



● PERSPECTIVE

## Fine-tuning the response of growth cones to guidance cues: a perspective on the role of microRNAs

In the development and regeneration of the nervous system, neurons face the complex task of establishing and/or repairing neuronal connections and contacts. The formation of these neuronal circuits is largely coordinated by tightly regulated temporal and spatial changes in mRNA translation, which enables incredibly precise control over protein expression and localization (Jung and Holt, 2011). Local mRNA translation in specific cellular compartments appears to play a role in many processes that are important to nervous system development and regeneration, including: cell survival, migration, growth cone guidance, and synaptogenesis (Jung and Holt, 2011).

In the past decade, numerous studies have provided evidence in support of a critical role for microRNAs (miRNAs) in the regulation of mRNA translation during neuronal development and regeneration (Bhalala et al., 2013). miRNAs are conserved, small non-coding RNAs that post-transcriptionally repress protein expression by directly targeting mRNAs. As individual miRNAs may target multiple mRNAs, they are particularly adept at the rapid fine-tuning and coordination of complex patterns of protein expression in a variety of both vertebrate and invertebrate cells, including neurons and glia. As such, recent studies have begun to explore the compartmental distribution of specific miRNAs, and their role in regulating mRNA translation during the establishment of neuronal connections. In the following brief perspective, we will examine recent evidence that miRNAs may regulate the translation of proteins involved in both intrinsic axonal growth and axonal guidance in response to specific guidance cues.

**miRNAs in neuronal growth cones:** To establish and/or repair neuronal circuits, distal axons navigate towards their synaptic targets utilizing a specialized, dynamic structure known as the growth cone. The growth cone senses and interprets complex sets of extrinsic guidance cues, and in response, rapidly induces changes in the direction of axonal outgrowth (Stoecki, 2018). To achieve such rapid turning behaviours, growth cones require the immediate production or degradation of specific proteins to initiate local, external cue-directed changes in the assembly and disassembly of microfilaments and microtubules (Stoecki, 2018). Growth cones are usually a significant distance from their cell bodies, and their rapid responses to guidance cues thus require local translation of select mRNAs known to be compartmentalized within distal axons and growth cones (Jung and Holt, 2011). Thus, miRNAs within neuronal growth cones may provide precise temporal and spatial control of compartmentalized mRNA translation during axonal pathfinding.

Recently, studies have begun to unravel the compartmental distribution of miRNAs within the cell body and distal axon (Hancock et al., 2014; Bellon et al., 2017). In neuronal cell cultures of the mouse (Hancock et al., 2014) and *Xenopus* (Bellon et al., 2017), unbiased RNA-Sequencing and qPCR analyses have uncovered a rich repertoire of miRNA activity within axons and growth cones, and have identified miRNAs that are enriched and/or depleted in comparison to the cell body. These studies have marked an important breakthrough in miRNA expression profiling, indicating potentially hundreds of growth cone-enriched miRNAs that may play a role in mediating axonal pathfinding behaviours.

**miRNAs regulating growth cone behaviors:** After identifying such an abundance of growth cone-enriched miRNAs, recent work has begun to disentangle the individual roles of specific miRNAs in mediating growth cone turning decisions. During development, miR-124 (Baudet et al., 2012), miR-132 (Hancock et al., 2014), miR-134 (Han et al., 2011) and miR-182 (Bellon et al., 2012) are expressed in vertebrate growth cones, and have been recently shown to play critical roles in axon pathfinding behaviors. For example, miR-134 regulates local protein synthesis-dependent turning behaviours of *Xenopus* retinal ganglion cell growth cones towards brain-derived neurotrophic factor (BDNF). However, miR-134 appears to have no effect on the turning behaviour of these same growth cones towards bone morphogenetic protein-7, a response that does not depend on local protein synthesis (Han et al., 2011). Interestingly, other miRNAs regulating local protein syn-

thesis-dependent turning behaviours have also been identified. These include miR-124 regulating *Xenopus* retinal ganglion cell growth cone responsiveness to Sema3A (Baudet et al., 2012), miR-182 regulating repulsive responses of *Xenopus* retinal ganglion cell growth cones to Slit (Bellon et al., 2017), and the miRNA lin-4 in regulating attractive turning behaviours of *C.elegans* anterior ventral neurons towards UNC-6 (Zou et al., 2013). Collectively, these studies may indicate that these miRNAs mediate fast-acting changes in axon guidance in response to guidance cues that rely on local mRNA translation.

**miRNAs in regenerating invertebrate growth cones:** Our current understanding of the role of miRNAs in regulating local protein synthesis in growth cones in response to guidance cues has largely been based on evidence obtained from *in vitro* studies on developing vertebrate nervous systems (Han et al., 2011; Baudet et al., 2012; Hancock et al., 2014; Bellon et al., 2017). Much less is known regarding the function of miRNAs in growth cones in a regenerative context, particularly within regeneration-competent systems such as those seen in many invertebrates.

Recently, miR-124 has been identified within regenerating growth cones of isolated motorneurons of the pond snail, *Lymnaea stagnalis*, indicating that miRNAs may exhibit similar functions across species (Walker et al., 2018). In support of this, miR-124 was shown to be enriched within the adult snail central nervous system, as is the case in some vertebrates. Moreover, miR-124 was up-regulated in the snail central nervous system following treatment with retinoic acid (Walker et al., 2018), which is a non-canonical guidance cue that can also promote neurite elongation. Interestingly, the attractive turning response of *Lymnaea* growth cones to a focal application of retinoic acid has been shown to rely on local protein synthesis and calcium influx. Thus, it appears that one universal function of miRNAs in both developing and regenerating neurons and growth cones of both vertebrates and invertebrates, is the regulation of local protein synthesis in a rapid response to a variety of canonical and non-canonical guidance molecules.

### What are the mRNA targets of these compartmentalized miRNAs?

Growth cones respond to numerous cues in their extracellular environments, often to many simultaneously, by integrating the responses of cue/receptor interactions to initiate rapid cytoskeletal changes. In order to mediate growth cone turning by attractive cues, cytoskeletal dissolution occurs on the opposing side of the growth cone from the cue, while rapid assembly ensues on the side facing the cue. The sequence of events occurring between the initial binding of a specific cue with its receptor and the ultimate alteration of the cytoskeleton leading to a directional change in growth, is only beginning to be understood (Stoeckli, 2018). Clearly, growth cone compartmentalized miRNAs may mediate the translation of mRNAs required at various levels of the signal transduction pathways activated. These could include everything from the RNA binding proteins needed to transport miRNAs and mRNAs to the growth cone, to guidance cue receptors and their downstream effectors such as Rho-GTPases. They may also be required for regulating the production of microtubule and microfilament binding proteins associated with dissolution and assembly of the local cytoskeleton.

The Rho-GTPases play a critical role in transducing many growth cone responses to canonical guidance cues. The most well understood and thoroughly studied members of the Rho-GTPase family include RhoA, Rac and Cdc42. Each protein plays a critical role in axonal pathfinding, with Rac and Cdc42 promoting outgrowth by facilitating actin polymerization within the lamellipodia and filopodia, respectively. Alternatively, Rho is activated in response to repulsive cues to restrict outgrowth and induce retraction. In order to regulate cue-specific responses, it is probable that some miRNAs may not directly regulate the Rho-GTPases themselves, but instead their upstream regulators or their downstream effectors. Numerous regulators act upstream of the Rho-GTPases, including GTPase activating proteins, guanine nucleotide exchange factors and guanine nucleotide dissociation inhibitors. Indeed, BDNF-induced branching of axons in the mouse retina is associated with increased level of miR-132, which inhibits the translation of a GTPase activating protein, p250GAP (Marler et al., 2014).

Other miRNAs have been shown to regulate translation of mRNAs associated with effectors downstream of the Rho-GTPases. For example, miR-134 was shown to regulate attractive turning behaviours of *Xenopus* spinal neurons by targeting LIM domain kinase 1, which mediates the expression of actin regulatory proteins to alter the growth cone cytoskeleton (Han et al., 2011). It has also been shown that the local translation of a microtubule associated protein, critical for axo-

**Table 1 Regulation of local protein synthesis-dependent guidance cues by miRNAs within neuronal growth cones**

Guidance cue	Organism	Cell type	miRNA locally regulating interpretation of guidance cue	miRNA target	Relation to Rho-GTPases (upstream or downstream)
Netrin	<i>Xenopus</i>	RGC	Lin-4	Lin-14	Upstream
	Rat	DRG			
Slit	<i>Xenopus</i>	RGC	miR-182	Cofilin-1	Downstream
Sema3A	<i>Xenopus</i>	RGC	miR-124	CoREST	Upstream
Engrailed	<i>Xenopus</i>	RGC	–	–	–
PACAP	<i>Xenopus</i>	Dissociated neurons from the neural tube	–	–	–
NGF	Rat	DRG	–	–	–
	Mouse	DRG	miR-181	MAP1B	Downstream
BDNF	Mouse	Cortical	miR-9	MAP1B	Downstream
	Mouse	RGC	miR-132	P250GAP	Upstream
	<i>Xenopus</i>	RGC	miR-134	LIMK1	Downstream
Neurotrophin3	Chick	Forebrain neurons	–	–	–
	<i>Xenopus</i>	Dissociated neurons from the neural tube	–	–	–
Retinoic acid	<i>Lymnaea stagnails</i>	Motoneuron	miR-124(?)	–	–

RGC: Retinal ganglion cell; DRG: dorsal root ganglion; PACAP: pituitary adenylate cyclase activating peptide; NGF: nerve growth factor; BDNF: brain derived neurotrophic factor.

nal outgrowth and branching, is regulated by different miRNAs (miR-9 and miR-181) in different neurons (cortical and dorsal root ganglia neurons, respectively) of the mouse (Dajas-Bailador et al., 2012; Wang et al., 2015). The levels of miR-9 are regulated by BDNF in cortical neurons (Dajas-Bailador et al., 2012) and the levels of miR-181 by nerve growth factor in dorsal root ganglia neurons (Wang et al., 2015). Collectively, these results may indicate an additional level of complexity in miRNA activity.

**What is the role of miRNAs in regulating turning responses to guidance cues that do not require local protein synthesis?** In this perspective, we have discussed the roles of various miRNAs in mediating growth cone turning decisions in response to guidance cues that require local protein synthesis (Table 1). The list is far from exhaustive, and clearly, many guidance cues can elicit turning behaviors even in the presence of translational inhibition. No studies have yet demonstrated changes in the distribution or expression of growth cone-localized miRNAs in neurons that respond to guidance cues in the presence of generalized translational inhibition. Certainly, many turning responses require the inhibition of translation of specific proteins by individual miRNAs, thus the effects of a generalized inhibition of translation may, at least initially, be ineffectual in preventing a response. It will be useful to determine which, if any, miRNAs mediate turning responses to cues in the presence of translation inhibitors. It will also be necessary to determine whether there are differences in the requirement for local protein synthesis to respond to guidance cues among different types of neurons (species differences, or peripheral versus central neurons) and in different contexts (development versus regeneration).

**Conclusions:** We are just beginning to understand the role of miRNAs in mediating the rapid responses of growth cones to individual guidance cues. Virtually all work has been done on identified peripheral neuronal types *in vitro* with the application of a single guidance cue. Thus, our understanding of the integration of responses that must occur when a growth cone navigates the local environment of a developing or regenerating brain or spinal cord is sorely lacking. The use of simpler model organisms, such as the snail, as well as a multidisciplinary approach may enable us to begin to unravel the complex signaling events and array of miRNA functions regulating the establishment of neural networks *in vivo*.

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