

Getting a grip on calcium regulation

Mordecai P. Blaustein*^{†‡§}, Thomas H. Charpentier[¶], and David J. Weber[¶]

Departments of *Physiology, [†]Medicine, and [¶]Biochemistry and Molecular Biology, and [‡]Center for Heart, Hypertension, and Kidney Disease, University of Maryland School of Medicine, Baltimore, MD 21201

Temporally and spatially regulated Ca^{2+} signals control numerous physiological processes. In most cells in higher animals, the plasma membrane (PM) Na/Ca exchanger (NCX) helps manage the cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{CYT}}$) and Ca^{2+} stored in the sarco/endoplasmic reticulum (S/ER), thereby influencing Ca^{2+} signaling (1–3). This tightly regulated transporter can move one Ca^{2+} ion either out of or into cells in exchange for three Na^+ . The direction of net Ca^{2+} movement depends on the prevailing membrane potential (V_M) as well as the Na^+ and Ca^{2+} concentration gradients because there is net charge transfer. Cardiac myocytes have large dynamic swings in V_M and $[\text{Ca}^{2+}]_{\text{CYT}}$ during each heartbeat, and NCX plays an especially interesting role: It can mediate Ca^{2+} entry during the upstroke of the action potential, help maintain the elevated $[\text{Ca}^{2+}]_{\text{CYT}}$ and contraction during the action potential plateau, and then extrude Ca^{2+} during repolarization and diastole (1). It is noteworthy that NCX is regulated by cytosolic Na^+ and Ca^{2+} at sites that do not directly participate in the ion translocation (4, 5). A rise in cytosolic Na^+ rapidly stimulates and then inactivates the exchanger (6); in contrast, cytosolic Ca^{2+} activates the exchanger and relieves the Na^+ -dependent inactivation (4, 5). Two elegant studies by Philipson, Abramson, and colleagues, including one in this issue of PNAS (7), provide novel insight into how Ca^{2+} binds to and alters the conformation of the Ca^{2+} regulatory sites in the cardiac/neuronal NCX (NCX type-1) (7, 8).

The mature NCX1 (2, 3) consists of nine transmembrane (TM) helices with an extracellular N terminus and a cytosolic C terminus (Fig. 1A). An inwardly directed reentrant loop between TM2 and TM3 and an outwardly directed loop between TM7 and TM8 are believed to be involved in ion translocation. A large intracellular loop between TM5 and TM6 contains several critical regulatory domains and an alternatively spliced region that is tissue-specific. An amphipathic sequence, located adjacent to the intracellular end of TM5, has been implicated in regulation by Na^+ and acidic phospholipids (4, 9). It is called the XIP (for eXchanger Inhibitory Peptide) region because a peptide with this sequence, when added to the

cytosol, inhibits Na/Ca exchange. A little farther along the intracellular loop are two β -repeats and the homologous Ca^{2+} -binding domains CBD1 and CBD2. Each repeat consists of a seven-strand β -sandwich with two antiparallel β -sheets. A tissue-specific, alternatively spliced region of NCX1 is located within CBD2. The cardiac/neuronal NCX1 isoforms studied here (7) are regulated by 1–10 μM Ca^{2+} , whereas the kidney isoform is not (10, 11). Key questions are: How does Ca^{2+} bind to these cytoplasmic domains? Also, how is this information transmitted to the ion transport domains of NCX1 in the TM domains? The new structure/function studies of CBD1 and CBD2 (7, 8) provide some answers.

High-resolution x-ray and NMR structures of CBD1 and CBD2 indicate that both Ca^{2+} -binding domains have an Ig-like fold with Ca^{2+} ion binding sites in the distal loops (7, 8, 12). CBD1 and CBD2 are similar, in some ways, to the well characterized C_2 domains, such as those in synaptotagmin and protein kinase C (13, 14), which also bind Ca^{2+} in a clustered fashion. Interestingly, in CBD1, the cluster of four Ca^{2+} is arranged in a parallelogram-like configuration, with distances of only 3.9–4.4 Å between the Ca^{2+} ions in consecutively oriented sites (8). Despite their similar fold, CBD1 binds four Ca^{2+} ions with high affinity, whereas CBD2 binds only two Ca^{2+} ions, and with lower affinity. In CBD1, this intricate coordination of Ca^{2+} is necessary to bring so many divalent cations into such close proximity. For example, aspartate and glutamate carboxylate oxygens participate in bidentate ligands that coordinate two (Asp-500) or even three (Glu-451) Ca^{2+} ions simultaneously. Also, although CBD2 contains only two Ca^{2+} binding sites, these sites are very close (5.47 Å apart), and are “bridged” by the two carboxylate oxygens of a single aspartate side chain moiety (Asp-578) (Fig. 1B).

Another major difference between the two CBDs is detected when Ca^{2+} is absent. CBD1 is unfolded in the apo-state; it rearranges into a well structured protein only upon the addition of Ca^{2+} (8). CBD2, however, is a fully folded protein, even in the absence of Ca^{2+} (7). The side chain of Lys-585, which forms a salt bridge with Asp-552 when Ca^{2+} is dissociated, stabilizes the apo-state and maintains the CBD2 structure (7). This

salt bridge may be functionally important because the conservation of overall structure in apo-CBD2 could facilitate rapid rebinding of Ca^{2+} to this site (7).

An important question addressed by Besserer *et al.* (7) is: How are the CBDs in NCX1 linked to the ion transport activity? Point mutations provide part of the answer. Previously, mutations of key acidic amino acid residues in CBD1 were found to decrease the affinity for Ca^{2+} but not eliminate Ca^{2+} -dependent regulation (5, 15). In contrast, mutation of key acidic residues in the higher-affinity “primary” Ca^{2+} site in CBD2 (site 1; Fig. 1B), Asp-516 or Asp-578, abolishes Ca^{2+} regulation altogether (7). Moreover, the alternatively spliced kidney NCX1 isoform, which is not regulated by Ca^{2+} , has an arginine at position 578, and a D578R mutation in the brain isoform abolishes Ca^{2+} regulation (10, 11). On the other hand, mutations that interfere with binding of the secondary Ca^{2+} site in CBD2 (such as Asp-552; site 2) do not prevent Ca^{2+} regulation. These data indicate that Ca^{2+} binding to site 1 in the CBD2 domain is required to sense the intracellular Ca^{2+} concentration and signal this information to the TM segments to regulate ion transport.

Now that the critical role of the primary Ca^{2+} in CBD2 and the mechanism of Ca^{2+} binding have been elucidated (7), we eagerly await the next chapters: How do CBD1 and CBD2 interact with one another when Ca^{2+} binds to CBD2? Also, how does this Ca^{2+} binding influence other parts of the intracellular loop to activate NCX and relieve Na^+ -dependent inhibition? At this point, the structural data provide a basis for speculation. One idea is that the clusters of Ca^{2+} -binding sites in CBD1, like those in C_2 domains, may be important for moving either one or both NCX1 CBDs to the region adjacent to the TM domains. For example, one can envision negatively charged phospholipids at the PM coordinating the two weaker binding sites in CBD1 and thus attaching

Author contributions: M.P.B., T.H.C., and D.J.W. wrote the paper.

The authors declare no conflict of interest.

See companion article on page 18467.

[§]To whom correspondence should be addressed. E-mail: mblaustein@som.umaryland.edu.

© 2007 by The National Academy of Sciences of the USA

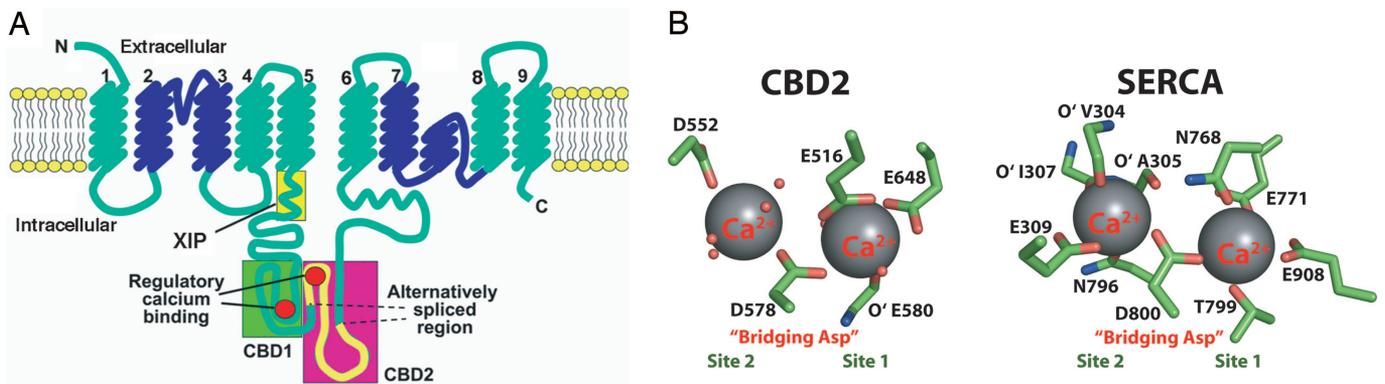


Fig. 1. Topology of NCX1 and comparison of Ca^{2+} ion coordination in CBD2 with a similar Ca^{2+} -binding domain in SERCA. (A) Overall topology of NCX1. The N and C termini, reentrant loops (in blue), XIP region, and CBD1 and CBD2, including the alternatively spliced region, are indicated; also see details in text. The image was modified from Lytton (3). (B) Ca^{2+} binding motifs of CBD2 (Left) and SERCA (Right). In both motifs, site 1 is the higher-affinity Ca^{2+} site. CBD2 and SERCA bind Ca^{2+} cooperatively and have a “bridging aspartate” residue that coordinates the two Ca^{2+} ions in the adjacent sites simultaneously.

CBD1 (or perhaps both CBDs) directly to the PM cytoplasmic surface, as is the case for several proteins containing C_2 Ca^{2+} -binding domains (16–18). Arguments for such a mechanism are strengthened when one considers the regulatory role of the XIP region adjacent to TM5 (Fig. 1A). However, one cannot ignore the new data (7), which reveal an important role for the CBD2 domain in Na/Ca exchange.

As we have learned (7), the CBD2 domain has two Ca^{2+} -binding sites: the primary site (site 1) has a greater affinity for Ca^{2+} than the secondary site (site 2), and the sites bind Ca^{2+} in a cooperative manner (Fig. 1B). These features of CBD2 are reminiscent of the two Ca^{2+} -binding sites in the SERCA pump, another Ca^{2+} transporter. Although the helical topology of the residues surrounding the Ca^{2+} sites in

SERCA (20) looks nothing like the β -strand structure surrounding the CBD2 sites, it is striking that the Ca^{2+} -binding sites themselves in SERCA and CBD2 are similar: two adjacent Ca^{2+} ions (5.95 vs. 5.47 Å apart, respectively) bridged by an aspartate (Asp-800 in SERCA and Asp-578 in CBD2; Fig. 1B). In SERCA, the primary (site 1) Ca^{2+} is coordinated by seven ligands, including the side chain oxygens of Asn-768, Glu-771, Thr-799, Asp-800, and Glu-908; both Glu-771 and Glu-908 form bidentate ligands, and Asp-800 links site 1 to site 2 via an “aspartate bridge” (Fig. 1B). Also, as in CBD2, the weaker, site 2 Ca^{2+} in SERCA coordinates with a combination of backbone carbonyl oxygens (from Val-304, Ala-305, and Ile-307) together with side chain carboxylate oxygens (from Glu-309 and Asn-796) and the bridging Asp-800 (Fig. 1B).

Such an intricate hydrogen bonding network connecting these sites results in the cooperative binding of the two Ca^{2+} ions in both CBD2 and SERCA. Importantly, significant structural changes in SERCA upon the association/dissociation of Ca^{2+} are essential for the release of Ca^{2+} into the S/ER lumen and for opening a path for the entry of new Ca^{2+} ions from the cytoplasmic side (19, 20). Therefore, by analogy with SERCA, we speculate that Ca^{2+} association/dissociation in the CBD2 site 1 also is associated with a global conformational change that is necessary for function. Such a conformational change, induced by binding of Ca^{2+} to CBD2 site 1, together with movement toward the PM, via CBD1, provides a means for sensing Ca^{2+} and delivering this message to the PM. Resolution of these issues must await further research.

- Blaustein MP, Lederer WJ (1999) *Physiol Rev* 79:763–854.
- Philipson KD, Nicoll DA (2000) *Annu Rev Physiol* 62:111–133.
- Lytton J (2007) *Biochem J* 406:365–382.
- DiPolo R, Beauge L (2006) *Physiol Rev* 86:155–203.
- Matsuoka S, Nicoll DA, Hryshko LV, Levitsky DO, Weiss JN, Philipson KD (1995) *J Gen Physiol* 105:403–420.
- Hilgemann DW, Matsuoka S, Nagel GA, Collins A (1992) *J Gen Physiol* 100:905–932.
- Besserer GM, Ottolia M, Nicoll DA, Chaptal V, Cascio D, Philipson KD, Abramson J (2007) *Proc Natl Acad Sci USA* 104:18467–18472.
- Nicoll DA, Sawaya MR, Kwon S, Cascio D, Philipson KD, Abramson J (2006) *J Biol Chem* 281:21577–21581.
- Matsuoka S, Nicoll DA, He Z, Philipson KD (1997) *J Gen Physiol* 109:273–286.
- Dyck C, Omelchenko A, Elias CL, Quednau BD, Philipson KD, Hnatowich M, Hryshko LV (1999) *J Gen Physiol* 114:701–711.
- Dunn J, Elias CL, Le HD, Omelchenko A, Hryshko LV, Lytton J (2002) *J Biol Chem* 277:33957–33962.
- Hilge M, Aelen J, Vuister GW (2006) *Mol Cell* 22:15–25.
- Nalefski EA, Falke JJ (1996) *Protein Sci* 5:2375–2390.
- Shao X, Davletov BA, Sutton RB, Sudhof TC, Rizo J (1996) *Science* 273:248–251.
- Levitsky DO, Nicoll DA, Philipson KD (1994) *J Biol Chem* 269:22847–22852.
- Verdaguer N, Corbalan-Garcia S, Ochoa WF, Fita I, Gomez-Fernandez JC (1999) *EMBO J* 18:6329–6338.
- Jerala R, Almeida PF, Ye Q, Biltonen RL, Rule GS (1996) *J Biomol NMR* 7:107–120.
- Nalefski EA, Slazas MM, Falke JJ (1997) *Biochemistry* 36:12011–12018.
- Zhang Z, Lewis D, Strock C, Inesi G, Nakasako M, Nomura H, Toyoshima C (2000) *Biochemistry* 39:8758–8767.
- Toyoshima C, Nakasako M, Nomura H, Ogawa H (2000) *Nature* 405:647–655.