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Translocation of the Neuronal Cytoskeleton and Axonal Locomotion [and Discussion]

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Translocation of the neuronal cytoskeleton and axonal locomotion

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Recent studies of axonal transport indicate that cytoskeletal proteins are assembled into polymers in the neuron cell body and that these polymers move from the cell body toward the end of the axon. On the other hand, membranous elements appear to be inserted into the axonal plasma membrane preferentially at the end of the axon. These new observations are explored in relation to our current understanding of axonal elongation.

Introduction

Neurons exhibit a remarkable form of locomotion when they extend axons over great distances without moving the nucleated cell body. This capacity of neurons to extend axons independently of the movement of the perikaryon is one of the distinctive properties of the neuronal lineage, because it distinguishes neurons from other migratory cell types. For example, when a fibroblast locomotes, it first extends its leading edge and subsequently moves the nucleated region and trailing tail forward (figure 1). By contrast, when the axon locomotes, it also extends its leading edge, but the perikaryon remains stationary and is usually not towed along. How do neurons accomplish this feat, which enables their axons to extend over essentially unlimited distances within the organism? This question has been thoughtfully considered by Wessels *et al.* (1973) and Bray & Bunge (1973) in separate papers that appeared in a volume devoted to cell locomotion. They note that locomotion of migratory cells involves two important steps: (i) extension of filopodia from the microfilament-rich periphery of the cell, and (ii) a subsequent contraction that pulls the rear of the cell forward (figure 1). Neurons also undergo the extension phase of locomotion at the leading edge of the growth cone, and in this respect neurons are similar to migratory cells. However, the subsequent steps in axonal growth are less clear. Wessels *et al.* (1973) suggest that axons are radically different from migratory cells at this point in their behaviour. Whereas the migratory cells contract to tow the trailing part of the cell forward, they suggest that axonal elongation occurs by the addition of new material to the structure of the axon. Most authors have proposed that this additional material is added to the end of the axon after it is conveyed from the perikaryon by axonal transport (Wessels *et al.* 1973; Bray & Bunge 1973). Addition of new material to the end of the axon has been established for the plasma membrane (Tessler *et al.* 1980; Griffin *et al.* 1981), but not for the cytoskeletal polymers that compose the backbone of the axon. Instead, a growing body of evidence indicates that the axonal cytoskeleton is assembled principally in the perikaryon (Lasek & Hoffman 1976; Black & Lasek 1980; Lasek 1981; Lasek & Brady 1982). This evidence comes from studies of axonal transport of radioisotopically labelled cytoskeletal proteins in axons of neonatal and adult mammals (Hoffman & Lasek 1980). The axonal transport studies suggest that many of the basic processes, which are involved in the extension of the cytoskeleton during axonal growth, continue to operate in the mature axon. Furthermore, they raise the possibility that during

axonal growth the axonal cytoskeleton moves within the axon in an orderly relation with the growth cone. The continuous movement of the axonal cytoskeleton during the entire lifespan of a neuron may provide both for its renewal and the capacity of the axon to regenerate after injury (Lasek & Hoffman 1976).

The ability of the axonal cytoskeleton to move without the coordinate movement of the cell body could be the critical feature that differentiates axonal elongation from the locomotion of

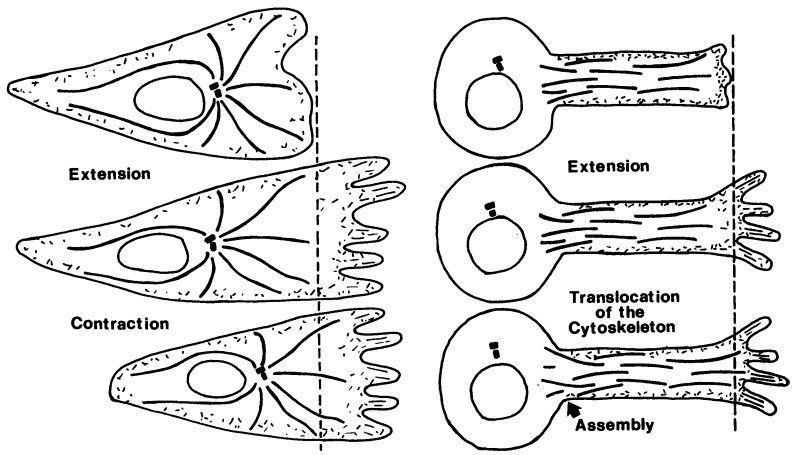

FIGURE 1. A schematic of the major steps in the locomotion of migratory cells (left panel) and axons (right panel). In the migratory cell the microtubules are organized around the centrosome. In the neuron the axonal microtubules are considered to be separate from the centrosome and it is proposed that the cytoskeleton moves independently of the cell body.

other migratory cell types. I shall explore this possibility by comparing the relevant information regarding axonal growth and axonal transport. One interesting feature of such a comparison is that studies of these two processes are based on different but complementary methods of analysis. While most of the observations on axonal growth have involved cytology and pharmacology, studies of axonal transport have emphasized analyses of the axonal proteins and their dynamics.

Axonal growth

Much of our current conception of axonal growth is based on light microscopic observations of the behaviour of axons *in vivo* and *in vitro*. Initially these observations were made on sectioned material from embryos and regenerating nerves in which the axons were selectively stained. Anyone with a serious interest in this subject should refer to the classic studies of Cajal (1928), which have been confirmed repeatedly as techniques have improved. A particularly important advance in the study of axonal growth occurred when Harrison (1910) employed tissue culture techniques so that growing axons could be observed directly. The validity of these *in vitro* observations was confirmed in an important set of studies by Speidel (1933) who used the light microscope to observe growing axons in the tail fin of living tadpoles. These and many other studies of the behaviour of growing axons have provided a great deal of information about the interactions between the growing axon and its environment (Katz & Lasek 1980). This has been particularly useful in studies of axonal guidance and termination. However, only a limited amount of information about the mechanisms of axonal growth can be gleaned from light microscopic observations of growing axons. The limitation of these analyses is that they have

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necessarily focused on the external form of the axon. The electron microscope partly overcomes the limitations of the light microscope by resolving the internal structure of the axon in considerable detail. Much of the fundamental information that has been gained from electron microscopic studies can be obtained from the important paper by Yamada *et al.* (1971).

The basic features of the structure of a growing axon are summarized in figure 2. The growth cone and axon proper are continuous and they have many structural similarities. For example,

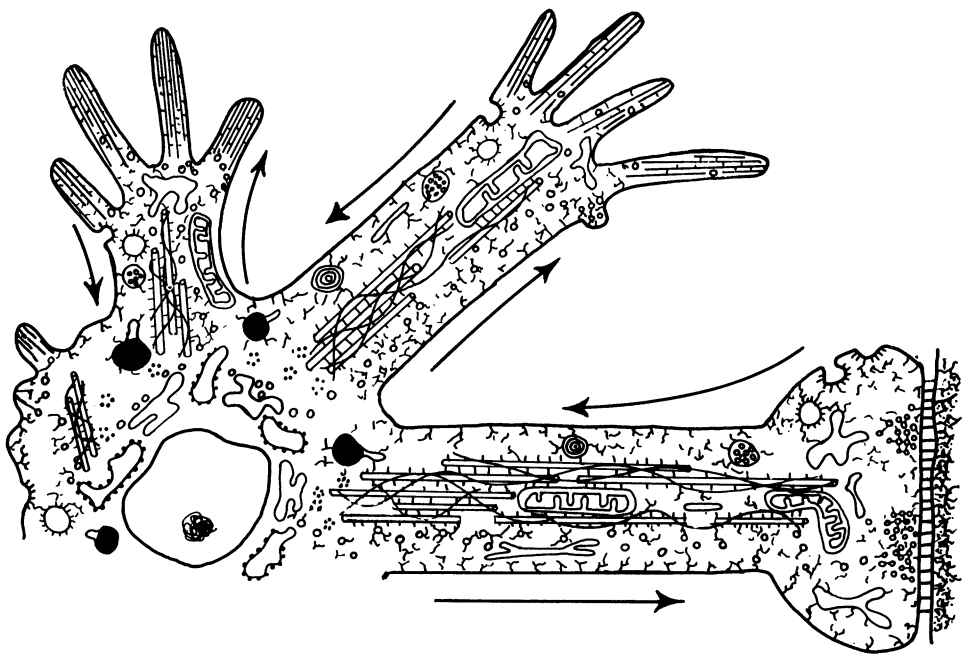

FIGURE 2. A schematic of the steps involved in axonal growth. The arrows denote the direction of movement of the vesicular structures that supply and recover membranes from the axon terminal. Small vesicles, which are products of the Golgi complex, carry membranes to the end of the axon. Multivesicular and multilamellar bodies carry membranes from the axon terminal to the cell body. The cortical microfilament meshwork is shown, together with the microtubules and neurofilaments.

the leading edge of the growth cone is a specialization of the cortex that is present along the entire surface of the axon. This region is characterized by a polygonal meshwork of microfilaments associated directly with the plasma membrane. The leading edge of the growth cone is characterized by the active extension and retraction of filopodia. Although the lateral surface of the axon is much less active than the leading edge of the growth cone, filopodia are found along the surface of the axon *in vitro*. Furthermore, when myelinated axons are severed *in vivo*, the new sprouts frequently arise from nearby nodes of Ranvier rather than from the transected end of the axon (Cajal 1928). Thus, the leading edge of the growth cone appears to be a modified region of the axonal cortex.

In addition to microfilaments, the growth cone contains two other cytoskeletal elements. Microtubules extend into the growth cone where they interdigitate with the polygonal meshwork (Yamada *et al.* 1971). However, the microtubules do not contact the plasma membrane directly. Neurofilaments tend to be concentrated at the core of the axon and extend only to the base of the growth cone. The cytoskeletal elements at the growing end of the axon are arranged in a serial progression. Microfilaments compose the vanguard at the leading edge of the axon.

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They are followed by microtubules and finally by the neurofilaments. Immunocytochemical localization of actin, tubulin and neurofilaments provide additional evidence for the serial progression of the cytoskeleton in the growing axon (Spooner & Holladay 1981; Shaw *et al.* 1981). Actin is preferentially associated with the cortex of the axon and is concentrated at the leading edge of the growth cone. Microtubules stained with antibodies against tubulin splay out into the body of the growth cone. Finally, the neurofilament proteins are localized at the core of the axon and extend only to the base of the growth cone. This progression of actin, tubulin and neurofilament protein from the front of the growth cone back into the axon may be related to the order in which these proteins move in the axon, because a similar arrangement has been noted for the transport of these proteins in the axon. That is, actin is transported faster than tubulin, which may be transported ahead of the neurofilament proteins (Hoffman & Lasek 1980; Mori *et al.* 1979).

The end of the cytoskeleton is the end of the axon

The primary difference between the growth cone and the axon proper may be that the growth cone is at the end of the axon. Materials conveyed by axonal transport tend to accumulate at the axon end (Grafstein & Forman 1980). This applies both to natural axon terminals, such as growth cones and presynaptic terminals, and to artificially induced endings, such as those produced by cutting the axon. All of the structural materials that compose the growth cone are conveyed through the axon from the cell body. For a new branch to form, a new terminal structure must arise from the lateral surface of the axon. Usually this happens when the end of the axon bifurcates, although in some cases new branches form from the lateral surface of the axon. In all cases, the formation of a new branch requires the extension of microfilament-rich filopodia followed by the introduction of the microtubules into the sprout (Yamada *et al.* 1970). One of the obvious functions of the microtubules is that they provide structural support for the axon. Microtubules also have an important role in the organization of intracellular transport and the orientation of particle movements in the axon. For example, observations of particle movement in axons suggest that the particles move along intracellular pathways that are organized by the microtubules (Smith 1980). The particles, which correspond to membranous vesicles, do not contact the microtubules directly but are surrounded by a filamentous matrix that makes contacts with the microtubules (Ellisman & Porter 1980). When the microtubules are disrupted with colchicine the particles continue moving but their movement is disordered (Chang 1972). Further support for the role of microtubules in the organization of vesicular traffic in axons is provided by observations of particle movements in isolated axoplasm from the squid giant axon (Brady *et al.* 1981). In this preparation, the particles move along pathways aligned parallel to the long axis of the axon. If the structure of the axoplasm is disrupted by stirring it with a needle, the cytoskeleton is fragmented into shorter pathways, which course at various angles to the original axis of the axon (Brady *et al.* 1982). The particles appear to follow these disrupted pathways.

In the axon proper, the microtubules are aligned parallel to the long axis of the axon. The orientation of intra-axonal traffic is aligned along this axis, and materials moving in the orthograde direction will be directed toward the end of the cytoskeleton, which is located in the growth cone. Thus a crucial difference between the end of the axon and the axon proper may simply be that the end of the axon contains the end of the orienting cytoskeletal network.

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When microtubules are recruited into a collateral sprout, some of the material that would have previously passed through the region containing the collateral sprout will be diverted into the new sprout.

The general view of axonal growth that has emerged from studies of the behaviour of growing axons and their structure is summarized in figure 2. Initially, the neuron extends filopodia from one or more regions of its surface, and this is followed by the emergence of a specialized motile structure, the growth cone. The importance of the growth cone in axonal growth is verified by the fact that if a growing axon is severed, a new growth cone must form before the axon can resume elongation (Shaw & Bray 1977; Wessels *et al.* 1978). When the growth cone reaches an appropriate target site, it stops advancing and is converted into a presynaptic terminal.

Surface membrane is added at the growth cone

Clearly, axonal elongation involves the addition of macromolecules to a growing structure. All of the macromolecules and many of the other materials that constitute the axon are provided by the neuronal cell body (Grafstein & Forman 1980). Where are these materials added to the fabric of the axon during axonal growth? One possibility is terminal addition. That is, the proteins might be conveyed to the end of the axon where they are added to the existing structure. On the other hand, materials might be added to the axon by proximal addition at the root of the axon where it joins the cell body. Theoretically, either or both of these mechanisms might operate during the growth of the axon. Observations of the behaviour of growing axons have shed very little light on this question, with the exception of one very interesting experiment by Bray (1970). By placing a carmine particle on the surface of a growing axon in tissue culture, he was able to determine whether the surface of the axon moves in step with the growth cone. The carmine particle remained stationary in position as the axon continued to elongate. Bray proposed that the surface of the growing axon does not move along with the growth cone and that additional membrane is inserted at the end or along the length of the axon. Recent radioisotopic labelling studies coupled with electron microscopic autoradiography of regenerating axons support this hypothesis (Tessler *et al.* 1980; Griffin *et al.* 1981). Membrane proteins labelled with [^{3}H]fucose are conveyed in the fast component of axonal transport at rates of about 400 mm/day. The proteins are preferentially inserted into the plasma membrane at the end of the growing axon. As the growth cone advances the inserted proteins remain behind in the plasma membrane of the axon proper. Although the membrane proteins are preferentially transported to the end of the axon and inserted at the growing tip, some membrane proteins are also added to the lateral surface of the axolemma along the length of the axon. Lateral addition of membrane components to the surface of the axon may be involved in the maturation of the axon when it increases in diameter.

Although terminal addition of membrane components is an important process in axonal elongation, the surface membrane is only the covering of the axon. The bulk of the structure of the axon is defined by the cytoskeleton and its associated matrix. To understand the mechanisms of axonal growth it is necessary to determine the mechanisms by which the cytoskeleton elongates. Most information relating to the dynamics of the axonal cytoskeleton has been obtained by studying the slow component of axonal transport. Therefore, before analysing the issue of cytoskeletal elongation in axons, I shall briefly review the relevant information on slow axonal transport.

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Axonal transport and the cytoskeleton

The transport characteristics of the primary subunits of the axonal cytoskeleton (i.e. tubulin, neurofilament protein and actin) have been carefully analysed by radioisotopic labelling (Black & Lasek 1980). The cytoskeletal proteins are conveyed exclusively in slow transport and are not found in the fast component of axonal transport (Tytell *et al.* 1981). In fact, none of the slowly transported proteins have been detected moving with the fast component. The fast and

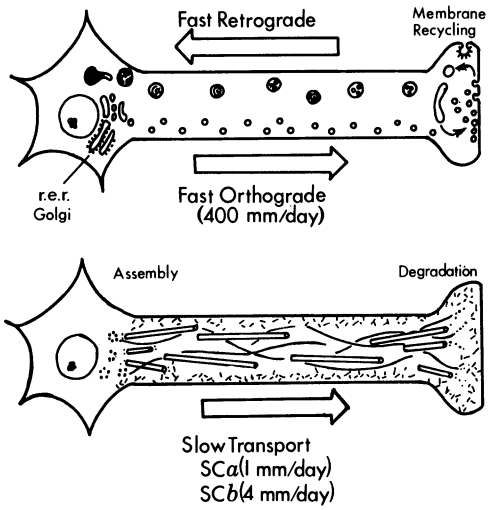

Figure 3. The major features of axonal transport are illustrated. Fast transport is divided into two components. The orthograde fast component conveys membranous elements from the Golgi complex to the axon terminal. These materials are transported in small vesicles that ultimately resupply the neurotransmitter release system at the end of the axon. The retrograde fast component recovers membranes from the terminal by endocytosis and conveys these materials in the form of multivesicular bodies to the lysosomal system in the cell body. Slow transport appears to be unidirectional and consists of two subcomponents, SC*a* and SC*b*. SC*a* corresponds to the microtubule–neurofilament network and SC*b* corresponds to the actin-containing axoplasmic matrix.

slow components of axonal transport represent two completely different classes of protein (figure 3). Table 1 contains a list of many of the proteins that have been identified in fast and slow transport. The fast component contains proteins associated with the vesicular structures that are products of the rough endoplasmic reticulum and the Golgi complex. Fast transport can be divided into the orthograde component, which conveys materials from the cell body to the axon terminal, and the retrograde component moving materials back toward the cell body (Tsukita & Ishikawa 1980; Smith 1980). Orthograde transport is made up of small tubulovesicular structures, of which many are 30–50 nm vesicles. Retrograde transport conveys membranous materials in the form of multivesicular bodies and multilamellar bodies. These are recovered from the axon terminal by endocytosis. The retrogradely transported structures are prelysosomal and represent the degradative pathway for membrane materials as well as a route for the transport of extracellular molecules such as nerve growth factor from the end of the axon to the cell body (Grafstein & Forman 1980).

The slowly transported proteins can be divided into two major subcomponents, called slow component *a* and *b* or SC*a* and SC*b* (Black & Lasek 1980). In pulse-labelling experiments, SC*a* and SC*b* appear as two distinct waves of radioactive protein differing both in rate and composi-

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tion (table 1). In some neurons, such as mammalian retinal ganglion cells, the two waves can be separated completely because most of the SC*b* proteins move faster than the SC*a* proteins. The transport properties of these proteins can be explained if these proteins are transported in the form of structural complexes. We have formalized this proposal as 'the structural hypothesis of axonal transport' (Tytell *et al.* 1981). This hypothesis holds that proteins are actively transported in the axon as component parts of cytological structures. For SC*a*, which contains

TABLE 1. MAJOR RATE COMPONENTS OF AXONAL TRANSPORT AND THEIR RELATION TO CYTOLOGICAL STRUCTURES

name	rate (mm/day)	structures	composition
fast			
orthograde	200–400 (1–3 μm/s)	vesicular–tubular elements, neurosecretory granules	membrane proteins, secretory proteins and peptides
retrograde	200	prelysosomal vesicles (multivesicular bodies, multilamellar bodies)	lysosomal hydrolases, materials obtained by endocytosis
slow			
SC*b*	2–4 (3 μm/min)	matrix: microfilaments, clathrin complex, metabolic enzyme complexes	actin, clathrin, fodrin calmodulin, enolase, CPK, aldolase, pyruvate kinase
SC*a*	0.2–1	microtubule–neurofilament network	tubulin, neurofilament proteins, certain tau proteins

tubulin and the neurofilament triplet, we propose that these proteins move preferentially in the form of microtubules and neurofilaments (Lasek & Hoffman 1976; Black & Lasek 1980). The presence of actin in SC*b* suggests that microfilaments may be one of the structures moving in SC*b* (Willard *et al.* 1979; Black & Lasek 1979). However, SC*b* consists of more than 200 different proteins. It seems likely that SC*b* will have an extremely complex structure and consist of many subassemblies in addition to microfilaments (Brady & Lasek 1981; Brady *et al.* 1981; Garner & Lasek 1981).

CYTOSKELETAL PROTEINS ARE TRANSPORTED IN THE FORM OF POLYMER

Evidence supporting the hypothesis that cytoskeletal proteins are transported in the form of polymers rather than as monomers is strongest for neurofilaments. The neurofilament proteins are essentially all assembled under physiological solution conditions, and no free monomer has been detected in the axon (Morris & Lasek 1982). This was demonstrated by using axoplasm extruded from the squid giant axon. When axoplasm is extracted with a buffered solution that mimics the internal solution conditions of the axon, the neurofilament proteins remain associated with the axoplasm, and electron microscopic analyses indicate that the neurofilaments remain assembled. Similarly, analyses of the solubility properties of radioactively labelled neurofilament proteins obtained in axonal transport experiments suggest that these proteins are in the polymerized form (S. T. Brady, unpublished observation).

The property of neurofilament proteins to form stable polymers in the axon explains the behaviour of these proteins in axonal transport experiments. When the neurofilament proteins

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are pulse-labelled they are distributed in a bell-shaped wave that moves along the axon (Hoffman & Lasek 1975). In certain neuronal systems this bell-shaped wave has a constant shape as it moves along the axon. Figure 4 shows the SC*a* wave, which contains the labelled neurofilament proteins in axons of the phrenic nerve at 15, 30 and 60 days after labelling. The wave retains the same shape between 30 and 60 days, though it has progressed 30 mm along the axon. Observations on the properties of the neurofilament protein therefore support the hypothesis that these proteins are axonally transported in the form of assembled polymers.

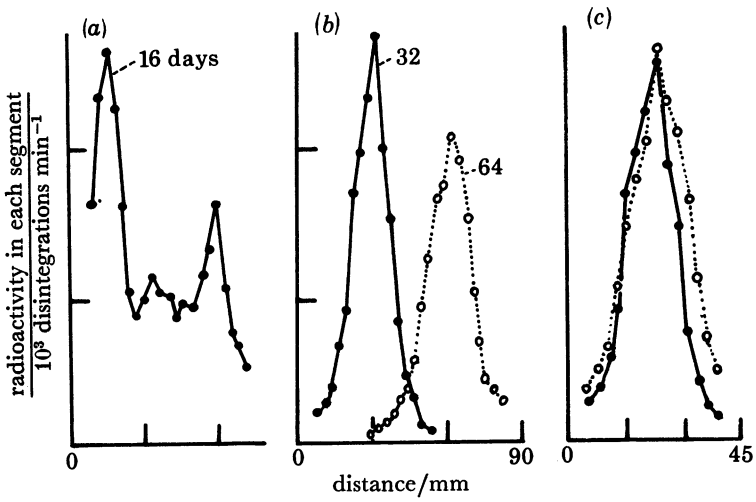

FIGURE 4. The movement of slowly transported proteins is illustrated in axons of guinea pig phrenic nerves. These data demonstrate the orderly progression of the SC*a* wave in phrenic axons during a period encompassing two months. The SC*a* wave does not spread as it progresses along the axons. A mixture of [^{3}H]lysine and proline were injected into the cervical spinal cord to label the phrenic motor neurons. Subsequently the distribution of slowly transported proteins was measured by analysing consecutive 3 mm segments of the phrenic nerve. Transport profiles are shown at (*a*) 16 and (*b*) 32 and 64 days after injection. At 16 days both the faster-moving SC*b* wave and the slower-moving SC*a* wave are apparent. At later periods only the SC*a* wave, which contains tubulin and neurofilament proteins, is present in the nerve. To compare more effectively the distributions of the radioactivity in SC*a* at 32 and 64 days, the data were normalized and plotted so that the peaks of the waves coincided (*c*). Each point is the mean of five determinations. The results are reproduced with permission from Mark Black (1978).

In some axons, such as those of adult mammalian retinal ganglion cells, tubulin and neurofilament protein are transported coordinately (Black & Lasek 1980). It can be argued by analogy with the neurofilaments that tubulin is also transported in the form of microtubules. However, both the properties of axonal tubulin and the detailed transport kinetics indicate that the dynamics of axonal tubulin are much more complex than the neurofilament proteins. Analyses of the solubility properties of axoplasmic tubulin in the squid giant axon demonstrate that tubulin exists in three forms, monomer, soluble polymer and stable polymer, comprising 23, 60 and 17 % of the total axoplasmic tubulin respectively (Lasek & Morris 1982). Soluble polymer represents that fraction of the microtubules that depolymerize when the monomer–polymer equilibrium is shifted by decreasing the available monomer concentration in axoplasm. Stable polymer remains assembled when the monomer concentration is reduced. Whereas subunits can exchange between the monomer pool and one or both ends of the soluble microtubules, the stable regions of microtubules do not appear to be in a simple equilibrium with monomer. Thus stable microtubules may be comparable with neurofilaments in that both of these polymers probably have a very slow exchange rate with monomer.

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Axonal microtubules appear to be more stable than other cytoplasmic microtubules (Dustin 1978). In the squid giant axon approximately one-quarter of the microtubules are stably polymerized and in mammalian retinal ganglion cells as much as 60 % of the axonally transported tubulin may be in the form of stable polymer (Brady 1981). Analyses of the solubility properties of labelled tubulin transported in the slow component demonstrate that more than 60 % of the transported tubulin is insoluble under conditions that normally depolymerize microtubules. Stable microtubules also appear to be present in the axons of growing embryonic neurons. Black & Greene (1980) have demonstrated that the fraction of microtubules insensitive to colchicine is increased when neuron-like tissue culture cells extend neurites in culture. Thus, stable microtubules appear to be present in both growing and mature axons and the axonal transport studies indicate that these microtubules move with the slow component of axonal transport.

The observation that axonal cytoskeletal proteins exist in the form of stable polymers, which have a low exchange rate with monomer in solution, supports the hypothesis that these proteins are assembled in or near the perikaryon and are then conveyed in the assembled form toward the axon terminal. For neurofilament proteins the transport kinetics are relatively simple because they apparently exist exclusively in the form of stable polymer. However, for tubulin the transport kinetics may be far more complex, because a significant fraction of the axonal tubulin exists in the form of monomer, probably in equilibrium with the soluble microtubules. In the simplest case, tubulin molecules exist in one of two states, either as monomer or soluble polymer. However, it appears that some tubulin is in the form of stable polymer and it seems likely that monomeric tubulin has the potential to interact with many other structures in the axon besides the microtubules and can therefore exist in a fourth state in which it is bound to the proteins of the axoplasmic matrix. This could explain the observation that the wave of labelled tubulin in slow axonal transport experiments can have a much broader distribution than that of the neurofilament proteins. For example, the tubulin wave is particularly broad in alpha motor neurons (Hoffman & Lasek 1980) and spinal ganglion cells (Mori *et al.* 1979). In these axons a very large fraction of the tubulin travels ahead of the neurofilament proteins. The motion of this faster-moving tubulin approaches the rates exhibited by SC*b* proteins such as actin and clathrin but it never exceeds the rate of these proteins (about 4 mm/day). Clearly, the transport properties of proteins such as tubulin, which exist both in the form of polymer and monomer, is a complex function of a number of variables. One of the most important is the fraction of total tubulin that is in the form of monomer. If the monomer fraction is very high, the probability that tubulin transport will be influenced by other structures in the axon will be high. However, if the amount of monomer is low, the tubulin will exhibit transport kinetics characteristic of the stable polymers. This appears to be so in mammalian retinal ganglion cell axons because the kinetics of tubulin and neurofilament protein are nearly identical and very little tubulin moves coordinately with the faster-moving SC*b* proteins (Black & Lasek 1980).

Assembly of the cytoskleton occurs in or near the cell body

Although the evidence remains indirect, these studies on the transport of tubulin and neurofilament protein indicate that polymers are assembled near the site of synthesis in the cell body and then conveyed along the axon. The exact location of polymerization has not been ascertained. In the neurofilaments it seems probable that the subunits are assembled near the polysomes where they are synthesized (Lasek 1981; Lasek & Brady 1982). Neurofilament

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proteins are highly asymmetric rod-shaped molecules 50 nm in length. It is unlikely that these molecules diffuse over any distance before assembly. Furthermore, the assembly reaction is highly favoured for neurofilament protein. Proximal addition of subunits to the cytoskeleton in the cell body therefore seems likely for neurofilaments. Komiya & Kurokawa (1980) have provided evidence that tubulin must be assembled into microtubules for it to be transported from the perikaryon into the axon. They demonstrated that colchicine specifically inhibited the transport of newly synthesized tubulin from the spinal ganglion into the sciatic nerve without blocking synthesis of tubulin. This result suggests that colchicine inhibits the transport of tubulin by interfering with the polymerization of tubulin into microtubules.

The proximal addition of subunits to the cytoskeleton is consistent with the observation that the diameter of the axon increases during maturation in a proximo-distal direction from the cell body toward the axon terminal (Friede & Samorajski 1970). Furthermore, the diameter of the axon appears to be related to the amount of cytoskeletal protein exported from the cell body. For example, during the response to injury of the axon, rat motor neurons reduce the amount of neurofilament protein exported into the axon (Hoffman & Lasek 1980). The diameter of the axon is directly related to the number of neurofilaments and microtubules in the axon at any point along its length. Thus it is expected that the decrease of neurofilament protein in the axon should lead to a decrease in the diameter of the axon. Hoffman *et al.* (1980) found that this is so and that the decrease in diameter first appeared near the cell body. Subsequently, the decrease in diameter progressed along the axon from the cell body at a rate consistent with that of slow transport. This observation strongly supports proximal addition of subunits to the neurofilament network at the cell body and the movement of these subunits in the form of neuofilaments.

Although studies of axonal transport suggest that subunits are added to the cytoskeleton at the perikaryon, they do not identify whether the subunits are added to the proximal end or the distal end of the polymers themselves. In microtubules, subunits could theoretically be added at either end. Serial reconstruction studies of axons demonstrate that the microtubules are short when compared with the length of the axon. For example, in growing embryonic neurons in culture the microtubules are approximately 100 μm in length (Bray & Bunge 1981). Tubulin could be added either at the proximal or the distal end of these microtubules during assembly.

Microtubules are polar structures, and the rates of assembly at the ends of the microtubules differ. The end at which subunits are added most rapidly is called the plus end and the slower-growing end is called the minus end. Heideman *et al.* (1981) have demonstrated that the plus end of the microtubules in axons is on the distal end of the microtubules. Although the plus end of the microtubules is on the distal end of microtubules, subunits could be added to microtubules at the proximal or minus end because the rate of assembly is determined by the microenvironment surrounding each end. The actual direction of microtubule growth has been established for only one system, the axoneme of cilia and flagella. In the axoneme the distal end of the microtubules is the plus end and subunits are added to the distal end during growth of the cilia. In the neuron, addition of subunits to the plus end may also be favoured. However, specializations have been noted at both the proximal and distal ends of neuronal microtubules and the presence of these structures suggests that the addition of subunits at one or both ends of the microtubules may be regulated (Chalfie & Thomson 1979).

The hypothesis that neurofilament proteins and tubulin are assembled into polymers in the perikaryon and that subsequently these polymers are transported in the axon is derived princi-

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pally from studies on mature axons that are not elongating. Does this mechanism of cytoskeletal elongation also occur in growing axons? Recent analyses of axonal transport in the elongating portion of regenerating motor axons indicate that it may. The transport kinetics for tubulin and neurofilament protein in new daughter axons are very similar to the kinetics which have been described for the mature parent axons (McQuarrie *et al.* 1981). This observation suggests that the properties of cytoskeletal translocation that have been defined in mature axons continues into the newly formed axonal sprouts. Thus the information that has been obtained about the assembly and translocation of cytoskeletal proteins in adult neurons may apply to growing axons.

Cytoskeletal translocation and axonal growth

If microtubules and neurofilaments are assembled in the perikaryon and conveyed within the axon, how do these structures move in the axon? The simplest possibility is that they are extruded from the perikaryon as a result of the assembly process, as Young (1945) suggested. This hypothesis fails for many reasons, which were originally presented by Weiss (1969). For example, extrusion from the cell body cannot possibly account for the movement of the cytoskeleton in long axons, which are centimetres or even metres in length. Furthermore, the recent observation that microtubules are discontinuous, averaging about 0.1 mm in length, is also inconsistent with an extrusion model (Bray & Bunge 1981). The forces that move the cytoskeleton within the axon are probably not limited to the perikaryon or the axon terminal. Instead, it is likely that the machinery that translocates the cytoskeleton is located along the entire length of the axon. This has been shown definitively for the membranous elements that move rapidly in the axon (Ochs 1975). The fast component requires ATP and if the source of ATP is blocked locally at any point along the axon, fast transport is blocked exclusively at that point. Similar experiments have not yet been carried out for slow transport. However, if the axon is cut or compressed the slowly transported proteins accumulate specifically at the cut end of the axon (Weiss & Pillai 1965; M. M. Black & R. J. Lasek, unpublished observations).

An important clue about the movement of the cytoskeleton is provided by an interesting observation that Shaw & Bray (1977) made on cultured neurons. They noted that when a growing neurite is disconnected from its perikaryon, the growth cone stops advancing. However, the disconnected portion of the neurite, which trails behind the growth cone, often continued to progress forward so that it ultimately collapsed into the growth cone. This process is energy dependent, suggesting that it involves an active mechanism. Furthermore, it is not simply a degenerative process, because the expanded growth cone continues to exhibit activity and may extend neurites equal in length to the original neurite.

The observation that the severed neurite collapses forward into the growth cone suggests that the cytoskeletal elements in the neurite also progress forward into the growth cone during this process. The collapse of the neurite into the growth cone is reminiscent of the behaviour of migratory cells during locomotion (figure 1). Shaw & Bray (1977) may have revealed the secondary phase of axonal locomotion by cutting the axon from the cell body. That is, the progression of the cytoskeleton forward, when it is disconnected from the cell body, may represent the normal tendency of the cytoskeleton to move forward in the axon.

The forward progression of the cytoskeleton in the neurite apparently requires that the proximal end of the cytoskeleton is free to advance. In a set of experiments comparable with those of

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Shaw & Bray (1977), Wessels *et al.* (1978) found that transected axons rarely collapsed forward into the growth cone. Instead, many of the severed axons in their experiment formed a growth cone at the trailing end of the cut axon. The primary difference between these two sets of experiments appears to be that Wessels *et al.* (1978) employed an extremely adhesive substrate and that Shaw & Bray (1977) employed one of the least adhesive substrates that supports axonal growth. In the experiments of Wessels *et al.* (1978) the fact that the proximal end of the cut axon became firmly attached to the substrate may have counteracted the forces required to move the cytoskeleton forward. In the experiments of Shaw & Bray (1977) the proximal end of the axon was obviously free to move forward. A comparable situation may exist in intact neurons because the proximal end of the cytoskeleton is in the perikaryon where additional materials can be added to compensate for the advance of the cytoskeleton.

Having made these observations regarding the assembly and translocation of the cytoskeleton in the axon we can return to the question originally proposed in this paper. What is the crucial difference between neurons and other migratory cells that enables the neuron to extend processes without towing the cell body along? I propose that the critical feature of neurons is their ability to translocate the axonal cytoskeleton independently of the perikaryon, and that this is accomplished by the continuous addition of cytoskeletal proteins at the proximal end of the cytoskeleton in the perikaryon. This proposal does not rule out the possibility that some remodelling of the cytoskeleton may occur at the distal end of the axon. It seems likely that changes in the lengths of microtubules may occur at the end of the axon and that subunits are either added or subtracted from the microtubules in the growth cone. These alterations in the distal end of the cytoskeleton could be involved in relatively small changes in axonal length, such as local retraction or sprouting.

If this proposal is correct, one prediction is that the cytoskeletons of neurons differ from those of other migratory cell types. The cytoskeletons in migratory cells must link the microfilament-rich leading edge of the cell with the trailing portion of the cell. As Schliwa (this symposium) has demonstrated, the centriole may play an important role in this linkage because it organizes the microtubules that radiate out to the cell periphery (Brinkley *et al.* 1981). In neurons the centrioles are not directly related to the axonal cytoskeleton during the initial phases of axonal differentiation (Hinds & Hinds 1974; Lasek *et al.* 1981), and thus the crucial difference between neurons and migratory cells may be that in migratory cells the cytoskeleton is organized around a centralized structure located in association with the nucleus and that in neurons the axonal cytoskeleton is organized independently of the centrioles. In fact, recent evidence suggests that the microtubules of the axon are different from those of the perikaryon and dendrites (Matus *et al.* 1981; Tytell *et al.* 1980). These differences in the cytoskeleton of the axon and perikaryon provide additional evidence for the separation of the axonal cytoskeleton from that of the neuronal perikaryon (Lasek & Brady 1982).

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Discussion

R. J. Adams (*M.R.C. Cell Biophysics Unit, London, U.K.*). I should like, briefly, to report some results that relate to the observations made by Dr Lasek on the rapid transport of organelles in squid axoplasm. The approach we have taken to investigate the problem of how these rapid movements are produced in axons is to make a model system where transport is seen to continue in axoplasm made freely permeable to a defined solution (Adams 1982; Adams *et al.* 1982). The preparation we have used is of single giant axons (about 30 μm in diameter) dissected from the walking legs of the shore crab *Carcinus maenas*. *In vitro* these axons will continue to exhibit transport, as seen by phase contrast microscopy, for at least 2 h. Axons are rendered permeable by subjecting them to brief intense electric fields, causing holes to be produced by the dielectric breakdown of the plasma membrane (Baker & Knight 1978). A series of holes made along a length of axon in this way results in the equilibration of the axoplasm with the bathing solution, which is in large excess to the volume of the axon, and so defines its composition. When axons are made permeable, in a suitable buffer lacking ATP, movement of the organelles ceases and a stable 'rigor'-like state is seen in which the organelles are suspended in the axoplasmic gel and show little or no Brownian motion. Upon the addition

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of ATP, above a threshold concentration of 500 μM, transport resumes at velocities approaching those seen in intact axons. The movement is not, however, supported by the non-hydrolysable ATP analogue, AMPPNP. The process appears to be independent of Ca^{2+} ions, continuing for 1 h in 50 mM EGTA and insensitive to 0.5 mM Ca^{2+} for at least 30 min if the bathing solution is supplemented with protease inhibitors.

Thus this system allows the ionic and metabolic requirements of rapid axoplasmic transport to be defined and, further, the manipulation of the axoplasmic environment and introduction of antagonists of transport may be used to identify the mechanical process involved.

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