Identification of physiological substrates for depalmitoylating enzyme ABHD17 in neurons

Egawa, Takahiko

Doctor of Philosophy

The Graduate University for Advanced Studies, SOKENDAI

School of Life Science

Department of Physiological Sciences

2024

Introduction

Posttranslational modifications including phosphorylation, ubiquitination, and lipidation regulate protein functions beyond genomic information. Lipidation such as palmitoylation (El-Husseini and Bredt, 2002; Resh, 2006; Linder and Deschenes, 2007; Fukata and Fukata, 2010), prenylation (Zhang and Casey, 1996) and myristoylation (Resh, 1999) increases the hydrophobicity of proteins to target them to various intracellular and plasma membranes. Palmitoylation is the addition of a 16-carbon saturated fatty acid to a specific cysteine residue of a protein via the labile thioester linkage. Unlike other lipid modifications, palmitoylation is a reversible reaction, allows proteins to shuttle between intracellular compartments and alters their activity bidirectionally. Classic representative palmitoylated proteins include small GTPases H/N-Ras (Hancock et al., 1989), heterotrimeric G-protein α subunits (Gα) (Linder et al., 1993), postsynaptic scaffolding protein PSD-95 (Topinka and Bredt, 1998), and endoplasmic reticulum chaperon protein Calnexin. In addition, transmembrane proteins, such as LRP6, LAT, and AMPA receptor, are also palmitoylated at the cytoplasmic or juxtamembrane cysteine residue (Zhang et al., 1998; Hayashi et al., 2005; Abrami et al., 2008).

The palmitoylation-depalmitoylation cycling is mediated by palmitoyl-acyl transferases (PATs) and palmitoyl-protein thioesterases (PPTs). Firstly, PATs were identified in yeast by a forward genetics screening (Lobo et al., 2002). PATs share the conserved cysteine rich (CR) domain followed by DHHC (Asp-His-His-Cys) domain. The DHHC domain and its surrounding sequence are essential for their enzymatic activity. Based on the CR-DHHC domain homology, a subsequent study identified a family of mammalian 23 PATs (Fukata et al., 2004), currently termed zinc finger DHHC domain containing (ZDHHC) proteins. A series of studies on palmitoyl-proteomics and PAT-substrate screening have led to establishment of the protein

palmitoylation database, SWISS-Palm, which currently encompasses ~7,000 palmitoylated proteins (Blanc et al., 2015). On the other hand, studies about proteins undergoing depalmitoylation are limited to a few of proteins, such as H/N-Ras (Hancock et al., 1989), Gα (Linder et al., 1993), and PSD-95 (Topinka and Bredt, 1998). Although APT1/2 and PPT1, were biochemically purified with depalmitoylating activity for H-Ras and Gα (Camp and Hofmann, 1993; Duncan and Gilman, 1998; Tomatis et al., 2010; Tian et al., 2012), it remained unknown how relevant their depalmitoylating activities are in cells and whether they depalmitoylate other palmitoylated proteins, such as PSD-95, or not.

In 2016, the laboratory I belong to identified ABHD17A, 17B, and 17C as PSD-95 depalmitoylating enzymes (Yokoi et al., 2016). The ABHD17 family proteins (ABHD17s) have the strongest depalmitoylating activity to PSD-95 when compared with 38 serine hydrolase candidates including APT1/2 and PPT1. Based on the phylogenetic tree, ABHD17B is considered as the prototype for ABHD17s. Importantly, the N-terminal cysteine cluster of ABHD17s is reported to be palmitoylated and is required for its targeting to the plasma membrane (Martin and Cravatt, 2009; Yokoi et al., 2016). A palmitoylation-deficient ABHD17B mutant is diffused into the cytoplasm and fails to depalmitoylate PSD-95 and N-Ras. Considering previous reports that APT1, APT2, and PPT1 are mainly localized to the mitochondria (Kathayat et al., 2018), Golgi apparatus (Vartak et al., 2014), and lysosome (Verkruyse and Hofmann, 1996), respectively, ABHD17s are thought to be responsible for depalmitoylation on the plasma membrane.

Therefore, to reveal the physiological functions of ABHD17s, I attempted to identify novel substrates for ABHD17s. Taking advantage of knock-in (KI) mice in which EGFP was introduced at the C-terminus of ABHD17B, I comprehensively identified 263 proteins

specifically interacting with ABHD17B in the mouse brain. From these proteins, I selected 11 proteins as substrate candidates for ABHD17s, and asked if ABHD17s physiologically depalmitoylates these proteins.

Materials and Methods

Animal experiments

All animal studies were reviewed and approved by the ethic committees at National Institute of Natural Sciences and were performed according to the institutional guidelines concerning the care and handling of experimental animals.

Generation of ABHD17B-EGFP knock-in (KI) mice

To generate KI mice in which ABHD17B was tagged with enhanced green fluorescent protein (EGFP) at the C-terminus (referred to as ABHD17B-EGFP KI mice), the sgRNA targeting the sequence of mouse *Abhd17b* (NM_146096), 5'-TTAAAACAGTTCGTGTCAC-3', was subcloned into pX330 vector. For a homology-directed repair (HDR) template DNA supplied in the targeting vector, the ~1.7-kb homology sequence flanking both sides of the target sequence for mouse *Abhd17b* was isolated by genomic PCR and then the sequence encoding an EGFP-tag was inserted before the stop codon of the mouse *Abhd17b* coding sequence. By Dr. Hirabayashi's group (Section of Mammalian Transgenesis, NIPS), the pX330 plasmid harboring sgRNA and linearized targeting vector were co-injected into fertilized eggs, collected from superovulated B6D2F1 female mice (F1 hybrid between C57BL/6 and DBA/2) mated with C57BL/6 male mice. To select the desired founder mice, obtained F0 pups were screened by the genomic PCR and subsequent direct sequencing analyses using primer pairs located outside the homology arms. Then, the founder mice harboring the KI allele were backcrossed with C57BL/6 mice for six generations. No genomic integration of the *Cas9*-cassette was confirmed by genomic PCR. Genotyping was performed by PCR using two forward primers and one reverse primer: Forward primers were 5'-ACGGCAACACATCCTGGGGCACAAG-3', and 5'-GGACTGAAGATGAAGTCATTGAC-3'; Reverse primer was 5'-

ACAGCCTGACTGCATGATACTGC-3' for ABHD17B-EGFP KI mice. Wild-type (WT) and KI alleles give 378- and 525-bp PCR products, respectively.

Immunoprecipitation (IP)

Mice were killed by neck dislocation and brains were dissected and frozen at -80°C immediately. Five whole brains from WT or ABHD17B-EGFP KI mice (P70) were homogenized in 12.5 ml of buffer H1 [20 mM Tris-HCl (pH 8.0), 2 mM EDTA, 320 mM sucrose, and 100 μg/ml PMSF] on ice. The homogenates were centrifuged at 20,000 *g* for 1 hour at 4°C. Supernatants (cytosolic fraction; S2) were removed, and pellets (crude membrane fraction; P2) were resuspended with 12.5 ml of extraction buffer [1.3% Triton X-100, 20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 100 mM NaCl, and 50 μg/ml PMSF] and incubated for 1 hour. The lysates were centrifuged at 100,000 *g* for 1 hour to divide into detergent-soluble (P2-soluble) and -insoluble (P2-insoluble) fractions. P2-soluble fractions were incubated with the GFP-trap beads (50 μl bed volume/5 brains) at 4°C overnight and washed with IP buffer [20 mM Tris-Cl (pH 7.5), 1 mM EDTA, 100 mM NaCl, 1% TritonX-100, and 50 μg/ml PMSF] four times. For SDS-PAGE followed by WB and silver staining, the 50% of beads were suspended in SDS-sample buffer and boiled at 100°C for 5 minutes, and bound proteins were separated from beads by microfiltration. The rest of beads were suspended in denaturing buffer [7 M guanidine hydrochloride, 0.5 M Tris-Cl (pH 8.5), and 10 mM EDTA] for shot-gun liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

Mass spectrometry and label-free quantification

Mass spectrometry analyses were supported by Functional Genomics Facility, NIBB Core Research Facilities. For proteomic analysis, the proteins on beads suspended in 150 μl of denaturing buffer were reduced with 5 mM DTT for 30 minutes at room temperature and

alkylated with 10 mM iodoacetamide for 1 hour at room temperature in the dark. Then, proteins were separated from the beads by microfiltration and concentrated by chloroform-methanol (CM) precipitation. The protein precipitants were solubilized in urea buffer [1.2 M urea and 0.1 M Tris-Cl (pH 8.5)] and digested with trypsin at 37°C for overnight (the in-solution digest). The obtained peptides were separated via nano-flow liquid chromatography (EASY-nLC1000, Thermo Fisher Scientific) using a reverse-phase C18 column (0.075 × 125 nm; Nikkyo Technos). The liquid chromatography eluent was coupled to a nano ion spray source attached to an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific). For protein identification, label-free quantification (LFQ) and volcano plot analyses, I used the Mascot2.6.1 (Matrix Science) and Proteome Discoverer2.2 software (Thermo Fisher Scientific). For proteomic analysis, three biological replicates were injected twice on the mass spectrometer to obtain technical replicates. Protein and peptide identifications were validated by protein false discovery rate (FDR). Obtained protein list with a high FDR confidence (< 0.01) from WT and ABHD17B-EGFP KI mice was used for the statistical analysis to compare protein abundances based on the peak area. The *p*-value of abundance ratio (KI/WT) was calculated by the Benjamini-Hochberg correction.

In silico prediction of palmitoylated protein analysis

For the prediction of palmitoylated proteins with their sites, I used the palmitoyl-protein database SwissPalm (Blanc et al., 2015; https://swisspalm.org) and the software CSS-Palm 4.0 (Palmitoylation Site Prediction with a Clustering and Scoring Strategy) (Ren et al., 2008; http://csspalm.biocuckoo.org/index.php). SwissPalm is based on published palmitoyl-protein proteomics data. I uploaded the list of ~13,800 proteins expressing in the mouse brain (Sharma et al., 2015) and ABHD17B-interacting proteins to examine if each protein is reported as a palmitoylated protein. On the other hand, the CSS-palm 4.0 algorithm is based on

experimentally verified palmitoylation sites: 263 palmitoylation sites from 109 distinct palmitoylated proteins. The mouse protein sequences downloaded from the UniProt database (http://www.uniprot.org/) were uploaded to CSS-Palm 4.0 to predict if each protein is a palmitoylated protein.

***In silico* gene ontology (GO) analysis**

Protein annotation through evolutionary relationship (PANTHER) software (Mi et al., 2019; http://pantherdb.org) was used for GO analysis. GO is a tool used to annotate genes, collect, and analyze information according to cellular component, biological process, and molecular function terms. I uploaded the list of ABHD17B-interacting proteins to PANTHER. GO enrichment analysis was performed by using ~13,800 proteins expressing in the mouse brain (Sharma et al., 2015) as a background comparison set.

Cell culture and transfection

COS7 cells and HEK293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich) at 37°C with 5% CO_2. COS7 cells and HEK293T cells were transfected with plasmid DNAs by Lipofectamine and plus reagent system (Life technologies).

Primary cultures of rat hippocampal neurons were prepared from rat embryonic day 19 embryos. Pregnant Wister/ST rats were purchased from Japan SLC, Inc. and used on the day the rats were received. A pregnant rat with embryonic rats was killed by CO_2 inhalation and then embryos were removed and decapitated. Hippocampi were dissected from embryonic rat brains and placed on ice with Ca^{2+}- and Mg^{2+}-free Hanks-buffered saline solution [CMF-HBSS; 10 mM glucose and 10 mM Hepes (pH 7.3 with NaOH) in Hanks' Balanced Salt solution (Nacalai

Tesque)]. To dissociate hippocampal neurons, hippocampi were treated with 30 units papain (Worthington Biochemical) in 3 ml buffer [5 mM L-Cysteine hydrochloride (Thermo Fisher Scientific), 1 mM EDTA, and 5 mg/ml bovine serum albumin (BSA; Nacalai Tesque) in CMF-HBSS] for 10 minutes at 37°C. Dissociated neurons were plated onto polyethyleneimine coated 6-well plates or poly-L-lysine coated 12-mm glass coverslips in 24-well plates with a plating medium [neuro basal medium (NBM) containing 10% FBS and 10 mM Hepes (pH 7.3 with NaOH)]. Neurons were incubated at 37°C and 5% CO_2 for 3 hours, and then the medium was replaced by a neurobasal medium supplemented with B-27, 2 mM GlutaMAX, and 10 mM Hepes (pH7.3 with NaOH). Half of the medium was removed and replaced with flesh medium every 7 days.

Acyl-biotin exchange (ABE) assay

The ABE assay was performed as described (Kang et al., 2008; Roth et al., 2006) with some modifications. A WT mouse brain was homogenized in 2.5 ml of buffer H2 (1 mM HEPES, 320 mM sucrose, 50 μg/ml PMSF, 10 μg/ml leupeptin, and 10 μg/ml pepstatin A). After sonication and centrifugation at 100,000 *g* for 15 minutes, the supernatant was diluted to 0.4 mg/ml protein concentration with Buffer A (4% SDS, 5 mM EDTA, 50 μg/ml PMSF, 10 μg/ml leupeptin, and 10 μg/ml pepstatin A in PBS) containing 8 M urea. The supernatant (0.4 mg/ml, 1800 μg) was reduced with 25 mM TCEP for 1 hour at room temperature. Rat cultured neurons (14 DIV) were treated with 2-BP (100 μM) alone or in combination with ABD957 (1 μM) or HDFP (20 μM) for 4 hours at 37°C. After treatment, neurons were harvested with Buffer A. Neuron lysates (0.3-0.4 mg/ml, up to 180 μg) were reduced with 25 mM TCEP for 1 hour at 55°C. The brain supernatant or neuron lysates were rotated with 50 mM NEM for 3 hours at room temperature in the dark to protect free-thiol groups. Then, unreacted NEM was removed from samples by CM precipitation. Precipitates were dissolved in 125 μl of 4% SDS buffer [4% SDS, 5 mM

EDTA, 50 mM Tris-HCl (pH 7.5), 4 M NaCl, 10 μg/ml leupeptin, and 10 μg/ml pepstatin A] and incubated for 1 hour at 37°C with 375 μl of HA1 buffer [1 M HA (pH 7.5), 150 mM NaCl, 0.2% Triton X-100, and 26% *N*,*N*-dimethylformamide] containing 1.3 mM Biotin-HPDP or Tris1 buffer [1 M Tris-HCl (pH 7.5), 150 mM NaCl, 0.2% Triton X-100, and 26% *N*,*N*-dimethylformamide] containing 1.3 mM Biotin-HPDP as a negative control. After CM precipitation, precipitates were dissolved in 100 μl of 2% SDS buffer [2% SDS, 5 mM EDTA, 50 mM Tris-HCl (pH 7.5), 4 M NaCl, 10 μg/ml leupeptin, and 10 μg/ml pepstatin A] and diluted to 0.2% SDS. The solutions were incubated with 30 μl of NeutrAvidin Agarose for 1 hour at 4°C. The beads were washed with wash buffer [0.1% SDS, 0.2% Triton X-100, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, and 150 mM NaCl] five times and incubated at 37°C for 20 minutes with 150 μl of wash buffer containing 2% β-mercaptoethanol to reduce the disulfide bond between palmitoylated cysteine residue and biotin. After microfiltration, eluates were subjected to SDS-PAGE followed by WB.

17-octadecynoic acid (17-ODYA) labeling and click chemistry

17-ODYA labeling and click chemistry were performed as described previously (Martin and Cravatt, 2009) with some modifications. HEK293T cells were seeded onto 6-well plates (5.0×10^5 cells/well), cultured overnight, and co-transfected with Flag-tagged ABHD17B and HA- or EGFP-tagged substrate candidates. For transfection, 1 μg plasmid of each construct was used. After 24 hours, the cells were incubated in DMEM containing 5 mg/ml fatty-acid free bovine serum albumin (BSA) for 30 minutes at 37°C, and the cells were labeled with 40 μM 17-ODYA for 4 hours at 37°C. The cells were washed with PBS buffer, lysed on ice with 1.5 ml of Lysis buffer (0.1% SDS and 1.0% TritonX-100 in PBS), and centrifuged at 100,000 *g* for 30 minutes. For HA-tagged substrate candidates, supernatants were rotated with anti-HA antibody for 1 hour at 4°C, and then Protein G-Sepharose was added and incubated for 1 hour at 4°C. For

GFP-tagged substrate candidates, supernatants were rotated with the GFP-trap beads for 2 hours at 4°C. IPs were washed with PBS buffer containing 1.0% TritonX-100 at three times. The click reaction was performed on beads with 2.5 μM IRDye 800CW Azide Infrared Dye for 1 hour at room temperature using Click-iT Protein Reaction Buffer Kit according to manufacturer's protocol. Beads were washed with PBS buffer containing 1.0% TritonX-100 at five times, suspended with SDS-sample buffer containing 10 mM DTT, and heated at 95°C for 5 minutes. The eluates were subjected to SDS-PAGE followed by WB and in-gel fluorescence analysis. In-gel fluorescence was detected using Odyssey CLX.

Immunofluorescence analysis

Rat cultured hippocampal neurons (0.4×10^5 cells/well) were fixed with 4% paraformaldehyde for 10 minutes at room temperature followed by permeabilization with 0.1% TritonX-100 for 10 minutes on ice. After blocking with PBS containing 1% BSA for 10 minutes on ice, neurons were stained with indicated antibodies and visualized with Alexa Fluor488, Cy3 or/and Alexa Fluor647-conjugated secondary antibodies. Fluorescent images were taken with a confocal laser scanning microscopy system (TCS SP5II; Leica) equipped with a HCX PL APO 63×/1.40-0.60 oil immersion lens. Microscope control and all image analyses were performed with Leica LAS AF software. The intensity of the fluorescence was calculated using Leica LAS AF software.

Acyl-PEGyl exchange gel shift (APEGS) assay

Rat hippocampal neurons were seeded at a density of 5.0×10^5 cells onto polyethyleneimine coated 6-well dishes and cultured in NBM supplemented with B-27 and 2 mM GlutaMAX. Cultured rat hippocampal neurons were harvested with Buffer A. After sonication and centrifugation at 200,000 *g* for 15 minutes, neuron lysates (0.3-0.4 mg/ml, up to 180 μg) were

reduced with 25 mM TCEP for 1 hour at 55°C in water bath and incubated with 50 mM NEM for 3 hours at room temperature in the dark. After CM precipitation, resuspended proteins in 125 μl of 4% SDS buffer were incubated in 375 μl of HA2 buffer [1.3 M HA (pH 7.5), 5 mM EDTA, and 0.2% Triton X-100 in PBS] or Tris2 buffer [1.3 M Tris-HCl (pH 7.5), 5 mM EDTA, and 0.2% Triton X-100 in PBS] for 1 hour at 37°C. After CM precipitation, resuspended proteins in 4% SDS buffer were PEGylated with 200 mM mPEG-2k or 100 mM mPEG-5k for 1 hour at room temperature to label newly exposed cysteinyl thiol groups. After CM precipitation, precipitates were resuspended with 60 μl of SDS-sample buffer and incubated at 37°C for 20 minutes. After protein concentrations were measured by BCA protein assay, samples were added β-mercaptoethanol and boiled at 100°C for 3 minutes. For WB, the EzFastBlot HMW transfer buffer (ATTO) was used for high transfer efficiency.

Results

In this study, to reveal the physiological functions of ABHD17s, I attempted to identify novel substrates for ABHD17s and clarify the regulatory mechanism for ABHD17s through the comprehensive identification of ABHD17B-interacting proteins. Because the generation of specific ABHD17s antibodies was difficult, I decided to use the KI mouse in which ABHD17B was tagged with enhanced green fluorescent protein (EGFP) at the C-terminus (referred to as ABHD17B-EGFP). After confirming that ABHD17B-EGFP is functional *in vitro*, the KI mouse carrying an EGFP cDNA before the stop codon in exon 4 of the *Abhd17b* gene was generated. Western blotting with anti-GFP antibody detected a strong band with molecular mass of about 60-kDa (p60) in the brain of heterozygous (KI/+) or homozygous KI (KI/KI) mice, but not in that of WT (+/+) mice. The observed molecular weight was compatible with the estimated molecular weight of ABHD17B-EGFP. Then, I purified ABHD17B-EGFP and comprehensively identified proteins co-purified with ABHD17B-EGFP by label-free quantitative proteomic analysis using LC-MS/MS analysis. I identified 263 ABHD17B-interacting proteins. I next classified ABHD17B-interacting proteins by the expected function according to GO enrichment analysis, and found that ABHD17B-interacting proteins are significantly enriched in biological processes including G protein-coupled signaling and cAMP mediated signaling pathways. By palmitoyl-protein database analysis, I found that palmitoylated proteins are enriched in ABHD17B-interacting proteins. From these 263 proteins, I selected 11 proteins as substrate candidates and explored if selected proteins are palmitoylated in the mouse brain by the ABE assay. I detected the palmitoylation of seven out of 11 selected proteins. Subsequent analyses including the metabolic labeling followed by the click reaction and the APEGS assay identified proteins that are depalmitoylated by ABHD17B in transfected heterologous cells and in cultured neurons from selected ABHD17B-interacting proteins.

Furthermore, I found that these substrate proteins physiologically undergo the palmitate cycling mediated by ABHD17s and localize at the plasma membrane in a palmitoylation-dependent manner in neurons.

Conclusion

In my thesis, leveraging the ABHD17B-EGFP KI mouse, I comprehensively identified ABHD17B-interacting proteins in the mouse brain and selected 11 substrate candidates for ABHD17B substrates. Among them, I found the proteins that are depalmitoylated by ABHD17B and observed that ABHD17B regulates their localization in heterologous cells. Importantly, I found that two of these proteins continuously undergo palmitoylation-depalmitoylation cycles in cultured hippocampal neurons, and ABHD17s are responsible for their depalmitoylation. Thus, I identified the novel physiological, neuronal substrates for ABHD17s.

References

Abrami L, Kunz B, Iacovache I, van der Goot FG (2008) Palmitoylation and ubiquitination regulate exit of the Wnt signaling protein LRP6 from the endoplasmic reticulum. Proc Natl Acad Sci U S A 105:5384-5389.

Blanc M, David F, Abrami L, Migliozzi D, Armand F, Burgi J, van der Goot FG (2015) SwissPalm: Protein Palmitoylation database. F1000Res 4:261.

Camp LA, Hofmann SL (1993) Purification and properties of a palmitoyl-protein thioesterase that cleaves palmitate from H-Ras. J Biol Chem 268:22566-22574.

Duncan JA, Gilman AG (1998) A cytoplasmic acyl-protein thioesterase that removes palmitate from G protein alpha subunits and p21(RAS). J Biol Chem 273:15830-15837.

Fukata M, Fukata Y, Adesnik H, Nicoll RA, Bredt DS (2004) Identification of PSD-95 palmitoylating enzymes. Neuron 44:987-996.

Hancock JF, Magee AI, Childs JE, Marshall CJ (1989) All ras proteins are polyisoprenylated but only some are palmitoylated. Cell 57:1167-1177.

Hayashi T, Rumbaugh G, Huganir RL (2005) Differential regulation of AMPA receptor subunit trafficking by palmitoylation of two distinct sites. Neuron 47:709-723.

Kang R, Wan J, Arstikaitis P, Takahashi H, Huang K, Bailey AO, Thompson JX, Roth AF, Drisdel RC, Mastro R, Green WN, Yates JR, 3rd, Davis NG, El-Husseini A (2008) Neural palmitoyl-proteomics reveals dynamic synaptic palmitoylation. Nature 456:904-909.

Linder ME, Middleton P, Hepler JR, Taussig R, Gilman AG, Mumby SM (1993) Lipid modifications of G proteins: alpha subunits are palmitoylated. Proc Natl Acad Sci U S A 90:3675-3679.

Lobo S, Greentree WK, Linder ME, Deschenes RJ (2002) Identification of a Ras palmitoyltransferase in Saccharomyces cerevisiae. J Biol Chem 277:41268-41273.

Martin BR, Cravatt BF (2009) Large-scale profiling of protein palmitoylation in mammalian cells. Nature Methods 6:135-138.

Ren J, Wen L, Gao X, Jin C, Xue Y, Yao X (2008) CSS-Palm 2.0: an updated software for palmitoylation sites prediction. Protein Eng Des Sel 21:639-644.

Resh MD (1999) Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. Biochimica et Biophysica Acta 1451:1-16.

Roth AF, Wan J, Bailey AO, Sun B, Kuchar JA, Green WN, Phinney BS, Yates JR, 3rd, Davis NG (2006) Global analysis of protein palmitoylation in yeast. Cell 125:1003-1013.

Sharma K, Schmitt S, Bergner CG, Tyanova S, Kannaiyan N, Manrique-Hoyos N, Kongi K, Cantuti L, Hanisch UK, Philips MA, Rossner MJ, Mann M, Simons M (2015) Cell type- and brain region-resolved mouse brain proteome. Nat Neurosci 18:1819-1831.

Tian L, McClafferty H, Knaus HG, Ruth P, Shipston MJ (2012) Distinct acyl protein transferases and thioesterases control surface expression of calcium-activated potassium channels. J Biol Chem 287:14718-14725.

Tomatis VM, Trenchi A, Gomez GA, Daniotti JL (2010) Acyl-protein thioesterase 2 catalyzes the deacylation of peripheral membrane-associated GAP-43. PLoS One 5: e15045.

Topinka JR, Bredt DS (1998) N-terminal palmitoylation of PSD-95 regulates association with cell membranes and interaction with K+ channel, Kv1.4. Neuron 20:125-134.

Vartak N, Papke B, Grecco HE, Rossmannek L, Waldmann H, Hedberg C, Bastiaens PI (2014) The autodepalmitoylating activity of APT maintains the spatial organization of palmitoylated membrane proteins. Biophys J 106:93-105.

Verkruyse LA, Hofmann SL (1996) Lysosomal targeting of palmitoyl-protein thioesterase. J Biol Chem 271:15831-15866.

Yokoi N, Fukata Y, Sekiya A, Murakami T, Kobayashi K, Fukata M (2016) Identification of PSD-95 Depalmitoylating Enzymes. J Neurosci 36:6431-6444.

Zhang FL, Casey PJ (1996) Protein prenylation: molecular mechanisms and functional consequences. Annu Rev Biochem 65:241-269.