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Review Article

A sticky situation: regulation and function of protein palmitoylation with a spotlight on the axon and axon initial segment

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In neurons, the axon and axon initial segment (AIS) are critical structures for action potential initiation and propagation. Their formation and function rely on tight compartmentalisation, a process where specific proteins are trafficked to and retained at distinct subcellular locations. One mechanism which regulates protein trafficking and association with lipid membranes is the modification of protein cysteine residues with the 16-carbon palmitic acid, known as S-acylation or palmitoylation. Palmitoylation, akin to phosphorylation, is reversible, with palmitate cycling being mediated by substrate-specific enzymes. Palmitoylation is well-known to be highly prevalent among neuronal proteins and is well studied in the context of the synapse. Comparatively, how palmitoylation regulates trafficking and clustering of axonal and AIS proteins remains less understood. This review provides an overview of the current understanding of the biochemical regulation of palmitoylation, its involvement in various neurological diseases, and the most up-to-date perspective on axonal palmitoylation. Through a palmitoylation analysis of the AIS proteome, we also report that an overwhelming proportion of AIS proteins are likely palmitoylated. Overall, our review and analysis confirm a central role for palmitoylation in the formation and function of the axon and AIS and provide a resource for further exploration of palmitoylation-dependent protein targeting to and function at the AIS.

Introduction

Neurons are large, complex, polarised cells. Their asymmetry allows the directional flow of information received on dendrites and cell body to the axon and subsequent downstream neuron. The compartmentalisation of subcellular regions with distinct functions is critical for neuronal activity, translating into complex behaviours such as learning and memory. Proteins and organelles must be sorted and trafficked to specific sites, often over long distances, each uniquely important for neuronal signaling and function. One way in which neurons precisely regulate protein trafficking is via post-translational modifications. In recent years, S-acylation has attracted significant attention due to its reversible nature, akin to that of phosphorylation, allowing for dynamic changes in palmitoylation and, subsequently, protein localisation in response to neuronal activity or signaling. Indeed, S-acylation is the most observed protein lipid modification of neuronal proteins [1] and mutations in or loss of S-acylating enzymes *in vivo* in many cases predominantly present with neurological phenotypes [2–13].

A primer on palmitoylation

S-acylation is the reversible addition of long-chain fatty acids to cysteine residues via a thioester bond (Figure 1). Saturated and unsaturated fatty acids of various lengths can be utilised, including palmitic, myristic, oleic, stearic, and arachidonic acids. However, the 16-carbon, saturated fatty acid palmitate is the
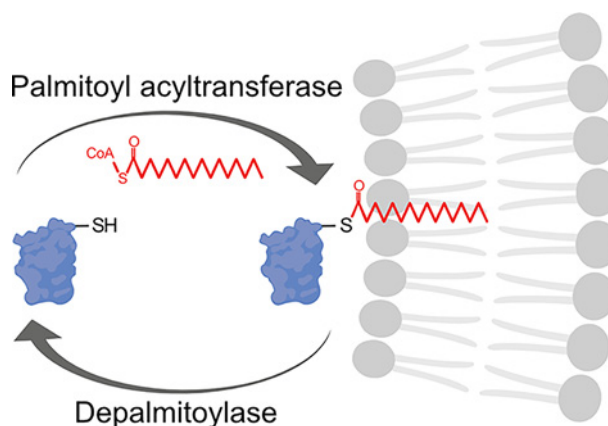
Figure 1. Dynamic protein palmitoylation
Long-chain fatty acids (red; commonly the 16-carbon saturated palmitic acid) are added to cytosolic cysteine residues (–SH) of proteins by palmitoyl acyltransferases via a thioester bond. This increases the binding affinity of palmitoylated proteins for membranes (grey phospholipid bilayer) and/or membrane subdomains and can regulate protein–protein interactions, protein stability, other post-translational modifications, and protein function. The reverse reaction is catalyzed by depalmitoylases including acyl protein thioesterases, palmitoyl protein thioesterases, and α/β hydrolase domain-containing serine hydrolases. Figure created with BioRender.com.

most common fatty acid added to proteins, therefore, S-acylation is often referred to as S-palmitoylation or, simply, palmitoylation and will be referred to as such in this review [14–16]. More than 10% of the human proteome is predicted to be palmitoylated [17,18], including proteins involved in a diverse array of biological processes, some of which include synaptic plasticity [19,20], cardiac contractility [21], immune response [22], and pathogen–host interactions and infection [23–25].

The hydrophobic nature of fatty acids increases the binding affinity of palmitoylated proteins for membranes and can regulate protein–protein interactions, protein stability, and other post-translational modifications [26]. The localisation and function of some soluble, cytosolic proteins require palmitoylation for stable membrane association [27]. Transmembrane proteins can also be modified. Palmitoylation can alter the conformation of protein transmembrane domains or cytosolic regions, regulate protein–protein interactions, and promote association with specific membrane microdomains [28–31]. In neurons, palmitoylation is best known to regulate synaptic expression and clustering of adhesion molecules, vesicle release machinery, signaling molecules, and neurotransmitter receptors, as well as their scaffolds and kinases [32–44] (reviewed in [1,20,45–47]). Indeed, previous estimates suggest that 41% of all synaptic proteins are substrates for palmitoylation [17]. However, palmitoylation-dependent regulation of protein trafficking and targeting to other neuronal locations, particularly within the axon, is not as well understood compared with what is known at the synapse, and for this reason, will be highlighted in this review.

Palmitoyl acyltransferases

Palmitoylation is catalyzed by the ZDHHC family of palmitoyl acyltransferases (PATs; Figure 1) that contain a conserved Asp–His–His–Cys (DHHC) active site motif within a zinc-finger cysteine-rich domain (CRD) [48]. The DHHC active site motif catalyzes palmitoylation and the CRD coordinates two zinc atoms in a zinc-finger motif, crucial for structural stability [49–53]. The PAT gene family is conserved throughout eukaryotes and the 23 mammalian ZDHHC PATs demonstrate distinct but overlapping substrate specificity [16,54].

PATs can be found throughout the endomembrane system, particularly the Golgi apparatus, endoplasmic reticulum (ER), endosomes, and plasma membrane, allowing substrates to be modified at different stages of their life [55,56]. Additionally, proteins that contain multiple palmitoylation sites can be modified at different intracellular locations by various PATs [57,58]. PATs are integral membrane proteins that contain four to six transmembrane domains with the conserved DHHC-CRD oriented to face the cytosol [16,59]. In addition to the DHHC-CRD, PATs have three other conserved motifs. The DPG (Asp–Pro–Gly) motif located amino (N)-terminal to the DHHC-CRD with unknown function. The TTxE (Thr–Thr–X–Glu, where X is any amino acid) and PaCCT (palmitoyltransferase conserved carboxyl (C)-terminus) motifs are located C-terminal to the DHHC-CRD and are critical for the enzyme's structural integrity and activity [51,60,61]. PATs use a two-step 'ping-pong' mechanism to palmitoylate substrates

where the cysteine residue in the DHHC motif is first autopalmitoylated and then palmitate is transferred to the substrate protein [49–51].

Despite the high similarity of the DHHC-CRD among the PATs, the N- and C-termini of these enzymes are poorly conserved, having diverse sequences and sizes contributing to substrate selectivity [16,54]. ZDHHC13 and ZDHHC17 have N-terminal ankyrin repeat domains that interact with many of their substrates' zDHHC ankyrin-binding motifs to allow palmitoylation [62–67]. Eight of twenty-three human PATs contain a C-terminal PSD95/discs large/ZO-1 (PDZ) ligand that binds PDZ domain-containing proteins, many of which are involved in synapse formation and neuronal regulation [68]. ZDHHC14 has a C-terminal type I PDZ motif that binds its substrate the type-I PDZ domain-containing Membrane-associated Guanylate Kinase (MaGUK) family scaffold protein PSD93 (postsynaptic density 93/chapsyn 110/DLG2) [68,69]. In contrast, ZDHHC3, ZDHHC5, ZDHHC7, ZDHHC8, ZDHHC16, ZD-HHC17, ZDHHC20, and ZDHHC21 have C-terminal type II PDZ motifs that have been shown to or are predicted to bind their type II domain-containing substrates [38,44,68]. Finally, the C-terminus of ZDHHC6 is unique in that it contains an Src homology 3 domain, which allows it to bind to selenoprotein K [70].

Depalmitoylating enzymes

Due to the labile nature of the thioester bond, palmitoylation is reversible, with many proteins undergoing dynamic cycles of palmitoylation and depalmitoylation (Figure 1) [71–73]. Dynamic palmitoylation of the transferrin receptor, ankyrin, and N-RAS was first reported in the 1980s [74–76]. Even though the first depalmitoylating enzymes were discovered years before the first PAT in the 1990s [77,78], depalmitoylation is not as well understood. Acyl protein thioesterases (APTs), palmitoyl protein thioesterases (PPTs), and α/β hydrolase domain-containing proteins (ABHDs) are the three known types of depalmitoylating enzymes, all belonging to a larger family of metabolic serine hydrolases [77–85]. These three classes of depalmitoylating enzymes all contain an α/β hydrolase fold with a Ser–His–Asp catalytic triad and active serine residue consensus motif of Gly–X–Ser–X–Gly [79,82,86–90].

PPT1 was the first depalmitoylating enzyme discovered in 1993 [78] and is predominantly a lysosomal enzyme mediating depalmitoylation prior to protein degradation [91]. Mutations in the gene coding for PPT1 cause a lysosomal storage neurodegenerative disease known as infantile neuronal ceroid lipofuscinosis [92]. There is also some evidence of a cytoplasmic role for PPT1 at synapses [93–95].

APT1 (LYPLA1) and APT2 (LYPLA2) are the most well-studied cytosolic depalmitoylating enzymes, but APT1-like (APT1L; LYPLAL1) as well as the ABHD17 family (A–C) have also been shown to have cytoplasmic depalmitoylating activity [77,79,80,83,85]. ABHD10 is a mitochondrial resident depalmitoylating enzyme [82] and while APT1 has also been observed in mitochondria, its role in depalmitoylating mitochondrial proteins is unclear [96]. The consensus is that to mediate depalmitoylation of their substrates the cytoplasmic depalmitoylating enzymes need to come into proximity with the membrane. Indeed, ATP1, APT2, and ABHD17A–C have all been shown to be palmitoylated themselves [97–99] and an interesting new mechanism of membrane binding was just identified for APT2 [100]. This study from the van der Goot group showed that APT2 binds membranes in a three-step process where long-range electrostatic interactions attract APT2 to the lipid membrane. This then allows the β-tongue of APT2 to dip into the membrane, temporarily holding APT2 in place, allowing its subsequent palmitoylation by ZD-HHC3 or ZDHHC7, thereby stably binding it to the membrane [100]. After APT2 identifies a target protein, it extracts palmitate from the membrane into its catalytic hydrophobic pocket for hydrolysis [100]. It will be interesting to see if the other depalmitoylating enzymes use similar mechanisms. A key outstanding question is how depalmitoylating enzymes recognise their substrates and how they are regulated by neuronal activity or signaling events.

Palmitoylation in neurological disorders

Disrupted palmitoylation is associated with several neurological disorders in humans, including X-linked intellectual disability, bipolar disorder, schizophrenia, and neuronal ceroid lipofuscinosis (reviewed in [64,101]). Mutations in the *ZDHHC9* gene are associated with X-linked intellectual disability [5,102–105], sometimes with epilepsy [12]. Consistent with this, *Zdhhc9*-deficient mice display altered excitatory/inhibitory synapse balance, seizure-like activity [34], and altered hippocampal-based learning and memory [106].

Two closely related PATs, ZDHHC5 and ZDHHC8, have been associated with schizophrenia. Chromosomal deletions in the region including *ZDHHC5* are linked with schizophrenia as well as bipolar disorder [107,108], and a *de novo* missense mutation in *ZDHHC5* has been identified in patients with schizophrenia [109]. Significant associations between schizophrenia and small nucleotide polymorphisms in the *ZDHHC8* gene, within the schizophrenia risk 22q11 microdeletion, have been identified in multiple populations [8,110–112] but not in others [113–118]. However, consistent with a role for ZDHHC8 in schizophrenia, 22q11-deletion mice have reduced dendritic growth,

spine density, and glutamatergic synapses that can be rescued with exogenous expression of ZDHHC8. Also, *Zdhhc8*-deficient mice display similar dendritic and synaptic changes and schizophrenia-like phenotypes [8,9]. Notably, a widespread reduction in palmitoylation was also observed in post-mortem dorsolateral prefrontal cortex of patients with schizophrenia, as well as reduced palmitoylation of vesicular glutamate transporter 1 (VGLUT1), RAS, and myelin basic protein [119].

Finally, mutations in genes encoding the depalmitoylating enzyme PPT1 and the palmitoylated cysteine string protein (CSP; DNAJC5) cause neuronal ceroid lipofuscinosis a lysosomal-storage neurodegenerative disease [120,121].

Evidence from animal models and cell culture systems also suggests a role for aberrant palmitoylation in various neurodegenerative diseases, including Huntington disease (HD), amyotrophic lateral sclerosis (ALS), Parkinson disease (PD), and Alzheimer disease (AD). HD is a neurodegenerative disorder caused by a CAG repeat expansion in the *HTT* gene coding for an elongated polyglutamine tract in the huntingtin (HTT) protein [122]. HTT is normally palmitoylated at Cys^{214} by ZDHHC17 and ZDHHC13 (also known as Huntingtin Interacting Proteins [HIPs] 14 and 14-like [HIP14 and HIP14L], respectively) [3,123], but mutant HTT is less palmitoylated in HD patient-derived cells and mouse models of HD [123,124]. Recent exciting findings from the Hayden and Saudou groups show that increasing palmitoylation by inhibiting the HTT depalmitoylating enzymes *in cellulo* and *in vivo* reduces cytotoxicity, mutant HTT aggregation, and restores trafficking deficits in cell culture and rescues behaviour and neuropathology in HD mice [124,125]. These two studies provide strong evidence for a role of palmitoylation in HD and rationale for targeting depalmitoylating enzymes to treat HD.

The evidence for a role of palmitoylation in other neurodegenerative diseases, including ALS, PD, and AD is not as strong but is briefly discussed here (reviewed in more depth in [64,101]). Familial ALS-linked superoxide dismutase 1 (SOD1) mutants are more palmitoylated than wildtype SOD1 in ALS-mouse models and human spinal cord. Palmitoylation prevents SOD1 disulphide bonding and, in turn, its maturation, which may contribute to the pathogenesis of ALS [126,127].

In a recently published study, Ho et al. showed that palmitoylation may play a role in PD for the first time. Inhibiting depalmitoylation reduces α-synuclein inclusions and phosphorylation at Ser^{129} (a PD neuropathological marker) as well as decreases neurotoxicity in human neuroblastoma cells, rat neurons, and induced pluripotent stem cell-derived PD patient neurons [128]. These protective effects are proposed to be due to restoring palmitoylation of microtubule-associated protein 6 (MAP6) [128]. It will be interesting to see the results of depalmitoylation inhibitor treatment in a longer term preclinical study in animal models of PD, such as was recently done in HD [125].

AD is linked to the formation of β-amyloid (Aβ) plaques derived via aberrant cleavage of the amyloid precursor protein (APP) by β-secretase (predominantly β-site APP cleaving enzyme 1 or BACE1) and γ-secretase enzymes [129]. BACE1 and γ-secretase subunits Nicastrin and APH-1 (anterior pharynx-defective 1), as well as APP itself are all palmitoylated [130–133]. While the role of BACE1 palmitoylation in pathological APP processing remains controversial (discussed in [101]), APP palmitoylation enhances amyloidogenic processing of APP to Aβ [131,134] and expressing palmitoylation defective Nicastrin and APH-1 in mice decreased amyloid deposits in the brain [135]. Additional investigations are needed to determine which palmitoylation event is the most critical for Aβ production and if targeting PATs or depalmitoylating enzymes would be a viable treatment approach.

Palmitoylation-dependent axonal protein trafficking and targeting

While the importance of palmitoylation-dependent regulation of protein trafficking and targeting to the synapse is well established, palmitoylation-dependent protein targeting to other neuronal locations, particularly within the axon, is less well understood. Axons are long, thin projections that project up to a meter away from the cell body or soma in humans and are much longer than dendrites. The correct connection of axons to their target during development and long-term integrity of mature axons is essential for nervous system function. Indeed, axonal degeneration and axon transport deficits is a hallmark of many neurodegenerative diseases, including during acute nervous system injury [136] as well as in chronic neurodegenerative diseases such as AD, HD, and chemotherapy- and diabetes-associated neuropathies [137–139]. Thus, the efficient, precise control of protein trafficking and targeting in axons presents a significant challenge for the neuron. As with the synapse, palmitoylation of axonal proteins could provide a dynamic mechanism to control protein targeting and function. We will discuss below what is already known regarding palmitoylation-dependent protein trafficking and localisation during neuronal development and retrograde signaling from the distal axon back to the cell body or soma as well as to the cytoskeleton and presynaptic compartment. In addition, an interesting new role for palmitoylation in the regulation of protein clustering at another axonal compartment, the axon initial segment (AIS) is emerging, and we will expand on that here as well.

Neuronal development

During neuronal development, neurite outgrowth as well as axon determination, the establishment of polarity, and axon pathfinding rely on several palmitoylated proteins (reviewed in [140]). Palmitoylation of neural cell adhesion molecule (NCAM) by ZDHHC7 directs it to lipid rafts in growth cones promoting neurite outgrowth and axon pathfinding [141–143]. Interestingly, palmitoylation of NCAM is induced by activation of the fibroblast growth factor (FGF) receptor by FGF2 treatment which stimulates neurite outgrowth in hippocampal neurons [142]. Palmitoylation of CDC42 by ZDHHC8 promotes axon growth and branching. Palmitoylated CDC42 is targeted and transported to the tip of the axon, where it modulates axon branching and length [8,144]. Rapid and dynamic palmitoylation of c-Jun N-terminal kinase (JNK) 3 is essential for axonal branching and growth during development. Unlike NCAM, palmitoylation does not regulate lipid raft association of JNK3 and instead reduces its translocation to the actin cytoskeleton to inhibit axonal branching and axon length. Thus, palmitoylation-deficient JNK3 increases axonal branching and motility of axonal filopodia [145]. Finally, GAP43 is another palmitoylated protein [146] that plays an important role in neuronal growth by modulating levels of the phospholipid phosphatidylinositol 4,5-bisphopsphate to regulate the assembly of actin-based structures and promote neurite outgrowth [147]. Targeting GAP43 to axonal membranes and to growth cones via detergent-insoluble glycolipid-enriched complexes depends on its palmitoylation [146,148,149].

Palmitoylation is also involved in the bifurcation of somatosensory afferents from the dorsal root ganglia (DRG) neuron [150]. The ligand C-type natriuretic peptide (CNP) mediates a cyclic guanosine monophosphate (cGMP)-dependent signaling pathway that promotes neurite elongation and DRG growth cone expansion. This CNP-mediated growth cone enlargement is potentiated by thioesterase inhibitors and blocked by broad-spectrum palmitoylation inhibitors. As such, cGMP-stimulated palmitoylation of proteins is thought to be implicated in axon bifurcation [150]. These findings are all consistent, demonstrating the important role of palmitoylation during neuronal development.

Several cytoskeleton proteins, molecular motors, and their scaffolds are also known to be palmitoylated and their palmitoylation plays an important role during neuronal polarisation, axon development, and maintenance. Axons and dendrites have different patterns of microtubule orientations, which is important for the development of neuronal polarity and axon versus dendritic sorting of proteins. In axons, microtubules are oriented with plus ends out whereas in dendrites microtubule orientations are mixed [151]. The stabilisation of microtubules in axons during the establishment of neuronal polarity requires the axon-enriched MAP6 [151,152]. Palmitoylation is required for MAP6 tethering to Golgi membranes and targeting of subsequent secretory vesicles to axons. Once in the axon, MAP6 is depalmitoylated by ABHD17A–C, allowing it to detach from Golgi vesicles and become enriched on microtubules within newly formed axons [152].

Furthermore, several subunits of dynein and kinesin motor proteins involved in anterograde (away from the soma) and retrograde (towards the soma) trafficking are known palmitoyl proteins or have been identified in palmitoyl proteomic studies [17,18]. Both evolutionarily conserved NUDE proteins, Nde1 and Ndel1 of the LIS1–Ndel1–Nde1 complex are palmitoylated. This complex positively regulates the retrograde motor cytoplasmic dynein. Nde1 interacts with LIS1 and dynein light and intermediate chains, and Ndel1 also interacts with LIS1 and dynein heavy and intermediate chains. Interestingly, blocking palmitoylation of Ndel1 increases its interaction with dynein as well as increases dynein activity and axonal transport [153]. Palmitoylation of dynein intermediate chain (DYNC1I1) was verified in a low throughput study [144]. DYNC1I1 plays a role in retrograde trafficking and mediates binding to its accessory factor dynactin via p150^{Glued}. However, while DYNC1I1 palmitoylation was confirmed, no studies were performed to determine the role of palmitoylation in regulating DYNC1I1 function. In addition, no low throughput and functional follow-up studies have been performed on palmitoylation of other dynein and kinesin motor protein subunits identified in palmitoyl proteomic studies. Furthermore, it would be interesting to determine if subunits of the final, working motor complexes are palmitoylated and, if so, how that regulates motor function, speed, and binding to membranous cargo. Alternatively, palmitoylation may negatively regulate complex formation preventing assembly of the mature, working motor complex. These questions would be an interesting area for future investigation.

Presynaptic protein targeting

Palmitoylation is important for the presynaptic targeting of several proteins critical for synaptic transmission. γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system and is produced in GABAergic inhibitory neurons [154,155]. The GABA-synthesising enzyme glutamate decarboxylase 65 (GAD65)

is a hydrophilic cytosolic protein primarily localised at presynaptic clusters within axons [156,157]. Both palmitoylation of GAD65 and the subsequent binding of GAD65 to Golgi membranes are critical for its trafficking to presynaptic clusters [158]. When GAD65 is palmitoylated, it colocalises with a small guanosine triphosphate-binding protein known as Rab5a within axonal but not with somatodendritic endosomes. In contrast, palmitoylation-deficient GAD65 is absent from presynaptic clusters despite the presence of Rab5a [156].

Several synaptic vesicle release machinery proteins are also palmitoylated. Synaptotagmin I (SYT1) is localised to synaptic vesicles where it binds Ca^{2+} ions to trigger the release of neurotransmitters from synaptic vesicles. Palmitoylation of SYT1 is required for its sorting to the presynaptic vesicle pool [144,159]. Synaptosome-associated protein of 25 kDa (SNAP25) is a target SNARE (synaptic soluble *N*-ethylmaleimide-sensitive fusion attachment protein receptor) that complexes with the vesicle SNARE vesicle-associated membrane protein (VAMP) and another target SNARE syntaxin 1. This complex mediates synaptic vesicle fusion with the plasma membrane during neurotransmitter release. While syntaxin 1 and VAMP are integral membrane proteins, SNAP25 is palmitoylated [160,161] and its palmitoylation is required for membrane binding, syntaxin-independent trafficking to the plasma membrane, and SNARE complex disassembly and exocytosis [162,163].

Axon integrity and degeneration

In addition to its roles in neuronal development and presynaptic protein targeting and function, palmitoylation is a critical regulator of axon-to-soma signaling, axon integrity, and axon degeneration. Axotomy in cultured neurons or nerve transection or nerve crush *in vivo* are models of traumatic axon injury that activate intrinsic axon degeneration pathways both proximal and distal to the injury site. Palmitoylation of dual leucine-zipper kinase (DLK; also known as mitogen-activated protein kinase kinase kinase 12 [MAP3K12]) plays a critical role in axon injury pro-degeneration response in the proximal axon and soma [164,165]. Axon injury induces DLK-dependent axon and soma degeneration in sensory and motor neurons. DLK activation triggers an MAPK cascade involving its direct MAP2K targets MKK4 and MKK7 and the MAPK JNK, resulting in phosphorylation of the transcription factor c-Jun [166–168]. When DLK is palmitoylated, it localises to axonal transport vesicles where it complexes with JIP3, MKK4, MKK7, and JNK3 to allow retrograde pro-degenerative signaling in response to injury (Figure 2A,B) [164,165,169]. Blocking palmitoylation of DLK completely prevents JNK activation and degeneration both in cultured neurons and *in vivo* [164,165]. Indeed, blocking palmitoylation of JNK3 also blocks pro-degenerative phosphorylation of c-Jun following axonal injury [169]. These findings highlight the critical role palmitoylation plays in neurodegeneration and provide a strong rationale for identifying compounds that inhibit DLK palmitoylation to treat chronic neurodegeneration and acute neuronal injury [170].

Conversely, palmitoylation also regulates two important axon survival factors critical for Wallerian degeneration [140]. Wallerian degeneration is the degeneration of the axon distal to the injury site and is important in the peripheral nervous system to clear axonal debris to promote regeneration. However, 'Wallerian-like' degeneration is also observed in chronic degenerative conditions, so there is considerable interest in the mechanisms underlying this type of degeneration [136,171,172]. The microtubule-stabilising protein superior cervical ganglion 10 (SCG10; also known as stathmin-2 [STMN2]) is a short-lived protein that is constantly supplied to the axon by fast axonal transport and is implicated in TDP-43 neuropathies, in particular ALS [173]. Rapid degradation of SCG10 in healthy and injured axons is JNK dependent. Knockdown of SCG10 accelerates degeneration, whereas blocking JNK-dependent phosphorylation and subsequent degradation of SCG10 delays axon degeneration [174]. SCG10 is palmitoylated and transported anterogradely in axons in a palmitoylation-dependent manner by fast axonal transport (Figure 2C) [175,176]. Interestingly, its degradation also depends on palmitoylation and the activation of DLK/JNK [175]. These findings suggest that palmitoylation tethers SCG10 to transport vesicles to maintain a constant supply in distal axons but also plays a role in its labile nature (Figure 2C,D). This means that rate-limiting amounts of SCG10 are maintained in distal axons such that in the event of an axonal injury, SCG10 levels fall quickly so Wallerian degeneration can occur.

Nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2) is another short-lived survival factor (half-life <1 h in cultured neurons). NMNAT2 is a nicotinamide adenine dinucleotide (NAD^+)-synthesising enzyme that must be constantly supplied to the axon as a limiting factor for axon survival [177–179]. NMNAT2 knockdown causes axon degeneration in the absence of injury and, in injured neurons, NMNAT2 levels in the distal axon drop rapidly, inducing Wallerian degeneration [180]. This degeneration can be prevented by overexpression of NMNAT2 or expression of Wld^S (a stable, partially cytosolic form of NMNAT1, a paralogue of NMNAT2) that substitutes for NMNAT2 [177,178]. NMNAT2 is palmitoylated and transported anterogradely in axons in a palmitoylation-dependent manner by fast axonal transport (Figure 2C) [181,182]. However, palmitoylation-deficient NMNAT2 is less ubiquitinated, more stable, and, subsequently, more protective against axon degeneration when overexpressed in cultured
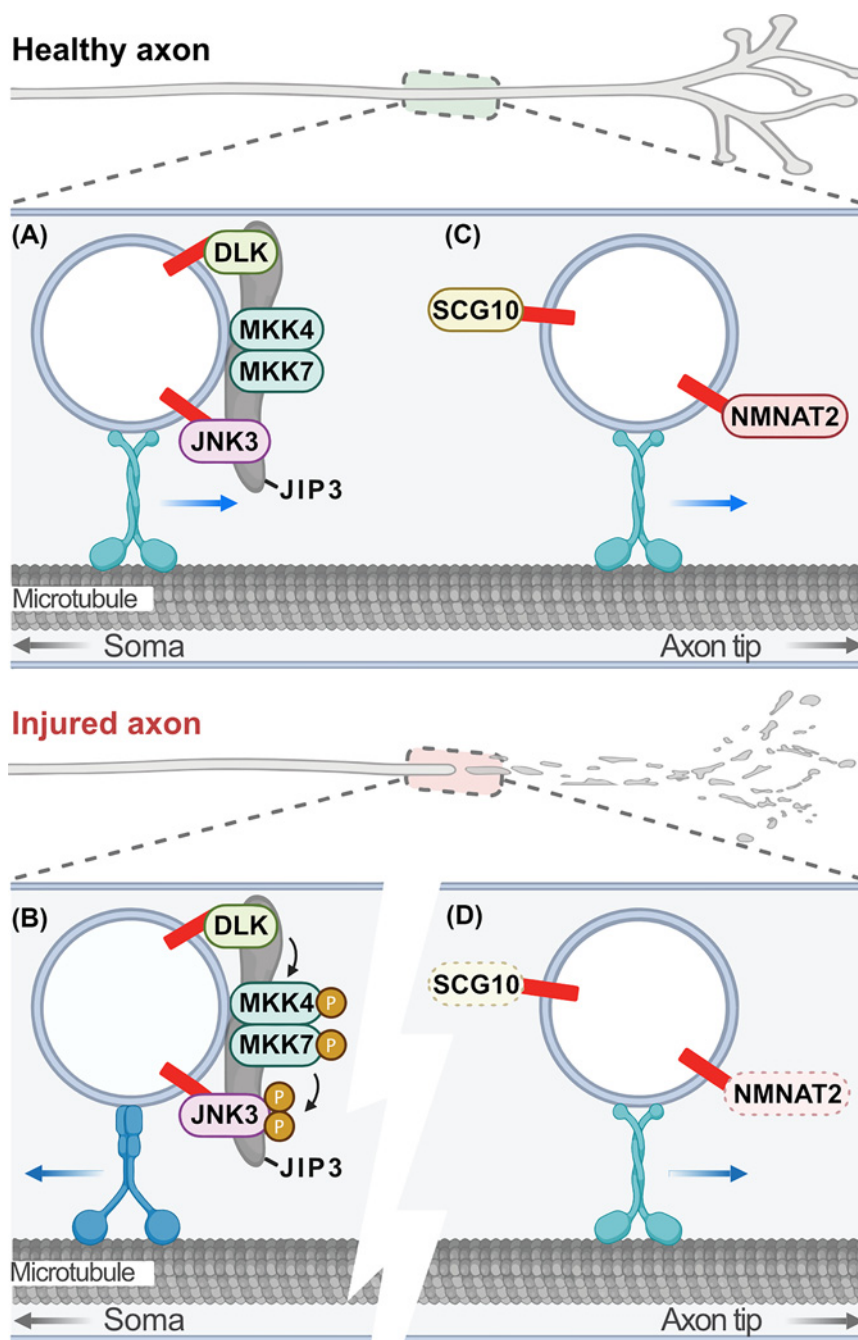
Figure 2. Palmitoylation-dependent regulation of axon survival and degeneration

(**A**) In healthy peripheral projecting sensory or retinal ganglion axons DLK and JNK3 are trafficked on axon transport vesicles into distal axons in a palmitoylation-dependent manner along with MKK4/7 and the scaffold JIP3. (**B**) Following axonal injury DLK is activated on vesicles proximal to the injury site where it phosphorylates its direct targets MKK4/7, which then phosphorylates JNK3. This pro-degenerative signaling transport vesicle traffics retrogradely back to the soma in a palmitoylation-dependent manner to induce neurodegeneration. (**C**) Also in healthy axons, the labile, pro-survival factors NMNAT2 and SCG10 are continually supplied on axon transport vesicles into distal axons in a palmitoylation-dependent manner. (**D**) Following axonal injury, the constant supply of SCG10 and NMNAT2 from the soma into distal axons is blocked, and SCG10 and NMNAT2 are quickly degraded, inducing Wallerian degeneration. Figure created with BioRender.com. Abbreviation: NMNAT2, nicotinamide mononucleotide adenylyltransferase 2; SCG10, superior cervical ganglion 10.

neurons than wildtype palmitoylated NMNAT2 [182]. Interestingly, the DiAntonio group recently found that different populations of NMNAT2 are targeted for degradation by different mechanisms [175,178]. Palmitoylated NMNAT2 degradation is DLK/JNK dependent, whereas non-palmitoylated NMNAT2 degradation is dependent on the Phr1/Skp1a/Fbxo45 E3 ligase complex [175,178]. Like SCG10, palmitoylation of NMNAT2 likely provides a mechanism for its delivery over long distances to distal axons while also contributing to its labile nature to ensure it is present in distal axons in rate-limiting amounts to induce Wallerian degeneration in the event of an injury (Figure 2D).

Until recently, the PATs that mediate NMNAT2 and DLK palmitoylation in neurons were unknown. Interestingly, the Thomas group identified ZDHHC17 as the enzyme responsible for palmitoylation of both NMNAT2 and DLK [165]. Acute loss of ZDHHC17 blocks DLK-dependent pro-degenerative signaling and the induction of apoptosis following injury but the long-term loss of ZDHHC17 results in NMNAT2-dependent degeneration of distal axons. This finding is intriguing, but why would the same PAT be used to palmitoylate pro-degenerative and pro-survival proteins? It was hypothesised that palmitoylation of DLK and NMNAT2 by ZDHHC17, localised to the Golgi, forms a 'trust but verify' system. In this system, palmitoyl-NMNAT2 and palmitoyl-DLK are delivered to distal axons where palmitoyl-NMNAT2 will serve a neuroprotective role and palmitoyl-DLK is present to respond to axonal injury (Figure 2). Additionally, the regulation of NMNAT2 and DLK by a single PAT is advantageous as it allows for coordinated regulation of expression and function of DLK and NMNAT2 in the distal axon [165].

The DLK pro-degenerative signaling complex is not the only retrogradely trafficked complex regulated by palmitoylation. The palmitoylated glycoprotein 130 (Gp130, gene name *IL6ST*) is a critical component of another retrograde signaling complex that plays a role in neurogenesis, stem cell fate, and response to injury [56]. Gp130 receptor complexes respond to neuropoietic cytokines, including ciliary neurotrophic factor (CNTF), cardiotrophin-1, and leukemia inhibitory factor, to activate the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signal transduction pathway [183]. Knockdown of axonal PATs ZDHHC5 and ZDHHC8 decreases Gp130 palmitoylation and surface expression as well as significantly attenuates retrograde signaling in DRG neurons by the Gp130/JAK/STAT3 pathway in response to CNTF stimulation [56]. Interestingly, JAK1 is also palmitoylated by ZDHHC3 and ZDHHC7, and its palmitoylation is required for downstream phosphorylation of STAT3 and neuronal survival [184].

Palmitoylation-dependent AIS protein targeting

An exciting new role for palmitoylation-dependent regulation of protein trafficking and targeting to another site within axons, the AIS, has recently emerged. The AIS is proximal to the soma and is the site of integration of synaptic input and generation of action potentials (Figure 3). The scaffold protein Ankyrin G (AnkG; ANK3) is highly enriched in the submembrane at this site along with voltage-gated sodium (Na_v1) and potassium channels (K_v7, and K_v1) [185–188]. Anchoring of Na_v1 and K_v7 channels at the AIS critically requires AnkG [189–192]. Interestingly, AnkG is palmitoylated on a cysteine residue in the membrane-binding domain, which is required for its membrane association and polarisation in epithelial cells and its targeting to and scaffolding ability at the AIS in neurons (Figure 3) [193,194]. This was the first example of palmitoylation-dependent regulation of protein targeting at the AIS. AnkG AIS-binding partners Na_v1 and K_v7 channels are known or are predicted to be palmitoylated as well [18,195,196]. Whether or not palmitoylation plays a direct role in their AIS clustering is unknown, but this would be an interesting area for future investigation.

A subset of voltage-gated potassium channels, $K_v1.1$, $K_v1.2$, and $K_v1.4$, are also present at the AIS but lack an AnkG-binding motif and do not associate with AnkG. How these proteins are targeted to the AIS has remained poorly understood [197,198]. One mechanism aiding in K_v1 channel localisation to the AIS is through the association with the scaffold protein PSD93 [199,200]. PSD93 is a well-known palmitoyl protein, but until recently, the function of its palmitoylation was unclear [41,201,202]. PSD93 localises to postsynaptic sites, but palmitoylation is not required for its synaptic targeting [41]. Of the MaGUK family, PSD93 is the only one that enriches at the AIS [199] and, interestingly, its palmitoylation is required for its clustering at this site (Figure 3) [69].

The only PAT that contains a C-terminal type-I PDZ ligand (S/T-X-V) that would be predicted to bind to PSD93's type-I PDZ domains is ZDHHC14 [68]. Indeed, the C-terminal PDZ ligand of ZDHHC14 binds PSD93's third PDZ domain. The subsequent palmitoylation of PSD93 by ZDHHC14 also requires ZDHHC14's PDZ ligand, suggesting that the ligand is important not only for PSD93 binding, but also for substrate recognition [69]. Indeed, ZDHHC14 is the predominant PSD93 PAT in neurons where loss of ZDHHC14 significantly reduces PSD93 palmitoylation and targeting to the AIS [69]. Consistent with this, expression of a palmitoylation deficient PSD93 in neurons also reduces PSD93 targeting to the AIS, albeit less intensely than with loss of ZDHHC14.
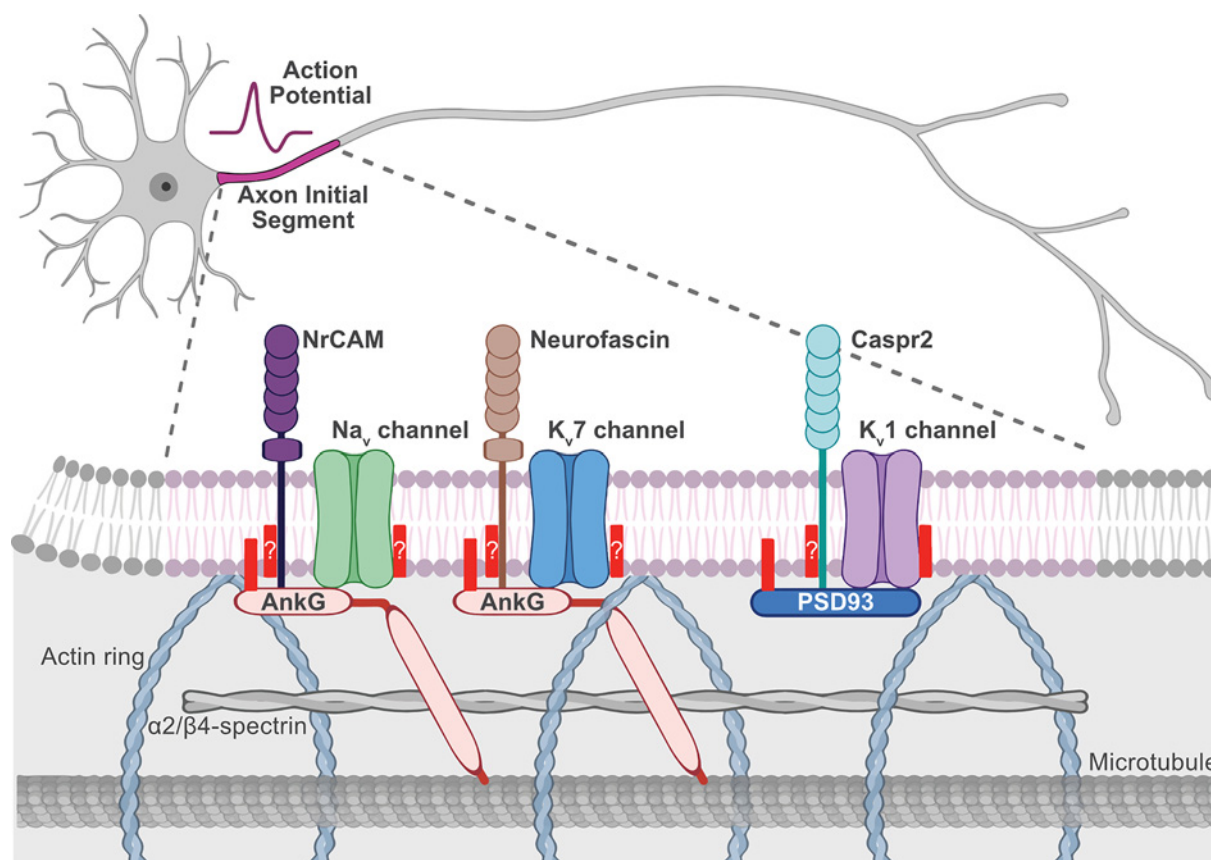
Figure 3. Palmitoylation-dependent regulation of protein targeting to the AIS

The AIS is in the proximal part of the axon (pink) and is the site of action potential generation. Action potential firing is dependent on the high density of voltage-gated sodium (Na_v; green) and potassium channels (K_v7 [light blue] and K_v1 [light purple]) at this site. These ion channels are clustered at the AIS via interactions with their scaffolds AnkG (light red) and PSD93 (dark blue) that also interact with the cell adhesion molecules NrCAM (dark purple), Neurofascin (brown), and Capsr2 (teal) and with the underlying actin, $\alpha2/\beta4$-spectrin, and microtubule cytoskeleton. All these AIS components are either targeted to the AIS in a palmitoylation-dependent manner (solid red bar, AnkG, PSD93, and K_v1 channels) or are palmitoylated, but the role of palmitoylation in AIS targeting is currently unknown (red bar with a question mark). Figure created with BioRender.com. Abbreviation: NrCAM, neuronal cell adhesion molecule.

ZDHHC14 is a very interesting PAT and very little is known regarding its role in mammalian cells. It is highly expressed in the hippocampus [203,204] and is intolerant to loss of function genetic mutations in humans [205] and may play a role in cancers [206,207]. There were no known unique ZDHHC14 substrates prior to the identification of PSD93. One study identified the human β2-adrenergic receptor (β2AR) as a redundant substrate of ZDHHC9, ZDHHC14, and ZDHHC18. However, the specific contribution of ZDHHC14 to β2AR palmitoylation, particularly in a relevant cell type without overexpression is unclear [208].

At the AIS, PSD93 acts as a scaffold for K_v1 channels [199]. K_v1 clustering likely involves binding of the second PDZ domain of PSD93 to the C-terminal type-I PDZ ligands of $K_v1.1/1.2/1.4$ [200,209]. K_v1 channels are also palmitoylated on a cysteine residue in a highly conserved region in the loop between their second and third transmembrane domains [69,210]. Interestingly, the developmental expression of ZDHHC14 mirrors that of PSD93 and $K_v1.1/1.2/1.4$ with expression first detectable at day *in vitro* (DIV) eight and increasing to DIV16, suggesting a shared function in hippocampal neurons. Indeed, palmitoylation of K_v1 channels is also ZDHHC14-dependent. Furthermore, ZDHHC14 also regulates AIS targeting of K_v1 channels themselves, such that loss of ZDHHC14 reduces AIS targeting of K_v1 channels [69]. Like many other PATs, ZDHHC14 is predominantly localised to the ER and Golgi apparatus [55,56,69] but has also been observed on the plasma membrane in polarised endothelial cells [193] and on dendritic punctate structures and in axons, but not enriched at the AIS, in hippocampal neurons [69]. This suggests that

palmitoylation of PSD93 and K_v1 channels likely occurs early in their trafficking route prior to insertion in the AIS. However, these studies used overexpressed epitope-tagged ZDHHC14, so the true endogenous localisation and site of K_v1/PSD93 palmitoylation are still unclear.

Intriguingly, ZDHHC14-deficient hippocampal neurons are hyperexcitable with a dramatic decrease in the density of outward currents as well as increased action potential firing [69]. These neurophysiological changes are consistent with a loss of K_v1 channels at the AIS, although the effects of loss of ZDHHC14 on neuronal excitability are more dramatic than changes in AIS targeting of any individual K_v1 channel [69]. This raises the intriguing possibility that ZDHHC14 may be responsible for palmitoylating other AIS components that may be contributing to the hyperexcitability phenotype.

Although loss of ZDHHC14 significantly reduces palmitoylation of PSD93 and K_v1 channels, it does not entirely abolish palmitoylation. Another study suggested that ZDHHC17 palmitoylates the zebrafish orthologue of $K_v1.1$, but the effects of endogenous neuronal $K_v1.1$ after loss of ZDHHC17 were not examined [211]. Additionally, ZD-HHC17 lacks a Type I-PDZ ligand, making it less likely to mediate residual PSD93 palmitoylation and, indeed, loss of ZDHHC17 *in vivo* in mice does not alter PSD93 palmitoylation [212]. However, whether other PATs contribute to palmitoylation of PSD93/K_v1 channels is possible and warrants further investigation.

In addition to palmitoylation-dependent regulation of AIS targeting of AnkG, PSD93, and K_v1 channels, we noticed that several other main AIS components are known to be palmitoylated or have been identified in palmitoyl proteomic studies, including Na_v and K_v7 channels, as mentioned above, and AIS cell adhesion molecules neurofascin (NF186), neuronal cell adhesion molecule (NrCAM), and contactin-associated protein-like 2 (Caspr2; Figure 3). NF186 is palmitoylated and its palmitoylation is required for sorting to specialised membrane domains but not for AnkG binding, but whether palmitoylation regulates its AIS targeting is not clear [213]. NrCAM has been identified in one palmitoyl proteome [18] and has a cysteine in a homologous sequence to that of NF186 [213], so it is likely also palmitoylated, but no follow-up studies have been performed. Caspr2 has also been identified in one palmitoyl proteome study [18], but again, no follow-up studies have been performed.

To further understand the importance of palmitoylation in regulating the clustering and function of AIS proteins, we sought to determine whether palmitoylation is a prominent modification in the AIS proteome. Even though many individual proteins are well-known to be localised to the AIS, until recently there had been no systematic studies to define the entire AIS proteome [186]. However, a recent study from the Rasband group used BioID (multiplexed proximity biotinylation followed by mass spectrometry) to map proteins localised to the AIS [214,215]. They used BirA* biotin ligase fused to NF186, Ndel1, and Trim46 to identify the AIS membrane-proximal, shallow cytoplasm, and microtubule-associated proteins, respectively.

Using the proteins that showed the most significant change between control and experimental conditions (i.e., Figure 2A and Figure 7F, and Supplemental Figure 6B of [214]; see reference for details), we compiled a single, non-redundant list of putative AIS proteins. The BioID study did not identify several known AIS proteins, including $Na_v1.6$, K_v1 channels, Na_v and K_v1 β subunits, PSD93, $K_v7.2$, $K_v7.3$, NrCAM, and Caspr2. We manually added these proteins to the NF186 proteome and obtained a cumulative AIS proteome dataset comprised of 172 genes (Supplementary Table S1). This dataset was then uploaded to the palmitoyl protein database SwissPalm and compared with all available human, mouse, and rat palmitoyl proteomic datasets (54 in total) [18]. Due to the rat nomenclature of the proteins used in the BioID dataset, some were not recognised in the palmitoyl-proteome comparison, so all negative hits in the AIS dataset, i.e., not found in any study or found in only one palmitoyl proteome, were screened manually in SwissPalm. All proteins/orthologues identified in at least two palmitoyl proteomic studies or one targeted study were counted as palmitoylated. As a control comparison, the synaptic gene set (SynGO release 1.1, all genes) was downloaded from the SynGO database and subjected to the same comparison using SwissPalm [18,216].

Surprisingly, our new analysis revealed that a staggering 77.9% of the AIS proteome is likely to be palmitoylated (Figure 4A, Supplementary Table S2). This was much higher than the 48.4% of palmitoylated proteins at the synapse, a well-known palmitoylation hotspot (Figure 4A) [17,20]. Further analysis of palmitoylation in each proteome revealed that low-throughput targeted studies frequently verified proteins identified in the NF186 proteome, but some were only rarely found in high-throughput palmitoyl proteomic studies (Figure 4B). In contrast, those proteins identified in the Trim46 (microtubule-associated) proteome were commonly identified in high-throughput palmitoyl proteomic studies, but rarely verified by low-throughput targeted studies (Figure 4D). The Ndel1 (shallow cytoplasm) proteome was the least enriched for palmitoylated proteins (Figure 4C).

The NF186 proteome contained the highest number of known palmitoylated AIS proteins, such as AnkG, NF186, and $Na_v1.6$ [193,196,213]. It was also the proteome most enriched in low-throughput targeted verification studies. These observations indicate that palmitoylation of the AIS membrane-proximal proteome is the most well-verified and likely to be highly significant to the function of the AIS. Proteins identified by Trim46, despite showing a high
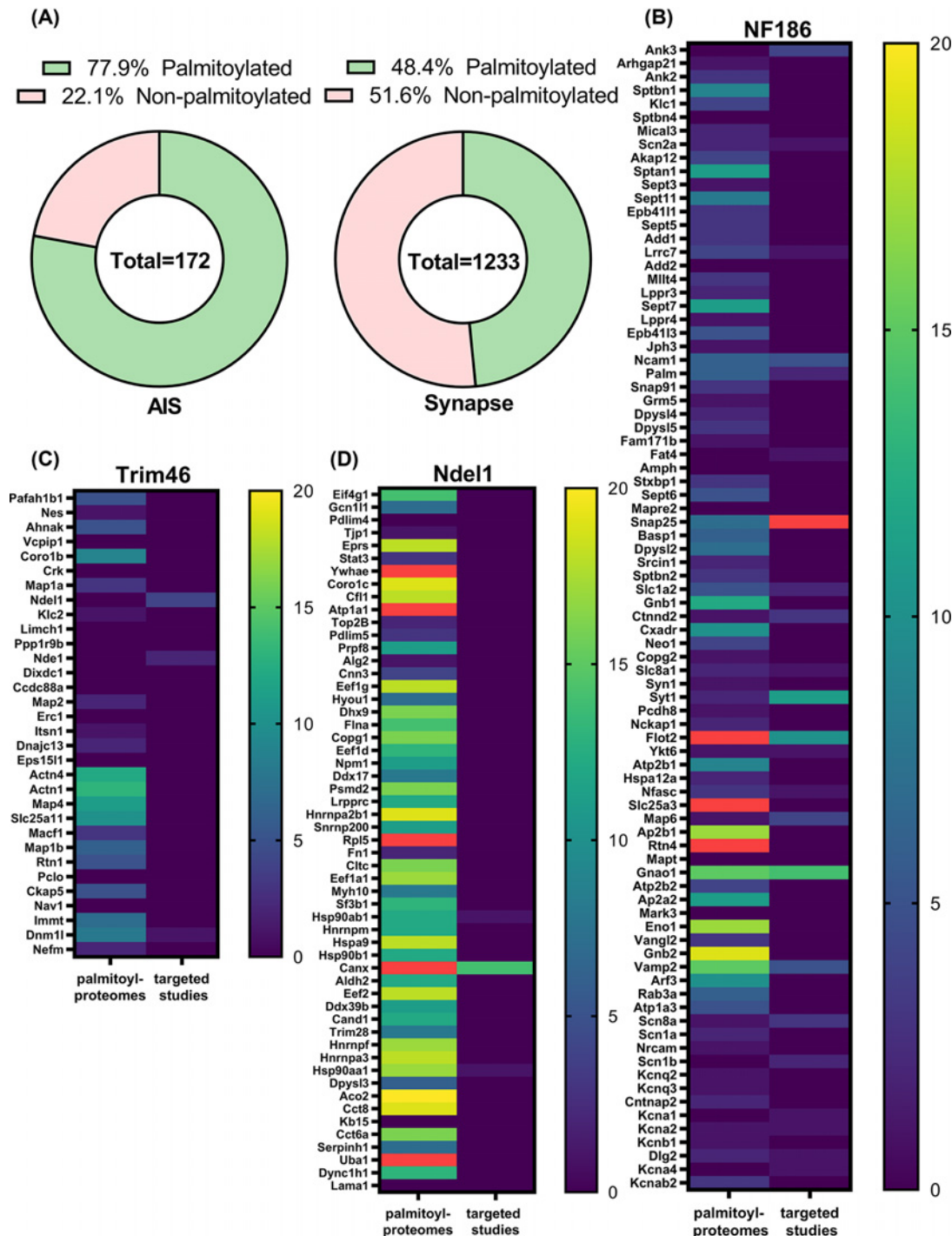
Figure 4. Palmitoyl-proteome comparison analysis reveals the prevalence of palmitoylation among AIS proteins

(**A**) Donut charts showing the percentage of palmitoylated proteins in AIS or synaptic proteomes. The totals indicate the number of proteins in each dataset. The AIS proteome gene list was assembled from the highest confidence AIS proteins identified by Hamdan et al. ([214]) and several known AIS proteins missed in the Hamdan study. Synaptic proteome genes were extracted from the SynGO database (SynGO release 1.1-all genes [216]). Both gene sets were subjected to palmitoyl-proteome comparison analysis in SwissPalm using all 54 available mouse, human, and rat proteomes. All proteins found in at least two palmitoyl proteomes or one targeted study were designated as palmitoylated. (**B–D**) Heat maps showing all genes in membrane-proximal (NF186, B), microtubule-associated (Trim46, C), and shallow cytoplasm (Ndel1, D) and AIS proteomes and the number of palmitoyl proteome and targeted studies for each gene. Red colour designates values above 20. The data on the number of studies were extracted from the palmitoyl-proteome comparison analysis described above.

enrichment in high-throughput palmitoyl proteomic studies, have been largely missed by low-throughput targeted investigations (except for calnexin). This proteome contains several families of proteins, such as ribonuclear proteins, chaperones, chaperonins, and Dihydropiriminidase-related proteins, which have been previously unknown to localise to the AIS or to be verified as palmitoylated. Given these observations, one promising avenue for further investigation would be to study palmitoylation of AIS microtubule-associated and cytoplasmic proteins.

Conclusions and future directions

The bulk analysis of the AIS proteome for palmitoylated proteins reveals that palmitoylation is likely to be a crucial modification of AIS proteins, even more so than at the synapse. Our review provides an extensive basis for follow-up study. Indeed, the only AIS proteins known to localise to the AIS in a palmitoylation-dependent manner are AnkG, PSD93, and K_v1 channels. The vast enrichment of the AIS proteome for palmitoylated proteins is an intriguing finding and may suggest that palmitoylation provides a broad mechanism to anchor AIS-resident proteins at this site. Indeed, the AIS is a very structurally dense subcellular compartment (i.e., high density of proteins localised to a relatively small and narrow area) that acts as a trafficking gateway to the rest of the axon [217–219]. Thus, it is plausible that in such a dense environment with a lot of cargo passing by firm attachment and anchorage to the AIS membrane is provided by palmitoylation for many AIS proteins. Alternatively, palmitoylation could be a mechanism that is required for tethering proteins to transport vesicles or motor protein subunits and/or interaction with scaffold proteins during trafficking to the AIS [164,220].

Though our new analysis and the studies discussed here suggest that palmitoylation may participate in trafficking and retention of proteins at the AIS, several questions remain unanswered in the current literature. Is palmitoylation necessary and/or sufficient for AIS targeting or do protein–protein interactions dominate in determining AIS targeting? Are palmitoylated AIS proteins modified with a particular fatty acid that is preferentially inserted into the AIS plasma membrane? Is there a unique aspect about the number or position of acyl modifications that serves as the trafficking/retention signal? Even in the case of K_v1 channels and PSD93 it is unknown if palmitoylation of one or both are required for their interaction and whether this interaction occurs on the Golgi membrane or if PSD93 captures and retains K_V1 channels at the AIS. Answering these questions will be crucial for identifying the factors that determine how palmitoylation participates in establishing and organising axonal subcompartments.

Data Availability

All associated datasets are available as supplementary data files.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

ABHD, α/β hydrolase domain-containing protein; AD, Alzheimer disease; AIS, axon initial segment; ALS, amyotrophic lateral sclerosis; AnkG, Ankyrin G; APH-1, anterior pharynx-defective 1; APP, amyloid precursor protein; APT, acyl protein thioesterase; Aβ, β-amyloid; BACE1, β-site APP cleaving enzyme 1; Caspr2, contactin-associated protein-like 2; cGMP, cyclic guanosine monophosphate; CNTF, ciliary neurotrophic factor; CNP, C-type natriuretic peptide; CRD, cysteine-rich domain; DLK, dual leucine-zipper kinase; DRG, dorsal root ganglia; GABA, γ-aminobutyric acid; GAD65, glutamate decarboxylase 65; Gp130, glycoprotein 130; HD, Huntington disease; HTT, huntingtin; JAK/STAT, Janus kinase/signal transducer and activator of transcription; JNK, c-Jun N-terminal kinase; MaGUK, membrane-associated guanylate kinase; MAP6, microtubule-associated protein 6;

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NCAM, neural cell adhesion molecule; NMNAT2, nicotinamide mononucleotide adenylyltransferase 2; NrCAM, neuronal cell adhesion molecule; PAT, palmitoyl acyltransferase; PD, Parkinson disease; PDZ, C-terminal PSD95/discs large/ZO-1; PPT, palmitoyl protein thioesterase; PSD93, postsynaptic density 93/chapsyn 110/DLG2; SCG10, superior cervical ganglion 10; SNAP25, synaptosome-associated protein of 25 kDa; SNARE, synaptic soluble *N*-ethylmaleimide-sensitive fusion attachment protein receptor; SOD1, superoxide dismutase 1; VAMP, vesicle-associated membrane protein; β2AR, β2-adrenergic receptor.

References

1 Fukata, Y. and Fukata, M. (2010) Protein palmitoylation in neuronal development and synaptic plasticity. *Nat. Rev. Neurosci.* **11**, 161–175, https://www.nature.com/articles/nrn2788, https://doi.org/10.1038/nrn2788

2 Li, Y., Hu, J., Hofer, K., Wong, A.M.S., Cooper, J.D., Birnbaum, S.G. et al. (2010) DHHC5 interacts with PDZ domain 3 of post-synaptic density-95 (PSD-95) protein and plays a role in learning and memory. *J. Biol. Chem.* **285**, 13022–13031, https://doi.org/10.1074/jbc.M109.079426

3 Sutton, L.M., Sanders, S.S., Butland, S.L., Singaraja, R.R., Franciosi, S., Southwell, A.L. et al. (2013) Hip14l-deficient mice develop neuropathological and behavioural features of Huntington disease. *Hum. Mol. Genet.* **22**, 452–465, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=23077216&retmode=ref&cmd=prlinks, https://doi.org/10.1093/hmg/dds441

4 Singaraja, R.R., Huang, K., Sanders, S.S., Milnerwood, A.J., Hines, R., Lerch, J.P. et al. (2011) Altered palmitoylation and neuropathological deficits in mice lacking HIP14. *Hum. Mol. Genet.* **20**, 3899–3909, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=21775500&retmode=ref&cmd=prlinks, https://doi.org/10.1093/hmg/ddr308

5 Raymond, F.L., Tarpey, P.S., Edkins, S., Tofts, C., O'Meara, S., Teague, J. et al. (2007) Mutations in ZDHHC9, which encodes a palmitoyltransferase of NRAS and HRAS, cause X-linked mental retardation associated with a Marfanoid Habitus. *Am. J. Hum. Genet.* **80**, 982–987, http://linkinghub.elsevier.com/retrieve/pii/S0002929707609549, https://doi.org/10.1086/513609

6 Mansouri, M.R., Marklund, L., Gustavsson, P., Davey, E., Carlsson, B., Larsson, C. et al. (2005) Loss of ZDHHC15 expression in a woman with a balanced translocation t(X;15)(q13.3;cen) and severe mental retardation. *Eur. J. Hum. Genet.* **13**, 970–977, http://www.nature.com/doifinder/10.1038/sj.ejhg.5201445, https://doi.org/10.1038/sj.ejhg.5201445

7 Sanders, S.S., Parsons, M.P., Mui, K.K.N., Southwell, A.L., Franciosi, S., Cheung, D. et al. (2016) Sudden death due to paralysis and synaptic and behavioral deficits when Hip14/Zdhhc17 is deleted in adult mice. *BMC Biol.* **14**, 108–113, http://bmcbiol.biomedcentral.com/articles/10.1186/s12915-016-0333-7, https://doi.org/10.1186/s12915-016-0333-7

8 Mukai, J., Liu, H., Burt, R.A., Swor, D.E., Lai, W.-S., Karayiorgou, M. et al. (2004) Evidence that the gene encoding ZDHHC8 contributes to the risk of schizophrenia. *Nat. Genet.* **36**, 725–731, http://www.nature.com/doifinder/10.1038/ng1375, https://doi.org/10.1038/ng1375

9 Mukai, J., Dhilla, A., Drew, L.J., Stark, K.L., Cao, L., MacDermott, A.B. et al. (2008) Palmitoylation-dependent neurodevelopmental deficits in a mouse model of 22q11 microdeletion. *Nat. Neurosci.* **11**, 1302–1310, http://www.nature.com/doifinder/10.1038/nn.2204, https://doi.org/10.1038/nn.2204

10 Mukai, J., Tamura, M., Fénelon, K., Rosen, A.M., Spellman, T.J., Kang, R. et al. (2015) Molecular substrates of altered axonal growth and brain connectivity in a mouse model of schizophrenia. *Neuron* **86**, 680–695, https://doi.org/10.1016/j.neuron.2015.04.003

11 Kilpatrick, C.L., Murakami, S., Feng, M., Wu, X., Lal, R., Chen, G. et al. (2016) Dissociation of Golgi-associated DHHC-type zinc finger protein (GODZ) and Sertoli cell gene with a zinc finger domain-β (SERZ-β)-mediated palmitoylation by loss of function analyses in knockout mice. *J. Biol. Chem.*, . http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=27875292&retmode=ref&cmd=prlinks, https://doi.org/10.1074/jbc.M116.732768

12 Baker, K., Astle, D.E., Scerif, G., Barnes, J., Smith, J., Moffat, G. et al. (2015) Epilepsy, cognitive deficits and neuroanatomy in males with ZDHHC9 mutations. *Ann. Clin. Transl. Neurol.* **2**, 559–569, https://doi.org/10.1002/acn3.196

13 Mejias, R., Rodriguez-Gotor, J.J., Niwa, M., Krasnova, I.N., Adamczyk, A., Han, M. et al. (2021) Increased novelty-induced locomotion, sensitivity to amphetamine, and extracellular dopamine in striatum of Zdhhc15-deficient mice. *Transl. Psychiatry* **11**, 1–13, https://doi.org/10.1038/s41398-020-01194-6

14 Smotrys, J.E. and Linder, M.E. (2004) Palmitoylation of intracellular signaling proteins: regulation and function. *Annu. Rev. Biochem.* **73**, 559–587, http://www.annualreviews.org/doi/abs/10.1146/annurev.biochem.73.011303.073954, https://doi.org/10.1146/annurev.biochem.73.011303.073954

15 Hallak, H., Muszbek, L., Laposata, M., Belmonte, E., Brass, L.F. and Manning, D.R. (1994) Covalent binding of arachidonate to G protein alpha subunits of human platelets. *J. Biol. Chem.* **269**, 4713–4716, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=8106438&retmode=ref&cmd=prlinks, https://doi.org/10.1016/S0021-9258(17)37602-0

16 Malgapo, M.I.P. and Linder, M.E. (2021) Substrate recruitment by zDHHC protein acyltransferases. *Open Biol.* **11**, 210026, https://doi.org/10.1098/rsob.210026

17 Sanders, S.S., Martin, D.D.O., Butland, S.L., Lavallée-Adam, M., Calzolari, D., Kay, C. et al. (2015) Curation of the mammalian palmitoylome indicates a pivotal role for palmitoylation in diseases and disorders of the nervous system and cancers. *PLoS Comput. Biol.* **11**, e1004405–1004420, http://dx.plos.org/10.1371/journal.pcbi.1004405, https://doi.org/10.1371/journal.pcbi.1004405

18 Blanc, M., David, F., Abrami, L., Migliozzi, D., Armand, F., Bürgi, J. et al. (2015) SwissPalm: Protein Palmitoylation database. *F1000Res.* **4**, 261, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=26339475&retmode=ref&cmd=prlinks, https://doi.org/10.12688/f1000research.6464.1

19 Ji, B. and Skup, M. (2021) Roles of palmitoylation in structural long-term synaptic plasticity. *Mol. Brain* **14**, 1–27, https://doi.org/10.1186/s13041-020-00717-y

20 Matt, L., Kim, K., Chowdhury, D. and Hell, J.W. (2019) Role of palmitoylation of postsynaptic proteins in promoting synaptic plasticity. *Front. Mol. Neurosci.* **12**, 23119–23219, https://www.frontiersin.org/article/10.3389/fnmol.2019.00008/full, https://doi.org/10.3389/fnmol.2019.00008

21 Essandoh, K., Philippe, J.M., Jenkins, P.M. and Brody, M.J. (2020) Palmitoylation: a fatty regulator of myocardial electrophysiology. *Front. Physiol.* **11**, 108, https://www.frontiersin.org/articles/10.3389/fphys.2020.00108/full, https://doi.org/10.3389/fphys.2020.00108

22 Das, T., Yount, J.S. and Hang, H.C. (2021) Protein S-palmitoylation in immunity. *Open Biol.* **11**, 200411, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=33653086&retmode=ref&cmd=prlinks, https://doi.org/10.1098/rsob.200411

23 Li, Y. and Chunfu, Z. (2020) The emerging roles of ZDHHCs-mediated protein palmitoylation in the antiviral innate immune responses. *Crit. Rev. Microbiol.* **0**, 1–10

24 Veit, M. (2012) Palmitoylation of virus proteins. *Biol. Cell* **104**, 493–515, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=22548323&retmode=ref&cmd=prlinks, https://doi.org/10.1111/boc.201200006

25 Brown, R.W.B., Sharma, A.I. and Engman, D.M. (2017) Dynamic protein S-palmitoylation mediates parasite life cycle progression and diverse mechanisms of virulence. *Crit. Rev. Biochem. Mol.* **52**, 1–18, https://doi.org/10.1080/10409238.2017.1287161

26 María-Eugenia, Z. and Goot, F.G.v.d. (2018) The molecular era of protein S-acylation: spotlight on structure, mechanisms, and dynamics. *Crit. Rev. Biochem. Mol. Biol.* **53**, 420–431

27 Shahinian, S. and Silvius, J.R. (1995) Doubly-lipid-modified protein sequence motifs exhibit long-lived anchorage to lipid bilayer membranes. *Biochemistry* **34**, 3813–3822, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=7893678&retmode=ref&cmd=prlinks, https://doi.org/10.1021/bi00011a039

28 Yang, X., Kovalenko, O.V., Tang, W., Claas, C., Stipp, C.S. and Hemler, M.E. (2004) Palmitoylation supports assembly and function of integrin-tetraspanin complexes. *J. Cell Biol.* **167**, 1231–1240, http://jcb.rupress.org/content/167/6/1231.full, https://doi.org/10.1083/jcb.200404100

29 Blaskovic, S., Blanc, M. and Goot, F.G.V.D. (2013) What does S-palmitoylation do to membrane proteins? *FEBS J.* **280**, 2766–2774, https://febs.onlinelibrary.wiley.com/doi/full/10.1111/febs.12263, https://doi.org/10.1111/febs.12263

30 Jeffries, O., Tian, L., McClafferty, H. and Shipston, M.J. (2012) An electrostatic switch controls palmitoylation of the large conductance voltage- and calcium-activated potassium (BK) channel. *J. Biol. Chem.* **287**, 1468–1477, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=22084244&retmode=ref&cmd=prlinks, https://doi.org/10.1074/jbc.M111.224840

31 Levental, I., Lingwood, D., Grzybek, M., Coskun, Ü. and Simons, K. (2010) Palmitoylation regulates raft affinity for the majority of integral raft proteins. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 22050–22054, https://doi.org/10.1073/pnas.1016184107

32 Shimell, J.J., Globa, A., Sepers, M.D., Wild, A.R., Matin, N., Raymond, L.A. et al. (2020) Regulation of hippocampal excitatory synapses by the Zdhhc5 palmitoyl acyl transferase. *J. Cell Sci.* **6**, 8200–8227, https://doi.org/10.1101/2020.09.11.294397

33 Brigidi, G.S., Sun, Y., Beccano-Kelly, D., Pitman, K., Mobasser, M., Borgland, S.L. et al. (2014) Palmitoylation of δ-catenin by DHHC5 mediates activity-induced synapse plasticity. *Nat. Neurosci.* **17**, 522–532, http://www.nature.com/doifinder/10.1038/nn.3657, https://doi.org/10.1038/nn.3657

34 Shimell, J.J., Shah, B.S., Cain, S.M., Thouta, S., Kuhlmann, N., Tatarnikov, I. et al. (2019) The X-linked intellectual disability gene Zdhhc9 is essential for dendrite outgrowth and inhibitory synapse formation. *Cell Rep.* **29**, 2422.e8–2437.e8, https://doi.org/10.1016/j.celrep.2019.10.065

35 Brigidi, G.S., Santyr, B., Shimell, J., Jovellar, B. and Bamji, S.X. (2015) Activity-regulated trafficking of the palmitoyl-acyl transferase DHHC5. *Nat. Commun.* **6**, 8200, https://www.nature.com/articles/ncomms9200, https://doi.org/10.1038/ncomms9200

36 Hayashi, T., Rumbaugh, G. and Huganir, R.L. (2005) Differential regulation of AMPA receptor subunit trafficking by palmitoylation of two distinct sites. *Neuron* **47**, 709–723, http://linkinghub.elsevier.com/retrieve/pii/S0896627305006458, https://doi.org/10.1016/j.neuron.2005.06.035

37 Hayashi, T., Thomas, G.M. and Huganir, R.L. (2009) Dual palmitoylation of NR2 subunits regulates NMDA receptor trafficking. *Neuron* **64**, 213–226, http://linkinghub.elsevier.com/retrieve/pii/S0896627309006291, https://doi.org/10.1016/j.neuron.2009.08.017

38 Thomas, G.M., Hayashi, T., Huganir, R.L. and Linden, D.J. (2013) DHHC8-dependent PICK1 palmitoylation is required for induction of cerebellar long-term synaptic depression. *J. Neurosci.* **33**, 15401–15407, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=24068808&retmode=ref&cmd=prlinks, https://doi.org/10.1523/JNEUROSCI.1283-13.2013

39 Keith, D.J., Sanderson, J.L., Gibson, E.S., Woolfrey, K.M., Robertson, H.R., Olszewski, K. et al. (2012) Palmitoylation of A-kinase anchoring protein 79/150 regulates dendritic endosomal targeting and synaptic plasticity mechanisms. *J. Neurosci.* **32**, 7119–7136, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=22623657&retmode=ref&cmd=prlinks, https://doi.org/10.1523/JNEUROSCI.0784-12.2012

40 Purkey, A.M., Woolfrey, K.M., Crosby, K.C., Stich, D.G., Chick, W.S., Aoto, J. et al. (2018) AKAP150 palmitoylation regulates synaptic incorporation of Ca^{2+}-permeable AMPA receptors to control LTP. *Cell Rep.* **25**, 974.e4–987.e4, https://doi.org/10.1016/j.celrep.2018.09.085

41 Firestein, B.L., Craven, S.E. and Bredt, D.S. (2000) Postsynaptic targeting of MAGUKs mediated by distinct N-terminal domains. *Neuroreport* **11**, 3479–3484, https://insights.ovid.com/crossref?an=00001756-200011090-00016, https://doi.org/10.1097/00001756-200011090-00016

42 El-Husseini, A.E., Craven, S.E., Chetkovich, D.M., Firestein, B.L., Schnell, E., Aoki, C. et al. (2000) Dual palmitoylation of PSD-95 mediates its vesiculotubular sorting, postsynaptic targeting, and ion channel clustering. *J. Cell Biol.* **148**, 159–172, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=10629226&retmode=ref&cmd=prlinks, https://doi.org/10.1083/jcb.148.1.159

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43 Fukata, Y., Dimitrov, A., Boncompain, G., Vielemeyer, O., Perez, F. and Fukata, M. (2013) Local palmitoylation cycles define activity-regulated postsynaptic subdomains. *J. Cell Biol.* **202**, 145–161, https://rupress.org/jcb/article/202/1/145/37373/Local-palmitoylation-cycles-define, https://doi.org/10.1083/jcb.201302071

44 Thomas, G.M., Hayashi, T., Chiu, S.-L., Chen, C.-M. and Huganir, R.L. (2012) Palmitoylation by DHHC5/8 targets GRIP1 to dendritic endosomes to regulate AMPA-R trafficking. *Neuron* **73**, 482–496, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=22325201&retmode=ref&cmd=prlinks, https://doi.org/10.1016/j.neuron.2011.11.021

45 Naumenko, V.S. and Ponimaskin, E. (2018) Palmitoylation as a functional regulator of neurotransmitter receptors. *Neural Plast.* **2018**, 1–18, https://www.hindawi.com/journals/np/2018/5701348/, https://doi.org/10.1155/2018/5701348

46 Thomas, G.M. and Huganir, R.L. (2013) Palmitoylation-dependent regulation of glutamate receptors and their PDZ domain-containing partners. *Biochem. Soc. Trans.* **41**, 72–78, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=23356261&retmode=ref&cmd=prlinks, https://doi.org/10.1042/BST20120223

47 Globa, A.K. and Bamji, S.X. (2017) Protein palmitoylation in the development and plasticity of neuronal connections. *Curr. Opin. Neurobiol.* **45**, 1–11, https://doi.org/10.1016/j.conb.2017.02.016

48 Putilina, T., Wong, P. and Gentleman, S. (1999) The DHHC domain: a new highly conserved cysteine-rich motif. *Mol. Cell. Biochem.* **195**, 219–226, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=10395086&retmode=ref&cmd=prlinks, https://doi.org/10.1023/A:1006932522197

49 Jennings, B.C. and Linder, M.E. (2012) DHHC protein S-acyltransferases use similar ping-pong kinetic mechanisms but display different acyl-CoA specificities. *J. Biol. Chem.* **287**, 7236–7245, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=22247542&retmode=ref&cmd=prlinks, https://doi.org/10.1074/jbc.M111.337246

50 Mitchell, D.A., Mitchell, G., Ling, Y., Budde, C. and Deschenes, R.J. (2010) Mutational analysis of Saccharomyces cerevisiae Erf2 reveals a two-step reaction mechanism for protein palmitoylation by DHHC enzymes. *J. Biol. Chem.* **285**, 38104–38114, http://www.jbc.org/cgi/doi/10.1074/jbc.M110.169102, https://doi.org/10.1074/jbc.M110.169102

51 Rana, M.S., Kumar, P., Lee, C.-J., Verardi, R., Rajashankar, K.R. and Banerjee, A. (2018) Fatty acyl recognition and transfer by an integral membrane S-acyltransferase. *Science* **359**, 1–12, https://doi.org/10.1126/science.aao6326

52 Montoro, A.G., Quiroga, R. and Taubas, J.V. (2013) Zinc coordination by the DHHC cysteine-rich domain of the palmitoyltransferase Swf1. *Biochem. J.* **454**, 427–435, https://doi.org/10.1042/BJ20121693

53 Gottlieb, C.D., Zhang, S. and Linder, M.E. (2015) The cysteine-rich domain of the DHHC3 palmitoyltransferase is palmitoylated and contains tightly bound zinc*. *J. Biol. Chem.* **290**, 29259–29269, https://doi.org/10.1074/jbc.M115.691147

54 Huang, K., Sanders, S., Singaraja, R., Orban, P., Cijsouw, T., Arstikaitis, P. et al. (2009) Neuronal palmitoyl acyl transferases exhibit distinct substrate specificity. *FASEB J.* **23**, 2605–2615, http://www.fasebj.org/cgi/doi/10.1096/fj.08-127399, https://doi.org/10.1096/fj.08-127399

55 Ohno, Y., Kihara, A., Sano, T. and Igarashi, Y. (2006) Intracellular localization and tissue-specific distribution of human and yeast DHHC cysteine-rich domain-containing proteins. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **1761**, 474–483, http://linkinghub.elsevier.com/retrieve/pii/S1388198106000709, https://doi.org/10.1016/j.bbalip.2006.03.010

56 Collura, K.M., Niu, J., Sanders, S.S., Montersino, A., Holland, S.M. and Thomas, G.M. (2020) The palmitoyl acyltransferases ZDHHC5 and ZDHHC8 are uniquely present in DRG axons and control retrograde signaling via the Gp130/JAK/STAT3 pathway. *J. Biol. Chem.* **295**, 15427–15437, https://doi.org/10.1074/jbc.RA120.013815

57 Kang, R., Wang, L., Sanders, S.S., Zuo, K., Hayden, M.R. and Raymond, L.A. (2019) Altered regulation of striatal neuronal N-Methyl-D-aspartate receptor trafficking by palmitoylation in Huntington Disease Mouse Model. *Front. Synaptic Neurosci.* **11**, 3, https://www.frontiersin.org/article/10.3389/fnsyn.2019.00003/full, https://doi.org/10.3389/fnsyn.2019.00003

58 Tian, L., McClafferty, H., Jeffries, O. and Shipston, M.J. (2010) Multiple palmitoyltransferases are required for palmitoylation-dependent regulation of large conductance calcium- and voltage-activated potassium channels. *J. Biol. Chem.* **285**, 23954–23962, http://www.jbc.org/cgi/doi/10.1074/jbc.M110.137802, https://doi.org/10.1074/jbc.M110.137802

59 Politis, E.G. (2005) Transmembrane topology of the protein palmitoyl transferase Akr1. *J. Biol. Chem.* **280**, 10156–10163, http://www.jbc.org/cgi/doi/10.1074/jbc.M411946200, https://doi.org/10.1074/jbc.M411946200

60 Mitchell, D.A., Vasudevan, A., Linder, M.E. and Deschenes, R.J. (2006) Protein palmitoylation by a family of DHHC protein S-acyltransferases. *J. Lipid Res.* **47**, 1118–1127, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=16582420&retmode=ref&cmd=prlinks, https://doi.org/10.1194/jlr.R600007-JLR200

61 Montoro, A.G., Quiroga, R., Maccioni, H.J.F. and Taubas, J.V. (2009) A novel motif at the C-terminus of palmitoyltransferases is essential for Swf1 and Pfa3 function in vivo. *Biochem. J.* **419**, 301, http://www.biochemj.org/bj/419/bj4190301.htm, https://doi.org/10.1042/BJ20080921

62 Roth, A.F. (2002) The yeast DHHC cysteine-rich domain protein Akr1p is a palmitoyl transferase. *J. Cell Biol.* **159**, 23–28, http://www.jcb.org/cgi/doi/10.1083/jcb.200206120, https://doi.org/10.1083/jcb.200206120

63 Singaraja, R.R., Hadano, S., Metzler, M., Givan, S., Wellington, C.L., Warby, S. et al. (2002) HIP14, a novel ankyrin domain-containing protein, links huntingtin to intracellular trafficking and endocytosis. *Hum. Mol. Genet.* **11**, 2815–2828, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=12393793&retmode=ref&cmd=prlinks, https://doi.org/10.1093/hmg/11.23.2815

64 Young, F.B., Butland, S.L., Sanders, S.S., Sutton, L.M. and Hayden, M.R. (2012) Putting proteins in their place: palmitoylation in Huntington disease and other neuropsychiatric diseases. *Prog. Neurobiol.* **97**, 220–238, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed=id=22155432&retmode=ref&cmd=prlinks, https://doi.org/10.1016/j.pneurobio.2011.11.002

65 Lemonidis, K., Sanchez-Perez, M.C. and Chamberlain, L.H. (2015) Identification of a novel sequence motif recognised by the ankyrin-repeat domain of zDHHC17/13 S-acyl-transferases. *J. Biol. Chem.* **290**, 21939–21950, https://doi.org/10.1074/jbc.M115.657668

66 Lemonidis, K., MacLeod, R., Baillie, G.S. and Chamberlain, L.H. (2017) Peptide array based screening reveals a large number of proteins interacting with the ankyrin repeat domain of the zDHHC17 S-acyltransferase. *J. Biol. Chem.* **292**, 17190–17202, https://doi.org/10.1074/jbc.M117.799650

67 Verardi, R., Kim, J.-S., Ghirlando, R. and Banerjee, A. (2017) Structural basis for substrate recognition by the Ankyrin repeat domain of human DHHC17 palmitoyltransferase. *Structure* **25**, 1–18, https://doi.org/10.1016/j.str.2017.06.018

68 Thomas, G.M. and Hayashi, T. (2013) Smarter neuronal signaling complexes from existing components: how regulatory modifications were acquired during animal evolution: evolution of palmitoylation-dependent regulation of AMPA-type ionotropic glutamate receptors. *Bioessays* **35**, 929–939, https://onlinelibrary.wiley.com/doi/full/10.1002/bies.201300076, https://doi.org/10.1002/bies.201300076

69 Sanders, S.S., Hernandez, L.M., Soh, H., Karnam, S., Walikonis, R.S., Tzingounis, A.V. et al. (2020) The palmitoyl acyltransferase ZDHHC14 controls Kv1-family potassium channel clustering at the axon initial segment. *eLife* **9**, 1–32, https://doi.org/10.7554/eLife.56058

70 Fredericks, G.J., Hoffmann, F.W., Rose, A.H., Osterheld, H.J., Hess, F.M., Mercier, F. et al. (2014) Stable expression and function of the inositol 1,4,5-triphosphate receptor requires palmitoylation by a DHHC6/selenoprotein K complex. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 16478–16483, http://www.pnas.org/cgi/doi/10.1073/pnas.1417176111

71 Chen, J.J., Fan, Y. and Boehning, D. (2021) Regulation of dynamic protein S-acylation. *Front. Mol. Biosci.* **8**, 656440, https://www.frontiersin.org/articles/10.3389/fmolb.2021.656440/full, https://doi.org/10.3389/fmolb.2021.656440

72 Conibear, E. and Davis, N.G. (2010) Palmitoylation and depalmitoylation dynamics at a glance. *J. Cell Sci.* **123**, 4007–4010, http://jcs.biologists.org/cgi/doi/10.1242/jcs.059287, https://doi.org/10.1242/jcs.059287

73 Won, S.J. and Martin, B.R. (2018) Temporal profiling establishes a dynamic S-palmitoylation cycle. *ACS Chem. Biol.*, http://pubs.acs.org/doi/10.1021/acschembio.8b00157, https://doi.org/10.1021/acschembio.8b00157

74 Omary, M.B. and Trowbridge, I.S. (1981) Covalent binding of fatty acid to the transferrin receptor in cultured human cells. *J. Biol. Chem.* **256**, 4715–4718, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=6262300&retmode=ref&cmd=prlinks, https://doi.org/10.1016/S0021-9258(19)69309-9

75 Staufenbiel, M. (1987) Ankyrin-bound fatty acid turns over rapidly at the erythrocyte plasma membrane. *Mol. Cell. Biol.* **7**, 2981–2984, https://doi.org/10.1128/MCB.7.8.2981

76 Magee, A.I., Gutierrez, L., McKay, I.A., Marshall, C.J. and Hall, A. (1987) Dynamic fatty acylation of p21N-ras. *EMBO J.* **6**, 3353–3357, https://doi.org/10.1002/j.1460-2075.1987.tb02656.x

77 Duncan, J.A. and Gilman, A.G. (1998) A cytoplasmic acyl-protein thioesterase that removes palmitate from G protein alpha subunits and p21(RAS). *J. Biol. Chem.* **273**, 15830–15837, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=9624183&retmode=ref&cmd=prlinks, https://doi.org/10.1074/jbc.273.25.15830

78 Camp, L.A. and Hofmann, S.L. (1993) Purification and properties of a palmitoyl-protein thioesterase that cleaves palmitate from H-Ras. *J. Biol. Chem.* **268**, 22566–22574, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=7901201&retmode=ref&cmd=prlinks, https://doi.org/10.1016/S0021-9258(18)41567-0

79 Lin, D.T.S. and Conibear, E. (2015) ABHD17 proteins are novel protein depalmitoylases that regulate N-Ras palmitate turnover and subcellular localization. *eLife* **4**, e11306, https://elifesciences.org/articles/11306, https://doi.org/10.7554/eLife.11306

80 Tomatis, V.M., Trenchi, A., Gomez, G.A. and Daniotti, J.L. (2010) Acyl-protein thioesterase 2 catalyzes the deacylation of peripheral membrane-associated GAP-43. *PLoS ONE* **5**, e15045, http://dx.plos.org/10.1371/journal.pone.0015045.g006, https://doi.org/10.1371/journal.pone.0015045

81 Soyombo, A.A. and Hofmann, S.L. (1997) Molecular cloning and expression of palmitoyl-protein thioesterase 2 (PPT2), a homolog of lysosomal palmitoyl-protein thioesterase with a distinct substrate specificity. *J. Biol. Chem.* **272**, 27456–27463, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=9341199&retmode=ref&cmd=prlinks, https://doi.org/10.1074/jbc.272.43.27456

82 Cao, Y., Qiu, T., Kathayat, R.S., Azizi, S.-A., Thorne, A.K., Ahn, D. et al. (2019) ABHD10 is an S-depalmitoylase affecting redox homeostasis through peroxiredoxin-5. *Nat. Chem. Biol.* **15**, 1232–1240, http://www.nature.com/articles/s41589-019-0399-y, https://doi.org/10.1038/s41589-019-0399-y

83 Yokoi, N., Fukata, Y., Sekiya, A., Murakami, T., Kobayashi, K. and Fukata, M. (2016) Identification of PSD-95 depalmitoylating enzymes. *J. Neurosci.* **36**, 6431–6444, http://www.jneurosci.org/content/36/24/6431.full, https://doi.org/10.1523/JNEUROSCI.0419-16.2016

84 Zeidman, R., Jackson, C.S. and Magee, A.I. (2009) Protein acyl thioesterases (Review). *Mol. Membr. Biol.* **26**, 32–41, http://informahealthcare.com/doi/abs/10.1080/09687680802629329, https://doi.org/10.1080/09687680802629329

85 Tian, L., McClafferty, H., Knaus, H.-G., Ruth, P. and Shipston, M.J. (2012) Distinct acyl protein transferases and thioesterases control surface expression of calcium-activated potassium channels. *J. Biol. Chem.* **287**, 14718–14725, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=22399288&retmode=ref&cmd=prlinks, https://doi.org/10.1074/jbc.M111.335547

86 Bellizzi, J.J., Widom, J., Kemp, C., Lu, J.Y., Das, A.K., Hofmann, S.L. et al. (2000) The crystal structure of palmitoyl protein thioesterase 1 and the molecular basis of infantile neuronal ceroid lipofuscinosis. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 4573–4578, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=10781062&retmode=ref&cmd=prlinks, https://doi.org/10.1073/pnas.080508097

87 Camp, L.A., Verkruyse, L.A., Afendis, S.J., Slaughter, C.A. and Hofmann, S.L. (1994) Molecular cloning and expression of palmitoyl-protein thioesterase. *J. Biol. Chem.* **269**, 23212–23219, https://doi.org/10.1016/S0021-9258(17)31641-1

88 Devedjiev, Y., Dauter, Z., Kuznetsov, S.R., Jones, T.L. and Derewenda, Z.S. (2000) Crystal structure of the human acyl protein thioesterase I from a single X-ray data set to 1.5 A. *Structure* **8**, 1137–1146, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=11080636&retmode=ref&cmd=prlinks, https://doi.org/10.1016/S0969-2126(00)00529-3

89 Smith, M.A., Phillips, W.K., Rabin, P.L. and Johnson, R.J. (2018) A dynamic loop provides dual control over the catalytic and membrane binding activity of a bacterial serine hydrolase. *Biochim. Biophys. Acta Proteins Proteomics* **1866**, 925–932, https://doi.org/10.1016/j.bbapap.2018.05.012

90 Blankman, J.L., Simon, G.M. and Cravatt, B.F. (2007) A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem. Biol.* **14**, 1347–1356, https://doi.org/10.1016/j.chembiol.2007.11.006

91 Hellsten, E., Vesa, J., Olkkonen, V.M., Jalanko, A. and Peltonen, L. (1996) Human palmitoyl protein thioesterase: evidence for lysosomal targeting of the enzyme and disturbed cellular routing in infantile neuronal ceroid lipofuscinosis. *EMBO J.* **15**, 5240–5245, https://doi.org/10.1002/j.1460-2075.1996.tb00909.x

92 Vesa, J., Hellsten, E., Verkruyse, L.A., Camp, L.A., Rapola, J., Santavuori, P. et al. (1995) Mutations in the palmitoyl protein thioesterase gene causing infantile neuronal ceroid lipofuscinosis. *Nature* **376**, 584–587, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=7637805&retmode=ref&cmd=prlinks, https://doi.org/10.1038/376584a0

93 Heinonen, O., Kyttälä, A., Lehmus, E., Paunio, T., Peltonen, L. and Jalanko, A. (2000) Expression of palmitoyl protein thioesterase in neurons. *Mol. Genet. Metab.* **69**, 123–129, http://linkinghub.elsevier.com/retrieve/pii/S1096719200929615, https://doi.org/10.1006/mgme.2000.2961

94 Lehtovirta, M., Kyttälä, A., Eskelinen, E.L., Hess, M., Heinonen, O. and Jalanko, A. (2001) Palmitoyl protein thioesterase (PPT) localizes into synaptosomes and synaptic vesicles in neurons: implications for infantile neuronal ceroid lipofuscinosis (INCL). *Hum. Mol. Genet.* **10**, 69–75, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=11136716&retmode=ref&cmd=prlinks, https://doi.org/10.1093/hmg/10.1.69

95 Cho, S. and Dawson, G. (2000) Palmitoyl protein thioesterase 1 protects against apoptosis mediated by Ras-Akt-caspase pathway in neuroblastoma cells. *J. Neurochem.* **74**, 1478–1488, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=10737604&retmode=ref&cmd=prlinks, https://doi.org/10.1046/j.1471-4159.2000.0741478.x

96 Kathayat, R.S., Cao, Y., Elvira, P.D., Sandoz, P.A., Zaballa, M.-E., Springer, M.Z. et al. (2018) Active and dynamic mitochondrial S-depalmitoylation revealed by targeted fluorescent probes. *Nat. Commun.* **9**, 334, https://www.nature.com/articles/s41467-017-02655-1, https://doi.org/10.1038/s41467-017-02655-1

97 Kong, E., Peng, S., Chandra, G., Sarkar, C., Zhang, Z., Bagh, M.B. et al. (2013) Dynamic palmitoylation links cytosol-membrane shuttling of acyl-protein thioesterase-1 and acyl-protein thioesterase-2 with that of proto-oncogene H-Ras product and growth associated protein-43. *J. Biol. Chem.* **288**, 9112–9125, https://doi.org/10.1074/jbc.M112.421073

98 Vartak, N., Papke, B., Grecco, H.E., Rossmannek, L., Waldmann, H., Hedberg, C. et al. (2014) The autodepalmitoylating activity of APT maintains the spatial organization of palmitoylated membrane proteins. *Biophysj* **106**, 93–105, https://doi.org/10.1016/j.bpj.2013.11.024

99 Yang, W., Vizio, D.D., Kirchner, M., Steen, H. and Freeman, M.R. (2010) Proteome scale characterization of human S-acylated proteins in lipid raft-enriched and non-raft membranes. *Mol. Cell. Proteomics* **9**, 54–70, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=19801377&retmode=ref&cmd=prlinks, https://doi.org/10.1074/mcp.M800448-MCP200

100 Abrami, L., Audagnotto, M., Ho, S., Marcaida, M.J., Mesquita, F.S., Anwar, M.U. et al. (2021) Palmitoylated acyl protein thioesterase APT2 deforms membranes to extract substrate acyl chains. *Nat. Chem. Biol.* **17**, 438–447, https://doi.org/10.1038/s41589-021-00753-2

101 Cho, E. and Park, M. (2016) Palmitoylation in Alzheimer's disease and other neurodegenerative diseases. *Pharmacol. Res.* **111**, 133–151, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=27293050&retmode=ref&cmd=prlinks, https://doi.org/10.1016/j.phrs.2016.06.008

102 Han, J.Y., Lee, I.G., Shin, S., Kim, M., Jang, J.H. and Park, J. (2017) The first patient with sporadic X-linked intellectual disability with de novo ZDHHC9 mutation identified by targeted next-generation sequencing. *Eur. J. Med. Genet.* **60**, 499–503, https://doi.org/10.1016/j.ejmg.2017.07.002

103 Tzschach, A., Grasshoff, U., Beck-Woedl, S., Dufke, C., Bauer, C., Kehrer, M. et al. (2015) Next-generation sequencing in X-linked intellectual disability. *Eur. J. Hum. Genet.* **23**, 1513–1518, https://doi.org/10.1038/ejhg.2015.5

104 Mitchell, D.A., Hamel, L.D., Reddy, K.D., Farh, L., Rettew, L.M., Sanchez, P.R. et al. (2014) Mutations in the X-linked intellectual disability gene, zDHHC9, alter autopalmitoylation activity by distinct mechanisms*. *J. Biol. Chem.* **289**, 18582–18592, https://doi.org/10.1074/jbc.M114.567420

105 Masurel-Paulet, A., Kalscheuer, V.M., Lebrun, N., Hu, H., Levy, F., Thauvin-Robinet, C. et al. (2014) Expanding the clinical phenotype of patients with a ZDHHC9 mutation. *Am. J. Med. Genet. A* **164**, 789–795, https://doi.org/10.1002/ajmg.a.36348

106 Kouskou, M., Thomson, D.M., Brett, R.R., Wheeler, L., Tate, R.J., Pratt, J.A. et al. (2018) Disruption of the Zdhhc9 intellectual disability gene leads to behavioural abnormalities in a mouse model. *Exp. Neurol.* **308**, 35–46, https://doi.org/10.1016/j.expneurol.2018.06.014

107 Ripke, S., Walters, J.T., O'Donovan, M.C. and Consortium TSWG of the PG (2020) Mapping genomic loci prioritises genes and implicates synaptic biology in schizophrenia. *medRxiv* 1–50, https://www.medrxiv.org/content/10.1101/2020.09.12.20192922v1.abstract, https://doi.org/10.1101/2020.09.12.20192922v1.abstract

108 Fallin, M.D., Lasseter, V.K., Wolyniec, P.S., McGrath, J.A., Nestadt, G., Valle, D. et al. (2004) Genomewide linkage scan for bipolar-disorder susceptibility loci among Ashkenazi Jewish families. *Am. J. Hum. Genet.* **75**, 204–219, https://doi.org/10.1086/422474

109 Fromer, M., Pocklington, A.J., Kavanagh, D.H., Williams, H.J., Dwyer, S., Gormley, P. et al. (2014) De novo mutations in schizophrenia implicate synaptic networks. *Nature* **506**, 179–184, https://doi.org/10.1038/nature12929

110 Liu, H., Abecasis, G.R., Heath, S.C., Knowles, A., Demars, S., Chen, Y.-J. et al. (2002) Genetic variation in the 22q11 locus and susceptibility to schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 16859–16864, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=12477929&retmode=ref&cmd=prlinks, https://doi.org/10.1073/pnas.232186099

111 Shin, H.D., Park, B.L., Bae, J.S., Park, T.J., Chun, J.Y., Park, C.S. et al. (2010) Association of ZDHHC8 polymorphisms with smooth pursuit eye movement abnormality. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **153B**, 1167–1172, http://onlinelibrary.wiley.com/doi/10.1002/ajmg.b.31083/full

112 Chen, W.-Y., Shi, Y.-Y., Zheng, Y.-L., Zhao, X.-Z., Zhang, G.-J., Chen, S.-Q. et al. (2004) Case-control study and transmission disequilibrium test provide consistent evidence for association between schizophrenia and genetic variation in the 22q11 gene ZDHHC8. *Hum. Mol. Genet.* **13**, 2991–2995, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=15489219&retmode=ref&cmd=prlinks, https://doi.org/10.1093/hmg/ddh322

113 Xu, M., Clair, D.S. and He, L. (2010) Testing for genetic association between the ZDHHC8 gene locus and susceptibility to schizophrenia: An integrated analysis of multiple datasets. *Am. J. Med. Genet.* **153B**, 1266–1275, https://doi.org/10.1002/ajmg.b.31096

114 Demily, C., Legallic, S., Bou, J., Houy-Durand, E., Amelsvoort, T.V., Zinkstok, J. et al. (2007) ZDHHC8 single nucleotide polymorphism rs175174 is not associated with psychiatric features of the 22q11 deletion syndrome or schizophrenia. *Psychiatr. Genet.* **17**, 311–312, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=17728672&retmode=ref&cmd=prlinks, https://doi.org/10.1097/YPG.0b013e328133f369

115 Glaser, B., Moskvina, V., Kirov, G., Murphy, K.C., Williams, H., Williams, N. et al. (2006) Analysis of ProDH, COMT and ZDHHC8 risk variants does not support individual or interactive effects on schizophrenia susceptibility. *Schizophr. Res.* **87**, 21–27, http://linkinghub.elsevier.com/retrieve/pii/S0920996406002507, https://doi.org/10.1016/j.schres.2006.05.024

116 Glaser, B., Schumacher, J., Williams, H.J., Jamra, R.A., Ianakiev, N., Milev, R. et al. (2005) No association between the putative functional ZDHHC8 single nucleotide polymorphism rs175174 and schizophrenia in large European samples. *Biol. Psychiatry* **58**, 78–80, http://linkinghub.elsevier.com/retrieve/pii/S0006322305003574, https://doi.org/10.1016/j.biopsych.2005.03.017

117 Otani, K., Ujike, H., Tanaka, Y., Morita, Y., Kishimoto, M., Morio, A. et al. (2005) The ZDHHC8 gene did not associate with bipolar disorder or schizophrenia. *Neurosci. Lett.* **390**, 166–170, http://linkinghub.elsevier.com/retrieve/pii/S0304394005009249, https://doi.org/10.1016/j.neulet.2005.08.019

118 Saito, S., Ikeda, M., Iwata, N., Suzuki, T., Kitajima, T., Yamanouchi, Y. et al. (2005) No association was found between a functional SNP in ZDHHC8 and schizophrenia in a Japanese case-control population. *Neurosci. Lett.* **374**, 21–24, http://linkinghub.elsevier.com/retrieve/pii/S0304394004012674, https://doi.org/10.1016/j.neulet.2004.10.015

119 Pinner, A.L., Tucholski, J., Haroutunian, V., McCullumsmith, R.E. and Meador-Woodruff, J.H. (2016) Decreased protein S-palmitoylation in dorsolateral prefrontal cortex in schizophrenia. *Schizophr. Res.* **177**, 78–87, https://doi.org/10.1016/j.schres.2016.01.054

120 Nosková, L., Stránecký, V., Hartmannová, H., Přistoupilová, A., Barešová, V., Ivánek, R. et al. (2011) Mutations in DNAJC5, encoding cysteine-string protein alpha, cause autosomal-dominant adult-onset neuronal ceroid lipofuscinosis. *Am. J. Hum. Genet.* **89**, 241–252, https://doi.org/10.1016/j.ajhg.2011.07.003

121 Benitez, B.A., Alvarado, D., Cai, Y., Mayo, K., Chakraverty, S., Norton, J. et al. (2011) Exome-sequencing confirms DNAJC5 mutations as cause of adult neuronal ceroid-lipofuscinosis. *PLoS ONE* **6**, e26741, https://doi.org/10.1371/journal.pone.0026741

122 Group THDCR (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* **72**, 971–983, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=8458085&retmode=ref&cmd=prlinks, https://doi.org/10.1016/0092-8674(93)90585-E

123 Yanai, A., Huang, K., Kang, R., Singaraja, R.R., Arstikaitis, P., Gan, L. et al. (2006) Palmitoylation of huntingtin by HIP14is essential for its trafficking and function. *Nat. Neurosci.* **9**, 824–831, http://www.nature.com/doifinder/10.1038/nn1702, https://doi.org/10.1038/nn1702

124 Lemarié, F.L., Caron, N.S., Sanders, S.S., Schmidt, M.E., Nguyen, Y.T.N., Ko, S. et al. (2021) Rescue of aberrant huntingtin palmitoylation ameliorates mutant huntingtin-induced toxicity. *Neurobiol. Dis.* **158**, 105479, https://doi.org/10.1016/j.nbd.2021.105479

125 Virlogeux, A., Scaramuzzino, C., Lenoir, S., Carpentier, R., Louessard, M., Genoux, A. et al. (2021) Increasing brain palmitoylation rescues behavior and neuropathology in Huntington disease mice. *Sci. Adv.* **7**, eabb0799, https://advances.sciencemag.org/content/7/14/eabb0799.abstract, https://doi.org/10.1126/sciadv.abb0799

126 Antinone, S.E., Ghadge, G.D., Lam, T.T., Wang, L., Roos, R.P. and Green, W.N. (2013) Palmitoylation of superoxide dismutase 1 (SOD1) is increased for familial amyotrophic lateral sclerosis-linked SOD1 mutants. *J. Biol. Chem.* **288**, 21606–21617, http://www.jbc.org/cgi/doi/10.1074/jbc.M113.487231, https://doi.org/10.1074/jbc.M113.487231

127 Antinone, S.E., Ghadge, G.D., Ostrow, L.W., Roos, R.P. and Green, W.N. (2017) S-acylation of SOD1, CCS, and a stable SOD1-CCS heterodimer in human spinal cords from ALS and non-ALS subjects. *Sci. Rep.* **7**, 41141, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=28120938&retmode=ref&cmd=prlinks, https://doi.org/10.1038/srep41141

128 Ho, G.P.H., Ramalingam, N., Imberdis, T., Wilkie, E.C., Dettmer, U. and Selkoe, D.J. (2020) Upregulation of cellular palmitoylation mitigates α-synuclein accumulation and neurotoxicity. *Mov. Disord.* **91**, https://onlinelibrary.wiley.com/doi/10.1002/mds.28346, https://doi.org/10.1002/mds.28346

129 Selkoe, D.J. (2001) Alzheimer's disease: genes, proteins, and therapy. *Physiol. Rev.* **81**, 741–766,
http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=11274343&retmode=ref&cmd=prlinks,
https://doi.org/10.1152/physrev.2001.81.2.741

130 Benjannet, S., Elagoz, A., Wickham, L., Mamarbachi, M., Munzer, J.S., Basak, A. et al. (2001) Post-translational processing of beta-Secretase
(beta-amyloid-converting enzyme) and its ectodomain shedding. *J. Biol. Chem.* **276**, 10879–10887, http://www.jbc.org/content/276/14/10879.full,
https://doi.org/10.1074/jbc.M009899200

131 Bhattacharyya, R., Barren, C. and Kovacs, D.M. (2013) Palmitoylation of amyloid precursor protein regulates amyloidogenic processing in lipid rafts. *J. Neurosci.* **33**, 11169–11183, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=23825420&retmode=ref&cmd=prlinks,
https://doi.org/10.1523/JNEUROSCI.4704-12.2013

132 Vetrivel, K.S., Meckler, X., Chen, Y., Nguyen, P.D., Seidah, N.G., Vassar, R. et al. (2009) Alzheimer disease Abeta production in the absence of
S-palmitoylation-dependent targeting of BACE1 to lipid rafts. *J. Biol. Chem.* **284**, 3793–3803,
http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=19074428&retmode=ref&cmd=prlinks,
https://doi.org/10.1074/jbc.M808920200

133 Cheng, H., Vetrivel, K.S., Drisdel, R.C., Meckler, X., Gong, P., Leem, J.Y. et al. (2009) S-palmitoylation of gamma-secretase subunits nicastrin and
APH-1. *J. Biol. Chem.* **284**, 1373–1384,
http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=19028695&retmode=ref&cmd=prlinks,
https://doi.org/10.1074/jbc.M806380200

134 Bhattacharyya, R., Fenn, R.H., Barren, C., Tanzi, R.E. and Kovacs, D.M. (2016) Palmitoylated APP forms dimers, cleaved by BACE1. *PLoS ONE* **11**,
e0166400, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=27875558&retmode=ref&cmd=prlinks,
https://doi.org/10.1371/journal.pone.0166400

135 Meckler, X., Roseman, J., Das, P., Cheng, H., Pei, S., Keat, M. et al. (2010) Reduced Alzheimer's disease -amyloid deposition in transgenic mice
expressing S-palmitoylation-deficient APH1aL and Nicastrin. *J. Neurosci.* **30**, 16160–16169,
http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.4436-10.2010, https://doi.org/10.1523/JNEUROSCI.4436-10.2010

136 Gerdts, J., Summers, D.W., Milbrandt, J. and DiAntonio, A. (2016) Axon self-destruction: new links among SARM1, MAPKs, and NAD+ metabolism.
Neuron **89**, 449–460, https://linkinghub.elsevier.com/retrieve/pii/S0896627315011253, https://doi.org/10.1016/j.neuron.2015.12.023

137 Adalbert, R. and Coleman, M.P. (2013) Review: axon pathology in age-related neurodegenerative disorders. *Neuropath. Appl. Neuro.* **39**, 90–108,
https://doi.org/10.1111/j.1365-2990.2012.01308.x

138 Cashman, C.R. and Höke, A. (2015) Mechanisms of distal axonal degeneration in peripheral neuropathies. *Neurosci. Lett.* **596**, 33–50,
https://doi.org/10.1016/j.neulet.2015.01.048

139 Perlson, E., Maday, S., Fu, M., Moughamian, A.J. and Holzbaur, E.L.F. (2010) Retrograde axonal transport: pathways to cell death? *Trends Neurosci.*
33, 335–344, https://doi.org/10.1016/j.tins.2010.03.006

140 Holland, S.M. and Thomas, G.M. (2017) Roles of palmitoylation in axon growth, degeneration and regeneration. *J. Neurosci. Res.* **95**, 1528–1539,
https://doi.org/10.1002/jnr.24003

141 Little, E.B., Edelman, G.M. and Cunningham, B.A. (2009) Palmitoylation of the cytoplasmic domain of the neural cell adhesion molecule N-CAM serves
as an anchor to cellular membranes. *Cell Commun. Adhes.* **6**, 415–430, https://doi.org/10.3109/15419069809109150

142 Ponimaskin, E., Dityateva, G., Ruonala, M.O., Fukata, M., Fukata, Y., Kobe, F. et al. (2008) Fibroblast growth factor-regulated palmitoylation of the
neural cell adhesion molecule determines neuronal morphogenesis. *J. Neurosci.* **28**, 8897–8907,
http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.2171-08.2008, https://doi.org/10.1523/JNEUROSCI.2171-08.2008

143 Cremer, H., Chazal, G., Goridis, C. and Represa, A. (1997) NCAM is essential for axonal growth and fasciculation in the hippocampus. *Mol. Cell.
Neurosci.* **8**, 323–335, https://doi.org/10.1006/mcne.1996.0588

144 Kang, R., Wan, J., Arstikaitis, P., Takahashi, H., Huang, K., Bailey, A.O. et al. (2008) Neural palmitoyl-proteomics reveals dynamic synaptic
palmitoylation. *Nature* **456**, 904–909, https://doi.org/10.1038/nature07605

145 Yang, G., Liu, Y., Yang, K., Liu, R., Zhu, S., Coquinco, A. et al. (2012) Isoform-specific palmitoylation of JNK regulates axonal development. *Cell Death
Differ.* **19**, 553–561, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=21941371&retmode=ref&cmd=prlinks,
https://doi.org/10.1038/cdd.2011.124

146 Skene, J.H. and Virág, I. (1989) Posttranslational membrane attachment and dynamic fatty acylation of a neuronal growth cone protein, GAP-43. *J.
Cell Biol.* **108**, 613–624, https://doi.org/10.1083/jcb.108.2.613

147 Laux, T., Fukami, K., Thelen, M., Golub, T., Frey, D. and Caroni, P. (2000) GAP43, MARCKS, and CAP23 modulate PI(4,5)P(2) at plasmalemmal rafts,
and regulate cell cortex actin dynamics through a common mechanism. *J. Cell Biol.* **149**, 1455–1472,
http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=10871285&retmode=ref&cmd=prlinks,
https://doi.org/10.1083/jcb.149.7.1455

148 El-Husseini, A.E.D. (2001) Polarized targeting of peripheral membrane proteins in neurons. *J. Biol. Chem.* **276**, 44984–44992,
http://www.jbc.org/cgi/doi/10.1074/jbc.M103049200, https://doi.org/10.1074/jbc.M103049200

149 Liu, Y., Fisher, D.A. and Storm, D.R. (1993) Analysis of the palmitoylation and membrane targeting domain of neuromodulin (GAP-43) by site-specific
mutagenesis. *Biochemistry* **32**, 10714–10719, https://doi.org/10.1021/bi00091a023

150 Dumoulin, A., Dagane, A., Dittmar, G. and Rathjen, F.G. (2018) S-palmitoylation is required for the control of growth cone morphology of DRG neurons
by CNP-induced cGMP signaling. *Front. Mol. Neurosci.* **11**, 345, https://www.frontiersin.org/article/10.3389/fnmol.2018.00345/full,
https://doi.org/10.3389/fnmol.2018.00345

151 Kapitein, L.C. and Hoogenraad, C.C. (2015) Building the neuronal microtubule cytoskeleton. *Neuron* **87**, 492–506,
https://doi.org/10.1016/j.neuron.2015.05.046

152 Tortosa, E., Adolfs, Y., Fukata, M., Pasterkamp, R.J., Kapitein, L.C. and Hoogenraad, C.C. (2017) Dynamic palmitoylation targets MAP6 to the axon to promote microtubule stabilization during neuronal polarization. *Neuron* **94**, 809.e7–825.e7, https://doi.org/10.1016/j.neuron.2017.04.042

153 Shmueli, A., Segal, M., Sapir, T., Tsutsumi, R., Noritake, J., Bar, A. et al. (2010) Ndel1 palmitoylation: a new mean to regulate cytoplasmic dynein activity. *EMBO J.* **29**, 107–119, https://doi.org/10.1038/emboj.2009.325

154 Erlander, M.G., Tillakaratne, N.J.K., Feldblum, S., Patel, N. and Tobin, A.J. (1991) Two genes encode distinct glutamate decarboxylases. *Neuron* **7**, 91–100, https://doi.org/10.1016/0896-6273(91)90077-D

155 Erlander, M.G. and Tobin, A.J. (1991) The structural and functional heterogeneity of glutamic acid decarboxylase: a review. *Neurochem. Res.* **16**, 215–226, https://doi.org/10.1007/BF00966084

156 Kanaani, J., Diacovo, M.J., El-Husseini, A.E.-D., Bredt, D.S. and Baekkeskov, S. (2004) Palmitoylation controls trafficking of GAD65 from Golgi membranes to axon-specific endosomes and a Rab5a-dependent pathway to presynaptic clusters. *J. Cell Sci.* **117**, 2001–2013, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=15039456&retmode=ref&cmd=prlinks, https://doi.org/10.1242/jcs.01030

157 Kanaani, J., Lissin, D., Kash, S.F. and Baekkeskov, S. (1999) The hydrophilic isoform of glutamate decarboxylase, GAD67, is targeted to membranes and nerve terminals independent of dimerization with the hydrophobic membrane-anchored isoform, GAD65*. *J. Biol. Chem.* **274**, 37200–37209, https://doi.org/10.1074/jbc.274.52.37200

158 Kanaani, J., El-Husseini, A.E.-D., Aguilera-Moreno, A., Diacovo, J.M., Bredt, D.S. and Baekkeskov, S. (2002) A combination of three distinct trafficking signals mediates axonal targeting and presynaptic clustering of GAD65. *J. Cell Biol.* **158**, 1229–1238, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=12356867&retmode=ref&cmd=prlinks, https://doi.org/10.1083/jcb.200205053

159 Chapman, E.R., Blasi, J., An, S., Brose, N., Johnston, P.A., Südhof, T.C. et al. (1996) Fatty acylation of synaptotagmin in PC12 cells and synaptosomes. *Biochem. Biophys. Res. Commun.* **225**, 326–332, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=8769138&retmode=ref&cmd=prlinks, https://doi.org/10.1006/bbrc.1996.1174

160 Lane, S.R. and Liu, Y. (1997) Characterization of the palmitoylation domain of SNAP-25. *J. Neurochem.* **69**, 1864–1869, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=9349529&retmode=ref&cmd=prlinks, https://doi.org/10.1046/j.1471-4159.1997.69051864.x

161 Veit, M., Söllner, T.H. and Rothman, J.E. (1996) Multiple palmitoylation of synaptotagmin and the t-SNARE SNAP-25. *FEBS Lett.* **385**, 119–123, https://doi.org/10.1016/0014-5793(96)00362-6

162 Washbourne, P., Cansino, V., Mathews, J.R., Graham, M., Burgoyne, R.D. and Wilson, M.C. (2001) Cysteine residues of SNAP-25 are required for SNARE disassembly and exocytosis, but not for membrane targeting. *Biochem. J.* **357**, 625–634, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=11463334&retmode=ref&cmd=prlinks, https://doi.org/10.1042/bj3570625

163 Loranger, S.S. and Linder, M.E. (2002) SNAP-25 traffics to the plasma membrane by a syntaxin-independent mechanism*. *J. Biol. Chem.* **277**, 34303–34309, https://doi.org/10.1074/jbc.M202125200

164 Holland, S.M., Collura, K.M., Ketschek, A., Noma, K., Ferguson, T.A., Jin, Y. et al. (2016) Palmitoylation controls DLK localization, interactions and activity to ensure effective axonal injury signaling. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 763–768, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=26719418&retmode=ref&cmd=prlinks, https://doi.org/10.1073/pnas.1514123113

165 Niu, J., Sanders, S.S., Jeong, H.-K., Holland, S.M., Sun, Y., Collura, K.M. et al. (2020) Coupled control of distal axon integrity and somal responses to axonal damage by the palmitoyl acyltransferase ZDHHC17. *Cell Rep.* **33**, 108365, https://doi.org/10.1016/j.celrep.2020.108365

166 Shin, J.E., Cho, Y., Beirowski, B., Milbrandt, J., Cavalli, V. and DiAntonio, A. (2012) Dual leucine zipper kinase is required for retrograde injury signaling and axonal regeneration. *Neuron* **74**, 1015–1022, https://doi.org/10.1016/j.neuron.2012.04.028

167 Ghosh, A.S., Wang, B., Pozniak, C.D., Chen, M., Watts, R.J. and Lewcock, J.W. (2011) DLK induces developmental neuronal degeneration via selective regulation of proapoptotic JNK activity. *J. Cell Biol.* **194**, 751–764, https://doi.org/10.1083/jcb.201103153

168 Xiong, X., Wang, X., Ewanek, R., Bhat, P., DiAntonio, A. and Collins, C.A. (2010) Protein turnover of the Wallenda/DLK kinase regulates a retrograde response to axonal injury. *J. Cell Biol.* **191**, 211–223, https://doi.org/10.1083/jcb.201006039

169 Niu, J., Holland, S.M., Ketschek, A., Collura, K.M., Hayashi, T., Gallo, G. et al. (2020) Palmitoylation couples DLK to JNK3 to facilitate pro-degenerative axon-to-soma signaling. *bioRxiv* 1–36, https://doi.org/10.1101/2020.11.17.387191

170 Martin, D.D.O., Kanuparthi, P.S., Holland, S.M., Sanders, S.S., Jeong, H.-K., Einarson, M.B. et al. (2019) Identification of novel inhibitors of DLK palmitoylation and signaling by high content screening. *Sci. Rep.* **9**, 1–12, https://doi.org/10.1038/s41598-019-39968-8

171 Conforti, L., Gilley, J. and Coleman, M.P. (2014) Wallerian degeneration: an emerging axon death pathway linking injury and disease. *Nat. Rev. Neurosci.* **15**, 394–409, https://doi.org/10.1038/nrn3680

172 Wang, J.T., Medress, Z.A. and Barres, B.A. (2012) Axon degeneration: molecular mechanisms of a self-destruction pathway. *J. Cell Biol.* **196**, 7–18, https://doi.org/10.1083/jcb.201108111

173 Klim, J.R., Pintacuda, G., Nash, L.A., Juan, I.G.S. and Eggan, K. (2021) Connecting TDP-43 pathology with neuropathy. *Trends Neurosci.* **44**, 424–440, https://doi.org/10.1016/j.tins.2021.02.008

174 Shin, J.E., Miller, B.R., Babetto, E., Cho, Y., Sasaki, Y., Qayum, S. et al. (2012) SCG10 is a JNK target in the axonal degeneration pathway. *Proc. Natl. Acad. Sci. U.S.A.* **109**, E3696–E3705, https://doi.org/10.1073/pnas.1216204109

175 Summers, D.W., Milbrandt, J. and DiAntonio, A. (2018) Palmitoylation enables MAPK-dependent proteostasis of axon survival factors. *Proc. Natl. Acad. Sci. U.S.A.* **11**, 201806933–201806939, https://doi.org/10.1073/pnas.1806933115

20

176 Paolo, G.D., Lutjens, R., Pellier, V., Stimpson, S.A., Beuchat, M.-H., Catsicas, S. et al. (1997) Targeting of SCG10 to the area of the golgi complex is mediated by its NH2-terminal region*. *J. Biol. Chem.* **272**, 5175–5182, https://doi.org/10.1074/jbc.272.8.5175

177 Gilley, J. and Coleman, M.P. (2010) Endogenous Nmnat2 is an essential survival factor for maintenance of healthy axons. *PLoS Biol.* **8**, http://dx.plos.org/10.1371/journal.pbio.1000300, https://doi.org/10.1371/journal.pbio.1000300

178 Yamagishi, Y. and Tessier-Lavigne, M. (2016) An atypical SCF-like ubiquitin ligase complex promotes wallerian degeneration through regulation of axonal Nmnat2. *Cell Rep.* **17**, 774–782, https://doi.org/10.1016/j.celrep.2016.09.043

179 Coleman, M.P. and Freeman, M.R. (2010) Wallerian degeneration, WldS, and Nmnat. *Annu. Rev. Neurosci.* **33**, 245–267, https://doi.org/10.1146/annurev-neuro-060909-153248

180 Gilley, J., Mayer, P.R., Yu, G. and Coleman, M.P. (2018) Low levels of NMNAT2 compromise axon development and survival. *Hum. Mol. Genet.* **28**, 448–458, https://academic.oup.com/hmg/article/28/3/448/5124577, https://doi.org/10.1093/hmg/ddy356

181 Mayer, P.R., Huang, N., Dewey, C.M., Dries, D.R., Zhang, H. and Yu, G. (2010) Expression, localization, and biochemical characterization of nicotinamide mononucleotide adenylyltransferase 2. *J. Biol. Chem.* **285**, 40387–40396, http://www.jbc.org/content/285/51/40387.full, https://doi.org/10.1074/jbc.M110.178913

182 Milde, S., Gilley, J. and Coleman, M.P. (2013) Subcellular localization determines the stability and axon protective capacity of axon survival factor Nmnat2. *PLoS Biol.* **11**, e1001539, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=23610559&retmode=ref&cmd=prlinks, https://doi.org/10.1371/journal.pbio.1001539

183 Bauer, S., Kerr, B.J. and Patterson, P.H. (2007) The neuropoietic cytokine family in development, plasticity, disease and injury. *Nat. Rev. Neurosci.* **8**, 221–232, https://doi.org/10.1038/nrn2054

184 Hernandez, L.M., Montersino, A., Niu, J., Guo, S. and Thomas, G.M. (2020) JAK1 palmitoylation by ZDHHC3/7 is essential for neuropoietic cytokine signaling and DRG neuron survival. *bioRxiv* 1–29, https://doi.org/10.1101/ 2020.11.16.385971

185 Kordeli, E., Lambert, S. and Bennett, V. (1995) AnkyrinG. A new ankyrin gene with neural-specific isoforms localized at the axonal initial segment and node of Ranvier. *J. Biol. Chem.* **270**, 2352–2359, http://www.jbc.org/lookup/doi/10.1074/jbc.270.5.2352, https://doi.org/10.1074/jbc.270.5.2352

186 Leterrier, C. (2018) The axon initial segment: an updated viewpoint. *J. Neurosci.* **38**, 2135–2145, http://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.1922-17.2018, https://doi.org/10.1523/JNEUROSCI.1922-17.2018

187 Angelides, K.J., Elmer, L.W., Loftus, D. and Elson, E. (1988) Distribution and lateral mobility of voltage-dependent sodium channels in neurons. *J. Cell Biol.* **106**, 1911–1925, https://doi.org/10.1083/jcb.106.6.1911

188 Davis, J.Q., Lambert, S. and Bennett, V. (1996) Molecular composition of the node of Ranvier: identification of ankyrin-binding cell adhesion molecules neurofascin (mucin+/third FNIII domain-) and NrCAM at nodal axon segments. *J. Cell Biol.* **135**, 1355–1367, https://doi.org/10.1083/jcb.135.5.1355

189 Pan, Z., Kao, T., Horvath, Z., Lemos, J., Sul, J.-Y., Cranstoun, S.D. et al. (2006) A Common Ankyrin-G-based mechanism retains KCNQ and NaV channels at electrically active domains of the axon. *J. Neurosci.* **26**, 2599–2613, https://doi.org/10.1523/JNEUROSCI.4314-05.2006

190 Garrido, J.J., Giraud, P., Carlier, E., Fernandes, F., Moussif, A., Fache, M.-P. et al. (2003) A targeting motif involved in sodium channel clustering at the axonal initial segment. *Science* **300**, 2091–2094, https://doi.org/10.1126/science.1085167

191 Xu, M. and Cooper, E.C. (2015) An Ankyrin-G N-terminal gate and protein kinase CK2 dually regulate binding of voltage-gated sodium and KCNQ2/3 potassium channels*. *J. Biol. Chem.* **290**, 16619–16632, https://doi.org/10.1074/jbc.M115.638932

192 Cooper, E.C. (2011) Made for "anchorin": Kv7.2/7.3 (KCNQ2/KCNQ3) channels and the modulation of neuronal excitability in vertebrate axons. *Semin. Cell Dev. Biol.* **22**, 185–192, https://doi.org/10.1016/j.semcdb.2010.10.001

193 He, M., Jenkins, P. and Bennett, V. (2012) Cysteine 70 of ankyrin-G is S-palmitoylated and is required for function of ankyrin-G in membrane domain assembly. *J. Biol. Chem.* **287**, 43995–44005, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=23129772&retmode=ref&cmd=prlinks, https://doi.org/10.1074/jbc.M112.417501

194 Tseng, W.C., Jenkins, P.M., Tanaka, M., Mooney, R. and Bennett, V. (2015) Giant ankyrin-G stabilizes somatodendritic GABAergic synapses through opposing endocytosis of GABAA receptors. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 1214–1219, https://doi.org/10.1073/pnas.1417989112

195 Bosmans, F., Milescu, M. and Swartz, K.J. (2011) Palmitoylation influences the function and pharmacology of sodium channels. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 20213–20218, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=22123950&retmode=ref&cmd=prlinks, https://doi.org/10.1073/pnas.1108497108

196 Pan, Y., Xiao, Y., Pei, Z. and Cummins, T.R. (2020) S-palmitoylation of the sodium channel Nav1.6 regulates its activity and neuronal excitability. *J. Biol. Chem.* **295**, 6151–6164, https://doi.org/10.1074/jbc.RA119.012423

197 Kole, M.H.P., Letzkus, J.J. and Stuart, G.J. (2007) Axon initial segment Kv1 channels control axonal action potential waveform and synaptic efficacy. *Neuron* **55**, 633–647, https://linkinghub.elsevier.com/retrieve/pii/S0896627307005764, https://doi.org/10.1016/j.neuron.2007.07.031

198 Ogawa, Y. and Rasband, M.N. (2008) The functional organization and assembly of the axon initial segment. *Curr. Opin. Neurobiol.* **18**, 307–313, http://linkinghub.elsevier.com/retrieve/pii/S0959438808000810, https://doi.org/10.1016/j.conb.2008.08.008

199 Ogawa, Y., Horresh, I., Trimmer, J.S., Bredt, D.S., Peles, E. and Rasband, M.N. (2008) Postsynaptic density-93 clusters Kv1 channels at axon initial segments independently of Caspr2. *J. Neurosci.* **28**, 5731–5739, http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.4431-07.2008, https://doi.org/10.1523/JNEUROSCI.4431-07.2008

200 Kim, E., Cho, K.O., Rothschild, A. and Sheng, M. (1996) Heteromultimerization and NMDA receptor-clustering activity of Chapsyn-110, a member of the PSD-95 family of proteins. *Neuron* **17**, 103–113, https://linkinghub.elsevier.com/retrieve/pii/S0896627300802846, https://doi.org/10.1016/S0896-6273(00)80284-6

201 El-Husseini, A.E., Topinka, J.R., Lehrer-Graiwer, J.E., Firestein, B.L., Craven, S.E., Aoki, C. et al. (2000) Ion channel clustering by membrane-associated guanylate kinases. Differential regulation by N-terminal lipid and metal binding motifs. *J. Biol. Chem.* **275**, 23904–23910, http://www.jbc.org/lookup/doi/10.1074/jbc.M909919199, https://doi.org/10.1074/jbc.M909919199

202 Topinka, J.R. and Bredt, D.S. (1998) N-terminal palmitoylation of PSD-95 regulates association with cell membranes and interaction with K+ channel Kv1.4. *Neuron* **20**, 125–134, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=9459448&retmode=ref&cmd=prlinks, https://doi.org/10.1016/S0896-6273(00)80440-7

203 Cembrowski, M.S., Wang, L., Sugino, K., Shields, B.C. and Spruston, N. (2016) Hipposeq: a comprehensive RNA-seq database of gene expression in hippocampal principal neurons. *eLife* **5**, e14997, https://elifesciences.org/articles/14997, https://doi.org/10.7554/eLife.14997

204 Zeisel, A., Hochgerner, H., Lönnerberg, P., Johnsson, A., Memic, F., van der Zwan, J. et al. (2018) Molecular architecture of the mouse nervous system. *Cell* **174**, 999.e22–1014.e22, https://doi.org/10.1016/j.cell.2018.06.021

205 Lek, M., Karczewski, K.J., Minikel, E.V., Samocha, K.E., Banks, E., Fennell, T. et al. (2016) Analysis of protein-coding genetic variation in 60,706 humans. *Nature* **536**, 285–291, https://doi.org/10.1038/nature19057

206 Yeste-Velasco, M., Linder, M.E. and Lu, Y.-J. (2015) Protein S-palmitoylation and cancer. *Biochim. Biophys. Acta Rev. Cancer* **1856**, 107–120, https://doi.org/10.1016/j.bbcan.2015.06.004

207 Oo, H.Z., Sentani, K., Sakamoto, N., Anami, K., Naito, Y., Uraoka, N. et al. (2014) Overexpression of ZDHHC14 promotes migration and invasion of scirrhous type gastric cancer. *Oncol. Rep.* **32**, 403–410, https://doi.org/10.3892/or.2014.3166

208 Adachi, N., Hess, D.T., McLaughlin, P. and Stamler, J.S. (2016) S-Palmitoylation of a novel site in the β2-adrenergic receptor associated with a novel intracellular itinerary*. *J. Biol. Chem.* **291**, 20232–20246, https://doi.org/10.1074/jbc.M116.725762

209 Kim, E., Niethammer, M., Rothschild, A., Jan, Y.N. and Sheng, M. (1995) Clustering of Shaker-type K+ channels by interaction with a family of membrane-associated guanylate kinases. *Nature* **378**, 85–88, http://www.nature.com/articles/378085a0, https://doi.org/10.1038/378085a0

210 Gubitosi-Klug, R.A., Mancuso, D.J. and Gross, R.W. (2005) The human Kv1.1 channel is palmitoylated, modulating voltage sensing: identification of a palmitoylation consensus sequence. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 5964–5968, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=15837928&retmode=ref&cmd=prlinks, https://doi.org/10.1073/pnas.0501999102

211 Nelson, J.C., Witze, E., Ma, Z., Ciocco, F., Frerotte, A., Randlett, O. et al. (2020) Acute regulation of habituation learning via posttranslational palmitoylation. *Curr. Biol.* **30**, 2729–2738, https://doi.org/10.1016/j.cub.2020.05.016

212 Wan, J., Savas, J.N., Roth, A.F., Sanders, S.S., Singaraja, R.R., Hayden, M.R. et al. (2013) Tracking brain palmitoylation change: predominance of glial change in a mouse model of Huntington's Disease. *Chem. Biol.* **20**, 1421–1434, http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=24211138&retmode=ref&cmd=prlinks, https://doi.org/10.1016/j.chembiol.2013.09.018

213 Ren, Q. and Bennett, V. (1998) Palmitoylation of neurofascin at a site in the membrane-spanning domain highly conserved among the L1 family of cell adhesion molecules. *J. Neurochem.* **70**, 1839–1849, https://doi.org/10.1046/j.1471-4159.1998.70051839.x

214 Hamdan, H., Lim, B.C., Torii, T., Joshi, A., Konning, M., Smith, C. et al. (2019) Mapping axon initial segment structure and function by multiplexed proximity biotinylation. *Nat. Commun.* **11**, 1–17

215 Roux, K.J., Kim, D.I., Raida, M. and Burke, B. (2012) A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. *J. Cell Biol.* **196**, 801–810, https://doi.org/10.1083/jcb.201112098

216 Koopmans, F., van Nierop, P., Andres-Alonso, M., Byrnes, A., Cijsouw, T., Coba, M.P. et al. (2019) SynGO: an evidence-based, expert-curated knowledge base for the synapse. *Neuron* **103**, 217.e4–234.e4, https://doi.org/10.1016/j.neuron.2019.05.002

217 Rasband, M.N. (2010) The Axon Initial Segment and the Maintenance of Neuronal Polarity. *Nat. Rev. Neurosci.* , vol. **11**, pp. 552–562, https://doi.org/10.1038/nrn2852

218 Leterrier, C. and Dargent, B. (2014) No Pasaran! Role of the axon initial segment in the regulation of protein transport and the maintenance of axonal identity. *Semin. Cell Dev. Biol.* **27**, 44–51, https://doi.org/10.1016/j.semcdb.2013.11.001

219 Goethals, S. and Brette, R. (2020) Theoretical relation between axon initial segment geometry and excitability. *eLife* **9**, e53432, https://doi.org/10.7554/eLife.53432

220 Jensen, C.S., Watanabe, S., Stas, J.I., Klaphaak, J., Yamane, A., Schmitt, N. et al. (2017) Trafficking of Kv2.1 channels to the axon initial segment by a novel nonconventional secretory pathway. *J. Neurosci.* **37**, 11523–11536, https://doi.org/10.1523/JNEUROSCI.3510-16.2017