Local translation in neuronal processes
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Neurons exhibit a unique degree of spatial compartmentalization and are able to maintain and remodel their proteomes independently from the cell body. While much effort has been devoted to understanding the capacity and role for local protein synthesis in dendrites and spines, local mRNA translation in mature axons, projecting over distances up to a meter, has received much less attention. Also, little is known about the spatio-temporal dynamics of axonal and dendritic gene expression as function of mRNA abundance, protein synthesis and degradation. Here, we summarize key recent findings that have shaped our knowledge of the precise location of local protein production and discuss unique strategies used by neurons to shape presynaptic and postsynaptic proteomes.

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Introduction
Memories may persist for the lifetime of animals but the proteins that compose synapses undergo constant synthesis and degradation [1], enabling neurons to dynamically adjust their proteome in response to different contexts. More than 75% (1714 μm³) of a neuron’s entire volume (2252 μm³) consists of dendrites that make several thousand synaptic contacts (morphometric data were obtained from rat mature granule cell reconstructions [2] using the TREES toolbox [3]). Within each synapse, there are between 1000–3000 different proteins [4] that exist in copy numbers ranging from 150 to 20,000 [5–7]). This highly polarized morphology poses a major challenge for the homeostatic regulation of protein concentration (proteostasis) in synapses. The maintenance of presynaptic protein homeostasis is particularly demanding as axons provide long-range projections that can reach up to a meter in length, meaning for motoneurons and dorsal root ganglion cells the axonal volume can be up to 160 million μm³ [8]. In part, neurons overcome these distance constraints by localizing mRNAs to synapses and locally producing proteins (see Refs. [9,10]). This strategy provides unique advantages for the optimization of local proteostasis, allowing tight and rapid regulation of synaptic protein abundance in space and time. Initially, it was believed that local protein synthesis was a minor source of synaptic protein and was used only in certain circumstances during synaptic plasticity (see Ref. [11] for review). This assumption was challenged by the discovery of an astonishing number (~2500) mRNA species in the hippocampal neuropil (a.k.a. the local transcriptome) [12,13] and in neurites [14,15]. These observations changed our notion of the capacity for mRNA localization and local translation and raise new intriguing questions: Where exactly are proteins being locally produced? How can a neuron translate a given mRNA repertoire at a synaptic site in response to one cue but not another? And, how efficiently do localized transcripts need to be translated in order to meet presynaptic and postsynaptic demand under basal and synaptic stimulation plasticity conditions? This review summarizes the most recent work shaping our understanding of local mRNA translation in neurons, and highlight remaining unanswered questions for the field.

Where and when does a neuron produce protein?
Over the past ~30 years, many labs have contributed to dissecting the function of local translation at the synapse, especially in the postsynaptic compartment. Studies utilizing electron microscopy (EM) visualized polyribosomes (two or more ribosomes translating a single mRNA) present in dendritic shafts as well as at the base and interior of dendritic spines [16–18]. These localized pools of ribosomes are believed to play a vital role in protein synthesis-dependent synaptic plasticity (for reviews of local translation in plasticity please see Refs. [9,10]). While the role for local translation in the postsynapse is well documented, the role for it on the presynaptic side is much less understood.

Classically, roles for presynaptic translation have been assigned to developing and damaged axons (for review see Refs. [19–21]). A role for local translation in the mature axon or presynaptic terminal has been less clear and indeed the often prevailing view has been that little to no translation occurs in mature axons [22]. This view has arisen in large part from an apparent inability to detect either ribosomal RNA [23] or ribosomes themselves by EM [24]. Earlier work, however, demonstrating presynaptic incorporation of isotope labelled amino acids
[25–27] and recent studies showing clustered ribosomes associated with actin-rich patches along the length of mature axons [28] has received less attention.

Despite the above evidence indicating mature axons have the capacity for protein synthesis, the small size of axons and their terminals as well as the tight association with both glia and the postsynapse, has made the isolation of a transcriptome (all mRNAs present) or a translatome (mRNAs bound by ribosomes) extremely challenging. This limitation was recently overcome using cell-type-specific ribosome tagging [29] of the axonal projections of retinal ganglion cells in the optic tectum [30]. Shigeoka et al. identified differences in the translatome over various developmental stages, including phases of axonal growth vs maintenance. A localized pool of axonal mRNAs, present in pre-synaptically enriched synaptosomes was also recently described in the work of Hafner et al. [31]. In both studies, mRNAs for a number of synaptic proteins (Sx6, RIM1-3, SNAP25, etc.) which function in the active zone were detected. This indicates that, like in the postsynaptic compartment, translation likely serves a functional role in maintenance and modulation of the presynapse. Indeed, such a functional role has been reported for beta-actin mRNA in developing axons, where its local translation drives axonal branching [32] and its mRNA and protein localization to developing axonal branches was seen to predict longer lived branching events in developing axons [33**]. In addition, work by Batista et al. demonstrated that axonal synthesis of SNAP25 is required for proper formation of presynaptic boutons [34*]. Recent work has also described axonal RNAs are co-transported with late endosomes, and suggests that late endosomes function as platforms for local synthesis of proteins responsible for axonal integrity and survival [35]. Interestingly, disruption of endosomal RNA association lead to dysfunction within mitochondria. Given recent work demonstrating the importance of mitochondria in driving protein synthesis in the dendrite [36], this suggests that mitochondria themselves may be dependent on local production of proteins. Additionally, a number of axonopathies include alterations in axonal protein synthesis homeostasis [37,38,39]. Additionally, diseases such as Fragile X Syndrome with a known protein synthesis homeostasis defect also present with presynaptic defects [40]. These studies suggest that translation at the presynapse likely functions in a manner similar to the postsynapse, in modulating and remodelling the local proteome.

Production of transmembrane proteins: intraxon synthesis of transmembrane proteins

Interestingly, mRNAs encoding a number of transmembrane proteins, including ion channels, are found within the presynaptic transcriptome [30,31,41]. In addition, a recent work in squid giant axons has demonstrated that mRNA encoding for ion channels injected into the axoplasm can change the electrical properties of the axon, consistent with translation and incorporation into the plasma membrane [42]. Given rough ER and Golgi are noticeably absent within the axon [43], this presents a unique challenge in understanding presynaptic translation. Without a classical secretory pathway, how can transmembrane proteins be translated, processed, and incorporated into the plasma membrane? While it is true that rough ER is apparently absent in EM analyses, the expectation of EM detection of rough ER assumes that ribosomes are stably associated with the ER. Importantly, it undervalues the role of co-translational targeting of ribosomes and mRNA to the ER via the SRP pathway [44], where ribosomes only transiently dock on the ER during translation. According to this idea, rough ER could transiently exist, with either single or small multiples of ribosomes. Components of the SRP pathway are readily detected within both developing and mature axons [41,42,45–47].

While overlooked for many decades, recent studies have changed our notion of the capacity for mRNA localization and local translation in the presynaptic compartment. Not only dendrites, but also axons contain a remarkably diverse set of localized mRNAs, implying that presynaptic and postsynaptic protein synthesis is the rule rather than the exception [12,30]. Yet, little is known about the spatio-temporal dynamics of axonal and dendritic gene expression as function of mRNA abundance, protein synthesis and degradation.

How does the neuron regulate its proteome?

Protein life-cycle measurements using a dynamic SILAC approach (stable isotope labelling with amino acids in cell culture) have revealed the lifetimes of neuronal and synaptic proteins [1]. For example, Dorrbaum et al. reported a median half-life of synaptic proteins of ~5 days in mature primary hippocampal neurons [48]. Within synapses, some synaptic proteins are present at a very low copy number (e.g. 60 copies of the AMPAR subunits per synapse) [3–7,49] and display exceptionally long lifetimes exceeding 20 days [50]. Does the relatively small size of the synaptic compartment and the resulting low protein copy number change the landscape of how translation is accomplished? In yeast, monosomes (a single ribosome loaded onto an mRNA) are translationally active [51], raising the possibility that the production of a subset of low abundant synaptic proteins might be accomplished by single ribosomes. This assumption is supported by numerous EM studies, reporting a paucity or even lack of visible polysomes in some dendrites and axons. While the polyribosome scarcity would apply considerable constraints on the variety of locally synthesized proteins, translation by monosomes could allow for the production of a more diverse set of localized proteins, potentially only required at low stoichiometry per synapse [5–7,49] (Figure 1a).

It is now well appreciated that synaptic plasticity requires local translation [52–55]. One hallmark of local protein synthesis at the postsynapse is its dynamic nature, and it is regulated by various forms of synaptic
Local translation in neuronal processes – a conceptual view.

(a) While translation of specific mRNAs by single or few ribosomes may suffice to fuel synapses with long-lived and low abundant proteins, increased ribosome recruitment could be required for maintaining the local homeostasis of abundant, less stable proteins.

(b) 5' UTR diversity can provide means to differentially regulate the translational efficiency of the same transcript species. Cis-regulatory elements embedded within the 5' UTR dynamically interact with trans-acting factors to regulate the rate and the site of translation initiation. Initiation from alternative translation start sites could promote the production of various protein isoforms that may differ in their function or localization.

(c) Serving as platform for microRNAs and RBPs, 3' UTR isoforms may expand the potential for differential translational regulation of the same mRNA species. Synaptic stimulation could induce a local 3' UTR shortening of selective mRNAs, which may enhance their translational efficiency.

(d) Localized translation of ribosomal proteins may allow in-situ tailoring of the ribosome composition. This direct specialization of the translational machinery itself could control the translation of selective transcript subsets in response to specific synaptic stimuli.

plasticity and neuronal activity itself [56]. Recent studies suggest a similar regulation in the presynaptic compartment. For example, Scarnati et al. recently demonstrated an inverse relationship between activity and axonal protein synthesis in the calyx of Held: inhibition of protein synthesis enhances the spontaneous release of vesicles [57]. This suggests active protein synthesis may limit the pool of releasable presynaptic vesicles. In addition, endocannabinoid-mediated inhibitory LTD (iLTD) requires axonal protein synthesis within inhibitory terminals and translation is enhanced by endocannabinoid stimulation [58]. Expanding upon this, Hafner et al. demonstrate that both inhibitory and excitatory presynaptic terminals as well as the postsynapse can be engaged simultaneously or separately at the translational level during neuromodulation [31].

Studies investigating the effect of ongoing synaptic transmission and plasticity on protein synthesis have been limited to the detection of the synthesis of a few proteins such as Arc [59,60], Camk2a [55] or beta-actin [61]; proteome-wide changes in expression levels following synaptic stimulation remain largely unexplored. Using metabolic labelling of newly synthesized proteins with BONCAT (bioorthogonal noncanonical amino acid tagging), Schanzenbacher et al. showed that up or down scaling of neuronal
activity regulates the de novo synthesis of ~300 proteins, some of which were upregulated after just two hours of treatment [62,63]. Whether these newly synthesized proteins arise from a local source, remains a technically challenging issue that can only be addressed by metabolic labelling after physical separation of neuronal processes from cell bodies. Hodas et al. took advantage of the layered architecture of the CA1 hippocampus to separate stratum pyramidale (comprising largely pyramidal neuron cell bodies) from stratum radiatum and lacunosum-moleculare (containing mostly dendrites and axons of pyramidal neurons) and used BONCAT to detect 300 de novo synthesized proteins specific to axons and dendrites following neuronal activation [64]. As the neuropil is a composite tissue, some of the identified newly synthesized proteins may originate from glia and interneurons rather than dendrites or axons from pyramidal neurons. Recently, Alvarez-Castelao et al. developed a transgenic mouse line where Cre-recombinase induced expression of a mutant methionyl-tRNA synthetase enables the cell-type-specific labelling of nascent proteins with a non-canonical amino acid [65]. With this advanced technology, future studies will allow even more precise identification of the local nascent proteome in vivo.

Regulating protein levels: at the transcript or translational level or both?

Given the impressive ability of neuronal processes to rapidly alter protein synthesis in response to activity, a question arises: What is the major determinant of the local proteome, the localization or the translation of mRNAs? At locations remote from the transcription source (the nucleus), the regulation of gene expression at the translational level could provide a means to modulate the proteome with higher spatial and temporal fidelity. The translational status of an mRNA is thought to be reflected by the number of loaded ribosomes, which can be assessed at a genome-wide level by ribosome profiling. Deep sequencing of ribosome protected mRNA fragments (footprints) enables the measurement of translational efficiency, which is evaluated by the ratio of footprints relative to total transcript abundance [66]. Zappulo et al. performed independent translational and proteomic analysis of neuronal compartments after growing young neurons differentiated from mESCs on a microporous membrane where the neurites extend to the lower side of the membrane. The comparison of neurite ribosome profiling data with the transcriptome and proteome showed a surprisingly good correlation between RNA localization and local protein production, indicating that mRNA localization is a major determinant of the neurite enriched proteome in this developing system [14]. While translation at a low rate might suffice to fuel the constitutive local proteome, increases in local translational efficiency might be important to remodel the proteome in response synaptic stimulation (Figure 1a). In support of this hypothesis, two recent EM studies reported enhanced polyribosome detection at synapses in response to synaptic activity [18,67].

What determines the translational rate of a transcript in neuronal processes?

One major factor affecting the translatability of an mRNA is information contained the 5′ and 3′ untranslated regions (UTRs), which can differ between transcript isoforms [68–70]. Several studies have demonstrated that transcripts localized to neuronal processes display longer 5′ and/or 3′ UTR isoforms [13,15,30,71]. In the 5′ transcript leader, ribosomes initiate translation at canonical but also alternative start sites [72]. Generally, translation of a short upstream open reading frame (uORFs) captures scanning ribosomes, repressing translation levels of downstream protein-coding genes in various cell types [68,73], including neurons [74]. Thus, neurons could fine-tune their local translation rate through selective targeting of competitive 5′ UTR isoforms between compartments (Figure 1b). Alternatively, the differential usage of translation start sites may allow for the production of protein isoforms that could vary in their function or localization from the same transcript at different subcellular locations [75,76].

In neurons, 3′ UTR length has been negatively correlated with the translation rate of an mRNA, presumably owing to increased potential for interaction with trans-acting regulatory factors like miRNAs [74]. Recently, Tushev et al. demonstrated that 3′ UTR isoforms of transcripts localized to neuronal processes are significantly longer than 3′ UTRs of non-localized transcripts and often code for proteins associated with axons, dendrites and synapses [13]. Do transcripts regulating synaptic function display enhanced potential for translational regulation? In support, Tushev et al. observed increased structural complexity and the differential usage of RNA binding protein (RBPs) and microRNA (miRNAs) binding sites in localized 3′ UTR isoforms. This study also proposed the thought-provoking concept that neuronal activity could induce a local shortening of 3′ UTRs [13]. Therefore, long localized 3′ UTR isoforms may potentially translate poorly under basal conditions, and through activity-induced shortening become more efficient in their translation (Figure 1c).

Regulating the translational machinery: ribosome heterogeneity

An extra layer of spatio-temporal control of local translation could be added by the dynamic interaction of UTR regulons with highly specialized translational machinery. There is now emerging evidence that ribosomes are not as monolithic as typically assumed, and can accommodate multiple compositions, with respect to core ribosomal proteins (RPs) and ribosome-associated proteins (reviewed in Ref. [77]). Neuronal ribosomes display substantial variability in the half-life of their constituents ranging from 4 to 10 days [48]. As ribosomes are
Outstanding questions.
(a) How much of the proteome is synthesized locally? Over past two decades the number of identified localized transcripts increased from a handful to thousands. Still, it remains unknown the relative contribution of different neuronal compartments to the total proteome.
(b) How heterogeneous are individual synaptic compartment (dendritic and axonal) transcriptomes? Do they change in response to synaptic plasticity? With the wealth of current studies being derived from pooled samples, it still remains unknown what the transcriptome profiles of individual synaptic compartments look like.
(c) How are ribosomes targeted to the synapse? Given the abundant pool of localized ribosomes [16,18,27,31,67] in both the presynaptic and postsynaptic compartments, it is not known if they are transported specifically or through association with mRNA transcript in translationally-repressed RNA granules.
(d) How is differential translation of mRNAs achieved locally? Large-scale studies of localized RBPs, their mRNAs targets and potentially associated mRNAs thus far suggest a complex if not chaotic competition for regulatory elements.
(e) Is there crosstalk between the presynaptic and postsynaptic compartments at translational level? With recent work on the Arc transcript demonstrating the capacity for transmission from one side of the synapse to the other [78,79], it is an intriguing possibility that these two compartments could ‘talk’ and influence the proteome of each other.
(f) What is the relative contribution of presynaptic or postsynaptic translation to the long lasting molecular and behavioural changes induced by synaptic plasticity? The development of tools enabling spatio-temporal control over protein synthesis inhibition at the subcellular level, potentially through the use of a light-inducible system, would allow us to address this question.

assembled ‘for life’ during their biogenesis in the nucleus, the latter observation suggests that RPs could be exchanged for repair or specialization in neurons. The localization of transcripts coding for RPs to dendrites and axons [12,30] as well as their local translation [64] beg the question as to whether ribosomes could be specialized in situ (Figure 1d). For example, plasticity customized ribosomes could direct a specific local translational program required for changes in synaptic strength.

Conclusion
The need of a neuron to tightly and dynamically regulate its proteome is, in part, met at the local level through control of when and where proteins are made. While
appreciated for decades in both developing axons and dendrites, local protein synthesis has largely been overlooked in mature axons. However, with a recent renaissance in assessing local translation in the mature axon, we’re learning not only is it important in axonal homeostasis but for synaptic plasticity as well. Furthermore, the complexity of localized translational control lies in the dynamic interaction between regulatory elements embedded in the transcript and a rich toolbox of trans-acting factors, including translational machinery components, RNA binding proteins and miRNAs. Signaling pathways in response to activity and stimuli will alter the synaptic expression, distribution and functional state of these trans-factors, thereby allowing for the regulation of local protein production with high spatial and temporal fidelity. This level of localized regulation and complexity undoubtedly underlies the unique dynamic properties of the neuron. Importantly, for as much as we have learned over the past decades, several unresolved questions remain (Figure 2).

**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


Using 3’ end RNA sequencing of microdissected rat brain slices, this study discovered an impressive diversity in mRNA 3’ UTRs, with many transcripts showing enrichment for a particular 3’ UTR isoform in either the soma or the neuropil. The 3’ UTR isoforms of localized transcripts are significantly longer than the 3’ UTRs of non-localized transcripts and often code for proteins associated with axons, dendrites, and synapses.


This study investigates the relative contribution of protein transport, mRNA localization and local translation to polarity in neurons differentiated from mouse embryonic stem cells. The findings demonstrate that mRNA localization is the primary determinant of protein localization in neurites.


In this study, the authors using a FRAP-based translational reporter to visualize in real time, for the first time, ongoing local protein synthesis in developing axons in vivo.


In this study, the authors provide compelling evidence that synthesis of one particular presynaptic component, SNAP25, is a required step in the development and maturation of presynaptic terminals.


This study, using in vivo metabolic labeling, could see decreased metabolic labeling incorporation within the sciatic nerve of mutant FUS expressing mice. Providing the first, potential, demonstration of impaired local protein synthesis in an axonopathy in vivo.


By using in vivo SILAC labeling, this study measured accurate lifetime of around 3500 proteins in various regions of the brain and in isolated synapses.


In this study the authors found that enhanced neuronal activity, not just plasticity, could drive enhanced protein synthesis of neurogranin, and that this synthesis is required for contextual memory formation.


This study, taking advantage of the large Calyx of Held synapse, provided the first compelling visualization of local protein synthesis in a mature presynapse in brain sections.

This study provided the first compelling evidence for local protein synthesis occurring in inhibitory presynaptic terminals and being regulated by synaptic plasticity.


This study reports the first method to isolate cell-type-specific proteomes in vivo. The authors describe the development of a transgenic mouse line where Cre-recombinase-induced expression of a mutant methionyl-RNA synthetase (L274G) enables the cell-type-specific labeling of nascent proteins with a non-canonical amino-acid and click chemistry.


