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Review

Molecular basis of multidrug transport by ABC transporters

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ABSTRACT

Multidrug ABC transporters such as the human multidrug resistance P-glycoprotein (ABCB1) play an important role in the extrusion of drugs from the cell and their overexpression can be a cause of failure of anticancer and antimicrobial chemotherapy. These transport systems contain two nucleotide-binding domains (NBDs) where ATP is bound and hydrolyzed and two membrane domains (MDs) which mediate vectorial transport of substrates across the cell membrane. Recent crystal structures of the bacterial ABCB1 homologues Sav1866 from *Staphylococcus aureus* and MsbA from *Salmonella typhimurium* and other organisms shed light on the possible conformational states adopted by multidrug ABC transporters during transport. These structures help to interpret cellular and biochemical data gathered on these transport proteins over the past three decades. However, there are contradictory views on how the catalytic cycle of ATP binding and hydrolysis by the NBDs is linked to the change in drug binding affinity at the MDs, which underlies the capture (high affinity) of the transported drug on one side of the membrane and its release (low affinity) on the other. This review provides an overview of the current evidence for the different transport models and establishes the most recent structure-function relationships in multidrug ABC transporters.

1. Introduction

ABC transporters represent a very large superfamily of membrane proteins, which mediate the transport of a multitude of molecules across phospholipid bilayers in every living organism [1]. In bacteria, ABC transporters are predominantly involved in the import of nutrients. These importers act together with periplasmic binding proteins which deliver the cargo to the transporter at the outside surface of the membrane. Whereas ABC importers appear to be restricted to prokaryotes, ABC exporters are found in all living organism and are responsible for the active efflux of a wide range of different molecules such as hydrophobic drugs, lipids, peptides and even proteins including toxins such as hemolysin [2].

The functional entity of ABC transporters features four domains, two membrane domains (MDs) and two nucleotide-binding domains (NBDs), also named the *A*TP *b*inding *c*assettes domains. Whereas the NBDs are responsible for the binding and hydrolysis of ATP, and consequently for the generation of motional force, the MDs form the translocation pathway for transported substrates across the membrane. Due to their common substrate (ATP), the NBDs exhibit a number of conserved sequence motifs, including Walker A, Walker B and the ABC signature, which is the hallmark of the ABC superfamily. In contrast, the MDs are more diverse in sequence, reflecting the large diversity of transported substrates. Importers consist of two NBDs and two MDs which are present as separate polypeptide chains. In bacterial exporters, an MD fused to an NBD forms a "half-transporter", which homo- or heterodimerizes with another "half-transporter" in order to form the functional full-size transporter [3]. In eukaryotes, many ABC transporters contain the four domains fused on a single polypeptide chain. A recent review raised a comprehensive inventory of (bacterial) ABC systems (including those devoid of transmembrane domains) and we refer to this review for further information on the evolution and domain organization of ABC transporters [2]. In this paper, we shall focus on bacterial and eukaryotic ABC exporters and summarize current insights into the underlying mechanisms by which their NBD and MD domains cooperate in the transport reaction that is catalyzed by these systems.

2. Nucleotide-binding domains

2.1. Structural features

NBDs consist of a larger subdomain found in many RecA-like motor ATPases and a smaller helical subdomain, and dimerize in a sandwich-like fashion. Numerous crystallographic structures of isolated NDB monomers and dimers have been solved over the past 10 years (reviewed in [4]) [5–9], which gave insight into the molecular basis of nucleotide binding at the NBD:NBD interface. Stable NBD dimers could only be obtained for mutants which could not hydrolyze ATP [10,11]. The NBDs of all ABC systems display a series of highly conserved sequence motifs, which play indispensable roles in the catalytic cycle of ATP binding, hydrolysis and ADP/Pi release (Fig. 1). These motifs include the Walker A (also called P-loop) and the Walker B motif, which indicate the

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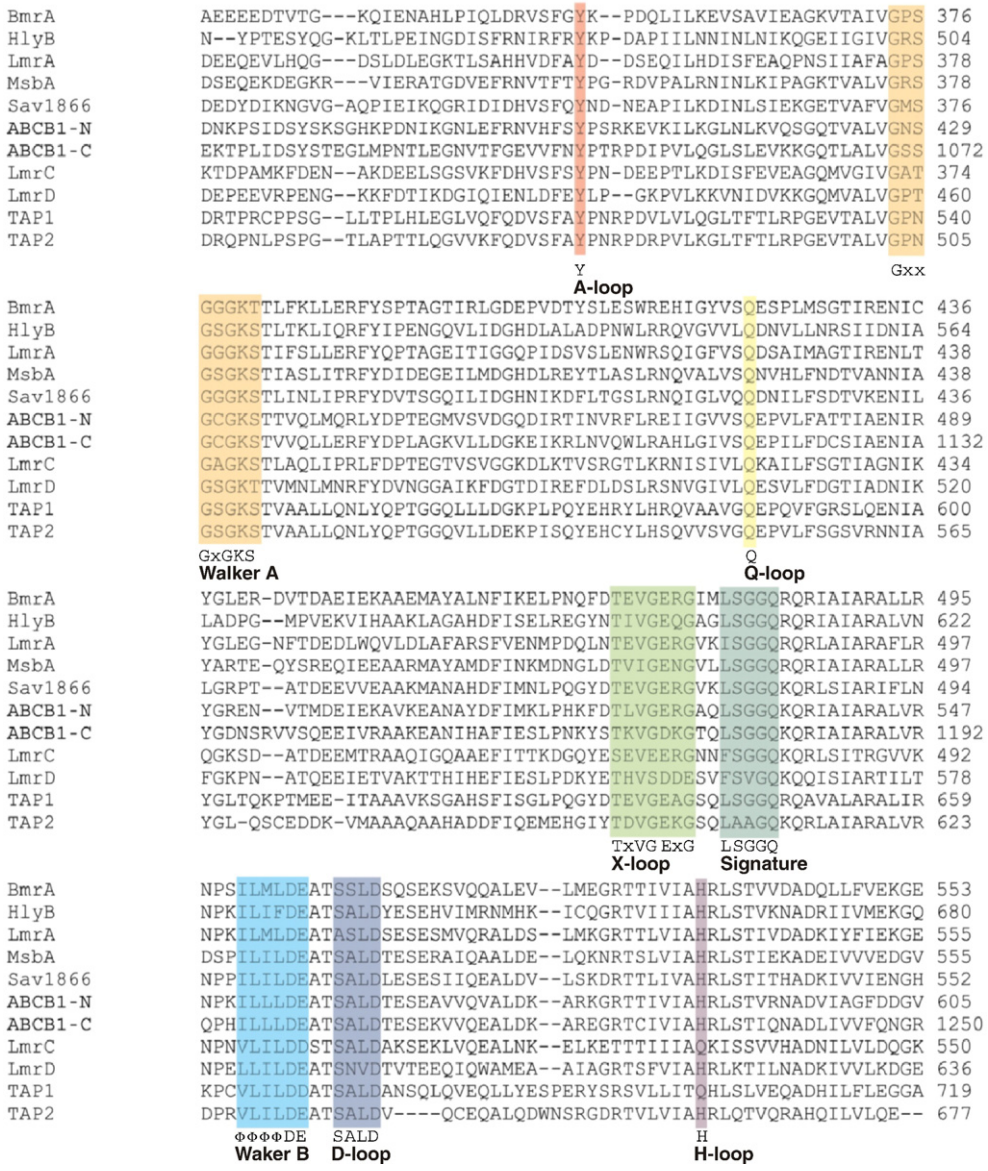

Fig. 1. Alignment of conserved NBD sequence motifs of well-characterized ABC exporters, which are dealt with in the review. The following color code was used: A-loop (red), Walker A (orange), Q-loop (yellow), X-loop (light green), Signature (dark green), Walker B (light blue), D-loop (dark blue), H-loop (purple). The consensus sequence for each motif is depicted below the alignment. X represents any amino acid, Φ stands for hydrophobic amino acids. The alignment was generated using the ClustalW algorithm.

presence of a nucleotide-binding site [12]. The signature motif (also called C-loop or LSGGQ motif) is the hallmark of ABC proteins. Together with the Walker A motif of the *cis*-NBD in the NBD dimer, the signature motif of the *trans*-NBD sandwiches the γ-phosphate moiety of the ATP and contributes to the formation of a composite catalytic site (Fig. 2A). Additional motifs such as the A-loop, Q-loop, D-loop and H-loop (also called the switch loop) only harbor a single conserved residue. The A-loop comprises an aromatic residue, usually a tyrosine, which was shown to stack against the adenine ring of ATP and to provide an essential contribution to the nucleotide-binding affinity of the NBDs [6,9,13,14]. Whereas the conserved glutamine in the Q-loop interacts with ATP's γ-phosphate via a structured water molecule, the histidine of the H-loop directly interacts with the γ-phosphate and was reported to play the role of a linchpin residue in HlyB, which is essential part of the type I secretion machinery of *Escherichia coli* that is involved in the efflux of hemolysin (HlyA) across the cell envelope [9,15]. In structures of full-length ABC transporters, the Q-loop is engaged in contacts with the MDs and the γ-phosphate of ATP and is thought to play a central role in the conformational coupling between the NBDs and MDs [16–21]. Residues in the D-loop in the *cis*-NBD make contact with residues in the *trans*-NBD (the aspartate of the *cis*-D-loop interacts with the *trans*-Walker A motif whereas the backbone of a *cis*-alanine upstream of the aspartate interacts with the *trans*-linchpin histidine), and *vice versa*, and are therefore suggested to play an important role in the communication between the two catalytic sites [22]. In ABC transporters a highly conserved glutamate at the C-terminus of the Walker B motif, which we will denote "Walker B glutamate" for clarity of discussion, is located immediately downstream of the conserved Mg^{2+}-coordinating aspartate in this motif. This Walker B glutamate is important for the proper positioning of the γ-phosphate moiety of the ATP and was proposed to play the central role in the ATP hydrolysis reaction by acting as a general base [23]; this hypothesis has recently been questioned (see Section 5 "Mutational studies in the nucleotide-binding domains").

2.2. Drug-stimulated ATPase activity

The stimulation of ATP hydrolysis by multiple drugs is widely used as an indicative tool to identify ABC multidrug transporters. A link between the ATP hydrolysis and drug binding by ABCB1, one of the best studied ABC exporter involved in the active efflux of chemotherapeutics