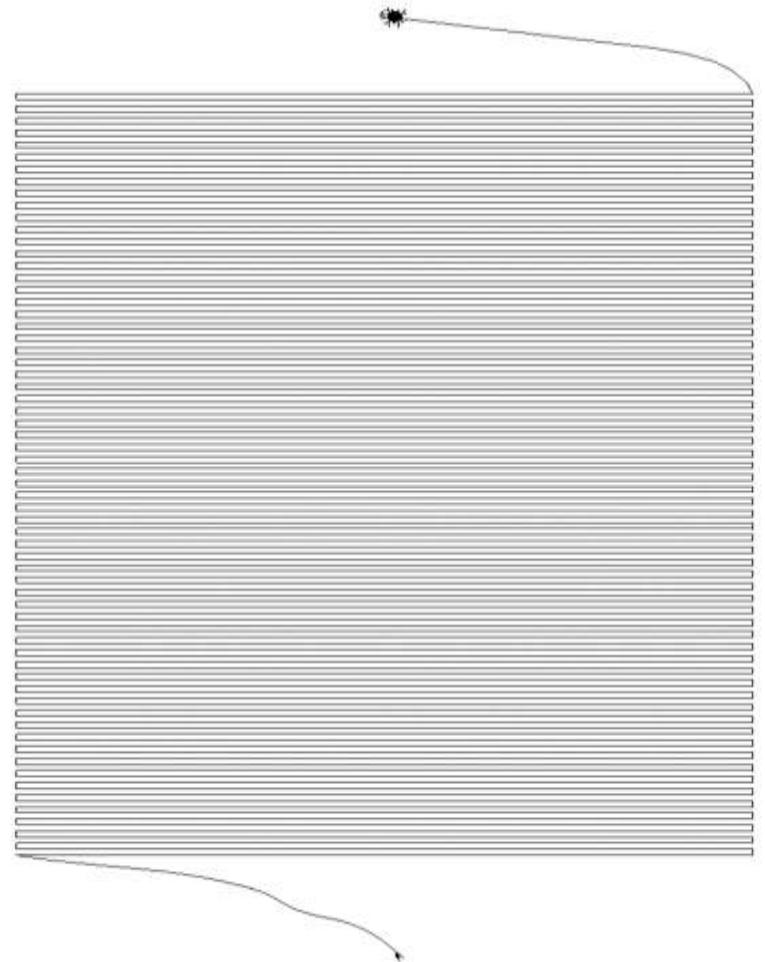




Historical Perspectives and Current Questions

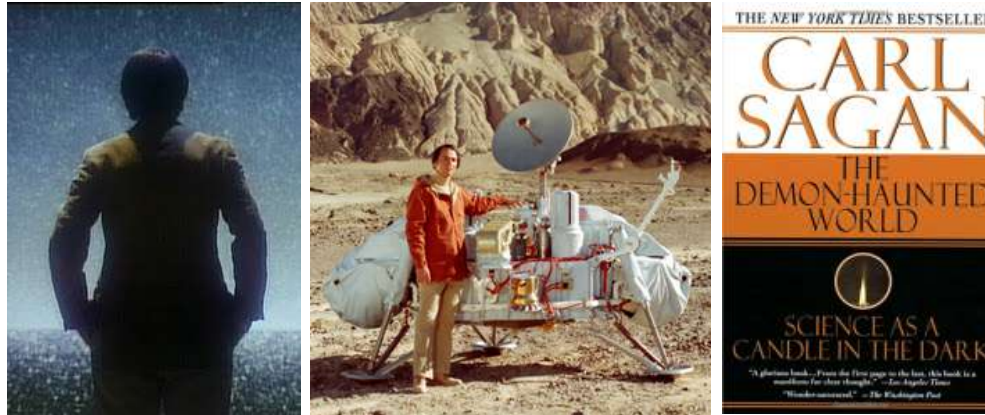
Peter J. Hollenbeck
Dept of Biological Sciences
Purdue University
West Lafayette, IN

School lecture 13
ICTS-TIFR Advanced School on Axonal
Transport & Neurodegenerative Disorders
18 January 2013



The scientific method is our greatest “Baloney Detector”

Carl Edward Sagan (1934-1996)
Professor of Astronomy, Cornell University



“Somewhere, something incredible is waiting to be known.”

“For me, it is far better to grasp the Universe as it really is than to persist in delusion, however satisfying and reassuring.”

On-line version of Sagan’s Baloney Detector:

<http://users.tpg.com.au/users/tps-seti/baloney.html>

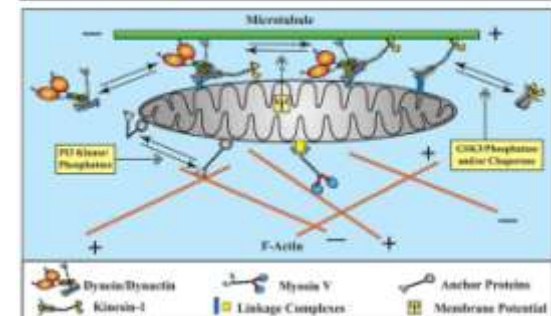
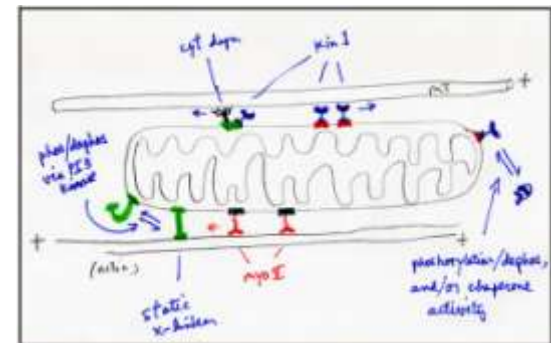
A more recent issue: lots of papers with lots of data, from lots of labs...

When is a body of work and its interpretation “fully cooked”?

When do we view something as settled science and draw the cartoon?

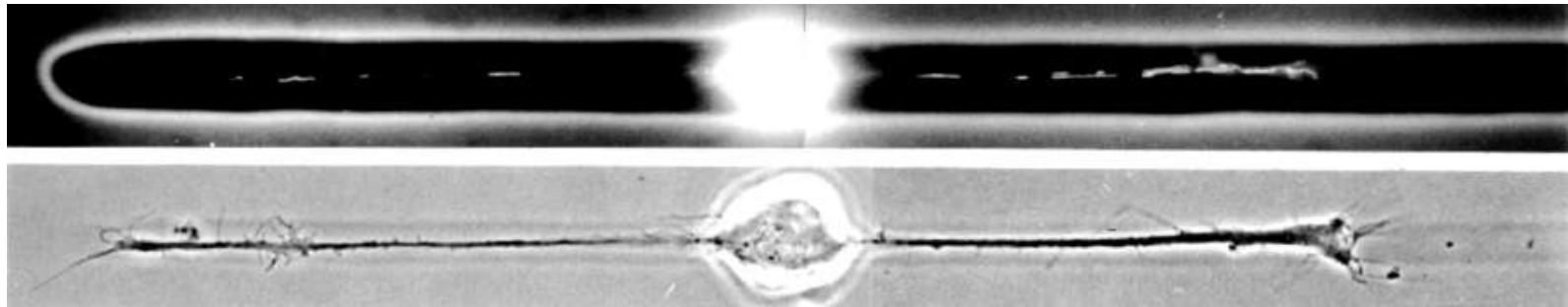


When?

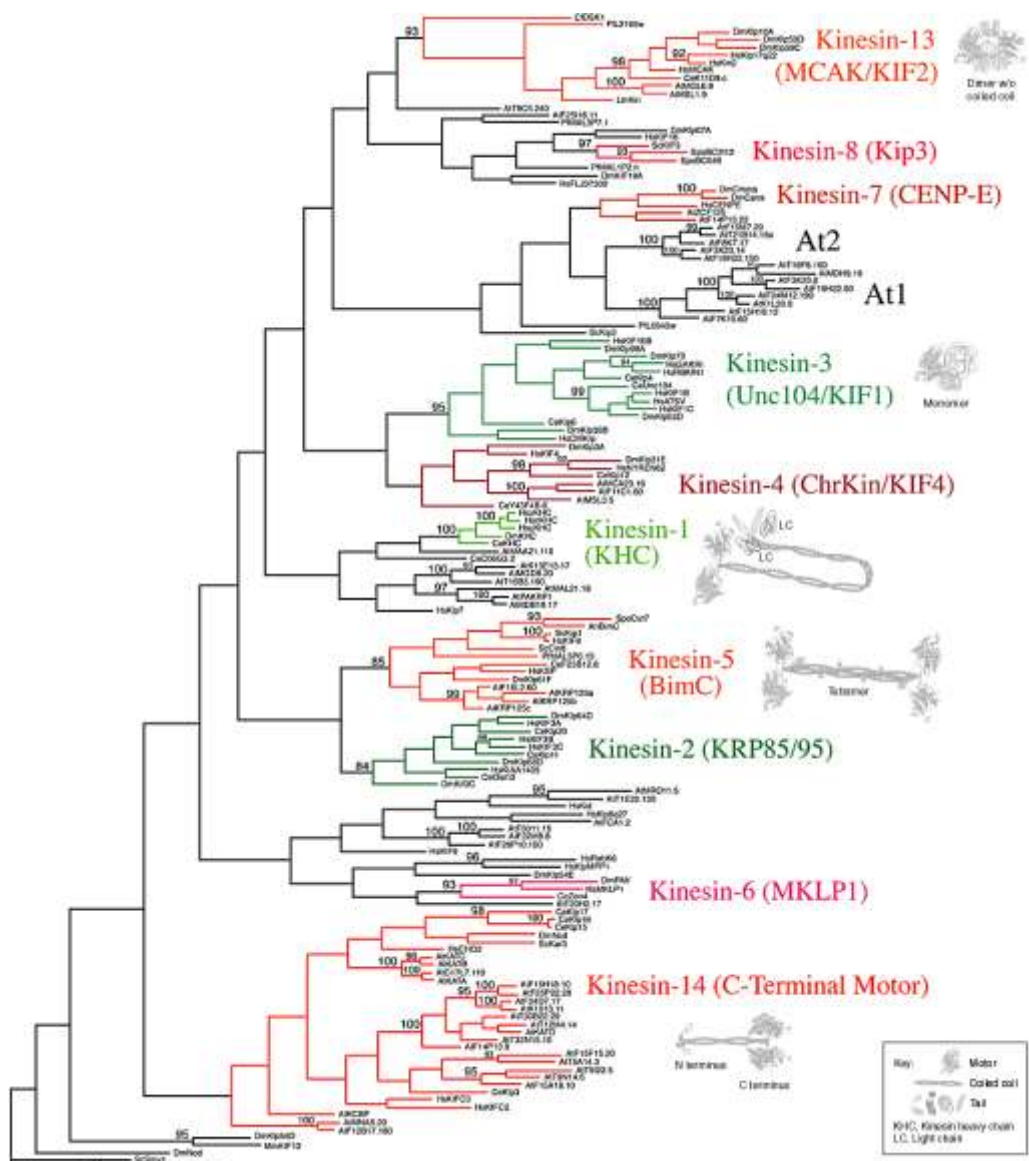


Are there facets of axonal organelle transport that are already in the “cartoon” stage but are perhaps still under-cooked?

- (1) Motor diversity and cargo diversity
- (2) Motor-organelle adaptor/regulator proteins
- (3) Neuronal polarity and organelle traffic

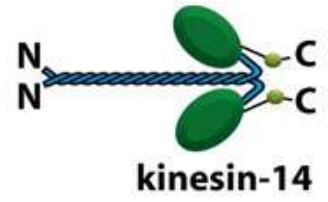
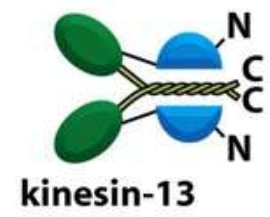


Kinesin: 14 families, plus orphans

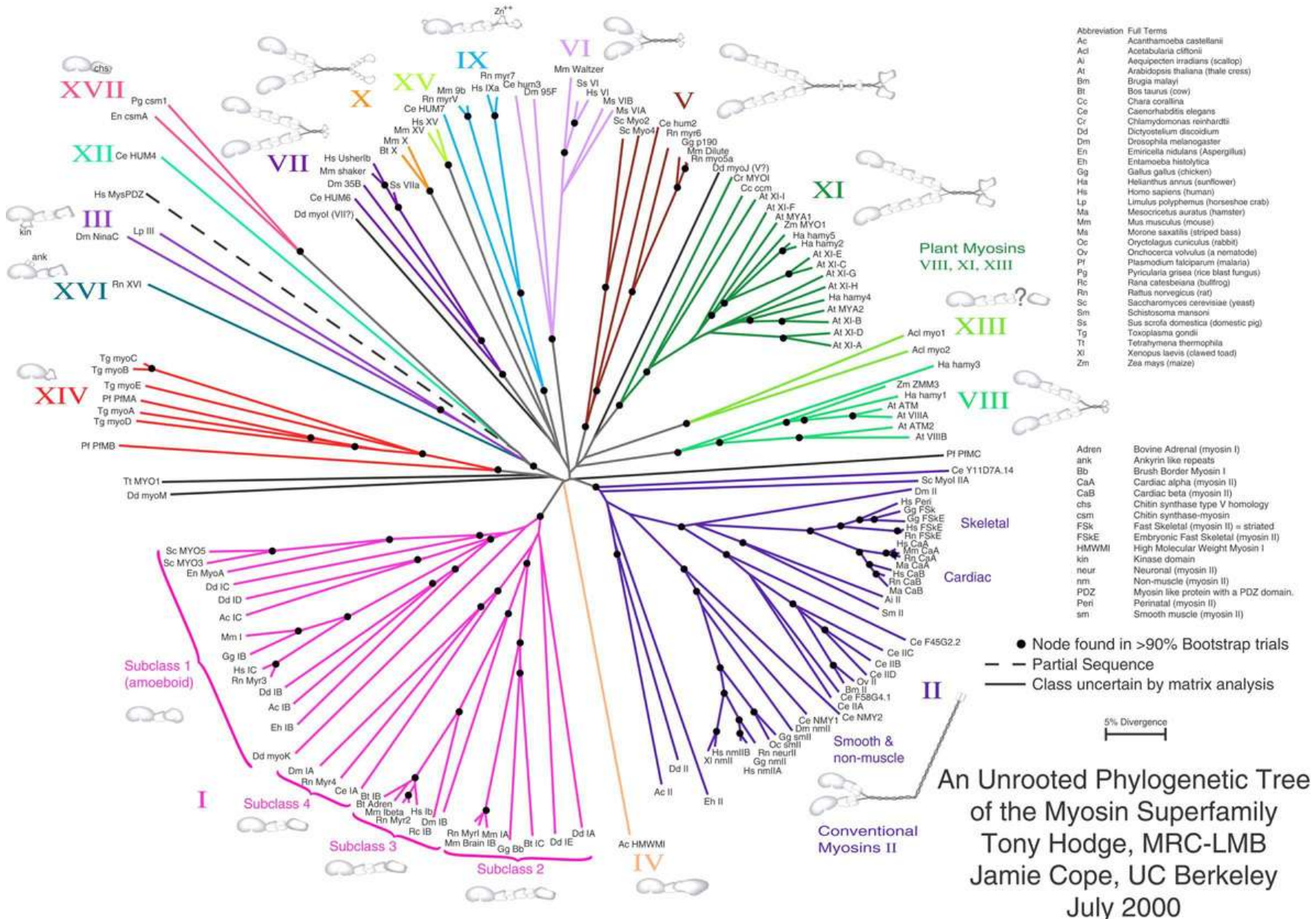


— 50 changes

Elise M. Dagenbach & Sharyn A. Endow



Myosin: 17-19 families



Myosin: domain structures



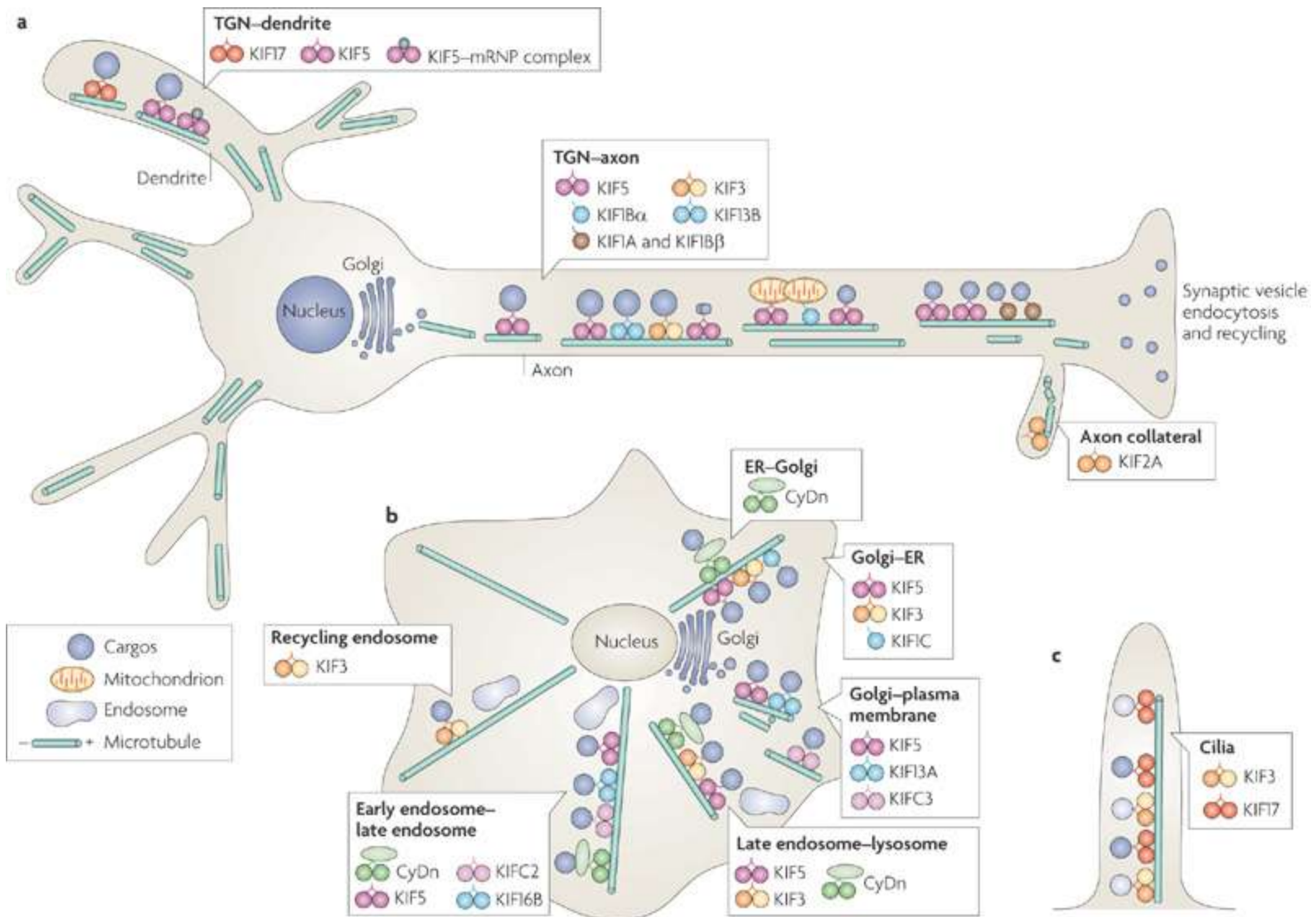
Mark Mooseker
Ross Granville Harrison Professor of MCD Biology
Yale University School of Medicine



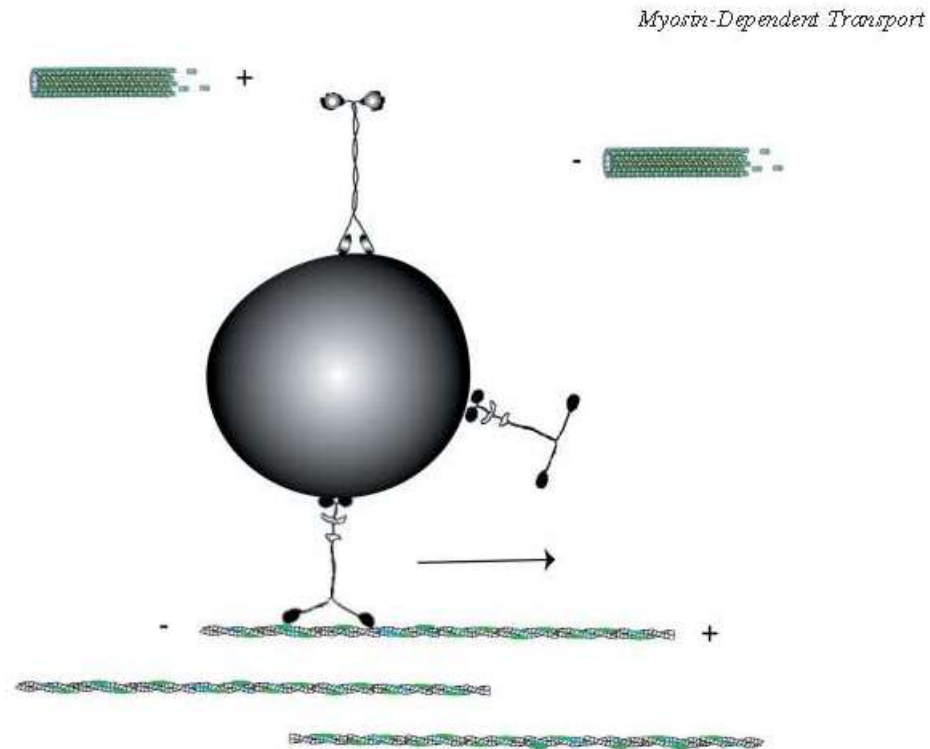
1990s: PCR approach revealed multitude of myosins

“Are you kidding? I’m miserable. The interesting part is over. With this many myosins, there’ll be a generation of nothing but spade-work in cell biology.”

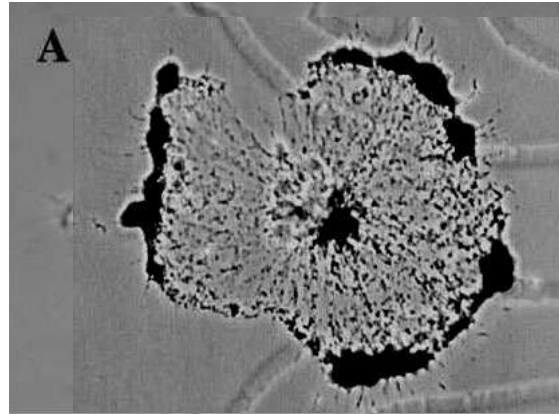
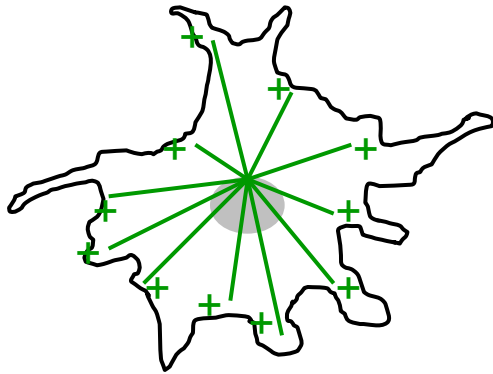
--After a seminar at HMS, around 1996



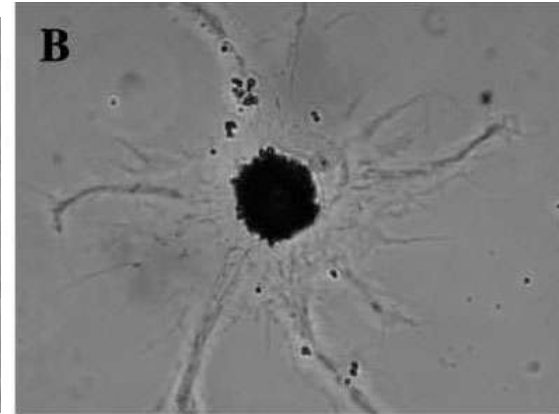
Turn-of-the-millennium model:
how do actin- and MT-based transport cooperate to drive FAT?



Pigment cells: tug of war between actomyosin- and MT-based movements

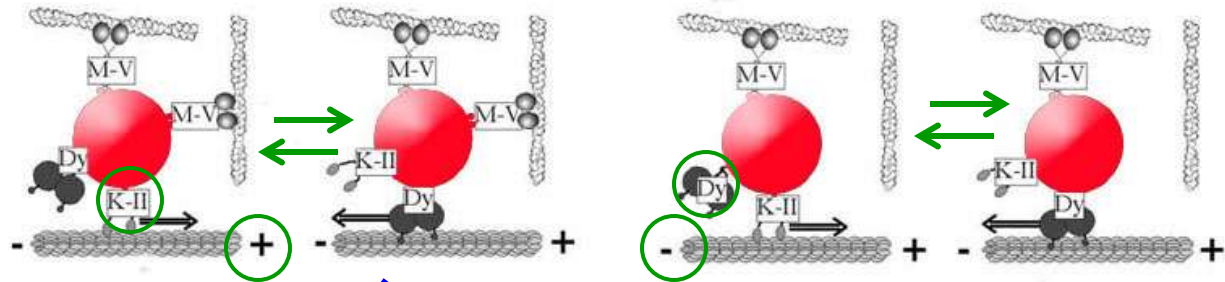


DISPERSION



AGGREGATION

Kinesin/dynein transport on MTs

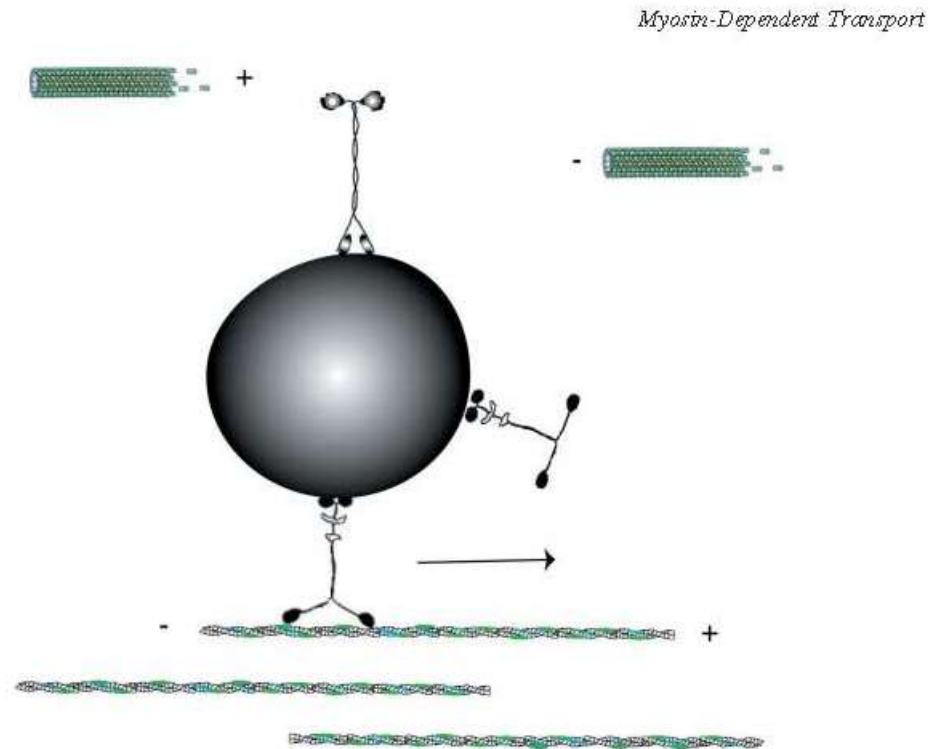


During dispersion, myosin V interrupts dynein, gives transport on actin



Myosin V can't interrupt MT-based transport during aggregation

Turn-of-the-millennium model:
how do actin- and MT-based transport cooperate to drive FAT?

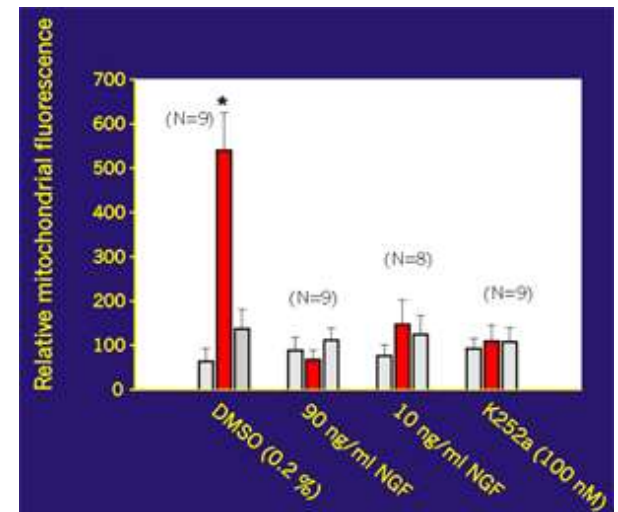
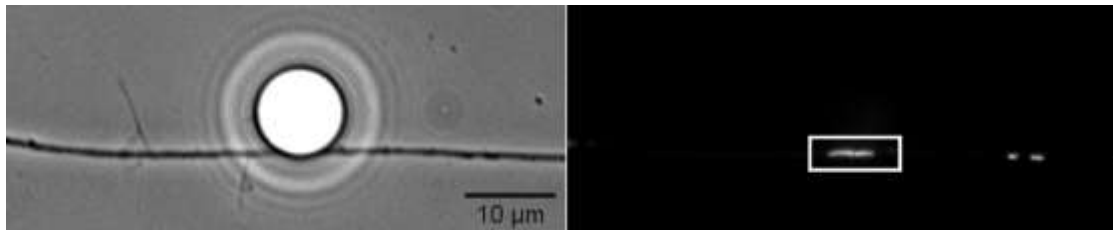


Mitochondrial movement and docking

Chick DRG neurons in culture:

When axonal actin is extensively **depolymerized**, mitochondria move **faster** and **more persistently**. (Morris & Hollenbeck, 1995)

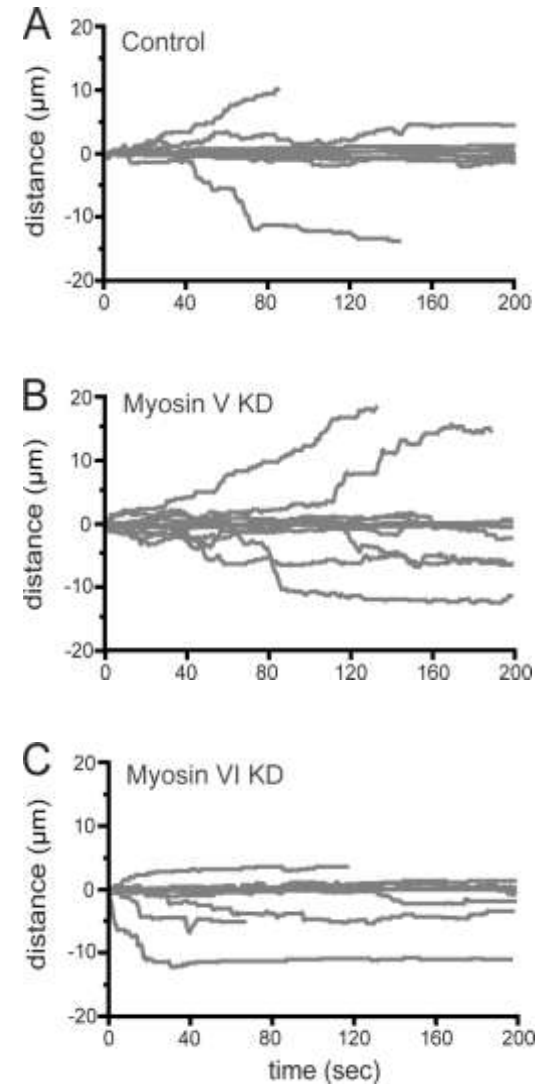
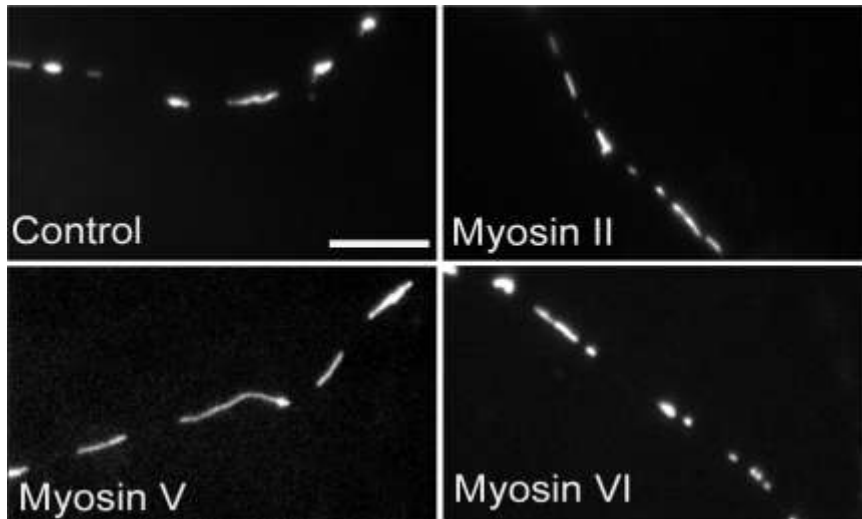
Mitochondria can be **halted** along the axon in response to local NGF/TrkA signaling, with a role for PI3K signaling downstream. However, in the **absence of F-actin**, mitochondria do not dock (Chada & Hollenbeck, 2003, 2004).

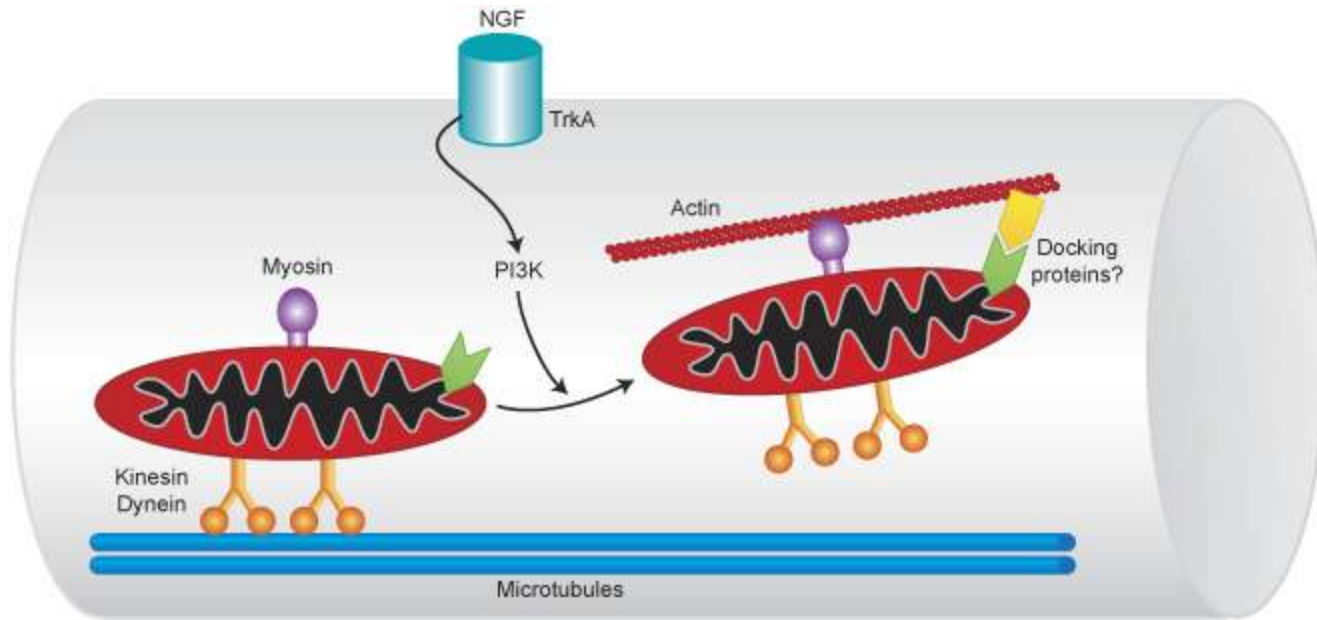


Mitochondrial movement and docking

Drosophila neurons in culture:

RNAi knock down of myosin V results in **increased flux** of axonal mitochondria, which move faster, more of the time, and more persistently than when normal myosin levels are present (Pathak et al, 2010).





Reynolds & Rintoul, *Science STKE*, 2004

Before we become satisfied that the science is settled, and rely on a cartoon mechanism, we need to have first-rate studies from :

more than one system, cell type or species.

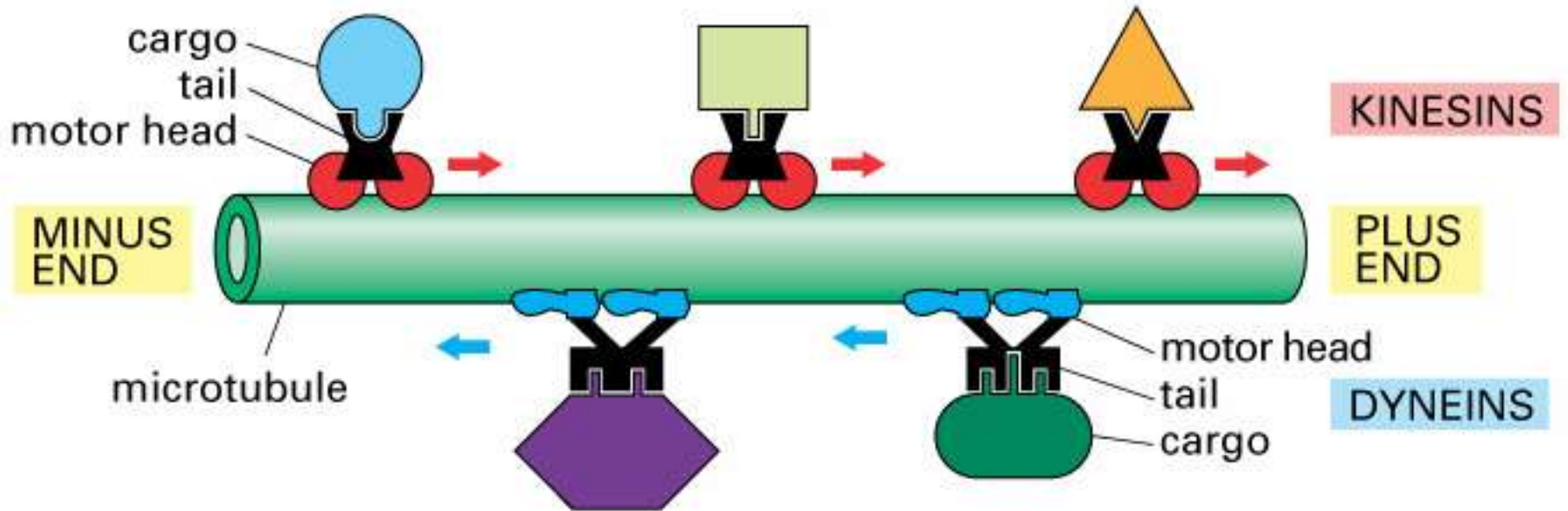
- vertebrates, invertebrates – with different features
- CNS, PNS, special senses, etc

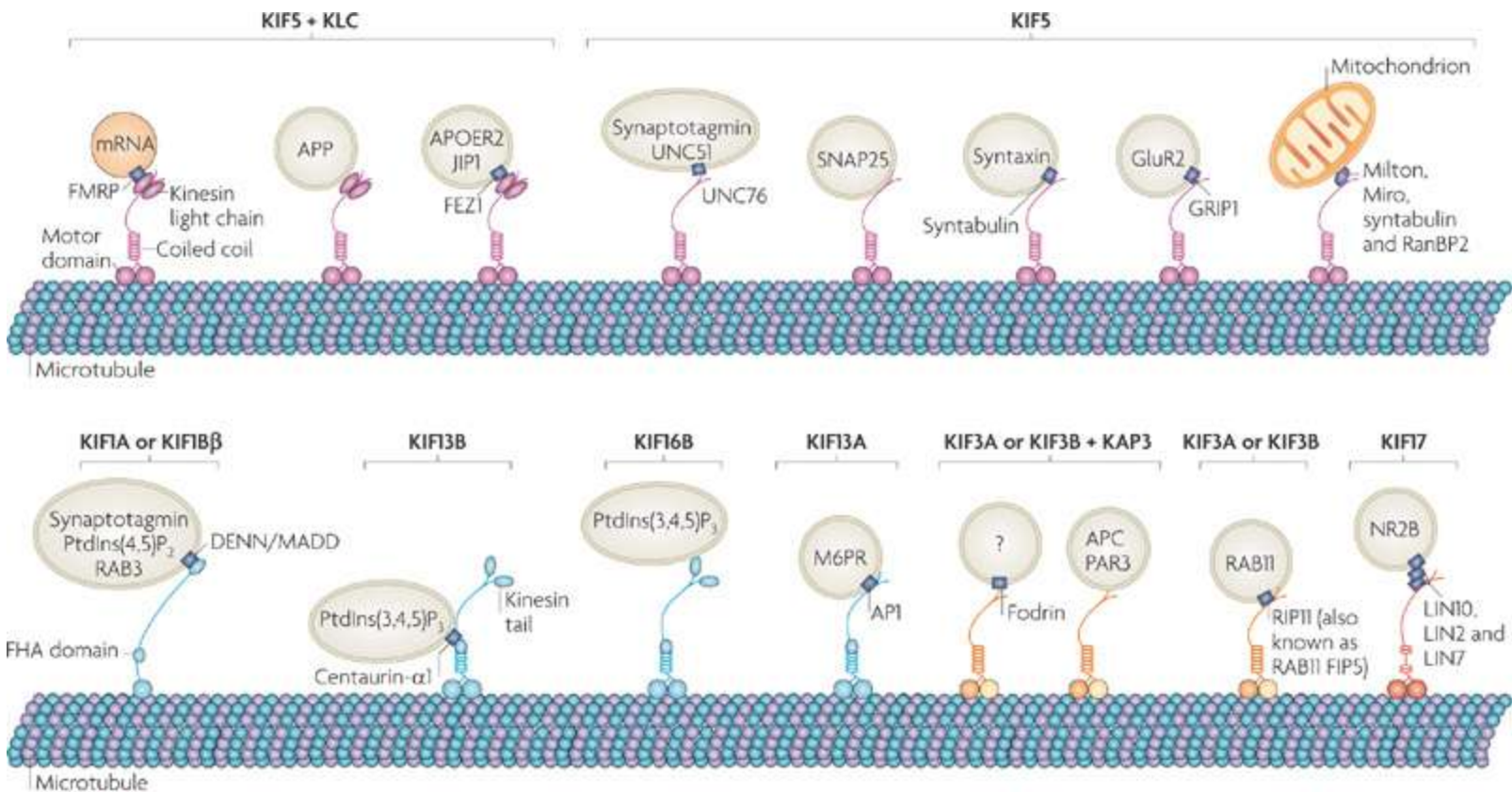
more than one technical approach.

- anatomy, EM, LM
- quantitative cellular phenotype
- in vitro systems
- biochemistry & physiology
- genetics

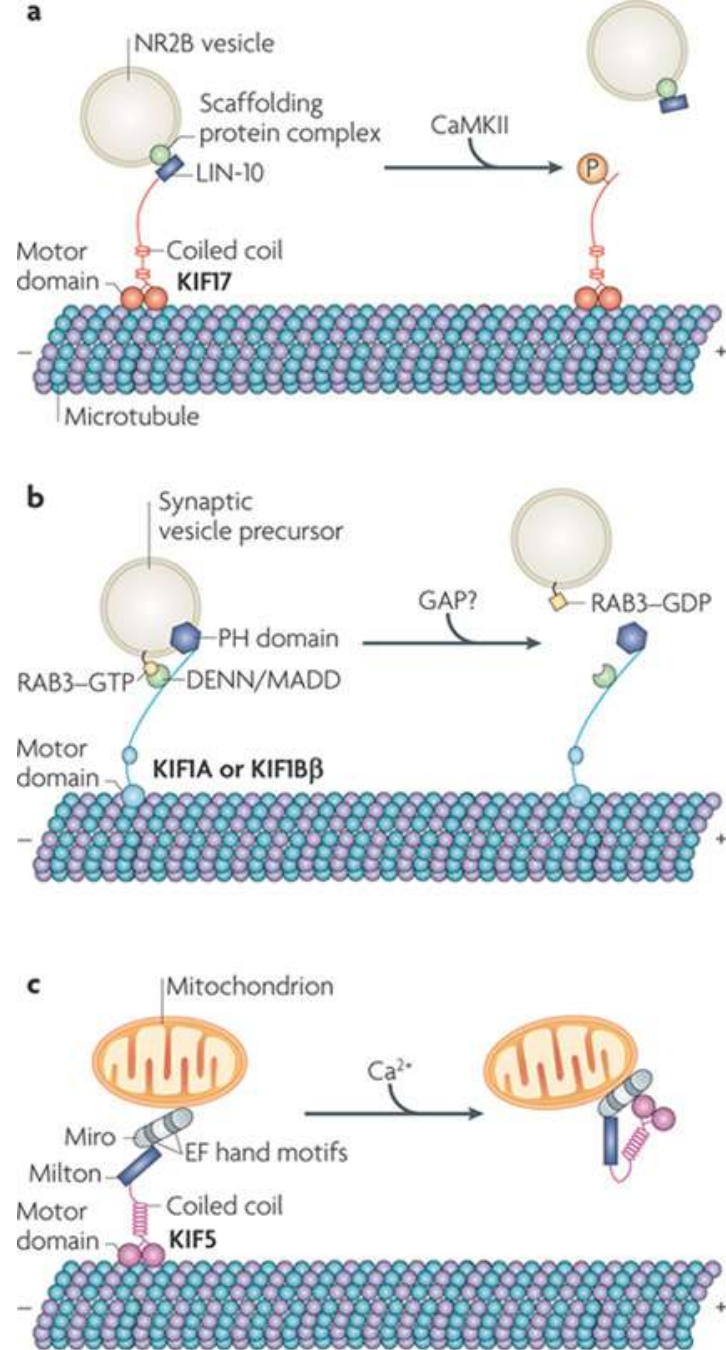
more than one laboratory.

Motor-organelle adaptor proteins: getting the right motor on the right cargo





Nature Reviews | Molecular Cell Biology



Example of a recent, exciting result: a complex that serves as a motor-mitochondrial adaptor and transport regulator: Miro/Milton and Ca regulation of FAT

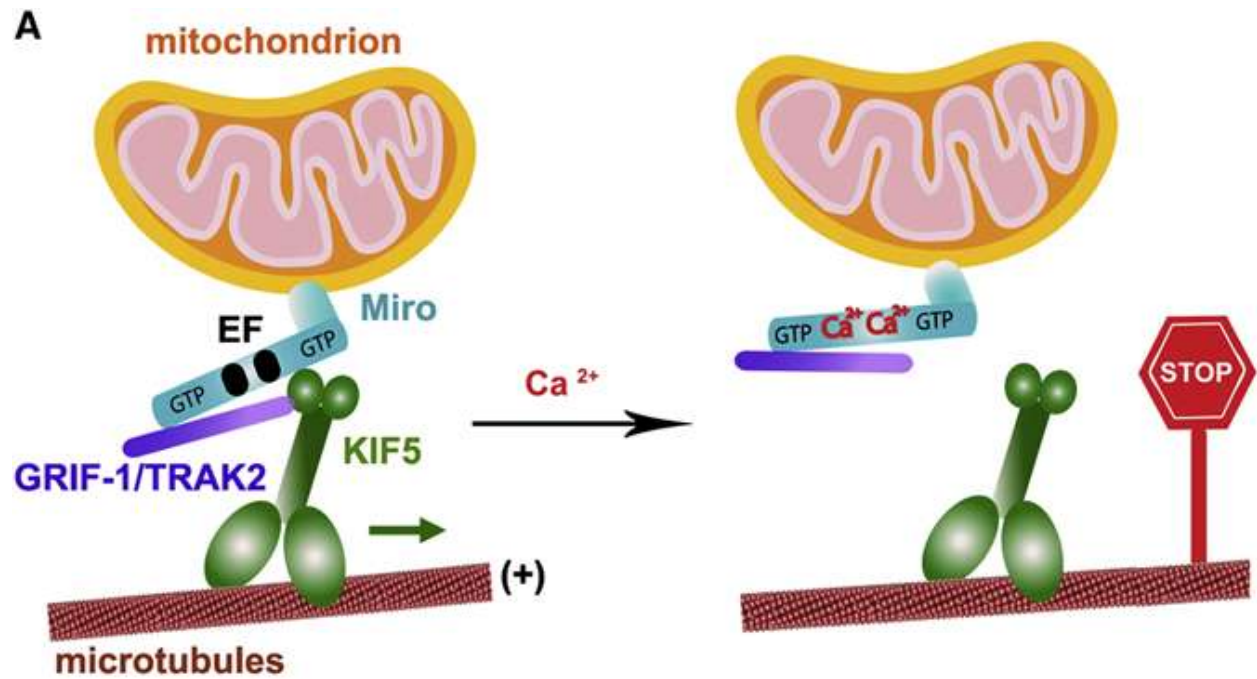
Milton mutant fly: fails to deliver mitochondria to distal axon (Schwarz lab)

Miro mutant fly: fails to deliver mitochondria to synapses (Zinsmaier lab)

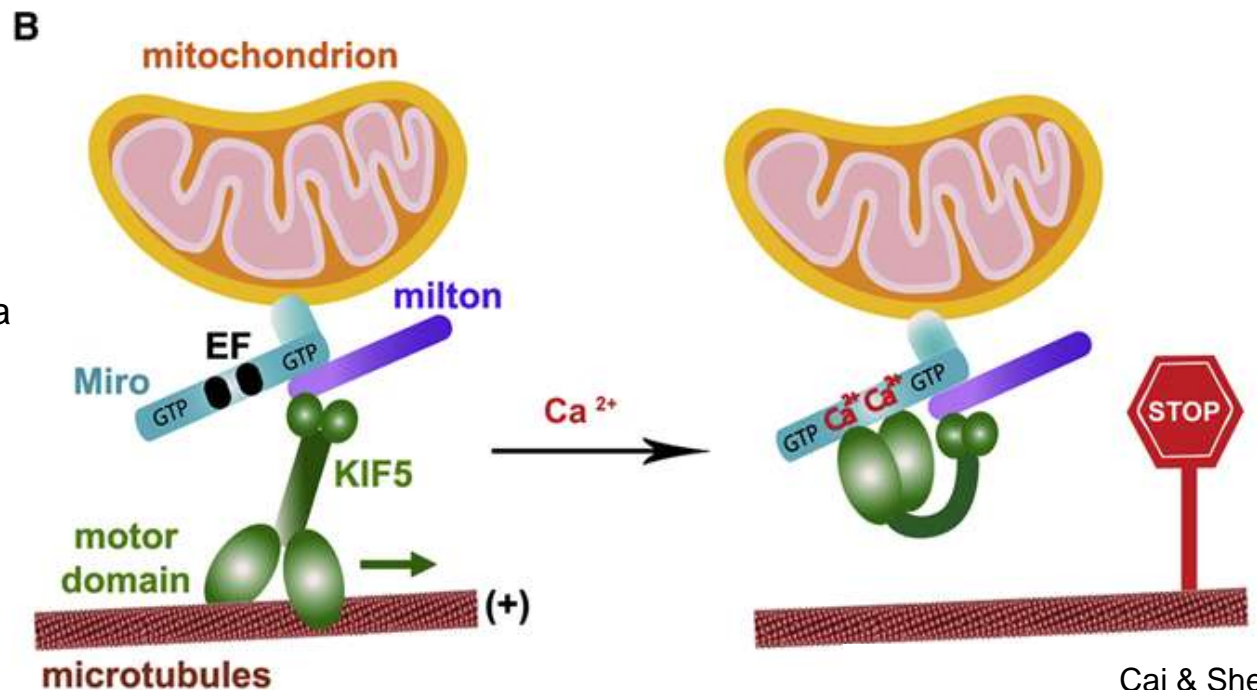
Studies of GRIF1/Milton and Miro-1 in vertebrate neurons (Kittler lab)

Studies of Milton and Miro in fly NS and various cell lines and neurons (Schwarz lab)

MacAskill & Kittler --
vertebrate cells



Schwarz et al – *Drosophila*
NS and immortalized cells



Other organelle-kinesin adaptors and regulators “in play”

- JIP scaffolding proteins (kinesin-vesicle)
- RanBP2 (kinesin-vesicle etc)
- APP (kinesin-vesicle, contentious)
- Kinectin (kinesin-vesicles, gone but not forgotten :-)

- Syntabulin (kinesin-vesicle or mitochondria?)
- Syntaphilin (docking, mitochondria)
- HUMMR (anterograde regulator, mitochondria)

- GluR2-receptor interacting protein (GRIP1, kinesin-vesicle, dendrites)

- Dynactin-dynein (any cargo specificity?)

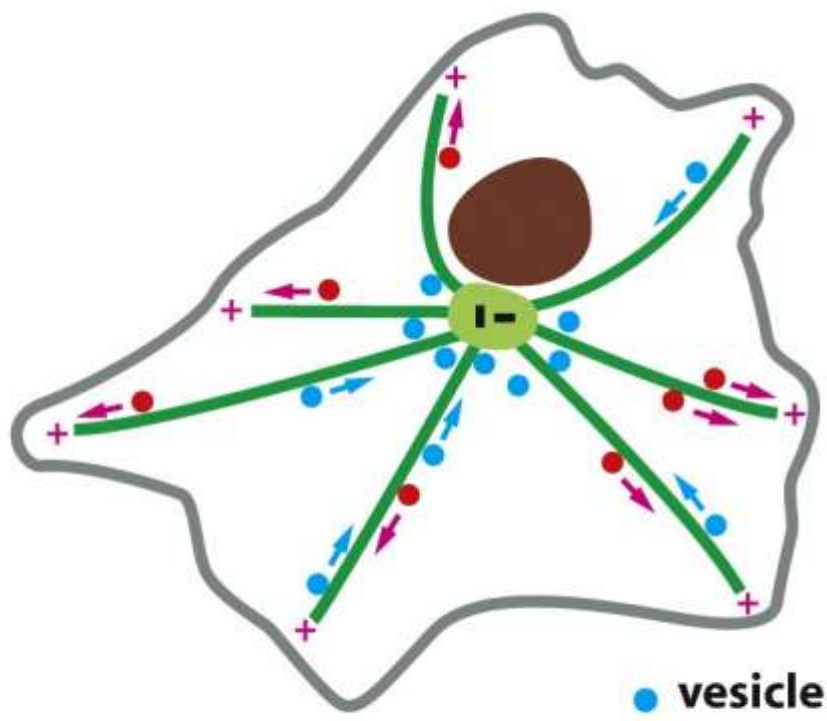
Neuronal polarity and organelle transport

Axons vs dendrites: almost uniform (axon) vs mixed (dendrite) polarity of MTs is now bankable.

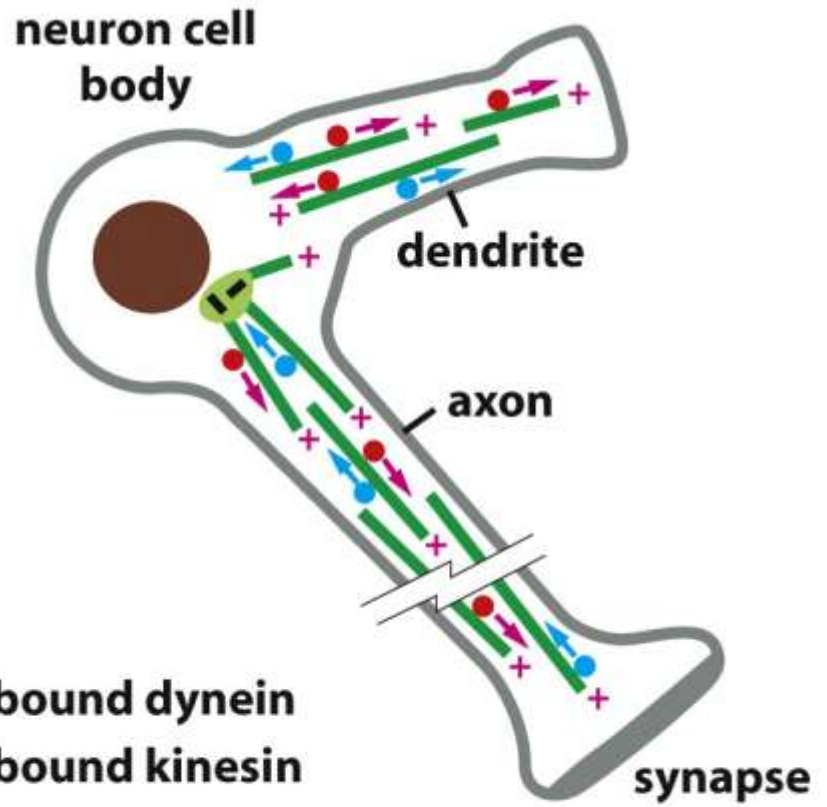
But what does it mean for organelle traffic? Can MT polarity and motors keep some organelles in or out of axons, or dendrites?

Epithelial cells vs neurons: existing models posit the cell polarity of these two v different cell types to be analogous – the distal axon being equivalent to the apical domain of epithelial cells.

There are similarities, but organelle traffic on MTs cannot be one of them – epithelia in real tissues have their (-) ends apical!

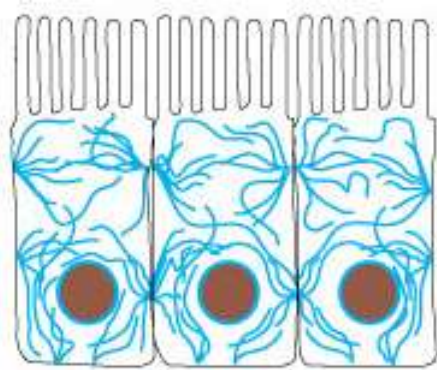


(A) **FIBROBLAST**



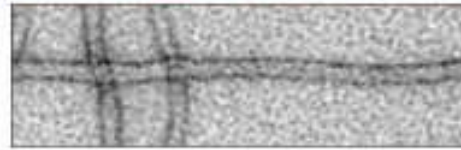
(B) **NEURON**

- vesicle with bound dynein
- vesicle with bound kinesin
- microtubule

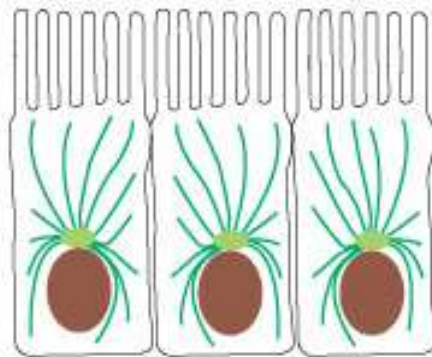


25 μm

INTERMEDIATE FILAMENTS

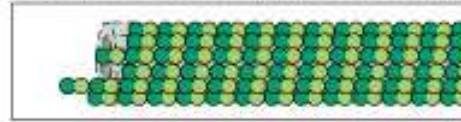


25 nm

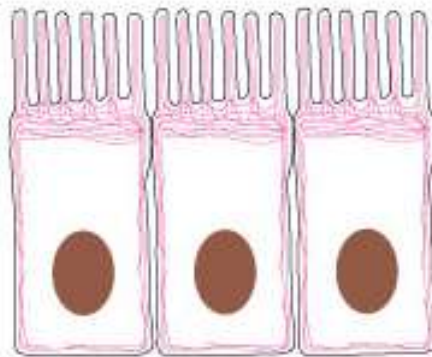


25 μm

MICROTUBULES



25 nm

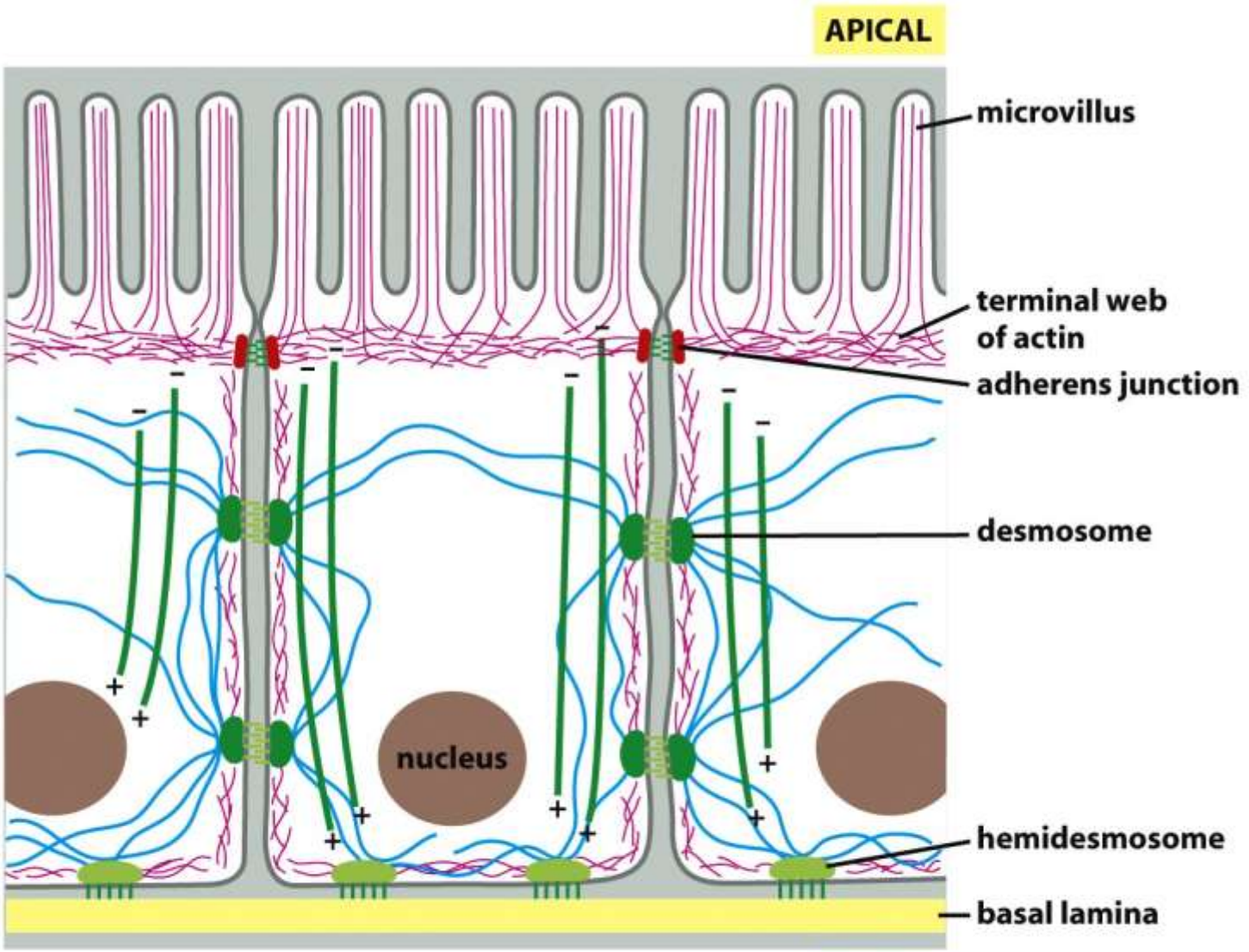


25 μm

ACTIN FILAMENTS



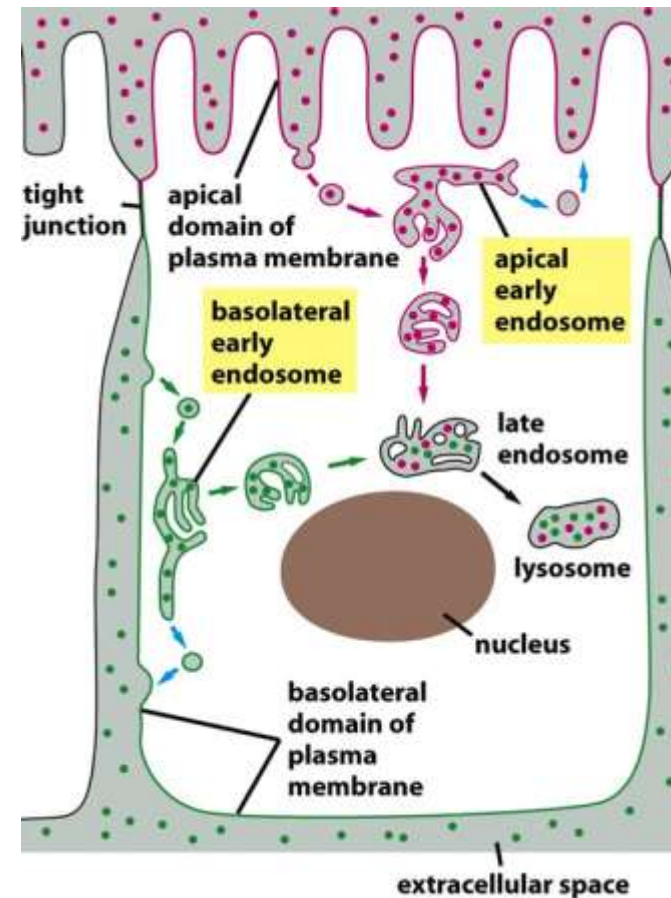
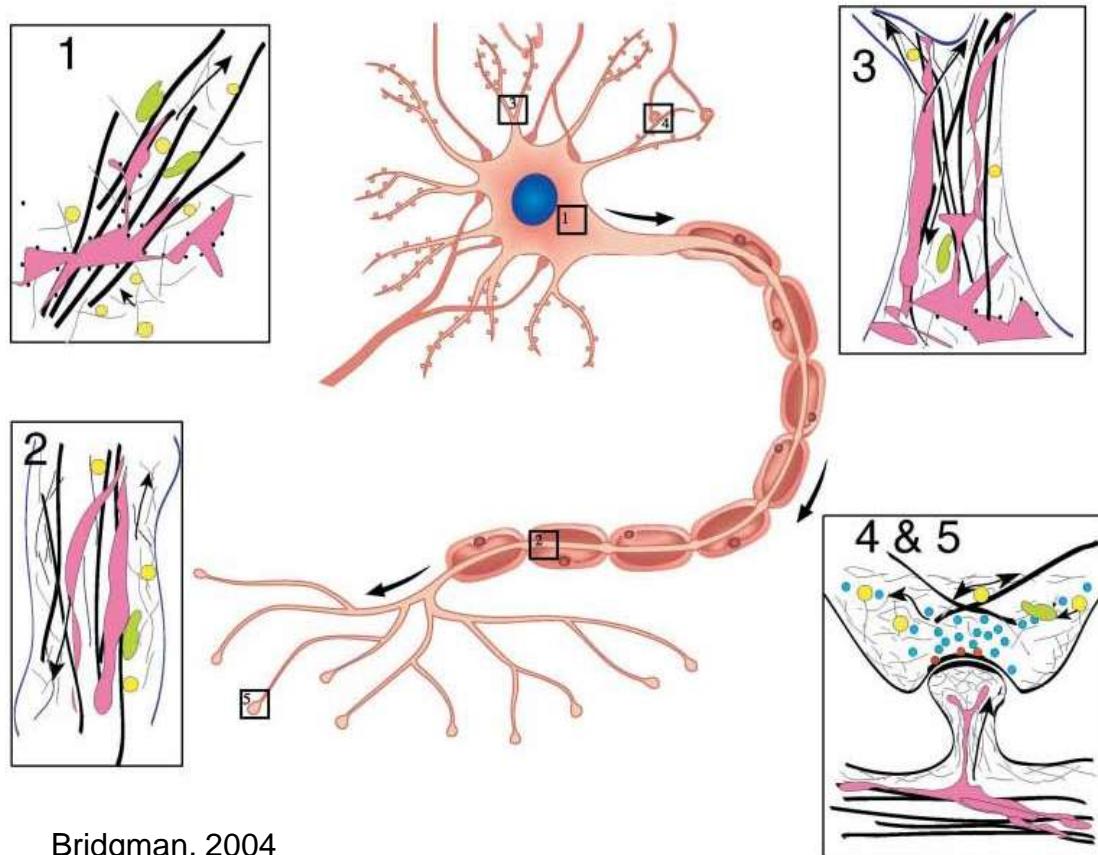
25 nm



- intermediate filaments
- microtubules
- actin microfilaments

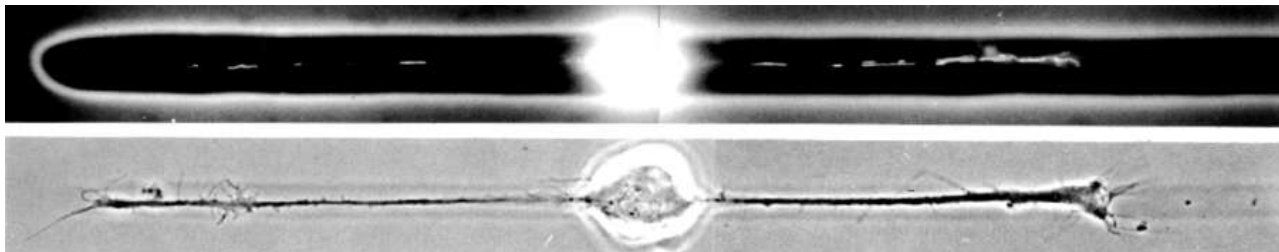
Some features of organelle traffic, secretion, endocytosis, etc are similar between the apical domains of epithelia the growth cone of synapse of a neuron.

Others are not!



Other exciting and “in play” axonal transport areas:

- Regulation of motor activity & function (refer to STB talk!)
- Motor cooperativity / tug of war
- Slow axonal transport of “soluble” proteins (ask SR!)
- (Any you’d like to add?)



Before we become satisfied that the science is settled, and rely on a cartoon mechanism, we need to have first-rate studies from :

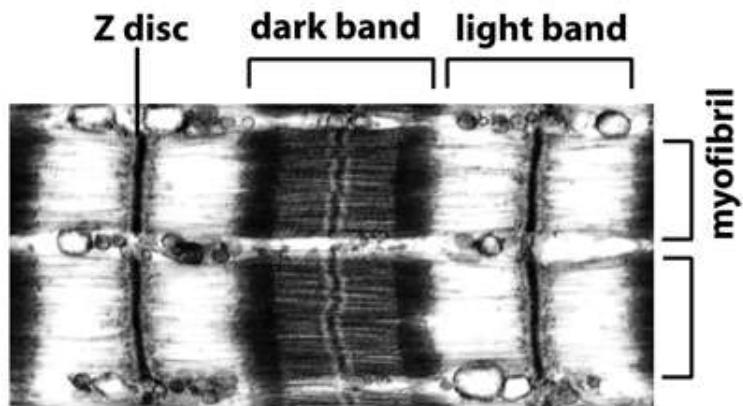
more than one system, cell type or species.

- vertebrates, invertebrates – with different features
- CNS, PNS, special senses, etc

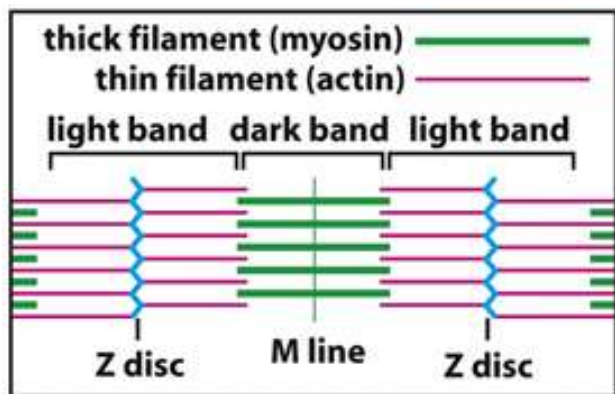
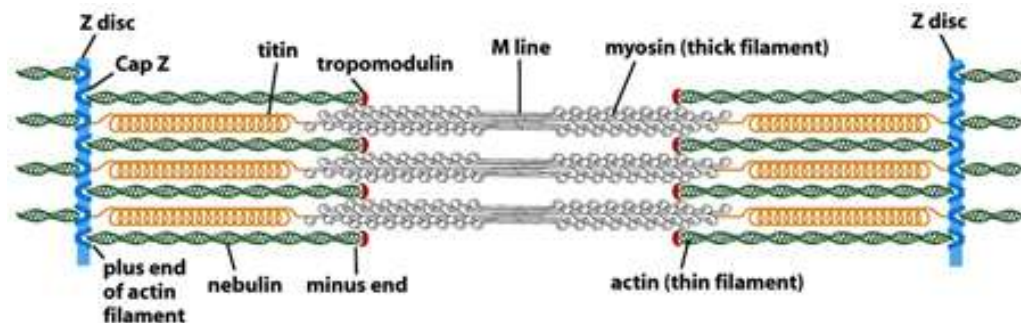
more than one technical approach.

- anatomy, EM, LM
- quantitative cellular phenotype
- in vitro systems
- biochemistry & physiology
- genetics

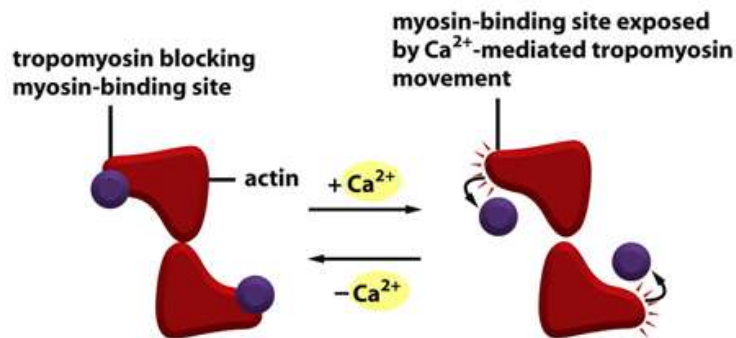
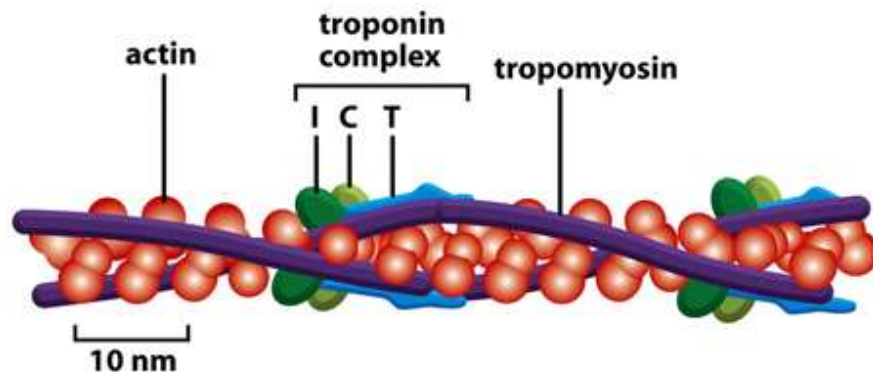
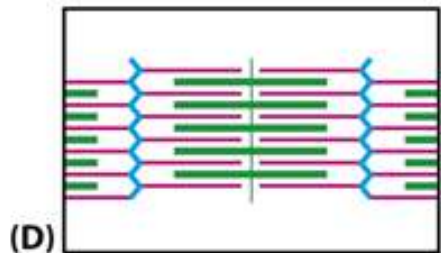
more than one laboratory (many more).



← **one sarcomere** →



(C)





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