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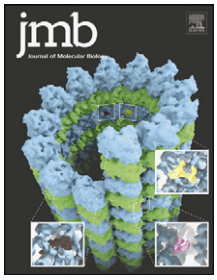

Mechanism of Action of ABC Importers: Conservation, Divergence, and Physiological Adaptations

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Abstract

The past decade has seen a remarkable surge in structural characterization of ATP binding cassette (ABC) transporters, which have spurred a more focused functional analysis of these elaborate molecular machines. As a result, it has become increasingly apparent that there is a substantial degree of mechanistic variation between ABC transporters that function as importers, which correlates with their physiological roles. Here, we summarize recent advances in ABC importers' structure–function studies and provide an explanation as to the origin of the different mechanisms of action.

The Different Classes of ABC Transporters

ATP binding cassette (ABC) transporters comprise a large superfamily of membrane proteins. In bacteria, fungus, plants, and animals, they transport molecules through the permeability barriers of cell membranes [1–4]. Cargo molecules are extremely diverse, including ions, small-to-medium-sized molecules such as sugars, amino acids, lipids, ionic metals, and large, bulky compounds including peptides, proteins, organo-metal complexes, and antibiotics. They are involved in many important physiological processes, such as plant development and growth, nutrient import, cellular detoxification, lipid homeostasis, signal transduction, antiviral defense, and antigen presentation [3,5–15]. From a clinical perspective, ABC transporters are of great interest as they are directly involved in tumor resistance to chemotherapeutics [16–18], drug resistance of parasites [19–21], fungal drug resistance [22], bacterial multidrug resistance, and bacterial virulence and pathogenesis [23–25]. All ABC transporters share a basic architecture comprising at least two intracellular nucleotide-binding domains (NBDs) and two transmembrane domains (TMDs). The NBDs supply the energy via ATP binding and hydrolysis, while the TMDs form the transmembrane permeation pathway. An ABC transporter may function either as an importer or an exporter with the exporters present in all kingdoms and the importers present solely in bacteria and plants [26,27]. In bacteria, ABC importers require an additional protein partner: a substrate-binding protein (SBP) [28–32] that binds the substrate and delivers it to the TMDs. For many years, this large family of proteins was considered both mechanistically and structurally uniform yet, as more systems are characterized, cracks are beginning to appear is this assumption. For example, four significantly different 3D folds have been observed (Fig. 1), yielding the Type I and Type II exporter and the Type I and Type II importer classifications [33–36].

More recently, an additional structurally divergent group has been identified, the Energy-Coupling Factor transporters [37–44], which are referred to as Type III ABC importers. The structure and function of ECF transporters has been reviewed elsewhere [45–48] and will not be discussed here. The structural divergence of ABC transporters is accompanied by significant mechanistic variation, and several alternative mechanistic models have recently been suggested [33,49–52].

Herein, we focus on Type I and Type II ABC importers, present our perspective of their mechanistic conservation and diversification, and suggest a

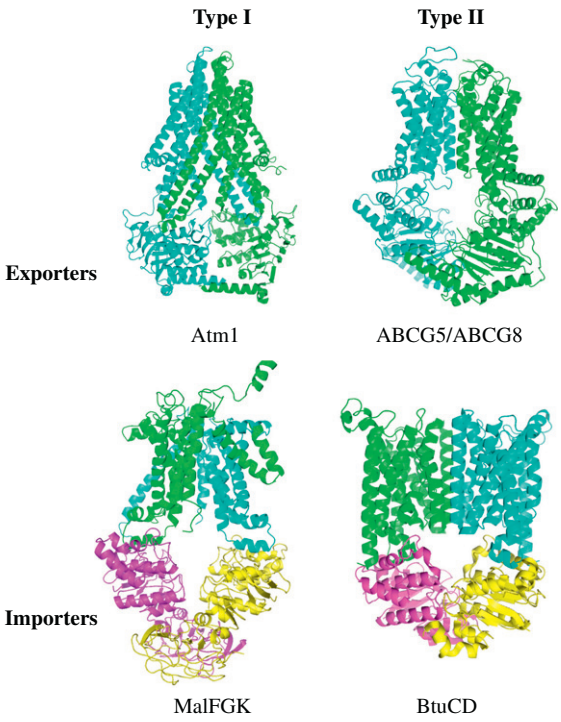

Fig. 1. The different folds of ABC transporters. Shown is the cartoon representation of the Type I metal-glutathione exporter Atm1 (PDB ID: 4MRV), the Type II sterol exporter (PDB ID: 5DO7), the Type I maltose importer MalFGK (PDB ID: 3FH6), and the Type II vitamin B_{12} importer BtuCD (PDB ID: 1L7V).

"biological logic" that explains the observed mechanistic speciation.

Common Ground: The NBDs and Mechanism of ATP Hydrolysis

All ABC proteins use the same motor to provide the driving force for the task they perform. Regardless of their physiological role, the 3D organization of this motor domain, or NBD, is highly conserved. For example, the RNase-L inhibitor is an ABC protein involved in ribosome biogenesis, formation of translation preinitiation complexes ,and assembly of HIV capsids, yet the structure of its NBD is extremely similar (RMSD 1.3 Å over 708 Cα atoms) to that of the vitamin B_{12} importer BtuCD (Fig. 2). The structural organization of the NBDs and the ATP binding sites has been extensively reviewed [34,50,53]. Briefly, two ATP binding sites are formed at the dimer interface between two NBDs, where residues from each monomer contribute to ATP binding and hydrolysis. The residues participating in ATP coordination and catalysis are highly conserved and are located in canonical motifs, including the Walker A/B and signature motifs and the Q, H, and D loops that define the superfamily of ABC proteins. These conserved motifs come together in

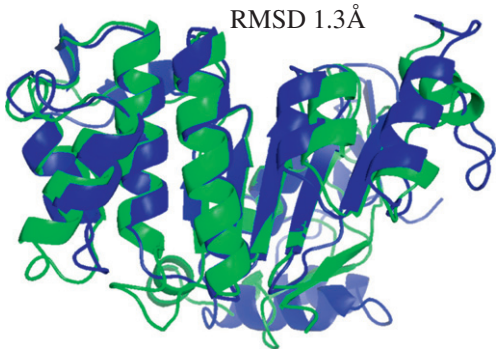

Fig. 2. Structural alignment between nucleotide-binding domains (NBDs) of two functionally unrelated ABC proteins. A single NBD from RNase-L inhibitor (blue; PDB ID: 1YQT) was aligned with a BtuD monomer (green; PDB ID: 1L7V), resulting in an RMSD value of 1.3 Å (over 708 Cα atoms).

the folded NBDs to form the ATP binding sites. The importance, and notably, the specific roles of these residues/motifs in binding and hydrolysis of ATP and to power transmission, appears to be highly conserved. One noteworthy example is the glutamate of the Walker B motif that serves as the catalytic base for ATP hydrolysis. The essential role of this glutamate has been demonstrated for prokaryotic and eukaryotic ABC transporters and for exporters and importers alike [54–61]. Other examples include the aspartate of the Walker B motif, the histidine in the H-loop, and the glutamine of the Q loop [59,62,63].

To hydrolyze ATP, the dimeric NBD's must come together to form a tight head-to-tail dimer sandwich [55], and this conformational change depends on ATP binding. This relationship between binding of ATP, the closure of the NBD dimer interface, and ATP hydrolysis is another feature that is conserved in all ABC proteins. It is often overlooked that ABC transporters can bind, hydrolyze, and drive transport by using nucleotides other than ATP. Several studies have shown that the affinities to CTP, GTP, and UTP are quite similar to those for ATP [64,65], which may be of importance, considering the high intracellular concentrations of all nucleotides [66,67].

First Signs of Divergence: Affinity, Kinetics, and Cooperativity of ATP Hydrolysis

The affinity (K_D, dissociation constant) of ABC transporters to ATP is rarely measured directly and is mostly inferred by the kinetic determination of the K_m of ATP hydrolysis. The K_m values differ over 3 orders of magnitude among the characterized ABC transporters, from the micromolar (e.g., BtuCD, MalFGK) to millimolar (e.g., Sav1866, HisPQM, Pgp) range [65,68–70], which was unexpected given the high 3D uniformity