T3SS cytoplasmic chamber preserves its secrets. But, for how long?

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Transporters Revealed

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The subfamily C ATP-binding cassette (ABCC) transporters mediate multidrug resistance and ion conductance regulation. Recent atomic or near-atomic resolution structures of three physiologically significant ABCC transporters (MRP1, SUR1, and CFTR), determined by using single-particle cryo-electron microscopy (cryo-EM), reveal structural details that help explain the wide functional diversity of this ABC transporter subfamily.

ATP-binding cassette (ABC) transporters are physiologically important membrane proteins that mediate ATP-driven transport of diverse substrates across cellular membranes. ABC transporters exist in all phyla, including 48 human proteins classified into seven subfamilies (A–G) (Dean et al., 2001). Subfamily C (ABCC) transporters are associated with multidrug resistance or hormone and metabolite secretion (ABCC1, ABCC2, ABCC3), ectopic mineralization (ABCC6), and ion conductance regulation (ABCC7, ABCC8, ABCC9). Analysis of the primary sequences of ABCC transporters predicts a conserved domain organization and topology; however, it's not clear how the functional diversity of this protein family arises. Recent breakthroughs have provided high-resolution models of three ABCC transporters—the multidrug resistance protein 1 (MRP1 or ABCC1) (Johnson and Chen, 2017 [this issue of Cell]), the sulfonylurea receptor 1 (SUR1 or ABCC8) of the K_ATP channel complex (Li et al., 2017; Martin et al., 2017), and the cystic fibrosis transmembrane conductance regulator (CFTR or ABCC7) (Zhang and Chen, 2016). These structures represent a leap forward in understanding the molecular basis of ABCC transporter function.

Over the past two decades, atomic models of ABC transporters were primarily obtained by X-ray crystallography and were limited to bacterial importers or B-type exporters (human ABCB homologs) (Locher, 2016). Progress on other ABC subfamilies has been hindered by the challenge of obtaining diffracting protein crystals, particularly for eukaryotic proteins; as an example, the first ABCG transporter crystal structure (human ABCG5/ABCG8) was only recently solved (Lee et al., 2016). In this issue of Cell, Johnson and Chen use cryo-electron microscopy (cryo-EM) to determine the structure of MRP1, which transports endogenous small molecules such as leukotrienes and is a major mediator of drug resistance. This data represents the first ABCC atomic model and the second multidrug resistance ABC transporter to be structurally characterized at high resolution, achieved by a technical tour de force in applying single-particle cryo-EM to a membrane protein as small as 190 kD.

Not surprisingly, MRP1 shares structural features with SUR1 and CFTR. Both MRP1 and SUR1 include two nucleotide-binding domains (NBDs) and three transmembrane domains (TMDs) that alternate from N to C termini, with a cytoplasmic L0 polypeptide linker connecting TMD0 and TMD1. CTFR is missing TMD0, but
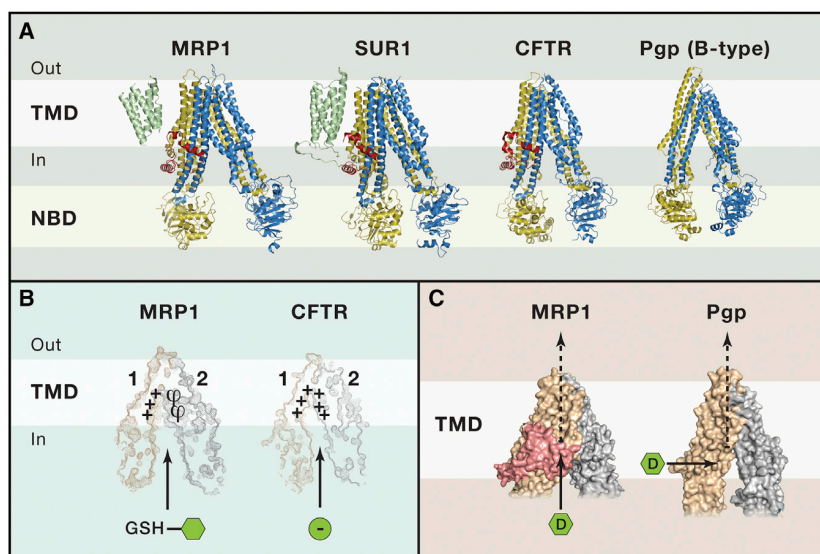
Figure 1. Structural Features and Putative Substrate Translocation Mechanisms of ABCC Transporters

(A) The structures of MRP1 (5UJ9), SUR1 (5WUA), and CFTR (5UAR) share a similar fold in the transmembrane domains (TMDs) to B-type exporter Pgp (3G5U). Light green, TMD0; red, L0 (lasso motif); yellow, TMD1 and NBD1; blue, TMD2 and NBD2.

(B) MRP1 shows a bipartite surface along the substrate translocation pathway to accommodate large, amphipathic compounds (e.g., GSH-conjugated leukotriene LTC_4, green hexagon). In CFTR, a positively charged translocation pathway may facilitate permeation of negatively charged chloride ions (green negative circle). 1, TM helical bundle 1 (TM6, TM7, TM8, TM11, TM15, TM16 in MRP1; TM1, TM2, TM3, TM6, TM10, TM11 in CFTR). 2, TM helical bundle 2 (TM9, TM10, TM12, TM13, TM14, TM17 in MRP1; TM4, TM5, TM7, TM8, TM9, TM12 in CFTR).

(C) MRP1 effluxes drugs by directly binding substrates from the cytoplasm, which is different from ABCB transporter Pgp, which can uptake drugs from bilayer membranes.

contains a regulatory (R) domain between NBD1 and TMD2. The core transmembrane domains (TMD1 and TMD12) common to all three cryo-EM structures adopt the same structural fold as those in the B-type exporters (Figure 1A), while a vestibule between the two TM bundles opens to the cytoplasm and penetrates halfway into the lipid bilayer (Figure 1B). The TMD0 bundle in MRP1 and SUR1 contains five transmembrane α helices, and while the role of TMD0 is unknown in MRP1, the SUR1 TMD0 engages in a direct protein-protein interaction with the inward rectifier potassium channel Kir6.2, forming the K_{ATP} potassium channel complex (Li et al., 2017; Martin et al., 2017). In all cases, the L0 linker (also called the lasso in CFTR) forms an extended structural motif between the membrane and the cytosol and packs against three transmembrane helices (TM2, TM10, and TM11 in CFTR; TM7, TM15, and TM16 in MRP1 or SUR1). The importance of this linker is underscored

by the presence of several cystic fibrosis mutations in this region (Zhang and Chen, 2016), its essentiality in MRP1 folding and function (Johnson and Chen, 2017), and its interaction with channel inhibitors of K_{ATP} (Li et al., 2017).

How do the distinct features of MRP1's structure help explain its unique functions? In the current study, Johnson and Chen describe distinct surface features along the substrate translocation pathway embedded between the two TM α-helical bundles (Figure 1B). MRP1 has the unusual property of binding both organic acids and hydrophobic substrates. The bipartite substrate-binding cavity at the inner leaflet contains a positively charged P-pocket (to help bind negatively charged acids) and a hydrophobic H-pocket, which permits recognition of GSH-containing hydrophobic compounds, as shown in the leukotriene C_4 (LTC_4)-bound structure (Johnson and Chen, 2017). CFTR, by contrast, forms a funnel-shaped central pathway with multiple positive

charges, which allows permeation of negatively charged chloride ions (Zhang and Chen, 2016). In line with this observation, the potassium channel complexes reveal an inward opening in the helical bundles of TMD1 and TMD2 of SUR1, although the current resolution (5–6 Å) does not allow for reliable positioning of surface charges (Li et al., 2017 and Martin et al., 2017). SUR1 is known to function as a receptor for sulfonylureas or K_{ATP} inhibitory drugs. Whether the TMD cavity plays a role in this function is yet to be determined. Nevertheless, differences in surface properties along the translocation pathway help to explain the diversity of substrates transported by ABCC family members.

Multidrug resistance, which often underlies the failure of chemotherapy in cancer patients, can be mediated by ABC transporters such as P-glycoprotein (Pgp, also known as MDR1 or ABCB1), MRP1, or ABCG2 (also known as breast cancer resistance protein [BCRP]) (Leslie et al., 2005). Utilizing energy from ATP hydrolysis by the cytoplasmic NBDs, these transporters engage in efflux of multiple classes of anticancer drugs from cancer cells through the substrate translocation TMDs. Previously, structural studies of B-type drug exporters have indicated that substrate polyspecificity may be mediated through a flexible and hydrophobic translocation pathway (Aller et al., 2009), and that such transporters change from inward- to outward-facing TMD conformations after nucleotide binding at the NBDs (Locher, 2016). Johnson and Chen expand on these findings by comparing the apo- and LTC_4-bound MRP1 structures (Johnson and Chen, 2017). The bipartite substrate-binding site is distinct from the largely hydrophobic cavity in Pgp, helping to explain why MRP1 transports large and amphipathic substrates, whereas Pgp mainly transports hydrophobic drugs. In addition, unlike Pgp, where the translocation pathway opens to the inner leaflet of the membranes, the translocation pathway in MRP1 is shielded from both leaflets (Figure 1C). Hence, while Pgp can bind substrates from within the membrane, MRP1 likely takes up transport substrates directly from the cytoplasm.

While it is known that substrate binding is required for ATP hydrolysis in MRP1,

the new analysis provides insight into how that happens, revealing that upon LTC$_4$ binding both TM bundles move inward (particularly around the substrate-binding site), bringing the separation of the two NBDs to a distance that is believed to prime the protein for ATP hydrolysis, similar to what is seen for bacterial maltose transporter (Oldham and Chen, 2011).

The recent structures of ABCC transporters have provided new insights into the functional diversity of these biomedically important membrane proteins. Using the complementary tools of X-ray crystallography and high-resolution single-particle cryo-EM, the next challenges will be to determine structures of these proteins in nucleotide-bound and outward-open conformations in order to fully understand the structural changes underlying the transport cycle. For these highly disease-relevant membrane proteins, such knowledge will have major implications for drug discovery.

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Energy in Ancient Metabolism

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Modern metabolism would not work without ATP and phosphate, but in primordial biochemical networks, energy currencies might have been simpler. Goldford et al. report a novel systems approach to reconstructing energetics in ancient metabolism, with very interesting results.

Life is a chemical reaction. Metabolism and life move forward only if energy is released in the overall reaction, as stipulated by the second law of thermodynamics, which permits no exceptions. Because many individual metabolic reactions are energetically uphill (endergonic), the biochemical pathways of a cell are all linked to a central energy supply. At the core of every cell's biochemistry there is thus a main energy releasing (exergonic) reaction that generates a diffusable energetic currency, usually adenosine triphosphate (ATP). Hydrolysis of ATP to ADP and phosphate (P$_i$) releases energy, such that coupling of ATP hydrolysis to uphill steps can energetically pull the reaction forward. ATP, phosphate, and coupling are universal to life today, but at the evolutionary onset of metabolism 4 billion years ago, the chemistry had to be simpler. What came before ATP? In innovative computer work in this issue of Cell, Goldford et al. (2017) remove all of the reactions from metabolism that are ATP-dependent or that even involve phosphate-containing cofactors to see if anything remains and whether what remains might hold clues about ancient metabolism. They find a connected reaction network of small molecular weight carbon compounds, a prevalence of thioesters, and an enrichment of FeS-dependent enzymes. Their findings shed light on the nature of chemical energy currencies in early evolution.

Of life's energy currencies, ATP is the most familiar and the most widely used, but that does not mean that it is also the most ancient. Where does ATP come from in metabolism? At the most basic level, there are only two ways in which cells synthesize ATP, both require sources of environmental energy.

The evolutionarily more advanced mechanism of ATP synthesis is electron transfer phosphorylation, or chemiosmotic coupling. In chemiosmotic coupling, exergonic reactions at the plasma membrane are coupled to the pumping of ions from the inside of the cell to the outside, generating electrochemical ion gradients that can be harnessed by highly complex multisubunit proteins, rotor stator type