Published in final edited form as:

J Neurosci Res. 2017 August ; 95(8): 1528–1539. doi:10.1002/jnr.24003.

Roles of Palmitoylation in Axon Growth, Degeneration and Regeneration

Sabrina M. Holland¹ and Gareth M. Thomas^{1,2,*}

¹Shriners Hospitals Pediatric Research Center (Center for Neurorehabilitation and Neural Repair), Temple University School of Medicine, 3500 N. Broad Street, Philadelphia, PA 19140

²Department of Anatomy and Cell Biology, Temple University School of Medicine, 3500 N. Broad Street, Philadelphia, PA 19140

Abstract

The protein-lipid modification palmitoylation plays important roles in neurons, but most attention has focused on roles of this modification in the regulation of mature pre- and post-synapses. However, exciting recent findings suggest that palmitoylation is also critical for both the growth and integrity of neuronal axons and plays previously unappreciated roles in conveying axonal anterograde and retrograde signals. Here we review these emerging roles for palmitoylation in the regulation of axons in health and disease.

Introduction

Axons are specialized, thin projections that often extend vast distances (up to 1 meter in humans) away from neuronal cell bodies. Regulation of axonal growth and integrity is essential for virtually all aspects of nervous system function. For example, axonal growth and branching must be precisely controlled to ensure that developing axons connect with their correct targets, while the integrity of mature axons is essential for appropriate cellular responses to an array of physiological and pathological stimuli (Kalil and Dent 2014; Kolodkin and Tessier-Lavigne 2011; Pease and Segal 2014). Conversely, functional deficits and/or physical breakdown of axons are hallmarks of numerous neurodegenerative conditions (Gerdtz et al. 2016; Neukomm and Freeman 2014; Wang et al. 2012). Axonal breakdown can result from acute severing, for example as a result of a nerve injury (Gerdtz et al. 2016), but is also observed in more chronic conditions, including diabetes-associated and chemotherapy-induced peripheral neuropathies, as well as Huntington and Alzheimer's Diseases (Adalbert and Coleman 2013; Cashman and Hoke 2015; Perlson et al. 2010). At least initially, axons in these chronic conditions remain intact and physical breakdown is thought to be a consequence of impaired axonal transport. Both anterograde and retrograde axonal protein transport is critical for normal axon development and function (Abe and Cavalli 2008; Harrington and Ginty 2013; Maday et al. 2014). Thus, an improved knowledge of the mechanisms used by neurons to transport proteins to and from distal axons may both

*Corresponding author: gareth.thomas@temple.edu.

The authors confirm that they have no conflict of interest.

clarify our understanding of normal physiological neuronal regulation and also provide insight into a variety of neuropathological conditions.

Regulation of axonal transport involves a battery of post-translational regulatory mechanisms (Harrington and Ginty 2013; Lu et al. 2014; Maday et al. 2014). Among these mechanisms, emerging evidence highlights the functional importance of S-palmitoylation, the modification of protein cysteine residues with palmitate (Tom and Martin 2013), in regulating diverse aspects of axonal function.

Palmitoylation plays key roles in protein targeting, trafficking and function in all eukaryotic cells, but is likely to be especially important in neurons, in which precise localization of proteins to specialized subcellular compartments is required to maintain cellular polarity and asymmetrical synaptic structures (Fukata and Fukata, 2010). Consistent with this notion, genetic mutation or targeted knockout of a number of palmitoyl acyltransferases (PATs, the enzymes that catalyze palmitoylation) results in predominantly neuropathological phenotypes (Li et al. 2010; Mansouri et al. 2005; Raymond et al. 2007; Singaraja et al. 2011).

Over the last decade, studies of neuronal palmitoylation have revealed key roles for this modification in the formation, maintenance and functional regulation of mature synapses, particularly in the targeting of proteins to pre- and post-synaptic sites (Fukata and Fukata 2010; Prescott et al. 2009; Thomas and Huganir 2013). In addition to its roles at synapses, though, recent findings suggest that palmitoylation plays a critical role in several aspects of axonal biology.

Roles of Palmitoylation in Axonal Growth During Development

Key roles for palmitoylation in axons were first suggested years before the identification of PATs. Live imaging of developing sensory axons revealed that acute treatment with the palmitoylation inhibitor tunicamycin caused rapid stalling of axonal growth, quickly followed by collapse of the axonal growth cone (Patterson and Skene 1994). Although tunicamycin also affects protein glycosylation and synthesis, its effects on axon extension were likely to be due to inhibition of palmitoylation because tunicamycin homologs that potently inhibit glycosylation, but not palmitoylation, only minimally affected growth cone function (Patterson and Skene 1994). Moreover, inhibition of axon extension was also observed following acute treatment with nitric oxide (NO) donors, an effect that was ascribed to blocking of palmitoylation sites by NO (Hess et al. 1993)

The rapid effects of tunicamycin and NO donors on growth cone collapse (evident within 10 minutes of treatment) strongly suggested that these compounds acted directly on the distal axon/ growth cone, rather than affecting long distance supply of proteins from the neuronal soma. If so, then this would suggest that dynamic protein palmitoylation in distal axons is necessary for normal axonal growth, an extremely exciting possibility. However, despite these dramatic functional effects, the palmitoyl-protein(s) acutely targeted by these reagents have still not been identified. Nonetheless, several proteins with key roles in axonal growth are now known to be palmitoylated. For example, Growth-associated Protein 43 kDa

(GAP-43), one of the first identified markers of rapidly extending axons (Skene and Willard 1981), was also one of the first described neuronal palmitoyl-proteins (Skene and Virag 1989). GAP-43 is highly expressed during axonal development and also in regenerating axons post-injury and is normally targeted to growth cones and growing axon tips (Gordon-Weeks 1989; Skene and Willard 1981) (Fig. 1). Mutation of two adjacent N-terminal cysteine residues, which are sites of palmitoylation, prevents this targeting (Liu et al. 1991; Skene and Virag 1989). Despite its robust palmitoylation, though, turnover of GAP-43 palmitoylation (Baker and Storm 1997; Holland et al. 2016) is too slow to account for the rapid effects of tunicamycin and NO donors reported by Skene and colleagues.

At the molecular level, palmitoylation reduces GAP-43's ability to stimulate G proteins (Nakamura et al. 1998), and can also augment GAP-43's ability to stimulate rises in intracellular calcium induced by low osmolarity (Caprini et al. 2003). Palmitoyl-GAP-43 is also targeted to lipid microdomains, where it can sequester the lipid PI(4,5)P₂ (Laux et al. 2000), which may in turn modulate recruitment and assembly of actin-based structures within growth cones (Caprini et al. 2003). However, it remains to be determined how these distinct molecular roles of GAP-43 palmitoylation might contribute to the known roles of GAP-43 in nervous system development and/or function (Donovan et al. 2002; Maier et al. 1999; Strittmatter et al. 1995).

Another palmitoyl-protein recently linked to the regulation of axon growth is the small G protein Cdc42 (Mukai et al. 2015). A palmitoylated splice variant of Cdc42 (Cdc42-palm) is present in growing axon tips and both palmitoylation and axonal localization of Cdc42-palm are controlled by the PAT ZDHHC8. Cdc42 palmitoylation is functionally important because axon growth is reduced by specific knockdown of Cdc42-palm, both in cultured neurons and *in vivo*, and is also reduced in neurons from both *ZDHHC8*^{-/-} and *ZDHHC8*^{+/+} mice (Mukai et al. 2015). Interestingly, Mukai et al. also reported that axon growth is reduced by the palmitoylation inhibitor 2-Bromopalmitate (2-Br; (Jennings et al. 2009)). This finding is reminiscent of the prior reports of stalling of axonal growth by inhibitors of palmitoylation (Hess et al. 1993; Patterson and Skene 1994). However, Mukai and colleagues only examined the effects of prolonged, not acute, 2-Br treatment. Thus, the key protein(s) that are dynamically palmitoylated in axons to control axon growth remain to be discovered. The identification of such protein(s) is an interesting avenue for future research.

Neurodevelopmental roles of palmitoylation are unlikely to be limited to axon growth, though. For example, another important developmental event is the establishment of axon-dendrite polarity. This process requires specific localization of membrane proteins to axonal growth cones, where they act as markers for the delivery of new membrane and proteins *via* axonal transport (Barnes and Polleux 2009). Several proteins implicated in neuronal polarization, including the small G proteins Rab10 and Cdc42 and the SAD protein kinases, are likely to be palmitoylated (Barnes et al. 2007; Blanc et al. 2015; Garvalov et al. 2007; Montersino and Thomas 2015). These findings raise the possibility that polarization cues may induce palmitoylation-dependent changes in the localization and/or turnover of these proteins.

A third neurodevelopmental process that is likely palmitoylation-dependent is the control of axon guidance. Both targeted and proteomic studies have identified numerous regulators of axon guidance as palmitoyl-proteins, including the netrin receptor *Deleted in Colorectal Cancer* (DCC; (Moore et al. 2007)), whose palmitoylation is critical for its ability to activate downstream signaling (Herincs et al. 2005). In addition, the DCC-related protein neogenin, as well as multiple members of the plexin, neuropilin and ephrin families of axon guidance molecules are all likely palmitoylated (Blanc et al. 2015; Kang et al. 2008; Sanders et al. 2015). How palmitoylation of these axon guidance proteins affects nervous system development is a key outstanding question.

Roles of Palmitoylation in the Maintenance and Integrity of Mature Axons

In addition to its neurodevelopmental roles, palmitoylation is also important in the mature nervous system. One key example of this importance is in the control of responses to nerve injury. Following injury, connections between distal axons and neuronal cell bodies are often severed, triggering a breakdown process in the severed distal axon termed Wallerian degeneration (Conforti et al. 2014; Gerdts et al. 2016; Waller 1850; Wang et al. 2012). In the Peripheral Nervous System (PNS), the rapid breakdown and clearance of axonal debris *via* Wallerian degeneration is thought to promote effective regrowth of the proximal axon stump (Vargas and Barres 2007).

There is intense current interest in Wallerian degeneration mechanisms, spurred in part by a desire to better understand PNS regeneration post-injury. However, similar “Wallerian-like” degeneration is seen in several neuropathological conditions, including chemotherapy-induced peripheral neuropathy (CIPN) and diabetic peripheral neuropathy, as well as both initial HIV infection and subsequent antiretroviral drug therapy (Cashman and Hoke 2015; Coleman and Freeman 2010; Gerdts et al. 2016). In these situations, distal axons remain connected to the cell body (at least initially), but undergo a “dying-back” degeneration. Wallerian-like / dying-back degeneration proceeds more slowly than ‘classic’ injury-induced Wallerian degeneration, but these two processes share common mechanisms, including key roles for multiple palmitoyl-proteins.

Wallerian and Wallerian-like degeneration were initially thought to occur passively, due to a general loss of nutrients ordinarily supplied by the cell body. However, genetic studies now suggest that both these forms of degeneration are instead actively triggered in distal axons by a lack of a specific survival/maintenance factor(s) that is normally supplied from cell bodies (Cashman and Hoke 2015; Coleman and Freeman 2010; Lunn et al. 1989). There is now strong evidence that this factor is the enzyme nicotinamide mononucleotide adenylyltransferase-2 (NMNAT2) and that palmitoylation is critical for NMNAT2-dependent regulation of axon integrity.

When axons of cultured sympathetic neurons are cut (an *in vitro* model of nerve injury), NMNAT2 levels in the severed distal axons drop rapidly and the axons degenerate. This degeneration is prevented by over-expressing NMNAT2 (Gilley and Coleman 2010). Conversely, RNA interference (RNAi)-mediated NMNAT2 depletion triggers rapid axonal breakdown in the absence of injury (Gilley and Coleman 2010).

Enzymatically, NMNAT2 catalyzes conversion of nicotinamide mononucleotide (NMN) to the key metabolite nicotinamide adenine dinucleotide (NAD⁺). Whether axonal breakdown in the absence of NMNAT2 is triggered by rapid NAD⁺ loss, rapid NMN build-up, or a combination of these factors is an area of intense investigation (Gerdtts et al. 2016). Readers interested in this issue, and in ‘downstream’ mechanisms that trigger axon degeneration following NMNAT2 loss are directed to this and other recent reports (Di Stefano et al. 2015; Gerdtts et al. 2015; Gerdtts et al. 2016; Summers et al. 2016; Yang et al. 2015). Here, though, we focus on how NMNAT2 itself is regulated and why it is so critical for axon maintenance.

In cultured neurons NMNAT2 is highly labile (half life <1 hour), and must thus be constantly supplied from neuronal cell bodies to preserve axon viability (Milde et al. 2013b). This constant supply likely involves fast axonal transport (FAT) of NMNAT2 on Golgi-derived vesicles, and NMNAT2 vesicle targeting requires palmitoylation (Milde and Coleman 2014; Milde et al. 2013b) (Fig. 1). Genetic studies also implicate the *Drosophila* NMNAT ortholog, DmNmnat, in the control of axonal integrity (Fang et al. 2012; Xiong et al. 2012) and a novel DmNmnat splice variant was recently reported to contain additional cysteines that are predicted to be palmitoylated. Moreover, this DmNmnat splice form localizes to neuronal axons and protects axon integrity under conditions of stress (Ruan et al. 2015). Palmitoylation-dependent regulation of NMNAT localization may thus be an evolutionarily conserved mechanism that is important for axonal integrity.

However, although palmitoylation is required for NMNAT2 vesicular transport, this modification negatively impacts NMNAT2 stability (Milde et al. 2013b). Indeed, when highly over-expressed, NMNAT2 mutants that cannot be palmitoylated protect severed axons in culture more effectively than does wild type NMNAT2 (Milde et al. 2013b). If non-palmitoylated NMNAT2 protects axons more effectively, then why do neurons express the palmitoyl form of NMNAT2 at all? One explanation stems from the finding that non-palmitoylated NMNAT2 mutants are only protective when highly over-expressed, and only in cultured neurons, in which axons are relatively short (Milde et al. 2013b). Under these conditions, cytosolic NMNAT2 mutants, which likely only reach axons by passive diffusion, might still accumulate sufficiently in distal axons to be protective. However, only palmitoyl-NMNAT2 may be sufficient to protect distal axons *in vivo*, due to its ability to reach these distal locations using vesicle-based FAT (Milde et al. 2013b).

NMNAT2's instability raises the question of how its degradation is controlled. Findings from both mammals and *Drosophila* suggest that NMNAT2 is targeted for ubiquitin-dependent degradation (Babetto et al. 2013; Milde et al. 2013b; Xiong et al. 2012). If palmitoylation both targets NMNAT2 to vesicle membranes and facilitates NMNAT2 degradation, it appears likely that palmitoyl-NMNAT2 is ubiquitinated by a membrane-associated E3 ubiquitin ligase(s). One candidate enzyme to perform this role is Phr1 (gene name *MYCBP2*) because Phr1 conditional knockout (CKO) axons are markedly (though not completely) preserved following injury *in vivo* or in culture (Babetto et al. 2013). Moreover, protection of severed Phr1 CKO axons in culture correlates with increased NMNAT2 levels and is prevented by NMNAT2 knockdown (Babetto et al. 2013). Further evidence suggests that Phr1 acts as part of an atypical Skp/Cullin/F-box (SCF)-type E3 ubiquitin ligase complex, because a core component of such complexes, Skp1a, was also recently found to

be critical for the control of NMNAT2 protein levels (Yamagishi 2016) and for the regulation of axon stability in *Drosophila* (Brace et al. 2014).

How might Phr1/Skp1a complexes localize close to NMNAT2? Intriguingly, both Phr1 and Skp1a have been reported to associate with vesicles (Abe et al. 2009; Debaisieux et al. 2016). Moreover, vertebrate Phr1 orthologs contain a predicted palmitoylated motif (Zhou et al. 2006) and Skp1a was reported to be palmitoylated in a proteomic study (Serwa et al. 2015). Palmitoylation may thus target Phr1 and/or Skp1a to vesicles to facilitate ubiquitin-dependent degradation of palmitoyl-NMNAT2 (Fig. 1). However, while this model is intriguing, it should be noted that it currently lacks experimental support. Moreover, Ubiquitin Proteasome System (UPS) inhibitors can protect injured sympathetic neurons from Wallerian degeneration but have little effect on injured sensory axons (Yang et al. 2013). Mechanisms of NMNAT2 degradation, and subsequent axon degeneration, may thus depend on the neuronal type.

A second palmitoyl-protein involved in the switch between axon integrity and degeneration is Superior Cervical Ganglion-10 (SCG10). SCG10 is highly expressed in both developing and regenerating sensory axons, and effectively marks the extent of sensory axon regeneration post-injury (Shin et al. 2012a; Shin et al. 2014). SCG10 (gene name *STMN2*), the homologous SCG10-Like Protein (SCLIP/*STMN3*) and *STMN5* (previously known as RB3) are neural-specific members of the stathmin family of microtubule regulatory proteins, all of which can bind tubulin heterodimers and thus disrupt microtubules (Charbaut et al. 2001). This activity is thought to counterbalance the microtubule stabilizing activity of Microtubule Associated Proteins (MAPs), thus increasing microtubule dynamics during periods of neuronal growth and regeneration (Mori and Morii 2002).

SCG10 and all other neuronal stathmin family proteins contain an N-terminal region that is absent in the more ubiquitously expressed stathmin (*STMN1*) itself (Mori and Morii 2002). Two cysteines within this N-terminal region are palmitoylated (Levy et al. 2011). Roles of palmitoylation have been best described for SCG10, for which this modification promotes Golgi localization and also growth cone targeting (Di Paolo et al. 1997; Levy et al. 2011; Lutjens et al. 2000). Moreover, live imaging studies reveal that wild type SCG10 moves anterogradely in axons on vesicle-like structures (Shin et al. 2012b). While live imaging studies of SCG10 palmitoyl mutants have not been reported, Golgi targeting may thus facilitate palmitoylation of SCG10 and subsequent anterograde trafficking *via* fast axonal transport, as is likely the case for NMNAT2 (Fig. 1). Several PATs can increase palmitoylation of cotransfected SCG10 (Levy et al. 2011), but which of these enzyme(s) palmitoylates SCG10 in neurons is not yet known.

Interestingly, like NMNAT2, SCG10 is unstable (half-life approx. 1 hour in cultured sensory neurons (Shin et al. 2012b)) and SCG10 levels in axons are continuously replenished by supply from neuronal cell bodies. Following axotomy, SCG10 in distal axons is rapidly degraded, again *via* the proteasome (Shin et al. 2012b) (Fig. 1). One key trigger for degradation of both SCG10, and of axons themselves, is SCG10 phosphorylation by c-Jun N-terminal Kinase (JNK). An over-expressed mutant SCG10 in which two JNK phosphorylation sites are mutated to alanine (SCG10-AA) is more stable than wild type

SCG10 following axotomy, and also significantly slows the degeneration of severed axons (Shin et al. 2012b). It is unknown how palmitoylation affects SCG10 stability in both healthy axons and post-axotomy, but this is an interesting area for future study.

SCG10 and NMNAT2 are thus both small, unstable palmitoyl-proteins linked to axon degeneration. NMNAT2 loss directly triggers degeneration of uninjured axons, while SCG10 loss does not affect healthy axons, but instead accelerates breakdown of injured axons. In both cases, though, palmitoylation-dependent targeting of these proteins appears critical for their roles in the regulation of axon integrity. It will be interesting to determine whether other regulators of microtubule dynamics and/or axonal integrity are targeted to axons by palmitoylation. Given the importance of axon degeneration in several neuropathological conditions, the identification of such proteins, and the PATs and thioesterases that regulate them, are key areas for future study.

Palmitoylation-dependent Axonal Retrograde Signaling

Palmitoylation is thus important for the anterograde transport of proteins linked to axonal growth and viability. However, recent work suggests an equally important role for palmitoylation in the control of retrograde signals from distal locations back to neuronal cell bodies. In the PNS, such signals are critical to stimulate pro-regenerative transcription following nerve injury (Abe and Cavalli 2008; Rishal and Fainzilber 2014).

One retrograde signaling protein that is important for pro-regenerative transcription is Dual Leucine-zipper Kinase (DLK), an upstream activator of JNK family Mitogen-activated Protein Kinases (MAPKs) (He and Jin 2016; Tedeschi and Bradke 2013; Xiong et al. 2010). DLK is predicted to be a freely diffusible, cytosolic protein, but nonetheless is critical to convey long distance directional signals in axons (Ghosh et al. 2011; Shin et al. 2012a). This ability of DLK was recently reported to be due to its palmitoylation, because shRNA 'knockdown' of DLK in sensory neurons prevented retrograde axonal injury signals, which could only be 'rescued' by wild type (palmitoylated) DLK, and not a DLK palmitoyl-site mutant (Holland et al. 2016). One major role of palmitoylation is to target DLK to vesicle membranes so that it can 'hitchhike' on axonal transport vesicles (Fig. 2). Interestingly, though, palmitoylation is also important for DLK to bind its downstream substrate MKK4, and also the scaffold protein JNK-interacting Protein-3 (JIP3), which itself is closely linked to axonal retrograde signaling (Cavalli et al. 2005; Ghosh et al. 2011; Holland et al. 2016). Palmitoylation is thus also essential for the assembly of DLK-dependent retrograde signaling complexes (Fig. 2).

Perhaps most strikingly, though, palmitoylation is also essential for DLK's ability to activate JNK pathway signaling. DLK palmitoyl-site mutation, or treatment of cells with 2-Br, greatly reduces DLK-dependent phosphorylation of MKK4, and also phosphorylation of DLK's downstream effector JNK3 (Holland et al. 2016). This effect on DLK's kinase activity is unlikely to be a secondary effect of subcellular localization, because the DLK palmitoyl-site mutant is also far less active towards MKK4 in *in vitro* assays (Holland et al. 2016). The picture emerging from these studies is that, by simultaneously controlling DLK localization, interactions and activity, palmitoylation ensures that only vesicle-bound DLK is

active in neurons. Palmitoyl-DLK is thus unlikely to inappropriately phosphorylate cytosolic MAP2Ks, perhaps explaining how DLK can control retrograde signals without affecting physiological JNK signaling (Ghosh et al. 2011). Indeed, evidence from multiple other neuron types suggests that a key role for palmitoylation of neuronal kinases is to ensure their signaling specificity (Monterisino and Thomas 2015).

Several questions arise from these findings. For example, DLK is also implicated in axonal retrograde signals from the injured optic nerve back to the cell bodies of Retinal Ganglion Cells (Watkins et al. 2013; Welsbie et al. 2013). It will thus be of interest to determine whether palmitoylation is also critical for DLK-dependent signaling in CNS axons. A related question is whether palmitoylation is necessary for two other injury responses that require DLK, but which are not thought to involve axonal retrograde trafficking (excitotoxic hippocampal neurodegeneration, and Wallerian degeneration of injured distal axons (Miller et al. 2009; Pozniak et al. 2013; Yang et al. 2015)). If these injury responses also require palmitoyl-DLK then this would further support the hypothesis that this modification controls DLK-dependent signaling, in addition to its role in DLK trafficking.

More broadly, other retrograde signaling pathway proteins (Cavalli et al. 2005; Chesarino et al. 2014; Ren et al. 2013; Yang et al. 2012) as well as both core components and regulators of the dynein/dynactin retrograde motor protein complex (Kang et al. 2008; Shmueli et al. 2010) are also reported to be palmitoylated (Fig. 2). In addition, dynein itself was recently reported to cluster in lipid microdomains on phagosomes in non-neuronal cells (Rai et al. 2016). Palmitoylation frequently controls protein targeting to lipid microdomains (Fukata and Fukata 2010), so investigations into whether palmitoylation plays broader roles in the regulation of dynein-dependent retrograde transport and signaling are eagerly awaited.

Additional Physiological Roles for Axonal Palmitoylation?

Palmitoylation is thus critical for axonal growth, integrity and retrograde signaling, but may also regulate other key aspects of axonal biology. For example, many proteins that cluster at the axon initial segment (AIS) and at nodes of Ranvier, are likely palmitoylated. Of these AIS-/node-enriched palmitoyl-proteins, the best studied is Ankyrin-G (AnkG). In AnkG 'knockdown' neurons, a transfected AnkG palmitoyl-site mutant fails to cluster at the AIS, and two other key AIS proteins, Neurofascin and voltage-gated sodium channels, are no longer AIS-enriched (He et al. 2012). Interestingly, the AnkG palmitoylation site is conserved in all vertebrate ankryins, suggesting that it may be important in other roles of this protein family. In non-neuronal cells, Ankyrin palmitoylation is controlled by two related PATs, ZDHHC5 and ZDHHC8 (He et al. 2014), though whether these PATs regulate Ankyrin palmitoylation at the AIS is unknown.

An additional possibility is that AnkG must also be palmitoylated to localize to nodes of Ranvier, and in this regard it is striking that several node-enriched proteins are palmitoylated. These include the node-enriched form of Neurofascin (Neurofascin-186) (Ren and Bennett 1998) and NrCAM, a node protein homologous to Neurofascin that contains an almost identical palmitoylation site (Wei et al. 2014). Multiple proteins that form complexes in the adjacent paranodes have also been identified in palmitoyl-proteomic

studies. Paranodal palmitoyl-proteins include Contactin and Contactin-associated protein-1, also known as Caspr (Ren et al. 2013; Wan et al. 2013), and Neurofascin-155, a paranode enriched Neurofascin isoform that contains the same palmitoylation site identified in Neurofascin-186. Whether and how palmitoylation of these different node and paranode proteins affects synaptic transmission, plasticity and neuronal circuit function merits further investigation.

Possible Roles of Axonal Palmitoylation in Neuropsychiatric and Neurodegenerative Disease

It is also an intriguing possibility that aberrant axonal palmitoylation contributes to pathogenesis in neuropsychiatric and/or neurodegenerative conditions. In support of this notion, the *ZDHHC8* gene has been implicated in schizophrenia (Mukai et al. 2008; Mukai et al. 2004), but see (Otani et al. 2005, Demily et al., 2007). Initial characterization of *ZDHHC8* knockout mice focused on postsynaptic deficits in dendritic spine morphology (Mukai et al. 2008), suggesting that this PAT might predominantly be involved in postsynaptic regulation. Indeed, a postsynaptic role for ZDHHC8 is supported by other reports (Moutin et al. 2016; Thomas et al. 2012). However, a recent study also revealed a role for ZDHHC8 in axonal development, which was linked to impaired functional connectivity and behavioral impairments (Mukai et al. 2015). These findings raise the possibility that impaired ZDHHC8-dependent axon growth or connectivity might contribute to schizophrenia.

Impaired axonal palmitoylation may also play a role in the devastating neurodegenerative condition Huntington Disease (HD), which is caused by poly-glutamine expansions in the protein Huntingtin (Htt). Gain-of-function effects of mutant Htt, in particular the formation of neurotoxic inclusions, are thought to be central to HD pathogenesis, but loss-of-function of wild type Htt is also thought to contribute to disease progression (Schulte and Littleton 2011). Htt is a palmitoyl-protein that normally localizes to neuronal axons and may regulate multiple aspects of axonal transport (Caviston and Holzbaur 2009; Caviston et al. 2007; Fu and Holzbaur 2014). Interestingly, mutation of Htt's palmitoylation site exacerbates formation of neurotoxic inclusions of both wild type and disease-associated Htt variants (Yanai et al. 2006). Palmitoylation of Htt is likely controlled by Huntingtin-interacting Protein 14 (HIP14), now identified as the PAT ZDHHC17 (Huang et al. 2004; Yanai et al. 2006), and the closely related HIP14-like, also known as ZDHHC13 (Huang et al. 2009). Moreover, mice with a *ZDHHC17* gene disruption develop neuropathological deficits reminiscent of HD (Singaraja et al. 2011). Together, these findings suggest that the normal function of Htt requires its palmitoylation and that impaired Htt palmitoylation likely contributes to HD pathogenesis. The extent to which impaired palmitoylation of other axonal proteins is important for the progression of other neuropsychiatric and/or neurodegenerative conditions is worthy of further study.

Outstanding Questions

Despite the recent progress in the identification of axonal palmitoyl-proteins and the roles of this modification in axons, many questions remain. In particular, one key issue is whether

palmitoylation of axonal proteins is dynamically regulated and, if so, which extracellular signals trigger acute increases or decreases in axonal palmitoylation. An initial report suggested that many axonal proteins appeared to be extremely stably palmitoylated, in contrast to the rapid palmitate turnover reported for several synapto-dendritic proteins (Kang et al. 2008). However, more recent studies have identified several axonal palmitoyl-proteins that undergo rapid palmitate turnover (Holland et al. 2016; Milde and Coleman 2014). Indeed, the rapid turnover of palmitoylation on NMNAT2 and the roles of this protein in axonal growth (Milde and Coleman 2014; Milde et al. 2013a) suggest that it could account for the acute effects of palmitoylation inhibitors on axonal growth cone stalling (Hess et al. 1993; Patterson and Skene 1994). While this issue has yet to be experimentally addressed, it is nonetheless an extremely exciting possibility that dynamic palmitoylation of proteins in axon tips may be critical for responses to guidance cues and/or signals that promote axonal survival or degeneration.

However, while palmitoylation of axonal proteins can certainly be dynamic, evidence that it is rapidly regulated in axons in response to stimuli remains scarce. Part of the reason for this absence of evidence is simply that studies to directly identify such proteins have not yet been performed. For example, proteomic studies reveal many axonal proteins that are likely palmitoylated (Kang et al. 2008; Pinner et al. 2016; Wan et al. 2013), but few follow-up investigations on the regulation of palmitoylation of these proteins have been carried out. Recently, though, stimulus-dependent changes in palmitoylation of specific proteins in both neurons and non-neuronal cells have been reported (Akimzhanov and Boehning 2015; Brigidi et al. 2014; Wei et al. 2014). Additional reports of agonist-induced palmitoylation changes in neurons, particularly those focusing on axonal proteins, are thus eagerly awaited.

There is also little current knowledge regarding the specific PATs and thioesterases that are critical for regulation of axonal substrates. Indeed, despite considerable recent progress in elucidating roles for specific members of these enzyme families in neuronal dendrites (Brigidi et al. 2015; Thomas et al. 2012; Yokoi et al. 2016), almost nothing is known regarding which PATs and thioesterases are present in axons and how the activity and/or localization of these enzymes is controlled. Further research into this area may reveal why some axonal/pre-synaptic proteins are very stably palmitoylated, while others exhibit rapid palmitate turnover (Holland et al. 2016; Kang et al. 2008; Milde and Coleman 2014) and may identify 'druggable' therapeutic targets to decrease axon degeneration and/or improve regeneration.

Another key question regards the extent to which alternative membrane targeting signals can substitute for palmitoylation. Current evidence, based on a limited number of studies, suggests that the answer depends very much on the protein in question. For example, heterologous membrane targeting sequences can substitute for palmitoylation in the case of NMNAT2, but not DLK, at least in the assays so far performed (Holland et al. 2016; Milde et al. 2013b). One possible reason for the unique role of palmitoylation for DLK is if this lipid attachment serves not only to control membrane attachment, but also to regulate DLK interactions and/or activity, as was recently reported (Holland et al. 2016). In such a case, adding a heterologous membrane targeting sequence to a different region of the protein might not be predicted to phenocopy palmitoylation.

However, further studies will be required to determine whether findings with DLK represent a ‘one-off’ result or whether palmitoylation uniquely regulates other axonal palmitoyl-proteins.

Finally, we have focused this review on palmitoyl-proteins expressed within axons themselves. However, there is almost nothing known regarding roles of palmitoylation in the oligodendrocytes and Schwann cells that ensheath and myelinate axons in the CNS and PNS, respectively. The specialized membrane morphology of oligodendrocytes / Schwann cells suggests that precise subcellular protein localization is likely also critical within these ensheathing cells and hence that palmitoylation may play important roles in their development and/or physiological function (Schneider et al. 2005). Moreover, Ribotag approaches to identify actively translating mRNAs reveals that oligodendrocytes express only a specialized subset of PATs, suggesting that palmitoylation in these cells may be regulated in a very different manner than within neurons (Doyle et al. 2008). The increasing appreciation that oligodendrocytes and Schwann cells also provide metabolic support for axons and play extensive roles in axon regeneration and degeneration (Jessen and Mirsky 2016; Kang et al. 2013; Simons and Nave 2016; Tognatta and Miller 2016) suggests that defining roles of palmitoylation in these cells could provide new insights into physiological nervous system regulation and into multiple disease conditions.

In summary, numerous axonal proteins are likely palmitoylated and this modification plays key roles in several aspects of axonal biology. Axonal proteins for which roles of palmitoylation have already been defined are listed in Table 1, but we expect this list to grow rapidly in the years to come. As knowledge of the roles of axonal palmitoylation and of the enzymes that regulate it increases, so will our understanding of axonal function in both health and disease. Further insights into these issues are eagerly awaited.

Acknowledgments

We thank Drs. Francesca DeSimone and Jingwen Niu (both Thomas Lab) and Dr. Chih-Ying Su (UC San Diego) for comments on the manuscript. Supported by grants from NIH (R01NS094402) and Shriners Hospitals for Children (85600) to G.M.T.

References

- Abe N, Almenar-Queralt A, Lillo C, Shen Z, Lozach J, Briggs SP, Williams DS, Goldstein LS, Cavalli V. Sunday driver interacts with two distinct classes of axonal organelles. *J Biol Chem*. 2009; 284(50):34628–34639. [PubMed: 19801628]
- Abe N, Cavalli V. Nerve injury signaling. *Curr Opin Neurobiol*. 2008; 18(3):276–283. [PubMed: 18655834]
- Adalbert R, Coleman MP. Review: Axon pathology in age-related neurodegenerative disorders. *Neuropathol Appl Neurobiol*. 2013; 39(2):90–108. [PubMed: 23046254]
- Akimzhanov AM, Boehning D. Rapid and transient palmitoylation of the tyrosine kinase Lck mediates Fas signaling. *Proc Natl Acad Sci U S A*. 2015; 112(38):11876–11880. [PubMed: 26351666]
- Babetto E, Beirowski B, Russler EV, Milbrandt J, DiAntonio A. The Phr1 ubiquitin ligase promotes injury-induced axon self-destruction. *Cell Rep*. 2013; 3(5):1422–1429. [PubMed: 23665224]
- Baker LP, Storm DR. Dynamic palmitoylation of neuromodulin (GAP-43) in cultured rat cerebellar neurons and mouse N1E-115 cells. *Neurosci Lett*. 1997; 234(2–3):156–160. [PubMed: 9364521]

- Barnes AP, Lilley BN, Pan YA, Plummer LJ, Powell AW, Raines AN, Sanes JR, Polleux F. LKB1 and SAD kinases define a pathway required for the polarization of cortical neurons. *Cell*. 2007; 129(3): 549–563. [PubMed: 17482548]
- Barnes AP, Polleux F. Establishment of axon-dendrite polarity in developing neurons. *Annu Rev Neurosci*. 2009; 32:347–381. [PubMed: 19400726]
- Blanc M, David F, Abrami L, Migliozi D, Armand F, Burgi J, van der Goot FG. SwissPalm: Protein Palmitoylation database. *F1000Res*. 2015; 4:261. [PubMed: 26339475]
- Brace EJ, Wu CL, Valakh V, DiAntonio A. SkpA Restrains Synaptic Terminal Growth during Development and Promotes Axonal Degeneration following Injury. *Journal of Neuroscience*. 2014; 34(25):8398–8410. [PubMed: 24948796]
- Brigidi GS, Santyr B, Shimell J, Jovellar B, Bamji SX. Activity-regulated trafficking of the palmitoyl-acyl transferase DHHC5. *Nat Commun*. 2015; 6:8200. [PubMed: 26334723]
- Brigidi GS, Sun Y, Beccano-Kelly D, Pitman K, Mobasser M, Borgland SL, Milnerwood AJ, Bamji SX. Palmitoylation of delta-catenin by DHHC5 mediates activity-induced synapse plasticity. *Nat Neurosci*. 2014; 17(4):522–532. [PubMed: 24562000]
- Caprini M, Gomis A, Cabedo H, Planells-Cases R, Belmonte C, Viana F, Ferrer-Montiel A. GAP43 stimulates inositol trisphosphate-mediated calcium release in response to hypotonicity. *Embo J*. 2003; 22(12):3004–3014. [PubMed: 12805215]
- Cashman CR, Hoke A. Mechanisms of distal axonal degeneration in peripheral neuropathies. *Neurosci Lett*. 2015; 596:33–50. [PubMed: 25617478]
- Cavalli V, Kujala P, Klumperman J, Goldstein LS. Sunday Driver links axonal transport to damage signaling. *J Cell Biol*. 2005; 168(5):775–787. [PubMed: 15738268]
- Caviston JP, Holzbaur EL. Huntingtin as an essential integrator of intracellular vesicular trafficking. *Trends Cell Biol*. 2009; 19(4):147–155. [PubMed: 19269181]
- Caviston JP, Ross JL, Antony SM, Tokito M, Holzbaur EL. Huntingtin facilitates dynein/dynactin-mediated vesicle transport. *Proc Natl Acad Sci U S A*. 2007; 104(24):10045–10050. [PubMed: 17548833]
- Charbaut E, Curmi PA, Ozon S, Lachkar S, Redeker V, Sobel A. Stathmin family proteins display specific molecular and tubulin binding properties. *J Biol Chem*. 2001; 276(19):16146–16154. [PubMed: 11278715]
- Chesarino NM, Hach JC, Chen JL, Zaro BW, Rajaram MV, Turner J, Schlesinger LS, Pratt MR, Hang HC, Yount JS. Chemoproteomics reveals Toll-like receptor fatty acylation. *BMC Biol*. 2014; 12(1):91. [PubMed: 25371237]
- Coleman MP, Freeman MR. Wallerian degeneration, wld(s), and nmnat. *Annu Rev Neurosci*. 2010; 33:245–267. [PubMed: 20345246]
- Conforti L, Gilley J, Coleman MP. Wallerian degeneration: an emerging axon death pathway linking injury and disease. *Nat Rev Neurosci*. 2014; 15(6):394–409. [PubMed: 24840802]
- Debaisieux S, Encheva V, Chakravarty P, Snijders AP, Schiavo G. Analysis of Signaling Endosome Composition and Dynamics Using SILAC in Embryonic Stem Cell-Derived Neurons. *Molecular & Cellular Proteomics*. 2016; 15(2):542–557. [PubMed: 26685126]
- Demily C, Legallie S, Bou J, Houy-Durand E, Van Amelsvoort T, Zinkstok J, Manouvrier-Hanue S, Vogels A, Drouin-Garraud V, Philip N, Philippe A, Héron D, Sarda P, Petit M, Thibaut F, Frébourg T, Campion D. ZDHHC8 single nucleotide polymorphism rs175174 is not associated with psychiatric features of the 22q11 deletion syndrome or schizophrenia. 2007; 17(5):311–312.
- Di Paolo G, Lutjens R, Pellier V, Stimpson SA, Beuchat MH, Catsicas S, Grenningloh G. Targeting of SCG10 to the area of the Golgi complex is mediated by its NH2-terminal region. *J Biol Chem*. 1997; 272(8):5175–5182. [PubMed: 9030585]
- Di Stefano M, Nascimento-Ferreira I, Orsomando G, Mori V, Gilley J, Brown R, Janeckova L, Vargas ME, Worrell LA, Loreto A, Tickle J, Patrick J, Webster JR, Marangoni M, Carpi FM, Pucciarelli S, Rossi F, Meng W, Sagasti A, Ribchester RR, Magni G, Coleman MP, Conforti L. A rise in NAD precursor nicotinamide mononucleotide (NMN) after injury promotes axon degeneration. *Cell Death Differ*. 2015; 22(5):731–742. [PubMed: 25323584]

- Donovan SL, Mamounas LA, Andrews AM, Blue ME, McCasland JS. GAP-43 is critical for normal development of the serotonergic innervation in forebrain. *J Neurosci*. 2002; 22(9):3543–3552. [PubMed: 11978831]
- Doyle JP, Dougherty JD, Heiman M, Schmidt EF, Stevens TR, Ma G, Bupp S, Shrestha P, Shah RD, Doughty ML, Gong S, Greengard P, Heintz N. Application of a translational profiling approach for the comparative analysis of CNS cell types. *Cell*. 2008; 135(4):749–762. [PubMed: 19013282]
- Fang Y, Soares L, Teng X, Geary M, Bonini NM. A novel *Drosophila* model of nerve injury reveals an essential role of *Nmnat* in maintaining axonal integrity. *Curr Biol*. 2012; 22(7):590–595. [PubMed: 22425156]
- Fu MM, Holzbaur EL. Integrated regulation of motor-driven organelle transport by scaffolding proteins. *Trends Cell Biol*. 2014; 24(10):564–574. [PubMed: 24953741]
- Fukata Y, Fukata M. Protein palmitoylation in neuronal development and synaptic plasticity. *Nat Rev Neurosci*. 2010; 11(3):161–175. [PubMed: 20168314]
- Garvalov BK, Flynn KC, Neukirchen D, Meyn L, Teusch N, Wu X, Brakebusch C, Bamberg JR, Bradke F. Cdc42 regulates cofilin during the establishment of neuronal polarity. *J Neurosci*. 2007; 27(48):13117–13129. [PubMed: 18045906]
- Gerds J, Brace EJ, Sasaki Y, DiAntonio A, Milbrandt J. SARM1 activation triggers axon degeneration locally via NAD(+) destruction. *Science*. 2015; 348(6233):453–457. [PubMed: 25908823]
- Gerds J, Summers DW, Milbrandt J, DiAntonio A. Axon Self-Destruction: New Links among SARM1, MAPKs, and NAD+ Metabolism. *Neuron*. 2016; 89(3):449–460. [PubMed: 26844829]
- Ghosh AS, Wang B, Pozniak CD, Chen M, Watts RJ, Lewcock JW. DLK induces developmental neuronal degeneration via selective regulation of proapoptotic JNK activity. *J Cell Biol*. 2011; 194(5):751–764. [PubMed: 21893599]
- Gilley J, Coleman MP. Endogenous *Nmnat2* is an essential survival factor for maintenance of healthy axons. *PLoS Biol*. 2010; 8(1):e1000300. [PubMed: 20126265]
- Gordon-Weeks PR. GAP-43--what does it do in the growth cone? *Trends Neurosci*. 1989; 12(10):363–365. [PubMed: 2479132]
- Harrington AW, Ginty DD. Long-distance retrograde neurotrophic factor signalling in neurons. *Nat Rev Neurosci*. 2013; 14(3):177–187. [PubMed: 23422909]
- He M, Abdi KM, Bennett V. Ankyrin-G palmitoylation and betaII-spectrin binding to phosphoinositide lipids drive lateral membrane assembly. *J Cell Biol*. 2014; 206(2):273–288. [PubMed: 25049274]
- He M, Jenkins P, Bennett V. Cysteine 70 of ankyrin-G is S-palmitoylated and is required for function of ankyrin-G in membrane domain assembly. *J Biol Chem*. 2012; 287(52):43995–44005. [PubMed: 23129772]
- He Z, Jin Y. Intrinsic Control of Axon Regeneration. *Neuron*. 2016; 90(3):437–451. [PubMed: 27151637]
- Herincs Z, Corset V, Cahuzac N, Furne C, Castellani V, Hueber AO, Mehlen P. DCC association with lipid rafts is required for netrin-1-mediated axon guidance. *J Cell Sci*. 2005; 118(Pt 8):1687–1692. [PubMed: 15811950]
- Hess DT, Patterson SI, Smith DS, Skene JH. Neuronal growth cone collapse and inhibition of protein fatty acylation by nitric oxide. *Nature*. 1993; 366(6455):562–565. [PubMed: 8255294]
- Holland SM, Collura KM, Ketschek A, Noma K, Ferguson TA, Jin Y, Gallo G, Thomas GM. Palmitoylation controls DLK localization, interactions and activity to ensure effective axonal injury signaling. *Proc Natl Acad Sci U S A*. 2016; 113(3):763–768. [PubMed: 26719418]
- Huang K, Sanders S, Singaraja R, Orban P, Cijssouw T, Arstikaitis P, Yanai A, Hayden MR, El-Husseini A. Neuronal palmitoyl acyl transferases exhibit distinct substrate specificity. *FASEB J*. 2009; 23(8):2605–2615. [PubMed: 19299482]
- Huang K, Yanai A, Kang R, Arstikaitis P, Singaraja RR, Metzler M, Mullard A, Haigh B, Gauthier-Campbell C, Gutekunst CA, Hayden MR, El-Husseini A. Huntingtin-interacting protein HIP14 is a palmitoyl transferase involved in palmitoylation and trafficking of multiple neuronal proteins. *Neuron*. 2004; 44(6):977–986. [PubMed: 15603740]
- Jennings BC, Nadolski MJ, Ling Y, Baker MB, Harrison ML, Deschenes RJ, Linder ME. 2-Bromopalmitate and 2-(2-hydroxy-5-nitro-benzylidene)-benzo[b]thiophen-3-one inhibit DHHC-mediated palmitoylation in vitro. *J Lipid Res*. 2009; 50(2):233–242. [PubMed: 18827284]

- Jessen KR, Mirsky R. The repair Schwann cell and its function in regenerating nerves. *J Physiol*. 2016
- Kalil K, Dent EW. Branch management: mechanisms of axon branching in the developing vertebrate CNS. *Nat Rev Neurosci*. 2014; 15(1):7–18. [PubMed: 24356070]
- Kang R, Wan J, Arstikaitis P, Takahashi H, Huang K, Bailey AO, Thompson JX, Roth AF, Drisdell RC, Mastro R, Green WN, Yates JR 3rd, Davis NG, El-Husseini A. Neural palmitoyl-proteomics reveals dynamic synaptic palmitoylation. *Nature*. 2008; 456(7224):904–909. [PubMed: 19092927]
- Kang SH, Li Y, Fukaya M, Lorenzini I, Cleveland DW, Ostrow LW, Rothstein JD, Bergles DE. Degeneration and impaired regeneration of gray matter oligodendrocytes in amyotrophic lateral sclerosis. *Nat Neurosci*. 2013; 16(5):571–579. [PubMed: 23542689]
- Kolodkin AL, Tessier-Lavigne M. Mechanisms and molecules of neuronal wiring: a primer. *Cold Spring Harb Perspect Biol*. 2011; 3(6)
- Laux T, Fukami K, Thelen M, Golub T, Frey D, Caroni P. 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*. 2000; 149(7):1455–1472. [PubMed: 10871285]
- Levy AD, Devignot V, Fukata Y, Fukata M, Sobel A, Chauvin S. Subcellular Golgi localization of Stathmin family proteins is promoted by a specific set of DHHC palmitoyl transferases. *Mol Biol Cell*. 2011
- Li Y, Hu J, Hofer K, Wong AM, Cooper JD, Birnbaum SG, Hammer RE, Hofmann SL. 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*. 2010; 285(17):13022–13031. [PubMed: 20178993]
- Liu YC, Chapman ER, Storm DR. Targeting of neuromodulin (GAP-43) fusion proteins to growth cones in cultured rat embryonic neurons. *Neuron*. 1991; 6(3):411–420. [PubMed: 1825782]
- Lu Y, Belin S, He Z. Signaling regulations of neuronal regenerative ability. *Curr Opin Neurobiol*. 2014; 27:135–142. [PubMed: 24727245]
- Lunn ER, Perry VH, Brown MC, Rosen H, Gordon S. Absence of Wallerian Degeneration does not Hinder Regeneration in Peripheral Nerve. *Eur J Neurosci*. 1989; 1(1):27–33. [PubMed: 12106171]
- Lutjens R, Igarashi M, Pellier V, Blasey H, Di Paolo G, Ruchti E, Pfulg C, Staple JK, Catsicas S, Grenningloh G. Localization and targeting of SCG10 to the trans-Golgi apparatus and growth cone vesicles. *Eur J Neurosci*. 2000; 12(7):2224–2234. [PubMed: 10947801]
- Maday S, Twelvetrees AE, Moughamian AJ, Holzbaur EL. Axonal transport: cargo-specific mechanisms of motility and regulation. *Neuron*. 2014; 84(2):292–309. [PubMed: 25374356]
- Maier DL, Mani S, Donovan SL, Soppet D, Tessarollo L, McCasland JS, Meiri KF. Disrupted cortical map and absence of cortical barrels in growth-associated protein (GAP)-43 knockout mice. *Proc Natl Acad Sci U S A*. 1999; 96(16):9397–9402. [PubMed: 10430954]
- Mansouri MR, Marklund L, Gustavsson P, Davey E, Carlsson B, Larsson C, White I, Gustavson KH, Dahl N. 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*. 2005; 13(8):970–977. [PubMed: 15915161]
- Milde S, Coleman MP. Identification of palmitoyltransferase and thioesterase enzymes that control the subcellular localization of axon survival factor nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2). *J Biol Chem*. 2014; 289(47):32858–32870. [PubMed: 25271157]
- Milde S, Gilley J, Coleman MP. Axonal trafficking of NMNAT2 and its roles in axon growth and survival in vivo. *Bioarchitecture*. 2013a; 3(5):133–140. [PubMed: 24284888]
- Milde S, Gilley J, Coleman MP. Subcellular localization determines the stability and axon protective capacity of axon survival factor Nmnat2. *PLoS Biol*. 2013b; 11(4):e1001539. [PubMed: 23610559]
- Miller BR, Press C, Daniels RW, Sasaki Y, Milbrandt J, DiAntonio A. A dual leucine kinase-dependent axon self-destruction program promotes Wallerian degeneration. *Nat Neurosci*. 2009; 12(4):387–389. [PubMed: 19287387]
- Montersino A, Thomas GM. Slippery signaling: Palmitoylation-dependent control of neuronal kinase localization and activity. *Mol Membr Biol*. 2015; 32(5–8):179–188. [PubMed: 27241460]
- Moore SW, Tessier-Lavigne M, Kennedy TE. Netrins and their receptors. *Adv Exp Med Biol*. 2007; 621:17–31. [PubMed: 18269208]

- Mori N, Morii H. SCG10-related neuronal growth-associated proteins in neural development, plasticity, degeneration, and aging. *J Neurosci Res.* 2002; 70(3):264–273. [PubMed: 12391585]
- Moutin E, Nikonenko I, Stefanelli T, Wirth A, Ponimaskin E, De Roo M, Muller D. Palmitoylation of cdc42 Promotes Spine Stabilization and Rescues Spine Density Deficit in a Mouse Model of 22q11.2 Deletion Syndrome. *Cereb Cortex.* 2016
- Mukai J, Dhillia A, Drew LJ, Stark KL, Cao L, MacDermott AB, Karayiorgou M, Gogos JA. Palmitoylation-dependent neurodevelopmental deficits in a mouse model of 22q11 microdeletion. *Nat Neurosci.* 2008; 11(11):1302–1310. [PubMed: 18836441]
- Mukai J, Liu H, Burt RA, Swor DE, Lai WS, Karayiorgou M, Gogos JA. Evidence that the gene encoding ZDHHHC8 contributes to the risk of schizophrenia. *Nat Genet.* 2004; 36(7):725–731. [PubMed: 15184899]
- Mukai J, Tamura M, Fenelon K, Rosen AM, Spellman TJ, Kang R, MacDermott AB, Karayiorgou M, Gordon JA, Gogos JA. Molecular substrates of altered axonal growth and brain connectivity in a mouse model of schizophrenia. *Neuron.* 2015; 86(3):680–695. [PubMed: 25913858]
- Nakamura F, Strittmatter P, Strittmatter SM. GAP-43 augmentation of G protein-mediated signal transduction is regulated by both phosphorylation and palmitoylation. *J Neurochem.* 1998; 70(3):983–992. [PubMed: 9489717]
- Neukomm LJ, Freeman MR. Diverse cellular and molecular modes of axon degeneration. *Trends Cell Biol.* 2014; 24(9):515–523. [PubMed: 24780172]
- Otani K, Ujike H, Tanaka Y, Morita Y, Kishimoto M, Morio A, Uchida N, Nomura A, Kuroda S. The ZDHHHC8 gene did not associate with bipolar disorder or schizophrenia. *Neurosci Lett.* 2005; 390(3):166–170. [PubMed: 16150541]
- Patterson SI, Skene JH. Novel inhibitory action of tunicamycin homologues suggests a role for dynamic protein fatty acylation in growth cone-mediated neurite extension. *J Cell Biol.* 1994; 124(4):521–536. [PubMed: 8106550]
- Pease SE, Segal RA. Preserve and protect: maintaining axons within functional circuits. *Trends Neurosci.* 2014; 37(10):572–582. [PubMed: 25167775]
- Pelerson E, Maday S, Fu MM, Moughamian AJ, Holzbaur EL. Retrograde axonal transport: pathways to cell death? *Trends Neurosci.* 2010; 33(7):335–344. [PubMed: 20434225]
- Pinner AL, Tucholski J, Haroutunian V, McCullumsmith RE, Meador-Woodruff JH. Decreased protein S-palmitoylation in dorsolateral prefrontal cortex in schizophrenia. *Schizophr Res.* 2016
- Pozniak CD, Sengupta Ghosh A, Gogineni A, Hanson JE, Lee SH, Larson JL, Solanoy H, Bustos D, Li H, Ngu H, Jubb AM, Ayalon G, Wu J, Searce-Levie K, Zhou Q, Weimer RM, Kirkpatrick DS, Lewcock JW. Dual leucine zipper kinase is required for excitotoxicity-induced neuronal degeneration. *J Exp Med.* 2013; 210(12):2553–2567. [PubMed: 24166713]
- Prescott GR, Gorleku OA, Greaves J, Chamberlain LH. Palmitoylation of the synaptic vesicle fusion machinery. *J Neurochem.* 2009; 110(4):1135–1149. [PubMed: 19508429]
- Rai A, Pathak D, Thakur S, Singh S, Dubey AK, Mallik R. Dynein Clusters into Lipid Microdomains on Phagosomes to Drive Rapid Transport toward Lysosomes. *Cell.* 2016; 164(4):722–734. [PubMed: 26853472]
- Raymond FL, Tarpey PS, Edkins S, Tofts C, O'Meara S, Teague J, Butler A, Stevens C, Barthorpe S, Buck G, Cole J, Dicks E, Gray K, Halliday K, Hills K, Hinton J, Jones D, Menzies A, Perry J, Raine K, Shepherd R, Small A, Varian J, Widaa S, Mallya U, Moon J, Luo Y, Shaw M, Boyle J, Kerr B, Turner G, Quarrell O, Cole T, Easton DF, Wooster R, Bobrow M, Schwartz CE, Gecz J, Stratton MR, Futreal PA. Mutations in ZDHHHC9, which encodes a palmitoyltransferase of NRAS and HRAS, cause X-linked mental retardation associated with a Marfanoid habitus. *Am J Hum Genet.* 2007; 80(5):982–987. [PubMed: 17436253]
- Ren Q, Bennett V. Palmitoylation of neurofascin at a site in the membrane-spanning domain highly conserved among the L1 family of cell adhesion molecules. *J Neurochem.* 1998; 70(5):1839–1849. [PubMed: 9572267]
- Ren W, Jhala US, Du K. Proteomic analysis of protein palmitoylation in adipocytes. *Adipocyte.* 2013; 2(1):17–28. [PubMed: 23599907]
- Rishal I, Fainzilber M. Axon-soma communication in neuronal injury. *Nat Rev Neurosci.* 2014; 15(1):32–42. [PubMed: 24326686]

- Ruan K, Zhu Y, Li C, Brazill JM, Zhai RG. Alternative splicing of *Drosophila* Nmnat functions as a switch to enhance neuroprotection under stress. *Nat Commun.* 2015; 6:10057. [PubMed: 26616331]
- Sanders SS, Martin DD, Butland SL, Lavalley-Adam M, Calzolari D, Kay C, Yates JR 3rd, Hayden MR. Curation of the Mammalian Palmitoylome Indicates a Pivotal Role for Palmitoylation in Diseases and Disorders of the Nervous System and Cancers. *PLoS Comput Biol.* 2015; 11(8):e1004405. [PubMed: 26275289]
- Schneider A, Lander H, Schulz G, Wolburg H, Nave KA, Schulz JB, Simons M. Palmitoylation is a sorting determinant for transport to the myelin membrane. *J Cell Sci.* 2005; 118(Pt 11):2415–2423. [PubMed: 15923654]
- Schulte J, Littleton JT. The biological function of the Huntingtin protein and its relevance to Huntington's Disease pathology. *Curr Trends Neurol.* 2011; 5:65–78. [PubMed: 22180703]
- Serwa RA, Abaitua F, Krause E, Tate EW, O'Hare P. Systems Analysis of Protein Fatty Acylation in Herpes Simplex Virus-Infected Cells Using Chemical Proteomics. *Chem Biol.* 2015; 22(8):1008–1017. [PubMed: 26256475]
- Shin JE, Cho Y, Beirowski B, Milbrandt J, Cavalli V, DiAntonio A. Dual leucine zipper kinase is required for retrograde injury signaling and axonal regeneration. *Neuron.* 2012a; 74(6):1015–1022. [PubMed: 22726832]
- Shin JE, Geisler S, DiAntonio A. Dynamic regulation of SCG10 in regenerating axons after injury. *Exp Neurol.* 2014; 252:1–11. [PubMed: 24246279]
- Shin JE, Miller BR, Babetto E, Cho Y, Sasaki Y, Qayum S, Russler EV, Cavalli V, Milbrandt J, DiAntonio A. SCG10 is a JNK target in the axonal degeneration pathway. *Proc Natl Acad Sci U S A.* 2012b; 109(52):E3696–E3705. [PubMed: 23188802]
- Shmueli A, Segal M, Sapir T, Tsutsumi R, Noritake J, Bar A, Sapoznik S, Fukata Y, Orr I, Fukata M, Reiner O. Ndel1 palmitoylation: a new mean to regulate cytoplasmic dynein activity. *Embo J.* 2010; 29(1):107–119. [PubMed: 19927128]
- Simons M, Nave KA. Oligodendrocytes: Myelination and Axonal Support. *Cold Spring Harb Perspect Biol.* 2016; 8(1):a020479.
- Singaraja RR, Huang K, Sanders SS, Milnerwood AJ, Hines R, Lerch JP, Franciosi S, Drisdell RC, Vaid K, Young FB, Doty C, Wan J, Bissada N, Henkelman RM, Green WN, Davis NG, Raymond LA, Hayden MR. Altered palmitoylation and neuropathological deficits in mice lacking HIP14. *Hum Mol Genet.* 2011; 20(20):3899–3909. [PubMed: 21775500]
- Skene JH, Virag I. Posttranslational membrane attachment and dynamic fatty acylation of a neuronal growth cone protein, GAP-43. *J Cell Biol.* 1989; 108(2):613–624. [PubMed: 2918027]
- Skene JH, Willard M. Axonally transported proteins associated with axon growth in rabbit central and peripheral nervous systems. *J Cell Biol.* 1981; 89(1):96–103. [PubMed: 6164683]
- Strittmatter SM, Fankhauser C, Huang PL, Mashimo H, Fishman MC. Neuronal pathfinding is abnormal in mice lacking the neuronal growth cone protein GAP-43. *Cell.* 1995; 80(3):445–452. [PubMed: 7859286]
- Summers DW, Gibson DA, DiAntonio A, Milbrandt J. SARM1-specific motifs in the TIR domain enable NAD(+) loss and regulate injury-induced SARM1 activation. *Proc Natl Acad Sci U S A.* 2016; 113(41):E6271–E6280. [PubMed: 27671644]
- Tedeschi A, Bradke F. The DLK signalling pathway--a double-edged sword in neural development and regeneration. *EMBO Rep.* 2013; 14(7):605–614. [PubMed: 23681442]
- Thomas GM, Hayashi T, Chiu SL, Chen CM, Hagan RL. Palmitoylation by DHHC5/8 targets GRIP1 to dendritic endosomes to regulate AMPA-R trafficking. *Neuron.* 2012; 73(3):482–496. [PubMed: 22325201]
- Thomas GM, Hagan RL. Palmitoylation-dependent regulation of glutamate receptors and their PDZ domain-containing partners. *Biochem Soc Trans.* 2013; 41(1):72–78. [PubMed: 23356261]
- Tognatta R, Miller RH. Contribution of the oligodendrocyte lineage to CNS repair and neurodegenerative pathologies. *Neuropharmacology.* 2016
- Tom CT, Martin BR. Fat chance! Getting a grip on a slippery modification. *ACS Chem Biol.* 2013; 8(1):46–57. [PubMed: 23215442]

- Vargas ME, Barres BA. Why is Wallerian degeneration in the CNS so slow? *Annu Rev Neurosci*. 2007; 30:153–179. [PubMed: 17506644]
- Waller A. Experiments on the Section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structures of their primitive fibres. *Philos Trans R Soc Lond*. 1850; 140:423–429.
- Wan J, Savas JN, Roth AF, Sanders SS, Singaraja RR, Hayden MR, Yates JR 3rd, Davis NG. Tracking brain palmitoylation change: predominance of glial change in a mouse model of Huntington's disease. *Chem Biol*. 2013; 20(11):1421–1434. [PubMed: 24211138]
- Wang JT, Medress ZA, Barres BA. Axon degeneration: molecular mechanisms of a self-destruction pathway. *J Cell Biol*. 2012; 196(1):7–18. [PubMed: 22232700]
- Watkins TA, Wang B, Huntwork-Rodriguez S, Yang J, Jiang Z, Eastham-Anderson J, Modrusan Z, Kaminker JS, Tessier-Lavigne M, Lewcock JW. DLK initiates a transcriptional program that couples apoptotic and regenerative responses to axonal injury. *Proc Natl Acad Sci U S A*. 2013; 110(10):4039–4044. [PubMed: 23431164]
- Wei X, Song H, Semenkovich CF. Insulin-regulated protein palmitoylation impacts endothelial cell function. *Arterioscler Thromb Vasc Biol*. 2014; 34(2):346–354. [PubMed: 24357059]
- Welsbie DS, Yang Z, Ge Y, Mitchell KL, Zhou X, Martin SE, Berlinicke CA, Hackler L Jr, Fuller J, Fu J, Cao LH, Han B, Auld D, Xue T, Hirai S, Germain L, Simard-Bisson C, Blouin R, Nguyen JV, Davis CH, Enke RA, Boye SL, Merbs SL, Marsh-Armstrong N, Hauswirth WW, DiAntonio A, Nickells RW, Inglese J, Hanes J, Yau KW, Quigley HA, Zack DJ. Functional genomic screening identifies dual leucine zipper kinase as a key mediator of retinal ganglion cell death. *Proc Natl Acad Sci U S A*. 2013; 110(10):4045–4050. [PubMed: 23431148]
- Xiong X, Hao Y, Sun K, Li J, Li X, Mishra B, Soppina P, Wu C, Hume RI, Collins CA. The highwire ubiquitin ligase promotes axonal degeneration by tuning levels of nmnat protein. *PLoS Biol*. 2012; 10(12):e1001440. [PubMed: 23226106]
- Xiong X, Wang X, Ewanek R, Bhat P, Diantonio A, Collins CA. Protein turnover of the Wallenda/DLK kinase regulates a retrograde response to axonal injury. *J Cell Biol*. 2010; 191(1):211–223. [PubMed: 20921142]
- Yamagishi Y, Tessier-Lavigne M. An Atypical SCF-like Ubiquitin Ligase Complex Promotes Wallerian Degeneration through Regulation of Axonal Nmnat2. *Cell Rep*. 2016; 17(3):774–782. [PubMed: 27732853]
- Yanai A, Huang K, Kang R, Singaraja RR, Arstikaitis P, Gan L, Orban PC, Mullard A, Cowan CM, Raymond LA, Drisdell RC, Green WN, Ravikumar B, Rubinsztein DC, El-Husseini A, Hayden MR. Palmitoylation of huntingtin by HIP14 is essential for its trafficking and function. *Nat Neurosci*. 2006; 9(6):824–831. [PubMed: 16699508]
- Yang G, Liu Y, Yang K, Liu R, Zhu S, Coquinco A, Wen W, Kojic L, Jia W, Cynader M. Isoform-specific palmitoylation of JNK regulates axonal development. *Cell Death Differ*. 2012; 19(4):553–561. [PubMed: 21941371]
- Yang J, Weimer RM, Kallop D, Olsen O, Wu Z, Renier N, Uryu K, Tessier-Lavigne M. Regulation of axon degeneration after injury and in development by the endogenous calpain inhibitor calpastatin. *Neuron*. 2013; 80(5):1175–1189. [PubMed: 24210906]
- Yang J, Wu Z, Renier N, Simon DJ, Uryu K, Park DS, Greer PA, Tournier C, Davis RJ, Tessier-Lavigne M. Pathological axonal death through a MAPK cascade that triggers a local energy deficit. *Cell*. 2015; 160(1–2):161–176. [PubMed: 25594179]
- Yokoi N, Fukata Y, Sekiya A, Murakami T, Kobayashi K, Fukata M. Identification of PSD-95 Depalmitoylating Enzymes. *J Neurosci*. 2016; 36(24):6431–6444. [PubMed: 27307232]
- Zhou F, Xue Y, Yao X, Xu Y. CSS-Palm: palmitoylation site prediction with a clustering and scoring strategy (CSS). *Bioinformatics*. 2006; 22(7):894–896. [PubMed: 16434441]

Figure 1. Palmitoylated regulators of axon growth and integrity

Upper panel: GAP-43, NMNAT2 and SCG10 are all likely palmitoylated on Golgi membranes and subsequently trafficked anterogradely down axons *via* fast, vesicle-based transport. NMNAT2 is intrinsically unstable and is subject to rapid ubiquitination-dependent turnover, even in healthy axons. **Lower panel:** Following axon injury, rapid degradation of NMNAT2 triggers axon degeneration. It is an intriguing possibility that the ubiquitin E3 ligase Phr1, which is reported to regulate NMNAT2 degradation, may be targeted to vesicles by palmitoylation. Simultaneously, JNK-dependent phosphorylation accelerates SCG10 degradation, which also contributes to axon degeneration.

Figure 2. Roles of palmitoylation in axonal retrograde transport and signaling

Palmitoylation targets the protein kinase DLK to axonal vesicles. Palmitoylation is also essential for the assembly of signaling complexes containing DLK, the kinase MKK4 and the scaffold protein JIP3, and for DLK to activate JNK pathway signaling. Any or all of these roles of palmitoylation may account for the critical role of palmitoyl-DLK in conveying retrograde injury signals that result in nuclear responses, including phosphorylation of the transcription factor c-Jun. The retrograde motor protein dynein itself has been identified as a palmitoyl-protein in several independent studies, as have subunits of the dynactin complex, and the dynein regulatory protein Ndel1 {Blanc et al., 2015; Kang et al., 2008; Sanders et al., 2015; Shmueli et al., 2010). Palmitoylation may thus affect the localization and/or activity of multiple proteins involved in axonal retrograde signaling.

Table 1

Axonal palmitoyl-proteins for which the cellular role of palmitoylation has been defined in targeted studies.

Axonal Palmitoyl-Protein	Initial Report of Palmitoylation	Likely Function of Palmitoylation in Axons	Possible PAT(s)
Ankyrin-G (AnkG)	He <i>et al.</i> 2012	Targeting to membranes and axon initial segment (He <i>et al.</i> 2012)	ZDHHC5, ZDHHC8? (He <i>et al.</i> , 2014)
Cdc42 et al	Kang <i>et al.</i> , 2008	Localization to axon tip, axonal branching (Mukai <i>et al.</i> 2015)	ZDHHC8 (Mukai <i>et al.</i> 2015; Moutin <i>et al.</i> , 2016)
Deleted in Colorectal Cancer (DCC)	Herincs <i>et al.</i> 2005	Localization to lipid rafts; netrin-1-induced ERK activation (Herincs <i>et al.</i> 2005)	Unknown
Dual Leucine-zipper Kinase (DLK)	Holland <i>et al.</i> , 2016	Localization to vesicles, also necessary for protein-protein interactions and kinase activity (Holland <i>et al.</i> 2016)	Multiple (Holland <i>et al.</i> , 2016)
Growth-associated Protein 43 kDa (GAP-43)	Skene and Virag, 1989	Growth cone targeting (Liu <i>et al.</i> 1991); G protein activation (Nakamura <i>et al.</i> 1998); osmosensitivity (Caprini <i>et al.</i> 2003)	ZDHHC2, 3, 7, 15 (Fukata <i>et al.</i> 2004)
Huntingtin (Htt)	Huang <i>et al.</i> , 2004	Prevention of Htt aggregation (Yanai <i>et al.</i> , 2006)	ZDHHC17 (HIP14), ZDHHC13 (Huang <i>et al.</i> , 2004; Huang <i>et al.</i> , 2009)
Neurofascin (Neurofascin- 186)	Ren and Bennett 1998	Localization to membrane microdomains (Ren and Bennett 1998)	Unknown
Nicotinamide mononucleotide adenylyltransferase-2 (NMNAT2)	Mayer <i>et al.</i> , 2010	Golgi and vesicle targeting; negatively impacts axon integrity (Mayer <i>et al.</i> , 2010; Milde and Coleman 2014; Milde <i>et al.</i> 2013b)	ZDHHC17? (Milde and Coleman, 2014)
Superior Cervical Ganglion-10 (SCG10)	Di Paolo <i>et al.</i> , 1997	Localization to Golgi (Di Paolo <i>et al.</i> 1997, Levy <i>et al.</i> 2011, Lutjens <i>et al.</i> 2000) and growth cones (Lutjen <i>et al.</i> 2000)	ZDHHC2,3, 7, 15, 17 and 21 (Levy <i>et al.</i> 2011)