Minireview

Membrane topology distinguishes a subfamily of the ATP-binding cassette (ABC) transporters

Gábor E. Tusnády[a], Éva Bakos[a], András Váradi[a], Balázs Sarkadi[b,*]

[a] *Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, H-1113 Budapest, Hungary*
[b] *National Institute of Haematology and Immunology, and Membrane Research Group of the Hungarian Academy of Sciences, Daróczi u. 24, H-1113 Budapest, Hungary*

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Abstract A group of ATP-binding cassette (ABC) transporters, including the yeast cadmium transporter (YCF1), the mammalian multidrug resistance-associated protein (MRP), the multispecific organic anion transporter and its congener (MOAT and EBCR), as well as the sulfonylurea receptor (SUR), group into a subfamily by sequence comparison. We suggest that these MRP-related proteins are also characterized by a special, common membrane topology pattern. The most studied ABC transporters, the cystic fibrosis transmembrane conductance regulator (CFTR) and the multidrug resistance (MDR) proteins, were shown to contain a tandem repeat of six transmembrane helices, each set followed by an ATP-binding domain. According to the present study, in contrast to various membrane topology predictions proposed for the different MRP-related proteins, they all seem to have a CFTR/MDR-like core structure, and an additional, large, N-terminal hydrophobic region. This latter domain is predicted to contain 4–6 (most probably 5) transmembrane helices, and is occasionally glycosylated on the cell surface. Since all the MRP-related transporters were shown to interact with anionic compounds, the N-terminal membrane-bound domain may have a key role in these interactions.

Key words: Membrane topology; ABC transporter; MRP; SUR; YCF1; cMOAT

1. Introduction – the ABC transporters

The so-called ATP-binding cassette (ABC) transporters, present from bacteria to man, are involved in the ATP-dependent transport of a great variety of compounds, ranging from inorganic ions to large polypeptides. In most cases these proteins were demonstrated to function as transport ATPases, that is hydrolyzing ATP in conjunction with transporting their substrate molecules through cellular or intracellular membranes [1–3]. The ABC transporters, forming one of the largest known protein family, are built from combinations of conservative domains, that is ATP- (nucleotide-) binding ABC units, and characteristic membrane-bound regions.

The ABC units harbor the highly conserved 'Walker A' and 'Walker B' sequences, most probably directly responsible for the interaction with ATP or other nucleotides [4]. These Walker sequences are separated by a stretch of about 120–170 amino acids, including a short (12–13 amino acid) peptide motif, called the ABC transporter 'signature' region, which is in fact diagnostic for these proteins [5,6]. The membrane-bound domains of the ABC transporters in most cases were predicted to contain six transmembrane (TM) helices, and in some bacterial and in all eukaryotic ABC transporters at least one ABC unit and one transmembrane domain are encoded by a single gene [1–3,6].

Characteristic eukaryotic ABC transporters are the yeast pheromone transporter, STE6, the mammalian multidrug resistance (MDR) proteins, producing a drug resistance phenotype in cancer cells, and the human cystic fibrosis transmembrane conductance regulator (CFTR), the mutations of which are causative in the lethal disease of cystic fibrosis. These proteins were all suggested to consist of a tandem repeat of six TM helices, each set of helices followed by an ABC unit. Such a basic structure and membrane topology with 6+6 TM helices in CFTR and MDR has already been experimentally confirmed [7–9]. There are several examples of the expression of 'half-MDR'-like proteins, which contain only one transmembrane region (with probably six TM helices) and one ABC unit. Still, e.g. the mammalian peptide transporter proteins of this type, involved in the MHCI-dependent antigen presentation process (TAP1 and TAP2), have already been shown to form functional heterodimers in the membrane environment [10].

2. The MRP family

In 1992, Cole at al. [11] reported the molecular cloning and characterization of the human multidrug resistance-associated protein (MRP), a large (190 kDa) membrane glycoprotein, which belongs to the group of ABC transporters and causes a multidrug resistance phenotype in cancer cells. MRP probably transports both hydrophobic anticancer agents and anionic (e.g. glutathione) drug conjugates, and its physiological functioning may provide a wide range of xenobiotic resistance [12–15]. During the past few years numerous homologs of MRP have been identified. These include the yeast cadmium factor (YCF1), which transports metallothionein-conjugated metal ions [16] and glutathione conjugates [17], and the mammalian sulfonylurea receptors (SUR1 and SUR2), which are glycoproteins present in pancreatic β cells and in cardiac and skeletal muscle, respectively, and are involved in the regulation of ATP-sensitive K^+ channels [18,19]. In 1996, the discovery of the MRP-related liver canalicular multispecific organic anion transporter (cMOAT) [20,21], exporting conjugated bile salts, and its close homolog, the rabbit epithelial basolateral chloride conductance regulator (EBCR), indicated to regulate specific chloride channels [22], was reported.

According to the original sequence analysis [11], MRP was predicted to contain 8+4 TM helices, each set of helices fol-

*Corresponding author. Fax: (36) (1) 185-2234.

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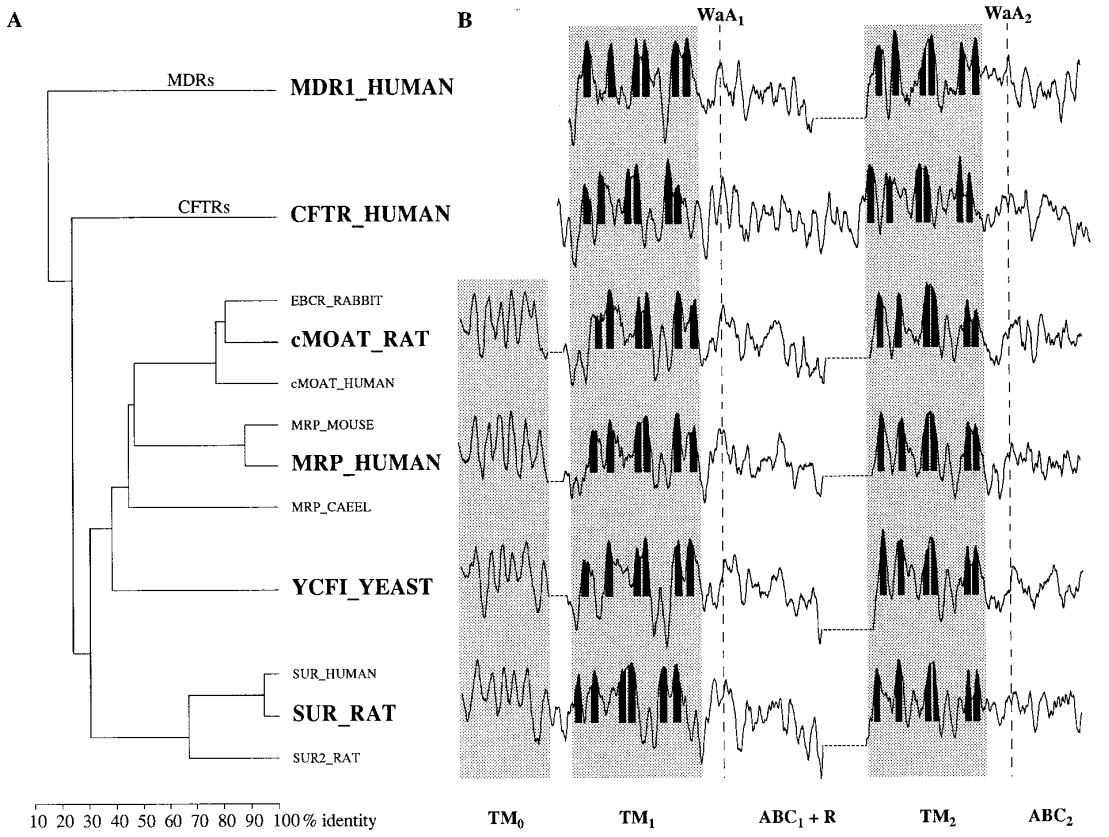

Fig. 1. Relative similarity dendrogram (A) and aligned hydrophobicity plots (B) of selected ABC transporter proteins. A: The relative similarity dendrogram was constructed using the PILEUP program. For the sake of better visualization MDR and CFTR sequences are represented as single branches on the tree. B: Hydrophobicity plots were generated according to von Heijne [25]. The plots are aligned in a way that the highly conserved Walker A consensus sequence motifs in the N-terminal (WaA_1) and in the C-terminal (WaA_2) ABC units are in register. The shaded areas (TM_0, TM_1 and TM_2) indicate hydrophobic, membrane-bound regions; black areas show transmembrane helices with locations experimentally confirmed in human CFTR and human MDR1. The membrane-bound domains, the two conserved ATP-binding cassette (ABC_1 and ABC_2) regions, and the regulatory (R) domain of CFTR, are indicated. Gaps are shown as horizontal dashed lines. The GenBank (EMBL) accession numbers for the proteins used in this comparison are the following: M14758 (MDR1_HUMAN); M28668 (CFTR_HUMAN); Z49144 (EBCR_RABBIT); L49379 (cMOAT_RAT); X96395 (cMOAT_HUMAN); 1488428 (MRP_MOUSE); L05628 (MRP_HUMAN); U66261 (MRP_CAEEL); L35237 (YCF1_YEAST); L78207 (SUR_HUMAN); L40624 (SUR_RAT); D83598 (SUR2_RAT). *Note:* In the same kind of search using the BLAST program [27] in the non-redundant combination of databases provided by NIH, we have also found several other expressed or probable protein sequences with as yet unknown functions, resulting from genome sequencing projects, which may belong to this MRP-like subfamily. These include both nematode (Genbank accession number: Z68113, Z81016 and U41554) and yeast (X97560, Z73153, Z69369, U41554, and Z 28328+Z 28329) sequences.

lowed by an ABC unit, and possible glycosylation sites could be found on the extracellular loops between helices 3–4, 9–10, and 11–12. Interestingly, in the original sequence analyses, YCF1 was predicted to contain 6+6, SUR 9+4, and cMOAT and EBCR 8+4 TM helices, again each set of helices followed by an ABC unit.

3. Membrane topology of MRP

Recently, based on antibody binding and limited proteolysis experiments [23], we have demonstrated that the human MRP contains three major membrane-bound domains, separated by two cytoplasmic loops. The first and last membrane-bound domains were found to be glycosylated when MRP was expressed in mammalian cells. These data and the hydrophobicity analyses of the aligned sequences of MRP and its close relative, the human CFTR, suggested a different membrane topology model than originally predicted by Cole et al. [11]. We proposed that MRP contains an N-terminal hydrophobic, membrane-bound, glycosylated domain (with 4–5 TM helices), which is fused with a CFTR-like region, containing the tandem repeat of the conservative six TM helices, each set of helices followed by an ABC unit. A recent communication by Cole et al. [24] suggests a similar membrane topology pattern both for the human and the mouse MRP. These results raised the question if a similar membrane topology pattern may be present in the other MRP-related proteins as well.

4. Similarity dendrogram and hydrophobicity plots of MRP-related proteins

In order to identify all probable MRP-related proteins, we have estimated the evolutionary relationships by sequence comparisons within the ABC transporter family. A relative similarity dendrogram (Fig. 1A) has been constructed using the PILEUP program and a non-redundant combination of databases provided by NIH, considering the amino acid sequences of all ABC transporters which have at least one ABC unit and one transmembrane domain encoded within the same gene. This analysis indicated that the MDR and CFTR sequences from numerous species group into separate subfamilies, while clustering around the MRP we have found a

recognizable subfamily, containing all the expected MRP-related proteins. As indicated in the legend to Fig. 1, several other uncharacterized protein sequences also grouped into this subfamily.

In the following we have generated hydrophobicity plots from the sequences of all the analyzed ABC transporter proteins, according to the method of von Heijne [25]. These plots were then aligned in a way that the highly conserved Walker A consensus sequence motifs in the N-terminal (WaA_1) and in the C-terminal (WaA_2) ABC units were in register. In Fig. 1B we present the aligned hydrophobicity plots of some of the key proteins (printed bold) from each group.

As emphasized by the shaded area in Fig. 1, the basic 6 TM helices–ABC unit–6 TM helices–ABC unit structure can be recognized in each protein presented. However, in addition to this basic pattern, the hydrophobicity plots in the MRP-related subfamily indicate that all these proteins contain an N-terminally located, large, hydrophobic, most probably membrane-bound domain, with 4–6 helices (labeled here TM_0), not present in MDRs or CFTRs. These data suggest that the previous membrane topology predictions for MRP, cMOATs and EBCR (8+4 helices), YCF1 (6+6 helices), or SURs (9+4 TM helices), yielded variable numbers because of including and/or missing some TM helices both from the CFTR/MDR-like core structure and the N-terminal hydrophobic domain.

From the data currently available, the actual number of TM helices in the N-terminal membrane-bound domain of the MRP-related proteins is hard to predict. Although the sequence analysis of the TM_0 domains shows a relatively low level of similarity (11–25%), due to the exchangeability of the hydrophobic amino acids in the TM regions, the membrane insertion and folding patterns are most probably better conserved than the actual amino acid sequences. In the case of SUR1, a possible *N*-glycosylation site in position 10 was shown to be indeed glycosylated, placing the N-terminal region of this protein to the external surface of the cell membrane [18]. Also, all the mammalian proteins in this group contain one or two possible *N*-glycosylation sites within the first 25 residues. These observations suggest that the N-terminal regions of the mammalian MRP-related proteins are extracellularly located, and the TM_0 region may contain 5 TM helices.

5. Conclusions

The data presented here indicate that by a proper alignment of the protein sequences of the ABC transporters, a characteristic modular membrane topology arrangement becomes apparent for the MRP-related subfamily. These proteins seem to contain a CFTR/MDR-like domain arrangement, and an additional, N-terminal hydrophobic region, with a probably conserved membrane insertion pattern, but with a low level of sequence similarity. When this membrane topology pattern was visually compared to that of other ABC transporters, we observed that some 'half-MDR'-like proteins contain a similar, relatively large N-terminal hydrophobic region. This was especially apparent in the case of the yeast metal resistance ABC transporter protein, HMT1, transporting anionic metal complexes [26]. Since all the MRP-related proteins interact with and/or transport large organic anions, a common functional role of the N-terminal membrane-spanning region may involve such an interaction.

We suggest that in the case of membrane proteins, in addition to sequence comparisons, topology pattern analysis may significantly help the search for characteristic domains. Mechanistic application of the widely available computerized membrane topology prediction programs may be misleading, while alignments according to strongly conserved domains and considering experimentally confirmed structures may significantly improve such predictions.

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