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Review

The axonal endoplasmic reticulum and protein trafficking: Cellular bootlegging south of the soma

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ABSTRACT

Neurons are responsible for the generation and propagation of electrical impulses, which constitute the central mechanism of information transfer between the nervous system and internal or external environments. Neurons are large and polarized cells with dendrites and axons constituting their major functional domains. Axons are thin and extremely long specializations that mediate the conduction of these electrical impulses. Regulation of the axonal proteome is fundamental to generate and maintain neural function. Although classical mechanisms of protein transport have been around for decades, a variety newly identified mechanisms to control the abundance of axonal proteins have appeared in recent years. Here we briefly describe the classical models of axonal transport and compare them to the emerging concepts of axonal biosynthesis centered on the endoplasmic reticulum. We review the structure of the axonal endoplasmic reticulum, and its role in diffusion and trafficking of axonal proteins. We also analyze the contribution of other secretory organelles to axonal trafficking and evaluate the potential consequences of axonal endoplasmic reticulum malfunction in neuropathology.

Contents

1. Introduction: spatio-temporal control in the nervous system

The nervous system allows multicellular animals to establish and adapt their interactions with the environment by transmitting information in the form of electrical impulses. Long distance and direction are two fundamental aspects of the system's functionality, and to improve performance neurons have evolved into large cells. Additionally, morphology has become significantly more complex than in other cell types, with polarized axons and dendrites mediating communication among local or with distant neural circuits (Fig. 1a). It is undeniable that the length of axons in large animals, such as living or extinct mammals, and reptiles including dinosaurs is an awesome evolutionary achievement of the cellular machinery (Fig. 1b). To illustrate this point the longest neurons in whales may exceed 30 m, while the recurrent laryngeal nerve in long-necked

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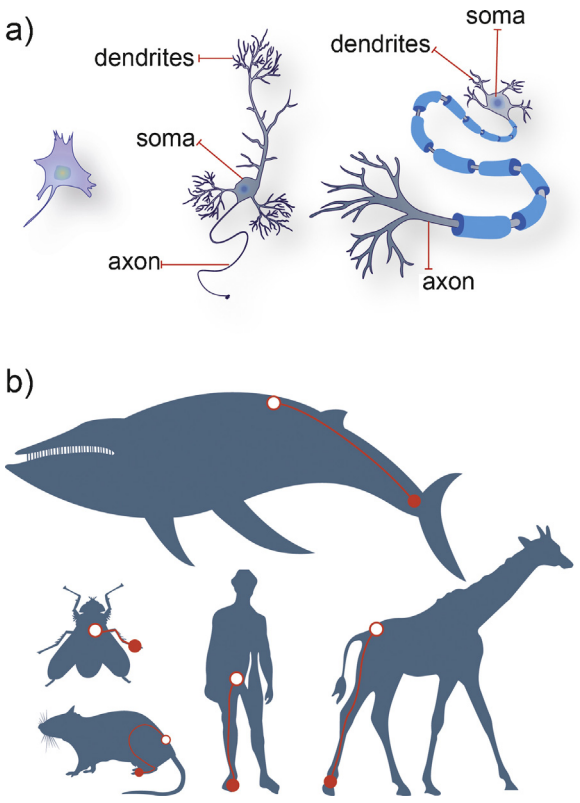

Fig. 1. Architecture and size are defining properties of neurons. (a) Comparative morphology and size of a non-polarized cell and neurons. Compared to most eukaryotic cells neurons from the central and peripheral nervous system are highly polarized with dendrites and axons extending long distances from the cell body. They have also evolved surface areas that may be up to 10,000 times greater than other cell types. Axons of the central and peripheral nervous system may be unmyelinated (center) or myelinated (right). Myelination (blue) increases the conduction velocity of electrical impulses. The drawings represent a non-polarized cell (left), a typical unmyelinated pyramidal neuron of the mammalian central nervous system (center) and a myelinated neuron such as those found in sensory and motor fibers of mammalian peripheral nerves (right). (b) Axonal length in different animals. Axonal length is highly variable within the nervous system of an organism. In addition, axons in different animals range from a few microns to meters in large mammals. Lengthy nerves in mammals include the sciatic nerve that run from the lower spinal cord to the lower extremity and is responsible for motor and sensory conduction (red). These can measure in the order of cms in small mammals like the rat, close to a meter in humans and several meters in large mammals like the giraffe and large cetaceans. Another extreme example is the left recurrent laryngeal nerve of the giraffe, which can measure up to 5 m.

mammals such as giraffes approaches 5 m and may have reached 38 m in long-necked sauropods [1].

Considering neuronal size and architecture, it is reasonable to postulate that crucial functions such as intercellular communication, intracellular signaling, energy production and consumption, and transport across the cell membrane will be regulated in a domain and temporal-specific manner. Regulating the abundance of protein, RNA, lipids and other macromolecules locally certainly impinges on these compartmentalized cellular functions during development, maintenance, plasticity and repair of neural tissue.

Axons are constituted by distinct morphological and functional domains that include the axon initial segment, the axonal shaft, Nodes of Ranvier (and the multiple specializations that encompass the nodal and internodal regions in myelinated axons), and the axon terminal or presynaptic specialization [2]. In this review we will concentrate on the control of the axonal proteome as a fundamental cellular process. Although in strict sense the proteome constitutes the entire repertoire of proteins present at a given moment within a specific domain, we will refer mostly to that part of the proteome that includes secreted and membrane proteins. Importantly, endocytosis and recycling of axonal components such as receptors, signaling molecules and synaptic vesicles [3–5] also play fundamental roles in regulating the proteome and are still topics of intense research, but is mostly anterograde protein trafficking that has continued to challenge the classical views of domain-specific regulation. Thus, we will focus on the mechanisms that allow the control of the proteome *via* direct transport and the contribution of the axonal endoplasmic reticulum (ER) to axonal biosynthesis and trafficking. Since these may operate independently of somatic or dendritic mechanisms we will refer to them as local regulation. We will also analyze the implications for pathology. We are aware that glial cells such as oligodendrocytes and Schwann cells play crucial roles in the control of local neuronal function in the central and peripheral nervous systems, but these topics have been carefully analyzed elsewhere and will not be directly considered here (reviewed in [6,7]).

2. Regulation of the axonal proteome

2.1. Axonal transport: classical transport models

A tight regulation of the axonal proteome is necessary for diverse processes such as the establishment and maintenance of neuronal polarity [8,9], axon growth and guidance [10], synapse formation and plasticity [11] and nerve regeneration [12–14]. One plausible mechanism to exert control over the abundance of axonal proteins is by transport of ready-made components.

Axonal transport is the intracellular movement of material away or toward the cell body that starts during development and continues throughout the life of the neuron. The study of axonal transport began more than 50 years ago fueled by the need to understand the intracellular dynamics that support growth, maintenance and repair of nerve fibers [13–15]. It was widely accepted initially that subcellular organelles and membrane components in axons and dendrites originated exclusively in the cell body. Classical studies of anterograde transport revealed the rate of transport of *de novo* synthesized proteins that reached the optic tectum from the retina by injecting radioactive leucine into the eyes of goldfish [16]. This knowledge gave rise to the influential concepts of fast and slow axonal transport that regulate the availability of axonal components *via* anterograde and retrograde mobility.

Fast axonal transport corresponds to the anterograde, retrograde and bidirectional movement of membrane bound cargoes propelled by the action of molecular motors along microtubules. The average velocities of fast axonal transport range between 50–400 mm/day (approximately 0.6–5 μm/s) [17]. Fast axonal transport is supported by microtubules arranged longitudinally with their plus ends pointing away from the neuronal soma [18]. This organization favors the directional mobility of molecular motors such as kinesins and the dynein complex [19], which transport and deliver cargoes to microtubule plus or minus ends in an ATP-dependent manner. A compelling body of evidence indicates that kinesins are involved in the anterograde transport of organelles, such as mitochondria, protein complexes and ribonuclear particles, whereas dynein promotes the retrograde transport of organelles such as endosomes, mitochondria, the Golgi apparatus, lysosomes and autophagosomes [19,20] (Fig. 2a).

Slow axonal transport corresponds to the movement of cytoskeletal polymers, cytosolic protein complexes and ribosomes at averages velocities of 0.2–8 mm/day (approximately 0.002–0.09 μm/s) [17]. Examination by live-cell imaging of cytosolic proteins enriched at the presynaptic terminal, such as synapsin and CamKIIa, has recently clarified the molecular

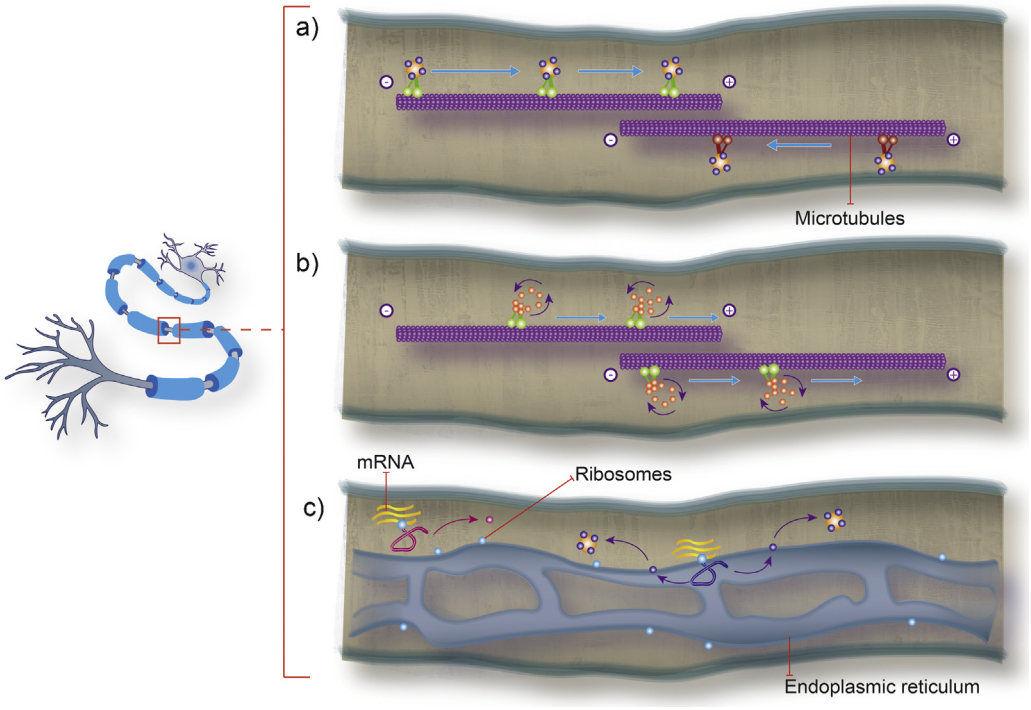

Fig. 2. Transport and trafficking as alternative routes to regulate the axonal proteome. (a) Fast axonal transport. Transport of biosynthetic secretory vesicles, synaptic vesicle precursors, ribonuclear particles, endosomes, mitochondria, the Golgi apparatus, lysosomes and autophagosomes (orange carriers with purple structures) is mediated by molecular motors of the kinesin superfamily (green) or the dynein complex (ochre) along polarized microtubules (magenta) at 50–400 mm/day (approximately 0.6–5 μm/s). (b) Slow axonal transport. Transport of cytoskeletal polymers, cytosolic protein complexes and ribosomes (orange) possibly as a consequence of assembly and disassembly of transient complexes between cytosolic cargo and fast-transport carriers, which result in averages velocities of 0.2–8 mm/day (approximately 0.002–0.09 μm/s). The transport of ready-made components *via* fast and slow axonal transport is widely used to regulate the availability of axonal components. (c) Cytosolic (red) and secretory (purple) proteins may also be synthesized in axons from local mRNAs (yellow) in free ribosomes or ER-associated ribosomes respectively. Locally synthesized soluble proteins directly alter the composition of the axonal proteome. Membrane or secreted proteins may subsequently be exported from axonal ER exit sites (orange carriers with purple structures) thus providing an alternative mechanism to regulate the composition of the axonal membrane or the secretory capacity.

mechanisms responsible for slow transport in axons of cultured hippocampal neurons [21,22]. Mobility of these components depends on microtubules, kinesin-1 and association to transport vesicles, and possibly results from the probabilistic assembly and disassembly of transient complexes between cytosolic proteins and vesicular carriers moving in fast axonal transport. These and other studies have contributed to establish that fast and slow axonal transport share a molecular basis that produces a variety of average velocities by differentially regulating the duration of the transport-competent structures (Fig. 2b). Importantly, the fundamental mechanisms that generate slow axonal transport *in vivo* and whether they operate equally in axons of different lengths and calibers remain to be elucidated.

Although anterograde transport provides an attractive solution to the supply of material to the axon and the nerve terminal, an alternative mechanism is to produce and process these components in the proximity of specialized domains (Fig. 2c). Accumulated evidence indicates that this is indeed the case for a number of soluble, membrane and secreted proteins that contribute to axonal autonomy through an exquisite spatiotemporal control of their abundance in response to growth, intrinsic and extrinsic cues or injury. These mechanisms will be analyzed in the next sections. To accurately differentiate between related concepts we will use "axonal transport" to describe the movement of carriers or soluble material, whereas we will utilize "axonal biosynthesis and trafficking" to describe the sequential progression of secreted or membrane proteins through the secretory pathway that starts with translation. In both cases proteins subsequently rely on targeting and clustering motifs that contribute to their specific insertion or selective anchoring at the plasma membrane.

2.2. *mRNA localization and transport support local synthesis*

The cell body has been historically considered the main source of newly synthesized proteins in neurons. Nevertheless, protein transport may not be sufficient to control the immediate requirements of the proteome at distant locations. Therefore, a biosynthetic solution based on mRNA transport and localization, storage centers and local protein synthesis provide the axon with a convenient alternative to respond rapidly to intracellular or extracellular demands that arise locally [23,24]. The advantages of this strategy include the generation of multiple copies from a single mRNA molecule, the avoidance of undesirable protein activities during transport and the use of targeting signals that do not compromise the structure or function of the protein [25]. Additionally, these mechanisms may circumvent limitations imposed by short half-lives and transport times of relevant axonal proteins [26] along lengthy nerves such as the sciatic nerve in mammals, a concept that serves to illustrate a fundamental problem in biology. Finally they provide additional stages of control for the assembly of multimeric complexes, typical configurations of ion channels and neurotransmitter receptors.

The distal localization of hundreds of mRNAs in squid giant axons, *Aplysia*, *Xenopus*, and mammals strongly supports axonal protein synthesis. Using proteomics and cDNA hybridization to examine gene expression profiles, mRNAs for protein synthesis factors like elongation and ribosomal proteins, for proteins of the endomembrane system including ER, mitochondrial and endocytic components, for transmembrane proteins like ion channels and membrane receptors, for enzymes, proteasome and cytosolic proteins, and actin, neurofilament and microtubule cytoskeletal

components have been identified in axons of normal or injured-conditioned dorsal root ganglia (DRG), which contain the cell bodies of afferent neurons that transmit sensory information from the periphery to higher integration centers in the spinal cord and the brain [27,28].

Fast axonal transport does play a role in mRNA localization and, consequently, is a functional determinant of axonal biosynthesis and trafficking. mRNAs are transported *via* their association with RNA binding proteins that assemble in ribonucleoprotein particles (RNP) [29]. These are generated within the cell body and travel along the axon using molecular motors in a microtubule-dependent fashion [30–32]. The cis-acting elements that mediate the axonal targeting of mRNAs are usually localized in the 3′ untranslated region (UTR). In this context the 3′ UTR of β-actin is sufficient to guide the localization of the mRNA in myelinated peripheral axons [33]. Other examples of mRNA localization signals in axons include the 3′ UTR of tau and importin β1 [34,35]. Although it is still largely unknown which proteins are synthesized in periodic accumulations of ribosomes in the cortical region of the axoplasm known as periaxoplasmic plaques and how they are produced from RNPs, the transport and localization of axonal mRNAs strongly support local protein synthesis. Indeed, translation has been convincingly demonstrated in a variety of vertebrate and invertebrate axonal models [36–41].

Importantly, the localization of axonal mRNAs is regulated by extracellular stimuli. For instance, growth-promoting neurotrophins such as nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 increase or decrease the levels of endogenous mRNAs in axons of injury-conditioned DRG neurons. Furthermore, growth-inhibiting factors such as myelin-associated glycoprotein and semaphorin 3A control the abundance of a different group of mRNAs and have inverse effects to neurotrophins on a proportion of transcripts [27]. In agreement with the use of a fast transport system changes in mRNA levels in response to external stimuli are microtubule-dependent [27].

If axonal mRNAs play a role in regulating the proteome the localization of ribosomes in the axon is an unavoidable requirement. The limited distribution of ribosomes to the axon initial segment and their small number in peripheral axons compared to those in dendrites initially suggested that axonal protein synthesis did not exist, or that at least was not robust enough to supply distant regions with locally synthesized protein [42]. However, a number of electron microscope studies have established the unambiguous presence of ribosomes and ribosome-like particles along immature and mature axons in central and peripheral axons *in vitro* and *in vivo* [43–48]. Periaxoplasmic plaques are visible in myelinated axons of the vertebrate sciatic nerve [30,32]. Additionally, exogenous ribosomal proteins localize in the vicinity of Nodes of Ranvier and participate in the local synthesis of heterologous proteins [49].

2.3. Function and regulation of local protein synthesis

Local protein synthesis plays a role in the development and maintenance of axons *in vitro* and *in vivo*, and occurs upon demand [29]. For example, it controls the growth of the axon tip as it responds to attractive and repulsive extracellular cues [50,51]. Here, the precise translational regulation of actin, molecules responsible for actin polymerization/depolymerization, and receptors to guidance cues contributes to axon guidance, providing a rapid autonomous response to external stimuli such as those that originate when axons reach the spinal cord midline [50,52–54]. Local protein synthesis also controls the abundance of receptors and ion channels, axon growth, axon survival, the establishment and maintenance of synapses, neurotransmitter biogenesis, and axon regeneration (reviewed in [25]). The mainstream view proposes that axons utilize external cues to temporally and spatially regulate the cellular machineries responsible for transport and translation of mRNAs *via* signal transduction cascades. However, an alternative scenario particularly studied in injured and regenerating axons, indicates that the neural environment not only provides external cues to modulate the rates of transport and translation, but also contributes with the translation machinery *per se*. As a matter of fact, the intercellular communication between Schwann cells and peripheral nerve cells allows the transfer of ribosomes and mRNAs from the glia to the axon, thus providing a non-cell autonomous mechanism to orchestrate the local response [55,56].

As mentioned above, many developmental and plastic processes in the axon require the production of membrane and secreted proteins such as adhesion molecules, neurotransmitter receptors, and secreted factors [52]. An essential condition for the local biosynthesis of these molecules is the existence of functional stations of protein processing including the ER, the ER-to-Golgi intermediate compartment (ERGIC) and the Golgi apparatus. These will be examined next.

2.4. The axonal ER

2.4.1. The structure of the ER in axons

The ER is highly conserved in eukaryote cells. It is responsible for the synthesis and modification of the majority of membrane and secreted proteins, lipid synthesis, calcium signaling and glucose homeostasis. Structurally, the ER is a continuous organelle that includes the nuclear membrane and the ribosome-rich rough ER (RER), both composed primarily of sheets, and the smooth ER (SER) that forms a tubular network containing sparse polyribosomes (Fig. 3a). ER sheets are enriched in proteins of the translocon family, such as Dad1, TRAPα and Sec61β, and proteins that contain a single transmembrane segment with a coiled–coil domain such as Climp-63, p180 and Kinectin [57]. Climp-63 forms intramolecular bridges that maintain a 50 nm distance between ER sheets in mammalians cells [57]. In contrast, ER tubules are enriched in reticulons and DP1/Yop1p, which form a hydrophobic wedge that spans the lipid membrane and curves the ER tubule maintaining its diameter around 60–100 nm in mammals. They also provide the curvature to the bent edges of ER sheets [58,59]. The relative abundance of reticulons or sheet-associated proteins may define the proportion of sheets and tubules within a given cell type or cell stage. As a consequence, the subcellular distribution and proportion of RER and SER are variable depending on cell function. The neuronal RER is abundant in the soma and the surrounding somatodendritic volume [60], whereas the SER invades distal dendrites and the axon forming a network of longitudinal tubules, connecting regions and irregularly spaced cisternae, all of which define regions of variable structural complexity [61–66].

The first reports of the ER in axons date back to the 1970s. Using electron microscopy, the SER was described as a longitudinal network frequently running proximal to the plasma membrane in axons of mouse sciatic and phrenic nerves and chick ciliary ganglia neurons, which control the pupilar diameter [66,67]. Consistently, it was reported as a continuous three-dimensional network of tubules that runs longitudinally along the axonal axis in myelinated peripheral axons with occasional subaxolemmal plates of smaller interconnected tubules. The ER is most likely the continuation of the somatic organelle, and is composed of tubules measuring 20–40 nm, free endings, beaded areas and larger interconnected cisternae in myelinated and unmyelinated axons [68,69] (Fig. 3b). Additional studies indicate that the ER, wrapped in other tubovesicular structures, is the predominant membranous organelle in the axon, and molecular evidence supports the existence of a variety of resident and functional components in central and peripheral neurons. The ER chaperone proteins grp78/BIP, calreticulin and ERp29, the glycoprotein ribophorin II, the signal recognition particle SRP54

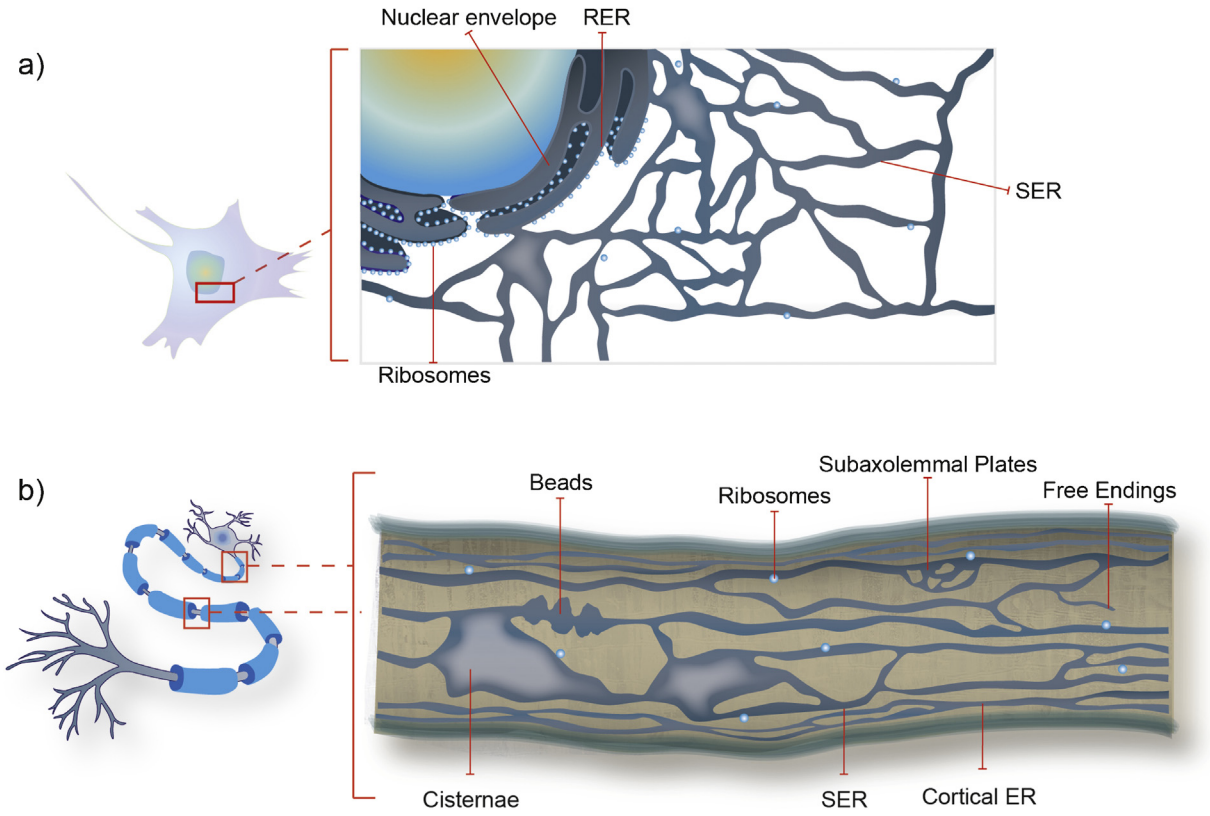

Fig. 3. The structure of the ER in axons. (a) The structure of the ER in non-polarized cells. In a typical non-polarized cell the ER (purple) is a continuous membranous organelle that distributes throughout the cellular volume, and includes the nuclear envelope, the rough ER (RER), which is rich in ribosomes (light blue), and the smooth ER (SER) that forms a tubular network containing fewer polyribosomes. The proportion of RER and SER are variable depending on cell type, cell stage and cell function. The SER is mostly an interconnected network of tubules containing a continuous lumen and three-way junctions. (b) The structure of the ER in axons. In axons the SER is the continuation of the somatic organelle and is composed primarily of a three-dimensional longitudinal network of tubules and larger interconnected cisternae. The network is associated to sparse ribosomes, contains occasional subaxolemmal plates, free endings, beaded areas and in some regions runs close to the plasma membrane.

and the translocon-associated protein TRAPα are some of the proteins found in axons of DRG cultures [27,28]. Likewise markers for the signal recognition particle (SRP54) and the ER (Robophorin II, TRAP-α, calreticulin, protein disulfide isomerase, SERCA, ERp29 and KDEL-receptor) have been described in peripheral axons *ex vivo* [70]. In dendrites the ER is associated primarily to the microtubule cytoskeleton *via* MAP2 and Climp-63 [71], whereas in axons multiple components of the cytoskeleton including microtubules, neurofilaments and actin, and adaptor proteins such as p600 may participate in maintaining the integrity of the ER [71–73].

2.4.2. A biosynthesis and trafficking-competent ER in axons

In addition to the meticulous morphological description of the axonal ER [66–68] compelling evidence now supports some of its distinguishing functions. For example, glucose-6-phosphatase-positive cytochemistry in salt-stressed mice suggests that the ER participates in glucose metabolism in axons [68]. More relevant for the present discussion a number of observations indicate that the axonal ER participates in biosynthetic trafficking. The existence of ER associated ribosomes has been reported in the axon initial segment (AIS), internodes and nodes of peripheral myelinated axons [44]. Sar1, a small GTPase involved in protein export from the ER through the formation of the cytosolic coat protein complex (COPII), localizes to axons of hippocampal neurons [74]. Decrease in Sar1 activity generated smaller axons while its overexpression produced longer axons. In agreement with the presence of functional ER export sites (ERES) in axons, other COPII components such as sec12, sec23 and Yip1a have also been reported [74]. Combined, these observations strongly suggest that the ER carries out functions related to protein synthesis and trafficking in axons. Whether these functions are subject to local regulation in specialized domains such as the axon initial segment or Nodes of Ranvier remains for the most part unexplored.

2.4.3. Does ER diffusion contribute to the production of membrane and secreted proteins?

Given the length of axons and the continuity of the ER, diffusion of ER luminal and membrane proteins, and not only local synthesis, may supply export sites with *de novo* synthesized protein, and therefore may be considered a central aspect of protein trafficking. Several explanations have been put forward to describe the diffusion of proteins along the axonal ER. Indeed, in dendrites ER diffusion, network complexity and ER residency times play a role in the trafficking of NMDA receptors [65]. Regulation of these ER-related parameters may define exploration volumes for *de novo* synthesized proteins prior to ER export, thus correlating ER confinement to local trafficking [75]. Whether similar mechanisms operate within the axonal ER is currently unknown. Initially, analysis of [3H]-labeled material in frog DRG and associated nerves indicated that all newly synthesized proteins were transported along the axon in post-Golgi vesicles [76]. In agreement with these observations brefeldin-A (BFA), a drug that interferes with COPI vesicles and results in the accumulation of proteins in the ER, significantly reduced the levels of fast axon transport-dependent protein [77]. However, as in dendrites, ER proteins may also diffuse along the axonal ER. The viral outer capsid protein VP7 localizes to axons in DRG and spinal cord neurons even in the presence of BFA, suggesting that VP7 is transported along the axonal ER [78]. A similar mechanism is supported by the axonal trafficking of the epidermal

growth factor receptor ligand in the visual system of *Drosophila* [79].

Importantly, accumulated evidence suggests that mobility of ER proteins is different from fast and slow axonal transport, and may be controlled by an active component in addition to diffusion. In cultured chick DRG neurons, the axonal mobility of two ER membrane proteins, sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and inositol trisphosphate receptor (IP_3R), is bidirectional and mostly, but not exclusively, non-vesicular with average velocities of 0.1 μm/s requiring microtubule tracks and dynamic microtubules [80]. In agreement with these findings, alteration of the microtubule cytoskeleton impairs the transport of $GABA_BRs$ along the axonal ER [81]. Although conclusive evidence is still lacking, the kinesin-dependent localization of axonal $GABA_BRs$ suggest that mobility is motor dependent. Even though the limitations of a motor pulling along a lipid bilayer must be considered, it is tempting to speculate that molecular motors may couple to ER cargo directly or *via* adaptor proteins and produce the sliding of cargo along the continuous ER membrane. Alternatively, discrete and transient tubo-vesicular structures may contribute to protein transport. Combined these studies suggest that the axonal ER is transport-competent and that the mobility of axonal proteins is not explained satisfactorily by diffusion alone, but may include an active component.

Interestingly, the transport of ER membranes itself may also contribute to protein mobility. The tubular ER network is highly dynamic, a property that has been observed in multiple eukaryote cells that allow live visualization at sufficient spatial resolution [82]. ER tubules constantly elongate, retract, and anastomose. Tubule elongation occurs by fast kinesin-1 and dynein-dependent sliding, preferentially along acetylated microtubules [83,84], or by slower tubule extension associated to the tip attachment complex (TAC) at the plus-end of growing microtubules [85]. Atlastin, a highly conserved GTPase present in the ER and to a lesser degree in the Golgi apparatus, promotes homotypic ER membrane fusion, a process necessary for the continuous shaping of the ER network [86]. Although current research in ER dynamics has concentrated in large and flat non-neuronal cells, primarily due to the fact that available techniques fail to visualize the live ER network in more limited spaces such as the axon, an emerging body of evidence suggests that ER dynamics allows the rearrangement of specific ER domains in dendrites and axons [71]. Classical radioactive injection studies indicate that profiles of labeled ER membranes accumulate in the proximity of compressed ciliary ganglia 3 h after cerebral injection, suggesting that ER membranes are conveyed *via* fast axonal transport [67]. Likewise, abundant tubo-vesicular structures, most likely derived from the SER, accumulate after blocking transport with a rapid cooling method in mouse sensory nerves suggesting that proteins may move together with the axonal ER membranes and not through an ER pipe [87]. However, the interpretation of these results may be confounded by the close association of ER membranes and post-Golgi vesicles that occurs along the axon [69]. Indeed, other studies employing cold blocks and three-dimensional analysis in the same fibers suggest that the axonal mobility of SER is not related to fast axonal transport and that accumulated membrane profiles are not continuous with the SER, establishing an unsettled controversy regarding the mechanism and implications of axonal ER transport [88]. These discrepancies may have originated from labeling procedures that did not mark the compartments selectively or that were obscured by labeled molecules changing compartments during transport. However, more recent reports support the transport of axonal ER membranes. For example, myosin Va is necessary for axonal ER localization uncovering the significance of motor-based dynamics in axonal ER membrane transport [72]. Furthermore, disruption of the ER-microtubule association *via* knockdown of p600 produces abnormalities in neuritogenesis [89], and knock-down of atlastin-1 impairs axonal elongation in developing cultures of cortical neurons [90]. Together these observations strongly suggest that ER membrane transport contributes to axonal function and may help neurons respond rapidly to internal and/or external signals. Whether they necessarily imply protein transport needs to be addressed, especially considering that ER membrane transport may also contribute to establish a local supply of Ca^{2+}, mitochondria and possibly other organelles [83,91,92].

In summary, our current understanding of the axonal ER, local protein synthesis, diffusion and active mobility, and ER export support a local secretory pathway. However, the relevance of each of these processes for axon development, maintenance, plasticity and regeneration await discovery.

2.5. Other early secretory organelles provide additional machinery for axonal translation

Besides the ER, other early secretory organelles such as the ERGIC and Golgi apparatus are essential for the processing and sorting of membrane and secreted proteins. Importantly, specific markers of these organelles have been observed in central and peripheral axons [70,81,93] and accumulating evidence suggests they contribute to axonal function [4]. Surprisingly, the role of the ERGIC in axons, and generally in neurons, has received little attention. In non-neuronal cells the ERGIC is a highly dynamic organelle that conveys cargo from ERES to the cis-Golgi, but the characterization of this compartment in axons is still lacking [94]. Regarding the Golgi apparatus, the paradox seems to reside not in finding Golgi components in the axon but in the failure to identify archetypical stacked structures, or Golgi outposts, such as those observed in dendrites [95]. In *Drosophila*, Golgi outposts accumulate in the axon in a dynein mutant [96]. These results suggest that in the absence of dynein Golgi outposts enter the axon by a kinesin-dependent default mechanism. Despite the fact that important differences exist between the organization of the neuronal cytoskeleton in *Drosophila* and mammals [97], they do not exclude that under normal conditions Golgi structures may be rapidly shipped in and out of the axon.

Clearly, the axonal localization of some, but not all the organelles of the secretory route represents a major challenge for the local biosynthetic hypothesis. However, several considerations must be taken into account before discarding this postulate. First, some protein modification and sorting functions may be carried out by organelles with mixed identity. The spine apparatus, a specialized form of the ER present in some dendritic spines, is an example of a neuronal organelle that combines typical ER functions, such as Ca^{2+} metabolism and membrane protein translocation, with specialized activities associated to the Golgi apparatus, like those carried out by giantin and α-mannosidase II [98–100]. Second, concerning the Golgi apparatus, the morphology of a putative axonal organelle may less conspicuous, or the organelle may be a transient structure that has escaped detection (Fig. 4). The current evidence in neurons, together with the diverse organization of secretory organelles in other animal cells during mitosis or in response to signaling cascades [101–106] and in plant cells [107] argues that size, morphology, and topological organization of biosynthetic organelles are more plastic than previously thought. Therefore, the lack of a conventional secretory ensemble is not sufficient to refute the axonal secretory pathway hypothesis.

3. The axonal ER in pathology

The integrity of the axonal ER may be compromised in devastating neurodegenerative disorders, but is still unknown whether they

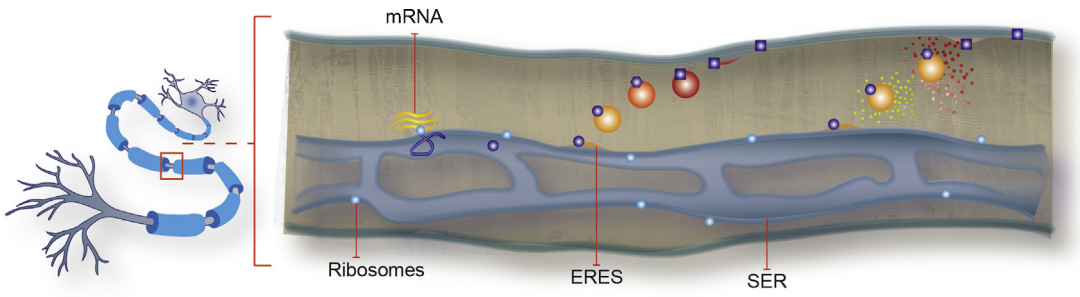

Fig. 4. Axonal ER trafficking. The ER in axons is associated to ribosomes and assembles functional ER exit sites (ERES, orange). A controversy still remains regarding other secretory organelles. Little information is available of the ERGIC and no conventional stacked Golgi structures have been observed. However, many resident proteins of secretory organelles have been reported, which suggest that a non-conventional secretory arrangement of the secretory route operates in axons. Two speculative models are envisioned to incorporate these observations. First, proteins may leave the ER and may be modified and sorted through organelles with mixed identity that mature along the final trafficking stages (left). Alternatively, proteins may leave the ER in cargo vesicles that encounter small and less conspicuous organelles (right).

do so by modifying the axonal proteome through local mechanisms. Axons of an Alzheimer's disease mouse model that expresses the swAPPprp mutation show varicosities and accumulation of vesicular profiles that may result from secretory malfunction [108]. Additionally, cerebellar specific Atg5-null mice that exhibit motor damage accumulate large amounts of ER membranes in axons, which form highly stacked and lamellar structures [109]. It also remains to be established whether the axonal ER or other local secretory compartments contribute to the trafficking defects produced by the ataxia3 mutation of voltage-gated sodium channels [110]. Interestingly, a mutation associated to a familial form of amyotrophic lateral sclerosis (ALS) reorganizes the ER in cultured cells [111]. In agreement with these findings loss of the ER shaping protein reticulon-4A in the superoxide dismutase 1 (SOD1) G93A transgenic mouse model of ALS accelerates motor neuron degeneration [112].

Despite the potential relevance of axonal ER defects in prevalent disorders such as Alzheimer's disease or ALS, hereditary spastic paraplegias (HSPs) represent perhaps the most direct association between pathology and the axonal ER. HSPs constitute a group of diseases characterized by progressive stiffness and spasticity of the lower limbs, and are the result of axonal degeneration, affecting mainly the long motor tracts. Because the molecular causes are heterogeneous symptoms include cataracts, ataxia, epilepsy, cognitive impairment, peripheral neuropathy and deafness [113]. Interestingly, a predominant group of genes associated with HSPs, spastin, atlastin-1, and REEP1 encode proteins required for ER morphogenesis and dynamics [114,115]. Spastin, a gene associated with 45% of patients with autosomal dominant (AD) HSP, controls the association of the ER to the microtubule cytoskeleton [116–118]. Atlastin-1, a gene with AD-HSP 10–15% average prevalence, mediates homotypic fusion of membranous tubules of the ER network and loss of function inhibits axonal elongation [86,90,119,120]. Finally, REEP1 is an ER morphogen [121,122]. A fundamental question that remains largely unexplored is whether the HSP-associated mutations affect specific functions of ER such as intracellular transport and trafficking.

At first glance it seems paradoxical that mutations affecting the biogenesis and dynamics of a conserved organelle will alter primarily the function of axonal tracts, and not the function of the rest of the cellular repertoire, which rely, as neurons do, on the ER for secretory functions, lipid synthesis and Ca^{2+} metabolism. However, it is reasonable to propose that mutations that alter the function of the ER will preferably perturb large and highly polarized cells such as neurons because subtle malfunctions will be amplified over long distances. The recent identification of mutations in reticulon 2 that cause of HSPs further supports this hypothesis [123]. Therefore, long distance, which is a fundamental property of the nervous system and has provided great advantages to large animals is also an Achilles' heels which renders the system particularly sensitive to defects in subcellular organelles [124].

4. Perspectives

To advance our current understanding of the regulation of the axonal proteome a conclusive demonstration of protein synthesis, processing and trafficking needs to be established. Additionally several conceptual and technical limitations must be overcome. First, it is for the most part unknown which membrane and secreted proteins traffic through an axonal trafficking route. Second, no specific axonal components that control trafficking steps, such as ER or Golgi export, have been indentified, making it difficult to dissect an axonal secretory pathway. Third, EM studies have generated a convincing structural representation of the axonal ER, but the small diameter of axons has limited the precise analysis of ER components by light microscopy, antibody labeling and fluorescent reporters. New super-resolution microscopy techniques provide an attractive methodology to re-explore the axonal secretory pathway [125]. These tools may be particularly useful in identifying the repertoire of secretory components in peripheral axons, whose diameter of approximately 1 μm make them especially amenable to analysis at 25–100 nm resolution. Although these techniques do not yet provide a solution for fast temporal analysis they may allow a more in depth characterization of the structural components. Interfering with these secretory components in combination with conventional live cell imaging may subsequently be used to dissect the function axonal protein trafficking.

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