Lesson (11)

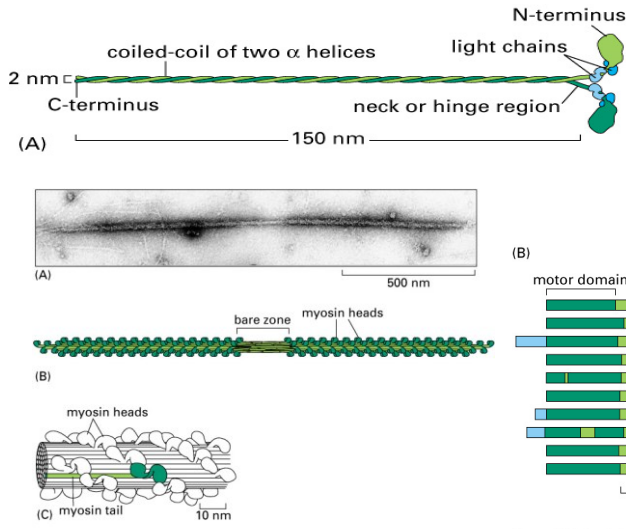
Inside the neuron – Molecular Neurobiology I:

Cellular transport and the molecular motors

MOLECULAR MOTORS

- **There Are Two Types of Microtubule Motor Proteins: Kinesins and Dyneins**
- **One Type of Actin-based Motor Proteins : Members of the Myosin Superfamily**
- **Motor Proteins Generate Force by Coupling ATP Hydrolysis to Conformational Changes**
- **Cilia and Flagella Are Motile Structures Built from Microtubules and Dyneins**

Actin-based Motor Proteins Are Members of the Myosin Superfamily



- **Myosin II is the muscle motor**
- **Other myosins have other functions**

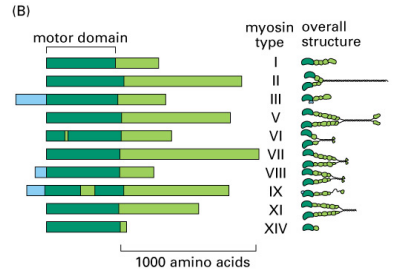


Figure 16–52. Molecular Biology of the Cell, 4th Edition.

Figure 16–54 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

There Are Two Types of Microtubule Motor Proteins: Kinesins and Dyneins

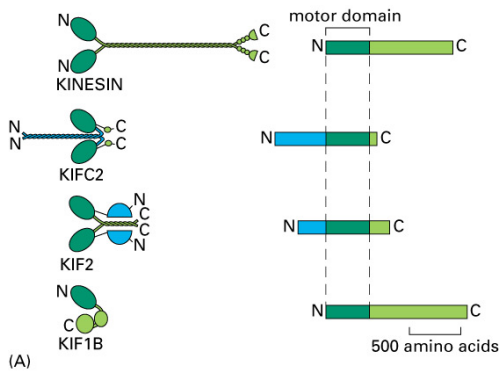


Figure 16–55. Molecular Biology of the Cell, 4th Edition.

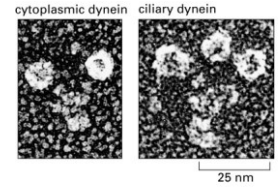


Figure 16–56. Molecular Biology of the Cell, 4th Edition.

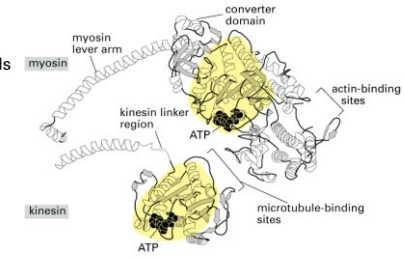


Figure 16–57. Molecular Biology of the Cell, 4th Edition.

Motor Proteins Generate Force by Coupling ATP Hydrolysis to Conformational Changes

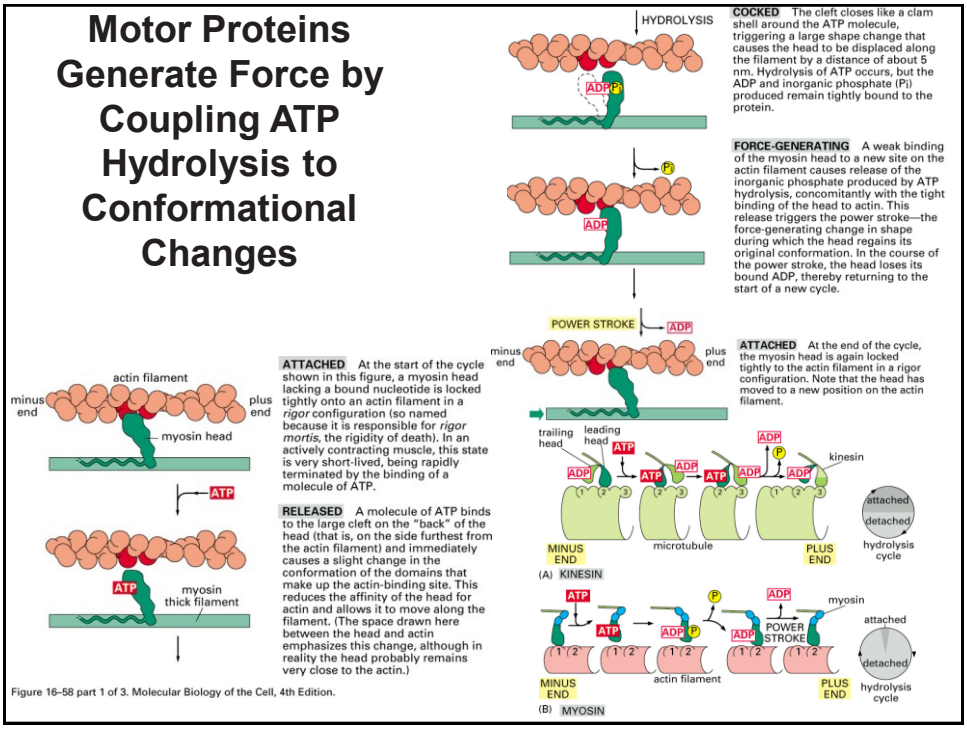
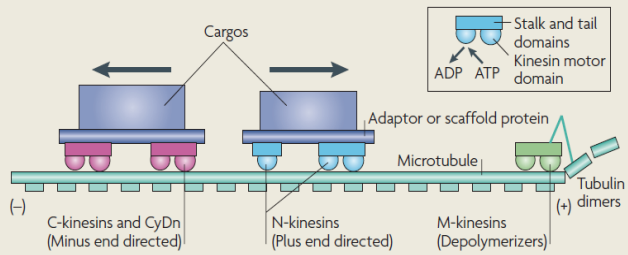


Figure 16–58 part 1 of 3. Molecular Biology of the Cell, 4th Edition.

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.

Different motors can run in opposite directions

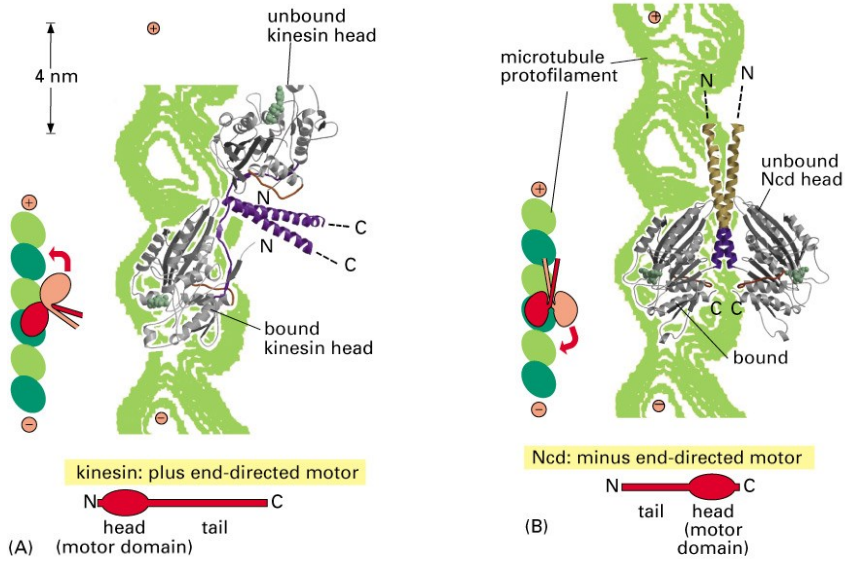


Figure 16–60 part 1 of 2. Molecular Biology of the Cell, 4th EditiFigure 16–60 part 2 of 2. Molecular Biology of the Cell, 4th

Cilia and Flagella Are Motile Structures Built from Microtubules and Dyneins

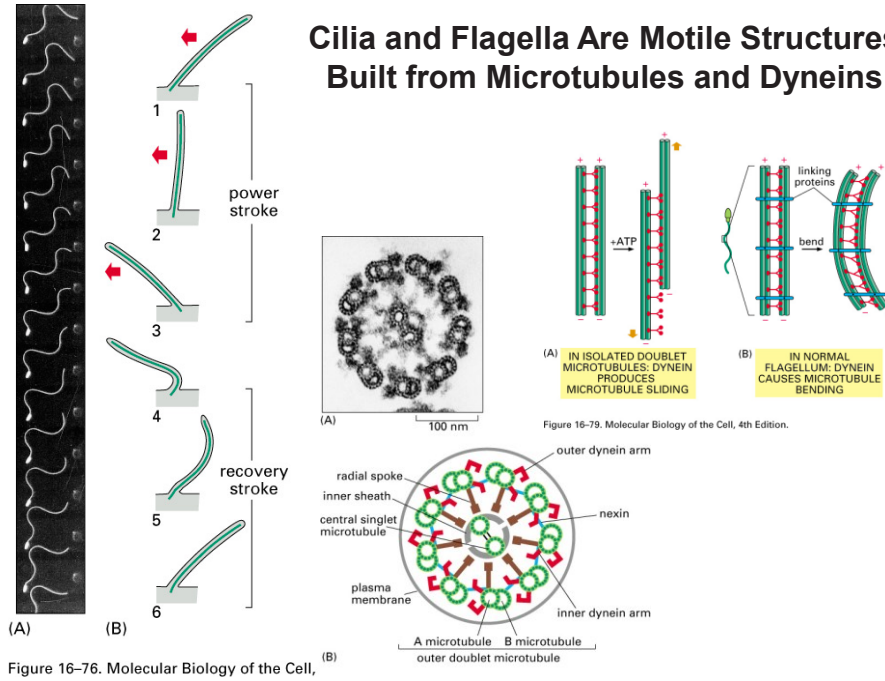
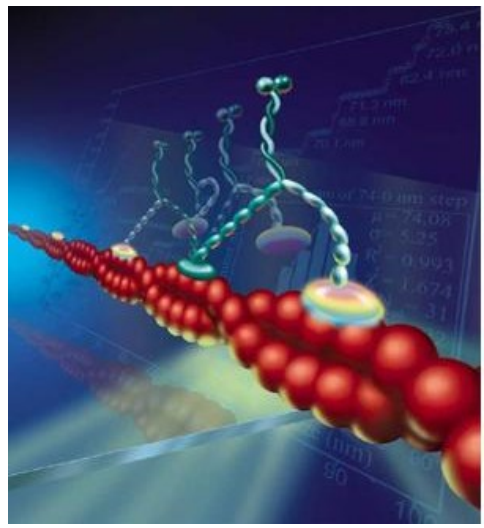


Figure 16–76. Molecular Biology of the Cell,

Figure 16–79. Molecular Biology of the Cell, 4th Edition.

Figure 16–77. Molecular Biology of the Cell, 4th Edition.

Motors Walk Along Filaments



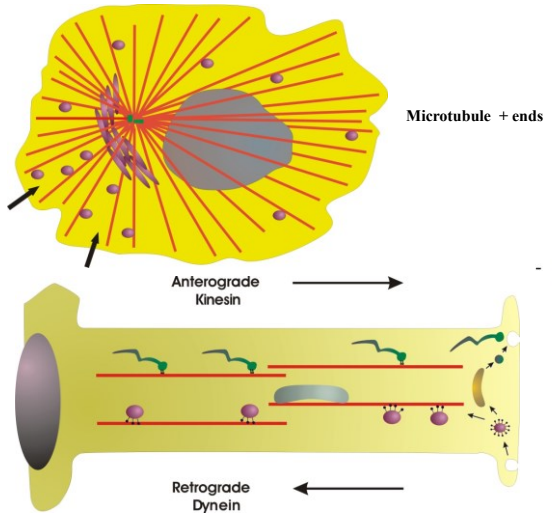
https://www.youtube.com/watch?v=B_zD3NxSsD8

Yildiz *et al.*
Science 2003.

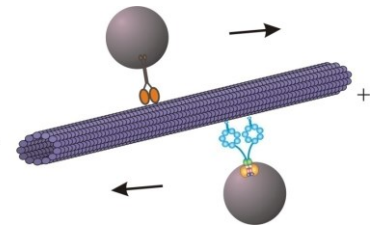
Motor proteins

- Move unidirectionally
- Stepwise
- Series of conformational changes
  - A *mechanical cycle*
  - Coupled to chemical cycle – Energy
    - Steps –
      - » ATP binding to motor
      - » Hydrolysis of ATP
      - » Release of ADP and P_i
      - » Binding of new ATP

Fast Axonal Transport Uses the Same Mechanisms as General Membrane Vesicle Trafficking



Kinesin motors
To cell periphery



Cytoplasmic Dynein
To cell center

REVIEWS

There is a large family of kinesins for different microtubule-plus end directed movements in axons.

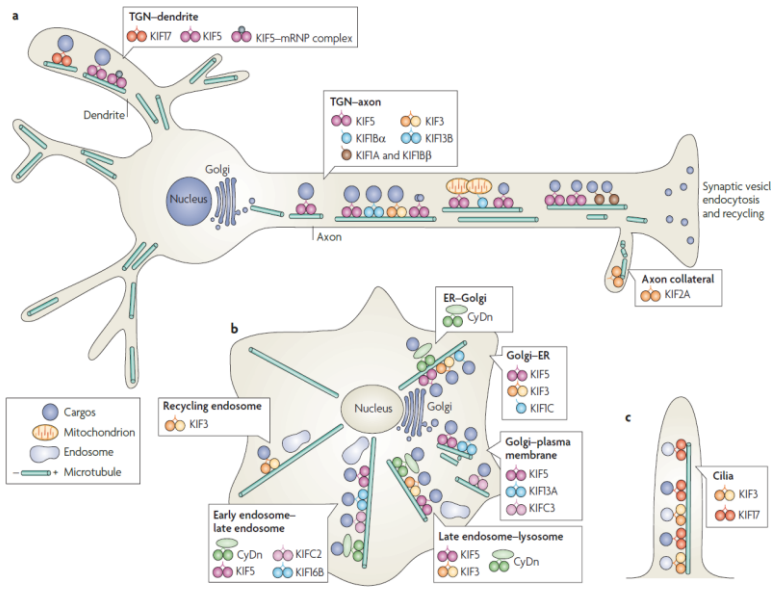
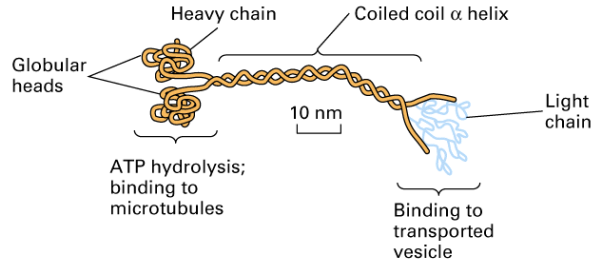


Figure 2 | **Intracellular transport by molecular motors in neurons, non-neuronal cells and cilia. a** | In neuronal

Hirokawa2009

Kinesin: Polypetides and Structure



Tetramer

2 identical heavy and 2 identical light chains

Functional domains

Pair of globular heads

Bind microtubule

ATP-hydrolysing

Neck / stem and tail

Tail binds cargo

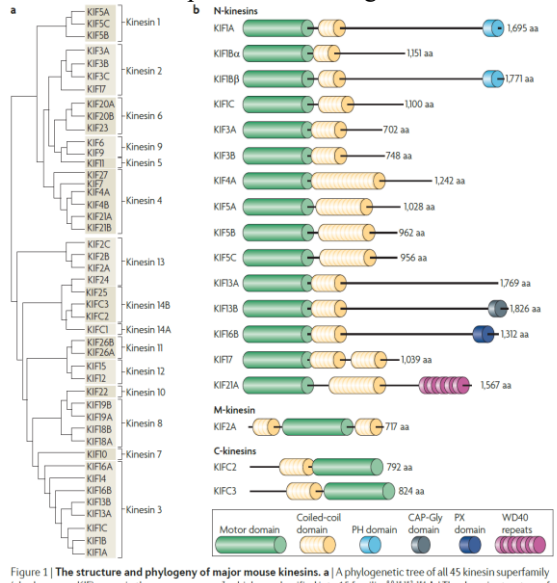
Move toward plus end of microtubule

Plus end directed

Kinesins: a family of ~45 members in mammals

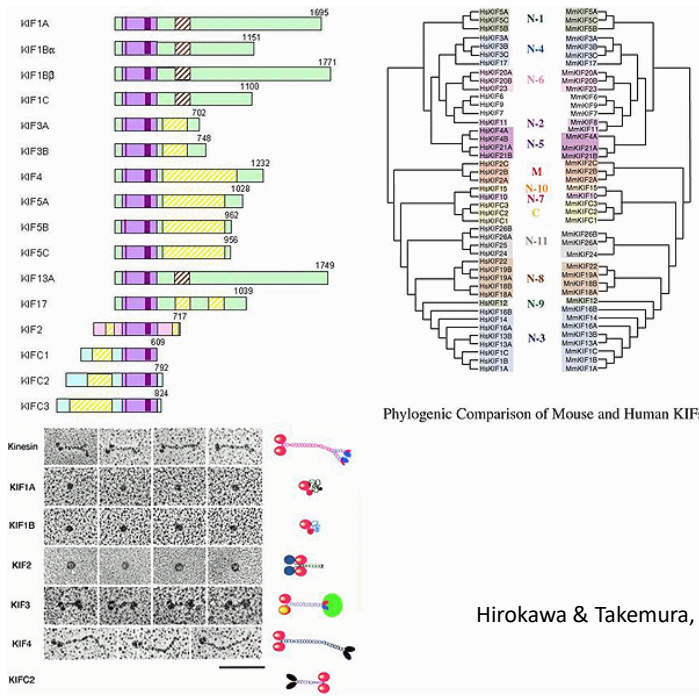
Conserved motor domain fused to variable domains

almost more kinesin proteins than cargo – and redundancy



Hirokawa, 2009

Figure 1 | **The structure and phylogeny of major mouse kinesins. a** | A phylogenetic tree of all 45 kinesin superfamily (also known as KIF) genes in the mouse genome[7], which are classified into 15 families[8,9,15,152–154]. **b** | The domain structure of



Phylogenic Comparison of Mouse and Human KIFs

Hirokawa & Takemura, 2005

Different kinesins to transport different cargoes in axonal and dendritic transport

Hirokawa • Motors and mRNA Transport in Dendrites
J. Neurosci., July 5, 2006 • 26(27):7139 –7142

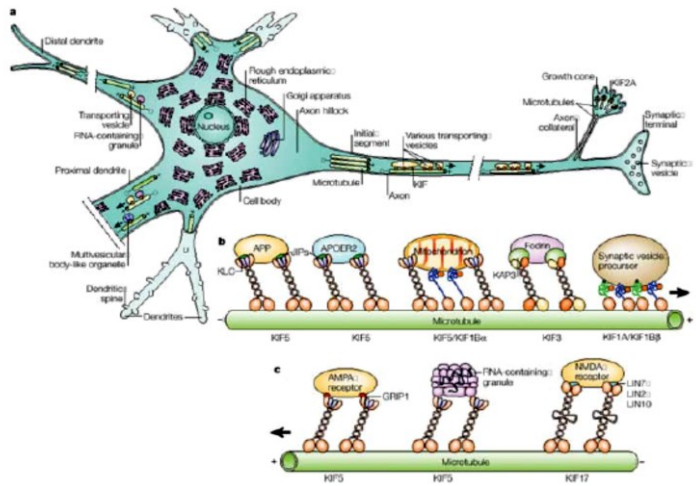
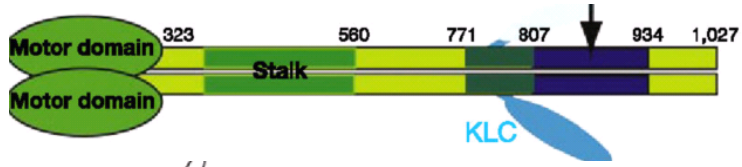
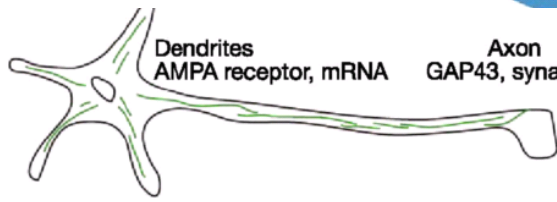
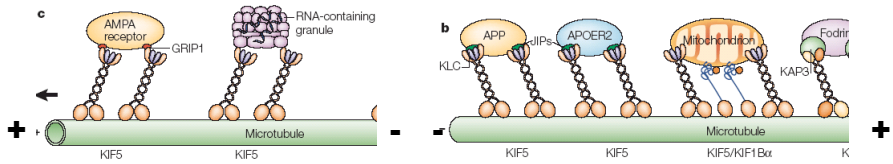


Figure 1. KIFs and cargoes for axonal and dendritic transport. ***a***, Microtubule polarity in the axon and dendrites. ***b***, KIFs and their cargoes in the axon. ***c***, KIFs and their cargoes in dendrites. mRNA and granules composed of a large number of proteins are transported by KIF5. Reproduced with permission from Hirokawa and Takemura (2005).

Model proposed by Hirokawa *(Nat Rev Neurosci. 2005 6:201-214)*







Dendrite **vs** **Axon**

Transport independent from KLC — Attached cargo via KLC

Adaptor-proteins for cargoes transport by kinesins

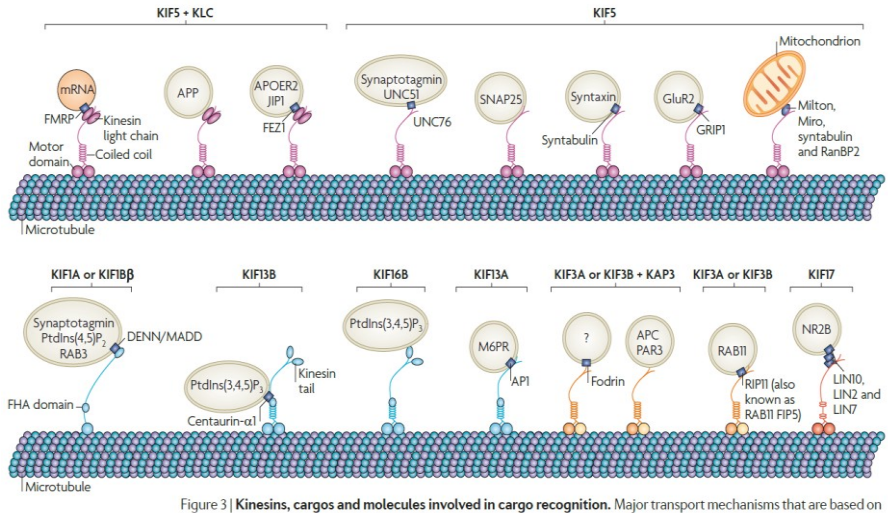


Figure 3 | **Kinesins, cargos and molecules involved in cargo recognition.** Major transport mechanisms that are based on

Horokawa, 2009

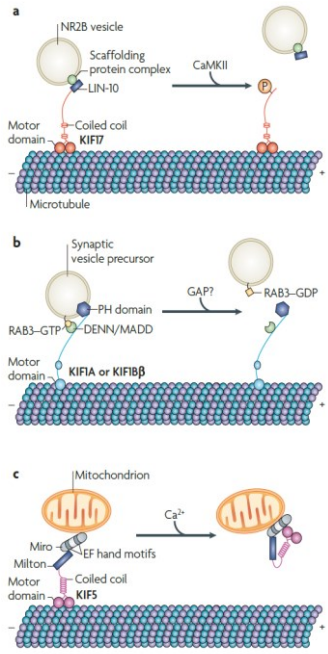


Figure 4 | **Regulation of kinesin–cargo binding by three different mechanisms. a** | Phosphorylation. The kinesin 2 family motor KIF17 (also known as OSM3) transports vesicles that carry NMDA (*N*-methyl-D-aspartate) receptor subunit 2B (NR2B) towards the plus end of microtubules through a scaffolding protein complex[65]. Phosphorylation of KIF17 by the Ca^{2+}/calmodulin-dependent protein kinase CaMKII disrupts the direct interaction between KIF17 and its interacting partner LIN10 (the mouse homologue of *Caenorhabditis elegans* LIN-10), leading to the unloading of NR2B-carrying vesicles[102]. **b** | The Rab GTPase cycle. The kinesin 3 family motors KIF1A and KIF1Bβ transport RAB3-carrying synaptic vesicle precursors through the adaptor protein mitogen-activated protein kinase-activating death domain (MADD; also known as DENN and shown as DENN/MADD)[115]. DENN/MADD recognizes the GTP-bound form of RAB3 on vesicles. Because the membrane-binding capacity of the pleckstrin homology (PH) domain of KIF1A and KIF1Bβ is insufficient to transport cargo[116] when RAB3 is inactivated by GTPase-activating proteins at the axon terminus, RAB3–GDP-carrying vesicles are released. **c** | Calcium. The kinesin 1 family motor KIF5 transports mitochondria through interaction with the Milton–Miro complex[29]. Miro has two EF hand motifs, which sense intracellular Ca^{2+} levels. In response to high Ca^{2+} influx, these motifs bind to the motor domain of KIF5 to inhibit its activity. As a result, mitochondria motility is inhibited.

Figure 4 | **Regulation of kinesin–cargo binding by three different mechanisms. a** | Phosphorylation. The kinesin 2

KIF1A and KIF1B transport synaptic vesicle precursors containing synaptic vesicle proteins such as synaptotagmin, synaptophysin, and Rab 3A.

KIF1B and KIF5 (KIF5A, KIF5B, and KIF5C) transport mitochondria in the anterograde direction

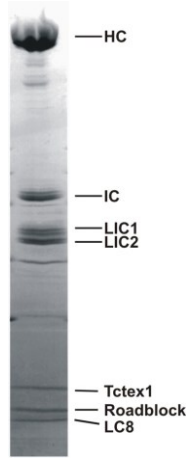
KIF5 also transports other cargoes, including vesicles that contain APPs (amyloid precursor proteins) and vesicles containing APOER2 (apolipoprotein E receptor 2)

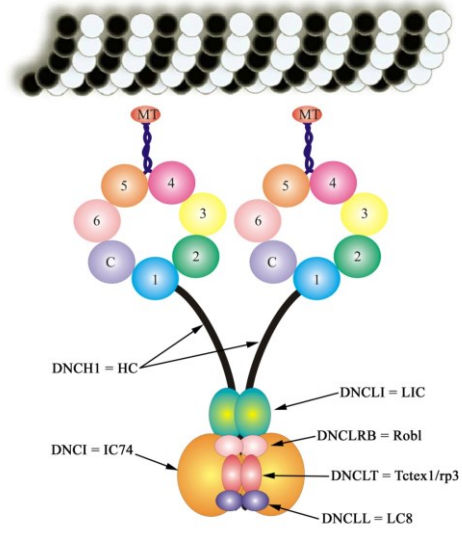
KIF5 participates in slow axonal transport of cytoskeletal proteins

KIF3, mainly composed of KIF 3A/KIF3B heterodimers and an associated protein KAP3, transports vesicles associated with fodrin and is important for neurite extension.

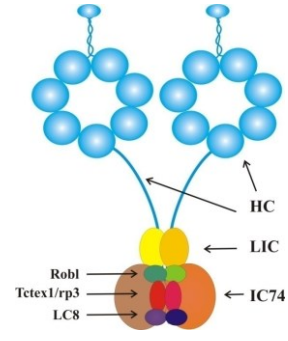
In dendrites, transport of NMDAreceptors is mediated by the molecular motor **KIF17**;

Motor Proteins:
Cytoplasmic Dynein: Polypeptides and Structure





One Type of Cytoplasmic Dynein Motor Domain (HC) But Multiple Mammalian Cargo Binding Subunits

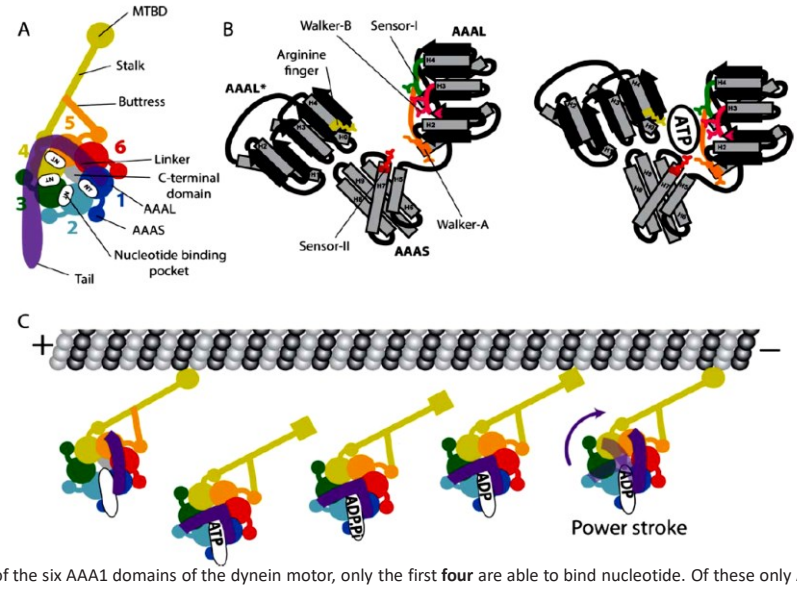


Intermediate Chains (dimer) – 2 genes
- 5 - 6 alternative splice variants
- Phosphorylated isoforms

Light Intermediate Chains (dimer) - 2 genes
- Phosphorylated isoforms
- May be alternative splice variants

Heavy Chains
- **Tctex1 Family** (dimer) - 2 genes
- **Roadblock Family** (dimer) - 2 or more genes
- **LC8 Family** (dimer) - 2 or more genes
- No evidence for cytoplasmic dynein LC phosphorylation

Structure and Mechanism of the Dynein Motor ATPase
Helgo Schmidt and Andrew P. Carter Biopolymers 105: 557–567, 2016

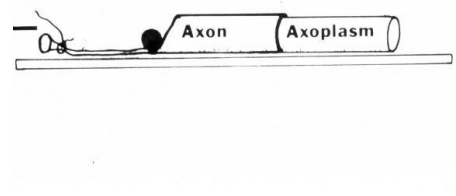


Out of the six AAA1 domains of the dynein motor, only the first **four** are able to bind nucleotide. Of these only AAA1 is strictly required for motility and in all dynein isoforms, it harbours the complete set of catalytic residues. The other nucleotide binding sites show a varying degree of conservation and seem to have accessory or regulatory roles for dynein motility. AAA5 and AAA6 have lost all catalytic residues and mainly function as a structural base for the stalk buttress.

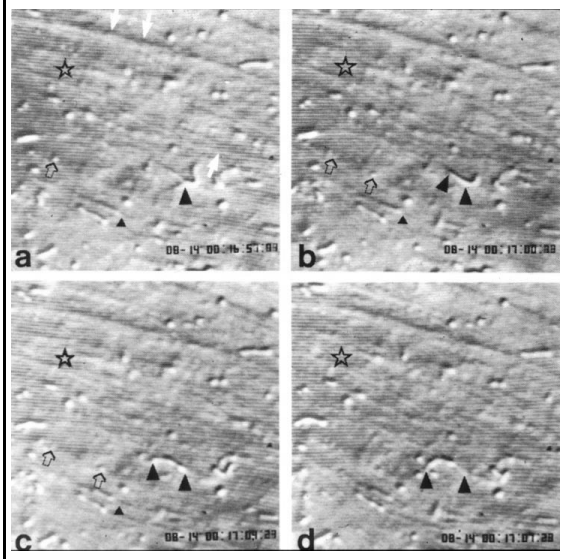
MICROTUBULE-BASED MOTOR PROTEINS & THE MECHANISM FOR FAST AXONAL TRANSPORT

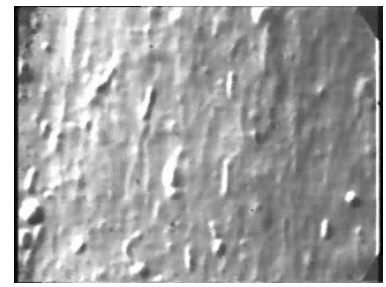
Fast Axonal Transport

Extrusion of Axoplasm from squid giant axon



1. Time Lapse analysis of extruded Axoplasm
(**VEC=Video-enhanced contrast Images**)

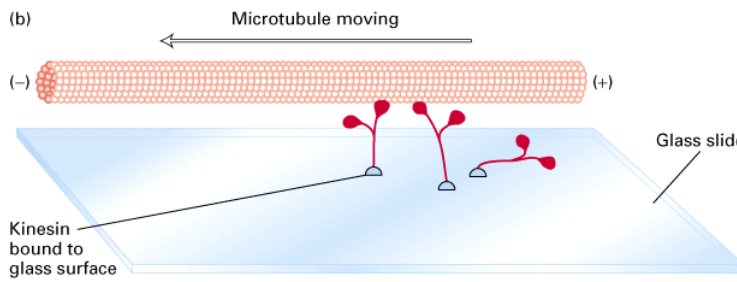




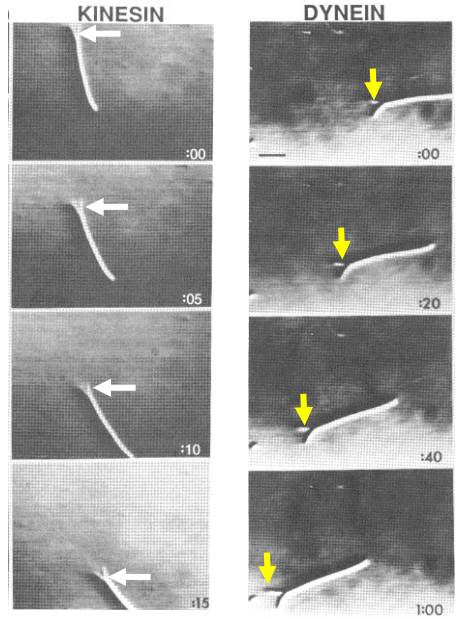
Extruded Squid Axoplasm

In Vitro Motility Assay

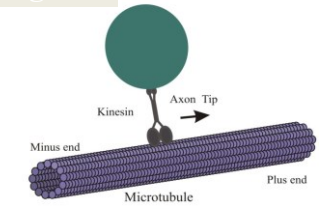
MTs move (almost glide) over surface of a motor protein coated coverslip (requires ATP).



In Vitro Motility Assay (VEC analyisis)

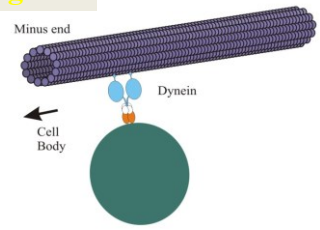


Anterograde



FC

Retrograde

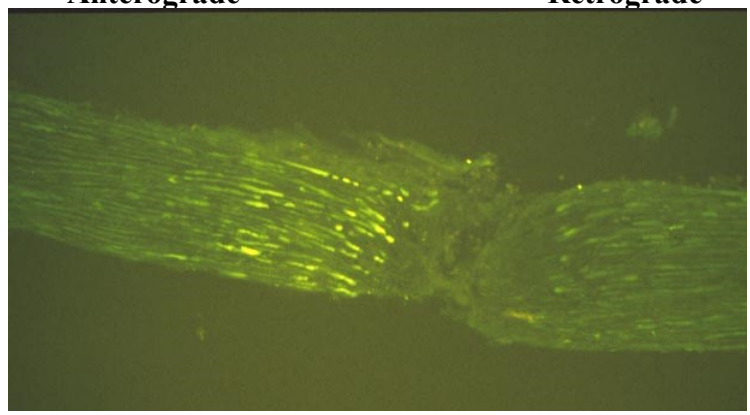


Retrograde

Kinesin – mostly seen on anterograde side

Staining of a nerve so there are many axons

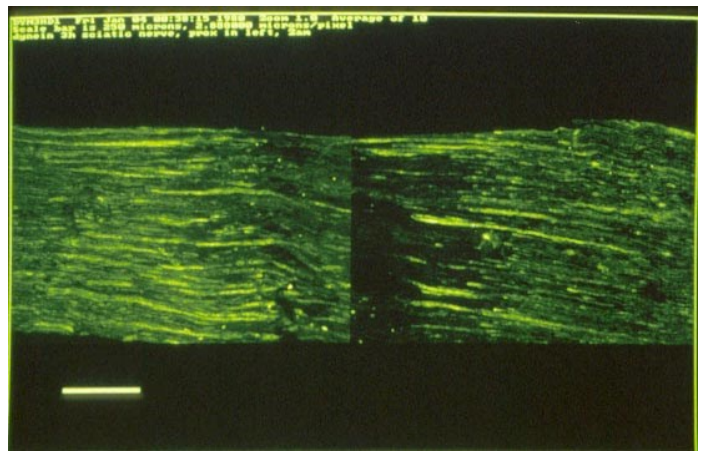
Anterograde **Retrograde**



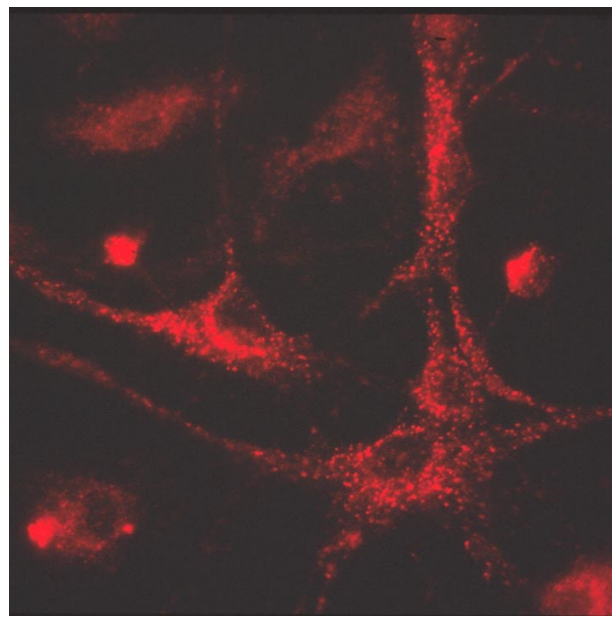
Most marker proteins are seen on both sides

Cytoplasmic Dynein seen on both sides

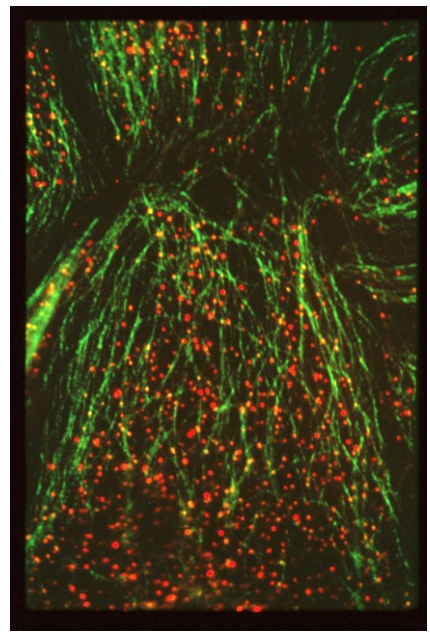
Anterograde Retrograde



Kinesin in Cultured Glia



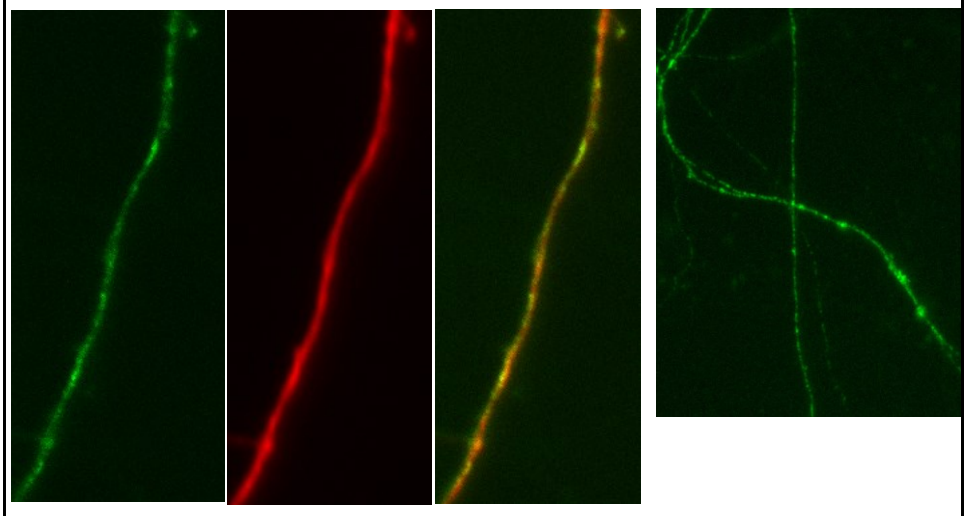
Fibroblasts:



Kinesin
MTs

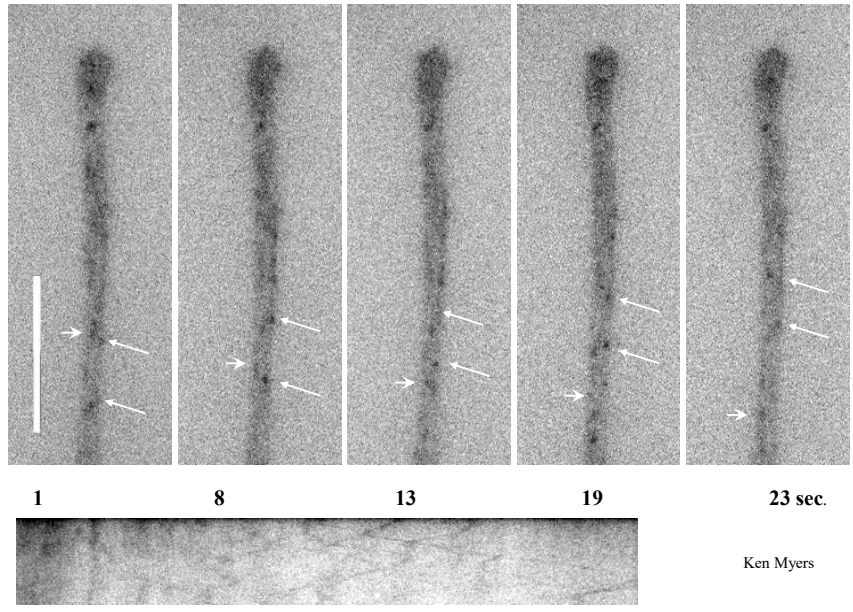
Axon: Cultured Rat Hippocampal Neuron

GFP-Dynein | mRFP | Overlay | GFP-Dynein



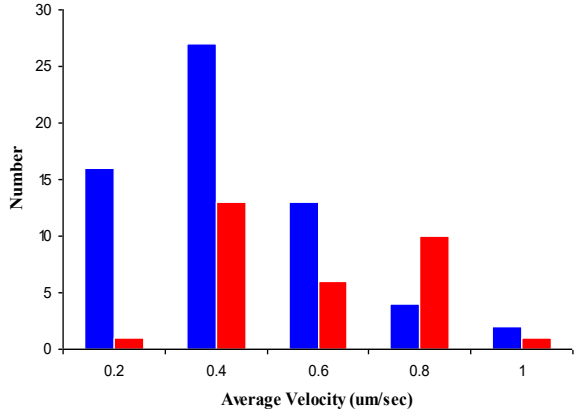
Junghoon Ha

Movement of GFP-Dynein Puncta in Both Directions



Ken Myers

Characterization of Dynein Puncta Motility in PC12 Cell Neurites



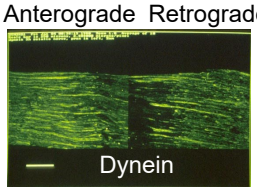
	Anterograde	Retrograde
V_{avg} µm/sec	0.4 ± 0.05	0.6 ± 0.05
Range µm/sec	0.1 - 1.3	0.1 - 1.5
N	62	31
%	67	33

Anterograde
Retrograde

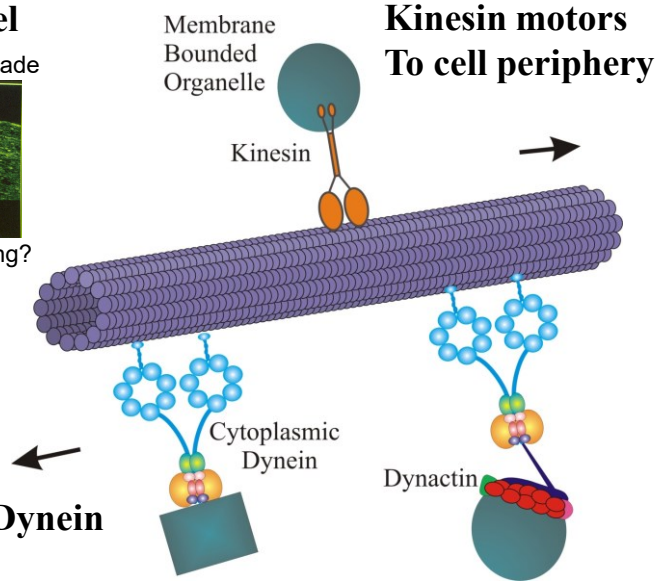
Junghoon Ha

General Model

Anterograde Retrograde



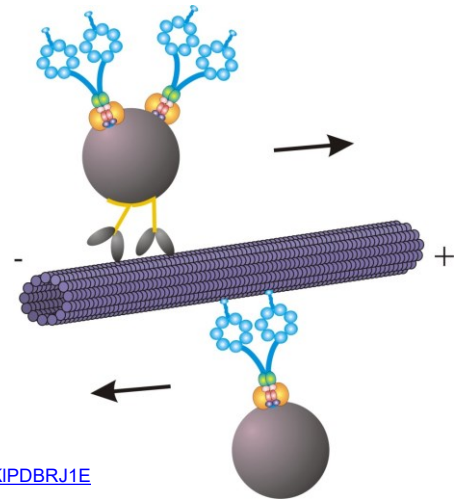
What about this finding?



Kinesin motors
To cell periphery

Cytoplasmic Dynein
To cell center

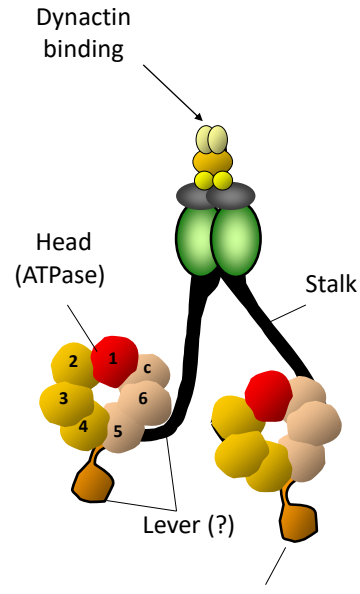
Revised Model



http://www.youtube.com/watch?v=tMKlPDBRJ1E

Dynein Can Shift Gears

Dynein

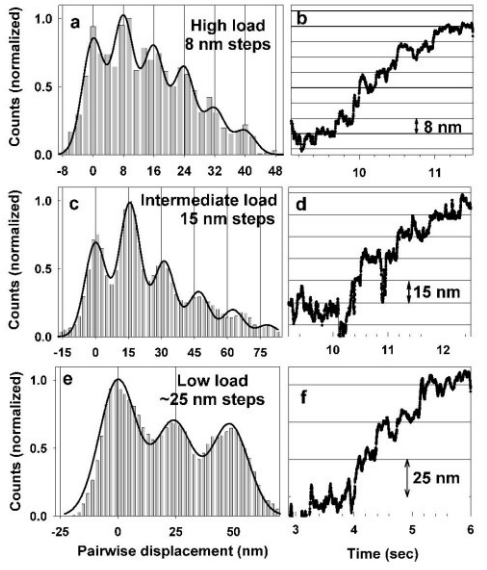


Roop Mallik[1], Brian Carter[1], Stephanie Lax[2], Stephen King[2], Steven Gross[1]

[1]UC Irvine

[2]Univ. of Missouri-Kansas City

Step Size as a Function of Load at High [ATP]



High load
8nm steps

Intermediate load
15 nm steps

Low load
~25 nm steps

Complication !

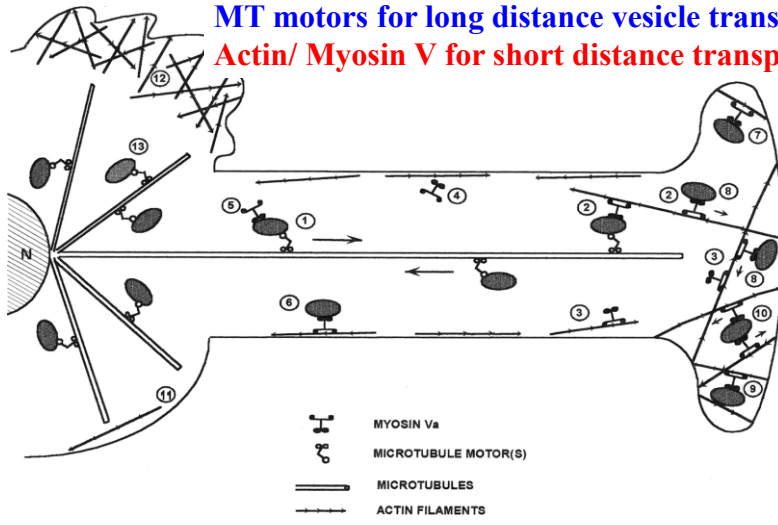
Mouse "dilute" Mutation is Myosin V

It has neurological defects

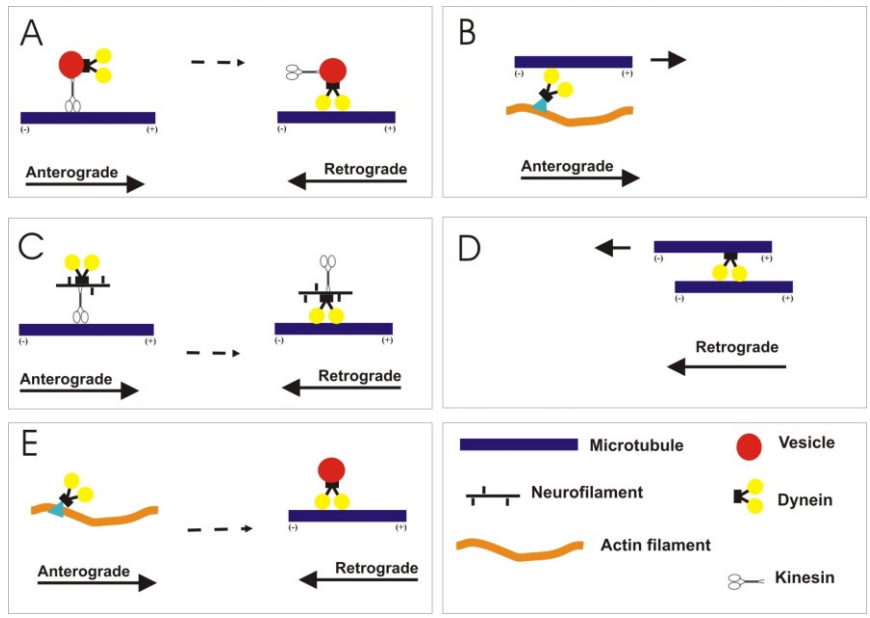
Revised Model:

MT motors for long distance vesicle transport

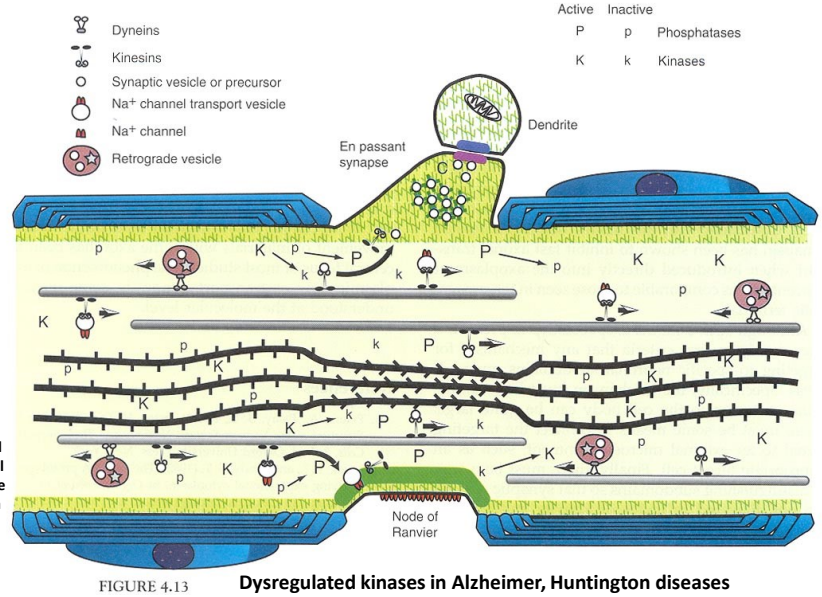
Actin/ Myosin V for short distance transport



Exercise: what is Fast and what is Slow Transport ?



Changes in NF phosphorylation may change NF packing/ spacing and <u>delivery of the cargo at synapses</u>



From: L.Squire et al Fundamental Neuroscience Third edition

FIGURE 4.13

Dysregulated kinases in Alzheimer, Huntington diseases

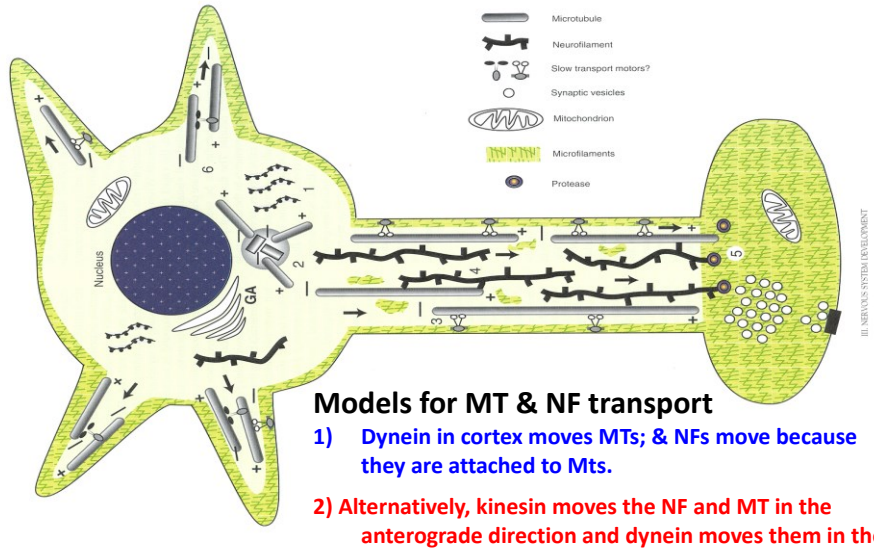
Summary: Axonal Transport 1

- Many proteins made in soma must be transported to axon and axon terminal
  - repair axolemma, for gated ion channel proteins, as enzymes or neurotransmitters
- Fast anterograde axonal transport
  - either direction up to 400 mm/day for organelles, enzymes, vesicles and small molecules

Summary Axonal Transport 2

- Fast retrograde for recycled materials and pathogens
- Slow axonal transport or axoplasmic flow
  - moves cytoskeletal and new axoplasm at 1-6 mm/day for normal housekeeping, and also for repair and regeneration in damaged axons

Roles of Dyneins and Kinesins in MT&NF transport



Models for MT & NF transport

1) **Dynein in cortex moves MTs; & NFs move because they are attached to Mts.**

2) **Alternatively, kinesin moves the NF and MT in the anterograde direction and dynein moves them in the retrograde direction.**

3) **Or a combination of both models.**