Hold the door: hPMCA1/neuroplastin interactions regulate Ca^{2+}-binding site accessibility

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Abstract

In a September 2018 paper published in Nature Communications, Gong *et al.* identified the domains through which human PMCA1 and neuroplastin (NPTN) interact. Upon binding, hPMCA1 TM domains separate T110 in TM1 and A370 in TM3 to reveal the Ca^{2+}-binding site. Thus, NPTN is able to directly modulate the accessibility of cytosolic Ca^{2+} to PMCA.

Graphical Abstract
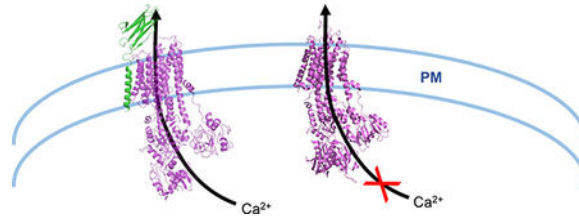
In recent years, our understanding of the regulation of Ca^{2+} clearance has expanded beyond calmodulin binding and multimerization to the concept that native PMCAs act as part of a complex requiring other modulators to function in a physiological context. Recent investigations have revealed the extracellular immunoglobulin superfamily members neuroplastin (NPTN) and close paralog basigin (BASI) as key regulators of PMCA complexes. Supporting this, double knockout studies of NPTN and BASI demonstrated decreased Ca^{2+} clearance[1]. In a paper by Gong *et al.* published in Nature Communications in September 2018, a new structure of human PMCA1d (hPMCA1) in complex with the recently identified regulator neuroplastin is presented[2]. Utilizing cryo-electron microscopy, Gong *et al.* resolved hPMPCA1/NPTN to 4.1 Å. This revealed that hPMCA1 and NPTN primarily interact via the NPTN TM and hPMCA1 TM10, which is distal from TM1–9, with additional stabilizing interactions along the TM[8–9] linker. Additionally, NPTN binding alters PMCA conformation. As hPMCA1 TM7 and TM10 rotate toward NPTN TM, TM1 and

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TM4 are moved toward TM2, exposing the hPMCA1 cytosolic Ca^{2+} binding site. Thus, while NPTN interactions may be limited to hPMCA1 TM10, NPTN binding to hPMCA1 induces major structural changes throughout the hPMCA1 TM domain that ultimately affect Ca^{2+} binding. Finally, hPMCA1 was found in these studies to engage in a gate-locking mechanism between residues T110 in TM1 and A370 in TM3 to control access to the Ca^{2+} binding site. The studies by Gong *et al.* not only offer valuable structural information to NPTN/PMCA interactions, but also reveal a novel binding pattern between a P-type ATPase and a partner protein. Furthermore, the degree of homology between NPTN and BASI open the possibility that these structural findings could extend to molecular modeling to predict how BASI interacts with PMCA.

The differences in hPMCA1 and hPMCA1-NPTN raise the question of what differences may exist between purified PMCA structure and *in vivo* conformation in the presence of endogenous PMCA modulators. The data presented by Gong *et al.* show purified hPMCA1 required NPTN for ATPase activity *in vitro,* supporting a model in which PMCA activity is NPTN-dependent[2]. Data from an *in vivo* study by Korthals *et al.* show NPTN co-localized with both PMCA1 and PMCA4 in T cells, suggesting that NPTN may modulate multiple PMCA isoforms in other tissue types[3]. This extends to evidence of a physiological role for NPTN-mediated regulation, as T cells from *Nptn–/–* mice displayed elevated resting Ca^{2+} and Th1 bias. Notably, although *Nptn–/–* T cells exhibit an elevated baseline and diminished Ca^{2+} clearance, PMCA expression was downregulated over 70%. Thus, how much NPTN modulates Ca^{2+} signals by regulating PMCA expression versus as a direct binding partner that induces a conformational change in PMCA remains to be defined. Additionally, PMCA2 expression restores partial Ca^{2+} clearance, suggesting not every isoform is dependent on the same regulatory partners, and some may not require partners for Ca^{2+} transporting activity. The host of proteins involved in the regulation of PMCAs may prove as multi-faceted as PMCA splice variants themselves, demonstrating the continued importance of studies such as by Gong *et al.* to define how physical interactions with regulatory proteins control PMCA conformation and function. Previous investigations by us and others have revealed regulation of PMCA activity by the ER membrane proteins STIM1 and POST[4,5]. The extent to which these phenomena are co-dependent has not been assessed, however, it is tempting to speculate that changes in PMCA function due to association with proteins like STIM1 or POST might result from changes in PMCA/neuroplastin association, a concept presented in figure 1.

NPTN was first defined in neurons, which express different PMCA isoforms between different neuronal populations (reviewed in [6]). In the adult rat brain, for example, PMCA1, although ubiquitously expressed, is enriched in the cortex and hippocampus, PMCA2/3 in the cerebellum and forebrain, and PMCA4 in the cortex[7]. While PMCA2 may be less reliant on NPTN, this could vary across PMCA isoform and therefore across cell type. Therefore, the ability of NPTN to alter basal Ca^{2+} could have important ramifications for understanding neuronal excitotoxicity, synaptic function, and neurological disease[8]. The clinical importance of BASI as a marker of resistance and potential therapeutic target in a variety of cancers also continues to gain momentum (reviewed in [9]). Others have forayed into defining mechanisms of how BASI contributes to invasiveness, namely through modulation of glycolysis and matrix metalloproteinase expression by tumor cells and stromal cells[10,11].

Mechanistic studies into whether regulation of PMCA contributes to these processes will be valuable for understanding how BASI/NPTN-mediated signaling may be targeted. Ultimately, future studies may prove critical to define how physical associations between PMCA isoforms and its regulators control PMCA function to integrate control of Ca^{2+} clearance with other aspects of Ca^{2+} signal generation.

References

1. Schmidt N et al. Neuroplastin and Basigin Are Essential Auxiliary Subunits of Plasma Membrane Ca(2+)-ATPases and Key Regulators of Ca(2+) Clearance. Neuron 96, 827–838.e829, doi:10.1016/j.neuron.2017.09.038 (2017). [PubMed: 29056295]

2. Gong D et al. Structure of the human plasma membrane Ca(2+)-ATPase 1 in complex with its obligatory subunit neuroplastin. Nat Commun 9, 3623, doi:10.1038/s41467-018-06075-7 (2018). [PubMed: 30190470]

3. Korthals M et al. A complex of Neuroplastin and Plasma Membrane Ca(2+) ATPase controls T cell activation. Scientific reports 7, 8358, doi:10.1038/s41598-017-08519-4 (2017). [PubMed: 28827723]

4. Krapivinsky G, Krapivinsky L, Stotz SC, Manasian Y & Clapham DE POST, partner of stromal interaction molecule 1 (STIM1), targets STIM1 to multiple transporters. Proc Natl Acad Sci U S A 108, 19234–19239, doi:1117231108 [pii 10.1073/pnas.1117231108 (2011). [PubMed: 22084111]

5. Ritchie MF, Samakai E & Soboloff J STIM1 is required for attenuation of PMCA-mediated Ca^{2+} clearance during T-cell activation. EMBO J 31, 1123–1133, doi:10.1038/emboj.2011.495 (2012). [PubMed: 22246182]

6. Krebs J The plethora of PMCA isoforms: Alternative splicing and differential expression. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 1853, 2018–2024, doi:10.1016/j.bbamcr.2014.12.020 (2015). [PubMed: 25535949]

7. Burette A, Rockwood JM, Strehler EE & Weinberg RJ Isoform-specific distribution of the plasma membrane Ca2+ ATPase in the rat brain. 467, 464–476, doi:10.1002/cne.10933 (2003).

8. Strehler EE & Thayer SA Evidence for a role of plasma membrane calcium pumps in neurodegenerative disease: Recent developments. Neuroscience Letters 663, 39–47, doi:10.1016/j.neulet.2017.08.035 (2018). [PubMed: 28827127]

9. Xin X et al. CD147/EMMPRIN overexpression and prognosis in cancer: A systematic review and meta-analysis. Scientific reports 6, doi:10.1038/srep32804 (2016).

10. Huang P et al. RNA interference targeting CD147 inhibits the proliferation, invasiveness, and metastatic activity of thyroid carcinoma cells by down-regulating glycolysis. International Journal of Clinical and Experimental Pathology 8, 309–318 (2015). [PubMed: 25755717]

11. Grass GD & Toole BP How, with whom and when: an overview of CD147-mediated regulatory networks influencing matrix metalloproteinase activity. Bioscience Reports 36, doi:10.1042/bsr20150256 (2016).
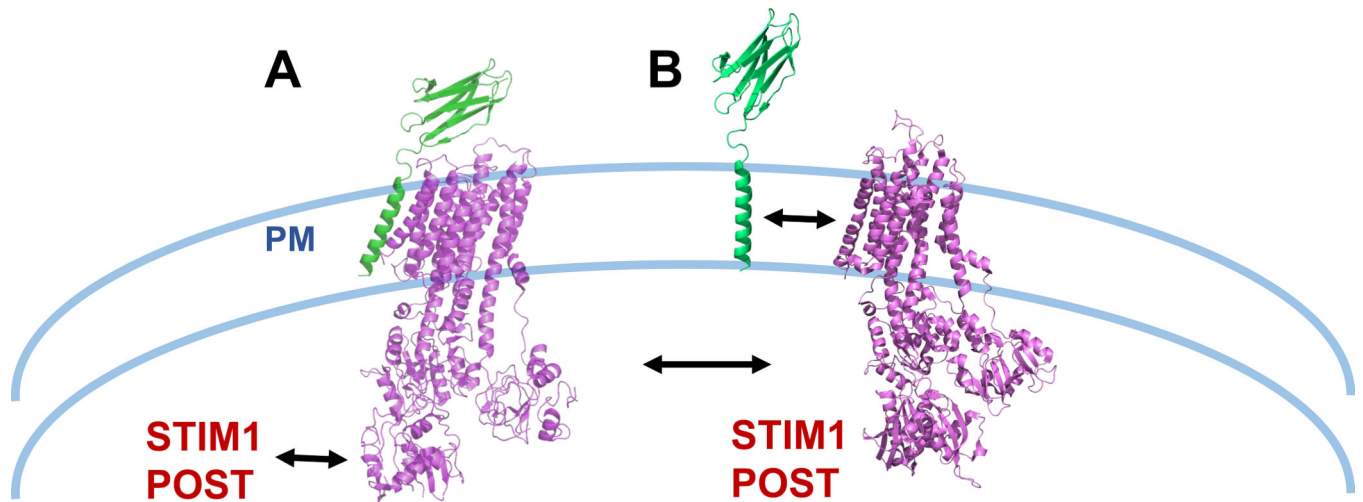
Figure 1. Regulation of PMCA by protein interactions.
(**A**) As shown in Gong et al (2018)[2], PMCA directly associates with neuroplastin via its TM10 domain (PDB: 6A69). STIM1 has also been shown to associate with STIM1[5] and POST[4]. (**B**) The possibility that association between STIM1 and/or POST with PMCA (PDB: 3W5C) may negatively regulate its association with neuroplastin (modified PDB: 6A69) is presented.