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New approaches for solving old problems in neuronal protein trafficking

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ABSTRACT

Fundamental cellular properties are determined by the repertoire and abundance of proteins displayed on the cell surface. As such, the trafficking mechanisms for establishing and maintaining the surface proteome must be tightly regulated for cells to respond appropriately to extracellular cues, yet plastic enough to adapt to ever-changing environments. Not only are the identity and abundance of surface proteins critical, but in many cases, their regulated spatial positioning within surface nanodomains can greatly impact their function. In the context of neuronal cell biology, surface levels and positioning of ion channels and neurotransmitter receptors play essential roles in establishing important properties, including cellular excitability and synaptic strength. Here we review our current understanding of the trafficking pathways that control the abundance and localization of proteins important for synaptic function and plasticity, as well as recent technological advances that are allowing the field to investigate protein trafficking with increasing spatiotemporal precision.

1. Introduction

Fundamental cellular properties are determined by the repertoire and abundance of proteins displayed on the cell surface. As such, the trafficking mechanisms for establishing and maintaining the surface proteome must be tightly regulated for cells to respond appropriately to extracellular cues, yet plastic enough to adapt to ever-changing environments. Not only are the identity and abundance of surface proteins critical, but in many cases, their regulated spatial positioning within surface nanodomains can greatly impact their function. In the context of neuronal cell biology, surface levels and positioning of ion channels and neurotransmitter receptors play essential roles in establishing important properties, including cellular excitability and synaptic strength. These properties are subject to potent regulation by neural activity, which directly influences the behavior of cellular trafficking processes. For example, the molecular composition and organization of the post-synaptic plasma membrane is regulated by plasticity-stimuli that trigger long-lasting forms of synaptic plasticity including long-term potentiation, depression (LTP and LTD) and homeostatic scaling. These diverse and important forms of neural plasticity are largely controlled both by mobilization of ion channels and receptors from intracellular stockpiles to the cell surface and regulated positioning of existing surface proteins within functional nanodomains at synaptic sites (Hayashi et al., 2000; Lledo et al., 1998; Luscher et al., 1999; MacGillavry et al., 2013; Nair et al., 2013; Park et al., 2004; Penn et al., 2017; Sinnen et al., 2017; Tang et al., 2016). Given the central importance of protein trafficking for neuronal function and plasticity, it is not surprising this has remained an area of intense investigation. Here we review our current understanding of the trafficking pathways that control the abundance and localization of proteins important for neuronal function and plasticity, as well as recent technological advances that are allowing the field to investigate protein trafficking with increasing spatiotemporal precision. We begin by reviewing our current understanding of the organization and function of the neuronal secretory pathway for trafficking newly synthesized proteins at remote sites in dendrites. We then review recent studies investigating post-secretory trafficking of synaptic proteins through endocytic recycling and lateral diffusion. Finally, we highlight new molecular tools that hold promise for addressing challenging problems in neuronal protein trafficking.

1.1. Organization of the dendritic secretory pathway for remote protein trafficking

Compared to other cell types, neurons place extreme demands on the protein biosynthetic pathway due to their large size, intricate morphology and requirement for precise temporal control of protein levels within diverse cellular domains and structures (Fig. 1). How the correct proteins are delivered in the appropriate amounts to remote sites in the dendritic arbor has remained a persistent and challenging question in the field. This issue is epitomized by modeling studies that estimate trafficking from the neuronal cell body to specific sites within the dendritic arbor could take several hours to days (Williams et al.,

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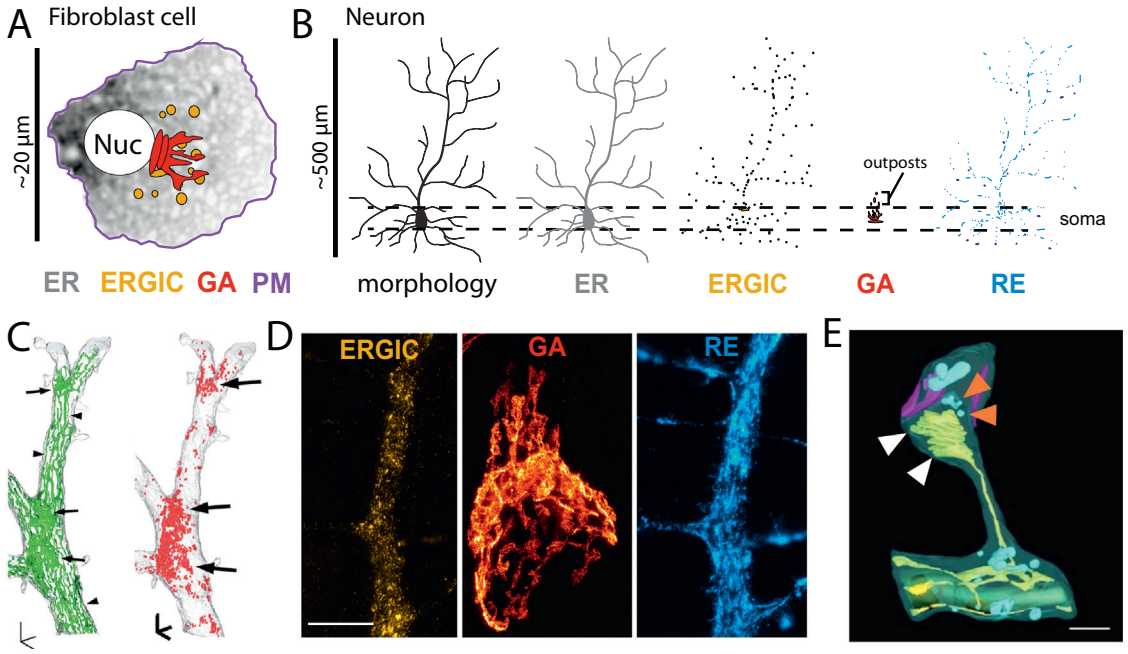

Fig. 1. Organization of the secretory network.
(A) Schematic of secretory organelles organization in a fibroblast cell.
(B) Schematic of secretory organelle organization in a neuron.
(C) Three-dimensional reconstructions of serial electron micrographs showing the distribution of the ER (green, *left*) and ER-bound ribosomes (red, *right*) in a dendritic segment of a hippocampal CA1 pyramidal neuron. *Left*: Elongated ER tubules in straight dendritic segments (*arrowheads*) whereas more complex ER cisternal sheets are found at dendritic branch points (*arrows*). *Right*: The density of ER-bound ribosomes at branch points (*arrows*) corresponds to the sites of ER complexity. Scale bars, 1 µm. Adapted from Cui-Wang et al. (2012) and reprinted with permission from Elsevier, copyright 2012.
(D) Stimulated emission depletion (STED) microscopy images of cultured cortical neurons expressing ERGIC-GFP (*left panel*) and TfR-mCh (*right panel*) to label dendritic ERGIC and recycling endosomes respectively. The middle panel shows the soma of a cultured cortical neuron stained with the *cis*-Golgi marker GM130 (*middle panel*). Scale bar, 5 µm.
(E) Three-dimensional model of organelles in a dendritic spine (from focused ion beam-scanning electron microscopy [FIB-SEM] image stacks, mouse cerebral cortex). ER/spine apparatus (*yellow*), endosomes/transport vesicles (*light blue*), synaptic interface (*magenta*), mitochondria (*green*). White arrowheads mark two ER-PM contact sites. Note the presence of endosomes/transport vesicles at the tip of the spine apparatus (*orange arrowheads*). Scale bar, 400 nm. Adapted from Wu et al. (2017) and reprinted with permission from the National Academy of Sciences.

2016), yet diverse forms of protein synthesis-dependent plasticity operate with synapse-level accuracy on significantly shorter timescales (Buffington et al., 2014; Hanus and Schuman, 2013; Mameli et al., 2007; Sutton et al., 2006; Sutton and Schuman, 2006). This problem has been recognized and investigated for decades, with early studies providing a crucial piece of the puzzle by localizing components of the biosynthetic machinery, including polyribosomes, near synaptic sites within proximal and distal dendrites (Bodian, 1965; Steward and Fass, 1983). Later studies confirmed the presence of diverse mRNAs within dendrites supporting a model where local protein synthesis supports a significant fraction of the dendritic proteome (Holt and Schuman, 2013).

1.2. Organization of the dendritic ER

Intriguingly, many of the mRNAs identified in dendrites encode integral membrane proteins and secreted factors that require specialized processing through the cellular secretory network, which primarily consists of the endoplasmic reticulum (ER) and Golgi apparatus (GA) (Cajigas et al., 2012; Zhong et al., 2006). The endoplasmic reticulum (ER) is broadly distributed throughout the dendritic arbor (Fig. 1B,C) (Spacek and Harris, 1997; Wu et al., 2017). Signal recognition particle (SRP), which recognizes and targets nascent polypeptides and their associated ribosomes to the ER is also present within dendrites (Tiedge and Brosius, 1996). Accordingly, ribosome-associated, or "rough", ER is observed at remote dendritic sites, confirming decentralized translation of integral membrane and secreted factors (Bodian, 1965; Cui-Wang et al., 2012; Gardiol et al., 1999; Pierce et al., 2000; Tiedge and Brosius, 1996). Following translation, nascent proteins can laterally diffuse within the ER membrane (or in the case of soluble secreted proteins, within the ER lumen) prior to ER exit (Cui-Wang et al., 2012; Fukatsu et al., 2004). Lateral mobility within the ER is an especially important consideration for many neurotransmitter receptors, which may linger in the ER for tens of minutes to hours (Cui-Wang et al., 2012; Greger et al., 2002; Valenzuela et al., 2014). This raises the question of whether these proteins could be locally trafficked to synaptic sites near their birthplace, or if spatial information is blurred or completely lost as they laterally diffuse within the ER, away from their sites of biogenesis. To quantitatively address this issue, Cui-Wang et al. used a combination of photoactivation and photobleaching, to show that specialized regions of ER adopt a complex and convoluted morphology (usually near dendritic branch points) that act as diffusion barriers for nascent proteins within the ER (Fig. 1C). Intriguingly, metabotropic glutamate receptor activation, which stimulates local translation, also increased ER complexity by regulating its dissociation from dendritic microtubules (Cui-Wang et al., 2012). The degree of ER complexity correlated with the density of ER-attached ribosomes (Fig. 1C), further supporting a model where morphological plasticity of ER structure could spatially confine cargoes during synthesis for targeted delivery to nearby synapses. Although no studies have directly investigated the spatial relationship between where a cargo exits the ER and where it appears at the plasma membrane, it is tempting to speculate that local synthesis and forward trafficking of ion channels and receptors could play a role in defining and maintaining subcellular excitability and synaptic properties over spatial scales ranging from dendritic branches to individual synapses (Béïque et al., 2011; Lee