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mRNA Transport and Local Translation in Glia

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Abstract

Though mRNA transport and local translation are extensively studied in neurons, emerging evidence supports that these cellular processes are also abundant in non-neuronal glial cells. Here, we explore mechanisms of mRNA transport and local translation in oligodendrocytes, astrocytes, microglia, radial glia, and their functions in development, structure, and intercellular interactions.

Keywords

mRNA transport; local translation; oligodendrocytes; astrocytes; microglia; radial glia

Subcellular localization of mRNA is an evolutionarily ancient mechanism for subcellular specification. In budding yeast, myosin transports *Ash1* mRNA to the daughter cell. In *Drosophila*, specific localization of *bicoid* and *oskar* mRNAs is crucial for embryonic patterning. In mammals, mRNA transport, which occurs extensively in neurons, requires associated RBPs (RNA-binding proteins) and/or motifs spanning few to hundreds of base pairs (bps) located either in the 5' or 3' UTR (untranslated region).

Emerging literature has uncovered that mRNA transport is important for local translation in oligodendrocytes, astrocytes, microglia, and radial glia. As highly ramified cells with long processes and specialized functions [1], glia use local translation to achieve spatial and temporal resolution. Spatially, local translation supports unique subcellular environments, such as myelin sheaths and astrocytic endfeet. Temporally, local translation is rapid, producing more protein in a relatively short time; this speed would be difficult to achieve via vesicular delivery from the cell body. Here, we review the latest evidence that mRNA transport and local translation are important for development and function in glia.

Oligodendrocytes

Oligodendrocytes are highly arborized cells that extend long projections from their cell bodies that wrap around axons to form myelin sheaths. Recent publications have further

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clarified mechanisms for mRNA transport, identified additional mRNAs enriched in myelin, and begun to elucidate the contributions of the epitranscriptome.

One of the first mRNAs characterized to be transported and locally translated is myelin basic protein (*Mbp*). *Mbp* is the most abundant mRNA in oligodendrocytes and is crucial for the formation of compact myelin sheaths. As early as 1982, local translation was demonstrated by *in vitro* translating MBP protein from mRNA isolated from myelin. By 1997, John Carson's lab visualized *Mbp* mRNA transport along oligodendrocyte processes and characterized this as a microtubule-dependent phenomenon [2]. More than a decade later, investigations found that both the anterograde kinesin motor KIF1B and the retrograde motor complex dynein/dynactin [3] are necessary (Fig. 1A). In pursuit of the mechanism of *Mbp* localization, regions in the 3' UTR necessary for transport have been identified. The RNA transport signal (RTS) region allows for association with key constituents of *Mbp* mRNA granules, including hnRNP A2 and CBF-A, while the RNA localization region (RLR) is hypothesized to act via its secondary structure. Other proteins associated with *Mbp* mRNA granules include hnRNP K, hnRNP F, and Quaking [2].

Additional mRNAs are also enriched in the myelin sheath. Myelin fractions obtained from mouse brain are enriched in *Mobp*, *Fth1*, myelin lipid-enriched enzymes, and transcripts with unclear function (*Plekhb1*, *Bcas1*, *Trp53inp2*, and *Ptgds*) [4]. A brand-new study in zebrafish confirmed two additional sheath-enriched mRNAs (*fmr1* and *eif4ebp2*; Table 1) and identified an additional 26-bp "motif 2" in the *Mbp* 3' UTR that is also enriched in the myelin transcriptome [5].

Finally, a new publication demonstrated the importance of the epitranscriptome in the appropriate development of oligodendrocytes. Oligodendrocytes defective for mRNA methylation had decreased arborization and expression of myelin proteins, including MBP. Though *Mbp* mRNA was found outside the cell body [6], it is unclear whether direct interactions with transport adaptors were affected by lack of mRNA methylation.

Astrocytes

Astrocytes are aptly named, because their many processes form star-like shapes. These processes are multifunctional: those terminating in "endfeet" contact blood vessels at the blood-brain barrier while others contact neuronal synapses to form a tripartite synapse and regulate neuronal signaling. Local translation is likely important for establishing these critical and unique roles at distances far from the cell body.

Recent studies have used diverse techniques to solidify that local translation occurs along astrocytic processes and at endfeet. In mice expressing EGFP-tagged ribosomal protein RPL10A specifically in astrocytes (driven by the *Aldh1l1* promoter), astrocyte processes contain ribosomes *in vivo*. Ultrastructurally, electron micrographs of astrocytic processes contain clusters of ribosomes in close apposition to the astrocyte-neuron tripartite synapse. Super-resolution microscopy further validated that these synapses contain both pre- and post-synaptic markers. In addition, in acute brain slides, immunostaining with puromycin to label actively translating ribosomes localized to astrocyte peripheral processes [7]. In

isolated astrocyte endfeet that contact blood vessels, local translation was visualized using a methionine analog, HPG (homopropargylglycine), which incorporates into nascent proteins and can be visualized using click chemistry to ligate fluorescent dyes [8]. Thus, multiple techniques have confirmed local translation in astrocyte processes that contact synapses as well as blood vessels.

A number of RNA-seq studies identified mRNAs enriched in astrocyte processes. Using compartmentalized Boyden chamber cultures, a 2013 study from Anders Nielsen's lab homed in on over 2000 enriched mRNAs, which include *Nestin* and *Rab13* mRNA. At synapses, ribosomes isolated from mice expressing EGFP-RPL10A in astrocytes were enriched for 244 transcripts encoding motor proteins, glutamate transporters, proteins involved in synapse phagocytosis or synaptogenesis, and lipid processing proteins [7]. Endfeet mechanically isolated from blood vessels using enzymatic removal of the basal lamina were enriched with transcripts encoding secreted proteins and plasma membrane proteins (Fig. 1B, Table 1) [8]. Thus, many putative locally translated mRNAs identified by RNA-seq likely have specialized functions at synapses or at blood vessels.

Astrocytes may share some of the mRNA transport machinery used by other brain cell types. Bioinformatic analysis indicated that 3' UTRs of mRNAs localized along astrocyte processes are enriched for Quaking response elements (QREs). Quaking is a RBP involved in *Mbp* mRNA nuclear export in oligodendrocytes [2]. In immunoprecipitations, *Sparc* mRNA associates with Quaking subunits. In addition, fusion constructs expressing a fluorescent reporter protein with *Sparc* 3' UTR showed that QREs are necessary for nuclear export [7].

Microglia

Microglia, the resident immune cells of the brain, respond to insults through a variety of mechanisms, including phagocytosis and chemical signaling. In their resting surveillance state, they are highly ramified with dynamic processes, but can transform to amoeboid or other cell shapes when activated.

Emerging evidence suggests that mRNA transport and local translation also occurs in microglia. In hippocampal sections, Iba1-positive microglia processes contain mRNAs encoding the ribosomal protein RPL4, suggesting that ribosomal proteins that participate in local translation may be themselves locally translated [9]. In a pre-print manuscript, mice expressing RPL10a-GFP in microglia via the CX3CR1 promoter contain ribosomes localized along microglial processes. Puromycin labeling indicates that active translation is occurring along microglial processes. RNA-seq data found 258 mRNAs enriched in microglial processes, including those involved in pathogen defense, motility, and phagocytosis (Fig. 1C, Table 1). Some of these mRNAs share motifs for binding to RBPs, such as TIA1 and IGF2BP2 (IMP2). Blocking translation led to functional deficits, including decreased phagocytic cup size and impaired lysosomal protein localization [10]. Together, these results indicate that many transcripts are found in microglial processes and they likely play important roles in local subcellular functions, like phagocytosis.

Radial Glia

In the developing embryonic brain, neural stem cells called radial glia span the cortex to function as scaffolds for migrating neurons. Radial glia have apical-basal polarity with a short apical process reaching inwards towards the ventricles, and a 100–450-μm long basal process that projects outwards towards the cortical surface. The basal process terminates in an endfoot that interacts with the basal lamina and responds to extrinsic cues [11]. Thus, this subcellular compartmentalization and specialization provides rationale for radial glia to rely on mRNA transport and local translation.

In the apical process of radial glia, the RBP Staufen 2 (Stau2) mediates mRNA localization and subsequent cell fates after asymmetric division. After asymmetric division, radial glia give rise to a new radial glia and either a terminally differentiated neuron or an intermediate progenitor cell. Stau2 is enriched in the apical endfeet, where it forms a complex with Pumilio 2 and DDX1 and mediates the localization of mRNAs, including *β-actin*, *prospero*, and *prox1* (Fig. 1D, Table 1). *In vivo*, depletion of Stau2 increases PROX1 protein, indicating a role for Stau2 in translational repression, and shifts the outcomes of asymmetric division to favor terminally differentiated neurons [11].

In basal processes, many mRNAs are transported then locally translated in the basal endfeet. These include *Nestin*, *Arhgap11a*, and *Ccnd2* (cyclin D2). Early work from Noriko Osumi's lab showed that *Ccnd2* localization is important during asymmetric division. In a novel *ex vivo* system of acute slice cultures obtained after *in utero* electroporation, these mRNAs were visualized moving along basal processes using the MS2 reporter system. Local translation was also confirmed by microdissecting endfeet (i.e., no cell bodies attached), then photoconverting the fluorescent reporter mDendra, which was expressed off of a fusion construct with *Ccnd2* 3' UTR or *Arhgap11a* 5' UTR; this allows newly translated mDendra to be visualized. Of several RBPs surveyed, FMRP (fragile X mental retardation protein) localized most strongly to basal endfeet. FMRP was targeted for RNA immunoprecipitation in microdissected endfeet, where microarrays identified 115 FMRP-associated mRNAs. Many of these mRNAs encode cytoskeletal or motor proteins, including *Camsap2*, *Apc*, the tubulin carboxypeptidase *Vash1*, and the unconventional kinesin-11 *Kif26a*. *Kif26a* mRNA transport is severely abrogated in FMRP-null radial glia, with decreased velocity, run length, and processivity [12]. Thus, many classes of transcripts are transported along basal processes in radial glia.

While major strides have been made in understanding mRNA transport and local translation in glia, much work remains to be done. The functions of many highly enriched mRNAs in glial processes are unknown. For most of these mRNAs, the precise mechanisms for their transport, including what adaptor proteins link mRNAs to motors, have yet to be defined. In addition, emerging concepts across different glial cell types indicate that motor proteins as well as local translation machinery may be themselves locally translated; these results parallel similar discoveries in neurons made by Erin Schuman's lab in 2020. Thus, this chicken-and-egg paradox opens many questions on regulation, feedback, and spatio-temporal coordination. Undoubtedly, it is an exciting time to study mRNA transport and local translation in glia.

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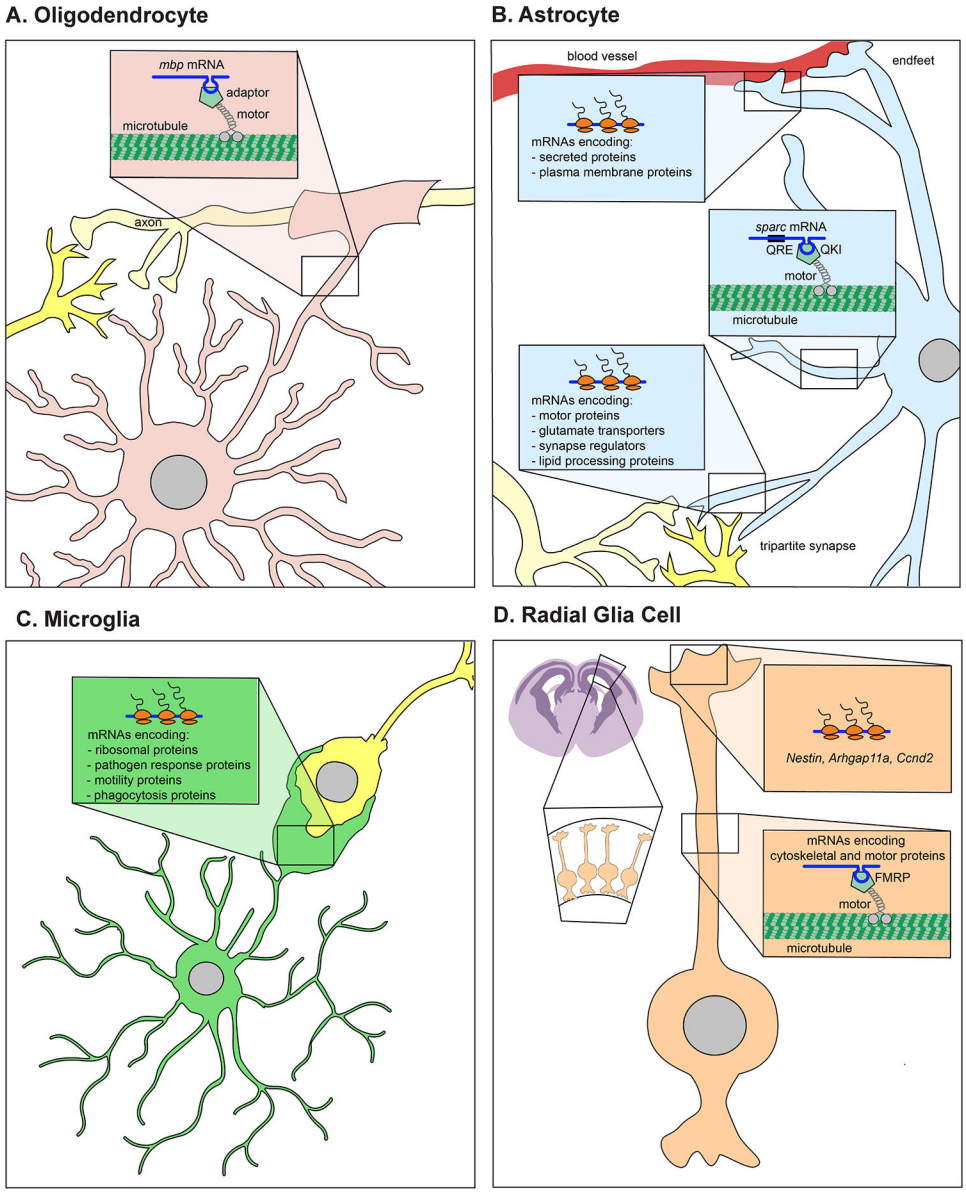

Figure 1 - mRNA Transport and Local Translation in Glial Functions

A. In oligodendrocytes (pink), many mRNA cargos have now been demonstrated to be transported to myelin sheaths that surround neuronal axons (yellow). A canonical mRNA cargo is *Mbp* (myelin basic protein, inset), which is the most abundant mRNA in this cell type. *Mbp* mRNAs are transported along microtubules by the kinesin KIF1B and the dynein/dynactin motor complex to myelin sheaths. Multiple motifs (not pictured), such as “motif 2” and RTS (RNA transport signal), are important for *Mbp* mRNA localization.

B. In astrocytes (blue), mRNAs are transported along processes and eventually arrive at either endfeet that contact blood vessels (red) or at terminals that contact the tripartite synapse (neuronal synapses picture in yellow). For example, the mRNA transcript *Sparc* is transported along microtubules in a complex that contains the RBP (RNA-binding protein) Quaking (QKI) (middle inset). At the endfeet (top inset), locally translated transcripts encode secreted proteins and plasma membrane proteins. At tripartite synapses (bottom

inset), locally translated transcripts encode motor proteins, glutamate transporters, synapse regulators, and lipid processing proteins.

C. In microglia (green), distal processes contain mRNAs that encode ribosomal proteins and that are involved in pathogen defense, motility, and phagocytosis (inset).

D. Radial glia cells (orange), which are found in the cortex of the developing brain (left), rely on local translation in both apical and basal endfeet to define cell fates after asymmetric division. For example, at the basal endfeet (top inset), the transcripts *Nestin*, *Arhgap11a*, and *Ccnd2* are locally translated. The RBP FMRP (fragile X mental retardation protein) participates in the transport of many mRNAs encoding cytoskeletal and motor proteins along the basal processes.

Table 1 -

Transported or Process-enriched mRNAs in Glia

Cell Type	Subcellular Location	mRNA	Protein Type / Function	Reference
Oligodendrocyte	Processes, Myelin Sheath	*Mbp*	Myelin	[2–5]
	Myelin	*Mobp*	Myelin	[4]
		Fth1	Iron storage	[4]
		Bcas1, Plekhb1, Ptgds, Trp53inp2	Unclear	[4]
		eif4ebp2, fmr1	Unclear	[5]
Astrocyte	Processes	*Gfap*	Intermediate filament	[9]
		Rpl4	Translation	[9]
		Kif1c, Myo10, Myo1D	Motor proteins	[7]
		Slc1a2, Slc1a3	Glutamate transporters	[7]
		Mertk, Sparc, Thbs4	Synapse phagocytosis or synaptogenesis	[7]
		Apoe, Elovl5, Fads2, Hadha, Scd1, Scd2	Lipid processing	[7]
	Endfeet	*Agt*	Angiotensinogen (secreted protein)	[8]
		Aqp4	Aquaporin (membrane protein)	[8]
		Ptprz1	Protein tyrosine phosphatase receptor (transmembrane protein)	[8]
Microglia	Processes	*Rpl4*	Translation	[9]
		CD68, P2ry12, Trem2	Microglia-neuron signaling and phagocytosis	[10]
		Ctss	Lysosome	[10]
Radial Glia	Apical endfeet	*β-actin*	Actin cytoskeleton	[11]
		Prox1	Asymmetric division	[11]
	Basal endfeet	*Nestin*	Intermediate filament	[11,12]
		Arhgap11a	Rho GTPase signaling	[11,12]
		Ccnd2	Cell cycle, asymmetric division	[11,12]