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Inroads into Membrane Physiology through Transport Nanomachines

A key event in the origin of life was the development of a lipid membrane that divided the inside of the cell from the outside world. Although this hydrophobic line of demarcation ensures that the contents of the cell stay confined and do not simply diffuse away, its very nature creates a fundamental problem for ions, metabolites, and waste products that must find passage across this hydrophobic barrier. To meet this challenge, Nature has devised a multitude of diverse transport nanomachines that shuttle different classes of ions, small molecules, lipids, and even membrane proteins themselves across or into this greasy boundary between 'in' and 'out'. In this Special Issue of *Journal of Molecular Biology*, we have collected a set of reviews and original research articles that focus on different types of membrane transport proteins. Transporters come in many structural flavors and modes of operation, but several key molecular questions are common to all, such as how they change shape to move substrates from one side of the membrane to the other, how they recognize substrates, and the role of allostery in coupling binding to protein conformation. Another connection across all membrane proteins, not just transporters, is the membrane itself. How do transporters get into the membrane in the first place? How do they find their key partners, whether through homooligomerization or binding auxillary subunits? And how is transporter activity regulated through specific lipid binding or through more general colligative properties of the host membrane? The advances in structural techniques over the last few years have favorably impacted the transporter field, revealing the structures of long sought-after transporters for the first time, capturing many snapshots of the same transporter in different states, and obtaining better, higher-resolution images that have resolved outstanding questions. By coupling these structural advances with careful biochemistry, functional assays, and cell-based studies, the field has made significant progress in answering these questions about conformation, recognition, and allostery. The articles presented here have been thoughtfully collected to touch on all these ideas and provide both novice and expert readers with a flavor for where the transporter field currently stands and where it is heading.

One original research article provides an example of the continued progress in structural techniques. Many transporter families are composed of proteins that are quite small (<100 kDa) and almost completely embedded in the membrane. This situation poses major challenges for structure determination. Randy Stockbridge, Melanie Ohi, Yen-Ting Lai, and colleagues[1] detail the development of a generalizable N-terminal transmembrane helix epitope tag (MPER) from the gp41 transmembrane subunit of HIV that is recognized by a well-characterized set of antibodies. They show that this tag, together with the antibodies, can be used as a crystallization chaperone for small membrane transporters and can also be used as a fiducial marker for cryo-EM studies.

Many transporters use ATP as an energy source to accomplish their task. Poul Nissen and colleagues[2] contribute two original research articles that focus on understanding the mechanisms of the large P-type ATPase superfamily. Members of this superfamily are responsible for creating the ion gradients that power the bioelectric signals of ion channels as well as maintaining the asymmetric lipid compositions of cell membranes. In work studying a Ca^{2+}-ATPase from *Lysteria monocytogenes*, Hansen *et al*. report three new crystal structures, including one state predicted by prior molecular dynamics simulations that helps elucidate a proton counter-transport mechanism. Timcenko and colleagues[3] present four cryo-EM structures that reveal the transport cycle of a P4-ATPase lipid flippase, Drs2p-Cdc50p, from yeast. These structures together with previous work provide a complete picture of the lipid transport cycle that highlights key differences between ATPases that transport ions and those that transport lipids. In an original research article, Thomas Tomasiak and colleagues[4] study an underappreciated feature of ATP-binding cassette (ABC) transporters – the largest family of ATP-dependent transporters with representatives in every branch of the tree of life. The authors find that a sequence motif in the first intracellular loop of each six-helix transmembrane domain, which they coin the "GRD" motif because

it contains conserved glycine, arginine, and aspartate residues, is evolutionarily coupled to other well-known motifs conserved in ABC transporters. Using the bacterial peptide exporter TmrAB as a model system, they provide evidence that the GRD motif forms a conserved allosteric communication pathway between the substrate binding site and the ATPase sites.

Not all transporters run on ATP, and secondary active transporters have evolved to harness the energy source at hand whether it be a proton gradient, sodium gradient, or something else. Here we focus on three members of the amino acid-polyamine-organocation (APC) superfamily. First, Rachelle Gaudet and Aaron Bozzi[5] review the molecular mechanism of Nramp family transporters, which use proton gradients to enable transition metal transport across all kingdoms of life. They show how a common transporter fold has adapted to a non-canonical mechanism for symport, in which the interplay of membrane potential and proton gradient with a constellation of charged residues tunes substrate specificity and transport kinetics. Second, the last two years have brought a wealth of structures for the cation-chloride cotransporters, including the second APC member we highlight. Representatives of five subtypes, including $Na^+/K^+/Cl^-$ co-transporter NKCC1 and K^+/Cl^- cotransporters KCC1, KCC2, KCC3, and KCC4, have been resolved since 2019. Liang Feng and colleagues[6] review the burgeoning insights into architecture, oligomerization, and ion binding sites of this family, which is among the newest of transporter families to be welcomed to the structural era. Third, the Nucleobase Ascorbate Transporter (NAT) family is bifurcated into two subtypes that import strikingly different substrates – nucleobases in some cases, and ascorbate in others. In a research article, George Diallinas and colleagues[7] investigate the molecular basis of selectivity in the purine importer from the filamentous fungus *Aspergillus nidulans.* They find that although it is not trivial to transform a purine importer into an ascorbate importer, limited mutagenesis shifts the specificity of the transporter to different purines and pyrimidines, showing how simple modifications may adapt a transporter to different physiological roles.

Pumping toxic substances out is an important general function for all cells and a key resistance mechanism by which pathogens evade the effects of drugs. This process relies on small molecule transporters from diverse superfamilies that are highlighted in two reviews. One review by Filippo Mancia and colleagues[8] recounts the diverse classes of transporters that serve this purpose in pathogens. They note that despite having different structures, oligomerization states, and mechanisms, all these rather nonselective transporters house binding sites that have common hydrophobic and charged features that enable the recognition and expulsion of compounds having diverse chemical properties. The second contribution from Hassane Mchaourab and colleagues[9] focuses on the very well characterized multidrug and toxin exclusion family (MATE). Their in-depth review of the mechanisms and energy landscapes traversed by this family underscores the diversity of transport mechanisms that can arise from a common scaffold and highlights the importance that lipids play in shaping the function of this transporter class.

Three articles take a closer look at the role of the membrane in transporter activity. Although the word 'transporter' often calls to mind movement of ions, metabolites, or small molecules, one other biologically important class of molecules that needs to be moved across or into membranes are the transporters themselves. Istvan Botos and colleagues[10] provide a review of one of the best understood systems for protein transport, the barrel assembly machinery (BAM) from bacteria and related sorting and assembly machinery (SAM) from mitochondria that insert b-barrel proteins into their target membranes. The authors offer a perspective on the mechanistic commonalities shared by these two systems, how the SAM complex may couple with the translocase of the outer membrane (TOM) complex, and the challenges in understanding how this fascinating dance of proteins occurs in these systems and may also work in chloroplasts. Melanie Ernst and Janice Robertson[11] then remind us that the role of the membrane should not be neglected during the final stages of protein assembly nor when considering the stability of membrane proteins. They describe the emerging evidence, derived from computational, structural, and functional studies of multiple transporter families, that the membrane is an intimate partner that makes major energetic contributions to transporter stability and function. Finally, as the review title by Cristina Paulino, Valeria Kalienkova and colleagues[12] indicates, membrane proteins from the TMEM16 family are "groovy", quite literally! TMEM16 proteins can be either (or both) ion channels and scramblases. Scramblases are membrane proteins that catalyze the flipping and flopping of lipids between the two bilayer leaflets, and TMEM16 proteins do this via membrane-spanning grooves at the edge of the protein that are favorable for polar headgroup passage. These same grooves, depending on the family member, or depending on the conformational state of a particular member, can also allow ions to flow down their electrochemical gradients. Paulino and colleagues[12] review the varied physiological roles of these proteins, the recent burst of structures, and the emerging mechanistic models based on structures, computation, and biochemistry, ending with a summary of key open questions in the field.

A beneficial consequence of this expansion of our molecular understanding of membrane transport processes – including both transporters and ion channels, the focus of a partner Special

Issue – is that we are in a better position to zoom out and consider the cellular context in which these complex membrane nanomachines work with each other and their environment to regulate ionic homeostasis. In that spirit of zooming out to an integrated cellular view, Inga Hänelt and Randy Stockbridge[13] teamed up, with contributions from lab members, to provide an updated and comprehensive overview of the physiology of potassium homeostasis in bacteria that will surely serve as a go-to reference for both experts and newcomers to the field. After introducing concepts of cell physiology, the authors review the molecular mechanisms of the principal ion channels, primary and secondary transporters responsible for potassium fluxes across bacterial membranes. The complexity of even this relatively simple homeostatic problem – one ion, one cell, one compartment – is humbling, and yet the exciting recent progress in our understanding of potassium homeostasis in bacteria illustrates a path for continued fruitful advances in the field of ion homeostasis in general.

Many transporters shape the electrochemical gradients that keep cells alive, and others use those electrochemical gradients to shuttle nutrients in, and signals and toxins out. The articles assembled in this Special Issue of the *Journal of Molecular Biology* highlight many recently developed approaches and conceptual advances. It should be noted that due to the rapid progress in this field, the journal has now created a specific category for papers in this area 'Receptors, channels, and transporters'. We hope that this new category focus will attract further exciting reports to *JMB* as the field uncovers the rich details of the molecular structure, dynamics and energetics of transport proteins and the membranes in which they are embedded.

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