Trends in Neurosciences

CellPress OPEN ACCESS

Review

Proteostatic regulation in neuronal compartments

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Neurons continuously adapt to external cues and challenges, including stimulation, plasticity-inducing signals and aging. These adaptations are critical for neuronal physiology and extended survival. Proteostasis is the process by which cells adjust their protein content to achieve the specific protein repertoire necessary for cellular function. Due to their complex morphology and polarized nature, neurons possess unique proteostatic requirements. Proteostatic control in axons and dendrites must be implemented through regulation of protein synthesis and degradation in a decentralized fashion, but at the same time, it requires integration, at least in part, in the soma. Here, we discuss current understanding of neuronal proteostasis, as well as open questions and future directions requiring further exploration.

Highlights

Dynamic local regulation of protein synthesis and degradation allows neuronal synapses to modify their proteome autonomously during plasticity.

While our understanding of how mRNAs are localized to different neuronal compartments has rapidly advanced, a comprehensive and mechanistic understanding of how synaptic activity triggers translation of certain transcripts over others is still lacking.

The UPS degrades protein substrates selectively and specifically, but the local role of the UPS in neuronal compartments remains underexplored. Recent efforts in chemical biology and proteomics have established new promising tools and techniques to bridge this knowledge gap.

PTMs are a rich regulatory resource in cells. Future efforts should focus on broadening our knowledge of local regulation of PTMs in response to neuronal activity and how specific PTMs contribute to neuronal proteostasis.

The challenge of regulating a distant proteome

Most catalyzed chemical reactions inside cells depend on protein levels, and fine-tuning protein concentrations is key to ensure proper cellular function. Because throughout the cellular lifespan proteins accumulate damage, become dysfunctional and need to be replaced continually, protein synthesis and protein turnover are central to cellular physiology and function. The dynamic regulation of a balanced and functional proteome (i.e., proteostasis) concerns all proteins whose levels need to be adjusted in space and time in response to intracellular and extracellular cues. Thus, the specific parameters of cellular proteostasis vary across cell types and states. However, cellular proteostasis invariably relies on the precise control of protein synthesis, folding and conformational maintenance, post-translational modifications (PTMs), degradation, and secretion [1]. Precise control of these parameters is already challenging in cells that have little or no polarity, but becomes a particularly impressive feat for highly polarized cells, such as neurons.

Neurons stand out from all other cell types in their unique morphology and high degree of compartmentalization; characteristics that are central to neuronal computation. Often, extrinsic signals are spatially localized so that only a confined portion of the neuron receives a certain signal, with the neuronal portion being, for instance, a cluster of dendrites (influenced by a neuromodulator), a single dendritic branch, a synaptic neighborhood, or even an individual synapse. How these local signals are transmitted to the somata remains largely unknown. Work over the last two decades has shown that neurons have the capacity to tune their proteome locally through regulation of local protein synthesis, degradation, and PTMs to regulate multiple aspects of dendritic and axonal biology [2]. Nevertheless, to date, we still lack a comprehensive understanding of how different cellular degradation pathways interact with the protein synthesis machinery to shape and maintain the local proteome.

In this review, we discuss current understanding of neuronal local proteostatic regulation, and key knowledge gaps in the field. We also comment on present technological limitations and promising recent advances to address some of the outstanding questions.

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What roles do mRNA transport and local translation play in synaptic proteostasis?

Studies in primary cultured neurons and mathematical modeling have estimated that ~0.7% of the synaptic protein content of a neuron is turned over every hour at glutamatergic postsynaptic densities [3]. Although this figure is likely to change depending on parameters such as neuron type, number of synapses, and activity, it suggests that maintaining the protein content of synapses places a considerable demand on the cellular protein synthesis machinery. Recent proteomic studies have shown that, at a population level, protein turnover rates change depending on the activity state of neurons [4]. At the level of an individual neuron, this likely translates into subsets of synapses differentially regulating protein turnover rates according to their activity state [5]. Alterations in protein turnover are accomplished by changes in the activity of protein degradation and protein synthesis machinery in the soma and, importantly, in neuronal processes and near synapses. The first data suggesting the capacity of neuronal processes to synthesize proteins came from early electron microscopy (EM) studies, showing polyribosomes in dendritic spines and in their near vicinity [6]. These experiments, however, lacked the ability to demonstrate the translational activity of these distally located ribosomes. Later, work on the CA1 hippocampal neuropil and retinal ganglion cell axons showed that the mRNA population in axons and dendrites is not limited to a small number of mRNAs but rather is a large and diverse group, containing up to ~5000 different mRNA species [7–11]. Nevertheless, only recently, using a combination of immunogold-labelling EM and expansion microscopy, it has been possible to demonstrate that mRNA translation is ongoing during basal synaptic states and is differentially regulated in pre- and post-synaptic compartments depending on the form of plasticity elicited [12,13] (Figure 1).

While these findings support the importance of local protein synthesis in shaping the local proteome with spatial and temporal precision, they also raise a number of questions related to the interplay between the complement of mRNAs in neuronal processes and the local translation machinery. So far, the axonal and dendritic transcriptomes have been investigated primarily by bulk-sequencing approaches on microdissected tissue, which fail to capture cell-to-cell and process-to-process heterogeneity. Recently, the combination of laser capture microdissection and droplet-based sequencing on dendrites and somata has revealed interesting differences and similarities between the dendritic and somatic transcriptomes and between excitatory and inhibitory neurons [14]. Nevertheless, laser capture microdissection is not precise enough to probe the spatial distribution of a large population of mRNAs relative to dendritic spines and synaptic boutons. In this regard, microscopy based approaches such as seqFISH+ [15] and ExSeq [16] hold promise for furthering our understanding of the subcellular distribution of mRNAs in neurons on a transcriptome-wide scale under basal conditions and during plasticity.

A more complete understanding of how mRNAs map across neurons needs to be complemented with information on mRNA transport and release dynamics under baseline conditions and following changes in activity that promote plasticity. The 3′ and 5′untranslated regions (UTRs) contain elements important for the binding, transport, localization, and regulated translation of mRNAs [17]. Pointing to the importance of translational regulation in neurons, the 3′ UTRs of neuronal mRNAs, particularly those localized to axons and dendrites, are significantly longer than those of other cell types [18]. An important tier of regulation likely exists at the level of how mRNAs are packaged and combined together before being trafficked to distal locations [17]. Work in yeast has shown that mRNA transport granules can comprise multiple functionally related RNAs in, what effectively represent, instruction packages for coordinated metabolic responses [19]. Biochemical analysis of neuronal RNA granules has led to the identification of hundreds of granule-associated transcripts [20], and both multiplexed [21] and multicopy [22] targeting of different mRNAs in neuronal dendrites have been reported. Other reports have suggested that RNA transport granules in neurons contain single mRNAs [23]. A potential explanation for this discordance may be that these

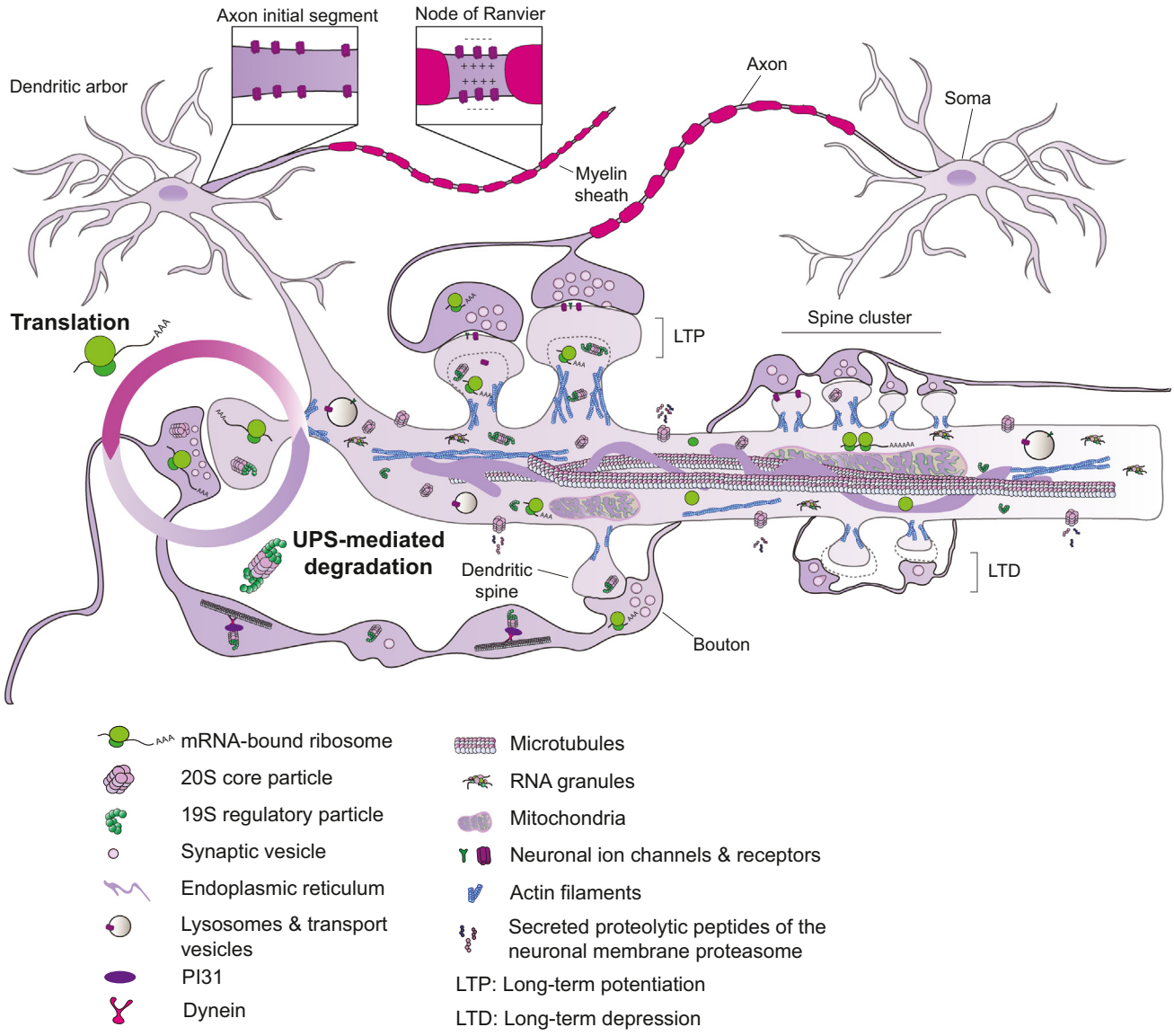

Figure 1. A decentralized proteostatic system maintains synaptic function and fuels neuronal plasticity. The schematic illustrates a distal dendrite harboring spines with juxtaposed axonal boutons. The presynaptic terminal is characterized by the presence of neurotransmitter vesicles, while the postsynaptic spine hosts transmembrane receptors that are activated upon neurotransmitter release – both compartments contain ribosomes and ubiquitin–proteasome system (UPS) components important for local proteostatic regulation. In both axons and dendrites, the translation machinery is supported by a network of actin, microtubules, and motor proteins that transport and deliver mRNAs and proteins to the distal parts of the cell. In axons, the proteasome chaperone PI31 serves as an adaptor for dynein-dependent transport of proteasomes. Mitochondria are found in both axons and dendrites, and represent an important local energy source under basal conditions and in the context of synaptic plasticity. Different types of plasticity such as long-term potentiation (LTP) and long-term depression (LTD) as well as synaptic upscaling and downscaling depend upon the coordinated action of the protein synthesis machinery and the UPS. The neuronal membrane proteasome localizes to the plasma membrane and is thought to degrade ribosome-associated nascent proteins to produce extracellular peptides important for activity-induced calcium signaling. Although a lot is known about how translation and UPS-mediated protein degradation are regulated, we are missing a comprehensive picture of the cell-biological and biochemical mechanisms that are implemented locally to maintain and modify on-demand the protein composition of synapses.

studies were performed on a small cohort of mRNAs. The degree of single versus multiplex mRNA packaging within single transport granules remains to be investigated on an transcriptome-wide scale. In addition to mRNAs, RNA granules contain ribosomes along with translational repressors,

and their integrity can be modulated in response to neuronal depolarization [24]. Studies of long-term potentiation (LTP) and long-term depression have shown that, following plasticity, RNAs are rapidly released from RNA granules, accumulate in the vicinity of dendritic spines, and undergo 3′ UTR cleavage, polyadenylation and on-site translation [22,25,26] (Figure 1). Conversely, local translational repression can be achieved through the release and maturation of pre-miRNAs following N-methyl-D-aspartate (NMDA) receptor stimulation [27]. The combination of these mechanisms allows for the decoding of spatially complex plasticity patterns into specific translational profiles that precisely remodel the synaptic proteome.

At first glance, the abundance of RNA transcripts in neuronal processes might seem to contrast with the low abundance of polyribosomes in axons and dendrites [28]. The notion that neuronal processes, and in particular axons, are depleted of ribosomes historically has come from histological analyses by Nissl staining and EM [29]. However, recent work has reaffirmed what was inferred from the robust translational capacity and shown that ribosomes are found ubiquitously in vertebrate pre- and post-synaptic termini [12,13]. A potential reason for this apparent discrepancy is that, while polyribosomes (recognized as 3+ ribosomes) can be detected with confidence by EM, monosomes cannot be unambiguously distinguished from other electron-dense cytoplasmic particles. Resolving these apparently contrasting observations, it was recently shown that monosomes are predominant in neuronal processes and are actively translating [30] (Figure 1). Historically, monosome translation has been considered negligible in mammalian cells (see [31] for a study in yeast) but in neurons it appears to be a key process. The advantage of monosome over polysome translation in neurons remains unclear, it could represent an adaptation to the small compartment size or a means to diversify the protein products with a limited ribosome population; alternatively, it could be adapted to translate specific mRNAs present in dendrites.

Another important aspect of mRNA translation concerns the ribosomes themselves – the protein and RNA composition of the ribosome complex and their PTMs. It is still not fully understood how heterogeneous individual ribosomes in neuronal processes are in terms of their composition. It is well established that the 40S and 60S ribosomal subunits form and mature in the nucleolus and nucleoplasm prior to nuclear export and cytoplasmic assembly into the 80S ribosome. However, several sequencing studies have detected a large number of mRNAs coding for ribosomal proteins (RPs) in neuronal processes [10,13,18], suggesting local translation. Furthermore, in cultured neurons the RPs half-lives are variable, raging from 3 to 9 days [4], thus suggesting that their degree of incorporation into fully assembled ribosomes may vary. This is consistent with the extra ribosomal function of certain RPs [32], but it also raises the possibility that ribosome composition in neuronal compartments may be more heterogeneous than previously thought. Related to this, recent work suggests that RP mRNAs are translated in dendrites and axons and has identified a population of nascent ribosomal proteins that dynamically exchange with mature, preassembled ribosomes [33]. Another study has showed that axonally translated ribosomal subunits are incorporated into axonal ribosomes in a nucleolus-independent fashion and that interfering with this process reduces branching of *Xenopus* retinal ganglion cell axons [34]. Arguments against the view of a ribosome with invariant composition are also based on the observation that ribosomopathies caused by mutations in different RPs lead to specific phenotypes across different tissues [35]. These observations have led to the idea of specialized ribosomes that, by incorporating different RPs, may display distinct properties such as transcript preference and translation rate [36]. However, direct evidence in support of the specialized ribosome hypothesis, in particular due to heterogeneity in RP composition, remains limited [35]. Its conclusive demonstration will likely require purification of ribosomes with different compositions followed by assays showing different properties or different populations of mRNAs translated. Future

efforts in this context should focus on addressing three main questions. First, mechanistically, how are these ribosomes remodeled outside the nucleus? For example, are established ribosome biogenesis factors locally recruited to remodel and regulate ribosomes? Second, how prevalent and diverse are locally remodeled ribosomes? Is their assembly elicited in response to changes in neuronal activity? Third, is there a specific translational pattern associated with different ribosome types?

How is protein degradation regulated in neuronal processes?

There is a well-established and increasingly growing literature pointing to local protein synthesis as a main contributor to the synaptic proteome, along with protein transport from somata. By contrast, what happens to proteins after they have been synthesized or localized to their ultimate subcellular compartment remains poorly understood. Are these proteins transported back to the soma to be degraded? Are they locally degraded? Or, are they secreted? The general consensus is that most cytosolic and nuclear proteins, short-lived and misfolded proteins are degraded by the proteasome, while long-lived and membrane proteins are commonly degraded by the lysosomal pathway [37]. The proteasome, a multiprotein macromolecular machine, degrades substrates that are usually tagged by ubiquitin chains; collectively this system is referred to as the ubiquitin–proteasome system (UPS). For an in depth review on proteasome biology and its function in neurons, see [38].

The proteasome plays a key role in the maintenance of neuronal function [39,40] and should be considered as an equal partner to protein synthesis in sculpting the neuronal proteome. Pointing to the importance of the UPS in many aspects of neuronal physiology, UPS activity is involved in the growth of dendritic spines and synapse formation on isolated axons [41]. It has been proposed that accumulation of K11 and K48 polyubiquitinated proteins following local proteasome inhibition may serve as a trigger for the assembly of pre-synaptic clusters [42]. Furthermore, the UPS was shown to regulate the levels of pre-synaptic vesicle components [43,44], post-synaptic responses [41,45–47] and synaptic plasticity [48]. One of the first reports describing the role of dendritic protein degradation in plasticity showed NMDA-receptor-dependent translocation of proteasomes from dendritic shafts to spines in response to KCl-induced depolarization [43] (Figure 1). A follow-up study showed that the activity-dependent proteasome translocation into spines is driven by interaction of the proteasome with CaMKIIα [49]. Furthermore, CaMKIIα redistribution into post-synaptic termini was shown to be required for activity-dependent degradation of ubiquitylated proteins at post-synaptic sites. Underscoring the importance of the spatial dimension for synaptic protein turnover, one possible explanation for this surprising translocation mechanism might be that it keeps proteasomes away from synaptic substrates under basal conditions and then rapidly recruits them following changes induced by neuronal activity. Because ubiquitination is necessary for substrate degradation by the 26S proteasome, its translocation into spines might represent an additional layer of regulation specific to this cell type.

The findings discussed above indicate a role for local UPS activity in synapse biology and physiology. Nevertheless, to date we are missing a full understanding of how ubiquitin activating and conjugating enzymes, ligases, deubiquitinating enzymes (DUBs), and the proteasome coordinate to regulate synaptic proteins under basal conditions and upon changes elicited by activity. Recent evidence indicates that proteasome abundance and assembly can be adapted to meet cellular challenges [50]. This mechanism and its high degree of evolutionary conservation point to the fact that the proteasome itself is an important regulatory hub of the UPS. The factors involved in proteasome biogenesis are well characterized from yeast to mammals [51,52], but in neurons it remains unclear where in the cell and in response to what cues assembly of 20S, 19S, and 26S particles occurs. In addition, we do not know the extent of unincorporated subunits and assembly

intermediates in neuronal compartments, the contribution of different regulatory particles (i.e., 11S and Blm10/PA200) to the degradation by the 20S core particle, or the relative contribution of the uncapped 20S complex to overall degradation in neurons. The 20S proteasome is known to degrade oxidatively damaged and disordered proteins [53,54], including important neuronal proteins such as c-Fos [55], tau [56] and α-synuclein [57]. Because degradation by the uncapped 20S proteasome is believed to occur in an ubiquitin-independent manner, it will be essential to define how target recognition by the 20S occurs *in vivo*. Recently, it was shown that in neurons up to 40% of the cellular 20S proteasomes exist in a membrane-associated form, breaking down intracellular proteins into peptides that are released directly into the extracellular space and may regulate neuronal activity (Figure 1) [58,59]. At present it remains unclear how the neuronal membrane proteasome is able to interact with membranes, whether it is present as a peripheral membrane protein closely interacting with a channel able to release peptides into the extracellular space or whether it is inserted into the membrane during its biogenesis process. In addition to the 19S cap, the 20S core particle can interact with other regulatory complexes such as the 11S regulatory particle (PA28αβ, PA28γ) [60], and Blm10/PA200 [61]. These alternative caps function in an ATP- and ubiquitin-independent manner, and tend to be recruited under conditions of stress [38]. However, their role in neuronal physiology and potential involvement in neuroprotection remain to be examined in detail. Not only the activity, but also the intracellular sorting of proteasomes can be influenced by protein–protein interactions. A prime example of this is Pl31, which was initially suggested to act as an inhibitor of 26S proteasome assembly, and was recently shown to also act as an adaptor for microtubule-based transport of proteasomes in axons (Figure 1) [62,63]. These findings underscore how dynamic the proteasome composition is and how different interactors can achieve different outcomes. How the complement of proteasome interactors is modulated during development, conditions of stress and cell state changes are exciting research directions that remain largely unexplored.

One of the main hurdles in studying UPS function is the rapid turnover rates of proteins degraded by the proteasome, which makes their identification very difficult by mass spectrometry (MS) approaches, especially in the case of low-abundance proteins [64]. In addition, the spatial dimension (which compartment) must also be considered in the case of morphologically complex cells such as neurons. Lastly, to disentangle the interplay between protein synthesis and degradation, generally, pharmacological manipulation of either pathway is required, hindering the interpretation of the results [65]. One way to overcome this problem is to use metabolic labeling of proteins by radioactivity, by introduction of artificial amino acids like ANL and AHA, or nonradioactive isotopes for stable isotope labeling by amino acids in cell culture (SILAC) analyses [66,67]. These approaches allow one to monitor the global behavior of proteins, or study specific candidate factors. Nevertheless, because of the amount of starting material required, implementing these techniques in small structures, such as dendritic spines, for unbiased bulk protein identification remains challenging. Recently, using dynamic triple SILAC it was possible to dissect the changes in protein synthesis and degradation rates following bicuculline-induced synaptic downscaling and tetrodotoxin-induced synaptic upscaling [68]. This study found a set of plasticity proteins common to both manipulations, and a set of distinct polarity proteins, unique to the specific manipulation. The study also found proteins for which abundance did not change but turnover did, and it is likely that this type of regulation also plays an important role in synaptic activity. Thus, dynamic SILAC allows the study of protein synthesis and degradation simultaneously within the same preparation. This is particularly relevant in view of the emerging idea that protein biosynthesis and degradation pathways are tightly coupled and coordinately regulated, so that perturbation of one elicits feedback mechanisms on the other [64]. For example, it was recently shown that in neurons, proteasomal inhibition leads to rapid upregulation in the levels of the protein kinase heme-regulated inhibitor (HRI), which phosphorylates eukaryotic initiation factor 2α, thereby inhibiting translation and restoring protein homeostasis [69].

Similarly, a high degree of integration is likely present across all cellular anabolic and catabolic pathways so that, for example, proteasome inhibition may impact autophagy, amino acid synthesis and many other metabolic pathways. How such integration is achieved at synapses is an exciting research avenue that remains largely unexplored.

How to map the distribution and activity of UPS components across neuronal compartments?

Recent developments in proximity-based labeling can aid in identifying transient interactions between UPS components and substrate proteins [70]. Such approaches, however, often rely on transgene overexpression, which, by increasing the levels of the bait above physiological expression levels, can generate false positives. Furthermore, it is important to consider that exogenous complex subunits may display varying rates of incorporation into active complexes, which is the case for the proteasome [71], and this will likely affect the identified interactors. An alternative approach to studying compartment-specific regulatory events is to isolate somata from processes either via dissection [30] or by using microfabricated cell culture inserts [72]. Such approaches have been used extensively for sequencing-based experiments and can be used for proteomics studies, although the latter approach requires large amounts of starting material. Sample availability is also a hurdle for smaller-scale targeted biochemical analyses of the UPS in neuronal compartments. Promising novel tools to study the UPS in an intact cellular context are activity-based probes (ABPs). In recent years, a number of ABPs have been developed to study the ubiquitylation cascade [73], DUBs [74], and the proteasome [75,76]. Although potentially powerful, several ABPs suffer from limitations such as limited cell permeability and irreversible inhibition of the target enzymes, which means they only provide a snapshot of the enzymatic activity queried at any given time. Destabilized fluorescent proteins have been extensively used to study degradation kinetics in living cells [77] but cannot indicate what components of the UPS are effecting such changes. Although the degradation kinetics of fluorescent reporters can vary depending on cell type and degron used for destabilization, they generally tend to be too slow to monitor the rapid changes in degradation relevant for neuronal plasticity. Furthermore, due to the confounding effects of diffusion, a number of controls need to be included to precisely assess compartment-specific changes in UPS activity. Lastly, blockade of proteasome activity by knock out of 20S components is lethal in mammals, and pharmacological inhibition of proteasome activity remains essential for the study of proteasome function. However, the use of such compounds comes with limitations; they can nonspecifically block other cellular proteases, thus generating off-target effects, and they inhibit all cellular proteasomes, regardless of their subcellular location or composition.

How do PTMs influence protein degradation?

Pioneering electron cryotomography work on proteasome assembly and activity in intact neurons has reported that there is a significant fraction of 26S proteasome that is not engaged in degradation [78]. This finding suggests that the amount of assembled 26S proteasomes is not a limiting factor for the degradation of substrates under basal conditions. Proteasomes in the substrate-accepting ground state could be in a standby mode, waiting for metabolic cues that require increased catalytic activity. Recruitment of 26S proteasomes from a ground state might be mediated by PTMs or by interaction with accessory factors. This would be an efficient way to rapidly alter proteasomal degradation in the cell, especially considering that proteasome complex assembly can take several hours depending on the cell type [79,80]. Recently, a meta-analysis of yeast proteasomal PTMs has described 11 types of modifications, distributed across 245 sites [81]. Some sites can be targeted by multiple PTMs, pointing to the possibility of crosstalk among modifications [82]. Proteasomal PTMs can either activate or repress proteasomal degradation [83]. For example, phosphorylation of Rpt6 at Ser120 by CaMKIIα locally increases

proteasomal degradation at synapses [46,49], and proteasome translocation into postsynaptic termini in response to neuronal activity is driven by interaction with CaMKIIα [49]. These findings underscore the importance of spatially restricted PTMs for synaptic protein turnover. Proteasomal PTMs are often coupled with changes in the regulatory complexes bound to the 20S core particle. For example, ADP ribosylation of the proteasome regulator PI31 allosterically relieves 20S proteasome repression and at the same time promotes 26S proteasome assembly [84]. Inactivation of PI31 in Purkinje cells disrupts neuronal proteostasis and leads to neuronal degeneration [62]. Further highlighting the potential of PTMs to regulate the proteasome, Rpn1 phosphorylation on Ser361 was recently shown to promote assembly of the 26S proteasome [85].

The ubiquitin molecule itself can carry PTMs. Two main PTMs have been described for ubiquitin: acetylation and phosphorylation. Six out of its seven lysine residues can be acetylated, and this modification generally favors monoubiquitination of substrates over the formation of polyubiquitin chains [86]. There are nine identified phosphorylation sites on ubiquitin. One of them – Ser65 – is involved in the modulation of mitophagy in dopaminergic neurons [87]. There are also two positions that can be SUMOylated. Summing up these modifications, 22% of the amino acids within the ubiquitin molecule can be modified, suggesting the possibility of extensive regulation of substrate ubiquitination; not only by the already complex enzymatic cascade necessary for attachment of ubiquitin to substrates, but also by modifications in the ubiquitin molecule itself [88]. Adding complexity to the system, it is important to note that some times the same protein can be degraded in both a ubiquitin-dependent and -independent manner [89], and that several proteins are degraded by the proteasome in a ubiquitin-independent manner [90] with the aid of factors such as NQO1 or DJ-1 [91,92]. These two proteins have been implicated in cancer and neurodegeneration, which are both closely linked to proteasome dysfunction.

It is clear that PTMs can regulate the UPS at several points. In addition, PTMs can modulate the localization, activity, interaction, and turnover of proteins that are degraded by other pathways. For example, phosphorylation of tau regulates its solubility, which in turn is linked to its aggregation and formation of the neurofibrillary tangles found in Alzheimer's disease [93]. PTMs are also essential for the modulation of activity-dependent processes related to learning and memory [94,95]. Despite an increasing understanding of UPS regulation, many open questions remain, especially in the context of neurons, and little is known about local PTMs of ribosomes or UPS components, which likely play an important role in targeted protein synthesis and degradation.

Concluding remarks and future perspectives

Over the last two decades our molecular-level understanding of how synapses form, are maintained, and undergo plasticity has radically changed. This was driven in large part by advances in sequencing and proteomics, which allow broad and holistic studies of neuronal molecular composition. Initially, these omics studies were conducted at the bulk level but more recently the focus has shifted towards understanding the diversity within cell populations using single-cell techniques [14,96]. Research perspectives have also expanded to gain information on transcript identity and spatial localization with nanoscale precision, on a transcriptome-wide scale [16]. It is expected that these efforts to further resolve the complex organization of cells will eventually produce a comprehensive description of proteins and mRNAs present across neuronal compartments (Box 1). Such datasets will represent a rich resource to be mined by further functional studies aimed at understanding the interplay of different proteostatic pathways. Within this framework, pharmacological inhibition represents a rapid and efficient way to manipulate neurons and identify genes involved in proteostatic regulation and metabolic integration. More refined and targeted genetic manipulation can then be used to study the function of these genes in the

Outstanding questions

How are certain protein species selectively and locally up-/downregulated following specific plasticity paradigms? Do PTMs in RPs, mRNA binding proteins, or translation factors play a role in regulation of local protein synthesis? Do PTMs of UPS components or in the substrates themselves, play a role in regulation of local degradation near synapses?

What are the specific sequence elements in mRNAs responsible for their targeting to neuronal compartments? How are the necessary mRNA molecules made delivered, sequestered, and degraded? What is the relative contribution of active transport and diffusion to mRNA targeting in neuronal processes?

How are the protein synthesis and degradation machineries coordinately regulated to effect local proteome remodeling? Are there broad proteostatic modules subject to regulation or do these balancing mechanisms operate on a single substrate level?

What fraction of neuronal proteostasis, in general, and synaptic proteostasis, more specifically, is covered by local mechanisms? Can local protein synthesis account for the bulk of peripheral proteostasis or is the soma still a major contributor?

To what extent is the regulation of protein synthesis and degradation in neuronal processes independent of regulation in the somata? Some proteins can be transported from the somata and also locally synthesized: how is the ratio of both species controlled? Are these species different in terms of their PTMs and functionality?

How is the local synaptic proteome maintained over time, and how is the influx of somatic proteins countered?

What is the minimal compartment at which independent proteostatic regulation is observed – is it at the level of single dendritic spines or at a higher hierarchical level?

The half-lives of synaptic proteins and basal translation rates are co-regulated

Box 1. Neuronal compartments

Classically, neurons have been defined by three main structural compartments: the cell body, dendrites, and the axon (see Figure 1 in main text). This classification, however, does not take into account molecular or functional subcompartments. Synapses, for example, display a considerable degree of heterogeneity owing to their recruitment of different RNA and protein complements. Signals are also processed differently across synapses. This can vary depending on several factors, for example, the dendritic branch where they are located, but even within the same branch specific stretches of synapses can share a similar capacity to integrate electrical impulses, forming a synapse cluster involved in cooperative plasticity. Developing axons can be subdivided into axon shaft and growth cone. While the former compartment has been proposed to have predominantly a structural role and is enriched in cytoskeletal components and tubular endoplasmic reticulum [105], the latter compartment, which senses and responds to guidance cues, is characterized by a dynamic actin cytoskeleton, the presence of mRNA, polysomes, UPS components, endo- and exocytic vesicles [106,107].

Mature axons are generally considered to be morphologically more homogeneous than dendrites, but display different proteins as a function of their distance from the somata. The axon initial segment bears a high density of ion channels and plays an important role in the initiation of action potentials. Its specific protein composition acts as a selective barrier that counters diffusion between the soma and distal parts of the axon. Similarly, the nodes of Ranvier are rich in voltage-gated sodium channels that are essential for the saltatory conduction of action potentials (see Figure 1 in main text). Similar to axons, dendrites can also display differential enrichment in certain proteins as a function of the distance from the soma. For example, the CA1 region of the hippocampus and the distal dendrites of CA1 pyramidal neurons (in the stratum lacunosum-moleculare) are enriched in a specific array of proteins and ion channels important for the regulation of dendritic activity [108].

Subcompartments can be defined by specific energy demands and allocation of resources. For example, mitochondria have a different distribution and morphology in dendrites and axons. In dendrites, mitochondria tend to be positioned under clusters of synapses [109], perhaps grouping them into the same computational unit, while other synapses lack mitochondria (see Figure 1 in main text) and might rely on diffusing ATP from adjacent regions or alternative energy sources such as glycolysis. Recent work has proposed that during synaptic scaling, independent allocation of nascent proteins occurs at the level of synaptic neighborhoods of ~10 μm in size [110]. However, in certain cases, compartmentalization can gain resolution and get to the level single spines. For example, CaMKIIα can be activated in response to calcium microdomains under a single spine potentiated by LTP, with the rapid on/off transition of the enzyme preventing neighboring spines to be affected by its diffusion [111].

context of synaptic function and plasticity. In this regard, the CRISPR-Cas system has dramatically changed our ability to interrogate gene function both at the level of single genes and genome-wide [97–99].

One could argue, however, that compared to other fields (such as cancer biology), molecular neuroscience has not capitalized on advances in genome editing. One reason for this is that neurons, being postmitotic, are not amenable to the classic gene editing workflow, which relies on expansion and screening of cells carrying the correct edits. This makes it virtually impossible to generate knockouts and establish a direct genotype–phenotype link. More broadly, introduction of nonfluorescent tags or degrons suffers from limitations, and downstream analyses are by and large limited to microscopy. Owing to their improved reproducibility [100,101], consistent regional identity [102] and ability to capture the physiology of mature neurons [103,104] recently developed *in vitro* neuronal differentiation protocols, combined with genome-editing workflows, have the potential to complement more traditional *ex vivo* preparations. How cells regulate their proteome in response to external cues is intrinsically tied to aspects such as species, cell type, and cell states, and thus represents a broad and diverse area of research (see Outstanding questions). Most of the fundamental pathways underlying proteostatic regulation are likely conserved, but it seems reasonable to expect that neurons display specific adaptations endowing them with fine-grained control over their local proteome.

to maintain the structural integrity and balanced molecular composition of synapses. Plasticity leads to changes in protein steady state levels and protein turnover rates. Because the synapse is a very complex supramolecular assembly, the maturation, membrane insertion, anchoring and degradation of proteins have to be precisely coordinated. Do changes that alter the rates of synthesis and degradation while leaving protein steady state levels unaltered lead to functional changes at synapses?

Acknowledgments

The authors would like to thank members of the Schuman laboratory for helpful discussions. S.L.G. is supported by a FEBS Long-Term Fellowship. B.A.C is funded by the Madrid Community (Spain) (Atraccion de Talento-2019T1/BMD-14057) and Spanish Ministry of Science and Innovation (Ramón y Cajal- RYC2018-024435-I and PID2020-113270RA-I00). E.M.S. is

funded by the Max Planck Society, an Advanced Investigator award from the European Research Council (grant 743216), DFG CRC 1080: Molecular and Cellular Mechanisms of Neural Homeostasis, and DFG CRC 902: Molecular Principles of RNA-based Regulation.

Declaration of interests

The authors declare no competing interests in relation to this work

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