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Axonal mRNA localization and local protein synthesis in nervous system assembly, maintenance and repair

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Abstract

mRNAs can be targeted to specific neuronal subcellular domains, which enables rapid changes in the local proteome through local translation. This mRNA-based mechanism links extrinsic signals to spatially restricted cellular responses and can mediate stimulus-driven adaptive responses such as dendritic plasticity. Local mRNA translation also occurs in growing axons where it can mediate directional responses to guidance signals. Recent profiling studies have revealed that both growing and mature axons possess surprisingly complex and dynamic transcriptomes, thereby suggesting that axonal mRNA localization is highly regulated and has a role in a broad range of processes, a view that is increasingly being supported by new experimental evidence. Here, we review current knowledge on the roles and regulatory mechanisms of axonal mRNA translation and discuss emerging links to axon guidance, survival, regeneration and neurological disorders.

Cell–cell signalling relies on the ability of cells to adjust their local proteome (protein abundance and complexity) with high spatiotemporal precision in response to extracellular signals. Thousands of mRNAs exhibit specific subcellular localization patterns in mammals[1] and in *Drosophila melanogaster*[2], which suggests that local mRNA translation may have a general role in controlling the local proteome. Conceptually, local protein synthesis provides several advantages over the transport of pre-existing proteins from one part of the cell to another[3]. First, translationally silent forms of mRNAs can be stored locally and used to make many copies of a protein when needed, thereby providing economic advantages. Second, the ectopic presence of proteins in other parts of the cell during protein transport is avoided. Third, mRNAs can be targeted to different subcellular localizations using ‘address’ information in their untranslated regions (UTRs) without changing the structure and function of the proteins they encode. Finally, properties that are unique to newly made proteins (such as minimal post-translational modification) may provide an additional layer of signalling information.

Intuition suggests that highly polarized cells like neurons would benefit greatly from local mRNA translation. Indeed, local mRNA translation is known to mediate long-lasting synaptic plasticity in dendritic spines, and studies in dendrites have provided significant mechanistic and functional insights into how extrinsic signals regulate local mRNA translation[4,5]. Although evidence for axonal protein synthesis in mammals dates back to the 1960s[6–8], surprisingly little is known about local mRNA translation in axons. The general view of the axon as a passive transmitter of information may have contributed to this

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neglect. Nevertheless, axons — like dendrites — are signal-receiving compartments. Growing axons require extrinsic signals to regulate their growth, navigation and synapse formation and mature axons depend on external cues for maintenance and repair. Thus, there is clearly a conceptual need for local protein synthesis in distal axons[9]. Indeed, stimulus-induced responses of axons in cell culture — such as chemotropic responses[10] and regeneration following injury[11] — require axonal mRNA translation, and evidence suggests that some of these findings could be applicable *in vivo*[12–14].

Thus, local mRNA translation can be regarded as a common mechanism for the regulation of local proteomic homeostasis in response to extracellular signals in axons and dendrites. Both developing[15,16] and mature[17,18] axons contain complex and dynamic mRNA repertoires, and recent studies have provided insights into the regulation of axonal mRNA translation. Here, we aim to provide a conceptual framework for this emerging field of active research by reviewing our understanding of the current literature and discussing future directions.

Evidence for axonal mRNA translation

The initial lack of interest in local axonal mRNA translation can be traced back to findings in the 1970s using the squid giant axon. It was suggested that protein synthesis is unlikely to occur in mature axons as little or no ribosomal RNA (as measured by their optical densities on polyacrylamide gels) was observed in the axoplasm[19]. This interpretation was, however, later disputed by evidence obtained using more sensitive biochemical methods showing the presence of ribosomal RNAs[20], mRNAs[21] and actively translating polysomes[22] in squid giant axons. In mammals, ribosomes were identified by electron microscopy in embryonic cortical[23] and sympathetic[24] neuronal axons in cell culture, and in embryonic peripheral sensory axons *in vivo*[25]. Recent immuno-electron microscopy has shown specific immunoreactivity to the ribosomal protein S6 in the axons of cultured embryonic sympathetic and hippocampal neurons[26]. Notably, axonal ribosomes rarely form polysomes *in vivo*[24,25,27], unlike in culture conditions[23], which suggests that monosomal translation may predominate or that translational activation is spatiotemporally restricted in axons in normal conditions.

In an electron microscopy study using adult rats, electron-dense ribosomal rosettes — the typical ultrastructural appearance of polysomes — were limited to the axon initial segment and not detectable in the axon shaft of CNS neurons[28], thus leading to speculation that axonal protein synthesis only occurs during development. Immunohistological studies, however, showed that mature PNS axons contain ribosomal proteins and RNAs, which form intermittent ribosomal plaques that are distributed unevenly in the peripheral axoplasm close to the plasma membrane[29–32]. These observations were consistent with an overlooked electron microscopy study from 1970 (REF. 33). The peripheral localization of ribosomes also agrees with a recent finding that a direct interaction between cell surface receptors and ribosomes spatially restricts mRNA translation to the site at which an extrinsic cue is received[26]. The specialized morphology and distribution of axonal ribosomes may thus have made their ultrastructural identification difficult[28]. Indeed, immuno-electron microscopy evidence from transgenic mice showed that an enhanced green fluorescent protein (EGFP)-tagged ribosomal protein L10a (EGFP–L10a) localizes to the nodes of Ranvier in corticospinal tract axons *in vivo*[34]. Although some EGFP–L10a may be present outside the ribosome (as it probably competes with endogenous L10a for ribosomal occupancy), this nonetheless supports the idea that mature axons contain intermittent ribosomal plaques that are not readily identifiable by conventional ultrastructural criteria[29–33].

Compelling evidence for axonal protein synthesis came from metabolic labelling experiments that showed that unmyelinated axons without somas are capable of mRNA translation-dependent protein synthesis in vertebrates[7,35–39] and invertebrates[6,21]. Another key finding was that exogenous mRNA can be translated when injected into mollusc axons without somas[40]. The list of axonally localizing and translatable mRNAs is rapidly growing and includes those encoding membrane-targeted proteins such as ephrin type-A receptor 2 (EPHA2)[41] and κ-type opioid receptor (KOR1; also known as OPRK1)[42].

Several electron microscopy studies have failed to identify rough endoplasmic reticulum (RER) and Golgi apparatus in vertebrate axons[24,25,27,43], thus raising the question of whether vertebrate axons have the capacity to process proteins. However, a recent study using the severed axons of cultured peripheral sensory neurons showed that metabolically labelled, newly synthesized proteins are trafficked to the plasma membrane[44], thus suggesting that the functional equivalent of RER and Golgi apparatus exists in these axons. ER-associated and Golgi-associated proteins have been detected immunocytologically in the axons of vertebrates[44], but the morphology of the axonal ER is distinct from that found in the soma[44,45]. These findings are consistent with those seen in invertebrates. Mollusc axons are capable of local protein targeting but do not appear to possess ultrastructurally identifiable RER and Golgi apparatus[46]. Nevertheless, proteins residing in the ER and Golgi can be detected immunocytologically[47]. It will be interesting to explore whether the axonal RER and Golgi adopt specialized morphologies, perhaps similar to those of primitive parasites[48]. Together, these findings show that both growing and mature axons possess protein synthesis and processing machinery, but the unconventional nature of their histological structure presents a puzzle.

Function of axonal mRNA translation

Growing axons receive continuous guidance information from their environment as they travel along long stereotypical paths to reach their synaptic partners. The distal tip of a growing axon, a specialized structure known as the growth cone, must quickly process this information, often without enough time to communicate with its soma. Indeed, axons severed from their cell bodies can correctly pathfind *in vivo*[49], and the growth cone can respond to guidance cues without the cell body *in vitro*[10,50].

Local mRNA translation is a key mechanism in this autonomous signalling, and protein synthesis inhibitors block the ability of growth cones that are severed from their somas to respond to several guidance cues. These guidance cues include netrin 1 (REFS 51–54), semaphorin 3A (SEMA3A)[10,55], SLIT2 (REF. 56), engrailed 1 and engrailed 2 (EN1 and EN2)[57–59], pituitary adenylate cyclase-activating polypeptide (PACAP; also known as ADCYAP1)[60], nerve growth factor (NGF)[52,61], brain-derived neurotrophic factor (BDNF)[54] and neurotrophin 3 (NT3)[62,63]. Increasing evidence shows that axonal mRNA translation continues to play roles in adulthood, particularly during plastic responses such as injury-induced axon regeneration[11,13,14,64–66]. These extrinsic factors rapidly induce local protein synthesis by activating the mammalian target of rapamycin (mTOR) pathway (TABLE 1).

Chemotropic responses of growth cones

One of the first identified functions of axonal protein synthesis was its ability to mediate netrin 1-induced and sema3a-induced chemotropic responses in cultured growth cones[10] (FIG. 1). *Xenopus laevis* retinal growth cones without somas turn towards netrin 1 gradients and away from sema3a gradients in culture[10]. Application of these cues to soma-less growth cones increases mTOR activity (as measured by phosphorylation of its substrate eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4e-bp1)) and axonal protein synthesis (as measured by ^{3}H-leucine incorporation) within minutes. In addition, inhibiting

mTOR activation (with rapamycin) or ribosome function (with cycloheximide or anisomycin) blocks both local protein synthesis and the chemotropic responses[10]. Inhibition of mTOR or protein synthesis also blocks sema3a-induced growth cone collapse (the rapid withdrawal of filopodia in response to uniformly applied repulsive cues)[10]. Intriguingly, a phosphoinositide 3-kinase (PI3K) inhibitor blocks the growth cone's response to netrin 1, whereas the response to sema3a is not blocked. This suggests that different signalling pathways downstream of netrin 1 and sema3a converge on mTOR[10]. Lysophosphatidic acid-induced growth cone collapse is not affected by any of these inhibitors, and lysophosphatidic acid application increases neither mTOR activity nor local protein synthesis[10]. Other cues that do not induce protein synthesis include ephrin B[67] and sphingosine 1-phosphate[68].

Interestingly, SEMA3A-induced, protein synthesis-dependent growth cone collapse occurs at low, but not at high, concentrations of SEMA3A[69]. This agrees with the finding that growth cones lose their physiological cue selectivity when the cues are applied in high concentrations[69]. For example, spinal motor neuron growth cones show neuronal subtype-specific collapse responses to either SEMA3A or SEMA3F in culture, and these responses are blocked by protein synthesis inhibitors[69]. When these cues are applied in high concentrations, the growth cones lose both cue specificity and protein synthesis dependence[69]. Differences in the concentration and/or bioactivity of SEMA3A may therefore account for discrepancies in the literature regarding the protein synthesis dependence of SEMA3A-induced growth cone collapse[10,55,70].

Although the detailed mechanisms vary, most protein synthesis-inducing cues seem to activate the translational machinery through the mTOR pathway[10,56,58] and the direction of turning responses by growth cones is determined by the specific mRNAs that are translated. For example, stimulating growth cones with a netrin 1 or BDNF gradient results in rapid trafficking of β-actin mRNA to the side of the growth cone nearest the gradient, resulting in asymmetric β-actin synthesis that is rapamycin-sensitive[53,54]. Zipcode binding protein 1 (ZBP1; also known as VG1RBP), an RNA-binding protein (RBP), transports β-actin mRNA in a translationally silent state by binding to a *cis*-acting element zipcode in its 3′-UTR[53,54,63] and repressing its translation[71]. Attractive growth cone turning towards netrin 1 or BDNF can be prevented by inhibiting β-actin synthesis using antisense oligonucleotides that block translation[53] and by disrupting the β-actin mRNA–ZBP1 interaction with antisense zipcode oligonucleotides[54] or sense zipcode oligonucleotides that competitively inhibit endogenous β-actin mRNA[13]. Attractive growth cone turning can also be prevented by genetically deleting ZBP1 (REF. 72); these all suggest that cue-induced β-actin synthesis is required for this process in culture. By contrast, the repulsive cues SLIT2 and SEMA3A do not elicit β-actin synthesis, but induce rapid axonal synthesis of proteins that promote actin disassembly such as cofilin[56] and RHOA[55]. SEMA3A-induced growth cone collapse can be blocked by inhibiting axonal *RHOA* mRNA translation[55].

These findings led to the development of the differential translation model (FIG. 1), whereby the direction of growth cone turning is determined by the localized synthesis of proteins that promote either assembly (mediating attraction) or disassembly (mediating repulsion) of the cytoskeleton[73]. This agrees with studies in fibroblasts that show a requirement for the localization of β-actin mRNA to the leading lamellipodia for cell polarization and motility[74,75]. Given that pre-existing β-actin monomers are abundant in growth cones and that local mRNA translation in fibroblast lamellipodia gives no clear temporal advantage over transporting pre-existing β-actin monomers, the primary role of *de novo* β-actin synthesis probably lies in the spatial bias it provides to the polarization of cytoskeletal dynamics. For example, newly synthesized β-actin that lacks post-translational modifications (such as arginylation[76] and glutathionylation[77]) could provide a more efficient

nucleation signal than pre-existing monomers and thus seed actin polymerization on the nearside of the growth cone in an attractive gradient, thus driving growth in this direction. Conversely, polarized disassembly of the cytoskeleton on the nearside of a repulsive gradient would steer axons away from the gradient; however, this idea has not yet been tested. Nevertheless, it is noteworthy that cytoskeletal-associated molecules are highly represented in the axonal transcriptome[15–18] (TABLE 2).

It is not yet clear what specific aspects of axonal pathfinding *in vivo* correspond to these *in vitro* findings. Although there is no reported axon guidance defect in mice with only one functional *Zbp1* allele[72], the peripheral sensory neurons of these mice, as well as those of mice overexpressing zipcode competitive inhibitors, exhibit reduced axon branching in culture[13]. Similarly, blocking the ubiquitin–proteasome system *in vivo* results in cell intrinsic defects in retinal axon branching but does not affect long-range pathfinding[78]. However, chemotropic responses of cultured growth cones to netrin 1 are blocked either by a proteasome or translation inhibitor alone[10]. Therefore, growth cone chemotropic responses *in vitro* may reflect short-range, cue-induced events, such as axon branching. It remains to be seen which particular processes are regulated by axonal mRNA translation *in vivo*.

Growth cone adaptation and gradient sensing

Growth cones must sense chemical gradients in order to properly guide their axons[79]. Growth cone adaptation, which is crucial for gradient sensing, involves successive cycles of desensitization and resensitization of the growth cone to an extracellular signal, and the resensitization step requires axonal protein synthesis[50,80]. Cultured *X. laevis* embryonic spinal neuronal growth cones lose their ability to respond to netrin 1 or BDNF after preincubation with the cue but regain responsiveness after 60–90 minutes in a manner that is sensitive to a translational inhibitor[50]. This enables these growth cones to respond to a netrin 1 or BDNF gradient many times in a lengthy period of exposure, which results in a zigzag pattern of growth[50]. This homeostatic reset mechanism is likely to be efficient for rapidly elongating axons that need to take only intermittent bearings to steer towards a distant cue.

Adaptation can also occur on a shorter timescale[80]. Cultured *X. laevis* retinal axons briefly preincubated with a low dose of repulsive sema3a or netrin 1 (netrin 1 can be either attractive or repulsive depending on the context[81,82]) lose their ability to respond to a collapse-inducing dose of the same cue[80]. This rapid (1–2 minutes) desensitization is cue-specific and mediated by ligand-induced receptor endocytosis[80]. Desensitized growth cones regain responsiveness in 4 minutes in a translation-dependent manner, but a higher dose of netrin 1 or sema3a is now required to induce the same degree of growth cone collapse[80]. This rapid recalibration mechanism allows advancing growth cones to readjust their sensitivity quickly and to compare minute differences in a concentration gradient. Interestingly, both receptor endocytosis and translation are spatially polarized to the gradient nearside of growth cones[83], which suggests that the two processes are intimately linked.

Changes at intermediate targets

Growth cones encounter different guidance cues along their pathway and change cue responsiveness while they travel. For example, *X. laevis* retinal axons are attracted towards netrin 1 expressed in the optic nerve head, but netrin 1 becomes repellent upon exit from the eye[81,82]. Such switches might be regulated by local translation in distal axons.

In chick commissural axons transfected with a fluorescent reporter protein mRNA — the translation of which is regulated by the 3′-UTR of the ephrin A receptor *epha2* mRNA (BOX 1) — increased reporter expression was observed in distal axons that had passed the midline of the spinal cord[41]. Although it is unknown whether endogenous *epha2* mRNA is

localized and regulated in this manner, this suggests the possibility that intermediate targets regulate the future responsiveness of axons by stimulating local synthesis of new guidance cue receptors. The expression of robo3, a receptor for slits that repels axons at the midline, is also regulated at the translational level[84]. It will be interesting to determine whether mRNAs encoding this and other receptors are axonally translated and regulate axon guidance *in vivo*.

Axon elongation

The short-term basal growth of axons does not require axonal protein synthesis[36], and severed axons grow normally for 2–5 hours in the presence of protein synthesis inhibitors in culture[10,53,85]. Cue-induced axon growth promotion does, however, require axonal protein synthesis[52,86]. NGF and netrin 1, when applied acutely to cultured mammalian sensory and commissural neurons, respectively, promote axon outgrowth. Axonal synthesis of proteinase-activated receptor 3 (PAR3), a component of a complex that controls cytoskeletal dynamics, is required for NGF-induced and netrin 1-induced axon outgrowth[52]. β-thymosin, which prevents actin polymerization, is locally synthesized in cultured mollusc neurites treated with brain lysate, and inhibiting the translation of β-thymosin mRNA in isolated neurites promotes their elongation[86]. These studies suggest that extrinsic signals might shape the pattern of axonal outgrowth by regulating local synthesis of positive and negative regulators of cytoskeletal dynamics.

Synapse formation

Upon reaching its target, the growth cone transforms into the presynaptic terminal, which is highly branched and enriched with synaptic vesicles. Comparative subcellular mRNA profiling analysis has revealed that mRNAs encoding proteins implicated in this process (branch-promoting proteins and synaptic vesicle proteins) become specifically enriched in the growth cone as *X. laevis* retinal ganglion cells mature in culture[16]. Thus, these mRNAs are trafficked to the growth cone to coincide with target arrival and might be translated in response to target-derived cues. Indeed, presynaptic protein synthesis is required for BDNF-induced and NT3-induced potentiation of synaptic vesicle release in cultured *X. laevis* lower motor neurons[62,87]. Presynaptic protein release is also required for an increase in synaptic vesicle number with synaptic maturation in cultured mouse hippocampal neurons[88].

This finding is in accordance with studies using mollusc neurons, which show that presynaptic protein synthesis[89] is required for synapse formation[47,90–92]. It is also in agreement with a study of the *Drosophila melanogaster* neuromuscular junction, which shows that 3-phosphoinositide-dependent protein kinase 1 (PDK1) and ribosomal protein S6 kinase (S6K) — which are both components of the mTOR pathway — control synaptic bouton size, active zone number and synaptic function, although translation-dependency has not been tested in this case[93]. Furthermore, fragile X mental retardation protein (FMRP), a known translational regulator and mediator of synaptic plasticity in dendritic spines[94,95], is found in axons and growth cones[96–98], and its loss leads to a cell autonomous defect in the formation of presynaptic terminals in organotypic mouse hippocampal slices[99]. Other translational regulators such as survival of motor neuron (SMN1)[100,101] and Hu-antigen D (HUD; also known as ELAVL4)[102,103] also localize to axons and growth cones, thus indicating a probable broad presynaptic role of translation in aspects of synapse formation.

Transmitter biogenesis

Nerve terminals that secrete neurotransmitters remotely from their cell bodies might benefit from local mRNA translation. Indeed, a metabolic labelling study carried out in the 1960s using rat brains showed that newly (15–90 minute) synthesized proteins localize to nerve endings[104]. There is evidence suggesting that enzymes that regulate neurotransmitter metabolism, such as tyrosine hydroxlase (a key regulator of catecholamine biogenesis) and

acetylcholinesterase (AChE; an enzyme that degrades acetylcholine), might be synthesized in nerve terminals.

A drug-induced increase in tyrosine hydroxlase activity[105] and the reappearance of AChE activity after irreversible pharmacological inactivation[106] occur in nerve terminals at speeds that cannot be explained by axonal transport from the cell bodies. Tyrosine hydroxlase mRNAs can be detected in the striatum by reverse transcription-polymerase chain reaction and *in situ* hybridization and is decreased by pharmacological lesion to the nigrostriatal pathway[107].

Neuropeptides and hormones might also be synthesized in nerve terminals. Oxytocin and vasopressin mRNAs can be detected in the neurohypophysis by reverse transcription-polymerase chain reaction, *in situ* hybridization and northern blot[108–112], and several invertebrate hormones can be locally synthesized in isolated axons[47,90–92,113]. However, whether these proteins are synthesized in the nerve endings *in vivo* and whether axonally synthesized proteins can be secreted is unknown.

Cell survival and axon maintenance

The promotion of axon survival by target-derived trophic factors can also be considered an adaptive response to extrinsic cues, and appears to involve axonal protein synthesis. For example, NGF supports the survival of cultured peripheral sensory and sympathetic neurons in a translational inhibitor-sensitive manner[15,61,114]. In cultured sympathetic neurons, inositol monophosphatase 1 (IMPA1) is axonally synthesized in response to NGF, and initiates an unknown signalling cascade that relays a survival signal to the nucleus[15] (FIG. 2a). In cultured peripheral sensory neurons, the transcription factor cyclic AMP responsive element-binding protein (CREB) is axonally synthesized in response to NGF and retrogradely transported to the nucleus where it initiates transcription-dependent cell survival mechanisms[61] (FIG. 2a). Interestingly, axonally synthesized CREB is specifically required for the NGF-induced survival signal: CREB synthesized in the cell body is unable to mediate NGF-induced cell survival[61]. This effect may be cell type-specific, because *CREB* mRNAs are found exclusively in peripheral sensory neurons[17,61].

Axonally synthesized proteins can also support axon survival locally by promoting mitochondrial function. Many axons contain nuclear-transcribed mRNAs encoding mitochondrial proteins[115–117] or proteins involved in mitochondrial function[15–18] (TABLE 2). Axon-specific inhibition of mRNA translation or mitochondrial protein import decreases mitochondrial membrane potential in cultured sympathetic neurons[117], thus suggesting that nuclear-encoded mitochondrial proteins are locally synthesized and imported to axonal mitochondria to support their function. New evidence shows that this mechanism may operate *in vivo*[118]. In cultured *X. laevis* retinal axons, target-derived cues such as en1 stimulate the local synthesis of lamin B2, a component of the nuclear lamina, which, surprisingly, then localizes to axonal mitochondria[118] (FIG. 2a). Sustained axonal translation of lamin B2 mRNA is required for mitochondrial function *in vitro* and axon maintenance *in vivo*[118]. Interestingly, en1 also induces the synthesis of nuclear-encoded mitochondrial proteins in midbrain dopaminergic neurons in mice and supports their survival[57]. Therefore, stimulating the local synthesis of proteins involved in mitochondrial function might be a common mechanism by which target-derived cues support axon maintenance. Intriguingly, mutations in either mitochondrial proteins[119] or lamins[120] can cause Charcot–Marie–Tooth disease type 2, a neuropathic disorder that is characterized by chronic axonal degeneration. Together, these suggest links among mitochondria, lamins and axon maintenance.

Response to nerve injury and axon regeneration

As local mRNA translation mediates adaptive responses to extracellular signals, it is not surprising that mRNA translation can occur even in mature axons, especially during plastic responses such as regeneration. Generally, the ability of axons to synthesize proteins decreases as they mature, which has been associated with a coincident reduction in their ability to re-grow after axotomy[121,122]. Indeed, axons preconditioned with nerve injury *in vivo* show increased local protein synthesis[11,38,39,43,64] and regeneration[11] *in vitro*, and further increasing mTOR activity at the injury site (by locally applying phosphatase and tensin homologue (PTEN) short interfering RNA (siRNA) or the PTEN inhibitor bisperoxovanadium) promotes regeneration *in vivo*[123]. Intriguingly, there is evidence suggesting that axonal protein synthesis might be augmented after nerve injury by an unconventional mechanism of transceullar ribosomal delivery from glial cells[124,125]. Critically, blocking mRNA translation in severed axons inhibits the regeneration of new growth cones in culture[11], and mice with reduced activity of ZBP1 exhibit attenuated axon regeneration after nerve injury *in vivo*[13]. These findings suggest that the ability of axons to locally synthesize proteins is related to their ability to regenerate. Indeed, olfactory sensory neurons, which naturally regenerate their axons, contain multiple axonal mRNAs[126,127], some of which associate with axonal polysomes *in vivo*[128].

In peripheral sensory neurons, nerve injury generates a cascade of retrograde signalling events that ultimately activates transcription of genes required for axon survival and regeneration[129] (FIG. 2b). Local synthesis of transcription factors, such as signal transducer and activator of transcription 3 (STAT3), at the injury site is required for generating this signal[14]. Newly synthesized STAT3 is locally phosphorylated and retrogradely transported in an active state[14]. This is similar to CREB, which is locally synthesized and activated by NGF[58]. Intriguingly, the retrograde transport itself is also regulated by local mRNA translation. Ran GTPase, a regulator of nuclear transport, localizes to axons in a GTP-bound state in normal conditions, preventing cargo proteins from binding to the importin-α–dynein motor complex that is involved in retrograde transport[66]. Nerve injury induces local synthesis of Ran-specific GTPase-activating protein (RANBP1)[66] and importin-β1 (REF. 64), which displaces Ran GTPase and directly binds to importin-α, respectively. The importin-α–importin-β1–dynein motor complex binds to transcription factors bearing nuclear localization signals, thus enabling their retrograde transport. Vimentin, which is also locally synthesized in response to injury, also directly binds to importin-β1 and other signalling molecules such as extracellular signal-regulated kinase 1 and 2 (ERK1/2), thereby facilitating the relay of complex information to the cell body[65].

Surprisingly, local application of membrane-permeable nuclear localization signal peptides — which prevent axonally synthesized transcription factors from binding to importin-β1 — at the time of nerve injury abolishes the positive effect of preconditioning lesion to axon regeneration *in vitro*[64] and neuronal survival *in vivo*[14]. Local translation of transcription factors might be an evolutionarily conserved mechanism to protect axons, because axonal translation of CAAT enhancer binding protein 1 (*cebp-1*) mRNA is required for axon regeneration after axotomy in *Caenorhabditis elegans*[130].

In the CNS, axons lose their ability to regenerate in adulthood. Intriguingly, increasing protein synthesis might restore their regenerative potential[131]. In mice, global translational activity (as measured by the phosphorylation of the ribosomal protein S6) in retinal ganglion cells decreases with age[132,133]. In contrast to peripheral axons[11], injury to postnatal retinal axons downregulates global translational activity in retinal ganglion cells *in vivo*[131]. Intriguingly, genetic deletion of the negative regulators of mTOR, PTEN and tuberous sclerosis protein 1 (TSC1), prevents this decrease in translational activity[131]. Remarkably, these rejuvenated neurons can regenerate axons, although whether axonal or somal protein

synthesis plays a more important role in this effect is unknown[131]. Thus, different translational responses to nerve injury might account for the different abilities of CNS and PNS neurons to regenerate axons.

Receptor expression and pain regulation

Some evidence suggests that local mRNA translation may regulate pain sensitivity. mRNAs encoding transient receptor potential cation channel subfamily V member 1 (TRPV1), a capsaicin-gated and high temperature-gated ion channel, are detected in the axons of peripheral nociceptors in adult mice[134]. Interestingly, inflammatory insults increase the axonal transport of *Trpv1* mRNA to central endings in the spinal cord, and intrathecal delivery of *Trpv1* antisense oligodeoxynucleotides reduces the inflammation-induced TRPV1 hypersensitivity in spinal cord slices[134]. Nerve injury also induces specific accumulation of tetrodotoxin-resistant voltage-gated sodium channel 1.8 (Nav1.8) mRNA in injured axons *in vivo*[135]. Subcutaneous injection of Nav1.8 siRNA into areas in which peripheral endings terminate specifically reduces Nav1.8 mRNA in the axon but not in the cell body, and reduction of axonal Nav1.8 mRNA correlates with the reversal of neuropathic pain in mice[135]. Furthermore, delivery of inhibitors of protein synthesis either intrathecally (rapamycin[136] and cycloheximide[134]) or intraplantarly (rapamycin[137] and anisomycin[138]) alleviates pathological pain in mice, although these effects may indirectly result from the decreased production and release of pro-inflammatory cytokines from neighbouring cells. The axons of cultured peripheral sensory neurons also contain mRNAs encoding KOR1, a GPCR that generates an antinociceptive signal, and the axonal localization of *Kor1* mRNA is enhanced by depolarization in culture[42,139]. Direct evidence showing that these receptors are trafficked to the plasma membrane is still lacking, although these axons have functional machinery to target proteins to the cell surface[44] and mollusc axons can target locally synthesized conopressin receptor to the plasma membrane[46].

Neurodevelopmental and neurodegenerative disorders

Numerous neurodevelopmental disorders, such as fragile X mental retardation and autism spectrum disorders, seem to share dysregulated protein synthesis as an underlying cause of clinical pathologies[140]. Although dendrites are well-documented sites of synaptic pathology[140], there is less evidence for axons. Recent evidence, however, shows that translational regulators such as FMRP, also localize to axons and regulate presynaptic function[99]. Therefore, dysregulated axonal mRNA translation may, in future, be found to contribute to clinical pathologies of neurodevelopmental disorders[141].

Recent evidence links axonal mRNA translation to neurodegenerative diseases, such as spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). SMA is characterized by lower motor neuron degeneration and muscular atrophy and is caused by deletions in *SMN1* (REF. 142). SMN1 associates with small nuclear ribonucleoproteins (snRNPs) in the nucleus to regulate pre-mRNA splicing[143] but also localizes to axons and growth cones[144,145]. In the cytoplasm, SMN1 regulates mRNA translation by forming RNA granules with FMRP[146], thereby suggesting that defective axonal mRNA translation may contribute to SMA. Indeed, SMN1 positively regulates the translation of candidate plasticity-related gene 15 (*cpg15*; also known as NRN1) mRNA in axons by interacting with HUD, and, remarkably, overexpressing cpg15 partially rescues SMN1 loss-of-function phenotype in zebrafish[12]. Therefore, the regulation of axonal mRNA translation by SMN1 may be required for the maintenance of these axons. ALS is the most common motor neuron disease in adults[147] and is associated with mutations in genes encoding RBPs such as TAR DNA-binding protein 43 (TDP43), RNA-binding protein FUS (FUS), and angiogenin[148,149]. These mutations commonly increase the numbers of stress granules, which reversibly sequester mRNAs and repress their translation[150]. Because sustained translation of axonal

mRNAs is required for axon maintenance[12,117,118], decreased axonal mRNA translation caused by these mutations might contribute to axon degeneration in ALS.

Regulation of axonal mRNA translation

Global translational activity

The rate of protein synthesis in cells is tightly coupled to their nutritional status: cells increase protein synthesis when they receive growth-promoting signals. This cellular homeostasis is regulated by mTOR, which activates translational initiation by phosphorylating its two major substrates 4E-BP1 and S6K[132,133]. Accumulating evidence shows that local protein synthesis in axons and growth cones is also regulated through mTOR. Protein synthesis-inducing cues activate mTOR in a concentration-dependent manner, which results in the asymmetric activation of the protein synthesis machinery within the growth cone[10,53,56,151].

mTOR activity is positively regulated by a GTP-bound form of RHEB GTPase, which is inactivated by its GTPase-activating proteins TSC1 and TSC2 (REFS 132,133). TSC1 and TSC2 are negatively regulated by AKT and ERK1/2 (REFS 132,133). Protein synthesis-inducing cues can activate translation through the PI3K–AKT–mTOR pathway by increasing PI3K or decreasing PTEN activities. For example, netrin 1 activates PI3K and targets PTEN for ubiquitin-mediated proteolysis by activating the E3 ubiquitin-protein ligase NEDD4 (REF. 78), but also through the mitogen-activated protein kinase (MAPK)–TSC2–RHEB–mTOR pathway by activating MAPK ERK1/2 (in cases of netrin 1 (REF. 151), BDNF[50] and SEMA3A[151]). By contrast, ephrin A reduces the activity of ERK1/2, which results in a decreased inhibition of TSC2 and a decreased mTOR activity[152]. This raises the interesting possibility that translational activity in growth cones could be regulated through spatial and temporal integration of diverse receptor-mediated signalling pathways converging on mTOR (FIG. 3). Cell contact-dependent changes in the cytoskeletal network may also regulate translational activity, as the cytoskeleton directly associates with the protein synthetic machinery and regulates mTOR activity[153].

In addition to regulating mTOR, guidance cues may directly regulate ribosomes[26]. DCC (deleted in colorectal carcinoma), a netrin 1 receptor, directly binds to ribosomal protein L5, a component of the 60S ribosomal subunit[30]. Binding of netrin 1 to DCC activates translational initiation and subsequently releases the ribosome–mRNA complex from DCC, thereby allowing more ribosomes to form polysomes in the vicinity of receptor activation. This provides a crucial mechanism for the localized control of mRNA translation near the site of signal activation and may also prevent unnecessary translation in the basal state by sequestering ribosomes. It will be of interest to determine whether other receptors show similar interactions with the translational machinery.

mRNA transport and axonal mRNA repertoire

Axonal transcriptome analyses from diverse neurons — including microarrays studies in *X. laevis,* mouse embryonic retinal ganglion cells[16], rat embryonic and perinatal cortical and hippocampal neurons aged to maturity in culture[18] and rat embryonic and adult peripheral sensory neurons[17], and a sequential analysis of gene expression study in rat perinatal sympathetic neurons[15] — identified thousands of different mRNAs in their axons. Notably, these axons contain common mRNAs, mainly those encoding protein synthesis machinery, mitochondrial proteins and components of the cytoskeleton (TABLE 2). However, some mRNAs were enriched in axons of specific cell types (for example, *Impa1* mRNA is found in peripheral[15,17] but not central[16,18] neuronal axons, and *Creb* mRNAs are found exclusively in peripheral sensory neuronal axons[17,61]). Comparative bioinformatics analyses revealed that specific anterograde transport is likely to account for mRNA enrichment in

axons[15] and growth cones[16]. Axonal transcriptomes change dynamically during development, even in the same axons[16], which suggests that axons may recruit specific and biologically relevant mRNAs using active transport.

The *cis*-acting localization elements that target mRNAs to axons have been identified only for a few mRNAs, such as β-actin mRNA[154,155]. Recent evidence shows that alternative transcription termination produces mRNAs that are targeted to distinct neuronal subcellular compartments (FIG. 4). Notably, a longer 3′-UTR targets *Ranbp1* mRNA to adult sciatic nerve[66], and a distinct 3′-UTR element targets *Impa1* mRNA into developing sympathetic axons in response to NGF stimulation[15]. Furthermore, *cis*-acting elements can be differentially regulated by extrinsic cues. In cultured adult peripheral sensory neurons, for example, NGF, BDNF, NT3, SEMA3A and myelin-associated glycoprotein (MAG) stimulation results in cue-specific mRNA axonal repertoires, thereby suggesting that extrinsic stimuli recruit specific mRNAs[156]. RBPs directly associate with target mRNAs to form transport ribonucleo-protein particles (RNPs), the components of which may directly associate with molecular motors to mediate bidirectional axonal transport on microtubules[157]. For example, the RBP La binds and promotes translation of mRNAs containing the 5′-terminal oligopyrimidine tract (TOP) sequence in their 5′-UTRs[158,159]. In axons, unmodified La interacts with the kinesin motors and mediates anterograde transport of RNPs, but La switches to interact with the retrograde dynein motor upon sumoylation[158]. Short-range RNP movements are likely to be mediated by myosin on actin filaments[53,54,160] and can be regulated by extracellular signals[53,54,156]. RNA granules may also switch between microtubule and microfilament tracks[141]. mRNAs are translationally repressed during transport and remain silent in RNA granules until released for translation[161]. Co-translational transport mechanisms might regulate subcellular localization of mRNAs according to the properties of proteins they encode, as seen in budding yeasts, in which actively translating *ABP140* mRNA is tethered to actin filaments[162]. Upon completion of translation, mRNAs can again associate with different RNA granules, such as transport RNPs, stress granules that sequester non-essential mRNAs upon cellular stress, and processing bodies (P bodies) where mRNAs are stored or degraded[163].

mRNA-specific translation

Protein synthesis-inducing cues increase global translational activity but initiate translation of only a subset of mRNAs. For example, netrin 1 and bdnf, but not en1, induce axonal translation of β-actin mRNA in *X. laevis* retinal axons, whereas all three cues increase global translational activity[53,54,118]. Furthermore, cues that increase global protein synthesis can also repress translation of some mRNAs. For example, en1 increases overall axonal protein synthesis in cultured *X. laevis* retinal axons but decreases the translation of heat shock protein 70 (*hsp70*) mRNA[118]. How different extracellular signals activate the translation of functionally coherent subsets of mRNAs is largely unknown.

Most known mRNA *cis*-acting elements reside in 3′-UTRs, including those in β-actin, *RHOA*, *EPHA2*, cytochrome *c* oxidase subunit IV isoform 1 (*COX4I1*) and *IMPA1* mRNAs[15,23,41,116,154], but they can also be localized to 5′-UTRs (such as that of *KOR1* mRNA)[164], protein coding regions (such as that of *ROBO3* mRNA)[84] and potentially to introns (as found in some dendritically targeted RNAs)[165]. Intron-retaining mRNAs in axons (such as *ROBO3* mRNA)[166] may be prone to nonsense-mediated decay in P bodies, a mechanism that is also regulated by extracellular cues[167].

Trans-acting molecules regulate mRNA transport, stability and translation by reversibly associating with specific mRNAs to form RNA granules[168]. Components of RNA granules, such as RBPs and microRNAs, localize to axons and growth cones, and can be regulated by extrinsic cues. For example, SRC-dependent phosphorylation of ZBP1 (REF. 71) is required

for BDNF-induced β-actin synthesis and growth cone turning[169]. Conformational changes induced by ZBP1 binding[170] or translational de-repression from ZBP1 (REF. 71) may promote the translation of β-actin mRNA.

FMRP is an RBP that recognizes target mRNAs based on their secondary structure[171] and represses their translation. The translational repression by FMRP is relieved by its dephosphorylation by protein phosphatase 2A, which can be activated by neuronal activity[172]. FMRP localizes to axons and growth cones and regulates cue-responsive local mRNA translation in axons[96,97].

Cytoplasmic polyadenylation element binding proteins (CPEB1, CPEB2, CPEB3 and CPEB4) are translational repressors that recognize a specific sequence element[173]. CPEB1 is required for NT3-induced axonal translation of β-catenin mRNA and axon branching in cultured rat hippocampal neurons[174]. Blocking CPEB function by overexpressing a dominant negative mutant impairs elongation of *X. laevis* retinal axons *in vivo*, but knocking down CPEB1 expression does not, suggesting tissue-specific function of CPEB isoforms[175]. These findings are consistent with a recent study using transgenic mice, which showed that CPEB1 inhibits the translation of *Pten* mRNA[176].

MicroRNAs are another class of *trans*-acting elements that could regulate mRNA translation in a sequence-specific manner. Axons and growth cones contain functional RNA interference machinery[55], and microRNAs repress the translation of their target mRNA in RNA granules until stimulated[177]. Recent evidence indicates that a single microRNA can regulate the translation of functionally related mRNAs, as miR-338 inhibits axonal translation of mRNAs encoding COX4I1 (REF. 178) and ATP5G1 (ATP synthase, H^+ transporting, mitochondrial Fo complex, subunit C1 (subunit 9))[117], the components of mitochondrial complexes IV and V, respectively. These translational regulators are likely to interact, as FMRP associates with ribosomes[179], microRNAs[180], and other RBPs such as SMN1 (REF. 146).

Finally, receptor–ribosome interactions could potentially regulate mRNA-specific translation. Expression of individual ribosomal proteins shows tissue specificity[181], which suggests that variable compositions of ribosomes may exist. Intriguingly, each composition may preferentially translate specific mRNAs, as mutant mice that are hypomorphic for the ribosomal protein L22 show specific defects in translation of related *Hox* mRNAs[181]. Considering that guidance cue receptors may regulate mRNA translation by sequestering ribosomes[26], it is tantalizing to speculate that different extracellular signals might release different ribosomes that are pre-tuned to translate a specific subset of mRNAs. It is intriguing that axons and growth cones contain mRNAs encoding most ribosomal proteins[15–18,156], which themselves are regulated by mTOR through their 5′-TOP sequences[159], and there is evidence that at least some ribosomal proteins can be exchanged in the cytoplasm[182]. Thus, axons might be able to fine-tune mRNA selectivity by dynamically modifying ribosomal compositions using locally synthesized ribosomal proteins.

Summary and future directions

Local mRNA translation is widely used to maintain subcellular autonomy in axons and dendrites. Here, we have focused our attention on various stimulus-driven axonal responses, such as growth cone chemotropic responses and axon regeneration, but evidence also suggests that local mRNA translation might contribute to the maintenance of the steady state of the local proteome in the distal axons[9]. Axonal mRNA translation occurs during development and in adulthood, but the ability of axons to activate translational machinery is closely associated with their ability to regenerate. Axons store neuronal subtype-specific and

age-specific repertoires of local mRNAs, and translation of a specific subset of mRNAs is likely to determine the nature of extrinsic cue-induced axonal responses (FIG. 5).

Recent advances in compartmentalized cell culture techniques[36,183–185] (BOX 1) have provided tools to study local mRNA translation in axons, and several candidate-based approaches have revealed crucial insights into the role of axonal mRNA translation. The unexpected complexity of axonal transcriptomes[15–18] indicates that many biological pathways are post-transcriptionally regulated in axons. In order to understand the roles that local mRNA translation may have *in vivo*, it will be important to investigate which of these axonally localized mRNAs are translated. It is possible to isolate axonally translating mRNAs by affinity purification and axonally synthesized proteins by metabolic labelling (BOX 1), although the minute amounts of these mRNAs and proteins makes it technically challenging. Genome-wide correlative analyses of axonal transcriptomes and translatomes in the context of extrinsic cue stimulation would provide insights into both axonal mRNA translation and cue-mRNA specificity.

Another question to investigate is whether the complex axonal mRNA repertoire is a property of individual axons or a collective property of diverse axons. Transcriptome analyses have shown that axons of similar origins (such as dorsal root ganglia neurons, sympathetic ganglia neurons or retinal ganglion cells) contain thousands of different mRNAs, whereas immunocytological and ultrastructural evidence indicates that few polysomes are present in individual axons. Information regarding the number and distribution of mRNAs and protein synthesis machinery at the single axon level is needed to improve our basic understanding of how mRNA translation is regulated in axons and to gain insights into the puzzling paucity of polysomes and the apparent unconventional nature of the axonal ER and Golgi apparatus.

Although candidate studies have identified several *cis*-acting elements that regulate axonal mRNA localization, our knowledge about how specific mRNAs are selected for axonal transport is limited. Most screening studies have used microarray techniques to identify axonally localizing mRNAs, followed by rapid amplification of cDNA ends of individual candidates to explore whether UTR diversity mediates selective axonal mRNA transport. Recent advances in RNA sequencing technologies will provide new opportunities for unbiased genome-wide screens for novel regulatory *cis*-acting elements that are not limited to the UTRs.

Recent evidence using transgenic mice bearing a local translational reporter (BOX 1) demonstrated that axonal mRNA translation occurs *in vivo*[186], and the studies using genetically modified mice with compromised functions of axonally localizing translational regulators[12,13,72,99,174] revealed potential roles that axonal mRNA translation may play *in vivo*. However, direct evidence linking axonal mRNA translation to a specific function *in vivo* is still scarce, because it is technically challenging to inhibit mRNA translation specifically in axons in intact animals. One approach to address this point in candidate genes is to introduce targeted mutations to known axon-localization elements in the UTRs, which would result in normal protein expression but the specific loss of axonal mRNA localization and translation. This is analogous to an *in vitro* study, in which an axon-targeted isoform of *Impa1* mRNA was selectively targeted by siRNA[15]. Another candidate approach involves delivering caged translation-blocking antisense oligonucleotides, which can be activated by local light stimulation in axons[187], to specific neurons. These methods may provide ways to inhibit the translation of a specific mRNA in axons in live animals. Intriguingly, a novel method to inhibit translation of all mRNAs in presynaptic terminals in mouse brain slices was recently developed: an inactive chemically inducible, genetically encoded translational inhibitor was expressed in a specific subset of neurons and the activating chemical was

locally applied to their axons[62,188]. Imaginative new approaches along these lines that facilitate axon-specific inhibition of mRNA translation *in vivo* are needed to fully uncover the role of local synthesis in nervous system assembly, maintenance and repair.

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Glossary

Polysomes	Strings of 80S ribosomes bound to mRNA molecules.
Ribosomes	Large RNA–protein complexes (80S) at which mRNA translation occurs. They contain 4 rRNAs and more than 79 proteins and are composed of a large (60S) subunit and a small (40S) subunit.
RNA-binding protein (RBP)	A protein that binds RNAs. Most RBPs have modular structures containing specific RNA-binding domains, catalytic domains and/or protein-binding domains.
Small nuclear ribonucleoproteins (snRNPs)	Complexes that are composed of a small nuclear RNA and a specific set of proteins.
RNA granules	Intermediate RNA–protein complexes that regulate RNA transport, translation and degradation. RNA granules include transport ribonucleoproteins, stress granules and processing bodies.
Stress granules	Dense cytosolic proteins and RNA aggregations that appear under conditions of cellular stress. The RNA molecules are thought to be stalled translation pre-initiation complexes.
MicroRNAs	Non-coding RNA molecules of 21–24 nucleotides in length that inhibit mRNA expression.

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labelling, these studies showed that vertebrate and invertebrate axons without somas are capable of translation-dependent protein synthesis.

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Box 1

Methods used to understand axonal mRNA translation and transport

Axonal mRNA isolation and transcriptome analysis

The accurate description of subcellular compartment-specific mRNA repertoires[16–18] provides invaluable information regarding axonal mRNA transport and translation. The axons of cultured neurons are separated from their cell bodies using compartmentalized culture systems[15,17,18] or laser-capture microdissection[16]. The Campenot chamber (see the figure, part **a**)[185,189], made of a Teflon divider attached to a petri dish, has two compartments with distinct fluid environments. The proximal compartment contains cell bodies, dendrites and proximal axons, whereas the distal compartment contains distal axons. Typically, the distal compartment is supplemented with nerve growth factor (NGF), which promotes the growth of peripheral sensory[61] and sympathetic neurons[15]. The microfluidic culture platform (see the figure, part **b**)[18,183] has two mirror-imaged compartments. Dissociated neurons are added to the somal compartment, and axons grow into the axonal compartment through microgrooves. In laser-capture microdissection (see the figure, part **c**)[16], cultured neurons labelled with a fluorescent lipophilic dye are fixed, and then axons or growth cones (indicated by white arrows in the figure) are microdissected individually. Because the amount of RNA obtained is minute, amplification is required before microarray analysis. This method, however, enables subcellular compartment-specific comparison, which is not possible in compartmentalized culture systems.

Quantitative immunofluorescence

The relative amount of a specific protein in cultured axons can be measured using quantitative immunofluorescence. Cultured axons without somas are treated with a protein synthesis-inducing cue with or without translational inhibitors and then fixed. The protein of interest is visualized by immunofluorescence. Background-subtracted average fluorescent intensity per unit area in axons or growth cones represents the relative protein amount[10]. An increase in protein quantity in axons without somas that is blocked by a translational inhibitor probably results from axonal mRNA translation.

Live imaging of translational reporter fluorescence

For mRNAs whose *cis*-acting translational regulatory element is known, translation can be visualized in real-time using a fluorescent reporter whose translation is regulated by the same *cis*-acting element. Diffusion-limited and short-lived fluorescent proteins are common translational reporters and their appearance indicates local mRNA translation[61,186]. Another class of reporter molecules is photoconvertible fluorescent proteins: the appearance of new fluorescent proteins after irreversible photoconversion of pre-existing proteins indicates local mRNA translation[53,190].

Visualization of mRNAs and RNA-binding proteins

An mRNA can be directly visualized by delivery of *in vitro*-transcribed mRNAs that have incorporated fluorescent nucleotides[191] into neurons by electroporation or microinjection. Alternatively, mRNAs can be visualized indirectly using the sequence-specific interaction of MS2 bacteriophage RNA hairpin and capsid protein (known as MS2 tagging)[191]. An mRNA fused to the MS2 binding site is co-expressed with a fluorescent MS2 protein, and the mRNA is visualized by MS2 fluorescence in axons[192]. MS2 tagging enables endogenous mRNAs to be labelled by generating MS2 binding site-containing knock-in mice[193]. RNA-binding proteins fused to a fluorescent protein can also be visualized[53]. Extracellular cue-induced changes in localization of mRNAs and RNA-binding proteins can be visualized in real-time or after fixation, and are commonly

quantified by examining the behaviours of individual fluorescent particles or the distribution of total fluorescent particles[53].

Unbiased screening of *de novo* proteome or translatome

De novo proteins in axons that do not have somas or glia in culture can be metabolically labelled (for example, using radioactive methionine), separated by two-dimensional electrophoresis, and identified by mass spectrometry. A challenge is to obtain sufficient amounts of axonally synthesized proteins, which are estimated to account for less than 5% of total protein synthesis in cultured sympathetic neurons[194]. Recently developed click chemistry enables extremely sensitive labelling of newly synthesized proteins[195] and was successfully applied to visualize axonally synthesized proteins[118]. Alternatively, the translatome can be described[196]. Axonally translating mRNAs can be isolated by immunoprecipitating ribosomes and associated mRNAs specifically from axons[118]. For example, a tagged ribosomal protein can be specifically expressed in the eye, and ribosome–mRNA complexes can be isolated from the brain, in which the only source of a tagged ribosome is the retinal axons[118].

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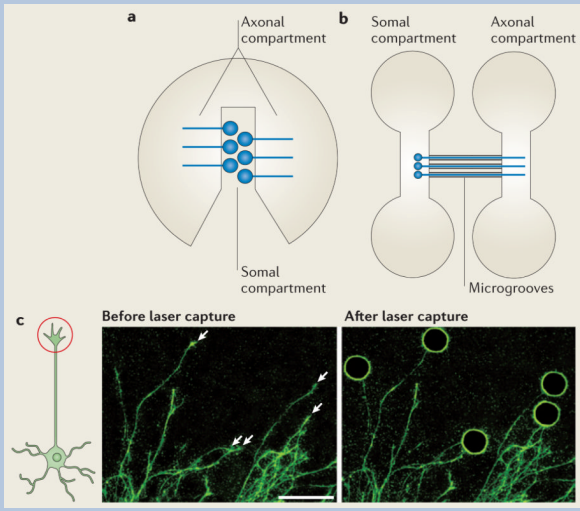

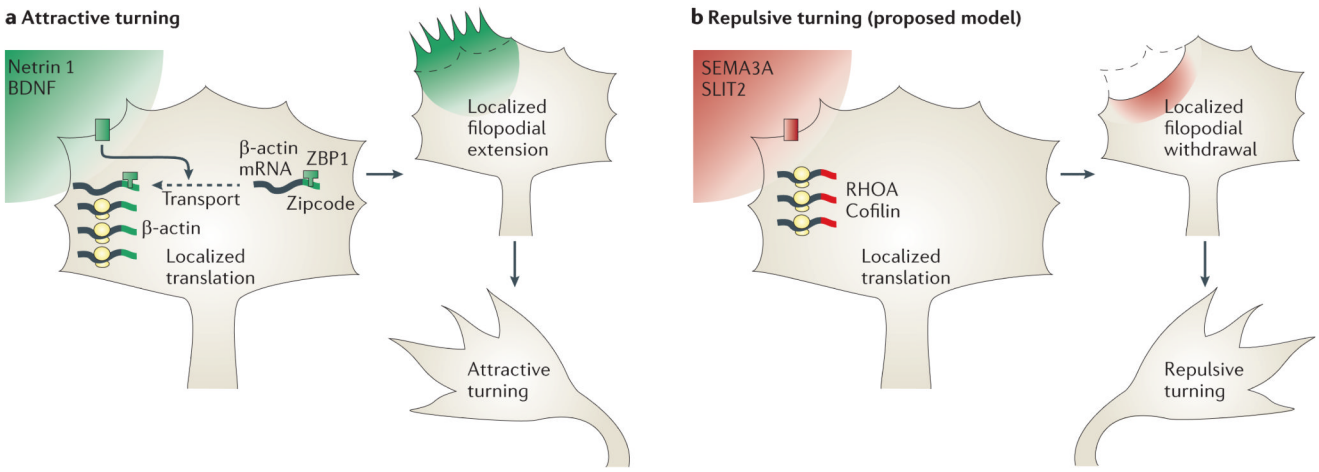

Figure 1. Growth cone turning regulated by differential mRNA translation

Gradients of protein synthesis-inducing guidance cues commonly activate global translational activity on the side of the growth cone nearest to the gradient by activating mammalian target of rapamycin (mTOR). However, the specific mRNA translated in response to the cue differs depending on whether it is an attractive or repulsive cue and determines the direction of growth cone turning. **a** | Stimulation by attractive cues, such as netrin 1 and brain-derived neurotrophic factor (BDNF), leads to asymmetric synthesis of β-actin on the side near to the source of the gradient, which is mediated by β-actin mRNA transport to this region by zipcode-binding protein 1 (ZBP1)[53,54]. Spatially restricted synthesis of β-actin may lead to actin polymerization, cytoskeletal assembly and attractive turning of the growth cone. **b** | By contrast, repulsive cues, such as semaphorin 3A (SEMA3A) and SLIT2, activate the axonal translation of the actin-depolymerizing proteins RHOA[55] and cofilin[56] when uniformly applied in cell culture. A proposed model is shown, in which localized cytoskeletal disassembly may result in repulsive turning through polarized filopodial collapse. However, whether these molecules are translated asymmetrically in a repulsive gradient has not yet been tested.

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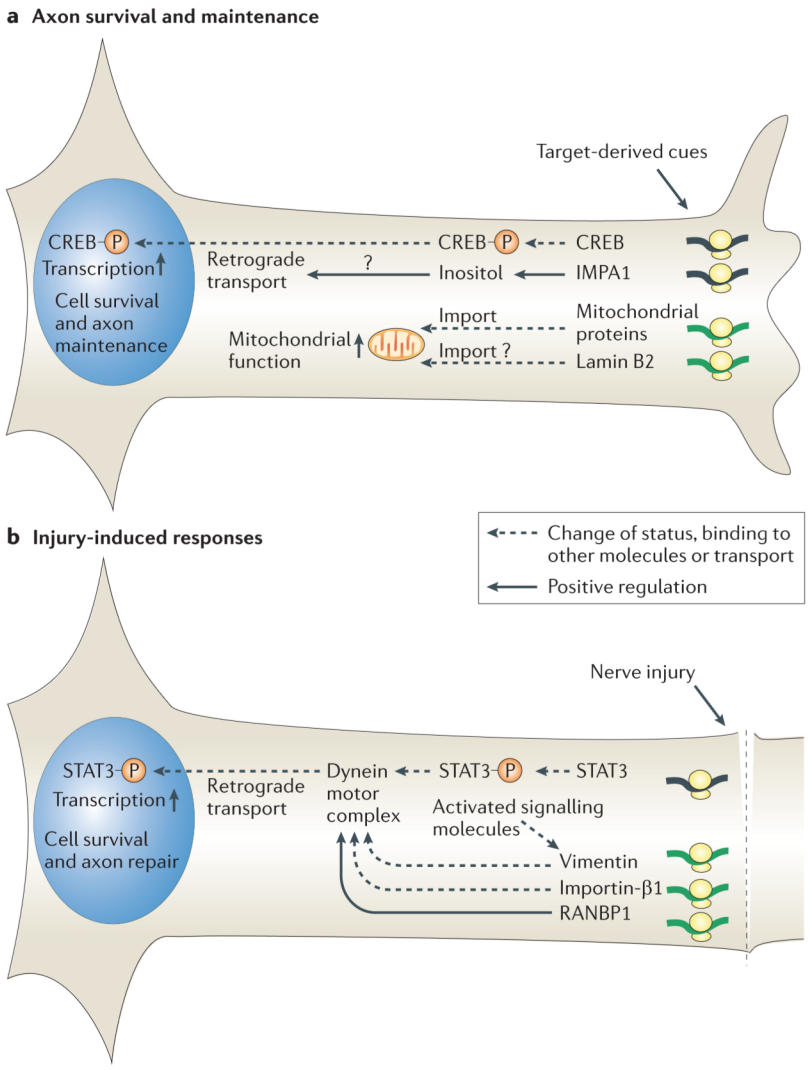

Figure 2. Axon survival, maintenance and injury-induced responses regulated by local protein synthesis

a | Distal axons receive target-derived trophic factors. These target-derived cues activate local synthesis of mitochondrial, signalling or nuclear proteins required for axon maintenance and cell survival. Stimulation of axons with nerve growth factor (NGF) results in local synthesis of cyclic AMP responsive element-binding protein (CREB), which is then locally phosphorylated[61]. This active form of axonal CREB is transported into the nucleus and is required for axon survival in cultured sensory neurons[61]. NGF also stimulates the axonal synthesis of inositol monophosphatase 1 (IMPA1), an enzyme that regulates the inositol cycle. Axonally synthesized IMPA1 may regulate retrogradely transporting vesicles and is required for axon survival in cultured sympathetic neurons[15]. Distal axons also synthesize nuclear-encoded mitochondrial proteins, and sustained local synthesis and mitochondrial import of such proteins are required for axon maintenance in cultured neurons[117]. Lamin B2, a known nuclear envelope component, is also axonally synthesized and localized to mitochondria in distal axons, and sustained axonal synthesis of lamin B2 is required for axon maintenance *in vitro* and *in vivo*[118]. **b** | Nerve injury generates a retrograde survival and/or repair signal, and local protein synthesis is required for its generation and relay to the nucleus. The retrograde dynein motor complex is inactivated by

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Ran GTPase in normal conditions. Nerve injury stimulates local synthesis of Ran-specific GTPase-activating protein (RANBP1), which displaces Ran from the dynein motor[66]. Importin-β1 is also locally synthesized in response to nerve injury and binds to the Ran-free dynein motor complex, which results in the activated dynein motor complex[64]. Transcription factors, such as signal transducer and activator of transcription 3 (STAT3), are also locally synthesized and activated in injured axons and bind to the activated dynein motor complex using the nuclear localization signal[14]. A type III intermediate filament vimentin is also locally synthesized in injured axons and co-transported with other signalling molecules that bind to it[65].

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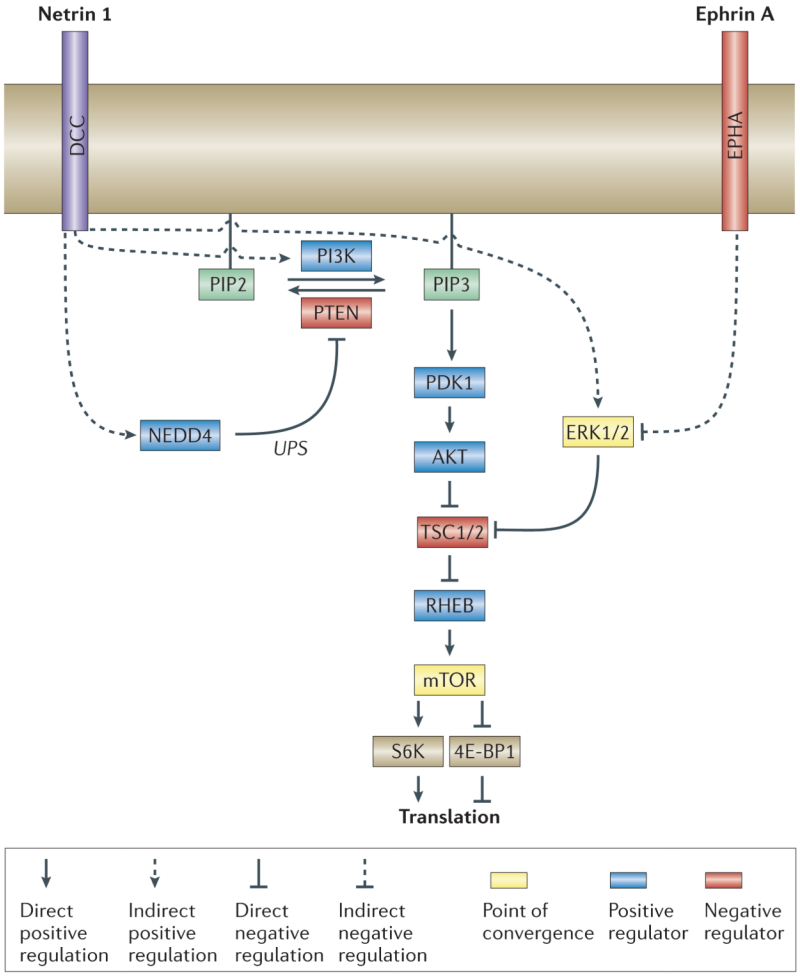

Figure 3. Regulation of global translational activity through mTOR

Cues that induce and inhibit protein synthesis antagonistically regulate the activity of mammalian target of rapamycin (mTOR), which regulates cap-dependent mRNA translation by phosphorylating its two major targets: eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1) and ribosomal protein S6 kinase (S6K). Protein synthesis-inducing cues, such as netrin 1 or brain-derived neurotrophic factor (BDNF), may increase mTOR activity through the AKT–mTOR pathway by activating phosphoinositide 3-kinase (PI3K)[10] or by promoting ubiquitin–proteasome system (UPS)-mediated degradation of phosphatase and tensin homologue (PTEN)[78], or through the mitogen-activated protein kinase (MAPK)–mTOR pathway by activating MAPK extracellular signal-regulated kinase 1 and 2 (ERK1/2)[50,151] that inhibits the mTOR negative regulators tuberous sclerosis protein 1 (TSC1) and TSC2. Some cues, such as ephrin A, can inhibit protein synthesis by inhibiting ERK1/2 leading to TSC1/2 activation and mTOR inhibition[152]. Spatiotemporal summation of cue-induced signals converging on mTOR might lead to asymmetric translational activity. DCC, deleted in colorectal carcinoma (netrin 1 receptor); PDK1, 3-phosphoinositide-dependent protein kinase 1; PIP2, phosphatidylinositol-4,5-bisphosphate (also known as PtdIns(4,5)P_2); PIP3, phosphatidylinositol-3,4,5-triphosphate (also known as PtdIns(3,4,5)P_3).

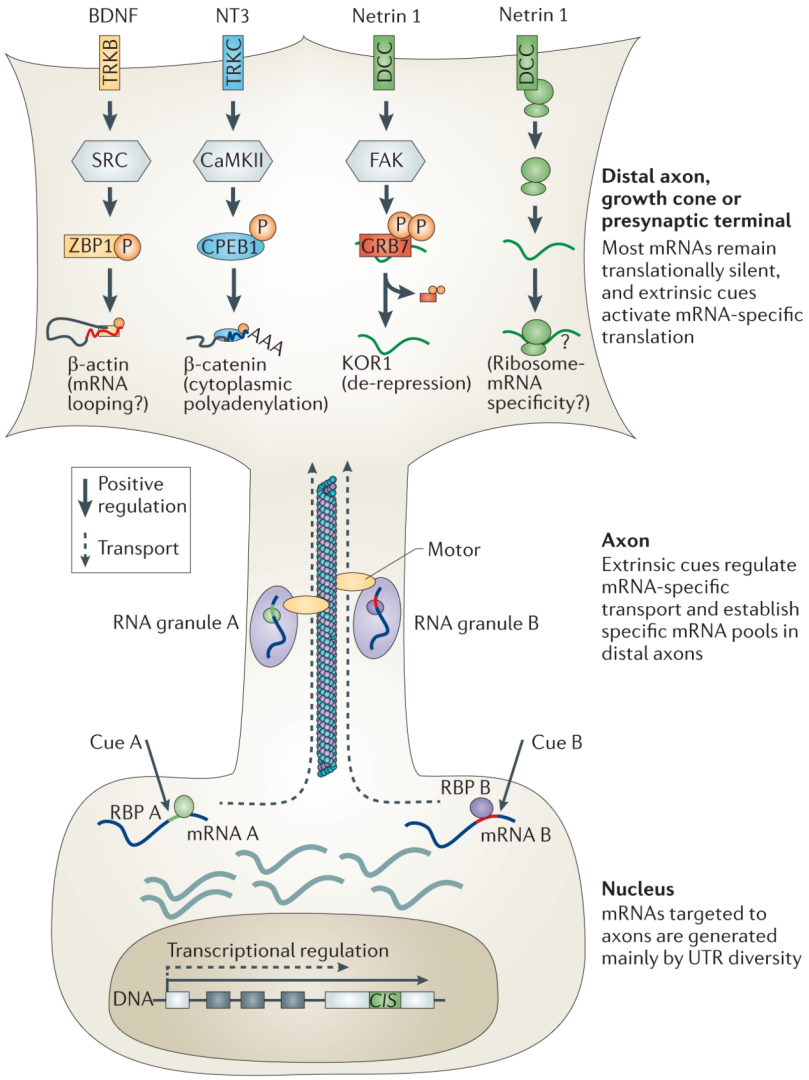

Figure 4. RNA-specific transport and translation

Axonal targeting of mRNAs is directed by *cis*-acting elements that are mainly localized to the 3′-untranslated regions (UTRs) of mRNAs. Retention of these axon-targeting *cis*-acting elements is commonly regulated by the use of different transcriptional termination sites[15,66]. Extrinsic cues influence axonal mRNA repertoires by promoting transport of specific mRNAs[156]. Axonally targeted mRNAs are recruited to RNA granules (transport ribonucleoproteins (RNPs)) by specific RNA-binding proteins (RBPs) and are transported along microtubules probably by kinesin motors[157]. mRNAs remain translationally silent during transport[21]. Extracellular signals activate the translation of specific mRNAs mainly by regulating RBPs. For example, neurotrophins and guidance cues activate the kinases SRC[71], calcium/calmodulin-dependent protein kinase II (CaMKII)[174] and focal adhesion kinase (FAK)[164], which phosphorylate the RBPs, zipcode binding protein 1 (ZBP1), cytoplasmic polyadenylation element binding protein (CPEB1), and growth factor receptor-bound protein 7 (GRB7), respectively. Cell surface receptors might regulate mRNA-specific translation by directly regulating ribosomes. For example, unstimulated netrin receptor DCC directly binds to ribosomes and inhibits translation[26], and ribosome composition influences mRNA selectivity[181]. Different receptors may bind to ribosomes that are pre-tuned to

specific mRNAs, and ligand stimulation might release such ribosomes and result in mRNA-specific translation. BDNF, brain-derived neurotrophic factor; KOR1, κ-type opioid receptor; NT3, neurotrophin 3; TRK, tyrosine kinase receptor.

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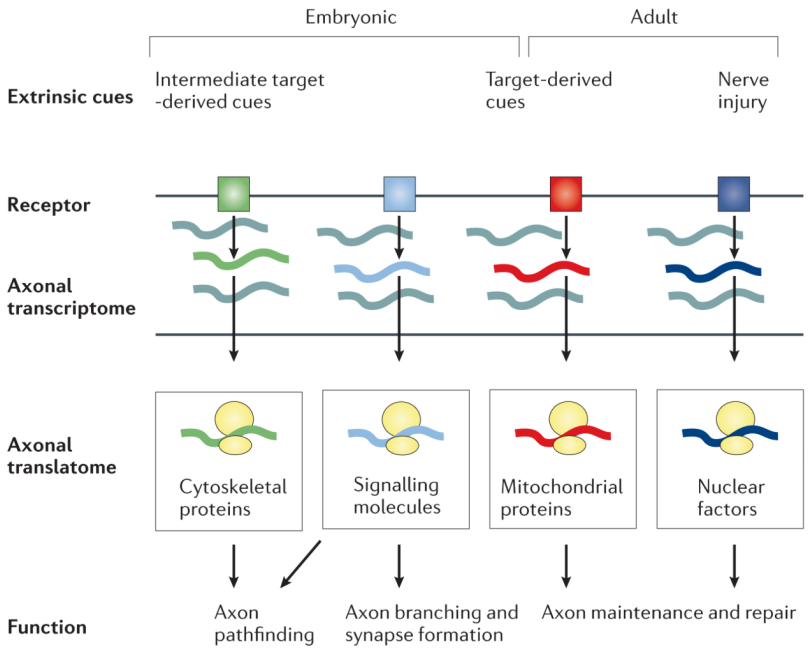

Figure 5. Local mRNA translation as a mediator of stimulus-induced axonal responses

A proposed model for the function and mechanism of axonal mRNA translation. Neuronal axons contain a complex and dynamic transcriptome, and many mRNAs remain translationally silent. Various extrinsic cues stimulate translation of a distinct subset of mRNAs during development and in adulthood. For example, guidance cues induce local synthesis of cytoskeletal proteins in growing axons and regulate axon guidance and branching. Target-derived trophic factors promote local synthesis of proteins required for mitochondrial function and support the survival of distal axons. Nerve injury in adulthood stimulates local synthesis of nuclear factors that activate repair mechanisms.

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Table 1

Signals that regulate axonal mRNA translation

Signal	Neuronal type	Target mRNA	Regulator	Function	Refs
Signals that increase axonal protein synthesis					
Netrin 1	RGC *	β-actin	ZBP1	GC turning	53
	P19	KOR1	GRB7	Analgesia	164
	DRG ‡	PAR3	Unknown	Axon elongation	52
Semaphorin 3A	DRG ‡	RHOA	Unknown	GC collapse	55
	DRG ‡	Calreticulin	Unknown	Axon regeneration	156
SLIT2B	RGC *	Cofilin	Unknown	GC collapse	56
Nerve growth factor	PC12	Unknown	Unknown	Neurite regeneration	114
	PC12	RPL4	Unknown	Neurite regeneration	197
	Sympathetic §	β-actin, ADF, NF	Unknown	Axon guidance ¶	194
	Sympathetic ‡	IMPA1	Unknown	Axon maintenance	15
	Hippocampal ‡, CTX ‡	β-actin	Unknown	Axon guidance ¶	63
	DRG ‡	β-actin	Unknown	Axon regeneration	156
	DRG ‡	CREB	Unknown	Cell survival	61
	DRG ‡	PAR3	Unknown	Axon elongation	52
	SCG ‡	COX4I1	miR-338	Axon maintenance	116,178
	SCG ‡	ATP5G1	miR-338	Axon maintenance	198
	PC12	GAP43	HUD	Axon guidance ¶	103
	SMN ‡	CPG15	SMN and HUD	Axon maintenance	12
Brain-derived neurotrophic factor	Spinal cord *	β-actin	ZBP1	GC turning	54
	Hippocampal ‡, CTX ‡	β-actin	ZBP1	Axon guidance ¶	63
	DRG ‡	β-actin	ZBP1	Axon regeneration	156
	Spinal cord ‡, SMN ‡	CPG15	SMN and HUD	Axon maintenance	12
Neurotrophin 3	Hippocampal ‡	β-catenin	CPEB1	Axon branching	174
	DRG ‡	β-actin	ZBP1	Axon regeneration	156
	DRG ‡, CTX ‡	Calreticulin	Unknown	Axon guidance	155
Pituitary adenylate cyclase-activating polypeptide	Spinal cord *	Unknown	Unknown	GC turning	60
5-hydroxytryptamine (serotonin)	Sensory //	eEF1A	Unknown	Long-term facilitation	199
Myelin-associated glycoprotein	DRG ‡	αB crystallin	Unknown	Axon regeneration	156
	DRG ‡, CTX ‡	Calreticulin	Unknown	Axon guidance ¶	155
Engrailed 1 and 2	RGC *‡§	Unknown	Unknown	Topographic mapping	58,59
	RGC *	Lamin B2	Unknown	Axon maintenance	118

Signal	Neuronal type	Target mRNA	Regulator	Function	Refs
	Midbrain dopaminergic‡	COX1 components	Unknown	Cell survival	57
Axotomy	DRG‡	Importin-β	Unknown	Axon regeneration	64
	DRG‡	Vimentin	Unknown	Axon regeneration	45,65
	DRG‡	STAT3A	Unknown	Axon regeneration	14
	DRG‡	NF-L, NF-M, NF-H	Unknown	Axon regeneration	200
Differentiation	P19	Tau	Unknown	Axon elongation	201
Unknown	OSN‡	Odorant receptors	Unknown	Axon regeneration	128
Unknown	*Caenorhabditis elegans*	C/EBP1	MAPK	Axon regeneration	130
Depolarization	DRG‡	KOR1	Unknown	Analgesia	42
Inflammation	DRG‡	TRPV1¶	Unknown	Hyperalgesia	134
Injury	DRG‡	Nav1.8	Unknown	Hyperalgesia	135,137
Signals that do not increase axonal protein synthesis					
Ephrin B	RGC*	Not applicable	Not applicable	Not applicable	67
Lysophosphatidic acid	RGC*	Not applicable	Not applicable	Not applicable	10
Signals that decrease axonal protein synthesis					
Ephrin A	RGC‡	Unknown	ERK1/2 and TSC1/2	Axon guidance	152

ADF, actin depolymerizing factor; ATP5G1, ATP synthase, H^+ transporting, mitochondrial Fo complex, subunit C1 (subunit 9); C/EBP1, CAAT enhancer binding protein 1; COX1, cyclooxygenase 1; COX4I1, cytochrome c oxidase subunit IV isoform 1; CPEB1, cytoplasmic polyadenylation element binding protein 1; CPG15, translation of candidate plasticity-related gene 15 (also known as NRN1); CREB, cyclic AMP responsive element-binding protein; CTX, cerebral cortical; DRG, dorsal root ganglion; eEF1A, eukaryotic elongation factor 1α; ERK, extracellular signal-regulated kinase; GAP43, growth associated protein 43; GC, growth cone; GRB7, growth factor receptor-bound protein 7; HUD, Hu-antigen D (also known as ELAVL4); IMPA1, inositol monophosphatase 1; KOR1, κ-type opioid receptor; MAPK, mitogen-activated protein kinase; miR, microRNA; Nav1.8, sodium channel 1.8; NF (L, M, H), neurofilament protein (light, medium, heavy); OSN, olfactory sensory; PAR3, proteinase-activated receptor 3; RGC, retinal ganglion cell; RPL4, ribosomal protein L4; SCG, superior cervical ganglion; SMN, spinal motor neuron; STAT3A, signal transducer and activator of transcription 3A; TRPV1, transient receptor potential cation channel subfamily V member 1; TSC, tuberous sclerosis protein; ZBP1, zipcode binding protein 1.

**Xenopus laevis*.

‡Mouse or rat.

§Chicken.

‖Aplaysia.

¶Indicates that the finding was not directly addressed in the study.

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Table 2

Selected functional categories of axonally enriched mRNAs

Neuronal type	Subcellular compartment	Protein synthesis	Mitochondrial	Cytoskeletal	Cell–cell signalling	Ref.
Rat						
Embryonic SCG	Axons	Yes	Yes	Yes	No	64
Embryonic DRG	Axons	Yes	Yes	Yes	No	65
Adult DRG	Young growth cones	Yes	Yes	Yes	Yes	65
Neonatal cerebral cortical	Axons (cultured to maturity)	Yes	Yes	Yes	No	66
Xenopus laevis						
Embryonic RGC	Young growth cones	Yes	Yes	Yes	No	67
Embryonic RGC	Old growth cones	Yes	Yes	Yes	Yes	67
Mouse						
Embryonic RGC	Growth cones	Yes	Yes	Yes	No	67

DRG, dorsal root ganglion; RGC, retinal ganglion cell; SCG, superior cervical ganglion.