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REVIEW

Local translation in perisynaptic and perivascular astrocytic processes – a means to ensure astrocyte molecular and functional polarity?

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ABSTRACT

Together with the compartmentalization of mRNAs in distal regions of the cytoplasm, local translation constitutes a prominent and evolutionarily conserved mechanism mediating cellular polarization and the regulation of protein delivery in space and time. The translational regulation of gene expression enables a rapid response to stimuli or to a change in the environment, since the use of pre-existing mRNAs can bypass time-consuming nuclear control mechanisms. In the brain, the translation of distally localized mRNAs has been mainly studied in neurons, whose cytoplasmic protrusions may be more than 1000 times longer than the diameter of the cell body. Importantly, alterations in local translation in neurons have been implicated in several neurological diseases. Astrocytes, the most abundant glial cells in the brain, are voluminous, highly ramified cells that project long processes to neurons and brain vessels, and dynamically regulate distal synaptic and vascular functions. Recent research has demonstrated the presence of local translation at these astrocytic interfaces that might regulate the functional compartmentalization of astrocytes. In this Review, we summarize our current knowledge about the localization and local translation of mRNAs in the distal perisynaptic and perivascular processes of astrocytes, and discuss their possible contribution to the molecular and functional polarity of astrocytes.

KEY WORDS: Local translation, mRNA distribution, Astrocyte

Introduction

Although it was long thought that translation occurs in the vicinity of the nucleus, it is now known that mRNAs can also be transported to and translated in distal cell compartments as part of a process that helps to regulate protein delivery in space and time (Holt and Schuman, 2013). The first evidence for compartmentalized mRNA localization was published in 1983; it was found that actin mRNA was present in different regions of the ascidian egg (Jeffery et al., 1983). Since then, mRNA localization and local translation have been observed in a number of cell types, and particularly in cells with complex morphologies. The best-characterized example is the neuron, which can grow an axon of up to 1 m in length (Biever et al., 2019; Glock et al., 2017; Holt et al., 2019). mRNA localization and local translation has been observed at active synapses in neurons of the sea slug *Aplysia* and were shown to contribute to synaptic plasticity (Si et al., 2003). Local protein translation has also been observed in dissected squid giant axons (Mathur et al., 2018). In *Drosophila*, mRNA transport in synapses is linked to synaptic plasticity (Kuklin et al., 2017). In vertebrates, local translation was first described in isolated axons from rabbits and cats (Koenig, 1965a, b, 1967a,b), and has been intensively studied in recent years – notably after the development of a number of techniques for tracking local translation events *in vitro* and *in vivo* (Holt et al., 2019). Overall, the diversity observed in animal models suggests that local translation is an evolutionarily conserved mechanism for the functional polarization of cells.

In glial cells, mRNA localization was first observed in the 1980s; through *in situ* hybridization, the distal distribution of mRNAs coding for myelin-binding protein (which is crucial for building myelin sheaths) was observed in spinal cord sections from mice infected with a demyelinating virus (Kristensson et al., 1986). A later study demonstrated the presence of carbonic anhydrase II mRNA in the processes of primary cultured oligodendrocytes (Ghandour and Skoff, 1991). mRNA isoform transcripts encoding the amyloid precursor protein and Tau protein (both implicated in the pathology of Alzheimer's disease) have also been detected in primary oligodendrocyte processes (Garcia-Ladona et al., 1997; LoPresti et al., 1995). More recently, several research groups have demonstrated that mRNA localization and local translation also occur in astrocytes – the most abundant population of glial cells in the mammalian brain (Boulay et al., 2017; Sakers et al., 2017; Mazare et al., 2020a).

Astrocytes are voluminous, morphologically complex cells. They are highly ramified and polarized, and bear processes that form branches, secondary branches and terminations in contact with blood vessels and neurons (Fig. 1). In the CA1 region of the hippocampus, between 60% and 90% of the synapses are contacted by extremely thin (<50 nm) perisynaptic astrocytic processes (PAPs) (Reichenbach et al., 2010). The number of PAPs varies from one region of the brain to another (Ventura and Harris, 1999). At the synaptic interface, astrocytes regulate synaptic transmission (Dallerac et al., 2013; Ghezali et al., 2016). In fact, PAPs can sense changes in the composition of the perisynaptic extracellular space and thus can prevent prolonged neuronal activation and excitotoxicity by clearing ions and neurotransmitters that are released from the synapse (Dallerac et al., 2018). PAPs are equipped with transporters (such as glutamate transporters) and channels, which tightly control perisynaptic homeostasis (Murphy-Royal et al., 2017). PAPs also release neuroactive factors and influence synaptic functions by dynamically modulating synaptic coverage (Bernardinelli et al., 2014; Pannasch et al., 2011). Finally, PAPs orchestrate synaptogenesis during development and in the

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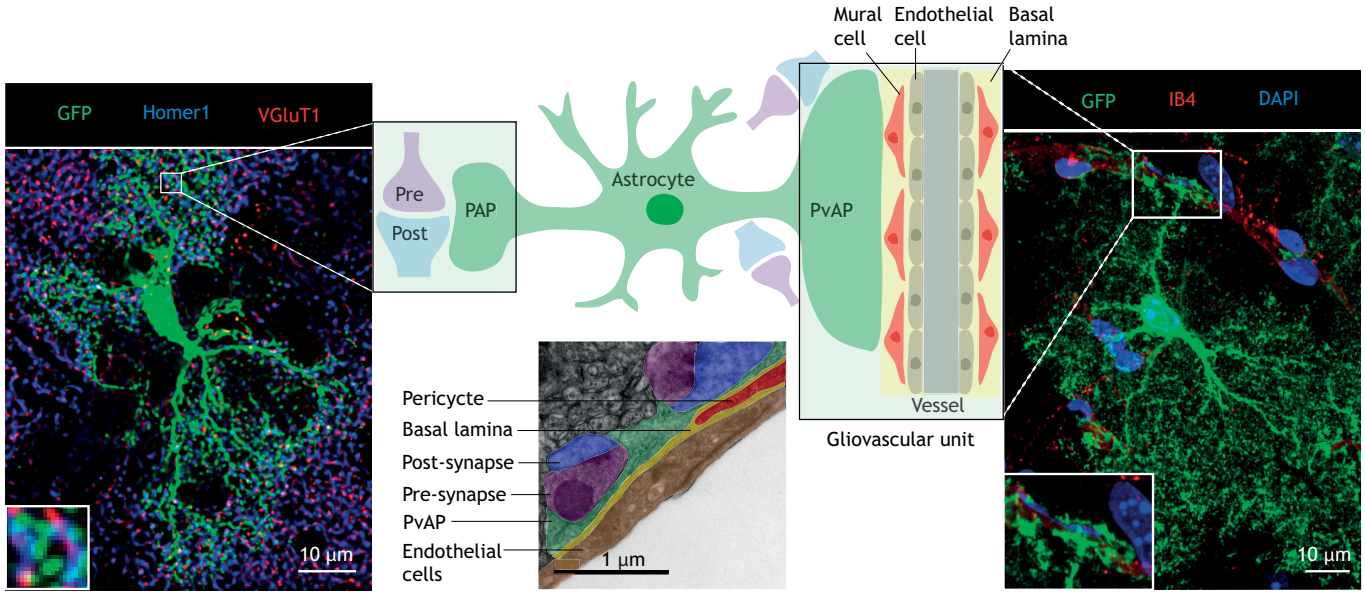

Fig. 1. Comparison of perivascular and perisynaptic astrocytic processes. Schematic representation of an astrocyte extending PAPs towards a synapse, comprising the pre- and the post-synapse. Astrocytes also send PvAPs towards blood vessels; together with mural cells, endothelial cells and the basal lamina, the PvAPs form the gliovascular unit. Synapses can be located adjacent to PvAPs (centre). A confocal microscopy image of an astrocyte filled with eGFP is shown on the left. The astrocyte comes from the pyramidal layer of the hippocampus in a transgenic mouse line expressing GFP under the control of the astrocyte-specific Gfap promoter. The synapses are labelled with the pre- and post-synaptic markers VGluT1 and Homer1. Scale bar: 10 µm. The inset shows PAPs in contact with synapses. On the right, a confocal microscopy image of a hippocampal astrocyte from a transgenic mouse expressing eGFP under the control of the Gfap promoter is shown. The astrocyte extends a PvAP (boxed area) that wraps around the blood vessel (labelled with isolectin B4; IB4). Scale bar: 10 µm. The inset highlights an astrocyte PvAP surrounding the blood vessel. The electron micrograph below the scheme shows a PvAP contiguous with PAPs: two synapses are abutting a PvAP around a blood vessel. Confocal images were taken by N.M.

mature brain (Allen and Eroglu, 2017; Chung et al., 2015; Stogsdill and Eroglu, 2017). At the vascular interface, perivascular astrocytic processes (PvAPs, often called endfeet) form a continuous layer around the brain vessels (Mathiisen et al., 2010; McCaslin et al., 2011). The dimensions of PvAPs vary greatly (from 8 to 198 µm^2) – even along the same vessel (Wang et al., 2020). On average, each astrocyte has 3.5 PvAPs (with a range from 1 to 7), which originate from one or more ramifications and that wrap around vessels (Bindocci et al., 2017). Via the PvAPs, astrocytes control several brain vascular functions, including the integrity of the blood–brain barrier, the homeostasis between the brain and the immune system, the transfer of metabolites and the regulation of cerebral blood flow (Alvarez et al., 2013; Cohen-Salmon et al., 2020). As in PAPs, most of the perivascular functions of astrocytes rely on a specific molecular repertoire that is enriched in PvAPs (Cohen-Salmon et al., 2020). For instance, the water channel aquaporin 4 (Aqp4) and the inward-rectifying K^+ channel Kir4.1 (encoded by *KCNJ10*) have critical roles in the regulation of perivascular homeostasis (Amiry-Moghaddam and Ottersen, 2003; Cohen-Salmon et al., 2020). Interestingly, PvAPs are sometimes contiguous with PAPs; this proximity might be critical for coupling the neuronal and vascular activities of astrocytes (Boulay et al., 2017) (Fig. 1).

The way astrocytes develop and maintain their high level of polarity has not been characterized. The recent discovery of local translation in the distal compartments of astrocytes strongly suggests that (as in neurons) this mechanism might underpin their functional polarity. Local translation requires mRNA transport, mRNA binding to the translation machinery and (for membrane and secreted proteins) proper folding and post-translational modifications. Here, we review the literature on RNA distribution, the detection of local translation in astrocytes, the subcellular organization of astrocytes at the perineuronal and perivascular interfaces, and the machinery for local translation. We conclude with a discussion on open questions in this new field of research.

Detection of mRNA in distal areas of the astrocyte

Several examples of local mRNA distribution in astrocytes had been described prior to the discovery of local translation. The analysis of protrusions obtained from primary cultures of astrocytes in a Boyden chamber (a cell culture device allowing cells to extend processes *in vitro*) provided an initial genome-wide assessment of mRNA localization in these structures (Thomsen et al., 2013a). *Glt1a* and *Glt1b* (also known as *Slc1a2a* and *Slc1a2b*) mRNAs were found to be differentially distributed; these mRNAs encode isoforms of the glutamate transporter 1 (GLT1), the most prominent glutamate transporter in astrocytes and which is responsible for glutamate uptake from the extracellular space in the brain (Murphy-Royal et al., 2017). Elevated amounts of *Glt1a* mRNA were found in the processes, whereas the *Glt1b* isoform was more restricted to the cell soma (Berger et al., 2005), suggesting that the composition and functions of GLT1 oligomers might differ in these two regions of the cell (Berger et al., 2005; Chen et al., 2004). A diurnal change in the distribution of fatty acid binding protein 7 (*Fabp7*) mRNA has been detected in mouse hippocampal PAPs, indicating that FABP7 might mediate diurnal changes in neuronal plasticity (Gerstner et al., 2012). mRNA encoding the glial fibrillary acidic protein (GFAP)-α isoform was preferentially detected in primary astrocyte protrusions, whereas mRNA encoding the GFAP-δ isoform was found in the soma (Thomsen et al., 2013b; Moeton et al., 2016). We recently confirmed these results in GFAP-immunolabelled hippocampal sections by combining mRNA detection (via *in situ* hybridization) with an *in silico* approach to quantify mRNAs in the somata, large processes and fine processes (Oudart et al., 2020). Changes in the distribution and density of *Gfap* mRNAs have also

been detected in a mouse model of Alzheimer's disease, suggesting that astrocyte mRNA transport is dysregulated in this pathology (Oudart et al., 2020). Interestingly, the assembly of GFAP-δ with GFAP-α promotes intermediate filament aggregation and dynamic changes (Moeton et al., 2016; Perng et al., 2008). Thus, the differential distribution of GFAP-α versus -δ-encoding mRNA might regulate intermediate filament dynamics in distal astrocyte processes.

The motility of mRNAs was recently assessed *in vivo* in radial glial cells (Pilaz et al., 2016). The latter are the progenitors of both neurons and astrocytes, and possess a basal process that emanates from the cell body, extends up to 450 µm away and terminates in an endfoot in contact with the meninges (Rakic, 2007). This basal process serves as a scaffold for the migration of excitatory neurons during early development (Nadarajah et al., 2001). The Ms2 system, which allows the movement of mRNA to be tracked *in vivo* (Bertrand et al., 1998), was used to study *Ccnd2* mRNA (encoding cyclin D2) in organotypic slices from embryonic mice (Pilaz et al., 2016). This work showed the active localization of the *Ccnd2* mRNA in the radial glia endfeet. Finally, three later studies used high-resolution *in situ* hybridization and RNA sequencing to demonstrate the presence of mRNAs in purified PvAPs (Boulay et al., 2017) and PAPs (Sakers et al., 2017; Mazare et al., 2020a) (see below).

Detection of local translation events in astrocytes

The localization of mRNAs in astrocytic distal processes raises the question of their translational status. To address this issue, Pilaz et al. linked a Dendra2 photoconvertible reporter to the *Ccnd2* 3′ untranslated region (UTR) and tracked the translation of this mRNA in radial glia endfoot preparations; green Dendra2 was irreversibly photoconverted to red, and time-lapse imaging over the following 45 min revealed a steady increase in green fluorescence recovery in the endfeet, suggesting *de novo* synthesis (Pilaz et al., 2016). Local translation of the *Gja1* mRNA, which encodes connexin 43 (Cx43; an astrocyte gap junction protein strongly expressed in PvAPs), has been measured in an *ex vivo* assay (Boulay et al., 2017). Cx43 is known to have a very dynamic life cycle, with a turnover time of 1.5 to 5 h (Fallon and Goodenough, 1981; Laird et al., 1995, 1991). Freshly isolated PvAPs attached to the surface of mechanically purified brain vessels (Boulay et al., 2015) were treated with cycloheximide (an inhibitor of protein synthesis) for 6 h. The level of Cx43 (assessed via western blots) was lower upon cycloheximide treatment than in untreated samples, indicating that Cx43 turnover in PvAPs relies on local translation (Boulay et al., 2017). Other recently developed techniques for visualizing local translation in astrocytes include the use of modified amino acid analogues, such as homopropargylglycine (HPG), or tRNA analogs (e.g. puromycin). The methionine analogue HPG inserts into the nascent protein chain and can be subsequently detected by a chemoselective ligation ('click') reaction with a fluorescent protein reporter (Horisawa, 2014). The aminoglycoside antibiotic puromycin, which can be detected by immunofluorescence, mimics tRNA-Tyr; it incorporates into the ribosome A binding site and induces the premature termination of translation by ribosome-catalysed covalent incorporation into the C-terminal of the nascent peptide (Schmidt et al., 2009; Pestka, 1971; Pestka and Brot, 1971). An HPG protein synthesis assay of freshly purified brain vessels gave a strong signal in the co-purified PvAPs (Boulay et al., 2017). Another study reported that, after incubating acute brain slices with puromycin, ∼73% of the puromycin puncta were located more than 9 µm away from the centre of the cortical astrocyte nucleus, suggesting that translation occurs more in distal processes than in the soma (Sakers et al., 2017). More recently, quantification of the puromycin signal in hippocampal PAPs on acute brain sections showed that immunolabelled synapses that are found within 1 µm of puromycin- and GFP-labelled astrocytic ribosome signals accounted for ∼3% of all synapses contacted by eGFP-labelled astrocytic ribosomes. Although this proportion is not large and might be due to technical issues related to the low level of puromycin incorporated into astrocytes, these results indicate the presence of local translation in PAPs (Mazare et al., 2020a).

Identification of ribosome-bound mRNAs in astrocyte processes

To further analyse translation in PvAPs and PAPs, several recent studies focused on ribosome-bound mRNAs in astrocyte processes (Fig. 2). All the studies were based on the use of a transgenic mouse expressing the chimeric ribosomal protein L10A tagged to GFP under the control of the Aldh1l1 astrocyte-specific promoter (Aldh1l1:L10A–eGFP) and the purification of eGFP-tagged polysomes by so-called translating ribosome affinity purification (TRAP) (Doyle et al., 2008; Heiman et al., 2014, 2008). Importantly, this transgenic model has also been instrumental in the visualization of ribosomes in PAPs and PvAPs (Boulay et al., 2017; Mazare et al., 2020a; Sakers et al., 2017). Our initial study enabled the identification of the most abundant ribosome-bound mRNAs in PvAPs from whole brain (Boulay et al., 2017) (Fig. 2A). First, we extracted total mRNAs from purified brain vessels and brain vessels that had been partially depleted of PvAPs by basal lamina digestion. The comparison of these two samples allowed us to identify mRNAs that were relatively abundant in PvAPs, compared to the vascular compartment. Second, we investigated the ribosome-bound status of these mRNAs by comparing ribosome-bound mRNAs from whole astrocytes or PvAPs that were extracted by TRAP from either whole-brains or purified brain vessels from Aldh1l1:L10A–eGFP mice. Only mRNAs detected in both preparations were considered, since all mRNAs present in PvAPs should also be detected in whole astrocytes. The intersection between the total mRNA preparation and the TRAP preparation allowed us to identify 28 mRNAs that constituted the 'endfeetome', that is, the pool of most abundant ribosome-bound mRNAs in PvAPs (Boulay et al., 2017). Some of these mRNAs encoded proteins involved in vascular functions, such as Aqp4, Kir4.1 and Cx43 – all of which are transmembrane proteins known to have crucial roles in blood–brain barrier homeostasis. More details on the functions of these proteins can be found in a recent review (Cohen-Salmon et al., 2020). Interestingly, a comparison of total mRNA versus ribosome-bound mRNAs in PvAPs indicated that some PvAP mRNAs were not bound to ribosomes and so might remain silent after their transport (Boulay et al., 2017). Although the ribosome-bound status of an mRNA does not necessarily reflect its translation, as ribosome-bound mRNAs can also be silent if they are compacted in granules, our results were the first to highlight potential translation events in a distal compartment of mature astrocytes. Given that transcripts in the endfeetome are known to have critical roles in the regulation of the brain vascular systems, we hypothesized that local translation may be crucially involved in maintaining the vascular functions of astrocytes (Boulay et al., 2017). In a second study, a similar subtractive approach was used to identify ribosome-bound mRNAs that were abundant in PAPs from the cortex (Sakers et al., 2017) (Fig. 2B). Here, the pool of ribosome-bound mRNAs in PAPs was extracted by performing TRAP on Aldh1l1:L10A–eGFP purified cortical synaptoneurosomes, which in addition to PAPs comprise the pre- and post-synaptic compartments of neurons

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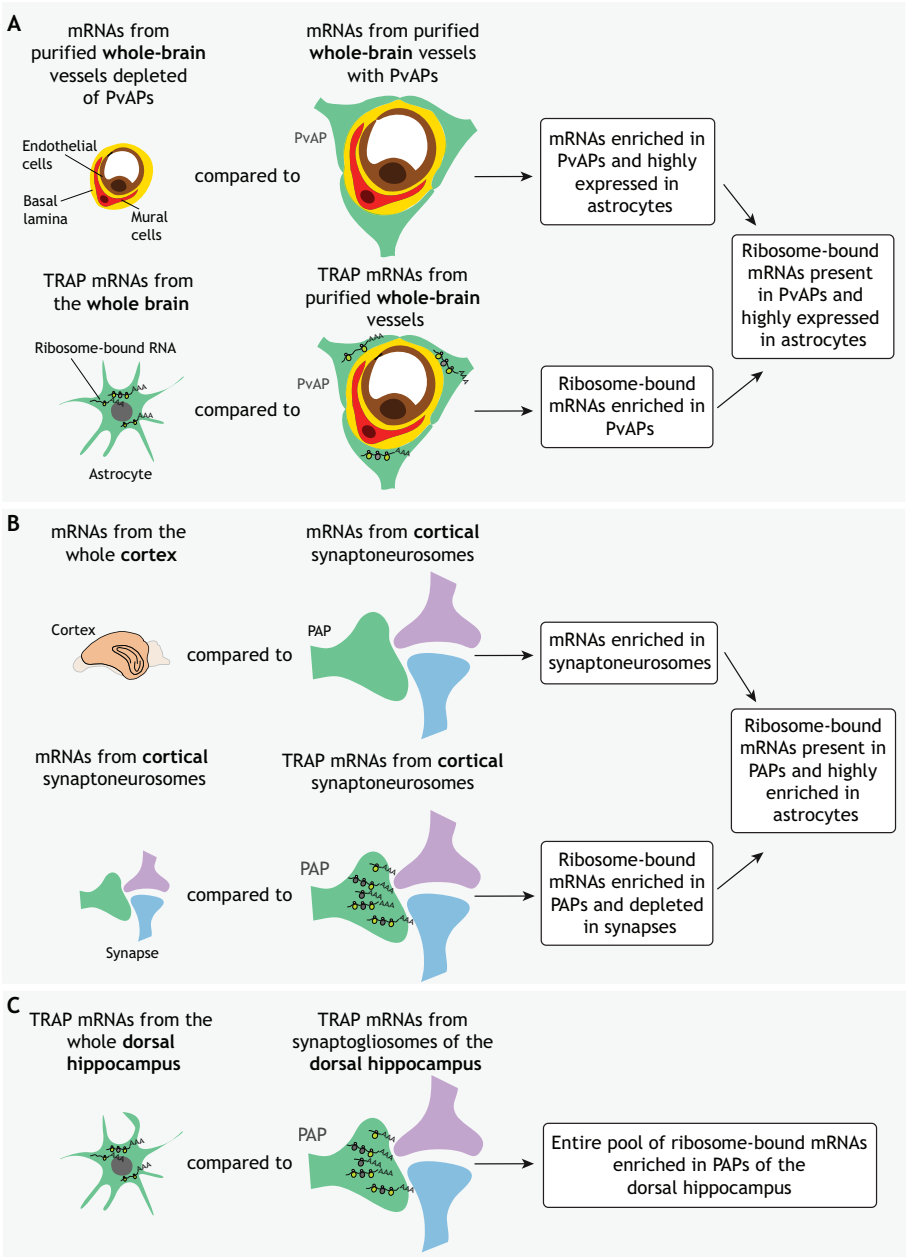

Fig. 2. Identification of ribosome-bound mRNAs in the perivascular and perisynaptic processes of astrocytes. (A) Identification of ribosome-bound mRNAs present in PvAPs and highly expressed in astrocytes in the whole brain. The experimental set up used in Boulay et al. (2017) to analyse ribosome-bound mRNAs in PvAPs from the whole brain. mRNAs from blood vessels (including the basal lamina, endothelial cells and mural cells) were mechanically purified from whole brain and thereby retained the PvAPs. They were then compared with mRNAs extracted from blood vessels after the basal lamina was enzymatically digested to remove PvAPs. mRNAs found to be more abundant in the preparation that retained PvAPs were identified. In parallel, translating ribosome affinity purification (TRAP) was applied to mechanically isolated blood vessels from whole brains of Aldh1l1:L10a–eGFP mice, which express an eGFP fusion to the ribosomal protein L10A specifically in astrocytes, in order to extract ribosome-bound mRNAs from PvAPs (second row). This preparation was then compared with the set of ribosome-bound mRNAs from whole astrocytes (extracted from whole brains using TRAP). Ribosome-bound mRNAs that were more abundant in PvAPs than in whole astrocytes were selected and then compared with ribosome-bound mRNAs in PvAPs. The overlapping mRNAs constituted the endfeetome, a set of highly expressed, ribosome-bound mRNAs in PvAPs. (B) Identification of ribosome-bound mRNAs present in PAPs and highly enriched in the synaptoneurosomes of the cortex. The experimental design used by (Sakers et al., 2017) to analyse ribosome-bound mRNAs in perisynaptic processes (PAPs) from the cortex. mRNAs from cortical synaptoneurosomes (including the PAP, pre-synapse and postsynapse) were purified and compared with mRNAs from whole cortices and the mRNAs that were more abundant in synaptoneurosomes were determined. In parallel, TRAP was used to extract the set of ribosome-bound mRNAs from cortical PAPs (the PAP-TRAP fraction, second row), and the latter were compared with mRNAs from whole cortical synaptoneurosomes to determine the mRNAs that are more abundant in the PAP-TRAP fraction. Next, mRNAs that were more abundant in synaptoneurosomes were compared with ribosome-bound mRNAs from PAPs; and overlap between the two sets yielded a set of ribosome-bound mRNAs present in PAPs and highly enriched in astrocytes. (C) Identification of the entire pool of ribosome-bound mRNAs enriched in PAPs of the dorsal hippocampus. The experimental design used by Mazaré et al. (2020a) to analyse ribosome-bound mRNAs that are more abundant in PAPs compared with their levels in whole astrocytes from the dorsal hippocampus. Starting with synaptogliosomes from dorsal hippocampi and whole dorsal hippocampi, a refined TRAP protocol was used to collect ribosome-bound mRNAs present in PAPs or whole astrocytes. The overlap between the two sets yielded the entire pool of ribosome-bound mRNAs that were enriched in PAPs from the dorsal hippocampus.

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(Sakers et al., 2017). The authors first compared the transcriptomes of total brain cortex and synaptoneurosomes, in order to identify mRNAs that were abundant in the latter structures. In parallel, they performed TRAP extraction on Aldh1l1:L10A–eGFP synaptoneurosomes and compared purified mRNAs with total mRNAs extracted from synaptoneurosomes, in order to identify ribosome-bound mRNAs that were highly abundant in PAPs (Sakers et al., 2017). Comparison of the two lists allowed the identification of 224 abundant ribosome-bound mRNAs in astrocyte PAPs (Sakers et al., 2017). These mRNAs coded for (1) proteins involved in glutamate metabolism, GABA metabolism and the biosynthesis of unsaturated fatty acids, (2) cytoskeletal proteins, such as ezrin, which might have a role in PAP remodelling (Lavialle et al., 2011), and (3) synaptogenic factors, such as the secreted protein acidic and rich in cysteine (Sparc), which regulates the synapse number (Lopez-Murcia et al., 2015). Taken as a whole, these two studies identified a set of ribosome-bound mRNAs that were more abundant in PvAPs and PAPs than in whole astrocytes. The results thus suggest that mRNA distribution and local translation could sustain the polarity of astrocytes at the perisynaptic and perivascular interfaces (Boulay et al., 2017; Sakers et al., 2017).

Despite these promising results, these initial studies focused solely on mRNAs that were either highly expressed in or were specific to astrocytes. In fact, the technical limitations of the TRAP protocol prevented the detection of ubiquitous transcripts. Further work was thus required to characterize the entire pool of local ribosome-bound mRNAs at the perisynaptic and perivascular interfaces. To overcome limitations of the two previous studies, we recently refined the TRAP protocol by adding additional precleaning and blocking steps that reduced the background noise caused by unspecific mRNA binding (Mazaré et al., 2020a) (Fig. 2C). The use of this protocol eliminated contamination by neuronal mRNA and enabled us to extract a pool of 844 astroglial ribosome-bound mRNAs in PAPs from dorsal hippocampus synaptogliosomes. It should be noted that our extraction protocol differed slightly from that used in the synaptoneurosome study by Sakers et al. because we did not include ultracentrifugation on discontinuous Percoll–sucrose density gradients (Westmark et al., 2011). By analogy with the above-mentioned ‘endfeetome’ in PvAPs, we referred to this repertoire as the ‘PAPome’ (Mazare et al., 2020a). Interestingly, our study revealed a wholly new, and unexpected, set of enriched ribosome-bound transcripts in PAPs compared to those found previously (Sakers et al., 2017); the most abundant mRNAs encoded ubiquitous proteins involved in iron homeostasis, translation, the cell cycle and the cytoskeleton [notably ezrin, as also identified by Sakers et al. (2017)] (Mazaré et al., 2020a). Remarkably, a large proportion of ribosome-bound transcripts in PAPs encoded ribosomal proteins and elongation factors (Mazaré et al., 2020a), which is reminiscent of observations in neuronal processes (Deglincerti and Jaffrey, 2012; Giustetto et al., 2003; Moccia et al., 2003; Shigeoka et al., 2019). Overall, our results strongly suggest that local translation in PAPs might be sustained by local synthesis of the translation machinery, with either the *de novo* assembly of translational complexes or the replacement of damaged proteins, as recently suggested for ribosomal proteins in axons (Shigeoka et al., 2019).

The subcellular organization of local protein synthesis and maturation in astrocytes

To become functional, most secreted and membrane proteins must undergo post-translational modifications. In PvAPs, most of the endfeetome mRNAs encode membrane proteins that require folding and/or glycosylation as they pass through the endoplasmic reticulum (ER) and the Golgi. Accordingly, we observed smooth, rough and mixed ERs in all PvAPs (Fig. 3). A full Golgi was also detected in 7% of PvAPs, and these specific PvAPs might therefore contain the same canonical translation machinery as the soma (Boulay et al., 2017). PAPs are extremely thin structures (<50 nm in diameter) (Reichenbach et al., 2010), and their subcellular organization has not yet been fully explored. However, by using

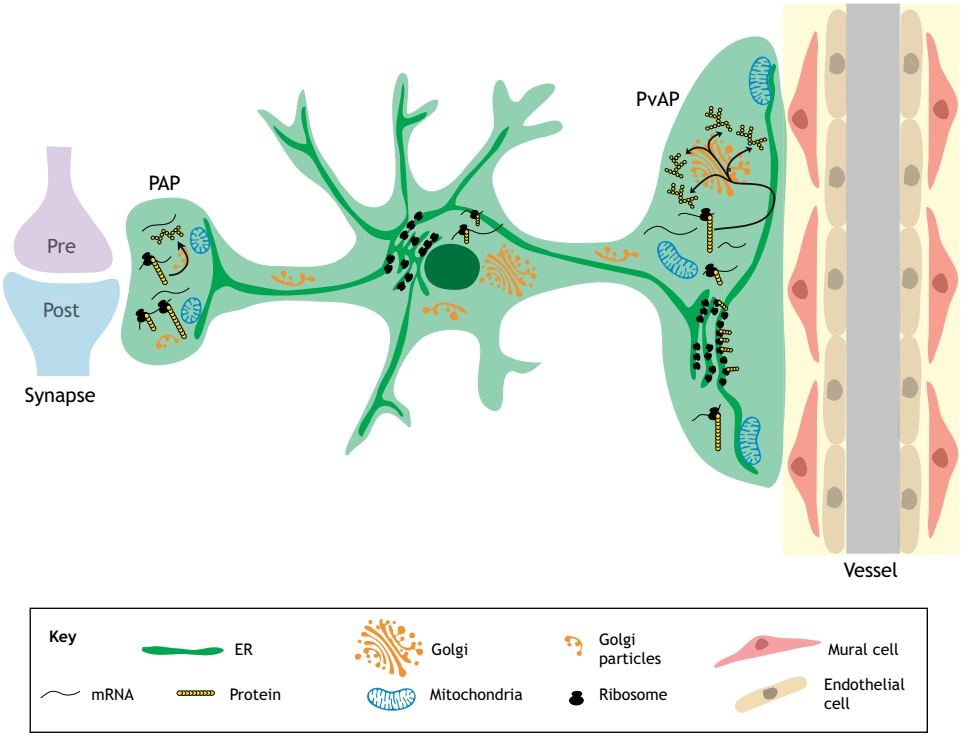

Fig. 3. Subcellular organization of perisynaptic and perivascular astrocytic processes. Astrocytes extend PAPs towards the synapse and PvAPs towards blood vessels. The astrocytic ER forms a continuous network that is in contact with both PAPs and PvAPs. PvAPs also contain rough ER to which ribosomes are bound, as well as mitochondria, ribosomes, mRNAs and mRNA-ribosome complexes that translate new proteins. A Golgi is observed in ∼7% of the PvAPs in the cortex and the hippocampus. In contrast, PAPs only contain small Golgi particles (pGolt particles), and small round mitochondria can also be observed.

an adeno-associated virus strategy, we were able to show that 32% of the hippocampal glutamatergic synapses in an astrocyte territory are contacted by PAPs containing ERGIC-53 (also known as LMAN1), an integral membrane protein located in the intermediate region between the ER and the Golgi (Mazare et al., 2020a). Furthermore, expression of the Golgi tracker pGolt (Mikhaylova et al., 2016) was detected in PAPs surrounding 38% of the glutamatergic synapses, suggesting the existence of detached Golgi particles (possibly outposts) in PAPs (Mazare et al., 2020a) (Fig. 3). A recent electron microscopy study provided evidence for ER in PvAPs and PAPs, and showed that the contact between the ER and mitochondria in PvAPs changed upon brain injury (Göbel et al., 2020). The authors suggested that the subcellular organization of astrocyte processes might be dynamically regulated (Göbel et al., 2020). Finally, to go further, it would be useful to determine whether polysomes and/or monosomes are present in PvAPs as well as PAPs. Indeed, monosomes display specific translational properties (Heyer and Moore, 2016) and have been shown to translate key synaptic transcripts in dendrites and axons (Biever et al., 2020).

Overall, the subcellular organization of PvAPs and PAPs is heterogenous; it is likely that the full functional diversity of translational and post-translational mechanisms in these structures has yet to be discovered (Rangaraju et al., 2017).

The molecular bases of translation in astrocytes

Local translation is mediated by *cis*-acting elements that include RNA motifs and secondary structures influencing the binding of *trans*-acting proteins, also known as RNA-binding proteins (RBPs) (Harvey et al., 2018). RNA-binding motifs are mostly present in UTRs in general and the 3′UTR in particular. Interestingly, a gene can give rise to different 3′UTR isoforms through alternative polyadenylation, which therefore modulates the ability of an mRNA to bind to an RBP. mRNAs bind to RBPs in the nucleus and soma to form ribonucleoparticles (RNPs, also referred to as granules). Depending on the nature of the *cis*- and *trans*-acting elements, the RNPs are transported along the cytoskeleton as cargo by kinesin and dynein molecular motor complexes (Pushpalatha and Besse, 2019). Although transcriptomic data indicate that RBPs are expressed in astrocytes (sometimes more strongly than in neurons), their roles in astrocytes have been poorly explored (Zhang et al., 2014) (Table 1). *Fmr1* encodes the fragile X mental retardation protein (FMRP) and is mutated in fragile X syndrome (FXS, a neurodevelopmental disorder resulting in intellectual disability and autism) (Penagarikano et al., 2007). One of the several possible functions of FMRP in neurons is its ability to act as an RBP and silence the translation of mRNAs encoding synaptic proteins (Darnell et al., 2011). Interestingly, FMRP might be more strongly expressed in astrocytes than in neurons (Table 1) (Zhang et al., 2014). It has been shown that FMRP controls mRNA transport in radial glia PvAPs *in vivo* and binds to mRNAs that encode autism-related signalling proteins and cytoskeletal regulators (Pilaz et al., 2016). In astrocytes, the selective loss of FMRP has been shown to dysregulate protein synthesis in general and expression of the glutamate transporter GLT1 (impairing neurotransmission and astrocytic glutamate uptake) in particular (Higashimori et al., 2016). In astrocytes, the expression of a pathological form of FMRP linked to late-onset FXS/ataxia syndrome has been found to impair motor performance in the mouse (Wenzel et al., 2019). These results strongly suggest that translational control by astroglial FMRP is involved in the pathogenesis of FXS.

The RBP quaking (QKI) is also strongly expressed in glial cells (Zhang et al., 2014). QKI was initially implicated in the regulation of *Mbp* mRNA transport in oligodendrocyte processes (Li et al., 2000; Doukhanine et al., 2010; Larocque et al., 2009; Wang et al., 2010). Furthermore, the QKI-7 cytosolic isoform was shown to regulate *Gfap* mRNA translation in human primary astrocytes (Radomska et al., 2013). More recently, it has been demonstrated that the ribosome-bound mRNAs that are preferentially found in PAPs contain a larger number of QKI-binding motifs (Sakers et al., 2017). This study also suggested that the inactivation of QKI-6 (another cytosolic isoform of QKI) in astrocytes altered the binding of certain mRNAs (e.g. *Sparc*) to ribosomes and thus influenced translation (Sakers et al., 2020 preprint). The role of QKI in brain development was recently studied in a conditional knockout (KO) model of *Qki* in neural stem cells (NSCs), which showed that QKI regulates the differentiation of NSCs into glial precursor cells by upregulating several genes involved in gliogenesis (Takeuchi et al., 2020). Finally, QKI has been reported as a candidate gene for schizophrenia susceptibility (Aberg et al., 2006). Thus, as with FXS, the regulation of QKI-mediated translation in astrocytes might be involved in schizophrenia.

Human antigen R (HuR, also referred to as ELAV-like protein 1) is one of the best-known RBPs in astrocytes. It binds to AU-rich elements (AREs) and stabilizes mRNAs (Brennan and Steitz, 2001). Although HuR is predominantly located in the nucleus, it translocates to the cytoplasm, transports the bound mRNAs to polysomes, and promotes their translation and stabilization (Fan and Steitz, 1998). This mode of translational regulation has been observed *in vivo* following spinal cord injury (Kwan et al., 2017a) and *in vitro* in a stretch injury model of primary astrocytes, in which HuR activated the expression of inflammatory mediators such as interleukin-1β, tumour necrosis factor (TNF), and matrix metalloproteinase 12 (Kwan et al., 2017b). Furthermore, translocation of HuR from the nucleus to the cytoplasm has been found to upregulate the translation of the cysteine-glutamate antiporter (Slc7a11, also known as the xCT system), following the treatment of mouse cortical primary astrocytes with interleukin-1β (Shi et al., 2016). HuR might therefore be a key factor for astrocyte translation in inflammatory contexts. Interestingly, HuR in primary astrocytes was also found to bind to the 3′UTR of *Tardbp*, which encodes TDP-43, an RBP linked to amyotrophic lateral sclerosis (ALS) (Lu et al., 2014). Finally, experiments in a glioma cell line showed that HuR bound to and stabilized the mRNA encoding B-cell lymphoma 2 (*Bcl2*, an important regulator of cell death) by activating its translation (Filippova et al., 2011). Consistent with this, the cytoplasmic level of HuR is positively correlated with the tumour grade in human glioma tissues (Bolognani et al., 2012).

Other known RBPs have been less extensively studied with regard to their possible effects on translation in astrocytes. This is the case for the cytoplasmic polyadenylation element binding protein 1 (CPEB1), which regulates poly(A) tail length and binds cytoplasmic polyadenylation elements (CPEs) in mRNA (Hake and Richter, 1994). CPEB1 also regulates synaptic plasticity (Alarcon et al., 2004) and binds to RNA granules in dendrites (Ohashi and Shiina, 2020). In primary astrocytes, *Fabp7* mRNA (containing CPEs in its 3′UTR) co-immunoprecipitates with CPEB1 (Gerstner et al., 2012). Interestingly, the length of the poly(A) tail in *Fabp7* mRNA in mouse brain samples varies with the time of day, and CPEB1 might thus have a role in the astrocyte-mediated neuronal plasticity linked to circadian rhythm, as previously suggested (Gerstner et al., 2012). Furthermore, CPEB1 controls the division of rat primary astrocytes (Kim et al., 2011). Upon the stimulation of cell division, CPEB1 is phosphorylated, binds to cyclin B1 mRNA and lengthens the mRNA poly(A) tail; in turn, this increases the rate

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Table 1. RNA-binding proteins in astrocytes

RBP	Gene	Expression level in astrocytes/ neurons*	Known roles	Astrocyte studies	Results	Reference(s)
FMRP	*Fmr1*	28.91/13.80	Alternative mRNA splicing; mRNA stability; mRNA transport (for a review, see Davis and Broadie, 2017)	*In vivo* mouse radial glia endfeet	FMRP binds autism-related mRNAs and controls active mRNA transport in radial glia.	Pilaz et al., 2016
				Inducible astrocyte-specific *Fmr1*-KO mouse	Decrease in GLT1 protein and glutamate uptake, resulting in increased neuronal excitability.	Higashimori et al., 2016
QKI	*Qki*	319.64/32.87	mRNA export; pre-mRNA splicing (for a review, see Darbelli and Richard, 2016)	*In vitro* primary human cortical astrocytes	QKI7 isoform binds *Gfap* mRNA; *Gfap* mRNA contains QKE. QKI increase leads to an elevated level of GFAP.	Radomska et al., 2013
				Astroglial QKI6 KO mouse	Impaired translation of *Sparc* mRNA. Increased synapse formation and delay in astrocyte maturation.	Sakers et al., 2020 preprint
				Quaking NSC-specific KO mouse	Quaking influences glial differentiation of NSCs by upregulating the expression of astrocyte and oligodendrocyte genes.	Takeuchi et al., 2020
HuR	*Elavl1*	10.23/11.16	mRNA stability (for a review, see Meisner and Filipowicz, 2011)	*In vivo* mouse spinal cord injury and *in vitro* stretch model of astrocytes	HuR translocates into the astrocyte cytoplasm upon injury. HuR increases the level of cytokine mRNAs.	Kwan et al., 2017a,b
				In vitro primary cortical astrocytes	IL-1 promotes HuR translocation to the cytoplasm. Binding of HuR to the 3′UTR of *xCT* mRNA increases the latter's half-life, protein level, and functions linked to the xCT system.	Shi et al., 2016
				In vitro primary cortical astrocytes from the G93A SOD1 mouse	HuR's binding to the 3′UTR of *Tardbp and Fus* mRNAs controls their translational efficiency. HuR KO in astrocytes leads to neuronal toxicity.	Lu et al., 2014
				In vitro U251 cells and glioblastoma xenograft	HuR upregulates *Bcl-2* mRNA translation and promotes cell survival.	Filippova et al., 2011
				Human glioma tissue and cell lines	The HuR protein level is increased in gliomas and correlates with the tumour grade.	Bolognani et al., 2012
CPEB1	*Cpeb1*	7.72/14.81	mRNA cytoplasmic polyadenylation mRNA transport (for a review, see Richter, 2007)	*In vivo* and *in vitro* PAPs from astrocytes in the mouse hippocampus and primary cortical	CPEB1 controls *Fabp7* mRNA translation in astrocytes via poly(A) tail length regulation in a time-of-day dependent manner.	Gerstner et al., 2012
				In vitro rat primary cortical astrocytes	CPEB1 regulates cyclin B1 translation and cell proliferation. CPEB1 KO enhances proliferation.	Kim et al., 2011
KSRP	*Khsrp*	14.17/19.78	Pre-mRNA splicing, mRNA decay, microRNA biogenesis (for a review, see Briata et al., 2016)	*In vitro* primary cortical astrocytes from KSRP-KO and WT mice	KSRP downregulates some cytokine mRNAs and mediates an inflammatory response in astrocytes.	Li et al., 2012

NSC, neural stem cell; WT, wild type.
*Expressed as the mean fragments per kilobase million (FPKM) (see Zhang et al., 2014).

of cyclin B1 translation and activates cell proliferation (Kim et al., 2011). The far upstream element-binding protein 2 [FUSE-binding protein 2 (FUBP2), also known as KH type-splicing regulatory protein (KSRP)] is an RBP that destabilizes ARE-containing mRNAs (Bird et al., 2013). In an *in vitro* luciferase reporter assay, FUBP2 was shown to downregulate the translation of cytokines. The knockdown of FUBP2 in rat primary astrocytes induces cortical neuron toxicity and astrocyte migration (Li et al., 2012).

In conclusion, only a few *in vivo* studies have been conducted regarding astrocytic RBPs, and data on their mode of action and their function in astrocytes are scarce. In particular, their possible role in the regulation of local translation remains to be addressed.

Conclusions and perspectives

Local translation in astrocytes is an emerging field of research, and many questions have yet to be addressed. First, the role of local translation in the establishment and maintenance of the functional polarity of astrocytes at their perivascular and perisynaptic interfaces is still an open question. Astrocyte heterogeneity constitutes a technical challenge in this respect. Indeed, astrocytes are diverse in terms of both morphology – for instance with voluminous, bushy, protoplasmic astroglia in the grey matter compared to elongated, fibrous astroglia in the white matter – and functionality, particularly with regard to their neuronal and vascular microenvironment (Farmer and Murai, 2017; Miller, 2018). For instance, the gliovascular interface greatly differs from one region of the brain to another and from one type of vessel to another, that is, capillaries, arteries or veins (Cohen-Salmon et al., 2020). The same reasoning applies to PAPs, which display functional and morphological differences throughout the brain. Another inherent difficulty in studies of astrocytes (compared to neurons) lies in the absence of *in vitro* systems in which astrocytes can reliably develop polarized

interfaces with vessels and/or neurons. The emergence of new *in vitro* microfluidic or organoid methods might offer greater opportunities in the future.

If local translation does sustain astrocyte polarity, it might occur differently in the soma and in the various processes. As mentioned above, the three TRAP studies found clear differences in the repertoire of ribosome-bound mRNAs between astrocyte soma and the processes, suggesting that local translation might sustain molecular polarity in PAPs and PvAPs (Boulay et al., 2017; Mazare et al., 2020a; Sakers et al., 2017). One of the most striking results obtained in our latest TRAP study of PAPs from the dorsal hippocampus is the enrichment in several ribosome-bound mRNAs encoding proteins of the large and small ribosomal subunits in PAPs compared to level of these mRNAs found in whole astrocytes (Mazare et al., 2020a). These results suggest that the ribosomal compositions of the astrocyte soma and PAPs could differ. Ribosomal proteins exhibit different functions in ribosomes (Castello et al., 2012), and mutations in individual ribosomal proteins give rise to distinct qualitative effects rather than an overall loss of protein synthesis (Shi and Barna, 2015). Hence, differences in ribosomal protein composition between astrocytic soma and PAPs might have important functional consequences. This question has yet to be resolved and will require a detailed proteomics study. The comparison of astrocytic interfaces might be an important way to determine the role of local translation in astrocyte polarity. PvAPs and PAPs differ in their subcellular organization; they both contain ER, but a full Golgi is observed only in ∼7% of PvAPs, suggesting that protein maturation is regulated differently (even among PvAPs) (Boulay et al., 2017). Interestingly, studies of dendrites have shown that membrane proteins bypassing the Golgi display atypical N-glycosylation profiles and thus probably have specific properties (Hanus et al., 2016). A link between Golgi outposts, microtubule branching, cell polarity and myelination was recently observed in oligodendrocytes (Fu et al., 2019). These findings suggest that the differences in ultrastructural organization between PvAPs and PAPs might underpin a diversity of translational, post-translational mechanisms and perhaps other functions yet to be discovered. Finally, and if local translation indeed sustains astrocyte polarity, PvAPs and PAPs might differ in the repertoire of ribosome-bound mRNAs. This question is still unresolved because the endfeetome has been characterized in whole brain and only covered transcripts that were abundant in astrocytes (Boulay et al., 2017), and the full ribosome-bound repertoire in PAPs was assessed specifically in the dorsal hippocampus (Mazaré et al., 2020a). Our recent refinement of the TRAP protocol now makes it possible to compare ribosome-bound mRNAs in PAPs and PvAPs from a given region of the brain (Mazaré et al., 2020a,b). Incidentally, this technical refinement might also be useful for reinvestigating ribosome-bound mRNAs in axons and dendrites because the earlier TRAP preparations of synapses or neurites contained astrocyte-specific mRNAs (Ouwenga et al., 2017; Shigeoka et al., 2016). Interestingly, our study of some of the most abundant mRNAs in the PAPome revealed differences between hippocampal PAPs and PvAPs, suggesting that local translation might indeed govern functional polarity (Mazaré et al., 2020a). One of the ribosome-bound mRNAs found to be more abundant in PAPs than in PvAPs was *Rplp1*, which encodes a ribosomal protein, suggesting that PvAPs and PAPs might also differ in the composition of their ribosomes, which might give rise to specific translational properties (Castello et al., 2012; Shi and Barna, 2015).

Besides polarity, there is a need for further investigations into the physiological and pathophysiological relevance of local translation in astrocytes. Interestingly, we observed that fear-conditioning in mice altered the levels of several enriched ribosome-bound mRNAs in dorsal hippocampal PAPs; this hints at a physiological role of astrocyte local translation in memory and learning and, more generally, in the cellular response to environmental cues (Mazaré et al., 2020a). During development, local translation in axons has a preponderant role in growth cone guidance, axon elongation and membrane remodelling (Wu et al., 2005; Yao et al., 2006; Campbell and Holt, 2001; Leung et al., 2006; Ming et al., 2002; Piper et al., 2006; Cagnetta et al., 2018; Hengst et al., 2009; Gracias et al., 2014). Results on axonal growth cone raised the intriguing possibility that local translation conditions the growth and formation of PvAPs and PAPs. Dysregulation of local translation in neurons has been linked to diseases such as FXS (Kao et al., 2010), spinal muscular atrophy (Jablonka et al., 2001; Zhang et al., 2003), ALS (Alami et al., 2014; Fallini et al., 2012) and Alzheimer's disease (Baleriola et al., 2014; Kobayashi et al., 2017; Li and Gotz, 2017; Walker et al., 2018). Furthermore, local translation might also be essential for the restoration of axon outgrowth after axon injury (Koley et al., 2019). With regard to the glia, translation of myelin basic protein in oligodendrocyte distal cell processes *in vitro* is impaired by exposure to amyloid β-peptide (Quintela-Lopez et al., 2019). We recently observed that the distribution of mRNAs encoding the GFAP isoforms α and δ differed in an Alzheimer's disease mouse model, particularly in astrocytes close to amyloid deposits (Oudart et al., 2020). As discussed above, most of the RBPs linked to neuronal dysfunction are also expressed in astrocytes. Alteration of astrocyte polarity is a hallmark in neuropathology (Cohen-Salmon et al., 2020; Dossi et al., 2018). Hence, changes in local translation in astrocytes are likely to be linked to diseases by altering the perivascular and perisynaptic functions of astrocytes.

In conclusion, the discovery of local translation in astrocytes raises a new repertoire of questions, in particular regarding the way astrocytes regulate their high level of polarity in normal and pathological contexts. Given the critical functions of astrocytes in the regulation of synaptic and vascular functions, characterization of local translation in these cells might also reveal important and novel aspects of the brain physiology.

Competing interests
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