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The Curious Case of the Soluble Protein

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How neurons tackle the challenge of soluble protein delivery to the distal axon has long puzzled neuroscientists. Reporting in *Neuron*, Scott et al. (2011) show that this axonal transport occurs through motor-dependent formation of dynamic heterogeneous protein complexes that pause upon complex disassembly and regain motility upon reassembly.

Most of us were introduced to protein chemistry through their categorization as membrane-associated, cytoskeleton-associated, or soluble. Soluble proteins—such as glycolytic enzymes or ubiquitous regulatory proteins like calmodulin—were the easiest to manipulate and understand in vitro: they entered our buffer solutions readily, exhibited proper enzymatic properties, and generally behaved well. However, when we looked at these proteins in a cellular context, their behavior was anomalous. For example, glycolytic enzymes and related proteins are not uniformly distributed in cells. Rather, they are preferentially enriched at the i-bands of muscle, and they exhibited colocalization (Sullivan et al., 2003). Understanding the behavior of these proteins in neurons presented another set of problems. Relative to other cells, neurons are big: some human neurons may be a meter or more in length. They are also highly polarized, such that all or nearly all of the proteins are synthesized in the cell body, but many soluble proteins are localized to the distal axon and synaptic terminals. How were these proteins delivered to the distal axon? In a study published in a recent issue of *Neuron*, Scott et al. (2011) use a combination of modeling and innovative imaging to shed new light on this question.

Early studies using pulse-chase labeling of neuronal proteins with radiolabeled amino acids found that soluble cytoplasmic proteins moved in an anterograde direction (away from the cell body) at roughly 2–4 mm/day, corresponding to one of the slow components of axonal transport, slow component b (SCb) (Brady and Lasek, 1981; Garner and Lasek, 1982). In contrast, cytoskeletal components like neurofilament and microtubule proteins moved more slowly (0.1–1 mm/day) as a part of slow component a (SCa), and membrane-associated proteins moved in fast axonal transport, with a rate that was two orders of magnitude faster (250–400 mm/day). Detailed analysis of changes in the distribution of labeled cytoplasmic proteins over days did not match predictions for a freely diffusible protein (Garner and Lasek, 1982). Soluble cytosplasmic proteins moved away from the cell body and remained as a discrete slow-moving peak of labeled protein for days. Some proteins showed little broadening of the peak over days or weeks while continuing to march toward terminals. Others exhibited a comparable overall rate, but a fraction trailed behind, presumably being deposited in the axon. Unfortunately, tools for analyzing soluble proteins in SCb at a higher temporal or spatial resolution were unavailable at the time. Researchers were left to speculate about the possibility that soluble proteins formed a complex, perhaps involving actin filaments, that could be moved. However, testing this model was a challenge that went unmet for years.

The real breakthrough in the analysis of single-molecule dynamics in living cells came with the demonstration that green fluorescent protein (GFP) could be introduced and visualized in living cells (Chalfie et al., 1994). Monomeric GFP behaved like a true, freely diffusible protein in the cytoplasm, filling the available volume and rapidly equilibrating. However, GFP fused with endogenous soluble proteins often behaved very differently, exhibiting discrete localizations and limited mobility. Refinement of live-cell imaging and labeling methods allowed investigators to revisit slow axonal transport. Initially, the focus was on movement of cytoskeletal proteins like neurofilament and microtubule proteins associated with SCa. Surprisingly, once the sensitivity of methods allowed for the acquisition of images at higher frame rates (Brown, 2003), we could not only see movement but could also start to identify motor proteins responsible for movement. Remarkably, microtubules and neurofilaments moved rapidly at rates comparable to fast axonal transport but did so infrequently, thus leading to a slower net rate.

Questions still remained about how soluble proteins of SCB might be transported. Some studies began addressing these questions by using lessons learned from the work on microtubules and neurofilaments in SCa to develop methods for the analysis of soluble, cytoplasmic proteins like fluorescently tagged GAPDH, α-synuclein, and synapsin-1 (Roy et al., 2007; Roy et al., 2008). The results were instructive: all three proteins moved rapidly but infrequently, with long pauses during axonal transport. Movements were analogous to, but distinct from, cytoskeletal protein and vesicle movements. The tagged SCb proteins could often be seen to move coordinately as an apparent complex, but the putative complexes were not always observed (Roy et al., 2007). Surprisingly, this movement was not affected by changes in axonal actin filaments (Roy et al., 2008), which had been proposed to serve as a scaffold for SCb based on the presence of actin in SCb slow axonal transport and analogies to muscle cells (Clarke and Masters, 1975). Thus, the old puzzle of how neurons organize and move soluble proteins continued to confound us.

The observations of Scott et al. (2011) now begin to offer clues to solving this puzzle. The authors used photoactivatable-GFP (PA-GFP) constructs to tag cytosolic proteins in different locations and facilitate analysis. Using this approach, Scott et al. (2011) saw that

monomeric PA-GFP behaved just like a freely diffusible protein should, exhibiting rapid symmetrical dispersion. In contrast, PA-GFP forms of synapsin and camodulin kinase IIa (CamKIIa) behave very differently: they disperse slowly as a plume, with a distinct anterograde bias. Interestingly, these proteins exhibit a granular appearance that is more suggestive of particles than the uniform distribution seen with freely diffusing GFP. As in previous studies, these ''particles'' moved rapidly but infrequently.

Significantly, the anterograde bias was abolished by treatments that interfere with motor function (N-ethylmaleimide [NEM]). NEM treatments do not convert the PA-GFP-tagged synapsin or CamKIIa into freely diffusing proteins but instead appear to limit mobility of the PA-GFP SCb proteins without affecting the diffusion of monomeric PA-GFP. Treatments to inhibit ATP production and disrupt microtubules similarly inhibit the anterograde bias. These observations indicate that the SCb movement, like SCa and fast axonal transport, are dependent on microtubule-based motor proteins.

The movement of these proteins is not a case of soluble proteins piggybacking on membrane vesicles. Indeed, the authors observed that the movement of integral membrane proteins is distinct from the plumes of anterograde SCb transport. Furthermore, analysis of the small fraction of synapsin that moves conjointly with synaptophysin-containing vesicles showed that these particles moved independently of the larger SCb pool.

Based on their observations, Scott et al. (2011) reached the conclusion that cytosolic proteins dynamically form multiprotein, heterogeneous complexes in the axon. These complexes move in SCb through interactions with microtubule-based motor proteins, pausing when the complexes disassemble, only to reassemble later for additional movements. This model of dynamic assembly and disassembly is a fresh approach to explaining how soluble proteins are organized and moved down the axon. Such dynamic complexes may have more general implications for organizing metabolic units within the cell, because this dynamic behavior would allow rapid exchange of these proteins with different compartments and partners to create distinct functional units. Understanding the relevant time constants and regulation of complex formation will help us better understand the dynamic organization of the cell, giving new insights into how one would build and maintain a neuron.

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