Structure of a transporter domain emerges

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Magnesium homeostasis relies on transporters like the CNNM family, but little information on these proteins’ structure and regulation limits our understanding of their biology and functions in disease. New characterization of a conserved cytoplasmic domain now confirms the presence of a self-ligated architecture that is indispensable for Mg$^{2+}$ efflux and suggests a possible role for a dimeric assembly.

Magnesium trafficking across the cell membrane requires dedicated transporters due to the ion’s unusual radius compared with other divalent cations. One important class of Mg$^{2+}$ transporters is the family of cystathione $\beta$-synthase domain divalent metal cation transport mediators (CNNMs), including CNNM1–CNNM4. Topologically, CNNM transporters contain an N-terminal extracellular domain, a transmembrane domain, and a large cytosolic sequence composed of two discrete domains. However, structural data for the CNNM transporters are sorely lacking, and important questions remain unanswered. What is the overall molecular architecture of the CNNM transporters? What are the functional roles of the extracellular and cytoplasmic regulatory domains? What is the gating and ion permeation mechanism in this family of transporters? Obtaining answers to these questions is important, not only to better understand the fundamental molecular mechanisms of CNNM transporter function, but also to provide strategies for drug design and to gain better insight into disease mutations associated with these transporters. Indeed, mutations in CNNM genes are associated with a range of inherited diseases, including familial dominant hypomagnesemia or Jalili syndrome, characterized by recessive enamel abnormalities and cone-rod dystrophy (1). A new study by Chen et al. (2) provides tentative but intriguing answers to one of these questions in their structural, biochemical, and cellular analysis of one of the cytosolic sequences, the cyclic nucleotide–binding homology domain (CNBD).

Cyclic nucleotides such as cAMP and cGMP act as universal second messengers. Cyclic nucleotide–sensitive proteins respond to fluctuations of these messengers via a cyclic nucleotide–binding domain (CNBD). The molecular architecture of the CNBD is highly conserved, from the catabolite gene activator protein (CAP) in *Escherichia coli* (3), the regulatory subunit of cAMP- or cGMP-dependent protein kinases (4), and Epac proteins (exchange proteins directly activated by cAMP) (5), to the eukaryote cyclic nucleotide-gated (CNG) (6) and hyperpolarization-activated/cyclic nucleotide–sensitive (HCN) channels (7). In certain ion channels, such as the hERG (8) and EAG channels (9), the CNBD has become “self-ligated,” which means that part of the CNBD sequence occupies the cyclic nucleotide–binding site and prevents access by external cAMP or cGMP. Because of their structural resemblance to CNBDs, these domains are referred to as cyclic nucleotide–binding homology domains or CNBHDs. Because cyclic nucleotide binding is impaired in CNBHDs, these domains have evolved to fulfill different roles. For example, in EAG channels, the CNBHD forms a binding interface with calmodulin, which controls channel gating (9). However, it is important to confirm that the putative CNBHDs are indeed self-ligated, and, furthermore, deciphering what new role they might have adopted can be a complicated task.

Because determining the structural details of integral membrane proteins remains a substantial challenge, Chen et al. (2) focused their attention on the isolated CNBHDs of two CNNM Mg$^{2+}$ transporters in a divide-and-conquer approach. Specifically, the authors obtained the first structures of the CNNM3 and CNNM2 CNBHDs, solved at relatively high resolution (1.9 and 2.6 Å, respectively) and revealing important structural detail. As expected, the CNBHD structures closely resemble those of previously solved CNBD structures (Fig. 1). The structural conservation is especially strong for the $\beta$-roll region, which is the site of cyclic nucleotide binding in CNBDs. Differences exist in certain loop regions, which are longer in CNNM transporters. The main difference is in the C-terminal $\alpha$C-helix, which contacts the bound cyclic nucleotide in CNBHDs, but it is unstructured in the CNNM CNBHDs. Additionally, the CNBHD in CNNM transporters is self-ligated, similar to other CNBHDs, as a tyrosine residue (Tyr-628) blocks access to the nucleotide–binding site via steric hindrance. The authors use thermal shift assays and NMR spectroscopy to further confirm the absence of nucleotide binding to all four CNNM CNBHDs. If cyclic nucleotide binding is impaired, then what is the role of the CNBHD in CNNM transporters?

A possible answer to this question comes from the observation that the CNBHDs form dimers in the crystal structures. The authors confirmed this dimerization was not just a crystalization artifact, as the CNBHD dimers were also detected using size exclusion chromatography and analytical ultracentrifugation. Furthermore, the authors identified a region in the $\beta$-roll...
as the dimerization interface; mutation of residues at this interface disrupted assembly in vitro. Amino acid interactions involved at the subunit interface are mainly hydrophobic in nature and mostly conserved between different CNNM members, suggesting an important functional role.

To further investigate, the authors employed a Mg$^{2+}$ efflux assay. They observed that deletion of the CNBHD in CNNM4 transporters impaired Mg$^{2+}$ efflux without affecting membrane localization. This result suggests that CNBHD deletion mutants are still targeted to the membrane but become nonfunctional. In contrast, one of the single point mutants that disrupt the dimer interface retained close to WT Mg$^{2+}$ efflux activity, suggesting that dimerization is not essential for transport function. However, the authors note an interesting correlation, which is that the isoforms that form less stable dimers are intrinsically more active proteins, leading them to propose that dimerization could be an inhibitory mechanism for the CNNM proteins.

Together, the results from this study bring us one step closer to understanding the structure and regulation of CNNM Mg$^{2+}$ transporters. First, the high resolution CNBHD structures will be extremely valuable in aiding the structural elucidation of the intact transporter, particularly if that structure is obtained at medium to low resolution. Second, it will be important to confirm whether the CNBHDs in CNNM transporters also form dimers versus other oligomeric states in their physiological environments. The importance of this point is driven home by a prior cautionary tale: Structures reported in 2012 of the isolated CNBHD of EAG-like K$^+$ channels (10) also showed dimers, while reports in 2016 and 2017 indicated the CNBHDs in the related hERG (8) and EAG channels (9) form tetramers in the integral channels (Fig. 1), requiring reinterpretation of several models. Finally, these new results open up a host of important questions. If the dimer assembly is an intermediate in the gating or transport cycle, how is it regulated and how does it control the overall protein? Can a comparison between CNBHDs such as those in the hERG and EAG channels provide any insights into new hypotheses regarding oligomer assembly and function? Can a more detailed comparison of the CNNM family members’ dimerization interface provide insights to enable specificity for drug discovery efforts among these ubiquitous domains? What will the integral protein structure teach us about the true role of CNBHDs in transport function? With this, the race is on to explain how a self-liganding domain got tangled up in Mg$^{2+}$ transport.

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