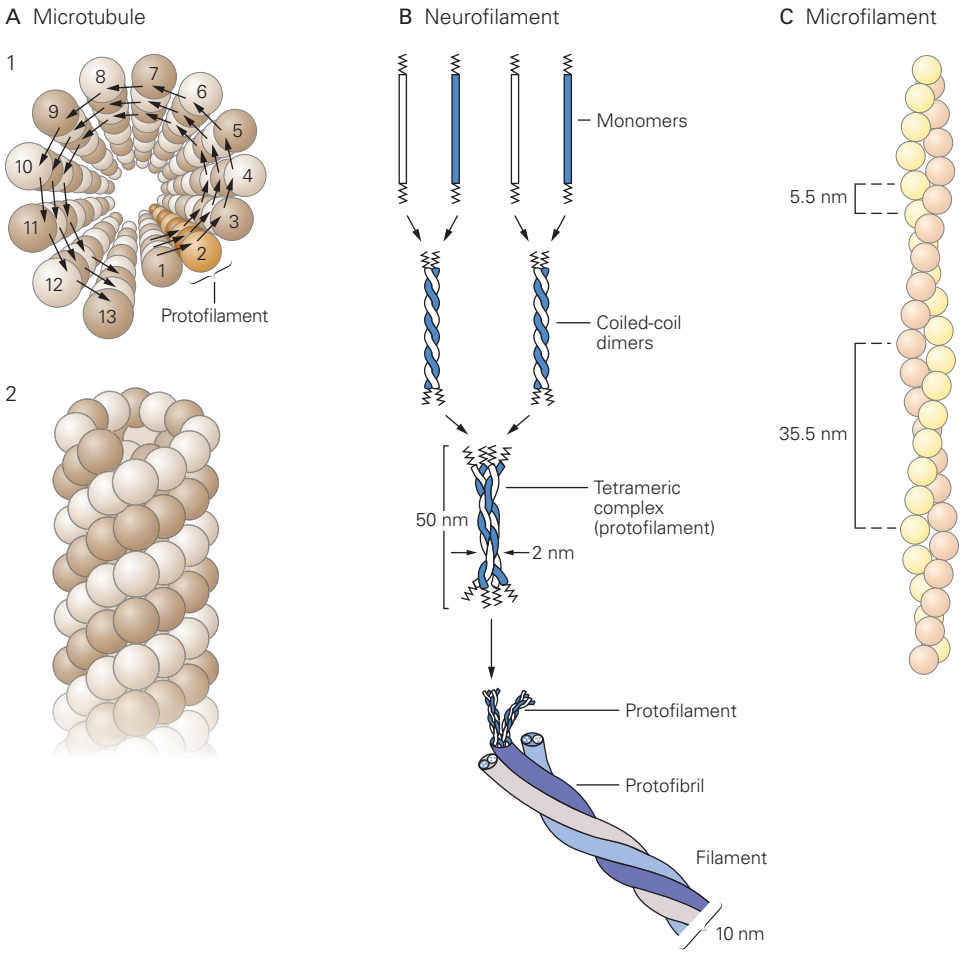


Figure 7–6 Atlas of fibrillary structures.

A. Microtubules, the largest-diameter fibers (25 nm), are helical cylinders composed of 13 protofilaments, each 5 nm in width. Each protofilament is made up of a column of alternating α- and β-tubulin subunits; each subunit has a molecular weight of approximately 50,000 Da. Adjacent subunits bind to each other along the longitudinal protofilaments and laterally between subunits of adjacent protofilaments.

A tubulin molecule is a heterodimer consisting of one α- and one β-tubulin subunit. **1.** View of a microtubule. The **arrows** indicate the direction of the right-handed helix. **2.** A side view of a microtubule shows the alternating α- and β-subunits.

B. Neurofilaments are built with fibers that twist around each other to produce coils of increasing thickness. The thinnest units are monomers that form coiled-coil heterodimers. These dimers form a tetrameric complex that becomes the protofilament. Two protofilaments become a protofibril, and three protofibrils are helically twisted to form the 10-nm diameter neurofilament. (Adapted, with permission, from Bershadsky and Vasiliev 1988.)

C. Microfilaments, the smallest-diameter fibers (approximately 7 nm), are composed of two strands of polymerized globular actin (G-actin) monomers arranged in a helix. At least six different (but closely related) actins are found in mammals; each variant is encoded by a separate gene. Microfilaments are polar structures because the globular monomers are asymmetric.

time, approximately half of the total actin in a cell can exist as unpolymerized monomers. The state of actin is controlled by binding proteins, which facilitate assembly and limit polymer length by capping the rapidly growing end of the filament or by severing it. Other binding proteins crosslink or bundle actin filaments. The dynamic state of microtubules and microfilaments permits a mature neuron to retract old axons and dendrites and extend new ones. This structural plasticity is thought to be a major factor in changes of synaptic connections and efficacy and, therefore, cellular mechanisms of long-term memory and learning.

Box 7–1 Abnormal Accumulations of Proteins Are Hallmarks of Many Neurological Disorders

Tau is a microtubule-binding protein normally present in nerve cells. In Alzheimer disease, abnormal aggregates of tau are visible in the light microscope in neurons and glia as well as in the extracellular space. Highly phosphorylated tau molecules arranged in long, thin polymers wind around one another to form paired helical filaments (Figure 7–7A and Chapter 64). Bundles of the polymers, known as *neurofibrillary tangles*, accumulate in neuronal cell bodies, dendrites, and axons (Figure 7–7A).

In normal neurons, tau is either bound to microtubules or free in the cytosol. In the tangles, it is not bound to microtubules but is highly insoluble. The tangles form at least in part because tau is not proteolytically degraded. The accumulations disturb the polymerization of tubulin and therefore interfere with axonal transport. Consequently, the shape of the neuron is not maintained.

Tau accumulations are also found in neurons of patients with progressive supranuclear palsy, a movement disorder, and in patients with frontotemporal dementias, a group of neurodegenerative disorders that affect the frontal and temporal lobes (Chapter 63). The familial forms of frontotemporal dementias are caused by mutations in the *tau* gene. Abnormal aggregates are also found in glial cells, both astrocytes and oligodendrocytes, in progressive supranuclear palsy, cortico-basoganglionic degeneration, and frontotemporal dementias.

The peptide *β-amyloid* also accumulates in the extracellular space in Alzheimer disease (Figure 7–7B and Chapter 64). It is a small proteolytic product of a much larger integral membrane protein, amyloid precursor protein, which is normally processed by several proteolytic enzymes associated with intracellular membranes. The proteolytic pathway that generates β-amyloid requires the enzyme β-secretase.

For unknown reasons, in Alzheimer disease, abnormal amounts of the amyloid precursor are processed by β-secretase. Some patients with early-onset familial Alzheimer disease either have mutations in the amyloid precursor gene or in the genes coding for the membrane proteins presenilin 1 and 2, which are closely associated with secretase activity.

In Parkinson disease, abnormal aggregates of α-synuclein accumulate in cell bodies of neurons. Like tau, *α-synuclein* is a normal soluble constituent of the cell. But in Parkinson disease, it becomes insoluble, forming spherical inclusions called *Lewy bodies* (Figure 7–7C and Chapter 63).

These abnormal inclusions also contain ubiquitin. Because ubiquitin is required for proteasomal degradation of proteins, its presence suggests that affected neurons have attempted to target α-synuclein or other molecular constituents for proteolysis. Apparently, degradation does not occur, possibly because of misfolding or the abnormal aggregation of the proteins or because of faulty proteolytic processing in the cell.

Do these abnormal protein accumulations affect the physiology of the neurons and glia? On the one hand, the accumulations may form in response to altered posttranslational processing of the proteins and serve to isolate the abnormal proteins, permitting normal cell activities. On the other hand, the accumulations may disrupt cellular activities such as membrane trafficking, axonal and dendritic transport, and the maintenance of synaptic connections between specific classes of neurons. In addition, the altered proteins themselves, aside from the aggregations, may have deleterious effects. With β-amyloid, there is evidence that the peptide itself is toxic.

(*continued*)

In addition to serving as the cytoskeleton, microtubules and actin filaments act as tracks along which organelles and proteins are rapidly driven by molecular motors. The motors used by the actin filaments, the *myosins*, also mediate other types of cell motility, including extension of the cell's processes and translocation of membranous organelles from the bulk cytoplasm to the region adjacent to the plasma membrane. (Actomyosin is responsible for muscle contraction.) Because the microtubules and actin filaments are polar, each motor drives its organelle cargo in only one direction.

As already mentioned, microtubules are arranged in parallel in the axon with plus ends pointing away from the cell body and minus ends facing the cell body. This regular orientation allows some organelles to move toward and others to move away from nerve endings, the direction being determined by the specific type of molecular motor, thus maintaining the distinctive distribution of axonal organelles (Figure 7–8). In dendrites, however, microtubules with opposite polarities are mixed together, explaining why the organelles of the cell body and dendrites are similar.

Box 7–1 Abnormal Accumulations of Proteins Are Hallmarks of Many Neurological Disorders (continued)

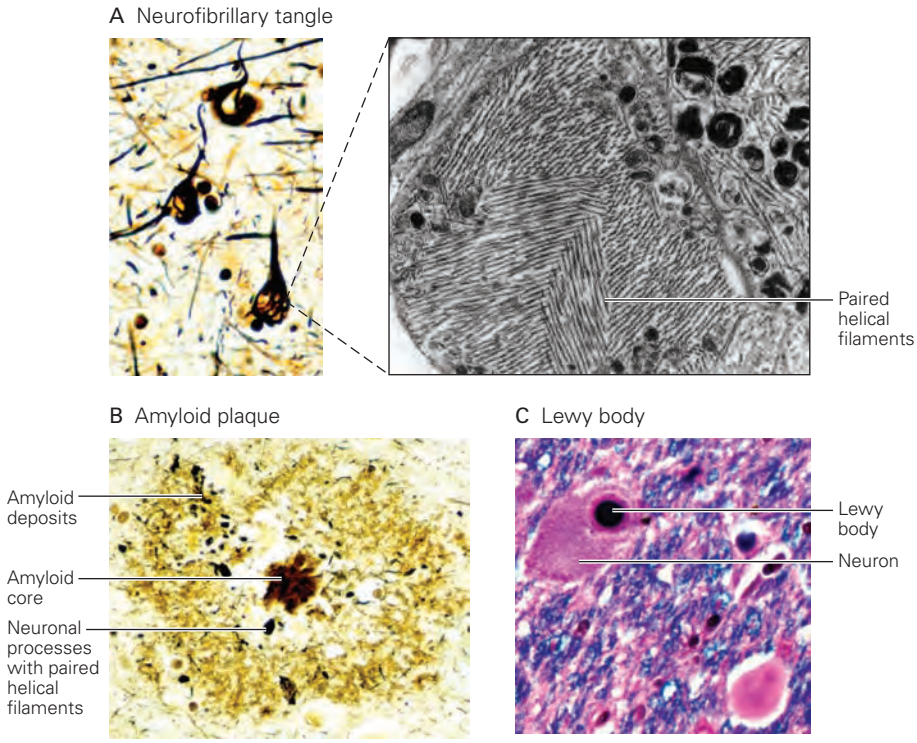


Figure 7–7 Abnormal aggregates of proteins inside neurons in Alzheimer and Parkinson diseases.

A. *Left:* Intracellular neurofibrillary tangles of Alzheimer disease, labeled here with a dark silver stain. (Reproduced, with permission, from J.P. Vonsattel.) *Right:* An electron micrograph of a tangle shows bundles of abnormal filaments, filling a dendrite. The filaments are composed of altered tau protein. (Used, with permission, from Dr. L. Carrasco, formerly of Runwell Hospital, Wickford, UK.)

B. In Alzheimer disease, amyloid plaque is created by extracellular deposits of polymerized β-amyloid peptides. The plaque shown here has a dense core of amyloid as well as a surrounding halo of deposits. Some neuronal processes in the plaque exhibit tangle pathology. (Reproduced, with permission, from J.P. Vonsattel.)

C. A Lewy body in the substantia nigra of a patient with Parkinson disease contains accumulations of abnormal filaments made up of α-synuclein, among other proteins. (Reproduced, with permission, from J.P. Vonsattel.)

Protein Particles and Organelles Are Actively Transported Along the Axon and Dendrites

In neurons, most proteins are made in the cell body from mRNAs. Important examples are neurotransmitter biosynthetic enzymes, synaptic vesicle membrane components, and neurosecretory peptides. Because axons and terminals often lie at great distances from the cell body, sustaining the function of these remote regions presents a challenge. Passive diffusion would be far too slow to deliver vesicles, particles, or even single macromolecules over this great distance.

The axon terminal, the site of secretion of neurotransmitters, is particularly distant from the cell body. In a motor neuron that innervates a muscle of the leg in humans, the distance of the nerve terminal from the cell body can exceed 10,000 times the cell body diameter. Thus, membrane and secretory products formed in the cell body must be actively transported to the end of the axon (Figure 7–9).

In 1948, Paul Weiss first demonstrated axonal transport when he tied off a sciatic nerve and observed that axoplasm in the nerve accumulated with time on the proximal side of the ligature. He concluded that axoplasm

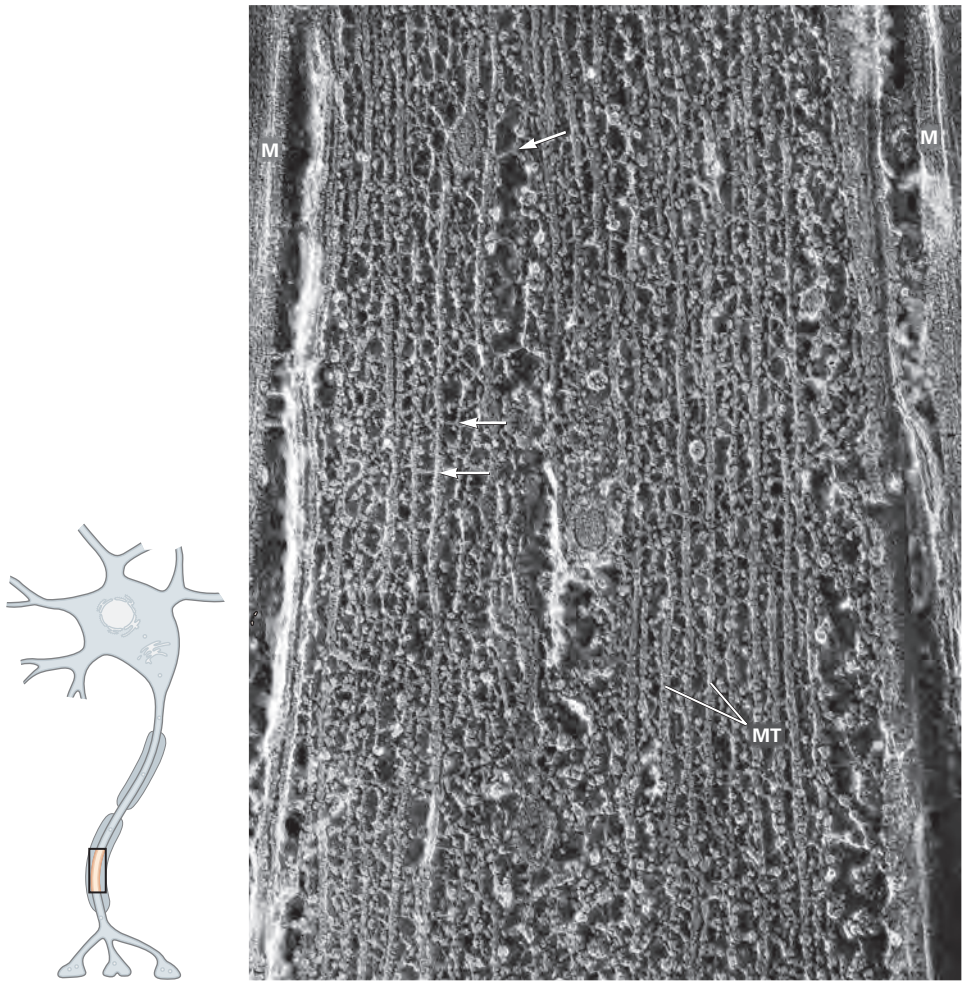


Figure 7–8 The cytoskeletal structure of an axon. The micrograph shows the dense packing of microtubules and neurofilaments linked by cross bridges (**arrows**). Organelles are transported in both the anterograde and retrograde directions in the microtubule-rich domains. Visualization in the micrograph was achieved by quick freezing and deep etching. **M**, myelin sheath; **MT**, microtubules. ×105,000. (Adapted, with permission, from Schnapp and Reese 1982. Copyright © 1982 Rockefeller University Press.)

moves at a slow, constant rate from the cell body toward terminals in a process he called *axoplasmic flow.* Today we know that the flow Weiss observed consists of two discrete mechanisms, one fast and the other slow.

Membranous organelles move toward axon terminals (anterograde direction) and back toward the cell body (retrograde direction) by *fast axonal transport*, a form of transport that is up to 400 mm per day in warm-blooded animals. In contrast, cytosolic and cytoskeletal proteins move only in the anterograde direction by a much slower form of transport, *slow axonal transport.* These transport mechanisms in neurons are adaptations of processes that facilitate intracellular movement of organelles in all secretory cells. Because all these mechanisms operate along axons, they have been used by neuroanatomists to trace the course of individual axons as well as interconnections between neurons (Box 7–2).

Fast Axonal Transport Carries Membranous Organelles

Large membranous organelles are carried to and from the axon terminals by fast transport (Figure 7–11). These organelles include synaptic vesicle precursors, large dense-core vesicles, mitochondria, elements of the smooth endoplasmic reticulum, and protein particles carrying RNAs. Direct microscopic analysis reveals that fast transport occurs in a stop-and-start (saltatory) fashion along linear tracks of microtubules aligned with the main axis of the axon. The saltatory nature of the movement results from the periodic dissociation

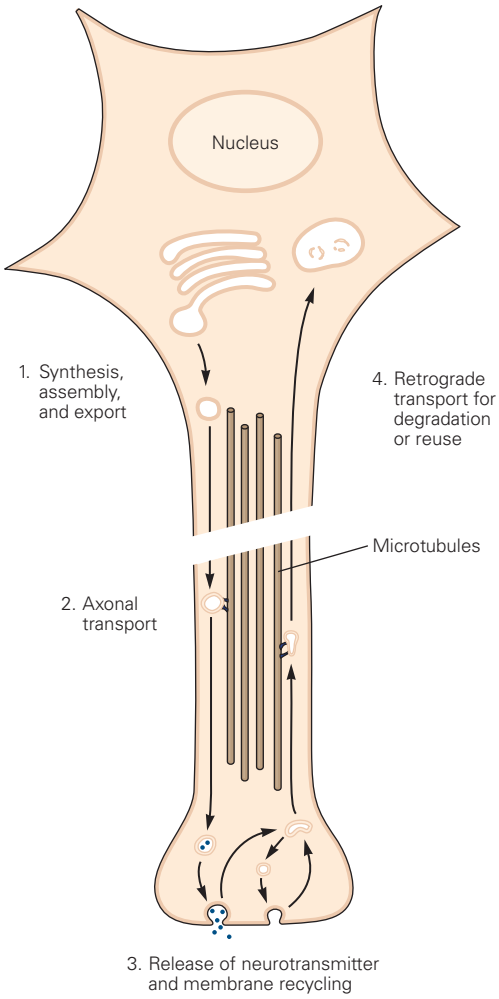


Figure 7–9 Membrane trafficking in the neuron. **1.** Proteins and lipids of secretory organelles are synthesized in the endoplasmic reticulum and transported to the Golgi complex, where large dense-core vesicles (peptide-containing secretory granules) and synaptic vesicle precursors are assembled. **2.** Large dense-core vesicles and transport vesicles carry synaptic vesicle proteins down the axon via axonal transport. **3.** At the nerve terminals, the synaptic vesicles are assembled and loaded with nonpeptide neurotransmitters. Synaptic vesicles and large dense-core vesicles release their contents by exocytosis. **4.** Following exocytosis, large dense-core vesicle membranes are returned to the cell body for reuse or degradation. Synaptic vesicle membranes undergo many cycles of local exocytosis and endocytosis in the presynaptic terminal.

of an organelle from the track or from collisions with other particles.

Early experiments on dorsal root ganglion cells showed that anterograde fast transport depends critically on ATP, is not affected by inhibitors of protein synthesis (once the injected labeled amino acid is incorporated), and does not depend on the cell body, because it occurs in axons severed from their cell bodies. In fact, active transport can occur in reconstituted cell-free axoplasm.

Microtubules provide an essentially stationary track on which specific organelles can be moved by molecular motors. The idea that microtubules are involved in fast transport emerged from the finding that certain alkaloids that disrupt microtubules and block mitosis, which depends on microtubules, also interfere with fast transport.

Molecular motors were first visualized in electron micrographs as cross bridges between microtubules and moving particles (Figure 7–8). More advanced fluorescence time-lapse microscopy techniques are able to visualize the dynamics of axon transport for specific cargos such as mitochondria and synaptic vesicles. The motor molecules for anterograde transport are plus-end-directed motors called *kinesin* and a variety of kinesin-related proteins. The kinesins represent a large family of adenosine triphosphatases (ATPase), each of which transports different cargos. Kinesin is a heterotetramer composed of two heavy chains and two light chains. Each heavy chain has three domains: (1) a globular head (the ATPase domain) that acts as the motor when attached to microtubules, (2) a coiled-coil helical stalk responsible for dimerization with the other heavy chain, and (3) a fan-like carboxyl-terminus that interacts with the light chains. This end of the complex attaches indirectly to the organelle that is moved through specific families of proteins referred to as cargo adapters.

Fast retrograde transport primarily moves endosomes generated by endocytic activity at nerve endings, mitochondria, and elements of the endoplasmic reticulum. Many of these components are degraded through fusion with lysosomes. Fast retrograde transport also delivers signals that regulate gene expression in the neuron's nucleus. For example, activated growth factor receptors at nerve endings are taken up into vesicles and carried back along the axon to the nucleus. Transport of transcription factors informs the gene transcription apparatus in the nucleus of conditions in the periphery. Retrograde transport of these molecules is especially important during nerve regeneration and axon regrowth (Chapter 47). Certain toxins (tetanus toxin) as well as pathogens (herpes simplex, rabies, and polio viruses) are also transported toward the cell body along the axon.

The rate of retrograde fast transport is approximately one-half to two-thirds that of anterograde fast transport. As in anterograde transport, particles move along microtubules during retrograde flow. The motor molecules for retrograde axonal transport are minus-end-directed motors called *dyneins*, similar to those found in cilia and

Box 7–2 Neuroanatomical Tracing Makes Use of Axonal Transport

Neuroanatomists typically locate axons and terminals of specific nerve cell bodies by microinjection of dyes; expression of fluorescent proteins; or autoradiographically tracing specific proteins soon after administering radioactively labeled amino acids, certain labeled sugars (fucose or amino sugars, precursors of glycoprotein), or specific transmitter substances.

Similarly, particles, proteins, or dyes that are readily taken up at nerve terminals by endocytosis and transported back to cell bodies are used to identify the cell bodies. The enzyme horseradish peroxidase has been most widely used for this type of study because it readily undergoes retrograde transport and its reaction product is conveniently visualized histochemically.

Axonal transport is also used by neuroanatomists to label material exchanged between neurons, making it possible to identify neuronal networks (Figure 7–10).

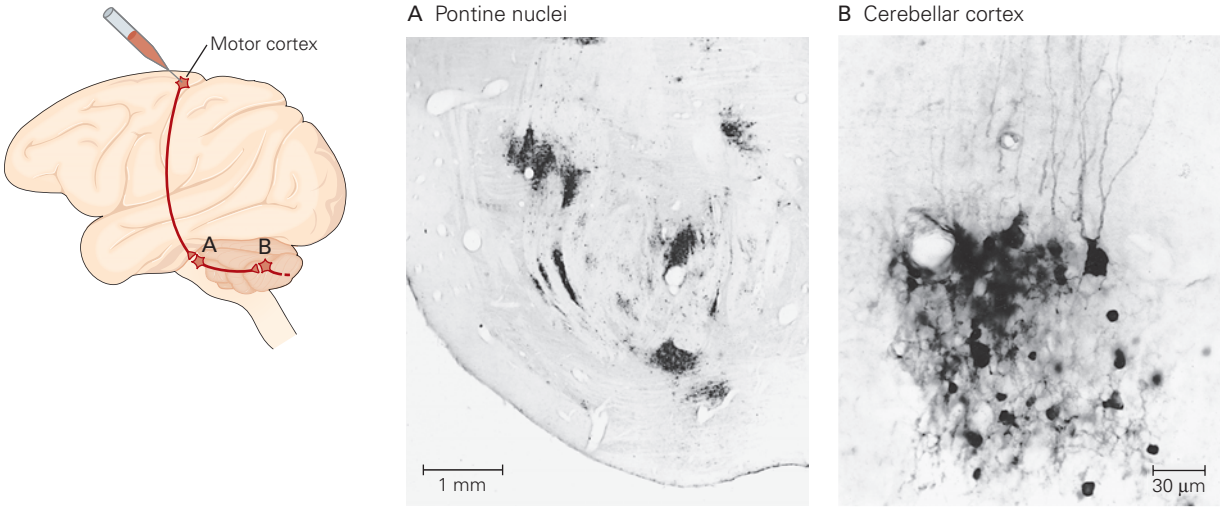


Figure 7–10 Axonal transport of the herpes simplex virus (HSV) is used to trace cortical pathways in monkeys. Depending on the strain, the virus moves in the anterograde or retrograde direction by axonal transport. In either direction, it enters a neuron with which the infected cell makes synaptic contact. Here the projections of cells in the primary motor cortex to the cerebellum in monkeys were traced using an anterograde-moving strain (HSV-1 [H129]). Monkeys were injected in the region of the primary motor cortex that controls the arm. After 4 days, the brain was sectioned and immunostained for viral antigen. Micrographs show the virus was transported from the primary motor cortex to second-order neurons in pontine nuclei (**A**) and then to third-order neurons in the cerebellar cortex (**B**). (Reproduced, with permission, from P.L. Strick.)

flagella of nonneuronal cells. They consist of a multimeric ATPase protein complex with two globular heads on two stalks connected to a basal structure. The globular heads attach to microtubules and act as motors, moving toward the minus end of the polymer. As with kinesin, the other end of the complex attaches to the transported organelle through specialized cargo adapters.

Microtubules also mediate anterograde and retrograde transport of mRNAs and ribosomal RNA carried in particles formed with RNA-binding proteins. These proteins have been characterized in both vertebrate and invertebrate nervous systems and include the cytoplasmic polyadenylation element binding protein (CPEB), the fragile X protein, Hu proteins, NOVA, and Staufen. The activities of these proteins are critical. For example, CPEB keeps select mRNAs dormant during transport from the cell body to nerve endings; once there (upon stimulation), the binding protein can facilitate the local translation of the RNA by mediating polyadenylation and activation of the messenger. Both CPEB and Staufen were discovered in *Drosophila*, where they maintain maternal mRNAs dormant in

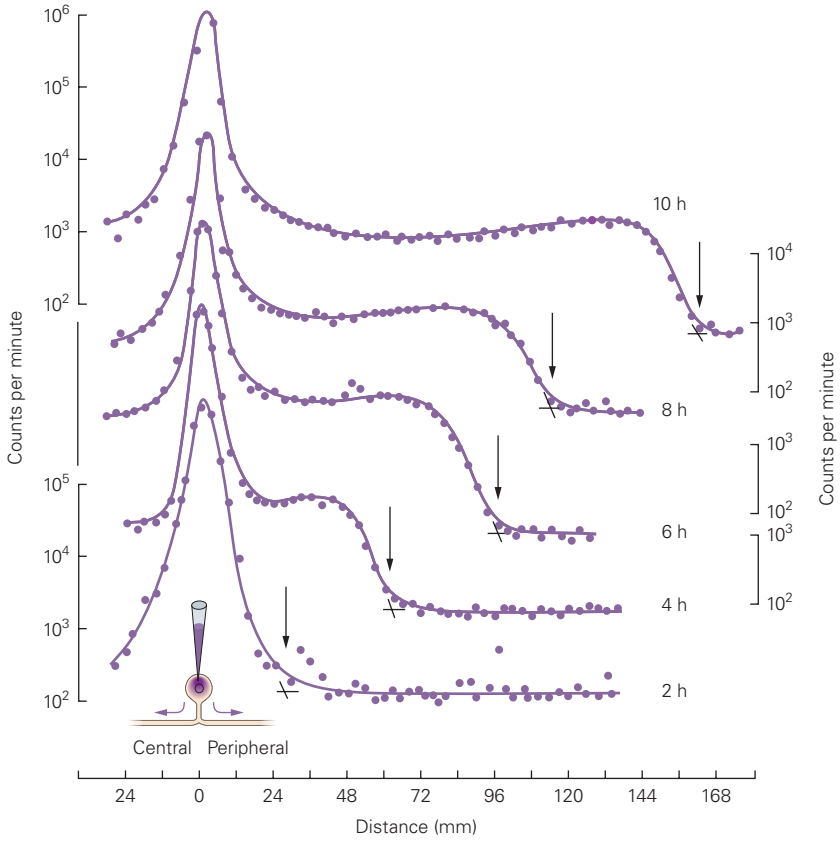


Figure 7–11 Early experiments on anterograde axonal transport used radioactive labeling of proteins. In the experiment illustrated here, the distribution of radioactive proteins along the sciatic nerve of the cat was measured at various times after injection of [^{3}H]-leucine into dorsal root ganglia in the lumbar region of the spinal cord. To show transport curves for various times (2, 4, 6, 8, and 10 hours after the injection) in one figure, several ordinate scales (in logarithmic units) are used. Large amounts of labeled protein stay in the ganglion cell bodies but, with time, move out along axons in the sciatic nerve, so the advancing front of the labeled protein is progressively farther from the cell body (**arrows**). The velocity of transport can be calculated from the distance of the front at the various times. From experiments of this kind, Sidney Ochs found that the rate of fast axonal transport is constant at 410 mm per day at body temperature. (Adapted, with permission, from Ochs 1972. Copyright © 1972 AAAS.)

unfertilized eggs and, upon fertilization, distribute and localize mRNA to various regions of the dividing embryo. Loss-of-function mutations in the fragile X (*FMR1*) gene lead to a severe form of mental retardation.

Proteins, ribosomes, and mRNA are concentrated at the base of some dendritic spines (Figure 7–12). Only a select group of mRNAs are transported into the dendrites from the soma. These include mRNAs that encode actin- and cytoskeletal-associated proteins, MAP2, and the α-subunit of the Ca^{2+}/calmodulin-dependent protein kinase. They are translated in the dendrites in response to activity in a presynaptic neuron. This local protein synthesis is thought to be important in sustaining the molecular changes at the synapse that underlie long-term memory and learning. Likewise, the mRNA for myelin basic protein is transported to the distant ends of the oligodendrocytes, where it is translated as the myelin sheath grows, as discussed later in this chapter.

Slow Axonal Transport Carries Cytosolic Proteins and Elements of the Cytoskeleton

Cytosolic proteins and cytoskeletal proteins are moved from the cell body by slow axonal transport. Slow transport occurs only in the anterograde direction and consists of at least two kinetic components that carry different proteins at different rates.

The slower component travels at 0.2 to 2.5 mm per day and carries the proteins that make up the fibrillar elements of the cytoskeleton: the subunits of

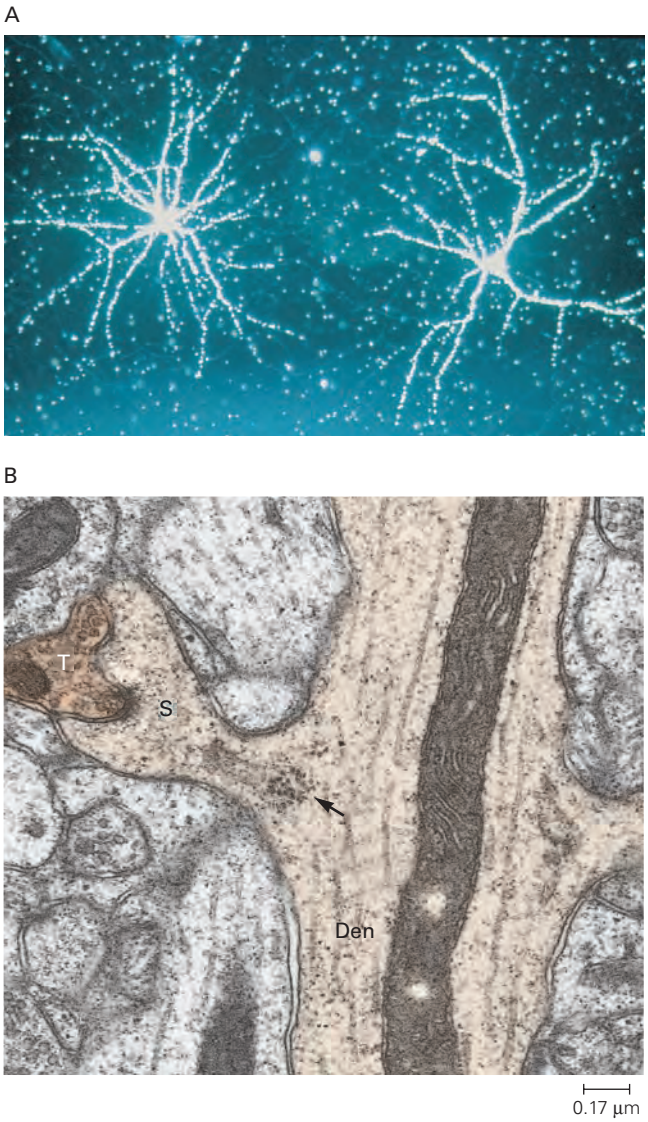


Figure 7–12 Ribosomes in the dendritic arbor. (Images reproduced, with permission, from Oswald Steward.)

A. Some ribosomes are dispatched from the cell body to dendrites where they are used in local protein synthesis. This autoradiograph shows the distribution of ribosomal RNA (rRNA) in hippocampal neurons in low-density cultures using in situ hybridization. The image was made with dark field illumination, in which silver grains reflect light and thus appear as bright spots. Silver grains, denoting the rRNA, are heavily concentrated over cell bodies and dendrites but are not detectable over the axons that crisscross among the dendrites.

B. Ribosomes in dendrites are selectively concentrated at the junction of the spine and main dendritic shaft (**arrow**), where the spine contacts the axon terminal of a presynaptic neuron. This electron micrograph shows a mushroom-shaped spine of a neuron in the hippocampal dentate gyrus. Note the absence of ribosomes in the dendritic shaft. **S**, spine head; **T**, presynaptic terminal; **Den**, main shaft of the dendrite containing a long mitochondrion. ×60,000.

neurofilaments and α- and β-tubulin subunits of microtubules. These fibrous proteins constitute approximately 75% of the total protein moved in the slower component. Microtubules are transported in polymerized form by a mechanism involving microtubule sliding in which relatively short preassembled microtubules move along existing microtubules. Neurofilament monomers or short polymers move passively together with the microtubules because they are crosslinked by protein bridges.

The other component of slow axonal transport is approximately twice as fast as the slower component. It carries clathrin, actin, and actin-binding proteins as well as a variety of enzymes and other proteins.

Proteins Are Made in Neurons as in Other Secretory Cells

Secretory and Membrane Proteins Are Synthesized and Modified in the Endoplasmic Reticulum

The mRNAs for secretory and membrane proteins are translated through the membrane of the rough endoplasmic reticulum, and their polypeptide products are processed extensively within the lumen of the endoplasmic reticulum. Most polypeptides destined to become proteins are translocated across the membrane of the rough endoplasmic reticulum during synthesis, a process called *cotranslational transfer.*

Transfer is possible because ribosomes, the site where proteins are synthesized, attach to the cytosolic surface of the reticulum (Figure 7–13). Complete transfer of the polypeptide chain into the lumen of the reticulum produces a secretory protein (recall that the inside of the reticulum is related to the outside of the cell). Important examples are the neuroactive peptides. If the transfer is incomplete, an integral membrane protein results. Because a polypeptide chain can thread its way through the membrane many times during synthesis, several membrane-spanning configurations are possible depending on the primary amino acid sequence of the protein. Important examples are neurotransmitter receptors and ion channels (Chapter 8).

Some proteins transported into the endoplasmic reticulum remain there. Others are moved to other compartments of the vacuolar apparatus or to the plasmalemma, or are secreted into the extracellular space. Proteins that are processed in the endoplasmic reticulum are extensively modified. One important modification is the formation of intramolecular disulfide linkages (Cys-S-S-Cys) caused by oxidation of pairs of free sulfhydryl side chains, a process that cannot occur

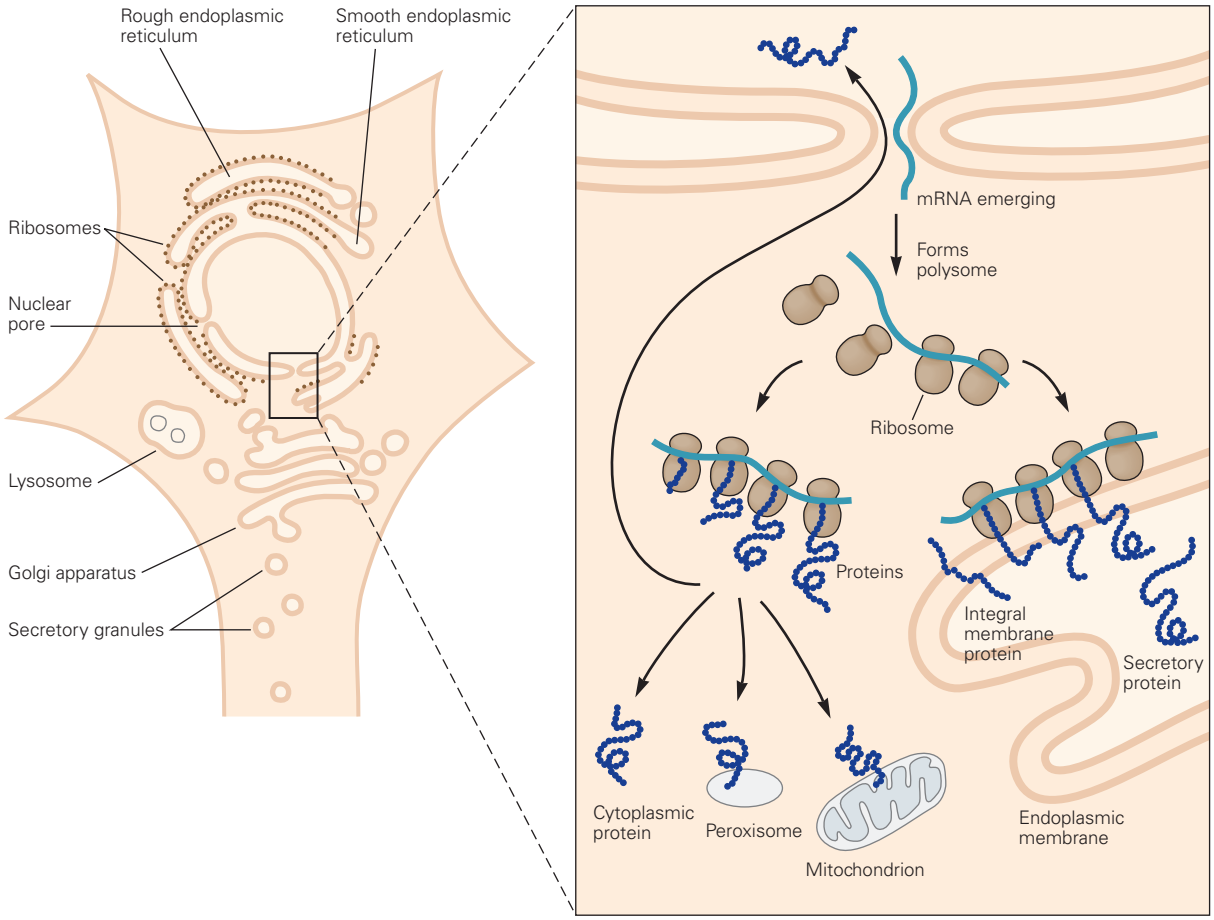
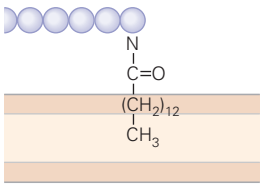


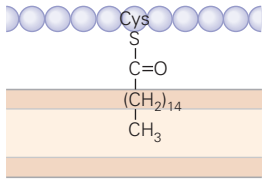
Figure 7–13 Protein synthesis in the endoplasmic reticulum. Free and membrane-bound polysomes translate mRNA that encodes proteins with a variety of destinations. Messenger RNA, transcribed from genomic DNA in the neuron's nucleus, emerges into the cytoplasm through nuclear pores to form polyribosomes (see enlargement). The polypeptides that become secretory and membrane proteins are translocated across the membrane of the rough endoplasmic reticulum.

in the reducing environment of the cytosol. Disulfide linkages are crucial to the tertiary structure of these proteins.

Proteins may be modified by cytosolic enzymes either during synthesis (cotranslational modification) or afterward (posttranslational modification). One example is *N*-acylation, the transfer of an acyl group to the N-terminus of the growing polypeptide chain. Acylation by a 14-carbon fatty acid myristoyl group permits the protein to anchor in membranes through the lipid chain.



Other fatty acids can be conjugated to the sulfhydryl group of cysteine, producing a thioacylation:



Isoprenylation is another posttranslational modification important for anchoring proteins to the cytosolic side of membranes. It occurs shortly after synthesis of the protein is completed and involves a series of enzymatic steps that result in thioacylation by one of two long-chain hydrophobic polyisoprenyl moieties (farnesyl, with 15 carbons, or geranyl-geranyl, with 20) of the sulfhydryl group of a cysteine at the C-terminus of proteins.