Axonal Transport of the Cytoplasmic Matrix

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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 microscopic 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 radioisotopic labeling methods can be employed to study the synthesis, transport, and metabolism of axonal cytoplasmic matrix proteins (11, 32). Labeled precursors, such as [35S]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,
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).

| Rate component | Rate | Protein composition | Cytological structure |
|----------------|------|---------------------|-----------------------|
| **Fast**       |      |                     |                       |
| Orthograde     | 50–400| Membrane-associated materials | Mitochondria |
| Mitochondria   | 200 (1–3 μm/s) | Na⁺-K⁺ ATPase, transmitter-associated enzymes, GAPs | Mitochondria |
| Retrograde     | 50–100| f1 ATPase, a small amount of spectrin lysosomal hydrolases, NGF, and other materials obtained by endocytosis | Multivesicular and multilamellar bodies |
| **Slow**       |      |                     |                       |
| SCb            | 0.2–8 | Cytoskeletal and associated proteins | Cytomatrix |
| SCa            | 2–8   | Actin, clathrin, spectrin, myosinlike proteins, NSE, CK, calmodulin, aldolase, pyruvate kinase | Microfilaments, clathrin complex, metabolic enzyme complex, carrier complex (?) |
|                | 0.2–1 | Tubulin, neurofilament triplet, tau proteins, spectrin | Microtubule-neurofilament network |

TABLE I

**Rate Components of Axonal Transport and Cytological Structures**

**Fast 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 axonically 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 microscopy 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.

**Slow axonal transport** 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 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:

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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 (3).

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 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, \(\beta\)-iminodipropionitrile; 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; a 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 the 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 EXEMPLARY 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...
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 cyto-
plasmic 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 microtu-
bules 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 be-
tween 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, bio-
chemical 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 pri-
marily in the form of microtubules and that there is a rela-
tively 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 com-
prising the cytoplasmic matrix of the axon are extremely
stable.

**MICROTUBULES AND NEUROFILAMENTS FORM A RELATIVELY STABLE NETWORK IN THE AXON:** In ret-
inal ganglion cells, the labeled tubulin wave moves coordinate-
ly with that of the labeled neurofilament proteins, sug-
gest 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 neurofil-
aments 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 move-
ment 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 struc-
tures 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 neuro-
filament 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 neu-
rofilament 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 be-
tween 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'-IMINODIPROPIONITRILE 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 ex-
ample, the neurotoxin β,β'-iminodipropionitrile (IDPN)
causes the neurofilaments and microtubules to segregate
(26, 27, 49). The neurofilaments become located almost exclu-
sively 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).
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 AXONOPHASM: 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 asymmetric transport wave. For example, actin has a very broad distribution and a long tail that follows the moving wave (Fig. 5).

The asymmetry 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

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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 phospho-
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.

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 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 non-diffusible. Biochemical analyses of axonally transported clathrin support this suggestion (20; Heriot, K., and R. J. Lasek, unpublished observations).
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 Mr 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 mechanicochemoic "engine" that both generates energy and converts it to the forces required to move the cytoplasmic matrix through the axon.

REFERENCES

1. Allen, R. D., J. Metziziai, I. Yasaki, S. T. Brady, and S. Gilbert. 1982. Fast axonal transport in squid giant axon. Science (Wash. DC) 218:1127-1129.
2. Alonso, G., J. Gabrion, E. Travers, and I. Assenmacher. 1981. Ultrastructural organization of actin filaments in neurosecretory axons of the rat. Cell Tissue Res. 214:323-341.
3. Baitinger, C., J. Levine, T. Lorenz, C. Simon, P. Skene, and M. Willard. 1982. Characteristics of axonally transported proteins. In Axoplasmic Transport. D. G. Weiss, editor. Springer-Verlag, Berlin. 119-120.
4. Black, M. M., and R. J. Lasek. 1979. Axonal transport of actin: slow component b is the principal source of actin for the axon. Brain Res. 171:401-413.
5. Black, M. M., and R. J. Lasek. 1980. Slow components of axonal transport: two cytoskeletal networks. J. Cell Biol. 86:616-623.

Black, M. M. 1978. Axonal Transport of Cytoskeletal Proteins. Ph.D. Thesis. Case Western Reserve University, Cleveland, Ohio.
6. Bloom, G., T. Schoenfeld, and R. Valle. 1984. Widespread distribution of the major polypeptide component of MAP 1 (microtubule-associated protein 1) in the nervous system. J. Cell Biol. 98:320-330.
7. Brady, S. T. 1981. Biochemical and solubility properties of axonal tubulin. J. Cell Biol. 91(2), Pt. 2:333a. (Abstr.)
8. Brady, S. T. 1981. Nerve-specific enolase and creatine phosphokinasenew transport in squid axon. Brain Res. 239:515-523.
9. Brady, S. T., M. Tytell, K. Heriot, and R. J. Lasek. 1981. Axonal transport of calmodulin: a physiologic approach to identification of long-term associations between proteins. J. Cell Biol. 89:607-614.
10. Brady, S. T., R. J. Lasek. 1982. The slow components of axonal transport: movement, composition and organization. In Axoplasmic Transport. D. G. Weiss, editor. Springer-Verlag, Berlin. 207-217.
11. Brady, S. T., and R. J. Lasek. 1982. Axonal transport: a cell biological method for studying proteins that associate with the cytoskeleton. Methods Cell Biol. 25:365-398.
12. Brady, S. T., R. J. Lasek, and R. D. Allen. 1983. Fast axonal transport in extruded axon from squid giant axon. Science (Wash. DC) 218:1125-1131.
13. Brady, S. T. Basic properties of fast axonal transport and the role of fast axonal transport in axonal growth. Adv. Neurochem. In press.
14. Brady, S. T., M. Tytell, and R. J. Lasek. 1984. Axonal tubulin and axon microtubules: biochemical evidence for cold stability. J. Cell Biol. In press.
15. Burridge, K., and P. Maddox. 1984. Actin-membrane interaction in fibroblasts: what proteins are involved in this association? J. Cell Biol. 90(1), Pt. 2:1094-1098.
16. Cheng, T. P., and J. G. Wood. 1982. Compartmentalization of clathrin in synaptic terminals. Brain Res. 239:201-212.
17. Ellisman, M. H., and K. R. Porter. 1980. Microtubular structure of the axoplasmic matrix: visualization of crosslinking structures and their distribution. J. Cell Biol. 87:464-479.
18. Fath, K., and R. J. Lasek. 1983. Actin microfilaments from axoplasm of the squid giant
43. Metuzals, J. 1969. Configuration of a filamentous network in the axoplasm of the squid (Loligo pealei L.) giant nerve fiber. J. Cell Biol. 43:480-495.
44. Metuzals, J., A. J. Hodge, R. J. Lasek, and I. R. Kaiserman-Abramof. 1983. Neurofilamentous network and filamentous matrix preserved by different techniques from squid giant axon. Cell Tissue Res. 228:415-432.
45. Metuzals, J., and W. E. Mushynska. 1974. Electron microscope and experimental investigations of the neurofilamentous network in Deiter's neurons. J. Cell Biol. 61:701-722.
46. Mori, H., Y. Komiyama, and M. Kurokawa. 1979. Slowly migrating axonal polypeptides. Inequalities in their rate and amount of transport between two branches of bifurcating axons. J. Cell Biol. 82:174-184.
47. Morris, J. R., and R. J. Lasek. 1982. Stable polymers of the axonal cytoskeleton: the neurofilamentous ghost. J. Cell Biol. 92:192-198.
48. Morris, J. R., and R. J. Lasek. 1984. Monomer-polymerequilibria in the axon: direct measurement of tubulin and actin as polymer and monomer in axoplasm. J. Cell Biol. 98:2004-2076.
49. Padovoccomenez, S. H., L. Auglio-Gambetti, and P. Gambetti. 1981. Reorganization of neurofilamentary filaments following iminodipropionitrile administration. J. Cell Biol. 91:866-871.
50. Pani, L. H., and M. C. Willingham. 1981. Journey to the center of the cell: the role of the microtubuli. Science (Wash. DC.). 212:971-972.
51. Pena, B. M. F. 1976. Clathrin: a unique protein associated with intracellular transfer of membrane by coated vesicles. Proc. Natl. Acad. Sci. USA. 73:1255-1259.
52. Pastan, I. H. 1983. Neurofibrillogenesis and morphogenesis in the nucleus. Science (Wash. DC.). 221:9-16.
53. Peters, A., S. L. Pala, and H. L. de Webster. 1976. The Fine Structure of the Nervous System: The Neurons and Supporting Cells. W. B. Saunders Co., Philadelphia.
54. Sabreth, Z. and S. Brady. 1983. Morphologic evidence for stable regions on axonal microtubules. J. Cell Biol. 97:II:210a (Abstr.)
55. Schnapp, B. J., and T. S. Reese. 1982. Cytoplasmic structure in rapid-frozen axons. J. Cell Biol. 94:679-679.
56. Schook, W., S. Pseukin, W. Bloom, C. Ores, and S. Kowka. 1979. Mechanochanical properties of brain clathrin: Interactions with actin and ATP and polymerization into basketlike structures of filaments. Biochemistry. 18:116-120.
57. Shelanski, M., J.-F. LeTerrier, and R. K. Liem. 1981. Evidence for interactions between neurofilaments and microtubules. Neurosci. Res. Program Bull. 19:32-43.
58. Stone, G. C., D. L. Wilson, and M. E. Hall. 1979. Two-dimensional gel electrophoresis of proteins in rapid axoplasmic transport. Brain Res. 144:287-302.
59. Tashiro, T., and Y. Komiyama. 1983. Two distinct components of tubulin in sensory neurons of the rat recognised by specific sulfite treatment. Brain Res. 443:450-450.
60. Tytell, M., S. T. Brady, and R. J. Lasek. 1984. Axonal transport of a subcell of Tau proteins: evidence for the regional differentiation of microtubules in neurons. Proc. Natl. Acad. Sci. USA. 81:1570-1574.
61. Tytell, M., M. M. Black, J. Garner, and R. J. Lasek. 1981. Axonal transport: each rate component reflects the movement of distinct macromolecular complexes. Science (Wash. DC.). 214:179-181.
62. Valler, R. 1982. A taxol-dependent procedure for the isolation of microtubules and microtubule-associated proteins (MAPs). J. Cell Biol. 92:425-442.
63. Willard, M. J. 1977. The identification of two intra-axonally transported polypeptides resembling myosin in some respects in the rabbit visual system. J. Cell Biol. 75:11-11.
64. Willard, M., W. M. Cowan, and P. R. Vaugelas. 1974. The polypeptide composition of intra-axonally transported proteins: evidence for four transport velocities. Proc. Natl. Acad. Sci. USA. 71:2183-2187.
65. Willard, M., and C. Simon. 1981. Antibody decoration of neurofilaments. J. Cell Biol. 98:198-205.
66. Wujek, J. R., and R. J. Lasek. 1983. Correlation of axonal regeneration and slow component b in two branches of a single axon. J. Neurosci. 3:243-251.
67. Zarras, R., W. Didier, P. Steinert, and R. Goldman. 1982. In vitro assembly of intermediate filaments from mammalian neurofilament triplet polypeptides. Proc. Natl. Acad. Sci. USA. 79:575-575.