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Local translation and directional steering in axons

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The assembly of functional neural circuits in the developing brain requires neurons to extend axons to the correct targets. This in turn requires the navigating tips of axons to respond appropriately to guidance cues present along the axonal pathway, despite being cellular 'outposts' far from the soma. Work over the past few years has demonstrated a critical role for local translation within the axon in this process *in vitro*, making axon guidance another process that requires spatially localized translation, among others such as synaptic plasticity, cell migration, and cell polarity. This article reviews recent findings in local axonal translation and discusses how new protein synthesis may function in growth cone guidance, with a comparative view toward models of local translation in other systems.

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Introduction

In the developing nervous system, growing axons are guided to their correct targets by a motile structure at their tips called the growth cone, which turns in response to attractive or repulsive cues in the extracellular environment. Much progress has been made on identifying guidance cues and their receptors, including the four 'classical' families, the netrins, ephrins, slits, and semaphorins, as well as many others. However, relatively little is known about the intracellular signaling mechanisms by which growth cones convert these signals into directional decisions. Recent studies have revealed that local axonal translation is involved in this process, helping to overturn the prevailing dogma that axons are incapable of protein synthesis (Koenig and Giuditta, 1999; Giuditta *et al*, 2002; Piper and Holt, 2004; Twiss and van Minnen, 2006).

Guidance cues induce chemotropic responses in growth cones *in vitro*; for example, bath application of repellents causes growth cones to withdraw filopodia and 'collapse', while gradients of guidance cues can cause either attractive or repulsive turning. Initial experiments revealed that axons isolated from their cell body still exhibit appropriate chemotropic responses *in vitro* to netrin-1 and Semaphorin3A, but these responses are blocked by protein synthesis inhibitors, indicating that local translation within the axon is required (Campbell and Holt, 2001). Later studies identified other guidance cues that also require local axonal translation to induce chemotropic responses: Slit2 (Piper *et al*, 2006), pituitary adenylate cyclase-activating peptide (PACAP) (Guirland *et al*, 2003), brain-derived neurotrophic factor (BDNF) (Yao *et al*, 2006), and Engrailed-2 (Brunet *et al*, 2005). The requirement for local translation is found in both *Xenopus* and mammals (Campbell and Holt, 2001; Wu *et al*, 2005), though not for all guidance cues (see below). Notably, guidance cues induce growth cone responses very quickly *in vitro*—collapse within 10 min, and early signs of axon turning within 15–20 min. Correspondingly, guidance cues induce translation quickly as well, with translation initiation activated within 5 min and significant radiolabeled amino acid incorporation within 10 min (Campbell and Holt, 2001). Moreover, local translation is not required for axon extension (Eng *et al*, 1999; Campbell and Holt, 2001), even over 48 h (Blackmore and Letourneau, 2007), suggesting a specific role in responding to guidance cues.

Local translation is also involved in growth cone adaptation during chemotaxis. Growth cones undergo cycles of desensitization and resensitization that serve to continuously reset their sensitivity as they 'climb up' a gradient, and the resensitization step (but not desensitization) requires protein synthesis (Ming *et al*, 2002; Piper *et al*, 2005). In addition, axons are often directed to intermediate targets or 'guideposts' (e.g., the midline) en route to their final destination, and in order to get there but not stay there, they need to switch from being attracted to the intermediate target to being repelled. Local synthesis of new receptors may be a mechanism to rapidly change their responsiveness to guidance cues for the next leg of their journey (Brittis *et al*, 2002). Finally, once growth cones arrive at the target, they must respond to synaptogenic signals from the target cell, such as BDNF. Here, too, BDNF-induced potentiation of presynaptic neurotransmitter release requires local axonal translation (Zhang and Poo, 2002). Beyond development, local axonal translation has a role in axonal regeneration (Zheng *et al*, 2001; Verma *et al*, 2005; Twiss and van Minnen, 2006; Willis and Twiss, 2006). These initial studies raised several questions: what proteins are synthesized in axons, how is their synthesis regulated, and why is their *de novo* synthesis important for growth cone chemotropic responses?

Cue-induced axonal translation

An emerging theme is that guidance cues induce rapid translation of cytoskeletal proteins or regulators based on

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whether they are attractive or repulsive: proteins induced by attractive cues build up the cytoskeleton, whereas proteins induced by repulsive cues break it down. For example, an attractive gradient of netrin-1 or BDNF induces asymmetrical translation of β-actin in axonal growth cones within 5 min (Leung *et al*, 2006; Yao *et al*, 2006). Attractive turning toward netrin-1 or BDNF is prevented by morpholino-based blockade of β-actin translation or de-regulation of β-actin translation by antisense binding to the β-actin 3′ untranslated region (UTR). In contrast, the repellent Slit2 induces a protein synthesis-dependent increase in growth cone cofilin-1, an actin depolymerizing factor, within 5 min (Piper *et al*, 2006). Another repellent, Semaphorin3A (Sema3A), induces axonal synthesis of the small GTPase RhoA, which is required for Sema3A-induced growth cone collapse (Wu *et al*, 2005). RhoA mediates neurite retraction through regulation of the actin cytoskeleton (Luo, 2000). Finally, local translation of β-thymosin, an actin monomer sequestering protein, reduces neurite length in *Lymnaea* neurons (van Kesteren *et al*, 2006).

Since both attractive and repulsive chemotropic responses can require protein synthesis (Campbell and Holt, 2001), the identity of proteins whose synthesis is activated may help determine the polarity of the response. By this model, attractive and repulsive turning are not 'mirror-symmetrical' phenomena where attractants promote extension on the 'near' side while repellents promote extension on the 'far' side, rather, attractants and repellents both act on the near side, but have opposite effects (Figure 1). In a similar example of attractive and repulsive turning involving distinct mechanisms, recent findings indicate that attractive, but not repulsive, turning requires asymmetrical exocytosis (Tojima *et al*, 2007). However, it remains to be seen whether repulsive gradients induce asymmetrical synthesis of proteins like RhoA, cofilin-1, or β-thymosin.

In addition to rapid synthesis of cytoskeletal proteins for immediate chemotropic responses, axons also locally translate transmembrane and secreted proteins to modulate future responsiveness, most likely over a longer timescale. The EphA2 receptor is upregulated in the post-midline segment of chick commissural axons, as is a translational reporter controlled by the 3′UTR of EphA2, suggesting that signals at the midline induce axons to locally translate EphA2 to change responsiveness to guidance cues after crossing (Brittis *et al*, 2002). Recent studies show that dorsal root ganglion (DRG) neurons translate κ-opioid receptor locally in axons, in response to KCl depolarization (Bi *et al*, 2006), and at least in the cell body in response to netrin-1 (Tsai *et al*, 2006), although it remains unclear what function local modulation of opioid responsivity serves. Finally, after the axon arrives at the target, synapse formation between *Aplysia* sensory and motor neurons requires local translation in the presynaptic terminal of sensorin (Lyles *et al*, 2006), a secreted neuropeptide that regulates presynaptic growth and synapse stabilization in an autocrine response (Hu *et al*, 2004).

These findings present a general cell biological puzzle with regard to how new proteins are processed. As the growth cone travels to its target, it increases the distance between itself and the cell body and becomes a cytoplasmic 'outpost' remote from the Golgi apparatus. Ultrastructural studies have not reported the classical signs of protein processing machinery in axons, such as membrane-bound ribosomes or Golgi stacks, although they have identified ribosomes and smooth endoplasmic reticulum (ER) (Tennyson, 1970; Yamada *et al*, 1971; Bunge, 1973; Zheng *et al*, 2001). However, ribosomes might be bound to membranes rarely, if axons only synthesize membrane proteins occasionally, and axonal protein export machinery may exhibit non-classical morphology, as is seen in the primitive eukaryote

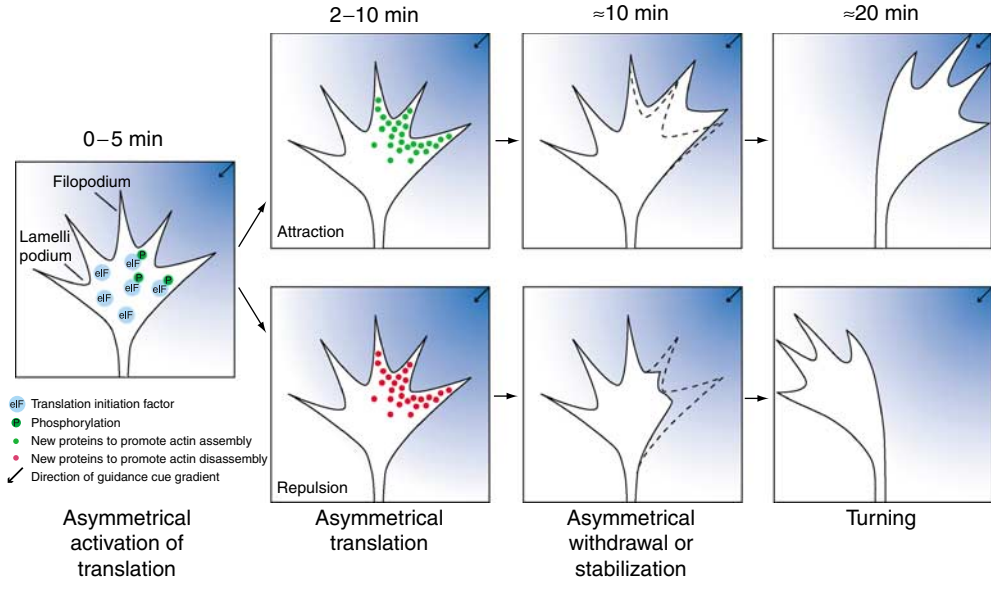

Figure 1 Hypothesis for cue-induced asymmetrical synthesis of cytoskeletal proteins. A guidance cue gradient causes an asymmetrical activation of translation initiation, 'opening the gates' to translation asymmetrically. mRNAs are selected for translation according to whether the guidance cue is attractive or repulsive, which may also depend on the internal state of the growth cone. For an attractive guidance cue, proteins that promote actin assembly are asymmetrically synthesized (green dots), whereas for a repulsive guidance cue, proteins that promote actin disassembly (red dots) are asymmetrically synthesized.

Giardia lamblia (Lujan *et al*, 1995). ER markers have been detected immunocytochemically in DRG axons (Willis *et al*, 2005) and both ER and Golgi markers are seen in *Aplysia* neurites (Lyles *et al*, 2006), but it is not yet clear whether these markers are localized to intracellular membranes. Moreover, vertebrates show sharper distinctions between axons and dendrites than invertebrates, and there are as yet no published accounts of axonal Golgi complex in vertebrates.

Regulation of translation in the growth cone

Protein synthesis can be regulated at a global level by translation initiation, and at an mRNA-specific level by transport, repression, and activation by RNA-binding proteins and microRNAs. We suggest that guidance cues activate translation initiation to 'open the gates' to translation, and use mRNA-specific regulation by RNA-binding proteins and microRNAs to select the appropriate proteins to synthesize for a given guidance cue response (Figures 1 and 2).

Global regulation of translation is achieved by translation initiation factors. Eukaryotic initiation factor 4E (eIF-4E) binds the 5′ cap of mRNAs and is the rate-limiting factor for cap-dependent translation. Hypophosphorylated eIF-4E-binding protein (eIF-4EBP) sequesters eIF-4E, preventing the recruitment of the rest of the translation initiation complex, while phosphorylation of eIF-4EBP releases eIF-4E, thus activating translation (Gebauer and Hentze, 2004). Netrin-1 and Sema3A induce phosphorylation of eIF-4EBP via MAP kinases and mammalian target of rapamycin (mTOR) (Campbell and Holt, 2001, 2003), and a netrin-1 gradient induces asymmetrical eIF-4EBP phosphorylation (Leung *et al*, 2006). Guidance cues also activate eIF-4E by phosphorylation, via MAP kinases and Mnk-1 (Campbell and Holt, 2003; Piper *et al*, 2006). Similar mechanisms have been described in local dendritic translation (Klann and Dever, 2004). Once translation is activated, how does the growth cone select which mRNAs to translate?

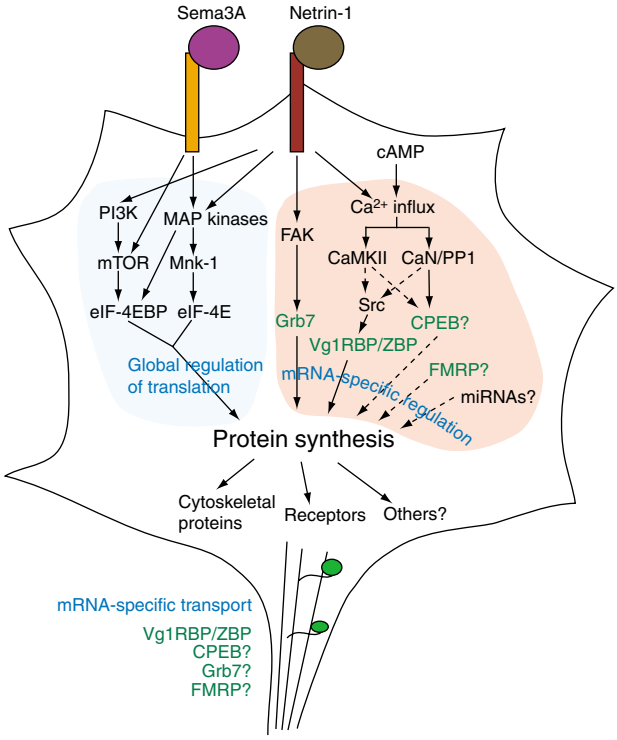

Figure 2 A model for regulation of translation in axonal growth cones. Guidance cues induce global activation of cap-dependent translation by activating translation initiation factors (left, 'global activation of translation'). This activation is largely 'permissive,' as translation of most mRNAs is also controlled by RNA-binding proteins and possibly microRNAs. By regulating these factors, different guidance cues—modulated by the internal state of the growth cone (e.g., cAMP levels)—can cause different effects by activating the translation of different mRNAs (right, 'mRNA-specific regulation'). Translation is also regulated by the differential transport of specific mRNAs to the growth cone, which is also controlled by RNA-binding proteins (bottom, 'mRNA-specific transport'). Dotted lines indicate hypothetical connections.

mRNA-specific regulation is achieved in part by which mRNAs are present in the growth cone. Neurotrophins induce specific transport of β-actin, peripherin, and vimentin mRNAs into axonal growth cones (Zhang *et al*, 1999, 2001; Willis *et al*, 2005). mRNA localization is even regulated within the growth cone, a structure only 5–10 μm across: a gradient of BDNF induces asymmetrical localization of β-actin mRNA to the side experiencing more BDNF (Yao *et al*, 2006). mRNAs are typically localized by *cis*-acting sequences at their 5′ or 3′UTRs. β-actin mRNA localization is controlled by a so-called 'zipcode' in the 3′UTR (Kislauskis *et al*, 1994), and the RhoA 3′UTR drives axonal localization of a reporter mRNA (Wu *et al*, 2005).

mRNA transport and translation are coupled and regulated by RNA-binding proteins, which transport mRNAs in 'granules', large ribonucleoprotein (RNP) complexes that hold mRNAs repressed at the initiation or elongation stage (Sossin and DesGroseillers, 2006). To activate translation of the transported mRNA, for example, in response to guidance cues, RNA-binding proteins typically release the RNA cargo to polysomes (Krichevsky and Kosik, 2001). Currently, the best studied axonal RNA-binding protein is zipcode-binding protein (ZBP), which binds to β-actin mRNA through the 3′UTR zipcode (Ross *et al*, 1997). The *Xenopus* homolog of ZBP, Vg1RBP, colocalizes with β-actin mRNA in axons, and moves asymmetrically within growth cones upon exposure to a netrin-1 or BDNF gradient (Leung *et al*, 2006; Yao *et al*, 2006). Biochemically, ZBP has been shown to repress β-actin translation until Src phosphorylates it, making it release β-actin mRNA, and thus activating β-actin translation (Huttelmaier *et al*, 2005). However, *in vivo*, BDNF actually *increases* ZBP colocalization with β-actin mRNA in growth cones, concomitant with an increase in β-actin translation (Yao *et al*, 2006); it may be that ZBP remains associated with translating polysomes after releasing its repressive grip on β-actin mRNA.

Several other RNA-binding proteins have also been proposed to regulate axonal translation. Grb7 represses κ-opioid receptor translation by binding to the 5′UTR of *kor* mRNA; netrin-1 stimulation causes focal adhesion kinase to phosphorylate Grb7, which then releases *kor* mRNA, allowing κ-opioid receptor translation (Tsai *et al*, 2007). In addition, axonal translation of EphA2 receptor requires a cytoplasmic polyadenylation element (CPE) sequence (UUUUUAU) in the 3′UTR (Brittis *et al*, 2002), suggesting a role for CPE-binding protein (CPEB), which has been shown to regulate dendritic

translation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) (Richter, 2007). Finally, the RNA-binding protein Fragile X Mental Retardation Protein (FMRP) has recently been found in axonal growth cones, where it is required for growth cone motility (Antar *et al*, 2006). FMRP regulates local translation in dendrites (Zalfa *et al*, 2006) and the 3′UTR of RhoA mRNA contains a possible binding site for FMRP (Wu *et al*, 2005).

RNA interference (RNAi) and microRNAs may also play a role in mRNA-specific regulation of axonal translation. Functional RNAi machinery is present in axons (Hengst *et al*, 2006) and the RhoA 3′UTR contains miRNA-binding sequences (Wu *et al*, 2005). In addition, miRNAs have been implicated in regulating local translation in dendrites, a system conceptually similar to axonal growth cones (see below). The microRNA miR-134 regulates dendritic spine morphology by inhibiting translation of Lim kinase 1, a protein that regulates actin dynamics by inhibiting cofilin (Schratt *et al*, 2006). In *Drosophila*, CaMKII translation in olfactory projection neuron dendrites is suppressed by miRNAs, until neuronal activity stimulates proteasomal degradation of RNA-induced silencing complex (RISC) proteins, relieving repression of CaMKII translation (Ashraf *et al*, 2006). RNAi is partly carried out in distinct RNA granules called processing bodies, or P bodies, which degrade mRNAs and store repressed mRNAs (Anderson and Kedersha, 2006); it is not yet clear whether axonal growth cones contain P bodies.

How might attractive versus repulsive guidance cues induce translation of different proteins? One hint comes from attraction and repulsion induced by Ca^{2+} release, a candidate integrator of growth cone signals. Artificial asymmetrical elevation of Ca^{2+} levels causes growth cone attraction or repulsion if the Ca^{2+} release is moderate or small, respectively (Zheng, 2000; Henley and Poo, 2004; Gomez and Zheng, 2006). Likewise, Ca^{2+}-dependent guidance cues like netrin-1, BDNF, and myelin-associated glycoprotein (MAG) induce Ca^{2+} release in the growth cone, and the polarity of the growth cone's response can be reversed by modulating the size of this Ca^{2+} release (Hong *et al*, 2000; Henley *et al*, 2004). Indeed, polarity reversals induced by substrate molecules (Hopker *et al*, 1999) and cyclic nucleotides (Song *et al*, 1998) appear to act through modulation of Ca^{2+}-induced Ca^{2+} release from internal stores (Ooashi *et al*, 2005). Attraction induced by moderate Ca^{2+} release is mediated by CaMKII, while repulsion induced by small Ca^{2+} release is mediated by calcineurin (CaN)-phosphatase-1 (PP1) (Wen *et al*, 2004), suggesting that the balance of activity between CaMKII and CaN/PP1 may act as a 'switch' between attraction and repulsion. Interestingly, in hippocampal neurons, CaMKII phosphorylates CPEB and thereby activates CPE-dependent translation (Atkins *et al*, 2004), and during cell cycle progression, the CPEB-binding translational inhibitor maskin is dephosphorylated by CaN, inducing translational repression (Cao *et al*, 2006), raising the intriguing possibility that selective translation of CPE-containing mRNAs may mediate the Ca^{2+} 'switch'. Also, Src can be asymmetrically activated or inhibited by attractive or repulsive Ca^{2+}-dependent cues, respectively (Yao *et al*, 2006), suggesting that Vg1RBP/ZBP and its target mRNAs, like β-actin, could help mediate the Ca^{2+} 'switch'. These conjectures are supported by findings that protein synthesis is required downstream of Ca^{2+} influx for both attractive and repulsive Ca^{2+}-induced turning (Yao *et al*, 2006). However, it should be noted that some guidance cues that act through local translation, such as Sema3A and PACAP, do not rely on Ca^{2+} (Song *et al*, 1998; Guirland *et al*, 2003; Wen *et al*, 2004).

Why is local translation used for growth cone turning?

Local axonal translation as a mechanism for growth cone guidance may be puzzling at first glance. To cite one example, β-actin translation seems unlikely to have a substantial impact on actin polymerization, given that in migrating fibroblasts the rate of β-actin translation is only 7% or less of the rate of consumption of actin monomers by actin polymerization (Condeelis and Singer, 2005), and given the large supply of pre-existing actin monomers and the varied array of actin-binding proteins that regulate actin polymerization (dos Remedios *et al*, 2003). In this section, we propose possible rationales for local axonal translation.

Macromolecular crowding and protein turnover

Why regulate protein activity by translation rather than post-translational modulations like phosphorylation? From a strictly theoretical standpoint, cells have limited volume, and it has been estimated that 20–30% of that volume is occupied by macromolecules (Ellis, 2001); further crowding might slow diffusion or alter reaction rates unacceptably. Since an mRNA can be a template for theoretically unlimited translation, it may be more efficient in the face of this biophysical limit to store mRNA rather than inactive proteins. Indeed, netrin-1-induced turning requires both translation and proteasomal protein degradation (Campbell and Holt, 2001), suggesting a constant turnover of proteins that tightly regulates the levels of specific proteins. A similar recycling of proteins may occur in synaptic plasticity: translation inhibitors and proteasomal inhibitors each block long-term potentiation (LTP), while both applied together do not (Fonseca *et al*, 2006).

RNA flexibility

In addition, regulation of proteins by mRNA translation rather than protein modification provides more flexibility, because the activity of a protein can be regulated by arbitrary mRNA sequences rather than constituent domains of the protein. Indeed, proteins do not always contain the information necessary for their localization (see discussion of tau, below). Moreover, alternative splicing can create mRNAs with different regulatory sequences. Cytoplasmic mRNA splicing has been demonstrated in anucleate platelets (Denis *et al*, 2005) and isolated dendrites (Glanzer *et al*, 2005). One can speculate that axonal mRNA splicing might provide an additional layer of regulation for axonally translated proteins.

Decentralization

A corollary of the idea that proteins sometimes need to be regulated at the mRNA translation level is that proteins should be formed locally. Axonal growth cones are often far from the cell body, and it would be temporally and energetically inefficient to wait for protein delivery from the soma,

not to mention that in very long axons, the protein might not even survive the journey (Alvarez *et al*, 2000). Indeed, growth cones can navigate correctly even when the soma has been removed, both *in vivo* and *in vitro* (Harris *et al*, 1987; Campbell and Holt, 2001), suggesting that the 'devolution' of decision making from the soma to the growth cone is a likely function for local axonal translation.

Axonal fate

Local translation has long been known to play a role in cell polarity, for example in anterior–posterior axis determination in *Drosophila* oocytes (Johnstone and Lasko, 2001). In this case, local translation is important for localizing transcription factors and hence for fate determination in daughter cells. However, one may also consider polarity in differentiated cells as 'fate determination' of cellular compartments, for example in specifying neurites as axons or dendrites. Axonal targeting of tau mRNA by its 3′UTR is required for axonal targeting of tau protein (Aronov *et al*, 2001). Tau binds to microtubules and promotes microtubule assembly (Johnson and Stoothoff, 2004), and plays a role in forming and maintaining an axonal phenotype (Caceres and Kosik, 1990), perhaps by inducing specifically axonal microtubule organization. As tau associates with all microtubules, axonal translation of tau mRNA may be required to prevent mislocalization of nascent tau protein and hence disruption of neuronal polarity (Aronov *et al*, 2001). This suggests that other axonally translated proteins may also be required for the expression or maintenance of axonal (rather than dendritic) fate.

'Microdomains' and asymmetry

In the case of β-actin or other cytoskeletal proteins, the large amount of pre-existing protein suggests that local translation of cytoskeletal proteins regulates not the presence or absence of protein, but site of translation. This is supported by findings that guidance cue gradients induce asymmetrical translation of β-actin (Leung *et al*, 2006; Yao *et al*, 2006), and that local translation is required for directional turning, not elongation (Campbell and Holt, 2001). The rate-limiting step in actin polymerization is nucleation, and the concentrated local synthesis of β-actin in a confined cellular compartment could contribute to actin nucleation (see also next paragraph). Asymmetrical actin nucleation would lead to asymmetrical filopodial and lamellopodial protrusion and eventually turning. A similar mechanism has been proposed for β-actin translation at the leading edge of motile cells (Shestakova *et al*, 2001; Condeelis and Singer, 2005), a system intuitively akin to motile growth cones (Figure 3). Interestingly, it has been suggested that the source of Ca^{2+} influx—through the plasma membrane or from internal stores—controls the polarity of the growth cone response (Ooashi *et al*, 2005), and Gomez and Zheng (2006) have highlighted the potential importance of Ca^{2+} 'microdomains,' local Ca^{2+} signals generated by a cluster of Ca^{2+} channels, where the Ca^{2+} sensor is less than 1 μm from the Ca^{2+} channels. It can be envisaged that Ca^{2+} microdomains regulate similar microdomains of protein synthesis.

Distinct properties of nascent proteins

Nascent proteins are presumably free of post-translational modifications that may mark 'older' proteins. For example,

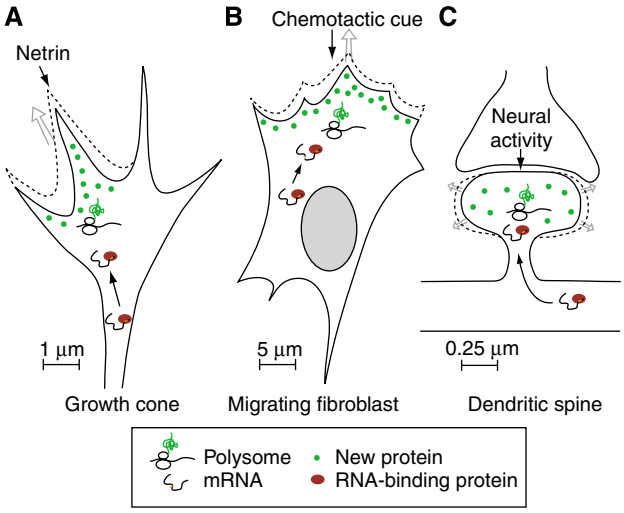

Figure 3 Comparison of models of stimulus-induced local translation in axon guidance, cell migration, and synaptic plasticity. mRNAs are transported to and within the growth cone (**A**), to the leading edge of migrating cells (**B**), and into dendrites and dendritic spines (**C**). Impinging signals stimulate translation of specific mRNAs, resulting in the formation of new proteins (green dots) in the appropriate location, thus changing the morphology or function of a localized subcellular compartment. Note that local translation occurs on a similar spatial scale across these systems, in subcellular compartments of the order of microns.

β-actin can be arginylated, which prevents actin filament clustering (Karakozova *et al*, 2006), or glutathionylated, which restricts actin polymerization (Wang *et al*, 2001). However, both arginylation and glutathionylation are thought to be reversible, like most post-translational modifications, and it is unclear why cells should make new proteins rather than simply removing post-translational modifications, as with dephosphorylation. New proteins should also lack the random oxidative damage that presumably accumulates on older proteins; however, the fact that axon outgrowth does not require local axonal translation suggests that proteins transported from the cell body are 'fresh' enough to function properly.

A more conceptually appealing possibility lies in chaperones, which associate with nascent proteins to assist proper folding (Hartl and Hayer-Hartl, 2002). The chaperone prefoldin aids β-actin folding by stabilizing intermediate folding states and transfers nascent β-actin to cytosolic chaperonin (CCT) (Hansen *et al*, 1999), which also catalyzes β-actin folding (Pappenberger *et al*, 2006) and associates with F-actin (Grantham *et al*, 2002). It has been suggested that CCT binding is required to aid actin polymerization by stabilizing vulnerable intermediates between the monomeric and polymerized states and protecting them from inappropriate aggregation in the crowded *in vivo* environment (Grantham *et al*, 2002). This model could explain why new translation of β-actin is required in cell motility and growth cone turning. Intriguingly, several chaperones are locally translated in injury-conditioned DRG axons (Willis *et al*, 2005). Perhaps analogous to local dendritic synthesis of translation factors to increase dendritic translation capacity (Tsokas *et al*, 2005), local synthesis of chaperones may support the continued function of newly translated proteins.

Comparison with local dendritic translation and synaptic plasticity

At the other end of the neuron in the post-synaptic compartment, dendritic protein synthesis and its role in synaptic plasticity has been the focus of intense study over the last 20 years (Sutton and Schuman, 2005). Many informative parallels can be drawn with axonal protein synthesis, as both axonal growth cones and dendritic spines are cellular compartments distant from the soma that need to respond quickly and autonomously to impinging signals. Just as axonal growth cones change shape in response to guidance cues to turn and guide the axon, dendritic spines change shape in response to stimulation to modulate synaptic efficacy (Figure 3), a process that involves local translation of proteins such as FMRP, Lim kinase, CaMKII, β-actin, and postsynaptic density-95 (Grossman *et al*, 2006; Schratt *et al*, 2006). Long-term potentiation and depression (LTP and LTD) might be considered analogous to attractive and repulsive turning, and a Ca^{2+}-dependent kinase/phosphatase balance similar to that suggested above in growth cones has been proposed to underlie the switch between LTP and LTD (Lisman and Zhabotinsky, 2001). In addition, translation-dependent re-sensitization of axonal growth cones (Ming *et al*, 2002; Piper *et al*, 2005) resembles translation-dependent homeostatic regulation of synaptic strength by mini-EPSP frequency (Sutton *et al*, 2006), suggesting that local translation is a general mechanism for maintaining a dynamic range of responsivity. Beyond potential parallels in axonal and dendritic translation already described, other regulatory mechanisms described in dendrites will likely prove important in axons as well, such as regulation of internal ribosomal entry (Pinkstaff *et al*, 2001) and mRNA stability (Perrone-Bizzozero and Bolognani, 2002).

Axonal translation may provide insights for synaptic plasticity as well. Studies on local translation in synaptic plasticity in vertebrates have hitherto focused on the post-synaptic side. However, activity-dependent refinement of synapses requires presynaptic responses to retrograde signals like BDNF (Schmidt, 2004). *In vitro* synapse formation in *Aplysia* (Lyles *et al*, 2006) and *Xenopus* (Zhang and Poo, 2002) requires translation in the presynaptic compartment. Moreover, recent evidence suggests that presynaptic translation may be required for LTD at the corticostriatal synapse in brain slices cut to exclude pre-synaptic cell bodies, although glial protein synthesis has not been excluded (Yin *et al*, 2006). Although the focus on axonal translation thus far has been on developing axons, adult mammalian axons are also capable of protein synthesis (Piper and Holt, 2004). In light of these initial studies, it may be time to consider a role in synaptic plasticity for local translation in mature vertebrate presynaptic terminals.

Future directions and questions

Future work on axonal translation will be aided by new tools to block translation of specific genes of interest in axons only, such as microfluidic compartmentalized cultures (Taylor *et al*, 2005) and axonal application of siRNA (Hengst *et al*, 2006). Axonal translation can be visualized using photoconvertible fluorescent reporters such as Kaede (Leung *et al*, 2006; Raab-Graham *et al*, 2006), or tetracysteine tags and the biarsenial dyes FlAsH and ReAsH (Rodriguez *et al*, 2006). It will be important, although technically challenging due to the small amounts of material obtainable from axons, to develop screens to uncover whole populations of proteins translated in response to specific guidance cues, which may form 'functionally coherent' groups (Ule and Darnell, 2006), such as 'attractive' or 'repulsive' proteins. If such functionally coherent groups exist, it will be interesting to see whether each group is associated with a specific RNA-binding protein. It will also be interesting to investigate whether axons contain distinct types of RNA granules such as stress granules and P bodies (Kiebler and Bassell, 2006), and what roles these diverse granules may play in axon guidance.

It is unclear why guidance cues that appear to have the same effect have different requirements for protein synthesis. For example, Sema3A, lysophosphatidic acid, and EphB2 all cause retinal growth cone collapse, but only Sema3A requires local translation (Campbell and Holt, 2001; Mann *et al*, 2003). Moreover, RhoA activity is also required for translation-independent LPA-induced neurite retraction (Yuan *et al*, 2003), suggesting that RhoA does not necessarily have to be translated to induce collapse. One possible rationale is that translation-requiring guidance cues might take higher (or lower) priority than non-translation-requiring guidance cues, when growth cones encounter multiple guidance cues at once. Another explanation is that growth cones *in vitro* have a limited repertoire of 'behaviors'—turning, outgrowth, collapse, branching—making *in vitro* assays too crude to distinguish specific *in vivo* behaviors that differentially require protein synthesis. Local translation may be required for behaviors such as topographic mapping and target selection, which do not have obvious *in vitro* analogies. Although recent studies have identified roles for translation and translational regulators in axon guidance and arborization *in vivo* in *Drosophila* (Chihara *et al*, 2007; Lee *et al*, 2007), the role of *local* axonal translation in axon guidance *in vivo* remains unknown. Answering this important question will require new tools such as translational inhibitors that act only in axons. A final area of interest is to understand what special, functionally important properties new, axonally synthesized proteins have, a question for which this review has provided only speculative answers.

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