Trends in Neurosciences

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Spotlight

Local Sourcing of Secretory Proteins in Faraway Places

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A recent paper by Carter *et al.* identifies a novel organelle, the ribosome-associated vesicle (RAV), that might serve as a portable, local factory for producing proteins destined for the secretory pathway. The appearance of RAVs in dendrites suggests they may serve to generate membrane and secreted proteins in distal processes.

The endoplasmic reticulum (ER) is the largest intracellular organelle and is comprised of an extensive continuous membrane throughout the cell. The ER is broadly separated into two regions: rough ER (RER), named for the presence of ribosomes, and smooth ER (SER), without ribosomes. Together these regions perform important cellular functions, including translation of membrane and secreted proteins, lipid homeostasis, protein glycosylation, and Ca^{2+} homeostasis through storage and release of this ion [1]. In neurons, the ER extends throughout the cell, including dendritic shafts, spines, axons, and nerve terminals, although only the SER is thought to be present in distal locations, while the RER is found in proximal dendrites and the cell soma (Figure 1A) [2,3]. Disruptions in ER morphology have been linked to neurodegenerative diseases, such as hereditary spastic paraplegia [4].

In a recent paper, Carter *et al.* report the discovery of a new organelle that they name the ribosome-associated vesicle (RAV) [5]. There are many challenges to identifying a *bona fide* new organelle in a microscope. Among the biggest challenges is not being fooled by the limits of resolution or labeling. This is illustrated, for instance, in Carter *et al.*'s analyses that led to the identification of RAVs.

The KDEL retention sequence and its receptor ensure that ER-resident proteins do not escape into the secretory pathway. Carter *et al.* noticed that in the numerous secretory cells they examined, expression of an mNeon-KDEL led to complete labeling of the reticular ER network but also to the appearance of mobile fluorescent puncta, particularly near the cell periphery. Such puncta could result from overexpression of such a fluorescent tag overwhelming the retention system, thereby allowing the label to escape into the secretory pathway. However, the authors deployed a battery of approaches to identify unequivocally that these mobile puncta are actually ~400-nm spherical vesicles derived from the ER. Furthermore, use of both correlative cryo-electron microscopy (EM) and EM tomography showed that these vesicles are studded with 80S ribosomes, so many of them that it allowed single particle averaging from cryo-EM tomograms of the RAVs to obtain a ~15 Å structure of the ribosome. These ribosomes were arranged in a manner indicative of polysomes, implying that they were translationally active (Figure 1B) [5]. These findings suggest that RAVs are a mechanism for secretory cells to control protein translation at the cell periphery.

In neurons, RAVs were found to be present in dendrites, which suggests a role in local translation. Strong evidence supports local translation as a mechanism to control the local proteome of distal regions of a neuron in response to changing conditions. However, questions remain about the trafficking and biogenesis of ribosomes in these regions [6]. RAVs, a mobile and ribosome-containing subcompartment of the cell, could prove to be a new mechanism to recruit translation machinery on demand, as neurons may use RAV trafficking to position the machinery necessary for local translation in dendrites. Our understanding of local translation in neuronal processes is still relatively primitive. For example, it is unclear if local protein synthesis is called upon for simple regular maintenance of local function due to protein turnover or if it is specifically engaged during periods where rapid changes in synapse function are needed. It will be interesting in the future to see if RAVs are engaged in either of these or perhaps other scenarios.

One of the best-studied organelle–organelle contacts is that between the ER and mitochondria. RAVs, like their parent ER, were also found to form tight contacts with mitochondria [5]. The role of specialized organelle–organelle contacts is an active area of research and, given that lipid handling proteins are often enriched in these contacts [7], RAV function may have a specific lipid requirement that depends on coordinated lipid exchange with mitochondria. It is also possible that the tethering to mitochondria simply helps ensure an adequate nearby supply of ATP. A recent study shows, for example, that the local protein synthesis required for synaptic plasticity in dendrites was powered by local mitochondria [8].

The unique structure of RAVs is an interesting hybrid of the sheet and tubule morphology of the ER. The RER is comprised mostly of long sheets that are connected by helicoidal motifs, a structure reminiscent of a parking garage [9]. These sheets contain very little curvature, except at their edges, and the presence of ribosomes have been suggested to stabilize this sheet morphology [1]. RAVs provide an interesting counterpoint to this hypothesis as they are highly curved structures containing ribosomes. Instead, RAVs share morphological similarity to ER tubules, which are highly dynamic and curved members of the ER but are typically devoid of ribosomes [1]. In neurons, both stacked ER sheets and ER tubules are present in

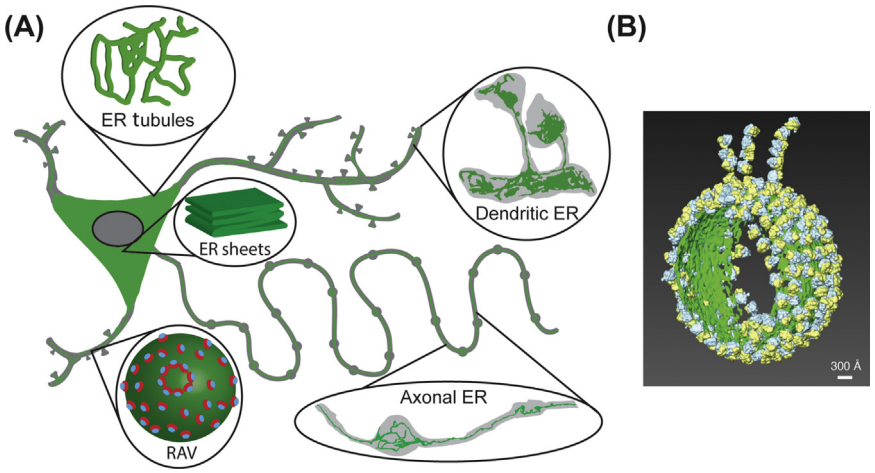

Figure 1. Morphology of the Endoplasmic Reticulum (ER) in Neurons. (A) The morphology of the ER (green) is shown in multiple regions of a neuron (plasma membrane shown in gray). Close to the center of the soma the ER is comprised mostly of sheets. Moving towards the periphery of the cell body, the ER is composed more of ER tubules and, potentially, ER matrices. The ER extends throughout the dendrites, including some dendritic spines, and throughout axons, sometimes forming elaborate structures in boutons. In dendritic regions, ribosome-associated vesicles (RAVs) like puncta were observed. RAV is shown with ribosomes (blue and red). The central rosette structure is indicative of polysomes of active translation. ER sheet morphology is based on [9] and axonal and dendritic ER morphology is based on [3]. (B) Cutaway showing polysome arrangement on RAVs. Note the chain of ribosomes approaching the RAV membrane. Yellow, 40S subunit; blue, 60S subunit; green, RAV membrane. Reproduced, with permission, from [5].

the cell body, though the stacked ER sheets are not as abundant as in other secretory cells (Figure 1A) [9]. These hybrid properties are likely a way for the secretory cells to harness the protein production ability of the RER combined with the mobility of the tubular SER. It will be interesting to see which of the known ER-shaping proteins may be involved in RAV formation.

The extended architecture of neurons, with processes far from the cell body, poses unique challenges for these faraway places. Carter *et al.*, with their characterization of RAVs, provide a potential solution to the question of how secreted and membrane proteins are produced in the dendrites of neurons. These ER-derived, ribosome containing, and highly mobile organelles suggest involvement in local translation, whereas the close contacts of RAVs with mitochondria implicate lipid trafficking between these organelles. At the cell soma, most proteins in the secretory pathway typically get glycosylated in their passage through the Golgi apparatus. By analogy, if RAVs are used for local secretory pathway protein production, it is likely coordinated with a local Golgi-like organelle as well, such as the Golgi outposts present in dendrites [10]. The unique architecture of RAVs, displaying a fusion of SER and RER properties, suggests an important role for RAVs in secretory cells. The discovery of RAVs is the beginning of what we expect will be a fruitful series of investigations into the role and function of RAVs in secretory cells and especially neurons.

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References

1. Schwarz, D.S. and Blower, M.D. (2016) The endoplasmic reticulum: structure, function and response to cellular signaling. *Cell. Mol. Life Sci.* 73, 79–94
2. Spacek, J. and Harris, K.M. (1997) Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. *J. Neurosci.* 17, 190–203
3. Wu, Y. *et al.* (2017) Contacts between the endoplasmic reticulum and other membranes in neurons. *Proc. Natl. Acad. Sci. U. S. A.* 114, E4859–E4867
4. Fowler, P.C. *et al.* (2019) NeurodegenERation: the central role for ER contacts in neuronal function and axonopathy, lessons from hereditary spastic paraplegias and related diseases. *Front. Neurosci.* 13, 1–20
5. Carter, S.D. *et al.* (2020) Ribosome-associated vesicles: a dynamic subcompartment of the endoplasmic reticulum in secretory cells. *Sci. Adv.* 6, eaay9572
6. Holt, C.E. *et al.* (2019) Local translation in neurons: visualization and function. *Nat. Struct. Mol. Biol.* 26, 557–566
7. Kumar, N. *et al.* (2018) VPS13A and VPS13C are lipid transport proteins differentially localized at ER contact sites. *J. Cell Biol.* 217, 3625–3639
8. Rangaraju, V. *et al.* (2019) Spatially stable mitochondrial compartments fuel local translation during plasticity. *Cell* 176, 1–12
9. Terasaki, M. *et al.* (2013) Stacked endoplasmic reticulum sheets are connected by helicoidal membrane motifs. *Cell* 154, 285–296
10. Valenzuela, J.I. and Perez, F. (2015) Diversifying the secretory routes in neurons. *Front. Neurosci.* 9, 358