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Review

The ABC gene family in arthropods: Comparative genomics and role in insecticide transport and resistance☆

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ABSTRACT

About a 100 years ago, the *Drosophila white* mutant marked the birth of *Drosophila* genetics. The *white* gene turned out to encode the first well studied ABC transporter in arthropods. The ABC gene family is now recognized as one of the largest transporter families in all kingdoms of life. The majority of ABC proteins function as primary-active transporters that bind and hydrolyze ATP while transporting a large diversity of substrates across lipid membranes. Although extremely well studied in vertebrates for their role in drug resistance, less is known about the role of this family in the transport of endogenous and exogenous substances in arthropods. The ABC families of five insect species, a crustacean and a chelicerate have been annotated in some detail. We conducted a thorough phylogenetic analysis of the seven arthropod and human ABC protein subfamilies, to infer orthologous relationships that might suggest conserved function. Most orthologous relationships were found in the ABCB half transporter, ABCD, ABCE and ABCF subfamilies, but specific expansions within species and lineages are frequently observed and discussed. We next surveyed the role of ABC transporters in the transport of xenobiotics/plant allelochemicals and their involvement in insecticide resistance. The involvement of ABC transporters in xenobiotic resistance in arthropods is historically not well documented, but an increasing number of studies using unbiased differential gene expression analysis now points to their importance. We give an overview of methods that can be used to link ABC transporters to resistance. ABC proteins have also recently been implicated in the mode of action and resistance to Bt toxins in Lepidoptera. Given the enormous interest in Bt toxicology in transgenic crops, such findings will provide an impetus to further reveal the role of ABC transporters in arthropods.

1. Introduction

All cells and organelles are separated from the external milieu by lipid membranes and need transporters to traffic a wide diversity of compounds across these membranes (Higgins, 1992). The importance of membrane transport is illustrated by the fact that in *Escherichia coli* and humans about 10% and 4% of the total number of genes encode transport-related proteins (Blattner et al., 1997; Hediger et al., 2013). Among the latter, the ABC (ATP-binding cassette)[1] protein family is one of the largest transporter families and is present in all kingdoms of life. The majority of these ABC proteins function as primary-active transporters, requiring the binding and hydrolysis of ATP to transport substrates across lipid membranes. A functional ABC transporter, previously also referred to as traffic ATPase, consists of two cytosolic nucleotide-binding domains (NBDs) that bind and hydrolyze ATP, and two integral transmembrane domains (TMDs) (Fig. 1A). The NBD domain contains several conserved sequences like a Walker A and Walker B motif, Q-loop, H-motif and the ABC-signature motif (LSGGQ-motif). The transmembrane domains consist of 5–6 transmembrane helices and

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[1] ABC, ATP-binding cassette; NBD, nucleotide-binding domain; TMD, transmembrane domain; FT, full transporter; HT, half transporter; BBB, blood–brain barrier; MRPs, multidrug-resistance associated proteins; MDR, multidrug-resistance protein, P-gp, P-glycoprotein; SUR, sulfonylurea receptor; TMR, tetramethylrosamine; Trp, Tryptophan; ML, macrocyclic lactone; OP, organophosphates; BPU, benzoylphenylureas.

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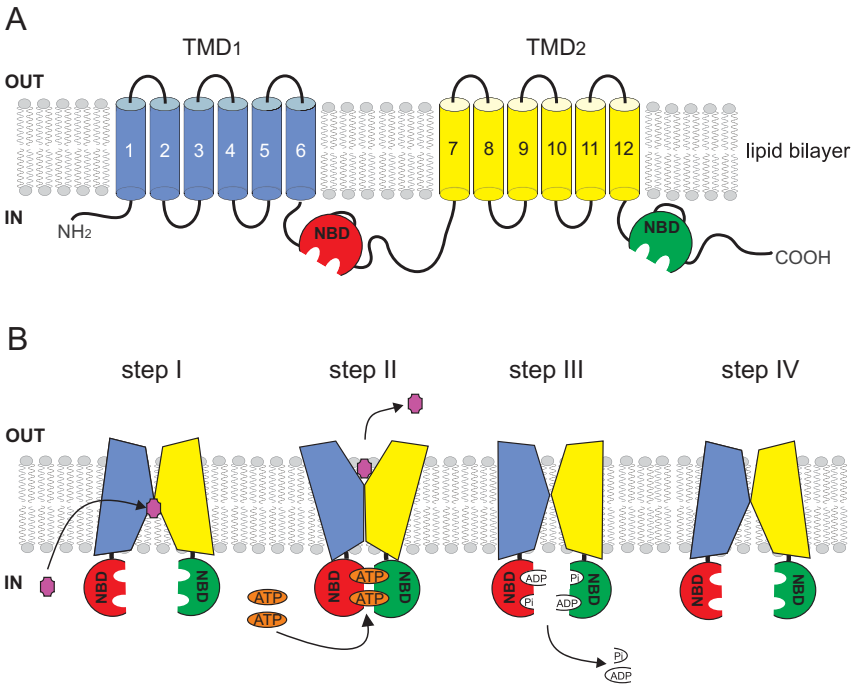

Fig. 1. ABC full transporter structure and the ATP-switch model for the transport cycle of an ABC transporter (exporter-type) A) Typical structure of an ABC full transporter containing two TMDs, TMD_1 (blue) and TMD_2 (yellow) each containing 6 transmembrane (TM) segments, and two NBDs, NBD_1 (red) and NBD_2 (green). In the case of "long" MRPs an additional TMD (TMD_0) is present at the N-terminus (Deeley et al., 2006). ABC half transporters have only one TMD and one NBD and need to homo or heterodimerize to form a functional unit. B) The ATP-switch mechanism (Higgins and Linton, 2004; Linton and Higgins, 2007). The transport cycle is started by binding of a substrate (purple cross) to a high-affinity pocket formed by the TMDs (blue and yellow pentagon). Subsequently, a conformational change is transmitted to the NBDs (green and red), facilitating ATP (orange oval) binding and closed NBD-dimer formation. The closed NBD dimer induces on its turn a major conformational change in the TMDs, with TMDs rotating and opening toward the outside, initiating substrate translocation (step II). ATP hydrolysis initiates dissolution of the closed NBD dimer, resulting in further conformational changes in the TMDs (step III). Finally, phosphate and ADP release restores the transporter to the open NBD-dimer conformation (step IV).

provide substrate specificity. The four domains of a functional transporter can be present in one protein (Full Transporter, FT) or spread over multiple proteins, for example one NBD and TMD per protein (half transporter, HT). In the case of the latter, ABC proteins need to homo or heterodimerize to form a functional ABC transporter (Higgins, 1992; Higgins and Linton, 2004; Rees et al., 2009). Based on the sequence similarity of the NBDs, the ABC protein family has been divided into eight subfamilies, denoted by the letters A–H (Fig. 2). Of particular note, the ABCH subfamily has at present only been identified in arthropod genomes and in zebrafish (Dean et al. 2001; Popovic et al., 2010). Eukaryotic ABC transporters mediate the efflux of compounds from the cytoplasm to the outside of the cell or into organelles, while bacterial ABC transporters can also facilitate import of substances. However, not all ABC proteins do play a role in transport. In the human ABC protein family, for example, ABC proteins acting as ion channel, receptor or with a role in translation, have been characterized (Dean et al., 2001; Rees et al., 2009).

Currently, the ATP-switch mechanism is the favored model for the transport cycle of ABC transporters (George and Jones, 2012; Higgins and Linton, 2004; Linton and Higgins, 2007). In this model, the transport cycle is started by binding of substrates to the TMDs. Subsequently a conformational change is transmitted to the NBDs, facilitating ATP binding and closed NBD-dimer formation. The closed NBD dimer in turn induces a major conformational change in the TMDs, with TMDs rotating and opening toward the outside, initiating substrate translocation. In a final step, ATP is hydrolyzed to initiate transition of the closed NBD dimer to the open NBD dimer and to return the transporter to its basal state (Fig. 1B). ABC-transporters traffic an array of substrates covering amino-acids, sugars, lipids, inorganic ions, polysaccharides, metals, peptides, toxic metabolites and drugs. Because of their ability to transport drugs, many human ABC members are also important players in multidrug resistance of cancer cells against chemotherapeutics: the multidrug-resistance proteins (MDRs) or P-glycoproteins (P-gps) belonging to the ABCB subfamily, the multidrug-resistance associated proteins (MRPs) from the ABCC subfamily and the breast cancer resistance protein (BCRP, ABCG subfamily member). The P-gp ABCB1 was in fact identified as the first ABC transporter to be overexpressed in multidrug resistant tumor cell lines (Kartner et al., 1985; Riordan et al., 1985). Compared to bacterial, nematode and human ABC transporters, the knowledge about arthropod ABC transporters is still limited. At present, the ABC families of seven arthropod species, spanning more than 400 arthropod ABC proteins, have been studied in detail (Broehan et al. 2013; Dean et al., 2001; Dermauw et al., 2013a; Liu et al., 2011; Roth et al., 2003; Sturm et al., 2009; Xie et al., 2012). Nevertheless, the precise function of only a few arthropod transporters has been unveiled. *Drosophila melanogaster* white and its insect orthologues are probably the most thoroughly characterized ABC transporters and are involved in the uptake of pigment precursors in the developing eye (Ewart and Howells, 1998).

We performed a thorough phylogenetic analysis with a dataset of well-annotated arthropod and human ABC transporters to infer orthologous relationships, which in turn can suggest the role and function of arthropod proteins. We further focus on biochemical and molecular methods that can be deployed to study arthropod ABC transporters, and give an overview of their documented role in xenobiotic transport and resistance.

2. Comparison of ABC subfamilies in arthropods

Despite the fact that more than 60 arthropod genomes have been sequenced, the ABC protein family was annotated and studied in detail in only seven species: the dipterans *D. melanogaster* and *Anopheles gambiae*, the honeybee *Apis mellifera*, the silk moth