Axonal Transport

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Contents

Abstract

Axons are long slender cylindrical projections of neurons that enable these cells to communicate directly with other cells in the body over long distances, up to a meter or more in large animals. Remarkably, however, most axonal components originate in the nerve cell body, at one end of the axon, and must be shipped out along the axon by mechanisms of intracellular motility. In addition, signals from the axon and its environment must be conveyed back to the nerve cell body to modulate the nature and composition of the outbound traffic. The outward movement from the cell body toward the axon tip is called anterograde transport and the movement in the opposite direction, back toward the cell body, is called retrograde transport. This bidirectional transport, known collectively as axonal transport, is not fundamentally different from the pathways of macromolecular

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and membrane traffic found in other parts of the neuron, or indeed in any eukaryotic cell, but it is unique for the volume and scale of the traffic required to maintain these long processes.

Keywords

Axonal transport • Accumulation techniques • Bidirectional • Cargo structures • Cargoes • Components • Cytoskeleton • Imaging technique • Microfilaments and microtubules • Molecular motor proteins • mRNA • Neurodegenerative diseases • Neurofilaments • Presynaptic development and plasticity • Pulse-labeling technique • Retrograde axonal transport • Ribonucleoprotein complexes

Brief History

The existence of axonal transport was inferred in the nineteenth and early twentieth centuries by pioneering neuroscientists such as Augustus V. Waller and Santiago Ramón y Cajal, but the first experimental evidence was described in a seminal paper by Paul Weiss and Helen Hiscoe in 1948. These authors used a clever surgical technique to apply a gentle and gradual constriction to regenerating axons in vivo. The axons gradually swelled proximal to the constriction (i.e., on the side closer to the cell body) over several weeks due to the accumulation of anterogradely transported materials (Fig. 1). When the constriction was released, the bolus of accumulated materials appeared to propagate distally along the axons (i.e., away from the cell body) at a rate of about a millimeter per day. Weiss and Hiscoe referred to this phenomenon as "damming," which was meant to conjure up the image of water upstream of a dam, and they reasoned that it was due to an "axomotile" mechanism. They termed this movement axonal flow, but it is now known as axonal transport. In later work, Weiss considered this movement to represent the bulk

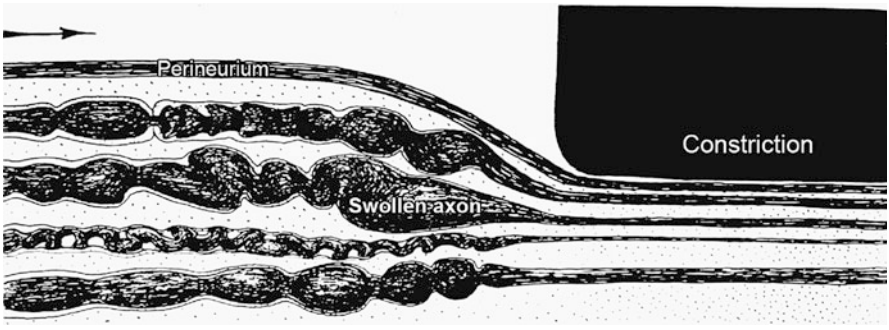

Fig. 1 *The original demonstration of axonal transport by Weiss and Hiscoe*. This is the original figure of Weiss and Hiscoe, published in 1948, with some labels added. It is a drawing of the axons in a constricted nerve and was originally entitled "Composite diagram of principal fiber deformations ('damming') proximal to a constriction." Proximal is left and distal is right: the *arrow* points toward the axon tip (Reproduced from Weiss and Hiscoe 1948)

movement of a "semirigid column" of cytoplasm, propelled by peristaltic contractions of the axolemma. Today, the movement is known to be far more complex and actually represents the independent movement of dozens if not hundreds of distinct intracellular cargo structures, but this should not detract from the seminal importance of the original work, which was published long before the era of modern cell biology. In fact, Weiss and Hiscoe's appreciation of the significance of axonal transport for the growth and maintenance of axons was remarkably prescient, and this study remains a landmark in the field of axonal transport.

Axonal Transport

Three Ways to Study Axonal Transport

Today, there are essentially three ways to study axonal transport experimentally: accumulation techniques, pulse-labeling techniques, and imaging techniques. Historically, the introduction of each of these techniques has revolutionized researchers' understanding of axonal transport, so the history of this topic is in many ways a history of these technical advances.

Accumulation Techniques

The oldest and simplest approach to the study of axonal transport is to block movement locally along an axon or nerve and then observe what cargo structures accumulate and the rate at which they do so. This method was used by Weiss and Hiscoe in their first description of axonal transport (see above), but variants on their approach are still used in laboratories today. The most common strategy is to ligate a nerve in vivo using surgical thread (Fig. 2). Anterogradely moving materials accumulate on the proximal side of the ligation and become depleted on the distal side, whereas retrogradely moving materials accumulate on the distal side and become depleted on the proximal side. A variant on this approach is the "cold block," in which local cooling is applied to a surgically exposed or isolated nerve. This method is technically more involved but eliminates the tissue damage that is associated with the ligation approach. Another approach, which has been applied to isolated axons teased apart from peripheral nerves and to single axons of cultured neurons, is to constrict axons locally with fine nylon or glass fibers (Fig. 3). A general advantage of accumulation techniques is their simplicity, but a major disadvantage is that they are nonselective (everything that moves accumulates) and can yield only limited molecular and kinetic information.

Pulse-Labeling Techniques

The original and most widely used pulse-labeling technique is radioisotopic pulse labeling, first applied to axonal transport in the late 1950s and 1960s. Most of what is known about the composition and kinetics of axonal transport has come from studies using this approach, and it remains to this day the preferred method for studying the composition and kinetics of slow axonal transport in vivo. Essentially, this technique

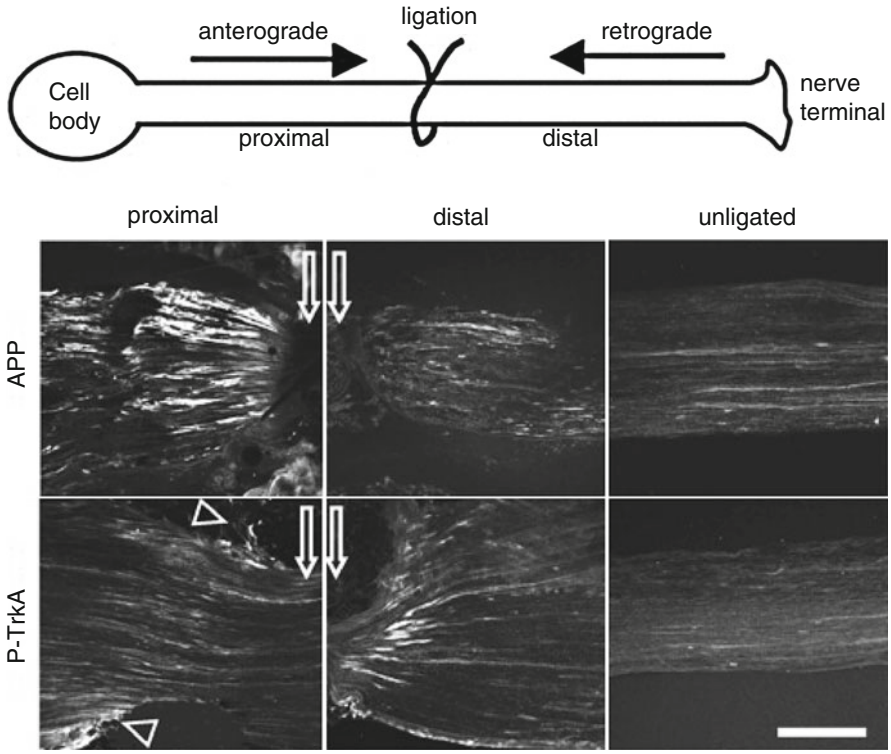

Fig. 2 *Accumulation of axonally transported cargoes at a sciatic nerve ligation*. Sciatic nerves of sedated mice were exposed and ligated with surgical thread. Several hours later, the mice were sacrificed and the nerves were removed and processed for immunofluorescence microscopy using antibodies specific for amyloid precursor protein (*APP*), which is present in a subpopulation of anterogradely transported organelles, and antibodies specific for the activated form of the neurotrophin receptor, phospho-TrkA, which is present in a subpopulation of retrogradely transported organelles. Amyloid precursor protein accumulates primarily on the proximal side of the ligation (note bright streaks, representing immunoreactivity in axons of the nerve), whereas phospho-TrkA accumulates primarily on the distal side. The *white arrows* point to the site of ligation. The *white arrowheads* (*lower left panel*) point to nonspecific staining of the perineurium. Scale bar = 100 μm (Adapted from Cavalli et al. 2005 © 2005 Rockefeller University Press. Originally published in J Cell Biol 168:775–787)

involves the injection of radiolabeled precursors of macromolecules (amino acids, sugars, or nucleotides) into the vicinity of neuronal cell bodies in an animal. Most published studies have used radiolabeled amino acids, which permit the movement of proteins to be investigated. The radiolabeled amino acids are taken up by the cell bodies, creating a pulse of radiolabeled proteins. Those proteins destined for the axon move into and along the axon in association with distinct cargo structures. By injecting numerous animals and sacrificing them at various time intervals, the kinetics of transport can be analyzed (each animal yields a single time point). The most powerful approach is to dissect out the nerves containing the radiolabeled

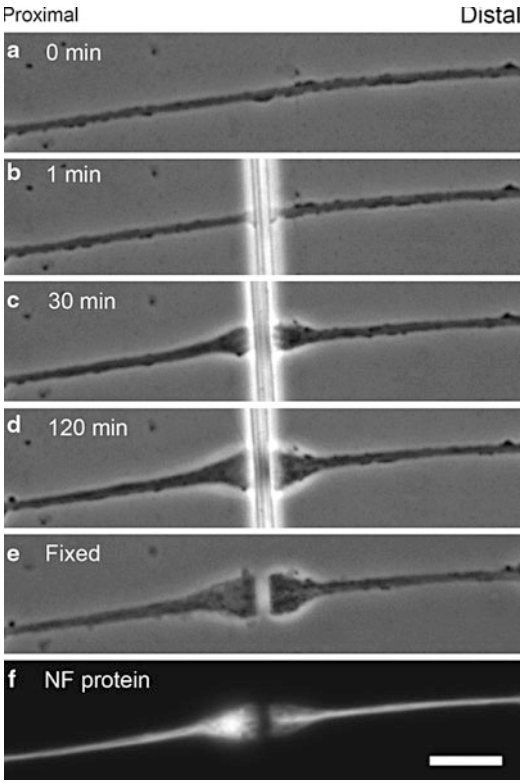

Fig. 3 *Constriction of an axon of a cultured nerve cell using a fine glass fiber*. (**a**–**d**) Phase contrast images of an axon of a cultured neuron immediately before constriction and after constriction for 1, 30, and 120 min. The swelling on both sides of the constriction is due to the accumulation of anterogradely and retrogradely moving cargoes. (**e**) Image of the axon after fixation and removal of the glass fiber. The axon appears to be broken under the glass fiber, but actually it is just flattened very thin. (**f**) Immunofluorescence microscopy of the axon using an antibody specific for neurofilaments, which are one cargo of axonal transport. Neurofilaments accumulate mostly on the proximal side of the constriction, which indicates that they are transported in a predominantly anterograde direction. Scale bar = 5 μm (Reproduced from Koehnle and Brown 1999 © 1999 Rockefeller University Press. Originally published in J Cell Biol 144:447–458)

proteins and then cut them into contiguous segments, permitting biochemical analysis of the radiolabeled proteins by subcellular fractionation, immunoprecipitation, and/or electrophoresis (Fig. 4).

Radioisotopic pulse-labeling studies of axonal transport can be performed on a variety of different nerve cell types. Those that are best suited are ones whose cell bodies are located in an anatomically discrete region, which facilitates reproducible injection of the radioisotope. In addition, it helps if the axons course within a nerve that can be dissected readily and that is long with minimal branching. Most radioisotopic pulse-labeling studies have been performed on rats or mice, and the most commonly used axons are the retinal ganglion cell axons of the optic nerve

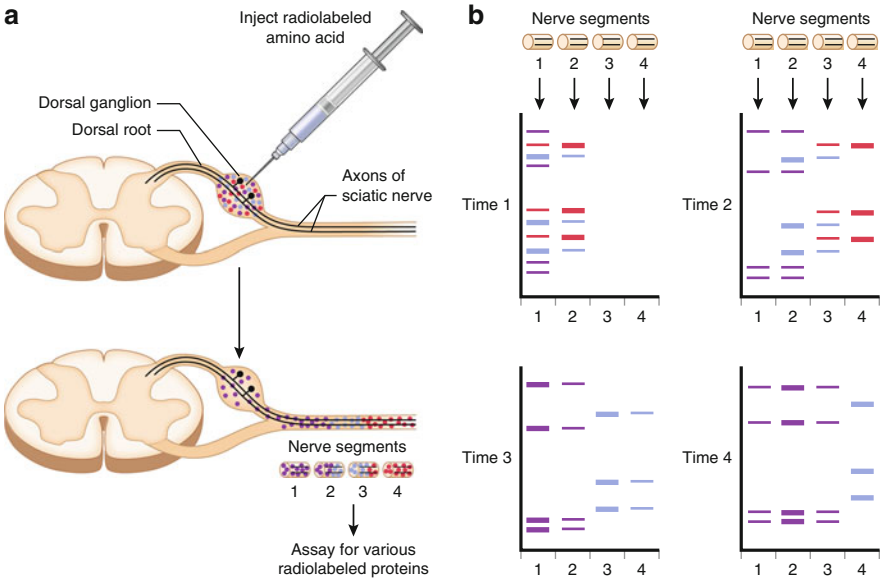

Fig. 4 *Schematic illustration of the radioisotopic pulse-labeling technique*. (**a**) Radiolabeled amino acids are injected into the vicinity of nerve cell bodies (dorsal root ganglion in this example). The amino acids are incorporated into proteins in the nerve cell bodies and the proteins move out along the axons in association with distinct cargo structures (shown here as *purple*, *blue*, and *red dots*), which move at different rates. At early times the faster and slower moving cargo structures overlap considerably, whereas at later times they become spatially separated. At various time points the animals are sacrificed and the nerves are cut into contiguous segments. (**b**) For each time point, the proteins in each segment are separated by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and the amount of radioactivity associated with each protein is quantified. Time points 1–4 represent four different injection-sacrifice intervals. Labeled proteins associated with the *purple*, *blue*, and *red* cargo structures in part **a** are shown as *purple*, *blue*, and *red bands*, respectively, in part **b** (Redrawn based on Lodish et al. 2000)

(radiolabeled precursor injected into the eye), the motor axons of the phrenic, and hypoglossal nerves (radiolabeled precursor injected into the cervical spinal cord or the hypoglossal nucleus of the medulla, respectively), and the motor and sensory axons of the sciatic nerve (radiolabeled precursor injected into the ventral horn of the lumbar spinal cord or into the lumbar dorsal root ganglia, respectively).

Imaging Techniques

The third approach to the study of axonal transport is direct observation of the movement of the cargo structures in living cells using light microscopy. The earliest reports of the movement of membranous organelles in axons date back to the 1920s, but it was not until the 1970s that this movement was understood to represent axonal transport. Most of these studies used phase contrast or differential interference contrast techniques, which permits detection of the largest and most refractile organelles. The movement of such structures was often described as saltatory,

primarily because large organelles face considerable resistance to movement in the dense environment of axoplasm and therefore often move in an intermittent or jerky manner.

A major breakthrough came in the early 1980s with the development of video-enhanced differential interference contrast microscopy (VEC-DIC) by Robert D. Allen. This light microscopic technique permitted the detection of small diffraction-limited vesicles and microtubules, which were previously undetectable by conventional light microscopy, and revealed movement in axons on a scale not previously appreciated (Video **1**). More recently, the advent of fluorescence microscopy has made it possible to observe the movement of specific cargo structures or molecules by fluorescent labeling. Fluorescence microscopy has permitted the characterization of distinct populations of membranous organelles based on their molecular composition, as well as the detection of nonmembranous cargoes that are too small to observe by transmitted light microscopy, such as cytoskeletal polymers, ribonucleoprotein particles, and cytosolic proteins.

The use of fluorescence microscopy for the study of axonal transport was revolutionized in the 1990s with the discovery of green fluorescent protein and the development of genetically engineered variants of green fluorescent protein. Today, the study of axonal transport is dominated by fluorescence microscopy of fluorescent fusion proteins in embryos or cultured nerve cells. The most common approach is to express fluorescent proteins in neurons, either by genetic manipulation of the organism or by transient transfection of cultured cells, and then to observe the fluorescently labeled cargo structures by time-lapse imaging (Video **2**). In the past few years, advances in deep imaging technologies such as two photon confocal microscopy have made it possible to image some axonally transported cargoes, such as mitochondria, in surgically exposed spinal cord in vivo as well as in peripheral nerve ex vivo preparations, and it is likely that the next decade will see exciting new developments in this area.

Axonal Cytoplasm Contains a Dynamic Network of Protein Polymers Called the Cytoskeleton

The cytoplasm of all eukaryotic cells is organized by a complex dynamic network of microscopic protein polymers known as the cytoskeleton. The organization and interactions of these polymer systems, which are coordinated by dozens if not hundreds of regulatory and interacting proteins, are critical for all aspects of cell shape and movement, including intracellular movement. These interacting proteins function to regulate the assembly and disassembly of the polymers as well as their interactions with each other and with other subcellular components.

In axons, the cytoskeleton is comprised of microtubules and microfilaments, which are found in all eukaryotic cells, and neurofilaments, which are the intermediate filaments of nerve cells (Box 1 and Fig. 5). Microtubules and neurofilaments are very long polymers which are aligned in a parallel overlapping array along the entire length of the axon (Fig. 6). Remarkably, serial sectioning studies in axons have yielded estimates of average microtubule and neurofilament lengths in vivo in excess of 350 and 100 μm, respectively. Microfilaments, in contrast, are much shorter and

may be orientated radially as well as axially within the axon. Microfilaments are particularly abundant beneath the axonal plasma membrane in a specialized zone called the submembrane cytoskeleton and also in the initial segment of myelinated axons. Microfilaments are also present in the vicinity of microtubules within axons, and they are enriched in growth cones and nerve terminals.

Box 1 A Primer on the Three Polymers of the Axonal Cytoskeleton

Microtubules are relatively rigid cylindrical polymers assembled from peanut-shaped heterodimers of alpha and beta tubulin (Fig. 5). The diameter of the microtubule is about 25 nm and its wall is composed of 13 protofilaments, each consisting of a head-to-tail string of α/β-tubulin heterodimers. The uniform orientation of the tubulin dimers gives rise to a structural polarity with distinct ends termed "plus" and "minus" that differ in their kinetics of assembly. The β-tubulin ends of the dimers are exposed at the "plus" end and the α-tubulin ends are exposed at the "minus" end. This structure is templated by gamma tubulin ring complexes, which are nucleating structures located primarily in the centrosome (the microtubule organizing center of the nerve cell). The centrosome is thought to be the sole site of formation of new microtubules in axons, which means that all axonal microtubules originate in the cell body and are transported out into the axons by the mechanisms of axonal transport.

Microfilaments are relatively flexible, two-stranded, filamentous polymers of actin proteins, principally beta and gamma actin in neurons (Fig. 5). Each strand is formed by the head-to-tail association of actin monomers, and the two strands twisted around each other to form a filament with a diameter of about 5–7 nm. Similar to microtubules, the uniform orientation of the actin monomers gives rise to a structural polarity with distinct ends termed "plus" and "minus" that differ in their kinetics of assembly. The subunit organization is templated by the Arp2/3 complex, which is the nucleating structure for microfilaments in cells. Arp2/3 complexes are present in axons and are abundant in the submembrane cytoskeleton, particularly in axon terminals and growth cones.

Neurofilaments, which are intermediate in size between microtubules and microfilaments, are flexible rope-like polymers with very high tensile strength. These polymers measure about 10 nm in diameter and are comprised of multiple neuronal intermediate filament proteins that coassemble with each other in varying stoichiometries. In mammals, these proteins are the neurofilament triplet proteins L, M, and H (low, medium, and high molecular weight, respectively), which are distinct gene products, in addition to internexin and, in peripheral neurons, peripherin. Some of these proteins are capable of forming homopolymers in vitro, but in vivo it appears that these proteins prefer to coassemble to form heteropolymers of two or more of these

(*continued*)

Box 1 (continued)
proteins. How these proteins are organized within the filament is not well understood. The precise composition of neurofilaments varies both temporally during development and spatially among different neuronal cell types.

In contrast to microtubules and microfilaments, the neurofilament protein subunits are elongated rather than globular in shape, comprised of alpha-helical coiled-coil rod-like domains that associate laterally and end-to-end in a staggered overlapping manner to form the backbone of the filament. By analogy with intermediate filament polymers in other cell types, neurofilaments are probably assembled from tetrameric subunits with approximately 32 polypeptides per filament cross section. Since the polypeptides in the tetrameric subunits have an antiparallel arrangement, the filaments have no structural polarity.

In electron micrographs, axoplasm is seen to be remarkably crowded with closely spaced cytoskeletal polymers and membranous organelles embedded in a dense granular matrix of cytosolic proteins (Fig. 7a). Extraction of soluble cytosolic proteins reveals extensive interconnections between the neurofilaments and microtubules, giving the impression of a rigidly cross-linked network (Fig. 7b). However, it is hard to reconcile this impression with the speed and volume of cargo traffic in axons, which suggest a much more fluid and dynamic environment. For this reason, it seems most likely that many of the interconnections that are observed between cytoskeletal polymers in electron micrographs of axons are weak or transient and that the axonal cytoskeleton may be more accurately described as a polymer solution rather than as a cross-linked network (Fig. 8).

Microfilaments and Microtubules Are Tracks for Axonal Transport

One of the breakthroughs made possible by direct imaging of axonal transport has been the discovery that microtubules and microfilaments serve as the tracks along which all cargoes move, and this is a fundamental feature of intracellular traffic in all eukaryotic cells. In addition, an important general principle of axonal microtubule organization, at least in vertebrate axons, is that all axonal microtubules are orientated with their minus ends pointing proximally, toward the cell body, and their plus ends pointing distally, toward the axon tip. This uniform polarity orientation has important implications for the directionality of axonal transport, which will be discussed below. The organization of axonal microfilaments is not known, but in contrast to microtubules it is unlikely that these short polymers have a uniform polarity orientation throughout the axon.

Because of their length and organization, it is generally assumed that microtubules are the tracks for long-range axial movements in axons, whereas microfilaments are the tracks for short-range movements, including lateral movements in the axon as well as movements in domains of axoplasm that lack microtubules such as close to the plasma membrane. However, it is important to note that even though

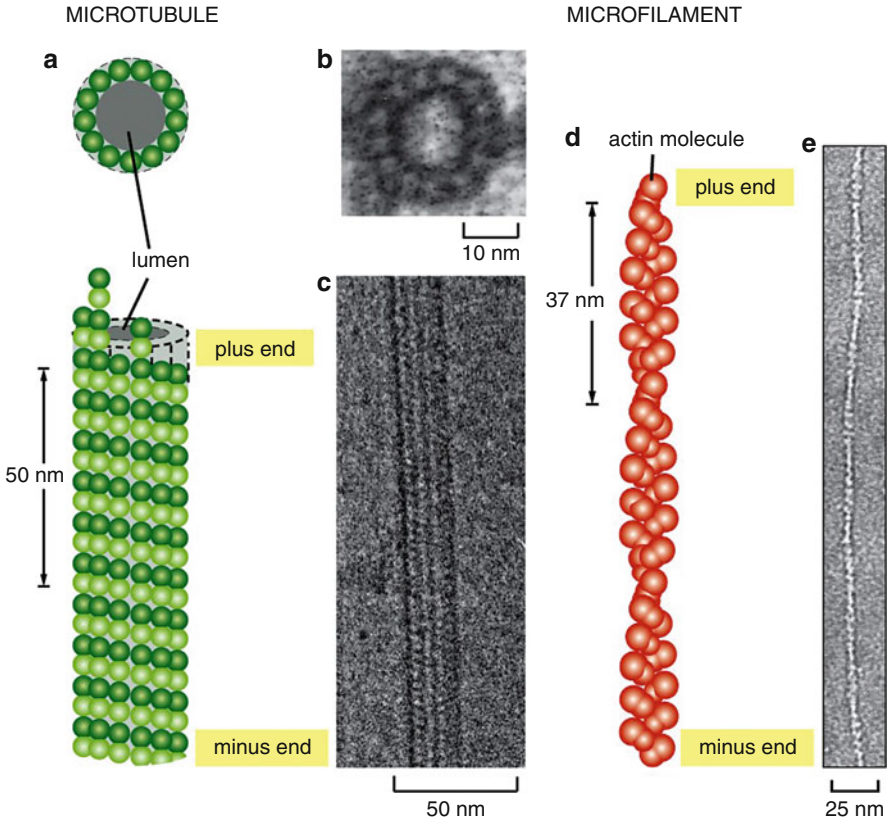

Fig. 5 *The structure of microtubules and microfilaments*. (**a**) Microtubules are hollow cylindrical polymers composed of 13 protofilaments, each formed by the head-to-tail association of α/β-tubulin heterodimers. α-tubulin is shown in *light green* and β-tubulin in *dark green*. All the subunits are in the same orientation, giving rise to a structural polarity (β-tubulin is exposed at the plus ends and α-tubulin at the minus ends). The stagger between adjacent protofilaments results in a helical pitch. (**b**) Electron micrograph of a microtubule in cross section. (**c**) Electron micrograph of a microtubule viewed from the side (negative staining). (**d**) Microfilaments are formed from monomers of actin, which associate head-to-tail to form two helically entwined strands. All the subunits are in the same orientation, giving rise to a structural polarity. (**e**) Electron micrograph of a microfilament (negative staining) (Adapted from Alberts et al. 2008 Reproduced by permission of Garland Science/Taylor & Francis Books, LLC. Micrograph images courtesy Richard Wade, Richard Linck, and Roger Craig)

microtubules can be very long, they do not extend for the entire length of the axon. Thus, the overlap of microtubules along axons is critical to establishing an uninterrupted highway from cell body to axon tip; gaps in this overlapping array obviously cannot occur because axonal transport is a lifeline for axons. Any interruption in the continuity of the overlapping microtubule array in axons would have profound and devastating consequences for the nerve cell.

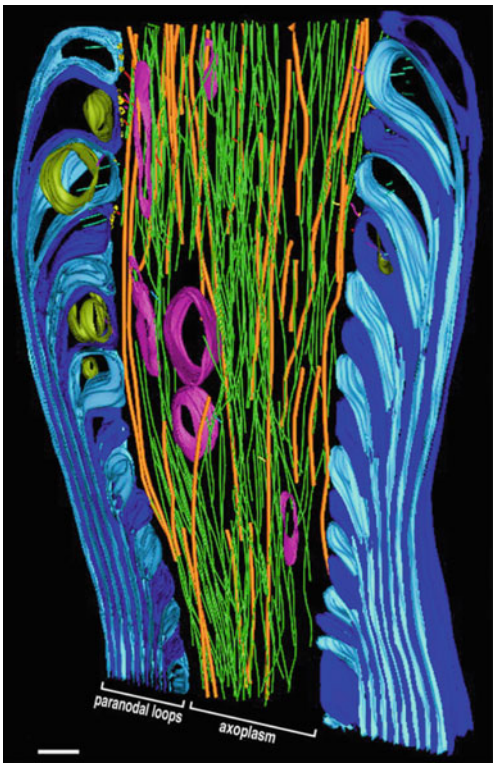

Fig. 6 *The long polymers of the axonal cytoskeleton*. (**a**) Electron tomographic reconstruction of microtubule and neurofilament polymers in the paranodal region of a myelinated axon adjacent to a node of Ranvier. In this short segment of axon there are 32 microtubules (*thicker orange threads*), 158 neurofilaments (*thinner green threads*), and eight mitochondria and other membranous organelles (*magenta*; only outer membrane shown). The paranodal loops of the myelin (alternating *blue* and *cyan*) contain tubules of endoplasmic reticulum called tubular cisternae (*dark green*). The adaxonal surfaces of the paranodal loops are linked to the axonal membrane (not shown here) by transcellular junctional complexes called paranodal junctional bridges (*yellow*), some of which are connected to neurofilaments on the axonal side by short fibrous linkers (*magenta*). Other paranodal and cytoplasmic cross-linkers are shown in *red* and *yellow*. Some microfilaments (*cyan*) are evident in the paranodal loops, but it would appear that these polymers are not well preserved by the methods used here because few are evident in the axoplasm in this tomogram. Scale bar = 200 nm (Reproduced from Perkins et al. 2008)

Neurofilaments Are Space-Filling Structural Elements That Maximize Axonal Caliber

In contrast to microtubules and microfilaments, neurofilaments do not serve as tracks for the movement of axonally transported cargoes. However, neurofilaments are the most abundant structures in large myelinated axons, where they can occupy most of the axonal cross-sectional area (Fig. 9a). Considerable evidence now indicates that these polymers have an important function as space-filling structures that maximize

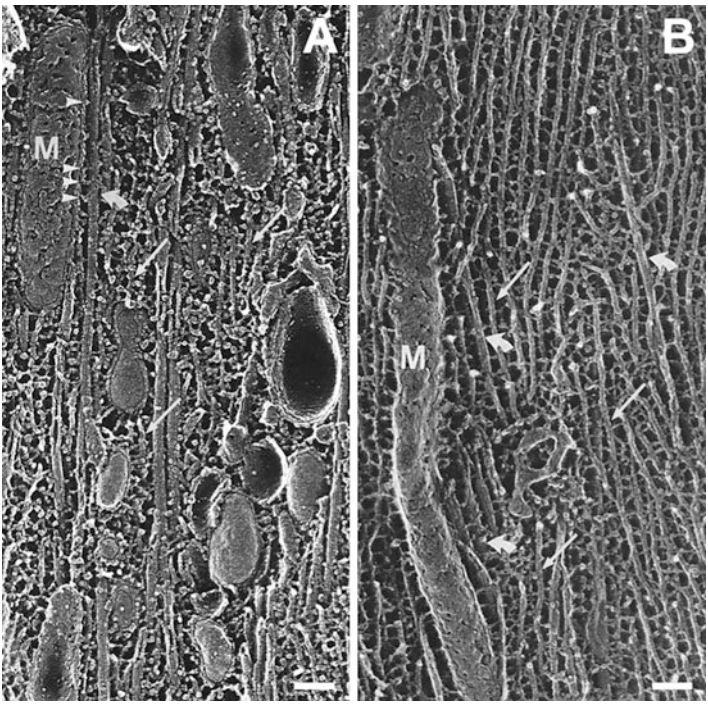

Fig. 7 *Rapid-freeze deep-etch electron microscopy of the axonal cytoskeleton*. Electron micrographs of the axoplasm of a frog axon in longitudinal section prepared using the rapid-freeze deep-etch platinum replica technique. (**a**) An intact axon that was frozen without prior permeabilization. Numerous membranous organelles including one mitochondrion (*M*) are interspersed among the microtubules (*thick arrows*) and neurofilaments (*thin arrows*), embedded in a dense granular matrix of cytosolic proteins. Note the cross-bridges connecting the membranous organelles to the microtubules (*arrowheads* on the mitochondrion). Some of these cross-bridges may represent molecular motor proteins, which are discussed below. (**b**) An axon that was permeabilized with saponin prior to freezing in order to extract the granular matrix of cytosolic proteins. This extraction procedure reveals the microtubules (*thick arrows*) and neurofilaments (*thin arrows*) more clearly. Note that the neurofilaments are spaced apart from each other by lateral projections called sidearms. A long mitochondrion (*M*) is visible but other membranous organelles are lost during the saponin treatment. Scale bars = 0.1 μm (Reproduced with some digital editing from Hirokawa 1982) Originally published in J Cell Biol 94:129–142)

the cross-sectional area of axons. This is important because the cross-sectional area of axons is an important determinant of the conduction velocity: larger axons can propagate action potentials more rapidly because the internal resistance to diffusion of ions is lower.

Electron microscopy of neurofilaments reveals that they are unique among intermediate filaments in that they possess lateral projections called sidearms (Fig. 9b, 9c), which are composed of the carboxy-terminal domains of the neurofilament proteins, particularly neurofilament proteins M and H. These sidearms appear to link adjacent filaments in electron micrographs, but the evidence suggests

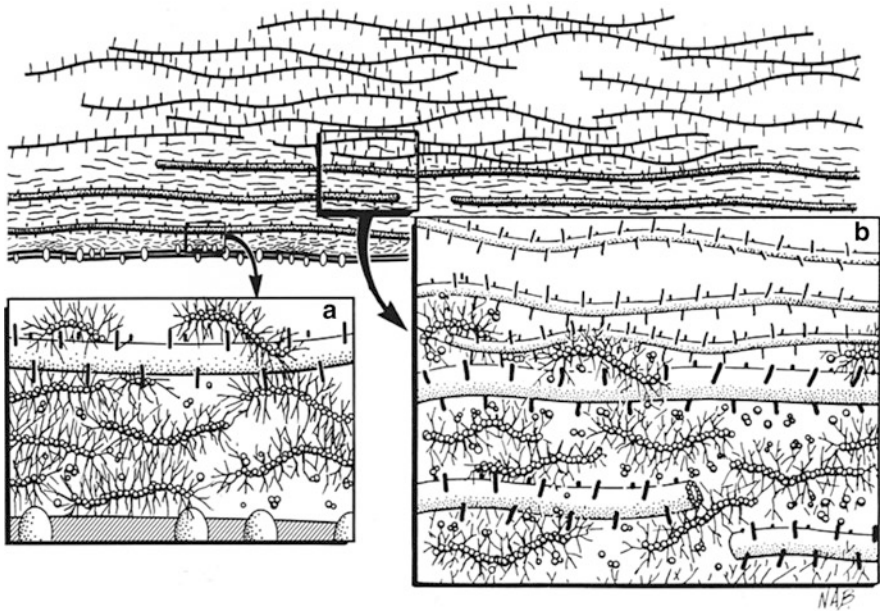

Fig. 8 *Cartoon diagram of the organization of the axonal cytoskeleton*. An artist's rendition of the organization of microtubules, neurofilaments, and microfilaments in an axon. Electron micrographs give the impression of a rigidly cross-linked network, but the extent of axonal transport in axons suggests that many of these interconnections are weak or transient and that the axonal cytoskeleton is better described as a polymer solution. (**a**) A view of the submembrane cytoskeleton, showing a single microtubule and multiple short microfilaments, some of which interact with integral membrane proteins in the plasma membrane. (**b**) A view of the cytoskeleton away from the plasma membrane, showing neurofilaments and microtubules aligned longitudinally, with microfilaments enriched in the vicinity of the microtubules (Reproduced from Lasek 1986)

that they actually function more as spacers than linkers and that their principal function is to keep adjacent neurofilaments at arm's length, thereby maximizing the space-filling properties of these polymers without creating a rigidly cross-linked network that would retard the movement of axonally transported cargoes. A striking illustration of the space-filling role of neurofilaments can be seen in mutant animals that lack neurofilaments; the axons in these animals fail to attain their normal caliber and have delayed conduction velocities.

Molecules Move in Association with Distinct Cargo Structures

A fundamental principle of axonal transport, first articulated by Raymond Lasek and colleagues in the 1980s, is that all transported molecules move in association with distinct cytologically identifiable structures. This hypothesis, originally termed the structural hypothesis, is now self-evident: of the hundreds of macromolecules conveyed by axonal transport, each moves in association with a distinct cargo

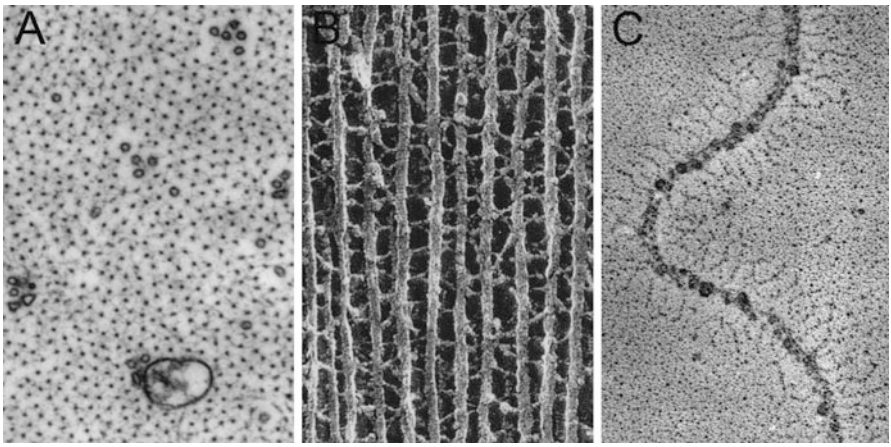

Fig. 9 *Three views of neurofilaments*. (**a**) Electron micrograph of a cross section through a large myelinated axon. In the cross section, the neurofilaments (*NF*) appear as *solid black dots* and the microtubules (*MT*) appear as hollow circular structures. Both the neurofilaments and microtubules are aligned in parallel to the long axis of the axon. The neurofilament sidearms appears as wispy spoke-like projections between the neurofilaments. (**b**) Electron micrograph of a longitudinal section of a myelinated axon prepared by the rapid-freeze deep-etch platinum replica technique. The axon was permeabilized with detergent prior to fixation, as described in Fig. 7b, in order to extract cytosolic proteins and expose the neurofilament sidearms, which project between adjacent neurofilaments like the rungs of a ladder (The image in **b** is reproduced from Hirokawa 1982 ©1982 Rockefeller University Press. Originally published in J Cell Biol 94:129–142). (**c**) Electron micrograph of an isolated neurofilament prepared by rotary shadowing. The sidearm projections give the neurofilament the appearance of a lamp brush or pipe cleaner (The image in **c** is reproduced from Hisanaga and Hirokawa 1988)

structure. For example, proteins that move in association with a membranous organelle may be integral membrane proteins embedded within the lipid bilayer, peripheral membrane proteins associated with the membrane surface, or soluble proteins contained within the luminal compartment. Each of these proteins is conveyed by axonal transport due to its association with moving organelles, much as passengers are conveyed by association with moving vehicles. The average rate of movement of each organelle (vehicle) is determined by its velocity and frequency of movement, but the average rate of movement of its molecular constituents (passengers) will depend on the proportion of the time that they spend in association with that organelle. Thus, two peripheral membrane proteins that associate with the same moving organelle could actually move at different average rates if their affinities for the moving organelle were different.

When researchers use fluorescence microscopy to observe axonal transport they typically label one protein, but it is important to remember that each protein moves in association with a cargo structure that may comprise dozens or even hundreds of different proteins. For example, Video **3** shows the movement of a synaptic vesicle precursor labeled with GFP-tagged synaptobrevin and Fig. 10 shows the protein composition of a synaptic vesicle. It can be seen that synaptobrevin is just one of

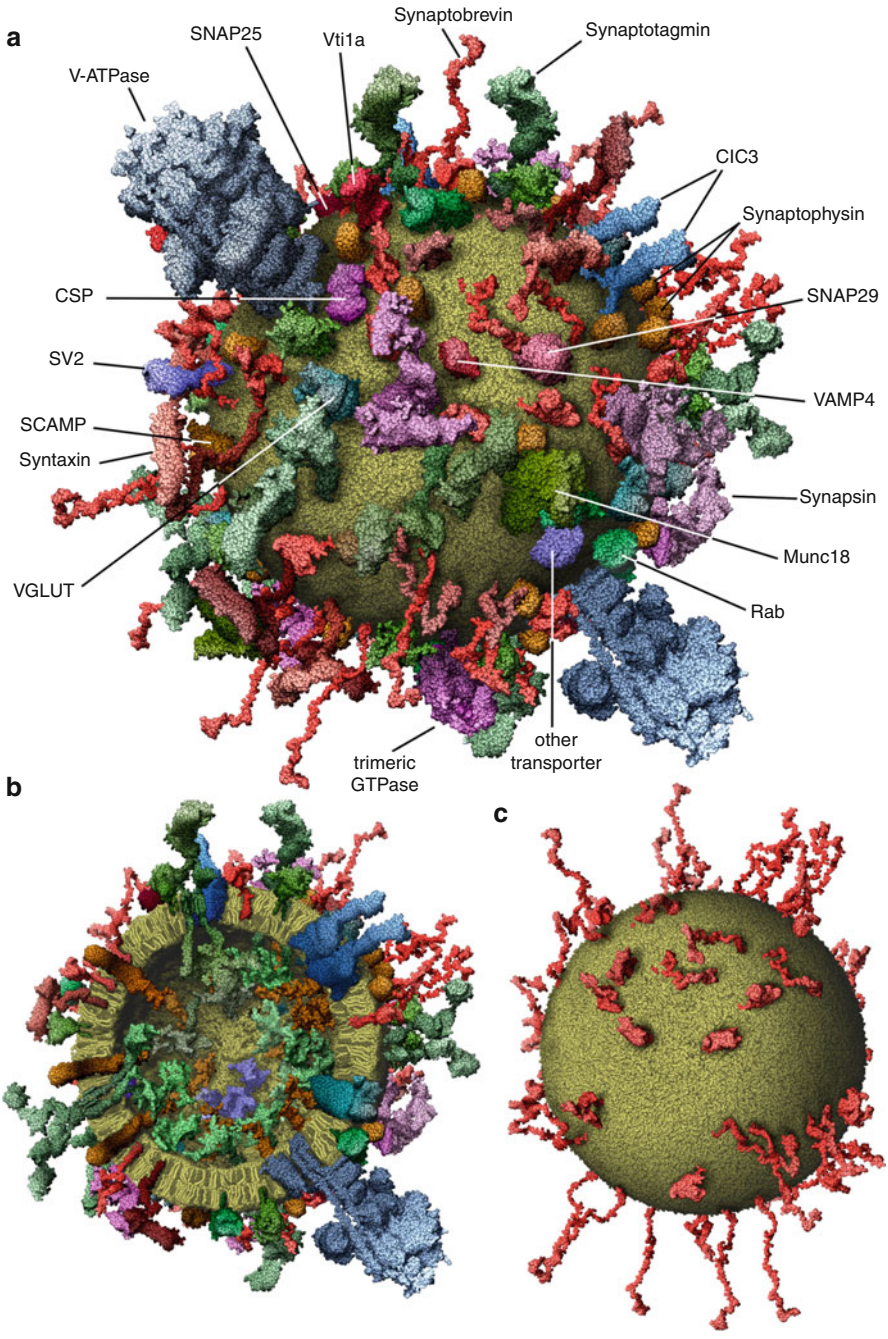

Fig. 10 *Molecular model of a synaptic vesicle*. This model was created based on proteomic and quantitative biochemical characterization of synaptic vesicles together with space-filling models of

many different kinds of macromolecules that comprise synaptic vesicles and their precursors. The full diversity of cargo structures in axons is not known, let alone their molecular composition. For example, there are multiple classes of Golgi-derived transport vesicles in axons such as the synaptic vesicle precursors shown in Video **3**, but their true molecular identity is not known (most often, these vesicles are identified by one or more "marker" proteins without knowing their complete molecular identity).

The Cargoes of Axonal Transport Are Very Diverse

Electron micrographs of axoplasm give a static impression of axonal cytoplasm, but these images belie an extremely fluid and dynamic state. In fact, it is probably no exaggeration to say that pretty much everything in axons moves, though many cargoes do not move continuously. For example, the membranous cargoes of axonal transport include Golgi-derived transport vesicles (of which there are probably many distinct types), as well as mitochondria, peroxisomes, lysosomes, signaling endosomes, and autophagosomes. There is also an extensive smooth endoplasmic reticulum in axons, though its transport is not well understood.

Beyond membranous cargo structures, it is clear that the cytoskeletal polymers and cytosolic protein complexes also move. This has been demonstrated most clearly for neurofilaments, which can be observed directly in cultured cells using fluorescent neurofilament fusion proteins (Video **4**). The neurofilament polymers move along microtubule tracks. The movement is fast but intermittent with each filament spending most of its time pausing between short bursts of rapid movement. There is also evidence for rapid intermittent movement of microtubules themselves in axons, but the tracks along which these polymers move is less clear.

An important question is how the movement of cytoskeletal polymers can be reconciled with their structural roles in axons. In the case of neurofilaments, it appears that the polymers spend the vast majority of their time pausing, so at any point in time only a small proportion of the polymers is actually being transported. The transport of microtubules and microfilaments is less clearly established, but it is possible that they move in a similar manner. It seems unlikely that microtubules or microfilaments could serve as tracks for the movement of other cargoes while they themselves are moving, but if they move infrequently then they could serve this function between their bouts of movement. In fact, it has been proposed that the very distribution and organization of neurofilaments, microtubules, and microfilaments in axons is generated and maintained by the axonal transport mechanisms that move these polymers.

Fig. 10 (continued) the macromolecules at near atomic resolution. (**a**) Outside view of a vesicle. Note the complexity and density of the proteins, which all move coordinately in axons due to their association with this cargo structure. (**b**) View of a vesicle in cross section (the *dark-colored* membrane components represent cholesterol). (**c**) Model containing only synaptobrevin to show the surface density of this abundant vesicle component (Reproduced from Takamori et al. 2006)

There Are Distinct Fast and Slow Components of Axonal Transport

In the early radioisotopic pulse-labeling experiments of the 1970s and 1980s, it was observed that the pulse of radiolabeled proteins synthesized in nerve cell bodies moves out along axons in several waves (see section "Pulse-Labeling Techniques" for a description of this technique). These waves were categorized as either fast or slow depending on their rate of propagation. Proteins in the fast component form sharp wave fronts with a broad trailing component. The wave fronts propagate at rates of hundreds of millimeters per day, which corresponds to micrometers per second, but the broad trailing component suggests that there is deposition of some of these cargoes along the axon during their transit down the axon. In contrast, proteins in the slow component form a roughly symmetrical bell-shaped wave that spreads as it propagates along the axon at rates on the order of millimeters per day, several orders of magnitude slower than fast axonal transport. The absence of a broad trailing component suggests that in contrast to the movement of membranous organelles, there is little "deposition" of these proteins during their transit (Fig. 11). The fast components consist of many proteins that are known to associate with membranous organelles, indicating that membranous organelles are the principal cargo structures, whereas the slow component consists of cytosolic proteins.

Detailed kinetic analyses of radioisotopic pulse-labeling studies have indicated that the slow component of axonal transport can actually be resolved into distinct subcomponents, called slow components "a" and "b" (Fig. 12a). Slow component "a" is slower (about 0.2–1 mm/day) and simpler in composition, being composed primarily of neurofilament proteins and tubulin. Other identified slow component "a" proteins include neuronal spectrin, tau protein, and calcium/calmodulin-dependent protein kinase IIβ. Slow component "b" is slightly faster (about 2–8 mm/day) and very complex in composition, consisting of hundreds of proteins that are generally described as "cytosolic" in nature, meaning that they are not membrane proteins and that they are not sequestered in membranous compartments or organelles. Among the proteins that have been identified in slow component "b" are cytoskeletal proteins such as actin, tubulin, cofilin, actin depolymerizing factor, profilin, and synapsin I; motor proteins such as dynein, dynactin, and myosin Va; metabolic enzymes such as aldolase, creatine phosphokinase, enolase, glyceraldehyde-3-phosphate dehydrogenase, phosphofructokinase, and superoxide dismutase 1; chaperone proteins such as heat shock protein hsp70, cytosolic chaperonin containing T-complex polypeptide 1 (CCT), and molecular chaperone hsc73; and numerous other cytosolic proteins including calmodulin, clathrin, clathrin uncoating protein hsc70, calcium/calmodulin-dependent protein kinase IIα, cyclophilin A, annexin VI, ubiquitin, and ubiquitin carboxyl-terminal hydrolase PGP 9.5.

The Cargo Structures of Slow Axonal Transport Are Largely Unknown

The sheer number and diversity of the proteins conveyed by slow axonal transport is truly remarkable, but equally remarkable is the fact that very little is known about the nature of the cargo structures. To date, the movement of neurofilament proteins, tubulin, and several cytosolic proteins have been observed in cultured nerve cells,

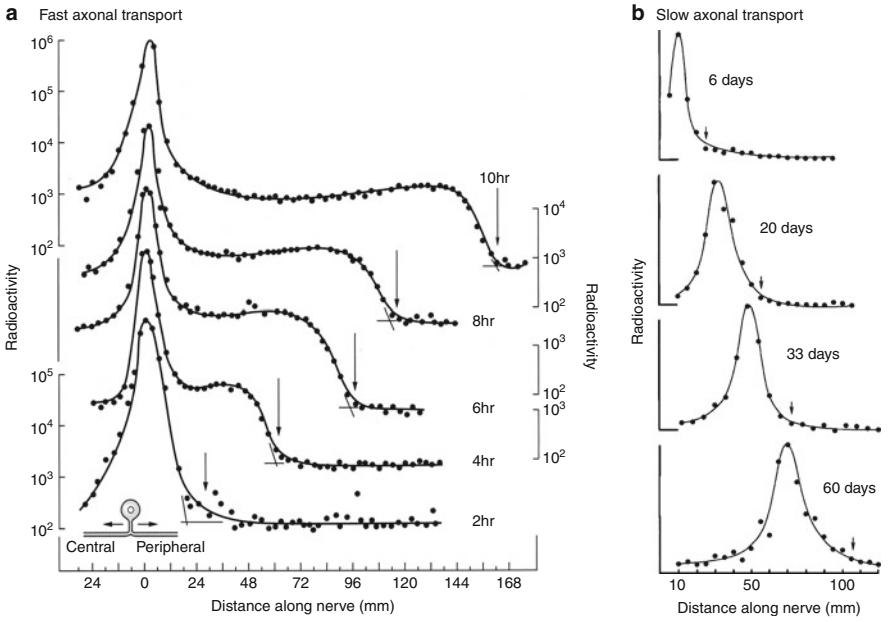

Fig. 11 *Typical radioisotopic pulse-labeling kinetics observed for fast and slow axonal transport*. Tritiated (tritium-labeled) amino acids were injected into the lumbar dorsal root ganglion or ventral horn of cats or rats and then the animals were sacrificed at intervals. The nerve roots and sciatic nerve were dissected out and cut into contiguous 3 or 5 mm segments, respectively, and the radioactivity in each segment was measured by scintillation counting. (**a**) Fast axonal transport kinetics in sensory axons of the cat sciatic nerve. The animals were sacrificed at 2-h intervals over a period of 10 h. The wave front advances at about 400 mm/day, which corresponds to about 4.5 μm/s. Note the broad plateau of radioactivity trailing behind the wave front, which suggests "deposition" of many axonally transported cargoes along the axon (Reproduced from Ochs 1981). (**b**) Slow axonal transport kinetics of a neurofilament protein in motor axons of the rat sciatic nerve. The animals were sacrificed at intervals over a period of 60 days. Note the fairly symmetrical wave of radioactivity, which advances at an average rate of about 1 mm/day, 400 times slower than the wave front of fast axonal transport. (Reproduced from Hoffman et al. 1985)

but these are just several of the many hundreds of proteins that are conveyed by slow axonal transport. It is clear that neurofilament proteins move in the form of assembled polymers and thus neurofilament polymers are one of the cargo structures of slow axonal transport. There is also evidence that tubulin moves in the form of microtubule polymers, although direct imaging of moving microtubules in axons has been more challenging.

The fact that the proteins in slow components "a" and "b" move together for days, weeks, or months as they travel down the axon suggests that they may move in the form of macromolecular complexes that either bind directly to motor proteins or indirectly via interactions with other moving structures. The future identification and characterization of these protein complexes may provide fundamental insights into the supramolecular interactions that organize the cytosolic compartment of

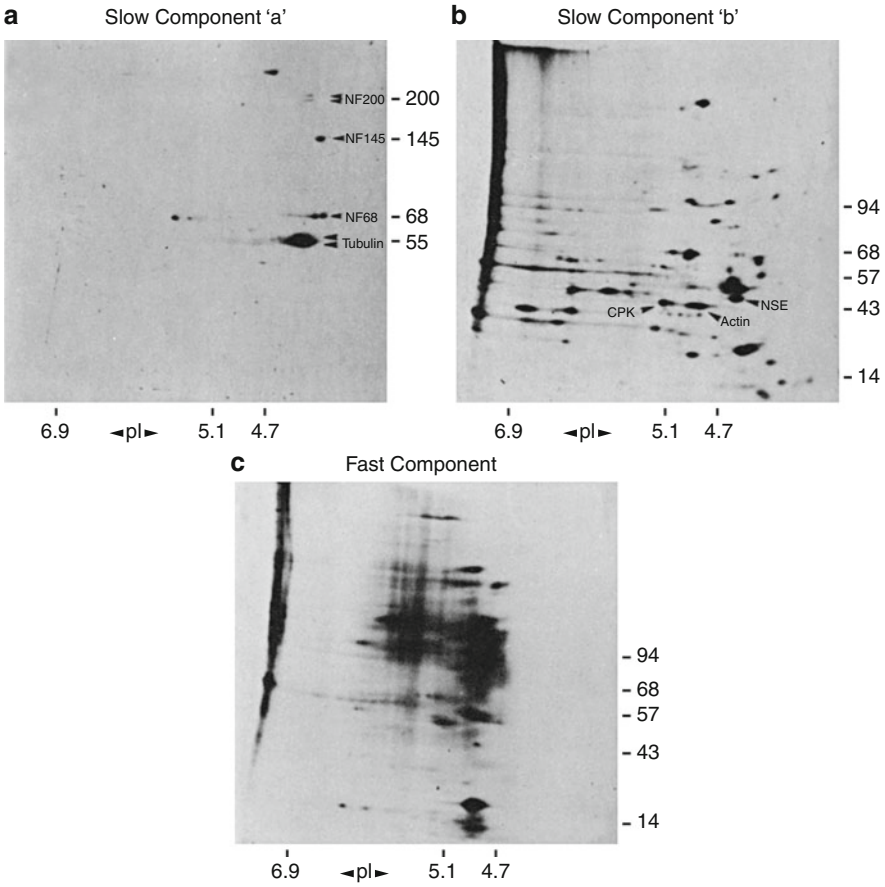

Fig. 12 *Two-dimensional electrophoresis of radiolabeled proteins in the fast and slow components of axonal transport*. Autoradiographs of radiolabeled proteins in mouse or guinea pig optic nerve separated by two-dimensional gel electrophoresis. The proteins were separated first according to isoelectric point in the horizontal dimension using isoelectric focusing and according to size in the vertical dimension using polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE). For each gel, isoelectric points (*pI*) are indicated on the *bottom* and molecular weights are indicated on the *right*. (**a**) Slow component "a" (SCa), which includes neurofilament triplet proteins (NF68, NF145, and NF 200; also known as NFL, M, and H), tubulin (α and β subunits not resolved), and some other proteins. (**b**) Slow component "b" (SCb), which includes actin, creatine phosphokinase (*CPK*), neuron-specific enolase (*NSE*), and many other proteins. (**c**) Fast component, which includes a complex subset of axonally transported proteins that includes many membrane proteins and is largely distinct from the slow components (Adapted from Brady and Lasek 1982)

cytoplasm, not just in axons but in all eukaryotic cells. One hypothesis is that cytoskeletal polymers may be carrier structures of slow axonal transport and that cytosolic proteins are transported by riding piggyback on the moving polymers. For example, many of the proteins in slow component "a" could move by virtue of their

association with neurofilaments. According to this hypothesis, the transport rate of each protein would be determined not only by the velocity and frequency of movement of the neurofilament "carriers" but also by the proportion of the time that the cargo proteins spent in association with those carriers. Presently, however, these ideas are all speculative.

The Cargoes of Fast and Slow Axonal Transport All Move Rapidly but Differ in Their Duty Ratio

For many years, the slow rate of slow axonal transport was vexing to cell biologists. How could dozens of diverse proteins move coordinately along axons at rates of just millimeters per day, which corresponds to just tens of nanometers per second? A resolution to this puzzle was provided in 2000 by the first direct observation of neurofilament transport in axons. Unexpectedly, the filaments were found to move at fast rates, comparable to the rate of membranous organelles, but the movements were also very infrequent. This led to the hypothesis that the slow rate of slow axonal transport is generated by short bouts of rapid movement interrupted by long pauses. Mathematical modeling of radioisotopic pulse-labeling experiments has indicated that such "stop and go" movements can explain the kinetics of slow axonal transport in vivo.

It is now clear that fast and slow axonal transport differ not in the actual rate of movement per se but rather in the manner in which the movements are regulated (Fig. 13). This can be expressed in terms of the duty ratio, which is the proportion of the time that the cargo structures spend moving (Table 1). Membranous organelles on the secretory and endocytic pathways, which function primarily to deliver membrane and protein components to sites along the axon and at the axon tip, move rapidly and continuously in a unidirectional manner, pausing for only brief periods of time. The high duty ratio of these organelles ensures that they are delivered rapidly to their destination. In contrast, cytoskeletal polymers, mitochondria, and possibly also endoplasmic reticulum have a low duty ratio. These cargoes move in an intermittent and bidirectional manner, pausing more often and for longer periods of time and sometimes reversing during their journey along the axon. Though these structures are referred to as cargoes, they are not simply the luggage of intracellular transport; these organelles and macromolecular assemblies are preassembled functional units that fulfill their architectural, physiological, and metabolic roles in the axon during their transit. For these cargoes, the journey is perhaps more important than the ultimate destination, and this may explain their unique motile behavior.

Axonal Transport Is Bidirectional

Since radioisotopic pulse-labeling selectively labels proteins that are synthesized at the site of injection, in the vicinity of nerve cell bodies, studies using this technique are inherently biased toward the detection of anterograde movement. However, if a

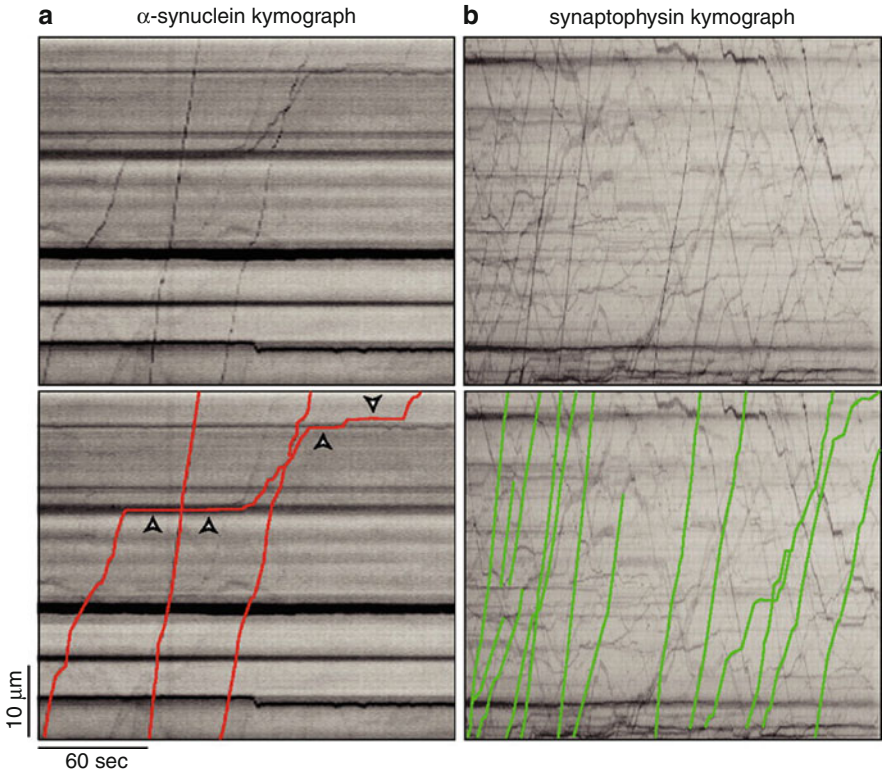

Fig. 13 *Comparison of the movement of proteins in fast and slow axonal transport*. This figure illustrates a kymograph analysis, which is a popular way to represent the movement of fluorescently labeled cargoes in axons. To construct a kymograph, a time-lapse video is acquired of the moving structures and then a line is drawn to obtain an axial linear intensity profile along the axon for each video frame. When the linear intensity profiles are arranged vertically and stacked side by side a two-dimensional image is obtained in which the horizontal axis represents elapsed time and the vertical axis represents distance. Note that the contrast of these kymographs has been inverted so that the fluorescent cargoes appear dark against a light background. Moving cargos trace diagonal trajectories across the kymograph and the slope of these lines yields the velocity. Stationary cargoes generate *horizontal lines*. (**a**) Kymograph showing the movement of α-synuclein, a cytosolic protein that is transported in slow component "b" of axonal transport, fused to red fluorescent protein (mRFP::-α-SYN). (**b**) Kymograph showing the movement of synaptophysin, an integral membrane protein that is transported in the fast component of axonal transport, fused to green fluorescent protein (SYP::GFP). In the lower kymographs, anterogradely moving particles are overlaid with *red* or *green lines* representing movement of α-synuclein and synaptophysin, respectively. Note that the α-synuclein moves in a more intermittent manner with frequent pauses (*arrowheads*), in contrast to the more persistent movements of synaptophysin. Synaptophysin is a component of presynaptic vesicles (see Fig. 10), but the cargo structure for α-synuclein is not known (Reproduced from Roy et al. 2007)

ligature or cold block is applied to axons to block axonal transport, cargoes are observed to accumulate on both sides of the site. This indicates that axonal transport is bidirectional with cargoes moving both forward (anterograde) and backward (retrograde) along the axonal highway.

Table 1 *The cargoes of fast and slow axonal transport all move rapidly, but differ in their duty ratio*. Each rate component of axonal transport corresponds to a distinct group of cargo structures that move either continuously or intermittently. The overall speed is the average speed determined on a time scale of hours, days, or weeks by radioisotopic pulse labeling, whereas the instantaneous speed is the actual speed of movement of the cargo structures (between pauses) determined on a time scale of seconds by direct imaging techniques such as fluorescence or differential interference contrast light microscopy. Note that these rates are approximate and that there is considerable variation between different cell types and different stages of development and maturation. The duty ratio is the proportion of the time that the structures spend moving, inferred by comparison of the overall and instantaneous rates. Other axonally transported cargos, such as endoplasmic reticulum, mRNAs, and ribonucleoprotein particles, are not included in this table because there is insufficient information about their overall rate of movement at this time (Adapted from Brown 2003)

Cargo structure	Overall speed	Instantaneous speed	Directionality	Duty ratio
Golgi-derived vesicles	Fast	0.5–5 μm/s	Anterograde	High
	50–400 mm/day			
	0.5–5 μm/s			
Endocytic vesicles, lysosomes, autophagosomes	Fast	0.5–3 μm/s	Retrograde	High
	50–250 mm/day			
	0.5–3 μm/s			
Mitochondria	Intermediate	0.3–0.7 μm/s	Bidirectional	Intermediate
	<70 mm/day			
	<0.8 μm/s			
Neurofilaments, microtubules, cytosolic protein complexes	Slow	0.2–3 μm/s	Bidirectional	Low
	0.1–10 mm/day			
	0.001–0.1 μm/s			

Ultrastructural studies of axons proximal and distal to a nerve ligation have revealed that the cargoes of anterograde and retrograde transport are structurally distinct (Fig. 14). For example, anterogradely moving membranous organelles, which rapidly accumulate proximal to the blockade, are predominantly small tubulovesicular organelles typical of the Golgi-derived transport vesicles found on the secretory pathway whereas retrogradely moving membranous organelles, which accumulate distal to the blockade, tend to be larger multilamellar and multivesicular organelles typical of the endocytic, lysosomal, and autophagosomal pathways. Thus, the axonal transport of Golgi-derived and endocytic membranous organelles is basically an exaggeration of the normal membrane cycling pathways that are found in all eukaryotic cells, with vesicles budded from the *trans* Golgi network moving outward toward the plasma membrane (axon and nerve terminals) on the secretory pathway, and with organelles formed peripherally moving back toward the cell center (Fig. 15).

While many cargoes in axons have a single preferred direction of movement, direct imaging studies in cultured neurons and ex vivo preparations have

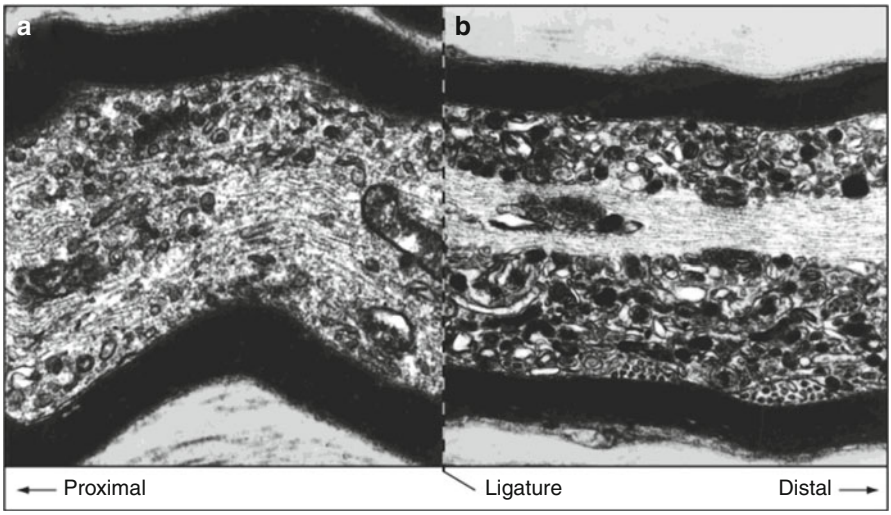

Fig. 14 *Accumulation of membranous organelles proximal and distal to a nerve ligation*. The saphenous nerve was ligated in a sedated mouse. Six to ten hours later, the animal was sacrificed and the nerve was fixed and processed for electron microscopy. (**a**) Anterogradely transported cargoes including tubulovesicular organelles and mitochondria accumulate proximal to the ligation and are depleted from the distal side. (**b**) Retrogradely transported cargoes including lysosomes and multivesicular bodies accumulate distal to the ligation and are depleted from the proximal side. Note that the anterogradely and retrogradely moving organelles are morphologically distinct (Reproduced from Hirokawa et al. 1990 ©1990 Rockefeller University Press. Originally published in J Cell Biol 111:1027–1037)

demonstrated that some cargoes exhibit persistent movement in both directions. For example, while the net direction of neurofilament and mitochondria transport in axons is normally anterograde, a significant proportion of these cargoes also move retrogradely (Videos **5**, **6**, and **7**). It is reasonable to ask why the neuron would go to the trouble of moving the same cargoes both forward and backward in axons. In the case of mitochondria, the balance of anterograde and retrograde movements and pauses is regulated during axon growth in order to recruit these organelles to sites of metabolic demand. Likewise, in the case of neurofilaments, the balance of anterograde and retrograde movements and pauses is likely to be the principal determinant of their steady state distribution along the axon, and thus the regulation of the axonal transport of these structures is probably essential for local and long-range remodeling of the neuronal cytoskeleton during axon growth and maturation. Thus, the purpose of axonal transport is not always to get a cargo from one end of the axon to the other; for those cargoes that have functions within the axon during their transit, axonal transport mechanisms function to dynamically recruit and redistribute these cargoes in response to the changing physiological and metabolic needs of the axon.

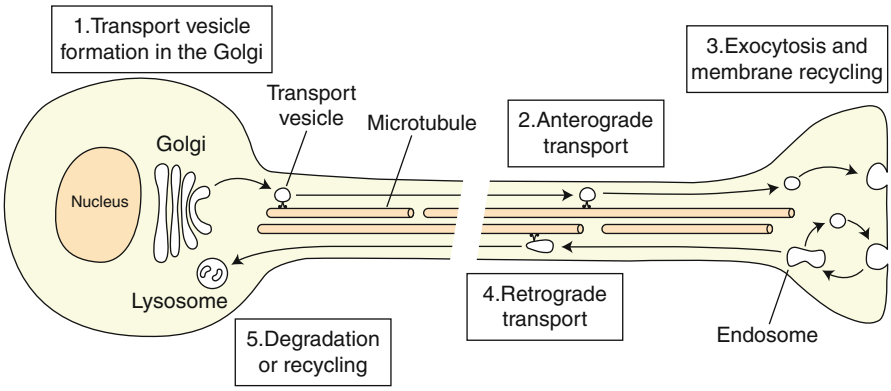

Fig. 15 *The pathways of axonal vesicular traffic on the secretory and endocytic pathways*. Many of the axonally transported vesicles and organelles in axons are intermediaries on the secretory and endocytic pathways, which are common to all eukaryotic cells. Secretory vesicles that are sorted and packaged in the trans-Golgi network are transported anterogradely to sites along the axon and at the axon tip where they fuse with the plasma membrane by exocytosis to release their luminal contents into the extracellular space and to insert their membrane components into the plasma membrane. At the same time, membrane retrieval by endocytosis gives rise to endosomal organelles which are transported retrogradely back towards the cell body where they may deliver their proteins to lysosomes for degradation or back to the Golgi for recycling. Based on Fig. 5.7 in Principles of Neural Science, 4th ed., by Kandel et al. McGraw-Hill, 2000

All Cargoes Are Propelled by Molecular Motors

The movement of cargoes inside cells is generated by molecular motor proteins: kinesins, dyneins, or myosins that move along cytoskeletal polymer tracks (see section "Microfilaments and Microtubules are Tracks for Axonal Transport"). These motors differ in the tracks with which they engage: dynein and kinesin motors move along microtubules, whereas myosins move along microfilaments (actin filaments). While there is considerable complexity to the structure and functions of these motors, the focus here will be on their commonalities. All three types of motor form multimeric protein complexes consisting of larger polypeptides (heavy chains) and smaller polypeptides (light chains and also, in the case of dynein, intermediate chains and light intermediate chains), which are distinct gene products with multiple isoforms. The heavy chains contain a globular head domain, a flexible neck linker, an alpha-helical coiled-coil stalk domain, and a globular tail domain (Fig. 16). The head domains (also known as the motor domains) interact with the polymer tracks, and the tail domains (or, less frequently, the stalk domains) interact with the cargo. The light chains interact with the tail or neck domains of the motor and function in cargo binding or regulation of motor activity.

All three types of motors are ATPases, which couple the binding, hydrolysis, and release of ATP within their the motor domains to a cycle of allosteric conformational changes that results in motion along the wall of the microtubule or microfilament. This cycle is often referred to as the cross-bridge cycle because the motors link

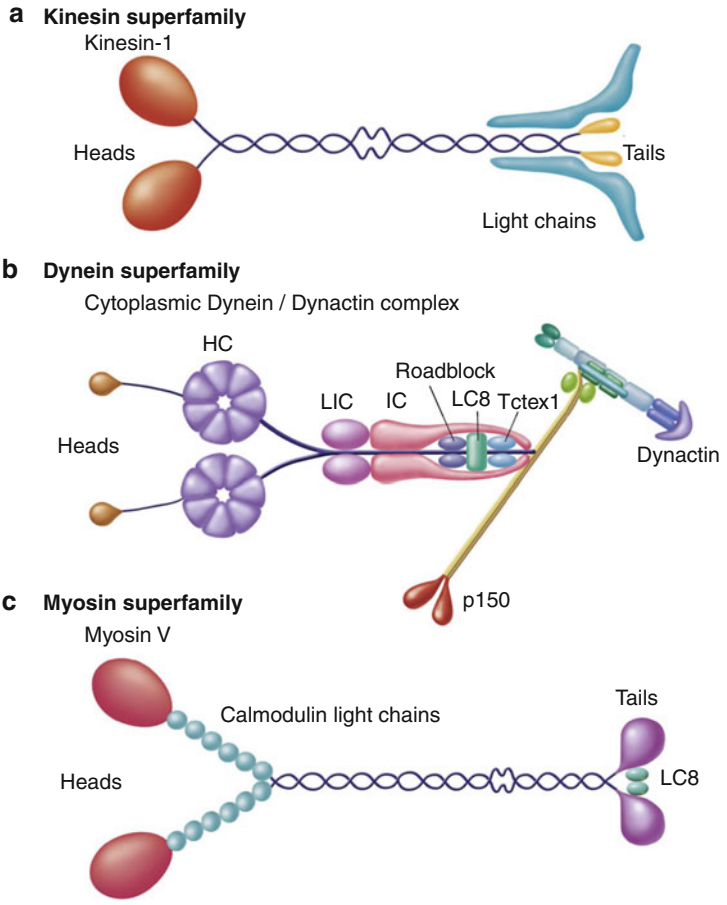

Fig. 16 *Molecular structure of three representative motors from the kinesin, dynein, and myosin superfamilies*. All three types of motor are multisubunit complexes consisting of heavy chains and light chains. The heavy chains consist of globular head domains (*left*) that contain the microtubule and ATP-binding sites, as well as flexible neck linkers, alpha-helical coiled-coil stalk domains (*middle*), and globular tail domains (*right*). (**a**) Kinesin-1 consists of two heavy chains and two light chains (*light blue*). The head domains are shown in *red* and the tail domains are shown in *yellow*. (**b**) Cytoplasmic dyneins consist of heavy chains (*HC*), intermediate chains (*IC*), light intermediate chains (*LIC*), and light chains (e.g., Roadblock, LC8, Tctex1). The microtubule-binding domains (*brown*) project from the head domains like the eyes of a snail. Dynein interacts with its cargoes via an adapter complex called dynactin, which consists of the protein p150 along with many other subunits. The p150 protein contains a microtubule-binding domain (*red*), which may interact with microtubules to enhance the processivity of the dynein motor. (**c**) Myosin-V consists of two heavy chains, which contain the head domains (*red*). Multiple calmodulin light chains (*light blue*) bind to the neck domain and light chains such as LC8 (*light blue*) bind to the tail domains (*purple*) (Adapted from Hirokawa et al. 2010)

(or bridge) their cargoes to the polymer tracks along which they move. Most motors contain two heavy chains, giving rise to two heads that can generate a walking motion along the polymer track. Many of these motors exhibit processivity, which is

the ability to take many successive steps along the polymer wall without dissociating; such behavior requires tight coordination of the two heads to ensure that at least one head is bound at all times. Some motors consist of a single heavy chain; such single-headed motors can only generate motion by acting in groups. An animated model for the processive movement of kinesin-1, also known as conventional kinesin, is shown in Fig. 17 and Video **8**.

Research in the last two decades has unveiled a remarkable diversity of molecular motors in neurons. For example, there are 45 different kinesin heavy chain genes in the human genome, which are grouped into 14 different families, and at least half of these are expressed in neurons. The human genome also contain at least 39 different myosin heavy chain genes, which are grouped into 18 different families, and at least five of these families are represented in neurons. There are only two cytoplasmic dynein heavy chain genes expressed in neurons, and only dynein heavy chain 1 appears to be present in axons, but dynein motors may be no less diverse than kinesins and myosins because there are numerous different isoforms of the dynein light, intermediate, and light intermediate chains.

Motor Proteins Move Unidirectionally Along Their Polymer Tracks

Another important general principle of motor protein function, which is a consequence of the structural polarity of microtubules and microfilaments, is that each motor binds to its track in a particular orientation and consequently moves unidirectionally along the polymer wall. Dyneins move toward the minus ends of microtubules, whereas most kinesins move toward the plus ends. Thus, the direction of movement of these motors is determined by the structural polarity of the polymer tracks. Since microtubules in axons are orientated with their plus-ends distal (toward the axon tip), this means that kinesins are responsible for anterograde transport in axons, and dyneins are responsible for retrograde transport. The one exception to this rule is the kinesin-14 family members, also known as C-type kinesins, which have the same directionality as dyneins and thus could potentially also serve as retrograde motors in axons. The directionality of most myosins is not known, but some are plus-end directed and one is known to be minus-end directed. Since microfilaments are short and are not orientated uniformly throughout the axon, the direction of movement generated by a particular myosin will depend on the microfilament organization at that location.

Due to the long length and axial orientation of microtubules within axons, kinesins and dyneins are thought to be responsible for most long-range movement within axons. By contrast, the abundance of microfilaments in the vicinity of microtubules and the plasma membrane suggests that myosin motors may be primarily responsible for short-range movements, perhaps facilitating the engagement of cargoes with microtubule tracks, or facilitating the movement of cargoes in regions of the cytoplasm that are devoid of microtubules.

Single Cargoes Interact with Multiple Types of Motors

A general theme that is emerging in the field of molecular motors and intracellular transport is that single cargoes associate with multiple distinct motors and that the activity of these motors is coordinated, either through physical or mechanical

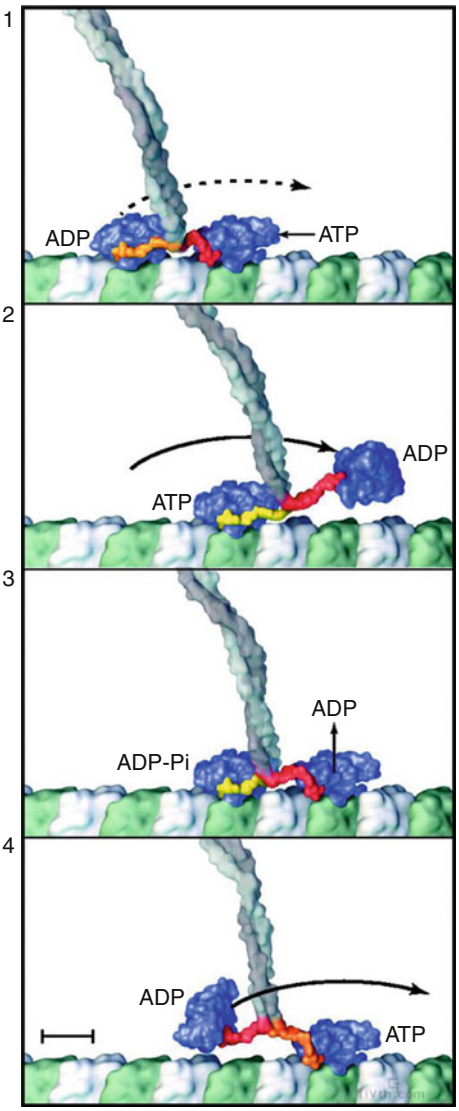

Fig. 17 *A model for the cross-bridge cycle of kinesin-1*. This schematic shows the heads, neck linkers, and a portion of the alpha-helical coiled-coil stalk domain of a dimeric kinesin-1 motor. The two heads (*blue*) work in a coordinated manner to move the motor and its attached cargo along a single protofilament of the microtubule track (each microtubule is made up of 13 protofilaments but only one is shown here). The motor moves toward the plus end of the microtubule, which is from left to right in this schematic. The alternating α-tubulin and β-tubulin subunits of the microtubule protofilament are shown in *white* and *green*, respectively. The alpha-helical coiled-coil of the stalk domain is shown in *gray*. The walking motion is generated by a precisely orchestrated series of conformational changes in the neck linkers and the heads, which is coupled to the binding and hydrolysis of ATP and the release of its hydrolysis products. The neck linkers are shown in different colors to distinguish their conformational states. *Frame 1*: The leading neck linker (*red*) is pointing

interactions, to permit seamless transitions between anterograde or retrograde movements on a particular track or to permit transitions between different tracks. The mechanism by which these motors are coordinated is currently a topic of great interest in the field of cell biology. An example of one class of cargoes that interacts with multiple different motors is mitochondria (Fig. 18). Kinesin-1 and dynein drive long-range anterograde and retrograde movements of these organelles along microtubules, whereas myosin drives short-range movements along microfilaments. However, myosin motors may influence the long-range transport behavior of mitochondria by delivering these organelles to their microtubule tracks or by moving them away. Myosin motors may also function as anchors by dynamically tethering their cargoes to microfilaments.

Motors Mostly Interact with Their Cargoes via Adapter Proteins

Most motors interact with their cargoes via adapter proteins which bind to receptors on the cargo. One example is the monomeric (single-headed) kinesin motor KIF1A, which transports a population of synaptic vesicles containing a membrane-anchored GTPase called Rab3. The interaction of KIF1A with Rab3-containing vesicles is mediated by a protein called Rab3 GDP/GTP exchange protein (Rab3GEP), also known as DENN/MADD. The death domain of DENN/MADD binds to the stalk domain of the kinesin motor and the MADD domain binds to Rab3, thereby linking the motor to the vesicle (Fig. 19). Another example is dynein, whose interaction with its cargoes requires a large complex of proteins called dynactin, which may in turn interact with cargo-specific adapter proteins. Dynactin is so critical for dynein function that the dynein motor is often referred to as the dynein/dynactin complex. The interaction of the dynein/dynactin complex with some vesicles is mediated by a protein called huntingtin, which is mutated in Huntington's disease (see below). One advantage of such adaptor proteins and adaptor protein complexes is that they represent additional signaling targets that increase the potential for independent regulation of motor-cargo interaction. In some cases, these adaptor proteins are

Fig. 17 (continued) backward (to the *left*) and the trailing neck linker (*orange*) is pointing forward. ATP binding to the leading head causes that head to bind tightly to the microtubule. The trailing head, which is bound to ADP, is weakly bound. *Frame 2*: The leading neck linker in Frame 1 (now shown in *yellow*) "zippers" up alongside its attached head. This conformational change flings the trailing ADP-bound head forward (*arrow*) toward the next tubulin-binding site. *Frame 3*: The head that is now leading docks weakly with its new location on the microtubule and the head that is now trailing, which is still bound tightly, catalyzes the hydrolysis of its bound ATP to form ADP and Pi (phosphate ion). *Frame 4*: Exchange of ADP for ATP on the leading head causes it to bind tightly to the microtubule. Subsequently, Pi dissociates from the trailing head causing it to weaken its hold on the microtubule. This allows the trailing head to be pulled forward by the conformational change in the leading neck linker (now shown in *orange*) as it begins to zipper up alongside its attached head to repeat the cycle. For each step in this cycle, the trailing head is flung forward by 16 nm (the stride length) and the stalk domain moves ahead by 8 nm (the step size), which is the length of a single tubulin dimer. Thus, kinesin-1 takes one 8 nm step for every ATP that it hydrolyzes. Scale bar = 4 nm (Reproduced from Vale and Milligan 2000) (*See* Video 8 *for animation*)

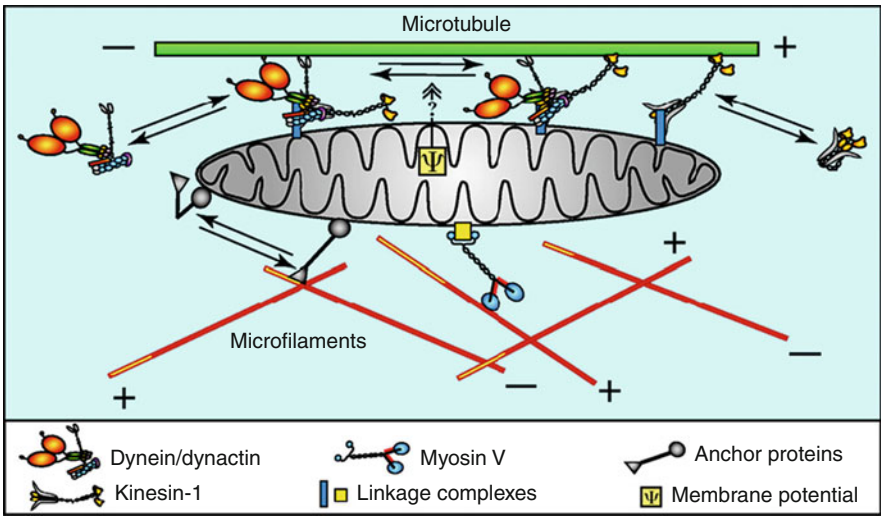

Fig. 18 *Mitochondria interact with multiple types of motors*. All three classes of motor proteins cooperate to move mitochondria in axons, though the mechanism of cooperation is not known. The motors interact with the outer membrane of the mitochondrion via adaptor protein complexes (referred to here as linkage complexes). Kinesin-1 and dynein drive long-range anterograde and retrograde movement along microtubules (*green*), and myosins drive short-range movements along microfilaments (*red*). It is not known whether these motors form multimotor complexes or whether they bind to mitochondria independently; in this schematic they are shown to bind independently. The directionality of movement is regulated by phosphorylation of the motors downstream of various intracellular signaling pathways and also by mitochondrial inner membrane potential. Mitochondria may be retained in particular regions of the axon by specific anchor proteins, which tether them to the microfilaments or microtubules; the one shown here is a hypothetical linker of mitochondria to microfilaments (Adapted from Hollenbeck and Saxton 2005)

signaling scaffolding proteins that can recruit signaling molecules such as protein kinases that are required for this regulation.

Even though there are many types of motors in neurons, there are even more types of cargoes. Thus, a single motor may often be called on to interact with multiple different cargoes. An important question is how the cell independently regulates these interactions. One possibility is that a given motor may require different types of adaptor proteins to interact with each type of cargo that it transports. For example, kinesin-1 motors have been implicated in the axonal transport of cargoes as diverse as mitochondria, neurofilaments, and a number of distinct classes of Golgi-derived transport vesicles. The interaction of kinesin-1 with mitochondria is mediated by the milton-miro complex, which will be discussed later. The interaction of kinesin-1 with a subclass of active zone precursor transport vesicles called piccolo-bassoon vesicles is mediated by syntabulin, whereas the interaction of this motor with a population of vesicles containing cell surface tyrosine receptor kinase B (TrkB) is thought to be mediated by the CRMP2-Slc1 protein complex. Kinesin-1 has also been proposed to interact with other classes of transport vesicle via an adapter complex comprised of JIP proteins (c-Jun N-terminal kinase interacting proteins),

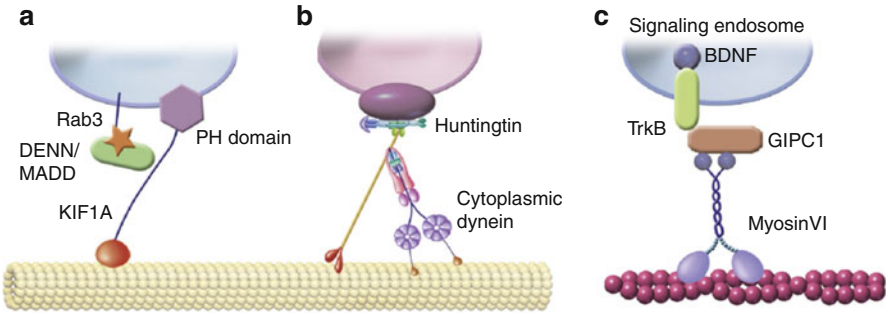

Fig. 19 *Some examples of adapter protein complexes that are thought to link motors to their cargoes*. (**a**) The interaction of KIF1A, a kinesin-3 motor, with Rab3-containing vesicles is mediated by the RAB3 GEP protein, also known as DENN/MADD. The tail of KIF1A also interacts directly with phospholipids in the vesicle membrane via a pleckstrin homology (*PH*) domain, but this interaction alone is not thought to be sufficient for cargo binding. (**b**) The interaction of dynein/dynactin with some vesicles is mediated by huntingtin. (**c**) The interaction of the myosin VI motor with BDNF-TrkB containing signaling endosomes is mediated by a protein called GIPC1 (Reproduced from Hirokawa et al. 2010)

which are a family of signal scaffolding proteins that function to recruit kinases involved in the MAP kinase-signaling cascade. Thus, kinesin-1 is an example of a motor that can move multiple distinct cargos depending on the adapter proteins that it interacts with (Fig. 20).

Some axonal cargoes are transported by motors that also transport dendritic cargoes, giving rise to the notion of "smart" motors, that is, motors that target cargoes to different cellular compartments based on the nature of the cargo that they are bound to. For example, kinesin-1 transports many cargoes selectively into axons, including synaptic vesicle precursors containing the synaptic protein VAMP2, but the same motor also transports vesicles containing AMPA-type glutamate receptors selectively into dendrites. This suggests that kinesin-1 transports cargoes to different locations in the nerve cell depending on the nature of the cargo.

Motors Are Targets for Axonal Transport Regulation

Axonal transport must be regulated to ensure that cargos are delivered to the correct destination within the axon or axon terminal and in the correct quantity at the correct time. The mechanisms that regulate axonal transport are not well understood, but it is likely that there are multiple mechanisms which act at multiple levels, including the cargo adaptors, the motor proteins, and the cytoskeletal tracks themselves. For example, neurons can regulate the posttranslational modification of axonal motor proteins and cargo adapters to affect the docking and release of motors with their cargoes and tracks. In addition, the subunit proteins of microtubules and microfilaments can be posttranslationally modified, and these modifications can confer selectivity for particular motors.

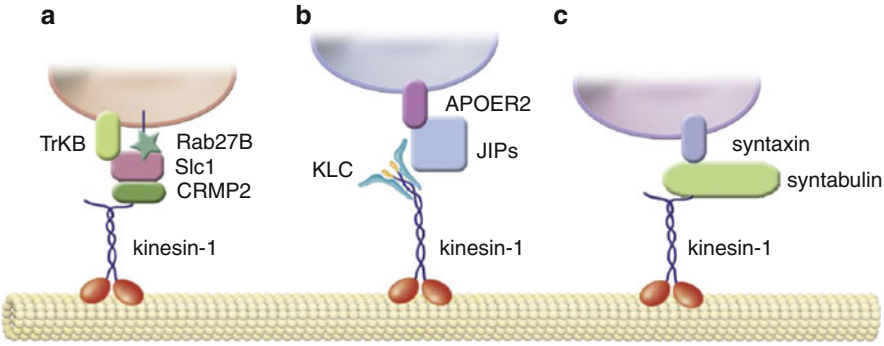

Fig. 20 *Adapter protein complexes allow a single motor to interact with different cargoes*. (**a**) The interaction of kinesin-1 with vesicles containing cell surface tyrosine receptor kinase B (*TrkB*) is thought to be mediated by the CRMP2-Slc1 protein complex, which interacts with the vesicle via TrkB and a glycophosphatidylinositol (*GPI*)-anchored GTPase called Rab27B. (**b**) The interaction of kinesin-1 with vesicles containing the apolipoprotein E receptor (*ApoER*) may be mediated by a complex of c-Jun N-terminal kinase (*JNK*) interacting proteins (*JIPs*), which are scaffolding proteins that function to recruit mitogen-activated protein kinases (*MAP kinases*) involved in intracellular signal transduction. (**c**) The interaction of kinesin-1 with syntaxin-containing vesicles, which convey protein components of the active zone of synapses, is mediated by a protein called syntabulin (Adapted from Hirokawa et al. 2010)

Adapter proteins are a common target of axonal transport regulation. One example is the anterograde transport of mitochondria in fruit flies by kinesin-1 motors. The calcium-dependent regulation of this movement is mediated by an adaptor complex composed of two proteins, milton and miro. According to one model, kinesin-1 is present on all mitochondria, whether they are moving anterogradely, pausing, or moving retrogradely. Miro is an integral membrane protein in the outer mitochondrial membrane. Milton binds directly to both miro and to the tail domain of kinesin-1, thereby recruiting kinesin-1 to mitochondria. In the presence of calcium ions, miro undergoes a conformational change that results in binding to the kinesin-1 head domains, competitively inhibiting their interaction with the microtubule track. Thus, the anterograde movement of mitochondria in fruit flies is regulated by altering the interaction of the motor with its track (Fig. 21).

Another important mechanism of axonal transport regulation is phosphorylation of motor proteins by specific kinases. For example, kinesin-1, which is a heterotetramer composed of two heavy chains and two light chains, is regulated by several different kinases including glycogen synthase kinase 3 (GSK3), casein kinase 2 (CK2), and c-Jun N-terminal kinase 3 (JNK3). Phosphorylation of the kinesin light chains in the kinesin-1 tail by GSK3 and CK2 causes the motor to detach from vesicular cargoes, whereas phosphorylation of the heavy chains in the head domains by JNK3 inhibits their interaction with microtubules (Fig. 22). Thus, phosphorylation can inhibit both motor activity and motor-cargo interactions.

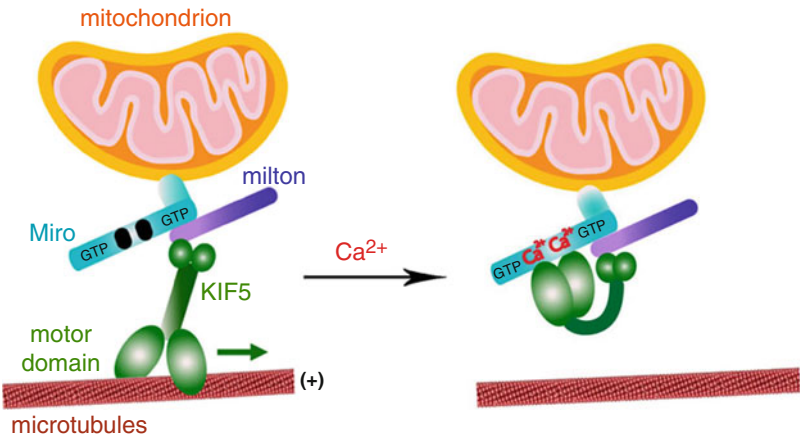

Fig. 21 *A model for the regulation of mitochondrial transport by the miro-milton adaptor complex*. Miro is an integral membrane GTPase embedded in the outer mitochondrial membrane, and it has two calcium-binding domains. Milton functions as a mitochondrial adapter protein that links kinesin-1 to miro. The activity of the kinesin-1 motor is regulated by calcium ions. In the presence of elevated intracellular calcium, miro binds to the kinesin-1 head domains causing the motor to dissociate from its microtubule track (Reproduced from Cai and Sheng 2009b)

The Axon Initial Segment May Function as a Gate Keeper for Axonally Transported Cargoes

The function of nervous systems in higher organisms is dependent on the polarization of nerve cells into axonal and somatodendritic (cell body and dendrite) compartments that have distinct electrophysiological properties. This requires selective sorting and retrieval mechanisms that enrich specific cargoes and molecular constituents within each compartment and then keep them segregated.

An important contributor to the sorting and segregation of axonal constituents is a specialized region at the proximal end of axons called the axonal initial segment (AIS), which is enriched in voltage-gated ion channels, cytoskeletal scaffolding proteins, and cell adhesion molecules. In addition to being the site of initiation of axonal action potentials, the axon initial segment also forms a diffusion barrier within the plasma membrane, preventing membrane proteins and lipids that are inserted into the axonal and somatodendritic membranes from mixing by lateral diffusion in the plane of the lipid bilayer. There is also evidence that the axon initial segment forms a barrier to the diffusion of cytosolic macromolecules and that it may function as a kind of molecular "gate keeper" for axonal transport, permitting the entry of axonal cargoes and rejecting the entry of dendritic cargoes. Such a mechanism could explain the existence of the so-called smart motors that can selectively transport cargoes to axonal or somatodendritic compartments depending on the nature of their cargo (see above). How the axon initial segment accomplishes this selectivity is not known, but it does appear to require microfilaments as well as components of the submembrane cytoskeleton such as ankyrin G and bIV spectrin (Fig. 23).

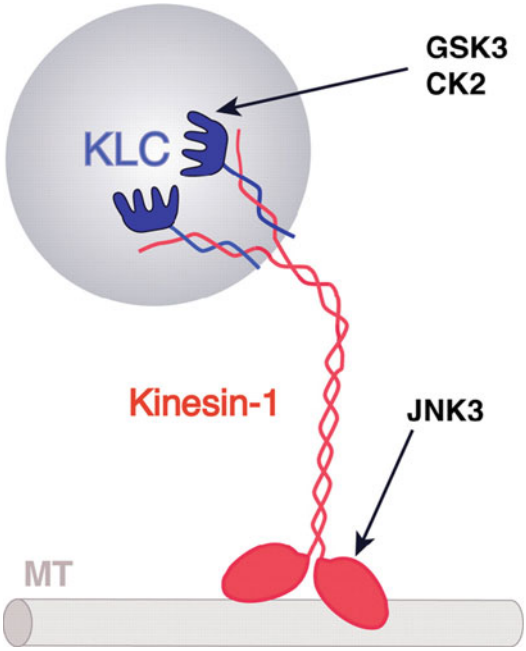

Fig. 22 *Differential regulation of kinesin-1 by phosphorylation of the head and tail domains*. Conventional kinesin is composed of two heavy chains (kinesin-1, in *red*) and two light chains (KLC, in *blue*). The light chains mediate the binding of kinesin-1 to some classes of membranous organelles. Phosphorylation of the light chains by glycogen synthase kinase 3 (*GSK3*) and casein kinase 2 (*CK2*) promotes detachment of the motor from the cargo, whereas phosphorylation of the heavy chains by c-Jun N-terminal kinase 3 (*JNK3*) inhibits binding to microtubules (*MT*) (Adapted from Morfini et al. 2009)

Axonal Transport Supplies mRNAs for Local Protein Synthesis

For many years, it was assumed that axons lack the capacity for protein synthesis and that the nerve cell body is the sole source of all axonal proteins. This opinion was reinforced by early reports that ribosomes are absent from axons. However, it is now known that axons can contain protein synthetic machinery including ribosomes, initiation and elongation factors, transfer RNAs (tRNA) and messenger RNAs (mRNA), as well as proteins and micro RNAs (miRNA) involved in the regulation of mRNA stability and translation. In addition, it is known that a specific subset of mRNAs in neurons can be transported into axons and translated locally. Thus, neurons have two mechanisms to deliver proteins to axons: they can synthesize the protein in the neuronal cell body and move the protein to its destination or they can move the mRNA for that protein and synthesize the protein locally. Though not the predominant mechanism, mRNA transport and local protein synthesis can be a very efficient means for delivering proteins to axons because the translation of a single mRNA can yield many thousands of copies of a protein.

The list of locally synthesized proteins that have been identified in axons is quite diverse and includes cytoskeletal proteins, heat shock proteins (protein chaperones), metabolic enzymes, and even some membrane proteins and secreted proteins. The fact that there are mRNAs for membrane and secreted proteins in axons is intriguing because it suggests that axons may be able to traffic locally synthesized proteins to membrane compartments. However, it is not clear how this could occur because axons lack Golgi apparatus and rough endoplasmic reticulum.

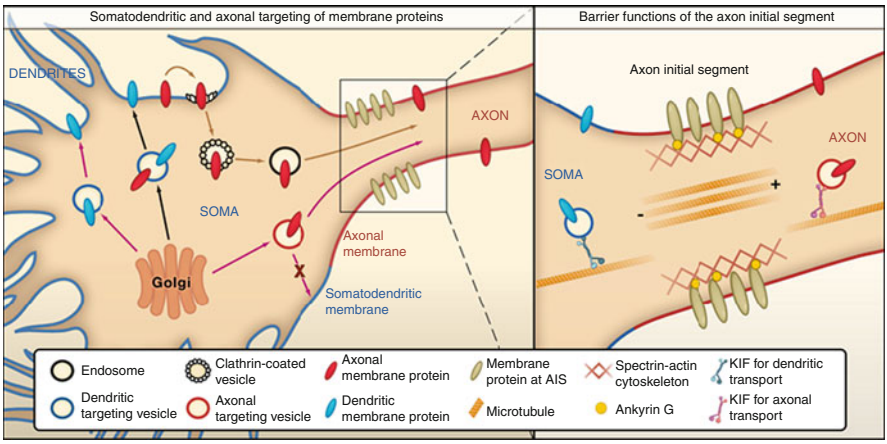

Fig. 23 *The role of the axon initial segment in the sorting and segregation of axonal membrane proteins in neurons*. Neurons establish and maintain distinct axonal (*red*) and somatodendritic (*blue*) compartments. (*Left*) Axonal and somatodendritic membrane proteins (*red and blue*, respectively) are sorted into distinct vesicles in the trans-Golgi network and delivered selectively to axons or dendrites (*purple arrows*). Alternatively, axonal membrane proteins can be targeted to the somatodendritic compartment along with dendritic proteins (*black arrows*), and then the axonal proteins can be redirected to the axonal plasma membrane by selective transcytosis (*gold arrows*). The segregation of these axonal and somatodendritic membrane proteins is maintained by a diffusion barrier in the plasma membrane of the axon initial segment. (*Right*) The axon initial segment also forms a cytoplasmic filter, which functions to prevent the entry of vesicles containing somatodendritic proteins (*blue*) into the axon while permitting the entry of vesicles containing axonal proteins (*red*). The plasma membrane of the axon initial segment is enriched in transmembrane proteins anchored to an actin-rich submembrane cytoskeleton by linker proteins such as ankyrin G. The axon initial segment also contains a core bundle of microtubules whose functional significance is not clear (Reproduced from Xiao and Jan 2009)

One limitation of axonal transport as a mechanism for delivering newly synthesized proteins to axons is that axons can be very long and therefore it can take a long time to deliver the proteins to where they are needed. For example, it can take many hours for vesicles in the nerve cell body to reach the distal end of the longest axons in the human body. An important advantage of local protein synthesis is that it can supply specific proteins rapidly to remote sites along axons without the delays inherent in axonal transport. In addition, mechanisms that localize the transcript and regulate the timing of its translation can provide axons with an additional level of control over the spatial and temporal localization of newly synthesized proteins. Thus, the mechanisms of mRNA transport and local protein synthesis give axons a level of autonomy from the nerve cell body that permits rapid and spatially restricted responses to local events. The extent to which local protein synthesis occurs in healthy mature axons remains unclear, but it is clear that it is important in axonal development and in the response of axons to injury.

Axonal mRNAs Are Transported as Ribonucleoprotein Complexes

mRNAs are transported into axons in association with RNA-binding proteins in the form of ribonucleoprotein particles, or RNPs, which are sometimes also referred to as RNA granules. These particles, which may also contain ribosomes and other components of the translational machinery, are dynamic structures that assemble in the nucleus and then recruit additional proteins, including motor proteins, after they are exported to the cytoplasm (Fig. 24). Within the axon they are transported along microtubules and microfilaments with the former guiding their long-range movements and the latter guiding their short-range movements. During their transport, the mRNAs in these complexes are translationally repressed by the action of RNA-binding proteins and regulatory RNAs, which may include microRNAs and associated translational silencing machinery. Once they reach their intended destination, the particles are thought to anchor to the cytoskeleton and become translationally derepressed. It is possible that many axonal mRNAs remain dormant until a particular developmental, physiological, or injury-derived signal triggers their use.

Axonal Transport of mRNAs Is Critical for Axonal Development

During axonal outgrowth in development, growth cones navigate through the complex environment of the developing embryo by making turning decisions in response to specific attractive and repulsive guidance cues, which are typically gradients of soluble factors in the extracellular matrix. Axonal mRNA transport

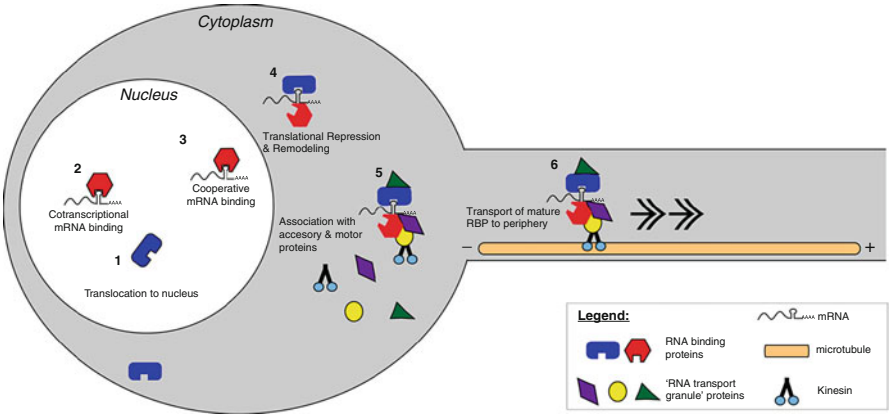

Fig. 24 *Sequential assembly of transport ribonucleoprotein particles through mRNA-protein interactions*. This schematic illustrates six stages of transport ribonucleoprotein particle (*RNP*) assembly. A nascent transcript in the nucleus recruits RNA-binding proteins (*red*) including ones that may shuttle between the nucleus and the cytoplasm (*dark blue*). The resulting complex is exported to the cytoplasm where remodeling may occur to ensure translational repression and to permit subsequent recruitment of additional RNP proteins (*purple*, *yellow*, and *green*) and kinesin motors (*light blue*). The resulting mature RNP complex can then be transported anterogradely into the axon along microtubule tracks (*light brown*) (Reproduced from Donnelly et al. 2010)

and local protein synthesis have recently emerged as important mechanisms in this process. Attractive and repulsive guidance cues stimulate spatially restricted translation of specific mRNAs leading to the local synthesis of proteins that are required for the cytoskeletal events involved in turning toward or away from the guidance cue. These events involve a constellation of accessory proteins that transport, target, and translate the mRNA, as well as signaling proteins that transduce the extracellular stimulus and regulate these processes.

One well-studied example is the mRNA for β-actin, which is an isoform of actin that is expressed in neurons as well as other cell types (Fig. 25). β-actin mRNA is transported to the growth cones of growing axons where it is locally translated. This mRNA forms transport ribonucleoprotein particles (RNPs) with a protein called zipcode-binding protein 1 (ZBP1). ZBP1 is a *trans*-acting RNA localization factor, which recognizes a 54 nucleotide sequence (called a zipcode sequence) in the 3′ untranslated region (3′ UTR) of the β-actin mRNA. The zipcode sequence is necessary and sufficient to target β-actin to axons. Binding of ZBP1 to the zipcode sequence is required for the axonal transport of β-actin mRNA, and it also

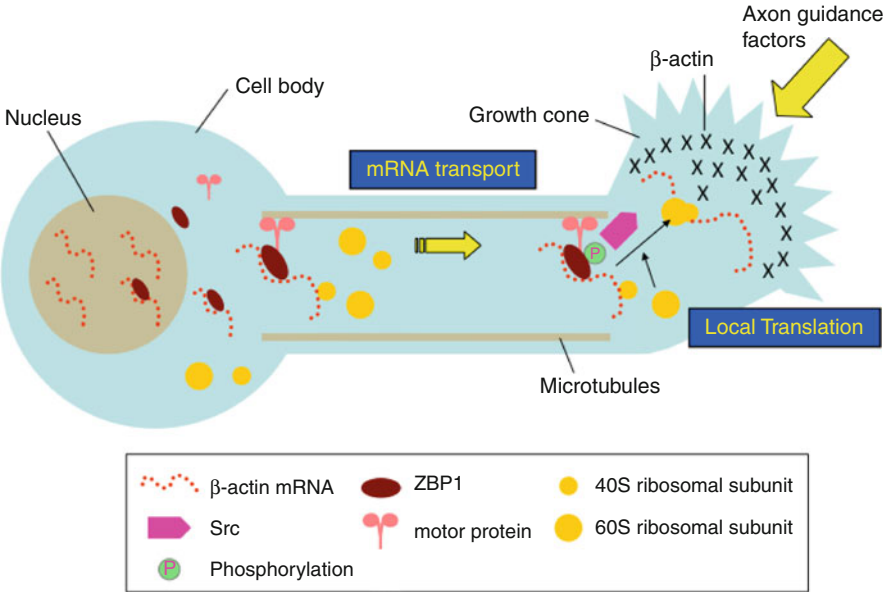

Fig. 25 *Axonal transport of β-actin mRNA is required for growth cone turning in response to attractive guidance cues*. Binding of zipcode-binding protein 1 (*ZBP1*) to a zipcode sequence in the 3' UTR of β-actin mRNA is required for nuclear export. In the cytoplasm, this complex recruits other RNA-binding proteins (not shown) to form a ribonucleoprotein particle (*RNP*). ZBP1 suppresses translation of the mRNA and recruits kinesin to transport the complex to the growth cone. In the growth cone, phosphorylation of ZBP1 by *src* kinase in response to an attractive guidance cue causes ZBP1 to dissociate from the β-actin mRNA, allowing it to be translated. This locally synthesized β-actin protein is required for growth cone turning in the direction of the attractant (Figure provided by Gary Bassell of Emory University)

suppresses translation. Phosphorylation of ZBP1 by *src* kinase in response to growth factor stimulation causes ZBP1 to dissociate from the β-actin mRNA, resulting in local activation of β-actin protein synthesis. It is possible that ZBP1 may also function as an adapter protein to link RNA transport particles containing β-actin mRNA to motor proteins. There is evidence that local synthesis of β-actin is required for growth cones to navigate in response to an attractive guidance cue and that repression of β-actin translation is required for growth cones to respond to a repulsive cue, but the role of β-actin in growth cone turning remains to be established. It is unlikely that the total amount of β-actin in the growth cone is rate limiting for growth cone turning, but it has been suggested that newly synthesized β-actin may be in some way functionally different from the preexisting endogenous β-actin pool, perhaps due to the nature or absence of certain posttranslational modifications.

Axonal Transport Regulates Presynaptic Development and Plasticity

Electrical communication in the nervous system involves the development of specialized contacts, called synapses, between axons and their target cells. Synapses typically consist of the presynaptic terminal of an axon closely apposed to a postsynaptic specialization of a target cell. The development and function of the presynaptic terminal requires the delivery and assembly of multiple components, which collectively allow the regulated formation, fusion, and recycling of synaptic vesicles that accompanies synaptic transmission.

To date, three distinct classes of membranous organelles have been identified that deliver critical components of presynaptic terminals: (1) active zone precursor vesicles, also known as piccolo-bassoon transport vesicles (PTVs), which contain active zone proteins such as piccolo, bassoon, syntaxin, and SNAP-25; (2) synaptic vesicle precursor vesicles; and (3) mitochondria. Whereas mitochondria and piccolo-bassoon transport vesicles are transported by kinesin-1 motors, synaptic vesicle precursor vesicles appear to be transported by kinesin-3 motors. In the case of mitochondria, kinesin-1 binds via the miro-milton adapter complex (Fig. 21), whereas in the case of active zone precursor vesicles, kinesin-1 appears to bind via an adaptor protein called syntabulin (see Fig. 20). The importance of axonal transport for the delivery of these components to presynaptic terminals is illustrated in fruit flies, where mutations in these motors or their adaptor proteins cause synaptic cargoes to be sequestered in the nerve cell bodies (Fig. 26).

Given the importance of axonal transport in synaptic development, it is likely that axonal transport also has important roles in the activity-dependent presynaptic changes that underlie learning and memory (Fig. 27).

Retrograde Axonal Transport Relays Signals from the Target Environment

The long length of axons in nervous systems means that the nerve cell body, which is the site of gene expression and the source of most axonally transported cargoes, can be far removed from the axon tip. To ensure that the axon receives the appropriate

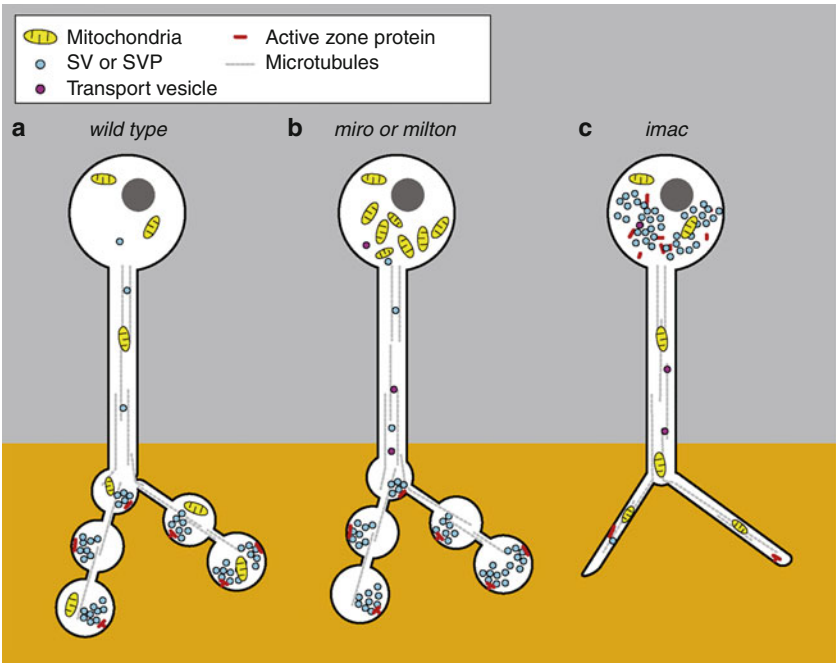

Fig. 26 *Axonal transport is critical for synapse formation in the fruit fly.* This schematic depicts motor neurons innervating skeletal muscle fibers, represented by the *colored zone* at the bottom. (**a**) In wild-type flies, mitochondria, active zone proteins, and synaptic vesicle precursors are all delivered to presynaptic terminals. (**b**) In *miro* or *milton* mutant flies, mitochondria fail to enter axons because *miro* and *milton* are adapters that link mitochondria to kinesin-1 motors. The axons grow normally but form presynaptic boutons that lack mitochondria. (**c**) In *immaculate connections (imac)* mutant flies, synaptic vesicle precursors fail to enter axons because the *immaculate connections* gene encodes for a kinesin-3 motor protein, which is the motor for synaptic vesicle precursors. The axons grow normally and innervate the muscle but fail to form synapses. Synaptic vesicle components accumulate in the nerve cell body but mitochondria are distributed normally (Adapted from Goldstein et al. 2008)

consignment of cargoes and molecules for its proper function, the nerve cell must modulate gene expression and protein trafficking in response to remote events in the axonal environment. Such long-range signaling is accomplished by retrograde axonal transport. The signals relayed in this manner can be target-derived survival factors, which indicate that the axon is innervating the appropriate target, or they can be stress factors, which indicate that the axon is injured or exposed to an adverse environment.

An example of retrograde signaling is the retrograde transport of neurotrophins. Neurotrophins are a family of proteins including nerve growth factor (NGF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), and brain-derived neurotrophic factor (BDNF) that regulate many aspects of neuronal function, including neuronal survival and differentiation, neuronal migration, and synaptic plasticity.

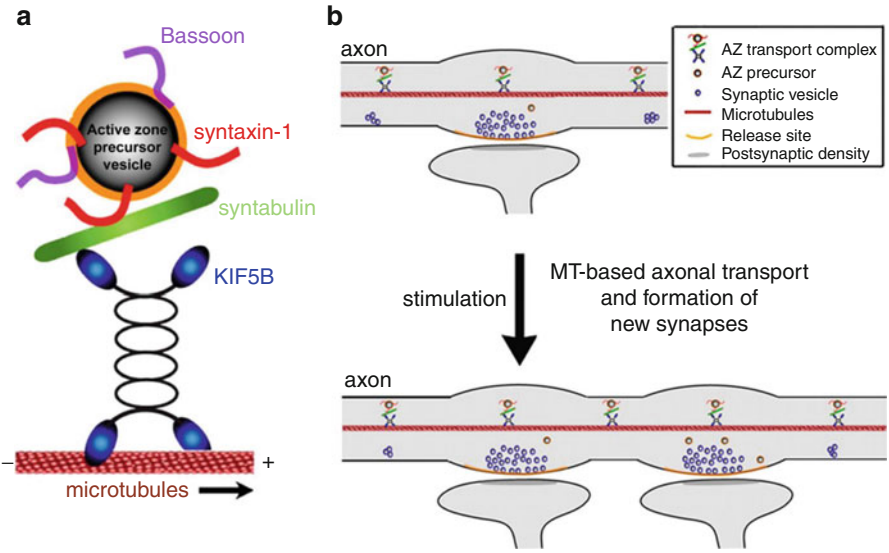

Fig. 27 *Axonal transport contributes to new synapse formation and presynaptic plasticity.* (**a**) Anterograde axonal transport of cargoes such as active zone (*AZ*) precursor transport vesicles (shown here) by kinesin-1 motors delivers synaptic components for presynaptic assembly. The interaction of the kinesin-1 motor with the syntaxin-1 receptor is mediated by the syntabulin adaptor protein. (**b**) Through this motor-adaptor complex, active zone precursor transport vesicles are delivered to nascent synapses where they participate in the formation of active zones, and thereby contribute to activity-induced presynaptic plasticity (Reproduced from Cai et al. 2007)

Neurotrophins are secreted by postsynaptic cells and they initiate signals at axon terminals by binding to specific cell surface tyrosine receptor kinase (Trk) receptors (pronounced "Trak" receptors), resulting in the activation of these receptors. The activated Trk/neurotrophin complexes are then internalized by endocytosis and sorted into a class of endosomes called signaling endosomes, which function as carriers. The signaling endosomes recruit dynein motors and are transported retrogradely to the cell body along microtubule tracks. The composition of these signaling endosomes has not been defined, but they appear to recruit downstream signaling intermediates such as extracellular signal-regulated kinase (Erk) and the transcription factor CREB. Within the cell body, these axon-derived retrograde signal effectors enter the nucleus where they modulate gene expression. Interestingly, the CREB that is recruited to these signaling endosomes is translated locally within the axon in response to NGF stimulation, which is another example of the importance of local protein synthesis in axonal development (Fig. 28).

Retrograde Transport of Locally Synthesized Proteins Is Important in the Axonal Response to Injury

Proteomic and mRNA profiling studies have demonstrated that when an axon is injured there is a local upregulation of mRNA transport and an increase in local

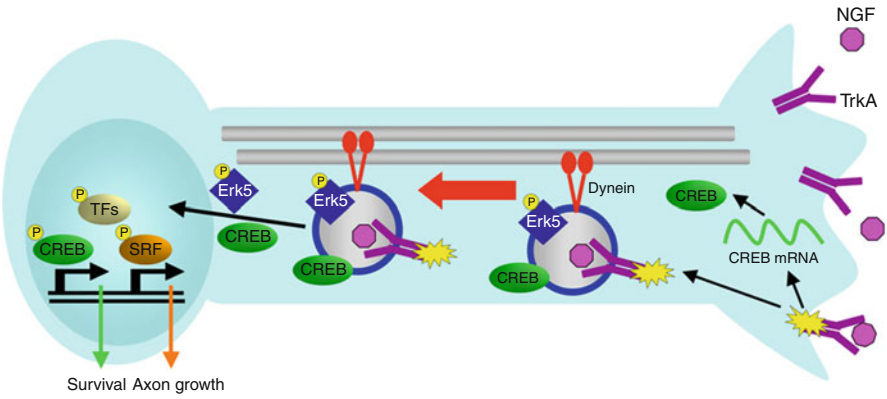

Fig. 28 *Retrograde neurotrophin signaling* via *signaling endosomes*. This schematic illustrates retrograde signaling by nerve growth factor (*NGF*), which is a target-derived neurotrophin. Binding of NGF to tyrosine receptor kinase A (*TrkA*) receptors results in receptor activation and subsequent internalization by endocytosis. TrkA activation also induces local phosphorylation (activation) of extracellular signal-regulated kinases (*Erk*) and local protein synthesis of the transcription factor CREB. The activated TrkA-NGF complex is sorted to signaling endosomes that recruit downstream signaling components (CREB and phosphorylated Erk) and are transported retrogradely along microtubules by dynein motors. In the nerve cell body, CREB and phosphorylated Erk are translocated into the nucleus where they modulate gene expression to mediate trophic responses. CREB induces a set of genes that promote cell survival, whereas phosphorylated Erk activates transcription factors (*TFs*), including serum response factor (*SRF*), which induce genes that promote axon growth (Reproduced from Cosker et al. 2008)

translation in axons which triggers events that are critical for the injury response and subsequent axon regeneration. Some of the axonally synthesized proteins function locally whereas others, which include transcription factors, are transported retrogradely back to the cell body where they may modulate gene expression. In this way, temporal control of local protein synthesis can provide long-distance communication between the site of injury and the neuronal cell body.

An example of the role of local protein synthesis in the response of axons to injury is the role of importins in retrograde axonal signaling in peripheral neurons. Importins are proteins that facilitate the entry of other proteins into the nucleus through nuclear pore complexes, but these proteins also function in processes other than nuclear import. In the classical nuclear import pathway, proteins that are destined for nuclear import contain a short amino acid sequence called a nuclear localization signal (NLS). Importins bind to the nuclear localization signal and mediate docking and translocation of the resulting complex across the nuclear pore.

There are two classes of importins, called importin α and importin β, which can form αβ heterodimers. Nuclear localization signal recognition can be mediated by importin β proteins alone or by importin α proteins when they are part of an αβ heterodimer (importin α proteins alone bind weakly to nuclear localization signals, but their affinity is increased greatly when they are bound to importin β). The stability of the resulting importin complex is regulated by Ran, which is a small GTPase.

Interestingly, importin α and Ran are present in axons, but importin β is absent or at very low levels. The axonal importin α is bound to RanGTP via an adapter protein called Cas, preventing the association of importin α with importin β. Upon injury, importin β mRNA in the axons is translated locally at the site of injury. In addition, the mRNA for a Ran-binding protein called RanBP1 is also translated. The newly synthesized RanBP1 interacts with a Ran GTPase-activating protein (RanGAP) to stimulate hydrolysis of RanGTP to RanGDP. This causes Ran and Cas to dissociate from importin α, allowing importin α to bind to the newly synthesized importin β. The resulting importin αβ heterodimer binds with high affinity to the nuclear localization signals of certain axonal signaling proteins resulting in a cargo complex that is transported retrogradely to the cell body along microtubules by dynein motors. For example, several axonal transcription factors are among the cargoes of this retrograde signaling complex, and their delivery to the neuronal cell body has the capacity to modulate gene expression directly, triggering a transcriptional and translational response that is critical for regeneration.

Local synthesis of importins can also mediate the retrograde transport of signaling proteins that do not contain classical nuclear localization signal sequences. For example, axonal injury has been shown to result in local translation of vimentin, an intermediate filament protein that is not normally expressed in mature neurons, as well as local phosphorylation (i.e., activation) of the extracellular signal-regulated kinases Erk1/2. Proteolytic cleavage of the locally synthesized vimentin by a calcium-activated protease called calpain results in the generation of a vimentin fragment that binds to importin β and to phosphorylated Erk1/2 kinases, thereby mediating the retrograde transport of these activated kinases by dynein motors. Thus, local synthesis of specific signal scaffolding and regulatory proteins in response to injury allows for rapid spatially restricted activation of a retrograde injury signaling pathway that initiates the neuron's injury response (Fig. 29).

Retrograde Axonal Transport Is Also a Pathway for Degradation and Recycling

In addition to relaying signals from the target environment back to the nerve cell body, retrograde transport also functions as a pathway for the recycling or degradation of membranous organelles and their macromolecular components. In the canonical membrane recycling pathway, membrane components retrieved from the axonal plasma membrane by endocytosis, either at the axon tip or along the length of the axon, enter early endosomes where they are sorted and either recycled to the plasma membrane by exocytosis or delivered to late endosomes. In addition, cytosolic proteins and membranous organelles such as mitochondria and peroxisomes, which are isolated from the pathways of Golgi and endosomal traffic, can be encapsulated by autophagocytosis into large vacuolar or multivesicular membranous organelles called autophagosomes. Late endosomes, prelysosomal organelles, and autophagosomes recruit dynein motors and are transported retrogradely along axons to the nerve cell body where they deliver their components to Golgi or lysosomal compartments in the cell body for degradation. The extent to which lysosomal

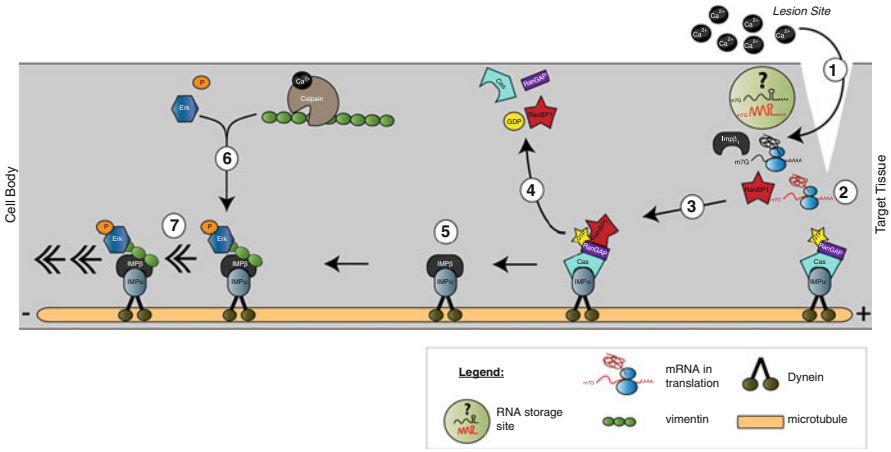

Fig. 29 *Role of local axonal protein synthesis in retrograde signaling of axon injury.* mRNAs for importin β (specifically, importin β1) and RanBP1 are present in uninjured axons, possibly in a putative "RNA storage site." Injury to the axon causes an increase in axoplasmic Ca^{++} due to influx from the extracellular space (*1*) and release from intracellular calcium stores (not shown). The elevated Ca^{++} triggers local synthesis of importin β (*black*) and RanBP1 (*red*) (*2*). Axonal importin α (*gray*) is bound to a complex consisting Cas (*light blue*), RanGAP (*purple*), RanGTP (*yellow star* labeled "GTP"), and dynein. The Cas-RanGAP-RanGTP complex prevents the interaction of importin α with importin β. The newly synthesized RanBP1 interacts with RanGAP (*3*). This interaction stimulates RanGTP hydrolysis to RanGDP (*yellow circle* labeled "GDP"), resulting in dissociation of the complex (*4*) and allowing importin α to heterodimerize with newly synthesized importin β (*5*). Meanwhile, the elevated calcium also activates calcium-dependent proteases, which cleave newly synthesized vimentin protein (*green*) (*6*). A fragment of the cleaved vimentin acts as a signal scaffolding protein to link phosphorylated (activated) Erk1/2 kinases (*blue hexagon*) to the importin α/β complex for retrograde transport to the cell body by dynein (*7*). The importin complex can also recruit axonal transcription factors and deliver them to the cell body, where they can modulate gene expression directly (see text) (Reproduced from Donnelly et al. 2010)

biogenesis and degradation occurs locally in axons, as well as the mechanism and regulation of autophagosome biogenesis in axons, remains unclear.

Axonal Transport Is Disrupted in Many Neurodegenerative Diseases

The long length of axons makes them critically dependent on axonal transport of proteins, lipids, mRNAs, and associated translational machinery for their development and maintenance. Thus, it is no surprise that axons are very vulnerable to disruptions of axonal transport and that axonal transport mechanisms are the direct or indirect targets of many disease mechanisms. Indeed, it is no exaggeration to say that axonal transport is probably disrupted in most neurodegenerative diseases, including Alzheimer's disease, motor neuron diseases, Huntington's disease, hereditary spastic paraplegias, spinal muscular atrophy, and Charcot-Marie-Tooth diseases. The challenge for axonal transport researchers is to determine if the axonal transport

abnormalities are an early and therefore potentially significant event in the etiology or progression of these diseases.

One group of neurodegenerative diseases for which axonal transport disruption is very likely to be a triggering event are those that are caused by mutations in axonal motor proteins. One example is Charcot-Marie-Tooth disease type 2A1, which is caused by mutations in KIF1Bβ, a member of the kinesin-3 family of kinesin motors. Another example is hereditary spastic paraplegia type 10 (SPG10), which is caused by mutations in kinesin-1A, also known as KIF5A, which is a member of the kinesin-1 family of kinesin motors.

A second group of neurodegenerative diseases in which axonal transport disruption is likely to be an important event are those that involve mutations in proteins that interact with motors, such as adapter proteins which mediate the interaction of motors with their cargoes. One example is *hereditary motor neuropathy type VIIB* (HMN7B), also known as spinal bulbar muscular atrophy (SBMA), which is caused by mutations in the p150 subunit of dynactin, also known as dynactin-1. Dynactin is an essential adapter for the interaction of dynein motors with their cargoes, and dynein/dynactin motor complexes appear to be responsible for most retrograde axonal transport in axons (see above). Another example is Huntington's disease, which involves mutations in a protein called huntingtin, which functions as an adapter for microtubule motors on some axonally transported vesicles (see above).

Finally, there are many neurodegenerative diseases that are not associated with mutations in motor proteins or their adapters, but which exhibit focal accumulations of axonally transported cargoes indicating that axonal transport mechanisms are disrupted. The impairment of axonal transport in these diseases is most likely caused by generalized alterations of intracellular signaling pathways resulting in aberrant posttranslational modification of the motors, cargoes, or tracks. An example of such a disease is amyotrophic lateral sclerosis (ALS), a form of motor neuron disease, which is characterized by massive swellings of the proximal axons of motor neurons in the spinal cord (Fig. 30). While some forms of motor neuron disease can be caused by mutations in motors or their adapters, most are not. The existence of axonal swellings in ALS suggests that axonal transport is impaired in this disease, and studies on laboratory animal models suggest that such impairments are an early and presymptomatic event in the disease progression.

Alterations in Retrograde Transport Can Cause Degeneration

One obvious mechanism by which changes in axonal transport could lead to neurodegenerative disease is by starving the axon of essential components. For example, defective anterograde transport of mitochondria could cause axons to be unable to meet their energy needs, leading to metabolic stress. However, there is now increasing evidence that changes in retrograde transport can also cause neuronal degeneration. One possible mechanism is a toxic accumulation of proteins or organelles in axons or axon terminals due to defective retrograde transport of lysosomes or autophagosomes. Another possible mechanism may be changes in retrograde signaling. In principle, neuronal degeneration could result from either the loss of a positive retrograde signal, such as prosurvival signaling by neurotrophins,

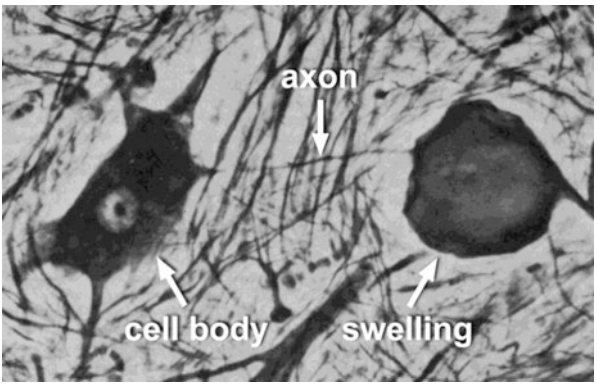

Fig. 30 *A massive focal swelling of a motor axon in motor neuron disease*. A hallmark pathological feature of patients with amyotrophic lateral sclerosis (Lou Gehrig's disease) is the presence of massive focal swellings of the motor axons in ventral horn of the spinal cord. In this example, a slender axon emerges from the nerve cell body (*left*) and then forms a giant balloon-like swelling (*right*). Termed spheroids by neuropathologists, these massive swellings are packed full of axonally transported cargoes including cytoskeletal elements and organelles. This suggests that there is a gross disruption of axonal transport in these patients. Several studies have suggested that disruptions of axonal transport are an early and possibly triggering event in the pathogenesis of this disease (Adapted from Carpenter 1968)

or the gain or a negative retrograde signal, such as the retrograde transport of activated stress kinases. While impairments in anterograde delivery or retrograde clearance are likely to be important, it is possible that changes in retrograde signaling may be more significant for the pathogenesis of many neurodegenerative diseases.

Outlook

Looking to the future, there are many important questions still faced by axonal transport researchers. In terms of the molecular mechanism of movement, the molecular identity of many axonal cargoes is still not known. For example, how many distinct vesicular cargoes are there, and what is their molecular architecture and composition? Also unknown are the identities of the motors that move most cargoes, how they are recruited to those cargoes, and how they are regulated. In the case of slow axonal transport, the nature of the cargo structures remains almost entirely unknown. Elucidating the structure and composition of these cytoskeletal and cytosolic macromolecular complexes is likely to provide fundamental insights into the nature and organization of axonal cytoplasm, and perhaps more generally for the cytoplasm of all cells.

A particularly fascinating problem is how motors interact to coordinate the bidirectional movement of cargoes. Many cargoes appear to have multiple motors bound to them, including both microfilament and microtubule motors. How many motors does it take to move a cargo in axons? How do microfilament and

microtubule motors cooperate to deliver cargoes to their correct destination? And when motors of opposing directionality are bound to the same cargo, do these motors engage in a tug-of-war, or is their activity coordinated so that only motors of one directionality are active at one time? To resolve these questions it will be necessary to combine direct imaging techniques with nanoscale force measurements in living axons.

Historically, progress in understanding axonal transport has largely paralleled the development of new techniques for studying this movement, and this is likely to continue in the future. For example, there is a pressing need for techniques that can enable direct imaging of real-time protein interactions in living cells and organisms with molecular resolution. Of particular interest are recent developments in in vivo imaging and super-resolution imaging, which are opening up new possibilities for experimentation on intracellular movement.

An exciting development in the field of axonal transport in recent years has been the widespread recognition of the importance of axonal protein synthesis for neuronal function. However, many questions still surround this issue. For example, the full inventory of locally synthesized proteins in axons is still not known, and little is known about the mechanisms that regulate the targeting, stability, and translation of axonal mRNA transcripts. Also, while it is now clear that mRNA transport and local protein synthesis are important events in axonal development, and in response to injury, it is not clear how important these processes are in mature and healthy neurons.

Last but not least, it is now clear that axonal transport is disrupted in many neurodegenerative diseases but an important challenge is to understand the mechanism of these disruptions and their significance for the disease pathogenesis. There is evidence that defects in axonal transport are an early event in the development of amyotrophic lateral sclerosis and Huntington's disease, but much still needs to be understood. While it is clear that some neurodegenerative diseases are caused by mutations in molecular motors, in most cases axonal transport disruption in disease is more likely to be a consequence of altered regulation, perhaps due to aberrant cell signaling. Such misregulation could target axonal transport at multiple levels including the cargos, adapters, motors, or their tracks. For those diseases in which alterations in axonal transport are a causative or exacerbating event, an even greater challenge will be to identify potential therapeutic strategies that ameliorate or reverse the disease progression.

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