Riding the rails – different modes for RNA complex transport in axons

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Neurons are highly polarized cells with axons that innervate distant targets. The distance of subcellular compartments from the nucleus requires sophisticated transport mechanisms and local action of vital processes for proper function and rapid responses to local stimuli (Terenzio et al., 2017). This is partially achieved by transport of mRNAs to subcellular locations and regulation of local translation for axonal growth, branching, synaptic plasticity, and regeneration, among other needs. Axonally synthesized proteins support neuronal survival, and axonal development, maintenance, and growth (Rishal and Fainzilber, 2014; Dalla Costa et al., 2021). Thus, understanding the mechanisms that promote RNA transport to subcellular locations in neurons will contribute to the development of novel strategies to enhance axon regeneration and survival.

In the axon, a variety of cargos are transported bidirectionally along microtubules to control neuronal functions such as neurite elongation and neuronal polarization. Microtubules are intrinsically polar, with a fast-growing plus end and an opposite slow-growing minus end. In axons, microtubule plus ends extend to the axon terminal while minus ends align towards the cell body. This polarity allows unidirectional movement in the axons with kinesins moving towards the plus end, mediating anterograde transport to axon tips, and cytoplasmic dynein moving towards the minus end, enabling retrograde signaling to cell bodies (Terenzio et al., 2017). The number of cargos far exceeds the number of molecular motors and ranges from membrane organelles through large macromolecular complexes to viruses (Salogiannis and Reck-Peterson, 2017). In addition, defects in microtubule-based transport and mutations in the motors can themselves lead to neurodevelopmental and neurodegenerative disorders (Sleigh et al., 2019).

The canonical view of microtubule-based transport is that specific cargo adaptors recruit molecular motors to cargos. However, an alternative mechanism of cargo motility, termed hitchhiking, was recently described. Evidence suggests some cargos can achieve motility by hitchhiking on organelles that are already linked to motors, rather than directly binding molecular motor complexes themselves. Thus, motile membranous organelles can provide platforms for the movement of other cargos (Salogiannis and Reck-Peterson, 2017). Here we review recent studies on RNA transport in neurons, revealing multiple modes of motility employed by different RNAs and RNA binding proteins (RBPs).

Hitchhiking modes of transport have been described for diverse types of RNAs in axons. microRNAs (miRNAs) are small noncoding regulatory RNAs, which have been identified in the axonal compartment together with their precursor miRNAs (pre-miRNA) (Corradi and Baudet, 2020). How are miRNAs transported and localized to axons? Gershoni-Emek et al. (2018) identified miRNAs, Dicer, and Argonaute-2 in motor neuron axons far from the perinuclear region and demonstrated that these silencing machinery components can be associated with mitochondria. Furthermore, live-cell imaging revealed that miR-124 is actively transported with acidic compartments in axons, and associates with staked mitochondria at growth cones and axonal branch points, where local translation was shown to occur (Gershoni-Emek et al., 2018). Another recent publication described axonal transport of pre-miRNAs on late endosomes/lysosomes (Corradi et al., 2020). These findings suggest that miRNAs and their precursors, and perhaps also other non-coding RNAs, can be transported to axons in association with different membranous organelles.

mRNAs are transported together with RBPs as ribonucleoprotein particles (RNP)s to distal subcellular locations for local translation. A recent study suggested that certain axonal RNA granules are also transported by associating with endosomes in axons (Cioni et al., 2019). Cioni et al. (2019) reported that RNPs associate with motile Rab7a endosomes along retinal ganglion cell axons, and that these RNP-bearing Rab7a endosomes also associate with ribosomes. In addition, they used live imaging to show that Rab7a endosomes often pause on mitochondria and that these contacts coincide with translational hotspots, suggesting that the RNP-bearing late endosomes are sites of local protein synthesis. Endosomes traffic diverse cargos within axons (Terenzio et al., 2017), and their usage as a platform for transport and regulation of RNPs may integrate different pathways.

A third example of a hitchhiking mechanism proposed lysosomes as another transport platform for RNA granules in neurons (Liao et al., 2019). These authors used a combination of proximity labeling proteomics, live-cell microscopy, and in vitro biophysical modeling to identify Annexin A11 as a molecular tether that can dynamically couple RNA granules with lysosomes in primary cortical neurons. Annexin A11 possesses a N-terminal low complexity domain, which promotes its phase separation into membrane-less RNA granules, and a C-terminal membrane-binding domain, which enables interactions with lysosomes. The findings suggest that lysosomes may also provide platforms for neuronal RNA transport, recruiting RNA granules through a molecular tether that links the granule to lysosome membranes. Moreover, since the endolysosomal system is very dynamic, the findings of Cioni et al. (2019) and Liao et al. (2019) may reflect a continuum of related membrane-bound organelles as platforms for RNA transport (Table 1). These platforms may enable the convergence of miRNAs, mRNA, and mitochondria for regulation of local translation by specific stimuli. The functional roles of such complexes will be of interest for future studies.

In contrast to the above examples of hitching a ride on organelles, other recent studies have described direct interactions of RNAs and their carriers with motors for axonal transport. Baumann et al. (2020) showed that adenomatous polyposis coli protein is linked to the heterotrimeric kinesin-2 KIF3A/B/KAP3 through the cargo adaptor KAP3 to drive the transport of specific axonal mRNA packages (Baumann et al., 2020). Using microscale thermophoresis and in vitro motility assays together with TIRF microscopy, Baumann et al. (2020) identified a minimal complex of proteins that can transport mRNAs with 3’UTRs enriched in guanine. Moreover, these G-motif-containing RNA sequences increase transport efficiency and balance access of different mRNAs to the transport system. Although this study has not yet been confirmed in vivo, the in vitro findings suggest that adenomatous polyposis coli can drive the transport of specific mRNA packages in axons by direct binding to motor proteins. It will be of interest to see if this mechanism is also exploited by other RBPs that bind guanine-rich RNA sequences.

A second motor-based transport system was identified in hippocampal neurons, where the APP tail-1 (PAT1) protein was shown to be involved in the transport of β-actin mRNA into dendrites, by direct binding to the kinesin-I motor complex and the RBP zipcode-binding protein 1 (ZBP1) (Wu et al., 2020). ZBP1 is the main RBP for β-actin mRNA, and the study used yeast two-hybrid to identify PAT1 as an adapter linking ZBP1 and KLC1 or KLC2 (KLC1/2), the cargo-binding subunits of kinesin. ZBP1 binds to the β-actin mRNA 3’UTR concomitantly with N terminal binding to PAT1, thereby bridging the complex to KLC1/2 and activating the kinesin-1 motor complex for microtubule-dependent β-actin mRNA transport. This study shows that motor proteins can transport RNA via membrane-free direct protein-protein and protein-mRNA binding interactions.

Fukuda et al. (2021) identified another system of RNA granule transport by direct binding to a kinesin motor. They used live-cell imaging of dorsal root ganglion sensory
Transport modes employed by different RNAs and RBPs

Table 1

| Transport molecules | Mechanism | Main cell type tested | Adaptor/platform | Reference |
|---------------------|-----------|-----------------------|------------------|-----------|
| miR-124, Dicer, Ago2 | Hitchhiking | Mouse motor neurons | Acidic compartments | Gershoni-Emek et al., 2018 |
| Axonal RNA granules | Hitchhiking | Xenopus retinal ganglion cells | Endosomes | Gioni et al., 2019 |
| pre-miRNAs | Hitchhiking | Rat cortical neurons | lysosomes via ANXA11 | Liao et al., 2019 |
| APC and associated mRNAs | Direct | Mammalian cell lines | KAP3 to kinesin-2 | Baumann et al., 2020 |
| β-Actin mRNA, RBP ZBP1 | Direct | Mouse hippocampal neurons | PAT1 to kinesin-1 | Wu et al., 2020 |
| nucleolin | Direct | Mouse DRG sensory neurons | KLC2 and KIF5A | Doron-Mandel et al., 2021 |
| SFPS and associated mRNAs | Direct | Rat DRG sensory neurons | KLC1 and KIF5A | Fukuda et al., 2021 |

negative has similar effects, in line with the predictions of our previously proposed length sensing and neuron growth control model (Rishal et al., 2012). Finally, the GAR domain also functions in plasma membrane association and nucleolar localization of nucleolin. We suggest that GAR domains may act as multifunctional subcellular localization elements for a range of RBPs, potentially explaining their implication in different neurodegenerative diseases.

In summary, a flurry of recent studies has reported multiple modes of transportation employed by different RNAs and RBPs in axons (Table 1). The studies highlight diverse carriers for mRNA transport to axons, utilizing both membrane-associated and membrane-free transport modalities. A number of questions arise, for example, if and how local translation might be regulated on or within these carriers? How do axons avoid or regulate the degradation of RNA or nascent proteins by lysosomes when those are part of the carrier complex? How can neurons regulate the recruitment of RBPs to motor proteins? Future studies on these and related questions will shed light on the dynamics and specificity of these different transport complexes and will be critical to devise specific perturbations to address their physiological importance in axonal maintenance and regeneration.

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