How are integral membrane proteins and secreted factors delivered to the appropriate neuronal domain, at the right time and in the right amounts?

Two main mechanisms:

1. Long-range protein trafficking (from cell body to proximal/distal dendrites, axon, presynaptic terminal, etc……)

2. Local RNA translation and trafficking (within **dendritic spines**, growth cones, immature axon terminals and regenerating axons)

RNA localization

Sorting of mRNAs to subcellular domains is an evolutionary conserved mechanism

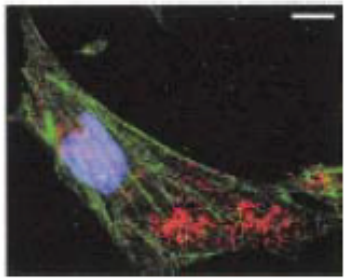
(from fungi to plants and animals)

local high-level production of the encoded protein at the site of mRNA localization

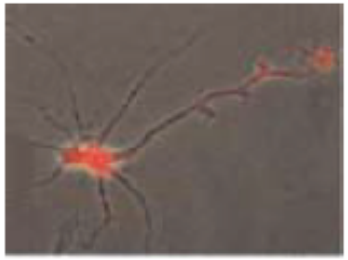
prevents production of the encoded protein in a region where it might have deleterious effects

Examples of Localized RNAs in Different Organisms and Cell Types

The localization of maternal *nanos* mRNA at the posterior of an activated, unfertilized *Drosophila* egg is the result of two different mechanisms: generalized degradation and local protection.



Colocalization of β-actin mRNA (red) in the leading lamellae of chicken fibroblast with phosphorylated myosin (green immunofluorescence). Nucleus stained blue with DAPI.



β -actin mRNA localization in the neurite and growth cones.

Mechanisms by which RNAs can be localized

1. random cytoplasmic diffusion and trapping,
2. general degradation and localized RNA stability,
3. vectorial transport from the nucleus to a specific target,
4. active directional transport of RNA on cytoskeletal elements,
5. combination of above mechanisms

In neurons, specific mRNAs are transported to dendritic spines

LOCALIZATION AND TRANSLATION OF mRNA IN DENDRITES AND AXONS

Christy Job and James Eberwine

The neurons of the brain extend axons and dendrites many hundreds of micrometres away from the cell body. The first electron microscope studies of these processes revealed that many of the structures that are found in the cell body are also present in dendrites. For example, particles resembling ribosomes and membrane structures like those of the endoplasmic reticulum (two structures that are important for protein synthesis) were seen in distal regions of dendrites, near synapses. Subsequent studies focused on identifying messenger RNAs in dendrites and providing evidence of dendritic protein synthesis. Transfection technologies have now been used to analyse translation within dendrites in response to pharmacological stimuli. These studies provide us with clues to the physiological role of the dendrite not just as a signal transducer, but also as a modulator of long-term synaptic efficacy.

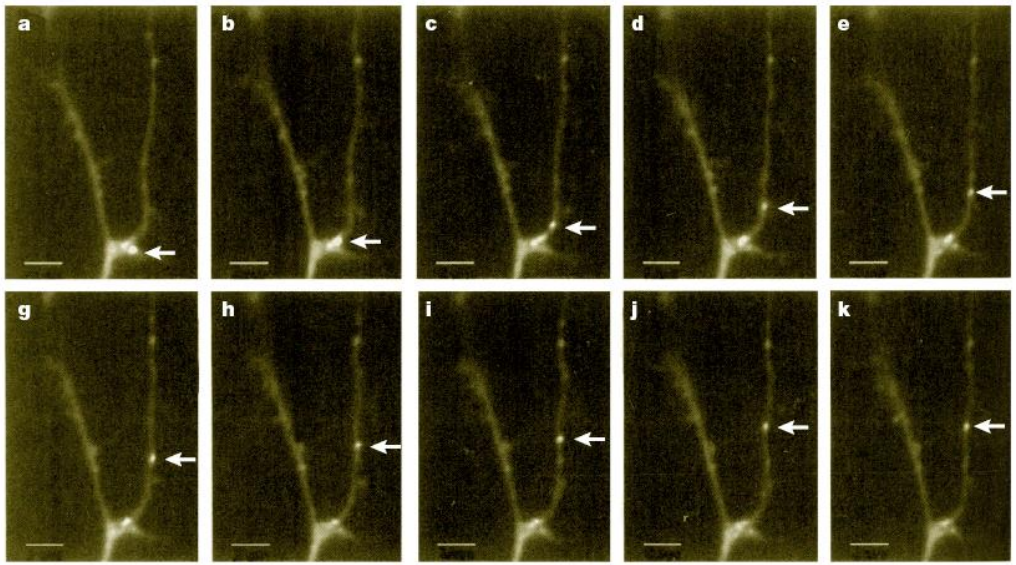
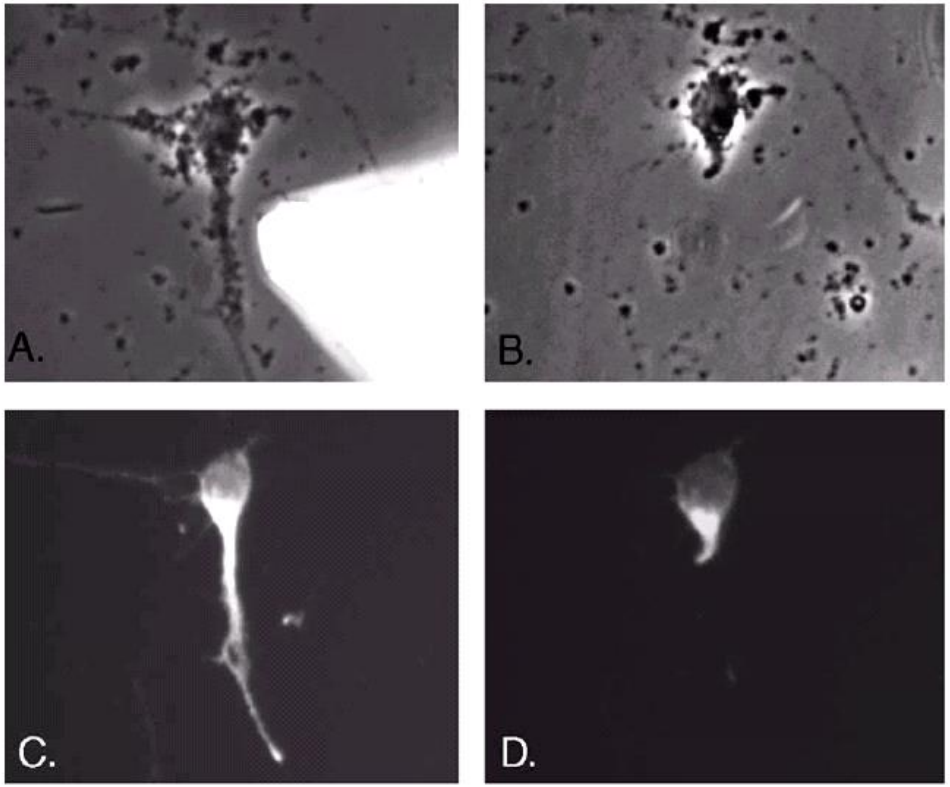


Figure 4 | **Movement of RNA-containing granules in dendrites of cultured neurons. a–k** | Time-lapse images, taken 20 s apart, of an anterograde-moving granule (arrow). The granule is detected by visualization of fluorescent SYTO-14, which binds to RNA. The granule moves more than 5 μm, with an average velocity of 0.04 μm s^{-1}. This movement was stimulated by depolarization. Reproduced with permission from REF. 34 © 2000 Society for Neuroscience.

Movement of **RNA-containing granules** in dendrites (visualized with the fluorescent dye SITO-14)

Job & Eberwine, 2001

Neuronal dendrites can be isolated by mechanical dissection, this preparation can be used for further molecular analysis



Hinkle et al., 2004

Fig. 2. The mechanical dissection of a neuronal dendrite. An individual neuron is shown in phase (panel A) and upon fluorescence from transfected GFP (panel C). The patch pipette used to sever the process is shown in panel A. Panels B and D show the remaining cell soma after the dendrite has been severed and harvested into the patch pipette. This mechanical severing is quite easy and can yield approximately 5 dendrites/min.

Which proteins can be synthesized locally in dendrites of mature neurons?

UNIVERSITÀ DI TRIESTE

Estimated 150-400 different mRNAs (~5% expressed genes)
(Eberwine et al, 2002, Zhong et al., 2006; Poon et al., 2007)

Category of mRNAs in CA1 dendrites	percentage
Receptors, ion channels, and postsynaptic molecules	7.8%
Cytoskeleton	7.8%
Extracellular matrix, cell adhesion, and immuno-molecules	20.1%
Signal transduction and Protein modification	16.9%
Translation factors and RNA-binding proteins	4.5%
Ribosomal proteins	16.2%
Peptide processing and degradation	7.8%
Protein transport, membrane trafficking, endocytosis, and exocytosis	6.5%
Molecular motor	0.6%
Growth factors	2.6%
Other	9.1%

Zhong, Zhang and Bloch, 2006 BMC Neuroscience

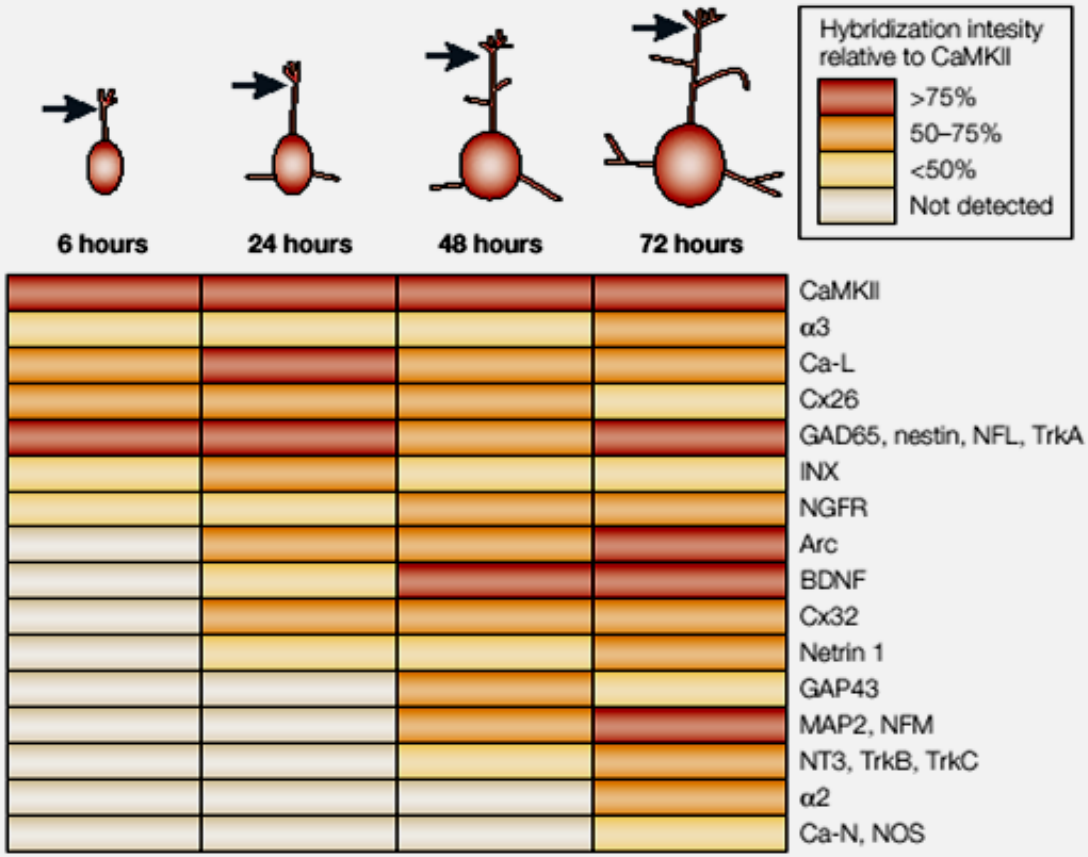


Figure 3 | **Time course of mRNA detection in dendritic growth cones in culture.** The messenger RNA complement of individual dendritic growth cones from cultured rat hippocampal neurons harvested on embryonic day 17 and cultured for 6, 24, 48 or 72 h was antisense RNA amplified and applied to macroarrays. The macroarrays contained various candidate mRNAs, as indicated on the right. Increasing abundance of the mRNA in the growth cone is indicated by the coloured gradient. All of the mRNAs were present in the cell soma at 6 h in culture, even if they are absent from the dendrite. Thirty other mRNAs were never found in dendrites. Arc, activity-regulated cytoskeletal-associated protein; BDNF, brain-derived neurotrophic factor; CaMKII, calcium/calmodulin-dependent protein kinase II; Cx26, connexin 26; Cx32, connexin 32; GAD65, glutamic acid decarboxylase 2; GAP43, growth-associated protein 43; MAP2, microtubule-associated protein 2; NFL, neurofilament light polypeptide; NFM, neurofilament medium polypeptide; NGFR, nerve growth factor receptor; NOS, nitric oxide synthase; NT3, neurotrophin 3; Trk, neurotrophic tyrosine kinase receptor. Reproduced with permission from REF. 30 © 1996 Elsevier Science.

Numerous mRNAs are detected in dendrites of cultured neurons and their abundance is regulated over time.

Job & Eberwine, 2001

mRNAs in dendrites are regulated by **synaptic activity**

Table 1 | **Neurotransmitter-regulated dendritic mRNAs in mammalian neurons**

Dendritic mRNA	RNA binding proteins/IRES	Neurotransmitter regulation			References
		NMDAR	mGluR	BDNF	
β-Actin	ZBP1	Increased dendritic transport	NA	Increased dendritic transport	11,12
Arc	IRES	Increased dendritic transport	NA	Increased dendritic transport and increased translation	28,29,40, 53,121
αCaMKII	CPEB1, FMRP, RNG105, IRES	Increased translation	Increased dendritic transport	Increased translation	35,42,45, 70,73,161
eEF1A	FMRP	NA	Increased translation	NA	59
FMR1	FMRP, CPEB1, IRES	NA	Increased dendritic transport and increased translation	NA	80,90
GluR1	NA	NA	Increased translation	NA	162
GluR2	CPEB3	NA	Increased translation	NA	64,162, 163
InsP3R	Hzf	NA	NA	Increased translation	164
LIMK1	NA	NA	NA	Increased translation	96
tPA	CPEB1	NA	Increased translation	NA	74

Known RNA-binding proteins for these mRNAs are listed, but only a few studies have linked neurotransmitter regulation to specific RNA-binding proteins. Several dendritic mRNAs also contain internal ribosomal entry sites (IRES), but the use of IRES-mediated translation in dendritic protein synthesis has not been shown. αCaMKII, calcium/calmodulin-dependent protein kinase II (α-subunit); Arc, activity-regulated cytoskeleton-associated protein; BDNF, brain-derived neurotrophic factor; CPEB1, cytoplasmic-polyadenylation-element-binding protein 1; eEF1A, eukaryotic translation elongation factor 1A; FMRP, fragile-X mental retardation protein; Hzf, hematopoetic zinc finger; InsP3R, inositol 1,4,5-trisphosphate receptor; LIMK, Lim-domain kinase 1; mGluR, metabotropic glutamate receptor; NA, not applicable; NMDAR, *N*-methyl-D-aspartate receptor; RNG105, RNA granule protein 105; tPA, tissue plasminogen activator; ZBP1, zip-code-binding protein 1.

Bramham, 2007

Epileptogenic seizures induce *in vivo* accumulation of BDNF mRNA in dendrites

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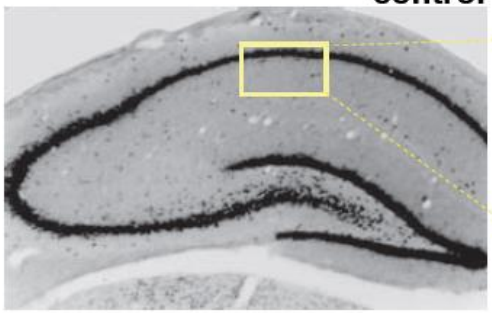
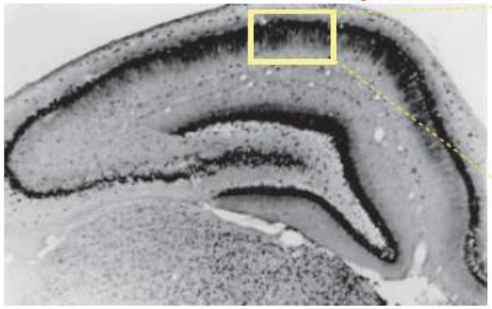
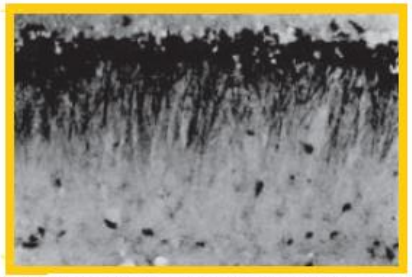
control

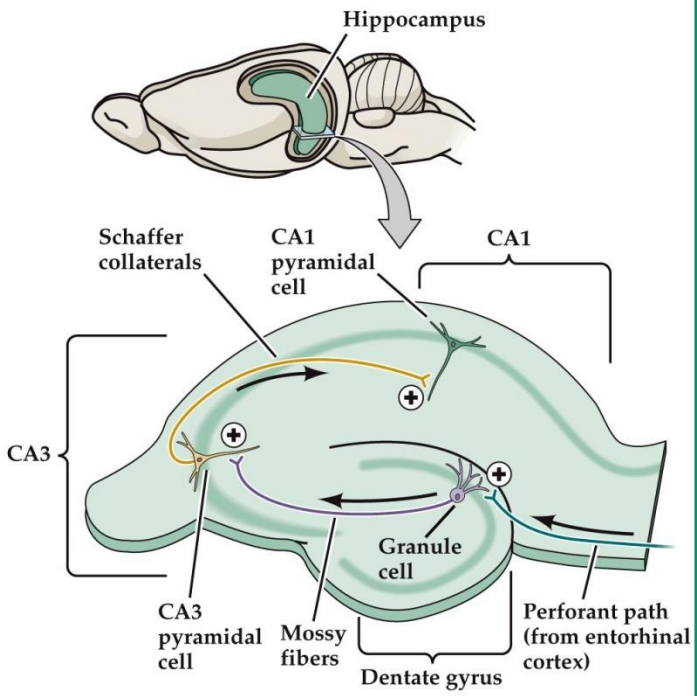


pilo 3h





Tongiorgi et al. The Journal of Neuroscience, July 28, 2004 • 24(30):6842–6852



NEUROSCIENCE 5e, Figure 8.6
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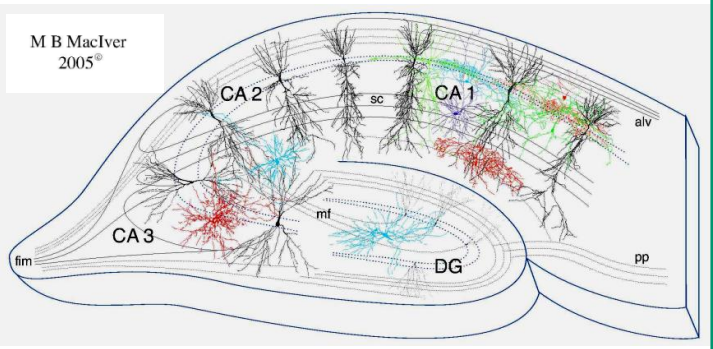
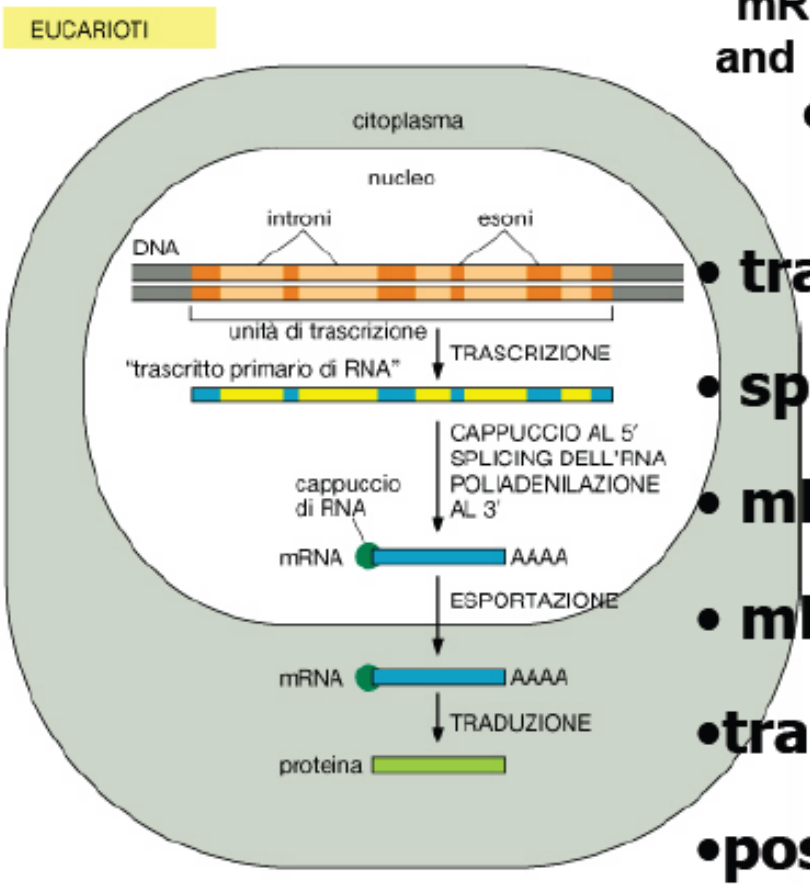


Spatially restricting gene expression by local translation at synapses

- ✓ **mRNA localization** and **regulated translation** provide a means of spatially restricting gene expression within each of the thousands of subcellular compartments made by a neuron, thereby vastly increasing the computational capacity of the brain.

- ✓ Recent studies reveal that local translation is regulated by stimuli that trigger neurite outgrowth and/or collapse, axon guidance, synapse formation, pruning, **activity-dependent synaptic plasticity**, and injury-induced axonal regeneration.

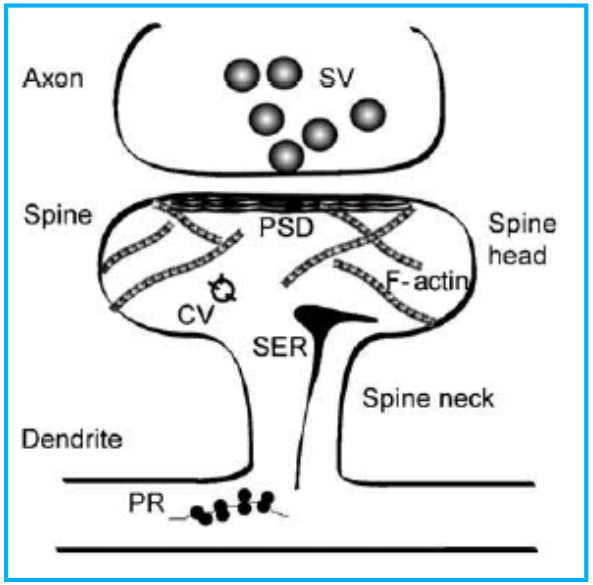
- ✓ Impairments in the local regulation of translation result in aberrant signaling, physiology and morphology of neurons, and are **linked to neurological disorders**.

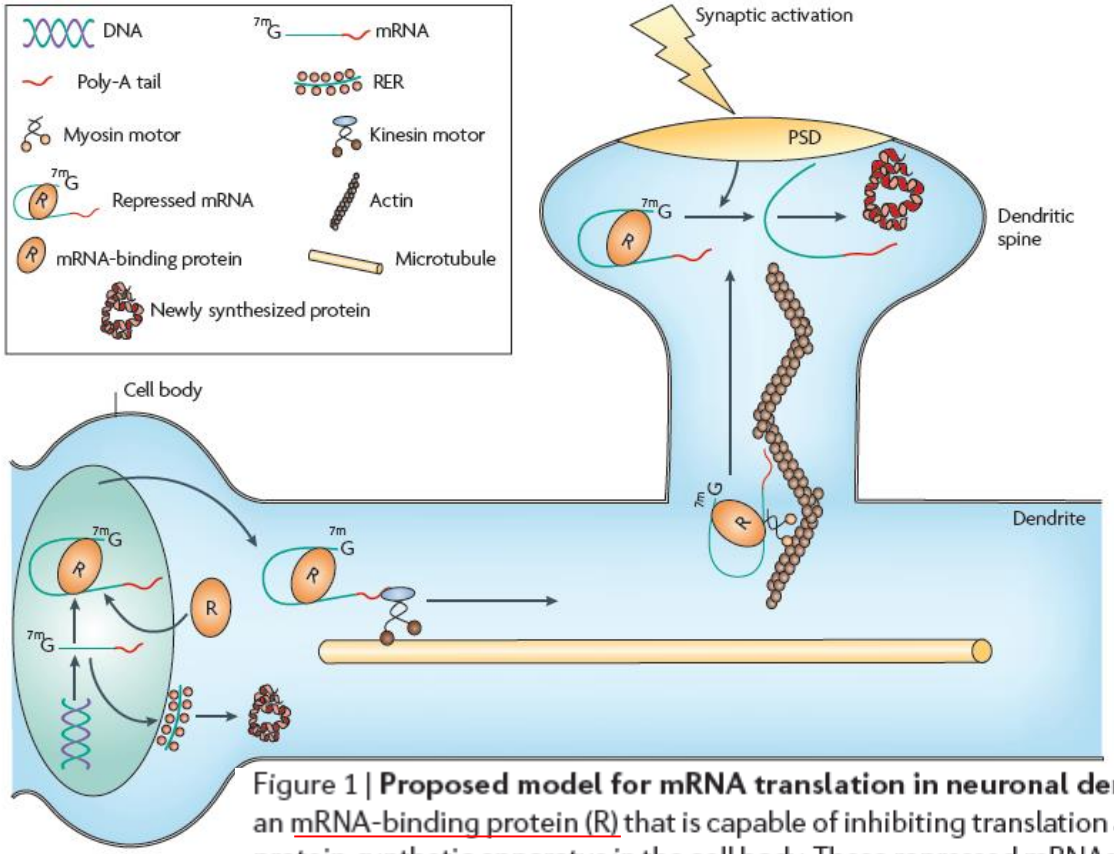


mRNAs are synthesized in the nucleus and undergo a series of regulatory steps each of which is tighly regulated

- **transcription**
- **splicing**
- **mRNA stability**
- **mRNA transport**
- **translation**
- **post-translational modifications**

Mechanisms of mRNA transport & translation in dendrites





Bramham et al., 2007

Figure 1 | **Proposed model for mRNA translation in neuronal dendrites.** Binding of a specific mRNA in the nucleus by an mRNA-binding protein (R) that is capable of inhibiting translation allows the mRNA to be sequestered away from the protein-synthetic apparatus in the cell body. These repressed mRNAs are then packaged into transport granules that are transported into the dendrite by kinesin motors on microtubules. Following synaptic activation, the granules are dispersed and the mRNA is localized to spines by the actin-based myosin motor proteins. Translation is activated in the synaptic compartment by neutralizing the repressive RNA-binding protein. ^{7m}G, 7-methyl-guanosine; PSD, postsynaptic density; RER, rough endoplasmic reticulum.

Wang et et al., 2010

Box 3. Outstanding questions in the field of local translation at the synapse

- What is the nature of the cis-acting RNA localization elements that target transcripts to specific subcellular compartments within a neuron?
- What are the RNA binding proteins that function to localize mRNAs within neurons and how do they mediate this localization?
- What is the composition of the RNPs that localize mRNAs to dendrites? What is the relationship between RNA transport granules, P-bodies and stress granules?
- What are the physiologically relevant stimuli that regulate local translation?
- Do/how do distinct stimuli regulate the translation of specific subsets of transcripts?
- How does the miRNA pathway contribute to local translation at synapses and thus synaptic plasticity?
- Are there differences in the mechanisms of translational regulation at the synapse as compared to in the soma?
- What mechanisms at synapses facilitate folding and maturation of the newly synthesized proteins?
- What is the nature of the secretory pathway, which is necessary for synthesis of membrane and secreted proteins, in distal dendrites?
- How does local translation of specific transcripts contribute to or alter the function of neural circuits?
- Is/how is stimulus-induced transcriptional regulation in the nucleus integrated with stimulus-induced local translation at the synapse?
- What are the proteins crucial to mGluR-LTD that accumulate at CA1 synapses of fragile X mice?
- Is PI3 kinase enhancer (PIKE) an FMRP target that is crucial for aberrant protein-synthesis-dependent synaptic plasticity observed in fragile X mice?

Fragile X Syndrome: a genetic intellectual disability based on silencing of the FMR1 gene encoding for an RNA-binding protein

Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability and also considered a **monogenic cause of Autism Spectrum Disorder**. FXS symptoms include neurodevelopmental delay, anxiety, hyperactivity, and autistic-like behavior.

The disease is due to the mutation or loss of the **FMRP (Fragile X Mental Retardation Protein)**, an RNA-binding protein involved in different steps of RNA metabolism, including mRNA decay, dendritic targeting of mRNAs, and protein synthesis.

FXS is due to triplet repeat expansion (90% of patients) or point mutations in the Fragile X mental retardation 1 (FMR1) gene. CGG expantion leads to hypermethylation of the CGG, transcriptional silencing, and abolished production of the Fragile X Mental Retardation Protein (FMRP)

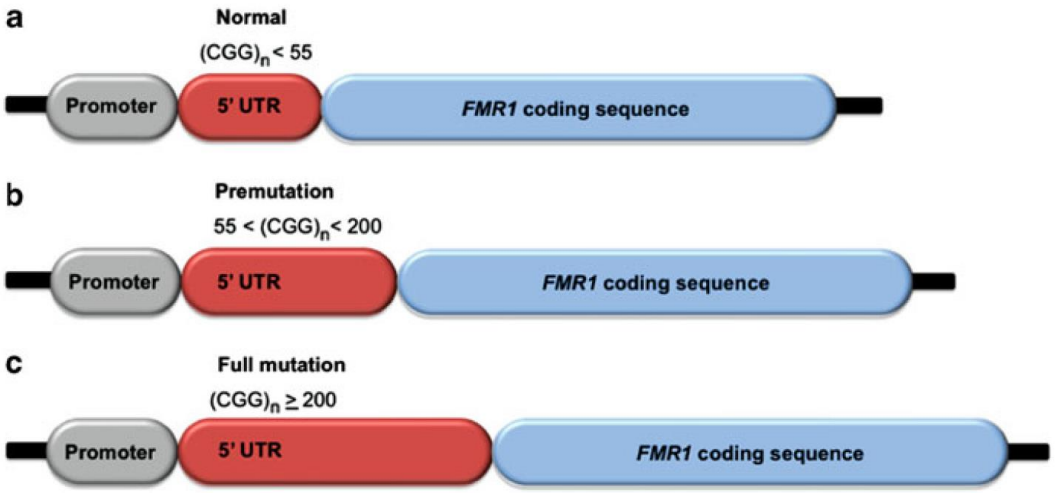


Fig. 23.1 Scheme of the *FMR1* gene which includes the promoter, the 5′ UTR, and the *FMR1*-coding sequence in a normal allele (5–44 CGG copies) (**a**), a premutated allele (55–200 copies) (**b**), and a full mutated allele (>200 repeats) (**c**)

In neurons lacking FMRP, a wide array of mRNAs encoding proteins involved in synaptic structure and function are altered. As a result of this complex dysregulation, in the absence of FMRP, **spine morphology and functioning is impaired**.

De Rubeis et al., 2012

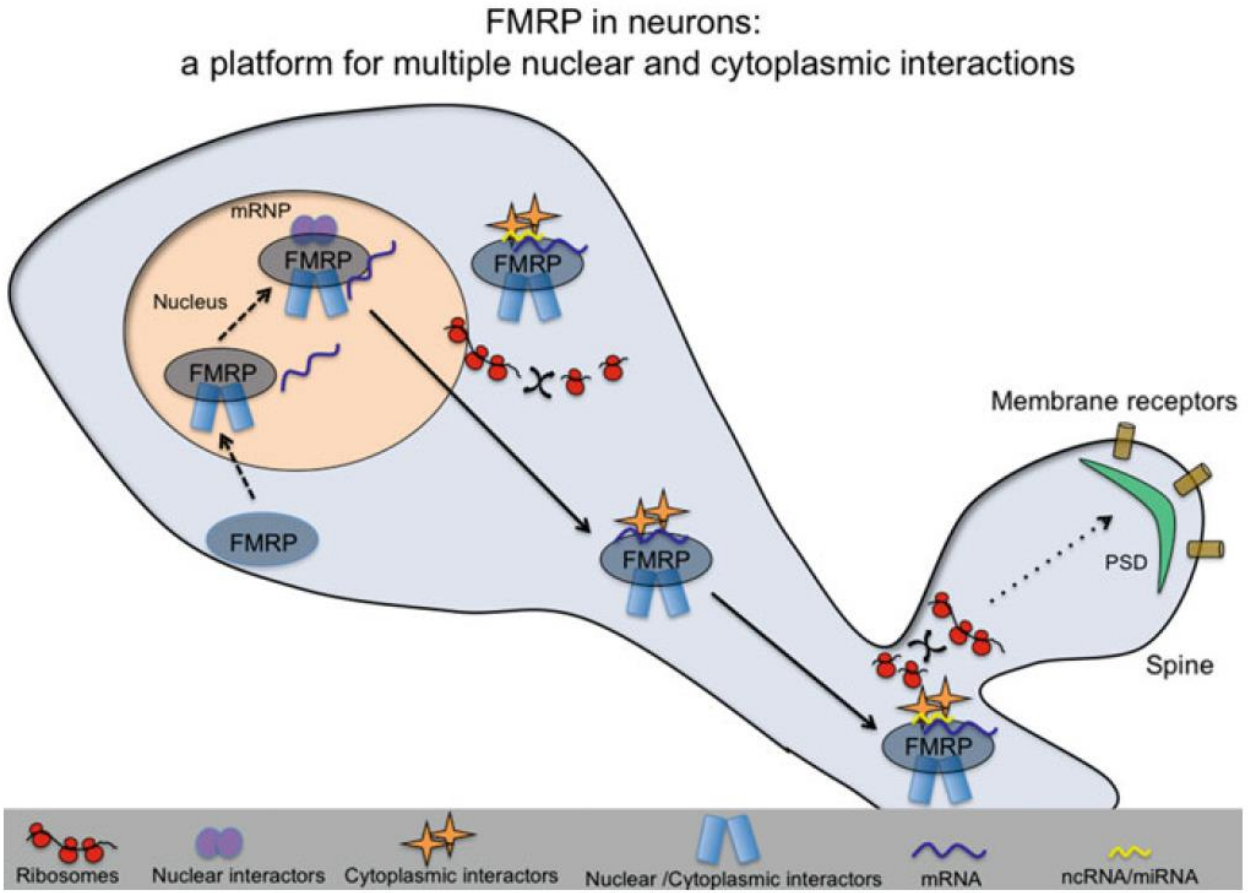


Fig. 23.3 FMRP forms part of a protein complex together with translationally arrested mRNAs. FMRP travels within an RNA–protein complex from the cell body to the synapses transporting dendritically localized mRNAs. After synaptic stimulation, FMRP liberates its mRNA targets allowing their local translation. The reversible translational repression and activation of the mRNA targets are regulated by a signaling pathway described in the text (see Sect. 23.3). Transported mRNAs are then locally translated in dendrites contributing to local protein synthesis and synaptic rearrangement that occurs after synaptic stimulation. FMRP can bind its mRNA targets through direct interaction or through noncoding RNAs such as BC1 RNA and microRNAs

De Rubeis et al., 2012

mRNA transport in axons?

Polyribosomes in axons?

Alvarez et al., 2000

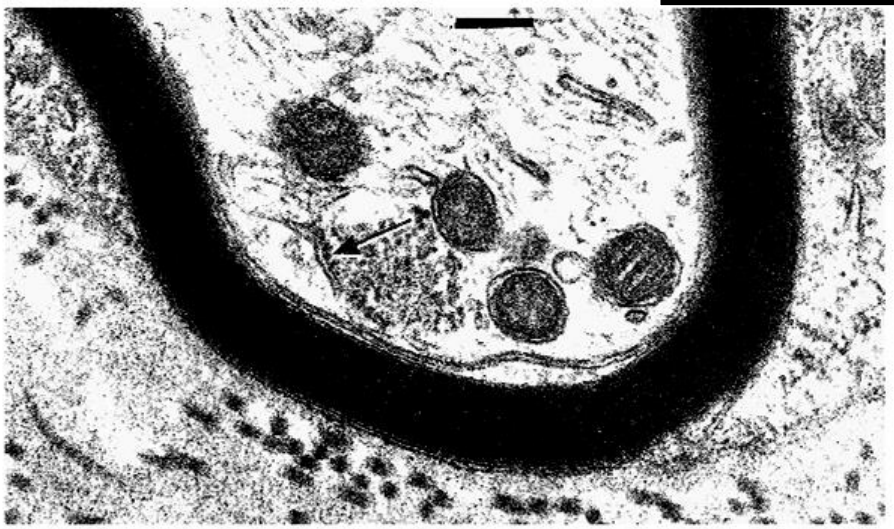


Fig. 12. A conventional electron micrograph kindly provided by Prof. Ennio Pannese (University of Milan), showing a myelinated axon from the sensory portion of a rabbit spinal nerve located close to the dorsal root ganglion, in which ribosomes are clustered near the axolemma. Note that some ribosomes appear attached to a tubular endoplasmic reticulum (arrow). Reproduced from Fig. 2a in Pannese and Ledda (1991), with permission of Nuova Immagine Editrice. Calibration, 0.2 μm.

400 Opinion TRENDS in Neurosciences Vol.25 No.8

Axonal and presynaptic protein synthesis: new insights into the biology of the neuron

Antonio Giuditta, Barry B. Kaplan, Jan van Minnen, Jaime Alvarez and Edward Koenig

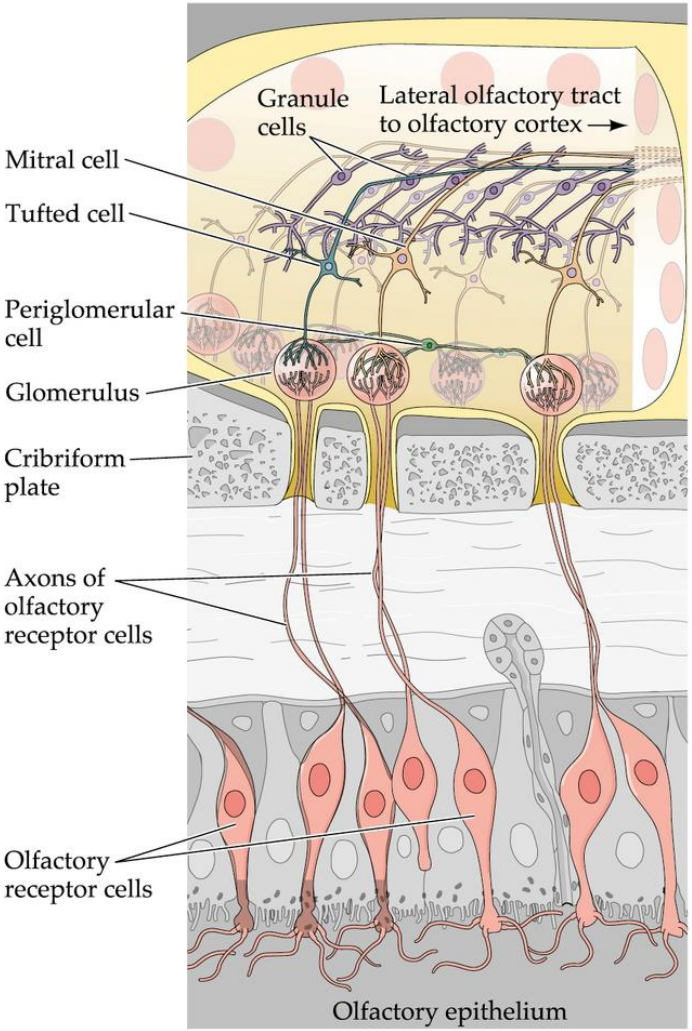
The presence of a local mRNA translation system in axons and terminals was proposed almost 40 years ago. Over the ensuing period, an impressive body of evidence has grown to support this proposal – yet the nerve cell body is still considered to be the only source of axonal and presynaptic proteins. To dispel this lingering neglect, we now present the wealth of recent observations bearing on this central idea, and consider their impact on our understanding of the biology of the neuron. We demonstrate that extrasomatic translation sites, which are now well recognized in dendrites, are also present in axonal and presynaptic compartments.

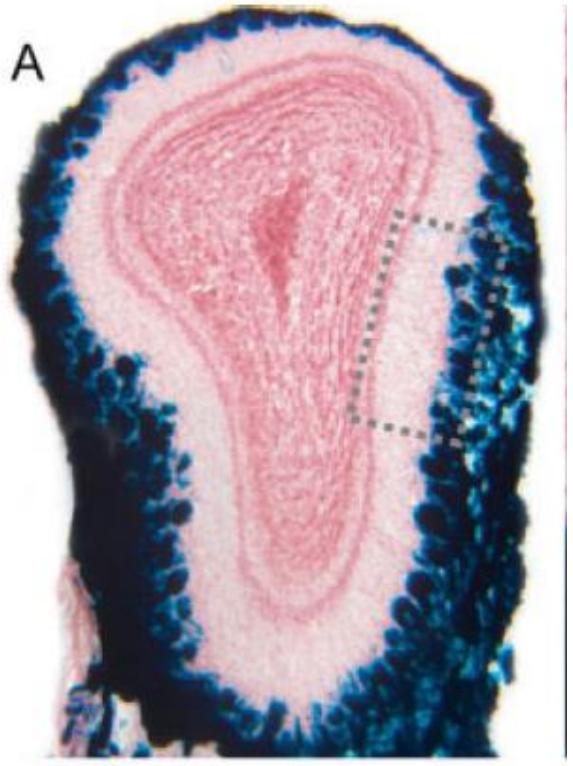
Giuditta et al., 2002

Early studies clearly indicated that presynaptic protein synthesis plays a part in the navigation of axonal growth cones in developing neurons, that presynaptic translation plays a part in invertebrate neurons and in specific classes of vertebrate neurons whose axonal/dendritic polarity might not be not fully established, and finally, that axonal protein synthesis is recruited during regeneration of injured axons.

However, in the last 2-3 years the idea that there is no mRNA transport and protein synthesis in normal mature axons has changed!!!

Transport of specific mRNAs along the axons of olfactory neurons

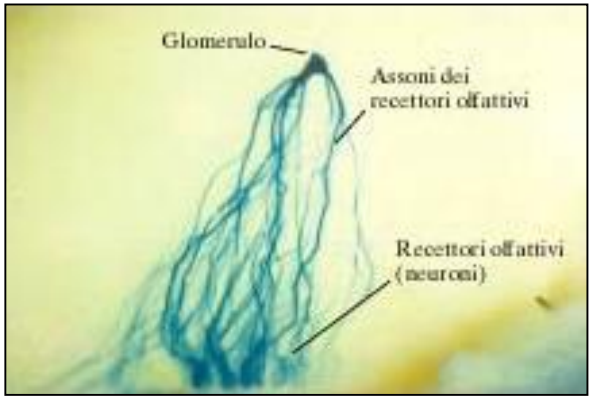


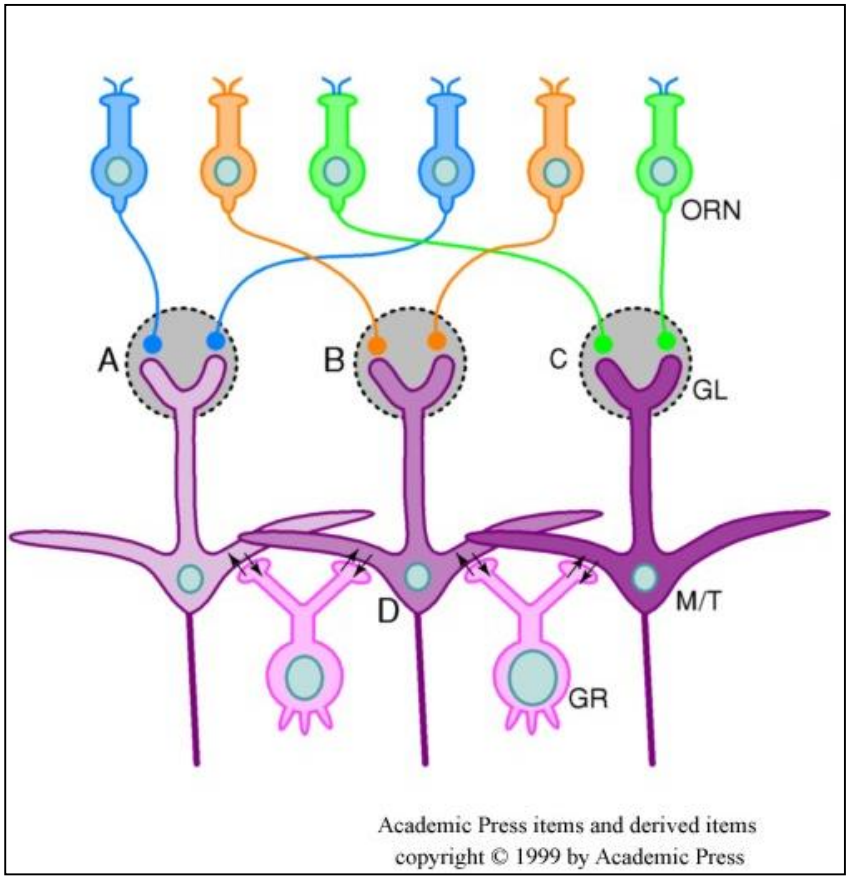


J Neurosci 1995 Jul;15(7 Pt 1):4827-37

Olfactory marker protein mRNA is found in axons of olfactory receptor neurons.
Wensley CH, Stone DM, Baker H, Kauer JS, Margolis FL, Chikaraishi DM.

The mRNA encoding each olfactory receptor is transported along the axon to a single glomerulus





Hypothesis: a regulated local translation of OR mRNAs in axons may allow controlling the distribution of ORs along OSN axons, which then may trigger the space-dependent sorting of OSN axons

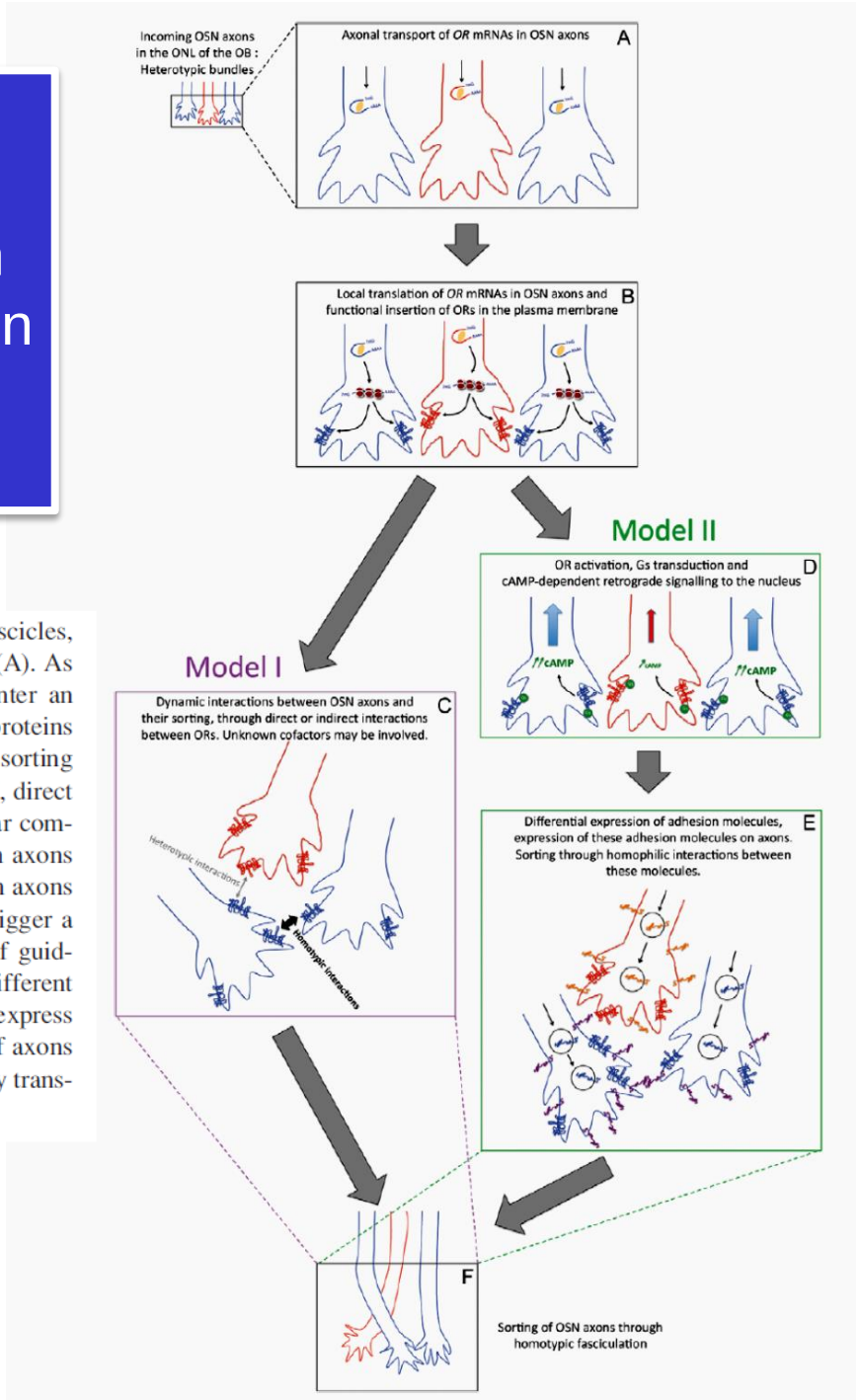


Figure 2 As they extend from the OE to the OB, growing OSN axons form heterotypic fascicles, where we propose that OSN growth cones contain *OR* mRNAs but little or no OR proteins (A). As they penetrate into the OB and cross its most superficial layer (ONL), they may encounter an extracellular signal activating *OR* mRNA translation and the subsequent trafficking of OR proteins to the plasma membrane (B). We postulate that neosynthesized ORs may then trigger the sorting and homotypic fasciculation of axons. Two non-exclusive models are envisaged. In Model I, direct or indirect trans-interactions between ORs (or peptides derived from ORs) in multimolecular complexes, which can be heterotypic or homotypic, may mediate differential adhesion between axons (C). If homotypic interactions lead to maximal adhesion, then dynamic interactions between axons may lead to their sorting (F). In Model II, neosynthesized axonal ORs are activated and trigger a Gs-cAMP-dependent retrograde signaling (D), which in turns activates the transcription of guidance/adhesion molecules (E). If axons expressing different ORs differentially activate different transcriptional programs, then each OSN population of a given OR identity will specifically express the same guidance/adhesion molecules, allowing the sorting and homotypic fasciculation of axons (F). A third model combining Models I and II, in which the Gs-cAMP cascade is activated by trans-interactions between complexes containing ORs, may also be proposed.

Dubacq et al., 2014

Local translation in growth cones

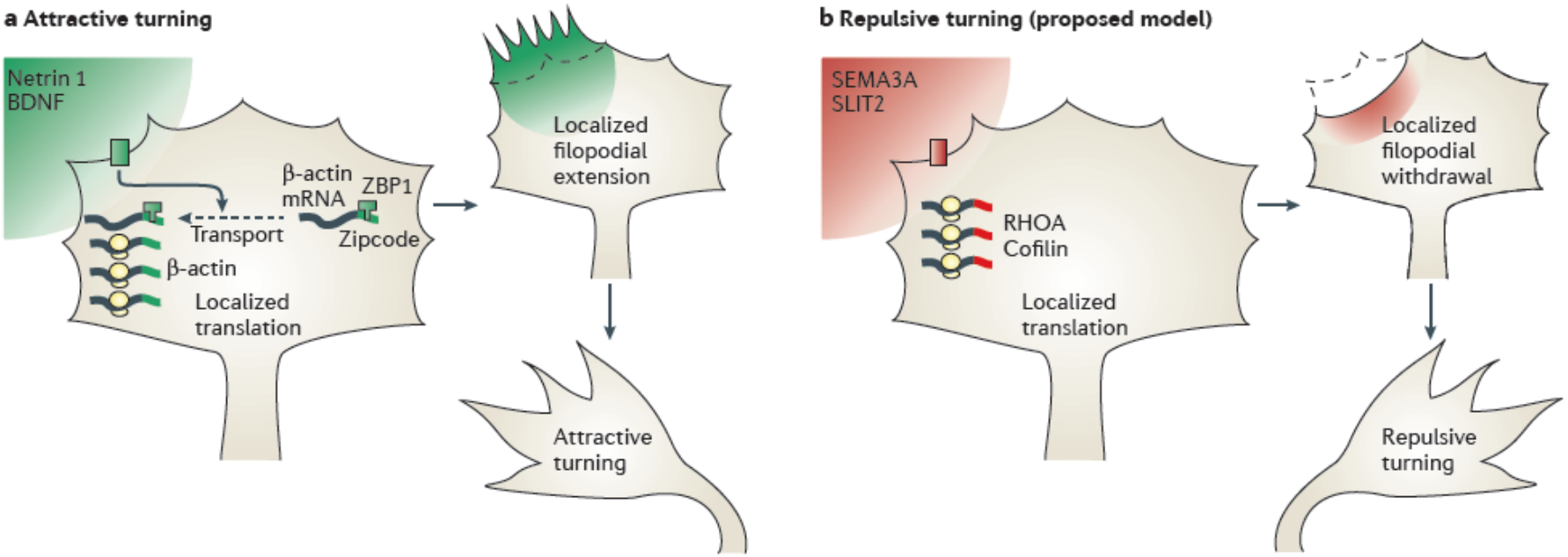
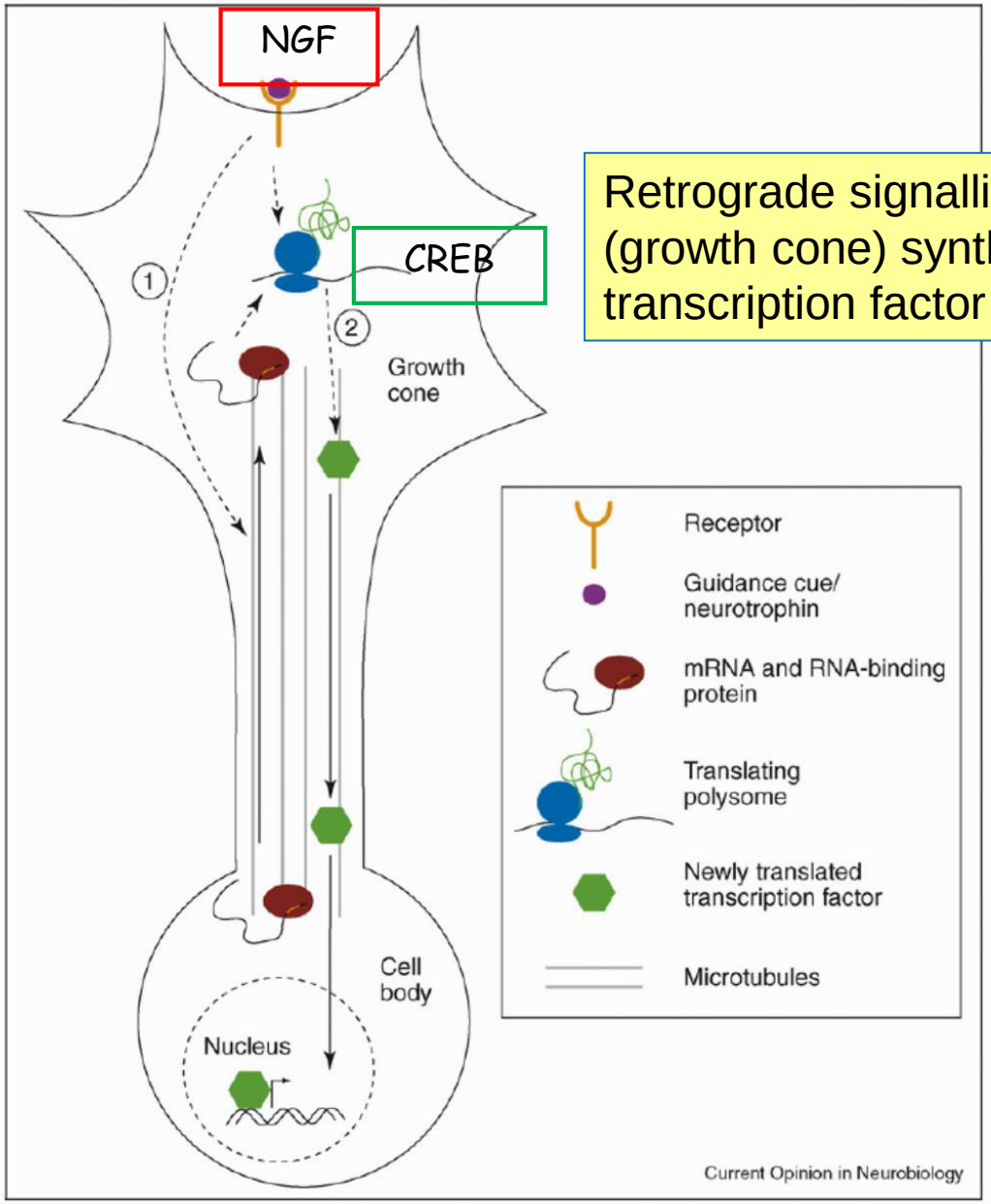


Figure 1 | **Growth cone turning regulated by differential mRNA translation.** Gradients of protein synthesis-inducing guidance cues commonly activate global translational activity on the side of the growth cone nearest to the gradient by activating mammalian target of rapamycin (mTOR). However, the specific mRNA translated in response to the cue differs depending on whether it is an attractive or repulsive cue and determines the direction of growth cone turning. **a** | Stimulation by attractive cues, such as netrin 1 and brain-derived neurotrophic factor (BDNF), leads to asymmetric synthesis of β-actin on the side near to the source of the gradient, which is mediated by β-actin mRNA transport to this region by zipcode-binding protein 1 (ZBP1)[53,54]. Spatially restricted synthesis of β-actin may lead to actin polymerization, cytoskeletal assembly and attractive turning of the growth cone. **b** | By contrast, repulsive cues, such as semaphorin 3A (SEMA3A) and SLIT2, activate the axonal translation of the actin-depolymerizing proteins RHOA[55] and cofilin[56] when uniformly applied in cell culture. A proposed model is shown, in which localized cytoskeletal disassembly may result in repulsive turning through polarized filopodial collapse. However, whether these molecules are translated asymmetrically in a repulsive gradient has not yet been tested.

http://biochemie.charite.de/en/research/signaling_mechanisms_in_brain_development_and_disease/projects/

http://www.youtube.com/watch?v=Wj3C6cLqXzY

Jung et al. , 2012



Retrograde signalling of locally (growth cone) synthesized transcription factor

Lin et al., 2008

Local translation and communication between the axon and cell body. (1) Stimulation of axons leads to transcription-independent differential localization of mRNAs to the axon through transport on microtubules, changing the population of mRNAs available for local axonal translation. (2) Newly synthesized transcription factors can be retrogradely transported on microtubules to the cell body where they influence transcription.

RNA-specific transport and translation in axons

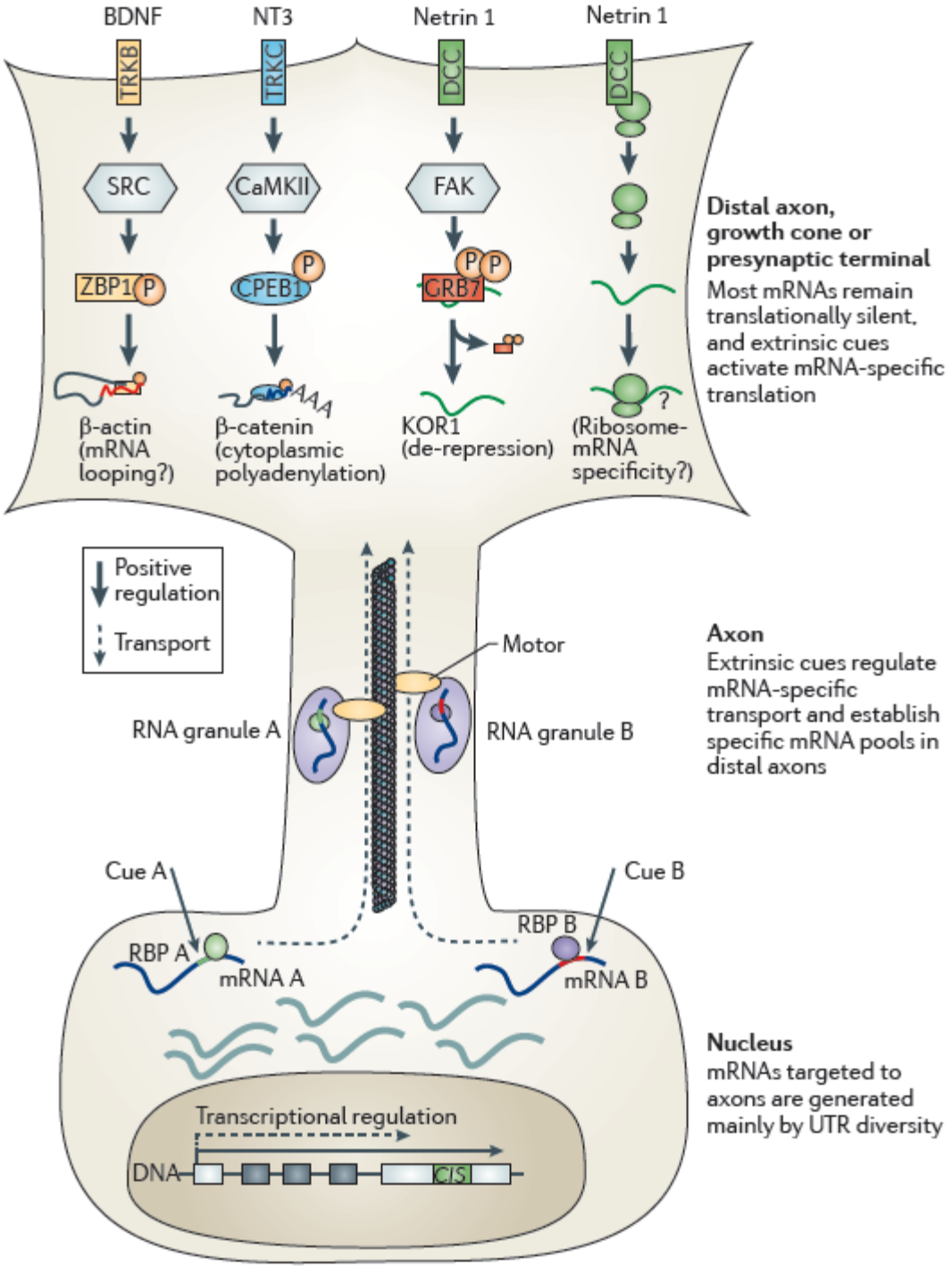


Figure 4 | **RNA-specific transport and translation.** Axonal targeting of mRNAs is directed by *cis*-acting elements that are mainly localized to the 3′-untranslated regions (UTRs) of mRNAs. Retention of these axon-targeting *cis*-acting elements is commonly regulated by the use of different transcriptional termination sites[15,66]. Extrinsic cues influence axonal mRNA repertoires by promoting transport of specific mRNAs[156]. Axonally targeted mRNAs are recruited to RNA granules (transport ribonucleoproteins (RNPs)) by specific RNA-binding proteins (RBPs) and are transported along microtubules probably by kinesin motors[157]. mRNAs remain translationally silent during transport[21]. Extracellular signals activate the translation of specific mRNAs mainly by regulating RBPs. For example, neurotrophins and guidance cues activate the kinases SRC[71], calcium/calmodulin-dependent protein kinase II (CaMKII)[174] and focal adhesion kinase (FAK)[164], which phosphorylate the RBPs, zipcode binding protein 1 (ZBP1), cytoplasmic polyadenylation element binding protein (CPEB1), and growth factor receptor-bound protein 7 (GRB7), respectively. Cell surface receptors might regulate mRNA-specific translation by directly regulating ribosomes. For example, unstimulated netrin receptor DCC directly binds to ribosomes and inhibits translation[26], and ribosome composition influences mRNA selectivity[181]. Different receptors may bind to ribosomes that are pre-tuned to specific mRNAs, and ligand stimulation might release such ribosomes and result in mRNA-specific translation. BDNF, brain-derived neurotrophic factor; KOR1, κ-type opioid receptor; NT3, neurotrophin 3; TRK, tyrosine kinase receptor.

Jung et al. , 2012

Model of stimulus-induced axonal translation

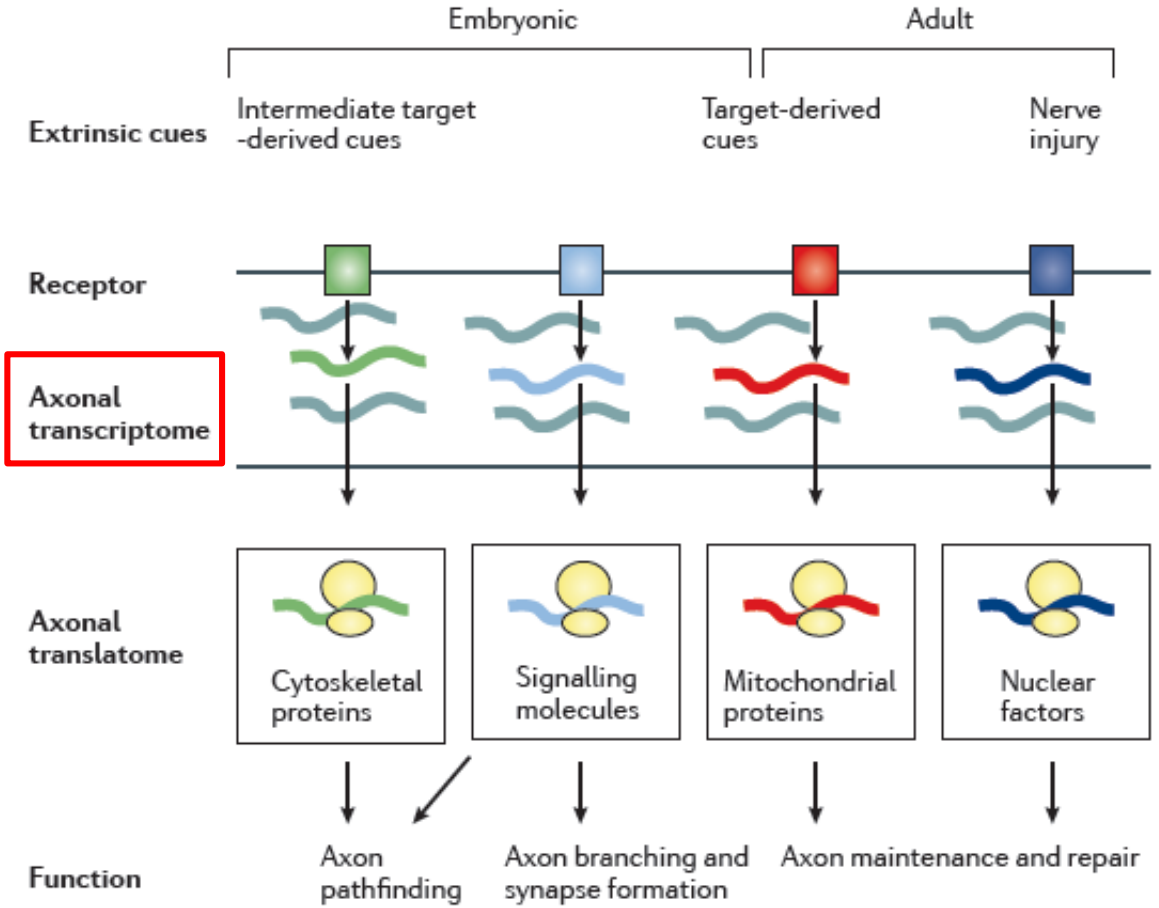


Figure 5 | **Local mRNA translation as a mediator of stimulus-induced axonal responses.** A proposed model for the function and mechanism of axonal mRNA translation. Neuronal axons contain a complex and dynamic transcriptome, and many mRNAs remain translationally silent. Various extrinsic cues stimulate translation of a distinct subset of mRNAs during development and in adulthood. For example, guidance cues induce local synthesis of cytoskeletal proteins in growing axons and regulate axon guidance and branching. Target-derived trophic factors promote local synthesis of proteins required for mitochondrial function and support the survival of distal axons. Nerve injury in adulthood stimulates local synthesis of nuclear factors that activate repair mechanisms.

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