Axonal transport: The orderly motion of axonal structures

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CHAPTER OUTLINE

Abstract

Axonal transport is a constitutive process that supplies the axon and axon terminal with materials required to maintain their structure and function. Most materials are supplied via three rate components termed the fast component, slow component a, and slow component b. Each of these delivers a distinct set of materials with distinct transport kinetics. Understanding the basis for how materials sort among these rate components and the mechanisms that generate their distinctive transport kinetics have been long-standing goals in the field. An early view emphasized the relationships between axonally transported cargoes and cytological structures of the axon. In this article, I discuss key observations that led to this view and contemporary studies that have demonstrated its validity and thereby advanced the current understanding of the dynamics of axonal structure.

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Axonal transport is the process by which proteins and other materials synthesized in the neuronal cell body are delivered to the axon and axon terminal. This is a constitutive process that occurs throughout the life of neurons, supplying axons with materials needed to maintain their structure and function. The notion that the axon depends on the cell body dates back to the nineteenth century, based on the observation that axons disconnected from their cell bodies degenerate (Ramon y Cajal, 1928). However, it was not until 1948 that movement of materials in axons was first revealed by Weiss and Hiscoe, who partially constricted axons and observed that axoplasm accumulated immediately proximal to the constriction, suggesting a proximal-to-distal movement of axonal materials. Upon release of the constriction, the accumulated axoplasm moved anterogradely at $\approx$1 mm/day, thus identifying what later came to be known as slow axonal transport.

Since this pioneering work, axonal transport has been studied extensively with two experimental approaches providing most of the current understanding. One uses radioactive precursors to pulse-label axonally transported materials and the other uses imaging techniques to directly observe transport in living axons. These two approaches provide distinct but complementary information (Brown, 2009). Pulse-chase approaches provide indirect information on movement of materials in axons in intact animals over time scales of hours to months whereas live-cell imaging directly visualizes axonal transport over time frames of seconds to hours. Below, I discuss contributions of these approaches to the current understanding of the cargoes that undergo axonal transport and their transport behavior as seen at short and long time scales.

1. PULSE-LABELING STUDIES OF AXONAL TRANSPORT

The pulse-labeling approach has revealed the kinetics of protein transport in axons over long time scales and the identity of many transported proteins. Typically, radioactive amino acids are injected into the environment surrounding the neuron cell bodies under study. The amino acids are taken into the neurons and incorporated into proteins, some of which are then transported into their axons. Because the amino acids are cleared relatively rapidly by the circulation, this procedure produces a pulse of labeling in vivo. To visualize the transport of the pulse-labeled proteins, the nerve containing them is cut into consecutive pieces of a few millimeters in length and the distribution of radioactivity along its length quantified. Also, the identity of specific radioactive proteins in the nerve segments has been determined using biochemical procedures. As each animal provides a single time point for analysis, multiple animals must be examined, each at different times after labeling. Comparing the results at the various times yields a detailed, though indirect, picture of the movement of proteins in axons.

This approach has been used with a variety of organisms and the essential results obtained are consistent among systems. The transported pulse-labeled

proteins are distributed along the axons as waves with distinct crests and fronts (Figure 1(A)). The positions and shapes of the waves change as a function of time after injection based on the transport behavior of the proteins. At time frames of hours, waves of pulse-labeled proteins are seen that advance at ≈50−400 mm/day (0.6−5 μm/s) (reviewed in Grafstein & Forman, 1980). This corresponds to the fast component (FC) of axonal transport. FC has both anterograde (soma toward axon tip) and retrograde (axon tip toward soma) components. There is also a slow component which moves at average rates of 0.2−10 mm/day (0.0002−0.1 μm/s). Slow axonal transport consists of two subcomponents, slow component a (SCa) and slow component b (SCb), that differ in specific protein composition and transport rate. SCa moves at modal rates of 0.2−3 mm/day, while SCb moves at 2.0−10 mm/day (the range in rates reflects variations among different populations of neurons). These three rate components provide most of the materials delivered to the axon by axonal transport.

Cell fractionation and electron microscopic autoradiographic studies (Di Giamberardino, Bennett, Koenig, & Droz, 1973; Droz, Koenig, Biamberardino, & Di Giamberardino, 1973; Lorenz & Willard, 1978) showed that fast and slow axonal transport deliver distinct materials to the axon. This result was confirmed by gel electrophoretic analyses of the proteins comprising FC, SCa, and SCb (Tytell, Black, Garner, & Lasek, 1981; Willard, Cowan, & Vagelos, 1974). FC and SCb each consists of hundreds of proteins, whereas SCa transports comparatively few, and strikingly very few proteins are present in more than one rate component (Figure 1(B) and (C)). Thus, the underlying mechanisms of axonal transport prevent the mixing of proteins as they move past each other in the axon. The structural hypothesis of axonal transport was put forth to explain this and other differences between FC, SCa, and SCb (Lasek, 1980; Lasek, Garner & Brady, 1984). This hypothesis posits that proteins are actively transported in the axon either as integral parts of moving cytological structures or in association with these structures. At the time, the strongest support was for FC for which multiple criteria showed was associated with membrane-bound organelles (Dahlström, Czernik, & Li, 1992; Droz et al., 1973; Di Giamberardino et al., 1973; Goldman, Kim, & Schwartz, 1976; Lorenz & Willard, 1978).

The evidence for cytological correlates of slow axonal transport based on the pulse-chase approach is much more limited. The principal proteins of SCa were tubulin and neurofilament proteins, the subunits of microtubules and neurofilaments, respectively (Black & Lasek, 1980; Hoffman & Lasek, 1975). Thus, it was hypothesized that SCa represented the transport of these cytoskeletal polymers. Based on the close similarity in transport kinetics of tubulin and neurofilament proteins, the initial suggestion was that microtubules and neurofilaments moved as a network of interacting polymers. However, as subsequent work revealed subtle differences between tubulin and neurofilament protein transport (McQuarrie, Brady, & Lasek, 1986) and structural studies indicated limited interactions between neurofilaments and microtubules (Brown & Lasek, 1993; Price, Paggi, Lasek, & Katz, 1988), the view of SCa evolved to the independent movement of microtubules and neurofilaments.

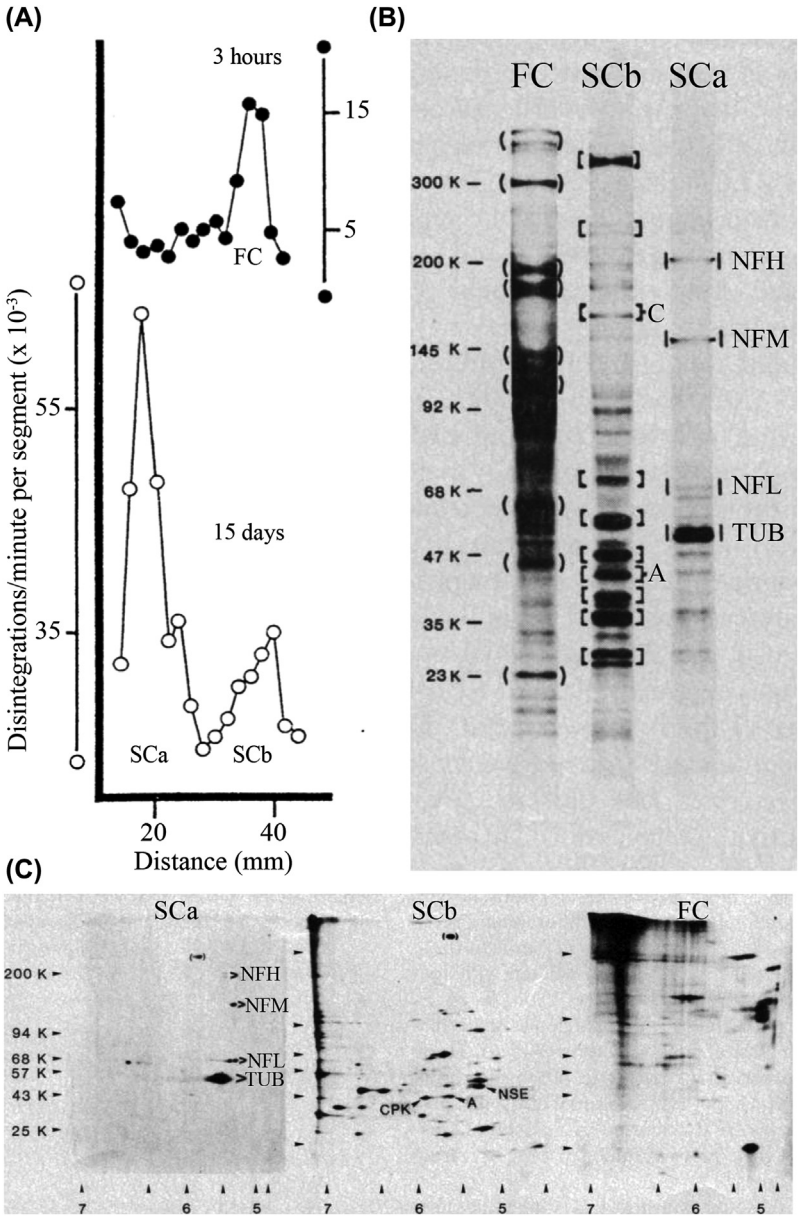

FIGURE 1

Axonal transport of proteins in hypoglossal and retinal ganglion cell axons of guinea pigs. *(These data are reprinted with permission from Tytell et al. (1981).)* Panel (A). The distribution of radioactive proteins in the hypoglossal nerves of guinea pigs 3 h (upper graph) or 15 days (lower graph) after injecting radioactive amino acids into the hypoglossal nucleus

The only additional evidence to support the hypothesis that neurofilaments moved in SCa was that neurofilament proteins were quantitatively assembled into neurofilaments in axons (Black, Keyser, & Sobel, 1986; Morris & Lasek, 1982). However, a small fraction of unassembled proteins could reasonably go undetected, thus limiting the power of this observation. If tubulin is transported in the form of microtubules, then microtubule-associated proteins should be cotransported with tubulin. In this regard, minor proteins move with tubulin in SCa that have mobilities similar to tau (Black & Lasek, 1980), a major axonal microtubule-associated protein. While an early study suggested that these may be tau (Tytell, Brady, & Lasek, 1984), subsequent analyses using two-dimensional gel electrophoresis indicated that they are chartins (Oblinger & Black, unpublished data), a family of microtubule-associated proteins distinct from tau. Thus, at least one microtubule-associated protein is cotransported with tubulin. However, other axonal microtubule-associated proteins move faster than tubulin at rates in the range of SCb (Ma, Himes, Shea,

(the location of the neuron cell bodies whose axons form the hypoglossal nerve). Distance is from the hypoglossal nucleus. At 3 h after injection, a well-defined wave which corresponds to the FC is apparent, while at 15 days, two waves are apparent which correspond to SCa and SCb. Panel (B). Comparison of the proteins comprising SCa, SCb, and FC of retinal ganglion cell axons of guinea pigs using one-dimensional polyacrylamide gel electrophoresis. Segments of the optic nerve and tract, which contain the retinal ganglion cell axons, were obtained at 6 h, 6 days, or 38 days for proteins of FC, SCb, or SCa, respectively. FC and SCb each consists of many polypeptides, whereas only five polypeptides account for the majority of material transport in SCa. Even by one-dimensional gel electrophoresis, it is apparent that any of the transported proteins appear in only one transport component (see the bands highlighted by brackets). Note: the radioactive bands below tubulin in the SCa profile are not transported in SCa but represent trailing proteins of SCb. Known polypeptides are indicated: C = clathrin, A = actin, NFL, NFM, NFH = low, middle, and heavy neurofilament subunits, TUB = tubulin. Apparent molecular weight is indicated on the left. Panel (C). Comparison of the proteins comprising SCa, SCb, and FC of retinal ganglion cell axons of the guinea pig using two-dimensional isoelectric focusing—polyacrylamide gel electrophoresis. The approximate pH gradient of each gel is indicated on the bottom and apparent molecule weight is indicated on the left. This high-resolution technique shows that with very few exceptions, each transported protein is present in only one rate component. The one exception is the protein spot highlighted with parentheses in the samples of SCa and SCb. Another protein present in more than one rate component is tubulin, which in peripheral motor and sensory neurons, is transported in SCa and SCb; however, in retinal ganglion cell axons, tubulin is only in SCa. Proteins of known identity when these data were originally published are identified in the figures and include neurofilament subunits (NFH, NFM, NFL) and tubulin (TUB), nerve-specific enolase (NSE), creatine phosphokinase (CPK), and actin (A). Note: clathrin heavy chain is not identified because it forms a streak that is too faint to be seen. The smearing of spots in the gel of FC is typical and is apparently due to the carbohydrate and lipid modifications common to FC proteins. FC, fast component; SCa, slow component a; SCb, slow component b.

& Fischer, 2000; Mercken, Fischer, Kosik, & Nixon, 1995). Interpretation of these data is not straightforward. First, tau, MAP1a, and MAP1b have multiple interacting partners in addition to tubulin, some of which (e.g., actin) move in SCb, and these interactions can be expected to impact their movement in axons. Second, live-cell imaging suggests that tau is cotransported with tubulin (Konzack, Thies, Marx, Mandelkow, & Mandelkow, 2007). However, when tau dissociates from microtubules, it diffuses quite rapidly, faster than the average rate of tubulin transport. Thus, the population of tau moves faster than tubulin. While the pulse-chase studies on transport of microtubule-associated proteins provide insights into the interactions between tubulin and microtubule-associated proteins in axons, they do not effectively address their transport form.

The structural correlates of SCb are unknown. This is in part due to its compositional complexity. Hundreds of diverse proteins move in SCb which include proteins of the actin and membrane cytoskeletons, enzymes of intermediary metabolism, proteins involved in membrane trafficking, and proteins that interact with synaptic vesicles. Actin was one of the first proteins identified in SCb (Black & Lasek, 1979; Willard, Wiseman, Levine, & Skene, 1979). It was suggested that actin filaments form a scaffold to which other SCb proteins bind and the resulting complex represents an SCb cargo. However, no direct data have been published to support this possibility.

An early insight into SCb derived from the observations that SCb proteins move together in a vectorial manner in axons and that they are also soluble components of axoplasm. Such a result would be difficult to explain if the proteins were freely diffusible. Thus, it was suggested that they existed as one or more assemblies that were conveyed by the transport machinery (Garner & Lasek, 1982; Tytell et al., 1981). This view is supported by cell fractionation analyses which show that many SCb proteins behave as large multiprotein complexes (Lorenz & Willard, 1978; Scott, Das, Tang, & Roy, 2011). In addition, immunoprecipitation analyses performed under nondenaturing conditions using antibodies specific for clathrin, an SCb protein (Garner & Lasek, 1981), isolated a complex that included clathrin, Hsc70, and several other minor SCb proteins (Black, Chestnut, Pleasure, & Keen, 1991). This complex may represent an SCb cargo. Finally, comparisons of the transport behavior of several individual SCb proteins have revealed three distinct transport profiles raising the possibility of three distinct cargoes (Garner & Lasek, 1982). While these studies support the idea that SCb proteins form higher order assemblies that undergo transport in axons, the identity of these complexes remains to be discovered.

This selected review has discussed some of the history that led to the structural hypothesis of axonal transport and the initial suggestions regarding structural correlates of FC, SCa, and SCb. Many of the suggestions were controversial sparking numerous studies using pulse-chase approaches that greatly enhanced knowledge of axonal transport. However, these studies did not resolve the controversy because they could not unambiguously reveal the identity of individual cargoes and the moment-to-moment details of their movements. To move forward on these issues,

new approaches based on live-cell imaging have been developed that provide direct visualization of the cargoes as they undergo transport in living axons. These new methods have provided compelling support for the structural hypothesis of axonal transport.

2. LIVE-CELL IMAGING OF AXONAL TRANSPORT

2.1 FC AND THE MOVEMENT OF VESICULAR CARGOES

Early studies using time-lapse optical imaging of living axons revealed the movement of mitochondria and heterogeneous populations of roughly spherical objects near the resolution limit of the light microscope (Forman, Padjen, & Siggins, 1977; Kirkpatrick, Bray, & Palmer, 1972). The rates of movement as well as their sensitivity to metabolic inhibitors suggested that these were fast transport cargoes. The introduction of video-enhanced contrast differential interference contrast microscopy revealed dramatically more movement than previously obtained because of its ability to detect structures as small as 30 nm. Early studies on axoplasm extruded from the squid giant axon revealed a large variety of structures moving at rates corresponding to FC (Brady, Lasek, & Allen, 1982). Subsequent studies using correlative electron microscopy identified many of the specific cargoes as a variety of membrane-bound structures, thereby confirming the view derived from pulse-chase studies (Miller & Lasek, 1985; Schnapp, Vale, Sheetz, & Reese, 1985). They also established that anterograde cargoes differed from those moving retrogradely, with the former including Golgi-derived vesicles and the latter including endocytic vesicles and prelysosomal structures. The squid axoplasm system also led to the discovery of kinesin, a microtubule motor that powers fast anterograde transport (Brady, 1985; Vale, Reese, & Sheetz, 1985) as well as the existence of a distinct motor that powered fast retrograde transport (Vale, Schnaapp, et al., 1985), which was later identified as cytoplasmic dynein. The reader is referred to numerous reviews on fast axonal transport and the motors that power this motility that have appeared in the intervening years.

Two points regarding FC will be highlighted. First, its anterograde and retrograde cargoes typically move persistently and unidirectionally, pausing infrequently during their transit in the axon. Second, while moving, their rates approximate both the maximum rates reported for FC using pulse-chase methods and the maximum rates reported for kinesin and dynein motors in vitro. Thus, fast axonal transport represents a system for efficiently moving vesicular structures between the cell body and axon tip. While much remains to be learned about regulatory mechanisms that control fast transport, the interactions of FC cargoes with the transport motors are relatively stable and the motors interact processively with the microtubule tracks upon which transport occurs.

Mitochondria, membrane-bound structures abundant in axons, exhibit very different transport behavior from typical FC cargoes. Mitochondria have much

slower average rates of transport compared to fast transport cargoes (Hollenbeck & Saxton, 2005). Live-cell imaging reveals that mitochondria pause frequently during their transport in the axon often remaining stationary for extended times and they can also undergo changes in direction (Saxton & Hollenbeck, 2012). Yet, mitochondria transport is powered by the same kinesin and dynein motors that translocate FC cargoes. Thus, differences in transport rate and behavior do not necessarily indicate fundamental differences in mechanism. It is the differences in the regulation of the transport machinery that allow the machinery to generate such distinctive transport behaviors (Brown, 2003; Saxton & Hollenbeck, 2012). This same theme will come up again in the discussion of slow axonal transport.

2.2 SLOW AXONAL TRANSPORT AND THE MOVEMENT OF CYTOSKELETAL POLYMERS

The first studies attempting to reveal microtubule and neurofilament transport specifically tested the hypothesis that these structures moved slowly and steadily from the cell body toward the axon tip at the modal rate of SCa as revealed by pulse-chase studies (Lim, Edson, Letourneau, & Borisy, 1990; Okabe & Hirokawa, 1990; Okabe, Miyasaka, & Hirokawa, 1993). The results failed to show such slow steady movement and were interpreted as evidence that microtubules and neurofilaments were not transported. However, given that these studies failed to reveal any movement at all including that known to occur, a more conservative interpretation would have been that microtubules and neurofilaments do not move in a slow steady manner. As discussed below, hints already existed from pulse-chase studies on slow axonal transport that the cargoes did not move in this manner.

2.3 NEUROFILAMENTS ARE TRANSPORTED IN AXONS

While pulse-chase studies showed that the bulk of neurofilament proteins moved slowly and steadily at a modal rate of $\approx$1 mm/day, the wave is quite broad, indicating that some SCa cargoes move faster and some slower than this. The SCa wave also broadens substantially over time, further indicating that SCa cargoes move at a distribution of rates. In a particularly detailed analysis of neurofilament protein transport, rates ranged from $<$0.01 mm/day to several tens of mm/day (Lasek, Paggi, & Katz, 1993). They suggested that the broad distribution of rates reflected a fundamental feature of the transport mechanisms in which neurofilament proteins moved with brief but rapid translocation steps interrupted by pauses. This is similar to the situation for mitochondria, but with pauses accounting for a much greater percentage of the transport behavior to account for the slow average rate of neurofilament protein transport. Although speculative at the time, this view presaged the findings of subsequent studies directly visualizing neurofilament protein transport in living axons.

Wang, Ho, Sun, Liem, and Brown (2000) were the first to directly visualize neurofilament transport in living axons, followed shortly thereafter by Roy et al. (2000).

Both groups expressed GFP-labeled neurofilament proteins in cultured sympathetic neurons. These neurons contain a relatively sparse neurofilament array in their axons, and many axons have regions along their length with no neurofilaments. By focusing on these gaps which have near zero background fluorescence, GFP-labeled neurofilaments, initially located outside of the gaps were observed to move into and through them. Detecting these movements required the use of imaging parameters to reveal fast but intermittent transport. The neurofilament proteins moved with generally brief bouts of relatively rapid transport ($\approx$0.5 μm/s) interrupted by prolonged pauses and unexpectedly, movement was bidirectional though the majority moved anterogradely. The moving proteins comprised linear structures of up to several tens of microns in length suggesting that they were moving as neurofilaments. Direct confirmation of this was subsequently provided by using correlative electron microscopy to show that the moving structures were indeed neurofilaments (Yan & Brown, 2005). Thus, the slow anterograde transport of neurofilament proteins in SCa actually reflects the average of brief episodes of rapid bidirectional transport of neurofilament polymers interspersed with prolonged pauses of little to no movement.

Subsequent studies showed that neurofilament transport is microtubule dependent (Francis, Roy, Brady, & Black, 2005) and uses the same motors that power fast axonal transport, with kinesin and dynein mediating anterograde and retrograde neurofilament transport, respectively (He, Francis, Myers, Yu, Black & Baas, 2005; Uchida, Alami, & Brown, 2009). Thus, neurofilament movement in slow transport does not represent a novel mechanism, but instead reflects a variation on the theme for the transport of vesicular cargoes. Specifically, fast motors propel neurofilaments within the axon, but the movement is not processive. Specialized regulatory mechanisms generate prolonged pauses in this movement, resulting in a slow rate when averaged over time. The specifics of this regulation are the subject of active investigation.

In the years since these studies first appeared, Brown and colleagues have continued to dissect neurofilament transport, revealing many novel details. One goal has been to determine whether the transport behavior of individual neurofilaments as observed in cultured neurons imaged over short time frames can explain the transport behavior of neurofilament proteins in axons observed over long time frames in vivo with the pulse-chase approach (Brown, Wang, & Jung, 2005; Li, Jung, & Brown, 2012). To address this, they developed computational models of neurofilament transport employing the parameters for neurofilament transport rate, directionality, and pausing observed in their studies. One essential feature of the model is that neurofilaments move linearly and independently within axons, mostly in the anterograde direction, but also retrogradely. In addition, individual filaments cycle between distinct states of active transport and pausing, such that they spend approximately 97% of their time pausing, while the remaining time, they move at relatively fast rates. The model recapitulates the in vivo transport kinetics with remarkable fidelity. Thus, the essential features of neurofilament protein transport seen with the pulse-chase approach can be fully explained by the known properties

of neurofilament polymer transport seen by live imaging of cultured neurons. Over the years, it has been suggested that neurofilament proteins may also undergo transport in a form other than as neurofilaments. While this remains a formal possibility, the available data indicate that neurofilaments constitute the principle transport form of neurofilament proteins.

2.4 MICROTUBULES AND SLOW AXONAL TRANSPORT

Several studies have demonstrated that microtubules can redistribute within growing neurons from the cell body into the axon and from the axon into the growth cone (Ahmad & Baas, 1995; Slaughter, Wang, & Black, 1997; Yu, Schwei, & Baas, 1996). Though not directly observed, it was inferred that active transport accounted for the redistribution. With the development of methods to reveal neurofilament transport, it was natural to apply them to the issue of microtubule transport. The methods clearly revealed tubulin moving in living axons, and like neurofilaments, the tubulin-containing cargo moved rapidly but intermittently with an average rate in the range reported for tubulin transport as seen in the pulse-chase studies (Hasaka, Myers, & Baas, 2004; He, Francis, Myers, Black, & Baas, 2005; Wang & Brown, 2002). The movement was bidirectional, though mostly anterograde, and during bouts of movement the rate was typical of that seen with fast motors, $\approx 1-2$ μm/s, but was interrupted by pauses. Strikingly, the moving structures were short, $\approx 1-5$ μm in length (average = 2.7 μm), and structures typical of the length of axonal microtubules (many tens of microns long), were not observed to move. It was thus suggested that short microtubules are conveyed rapidly but intermittently by slow axonal transport while long microtubules are stationary (Baas, Nadar, & Myers, 2006).

Several observations support the view that long microtubules are not transported in axons. For example, Chang, Svitkina, Borisy, and Popov (1999) used speckle microscopy to reveal individual axonal microtubules in living axons, and none of these polymers was observed to move. In another approach, microtubule plus ends were tagged with fluorescent tip-binding proteins and then imaged to see whether the polymers moved. It is expected that the plus ends will advance as the microtubules elongate. If they also undergo transport, then the rate of advance will exceed that due to microtubule elongation alone. However, in no case was this observed (Kim & Chang, 2006; Ma, Shakiryanova, Vardya, & Popov, 2004). As these studies imaged large numbers of microtubules, if microtubule transport occurred, even infrequently, it should have been detected. Thus, the conclusion that such transport does not occur is reasonable. However, this needs to be qualified as the studies did not restrict analyses to microtubules of particular length, but examined any polymer that could be detected. As most axonal microtubules are many tens of microns in length (Bray & Bunge, 1981), the findings reasonably apply to such long polymers. Whether they apply to short microtubules is unknown, and given the results by the Brown and Baas labs discussed above, they very well may not.

A key question in the studies by the Brown and Baas labs is whether the moving tubulin-containing structures are in fact short microtubules. Given that tubulin

assembles into microtubules, this seems reasonable. However, as this has not been directly tested by fixing tubulin-containing structures undergoing transport and imaging them by electron microscopy, uncertainty remains. The movement of tubulin-containing structures that are not microtubules has been reported (Hollenbeck & Bray, 1987). The majority move retrogradely, are spherical to oval in shape, and are associated with membrane-bound structures. Thus, they seem unrelated to the filamentous tubulin-containing structures of slow transport. Ma et al. (2004) have also observed short filamentous tubulin-containing structures move in axons, but have argued that these are not microtubules because they differ from microtubules in fluorescence intensity. However, this conclusion is not supported by their own data showing a transported tubulin-containing structure that is similar in fluorescence intensity to microtubules elsewhere in the same images (see their Figure 3(A)). Finally, it has been reported that brefeldin A blocks all slow axonal transport, including the movement of tubulin (Campenot, Soin, Blacker, Lund, Eng, & MacInnis, 2003). Because brefeldin A disrupts the Golgi complex and prevents the formation of Golgi-derived vesicles that are the cargoes of FC, it was suggested that slow axonal transport materials move by transient association with fast transport cargoes. Recent support for this idea has been obtained for some SCb proteins (see below), and thus it is a formal possibility for other slow transport cargoes such as neurofilaments and tubulin. However, in these experiments, brefeldin A treatment blocked the transport of all cargoes, including mitochondria. As mitochondria transport should not be affected by brefeldin A (Tang et al., 2013), the complete block of transport in the experiments by Campenot et al. (2003) raises concern of off target effects.

At present, the only independent evidence that these short tubulin-containing structures are microtubules derives from studies of tau (Konzack et al., 2007). These authors expressed various tau constructs in cultured neurons and examined tau diffusion, tau association with microtubules, and tau transport. The studies demonstrate that tau diffuses remarkably fast in axoplasm ($D \approx 3\ \mu m^2/s$) and that tau association with microtubules exhibits a high exchange rate ($t_{1/2} \approx 4$ s). Thus, diffusion is adequate to distribute tau throughout shorter axons (≈ 1 mm). However, as length increases beyond this, active transport is required to ensure delivery of tau to the distal axon. In terms of transport, the authors hypothesized that tau was transported in association with microtubules, and used procedures similar to that have revealed tubulin transport to visualize tau transport. Briefly, in neurons expressing fluorescent tau, photobleaching was used to create a gap in the fluorescence of tau along the axon, and then the gap was imaged to determine whether fluorescent tau located outside of the gap moved into and through the gap. When tau with four microtubule-binding repeats was expressed, transport of discrete structures was not observed. Given the short residence time of tau on microtubules combined with its rapid diffusion, this is expected; the fluorescent tau would spend too little time associated with microtubules to detect its movement. To increase the chances of detecting tau on moving microtubules, the authors also expressed tau engineered to contain eight repeats. The eight-repeat tau resided

significantly longer on axonal microtubules and when used in the transport assay, 2- to 6-μm long filamentous structures were seen to move into and through the gaps. The movement occurred in both anterograde and retrograde directions and exhibited stop-and-go characteristics with brief bouts of fast transport (0.2–2 μm/s) interrupted by pauses. The transport of these tau-containing structures strikingly resembles that of tubulin-containing structures. It is noteworthy that the manipulation that led to the detection of tau transport specifically involved increasing the number of microtubule-binding domains on tau. Thus, the most parsimonious explanation of the data on tubulin and tau transport is that tubulin is transported as short microtubules and tau transport reflects its association with transported microtubules.

It has been argued that microtubule transport is important for establishing the microtubule polarity pattern of the axon, for the expansion of the axonal microtubule array during growth and development and its maintenance in the adult (Baas, 2002; Baas & Ahmad, 1993; Black, 1994). Impairment of microtubule transport in axons may also be a factor in neurodegenerative diseases by compromising the axonal microtubule array and thereby the various transport processes that depend upon it (Baas & Mozgova, 2012). All of these ideas are based on the assumption that microtubules are transported in axons, and while a strong case for this can be made, some uncertainty remains. It is imperative to directly test whether these tubulin-containing structures are indeed microtubules and hopefully move past this lingering uncertainty. Methods are available for doing this using reagents that both fluoresce and can be seen using the electron microscope. While such experiments pose technical challenges, the effort will be worth the outcome because the issue will be resolved once and for all, and the outcome will provide essential direction for the field to move forward.

2.5 SCb AND THE MOVEMENT OF SOLUBLE PROTEINS OF AXOPLASM

In some respects, little progress has been made in deciphering SCb, whereas in other respects great strides have been made. With regard to the former, to explain the movement of the 200+ soluble proteins of SCb, it was hypothesized that they assemble into multiprotein complexes that are the cargoes of SCb (Garner & Lasek, 1982; Lorenz & Willard, 1978). While recent studies have provided further support for the hypothesis that SCb proteins assemble into multiprotein complexes (Scott et al., 2011; Tang, Das, Scott, & Roy, 2012), the identity of the complexes and their possible relationship to cytological structures of the axon remain unknown. On the other hand, substantial progress has been made in dissecting the transport mechanisms for these proteins. As described below, the theme of rapid but infrequent movements also figures important for SCb.

Initial studies expressed GFP-tagged SCb proteins (α-synuclein, synapsin-1, glceraldehyde-3-phosphate dehydrogenase) in cultured hippocampal neurons, and used imaging parameters to detect rapid but intermittent movements (Roy, Winton,

Black, Trojanowski, & Lee, 2007; Roy, Winton, Black, Trojanowski, & Lee, 2008). These studies focused on thin axons in which the GFP-tagged SCb proteins appeared as occasional discrete puncta above a more diffuse distribution. While many puncta remained stationary during imaging, some moved at rates comparable to FC ($\approx 1-2$ μm/s). Such movements were relatively infrequent and they were interrupted by pauses of variable duration. Furthermore, while most puncta moved anterogradely, some moved retrogradely. These results showed that SCb proteins can move bidirectionally in a stop-and-go manner. It was argued that the overall slow rate of transport reflected the average for the population of the time spent moving rapidly and the time spent pausing.

The key findings of these studies not fully appreciated at the time derived from direct comparisons of the transport behavior of SCb and FC cargoes in individual axons. Neurons coexpressing red-tagged α-synuclein (and later synapsin-1 (Tang et al., 2013)) and green-tagged synaptophysin, an integral membrane protein of FC, were imaged simultaneously to reveal their transport. Synaptophysin appeared as small puncta and exhibited typical FC behavior as reported by others, with synaptophysin puncta moving frequently and highly persistent. This was in marked contrast to the infrequent and less persistent movements of SCb puncta. However, SCb and synaptophysin puncta exhibited nearly identical transport velocities during bouts of movement, and furthermore, in dual imaging analyses, all moving SCb puncta moved together with synaptophysin. This latter finding was striking and suggestive of a linkage between the movement of SCb proteins and FC cargoes. Subsequent work by Roy and colleagues demonstrated the importance of this linkage to the transport of at least some SCb proteins.

To further dissect the mechanisms of SCb transport, Roy and colleagues developed an assay for SCb transport in cultured neurons using photoactivatable vectors in which bulk cargo movement and particle dynamics could be visualized with high resolution (Scott et al., 2011; Tang et al., 2012, 2013). These studies focused on three SCb proteins, synapsin-1a, calmodulin-dependent kinase IIa, and α-synuclein. While these proteins have distinctive transport kinetics, I will focus on their commonalities. The results obtained show that the bulk of these proteins moves with an anterograde bias at a rate of ≈ 0.01 μm/s, which is similar to the rates reported for SCb proteins based on pulse-labeling studies. The transport requires microtubules, microtubule motors, and ATP.

It has not been possible to visualize discrete movements within the bulk population of SCb proteins presumably because individual movements are too brief to capture and/or the vectorially moving proteins do not stand out from their neighbors that are just diffusing. However, a minor subpopulation of these SCb proteins appears as discrete particles that exhibit intricate transport kinetics. During their movement, transport rates are relatively fast, $\approx 1-2$ μm/s, but the duration of movement is variable, ranging from a few seconds to a few tens of seconds (the original live-cell imaging studies by Roy et al. (2007) focused on this minor subpopulation of SCb cargoes). It is assumed that movement within the wave exhibits transport kinetics similar to these particles, but for much shorter durations. Simulations were

developed to test specific mechanisms that could explain both the slow advance of the bulk of SCb proteins as well as the more persistent particle movements, focusing specifically on synapsin-1 transport. The model that best fit the data involved transient association of synapsin-1 with mobile units that moved persistently with a range of rates typical of microtubule motors and with an anterograde bias. The association of synapsin-1 with the mobile units occurred with a range of interaction strengths, such that most movements were of short duration ($\leq$1 s) and distance ($\leq$1 μm), although a minor fraction persisted for many seconds and moved many microns. Given the biochemical evidence suggesting that synapsin-1 along with other SCb proteins exist as multiprotein assemblies, this suggests a model in which complexes of SCb proteins containing synapsin-1 transiently engage with motors, either directly or indirectly, resulting in an overall slow anterograde advance within the axon.

Given the dual imaging analyses showing that SCb cargoes are cotransported with synaptophysin (Roy et al., 2007; Scott et al., 2011), the vesicular cargoes of FC that contain synaptophysin are logical candidates for the mobile units. Direct support for this possibility was obtained by showing that manipulations that suppressed FC similarly suppressed synapsin-1 transport (Tang et al., 2013). In addition, the transport of synapsin-1 was dependent on its domains that interact with vesicular structures. Refinements of the simulation parameters suggest a model in which synapsin-1 assembles into multiprotein complexes that have an affinity to vesicular cargoes of fast transport. The synapsin-1 complexes and vesicles interact stochastically, with most synapsin-1 complexes interacting transiently and thus advancing slowly within the axon, whereas a minor subset interacts for longer periods and so moves with FC.

While the extent to which this model applies to other SCb proteins is unknown, the finding that some of the synapsin-1 moves together with two other SCb proteins, α-synuclein and glceraldehyde-3-phosphate dehydrogenase (Roy et al., 2007), suggests some generality. However, SCb is compositionally very complex, containing 200+ different proteins, and these are likely organized into multiple cargo complexes (Black et al., 1991; Garner & Lasek, 1982; Lorenz & Willard, 1978; Roy et al., 2007). A number of SCb proteins are able to interact directly or indirectly with membranes (for example, α-synuclein, spectrin, actin, clathrin) and so may move via transient associations with FC cargoes in a manner resembling that of synapsin-1. However, it is also possible that SCb complexes are transported directly by molecular motors in a manner that results in an overall slow transport within the axon. Since its initial description as a discrete component of axonal transport (Black & Lasek, 1979; Garner & Lasek, 1982; Willard et al., 1974), SCb has been a mystery. Many of its proteins still remain to be identified and the current understanding of their organization in the axon is limited and has not advanced much beyond those of the early pulse-chase studies. However, the work of Roy and colleagues has provided a mechanistic understanding of the transport of select SCb proteins and the next several years promise to reveal many new insights into this still enigmatic component of axonal transport.

3. SUMMARY

In 1980, Raymond Lasek published an article on axonal transport entitled "Axonal Transport: A Dynamic View of Neuronal Structures" in which he emphasized the close relationship between axonal transport and the fine structure of the axon. He argued that the structures observed in axons by electron microscopy are the cargoes of axonal transport. In this view, studies of the fine structure of the axon and of axonal transport provide highly complementary information. Specifically, cytological studies provide a snapshot in time of the organization of axonal structures, whereas studies of axonal transport provide information on the orderly motion of these structures over time scales ranging from seconds to days, months, and longer. Combining this information provides a dynamic view of axonal structure. Based on information available at that time, specific hypotheses were proposed regarding the relationship between axonal transport and axonal structures. As new technologies were developed that provided increasingly higher resolution information on the motility of axonal components, it became possible to directly test these hypotheses, and some were proven correct, though often in very different ways from what was initially envisioned, whereas others were not. The contemporary picture of axonal transport is very different from that of three decades ago, but the fundamental premise that axonal transport provides dynamic information on axonal structures has been fully validated by contemporary studies. Indeed, this perspective is still at the heart of many studies of axonal transport. In many cases, the transported structures are well defined and the studies are aimed at more subtle issues of the regulation of transport. In other cases, the connection between the transported cargoes and axonal structure is still being defined. The increasing resolution with which these issues can now be examined promises answers to many of the currently outstanding questions and with these an increasingly sophisticated understanding of how axonal transport contributes to the elaboration and maintenance of axonal structure and function.

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