

SUBJECT

RNA targeting and translation in axons: A new acTOR steals the show

Local translation of axonal transcripts takes center stage during growth and regeneration

By Antonella Riccio

Neurons are amongst the largest and most complex cells in nature, often extending very long axons, which in adult mammals can reach up to one meter in length. These extraordinary morphological features pose a challenging problem as to how information codified in the nucleus can reach the periphery of the cell in a timely manner. Similar to virtually all eukaryotic cells, neurons have adopted the strategy of localizing RNA asymmetrically. The nature of the transcripts targeted to dendrites and axons have been extensively studied, however the mechanism underlying local translation has remained elusive. On page XXX of this issue, Terenzio *et al.* (1) add a new interesting piece to the puzzle and show that local translation of mTOR precedes the burst of protein synthesis associated with the regeneration of injured axons. mTOR, the mammalian Target of Rapamycin, is a serine/threonine kinase that plays a central role in regulating protein synthesis (2). The authors show that mTOR transcripts are transported and stored in sensory neuron axons where they undergo rapid translation at the lesion site.

Peripheral localization of transcripts is a widespread phenomenon that mediates a large number of cellular processes. In neurons, coding and non-coding RNA are targeted to dendrites and axons, where mRNAs are rapidly translated in response to extrinsic stimuli. Local protein synthesis has been shown to mediate synaptic development and plasticity in dendrites, whereas in axons, it is necessary for axon extension and steering in response to guidance cues (3). Although polyribosomes were visualized at the base of dendritic spines more than 30 years ago, the presence of the translational machinery in axons has been hotly debated. This was mostly due to the fact that in axons, ribosomes are found close to the plasma membrane, which makes the visualization using classical microscopy techniques difficult. Because of their localization, it has even been proposed that axons may “borrow” ribosomes from surrounding cells, such as

Schwann cells for example, with a mechanism that could help axons enhance local translation (4).

mRNA transcripts targeted to neuronal processes have been investigated using increasingly sensitive genome-wide techniques. Comparative analyses of RNA localized in either dendrites, axons or cell bodies showed expression patterns that only partially overlap and are different depending on the cell type and developmental stage (5-7). Interestingly, transcripts that are highly expressed in cell bodies are not necessarily enriched in axons or dendrites, indicating that RNAs do not reach the peripheral compartments by passive transport, but are sorted and delivered with an active mechanism. How do neurons select the transcripts that are meant to be transported to peripheral processes? At least two mechanisms must be taken into account, one intrinsic to the RNA and dependent on its structure and a second related to the extrinsic signals that trigger transcript localization. Although the information necessary for RNA transport can be stored anywhere along the transcript (exons and introns, 5' and 3' untranslated regions (UTRs)), most elements that regulate mRNA targeting are found within the 3'UTRs. The first localization element of a neuronal transcript was identified in the 3'UTR of the *β -actin* transcript and was named “zipcode” because it was necessary for delivering the mRNA to axons in response to neurotrophins (8). Following this important discovery, a number of localization elements have been found in the 3'UTR of transcripts transported to neuronal processes. In developing sympathetic neurons for example, a localization element within the 3'UTR of *Inositol Monophosphatase 1 (IMPA1)* is necessary for targeting the transcript to axons (5). Similarly, the long 3'UTR isoform of *importin- β 1* drives its localization to injured axons (9). Although the primary sequence of localization elements described so far show little resemblance, it is possible that the folding of the RNA may form secondary structures that share common localization domains.

The main aim of asymmetric distribution of mRNA is to create subcellular compartments where the translational machinery is

closely linked, and can respond rapidly to extrinsic signals. The guidance cue netrin-1 for example, induces localized synthesis of *β -actin* in growth cones, which mediates the steering of retinal axons toward the guidance cue (10). Interestingly, RNA localization and local protein synthesis is regulated with a high degree of signal specification. In developing sensory neurons for example, distinct transcripts are targeted to axons in response to different neurotrophins (11). A potential mechanism entails that each extrinsic signal activates specific RNA binding proteins (RBPs) that act as a hub to recruit and transport different sets of transcript. This has been demonstrated for the splicing factor glutamine-rich SFPQ, a RNA binding protein that regulates the transport of functionally related transcripts in response to neurotrophins (12).

A prototypical example of the advantages brought about by signal compartmentalization in neurons is provided by the regenerative response that follows nerve injury. Compared to developing neurons, adult neurons have fewer ribosomes in axons and lower levels of local protein synthesis are required for their maintenance. However, a sudden change of circumstances, such as a traumatic injury, has a profound impact on gene expression and dramatically increases RNA localization to axons (9), ensuring that regenerating axons receive a constant supply of newly synthesized proteins. The initial response to axon damage entails Erk activation and an increase of intracellular calcium at the site of the lesion. These rapid events are necessary for membrane resealing and although short-lived, they can also influence gene expression. Following the acute response, a sustained retrograde propagation of the injury signal to the nucleus induces the targeting of newly synthesized transcripts, including mTOR to the lesion site of the axons (1). The combination of increased transport of mRNA transcripts and higher levels of locally translated mTOR results in the synthesis of proteins necessary for nerve regeneration.

Despite a slow start chiefly due to technical difficulties, the biological significance of mRNA targeting and translation in axons is becoming clearer and research in the field is

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1 rapidly gaining pace. However, a number of
2 fundamental questions remain unanswered.
3 First, it is still unknown how RNAs are sorted
4 in the nucleus and “tagged” for transport to
5 dendrites and axons. A potential mechanism
6 may involve small non-coding RNAs and/or
7 the UTR of targeted transcripts that in combination
8 with specific RBPs, such as SFPQ,
9 could act as a scaffolding to tag mRNA for
10 transport. Indeed, the 3’UTR of the *CD47*
11 transcript physically interact with the encoded
12 protein, driving the localization to the
13 plasma membrane (13). New techniques
14 aimed at providing genome-wide analyses of
15 3’UTR expression, such as poly(A)-Seq and
16 3’end-Seq, will help to obtain a comprehensive
17 picture of the features shared among the
18 3’UTRs of peripherally localized transcripts.
19 Second, in addition to protein coding transcripts,
20 non-coding RNA, including miRNAs
21 are also present in both dendrites and axons.
22 It is not known whether their role is confined
23 to regulating mRNA stability and translation
24 of localized transcripts or if they may represent
25 the missing link between extrinsic signals
26 applied at the periphery of the cell and
27 nuclear functions. mRNAs encoding transcription
28 factors are known to be transported and translated
29 in both axons and dendrites (6, 14, 15). However,
30 their biological significance is uncertain, mostly
31 because it is difficult to understand how they could
32 elicit a transcriptional response distinct from the
33 one induced by the much larger fraction of the
34 same transcription factor residing in the nucleus.
35 It is possible that non-coding RNAs and perhaps
36 UTRs, provide a tag that determine the binding
37 of axon-derived transcription factors to specific
38 promoters in a manner akin to enhancer RNAs.
39 Third, the mechanistic links between extrinsic
40 signals and translational activation in developing
41 and regenerating axons are still largely unknown.
42 Although Terenzio et al. made the important
43 discovery that local translation of mTOR
44 regulates protein synthesis in response to injury
45 in adult axons, whether this is a mechanism
46 shared with other neurons and at different
47 developmental stages remains unclear. It should
48 be also noted that the incorrect processing and
49 delivering of mRNA has been linked to the
50 pathogenesis of many human neurological
51 disorders, to the point that it has been proposed
52 that most, if not all, neurodegenerative diseases
53 are fundamentally disorders of the metabolism
54 of the RNA. Further understanding of the basic
55 mechanisms underlying mRNA localizations in
56 dendrites and axons will lay the foundations
57 for developing new therapeutic approaches
58 for neural disorders.
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Figure 1: RNA transport and translation in developing and regenerating axons.

Neurotrophins (such as NGF) induce the transport and local translation in developing axons. Coding and non-coding RNAs may also be transported retrogradely within signaling endosomes to activate gene expression (upper panel).

Following injury, transcripts are rapidly transported to the lesion site where they are translated. Local synthesis of mTOR is necessary for supporting nerve regeneration (lower panel).