

Local translation in neuronal compartments: how local is local?

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Abstract

Efficient neuronal function depends on the continued modulation of the local neuronal proteome. Local protein synthesis plays a central role in tuning the neuronal proteome at specific neuronal regions. Various aspects of translation such as the localization of translational machinery, spatial spread of the newly translated proteins, and their site of action are carried out in specialized neuronal subcompartments to result in a localized functional outcome. In this review, we focus on the various aspects of these local translation compartments such as size, biochemical and organelle composition, structural boundaries, and temporal dynamics. We also discuss the apparent absence of definitive components of translation in these local compartments and the emerging state-of-the-art tools that could help dissecting these conundrums in greater detail in the future.

Keywords compartments; local translation; nascent protein; plasticity; spatial spread

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See the Glossary for abbreviations used in this article.

Introduction

Protein synthesis is essential for the maintenance and regulation of the cellular proteome. The discovery of the mechanism of protein synthesis 60 years ago led to the understanding that the decoding of information from mRNA to protein is carried out by “adaptor” RNAs and catalyzed by enzymes in cell extracts—later identified as tRNAs and ribosomes, respectively [1,2]. In the past 20–30 years, it has become clear that the translation of mRNA to protein is not only regulated temporally in a cell type-dependent manner, but also has a strong subcellular component. Once thought to occur exclusively in the somatic space close to the nucleus, protein translation has been demonstrated far from the central perinuclear region in decentralized local domains—a process referred to as local translation [3]. One cell type that has been studied extensively in the context of local translation is the neuron. Neurons are highly polarized cells

with specialized morphologies. Efficient neuronal function is mediated by the collection and integration of signals received by dendrites, processing, and “decision-making” in the soma, and then transmission of information to the axons (Fig 1). Axons communicate to adjacent neurons at synapses where chemical transmitters released from the presynaptic terminal bind to receptors at the postsynaptic terminal of a dendrite (Fig 1). A single neuron can receive signals at several thousand independent synapses, and the strength of the signal transmission can be regulated at the level of single inputs. The highly polarized morphology and function of neurons and the continuous demand to adapt to external stimuli make local translation a key process in the regulation of neuronal physiology [3].

Local protein synthesis provides a means to locally establish, maintain, and modify the synaptic proteome. Classical studies set the foundation for this idea by showing that protein synthesis constituents and machinery are present in or around synapses. Among the first players detected in dendrites were the mRNAs for microtubule-associated protein 2 (MAP2) [4], calcium/calmodulin-dependent protein kinase 2 alpha (CaMK2a) [5], activity-regulated cytoskeleton-associated protein (Arc) [6], and polyribosomes [7–9]. The demonstration of protein synthesis in severed neurites (axons and/or dendrites) and soma-free biochemical preparations further supported this concept [10–13]. Furthermore, the functional significance of these observations came with the demonstration that local protein synthesis is involved in some forms of synaptic plasticity and learning [14–17]. This led to the idea that local translation could drive the synthesis of a specific set of “plasticity-related proteins” (PRPs) and that their identity could be unraveled by the characterization of localized mRNAs—the local transcriptome.

Both high-throughput and single-molecule candidate approaches have been developed to characterize the local transcriptome and the newly synthesized proteome [18–29]. The characterization of local transcriptomes by RNA-seq and microarrays by various groups [18,30–37] revealed that the comprehensive set of localized mRNAs is as large as 2,550 mRNAs—in the neuropil alone [18]. Thus, it is important to also characterize the local translome—the fraction of mRNAs that get actively translated to carry out neuronal function. To address this question, high-throughput methods exploiting the association of ribosomes with mRNAs have been implemented [38–46]. In addition, the use of mRNA tracking along with nascent

Glossary

3'UTR	3' untranslated region
Acot7	acyl-coA thioesterase 7
AHA	azidohomoalanine
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
APV	amino-5-phosphonovaleric acid
Arc	activity-regulated cytoskeleton-associated protein
Arp	actin-related protein
ATF4	activating transcription factor 4
ATP	adenosine triphosphate
Aβ₁₋₄₂	amyloid β peptide 1-42
BDNF	brain-derived neurotrophic factor
CaMK2a	calcium/calmodulin-dependent protein kinase 2 alpha
CB1	cannabinoid receptor type 1
CHX	cycloheximide
DCC	deleted in colorectal cancer
DHPG	dihydroxyphenylglycine
DIV	days <i>in vitro</i>
DRG	dorsal root ganglion
E5	embryonic day 5
EphA2	ephrin type-A receptor 2
ER	endoplasmic reticulum
ERGIC	endoplasmic reticulum–Golgi intermediate compartment
FISH	fluorescence <i>in situ</i> hybridization
FMRP	fragile X mental retardation 1
FRAP	fluorescence recovery after photobleaching
FRET	fluorescence resonance energy transfer
FUNCAT	fluorescent non-canonical amino acid tagging
GFP	green fluorescent protein
GluA1	glutamate receptor subunit A1
KO	knock out
Kv3.1a	voltage-dependent potassium channel 3.1a
LTP	long-term potentiation
Lys	lysine
MAG	myelin-associated glycoprotein
MAP2	microtubule-associated protein 2
mGluR	metabotropic glutamate receptors
miRNA	micro ribonucleic acid
mRNA	messenger ribonucleic acid
NA	not applicable
NGF	nerve growth factor
NLS	nuclear localization signal
NMDAR	<i>N</i> -methyl-D-aspartate receptor
NT3	neurotrophin-3
PAGFP	photoactivatable green fluorescent protein
PALM	photoactivated localization microscopy
PRPs	plasticity-related proteins
PSD-95	postsynaptic density protein 95
Puro-PLA	puromylation-proximity ligation assay
RanBP1	Ran-specific guanosine triphosphatase activating protein
RNA	ribonucleic acid
RNA-seq	ribonucleic acid sequencing
Rpl35	ribosomal protein l35
Sema3A	semaphorin 3A
Thy1	thymus cell surface antigen 1
tRNA	transfer ribonucleic acid
WIN	(R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate

protein visualization has enabled single-molecule, real-time visualization of translation and its kinetics [22,23,26–29].

All of the above techniques have set the stage to probe different facets of local translation and address a new generation of

questions. For example, where exactly is a protein translated, what is its functional fate and what is its spatial range of action within subneuronal regions—in other words, what are the relevant *compartments*?

Space redefined: What are the relevant compartments?

We define the term *compartments* here as spatially restricted domains within which cell biological machines carry out a function. In the context of protein synthesis, we consider that if new proteins are synthesized locally in subneuronal regions, they should be spatially restricted to sustain their functional activity in a localized fashion. How does one define a relevant compartment for local translation (*translation compartment*)? And how might these cell biological compartments map onto functional compartments for information processing?

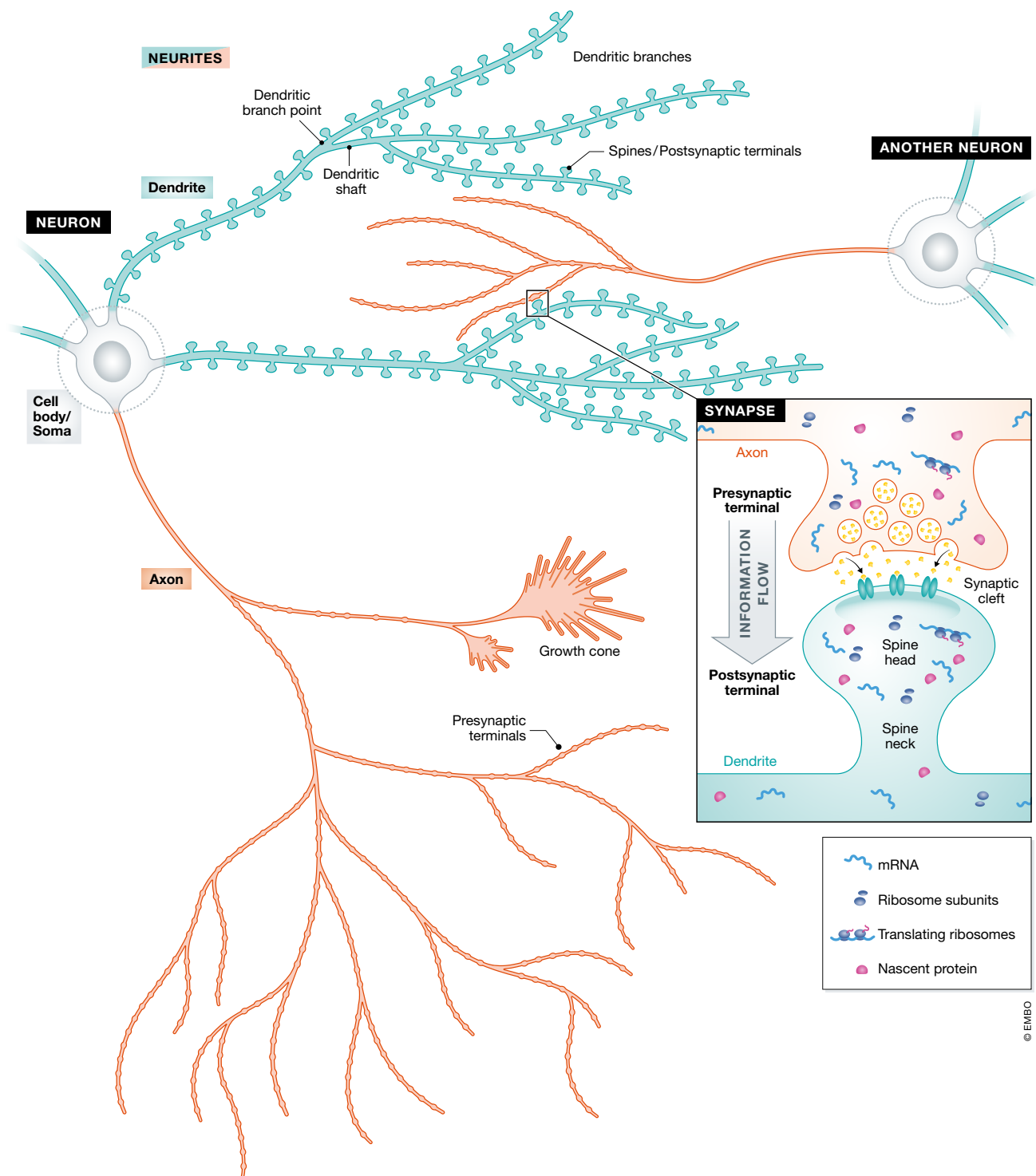
First, it is important to discriminate between the site of synthesis (*source compartment*) and the site of action of the nascent protein (*effector compartment*) (Fig 2A–E). The source and the effector compartments could be within a few microns of each other (e.g., within a dendritic branch) or hundreds of microns apart (Fig 2A–E). We also consider specific features of compartments: Do they possess defined structural boundaries? What roles do cellular organelles such as ribosomes, mitochondria, and secretory pathway machinery play? Are these compartments dynamic—do they form, adapt, and/or disassemble in response to local cues? All these characteristics operate in unison to constitute a functional outcome.

This review's focus is on the emerging picture of the spatio-temporal organization of such compartments relevant for local translation. In addition, we summarize some unsolved issues—the apparent absence of expected translational components in some compartments and the unexplored prerequisites such as local energy reserves for translation.

Translation compartments in dendrites

Local translation was initially studied by comparing the translational capacity of structurally defined classical compartments, namely the cell body and neurites. The protein synthesis observed in neurites was mainly attributed to dendrites, owing to the low levels of mRNAs and conflicting data on the presence of local translational machinery in axons [47,48]. Furthermore, the capacity of dendrites, isolated from the cell body, to independently synthesize new proteins upon stimulation strongly supported this view [10].

In order to understand how translation compartments are formed, it is important to describe the two general prerequisites that have to be fulfilled to allow localized translation: (i) the mRNA of interest and the translational machinery have to be present at the site of action, and (ii) the stimulus to induce translation has to be sensed and transferred to the translational machinery. The overlap of these prerequisites defines the site of translation. In some cases, while mRNA is widely distributed in the neurites by efficient mRNA transport, the presence of overlapping local signals leads to minor local redistribution of mRNA and its subsequent translation. In other cases, the mRNA localization itself is confined and targeted to specific subneuronal regions; in this case, even global stimulation would lead to only restricted sites of translation, despite the



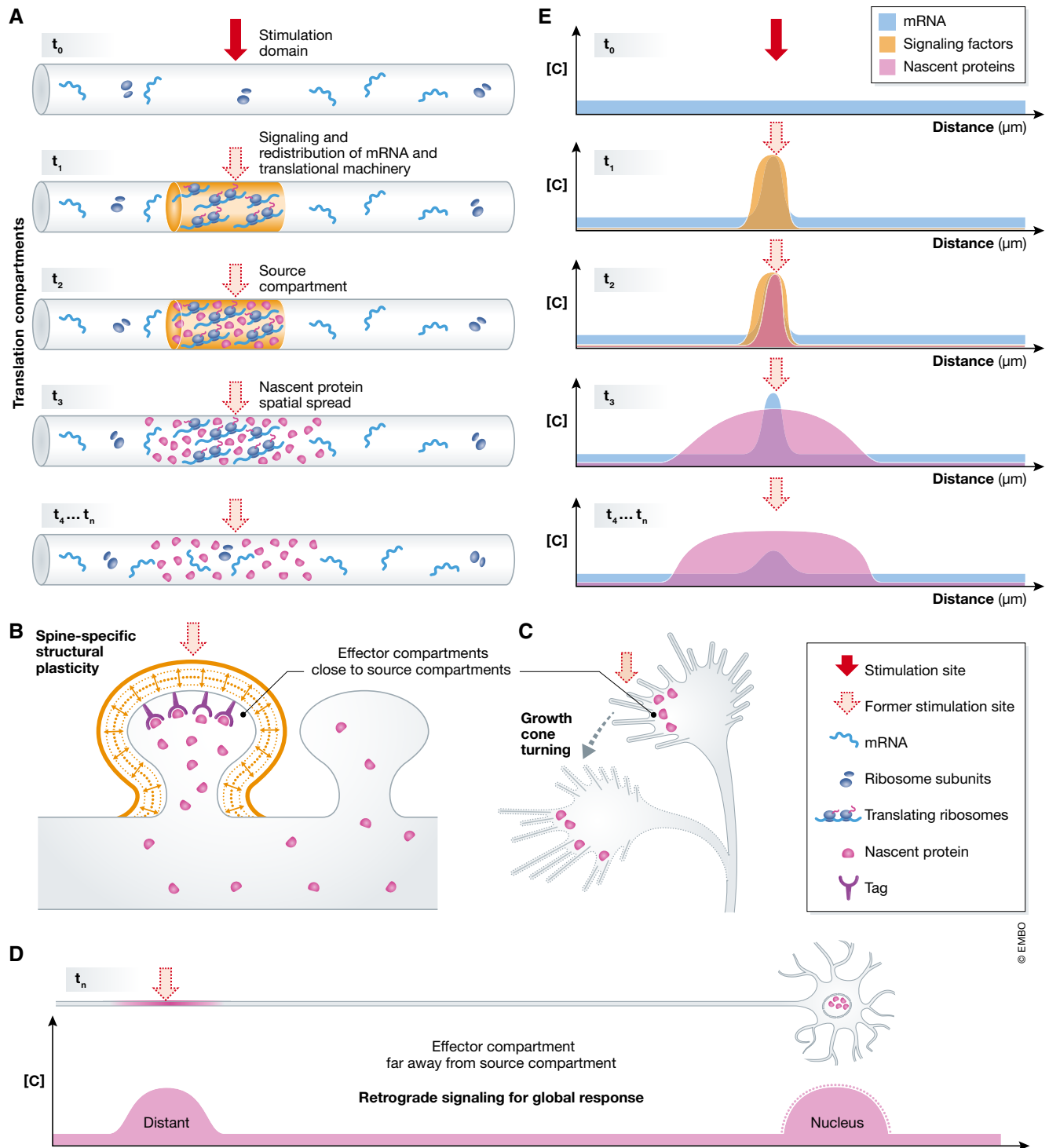
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Figure 1. Neuron and its structural compartments.

Morphology of the neuron showing its cell body (gray) and neurites—composed of dendrites (blue) and axons (red). The inset shows a synapse formed between the presynaptic terminal of one neuron (red) and the postsynaptic terminal of another neuron (blue).

widespread availability of receptors to sense the stimulus. One example is the spatial coding for the delivery of BDNF mRNAs to different parts of somata and dendrites. Under basal conditions, BDNF mRNA localization is mainly somatic [49,50], and the

translated BDNF protein distribution is regulated by the secretory pathway. Upon activity, BDNF mRNA levels can be upregulated, and their selective distribution to proximal or distal dendrites is achieved by a code in their 5' non-coding regions. This selective



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Figure 2. Local translation compartments.

(A) On receiving a focal stimulus (red filled arrow)—stimulation domain (t_0)—mRNA and translational machinery are redistributed to the stimulation site (t_1). This local redistribution of mRNA and translational machinery, on overlap with signaling events (orange) form the site of synthesis for nascent proteins (magenta)—source compartment (t_2)—near the former stimulation site (red dotted arrow). The nascent proteins quickly spread over time. This nascent protein spatial spread (t_3) gradually increases and might reach a compartment of stable size defined by unknown factors (t_4, t_n). The site of action of the nascent proteins—effector compartment—is restricted within a smaller region of the nascent protein spatial spread and can be either close to the source compartment as in a spine (B) and the growth cone (C) or hundreds of microns apart as in the nucleus (D). All these translation compartments operate in unison to elicit a functional outcome, for example, spine-specific structural plasticity (B), growth cone turning (C), and retrograde signaling for global response (D). (E) Graphical representation showing the concentration ([C]) of mRNA (blue), signaling factors (orange), and nascent proteins (magenta) plotted at various time points $t_0, t_1, t_2, t_3, t_4, t_n$.

distribution of BDNF mRNA results in restricted sites of putative BDNF translation in dendrites even upon global stimulation [49].

Dendrites comprise several recognizable structural compartments such as the dendritic spine, spine neck, dendritic shaft, branch points, and dendritic branches (Fig 1). Measured translation compartments, however, are often not limited to these structural boundaries. They seem to exist as a continuum of spatial domains either restricted within part of these structures or spanning across them. It is only beginning to be understood where specific proteins are synthesized, and what the limits of their spatial spread and subsequent function are in the context of these structural boundaries.

Spines

Spines are nodes where dendrites receive information from adjacent neurons (Fig 1). Spine heads, the sites of most excitatory synapses, are diffusionally and electrically restricted from their respective dendritic shafts by thin spine necks [51,52]. The spine neck acts as a diffusion barrier to proteins and small molecules, and the ease of diffusion is modulated by activity [53–56]. This compartmentalization is likely important for spine-specific synaptic modulation, as local stimulation of spine heads shows spine-specific structural plasticity [52,57,58]. It is not clear, however, if local translation of proteins is confined to spines (Fig 3A). During tetanic stimulation, the enrichment of polyribosomes in spines compared to dendritic shafts supports this view [59]. Moreover, upon a global increase in basal translation, translation hot spots were observed in some spines in addition to hot spots in dendritic shafts [60] (Table 1). In order to achieve local stimulation, a clever approach was recently developed to stimulate multiple adjacent spines and visualize β -actin mRNA and its translation simultaneously. This experiment revealed the recruitment of β -actin mRNAs and newly synthesized β -actin protein near the stimulated region. However, these translation hot spots were not spine-specific [29] (Table 1) and direct evidence for spine-specific translation is still lacking (Fig 3A). This is partly because translation has not yet been successfully monitored in the presence of localized single-spine-restricted stimulation. However, it is possible that the translation compartment measured upon single-spine stimulation is *not* restricted to a spine, but the functional outcome of the translation event is spine-specific. For instance, a stimulated spine could selectively trap a newly synthesized protein even if the respective mRNA and translation machinery are localized outside the spine [61–63] (Fig 3B). If that were the case, what would be the spatial spread of the nascent protein? And what influences the spatial limit of its action—the effector compartment?

Dendritic compartments

In order to probe the spatial spread of nascent proteins, several experiments have been conducted to monitor the translation compartment size in response to local stimulation of a dendritic stretch (Table 1). By translation compartment size, we mean the measured sizes (in microns) of different aspects of translation—particularly the spatial spread of nascent proteins and in some cases the redistribution of mRNA or translation markers (Fig 2A and Table 1). The measured sizes range from 3 to 60 μm and are dependent on the size of the locally perfused region, diffusivity of the perfused stimulating factor and duration of the perfusion [19,64–66]

(Table 1). In order to understand the spatial relationship between the stimulation domain size and the resulting translation compartment, we examined the size ratio of the translation compartment to the stimulation domain (*translation to stimulation domain ratio*, Table 1). The translation to stimulation domain ratio of nascent proteins ranges between 1 and 3 across different experiments, implying a close spatial relationship between the stimulation and its concomitant localized translation compartment. The reported translation compartments were measured using a wide range of methodologies, with some monitoring endogenous nascent proteins and the others relying on reporter constructs (Table 1). Interestingly, a recent study showed that for the same stimulation, the size of the nascent protein spatial spread ($\sim 18 \mu\text{m}$) is slightly larger than the mRNA redistribution domain ($\sim 6 \mu\text{m}$) [29] (Table 1, Fig 2A and E). This could be due to the fast spread of the nascent protein within a larger predestined spatial domain, compared to a narrower mRNA localization domain marking the point of translation. A similar observation was made following single-spine PSD-95 photoactivation, where the photoactivated proteins redistribute and stay “captured” in spines within a defined spatial range of 10–15 μm of a dendritic shaft [67] (Table 1). Moreover, measurements of the regulation of local translation by miRNAs during single-spine stimulation showed a $\sim 20 \mu\text{m}$ larger domain of protein repression compared to a 1–2 μm narrower domain of miRNA maturation [68] (Table 1). These experiments argue that the site of action of nascent proteins (effector compartment) is defined but not limited to the site of synthesis (source compartment) or structural boundaries. It is likely that the intensity of the stimulation, the amount of nascent protein made, the nature of the nascent protein (transmembrane, cytoskeletal, or cytosolic), its diffusional property, and the number of competing slots for trapping the nascent protein influence its spatial spread and functional outcome.

The functional consequence of the spatial spread of nascent proteins was first demonstrated in dendrites by experiments probing the “clustered plasticity” model [63,69]. According to this model, a spine receiving a stimulus that leads to long-term structural plasticity (late-LTP stimulus) (S1, Fig 3B) gets “tagged” in a protein synthesis-independent manner and also leads to the synthesis of PRPs. These newly synthesized PRPs are subsequently “captured” at the tagged spine leading to spine-specific structural changes (S1, Fig 3B). However, it was not clear if these PRPs were synthesized locally in the dendrites [63,69] or in the soma [61,62]. In order to address this, another spine was “tagged” by a subthreshold stimulus—insufficient to induce PRP synthesis by itself (S2, S3, Fig 3B). It was observed that the subthreshold-tagged spine (S2) was able to show spine-specific structural plasticity by capturing PRPs, only when it was clustered within a distance of $\sim 50 \mu\text{m}$ from the late-LTP-induced spine (S1) [63]. Spines distributed beyond this spatial distance (S3) did not show acquired plasticity, suggesting that PRP synthesis cannot be somatic but has to be local. This effective distance of the cluster is dependent on the number of neighboring tagged synapses that compete for the limited pool of PRPs. The more neighboring tagged synapses, the more promptly the PRPs are captured, thereby shortening the spatial spread of the nascent proteins and the size of the effector compartment. The observed time dependence of this clustering also suggests the transient nature of these compartments. The exact location where PRPs are synthesized in dendrites is still not known. Recent tools available to

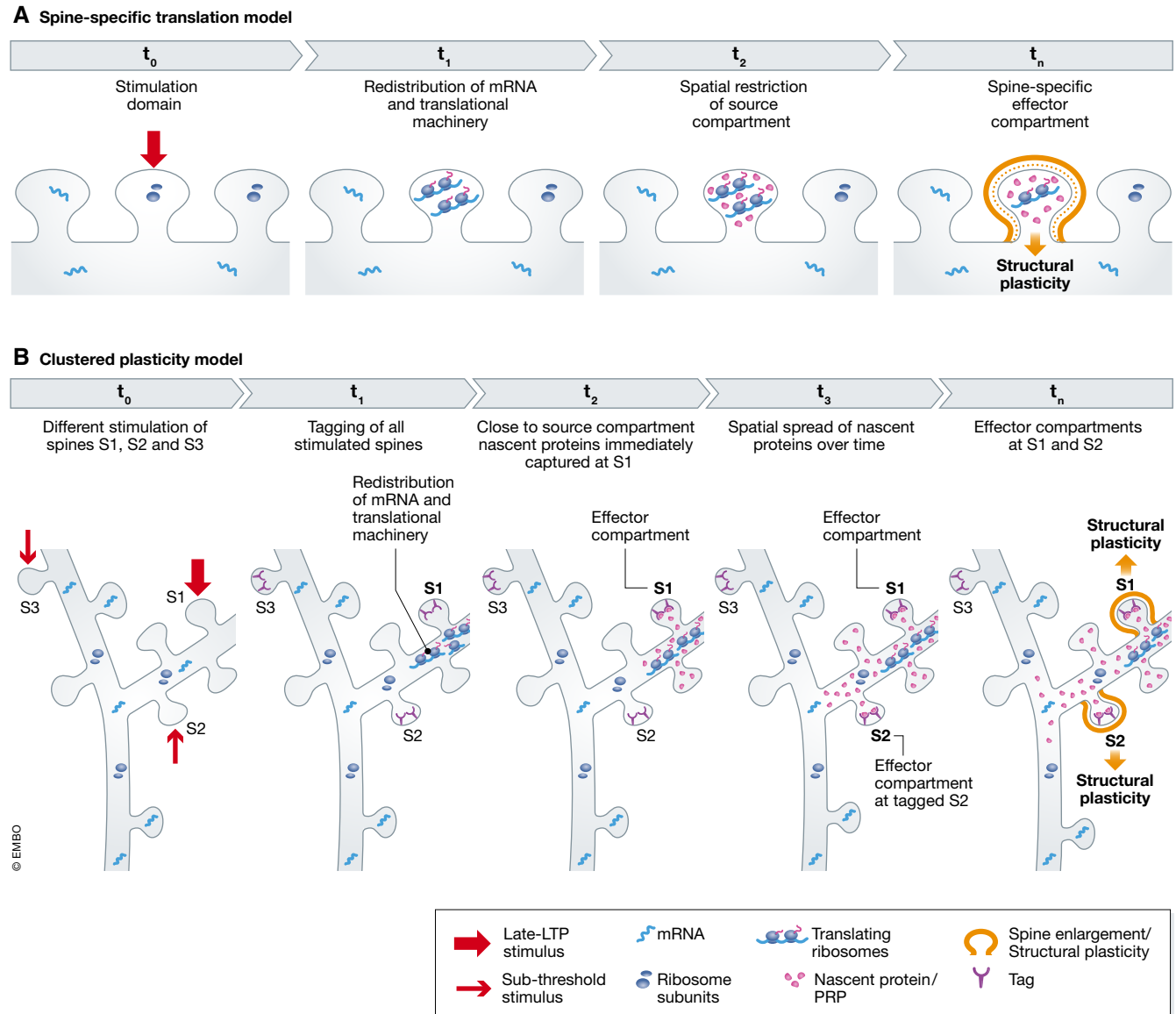


Figure 3. Subcompartments in dendrites.

(A) Concept of spine-specific translation: A spine-specific stimulation (t_0) could result in redistribution of mRNA and translational machinery to the stimulated spine (t_1). On overlapping with spine-specific signaling events, this would result in the synthesis of nascent proteins—source compartment—whose spatial spread is restricted within the spine, due to diffusional restriction by the spine neck (t_2). This would lead to a spine-specific effector compartment and subsequent functional outcome—structural plasticity (t_n). (B) Clustered plasticity model: Spine S1 receives a late-LTP stimulus, and spines S2 and S3 receive a subthreshold stimulus (t_0). All three spines get tagged but mRNA and translational machinery redistribute only close to spine S1 that received the late-LTP stimulus (t_1). The newly translated proteins—source compartment—are instantly captured at spine S1 (t_2) and with time, the spatial spread of the nascent proteins increases allowing for its additional capture at adjacent tagged spine S2 (t_3). Both the tagged spines S1 and S2 that capture nascent proteins—effector compartment—undergo spine-specific structural plasticity—functional outcome (t_n). However, only tagged spines clustered within a nascent protein spatial spread of $\sim 50 \mu\text{m}$ show this functional outcome—tagged spine (S3) present beyond this spatial spread does not.

simultaneously visualize mRNA and translation [22,26–29], in combination with a functional readout for synaptic clustering, should enable careful investigation of the dynamic spatial spread and functional outcome of these translation compartments.

Mapping functional compartments for information processing

Functional evidence for clustered plasticity is not only limited to observations in cultured neurons, but also has been observed in various animal model systems [70–72]. While it is not yet clear if

the observed clustered plasticity is protein synthesis-dependent, the observation of synaptic clustering during learning paradigms supports this view [73,74]. Synaptic clustering in a dendritic branch is thought to increase local spike initiation by non-linear summation resulting in enhanced dendritic excitability and effective information processing [51,75–80]. On the other hand, studies have also demonstrated that spines that respond to similar sensory inputs are widely distributed throughout the dendritic arbor and are therefore non-clustered [81–85]. While this is still a subject of debate [86], the

Table 1. Translation compartment sizes estimated from literature data.

Article	Condition	Stimulating factor	Readout (measure) ^a	Additional comments	Compartment size	Translation/stimulation domain ratio ^b
I. Dendrite						
[63]	Local glutamate uncaging 30 pulses, 0.5 Hz plus bath application with forskolin	Late-LTP induction	Spine volume change/structural plasticity (GFP fluorescence) (R)	Organotypic slice cultures from Thy1-GFP mice, 8–16 DIV; protein synthesis inhibitor sensitive	50 μ m	50
[19]	Spot perfusion 60 μ m for 30 min	BDNF—modulator of neuronal activity	Nascent proteins (fluorescent AHA) (E)	Rat hippocampal neurons 17 DIV	200 μ m; whole dendritic stretch imaged showed an increase	3
[29]	Local glutamate uncaging 10 pulses, 0.5 Hz, 6 μ m	Glutamate—AMPA activation	Nascent β -actin (halotag of β -actin detected by dye) (R)	Mouse hippocampal neurons 14–21 DIV; uncaging 100 μ m away from cell body	18 μ m	2
			β -actin mRNA (GFP tagged to β -actin mRNA binding protein) (R)		6 μ m	1
[64]	Spot perfusion 3–10 μ m for 15 min	Dihydroxydopamine—dopamine receptor agonist	Nascent proteins (fluorescent puromycin signal) (E)	Rat hippocampal neurons 14–21 DIV; perfusion 100 μ m away from cell body	3–10 μ m	1
[130]	Spot perfusion 25–59 μ m for 90–105 min	APV—NMDAR mini blockade	Surface GluR1 (immunofluorescence) (E)	Rat hippocampal neurons > 14 DIV	25–59 μ m	1
[66]	Local dendritic perfusion < 50 μ m for 10 min in microfluidic chambers	DHPG—mGluR activation	Enrichment of newly transcribed Arc mRNA (FISH) (E)	Rat hippocampal neurons 15–20 DIV	< 5 μ m	< 0.1
[10]	Bath application on optically isolated dendrites after photobleaching soma	BDNF—modulator of neuronal activity	Nascent myristoylated GFP with CaMK2a 3'UTR (GFP fluorescence) (R)	Rat hippocampal neurons 14–21 DIV	Whole dendritic stretch imaged ~150 μ m	NA
[60]	Bath application for 3 min	DHPG—mGluR activation	Nascent PSD-95 (Venus-PSD95 fluorescence super resolved by PALM) (R)	Mouse hippocampal neurons 12–16 DIV; newly synthesized PSD-95 did not colocalize with preexisting translational site	PSD-95 translation “hot spots” enriched in spines and dendritic shaft	NA
	FMRP-KO	Basal neuronal activity	Increase in basal translation			
[67]	None (visualized PSD95 redistribution following photoactivation for 60 min)	Basal neuronal activity in anesthetized mice	Photoactivated PSD95—not nascent protein (PAGFP fluorescence) (R)	Mouse pyramidal neurons of somatosensory cortex E16	~10–15 μ m	NA
[68]	Local glutamate uncaging 20 pulses, 1 Hz, 2 μ m ²	Dicer activation	miRNA maturation (FRET sensor ratio) (R)	Rat hippocampal neurons 14–21 DIV	1–2 μ m	1–2
			Nascent CaMK2a repression (Puro-PLA signal) (E, R)		~20 μ m	14
II. Axon						
[89]	Focal bead challenge 4.5 μ m overnight	NGF, NT3, MAG, Sema3A—growth factors/guidance cues	β -actin, peripherin, Kv3.1a mRNAs (FISH) (E)	Rat dorsal root ganglion cells injury conditioned <i>in vivo</i> for 7 days and cultured overnight	5–20 μ m	1–4

Table 1. (continued)

Article	Condition	Stimulating factor	Readout (measure) ^a	Additional comments	Compartment size	Translation/stimulation domain ratio ^b
[106]	Focal bead challenge 5 μ m in axonal compartment of microfluidic chambers for 24 h	Poly-D-Lys—for induction of presynaptic terminals	β -catenin mRNA (FISH) (E)	Rat hippocampal neuron axons > 10 DIV	5 μ m	1
	For 15 min–3 h		β -catenin protein (immunofluorescence) (E)	Protein synthesis inhibitor sensitive β -catenin increase	5 μ m	1
[113]	Nerve crush lesion <i>in vivo</i> ~1 mm	Injury	Newly synthesized NLS-binding protein (fluorescent NLS-peptide binding) (E)	Sciatic nerve crush generated nascent protein is retrogradely transported as a domain	~1 mm	~1
[108]	Local perfusion 150 μ m, 5 \times 5 min pulses, soma-free neurons	Serotonin—for long-term facilitation	Nascent dendra with sensorin 5'3'UTR (photoswitchable dendra fluorescence) (R)	Aplysia sensory neuron-motor neuron cocultures ~3 DIV; translational hot spots observed only in perfusion area	10–20 μ m hot spots	~0.07–0.13
[114]	None (visualized recovery of RanBP1 following photobleaching in isolated axons)	Basal neuronal activity	Nascent myristoylated GFP with RanBP1 3'UTRs (GFP FRAP) (R)	Transfected DRG neurons; reporter recovery after FRAP is sensitive to the UTR variant used and to protein synthesis inhibitors	5–10 μ m	NA
[110]	During axon branching, bath application	NGF—growth factor	Nascent myristoylated GFP with β -actin, cortactin, Arp 3'UTRs (GFP FRAP) (R)	Chicken dorsal root ganglion neurons 7 DIV; majority of GFP reporter hot spots colocalize with mitochondria but not all mitochondria localize with hot spots	~5 μ m	NA
			β -actin mRNA (FISH) (E)	β -actin mRNA accumulation along some mitochondria	~5 μ m	NA
[36]	Axonal compartment bath application in microfluidic chambers for 24 h and 48 h	A β ₁₋₄₂ —for induction of Alzheimer's pathogenicity	Nascent proteins (fluorescent AHA) (E)	Rat hippocampal neurons 9–10 DIV; measured hot spots sensitive to protein synthesis inhibitor	~5–10 μ m hot spots	NA
[109]	Bath application for 25 min	WIN—cannabinoid receptor CB1 agonist	Newly synthesized protein (FUNCAT) (E)	Hippocampal neuron culture; synthesis is increased in the whole neuron with hot spots visible around CB1-positive terminals	2–5 μ m	NA
[107]	Bath application for 24 h in soma-free neurons	Netrin 1—chemotropic factor	Sensorin protein (immunofluorescence) (E)	Aplysia sensory neuron-motor neuron cocultures 3 DIV	10 μ m hot spots	NA
III. Growth cone						
[104]	In tissue commissural axon midline crossing	Midline crossing—guidance cue	GFP with EphA2 3'UTR (GFP fluorescence) (R)	Commissural neurons; E5 chicken spinal cord; sharp 20 μ m domain upon experiencing the cue, translation domain widens as growth cone crosses	20 μ m; 80–100 μ m	NA

Table 1. (continued)

Article	Condition	Stimulating factor	Readout (measure) ^a	Additional comments	Compartment size	Translation/stimulation domain ratio ^b
[97]	Bath application for 5 min	Netrin-1—chemotropic factor	Nascent β -actin (immunofluorescence) (E)	<i>Xenopus</i> stage 24 retinal ganglion cell growth cone; β -actin hot spots correspond to growth cone filopodia; CHX-sensitive	5–10 μ m	NA
	Bath application for 10 min		Nascent kaede with β -actin 3'UTR (photoswitchable kaede fluorescence) (R)	Kaede hot spots in growth cone and filopodia	5–15 μ m	NA
	Asymmetric gradient, 90° to the direction of axon shaft for 5 min		β -actin protein accumulation (immunofluorescence) (E)	Protein synthesis inhibitor sensitive β -actin fluorescence; signal is higher near the gradient source	< 20 μ m domains	NA
[99]	Bath application for 5 min	Slit conditioned medium—guidance cue	Cofilin protein accumulation (immunofluorescence) (E)	<i>Xenopus</i> retinal explants culture stage 35/36; protein synthesis inhibitor-sensitive cofilin increase in filopodia	5–10 μ m	NA
[41]	None (visualized recovery of Acot7 following photobleaching)	Basal neuronal activity	Nascent myristoylated GFP with Acot7 axon-exon (GFP FRAP) (R)	<i>Xenopus</i> retinal ganglion cell growth cone	< 10 μ m	NA

^aTranslation compartments were measured either by monitoring endogenous nascent proteins (E) or reporter constructs (R).

^bRatio of the translation compartment size (protein or mRNA) to the stimulation domain (perfusion, local uncaging, focal stimulation, etc.). The calculated ratios have been rounded off to the closest whole number, where applicable. For Govindarajan *et al* [63], the stimulation domain size for uncaging was assumed as $1 \times 1 \mu\text{m}^2$. Literature listed in each segment—dendrite, axon, growth cone—is in decreasing order of the calculated ratio, followed by chronology.

investigation of local translation during learning paradigms in animal model systems should facilitate the understanding of the molecular mechanisms underlying the functional mapping of synaptic inputs and the subsequent information processing.

Translation compartments in axons

Significant developments in the ability to separate axons from dendrites [34–37,87–89] led to the demonstration of local translation in axons, in addition to dendrites. In fact, among the earliest evidences for neuronal local translation was the incorporation of radioactive amino acids into proteins in the isolated squid giant axon [11] and the detection of protein synthesis machinery in the squid axoplasm [90–92]. Also, the dependence of synaptic plasticity in *Aplysia* neurons on presynaptic local translation suggested that the axon is capable of protein synthesis [16]. Despite this evidence, there remained skepticism about generality of axonal translation with some arguing that these model systems represented special cases. The apparent low basal content of mRNA and protein synthesis machinery in axons also contributed to this skepticism. The current emerging theme is that axons make use of local translation primarily when they act as signal receivers in response to local cues [3,93–96]. For example, axonal translation is observed during development in response to guidance or maturation cues, under injury

conditions and presumably during synaptic activity. Where in the axon is a signal sensed and how does this relate to the site of local translation? What is the site of action of the newly synthesized proteins? Broadly speaking two opposing scenarios have been observed in axons for locally synthesized proteins in response to a local signal: (i) spatially restricted use for local response and (ii) distant use for global response.

Before discussing these local and global effects of local translation, it is important to look at the factors that influence the size of the axonal source compartment. In general, the dynamics of mRNA distribution depend not only on the transcript/protein of interest but also on the strength and type of stimulation [89,97]. When axonal segments were focally stimulated using ligand-coated beads, mRNAs were redistributed in both transcript- and cue-dependent manner. In one study, a growth factor NT3-dependent increase in β -actin mRNA was confined to a narrow domain of 5 μ m, corresponding to the bead size, while for other cues, it spread over wider regions (~20 μ m) of the axon around the stimulation site [89]. Interestingly, focal cues that stimulated the down-regulation of the transcript resulted in a wider domain size (> 20 μ m) than a cue that resulted in upregulation of the transcript (~5 μ m). This study also showed that the subsequent translation responses correlated with the regions of mRNA distribution. However, it was not addressed if axons use these nascent proteins for local function.

Local use of translated proteins: the case of growth cone guidance

Locally restricted use of axonally translated proteins was first observed in growth cones of the developing axon [94,97–101]. Growth cones are axon-end structures that pioneer axonal growth in response to attractive and repulsive chemical guidance cues. They fulfill this role even when axons are severed from cell bodies and the response to several cues is protein synthesis-dependent [94,97–101]. The growth cone is easy to identify morphologically, and excellent assays have been developed to measure growth cone responses. This has allowed researchers to address the spatial relationship between the compartment that senses the cue, the induced signaling compartment, the resulting source compartment (e.g., redistribution of mRNA and translation markers), spatial spread of nascent proteins, and the functional outcome (e.g., growth cone turning and collapse). Even with the bath application of guidance cues, the compartment sizes involved in cue sensing, local translation, and the functional outcome are often restricted to the growth cone itself (~40 μm) or to parts of the growth cone like filopodia (5–10 μm) [81,97,99] (Table 1). Application of cues as point source gradients on one side leads to a directional response of the growth cone [94,97–99]. For example, the introduction of Netrin-1 as point source resulted in the asymmetric distribution of translational markers for β -actin synthesis. Gradients within the growth cone concentrated the translational markers in a < 20 μm zone toward the cue and correlated with the direction of the growth cone response [97]. The overlap of signaling compartments and β -actin mRNA redistribution to filopodia resulted in asymmetric local translation of β -actin. However, the turning response was absent when β -actin mRNA was knocked down. Thus, these data suggest that the overlap of different compartments contributes to the direction-specific growth cone response.

A similar pattern has been observed for a number of other cues in which the growth cone responses involve the overlap of several different signals or processes [94,100,102]. Local translation, which is likely restricted to the growth cone, is also necessary for restoring ongoing growth cone responsiveness [98], axon elongation, and membrane remodeling during development [96,103]. Moreover, during axonal pathfinding, sharp upregulation of translation (within ~20 μm , Table 1) was observed upon contact of growth cones with an intermediate target [104]. Taken together, these data show that many local remodeling responses are mediated by local translation and its local use within compartments smaller than 20 μm , resulting in a fast, autonomous functional outcome in the growth cone.

Local use of translated proteins: branch maturation, synapse maturation, and synaptic plasticity

During growth, axons branch out as they navigate toward their postsynaptic targets and make contacts that eventually mature into presynaptic boutons. The axonally localized mRNA population is regulated during development and presynapse formation [34,35,37,41,105,106]. Focal bead-triggered presynapse formation was associated with enriched β -catenin mRNA within the newly formed terminal with a compartment size of ~5 μm , corresponding to the size of the artificial trigger [106]. A translation-dependent increase in β -catenin protein was also confined to the newly formed presynaptic terminals and was essential for regulation of vesicle recycling. Evidence that presynaptic local translation is involved in synaptic plasticity was first shown in *Aplysia* neurons during

long-term facilitation [16,107,108]. In soma-free neurons, translation hot spots of ~10–20 μm were observed in response to local perfusion or bath application of the stimulus, in spite of a neuron-wide distribution of mRNA (Table 1). Here, translation hot spots likely corresponded to presynaptic varicosities. In addition, in the adult hippocampus, the involvement of presynaptic local translation in synaptic plasticity was shown during cannabinoid signaling [109]. Translation markers were found within 1–2 μm of presynaptic terminals; however, the spatial spread of the nascent proteins was not determined. In cultured neurons, in addition to a global increase in protein synthesis after cannabinoid receptor stimulation, strikingly, an apparent enrichment of nascent proteins at cannabinoid receptor-positive terminals was observed in axons (~2–5 μm) [109] (Table 1).

Axon branching also requires local translation. Interestingly, mitochondria were recruited near the sites of branch point formation; the size (~5 μm , Table 1) and location of detected local translation compartments correlated with that of the mitochondria. This mitochondrial recruitment was found to be a prerequisite for local translation and subsequent branch stabilization [110,111].

Local source and global effector compartments: a case of retrograde signaling

As discussed so far, local translation provides a source for proteins that are used as close as 1–20 μm from their site of synthesis to elicit an effect. However, in other cases, the effector compartment can be more than hundreds of microns to millimeters away from the source compartment. Retrograde signaling to the nucleus involving locally synthesized proteins is used in different situations by axons to elicit global responses via transcriptional changes. The response to axonal injury is one well-studied example where the main retrograde signaling response relies on local translation near the injury site [112]. The initial size of the source compartment and the subsequent spatial spread of nascent proteins in response to injury are difficult to measure in tissue. In the peripheral nervous system, a smart binding assay reporting protein synthesis and subsequent retrograde transport labeled a compartment of 1 mm at the site of lesion in the sciatic nerve. The size of the compartment was unchanged after 6 h but was shifted several millimeters toward the cell body, indicating precise localization, timing, and transport of the newly synthesized protein [113]. In cell culture, monitoring translation of one of the injury-related mRNAs in isolated axons revealed a translation compartment as narrow as 5–10 μm [114].

In addition to lesion events, local translation with retrograde signaling is elicited in response to neurotrophic factors by growth cones and in neuronal subtype specification [115,116]. Evidence that axon-site-synthesis-to-nucleus signaling plays a role in mature central nervous system and Alzheimer's pathology came recently with the demonstration that injection of $A_{\beta_{1-42}}$ into the dentate gyrus resulted in increased levels of ATF4 mRNA and protein in forebrain neuronal axons that project to the dentate gyrus [36]. Local synthesis of the transcription factor ATF4, followed by retrograde transport and a nuclear transcriptional change, led to neurodegeneration. Consistent with other studies, hot spots of local translation with a compartment size of ~5–10 μm were apparent (Table 1).

Local translation of transcription factors and their retrograde transport to the nucleus partially explains how a global response in the neuron can be evoked [36,115,117,118]. Often non-transcription

factors are locally synthesized, triggering the assembly of defined transport complexes and their modification by coincident local signaling events [113,117,119,120]. This has led to the understanding that these locally synthesized non-transcription factors could carry a signature about the synthesis site to the nucleus. It should be noted that dendrites also employ synapse-to-nucleus signaling of transcription factors to elicit global responses [121–123]. There is evidence that local translation also plays a role in dendritic synapse-to-nucleus signaling [122].

In all of the above cases, the translation event (source compartment) and the elicited function (effector compartment) are uncoupled in space and time. This uncoupling might therefore facilitate the visualization of the various translation compartments, including the gradual changes in the spatial spread of nascent proteins, and their dynamics.

Unanswered questions

In the field of local translation, there remain some unresolved issues. For example, in many cases, components of the downstream processing machinery required for canonical protein translation are apparently missing in subneuronal compartments. It is important to address these issues, as they might serve as important factors in defining the site and size of local translation compartments. Besides, as described below for membrane protein processing, resolving such issues also sheds light on the potential function of local translation.

The membrane protein conundrum

There is ample evidence for the dendritic and axonal localization of mRNAs that code for membrane and secreted proteins [18]. Moreover, there is experimental data on their local translation, their potential to reach the plasma membrane, and the capacity of isolated dendrites to glycosylate them [25,64,104,124–130]. Membrane and secreted proteins require insertion into the endoplasmic reticulum (ER) for proper protein folding, followed by passage via the ER–Golgi intermediate compartment (ERGIC) and to the Golgi apparatus for extensive glycosylation. ER, ER exit sites, and ERGIC carriers have been found throughout dendrites and axons [131–133]. The ER network is highly non-uniform in dendrites with interspersed regions of high complexity and diffusion-restricted zones close to large spines and at branch points, indicating hot spots of local processing [134]. In addition, highly mobile carriers of the dendritic ERGIC system are restricted in their mobility by neuronal activity [132].

The Golgi apparatus, however, is localized primarily in the soma raising the question: How are membrane and secretory proteins processed locally in response to local stimuli? A specialized dendritic Golgi compartment termed “Golgi outposts” [132,135,136], consisting of discrete, static elements with a Golgi ministack morphology are occasionally observed at primary dendritic branch points. However, this compartment is scarce and found in < 20% of mature hippocampal neurons [136]. Consistent with immunoelectron microscopic studies describing undefined, heterogeneous membrane compartments near postsynaptic sites in distal dendrites [137,138], a tubulo-vesicular carrier system called

the “Golgi satellite” was recently characterized in dendrites [139]. The movement of Golgi satellites is controlled by synaptic activity. However, the lack of an entire Golgi enzyme repertoire in Golgi satellites was postulated to lead to altered glycosylation patterns and less efficient membrane delivery of membrane proteins. Indeed, a recent study demonstrated the large-scale presence of immature N-glycans at the neuronal plasma membrane [126]. The differential immature-to-mature glycosylation patterning in subsets of neuronal membrane proteins was found to be important for receptor function and protein turnover, indicating that locally synthesized membrane proteins are likely endowed with distinct features and a signature for the site of synthesis [126].

Ribosomes and their puzzling aspects

Ribosome localization in neuronal compartments is essential for local translation, but their biogenesis occurs in the nucleolus and nucleoplasm [140–142]. It has therefore been puzzling to observe a consistently large fraction of localized mRNAs coding for ribosomal proteins by various high-throughput approaches [18,31,34,41]. The functional significance and whether these mRNA populations are translated into proteins remain unknown. The synthesis of single ribosomal proteins could serve as replacement of specific ribosomal protein subunits for local repair or maintenance. On the other hand, the local translation of ribosomal proteins could result in distinct ribosomal species with different translational properties compared with the somatically synthesized and assembled ones. Evidence for ribosomal heterogeneity has been shown in yeast where mass spectrometric analysis revealed ribosomal heterogeneity that influences protein translation rate [143]. Ribosomal heterogeneity is also important for translational control of specific transcripts—*Rpl35* mutant mouse embryos showed a defect in the translation of a select group of transcripts important for skeletal patterning during development, while global protein expression remained unaffected [144]. Various other ribosomal proteins have also been shown to have tissue-specific functions [145]. Thinking further, this opens up the intriguing possibility of a spatially controlled regulatory step in local translation, achieved by the tethering of specific ribosomal species at select neuronal subcompartments. This is supported by evidence that ligand–receptor interactions (Netrin and its receptor DCC) can regulate the translational state of ribosomes in a localized fashion [146]. Tethering of ribosomes and select mRNAs to subcellular sites and organelles has been observed in non-neuronal cells [42,43,147,148]. While it is accepted that secretory and membrane protein mRNAs are translated by ribosomes associated with the ER, whether cytosolic protein mRNAs might also be translated at the ER is still a matter of debate [42,148–150]. It remains to be determined whether the contradictory findings of cytosolic protein translation at the ER could perhaps reflect an additional layer of translational regulation. Evidence also exists for cytosolic protein mRNAs tethered to the ER that do not undergo translation [151]. It is possible that the local heterogeneity of ribosomes and RNA-binding proteins at the ER could contribute to mRNA tethering and subsequent translation regulation. Similarly, other organelles like mitochondria and endosomes tether specific subsets of mRNAs and serve as translation platforms (discussed below), but the role of ribosome composition in this context has not yet been determined. The ability of all

these organelles to attach to the cellular transport machinery [147,152–154] could in addition contribute to the dynamic nature and size of source compartments. Careful characterization of unique ribosomal species from subcellular compartments and organelles will shed light on the structural consequence of their specific biochemical composition, their transcript preference, and their physiological significance in local translation.

In contrast to the observation of polyribosome redistribution in dendritic spines following tetanic stimulation [59], the paucity of polyribosomes in axons has long been a major detriment to the idea of axonal translation. Several findings have shed light on this enigma suggesting the need to reconsider long-standing concepts. In the sciatic nerve, axons contain low numbers of ribosomes. Upon sciatic nerve injury, Schwann cells (myelinating glial cells in the peripheral nervous system) deliver ribosomes including polyribosomes via membrane protrusions and multilamellar vesicles to the sciatic nerve axons, after which axonal ribosome numbers increase by orders of magnitude [155]. Consistent with this, an electron microscopy study suggested that spinal cord axons receive ribosomes from central nervous system glia [156]. The modes of delivery are not fully understood but broadly assumed to be from direct connections or exosome transfer [157]. The origin of local translation material from non-autonomous sources could also explain the observation of clustered, submembranous distribution of putative source compartments named periaxoplasmic ribosomal plaques, ~20 μm in size, in some axonal preparations [158–161].

In addition, recent studies have opened up the possibility of monosomes being more translationally active than previously assumed. This was revealed by monosome isolation and translome profiling in yeast, where most monosomes were translationally active with a bias toward the translation of low abundance regulatory proteins [162]. Real-time imaging of mRNA translation also showed that the fraction of mRNA translated by monosomes is highly transcript-dependent [22]. This new approach now provides the means to address whether axonal translation could be largely carried out by monosomes that might be present at higher copy numbers in axons.

The intercellular route—solving the delivery problem?

The demonstration of the intercellular delivery of ribosomes from Schwann cells to axons [155] as discussed above was one of the studies that indicated that other cells can serve as a source for translation machinery components, mRNAs, regulatory/processing molecules, or even newly synthesized proteins in axons or at synapses [157,163–170]. The intercellular exchange of material between cells by multivesicular body-derived exosomes and other extracellular vesicles is now regarded as an integral part of an organism's physiological and pathophysiological repertoire. Proteins, mRNAs, miRNAs, and multiprotein complexes like ribosomes represent extracellular vesicle cargoes. As such, it is not surprising that extracellular vesicles have been shown to complement the intercellular communication system in neurons [171,172]. At the *Drosophila* neuromuscular junction, multivesicular bodies fuse with the plasma membrane of presynaptic boutons at extrasynaptic sites and the expelled exosomes travel in the extracellular space to interact with receptors in the muscle's subsynaptic reticulum [166,167]. Schwann

cells and oligodendrocytes (myelinating cells in close contact with axons) also communicate with axons via exosomes. Dedifferentiated Schwann cells and oligodendrocytes release exosomes for enhancing axonal regeneration and neurotransmitter-triggered neuronal uptake, respectively [164,165,168,169]. Notably, neurons also release exosomes in an activity-dependent manner [173–175], and these exosomes bind to presynaptic terminals [176], potentially providing a source of material for local translation and source compartment formation. Intriguingly, active endocannabinoids, involved in regulating synaptic plasticity by targeting presynaptic cannabinoid receptors [109,177,178], were found associated with extracellular vesicles [179]. In fungi, Septin mRNAs were translated on the endosomal surface during transport [147,152] potentially serving as a means to sort newly translated proteins into exosomes.

In addition, tunneling nanotubes (direct connection between cells) have also been implicated as routes of material delivery between cells [180,181]. Suggestive evidence for trans-endocytosis of spinules (small structures extending mostly from postsynaptic spines into presynaptic terminals) provided by an electron microscopy study also offers a route for material transfer ideally suited for synaptic plasticity [182]. Spinules, likely containing ribosomes, showed activity-related changes in their numbers [181,182]. Future experiments combining genetic manipulation, highly sensitive detection methods, and sophisticated sample preparation will determine how such intercellular material transfer relates to local translation.

The energy question

Similar to ribosomal protein mRNAs, transcripts for nuclear-encoded mitochondrial proteins were also found in large numbers in local transcriptomes [18,183]. Although it is not clear if the full set of mitochondrial transcripts is translated locally, it has been demonstrated that the local translation of some of them is crucial for mitochondrial and neuronal function [183–185]. A large fraction of nuclear-encoded mitochondrial mRNAs and ribosomes associated with the mitochondrial outer membrane in yeast [43,186–190], and flies [191] indicate that the machinery required to carry out local changes in the mitochondrial proteome is present. Furthermore, proteomic analyses of neurons show that the mitochondrial proteome, especially the subset important for energy metabolism, is regulated during synaptic plasticity [192,193]. Moreover, a recent study of the mitochondrial translome in yeast reveals that the translation of proteins relevant for energy metabolism is dynamically regulated based on the nutrient source [194]. If the local mitochondrial proteome is indeed dynamically regulated according to local energy demands, distinct mitochondrial species unique to specific subneuronal compartments must exist. This is supported by the observation of a specially tuned mitochondrial proteome in synaptic mitochondria compared with non-synaptic mitochondria [195].

Given the availability of the translational machinery associated with mitochondria, including miRNAs for translational regulation [184,196,197], is it possible that translation of proteins on the mitochondrial surface is not just limited to mitochondrial proteins? In other words, could mitochondria serve as local platforms for the translation of other proteins? As discussed earlier, the localization of β -actin mRNA and its corresponding translation hot spots along the

Box 1: In need of answers

- (i) For a given stimulus:
 - (a) What are the specific sets of mRNAs recruited and the subsequent proteins newly made?
 - (b) How different are the spatial spreads of these recruited mRNAs and their respective nascent proteins?
 - (c) What are the temporal details of the following processes: redistribution of translational machinery, protein translation, the ensuing spatial spread of the nascent proteins, and their functional outcome?
- (ii) How does stimulus strength influence the size of the different translation compartments—source compartment, spatial spread of the nascent protein, effector compartment, and functional outcome?
- (iii) Is the site of local translation static, in other words, what is the contribution of dynamic structures such as mitochondria, endosomes, and polysomes in local translation?
- (iv) Do the proteins synthesized in local translation compartments carry special characteristics and what significance does it have on local biological processes? For instance, given that locally translated membrane proteins (especially receptors) are immaturely glycosylated [126], could this facilitate local modulation of their physiological properties when compared with their somatically synthesized counterparts?
- (v) Does local translation of ribosomal mRNA result in the synthesis of special subsets of ribosomal subunits? If yes, do translational compartments possess specialized ribosomes made of special subsets of ribosomal subunits?
- (vi) What is the contribution of translational machinery delivered from adjacent cellular sources [155] in local translation?
- (vii) How are the energy demands of various translation compartments met? Do compartmentalized energy sources exist? If yes, what is the spatial compartment they serve and what is their significance in local translation?

length of mitochondria during axon branching is consistent with this view [110] (Table 1).

Another important facet of mitochondria that is largely unexplored in the context of local translation is its potential to supply energy. Proteins are turned over at high rates at synapses. At the glutamatergic postsynaptic density and synaptic vesicle pool alone, about 2,670 and 13,800 proteins are turned over per neuron per minute, respectively [198]. Considering such high protein turnover rates, the energy required to synthesize these proteins at a rate of 4 ATP molecules per peptide bond [199] represents a high local energy demand indicating the need for compartmentalized energy sources. Considerable evidence suggests that mitochondria function in spatially defined compartments to power local energy demands. For example, mitochondrial motility is regulated by neuronal activity in dendrites [200] and axons [154] and they redistribute into active spines during spine morphogenesis and plasticity [201]. Most importantly, stalling of mitochondria near the base of axonal branches is essential for local translation [110]. If mitochondria power local translation, it is not clear if they function as compartmentalized entities serving specific translation compartments. The stabilization of mitochondrial localization at presynaptic sites [202,203] and dendritic branch points [204] during development and plasticity supports this view. Careful examination of such stable mitochondrial compartments that might power local translation is warranted in the future. On the other hand, the

importance of local translation for mitochondrial function [183,185] reveals a striking mutual dependence between local translation and mitochondrial function.

Although mitochondria produce ATP at a higher yield than glycolysis [205], during high energy demands glycolysis seems to play a complementary role [206–209]. Given the cytosolic nature of glycolytic enzymes, local energy provision by glycolysis requires their spatial compartmentalization in local subcellular regions—called the “glycolytic metabolon”. These metabolons observed in synaptic vesicles [207,210,211], postsynaptic densities [212], and nerve endings [213] are thought to power local biological processes. More importantly, high energy demanding states such as neuronal stimulation, hypoxia, and inhibition of mitochondrial respiration drive the transient compartmentalization of these glycolytic metabolons within ~1–2 μm compartments of presynaptic terminals for fueling synaptic function [208]. In the context of local translation, it would therefore be useful to investigate whether these glycolytic metabolons exist in dendrites and whether they serve as energy supply.

Summary

Given their complex morphology, neurons have evolved mechanisms to independently remodel their local proteome for efficient neuronal function. Local translation and proteome remodeling is a complex phenomenon carried out in several phases. Each phase (mRNA redistribution, signaling events, translation of proteins) occupies its own spatial compartment ultimately operating in unison to result in a functional outcome. In some cases, translational hot spots of 5–20 μm size have been observed in dendrites and axons following global neuronal stimulation. Similar sizes of translation compartments have also been observed following local stimulation. The repeated observation of this apparent translation compartment size argues that it could be characteristic of local translation processes although what defines this size remains to be determined. In particular, it should be clarified if the measured size of the translation compartment is driven by the biology or the methodology used. It is certain that structural boundaries are not the sole defining parameter, as in most cases the translation compartments were spread over or restricted within a substructure. Current emerging tools should enable clarifying this question in the near future.

Conflict of interest

The authors declare that they have no conflict of interest.

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