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RNA-based mechanisms underlying axon guidance

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Axon guidance plays a key role in establishing neuronal circuitry. The motile tips of growing axons, the growth cones, navigate by responding directionally to guidance cues that pattern the embryonic neural pathways via receptor-mediated signaling. Evidence in vitro in the last decade supports the notion that RNA-based mechanisms contribute to cue-directed steering during axon guidance. Different cues trigger translation of distinct subsets of mRNAs and localized translation provides precise spatiotemporal control over the growth cone proteome in response to localized receptor activation. Recent evidence has now demonstrated a role for localized translational control in axon guidance decisions in vivo.

Introduction

During development, a neuron extends its axon through a complex and precise path to reach its final destination by sensing extracellular molecules called guidance cues. These cues are either locally tethered to, or diffuse from, intermediate or final targets and are sensed by the growth cone, a complex, motile structure at the leading edge of the extending axon. A wide range of extrinsic signals such as guidance molecules and growth factors are detected through specific receptors on the surface of growth cones and are integrated and translated into structural changes of the cytoskeleton that determine the rate and direction of extension (Hedgecock et al., 1990; Kennedy et al., 1994; McFarlane and Holt, 1996; Tessier-Lavigne and Goodman, 1996; Smith, 1988; Dickson, 2002).

The navigational movement of the growth cone has numerous parallels with the chemotactic migration of cells, except that growth cones rarely reverse direction, and many aspects of the underlying molecular mechanisms of migration are shared (Mortimer et al., 2008). However, the growth cones are often located far from their neuronal cell bodies (for example, retinal ganglion cells must send their axons several millimeters to the midbrain), and the spatial separation from the nucleus endows distinct characteristics to the neuronal growth cone. When a growth cone is severed from the cell body, it continues to exhibit chemotropic responses, indicating that the growth cone functions as a semi-autonomous apparatus that contains all the machinery required to sense and respond to the extracellular environment (Harris et al., 1987; Campbell and Holt, 2001). The finding that the cue-induced responses of severed axons are hampered by protein synthesis inhibitors provides strong evidence that the autonomous nature of the growth cone involves local mRNA translation (Campbell and Holt, 2001; Wu et al., 2005). In the last decade, scientific evidence has accumulated indicating that the local mRNA translation plays a potentially important role in axon guidance. Here, we review the basic roles and mechanisms of local protein synthesis in axon guidance and discuss recent findings that localized translational control is involved in guidance decisions in vivo. A comprehensive review of axon guidance is beyond the scope of this review, and the reader is referred to several excellent recent reviews that cover this topic (Mortimer et al., 2008; Quinn and Wadsworth, 2008; Geraldo and Gordon-Weeks, 2009; Bai and Pfaff, 2011; Kolodkin and Tessier-Lavigne, 2011; Tojima et al., 2011; Vitriol and Zheng, 2012; Dudanova and Klein, 2013).

Axon guidance and spatiotemporal protein distribution

A key process in axon guidance is the chemotropic response of growth cones, in which guidance cues control the growth cone motility through directed cytoskeletal remodeling. The four classic classes of guidance cues—netrins, semaphorins, slits, and ephrins—elicit attractive or repulsive responses in growth cones in vitro via specific receptors (Table 1; Kapfhammer and Raper, 1987; Kennedy et al., 1994; Serafini et al., 1994; Cheng et al., 1995; Drescher et al., 1995; Fan and Raper, 1995; Brose et al., 1999; Kidd et al., 1999). The list of guidance molecules has expanded enormously in recent years as other classes of molecules such as morphogens, growth factors, and cytokines have been shown to influence axon growth (McFarlane and Holt, 1996; Trousse et al., 2001; Chalasani et al., 2003; Lyuksyutova et al., 2003). Most cues seem to act bifunctionally in the sense that the response they elicit, attraction versus repulsion, depends on

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Abbreviations used in this paper: DRG, dorsal root ganglion; NMD, nonsense-mediated mRNA decay; RGC, retinal ganglion cell; Sema3A, semaphorin 3A; TOR, target of rapamycin.

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Table 1. **Guidance cues and local protein synthesis**

Guidance cue	Receptor	Effect on protein synthesis	Target mRNA involved in axon guidnace	Response mediated by protein synthesis	Neuron type	Reference
Netrin1	DCC, UNC-5	Induce	*β-actin*	Attractive	RGC	Campbell and Holt, 2001; Leung et al., 2006; Yao et al., 2006; Welshhans and Bassell, 2011
Sema3A	Neuropilin1, PlexinA	Induce	*Rho A* *NFPC*	Repulsive Caudal turn	DRG RGC	Campbell and Holt, 2001; Wu et al., 2005; Leung et al., 2013
Slit2	Robo3	Induce	*Cofilin*	Repulsive	RGC	Campbell and Holt, 2001; Piper et al., 2006
Ephrin A	EphA	Suppress	ND	Repulsive	RGC	Nie et al., 2010
Ephrin B	EphB	None	None	None	RGC	Mann et al., 2003
Engrailed	ND	Induce	ND	Attractive, Repulsive	RGC	Brunet et al., 2005
BDNF	TrkB	Induce	*β-actin*	Attractive, Repulsive	Spinal, hippocampal, and cortical	Zhang et al., 1999; Yao et al., 2006; Sasaki et al., 2010
SFRP1	Fz2	Induce	ND	Attractive	RGC	Rodriguez et al., 2005
NGF	TrkA	Induce	*Par3, β-actin* *WAVE1, cortactin, Arp2*	Elongation Branching	RGC DRG	Hengst et al., 2009 Spillane et al., 2012
CSPGs	NgR1, 3	Induce	*Rho A*	Axonal growth inhibition	DRG	Dickendesher et al., 2012; Walker et al., 2012

BDNF, brain-derived neurotrophic factor; SFRP1, secreted frizzled-related protein 1; NFPC, NF-protocadherin; NGF, nerve growth factor; CSPGs, chondroitin sulfate proteoglycans; RGC, retinal ganglion cell; DRG, dorsal root ganglion neuron. ND, not determined.

several modulating factors such as the intracellular level of second messengers, such as cAMP and cGMP (Ming et al., 1997; Song et al., 1998), the molecular composition of the extracellular microenvironment (Höpker et al., 1999), and the age of the growth cone (Campbell et al., 2001; Shewan et al., 2002). Axons of projection neurons grow long distances before reaching their final target and their pathway is broken up into molecularly distinct steps with intermediate targets acting as "stepping stones" (Bate, 1976; Zou et al., 2000). The floorplate at the midline of the spinal cord, for example, secretes netrin-1 and acts as an attractive intermediate target for commissural axons (Tessier-Lavigne et al., 1988; Kennedy et al., 1994). Remarkably, in order to progress beyond the floorplate, growth cones use an anti-linger mechanism which coordinately switches off attraction and turns on repulsion to the floorplate (Kidd et al., 1998; Zou et al., 2000). As discussed later, recent evidence indicates that local translation partly controls this switch (Colak et al., 2013).

An important aspect of mechanisms underlying axon guidance is the spatial and temporal control of protein distribution at the subcellular level. For example, an extracellular gradient of a guidance cue elicits not only the polarized activation or repression of components of intracellular signaling pathways, but also the asymmetric distribution of proteins including cytoskeletal proteins and cell surface receptors in the growth cone (Lin and Forscher, 1993; Zhou et al., 2002; Leung et al., 2006; Yao et al., 2006; Bouzigues et al., 2007). Furthermore, the spatiotemporally restricted expression of guidance cue receptors has been demonstrated to be essential for the switching of the growth cone response to the cues at intermediate targets (Chen et al., 2008). Localized patterns of protein distribution in cellular compartments occur mainly by three different mechanisms: protein transport (including exocytosis/endocytosis), protein degradation, and local protein synthesis. All of these mechanisms have been implicated in axon guidance in a nonredundant manner (Campbell and Holt, 2001; Guirland et al., 2004; Bouzigues et al., 2007).

Among these mechanisms, local protein synthesis has theoretical advantages in certain situations (Lin and Holt, 2007). Particularly, for example, when the ectopic presence of a protein is harmful to the axon/growth cone functions, then local translation coupled with the transport of translationally silenced mRNA is potentially advantageous. When a rapid (minutes) and local increase of a protein is required in growth cones during processes such as chemotropic responses, translation of mRNAs stored in the growth cone is a much faster way to express the genes than axonal transport of proteins from the cell body (the velocity of fast axonal transport is ∼1.0–5.0 µm/s [Stokin and Goldstein, 2006]). Thus, local translation provides a fast, "on demand" supply of a protein that, within the confines of a subcellular compartment where just a few de novo molecules (e.g., receptors) can make a functional difference, enables exquisite tuning of growth cones to their microenvironment.

Evidence for local mRNA translation and its physiological roles in axon guidance

The first evidence for protein synthesis in axons was reported more than 40 years ago using metabolic labeling techniques (Koenig, 1967; Giuditta et al., 1968). These initial findings were criticized at the time because the small amount of axonal labeling (only a small percentage of the cellular total) was thought to be due to low-level contamination by cell body material. Ultrastructural studies in the 1970s, however, reported the presence of ribosomes in cultured growth cones and young axons (Tennyson, 1970; Zelená, 1970; Bunge, 1973) and, in the last 15 years, biochemical and immunocytochemical approaches have confirmed the presence of axonal ribosomes (Koenig, 1979; Giuditta et al., 1991; Koenig and Martin, 1996; Bassell et al., 1998) along with other components of the translation machinery, including

translation initiation factors, mRNA, tRNA, aminoacyl-tRNA synthetases, elongation factors, Golgi, and endoplasmic reticulum proteins (Black and Lasek, 1977; Giuditta et al., 1977; Gioio et al., 1994; Giustetto et al., 2003; Merianda et al., 2009). Axonal translation was first linked with axon guidance by experiments done on isolated axons (severed from their cell bodies) showing that metabolic labeling representing new protein synthesis increased significantly after addition of the guidance cues netrin-1 and semaphorin 3A (Sema3A; Campbell and Holt, 2001).

The functional role(s) of local mRNA translation in axon guidance has been challenging to address, particularly in vivo, partly due to the technical difficulty of inhibiting protein synthesis exclusively in the axonal compartment. However, techniques such as compartmentalized cultures, antisense morpholinos, and small interfering (si)RNA are enabling the block of translation of specific mRNAs with increasing spatial and temporal precision. This was first demonstrated using chemotropic assays in *Xenopus* retinal ganglion cell (RGC) axons separated from their cell bodies. In these surgically isolated axons, translation inhibitors (anisomycin or cycloheximide) were found to block the attractive turning response of growth cones to a gradient of netrin-1 (Campbell and Holt, 2001). A similar dependence on protein synthesis was observed with the chemotropic "collapse" response of growth cones to repellents such as Sema3A or Slit2 (Campbell and Holt, 2001; Wu et al., 2005; Piper et al., 2006). In the growth cone collapse assay, global application of a repellent cue triggers the rapid (10 min) withdrawal of filopodia and lamellipodia causing growth cones to lose their expanded complex morphology and assume a cigar-shaped (collapsed) profile that is easily quantified. Subsequent in vitro studies, using pharmacological translational inhibitors or antisense morpholinos, which block translation of specific mRNAs, showed that local mRNA translation plays a prominent part in guidance processes that are regulated by a variety of extrinsic cues, such as Slit2, engrailed 1 and 2, and brain-derived neurotrophic factor (BDNF; Guirland et al., 2003; Brunet et al., 2005; Leung et al., 2006; Piper et al., 2006; Yao et al., 2006; Wizenmann et al., 2009). Ephrin-mediated growth cone collapse is not significantly affected by protein synthesis inhibition, indicating that not all cues trigger local translation (Mann et al., 2003).

Recently it has been discovered that the dependence of chemotropic responses of axonal growth cones on local protein synthesis varies according to the concentration of the guidance cue (Manns et al., 2012; Nédelec et al., 2012). This finding helps to resolve a controversy surrounding the role of local mRNA translation in axon guidance and brings fresh insight. The growth cones of mouse spinal motor neurons and chick dorsal root ganglion (DRG) neurons exhibit protein synthesis–dependent collapse only at low concentrations (<500 ng/ml) of Sema3A. Above this level, growth cone collapse is protein synthesis–independent and, in DRGs, depends on GSK-3β activation (Manns et al., 2012; Nédelec et al., 2012). This concentration dependence of axonal protein synthesis provides a simple and plausible explanation for the discrepant interpretation of results in a report using DRG neurons which stated that "protein synthesis in distal axons is not required for growth cone responses to guidance cues" (Roche et al., 2009), because a high concentration of Sema3A (1 µg/ml) was used for the collapse experiments, far beyond the protein synthesis–sensitive range in DRGs. It is noteworthy that all the other results reported in this study indicated that Sema3A does stimulate axonal protein synthesis (e.g., increased amino acid incorporation, activation of eIF4E). The differential requirement for protein synthesis at low versus high cue concentrations leading to the activation of distinct signaling pathways may enable growth cones to extract more information from a limited set of guidance cues and/or may be an important part of the mechanism underlying growth cone adaptation (Piper et al., 2006).

Roles of local mRNA translation in axon guidance

The studies using translational inhibitors led to the question of what proteins are the critical targets of local translation for axon guidance. β-Actin was the first mRNA to be visualized using in situ hybridization in rat cortical axonal growth cones (Bassell et al., 1998) and has subsequently been found to be universally present in growing axons. A gradient of netrin-1 induces asymmetric translation of β-actin on the side of the growth cone closest to the gradient source, and the suppression of local synthesis of β-actin by antisense oligonucleotides or morpholinos abolishes growth cone attraction in *Xenopus* (Fig. 1; Leung et al., 2006; Yao et al., 2006). On the other hand, repulsive cues such as Slit2 and Sema3A induce the axonal translation of cytoskeletal regulators such as RhoA and cofilin, both of which control actin polymerization, and the inhibition of RhoA translation impairs Sema3A-induced growth cone collapse (Fig. 2; Wu et al., 2005; Piper et al., 2006). This indicates that cue-induced local mRNA translation modulates guidance pathways through the spatiotemporal up-regulation of their components. Importantly, these local translation events are rapid, on the time scale of minutes, with de novo proteins appearing before overt collapse and turning occurs. These studies support the idea that the local synthesis of β-actin and its regulators mediate chemotropic responses of growth cones through promoting spatially polarized assembly or disassembly of the cytoskeleton (Fig. 1).

Recent studies conducted in vivo showed that local protein synthesis is also involved in regulatory mechanisms that orchestrate the chemotropic responses of growth cones to navigate precisely in the developing nervous system. During midline crossing, several receptors for repulsive guidance cues are expressed only on distal axon segments. This restricted expression pattern is important for neutralizing the repulsion between the midline intermediate targets, which express the repulsive cues, and precrossing axons. A previous study based on a fluorescent translation reporter analysis suggested that the spatially restricted expression of EphA2 in distal axon segments occurs through translational control (Brittis et al., 2002). Recent reports have revealed a novel RNA-based mechanism for the expression control in the midline crossing (Colak et al., 2013). The receptor Robo3.2, whose interaction with Slit mediates the repulsive response of axons, is selectively expressed on the post-crossing distal segment of commissural axons. This restricted expression is crucial for the axons to cross through the Slit-expressing midline (Chen et al., 2008). The Robo3.2 transcript has a premature termination codon (PTC) and is a potential target for nonsense-mediated mRNA decay (NMD), a translation-dependent quality-control

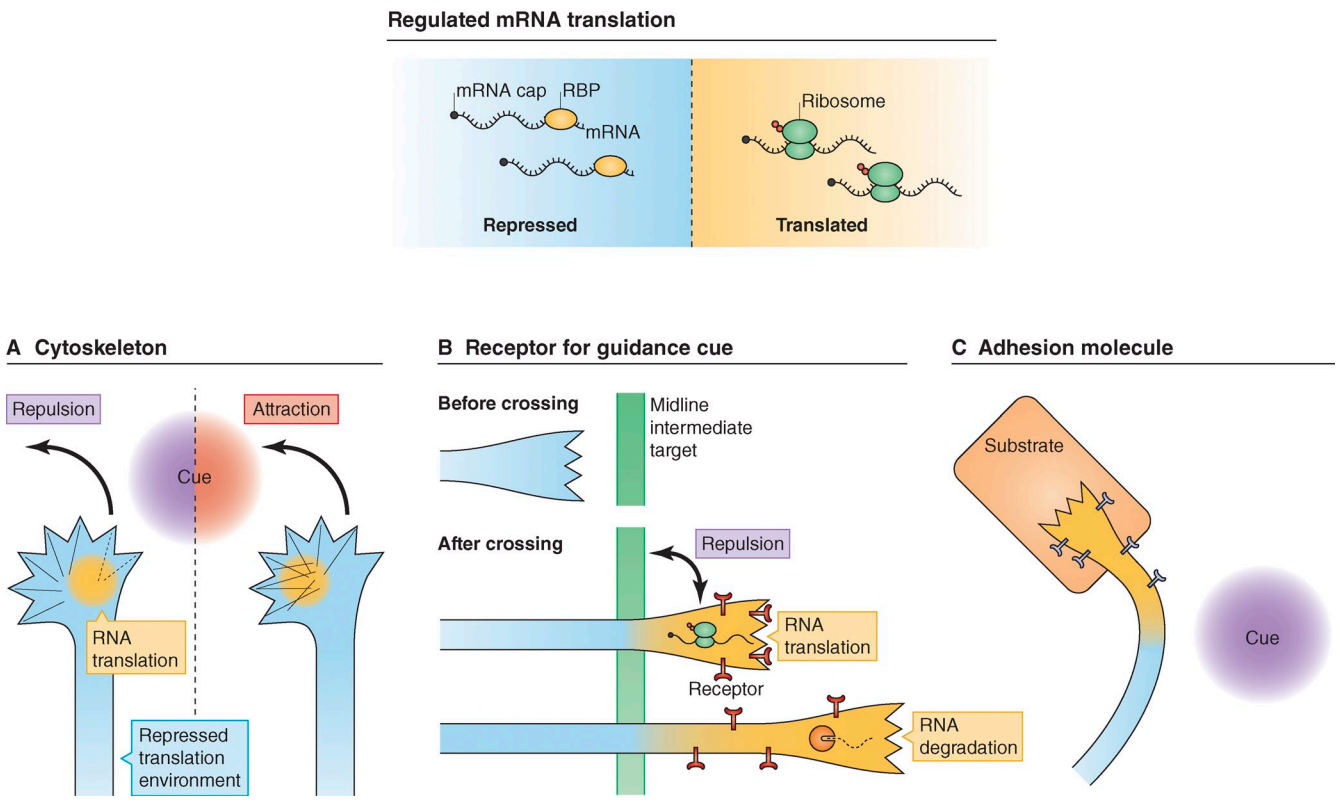

Figure 1. **How regulated mRNA translation mediates axon guidance.** (A) Several studies support a model in which guidance cue–induced asymmetrical synthesis of cytoskeletal proteins, or their regulators, mediates attractive/repulsive responses in growth cones through the polarization of cytoskeletal dynamics (Wu et al., 2005; Leung et al., 2006; Piper et al., 2006; Lin and Holt, 2007). (B) During midline crossing of axons, the receptor Robo 3.2 for the repulsive guidance cue Slit present at midline intermediate targets is expressed only on distal axon segments after crossing. This spatiotemporal expression pattern is formed through translational control to avoid the repulsion between the midline intermediate targets and axons that have not yet reached the midline (Colak et al., 2013). The Robo3.2 transcript, which harbors a PTC, is degraded by the NMD pathway after local translation, modulating its expression levels (Colak et al., 2013). (C) Axonal translation of an adhesion molecule, NF-protocadherin, is triggered by regionally expressed Sema3A in the visual pathway, resulting in increased adhesion between axons and the substrate and helping axons to turn correctly in vivo (Leung et al., 2013). RBP, RNA-binding protein; Cue, guidance cue.

mechanism for mRNAs (Maquat, 1995). The study shows that floorplate signals in the spinal cord midline induce the local translation of Robo3.2 in growing commissural axons, and the induced translation triggers the NMD-directed degradation of its own transcripts (Fig. 1; Colak et al., 2013). Conditional knockout mice that lack an essential NMD factor, Upf2, specifically in commissural neurons, has an increased level of the Robo3.2 protein in distal axon segments after crossing and exhibits defective guidance in this region, suggesting that NMD controls the Robo3.2 protein levels with regional precision for correct axon guidance (Fig. 1; Colak et al., 2013).

A cell adhesion molecule is another target of pathway region-specific translational control in growing RGC axons (Leung et al., 2013). This study showed that the pathfinding accuracy of RGC axons at the caudal turn in the mid-optic tract depends on axon–substrate interactions mediated by NF-protocadherin (NFPC, PCDH-7), a homophilic cell adhesion molecule, which is expressed in RGC axons and in the mid-to-dorsal segment of the optic tract neuroepithelium. Live translation-reporter imaging in vivo showed that NFPC translation is switched on in growth cones only when they reach the caudal turn. Sema3A, which lies adjacent to this turn, triggers rapid, protein synthesis–dependent increases of NFPC in RGC axons, suggesting that guidance cue–induced local synthesis of an adhesion molecule modulates the strength of axon–substrate adhesion, thereby controlling the direction of axon pathfinding (Fig. 1).

The above studies reveal two remarkably diverse mechanisms for place-specific translation in growth cones, one involving self-regulated destruction of existing message and the other involving a translation boost. Both mechanisms rely on regionally expressed cue-induced translation and provide a way to synchronize growth cone sensitivity with its progressively changing microenvironment.

Mechanisms underlying local mRNA translation in the growth cone

To regulate protein synthesis with spatial and temporal precision, the growth cone requires mechanisms that avoid unwanted mRNA translation. Importantly, most of the known RNA-binding proteins that mediate mRNA transport repress translation, and this coupling mechanism is considered to be crucial to prevent the premature translation of mRNAs before reaching their destination (Fig. 3). The best-studied RNA-binding protein involved in axon guidance is zip-code binding protein 1 (ZBP1; Vg1RBP in *Xenopus*, IMP1 in human), which binds the "zip-code", a cis element in the 3′ UTR of β-actin mRNA (Ross

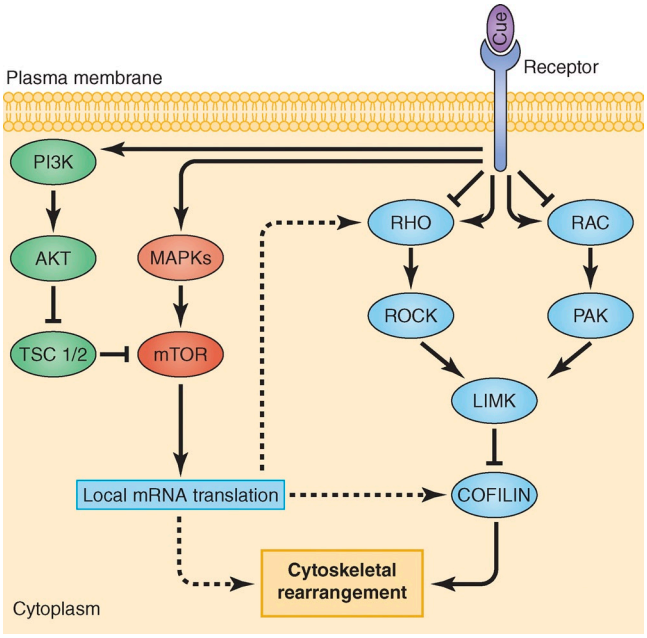

Figure 2. **Intracellular signaling pathways activated by guidance cues.** Members of the Rho subfamily of small GTPases, which includes Rac, Rho, and Cdc42, have been well established to mediate the chemotropic responses of growth cones through controlling cytoskeletal dynamics (Hall and Lalli, 2010). Rac and Rho activate LIM kinase (LIMK) through PAK and ROCK, and LIMK regulates the cofilin-mediated actin depolymerization. The mTOR pathway is also activated by several cue-induced responses through the PI3K and/or MAPKs pathways. mTOR regulates mRNA translation through phosphorylation of downstream targets (Brown et al., 1995; Brunn et al., 1997; Burnett et al., 1998). Cue-induced local mRNA translation modulates other guidance pathways through the spatiotemporal up-regulation of signaling components such as Rho A and cofilin (Wu et al., 2005; Piper et al., 2006). Arrows do not necessarily indicate direct interactions. Cue, guidance cue; Receptor, guidance cue receptor.

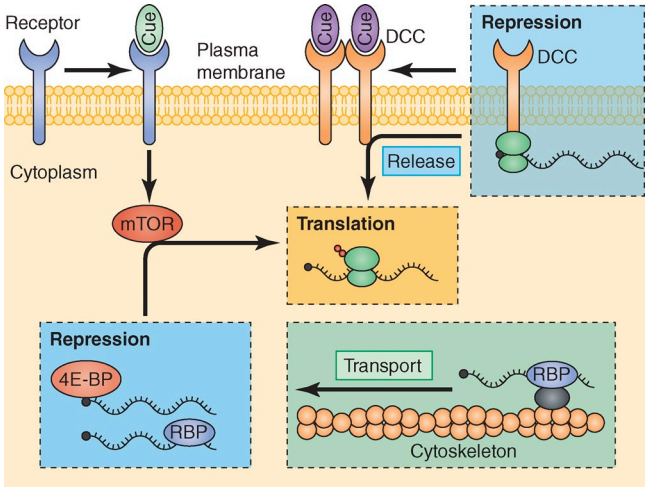

Figure 3. **Mechanisms linking local mRNA translation with guidance cue stimulation.** Most of the known RNA-binding proteins that mediate mRNA transport are responsible for repression of translation. Some guidance cues activate the mTOR pathway, and activated mTOR promotes mRNA translation through phosphorylation of downstream targets (Campbell and Holt, 2001). DCC, a receptor for netrin 1, is physically associated with the translation machinery including eukaryotic initiation factors and ribosomal large and small subunits to repress translation. Activation of DCC by netrin 1 triggers the release of the translation machinery to facilitate the translation (Tcherkezian et al., 2010). RBP, RNA-binding protein; Cue, guidance cue.

et al., 1997). When the β-actin mRNA–ZBP1 interaction is disrupted either by an antisense oligonucleotide targeting the zip-code sequence (Yao et al., 2006) or by the knock-out of the ZBP1 gene (Welshhans and Bassell, 2011), the cue-induced localization of β-actin mRNA in growth cones is significantly reduced, and the translation-dependent growth cone turning response is in turn abolished. These results suggest that ZBP1 interacts with the zip-code element to transport β-actin mRNA, and the interaction is important for growth cone turning. In vivo studies of Vg1RBP (ZBP1 homologue) and another RNA-binding protein, Hermes (RBPMS), in *Xenopus* and zebrafish retinal ganglion cells, respectively, show that loss of function of these genes causes severe defects in axon terminal arborization without affecting the long-range guidance from the eye to the tectum. These studies indicate that the translational regulation mediated by these RNA-binding proteins has a key role in the axon–target guided cell-specific interactions that lead to axon branching and selective synapse formation (Hörnberg et al., 2013; Kalous et al., 2013).

Fragile X mental retardation protein (FMRP), which is known to mediate mRNA delivery and translational repression of target mRNAs in dendrites (Zhang et al., 2001; Reeve et al., 2005; Dictenberg et al., 2008), was demonstrated to support Sema3A-induced growth cone collapse through the translational suppression of its binding partner, microtubule-associated protein 1B (MAP1B; Antar et al., 2006; Li et al., 2009). Transcripts that contain a cytoplasmic polyadenylation element (CPE) in their 3′UTR are recognized by CPE-binding protein (CPEB), and CPEB regulates their translation through cytoplasmic polyadenylation and localization (Hake and Richter, 1994; Richter and Klann, 2009; Nagaoka et al., 2012). The local synthesis of the guidance receptor EphA2 following axonal midline crossing as described above is implicated to be regulated by CPEB (Brittis et al., 2002). Besides RNA-binding proteins, initiation factor 4E (eIF4E)–binding protein 1 (4E-BP1; Pause et al., 1994), which is known to bind the eIF4E to repress translation initiation, has also been suggested to regulate axonal translation of β-actin mRNA (Fig. 3; Leung et al., 2006).

The mechanisms for translational repression need to be overcome to activate the protein synthesis when the proteins are required. One key pathway that mediates cue-induced protein synthesis in axons is the target of rapamycin (TOR) pathway, in which TOR regulates mRNA translation through phosphorylation of downstream targets (Fig. 2; Brown et al., 1995; Brunn et al., 1997; Burnett et al., 1998). TOR-dependent phosphorylation of 4E-BP1 causes the dissociation of 4E-BP1–eIF4E complex to activate the translation machinery, and the FMRP function is also regulated by the TOR pathway through the phosphorylation of the ribosomal protein S6 kinase (S6K; Narayanan et al., 2008). The inhibition of MAPKs prevents Netrin-1– and Sema3A-induced events including the phosphorylation of 4E-BP1, axonal protein synthesis, and the chemotropic responses, suggesting that the MAPKs link guidance cue signaling with the mammalian TOR (mTOR) pathway (Fig. 2; Campbell and Holt, 2003). The PI(3)-kinase (PI3K) pathway has also been demonstrated to mediate guidance cue signaling (Ming et al., 1999; Markus et al., 2002; Drinjakovic et al.,

2010), and components of the PI3K pathway, such as Akt and Tsc1/2, regulate the activation of the mTOR pathway (Fig. 2). Evidence from in vitro and in vivo studies indicate that dysregulation of the TOR pathway results in defects in several chemotropic responses. Rapamycin, an allosteric inhibitor of TOR, for example, blocks the Sema3A- and netrin-1–induced local protein synthesis and growth cone responses (Campbell and Holt, 2001). A recent study (Nie et al., 2010) demonstrated that Tsc2+/− mice, in which retinal mTOR activity is abnormally elevated, exhibit aberrant retinogeniculate projections, and EphA-mediated growth cone collapse is impaired in Tsc2+/− RGCs. Interestingly, this study also showed that EphA signaling depresses not only mTOR activities but also axonal protein synthesis, suggesting an important role of the regulation of mTOR-mediated local translation in the Ephrin A–induced chemotropic response (Nie et al., 2010). This finding, together with the observation that protein synthesis inhibition does not significantly affect the EphA-mediated growth cone collapse (Roche et al., 2009; Nie et al., 2010), opens the interesting possibility that other guidance cues previously classed as nonprotein synthesis-dependent (e.g., ephrinB/EphB; Mann et al., 2003) may also act to repress this master integrator of translational control. These findings support the view that the TOR pathway is a key component of the mechanism linking guidance cue signaling and translational control of mRNAs. Importantly, dysregulation of the mTOR pathway has been implicated in the pathophysiology of a number of neurological diseases, including autism, epilepsy, and other neurodevelopmental disorders (Wong, 2013). However, axons have been relatively neglected in this field, as the focus has been primarily on the post-synaptic compartment (dendrites and spines). It will be crucial in the future to examine the link between the axonal mRNA translation and human neurological diseases.

Another more direct mechanism that links guidance cue signaling to local mRNA translation has been reported by Flanagan and colleagues (Tcherkezian et al., 2010). This group has shown using coimmunoprecipitation that DCC, a transmembrane receptor for netrin-1, is physically associated with the translation machinery, including eukaryotic initiation factors, ribosomal large and small subunits. In response to netrin-1, DCC releases the translation machinery and promotes polysome-associated translation (Fig. 3). This attractive receptor–ribosomal coupling mechanism provides exquisite spatial control over translation such that activation of just a few receptors in a single filopodium could lead to highly localized proteomic changes and polarized axon growth. It will be important in the future to know whether this receptor–ribosomal mechanism is commonly used by other guidance receptors and whether it mediates mRNA-specific translation.

Future directions

For a long period of time, studies of mRNAs localized in axons were mainly focused on a small number of transcripts because of insufficient knowledge of axonal transcriptome. However, recent microarray studies identified thousands of transcripts in axons/growth cones (Zivraj et al., 2010; Gumy et al., 2011), indicating that the mRNA population in axons/growth cones is far more diverse and complicated than previously thought. Although not all of the mRNAs localized in axons are likely to have a role in axon guidance, knowledge of the mRNAs localized in axons provides new insight into how the local translation contributes to growth cone functions. One of the future challenges will be the systematic testing and identification of mRNAs whose translation in the growth cone contributes to axon guidance. As studies to date have indicated, most local protein synthesis involved in growth cone responses is stimulated by extrinsic cues. Therefore, it will be interesting to conduct studies based on deep sequencing for mRNAs co-purified with ribosomes (Sanz et al., 2009) or ribosome profiling technique (Ingolia et al., 2009) to detect the change in translation status of mRNAs during guidance cue responses. This approach could reveal whether signature sets of mRNAs are translated by specific cues and address whether there are overlapping sets of RNAs associated with common functions (e.g., mitochondrial). For the functional studies, local translation-blocking experiments through the axon-specific delivery of siRNA or morpholinos, or use of photo-activatable reagents, will be needed to discover the roles of candidate proteins synthesized de novo (Hengst et al., 2006; Shestopalov et al., 2007; Yoon et al., 2012). A particular challenge for the future will be to improve current methodology for conducting such subcellular (axon only) manipulations in vivo to gain an increased understanding of the precise physiological functions of axonal mRNA translation.

Another future challenge is to understand the sorting mechanisms that underlie the selective transport and translation of specific mRNAs. Recent transcriptome studies strongly suggested that the subcellular distribution of mRNAs in the neuron is formed in a highly selective manner (Andreassi et al., 2010; Zivraj et al., 2010). For example, a serial analysis of gene expression (SAGE) analysis (Andreassi et al., 2010) showed that, although transcripts encoding proteins related to cytoskeletal, synaptic, and nuclear functions were much more abundant in cell bodies than in axons, transcripts encoding mitochondrial proteins, ribosomal proteins, and proteins related to signal transduction were enriched in axons, indicating the presence of mechanisms for sorting of mRNAs. However, the underlying molecular mechanisms remain largely unknown. Discovery of growth cone–specific isoforms of mRNAs (e.g., splice variants) by RNA-seq studies would provide new clues for understanding the mechanisms governing specificity of the axonal mRNA transport. The future research tackling these challenges will have a significant impact on our understanding of the roles of local mRNA translation in axon guidance.

We apologize to the authors of papers we could not include in this review owing to space limitations. Illustrations were provided by Neil Smith, www.neilsmithillustration.co.uk.

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