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Dynein's Life in the Slow Lane

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Dynein synthesized in neuronal cell bodies is conveyed into the axon by slow transport, but underlying mechanisms are unclear. In this issue, Twelvetrees et al. (2016) propose a model where dynein is transported by direct—but transient—interactions with kinesin.

The retrograde motor dynein is famously involved in moving endosomes from the axon tip back to the cell body. However, like most neuronal proteins, dynein is synthesized in the soma and must be transported anterogradely up to the axon tip. Overall, anterograde axonal transport of dynein was characterized by pulse-chase radiolabeling studies in the 1990s, revealing that that the majority of somatically synthesized dynein is not conveyed with vesicles, but instead moves in a distinct rate-class called slow component b or SCb (Dillman et al., 1996a, 1996b). SCb is a heterogeneous transport group containing hundreds of soluble cytosolic proteins, sluggishly moving along axons at overall rates of ~2–8 mm/day. This movement is orders of magnitude slower than fast transport of membrane-anchored proteins at ~50–400 mm/day (reviewed in Roy, 2014; also see Figure 1A).

How is this slow, anterogradely biased motion of dynein generated? Although pulse-chase radiolabeling techniques can characterize the phenomenon of axonal transport, they are of limited use in dissecting mechanisms as they lack spatiotemporal resolution and are not amenable to experimental manipulations. To examine dynein motility in living axons, Twelvetrees et al. from Erika Holzbaur's lab imaged cultured hippocampal neurons from a knockin "dynein-GFP" mouse, where the GFP is tagged to the C terminus of an isoform of the neuron-specific dynein intermediate chain DIC1 (Zhang et al., 2013). The tagged DIC1 is expressed at endogenous levels and is functionally incorporated into the multi-subunit dynein complex, as shown previously (Ayloo et al., 2014; Zhang et al., 2013). Using these neurons, the authors saw a striking accumulation of dynein-GFP at axon tips, suggesting active targeting mechanisms. Upon photobleaching a small segment of the distal axon and growth cone, there was a slow recovery of fluorescence over several minutes, suggesting slow transport.

To directly visualize dynein-GFP dynamics in axon shafts, Twelvetrees et al. used a modification of a recently described technique to examine slow axonal transport of cytosolic SCb cargoes in living neurons. The essence of this method is to optically highlight a discrete pool of fluorescent-tagged cytosolic molecules within the axon shaft and evaluate the mobility of this fluorescent pool by live imaging. The overall fluorescence dispersion is anterogradely biased, thought to represent slow axonal transport. The biased motion is quantitatively analyzed by measuring net displacement of the highlighted molecules over time (see schematics in Figures 1B and 1C for logic of these experiments; see also Roy et al., 2012; Scott et al., 2011; Tang et al., 2013). Specific to their experiments, Twelvetrees et al. highlighted a pool of dynein-GFP in axons by photobleaching neighboring regions in an axon from the GFP-dynein knockin mice, creating a "photoprotected zone" of GFP flanked by bleached areas. Indeed there was a slow, anterogradely biased movement of GFP-dynein, and the overall velocities were in agreement with known rates of dynein, as determined by radiolabeling studies. Moreover, this movement was microtubule dependent (also seen with other SCb proteins; see Scott et al., 2011).

What is the mechanism underlying the slow, biased transit of dynein in axons? In previous studies, the Holzbaur lab had found direct interactions between DIC1 and kinesin (Ligon et al., 2004), and they wondered if these interactions were driving the slow axonal transport of dynein. Mapping the binding region of DIC1 to kinesin in COS cells, they show that DIC1 directly interacts with the TPR domains of kinesin light chains (KLCs), as well as the stalk region of kinesin heavy chain. Dynein and kinesin also co-segregated in brain fractions, suggesting in situ interactions. However, these experiments do not show that dynein-kinesin interactions are relevant for the slow transport of dynein. To address this, Twelvetrees et al. tested the effect of a peptide predicted to block DIC1-KLC interactions on dynein-GFP mobility in cultured hippocampal neurons using the "photoprotection assay." Indeed, incubating neurons with the peptide decreased the overall anterograde bias of dynein-GFP in their assay, suggesting a role for DIC1-kinesin interactions in dynein transport. Though difficult to visualize clearly, the authors also saw rapid but very transient movement of small dynein-GFP puncta in axons, suggesting that the overall slow movement was generated by rapid, short-range dynamics.

The authors propose a model where "unstable kinesin recruitment" by the dynein-SCb cargo leads to "short spontaneous runs followed by dissociation [of dynein] from the microtubule track and disassembly of the [dynein] transport complex." They call this model "kinesin limited," as dynein-SCb cargoes would have a limited ability to bind kinesin in this scenario. In principle, this model is conceptually similar to the "dynamic

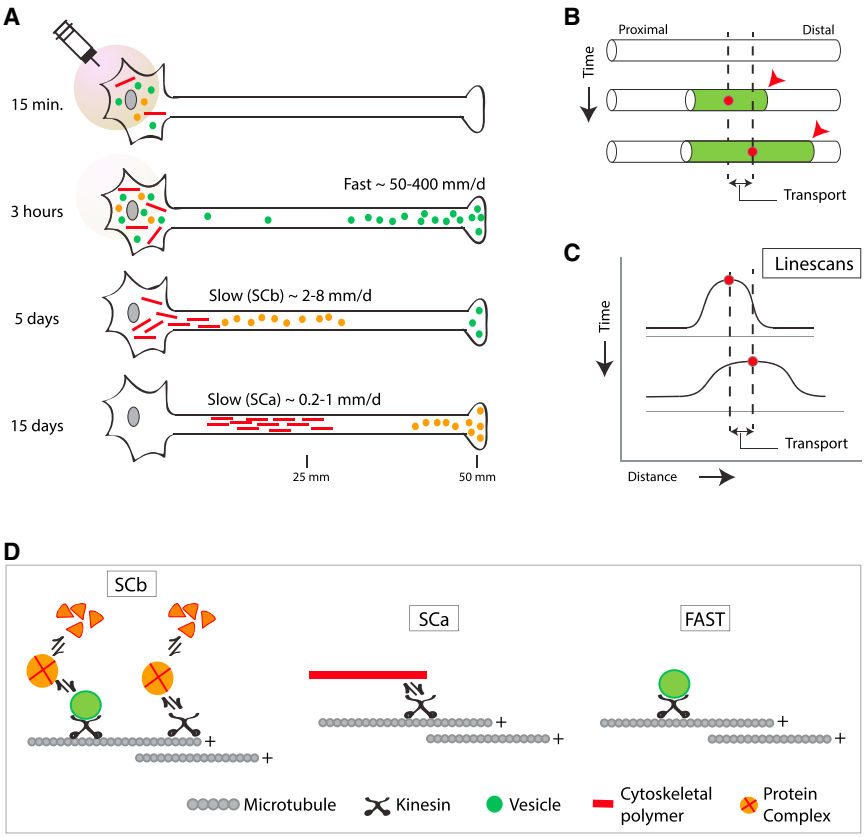

Figure 1. Mechanisms of Fast and Slow Axonal Transport

(A) Schematic of pulse-chase radiolabeling experiments. Note two overall rates: fast transport carrying vesicular proteins (green dots), and a slower movement carrying soluble/cytosolic and cytoskeletal proteins (orange dots and red bars, respectively; reproduced from Roy, 2014, with permission).

(B) Schematic of live imaging assays to study slow transport in cultured neurons. A discrete pool of soluble/cytosolic molecules (of a given SCb protein) is optically highlighted in axons (green), and the biased dispersion of highlighted pool is recorded by live imaging. Note anterogradely biased flow of highlighted molecules (leading tip marked by red arrowhead) and corresponding anterograde shift of fluorescence center (marked by red dots and vertical dashed lines).

(C) Illustrative intensity line scans of axons in (B).

(D) Working models of slow and fast axonal transport.

recruitment" model proposed for other SCb proteins like synapsin and calcium/calmodulin-dependent kinase, where clustering/dispersion of dynamic SCb cargo complexes and association with a persistently moving "mobile unit" would drive the cytosolic cargo in slow transport (Scott et al., 2011; also see Figure 1D, left). Both models posit that the sluggish movement of the overall population is achieved by restricting the duration for which the assembled SCb cargo complex associates with a persistently moving unit. However, in case of synapsin—a protein known to peripherally associate with vesicles—inhibiting vesicle transport led to a decreased synapsin transport as well, leading to the idea that the SCb cargo in this case transiently associates with anterogradely moving vesicles (Tang et al., 2013). Although a direct interaction between synapsin-SCb cargoes and kinesins cannot be ruled out, a difference between the "kinesin limited" and the "dynamic recruitment" models seems to be the way the SCb cargo interacts with the moving unit—directly to motors in one, but to anterogradely moving vesicles in the other (Figure 1D, left).

One possibility is that both mechanisms may be operating in SCb transport. Overall, SCb is an extremely heterogeneous transport group containing hundreds of proteins including metabolic enzymes, ubiquitins, clathrin, heat-shock proteins, motor proteins, actin/actin-related proteins, and miscellaneous cytosolic/soluble proteins that are difficult to categorize (reviewed in Roy, 2014). Thus, SCb is somewhat different from the other rate classes that are relatively homogenous in overall cargo composition. For instance, fast transport is the movement of membranes (vesicles), and slow component a (or SCa), the other "slow" rate class, is primarily composed of cytoskeletal proteins. The lack of a common "compositional theme" in SCb has been somewhat puzzling, since it is difficult to imagine how such a diverse group of seemingly unrelated proteins can be conveyed by a (presumably) common mechanism. But perhaps there is no such common mechanism.

Interestingly, radiolabeling studies that have examined SCb transport at a relatively higher spatial resolution in long axons have observed considerable variation in the transport rates of individual polypeptides within SCb, particularly within the crests and trails of the advancing radiolabeled "waves" (Garner and Lasek, 1982). Although these data have been interpreted as evidence for differential association of SCb cargoes with a common moving structure—the ones lagging behind associating for lesser times—they may also reflect distinct SCb transport mechanisms. While some SCb cargoes like synapsin, with innate peripheral associations with membranes, may move by transiently interacting with the latter, others like dynein might directly bind to the anterograde motor. Precise mechanisms that regulate the association and/or clustering of SCb cargoes to the persistently moving structure (kinesin or vesicle) are unknown, and will probably require characterization of the transport complexes.

The concept of sporadic transport by "fast" motors seems to be a common theme across all forms of slow transport, and this is undoubtedly a fundamental insight into the phenomenon (Brown, 2003; Roy et al., 2000; Wang et al., 2000; also see Figure 1D). The study by Twelvetrees et al. extends this idea and provides new mechanistic information regarding the delivery of a critical motor protein to its site of action in axons. It is interesting to consider why dynein would move in slow axonal transport. Wouldn't it make more sense to rapidly convey

this motor to the axon tip? One possibility is that the slow motion of dynein is related to its functionality in axon during transit. In that regard, SCb-dynein appears to be active, binding to microtubules in an ATP-dependent manner (Dillman et al., 1996b) and perhaps mediating axonal transport of short microtubules (He et al., 2005). Dynein is one of the most versatile motors known in biology, with many diverse roles, and it remains possible that this motor performs other, yet unknown roles as it slowly meanders along the axon. For instance, speculatively, the activated dynein, when free from the anterogradely bound kinesin in the "kinesin limited" model, might assist ongoing retrograde transport of vesicles. In principle, slow transport could provide a local pool of "on-board" active dynein molecules all along the length of the axon, making the overall retrograde transport more efficient (perhaps also helping to overcome potential transport blocks).

Unlike fast transport, where the basic mechanistic unit is clear (microtubule-motors-vesicles) and the field has moved on to dissect regulatory components, the arena of slow transport is riddled with mysteries. Despite the fact that slow transport is an established phenomenon, discovered over half a century ago, even the very basic tenets of this motion remain unclear. With the advent of new ways to visualize this motion and the development of model systems that can look at this motion at both the meso- and microscale, perhaps we can hope that some of these unknowns will be resolved in the near future.

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Melanopsin: The Tale of the Tail

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In this issue of *Neuron*, Mure et al. (2016) demonstrate that two mechanisms—phosphorylation of a C-terminal intracellular region, and mechanism involving the whole of the C terminus—oppositely shape the kinetics and sensitivity of the nonvisual photoreceptor melanopsin.

In 1980, Ebihara and Tsuji demonstrated that C3H mice—which are visually blind from the *rd1* mutation in Pde6b gene, causing severe outer retinal degeneration—could still synchronize their behavioral circadian rhythms to light:dark cycles as dim as 1 lux (Ebihara and Tsuji, 1980). Since enucleated animals cannot entrain their rhythms to lighting cycles, these results suggested the presence of a novel photoreceptor in the eye, which is spared in outer retinal degeneration. In 2002, Berson and colleagues discovered a novel class of photoreceptors, the intrinsically photosensitive retinal ganglion cells (ipRGCs), which mediate circadian entrainment (Berson et al., 2002). These cells were shown to express melanopsin, a then-orphan opsin expressed nearly exclusively in this small subpopulation of retinal ganglion cells. Melanopsin-driven light responses in ipRGCs underlie a wide range of behavior and physiology, including circadian entrainment, the pupillary light response, negative phototaxis in neonatal mice, and retinal vascular development, among others (Hattar