Published in final edited form as:

Cell Calcium. 2020 November ; 91: 102254. doi:10.1016/j.ceca.2020.102254.

Palmitoylation: A new mechanism for control of NCX1 function.

Elton Veseli¹, Jonathan Soboloff^{1,2}

¹Department of Fels Institute for Cancer Research and Molecular Biology

²Department of Medical Genetics & Molecular Biochemistry, Temple University School of Medicine, Philadelphia, PA, 19140, USA

Abstract

A paper by Gök et al., identified Zinc Finger DHHC-Type Palmitoyltransferase 5 (zDHHC5) and Acyl-Protein Transferase 1 (APT1) as the enzymes responsible for the dynamic palmitoylation of NCX1. Palmitoylation occurs at the cell surface and increases the affinity of NCX1 for lipid rafts. Additionally, they discovered that palmitoylation controls the affinity of NCX1 for exchange inhibitory protein (XIP) and regulates intracellular calcium concentration. These findings provide new insights into endogenous control of NCX1 function and will drive future investigations directed at understanding its full potential.

Maintenance of intracellular calcium concentration is essential for proper cellular function and survival. This is accomplished through the actions of multiple channels, pumps, and exchangers that work cooperatively to control calcium levels to drive multiple cellular processes. The sodium-calcium exchanger 1 (NCX1) utilizes the electrochemical gradient of sodium ions to clear cytosolic calcium [1]. Palmitoylation, a post-translational attachment of fatty acids to protein residues, is the only known reversible endogenous regulator of NCX1 activity. Specifically, palmitoylation attenuates NCX1 in the presence of elevated intracellular sodium levels and reduced intracellular calcium levels. Recently Gök et al., used Förster resonance energy transfer (FRET) to discover that NCX1 palmitoylation restructures the arrangement of the existing NCX1 dimer causing an increased FRET following palmitoylation [2]. After manipulating lipid raft formation and using palmitoylated and non-palmitoylated NCX1, they concluded that palmitoylation increases NCX1's affinity for lipid rafts. Affinity purification showed that palmitoylation increases the affinity of NCX1 for XIP by creating a binding site in the f-loop for the peptide as biotinylated XIP showed reduced ability to interact with unpalmitoylatable NCX1 as shown in Figure 1. Experiments with APT1 inhibitors showed increases in NCX1 palmitoylation indicating that APT1 is responsible for depalmitoylation of NCX1. The comparison of FRET signals between WT HEK cells and zDHHC5 KO HEK cells indicated that zDHHC5 palmitoylated NCX1 at the cell surface. Further, zDHHC5 KO HEK cells showed significant decreases in FRET signals relative to WT HEK cells. Figure 1 shows the effect of palmitoylation and depalmitoylation of NCX1 on calcium flux. Another paper by Gök et al.,

To whom correspondence should be addressed: Jonathan Soboloff, Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, 3307 North Broad Street, Philadelphia, PA 19140. Office: (215) 707-6567; Fax: (215) 707-2805
soboloff@temple.edu.

suggested palmitoylation is not required for NCX1 movement in the secretory pathway, but regulates NCX1 activity at the cell surface [3]. Identification of zDHHC5 as the enzyme responsible for palmitoylation confirms this suspicion.

It is widely accepted that palmitoyl transferase enzymes contain highly conserved DHHC cysteine rich domains required for palmitoylation [4]. The discovery of zDHHC5 as the enzyme responsible for NCX1 palmitoylation is unsurprising as it is a ubiquitous palmitoyl transferase found in many types of human tissue. For example, palmitoylation of NOD1/2 by zDHHC5 contributes to immune responses, while CIL56-induced cell death requires a complex of zDHHC5 and GOLGA7 [5, 6]. Generally, palmitoylation occurs in the Golgi apparatus where many post-translational modifications occur, but the fact that NCX1 is palmitoylated at the cell surface may reveal palmitoylation as a functional regulator of NCX1 function independent of trafficking [7]. Hence, zDHHC5 activity is itself regulated. Gök et al., suggested that zDHHC5 acts as the sensor that mediates dynamic palmitoylation of NCX1. This idea has enormous potential for future investigations focused on the association between zDHHC5 and control of intracellular calcium concentrations. A deeper understanding of the regulation of zDHHC5 would also help to provide the physiological context for this novel mechanism of action.

There are two major human acyl-protein thioesterases responsible for depalmitoylation of proteins in the cell: APT1 and APT2. Gök et al., identified APT1 as the sole NCX1 depalmitoylating enzyme since an APT1 inhibitor increased palmitoylation while an APT2 inhibitor had no effect. This discovery is interesting given the 68% homology between the enzymes [8]. Another study focused on APT1/2 as depalmitoylating enzymes that control calcium activated BK channels [9]. Depalmitoylation of BK channels prevents its translocation to the plasma membrane. This study also identified APT1 as the primary depalmitoylating enzyme. Little is known about the specific substrates of APT1/2, but the advantage that palmitoylation reversibility provides to the cell makes APT1/2 a valuable target for future research focused on controlling the localization and function of ion channels, pumps, and exchangers.

The dynamism of NCX1 palmitoylation suggests that this type of post-translational modification plays a pivotal and versatile role in a variety of cellular processes. While palmitoylation was shown to inactivate NCX1, palmitoylation can have the opposite effect, for example upon the P2X7 receptor [10]. Several residues in P2X7 are palmitoylated in order to anchor it to the plasma membrane and to inhibit desensitization. Mutations to the palmitoylated regions of the receptor resulted in complete desensitization whereas WT P2X7R showed no desensitization. Comparisons between NCX1 and P2X7 palmitoylation show that palmitoylation can contribute to the function of surface channels and exchangers in unique ways. While palmitoylation inactivates NCX1 to prevent calcium efflux, P2X7 palmitoylation prevents the desensitization of the P2X7 receptor, thereby increasing calcium influx. Hence, in both cases, palmitoylation tends to lead to an increase of intracellular calcium.

Palmitoylation remains a relatively novel new mechanism for controlling the function of calcium channels, pumps and exchangers. Future investigations focused on identifying

which enzymes are regulated by palmitoylation and how palmitoylation affects their function are needed before therapeutic advantage of this pathway will be possible. Further, zDHHC5 and APT1 are only two of the many enzymes capable of palmitoylation and depalmitoylation. Future studies focused on the identification of these enzymes may lead to novel new strategies for the manipulation of intracellular calcium content.

References

- [1]. DiPolo R, Beauge L, Sodium/calcium exchanger: influence of metabolic regulation on ion carrier interactions, *Physiol Rev*, 86 (2006) 155–203. [PubMed: 16371597]
- [2]. Gok C, Plain F, Robertson AD, Howie J, Baillie GS, Fraser NJ, Fuller W, Dynamic Palmitoylation of the Sodium-Calcium Exchanger Modulates Its Structure, Affinity for Lipid-Ordered Domains, and Inhibition by XIP, *Cell reports*, 31 (2020) 107697.
- [3]. Gok C, Fuller W, Regulation of NCX1 by palmitoylation, *Cell Calcium*, 86 (2020) 102158.
- [4]. Roth AF, Feng Y, Chen L, Davis NG, The yeast DHHC cysteine-rich domain protein Akr1p is a palmitoyl transferase, *J Cell Biol*, 159 (2002) 23–28. [PubMed: 12370247]
- [5]. Ko PJ, Woodrow C, Dubreuil MM, Martin BR, Skouta R, Bassik MC, Dixon SJ, A ZDHHC5-GOLGA7 Protein Acyltransferase Complex Promotes Nonapoptotic Cell Death, *Cell Chem Biol*, 26 (2019) 1716–1724 e1719. [PubMed: 31631010]
- [6]. Lu Y, Zheng Y, Coyaude E, Zhang C, Selvakumaran A, Yu Y, Xu Z, Weng X, Chen JS, Meng Y, Warner N, Cheng X, Liu Y, Yao B, Hu H, Xia Z, Muise AM, Klip A, Brumell JH, Girardin SE, Ying S, Fairn GD, Raught B, Sun Q, Neculai D, Palmitoylation of NOD1 and NOD2 is required for bacterial sensing, *Science*, 366 (2019) 460–467. [PubMed: 31649195]
- [7]. Guan X, Fierke CA, Understanding Protein Palmitoylation: Biological Significance and Enzymology, *Sci China Chem*, 54 (2011) 1888–1897. [PubMed: 25419213]
- [8]. Davda D, Martin BR, Acyl protein thioesterase inhibitors as probes of dynamic S-palmitoylation, *Medchemcomm*, 5 (2014) 268–276. [PubMed: 25558349]
- [9]. Tian L, McClafferty H, Knaus HG, Ruth P, Shipston MJ, Distinct acyl protein transferases and thioesterases control surface expression of calcium-activated potassium channels, *J Biol Chem*, 287 (2012) 14718–14725. [PubMed: 22399288]
- [10]. McCarthy AE, Yoshioka C, Mansoor SE, Full-Length P2X7 Structures Reveal How Palmitoylation Prevents Channel Desensitization, *Cell*, 179 (2019) 659–670 e613. [PubMed: 31587896]

Figure 1: The effect of palmitoylation and depalmitoylation of NCX1 on calcium efflux. Palmitoylation of NCX1 by zDHHC5 at the cell surface alters the structure of and inactivates NCX1 to prevent calcium efflux. APT1 depalmitoylates NCX1 and allows calcium to flow out of the cell.