REVIEW

# Targeting Axonal Protein Synthesis in Neuroregeneration and Degeneration

Jimena Baleriola · Ulrich Hengst

**Abstract** Localized protein synthesis is a mechanism by which morphologically polarized cells react in a spatially confined and temporally acute manner to changes in their environment. During the development of the nervous system intra-axonal protein synthesis is crucial for the establishment of neuronal connections. In contrast, mature axons have long been considered as translationally inactive but upon nerve injury or under neurodegenerative conditions specific subsets of mRNAs are recruited into axons and locally translated. Intra-axonally synthesized proteins can have pathogenic or restorative and regenerative functions, and thus targeting the axonal translatome might have therapeutic value, for example in the treatment of spinal cord injury or Alzheimer's disease. In the case of Alzheimer's disease the local synthesis of the stress response transcription factor activating transcription factor 4 mediates the long-range retrograde spread of pathology across the brain, and inhibition of local *Atf4* translation downstream of the integrated stress response might interfere with this spread. Several molecular tools and approaches have been developed to target specifically the axonal translatome by either overexposing proteins locally in axons or, conversely, knocking down selectively axonally localized mRNAs. Many questions about axonal translation remain to be answered, especially with regard to the mechanisms establishing specificity but, nevertheless, targeting the axonal translatome is a promising novel avenue to pursue in the development for future therapies for various neurological conditions.

**Key Words** Neuroregeneration · neurodegeneration · Alzheimer's disease · local translation · mRNA · stress signaling

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## Introduction

Neurons are by far the most polarized cells in the human body. Their neurites extend over distances reaching up to 10,000 times the diameter of the neuronal soma, and neurites make up the majority of the volume and cell surface of a neuron. This remarkable morphology challenges the overly simplified view of a cell as a uniform signaling entity and necessitates the investigation of neuronal signal transduction pathways in a compartmented fashion. The main morphological compartments of neurons are the soma, the dendrites, and the axon. While dendrites and soma form a continuous compartment with no clearly defined boundary separating their cytoplasms, the axon is separated from the cell body by the axonal hillock or initial segment, which acts as a diffusion barrier. The existence of this physical separation between axons and the somato-dendritic part of neurons emphasizes the need to investigate signaling events in axons separately from the other compartments. In many instances a cell's reaction to changes in its environment or to cytotoxic insults requires changes of the cellular proteome, and much research has been devoted to the understanding of the transcriptional mechanisms underlying these cellular adjustments. However, equally important are post-transcriptional mechanisms for the regulation of gene expression. mRNA sorting, silencing, transport, translation, and stability are all important mechanisms to adjust the proteome within a subcellular compartment in a temporally acute and spatially confined way [1]. This is especially true in the

case of neurons where mRNA transport into the periphery and signal-dependent translation of these transcripts is extensively utilized to fine tune gene expression in response to changes in the neurons' environment.

## Intra-axonal Protein Synthesis

During the development of the nervous system axons navigate vast distances towards their cognate synaptic targets, guided by extracellular guidance cues that act either as attractants or repellents [2, 3]. At any given moment a multitude of these chemotropic factors influences the growth behavior of developing axons. These signals are integrated and translated into changes of the axonal growth machinery, controlling principally the cytoskeleton but also membrane dynamics, within the growth cones [4]. In order for the extracellular signals to trigger changes in axonal growth behavior in a timely manner axons have long been proposed to rely on local, intra-axonal protein synthesis. In fact, by the end of the nineteenth century it had already been hypothesized that axons might 'depend, as would seem a priori much more likely, for the most part upon autochthonous metabolism' [5]. This theory of intra-axonal protein synthesis remained for a long time a hotly debated issue, even long after protein synthesis in dendrites had been widely accepted (for a historical perspective see [6]). Only at the beginning of this century did work by Holt's group conclusively demonstrate that local protein synthesis is required for the chemotropic responses of axons to attractive and repulsive guidance cues [7]. This work heralded an extremely productive phase of research on local translation in developing axons, and now intra-axonal protein synthesis is recognized to be crucial for growth cone behavior [7–14], axonal pathfinding [15–17], axon maintenance [18], and retrograde signaling in developing axons [19, 20]. However, far less is known about the extent and importance of localized protein synthesis in axons after the developmental period.

In fact, once the period of rapid axonal growth and pathfinding ceases, the levels of axonally localized mRNAs and ribosomes drop so much that mature axons have long been regarded as translationally inactive [21–23]. In contrast to this assumption, local synthesis of β-catenin has been found to regulate presynaptic vesicle dynamics in cultured hippocampal neurons [24], indicating important roles for intra-axonal translation beyond the developmental period. However, to determine definitively whether postdevelopmental neurons are indeed incapable of axonal protein synthesis it is necessary to perform this investigation in vivo as opposed to primary cultures. The latter approach is valuable for the study of developing or regenerating axons but it is unclear how well an in vitro approach can possibly model mature, postdevelopmental axons. Indeed, recent results from carefully designed in vivo studies are beginning to change the

assumption that mature axons are translationally inactive. For example, odorant receptor mRNAs are translated within axons at a high rate in immature axons but, importantly, the transcript coding for the olfactory marker protein, a protein specific to mature axons, is also translated within axons, albeit at a much lower level [25]. Further, a transgene mRNA containing the 3'untranslated region (UTR), that is the regulatory region, of β-actin mRNA localizes to and appears to be translated within axons of peripheral and central nervous system neurons in mice even well after neurodevelopment has been completed [26].

A functional requirement for intra-axonal protein synthesis has been provided in experiments in which the mRNA coding for lamin B2 has been selectively depleted in retinal axons in Xenopus laevis tadpoles [18]. Axons depleted of LB2 mRNA degenerate even after they have reached their targets, suggesting that they rely on intra-axonal synthesis of laminB2 not only during development, but also after maturation. Finally, interference with axonal transport of the nuclear-encoded mitochondrial mRNA of cytochrome C oxidase IV was found to impact on neuronal mitochondrial function leading to altered mouse behavior [27]. Together, these findings strongly suggest that the ability for protein synthesis persists in postdevelopmental axons in vivo. However, the lower number of mRNAs and ribosomes localized to axons, as well as the changed composition of the mature axonal transcriptome [28], indicates that its function and relevance might be distinct from the developmental period. For example, if one of the chief purposes of intra-axonal translation is the rapid and spatially restricted response to changes in the axon's environment, as is suggested by numerous developmental studies, then axons in the mature and inherently more stable nervous system might simply have a much lower need to synthesize proteins locally unless they are challenged. This concept is exemplified in the rapid upregulation of local protein synthesis following nerve injury and in the context of neurodegenerative disorders.

## Regeneration After Nerve Injury

Upon dissection of an axon its distal part undergoes Wallerian degeneration, while the proximal part forms a growth cone-like structure, the nerve bulb. The severed axons initially start to grow and react to attractive and repulsive cues in their environment [29]. During the development of the nervous system, guided axon growth requires intra-axonal mRNA translation, and it is thus not surprising that local protein synthesis is also crucial for axon regeneration. The formation of a new growth cone after axotomy of developing axons in vitro requires both local protein synthesis and degradation [30], and upon injury of mature axons, mRNAs and protein synthesis machinery are rapidly recruited into axons and intra-axonal translation is upregulated or re-activated within these

mature axons [31–34]. Locally synthesized proteins are required for communication from the injured axons to their soma and likely participate in the formation of the growth bulb at the site of injury [35, 36]. Several recent excellent reviews have been published covering the multifaceted role of local translation in injured axons [37–39]; here, we will focus on the question whether manipulation of the local translatome in injured axons might be of therapeutic value. The requirement for protein synthesis and degradation is strikingly similar to the requirements for axon elongation and growth cone turning in response to attractive guidance cues [7], and a comparison of the axonal mRNA pools present in uninjured matured and actively regeneration axons of cortical neurons revealed that axotomy triggers the recruitment of transcripts related to axonal targeting [40]. Importantly, while some of the axonally localized mRNAs have been shown to support axon outgrowth and elongation in response to attractive cues, such as $\beta$-actin [9, 11], Par3 [12], and TC10 [13], the translation of others, including RhoA [8] and cofilin [10], leads to growth cone collapse and axon retraction. Therefore, the axonally localized transcriptome in severed nerves might support the innate regenerative capacity of injured axons but at the same time also mediate the growth preventing effects from the inhibitory molecules in the nerve scar and myelin that ultimately prevent regenerative growth. The presence of both growth-promoting and growth-retarding mRNAs suggests that it might be possible to interfere with the axonal transcriptome or with intra-axonal protein synthesis in order to support regeneration of injured axons. Potentially, targeting the axonally localized transcriptome would have several advantages over more classical forms of gene therapy currently discussed for the treatment of nerve injury, especially following spinal cord trauma. Any gene transfer strategy that relies on DNA-based delivery of genes has to be targeted to the cell bodies of the injured axons. The soma of motor neurons are found interspersed in the upper spinal cord and the motor cortex in the brain, making it essentially impossible to specifically target them without affecting the millions of neurons surrounding them, whose axons have not been injured. In contrast, the trauma site where the injured axons are located is easily accessible. Several experimental strategies have been developed that could be used to enhance or suppress selectively the local expression of specific mRNAs at the site of injury. We will discuss them later in this review.

## Neurodegenerative Disorders

The reactivation of intra-axonal protein synthesis in response to nerve injury has been the first example for a role of local translation in a pathological situation [31]. Additionally, several recent reports support the idea that the axonal transcriptome and proteome are changed in amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). For example, ALS-causing mutations in the RNA-binding protein TDP-43 impairs axonal trafficking of mRNA granules to distal axons [41]. Similarly, reduced levels of survival of motor neuron (SMN) decrease axonal mRNA localization and human SMN1 mutations that alter the amount of functional protein are responsible for most SMA cases [42]. Reduced SMN levels, such as seen in SMA, cause increase expression of microRNA-138, which, in turn, leads to reduced local mammalian target of rapamycin synthesis and activity in axons [43]. For both these examples it has been suggested that impaired local translation of localized mRNAs at the neuromuscular junction contributes to the pathogenesis of ALS, SMA, and related disorders [44]; however, direct proof from in vivo studies is sparse.

Recently, our laboratory uncovered a crucial role of intra-axonal protein synthesis in the pathogenesis of Alzheimer's disease (AD) [45]. AD is the leading cause for age-related dementia and, in the context of an aging society, a pressing public health challenge. AD is a progressive disorder characterized by increasing cognitive decline and memory loss. In order to delay or eventually even stop disease progression it is necessary to understand the natural history of AD, beginning with the earliest pathogenic changes in the brain. A defining hallmark of AD is $\beta$-amyloid (A$\beta$) pathology [46], and, according to the amyloid hypothesis, the less abundant of two major $\beta$-amyloid peptides, A$\beta_{1-42}$, is causative for many neurodegenerative alterations in the AD brain. The size of the neuronal soma pales in comparison with the area covered by axons and dendrites, and consequently, elevated A$\beta_{1-42}$ levels in the central nervous system will most frequently first be encountered by neurites, and pathogenic signaling mechanisms will initially be triggered within axons and dendrites. Indeed, many pathogenic alterations in AD, including tau hyperphosphorylation and synaptic changes, occur first in axons or dendrites, respectively [47, 48]. However, the pathogenic changes elicited by locally sensed A$\beta_{1-42}$ are not restricted to neurites but are propagated retrogradely to the neuronal soma. For example, in an AD mouse model with amyloid plaques restricted to the cortex, subcortical neurons with cortical projections degenerate suggesting that A$\beta_{1-42}$ acting on axons alone is sufficient to trigger the amyloid cascade [49]. This finding is mirrored in observations from the brains of patients with AD, that monoaminergic neurodegeneration in the locus coeruleus occurs in the absence of local A$\beta$ pathology, again suggesting that axonal connections into areas with A$\beta$ pathology are sufficient to induce neurodegeneration [50–52]. Thus, pathogenic changes triggered within the axon in response to exposure to pathological levels of A$\beta_{1-42}$ have been proposed to initiate a pathogenic cascade affecting the entire neuron [53–55]. As neurodevelopment and neurodegeneration share similarity at the molecular level [56], we asked whether intra-axonal protein synthesis was activated

in response to $A\beta_{1-42}$ and functionally relevant for the retrograde transmission of neurodegenerative signals across brain regions. For example, the exposure of isolated axons to soluble oligomeric $A\beta$ might be sufficient to change the composition of the axonally localized transcriptome and to activate intra- axonal protein synthesis in a manner analogous to the changes observed in mature axons after injury. Indeed, specific axonal application $A\beta_{1-42}$ changed the composition of the axonal transcriptome through recruitment of a defined set of mRNAs, including the transcript coding for the transcription factor activating transcription factor 4 (ATF4) [45]. Treatment of axons with $A\beta_{1-42}$ triggered local synthesis of ATF4. The locally synthesized ATF4 is retrogradely transported in a dynein-dependent manner to the neuronal cell bodies, ATF4 levels and ATF4-dependent transcription in the neuronal soma increase, and ATF4-dependent expression of CCAAT-enhancer-binding protein homologous protein (CHOP) is up-regulated leading to cell death. Pathological changes in AD spread through the brain in a nonrandom manner that indicates propagation along connecting fiber tracts [57]. The molecular mechanisms driving the spread of pathology remain largely unknown. Two recent studies propose that (abnormal) tau protein can be transported trans-synaptically and can function prion-like in triggering tau tangle formation [58, 59]. Our findings support a model in which the retrograde transport of locally synthesized ATF4 causes transcriptional changes in the neuronal cell bodies that eventually lead to neurodegeneration and loss of neurons. In fact, we found increased ATF4 transcript and protein within axons in the brain of patients with AD [45], further supporting this model of long-range transmission of a neurodegenerative signal via locally synthesized ATF4. Together, our results establish that intra-axonal protein synthesis is functionally significant in a neurodegenerative disorder and indicatea potential therapeutic strategy to slow down or interrupt the long-range transmission of neurodegeneration across the brain in AD.

Importantly, ATF4 is not the only axonally recruited mRNA found in axons upon treatment with $A\beta$ [45]. When clustered in gene ontology term categories, the mRNAs found in vehicle and $A\beta_{1-42}$-treated axons overlap in many gene ontology terms, but 33 are unique to control axons and 54 to $A\beta_{1-42}$-treated axons. The mRNAs found in these unique clusters specific for $A\beta_{1-42}$-treated axons have functions in response to stimuli, cell death, transcription, and molecule biosynthesis (Table 1).

Embryonic hippocampal neurons were cultured in compartmentalized chambers for 9–10 days, the axons only were specifically treated with $A\beta_{1-42}$ for 24 h, and the composition of the axonal transcriptomes were determined by RNA-sequencing [45].

From this analysis it is obvious that exposure of axons to $A\beta_{1-42}$ does not merely lead to a reactivation or upregulation of the translational capacity in these post-developmental axons, but rather it causes a qualitative change in the composition of locally translated mRNAs. This is further exemplified in a comparison between the transcriptomes found in injured and in $A\beta_{1-42}$-treated axons [40, 45]. Nerve injury caused the recruitment of a greater number of mRNAs compared with treatment with $A\beta_{1-42}$ and, furthermore, the composition of the localized transcriptomes was distinct with very few mRNAs found in both datasets (Baleriola and Hengst, unpublished data). Thus, the transcriptome analysis established that exposure of axons to $A\beta_{1-42}$ triggers the recruitment of a specific cohort of mRNAs that is unique to the exposure of distal axons to oligomeric $A\beta_{1-42}$. For example, we found that $A\beta_{1-42}$-treated axons contained many mRNAs coding for proteins with known or proposed functions in AD or neurodegeneration, including 4 out of the current list of 20 AD susceptibility loci [60]: amyloid precursor protein (APP) [61], apolipoprotein E (ApoE) [62], clusterin (Clu) [63, 64], and fermitin family homolog 2 (FERMT2) [60]. The axonal localization of *App*, *Clu*, and *Fermt2* mRNAs is increased in response to $A\beta_{1-42}$. APP is the precursor for $A\beta_{1-42}$, and ApoE and Clu are involved in $A\beta_{1-42}$ metabolism [65], while FERMT2 has been described to be involved in tau pathology [66]. The fact that we find the axonal localization of these transcripts changed in response to $A\beta_{1-42}$ exposure, uncovers the existence of feedback signaling mechanisms in which oligomeric $A\beta_{1-42}$ post-transcriptionally controls the expression of several genes that, in turn, influence $A\beta_{1-42}$ abundance. Importantly, our findings point to the importance of analyzing the axonal or dendritic transcriptome separately from global transcriptional changes. Changes in localized mRNAs can evidently provide invaluable insight into pathological changes induced by neurodegenerative stimuli that, when focusing only on global modifications in gene expression, might be overlooked.

Intra-axonal translation might be part of the pathogenic pathways elicited by $A\beta_{1-42}$ that ultimately lead to neurodegeneration, as is, for example, the case for ATF4. However, conceivably, the protein product of other mRNAs, among them ApoE and Clu, might instead be part of the neuronal response to $A\beta_{1-42}$ and their local synthesis might serve to neutralize the neurotoxic or neurodegenerative effects of $A\beta_{1-42}$. Thus, in order to target intra-axonal protein synthesis as a therapeutic approach in AD, it is necessary to distinguish pathogenic from restorative mRNA translation events and to be able to target the former specifically. It is still largely unclear how specificity in local translation is being established, that is which cis- and trans-acting factors determine axonal recruitment, and which determine the translational state of axonally localized mRNAs. However, owing to its special translational regulation the discovery of ATF4 as a long-range, axonally-derived neurodegenerative signal provides unexpected insight in the molecular mechanisms that control the local translation of this and potentially other

**Table 1** Top ten functional gene ontology (GO) terms for mRNAs found in vehicle and Aβ$_{1-42}$-treated axons

| Vehicle-treated axons | Aβ1-42 |
| --- | --- |
| Intracellular signaling cascade (GO:0007242) | Response to organic substance (GO:0010033) |
| Phosphate metabolic process (GO:0006796) | Positive regulation of macromolecule metabolism (GO: 0010604) |
| Phosphorus metabolism (GO:0006793) | Regulation of cell death (GO:0010941) |
| Phosphorylation (GO:0016310) | Regulation of programmed cell death (GO:0043067) |
| Protein amino acid phosphorylation (GO:0006468) | Regulation of apoptosis (GO:0042981) |
| Cell projection organization (GO:0030030) | Regulation of transcription (GO:0045449) |
| Cellular component morphogenesis (GO:0032989) | Response to endogenous stimulus (GO:009719) |
| Cell morphogenesis (GO:0000902) | Intracellular signaling cascade (GO:0007242) |
| Neuron differentiation (GO:0030182) | Positive regulation of cellular biosynthesis process (GO: 0031328) |
| Cell motion (GO:0006928) | Positive regulation of biosynthetic process (GO:0009891) |

mRNAs and outlines a possible strategy to interfere with their translation.

## Stress Signaling in Axons

Cells have developed several mechanisms to react to stress elicited by extrinsic cues or by disturbances of protein synthesis and secretion. A group of these mechanisms, collectively referred to as the integrated stress response (ISR), share as a common, pivotal element the phosphorylation of the elongation initiation factor 2α (eIF2α) [67, 68]. The translation of *Atf4* mRNA is controlled by eIF2α phosphorylation, and it is the central component of the ISR and the related unfolded protein response (UPR). Activation of eIF2α by phosphorylation prevents the formation of the preinitiation complex but triggers the translation of a class of transcripts whose translation is regulated through the presence of inhibitory upstream open reading frames in their 5'UTR [69]. eIF2α phosphorylation shifts the cell's translation to genes with roles in stress responses thereby reducing the pressure on protein synthesis, folding, and secretion, and conserving limited resources, such as amino acids. Mammals have four different eIF2α kinases that are activated in response to distinct stress situations [68]: protein kinase doubled-stranded RNA-dependent (PKR) is activated in response to endoplasmic reticulum (ER) stress, viral infections, cytokines, and growth factors; PKR-like ER kinase (PERK) is mainly activated in response to accumulation of misfolded proteins in the ER; general control nonderepressible-2 (GCN2) is primarily activated in response to amino acid deprivation; and heme-regulated inhibitor (HRI) is a sensor in erythrocytes for heme and iron levels. The activation of any of the four kinases results in eIF2α phosphorylation, but whether the effect is ultimately pro- or antiapoptotic is heavily dependent on the nature, duration,

and level of the stress signal. A growing body of evidence indicates a functional involvement of several eIF2α kinases in the development of neurodegenerative disorders, including AD [70]. Arguably the best studied eIF2α kinase in relation to neurodegeneration is the ER stress sensor PERK, which triggers the UPR. The ER is a single cellular organelle required for many crucial cellular functions, including lipid synthesis, Ca$^{2+}$ homeostasis, and synthesis and post-translation modification of membrane-bound and secreted proteins. Depending on the cell type and the subcellular localization ER morphology is very different but 3 main classes can be defined: the nuclear envelope, the sheet-like polyribosome-bound rough ER predominant in cell bodies, and the smooth ER. The ER in axons is formed by a network of longitudinally running, narrow tubules of smooth ER (tubular ER) [71, 72], which is likely continuous with the somatodendritic ER [73]. A variety of insults and perturbations of normal cellular function, such as the accumulation of un- or misfolded proteins result in ER stress. A series of adaptive responses, known collectively as the UPR, are triggered in response to ER stress [74], which, depending on the duration and level of the stress, can either restore protein homeostasis or result in apoptosis [75]. In mammalian cells the UPR is controlled through 3 major ER stress sensors: PERK, inositol-requiring enzyme 1α/β, and ATF6 [76]. The role of the UPR in the nervous system in physiological or neurodegenerative conditions remains only partially understood and somewhat controversial (for a discussion see [77]), but recent evidence suggests that ER stress is a common neuronal response to injury or neurodegenerative stimuli and that the UPR is a general mechanism in neurodegeneration. Examples for neurodegenerative diseases for which a UPR contribution has been proposed include Parkinson's disease [78], Huntington's disease [79], and AD [80]. More specifically, a role of axonal ER stress or UPR has been is suggested

by the finding of elevated ER stress markers within motorneuron axons in a mouse model for ALS [81], and in response to axotomy [82] or ischemic injury [83]. Despite this evidence it is not known whether the UPR or other eIF2α kinases are activated within axons upon exposure to Aβ$_{1-42}$ or whether their activation necessarily leads to degeneration of the neuronal cell bodies. Our finding that axonal exposure to Aβ$_{1-42}$ triggers local *Atf4* translation indicates that either the ISR or the UPR is triggered locally within axons [45], and that the local activation of these stress responses sets signaling events in motion that ultimately lead to the retrograde degeneration of the neurons. Thus, targeting the intra-axonal ISR or UPR might present a novel therapeutic approach to prevent or slow down disease progression in AD by inhibiting the local synthesis of ATF4. For example, recently small molecule inhibitors of PERK or eIF2α have been described that could potentially be used to uncouple axonal stress responses from retrograde neurodegenerative signals [84, 85], thereby preventing the spread of AD pathology. By specifically targeting the UPR or eIF2α, these small molecule would not change the intra-axonal synthesis of potentially restorative proteins but instead act fairly selectively to suppress the translation of *Atf4* and thereby prevent the retrograde spread of pathology without interfering with local, potentially prosurvival translation events.

## Tools

Most methods to overexpress proteins in cells rely on the transfer of DNA into the cells, its transcription into mRNA within the nucleus followed by translation of the mRNAs into proteins. In morphologically highly polarized cells such neurons, this approach has several drawbacks, especially if the overexpressed protein is needed only in a specific subcellular compartment, such as an injured axon. An overexpressed protein will normally be present throughout the neuronal structure without any preference for a specific compartment. Thus, to reach functionally relevant levels of an overexpressed protein within axons, it is likely that the protein will be present at comparable levels in dendrites and the cell body with potentially unwanted side effects in those compartments. Indeed, our own findings suggest that axonally and globally (or dendritically) synthesized ATF4 have opposite effects on neurons in vivo [45], thus emphasizing the need to use overexpression or knockdown techniques that selectively modify the axonal transcriptome or translatome only. Further, in the context of traumatic spinal cord injury damage the location of the injured axons is easy to locate and access while the cell bodies from which the injured axons project are diffusely localized throughout the upper spinal cord and the motor cortex. Any gene therapy relying on the transfer of DNA has to target the neuronal cell bodies and, as a consequence, it would be necessary to transfect a great amount of neurons, many of which are unaffected by the injury to ensure the affected neurons are receiving the message. It is thus extremely difficult to establish spatial specificity, both on the organ and the subcellular levels. The development of a modified Sindbis virus expression system is a potential solution for both of these problems. The genome of Sindbis virus is a single m$^7$G-capped and polyadenylated RNA molecule, which is processed by the host cell translational machinery just like endogenous mRNAs [86]. The coat protein of Sindbis virus has been modified to establish neurotropism [87], and point mutations in its *nsP2* gene have been engineered to reduce neurotoxicity [87, 88]. In order to allow for direct expression of proteins off the viral genome, the encephalomyocarditis virus internal ribosome entry site (IRES) was introduced into the Sindbis virus expression plasmid SinRep(nsP2S$^{726}$) [87, 89], resulting in efficient transfection and protein expression in transfected axons in culture and in animals [8, 12, 13, 19, 90]. With the Sindbis–IRES approach it is possible to transact directly axons and to overexposes proteins locally, without any need for somatic contribution. By overexpressing proteins that positively regulate axon growth it is thus possible to enhance axonal elongation rates even on normally inhibitory substrates [90]. The modified Sindbis–IRES expression system is an easy way to change growth cone behavior by overexpressing growth-promoting proteins or dominant-negative proteins that can interfere with endogenous signaling pathways [12, 90]. This method to utilize axonal protein synthesis is complemented by the possibility of using RNA interference (RNAi) in axons to deplete selectively axons of specific mRNAs without changing the mRNA's abundance in other parts of the neurons. The RNAi is present and functional in developing axons of cultured dorsal root ganglion and superior cervical neurons [91, 92], and in sciatic nerves [93]. More recently, we have found that injection of small interfering RNA (siRNA) into in the brain of adult mice can be used to deplete axons of mRNA [45]. Thus, independent from the developmental state of an organism, RNAi appears to be a feasible method to knockdown axonally localized mRNAs. Importantly, the siRNA is not quantitatively retrogradely transported to the neuronal soma, and the knockdown effect is restricted to the transfected axons at least in vitro and likely in vivo [91]. The use of siRNA in axons has several advantages: far fewer mRNAs are found in axons than in whole cells. The lower abundance of the localized mRNAs makes siRNA-mediated knockdown very efficient and quick, often within 24 h [13, 45]. Additionally, as there are fewer mRNA species found in axons, the danger of off-target effects is proportionally reduced.

Essentially, all steps of an mRNA's life are controlled by cis- and trans-acting factors. Cis-acting factors are sequence or structural elements within the mRNA that serve as binding sites for other RNAs or proteins, the trans-acting factors, that

together regulate the transport, stability, and translation of the mRNA. All of these processes regulate the localization, abundance, and translation of mRNAs in axons, and it thus an attractive possibility to interfere with these processes in order to influence the axonal transcriptome. This approach is somewhat hampered by the relative scarcity of information about the cis-acting factors; very few sequence elements have been described. However, most if not all of these elements are localized to the 3'UTRs of mammalian mRNAs. Thus, in theory, it might be sufficient to overexpress the entire the 3'UTR of an mRNA of interest in order to interfere with its localization or translation in axons. Indeed, such an approach has been followed successfully in genetically modified mice [27].

## Perspective/Challenges

Currently, targeting of axonal protein synthesis is far from any clinical application. Studies on axonal regeneration and AD-related neurodegeneration have demonstrated in model systems that it is possible to influence axonal behavior or to inhibit the spread of pathology across the brain by changing the axonal translatome. However, currently, most studies concentrate on specific axonally localized mRNAs and it remains an open question what the role the other localized transcripts might be. Presumably, in an in vivo situation many localized transcripts are translated into proteins at any given time and the neuronal or axonal response is the result of the coordinated effect of the temporally and spatially restricted increase in their abundance. In fact, one of the advantages of local translation might be the coordinated activation of seemingly unconnected signaling pathways [13]. Thus, in order to target therapeutically axonal protein synthesis more knowledge is required about the axonal translatome, that is the cohort of actively translated mRNAs in axons under regenerative or degenerative conditions. For example, what is the cohort of mRNAs whose local translation supports axonal regrowth and which mRNAs make up the growth-preventing component in response to extracellular inhibitory cues? Next-generation sequencing techniques such as ribosome profiling might be adapted to determine translational activation of localized transcripts in response to injury or degenerative stimuli. Additionally, the molecular mechanisms establishing specificity remain largely unknown. For example, we do not know whether translation-activating signals act on the individual mRNA species or on ribonucleoprotein (RNP) complexes in which transcripts coding for proteins with complementary function are co-packaged. Association of RNA-binding proteins with discrete sets of mRNAs coding for functionally related proteins and co-existence of several mRNA species within shared RNP complexes has been unambiguously demonstrated in yeast [94, 95], and is proposed to exist in eukaryotic cells [96–98]. If these putative post-transcriptional RNA operons exist in axons it will be an interesting idea to target the formation or the axonal transport of the multifunctional RNP in order to interfere with intra-axonal protein synthesis of a cohort of functionally related proteins.

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