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Review

Mutational analysis of ABC proteins

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Abstract

The 49 human members of the ATP-binding cassette (ABC) family of proteins are involved in a wide range of activities such as active transport of compounds across membranes, extraction of compounds out of membranes, functioning as ion channels, or regulators of channel activity. Mutations and/or overexpression of many of the proteins can have adverse effects on health. A goal in the study of ABC proteins is to understand their mechanisms of action. This review will focus on the mutational approaches that have been used to study the structure and mechanisms of some ABC proteins.

Keywords: ABC proteins; P-glycoprotein; CFTR; MRP1; BCRP; Mutational analysis; ATPase activity; Cysteine mutagenesis; Disulfide cross-linking; Arginine mutagenesis

The ABC (ATP-binding cassette) family of transporters is the largest class of membrane transport proteins. The 49 human ABC proteins have received much attention because they play important physiological roles or are implicated in diseases (reviewed in [1]). Although ABC transporters share a similar domain organization and structure, the various members of this family can transport a diverse range of substrates such as ions, amino acids, proteins, sugars, charged molecules, and hydrophobic molecules. Some members show specificity for one substrate whereas others can transport a wide range of hydrophobic compounds that have diverse structures.

ABC transporters play an important role in protecting us from harmful agents. Drug transporters such as P-glycoprotein (P-gp; ABCB1), multidrug resistance protein 1 (MRP1; ABCC1)[1] and breast cancer resistance protein (BCRP; ABCG2) protect us from toxins in the diet. Some ABC transporters prevent accumulation of excess cholesterol (ABCA1, ABCG5/ABCG8) or long chain fatty acids (ABCD1) in tissues of the body. The TAP (transporter associated with antigen processing) peptide transporter helps to protect us from viral infections. They transport viral peptides into the endoplasmic reticulum where they bind to the MHC class I complex and are displayed on the cell surface to initiate an immune response. The chloride channel activity of the cystic fibrosis transmembrane conductance regulator (CFTR) protein in the lung helps to protect from bacterial infections by maintaining hydration of the mucus layers lining the airways. The ABCA4 transporter protects the eye from accumulation of toxic pigment metabolic products. The SUR1 protein prevents the pancreas from releasing too much insulin into the bloodstream. Mutations and/or overexpression of at least 19 of the 49 human ABC transporters have been linked to diseases such as cystic fibrosis (CFTR), cancer (overexpression of drug transporters), congenital hyperinsulinism (SUR1), progressive familial intrahepatic cholestasis (ABCB4, ABCB11), anemia (ABCB7) Tangier disease (ABCA1), Stargardt disease (ABCA4), age-related macular degeneration (ABCA4), immune deficiency (TAP trans-

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[1] *Abbreviations used:* P-gp, P-glycoprotein; NBD, nucleotide-binding domain; NBD1, NH_2-terminal nucleotide-binding domain; NBD2, COOH-terminal nucleotide-binding domain; TMD, transmembrane domain; TMD1, NH_2-terminal transmembrane domain containing TM segments 1–6; TMD2, COOH-terminal transmembrane domain containing TM segments 7–12; TM, transmembrane.

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porter), Dubin–Johnson syndrome (MRP2), adrenoleukodystrophy (ABCD1) and sitosterolaemia (ABCG5/ABCG8).

While the human proteins have received much attention in recent years, identification of this class of transporters emerged from fundamental studies on the growth of bacteria. ABC transporters were discovered over 30 years ago in studies on bacterial nutrient uptake systems [2]. The *Escherichia coli* histidine transporter was the first ABC protein to be cloned and sequenced [3] and it soon became apparent that the histidine transporter was a member of a large class of transport proteins [4,5]. The proteins were named ABC transporters because of the high amino acid identity in the predicted ATP-binding sites [5]. While many of the bacterial transporters catalyze the import of nutrients into the cells, all of the human ABC transporters mediate export of compounds. Not all human ABC transporters however, act as transporters. For example, CFTR acts as a chloride channel and SUR1 (sulfonylurea receptor) acts as a potassium channel regulator. Some of the functions of ABC transporters are shown in Fig. 1. Knowledge about the structure, mechanism and biosynthesis of human ABC transporters would provide clues about how to treat the diseases involving these proteins.

The basic structure of an ABC transporter consists of two cytoplasmic nucleotide-binding domains (NBDs) and two transmembrane domains (TMDs). Each NBD contains conserved sequences for ATP-binding such as the Walker A motif (also called P-loop), Q-loop (thought to sense the γ-phosphate of ATP), LSGGQ signature sequence (also called C-loop), Walker B motif, D-loop, and H-loop (reviewed in [6]). Each TMD generally consists of six transmembrane (TM) segments joined by short extracellular loops and larger intracellular loops. The TMDs show little amino acid homology among the different types of ABC transporters.

The mechanism of transport by an ATP transporter is unknown. It is predicted that the cytoplasmic domains form the 'engines' of the ABC proteins with the TMDs forming the substrate-binding sites and/or substrate translocation pathway through the membrane. Binding and hydrolysis of ATP in the cytoplasmic domains likely induces long-range conformational changes in the TMDs to control opening and closing of the translocation pathway and/or expose bound substrates to the opposite side of the membrane with alterations in substrate-binding affinity.

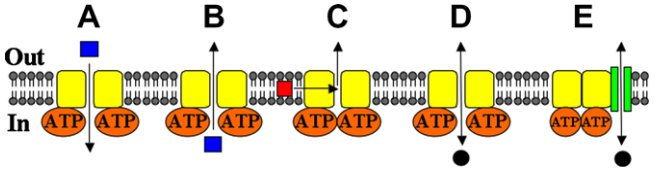

Fig. 1. Transport mediated by ABC transporters. Many prokaryotic ABC transporters catalyze the import of substrates into the cell (A) while most mammalian transporters catalyze export of substrates (B) or transport substrates out of the lipid bilayer (C). CFTR is different as it acts as an ion channel (D) whereas the SUR-type proteins act as channel regulators (E).

Mutational approaches have been used to study the structure and mechanism of ABC transporters. There are comprehensive reviews and books on ABC proteins that describe the results obtained with classical mutational approaches such as alanine-scanning mutagenesis [1]. The focus of this review will be to highlight some specific approaches that have been used to study human ABC proteins. The three approaches that will be highlighted are: (1) study of deletion mutants; (2) cysteine mutagenesis and modification with thiol-reactive compounds; and (3) arginine mutagenesis.

Classification of human ABC proteins

The human ABC proteins are often easier to study by mutagenesis than bacterial ABC transporters because the four core domains (two NBDs and two TMDs) are contained within a single polypeptide. In contrast, all bacterial ABC transporters are composed of multiple polypeptides. In human ABC transporters, there can be considerable variability in how the four core domains are organized and some have additional domains. To aid in the study of the 49 human ABC proteins, they have been divided into subfamilies depending on the order of the domains, sequence homology in the NBDs and TMDs and the presence of insertions or deletions to the protein [7,8]. Fig. 2 shows the organization of the various domains and the number of human members in each subfamily. The half-molecule forms of the transporters are predicted to form homo- or heterodimers to yield a functional complex.

A first step in understanding the mechanism of human ABC transporters was to determine the minimum functional unit that was required for activity. The genes of some transporters such as BCRP only encode a half-molecule form of an ABC transporter. In other cases, the proteins contain additional domains such as the R domain in CFTR or the extra 5 TM segments at the beginning of some MRP or SUR-type transporters. Therefore, deletion mutants were generated to study whether half-molecule forms of ABC proteins were functional, whether the extra domains were essential for activity, and whether removal of one ATP-binding site reduced activity by 50%.

Analysis of deletion mutants

The multidrug resistance P-gp was the first mammalian ABC transporter to be cloned and sequenced [9–11]. The protein had been discovered during efforts to understand how cancer cells developed resistance to chemotherapeutic drugs. In the early 1970s it was observed that a cancer cell line showed drug resistance due to the active outward transport of chemotherapeutic agents [12]. It was later shown that cancer cell lines selected for resistance to chemotherapeutic agents such as vinblastine or doxorubicin also showed cross-resistance to structurally different anti-