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Na⁺/Ca²⁺ exchange: it's all in the symmetry

Jeff Abramson, Aviv Paz, and Kenneth D. Philipson

The Department of Physiology and the Cardiovascular Research Laboratories, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095

Ca²⁺ signaling is an essential component of numerous cellular processes ranging from the first stages of a multicellular organism (fertilization) to its demise (apoptosis). Ca²⁺ homeostasis is critical to normal physiology and is achieved by tightly regulated and coordinated processes involving multiple organelles, hormones, channels, and transporters. Alterations in Ca²⁺ homeostasis are involved in many pathological conditions including stroke and heart failure (1). Following Ca²⁺ stimulation, the Na⁺/Ca²⁺ exchanger (NCX)—a ubiquitous Ca²⁺ export protein residing in the plasma membrane—participates in the restoration of resting levels of Ca²⁺ in excitable cells. This electrogenic process utilizes an inwardly directed Na⁺ gradient to exchange three Na⁺ ions for a single Ca²⁺ ion that is extruded against an electrochemical gradient. In this issue, Liao *et al.* report a structure of a prokaryotic homologue of NCX from *Methanococcus jannaschii* (NCX_Mj) revealing a symmetrical protein scaffold and ion-binding pocket that provides the first high resolution mechanistic clues for understanding the Na⁺/Ca²⁺ exchange process.

Popularized by the laboratories of Douglas Reese and Roderick Mackinnon, Liao *et al.* tested over 50 prokaryotic NCX homologues to screen for their ability to be expressed in an *E. Coli* host. NCX_Mj was the only ortholog to reach an expression level suitable for structural studies. After purification, the authors resolved the structure of NCX_Mj using both conventional as well as lipidic mesophase (Lipidic Cubic Phase) (2) crystallization techniques, delivering the first high-resolution structure from this class of proteins.

The NCX_Mj protomer is composed of 10 transmembrane (TM) helices formed from structural repeats of 5-TM helices with opposing topology (Figure 1A). 5-TM inverted repeat proteins have emerged as a vast and robust structural class of transmembrane transport proteins (3–5), however, the structure of NCX_Mj represents an entirely *new structural fold* yet utilize the same principals elements of structural symmetry to carry out Na⁺/Ca²⁺ exchange. Although the NCX sequences between species are broadly divergent, all NCX proteins are characterized by conserved α -repeats residing in transmembrane helices 2–3 (α -1) and 7–8 (α -2) (6–8), which are critical for transport function (9). The two α -repeats are packaged in the core of the protein forming an ion-binding pocket where three Na⁺-binding sites and a single Ca²⁺-binding site are observed (Figure 1A inset). As reported for various transporters, the substrate-coordinating helices have breaks in their secondary structure to accommodate substrates and provide flexibility for transport.

Centered in the core of the protein, four potential cation sites are arranged symmetrically in a “diamond” pattern (Figure 1A inset). Two high affinity Na⁺ sites are located closest to the extracellular (S_{ext}) and intracellular (S_{int}) surfaces and display nearly identical ligand coordination patterns. There is a two-fold rotational axis, centered on the plane connecting a low affinity Na⁺ site (S_{mid}) and a high affinity Ca²⁺ site (S_{Ca}) that coincides with the 5-TM inverted topology of the protein. This arrangement is consistent with a 3 Na⁺ to 1 Ca²⁺ stoichiometry. The caveat to this elegant cation coordination pattern is that while the precise location of the Ca²⁺ ion was determined experimentally by Ca²⁺ anomalous scattering, it is difficult to unequivocally identify the position of Na⁺ ions (even at 1.9 Å resolution).

Electron densities and ion-ligand coordination guided the positioning of the Na⁺ ions. The four sites are all in close proximity and have ideal ion coordination; furthermore all ligating residues reside in the two α -repeats in accord with biochemical data on NCX1 (9).

Consistent with kinetic data (10), the NCX_Mj structure suggests an alternating access mechanism of transport with a series of distinct conformational transitions such that the substrate-binding site is alternately accessible to either the extracellular or the intracellular face, but not to both sides simultaneously. The NCX_Mj structure resides in an outward-facing conformation with two independent hydrophilic cavities leading from the extracellular face and penetrating the ion-binding pocket (Figure 1B). Using the “rocking bundle” mechanism (11), Liao *et al.* modeled the inward-facing state by swapping the conformations of the 5-TM inverted repeats. The major changes are due to alterations in the peripheral helices (TMs 1–2 and TMs 6–7) generating two independent pathways facing the intracellular compartment. The inverted structural repeats, as modeled here and previously shown by Forrest and Rudnick (11), demonstrate a common mechanism for achieving alternating access that appears to be shared by many classes of transport proteins.

Combining the structural information with known functional data, Liao *et al.* propose an attractive mechanism for Na⁺/Ca²⁺ exchange by “a progressive antagonistic effect of multiple Na⁺ binding on Ca²⁺ affinity”. When we describe the reaction cycle beginning from an outward-facing conformation with Ca²⁺ bound at the high affinity S_{Ca} site, exchange is initiated as Na⁺—entering from the extracellular side—occupies the three Na⁺ sites (Na_{int}, Na_{ext} and Na_{mid}) in concentration dependent manner and competes for the Ca²⁺-coordinating residues. This reduces the Ca²⁺ affinity and causes its release to the extracellular surface. Upon Ca²⁺ release, the “rocking bundle” conformational change transitions the protein to the inward-facing conformation where the Na⁺ ions are exposed to the low intracellular Na⁺ concentration and released. As the Na⁺ ions exit NCX, the high affinity S_{Ca} site is restored.

In addition to solving the structure, Liao *et al.* convincingly demonstrate that NCX_Mj facilitates transmembrane movement of Ca²⁺ in a Na⁺-dependent manner consistent with Na⁺/Ca²⁺ exchange. However, characterization is relatively sparse and kinetic details are still required. At this initial stage, there is no evidence that NCX_Mj catalyzes an accumulation of Ca²⁺ beyond that which would be achieved by facilitated passive diffusion thus future comparisons of features such as electrogenicity with those of the well-characterized mammalian NCX1 will be of interest.

Beyond mechanistic insights, the NCX_Mj structure provides an opportunity to evaluate extensive biochemical studies performed on the mammalian NCX1 for which the most functional data exist. First, there is a striking discrepancy between the NCX_Mj structure and the inferred topology of NCX1. Biochemical approaches have indicated that NCX1 has 9, rather than 10, TM segments (12, 13). However, the sequence similarity between NCX_Mj and NCX1 outside of the α -repeat regions is nonexistent and there still may be some deviation between structures. It is clear the locations of the key ion binding residues within the α -repeat regions of NCX1 can be readily modeled with the structure of NCX_Mj and the mechanistic implications revealed in the NCX_Mj structure are directly applicable to the distantly related NCX1.

Second, there is extensive mutational analysis of the α -repeat regions of NCX1 (9, 14). Mutations of residues that are homologous to the ion-binding residues of NCX_Mj produce exchangers that are inactive or have low activity with an almost perfect correlation. Thus, the mutational data help confirm that the modeled Na⁺-binding sites of NCX_Mj are accurate. Although the conserved α -repeat regions of members of the NCX family are only

a small fraction of the proteins, it is quite striking that the reaction mechanism is so focused within these regions as indicated by both the new structure and by mutational analysis. Liao *et al.* has generated the first structural blue prints of an NCX homologue that will aid the position of biophysical probes and further direct mutational studies in both prokaryotic as well as their eukaryotic counterparts. As is the case with so many classes of transporters before it, the initial structure provides an exciting milestone and while many questions have been answered many more will certainly arise.

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