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Seeing the Unseen: The Hidden World of Slow Axonal Transport

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Abstract

Axonal transport is the lifeline of axons and synapses. After synthesis in neuronal cell bodies, proteins are conveyed into axons in two distinct rate classes—fast and slow axonal transport. Whereas fast transport delivers vesicular cargoes, slow transport carries cytoskeletal and cytosolic (or soluble) proteins that have critical roles in neuronal structure and function. Although significant progress has been made in dissecting the molecular mechanisms of fast vesicle transport, mechanisms of slow axonal transport are less clear. Why is this so? Historically, conceptual advances in the axonal transport field have paralleled innovations in imaging the movement, and slow-transport cargoes are not as readily seen as motile vesicles. However, new ways of visualizing slow transport have reenergized the field, leading to fundamental insights that have changed our views on axonal transport, motor regulation, and intracellular trafficking in general. This review first summarizes classic studies that characterized axonal transport, and then discusses recent technical and conceptual advances in slow axonal transport that have provided insights into some long-standing mysteries.

Keywords

cytosolic proteins, soluble proteins, slow axonal transport, transport packets, cargo complexes, diffusion

The term "axonal transport" typically invokes an image of tiny vesicles moving up and down axons—a view reinforced by YouTube videos. Though evocative, this is a limited portrait of axonal transport. Hidden from this picture, a deluge of cytoskeletal and soluble proteins are also moving along these very same axons, and their role in maintaining axonal and synaptic function is no less important than their vesicular counterparts. Known as slow axonal transport, these cargoes include microtubules, neurofilaments, actin/actin-related proteins, metabolic enzymes, chaperones, various soluble synaptic proteins involved in exo/endocytosis, and even molecular motors such as dynein and myosin—making slow transport indispensable for neuronal form and function. For decades, slow axonal transport has been recognized and studied but has remained difficult to visualize. However, significant advances in recent years have begun to reveal some of its secrets. This review first summarizes experiments that defined slow axonal transport and then outlines insights from recent studies that have visualized the phenomenon and uncovered new mechanistic details in the process.

What Is Fast and Slow Axonal Transport?

Our understanding of overall axonal transport is largely derived from classic pulse-chase studies, where newly synthesized perikaryal proteins are tagged by radiolabeled amino acids, and their movement into the axon is analyzed by autoradiography (see Fig. 1A and Roy and others, 2005). Although the bulk of these studies were done more than 30 years ago, they characterized the phenomenon in vivo and provide a template for interpreting contemporary experiments. Key insights from these studies in a variety of organisms (mice, rats, guinea pigs, rabbits, *Aplysia*, and others) are as follows. After perikaryal synthesis (1) a population of labeled proteins is conveyed rapidly at rates of ~50 to 200 mm/day (fast axonal transport); composed of membranous cargoes (Fig. 1B) and (2) a distinct pool is conveyed at much lower overall rates of ~0.2 to 10 mm/day (slow axonal transport); composed of *cytoskeletal proteins* (e.g., tubulin, neurofilaments protein, actin/actin-associated proteins, spectrin) as well as hundreds of *soluble* or *cytosolic proteins* (Fig. 1C). Examples of the latter include

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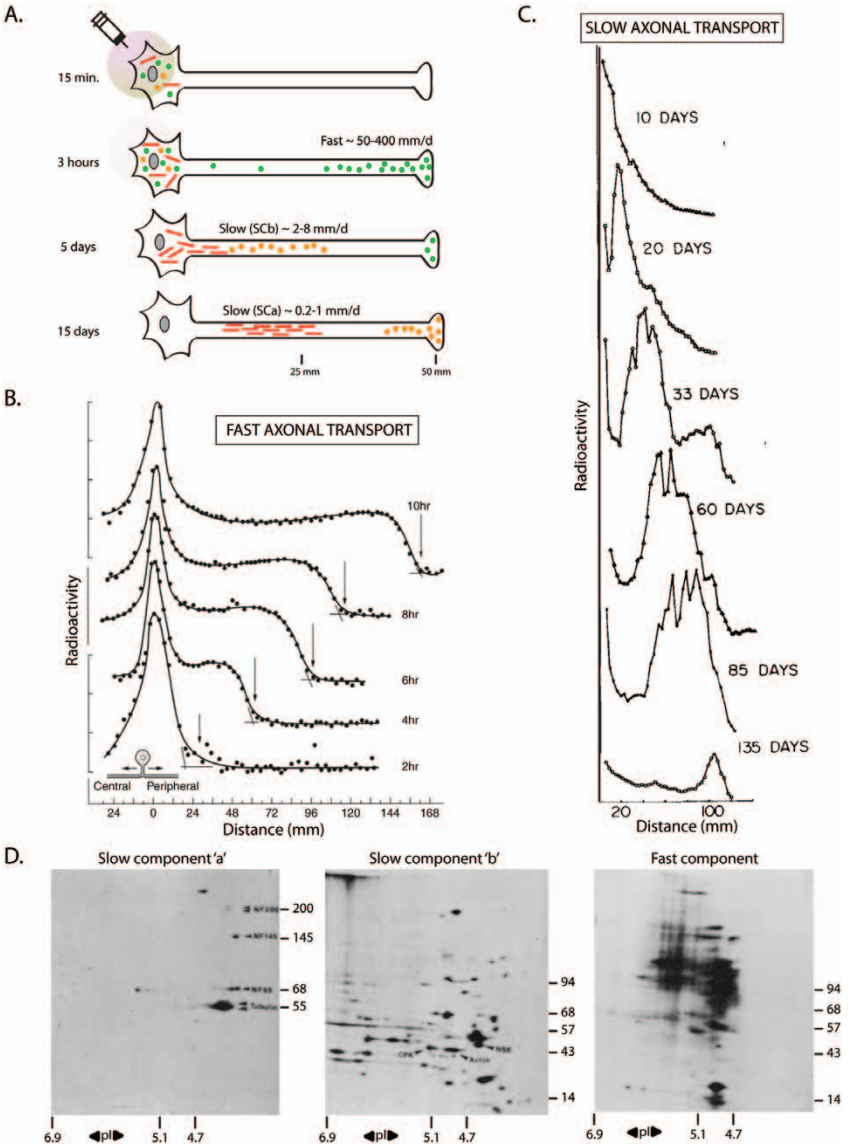

Figure 1. The pulse-chase radiolabeling paradigm to study axonal transport. (A) Radiolabeled amino acids injected in the vicinity of neuronal cell bodies of an adult animal are incorporated by newly synthesized proteins, and then transported into axons and distal synapses by endogenous processes. The movement of these proteins is then inferred by analyzing sequential axonal segments over incremental time-periods (for specifics of methods, see fig. 2 of Roy and others 2005). After labeling, a population of proteins (green circles) is rapidly conveyed into axons at rates of 50 to 400 mm/day ("fast component," vesicular cargoes). A second pool enters the axons at velocities that are several orders of magnitude lower at 0.2 to 8 mm/day ("slow component"). The slow component can be further resolved into two largely distinct "peaks" composed of cytosolic/soluble cargoes ("Slow Component b" or SCb—orange circles) or the major cytoskeletal cargoes ("Slow Component a" or SCa—red bars). (B) Kinetics of fast axonal transport in cat sensory axons accessed by pulse-chase radiolabeling. Note the rapid movement of the radiolabeled wave front along the peripheral axon over 10 hours (~4.5 μm/s). Also note the broad plateau behind the advancing 'front" suggesting deposition of cargoes (vesicles) during transit. (C) Kinetics of slow axonal transport in rat motor neurons accessed by pulse-chase radiolabeling. Note the extremely slow movement of the slow component wave front (~100 mm in over 100 days). (D) Two-dimensional polyacrylamide gel electrophoresis analysis of the three rate components. Transported radiolabeled proteins from mouse or guinea pig optic axons were separated by mass/charge, and analyzed by autoradiography. Some individual protein "spots" are identified by arrowheads on the gels—neurofilaments (NF) and tubulin (SCa, left); creatine phosphokinase (CPK), actin and nonspecific enolase (NSE, SCb, middle). Note the unique overall composition of the three rate classes. Isoelectric points are on the *x*-axis and molecular weights are on the *y*-axis. Figure (B) adapted from Ochs and others (1981); Figure (C) adapted from Hoffman and Lasek (1975); Figure (D) adapted from Brady and Lasek (1982)—all with permission.

metabolic enzymes (e.g., phosphofructokinase, creatine kinase, aldolase, enolase, glyceraldehyde-3-phosphate dehydrogenease [GAPDH], superoxide dismutase-1 [SOD-1]), heat shock proteins (e.g., hsp-70, hsc-73, chaperonin containing T complex protein-1 [CCT]), proteins involved in synaptic homeostasis (e.g., synapsin, α-synuclein, clathrin, calmodulin, calcium/calmodulin-dependent kinase [CamK]), motor proteins (dynein, dynactin, myosin), and several other cytosolic proteins such as ubiquitin, cyclophilin, annexin, as well as many that are not yet identified. In general, tubulin and neurofilament proteins move the slowest—at rates of ~0.2 to 1 mm/day (called "Slow Component a" or SCa)—whereas actin/actin-associated proteins and cytosolic/soluble proteins move a little faster, at rates of ~1 to 10 mm/day (called "Slow Component b" or SCb) (Baitinger and Willard 1987; Black and Lasek 1980; Brady and others 1981; Bray and others 1992; Dillman and others 1996b; Jensen and others 1999; Li and others 2004; Ma and others 2000; Nixon and others 1990; Sekimoto and others 1991; Willard and others 1974; Yuan and others 1999). Although soluble/cytosolic molecules have inherent diffusive properties, the coordinated movement of these proteins over large distances in axons is incompatible with free diffusion. In accordance with physical laws, free diffusion of molecules in axons exponentially decays over time and cannot explain any form of slow transport (Koike and Matsumoto 1985). Many other properties of cytosolic slow axonal transport are also incompatible with diffusion (for instance motor- and ATP-dependence, see below).

Cargoes Moving in Fast and Slow Axonal Transport

Although radiolabeling studies proved the existence of the two rate components, they could not visualize the transport directly, and cargo structures responsible for fast and slow transport remained unknown. Fast motile structures resembling vesicles were seen in early microscopic studies (reviewed in Grafstein and Forman 1980), and with advances in transmitted light microscopy and video imaging in the 1980s, rapidly moving vesicles were unequivocally seen in extruded squid axons (Allen and others 1982; Brady and others 1982). With subsequent discovery of the motor protein kinesin (Brady 1985; Vale and others 1985), it became obvious that the plethora of mobile vesicles was the visual correlate of "fast" radiolabel movement. Investigations spanning the next few decades—and continuing to this day—have provided numerous mechanistic insights into vesicle transport and this process is understood in detail (Twelvetrees and others 2012). But while tubulovesicular profiles of single vesicles could be pinpointed by their phase-dense silhouettes—or later, by live imaging of green fluorescent protein (GFP)/red fluorescent protein (RFP)–tagged membrane-spanning proteins—similar protocols could not be immediately applied to slow-component cargoes. The main reason was that the distribution of cytoskeletal and cytosolic proteins is typically continuous along axons, precluding visualization of individual moving structures. Thus in the absence of concrete visual evidence, the cargo structures conveyed in slow axonal transport was a subject of much debate throughout the 90s. In particular, the form in which cytoskeletal elements—actin, tubulin, and neurofilament protein—were conveyed was a source of contention, with some investigators favoring polymeric form and others a less-defined monomeric/oligomeric form (Baas and Brown 1997; Bray 1997; Hirokawa and others 1997; Vallee and Bloom 1991).

Axonal Transport of Cytoskeletal Polymers: The "Stop and Go" Model

The debate over whether cytoskeletal proteins could be transported as polymers or subunits showed its first signs of resolution in 2000, when moving neurofilament polymers were seen in axons (Roy and others 2000; Wang and others 2000). Though the neurofilament array in most axons is continuous as mentioned above, it happens to be naturally sparse in very thin axons of some cultured neurons, with "gaps" in distribution where there are no neurofilaments at all. When GFP-tagged neurofilaments were visualized in such axons by live imaging, single neurofilaments were seen to move in the "gaps." Studies over the past decade have resolved many mechanistic aspects of neurofilament transport (Li and others 2012), including ultrastructural demonstration that these moving assemblies are indeed single neurofilaments (Yan and Brown 2005), essentially settling reasonable doubt. But more important, seeing neurofilaments move revealed a long-standing secret of slow axonal transport.

Surprising at the time of initial discovery, neurofilaments moved rapidly with instantaneous velocities similar to moving vesicles. But compared with vesicles, neurofilament movement was very infrequent, and moreover, neurofilaments often paused during transit—unlike vesicles that move persistently. Thus, though a single neurofilament moved rapidly, the majority of neurofilaments in an axon (>90%) were paused at any given time (but could potentially move again). This infrequent and intermittent transport behavior of individual neurofilaments would, over time, expectedly result in an overall slow movement of the entire population. Christened the "Stop and Go" model, a substantial amount of evidence supports this concept (Brown, 2003; Brown and others

2005; Li and others 2012). An additional appeal of this model is the implication that the same kinds of "fast" motor proteins could drive both fast and slow axonal transport. This is important, as candidate "slow" motors, capable of producing the velocities of SCa or SCb by continuous engagement, were conspicuously absent from the avalanche of motor protein superfamilies discovered in the 1990s (Miki and others 2001).

Similarly, structures resembling short microtubules also move infrequently in cultured neurons (Wang and Brown 2002). This movement is motor dependent (He and others, 2005), suggesting that microtubules may also be transported in a "stop and go" manner (reviewed in Falnikar and Baas 2009). Microtubule movement in other cell types is well established, providing a precedent (Jolly and others 2010; Keating and others 1997). However, unlike neurofilament transport, where the evidence is compelling, some aspects of tubulin transport are still ambiguous. For example, it is not clear if microtubules also pause frequently, or how individual polymer movements seen in imaging experiments give rise to the overall slow transport of tubulin seen in radiolabeling studies. Also unlike neurofilaments that are almost entirely present as polymers in steady state, soluble pools of tubulin also exist in axons, and it has been proposed that soluble tubulin is transported (Galbraith and others 1999; Terada and others 2000).

Actin, the third major cytoskeletal protein in neurons, is also conveyed in slow axonal transport; largely in SCb (Black and Lasek 1979; Bray and others 1992; Willard and others 1979). Actin is a key protein in cellular homeostasis, involved in numerous physiologic events in neurons—structural, growth-cone/axon extension, axonal branching, cell signaling, and so on—yet surprisingly, mechanistic details of actin transport are almost entirely unknown. The reasons for this gap in knowledge is probably because of the technical difficulties of visualizing cytoskeletal transport as outlined above. Another unclear aspect is that cytoskeletal proteins can be dynamic—with subunits being added and removed from existing polymers—and prevailing models of axonal transport either do not take this into account, or assume a steady-state polymeric state. Though the latter is largely true for neurofilaments—validating the polymer transport model in this case—tubulin, and in particular actin, are unstable polymers (Mitchison and Kirschner 1984; Vavylonis and others 2005) and the interplay between dynamic instability and axonal transport may be an important aspect of its transport behavior in axons.

Cargoes of Soluble/Cytosolic Proteins: Insights from Radiolabeling Studies

Besides cytoskeletal proteins, cytosolic (or soluble) proteins are also conveyed in slow axonal transport—specifically in SCb. What are the mechanisms conveying these proteins? A look into radiolabeling studies offers three general insights. (1) Though the bulk of any given cytosolic protein is conveyed in SCb, small amounts of radiolabel (10% to 15%) are also seen in the fast component (Baitinger and Willard 1987; Garner and Lasek 1982; Jensen and others 1999; Lasek and others 1984; Lund and McQuarrie 2001, 2002; Paggi and Petrucci 1992; Petrucci and others 1991). Although this "fast pool" has been largely ignored, it may have important implications in understanding SCb transport, as discussed later. (2) A large number of proteins (more than 200 at least) move exclusively in SCb (see two-dimensional gels in Fig. 1D). Interestingly, the radiolabeled SCb pool entering synapses via axonal transport is quite large—about threefold greater than proteins conveyed by fast transport—and many are enriched at synapses (Fig. 2A). (3) Whereas cargo composition of SCa and fast component is largely thematic (cytoskeletal or vesicular cargoes, respectively), composition of SCb is disconcertingly varied—a potpourri of proteins (peripheral synaptic proteins, chaperones, metabolic enzymes, actin/actin-binding proteins, motors, etc.).

A look at individual SCb transport profiles also highlights an intriguing aspect of this rate class. When individual radiolabeled wave profiles of multiple SCb proteins are overlaid, there is a striking correspondence in overall waveforms, particularly in their "fronts" and the "peaks" (see Fig. 2B and Garner and Lasek 1982; Lasek and others 1984). This coherence is maintained even after several days of transit (note overlapping wave profiles of the same 20 SCb proteins at 4, 6, and 9 days in Fig. 2B). Despite the inherently limited resolution of radiolabeling studies, this is a remarkable phenomenon, invoking a model where diverse cytosolic SCb proteins associate with a common "carrier structure" (Garner and Lasek 1982). An extension of this idea is that the SCb proteins may themselves organize into cargo complexes, which would then bind to a common moving organelle. Indeed radiolabeling studies suggest that different SCb proteins are co-transported in axons. For instance actin is co-transported with several actin-binding proteins (Mills and others 1996), clathrin is co-transported with clathrin-binding proteins (Black and others 1991; de Waegh and Brady 1989), and dynein and dynactin proteins are also co-transported (Dillman and others 1996a; Susalka and others 2000). How are these sundry proteins transported in a common rate class, all of them creeping along the axon at rates of a few millimeters per day?

Visualization of Cytosolic/Soluble Protein Transport in Axons: A Tricky Business

It was generally thought that direct visualization of SCb transport would resolve mechanistic details of this rate

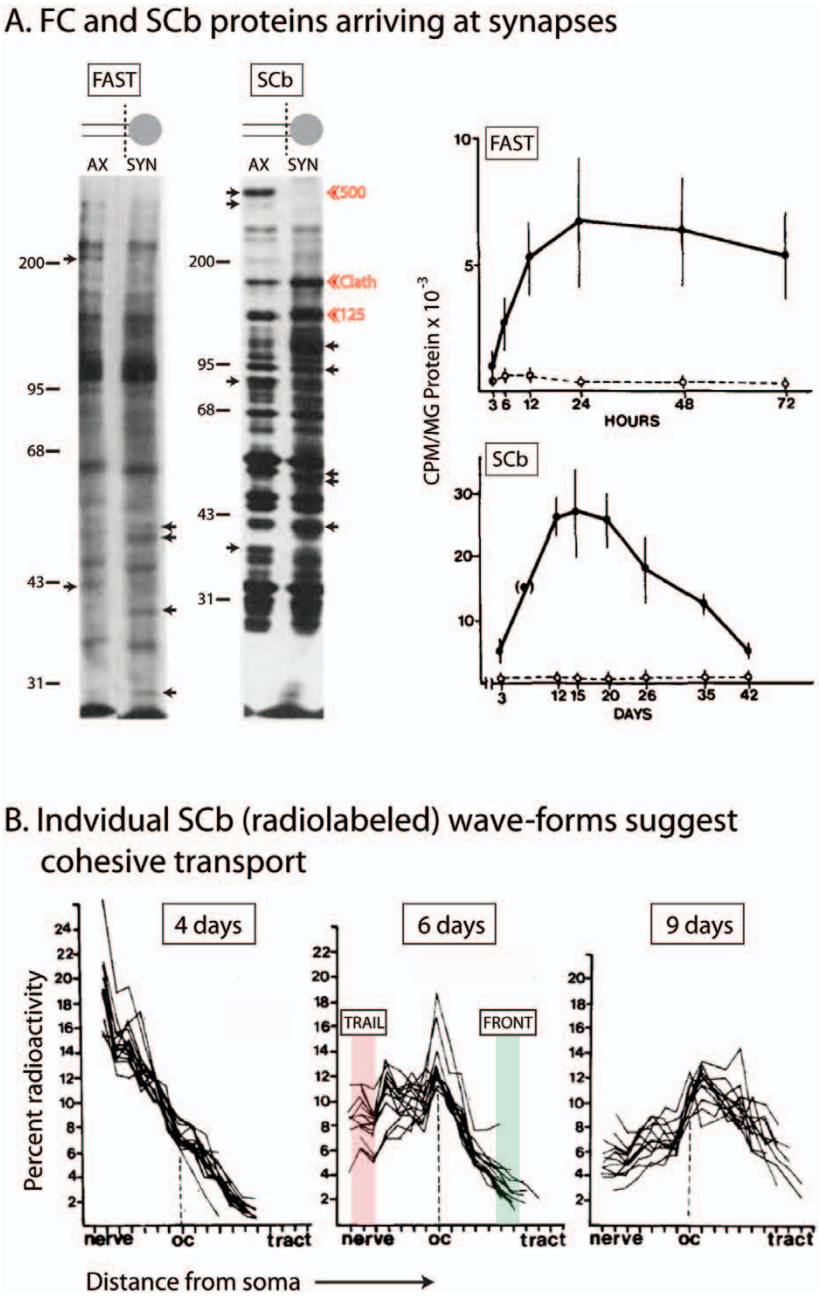

Figure 2. A detailed look at the overall kinetics of cytosolic/soluble cargoes moving in "Slow Component b" (SCb). (A) After somatic pulse-chase radiolabeling, terminal axon ("AX") or synaptosomal ("SYN') preparations from guinea pig retinal ganglion cells were analyzed at various time points to document the ingress of labeled proteins into distal axons (left lanes) and synapses (right lanes). Small arrows point to proteins that are selectively enriched in axons or synapses. Red arrowheads highlight clathrin and two other major unidentified SCb proteins (125 and 500 kDa). In the bottom panel, note that the total pool of transported SCb proteins is about threefold larger than proteins conveyed in the fast component (bottom panel). Also note the decay in radiolabeled SCb proteins after entry into synapses, suggesting turnover and/or retrograde transport at synapses. Figure adapted from Garner and Mahler (1987) with permission. (B) Overlaid radiolabeled "wave profiles" of 20 SCb proteins at 4, 6, and 9 days after somatic radiolabeling. Note the striking overlap between different SCb wave profiles suggesting association with a common "carrier." Also note the maintenance of the overall coherence of the "fronts" (shaded green) and the "peaks," even after several days of transit, and also the divergence in the radiolabeled "trails" (shaded pink) suggesting that the deposition of individual moving SCb proteins along the axon was variable. Figures adapted from Garner and Lasek (1982), with permission.

class, but this has proven to be trickier than expected. One way is to tag cytosolic proteins with GFP and visualize them in axons, using methods akin to those used for membrane-bound proteins. However, unlike vesicles that appear as discrete particles, a complication with most GFP-tagged SCb proteins is that they have inherent soluble pools that create a fluorescent background "haze." Thus, observations are invariably limited to thin distal axons, where putative particulate structures can be resolved over the background haze. We used such methods in our early studies on SCb, looking at the axonal transport of moving GFP:α-synuclein particles in thin, distal axons (Roy and others 2007, 2008). Particles containing α-synuclein moved rapidly with an anterograde bias, but the movements were much more infrequent than fast transport, and the particles also paused during transit. Accordingly, we suggested that cytosolic cargoes in SCb were also transported in a "stop and go" fashion, similar to neurofilaments moving in SCa. Several other groups have reported similar results with α-synuclein (Freundt and others 2012; Utton and others 2005; Yang and others 2010). Though the dynamics of α-synuclein particles in this assay likely represent aspects of its axonal transport in SCb, these methods may not provide a complete picture, as the transport behavior of the population is not analyzed.

To overcome these limitations, more recently we have used photoactivatable vectors to study SCb transport, analyzing the kinetics of axonal protein populations (Scott and others 2011; Tang and others 2012). The main advantage of this paradigm is that it allows dynamic visualization of the entire repertoire of soluble molecules in proximal/primary axons, including kinetics of rapidly mobile/freely diffusible fractions that could not be observed by our earlier methods. The basic design of these experiments is shown in Figure 3A; detailed protocols are published (Roy and others 2011). Briefly, cultured neurons are transfected with cytosolic proteins tagged to photoactivatable GFP (PAGFP) and a soluble red marker (to visualize the transfected axon). Thereafter, a discrete region within the axon is photoactivated, and the kinetics of the photoactivated molecules is followed over time by live imaging (Fig. 3A). Photoactivated SCb proteins were conveyed in a peculiar manner that superficially resembled diffusion but was very different from either untagged PAGFP or vesicular proteins. Specifically, cytosolic SCb proteins dispersed as a plume of fluorescence that had a distinct anterograde bias (Fig. 3B). However, the free diffusion of untagged PAGFP was rapid and unbiased (Fig. 3C), and when vesicular proteins were imaged using this paradigm, individual moving vesicles were seen as expected (Fig. 3D).

The estimated rate of biased flow of photoactivated SCb molecules—quantified by measuring the shift in overall fluorescence peaks over time (for details, see Roy and others 2011)—was within the range predicted by radiolabeling experiments (~0.01-0.03 μm/s or 1-3 mm/day), suggesting that this movement represented *bona fide* SCb transport (Scott and others 2011). Since cytosolic proteins have inherently diffusible fractions (unlike membrane-anchored proteins); in most cases, we also saw small pools that rapidly dispersed bidirectionally, in a manner resembling free diffusion. Note that some fluorescent-tagged cytosolic proteins have very large, rapidly diffusible pools, and may not be ideal for such analyses (Roy and others 2011).

Egress of Somatically Derived Cytosolic Proteins into Axons Reveals Mechanistic Details of Slow Transport

A variation of the photoactivation paradigm can also reveal the movement of soluble proteins from soma into axons, thus more closely simulating pulse-chase experiments. In these experiments, PAGFP-tagged SCb proteins at the neuronal soma are photoactivated, and then the ingress of this photoactivated pool into axons is visualized. An example with PAGFP:synapsin is shown in Figure 4A (also see Scott and others 2011). Interestingly, although the bulk of photoactivated synapsin molecules were transported into axons as a slow-moving "wave" as expected (Fig. 4A(i)), rapidly moving synapsin particles were also seen distally (Fig. 4A(ii)), suggesting that while the bulk of synapsin is conveyed as a slowly biased "wave" (in SCb), a fraction is also transported as persistent particles with kinetics similar to fast transport. The observations are also congruent with radiolabeling experiments where small pools (~10% to 15%) of synapsin and other SCb proteins move in the fast component (see above). Collectively, the data can be interpreted in the following way. If at any given time, cytosolic molecules in axons are moving both as a slow "wave" as well as fast-moving particles, imaging cytosolic proteins tagged to conventional GFP in thin axons would highlight the particle movement, whereas the slow "wave"—at steady state—would only appear as a diffuse background. However, when SCb proteins are imaged using the photoactivation paradigm where a large protein population is simultaneously photoactivated, the wave-like overall kinetics are highlighted while the fast-moving particles are only occasionally captured (see Tang and others 2012 and below).

Are cytosolic proteins conveyed as individual molecules, or do they organize into multiprotein complexes? If the latter is true, such complexes are likely to be transient, analogous to the on/off particle kinetics that we see in our photoactivation experiments (see Fig. 3B). Early

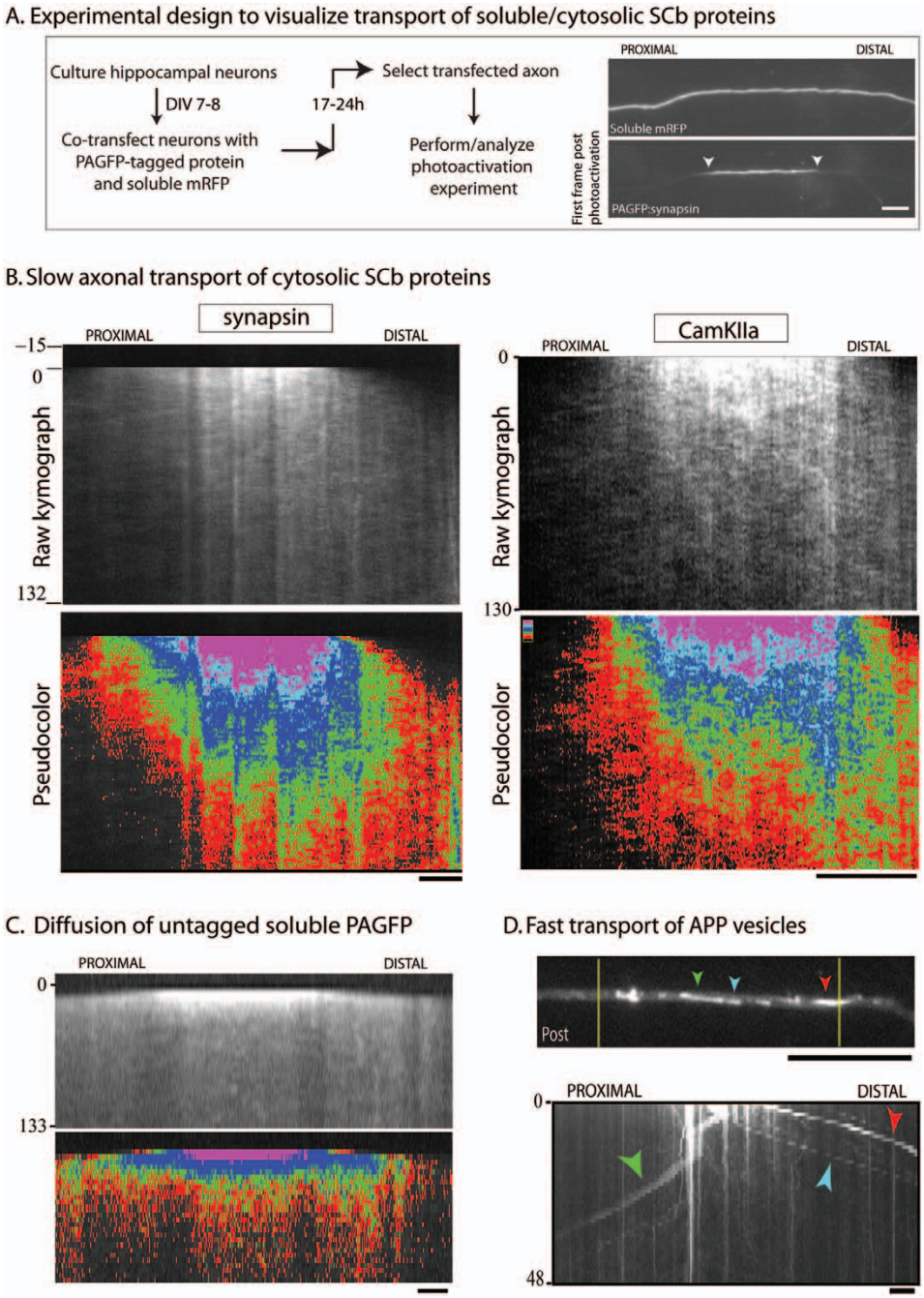

Figure 3. Photoactivation paradigm to visualize the slow axonal transport of cytosolic "Slow Component b" (SCb) protein populations. (A) Cultured neurons are co-transfected with a photoactivatable green fluorescent protein (PAGFP)–tagged protein of interest and untagged monomeric red fluorescent protein (mRFP; to identify transfected axons). A discrete axonal region of interest (ROI; ~20 μm) is photoactivated, and the dispersion of photoactivated molecules is visualized over time (examples of images with PAGFP:synapsin are shown). (B) Grayscale (above) and pseudo-colored (below) kymographs from two PAGFP-tagged SCb proteins synapsin and CamKIIa, imaged using the paradigm above (distance and time in kymographs are on the *x*- and *y*-axis respectively; time shown in seconds). Note the anterogradely biased plume of fluorescence. (C) Photoactivation of untagged PAGFP leads to a rapid and unbiased diffusion of fluorescence as expected, different from SCb proteins. (D) Photoactivation of amyloid precursor protein (APP)—a vesicle-associated fast-component protein—results in the stochastic bidirectional departure of individual vesicles; also different from SCb proteins (colored arrowheads mark the same vesicles in image/kymograph). Scale bar = 5 μm. Figure adapted from Scott and others (2011) and Tang and others (2012), with permission.

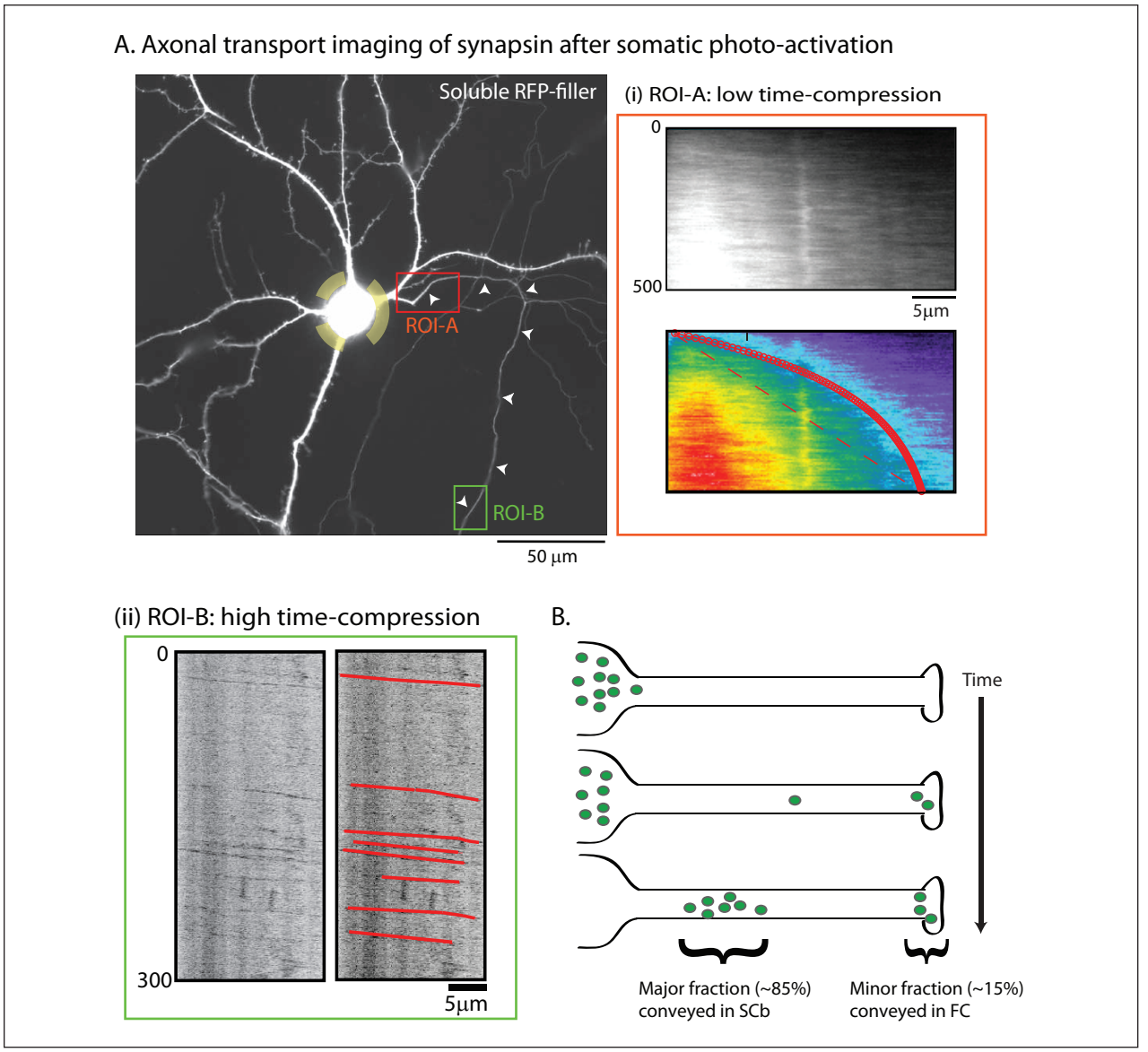

Figure 4. A fraction of "Slow Component b" (SCb) proteins are conveyed in the fast component. (A) To more closely simulate the radiolabeling paradigm, cultured neurons were transfected with GFP:synapsin and soluble mRFP (shown); the neuronal soma was photoactivated (yellow dashed ROI), and the egress of photoactivated molecules into the emergent axon was evaluated over time. While the bulk of synapsin molecules moved slowly into the proximal axon with kinetics expected for slow axonal transport (ROI-A, red box), rapidly moving particles of synapsin were also seen when the distal axon was imaged after somatic photoactivation (ROI-B, green box) (GFP = green fluorescent protein; mRFP = monomeric red fluorescent protein; ROI = region of interest). (B) The "dynamic recruitment" model for SCb transport. After synthesis in the soma, cytosolic molecules intermittently and probabilistically associate with "carriers" moving in fast axonal transport. As such, some molecules remain associated with these carriers for long periods, giving rise to a small population (~10% to 15%) that is rapidly transported to axons and synapses. However the majority of cytosolic molecules are slowly conveyed with kinetics resembling slow axonal transport. An implication of this model is that common transport "carriers" are responsible for conveying both fast component and SCb proteins. Figure adapted from Scott and others (2011), with permission.

biochemical studies suggested that cytosolic SCb proteins in neurons are organized into protein complexes (Lorenz and Willard 1978), and our biochemical data largely support this idea. For instance, brain cytosolic proteins exist in high-speed pellet fractions where they settle into high-density fractions (Scott and others 2011). This behavior would not be expected if these proteins were entirely soluble. Moreover, on careful observation, SCb kymographs occasionally have persistent "streaks" of fluorescence—representing fast vectorial motion

(Tang and others 2012)—suggesting that SCb particles accumulate into aggregates or complexes that subsequently associate with fast and persistent motile structures. Immunostaining of endogenous SCb proteins in axons is also invariably particulate (see Roy and others, 2007; Scott and others, 2012). However, the detailed composition of such complexes is as yet unknown.

The Search for SCb "Carriers" and the "Dynamic Recruitment" Working Model

The slow-moving wave-like kinetics seen in experiments with cultured neurons is likely the visual counterpart of slow axonal transport (SCb) in pulse-chase radiolabeling experiments. How is this slow movement achieved at a molecular level? One possibility is that cytosolic assemblies transiently associate with other persistent cargoes that are continuously moving in fast axonal transport. Because of the transient nature of these associations, the overall movement of the cytosolic population would be much slower than the overall movement of the fast cargoes that the cytosolic particles are associating with. In support of this, closer examination of SCb kymographs from photoactivation experiments reveal occasional persistent vectorial movements that resemble structures moving in fast axonal transport (Tang and others 2012). One possibility is that SCb molecules transiently associate with moving vesicles, and ongoing studies in our lab should clarify this issue.

We propose the following working-model for cytosolic/soluble proteins moving in slow axonal transport (the "dynamic recruitment" model). After synthesis in perikaryal-free ribosomes, cytosolic/soluble proteins assemble into multiprotein complexes that can dynamically associate with a mobile "carrier" that is conveyed persistently in fast axonal transport. As the carrier moves out of the cell body—moving persistently—the soluble/cytosolic protein assemblies are also transported into the axon by virtue of their intermittent associations with the carriers. However, as such associations are dynamic and probabilistic, the overall displacement of the cytosolic population is much slower than the fast-moving carrier, resulting in the slow overall rate seen in the pulse-chase radiolabeling experiments. Moreover, because of the probabilistic nature of such interactions, a small fraction of cytosolic molecules remain associated with mobile vesicles for long periods, and this pool represents the minor "fast population" observed in previous radiolabeling studies of SCb.

There are obvious parallels of the "dynamic recruitment" model to the "stop and go" model. However, unlike neurofilaments that exist as stable polymers, many cytosolic proteins are dynamic (see above and Tang and others 2012), introducing a variable in the model where the assembly/disassembly kinetics are a key determinant of overall kinetic behavior. An interesting prediction of our model is that SCb proteins that exist in a largely assembled form in axons *would* exhibit dynamics similar to the "stop and go" motion seen with neurofilaments (as the assembly/disassembly dynamics would only play a minor role in these cases); an idea that can be tested in the future. Given the heterogeneous nature of SCb, it is plausible that many different transport mechanisms at play; nevertheless the above scenario captures the essence of the available radiolabeling and imaging data and offers a testable working model.

Experiments in other model systems have also reported an anterogradely biased wave-like kinetics of soluble/cytosolic proteins. Terada and others (2000, 2010)colleagues injected a bolus of fluorescently labeled creatine kinase (a soluble protein conveyed in SCb) into the squid giant axon and saw a slow anterogradely biased movement of the labeled protein population at rates reminiscent of SCb. Using a *drosophila* model, Sadananda and others (2012) have also recently shown a kinesin-dependent slow, anterogradely biased flow of the soluble protein choline acetyltransferase in axons. Thus, it appears that the biased flow of cytosolic cargoes in slow axonal transport is a conserved phenomenon, and collective efforts in different model systems should provide clarity of underlying mechanisms in the future.

Coda

Though slow axonal transport conveys proteins that are critical in maintaining neuronal form and function, and several such proteins also play a role in neurodegeneration—tau, α-synuclein, SOD-1, for instance—our understanding of slow transport pales in comparison with that of its faster counterpart. Though this is likely because of the inherent difficulties in seeing the phenomenon, robust imaging paradigms are now available to directly visualize slow transport. Moreover, ongoing advances in development of fluorescent probes, new imaging tools, and innovative ways of integrating these tools should allow us to examine slow axonal transport of a larger repertoire of cargoes with higher fidelity, perhaps providing "trafficking rules" to decipher this enigmatic rate component. In the process, they will uncover new mysteries that are undoubtedly hidden within the depths of this historically "unseen" component of axonal transport.

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References

Allen RD, Metuzals J, Tasaki I, Brady ST, Gilbert SP. 1982. Fast axonal transport in squid giant axon. Science 218:1127–9.

Baas PW, Brown A. 1997. Slow axonal transport: the polymer transport model. Trends Cell Biol 7:380–4.

Baitinger C, Willard M. 1987. Axonal transport of synapsin I-like proteins in rabbit retinal ganglion cells. J Neurosci 7:3723–35.

Black MM, Chestnut MH, Pleasure IT, Keen JH. 1991. Stable clathrin: uncoating protein (hsc70) complexes in intact neurons and their axonal transport. J Neurosci 11: 1163–72.

Black MM, Lasek RJ. 1979. Axonal transport of actin: slow component b is the principal source of actin for the axon. Brain Res 171:401–13.

Black MM, Lasek RJ. 1980. Slow components of axonal transport: two cytoskeletal networks. J Cell Biol 86:616–23.

Brady ST. 1985. A novel brain ATPase with properties expected for the fast axonal transport motor. Nature 317:73–5.

Brady ST, Lasek RJ. 1982. Axonal transport: a cell-biological method for studying proteins that associate with the cytoskeleton. Methods Cell Biol 25:365–98.

Brady ST, Lasek RJ, Allen RD. 1982. Fast axonal transport in extruded axoplasm from squid giant axon. Science 218:1129–31.

Brady ST, Tytell M, Heriot K, Lasek RJ. 1981. Axonal transport of calmodulin: a physiologic approach to identification of long-term associations between proteins. J Cell Biol. 89:607–14.

Bray D. 1997. The riddle of slow transport—an introduction. Trends Cell Biol 7:379.

Bray JJ, Fernyhough P, Bamburg JR, Bray D. 1992. Actin depolymerizing factor is a component of slow axonal transport. J Neurochem 58:2081–7.

Brown A. 2003. Axonal transport of membranous and nonmembranous cargoes: a unified perspective. J Cell Biol 160:817–21.

Brown A, Wang L, Jung P. 2005. Stochastic simulation of neurofilament transport in axons: the "stop-and-go" hypothesis. Mol Biol Cell 16:4243–55.

de Waegh S, Brady ST. 1989. Axonal transport of a clathrin uncoating ATPase (HSC70): a role for HSC70 in the modulation of coated vesicle assembly in vivo. J Neurosci Res. 23:433–40.

Dillman JF III, Dabney LP, Karki S, Paschal BM, Holzbaur EL, Pfister KK. 1996a. Functional analysis of dynactin and cytoplasmic dynein in slow axonal transport. J Neurosci 16:6742–52.

Dillman JF III, Dabney LP, Pfister KK. 1996b. Cytoplasmic dynein is associated with slow axonal transport. Proc Natl Acad Sci. U S A 93:141–4.

Falnikar A, Baas PW. 2009. Critical roles for microtubules in axonal development and disease. Results Probl Cell Differ 48:47–64.

Freundt EC, Maynard N, Clancy EK, Roy S, Bousset L, Sourigues Y, and others. 2012. Neuron-to-neuron transmission of alpha-synuclein fibrils through axonal transport. Ann Neurol 72:517–24.

Galbraith JA, Reese TS, Schlief ML, Gallant PE. 1999. Slow transport of unpolymerized tubulin and polymerized neurofilament in the squid giant axon. Proc Natl Acad Sci U S A 96:11589–94.

Garner JA, Lasek RJ. 1982. Cohesive axonal transport of the slow component b complex of polypeptides. J Neurosci 2:1824–35.

Garner JA, Mahler. 1987. Biogenesis of presynaptic terminal proteins. J Neurochem 49:905–15.

Grafstein B, Forman DS. 1980. Intracellular transport in neurons. Physiol Rev 60:1167–283.

He Y, Francis F, Myers KA, Yu W, Black MM, Baas PW. 2005. Role of cytoplasmic dynein in the axonal transport of microtubules and neurofilaments. J Cell Biol 168:697–703.

Hirokawa N, Funakoshi ST, Takeda S. 1997. Slow axonal transport: the subunit transport model. Trends Cell Biol 7:384–8.

Hoffman PN, Lasek RJ. 1975. The slow component of axonal transport. Identification of major structural polypeptides of the axon and their generality among mammalian neurons. J Cell Biol 66:351–66.

Jensen PH, Li JY, Dahlstrom A, Dotti CG. 1999. Axonal transport of synucleins is mediated by all rate components. Eur J Neurosci 11:3369–76.

Jolly AL, Kim H, Srinivasan D, Lakonishok M, Larson AG, Gelfand VI. 2010. Kinesin-1 heavy chain mediates microtubule sliding to drive changes in cell shape. Proc Natl Acad Sci U S A 107:12151–6.

Keating TJ, Peloquin JG, Rodionov VI, Momcilovic D, Borisy GG. 1997. Microtubule release from the centrosome. Proc Natl Acad Sci U S A 94:5078–83.

Koike H, Matsumoto H. 1985. Fast axonal transport of membrane protein and intra-axonal diffusion of free leucine in a neuron of Aplysia. Neurosci Res 2:281–5.

Lasek RJ, Garner JA, Brady ST. 1984. Axonal transport of the cytoplasmic matrix. J Cell Biol 99(1 Pt 2):212s–21s.

Li W, Hoffman PN, Stirling W, Price DL, Lee MK. 2004. Axonal transport of human alpha-synuclein slows with aging but is not affected by familial Parkinson's disease-linked mutations. J Neurochem 88:401–10.

Li Y, Jung P, Brown A. 2012. Axonal transport of neurofilaments: a single population of intermittently moving polymers. J Neurosci 32:746–58.

Lorenz T, Willard M. 1978. Subcellular fractionation of intra-axonally transport polypeptides in the rabbit visual system. Proc Natl Acad Sci U S A 75:505–9.

Lund LM, McQuarrie IG. 2001. Calcium/calmodulin-dependent protein kinase IIalpha in optic axons moves with slow axonal transport and undergoes posttranslational modification. Biochem Biophys Res Commun 289:1157–61.

Lund LM, McQuarrie IG. 2002. Calcium/calmodulin-dependent protein kinase IIbeta isoform is expressed in motor neurons during axon outgrowth and is part of slow axonal transport. J Neurosci Res. 67:720–8.

Ma D, Himes BT, Shea TB, Fischer I. 2000. Axonal transport of microtubule-associated protein 1B (MAP1B) in the sciatic nerve of adult rat: distinct transport rates of different isoforms. J Neurosci 20:2112–20.

Mills RG, Minamide LS, Yuan A, Bamburg JR, Bray JJ. 1996. Slow axonal transport of soluble actin with actin depolymerizing factor, cofilin, and profilin suggests actin moves in an unassembled form. J Neurochem 67: 1225–34.

Mitchison T, Kirschner M. 1984. Dynamic instability of microtubule growth. Nature 312:237–42.

Miki H, Setou M, Kaneshiro K, Hirokawa N. 2001. All kinesin superfamily protein, KIF, genes in mouse and human. Proc Natl Acad Sci U S A 98:7004–11.

Nixon RA, Fischer I, Lewis SE. 1990. Synthesis, axonal transport, and turnover of the high molecular weight microtubule-associated protein MAP 1A in mouse retinal ganglion cells: tubulin and MAP 1A display distinct transport kinetics. J Cell Biol 110:437–48.

Ochs S. 1981. Characterization of fast orthograde transport. Neurosci Res Program Bull 20:19–31.

Paggi P, Petrucci TC. 1992. Neuronal compartments and axonal transport of synapsin I. Mol Neurobiol 6:239–51.

Petrucci TC, Macioce P, Paggi P. 1991. Axonal transport kinetics and posttranslational modification of synapsin I in mouse retinal ganglion cells. J Neurosci 11:2938–46.

Roy S, Coffee P, Smith G, Liem RK, Brady ST, Black MM. 2000. Neurofilaments are transported rapidly but intermittently in axons: implications for slow axonal transport. J Neurosci 20:6849–61.

Roy S, Winton MJ, Black MM, Trojanowski JQ, Lee VM. 2007. Rapid and intermittent cotransport of slow component-b proteins. J Neurosci 27:3131–8.

Roy S, Winton MJ, Black MM, Trojanowski JQ, Lee VM. 2008. Cytoskeletal requirements in axonal transport of slow component-b. J Neurosci 28:5248–56.

Roy S, Yang G, Tang Y, Scott DA. 2011. A simple photoactivation and image analysis module for visualizing and analyzing axonal transport with high temporal resolution. Nat Protoc 7:62–8.

Roy S, Zhang B, Lee VM, Trojanowski JQ. 2005. Axonal transport defects: a common theme in neurodegenerative diseases. Acta Neuropathol 109:5-13.

Sadananda A, Hamid R, Doodhi H, Ghosal D, Girotra M, Jana SC, and others. 2012. Interaction with a kinesin-2 tail propels choline acetyltransferase flow towards synapse. Traffic 13:979–91.

Scott DA, Das U, Tang Y, Roy S. 2011. Mechanistic logic underlying the axonal transport of cytosolic proteins. Neuron 70:441–54.

Sekimoto S, Tashiro T, Komiya Y. 1991. Two 68-kDa proteins in slow axonal transport belong to the 70-kDa heat shock protein family and the annexin family. J Neurochem 56:1774–82.

Susalka SJ, Hancock WO, Pfister KK. 2000. Distinct cytoplasmic dynein complexes are transported by different mechanisms in axons. Biochim Biophys Acta 1496:76-88.

Tang Y, Das U, Scott DA, Roy S. 2012. The slow axonal transport of alpha-synuclein-mechanistic commonalities amongst diverse cytosolic cargoes. Cytoskeleton (Hoboken) 69:506–13.

Terada S, Kinjo M, Aihara M, Takei Y, Hirokawa N. 2010. Kinesin-1/Hsc70-dependent mechanism of slow axonal transport and its relation to fast axonal transport. EMBO J 29:843–54.

Terada S, Kinjo M, Hirokawa N. 2000. Oligomeric tubulin in large transporting complex is transported via kinesin in squid giant axons. Cell 103:141–55.

Twelvetrees A, Hendricks AG, Holzbaur EL. 2012. SnapShot: axonal transport. Cell 149:950-950.e1.

Utton MA, Noble WJ, Hill JE, Anderton BH, Hanger DP. 2005. Molecular motors implicated in the axonal transport of tau and alpha-synuclein. J Cell Sci 118:4645–54.

Vale RD, Schnapp BJ, Reese TS, Sheetz MP. 1985. Movement of organelles along filaments dissociated from the axoplasm of the squid giant axon. Cell 40:449–54.

Vallee RB, Bloom GS. 1991. Mechanisms of fast and slow axonal transport. Annu Rev Neurosci 14:59-92.

Vavylonis D, Yang Q, O'Shaughnessy B. 2005. Actin polymerization kinetics, cap structure, and fluctuations. Proc Natl Acad Sci U S A 102:8543–8.

Wang L, Brown A. 2002. Rapid movement of microtubules in axons. Curr Biol. 12:1496–501.

Wang L, Ho CL, Sun D, Liem RK, Brown A. 2000. Rapid movement of axonal neurofilaments interrupted by prolonged pauses. Nat Cell Biol 2:137–41.

Willard M, Cowan WM, Vagelos PR. 1974. The polypeptide composition of intra-axonally transported proteins: evidence for four transport velocities. Proc Natl Acad Sci U S A 71:2183–7.

Willard M, Wiseman M, Levine J, Skene P. 1979. Axonal transport of actin in rabbit retinal ganglion cells. J Cell Biol 81:581–91.

Yan Y, Brown A. 2005. Neurofilament polymer transport in axons. J Neurosci 25:7014–21.

Yang ML, Hasadsri L, Woods WS, George JM. 2010. Dynamic transport and localization of alpha-synuclein in primary hippocampal neurons. Mol Neurodegener 5:9.

Yuan A, Mills RG, Bamburg JR, Bray JJ. 1999. Cotransport of glyceraldehyde-3-phosphate dehydrogenase and actin in axons of chicken motoneurons. Cell Mol.Neurobiol 19:733–44.