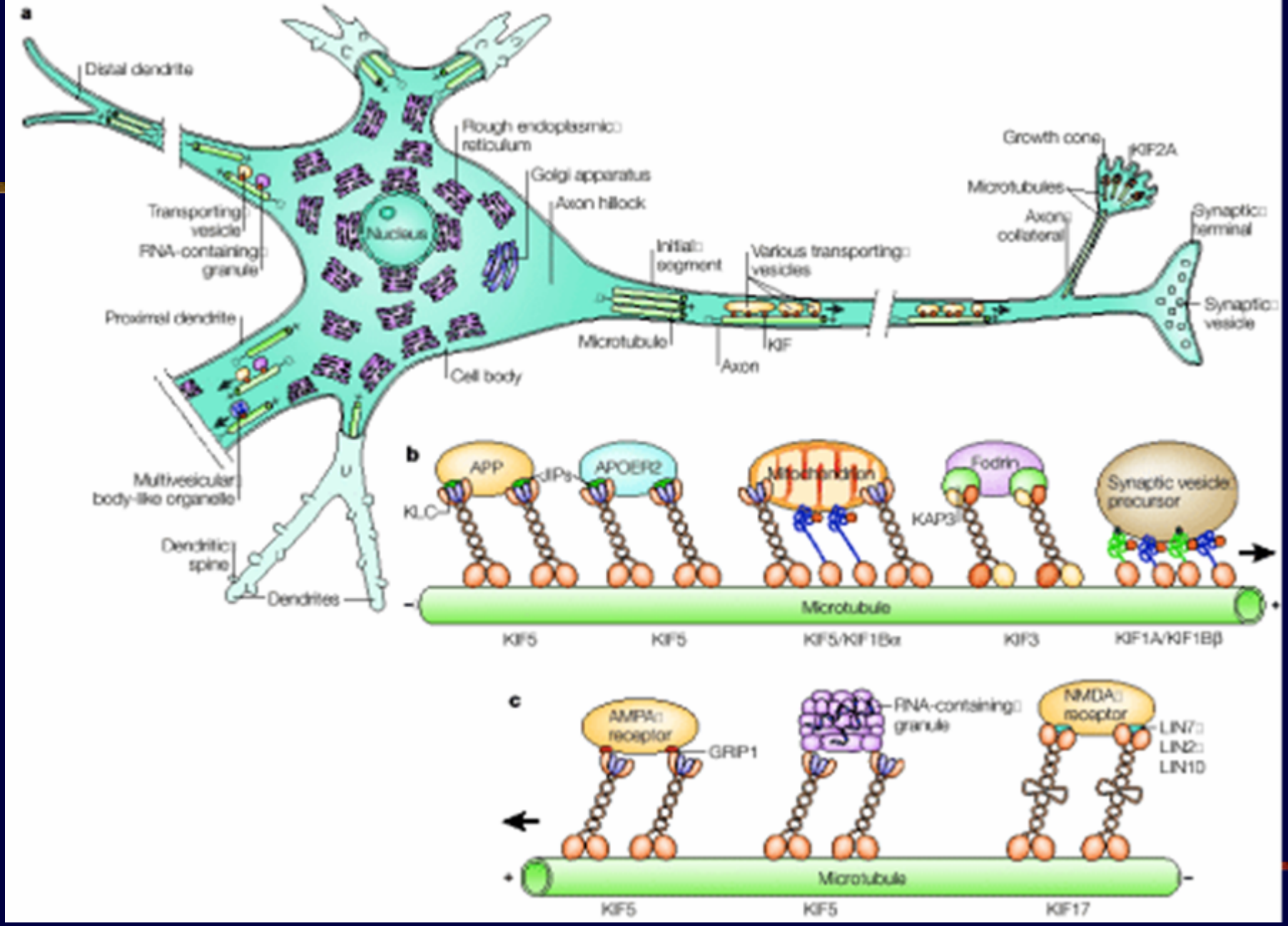
分子モーターから観た生命科学

脳、神経の働きと分子モーター

2006年11月13日

東京大学医学研究科　廣川信隆


a
Distal dendrite
Rough endoplasmic reticulum
Golgi apparatus
Axon hillock
Transporting vesicle
RNA-containing granule
Nucleus
Initial segment
Various transporting vesicles
Growth cone
KIF2A
Microtubules
Axon collateral
Synaptic terminal
Synaptic vesicle
Proximal dendrite
Microtubule
KIF
Axon
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

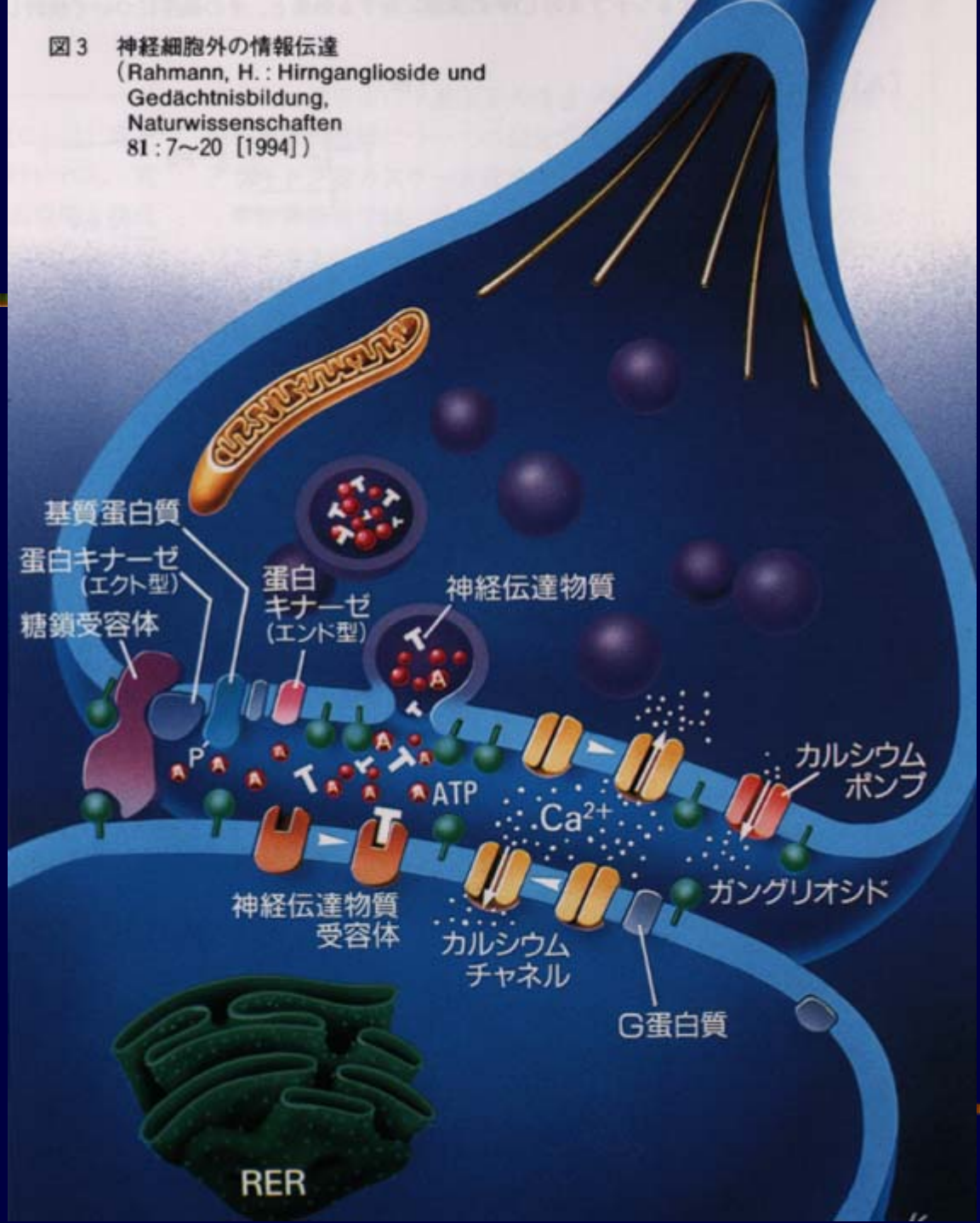
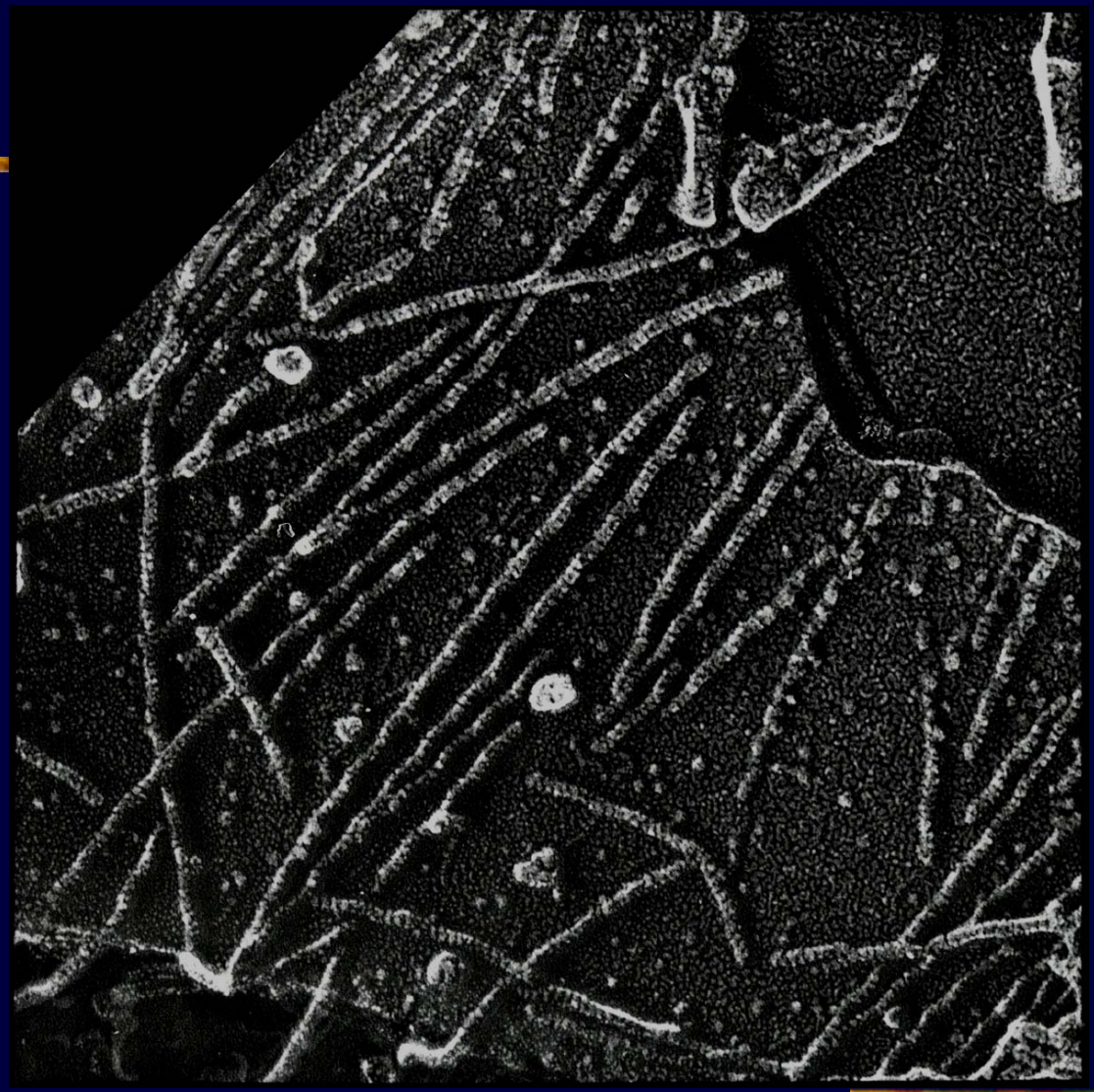
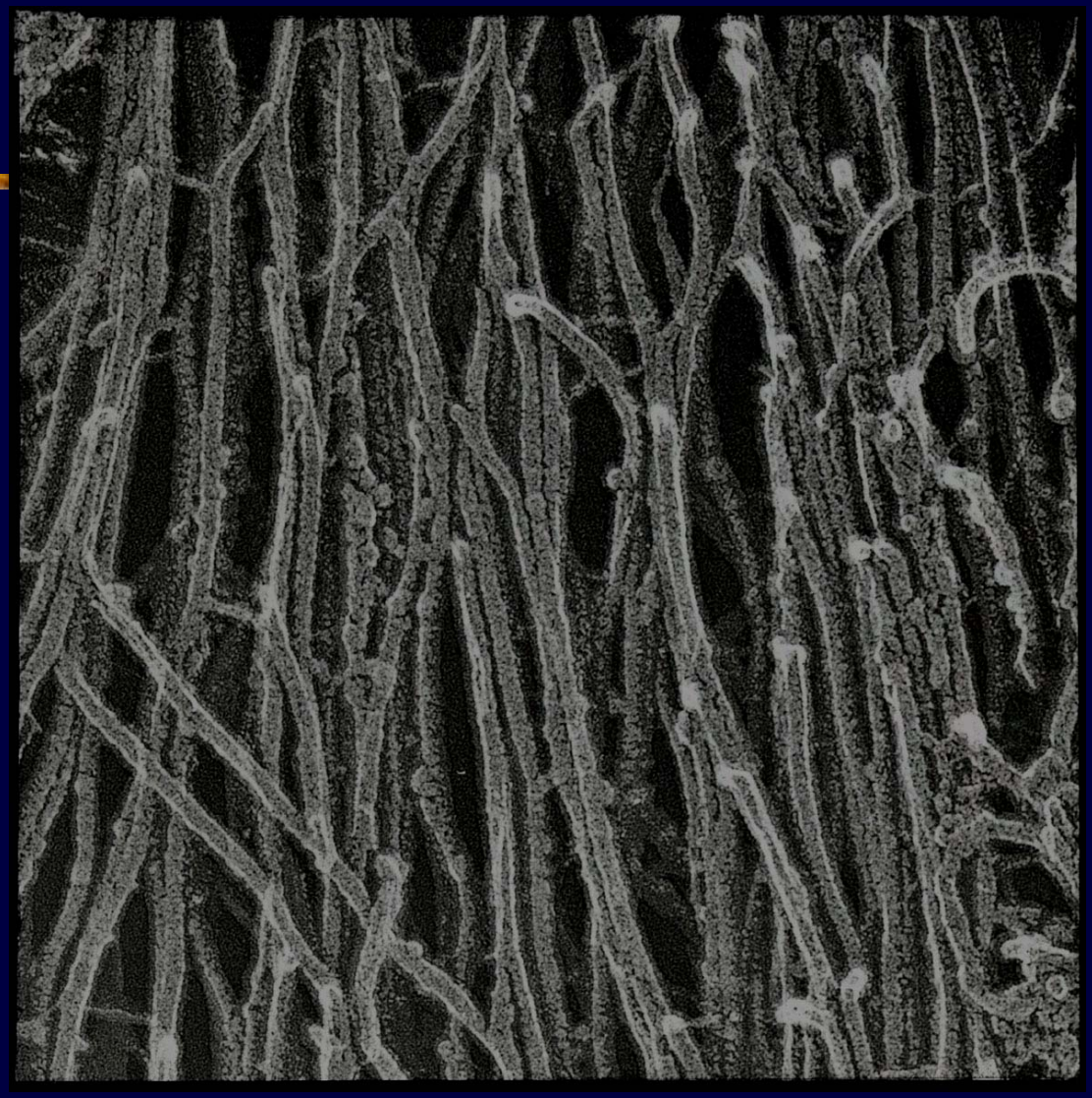


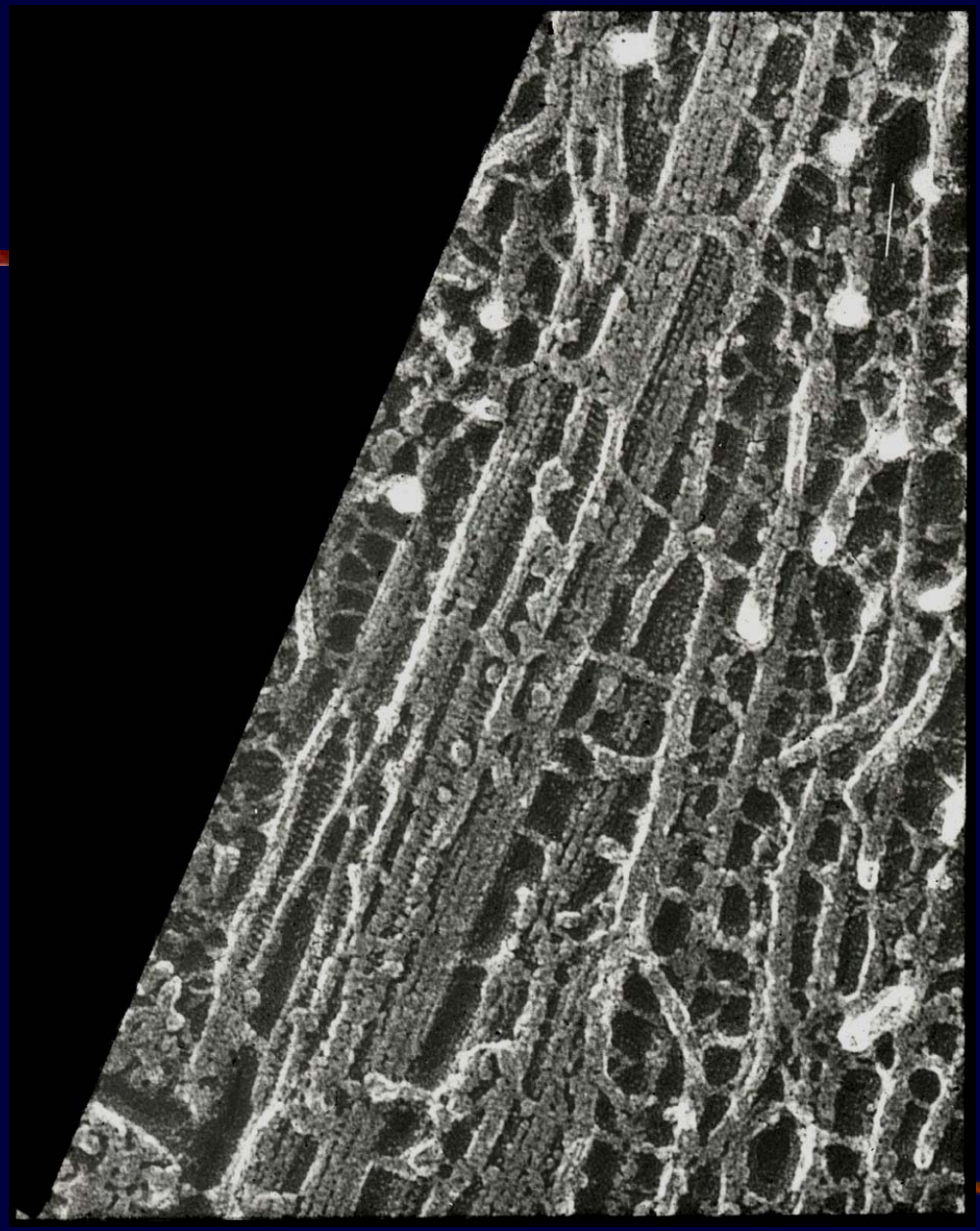
図3　神経細胞外の情報伝達
(Rahmann, H. : Hirnganglioside und Gedächtnisbildung, Naturwissenschaften 81 : 7～20 [1994])

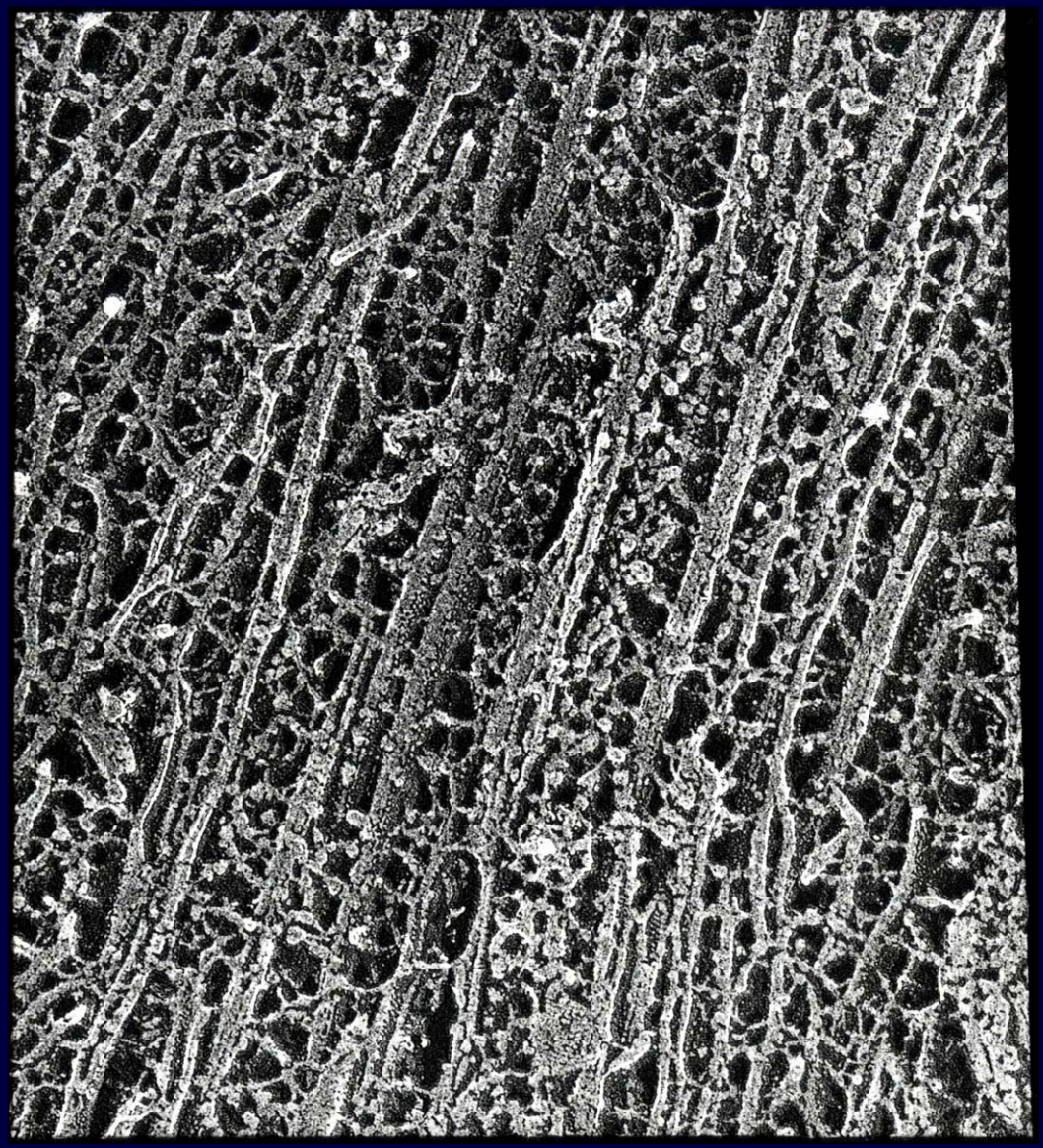
早石　修・伊藤正男編
『精神活動の流れを遡る』p. 135：図3 ‡

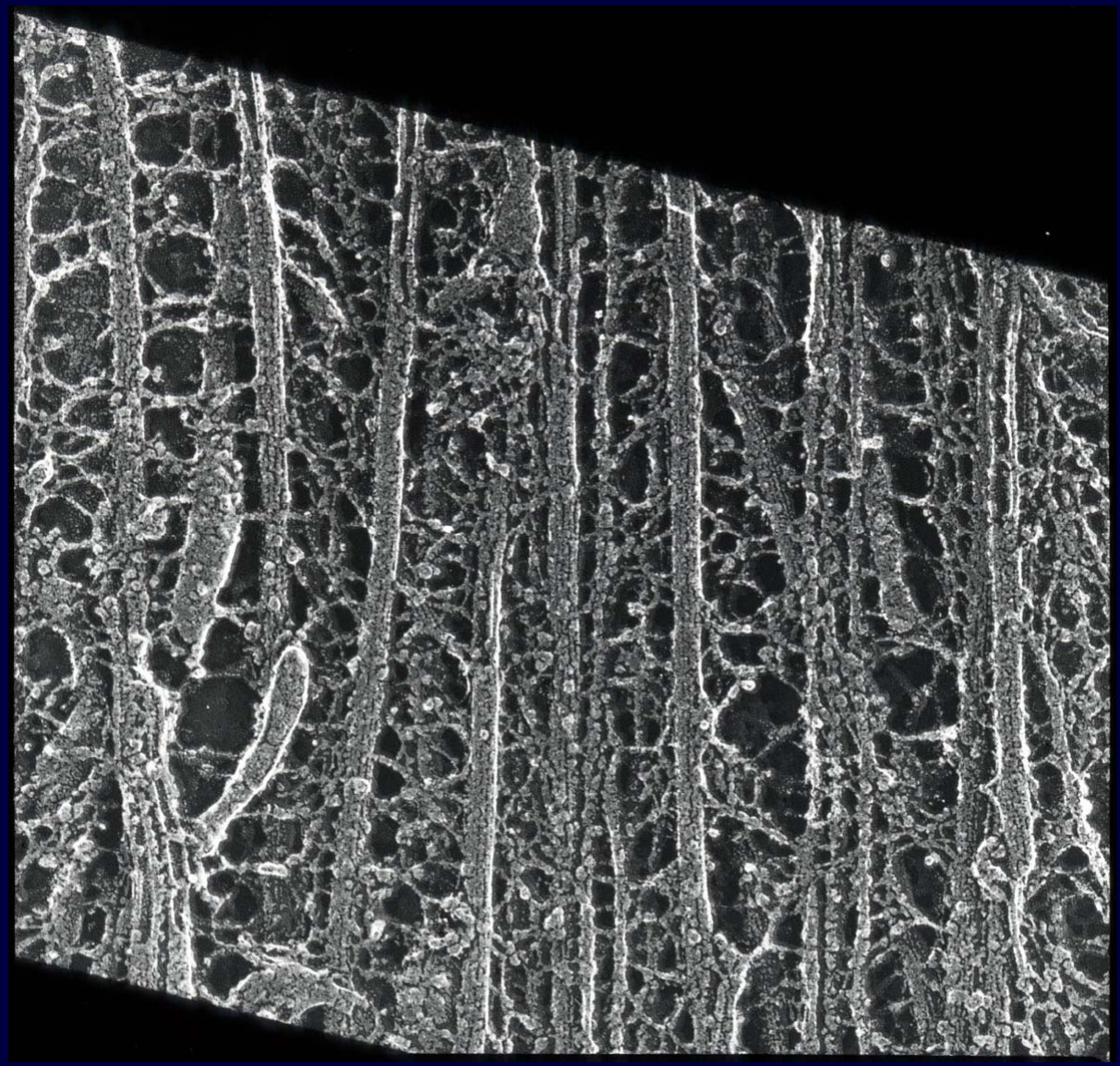
Cytoskeleton 細胞骨格

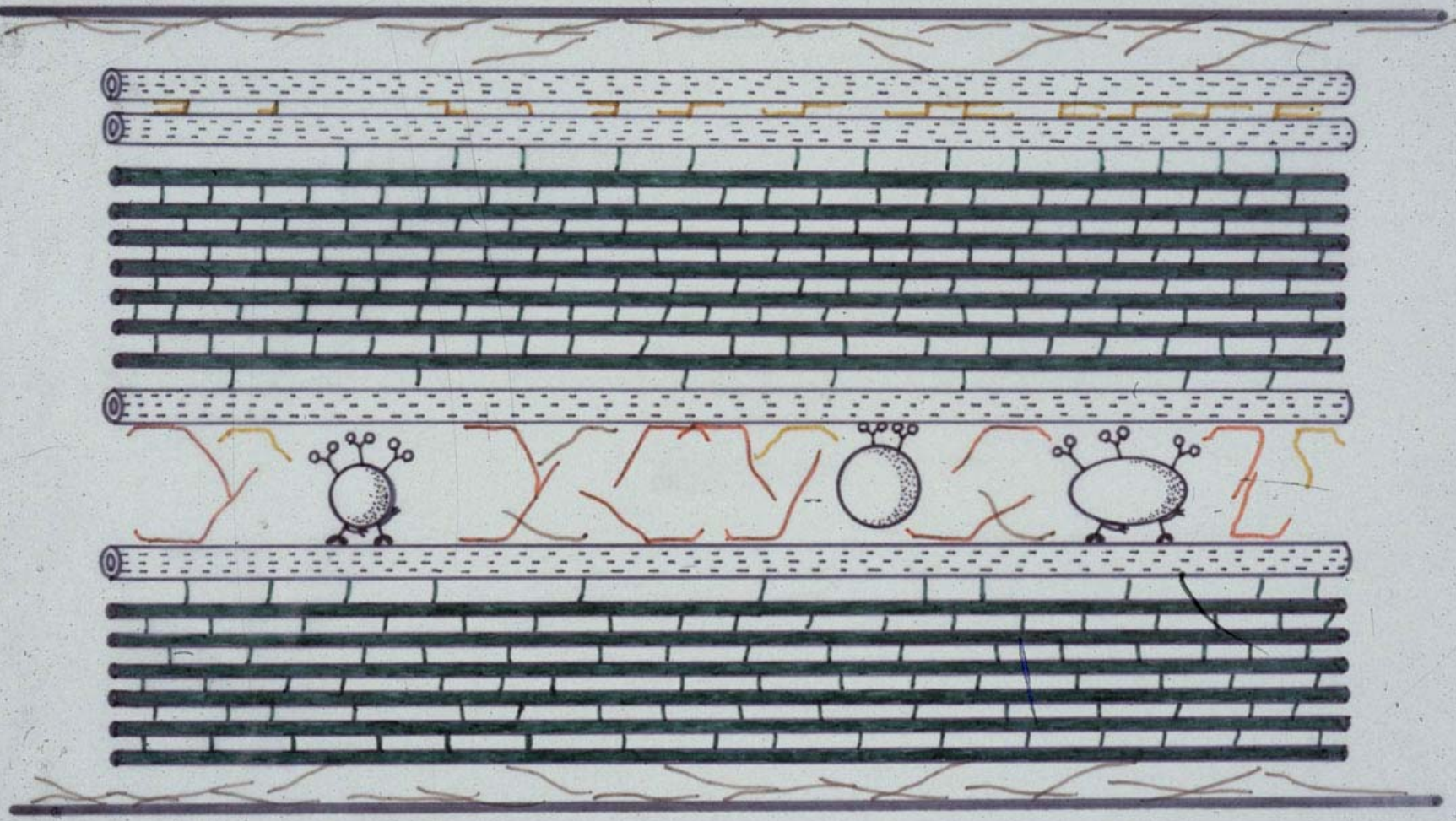




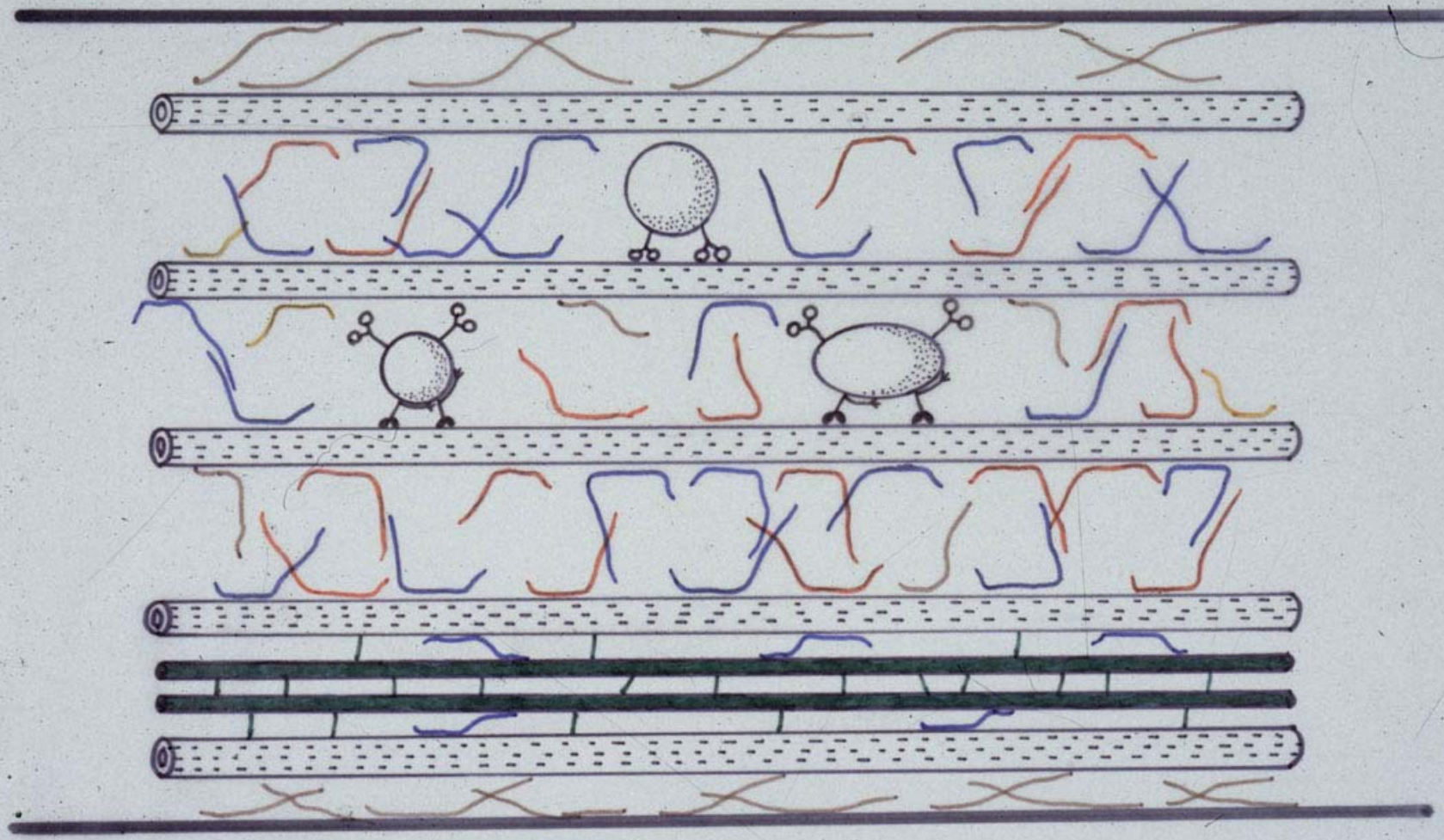








AXON



DENDRITE

Location of MAPs

Dendrite

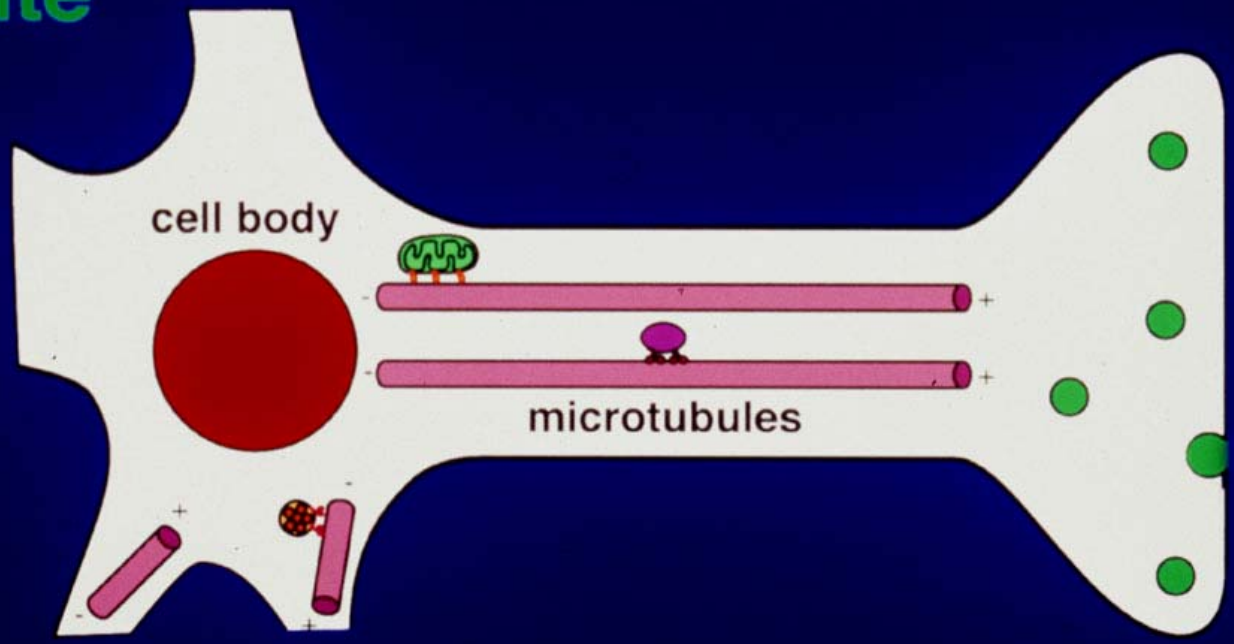
MAP1A

MAP1B

MAP2A

MAP2B

(Tau)



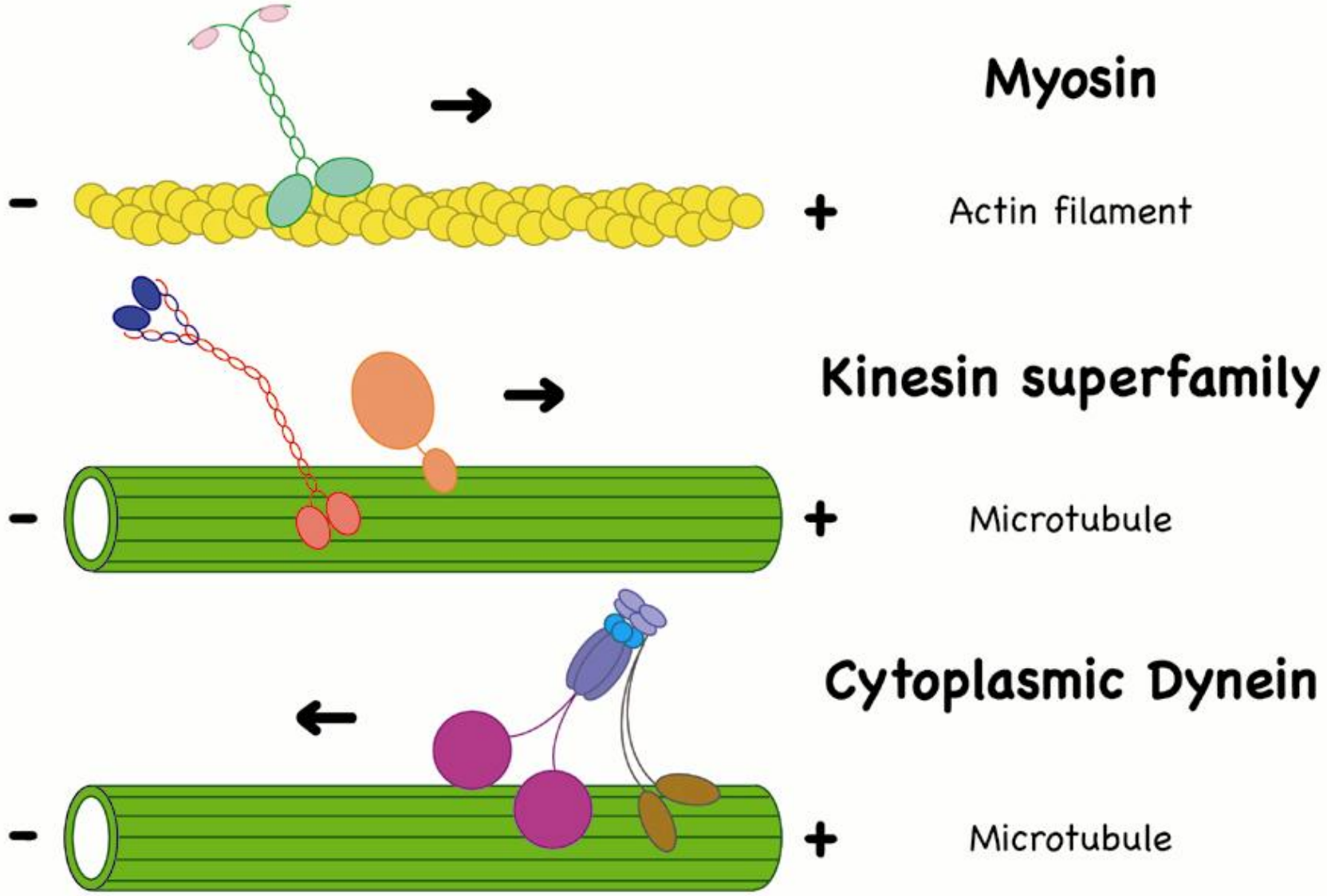
Axon

MAP1A

MAP1B

MAP2C

Tau

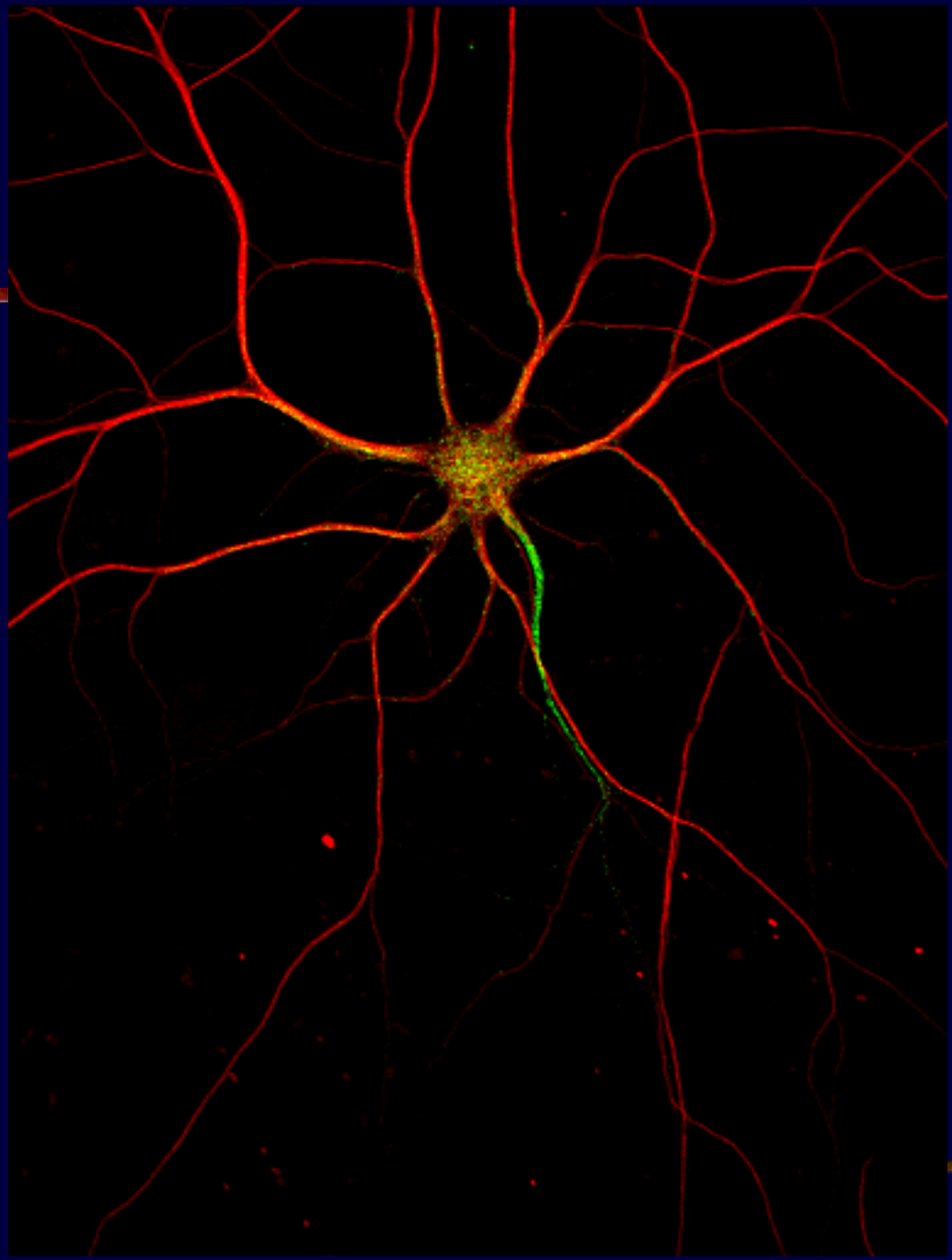

Myosin
-
+
Actin filament
Kinesin superfamily
-
+
Microtubule
Cytoplasmic Dynein
-
+
Microtubule

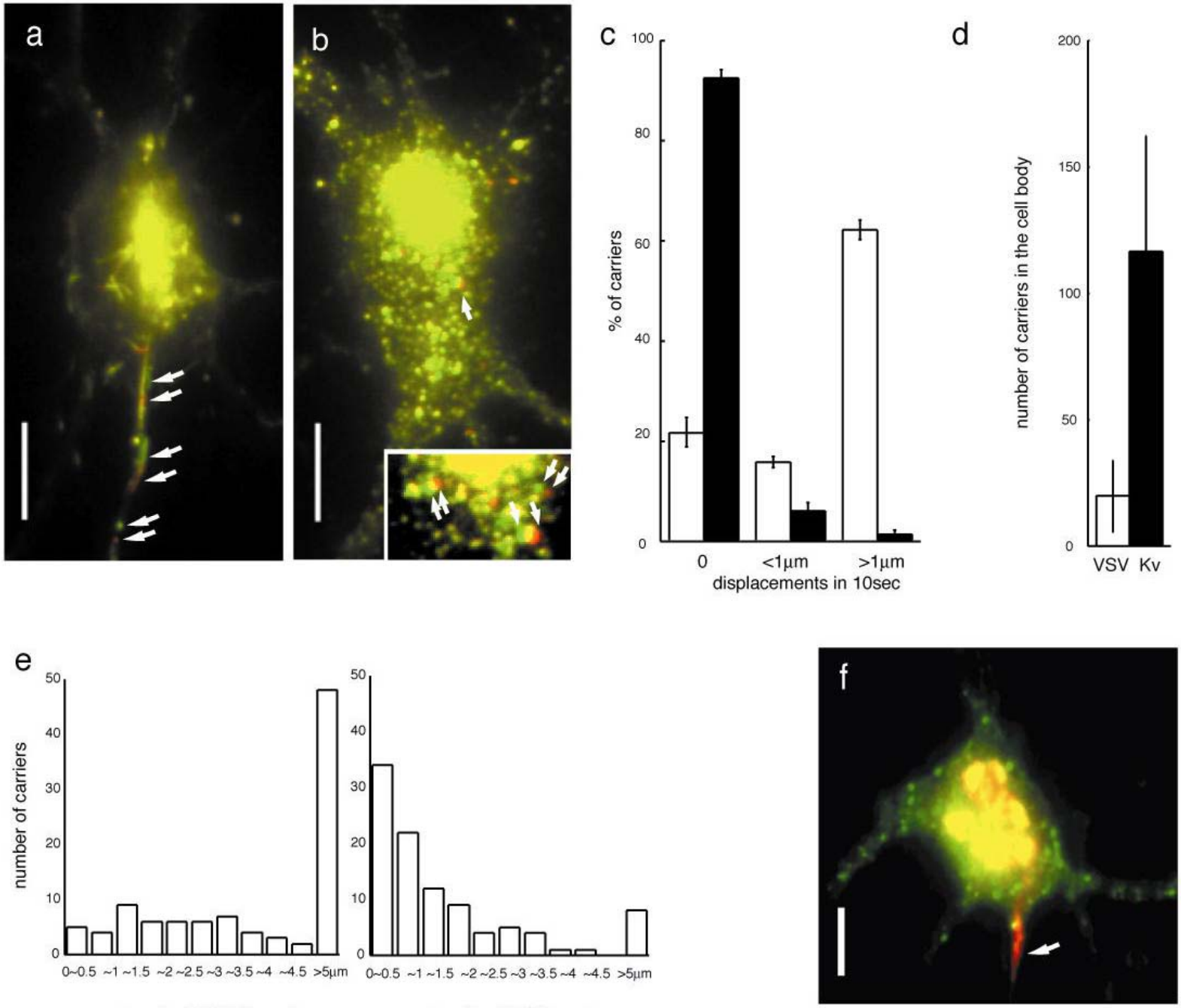
Intracellular Transport and Molecular Motors

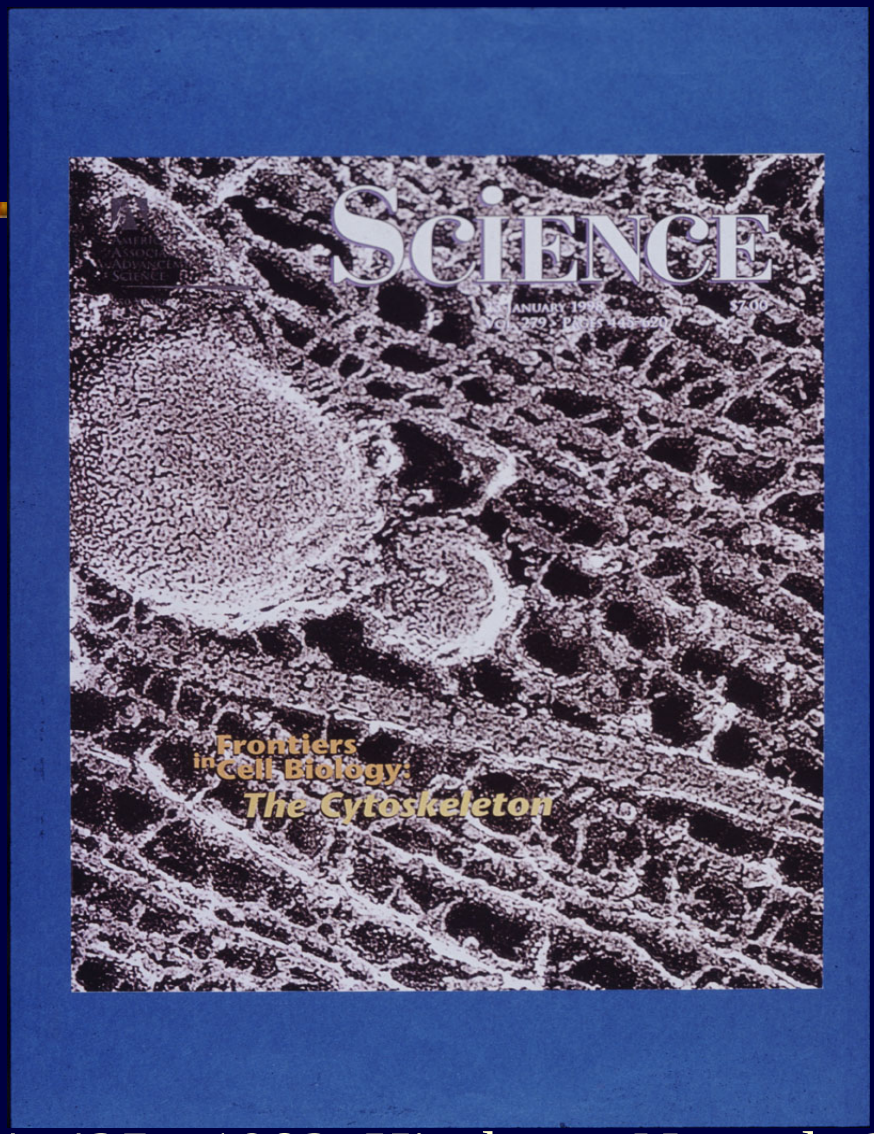
The Mechanism of Organelle Transport

Axonal Transport

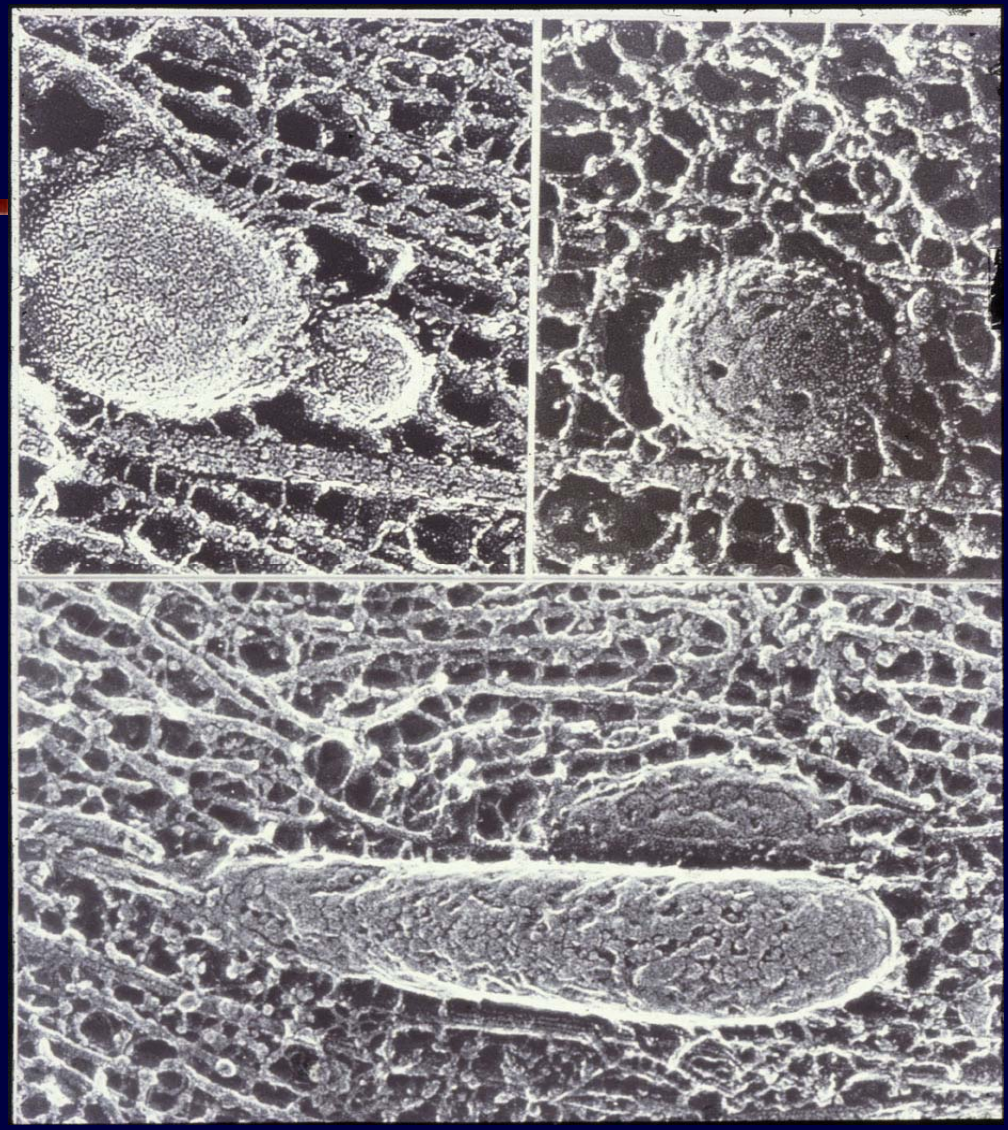
	Group	Velocity mm/day	composition
Fast	I	240	membrane organella
	II	60	membrane organella
	III	6	myosin like actin binding protein
Slow	IV (SCb)	2	actin, clathrin calmodulin
	V (SCa)	1	tubulin, neurofilament triplet proteins



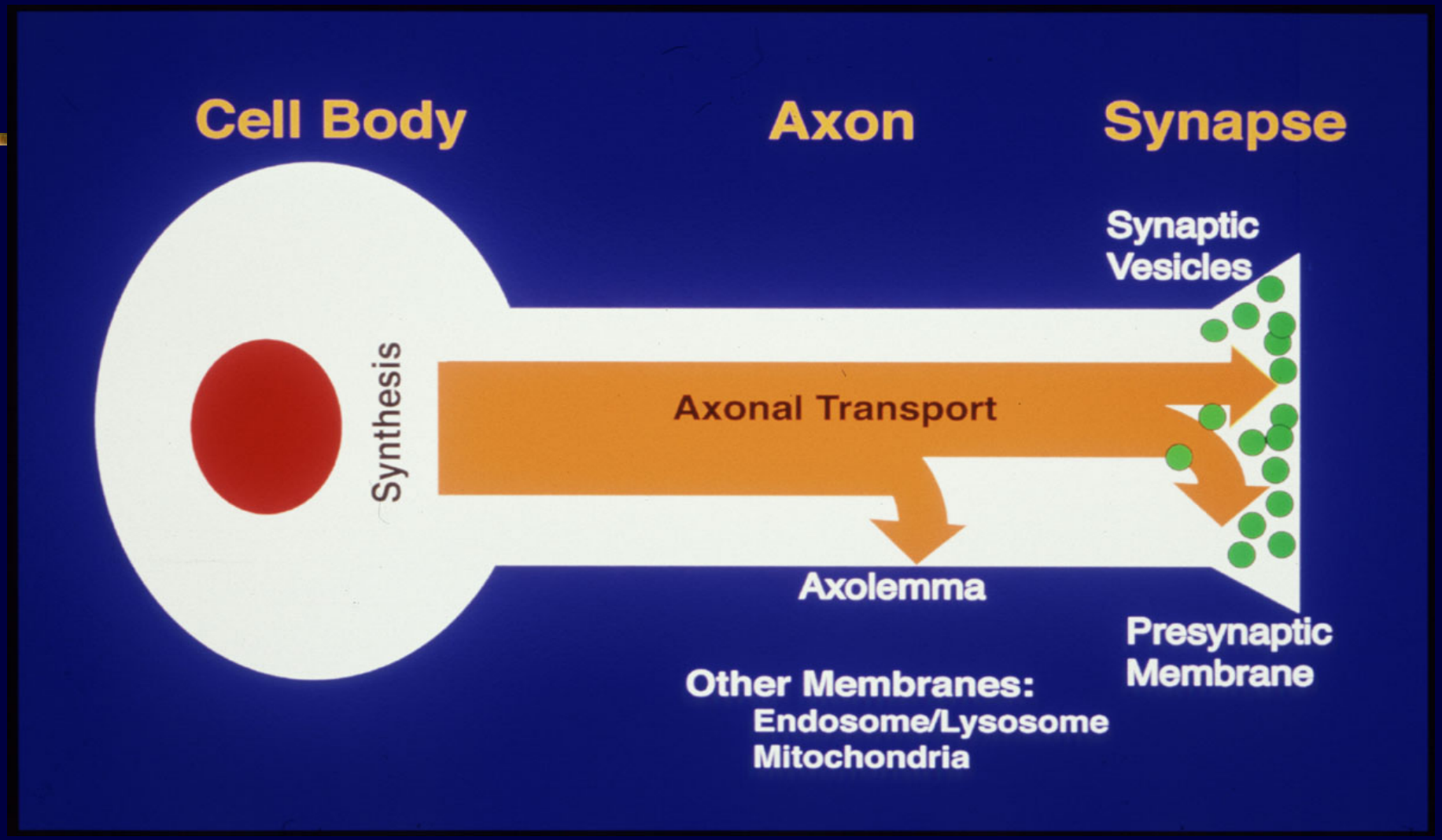

a
b
c
100
80
60
40
20
0
% of carriers
0
<1μm
>1μm
displacements in 10sec
d
200
150
100
50
0
number of carriers in the cell body
VSV
Kv
e
50
40
30
20
10
0
number of carriers
0~0.5 ~1 ~1.5 ~2 ~2.5 ~3 ~3.5 ~4 ~4.5 >5μm
run-length of VSV-G carriers
run-length of Kv2.1 carrier
f



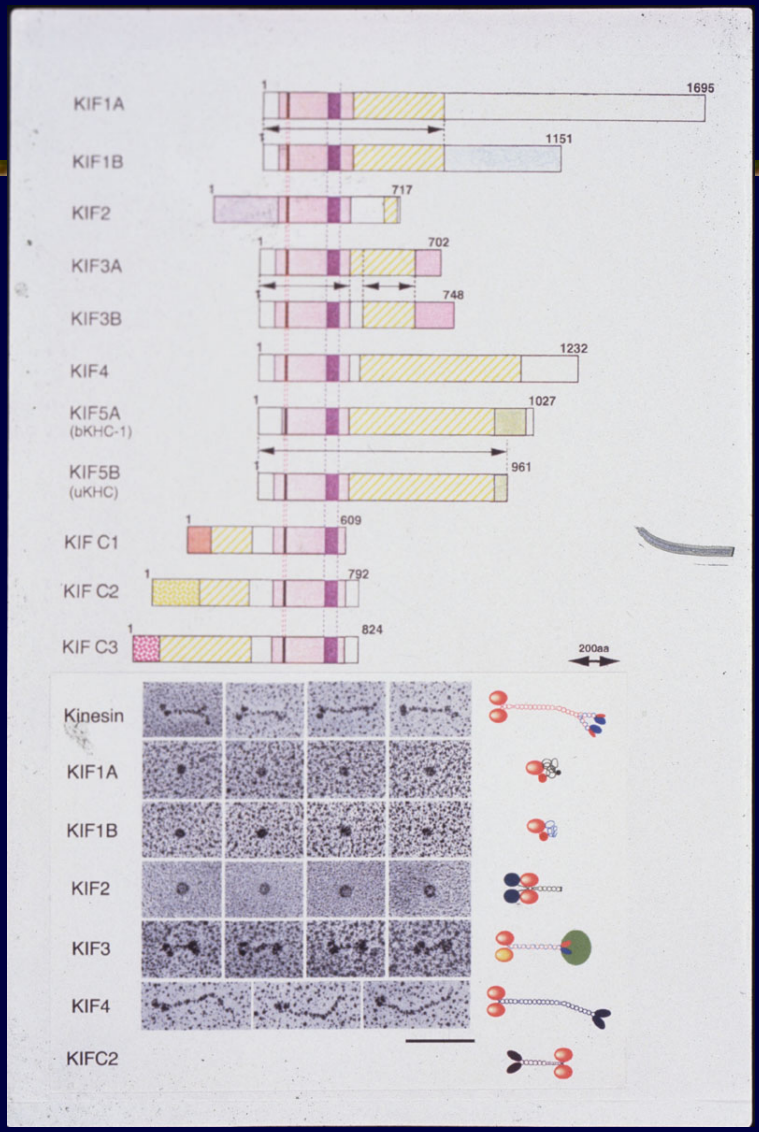
Hirokawa N. JCB 94: 425- ,1982, Hirokawa N. et al. Cell 56: 867- ,1989
Hirokawa N. Science 279: 519- ,1998



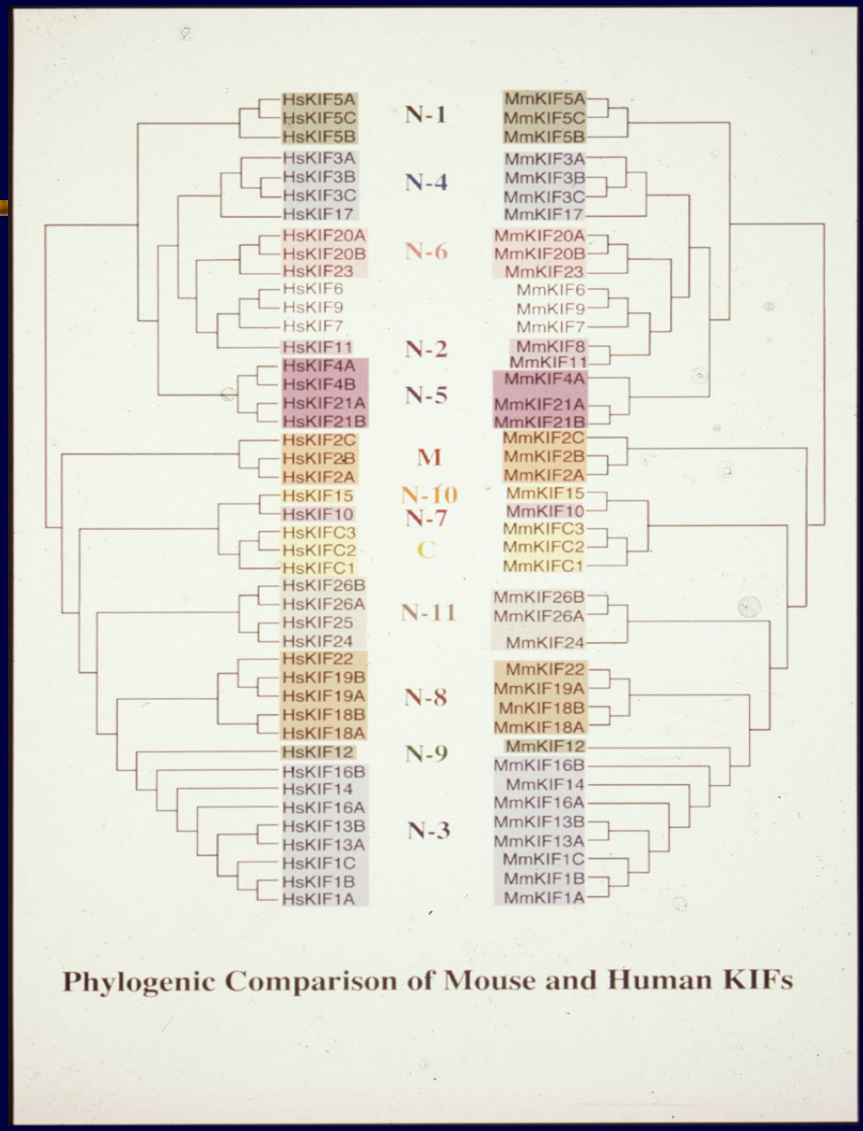
Hirokawa JCB 94:129－,1982; Hirokawa et al. Cell 56:867－,1989
Hirokawa Trends Cell Biol. 132:667－,1996


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

Kinesin Superfamily Proteins KIFs

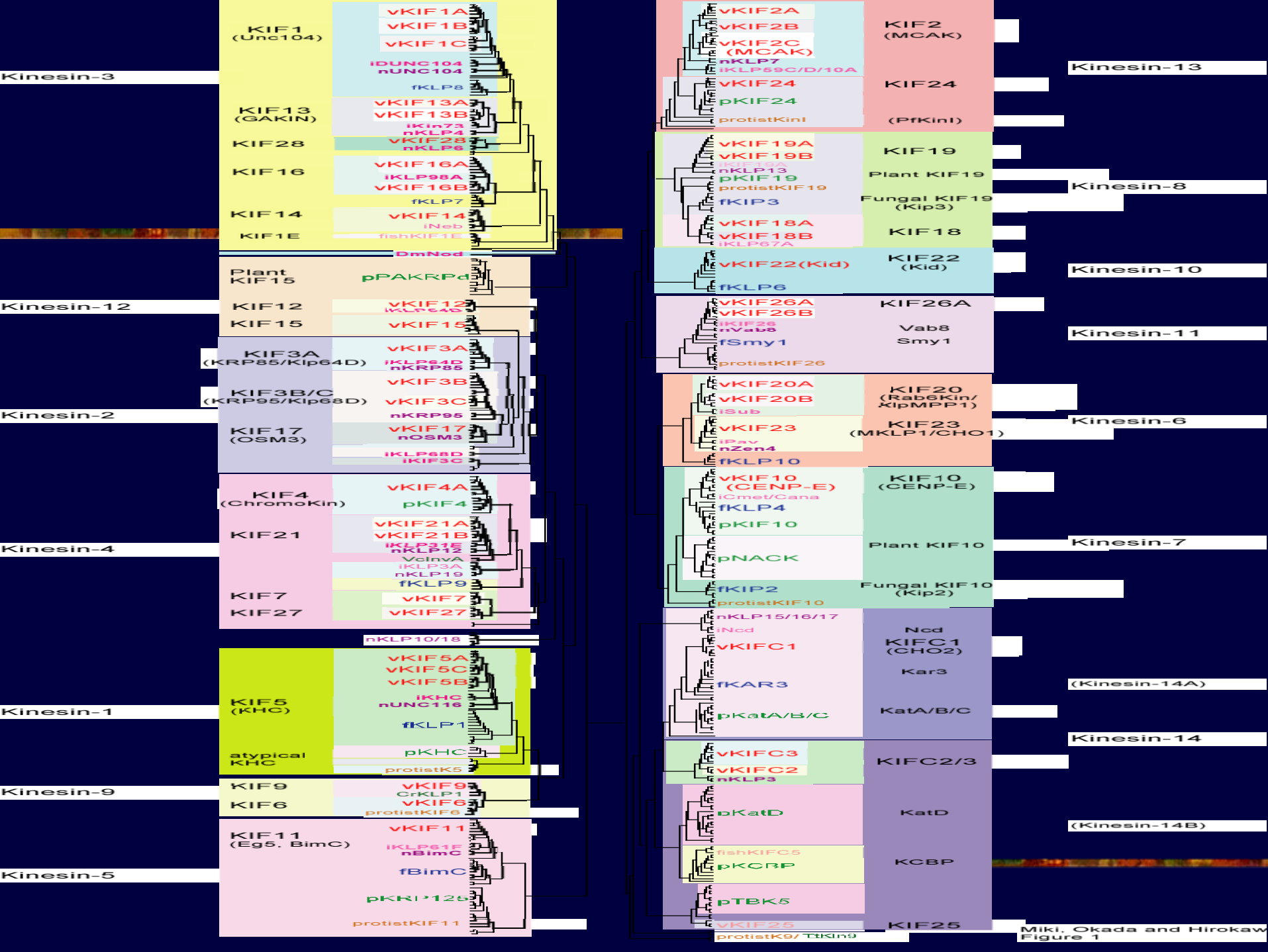


Hirokawa et al. Cell 56:867－,1989
Hirokawa et al. JCB 114:295－,1991
Aizawa et al. JCB 119:1287－,1992
Kondo et al. JCB 125:1095－,1994
Sekine et al. JCB 127:187－,1994
Nangaku et al. Cell 79:1209－,1994
Noda et al. JCB 129:157－,1995
Okada et al. Cell 81:769－,1995
Kikkawa et al. Nature 376:274－,1995
Yamazaki et al. JCB 130:1387－,1995
Nakata & Hirokawa JCB 131:1039－,1995
Hirokawa Trends Cell Biol. 132:667－,1996
Yamazaki et al. PNAS 93:8443－,1996
Saito et al. Neuron 18:425－,1997
Nakagawa et al. PNAS 94:9654－,1997
Hirokawa Science 279:519－,1998
Yonekawa et al. JCB 141:431－,1998
Tanaka et al. Cell 93:1147－,1998
Nonaka et al. Cell 95:829－,1998
Okada & Hirokawa Science 283:－,1999
Takeda et al. JCB 145:825－,1999
Kikkawa et al. Cell 100:241－,2000
Takeda et al. JCB 148:1255－,2000
Setou et al. Science 288:1796－,2000
Kanai et al. J.Neurosci 20:6374－,2000
Terada et al. Cell 103:141－,2000
Nakagawa et al. Cell 103:569－,2000
Kikkawa et al. Nature 411:439－,2001
Zhao et al. Cell 105:587－,2001
Miki et al. PNAS 98:7004－,2001
Noda et al.. JCB 155:77--,2001
Setou et al. Nature 417:83－,2002
Xu et al. JCB 158:293－,2002
Wong et al. PNAS 99:14500－,2002
Macho et al. Science 298:2388－,2002
Guillaud et al. J.Neurosci 23:131-,2003
Homma et al. Cell 114:229－,2003
Okada et al. Nature 424:574-,2003
Nakata & Hirokawa JCB 162:1045--,2003
Ogawa et al. Cell 116:591--,2004
Nitta et. al. Science 30:678--,2004
Kanai et. al. Neuron 43:513--,2004
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

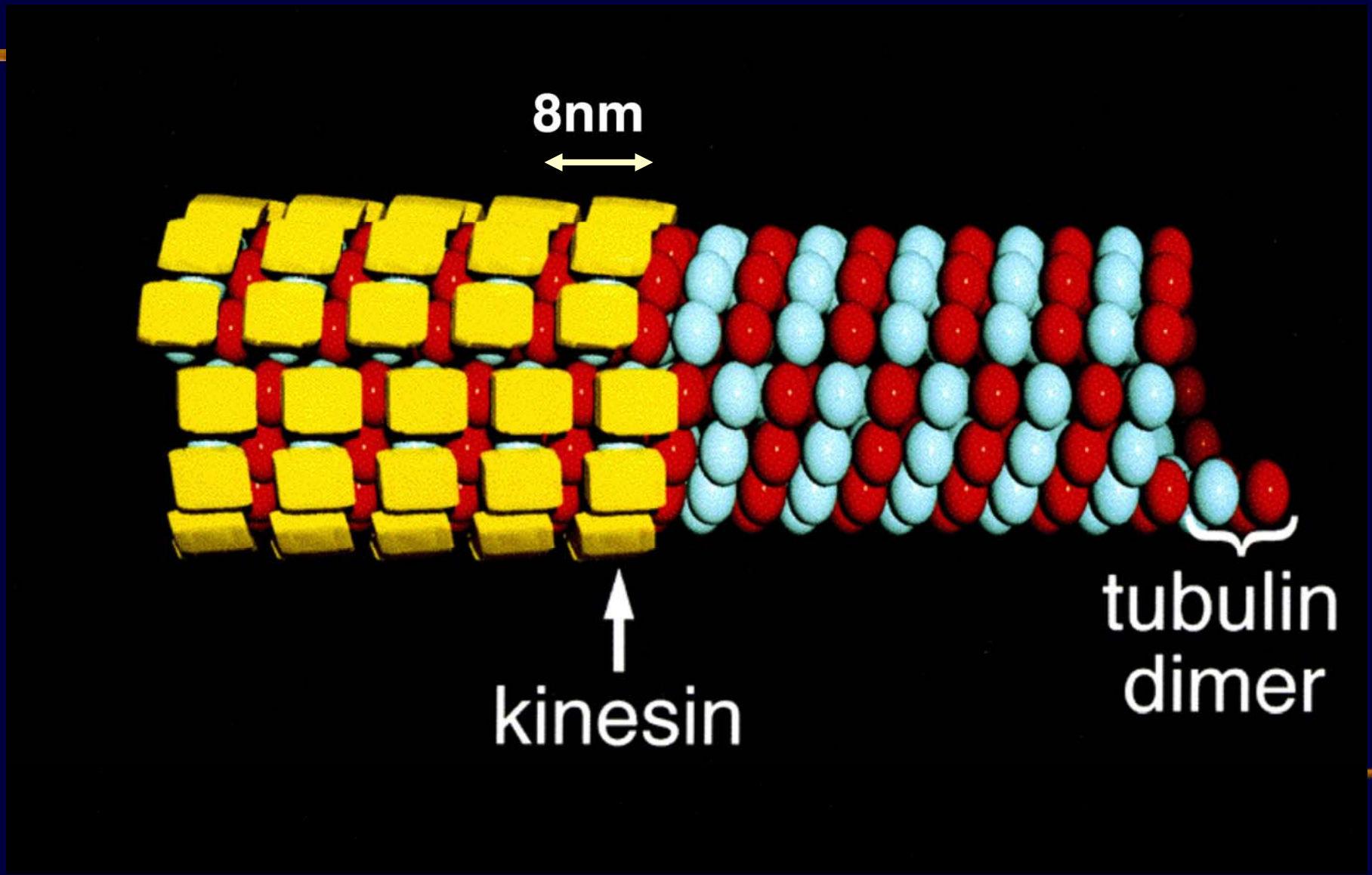
Miki et al. PNAS 98:7004－,2001

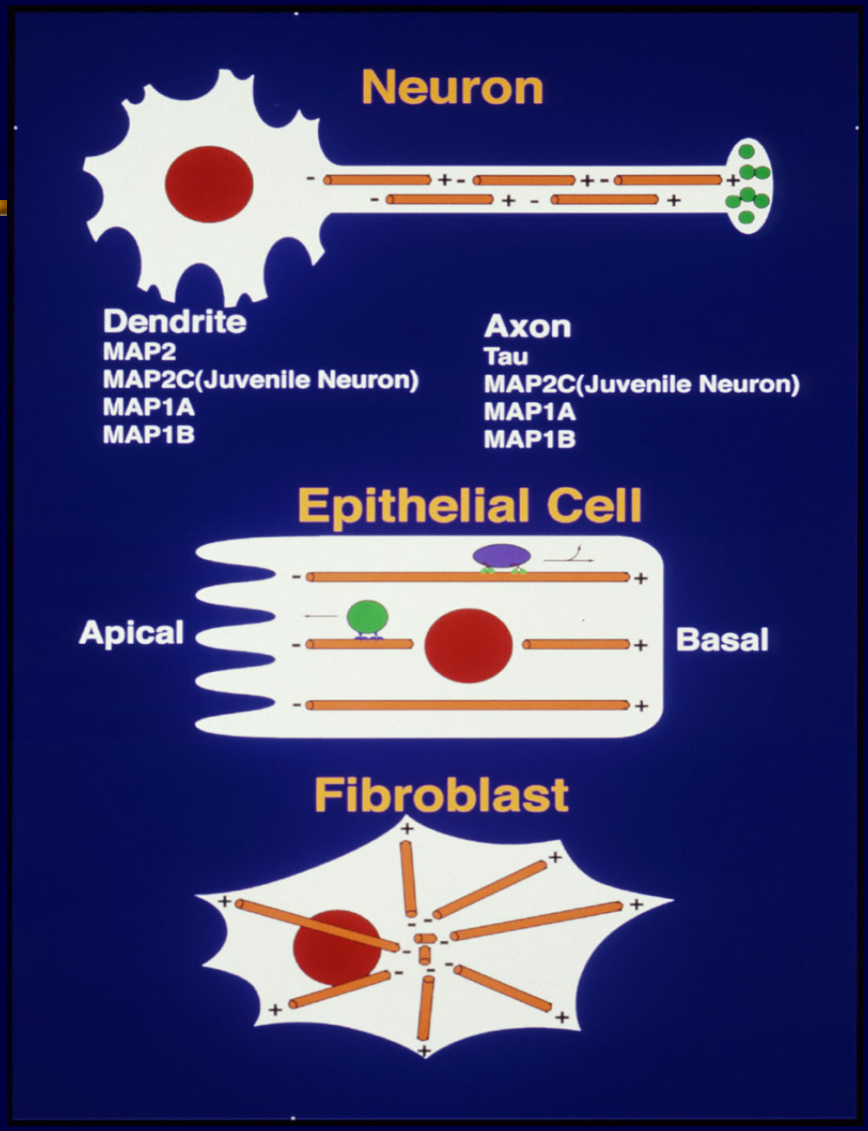


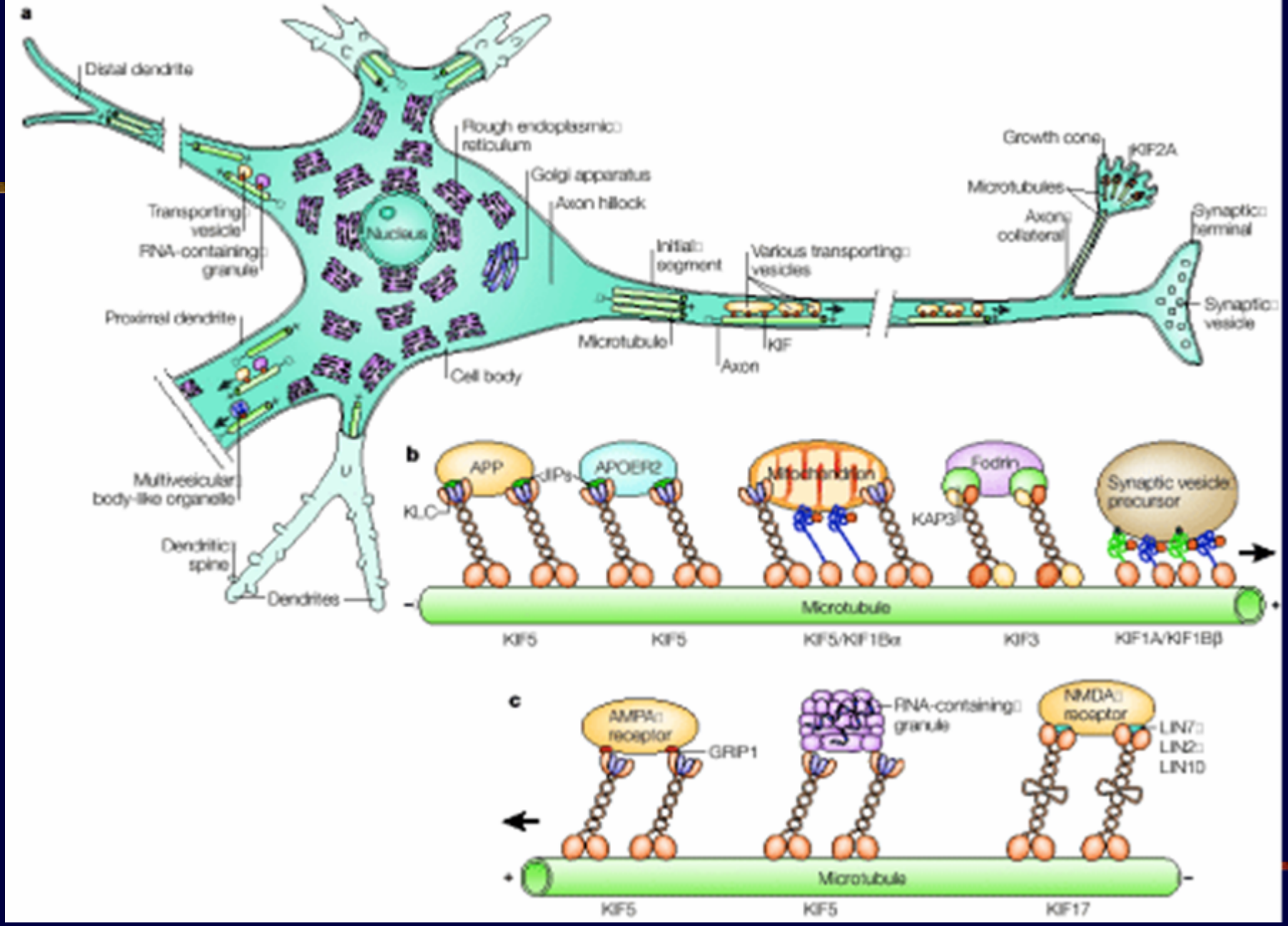
Miki, Okada and Hirokaw
Figure 1

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

キネシンのレール：微小管の構造




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
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Nucleus
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KAP3
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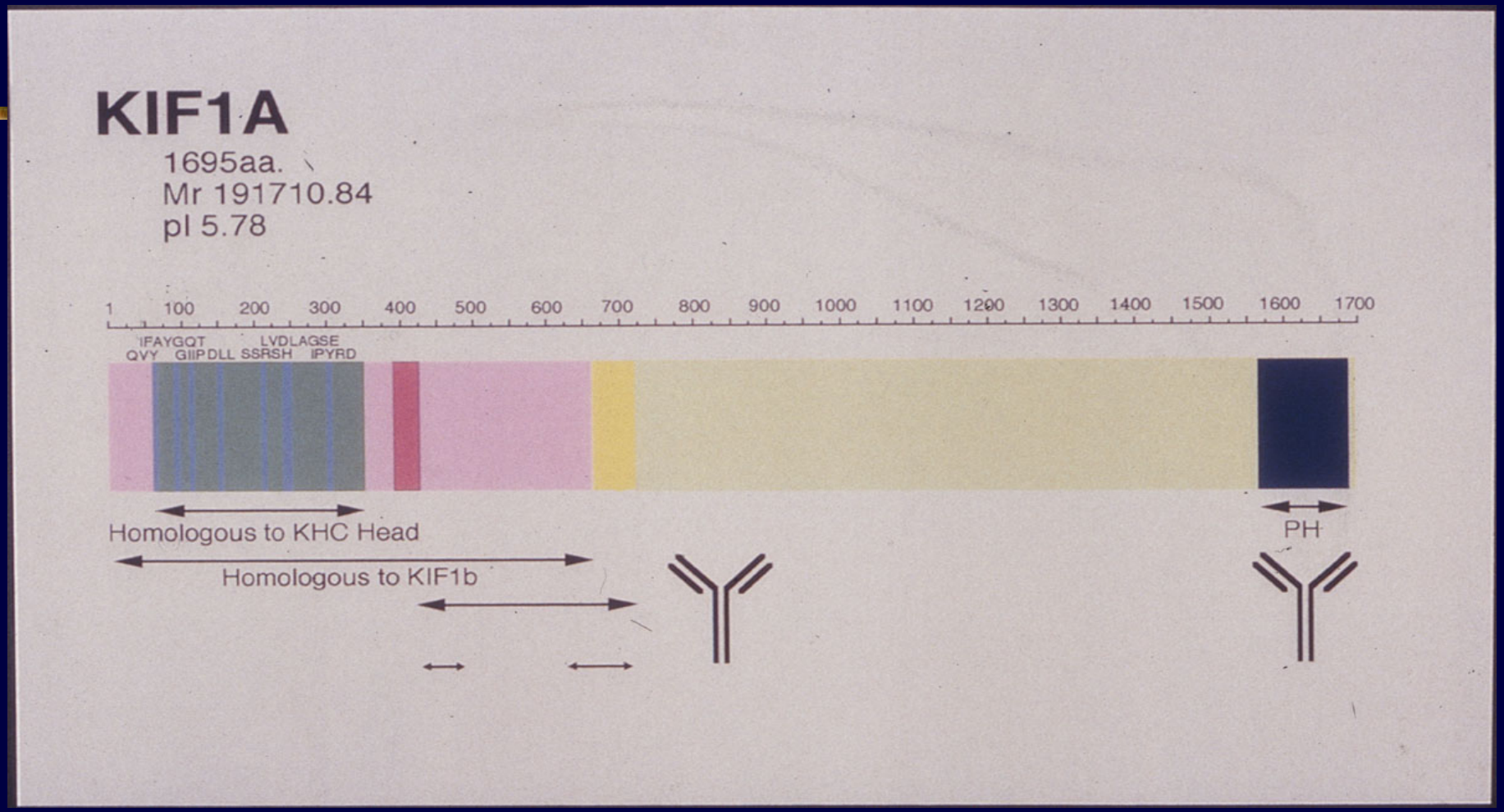
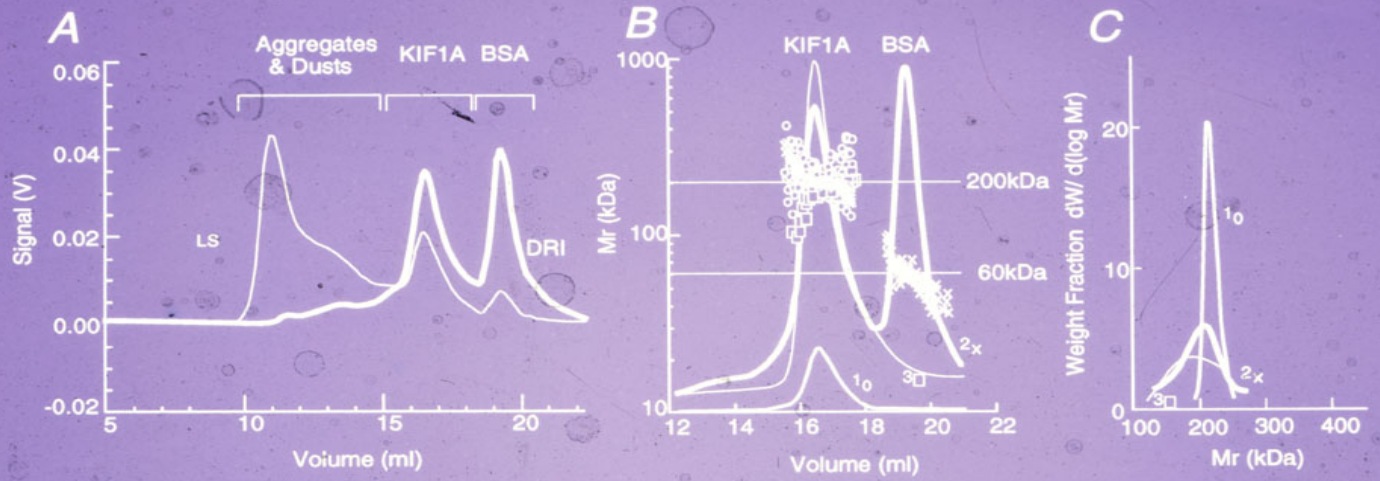
Microtubule
KIF5
KIF5
KIF5/KIF1Bα
KIF3
KIF1A/KIF1Bβ
c
AMPA receptor
GRIP1
RNA-containing granule
NMDA receptor
LIN7
LIN2
LIN10
Microtubule
KIF5
KIF5
KIF17



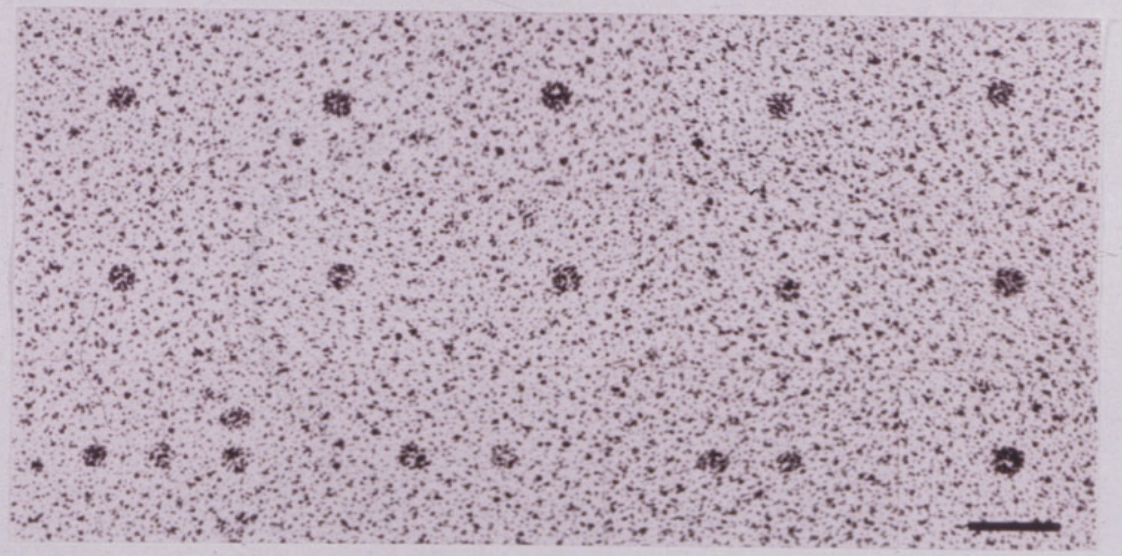
Aizawa et al. JCB 119:1287－, 1992
Okada et al. Cell 81:769－, 1995

Molecular Weight Determination by GPC-DLS

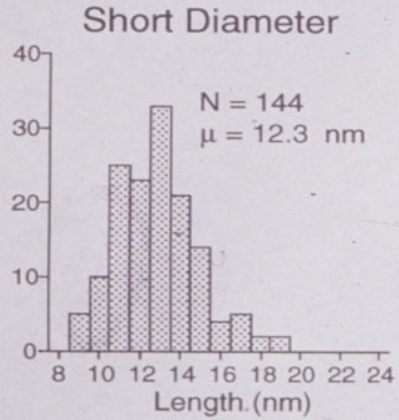


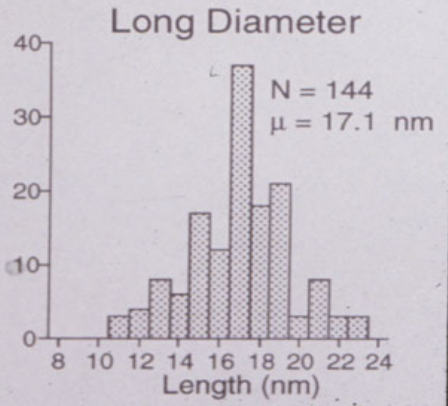
Molecular weight (Mr) of recombinant KIF1A protein was determined by gel permeation chromatography-differential laser light scattering (GPC-DLS), because this method absolutely determines Mr of polymer unbiased by its shape or other physical or chemical properties. *A* shows typical light scattering 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

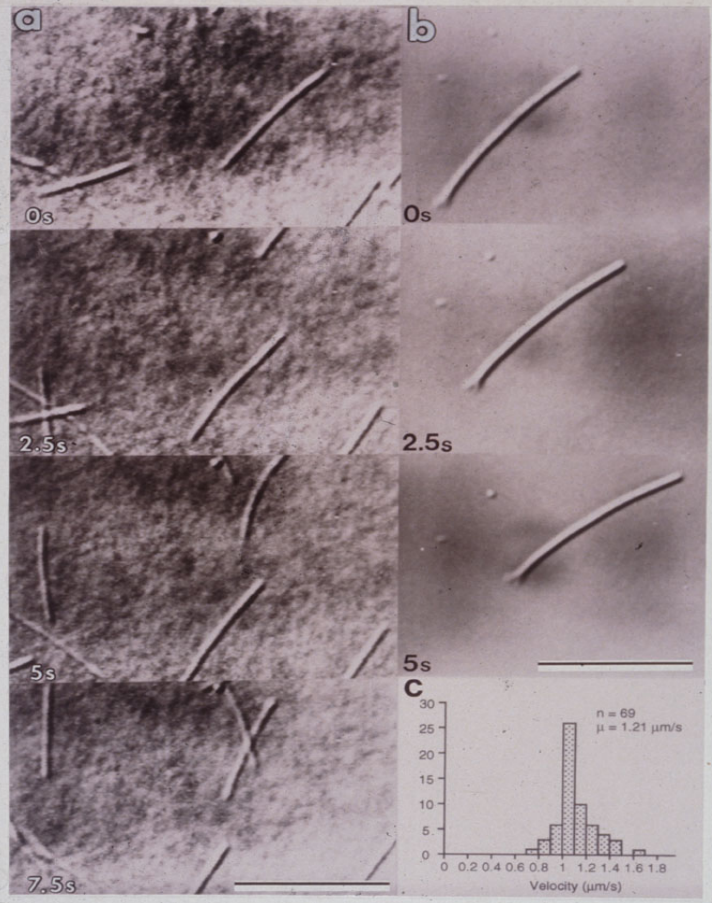


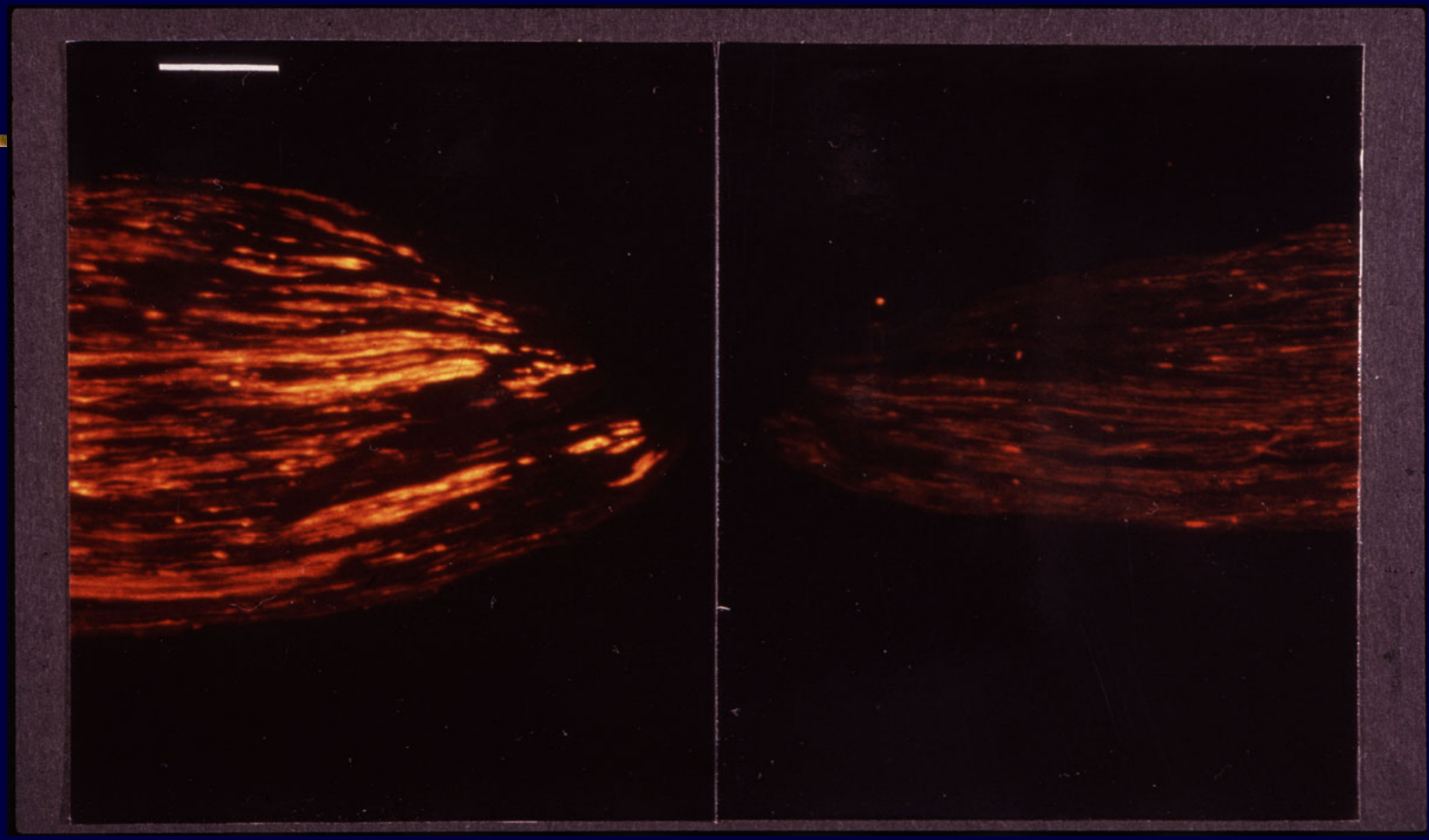


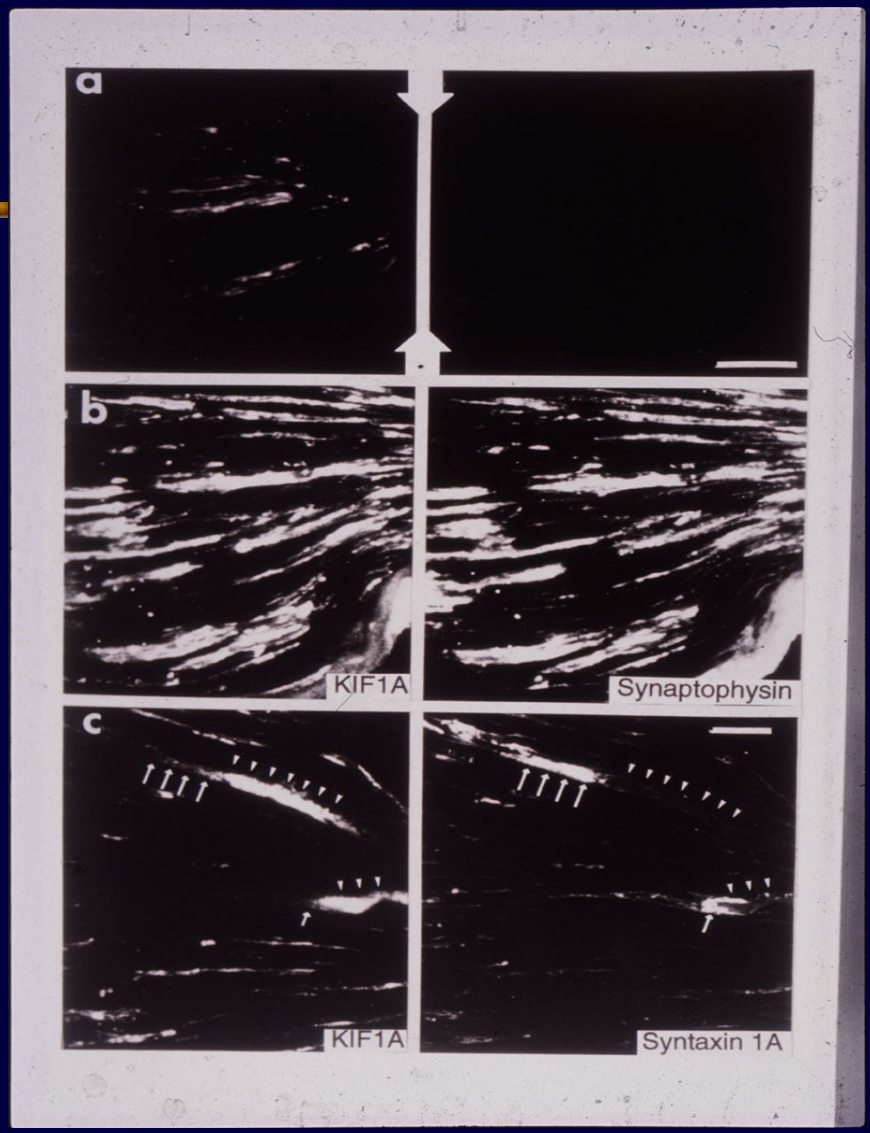
Unlike other KRMPs, KIF1A was a globular molecule. No clearly discernable tails were observed.,

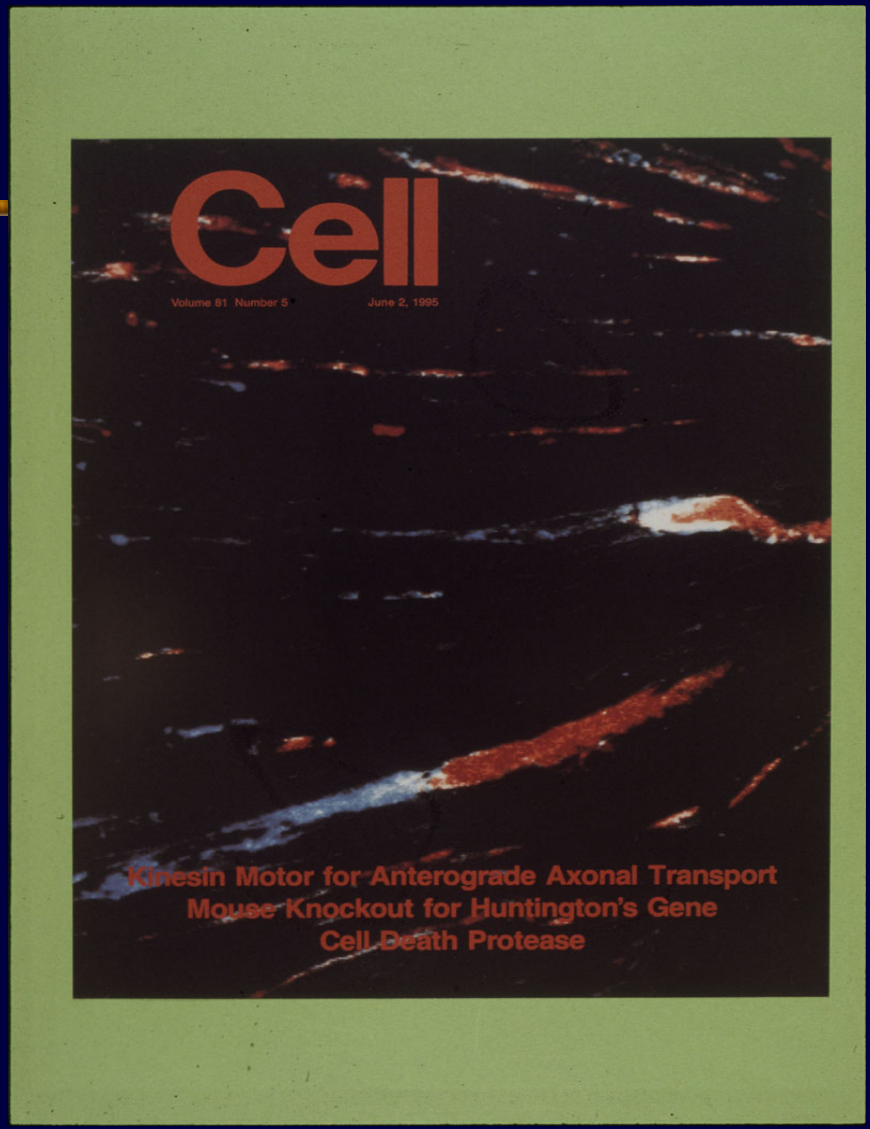
In vitro Motility Assay of Recombinant KIF1A

(~100 KIF1A monomers / μm^2)

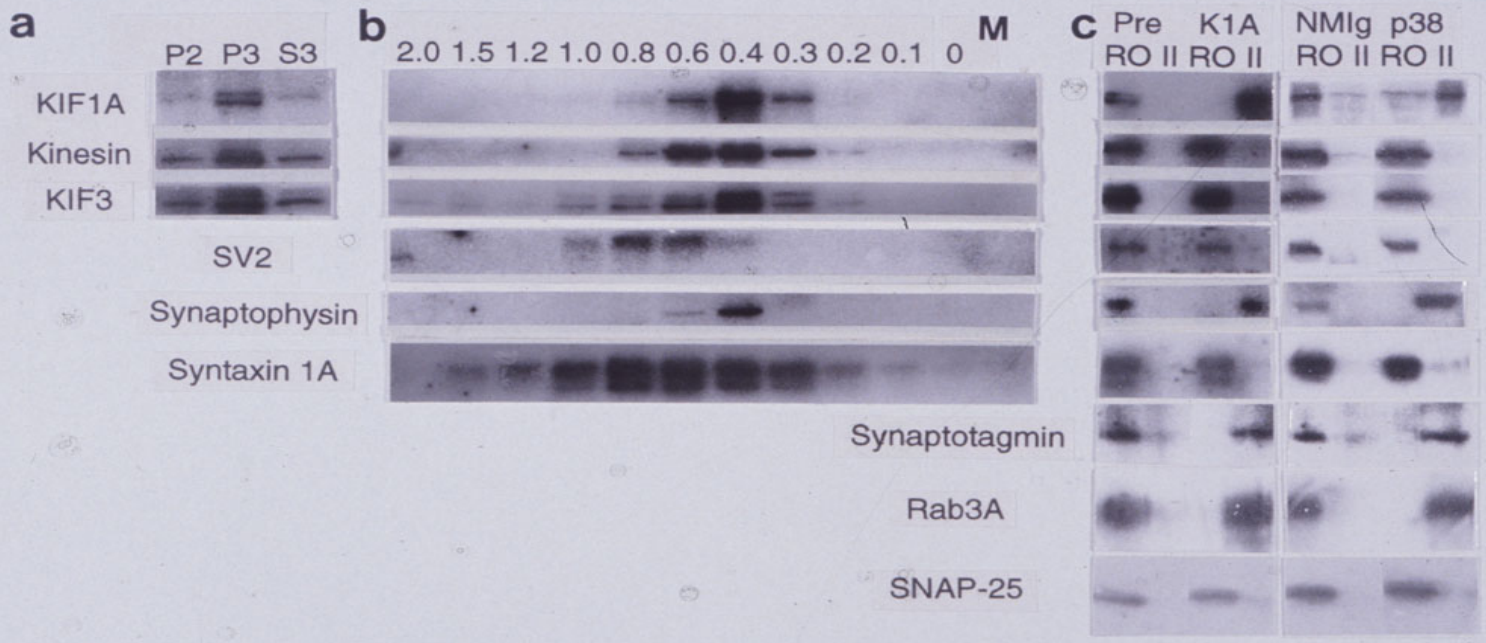


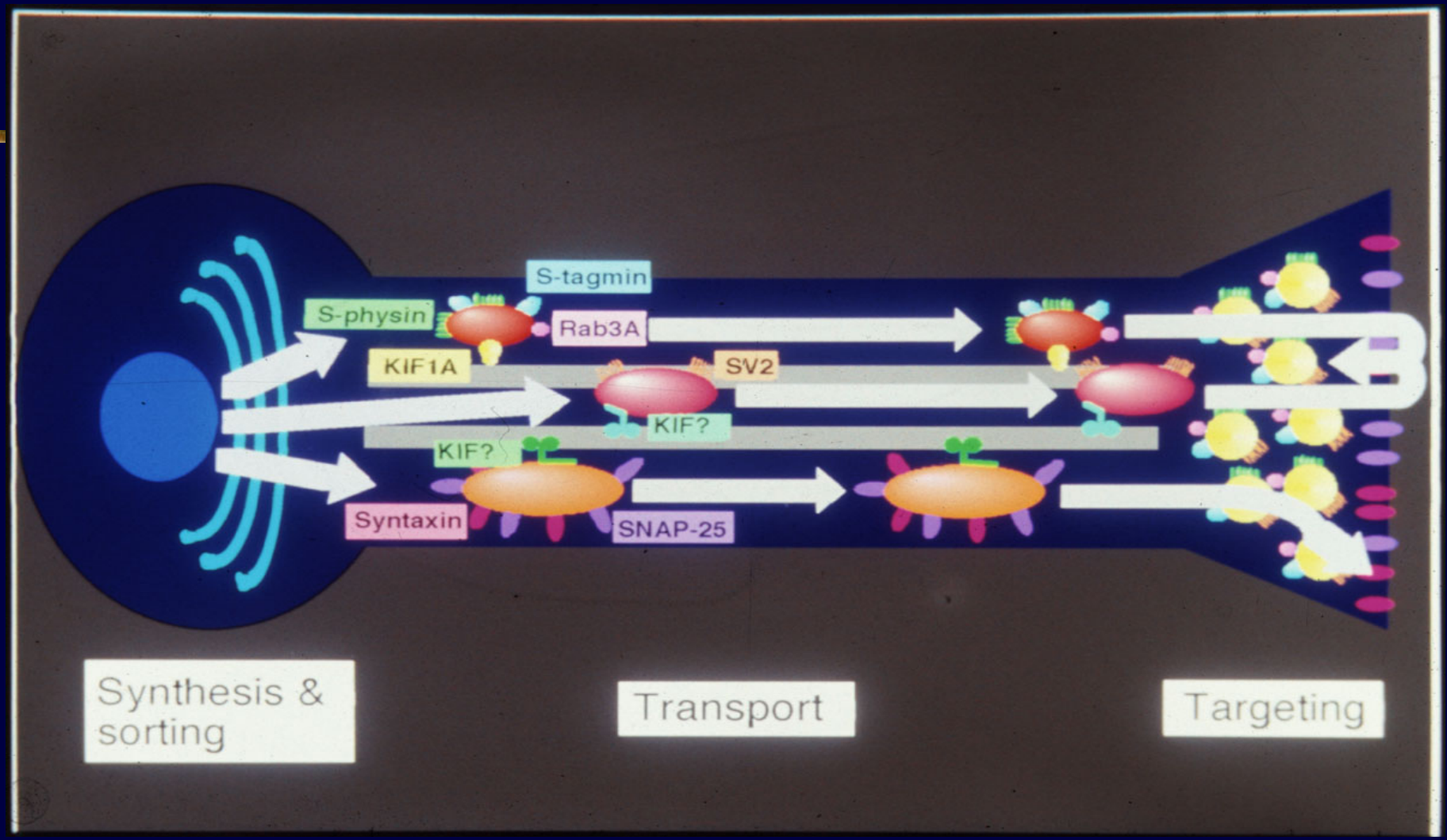


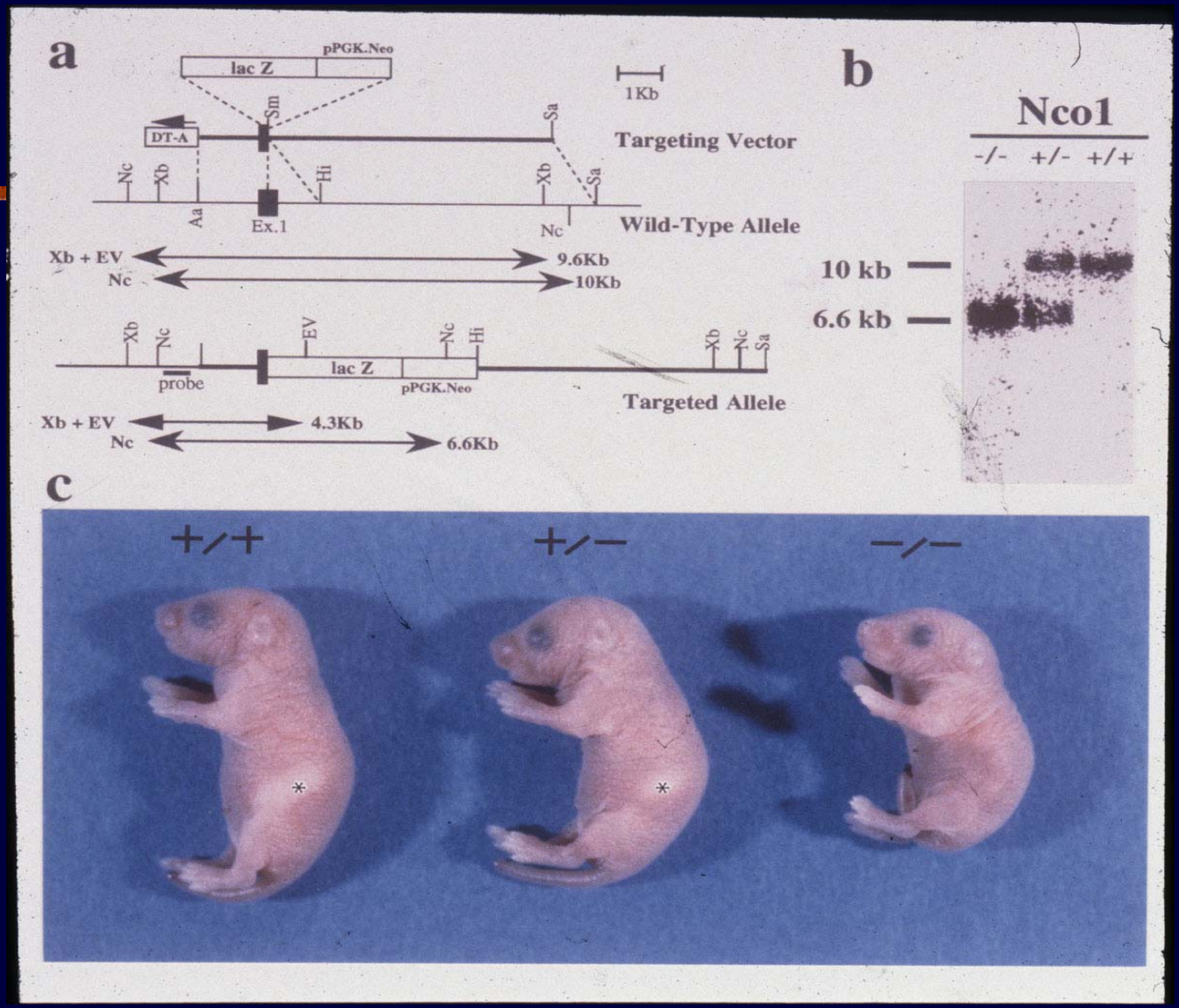

a
b
KIF1A
Synaptophysin
c
KIF1A
Syntaxin 1A



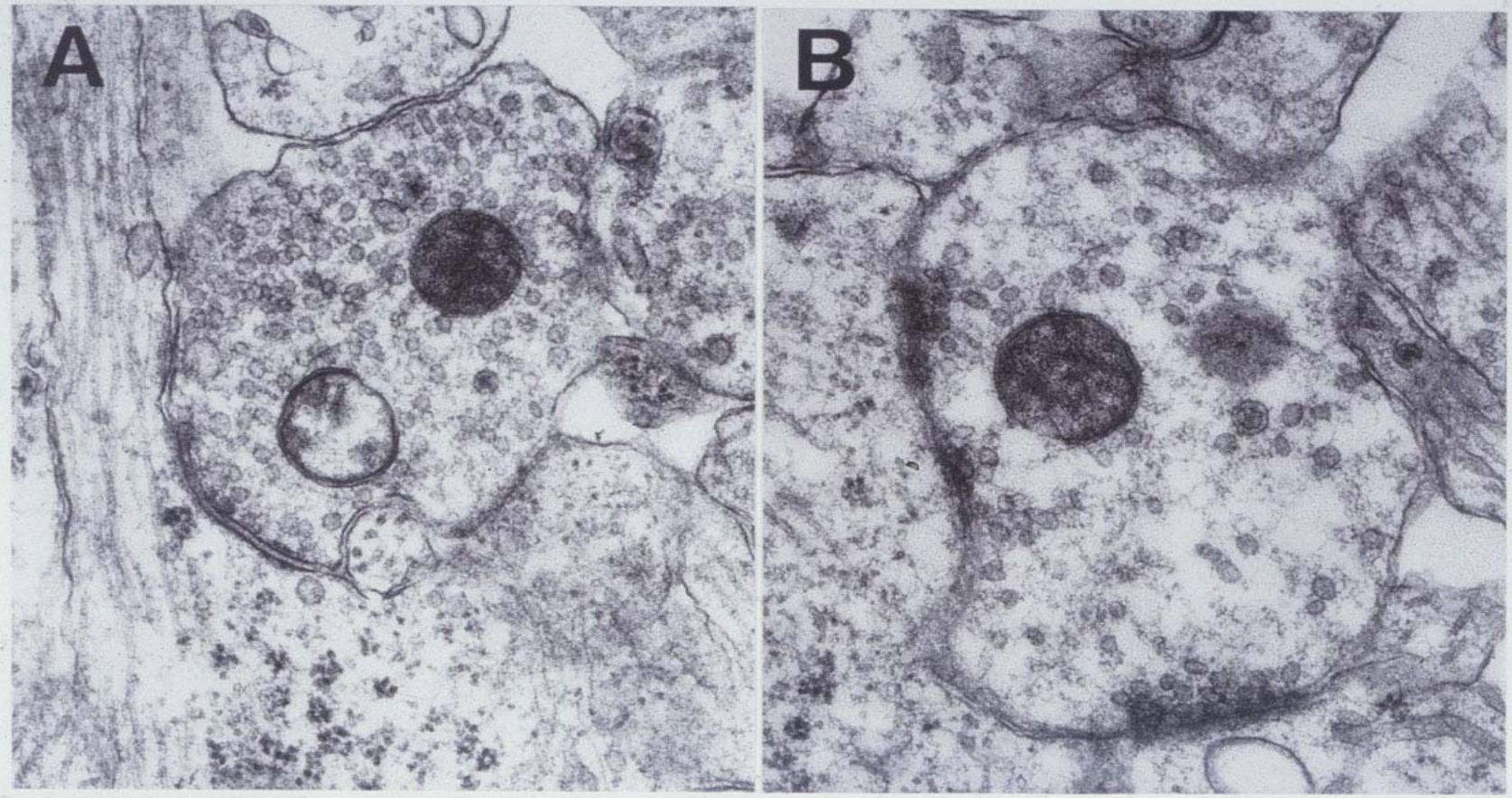
Okada et al. Cell 81:769-780, 1995

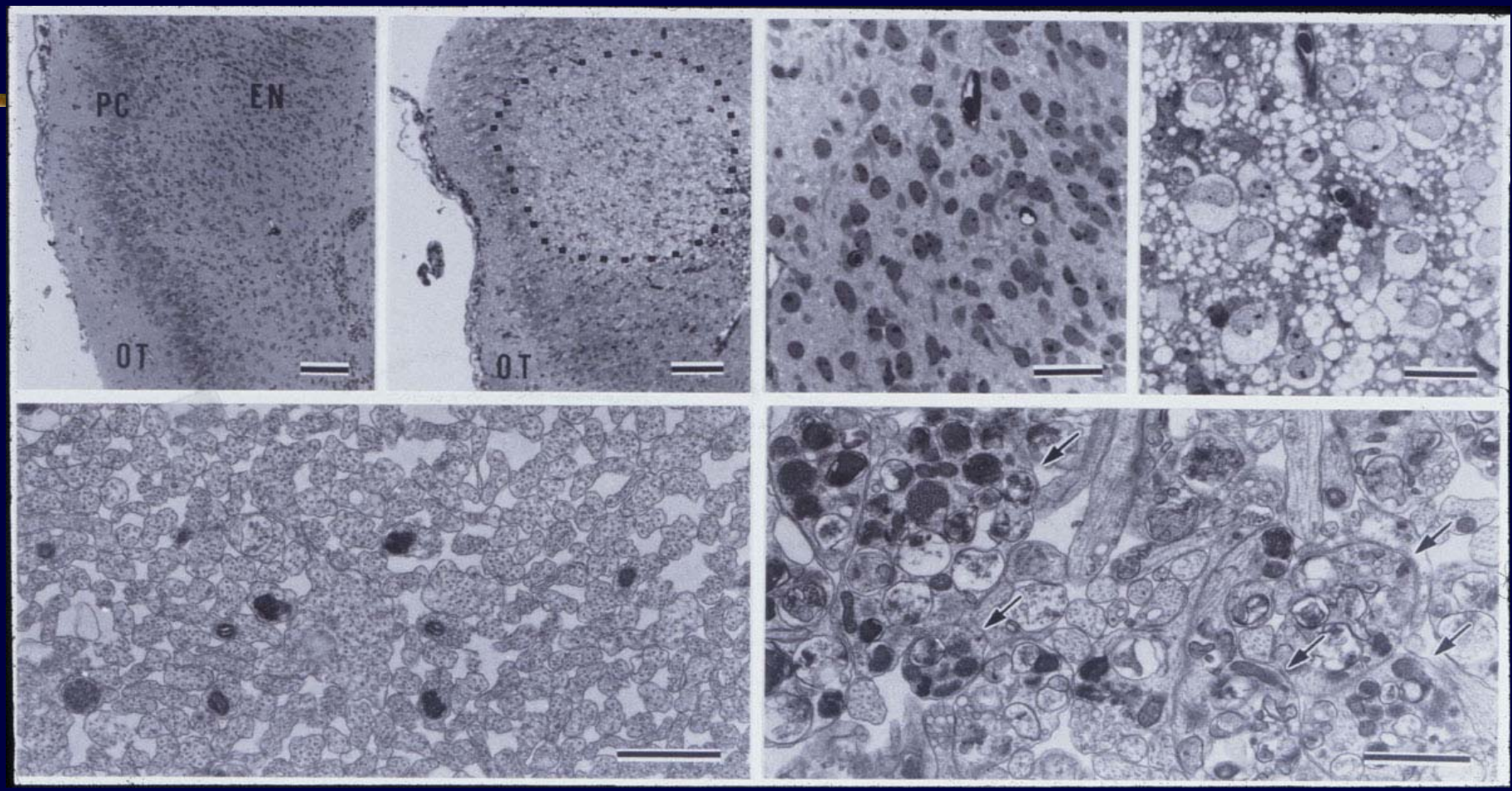

a
P2 P3 S3
KIF1A
Kinesin
KIF3
SV2
Synaptophysin
Syntaxin 1A
b
2.0 1.5 1.2 1.0 0.8 0.6 0.4 0.3 0.2 0.1 0
M
c
Pre K1A NMIg p38
RO II RO II RO II RO II
Synaptotagmin
Rab3A
SNAP-25

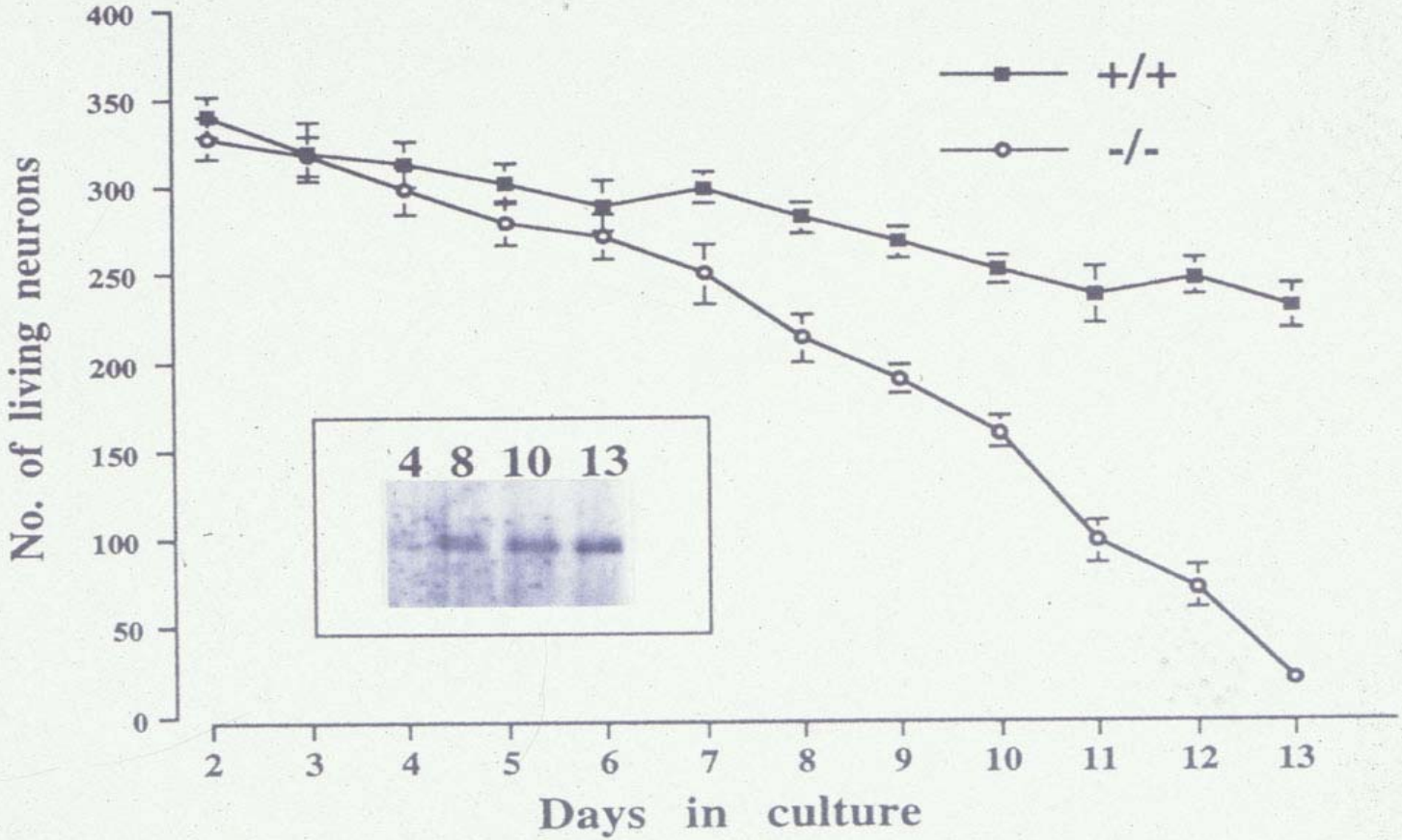

S-tagmin
S-physin
Rab3A
KIF1A
SV2
KIF?
KIF?
Syntaxin
SNAP-25
Synthesis & sorting
Transport
Targeting

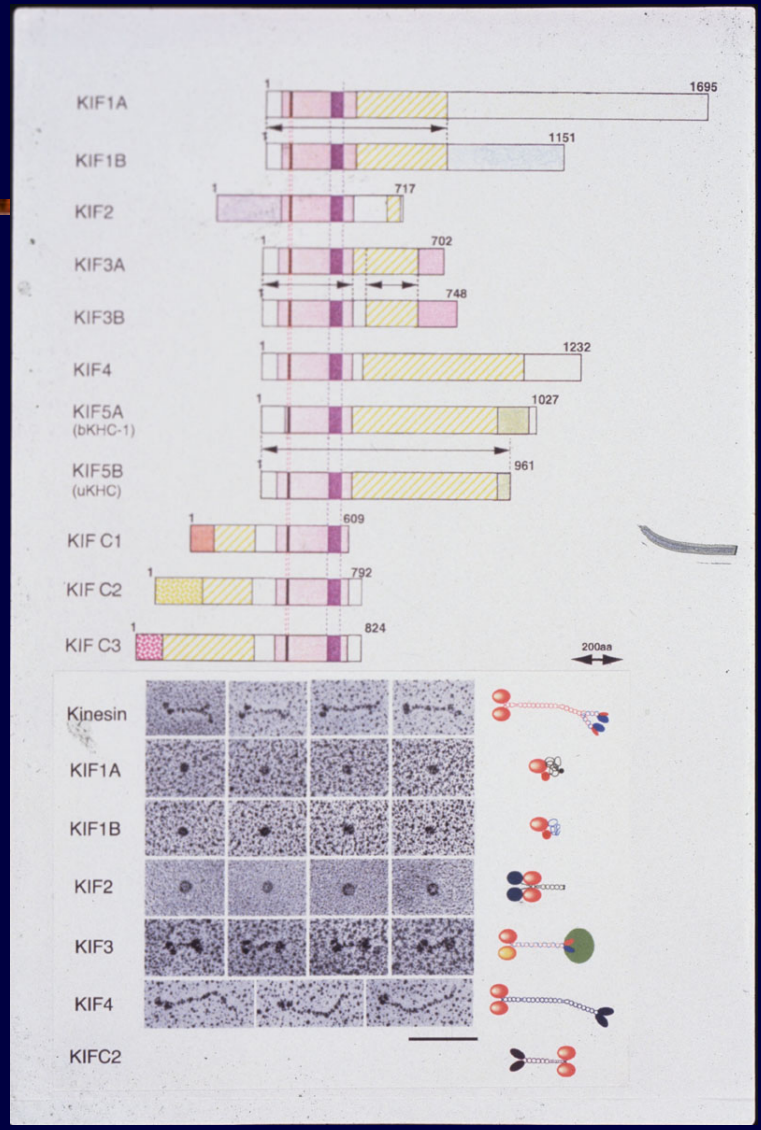


Yonekawa et al. JCB 141:431-, 1998

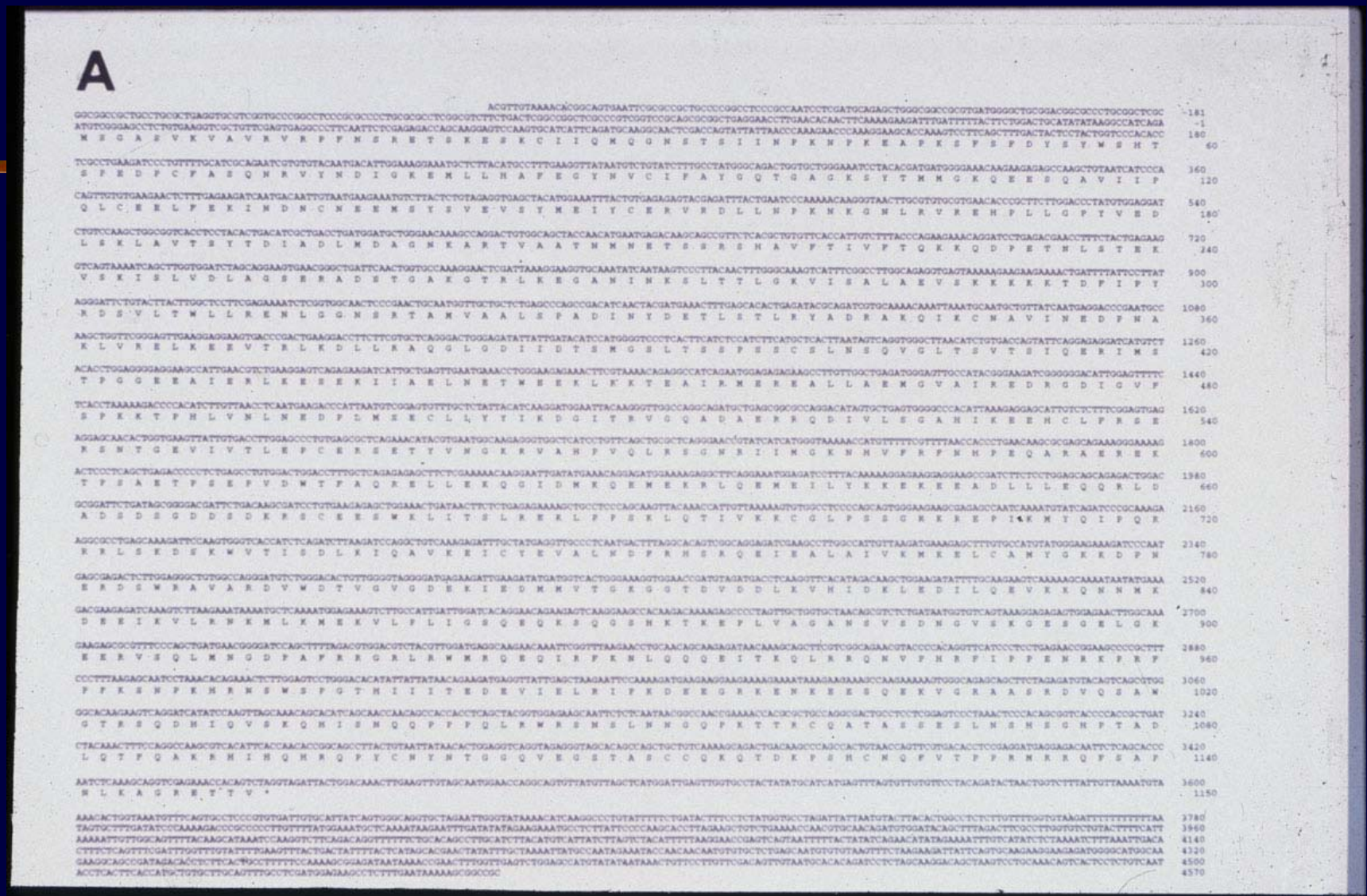

A
B

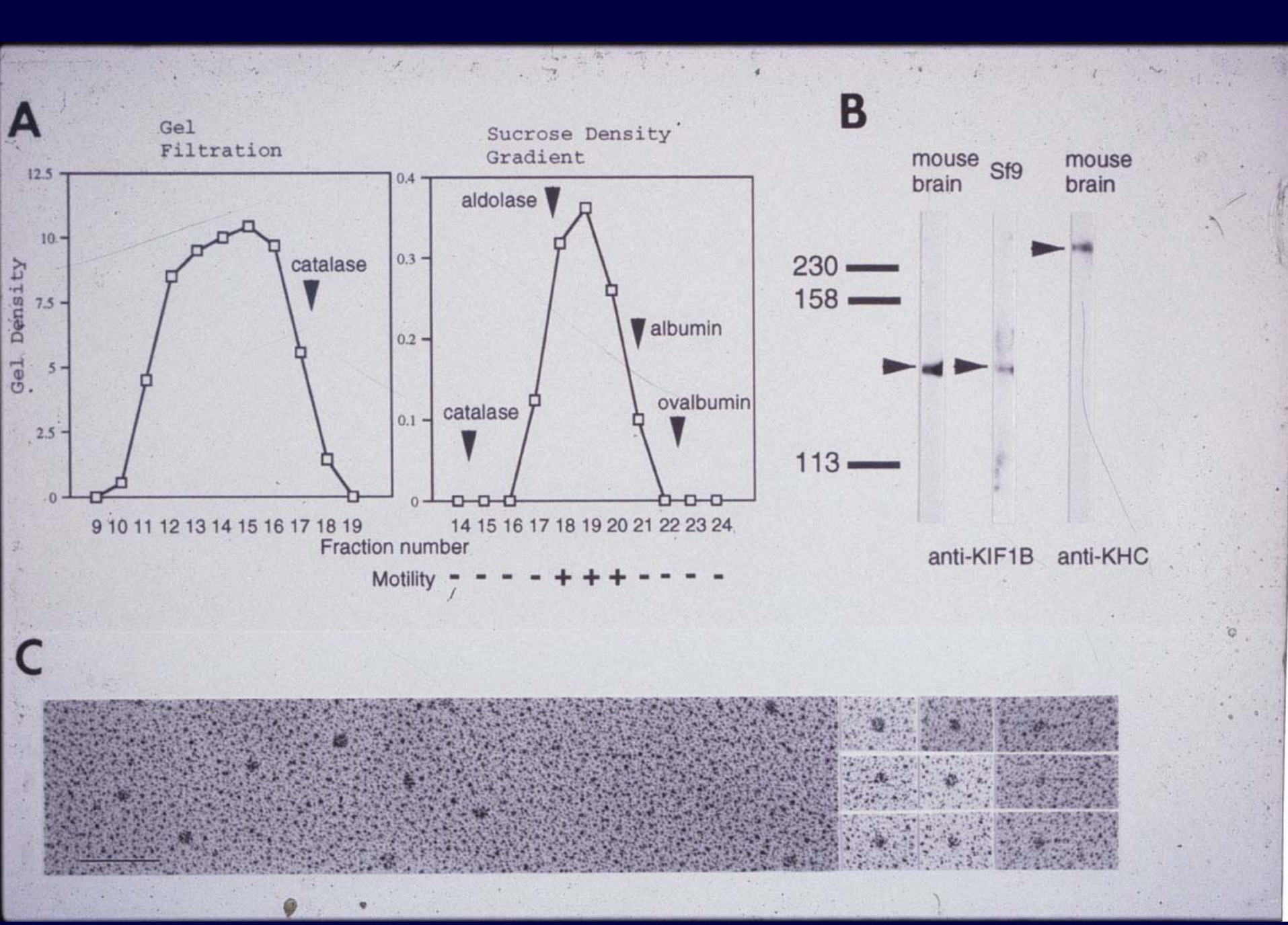

PC
EN
OT
OT


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

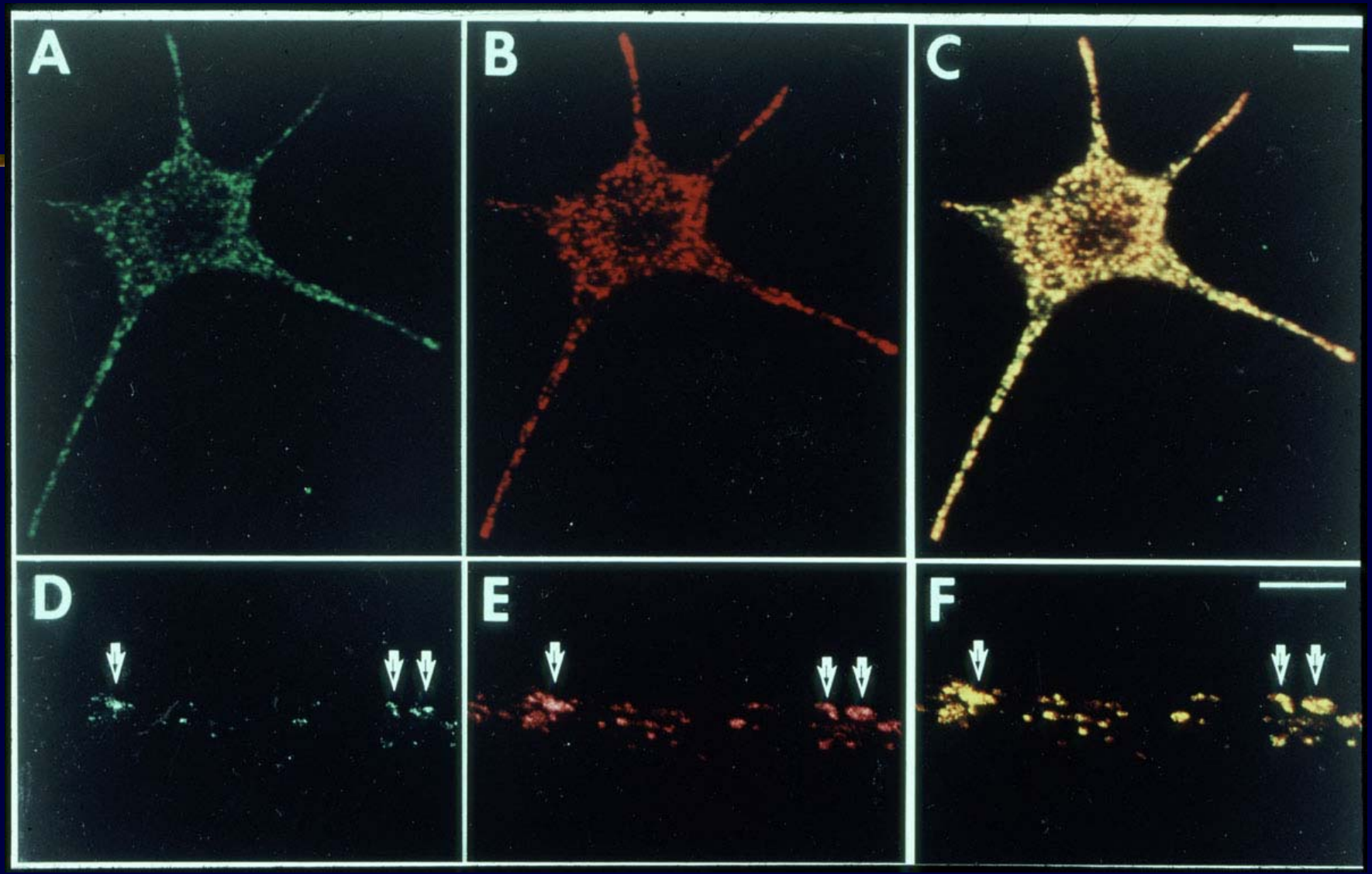


Nangaku, M. et al. Cell 79: 1209- , 1994

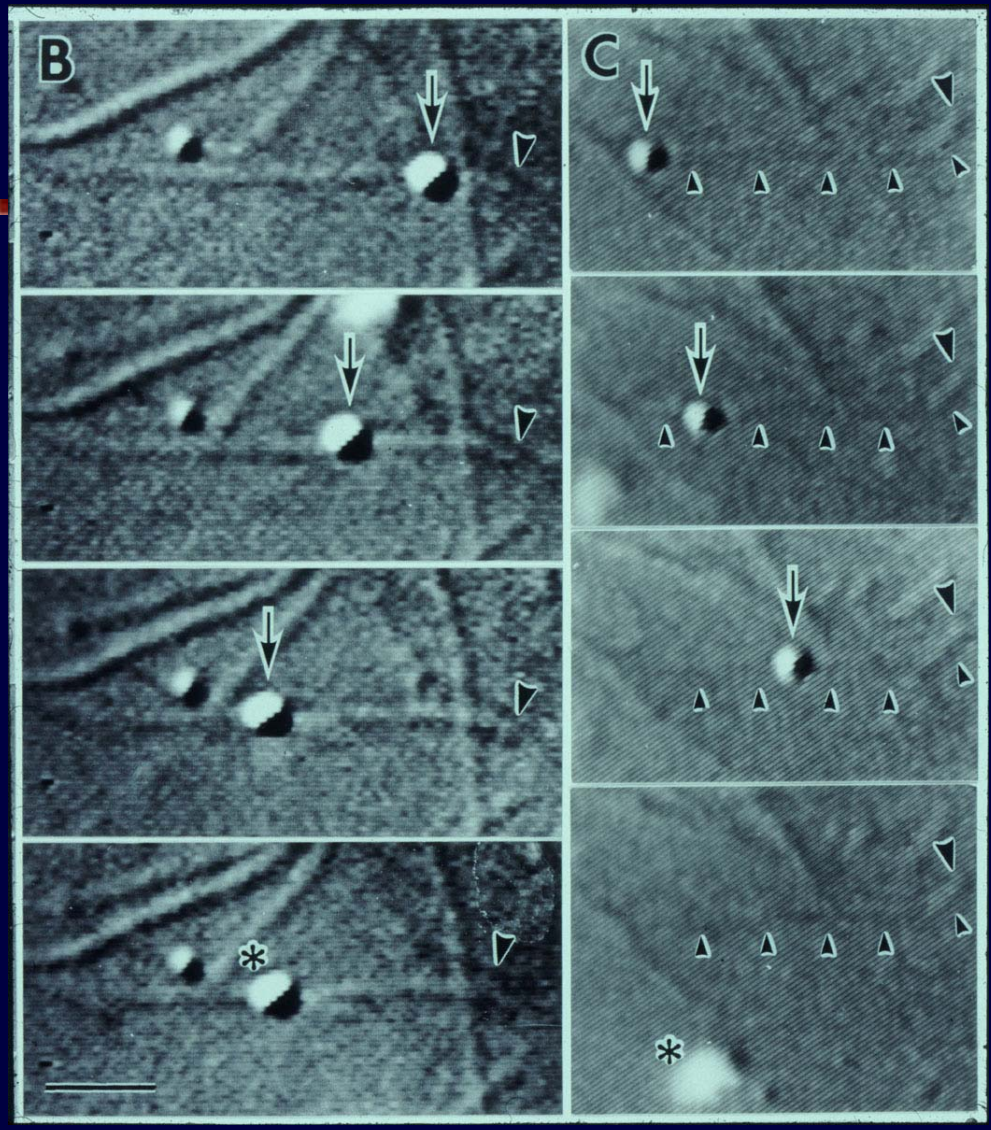

A

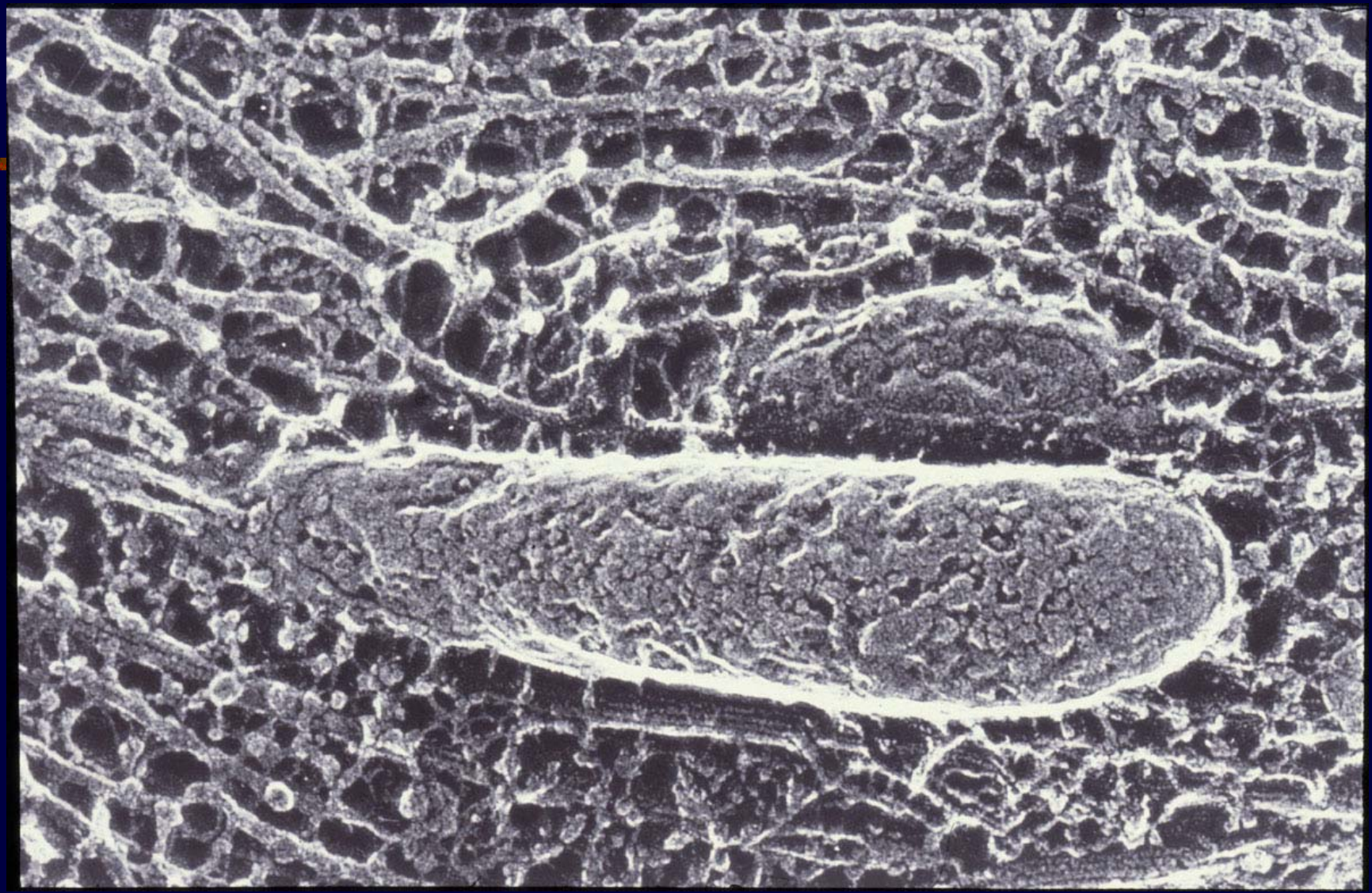

A
Gel Filtration
Sucrose Density Gradient
Gel Density
12.5
10
7.5
5
2.5
0
catalase
0.4
0.3
0.2
0.1
0
aldolase
albumin
catalase
ovalbumin
9 10 11 12 13 14 15 16 17 18 19
14 15 16 17 18 19 20 21 22 23 24
Fraction number
Motility - - - - + + + - - - -
B
mouse brain
Sf9
mouse brain
230
158
113
anti-KIF1B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

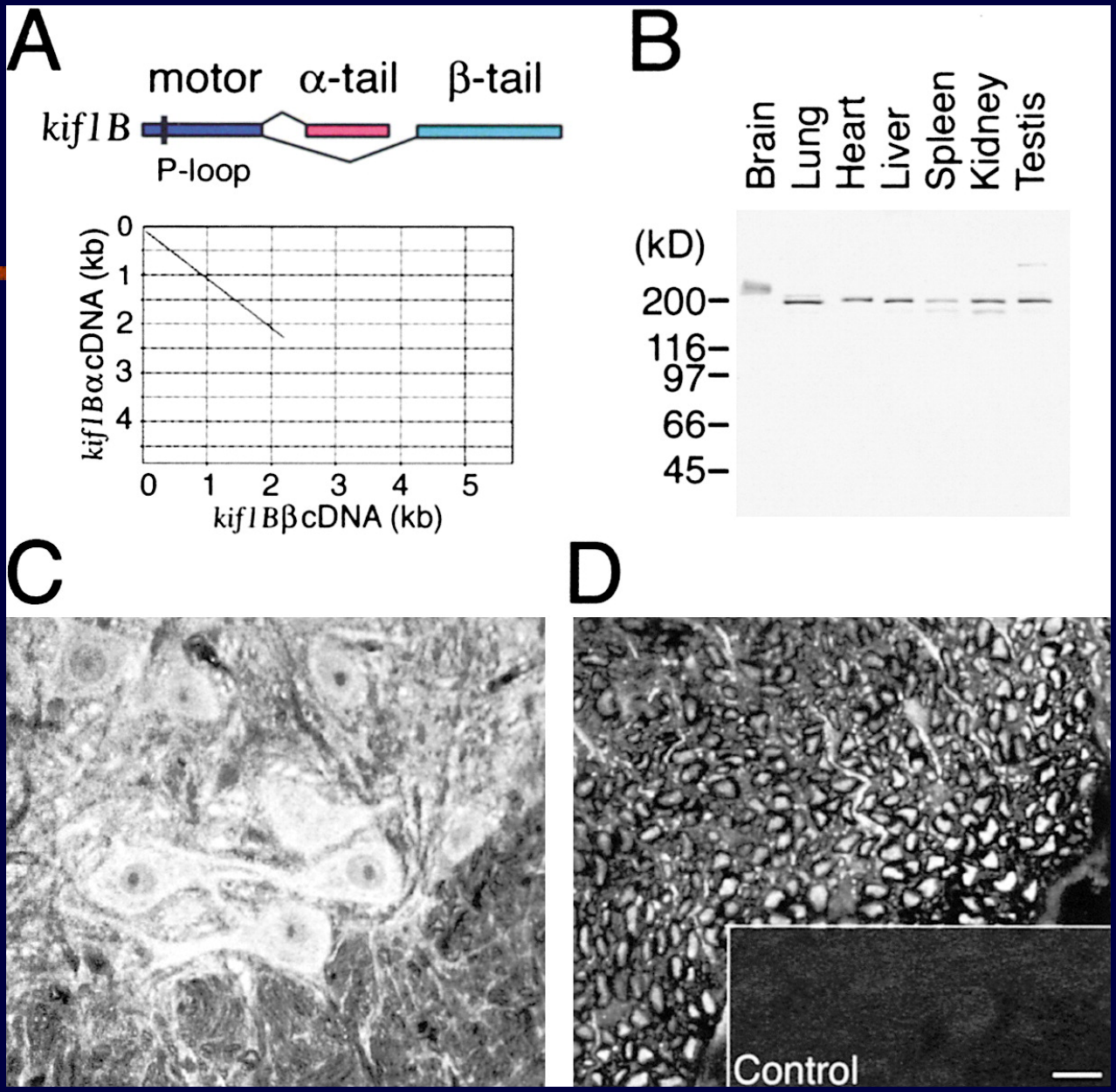

A


A
B
C
D
E
F

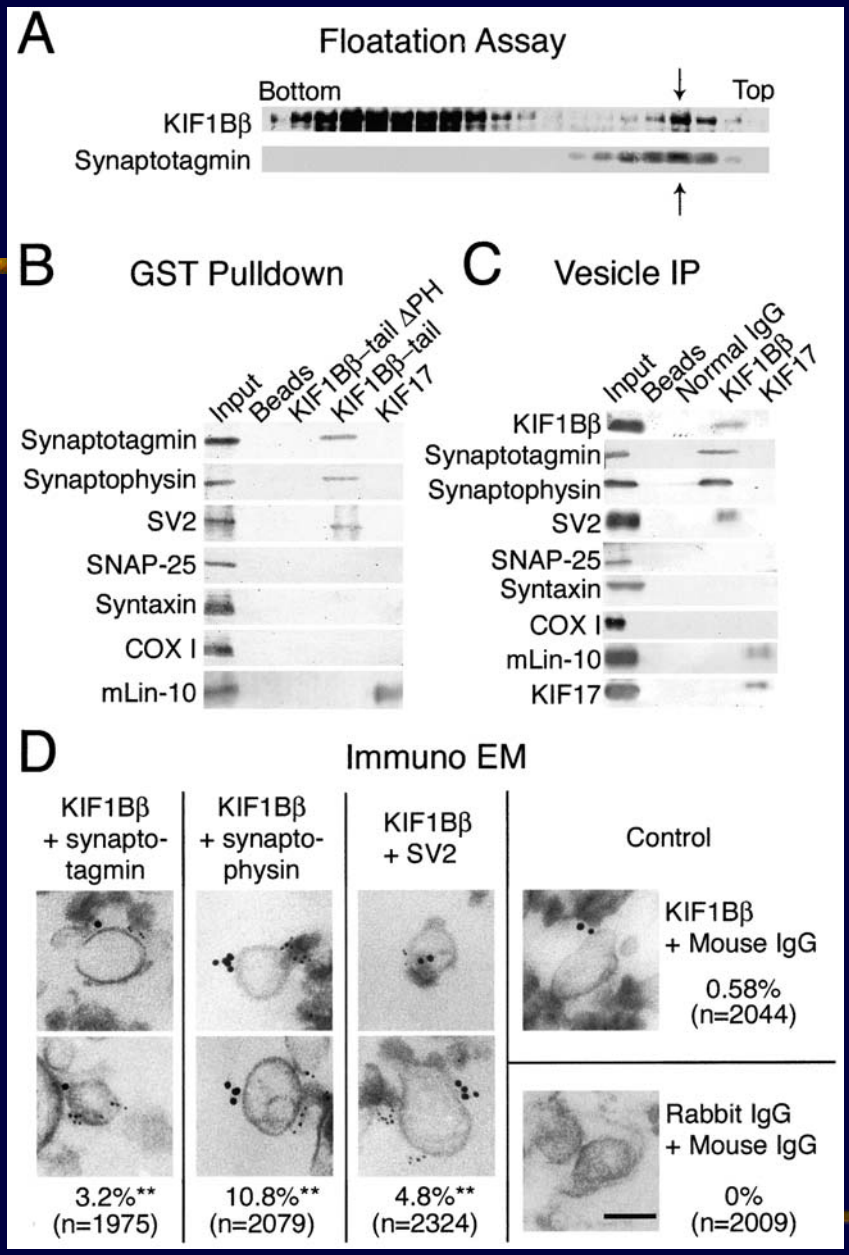

L
M
CE
S1
P1
S2
P2
P3
KIF1B
COX

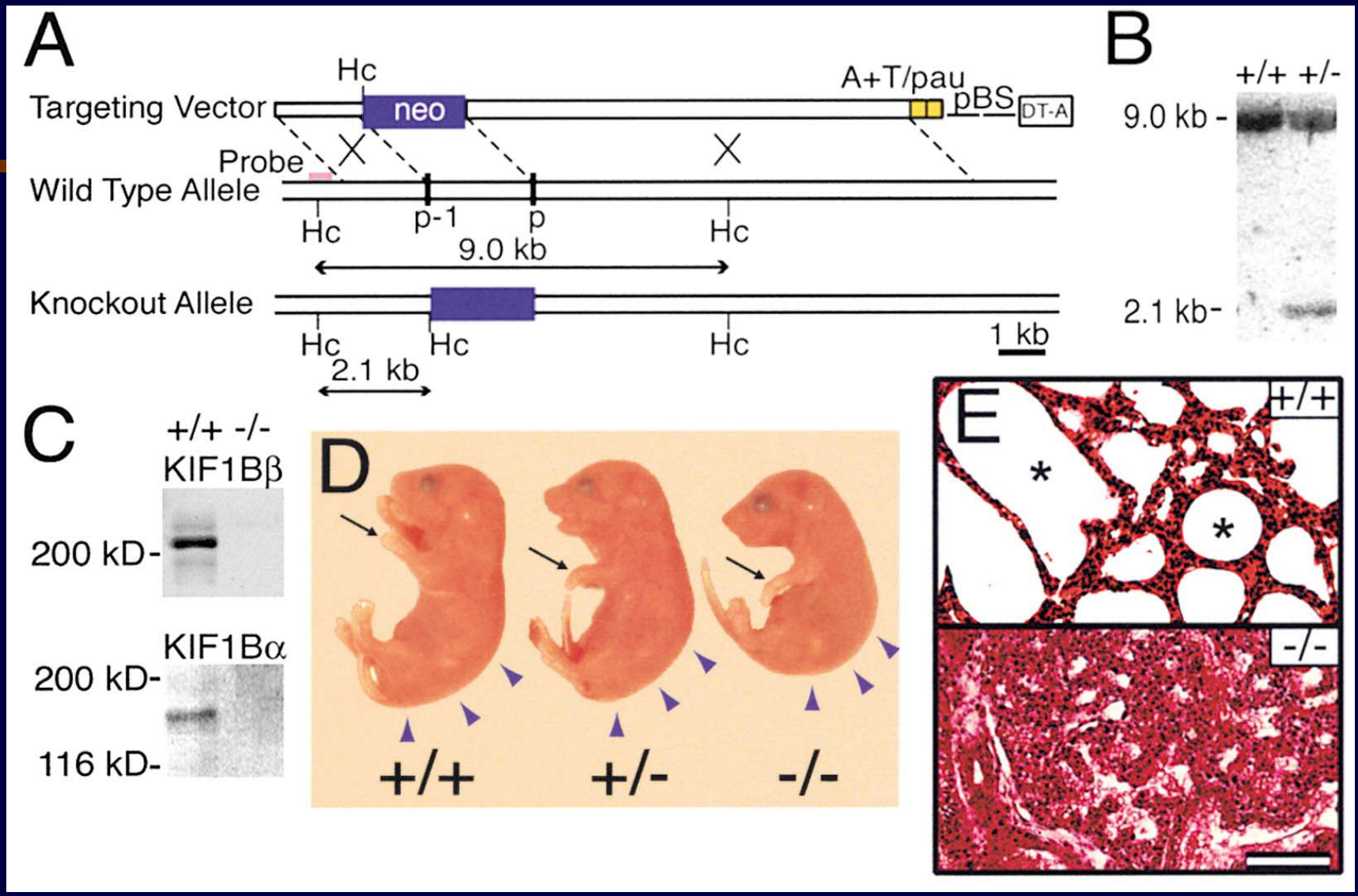

B
C

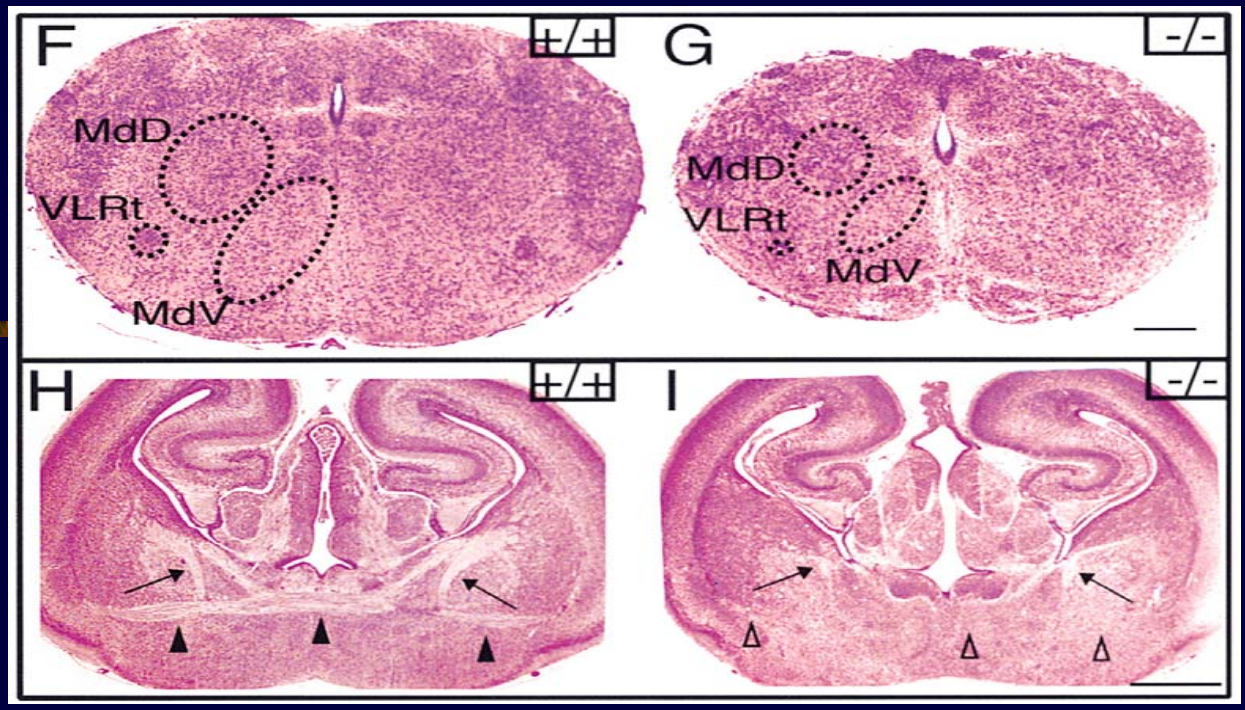


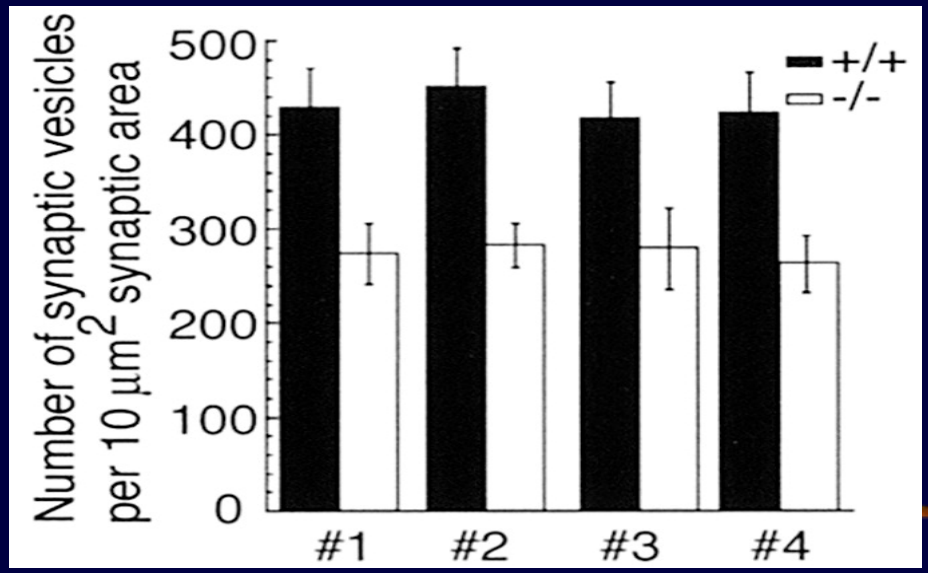


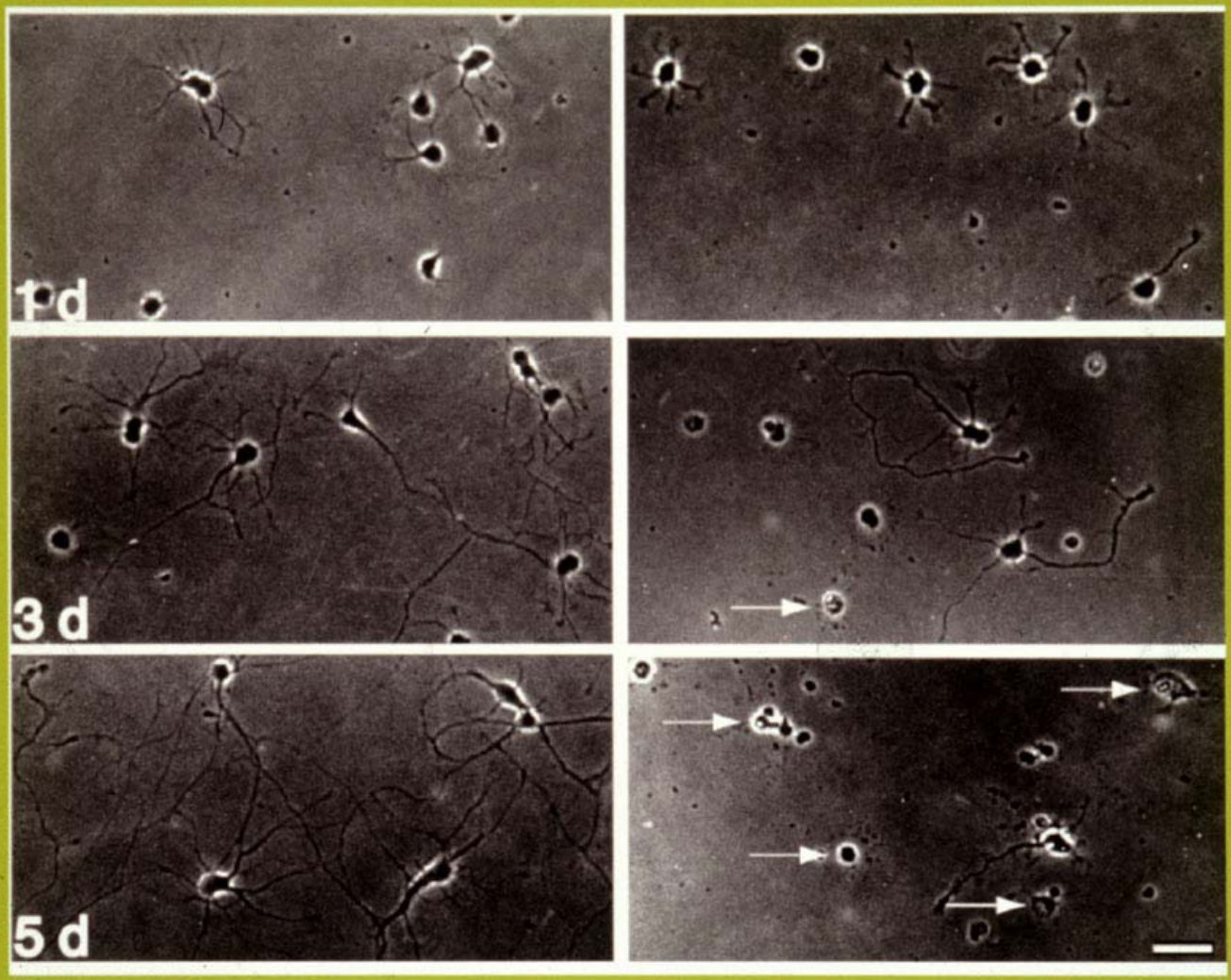
Zhao et al. Cell 105:587-, 2001

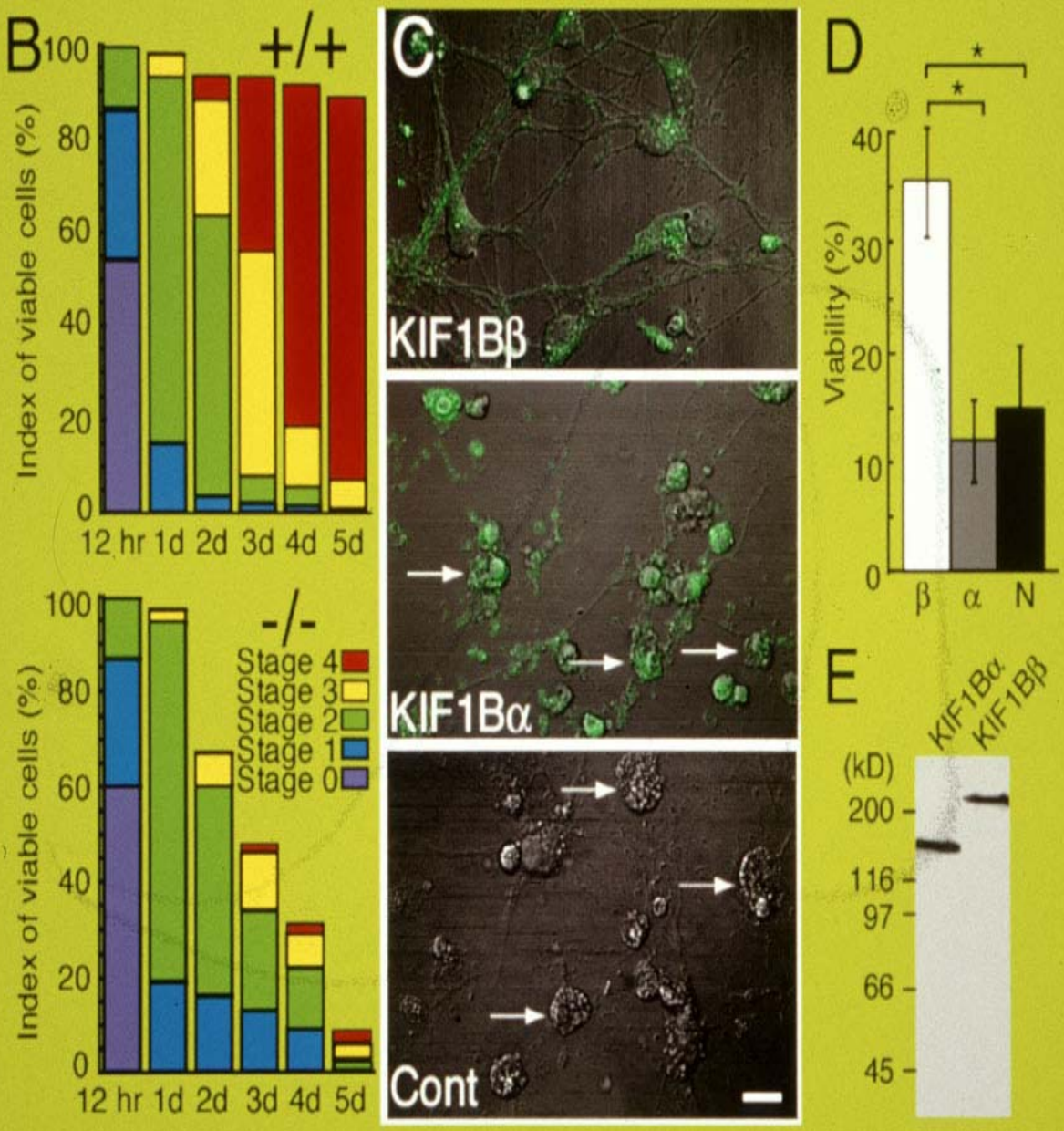

A
Floatation Assay
Bottom
Top
KIF1Bβ
Synaptotagmin
B
GST Pulldown
Input
Beads
KIF1Bβ–tail ΔPH
KIF1Bβ–tail
KIF17
Synaptotagmin
Synaptophysin
SV2
SNAP-25
Syntaxin
COX I
mLin-10
C
Vesicle IP
Input
Beads
Normal IgG
KIF1Bβ
KIF17
KIF1Bβ
Synaptotagmin
Synaptophysin
SV2
SNAP-25
Syntaxin
COX I
mLin-10
KIF17
D
Immuno EM
KIF1Bβ + synapto-tagmin
KIF1Bβ + synapto-physin
KIF1Bβ + SV2
Control
KIF1Bβ + Mouse IgG
0.58% (n=2044)
Rabbit IgG + Mouse IgG
0% (n=2009)
3.2%** (n=1975)
10.8%** (n=2079)
4.8%** (n=2324)


A
Hc
A+T/pau
Targeting Vector
neo
pBS
DT-A
Probe
Wild Type Allele
Hc
p-1
p
Hc
9.0 kb
Knockout Allele
Hc
Hc
Hc
1 kb
2.1 kb
B
+/+ +/-
9.0 kb -
2.1 kb-
C
+/+ -/-
KIF1Bβ
200 kD-
KIF1Bα
200 kD-
116 kD-
D
+/+
+/-
-/-
E
+/+
-/-

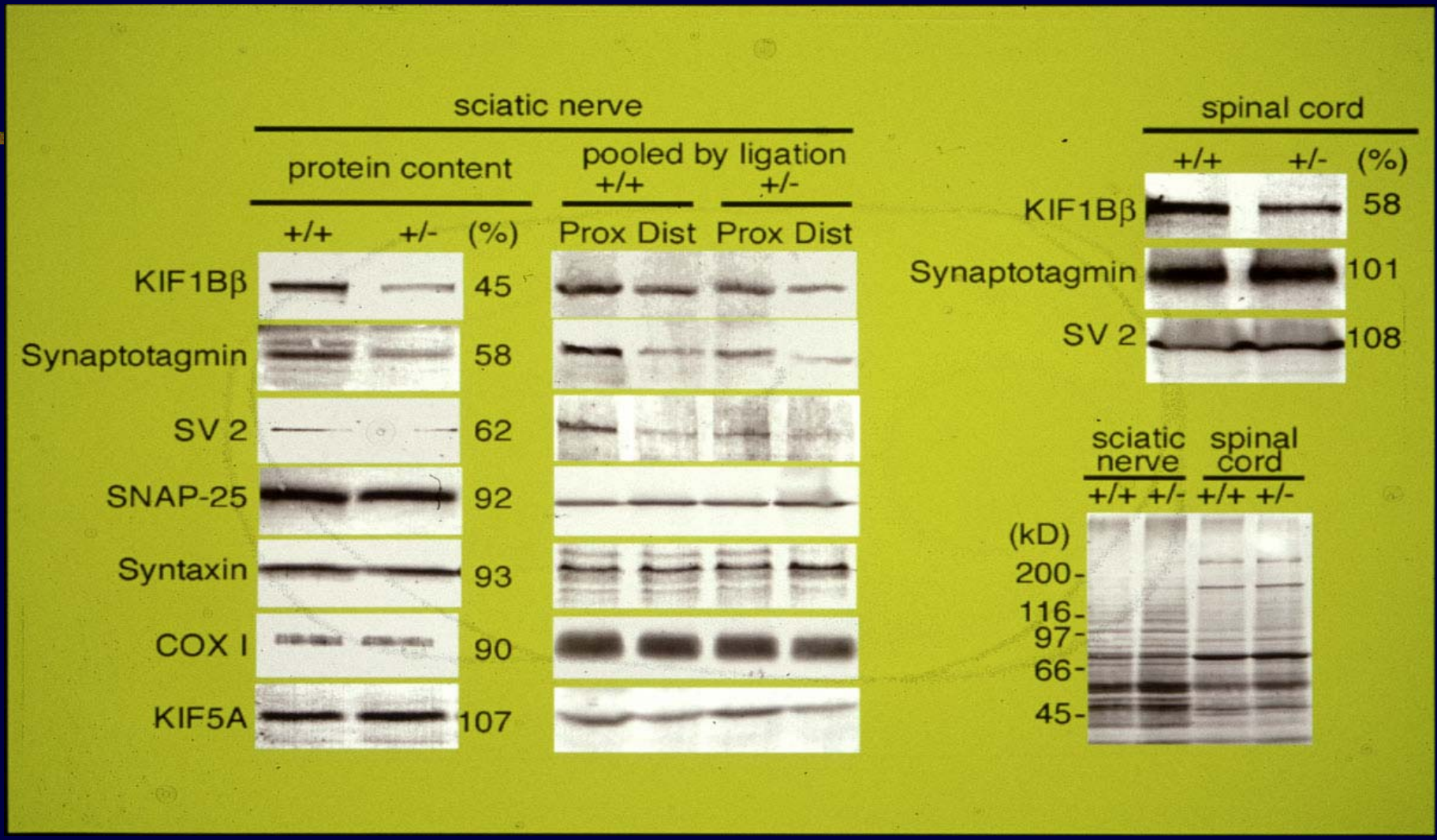

F
+/+
MdD
VLRt
MdV
G
-/-
MdD
VLRt
MdV
H
+/+
I
-/-

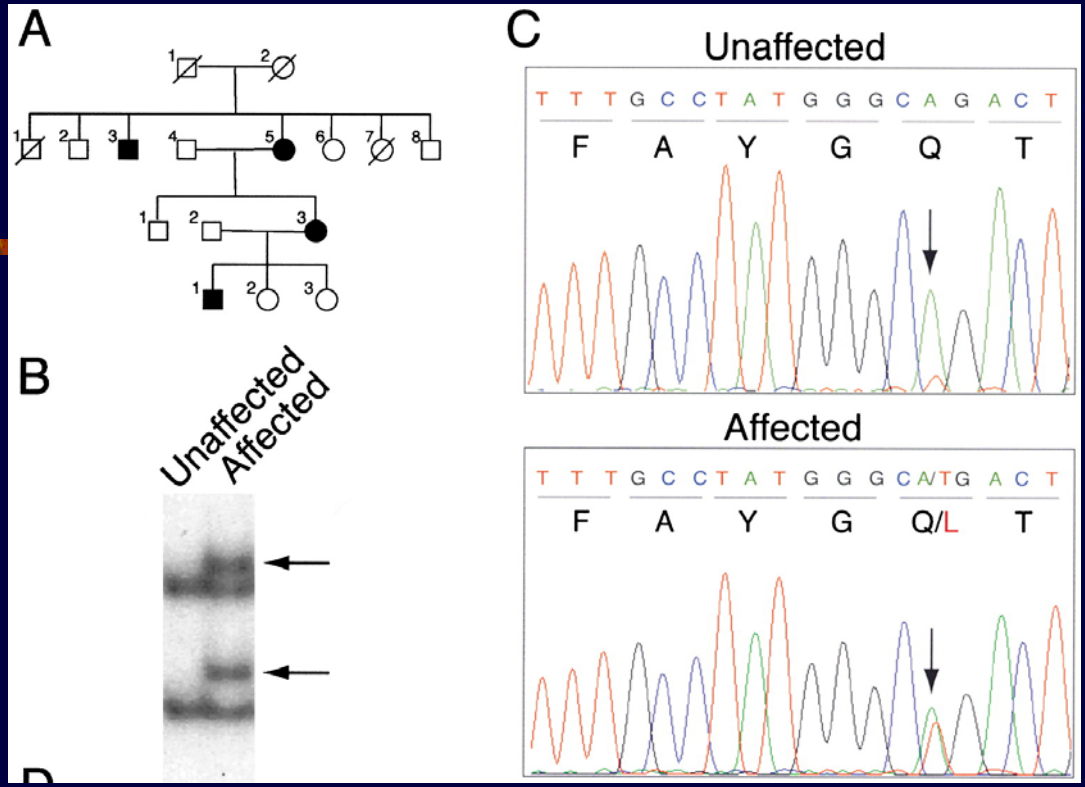

Number of synaptic vesicles per 10 μm2 synaptic area
500
400
300
200
100
0
+/+
-/-
#1
#2
#3
#4

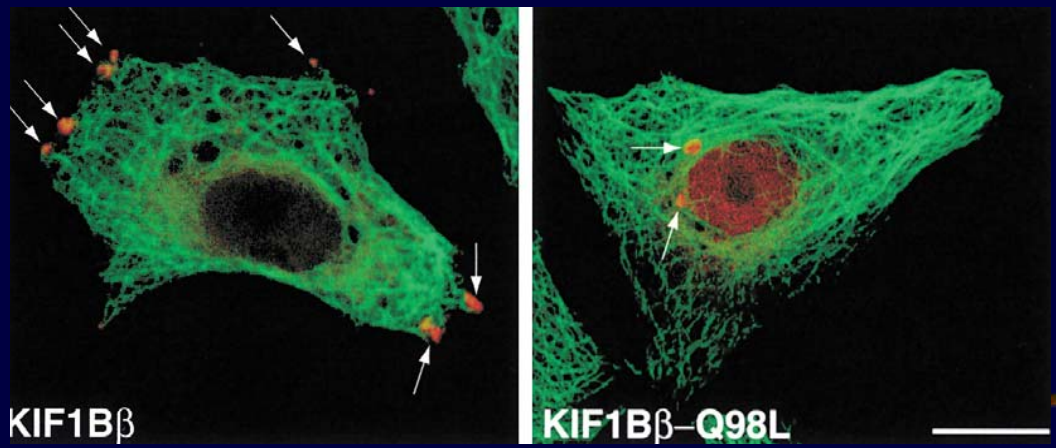

+/+
–/–
1 d
3 d
5 d

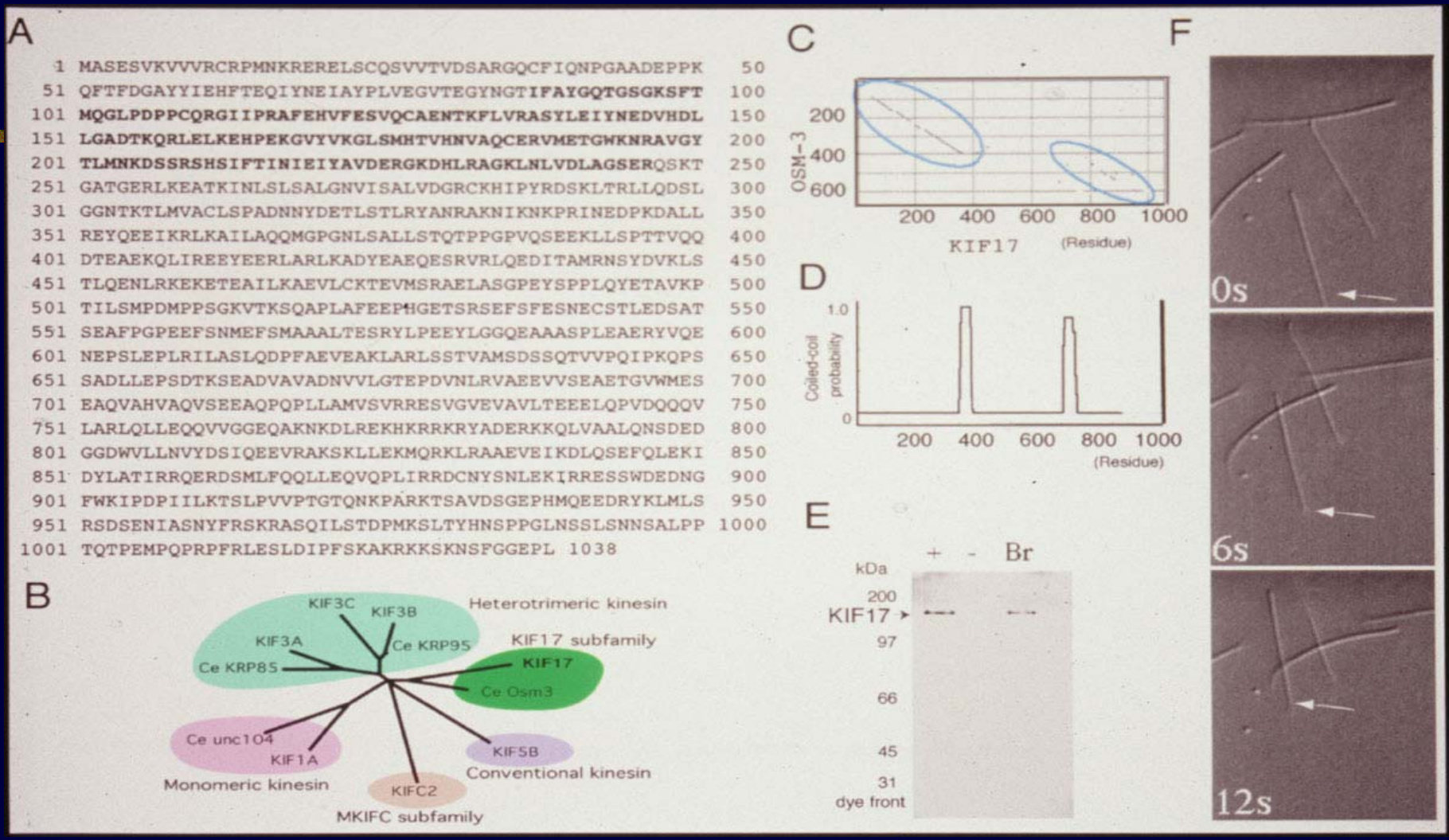

B
+/+
Index of viable cells (%)
100
80
60
40
20
0
12 hr 1d 2d 3d 4d 5d
-/-
Stage 4
Stage 3
Stage 2
Stage 1
Stage 0
C
KIF1Bβ
KIF1Bα
Cont
D
Viability (%)
40
30
20
10
0
β α N
E
KIF1Bα
KIF1Bβ
(kD)
200
116
97
66
45


Cell
+/+
+/−
Kinesin in Peripheral Neuropathy

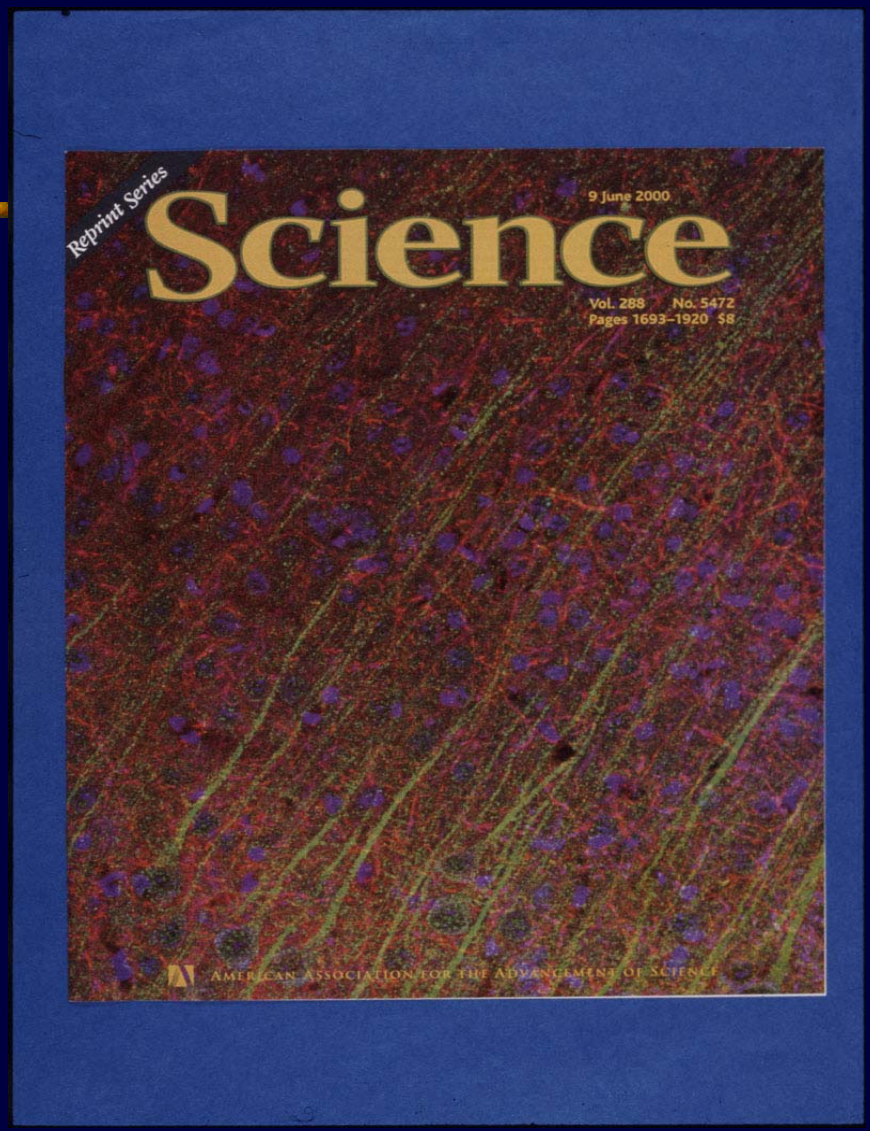

sciatic nerve
protein content
pooled by ligation
+/+
+/-
+/+
+/-
(%)
Prox Dist
Prox Dist
KIF1Bβ
45
Synaptotagmin
58
SV 2
62
SNAP-25
92
Syntaxin
93
COX I
90
KIF5A
107
spinal cord
+/+
+/-
(%)
KIF1Bβ
58
Synaptotagmin
101
SV 2
108
sciatic nerve
spinal cord
+/+ +/-
+/+ +/-
(kD)
200-
116-
97-
66-
45-


A
B
Unaffected
Affected
C
Unaffected
T T T G C C T A T G G G C A G A C T
F A Y G Q T
Affected
T T T G C C T A T G G G C A/T G A C T
F A Y G Q/L T


KIF1Bβ
KIF1Bβ–Q98L



Setou et al. Science 288:1796-, 2000


Reprint Series
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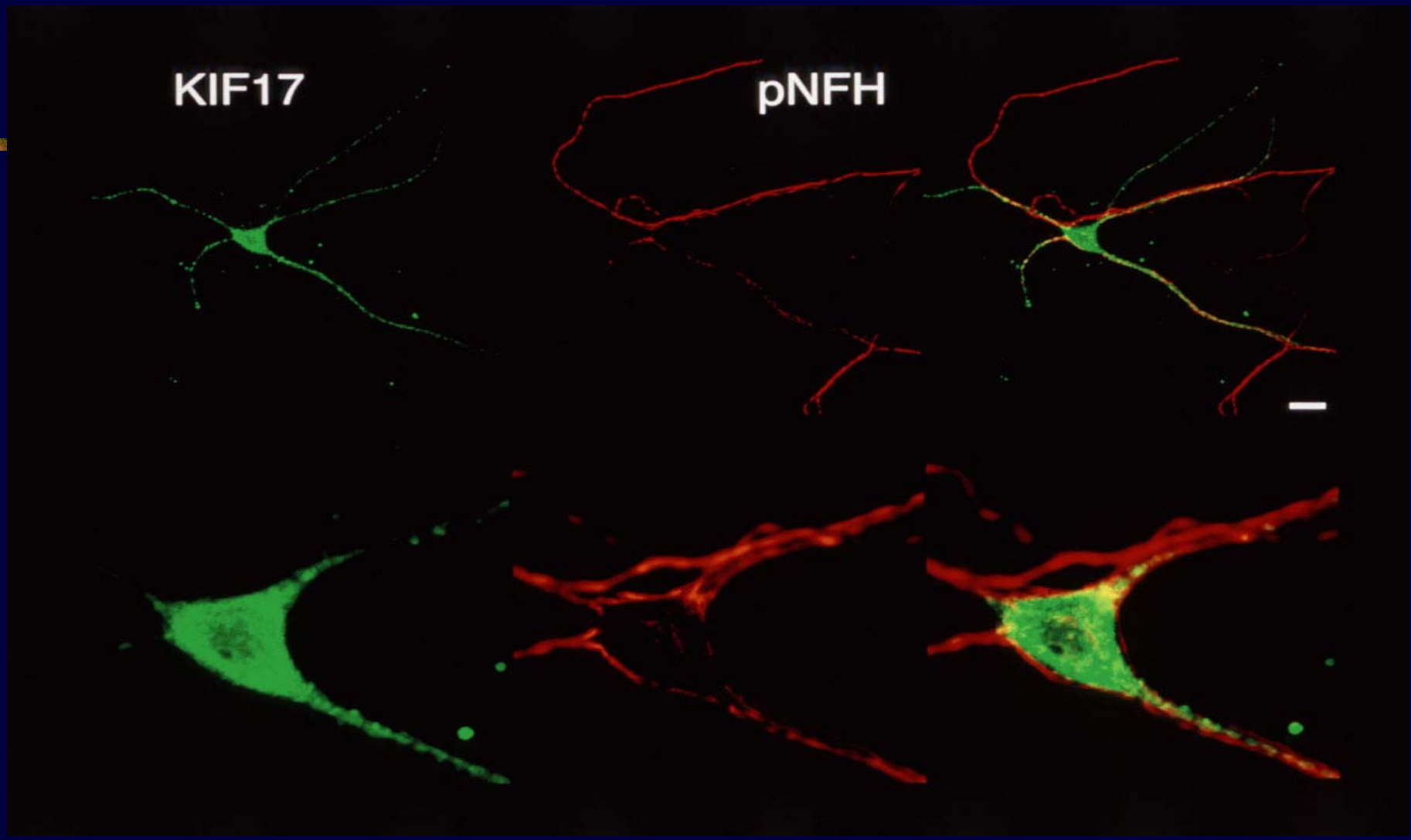
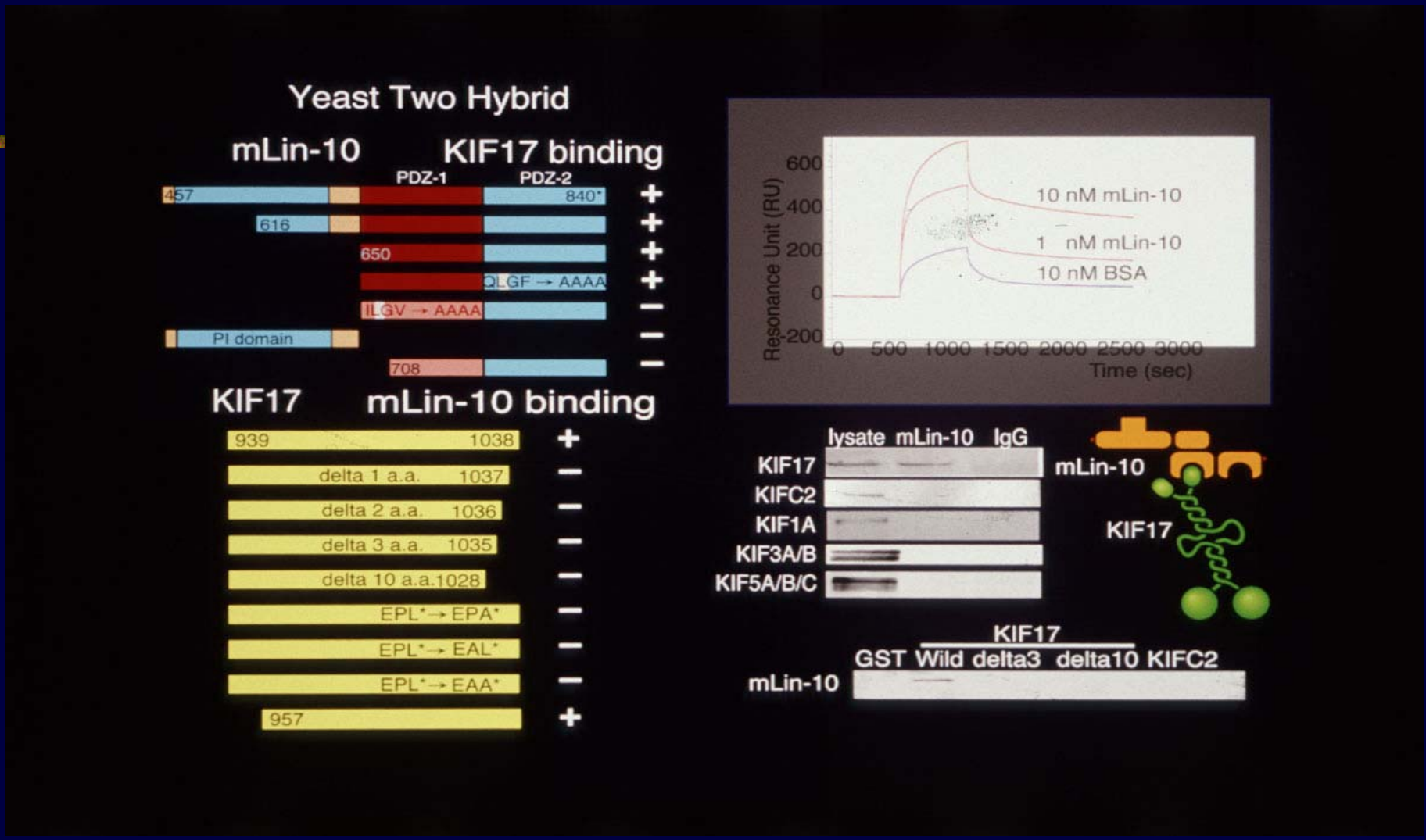
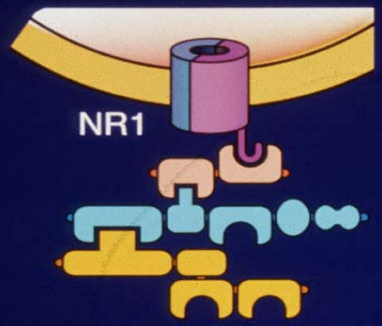
Science
Vol. 288 No. 5472
Pages 1693–1920 $8
AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

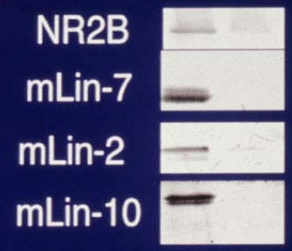

KIF17
pNFH


vesicles counts
25
20
15
10
5
0
KIF17
preimmune
10 20 30 40 50 60 70 80 90 100
vesicle size (nm)
control pH 7.4
0.5M KCl pH 7.4
0.1M Na2CO3 pH 11.5
ppt sup ppt sup ppt sup
KIF17

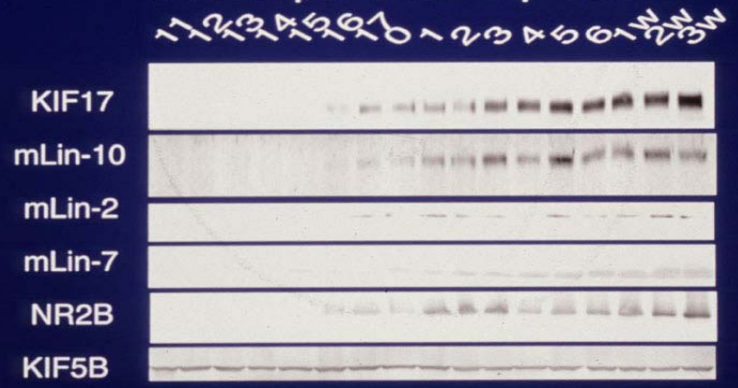

Yeast Two Hybrid
mLin-10
KIF17 binding
PDZ-1
PDZ-2
457
840*
616
650
QLGF → AAAA
ILGV → AAAA
PI domain
708
KIF17
mLin-10 binding
939
1038
delta 1 a.a. 1037
delta 2 a.a. 1036
delta 3 a.a. 1035
delta 10 a.a.1028
EPL* → EPA*
EPL* → EAL*
EPL* → EAA*
957
Resonance Unit (RU)
600
400
200
0
-200
0 500 1000 1500 2000 2500 3000
Time (sec)
10 nM mLin-10
1 nM mLin-10
10 nM BSA
lysate mLin-10 IgG
KIF17
KIFC2
KIF1A
KIF3A/B
KIF5A/B/C
mLin-10
KIF17
KIF17
GST Wild delta3 delta10 KIFC2
mLin-10

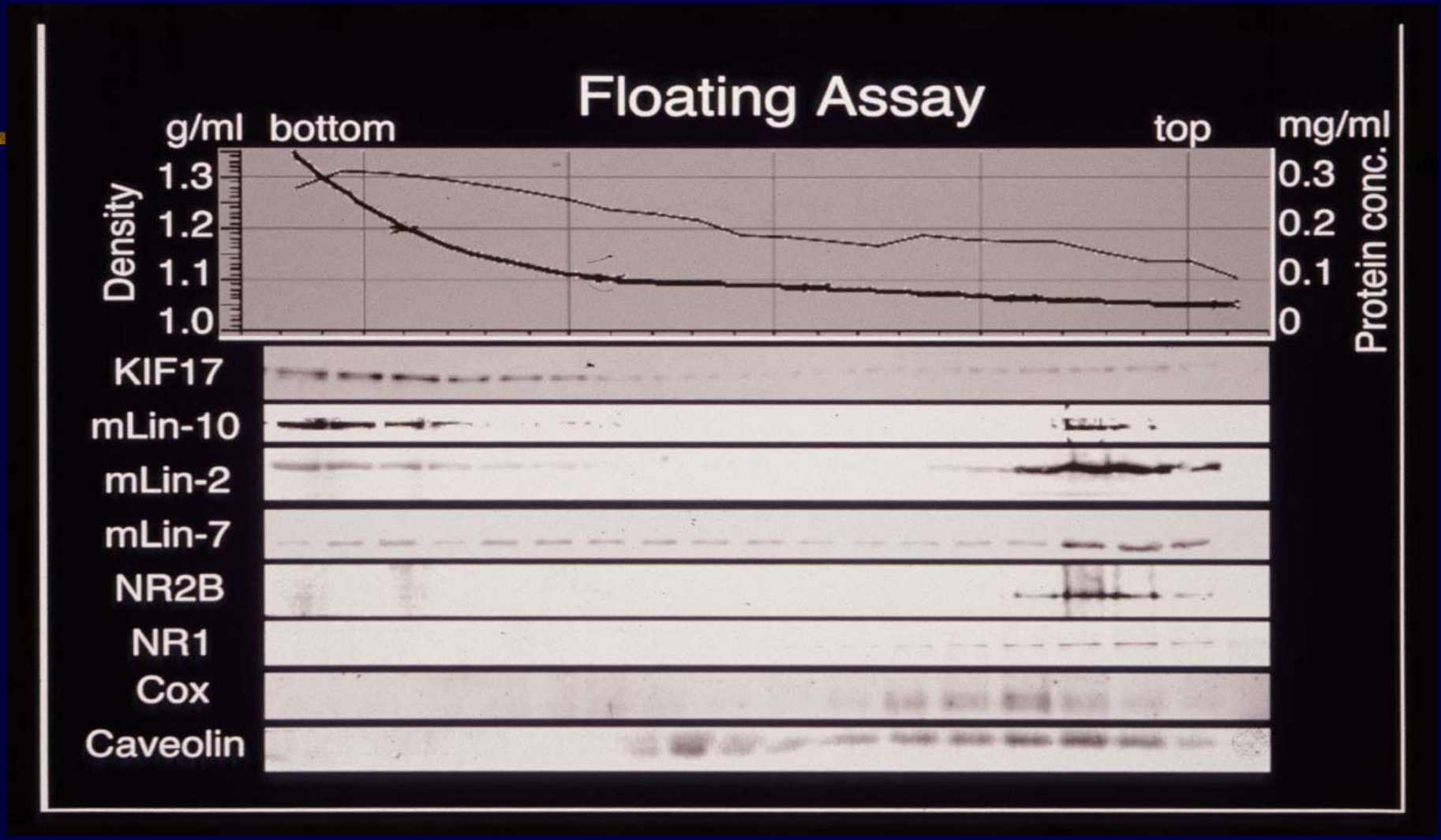
mLin-10 makes protein complex



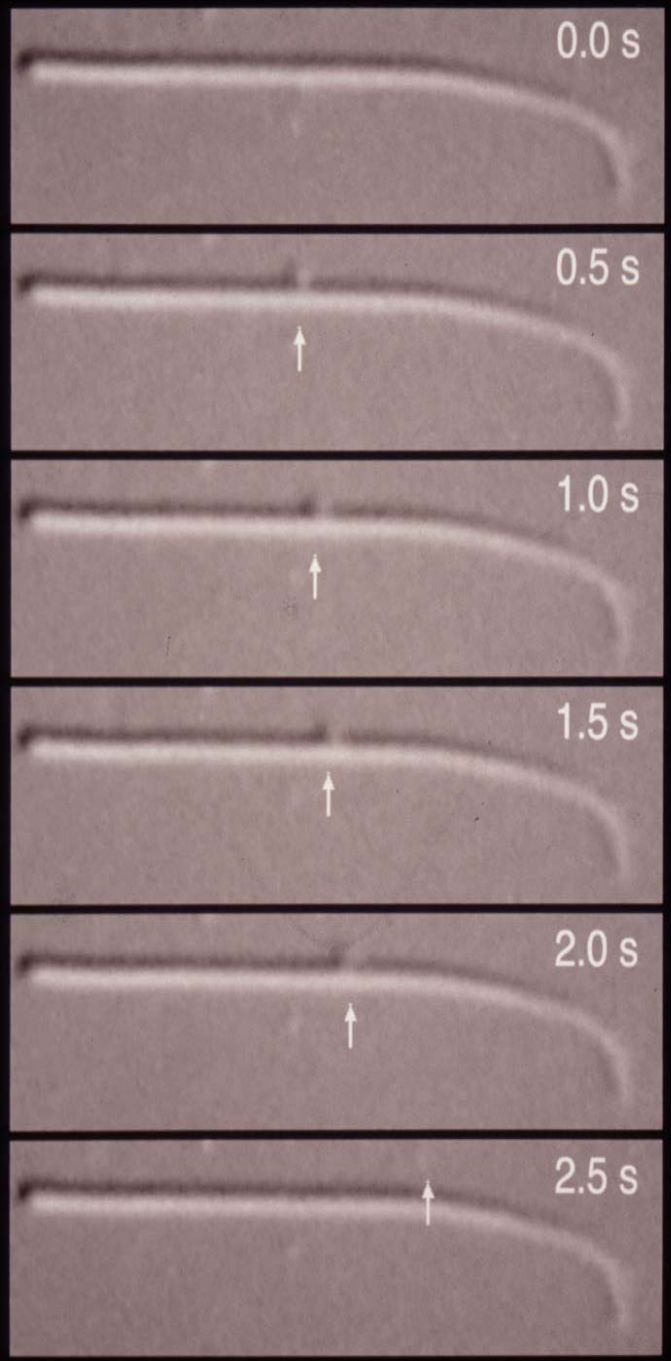


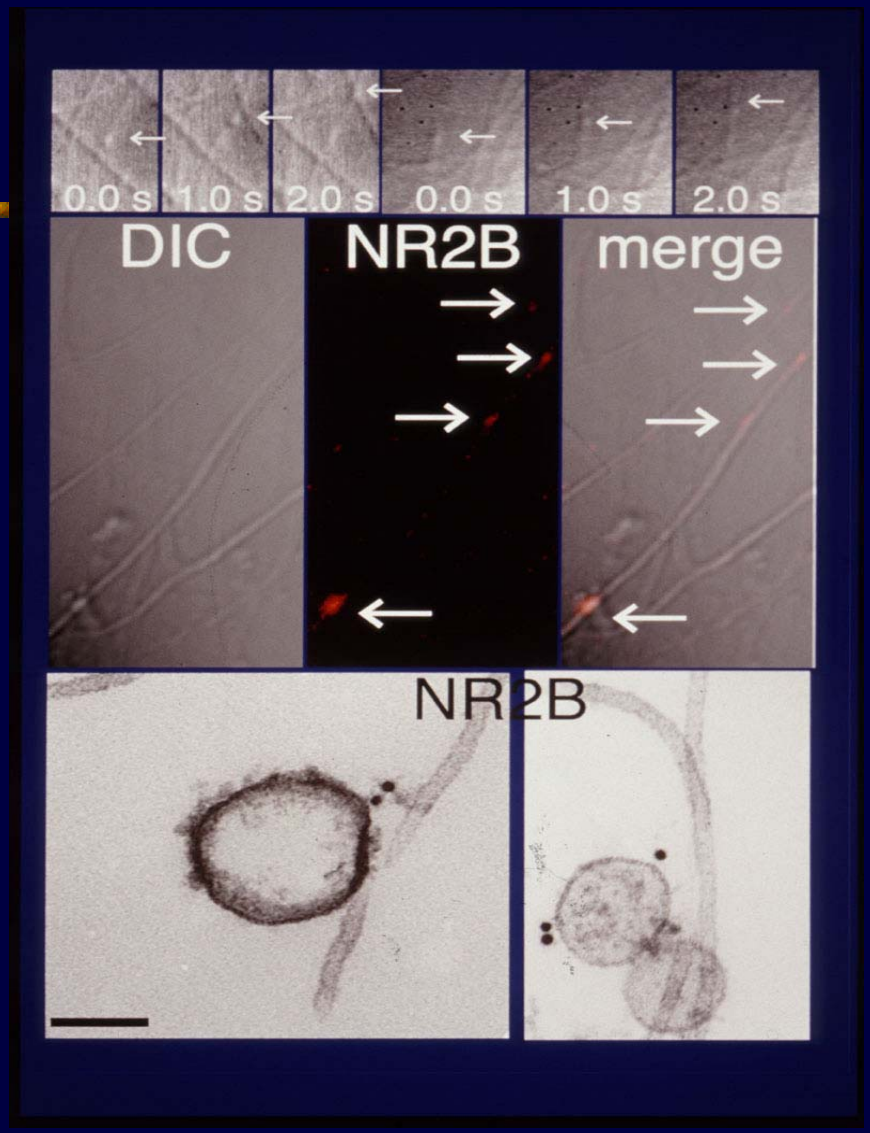
Developmental expression

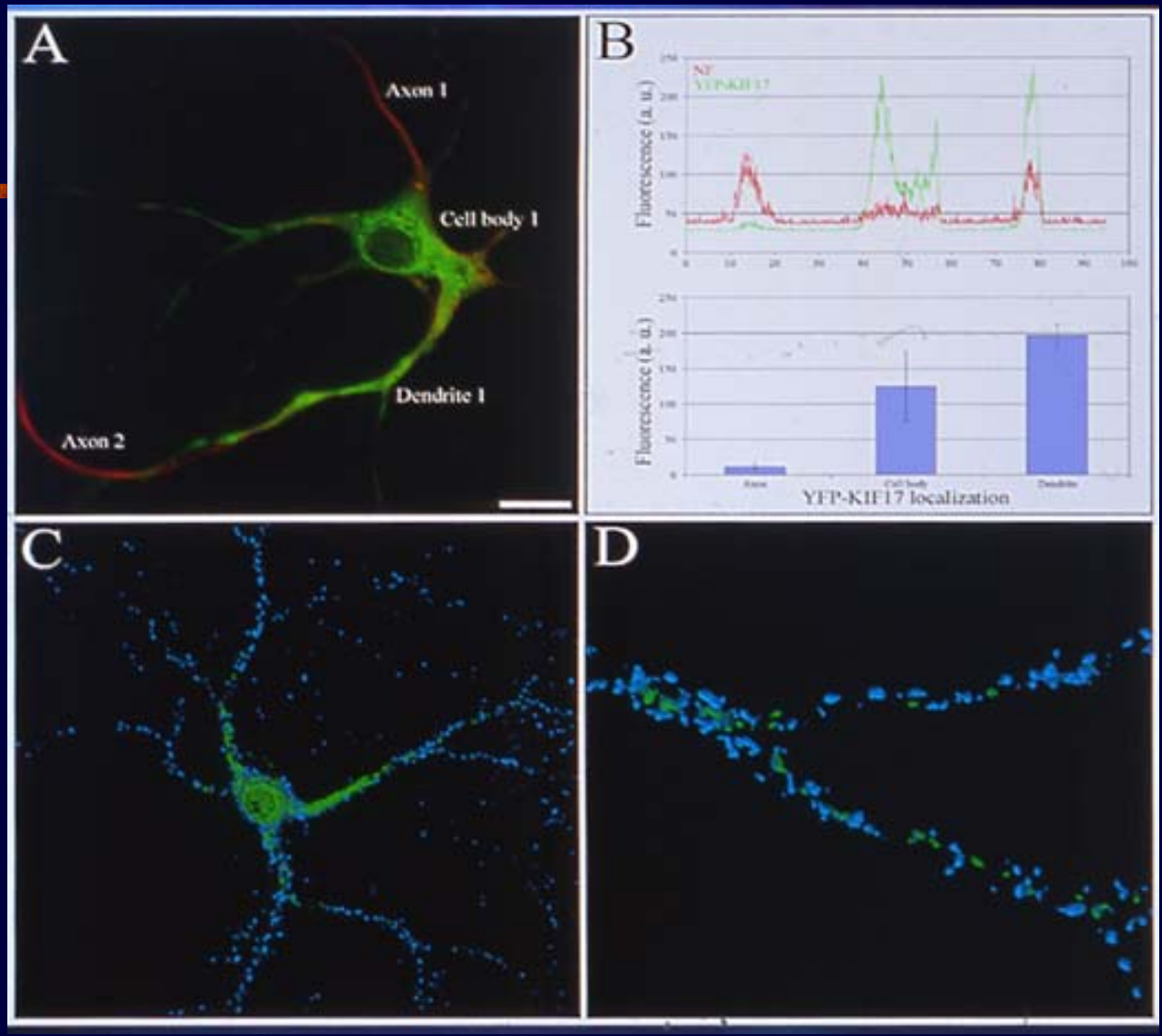


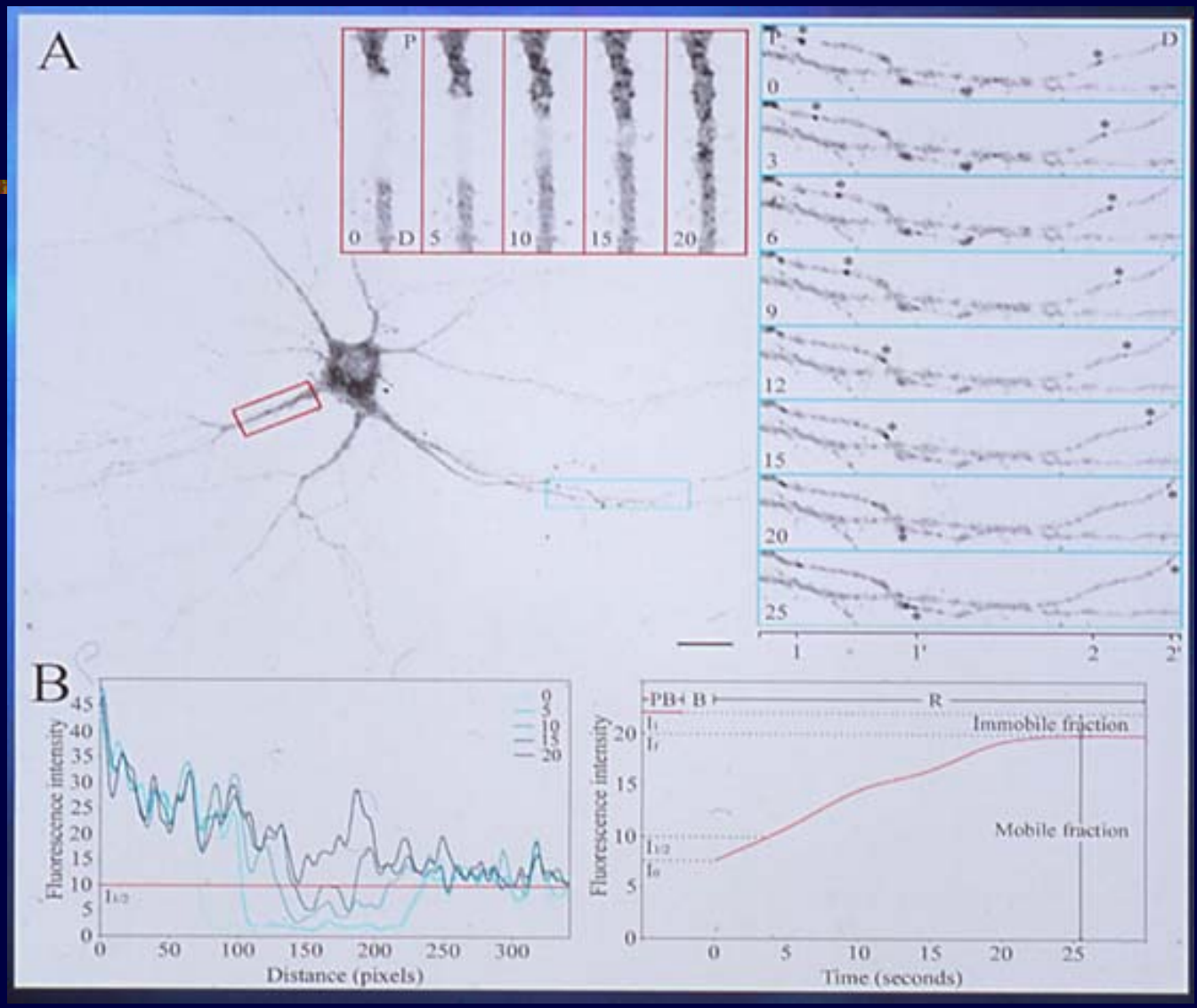

Floating Assay
g/ml
bottom
top
mg/ml
Density
1.3
1.2
1.1
1.0
0.3
0.2
0.1
0
Protein conc.
KIF17
mLin-10
mLin-2
mLin-7
NR2B
NR1
Cox
Caveolin

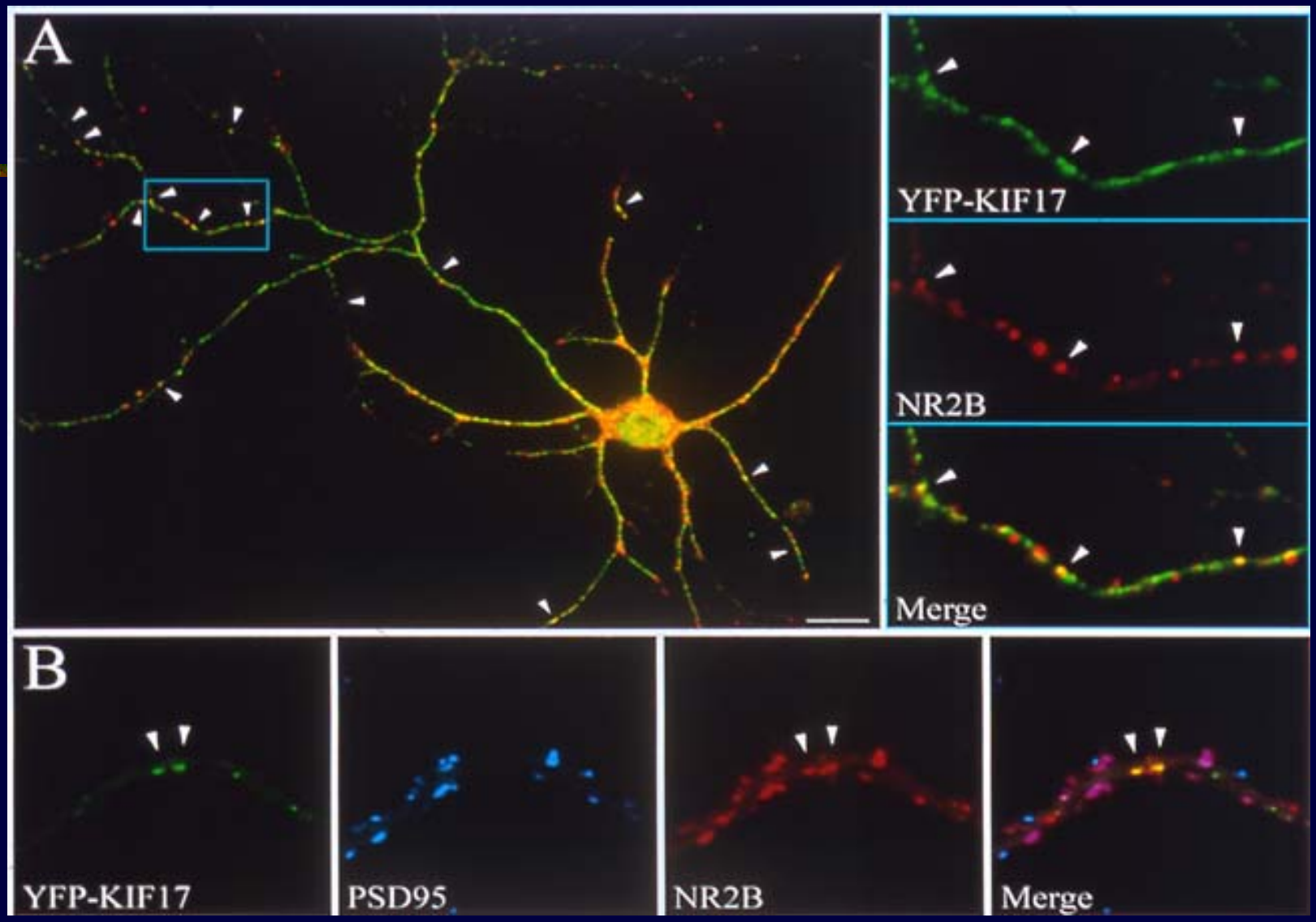
KIF17 makes large protein complex

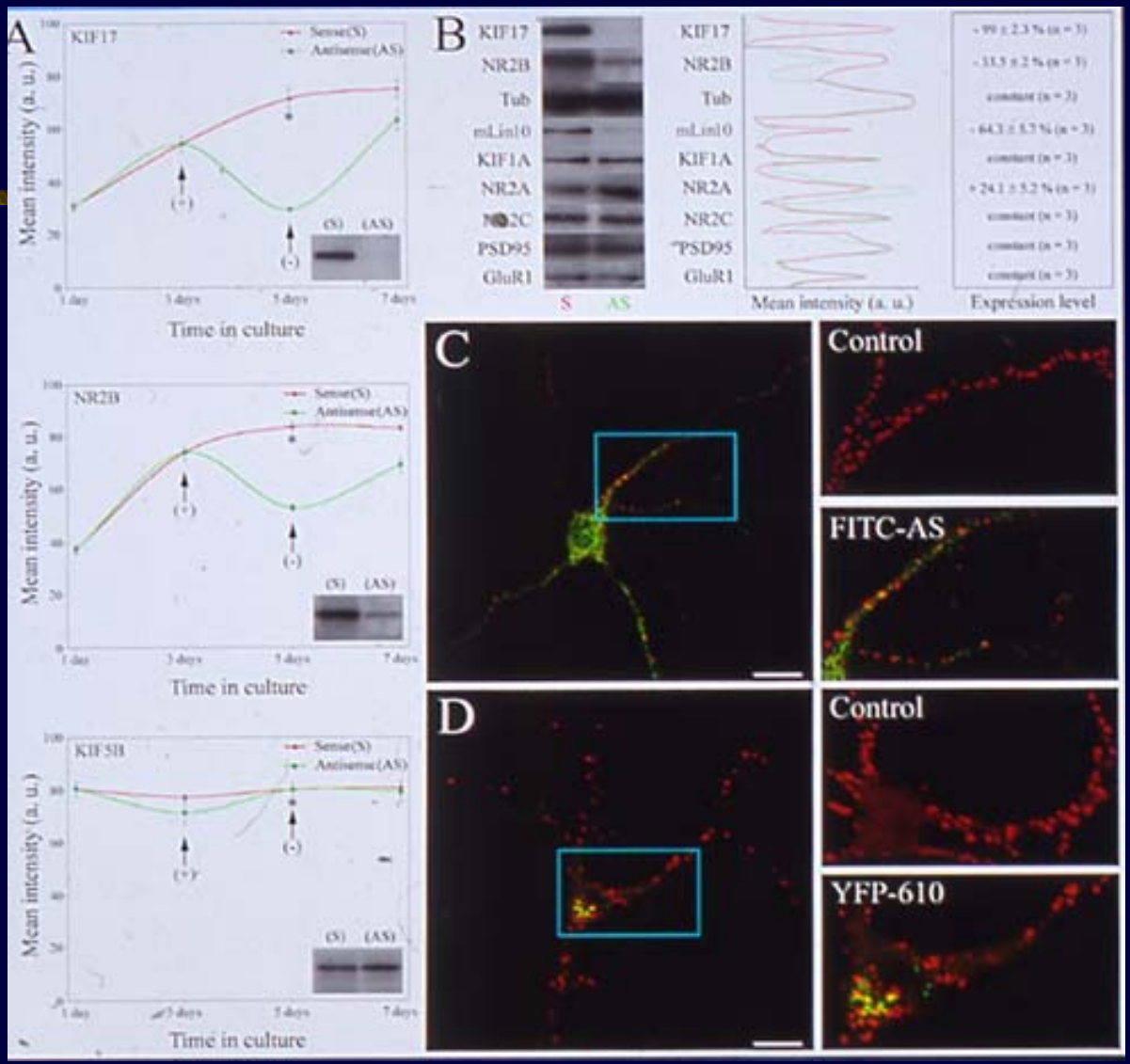

0.0 s
0.5 s
1.0 s
1.5 s
2.0 s
2.5 s

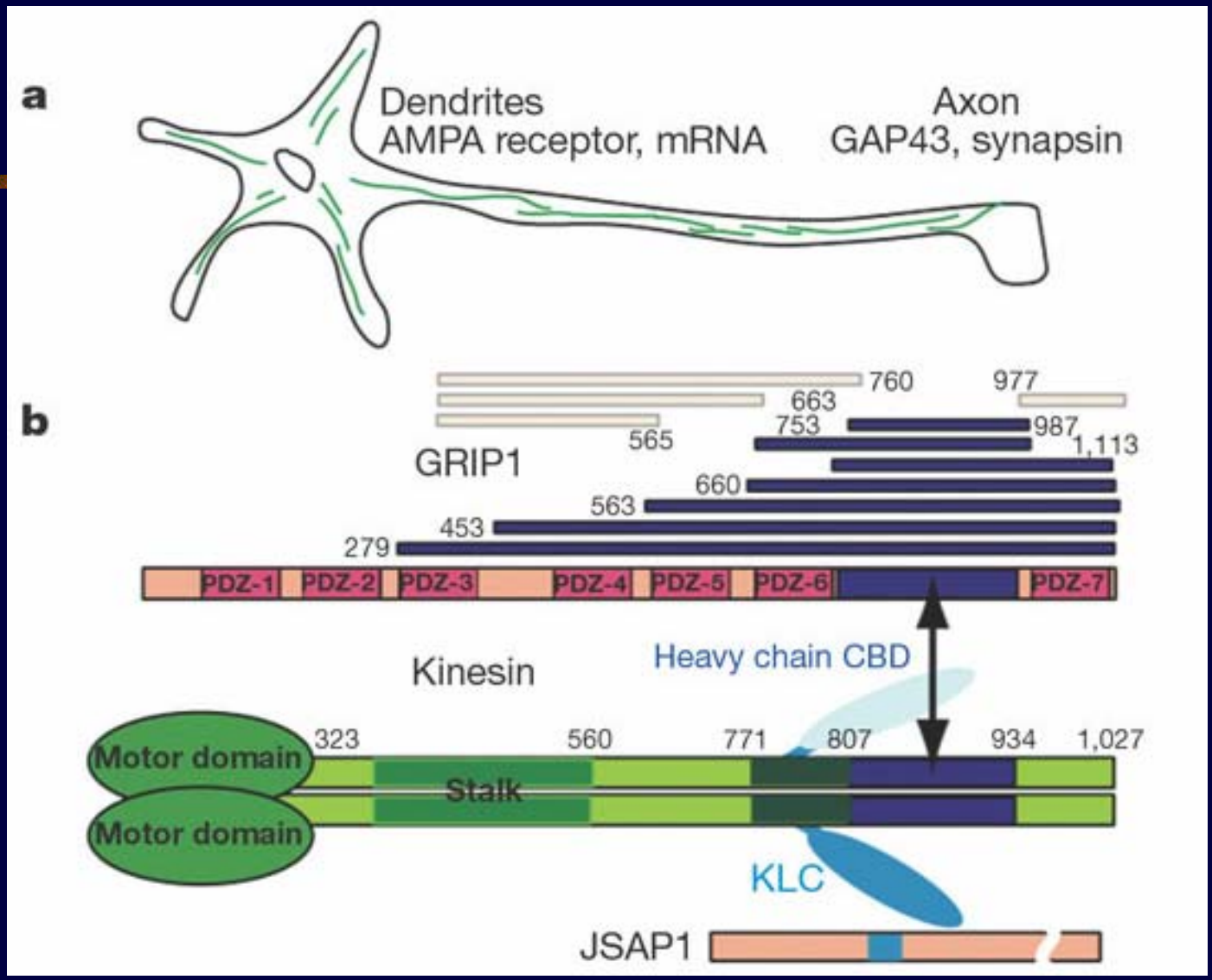

0.0 s
1.0 s
2.0 s
0.0 s
1.0 s
2.0 s
DIC
NR2B
merge
NR2B

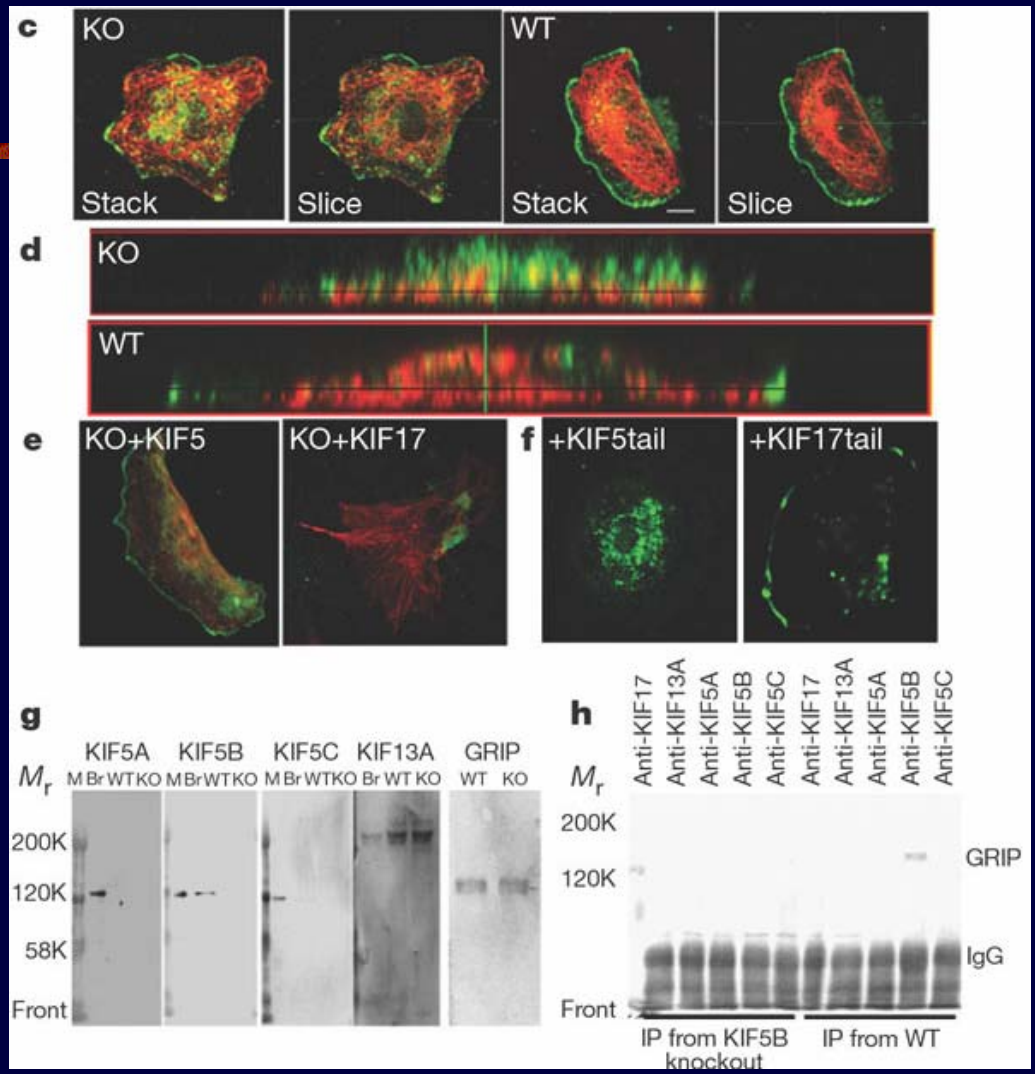

A
Axon 1
Cell body 1
Dendrite 1
Axon 2
B
Fluorescence (a. u.)
Fluorescence (a. u.)
YFP-KIF17 localization
C
D

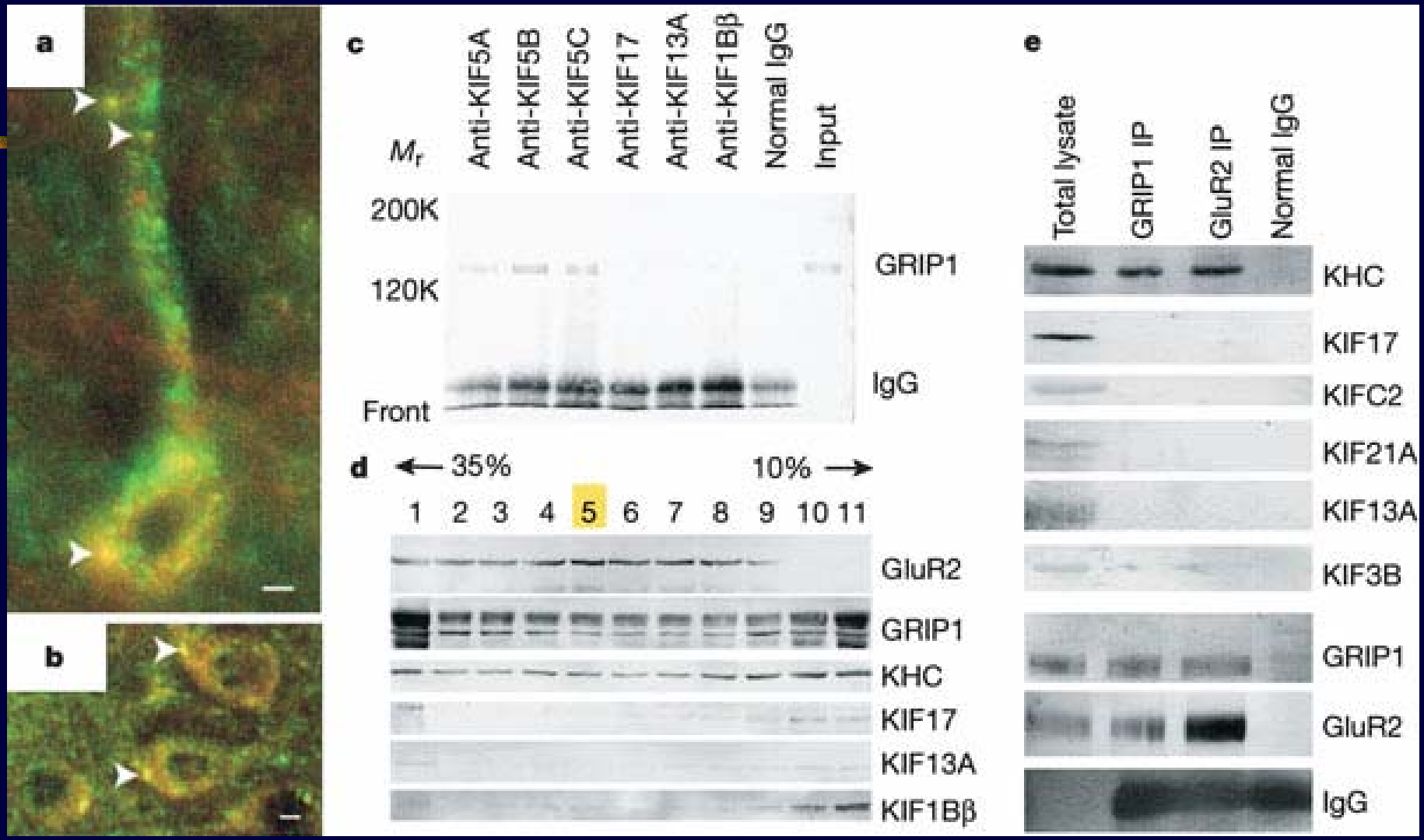

A
P
D
0
5
10
15
20
0
3
6
9
12
15
20
25
1
1'
2
2'
B
Fluorescence intensity
Distance (pixels)
PB
B
R
Immobile fraction
Mobile fraction
Time (seconds)

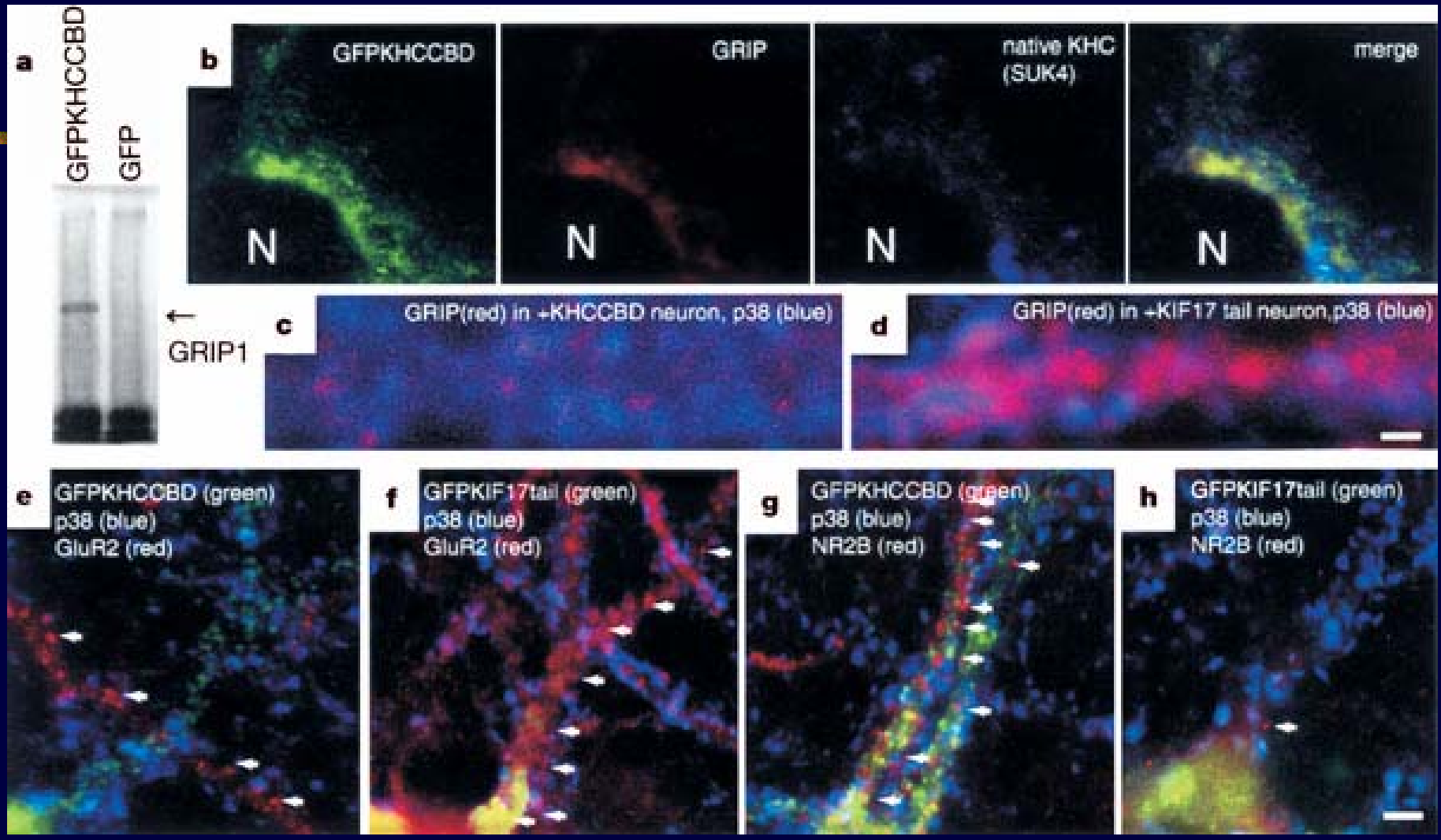

A
YFP-KIF17
NR2B
Merge
B
YFP-KIF17
PSD95
NR2B
Merge

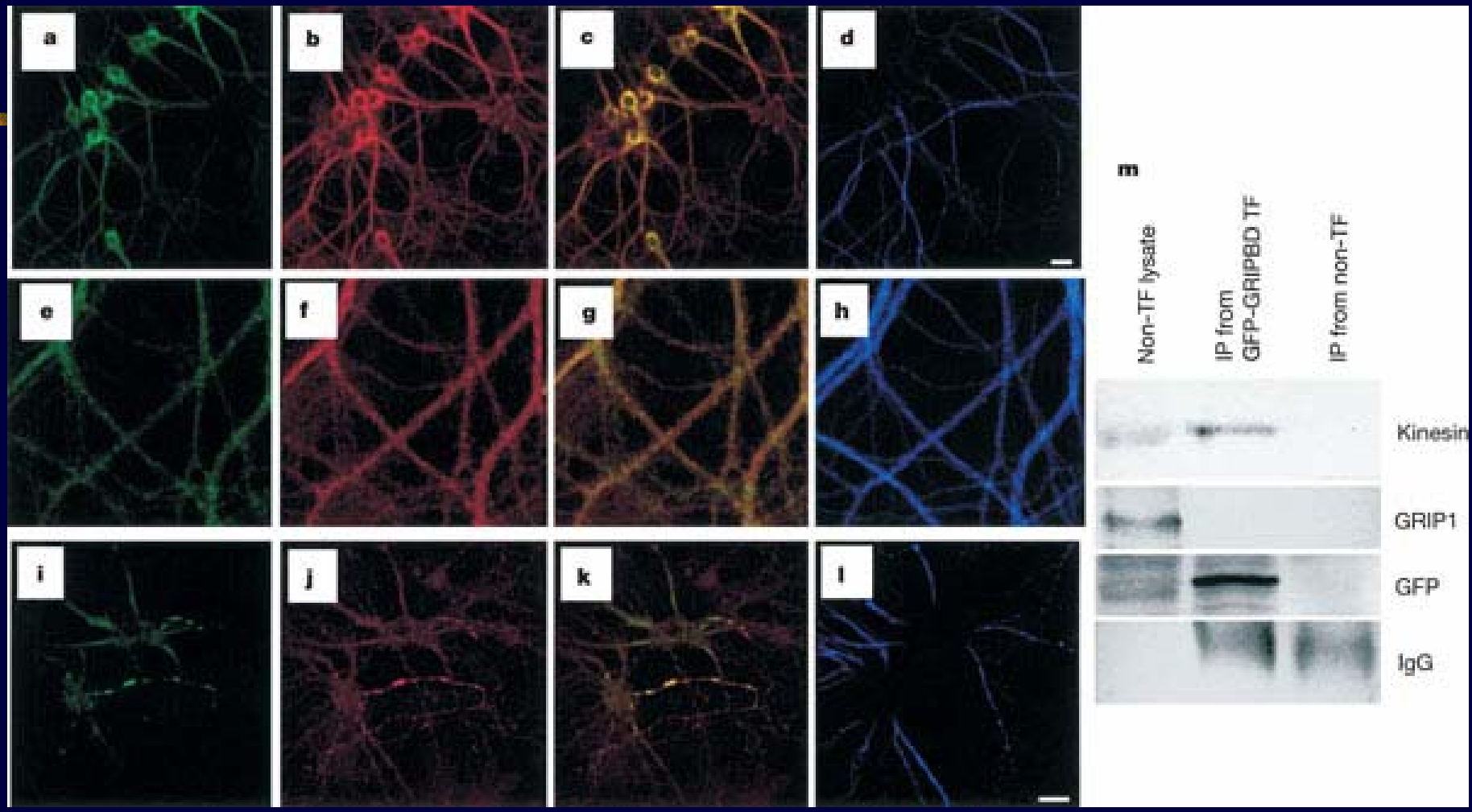

A
KIF17
Sense(S)
Antisense(AS)
Mean intensity (a. u.)
(+)
(-)
(S) (AS)
1 day
3 days
5 days
7 days
Time in culture
NR2B
Sense(S)
Antisense(AS)
Mean intensity (a. u.)
(+)
(-)
(S) (AS)
1 day
3 days
5 days
7 days
Time in culture
KIF5B
Sense(S)
Antisense(AS)
Mean intensity (a. u.)
(+)
(-)
(S) (AS)
1 day
3 days
5 days
7 days
Time in culture
B
KIF17
NR2B
Tub
mLin10
KIF1A
NR2A
NR2C
PSD95
GluR1
S AS
KIF17
NR2B
Tub
mLin10
KIF1A
NR2A
NR2C
PSD95
GluR1
Mean intensity (a. u.)
Expression level
C
Control
FITC-AS
D
Control
YFP-610

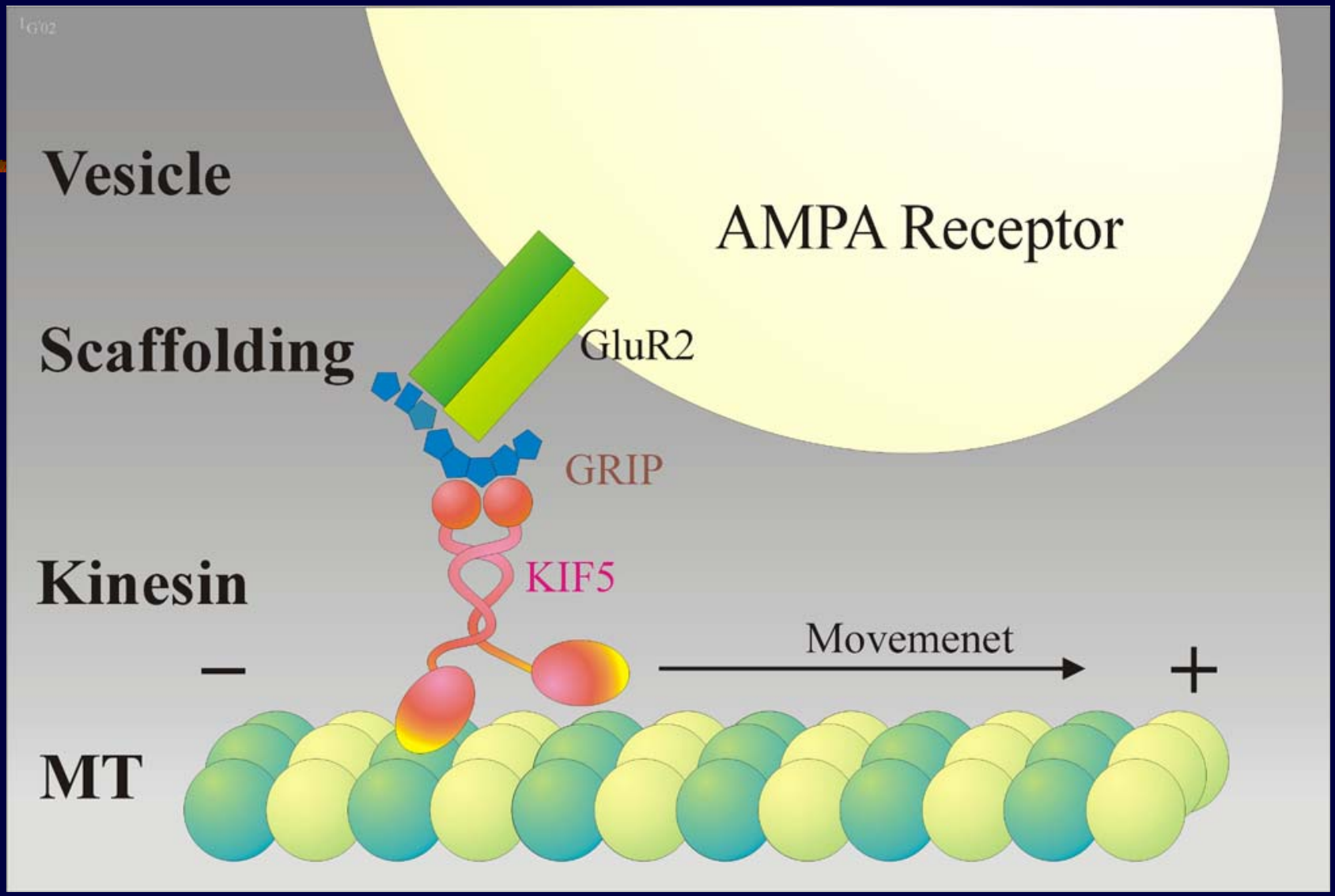

a
Dendrites
AMPA receptor, mRNA
Axon
GAP43, synapsin
b
760
977
663
753
987
565
1,113
GRIP1
660
563
453
279
PDZ-1
PDZ-2
PDZ-3
PDZ-4
PDZ-5
PDZ-6
PDZ-7
Kinesin
Heavy chain CBD
323
560
771
807
934
1,027
Motor domain
Stalk
Motor domain
KLC
JSAP1

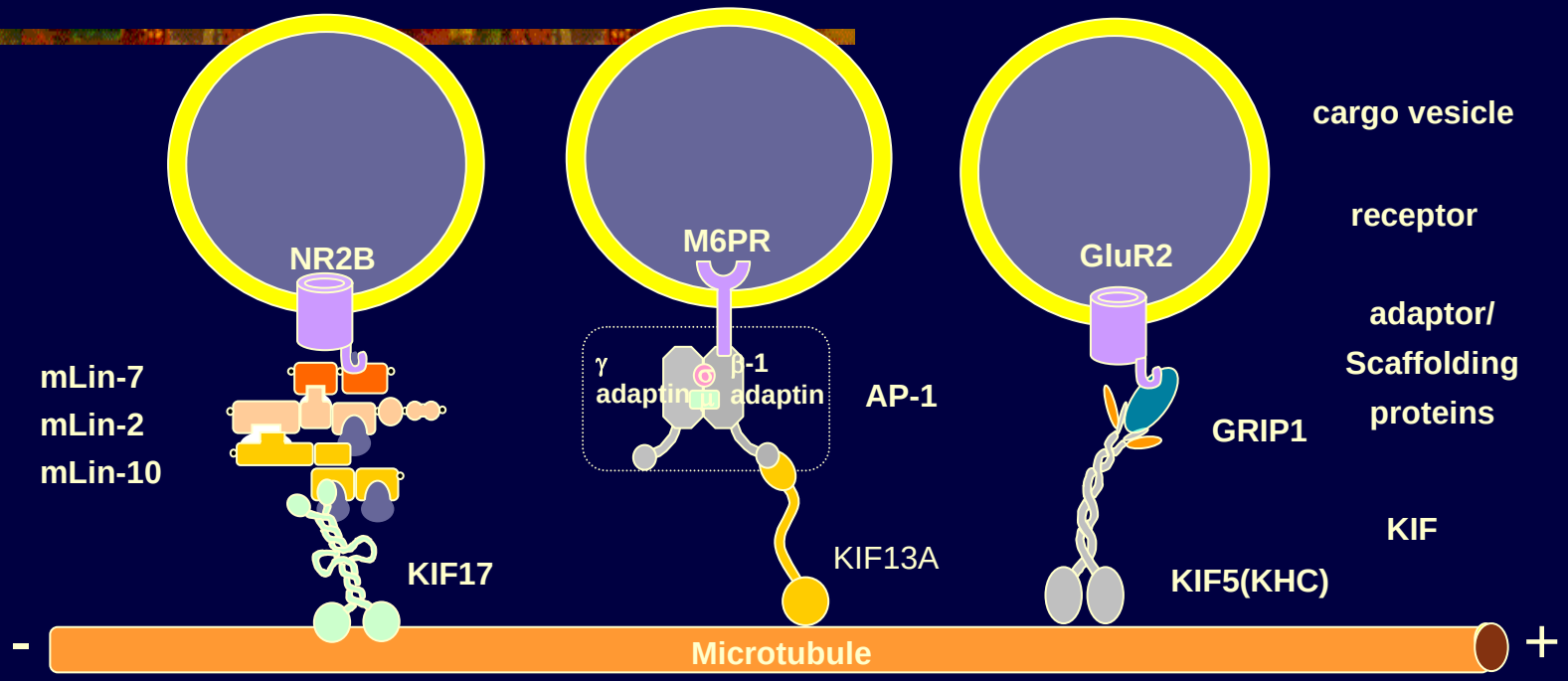

c
KO
Stack
Slice
WT
Stack
Slice
d
KO
WT
e
KO+KIF5
KO+KIF17
f
+KIF5tail
+KIF17tail
g
KIF5A
KIF5B
KIF5C
KIF13A
GRIP
M Br WT KO M Br WT KO M Br WT KO Br WT KO WT KO
M_r
200K
120K
58K
Front
h
Anti-KIF17
Anti-KIF13A
Anti-KIF5A
Anti-KIF5B
Anti-KIF5C
Anti-KIF17
Anti-KIF13A
Anti-KIF5A
Anti-KIF5B
Anti-KIF5C
M_r
200K
120K
Front
GRIP
IgG
IP from KIF5B knockout
IP from WT


a
b
c
Anti-KIF5A
Anti-KIF5B
Anti-KIF5C
Anti-KIF17
Anti-KIF13A
Anti-KIF1Bβ
Normal IgG
Input
M_r
200K
120K
Front
GRIP1
IgG
d
35%
10%
1 2 3 4 5 6 7 8 9 10 11
GluR2
GRIP1
KHC
KIF17
KIF13A
KIF1Bβ
e
Total lysate
GRIP1 IP
GluR2 IP
Normal IgG
KHC
KIF17
KIFC2
KIF21A
KIF13A
KIF3B
GRIP1
GluR2
IgG

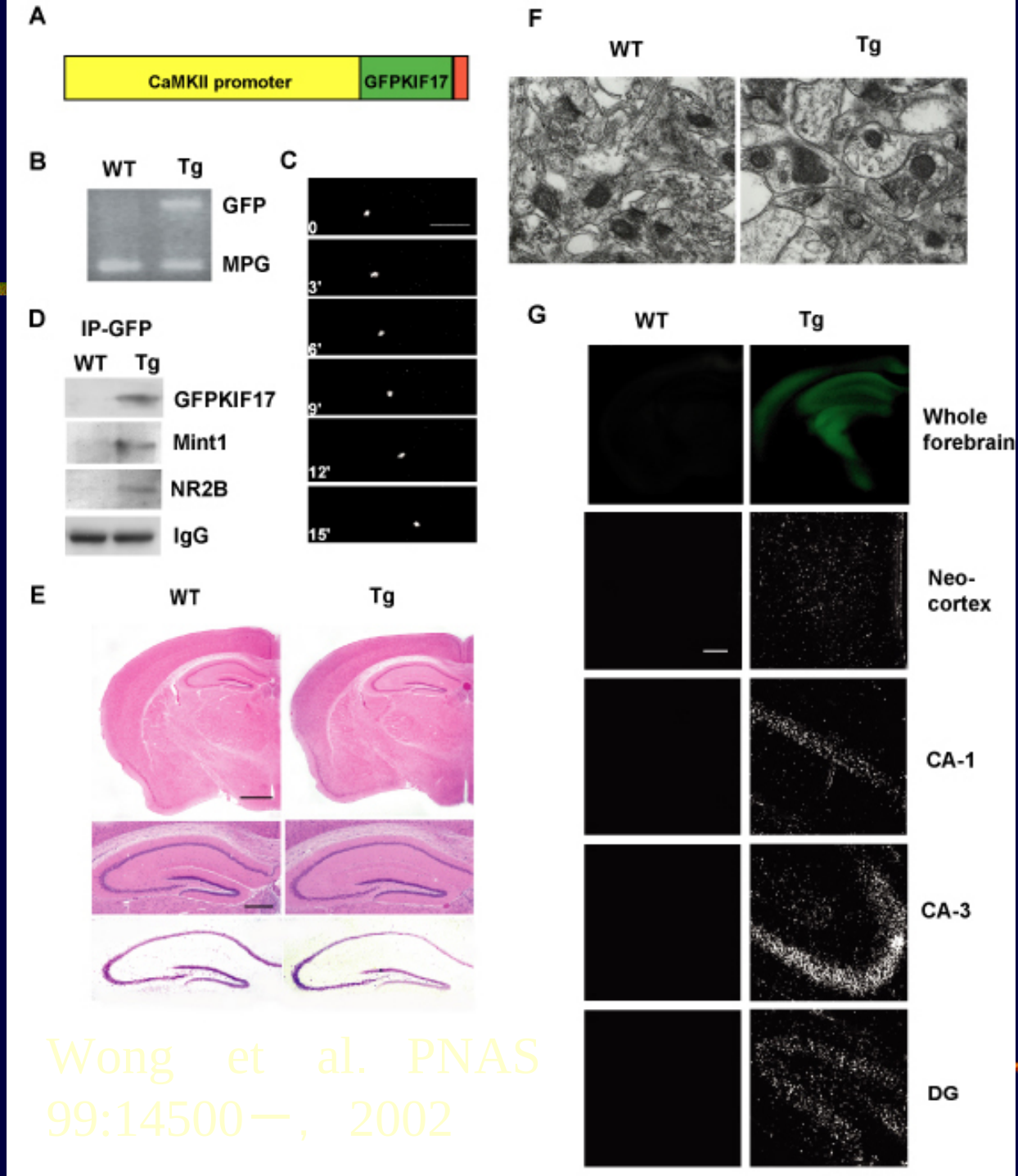

a
GFPKHCCBD
GFP
GRIP1
b
GFPKHCCBD
GRIP
native KHC
(SUK4)
merge
N
N
N
N
c
GRIP(red) in +KHCCBD neuron, p38 (blue)
d
GRIP(red) in +KIF17 tail neuron,p38 (blue)
e
GFPKHCCBD (green)
p38 (blue)
GluR2 (red)
f
GFPKIF17tail (green)
p38 (blue)
GluR2 (red)
g
GFPKHCCBD (green)
p38 (blue)
NR2B (red)
h
GFPKIF17tail (green)
p38 (blue)
NR2B (red)


a
b
c
d
e
f
g
h
i
j
k
l
m
Non-TF lysate
IP from GFP-GRIPBD TF
IP from non-TF
Kinesin
GRIP1
GFP
IgG


Vesicle
AMPA Receptor
Scaffolding
GluR2
GRIP
Kinesin
KIF5
Movemenet
–
+
MT



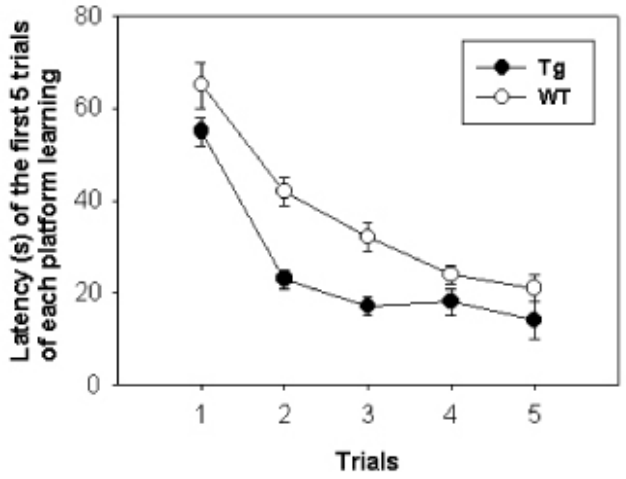
Setou et al. Science 288:1796－,2000
Nakagawa et al. Cell 103:569－,2000
Setou et al. Nature 417:83－,2002



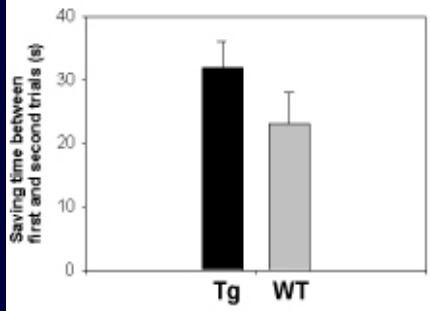
Wong et al. PNAS 99:14500-2002

Wong et al. PNAS 99:14500—, 2002

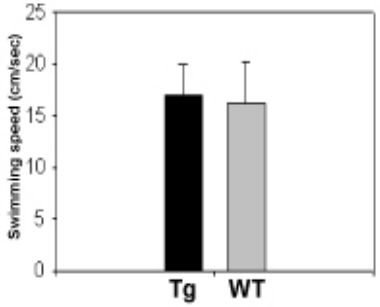
A Delay Matching-to-place test



B Saving time

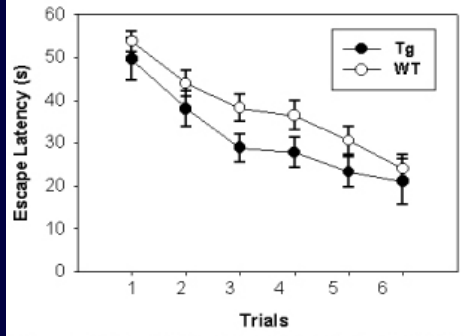


C Swimming speed

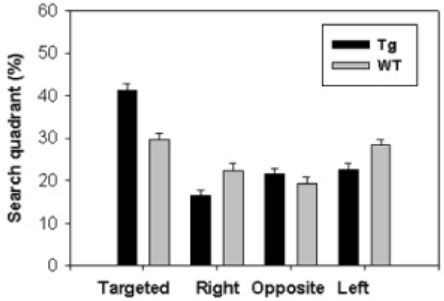


Working memory

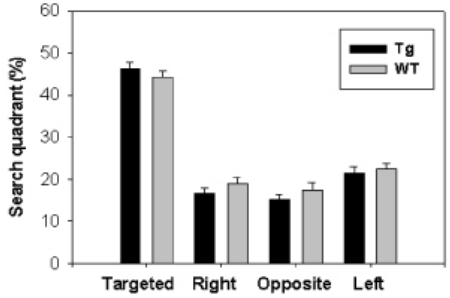
A Morris hidden platform water maze test



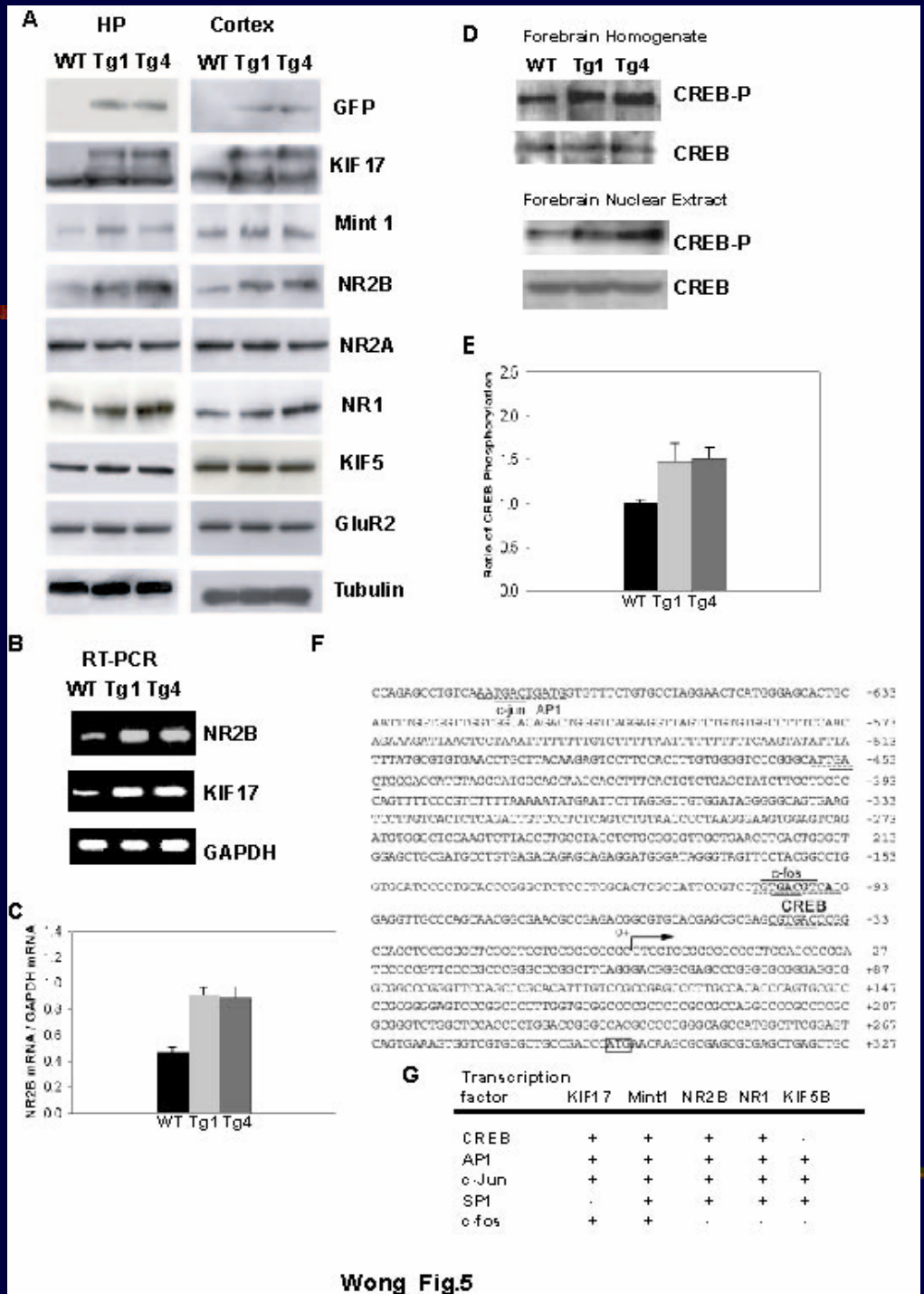
B Transfer test at the end of 3 trial



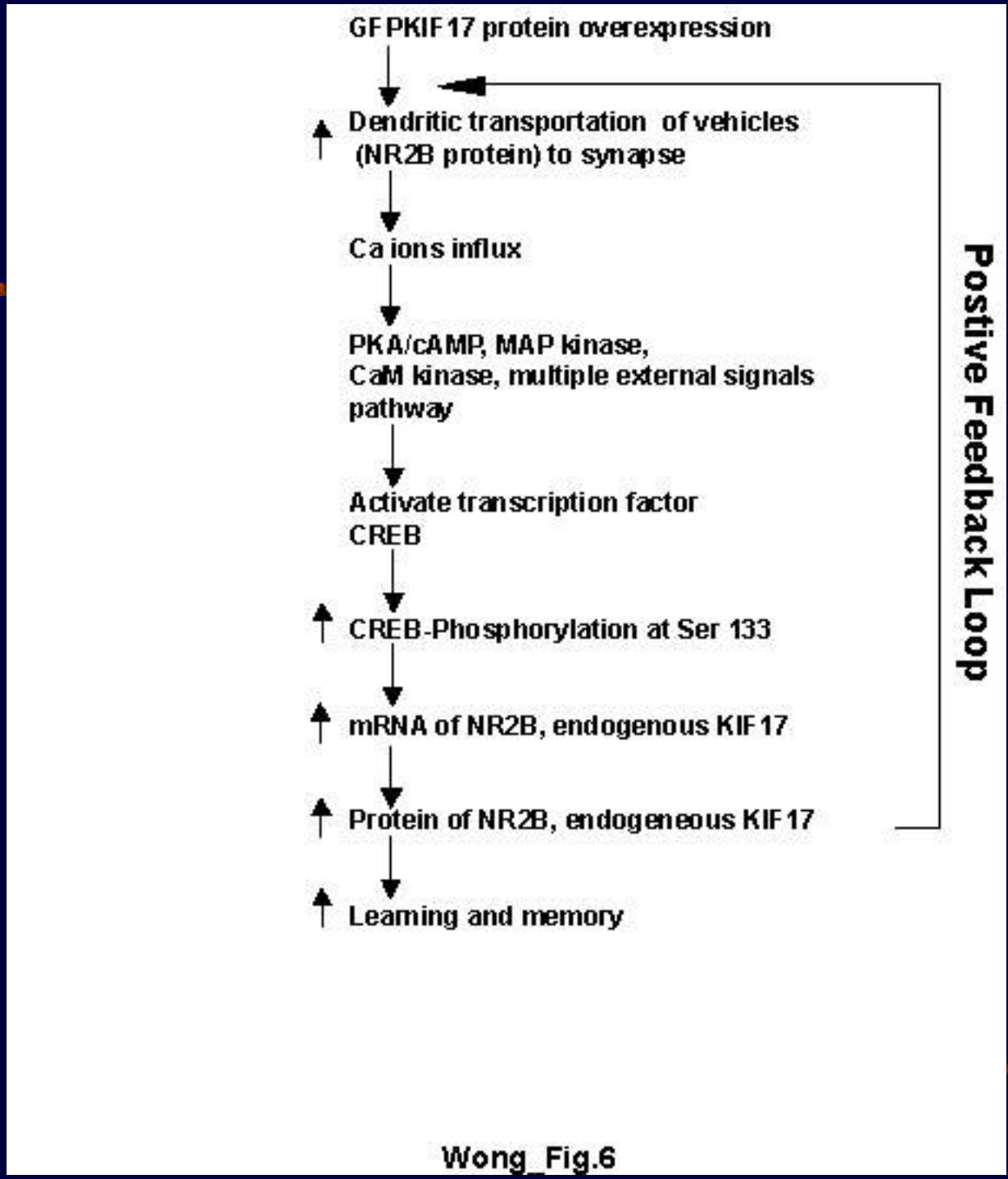
C Transfer test at the end of 6 trial



Spatial memory


A
HP
Cortex
WT Tg1 Tg4
WT Tg1 Tg4
GFP
KIF17
Mint 1
NR2B
NR2A
NR1
KIF5
GluR2
Tubulin
D
Forebrain Homogenate
WT Tg1 Tg4
CREB-P
CREB
Forebrain Nuclear Extract
CREB-P
CREB
E
Ratio of CREB Phosphorylation
2.0
1.5
1.0
0.5
0.0
WT Tg1 Tg4
B
RT-PCR
WT Tg1 Tg4
NR2B
KIF17
GAPDH
C
NR2B mRNA / GAPDH mRNA
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
WT Tg1 Tg4
F
c-jun
AP1
c-fos
CREB
G
Transcription factor
KIF17
Mint1
NR2B
NR1
KIF5B
CREB + + + + .
AP1 + + + + +
c-Jun + + + + +
SP1 . + + + +
c-fos + + . . .

Wong Fig.5

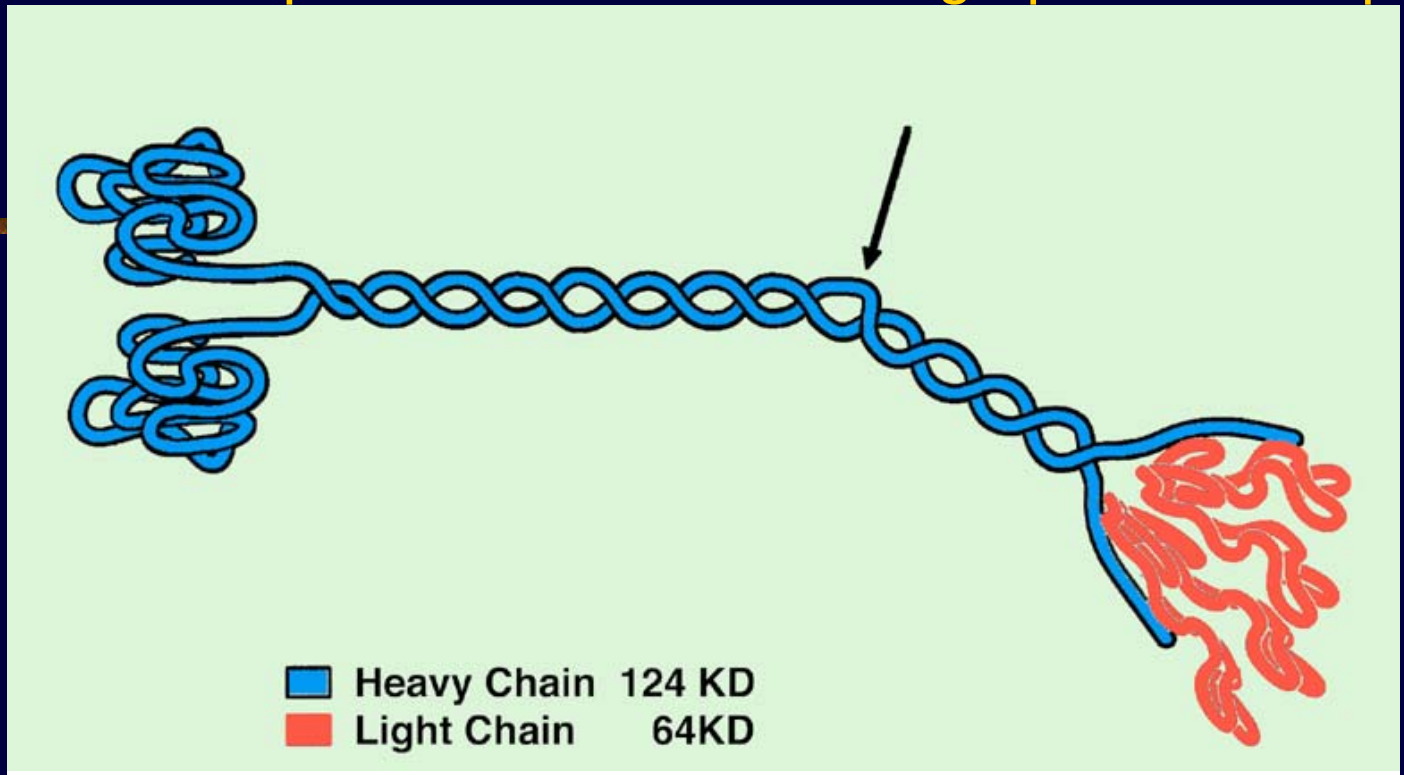

GFPKIF17 protein overexpression
Dendritic transportation of vehicles (NR2B protein) to synapse
Ca ions influx
PKA/cAMP, MAP kinase, CaM kinase, multiple external signals pathway
Activate transcription factor CREB
CREB-Phosphorylation at Ser 133
mRNA of NR2B, endogenous KIF17
Protein of NR2B, endogeneous KIF17
Learning and memory
Postive Feedback Loop

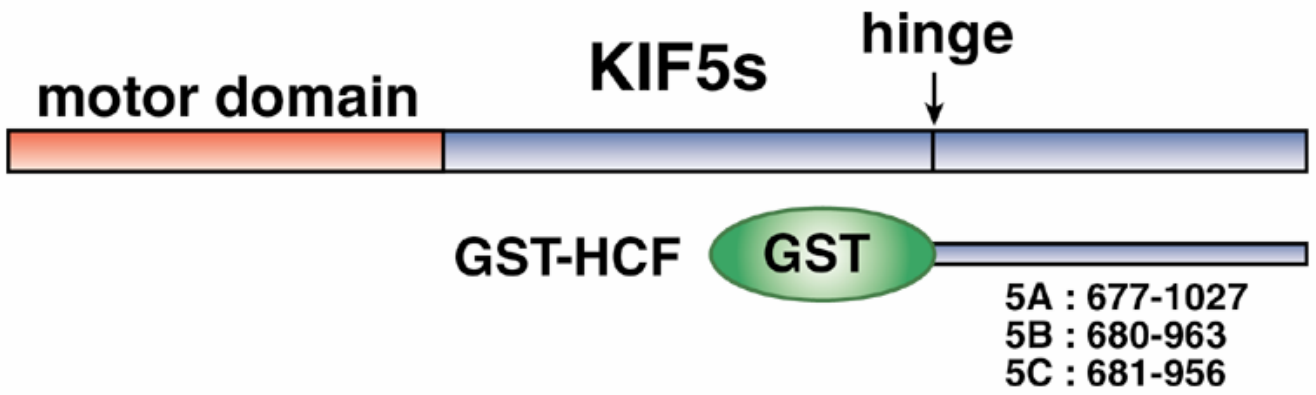
Wong_Fig.6

Kinesin transports RNA: Isolation and characterization of an RNA-transporting granule

Kanai, Y. et. al Neuron 43: 513- , 2004

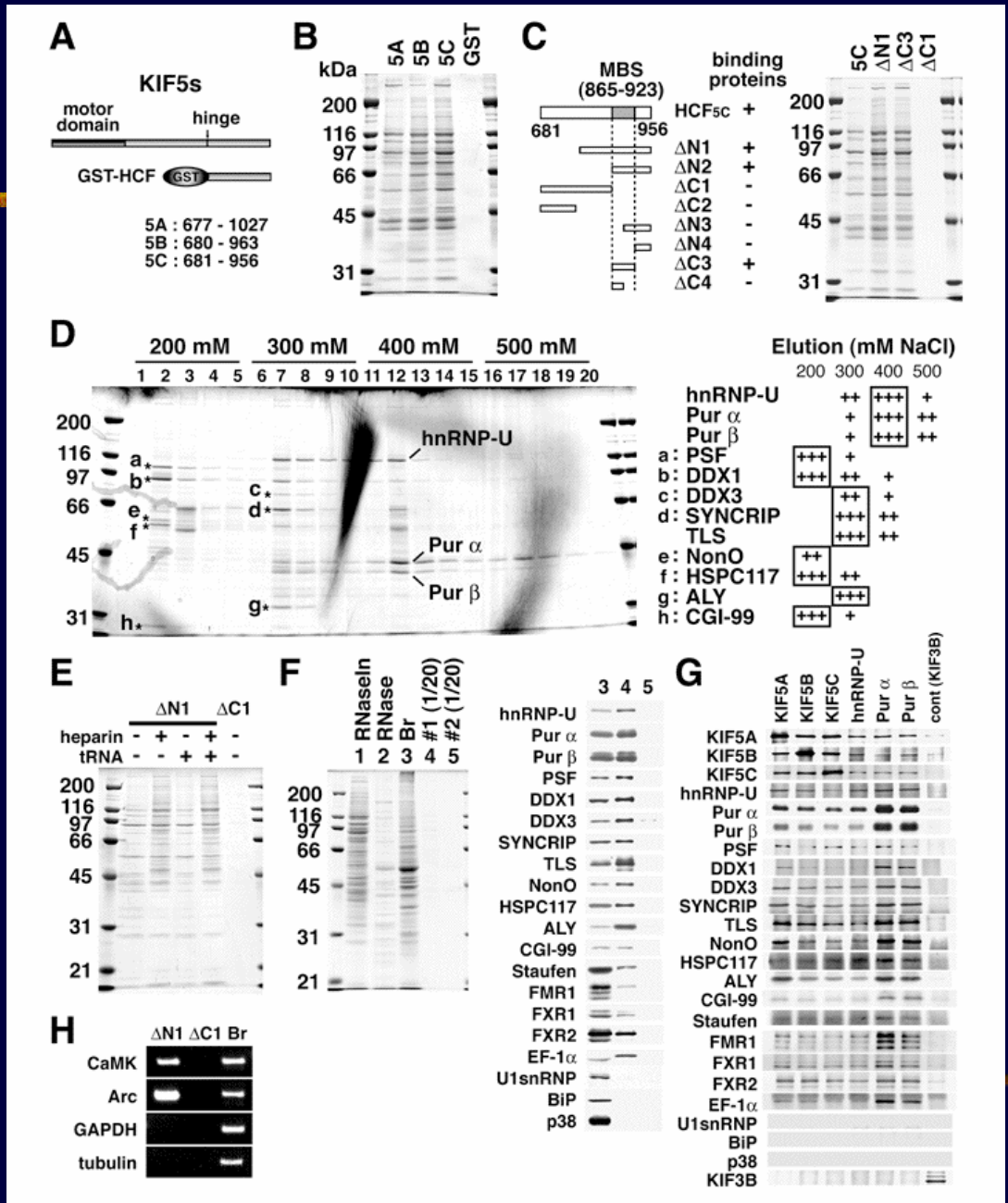
Kinesin transports mRNAs with a large protein complex



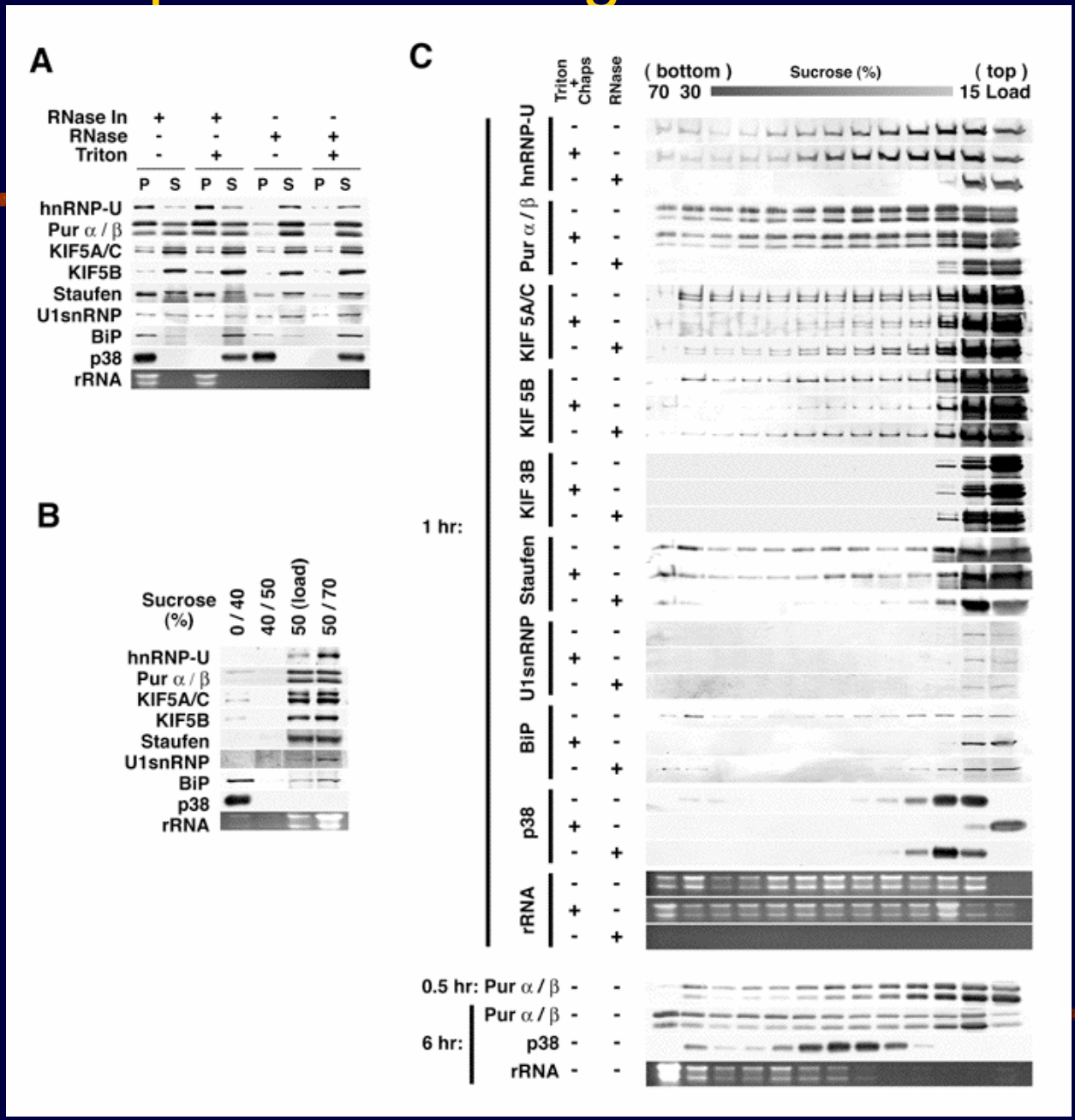


Kanai,Y. et al Neuron 43:513- ,2004

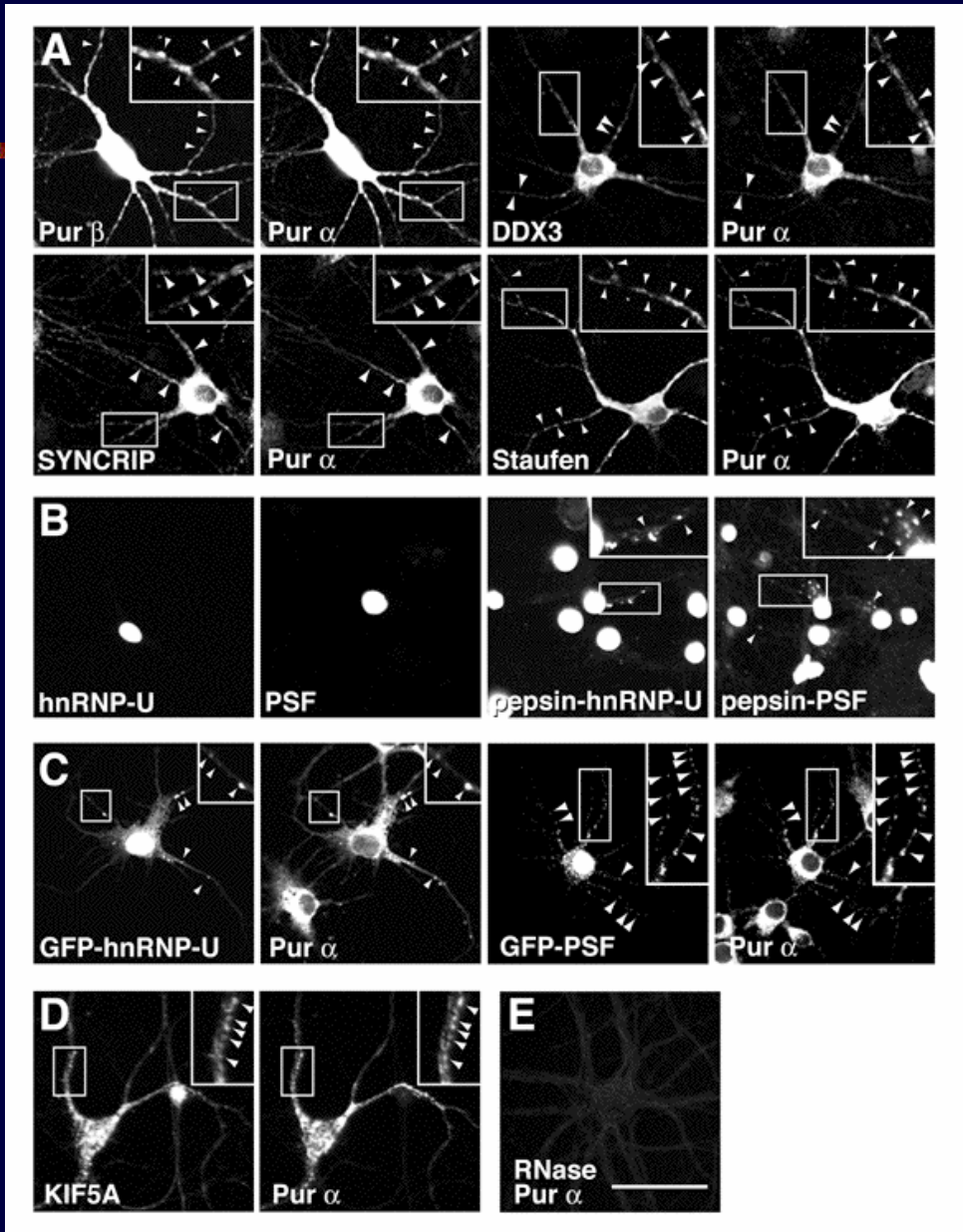
Isolation of a protein-RNA complex using KIF5 tail



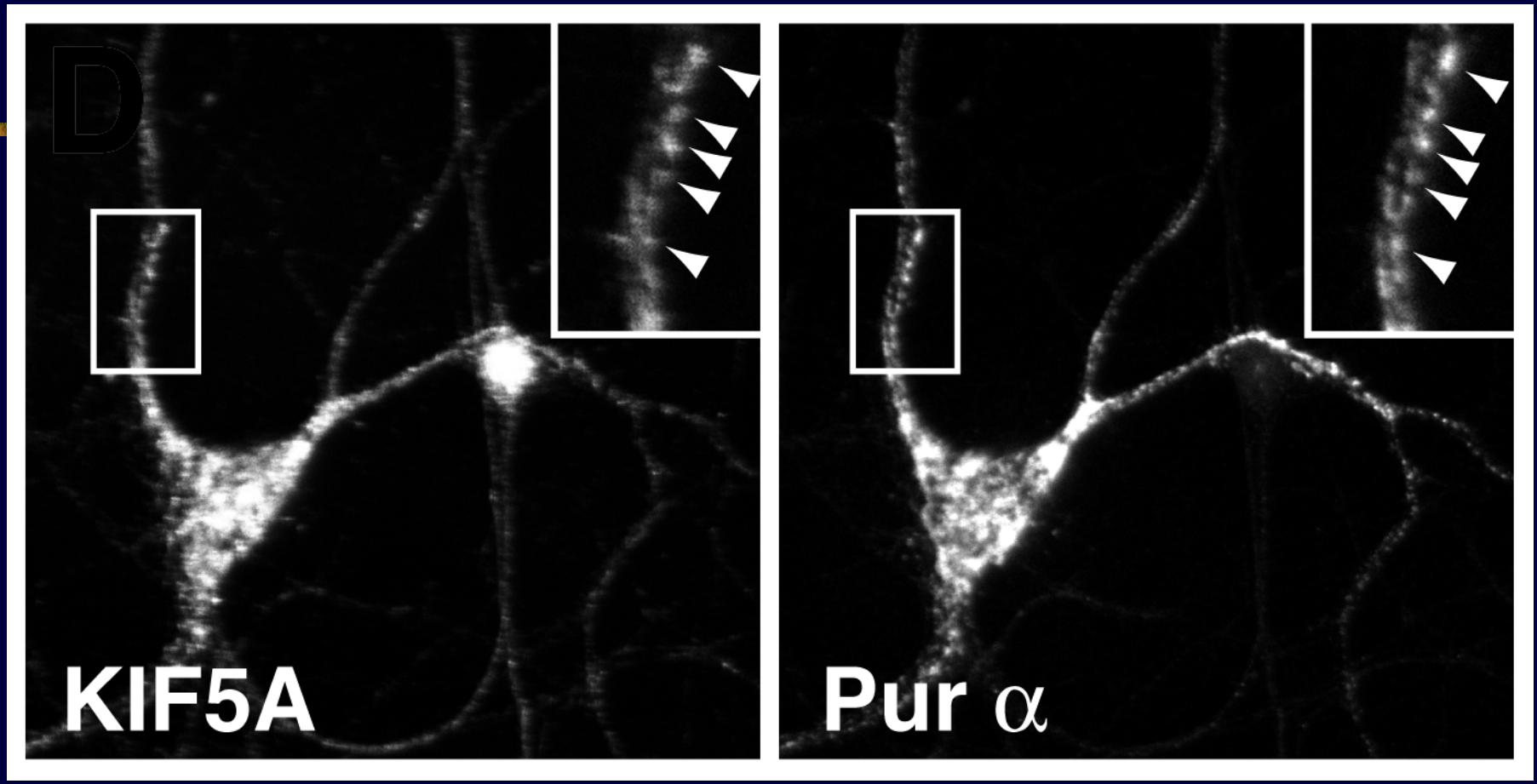
The complex has a large S-value of 1000~



Colocalization of identified proteins and KIF5 to the Pur α-containing granules in dendrites

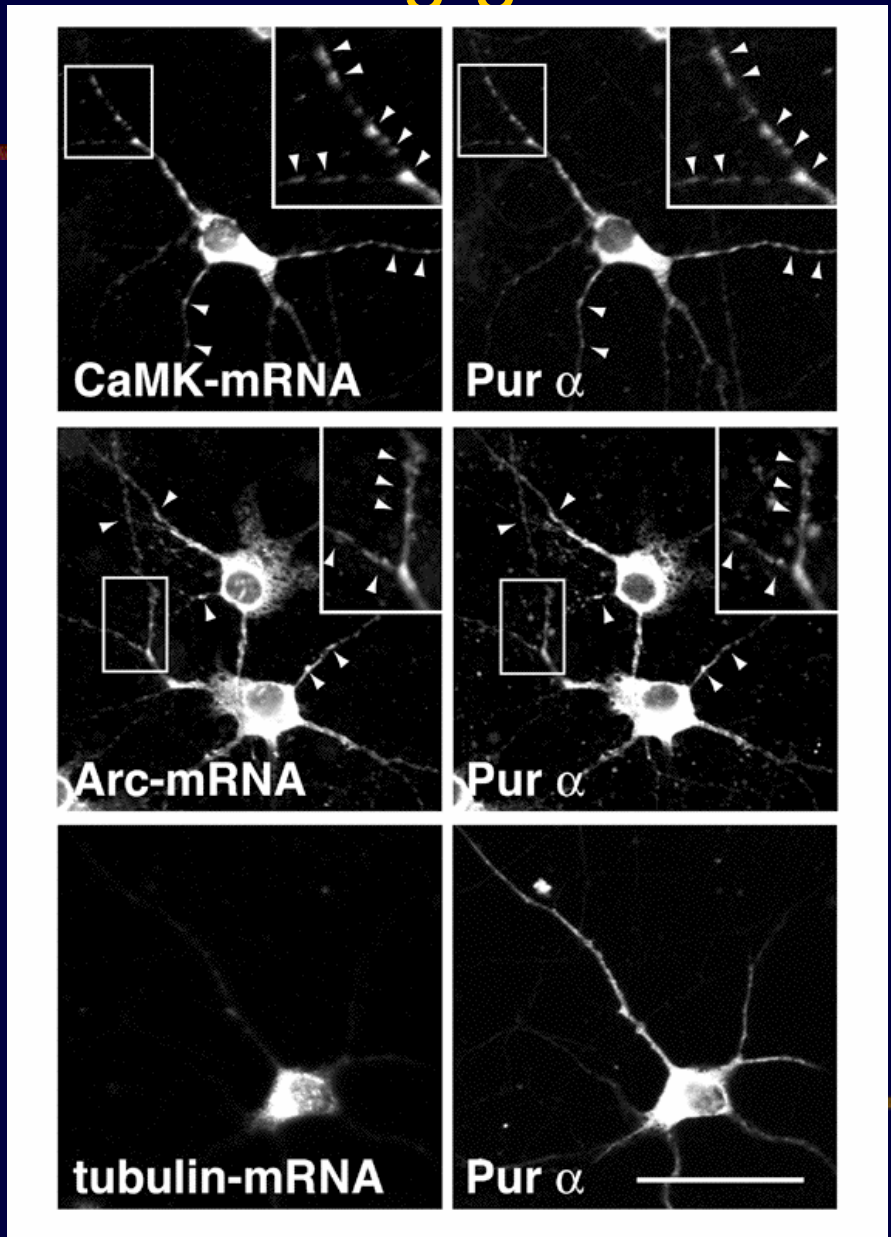


KIF5A in Triton-extracted cultured neurons

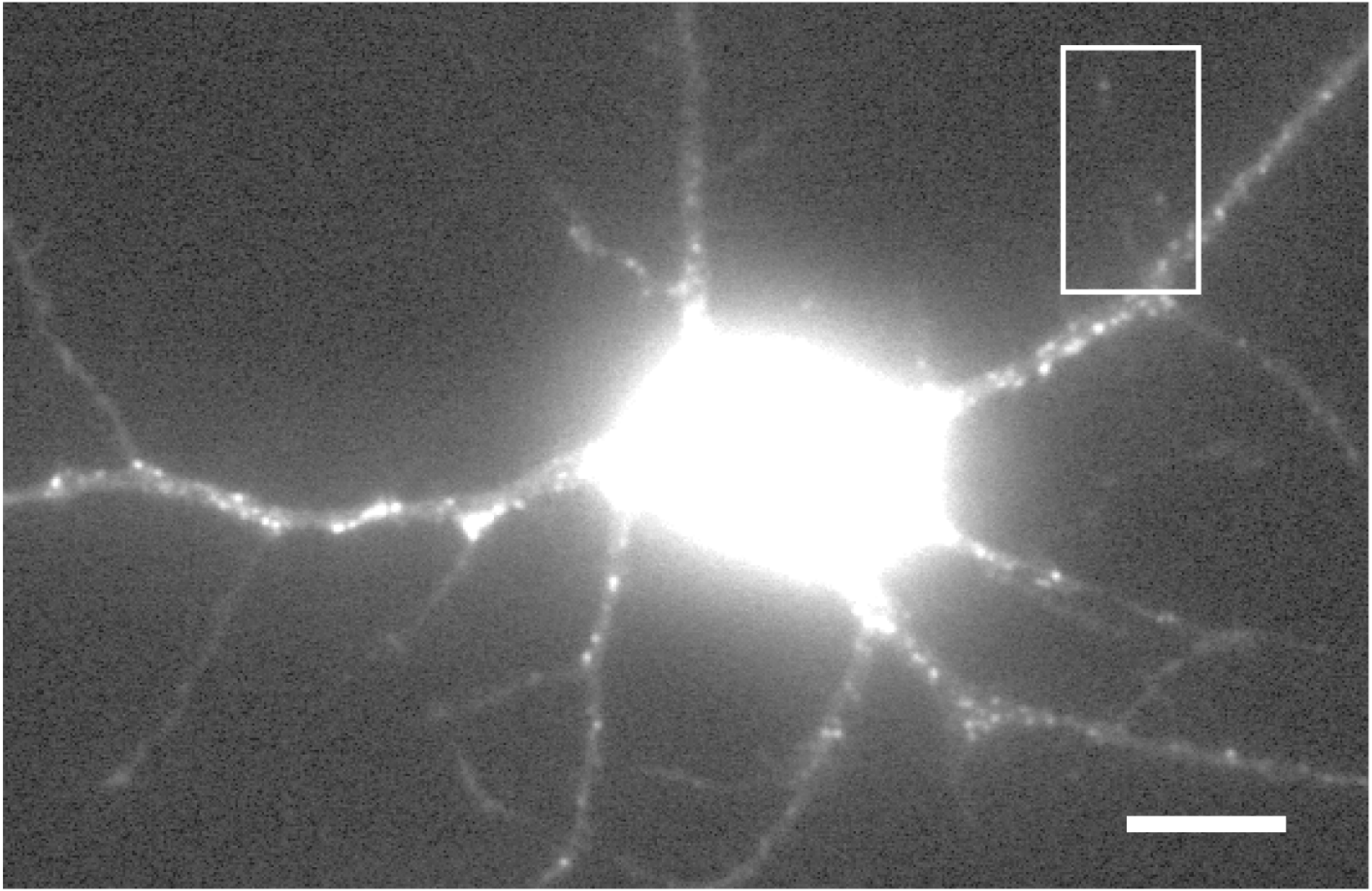


KIF5A colocalized to the Pur-α-containing granules in Triton-extracted neuron

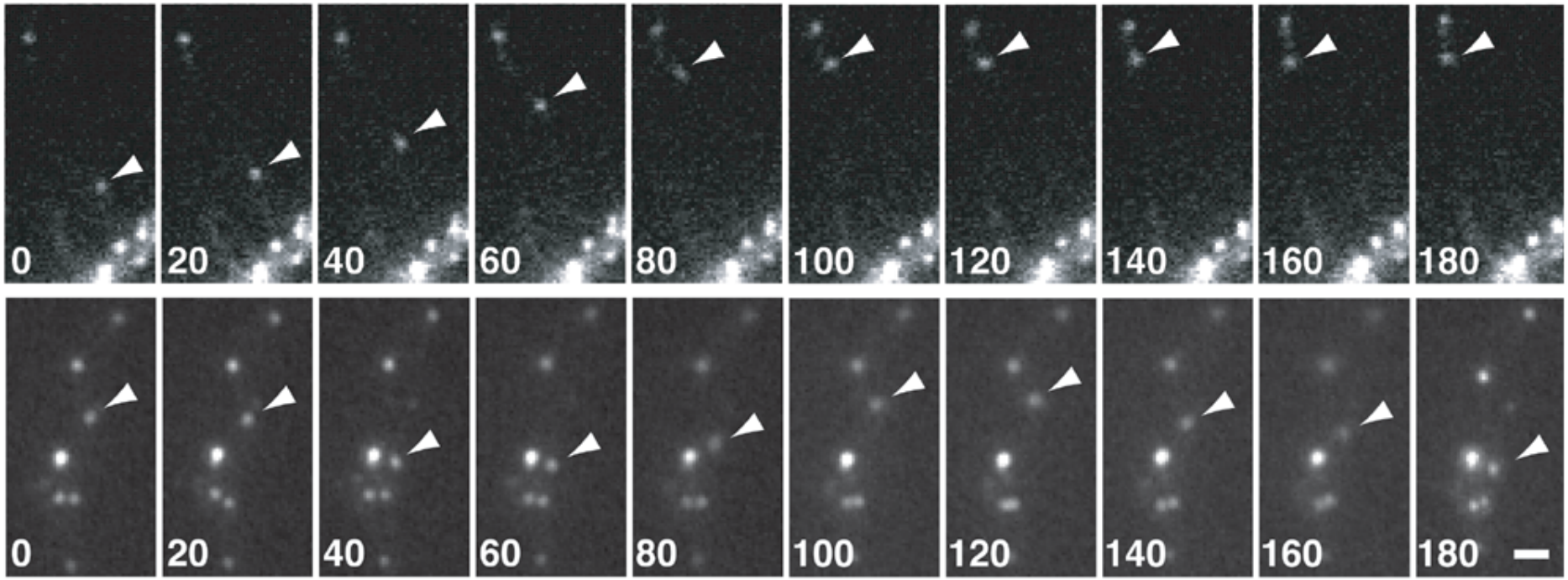
Colocalization of mRNAs for CaMKIIα and Arc to the Pur α-containing granules in dendrites

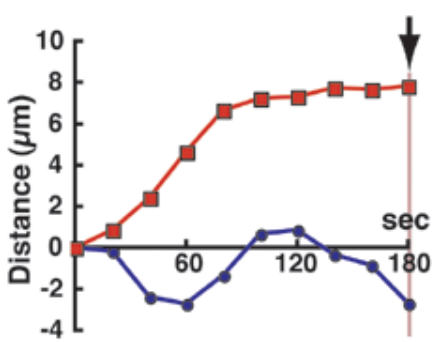


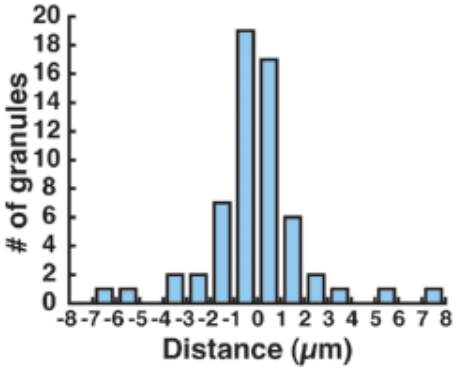
Movement of the complex（GFP-Pur α）

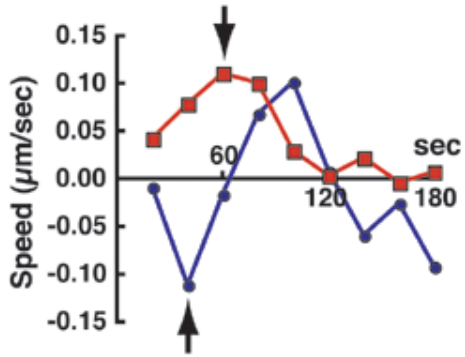


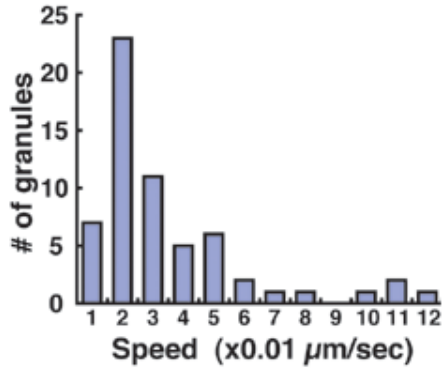
Movement of the complex（GFP-Pur α）



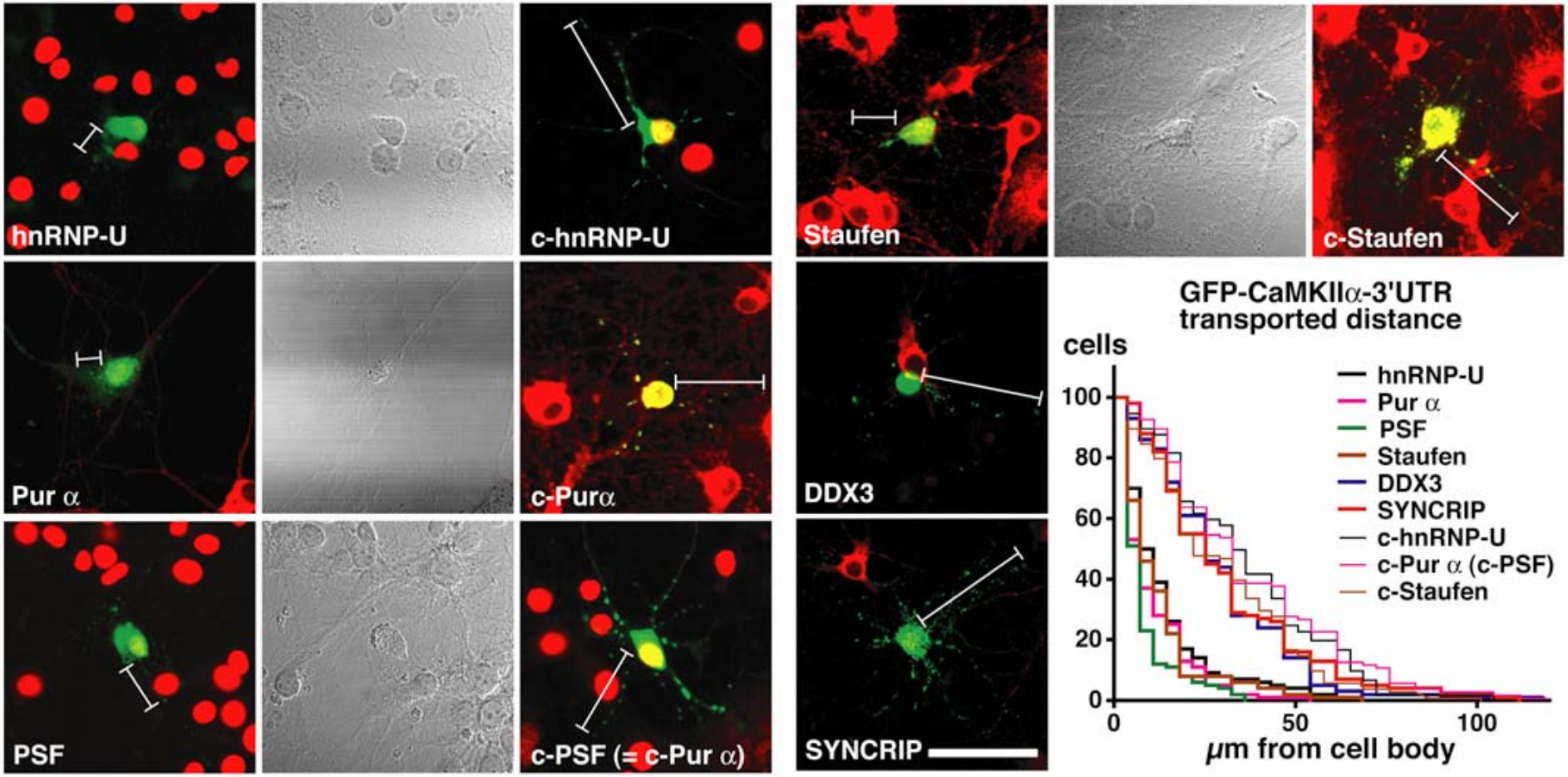




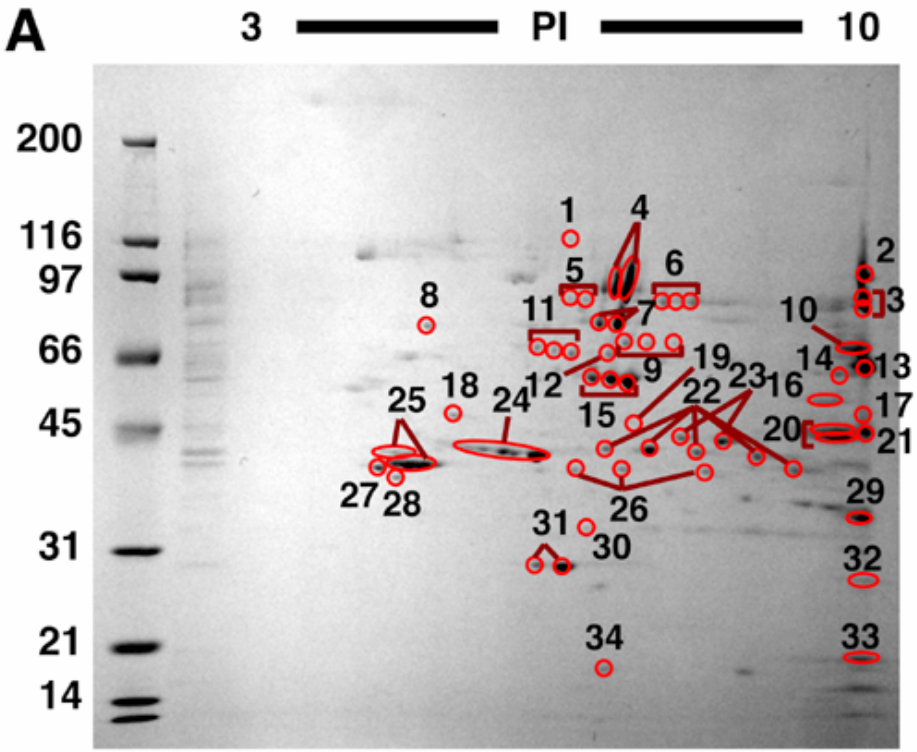




Knockdown of the identified proteins by RNAi



Proteomics analysis of the RNA-transporting granules



B

RNA transport: 6

name	accesion	spot
FMR1	NM_008031	**
FXR1	X90875	**
FXR2	NM_011814	**
Pur α	NM_008989	24*
Pur β	NM_011221	25*
staufen	NM_011490	**

Protein synthesis: 6

EF-1α	NM_007906	**
eIF2α	NM_019356	28
eIF2β	AK012817	18
eIF2γ	NM_012010	16
Hsp70	NM_031165	8
ribosomal protein L3	NC_003143	32

RNA helicases: 3

DDX1	NM_134040	4*
DDX3	NM_010028	7*
DDX5	NM_007840	10

hnRNPs: 5

hnRNP-A/B	NM_010448	26
hnRNP-A0	AK019388	29
hnRNP-A1	NM_010447	29
hnRNP-D	XM_194232	22
hnRNP-U	AF073992	*

Other RNA associated: 12

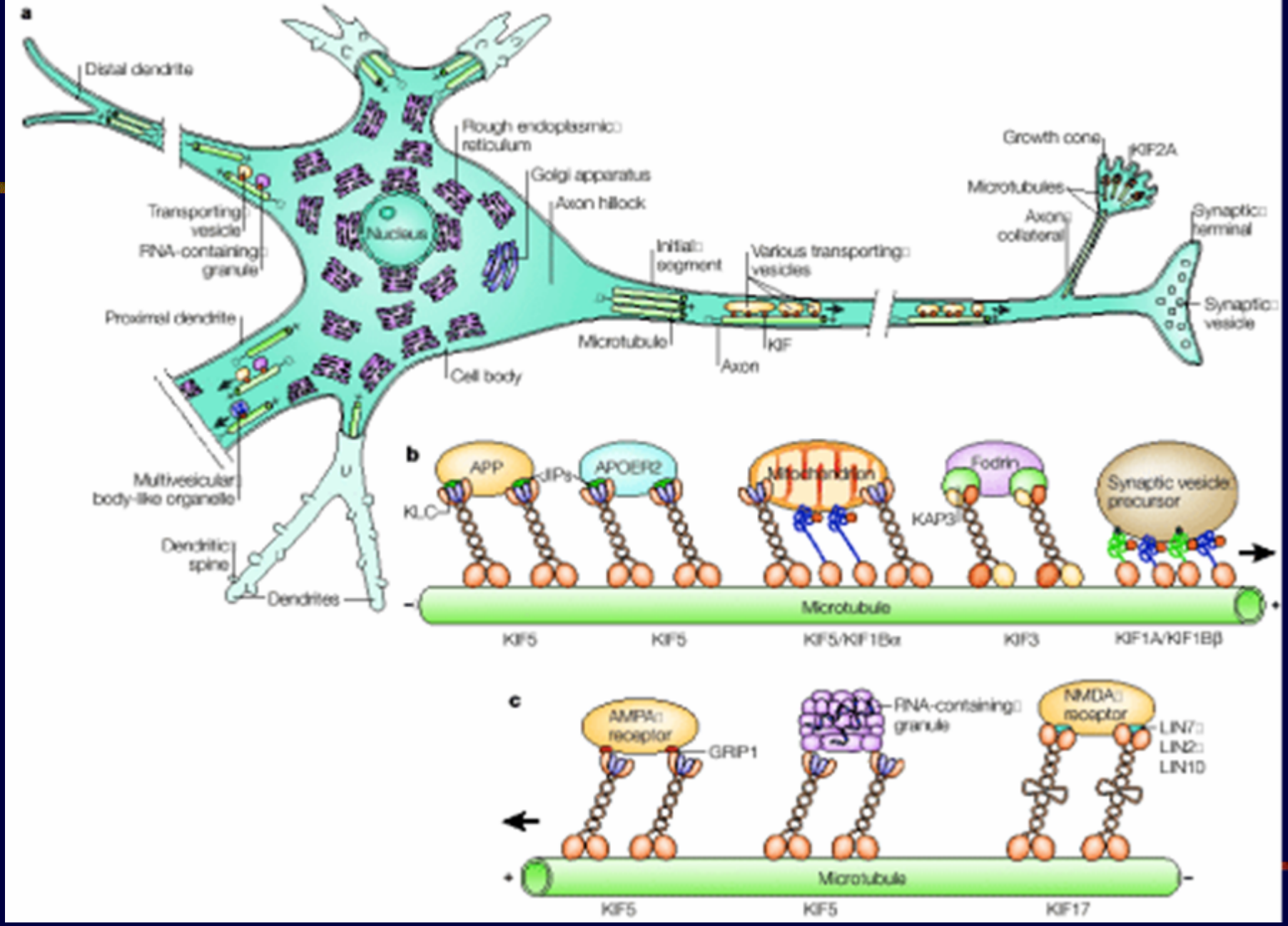
name	accesion	spot
ADP-ribosylation factor-guanine nucleotide factor 6	NM_015310	21
ALY	MMU89876	*
cold inducible RNA-binding protein	NM_007705	33
EWS	NM_007968	3
NonO	NP_075633	14*
Nucleolin	Q99K50	1
paraspeckle protein 1	BC026772	11
PSF	NM_023603	2*
RNA 3'-terminal-phosphate cyclase	NM_025517	23
RNA binding motif protein 3	BC059098	34
SYNCRIP	AB035725	9*
TLS	NM_139149	13*

Other known proteins: 3

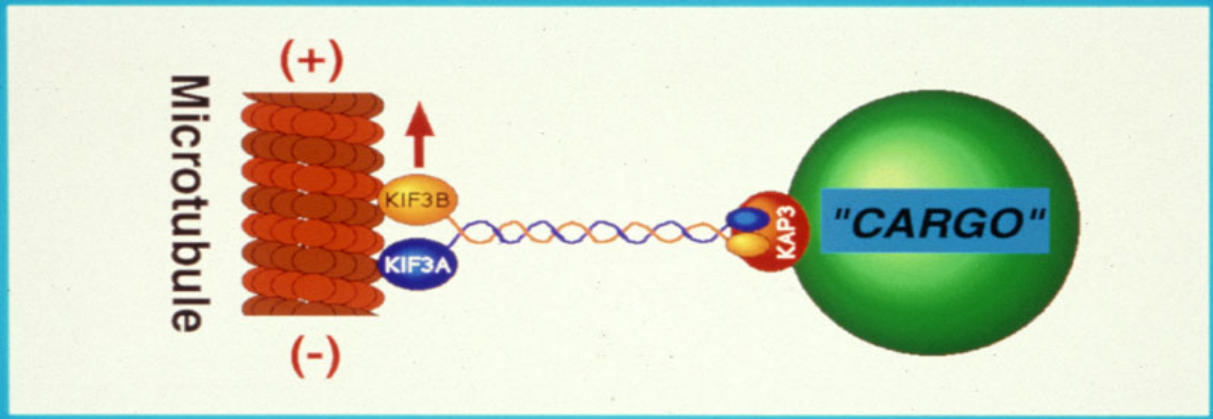
Ser / Thr kinase receptor-associated protein	NM_011499	27
TRIM2	NM_030706	5
TRIM3	NM_018880	6

Hypothetical proteins: 7

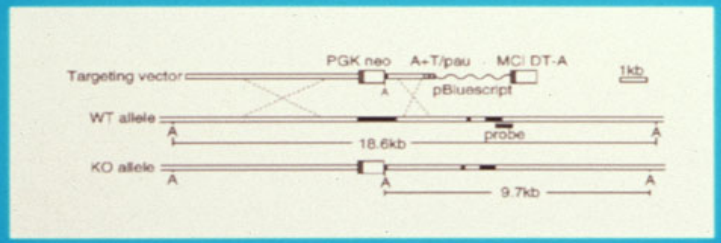
CGI-99	NM_026528	31*
FLJ38426	BC048087	20
HSPC-117	NM_145422	15*
zfp385	BC017644	17
2610528C06Rik	BC037640	12
5730436H21Rik	NM_134139	19
6720458F09Rik	NM_177374	30


a
Distal dendrite
Rough endoplasmic reticulum
Golgi apparatus
Axon hillock
Transporting vesicle
RNA-containing granule
Nucleus
Initial segment
Various transporting vesicles
Growth cone
KIF2A
Microtubules
Axon collateral
Synaptic terminal
Synaptic vesicle
Proximal dendrite
Microtubule
KIF
Axon
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

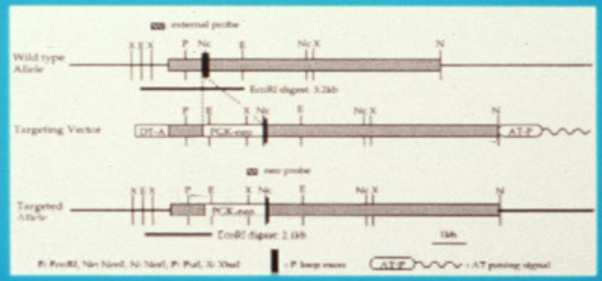
KIF3 Complex
= KIF3A + KIF3B + KAP3



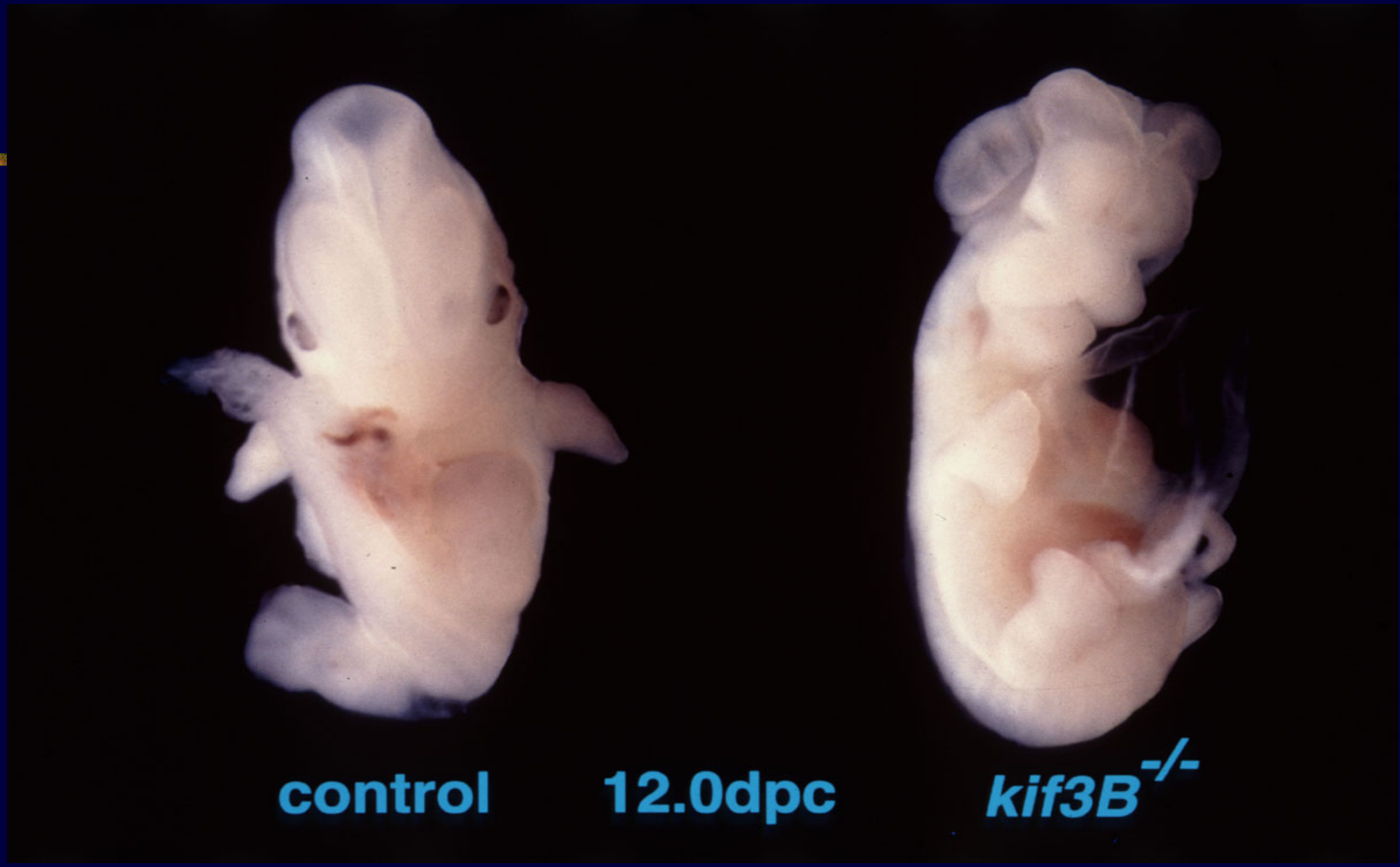
kif3B Knockout

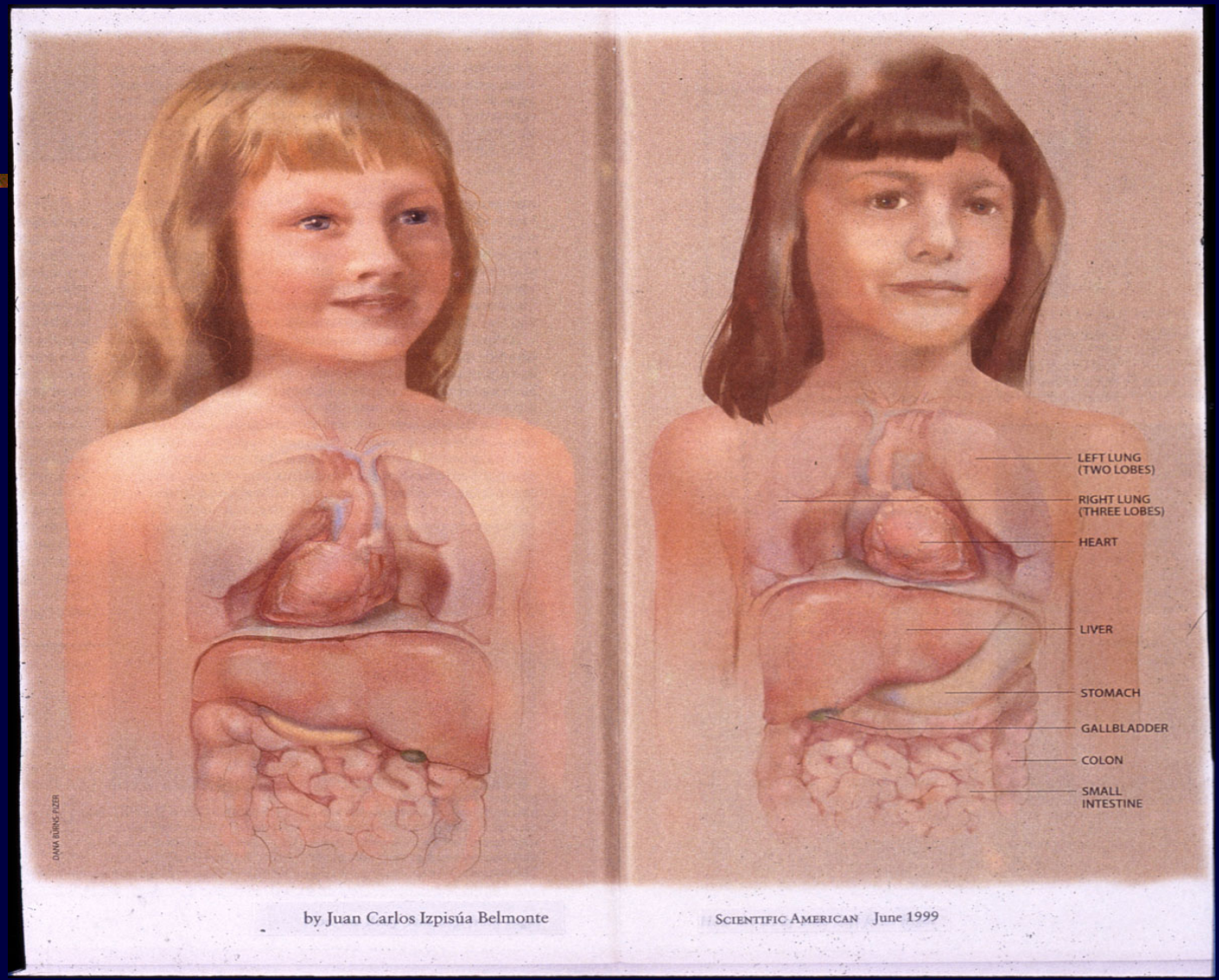


kif3A Knockout



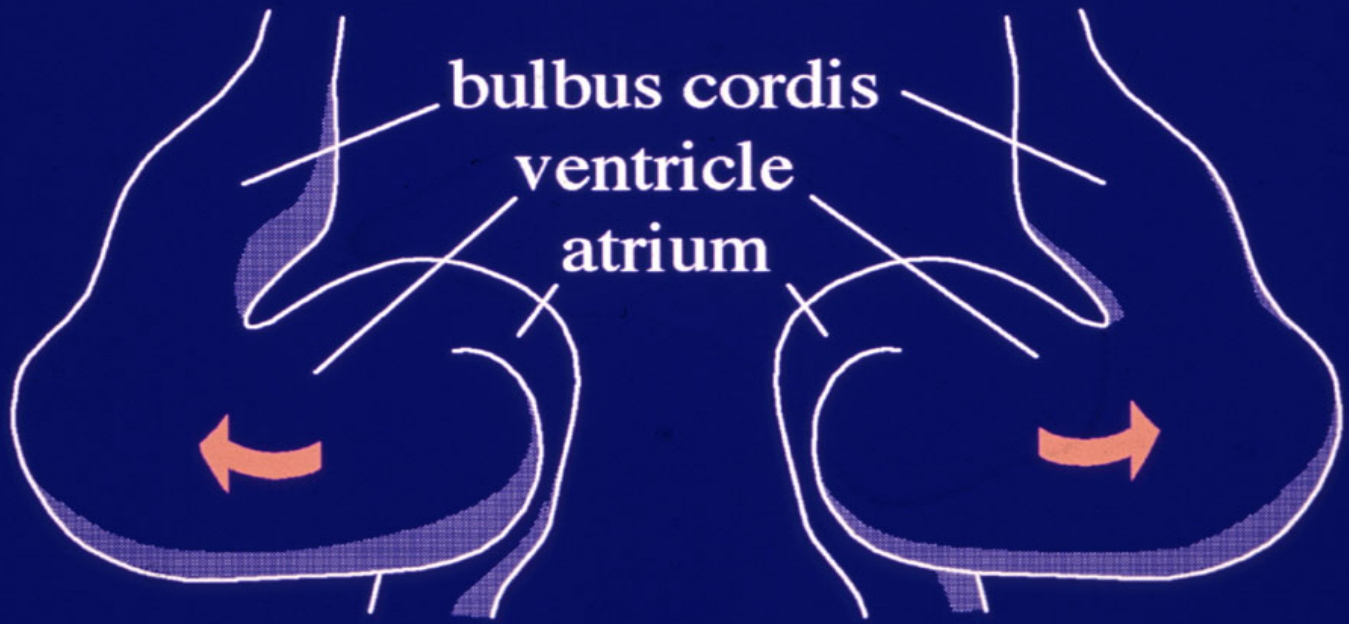
Aizawa et al. JCB 119:1287－, 1992; Yamazaki et al. JCB 130:1387－,1995
Nonaka et al. Cell 95:829－,1998; Takeda et al. JCB 145:825－,1999


control
12.0dpc
kif3B-/-

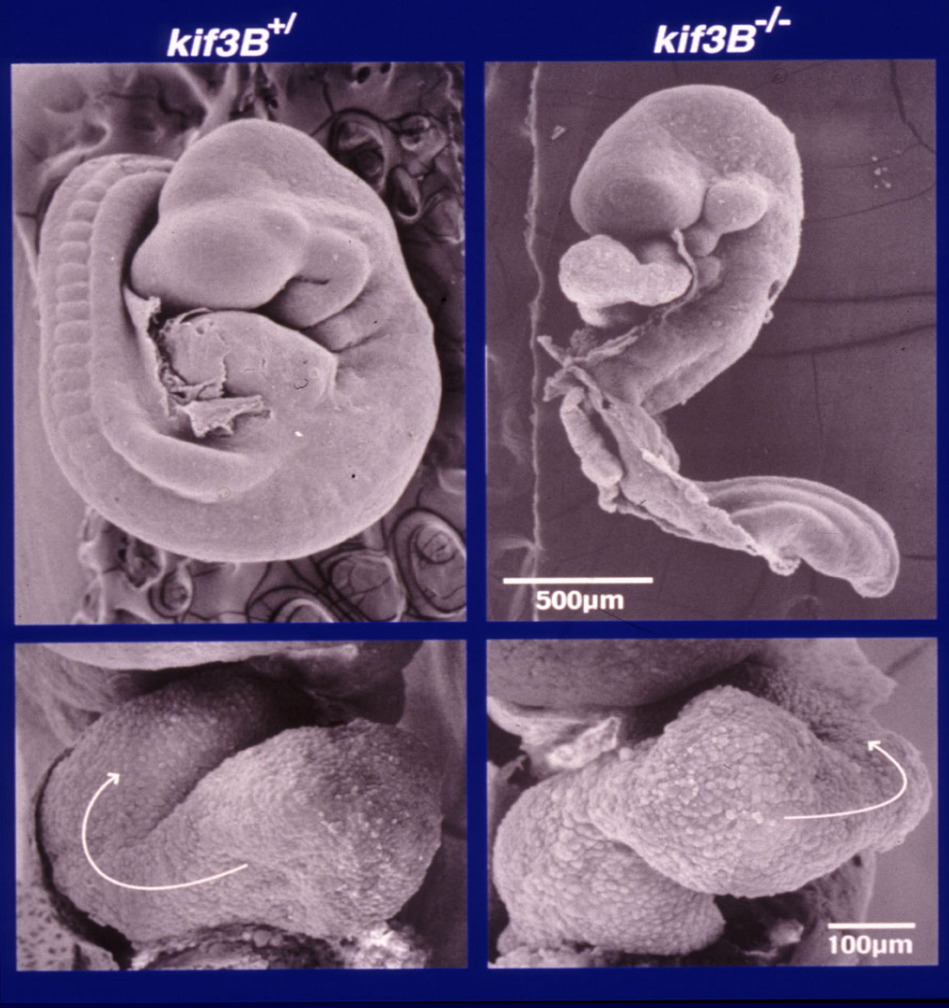


by Juan Carlos Izpisúa Belmonte

Cardiac Loop at 9.5dpc



Heart Looping & Tail Curling



L-R Determination Pathway

7.5 dpc
node

8.0 dpc
somitegensis

8.5 dpc
heart looping

9.0 dpc
embryonic turning

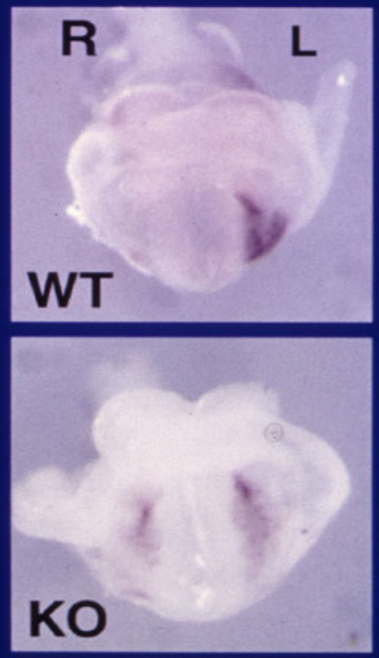
iv (randomization)
inv (inversion)

?

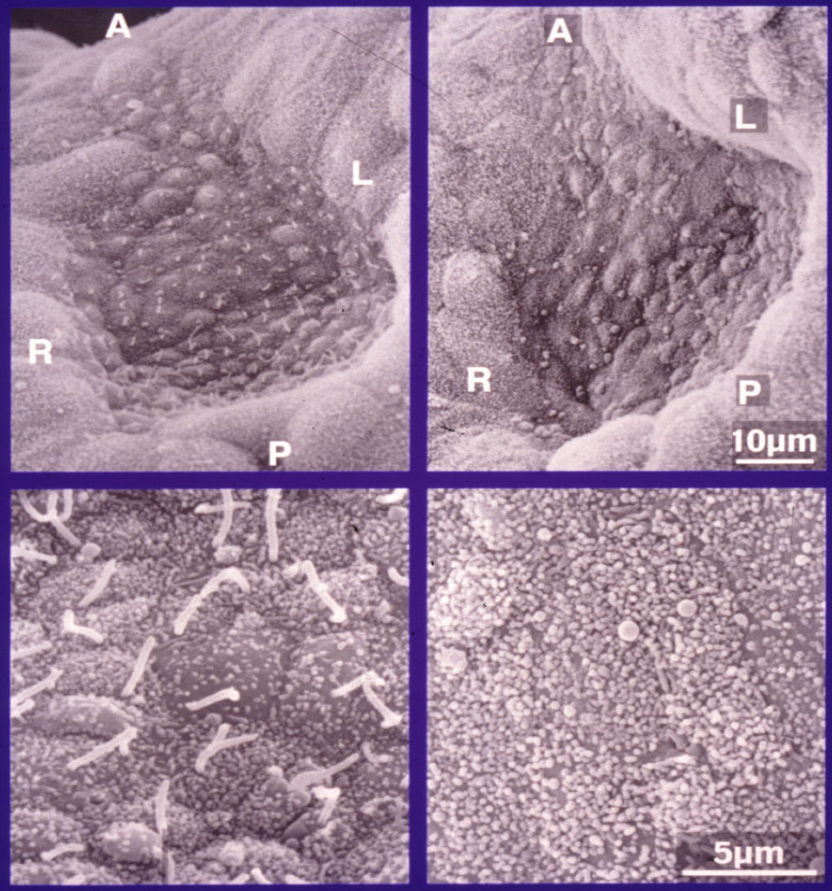
R L

nodal
lefty-1
lefty-2
Pitx2

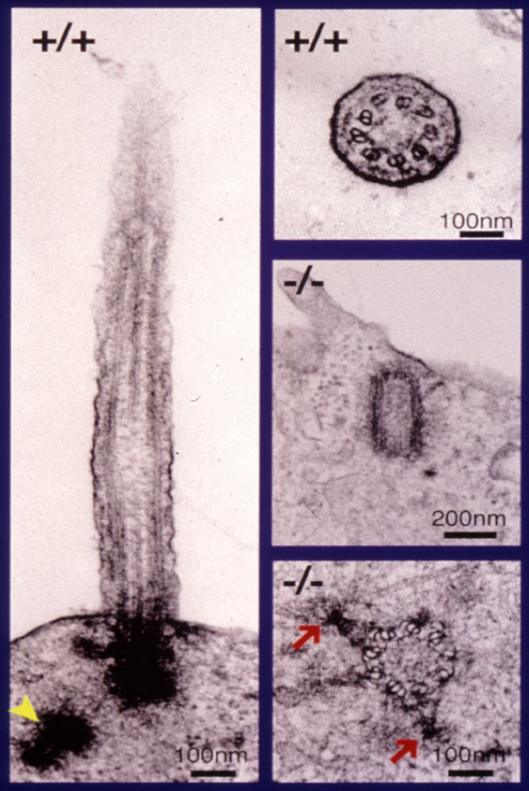
lefty-2 expression



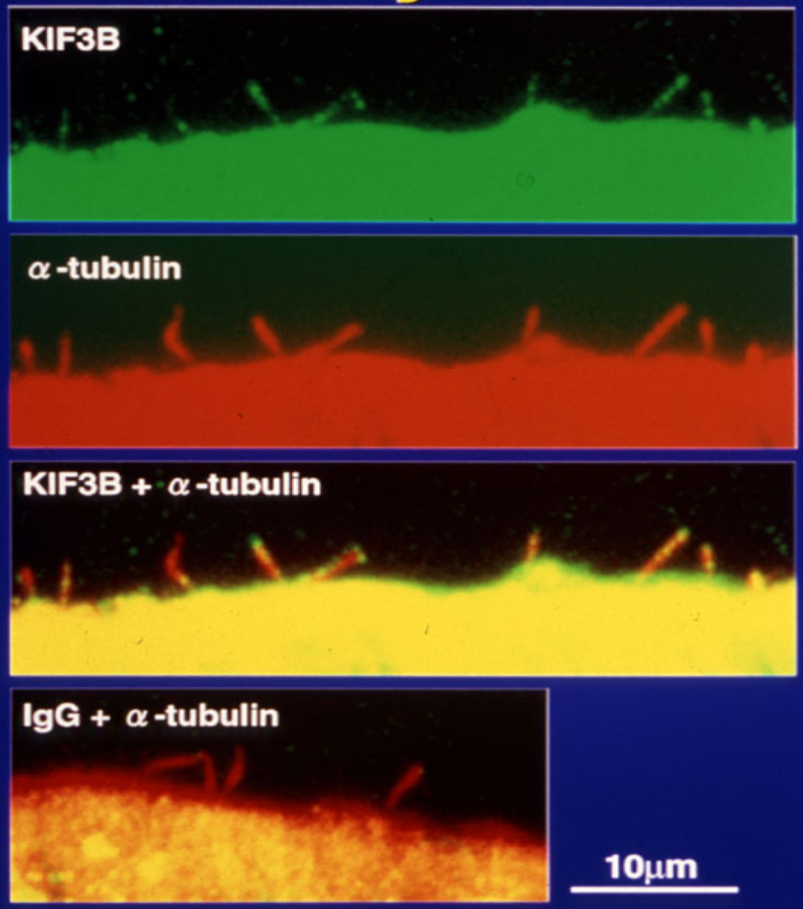
Monocilia of the Node

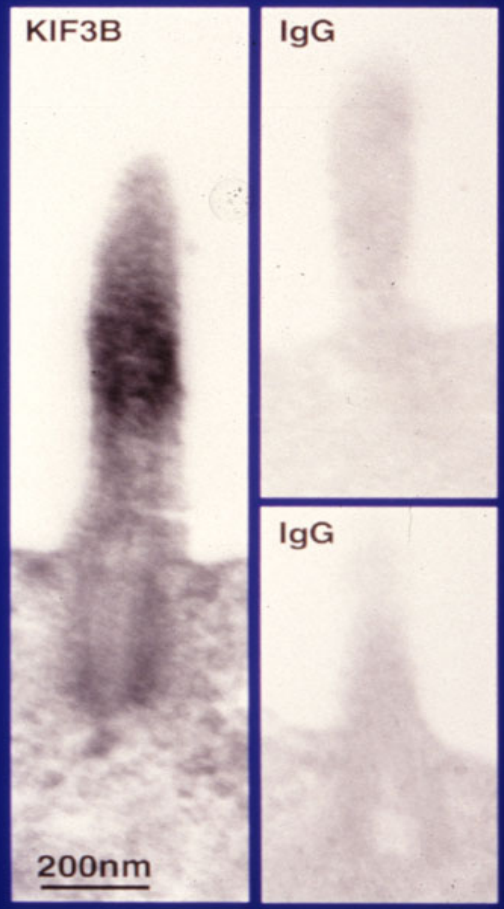


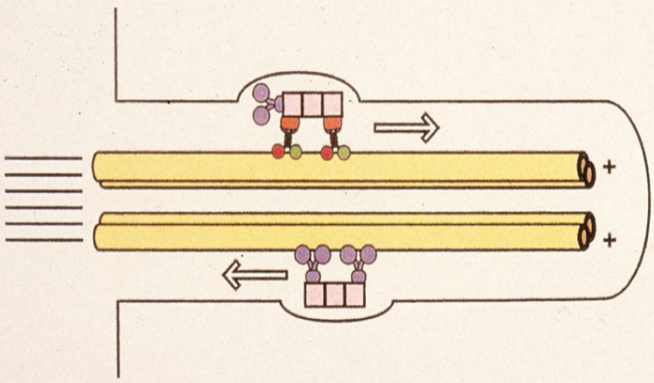
Loss of Primary Cilium Leaving Intact Basal Bodies



Ciliary Staining of KIF3B



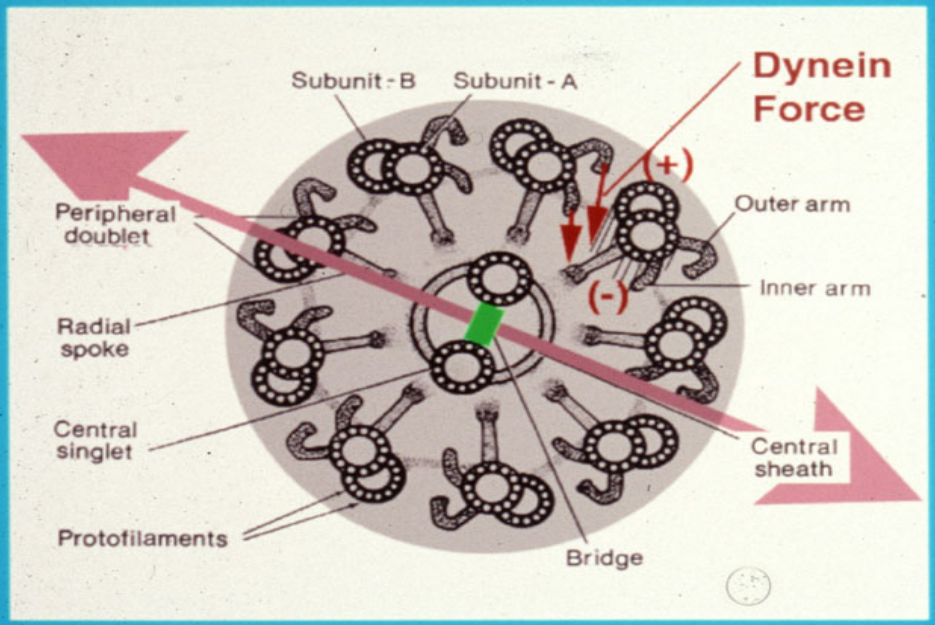




Primary cilium of nodal cell
Cilia, Flagella

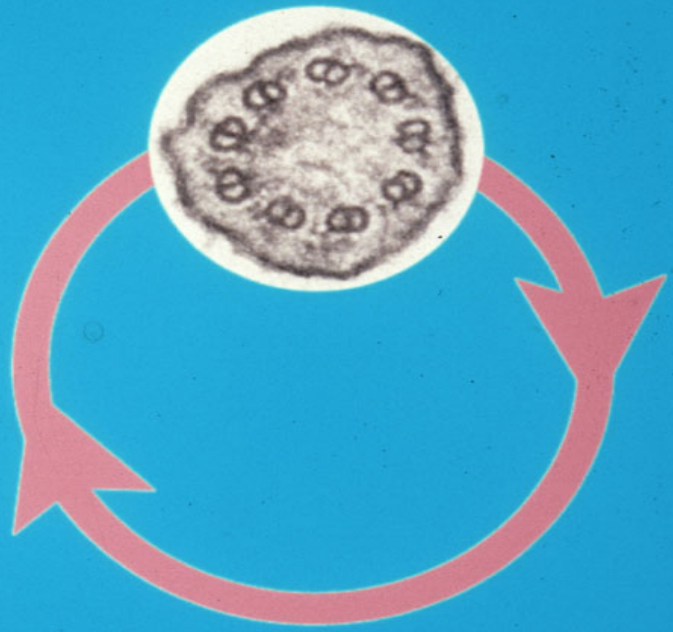
Cilia Have Clockwise Chirality

9+2: Beating
(Respiratory Cilia)



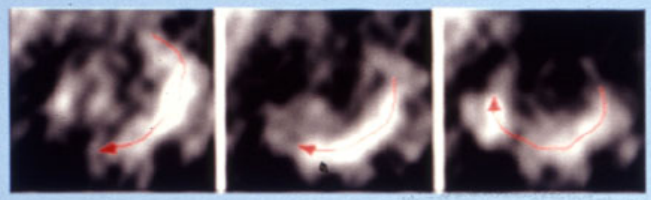
(Modified from Fawcett, *The Cell*, 1981)

9+0: Rotation
(Nodal Cilia)

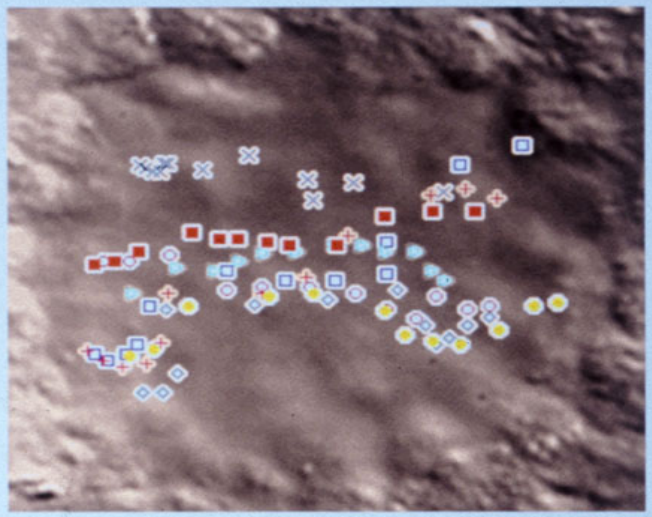


(Takeda et al., *J Cell Biol* 145:825, 1999)

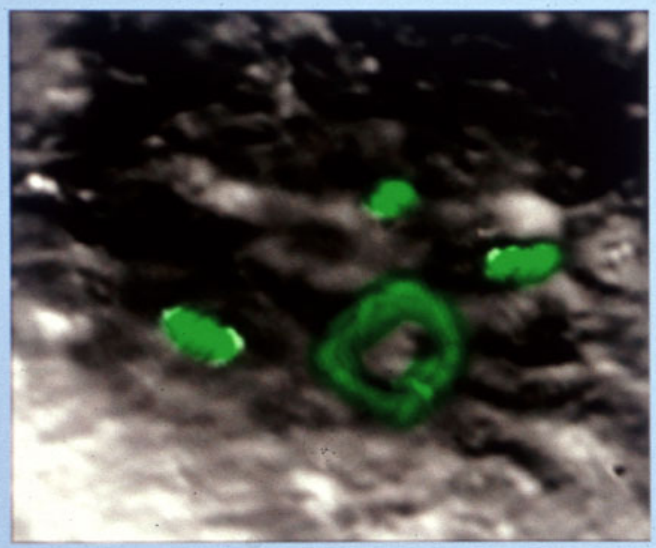
Rotation of Nodal Cilia & Leftward Nodal Flow



5μm



R L

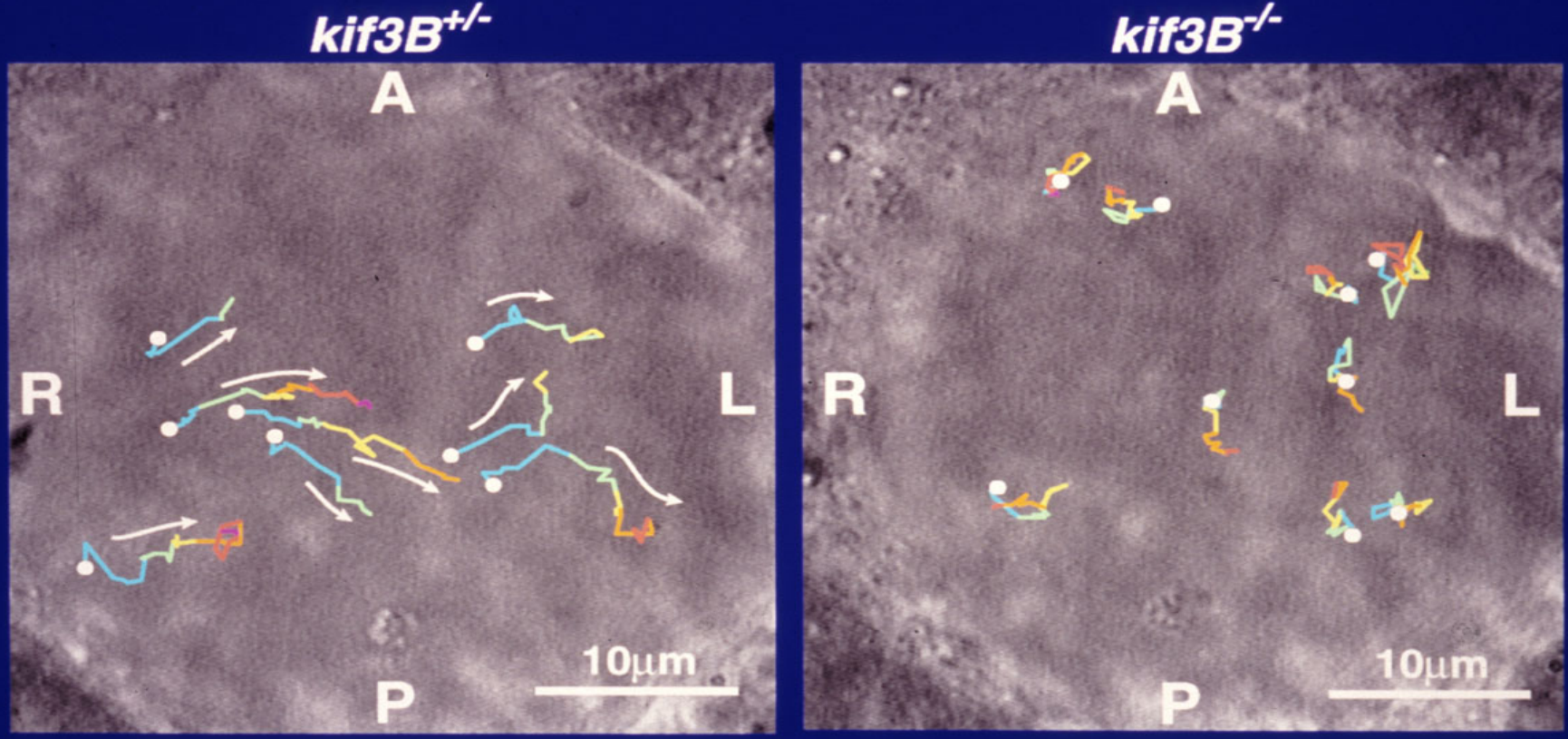


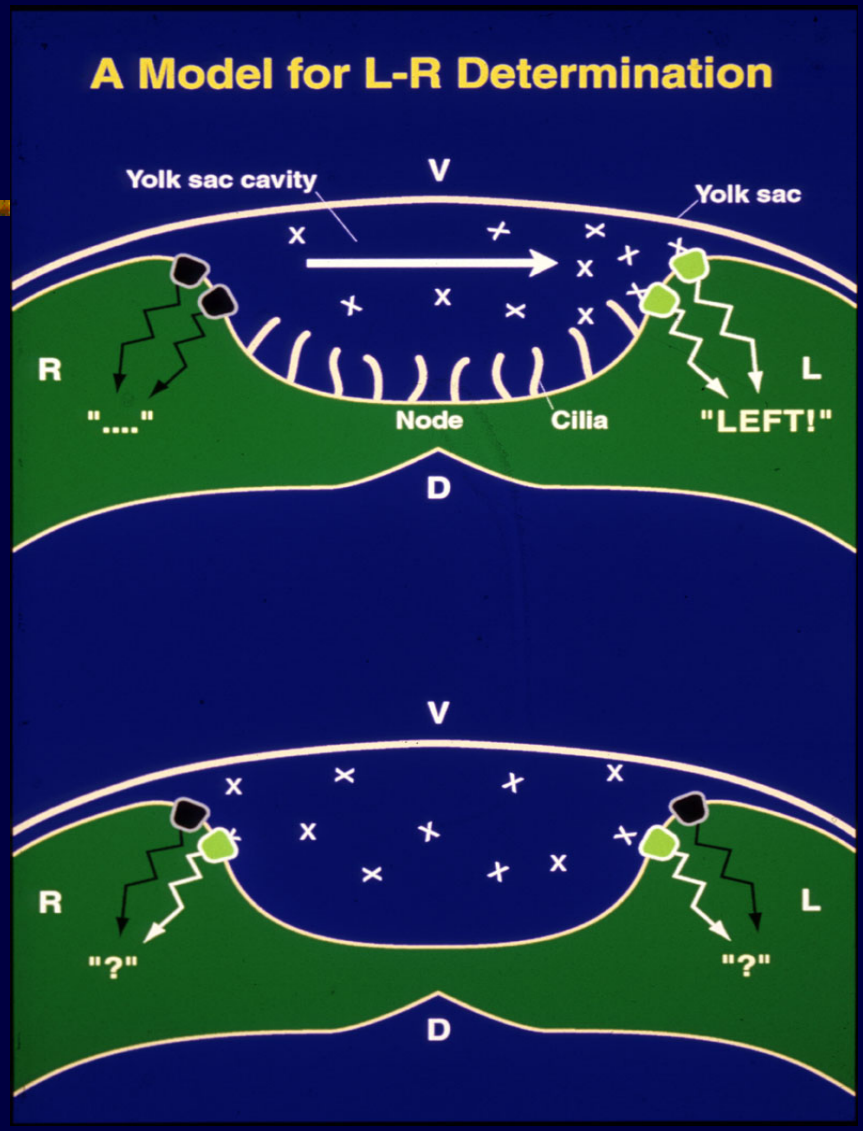
R L

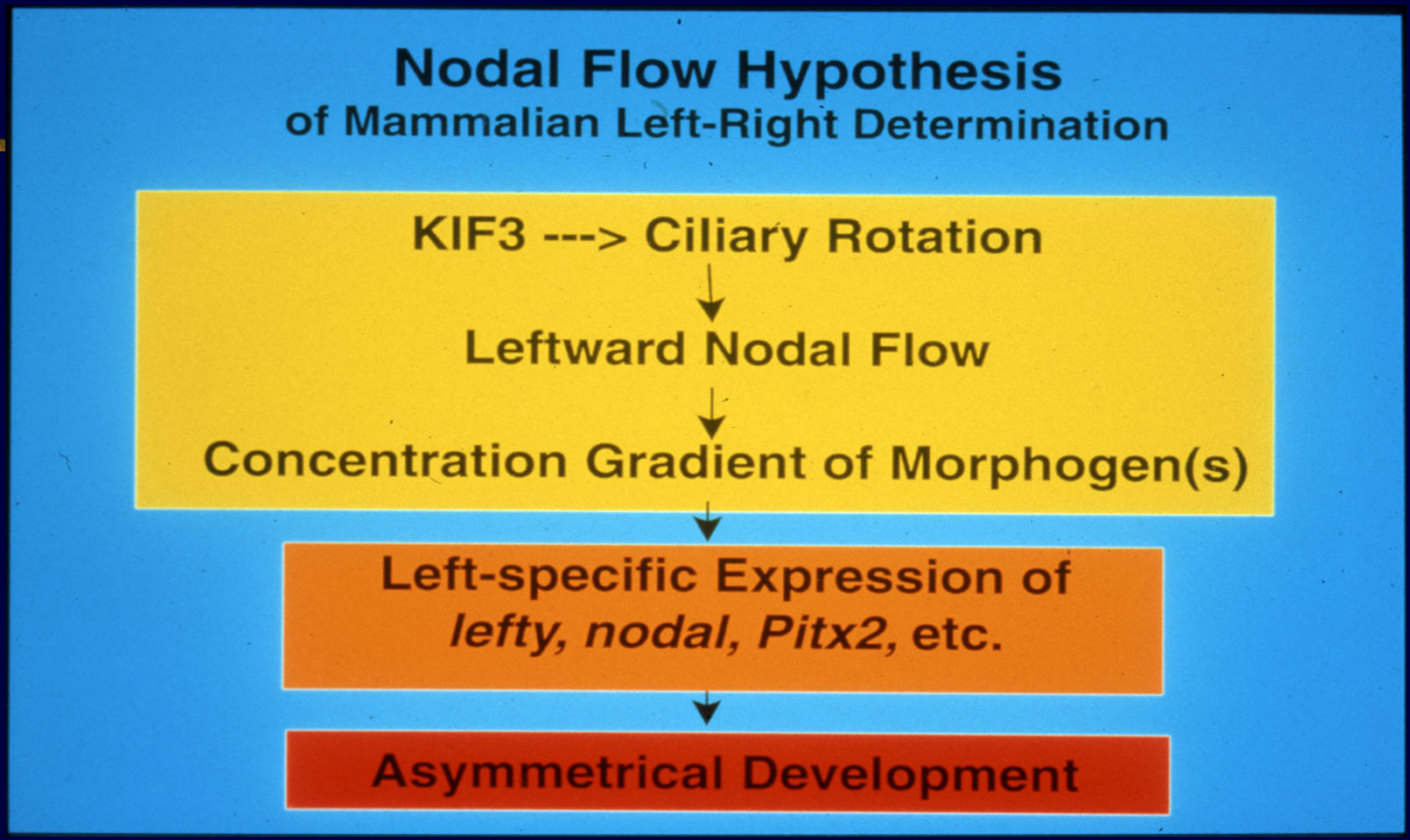
R L

20μm

KIF3B KO Disrupts The Nodal Flow




A Model for L-R Determination
Yolk sac cavity
V
Yolk sac
R
L
"...."
Node
Cilia
"LEFT!"
D
V
R
L
"?"
"?"
D


Nodal Flow Hypothesis
of Mammalian Left-Right Determination
KIF3 ---> Ciliary Rotation
Leftward Nodal Flow
Concentration Gradient of Morphogen(s)
Left-specific Expression of
lefty, nodal, Pitx2, etc.
Asymmetrical Development

Kartagener's Syndrome

Immotile Cilia syndrome

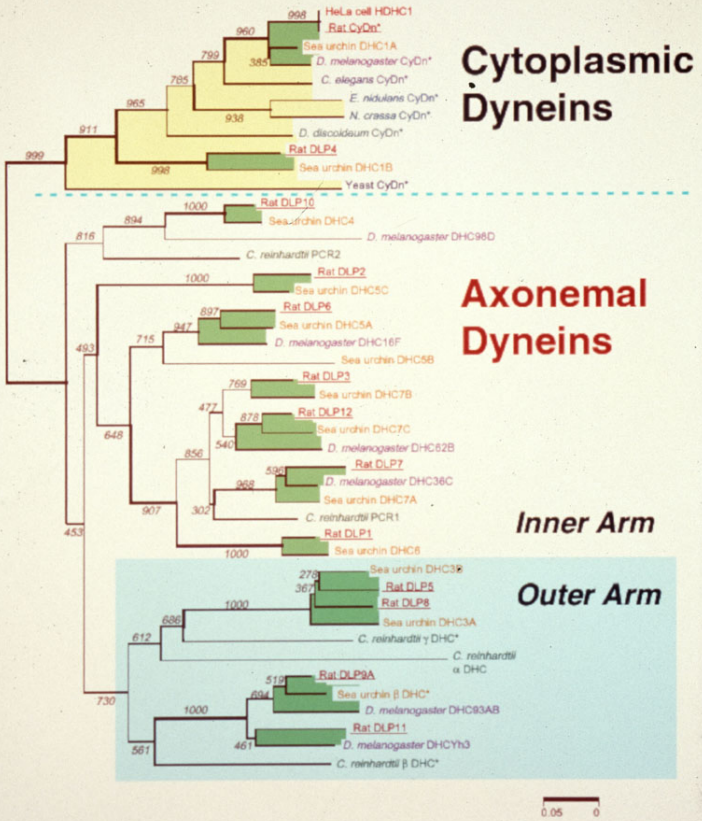
Immotile Cilia
(Male Infertility + Respiratory Failure etc.)

?

Situs Inversus

...Why don't the 9+0 Nodal Cilia MOVE?

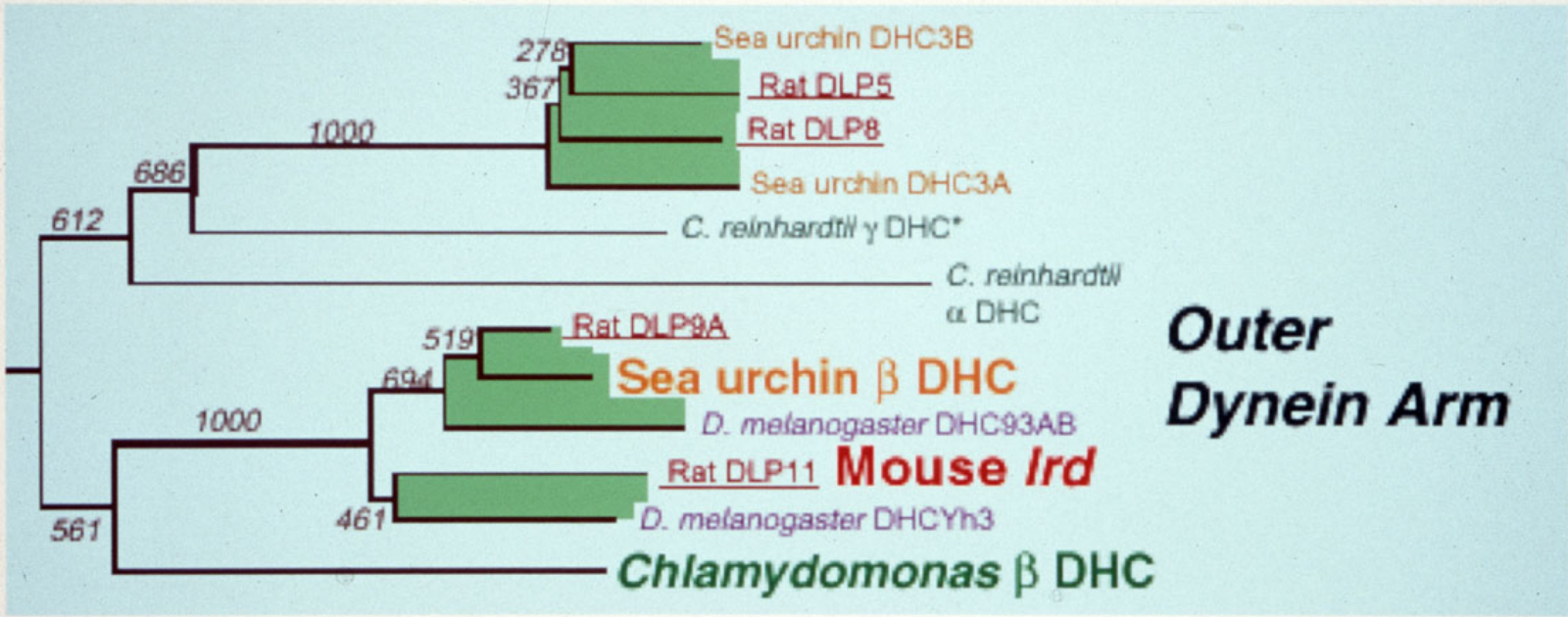
The Dynein Superfamily



Tanaka et al., *J Cell Sci* 108:1883 (1995)

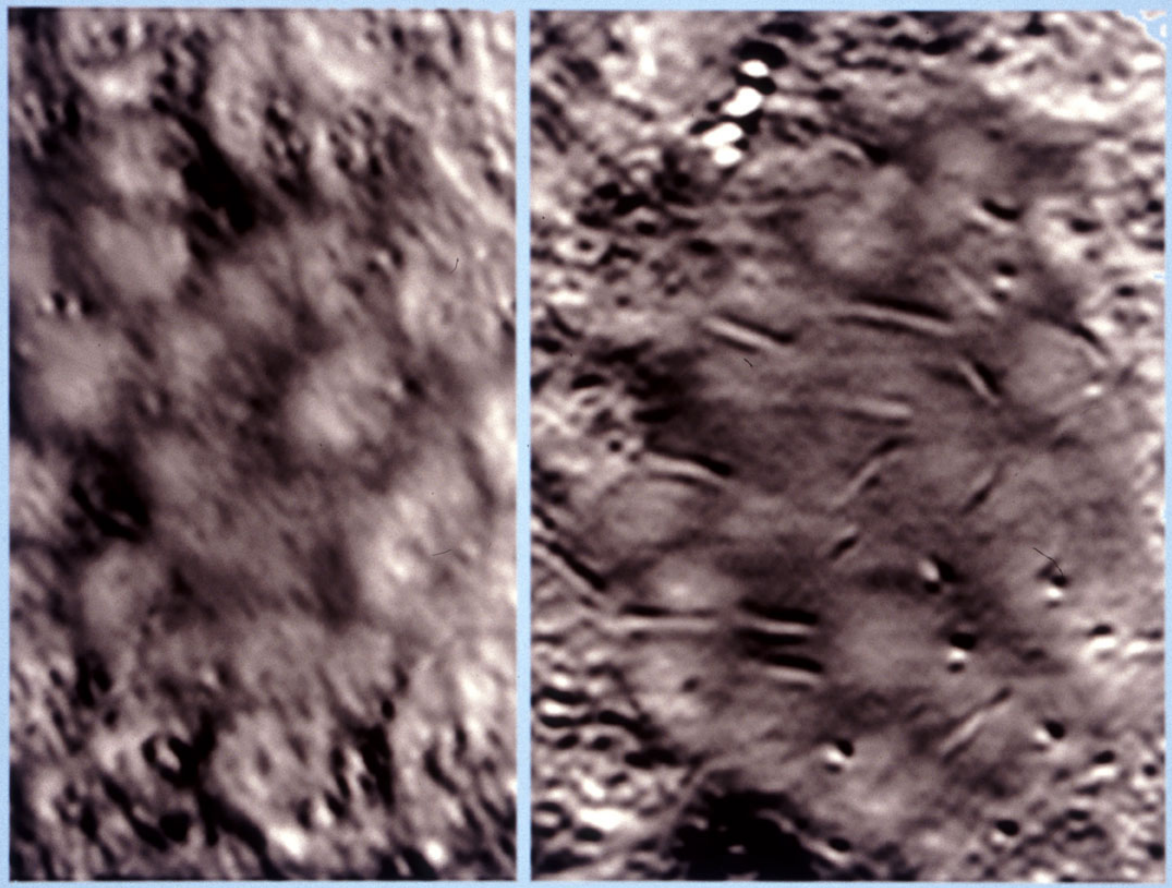
lrd/DLP11 Is An Outer-Arm β Dynein

(Supp et al., *Nature* 389:963, 1997)



Tanaka et al., *J Cell Sci* 105:1883 (1995)

Frozen Cilia in *iv*

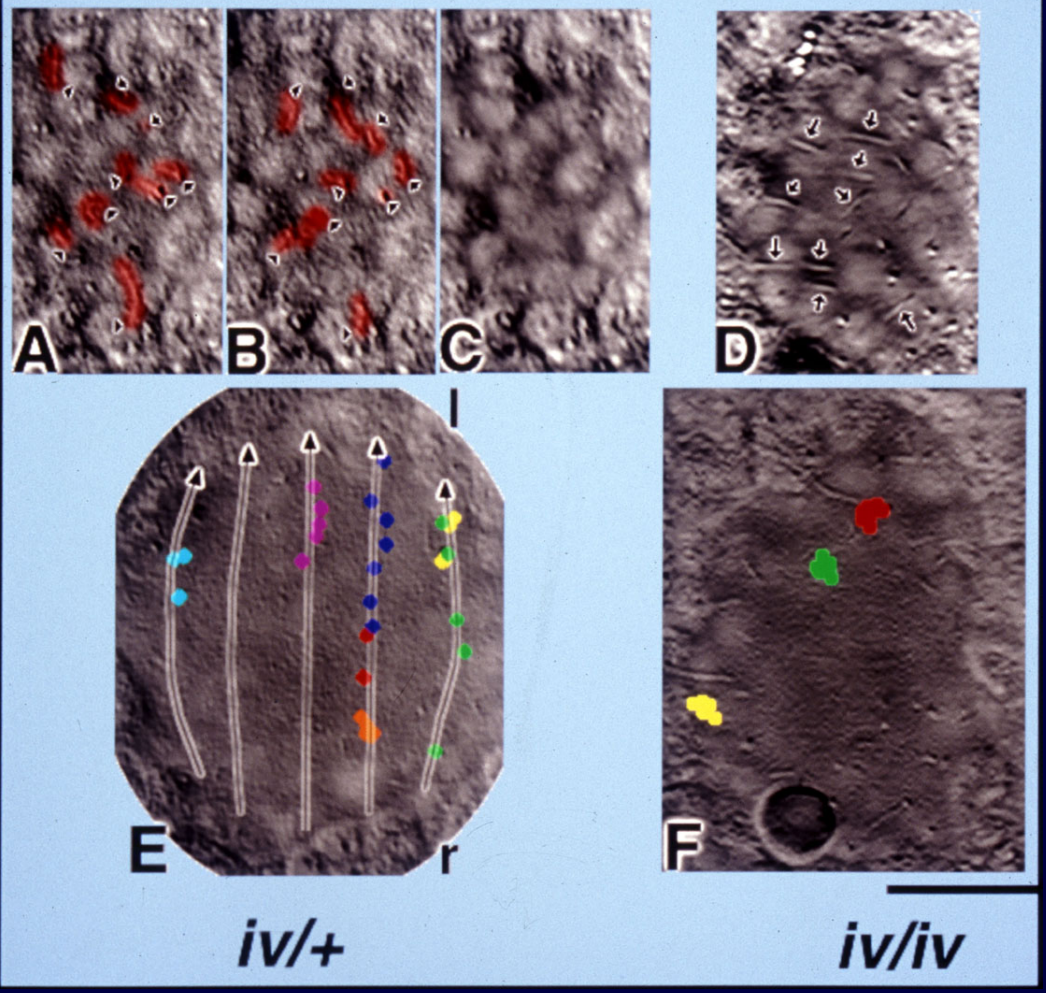


iv/+

iv/iv

2s exp

Frozen Cilia in *iv*



Kartagener's Syndrome

Immotile Cilia syndrome

Immotile Cilia
(Male Infertility + Respiratory Failure etc.)

?

Situs Inversus

...Why don't the 9+0 Nodal Cilia MOVE?

Why our hearts are on left?

- Left side is determined at the early stage of development.
- In some genetic diseases, patients have their hearts on right.
- In Kartagener’s syndrome, immotile cilia in airway epithelium and immotile sperm are linked to right heart.

Motile cilia are necessary for the L-R axis determination.

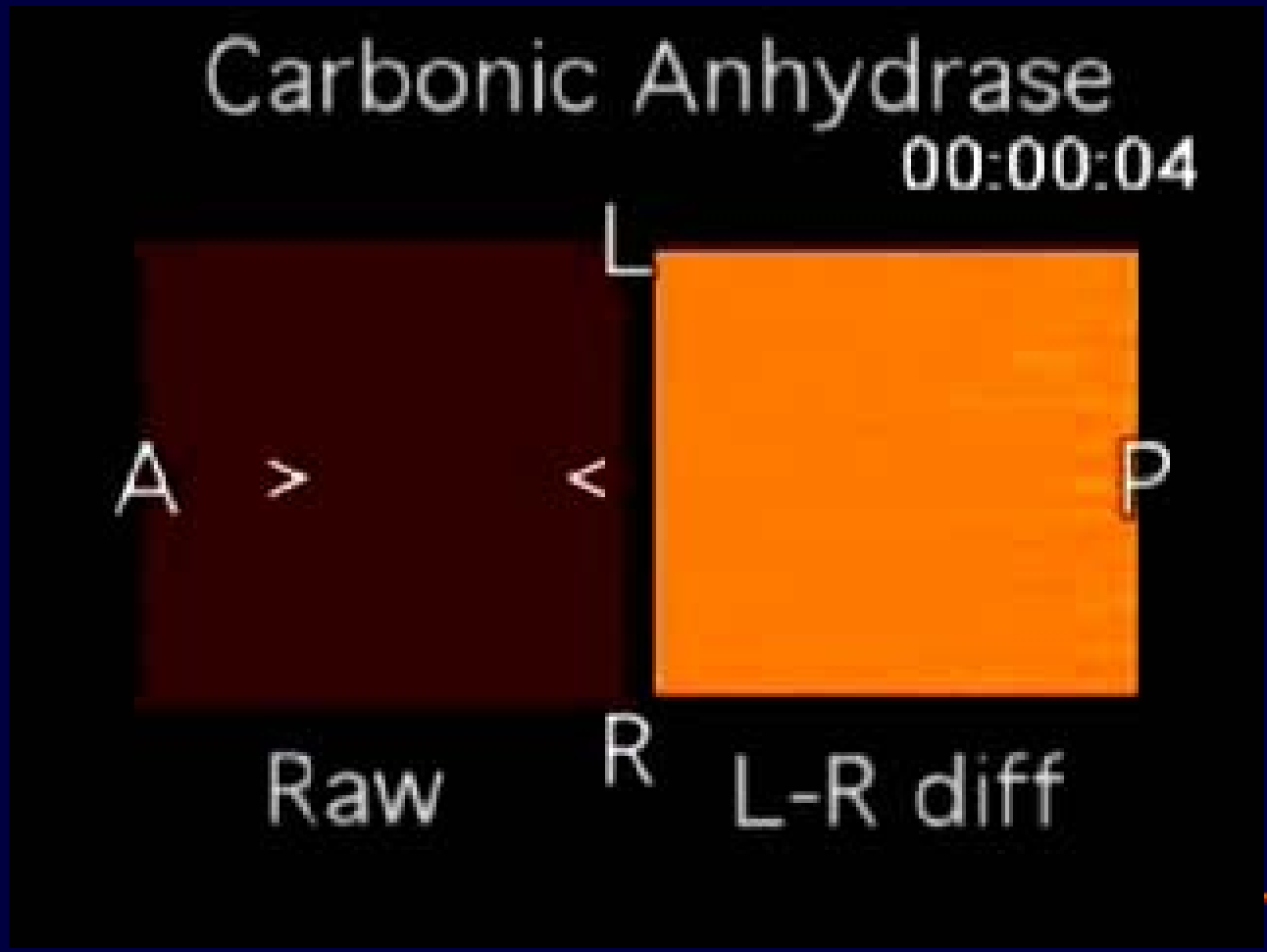
Randomization of the L-R axis in

1) immotile cilia syndrome (human)
2) ciliogenesis mutant mice ($KIF3^{-/-}$)

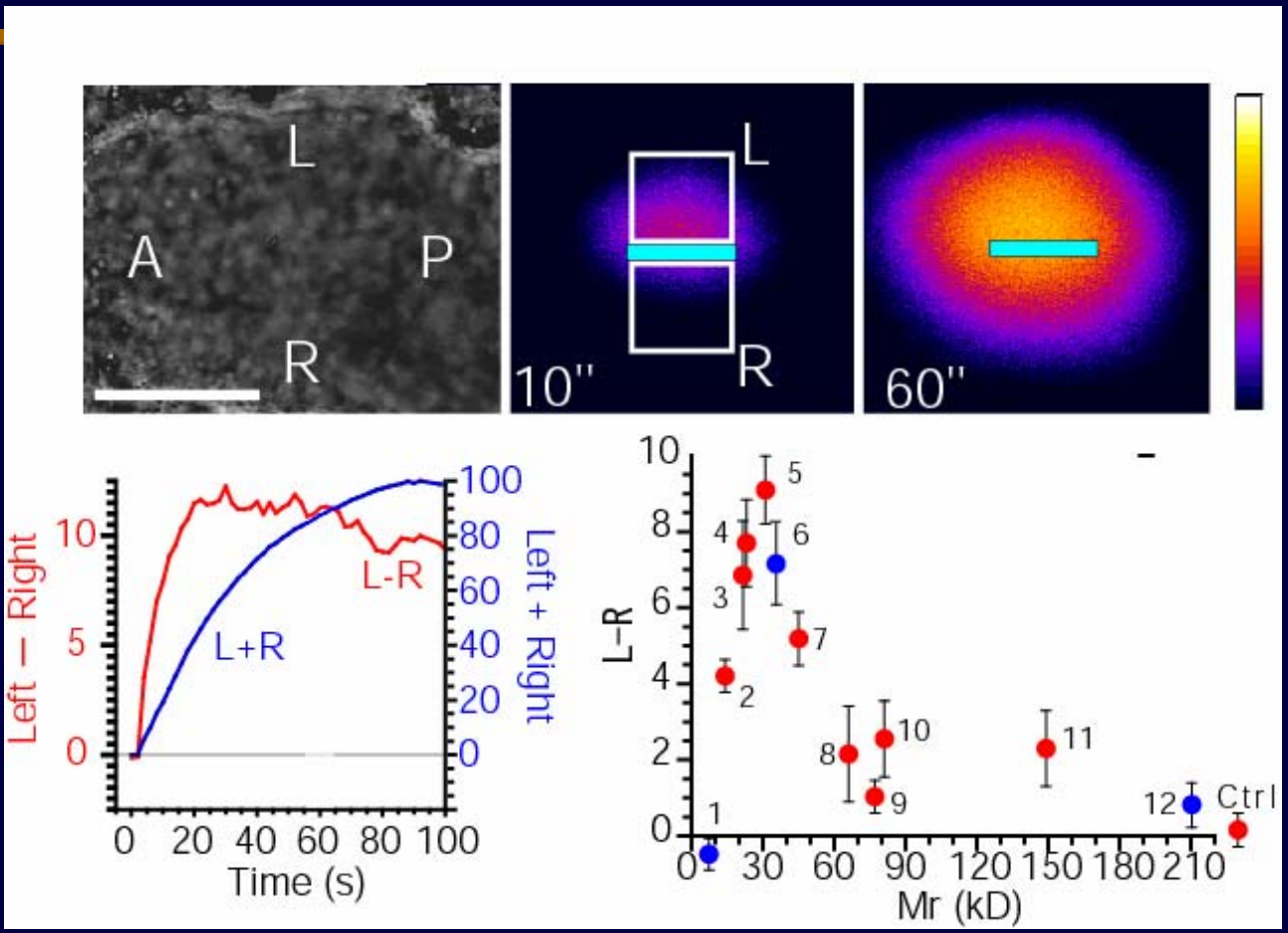
Where are the motile cilia?

How does the ciliary movement determine the L-R axis?

Asymmetric distribution of caged-fluorescently labeled protein after continuous uncaging at the middle of node.

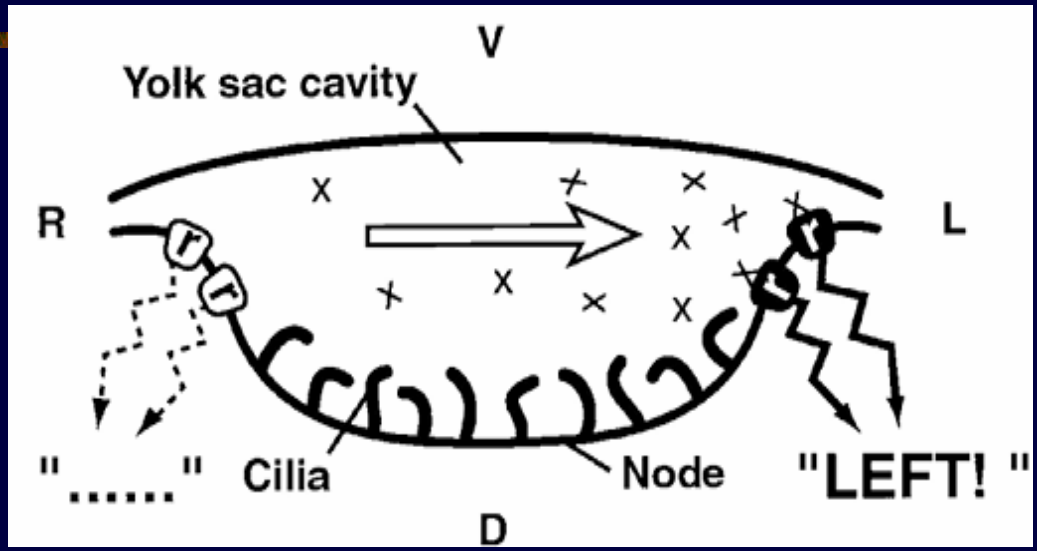


Asymmetric distribution of 20~40 kDa protein by nodal flow



Leftward nodal flow is rapid enough to generate stationary asymmetric distribution of soluble protein in the node.

Nodal Flow Hypothesis



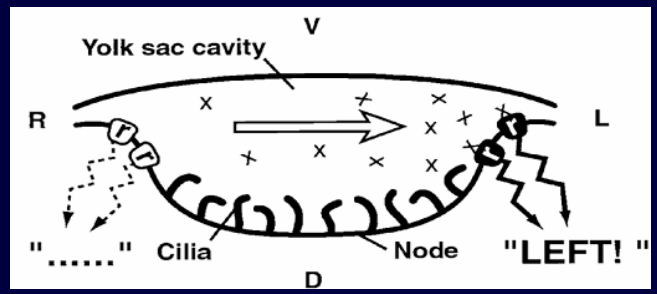
1. Clockwise rotation of cilia
2. Rapid leftward flow
3. Left-specific gene expression

Is nodal flow universal?

Mouse:

egg cylinder

nodal pit on the ventral surface



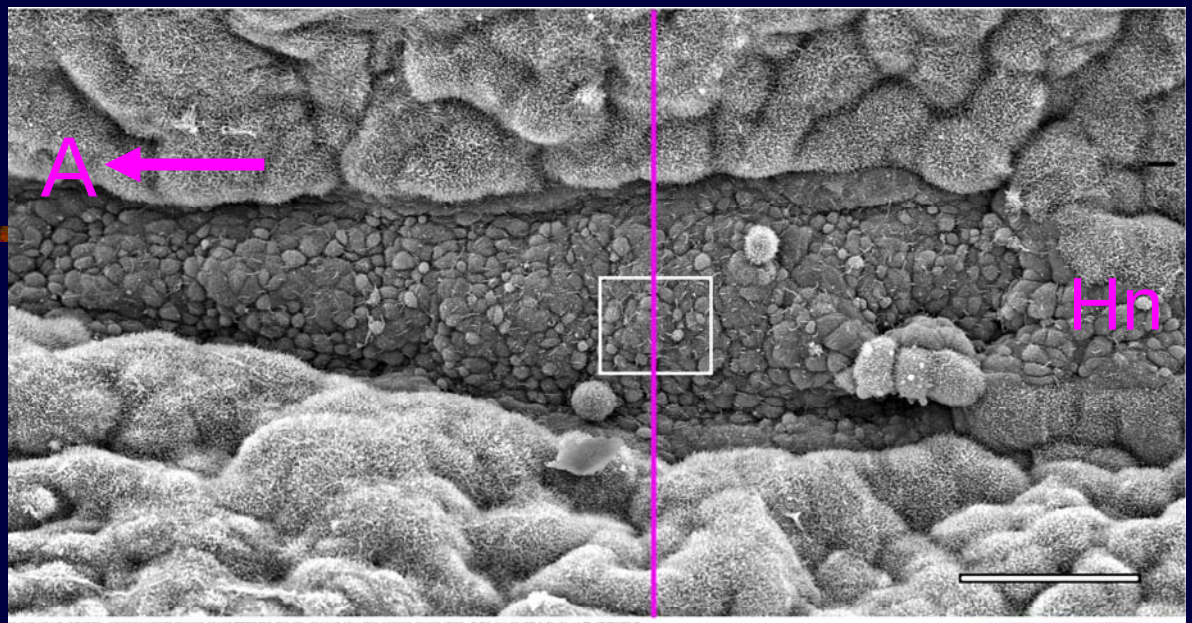
Higher vertebrates:

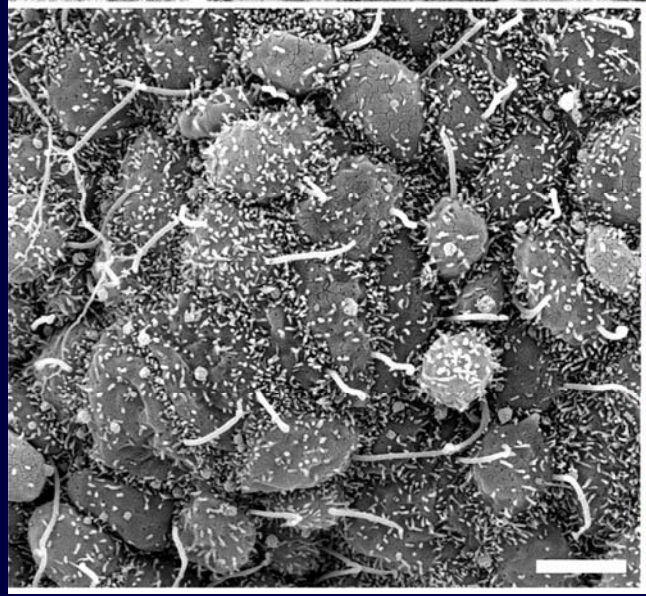
embryonic disc

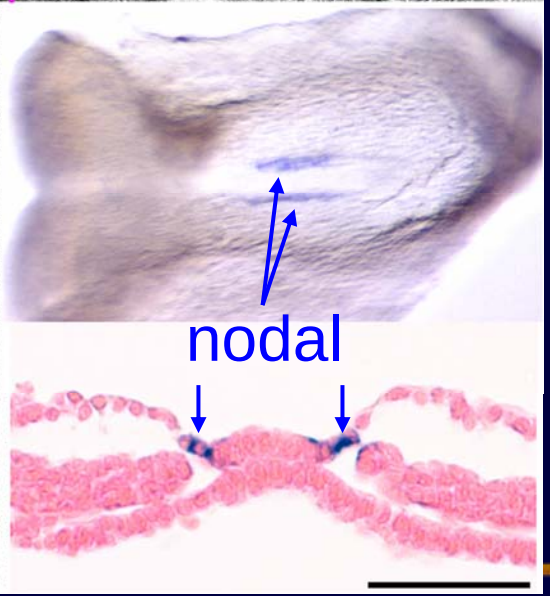
No nodal pit

Lower vertebrates:

ventral surface is embedded





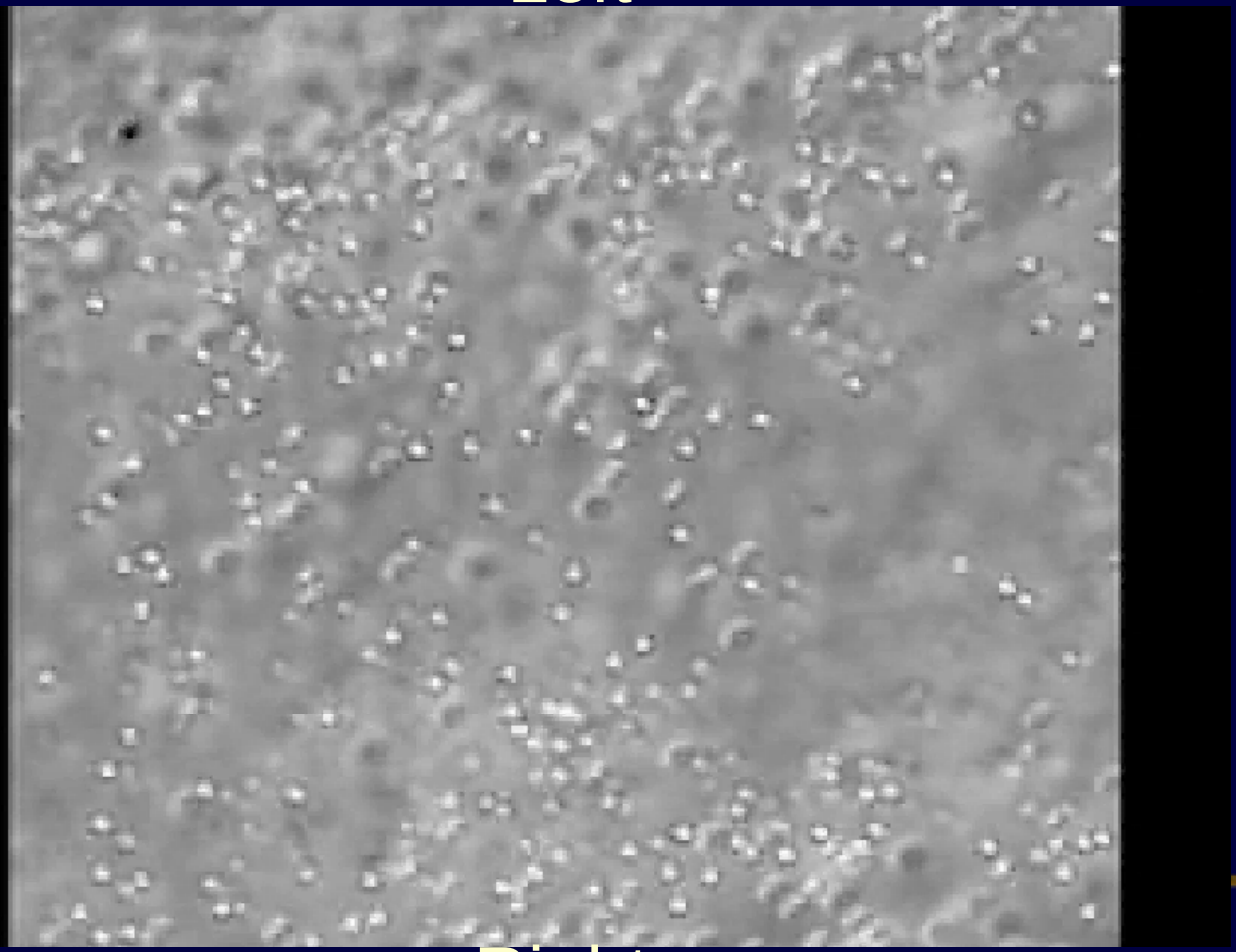


Monocilia on the ventral surface of the notochordal plate of rabbit embryo.

Probe for nodal: courtesy of Dr Hamada (Osaka Univ)

Leftward flow in the notochordal plate of the rabbit embryo

Left

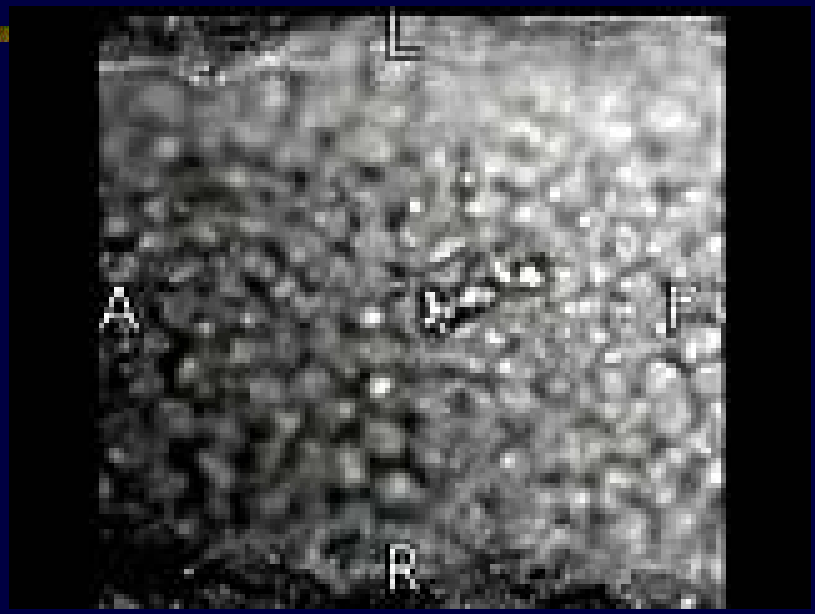


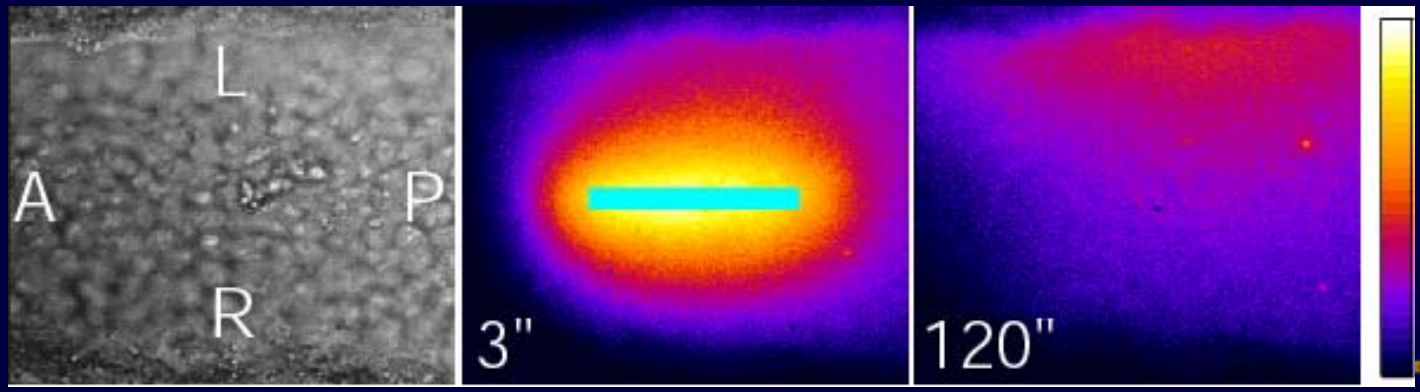
Right

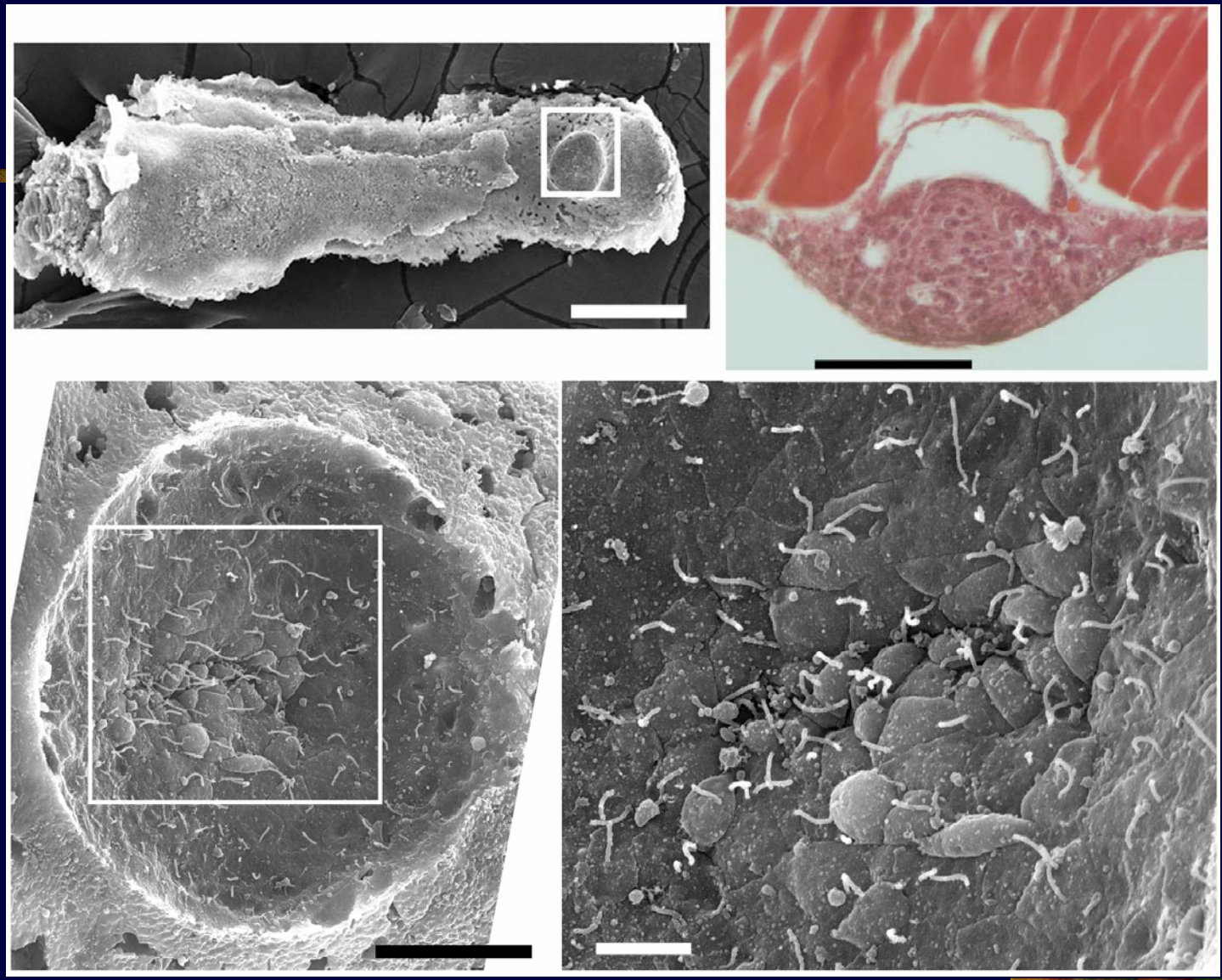
20 um

20x Time Lapse

Asymmetric distribution of caged-fluorescent dextran



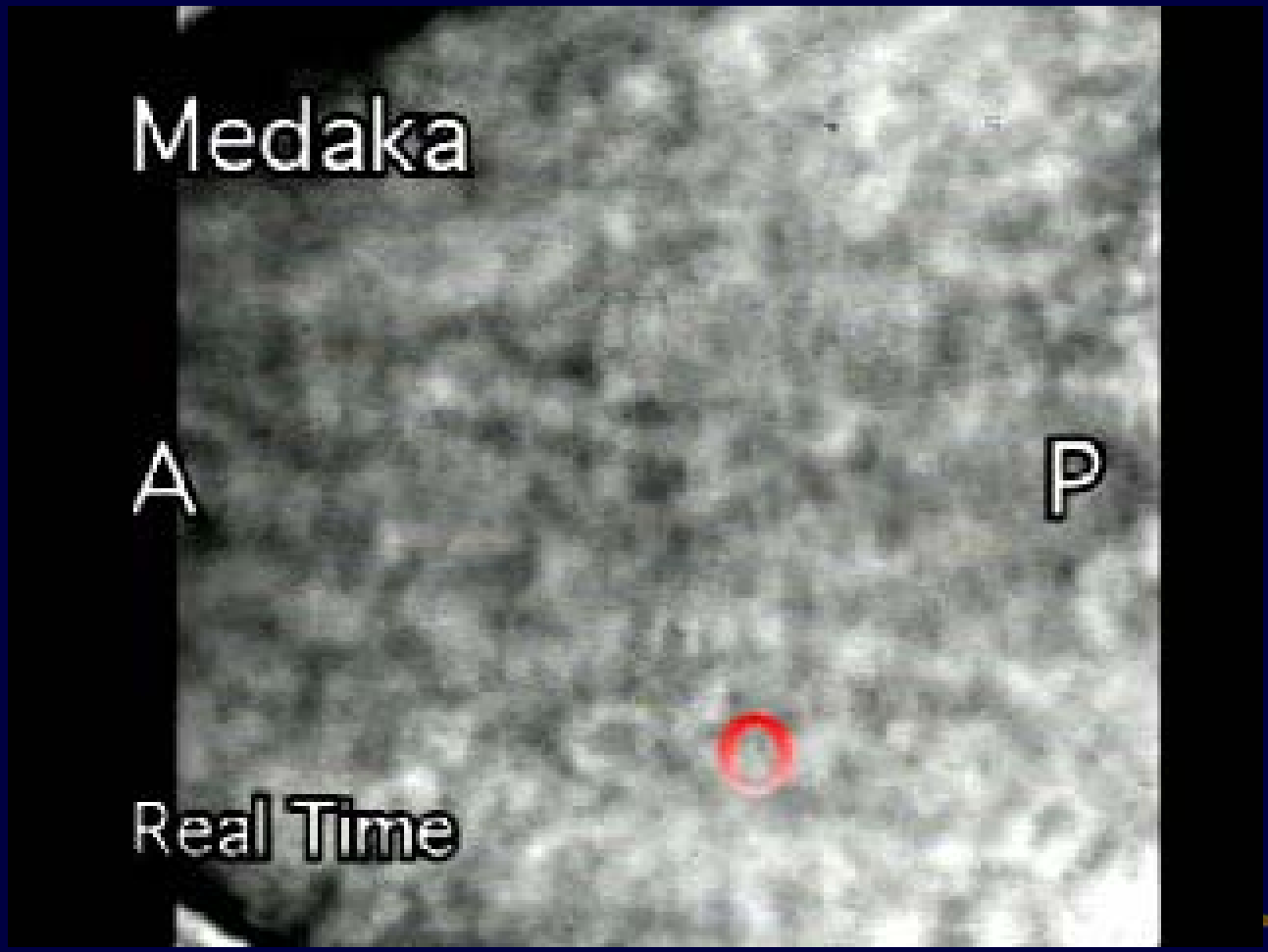




Monocilia on the inner surface of Kupffer's vesicle of medaka embryo.

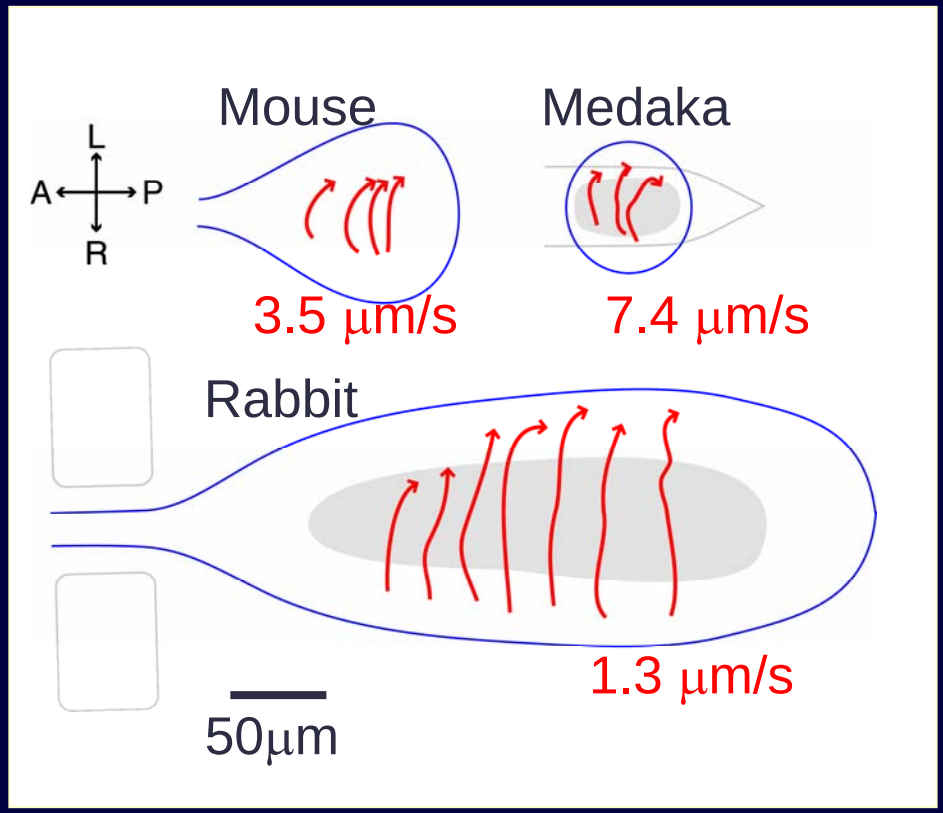
Medaka embryo: courtesy of Dr. Shima (Univ.Tokyo)

Leftward flow in Kupffer's vesicle of medaka embryo



Medaka embryo: courtesy of Dr. Shima (Univ.Tokyo)
Hatching enzyme: courtesy of Dr. Yasumasu (Sophia)

Leftward Flow in the Ventral Node



Conserved:

- Primary monocilia
- Clockwise rotation
- Leftward flow

Not conserved:

- Shape, size and position of the ciliated organ
- Velocity of the flow

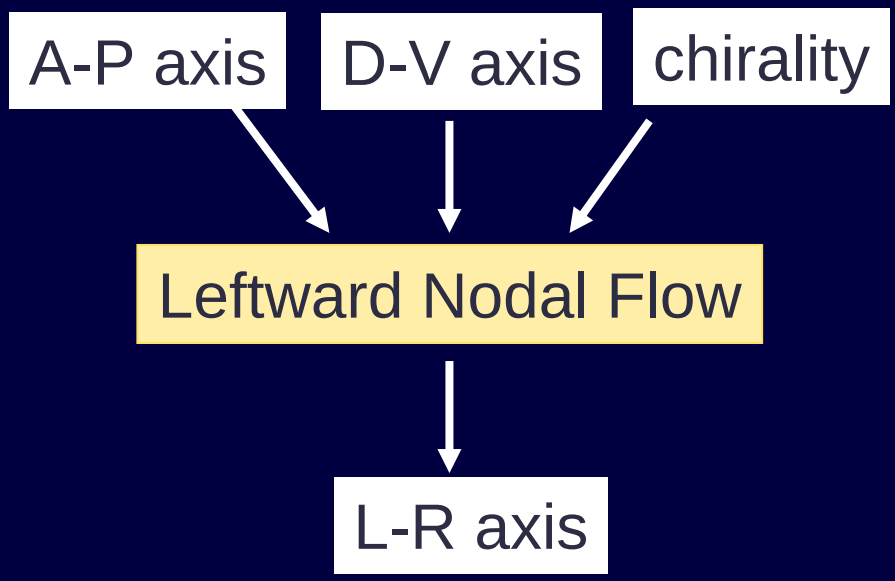
The Nodal Flow Hypothesis

1. Leftward Nodal Flow
2. Left-specific expression of master genes
3. Left/right asymmetric morphogenesis

Tautology!

Doesn’t answer why left is left.

Central Question:
What directs the flow to the left?

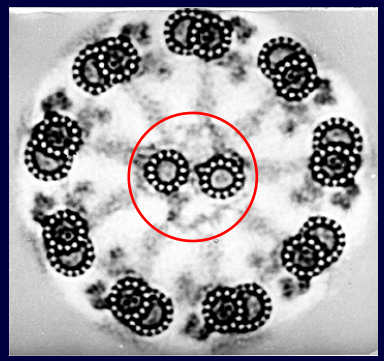


- How are the information of the A-P axis, D-V axis and the chirality integrated to determine the directionality of the nodal flow?

What produces the leftward flow?

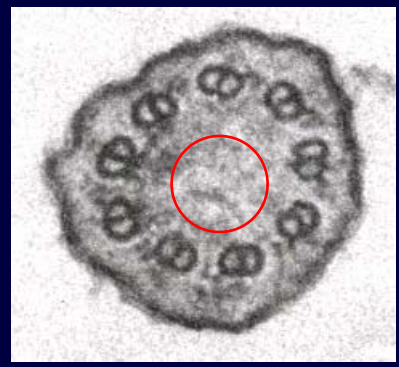
- Rotation of the primary cilia in the node.

Conventional “9+2” cilia



Regulation by the central pair microtubules enables the planar beating.

Primary “9+0” cilia in the node.



No central pair microtubules

→ unable to beat

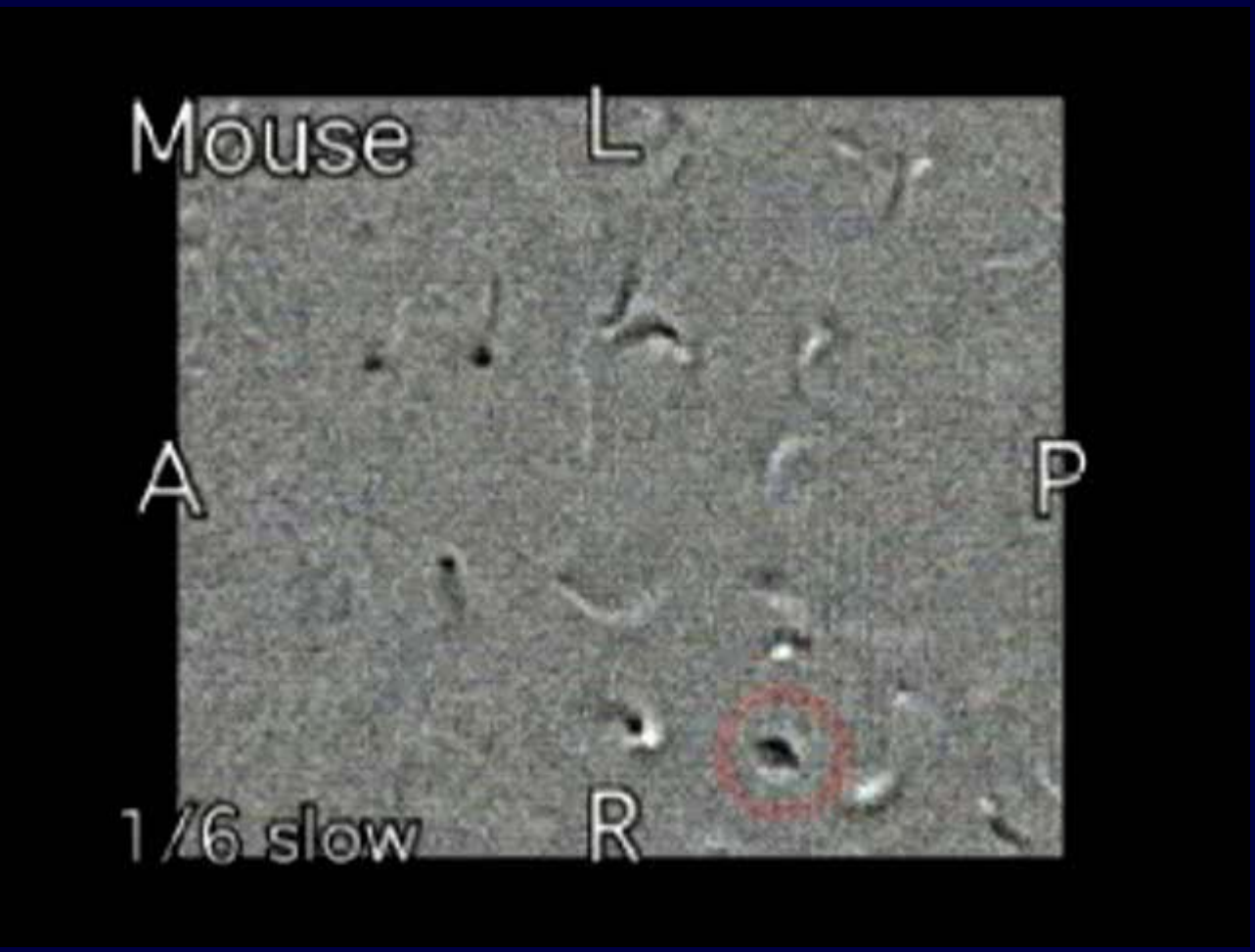
→ Clockwise rotation

What produces the leftward flow?

- Rotation of 9+0 cilia
- Rotation can only produce vortices.
- In *inv* mutant mice, and in the wild type embryos at the earlier stages, the flow is vortical and the leftward flow is not evident.

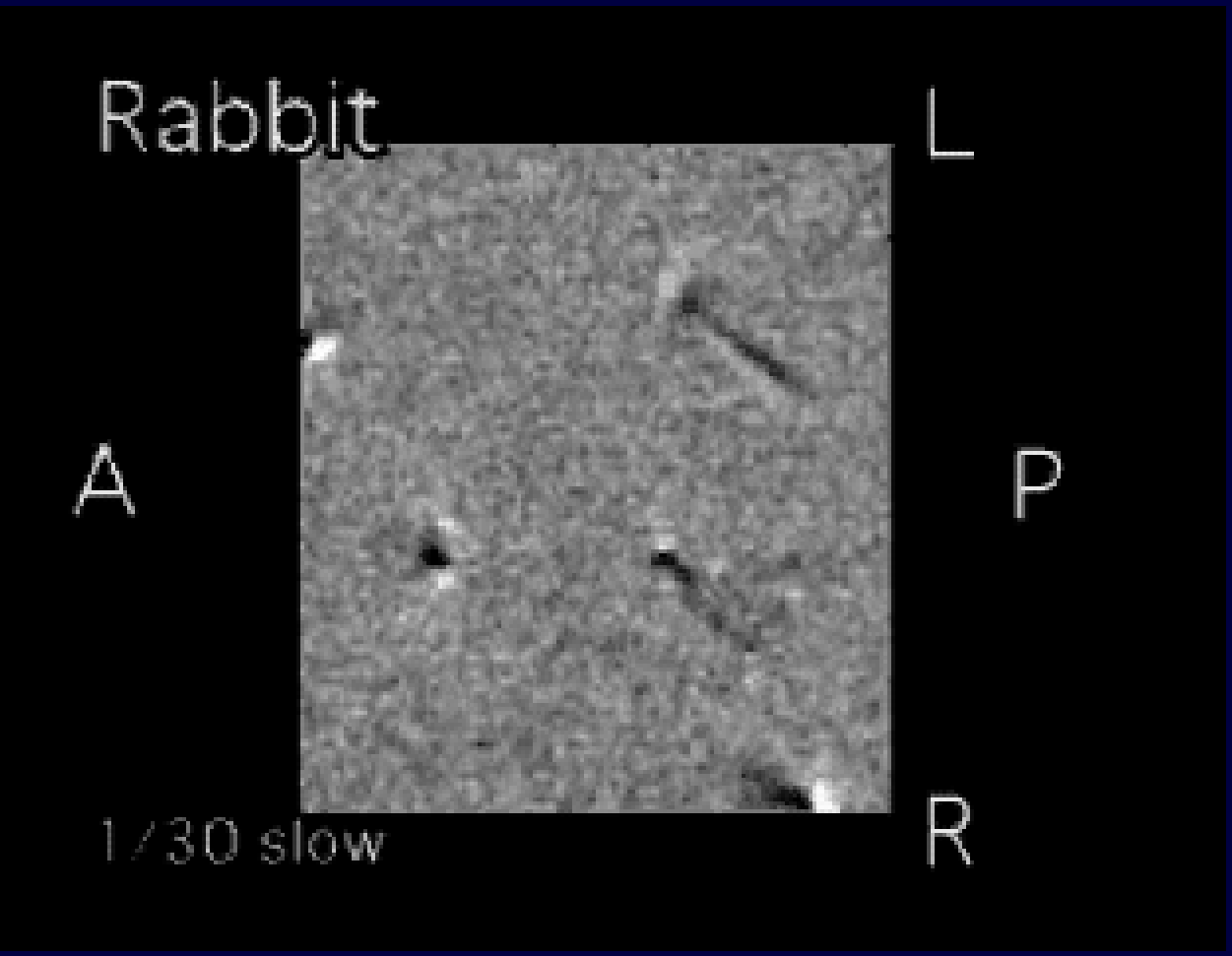
Some mechanism(s) exist for the conversion into the laminar leftward flow.

Rotation of Monocilia



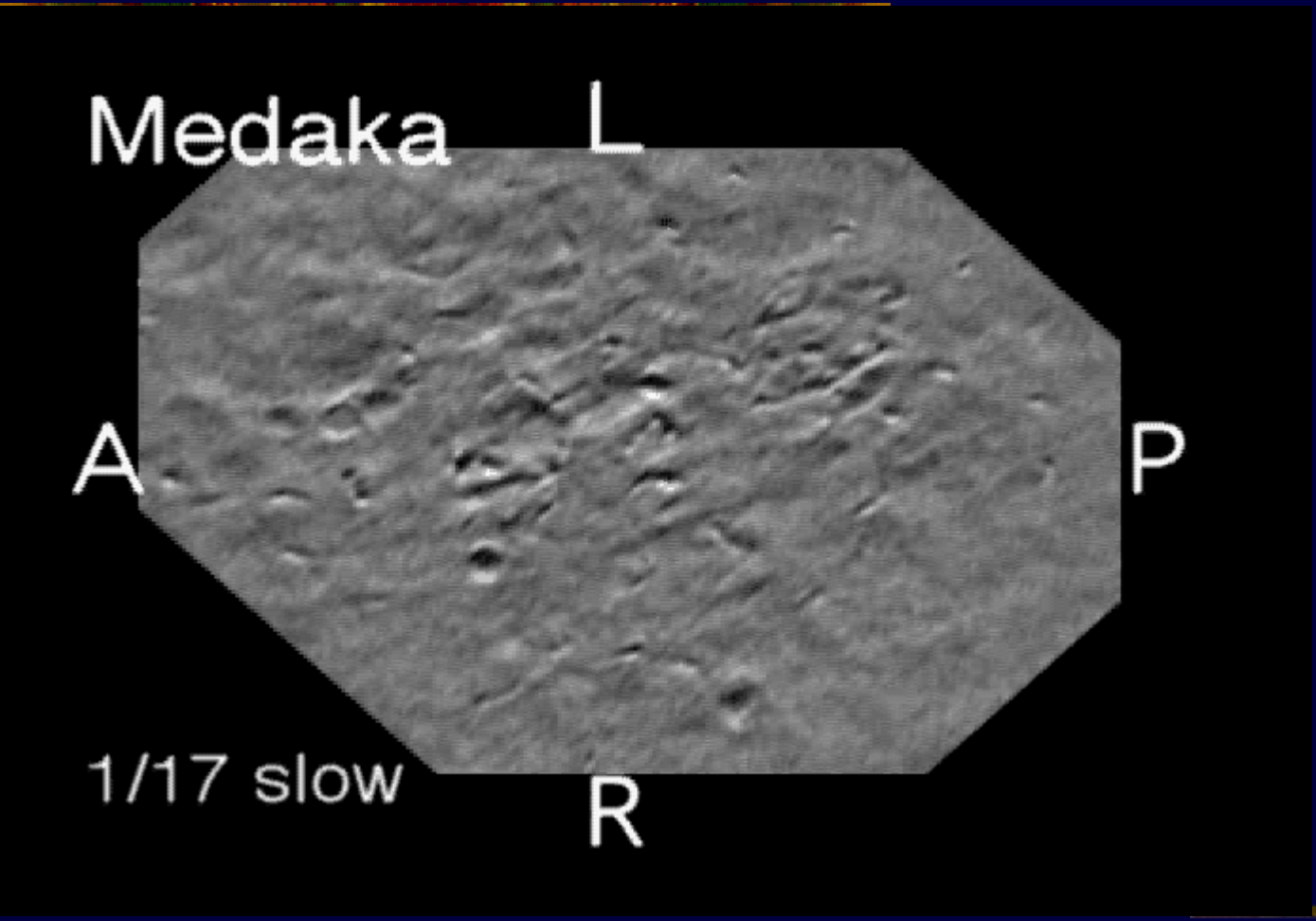
Recording frame rate:
500 frames / sec

Posteriorly Tilted Rotation of Monocilia



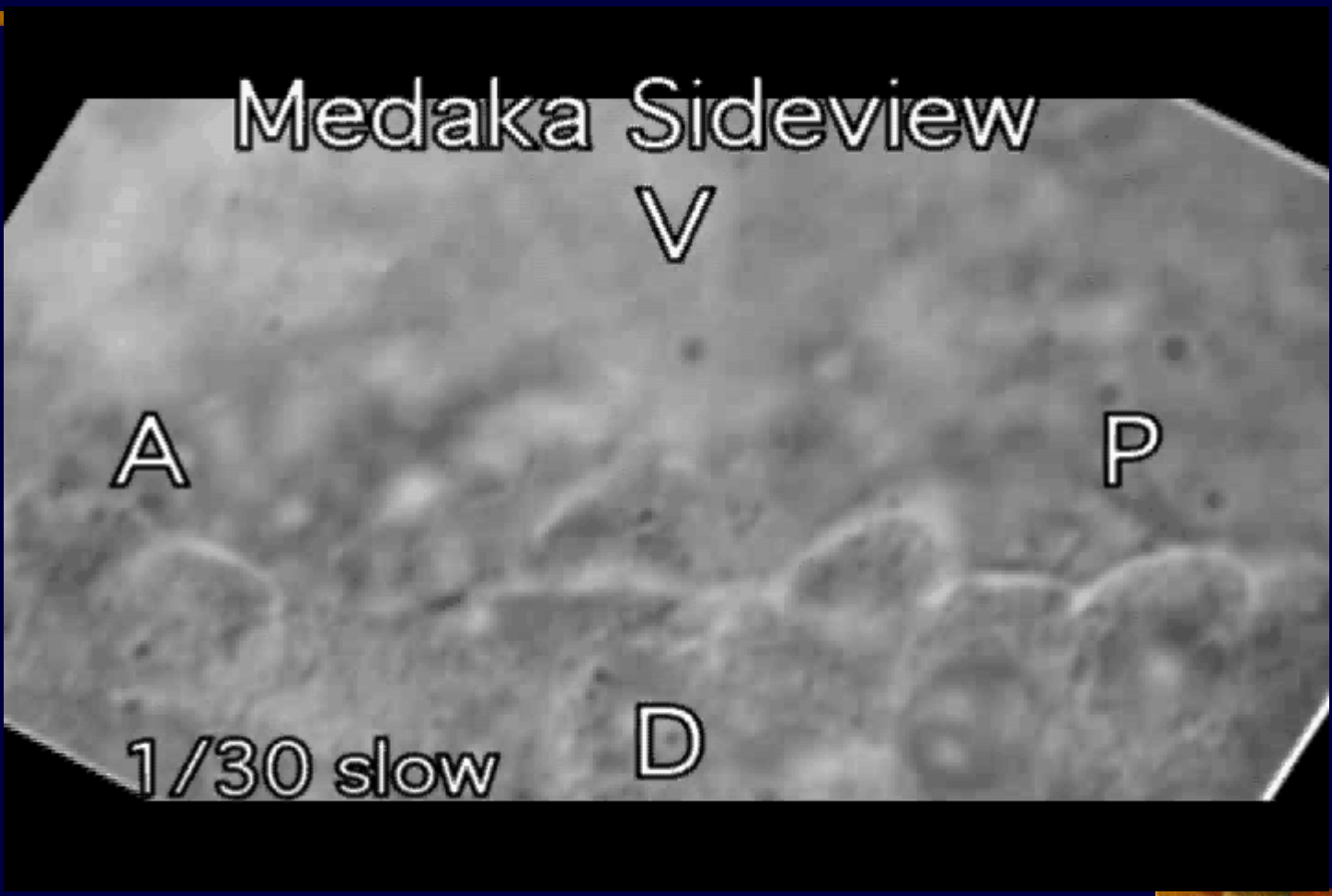
Recording frame rate:
500 frames / sec

Posteriorly Tilted Rotation of Monocilia



1000 frames / sec

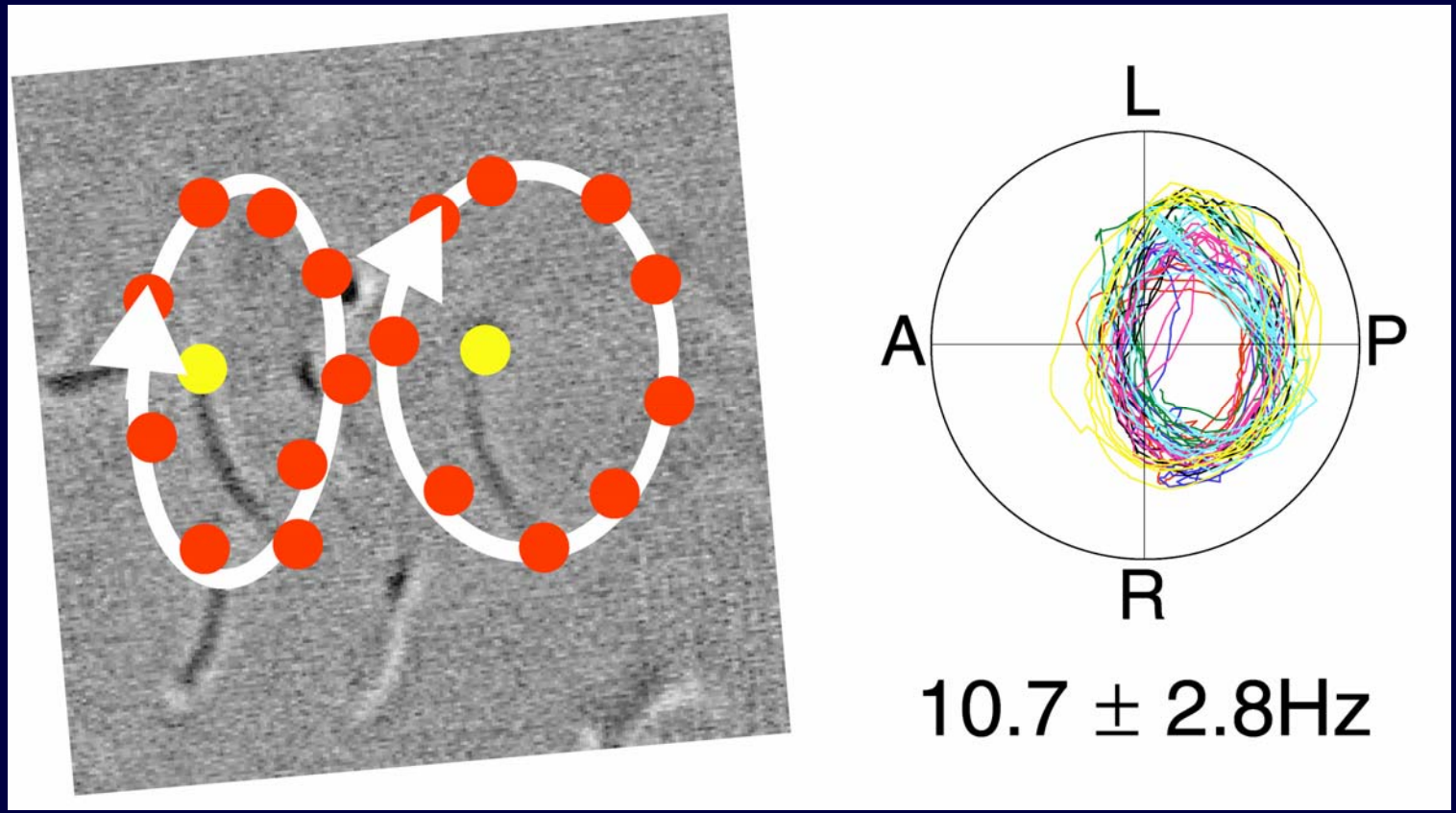
Posteriorly Tilted Rotation of Monocilia



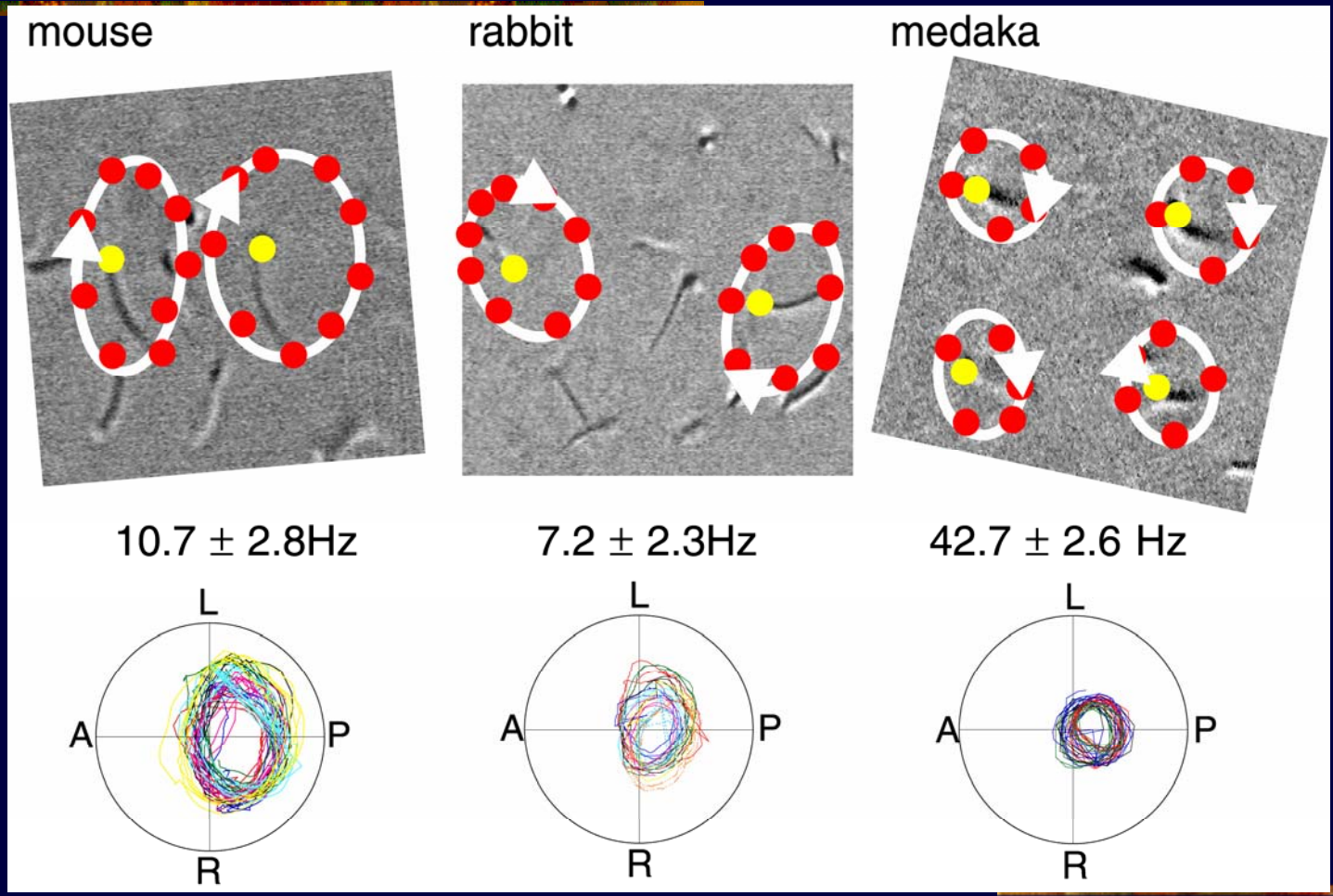
Side view

1000 frames / sec

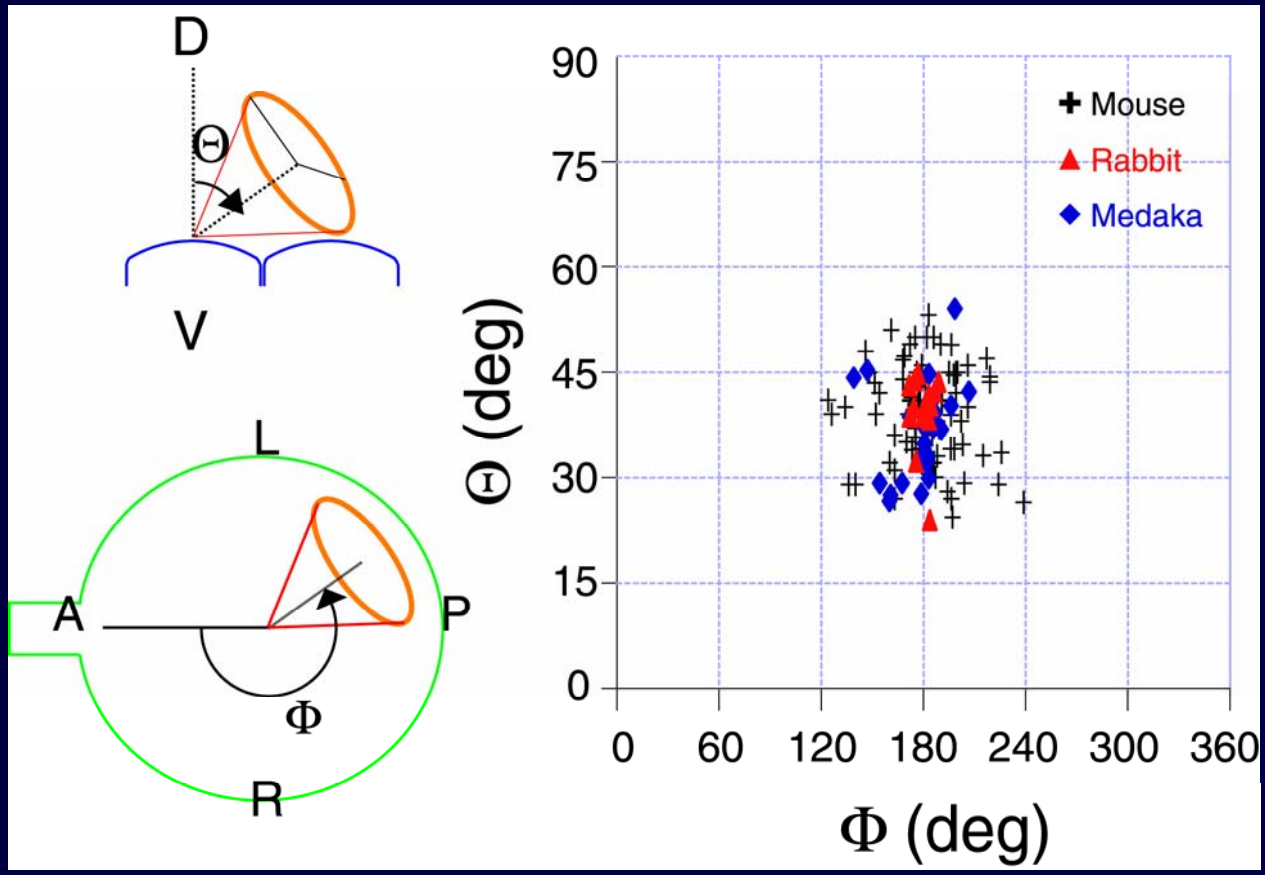
Posteriorly tilted rotation of cilia



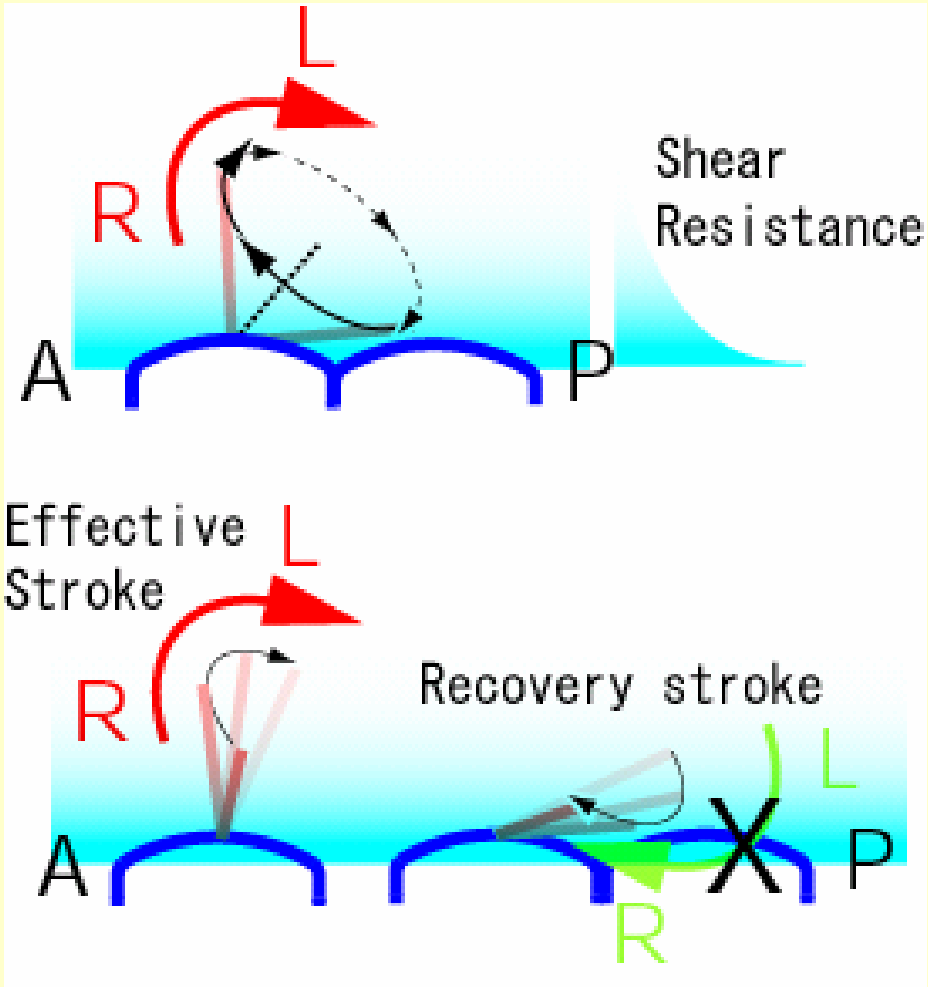
Posteriorly tilted rotation of cilia

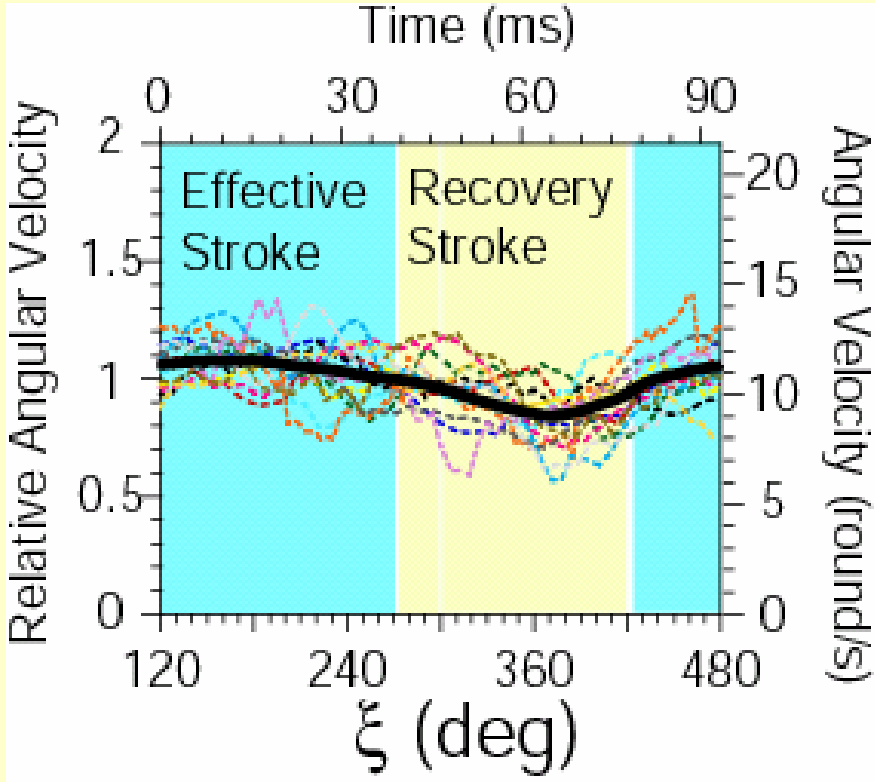


Axis of Rotation is Tilted ~40˚ to the Posterior.



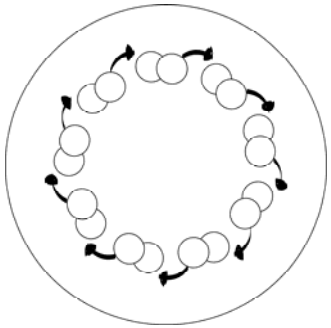
Hydrodynamic Mechanism of the Generation of the Leftward Flow





Cilia integrate the information of the axes and the chirality

Chiral Structure



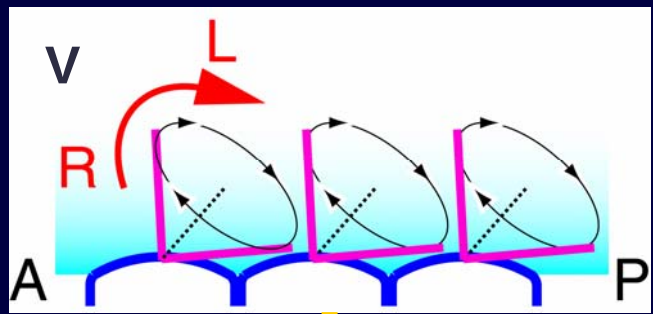
Clockwise rotation

DV axis

Ventral projection

AP axis

Posterior tilting

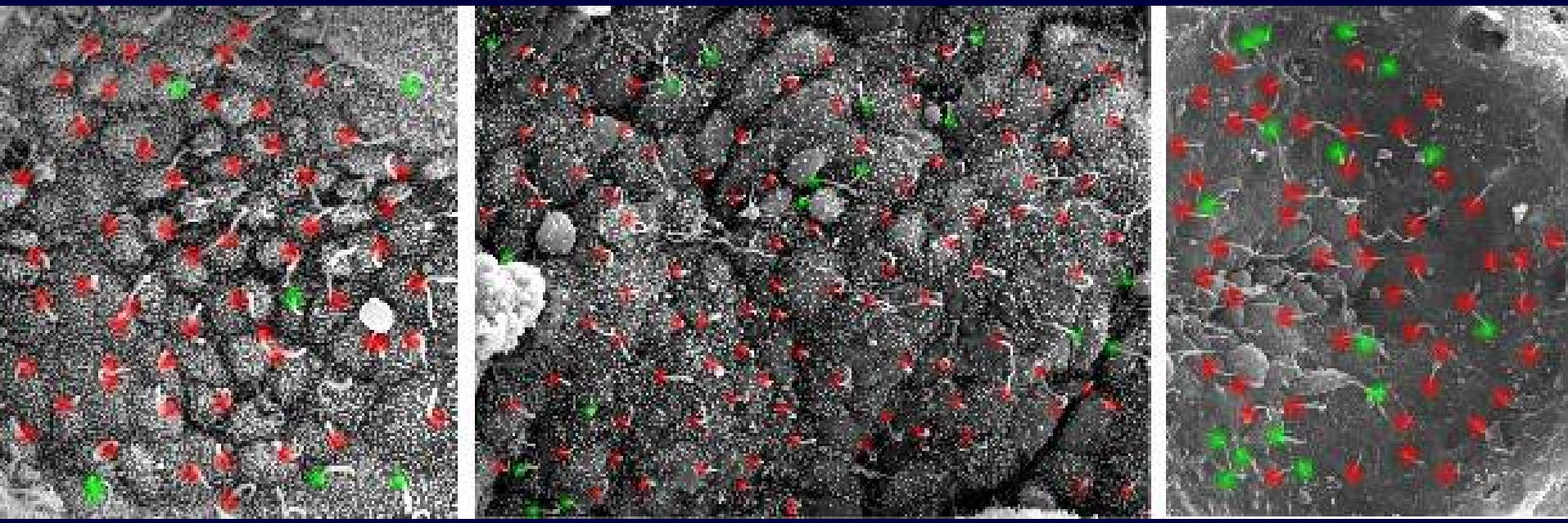


Leftward Flow

What determines the posterior tilting of the nodal cilia?

Posterior projection of nodal cilia

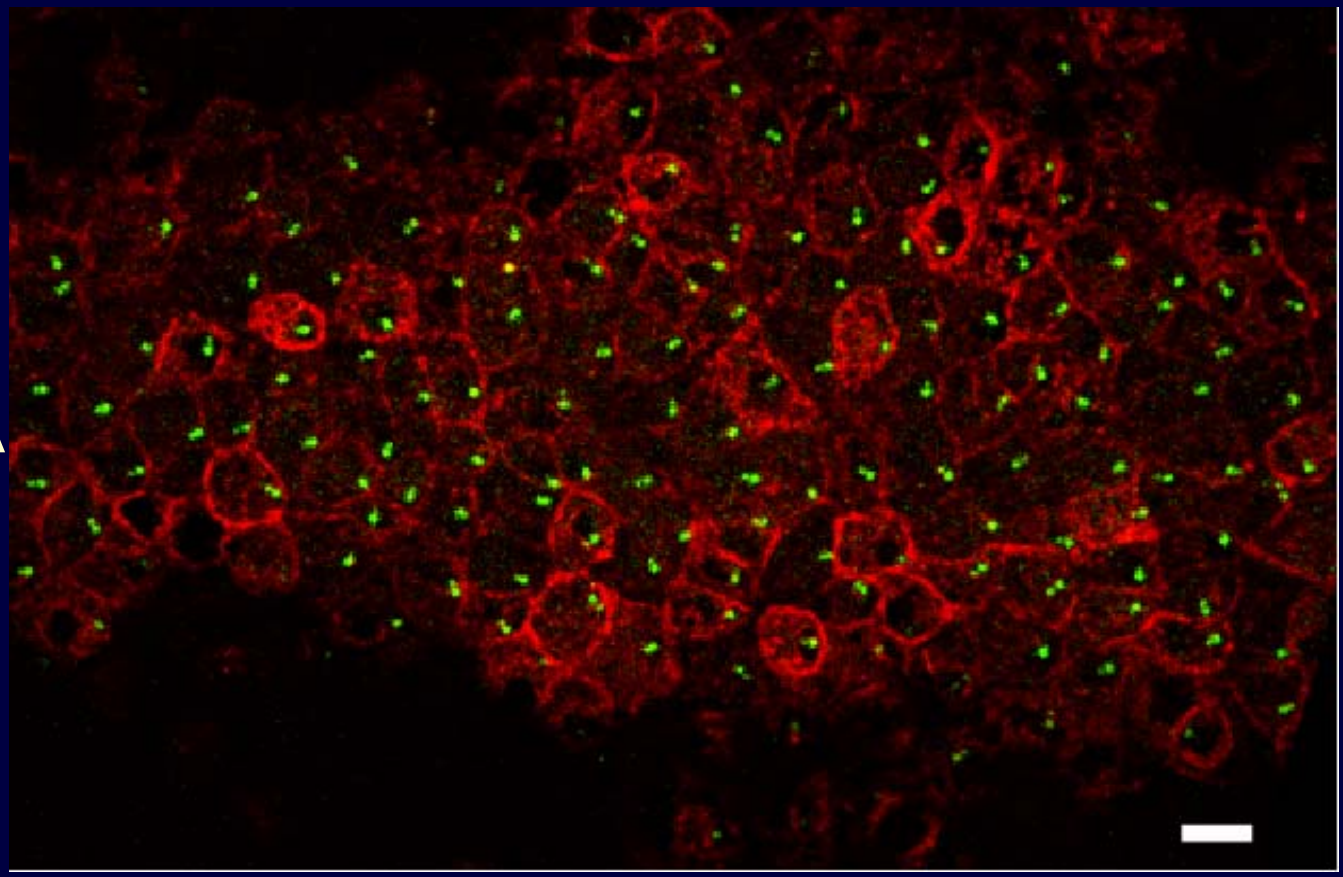
Mouse Rabbit Medaka



Red: Cilia projecting from posterior quadrant of the apical surface.
Green: Cilia projecting from other quadrants.

Posterior Positioning of Basal Body

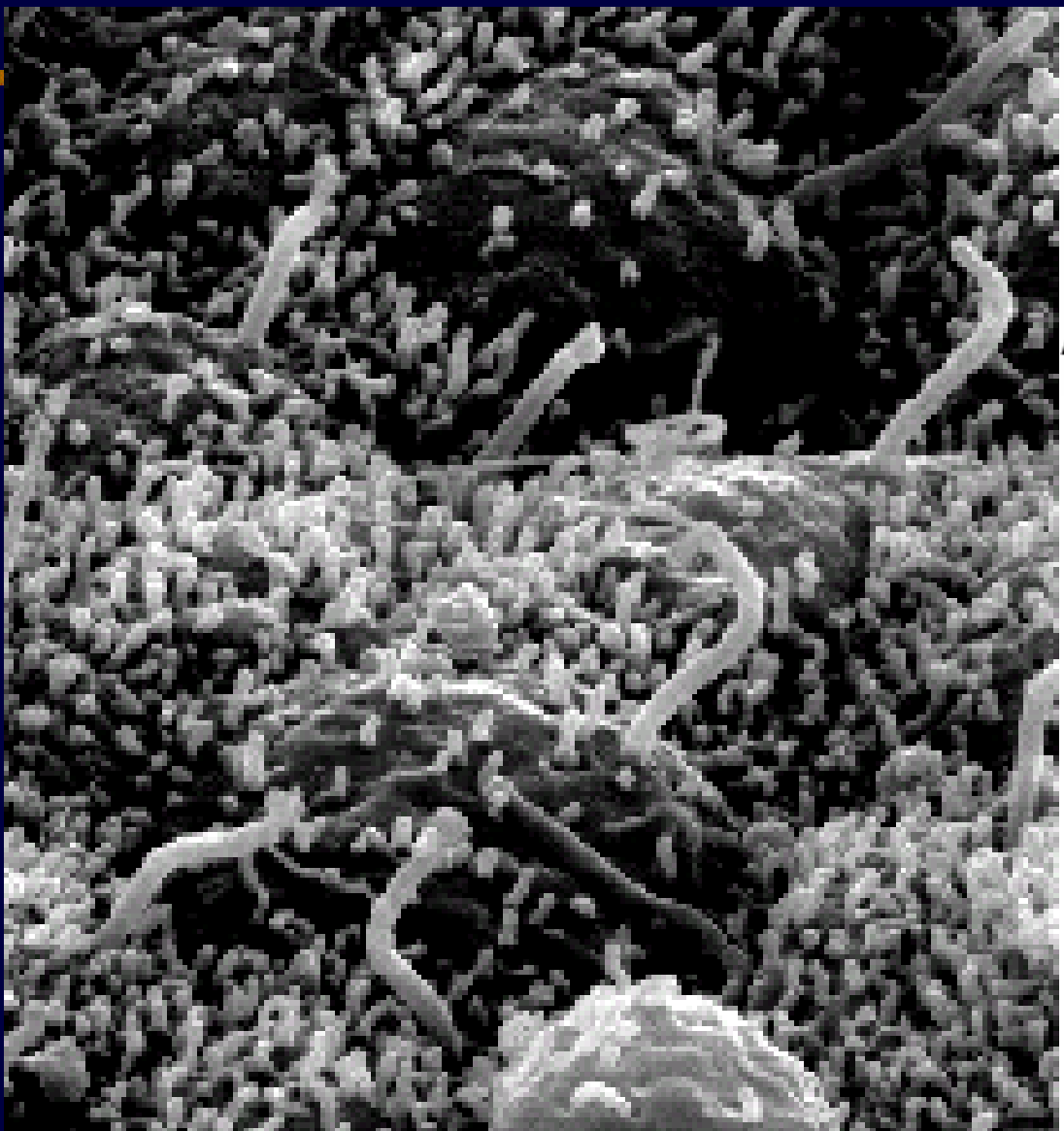
A

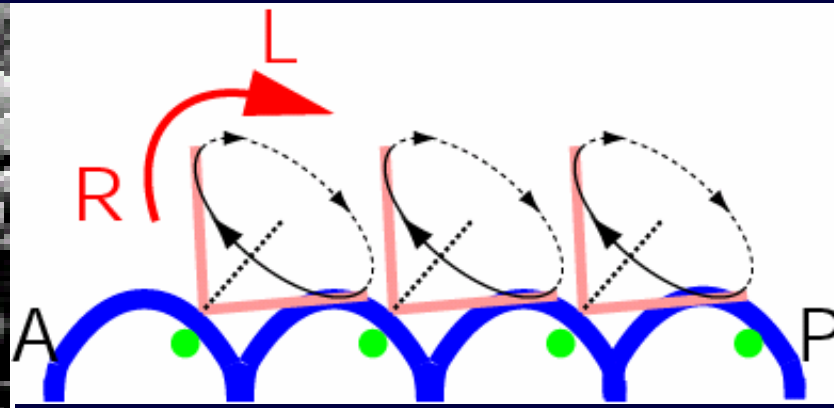


P

Green: γ–tubulin, Red: apical cell surface

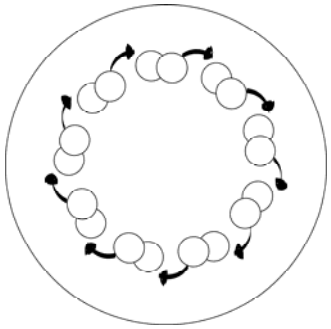
Potential link to planar cell polarity





Posterior positioning of basal body and the dome-like shape of the apical surface might determine the posterior tilting of nodal cilia.

Chiral Structure



DV axis

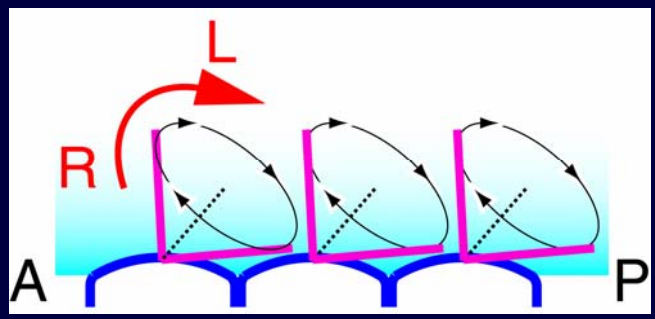
AP axis

PCP?

Ventral projection

Posterior tilting

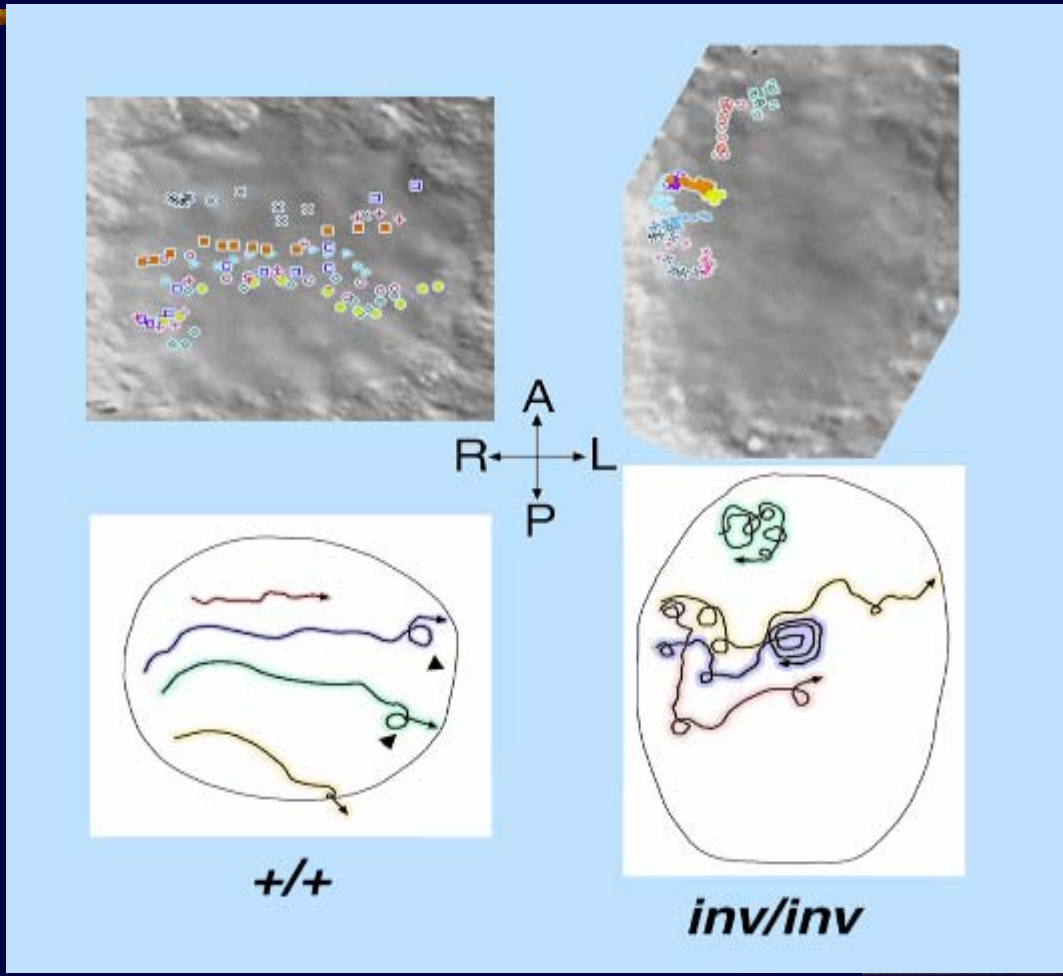
Clockwise rotation



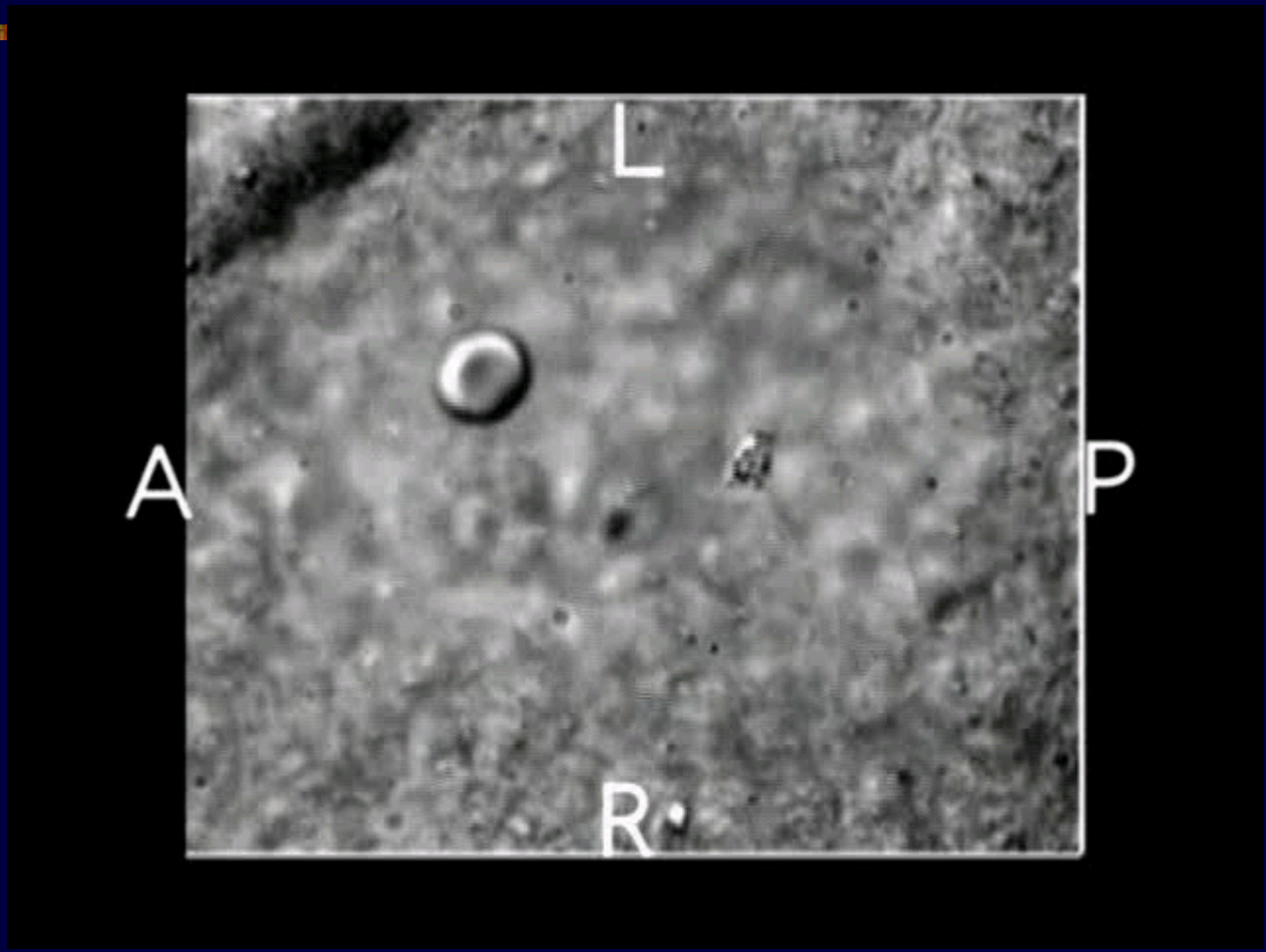
Hydrodynamic Mechanism

Leftward Flow

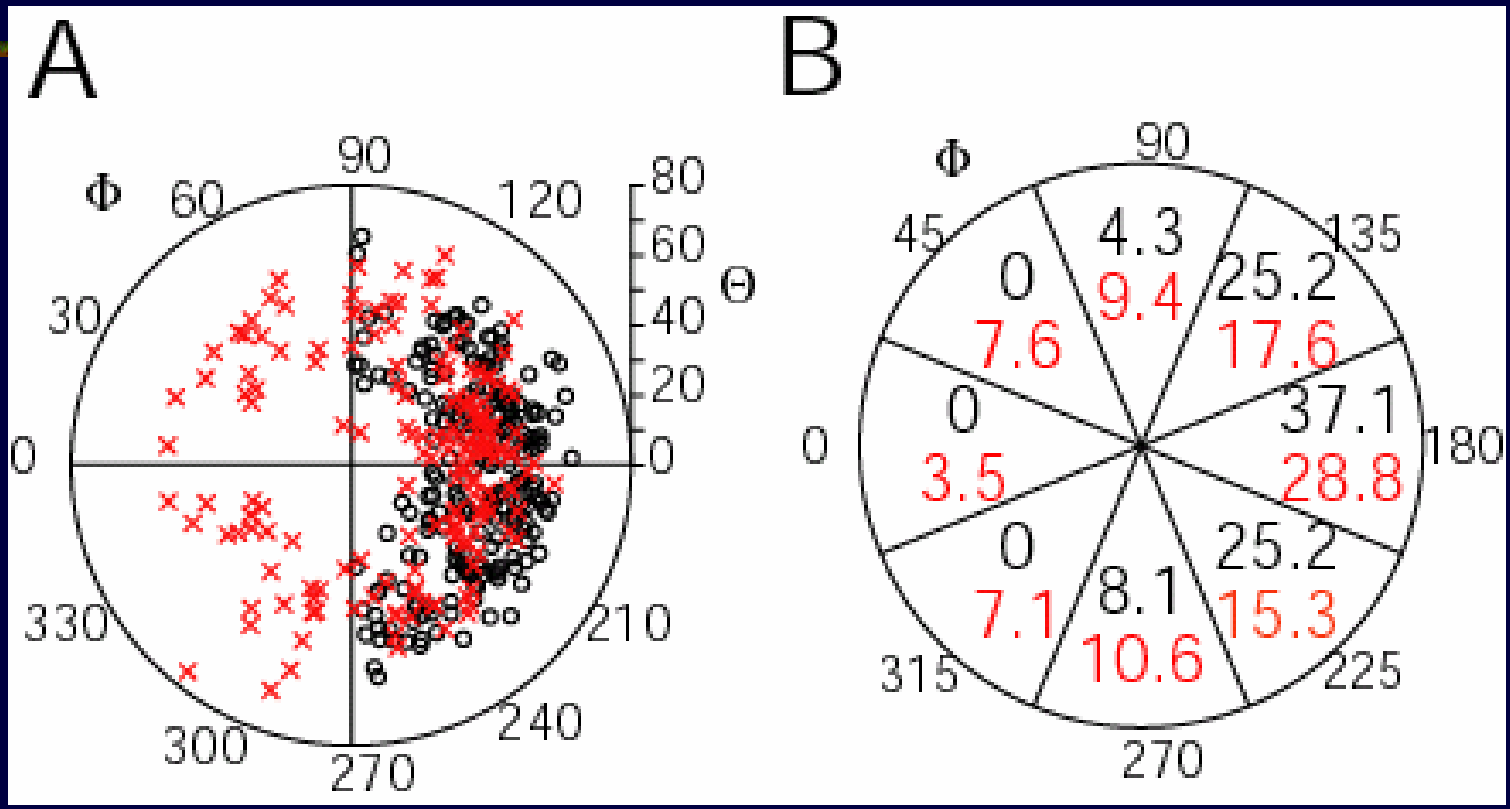
Slow leftward flow with meandering streamline in *inv* mutant mice.



Slow nodal flow and abnormal rotation of nodal cilia in *inv/inv* mouse



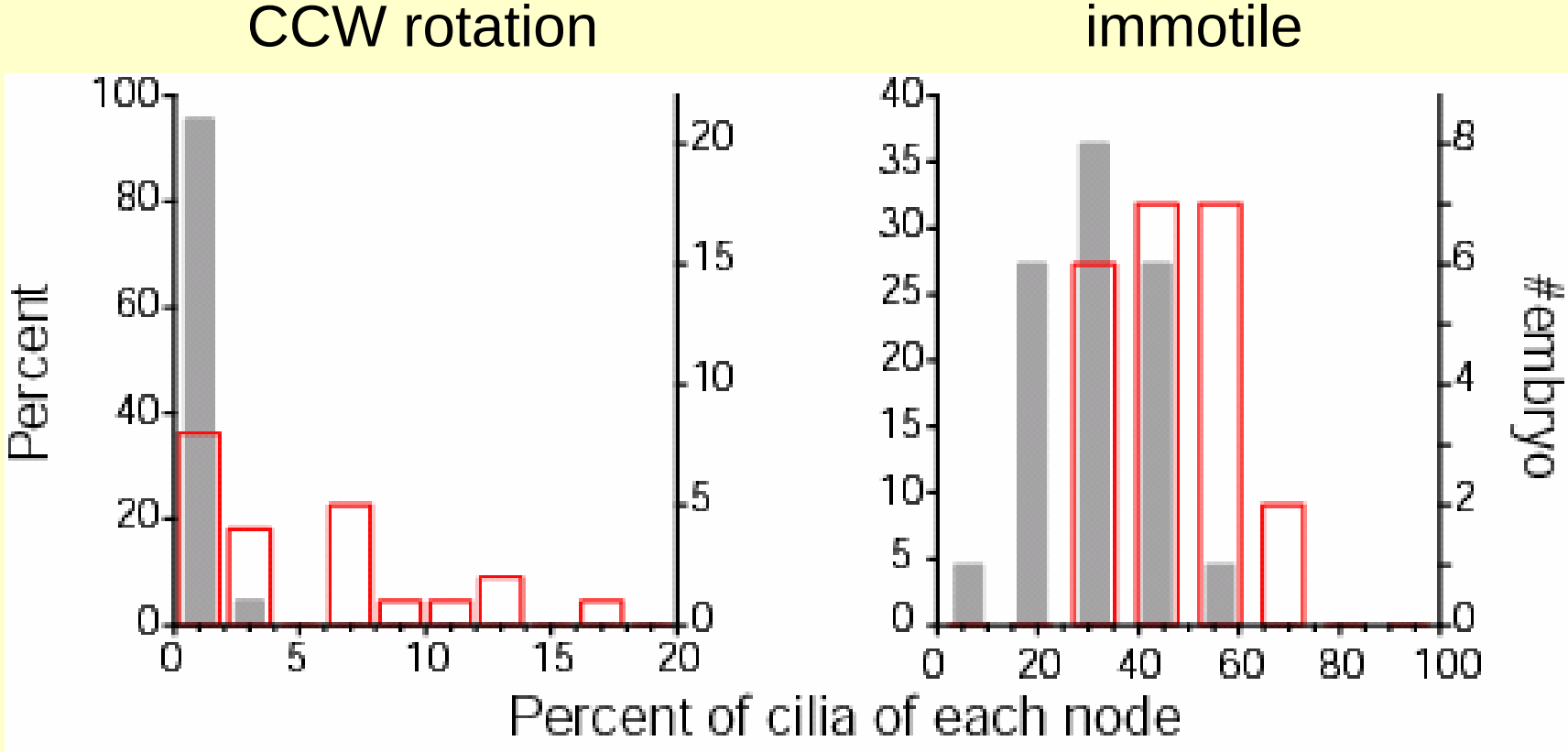
Wider distribution of the direction of the rotation axis in *inv* mutant.



Red: *inv/inv*, Black: *inv/+*

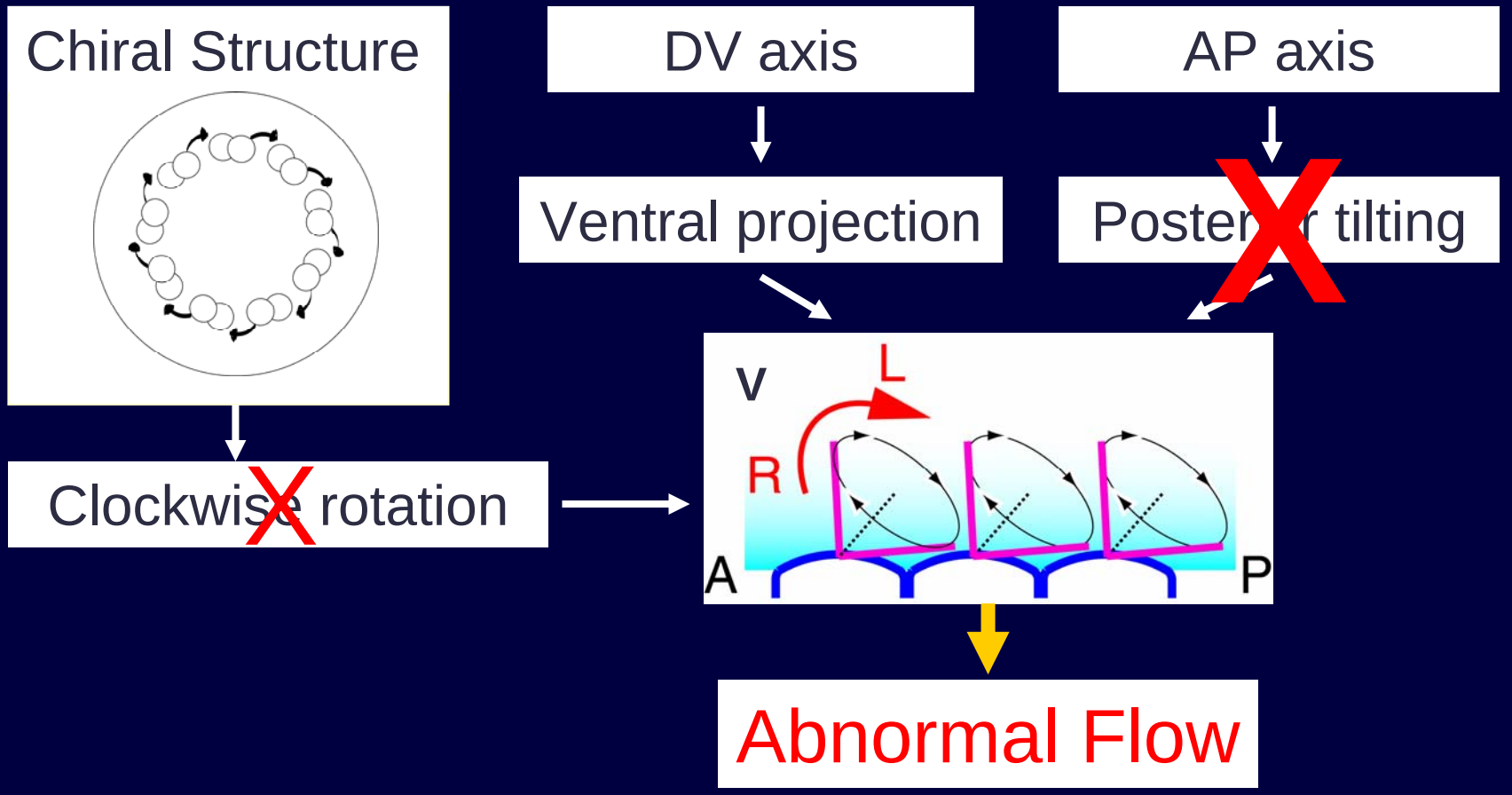
~20% of cilia were anteriorly tilted in *inv/inv* mice.

Abnormal rotation of nodal cilia in some *inv/inv* mutants



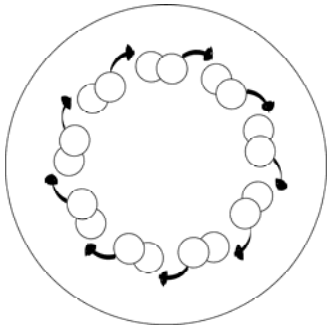
Red: *inv/inv*, Black: *inv/+*

Mechanism of abnormal flow in *inv* mutant mice.



Cilia integrates the information of the axes and the chirality

Chiral Structure



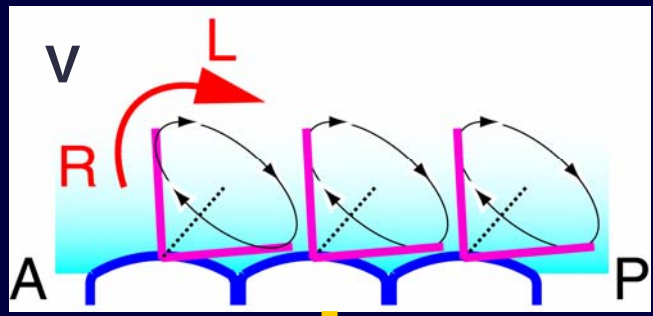
Clockwise rotation

DV axis

Ventral projection

AP axis

Posterior tilting



Leftward Flow

Kartagener's Syndrome

Immotile Cilia syndrome

Immotile Cilia
(Male Infertility + Respiratory Failure etc.)

?

Situs Inversus

...Why don't the 9+0 Nodal Cilia MOVE?

FGF-induced vesicular release of Sonic hedgehog and retinoic acid in leftward nodal flow is critical for left right determination

Nature 435:172-177, 2005

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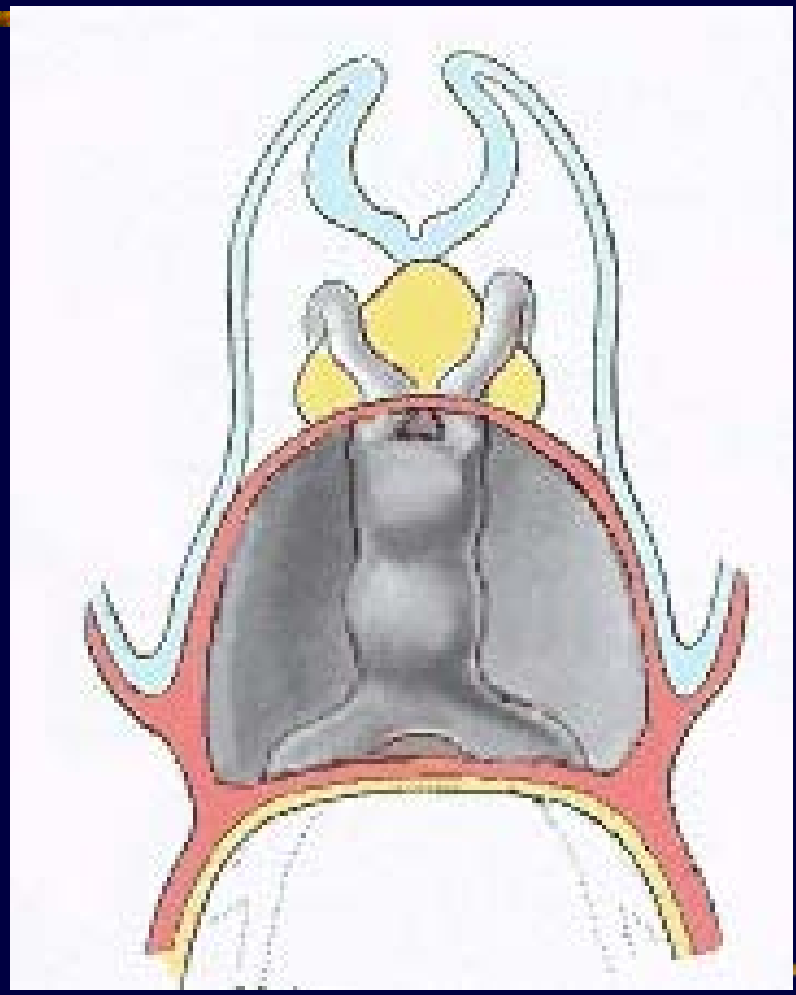
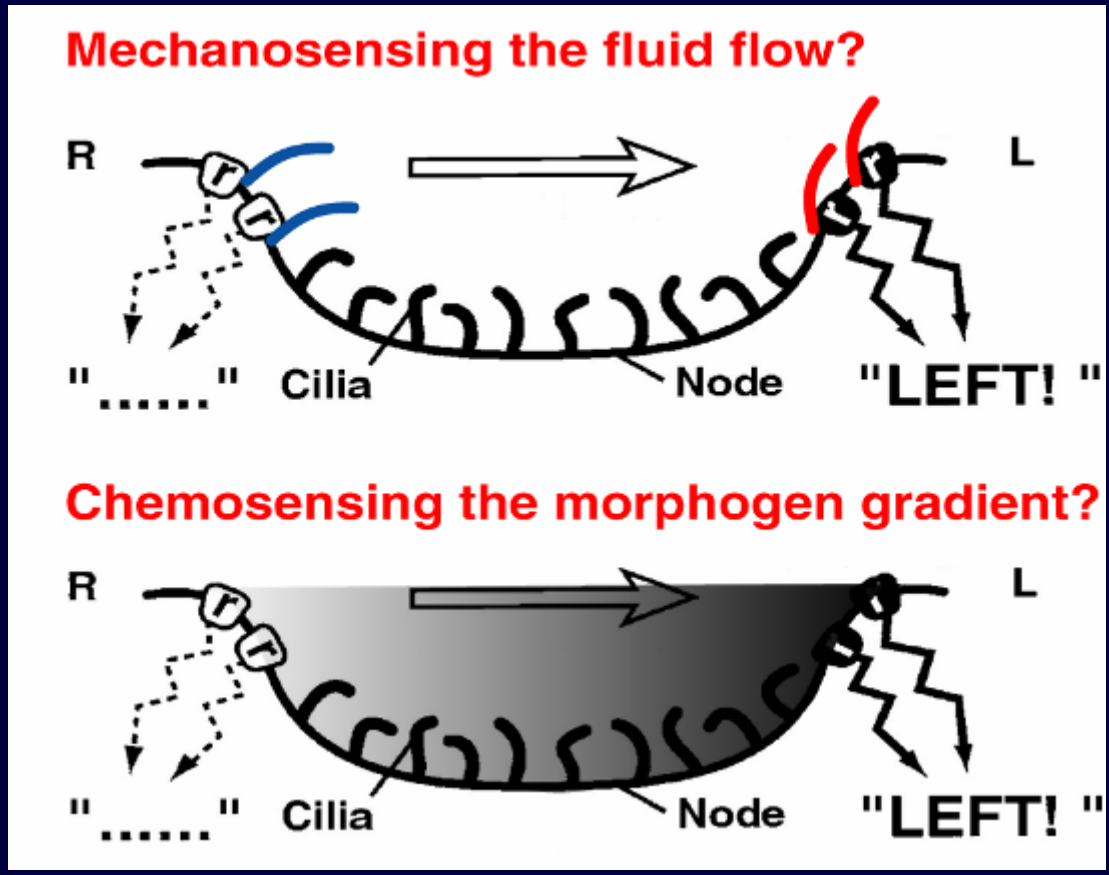
Symmetry breaking is essential for developing your internal organs

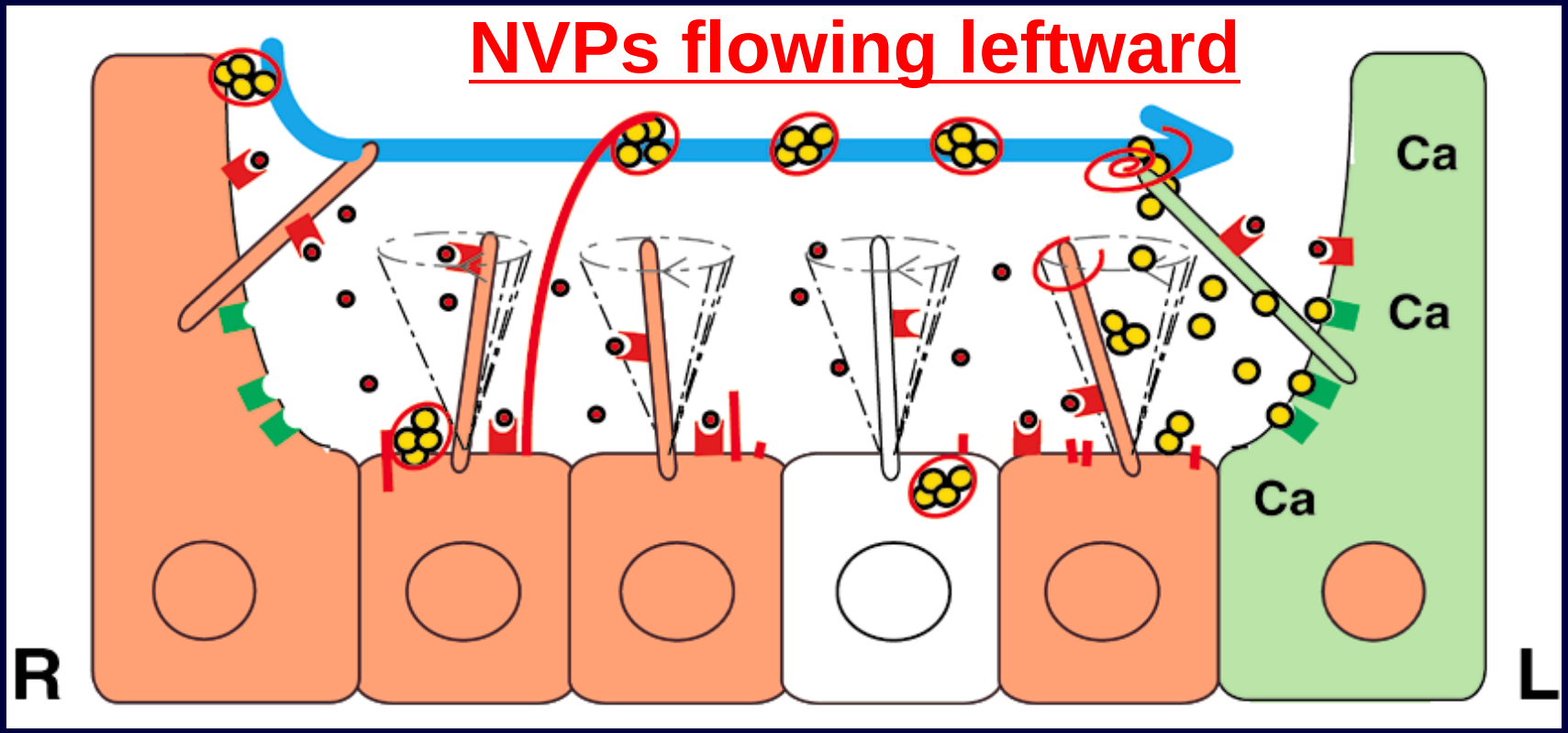


(William Larsen's Human Embryology website)

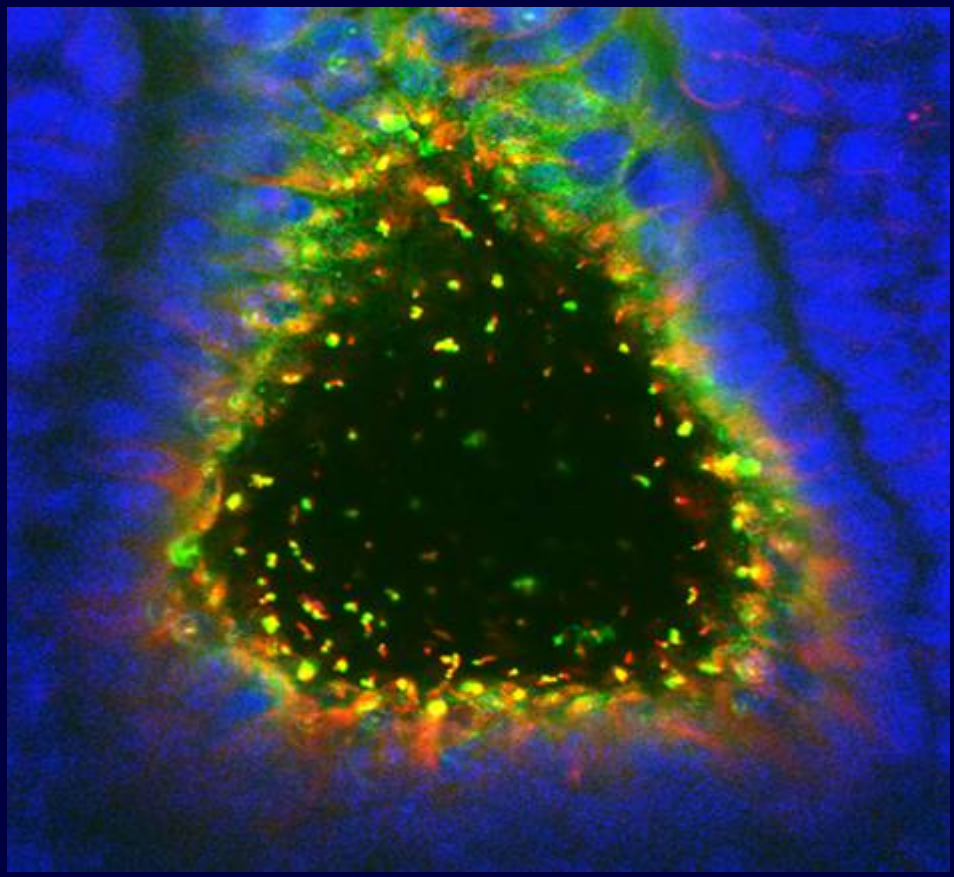
The sensing mechanism of nodal flow is very much controversial

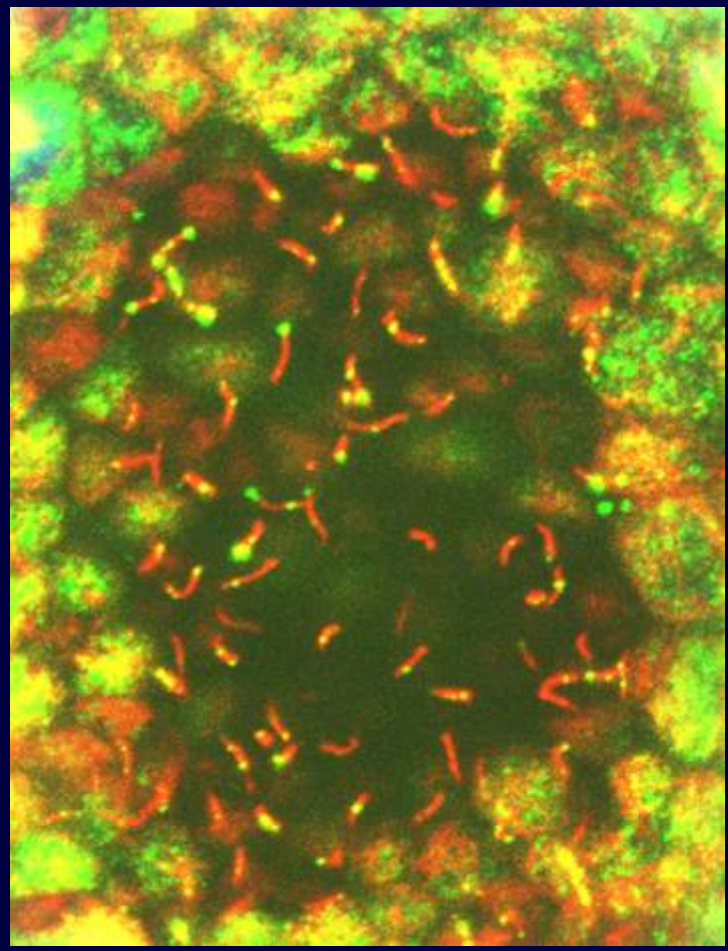


We identified morphogen-carrying vesicular parcels flowing to the left

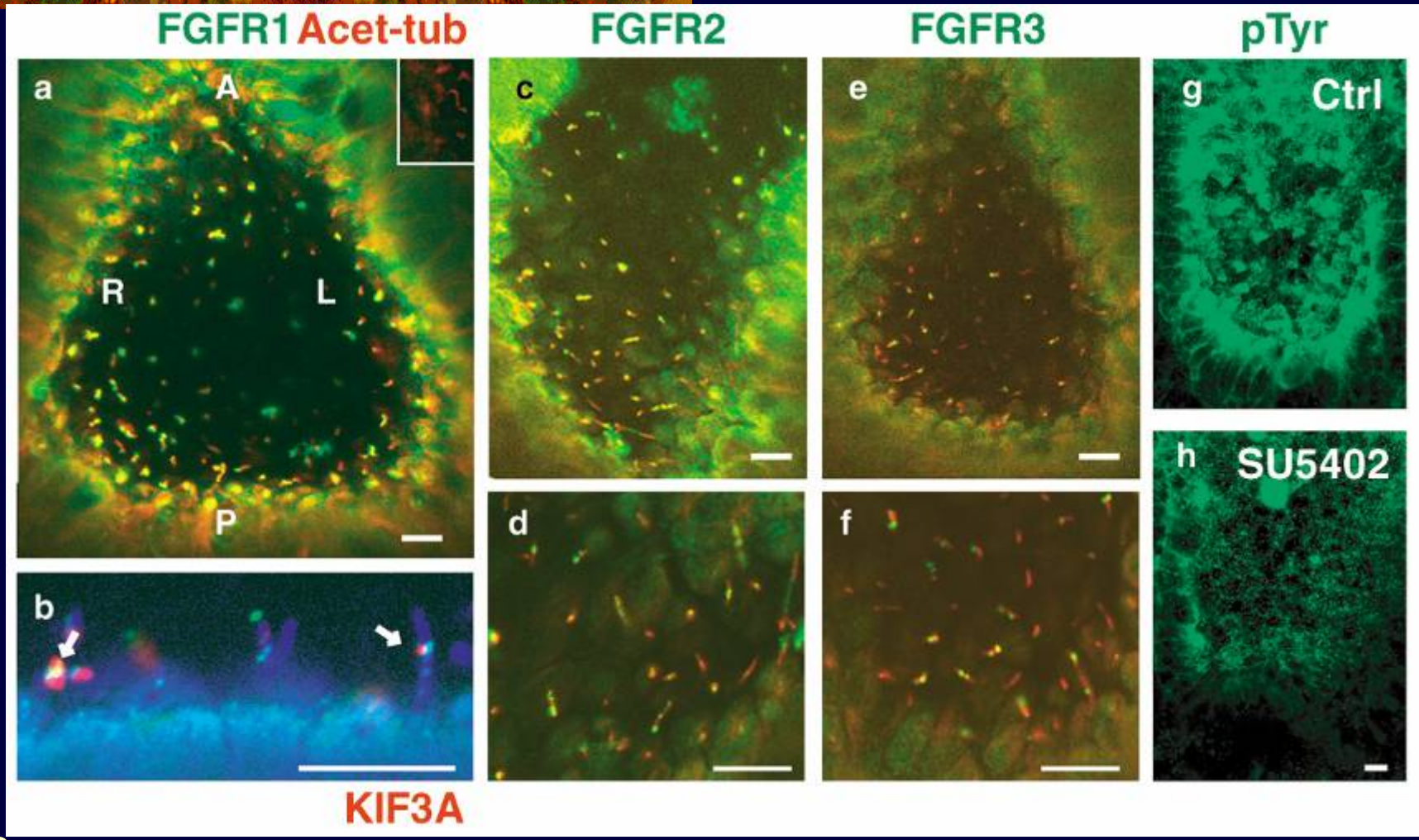


FGF receptors in ventral node

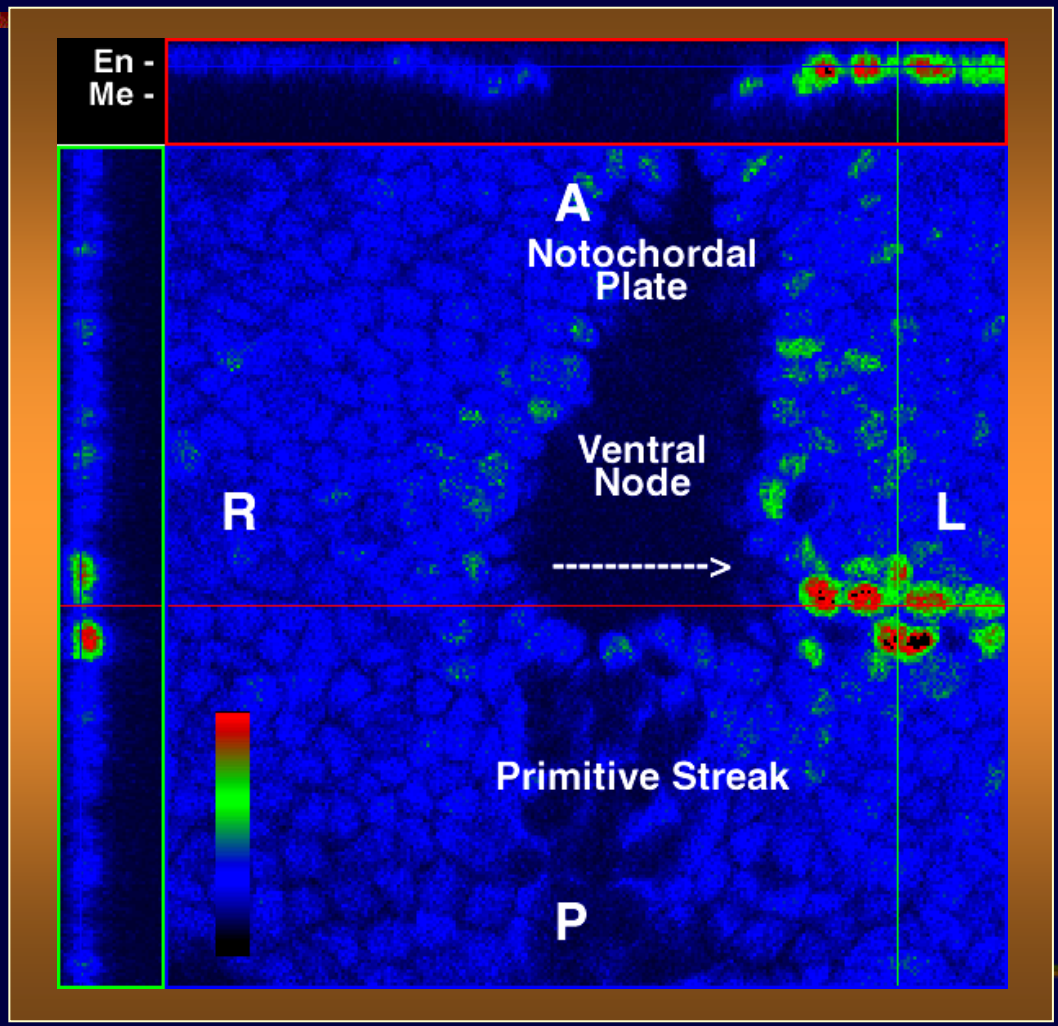




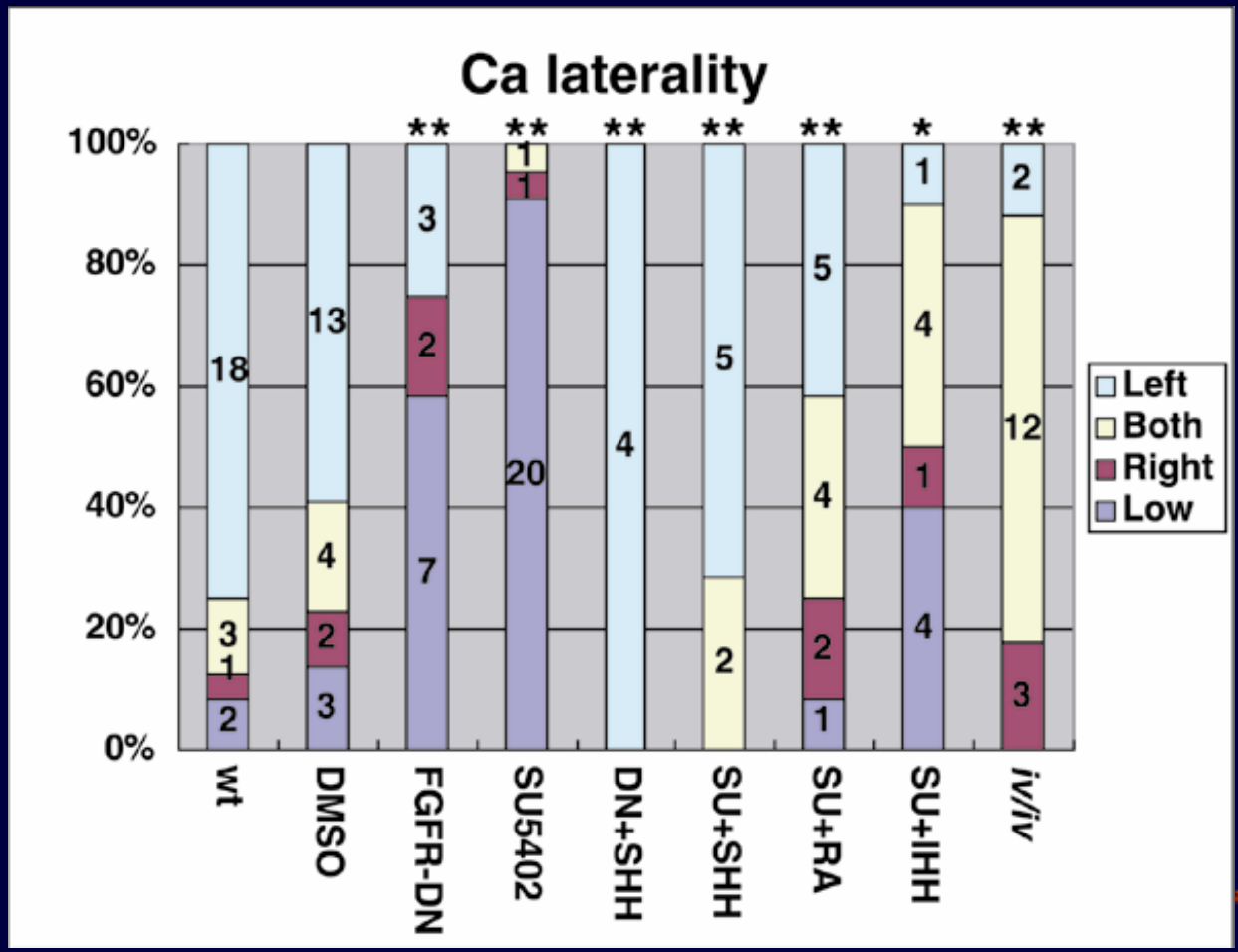
Active FGF signals in ventral nodal region



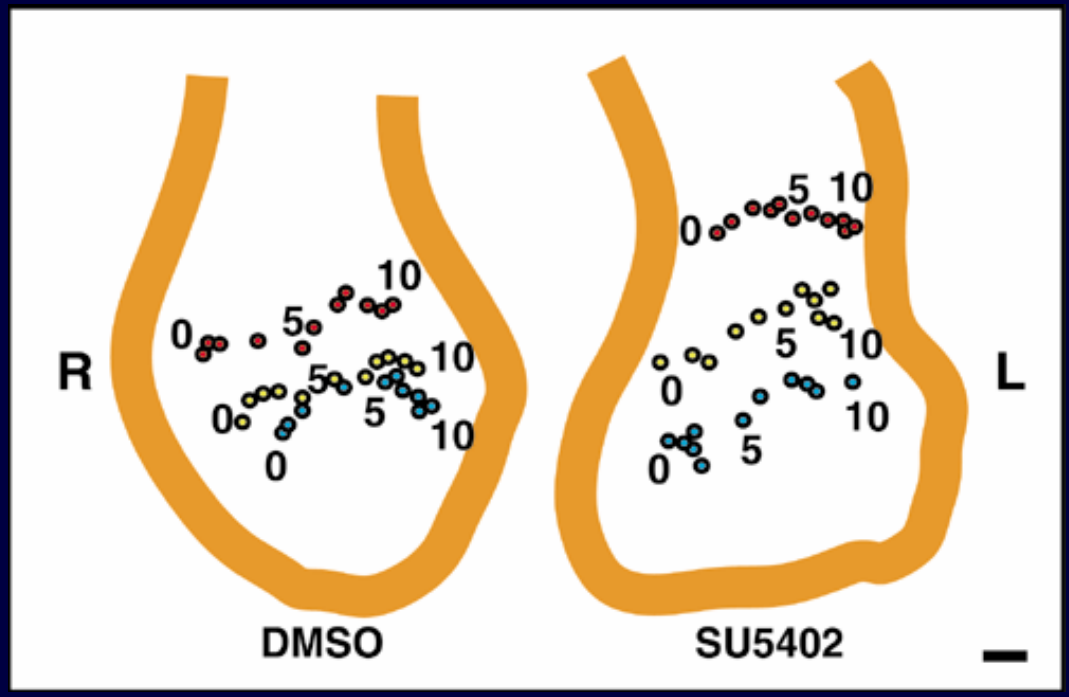
Ca elevation in left definitive endoderm

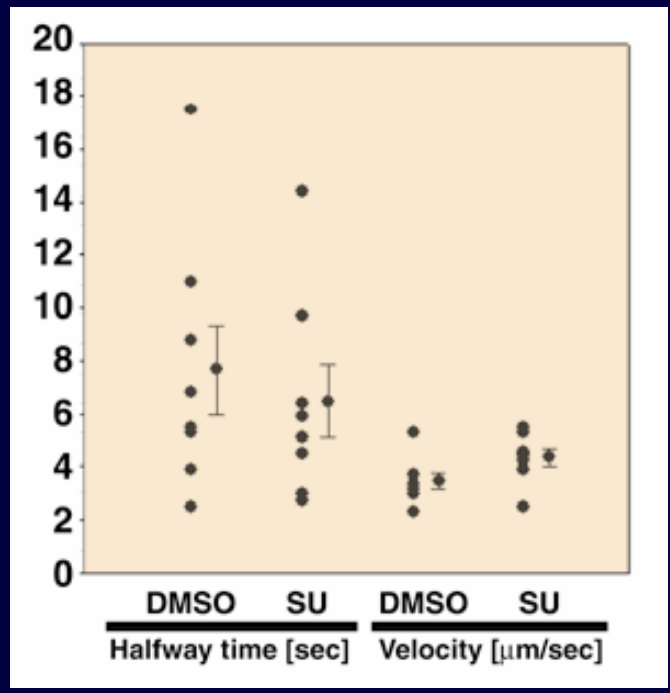


RA and SHH elevated Ca on the left side in the presence of SU5402

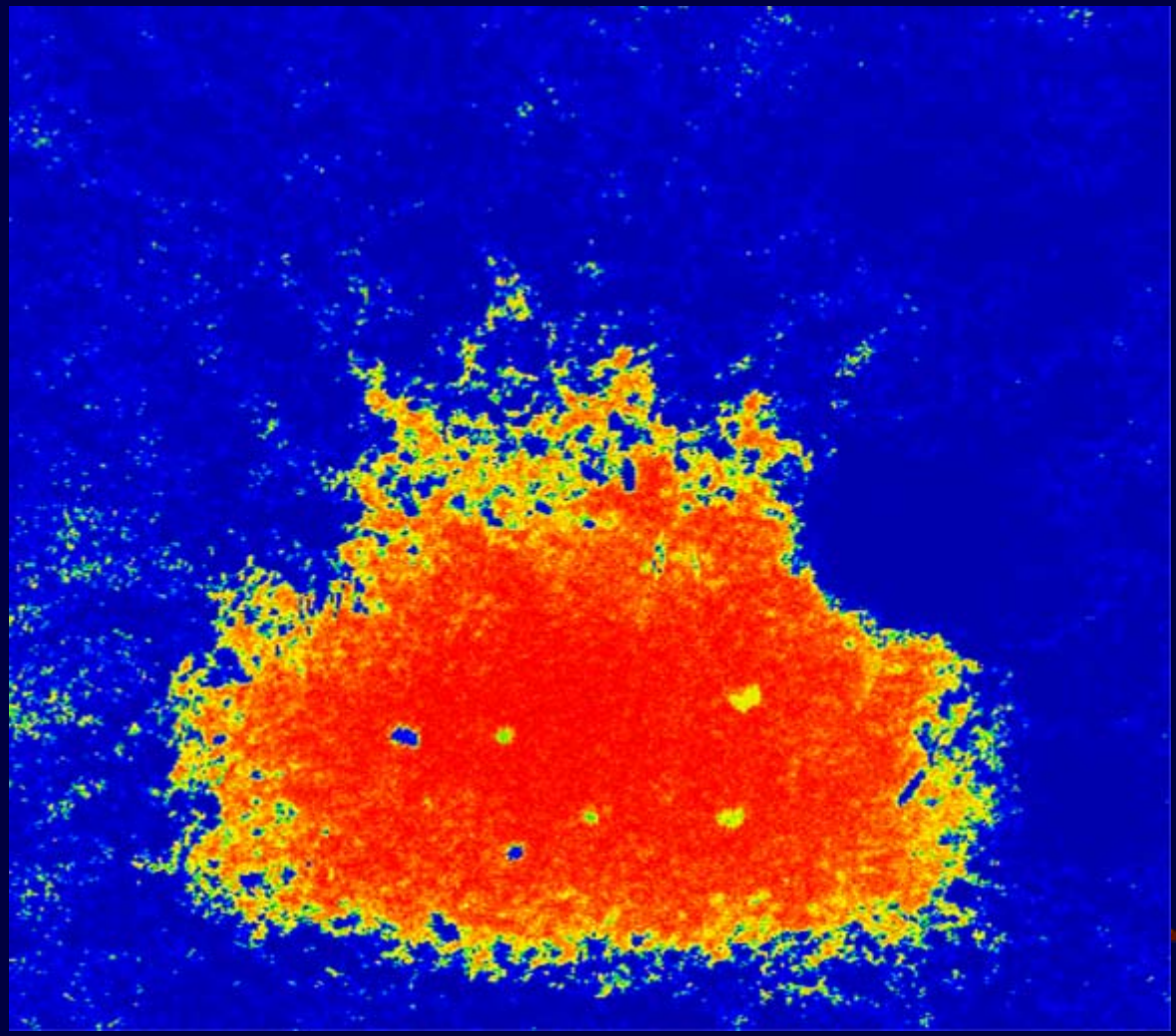


SU5402 did not apparently impair the fluid flow on 1-3 somite stage

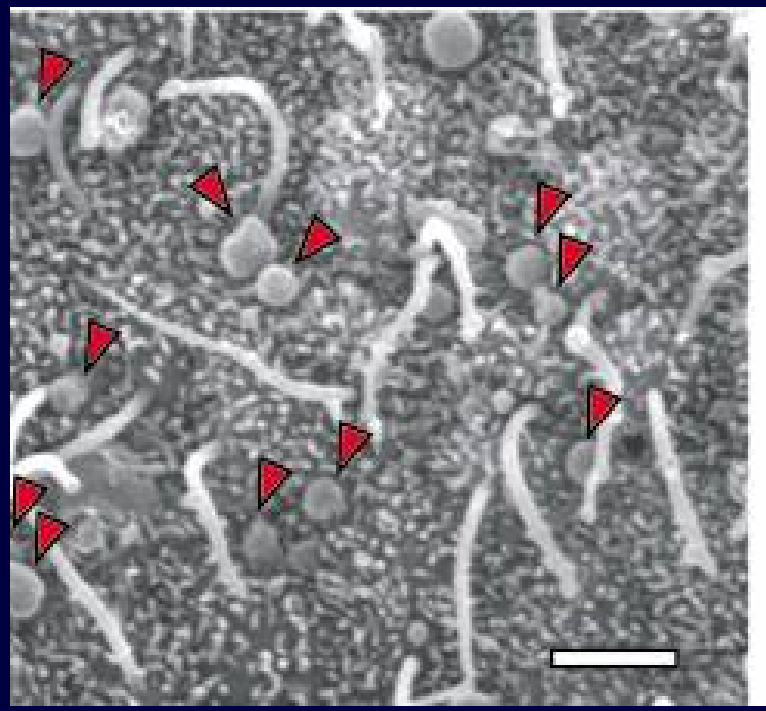


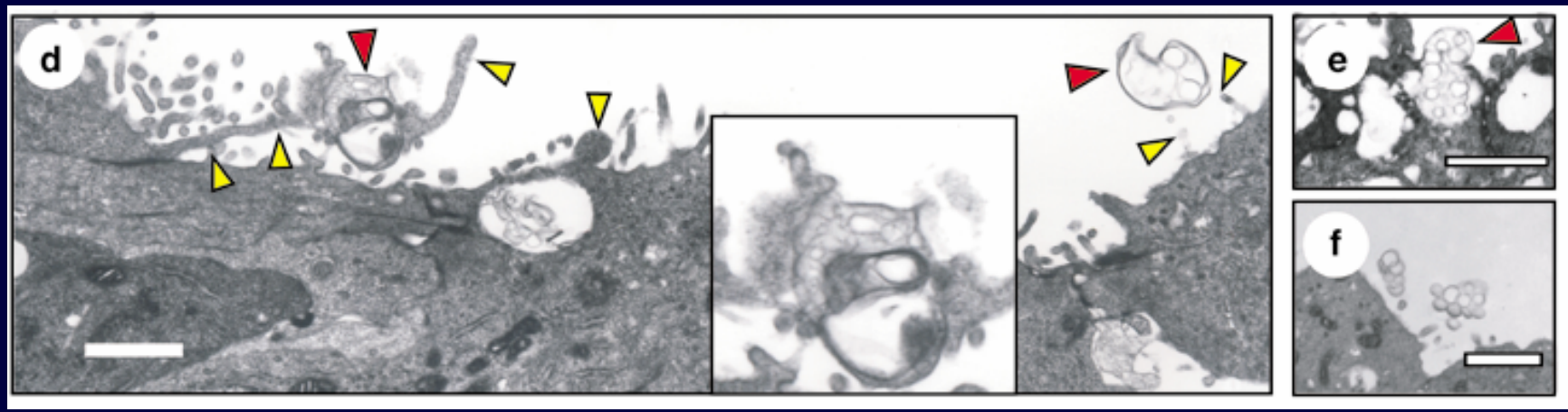


Nodal Vesicular Parcels (NVPs) flows from the right to the left



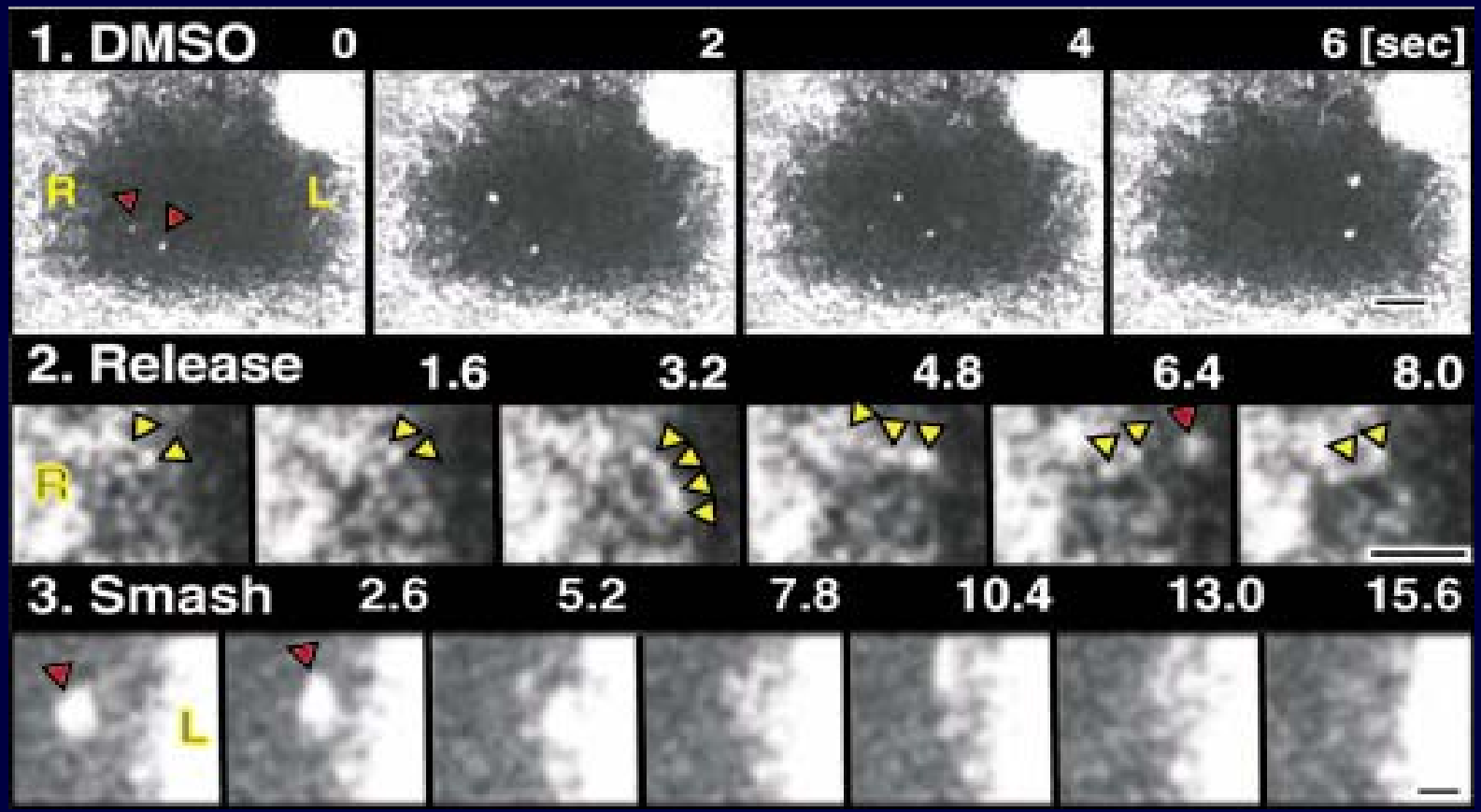
NVPs are associated with microvilli on its release



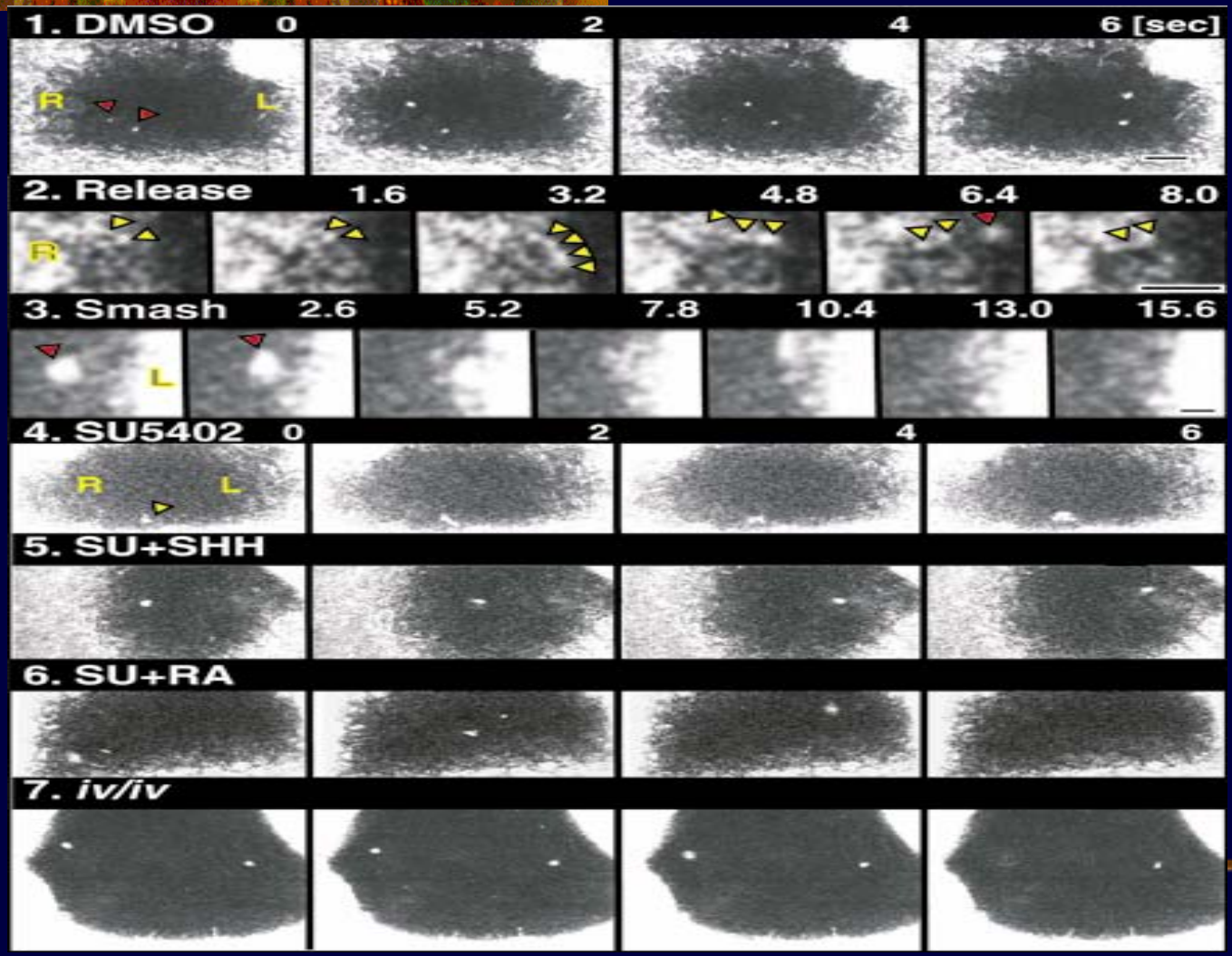


e & f, SU5402-treated

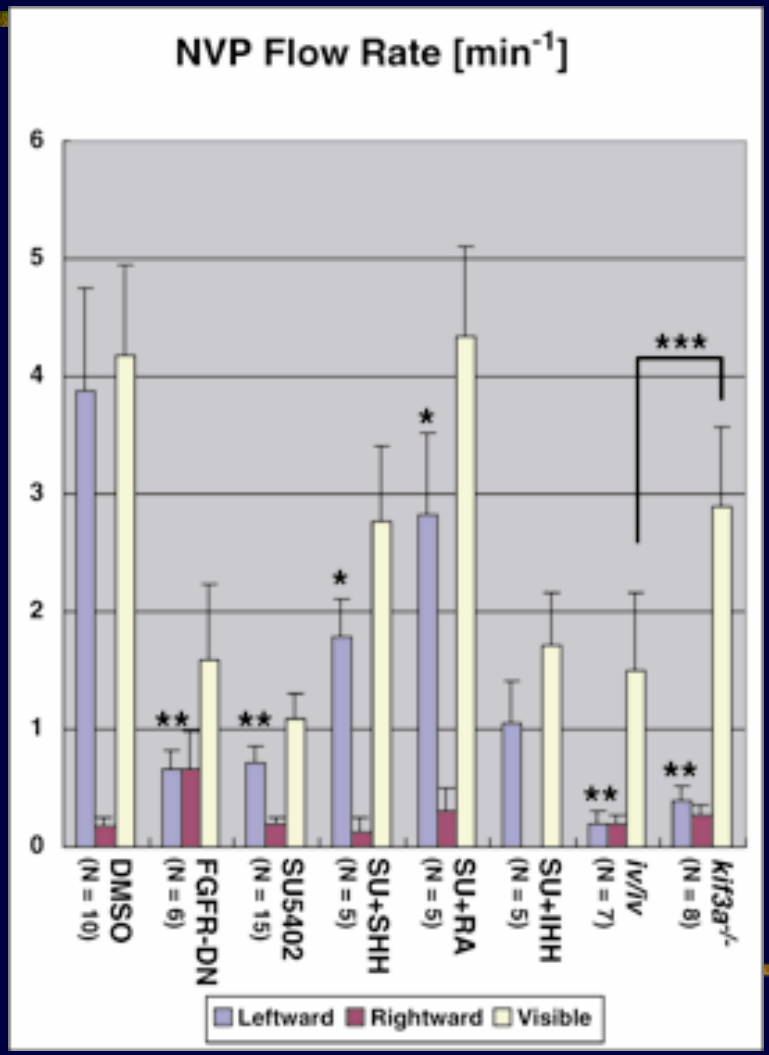
Unidirectionality of NVP flow is ensured by its fragmentation



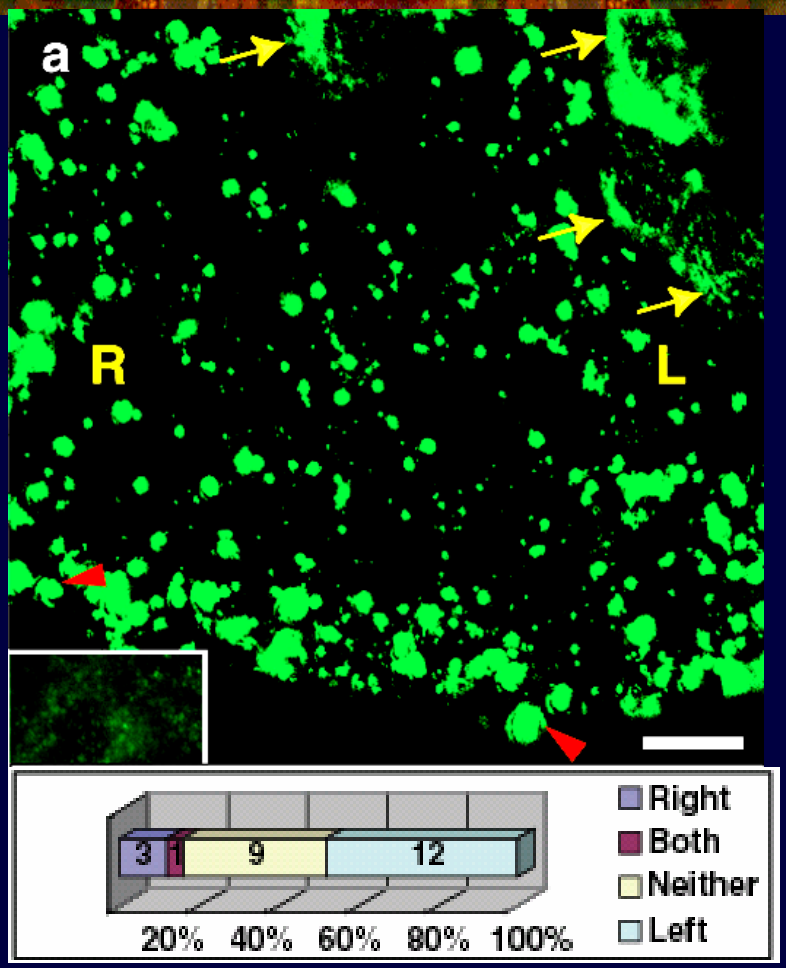
NVP flow can be modulated by pharmacological perturbations



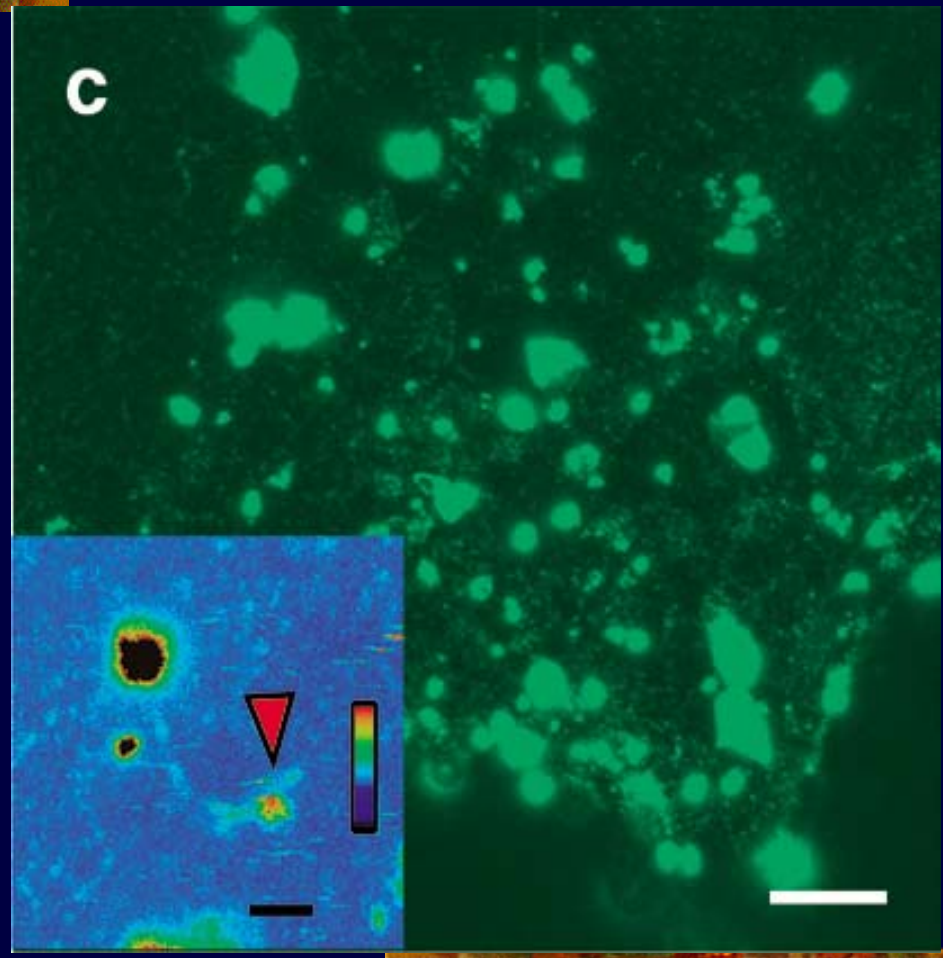
A non-ciliated node has more NVPs than a node with immotile cilia



SHH and RA localize on NVPs

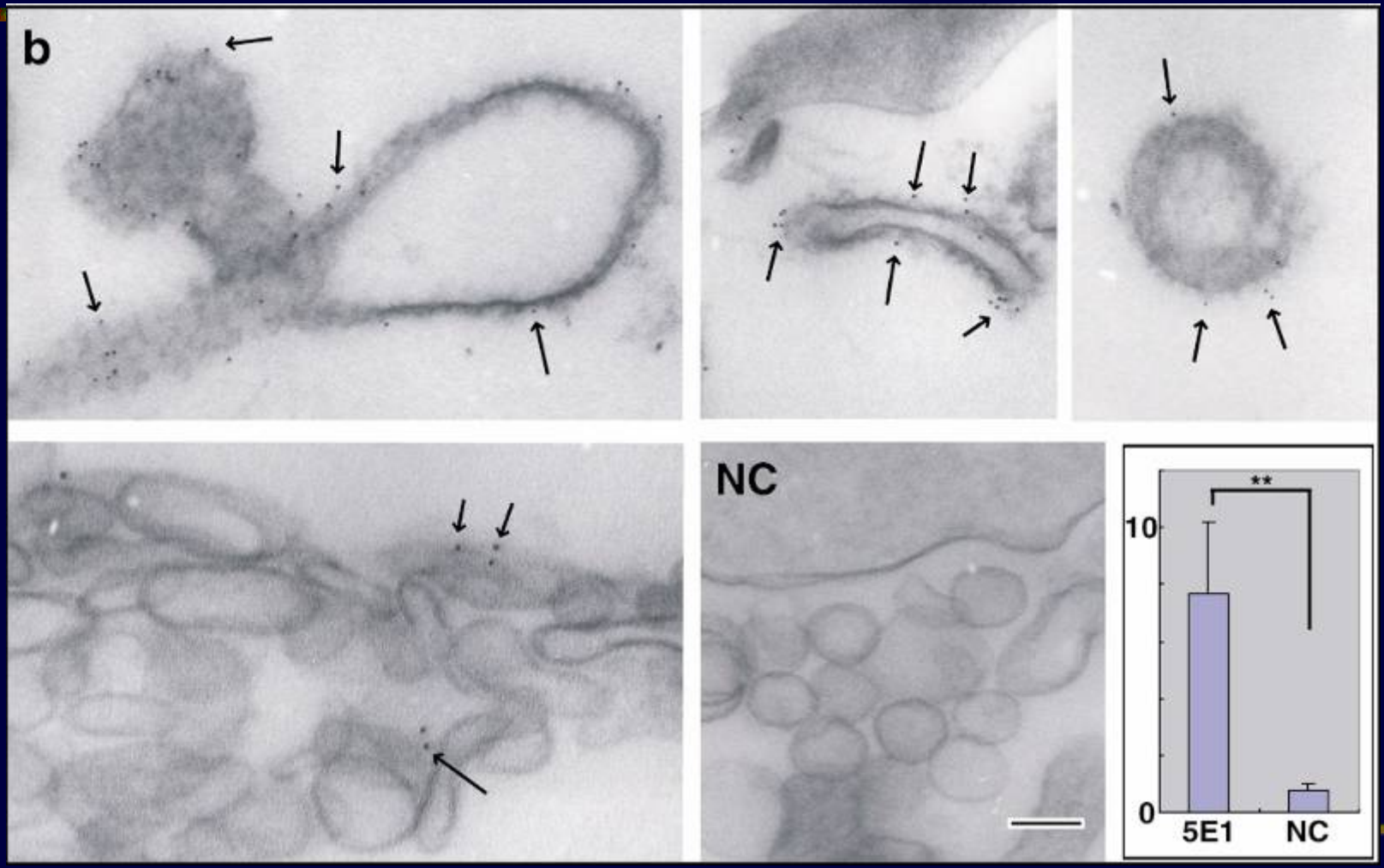


SHH

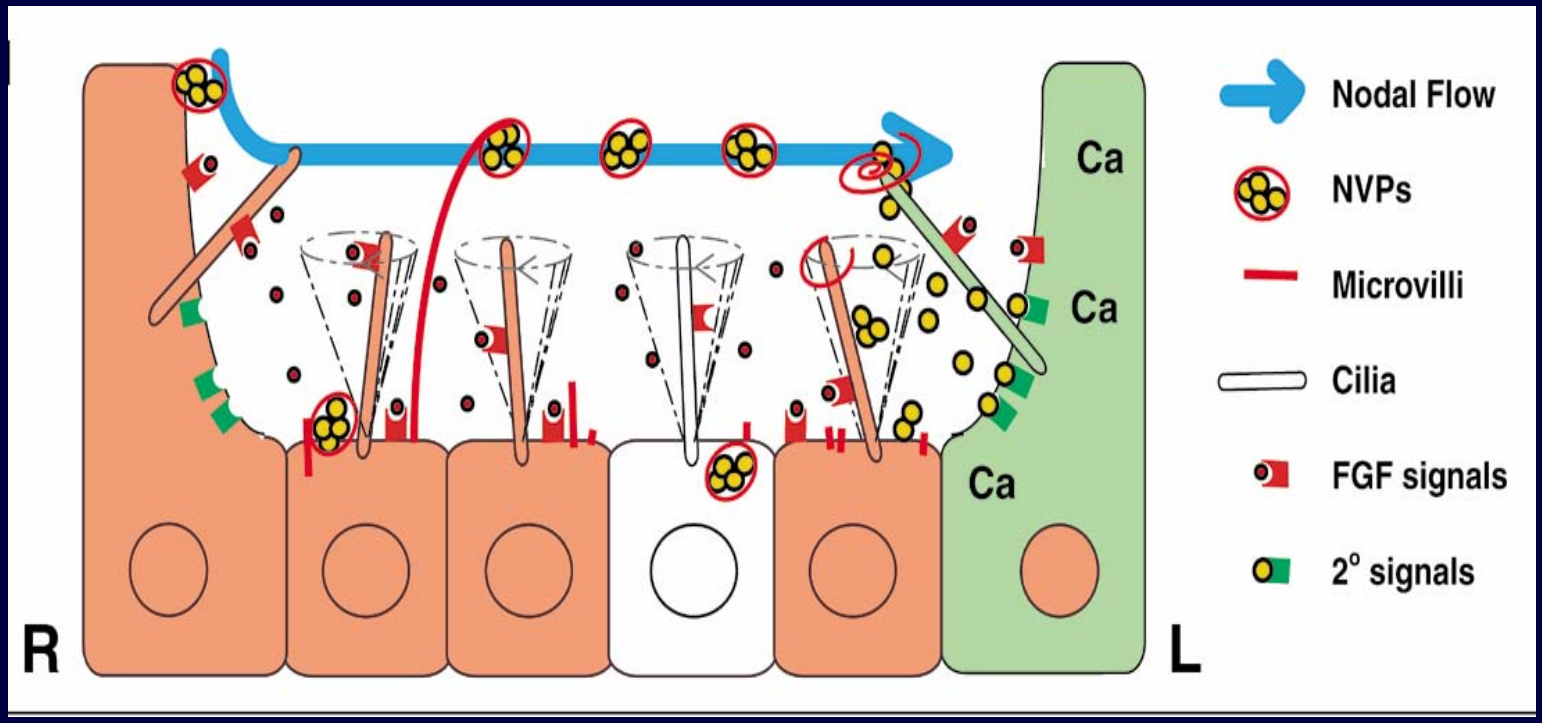


RA

ImmunoEM labeled against SHH on NVPs



FGF-induced NVP flow transports SHH and RA to the left



Nodal Flow
Microvilli
FGF signals
NVPs
Cilia
2° signals

Summary

- We detected a direct evidence of leftward morphogen transport in mouse ventral node on 1-3 somite stage as a molecular basis of forming concentration gradients along the left-right axis.
- Membrane-sheathed extracellularly secreted objects, the NVPs, were identified to be vehicles of SHH and RA, which flow to the left and trigger Ca elevation on the left periphery of the node.
- Dynamically protruding microvilli are involved in active release of NVPs, and nodal cilia appear to facilitate their fragmentation on the left, in addition to generating the fluid flow.
- FGF signaling in the nodal region facilitates NVP release and Ca elevation, but is not indispensable for generating the fluid flow. Thus fluid flow itself is not sufficient for Ca elevation.
- SHH or RA is sufficient to evoke the NVP release and Ca elevation even in the presence of FGFR inhibitor, suggesting a “shuttle bus model” on its releasing machinery that may sense the contents.