Life Science Seen from Molecular Motor

Functions of brain and nerves, and molecular motor

2006.11.13.

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Nobutaka Hirokawa

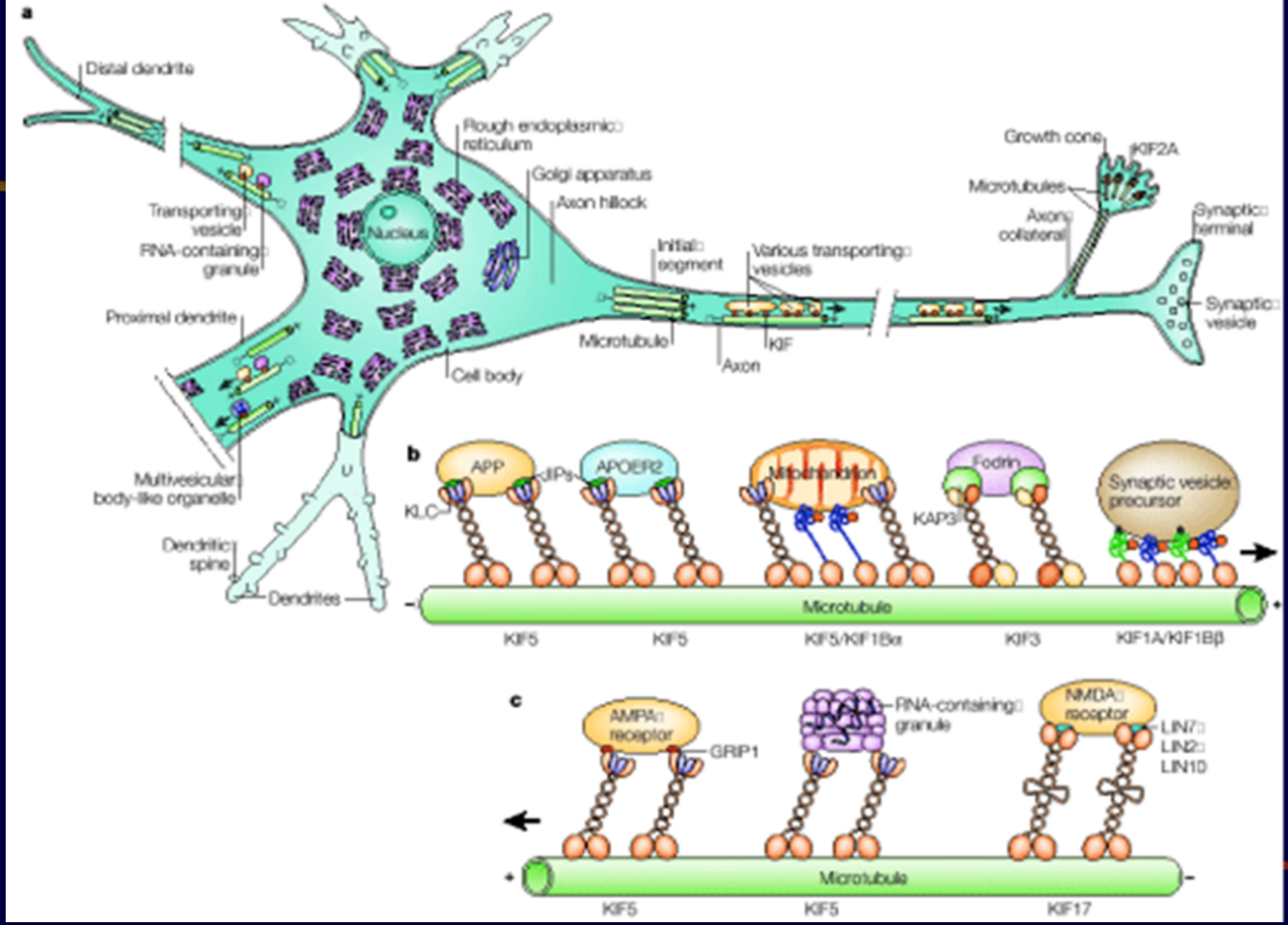
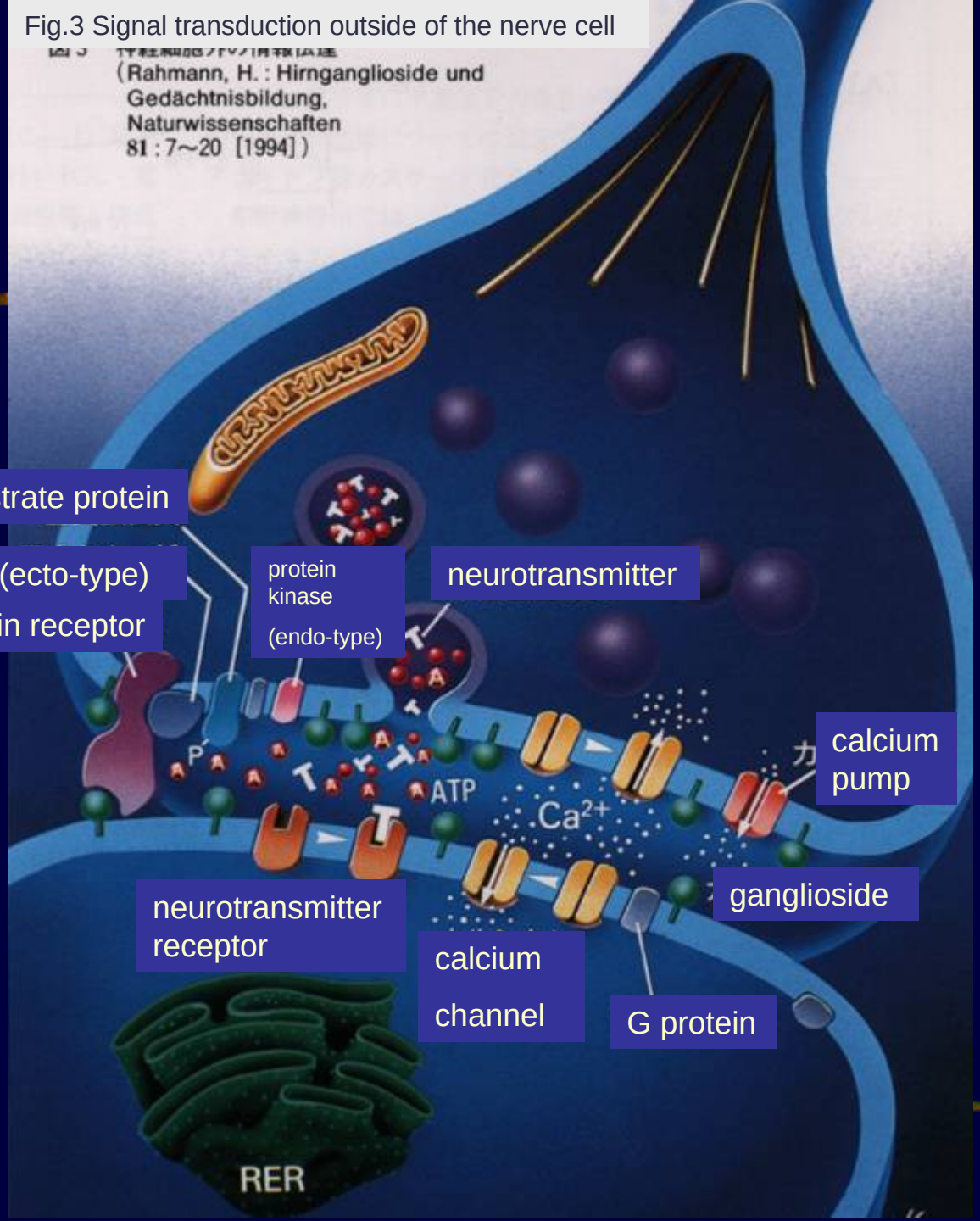
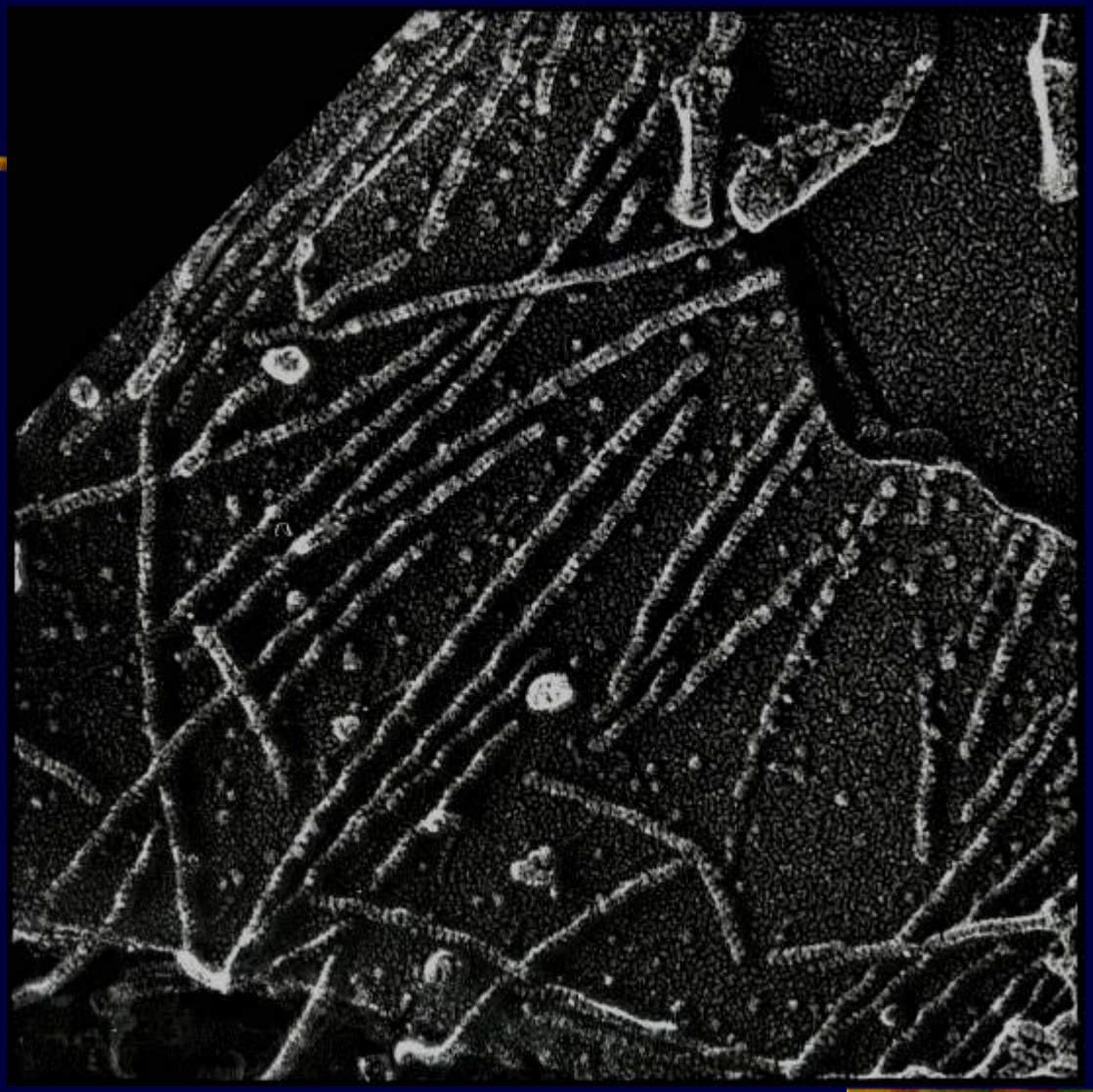
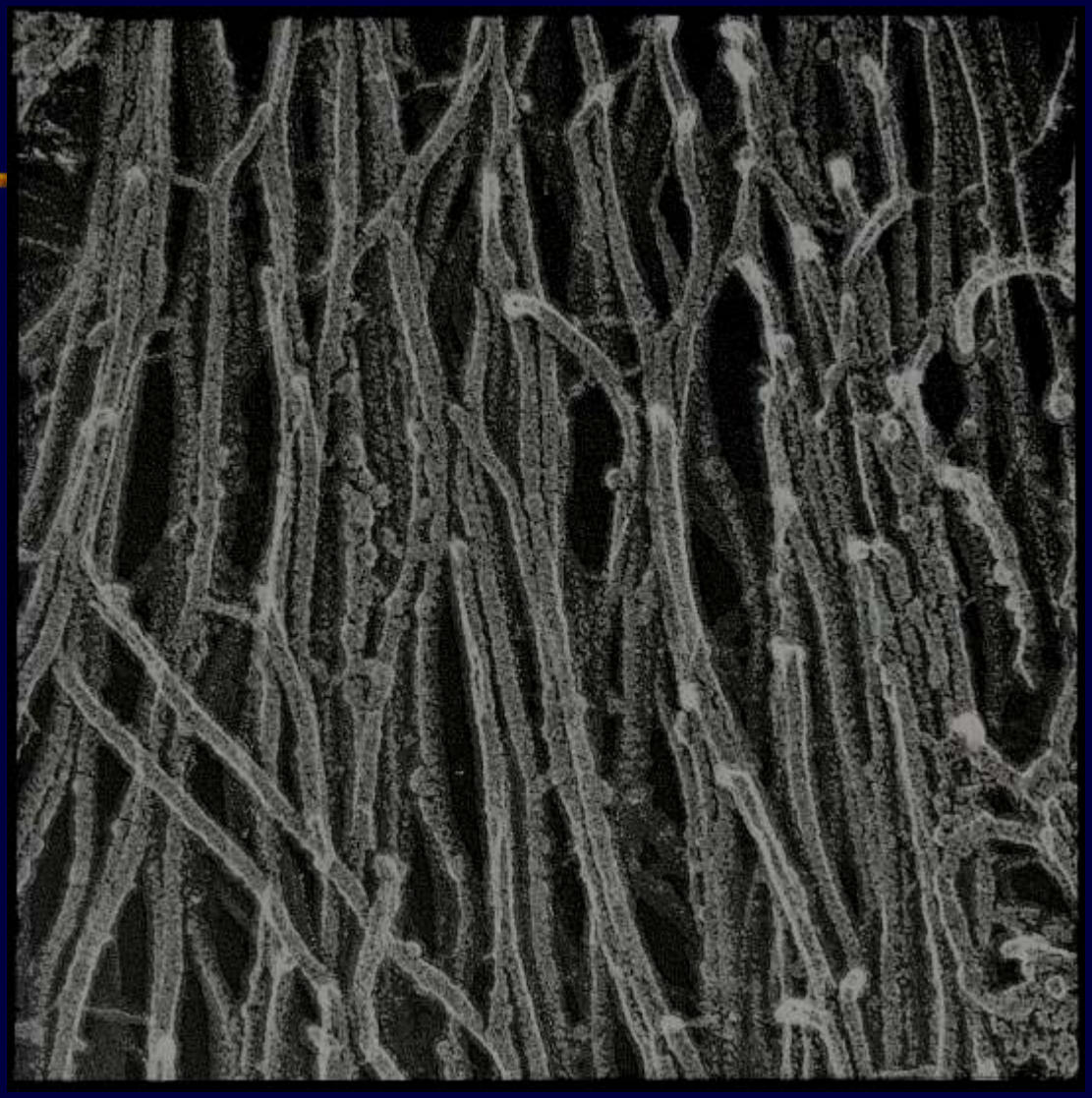

a
Distal dendrite
Rough endoplasmic reticulum
Golgi apparatus
Axon hillock
Transporting vesicle
RNA-containing granule
Nucleus
Growth cone
KIF2A
Microtubules
Axon collateral
Synaptic terminal
Initial segment
Various transporting vesicles
Proximal dendrite
Microtubule
KIF
Axon
Synaptic vesicle
Cell body
Multivesicular body-like organelle
Dendritic spine
Dendrites
b
APP
JIPs
APOER2
KLC
Mitochondrion
Fodrin
KAP3
Synaptic vesicle precursor
Microtubule
KIF5
KIF5
KIF5/KIF1Bα
KIF3
KIF1A/KIF1Bβ
c
AMPA receptor
GRIP1
RNA-containing granule
NMDA receptor
LIN7
LIN2
LIN10
Microtubule
KIF5
KIF5
KIF17

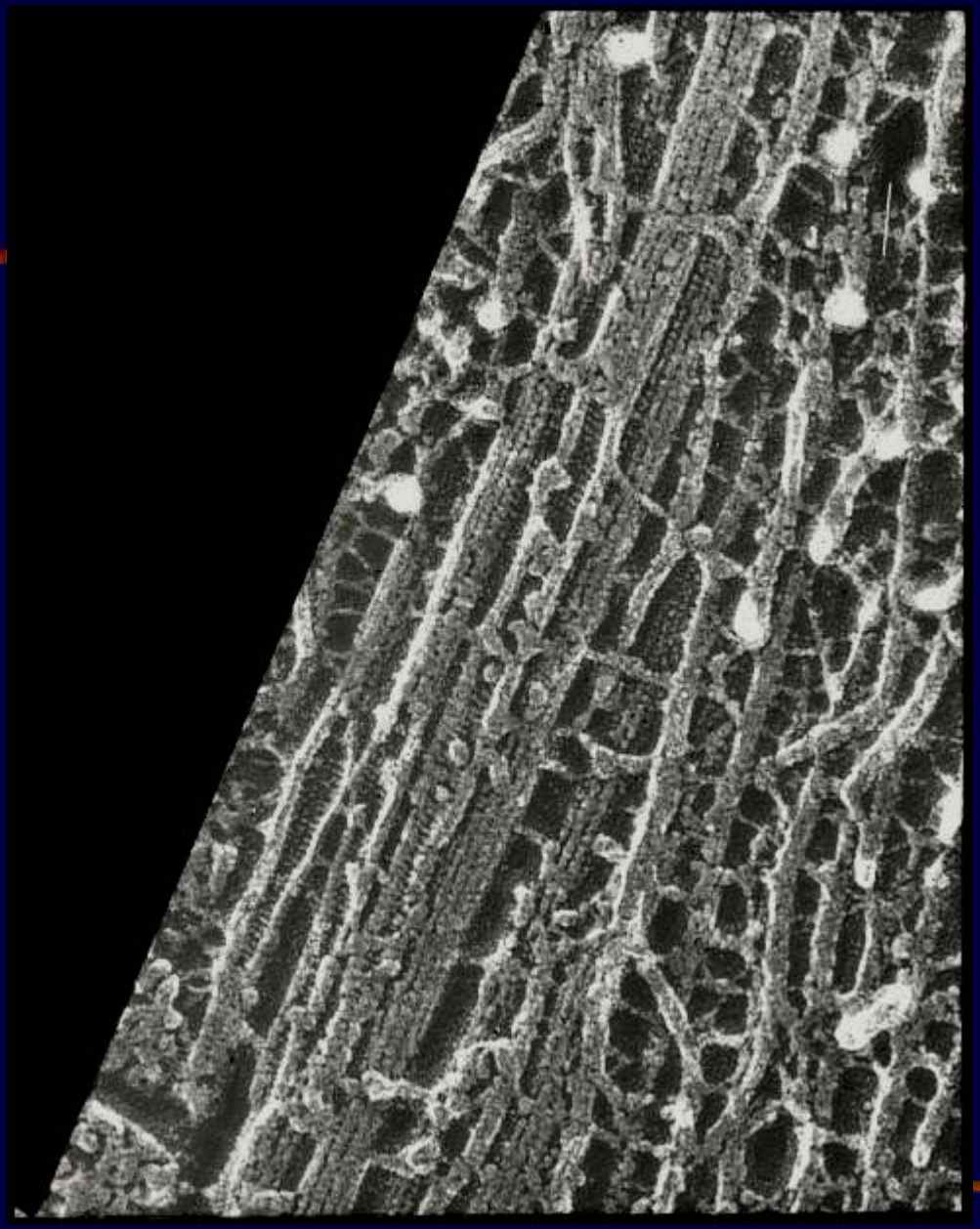
Fig.3 Signal transduction outside of the nerve cell

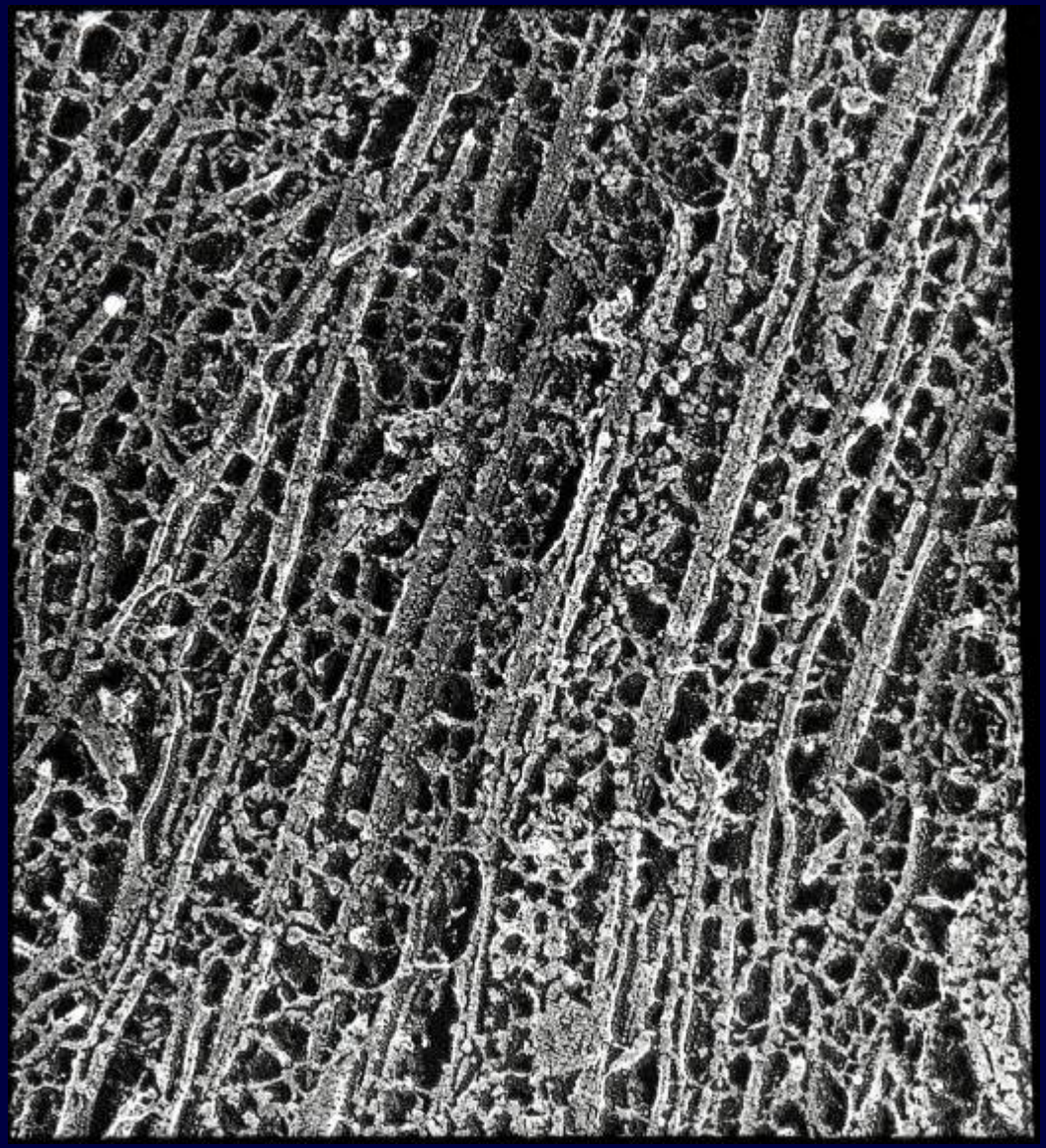


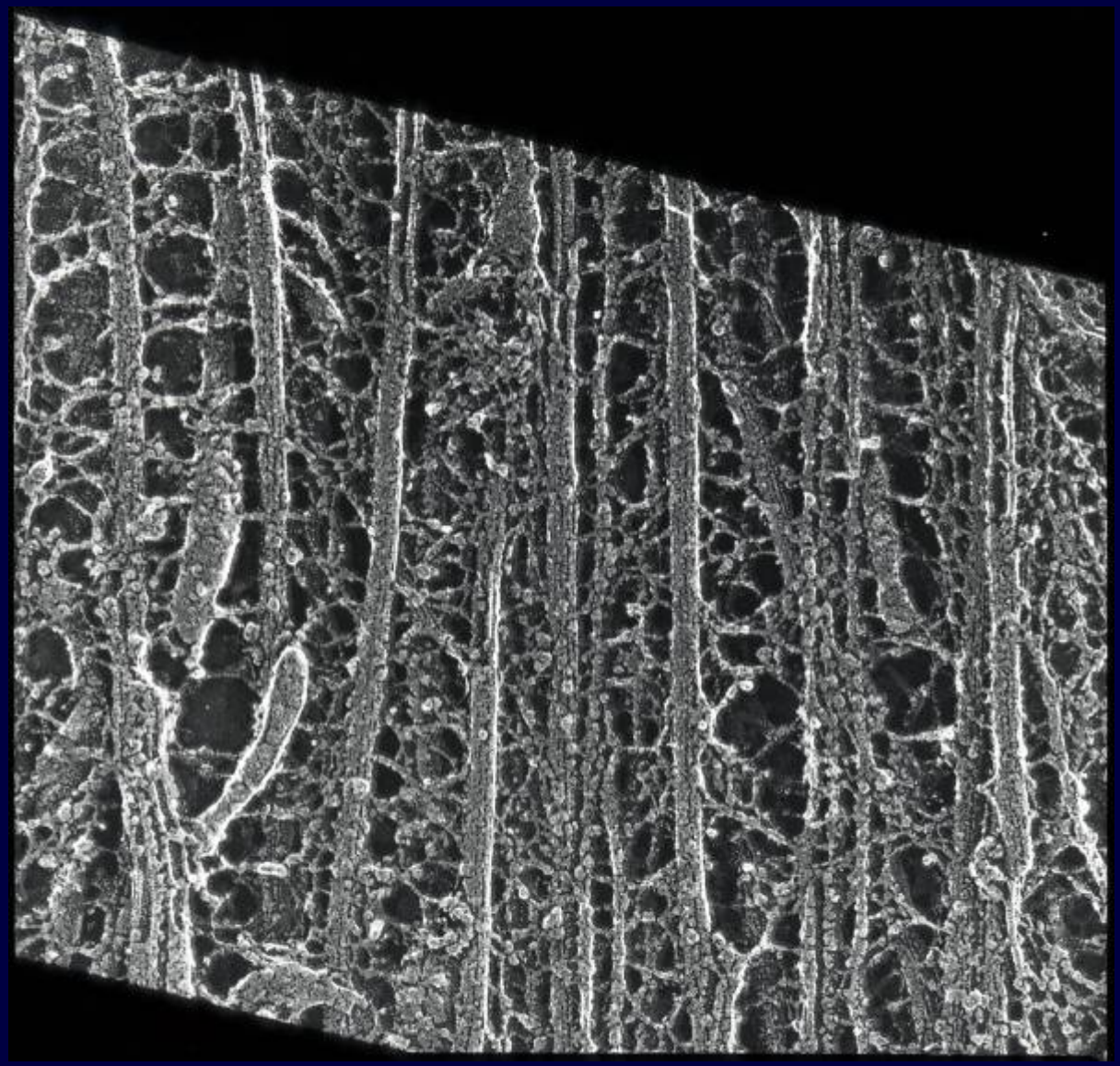
Cytoskeleton

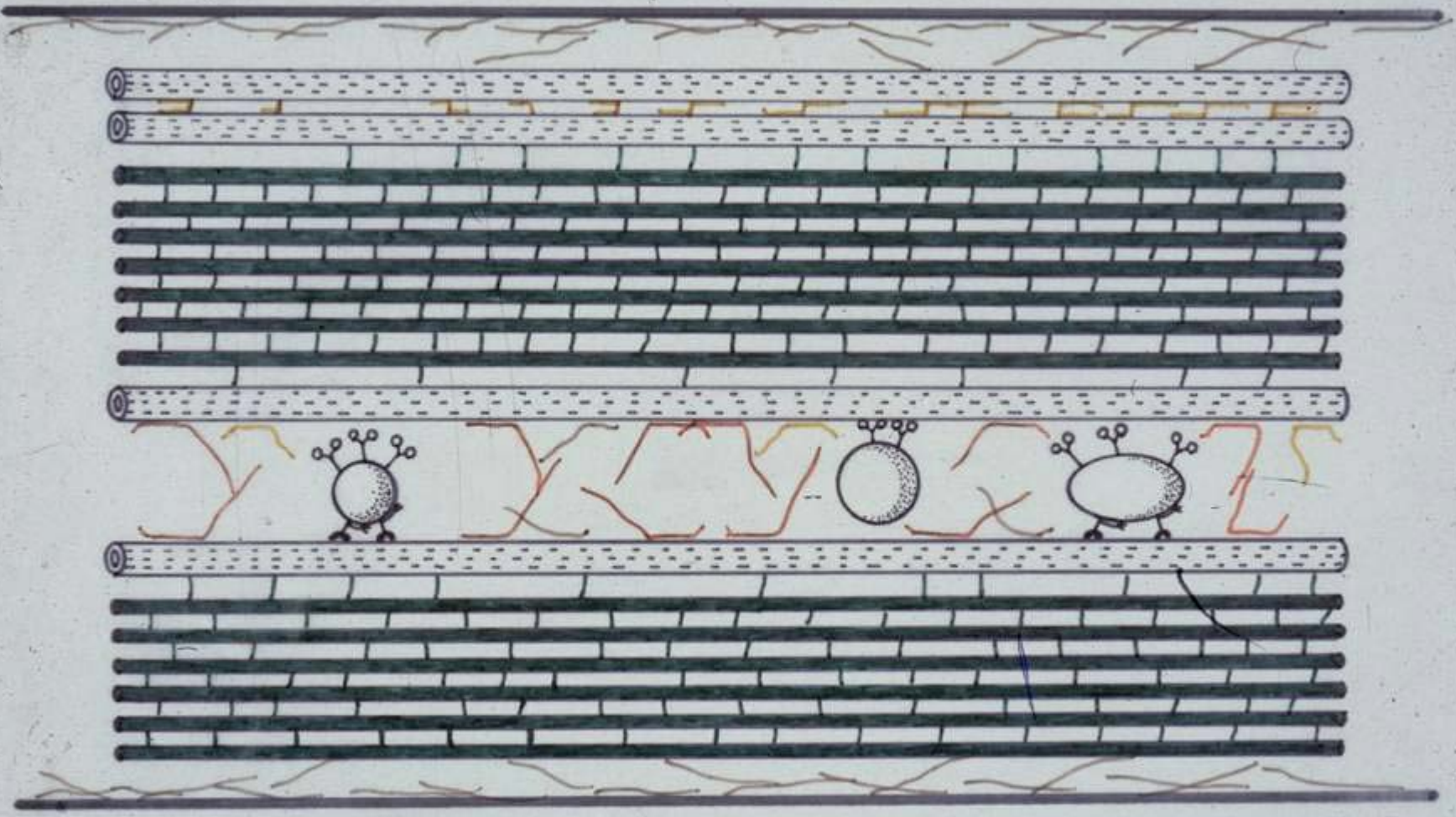




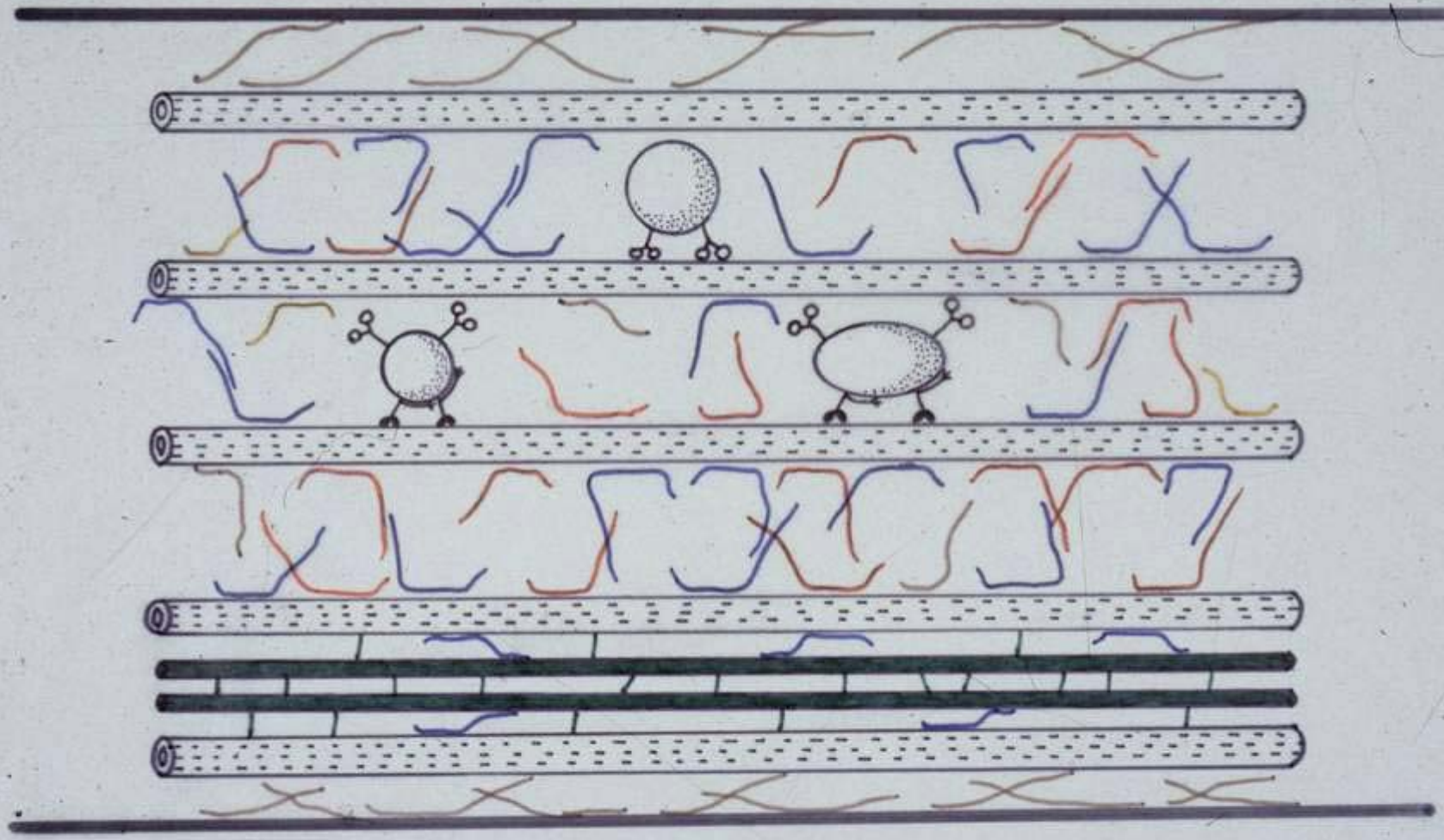








AXON



DENDRITE

Location of MAPs

Dendrite

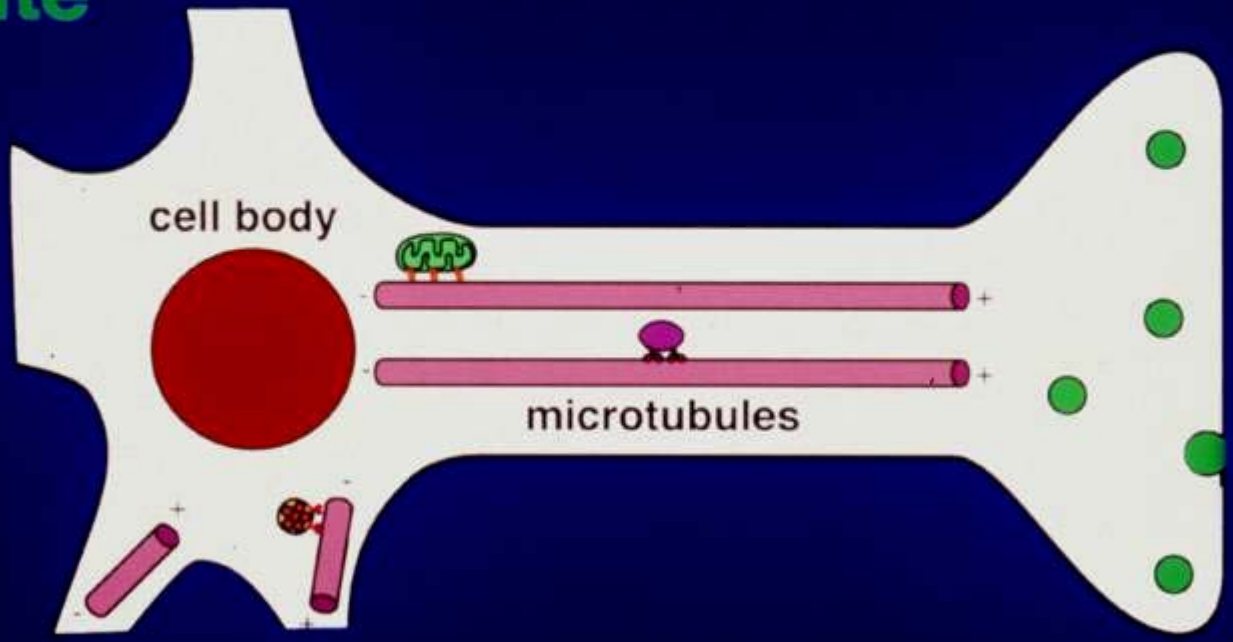
MAP1A

MAP1B

MAP2A

MAP2B

(Tau)



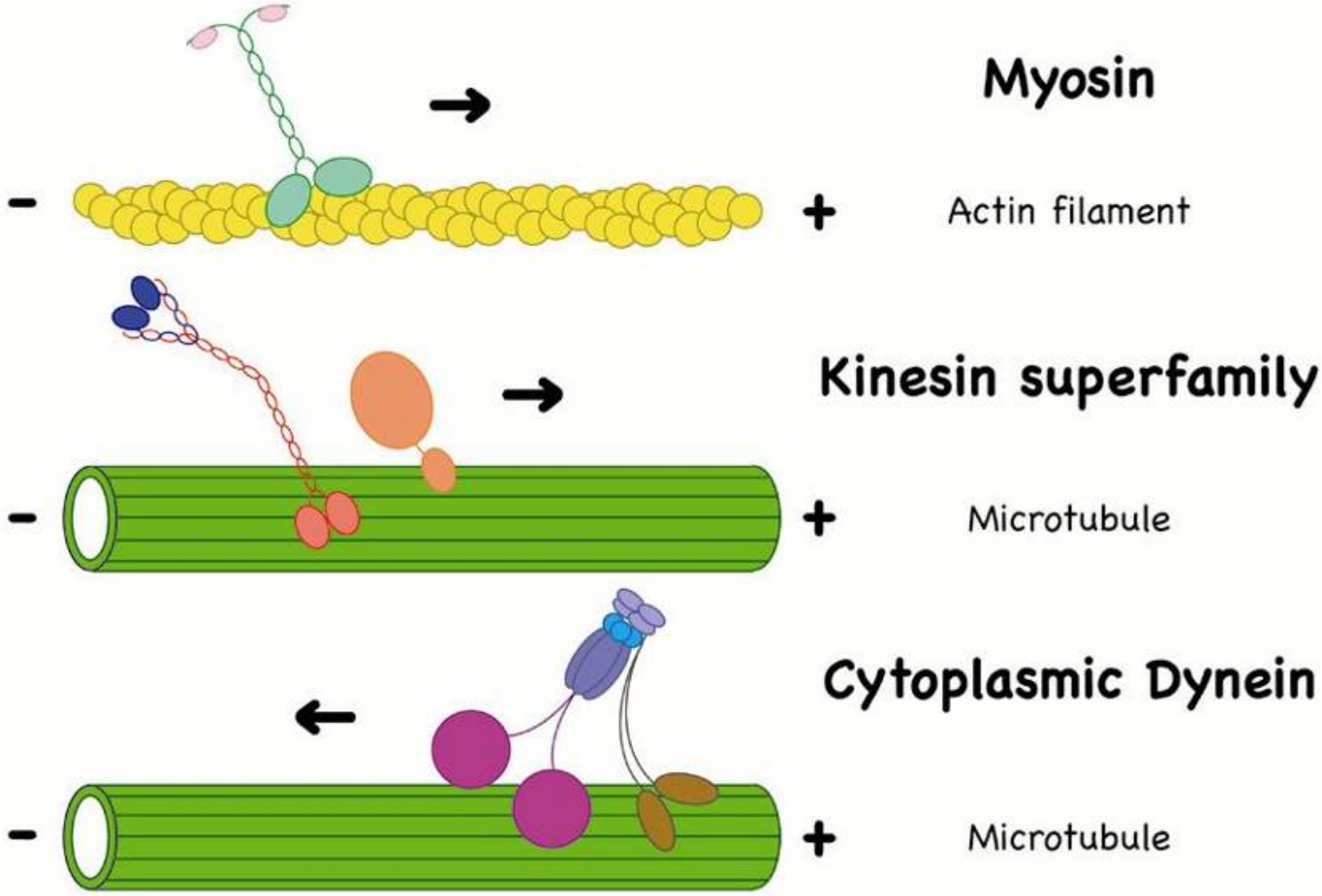
Axon

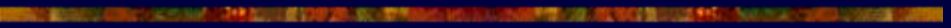
MAP1A

MAP1B

MAP2C

Tau


Myosin
-
+
Actin filament
Kinesin superfamily
-
+
Microtubule
Cytoplasmic Dynein
-
+
Microtubule

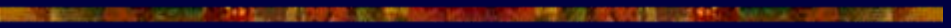


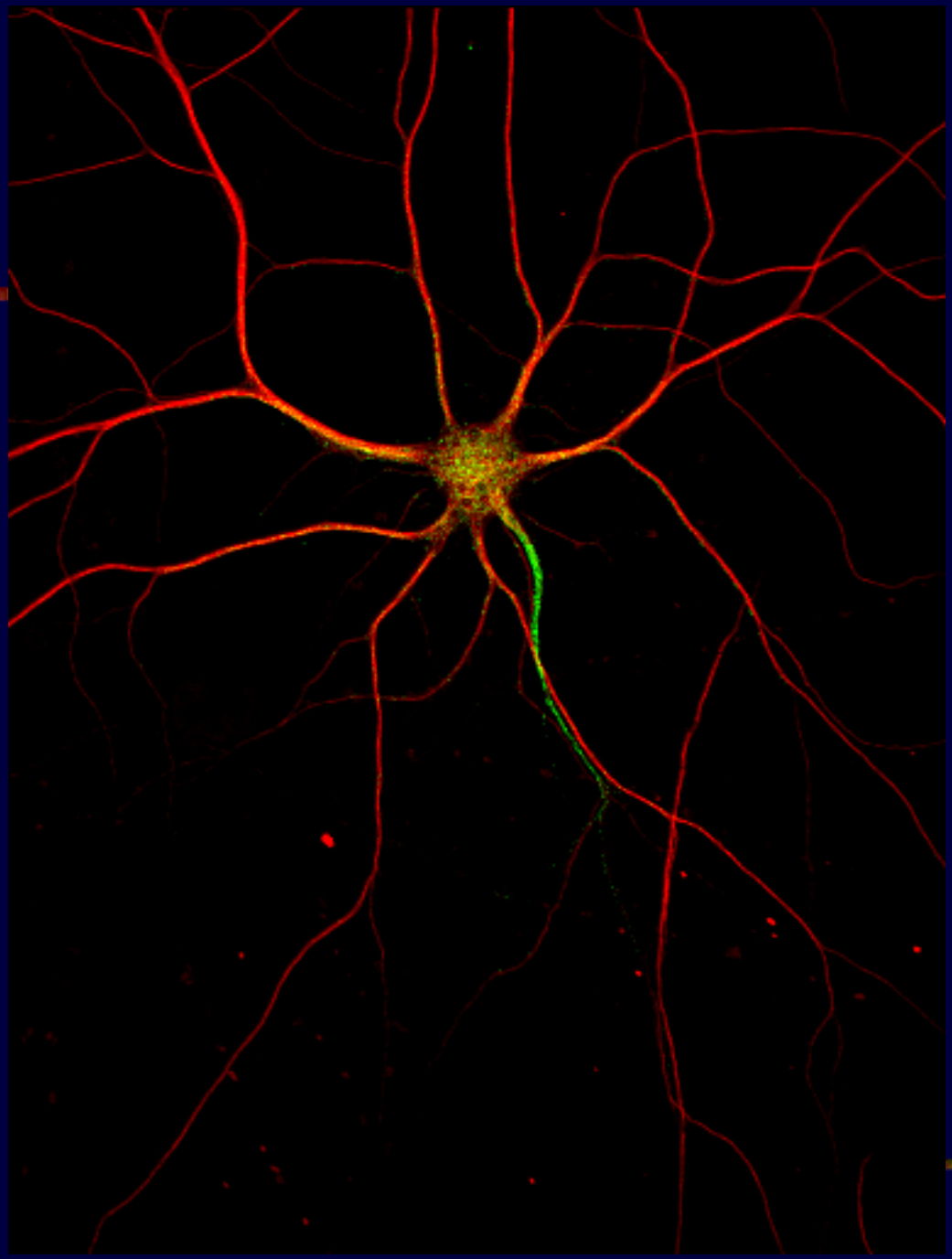
Intracellular Transport and Molecular Motors

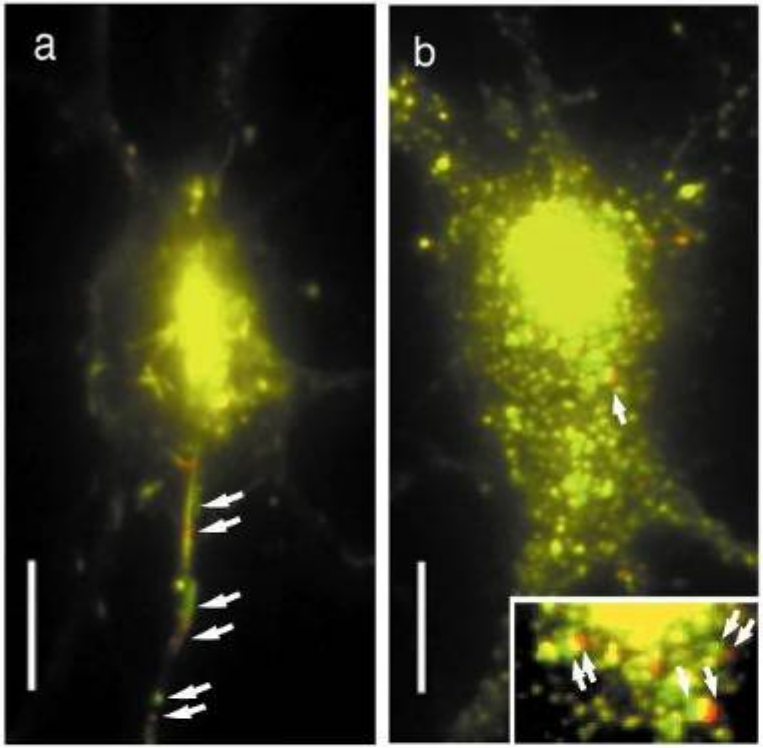
The Mechanism of Organelle Transport

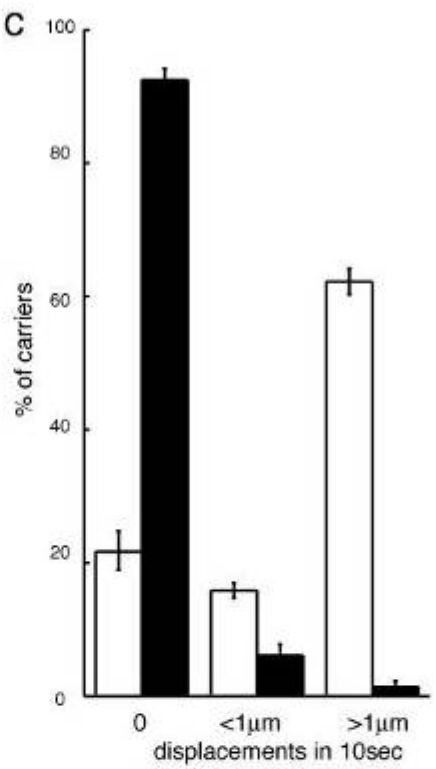
Axonal Transport

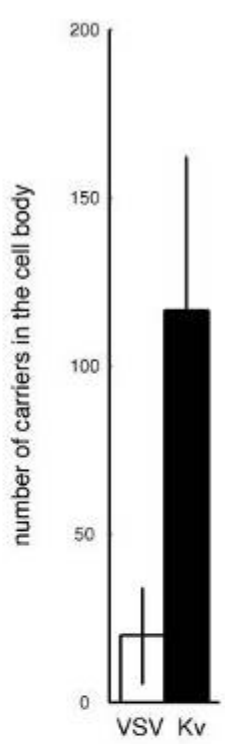
	Group	Velocity mm/day	composition
Fast	I	240	membrane organella
	II	60	membrane organella
	III	6	myosin like actin binding protein
Slow	IV (SCb)	2	actin, clathrin calmodulin
	V (SCa)	1	tubulin, neurofilament triplet proteins

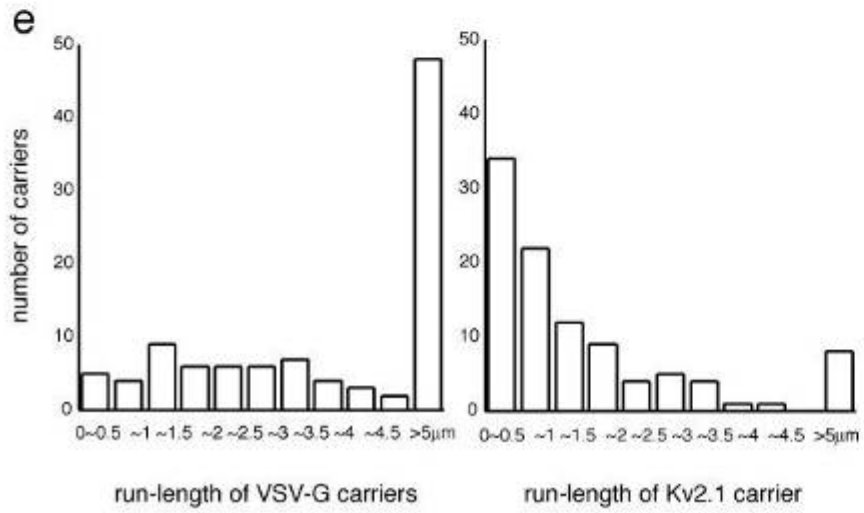


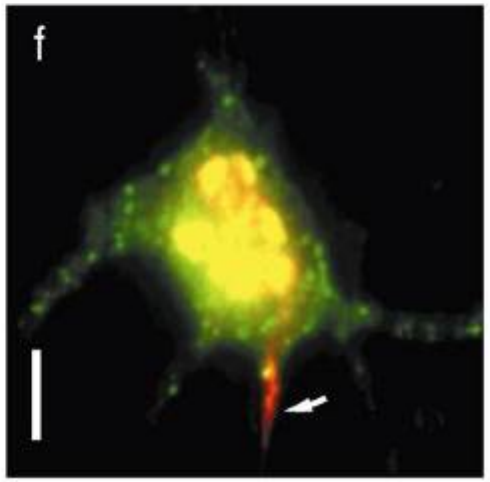


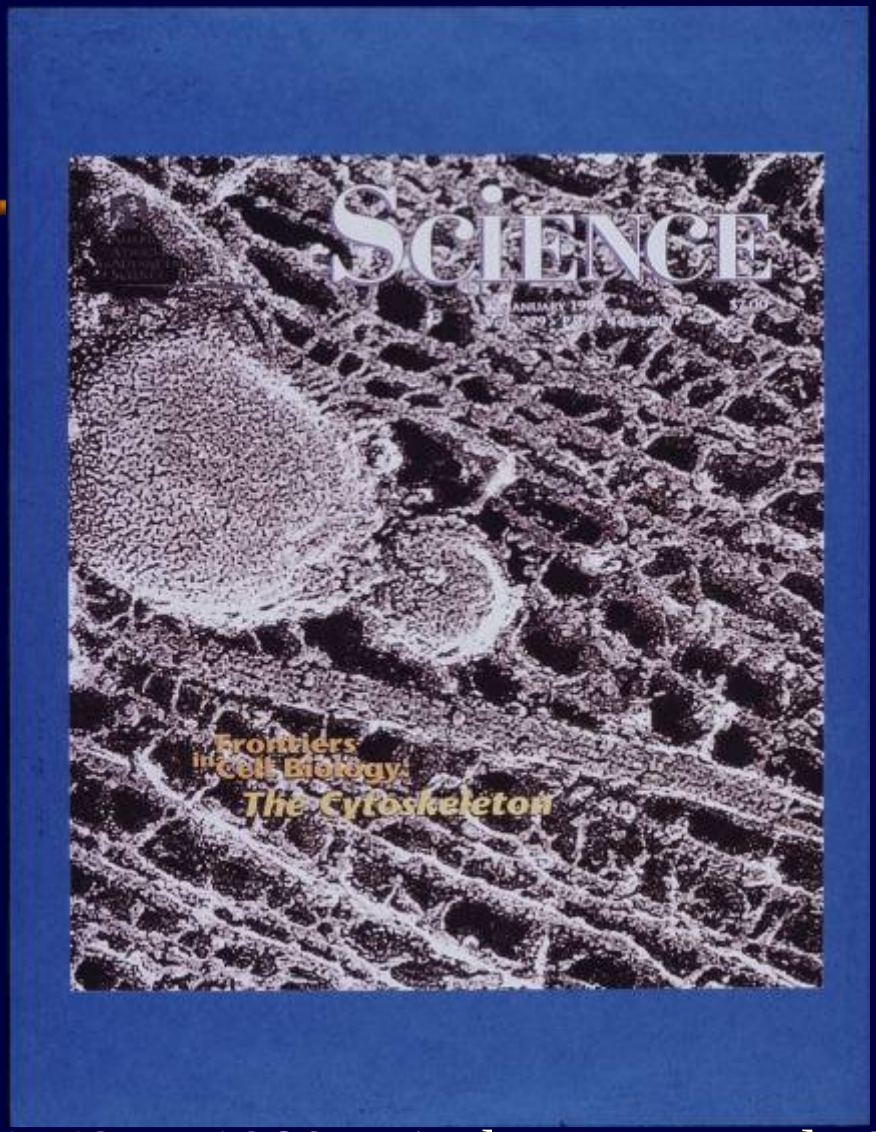

a
b


c
100
80
60
40
20
0
% of carriers
0
<1μm
>1μm
displacements in 10sec


d
200
150
100
50
0
number of carriers in the cell body
VSV
Kv

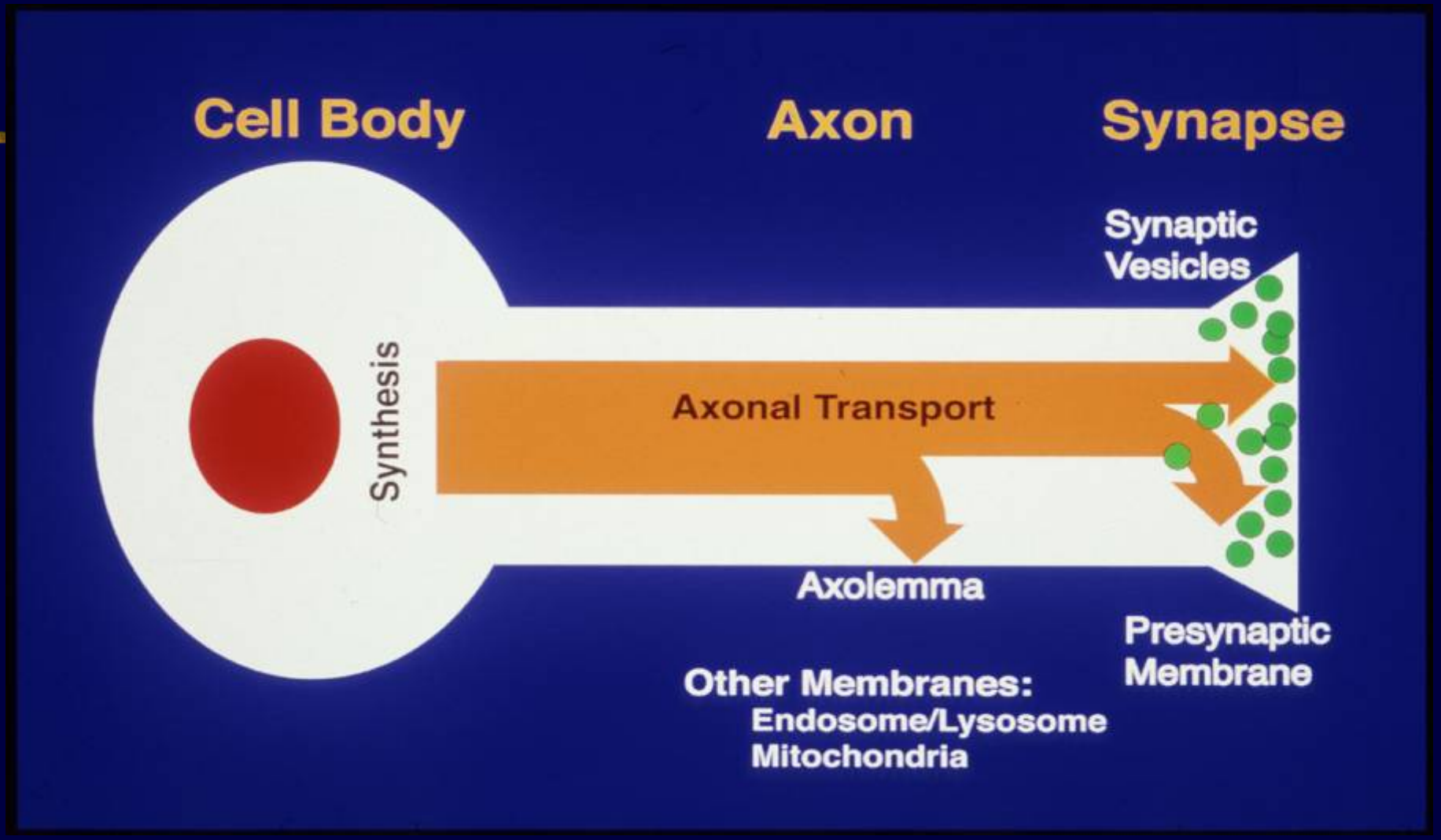

e
50
40
30
20
10
0
number of carriers
0~0.5 ~1 ~1.5 ~2 ~2.5 ~3 ~3.5 ~4 ~4.5 >5μm
run-length of VSV-G carriers
run-length of Kv2.1 carrier


f

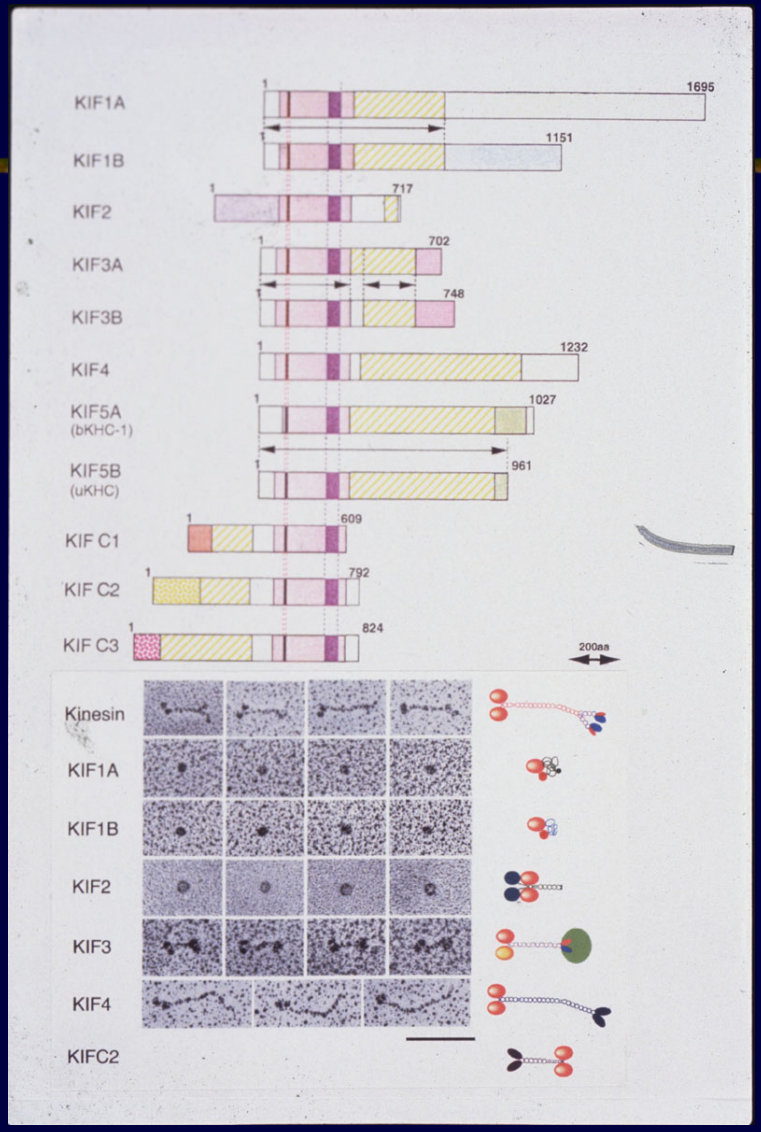


Hirokawa N. JCB 94: 425- ,1982, Hirokawa N. et al. Cell 56: 867- ,1989
Hirokawa N. Science 279: 519- ,1998

Hirokawa JCB 94:129－,1982; Hirokawa et al. Cell 56:867－,1989
Hirokawa Trends Cell Biol. 132:667－,1996


Cell Body
Axon
Synapse
Synaptic Vesicles
Synthesis
Axonal Transport
Axolemma
Presynaptic Membrane
Other Membranes:
Endosome/Lysosome
Mitochondria

Kinesin Superfamily Proteins KIFs



Hirokawa et al. Cell 56:867－,1989
Hirokawa et al. JCB 114:295－,1991
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Nitta et. al. Science 30:678--,2004
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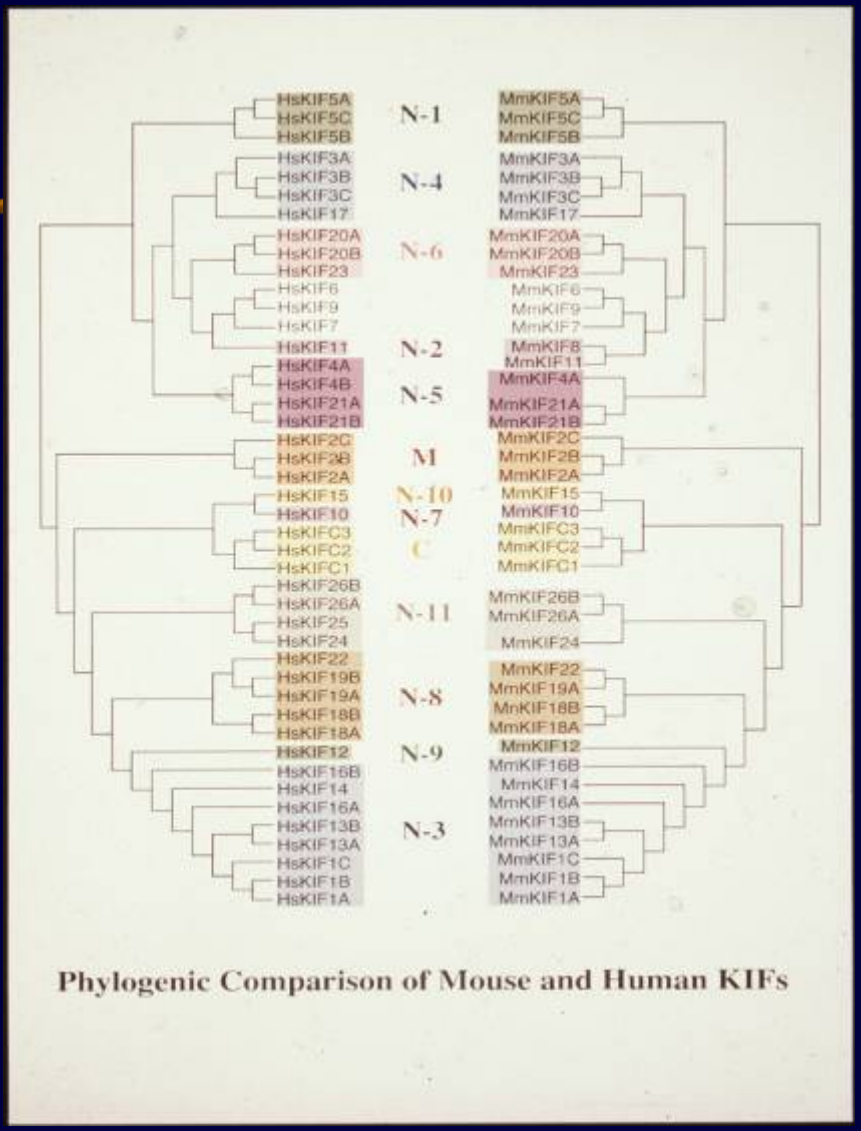
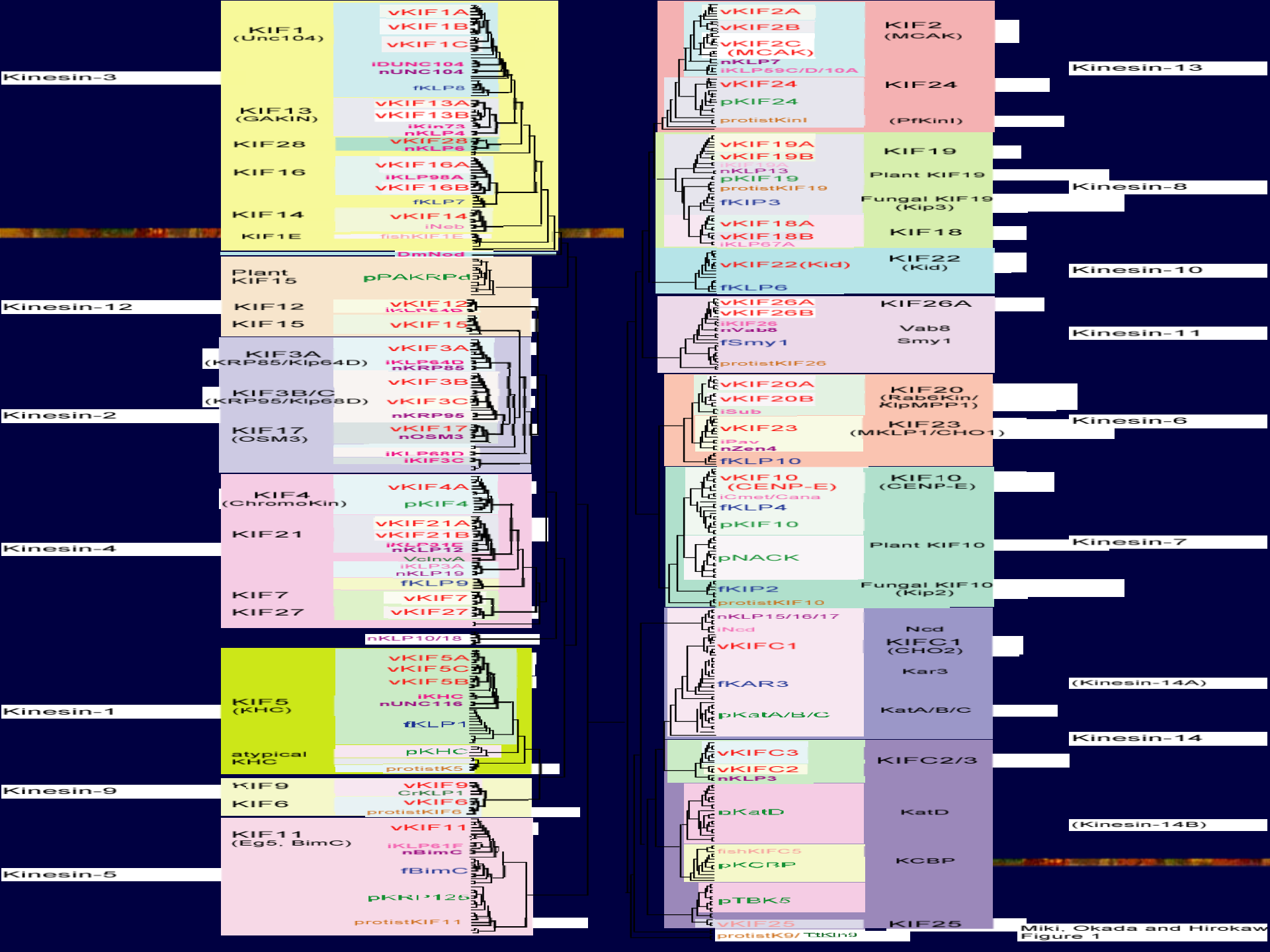
Tanaka et al. Nature in press, 2005
Teng et al. Nature Cell Biol., in press
Okada et al. Cell in press, 2005



Phylogenic Comparison of Mouse and Human KIFs

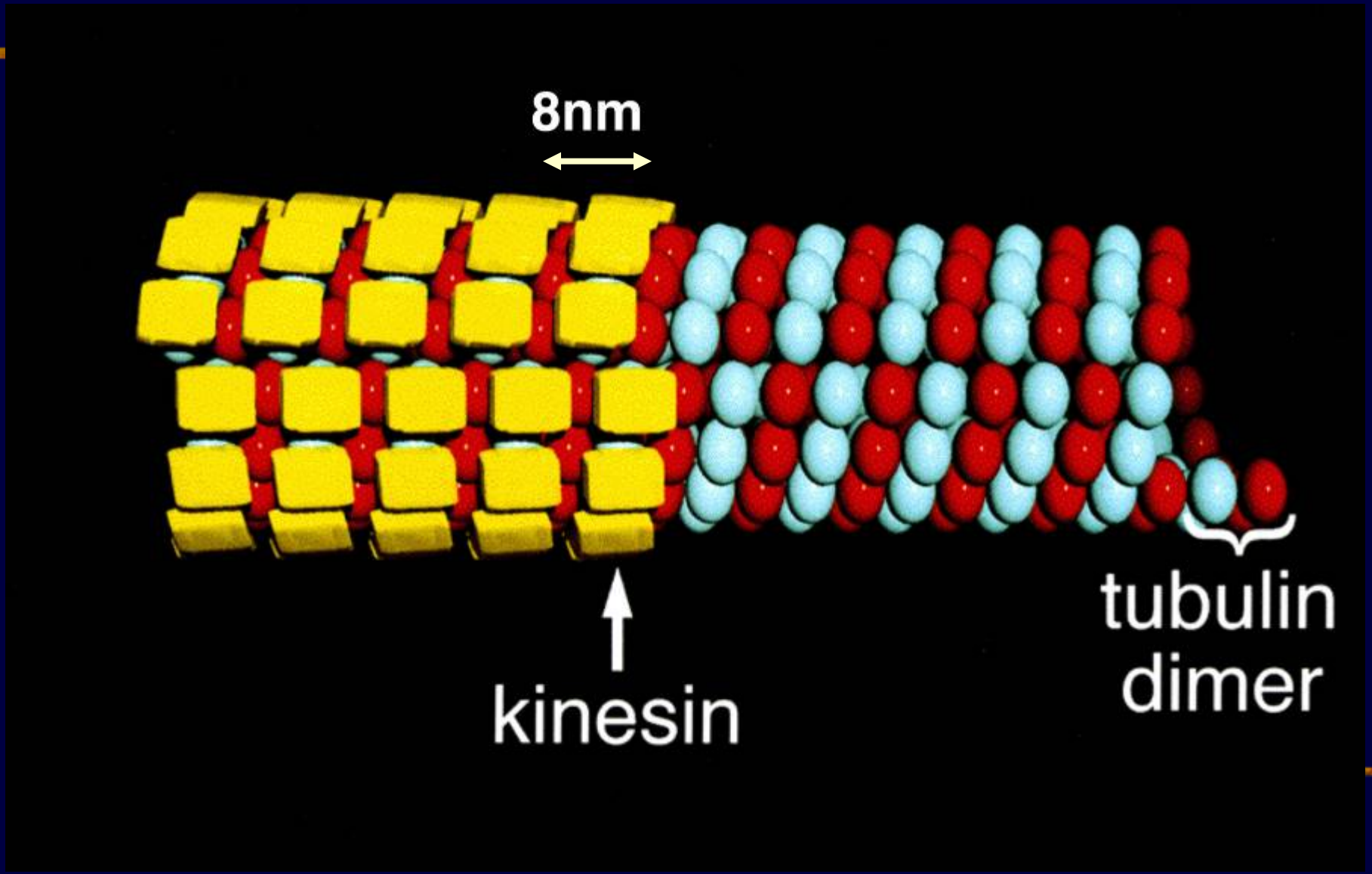
Miki et al. PNAS 98:7004－,2001

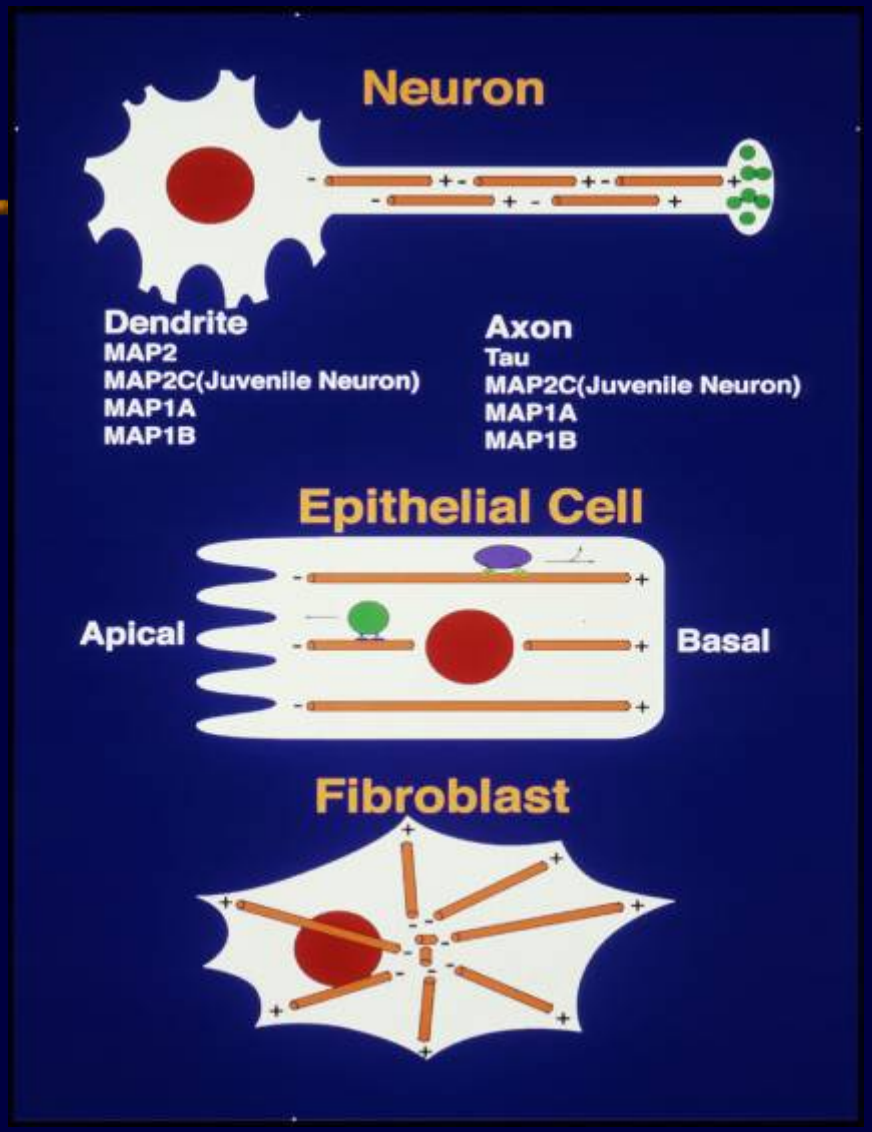


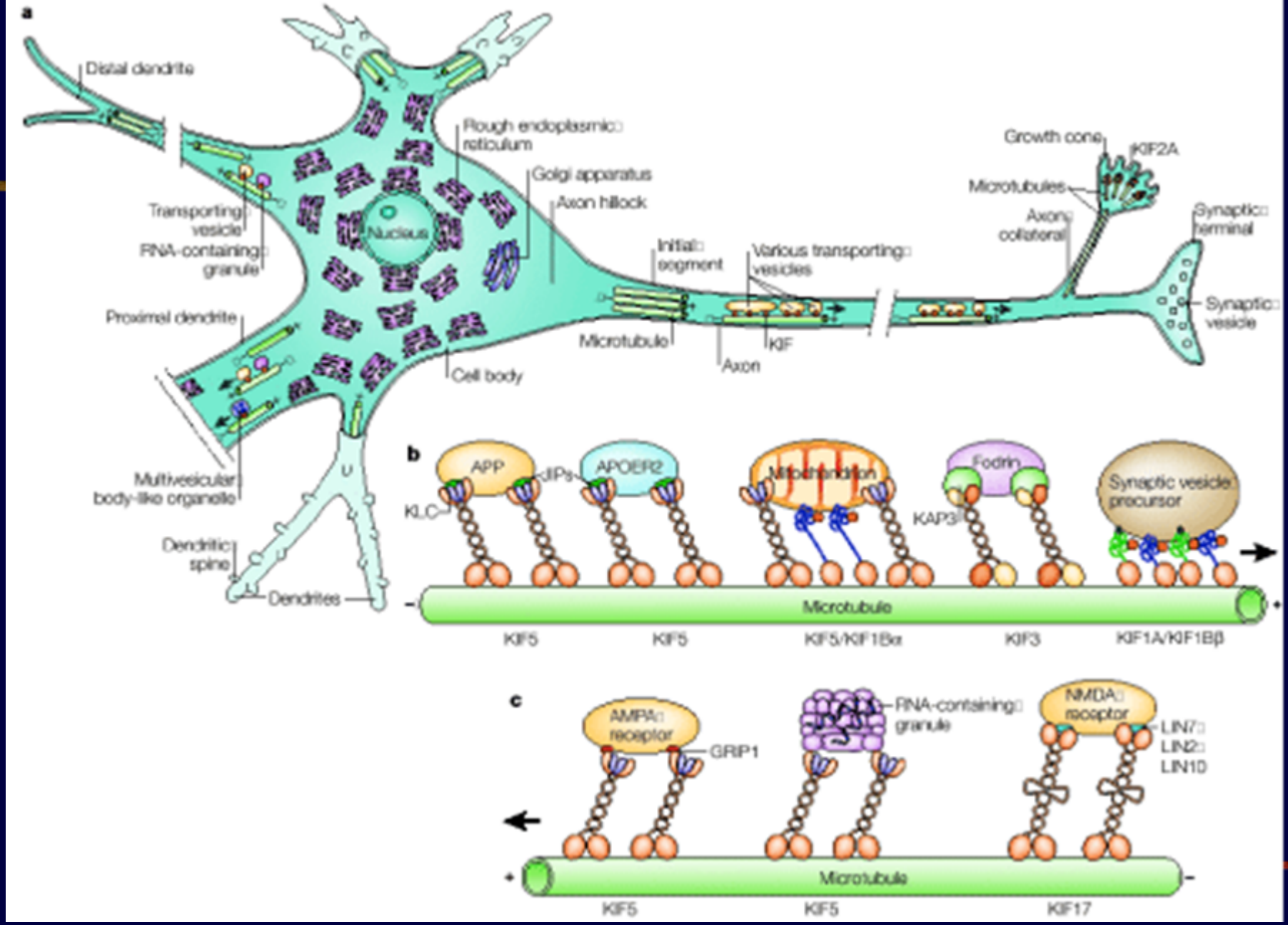
Miki, Okada and Hirokawa
Figure 1

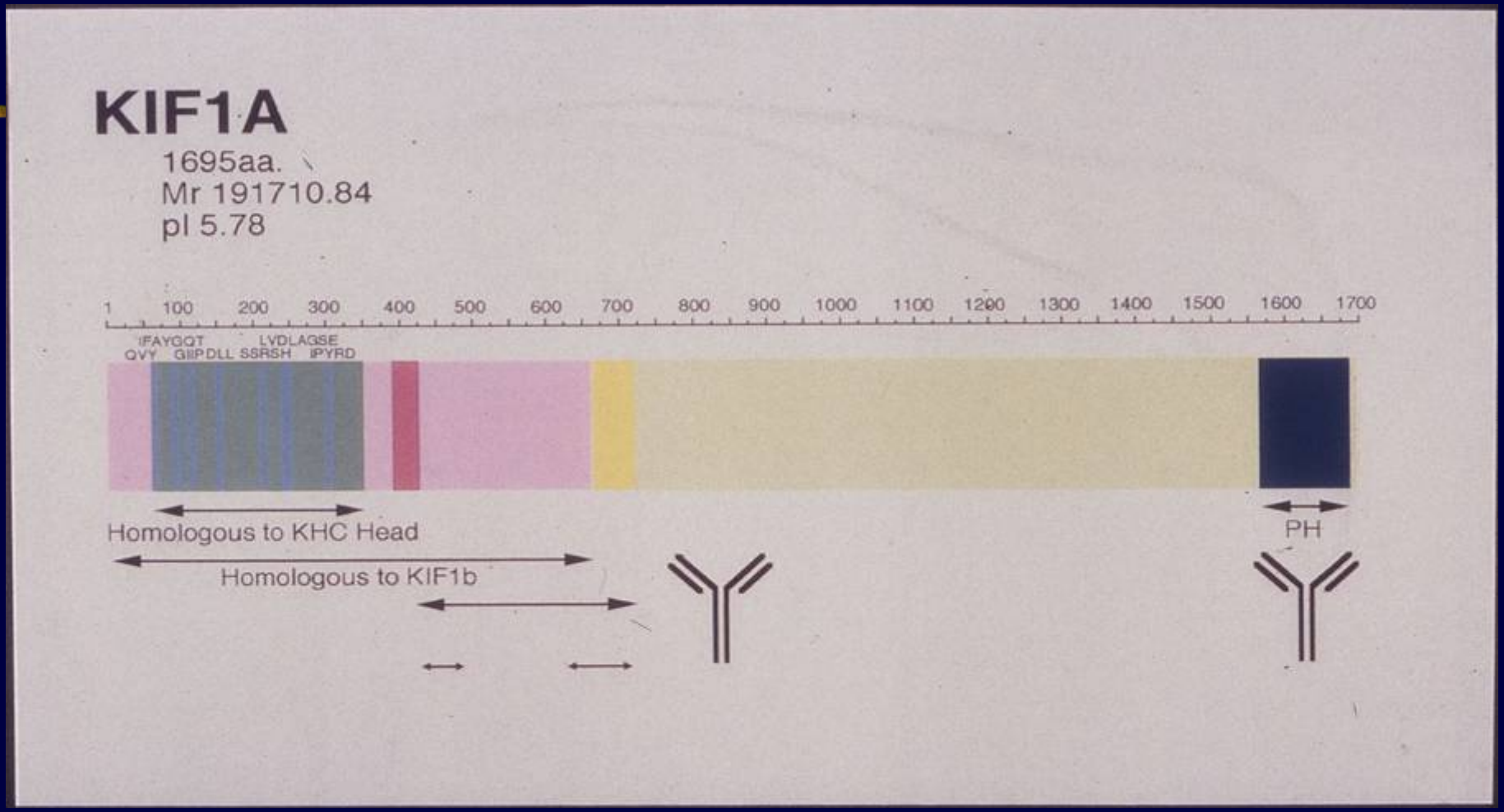
Name	Type	Cargo & Function	Reference
KIF1A	**N-KIF** Monomer	Synaptic vesicle precursor Essential for neuronal function and survival	Aizawa et al.**JCB**1992 Okada et al.**Cell**1995 Yonekawa et al.**JCB**1998 Okada et al.**Science**1999 Kikkawa et al.**Cell**2000 Kikkawa et al.**Nature**2001 Okada et al. **Nature** 2003 Nitta et. al. **Science**2004
KIF1Bα	**N-KIF** Monomer	Mitochondria	Nangaku et al. **Cell**1994
KIF1Bβ	**N-KIF** Monomer	Synaptic vesicle precursor Responsible gene of Charcot-Marie Tooth Type IIA Neuropathy	Zhao et al. **Cell** 2001
KIF2A **KIF2C**	**M-KIF** Homodimer	Expressed abundantly in Juvenile neurons Supression of axon collateral branch extension Microtubule Destabilizer	Aizawa et al. **JCB** 1992 Noda et al. **JCB** 1995 Homma et al. **Cell** 2003 Ogawa et al. **Cell** 2004
KIF3A **KIF3B**	**N-KIF** Heterodimer	Form heterotrimer composed of KIF3A, KIF3B, and KAP3 Vesicles associated with α-fodrin important for neurite extension Protein complexes to form cilia > Nodal flow > Left / Right determination, Transport of N cadherin and β catenin to suppress tumorigenesis	Aizawa et al. **JCB** 1992 Kondo et al. **JCB** 1994 Yamazaki et al **JCB**1995 Yamazaki et al.**PNAS** 1996 Nonaka et al. **Cell** 1999 Takeda et al. **JCB** 1999 Takeda et al. **JCB** 2000 Tanaka et al. **Nature** 2005 Teng et al. **NCB** 2005 Okada et al. **Cell** 2005 Hirokawa et al. **Cell** 2006
KIF4	**N-KIF** Homodimer	Expressed abundantly in Juvenile neurons Regulation of activity dependent neuronal survival through binding to PARP	Aizawa et al. **JCB** 1992 Sekine et al. **JCB** 1994 Midorikawa et al. **Cell** 2006
KIF5A **KIF5B** **KIF5C**	**N-KIF** Homodimer	Mitochondria, Lysosome, Tubulin oligomer GRIP1- AMPA type - glutamate receptor transport in dendrites RNA transport in dendrites	Hirokawa et al. **Cell** 1989 Hirokawa et al. **JCB** 1991 Aizawa et al. **JCB** 1992 Nakata et al. **JCB** 1995 Tanaka et al. **Cell**1998 Kanai et al. **J.Neurosci**.2000 Terada et al. **Cell** 2000 Setou et al. **Nature**2003 Kanai et. Al. **Neuron** 2004
KIF13A	**N-KIF** Homodimer	Adaptin - AP1 adaptor complex - Mannose 6 phosphate receptor vesicle	Nakagawa et al. **Cell** 2000
KIF17	**N-KIF** Homodimer	Transport of Mint1 - NMDA type glutamate receptor in dendrites Learning & Memory	Setou et al. **Science** 2000 Wong et al. **PNAS** 2002 Macho et al. **Science** 2002 Guillaud et al. **J.Neurosci.** 2003
KIFC2	**C-KIF** Homodimer	Transport of multivesicular body like organella in dendrites	Saito et al. **Neuron** 1997
KIFC3	**C-KIF** Homodimer	Apical transportor of cholesterol, Annexin III enriched vesicles Golgi complex integration and positioning	Noda et al. **JCB** 2001 Xu et al. **JCB** 2002

Kinesin rail : structure of microtubule



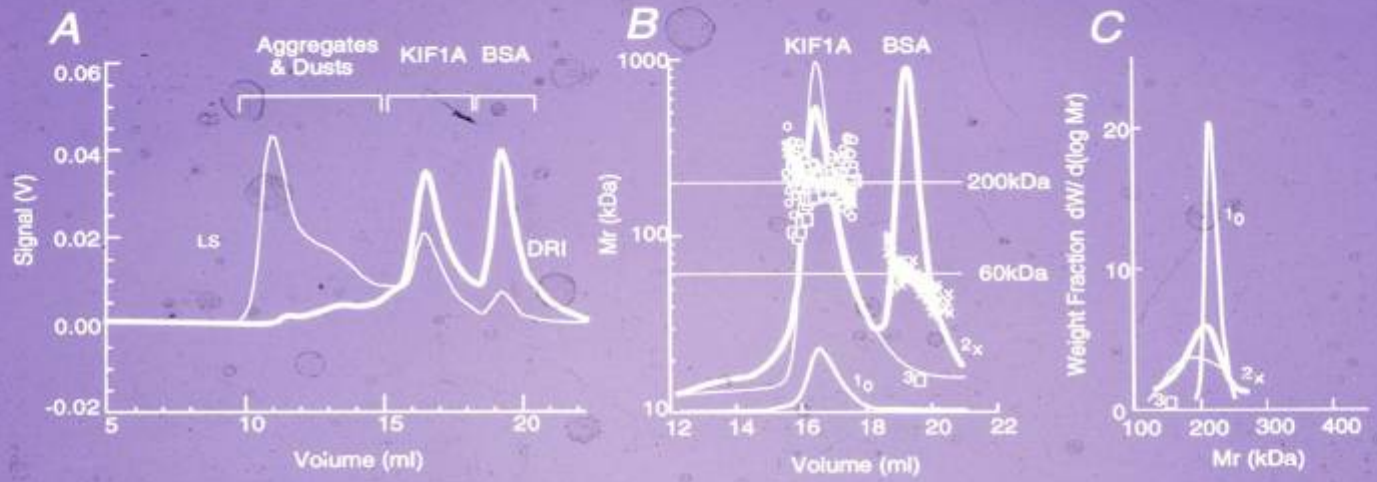

Neuron
Dendrite
MAP2
MAP2C(Juvenile Neuron)
MAP1A
MAP1B
Axon
Tau
MAP2C(Juvenile Neuron)
MAP1A
MAP1B
Epithelial Cell
Apical
Basal
Fibroblast


a
Distal dendrite
Rough endoplasmic reticulum
Golgi apparatus
Axon hillock
Transporting vesicle
RNA-containing granule
Nucleus
Growth cone
KIF2A
Microtubules
Axon collateral
Synaptic terminal
Initial segment
Various transporting vesicles
Proximal dendrite
Microtubule
KIF
Axon
Synaptic vesicle
Cell body
Multivesicular body-like organelle
Dendritic spine
Dendrites
b
APP
JIPs
APOER2
KLC
Mitochondrion
Fodrin
KAP3
Synaptic vesicle precursor
Microtubule
KIF5
KIF5
KIF5/KIF1Bα
KIF3
KIF1A/KIF1Bβ
c
AMPA receptor
GRIP1
RNA-containing granule
NMDA receptor
LIN7
LIN2
LIN10
Microtubule
KIF5
KIF5
KIF17



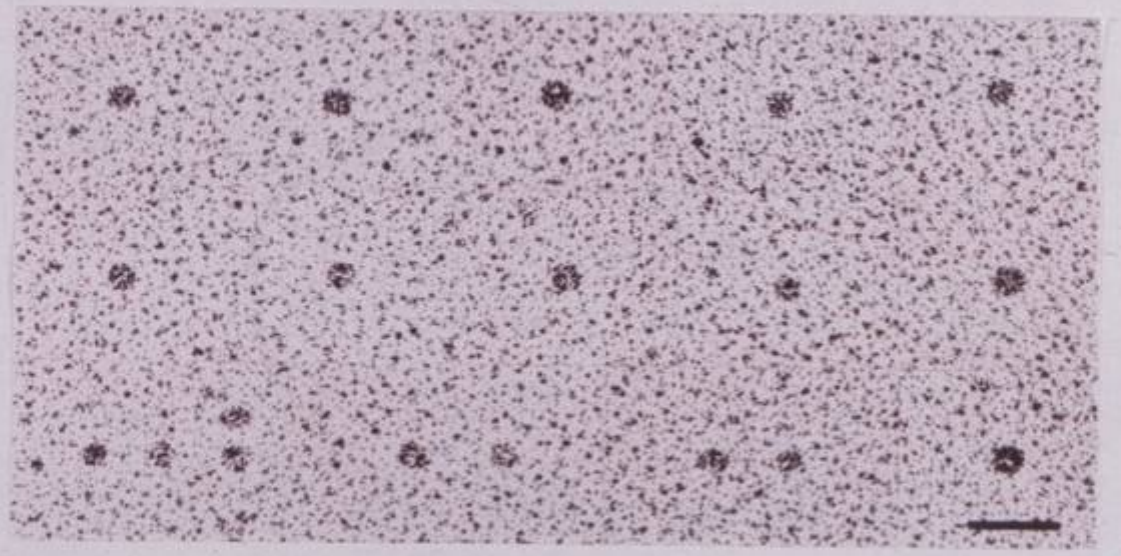
Aizawa et al. JCB 119:1287－, 1992
Okada et al. Cell 81:769－, 1995

Molecular Weight Determination by GPC-DLS

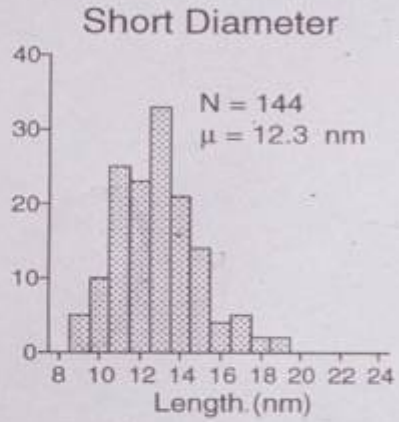


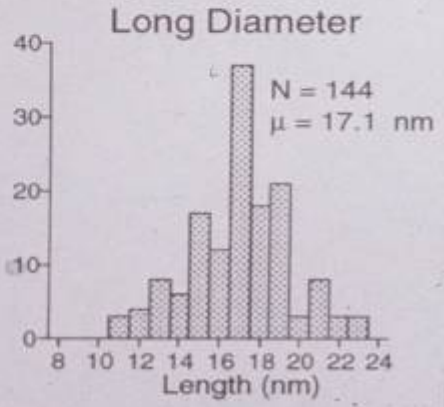
Molecular weight (Mr) of recombinant KIF1A protein was determined by gel permeation chromatography-differential laser light scattering (GPC-DLS), because this method absolutely determines Mr of polymer unbiased by its shape or other physical or chemical properties. *A* shows typical light scattering chromatogram at 90° (LS) contrasted with differential refractive index (DRI). *B* shows calculated Mr overlaid on DRI chromatogram. Results from three experiments are shown. These data were converted into differential Mr distribution (*C*). Thus, Mr of recombinant KIF1A was determined as 180-220 kDa, indicating that KIF1A is a monomer.

Single Molecule Structure of Recombinant KIF1A Revealed by Low-Angle Rotary-Shadowing EM



Bar: 50nm

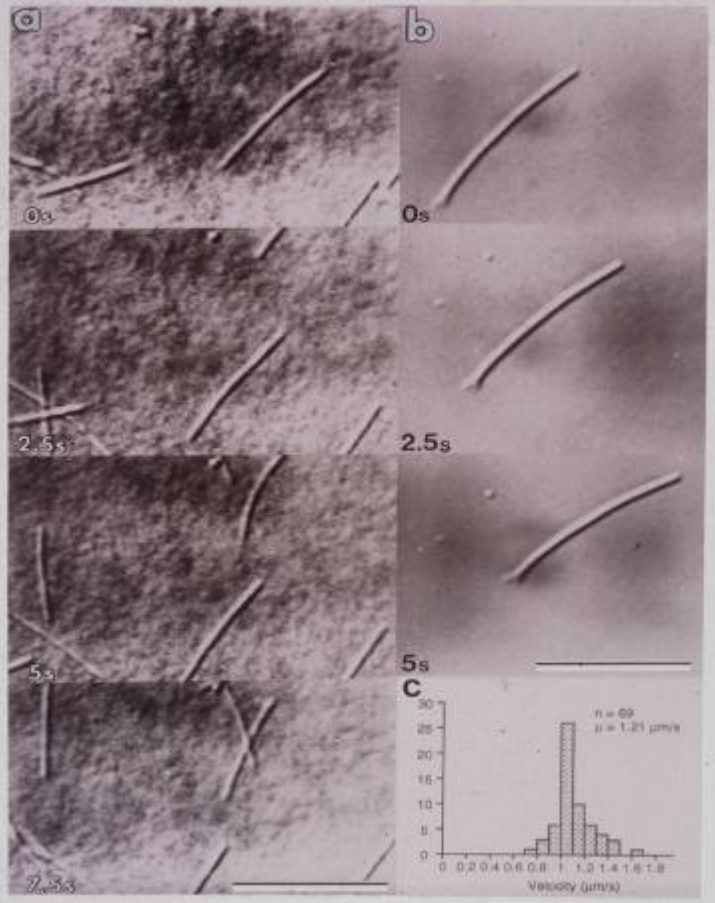


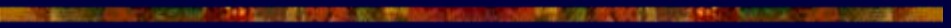


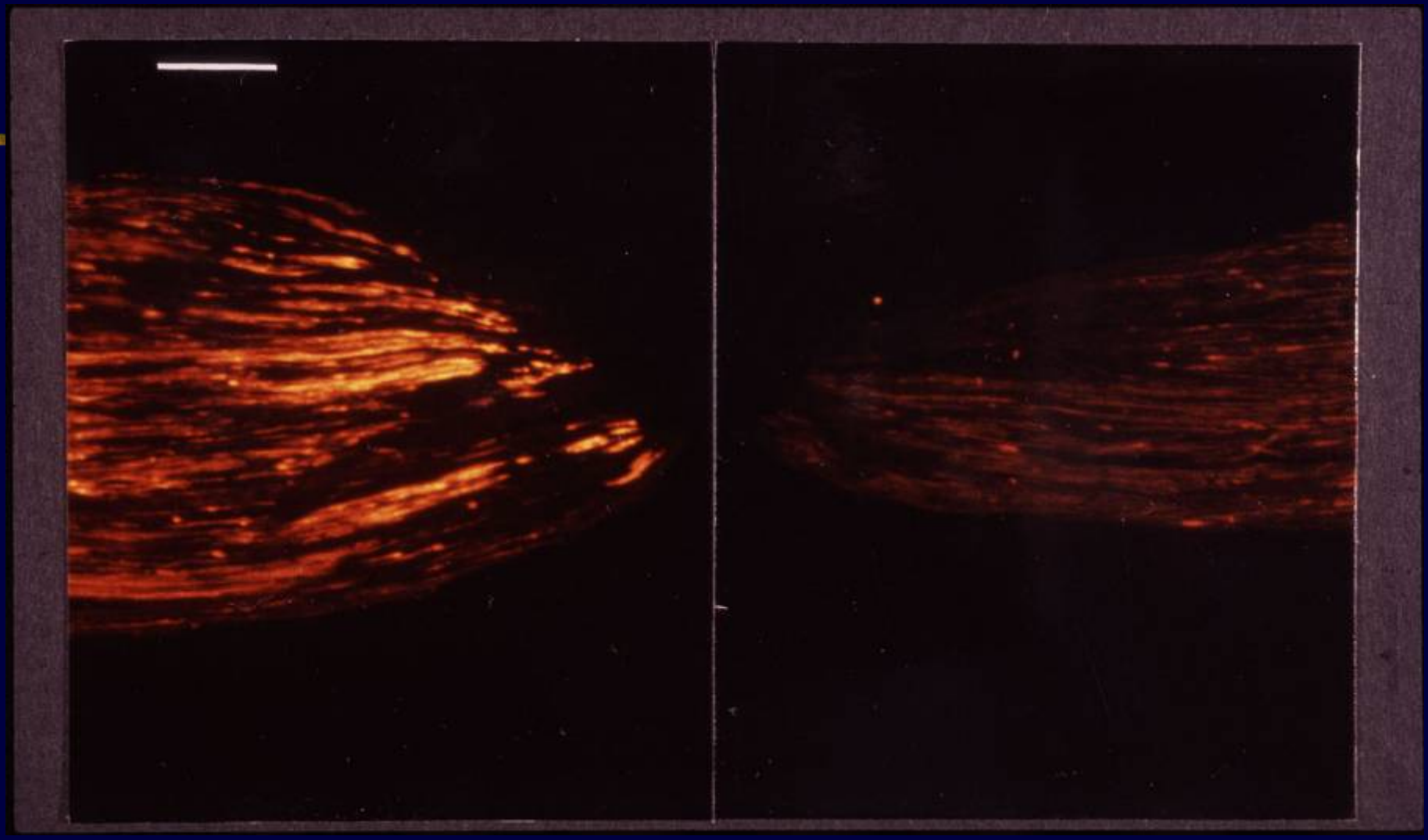
Unlike other KRMPs, KIF1A was a globular molecule. No clearly discernable tails were observed.,

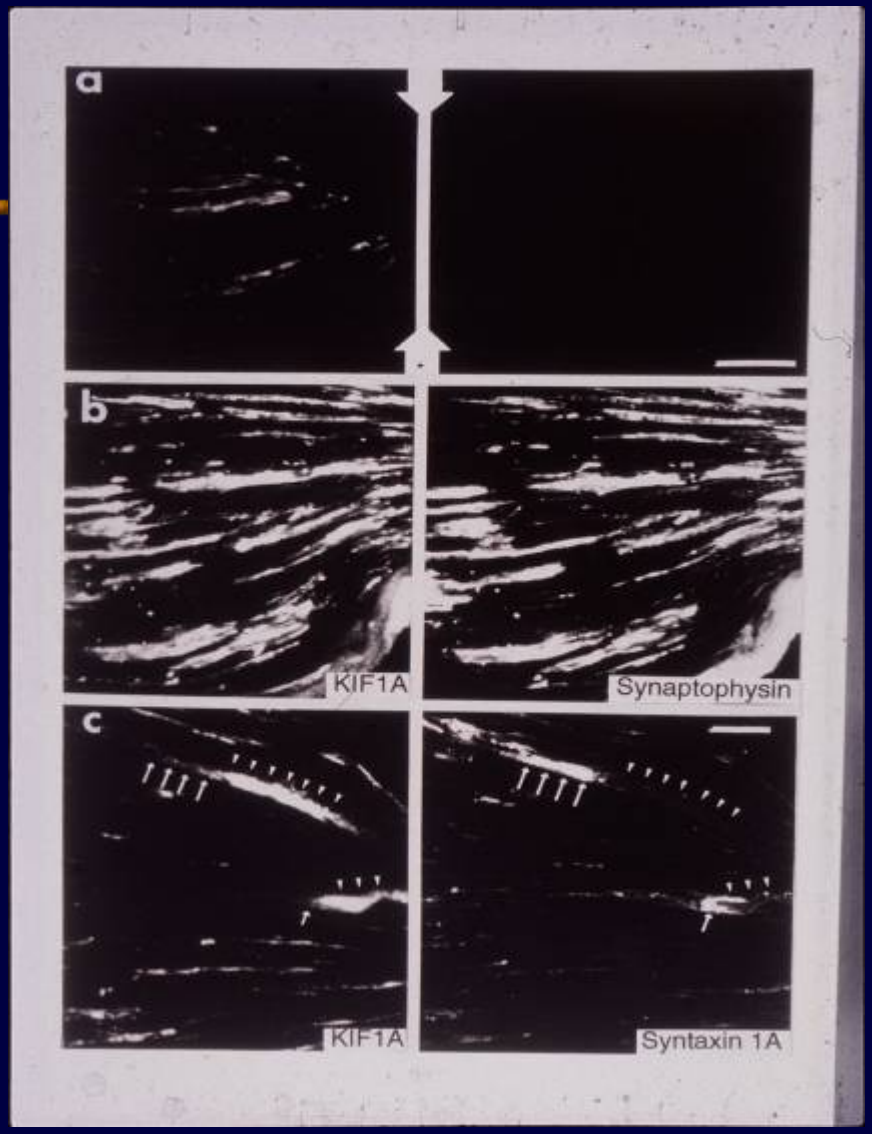
In vitro Motility Assay of Recombinant KIF1A

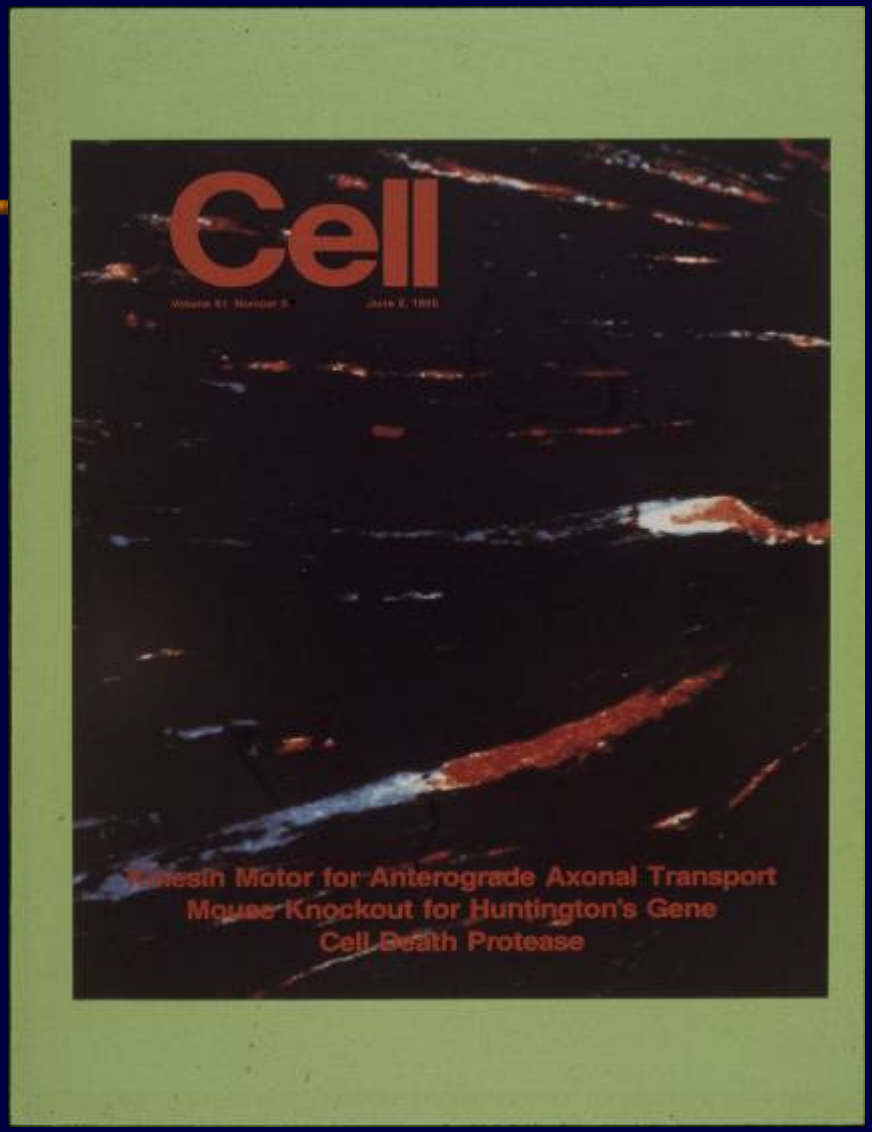
(~100 KIF1A monomers / μm^2)



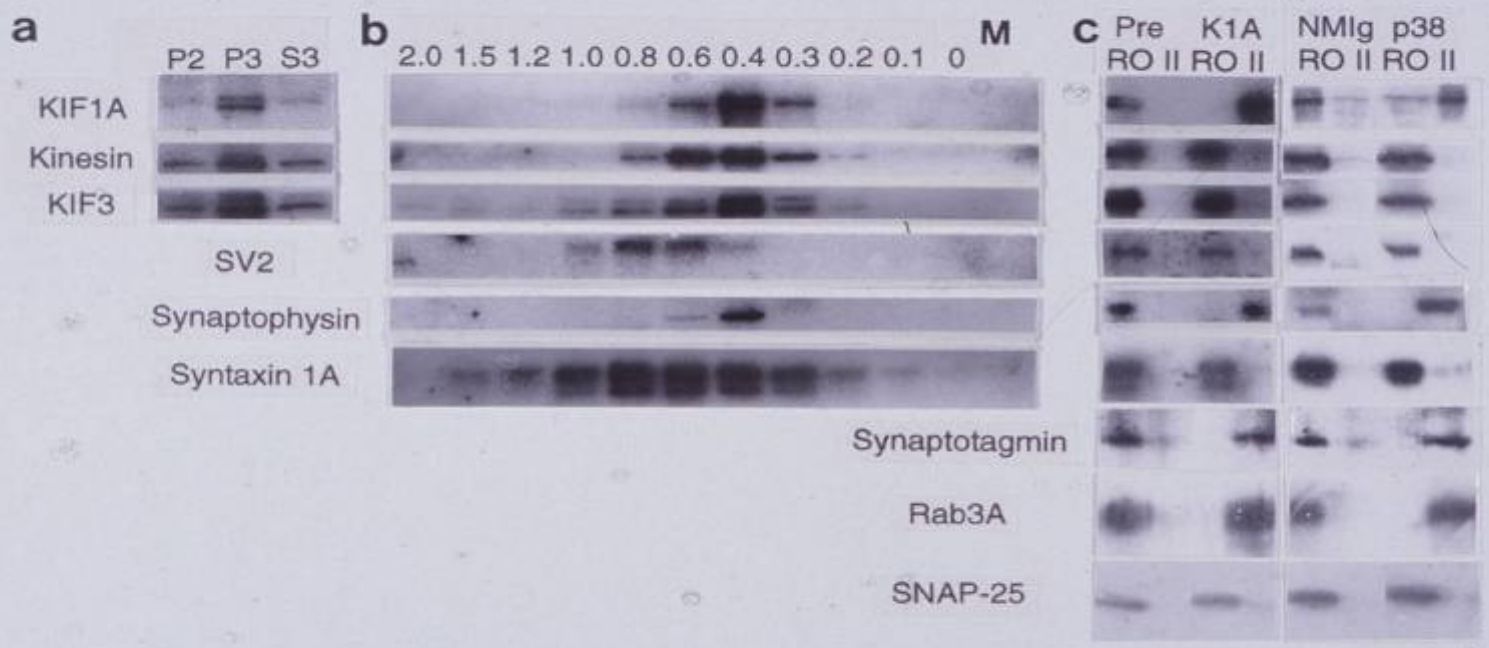


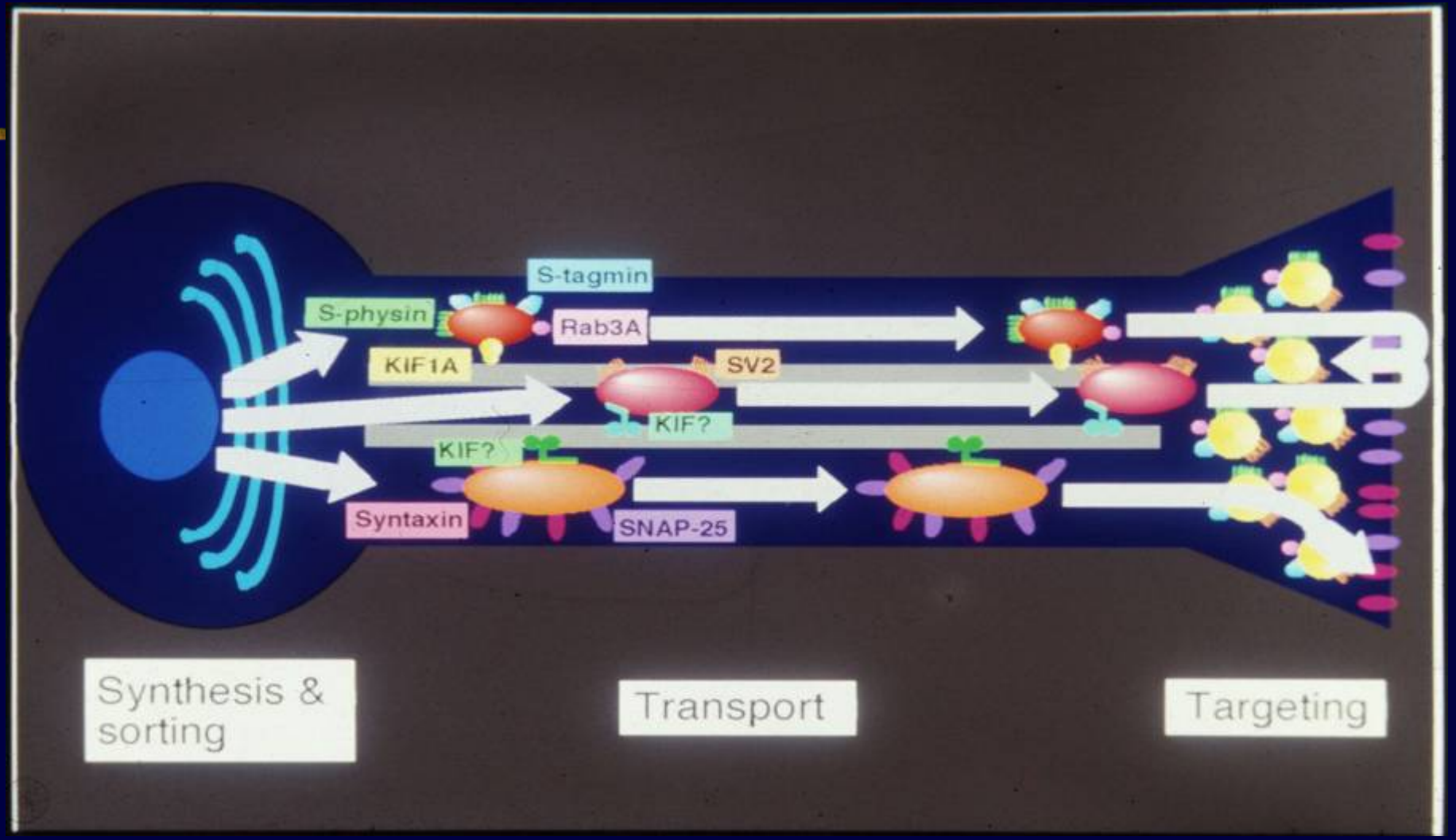


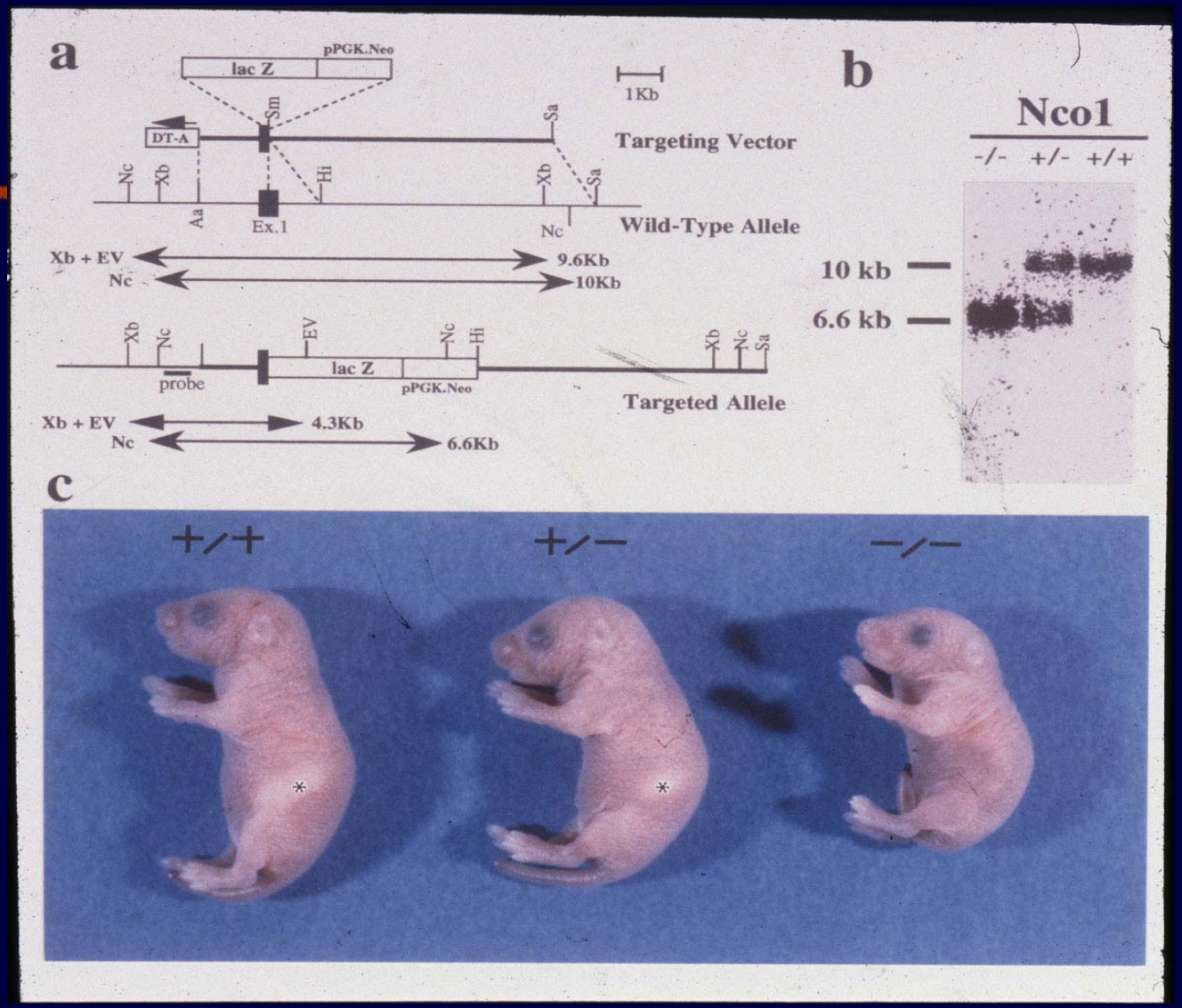

a
b
KIF1A
Synaptophysin
c
KIF1A
Syntaxin 1A



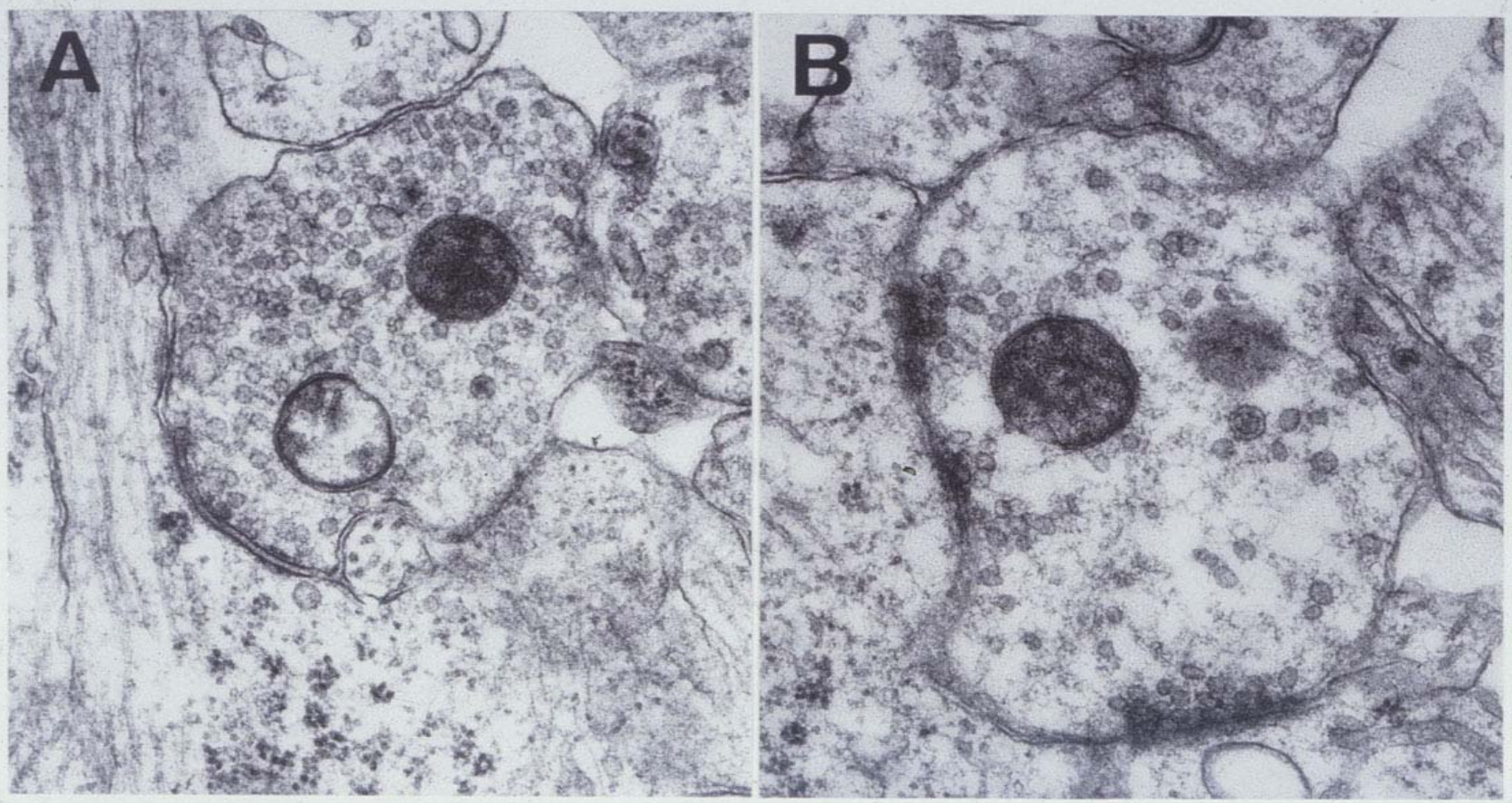
Okada et al. Cell 81:769-780, 1995

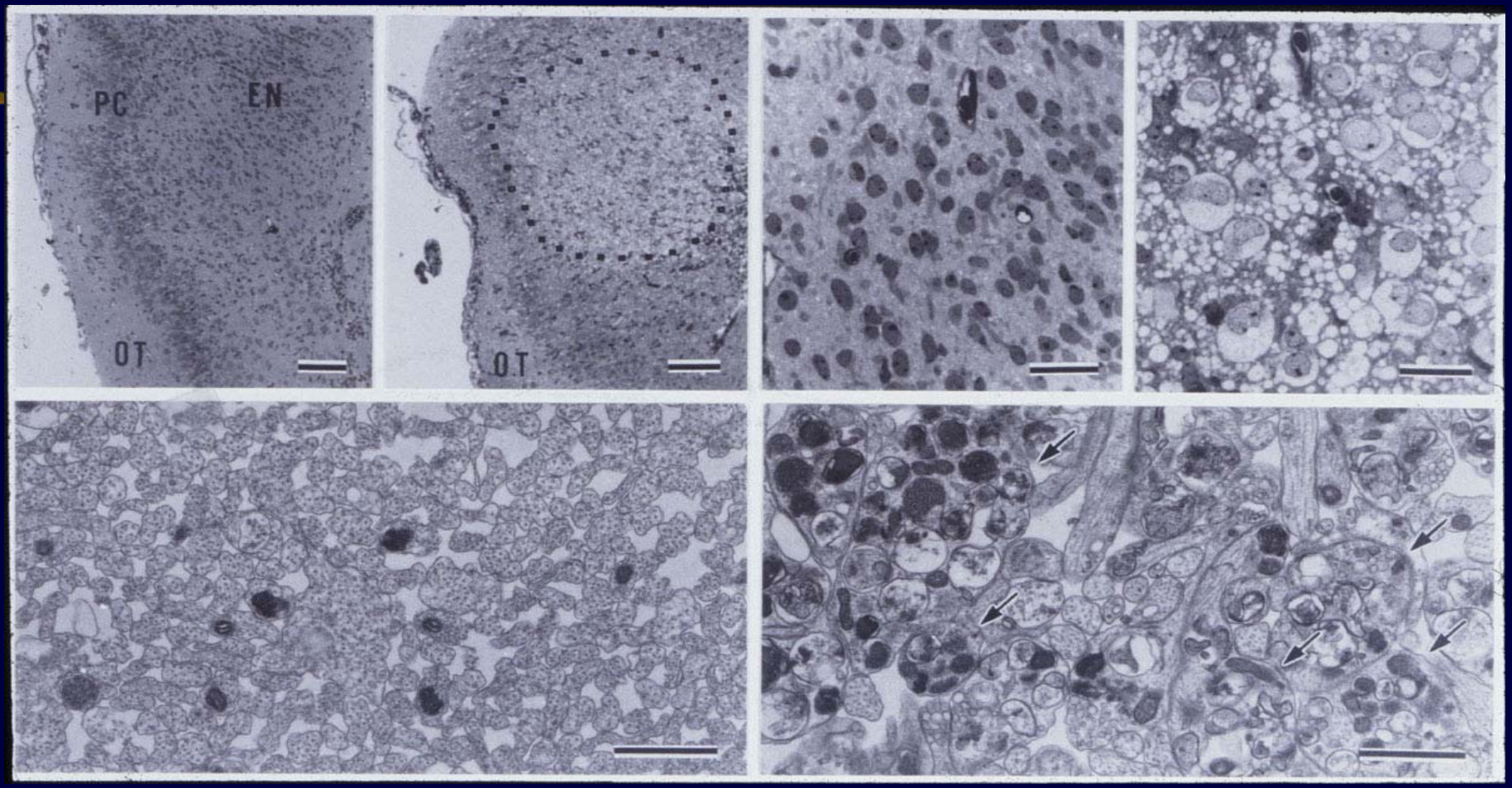

a
P2 P3 S3
KIF1A
Kinesin
KIF3
SV2
Synaptophysin
Syntaxin 1A
b
2.0 1.5 1.2 1.0 0.8 0.6 0.4 0.3 0.2 0.1 0
M
c
Pre K1A NMIg p38
RO II RO II RO II RO II
Synaptotagmin
Rab3A
SNAP-25

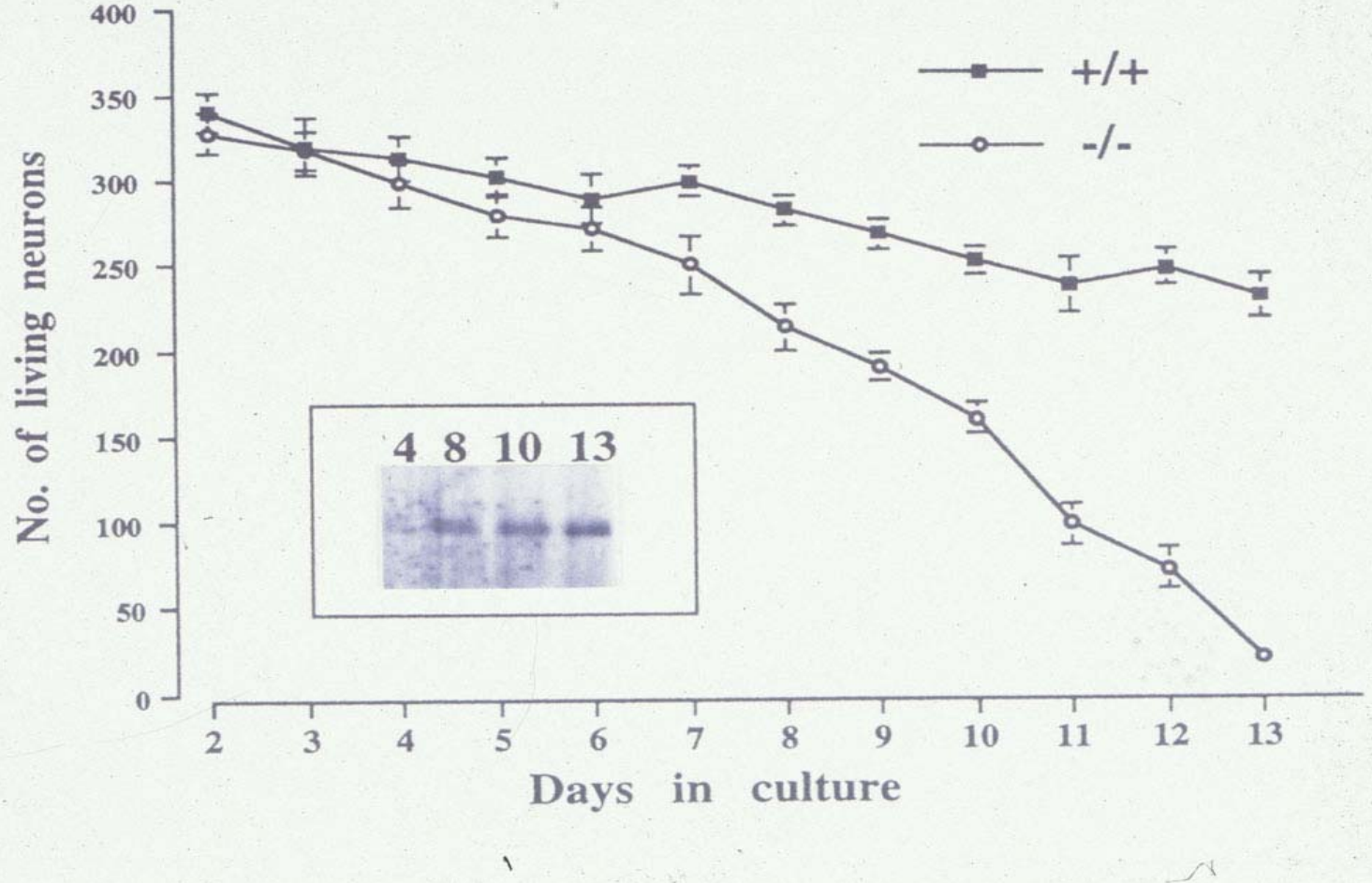

S-tagmin
S-physin
Rab3A
KIF1A
SV2
KIF?
KIF?
Syntaxin
SNAP-25
Synthesis & sorting
Transport
Targeting

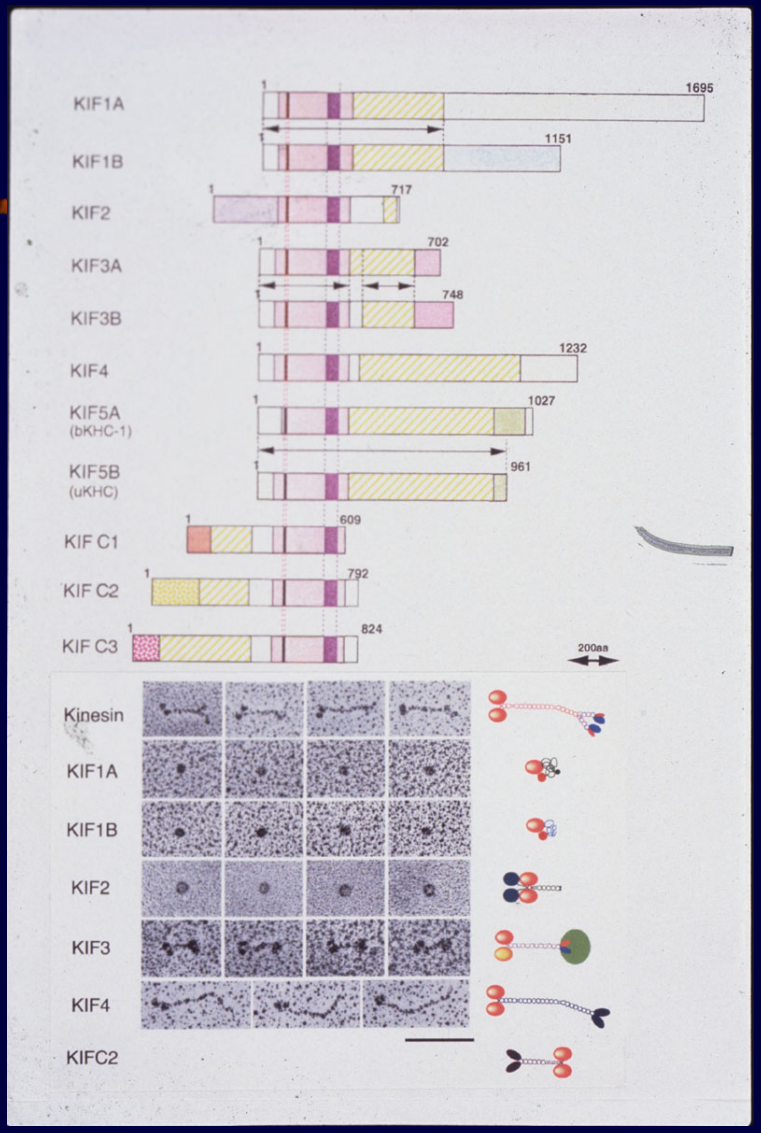


Yonekawa et al. JCB 141:431-, 1998


A
B


PC
EN
OT
OT


No. of living neurons
400
350
300
250
200
150
100
50
0
+/+
-/-
4 8 10 13
2 3 4 5 6 7 8 9 10 11 12 13
Days in culture



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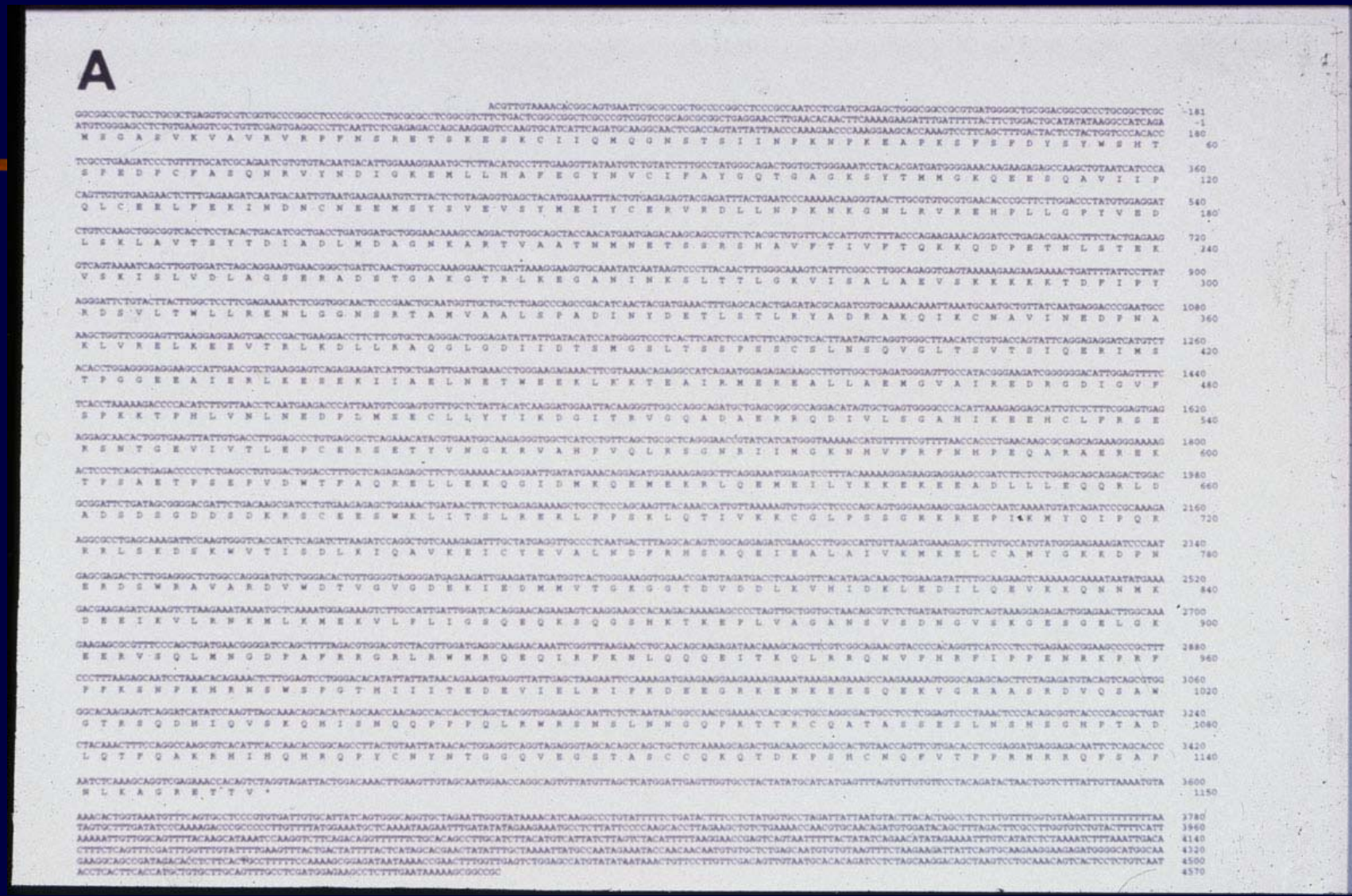
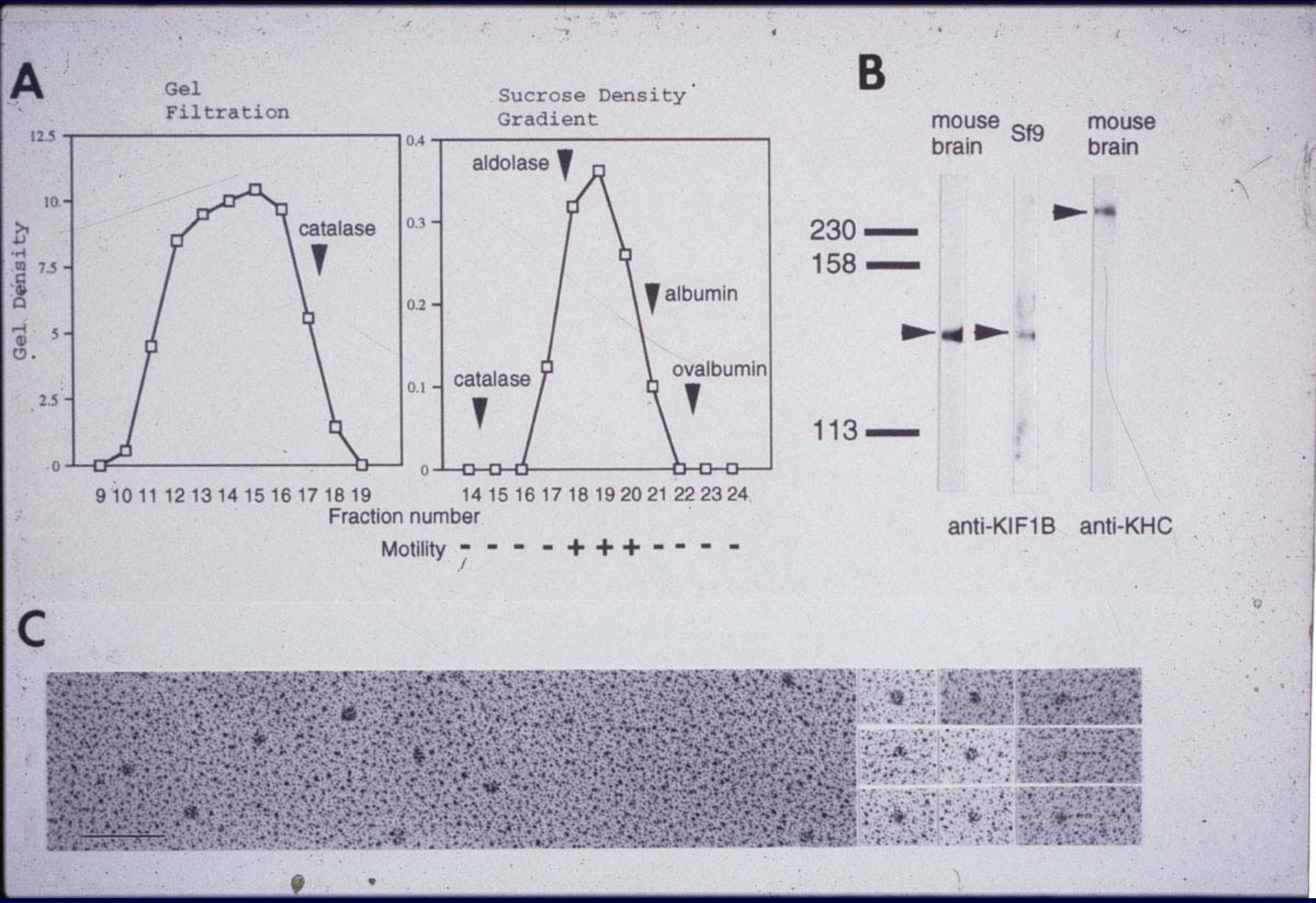
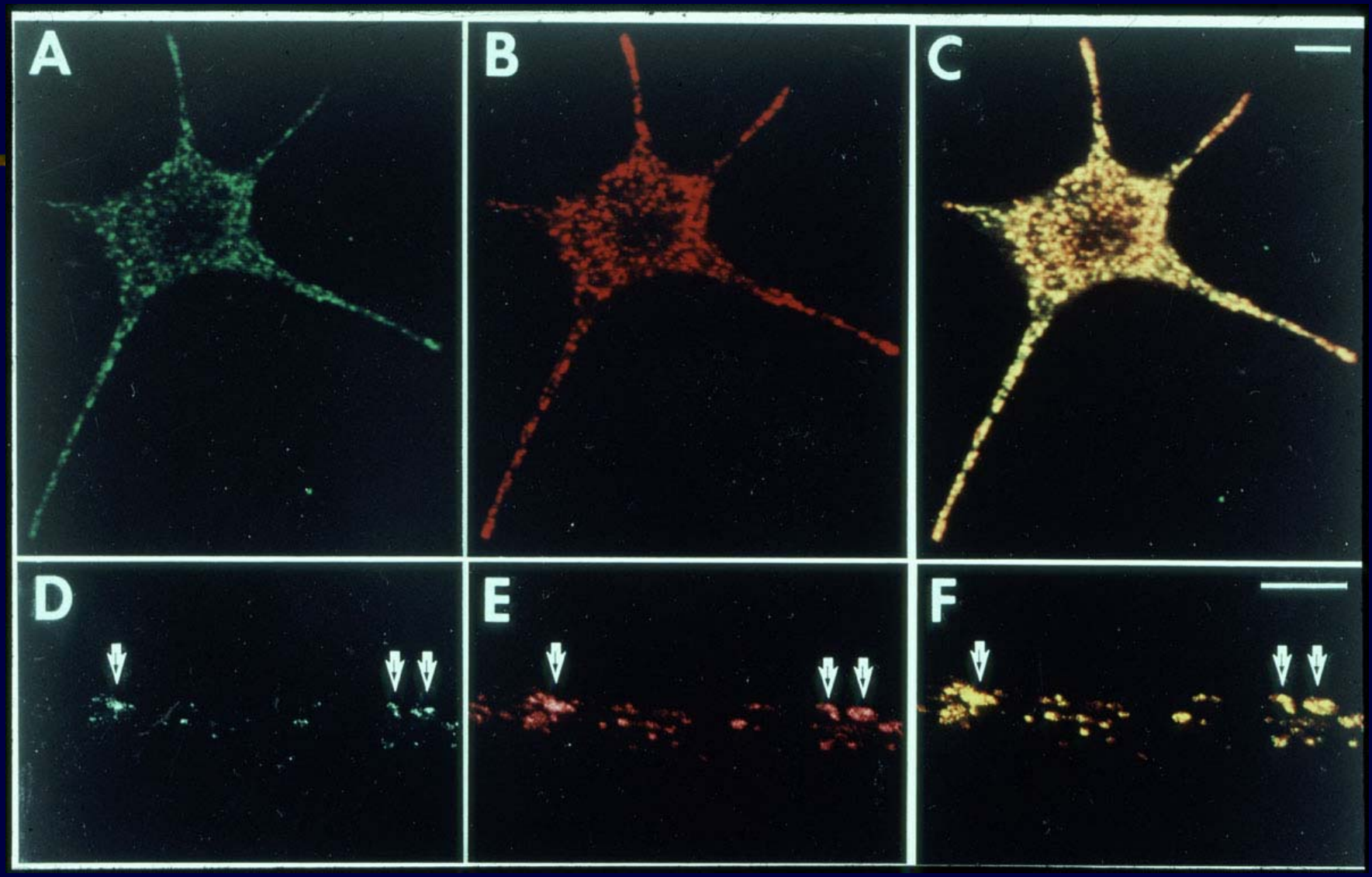
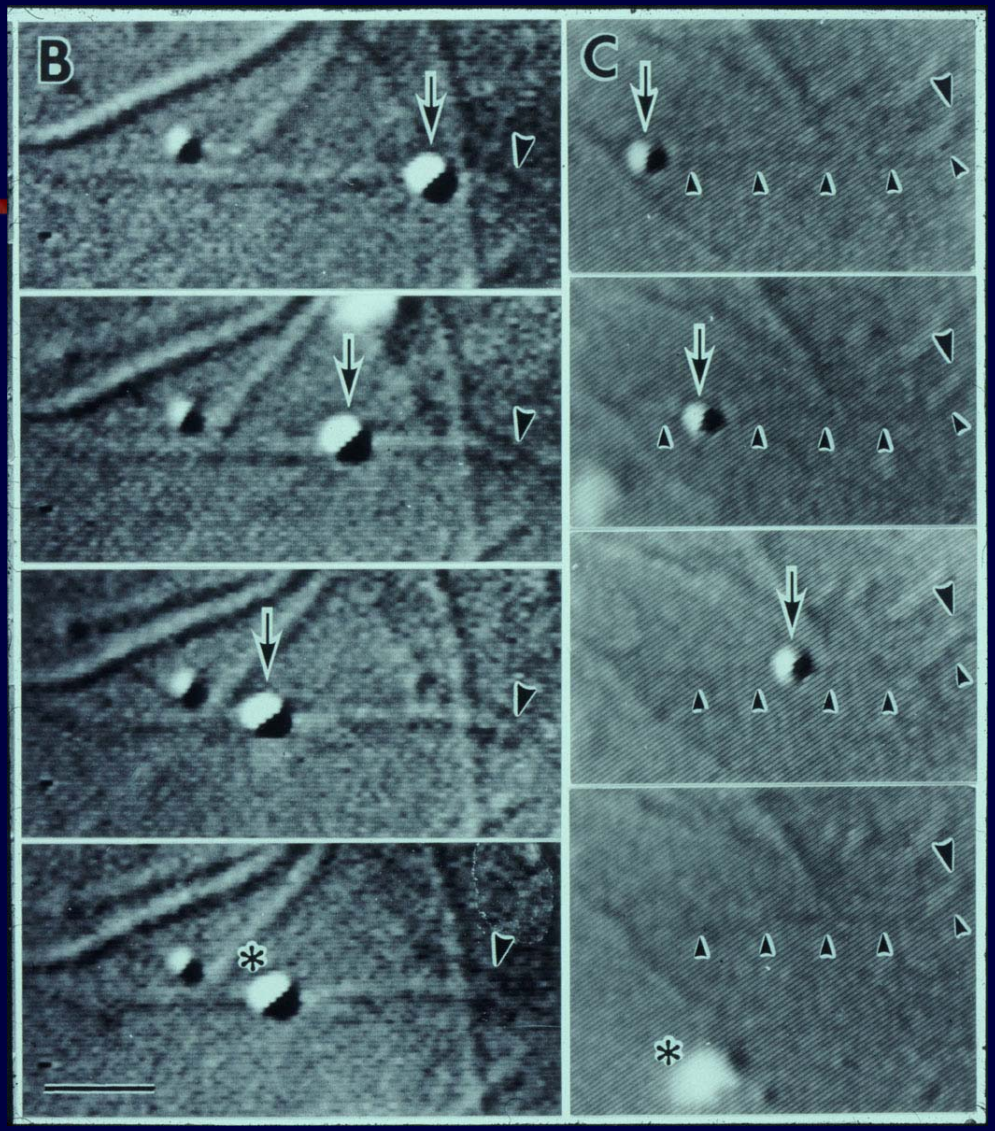

A


A
Gel Filtration
Sucrose Density Gradient
Gel Density
12.5
10
7.5
5
2.5
0
catalase
0.4
0.3
0.2
0.1
0
aldolase
albumin
catalase
ovalbumin
9 10 11 12 13 14 15 16 17 18 19
14 15 16 17 18 19 20 21 22 23 24
Fraction number
Motility - - - - + + + - - - -
B
mouse brain
Sf9
mouse brain
230
158
113
anti-KIF1B
anti-KHC
C


A


A
B
C
D
E
F


L
M
CE S1 P1 S2 P2 P3
KIF1B
COX


B
C