Signaling Over Distances*

Atsushi Saito‡ and Valeria Cavalli‡§

Neurons are extremely polarized cells. Axon lengths often exceed the dimension of the neuronal cell body by several orders of magnitude. These extreme axonal lengths imply that neurons have mastered efficient mechanisms for long distance signaling between soma and synaptic terminal. These elaborate mechanisms are required for neuronal development and maintenance of the nervous system. Neurons can fine-tune long distance signaling through calcium wave propagation and bidirectional transport of proteins, vesicles, and mRNAs along microtubules. The signal transmission over extreme lengths also ensures that information about axon injury is communicated to the soma and allows for repair mechanisms to be engaged. This review focuses on the different mechanisms employed by neurons to signal over long axonal distances and how signals are interpreted in the soma, with an emphasis on proteomic studies. We also discuss how proteomic approaches could help further deciphering the signaling mechanisms operating over long distance in axons. Molecular & Cellular Proteomics 15: 10.1074/mcp.R115.052753, 382–393, 2016.

Neurons have unique and highly polarized morphologies. The axon allows intracellular communication between the cell soma and the distantly located synaptic terminal. The extreme length of axons relative to the cell soma diameter poses great challenges for neuronal development, maintenance, and repair. Anterograde and retrograde axonal transport of proteins, vesicles, and RNA along the microtubule tracks in the axon is crucial to sustain the required signaling events underlying development and maintenance of the nervous system. The retrograde transport system is also used following axon injury to communicate information from the site of injury back to the soma. Communication between distant axon locations and the cell soma is also ensured by a more rapid signal encoded in calcium waves. Incoming signals from distant axon locations are interpreted by the soma to regulate gene expression for survival or repair. The neuronal epigenome is also influenced by incoming signals to appropriately coordinate transcriptional responses. In this review, we discuss the state of the field regarding the different mechanisms employed by neurons to signal intracellularly over long distances in axons and how signals are interpreted in the cell soma, with an emphasis on proteomic studies. We also discuss how the use of proteomics could further help in understanding long distance signaling system in axons. The communication employed by neurons to signal along dendrites has been reviewed in detail elsewhere (1) and will not be discussed here.

Calcium Influx, Back Propagation, and Long Distance Effects—Alterations in axonal calcium levels affect multiple and diverse signaling events, including local protein synthesis and degradation. These local signals can have long distance effects. Axon injury provides a powerful system to study the role of calcium in axonal signaling, because injury-induced calcium influx in the axon triggers important downstream events at the injury site and the soma (Fig. 1). Calcium influx following injury is critical for the control of local protein synthesis (2–4). The rapid increase in calcium concentrations in response to axotomy is also required for membrane resealing that is necessary for neuronal survival and regrowth after the injury. By controlling the activity of various members of the synaptotagmin family of proteins, calcium elevation promotes vesicle fusion events required for axonemal repair (5–8). Increased calcium levels in the axon also lead to protein degradation via the activation of the protease calpain, which regulates local repair events and the transport of retrograde injury signals (2, 3). Immediately following axon injury, activation of calpain by transient elevation of the intracellular calcium levels is critical for growth cone formation in Aplysia neurons via the cleavage of spectrin, one of the main components of the membrane skeleton (9, 10). The calpain-mediated cleavage of vimentin in injured peripheral nerves enables transport of phosphorylated extracellular signal-regulated kinases (ERKs)† from the injury site to the soma.

† The abbreviations used are: ERK, extracellular signal-regulating kinase; DLK, dual leucine zipper kinase; MAP3K, mitogen-activated protein kinase kinase kinase; ER, endoplasmic reticulum; RyR, ryanodine receptor; ROS, reactive oxygen species; mPTP, mitochondrial permeability transition pore; PTM, posttranslational modification; REEP, receptor expression enhancing protein; UPR, unfolded protein response; TeNT, tetanic neurotoxin; RVG, rabies virus envelope glycoprotein; JIP, JNK-interacting protein; MS, mass spectrometry; STAT3, signal transduction and activator of transcription 3; TTL, tubulin-tyrosine ligase; KHC, kinesin-1 heavy chain; NT-3, neurotrophin 3; BDNF, brain derived neurotrophic factor; RGC, retinal ganglion cell; Htt, huntingtin; HAP1, huntingtin associated protein 1; APP, amyloid precursor protein; BICDR-1, BICD-related protein 1; RNP, ribonucleaseprotein; snRNP, small nuclear ribonucleoprotein; SMN, survival motor neuron; NLS, nuclear localization signals; ATF, activating transcription factor; AD, Alzheimer’s disease; SCIRR89, spinal cord...
site to the cell body to regulate axon regeneration (11). In addition to linking ERK to the dynein retrograde complex, vimentin sterically protects ERK from dephosphorylation in a calcium-dependent manner (12), ensuring delivery of activated ERK to the cell soma. Activated calpain was also shown to cleave voltage-dependent sodium channels in axon in an in vitro model of traumatic brain injury (13). The proteolyzed sodium channels remain in the membrane, altering action potential initiation, propagation, and downstream signaling events (13). Calcium influx also activates axonal proteins independently of protein translation or degradation. For example, Dual leucine zipper kinase (DLK/MAP3K12), which belongs to the Mitogen-activated protein kinase kinase kinase (MAP3K) family of proteins, is activated in response to increased calcium levels. In normal conditions in C. elegans axons, DLK activity is blocked by the binding of an inhibitory short isoform of DLK, DLK-1S (14). A calcium dependent switch from heteromeric to homomeric protein complexes activates DLK-1 (14). A human DLK-1 homolog MAP3K13 (MLK) contains an identical C terminus that is required for calcium dependent regulation and can functionally complement DLK-1 mutants in C. elegans (14), suggesting that the calcium dependent activation of MAP3K family members may also occur in mammalian models of axon injury.

The precise mechanisms leading to elevated calcium levels following axon injury are being elucidated. In cultured cortical neurons, overactivation of sodium channels increases sodium load in the axon after injury, subsequently triggering reversal of the sodium/calcium exchanger and increased axonal calcium levels (15, 16). The calcium back-propagation to the cell soma then requires the activation of voltage gated sodium channels (15). In mouse sensory neurons, however, the retrograde propagation of the calcium wave toward the cell soma requires voltage gated calcium channels, but not voltage gated sodium channels, as well as calcium release from the

---

**Fig. 1. Long distance signaling initiated by calcium after axon injury.** Calcium influx following injury in distal is initiated by various factors including RyR and IP3 channels. The released calcium ion activates calpain, eventually enhancing the cleavage of vimentin, which sterically protects ERK from dephosphorylation and enables transport of phosphorylated ERKs from the injury site to the cell body. In the soma, phosphorylated ERKs may activate downstream signals such as ELK1. In addition, calcium ion outflow from axonal ER, which is organized by ER-microtubule-associated proteins REEPs, atlastin and spastin, via IP3R and RyR may induce ER stress after the injury, because the transient calcium depletion of the ER lumen leads to ER stress. In response to ER stress, the ER stress transducers including protein kinase R-like ER kinase (PERK), inositol requiring kinase 1 (IRE1) and activating transcription factor 6 (ATF6) activate downstream signals. The other ER stress transducer Luman is locally cleaved in response to ER stress. The processed N-terminal fragment retrogradely transported to promote axon regeneration. In the distal site, calcium release induces ROS production and also affects mitochondria. Mitochondrial calcium and ROS overload lead to activate mPTP, resulting in mitochondrial-derived apoptosis. The damaged axonal mitochondria is degraded by PTEN induced putative kinase 1 (PINK1) and Parkin-mediated mitophagy.
endoplasmic reticulum (ER) internal stores (17). Cortical and sensory neurons also display differences in the magnitude of cell body calcium changes, which are much larger both in amplitude and duration in sensory than in cortical neurons (15, 17). The role of internal calcium stores in calcium wave propagation has been further supported by studies in C. elegans ALM neurons. Mutation of ryanodine receptor (RyR) channels (Unc68) eliminates the injury-induced sustained calcium elevation, with greater effect in the axons and smaller effects in the cell soma (18). The injury-induced calcium outflow via RyR is crucial for stimulating both outgrowth and guidance of regenerating neurons (18). In C. elegans PLM sensory neurons, the amplitude of injury triggered calcium waves also correlates with the extent of regeneration (19). Whether calcium release from internal stores to sustain a back propagating calcium wave represents a specific property of regenerating neurons remains to be tested.

Interestingly, injury-induced propagation of the calcium wave occurs in axons both proximal to and distal to the injury site. Whereas propagation toward the cell body correlates with regenerative growth (17–19), propagation of the calcium wave away from the injury site toward the nerve terminal appears to correlate with axon degeneration (20). In mouse sensory neurons, at least one of the underlying factors for the early calcium increase distal to the injury site is the RyR and IP3 channels localized at ER membranes (18, 20). ER-derived calcium release promotes reactive oxidative species (ROS) production and also affects mitochondria, which are closely apposed to the ER in the axon (20). Mitochondria represent one of the critical convergence points for axon degeneration pathways (21). Mitochondrial calcium and ROS overload caused by ER-derived calcium release activate the mitochondrial permeability transition pore (mPTP), a key event for mitochondrial-derived apoptosis signaling (20). These studies reveal that axonal calcium levels can fine-tune bidirectional signaling to regulate both axon degeneration and regeneration. A key unanswered question remains: why is calcium back propagation on the proximal side not affecting axonal and mitochondria’s health? Perhaps the supply of anterogradely transported healthy mitochondria from the soma can balance the removal of damaged axonal mitochondria mediated by internalization into autophagosomes, a process known as mitophagy, shown to occur in distal axon of hippocampal axons (22) and sensory neurons (23).

The Axonal Endoplasmic Reticulum—The ER is responsible for calcium storage, lipid metabolism, protein synthesis and post-translational modification (PTM) of abundant secretory proteins and membrane proteins. This organelle has a single and continuous membrane, and can be divided into the nuclear envelope, the rough ER and the smooth ER. Whereas the ribosomes-enriched ER (rough ER) is observed in the cell soma, dendrites, and initial axon segments, the axon is filled with smooth ER (24, 25). The axonal ER network is a highly dynamic structure that is constantly remodeled and extended through interaction with microtubules, kinesin-1 and dynein molecular motors (26, 27).

The formation and maintenance of axonal ER is regulated by at least two protein families, Receptor expression enhancing proteins (REEP) and Reticulon proteins. The importance of axonal ER structure and morphology for proper neuronal function is highlighted by the fact that mutations in many ER-associated proteins, including REEP, atlastin, and spastin are associated with pathological conditions, such as hereditary spastic paraplegia, amyotrophic lateral sclerosis, and Charcot-Marie-Tooth disease (reviewed in (27)). The precise mechanisms by which ER alterations cause these disorders remain poorly understood, but given the role for the ER in axonal calcium homeostasis, lipid metabolism, and protein trafficking, defects in long range axonal signaling are likely to occur.

Multiple cellular abnormalities including disturbance of calcium homeostasis, expression of mutated proteins, or ischemic insults cause ER stress. Under ER stress, cells transduce unfolded protein response (UPR) signaling, which activates chaperones, attenuates protein translation, and degrades unfolded proteins to avoid cellular damage. Prolonged or excessive ER stress leads to apoptosis via UPR signaling, but mild or controlled ER stress may have beneficial outcomes. Indeed, a recent study has uncovered novel UPR long distance signaling functions in axon regeneration. The ER stress transducer Luman is expressed in axons of sensory neurons and is cleaved in response to axon injury (28). The N-terminal fragment is retrogradely transported toward the cell soma via importin α, where it acts as a transcription factor (28). The knockdown of Luman specifically in axon is sufficient to impair axon regeneration (28), further supporting the role of axonal Luman activation and retrograde transport in mediating long-distance effect in the cell soma. The UPR signaling activated by ER stress in axons is likely to elicit diverse signaling pathways. Further studies will uncover the precise mechanisms by which UPR signaling contribute to long-range signaling in axons and how it can have both beneficial and detrimental effects on axon health.

Retrograde Vesicular Transport: From Endosomes to Autophagosomes—Membrane trafficking, especially endocytosis, is important for neuronal development and survival (29, 30). Signaling endosomes are a specific pool of endocytic organelles, which travel along the axon back to the cell soma and transport neurotrophins and their signaling receptors (Fig. 2). These organelles have been studied extensively because of the crucial importance of neurotrophin signaling in neuronal development and disease and the state of the field has been comprehensively covered in recent reviews (31, 32).

Interestingly, several pathogens and virulence factors exploit the signaling endosome transport pathway in peripheral neurons to gain access to the central nervous system (33, 34). For example, the tetanus neurotoxin (TeNT) reaches the central nervous system by hijacking retrogradely moving endo-
The TeNT heavy chain has high affinity for neuronal surface and plays key roles in promoting TeNT entry into neurons, whereas the light chain suppresses motor neuron activity and triggers spastic paralysis. Using a proteomic approach and a purification strategy based on a fragment of TeNT conjugated to paramagnetic beads, the small GTPase Rab7 was identified as a functional marker of a specific pool of axonal retrograde carriers, which transport neurotrophins and their receptors (36). Whereas Rab5 is essential for an early step in TeNT sorting, Rab7 plays an essential role in long-range axonal retrograde transport. Endocytosis of TeNT was also recently shown to require the extracellular matrix proteins, Nidogens, at the neuronal surface (37). The endocytosed TeNT-Nidogen complex is retrogradely transported into the cell body to elicit its toxic effects. Inhibition of the TeNT-Nidogen interaction with small Nidogen-derived peptides or genetic ablation of Nidogens prevented the binding of TeNT to neurons and protected mice from TeNT-induced spastic paralysis (37).

Another pathogen, the rabies virus, reaches neuronal cell bodies of motor neurons via axonal retrograde transport (38). Recently, Hislop et al. showed that pseudotyping lentiviral vector with rabies virus envelope glycoprotein (RVG) mediates axonal retrograde transport through Rab5-positive endosomes and accumulation within the Rab7 compartment (39). Gluska et al. also showed that rabies virus not only hijacks the signaling endosome system but also accelerates the p75 receptor retrograde axonal transport machinery, representing a fast and efficient mechanism to gain access to the central nervous system (40). These studies indicate that pathogens have evolved to take advantage of long distance signaling mechanisms employed by neurons. On a positive side, these
pathogens offer great tools to better understand these long-distance signaling mechanisms regulating neuronal function.

In addition to neurotrophic signaling, retrograde vesicular axonal transport of kinases and transcription factors plays a major role in injury signaling (Fig. 2). The adapt protein JNK-interacting protein 3 (JIP3) links molecular motors to axonally transported vesicles (41–43). Using a vesicle immunopurification strategy followed by mass spectrometry (MS), JIP3 was found to be associated with at least two distinct pools of vesicles, small anterogradely moving vesicles and larger endosomal-like bidirectional vesicles (43). The large vesicles associated with JIP3 may be involved in retrograde injury signaling. Indeed, DLK/JNK kinases are retrogradely transported (44–46) together with JIP3, because JIP3 interacts with a component of dynein retrograde motor, dynactin (47). The retrograde transport of DLK/JNK then enables activation of c-Jun in cell soma, which is required for axon regeneration (45, 48, 49). In mice, stabilization of DLK protein allows propagation of injury signals (46). In an optic nerve crush model, elevation of DLK protein levels in the axon precedes elevation in cell bodies and modulates the propagation of downstream pro-apoptotic signaling (46). In mouse peripheral sensory neurons, DLK is activated by cytoskeletal disruption and can promote the retrograde transport of crucial factors for axon regeneration, such as Signal Transduction and Activator of Transcription 3 (STAT3) and c-Jun (49, 50). A recent study suggests that increased tyrosinated tubulin mediated by Tubulin-tyrosine ligase (TTL) at the site of injury recruits plus end tracking proteins and facilitates the retrograde transport of injury signals that are required to activate c-Jun in the soma (51).

To maintain organelle quality and cellular homeostasis across long axonal distances, neurons utilize the degradation machinery mediated by autophagosomes to remove damaged and malfunctioning organelles. Autophagosomes in sensory neurons are constitutively generated from ER membranes in the distal axon and collect soluble and organelle cargoes (52). These autophagosomes initially move bidirectionally by associating with both kinesin-1 and dynein motors, but transition to exclusive retrograde transport toward the cell soma (23). The exact mechanisms causing this transition in autophagosomes directionality are not fully understood, but depend in part on the scaffolding protein JIP1. JIP1 is recruited to autophagosomes by binding to the autophagosomal membrane protein LC3 (53, 54). Similarly to JIP3, JIP1 can bind both the kinesin-1 heavy chain (KHC) and the dynactin subunit p150Glued, but cannot simultaneously bind to both motor complexes. The switch of binding partners is regulated by the phosphorylation of JIP1 on serine 421 located within the KHC-binding domain (54). As autophagosomes move retrogradely along the axon they mature and fuse with lysosomes to effectively degrade the enveloped cargo (23, 53). Destruction of malfunctioning mitochondria by autophagosomes can occur locally, without a requirement for retrograde transport to the soma, a system that may provide rapid and effective neuroprotection from oxidative damage produced by malfunctioning mitochondria (22).

**Anterograde Vesicular Transport**—The anterograde transport and release of neurotrophins from axons via vesicle transport is regulated to support cell survival, development and axon growth (Fig. 2). Several neurotrophic factors including neurotrophin 3 (NT-3) and brain derived neurotrophic factor (BDNF) are transported via Golgi-derived vesicles and secreted at distal axonal locations. In retinal ganglion cells (RGCs), NT-3 is internalized in endosomes in tyrosine kinase receptor, TrkC-dependent manner, and anterogradely transported via transcytosis mediated by the Golgi-system (55). BDNF is also mainly anterogradely transported in the optic nerves. BDNF synthesized by RGC is transported in the anterograde direction along the axons of optic nerves to protect geniculate neurons (56, 57). Antibodies against the BDNF receptors TrkB, but not p75 receptor significantly reduces the anterograde transport of BDNF (57), indicating that TrkB receptor but not p75 receptor is necessary for the anterograde transport for BDNF. Mass spectrometry studies identified TrkB on small anterogradely moving JIP3 vesicles (43), consistent with the role of JIP3 in mediating TrkB anterograde axonal transport and enhancing BDNF signaling (58).

Huntingtin (Htt) and its interacting protein, Huntingtin associated protein 1 (HAP1), are also key factors for the anterograde transport of BDNF. HAP1 has the ability to interact with both the kinesin-1 motor complex and dynactin subunit p150Glued to transport vesicles in axons (59, 60). Htt phosphorylation is responsible for the directionality of vesicular transportation regulated by Htt-HAP1, with phosphorylated Htt recruiting kinesin-1 on the microtubules to promote the anterograde transport of BDNF vesicles (61, 62). One of the mechanisms of pathogenicity brought by polyglutamine-repeated Htt (PolyQ-Htt) in Huntington’s disease is the disruption of Htt-HAP1 interaction with microtubules and the decrease in the transport of BDNF-containing vesicles, consequently inducing the loss of BDNF release and an increase in neuronal death in cortical neurons (59).

Amyloid precursor protein (APP) is another pathogenic molecule that operates in the anterograde axonal transport pathway. The motility of APP vesicles is regulated by various scaffolding and motor associated proteins such as JIP1 (63), and HAP1 (60) and reduced APP transport induces axonopathy (64). APP may act as a signaling platform and disruption of APP transport and signaling may trigger the onset of neuronal dysfunction leading to Alzheimer pathology (65).

**Molecular Motor-Based Axonal Signaling**—Regulation of intracellular transport is important for neuronal development. Impairments in axonal transport contribute to the initiation or progression of several neurodegenerative diseases (66–69). Axonal transport defects can be caused by alterations in various components of the transport machinery, including molecular motors and cargo adaptors. The roles of antero-
grade and retrograde axonal transport pathways have been discussed recently (70). Beyond its role in delivering cargo to and from the axon terminal, axonal transport is emerging as a more sophisticated system to regulate signaling. A recent study revealed that regulation of kinesin-1 motility by JIP3 is critical for axon growth and regeneration (71). This study found that a JIP3 mutant that is unable to bind the motor subunit of kinesin-1 (KHC) (42) leads to slow moving kinesin-1 tetramer. When expressed in cultured hippocampal neurons this JIP3 mutant reduces axon growth despite being properly localized to axon tips (71). This study illustrates that in addition to JIP3-dependent localization at axon tips to mediate axon growth (72), JIP3 controls axon growth via a motor processivity-based mechanism. These observations are interesting in light of the proposed model of axon length-sensing (73) which posits a frequency-based signaling module, in which a neuron senses the length of its axon based on the frequency of an oscillating signal sent anterogradely to the axon tip and returned to the cell soma via retrograde transport. In this model, reducing the levels of either kinesin or dynein increases axon length (73) but if the amount of signal in the system is conserved, changing motor velocities might also affect axon length. It is important to bear in mind that motors might affect axon elongation in different manners (71, 74). For example, BICD-related protein 1 (BICDR-1) accelerates minus-end-directed vesicle movements, reducing the amount of Rab6 vesicle at neurite tip and reducing axon length in both dorsal root ganglia (DRG) and hippocampal neurons. This effect on axon growth is most likely because of the drastic redistribution of the Rab6 vesicles observed upon BICDR-1 expression from neurite tip to cell soma (74), implying that more than just the velocity of the dynein motors may be affected. Given that JIP3 and JIP1 associate with both kinesin-1 and dynein (41, 47, 63, 75), JIPs may be interesting candidates to elucidate further the signaling modules involved in axon growth control.

In addition to regulating the transport of signaling modules, molecular motors are also involved in moving cytoskeleton components such a short microtubules (76) and neurofilaments (77) along the axon length. Microtubule transport and assembly plays a role in axon growth (78). Reducing the levels of dynein in cultured sympathetic neurons leads to Golgi disruption, interruption of vesicle traffic. However, unlike vesicle transport, which is impaired in both directions, the transport of microtubules or neurofilaments is impaired in only one direction or the other (79). Dynein depletion suppresses retrograde neurofilament transport, whereas it reduces anterograde microtubule transport, supporting a sliding model for axonal microtubule transport (79). Notably the axon still grows and the microtubule array still advances when dynein is depleted (79). Recently, microtubules have received greater attention as a target to augment axon growth and regeneration in the central nervous system, with drugs stabilizing microtubule showing great promises (80–82). Targeting the molecular motor Kinesin-5 may offer another mean to boost axonal growth by acting on microtubules. Kinesin-5 is a very slow motor, roughly hundred times slower than cytoplasmic dynein, which acts as a brake on microtubule movements (83). Inhibiting kinesin-5 result in greater mobility of microtubules in the axon and an overall shift in the forces on the microtubule array (84). As a result, the axon grows faster and more readily enters environments that are inhibitory to axonal regeneration (85). These studies highlight that a detailed understanding of the roles of molecular motors in vesicular and cytoskeleton movements along the axons may hold promise for strategies aiming at promoting axon growth.

Local Translation and Axonal Roles for Nuclear Proteins—Although the role of axonal protein translation has long been debated, it is now clear that peripheral and central axons have the capacity to synthesize new proteins during development and in response to injury (4, 86–88). mRNAs in complex with multiple proteins form a ribonucleoprotein (RNP) that engage with motor proteins for transport to distal axonal locations (reviewed in (89, 90)). Evidence gained so far suggests that RNA is transported in complex with proteins that mediate association with molecular motors (90). A recent study suggests that splicing small nuclear ribonucleoproteins (snRNP) are transported on vesicles along axons (91). The biogenesis of splicing snRNPs is a complex process and this study used time-resolved quantitative proteomic analysis to identify proteins that associate preferentially with either newly assembled or mature splicing snRNPs. This revealed novel cytoplasmic vesicles containing Survival motor neuron (SMN), one of the pathogenic genes in spinal muscular atrophy, and SmB (91). These small SmB vesicles are highly mobile along microtubules in human neuroblastoma cells, and interact with dynein and the coatomer complex. How these small vesicles engage with molecular motors remains unknown. SMN performs an essential role in the maturation of snRNPs, promoting the addition of the core Sm proteins to the snRNP complex to form the spliceosome (92, 93), suggesting that mRNA processing may occur in axons. Given that mRNA localization is regulated by neurotrophin stimulation (94) and that translation of certain axonal mRNA can be up-regulated by injury (95), it will be interesting to know whether mRNA processing can also be differentially regulated by various stimuli that the axon receives.

Some of the axonally transported mRNAs encode proteins that return back to the cell soma. This is the case after axonal injury, which provides a mechanism to transmit information about the injury back to the soma and assist with the activation of a preregenerative program (2). Interestingly, some axonally translated proteins have traditional roles in the nucleus and include transcription factors and other proteins with nuclear functions (2, 96). The localized axonal synthesis of importin β strengthens the affinity between nuclear localization signals (NLS)-containing proteins and importin α and promotes retrograde transport of an NLS-containing complex.
via dynein (97, 98). Hence, the classical function of the importin system to promote transport through the nuclear pore has been harnessed by neurons to promote sophisticated regulation of retrograde signaling in neurons.

Transcription factors have also been reported to move along the axon in diverse physiological contexts. The appearance of activated STAT3 in injured axons before nuclei (99) suggested a potential role for STAT3 in retrograde injury signaling. A proteomic array approach later revealed that axon injury triggers the synthesis and phosphorylation of STAT3 (100). STAT3 retrograde transport mediated by interaction with importins and dynein constitutes a retrograde injury signal for neuronal survival and axon regeneration in sensory neurons (49, 100) and axonogenesis of hippocampal neurons (101).

Although the axon injury paradigm has led to the discovery of many nuclear-related factors involved in survival and repair mechanisms (2), a recent study revealed an active role for intra-axonal translation of activating transcription factor 4 (ATF4) as a mediator of neurodegeneration in Alzheimer’s disease (AD) pathology (102). The mRNA encoding the transcription factor ATF4 is recruited in axons of hippocampal neurons exposed to Amyloid β<sub>1–42</sub>, a critical factor in AD. ATF4 protein is then locally synthesized and retrogradely transported (102). Neurodegeneration of Amyloid β<sub>1–42</sub>-treated cells is inhibited by Atf4 knockdown, suggesting that axonally-derived ATF4 promote neurodegeneration (102). Downstream of PERK-ATF4, CHOP mediates degeneration (102). Two activators of ER stress, tunicamycin and thapsigargin, did not trigger axonal recruitment of Atf4 mRNA, suggesting that local ER stress does not phenocopy the effect of Amyloid β<sub>1–42</sub> oligomers on Atf4 mRNA recruitment.

Another transcription factor of the CREB/ATF family, spinal cord injury and regeneration-related gene 69 (SCIRR69) is activated in response to axon injury (103). The activation mechanism of SCIRR69 closely resembles that of Luman, that is, SCIRR69 localizes at ER membrane under normal condition, and is cleaved by site-1 protease and site-2 protease after the injury (103). The cleaved N-terminal fragment is translocated into the nucleus, subsequently inducing the expression of BDNF through the binding to BDNF promoter II region (103). Although it is unclear whether SCIRR69 N terminus is transported from the distal axon to the nucleus, these studies raise the possibility that CREB/ATF transcription factors may be involved in axon injury responses. In support of this idea, axonal translation and retrograde trafficking or CREB in a model of interleukin-6-induced mechanical hypersensitivity links locally-generated signals to long-term nociceptive sensitization (104).

The converse route, from nucleus to distant axonal locations is also used by neuronal protein usually believed to function in the nucleus. Following axon injury, histone deacetylase 5 (HDAC5) is exported from the nucleus, transported toward the site of injury via kinesin-1 where it regulates microtubule dynamics to promote axon regeneration (17, 105). HDAC3, a class I HDAC, also exits the nucleus in response to axon injury (17), but whether HDAC3 plays a role in the cytoplasm or axon has not been explored. The observation that HDAC3 localizes to microtubules in the mitotic spindle (106) suggests that HDAC3 may also function in axons to regulate the microtubule cytoskeleton. Another class I HDAC, HDAC1, which is believed to reside primarily in the nucleus where it is involved in transcriptional repression, was shown to exit the nucleus and impair mitochondrial transport in damaged neurons in a model of multiple sclerosis (107).

High mobility group (HMG) proteins represent another family of proteins that are concentrated in the nucleus, where they interact with chromatin. This family of proteins was shown to also play a role in axon growth, via the localization of their mRNA in axons and their local translation. For example, amphetamine (HMBG1) protein expression in axons increases following nerve injury, whereas the levels of amphetamine mRNA in the axons remain unaffected, suggesting that amphetamine is locally translated in axons in response to injury (95). Consistently, overexpression of axonally targeted amphetamine mRNA, but not cell body restricted mRNA increases axon outgrowth in cultured sensory neurons (95). Another study suggests that the localization and local translation of transcripts coding for high mobility group nucleosome binding domain 5 (HMGN5) links neurite outgrowth to chromatin regulation in the nucleus (108). The mRNA coding for HMGN5 localizes to growth cones via its 3'UTR and the HMGN5 protein can be retrogradely transported to influence chromatin structure in a differentiated neuroblastoma cell line and in cultured hippocampal neurons (108). Together these studies highlight that proteins typically known for their nuclear function as transcription factors or chromatin regulator also play important roles in axonal signaling, transducing information from distantly located axon to the nucleus to control neuronal development, survival, or repair.

**Identifying Novel Signaling Modules with Proteomic Approaches**—Proteomics approaches are ideal tools to identify novel signaling modules in neurons. Of particular interest is the use of proteomics to identify stimuli-induced PTMs that redirect traffic along axons. For example sumoylation serves as a mechanism to determine the directionality of transport of the RNA chaperone protein La from axon to cell soma (109). Proteomics studies could reveal whether other SUMO-modified proteins in axons exert control on somatic transcriptional responses. Ubiquitination was also shown to regulate transport of signals along axons. Phosphorylation of DLK in response to nerve injury results in increased DLK abundance via reduction of DLK ubiquitination, which is mediated by the E3 ubiquitin ligase PHR1 and the de-ubiquitinating enzyme ubiquitin-specific protease 9X (USP9X), allowing a DLK signal to propagate back to the nucleus to enable a transcriptional stress response (46). Given the presence of several HDACs in axons (105, 107, 110), acetylation and deacetylation mecha-
nisms are likely used in axons to regulate signaling. However, proteomics studies of subcellular compartments like axons, which is physiologically relevant for long-distance signaling present greater challenges because of the small amount of pure axonal material that can be analyzed. Indeed, whereas identifying axonal proteins has become relatively standard (96), identifying PTMs is more challenging, given the higher amount of protein needed to ensure good coverage of a given protein sequence.

The comparison of protein expression between intact and injured neurons represents a model of choice to identify novel signaling modules in neurons. In a recent study, multiplexed quantitative proteomic analysis, using tandem mass tags (TMT) 6-plex isobaric-labeled samples coupled to fluorescence-activated cell sorting (FACS), identified a novel molecular network of neuronal injury responses in retinal ganglion cells that includes c-myc (111). They showed that manipulations of c-myc resulted in significantly increased neuronal survival and axon regeneration after the optic nerve injury (111).

Following the arrival of axon-derived signals, a neuron changes the epigenome and reprograms itself to a new mode of gene expression to accommodate a new condition (Fig. 3). For example, in the case of axon injury, incoming calcium-encoded signals (17) or ERK-dependent signals (112) alter the neuronal epigenome, inducing changes in histone modifications that together regulate a proregenerative transcriptional response. In addition, a retrograde calcium wave elicits nuclear export of HDAC3 and HDAC5, which results in increased histone acetylation and activation of the proregenerative transcriptional response to the injury. Phosphorylated HDAC5 is also anterogradely transported along where it promotes deacetylation of microtubule in proximity of the injury site.

FIG. 3. Epigenetic responses to axonally-derived signals. The arrival of axon-derived signals leads to changes in the neuronal epigenome. Calcium influx in distal axon promotes retrograde transport of phosphorylated ERK toward cell soma. In the soma, phosphorylated ERK activates p300/CBP-associated factor (P/CAF) nuclear translocation, where P/CAF promotes the acetylation of histones that correlate with active transcription. In addition, a retrograde calcium wave elicits nuclear export of HDAC3 and HDAC5, which results in increased histone acetylation and activation of the proregenerative transcriptional response to the injury. Phosphorylated HDAC5 is also anterogradely transported along where it promotes deacetylation of microtubule in proximity of the injury site.

PTMs of histones by acetylation and methylation were first described nearly 50 years ago (118). Since then, a growing number of histone PTMs have been identified. These include phosphorylation, proline isomerization, ubiquitination, ribosylation, sumoylation, citrullination, and carbonylation (116). Most of the modifications known to affect interaction with chromatin reader proteins occur on the N-terminal tail of histones H3, H4 H2A, and H2B, but recent studies have also focused on identifying new PTMs sites on the histone globular
domain, which could impact nucleosomal structure (119). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses offer the great advantage to search for novel types of modifications, or map known modifications to new sites. Yet, analysis of histone PTMs can present some challenges including shorter peptides because of high content in lysine and arginine residues, inconsistent peptide length because of cleavage at modified residues, and false positive modified sites resulting from sample processing (116). The challenges becomes considerably greater when dealing with small amount of material, which is the case if one wants to study neuronal-specific modification, which requires pure neuronal preparation. The gold standard to understand how an incoming signal affects the epigenome and gene expression would be to elucidate the combinations of histone PTMs on nucleosomes associated with histone code reading proteins. Indeed, the importance of the combination of PTMs has been revealed by the identification of cell type-specific multivalent histone PTMs (120, 121). To answer these types of questions, chromatin immunoprecipitation with quantitative MS (ChIP-qMS) combined with deep sequencing to map the DNA sequences contained within these nucleosomes to their genomic locations can be performed (122–124), but whether sufficient pure material can be obtained from neurons remain to be shown. Providing genomic maps of where histone code reader proteins are bound and the combinations of modifications found on such nucleosomes would help understanding how the network of chromatin-associated protein translates these histone PTMs in response to a given axonally derived stimulus.

Concluding remarks—Understanding the mechanisms by which long-range signaling occur in neurons is important to our understanding of neuronal development, survival, and repair. Proteomic approaches can help elucidate protein sorting and trafficking along the axon. These types of approaches could provide insight into new therapeutic targets for multiple neurodegenerative diseases, which are associated with deficits in axonal transport (67, 68, 70). Proteomic approaches could also assist with deciphering how the epigenome is influenced by incoming signals and may help unravel how information about extracellular conditions or neuronal damage affects gene expression during development and in disease conditions.

Acknowledgments—We thank Dan Carlin and Dana Watt for critical reading of the manuscript. We apologize to those whose work could not be cited because of space limitation.

* This work was supported in part by grants from NIH (DE022000 and NS082446), and from the University of Missouri Spinal Cord Injuries Research Program (to VC). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

§ To whom correspondence should be addressed: Department of Anatomy and Neurobiology, Washington University School of Medicine, Campus Box 8108, 660 S. Euclid Ave, St. Louis, MO 63110–1093. Tel.: 314 362 3540; Fax: 314 362 3446, E-mail: cavalli@pcg.wustl.edu.

REFERENCES

1. Panayotis, N., Karpova, A., Kreutz, M. R., and Fainzilber, M. (2015) Macromolecular transport in synapse to nucleus communication. Trends Neurosci. 38, 108–116
2. Rishal, I., and Fainzilber, M. (2014) Axon-soma communication in neuronal injury. Nat. Rev. Neurosci. 15, 32–42
3. Bradke, F., Fawcett, J. W., and Spira, M. E. (2012) Assembly of a new growth cone after axotomy: the precursor to axon regeneration. Nat. Rev. Neurosci. 13, 183–193
4. Gumi, L. F., Tan, C. L., and Fawcett, J. W. (2010) The role of local protein synthesis and degradation in axon regeneration. Exp. Neurol. 233, 28–37
5. Detrait, E., Eddleman, C. S., Yoo, S., Fukuda, M., Nguyen, M. P., Bittner, G. D., and Fishman, H. M. (2000) Axolemmal repair requires proteins that mediate synaptic vesicle fusion. J. Neurobiol. 44, 382–391
6. Detrait, E. R., Yoo, S., Eddleman, C. S., Fukuda, M., Bittner, G. D., and Fishman, H. M. (2000) Plasmalemmal repair of severed neurites of PC12 cells requires Ca(2+) and synaptotagmin. J. Neurosci. Res. 62, 566–573
7. Tuck, E., and Cavalli, V. (2010) Roles of membrane trafficking in nerve repair and regeneration. Commun. Integr. Biol. 3, 209–214
8. Andrews, N. W. (2005) Membrane resealing: synaptotagmin VII keeps running the show. Science’s STKE: signal transduction knowledge environment 2005, pe19
9. Gitler, D., and Spira, M. E. (2002) Short window of opportunity for calpain induced growth cone formation after axotomy of Aplysia neurons. J. Neurobiol. 52, 267–279
10. Kamber, D., Erez, H., and Spira, M. E. (2009) Local calcium-dependent mechanisms determine whether a cut axonal end assembles a retarded endbulb or competent growth cone. Exp. Neurol. 219, 112–125
11. Perlson, E., Hanz, S., Ben-Yaakov, K., Segal-Ruder, Y., Seger, R., and Fainzilber, M. (2005) Vimentin-dependent spatial translocation of an activated MAP kinase in injured nerve. Neuron 45, 715–726
12. Perlson, E., Michaelievski, I., Kowalsman, N., Ben-Yaakov, K., Shaked, M., Seger, R., Eisenstein, M., and Fainzilber, M. (2006) Vimentin binding to phosphorylated Erk strictly hinders enzymatic dephosphorylation of the kinase. J. Mol. Biol. 364, 938–944
13. von Reyn, C. R., Spaethling, J. M., Mesfin, M. N., Ma, M., Neumar, R. W., Smith, D. H., Siman, R., and Meaney, D. F. (2009) Calpain mediates proteolysis of the voltage-gated sodium channel alpha-subunit. J. Neurosci. 29, 10350–10356
14. Yan, D., and Jin, Y. (2012) Regulation of DLK-1 kinase activity by calcium-mediated dissociation from an inhibitory isoform. Neuron 76, 534–548
15. Mandolesi, G., Madeddu, F., Bozzi, Y., Maffei, L., and Ratto, G. M. (2004) Acute physiological response of mammalian central neurons to axotomy: ionic regulation and electrical activity. FASEB J. 18, 1934–1936
16. Iwata, A., Stys, P. K., Wolf, J. A., Chen, X. H., Taylor, A. G., Meaney, D. F., and Smith, D. H. (2004) Traumatic axonal injury induces proteolytic cleavage of the voltage-gated sodium channels mediated by tetradotoxin and protease inhibitors. J. Neurosci. 24, 4605–4613
17. Cho, Y., Sloutsky, R., Naegle, K. M., and Cavalli, V. (2013) Injury-induced HDACs nuclear export is essential for axon regeneration. Cell 155, 894–908
18. Sun, L., Shay, J., McLeod, M., Roodhouse, K., Chung, S. H., Clark, C. M., Piri, J. K., Alkema, M. J., and Gabel, C. V. (2014) Neuronal regeneration in C. elegans requires subcellular calcium release by ryosolide receptor channels and can be enhanced by optogenetic stimulation. J. Neurosci. 34, 15947–15956
19. Ghosh-Roy, A., Wu, Z., Goncharov, A., Jin, Y., and Chisholm, A. D. (2010) Calcium and cyclic AMP promote axonal regeneration in Caenorhabditis elegans and require DLK-1 kinase. J. Neurosci. 30, 3175–3183
20. Villegas, R., Martinez, N. W., Lillo, J., Pihan, P., Hernandez, D., Twiss, J. L., and Court, F. A. (2014) Calcium release from intra-axonal endoplasmic reticulum leads to axon degeneration through mitochondrial dysfunction. J. Neurosci. 34, 7179–7189
21. Court, F. A., and Coleman, M. P. (2012) Mitochondria as a central sensor for axonal degenerative stimuli. Trends Neurosci. 35, 364–372
22. Ashraf, G., Schiehe, J. S., LaVoie, M. J., and Schwarz, T. L. (2014)
Mitophagy of damaged mitochondria occurs locally in distal neuronal axons and requires Pink1 and Parkin. *J. Cell Biol.* **206**, 655–670

Maday, S., Wallace, K. E., and Holzbaur, E. L. (2012) Autophagosomes initiate distally and mature during transport toward the cell soma in primary neurons. *J. Cell Biol.* **196**, 407–417

Tsukita, S., and Ishikawa, H. (1976) Three-dimensional distribution of smooth endoplasmic reticulum in myelinated axons. *J. Electron Microsc.* **25**, 141–149

Broadwell, R. D., and Cataldo, A. M. (1983) The neuronal endoplasmic reticulum: its cytochemistry and contribution to the endomembrane system. I. Cell bodies and dendrites. *J. Histochem. Cytochem.* **31**, 1077–1088

Renvoise, B., and Blackstone, C. (2010) Emerging themes of ER organization in the development and maintenance of axons. *Curr. Opin. Neurol.* **23**, pii:a020669

Valenzuela, J. I., Jaureguliberry-Braço, M., and Counge, A. (2011) Neuronal protein trafficking: emerging consequences of endoplasmic reticulum dynamics. *Mol. Cell. Neurosci.* **48**, 269–277

Ying, Z., Misra, V., and Verge, V. M. (2014) Sensing nerve injury at the axonal ER: activated Luman/CREB3 serves as a novel axonally synthesized retrograde regeneration signal. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 16148–16153

Humbert, S., Bryson, E. A., Cordelieres, F. P., Connors, N. C., Datta, S. R., Humbert, H. J., and Chen, Z. Y. (2011) JIP3 mediates TrkB axonal anterograde transport: travel updates on the molecular highway. *Semin. Cell Dev. Biol.* **22**, 37–43

Butowt, R., and von Bartheld, C. S. (2005) Anterograde axonal transport of kinesin Va and microtubule-based motors are required for fast axonal retrograde transport of tetanus toxin in motor neurons. *J. Cell Sci.* **118**, 453–460

Deinhardt, K., Salinas, S., Verastegui, C., and Schiavo, G. (2006) Rab5 and Rab7 control of tetanus. *Tetanus toxin entry. Nidogens are therapeutic targets for the prevention of neurotrophic toxicity. J. Neurosci.* **26**, 775–787

Lalli, G., Bohnert, S., Deinhardt, K., and Schiavo, G. (2003) The journey of tethers and botulinum neurotoxins in neurons. *Trends Microbiol.* **11**, 431–437

Bercsenyi, K., Giribaldi, F., and Schiavo, G. (2013) The elusive compass of endocytic sorting along the axonal retrograde transport pathway. *Neuron* **52**, 293–305

Bercsenyi, K., Schmie, N., Bryson, J. B., Wallace, M., Caccin, P., Gold, I., Zanotti, G., Greensmith, L., Nischt, R., and Schiavo, G. (2014) Tetanus toxin entry. Nidogens are therapeutic targets for the prevention of neurotrophic toxicity. *Science* **346**, 1118–1123

Chen, Z. Y., Humbert, S., and Saudou, F. (2004) Huntington controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell 118*, 127–138

McGuire, J. R., Rong, J., Li, S. H., and Li, X. J. (2006) Interaction of Huntington-associated protein-1 with kinesin light chain: implications in intracellular trafficking in neurons. *J. Biol. Chem.* **281**, 3552–3559

Humbert, S., Bryson, E. A., Cordelieres, F. P., Connors, N. C., Datta, S. R., Finkbeiner, S., Greenberg, M. E., and Saudou, F. (2002) The IGF-1/Akt pathway is neuroprotective in Huntington’s disease and involves Huntington phosphorylation by Akt. *Dev. Cell 2*, 831–837

Colin, E., Zala, D., Liot, G., Rangone, H., Borrell-Pages, M., Li, X. J., Saudou, F., and Humbert, S. (2008) Huntington phosphorylation acts as a molecular switch for anterograde/retrograde transport in neurons. *EMBO J.* **27**, 2124–2134

Fu, M. M., and Holzbaur, E. L. (2013) JIP1 regulates the directionality of APP axonal transport by coordinating kinesin and dynein motors. *J. Cell Biol.* **202**, 495–508

Stokin, G. B., Lillo, C., Falzone, T. L., Bruschi, R. G., Rockenstein, E., Mount, S. L., Raman, R., Davies, P., Masliah, E., Williams, D. S., and Goldstein, L. S. (2005) Axonopathy and transport deficits early in the pathogenesis of Alzheimer’s disease. *Science* **307**, 1282–1288
65. van der Kant, R., and Goldstein, L. S. (2015) Cellular functions of the amyloid precursor protein from development to dementia. Dev. Cell 32, 502–515
66. Miliecamps, S., and Julien, J. P. (2013) Axonal transport deficits and neurodegenerative diseases. Nat. Rev. Neurosci. 14, 161–176
67. Goldstein, L. S. (2012) Axonal transport and neurodegenerative disease: can we see the elephant? Prog. Neurobiol. 99, 186–190
68. De Vos, K. J., Grieron, A. J., Ackery, S., and Miller, C. C. (2008) Role of axonal transport in neurodegenerative diseases. Annu. Rev. Neurosci. 31, 151–173
69. Perlson, E., Maday, S., Fu, M. M., Moughamian, A. J., and Holzbaur, E. L. (2010) Retrorgressive axonal transport: pathways to cell death? Trends Neurosci. 33, 335–344
70. Maday, S., Twelvetrees, A. E., Moughamian, A. J., and Holzbaur, E. L. (2009) Cargo-specific mechanisms of motility and regulation. Neuroreport 20, 292–309
71. Dicht, R., and Cavalli, V. (2015) JIP3 activates kinesin-1 motility to promote axonal elongation. J. Biol. Chem. 290, 15512–15525
72. Sun, T., Yu, N., Zhai, L. K., Li, N., Zhang, C., Zhou, L., Huang, Z., Jiang, X. Y., Shen, Y., and Chen, Z. Y. (2013) c-Jun NH2-terminal kinase (JNK)-interacting protein-3 (JIP3) regulates neuronal axonal elongation in a kinesin-1 and JNK-dependent manner. J. Biol. Chem. 288, 14531–14543
73. Arimoto, M., Koushika, S. P., Choudhary, B. C., Li, C., Matsumoto, K., and Hisamoto, N. (2011) The Caenorhabditis elegans JIP3 protein UNC-16 functions as an adaptor to link kinesin-1 with cytoplasmic dynein. J. Neurosci. 31, 2216–2224
74. Wang, L., and Brown, A. (2002) Rapid movement of microtubules in axons. Curr. Biol. 12, 1496–1501
75. Yan, Y., and Brown, A. (2005) Neurofilament polymer transport in axons. J. Neurosci. 25, 7014–7021
76. Yu, W., Schwei, M. J., and Baas, P. W. (1996) Microtubule transport and assembly during axon growth. J. Cell Biol. 133, 151–157
77. He, Y., Francis, F., Myers, K. A., Yu, W., Black, M. M., and Baas, P. W. (2005) Role of cytoplasmic dynein in the axonal transport of microtubules and neurofilaments. J. Cell Biol. 168, 697–703
78. Hellal, F., Kursad, S., Ruschel, J., Flynn, K. C., Laskowski, C. J., Umlauf, M., Kapitein, L. C., Strikis, D., Lemmon, V., Bixby, J. J., Hoogenraad, C. C., and Bradke, F. (2011) Microtubule stabilization reduces scarring and causes axon regeneration after spinal cord injury. Science 331, 928–931
79. Sengottuvell, V., Leibinger, M., Phreimer, M., Andreadaki, A., and Fischer, D. (2011) Tau protein facilitates axon regeneration in the mature CNS. J. Neurosci. 31, 2689–2699
80. Ruschel, J., Hellal, F., Flynn, K. C., Dupraz, S., Elliott, D. A., Tadeschi, A., Bates, M., Slivinski, C., Brook, G., Dobrindt, K., Peitz, M., Brustle, O., Norenberg, M. D., Blesch, A., Weidner, N., Bunge, M. B., Mudgett, J. L., and Bradke, F. (2015) Axonal regeneration. Systemic administration of epothilone B promotes axon regeneration after spinal cord injury. Science 348, 347–352
81. Myers, K. A., and Baas, P. W. (2007) Kinesin-5 regulates the growth of the axon by acting as a brake on its microtubule array. J. Cell Biol. 178, 1081–1091
82. Kahn, O. I., Sharma, V., Gonzalez-Billault, C., and Baas, P. W. (2015) Effects of kinesin-5 inhibition on dendritic architecture and microtubule organization. Mol. Biol. Cell 26, 66–77
83. Xu, C., Klaw, M. C., Lemay, M. A., Baas, P. W., and Tom, V. J. (2015) Pharmacologically inhibiting kinesin-5 activity with a microtubule promotes axonal regeneration following spinal cord injury. Exp. Neurol. 263, 172–176
84. Yoo, S., van Niekerv, E. A., Merianda, T. T., and Twiss, J. L. (2010) Dynamics of axonal mRNA transport and implications for peripheral nerve regeneration. Exp. Neurol. 223, 19–27
85. Jung, H., Gokogkas, C. G., Sonenberg, N., and Holt, C. E. (2014) Remote control of gene function by local translation. Cell 157, 26–40
86. Jung, H., Yoon, B. C., and Holt, C. E. (2012) Axonal mRNA localization and local protein synthesis in nervous system assembly, maintenance and repair. Nat. Rev. Neurosci. 13, 308–324
87. Donnelly, C. J., Fainzilber, M., and Twiss, J. L. (2010) Subcellular communication through RNA transport and localized protein synthesis. Traffic 11, 1498–1505
88. Xing, L., and Bassell, G. J. (2013) mRNA localization: an orchestration of assembly, traffic and synthesis. Traffic 14, 2–14
89. Prescott, A. R., Bales, A., James, J., Trinkle-Mulcahy, L., and Steelman, J. E. (2014) Time-resolved quantitative proteomics implicates the core snRNP protein SmB together with SMN in neural trafficking. J. Cell Sci. 127, 812–827
90. Meister, G., Eggert, C., Buhler, D., Brahms, H., Kambach, C., and Fischer, U. (2001) Methyltransfer of splicing protein by a complex containing PRMTs and the putative U snRNP assembly factor pCln. Current Biol. 11, 1990–1994
91. Paushkin, S., Gubitz, A. K., Massenet, S., and Dreyfuss, G. (2002) The SMN complex, an assemblyosome of ribonucleoproteins. Curr. Opin. Cell Biol. 14, 305–312
92. Willis, D., Li, K. W., Zheng, J. Q., Chang, J. H., Smit, A. B., Kelly, T., Baleriola, J., Walker, C. A., Jean, Y. Y., Crary, J. F., Troy, C. M., Nagy, M., Nettzel, K. L., Devlin, B. K., and MacLennan, A. J. (2004) STAT3 phosphorylation in injured axons before sensory and motor neuron nuclei: potential role for STAT3 as a retrograde signaling transcription factor. J. Comp. Neurol. 474, 535–545
93. Ben-Yaakov, K., Dagan, S. Y., Segal-Ruder, Y., Shalem, O., Vuppalanchi, D., Willis, D. E., Yudin, D., Risbey, I., Rother, F., Mader, B., Blesch, A., Pipler, Y., Twiss, J. L., and Fainzilber, M. (2012) Axonal transcription factors act as signal retrogradely in lesioned peripheral nerve. EMBO J. 31, 1350–1363
94. Ohara, R., Fujita, Y., Hata, K., Nakagawa, M., and Yamashita, T. (2011) Axotomy induces axonogenesis in hippocampal neurons through STAT3. Cell Death Dis. 2, e175
95. Baleriola, J., Walker, C. A., Jean, Y. Y., Crary, J. F., Troy, C. M., Nagy, M. P., and Hengst, U. (2014) Axonally synthesized ATF4 transmits a neurodegenerative signal across brain regions. Cell 158, 1159–1172
96. Liu, Y., Que, H., Ma, Z., Yang, S., Ni, Y., Luo, Z., Tang, N., Yang, J., Jing, S., and Liu, S. (2013) Transcription factor SCIRF69 involved in the activation of brain-derived neurotrophic factor gene promoter II in mechanically injured neurons. Neuron. Med. 15, 605–622
97. Melemedjian, O. K., Tillu, D. V., Moy, K. J., Asiedu, M. N., Mandell, E. K., Ghosh, S., Dussor, G., and Price, T. J. (2014) Local translation and retrograde axonal transport of CREB regulates I/B-induced nociceptive plasticity. Mol. Pain 10, 45
98. Cho, Y., and Cavalli, V. (2012) HDAC5 is a novel injury-regulated tubulin deacetylase controlling axon regeneration. EMBO J. 31, 3063–3078
99. Ishii, S., Kurasawa, Y., Wong, J., and Yu-Lee, L. Y. (2008) Histone deacetylase 3 localizes to the mitotic spindle and is required for kinetochoore-microtubule attachment. Proc. Natl. Acad. Sci. U.S.A. 105, 4179–4184
100. Kim, J. Y., Shen, S., Dietz, K., He, Y., Howell, O., Reynolds, R., and Casaccia, P. (2010) HDAC1 nuclear export induced by pathological conditions is essential for the onset of axonal damage. Nat. Neurosci. 13, 180–189
101. Moretti, F., Rolando, C., Winkler, M., Ivanek, R., Rodriguez, J., Von Kriegsheim, A., Taylor, V., Bustin, M., and Perz, O. (2015) Growth cone
localization of the mRNA encoding the chromatin regulator HMGN5 modulates neurite outgrowth. Mol. Cell. Biol. 35, 2035–2050
109. van Niekerk, E. A., Willis, D. E., Chang, J. H., Reumann, K., Heise, T., and Twiss, J. L. (2007) Sumoylation in axons triggers retrograde transport of the RNA-binding protein La. Proc. Natl. Acad. Sci. U.S.A. 104, 12913–12918
110. Rivieccio, M. A., Brochier, C., Willis, D. E., Walker, B. A., D'Annibale, M. A., McLaughlin, K., Siddiq, A., Kozikowski, A. P., Jaffrey, S. R., Twiss, J. L., Ratan, R. R., and Langley, B. (2009) HDAC6 is a target for protection and regeneration following injury in the nervous system. Proc. Natl. Acad. Sci. U.S.A. 106, 19599–19604
111. Belin, S., Nawabi, H., Wang, C., Tang, S., Latremoliere, A., Warren, P., Schorle, H., Uncu, C., Woolf, C. J., He, Z., and Steen, J. A. (2015) Injury-induced decline of intrinsic regenerative ability revealed by quantitative proteomics. Neuron 86, 1000–1014
112. Puttagunta, R., Tedeschi, A., Soria, M. G., Hervera, A., Lindner, R., Rathore, K. I., Gaub, P., Joshi, Y., Nguyen, T., Schmandke, A., Lastowski, C. J., Boutillier, A. L., Bradke, F., and Di Giovanni, S. (2014) PCAF-dependent epigenetic changes promote axonal regeneration in the central nervous system. Nat. Commun. 5, 3527
113. Pelzel, H. R., Schlamp, C. L., and Nickells, R. W. (2010) Histone H4 deacetylation plays a critical role in early gene silencing during neuronal apoptosis. BMC Neurosci. 11, 62
114. Schmitt, H. M., Pelzel, H. R., Schlamp, C. L., and Nickells, R. W. (2014) Histone deacetylase 3 (HDAC3) plays an important role in retinal ganglion cell death after acute optic nerve injury. Mol. Neurodegen. 9, 39
115. Penney, J., and Tsai, L. H. (2014) Histone deacetylases in memory and cognition. Sci. Signal. 7, re12
116. Arnaudo, A. M., and Garcia, B. A. (2013) Proteomic characterization of novel histone post-translational modifications. Epigenet. Chromat. 6, 24
117. Brunner, A. M., Tweedie-Cullen, R. Y., and Mansuy, I. M. (2012) Epigenetic modifications of the neuroproteome. Proteomics 12, 2404–2420
118. Allfrey, V. G., Faulkner, R., and Mirsky, A. E. (1964) Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. Proc. Natl. Acad. Sci. U.S.A. 51, 786–794
119. Tropberger, P., and Schneider, R. (2010) Going global: novel histone modifications in the globular domain of H3. Epigenetics 5, 112–117
120. Bernstein, B. E., Mikkelsen, T. S., Xie, X., Kamal, M., Huebert, D. J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K., Jaenisch, R., Wagschal, A., Feil, R., Schreiber, S. L., and Lander, E. S. (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125, 315–326
121. Mikkelsen, T. S., Ku, M., Jaffe, D. B., Issac, B., Lieberman, E., Giannoukos, G., Alvarez, P., Brockman, W., Kim, T. K., Koche, R. P., Lee, W., Mendellath, E., O’Donovan, A., Presser, A., Russ, C., Xie, X., Meissner, A., Wernig, M., Jaenisch, R., Nusbaum, C., Lander, E. S., and Bernstein, B. E. (2007) Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature 448, 553–560
122. LeRoy, G., Chepelev, I., DiMaggio, P. A., Blanco, M. A., Zee, B. M., Zhao, K., and Garcia, B. A. (2012) Proteogenomic characterization and mapping of nucleosomes decoded by Brd and HP1 proteins. Genome Biol. 13, R68
123. Volgt, P., LeRoy, G., Drury, W. J., 3rd, Zee, B. M., Son, J., Beck, D. B., Young, N. L., Garcia, B. A., and Reinberg, D. (2012) Asymmetrically modified nucleosomes. Cell 151, 181–193
124. Shaob, M., Kulyyassov, A., Robin, C., Winczura, K., Tarlykov, P., Despas, E., Kanneuchi, P., Ramamurti, E., Lipinski, M., and Ogrzyko, V. (2013) PUB-NChIP—“in vivo biotinylation” approach to study chromatin in proximity to a protein of interest. Genome Res. 23, 331–340