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Local Translation and mRNA Trafficking in Axon Pathfinding

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Abstract

Axons and their growth cones are specialized neuronal sub-compartments that possess translation machinery and have distinct messenger RNAs (mRNAs). Several classes of mRNAs have been identified using candidate-based, as well as unbiased genome-wide-based approaches. Axonal mRNA localization serves to regulate spatially the protein synthesis; thereby, providing axons with a high degree of functional autonomy from the soma during axon pathfinding. Importantly, *de novo* protein synthesis in navigating axonal growth cones is necessary for chemotropic responses to various axon guidance cues. This chapter discusses the molecular components involved in regulating axonal mRNA trafficking, targeting, and translation, and focuses on RNA binding proteins (RBPs) and microRNAs. The functional significance of local mRNA translation in the directional response of growth cones to a gradient is highlighted along with the downstream signaling events that mediate local protein synthesis. The view that emerges is that local translation is tightly coupled to extracellular cues, enabling growth cones to respond to new signals with exquisite adaptability and spatiotemporal control.

1 Introduction

During development, axons often navigate significant distances to their synaptic targets and are guided by multiple molecular cues, including the netrins, slits, semaphorins, and ephrins (Dickson 2002). The growth cone at the tip of the axon senses the guidance cues and steers towards or away from them (Fig. 1a, b). Given the extreme distance that can separate growth cones from their cell bodies (sometimes centimeters), axon pathfinding presents a unique challenge to neurons in ensuring that growth cones respond properly and rapidly to complex extracellular stimuli encountered along the way.

Classically, it has been thought that the proteins required for directional steering originate in cell bodies, where they are synthesized and subsequently transported to growth cones to mediate responses to extracellular stimuli (for review, see Koenig and Giuditta 1999). However, a growing body of evidence challenges this view. For instance, the isolated growth cones of *Xenopus laevis* retinal ganglion cells (RGCs) not only survive for up to 3 h when detached from their cell bodies *in vitro* and *in vivo*, but also migrate and respond to guidance cues (Campbell and Holt 2001; Harris et al. 1987). Since chemotropic responses require precise regulation of protein levels and activity, the question arises as to how isolated growth cones are able to sustain their responses without contributions from their cell bodies. Recent evidence suggests that this functional autonomy seen in axonal growth cones is mediated, at least in part, by local protein synthesis. According to this view, subsets of neuronal mRNAs are trafficked into axons and growth cones, where they are locally

translated independent of cell bodies. This chapter will focus on the role of local protein synthesis in axon pathfinding during development as well as the regulators involved in this process.

2 Axonal mRNA Localization and Translation: Historical Perspectives

Cytoplasmic RNA localization is an evolutionarily conserved mechanism that contributes to spatially restricted protein synthesis. The first hint of RNA localization in neurons dates back to the 1960s when ribosomes associated with endoplasmic reticulum (ER), together known as “ergastoplasm,” were detected near dendritic synapses of spinal cord neurons in monkeys by electron microscopy (EM) (Bodian 1965). This anatomical finding suggested that these neurons employ protein synthesis of localized RNAs for their synaptic function. Several years later, the concept of RNA localization was established in various other cell types ranging from unicellular organisms such as yeasts to oocytes and embryos of *Drosophila* and *Xenopus* in addition to other vertebrate and invertebrate neurons (Palacios and St. Johnston 2001).

RNA localization is particularly relevant in neurons where the polarity of cell processes (dendrites and axons) is important for structure and function. Specific targeting of various molecules to dendrites and axons establishes subcellular compartmentalization within neurons. In addition to the well-known concept of targeting proteins, the identification of polyribosomes at the base of dendritic spines in the 1980s indicated that synapses have close access to protein synthesis machinery (Steward and Levy 1982). The concept of dendrites synthesizing new proteins for synaptic function was not only consistent with Bodian’s early observations, but also triggered a whole new area of research for neuroscientists. Shortly following this observation, several mRNAs were identified in dendrites, and the molecular mechanisms underlying dendritic mRNA localization and translation became a subject of intense interest. Local translation of mRNAs in dendrites is now relatively well characterized and is critically important for synaptic plasticity and memory (Bramham and Wells 2007).

While postsynaptic dendritic mRNA translation enjoyed the limelight, the concept of *de novo* protein synthesis in presynaptic axons remained controversial. Although EM studies in the embryonic rabbit spinal cord axons revealed the presence of polysomes in axons (Tennyson 1970; Yamada et al. 1971; Zelenà 1970), the failure to detect biochemically the protein translation machinery in axons hampered further investigation (Alvarez et al. 2000). However, the notion of axonal localization and translation of mRNAs gathered momentum with the identification of mRNAs and ribosomes in vertebrate axons (Koenig and Giuditta 1999; Koenig and Martin 1996; Koenig et al. 2000), along with the evidence for local protein synthesis of cytoskeletal proteins such as actin, tubulin, and neurofilament in both vertebrate and invertebrate axons (Bassell et al. 1998; Eng et al. 1999; Koenig and Giuditta 1999; Olink-Coux and Hollenbeck 1996).

The first direct evidence for axonal protein synthesis came from experiments in the 1960s with the demonstration of acetylcholinesterase (AChE) resynthesis in axons of cat cholinergic neurons, in which this particular enzyme was irreversibly inactivated (Koenig and Koelle 1960). Axonal protein synthesis was subsequently shown in rabbit spinal root neurons by metabolic labeling experiments wherein neurons were incubated with [³H]leucine, which was incorporated into newly synthesized proteins of isolated axons (Koenig 1967). Similar metabolic labeling experiments were done using the squid giant axon and Mauthner neurons of the carpfish (*Carassius carassius*; for review, see Giuditta et al. 2008). Moreover, isolated axons of goldfish RGCs and newborn rat sympathetic neurons are capable of synthesizing actin and β -tubulin (Koenig and Adams 1982; Eng et al. 1999).

In fact, it is estimated that the axonal protein synthesis capacity of chick sympathetic axons accounts for 5% of the total protein synthesis occurring in the cells (Lee and Hollenbeck 2003). Moreover, local synthesis of a reporter protein of the receptor EphA2, and its expression at the cell surface, has been demonstrated in chick spinal cord axons, indicating that axons and their growth cones can synthesize new proteins and export them to the cell surface (Brittis et al. 2002). This finding is also supported by recent evidence showing that active ER and Golgi components in axons are required for protein synthesis and secretion, and that axons can target locally synthesized proteins into secretory pathways (Merianda et al. 2009).

Collectively, these findings raise several questions regarding mRNA localization within axons and growth cones: What are the mRNAs that are targeted to axons and growth cones? How are they selectively transported into the axons from the cell body? Does mRNA localization and translation in axons and growth cones serve a functional role in axon guidance? These questions will be discussed in the following sections.

3 Identity of Axonal mRNAs

Specific mRNAs were first identified in invertebrate axons using biochemical fractionation and metabolic labeling in the 1960s. These studies revealed the presence of ribosomal RNAs, transfer RNAs, translation initiation and elongation factor mRNAs, and mRNAs encoding three cytoskeletal elements: actin, tubulin, and neurofilament (Giuditta et al. 2008; Koenig and Giuditta 1999). In vertebrates, similar studies using rat spinal ventral roots, components of the peripheral nervous system (PNS), identified the same three cytoskeletal protein mRNAs as the major newly synthesized proteins among several other smaller molecular weight proteins that were not identified (Giuditta et al. 2008). Some of the first mRNAs identified in vertebrate central nervous system (CNS) axons were the peptide hormones oxytocin (Jirikowski et al. 1990) and vasopressin (Trembleau et al. 1996). These were localized using *in situ* hybridization (ISH) and EM studies on neurons of the rat hypothalamus. Other vertebrate CNS axonal mRNAs include cytoskeletal proteins; namely, β -actin (Bassell et al. 1998; Leung et al. 2006; Olink-Coux and Hollenbeck 1996), β -tubulin (Eng et al. 1999), microtubule-associated protein 1b (MAP1b) (Antar et al. 2006), tau (Litman et al. 1993), neurofilament (Sotelo-Silveira et al. 2000; Weiner et al. 1996), and actin depolymerizing factor ADF/cofilin (Lee and Hollenbeck 2003). mRNAs encoding other functional proteins include cell signaling molecules such as RhoA (Wu et al. 2005), transcription factors like CREB (Cox et al. 2008), and transmembrane receptors like EphB2 (Brittis et al. 2002), the κ -opioid receptor (Bi et al. 2006), capsaicin receptor VR1 (Tohda et al. 2001), and the olfactory marker protein (Wensley et al. 1995).

Generation of pure axonal preparations followed by genome-wide analysis allows for the identification of axonally localized mRNA transcripts in an unbiased manner. Proteomic analysis and reverse-transcription polymerase chain reaction (RT-PCR) of injury-conditioned or regenerating dorsal root ganglion cell (DRG) axons have revealed the localization of several different mRNAs in these axons. The mRNAs identified include many cytoskeletal encoding transcripts, and also several mRNAs encoding heat-shock proteins, endoplasmic reticulum (ER) proteins, such as calreticulin, antioxidant proteins like peroxiredoxin 1 and 6, and metabolic proteins such as phosphoglycerate kinase 1 (PGK1) (Willis et al. 2005). Moreover, microarray analysis of injury-conditioned DRG axons expanded the list of axonal mRNAs to over 200 transcripts, revealing many previously unidentified mRNAs. These include several transmembrane proteins and components of the translational machinery such as ribosomal proteins (Willis et al. 2007). This suggests that axons contain a diverse repertoire of mRNAs that can be potentially translated similar to dendrites. Increasingly sensitive methods of mRNA isolation and new sequencing

technologies will doubtless reveal further complexity in the mRNA content and localization in axons.

4 Molecular Mechanism of Axonal mRNA Transport

The presence of mRNAs in axons raises the question of how they are transported. Messenger RNA localization in axons, as in nonneuronal cells and in dendrites (Kindler et al. 2005), involves *cis*-acting sequence elements within a given transcript that are recognized by specific *trans*-acting factors or RNBP. mRNAs bound to RNBPs along with other proteins, such as motor proteins, together constitute the ribonucleoprotein particles (RNPs) or RNA granules. These RNP complexes undergo dynamic transitions ranging from stress granules, P-bodies, transport particles and microRNA RNPs (miRNPs) or RNA interference silencing complexes (RISCs) (Sossin and DesGroseillers 2006). Importantly, the mRNA in the RNA granule is usually translationally silent during transport (Krichevsky and Kosik 2001; Fig. 2). Translational repression is regulated by RNBPs themselves or by miRNAs, a class of small noncoding RNAs in axons. Although, much of our current understanding of RNPs comes from dendrites, we will consider the role of each of these molecular components in axonal mRNA targeting and translation, and draw parallels with dendritic mRNA targeting.

4.1 Cis-Acting Factors

Cis-acting elements, present in the 3' or 5' untranslated region (UTR) of mRNAs, exist in the form of specific nucleotide sequences, or in the form of secondary or tertiary stem-loop structures (Bassell and Kelic 2004). The most well-characterized *cis*-acting element is the 54-nucleotide (nt) sequence in the 3'UTR of β -actin mRNA, referred to as the "zipcode" (Kislauskis et al. 1994; Zhang et al. 2001). Mutational analysis identified this 54-nt zipcode as the minimum *cis*-acting element essential and sufficient to target β -actin mRNA into growth cones of chick forebrain axons (Eom et al. 2003). Antisense oligonucleotides to this zipcode, as well as mutations disrupting its secondary structure, abolish the localization of β -actin mRNA to growth cones (Zhang et al. 2001). Similarly, the *cis*-acting axonal localization element of tau mRNA, which encodes a microtubule associated protein that is exclusively localized to the axonal compartment, is a distinct 240-nt AU-rich sequence in tau mRNA 3'UTR (Aronov et al. 1999). Thus, axonal mRNA *cis*-acting elements are complex and variable and, on the basis of primary sequence alone, cannot be used to predict reliably whether a particular mRNA will be targeted to the axon.

4.2 Trans-Acting Factors, or RNBPs: Dual Function in mRNA Targeting and Translational Regulation

Regulation of mRNA localization is mediated by the recognition of *cis*-acting elements by *trans*-acting RNBPs. The most well-studied *trans*-acting proteins contain either RNA recognition motifs (RRMs) and/or hnRNP K homology (KH) domains (Bassell and Kelic 2004). One example is the zipcode-binding protein-1 (ZBP1), which binds to β -actin mRNA's zipcode and represses β -actin translation (Ross et al. 1997). ZBP1 itself is regulated by src kinase, which phosphorylates ZBP1 and disrupts its binding to β -actin mRNA (Huttelmaier et al. 2005). Fragile-X mental retardation protein (FMRP), another RNA-binding protein, interacts with MAP1b mRNA in axons and dendrites (Antar et al. 2006). FMRP acts as a translational repressor in dendrites (Zalfa et al. 2006) and is required for filopodia formation and motility in axonal growth cones (Antar et al. 2006). Similarly, the cytoplasmic polyadenylation element (CPE)-binding protein 1, CPEB1, binds to the CPE sequence (consensus UUUUUAU) in mRNAs and regulates cytoplasmic polyadenylation of transcripts in oocytes and dendrites (Huang et al. 2003; Richter 1999). Control of polyA-tail length is a conserved mechanism for mRNA-specific translational regulation, and it will be

interesting to know whether it is involved in regulating translation in axons. In general, RNBP are involved in multiple aspects of mRNA localization in axons (e.g., trafficking, targeting, and anchoring) and also help to “silence” translation during transport.

4.3 MicroRNA-Mediated Translational Repression

MicroRNAs and RNA interference (RNAi) are recently described players in the regulation of mRNA translation in neurons. Several miRNAs identified in mammalian neurons are developmentally regulated and are also associated with polyribosomes (Kim et al. 2004; Kye et al. 2007). Moreover, FMRP interacts with miRNAs and with miRNA machinery, including Dicer and the mammalian orthologue of Argonaute (Jin et al. 2004). Functional RNA interference (RNAi) machinery is also present in axons (Hengst et al. 2006), and in fact, the 3' UTR of RhoA mRNA contains putative miRNA-binding sequences (Wu et al. 2005). MicroRNAs regulate translation in dendrites; for example, miR-134 inhibits the translation of Lim kinase 1 (LIMK1), which is involved in regulating actin cytoskeletal dynamics in dendrites by suppressing cofilin activity (Schratt et al. 2006). This in turn alters dendritic spine morphology, although it remains unknown whether this particular miRNA plays a role in regulating axonal mRNA translation. Recently, miR-338, the first axonal miRNA identified, was found in axons of sympathetic neurons. miR-338 targets cytochrome c oxidase IV mRNA in sympathetic axons and controls the local synthesis of cytochrome c oxidase IV; thereby, modulating energy metabolism in these axons in a soma-independent mechanism (Aschrafi et al. 2008). These findings suggest that miRNAs play a distinct role in regulating axonal mRNA translation, and future studies may uncover a role in axon guidance.

4.4 RNA Granule Formation

As soon as mRNAs are generated in the nucleus, they associate with several RNBP involved in mRNA processing and nuclear export. Evidence for the formation of RNP complexes in the nucleus comes from *Drosophila* and *Xenopus* oocytes (Hachet and Ephrussi 2004; Kress et al. 2004). RNBP regulating cytoplasmic RNA localization are also localized to the nucleus, implying that these proteins may serve as nucleocytoplasmic shuttling proteins that regulate nuclear and cytoplasmic mRNA targeting (Kindler et al. 2005). For example, zipcode binding protein 2 (ZBP2), also known as KSRP, MARTA1, or FBP2, is predominantly a nuclear protein (Gu et al. 2002; Min et al. 1997; Rehbein et al. 2002). It associates with β -actin mRNA in the nucleus to form the initial RNP and shuttles out into the cytoplasm. Additional RNA binding proteins like ZBP1 are required in the cytoplasm, while ZBP2 shuttles back into the nucleus (Gu et al. 2002). Thus, an RNP formed in the nucleus is commonly remodeled with additional transport factors once outside in the cytoplasm, resulting in large RNP complexes or RNA granules (Gu et al. 2002; Kindler et al. 2005). Biochemical characterization of these granules from neuronal extracts has revealed the presence of many mRNAs, RNBP, ribosomal proteins, proteins constituting the translational machinery, and motor proteins that move these granules along the cytoskeleton (Kanai et al. 2004). Hence, RNA granules are highly dynamic structures with multiple proteins orchestrating the targeting of a particular mRNA right from the time it is transcribed in the nucleus until its final destination in the cytoplasm.

4.5 Cytoskeletal Elements and Motor Proteins

RNA granules need to be actively transported along the cytoskeleton to reach the target site. Live-imaging studies of RNA granules in neurons show that these granules move with an average speed of $0.1\text{--}0.2\ \mu\text{m s}^{-1}$ (Knowles et al. 1996). While microfilaments are used to localize β -actin RNA along with ZBP1 in fibroblasts, the same RNP complex predominantly travels along microtubules in neuronal processes (Bassell et al. 1998). Microtubules are polarized polymers arranged in axons with the plus-ends oriented away from the cell body

(Baas et al. 1988). Moreover, microtubules are required for stimulus-induced changes in axonal localization of certain mRNAs (Willis et al. 2007). Although the motor proteins regulating mRNA transport in axons have not been studied, it is likely that as in neuronal dendrites, mRNAs are transported by kinesin and dynein molecular motor proteins, which are components of RNP particles (Hirokawa 2006). Dendritic CPEB granules, for example, contain both kinesin and dynein motor proteins; thereby, allowing bidirectional movement along microtubules (Huang et al. 2003). While kinesins are predominantly “plus-end” motor proteins carrying their cargo in the anterograde direction, axonal retrograde transport is mediated by the “minus-end” directed dynein motor proteins (Hirokawa and Takemura 2005).

In addition to microtubules, axonal growth cones have a fine meshwork of actin microfilaments in their peripheral domain. Several RNBP are detected in developing growth cones such as ZBP1 or Vg1RBP in *Xenopus*, and FMRP (Antar et al. 2006; Leung et al. 2006). Cytochalasin D treatment abolishes the movement of Vg1RBP granules in RGC axons and growth cones, indicating the essential role of actin microfilaments (Leung et al. 2006). Moreover, Vg1RBP co-localizes with β -actin mRNA in retinal growth cones, and netrin-1 stimulation induces the directed movement of Vg1RBP granules into filopodia (Leung et al. 2006). Hence, microtubules, as well as microfilaments, along with various molecular motors facilitate the transport of RNA granules in axons.

5 Local Protein Synthesis and Cue-Induced Chemotropic Guidance

Axonal growth cones navigate accurately over long distances through complex microenvironments. This directed migration or pathfinding requires the transduction of extracellular stimuli in the growth cone. A key question raised by the findings is: does mRNA localization and translation have any functional role in the directed migration of axons? Local protein synthesis could contribute to growth cone autonomy in responding to guidance cues, since it would allow a rapid regulation of proteins without requiring transport to and from cell bodies.

One of the earliest studies demonstrating that local protein synthesis plays a role in the cue-induced chemotropic responses of growth cones was done using developing RGCs of *Xenopus* (Campbell and Holt 2001). Global application of the guidance cues Semaphorin3A (Sema3A) or netrin-1 elicited rapid (within 10 min) protein synthesis in isolated axons, as measured by the incorporation of [³H]leucine. The guidance cue stimulation also resulted in rapid phosphorylation of translation initiation factor (eIF-4E) and initiation factor binding protein (eIF-4EBP1), which mark translation initiation. This translation pathway was shown to be functionally significant, as inhibition of protein synthesis with anisomycin or cycloheximide abolished the directional turning and collapse responses to Sema3A and the turning responses to netrin-1 in growth cones (Fig. 1c, d). The same results were obtained when the axon was cut, separating the growth cone from its soma, demonstrating that the translation was local (Campbell and Holt 2001). Subsequent studies have shown that other guidance cues, such as Engrailed-2, Slit-2, brain-derived neurotrophic factor (BDNF), and pituitary adenylate cyclase-activating polypeptide (PACAP), require local protein synthesis (Brunet et al. 2005 ; Guirland et al. 2003; Piper et al. 2006; Yao et al. 2006). Interestingly, 1- α -Lysophosphatidic acid (LPA)-induced growth cone collapse does not require local translation, neither ephrinB nor EphB, indicating that local protein synthesis is a differentially regulated mechanism employed by specific cues (Campbell and Holt 2001; Mann et al. 2003). These findings suggest that specific guidance cues elicit rapid, local protein synthesis in growth cones, and that this local translation is required for the chemotropic response *in vitro*.

6 Differential Translation of mRNAs

The finding that local protein synthesis is required for a growth cone's response to guidance cues raises several questions. For instance, what are the proteins that are synthesized in response to guidance cues? Are different mRNAs translated in response to different guidance cues? For chemotropic steering, growth cones need to sense a minute gradient of molecular cues across their width (Rosoff et al. 2004). Does local translation play a role in the detection of the chemotropic gradient? A recently proposed model, termed the "differential translation" model, suggests that different cues stimulate the translation of a specific subset of proteins, and that guidance cue gradients do so asymmetrically, resulting in growth cone steering (Fig. 3 and Table 1; Lin and Holt 2008).

6.1 Attractive Cues

Two recent studies support the idea of differential translation (Leung et al. 2006; Yao et al. 2006). Using immunostaining, Leung et al. (2006) showed that netrin-1, under attractive conditions, elicits a translation-dependent increase in β -actin levels locally within *Xenopus* RGC growth cones. The authors confirmed the immunostaining results using a reporter construct with the 3'-UTR of β -actin fused to the Kaede protein. Kaede is a green fluorescent protein that can be irreversibly converted to red fluorescence upon ultraviolet (UV) or violet irradiation (350–400 nm) (Ando et al. 2002). By photoconverting the pre-existing green Kaede to red and measuring the new green signal, the *de novo* Kaede signal can be quantified (Leung and Holt 2008). Upon netrin-1 application, the intensity of the green signal increased markedly, indicating that netrin-1, indeed, induces β -actin translation. In addition, preventing β -actin translation with antisense morpholino oligonucleotides targeted against the ATG site of β -actin mRNA disrupted the growth cone's steering in response to netrin-1. This indicates that β -actin translation is required for attractive turning to netrin-1. Interestingly, when growth cones are stimulated with a gradient of netrin-1, 4EBP phosphorylation and β -actin levels increase asymmetrically on the near-side of the growth cone, closest to the netrin-1 source. Vg1RBP was shown to interact directly with β -actin mRNA, raising the possibility that this interaction regulates asymmetrical translation of β -actin (Leung et al. 2006).

Similarly, a gradient of BDNF under attractive conditions elicits an asymmetric regulation of the β -actin level in growth cones of embryonic *Xenopus* spinal neurons (Yao et al. 2006). Fluorescent *in situ* hybridization (FISH) revealed that the intensity of the β -actin mRNA signal increases with BDNF stimulation. Furthermore, the co-localization of β -actin mRNA with ZBP1 increases with BDNF, suggesting that BDNF-induced β -actin mRNA localization occurs via ZBP1 binding. In a BDNF gradient, co-localization of β -actin mRNA and ZBP1, as well as β -actin protein expression increased rapidly (5 min) on the near-side of the growth cone closest to the BDNF source. A similar asymmetric rise was observed with the phosphorylated (active) form of src kinase. Interfering with the β -actin mRNA binding to ZBP1, using antisense oligonucleotides, abolished the growth cone's turning response to BDNF, signifying that the asymmetric β -actin translation is critical for BDNF-induced steering (Yao et al. 2006).

Together, these studies demonstrate that both netrin and BDNF-induced steering under attractive conditions is in part mediated by asymmetric translation of β -actin. Newly synthesized β -actin may mediate steering by stimulating actin polymerization that helps the growth cone to extend closer to the source of an attractive cue. Vg1RBP/ZBP1 may also be involved in this process by transporting β -actin mRNA and regulating its local translation (Lin and Holt 2008). Indeed, extracellular signals exert significant and selective effects on the transport of mRNAs in axons (Willis et al. 2007; Zhang et al. 1999).

6.2 Repulsive Cues

In contrast to attractive conditions, netrin-1 and BDNF under repulsive conditions do not elicit translation of β -actin (Leung et al. 2006; Yao et al. 2006). Furthermore, repulsive turning induced by BDNF was associated with an asymmetric decrease of β -actin on the near-side of the growth cone, closest to the BDNF source (Yao et al. 2006). Turning by netrin-1 under repulsive conditions is also not affected by morpholino-based blockade of β -actin translation (Leung et al. 2006). These findings indicate that attractive cues and repulsive cues elicit the translation of distinct subsets of mRNAs (Table 1), and that this helps to direct axon pathfinding.

Repulsive cues such as Semaphorin 3A and Slit-2 elicit steering away of growth cones from the source of the cue or collapse of growth cones, processes that require breakdown of the cytoskeleton. Indeed, the proteins identified as being synthesized in response to repulsive cues are mainly involved in cytoskeletal disassembly. For instance, Wu et al. (2005) showed that Semaphorin 3A stimulates the intra-axonal translation of RhoA mRNA, and that this local translation is necessary for growth cone collapse in embryonic rat DRG explant cultures (Wu et al. 2005). RhoA is a member of the Rho small guanosine triphosphatase (GTPase) protein known to regulate actin dynamics. In particular, RhoA-mediated signaling triggers growth cone collapse and neurite retraction by upregulating myosin II activity (for review, see Gallo and Letourneau 2004). To examine the dynamics of RhoA translation within axons and growth cones, Wu et al. expressed myristoylated-destabilized EGFP, regulated by RhoA 3'UTR. Myristoylation limits diffusion of the fluorescent protein, and destabilization of EGFP markedly reduces its half-life, facilitating visualization of *de novo* protein synthesis by reducing diffusion and persistence of existing proteins. When Semaphorin 3A was added, EGFP puncta significantly increased within 60 min, suggesting that Semaphorin 3A induces local translation of this RhoA reporter mRNA. Furthermore, intra-axonal knockdown of RhoA transcripts, using small interfering RNA (siRNA) directed against the 5'UTR of RhoA, significantly reduced Semaphorin 3A-mediated growth cone collapse (Hengst et al. 2006). Expression in these neurons of exogenous RhoA restored Semaphorin 3A-mediated growth cone collapse, indicating that RhoA translation is necessary for Semaphorin 3A's effect.

Other studies also identified proteins that are locally translated by repulsive cue stimulation. For instance, Slit elicits a rapid translation-dependent increase in actin-depolymerizing factor (ADF)/cofilin-1 (Piper et al. 2006). A selective knockdown of β -thymosin mRNA within axons was also shown to enhance neurite outgrowth in *Lymnaea* neurons (van Kesteren et al. 2006). β -Thymosin sequesters monomeric actin, thereby preventing actin polymerization (For review, see Huff et al. 2001). This finding suggests that locally translated β -thymosin may inhibit neurite outgrowth by preventing actin polymerization. However, it remains to be determined whether local translation of ADF/cofilin-1 and β -thymosin is necessary for growth cone steering or collapse.

All considered, these findings suggest that attractants and repellents stimulate the synthesis of distinct subsets of proteins. Furthermore, guidance cue gradients appear to induce spatially regulated mRNA translation. It has yet to be elucidated whether a polarized repulsive cue elicits spatially asymmetric protein synthesis in growth cones like an attractive cue; however, the differential translation model predicts that near-side synthesis of proteins involved in cytoskeletal disassembly would induce directional turning away from the cue (Fig. 3).

7 Modulation of Chemotropic Response

7.1 Adaptation to Cue Stimulation

A growth cone's response to guidance cues like netrin-1 and BDNF involves cycles of desensitization and resensitization, sometimes manifested by a zigzag pattern of migration towards a cue gradient (Ming et al. 2002). Such adaptation to guidance cues is critical for long-term navigation, enabling growth cones to respond to a dynamic range of cue concentrations during their journey (Ming et al. 2002). Growth cones of *Xenopus* spinal neurons have reduced, or desensitized, responses to micropipette-ejected netrin-1 in the presence of bath-applied netrin-1 within 30 min. Interestingly, they eventually recover from desensitization, or are resensitized, to the cue after an additional 90 min of incubation. Experiments with BDNF also showed similar results. When either anisomycin or cycloheximide was added to the isolated growth cones, they no longer exhibited resensitization, indicating that local protein synthesis is critical for the recovery of cue response (Ming et al. 2002).

Desensitization occurs on a rapid timescale in *Xenopus* retinal growth cones, as demonstrated by the finding that brief exposure (2 min) to a sub-threshold level of Sema3A prior to stimulation with full-collapse-inducing levels of Sema3A significantly reduces the collapse response (Piper et al. 2005). When the sub-threshold preincubation time is extended from 2 to 5 min, growth cones resensitize and exhibit full collapse in response to Sema3A. Netrin-1 under repulsive conditions elicits similar responses. Translation inhibitors abolish the resensitization phase of the response to both Sema3A and netrin-1. Interestingly, the desensitization step is not affected by protein synthesis inhibitors, but is sensitive to inhibitors of endocytosis, indicating that protein synthesis and endocytosis together coordinately regulate cue-driven guidance (Piper et al. 2005). Of note, local translation is also involved in sensitization of fast-nociceptive A-fibers from the DRG. This implies that local protein synthesis may have a broader role in fine-tuning the neuron's response to external stimuli (Jimenez-Diaz et al. 2008).

7.2 Intermediate Targets

During development, growth cones encounter multiple intermediate targets before reaching their final destination. One of the best-characterized examples is the midline of CNS. Developing commissural axons of vertebrates, insects, and nematodes, for example, initially are attracted to the midline by netrins. After crossing, however, they are repelled from the midline and project to the contralateral side (for review, see Tessier-Lavigne and Goodman 1996). This process is mediated in part by receptor–receptor “silencing” interactions between the netrin receptor, deleted in colorectal carcinoma (DCC), and the slit receptor Roundabout (Robo) (for review, see Stein and Tessier-Lavigne 2001). However, it is not yet clear how axons are capable of responding to a new set of guidance cues after crossing the midline.

Findings that L1 receptors are expressed exclusively on the contralateral segment of spinal cord commissural axons (Dodd et al. 1988) raise the possibility that new proteins are synthesized after midline crossing. Brittis et al. (2002) examined this issue using chick commissural axons and found that ephrinA receptor, EphA2, is upregulated in the post-midline segment of spinal cord commissural axons. EphA2 transcripts are present in these axons, and the expression of a reporter driven by EphA2 3'UTR is upregulated in growth cones only after they cross the midline. The EphA2 3'UTR contains a CPE sequence, and when CPE mutations were introduced, the increase of the reporter upregulation was markedly reduced (Brittis et al. 2002). Spatial restriction of EphA2 to the post-midline

segment of axons may thus be controlled by CPE-mediated polyadenylation and translation of localized mRNAs.

8 Intracellular Signaling Pathways Mediating Local Protein Synthesis

The transduction of extracellular stimuli requires highly regulated pathways involving multiple downstream effectors. Although the signaling processes that convert cue stimulation to local translation are not fully elucidated, evidence so far suggest that different cues are transduced via specific yet inter-related intracellular signaling pathways.

A common theme of the findings so far is that the cue-stimulated signal transduction utilizes pathways similar to those that regulate protein synthesis elsewhere. For instance, extracellular signals such as growth factors modulate protein synthesis via the phosphatidylinositol-3 kinase (PI3K) and mammalian target of rapamycin (mTOR)-dependent pathway. Both PI3K and mTOR activation leads to phosphorylation of eIF-4EBP1, which in turn leads to the release of eIF-4E and translation initiation (for review, see Gingras et al. 1999). Protein synthesis induced by Sema3A and netrin-1 is inhibited by rapamycin, a pharmacological inhibitor mTOR. Interestingly, a PI3K inhibitor, wortmannin, inhibits netrin-1, but not Sema3A-mediated protein synthesis, indicating that the two guidance cues work via similar but distinct signaling pathways (Campbell and Holt 2003).

Mitogen-activated protein kinases (MAPKs) have also been shown to be involved in the cue-mediated protein synthesis. The MAPK family includes signal transducing enzymes that are involved in many aspects of cellular responses, including cell growth and differentiation (p42/p44 MAPK), as well as stress responses (p38 and c-Jun N-terminal kinase/stress-activated protein kinase) (Chang and Karin 2001). MAPKs modulate protein translation by regulating MAP kinase-interacting protein kinase-1 (MNK1), which in turn phosphorylates eIF4E (for review, see Gingras et al. 1999). In embryonic *Xenopus* RGC axonal growth cones, netrin-1 and Sema3A activate p42/p44, and inhibition of p42/p44 abolishes cue-induced protein synthesis (Campbell and Holt 2003). The activation of translation initiation regulators (i.e., phosphorylation of eIF-4E, eIF-4EBP1, and Mnk-1) in growth cones is also dependent on p42/p44 and p38 MAPK. Interestingly, p38 is critical for netrin-1, but not for Sema3A-mediated protein synthesis, indicating that the two cues activate distinct pathways upstream of translation initiation (Campbell and Holt 2003). MAPK signaling is also critical for resensitization in *Xenopus* spinal axons (Ming et al. 2002).

It should be noted that these findings represent an incomplete view of the intracellular processes mediating local protein synthesis. Other second messenger molecules such as calcium (Ca^{2+}) are involved in this process, as Ca^{2+} -mediated bidirectional turning of *Xenopus laevis* growth cones is also dependent on protein synthesis (Yao et al. 2006). In addition, specific mRNAs are tightly regulated by various RNBP as discussed previously. Therefore, the regulation of cue-induced local protein synthesis involves multiple targets and mechanisms, many of which remain to be characterized.

9 Conclusion and Perspectives

mRNA trafficking and local protein synthesis have emerged as a critical mechanism through which axons respond and navigate through a complex environment. Messenger RNAs are targeted to axons and growth cones, and different guidance cues elicit translation of a distinct set of proteins in a spatially restricted manner. The entire process is tightly regulated by multiple mediators that act on mRNA transport and translation, as well as on signal transduction of extracellular stimuli. In this way, a neuron is able to rapidly and faithfully respond to the extracellular environment. Findings so far, however, represent only a snapshot of a highly complex and dynamic process. For instance, there remain many *cis*-

and *trans*-acting elements regulating mRNA transport and translation to be identified. Furthermore, more work is needed to determine exactly what proteins are synthesized at different stages of pathfinding, and to identify intracellular signals controlling this protein synthesis. Elucidating these mechanisms will not only help us understand the remarkable journey of an axon to its target, but also help us identify the potential therapeutic targets in diseased or injured conditions, when these carefully balanced processes go awry.

References

- Alvarez J, Giuditta A, Koenig E. Protein synthesis in axons and terminals: significance for maintenance, plasticity and regulation of phenotype. With a critique of slow transport theory. *Prog Neurobiol.* 2000; 62:1–62. [PubMed: 10821981]
- Ando R, Hama H, Yamamoto-Hino M, Mizuno H, Miyawaki A. An optical marker based on the UV-induced green-to-red photoconversion of a fluorescent protein. *Proc Natl Acad Sci USA.* 2002; 99:12651–12656. [PubMed: 12271129]
- Antar LN, Li C, Zhang H, Carroll RC, Bassell GJ. Local functions for FMRP in axon growth cone motility and activity-dependent regulation of filopodia and spine synapses. *Mol Cell Neurosci.* 2006; 32:37–48. [PubMed: 16631377]
- Aronov S, Marx R, Ginzburg I. Identification of 3'UTR region implicated in tau mRNA stabilization in neuronal cells. *J Mol Neurosci.* 1999; 12:131–145. [PubMed: 10527457]
- Aschrafi A, Schwechter AD, Mameza MG, Natera-Naranjo O, Gioio AE, Kaplan BB. MicroRNA-338 regulates local cytochrome c oxidase IV mRNA levels and oxidative phosphorylation in the axons of sympathetic neurons. *J Neurosci.* 2008; 28:12581–12590. [PubMed: 19020050]
- Baas PW, Deitch JS, Black MM, Banker GA. Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. *Proc Natl Acad Sci USA.* 1988; 85:8335–8339. [PubMed: 3054884]
- Bassell GJ, Kelic S. Binding proteins for mRNA localization and local translation, and their dysfunction in genetic neurological disease. *Curr Opin Neurobiol.* 2004; 14:574–581. [PubMed: 15464890]
- Bassell GJ, Zhang H, Byrd AL, Femino AM, Singer RH, Taneja KL, Lifshitz LM, Herman IM, Kosik KS. Sorting of beta-actin mRNA and protein to neurites and growth cones in culture. *J Neurosci.* 1998; 18:251–265. [PubMed: 9412505]
- Bi J, Tsai NP, Lin YP, Loh HH, Wei LN. Axonal mRNA transport and localized translational regulation of kappa-opioid receptor in primary neurons of dorsal root ganglia. *Proc Natl Acad Sci USA.* 2006; 103:19919–19924. [PubMed: 17167054]
- Bodian D. A suggestive relationship of nerve cell RNA with specific synaptic sites. *Proc Natl Acad Sci USA.* 1965; 53:418–425. [PubMed: 14294076]
- Bramham CR, Wells DG. Dendritic mRNA: transport, translation and function. *Nat Rev Neurosci.* 2007; 8:776–789. [PubMed: 17848965]
- Brittis PA, Lu Q, Flanagan JG. Axonal protein synthesis provides a mechanism for localized regulation at an intermediate target. *Cell.* 2002; 110:223–235. [PubMed: 12150930]
- Brunet I, Weinl C, Piper M, Trembleau A, Volovitch M, Harris W, Prochiantz A, Holt C. The transcription factor Engrailed-2 guides retinal axons. *Nature.* 2005; 438:94–98. [PubMed: 16267555]
- Campbell DS, Holt CE. Chemotropic responses of retinal growth cones mediated by rapid local protein synthesis and degradation. *Neuron.* 2001; 32:1013–1026. [PubMed: 11754834]
- Campbell DS, Holt CE. Apoptotic pathway and MAPKs differentially regulate chemotropic responses of retinal growth cones. *Neuron.* 2003; 37:939–952. [PubMed: 12670423]
- Chang L, Karin M. Mammalian MAP kinase signalling cascades. *Nature.* 2001; 410:37–40. [PubMed: 11242034]
- Cox LJ, Hengst U, Gurskaya NG, Lukyanov KA, Jaffrey SR. Intra-axonal translation and retrograde trafficking of CREB promotes neuronal survival. *Nat Cell Biol.* 2008
- Dickson BJ. Molecular mechanisms of axon guidance. *Science.* 2002; 298:1959–1964. [PubMed: 12471249]

- Dodd J, Morton SB, Karagogeos D, Yamamoto M, Jessell TM. Spatial regulation of axonal glycoprotein expression on subsets of embryonic spinal neurons. *Neuron*. 1988; 1:105–116. [PubMed: 3272160]
- Eng H, Lund K, Campenot RB. Synthesis of beta-tubulin, actin, and other proteins in axons of sympathetic neurons in compartmented cultures. *J Neurosci*. 1999; 19:1–9. [PubMed: 9870932]
- Eom T, Antar LN, Singer RH, Bassell GJ. Localization of a beta-actin messenger ribonucleoprotein complex with zipcode-binding protein modulates the density of dendritic filopodia and filopodial synapses. *J Neurosci*. 2003; 23:10433–10444. [PubMed: 14614102]
- Gallo G, Letourneau PC. Regulation of growth cone actin filaments by guidance cues. *J Neurobiol*. 2004; 58:92–102. [PubMed: 14598373]
- Gingras AC, Raught B, Sonenberg N. eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annu Rev Biochem*. 1999; 68:913–963. [PubMed: 10872469]
- Giuditta A, Chun JT, Eyman M, Cefaliello C, Bruno AP, Crispino M. Local gene expression in axons and nerve endings: the glia-neuron unit. *Physiol Rev*. 2008; 88:515–555. [PubMed: 18391172]
- Gu W, Pan F, Zhang H, Bassell GJ, Singer RH. A predominantly nuclear protein affecting cytoplasmic localization of beta-actin mRNA in fibroblasts and neurons. *J Cell Biol*. 2002; 156:41–51. [PubMed: 11781334]
- Guirland C, Buck KB, Gibney JA, DiCicco-Bloom E, Zheng JQ. Direct cAMP signaling through G-protein-coupled receptors mediates growth cone attraction induced by pituitary adenylate cyclase-activating polypeptide. *J Neurosci*. 2003; 23:2274–2283. [PubMed: 12657686]
- Hachet O, Ephrussi A. Splicing of oskar RNA in the nucleus is coupled to its cytoplasmic localization. *Nature*. 2004; 428:959–963. [PubMed: 15118729]
- Harris WA, Holt CE, Bonhoeffer F. Retinal axons with and without their somata, growing to and arborizing in the tectum of *Xenopus* embryos: a time-lapse video study of single fibres in vivo. *Development*. 1987; 101:123–133. [PubMed: 3449363]
- Hengst U, Cox LJ, Macosko EZ, Jaffrey SR. Functional and selective RNA interference in developing axons and growth cones. *J Neurosci*. 2006; 26:5727–5732. [PubMed: 16723529]
- Hirokawa N. mRNA transport in dendrites: RNA granules, motors, and tracks. *J Neurosci*. 2006; 26:7139–7142. [PubMed: 16822968]
- Hirokawa N, Takemura R. Molecular motors and mechanisms of directional transport in neurons. *Nat Rev Neurosci*. 2005; 6:201–214. [PubMed: 15711600]
- Huang YS, Carson JH, Barbarese E, Richter JD. Facilitation of dendritic mRNA transport by CPEB. *Genes Dev*. 2003; 17:638–653. [PubMed: 12629046]
- Huff T, Muller CS, Otto AM, Netzker R, Hannappel E. beta-Thymosins, small acidic peptides with multiple functions. *Int J Biochem Cell Biol*. 2001; 33:205–220. [PubMed: 11311852]
- Huttelmaier S, Zenklusen D, Lederer M, Dictenberg J, Lorenz M, Meng X, Bassell GJ, Condeelis J, Singer RH. Spatial regulation of beta-actin translation by Src-dependent phosphorylation of ZBP1. *Nature*. 2005; 438:512–515. [PubMed: 16306994]
- Jimenez-Diaz L, Geranton SM, Passmore GM, Leith JL, Fisher AS, Berliocchi L, Sivasubramaniam AK, Sheasby A, Lumb BM, Hunt SP. Local translation in primary afferent fibers regulates nociception. *PLoS ONE*. 2008; 3:e1961. [PubMed: 18398477]
- Jin P, Zarnescu DC, Ceman S, Nakamoto M, Mowrey J, Jongens TA, Nelson DL, Moses K, Warren ST. Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. *Nat Neurosci*. 2004; 7:113–117. [PubMed: 14703574]
- Jirikowski GF, Sanna PP, Bloom FE. mRNA coding for oxytocin is present in axons of the hypothalamo-neurohypophysial tract. *Proc Natl Acad Sci USA*. 1990; 87:7400–7404. [PubMed: 2268384]
- Kanai Y, Dohmae N, Hirokawa N. Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. *Neuron*. 2004; 43:513–525. [PubMed: 15312650]
- Kim J, Krichevsky A, Grad Y, Hayes GD, Kosik KS, Church GM, Ruvkun G. Identification of many microRNAs that copurify with polyribosomes in mammalian neurons. *Proc Natl Acad Sci USA*. 2004; 101:360–365. [PubMed: 14691248]

- Kindler S, Wang H, Richter D, Tiedge H. RNA transport and local control of translation. *Annu Rev Cell Dev Biol.* 2005; 21:223–245. [PubMed: 16212494]
- Kislauskis EH, Zhu X, Singer RH. Sequences responsible for intracellular localization of beta-actin messenger RNA also affect cell phenotype. *J Cell Biol.* 1994; 127:441–451. [PubMed: 7929587]
- Knowles RB, Sabry JH, Martone ME, Deerinck TJ, Ellisman MH, Bassell GJ, Kosik KS. Translocation of RNA granules in living neurons. *J Neurosci.* 1996; 16:7812–7820. [PubMed: 8987809]
- Koenig E. Synthetic mechanisms in the axon. IV. In vitro incorporation of [3H]precursors into axonal protein and RNA. *J Neurochem.* 1967; 14:437–446. [PubMed: 5336968]
- Koenig E, Adams P. Local protein synthesizing activity in axonal fields regenerating in vitro. *J Neurochem.* 1982; 39:386–400. [PubMed: 7086424]
- Koenig E, Giuditta A. Protein-synthesizing machinery in the axon compartment. *Neuroscience.* 1999; 89:5–15. [PubMed: 10051213]
- Koenig E, Koelle GB. Acetylcholinesterase regeneration in peripheral nerve after irreversible inactivation. *Science.* 1960; 132:1249–1250. [PubMed: 13757475]
- Koenig E, Martin R. Cortical plaque-like structures identify ribosome-containing domains in the Mauthner cell axon. *J Neurosci.* 1996; 16:1400–1411. [PubMed: 8778291]
- Koenig E, Martin R, Titmus M, Sotelo-Silveira JR. Cryptic peripheral ribosomal domains distributed intermittently along mammalian myelinated axons. *J Neurosci.* 2000; 20:8390–8400. [PubMed: 11069946]
- Kress TL, Yoon YJ, Mowry KL. Nuclear RNP complex assembly initiates cytoplasmic RNA localization. *J Cell Biol.* 2004; 165:203–211. [PubMed: 15096527]
- Krichevsky AM, Kosik KS. Neuronal RNA granules: a link between RNA localization and stimulation-dependent translation. *Neuron.* 2001; 32:683–696. [PubMed: 11719208]
- Kye MJ, Liu T, Levy SF, Xu NL, Groves BB, Bonneau R, Lao K, Kosik KS. Somatodendritic microRNAs identified by laser capture and multiplex RT-PCR. *Rna.* 2007; 13:1224–1234. [PubMed: 17592044]
- Lee SK, Hollenbeck PJ. Organization and translation of mRNA in sympathetic axons. *J Cell Sci.* 2003; 116:4467–4478. [PubMed: 13130093]
- Leung KM, Holt CE. Live visualization of protein synthesis in axonal growth cones by microinjection of photoconvertible Kaede into *Xenopus* embryos. *Nat Protoc.* 2008; 3:1318–1327. [PubMed: 18714300]
- Leung KM, van Horck FP, Lin AC, Allison R, Standart N, Holt CE. Asymmetrical beta-actin mRNA translation in growth cones mediates attractive turning to netrin-1. *Nat Neurosci.* 2006; 9:1247–1256. [PubMed: 16980963]
- Lin AC, Holt CE. Function and regulation of local axonal translation. *Curr Opin Neurobiol.* 2008; 18:60–68. [PubMed: 18508259]
- Litman P, Barg J, Rindzoonski L, Ginzburg I. Subcellular localization of tau mRNA in differentiating neuronal cell culture: implications for neuronal polarity. *Neuron.* 1993; 10:627–638. [PubMed: 8476613]
- Mann F, Miranda E, Weint C, Harmer E, Holt CE. B-type Eph receptors and ephrins induce growth cone collapse through distinct intracellular pathways. *J. Neurobiology.* 2003; 57:323–336.
- Merianda TT, Lin A, Lam J, Vuppalanchi D, Willis DE, Karin N, Holt CE, Twiss JL. A functional equivalent of endoplasmic reticulum and Golgi in axons for secretion of locally synthesized proteins. *Mol Cell Neurosci.* 2009; 40:128–142. [PubMed: 19022387]
- Min H, Turck CW, Nikolic JM, Black DL. A new regulatory protein, KSRP, mediates exon inclusion through an intronic splicing enhancer. *Genes Dev.* 1997; 11:1023–1036. [PubMed: 9136930]
- Ming GL, Wong ST, Henley J, Yuan XB, Song HJ, Spitzer NC, Poo MM. Adaptation in the chemotactic guidance of nerve growth cones. *Nature.* 2002; 417:411–418. [PubMed: 11986620]
- Olink-Coux M, Hollenbeck PJ. Localization and active transport of mRNA in axons of sympathetic neurons in culture. *J Neurosci.* 1996; 16:1346–1358. [PubMed: 8778286]
- Palacios IM, St. Johnston D. Getting the message across: the intracellular localization of mRNAs in higher eukaryotes. *Annu Rev Cell Dev Biol.* 2001; 17:569–614. [PubMed: 11687499]

- Piper M, Anderson R, Dwivedy A, Weinl C, van Horck F, Leung KM, Cogill E, Holt C. Signaling mechanisms underlying Slit2-induced collapse of *Xenopus* retinal growth cones. *Neuron*. 2006; 49:215–228. [PubMed: 16423696]
- Piper M, Salih S, Weinl C, Holt CE, Harris WA. Endocytosis-dependent desensitization and protein synthesis-dependent resensitization in retinal growth cone adaptation. *Nat Neurosci*. 2005; 8:179–186. [PubMed: 15643427]
- Rehbein M, Wege K, Buck F, Schweizer M, Richter D, Kindler S. Molecular characterization of MARTA1, a protein interacting with the dendritic targeting element of MAP2 mRNAs. *J Neurochem*. 2002; 82:1039–1046. [PubMed: 12358751]
- Richter JD. Cytoplasmic polyadenylation in development and beyond. *Microbiol Mol Biol Rev*. 1999; 63:446–456. [PubMed: 10357857]
- Rosoff WJ, Urbach JS, Esrick MA, McAllister RG, Richards LJ, Goodhill GJ. A new chemotaxis assay shows the extreme sensitivity of axons to molecular gradients. *Nat Neurosci*. 2004; 7:678–682. [PubMed: 15162167]
- Ross AF, Oleynikov Y, Kislauskis EH, Taneja KL, Singer RH. Characterization of a beta-actin mRNA zipcode-binding protein. *Mol Cell Biol*. 1997; 17:2158–2165. [PubMed: 9121465]
- Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M, Greenberg ME. A brain-specific microRNA regulates dendritic spine development. *Nature*. 2006; 439:283–289. [PubMed: 16421561]
- Sossin WS, DesGroseillers L. Intracellular trafficking of RNA in neurons. *Traffic*. 2006; 7:1581–1589. [PubMed: 17054760]
- Sotelo-Silveira JR, Calliari A, Kun A, Benech JC, Sanguinetti C, Chalar C, Sotelo JR. Neurofilament mRNAs are present and translated in the normal and severed sciatic nerve. *J Neurosci Res*. 2000; 62:65–74. [PubMed: 11002288]
- Stein E, Tessier-Lavigne M. Hierarchical organization of guidance receptors: silencing of netrin attraction by slit through a Robo/DCC receptor complex. *Science*. 2001; 291:1928–1938. [PubMed: 11239147]
- Steward O, Levy WB. Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus. *J Neurosci*. 1982; 2:284–291. [PubMed: 7062109]
- Tennyson VM. The fine structure of the axon and growth cone of the dorsal root neuroblast of the rabbit embryo. *J Cell Biol*. 1970; 44:62–79. [PubMed: 5409464]
- Tessier-Lavigne M, Goodman CS. The molecular biology of axon guidance. *Science*. 1996; 274:1123–1133. [PubMed: 8895455]
- Tohda C, Sasaki M, Konemura T, Sasamura T, Itoh M, Kuraishi Y. Axonal transport of VR1 capsaicin receptor mRNA in primary afferents and its participation in inflammation-induced increase in capsaicin sensitivity. *J Neurochem*. 2001; 76:1628–1635. [PubMed: 11259480]
- Trembleau A, Morales M, Bloom FE. Differential compartmentalization of vasopressin messenger RNA and neuropeptide within the rat hypothalamo-neurohypophysial axonal tracts: light and electron microscopic evidence. *Neuroscience*. 1996; 70:113–125. [PubMed: 8848117]
- van Kesteren RE, Carter C, Dissel HM, van Minnen J, Gouwenberg Y, Syed NI, Spencer GE, Smit AB. Local synthesis of actin-binding protein beta-thymosin regulates neurite outgrowth. *J Neurosci*. 2006; 26:152–157. [PubMed: 16399682]
- Weiner OD, Zorn AM, Krieg PA, Bittner GD. Medium weight neurofilament mRNA in goldfish Mauthner axoplasm. *Neurosci Lett*. 1996; 213:83–86. [PubMed: 8858614]
- Wensley CH, Stone DM, Baker H, Kauer JS, Margolis FL, Chikaraishi DM. Olfactory marker protein mRNA is found in axons of olfactory receptor neurons. *J Neurosci*. 1995; 15:4827–4837. [PubMed: 7623114]
- Willis D, Li KW, Zheng JQ, Chang JH, Smit A, Kelly T, Merianda TT, Sylvester J, van Minnen J, Twiss JL. Differential transport and local translation of cytoskeletal, injury-response, and neurodegeneration protein mRNAs in axons. *J Neurosci*. 2005; 25:778–791. [PubMed: 15673657]
- Willis DE, van Niekerk EA, Sasaki Y, Mesngon M, Merianda TT, Williams GG, Kendall M, Smith DS, Bassell GJ, Twiss JL. Extracellular stimuli specifically regulate localized levels of individual neuronal mRNAs. *J Cell Biol*. 2007; 178:965–980. [PubMed: 17785519]

- Wu KY, Hengst U, Cox LJ, Macosko EZ, Jeromin A, Urquhart ER, Jaffrey SR. Local translation of RhoA regulates growth cone collapse. *Nature*. 2005; 436:1020–1024. [PubMed: 16107849]
- Yamada KM, Spooner BS, Wessells NK. Ultrastructure and function of growth cones and axons of cultured nerve cells. *J Cell Biol*. 1971; 49:614–635. [PubMed: 4326456]
- Yao J, Sasaki Y, Wen Z, Bassell GJ, Zheng JQ. An essential role for beta-actin mRNA localization and translation in Ca²⁺-dependent growth cone guidance. *Nat Neurosci*. 2006; 9:1265–1273. [PubMed: 16980965]
- Zalfa F, Achsel T, Bagni C. mRNPs, polysomes or granules: FMRP in neuronal protein synthesis. *Curr Opin Neurobiol*. 2006; 16:265–269. [PubMed: 16707258]
- Zelenà J. Ribosome-like particles in myelinated axons of the rat. *Brain Res*. 1970; 24:359–363. [PubMed: 5490299]
- Zhang HL, Eom T, Oleynikov Y, Shenoy SM, Liebelt DA, Dictenberg JB, Singer RH, Bassell GJ. Neurotrophin-induced transport of a beta-actin mRNP complex increases beta-actin levels and stimulates growth cone motility. *Neuron*. 2001; 31:261–275. [PubMed: 11502257]
- Zhang HL, Singer RH, Bassell GJ. Neurotrophin regulation of beta-actin mRNA and protein localization within growth cones. *J Cell Biol*. 1999; 147:59–70. [PubMed: 10508855]

Fig. 1. Growth cone steering in response to guidance cues in the absence or presence of protein synthesis inhibitors. **(a)** Growth cone turns toward the gradient of an attractive cue. **(b)** Growth cone turns away from the gradient of a repulsive cue. **(c)** Attractive turning response is abolished when local protein synthesis is inhibited. **(d)** Repulsive turning response is also abolished when local protein synthesis is inhibited

Fig. 2.

Axonal mRNA localization in neurons. **(a)** Recognition of mRNA *cis*-acting elements (orange) by *trans*-acting factors (blue ovals) in the nucleus forming RNP complexes. **(b)** Nuclear export of RNP particles into the soma and recruitment of additional *trans*-acting factors (red ovals) into the complex. **(c)**. Transport of remodeled RNP or RNA granule along the cytoskeleton (light blue lines) with the help of motor proteins (black star) to the axon/growth cone. Translational machinery including ribosomes (grey ovals) attach to the RNA granule to initiate protein synthesis. Ribosomes may even attach to the RNA granule earlier during transport but begin translation only after reaching its final destination

Fig. 3. “Differential translational” model describing local protein synthesis and growth cone steering. A gradient of an attractive cue (e.g., netrin-1) elicits asymmetric phosphorylation of translation initiation factor and subsequent asymmetric synthesis of proteins that build up the cytoskeleton closer to the gradient. A gradient of a repulsive cue (e.g., slit2) elicits asymmetric synthesis of proteins that break down the cytoskeleton close to the gradient, resulting in growth cone steering away from the cue. Adapted from Lin and Holt (2008)

Table 1

Differential translation of axonal mRNAs in response to distinct cues

External cue	mRNA level	Reference
Netrin-1	β -Actin $\uparrow$	Leung et al. (2006)
	Enolase $\leftrightarrow$	
Sema3A	RhoA $\uparrow$	Wu et al. (2005)
Slit2	Cofilin $\uparrow$	Piper et al. (2006)
	β -Actin $\leftrightarrow$	
BDNF	β -Actin $\uparrow$	Yao et al. (2006)
Nerve growth factor (NGF)	cAMP-responsive element (CRE)-binding protein (CREB) $\uparrow$	Cox et al. (2008)
KCl depolarization	κ -Opioid receptor $\uparrow$	Bi et al. (2006)