Structural and functional diversity calls for a new classification of ABC transporters

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Abbreviations

ABC, ATP-binding cassette; cryo-EM, cryogenic electron microscopy; NBD, nucleotide-binding domain; TMD, transmembrane domain.
Members of the ATP-binding cassette (ABC) transporter superfamily translocate a broad spectrum of chemically diverse substrates. While their eponymous ATP-binding cassette in the nucleotide-binding domains (NBDs) is highly conserved, their transmembrane domains (TMDs) forming the translocation pathway exhibit distinct folds and topologies, suggesting that during evolution the ancient motor domains were combined with different transmembrane mechanical systems to orchestrate a variety of cellular processes. In recent years, it has become increasingly evident that the distinct TMD folds are best suited to categorize the multitude of ABC transporters. We therefore propose a new ABC transporter classification that is based on structural homology in the TMDs.

Keywords: ABC transporters; ATPases; cryo-EM; membrane proteins; molecular machines; phylogeny; primary active transporters; sequence alignment; structural biology; X-ray crystallography

We suggest a new classification of the ABC transporter superfamily that is based on the TMD fold. Historically, first hints of the ABC protein superfamily came from sequence alignments of bacterial proteins that revealed highly conserved motifs in their ATPase domains [1]. The superfamily of ABC proteins was subsequently divided into three main classes [2–4]: exporters, nontransporter ABC proteins, and a third class consisting primarily of importers. The mammalian ABC systems, in particular, were grouped into seven subfamilies (ABCA to ABCG), based on NBD and TMD sequence homology, gene structure, and domain order [5–7]. It should be noted that ABCE and ABCF are not transporters, but exist as twin-NBDs without TMDs and are involved in mRNA translation control [8]. Detailed membrane topology and sequence analyses of exporters uncovered that, in contrast to the NBDs, the TMDs are polyphyletic and can serve as references to categorize ABC transporters into three distinct types (ABC1-3) [9,10]. According to this classification, the cystic fibrosis transmembrane conductance regulator (CFTR), the transporter associated with antigen processing (TAP), and the drug efflux pump P-glycoprotein (P-gp) belong to the ABC1 transporters; ABCG2 and ABCG5/G8 are members of the ABC2 group, which also comprises importers; and the macrolide translocator MacB is categorized as an ABC3 system. Yet, another classification scheme currently in use differentiates between the three types of importers predominantly found in prokaryotes [11–14] and two types of exporters, exemplified by Sav1866 [15] and ABCG5/8 [16], in addition to the LptB2FG-type [17,18] and MacB-type [19–22] transporters.

Our motivation for proposing a revised nomenclature stems from the recent wealth of ABC transporter structures determined by X-ray crystallography and single-particle cryo-electron microscopy, which has unveiled a remarkable diversity of TMD folds and evolutionary relationships between bacterial and eukaryotic/mammalian transporters [16–21,23–26]. This affluence of structural information provides the opportunity to introduce a universal nomenclature that
combines previous phylogenetic analyses with the new findings coming from high-resolution structures. The nomenclature groups ABC transporters into distinct types, I–VII, based on their TMD fold (Fig. 1, Tables 1 and 2). This classification is supported by quantitative analyses using TM-scores based on pairwise structural alignment of TMDs (Tables S1–S6, Fig. S1). The classification focuses on the transporter-forming TMDs and does not consider additional membrane-integrated domains, as for example observed in TAP1/TAP2 [27,28].

As before, types I–III of the new nomenclature cover the three different importer architectures (Fig. 1, Table 1, Tables S2 and S3; TM-score for pairwise structural alignment between the type III systems CbiQ (PDB code 5X3X) and EcT from Lactobacillus brevis (PDB code 4HUQ): 0.736). It is noteworthy that prokaryotic importers typically operate with periplasmic, extracellular, or membrane-embedded substrate-binding proteins whose structural features correlate with the type of TMD fold [29].

Based on the characteristic structure of the founding member Sav1866, which includes a domain-swapped TMD arrangement, type IV members of the new nomenclature have previously been classified as type I ABC exporters [15]. However, a significant and growing number of these ABC proteins have nonexporter functions, i.e., the gated chloride channel CFTR, the regulatory KATP channel modules SUR1/2, the lysosomal cobalamin (vitamin B12) transporter ABCD4 [30], the bacterial siderophile importers YbtPQ and IrtAB, and the cobalamin/antimicrobial peptide importer Rv1819c [31–33], as well as several type IV systems with importer functions in plants [34–39]. This striking functional diversity mediated by the same structural framework (Fig. 1, Tables 1 and 2, Tables S4 and S5) makes the type IV ABC transporters stand out and is also the main reason why we suggest the more universal architecture can be universally applied to ABC transporters beyond their particular physiological functions and across the three domains of life. Hence, it allows any newly discovered transporter fold to be compared with the existing types and seamlessly incorporated into the classification scheme, possibly as a new type. Since the new nomenclature depends on TMD architecture, it requires structural information in order to classify new transporter systems. At the same time, we regard the nomenclature as a dynamic platform that can be upgraded, adjusted, or refined whenever necessary due to novel insights that add extra dimensions to our understanding of ABC systems.

The recent advances in structural mapping of the diverse superfamily of ABC transporters have revealed a vast area of mechanistically uncharted territory. One key objective of future research should be to fully comprehend how type IV systems perform so many different functions, i.e., as importer, exporter, lipid floppase, ion channel, and regulator, by employing a single structural scaffold. However, we do not exclude that other types might turn out to be as functionally diverse as type IV systems. Exploring the different modes of operation and accompanying conformational landscapes [49] and the dynamics of the multifarious ABC systems will require integrative experimental methods.
Table 1. Prokaryotic ABC transporters classified according to their TMD folds.

| TMD fold | TM helix organization | Experimentally determined structures | PDB codes* | Function |
|----------|-----------------------|--------------------------------------|------------|----------|
| Type I   | (5-6) + (5-6)/8<sup>b</sup> | MalFGK<sub>2</sub>-MalE: 2R6G, 3FH6, 3PUV, 3PUX, 3RLF, 4JBW; Mod<sub>B</sub>C<sub>2</sub>-ModB2C<sub>2</sub>-A: 2ONK, 3D31; MetNi-Q: 3DHW, 3TUJ; Art(QN): 4YMS, 4YMU, 4YMV, 4YMW | 2R6G, 3FH6, 3PUV, 3PUX, 3RLF, 4JBW | Maltose import |
|          |                       | Art(QN)2: 4YMS, 4YMU, 4YMV, 4YMW | 2ONK, 3D31 | Molybdate import |
|          |                       | AlgM1M2SS-Q2: 4TQU | 3DHW, 3TUJ | Methionine import |
|          |                       | BtuC<sub>D</sub>-BtuC: 4FI3, 4R9U | 4YMS, 4YMU, 4YMV | Amino acid import |
| Type II  | 10 + 10               | MoIBC: 1L7V, 2Q19, 4DBL, 4FI3, 4R9U | 5B57, 5B58 | Cobalamin import |
|          |                       | HmuUV: 4G1U | 2Q19, 4DBL, 4FI3 | Import of molybdate and tungstate |
|          |                       | BhuUvi(T): 5B57, 5B58 | 4G1U | Heme import |
| Type III | 4-8 (T) + 6-7 (S)     | EcfTAA'-FolT: 4HUQ, 5D3M, 5JSZ | 4H2U | Folate import |
|          |                       | EcTAA'-PdxU2: 4H2U | 4H2U | Pyridoxine import |
|          |                       | LeECF-PanT: 4RF5 | 4HUQ | Pantothenate import |
|          |                       | CbiMQO: 5X3X, 5X41 | 4H2U | Co<sup>2+</sup> import |
|          |                       | ECF-CbrT: 6FNS | 4H2U | Cobalamin import |
| Type IV  | 6 + 6                 | Sav1866: 2HYD, 2ONJ | 4MRM, 4MRV, 4MRN, 4MRP | Multidrug export |
|          | Homodimer             | MsbA: 3B60, 3BSY, 3BSZ, 5TV4, 6BPL, 6BPP, 6BL6, 6O3O, 6UZZ, 6U2L | 4MRM, 4MRV, 4MRN, 4MRP | Lipid A/LPS flopping |
|          | Heterodimer           | NaAtm1: 4Q4A, 4Q4H, 4Q4J, 6QU0, 6O0V, 6O0V, 6QV1, 6QQ2 | 4MRM, 4MRV, 4MRN, 4MRP | Export of GSH, GSH-related compounds, and metal-GSH complexes |
|          | Single chain          | TM287/288: 4Q4A, 4Q4H, 4Q4J, 6QU0, 6O0V, 6O0V, 6QV1, 6QQ2 | Daunorubicin export |
|          |                       | MocD: 4PL0, 5EG1, 5OFR | 4PL0, 5EG1, 5OFR | Antimicrobial peptide export |
|          |                       | PCAT1: 4RY2, 6V9Z | 4PL0, 5EG1, 5OFR | Peptide export |
|          |                       | PgiK: 5C76, 5C78, 5NBD, 6HRC | 4PL0, 5EG1, 5OFR | Polypeptide export |
|          |                       | TmrAB: 5MKK, 6RAF, 6RAJ, 6RAI, 6RAJ, 6RAI, 6RAJ, 6RAI, 6RAJ, 6RAI, 6RAI, 6RAI, 6RAI | 5MKK, 6RAF, 6RAJ, 6RAI, 6RAJ, 6RAI, 6RAI, 6RAI, 6RAI, 6RAI, 6RAI, 6RAI, 6RAI, 6RAI | Peptide export |
|          |                       | PrtD: 5L22 | 5L22 | Metal–siderophore export |
|          |                       | YtbPQ: 6P6I, 6P6J | 5L22 | Import of cobalamin and bleomycin |
|          |                       | Rv1819c: 6TQE, 6TQF | 6TQE, 6TQF | Iron–siderophore import |
|          |                       | IrAB: 6TEJ | 6TQE, 6TQF | Iron–siderophore import |

*PDB codes: MalFGK<sub>2</sub>-MalE: 2R6G [12]; BtuC<sub>D</sub>-BtuF: 4FI3 [50]; EcTAA'-FolT: 4HUQ [14]; Sav1866: 2HYD [15]; TmrAB: 5MKK [51]; TM287/288: 4Q4H [52]; MocD: 4PL0 [53]; PCAT1: 6V9Z [54]; Atm1: 4MYH [55]; MRP1: 5UJA [56]; PrtD: 5L22 [57]; P-gp: 4M1M [58]; TAP1/2: 5U1D [59]; ABCB4: 6S7P [60]; ABCB8: 5OCH; ABCB10: 3ZDO [61]; ABCB11: 6LR0 [62]; MsbA: 5TV4 [63]; PgiK: 6HRC [64]; YtbPQ: 6P6J [31]; IrAB: 6TEJ [32]; Rv1819c: 6TQE [33]; ABCG5/8: 5DO7 [16]; ABCBC1: 5XJY [23]; LptB-FG: 5X5Y [17]; MacB: 5LJ7 [21].
approaches that include electron paramagnetic resonance (EPR), nuclear magnetic resonance (NMR), single-molecule techniques, and single-turnover experiments. We are confident that future studies of such kind will provide major new insights into the mechanisms of these fascinating molecular machines.
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Author contributions

CT and RT wrote the manuscript with contributions from all coauthors. This review is the quintessence of a resumed discussion that started at the FEBS Advanced Lecture Course on the Biochemistry of Membrane Proteins in Budapest (2019) and continued at the FEBS Conference on ATP-Binding Cassette (ABC) Proteins in Innsbruck (2020). The discussion included a vivid exchange of thoughts via hundreds of emails and remote video sessions during the global COVID-19 pandemic. In addition to the authors listed, we received positive feedbacks on our proposed classification from several further leading scientists in the ABC transporter field. Yet, as they felt that their contribution was too small, they decided not to accept authorship.

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Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Phylogenetic tree based on TM-scores of structural TMD alignments.

Table S1. TM-scores based on pairwise structural alignment of representatives of the different TMD types.

Table S2. TM-scores based on pairwise structural alignment of type I TMDs.

Table S3. TM-scores based on pairwise structural alignment of type II TMDs.

Table S4. TM-scores based on pairwise structural alignment of type IV TMDs in inward-facing conformations.

Table S5. TM-scores based on pairwise structural alignment of type IV TMDs in (semi-) occluded/ outward-facing conformations.

Table S6. TM-scores based on pairwise structural alignment of type V, VI, and VII TMDs.

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