Molecular control of local translation in axon development and maintenance

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Highlights:

- **Many mRNAs are translated in developing and mature CNS axons *in vivo***
- **Axonal translation is critical for synaptogenesis, remodelling and survival**
- **RNA-binding proteins are essential for axonal mRNA localization**
- **Multiple mechanisms regulate the translation of axonal mRNAs**

Abstract

The tips of axons are often far away from the cell soma where most proteins are synthesized. Recent work has revealed that axonal mRNA transport and localised translation are key regulatory mechanisms that allow these distant outposts of the cell to respond rapidly to extrinsic factors and maintain axonal homeostasis. Here, we review recent evidence pointing to an increasingly broad role for local protein synthesis in controlling axon shape, synaptogenesis and axon survival by regulating diverse cellular processes such as vesicle trafficking, cytoskeletal remodelling and mitochondrial integrity. We further highlight current research on the regulatory mechanisms that coordinate the localization and translation of functionally linked mRNAs in axons.

Introduction

RNA localisation and localised translation are highly conserved mechanisms that confer spatial and temporal control of protein expression. This might be especially important for highly polarized and morphologically complex cells such as neurons [1]* in which local translation enables axons and dendrites, remote subcellular compartments, to remodel their proteome precisely in response to local demand.

The core components of the translation machinery are present in developing and mature axons, and axonal protein synthesis is involved in an increasing amount of physiological and disease-related processes [2-4]. With the combined progress made in the techniques of axonal isolation and next-generation sequencing (RNA-seq), thousands of mRNAs have now been detected in axons [5-7]. The identity of these mRNAs varies between neuronal subtypes [7], axonal subdomains [8] and throughout the axonal lifetime [8,9]. Recently, a cell-type specific genome-wide analysis of the axonal translatome further revealed the dynamic nature of local translation during the establishment and maintenance of neural wiring *in vivo,* and identified subsets of axonally translated mRNAs encoding functionally linked proteins that match the temporal needs of the axon [10]*. Here we review recent advances in the role of local translation as a regulator of axonal shape and maintenance and discuss the mechanisms by which coordinated spatiotemporal translational control of specific subsets of mRNAs in axons can be achieved.

Function of axonal protein synthesis

Steering:

The outgrowth and navigation of axons to the correct target area is mediated by surrounding extracellular guidance cues that are sensed by the highly motile, leading tip of the axon, the growth cone. Isolated axons separated from their cell bodies continue to grow properly when protein synthesis is acutely inhibited [11], but the cue-induced elongation or collapse response of an axon to several guidance cues is sensitive to local inhibition of translation [12]. Attractive cues such as netrin-1 and nerve growth factor (NGF) stimulate axonal protein synthesis of constituents of the cytoskeleton [13-15], whereas repulsive guidance cues like Sema3A and Slit2 induce local synthesis of proteins that promote the disassembly of the cytoskeleton [16,17]. Additionally, the asymmetrical synthesis of these proteins in growth cones exposed to a polarised cue gradient causes the local extension or withdrawal of filopodia and lamellipodia leading to steering towards or away from these *in vitro* gradients [13,14] (Figure 1a). Since these initial findings, various proteins have been shown to be locally synthesized during axon growth *in vitro* and *in vivo*, encoding cytoskeletal regulators, cell-adhesion molecules, guidance receptors and components of signaling pathways [2,10].

Evidence demonstrating a requirement for these locally synthesized proteins for guidance *in vivo* is sparse due to the technical challenges associated with blocking protein synthesis exclusively in the axonal compartment. Studies in the mammalian spinal cord provide evidence that specific receptors (e.g. EphA2, Robo3.2) are synthesised in growing axons at the midline suggesting an underlying role for local translation in the switches of commissural growth cone responsiveness along the pathway [18,19]. *In vivo* inhibition of an axonally synthesised cell adhesion molecule (NFPC) [20] or an mRNA translation regulator (microRNA) [21] causes subtle defects in pathfinding and target entry in small subsets of retinal axons. This may indicate a differential reliance among retinal axonal subpopulations *in vivo* for *de novo* synthesised proteins.

Branching:

Once axons have navigated to their targets, they branch to form terminal arbors bearing synapses and establish correct connections with their post-synaptic partners [23]. Translation machinery, as well as mitochondria for energy provision, is present at branching points and cue-induced local protein synthesis is required for axon branching *in vitro* [24-26]

(Figure 1b). Recent dynamic imaging studies *in vivo* in Xenopus retinal ganglion cell (RGC) axon terminals demonstrated that RNA granules dock at sites that predict branch emergence and where 'hotspots' of *de novo* β-actin synthesis accumulate [22]*. Moreover, local inhibition of *β-actin* translation in axon terminals diminished both the generation and stabilization of new branches leading to reduced axonal arborisation [22]*. These results demonstrate the importance of local protein synthesis for axon branching *in vivo* [22]* and suggest a wider role in plastic (signal-induced) cell shape remodelling. The molecular mechanisms underlying the coordinated docking of specific mRNAs, translation-associated machinery and organelles at precise axonal locations are not known and are an interesting area for future study.

Synapse formation and function:

Synaptogenesis also requires local protein synthesis in the pre-synaptic compartment, as highlighted by recent findings of Hengst and colleagues [27] (Figure 1c). In cultured embryonic hippocampal neurons, they show that rapid local synthesis of SNAP-25 and β-catenin occurs at sites of synapse formation and these axonally synthesized proteins are required for the assembly of presynaptic sites. Furthermore, repressing presynaptic translation affects synaptic vesicle recycling and blocking axonal translation of *SNAP-25* and *β-catenin* mRNAs impairs presynaptic vesicle release [27-29]. The relevance of these findings in the mature brain was also recently demonstrated by the finding that presynaptic local translation is needed for long-term plasticity of GABA release in established synapses [30]. Combined with the discovery that hundreds of mRNAs are translated in mature axons *in vivo* [10]*, these studies open exciting new areas of research on the role of axon translation in neurotransmission.

Surviving:

A well-established mechanism to promote neuronal survival is through neurotrophins that generate retrograde signals that travel from the axon to the nucleus, resulting in activation of anti-apoptotic transcriptional networks [31]. *In vitro* application of NGF triggers local synthesis of pro-survival transcription factors such as cAMP-responsive element (CRE)-binding protein (CREB) and its activator myo-inositol monophosphatase 1 (Impa-1) for retrograde transport, and the inhibition of this local synthesis results in an increase in neuronal and axonal degeneration [32,33] (Figure 2a). Axonal synthesis of the dynein regulators Lis1 and $p150^{Glued}$ was recently reported in response to NGF [34]. Their local translation mediates the transport of signaling vesicles that are presumed to contribute to axon survival. Interestingly, local synthesis of Lis1 was also shown to be necessary to induce retrograde transport of a pro-apoptotic signal upon NGF-deprivation [34]. These findings

suggest that the balance between neuronal death and survival can be achieved by precise control of local protein synthesis in the axon [6].

Local translation also mediates NGF-dependent regulation of axonal mitochondrial integrity and inhibition of its associated apoptotic signaling (Figure 2b). Target-derived cues trigger the local synthesis of LaminB2 in RGC axons, where the protein localizes to mitochondria and regulates mitochondrial integrity and axon maintenance *in vivo* [35]. In dorsal root ganglion axons, axonally-applied NGF stimulates the recruitment and translation of *Bcl-w* mRNA, which then interacts with Bax to promote axon survival by inhibiting the caspase-dependent apoptotic pathway [36]. Axonally synthesized Bcl-w also interacts with, and blocks, the ER-associated IP3-receptor, preventing intracellular calcium dysregulation which could otherwise lead to the activation of calpain proteases and subsequent axon degeneration [37]. Interestingly, mRNAs encoding proteins involved in axon degeneration, such as caspases and SARM1, are translated particularly highly *in vivo* during the axon pruning phase of development [10]*. The functional relevance of this in the selective maintenance and removal of branches still needs to be determined.

The constant import of cytosolic proteins into mitochondria is not only necessary to prevent apoptotic pathways but also to maintain its function in energy production and cellular metabolism [38]. Nuclear-encoded mitochondrial mRNAs have consistently been found enriched in axons *in vitro* regardless of the neuronal subtype and stage analysed [8,9,33,39] and they are translated in developing and mature axons *in vivo* [10]. Analysis of the axonal transcriptome of primary sympathetic neurons revealed more than 100 nuclear-encoded mitochondrial mRNAs, associated with a wide variety of mitochondrial functions [40]. Axonal synthesis of mitochondrial proteins such as ATP5G1 [41] or COXIV [42] was shown to be essential to maintain axonal mitochondrial membrane potential and to regulate ATP and Reactive Oxygen Species (ROS) production (Figure 2c). Thus, the local synthesis of mitochondrial-related proteins seems to be critical for axon viability *in vitro* and may be a major determinant of axon maintenance *in vivo*.

Axonal mRNA localization

The increasing diversity of functions attributed to axonally synthesized proteins at a precise developmental stage and in specific subdomains implies the existence of mechanisms to coordinate and control axonal translation according to local demand. This is partly due to the stage-specific changes in the axonal transcriptome. For instance, different

mRNAs are present in young growing axons versus target-arrived axons, with expression of many presynaptic mRNAs present exclusively in the latter [8,39] (Figure 3a). *In vivo*, axonal translation is at its peak during the branching stage in mouse RGC axons [10]*, and this is accompanied by a dynamic change in the translatome between the axon elongation and axon branching/pruning stages [10,43].

Transport, stability and translation of axonal mRNAs are primarily achieved through post-transcriptional functions of RNA-binding proteins (RBPs). RBPs can bind to specific mRNAs through cis-elements in their untranslated regions (UTRs), repressing their translation and allowing their subsequent targeting to the axon. In cortical neurons, fragile-X mental retardation protein (FMRP) prevents telomere repeat-binding factor 2 (TRF2-S) from binding to its mRNAs, thereby influencing the transport of TRF2-S mRNAs into axons [44]. RBPs can also compete for the same mRNA, influencing their stability, as has been shown for HuD and KH-type splicing regulatory protein (KSRP) [45] (Figure 3b). Moreover, mRNAs themselves can compete for binding to RBPs for their axonal localization. Exogenous expression of 3'UTR elements of *GAP-43* and *β-actin* suggest that these mRNA's compete for binding to ZBP1 [26], a competition mechanism also reported for endogenous *nrn1* and *GAP-43* mRNAs for binding to HuD [46] (Figure 3c). Interestingly, a recent study has shown that axonal transcripts have significantly longer 3'UTRs [47]. It has therefore been proposed that regulation of the abundance and diversity of mRNA alternative splice isoforms can regulate the transport in the axonal compartment by introducing specific cis-regulatory elements in the mRNA [10]*. Various RBPs have also been found to fulfil independent or shared functions in growth/guidance, branching and synapse formation [7,48]. For example, the translation of adenomatous polyposis coli (APC) target mRNAs is highest during the stage of axon elongation *in vivo* and decreases afterwards, whereas the translation of target mRNAs for FMRP peaks later, at the axon branching stage [10]*, suggesting a prominent role for particular RBPs at specific stages to coordinate RNA localization and translation of functionally linked mRNAs.

RBPs can also assemble and coordinate axonal transport of subsets of functionally linked mRNAs in specific ribonucleoprotein (RNP) complexes, or RNA regulons [49] (Figure 3d). A good example of such regulation in axons comes from the identification of a RNA regulon orchestrated by the RBP SFPQ (splicing factor proline and glutamine rich) [50]*. The authors show that SFPQ binds and regulates axonal localization of the functionally linked *creb1*, *impa-1*, *lmnb2* and *Bcl-w* mRNAs. SFPQ facilitates the co-assembly and co-trafficking of *lmnb2* and *Bcl-w* mRNAs and regulates neurotrophin-dependent axon survival. Interestingly, SFPQ was found to colocalize with ribosomes in close proximity to

mitochondria in axons. These results suggest that RBPs can precisely target mRNAs to favour direct coupling between local translation and use of the synthesized protein on-site.

Together, dynamic expression of distinct mRNAs, splice variants, RBPs and the formation of RNA regulons permit exact temporal and spatial control of the axonal transcriptome.

Local translational control

Despite clear spatiotemporal regulation of RNA localization, hundreds of different mRNAs reside in the same subcellular locations, such as the growth cone. This begs the question of how specific subsets of mRNAs are translated in response to specific cues. Axon guidance cues and neurotrophins stimulate kinases, inducing the subsequent phosphorylation of specific RBPs and release of their associated mRNAs for local translation [48,51] (Figure 4a). The same extracellular signals increase axonal protein synthesis through mTORC1 (mammalian target of rapamycin complex 1) activation of cap-dependent translation. Although mTORC1 controls global protein synthesis, it also selectively promotes the translation of subsets of mRNAs, including eIF4E-sensitive and 5' terminal olygopyrimidine (TOP) mRNAs [52,53]. The mRNA specificity of axonal translation might therefore require the spatial and temporal combination of signaling pathways. Indeed, it has been suggested that integration of multiple cues can result in the crosstalk of signaling by multiple pathways which could fine-tune local translation for specific developmental and physiological situations [54].

Regulation of local translation of specific mRNAs can also be achieved by axonal microRNAs (miRNA) (Figure 4b). Axons contain a vast diversity of miRNAs that differ between neuronal populations [55]. Since miRNAs are known to repress translation mainly by binding to 3'UTRs, the longer 3'UTRs found in axonal transcripts could allow for more stringent, or even axon-specific, regulation by miRNAs. Moreover, inhibition or activation of specific miRNAs by extracellular cues can lead to the selective stimulation or repression of subsets of mRNAs in axons. For example, miR-338 controls axonal synthesis of two functionally linked mRNAs for the nuclear-encoded mitochondrial proteins COXIV and ATP5G1 [42,56]. This process can modulate the global axonal proteome, but also respond to acute needs in restricted subdomains as was recently reported for miR-182 in response to Slit2 in RGC growth cones [21]. Specific precursors of miRNAs have also been detected in axons [57], but whether and how they are locally processed, as recently shown in dendrites [58], still needs to be determined.

Regulation of post-transcriptional modifications of mRNAs, of which n6-methyladenosine (m^6A) is the most prevalent one, has recently been suggested to participate in axonal translation [59]* (Figure 4c). Axonal *GAP-43* mRNA is modified by m^6A and is a substrate of the demethylase enzyme fat mass and obesity-associated protein (FTO). FTO itself can be axonally synthesized and depleting this enzyme in axons increases m^6A modification of *GAP-43* mRNA, thereby repressing its local translation [59]*. It would be interesting to investigate whether such processes can restrict the translation of subsets of mRNAs in response to extracellular cues. This work also opens the possibility for the involvement of other internal mRNA modifications, and the proteins involved in these modifications, in regulating axonal protein synthesis.

Another attractive mechanism for spatiotemporal regulation of translation is the direct coupling and cue-induced dissociation of specific axon guidance receptors and ribosomes [60] (Figure 4d). Netrin-1 induces the dissociation and release of ribosomes from deleted in colorectal cancer (DCC), thereby increasing local translation [60]. Ribosomal protein-coding mRNAs are amongst the most abundant mRNAs present in axons [8,9,39] and translated *in vivo* [10], and it has been proposed that they can possibly repair damaged ribosomes or alter the composition of pre-existing ribosomes for specific translation [51]. Indeed, an intriguing possibility is that specific receptors can bind to ribosomes that are themselves tailored, through local proteomic remodelling, to preferentially translate specific subsets of mRNAs [61]*.

Local translation is intimately linked with the cytoskeleton that may serve as a scaffold to organize the translational machinery [62], and acute disruption of local cytoskeletal dynamics affects cue-induced axonal translation [63]. These results lead to the idea that proteins involved in cytoskeletal dynamics can also regulate local translation by binding to specific subsets of mRNAs. Such a dual function was first proposed for APC, a plus-end microtubule binding protein, that was shown to act as a RBP regulating the axonal localization and translation of *β2B-tubulin* mRNA [64]. A more recent study has shown that the actin regulator Mena also binds to a specific set of mRNAs by associating with the RBPs hnRNPK and PCBP1 [65]*. Mena is required for both basal and BDNF-induced axonal translation of one of its target mRNAs. Moreover, Mena can bind to specific receptors opening the possibility for direct spatiotemporal restriction of its dual function after the arrival of an extracellular cue [66]. Even more intriguing is the finding that mRNAs binding to APC and Mena are enriched in functions related to APC and Mena, suggesting an interdependency and cross-talk between their direct role as regulators of cytoskeletal

dynamics and their involvement in mediating local translation of mRNAs important for the same function (Figure 4e). It will be very interesting to see whether this is a more common self-regulatory mechanism occurring in proteins with other functions.

Conclusions

Remarkable advances have been made in the recent years in our understanding of the functional importance of axonal protein synthesis and the associated regulatory mechanisms. It is now clear that axonal translation plays a key role in axon survival and is increasingly moving centre-stage in studies of neurodegenerative disease and mitochondrial disorders [3,51]. It is also clear that localised translation underlies structural plasticity providing a precise mechanism to remodel the proteome locally in response to extrinsic cues, cell contacts or activity. However, many exciting open questions remain. With the advent of novel and more sensitive techniques to identify the mRNAs present and translated [67], and new methods to obtain cell-type specific proteomes *in vivo* [68], the complexity of the axonal translatome and the diversity in functions of locally synthesized proteins in developing and mature axons will likely become even more evident and better understood. Moreover, further investigation into the underlying molecular events allowing the selective control and coordination of axonal translation may enable the design of new strategies for therapies aimed at neurodevelopmental and neurodegenerative diseases.

Conflict of interest statement

Nothing declared

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References and recommended reading

Papers of particular interest, published within the period of review have been highlighted as:

* of special interest

** of outstanding interest

Figure legends:

Figure 1: Axonal protein synthesis in shaping the axon.

(a) Cue-induced asymmetrical translation of mRNAs coding for cytoskeletal proteins, or their modulators, mediates growth cone responses. **(b)** Localized protein synthesis of cytoskeletal proteins mediates the emergence and stabilization of new axon branches in response to extracellular cues. **(c)** Local mRNA recruitment and translation is necessary for synaptogenesis and synapse vesicle release.

Figure 2: Axonal protein synthesis in maintaining the axon.

(a) Target-derived cues induce local protein synthesis of transcription factors and their regulators, mediating axon-soma communication and axon survival. **(b)** Axonal protein synthesis is necessary to maintain mitochondrial integrity and inhibition of anti-apoptotic pathways. **(c)** Axon survival relies on maintaining proper mitochondrial physiology by local synthesis of mitochondrial-related proteins.

Figure 3: Mechanisms regulating axonal mRNA localization.

(a) The axonal transcriptome changes over time thereby providing the correct mRNAs needed at each time point. **(b)** FMRP can bind to and sequester the RBP TFR2-S, which prevents TFR2-S from transporting its mRNAs into the axon (1). RBPs can also compete for binding to the same mRNA thereby influencing mRNA stability (2). **(c)** Different mRNAs can also compete for binding to the same RBP allowing axonal transport for only one of the two different mRNA isoforms. **(d)** A RNA regulon can be formed by the RBP SFPQ, which ensures axonal localization of functionally-related mRNAs.

Figure 4: Mechanisms of local translational control.

(a) Upon cue stimulation RBPs can be phosphorylated, which releases mRNAs making them available for translation. **(b)** microRNAs can bind and suppress translation of axonal mRNAs. Upon cue stimulation microRNAs can be released from their mRNA, increasing their translation (1) or microRNA levels can be upregulated and suppress local translation of specific mRNAs (2). **(c)** Post-transcriptional mRNA modification occurs in axons and regulates translation of axonal mRNAs. **(d)** Translational machinery can be directly coupled to a guidance cue receptor and cue stimulation releases this translational machinery and increases translation. **(e)** Cytoskeletal proteins APC and Mena have a dual function as RBPs regulating the axonal translation of mRNAs related to their own function.

Highlighted papers:

Zapullo et al., 2017 Nature Communications ** of outstanding interest:

This is the first study to perform a multi-omics approach to examine the contribution of protein transport, mRNA localization and local translation in neurons differentiated from mouse embryonic stem cells. The authors perform RNA-seq, Ribo-seq and mass spectrometry on neurites versus somas and find that local translation can account for almost half of the neurite-enriched proteome. Additionally, they determine translation rates by pSILAC proteomics and QuaNCAT.

Shigeoka et al., 2016 Cell ** of outstanding interest:
The authors utilize the Ribotag mouse to identify translating mRNA's in the axons of mouse RGCs *in vivo*. They identify the change of the axonal translatome during several developmental stages and show that axonal translation persists in mature axons.

Wong et al., 2017 Neuron * of special interest:
Wong et al. examine dynamics of RNA granules *in vivo* in RGC axons and show that these granules dock at sites of branch emergence that correlate with hotspots of protein synthesis. Furthermore, they show that *in vivo* inhibition of *β-actin* axonal translation specifically disrupts axon branching dynamics.

Villarin et al., 2016 Nature communication * of special interest:
This study is the first demonstration of a role for local translation in the modulation of axonal trafficking in response to extracellular cues. The authors also suggest a dual role for axonal translation in regulating axon survival and death.

Cosker et al. 2016 Nature Neuroscience * of special interest:
By identification of a SFPQ-dependent RNA regulon essential for axon maintenance, this study demonstrates that axon survival relies on the coassembly and spatial distribution of functionally-linked mRNAs by RNA-binding proteins.

Yu et al., 2017, Nucleic Acid Research * of special interest:
This work provides the first example of a role for mRNA modification in the regulation of axonal translation. Using mouse DRG cultures the authors show that FTO, an eraser of m^6A mRNA modifications, is present and locally translated in axons. siRNA knockdown of FTO in axons increases total m^6A levels and represses axon elongation. Depleting axonal FTO increases m^6A modification of *GAP-43* mRNA, which causes decreased axonal translation of *GAP-43*.

Shi et al., 2017 Molecular Cell * of special interest:

By using state-of-the-art proteomic techniques in mouse embryonic stem cells, the authors show the existence of heterogeneous ribosomes with different ribosomal protein composition. Furthermore, they show these 'specialized' ribosomes preferentially translate specific subsets of mRNA's.

Vidaki et al., 2017 Neuron * of special interest:
The authors uncover a novel role for the actin-binding protein Mena as a regulator of restricted translation in response to BDNF in axons. They show that Mena is a constituent of an axonal RNP complex containing specific RNA-binding proteins and a subset of mRNA's linked to Mena's function in cytoskeletal regulation, suggesting a cross-talk between actin polymerization and cue-dependent local protein synthesis in axons.

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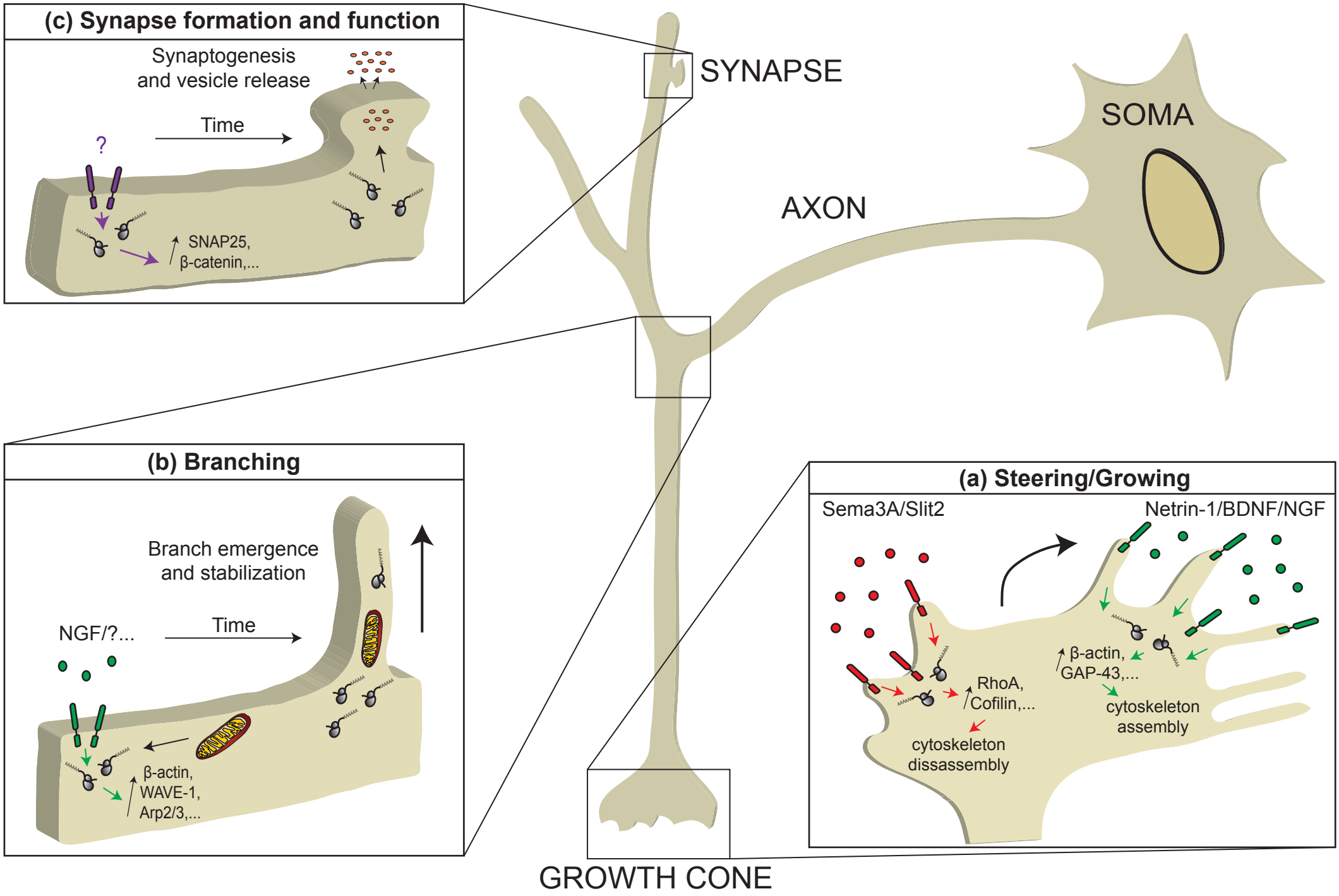
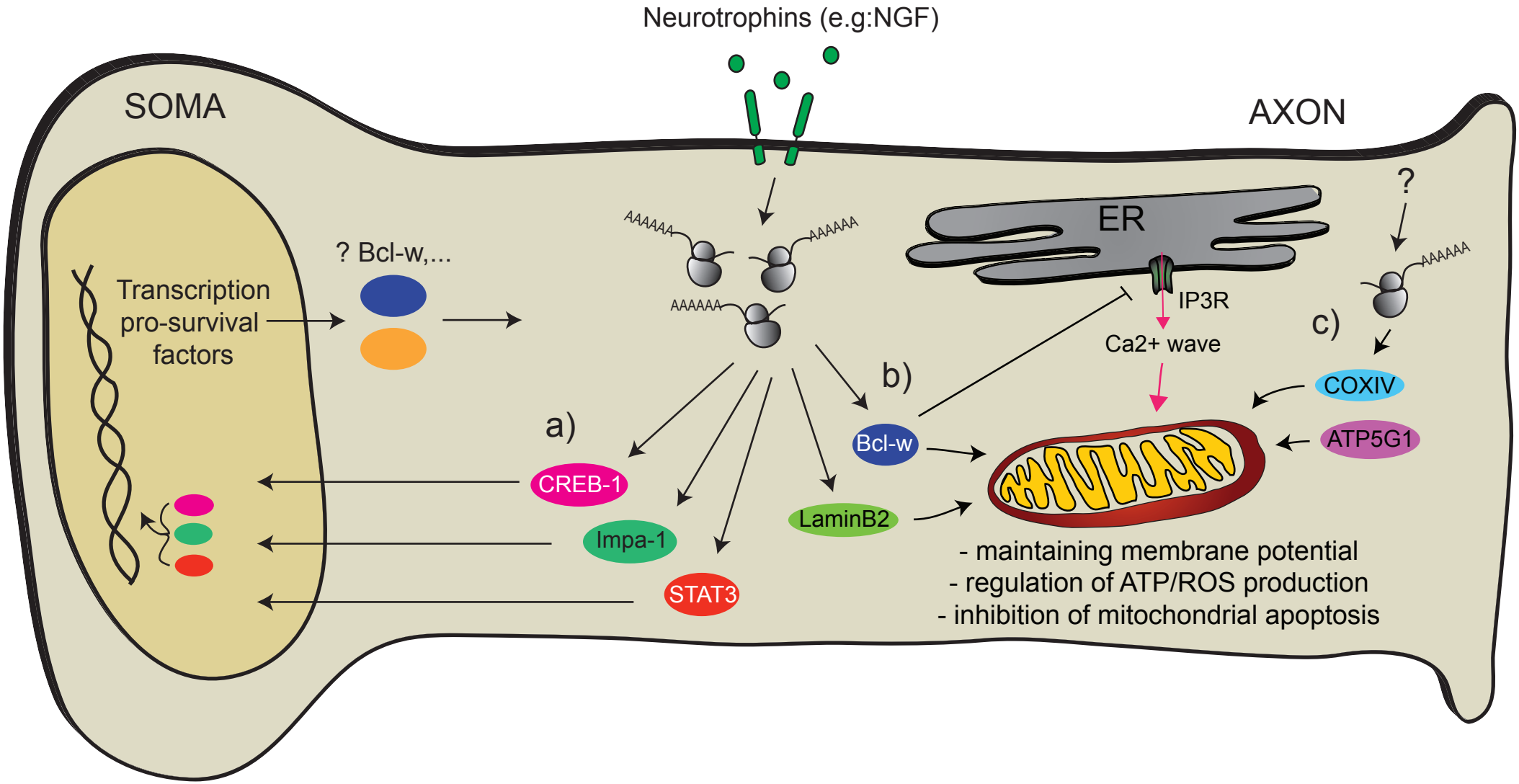
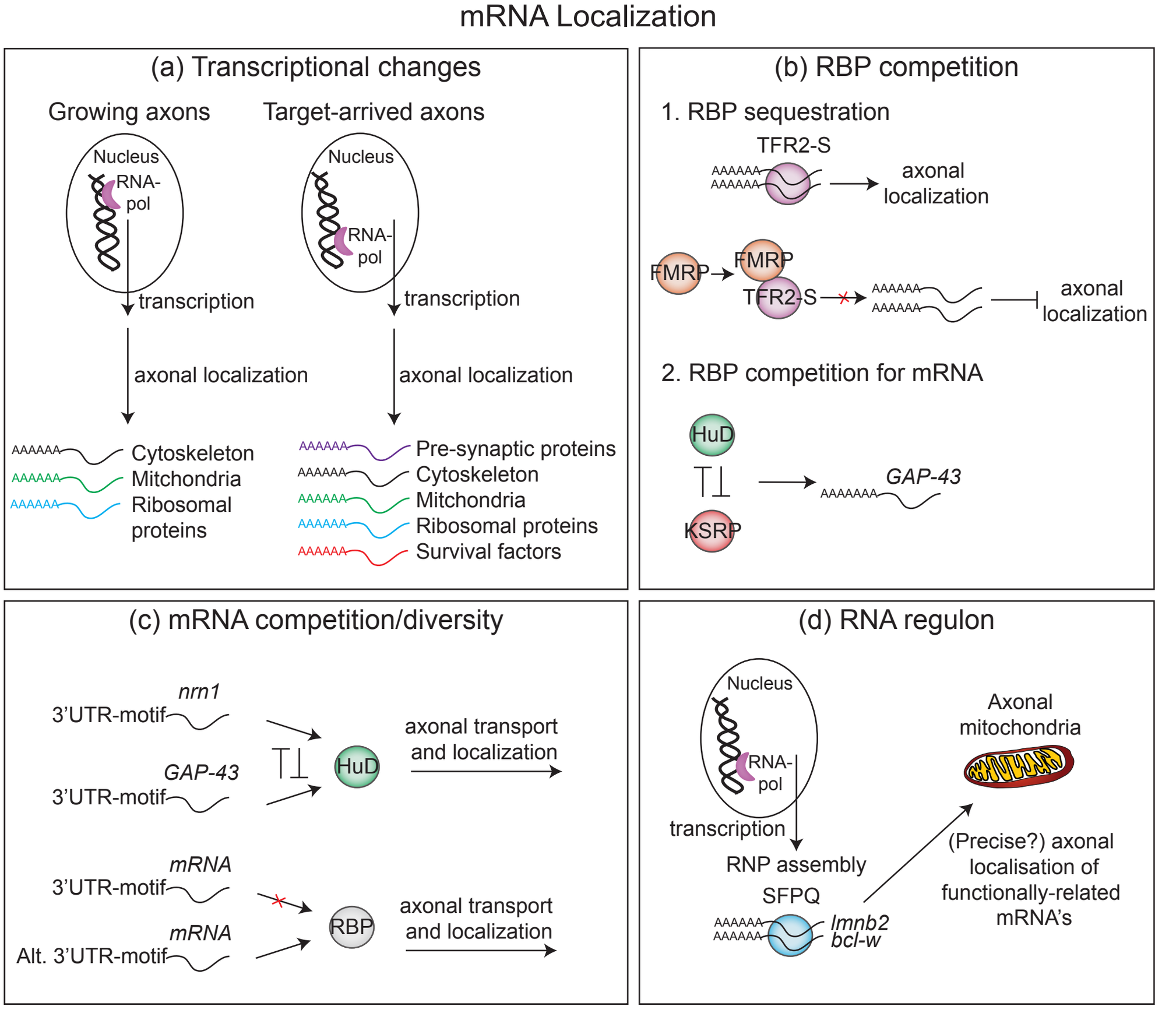
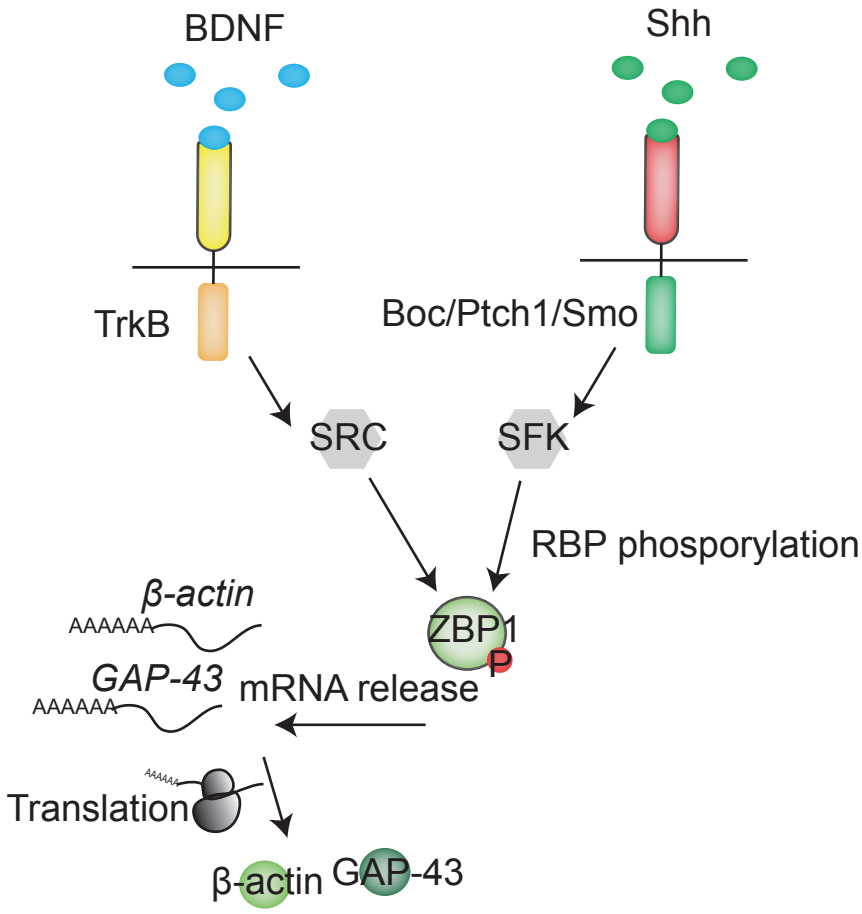

(c) Synapse formation and function
Synaptogenesis
and vesicle release
Time
?
SNAP25,
β-catenin,...
SYNAPSE
SOMA
AXON
(b) Branching
Branch emergence
and stabilization
NGF/?...
Time
β-actin,
WAVE-1,
Arp2/3,...
(a) Steering/Growing
Sema3A/Slit2
Netrin-1/BDNF/NGF
RhoA,
Cofilin,...
cytoskeleton
dissassembly
β-actin,
GAP-43,...
cytoskeleton
assembly
GROWTH CONE


Neurotrophins (e.g:NGF)
SOMA
AXON
Transcription
pro-survival
factors
? Bcl-w,...
AAAAAA
AAAAAA
AAAAAA
ER
IP3R
Ca2+ wave
?
AAAAAA
c)
COXIV
ATP5G1
b)
Bcl-w
a)
CREB-1
Impa-1
STAT3
LaminB2
- maintaining membrane potential
- regulation of ATP/ROS production
- inhibition of mitochondrial apoptosis

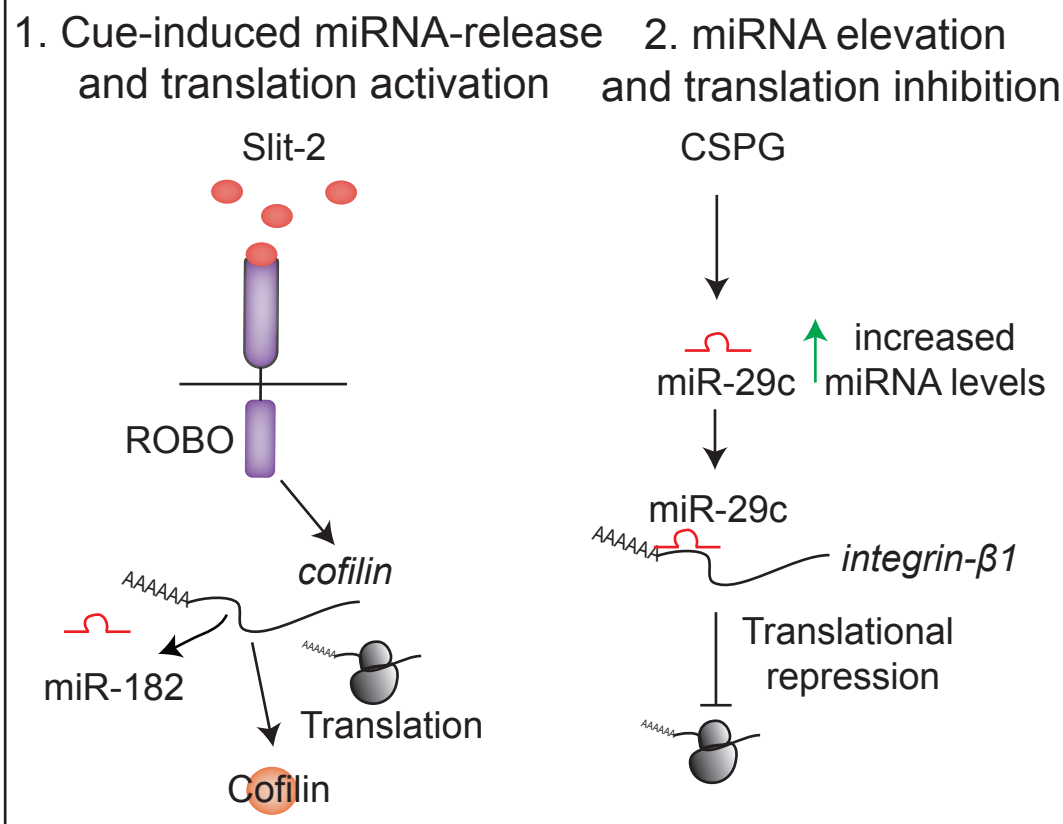

mRNA Localization
(a) Transcriptional changes
Growing axons
Target-arrived axons
Nucleus
RNA-pol
transcription
axonal localization
Cytoskeleton
Mitchondria
Ribosomal proteins
Pre-synaptic proteins
Cytoskeleton
Mitchondria
Ribosomal proteins
Survival factors
(b) RBP competition
1. RBP sequestration
TFR2-S
axonal localization
FMRP
TFR2-S
2. RBP competition for mRNA
HuD
KSRP
GAP-43
(c) mRNA competition/diversity
nrn1
3'UTR-motif
GAP-43
3'UTR-motif
axonal transport and localization
mRNA
3'UTR-motif
mRNA
Alt. 3'UTR-motif
RBP
(d) RNA regulon
Axonal mitochondria
RNP assembly
SFPQ
lmnb2
bcl-w
(Precise?) axonal localisation of functionally-related mRNA's

Local translational control

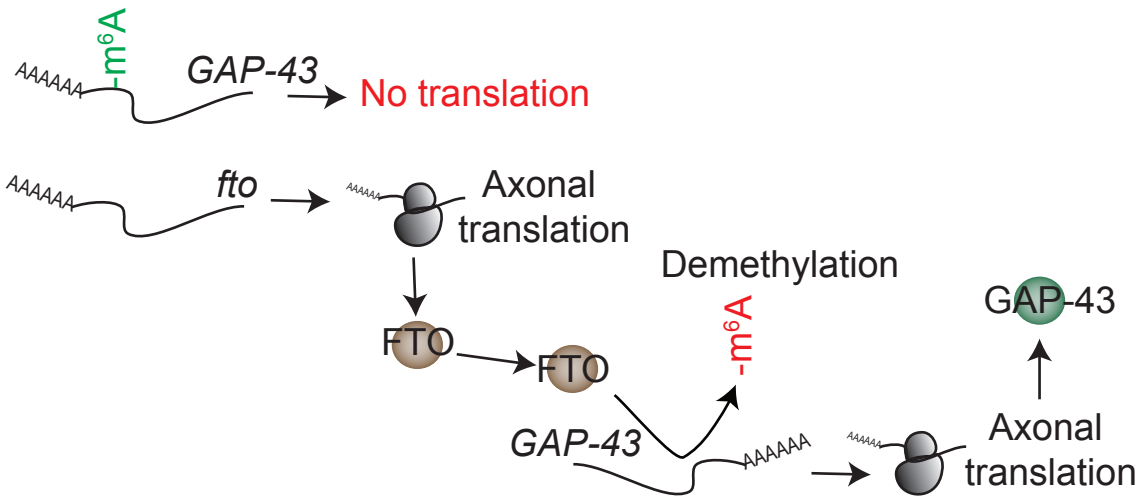
(a) RBP phosphorylation



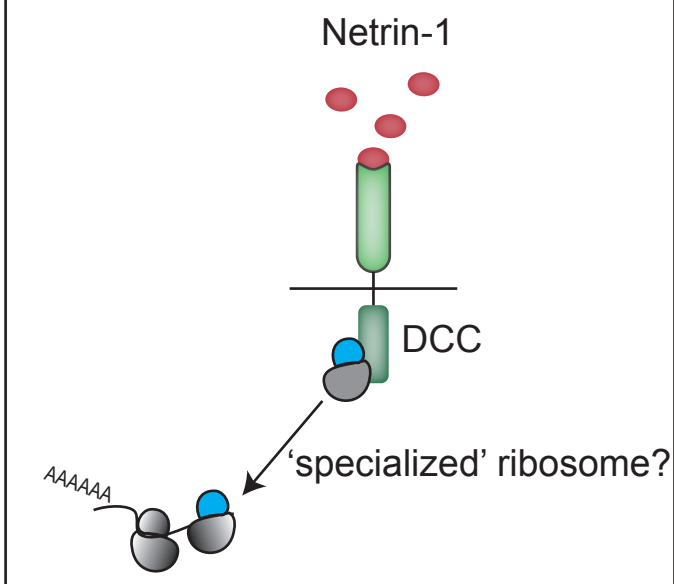
(b) micro-RNA regulation



(c) Post-transcriptional mRNA modification



(d) Receptor-ribosome coupling



(e) Functional self-regulation