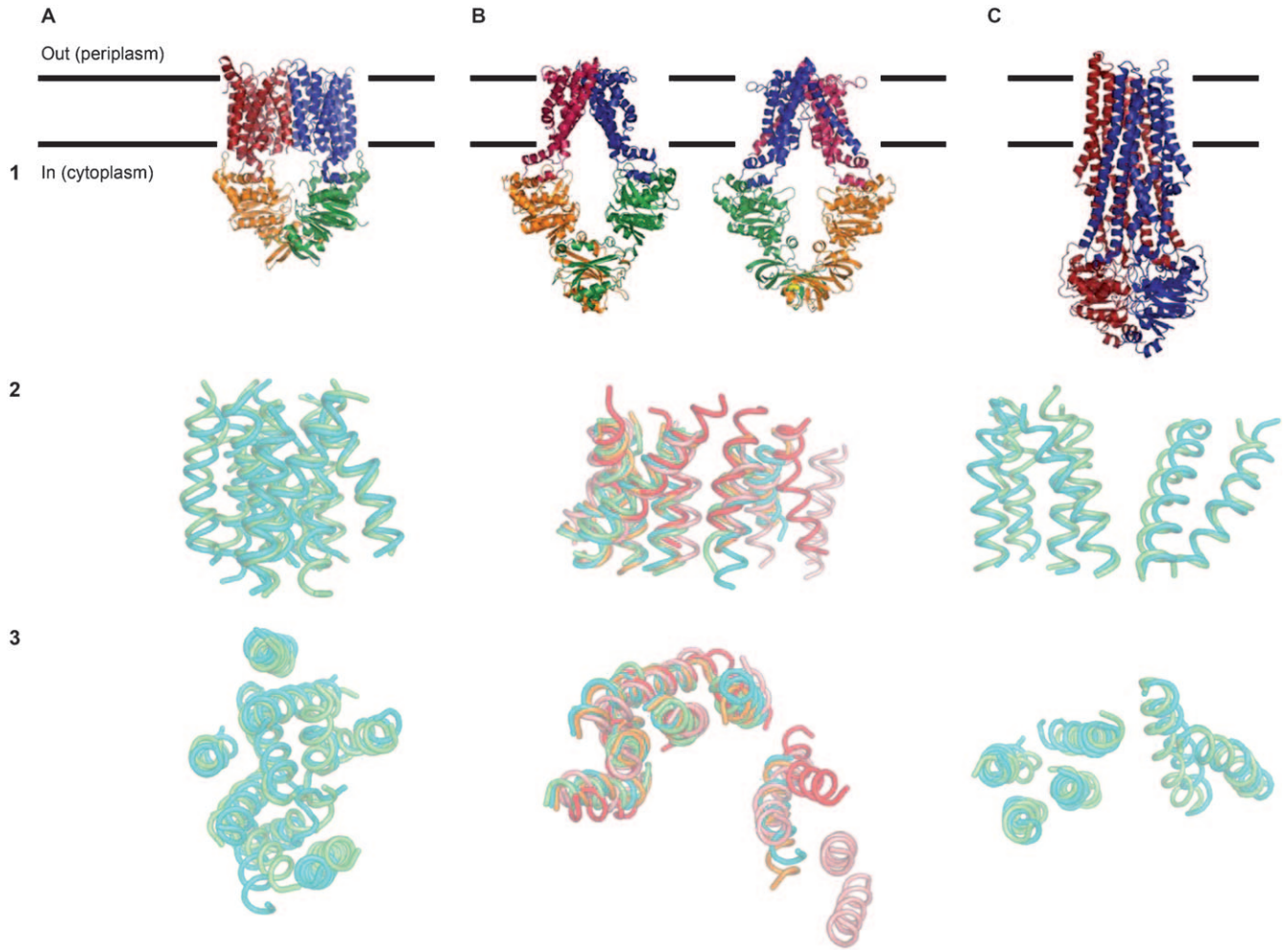

Figure 1. Classification of ATP transporters according to their transmembrane domain architecture: 1) Schematic representative structures; A) HI1470/71 (2NQ2.pdb[9]); B) MetNI (3DHW.pdb[2]) and maModBC (3D31.pdb[1]); C) Sav1866 (2HYD.pdb[15]). The lines show the putative membrane location; 2) superposition of trans-membrane helices from a single TMD subunit viewed parallel to the membrane plane; A) BtuCD (lime, 1 LV7.pdb[8]) and HI1470/71 (cyan); B) MetI (lime), afModBC (lime, 2ONK.pdb[6]), maModBC (orange), MalF (salmon, 2R6G.pdb), MalG (red, 2R6G.pdb[7]); C) The exporters Sav1866 (lime) and *S. typhimurium* MsbA (cyan, 3B60.pdb[11]); 3) the TMD helices overlaid as in panel 2 viewed normal to the membrane plane from the periplasmic side.

undesirable. The modulatory domain in ModC is similar to the protein ModE, which regulates transcription of the transporter, so both short-term and long-term regulation is possible. Interestingly, the amount of methionine required for half-inhibition of MetNI (30 μM) is higher than that of molybdate required for similar modulation of maModBC (~5 μM). This presumably reflects the higher risk to the cell due to toxicity of the metal. The maltose transporter from *E. coli* also possesses a similar domain, but strangely it does not appear to be used for regulation.

The translocation pathway: substrate binding and transport cycle

Regulation of transported solute is not required or present in many importers but clearly its recognition is required for selective transport. The likely mechanism for ABC (and other) transporters is an alternated access mechanism. In this scenario, the transporter TMD cycles between an outward facing and an inward facing conformation with the interchange driven by ATP binding and hydrolysis. For an importer it would be expected that the outward facing state binds substrate with high affinity, while the inward facing form binds substrates with low affinity. Substrates are picked up with high efficiency from outside the cell and deposited into the cytoplasm. Unfortunately, no single importer has been crystallized in both in- and outward facing conformations. However, enough structures are now available to tentatively propose a model describing the sites of substrate recognition during the cycle (Figure 2). In most importers, the substrate is presented to the outer face of the transporter by a high affinity binding protein.[4,12] Structures have shown that this protein binds the substrate by enclosing it between two lobes. In the absence of any substrate, the transporter maintains an inward-facing TMD conformation, as exemplified by the HI1470/71 structure.[9] In this structure, no nucleotide is found in the NBDs. However, this state is highly un-

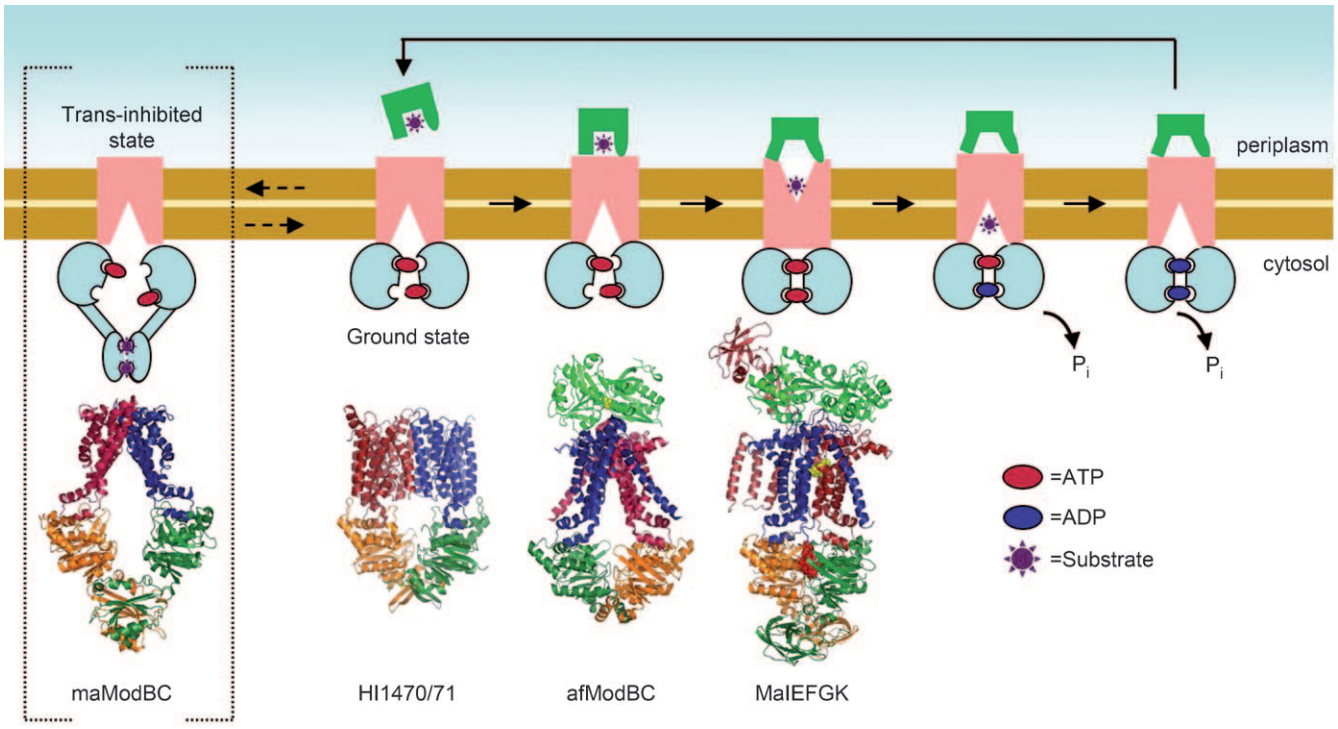

Figure 2. A schematic diagram illustrating a model of the ABC importer cycle. Where available, equivalent structures are shown beneath each putative intermediate. Transmembrane subunits: blue and red (protein structure), pink (schematic structure). NBD subunits: orange or dark green (protein structures), light blue (schematic structure). Substrate-binding domains: light green (protein structure), green (schematic structure). ATP and substrate (where present) are shown by red and yellow space-filling spheres, respectively in the protein structures. Note that only the structure of MalEFGK was solved with nucleotides bound.

likely to exist in vivo because ATP is normally present in the cytosol at concentrations well above the K_d value of the NBDs. Thus, the resting state is likely to have ATP bound, but with the NBDs still slightly separated. The substrate-binding protein then docks (or the binding protein remains bound waiting for substrate). This conformation is best represented by the afModBC-derived afModABC structure, where "A" designates the binding protein.[6] However, once again the structure contains no nucleotide. Docking and sensing of the substrate-loaded binding protein probably triggers closure of the NBDs followed by a conformational change. This means that the afModABC structure is likely to correspond to a normally short-lived intermediate. The conformational change induced by NBD closure results in a state represented by the *E. coli* maltose transporter; a complex of two TMDs, MalF and MalG, two MalK NBDs, and a substrate-binding protein.[7] Like the afModABC, this structure corresponds to an arrested transition state engineered by mutation of the catalytic glutamate (E159Q) in both NBDs, which is required for efficient ATP hydrolysis. Here, the substrate-binding protein is more open than in the substrate-bound form, and a loop from MalG protrudes into the maltose-binding groove. This causes the release of substrate into a cavity formed by the outward-facing TMDs, where it binds to a pocket close to the middle of the membrane. Importantly, the substrate is in an occluded state with access to neither outer nor intracellular environment. The transporter then undergoes a transition to the inward facing form, preceded or accompanied by ATP hydrolysis. In this state, the substrate binding may be weakened, or the substrate affinity may be sufficiently low to be released into the cytosol. To date, no crystal structures portraying this transition are available. The transporter must then return to the outward-facing conformation, possibly via an intermediate state as seen for BtuCDF, although it is not clear how this unusual structure fits into the cycle.[13,14]

Outlook

Although the general cycle of alternate access seems to hold true, there are many key questions as yet unanswered. For example, is the binding of ATP or hydrolysis sequential, alternating, or concerted, and at which step do these events take place? Is the cycle dependant on the substrate? Are the known crystal structures true representations of the cycle intermediates? In spite of these questions, some general principles can be drawn; it appears that conformational changes from the NBDs can be transmitted to the TMDs via coupling helices, which are intracellular loops of the TMD subunits.[3,15]

And what about the medically important ABC exporters? These proteins efficiently function without the help of binding proteins, so where does the substrate bind? A striking difference between the structures of importers and the two exporters solved to date, is the large cytosolic extension of some of the transmembrane helices (Figure 1C).

Recent work on the human TAP complex, the transporter protein associated with antigen processing, has suggested that the solute binds to a high affinity site in this region, and this could potentially represent a generalized substrate-binding domain function analogous to substrate-binding proteins of ABC importers,[16] although hydrophobic drugs have been suggested to bind via the membrane bilayer. These questions await yet more high-resolution structures before they can be definitively answered.

Keywords: medicinal chemistry · drug design · protein structures · multidrug resistance · membrane proteins

[1] S. Gerber, M. Comellas-Bigler, B. A. Goetz, K. P. Locher, *Science* **2008**, *321*, 246–250.
[2] N. S. Kadaba, J. T. Kaiser, E. Johnson, A. Lee, D. C. Rees, *Science* **2008**, *321*, 250–253.
[3] K. Hollenstein, R. J. Dawson, K. P. Locher, *Curr. Opin. Struct. Biol.* **2007**, *17*, 412–418.
[4] A. L. Davidson, E. Dassa, C. Orelle, J. Chen, *Microbiol. Mol. Biol. Rev.* **2008**, *72*, 317–364, table of contents.
[5] L. Schmitt, R. Tampé, *Curr. Opin. Struct. Biol.* **2002**, *12*, 754–760.
[6] K. Hollenstein, D. C. Frei, K. P. Locher, *Nature* **2007**, *446*, 213–216.
[7] M. L. Oldham, D. Khare, F. A. Quiocho, A. L. Davidson, J. Chen, *Nature* **2007**, *450*, 515–521.
[8] K. P. Locher, A. T. Lee, D. C. Rees, *Science* **2002**, *296*, 1091–1098.
[9] H. W. Pinkett, A. T. Lee, P. Lum, K. P. Locher, D. C. Rees, *Science* **2007**, *315*, 373–377.
[10] R. J. Dawson, K. P. Locher, *FEBS Lett.* **2007**, *581*, 935–938.
[11] A. Ward, C. L. Reyes, J. Yu, C. B. Roth, G. Chang, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 19005–19010.
[12] T. van der Heide, B. Poolman, *EMBO Rep.* **2002**, *3*, 938–943.
[13] R. N. Hvorup, B. A. Goetz, M. Niederer, K. Hollenstein, E. Perozo, K. P. Locher, *Science* **2007**, *317*, 1387–1390.
[14] D. Parcej, R. Tampé, *Structure* **2007**, *15*, 1028–1030.
[15] R. J. Dawson, K. P. Locher, *Nature* **2006**, *443*, 180–185.
[16] M. Herget, G. Oancea, S. Schrodt, M. Karas, R. Tampé, R. Abele, *J. Biol. Chem.* **2007**, *282*, 3871–3880.

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