Trends in Cell & Molecular Biology
Vol. 10, 2015

Review

Subcellular compartmentalization of neuronal RNAs: An overview

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ABSTRACT

The neuron is a highly polarized cell that processes and transmits signals among different components of neural circuitry. This coordinated signaling process relies, in part, on the complexity and relative abundance of the proteins in the different structural and functional domains of the neuron: dendrites, axons and presynaptic nerve terminals. Previously, it was hypothesized that the local proteome of each domain is maintained by the transport of proteins synthesized in the parental soma. However, accumulating evidence indicates that a variety of RNA molecules (protein-coding and non-coding) are transported and localized to specific neuronal compartments, where they regulate many fundamental neuronal functions. Furthermore, the compartment-specific targeting of mRNAs and their local translation plays important regulatory roles in neuronal processes including synapse formation and dendritogenesis, as well as axonal guidance and plasticity. Here, we review current knowledge of mRNA localization, the functional significance of local protein synthesis in neuronal subcellular domains and examine the regulation of mRNA transport and expression in various animal model systems.

KEYWORDS: mRNAs, microRNAs, axon, dendrite, local protein synthesis, translational regulation, mRNA trafficking, RNA intercellular transfer, RNA-binding proteins

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1. Introduction

The axon, dendrite and synaptic nerve endings are highly specialized domains of the neuron, which receive and process information locally with a high degree of autonomy from the cell body. For many years, however, it was assumed that the proteome of these subcellular compartments were maintained through the transport of proteins from the perikaryon. This view was initially supported and perpetuated by evidence from two key scientific discoveries: (1) the identification of nuclear DNA as the 'undisputed repository of protein primary structure and mRNA as its molecular envoy' and (2) the absence of Nissl bodies (i.e., ribosomes) and other protein synthetic machinery (i.e., Golgi apparatus and endoplasmic reticulum) in the distal structural and functional domains of the neuron [1]. Implicitly, the lack of Nissl bodies was equated to the lack of ribosomes and therefore the lack of RNA and protein synthesis. Together, this evidence reinforced the neuronal perikarya central dogma and further convinced investigators that the cell soma was the exclusive site for the synthesis of all proteins expressed in the neuron.

It bears note, however, that during the evolution of this conceptualization of basic neuronal cell function, evidence for the presence of ribosomes in the distal domains of the neuron was already extant in the literature. These early findings were derived from the use of electron microscopy and spectroscopic imaging techniques [2] that facilitated the visualization of 'ergastoplasm' (i.e., aggregates of ribosomes and rough endoplasmic reticulum)

and polyribosomes in the proximal dendrites [3, 4] and ribosomal RNA and intact ribosomes in axons. In the axons, these data were acquired from both invertebrate and vertebrate model systems [5, 6] and were confirmed by immunocytochemical studies [7]. Molecular markers concentrated in these subcortical, ribosome-rich axonal domains included β-actin mRNA, ZBP-1 protein, myosin Va and kinesin motor proteins. As such, these intermittent peri-axoplasmic domains (i.e., plaques) were thought to provide the structural basis for the local protein synthetic system [for review, see 8]. Biochemical evidence for the presence of a heterogeneous population of mRNAs, as well as biologically active polysomes in the axons and nerve terminals of invertebrate animal species was also present in the early literature [9-13]. A diverse population of mRNAs and a local protein synthetic system were also reported to exist in dendritic growth cones of cultured hippocampal neurons, and the relative abundances of these mRNAs were developmentally regulated [14]. Based on these findings, Crino and Eberwine hypothesized that the translation of these localized mRNAs contributed to the regulation of growth cone motility and dendrite outgrowth [14].

In addition to the above considerations, the failure to identify the endoplasmic reticulum (ER) and Golgi apparatus in axons also raised the question of whether axons could modify and process newly synthesized proteins, especially integral membrane and transmembrane proteins. In this regard, it is noteworthy that it was first shown by immuno-histochemistry that several protein components of the translational machinery are present within dendrites [15-17]. Recently, it has also been shown that functional equivalents of ER and Golgi are present in the axons of central and peripheral sensory neurons [18], and that axons can synthesize and insert functional neurotransmitter receptors into the axolemma [19].

Intuitively, the idea that the soma provided all the proteins for its peripheral compartments also manifested several theoretical difficulties. Most notable, the rate of axoplasmic flow was insufficient to account for effective delivery and maintenance of many proteins needed for the subcellular domains to carry out their specific functions and/or signaling responses [for discussion, see 20, 21]. This incongruence was further underscored by the fact that the combined cytoplasmic volume of the distal processes of the neuron is often far greater than that of the cell soma. Moreover, this issue raised the fundamental question: how does the cell body metabolically support and replenish the proteins in its peripheral compartments which are often located long distances from the cell soma and are often highly ramified? Studies, as early as 1960, suggested that the cell body satisfied this delivery requirement through anterograde transport. However, due to the length and asymmetry of the neuron, it was clear that the cell body-dependence theory did not adequately address the phenomenon.

Based on these empirical and theoretical considerations, it comes as somewhat of a surprise that the concept of local protein synthesis was so highly contested for such a long period of time. The localization of mRNA and its local translation in the peripheral compartments of the neuron provides significant advantages for these large, highly asymmetric cells. First, it is energetically favorable, since many copies of a protein can be translated from one mRNA, providing the domains with a locally renewable source of proteins. Second, local translation can rapidly provide a host of new proteins on demand at multiple subcellular sites for ‘immediate’ use. Third, translationally silent mRNA can be stored locally for long periods of time by mechanisms that allow moment-to-moment (real-time) regulation of the local protein composition in response to impinging external stimuli.

It is now clear that local translation of mRNAs plays a key role in the development of the neuron and function of the subcellular domains including axon guidance, synthesis of membrane receptors, axon regeneration, synapse formation, activity-dependent synaptic plasticity and long-term memory formation [for reviews, see 1, 22, 23]. Consequently, the phenomenon of RNA localization and local protein synthesis raises several fundamental cell biological questions: (1) How many and which RNAs are present in the distal processes? (2) What is the physiological function of the local translation of specific mRNA transcripts? (3) What mechanisms underlie the selective docking/localization of mRNAs to the

appropriate/specific subcellular compartments? (4) What regulatory controls are at play and how are they dependent on neuronal activity and extracellular signals?

In this article, we will review some of the key findings supporting local translation in the distal reaches of the neuron and address the exciting reports regarding its regulation. The roles/ implications of local mRNA translation in development, growth, regeneration and memory will also be discussed. The role of RNA may not be limited to the cells where it is synthesized and therefore, we will also address the intercellular transfer of RNA and the implication of these findings in regard to intercellular communication. In closing, we will address the methodological issues and experimental limitations that exist in the field and raise some fundamental questions for future directions for this rapidly growing field of research.

2. Identification and function of mRNAs in the distal processes of the neuron

2.1. mRNAs in dendrites

Steward and Levy's [4] identification of polyribosomes present at the base of dendritic spines markedly altered the thinking about local protein synthesis in the synaptodendritic neuronal domains. This novel observation led to a flurry of experiments aimed at illustrating the role of local protein synthesis in the function of dendrites. Using metabolic labeling experiments, the Steward and Davis groups were among the first to demonstrate *de novo* synthesis of proteins and the possibility of RNA transport to the dendrites of cultured hippocampal neurons [4, 24]. Following these findings, *in-situ* hybridization studies further demonstrated the presence of specific mRNAs localized to the dendrites [25, 26]. Subsequent transcriptome analyses of purified dendritic and/or synaptic compartments have generated lists of localized mRNAs numbering in the range of hundreds to thousands [27, 28]. These mRNAs encode a variety of proteins, including receptors, cytoskeletal elements, signaling molecules, integral membrane and translation regulatory proteins. This complex dendritic mRNA population suggests that specific biological processes are modulated through regulation of dendritic biology [29]. Most importantly, local translation of mRNAs in the dendrites is regulated in a synaptic activity-dependent manner allowing for the local modification of the postsynaptic densities [30, 31]. Taken together, these observations provide strong evidence supporting the belief that the dendrite is a semi-autonomous cellular compartment of the neuron. In this section, we will highlight some examples of well-studied dendritically localized mRNAs (Table 1) whose translation modulates dendritic spine shape, density and electrophysiological characteristics: processes that underlie the ability of the dendrite to receive and integrate presynaptic information.

2.1.1. Structural, receptor and cytoplasmic proteins

Among the dendritically localized mRNAs, the most intensively studied structural receptor and cytoplasmic proteins include β-actin [32, 57], microtubule-associated protein 2 (MAP2) [25, 58], microtubule-associated protein 1B (MAP1B), the activity-regulated cytoskeletal-associated protein (Arc) [59, 60], *N*-methyl-*D*-aspartate (NMDA) receptor, shank1 [36], tyrosine-related kinase B receptor (TrkB) [38], the inositol triphosphate receptor (InsP3) [61], Ca^{2+}/calmodulin-dependent protein kinase II alpha subunit (CaMKIIα) [26, 27] and brain-derived neurotropic factor (BDNF) [38, 62]. The localization of these mRNAs has been implicated in functions that contribute to dendrite growth, spine morphology and synaptic plasticity. Worthy of note, some of these structural and receptor proteins may work in combination to regulate dendritic function. In this regard, it has been proposed that dendritically synthesized CAMKIIα, shank1, InsP3 receptor and Arc proteins are integral for the formation of the multimolecular receptor-like structure termed the NMDAR complex (NRC) [63; for review, see 31], which is thought to promote the translocation of ribosomes from the base of dendritic spines to the spine head and closer to the postsynaptic density itself [64].

Local protein synthesis influences the distribution of β-actin, a major cytoskeletal component of dendritic spines [65]. Dendritic spines are small actin-rich protrusions that receive excitatory input from the presynaptic terminals and are the major sites of information processing in the neuron (Figure 1a). Live imaging studies of spine dynamics have also

Table 1. mRNAs in dendrites. Summary of mRNAs identified and enumerated in the dendrites of cultured neurons and/or *in vivo* at the individual level. Arc, activity-regulated cytoskeletal-associated protein; BDNF, brain-derived neurotropic factor; CaMKIIα, Ca^{2+}/calmodulin-dependent protein kinase II alpha subunit; CREB, cyclic AMP response element-binding protein; eEF1α, eukaryotic elongation factor 1α; Elk-1, E-26-like protein 1; FMR1, fragile X mental retardation 1; GluR, glutamate receptor 1 and 2; InsP3, inositol 1,4,5 triphosphate; LIMK-1, LIM domain kinase 1; LTD, long-term depression; LTP, long-term potentiation; MAP1B, microtubule-associated protein 1; MAP2, microtubule-associated protein 2; NMDAR, *N*-methyl-D-aspartate receptor; PKMζ, atypical protein kinase M zeta; Shank1, SH3 and multiple ankyrin repeat domains protein 1; TrkB, tyrosine-related kinase B receptor; *Zif* 268, zinc finger transcription factor. *Indicates mRNAs that have been identified but not studied at the individual level. $^{\Psi}$Indicates the finding was not addressed in this review. $^{\delta}$Indicates function was not directly addressed in the study.

Type of mRNA	Regulator(s)	Function	Species	Tissues
Structural and receptor				
β-actin	ZBP1	Dendrite spine growth$^{\delta}$	Rat	*Hippocampal* [32]
MAP2	CPEB1	Microtubule growth and assembly$^{\delta}$	Rat	*Hippocampal* [25, 33]
MAP1B	eEF2K	Dendrite morphology and density	Mouse	*Hippocampal* [34]
GluR1 and GluR2	Unknown	Neurotransmitter signalling$^{\delta}$	Rat	*Hippocampal* [35]
Shank1	Unknown	Scaffolding protein, Synaptic morphology$^{\delta}$	Rat	*Hippocampal and Purkinje Cells* [36]
NMDAR	Unknown	Synaptic transmission; LTP and LTD$^{\delta}$	Rat	*Hippocampal Neurons* [37]
TrkB receptor	Unknown	Protein secretion; Actin dynamics	Rat	*Hippocampal* [38, 39]
InsP3 receptor	Unknown	Cell signaling$^{\delta}$	Mouse	*Purkinje Cells* [40]
Arc	Unknown	Synaptic modifications and plasticity	Rat	*Hippocampal* [41, 42]
Protein kinases				
PKMζ	Unknown	Long-term facilitation, Dendrite spine length and maturation	Rat	*Cortical Neurons* [43]
LIMK-1	miR-134	Actin polymerization; Dendrite morphology and synaptic function; Spine structure	Mouse	*Hippocampal* [44]; *Hippocampal* [45-47]
Transcription and translation factors				
eEF1α	Unknown	Peptide synthesis, LTP and LTD	Rat, Mouse	*Hippocampal* [48, 49]
CREB	Unknown	Transcription regulator$^{\delta}$	Rat	*Hippocampal* [50]
zif 268*	Unknown	Transcription regulator$^{\delta}$	Rat	*Hippocampal* [50]
Elk-1	Unknown	Transcriptional regulation, Cell apoptosis	Rat	*Hippocampal* [51]
Others				
BDNF	eEF2K	Dendritic spine morphology	Rat	*Hippocampal* [52]
CaMKIIα	CPEB	Synaptic plasticity and LTP$^{\delta}$	Rat	*Hippocampal* [53, 54]
Sensorin	Unknown	Neurotransmitter$^{\delta}$	*Aplysia*	*Sensory Neurons* [55]
FMR1$^{\Psi}$	Unknown	Synaptic and cytoskeletal structure	Rat	*Hippocampal* [56]

revealed that the morphology of spines can be altered by neuronal activity *in vitro* and *in vivo*, suggesting that changes in dendritic spine morphology play an important role in synaptic plasticity and memory formation [66-68]. Therefore, it is no surprise that the localization and expression of β-actin mRNA in dendrites is correlated with spine shape and size. β-actin mRNA delivery to the dendrites is dependent on neuron activity and external stimulation [57], and is mediated by sequences in the 3' untranslated region (3'UTR) of β-actin mRNA. In fact, downregulation of β-actin mRNA and protein reduces the formation and maturation of dendritic spines, which indicates the importance of local β-actin mRNA translation in the developing dendrite [for review, see 69, 70].

In the context of long-term potentiation (LTP), a cellular mechanism thought to underlie learning and memory, an increase in the local synthesis of β-actin in dendrites contributes to synaptic modifications such as changes in spine head distribution and morphology, further indicating that regulation of β-actin mRNA expression plays an important role in spine synaptic plasticity and memory formation in the mature dendrite [69, 71]. Taken together, it can be asserted that long-lasting forms of activity-dependent synaptic plasticity, such as LTP, may require local protein synthesis within the dendrites.

The localization of MAP2, Arc and TrkB mRNAs is also essential for dendrite morphology and stability. During neuritogenesis and in response to neuronal activity, MAP2 mRNA localizes to the somatodendritic compartments in neurons, where its local translation promotes microtubule (MT) growth and assembly. Inhibition of MAP2 production by the addition of MAP2-antisense oligonucleotides in culture [72] or MAP2-deficient cultured neurons [73] resulted in the inhibition of neurite formation and reduction in MT density in dendrites. The expression of Arc mRNA in dendrites is also regulated by neuronal activity, physiological induction of LTP and long-term depression (LTD), as well as growth factors (e.g. BDNF). Link and colleagues demonstrated that the induction of LTP *in vivo* leads to dramatic increases in the expression of Arc mRNA and protein in the dendrites [74]. The decrease in Arc mRNA and protein in dendrites alters spine density and/or morphology and may also influence synaptic plasticity and LTP-dependent memory formation. Consistent with the findings from the Link group, Steward and colleagues have recently shown that newly synthesized Arc mRNA accumulates at activated synapses *in vivo* [42]. In addition, they also observed that synaptic activity also triggered Arc mRNA degradation in inactive dendritic domains. In a similar manner, enrichment of dendritic localization of mRNAs encoding the BDNF receptor TrkB, is also observed in response to neuronal activity. The local expression of TrkB could also play a crucial role in LTP at the synapse by mediating the release of secretory proteins and regulating actin dynamics in dendritic spines [38, 39].

The mRNA encoding the alpha subunit of CaMKII is also dendritically localized and translated in response to synaptic activation. Neural activity promotes the delivery of CaMKIIα mRNA to dendrites of hippocampal and cortical neurons, where it is rapidly translated [26, 75]. The CaMKIIα mRNA contains a localization element within the 3'UTR that is necessary for dendritic localization [76, 77]. The localization and translation of CaMKIIα is also important for LTP at synapses and memory consolidation. For example, in animal behavior studies, LTP induction in the hippocampus causes rapid accumulation of CaMKIIα mRNA in the dendrites and synaptic sites [78, 79]. *In vivo* disruption of CaMKIIα mRNA localization to the dendrites resulted in a dramatic reduction of CaMKIIα in the postsynaptic densities (PSDs) [77]. Most importantly, physiological and behavioral experiments conducted with these mutant mice showed deficits in LTP at hippocampal synapses and impairments of hippocampus-dependent memories, as evidenced by associative fear conditioning and object recognition tests [77, 80]. This finding is in accordance with studies using granule cells of mouse olfactory bulb. In mice devoid of CaMKIIα 3'UTR, Néant-Fery and colleagues demonstrated that dendritic localization of CaMKIIα mRNA is disrupted in the olfactory bulb and olfactory associative learning was severely impaired [81]. It will be interesting to determine

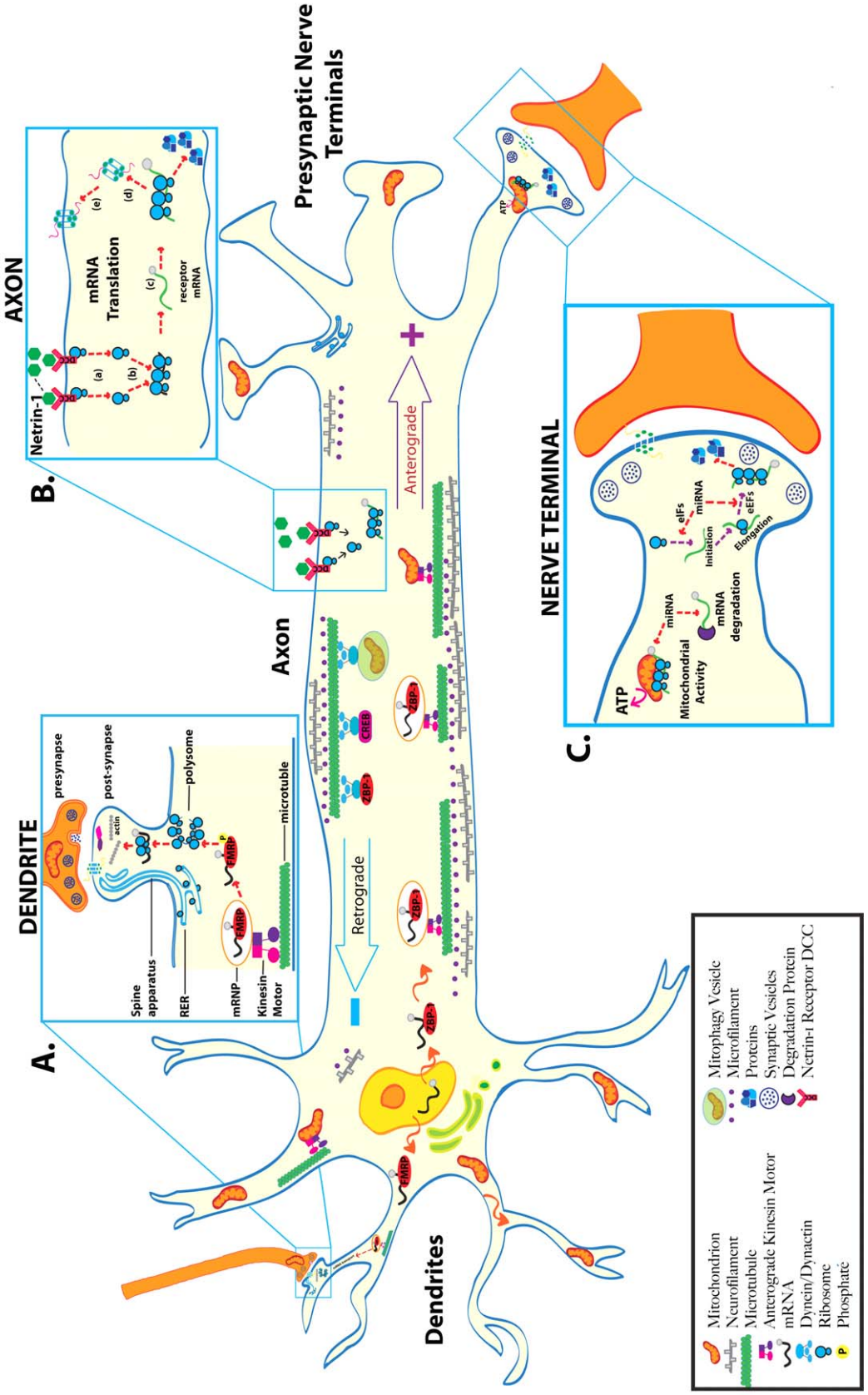
A.
DENDRITE
presynapse
post-synapse
polysome
actin
Spine apparatus
RER
mRNP
Kinesin Motor
microtuble
FMRP
B.
AXON
Netrin-1
mRNA Translation
receptor mRNA
(a)
(b)
(c)
(d)
(e)
Presynaptic Nerve Terminals
Axon
Retrograde
Anterograde
CREB
ZBP-1
Dendrites
C.
NERVE TERMINAL
ATP
Mitochondrial Activity
miRNA
mRNA degradation
eIFs
eEFs
Initiation
Elongation
Mitochondrion
Neurofilament
Microtubule
Anterograde Kinesin Motor
mRNA
Dynein/Dynactin
Ribosome
Phosphate
Mitophagy Vesicle
Microfilament
Proteins
Synaptic Vesicles
Degradation Protein
Netrin-1 Receptor DCC

Figure 1

whether the local translation of CaMKIIα mRNA in dendrites of other neuronal cell types will reveal functional modalities consistent with the above studies. Overall, from these studies, one can generalize that localization and translation of structural, receptor and cytoplasmic dendritic mRNAs are an essential determinant of nervous system assembly and long-term synaptic plasticity and that disruption of their localization and expression may have implications for cognitive function.

2.1.2. Protein kinase mRNAs

In addition to the localization of mRNAs coding for synaptodendritic structural, receptor and cytoplasmic proteins, various protein kinase mRNAs have also been identified in the synaptodendritic domain. Two well-studied protein kinase mRNAs include atypical protein kinase M zeta (PKMζ) and LIM kinase protein 1 (LIMK-1). While the mechanism by which PKMζ regulates neuronal plasticity and the process of memory remains to be elucidated, there is ample evidence suggesting roles for PKMζ in the regulation of dendritic spine structure, function, and long-term synaptic plasticity [for review, see 82]. Overexpression of exogenous PKMζ in rat cortical neurons grown in dissociated cultures resulted in reduced dendritic spine length and maturation [43]. Additionally, it has been proposed that PKMζ upregulates the trafficking of GluR2-containing AMPA receptors into postsynaptic sites, which may augment synaptic efficacy during neuronal activity [83, 84]. Serine protein kinase LIMK-1 localizes to dendritic growth cones during development. In the growth cones, LIMK-1 regulates actin polymerization by phosphorylating its substrates, actin-depolymerizing factor (ADF) and cofilin 1 [44]. In neurons, cofilin 1/ADF is required for proper actin turnover and the morphology of dendritic spines. Inhibition of LIMK-1 causes inhibition of cofilin 1 activity and results in altered dendritic spine morphology and synaptic function [45, 85]. Consistent with the structural and electrophysiological deficits, LIMK-1 knockout mice exhibited abnormalities in behavior, including altered fear responses and spatial learning [86].

2.1.3. Translation machinery proteins

Neuronal activity facilitates long-lasting changes in synaptic structure and function by regulating mRNA translation in dendrites. These activity-dependent events promote the synthesis of proteins integral to spine stability, synaptic modifications and synaptic plasticity. There is evidence to suggest that dendrite-localized mRNAs that encode components of the translational machinery (notably eEF1α) are involved in this process.

Legend to Figure 1. Subcellular transport and translation of mRNAs. Regulated translation and localization of mRNAs allow polarized cells like the neuron to spatially and temporally regulate gene expression. The site-specific targeting of mRNAs to the neuronal subcellular domains is regulated by *cis*-acting elements that are often localized to the 3' and 5' untranslated regions (UTRs) of the mRNA. These localization elements are recognized by *trans*-acting RNA-binding proteins (RBPs). RBPs directly associate to their specific mRNA targets to form ribonucleoprotein particles (RNPs), which are then transported to the distal compartments along microtubules by the anterograde kinesin molecular motor complex. mRNAs are transported in a translationally repressed state by the RBPs. **A. DENDRITE:** This distal domain contains all the cellular machinery necessary to synthesize proteins including ribosomes [4]. The presence of these components along with RBPs allows mRNAs to be transported and translated in the dendrites. **B. AXON:** Extracellular signals also activate the translation of mRNAs in the distal axons. The signaling of cell surface receptors by extracellular cues may also regulate the translation of mRNAs in the axon by directly regulating ribosomal activity [151]. In the inactivated state, the netrin-1 receptor DCC binds the ribosomes and thereby regulates translation **(a)**. After inactivation by netrin-1, DCC releases the ribosomes **(b)**, which are then made available to locally synthesize proteins **(c)** [151, 152]. In addition, the axons can synthesize receptor proteins and insert functional receptors **(d-e)** into the axolemma [111]. **C. NERVE TERMINAL:** In this inset, emphasis is placed on miRNA regulation of local translation. Neuronal miRNAs function at multiple levels within the neuronal gene expression system to modulate neuronal activity and function. It has been shown that miRNAs regulate the local post-transcriptional gene expression of specific target mRNAs that encode factors affecting mitochondrial activity, as well as axonal growth and branching. In addition, miRNAs can also modulate the translation of multiple mRNAs in the axon and nerve terminal by regulating local expression of eukaryotic translation factors (i.e., eEFs and elFs). Last, miRNA control of the local synthesis of cytoskeletal and/or motor proteins might facilitate the regulation of their own anterograde transport to their ultimate sites of function.

In vivo studies conducted by Steward and colleagues demonstrated that the mRNA for eukaryotic elongation factor 1 alpha (eEF1α) is present in the dendrites of neurons that exhibit LTP and LTD, and that eEF1α translation is locally regulated [48]. The mechanism by which eEF1α promotes mRNA translation has been elucidated. eEF1α facilitates the GTP-dependent binding of aminoacyl-tRNA to the ribosome during peptide synthesis and elongation. Therefore, the local synthesis of this component of the translational machinery in the dendrites provides an elegant mechanism for regulating overall translational capacity at dendrosynaptic sites [87]. Interestingly, however, since eEF1α is an elongation factor, one may expect that increases in eEF1α protein levels in the dendrites would signal a dramatic increase in local protein synthesis, but Huang and colleagues [48] did not observe detectable increases in overall translational activity (as measured by ^{3}H-leucine incorporation using autoradiographic techniques). This observation suggests that the increase in eEF1α protein levels may be important for something other than regulating translational capacity [48], such as regulating the translation of a select subset of proteins.

2.1.4. Transcription factor proteins

The local synthesis of transcription factor proteins in dendrites in response to synaptic activation and their subsequent transport to the nucleus provides an elegant mechanism that would allow the neuron to regulate gene expression, a process the Eberwine group termed 'dendritic imprinting' [for review, see 29]. Indeed, during neuronal differentiation, it has been shown that transcription factor proteins are locally synthesized, post-transcriptionally modified in the dendrites and transported to the nucleus, providing a direct signaling pathway between the distal dendrite and the nucleus [50]. Using radiolabeled anti-sense RNA and *in-situ* hybridization techniques, Eberwine and colleagues demonstrated that mRNAs encoding cyclic AMP response element-binding protein (CREB) and zinc finger transcription factor (*zif*) 268 (also known as egr-1) are localized to dendrites of rat hippocampal neurons and is locally synthesized in dendrites severed from their cell bodies [50]. *De novo* synthesized CREB is also translocated to the nucleus. Importantly, using an *in-situ* Southwestern assay these investigators demonstrated that dendritically synthesized CREB protein also interacts with the *cis*-acting cyclic response element (CRE) DNA sequence. This observation raises the possibility that dendritically synthesized CREB may influence transcription in the nucleus. Like CREB, E-26-like protein 1 (Elk-1) mRNA localizes to the dendrites and its translation product might be transported to the nucleus where it influences the transcription of various target genes. Interestingly, the introduction of Elk-1 mRNA into intact rat primary neurons and its subsequent translation in the dendrites caused cell apoptosis, whereas the translation of Elk-1 mRNA in cell bodies did not produce cell death [50]. Together, these findings suggest an important role for dendritically synthesized transcription factors in regulating neuronal function and viability at the level of transcription. In addition to the activity-dependent regulation of mRNAs, some dendritic mRNAs are also regulated during development. In this regard, some mRNAs localize to dendritic growth cones of developing neurons, but are absent in dendrites of mature neurons [14]. For example, Berry and Brown demonstrated that calmodulin (CaM I) mRNA is transiently targeted to the dendrites during early development of cortical and hippocampal pyramidal neurons, but is absent in mature neurons [88]. Additionally, it is important to note that most dendritic mRNAs that have been assessed, including CaMKIIα and Arc mRNA, are present at low levels during development and increase in abundance as dendrites mature. In contrast, EF1α mRNA was found to be present at high levels in developing dendrites but decreases with age [48]. Intuitively, the fact that some mRNAs, like EF1α, CREB and CaM I mRNA, are present at high levels in developing dendrites invites the speculation that local translation of such mRNAs plays an integral role in synaptic development. Therefore, identifying novel developmentally regulated dendritic mRNAs and the implications of their translation is a high priority, because they may also play a key role in synaptic plasticity in the adult nervous system.

2.2. mRNAs in axons

Consistent with the observations made in dendrites, there is a large number of gene transcripts localized in the axonal domain (nerve terminals

included) of the neuron (Table 2). Early observations, using RNA-cDNA hybridization analysis and shot-gun cDNA cloning of RNA derived from invertebrate model systems, indicated that the axon contained approximately 200-400 different mRNAs [9, 13]. More recent DNA microarray analyses have shown that the axon contains several hundred different mRNAs [89-91]. The list confirms the early observations of the presence of β-actin, β-tubulin and neurofilament mRNAs [92-94], as well as the mRNA encoding the heavy chain of kinesin in the squid giant axon [95]. Furthermore, these axonal mRNAs were also associated with polysomes, indicating that they were translationally active in the squid axon [10, 96]. Recent deep sequencing studies have revealed that growing axons contain between 1,000-4,500 mRNAs [89] in comparison to the dendrites where investigators suggest the presence of about 2,550 mRNA transcripts [91]. Early in development, the growth cones of Xenopus laevis retinal ganglion cells (RGCs) contain primarily mRNAs encoding translation machinery and cytoskeleton elements, while in later developmental stages, the growth cones harbor a more complex set of transcripts, including mRNAs encoding synaptogenesis-related proteins. For example, there is evidence to suggest that Ephrin B4 receptor mRNA is only localized to older growth cones even though its transcription is not altered during this development period [91]. Some of the mRNAs are compartment-specific. For example, tau and GAP43 mRNA selectively localizes to the axon but appears excluded from dendrites [97-99]. Alternatively, MAP2 and neuro-transmitter receptor subunits localize only to dendrites [23, 25, 100]. In addition, there are other mRNAs that are cell type-specific. For example, IMPA1 mRNA is found in peripheral [90, 101], but not central [102] neuronal axons and CREB mRNA is found exclusively in peripheral and sensory neuronal axons [90]. It would be interesting to further analyze the developmental switches that alter mRNA targeting and translational capacity as axons grow and mature.

2.2.1. Axon growth and turning

2.2.1.1. Structural proteins

Growing axons require extrinsic and/or guidance signals to regulate their growth and navigation to reach their distant synaptic partners [110, 141]. In developing neurons, axons receive continuous guidance information from their environment that is processed by a specialized structure known as the growth cone located at the distal tip of the growing neuron. The axonal growth cone is a highly motile structure that drives axon elongation and pathfinding by quickly processing information autonomously. Extracellular cues direct growth cones by inducing rapid changes in local protein expression, and developing axons contain the necessary translational machinery and specific mRNAs to support local protein synthesis. Thus, the developing axon strongly depends on the local synthesis and organization of structural and cytoskeletal proteins. Indeed, the proteins coordinating or comprising the cytoskeletal elements are locally synthesized and contribute to the growth cones path finding and turning apparatus [141]. For example, some of the first identified functions of axon protein synthesis were intricately linked to its ability to mediate local translation of mRNAs that encode cytoskeletal proteins [92, 93, 95].

Pertaining to cytoskeletal proteins, several studies using *Xenopus laevis* RGCs and rat dorsal root ganglion neurons (DRGs) have specified a role for the local translation of some of the well-studied axonally localized cytoskeletal proteins such as β-actin [57, 103], RhoA [136], ADF/cofilin [134], GAP43 [121] and MAP1B [107]. It is important to note that these mRNAs were elucidated in response to cue-induced axon guidance; such cues include Netrin-1, Semaphorin 3A (Sema3A), SLIT2, engrailed 1 and engrailed 2 (EN-1 and EN-2), pituitary adenylate cyclase activating polypeptide (PACAP), nerve growth factor (NGF), BDNF, and neurotrophin (NT3). It has also been shown that these extrinsic cues also promote the local synthesis of guidance receptor mRNAs such as Eph receptor B4 [91] and EphA2 [110]. While many aspects of this cue-induced turning remain to be elucidated (for instance, how does receptor activation promote mRNA recruitment and translation?), it is clear that extracellular guidance cues and growth factors are integral to developing and regenerating axons, and may also be critical in the wiring of neural circuits. These guidance cues have also been used to develop differential translation models for studying local protein synthesis in growing axons.

Table 2. mRNAs in Axons. Summary of mRNAs identified and enumerated in the axons of cultured neurons, invertebrate axons and/or *in vivo* at the individual level. ADF, actin depolymerizing factor; Atf4, activating transcription factor 4; ATP5G1, ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit C1 (subunit 9); CamKIIα, Ca^{2+}/calmodulin-dependent protein kinase II alpha subunit; CEBP-1, CCAAT/enhancer-binding protein; COX1, cyclooxygenase 1; COXIV, cytochrome c oxidase subunit IV isoform; CPEB1, cytoplasmic polyadenylation element binding protein 1; CPG15, Candidate plasticity-related gene 15 (also known as NRN1); CREB, cyclic AMP response element-binding protein; DRG, dorsal root ganglion; eEF1A, eukaryotic elongation factor 1α; EphA2, Ephrin type A receptor 2; Eph B4, Ephrin type B receptor 4; eIF2B2, eukaryotic initiation factor 2 beta 2; eIF4G2, eukaryotic initiation factor 4 gamma 2; GAP43, growth-associated protein 43; G/CPR-2, G-protein–coupled conopressin receptor; Hsp70; Heat shock protein 70 kilodalton; HuD, Hu-antigen D (also known as ELAV-L4); IMPA1, inositol monophosphatase 1; κOR; κappa opioid receptor; LIMK-1, LIM domain kinase 1; MAPK, mitogen-activated protein kinase; MAP1B, microtubule-associated protein 1 B; miR, microRNA; Nav 1.8, voltage-gated sodium channel; NF-H, neurofilament heavy chain; NF-L, neurofilament light chain; NF-M, neurofilament medium chain; PTEN, phosphatase and tensin homolog; RanBP1, Ran-specific binding protein 1; RGC, retinal ganglion cell; RPL4, ribosomal protein L4; ROS, reactive oxygen species; SMN, spinal motor neuron; STAT3, signal transducer and activator of transcription 3A; TrkB, tyrosine-related kinase B receptor; Trpv1, transient receptor potential vanilloid 1; ZBP1, zip-code binding protein 1. *Indicates mRNAs that have been identified but not studied at the individual level. $^{\Psi}$Indicates the finding was not discussed in this review. $^{\delta}$Indicates function was not directly addressed in the study.

Type of mRNA	Regulator	Function	Species	Tissue
Cytoskeletal and structural proteins				
β-actin	ZBP1	Axon guidance; GC turning	Rat	*Hippocampal* [103]; *RGC* [104]; *Spinal Cord* [105]
α and β tubulin	Unknown	Cytoskeletal membrane $^{\delta}$	Squid	*Giant Axon* [93]
Vimentin	Unknown	Axon regeneration and retrograde transport	Rat	*DRG* [106]
β-spectrin$^{\Psi}$	Unknown	Axon regeneration $^{\delta}$	Squid	*Photoreceptor Neurons* [12]
MAP1B	FMRP	Axon growth, Microtubule polymerization and stability	Rat	*PC 12 Cells* [107]
Tau	Unknown	Axon growth and elongation	N/A	*P19 Embryonic Cells* [108]
NF-L, NF-M, NF-H	Unknown	Axon regeneration and repair	Rat	*DRG* [109]
Membrane receptor proteins				
EphB4 receptor	Unknown	Axon guidance $^{\delta}$	Rat	*Hippocampal Neurons* [91]
EphA2 receptor	Unknown	Axon guidance	Chick	*Spinal Cord* [110]
G/CPR-2	Unknown	Neurotransmitter signaling	*Lymnaea stagnalis*	*Fresh Water Mollusc Lymnaea stagnalis Neurons* [111]
κOR	Unknown	Analgesia $^{\delta}$	Mouse, Rat	*DRG* [112]
Trpv1$^{\Psi}$	Unknown	Hyperalgesia	Rat	*DRG* [113]
Molecular motors				
Kinesin	Unknown	Molecular motor $^{\delta}$	Squid	*Giant Axon* [95]
MAP H1$^{\Psi}$	Unknown	Retrograde motor $^{\delta}$	Squid	*Giant Axon* [94]

Table 2 continued..

Ribosome-associated proteins				
S5,S6,S15,S16,S19,S29*[Ψ]	Unknown	Translation machinery [δ]	*Aplysia*	*Sensory Neurons* [13]
L7A, L8, L9, L11, L18 *[Ψ]	Unknown	Unknown	*Aplysia*	*Sensory Neurons* [13]
L22, L31, L36, L37 *[Ψ]	Unknown	Unknown	*Aplysia*	*Sensory Neurons* [13]
RPL4	Unknown	Neurite regeneration	Rat	*DRG; PC12 Cells* [114]
Transcription and translation factors				
eEIF1α	Unknown	Long-term-facilitation	Aplysia	*Sensory Neurons* [115]
Atf4	Unknown	Axon regeneration, Cell apoptosis	Rat	*Hippocampal* [116]
elF2B2	miR-16	Axon growth	Rat	*SCG* [117]
elF4G2	miR-16	Axon growth	Rat	*SCG* [117]
CREB	Unknown	Cell survival	Rat	*DRG* [118]
STAT3A	Unknown	Axon regeneration	Rat	*DRG* [119]
CEBP-1[Ψ]	MAPK	Axon regeneration	*C. elegans*	*Touch and Motor Neurons* [120]
Mitochondrial proteins and lamins				
COXIV-isoform 1	miR-338	Axon maintenance, Mitochondrial activity, ROS levels	Rat	*SCG* [121-124]
ATP5G1	miR-338	ATP synthesis	Rat	*SCG* [125]
Lamin B2	Unknown	Axon maintenance	*Xenopus laevis*	*RGC* [126]
COX1 components[Ψ]	Unknown	Cell survival	Mouse, Rat	*Midbrain Dopaminergic Neurons* [127]
Others				
Nav 1.8[Ψ]	Unknown	Hyperalgesia	Rat	*DRG* [128]
BDNF				
GAP43	ZBP1; HuD-ZBP1	Axon regeneration and repair	Rat	*DRG* [129]; *Adult Sciatic Nerve* [130]
PTEN	Unknown	Axon regeneration	Rat	Primary Cortical Neurons [131]
IMPA1	Unknown	Axon maintenance	Rat	*SCG* [101]
Importin-β-1	Unknown	Axon regeneration and repair	Rat	*DRG* [132]
β-catenin	CPEB1	Axon branching, Vesicle release	Rat	*Hippocampal* [133]
ADF/Cofilin	Unknown	GC collapse, Axon guidance	*Xenopus laevis*	*RGC* [134]

Table 2 continued..

β-thymosin	Unknown	Neurite elongation	*Lymnaea stagnalis*	*Lymnaea stagnalis* [135]
RhoA	Unknown	GC collapse/repulsion	Rat	*DRG* [136]
LIMK-1	Unknown	Actin polymerization, Axon guidance	*Xenopus laevis*	*RGC* [137]
CPG15	SMN1 and HuD	Axon branching	Mouse	*Spinal cord, SMN* [138]
*Hsp*70$^{\Psi}$	Unknown	Stress response$^{\delta}$	Squid; Rat	*Optic Lobe* [11]; *DRG* [139]
RanBP1	Unknown	Retrograde signaling, Axon regeneration	Rat	*Peripheral Sensory Neurons, DRG* [140]

Among the many mRNAs known to localize in the growth cones of developing axons, β-actin mRNA is the best characterized [57, 93, 136]. Reminiscent of the findings in the squid giant axon, β-actin mRNA is also found in the axons and growth cones of *Xenopus laevis* RGCs. Interestingly, in response to polarized netrin-1 gradients, β-actin mRNA is enriched and locally translated in the growth cones of developing RGCs [142]. Furthermore, local protein synthesis in the growth cones appears to be important for both repulsive and attractive guidance mechanisms. In RGC axons severed from their somas, the distal axon grows positively towards the netrin-1 gradient and negatively away from Sema3A gradients [104].

In addition to the localization and translation of β-actin mRNAs in the axons, the mRNAs for several actin-binding proteins (ABPs) are also translated in developing axons [143]. RhoA mRNA, which codes for a GTPase that regulates the growth of actin cytoskeleton, is localized to the growth cones of developing neurons. Wu and colleagues demonstrated that the axonal localization and translation of RhoA mRNA occurs in chemotropic response to Sema3A stimulation, and is necessary for growth cone collapse [136]. Furthermore, the 'negative' chemotrophic signals, SLIT2B [134, 144] and Sema3A gradients do not elicit β-actin synthesis, but instead induce the rapid axonal synthesis of proteins such as ADF/cofilin and RhoA that promote β-actin disassembly and collapse of the growth cones in culture. These findings are congruent with studies conducted using fibroblasts, which showed that β-actin mRNA localization and protein synthesis determines lampellipodia polarity and motility [145, 146]. In addition to ADF/cofilin and RhoA, the β-thymosin protein also negatively regulates actin polymerization. β-thymosin mRNA localizes to the growth cones of cultured *Aplysia* sensory neurons [13], and is locally synthesized in cultured *Lymnaea stagnalis* neurons where it regulates axon growth by preventing actin polymerization and formation of filamentous actin [135, 147]. Furthermore, van Kesteren and colleagues showed that double-stranded RNA (dsRNA) inhibition of β-thymosin mRNA translation in both the neurites of intact cells and in neurites isolated from snails promoted significant neurite outgrowth and elongation [135]. These studies support the hypothesis that local translation in the growth cones is an important regulator of cytoskeletal dynamics in the developing axon.

The local translation of microtubule associated protein-1-B (MAP1B) mRNA also contributes to the cytoskeletal and morphological changes of distal axons and growth cones in response to chemotrophic signals [107, 148]. Bassell and colleagues have shown that MAP1B mRNA is also localized to the distal axons and growth cones of hippocampal neurons and implicated its local translation in axon guidance and turning [148]. In addition, candidate plasticity-related gene 15 (cpg15; also known as neuritin) mRNA is also localized to the axons of mouse embryonic motor neurons [138] where the cpg15 protein is involved in motor neuron axon branching and neuromuscular synapse formation [138, 149]. β-catenin mRNA also localizes to distal axons and presynaptic terminals where it regulates synaptic vesicle release during the development of functional buttons [150]. The regulation of neurotransmitter exocytosis by the local translation of β-catenin mRNAs suggests that the release of neurotransmitters may also benefit from local synthesis of key enzymes in the neurotransmitter biosynthetic pathway in the distal axon and presynaptic nerve terminal of the neuron (see section on neurotransmitter proteins, 2.2.2.5).

2.2.2. Axon maintenance and viability

2.2.2.1. Structural, receptor and cytoplasmic proteins

In addition to the important role played by the local protein synthetic system in development, the localization of mRNAs and their translation may also be important in adulthood, during axonal response to nerve injury, and may participate in axon repair and neuron survival [22, 153]. For example, in the mature neuron, axonal injury triggers the local translation of structural proteins (e.g. tubulin, β-actin), receptor proteins (e.g. CPR-2) and cytoplasmic proteins (e.g. IMPA1), which appear to be essential in the promotion of axonal regeneration. The NGF-dependent synthesis of inositol monophosphate 1 (IMPA1) in the axons of cultured sympathetic neurons also participates in maintaining axon viability. Moreover, there is evidence to suggest that the local synthesis of

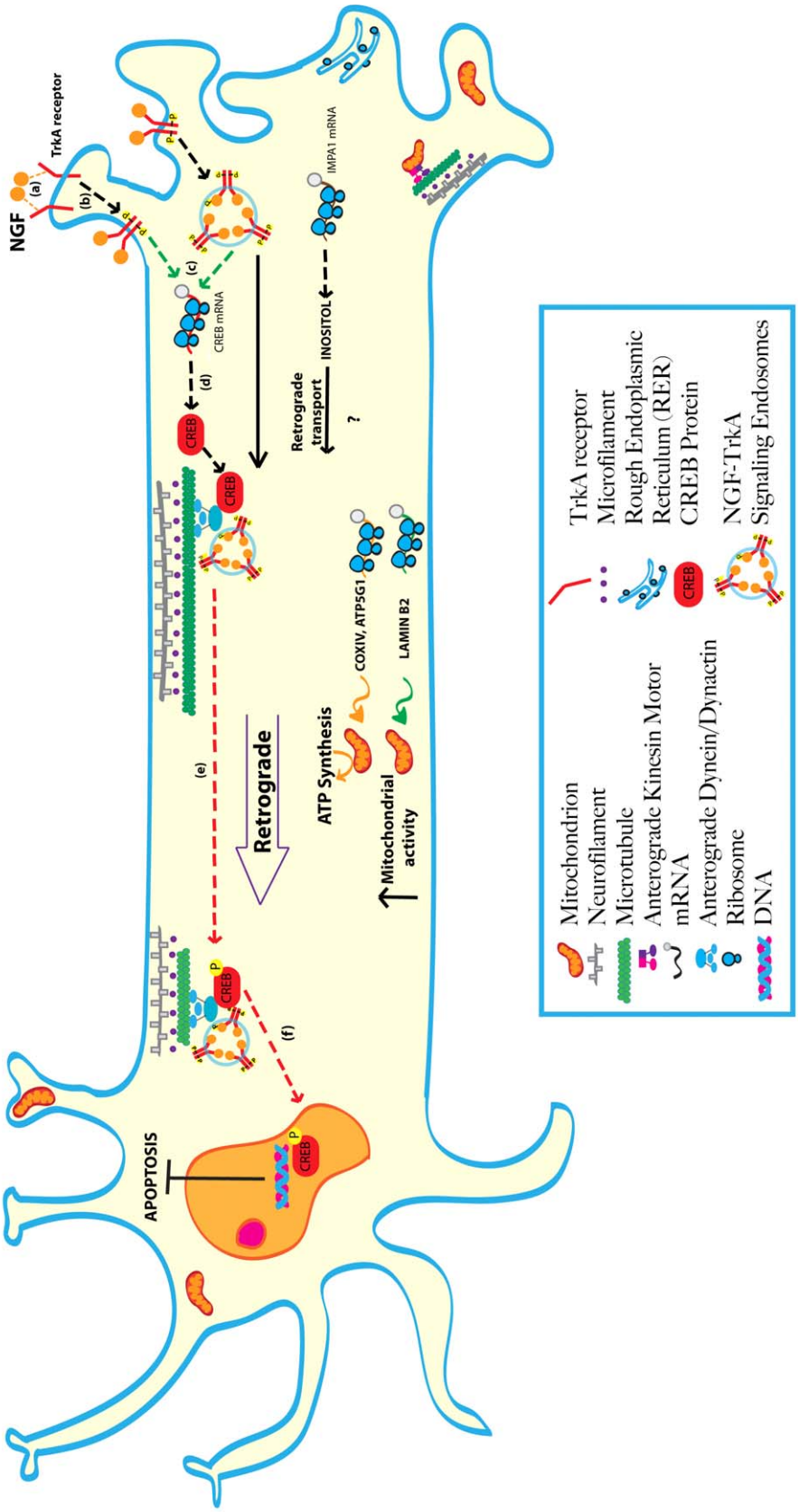
NGF
TrkA receptor
(a)
(b)
(c)
(d)
(e)
(f)
CREB mRNA
CREB
P
IMPA1 mRNA
INOSITOL
Retrograde transport
?
Retrograde
ATP Synthesis
COXIV, ATP5G1
LAMIN B2
Mitochondrial activity
APOPTOSIS
Mitochondrion
Neurofilament
Microtubule
Anterograde Kinesin Motor
mRNA
Anterograde Dynein/Dynactin
Ribosome
DNA
TrkA receptor
Microfilament
Rough Endoplasmic Reticulum (RER)
CREB Protein
NGF-TrkA Signaling Endosomes

Figure 2

IMPA1 initiates an unknown retrograde signaling cascade that relays a survival signal to the nucleus (Figure 2) [101]. In reference to these observations, it can be postulated that local translation is a protective mechanism employed by neurons to maintain axon viability in response to injury, and therefore a requisite for axon regeneration and survival. For further discussion on the postulated mechanisms of retrograde axonal transport of locally synthesized proteins see section 7.

Similar to dendrites, mRNAs encoding receptor proteins are also locally synthesized and regulated in the mature axon. Using immunocytochemistry and electrophysiological techniques, the van Minnen group provided the first evidence that isolated axons could synthesize, express and insert functional neurotransmitter receptor proteins [111]. Convincingly, they demonstrated that surgically isolated axons from the fresh water mollusc *Lymnaea stagnalis* injected with exogenous G-protein-coupled conopressin receptor (CPR-2) mRNA locally translated the messenger and integrated the receptor protein into their axons [111]. However, further experiments will be required to determine whether this capability also exists in the axons of vertebrates.

2.2.2.2. Mitochondrial proteins

One of the surprising features of the axonal mRNA population is the presence of a large number of nuclear-encoded mitochondrial mRNAs [11, 12]. Mounting evidence suggests that local synthesis of nuclear-encoded mitochondrial proteins (e.g., cytochrome c oxidase IV, COXIV and ATP synthase subunit 9, ATP5G1) in the axons and presynaptic nerve terminals is integral to axon survival and synaptic plasticity [123, 125, 154]. The first evidence of axonal mRNAs coding for nuclear-encoded mitochondrial proteins was observed in the distal compartments of neurons derived from invertebrates such as the squid giant axon and the presynaptic nerve terminals of squid retinal photoreceptor neurons [11, 12]. To date, and consistent with the findings from the squid, numerous nuclear-encoded mitochondrial mRNAs have also been found in the axons of the rat primary superior cervical ganglion (SCG) neurons [for review, see 123] Axonal mRNA translation supports axon survival by promoting mitochondrial function in the distal axons *in vitro* (Figure 2). For example, COXIV mRNA is localized in the axons of primary SCG neurons where its local translation is involved in the regulation of mitochondrial activity and axon elongation [121, 122, 124]. Additionally,

Legend to Figure 2. Axon survival and maintenance requires local synthesis and retrograde transport of proteins. Axonally localized synthesis of nuclear-encoded, mitochondrial, and signaling proteins is required for axon maintenance and viability. In cultured sensory neurons, the stimulation of the axon with nerve growth factor (NGF) results in the translation of cyclic AMP response element-binding protein (CREB) mRNA. The binding of NGF to TrkA receptors **(a)** results in dimerization and autophosphorylation of TrkA dimer complex **(b)**. Subsequently, the activated TrkA dimer complex leads to translation of axonal CREB mRNA **(c)** and the production of CREB protein **(d)**. The NGF-bound-activated TrkA receptors are internalized into endosomes and initiate the formation of a signaling complex that consists of downstream effectors and the motor protein dynein. The newly synthesized CREB protein associates with this NGF–pTrkA-signaling endosome and is retrogradely transported to the nucleus along microtubules **(e)**. In the cell soma, axonally synthesized CREB is phosphorylated by this NGF–pTrkA-signaling endosome. In the nucleus, activated CREB initiates the transcription of anti-apoptotic genes **(f)** leading to neuronal cell survival [118-143]. NGF-mediated synthesis of inositol monophosphatase 1(IMPA1) in the axon also supports axon survival. In cultured sympathetic neurons, the axonally localized synthesis of IMPA1 appears to be critical for the regulation of vesicle transport, and the internalization of NGF–TrkA complexes that initiate the retrograde signaling necessary for promoting axon growth and neuronal survival. It is unclear, however, whether the IMPA1 protein itself is retrogradely transported to the cell soma [22, 101]. In the distal axons of cultured sympathetic neurons, nuclear-encoded mitochondrial protein are synthesized and imported to the mitochondria. Such proteins include cytochrome c oxidase complex IV (COXIV) isoform 1 and ATP synthase 5G1 (ATP5G1). The axonal synthesis of both COXIV and ATP5G is important for mitochondrial activity and axonal growth [122, 125]. The axonal synthesis of Lamin B2 (lb2), a nuclear-envelope component, is also important for axon viability *in vitro* and *in vivo*. In response to extracellular engrailed, *lb2* localizes to mitochondria, regulating their size and activity [165].

the local translation of mRNA coding for mitochondrial protein ATP5G1, a key component of Complex V of the oxidative phosphorylation chain, is also important for ATP production and axonal elongation [125].

The axon-specific inhibition of COXIV mRNA translation using siRNAs attenuates the generation of ATP and axon growth and elongation. Likewise, the silencing of ATP5G1 translation decreases mitochondrial membrane potential, resulting in a reduction in ATP synthesis, as well as axonal growth. In a separate study the Kaplan group showed that transgenic mice over-expressing COXIV mRNA localization element resulted in the reduction of local ATP levels and increase in the levels of reactive oxygen species (ROS) [155]. In these transgenic animals, elevated levels of ROS were observed in the frontal cortex and behavioral tests revealed an 'anxiety-like' phenotype, suggesting an important role for the localization of nuclear-encoded mitochondrial mRNAs in neuronal physiology and behavior [155]. Together, these observations indicate that local protein synthesis in the distal axons is required for energy production and ultimately for axon growth and maintenance [124].

2.2.2.3. Lamins

In a similar fashion, the local synthesis of Lamin B2 (lb2) mRNA in *Xenopus laevis* RGC axons following their exposure to EN-1 protein also appears to promote axonal survival [126]. The *de novo* synthesized Ib2 protein associates with the mitochondria, regulating their size and function (Figure 2). In fact, inhibition of lb2 mRNA translation causes axonal degeneration *in vivo* and mitochondrial dysfunction and defects in axonal transport *in vitro* [126]. In line with these findings, it is well known that malfunctions in mitochondria function are implicated in several neuropsychiatric and neurodegenerative diseases [for review, see 156]. For example, the demonstration that the synthesis of mitochondrial proteins and lamins are important for axonal viability is consistent with studies that show that mutations in either mitochondrial proteins or lamins may lead to a Charcot-Marie-Tooth disease phenotype, a neuropathic disorder characterized by chronic axonal degeneration [22].

2.2.2.4. Ribosomal and translation regulatory elements

Findings derived from recent microarray analyses and deep sequencing established that mRNAs coding for translational factors and ribosomal proteins constitute one of the largest functional groups of mRNAs in the axon [89, 109]. Despite this observation, only one study has demonstrated the local synthesis of an individual ribosomal associated protein [114]. In this work, it was shown that the mRNA that codes for the ribosomal protein (RP) L4 is localized and translated in developing axons of rat PC 12 cells and axons of adult primary sensory neurons *in vitro* but decreases in the mature axon. In response to neurite injury, the translation of RPL4 is not suppressed in the presence of RNA synthesis inhibitors. In fact, Twiss and colleagues observed that activation of RPL4 translation promoted neurite regeneration in PC12 cells, as well as, rapid axonal growth and elongation in conditioned (cultured after sciatic nerve crush) rat DRG neurons *in vitro* [114]. Furthermore, inhibition of RPL4 mRNA translation using antisense oligonucleotides abolished these regeneration and elongation phenotypes. While the function of newly synthesized RPL4 protein in rapid growth of these neuronal processes has not been elucidated, it has been hypothesized that translation regulation of mRNAs encoding components of the translational apparatus likely provides neurons with an expeditious means to respond to injury [114]. In a similar fashion, the translation regulation of axonally localized initiation factors also influences axon growth and elongation [117]. Kar and colleagues have shown that mRNAs encoding eukaryotic translation initiation factors eIF2B2 and eIF4G2 are present in the axons of cultured rat sympathetic neurons and are locally translated [117]. siRNA-mediated knockdown and metabolic labeling studies showed that inhibition of axonal eIF2B2 and eIF4G2 expression markedly inhibited overall local protein synthesis and axon growth.

2.2.2.5. Neurotransmitter proteins

In addition to mRNAs coding for structural, mitochondrial and ribosomal proteins, as well as translation regulatory elements, there is considerable indirect evidence to suggest that mRNAs encoding proteins involved in neurotransmitter synthesis are present in the axon. For example, the presence of mRNA encoding tyrosine hydroxylase (TH), the

rate-limiting enzyme in the catecholamine neurotransmitter biosynthetic pathway, has been reported in the human neocortex and the rat striatum and cerebellum [157, 158]. These brain regions receive considerable catecholaminergic afferent innervation from the locus coeruleus and substantia nigra, but are devoid of catechoalamine-synthesizing cells *per se*. In the rat brain, lesion of the nigrostriatal pathway significantly decreased levels of TH mRNA in the striatum, suggesting that the message was being transported to the terminal fields. Interestingly, the administration of reserpine, a powerful catecholamine depleting agent, markedly enhanced TH mRNA levels in the cerebellum, a finding that raised the possibility that the local synthesis of TH might function to facilitate the restoration of neurotransmitter levels [158].

Not unexpectedly, expression of the TH gene is heavily regulated at both the transcriptional and post-transcriptional levels [for review, see 159, 160]. In midbrain dopaminergic neurons, TH synthesis can be regulated at the translational level and synthesis of the enzyme can be induced in a cyclic AMP-dependent manner in the absence of alteration in mRNA levels [161]. Consistent with this finding TH mRNA is present in axonal polysomes and its active translation has been observed in the axons of rat primary sympathetic neurons [162]. Taken together, these findings raise the intriguing possibility that the synthesis of the catecholamine neurotransmitters could be regulated locally in the terminal fields situated far distant from the parental cell soma.

At present, mRNAs encoding the biosynthetic enzymes regulating the synthesis of other amino acid-derived neurotransmitters is unknown. However, mRNAs coding for the neuropeptides, oxytocin, vasopressin and dynorphin are transported to the distal axons of peptidergic neurons comprising the hypothalamo-hypophyseal tract [163, 164]. Hence, it is possible that mRNAs coding for a wide variety of neurotransmitters and/or neuromodulatory substances can be functioning locally in the axon and presynaptic nerve terminal.

3. Trafficking of axonal mRNAs

3.1. Cell soma origin of axonal mRNA

As described above, axonal mRNA is a subset of the total neuronal transcriptome, some of which are enriched in the axon [89, 90, 101, 102, 166]. This asymmetrical distribution of mRNA within neurons is attributable, at least in part, to the active sorting and transport of mRNAs. mRNA sorting to the axon is facilitated by the presence of a localization signal. In addition, for an mRNA to reach the most distal domains of an axon, it is necessary that the mRNA is stable and that its translation is repressed.

The molecules that recognize the localization signal present in an mRNA, stabilize the mRNA and repress its translation during transport and/or storage are known as RNA-binding proteins (RBPs). A series of different RBPs bind mRNA from the moment of its transcription until its translation in designated cellular compartments. In the nucleus, RBPs direct the post-transcriptional modifications and processing of pre-mRNAs that lead to mature mRNA and the export of mature mRNA to the cytoplasm. In the cytoplasm, RBPs stabilize mRNA by preventing its degradation, mediate transport of the mRNA along the axon or to the dendrite, and repress premature mRNA translation during transport (Figure 1).

RBPs bind mRNA by recognizing motifs usually located in the 3'UTR, and less commonly in the 5'UTR or coding sequence (see below). These motifs are known as *cis*-acting elements or 'zip-codes' and contain localization signals. The motifs can be constituted by either the primary sequence of the mRNA, or its secondary or tertiary structure [167]. A common type of secondary structure recognized by RBPs is a hairpin stem-loop structure [122, 167]. Given the complexity of secondary and tertiary structures, attempts to predict localization signals have so far yielded disappointing results, and primary sequence analysis of mRNAs sorted to the axon has not revealed a common motif. Motif recognition by RBPs is based on a relatively small number of RNA-binding domains that usually recognize only a short RNA stretch. As a consequence, RBPs are not specific to one particular mRNA. For instance, ZBP1 binds β-actin and GAP43 [129], while HuD binds FMRP in dendrites and GAP43, tau and AchE mRNA in axons [168].

The complex formed by RNA and RBPs is known as ribonucleoprotein (RNP), the precise composition of which is a matter of debate. Until recently,

the prevalent hypothesis was that RNP granules transported a large number of different mRNAs [169, 170]. However, recent studies suggest that RNPs may contain single copies of a specific RNA. *In-situ* hybridization with differently tagged probes targeting the same transcript has shown little or no co-localization of the probes, implying that often only one transcript is present in an RNP [171, 172]. Similarly, simultaneous detection of pairs of mRNAs has shown only limited co-localization in the same RNP. Although these results were obtained in rat hippocampal dendrites [171] and *Drosophila* embryos [172], it is not unreasonable to hypothesize that the RNPs of mammalian axons may have a similar composition.

Once in the cytoplasm, RBPs bind to motor proteins and the RNP complex is transported along the cytoskeleton. The long-range transport along the axon relies on the action of the motor proteins, kinesins and dyneins, which are associated with microtubules. In the axon, RNPs often move bidirectionally. Evidence suggests that kinesins and dyneins can simultaneously bind the same RNP so that the overall direction of movement of an RNP depends on the ratio between its plus-end (kinesins) and minus-end (dyneins) directed microtubule motor proteins [173, 174]. Interestingly, the probability of a motor protein binding to a specific RNP may be regulated by the transported RNA. A recent study by Amrute-Nayak and Bullock conducted in the *Drosophila* syncytial blastoderm embryo has shown that the *cis*-acting localizing element on K10 mRNA increases the average number of dynein recruited to individual mRNPs, therefore increasing the frequency of minus-end directed movement and directing its localization to the apical cytoplasm [172].

Bidirectional movement of RNPs slows down particles and might appear to provide an inefficient mechanism for selectively transporting RNA. It has been suggested, however, that bidirectional movement facilitates the anchoring and enrichment of specific mRNAs to axonal subcompartments [174]. Indeed, in addition to RNA being sorted to axons, there is evidence that RNA sorting takes place within the axon. For example, β-catenin mRNA is found preferentially in newly formed presynaptic terminals along the axon [150]. The mechanism of intra-axonal sorting has not been fully elucidated; however, it is reasonable to hypothesize that it mimics intra-dendritic sorting, which depends on additional localizing signals present in either the 5' or 3'UTR of mRNAs. For instance, while the 3'UTR of sensorin mRNA is sufficient for localization of the RNA to distal neurites, the 5'UTR is necessary for accumulation of the mRNA at the synapses [55]. Similarly, the dendritic localization of protein kinase M zeta (PKMζ) mRNA is dependent on two cis-acting dendritic targeting elements (DTE), Mζ DTE1 and Mζ DTE2 [82]. Mζ DTE1 spans the 5'UTR and the open reading frame, and directs the export of the mRNA from the soma to the proximal dendrites, while Mζ DTE2 is located in the 3'UTR and is required for transport to the more distal aspects of the dendrites.

Tethering of RNPs to specific regions of the axon is believed to involve the action of myosin motors moving along actin filaments [175-177]. Accordingly, knockdown of myosin Va in hippocampal neurons is associated with an increase in the speed of anterograde axonal transport of ZBP1 [178]. In myelinated axons, actin filaments are enriched in periaxoplasmic ribosomal plaques (PARPs), where translational activity is believed to take place [5, 8, 152, 179].

Current understanding of the axonal transport of RNA is that kinesin and dynein moving along microtubules are involved in the long range longitudinal transport of RNPs, while myosins moving along actin filaments are involved in the short range radial transport and tethering of RNPs in the subcortical axoplasm. In addition, evidence suggests that MyoVa-actin system is also involved in the cell-to-cell transfer of RNA (see section 4).

β-actin, KOR and cpg15 mRNAs are three well-categorized axonally transported mRNAs. Nascent β-actin mRNA is bound by the nuclear protein zipcode binding protein 2 (ZBP2). ZBP2 binding facilitates subsequent recruitment of ZBP1 protein, a predominantly cytosolic protein that shuttles between the cytoplasm and the nucleus. Binding of ZBP1 to β-actin mRNA occurs through two KH-domains that recognize a *cis*-acting element in the 3'UTR of β-actin mRNA [180-182]. The ZBP1-β-actin mRNA complex translocates to the cytoplasm and moves to the axon. During transport, ZBP1 binding of β-actin prevents premature

translation. In response to neuronal activity, the translation of β-actin mRNA is initiated after Src protein kinase-mediated phosphorylation of a tyrosine residue in ZBP1, an event which is required for binding of ZBP1 to RNA [183].

In contrast to the β-actin mRNA, the KOR transcript is silenced by Grb7 protein, which binds to its 5'UTR. Translocation to the cytoplasm requires two proteins, exportin CRM and HuR [184]. Once in the cytoplasm, KOR mRNA may be translated, stored in processing-bodies (P-bodies) or stress granules, or transported to axons. Similarly to β-actin mRNA, release of KOR mRNA from Grb7 requires phosphorylation of two tyrosine residues on Grb7 by focal adhesion kinase (FAK).

Hu-antigen D (HuD, also known as ELAV-L4) is a neuron-specific RBP that also associates with mRNAs, including cpg15. In addition to HuD, survival motor neuron (SMN) protein 1 also interacts with cpg15 and promotes the trafficking of cpg15 mRNA to the axons of motor neurons. Twiss and colleagues demonstrated that HuD-SMN1 protein complex binds to cpg15 mRNA and may be important for axonal targeting or transport of cpg15 mRNA. In fact, SMN1 deficiency reduces cpg15 mRNA levels in the axons of mouse embryonic motor neurons and overexpression of cpg15 mRNA in neurons partially rescues SMN-deficiency in zebrafish [138]. Consistent with this, it has also been shown that SMN can form a complex with hnRNP-Q/R protein, an RBP that appears to regulate the axonal transport of β-actin mRNA in the axons and growth cones of motor neurons [185-187]. Moreover, considering the established role of SMN-hnRNP-β-actin mRNA interaction in the targeting of β-actin mRNA to axons, a similar mechanism appears to be integral to the transport of cpg15 mRNA [138].

4. Inter-cellular transfer of RNA

To date, the possibility that a portion of the RNA that is present in the distal extrasomatic domains of the neuron derives from the neighboring glial cells has received limited attention [for review, see 1; 188]. Early evidence for the 'glia-neuronal transfer' hypothesis derived from invertebrate model systems [189-190]. In these studies, newly synthesized RNA was isolated from the perfused, isolated squid giant axon. The RNAs present in the perfusate included small RNAs (i.e., tRNA), rRNA and a heterogeneous population of polyadenylated RNAs. The synthesis of these RNAs was strongly inhibited by actinomycin D, indicating that the incorporation of the radiolabel into RNA was transcription-dependent, and supported the contention that the newly synthesized RNA present in the isolated axon perfusate originated in the periaxonal glia. Importantly, the transfer of the newly synthesized RNA from the periaxonal glia to the isolated axon was markedly stimulated by depolarization of the axon, as well as exposing the axon to agonists of the glial glutamate and nicotinic acetylcholine receptor. Conversely, pretreatment of the axon with these receptor antagonists inhibited the transfer of the radiolabeled RNA. Taken together, these early findings raised the possibility that both the axon and nerve ending were endowed with a local inter-cellular RNA transport system [for discussion, see 1; 191].

Additional support for the 'glia-neuronal transfer' hypothesis was obtained from radiolabelling experiments conducted using the squid optic system (e.g. optic lobe tissue slices and large presynaptic nerve terminals of the retinal photoreceptor neurons) as a model sensory neuron experimental system [192]. Evidence to support the existence of such a phenomenon is also present in the vertebrate literature. For example, Court and colleagues have reported that Schwann cells transfer rRNA and polyribosomes to intact sciatic nerve axons. The levels of the transferred RNA increased markedly when the axons were desomatized (i.e., severed from their parental cell bodies) [193]. Most recently, Sotelo and colleagues demonstrated that after sciatic nerve injury a significant amount of newly synthesized RNA was transferred from Schwann cells to the axon [179, 188]. The cell-cell transfer of newly synthesized RNA is myosin Va- and F-actin-dependent, and appears to be mediated by a mechanism similar to that of vesicular transendocytosis [188]. In myelinated axons, the inter-cellular transfer of macromolecules is likely to occur at the Nodes of Ranvier or at Schmidt-Lanterman Clefts, as suggested by Twiss and Fainzilber [194].

At this juncture, it is important to note that the inter-cellular transfer of RNA is well documented in other cell systems. For instance, ovarian follicle

cells supply mRNA to oocytes [195] and mast cells transfer mRNAs between cells [for example, see 196]. Hence, the cell-to-cell transfer of mRNA and components of the translational machinery may prove to be a widespread phenomenon and in the nervous system may serve to regulate and integrate glial and neuronal cell function e.g., the 'glia-neuron unit' [1, 197].

5. Regulation of subcellular mRNA translation

The rate of protein synthesis in neuronal subcellular domains is intricately linked to the metabolic state of the neuron [22]. However, to date, the signaling pathways that mediate the selective local translation of mRNAs in the neuronal compartments are poorly understood. There is evidence to suggest that global regulation of protein synthesis in the distal processes of the neuron is regulated by the mammalian target of rapamycin (mTOR), a serine/threonine protein kinase, which influences cell growth, motility and synaptic plasticity [22, 49]. mTOR initiates activation of mRNA translation by phosphorylating its two major targets: eukaryotic initiation factor 4E-binding protein 1 (elF4E-BP1) and ribosomal protein S6 kinase (S6K) [for review, see 22, 198-199]. Evidence from several studies demonstrates that guidance cue-stimulated activation of the mTOR pathway by Netrin-1, Sema3a, and BDNF increases the translation activity of axon growth cones *in vitro,* while Ephrin A cues inhibit mTOR activity [104, 134, 142]. These guidance cues induce the phosphorylation of elF4E-BP [199], which recruits ribosomes to target mRNAs. A similar mechanism has been described for regulation of local translation in dendrites [198, 200]. However, the precise mechanism by which particular mRNAs are translated in response to specific cellular signaling is yet to be elucidated. This raises the fundamental question: what mechanism underlies the selective translation of specific mRNAs in the neuronal subcompartments?

5.1. Dendrites

While the exact molecular mechanism(s) that underlie dendrite-localized mRNA translation is still unclear, several studies have addressed this issue, albeit indirectly. In some cases it has been shown that translation of mRNAs in dendrites is regulated by external stimuli such as, acute depolarization [33], receptor activation [37] and cyclic AMP stimulation [57]. In other cases, it has been shown that modifying the transport/localization of mRNAs to the distal reaches of the neuron also represents an alternate means to regulate localized protein synthesis [201].

One well-studied example of receptor-dependent translation in dendrites is the ionotrophic glutamate receptors (NMDA, AMPA, or glutamate). Early studies by Marin and colleagues revealed that treatment of cortical neurons with ionotrophic glutamate receptor agonists inhibits overall protein synthesis [202]. Consistent with this observation, NMDA receptor activation decreased overall protein synthesis, but selectively induced the translation of CaMKIIα and β-actin mRNAs within dendritic synapses [33, 203, 204]. Interestingly, pharmacological activation of glutamate receptors in dendrites of hippocampal neurons with mGluR agonists triggers local synthesis of eEF1α *in vitro* and *in vivo* [48, 49]. In addition, studies have also shown that AMPA receptors are also synthesized in dendrites in response to stimulation [205], suggesting that endogenous neurotransmitter release can regulate the expression of postsynaptic mRNAs and receptors. These studies show that while several aspects of this receptor-dependent regulation of mRNA translation remains to be delineated, translation of particular mRNAs may be regulated in response to neurotransmitter signaling [200]. Thus, it remains to be shown, what specific stimuli activate the translation of distinct mRNAs?

RNA-binding proteins also seem to play a central role in translational regulation of mRNAs and therefore modulate protein expression in dendrites. Increasing evidence indicates that mRNA-binding proteins are potentially direct targets of glutaminergic-receptor-dependent regulation of dendritic mRNA translation [56]. Mendez and colleagues demonstrated that NMDAR activation might signal CaMKIIα mRNA recruitment by activating cytoplasmic polyadenylation-binding protein1 (CPEB1) [206]. Synaptic stimulation of the NMDA receptor permits an influx of Ca^{2+} that allows the subsequent phosphorylation of CPEB1, which in turn binds to the 5'UTR of CaMKIIα mRNA through its CPE binding element, leading

to the synthesis of CaMKIIα [31, 53]. Translation of dendritically localized MAP2 mRNA is also regulated by CPEB1 protein [33]. In a separate study, it has been shown that NMDA receptor activation leads to the phosphorylation of CPEB by aurora kinase and the activation of CaMKIIα translation [54]. Consistent with this finding, a metabolic glutamate-receptor (mGluR) signaling pathway that involves activation of protein kinase C and release of internal calcium reduces the localization of fragile X mental retardation protein (FMRP) in dendritic synapses [56]. Future studies are needed to identify how receptor-dependent phosphorylation of the mRNA-binding proteins affects the translation of specific mRNAs.

FMRP is a *trans*-acting RBP that reversibly associates with target mRNAs and represses their translation in the dendrites [207]. In fact, loss of FMRP results in abnormal spine structure, a hallmark of fragile X mental retardation syndrome [208]. FMRP acts as a repressor of translation of several dendritic mRNAs, including CaMKIIα, Arc, and β-actin [31]. Investigators have shown that FMRP interacts with target mRNAs via a non-coding pol3 RNA transcript called BC1 RNA, suggesting that BC1 mediates the repression of translation [209, 210]. The role of BC1 in translation repression is consistent with the evidence implicating BC1 as a regulator of translation initiation [211]. At a global level, BC1 RNA appears to repress initiation by stimulating eukaryotic initiation factor 4A (e1F4A), ATPase activity and disrupting the helicase activity of e1F4A [212]. Considering the classical means of translation regulation, Edelman and colleagues have hypothesized that dendritic mRNAs can be preferentially regulated via internal ribosomal entry sites [33, 208].

The activation of dendrite-localized eukaryotic Elongation Factor 2 Kinase (eEF2K; also known as CaM-dependent protein Kinase III) leads to dramatic reduction of local mRNA translation [52]. Specifically, RNAi knockdown of eEF2K results in a reduction of the overall synthesis of proteins in the dendrites and alteration in dendritic spine morphology [52]. Surprisingly, however, although general eEF2K activity and dendritic mRNA translation are negatively correlated, in the context of neuronal activity and synaptic plasticity paradigms, the translation rate of MAP1B, CaMKIIα and Arc actually increases [213]. Worthy of note is that experiments on the eEF2K effects on dendritic mRNA translation and protein activity are conducted in the presence of elevated levels of calcium, glutamate signaling, and NMDAR activation [for review, see 213] paradigms which have also been shown to regulate the localization and translation of CaMKIIα, Arc and MAP1B. Therefore, the apparent increase in the mRNAs encoding CaMKIIα, Arc and MAP1B may have resulted from these experimental confounds.

Accumulating evidence also indicates a role for microRNAs (miRNAs) in the translational regulation of mRNAs localized to the dendrites [for review, see 214]. Importantly, Kunes and colleagues demonstrated that components of miRNA processing machinery are also localized to dendritic synapses of *Drosphila* and that the RNA helicase protein, armitage, was degraded at the synapses [215]. In a separate study, it was shown that brain-specific microRNA-134 (miR-134) regulates translation in dendrites. For example, miR-134 inhibits the translation of LIMK-1 mRNA, which is involved in regulating actin cytoskeletal dynamics by suppressing cofilin activity [47]. Furthermore, miR-134 binds to the 3'UTR of LIMK-1 and modulation of miR-134 levels altered dendritic LIMK-1 protein expression and appears to affect dendritic spine development (for discussion, see section 6 on MicroRNAs).

5.2. Axons

In the axon, mRNA transcripts are also translated in response to extracellular stimuli and neuronal activity. Given the fact that the mRNAs encoding ribosomal proteins are also enriched in the axon [89], it has been hypothesized that compartmental regulation of ribosome synthesis/assembly mediates translational control of mRNAs. Recently, the Flanagan group has shown that cue-induced signals regulate local ribosomal activity that may also regulate local translation of mRNA [151]. These investigators provided the first evidence that guidance cue receptors can directly regulate ribosome activity (Figure 1b). For example, netrin-1 trans-membrane receptor DCC (deleted in colorectal carcinoma) binds and sequesters ribosomal protein L5, a component of the 60s ribosomal subunit, in an inactive state and regulates translation. The binding of netrin-1 releases these organelles from DCC and allows the ribosomes to form polysomes

in the vicinity of receptor activation. These observations are consistent with the observation that ribosomes are localized close to the axolemma in a configuration that can support axonal protein synthesis [216]. The interaction of DCC receptor with the translation machinery represents an elegant mechanism to regulate mRNA translation locally. It would be interesting to learn if other axon-specific membrane receptors also participate in the regulation of the translation machinery and if receptor-ribosome interactions mediate mRNA-specific translation.

In addition to the receptor-ribosome-dependent translation hypothesis, most of the evidence for regulating axonal mRNA translation points to the interactions between *cis*-acting signals in the 3'UTR of mRNAs and *trans*-acting RBPs. RBPs have been shown to facilitate the transport and stability of mRNAs and are therefore perfectly positioned as determinants of subcellular mRNA translation and regulation. A well-studied example of these *trans*-acting proteins is ZBP1. The ZBP1 protein binds to the 3'UTR of β-actin mRNA and represses the translation of β-actin. It has been shown that the decreasing availability of ZBP1 [103, 105], either through over-expression of an exogenous 3'UTR competitor of β-actin mRNA or deletion of one ZBP1 allele, decreases the *in vitro* and *in vivo* axonal levels of endogenous β-actin mRNA and protein [129]. In the growth cones of dissociated rat DRG neuronal cultures, extracellular netrin-1 binds to DCC, which activates Src kinases. Src phosphorylates ZBP1 resulting in dissociation of ZBP1 from β-actin mRNA, ribosome recruitment to β-actin mRNA and local β-actin protein synthesis. Using a similar experimental approach, axonal levels of GAP43 mRNA were also decreased in rat DRG neurons resulting in reduced axon elongation. Similar to the situation in the dendrites, FMRP also targets mRNAs in the axons and represses their translation [217]. FMRP localizes to axons and growth cones and regulates chemotropic-responsive local translation in axons. For example, FMRP binds to MAP1B mRNA that is localized in the growth cones of axons [34]. Moreover, in cultured neurons obtained from FMR1 knockout mice, excessive translation of MAP1B mRNA was observed [34].

Another molecule that may function as repressor of axonal mRNA translation is CPEB (CPEB1, CPEB2, CPEB3). The CPEB1 protein regulates mRNA translation by binding to its recognition element (CPE) located in the 5' or 3'UTR of its target mRNAs and keeps them in a translationally inactive state [206, 218, 219]. Phosphorylation of CPEB1 allows elF4E to facilitate recruitment of the 43S pre-initiation complex to the mRNA for translation. For example, CPEB1 has been shown to bind to β-catenin mRNA and regulates β-catenin mRNA translation in a NT3-dependent manner in growth cones of developing hippocampal neurons. Both the NT3-mediated rapid increase in β-catenin and process branching are abolished when CPEB1 function is inhibited [133, 220]. These findings are consistent with the recent demonstration that CPEB1 knockout mice exhibit aberrantly increased translation of phosphatase and tensin homolog (*PTEN*) and signal transducer and activator of transcription 3A (STAT3) mRNAs [221; see section 7 on retrograde transport for more on PTEN and STAT3 mRNAs].

In addition to RNA-binding proteins, regulation of mRNA translation also involves other *trans*-acting molecules such as non-coding RNAs (ncRNAs) (Figure 1c). Notably, increasing evidence shows that miRNAs regulate mRNA translation by associating with a complementary sequence present in the 3'UTR of target mRNA (see section 6 on MicroRNAs). The axonal translation of nuclear-encoded mitochondrial mRNAs, including COXIV and ATP5G1 mRNAs, is regulated by microRNAs. In these cases, local translation appears to be regulated by miR338, a brain-specific miRNA [121, 125]. In addition, microRNAs such as miR-16 have also been shown to mediate expression of mRNAs encoding eukaryotic translation initiation factors eIF2B2 and eIF4G2 in the axons of rat sympathetic neurons. In this instance, the binding of miR-16 to its target mRNAs, elF2B2 and elF4G2, greatly reduced the activity of the axonal protein synthetic system [117].

Surprisingly, chondrotin sulfate proteoglycans (CSPG), a component of the glial scar has also been identified as a novel regulator of axonal protein synthesis. In the growth cones of axons derived from rat DRG neurons, local CSPG expression results in increased synthesis of RhoA protein and collapse of the growth cones [222].

Finally, while the molecular events that mediate the local translation of specific mRNAs in the neuronal subcellular domains is poorly understood, it is clear that RBPs function in both the regulation of mRNA trafficking and translation, and that non-coding RNAs also play an important role in the control of local protein synthesis (see below).

6. MicroRNAs in the distal processes of the neuron

In addition to the diverse mRNA populations present in the different structural/function domains of the neuron, these regions also contain numerous miRNAs. These small, non-coding RNAs play an important role in the post-transcriptional regulation of gene expression as well as neuronal function [as reviewed in 214, 223]. Initial estimates of the complexity of these miRNA populations, derived from microarray analyses of total RNA from primary rat sympathetic neurons, suggested that the axon and nerve terminal contained as many as 130 different miRNAs [224]. This study also revealed a set of miRNAs that were highly enriched in the axon as compared to the parental cell body. Moreover, a number of miRNAs encoded by a common primary transcript (pri-miRNA) were differentially expressed in the distal axon. Similar results were obtained for the individual miRNA components of the miR-17-92 cluster in cortical neurons cultured in microfluidic chambers [131]. Taken together, these findings suggested that the neuron contained a differential processing and transport system for axonal miRNAs.

Several of the miRNAs identified in the Natera-Naranjo investigation were later shown to play an important regulatory role in the local protein synthetic system, as well as in axonal function and growth. For example, miR-338 was demonstrated to regulate the local expression of two nuclear-encoded mitochondrial mRNAs both of which coded for key components of the oxidative phosphorylation chain (i.e., COXIV isoform 1 and ATP5G1). Modulation of the levels of the axonal miR-338 by axonal transfection of anti-miR-338 greatly affected mitochondrial activity, resulting in a diminution in axonal ATP levels, increased levels of reactive oxygen species, and ultimately changes in growth rate of axons of sympathetic neurons in culture [121, 124]. The transfection of precursor miR-338 (pre-miR-338) resulted in the opposite effects. Interestingly, transfection of the SCG axons with pre-miR-338 resulted in a 10 to 42-fold increase in mature miR-338 levels compared with endogenous miR-338 levels in sham-transfected axons 1 and 4 hrs after transfection, respectively [121]. These findings demonstrated that the distal axon had the capability of processing miRNA precursors to the mature form of the molecules.

Two putative mRNA targets for another miRNA (miR-16) were subsequently shown to be present in the axon and coded for two translation initiation factors (i.e., eIFB2 and eIF4G2). Transfection studies employing chimeric reporter gene constructs containing the 3'UTR of these mRNAs established that the *cis*-acting regulatory elements present in the 3'UTR that bound miR-16 were indeed functional in the distal axons of cultured SCG neurons [117]. Transfection of the SCG axons with the precursor of miR-16 and anti-miR-16 established that this miRNA modulated the local levels of these translation initiation factors, as well as the activity of the local protein synthetic system itself. Again, the miR-16-mediated modulation of local protein synthesis via levels of eIFB2 and/or eIF4G2 markedly affected the growth of the axon [117].

The miRNA population has also been evaluated in the axons and growth cones of murine embryonic cortical neurons [225]. Consistent with the findings derived using postnatal SCG axons [224], approximately 105 different miRNAs were detected in these cortical axons, and six of these miRNAs were preferentially expressed in the axon. Again, in keeping with previous findings, the results of this study indicate that a mechanism(s) exists to localize and/or preferentially transport specific miRNAs to the axons and growth cone/nerve terminal.

That the presence and composition of the miRNA population can have profound effects on axonal development is now well documented. In this regard, the expression and alterations in the abundance of the miR-17-92 cluster in cultured rat embryonic cortical neurons has been demonstrated to regulate axonal outgrowth, perhaps through modulation of axonal PTEN protein levels [131]. The local expression of miR-9 has also been reported to regulate axon extension and branching in primary cortical neurons [226]. In this case, the

effects of the miRNA were mediated by regulating the axonal levels of MAP1B, a protein that serves as a functional target for the BDNF-dependent control of axonal growth.

A recent screen for axonal localization has identified a set of miRNAs that manifest high expression levels and preferential localization in the axons of developing DRG neurons [227]. In this study, local mRNA translation was regulated by miR-132, a miRNA that was highly enriched in the axon. The knockdown of miR-132 reduced extension of cultured DRG axons, whereas the over-expression of this miRNA increased extension of the axon. Mechanistically, miR-132 regulated the local expression of Rasa1, a protein that activates RasGTPase. Interestingly, the abundance of miR-132 in DRGs peaked in the period of maximum axon growth *in vivo*, an observation that is consistent with its effect on axon growth in cell culture.

Surprisingly, the miRNA populations delineated in developing DRG axons showed little overlap with the set of miRNAs present in the axons of developing cortical neurons or postnatal rat SCGs. This observation might derive from differences in the neuronal cell type, developmental stage, or the detection methodologies employed in the work. Alternatively, the lack of RNA sequence conservation within these miRNA populations could result, at least in part, from the significant redundancy present in the miRNA regulatory network. Hence, variation in the individual components of the population might have relatively little functional consequence(s).

Similar to the situation in the axon and nerve terminal, the dendritic domain also contains a highly diverse population of miRNAs. Results of a laser capture and PCR analysis of hippocampal neurons led to the identification of a subset of miRNAs that were differentially expressed in the dendrites of these neurons [228]. Early microarray analyses of synaptosomal preparations from adult mouse forebrain also established the presence of several miRNAs, the abundance of which was enriched in the synaptodendritic compartment of the neuron [229]. The function of several of these dendritic miRNAs has been recently reviewed [230]. The dendritic localization of this select subset of miRNAs is thought to play a key role in fine-tuning of the expression of synaptic proteins that regulate dendritogenesis, dendritic spine morphology, and the plasticity of the synaptodentric domain of the neuron [for example, see 231, 232]. Importantly, altered expression patterns of activity-regulated miRNAs (i.e., miR-132) have often been associated with neurological disorders and psychiatric disease [233].

7. Retrograde transport of local translation products

In addition to the anterograde transport of RNA from the cell body to the distal processes of the neuron, there exists a retrograde transport of mRNA translation products. This transport system may provide for a communication circuit(s) between the neuronal cell body/nucleus and the distal structural/function regions of large, highly asymmetric cell types. These lines of communication might prove particularly important in neuronal development and/or response to injury [234]. In this regard, a significant number of mRNAs encoding well-studied transcription factors are localized to the axon and dendrite [for review, see 235]. For example, four different transcription factors have been reported to be synthesized locally in the axon [118-120, 236]. However, evidence from recent axonal transcriptome analyses suggests that axons may contain a large number of other mRNAs encoding transcription factors [89, 90, 237], and the composition of this mRNA subset differs markedly during development [for example, see 90]. There is also compelling evidence, derived from lesioned peripheral nerve, to indicate that the local synthesis and retrograde transport of transcription factors (e.g. STAT3) is directly involved in axonal injury signaling [119].

As mentioned above, mRNAs coding for transcription factors (e.g., CREB) are also present in the dendrite [28, 50, 238], and the trafficking of CREB from the dendrites to the nucleus has been eloquently demonstrated in cultured hippocampal neurons [50, 51]. The retrograde transport of locally synthesized CREB from axon to cell body has also been reported [132, 239]. In axons of developing DRG neurons, the CREB protein is locally synthesized in an NGF-dependent manner and retrogradely transported back to the neuronal nucleus where it influences transcription of genes

regulating the survival of DRG neurons [118]. In contrast, the cell body synthesis of CREB was not found to mediate this NGF-induced cell survival mechanism. An importin-dependent, dynein motor complex mediates the intra-cellular trafficking of these translation products. Interestingly, some of the structural and regulatory components of the retrograde transport system itself, such as importin-β-1, appear to be locally synthesized [132], especially upon axonal injury [240]. Strikingly, axonal knockout of importin-β-1 attenuates cell body transcriptional responses to nerve injury and delays functional recovery *in vivo* (Figure 3) [241]. Signal transducer and activator of transcription 3 (STAT3), is also locally synthesized in response to axonal injury and retrogradely transported to the nucleus by binding to the activated dynein complex using the nuclear localization signal [119]. In a similar fashion, vimentin, which is also locally synthesized in the axon in response to injury, binds directly to importin-β-1 and concomitantly relays information to the cell body (Figure 3) [239]. It has been postulated that the retrograde transport of these locally synthesized transcription factors regulates the composition of the neuronal transcriptome in response to stimuli impinging on the distal processes of the cell. It is also noted that the retrograde transport of locally synthesized kinases and other signaling proteins could also play an important role in these intra-cellular communication pathways.

Interestingly, Hengst and associates have proposed that retrograde intra-axonal signaling pathways play an important role in the pathogenesis of neurodegenerative disease [116]. In this study, local exposure of the axons of rat embryonic hippocampal neurons, grown in tripartite microfluidic chambers, to Aβ1-42 initiated the local synthesis of a set of proteins that included the activating transcription factor 4 (Atf4), and resulted in increases in cell death within 48 hr. Inhibition of the local translation of Atf4 mRNA or its knockdown by siRNA application abolished the Aβ1-42-induced cell loss. Consistent with the findings obtained in these *in vitro* experiments, injection of Aβ1-42 into the dentate gyrus (DG) of mice resulted in the loss of forebrain neurons whose axons innervated this brain region. Based on these findings, the Hengst group postulated that the retrograde transport of the locally synthesized proteins (e.g. Atf4) might mediate the transmission of neurodegenerative signals across brain regions, and may be involved in the transmission of amyloid pathology in Alzheimer's disease [116].

8. Pathophysiology

Given that axonal RNA and local protein synthesis play a fundamental role in neuronal development and function, it is not unreasonable to expect that dysregulation of axonal RNA localization and/or the local protein synthetic system might lead to disease. For instance, mutation of RBPs with consequent altered localization of axonal mRNAs has been implicated in the pathogenesis of a series of neurodegenerative disease such as amyotrophic lateral sclerosis [243], frontotemporal lobar degeneration [243], spinal muscular atrophy [244, 245], and fragile X mental retardation syndrome [34]. Similarly, mutations in mRNA sequence that alter RBP binding of RNA have been linked to disease. For instance, microsatellite expansion disorders may cause sequestration of RBPs [246-248]. Hence, disruption of the local protein synthetic system may ultimately prove to represent new foci for neurodevelopmental disorder(s) or neuro-degenerative disease. However, at the present time, the molecular mechanism by which malfunction of these local neuronal systems ultimately affect brain function and behavior remains unclear. Moreover, whether these systems might serve as new targets for disease diagnosis or effective therapeutic targets remains a compelling subject for future investigation.

9. Methodological issues

At its inception, the study of subcellular compartment-specific expression and translation of mRNAs was hampered by several methodological issues and technical limitations [23]. One major impediment was the difficulty in obtaining pure and sufficient quantities of dendrites and axons for analyses [22]. While pioneering studies using metabolic labeling allowed researchers to demonstrate the local synthesis of proteins such as β-tubulin in the axons of invertebrates [249], due to technical limitations, it was not possible to totally eliminate the possibility that the signal arose from glial

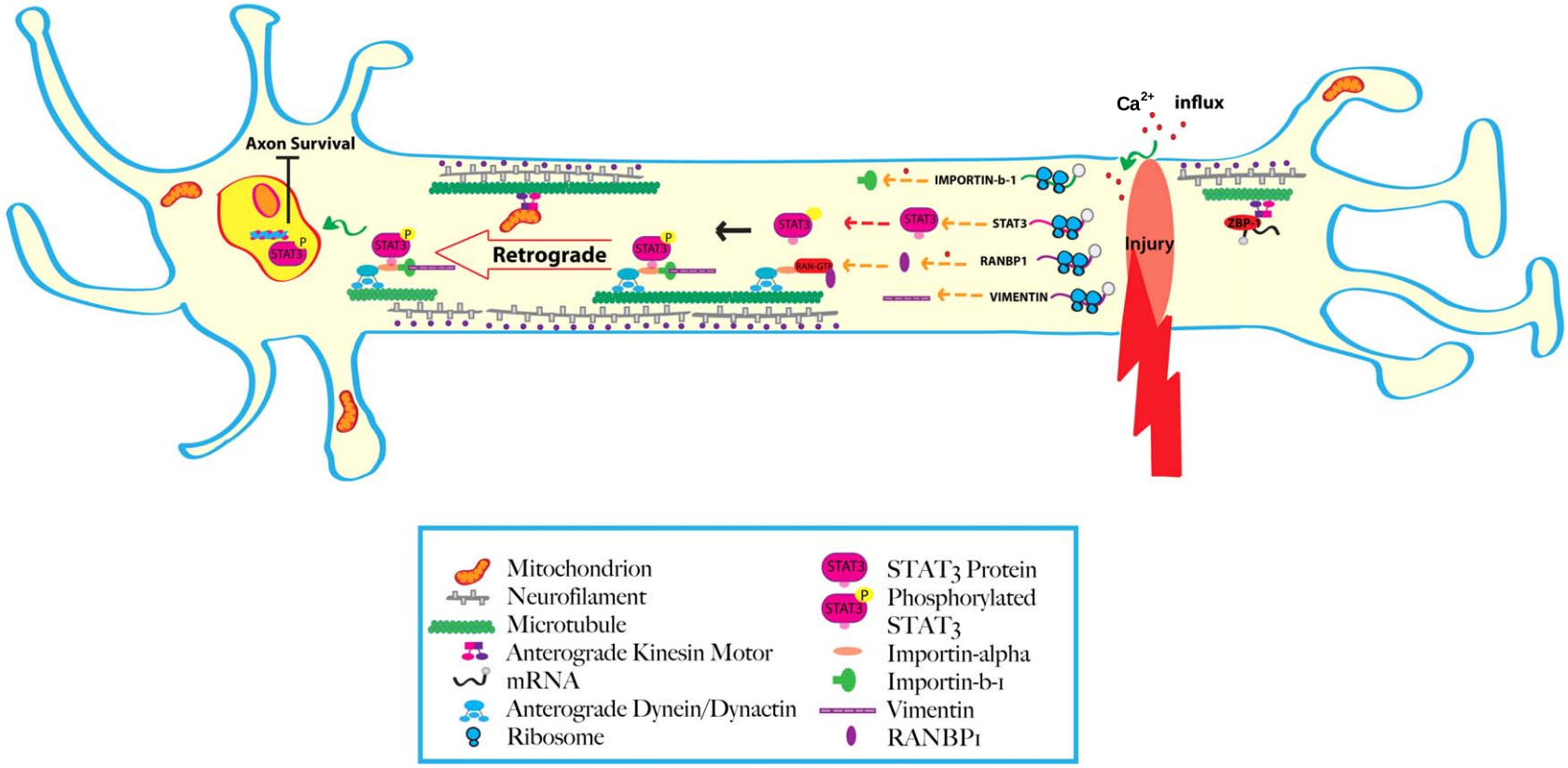

Figure 3. Local translation and retrograde transport in response to axonal injury. Axonal injury also induces local translation of mRNAs and possibly influences axon regeneration and repair via the retrograde transport of local translation products. Under normal conditions, RanGTPase inactivates the dynein-motor complex. Following peripheral nerve injury in DRG neurons, RanBP1, a Ran-specific GTPase-activating protein, is locally synthesized in the axon and subsequently displaces RanGTP from the dynein motor complex [22, 140]. The Importin-β-1 protein is also locally synthesized in response to axonal nerve injury and activates dynein motor complex via the Ran-free dynein motor protein [132]. Signal transducer activator of transcription 3 (STAT3) is locally synthesized in the axon where the newly synthesized STAT3 is activated by phosphorylation. The phosphorylated STAT3 binds to the activated retrograde dynein molecular motor complex via nuclear import factor α5. In the cell soma, the activated STAT3 protein activates transcription of target genes to induce an axonal survival program [119]. Vimentin, a type III intermediate filament, is axonally synthesized and activated in injured axons where it appears to participate in the activation of the retrograde signaling cascade by binding to importin-β-1 [242].

contamination. The confound of cell body contamination was eliminated by surgically severing the soma from its distal processes, allowing the local synthesis of proteins to be convincingly demonstrated [250, 251]. Recent compartmentalized cell culture systems [252-254], laser capture-micro dissection [89], and neuronal ball culture [255] have allowed researchers to culture axons and dendrites in distinct fluidic environments separate from their parental cell bodies. These techniques have allowed for recent subcellular profiling and transcriptome analyses of axons and dendrites at various stages of development [89].

Difficulty in visualizing and detecting *de novo* synthesized proteins was also a barrier for researchers seeking to demonstrate local protein synthesis intrinsic to the axon and dendritic compartment of the neuron. Due to steady-state bidirectional trafficking of proteins between the cell body and its distal domains it was also difficult to distinguish between existing and newly synthesized proteins [23]. To circumvent these difficulties, biorthogonal noncannonical amino acid tagging (BONCAT) and fluorescent noncanonical amino acid tagging (FUNCAT) have been successfully employed to selectively identify [256] and visualize locally *de novo* synthesized proteins [257]. Additionally, local translation of mRNAs can be demonstrated by immunoprecipitating polysomes and associated mRNAs. In this regard, Heiman and colleagues convincingly showed that axonally translating mRNAs could be isolated on ribosomes using the translating ribosome affinity purification (TRAP) technique. In this study, tagged ribosomal proteins were specifically expressed in retinal axons, and tagged ribosome-mRNA complexes were subsequently immunoprecipitated and characterized [258].

Although local translation has been demonstrated in axons and dendrites of neurons *in vitro*, it has been difficult to delineate what/if mRNAs are translated in subcellular compartments *in vivo*. To date, only a few studies in rodents have shed light on the functional significance of locally translated proteins in distal neuron processes [110, 119, 129, 222]. This is particularly difficult because it is technologically challenging to inhibit compartment-specific mRNA translation in intact animals. It has been hypothesized that a potential approach to address this issue in candidate genes would be to introduce targeted mutations to known axon-localization elements in the UTRs. The result would be normal expression of all other proteins, but specific dysregulation/loss in mRNA localization and translation of the desired protein [22]. Additionally, the site-specific delivery of caged translation-blocking antisense oligonucleotides, which can be activated locally by light stimulation in axons, may also provide ways to alter mRNA translation in intact animals [22]. Looking ahead, new technological advances are required for the investigation of compartment-specific mRNA translation *in vivo*.

10. Summary and future directions

The selective targeting of mRNAs to the subcellular domains of the neuron provides a means to spatially and temporally regulate the local protein composition in these regions. Here, we have highlighted some prominent findings of axonal and dendritically localized mRNAs, the classes of proteins translated locally, summarized the known regulatory mechanisms underlying RNA transport and its translation, and discussed intriguing evidence supporting the transcellular trafficking of RNA from glial cells to axons. We have also addressed the findings that the local translation of mRNA in neuronal processes is driven by stimuli ranging from chemotropic responses to axonal nerve injury. In addition, local translation of mRNAs is likely to depend on the state of activity of the neuron (i.e., during growth and prolonged activity of the neuron). In fact, regionally expressed cues have been shown to trigger the region-specific translation of proteins needed for growth cone path finding, as well as neuronal survival [22]. Looking forward, there are many questions about subcellular compartment-specific mRNA translation that remains to be addressed such as: what is the composition and function of the local mRNA populations? Is the composition of the population developmentally regulated? How is it affected by injury or acute insults? How is mRNA identification and targeting achieved (i.e., selective/differential transport mechanism(s))? What is the composition of the transport apparatus? What RBPs are responsible for transporting mRNAs? What are the regulatory mechanisms

that govern local translation of mRNAs in the distal neuronal compartments? Elucidation of these issues will greatly enhance our understanding of the biology of the distal reaches of the neuron (axon, dendrites and nerve terminals) and offer further insight into neural pathophysiology.

ACKNOWLEDGEMENTS

The Division of Intramural Research Programs of the National Institute of Mental Health (ZO1MH00276) supported the author's work. We thank Anthony E. Gioio, Amar N. Kar, Jobert Vargas, Cai Chen and Ching-Yu Sun for their invaluable contributions to the authors' research endeavors and critical review of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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