Axonal Transport of the Cytoplasmic Matrix

RAYMOND J. LASEK, JUDY A. GARNER, and SCOTT T. BRADY
Department of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, Ohio 44106

The Cytoplasmic Matrix in Neurons Is Specialized to Support the Elongate Shape of Neurites

The cytoplasmic matrix is often highly specialized, making it possible to clearly relate particular aspects of the cytoplasmic matrix to the specialized functions of cells. For example, in striated muscle cells the contractile components of the cytoplasmic matrix dominate the cell structurally and functionally. Neurons are another example of cells in which specializations of structure and function can be clearly related to particular aspects of the cytoplasmic matrix (36, 37). The primary function of neurons is to convey information from one location in the organism to another. Pathways for information transfer in the nervous system are provided by specialized neuronal extensions, the axons and the dendrites. Axons, in particular, are specialized to convey information over very long distances, meters in some cases. Accordingly, in the axon the cytoplasmic matrix is specialized to generate and support the extremely elongate shape of the axon during development, regeneration, and maturity.

To generate and maintain the great volume of cytoplasm within the axon, neurons must produce tremendous amounts of protein (32). Essentially all axonal proteins are synthesized in the neuron cell body and then conveyed into the axon by axonal transport, which provides a lifeline for the axon and its terminus (25, 36). Axonal transport is a process that is initiated when the axon first develops and that continues throughout the life of the neuron. To meet the needs of large animals, which require long axons, axonal transport has become one of the most highly developed mechanisms for the intracellular transport of materials in metazoan cells.

Studies of Axonal Transport with Radioisotopes Reveal Processes at the Microscopic Level

Although axonal transport occurs on a scale that is properly measured in micrometers, it is possible to study it by macroscopic methods in which the unit used for measurement is the millimeter (12). Axons are often grouped together in parallel bundles, and in long nerves a bundle of axons may extend for 10 cm or more. Axonal transport can be studied in these long axons by radioisotopic labeling methods.

Standard radioistopic labeling methods can be employed to study the synthesis, transport, and metabolism of axonal cytoplasmic matrix proteins (11, 32). Labeled precursors, such as [^{35}S]methionine, are microinjected in the vicinity of the neuronal cell bodies, where they are rapidly incorporated into neuronal proteins, producing a pulse of labeling in vivo. It then is possible to study the pulse-labeled proteins, which are selectively transported into the axon, by removing the nerve containing the labeled axons and dividing it into consecutive 1- to 3-mm segments (1). By this method the distribution of labeled proteins within the axons can be determined at various intervals after labeling (see Fig. 1). Biochemical methods can then be used to detect specific labeled proteins in the nerve segments. This approach has provided a rather complete picture of the kinetics of axonal transport (Table I).

The rates of transport of the radiolabeled proteins are measured in millimeters per day. However, these rates represent the average rate of processes that occur at the level of micrometers per second. For example, axonal transport of the proteins of the cytoplasmic matrix and fibroblast locomotion occur at about the same rate. When measured microscopically, this rate is 0.01 μm/s. In the axonal transport experiments the rate is 1 mm/d. In long axons it is possible to distinguish between rates of transport that differ by as little as 0.1 mm/d. When translated into microscopic terms, this means that it is possible to distinguish two processes that differ in their average rates by as little as 0.001 μm/s. Thus, the axonal transport paradigm can provide information about the dynamic properties of the cytoplasmic matrix at a level of resolution usually limited to microscopic methods.

Fast and Slow Axonal Transport

Studies of the transport of proteins in axons demonstrate that transported proteins fall into two distinct categories on the basis of their rates of transport (3, 25, 35). One group of proteins is carried by fast axonal transport at rates ranging from 50 to 400 mm/d (0.5–4 μm/s). The other group is transported by slow axonal transport at rates ranging from <1 to 5 mm/d (0.01–0.05 μm/s). Fast and slow axonal transport components differ biochemically and cytologically. Fast axonal transport conveys the membranous organelles of the axoplasm, and slow axonal transport conveys the components of the cytoplasmic matrix (Table I).

FAST AXONAL TRANSPORT: All proteins carried by fast axonal transport are components of membranous organelles,

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TABLE 1

Rate Components of Axonal Transport and Cytological Structures

Rate component	Rate	Protein composition	Cytological structure
	mm/d		
Fast	50–400	*Membrane-associated materials*	*Membranous organelles*
Orthograde	200–400 (1–3 μm/s)	Na^+-K^+ ATPase, transmitter-associated enzymes, GAPs	50-nm tubulovesicular structures, dense-core vesicles
Mitochondria	50–100	F1 ATPase, a small amount of spectrin	Mitochondria
Retrograde	200	Lysosomal hydrolases, NGF, and other materials obtained by endocytosis	Prelysosomal structures (multivesicular and multilamellar bodies)
Slow	0.2–8	*Cytoskeletal and associated proteins*	*Cytomatrix*
SCb	2–8	Actin, clathrin, spectrin, myosinlike proteins, NSE, CK, calmodulin, aldolase, pyruvate kinase	Microfilaments, clathrin complex, metabolic enzyme complex, carrier complex (?)
SCa	0.2–1 (0.002–0.01 μm/s)	Tubulin, neurofilament triplet, tau proteins, spectrin	Microtubule-neurofilament network

Studies on the kinetics of individual proteins in axonal transport have led to the identification of a number of axonal proteins and the rates at which they move. By looking at the proteins that move coherently in axonal transport, we can relate rate components of axonal transport to the composition of specific cytological structures.

For general reviews on the composition and organization of fast axonal transport, see references 3, 14, 25, 35, and 61. For slow axonal transport, see references 3, 5, 11, 21, 25, 34, 35, and 37. For transport of specific proteins, see selected references: actin (4, 6), myosinlike proteins (63), spectrin (fodrin) (39), neurofilament proteins (30), tubulin (8, 15, 59), MAPs (tau) (60), clathrin (20), growth-associated proteins (GAPs) (3), calmodulin (10), nerve-specific enolase (NSE), and creatine kinase (CK) (9).

such as small vesicles, secretory granules, dense bodies, multivesicular bodies, and mitochondria (14). The movement of these membranous organelles can be observed directly with the light microscope, and their rate of movement correlates exactly with that predicted from studies with radioisotopes (1, 13). The membranous organelles move bidirectionally in the axon, either orthogradely from the cell body toward the axon terminus or retrogradely from the axon terminus toward the cell body. Although individual membranous particles can move bidirectionally, each particle has a preferred direction. For example, vesicles that supply the presynaptic transmitter structures move orthogradely, and endocytic vesicles containing worn-out proteins from the axon terminus move retrogradely (14, 25).

SLOW AXONAL TRANSPORT: Slow axonal transport differs from fast axonal transport in its rate and the materials transported by it (3, 35, 64). No membranous proteins have been detected moving at the slow rates (61). Conversely, no proteins of the cytoplasmic matrix have been detected moving with the most rapidly moving membranous elements (58). All proteins of the cytoplasmic matrix, including the cytoskeletal proteins and the soluble proteins of the axon, move by slow transport (11, 12). This segregation of the axonally transported membranous elements from those of the cytoplasmic matrix provides new insights into the normal or physiological associations between the proteins of the cytoplasmic matrix and the membranous organelles. Many of these associations were not readily predicted from previous electron microscope and biochemical studies of axons.

ACTIN AND CLATHRIN ARE TRANSPORTED BY SLOW TRANSPORT AND NOT WITH THE MEMBRANOUS ELEMENTS BY FAST AXONAL TRANSPORT: Electron microscope analyses of intracellular architecture demonstrate an intimate association between the components of the cytoplasmic matrix and the membranous organelles (18). Two elements of the cytoplasmic matrix in particular, the actin microfilaments and the clathrin baskets, appear to have a particularly close association with membranous organelles (2, 51). However, studies of axonal transport have demonstrated unequivocally that neither actin nor clathrin is carried along with the rapidly moving membranous organelles in the axon. Instead, these proteins are carried exclusively by slow axonal transport (4, 20). This suggests that the interactions between these cytoplasmic matrix proteins and the rapidly moving membranous organelles must be transient. That is, the rapidly moving membranous organelles move through the cytoplasmic matrix without forming permanent associations with the cytoskeleton and without picking up any of the cytoskeletal proteins as they move along.

These observations are inconsistent with models of axonal transport that invoke cytoplasmic streaming or bulk transport of the axoplasm (9, 11, 35). If membranous organelles in the axoplasm were propelled by cytoplasmic streams, then soluble proteins, which are present in the aqueous phase, would be swept along in the streams that move the membranous organelles. However, no soluble proteins or any other proteins that are components of the cytoplasmic matrix are carried along with the membranous organelles. Instead, these proteins move entirely by slow axonal transport (9, 11). Some small molecules, such as amino acids, have been found moving by fast axonal transport, but they probably are contained within the rapidly transported membranous vesicles (23). Rather than by cytoplasmic streaming, it seems likely that the membranous organelles are moved through the axoplasm by specific force-generating structures present in the cytoplasmic matrix. These force-generating structures, which may include actin and myosin, probably act directly on the membranous organelles in cycles of attachment, contraction, and detachment (14, 22).

SCa AND SCb REPRESENT DIFFERENT STRUCTURAL COMPARTMENTS IN THE CYTOPLASMIC MATRIX: The membranes of the rapidly transported organelles act as a natural boundary that prevents mixing of proteins of the slowly transported cytoplasmic matrix with the rapidly transported organelles. However, there also are other natural boundaries within the axon that separate the elements of the cytoplasmic matrix into subcompartments. In studies on slow axonal transport, these subcompartments are distinguished by their coherent rates of movement. Two major rate subcomponents of slow axonal transport have been studied in detail:

slow component a (SCa)[1] and slow component b (SCb) (5, 11).

In Fig. 1, the slowly transported proteins are shown as two separate and distinct waves of radioactivity, SCa and SCb. SCa, which is the slower of the two moving waves, is transported at rates of about 1 mm/d. SCb generally moves five to ten times faster than SCa. Simple diffusion cannot account for the coherent movement of the proteins comprising the two different SCa and SCb waves (11). In radioisotopic labeling experiments, diffusion of proteins from the cell bodies would appear as an exponentially declining curve extending out along the axons. However, the labeled proteins in both SCa and SCb are distributed as separate waves that move unidirectionally away from the cell body. The separation between the waves increases with time because they move at different rates.

Analyses of the proteins contained within these waves by two-dimensional polyacrylamide gel electrophoresis (2D PAGE) demonstrate that the patterns of proteins in SCa and SCb differ greatly (Fig. 2). SCa has a relatively simple protein composition. By contrast, SCb has an extremely complex composition, and more than 200 polypeptides can be resolved. Another important feature that distinguishes SCa and SCb is that each transport component contains proteins that can be considered specific either to SCa or SCb. In fact, only a few proteins are transported coordinately with both SCa and SCb.[2]

SCa consists principally of tubulin and the neurofilament proteins. Certain tau proteins, which are a specialized subset of microtubule-associated proteins (MAPs) and brain spectrin (fodrin), are also found in SCa (Table I and Fig. 2). Except for brain spectrin, none of these SCa proteins is represented in SCb. In contrast, SCb contains actin, clathrin, calmodulin, spectrin, and a variety of metabolic enzymes, such as enolase, aldolase, and creatine phosphokinase (Table I and Fig. 2). Most of these SCb proteins are not detectable in SCa. The small number of SCb proteins, such as actin, present in SCa represent a trailing component of the SCb wave (see Fig. 5). Each of the waves in slow transport corresponds to a different class of proteins that are associated with each other but remain partitioned from the proteins within the other wave. This indicates that the SCa and SCb waves each correspond to a subcompartment in the cytoplasmic matrix (5).

What is the structural basis of the compartmentalization of the proteins of the cytoplasmic matrix? It seems that proteins of the cytoplasmic matrix are compartmentalized because of their affinity for particular structures within the cytoplasmic matrix (35, 61). The SCb proteins either are incorporated into these structures or bind to their surfaces. In the axon, the cytoplasmic matrix contains the cytoskeletal polymers (microtubules, neurofilaments, microfilaments) and a variety of less defined granulofilamentous structures that are organized

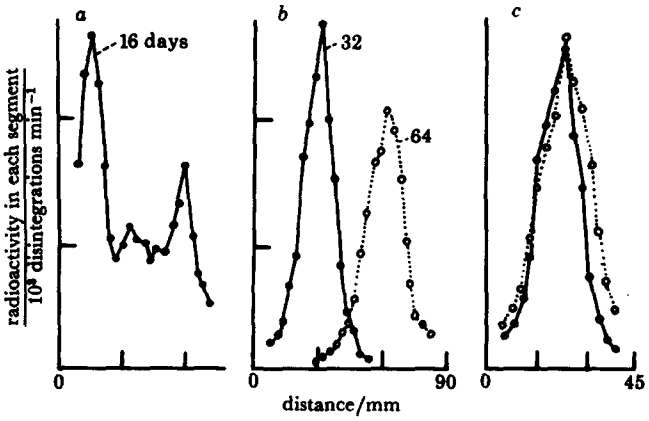

FIGURE 1 The movement of slowly transported proteins is illustrated in axons of guinea pig phrenic nerves. These data demonstrate the orderly progression of the SCa wave in phrenic axons over 2 months. The SCa wave does not spread as it progresses along the axons. A mixture of [^{3}H]lysine and proline was injected into the cervical spinal cord to label the phrenic motor neurons. Then the distribution of slowly transported proteins was measured by the analysis of consecutive 3-mm segments of the phrenic nerve. Transport profiles 16 (*a*), 32, and 64 d (*b*) after injection are shown. At 16 d, both the faster-moving SCb wave and the slower-moving SCa wave are apparent. Later only the SCa wave, which represents tubulin and neurofilament proteins, is seen. To more effectively compare the distributions of the radioactivity in SCa at 32 and 64 d, the data were normalized and plotted in such a way that the peaks of the waves coincide (*c*). Each point is the mean of five determinations. The results are reproduced with permission from M. Black (6).

by the primary cytoskeletal polymers (20, 55). Analysis of the axonal transport of the cytoplasmic matrix proteins has provided us with a reasonably detailed understanding of the dynamics of their association with the structural elements in the axoplasm.

Structural Hypothesis of Axonal Transport

The possibility that cytoplasmic matrix proteins move in the form of polymers and/or supramolecular complexes follows from the *structural hypothesis* of axonal transport (33, 35, 61). This hypothesis holds that proteins are actively transported in the axon either as an integral part of a moving cytological structure or in long-term association with these structures. For reviews of studies dealing with this hypothesis, see Lasek (34, 37), Brady and Lasek (11, 12), Lasek and Brady (35), and Tytell et al. (61). These studies correlate SCa and SCb with different cytological structures and demonstrate that each of these structures must have specific affinities for particular axonal proteins. The test of any hypothesis is that it is predictive and that the predictions generated are testable. In the following section we provide examples of some of the testable predictions that have been made from the structural hypothesis.

SPECIFIC PREDICTIONS OF THE STRUCTURAL HYPOTHESIS: The structural hypothesis predicts that proteins that are stably associated during their transit within the axon have an equally stable structural counterpart in the axon. This prediction can be tested for any set of proteins that have coordinate transport kinetics by analyzing the composition of structures in the axon. If the hypothesis is correct, then it should be possible to identify a stable axonal structure with

[1] *Abbreviations used in this paper:* 2D PAGE, two-dimensional polyacrylamide gel electrophoresis; IDPN, β-iminodiproprionitrile; MAPs, microtubule-associated proteins; SCa, slow component a; SCb, slow component b.

[2] The analyses of SCa and SCb presented in this paper focus primarily on one set of neurons, the retinal ganglion cells of adult mammals. In these neurons, SCa and SCb are completely separate, permitting the structural subcomplexes in SCa and SCb to be distinguished by their rates of movement. In some other neurons, the SCa and SCb waves are much broader and tend to overlap, causing the distinction between SCa and SCb to be blurred (30, 46, 66).

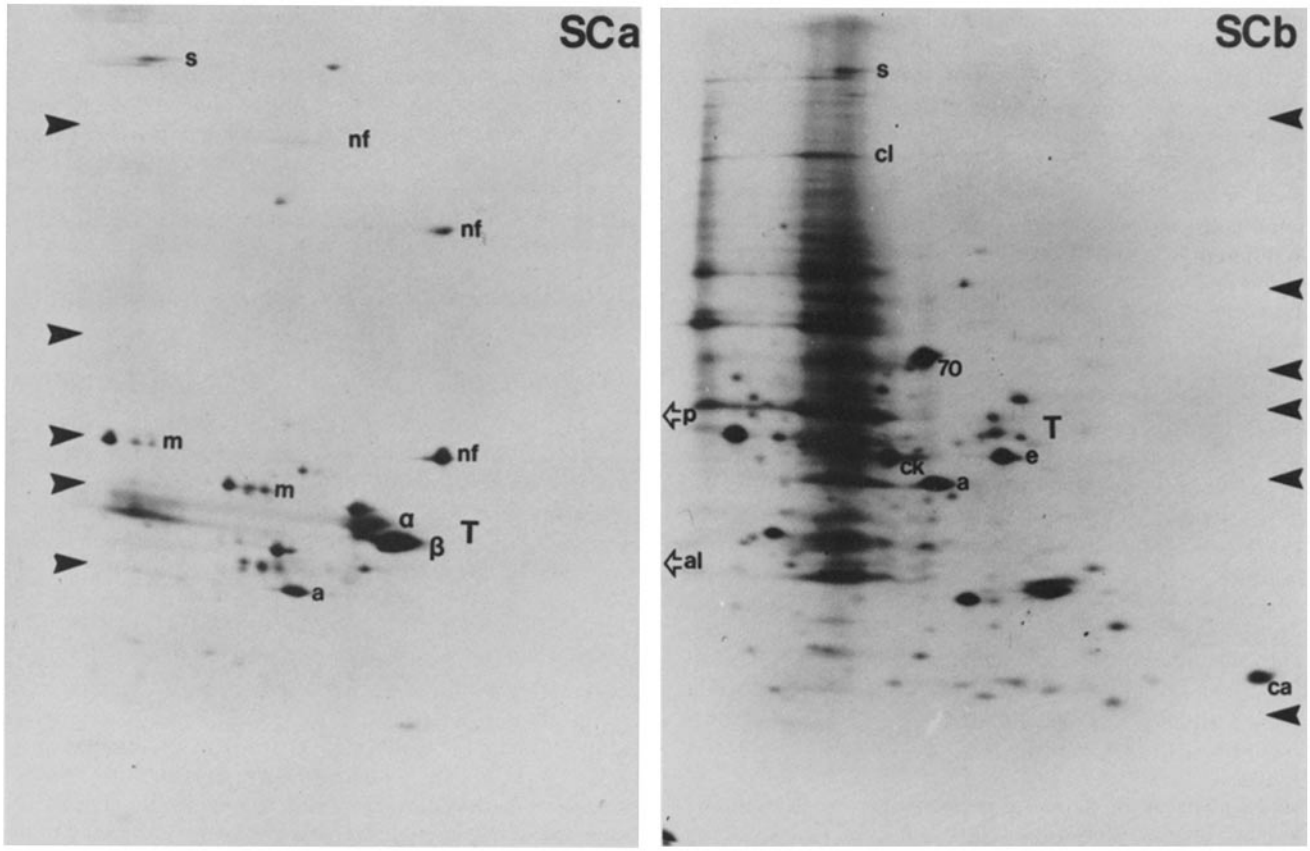

FIGURE 2 2D PAGE of the slow components of axonal transport. Fluorographs showing the major polypeptides of optic nerve resolved in 2D PAGE after labeling of the retina with [^{35}S]methionine with an injection sacrifice interval of 40 (SCa) or 6 d (SCb). Comparable segments of optic nerve were analyzed and processed identically. The major identified proteins are indicated for each rate component. SCa includes neurofilament proteins (*nf*), tubulin (*T*; α and β indicate alpha and beta subunits), MAPs (*m*), brain spectrin (fodrin) (*s*), and a small amount of actin (*a*) trailing behind the SCb wave. SCb includes many more polypeptides, but only a few have been identified. These are actin (*a*), calmodulin (*ca*), nerve-specific enolase (9e), creatine kinase (*ck*), clathrin (*cl*), and spectrin (*s*). In addition, two enzymes have been identified that are relatively basic proteins and are not resolved on standard two-dimensional gels, aldolase (*al*) and pyruvate kinase (*p*). The polypeptide complex indicated by the number 70 has not yet been identified. It is very strongly labeled with [^{35}S]methionine in SCb but does not trail into SCa significantly (see SCa fluorograph).

the same composition as that predicted by the axonal transport kinetics. Hoffman and Lasek (30) provided the first successful application of this hypothesis when they deduced the composition of the neurofilaments from studies of axonal transport. They demonstrated that SCa contains three proteins, the triplet, which move coordinately in the axon. They proposed that the triplet proteins are the subunits of the neurofilaments. This proposal was made on the basis of several factors, including the transport kinetics and the abundance of the triplet proteins in the axons. At the time of this proposal, the composition of neurofilaments was unknown, and the triplet proteins had not been previously reported in the literature. Subsequent research proved that the triplet proteins are the subunits of neurofilaments (40, 67).

In a reverse application, the structural hypothesis has also been useful in predicting the transport properties of proteins from knowledge of the composition of characterized axonal structures. If the composition of an axonal structure is known, the structural hypothesis predicts that all of the proteins that are stably associated with that structure will move coordinately in the axon. This prediction has been tested in microtubules. For example, microtubules consist of tubulin and MAPs, and the MAPs bind tightly to microtubules but not to monomeric tubulin (24). The structural hypothesis predicts that MAPs will move coordinately with tubulin in the axon if the tubulin moves in the form of microtubules. Tytell et al. (60) tested this prediction and found that tubulin and one set of MAPs, the tau proteins, are transported coordinately in retinal ganglion cells (see Fig. 2). This suggests that tubulin and tau proteins are transported as component parts of microtubules and provides support for the validity of the structural hypothesis. These observations also suggest that tau proteins are the most abundant MAPs on the microtubules of retinal ganglion cell axons. The axonal transport studies indicate that MAP 1 and MAP 2 are present in much smaller amounts than the tau proteins in these axons. This is consistent with immunocytochemical studies that demonstrate that MAP 2 is much more abundant in dendrites than axons (7, 42, 62).

We have demonstrated that it is possible to make specific predictions based on the structural hypothesis. Furthermore, when these predictions have been tested they have been found to be accurate. Although the hypothesis must undergo further analysis, our experience suggests that it can provide a theoretical framework for understanding how the multiplicity of structures that comprise the cytoplasmic matrix move in neurons and in other cells. One of the important aspects of the structural hypothesis is that it calls attention to the struc-

tural relationships of the transported proteins—i.e., to their associations with specific binding sites on structures of interest to cell biologists—rather than to the individual proteins themselves. Consequently, as confidence in the hypothesis has grown it has become increasingly clear that cytological structures, not individual proteins, are the biologically relevant units of axonal transport.

STRUCTURAL COUNTERPARTS OF SLOW AXONAL TRANSPORT: Table I provides a list of structures that may correspond to axonally transported proteins in the axon. It should be noted that the degree of confidence which should be attached to the structural relationship in this list varies. For example, it has been directly demonstrated that the rapidly transported proteins are conveyed in the membranous elements which are included in Table I (14, 25). The evidence supporting the proposal that neurofilament proteins, tubulin and actin, are transported in the form of polymers in SCa remains indirect but reasonably strong (5, 11, 32, 60). We are less certain about the cytological counterpart of many of the SCb proteins (9–11, 21).

SCb is far more heterogeneous in its composition than SCa (Fig. 2), suggesting that the SCb proteins comprise an equally heterogeneous set of structures. The microfilaments are probably one important set of structures that contribute to the organization of the SCb proteins, but there must be others. Recent electron microscope studies have identified a granulofilamentous matrix in the axon that could encompass the heterogeneous constellation of proteins that is present in SCb (20, 55). These structures are distributed in two locations. One set of granulofilamentous elements is associated with the cortical region of the axon underlying the plasma membrane, the other with the microtubule domains (20, 28, 55).

Axonal Transport Kinetics Provide Information about the Associations between Proteins and Structures in the Axon

The correlation of particular structures with SCa and SCb permits us to extract information about the relative movements of these structures within the axon (34). That is, the axonal transport paradigm provides an assay for the relative strength of associations among structures within the cytoplasmic matrix (10, 12). If the associations are relatively stable, the structures move coordinately as a single wave in the axon. In contrast, if the associations are transient, the structures can move separately and form a separate transport wave (5). For example, the microfilaments transported in SCb must move separately and more rapidly than the microtubules and neurofilaments in SCa, because SCa and SCb move separately in the axon (4). Although microfilaments probably interact with the microtubules and neurofilaments, these interactions must be relatively transient compared with the interactions between the microtubules and the neurofilaments, which move together in the axon (5, 11, 34).

Whereas the separation between transport waves can be related to the strength of associations between moving structures in the axon (34), changes in the shapes of the waves may provide information about the associations between proteins and structures that are transported in the waves (21). The strength of the associations between proteins and axonal structures ranges from extremely strong bonds, which are effectively nondissociable, to relatively weak bonds, which readily dissociate. Proteins that have strong affinities for axonal structures are generally classed as insoluble. In contrast, proteins that have weak affinities for structures in the axon can readily enter the aqueous phase and are classed as soluble. By analyzing the detailed wave shapes of axonally transported proteins it is possible to compare the relative affinities between these proteins and the transported structures. It is noteworthy that the information obtained in these analyses reflects the associations between proteins and structures under physiological conditions in situ (34).

Axonal Transport of SCa: Axonal Cytoskeletal Proteins Are Incorporated into Stable Polymers That Stabilize the Axonal Cytoplasmic Matrix

NEUROFILAMENT PROTEINS EXEMPLIFY PROTEINS THAT ARE STABLY ASSOCIATED WITH A TRANSPORTED STRUCTURE: The neurofilament proteins have been particularly useful for understanding the relationship between axonal transport kinetics and the structural associations of proteins. In axons, the neurofilament proteins are stably assembled into neurofilaments and there is little, if any, diffusible monomer (38, 47, 48). Thus, the neurofilament proteins exemplify the subset of axonal proteins that have an extremely high affinity for a transported structure in the axon.

The transport kinetics of the neurofilaments is relatively simple (5, 30). Radiolabeled neurofilament proteins move along the axon as a bell-shaped wave that exhibits the same properties as the SCa wave illustrated in Figs. 1 and 3. This wave maintains its shape as it moves along the axon and does not leave a trail behind it (32). The shape of the wave form apparently reflects the various steps in the incorporation of labeled precursor into neurofilaments before their transport in the axon. Once in the axon, the neurofilament proteins move unidirectionally toward the terminus, where they are specifically degraded (32, 52).

Neurofilaments have another property that affects their transport through the axon. They are linked to each other by cross-bridges and form a network (43, 44). The cross-bridges apparently are dynamic (i.e., they can detach from adjacent neurofilaments and reattach) (44, 45). Despite the dynamic nature of the individual cross-bridges, the hundreds or thousands of cross-bridges that are located along the length of the neurofilaments may restrict the capacity of neurofilaments to slide past each other. This may explain why the labeled neurofilament wave does not spread very much during the

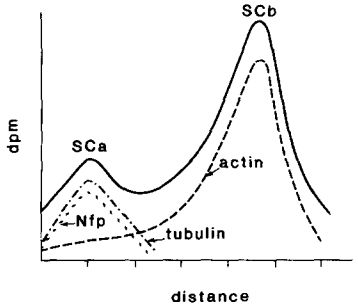

FIGURE 3 Illustration of the kinetics of SCa and SCb in rat and guinea pig retinal ganglion cells extrapolated from results obtained at a number of intervals after slowly transported proteins had been labeled in these cells. The distribution of radioactivity is shown as it would appear if the optic nerve and tract were 30 mm long and the animal were sacrificed 12 d after injection. The individual proteins were analyzed by the method described in Black and Lasek (5). The upper curve is the distribution of total transported radioactivity in SCa and SCb, and the individual distributions of neurofilament proteins (*Nfp*), tubulin, and actin are noted.

long periods that it takes the neurofilaments to move over distances of centimeters in the axon.

One of the most important functions of neurofilaments in the axon is their contribution to the stability of the cytoplasmic matrix (34, 38, 47). Neurofilaments provide structural stability because they remain polymerized and because of their tendency to form stable networks (44). This may be an example of a large class of mechanisms that maintain stability within the axonal cytoplasmic matrix. The tendency of the proteins in the axon to form stable structures may be a special adaptation related to the fact that structures of the cytoplasmic matrix are in transit for months and, in some cases, for years. Stable bonds in the cytoplasmic matrix ensure that these structures retain their integrity during their long journey from the cell body to the axon terminus.

STABLE MICROTUBULES ADD STABILITY TO THE AXONAL CYTOPLASMIC MATRIX: In retinal ganglion cells, tubulin exhibits axonal transport kinetics very similar to those of the neurofilament protein. The tubulin moves at the same rate as the neurofilament proteins, suggesting that microtubules and neurofilaments move coordinately (Fig. 3 and reference 5). Furthermore, the shapes of the tubulin wave and the neurofilament wave are very similar. The similarity between the shapes of the tubulin wave and that of the insoluble neurofilament proteins suggests that tubulin is relatively stably associated with the transported microtubules. In fact, biochemical analyses of axonally transported tubulin in retinal ganglion cells indicate that >60% of the axonally transported tubulin is insoluble under conditions that normally solubilize microtubules from brain (8, 15). Furthermore, this cold-insoluble tubulin appears to be in the form of microtubules (54). The stability of the axonal tubulin in the cold-insoluble microtubules may be due to the presence of an unusual isoform of α-tubulin that is biochemically different from soluble brain α-tubulin (15).

These observations are consistent with the prediction that axonally transported tubulin in retinal ganglion cells is primarily in the form of microtubules and that there is a relatively small pool of tubulin in the aqueous phase of these axons. Furthermore, the surprising observation that most of the polymerized tubulin in retinal ganglion cells is stably polymerized reinforces our proposal that the elements comprising the cytoplasmic matrix of the axon are extremely stable.

MICROTUBULES AND NEUROFILAMENTS FORM A RELATIVELY STABLE NETWORK IN THE AXON: In retinal ganglion cells, the labeled tubulin wave moves coordinately with that of the labeled neurofilament proteins, suggesting that microtubules and neurofilaments move together in the axon (Fig. 3). Electron microscope and biochemical studies indicate that these two sets of structures are linked by cross-bridges (53, 57). Neurofilaments tend to associate with other neurofilaments, and microtubules tend to associate preferentially with microtubules. However, the neurofilaments are also linked to the microtubules in those regions where the different polymers lie next to each other.

The cross-bridges between the linear polymers keep these structures aligned so that the polymers form an ordered network (18, 29). This network has two important properties: it defines the shape of the axon (44) and it directs the movement of particles so that they move parallel to the long axis (1, 13). Crosslinking between the elements of the cytoplasmic matrix also plays an important role in the translocation of the cytoplasmic matrix. It has been suggested that the components of the cytoplasmic matrix are moved by a force-generating system that is an integral part of the cytoplasmic matrix within the axon (3, 37). The presence of actin and a myosinlike protein in SCb suggests that SCb contains the force-generating structure for movement of the cytoplasmic matrix (4, 63). If this is the case, then the force must be distributed from the motile complex in SCb to the other components of the cytoplasmic matrix. The extensive system of cross-bridges between the microtubules and neurofilaments is probably important in distributing these forces from the motility structures to the components in the network (32).

The cross-bridges on the neurofilaments and microtubules consist of different proteins. The neurofilament cross-bridges are formed from regions of the neurofilament subunits that extend out from the primary structure of the neurofilament (65). In particular, the 200,000-dalton subunit of the neurofilament triplet has been shown to contribute to these cross-bridges. The cross-bridges on microtubules consist of the MAPs (24). The composition of the MAPs is different in different regions of the nervous system (7, 42, 62; Brady, S. T., unpublished observations). In retinal ganglion cells the most abundant MAPs are the tau proteins (60; see also Fig. 2). In contrast, MAP 2 is particularly abundant in dendrites and is much less abundant in axons (42, 60). Differences in the MAP composition in different regions of the neuron and in different neurons may influence the amount of crosslinking between the polymers in the axon.

Comparisons of the axonal transport kinetics in different neurons suggest that the amount of crosslinking between neurofilaments and microtubules varies substantially. For example, in retinal ganglion cells the transport distributions of tubulin and the neurofilament proteins are very similar, suggesting that these structures are tightly crosslinked (Fig. 3). In contrast, in ventral motor neurons and dorsal root ganglion cells, tubulin has a more complex distribution than the neurofilament proteins (30, 46). In these neurons, most of the tubulin is transported coordinately with the neurofilament proteins, but a significant fraction moves more rapidly, spreading the wave orthogradely toward SCb. This more rapidly moving tubulin may represent a distinct population of microtubules composed of a different set of isoforms than the tubulin moving with the neurofilaments (59; Brady, S. T., and Oblinger, unpublished observations). The differences between these two populations of microtubules may affect their affinity for neurofilaments, with the result that one population is more tightly crosslinked to the neurofilaments.

β,β'-IMINODIPROPRIONITRILE INTERFERES WITH THE COORDINATE MOVEMENT OF MICROTUBULES AND NEUROFILAMENTS IN THE AXON: The importance of the cross-bridges between microtubules and neurofilaments for the coordinate movement of these structures is further supported by studies with pharmacological agents that cause segregation of the neurofilaments and microtubules. For example, the neurotoxin β,β'-iminodiproprionitrile (IDPN) causes the neurofilaments and microtubules to segregate (26, 27, 49). The neurofilaments become located almost exclusively at the perimeter of the axon and the microtubules are located at the center of the axon (49). At appropriate doses, IDPN selectively blocks movement of the neurofilaments but has very little effect on the transport of tubulin and SCb proteins (26). The neurofilaments accumulate in large masses at the proximal end of the axon near the cell body (27).

Apparently, neurofilaments can be transported from the cell body into the axon but require an association with the microtubules to continue moving within the axon.

SPECTRIN CROSSLINKS STRUCTURES IN BOTH SCa AND SCb: Brain spectrin (fodrin) may be a particularly important crosslinking component in the cytoplasmic matrix of the axon. Spectrin has unusual transport kinetics because it is distributed in many different rate components (3, 39). A small amount of it moves in the axon at rates as fast as 50 mm/d, which would place it in association with the membranous elements. The bulk of the spectrin moves by slow transport and is distributed between SCa and SCb, suggesting that spectrin has a high affinity for structures in both SCa and SCb (39). It is not surprising that spectrin is in SCb, because actin microfilaments are major components of SCb and spectrin binds to microfilaments. However, more spectrin moves in SCa than in SCb (39).

The coordinate transport of spectrin with SCa suggests that spectrin makes stable associations with structures transported in SCa (i.e., microtubules or neurofilaments). This possibility was unexpected because neurofilaments and microtubules are distributed uniformly through the cross section of the axon, and immunocytochemical studies have suggested that spectrin is present primarily beneath the plasma membrane (39). The apparent inconsistency between the axonal transport results and the immunocytochemical results has recently been resolved. When spectrin is injected into cells it becomes concentrated in the regions containing intermediate filaments, suggesting that it binds to the intermediate filaments (16). Quantitative analyses of the distribution of spectrin in the squid giant axon indicate that most of the axonal spectrin (~80%) is present in the central axoplasm and that the remainder is located in the cortex (Fath, K., and R. J. Lasek, unpublished observations). These results indicate that, although spectrin is concentrated in the cortical axoplasm, most of it is more widely distributed throughout the axoplasm. This observation points up the limitations of immunocytochemical methods, which tend to focus attention on highly concentrated antigens while deemphasizing less concentrated antigens that may be more abundant. Furthermore, these observations provide another example of how axonal transport can provide novel insights into the structural associations between proteins and the cytoplasmic matrix.

Axonal Transport of SCb: Proteins That Actively Exchange between the Cytoplasmic Matrix and the Aqueous Phase

The transport of insoluble proteins such as neurofilament proteins is not complicated by the stochastic events that occur when proteins are free to enter the aqueous phase of the axoplasm. However, many proteins of the cytoplasmic matrix have a large soluble component (9, 10, 21). These proteins exist in equilibrium between the aqueous phase and binding sites located in the cytoplasmic matrix. When they are in the aqueous phase, they are subject to diffusion and potential interactions with other structures in the axon. These interactions tend to retard the movement of the proteins, with the result that the diffusible proteins fall behind the structure that conveys them through the axon (21).

Many of the proteins of SCb are soluble. For example, soluble enzymes of intermediary metabolism have been identified among the proteins of SCb (e.g., enolase, creatine phosphokinase, aldolase, pyruvate kinase), and it seems likely that many of the unidentified proteins in SCb correspond to the components of the pathways of intermediary metabolism (9, 11). Although many of the proteins of SCb are soluble, all are not. Proteins such as clathrin and spectrin are generally considered to be bound within the cytoplasm and not free to diffuse into the aqueous phase (20, 39). Thus, any model of the transport of the proteins in SCb must explain how proteins that are stably associated with the cytoplasmic matrix move coordinately with proteins that can exchange with the aqueous phase.

SCB CONTAINS A CARRIER STRUCTURE THAT ORGANIZES THE SOLUBLE PROTEINS OF THE AXOPLASM: Careful analysis of the kinetics of 20 different SCb proteins demonstrates that these proteins exhibit a great deal of coherence in the front of the axonally transported wave as it advances along the axon (Fig. 4). This front represents the average maximum rate at which each of the labeled proteins moves within the axon (21). The behavior of the SCb proteins suggests that they are transported by a common carrier structure. However, most of the SCb proteins appear to associate and dissociate from the carrier structure as it moves through the axon. These proteins have an assymetric transport wave. For example, actin has a very broad distribution and a long tail that follows the moving wave (Fig. 5).

The assymetry of the wave form exhibited by proteins such as actin suggests that the transport of these proteins is influenced by forces that retard their movement in the orthograde direction (21). This effect is expected if a relatively large proportion of these proteins is in the aqueous phase and if these proteins can exchange with the proteins that bind to the moving carrier structure. Actin has exactly these properties. In the squid giant axon, 50% of the axoplasmic actin is in the

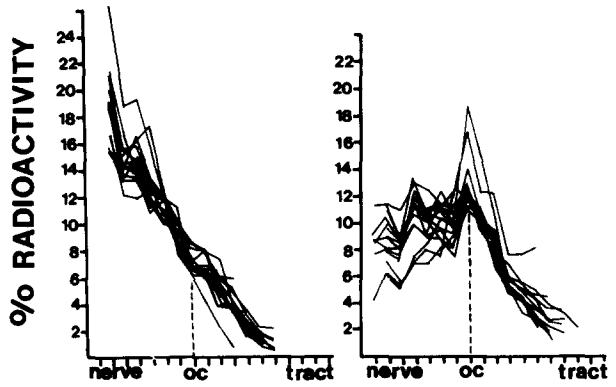

FIGURE 4 The distribution of radioactivity curves 4 (*left*) and 6 (*right*) d after intraocular injection for each of 20 SCb proteins have been superimposed to demonstrate the similarity of the distribution in the advancing front of the SCb wave and the range in variation of the curves trailing behind the SCb peak. The range in variation may reflect differences in the affinity the SCb proteins have for the carrier structure (see text). Guinea pig optic nerves, optic chiasma (OC), and tracts were cut into 1-mm segments and homogenized, and sequential segments were electrophoresed on gradient SDS polyacrylamide gels after the appropriate injection-sacrifice interval. Radioactivity present in individual SCb protein bands was determined as described by Garner and Lasek (21). The distribution of radioactivity in individual SCb proteins was determined as follows: first, the total radioactivity found in a particular SCb band was summed over the entire length of the visual system, then the amount of radioactivity in that SCb band in each segment was plotted as percent total radioactivity. Eight to ten nerves were used for each time point.

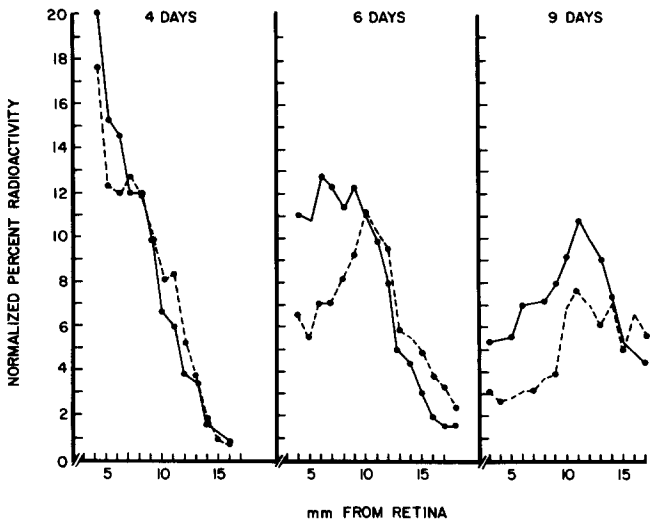

FIGURE 5 Radioactivity distribution curves of two identified SCb proteins, actin and clathrin, are characteristic of two major classes of distribution curves. Actin (solid lines) has a broad distribution, suggesting that it may be able to dissociate from the SCb carrier structure and transiently interact with other elements in the aqueous phase. Clathrin (broken lines), on the other hand, has a sharply triangular distribution, suggesting a stable association with the carrier structure (or an integral role). Distribution curves were plotted as described in the legend to Fig. 4. Percent radioactivity 9 d after injection was normalized to allow for the proportion of the curves that had exited the visual system.

form of diffusible monomer and the remainder of the actin is polymeric (48). These observations suggest that the shape of the transport wave is predictably related to the affinity of the protein for the carrier structure that conveys the SCb proteins. Furthermore, the coordinate transport of the soluble proteins with SCb and not with SCa indicates that the carrier structure has a higher affinity for these proteins than do the microtubules and neurofilaments.

The wave form of some of the SCb proteins, such as clathrin, suggests that these proteins make stable associations with the carrier structure for SCb. Clathrin has a triangular wave form resembling that of the neurofilament protein (cf. Figs. 1 and 5). The similarity of the transport wave of clathrin and that of the insoluble neurofilament proteins suggests that clathrin has a stable association with the carrier structure in SCb and that it is nondiffusible. Biochemical analyses of axonally transported clathrin support this suggestion (20; Heriot, K., and R. J. Lasek, unpublished observations).

IDENTIFICATION OF THE PROTEINS COMPRISING THE CARRIER STRUCTURE FOR SCB PROTEINS: Identification of the carrier structure (Fig. 6) that organizes the proteins of SCb would be an important step toward understanding the cytoplasmic components that organize the soluble proteins of the cytoplasm. The insoluble proteins that move coordinately with the front of SCb provide important clues to the composition of the carrier structure because these proteins are stably associated with the carrier and probably contribute to its structure. Clathrin is an interesting example

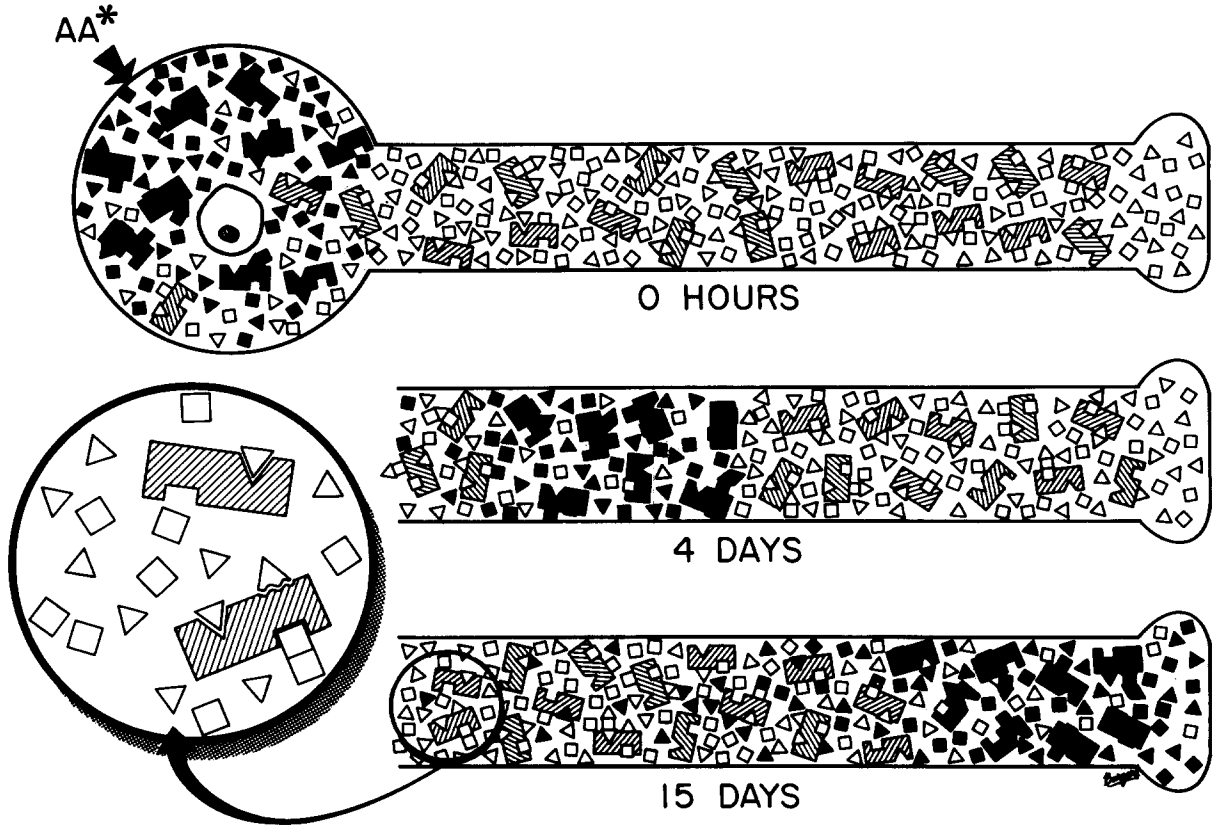

FIGURE 6 Schematic representation of the carrier structure within the axon as proposed in the text. The multidimensional blocks represent the carrier structure itself; the smaller geometric forms represent individual proteins of the cytoplasmic matrix that are carried by and associated with the moving structure. The colored forms illustrate the distribution of pulse-radiolabeled proteins and carrier structure as they move down the axon. As can be seen, the radiolabeled carrier structure units move as a much more discrete and cohesive wave than the other proteins. The distribution of the latter tends to spread as they move, to a degree correlated with their relative affinity for the moving complex.

of the proteins that remain stably associated with the carrier structure because it is multivalent. It not only binds with high affinity to actin microfilaments and other proteins (56) but also associates with specialized membranes (50, 51). Clathrin can self-assemble (31), and it may exist in more than one structural form (17, 56). These properties suggest that clathrin could be a multifunctional component of the carrier structure. One of these functions may be to provide a dynamic linkage with the plasma membrane.

Two other proteins have transport kinetics that suggest that they form part of the carrier structure (21). These proteins have nominal molecular weights of 200,000 and 500,000. The 500,000 M_r protein is one of the more prominent proteins in SCb, suggesting that it may be relatively abundant in the axon. Furthermore, its large subunit size suggests that it could have many binding domains. Proteins with many binding domains can structure many additional proteins and crosslink components of the cytoplasmic matrix.

Although actin has a large soluble component in the axoplasm, it may also contribute to the carrier structure. A stably polymerized form of actin has been identified in the axon (48). Furthermore, microfilaments bind many different cytoplasmic proteins, including metabolic enzymes such as aldolase, with relatively high affinity (41). Thus, microfilaments could provide a large number of sites for binding other SCb proteins to the carrier structure.

Summary and Conclusions

The cytoplasmic matrix of neurons consists of the same basic components that are found in the cytoplasmic matrix of other cells. However, during the evolution of the neuron many of the cytoplasmic matrix proteins became highly specialized. In many cases, these specializations appear to be directly related to the unique capacity of neurons to generate axons. In fact, some of the major cytoplasmic matrix proteins, such as the neurofilament proteins, appear to be targeted specifically for the axon.

Most of the specialized properties of the axonal cytoplasmic matrix can be related, either directly or indirectly, to one basic property of axons, their unusual length. It appears that during the evolution of neurons the basic mechanisms of cell locomotion have changed to allow the efficient movement of the axonal cytoplasmic matrix from the cell body through the axon. The cytoplasmic matrix is transported at rates similar to those of fibroblast locomotion and axonal elongation. It is likely that the mechanisms underlying the movement of the axonal cytoplasmic matrix have many similarities with those underlying fibroblast locomotion. For example, both of these processes probably involve the intracellular translocation of cytological structures. In axons, the transport of the cytoplasmic matrix is manifested externally only when the axon is elongating. However, studies with radioisotopic precursors reveal that this transport operates continually.

Slow transport is responsible for the translocation of the cytoplasmic matrix within the axon. Detailed analyses of this process indicate that it involves the concerted action of many distinct structures acting synergistically. Although the system is interactive, two subcomponents of slow transport, SCa and SCb, can be clearly distinguished on the basis of their rates of movement. SCa, which moves at about 1 mm/d, corresponds to the long cytoskeletal polymers, microtubules, and neurofilaments. SCb, which moves at about 4 mm/d, is a much more heterogeneous system consisting of microfilaments, clathrin, and a wide variety of soluble metabolic enzymes.

The principal function of SCa, the microtubule and neurofilament network, is to provide the stable scaffolding that supports the structure of the axon and gives it its dimensions. The extensive system of cross-bridges between the microtubules and the neurofilaments aligns the polymers and coordinates their movement through the axon. This aligned network then organizes axonal motility processes longitudinally within the axon.

SCb corresponds to a structural complex that has diverse functions in the axon, one important function being the efficient translocation of the many soluble proteins within the axon. The active transport of the soluble proteins appears to be mediated by a carrier structure that forms the stable core that organizes all of the proteins of SCb.

The axonal transport kinetics of SCb suggests that the soluble axonal proteins associate specifically but reversibly with the carrier structures and form supramolecular complexes that move coordinately through the axon. These supramolecular complexes are composed of a great number of proteins, including contractile proteins (actin and myosin), crosslinking proteins (clathrin and fodrin), regulatory proteins (calmodulin), and metabolic enzymes. It may be that one of the primary functions of these supramolecular complexes is to specifically associate a particular collection of diverse molecules. Besides allowing the coherent translocation of these molecules, the specific associations may create an integrated functional unit. Indeed, the composition of SCb suggests that these proteins form a mechanicochemical "engine" that both generates energy and converts it to the forces required to move the cytoplasmic matrix through the axon.

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