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Relationships Between the Rapid Axonal Transport of Newly Synthesized Proteins and Membranous Organelles

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Contents

Abstract

Rapid axonal transport is generally viewed as being exactly analogous to the secretory process in nonneuronal cells. The cell biology of rapid axonal transport is reviewed, the central concern being to explore those aspects that do not fit into the general secretory model and which may thus represent specific neuronal adaptations. Particular attention is paid to the relationship between the transport of newly synthesized proteins and of the membranous organelles that act as carriers. Sites in the transport sequence at which the behavior of axonal transport may differ from the secretory model are at the initiation of axonal transport at the trans-side of the Golgi apparatus, within the axon where molecules are deposited from the moving phase to a stationary phase, and at nerve terminals or axonal lesions where transport reversal takes place.

Index Entries: Axonal transport; protein; organelles.

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Introduction

In 1905 Scott observed that neurons resemble secretory cells in their arrangement of Nissl substance, and he also proposed that nerve cells are secretory cells that transport their secretions to nerve terminals in a particulate form. This prescient hypothesis is still a fair summary of our understanding of the mechanisms and function of rapid axonal transport. In addition, the argument that the transport of proteins and the flow of membrane within neurons closely resemble the same processes within secretory cells has proven to be of great predictive value. However, some significant peculiarities of nerve cells indicate that the analogy should not be extrapolated beyond the evidence. It is the purpose of this review to examine the relationship between newly synthesized proteins and the organelles that carry these proteins by rapid axonal transport. The extent to which the mechanisms of rapid axonal transport may differ from those involved in the secretory process will be a central concern. The large areas of agreement between evidence from nonneuronal secretory cells and nerve cells will be treated very briefly in order to place emphasis on those areas in which the study of nerve cells seems to be producing unique information. For general information on secretory cell mechanisms the reader is referred to recent reviews (Moore et al., 1988; Schwartz, 1990).

In all cells, proteins synthesized by ribosomes are directed by targeting sequences within the protein molecule to one of four routes: to the nucleus of the cell, to the cytoplasm, to mitochondria, or to the rough endoplasmic reticulum (RER). Some of the proteins that pass to the RER are destined for the secretory pathway. Transfer to the secretory pathway takes place from the RER via a vesicle shuttle to the cis-face of the Golgi apparatus. Within the Golgi apparatus proteins are modified by a series of glycosylation and sulfation reactions that depend on the specific enzyme content of the cis-, medial-, and trans-portions of the Golgi apparatus. Some of the proteins in the RER and Golgi apparatus are not moved through the membrane system because their further passage is prevented by the action of retention signals. Some proteins are moved from the trans-Golgi apparatus to lysosomes. Those proteins that contain neither retention signals nor the signal that destines them for lysosomes are packaged in the trans-Golgi apparatus into vesicles of two types: secretory vesicles belonging to what are known as the constitutive and the regulated pathways. Tartakoff and Vassalli (1978) first made the distinction between the two types of secretion on the basis of the behavior of different types of cells. Those cells that liberated secretions only in response to external stimuli were called regulated secretory cells in contrast to those cells that secreted without stimulation. It has since become clear that two secretory pathways, the regulated and the constitutive pathways (Burgess and Kelly, 1987), can coexist in the same cell. Constitutive secretion directs membrane and membrane-associated molecules to the plasmalemma; this is the default pathway of protein sorting through the endoplasmic reticulum and Golgi apparatus, the only signal required is the signal sequence controlling entry to the lumen of the RER. However, movement of proteins into the regulated pathway requires specific sorting signals.

The secretory vesicle carries a number of proteins in addition to the secretions that are destined for release at the plasma membrane. These include the integral membrane proteins, motor proteins that propel the vesicle, and proteins whose function is to code the vesicle for its final destination (Hammerschlag, 1983). Motor proteins drive vesicles through the cytoplasm by reacting against essentially stationary microtubules (Vale, 1987; Vallee and Bloom, 1991). Vesicle movement toward the plasma membrane is powered, at least in some identified cases, by the ATPase motor kinesin. Following retrieval of membrane from the cell surface by endocytosis and passage through an endosomal membranous compartment, vesicles are transported back toward the Golgi apparatus by a similar, but

oppositely polarized, microtubule-dependent motor system that, again in some cases, is represented by cytoplasmic dynein.

The brief account given above outlines the complex series of events that are involved in the movement of membrane and proteins in nonneuronal secretory cells. A simplifying hypothetical scheme states that a series of separate membrane compartments, the endoplasmic reticulum, the Golgi apparatus, the plasma membrane, and the endosome, act as stations at which sorting decisions are carried out. The transfer of membrane and membrane-associated molecules between these stations is accomplished by a directed transport of vesicles. This general hypothesis is not entirely uncontested (Heuser, 1989), but it is certainly a scheme within which axonal transport fits very well. If one considers the ontogenetic and phylogenetic development of nerve cells, from simple nearly spherical structures to the vastly extended and branched forms seen in adult animals, then it is reasonable to suppose that sorting stations are retained unaltered while the problems of distribution are handled merely by stretching the microtubule-dependent motor apparatus that is responsible for the delivery of secretory vesicles to terminal membranes (anterograde transport) and the return of membrane from the cell surface to the Golgi apparatus (retrograde transport). However, the development of nerve cells that have a highly extended geometry and that are required to function as rapidly responding signal lines has given rise to some modifications that are apparently unique to neurons and neuroendocrine cells (Clift-O'Grady et al., 1990). Enzymes for the synthesis of nonpeptide transmitters are delivered by rapid anterograde transport to nerve terminals where the synthesized transmitters are loaded into synaptic vesicles that cycle locally through an endosomal compartment (*see* review by Südhof, this issue). The local recharging of synaptic vesicles confers an obvious advantage in allowing a rapid and sustained synaptic response to nerve stimulation with considerable independence from the synthetic mechanisms in the cell body.

The Neuronal Cell Body: Protein Passage Through the Golgi Apparatus and the Initiation of Vesicle Transport

The evidence that proteins that undergo rapid axonal transport are obligated to move from the RER through the Golgi apparatus has been obtained by manipulation of various parts of the Golgi apparatus with inhibitors. Passage of rapidly transported proteins from the RER by vesicles that bud from the RER and fuse with the cis-face of the Golgi apparatus is consistent with the findings of Hammerschlag and coworkers (Lindsey et al., 1981; Stone and Hammerschlag, 1981; Hammerschlag, 1982) that movement of proteins through the Golgi apparatus is a calcium dependent process in which fusion of vesicles with the cis-Golgi apparatus can be blocked by calcium antagonists.

Obligatory movement through the Golgi apparatus of proteins destined for rapid axonal transport has been demonstrated by experiments in which the function of the Golgi apparatus was inhibited either by the ionophore monensin (Hammerschlag, 1982) or by use of brefeldin A (Smith et al., 1991), the latter an agent that causes disassembly of the cis- and medial-Golgi apparatus and inhibition of secretion in nonneuronal cells (Misumi et al., 1986). Neither of these inhibitors of Golgi apparatus function impaired protein synthesis, but rapid axonal transport was dramatically inhibited.

The rapid axonal transport of newly synthesized lipids is dependent upon the synthesis of proteins (Grafstein et al., 1975; Sherbany et al., 1979; Nichols et al., 1982) and, conversely, inhibition of phospholipid or cholesterol synthesis within the cell body produces a related inhibition of newly synthesized axonally transported protein (Longo and Hammerschlag, 1980). A study of the time relationships between the addition of protein synthesis inhibitors and the inhibition of the axonal transport of proteins or lipids (Nichols et al., 1982) suggests that protein syn-

thesis is parallel to rather than serial with lipid synthesis in the formation of an axonally transportable complex. These results suggest that rapid axonal transport requires the assembly of a protein—lipid complex. This interrelationship between protein and lipid transport seems to make sense since all current evidence indicates that the vehicle for the rapid anterograde transport of macromolecules is a membrane-bounded vesicle (Smith, 1980; Tsukita and Ishikawa, 1980; Fahim et al., 1985). As is the case in the formation of the vesicles of the regulated secretory pathway, at least some of the axonally transported vesicles are budded off the trans-Golgi apparatus as clathrin-coated vesicles (Stone et al., 1984a); the clathrin coats do not persist into the axon. Direct evidence for sorting of proteins into both constitutive and regulated secretory pathways within the same neuron has been presented by Jung and Scheller (1991) and the results (discussed below) of Morin et al. (1991) on the chemistry of rapidly transported vesicles may be interpreted in the same way.

The sequence of events described above for the initiation of rapid axonal transport is very similar, perhaps identical, to the initiation of secretion in all secretory cells. Recent work, however, has revealed some features of the final stages of the initiation of rapid axonal transport that may be unique to neurons.

It has been reported that incubating neuronal cell bodies with cobalt or monensin to inhibit radiolabeled protein transport by 95% caused little or no inhibition in the axonal transport of acetylcholinesterase or adenylate cyclase (Hammerschlag and Stone, 1987). This observation bears a remarkable similarity to other recent findings (Smith et al., 1991). In that work cycloheximide, puromycin, or brefeldin A was used to inhibit by two to three orders of magnitude below control values the axonal transport of labeled proteins in amphibian neurons. The anterograde and retrograde rapid transport of organelles in single myelinated axons was monitored by computer enhanced video microscopy (Smith, 1989). Surprisingly, in the face of the profound inhibition of protein transport, organelle traffic in the axons remained at control values for periods of 24 h or more.

There are three ways in which the discrepancy, under these experimental conditions, between the inhibition of the axonal transport of newly synthesized proteins and the transport of optically detected organelles might be explained. First, it is possible that metabolic labeling of newly synthesized proteins results in the labeling of a very small fraction of the anterogradely transported organelles. If this were the case, then an inhibition of the formation of the labeled species of organelles may not have been detected. There is, however, no evidence at all for such a highly restricted organelle labeling.

A second category of explanation is to postulate that organelles that are axonally transported rapidly in the anterograde direction are formed by recycling materials that are delivered to the trans-Golgi apparatus by retrograde axonal transport. This implies that organelle transport may be insensitive to protein synthesis in the short term, and that newly synthesized proteins may be loaded onto the preexisting flow of membranous vesicles. Such an explanation is consistent with the evidence that in secretory cells there is an intersection between endocytotic and secretory pathways (Fishman and Fine, 1987; Stoorvogel et al., 1988), as well as with evidence that receptor proteins may be recycled via retrograde transport to the neuronal cell body (Levy et al., 1990). It has been estimated that half the phospholipids in the endoplasmic reticulum are removed by vesicle transport every 10 min, while the synthesis of phospholipids is not sufficient to replace this loss (Weiland et al., 1987). Thus, vesicle traffic from internal membrane systems may be generally maintained by a process of membrane recycling, and furthermore this process may be independent of the transport of protein through the Golgi apparatus (Vance et al., 1991).

Recycling of membrane may be an attractive explanation for the independence of rapid organelle transport and the synthesis of proteins. But

the report that protein transport (estimated by enzyme assay) and protein synthesis are independent (Hammerschlag and Stone, 1987) may be explained by a third mechanism: the existence of a large buffer or storage pool of axonally transported materials so that even if the new synthesis of materials is suppressed, axonal transport may continue for considerable periods of time. Evidence for the existence of such a pool of lipids (Grafstein et al., 1975; Haley et al., 1979; Toews et al., 1979; Snyder et al., 1980) and proteins (Berry, 1980; Goodrum and Morell, 1982; Nichols et al., 1982) seems good, although it must be noted that if membrane recycling is taking place the estimation of the size of a lipid pool may be in considerable error. The available data on vertebrate neurons point to large pools of lipids with half lives extending to several days. However, the protein pools appear to have shorter half lives.

It is worth noting that this type of storage pool is probably not related to the pool of regulated secretory materials since there is no evidence that release from the neuronal pool is anything other than constitutive. However, it has been noted (Morin et al., 1991) that if release from such a pool were coupled to the cell's electrical activity, then an increase in demand for peptide transmitters could be met without requiring new protein synthesis.

Organelles that Carry Rapidly Transported Proteins

The morphological varieties of rapidly transported organelles in axons have been described in normal axons (Smith, 1980; Tsukita and Ishikawa, 1980; Ellisman and Lindsey, 1983; Fahim et al., 1985) and in axons in which the retrogradely moving organelles originated at an injury site (Smith and Snyder, 1991). Anterogradely moving organelles in a number of animal species are predominantly small vesicles averaging approximately 80 nm diameter, and tubulovesicular organelles. Larger secretory-type dense cored vesicles occur in varied proportions depending on the cell type. A small proportion of mitochondria and organelles that resemble lysosomes or prelysosomes is present in the anterogradely transported group, a finding that is consistent with the rapid anterograde transport of mitochondrial proteins and lipids and with the anterograde transport of lysosomal enzymes (Schmidt et al., 1980).

Retrogradely moving organelles are generally described as being larger than those of the anterogradely transported group, but this is only partly correct. The retrogradely transported group of organelles is distinct because it normally contains a high proportion of large multivesicular bodies and other organelles of the lysosomal series, as well as mitochondria. However, descriptions of the composition of the retrogradely transported population (Smith, 1980; Tsukita and Ishikawa, 1980; Smith and Snyder, 1991) also point out that a substantial proportion of the organelles is composed of vesicles that are morphologically indistinguishable from the anterogradely transported vesicles. In the case of preparations in which the retrogradely transported organelles are returning from the site of a lesion, the proportion of small vesicles is even larger than normal (Smith and Snyder, 1991).

A recent study of the biochemical nature of anterogradely transported organelles in rabbit optic nerve presented evidence for the heterogeneity of the organelles (Morin et al., 1991). Metabolically labeled protein was transported rapidly into the axons where it was associated with three sets of membranes. The first set was retained in the axons in an immobile state and hence represented deposited label (see below). This set of membranes contained most of the axonally transported glucose transporter and hence was judged to be axolemmal membrane. The second set of membranes was composed of 84 nm vesicles that contained glucose transporter, about half the transported synaptophysin, and the motor molecule kinesin. On this evidence the 84 nm vesicles were assumed to be rapidly transported

to the axolemma and the nerve terminal, and could be classified as constitutive secretory vesicles. The third set of membranes contained half the rapidly transported synaptophysin, 80% of the axonal tachykinins, and kinesin. Therefore, this last group of membranes probably represents material destined for the nerve terminal and is material that behaves as a regulated secretory component.

Transport Kinematics

To determine whether the axonal transport of proteins is similar to, or differs from, the transport of membrane-bounded organelles the kinematics of the two processes can be compared, but the comparison is not simple. On the one hand there is a considerable difference in the scale of the measurement of organelle transport and the transport of any molecular marker. Organelle transport is observed microscopically over distances of tens of micrometers on a time scale of seconds to minutes, whereas the transport of proteins and other molecules is measured over distances of tens of millimeters on a time scale of hours. An exact translation between the two approaches is impossible and some simplifying assumptions are necessary. In addition, it is not possible to assume that the characteristics of transport are similar between classes of animals, between different nerves in the same animal, or even between axons in the same nerve trunk. For instance, the rate of anterograde protein transport in the optic and the olfactory nerves of the garfish (Gross and Beidler, 1975; Cancalon, 1988) has a linear dependence on temperature over the ranges of 14–35°C and 10–28°C, respectively, in contrast to the nonlinear dependence reported for amphibian sciatic nerve over the range of 5.5–28°C (Edström and Hanson, 1973a). In addition, while the rates of anterograde protein transport in cat (Ochs and Smith, 1975) and rat sciatic nerve (Bisby and Jones, 1978) both have a nonlinear temperature dependence in the range of 13–38°C, the absolute rates differ markedly between the two animals so that anterograde transport rates in the rat may be as much as 50% less than those in the cat at low temperatures. A difference between transport in mammalian and nonmammalian nerve is the cessation of transport below 11–13°C in mammalian nerve (Ochs and Smith, 1975; Bisby and Jones, 1978), in contrast to continued transport to 10°C in the garfish (Gross and Beidler, 1975) and 5.5°C in the frog (Edström and Hanson, 1973a). In a single species (garfish) anterograde transport rates for protein in the optic nerve and the olfactory nerve are statistically different at the same temperature (Gross and Beidler, 1975; Cancalon, 1988). Finally, the mean rates of retrograde transport of organelles may differ between individual myelinated axons in amphibian sciatic nerve (Smith and Cooper, 1981).

Clearly, to arrive at any useful conclusion the kinematics of protein transport and organelle transport must be compared in the same animal and preferably in the same nerve. Very little appropriate information is available. In *Xenopus* sciatic nerve (Snyder, 1989) a pulse of radiolabeled protein moves in the range (slowest to fastest) of 1.64–1.98 μm/s in the anterograde direction and 0.93–1.71 μm/s in the retrograde direction (22.5°C). In the same preparation at the same temperature, vesicle transport monitored by computer enhanced video microscopy (Smith, 1989) has an anterograde rate of 1.92 ± 0.82 μm/s (± SD) and a retrograde rate of 1.66 ± 0.82 μm/s (Smith and Snyder, 1991). In rat (Sprague-Dawley) sciatic nerve (Bisby and Jones, 1978) the fastest rate of anterograde protein transport at 31°C is 2.06 μm/s. At an equivalent temperature, anterograde organelle transport rates of 1.62 ± 0.53 μm/s have been recorded in sciatic axons of Wistar rats (Abbate et al., 1991). Although limited in scope, these results show that there is a general agreement between the maximum rates of protein and organelle transport.

If the passage of a pulse of protein in a nerve trunk is monitored, the pulse is seen to widen as it progresses along the nerve. The observed dispersion of protein could reflect a general prop-

erty of the transport system, such as a dispersion of vesicle velocities, or it might mean that the rates of protein transport are not similar in different axons. The transport of a labeled transmitter in single *Aplysia* neurons has been studied (Goldberg et al., 1978). The researchers injected ^{3}H-serotonin into the cell body of the giant cerebral neuron and ligated the neuron near its cell body shortly after injection, creating a narrow pulse of transporting radiolabel. At 23°C the full-width at half-maximum of the peak broadened at a rate of 143 μm/mm of travel; the dispersion probably reflects the behavior of serotonin-containing vesicles that are transported in the anterograde direction. The value for a single molecular species in *Aplysia* is to be compared to the broadening of pulses of labeled protein in vertebrates: an anterograde peak to wavefront broadening (at 23°C) of 74 μm/mm in the garfish optic nerve (Cancalon, 1988), 97 μm/mm in the garfish olfactory nerve (Gross and Beidler, 1975), and a full-width at 90% maximum of the peak broadening of 183 μm/mm in *Xenopus* sciatic nerve (Snyder, 1989). These results suggest that a major part of the dispersion of transport in whole nerve trunks relates to intraaxonal, and not interaxonal, effects.

Whatever the source of the dispersion of protein transport, the effect compared to the dispersion of velocities of organelle transport is relatively small. Coefficients of variation of ±5–8% are reported for anterograde protein transport (Gross and Beidler, 1975; Goldberg et al., 1978; Cancalon, 1988; Snyder, 1989) and ±20% for retrograde protein transport (Snyder, 1989), while the coefficients of variation in the velocities of both anterogradely and retrogradely transported organelles are ±30–50% (Smith and Cooper, 1981; Schroer et al., 1985; Smith and Forman, 1988; Abbate et al., 1991; Smith and Snyder, 1991). The differences between protein and organelle transport might be explained by postulating that the short-term variation in organelle rates is averaged to a lower value with increased transport distance. However, the only attempt to record the behavior of individual axonally transported organelles over long periods of time (circa 60 min in the giant axon of *Myxicola*) does not support this possibility (Breuer et al., 1988).

The overall evidence from the study of the kinematics of the anterograde transport of proteins and organelles is that, though there is reasonable agreement between the maximum velocities of the two processes, the dispersion of velocities of organelles is much greater than the dispersion of protein transport. This could mean that recently synthesized proteins (those that were studied in all experiments on the dispersion of protein transport) are carried by a subset of axonally transported organelles. The subset would include the fastest organelles, which may be identified with the small anterogradely transported tubulovesicular organelles.

Even greater problems occur in comparing retrograde protein transport with retrograde organelle transport. This is largely the result of experimental difficulties encountered in the study of retrograde transport of protein. Most data concerning retrograde protein transport has resulted from one of two experimental approaches: (1) study of the transport of endogenous material after it has undergone transport reversal at an axon terminal or at an axonal lesion, and (2) by following endocytosed foreign material. Retrograde axonal transport is generally said to have a rate of approximately 50% that of anterograde transport (e.g., Lubinska and Niemierko, 1971; Ranish and Ochs, 1972; Edström and Hanson, 1973b; Bisby and Buchan, 1981). However, many of these estimates are inaccurate as a result of an inability to distinguish reversal or uptake times from transport times. There is some evidence for two retrograde rates, approximately 25% and 75–90% that of the anterograde rate, the former relating to exogenous material (*see* Schwab and Thoenen, 1983) and the latter to endogenous material (e.g., Brimijoin and Helland, 1976; Brimijoin and Wiermaa, 1977; Snyder, 1986, 1989). Retrograde organelle velocities, as previously noted, are sufficiently dispersed to account for the full range of protein transport rates.

Deposition of Protein Within the Axon

Not all rapidly transported proteins synthesized in the cell body reach the nerve ending; a substantial proportion of the total is deposited to a stationary phase within the axon (Gross and Beidler, 1975; Ochs, 1975). The fraction of moving proteins lost to the stationary phase generally increases with the total length of a nerve (Cancalon and Beidler, 1975; Gross and Beidler, 1975; Rulli and Wilson, 1987). In addition to proteins, glycoproteins (Goodrum and Morell, 1982; Harry et al., 1989), lipids (Haley and Ledeen, 1979; Bisby, 1985), and molecules such as serotonin (Goldberg et al., 1978), norepinephrine (Brimijoin and Wiermaa, 1977), and substance P (Brimijoin et al., 1980), whose known function is at the synaptic terminal, are deposited within the axon. It has also been demonstrated that proteins that are anterogradely transported, and that reverse their direction of transport at an axonal lesion to become retrogradely transported, are then deposited at essentially the same rate as anterogradely transported proteins (Snyder, 1989).

The deposition of molecules to the stationary phase within the axon is not a homogeneous process. Some classes of proteins appear to be preferentially deposited, and different lipids are deposited at different rates and at different places (Cancalon and Beidler, 1977; Baitinger et al., 1981; Goodrum and Morell, 1982; Toews et al., 1982, 1983; Stone et al., 1984b; Rulli and Wilson, 1987; Morin et al., 1991). The differential deposition of molecules could be explained if different classes of organelles were targeted toward different regions of the axon. Although evidence exists for at least two chemically distinct classes of anterogradely transported organelles (Morin et al., 1991), there is no evidence that a given class of organelles is directed specifically to the terminal. Indeed, the axonal deposition of molecules that are believed to have their specific function at the nerve terminal (Brimijoin and Wiermaa, 1977; Goldberg et al., 1978; Brimijoin et al., 1980) suggests that deposition may be a passive phenomenon resembling the leakage of material from a permeable container. A patterned, but in some respects nonspecific, deposition might be explained if molecules of different functions were transported by a common carrier and were deposited in structurally associated clusters. The site of deposition might then be controlled by one molecule of the cluster while the rest of the cluster, even though composed of molecules with no local function, was constrained to follow.

It is clear that protein synthesized in the cell body is rapidly transported to a final stationary stage in the axon, but it is not known whether this protein arrives uninterrupted or is initially deposited to an intermediate location and later moved to its final position. The identification of at least some of the stationary material as axolemmal (Morin et al., 1991) suggests that deposition does not represent a single process since most anterogradely moving organelles are physically well removed from the axolemma.

Some evidence suggests that in myelinated fibers the nodes of Ranvier play a role in the initial deposition of protein. It has been shown that anterogradely transported glycoprotein accumulates at nodes immediately following passage of a radiolabeled pulse (Armstrong et al., 1987). The glycoprotein gradually dispersed from the nodes over a period of days. The authors hypothesize that the increase results from rheological effects of axon constriction and from selective deposition of some transported molecules required in the nodal regions (that is, partly a passive and partly an active phenomenon). It is not clear how much of the total transported material accumulates at the nodes. If a significant fraction of the total protein accumulates, then some mechanism is required to distribute this material to the internodal regions. Diffusion could distribute even large molecules (approximately 500 kDa) over internodal distances within time periods of a few days (Gross et al., 1981).

In normal axons the amount of protein deposited at each location along the axon is proportional to the quantity of protein in the moving

phase (Gross and Beidler, 1975; Muñoz-Martínez et al., 1981). Thus, protein is lost exponentially from the moving phase as it traverses the nerve, suggesting that the deposition of protein is a passive event. Exponential deposition implies that there may be an oversupply of newly synthesized protein to the proximal regions and an undersupply to the distal regions of the axon. Undersupply could be avoided by initially delivering an excess of protein to the axon, the residue being returned from the axonal terminal to the cell body for degradation or recycling. Or, the supply of protein to the distal region of an axon could be increased through the deposition of rapidly transported protein that returns from the axonal terminal (Snyder, 1989). This potential mechanism for the supply of the distal axon is a possibility since the composition of retrogradely transported protein differs only slightly from that of anterogradely transported protein (Abe et al., 1974; Bisby, 1981; Martz et al., 1989).

Although deposition may be proportional to the quantity of protein in the moving phase, the magnitude of deposition appears to be regulated by local signals from the axon. Deposition of protein within regenerating nerve has been shown to be two to five times the normal rate (Griffin et al., 1981; Chan et al., 1989). One study (Harry et al., 1989) has shown that acrylamide neurotoxicity results in an increased rate of deposition of anterogradely transported glycoproteins, but not methionine-labeled proteins. In control preparations both proteins and glycoproteins exhibited similar transport patterns, enabling the deposition of a limited class of proteins to be experimentally modulated.

Various models have been proposed to account for the delivery of molecules that are synthesized in the cell body to their final destination. The most complete (Hammerschlag and Stone, 1986; Stone and Hammerschlag, 1987) views the protein/organelle complex as an irreducible unit once it leaves the soma. Docking receptors (address sites) exist within the axon or terminal, and either the organelle *in toto* fuses to stationary membrane or vesicles bud off and fuse to the membrane. One model was proposed (Toews et al., 1987) in which lipids exchange between the organelle and stationary membrane, either as a result of direct contact or via lipid transfer proteins (Ledeen, 1985), without membrane fusion. Another scenario in which small vesicles mediate the exchange of molecules between moving organelles and stationary structures (Stone et al., 1984a) was suggested. It is apparent from the foregoing description of the properties of axonal deposition that the models do not attempt to predict all the observed properties of deposition. It is also true that experimental work on axonal deposition has emphasized the phenomenology of molecular transport, while the role played by the carrier organelle has been essentially ignored.

The role of the organelle in the deposition process has been examined (Snyder et al., 1990) by testing the hypothesis that protein deposition can be accounted for by a loss of anterogradely transported organelles from the transport system. Using amphibian sciatic nerve, it was shown that protein is lost from an anterogradely moving pulse of radiolabeled protein at a rate of approximately 2%/mm of nerve. This implies that approximately 60% of the protein in the pulse is lost over a distance of 50 mm. An examination of organelle traffic in sciatic axons at two locations 60–75 mm apart showed that no reduction occurs in either the number or sizes of organelles.

The interpretation of this experiment depends on several assumptions. The first of these is that the majority of the anterogradely transported vesicles was detected by the optical system, and the evidence (Smith, 1989) indicates that this is a valid assumption. Second, it is assumed that most of the vesicles carried labeled protein. If the second assumption is valid then it must be concluded that protein deposition occurs without a decrease in either the number or the sizes of transported vesicles. This result could imply either that protein is lost from transport vesicles or that vesicles are lost from the anterograde stream and are then resupplied in equal numbers to the anterograde stream, perhaps by budding from axonal membrane systems. A completely com-

pensatory supply of anterogradely transported vesicles from previously stationary membrane systems would seem to be an unlikely occurrence with no useful function.

Reversal of Rapid Axonal Transport

Rapid axonal transport can be reversed. This means that molecules or organelles, known to have been moving in one direction at some initial time may be demonstrated to move in the opposite direction at a later time. Transport reversal may take place from either the anterograde-to-retrograde (A–R) direction or the retrograde-to-anterograde (R–A) direction. The reversal of rapid axonal transport may take place at any of four sites within the neuron. At normal nerve terminals proteins undergo A–R reversal (e.g., Bray et al., 1971; Abe et al., 1974; Bisby, 1976). At axonal lesions, both protein (e.g., Bisby and Bulger, 1977; Snyder, 1989) and organelles (Smith, 1988) have been demonstrated to undergo A–R reversal. Organelles also undergo R–A reversal on the distal side of axonal lesions, but R–A reversal of protein transport has not been demonstrated (*see* Schmidt et al., 1980). At the junction between the parent and the daughter axons of regenerating nerve A–R reversal of both protein and organelles may take place (Chan et al., 1989). This is an interesting case of transport reversal, since evidence was obtained that A–R but not R–A organelle reversal takes place at the junction, indicating that a junctional region does not merely act as a lesion but somehow causes the specific reversal of anterograde transport. The fourth site of reversal may be the cell body itself. Inhibitors of protein synthesis have been shown to prevent the export of newly synthesized proteins and phospholipids from the cell body, but not the export of organelles (Snyder and Smith, 1990). Similarly, brefeldin A blocks the export of newly synthesized proteins, but does not affect the anterograde transport of organelles (Smith et al., 1991). These findings imply that the organelles exported from the cell body may be derived from retrogradely transported organelles that have undergone transport reversal at the cell body, possibly by passage through the trans-Golgi apparatus (cf., Laduron, 1984; Levy et al., 1990).

Transport reversal is affected by local conditions at nerve endings or axonal lesions. One study (Bulger and Bisby, 1978) has shown that the quantity of protein undergoing A–R reversal at a crush site depends upon whether or not the site is allowed to regenerate. Another study (Sahenk and Mendell, 1980) reported that A–R reversal is suppressed by zinc pyridinethione toxicity, and a third (Jakobsen and Brimijoin, 1981) described a similar finding in the case of axonopathy induced by *p*-bromophenylacetylurea.

Inhibitors of calcium-activated proteases (leupeptin or E-64) prevent the A–R reversal of radiolabeled protein, cause an accumulation of anterograde organelles, and a reduction in what were believed to be retrograde organelles at severed axon tips (Sahenk and Lasek, 1988). From these results it was concluded that proteolysis is necessary for a morphological conversion of organelles that allows the organelles to undergo A–R reversal. In a closely related study (Smith and Snyder, 1991), it was shown that leupeptin inhibits the A–R reversal of newly synthesized protein at a lesion but does not prevent either A–R or R–A reversal of organelles, suggesting that though the reversal of organelle transport is necessary for the reversal of the transport of newly synthesized proteins, it is not a sufficient condition. It appears that newly synthesized proteins may be off-loaded from organelles when they undergo transport reversal and that a proteolytic process is important in allowing the association of these proteins with organelles that have undergone transport reversal. This interpretation is consistent with the minimum time required for newly synthesized proteins and organelles to reverse their direction of transport. Protein has been shown to require more than 1.5 h to undergo A–R reversal at a lesion (Bisby and Bulger, 1977; Sahenk and Mendell, 1980; Snyder, 1986, 1989), whereas organelles are capable of reversal within

less than 10 min following their arrival at a lesion (Smith 1988). The time relationships imply that molecules do not return from a site of reversal with the same organelle on which they arrived (Snyder et al., 1989). It has also been reported (Sahenk and Brown, 1991) that weak-based amines produce phenomena similar to protease inhibitors. Acidic membranous compartments within axon terminals are thought to be required for the A–R reversal of transport organelles. Thus, calcium-activated proteolysis and acid hydrolytic activity appear to play roles in reversal, but the relationship between the transported proteins and carrier organelles at the site of reversal is unclear.

It has been suggested that for an organelle to undergo transport reversal it must be converted from the morphological type of anterograde organelle to a morphological type that is typical of the retrogradely transported organelle (e.g., Fahim et al., 1985; Cheng and Reese, 1988; Sahenk and Lasek, 1988). This idea seems to depend on the published reports of morphological differences between anterograde and retrograde organelles in normal axons (e.g., Smith, 1980; Tsukita and Ishikawa, 1980; Fahim et al., 1985; Miller and Lasek, 1985; Smith and Snyder, 1991). The idea has only limited applicability since the published reports show that many retrogradely transported organelles in normal axons are morphologically indistinguishable from anterogradely transported organelles. In addition, we (Smith and Snyder, 1991) have shown in amphibian axons that organelles that reverse their direction of transport at a lesion are predominantly (>80%) small vesicles having mean diameters statistically equal to vesicles that traveled in the anterograde direction in the same preparations. This finding argues that a change in morphology is not a prerequisite for A–R transport reversal, at least at an axonal lesion. This conclusion is in keeping with the finding (Schroer et al., 1985) that foreign synaptic vesicles injected into squid axoplasm may travel in either the anterograde or the retrograde direction, and that the proportion of vesicles that travel in each direction depends upon their surface molecular properties. Video microscopic investigations into the mechanisms of A–R and R–A organelle reversal at axonal lesions (Smith, 1988) are also consistent with the idea that organelle reversal depends primarily on some change in the surface property of the organelles. In this work, the action of inhibitors of motor molecules provided evidence that transport reversal can take place by a process in which an individual organelle rapidly switches between two transport mechanisms. The two mechanisms had pharmacological sensitivities resembling those of kinesin (anterograde) and cytoplasmic dynein (retrograde). What controls the switch is unknown, but an important fact that emerges from this and other work (Smith and Snyder, 1991) is that an organelle may undergo several changes in direction, indicating that the switch is completely reversible and hence does not rely on any irreversible process such as proteolysis.

The molecular mechanisms underlying the reversal of axonal transport are almost completely unknown. One possibility that should be investigated is the involvement of protein phosphorylation/dephosphorylation reactions that have the required switch-like properties and have been shown to be a part of the mechanism of the control of the direction of organelle movement in nonneuronal cells (Lynch et al., 1986a,b; Rozdzial and Haimo, 1986). Another, perhaps related, avenue for investigation is the role of protein alterations at lesion sites or nerve endings. Recently a polypeptide, designated A25, has been reported to appear at a crush site within a few hours of crush (Perng et al., 1990). These results show that A25 appears immediately proximal to a cold-blocked region of nerve, that is, at a site where transported material would accumulate. However, A25 does not appear in either a crushed or uncrushed region of nerve maintained in the cold. Thus, cold alone does not result in the production of A25. The authors concluded that A25 is most likely produced from post-translational processing of a protein that is rapidly transported in the anterograde direction. This suggests that accumulation of rapidly trans-

ported material may in itself result in biochemical changes that could serve as part of the mechanism of reversal of rapid axonal transport.

Conclusion

There are similarities between the secretory process as it is generally understood and rapid axonal transport. Many of the proteins carried by rapid axonal transport make an obligatory passage from the rough endoplasmic reticulum through the Golgi apparatus, where organelles of types resembling regulated and constitutive secretory vesicles are formed and transported out into the axon by a microtubule-dependent process. The retrieval of membrane and membrane-associated molecules by retrograde axonal transport probably has many features in common with the retrieval of membrane in secretory cells, but these aspects of membrane flow are not well investigated in neurons. The rapid axonal transport of mitochondria and lysosomes shifts emphasis away from the secretory model of the transport system, but these properties also appear to be very similar to those existing in nonneuronal cells.

What appear to be unique features of the neuronal transport system involve the relationship between newly synthesized proteins (and other molecules) and axonally transported organelles. The independence of rapid organelle traffic from the passage of newly synthesized proteins through the Golgi apparatus is open to a number of interpretations. The first of two main possibilities is that anterograde organelle traffic is in the short term independent of protein passage through the Golgi apparatus because materials required for the formation of the organelles are available from recycled, retrogradely transported material. This possibility implies that newly synthesized proteins are loaded onto existing transport vesicles. The second possibility is that neurons contain, after the Golgi apparatus, a pool of rapidly transportable proteins and organelles that buffers the export of all rapidly transported material.

Deposition of rapidly transported material, protein, lipid, and even transmitter molecules to a stationary phase within the axon does not resemble any described property of secretory systems and may represent a neuronal adaptation. Deposition occurs from both rapid anterograde and retrograde transport and is puzzling because many of its properties suggest that it is a passive, nonspecific phenomenon. However, evidence also exists that specific types of proteins and lipids are deposited to certain regions of the axon, and the amount of deposition seems to be regulated by the functional state of the axon.

Some aspects of transport reversal resemble the circulation of material, from the Golgi apparatus to surface membrane and back to the Golgi apparatus, that is described so well in nonneuronal systems. The reversal of individual, rapidly transported, organelles appears to be a separate phenomenon that is also probably not unique to neurons. The reported loss of protein from organelles that undergo anterograde to retrograde reversal may, however, be uniquely neuronal.

The independence of somal organelle export from the export of recently synthesized proteins, the deposition of materials to the axon, and the loss of newly synthesized proteins from organelles during transport reversal may all be related phenomena that reflect a dynamic relationship between cargo proteins and carrier organelles.

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