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Intra-axonal protein synthesis in development and beyond

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Abstract

Proteins can be locally produced in the periphery of a cell, allowing a rapid and spatially precise response to the changes in its environment. This process is especially relevant in highly polarized and morphologically complex cells such as neurons. The study of local translation in axons has evolved from being primarily focused on developing axons, to the notion that also mature axons can produce proteins. Axonal translation has been implied in several physiological and pathological conditions, and in all cases it shares common molecular actors and pathways as well as regulatory mechanisms. Here, we review the main findings in these fields, and attempt to highlight shared principles.

1. Introduction

Asymmetric expression of proteins through the subcellular localization and translation of their mRNAs is an evolutionary conserved mechanism found in essentially all cells (Donnelly et al., 2010; Holt and Schuman, 2013; St Johnston, 2005). In the bacterium *Escherichia coli*, some transcripts are differentially localized between the cytoplasm and the inner membrane, according to the destination of their protein products (Nevo-Dinur et al., 2011). In *Drosophila* embryos, *in situ* hybridization analysis showed that 71% of the more than three thousand analysed transcripts were specifically localized, and their distribution was tightly correlated with the expression pattern of their protein products (Lécuyer et al., 2007). In rat hippocampal neurons an astonishing greater than 50% of the total mRNA content is estimated to be localized to neurites (Cajigas et al., 2012).

Neurons are highly specialized cells, able of conveying information over large distances. They typically possess several highly branched dendrites and a single long effector process, the axon. Due to this extremely polarized morphology, neurons are an ideal cell type to

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Conflict of Interest

The authors state that the present manuscript presents no conflict of interest.

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study the regulation and functional consequences of localized protein synthesis. In fact, their unique shape and the extreme distances covered by their neurites prompted already at the end of the 19th century the suggestion that axons might be able to synthesize at least parts of their materials in a manner independent of their cell bodies (Barker, 1899). Axons can span over distances thousands times the neuronal soma's diameter, and the fastest anterograde velocity reported in living neurons is a little less than 40 cm in a day (Kaether et al., 2000), with most cytoskeletal and cytosolic proteins moving only a few millimetres per day (Campanot and Eng, 2000). As a consequence, it can take days for somatically derived proteins to reach their targets in distal axons, raising the issue of protein stability during these long transport times. Slowly transported proteins are indeed degraded over time within mouse retinal ganglion axons (Nixon, 1980). Based on these considerations, the hypothesis was formulated that axonal protein synthesis was needed to overcome the slow transport rates of proteins from the neuronal cell bodies (Alvarez, 1992). Intensive research efforts aimed at understanding the functional requirements of individual axonally localized mRNAs, and more recently transcriptome analyses have changed this view. Instead of being merely an adaptation to slow axonal transport (Alvarez et al., 2000), local protein synthesis is now understood as a mechanism for the spatial-temporal regulation of gene expression, allowing neurons to react acutely to changes in their environment, to compartmentalize signalling events, and to communicate over long distances from the periphery to the cell body. Here we review the functional importance of local translation in developing axons and discuss the rapidly emerging evidence for the requirement of axonal translation beyond the developmental period.

2. Local translation in dendrites

Even though intuitively the longer length of axons means that they may benefit most from a local source of proteins, the existence of local translation in dendrites has been far less controversial, likely due to the ease with which it is detected. It has been known for more than 50 years that disrupting mRNA transcription or translation impedes the long-term storage of memory (Agranoff et al., 1967; Flexner et al., 1963). In the same decade, Bodian (1965) identified ribosome particles in proximal dendrites in monkey spinal cord motoneurons and speculated about a possible functional significance for local protein synthesis in synaptic function. Roughly twenty years later, using electron microscopy, polyribosomes were identified under the base of dendritic spines (Steward and Levy, 1982), and their number and localization showed changes following lesion and during synaptogenesis (Steward, 1983; Steward and Falk, 1986). Metabolic labelling studies in both synaptosomal fractions and compartmentalized cultures soon established that proteins could actually be produced outside of the neuronal soma (Rao and Steward, 1991; Torre and Steward, 1992; Weiler and Greenough, 1991). Initially, the number of proposed dendritically localized mRNAs was no more than a few dozens. New interest in the matter soon revealed an unexpectedly greater number of localized RNAs (Miyashiro et al., 1994), and large-scale microarray studies identified hundreds of transcripts (Poon et al., 2006; Zhong et al., 2006). More recently, RNA-sequencing analysis uncovered 2550 mRNAs localized to neurites of rat hippocampal neurons (Cajigas et al., 2012).

A first functional role for dendritic protein synthesis was revealed when it was found to be involved in a neurotrophin-dependent form of long-lasting synaptic enhancement in the hippocampus: neuropil isolated from its cell bodies could undergo brain-derived neurotrophic factor (BDNF)-induced long term plasticity, and, more importantly, this process was sensitive to inhibitors of protein synthesis (Kang and Schuman, 1996). Dendritic protein synthesis is now considered a requisite process in synaptic plasticity (Sutton and Schuman, 2005), especially for the late phase of long-term potentiation (LTP). LTP reflects a persistent strengthening of synaptic connections and is regarded as the main molecular mechanism underlying memory consolidation, or the conversion of short-term and working memory into long-term memory (Kandel, 2001; McGaugh, 2000). Accordingly, the dysregulation of dendritic protein synthesis has been implied in several neurological disorders (Swanger and Bassell, 2013).

3. Local translation in developing axons

3.1. Early evidence and controversy

Unlike dendritic translation, the existence of a localized system of protein synthesis in axons and nerve terminals was not easily accepted. The first experiments that argued against the exclusive somatic origin of axonal proteins were conducted on vertebrate neurons. If the soma was the only source of an axonal protein such as acetylcholinesterase (AChE), after an irreversible inactivation of AChE, its reappearance in axons should follow a proximal to distal gradient. However, in cat cholinergic neurons both proximal and distal axonal segments displayed an homogeneous recovery rate (Koenig and Koelle, 1960). Also, metabolic labelling studies showed incorporation of [^3H]leucine into proteins of desomatized rabbit axons (Koenig, 1967). But after initial enthusiasm and several studies conducted on synaptosomal fractions, the idea lost widespread support mainly because, differently from dendrites, only in very few studies were ribosomes identified in axons by electron microscopy (Peters et al., 1970; Zelená, 1972). Thus based mainly on the perceived absence of clearly identified ribosomes in electron micrographs of mature axons the source of newly synthesized proteins found in synaptosomal fractions was assumed to be exclusively glial, post-synaptic, free mitochondria and membranous contaminants (reviewed in Alvarez et al., 2000). On the contrary, ribosomes were easily identified in growth cones of developing dorsal root ganglion neurons (Tennyson, 1970; Yamada et al., 1971) and in the neurite endings of sympathetic neurons (Bunge, 1973). Brain synaptosomes prepared from young rats had a higher proportion of polyadenylated RNAs and seemed to show higher protein synthetic ability (DeLarco et al., 1975). Further evidence showed isolated axonal growth cones could incorporate radiolabelled amino acids (Davis et al., 1992). Polyribosomes were later identified in the growth cones of developing hippocampal axons by ultrastructural analysis (Deitch and Banker, 1993), even though at a much lower density. Indeed, this paucity of polyribosomes was pointed as one of the earliest and most reliable feature that distinguishing a developing axon from other minor processes (Deitch and Banker, 1993).

Evidence for axonal protein synthesis also started to accumulate from invertebrate models. The squid giant axon was found to possess ribosomal RNAs (Giuditta et al., 1980), mRNAs

(Giuditta et al., 1986), tRNAs (Ingoglia et al., 1983), and actively translating polysomes (Giuditta et al., 1991). More compelling still, squid axons separated from their soma could incorporate radioactive amino acids into newly synthesized proteins (Giuditta et al., 1968). Similar experiments detected protein synthesis in isolated large myelinated Mauthner goldfish axons (Edström and Sjöstrand, 1969), and in cultured mollusc axons separated from their soma (Van Minnen et al., 1997). Electron spectroscopy imaging together with immunohistological studies of axoplasm whole-mounts showed discrete, intermittent 'plaque-like' ribosome-containing structures closely associated with the plasma membrane in the Mauthner axons (Koenig and Martin, 1996). These same periaxoplasmic ribosomal plaques (PARPs) were also seen on mammalian peripheral nervous system (PNS) axons (Koenig et al., 2000; Kun et al., 2007). In fact, a very early report of few 'ribosome-like' particles close to the periphery of myelinated mammalian axons is in agreement with these later descriptions (Zelená, 1970). Individual RNAs were studied and *in situ* hybridization confirmed the presence of β -actin and β -tubulin mRNAs in squid giant axons (Kaplan et al., 1992), and of β -actin mRNA in the Mauthner axon, associated with the newly identified PARPs (Sotelo-Silveira et al., 2008). Several later studies additionally demonstrated the presence of ribosomal proteins and translation factors in vertebrate axonal growth cones (Bassell et al., 1998; Campbell and Holt, 2001). Taken together, these studies challenged the widely held view at the time that only dendrites but not axons were able to synthesize proteins locally, but it remained unclear to which extent local translation was required for axons.

3.2. Local translation in axonal growth and guidance

Elongating neurites terminate in specialized structures, the growth cones, responsible for directional outgrowth into the synaptic target. Growth cones are highly motile and react to extracellular cues in a strictly regulated temporally and spatially-specific way. Both microtubule dynamics and actin filament remodelling are involved in correct growth cone navigation (Dent et al., 2011), and cytoskeletal proteins were the first likely candidates for local synthesis. *In situ* hybridization analysis showed that β -actin mRNA localized to neurites and growth cones of cultured cortical neurons (Bassell et al., 1998), and both actin and tubulin were found to be synthesized in vertebrate growth cones (Eng et al., 1999). Nevertheless, inhibiting local synthesis did not inhibit axonal elongation (Eng et al., 1999), and its functional significance remained elusive. A major breakthrough was the discovery of the role of local translation in a growth cone's response to guidance cues. Cultured *Xenopus laevis* retinal growth cones severed from their cell body continue to grow and turn towards a netrin-1 gradient and away from semaphorin 3A gradient. Blocking local protein synthesis prevented these chemotropic responses (Campbell and Holt, 2001). In a series of studies motivated by this finding, individual mRNAs have been identified whose local translation is required for growth cone responses to extracellular guidance cues. The attractive guidance cues netrin-1 and nerve growth factor (NGF) trigger local synthesis of a variety of proteins including β -actin (Leung et al., 2006; Yao et al., 2006), Par3 (Hengst et al., 2009), and TC10 (Gracias et al., 2014). On the other hand, the repulsive guidance cues Slit2 and Sema3A induce the translation of actin destabilizing proteins, such as RhoA (Piper et al., 2006; Wu et al., 2005) and cofilin (Piper et al., 2006). A growing body of evidence connects local axonal translation with directional and stimulated growth during development, and disruption of

some pathways can lead to axonal morphological changes. β -catenin is synthesized in developing hippocampal growth cones following neurotrophin-3 (NT-3) stimulation and its translation is regulated by the cytoplasmic polyadenylation element-binding protein, whose inhibition affects axonal growth and branching (Kundel et al., 2009). Axonal branching in response to NGF in chicken embryonic sensory neurons is also dependent on intra-axonal synthesis of the actin nucleating Arp2/3 complex activator WAVE1 and the complex stabilizer cortactin (Spillane et al., 2012). In cultured rat DRG neurons, increasing axonal synthesis of β -actin protein while decreasing growth associated protein 43 (GAP-43) results in short, highly branched axons, while increasing GAP-43 axonal expression over β -actin increases the length and reduces branching of axons (Donnelly et al., 2013a). NGF-induced axon elongation is mediated by local synthesis of microtubule associated protein 1B and calmodulin (Wang et al., 2015). Axonal pathfinding often involves more complex environmental interactions and more demanding responses. While migrating axonal growth cones can be attracted to intermediate targets, the classical example being the central nervous system (CNS) midline, but upon reaching their intermediate targets, they need to change their responsiveness to the guidance cues, in order to progress. Besides losing responsiveness to the intermediate attractant, they need to gain responsiveness to a new set of cues, and axonal protein synthesis was suggested as a mechanism for the latter (Brittis et al., 2002). Axon growth is guided by extracellular gradients of guidance cues, and growth cones need to adapt successively to different concentrations, in order to respond to further increases or decreases in the signal. Indeed, cultured *Xenopus* spinal neurons show desensitization and resensitization in response to netrin-1 and brain-derived neurotrophic factor, and resensitization required local protein synthesis (Ming et al., 2002). It is worth noting that not all growth cone or axonal responses to extracellular factors are the result of local translation; for example L- α -lysophosphatidic acid-induced growth cone collapse is not abolished by protein synthesis inhibitors (Campbell and Holt, 2001). In another study, chick retinal, sympathetic and DRG neurons, and mouse DRG neurons, attractive responses to NGF and NT-3, as well as collapsing ephrin-A2, slit3 and sema3A effects were not dependent on local translation (Roche et al., 2009). Axonal protein synthesis was elicited, but its inhibition failed to affect growth cone responses for 24–48h. Factors such as a higher metabolic activity or differences in developmental stages might explain these discrepancies (Nédelec et al., 2012).

The stimulus-dependent nature of these local translation events does appear to be typical for intra-axonal protein synthesis. Severed axons grow normally for hours in the presence of protein synthesis inhibitors (Campbell and Holt, 2001), and basal axonal elongation does not require axonal protein synthesis (Hengst et al., 2009). Further, the vast majority of β -actin protein is transported anterogradely from the cell body (Eng et al., 1999), and yet disrupting axonal *β -actin* mRNA interaction with zipcode binding protein 1 (ZBP1) (Yao et al., 2006) or genetically deleting ZBP1 (Welshhans and Bassell, 2011) interferes with directional growth. ZBP1 is a RBP that is required for the trafficking and localized translation of *β -actin* mRNA in axons (Hüttelmaier et al., 2005). Thus, *β -actin* mRNA translation at the growth cone is clearly not merely a means to overcome long distances from the cell body and slow axonal transport, especially as a similar asymmetrical β -actin synthesis is also seen in lamellipodia

of fibroblast (Shestakova et al., 2001), and in these cells there is no obvious temporal advantage for *de novo* synthesis of β -actin.

Instead, local translation affords axons advantages that are specific to the protein products. For example, in the case of attractive guidance cues, both Par3 and TC10 are locally synthesized. Par3 is the major component of the PAR polarity complex regulating cytoskeletal dynamics while TC10 is a member of the exocyst complex, that is essential for the addition of new membrane to the plasma membrane in growing axons (Dupraz et al., 2009). The temporally and spatially coincident local synthesis of Par3 and TC10 downstream of attractive guidance cues is thus a mechanism to ensure the simultaneous and spatially restricted activation of these two pathways during stimulated axon outgrowth (Gracias et al., 2014). In this context it is especially interesting that the netrin-1 receptor DCC and the plus-end microtubule tracking protein APC can act as translational hubs (Preitner et al., 2014; Tcherkezian et al., 2010) that at least in the case of APC interact with a defined subset of localized mRNAs. Similarly, stalled axonal mitochondria can also generate hot spots of localized mRNA translation, with proven functional significance in axonal branching (Spillane et al., 2013), and, likely, in other axonal responses, given the high energy demand of translation. These local translation hubs might provide an answer to the question how the local translation of a specific cohort of localized mRNAs is triggered in response to the extracellular stimuli.

3.3. Local translation in neuron specification and survival

Besides influencing local morphological responses, neurotrophins acting at distal axonal tips can also signal retrogradely to influence neuronal survival or specification through changes of nuclear gene expression (Zweifel et al., 2005). An increasing body of evidence supports the notion that local translation within axons and growth cones is a crucial mechanism for retrograde communication from the cellular periphery to the nucleus. Local synthesis of retrogradely trafficked signals, especially transcription factors, is triggered by several neurotrophins. In response to axonal treatment with NGF, cAMP-responsive element (CRE)-binding protein (CREB) is locally synthesized and retrogradely transported to the nucleus in dorsal root ganglion (DRG) neurons, and suppression of this process markedly reduced NGF-dependent survival of DRGs in culture (Cox et al., 2008). It remains unclear why a transcription factor would be locally synthesized just to be transported back to the nucleus. A possible answer might be that local synthesis and retrograde transport of transcription factor allow to very tightly control a signalling event. For example, BDNF induces local synthesis of SMAD1/5/8 followed by their retrograde transport in trigeminal neurons. But importantly, these transcription factors were activated only in the cell bodies by retrogradely transported BMP4-induced signalling endosomes leading subtype specification (Ji and Jaffrey, 2012). This mechanism provides a means to ensure subtype specification of trigeminal neurons occurs only if two signals, BDNF and BMP-4 are detected by an axon at the same time.

4. Axonal translation after development

4.1. Local translation in nerve terminals and synapse formation

While the requirement of local translation in developing axons has been demonstrated in several instances during the last 15 years and is now widely accepted, its presence and significance during synaptogenesis and in mature axons has remained in comparison much more controversial. As the axon matures, and upon encountering proper connectivity targets, it forms specialized structures, the presynaptic terminals, that contains neurotransmitters inside synaptic vesicles, and upon a nerve impulse, release them near the postsynaptic cell. Each neuron makes thousands of synapses, and the capacity of the neurons to modulate the strength of their synapses in response to their environment is fundamental for several processes already highlighted, such as memory formation and learning (Bliss and Collingridge, 1993).

After the discovery of the enrichment of presynaptic nerve terminals in synaptosome preparations from vertebrate brains, the heterogeneity of these preparations raised doubts about the actual origin of the newly synthesized proteins, and efforts in identifying presynaptic translated proteins in mature vertebrate terminals using synaptosomal preparations were largely abandoned (Crispino et al., 2001). Again, studies in invertebrate systems provided early evidence for protein synthesis in nerve terminals and pre-synapses. Preparations from squid optic lobes generate large presynaptic nerve terminals with low level of contaminating structures (Crispino et al., 1997). Squid photoreceptor nerve terminals contain active polysomes (Crispino et al., 1997) and polysome-associated mRNAs encoding translation factors, cytoskeletal and ribosomal proteins, and nuclear-encoded mitochondrial proteins (Gioio et al., 2001; Gioio et al., 2004). The presynaptic terminals are capable of synthesizing approximately 80 different proteins (Jiménez et al., 2002), and this synthesis appears to be under the control of cell signalling pathways, as it is strongly modulated by cytosolic calcium (Crispino et al., 1993). In the giant marine snail *Aplysia*, a touch to the siphon elicits a reflexive gill withdrawal. This reflex shows different forms of learning, such as habituation, dishabituation, sensitization, and classical conditioning (Carew et al., 1971; Carew et al., 1981; Pinsker et al., 1970). It involves sensory neurons that make synapses with motor neurons and plasticity in these synapses accounts for the learning responses (Purves et al., 2012). In a culture system where a bifurcated sensory axon makes synapses with two different motor neurons, administration of serotonin specifically only to one synapse induces long term facilitation (LTF) at that synapse, but not on the other. The nerve terminal of the stimulated synapse shows enhanced protein synthesis when compared with the unstimulated terminal and inhibiting local protein synthesis abolished LTF (Casadio et al., 1999; Martin et al., 1997). LTF occurs in terminals separated from their cell bodies (Schacher and Wu, 2002), but transcriptional events are necessary for its sustained stabilization (Liu et al., 2003), suggesting that a retrograde signal might travel to the nucleus (Guan and Clark, 2006). The sensory cell-specific neurotransmitter sensorin is locally produced and both the mRNA and the peptide accumulate in nerve terminals after LTF. Interference with its local production does not decrease the protein levels at the presynapse, but it prevents synapse formation (Lyles et al., 2006). It is produced in response to interaction with specific post synaptic targets and as the synapse matures, its levels decline,

but it remains necessary for maintenance of synapse efficacy (Hu et al., 2004). These experiments in *Aplysia* established a role for local translation in synaptic plasticity, and its ability to modify synapses with great spatial specificity. They also hinted at a complex interaction between nerve terminals and cell body, requiring general transcriptional changes while maintaining branch-specific regulation, and they once again showed that local protein synthesis is far more than a way to maintain auto sufficiency, as presynthesized sensorin protein could not fulfil the same role as the locally translated one.

There are however fundamental differences between invertebrate and vertebrate neurons that complicate the comparison of these findings with the situation in vertebrate neurons. In invertebrate models, LTF is largely dependent on presynaptic protein synthesis. The regulation of the most intensively studied protein synthesis-dependent form of synaptic plasticity in mammalian neurons, late phase long-term potentiation (L-LTP), on the other hand, is largely postsynaptic (Colbran, 2015; Sutton and Schuman, 2006). Invertebrate neurons lack the strict polarity of vertebrate neurons, and their axons are both transmissive and receptive surfaces (Martin et al., 2000). LTF induction in *Aplysia* neurons is mediated by serotonin binding to receptors on the presynaptic neuron membrane, thus acting functionally as postsynaptic (Mohr and Richter, 2000).

As *Xenopus* retinal ganglion cell (RGC) growth cones mature in culture, their mRNA population changes, and specific presynaptic protein mRNAs, undetectable in young growth cones, become enriched (Zivraj et al., 2010). Local translation could provide a means for the developing nerve terminal to sense and respond to extrinsic signals, playing an essential role in synaptogenesis, synapse strengthening and elimination, and even relay signals to the cell soma and influence neuronal survival. Indeed, in *X. laevis* neuro-muscular junction neurons, presynaptic protein synthesis is needed for neurotrophin-induced synaptic potentiation of vesicle release (Je et al., 2011; Zhang and Poo, 2002) and studies on rodent neurons appear to confirm these findings. In compartmentalized cultures of mature rat hippocampal neurons, where poly-D -lysine-coated beads were added to induce the formation of functional presynaptic terminals (Lucido et al., 2009), 18S rRNA and β -catenin mRNA were detected within these terminals (Taylor et al., 2013). The β -catenin mRNA localized preferentially to newly formed terminals, was locally translated during terminal formation, and knockdown of it interfered with in synaptic vesicle release. A recent study proposed a role for axonal protein synthesis in regulating vesicle recycling pool size in developing CNS synapses (Hsiao et al., 2014). Another study suggested that presynaptic local translation, and not postsynaptic, is required for a type of long-term depression in rat striatum (Yin et al., 2006). In the fly visual system, taking advantage of the synchronous conversion of R cells' growth cones into synaptic terminals (Chen et al., 2014), global changes in gene expression during presynaptic differentiation were analyzed by tandem tagged ribosome affinity purification of RNA (Zhang et al., 2016). Presynaptic mRNAs showed lengthening of their 3' untranslated regions (UTRs) and enrichment in RBP binding motifs. Several RBPs known to regulate mRNA localization and translation were also dynamically regulated. Post-transcriptional regulation mechanisms could be especially important during this time period, and local translation might play a role.

4.2. Axonal synthesis of secreted and membrane proteins

Connected to the issue of a potential role of local protein synthesis in synapse formation and function is the question which protein are expressed in mature axons. A local pathway for the production of neuropeptides and hormones, or even neurotransmitters, could arguably have special benefits. Indeed, invertebrate examples for the production of secreted proteins or peptides in nerve terminals include the hormone sensorin in *Aplysia* sensory neurons (Hu et al., 2002; Lyles et al., 2006; Schacher et al., 1999), the egg-laying hormone in *Aplysia* peptidergic neurons (Lee et al., 2002), and the caudodorsal hormone in the central nervous system of the mollusc *Lymnae stagnalis* (Dirks et al., 1993). In vertebrates, olfactory marker protein mRNA is found in mature axons of the olfactory receptor neurons in rat (Wensley et al., 1995), and a significant amount of odorant receptors' mRNAs associate with polysomes in nerve terminals of axons of adult mouse bulb (Dubacq et al., 2009). Similarly, posterior hypophysis mammalian nerve terminals possess transcripts encoding vasopressin and oxytocin (Jirikowski et al., 1990; Mohr et al., 1991; Mohr and Richter, 1992; Trembleau et al., 1996), and *vasopressin* mRNA at the presynapse is differentially regulated by dehydration and rehydration (Trembleau et al., 1995). Mature DRG terminals express κ -*opioid receptor* mRNA, are able to translate it, and KCl depolarization can regulate transcript quantities (Bi et al., 2006).

These examples of locally produced secreted or membrane proteins are somewhat puzzling because of the lack of a conventional Golgi apparatus in axons that is normally required for the secretory pathway. Yet, also in developing axons several transcripts encoding membrane and secreted proteins localize to axons including for example Ephrin type-A receptor 2 (EphA2) (Brittis et al., 2002). Despite the absence of a Golgi apparatus, many Golgi and ER markers are present in axons and a yet poorly understood secretory mechanism appears to be available for locally produced proteins (González et al., 2016; Merianda et al., 2009).

4.3. Local protein synthesis and axonal maintenance

Local translation also mediates axon maintenance in both developing and mature axons. In cultured sympathetic neuron axons, myo-inositol monophosphatase 1 (Impa-1) is locally produced in response to NGF stimulation and inhibiting its synthesis resulted in axonal degeneration (Andreassi et al., 2010). This study, along with other axonal transcriptome analysis, identified several mitochondrial proteins (Andreassi et al., 2010; Gumy et al., 2011; Taylor et al., 2009; Zivraj et al., 2010). Nuclear-encoded mitochondrial proteins mRNAs are present in the nerve terminals of squid photoreceptor cells (Gioio et al., 2001), as well as in the axons of sympathetic vertebrate neurons (Aschrafi et al., 2010). Inhibition of axonal translation or axonal mitochondrial import of proteins decreases mitochondrial membrane potential in cultured sympathetic neurons (Hillefors et al., 2007). It also impairs the mitochondria ability to restore ATP levels in these neurons after stimulated depolarization. All this implies a function for local translation in maintaining mitochondrial function and axon viability. This idea has been validated *in vivo* by the finding that lamin B2, a protein commonly thought to be exclusively found at the nuclear membrane, is synthesized in *Xenopus* RGC axons in response to engrailed 1 (Yoon et al., 2012). Axonally derived lamin B2 associates with mitochondria and is necessary for mitochondrial maintenance and axon survival.

4.4. Mature axons and injury response

After the developmental period the quantity of rRNA and mRNA in axons decreases (Bassell et al., 1994; Hengst and Jaffrey, 2007; Kleiman et al., 1994). This decreased protein synthetic capacity has been associated with decreased ability to regrow after axotomy (Gumy et al., 2010). Central nervous system neurons show decreased regenerative potential as compared with PNS axons and this is related to their intrinsic protein synthetic ability (Verma et al., 2005). Indeed, preconditioning an axon *in vivo* with nerve injury, leads to enhanced protein synthesis and regeneration *in vitro* (Verma et al., 2005; Zheng et al., 2001). The signalling cascade induced by nerve injury has been extensively studied in peripheral axons and some of the locally synthesized proteins and signalling cascades have been uncovered (Gumy et al., 2010; Rishal and Fainzilber, 2010). An initial calcium wave triggers a first round of translation of several sensor mRNAs, forming a signalling complex that is retrogradely trafficked and influences transcription (Rishal and Fainzilber, 2014). Importin β , whose classical function is to facilitate nuclear import of nuclear localization signal (NLS)-bearing proteins, is locally translated in the injury site and a core component of the injury signalling complex. Its upregulation leads to the formation of a NLS binding complex that associates with the motor protein dynein and travels retrogradely to the cell body (Hanz et al., 2003). Deleting axonal localization sequence in the importin β 1 transcript in mice causes a subcellular loss of both importin β 1 mRNA and protein in axons, affects the transcriptional response, and delays functional recovery to nerve injury (Perry et al., 2012), confirming its significance *in vivo*. Once again the local synthesis of transcription factors, such as signal transducer and activator of transcription 3 (STAT 3), is a necessary requirement for the retrograde communication with from the periphery to the cell body, in this case leading to the mounting of an injury response (Ben-Yaakov et al., 2012). Boosting protein synthesis might restore the regenerative potential of CNS axons (Park et al., 2008), and importantly, mRNAs and protein synthetic machinery can localize to regenerating mature CNS axons when they are provided with a growth supporting substrate (Kalinski et al., 2015).

Tissue injury induces the release of pro-inflammatory mediators that are critical for the initiation of the repair and regeneration mechanisms, but also induce nociceptive sensitization, a mechanism which might seem maladaptive, but is crucial for organism survival (Price and Dussor, 2014). Inflammatory mediators such as interleukin-6 and NGF are released in response to tissue or nerve injury and sensitize nociceptors (Dina et al., 2008; Pezet and McMahon, 2006), by regulating local protein synthesis (Melemedjian et al., 2010). Increases in the sensory neurons' translational capacity are associated with persistent pain, and several mechanisms involved are similar to those seen in other axonal stimulated conditions, such as mTOR activation (Jiménez-Díaz et al., 2008), and local CREB synthesis and retrograde trafficking (Melemedjian et al., 2014). This nociceptive plasticity affects not only the peripheral pain sensing neurons, but also the central neurons that receive their projections, and this spinal cord central sensitization is also dependent upon local translation (Asante et al., 2009). Molecular mechanisms underlying nociceptive sensitization show remarkable resemblance with those involved in learning and memory processes, and chronic pain disorders are nervous system disorders, differing from acute pain syndromes, with treatment implications (Price and Inyang, 2015). Interfering with local axonal translation is a

promising treatment option (Mao-Ying et al., 2014; Price et al., 2007; Russe et al., 2013), but we are far from understanding the complexity of the pain pathway regulation and the identity of the locally relevant transcripts (Melemedjian and Khoutorsky, 2015).

4.5. Role in neurodevelopmental disorders and neurodegeneration

Several neurodevelopmental disorders such as fragile X mental retardation and autism spectrum disorders seem to have underlying local translation deficits (Bear et al., 2008; Kelleher and Bear, 2008). Fragile X mental retardation protein (FMRP) is a well documented plasticity regulator in dendritic spines (Bassell and Warren, 2008). It is found in growth cones and axons (Antar et al., 2006; Christie et al., 2009; Li et al., 2009), where it regulates the presynaptic proteome (Akins et al., 2012), and its loss affects the formation of presynaptic nerve terminals in organotypic mouse slices (Hanson and Madison, 2007).

It is also not at all surprising that changes in local translation have been proposed to play a role in several neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) (Liu-Yesucevitz et al., 2011). Two related RBPs have been associated with ALS, FUS and TDP-43, which impair axonal trafficking of mRNA granules (Alami et al., 2014). SMA is caused by reduced level of survival motor neuron (SMN) protein. SMN is present in all cell types, and its total deletion is lethal, but somehow motor neurons are more sensitive to its reduction (Fallini et al., 2012). This might be because SMN reduction causes the reduction in the axonal localization of several mRNAs (Rage et al., 2013), and inhibits mTOR activity in axons (Kye et al., 2014).

Perhaps more unexpected was the recent discovery for a role in axonal protein synthesis in response to β -amyloid ($A\beta$) stimulation, indicating a functional role for local translation in the pathogenesis Alzheimer's disease (AD) (Baleriola et al., 2014). In compartmentalized hippocampal cultures, application of oligomeric $A\beta_{1-42}$ specifically to axons elicited the recruitment and axonal translation of many mRNAs, including the transcript for the transcription factor activating transcription factor 4 (ATF4). Locally synthesized ATF4 is retrogradely transported to the cell soma, where it changes nuclear transcription, ultimately leading to cell death. Once more, a locally translated transcription factor mediates axon-soma communication. AD follows a stereotypical pattern of neuronal dysfunction (Braak and Braak, 1991), and several studies hypothesize that there might be transsynaptic spread of the pathology in neural circuits (Harris et al., 2010; Palop and Mucke, 2010). For example, synaptic changes precede neuronal dysfunction (Shankar and Walsh, 2009), and $A\beta$ oligomers interfere with presynaptic vesicle release machinery (Abramov et al., 2009; Nimmrich and Ebert, 2009). The discovery that $A\beta$ oligomers change intra-axonal protein synthesis could indicate that part of the presynaptic alterations might be caused by changes in intra-axonal translation.

The translation of *Atf4* mRNA is controlled by the phosphorylation of the elongation initiation factor 2 α (eIF2 α), which constitutes the pivotal component in the integrated stress response (Donnelly et al., 2013b; Harding et al., 2003). Brain ER stress (De Felice and Lourenco, 2015) and eIF2 α kinases have been implicated in AD (Ohno, 2014). Importantly, activation of the integrated stress response is not limited to AD, and it might be a possible therapeutic target not only in AD, but in other neurodegenerative disorders (Baleriola and

Hengst, 2015). In fact, the first oral treatment to have an effect in preventing neurodegeneration in a prion mouse model targeted one eIF2 α kinase (Moreno et al., 2013).

5. Challenges and future perspectives

Axonal transcriptome studies (Andreassi et al., 2010; Baleriola et al., 2014; Gumy et al., 2011; Minis et al., 2014; Taylor et al., 2009; Zivraj et al., 2010) identified thousands of transcripts, encoding a huge variety of proteins of very different functional classes, in both embryonic and mature, CNS and PNS, uninjured and regenerating axons (Deglincerti and Jaffrey, 2012). Local translation in the axonal compartment is far more complex than previously thought and changes dynamically in response to extracellular and developmental factors. It has been implied in several processes from development to degeneration. Contrary to what was previously thought, mature axons contain mRNAs and are able to rapidly activate their translational capacity in response to stimuli or stress. The elaborate neuronal environment, where nerve terminals, postsynaptic targets and glial cells all interact is one that is not accurately reproducible in culture. There is some evidence that glial cells could contribute with the transfer of translational machinery and even RNA to axons and nerve terminals (Giuditta et al., 2008; Twiss and Fainzilber, 2009). Also, many compartmentalized cultures lack postsynaptic targets, so they are inadequate for the study of the nerve terminal. The study of axonal protein synthesis is further hindered by the low levels of mRNAs in axons, when compared with the somatodendritic compartment. New sensitive tools with single-molecule resolution are needed to detect endogenous levels of expression of biomolecules in axons. These will also enable investigation into translation localized in even smaller sites such as organelle surfaces. An interesting question that remains is the role of local translation at the mature nerve terminal in vertebrates, specifically in presynaptic plasticity and the response to pathological and adaptive stimuli. The synapse being such a dynamic structure, and in light of recent evidence pointing for a possible role of local translation at the nerve terminal, it is likely that, similarly to what happens in dendrites, there are translationally regulated mechanisms at the presynapse. One cannot view the nerve terminal as a translational stagnant compartment, especially in stimulated/pathological conditions. Additionally, the vast diversity of neuronal populations in biological systems warrants more research into how different neuron types react to these stimuli. Our knowledge about local translation in mature axons is still limited and even general principles are not fully comprehended. To understand how the composition of the axonal transcriptome is regulated and how specificity both in mRNA recruitment and local translation is established, remain major research challenges.

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Highlights

- Intra-axonal protein synthesis supports axonal growth and guidance during development
- Retrogradely transported, locally synthesized proteins regulate neuron specification and survival
- Local translation in mature axons is upregulated following nerve injury and neurodegenerative insults
- A possible role for local translation in the presynapse is emerging

Figure.

In developing axons, local protein synthesis of cytoskeletal regulators supports: axons branching in response to growth factors (brown), axonal elongation and attractive turning triggered by attractive guidance cues (green), and growth cone collapse and axonal retraction induced by repulsive guidance cues (magenta). Local growth factor signalling triggers intra-axonal synthesis of transcription factors that are required for cell survival or specification (yellow). In mature neurons, local protein synthesis supports axon maintenance through the local synthesis of mitochondria-associated proteins (brown). Neurodegenerative insults and nerve injury activate local protein synthesis of transcription factors that can cause cell death or initiate a regenerative response, respectively (green, yellow). Local synthesis of in presynaptic terminals is involved in synapse formation but its role in synapse function or maintenance remains unknown (magenta).