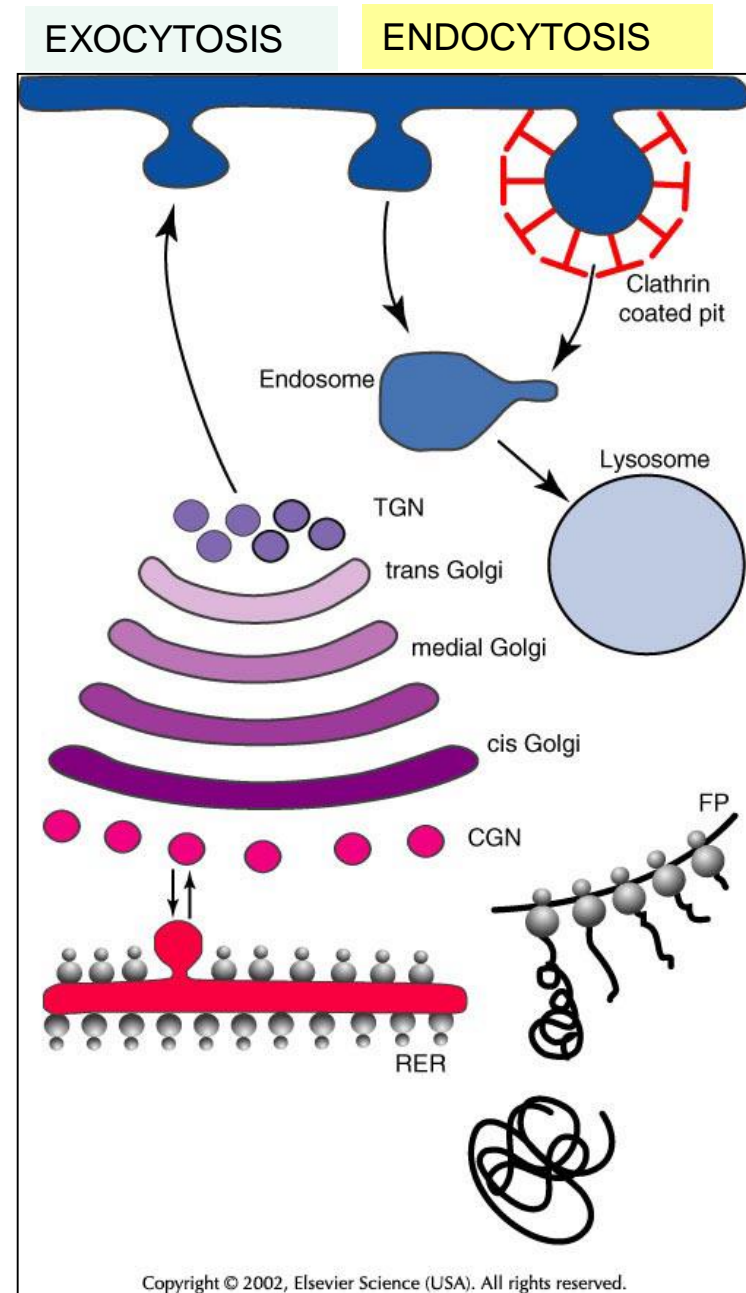
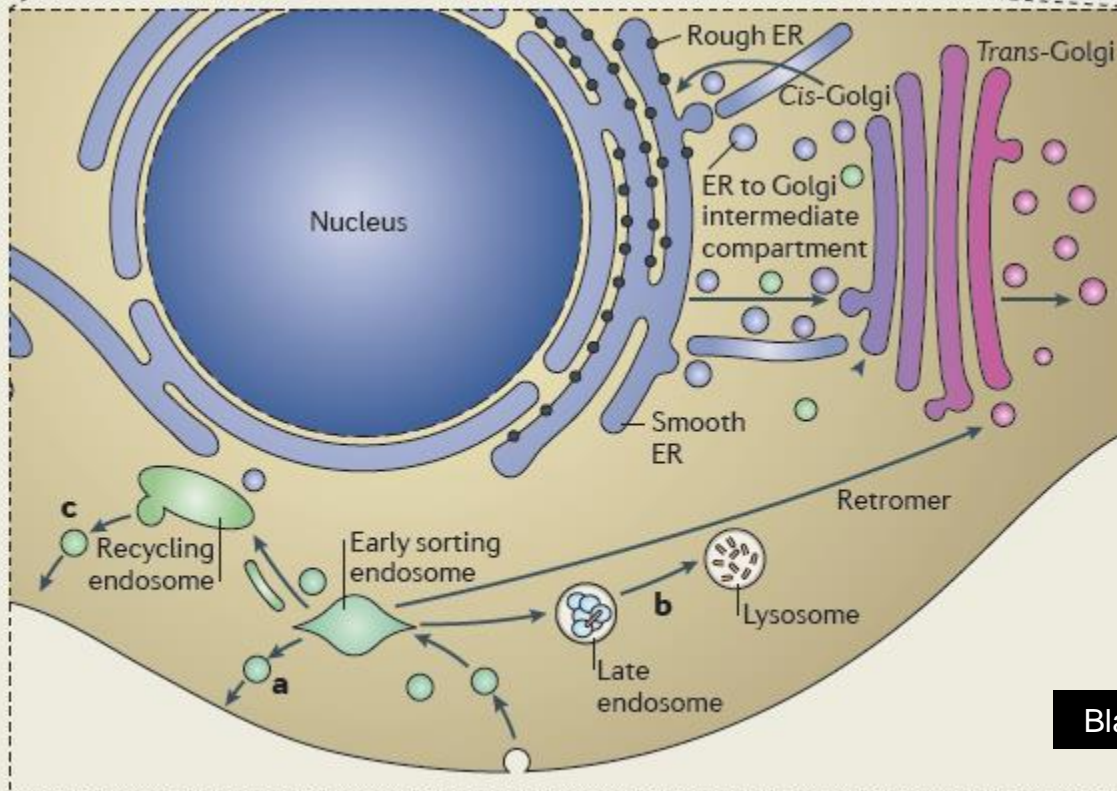
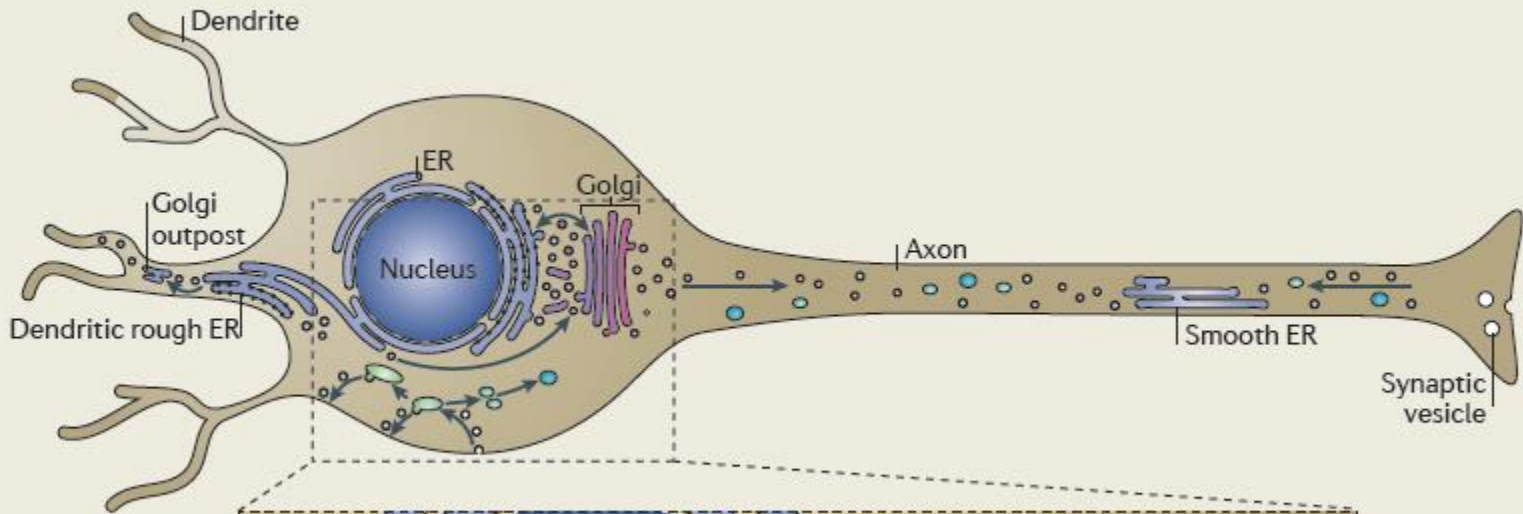


The neuron as a secretory cell

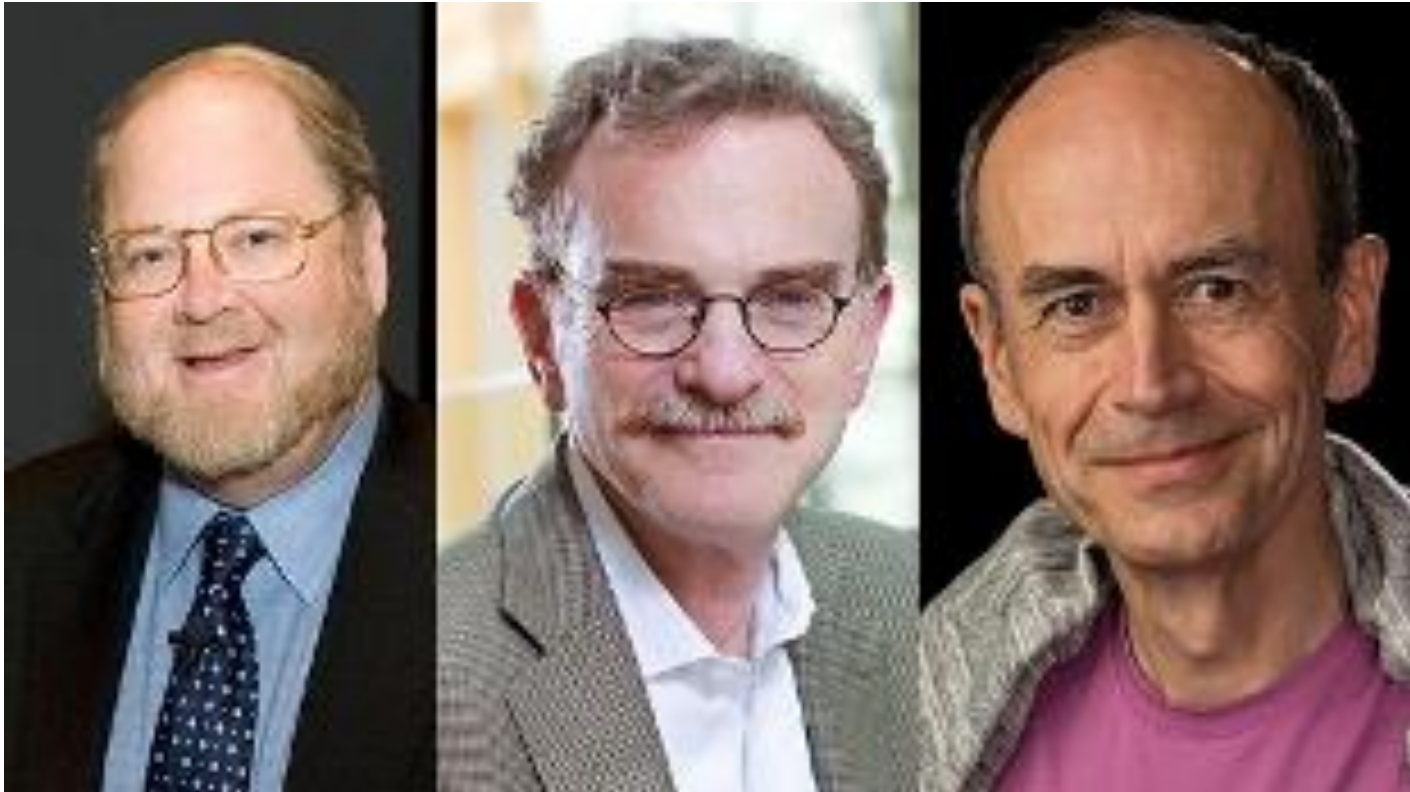
The secretory pathway. Transport and sorting of proteins in the secretory pathway occur as they pass through the Golgi complex before reaching the plasma membrane. Sorting occurs in the *cis*-Golgi network (CGN), also known as the intermediate compartment, and in the *trans*-Golgi network (TGN). Proteins exit from the Golgi complex at the TGN. The default pathway is the direct route to the plasma membrane. Proteins bound for regulated secretion or for transport to endosomes and from there to lysosomes are diverted from the default path by means of specific signals. In endocytosis, one population of vesicles is surrounded by a clathrin cage and is destined for late endosomes. Another population appears to be coated in a lace-like structure whose composition is yet to be defined.



Box 2 | Membrane traffic pathways in non-polarised cells and neurons.



The 2013 Nobel prizes in Medicine and Physiology

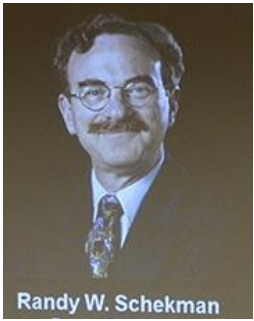


J.E. Rothman

R.W. Sheckman

T.C. Sudhof

*“for their discoveries of machinery regulating **vesicle traffic**, a major transport system in our cells”*



Randy W. Schekman

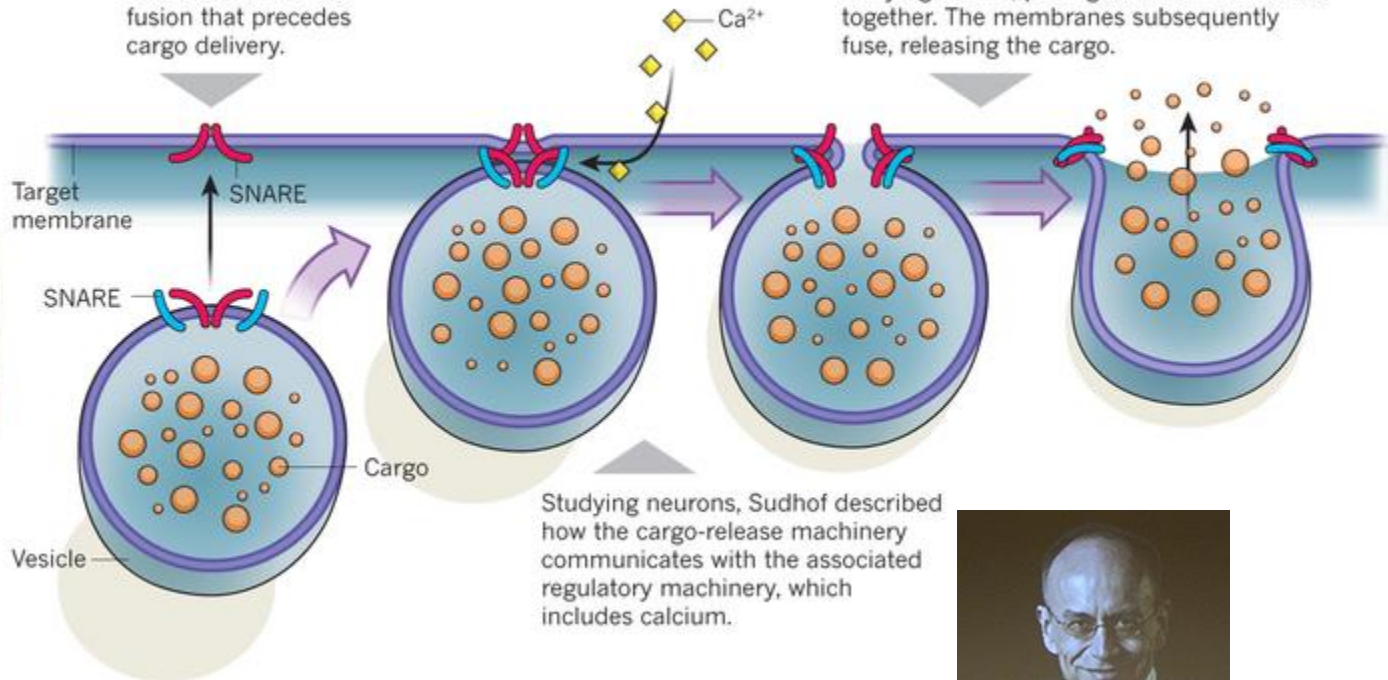
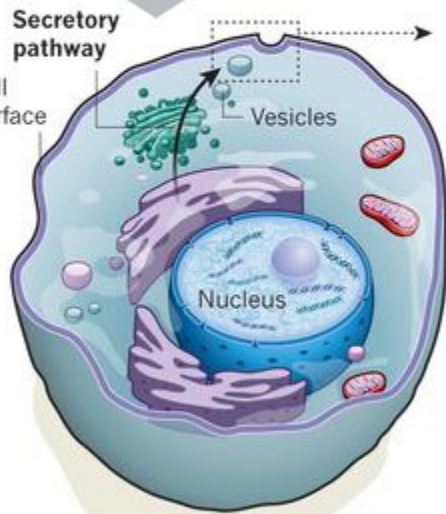
Schekman identified many of the genes that control intracellular transport of the cargo-carrying vesicles along the secretory pathway in yeast cells.



James E. Rothman

Rothman revealed that SNARE proteins mediate membrane fusion that precedes cargo delivery.

On the target membrane, SNARE proteins 'zip up' with specific SNAREs on the cargo-carrying vesicle, pulling the two membranes together. The membranes subsequently fuse, releasing the cargo.



Studying neurons, Südhof described how the cargo-release machinery communicates with the associated regulatory machinery, which includes calcium.



Thomas C. Südhof

Destiny of vesicles that exit from the *trans* Golgi network

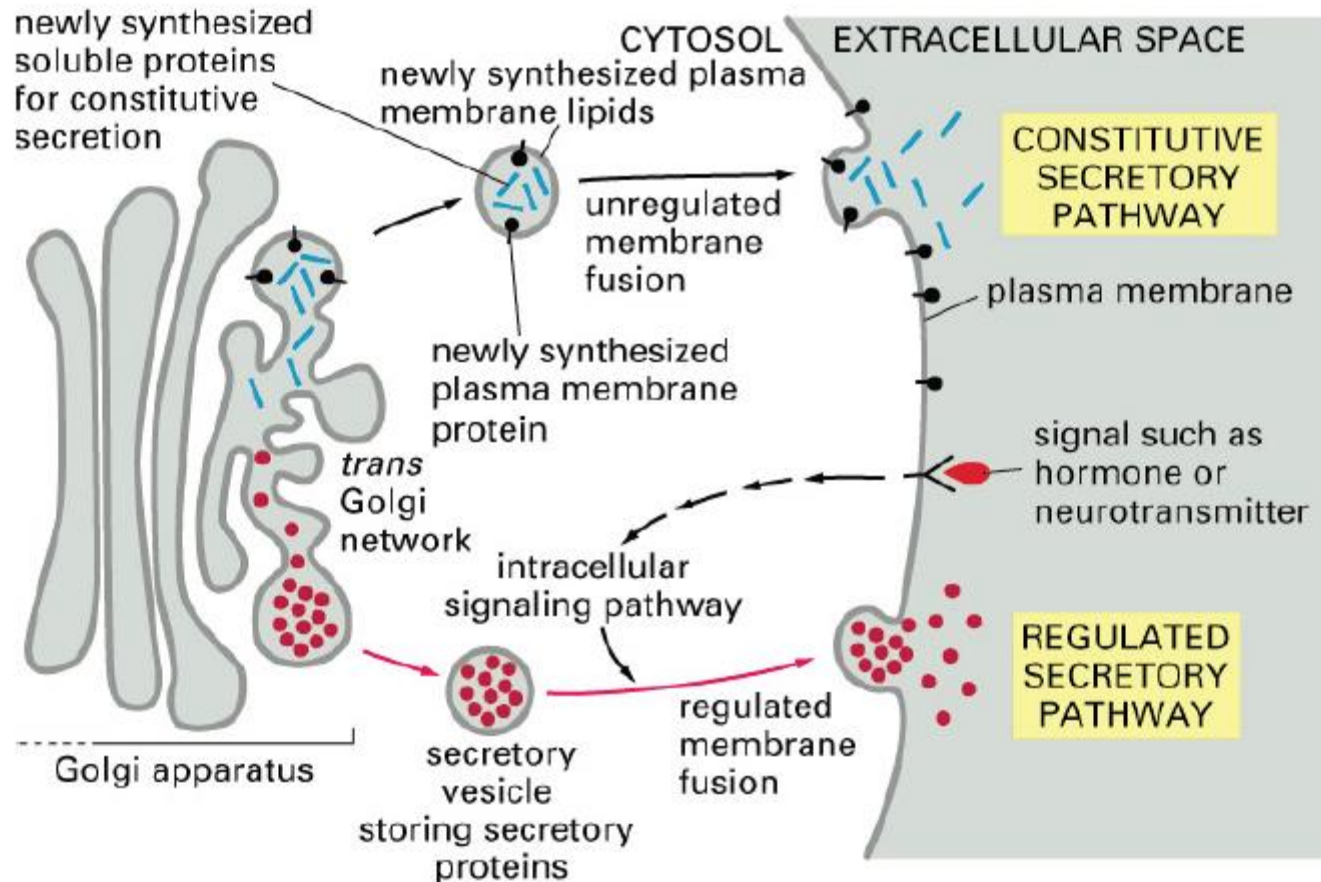
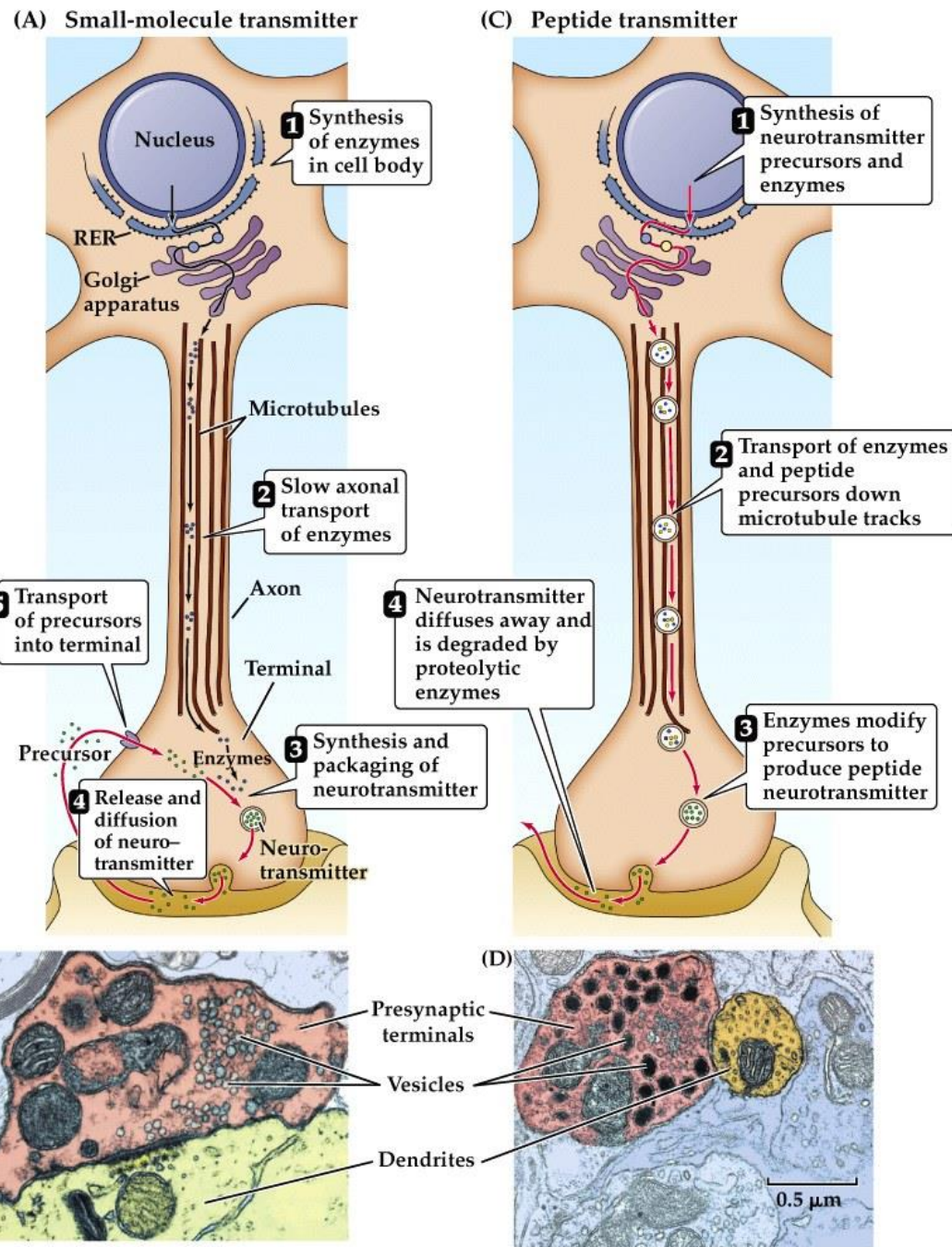


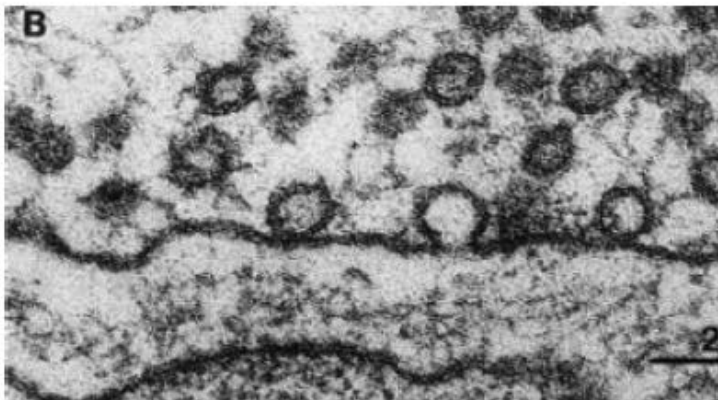
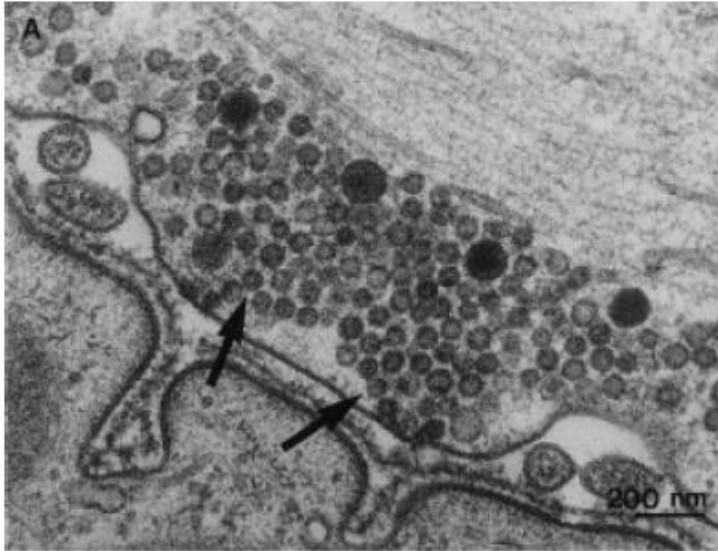
Figure 13-54. Molecular Biology of the Cell, 4th Edition.

**Synaptic vesicles for
small-molecule
(small/clear vesicles)
and peptide transmitters
(dense-core granules)**



NEUROSCIENCE 5e, Figure 5.5

Why store transmitters in vesicles?



- Protection from degradation - by proteases and esterases
- Allows for regulation
- Provides a storage system
- Can be docked at active zone
- Differ for classical transmitters (small, clear-core) vs. neuropeptides (large, dense-core)

Mechanisms of trafficking and targeting of neuronal proteins

How are integral membrane proteins and secreted factors delivered to the appropriate neuronal domain, at the right time and in the right amounts?

Two main mechanisms:

1. Long-range protein trafficking (from cell body to proximal/distal dendrites, axon, presynaptic terminal, etc.....)
2. Local RNA translation and trafficking (within **dendritic spines**, growth cones, immature axon terminals and regenerating axons)

Mechanisms of trafficking and targeting of neuronal proteins.....

Specific neurotransmitter receptors and ion channels must be delivered to specific subcellular compartments (axonal, somatodendritic, etc.)

What is the targeting mechanisms of these proteins? Is there only one fundamental targeting mechanism, or more than one exists?

Targeting of axonal and dendritic proteins is mediated by multiple mechanisms

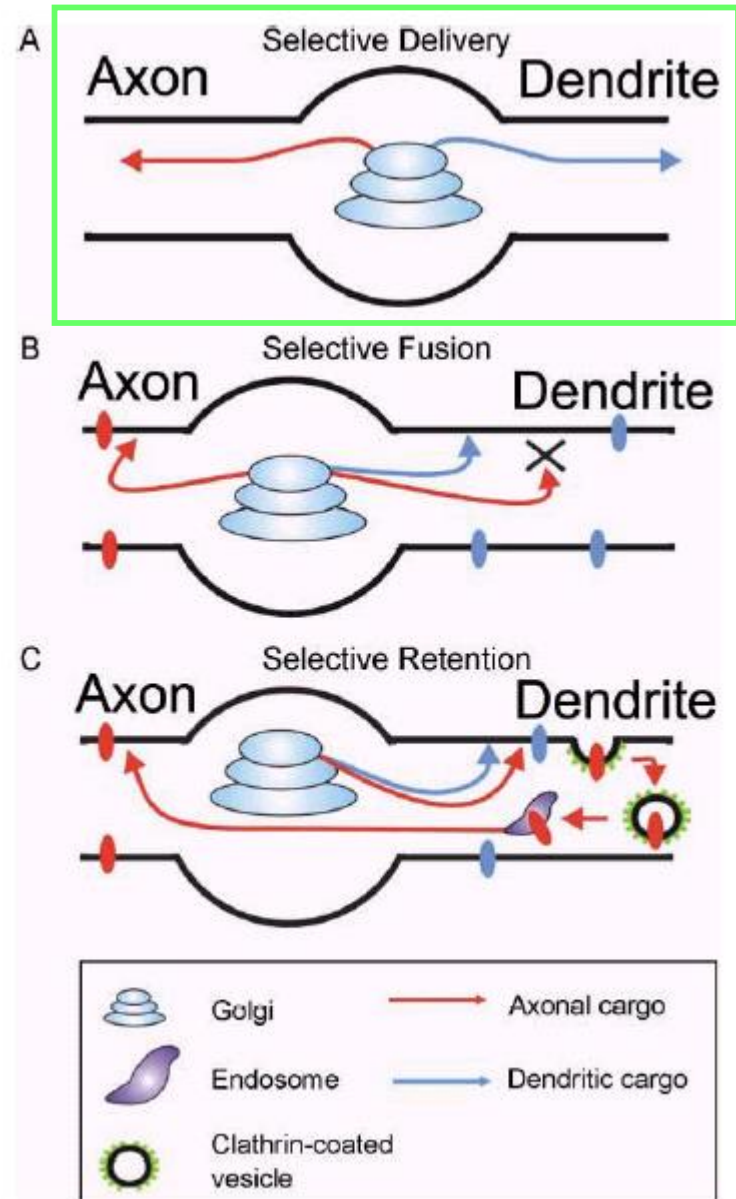
- a) direct polarized delivery from the trans-Golgi network
- b) non-polarized delivery followed by selective retrieval and retention
- c) indirect polarized delivery via endosomes (transcytosis)

Figure 4. Multiple Mechanisms Function in Neurons for the Targeting of Dendritic and Axonal Proteins

(A) In this schematized model, axonal and dendritic proteins are sorted at the level of the Golgi into carriers that preferentially deliver their cargo to axons or dendrites, respectively.

(B) In some cases, carriers of axonal proteins can traffic within dendrites but are not competent to fuse with the dendritic membrane, ensuring selective surface delivery only in the axon.

(C) In other cases, axonal proteins are delivered to the dendritic plasma membrane alongside dendritic proteins, either in distinct or perhaps in common post-Golgi carriers. After a transient period on the cell surface, axonal proteins are selectively endocytosed and trafficked via transcytosis to the axonal membrane.

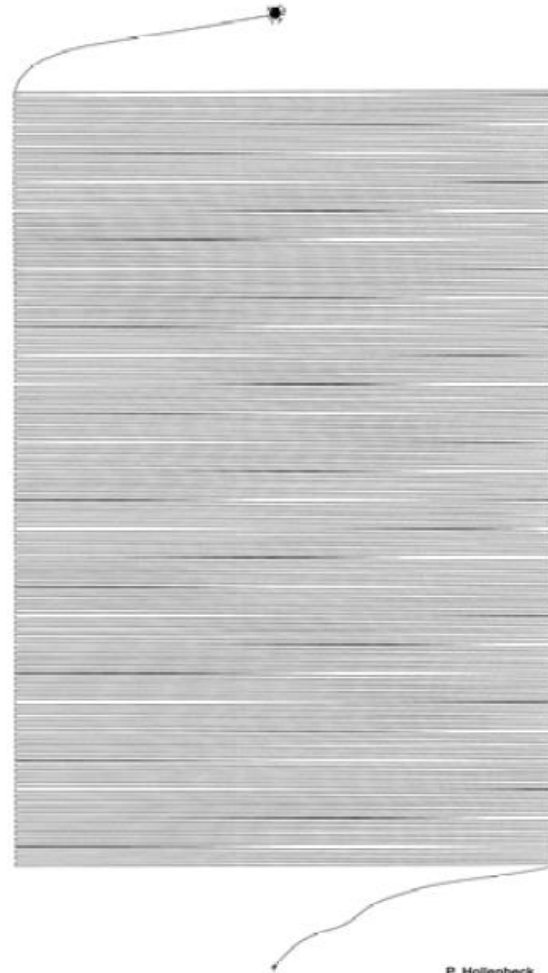


How are proteins and small organelles delivered to the **axon terminal**?

Axonal Transport

Axons can be >95% of total neuronal volume.

All proteins in the axon are made in cell body and must be transported into and along the axon



Roles of axonal transport:

ANTEROGRADE

- Establishing neuronal polarity
- Deliver growth and guidance molecules to the growth cone
- Deliver synaptic components
- Deliver cytoskeletal construction along the axon
- Neurotrophin transport
- Energy supply - mitochondrial transport
- Transport of mRNA

RETROGRADE

- Growth factor signaling
- Injury signaling
- Neurotoxin / viral transport
- Energy supply - mitochondrial transport

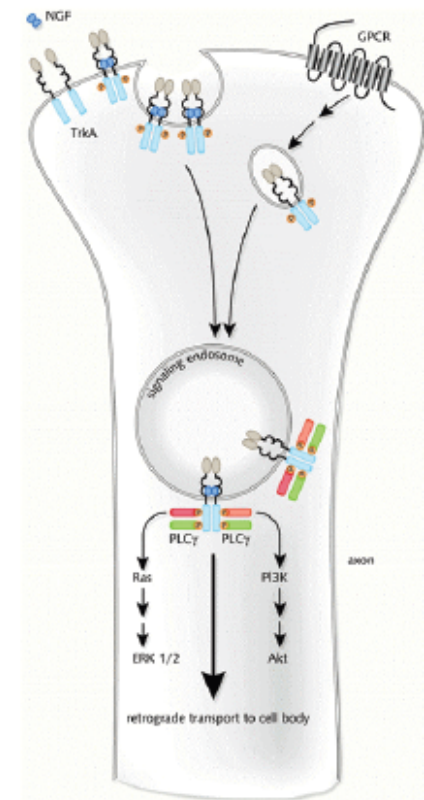
NGF retrograde transport:

-Many neurons in mammalian brain are dependent on trophic factors for survival

-Nerve Growth Factor (NGF) required for survival of sensory neurons

-NGF is secreted by the peripheral targets of sensory neurons (salivary glands in original isolation of NGF)

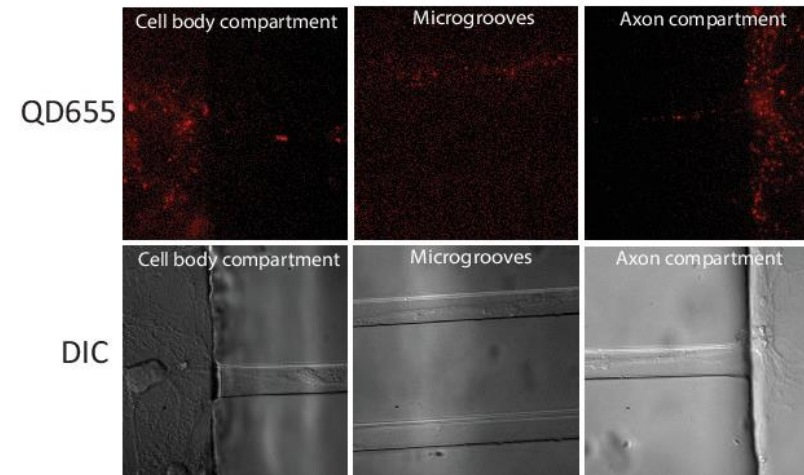
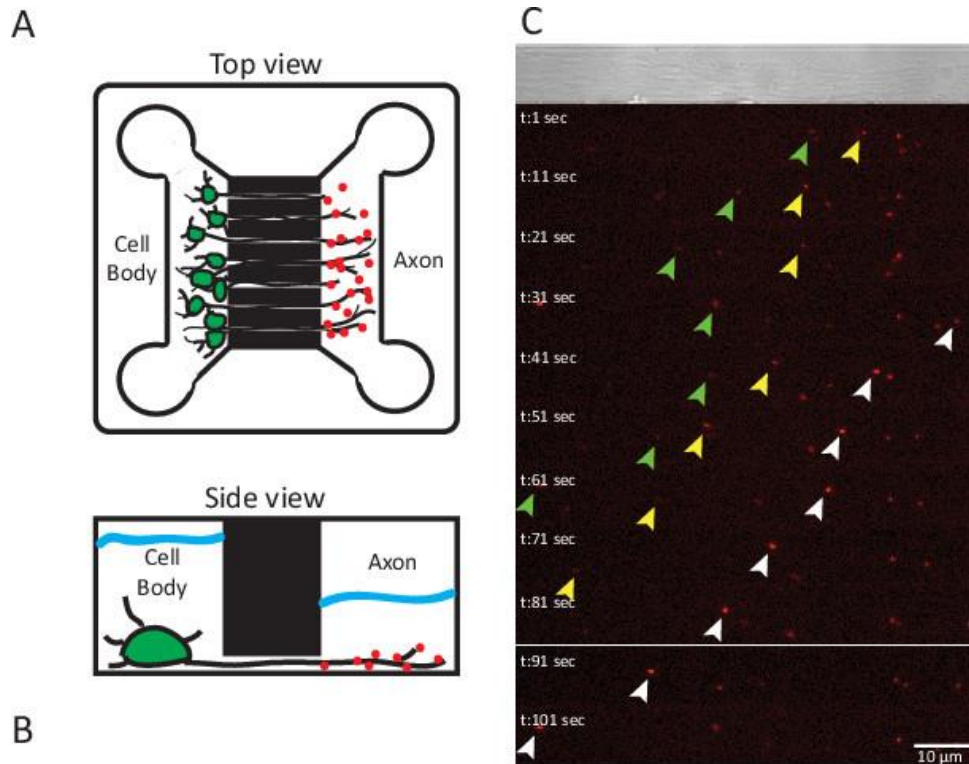
NGF-TrkA receptor complexes are endocytosed and are transported back to soma in signaling endosomes by dynein.



Real-time Imaging of Axonal Transport of Quantum Dot-labeled BDNF in Primary Neurons

Xiaobei Zhao¹, Yue Zhou², April M. Weissmiller¹, Matthew L. Pearn^{3,4}, William C. Mobley¹, Chengbiao Wu¹

<http://www.jove.com/video/51899/real-time-imaging-axonal-transport-quantum-dot-labeled-bdnf-primary>



Pathak et al. • Quantum Dot Labeling of Neurons and Glia

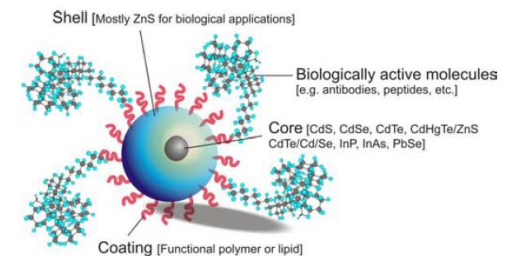


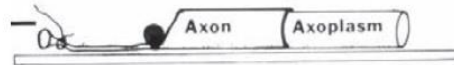
Figure 1. Structure of a semiconductor fluorescent quantum dot nanocrystal. The heavy metal core is responsible for the fluorescence properties of the quantum dot. The nonemissive shell stabilizes the core, whereas the coating layer provides anchor sites to organic and biological ligands such as antibodies, peptides, and other organic molecules.

How do we study axonal transport?

- ❑ Metabolic cell-labeling experiments in the 1960s demonstrated the rapid movement of newly synthesized proteins along the axon in a process termed “**cellulifugal transport**” (Weiss, 1967).
- ❑ Experiments with drugs that disrupt the cellular cytoskeleton demonstrated that **microtubules are required** for active transport along the axon (Kreutzberg, 1969).
- ❑ Pulse-chase labeling experiments led to the discovery of multiple phases of transport. Organelles were observed to move outward from the cell body at “**fast**” speeds of up to 400 mm/day, while cytoskeletal proteins and some soluble proteins were observed to move via “**slow**” transport, at speeds of <8 mm/day.
- ❑ The development of **live-cell imaging** allowed the direct observation of organelle motility (Allen et al., 1982; Brady et al., 1982). These observations led to the discovery of the microtubule motor **kinesin**, now known as kinesin-1; **cytoplasmic dynein** was discovered soon after.

Fast Axonal Transport

Extrusion of Axoplasm from squid giant axon



Video microscopy reveals that organelle movement can continue in apparently normal fashion in axons isolated from their cell bodies and divested of a cell membrane. The implication is that transport must be driven by **local energy-generating mechanisms**

The different transport components

Fast Anterograde Axonal Transport

100's of mm/day

(1-2 micron/sec)

Slow Component B Anterograde Axonal Transport

2-6 mm/day

(0.02 – 0.07 micron/sec)

Slow Component A Anterograde Axonal Transport

0.1–1 mm/day (=1000 days to reach the end of a meter-long axon!)

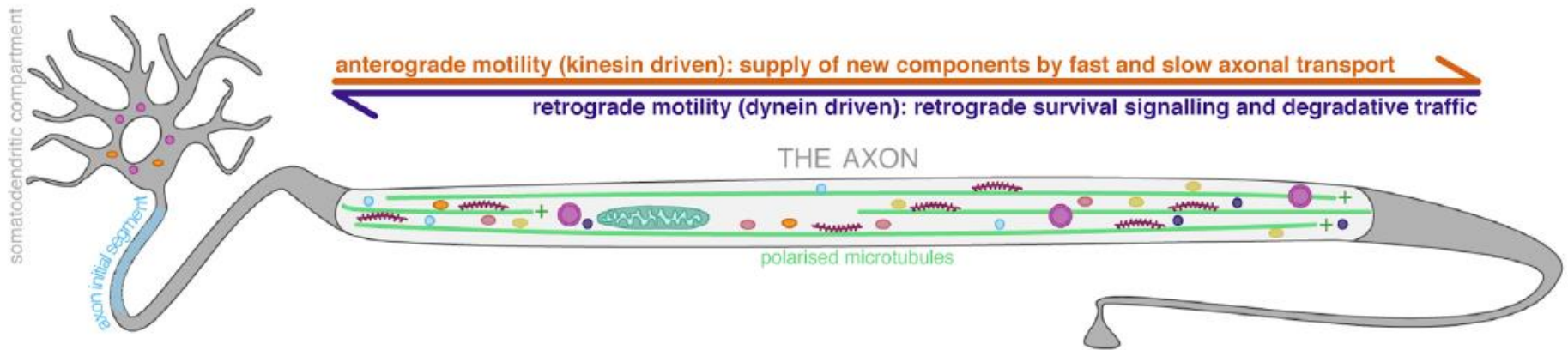
(0.01 – 0.001 micron/sec)

Retrograde Axonal Transport - Fast only

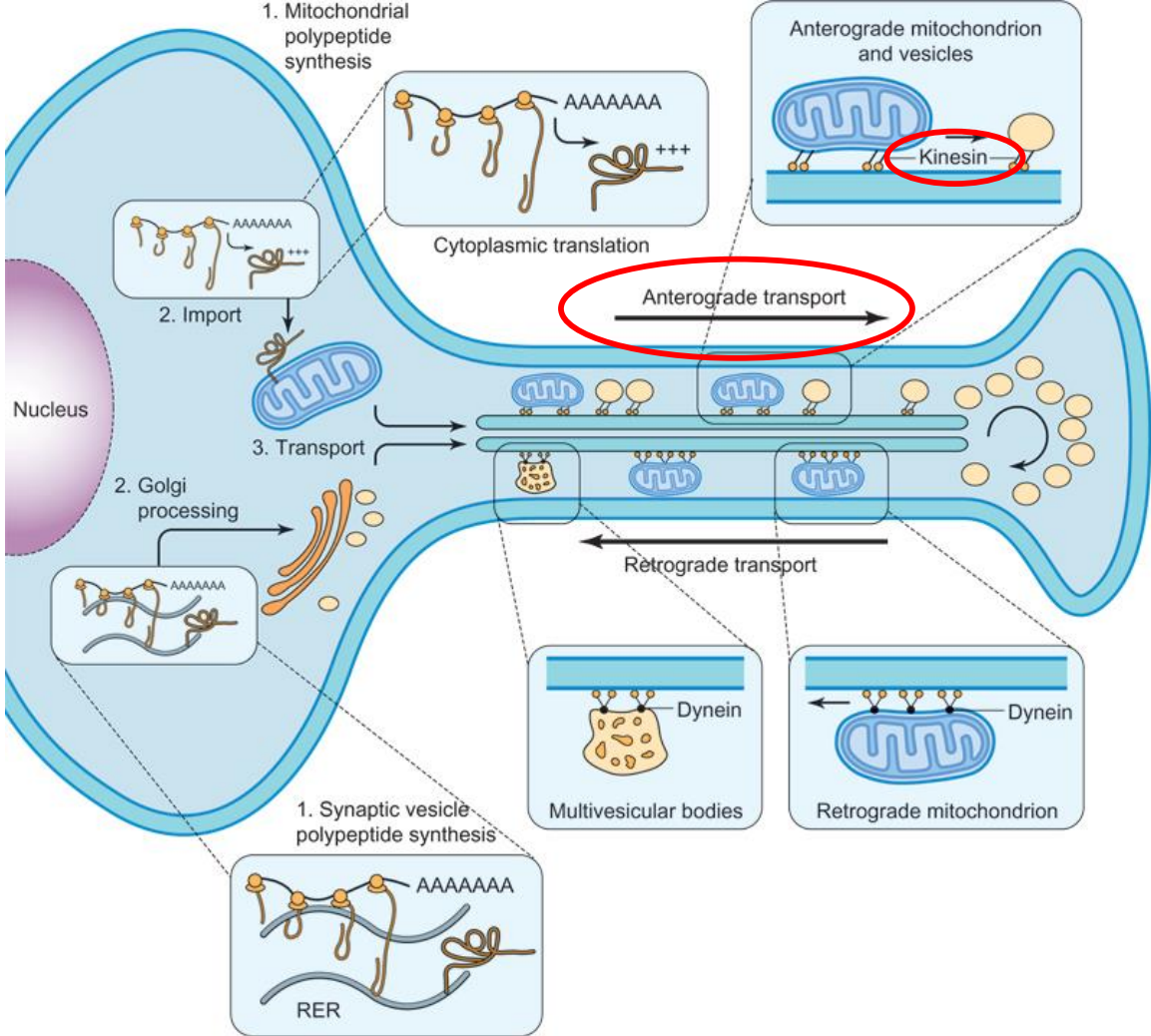
100s of mm/day

Molecular Mechanisms of Axonal Transport

Microtubule motor proteins **kinesin** and **dynein** drive the movement of organelles, vesicles, RNA granules, and proteins along the axon. Kinesins drive **anterograde** transport outward from the soma, and dynein drives **retrograde** transport back from distal axon. To avoid either distal accumulation or distal depletion of cellular components, anterograde and retrograde axonal transport must be in balance.



Fast axonal transport

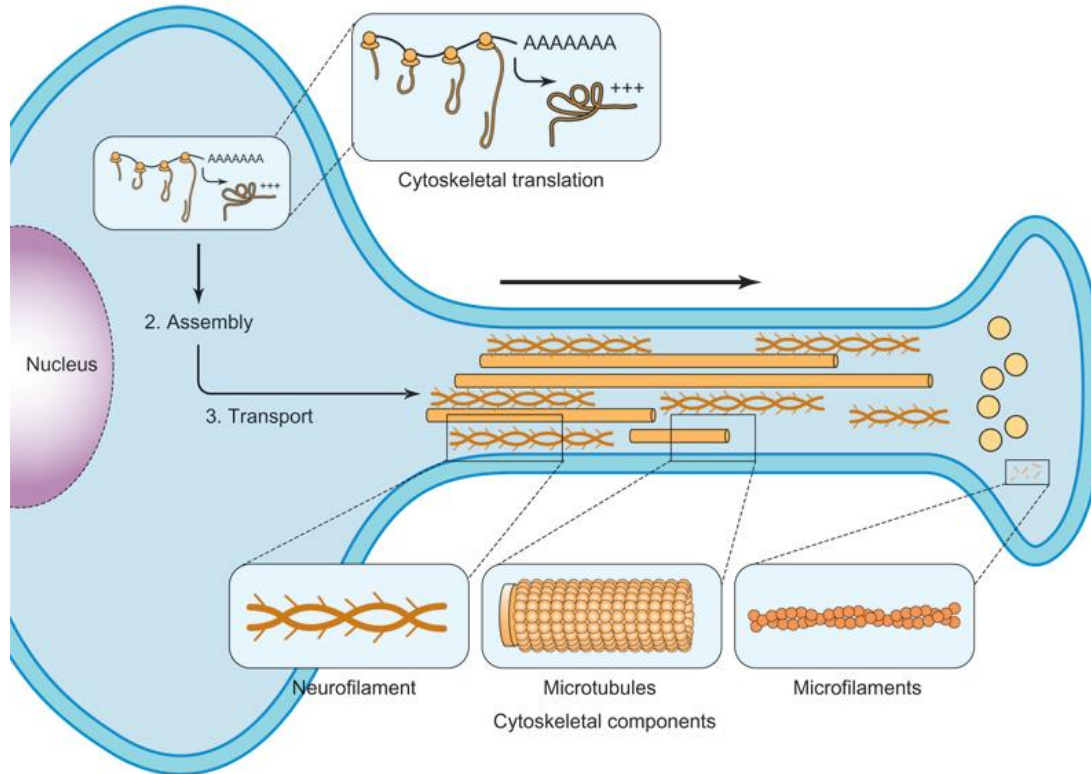


100-400 mm/day

Membranous organelles

**ANTEROGRADE
&
RETROGRADE**

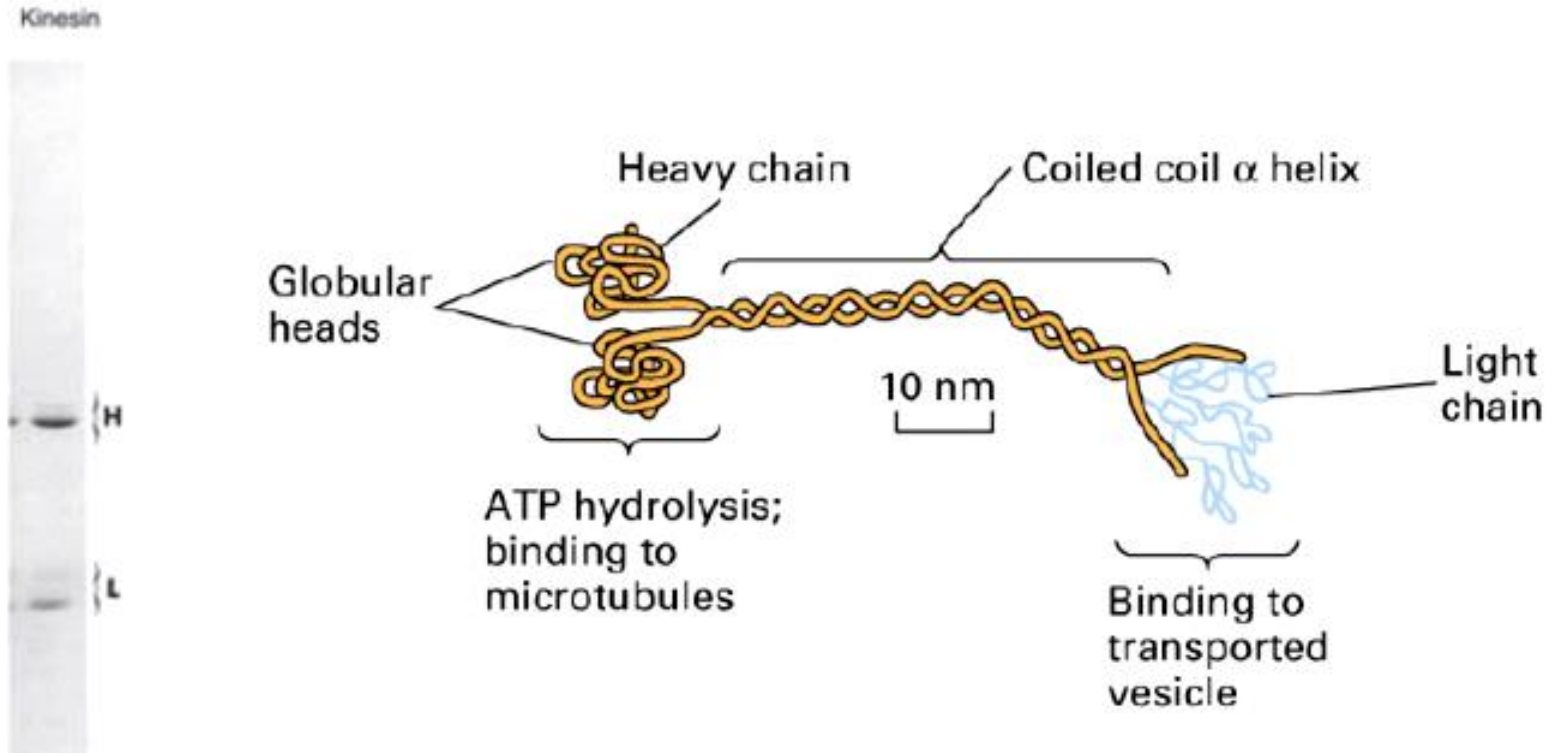
Slow axonal transport



~ 0.2-2.5 mm/day

Cytosolic and cytoskeletal proteins

Kinesins: motor, stalk and cargo binding

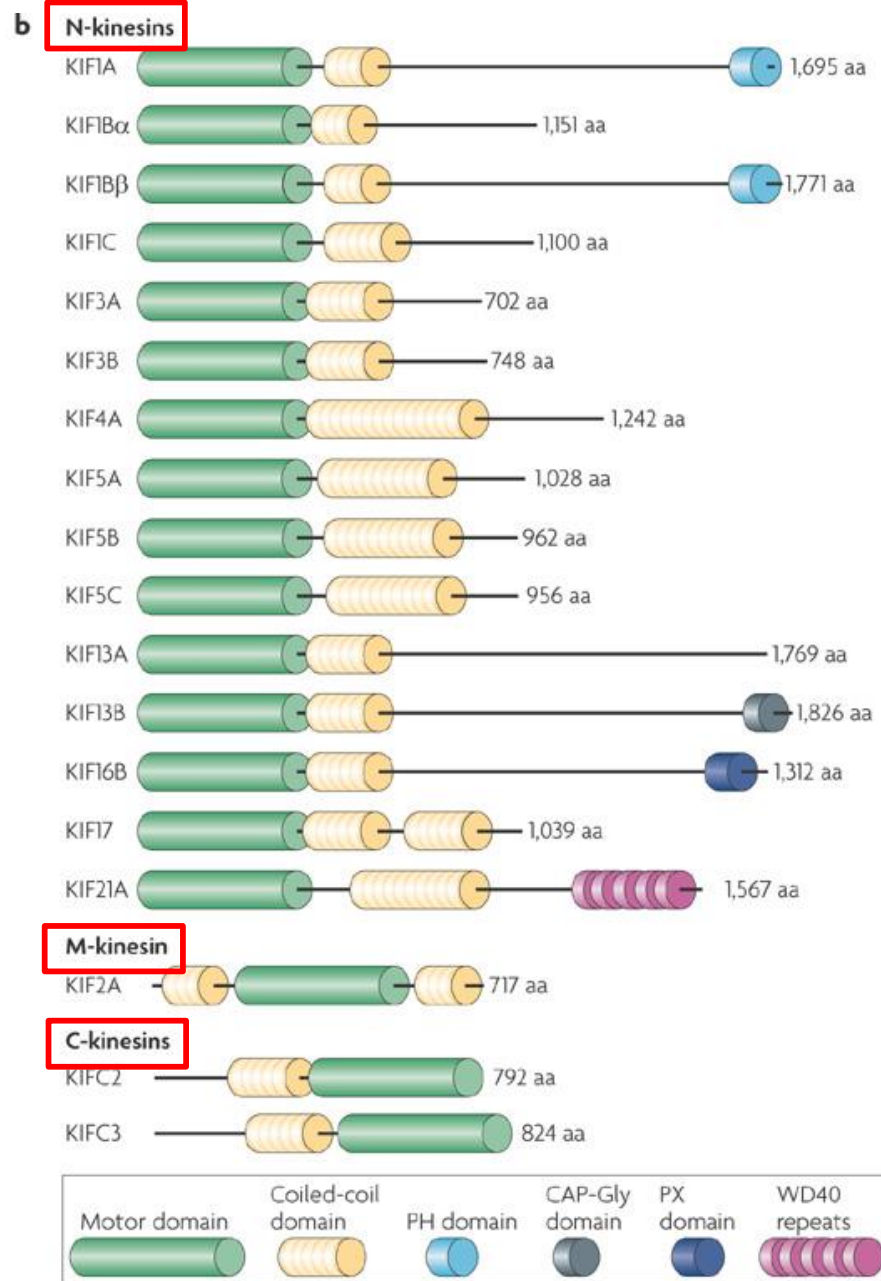
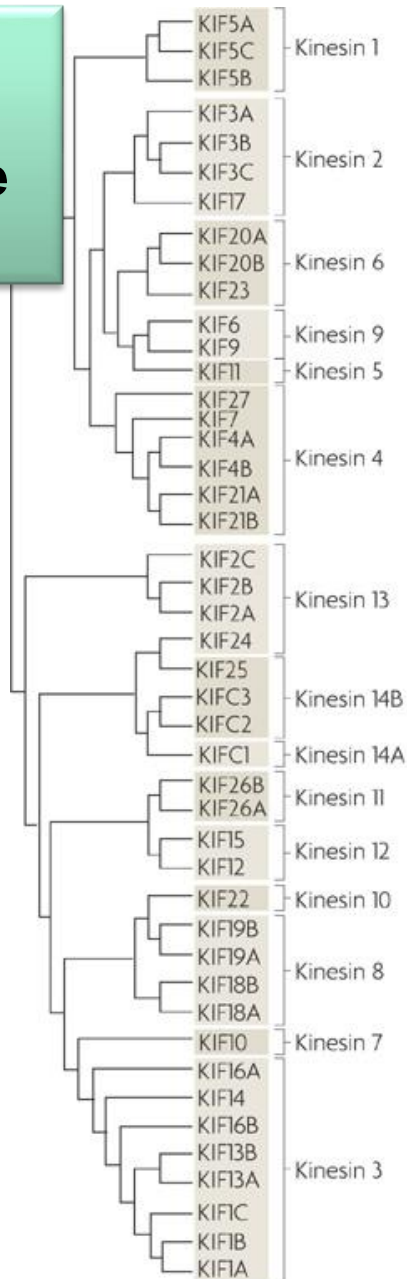


The autoinhibition of kinesin-1 is key to its regulation. **The binding of kinesin tail to the motor domain blocks motor function**; inhibition is relieved by specific binding partners such as the scaffolding proteins **JIP1 and JIP3**

The structure and phylogeny of major mouse kinesins

The kinesin superfamily constitutes **45 genes** in the mammalian genome, 38 of which are expressed in brain.

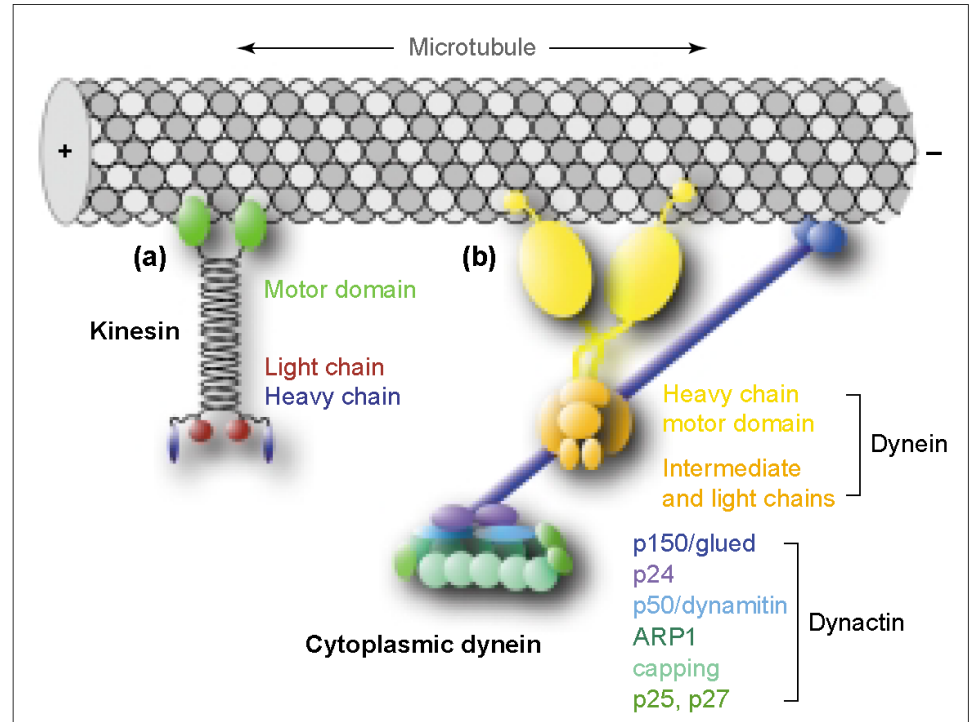
Kinesin genes can be grouped into **14 subfamilies** that share structural and functional similarities; motors from the kinesin-1, kinesin-2, and kinesin-3 families all contribute to axonal transport dynamics.



Cytoplasmic dynein: the minus end motor

One Dynein with many regulatory proteins.

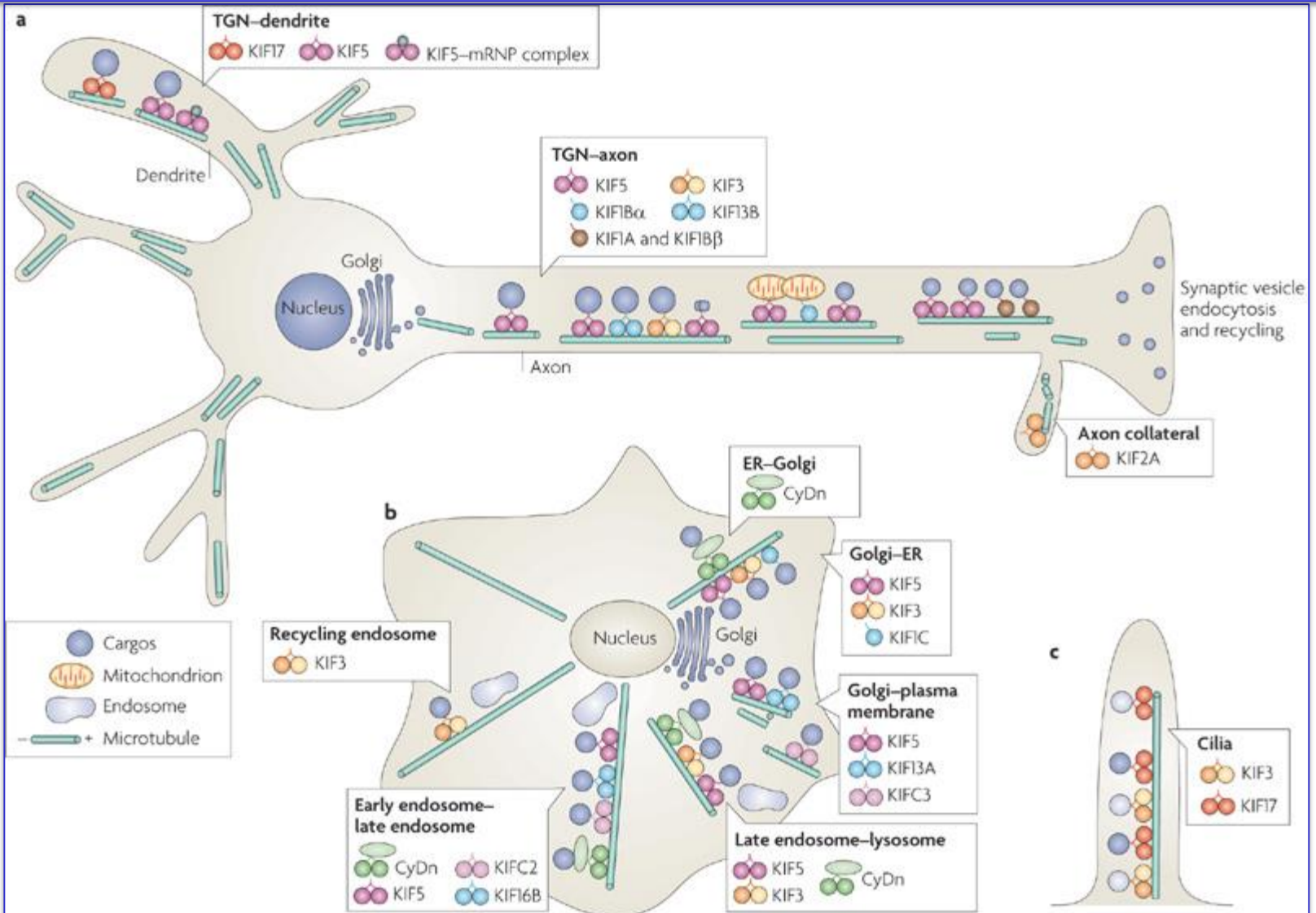
- Only one retrograde motor
- Many associated proteins that regulate dynein functions including cargo binding
- Retrograde transport clearly regulated by multiple distinct associated proteins



Hildegard Tekotte and Ilan Davis

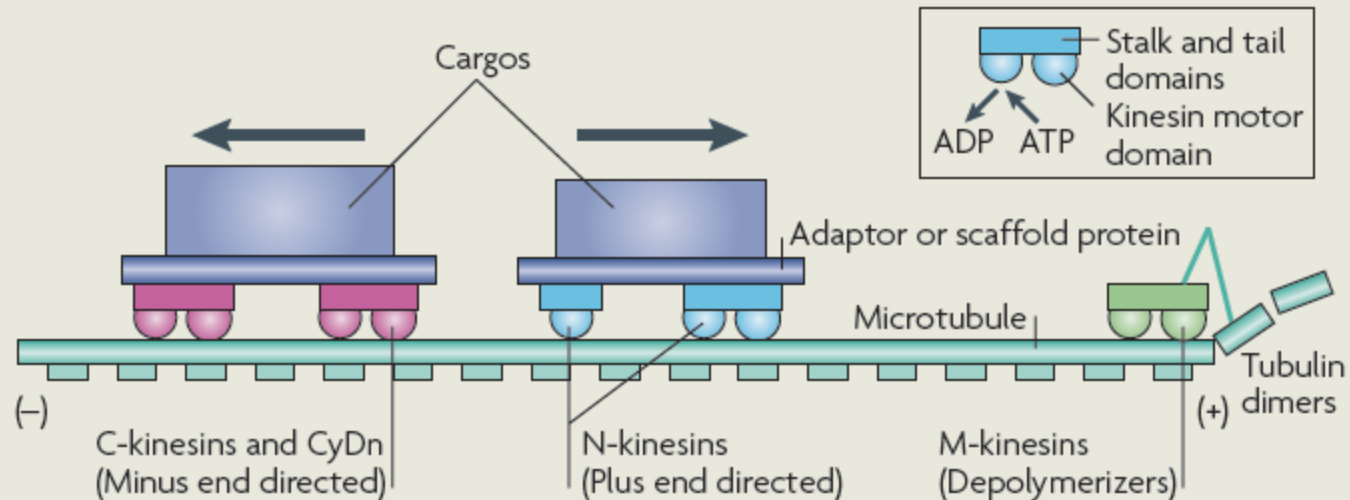
TRENDS in Genetics Vol.18 No.12 December 2002

Intracellular transport by molecular motors in neurons, non-neuronal cells and cilia.



Hirokawa 2009

Box 1 | General mechanism of kinesin-mediated cellular transport



Kinesins transport cargos along microtubules by a standard mechanism (depicted here using a freight train model; see the figure). A kinesin motor generally consists of a kinesin motor domain, which is conserved among kinesin superfamily proteins (KIFs), and unique stalk and tail domains that are used for kinesin dimerization and/or kinesin binding to cargos, adaptors or scaffold proteins. The kinesin motor domain generates force by hydrolysing ATP. Kinesins are largely classified as N-kinesins, M-kinesins or C-kinesins, which contain their motor domain at the amino terminus, in the middle or at the carboxyl terminus, respectively. N-kinesins generally provide plus end-directed motility that is anterograde towards the cell periphery or axon terminals in neurons. Some N-kinesins act as monomers and others act as dimers. C-kinesins, together with cytoplasmic dynein (CyDn), provide minus end-directed motility that is generally retrograde towards the cell centre. M-kinesins depolymerize microtubules. In some cases, adaptors and scaffolds provide a mechanistic link between kinesins and cargos, and they might also have regulatory roles in kinesin-driven intracellular transport, namely in the recognition of specific cargos and the regulation of cargo loading and unloading.

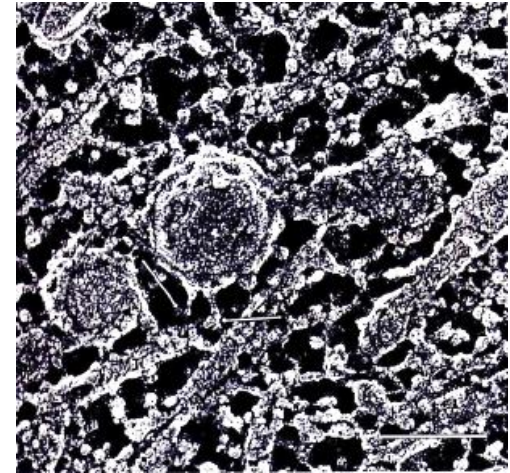
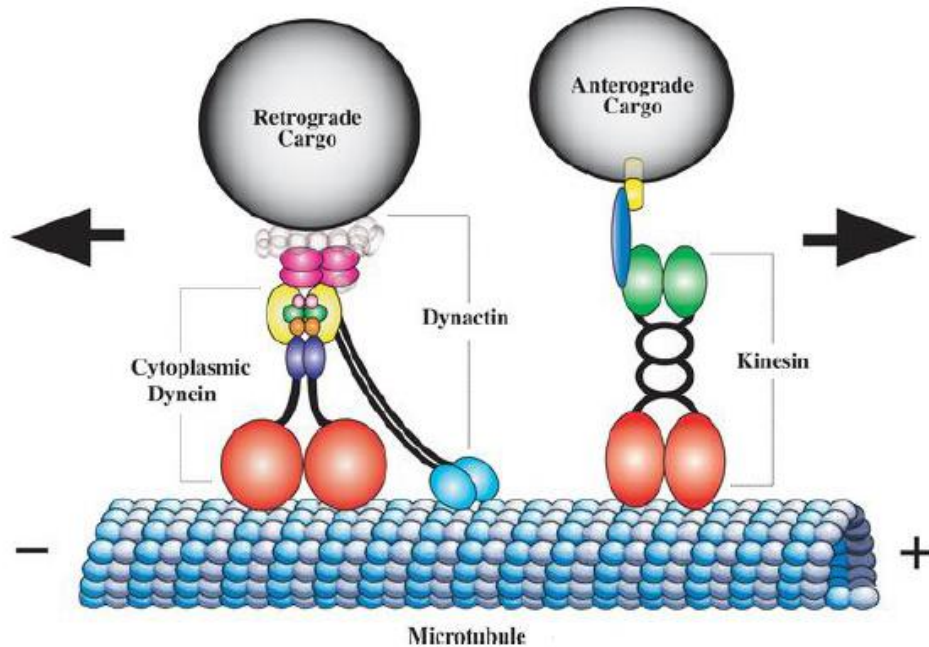
Genetic evidence confirms an essential role for active transport in the neuron, as defects in many of the proteins involved are sufficient to cause either neurodevelopmental or neurodegenerative disease

Table 1. Neurodevelopmental and Neurodegenerative Diseases Caused by Mutations in the Axonal Transport Machinery

Protein(s)	Gene(s) with Known Mutation	Disease(s)	References
Motor Proteins			
Dynein	<i>DYNC1H1</i>	CMT, SMA-LED, ID, MCD (Epilepsy)	Weedon et al., 2011; Tsurusaki et al., 2012; Harms et al., 2012; Willemssen et al., 2012; Poirier et al., 2013; Fiorillo et al., 2014
Kinesin-1	<i>KIF5A, KIF5C</i>	HSP (SPG10), ID, MCD	Ebbing et al., 2008; de Ligt et al., 2012; Poirier et al., 2013
Kinesin-13	<i>KIF2A</i>	CDCBM3/MCD	Poirier et al., 2013
Kinesin-3	<i>KIF1A, KIF1B, KIF1C</i>	HSP (SPG30), CMT2A, HSN, MR, SPAX	Erlich et al., 2011; Zhao et al., 2001; Rivière et al., 2011; Hamdan et al., 2011; Klebe et al., 2012; Dor et al., 2014; Novarino et al., 2014
Kinesin-4	<i>KIF21A</i>	CFEOM	Yamada et al., 2003
Motor Adaptors and Regulators			
Dynactin	<i>DCTN1</i>	Perry syndrome, MND	Puls et al., 2003; Farrer et al., 2009; Caroppo et al., 2014; Araki et al., 2014
BICD2	<i>BICD2</i>	SMA, HSP	Neveling et al., 2013; Peeters et al., 2013; Oates et al., 2013
Huntingtin	<i>HTT</i>	HD	HDCRG, 1993
Lis-1	<i>PAFAH1B1</i>	Lissencephaly	Dobyns et al., 1993; Reiner et al., 1993
NDE1	<i>NDE1</i>	Microcephaly, MHAC	Alkuraya et al., 2011; Bakircioglu et al., 2011; Paciorkowski et al., 2013
Rab7	<i>RAB7A</i>	CMT2B	Verhoeven et al., 2003
Cytoskeleton and Associated Proteins (e.g., MAPs)			
CLIP-170	<i>CLIP1</i>	ARID	Larti et al., 2014
Doublecortin	<i>DCX</i>	Lissencephaly	des Portes et al., 1998a, 1998b; Gleeson et al., 1998
Microtubules	<i>TUBA1A, TUBA8, TUBG1, TUBB3, TUBB2B</i>	Lissencephaly, MCD, microcephaly, polymicrogyria, CFEOM	Keays et al., 2007; Poirier et al., 2007, 2010, 2013; Jaglin et al., 2009; Abdollahi et al., 2009; Tischfield et al., 2010; Chew et al., 2013
Neurofilaments	<i>NEFL</i>	CMT	Mersiyanova et al., 2000
Spastin	<i>SPAST</i>	HSP (SPG4)	Hazan et al., 1999
Tau	<i>MAPT</i>	FTD, Pick disease, AD	Hutton et al., 1998; Murrell et al., 1999

Abbreviations are as follows: AD, Alzheimer's disease; ARID, autosomal recessive intellectual disability; CDCBM3, complex cortical dysplasia with other brain malformations-3; CFEOM, congenital fibrosis of the extraocular muscles; CMT, Charcot-Marie-Tooth disease; FTD, frontotemporal dementia; HD, Huntington's disease; HMN, hereditary motor neuropathy; HSN, hereditary sensory neuropathy; HSP, hereditary spastic paraplegia; ID, intellectual disability; MCD, malformations of cortical development; MHAC, microhydranencephaly; MND, motor neuron disease; MR, mental retardation; SMA, spinal muscular atrophy; SMA-LED, SMA-lower extremity dominant; and SPAX, spastic ataxia.

Cargo-bound motors are regulated by organelle-specific complements of scaffolding and adaptor proteins.

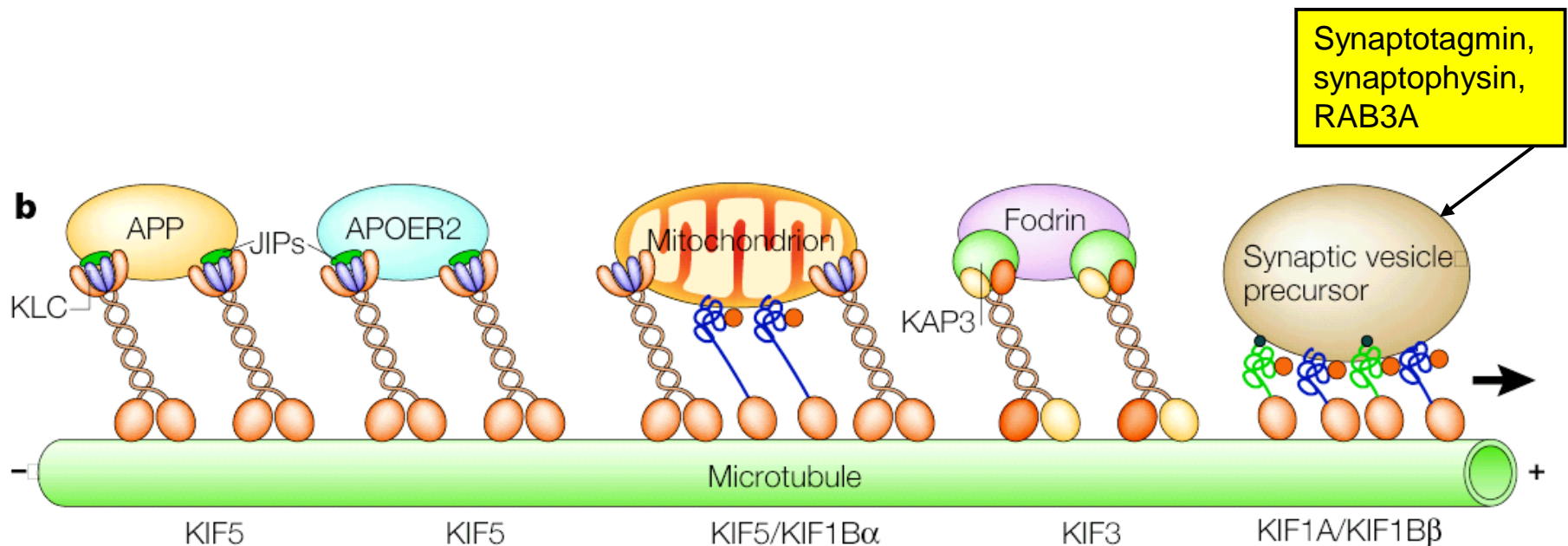


The complement of **motors**, **adaptors**, and **scaffolding** proteins bound to each cargo is organelle specific, leading to distinct patterns of motility and localization along the axon.

Thus, while broad themes emerge, the **specific mechanisms regulating the transport** of each organelle or protein complex may be unique.

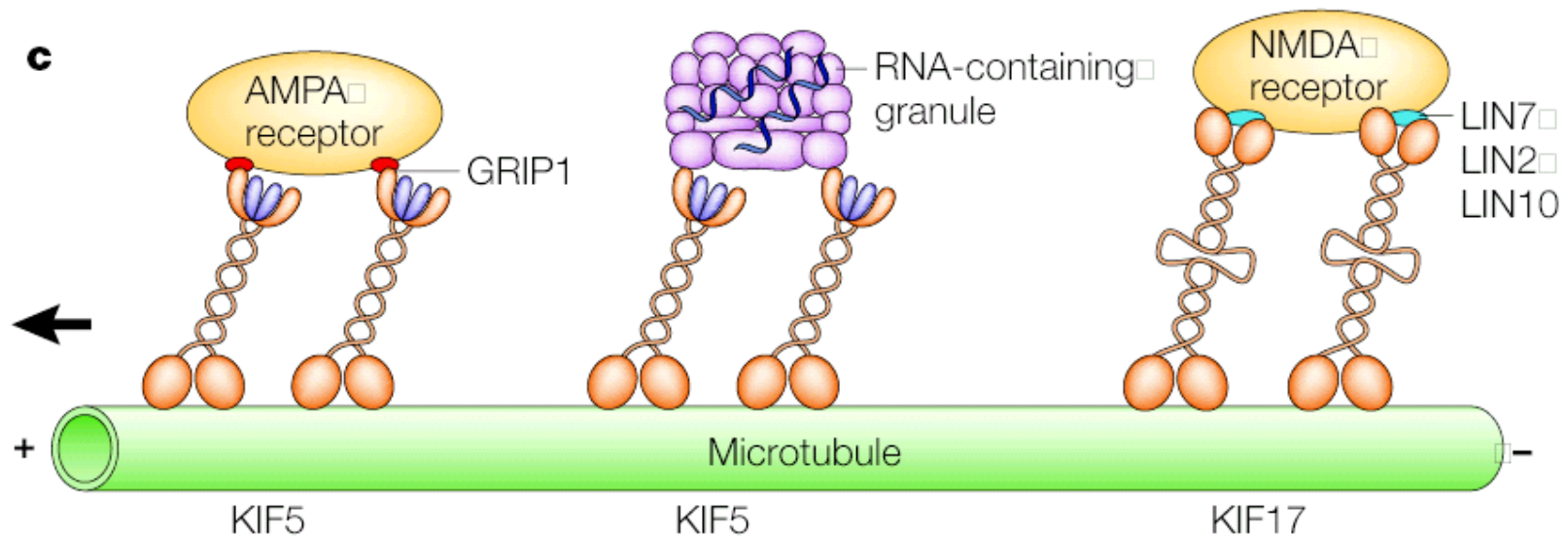
There is increasing evidence for the localized regulation of trafficking in **key zones** along the axon, such as the **axon initial segment** or in the **distal axon**.

Combinations of molecular motors and adaptors for anterograde transport of axonal cargoes



b | KIF5 transports vesicles containing APP (amyloid precursor protein) and APOER2 (apolipoprotein E receptor 2) by interacting with KLC (kinesin light chain)^{46,47,51,96,97}. Mitochondria are transported by KIF5 and KIF1B α ^{27,45}. KIF3 transports vesicles associated with fodrin⁵⁷. KIF1A and KIF1B β both transport synaptic vesicle precursors^{26,31,32}. JIPs, scaffolding proteins of the c-Jun amino (N)-terminal kinase (JNK) signalling pathway; KAP3, kinesin superfamily-associated protein 3.

Combinations of molecular motors and adaptors for transport of dendritic cargoes

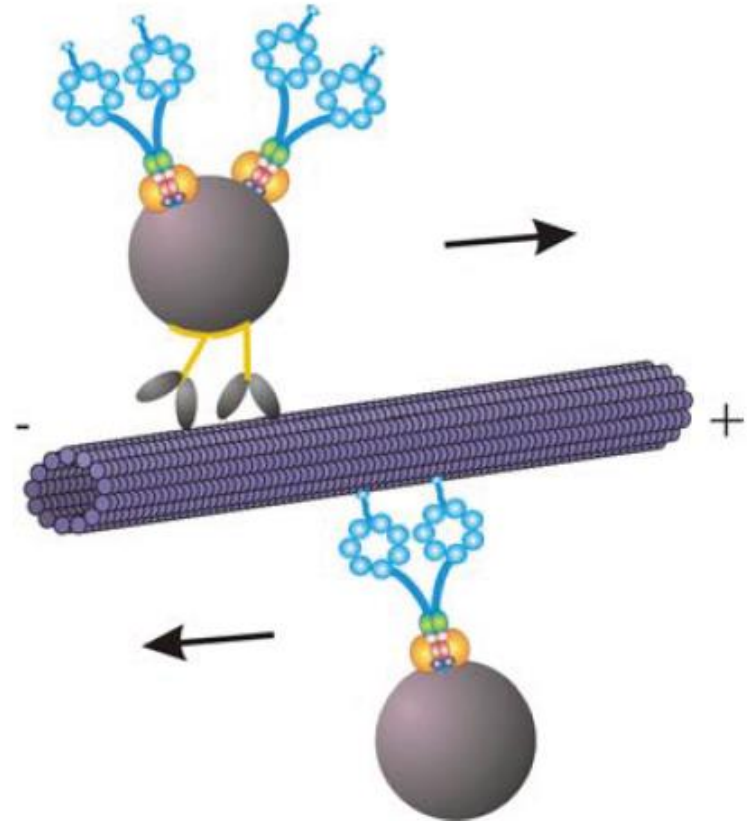


c | In dendrites, KIF5 transports vesicles containing AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors through an interaction between KIF5 and GRIP1 (glutamate receptor-interacting protein 1)⁶⁸. RNA-containing granules are also transported by interacting directly with KIF5 (REF. 77). KIF17 transports vesicles containing NMDA (*N*-methyl-D-aspartate) receptors by interacting through the LIN complex, a tripartite protein complex containing mammalian homologues of the *Caenorhabditis elegans* presynaptic density zone (PDZ) proteins LIN-2, LIN-7 and LIN-10⁶⁵.

Opposing motors bind simultaneously to cargos along the axon

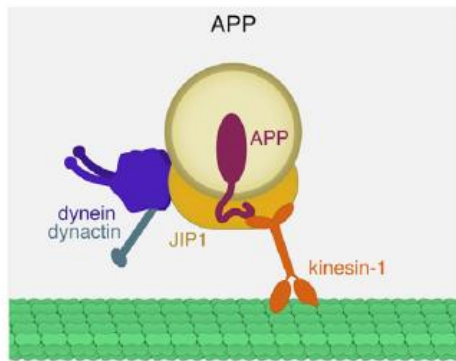
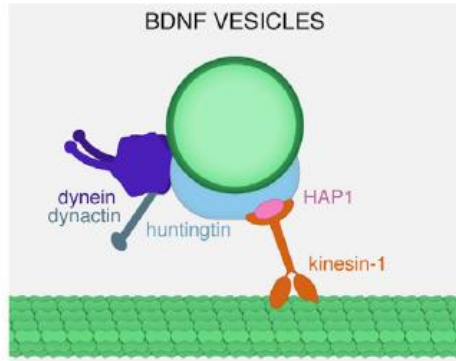
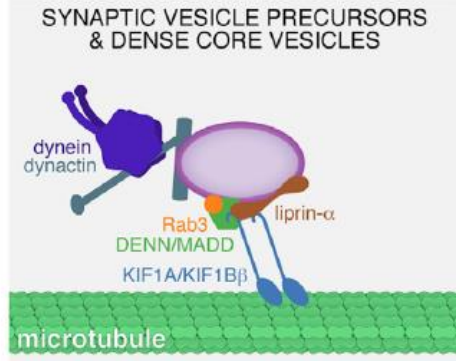
Many axonal cargos have multiple motor types bound simultaneously.

Quantitative analyses and live-cell trapping experiments suggest that 1–2 kinesins and 6–12 dyneins may act together to move a single organelle along the microtubule.

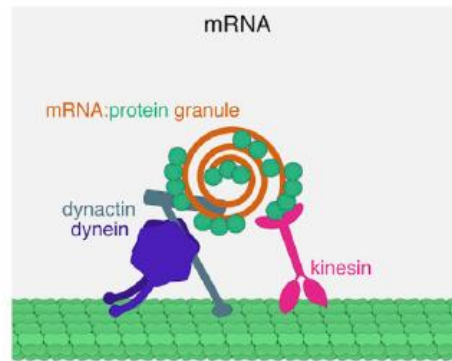
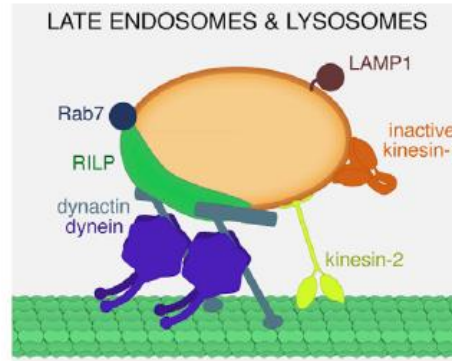
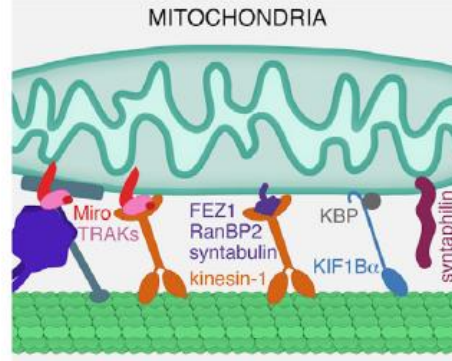


fast axonal transport

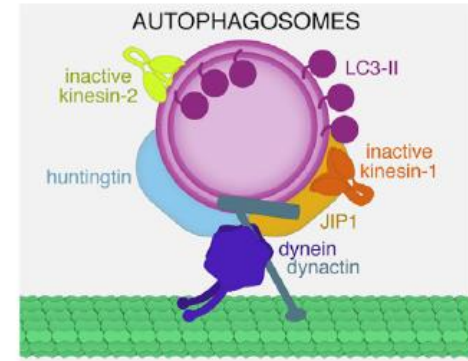
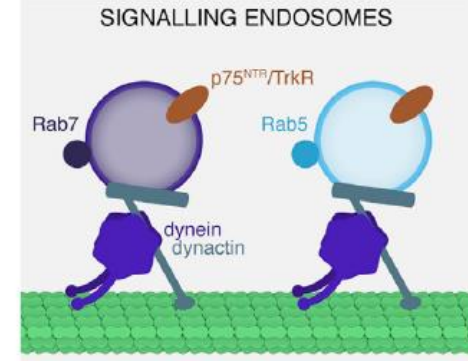
anterograde



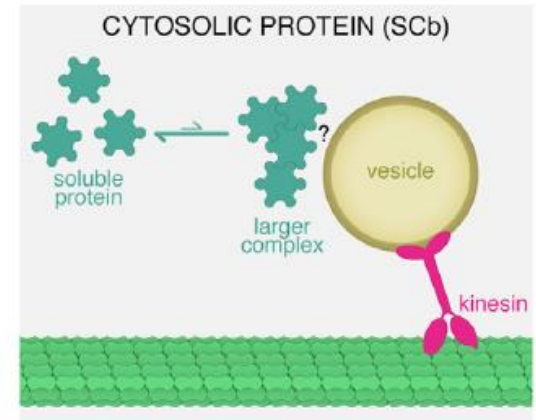
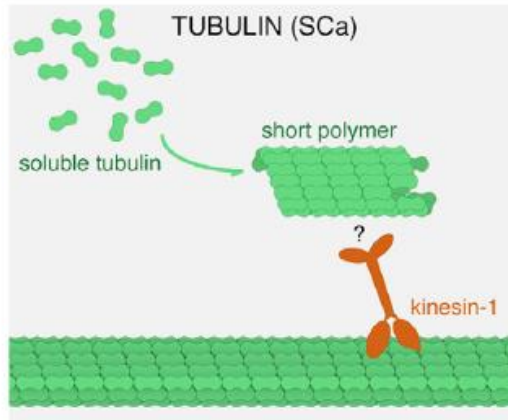
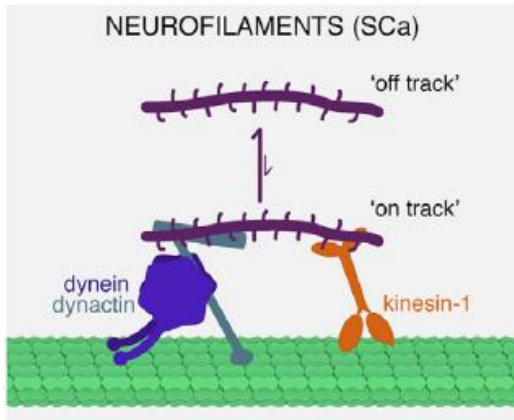
bi-directional



retrograde



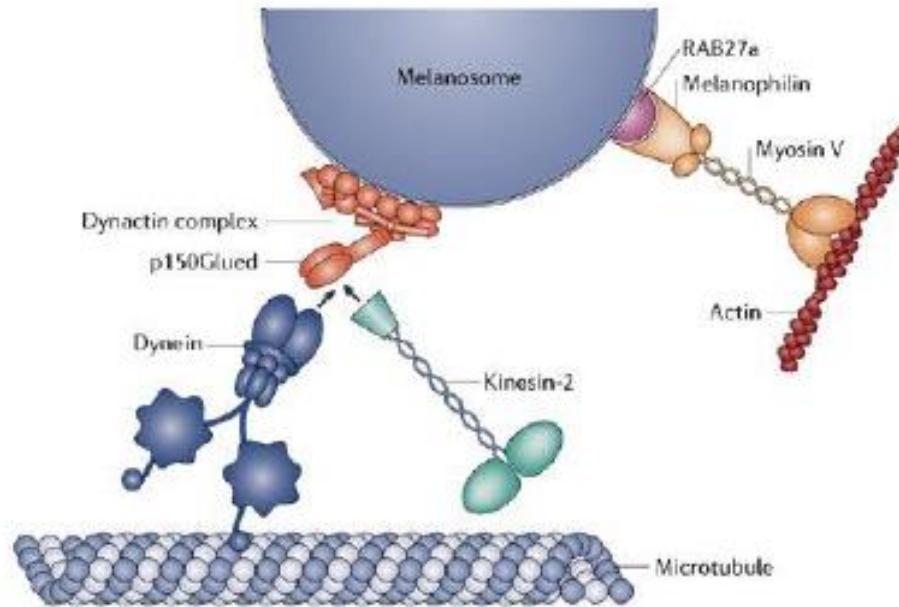
slow anterograde axonal transport



<http://dx.doi.org/10.1016/j.neuron.2014.10.019>

Maday, Neuron 84, October 22, 2014

Some cargos are 'handed off' from MTs to actin filaments for local transport



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Nature Reviews | Molecular Cell Biology

How is cargo transported along actin filaments?

Answer: Myosin motors

A model for dendritic targeting

The axonal initial segment contains an actin-based filter that prevents vesicles carrying dendritic transmembrane proteins to enter the axon. Selective axonal entry of KIF-driven vesicular carriers depends on both the efficacy of the motor and the specific cargos it carries.

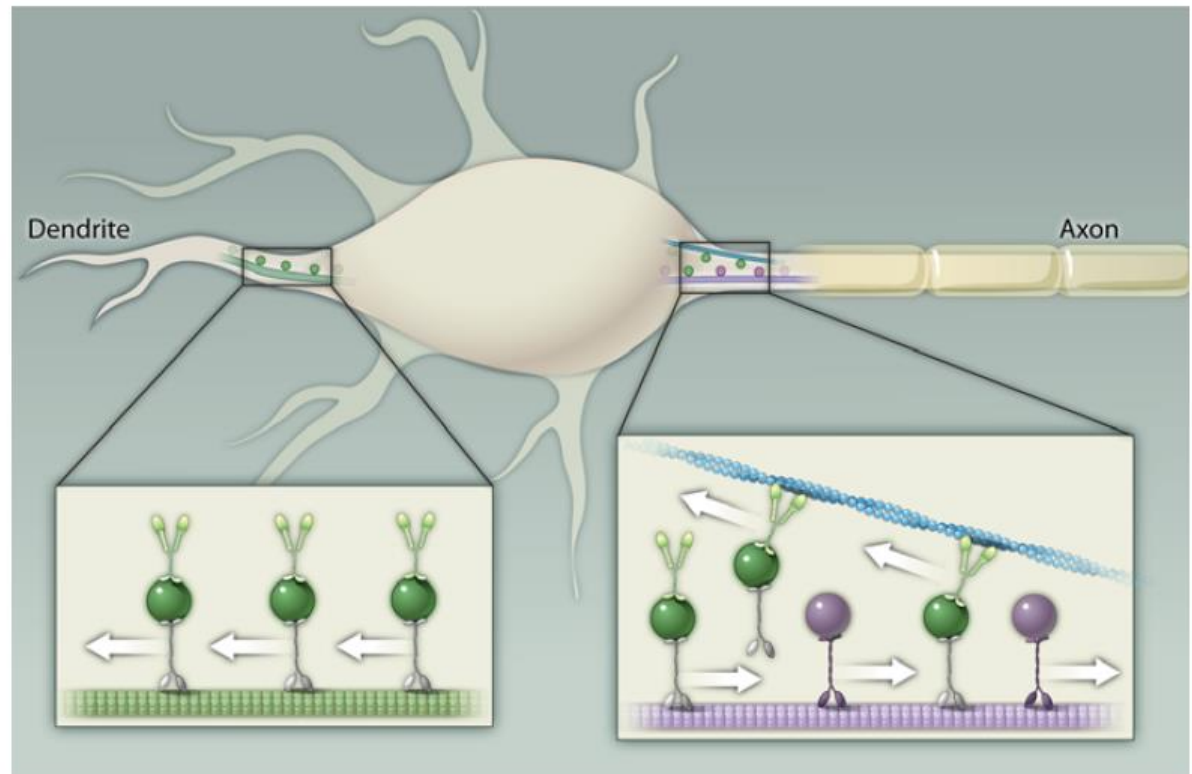


Fig. 1. A model for dendritic targeting. Vesicles containing dendritic proteins (dark green) associate with both kinesin motors and Myosin Va (light green) and move along axonally projecting microtubules (purple) until Myosin Va interacts with actin filaments (blue). The orientation of these filaments causes Myosin Va and the vesicle to which it is attached to move toward the cell body. Vesicles containing axonal proteins (dark purple) do not associate with Myosin Va and, thus, are able to move to the distal axon unimpeded. In the dendrite, the absence of a vesicle filter allows vesicles carrying dendritic proteins to move distally. Purple denotes deetyrosinated microtubules, which are found in the axon, and light green denotes tyrosinated microtubules, which are found in the dendrites. Kif5 is indicated in purple, other kinesins in gray.