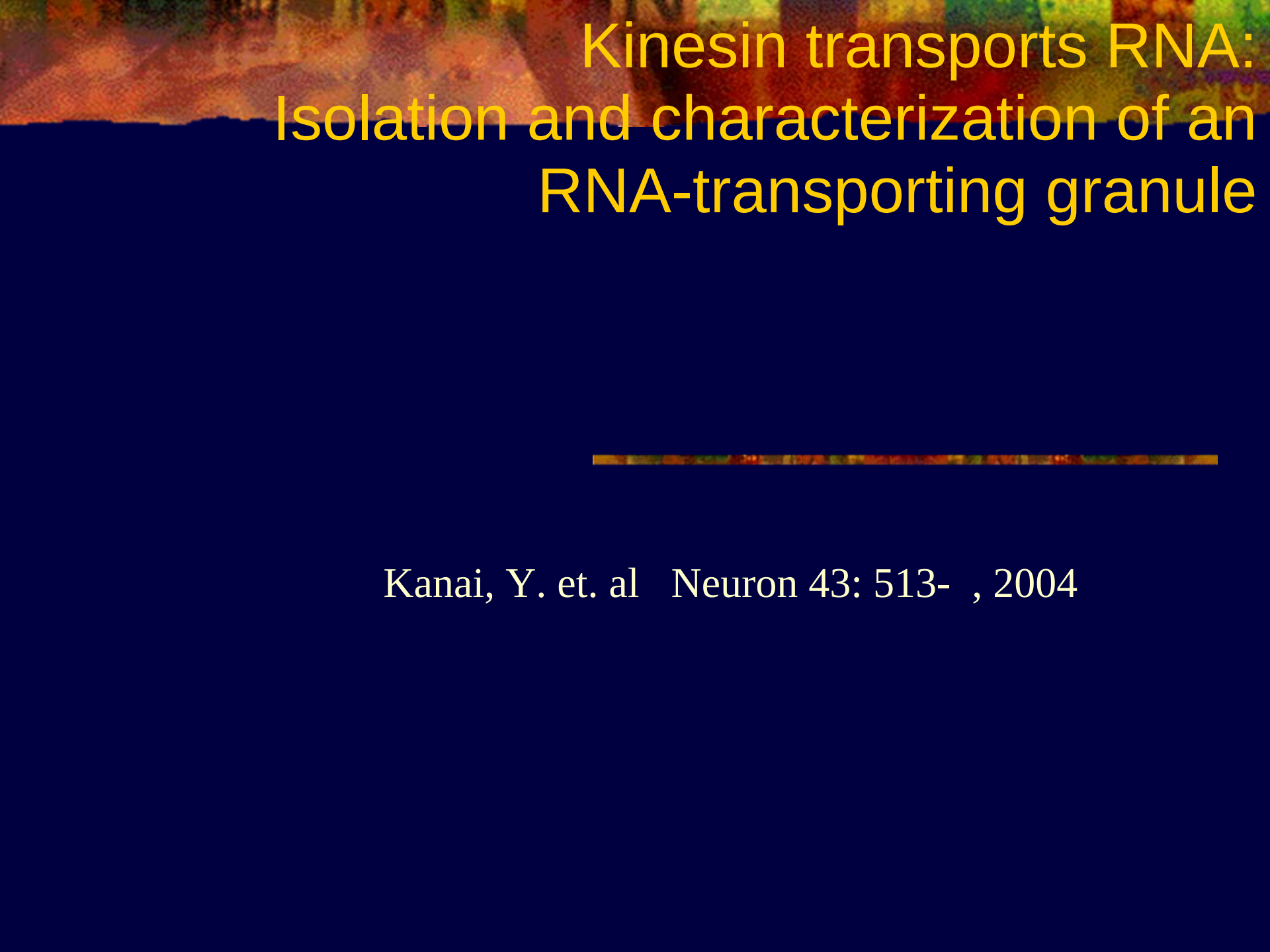
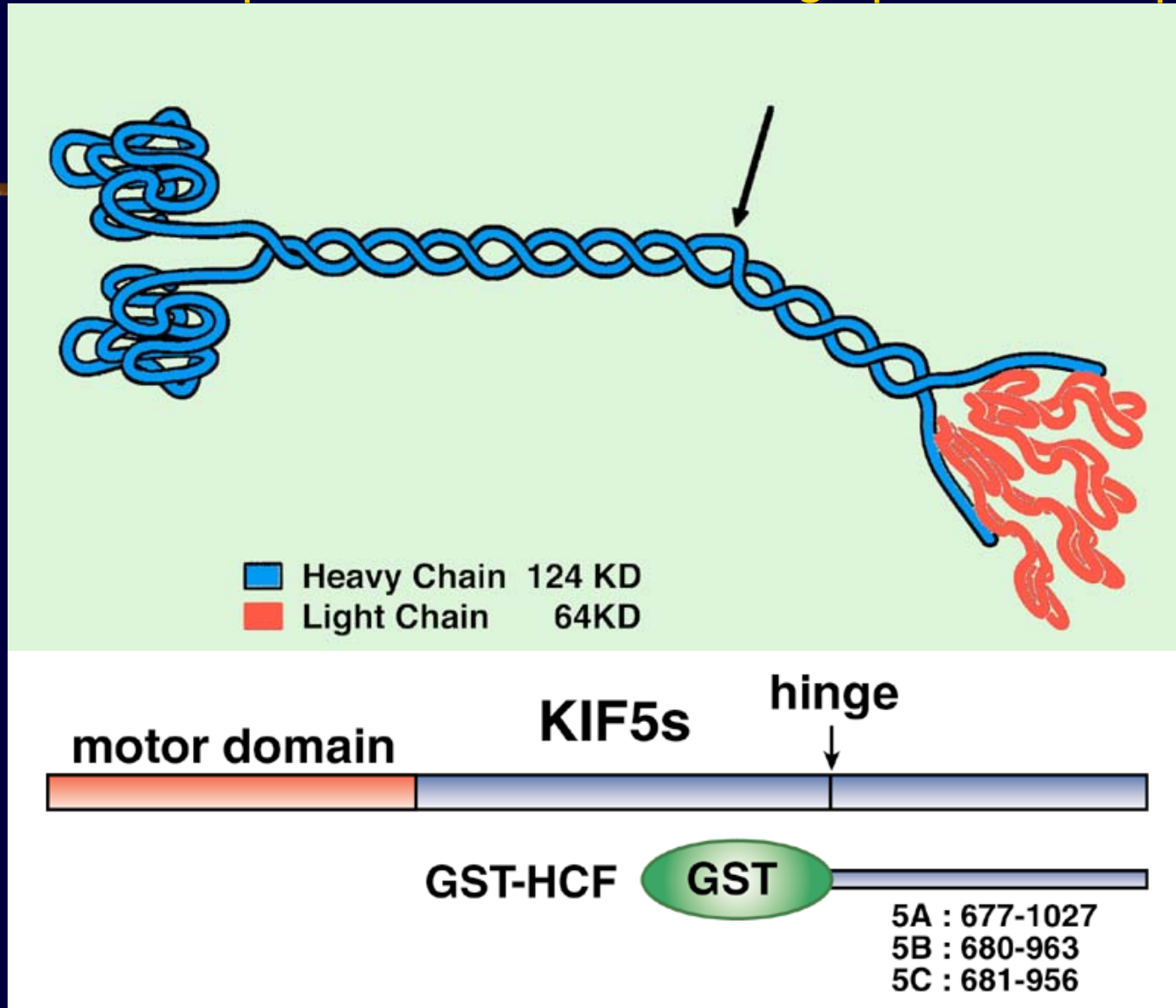




Kinesin transports RNA: Isolation and characterization of an RNA-transporting granule

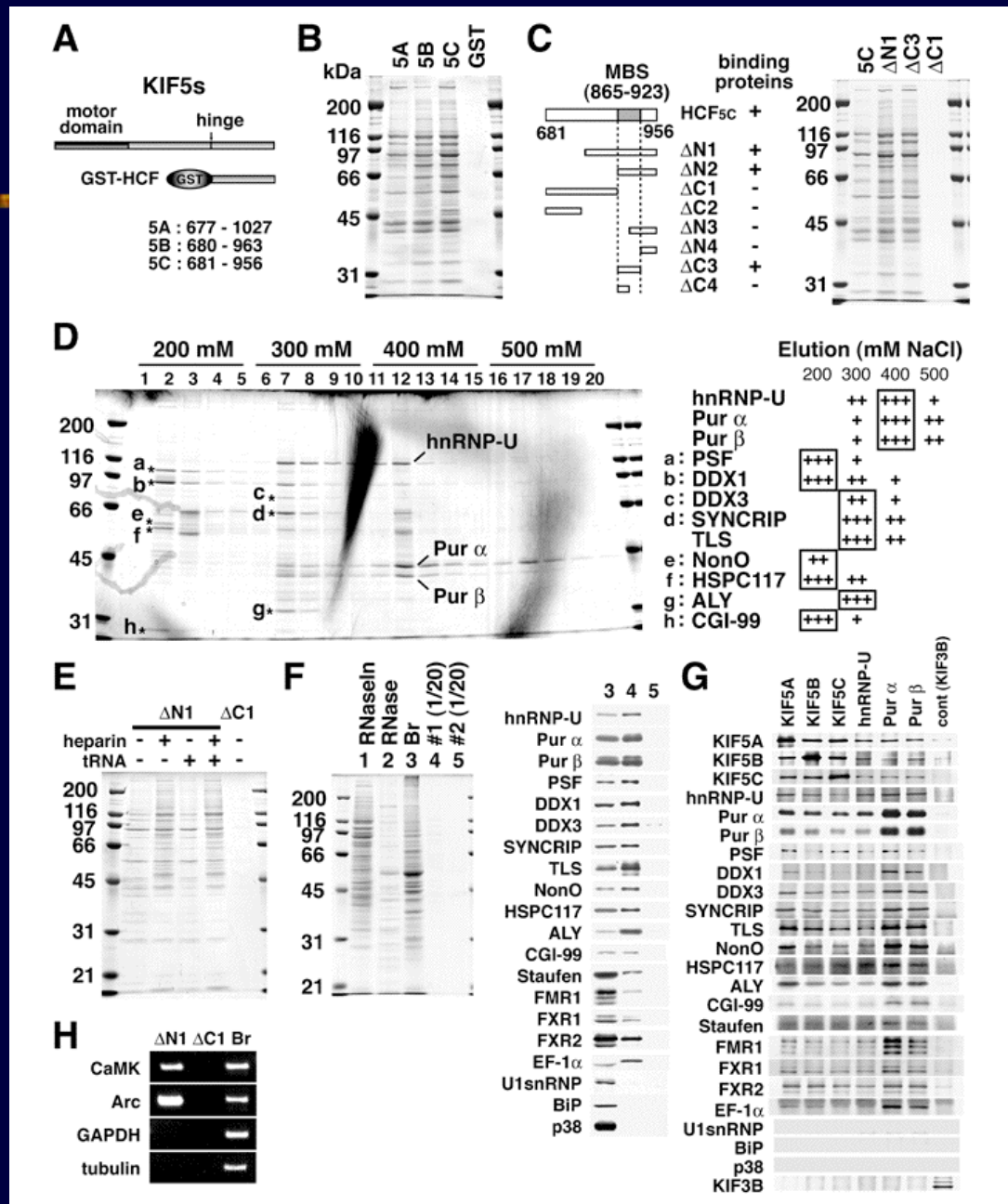
Kanai, Y. et. al Neuron 43: 513- , 2004

Kinesin transports mRNAs with a large protein complex

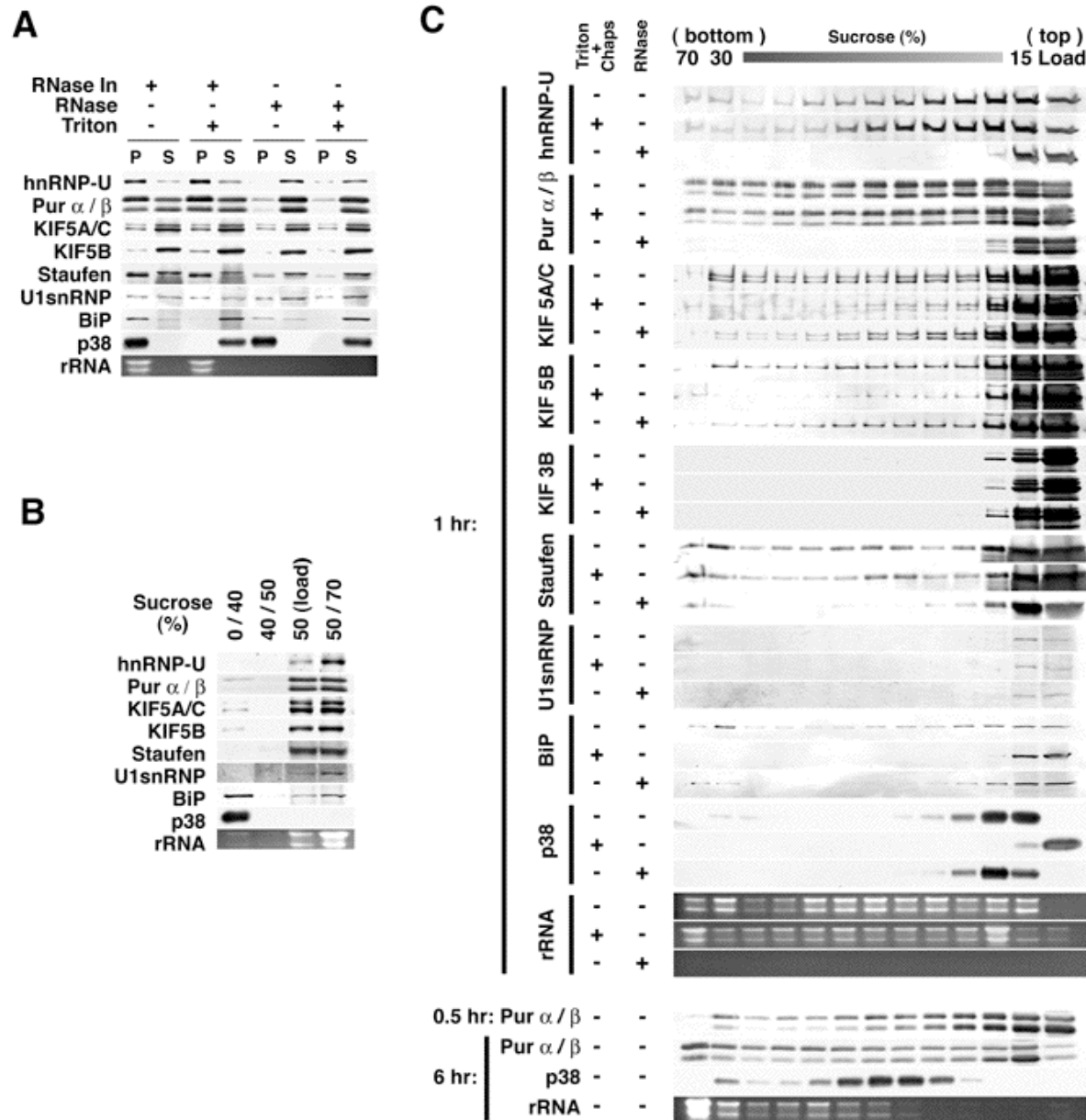


Kanai, Y. et al Neuron 43:513- , 2004

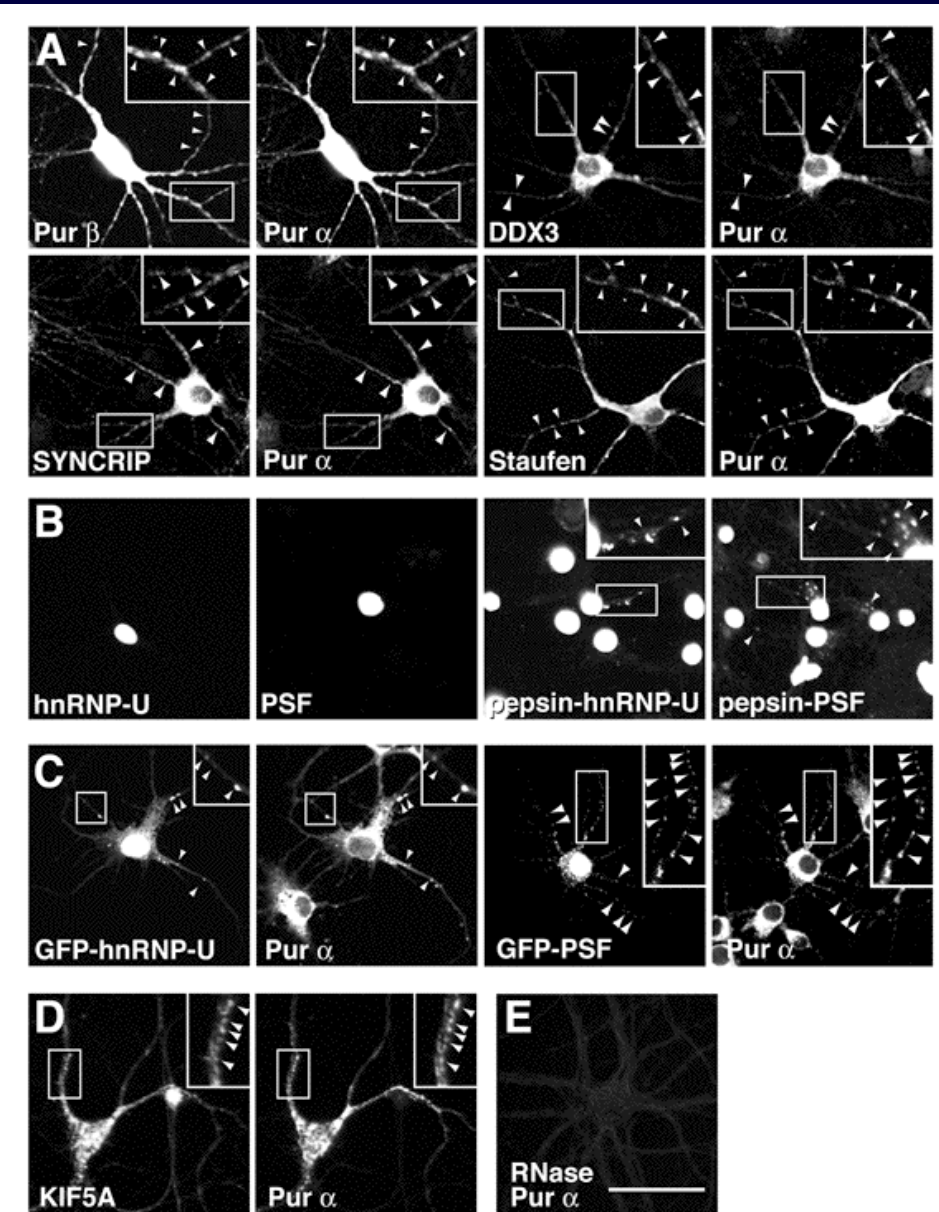
Isolation of a protein-RNA complex using KIF5 tail



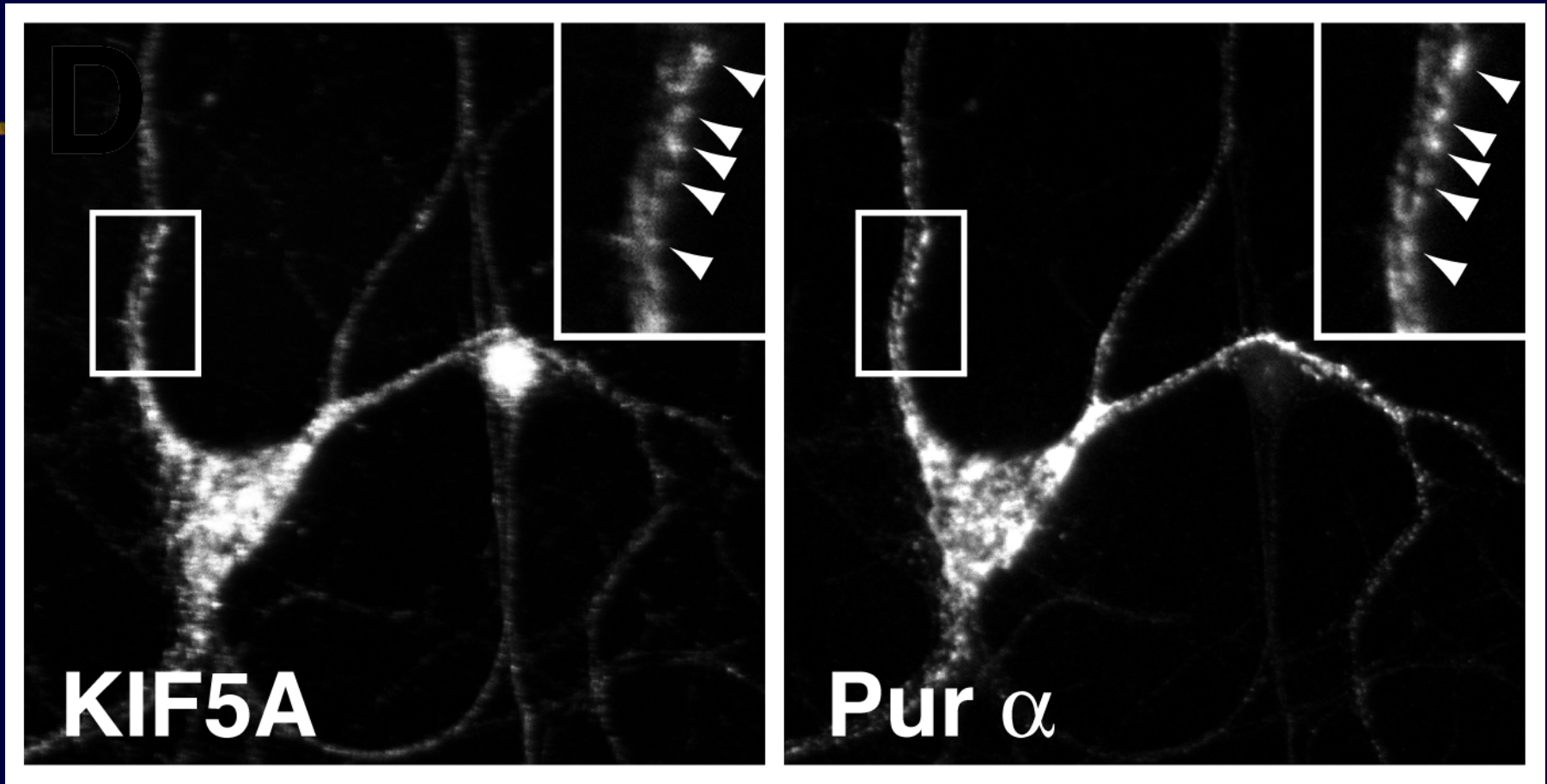
The complex has a large S-value of 1000~



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

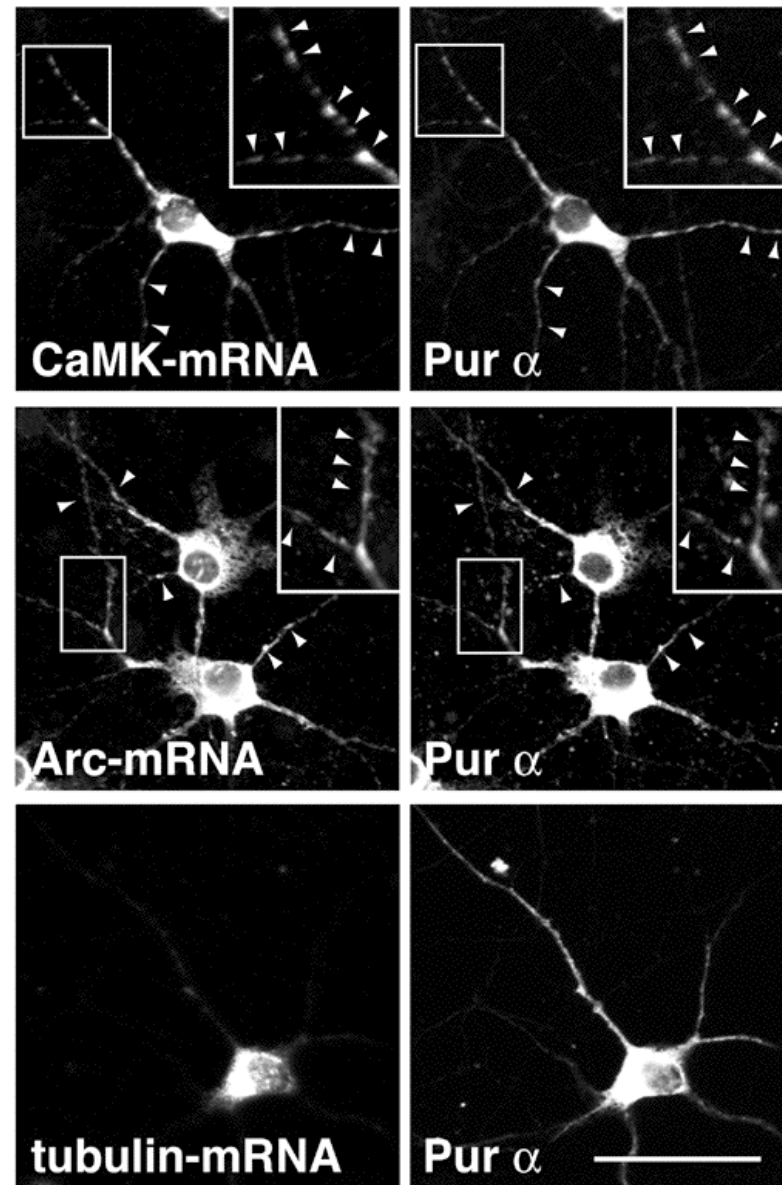


KIF5A in Triton-extracted cultured neurons

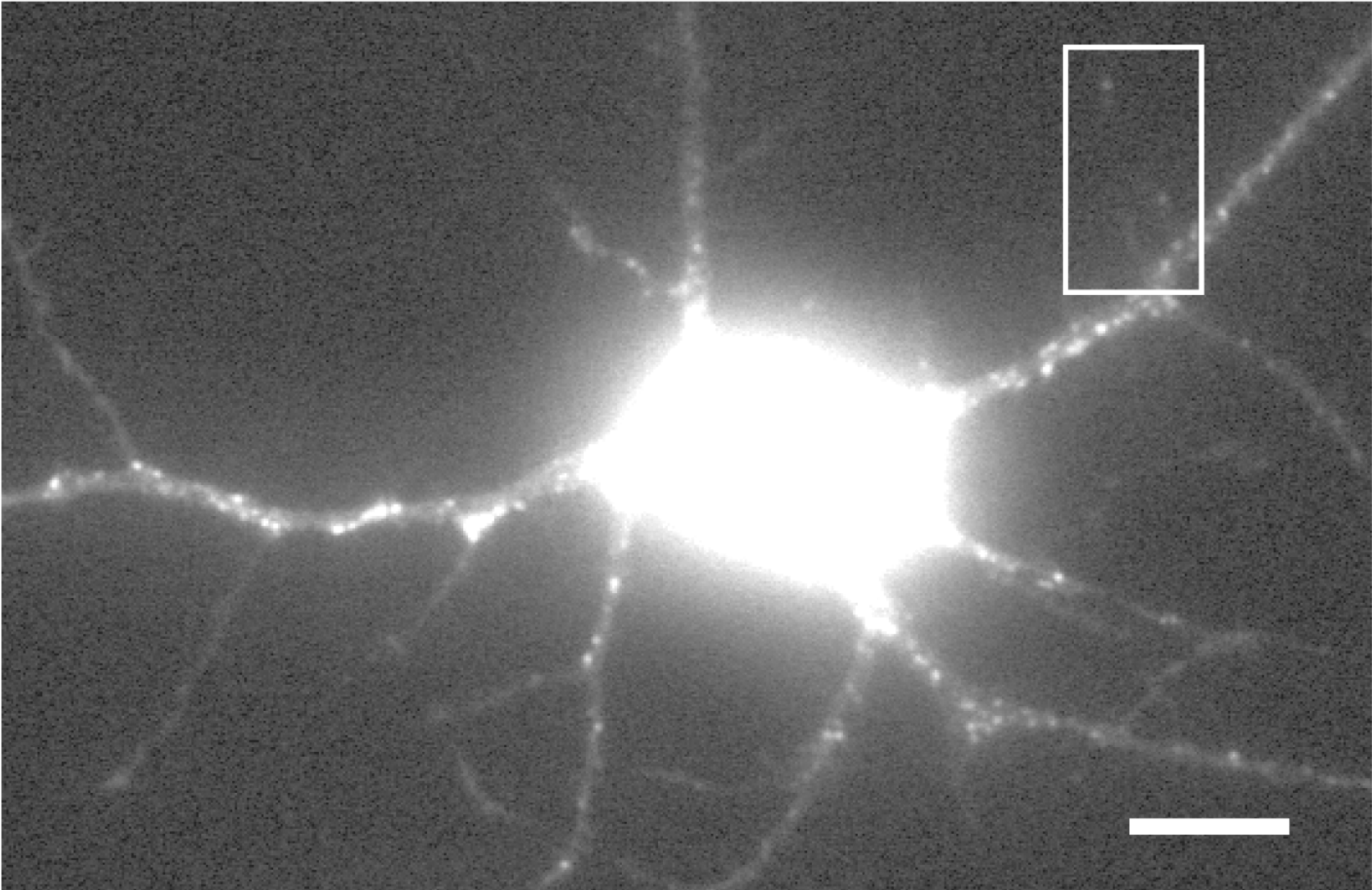


KIF5A colocalized to the Pur- α -containing granules in Triton-extracted neuron

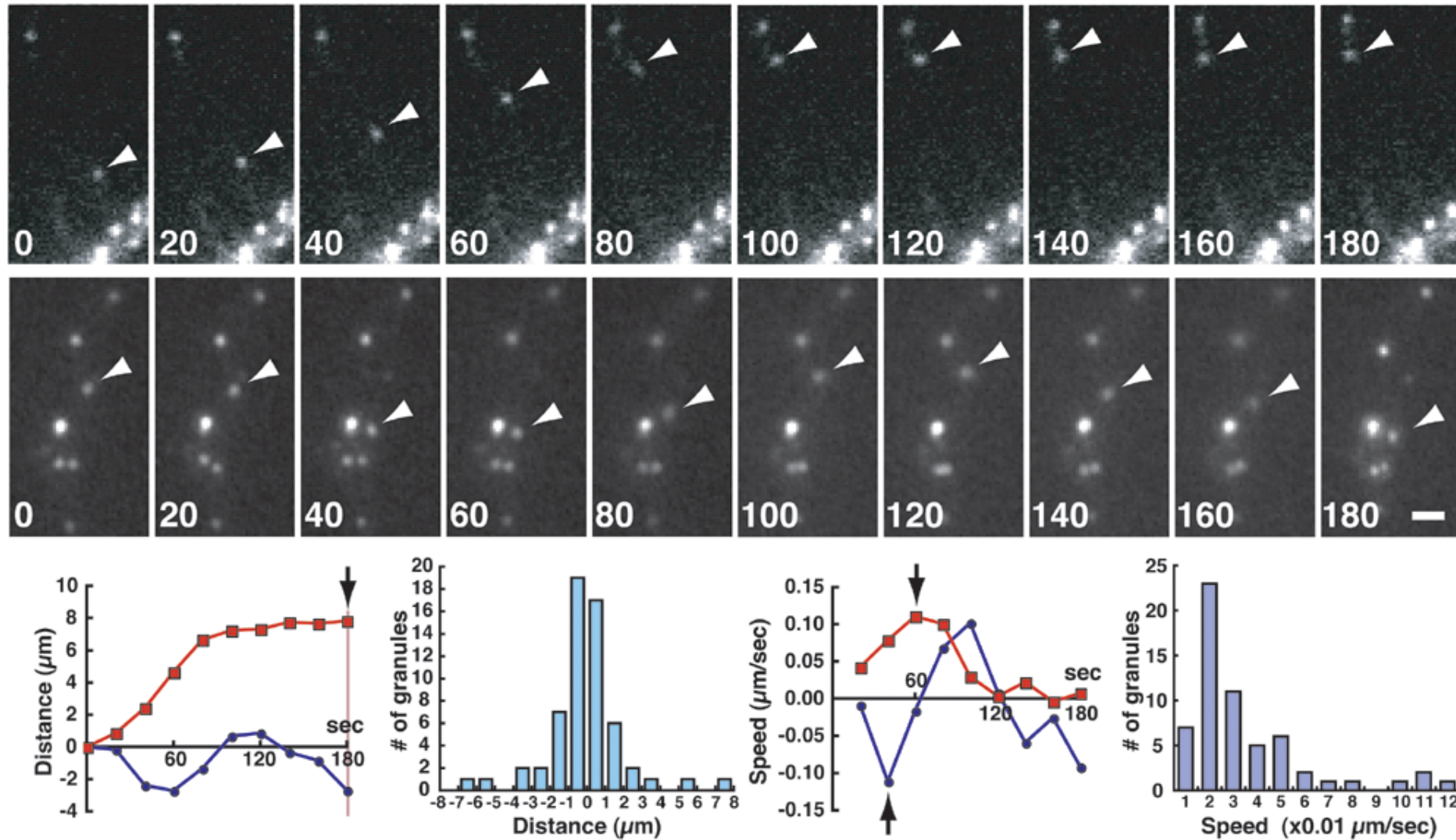
Colocalization of mRNAs for CaMKII α and Arc to the Pur α -containing granules in dendrites



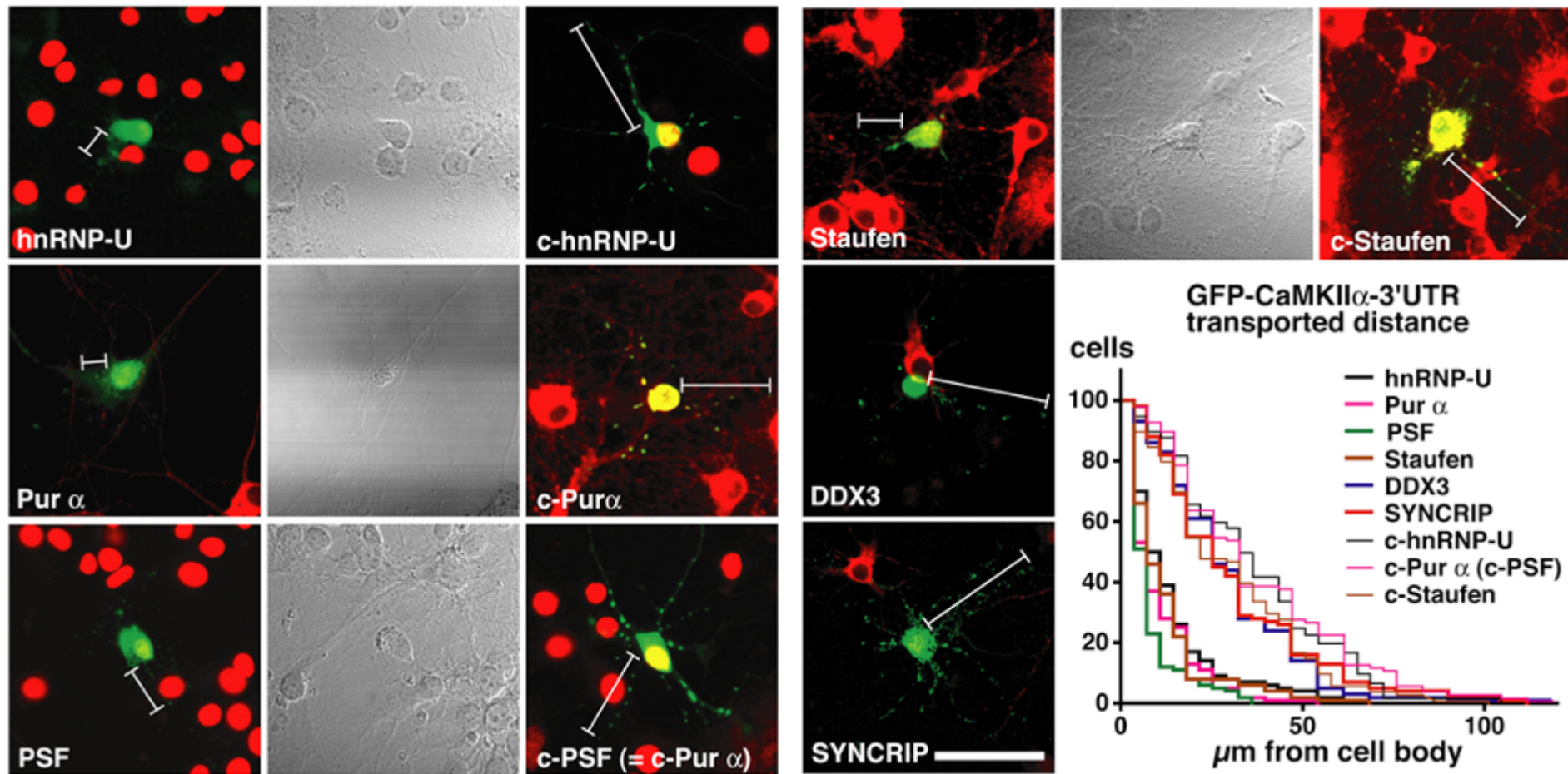
Movement of the complex (GFP-Pur α)



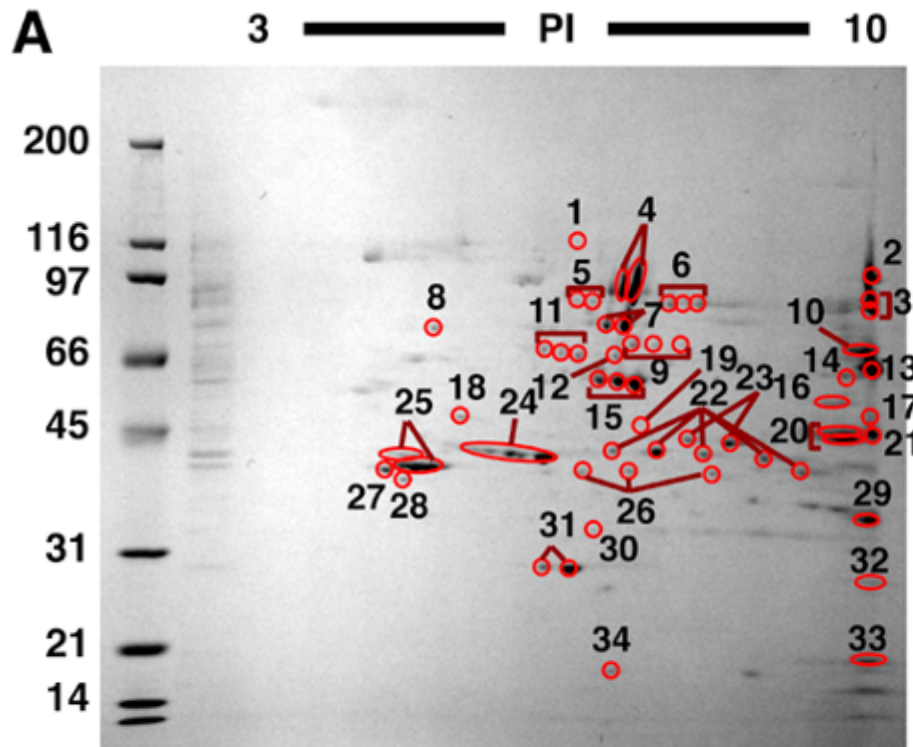
Movement of the complex(GFP-Pur α)



Knockdown of the identified proteins by RNAi



Proteomics analysis of the RNA-transporting granules



B

RNA transport: 6

name	accession	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-1 α	NM_007906	**
eIF2 α	NM_019356	28
eIF2 β	AK012817	18
eIF2 γ	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

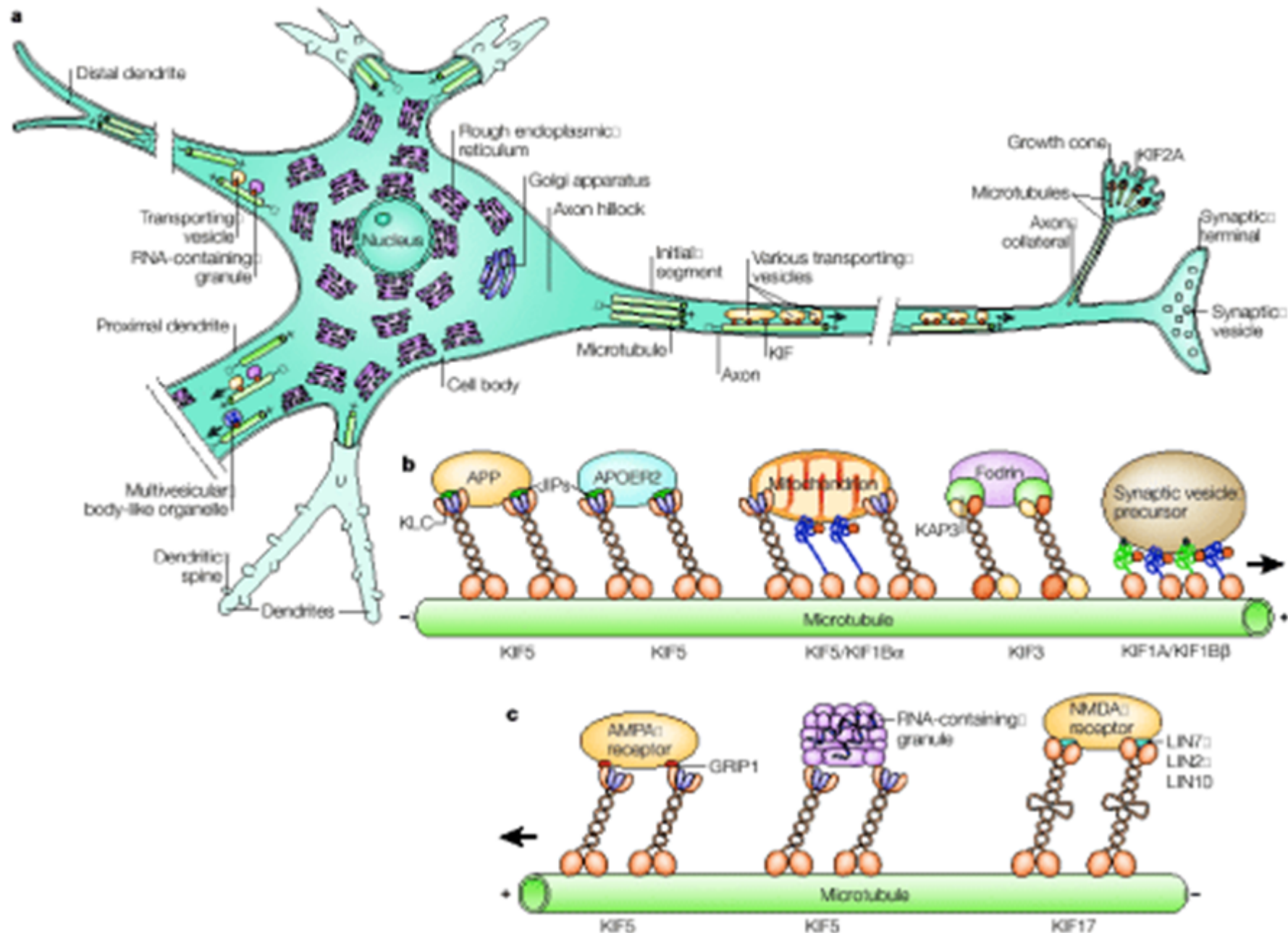
name	accession	spot
ADP-ribosylation factor-guanine nucleotide factor 6	NM_015310	21
ALY	MMU89876	*
cold inducible RNA-binding protein	NM_007705	33
EWS	NM_007968	3
NonO	NP_075633	14*
Nucleolin	Q99K50	1
paraspeckle protein 1	BC026772	11
PSF	NM_023603	2*
RNA 3'-terminal-phosphate cyclase	NM_025517	23
RNA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

Other known proteins: 3

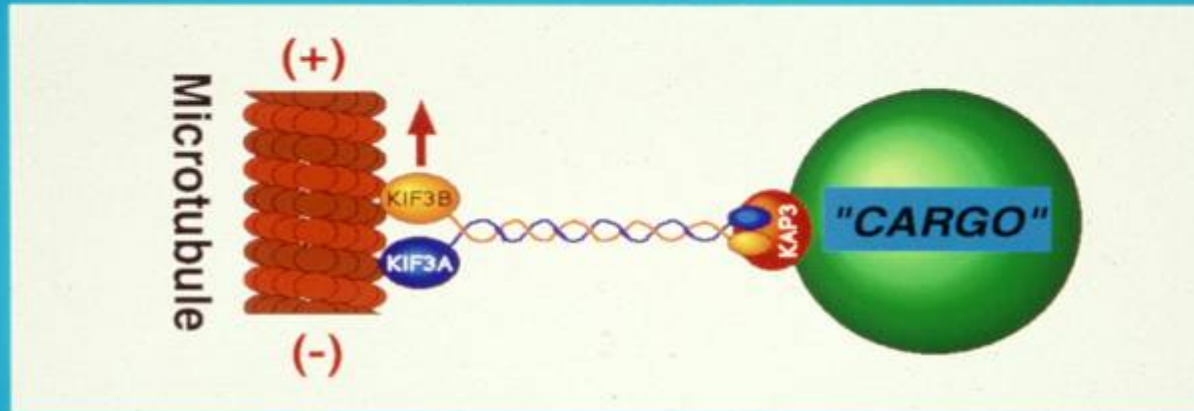
Ser / Thr kinase receptor-associated protein	NM_011499	27
TRIM2	NM_030706	5
TRIM3	NM_018880	6

Hypothetical proteins: 7

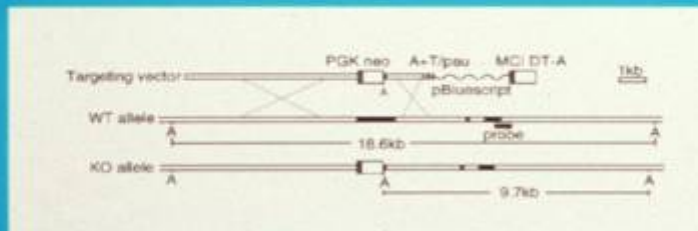
CGI-99	NM_026528	31*
FLJ38426	BC048087	20
HSPC-117	NM_145422	15*
zfp385	BC017644	17
2610528C06Rik	BC037640	12
5730436H21Rik	NM_134139	19
6720458F09Rik	NM_177374	30



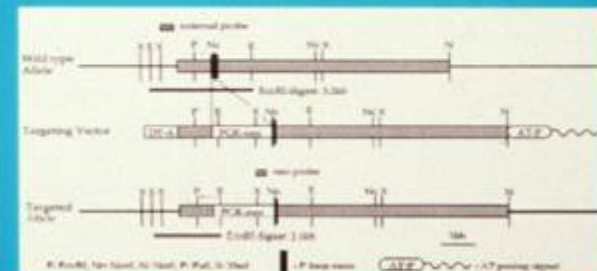
KIF3 Complex = KIF3A + KIF3B + KAP3



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

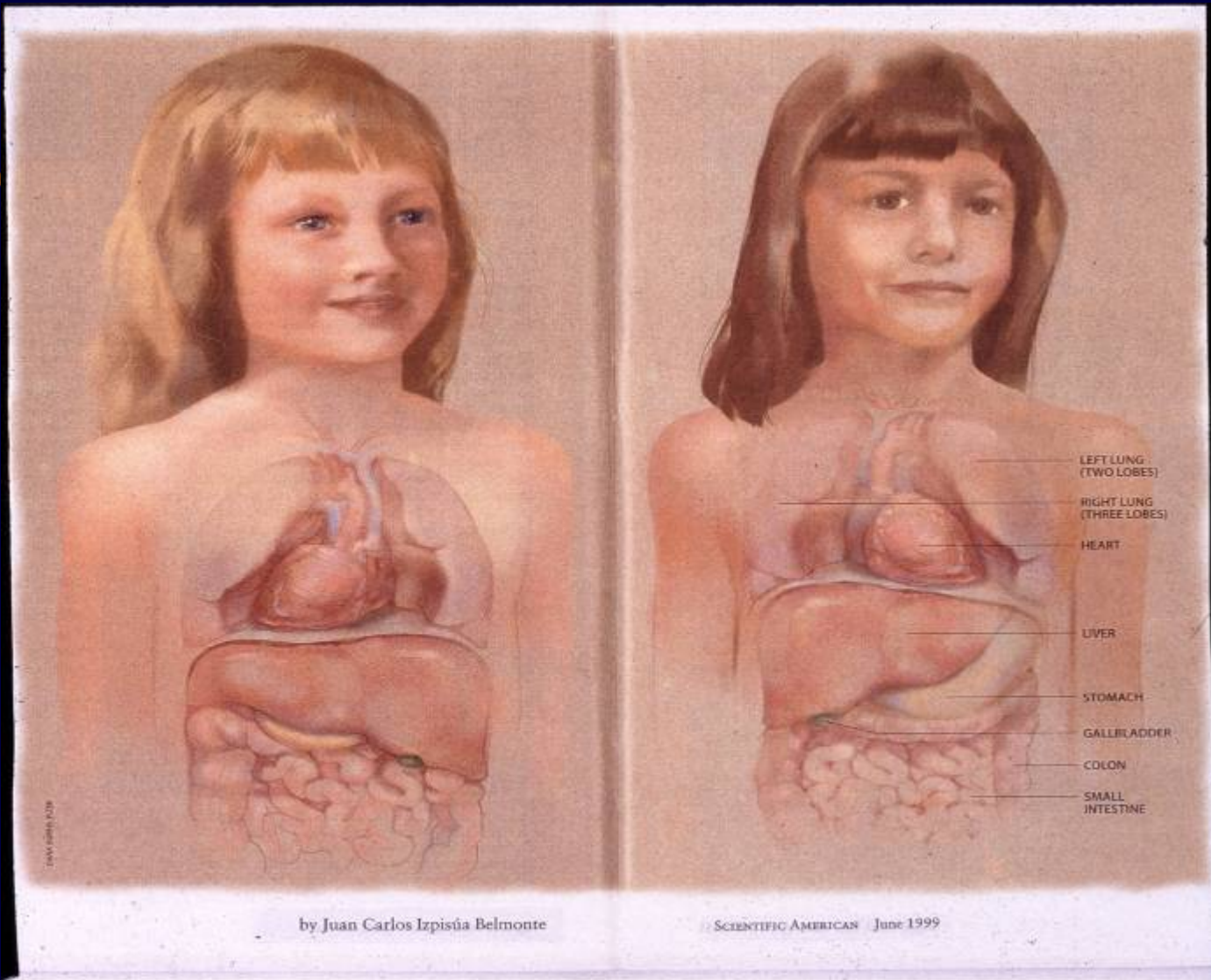


control

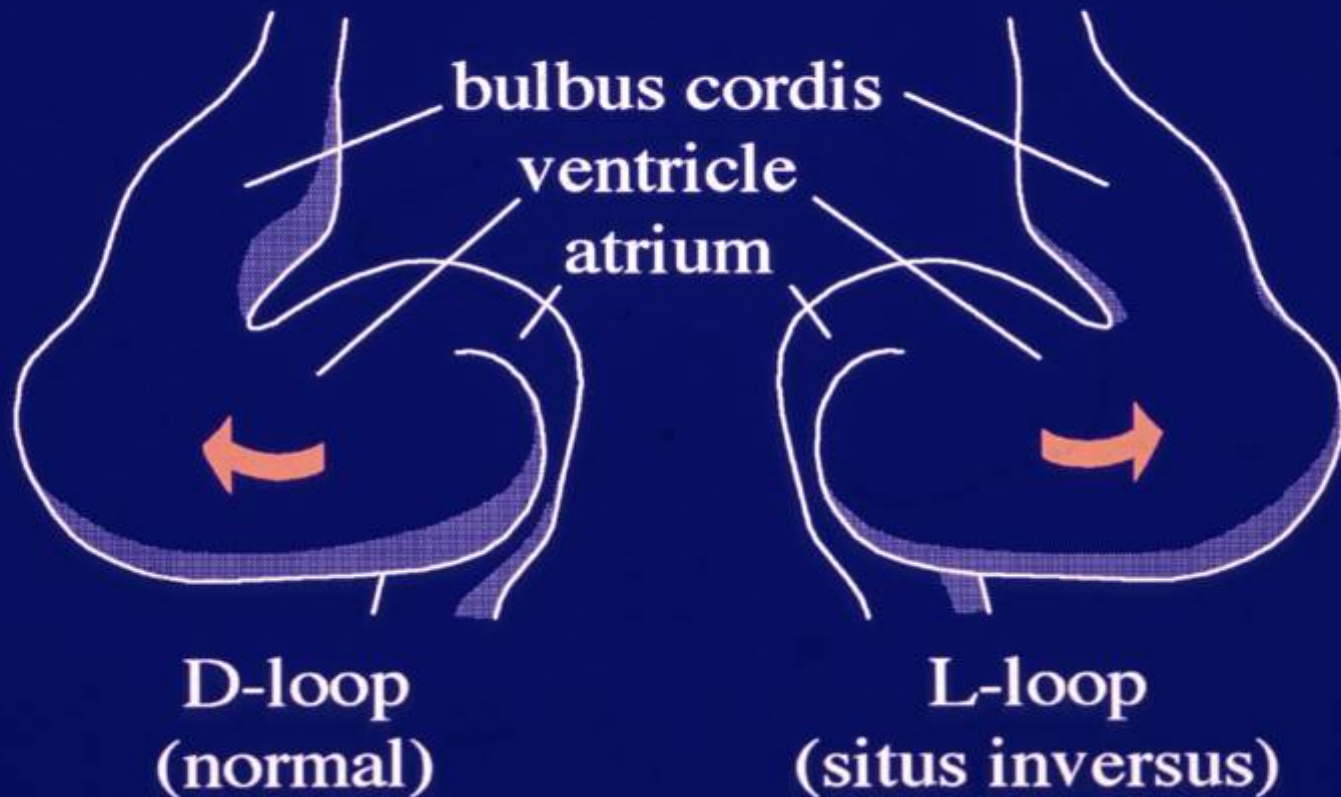
12.0dpc



***kif3B*^{-/-}**



Cardiac Loop at 9.5dpc

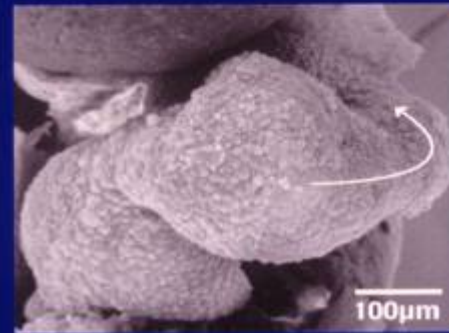
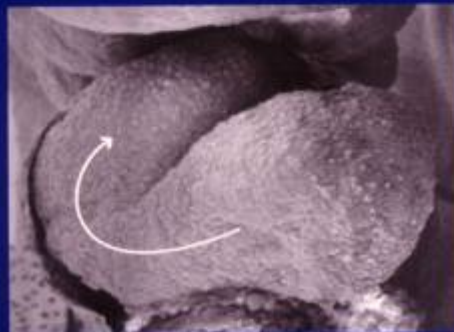


Heart Looping & Tail Curling

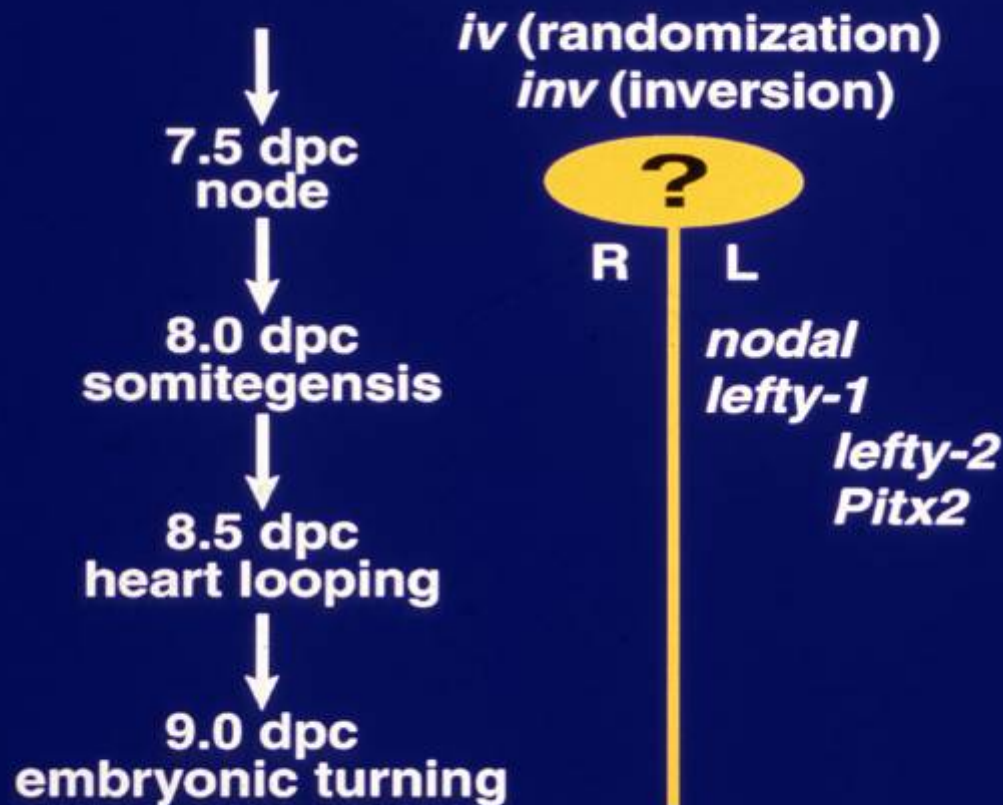
kif3B^{+/+}



kif3B^{-/-}



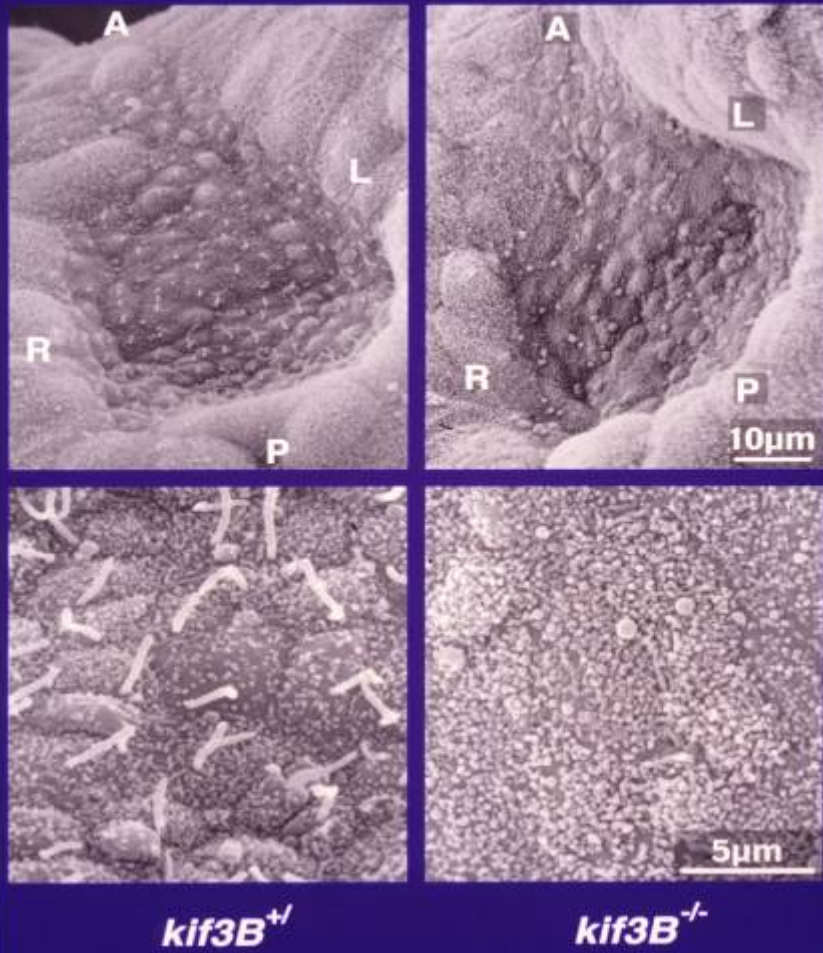
L-R Determination Pathway



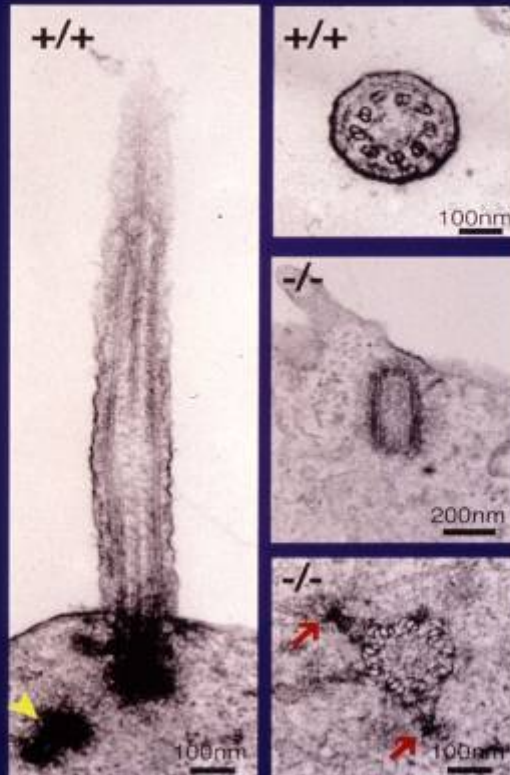
lefty-2 expression



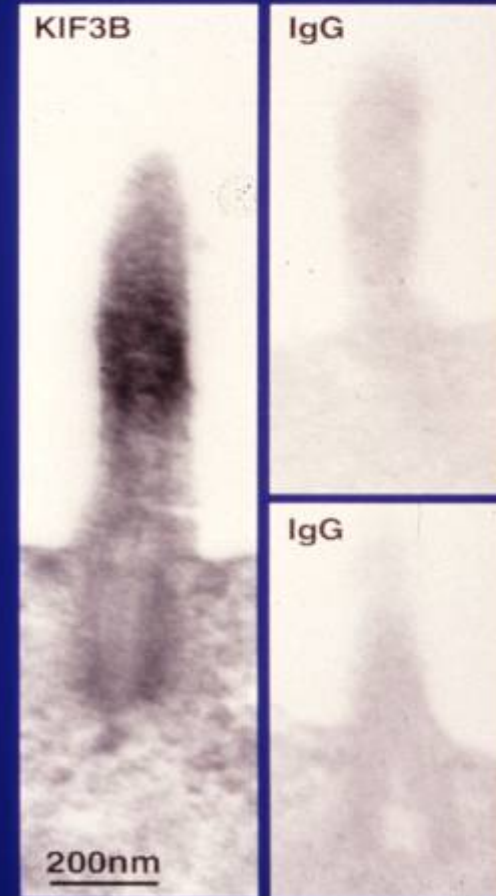
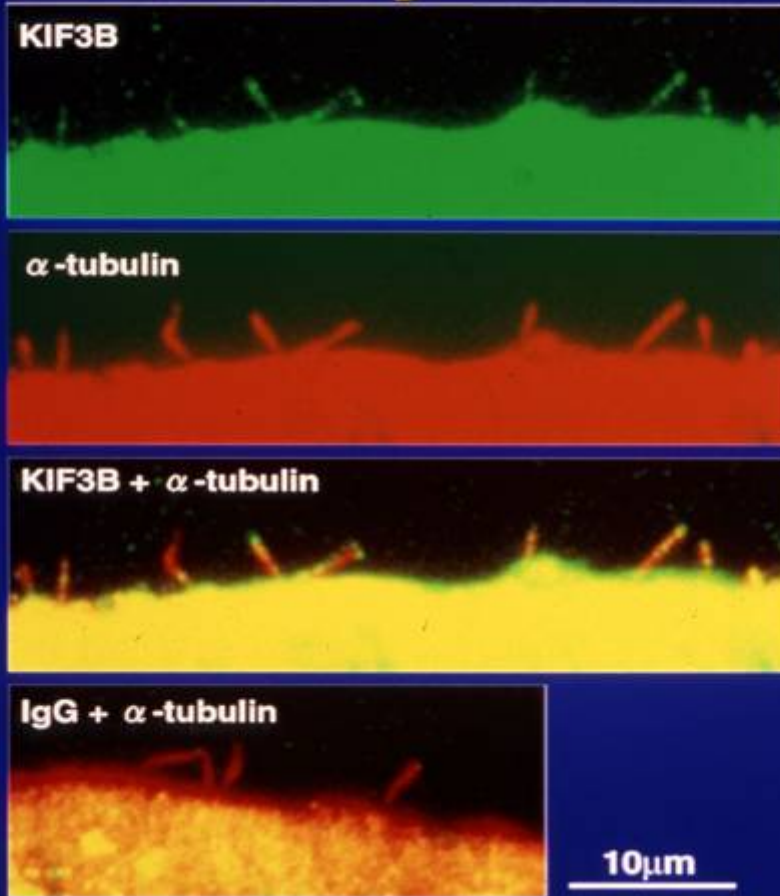
Monocilia of the Node

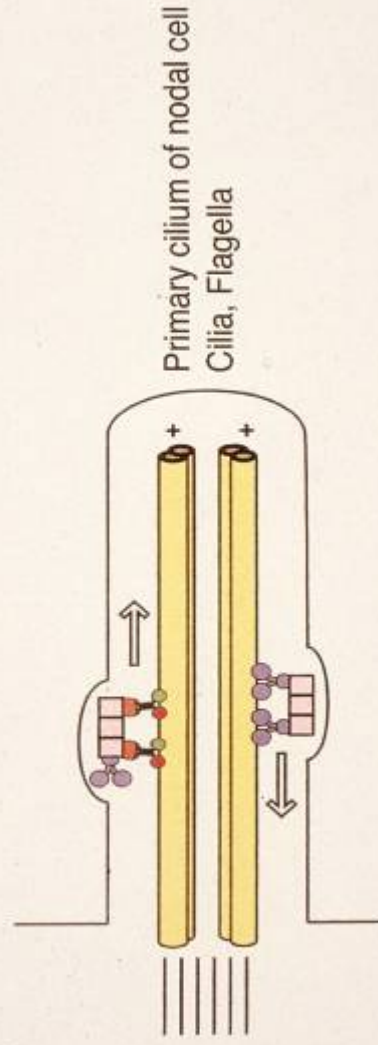


Loss of Primary Cilium Leaving Intact Basal Bodies



Ciliary Staining of KIF3B

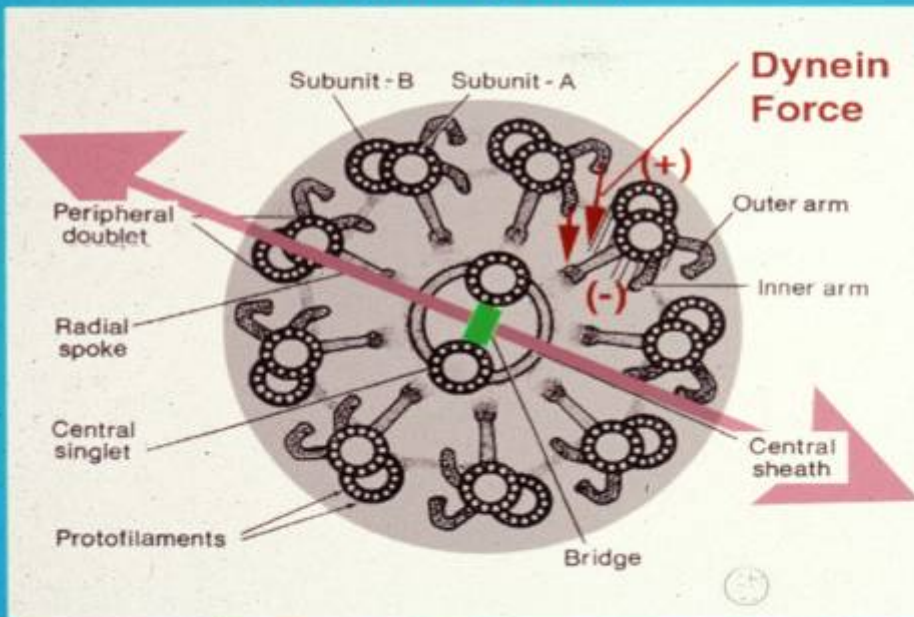




Primary cilium of nodal cell
Cilia, Flagella

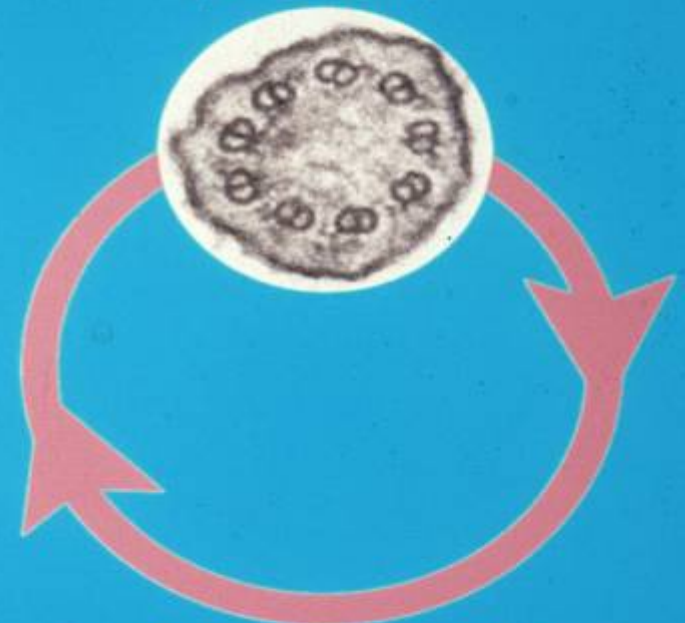
Cilia Have Clockwise Chirality

9+2: Beating (Respiratory Cilia)



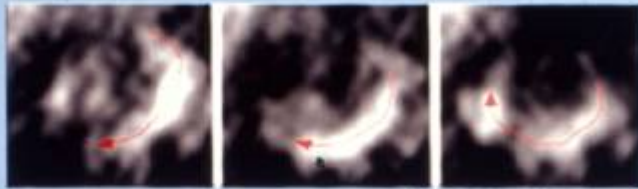
(Modified from Fawcett, *The Cell*, 1981)

9+0: Rotation (Nodal Cilia)



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

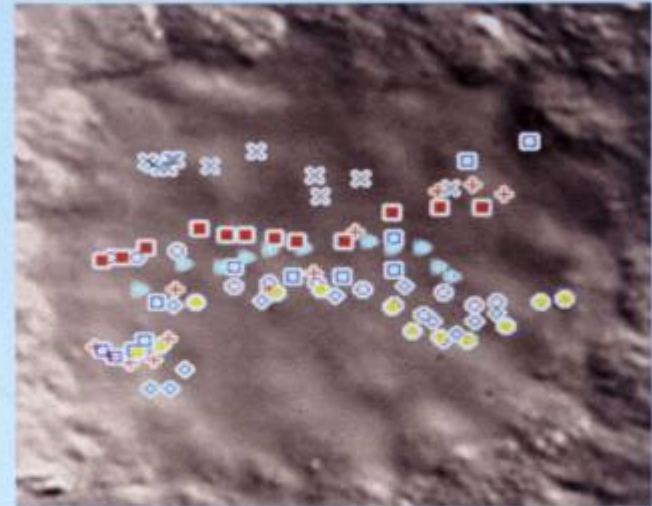
Rotation of Nodal Cilia & Leftward Nodal Flow



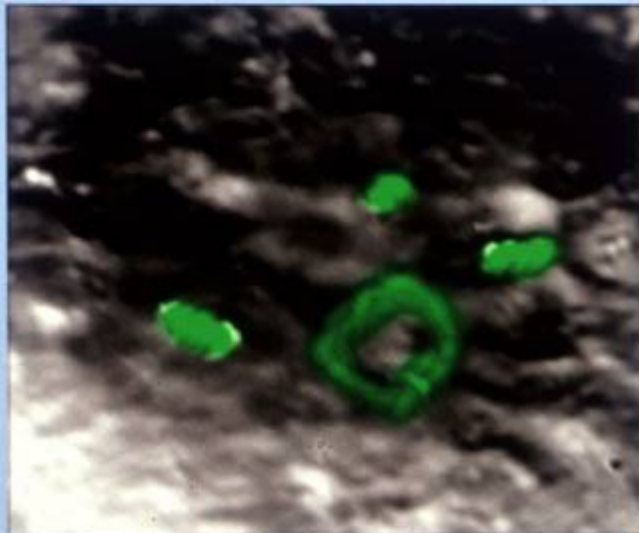
5μm

R

L



R



L

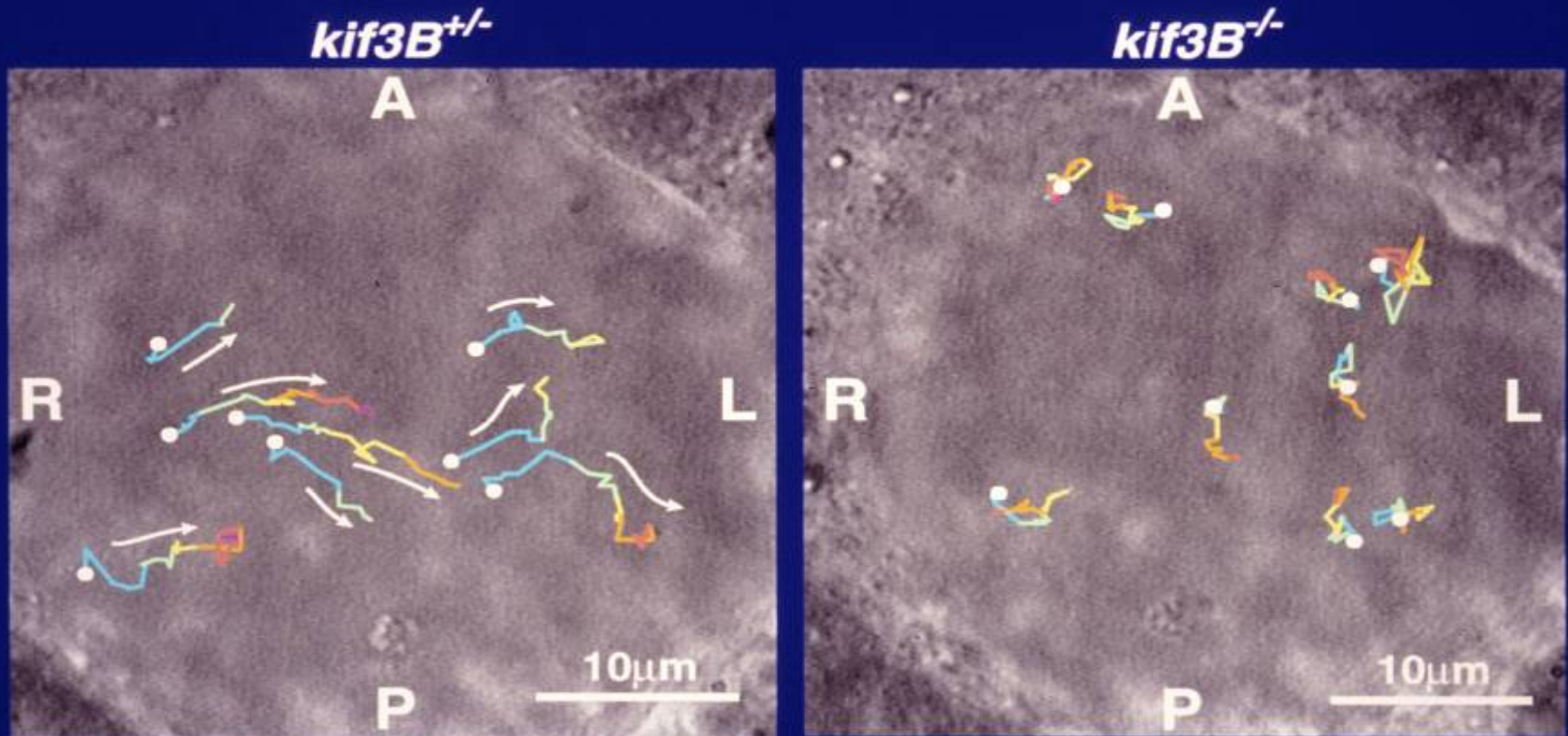
R

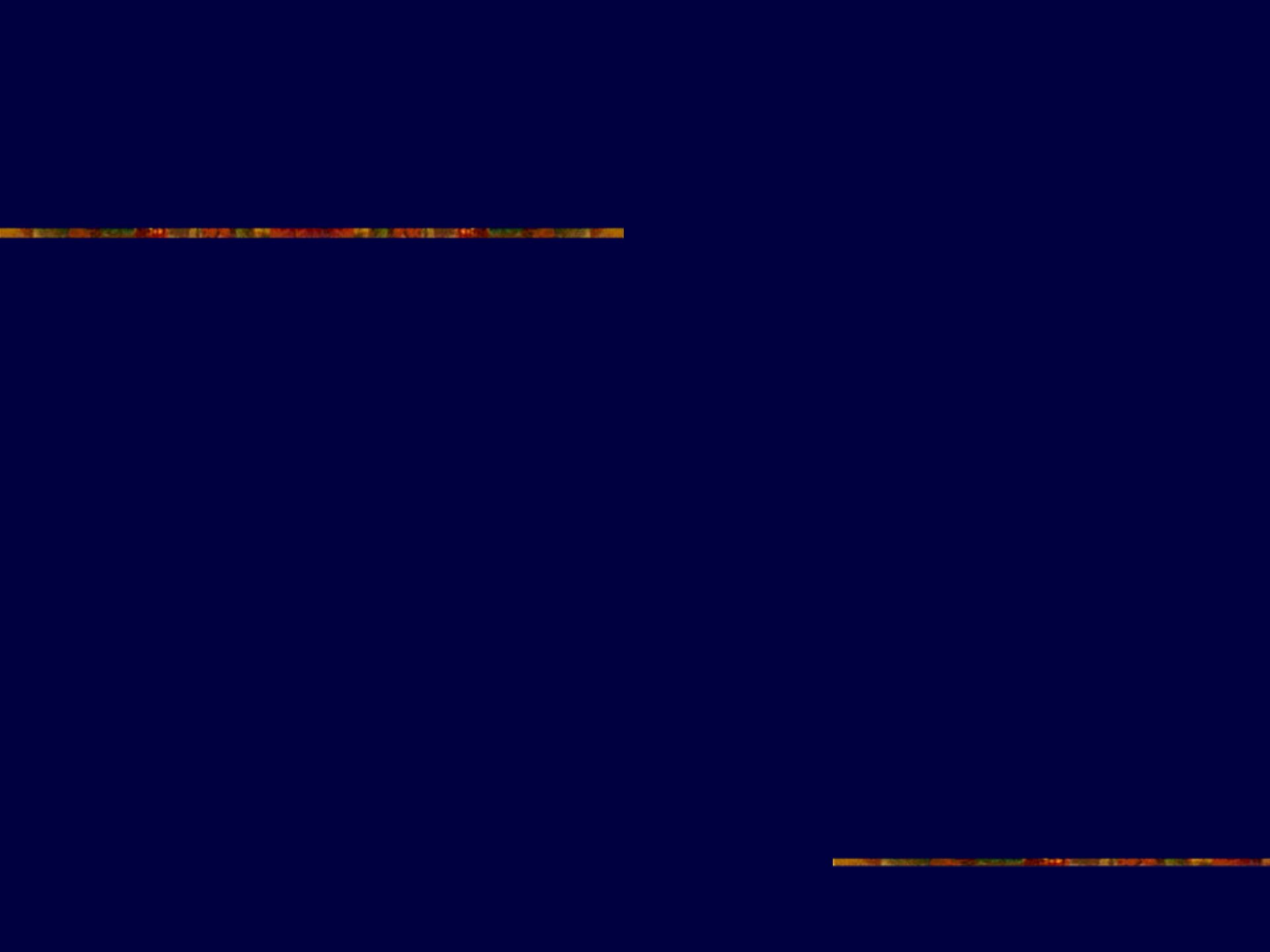


L

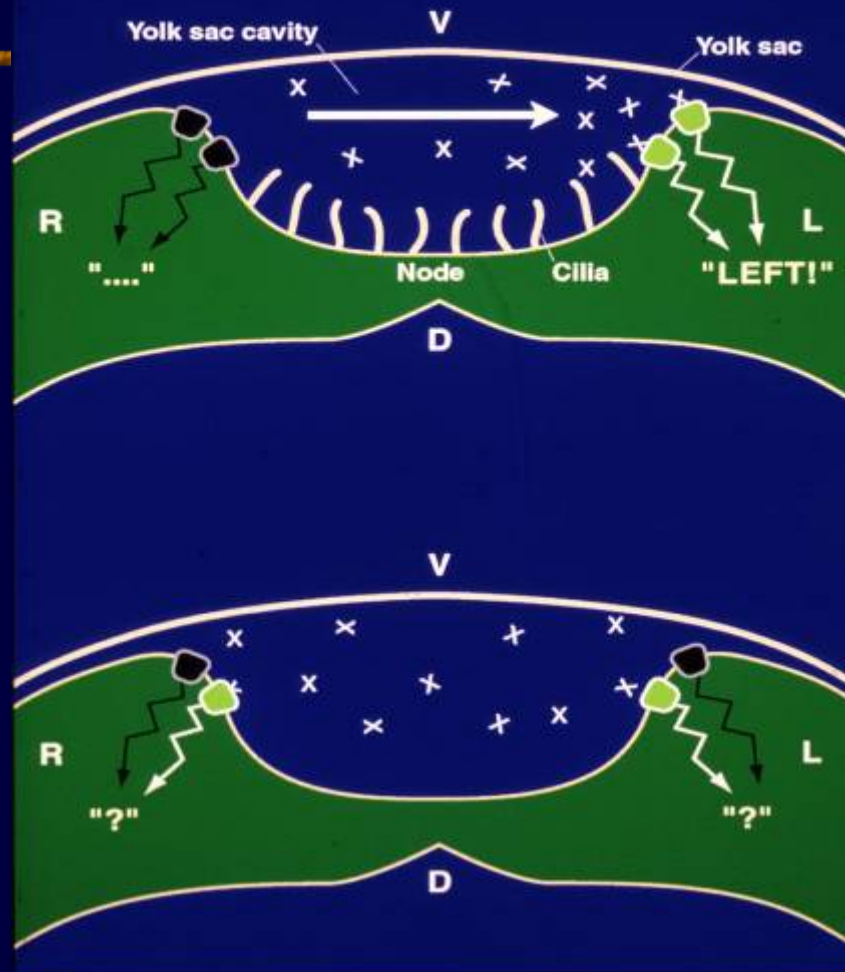
20μm

KIF3B KO Disrupts The Nodal Flow

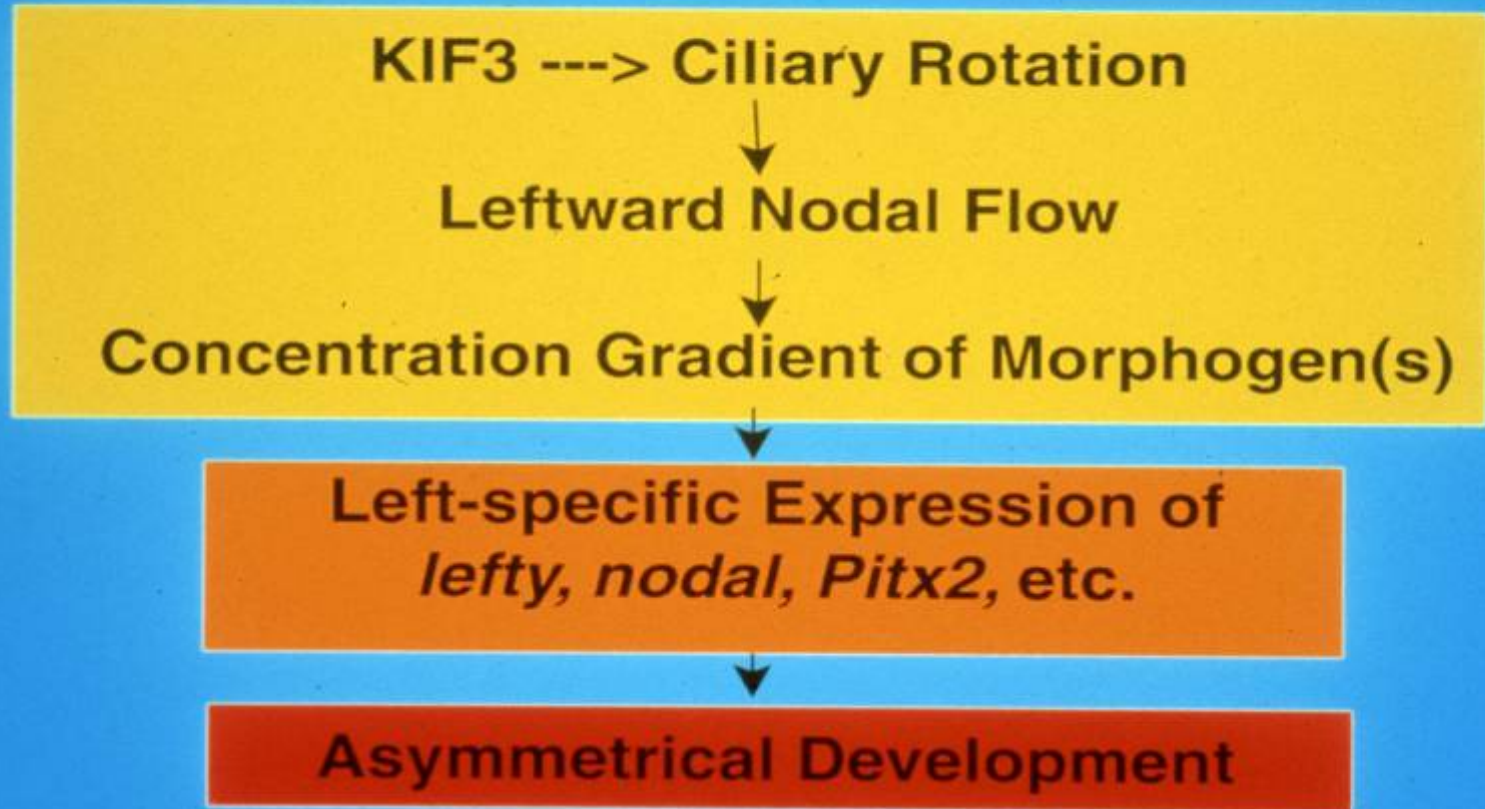




A Model for L-R Determination



Nodal Flow Hypothesis of Mammalian Left-Right Determination



Kartagener's Syndrome

Immotile Cilia syndrome

Immotile Cilia
(Male Infertility + Respiratory Failure etc.)

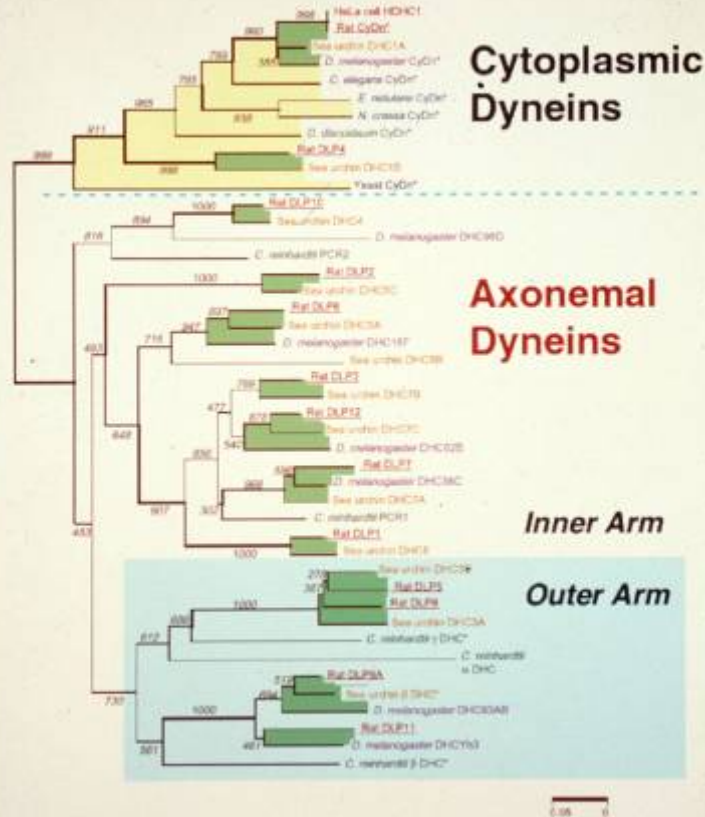
?

Situs Inversus



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

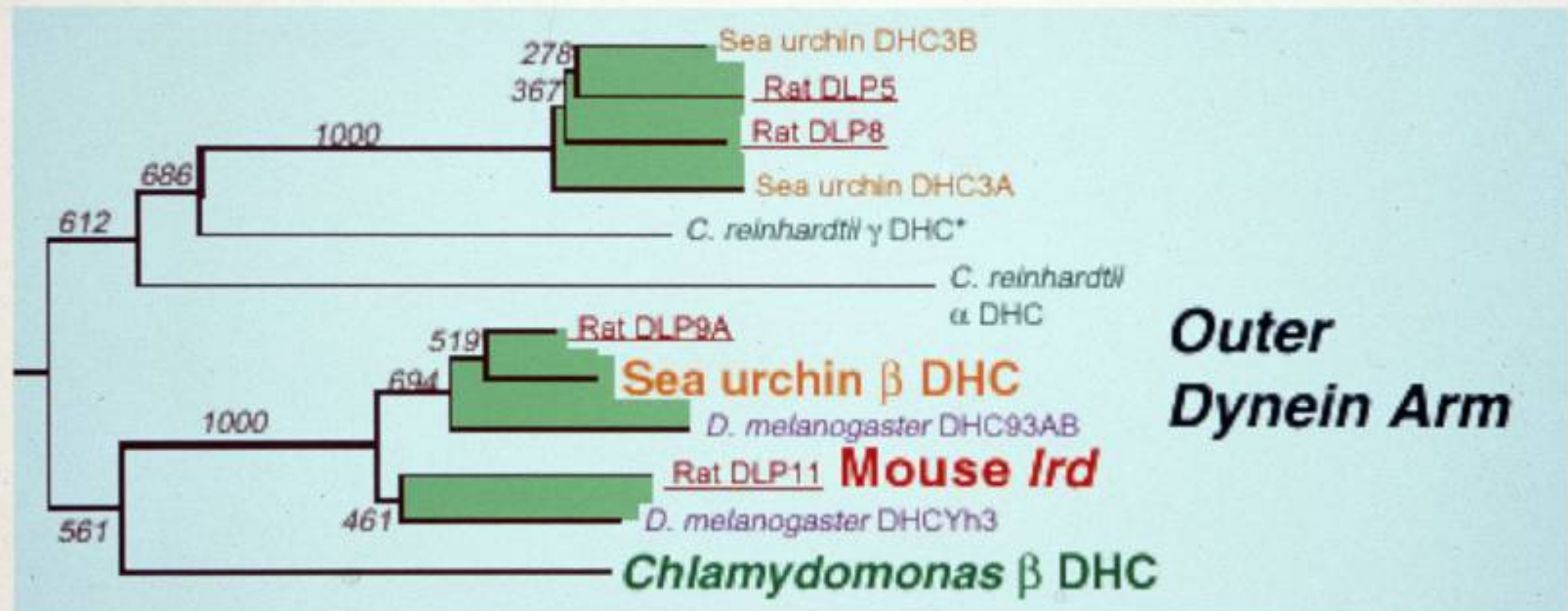
The Dynein Superfamily



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

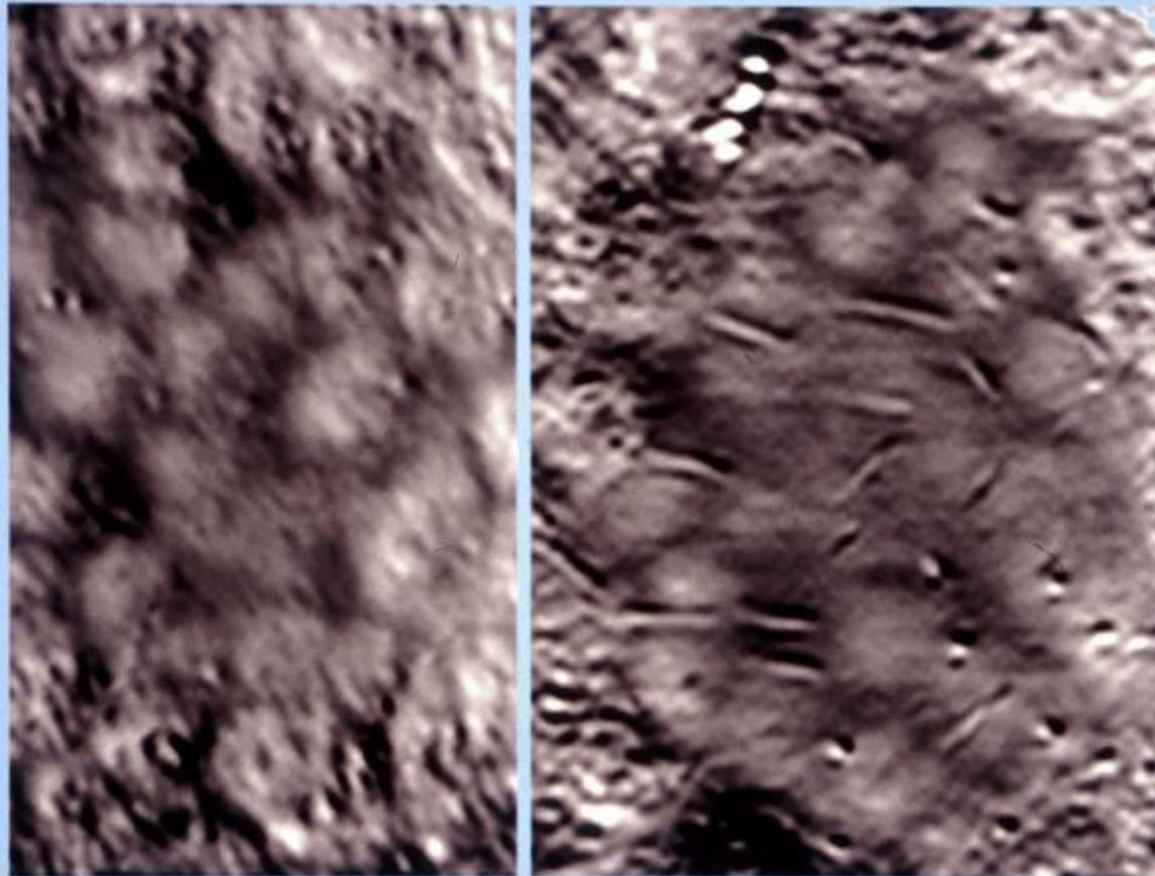
Ird/DLP11 Is An Outer-Arm β Dynein

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



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

Frozen Cilia in *iv*

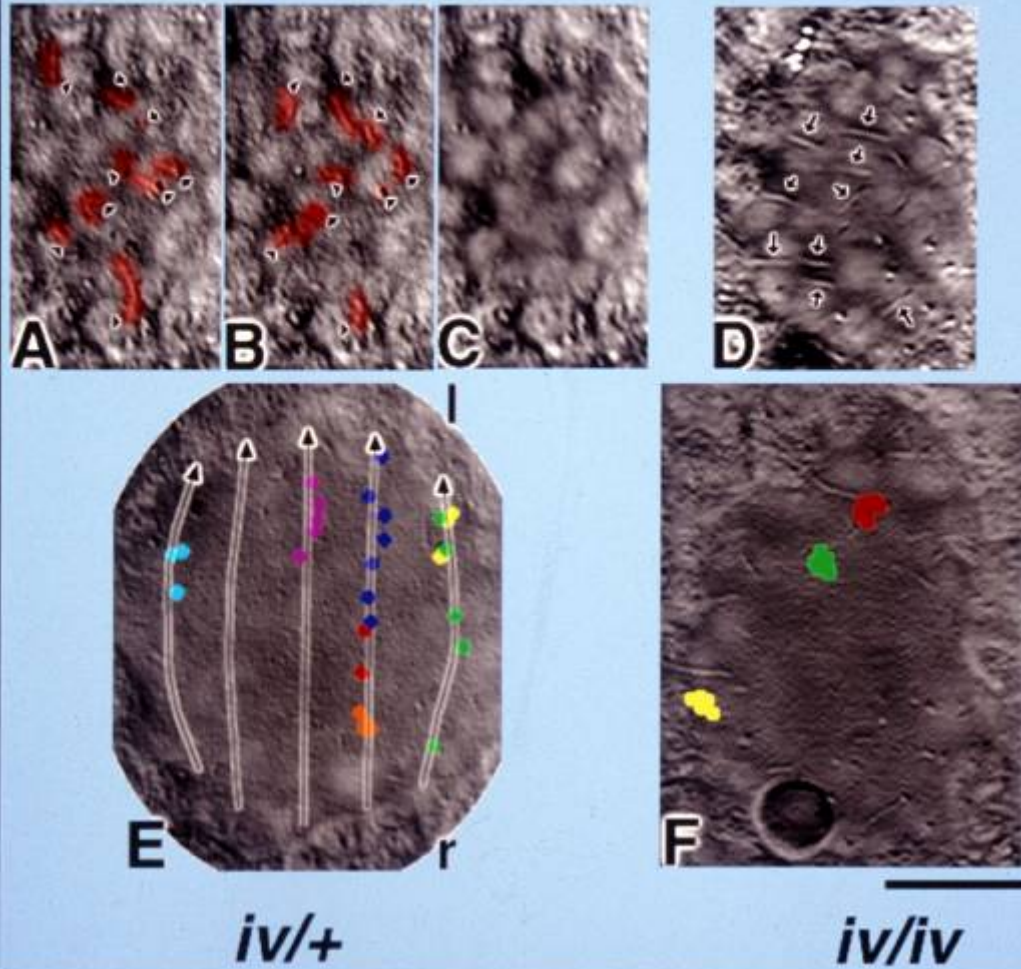


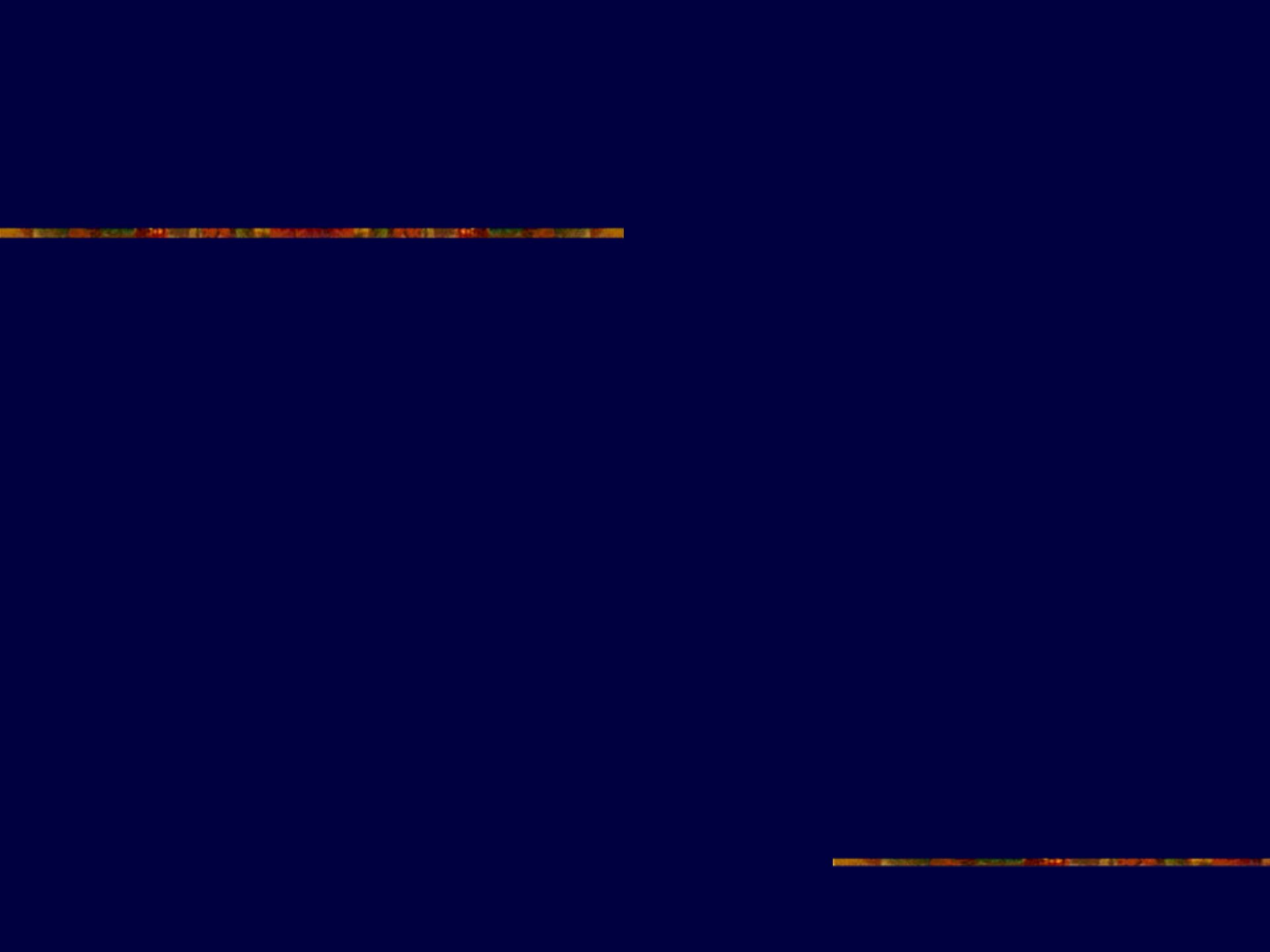
iv/+

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.

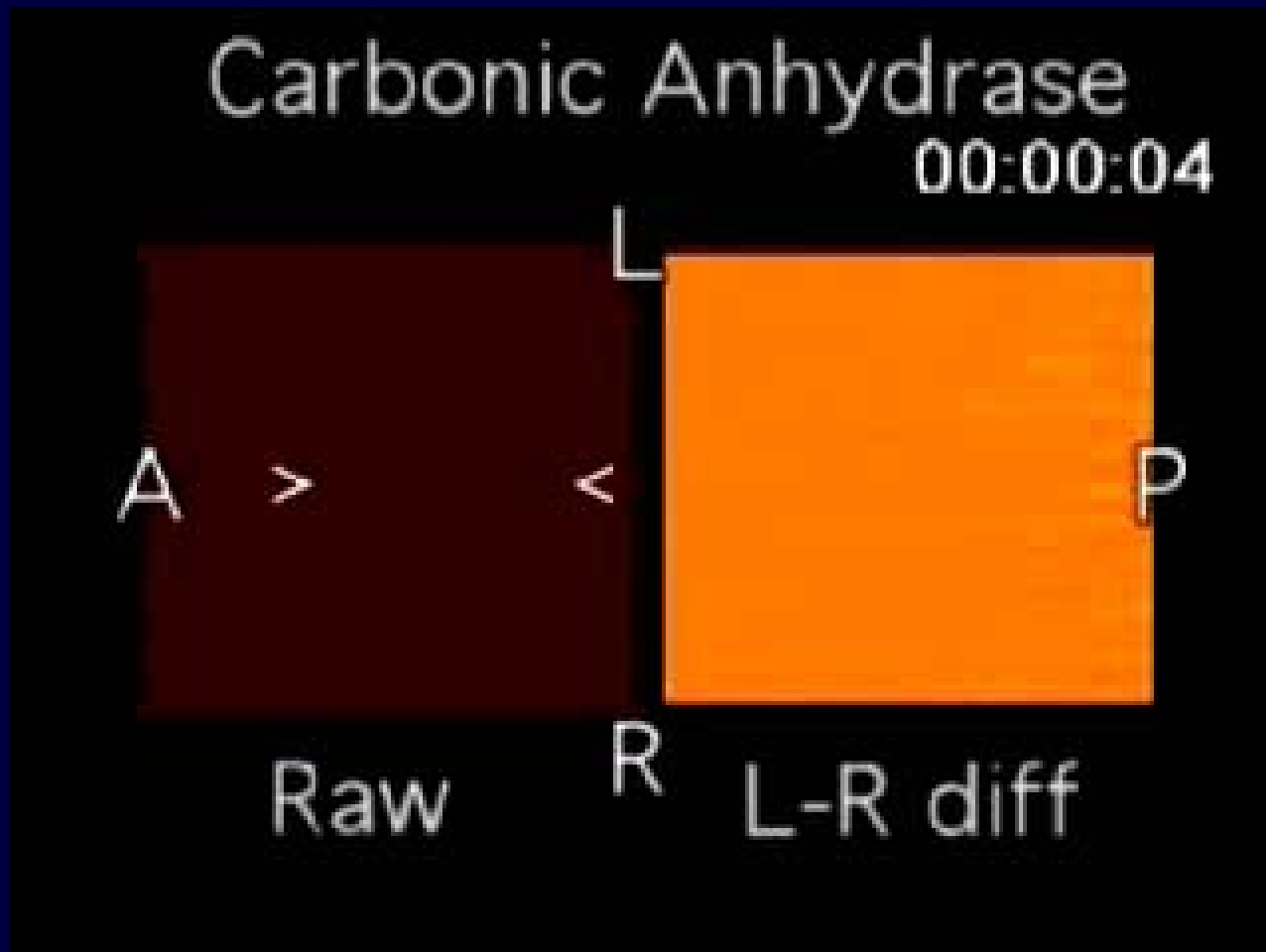
Randomization of the L-R axis in

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

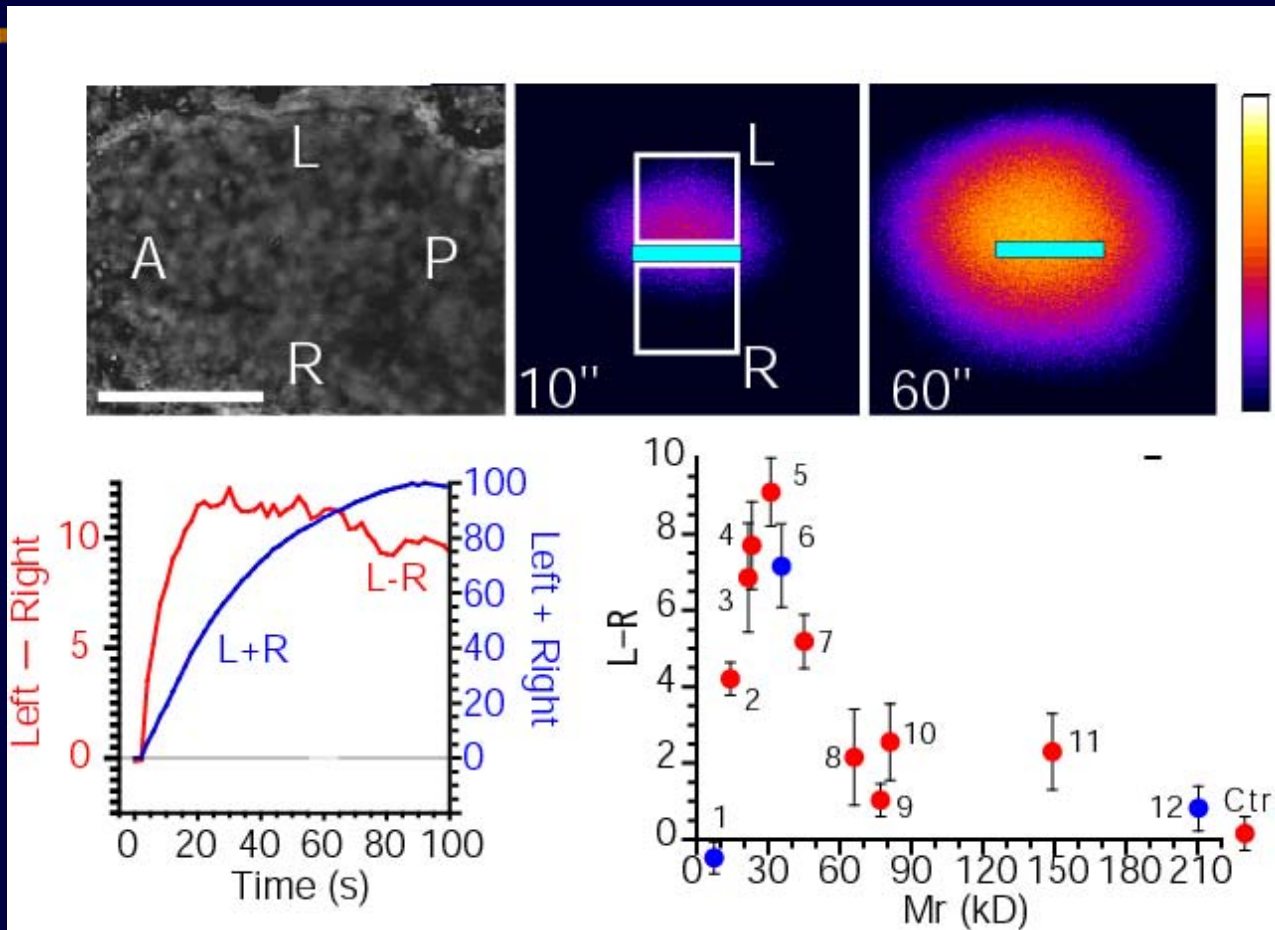
Where are the motile cilia?

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.

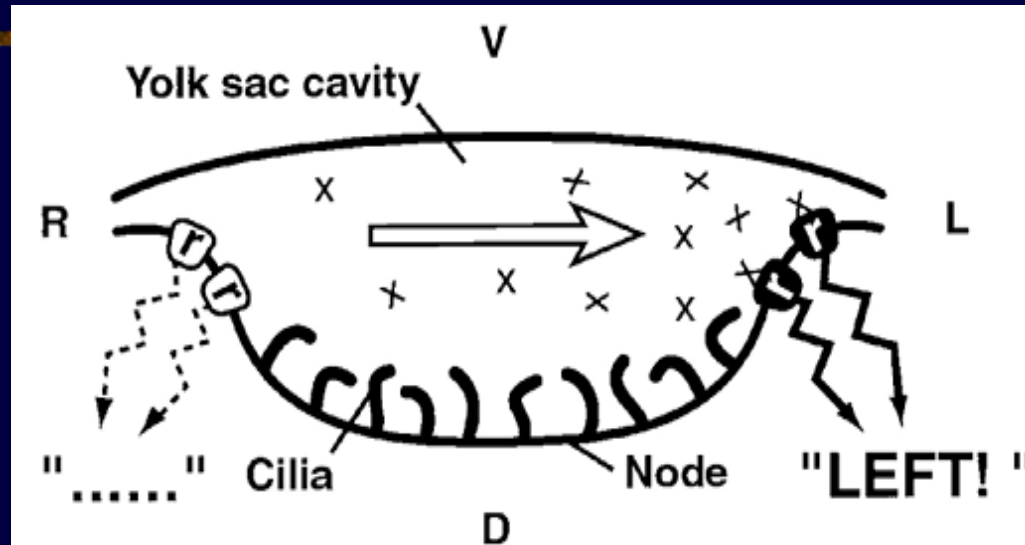


Asymmetric distribution of 20~40 kDa protein by nodal flow



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

Nodal Flow Hypothesis



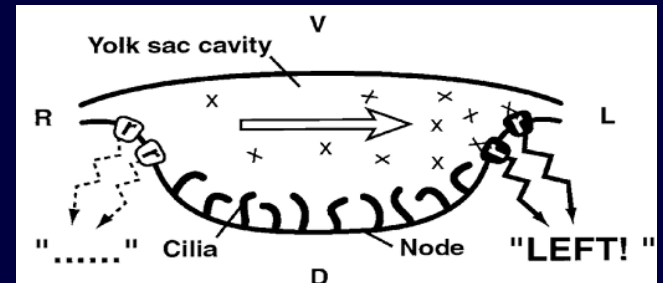
1. Clockwise rotation of cilia
2. Rapid leftward flow
3. Left-specific gene expression

Is nodal flow universal?

Mouse:

egg cylinder

nodal pit on the ventral surface



Higher vertebrates:

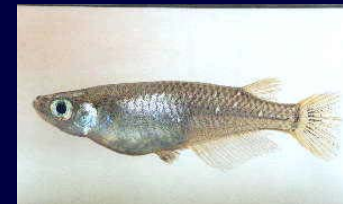
embryonic disc

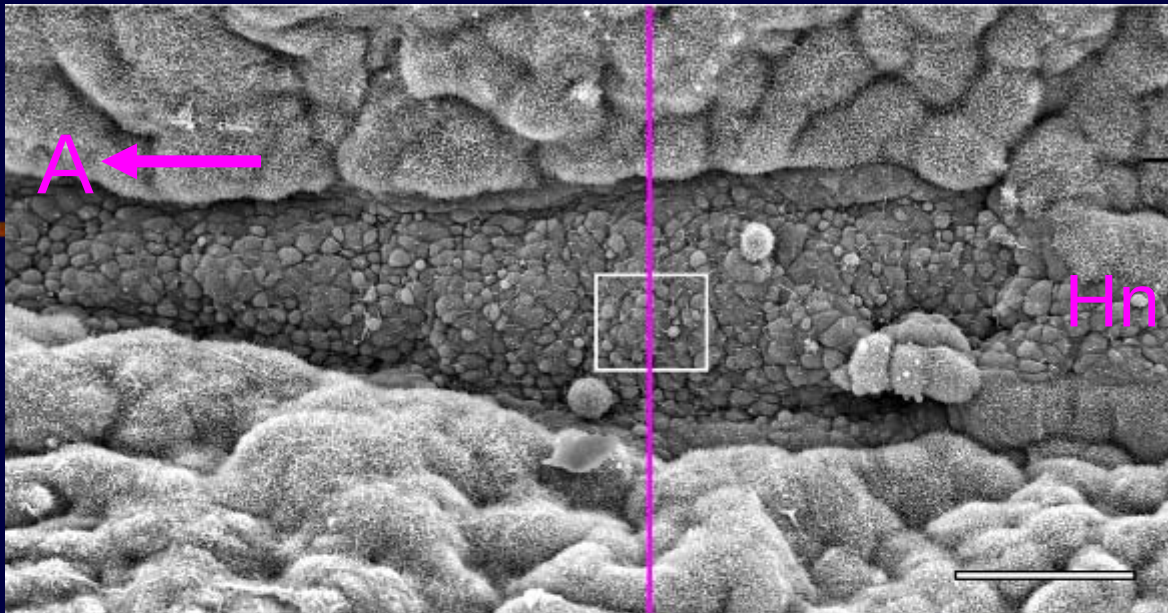
No nodal pit



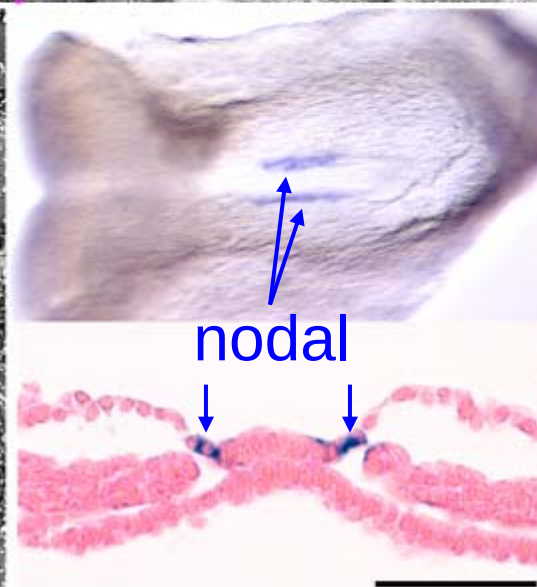
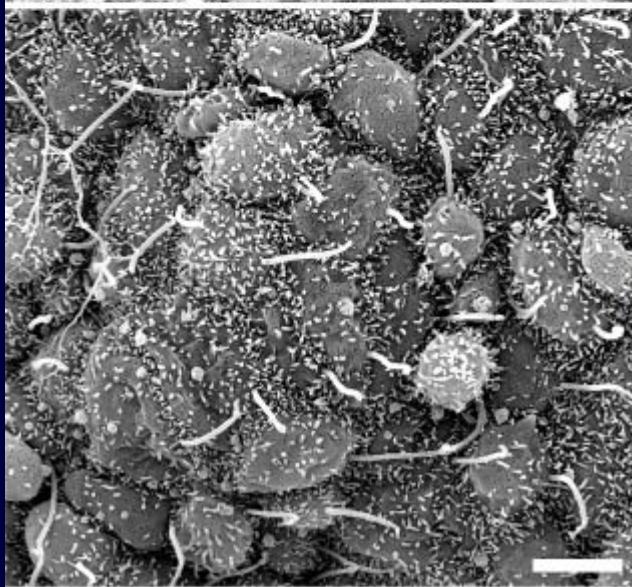
Lower vertebrates:

ventral surface is embedded





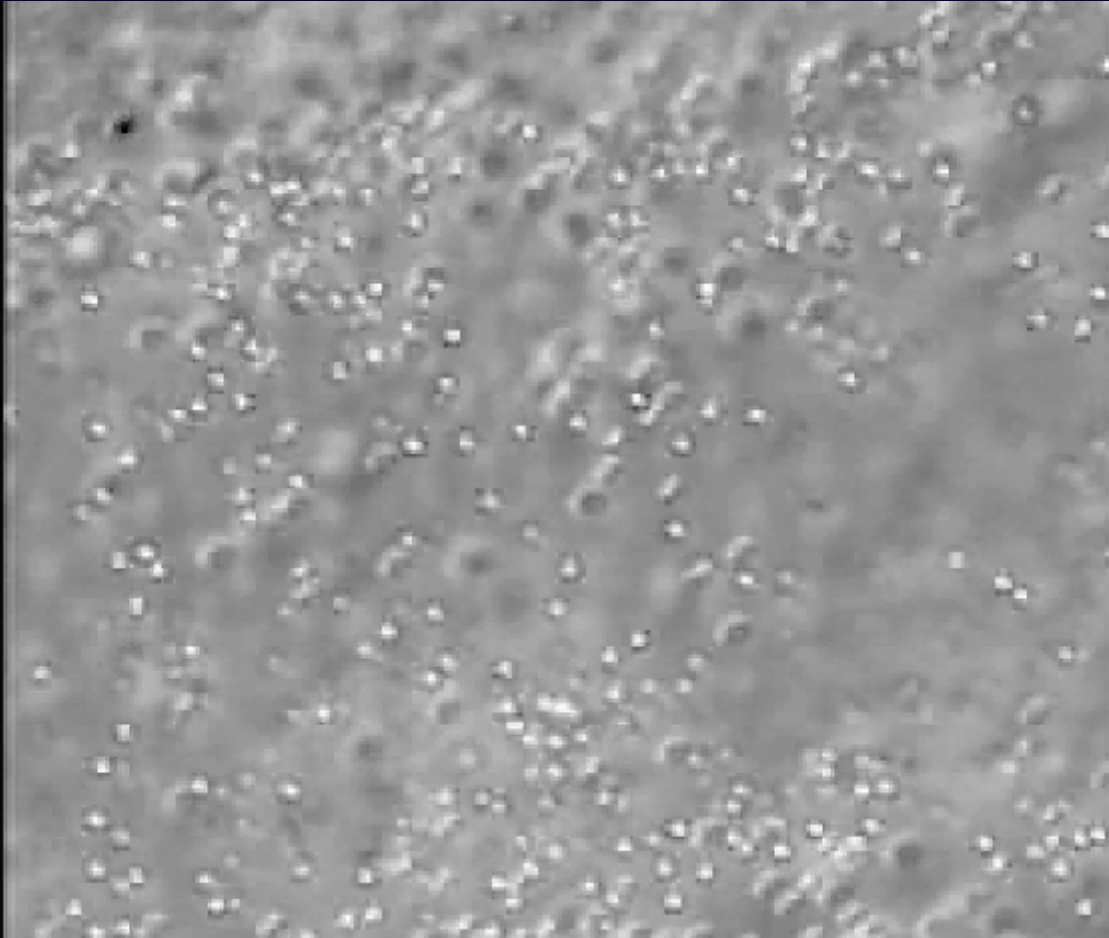
Monocilia on the ventral surface of the notochordal plate of rabbit embryo.



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

Leftward flow in the notochordal plate of the rabbit embryo

Left



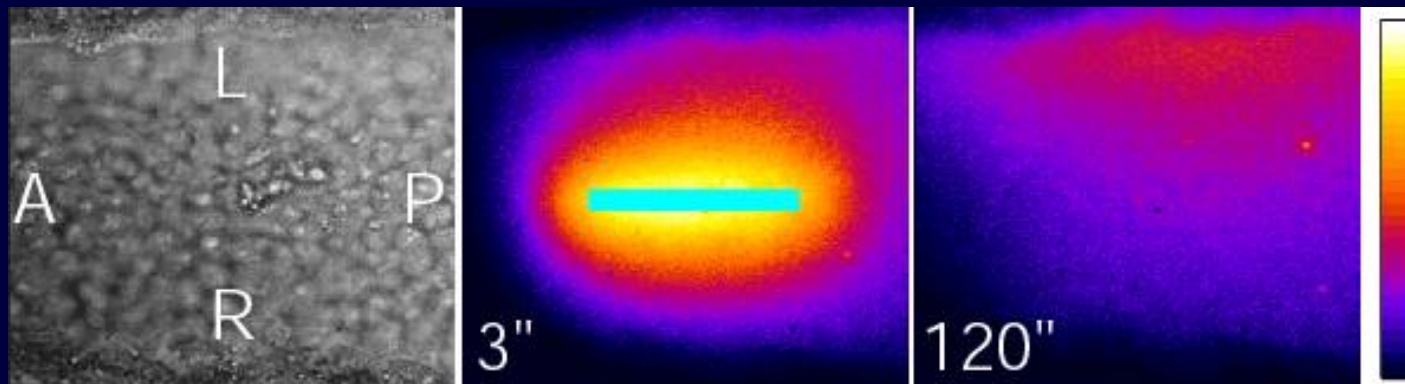
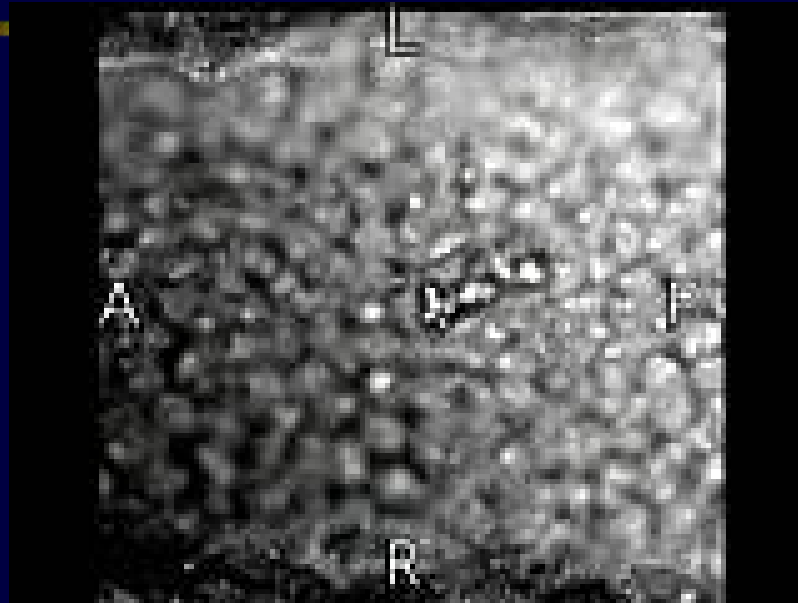
Right

20 μ m

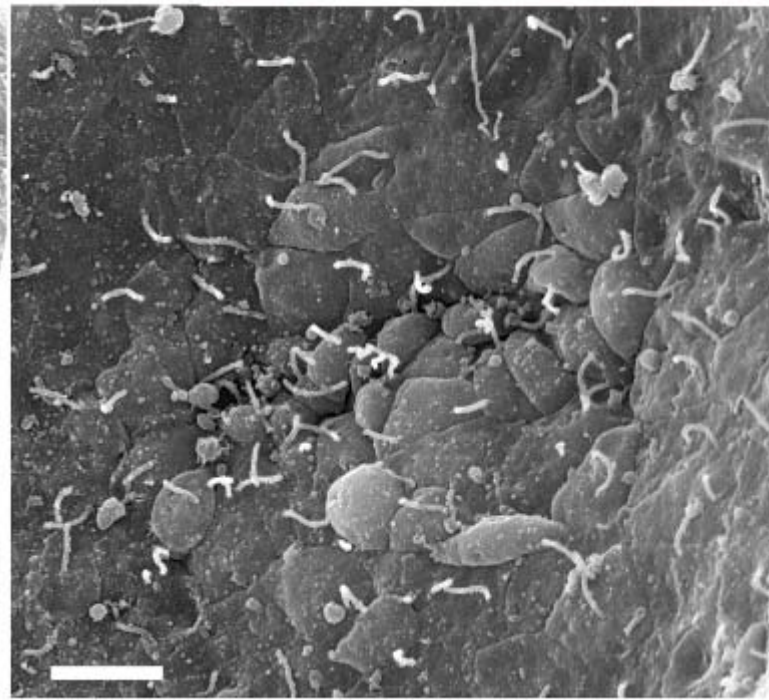
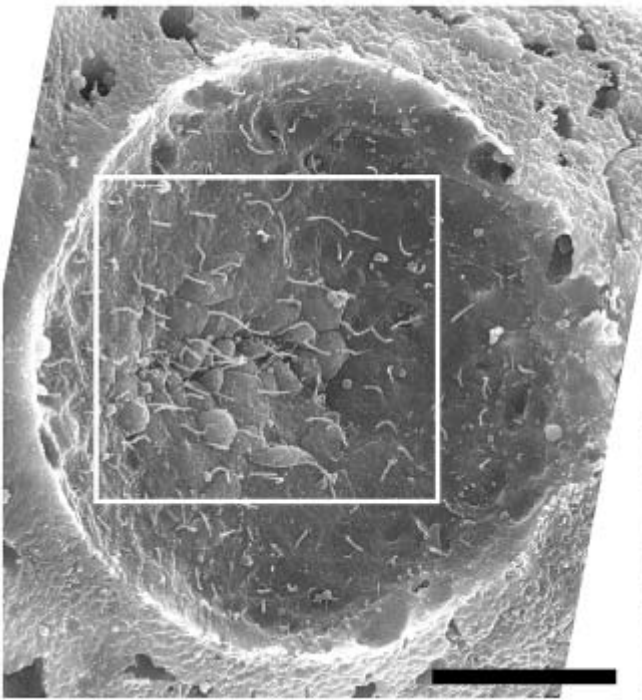
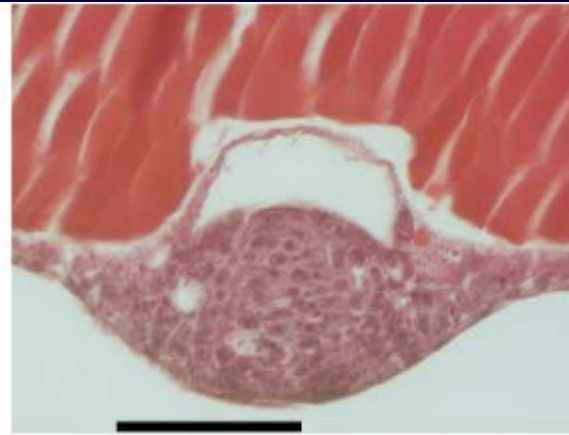
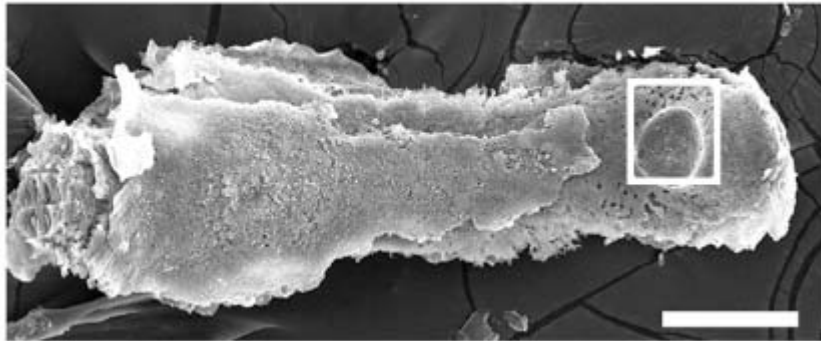


20x Time Lapse

Asymmetric distribution of caged-fluorescent dextran

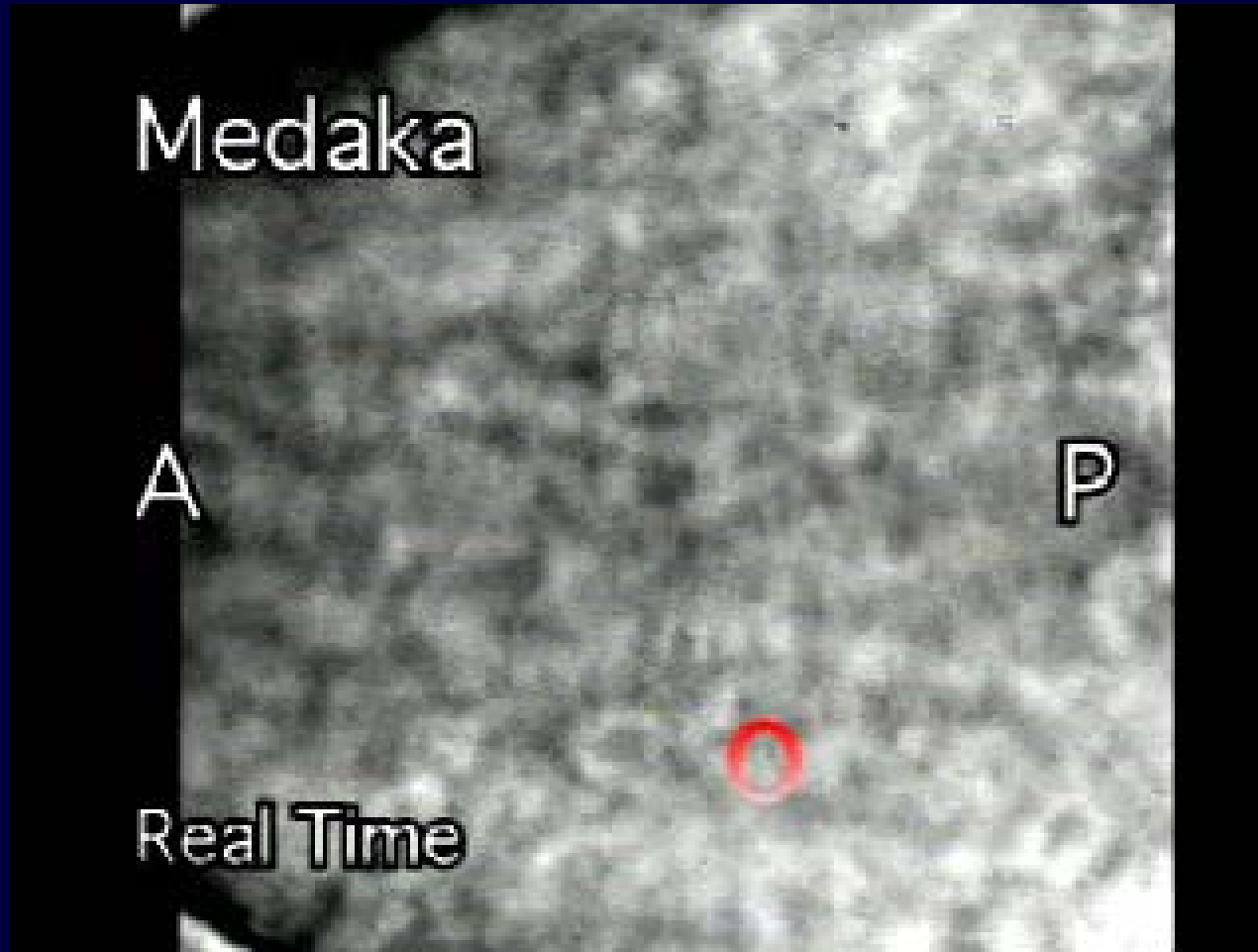


Monocilia
on the
inner
surface of
Kupffer's
vesicle of
medaka
embryo.



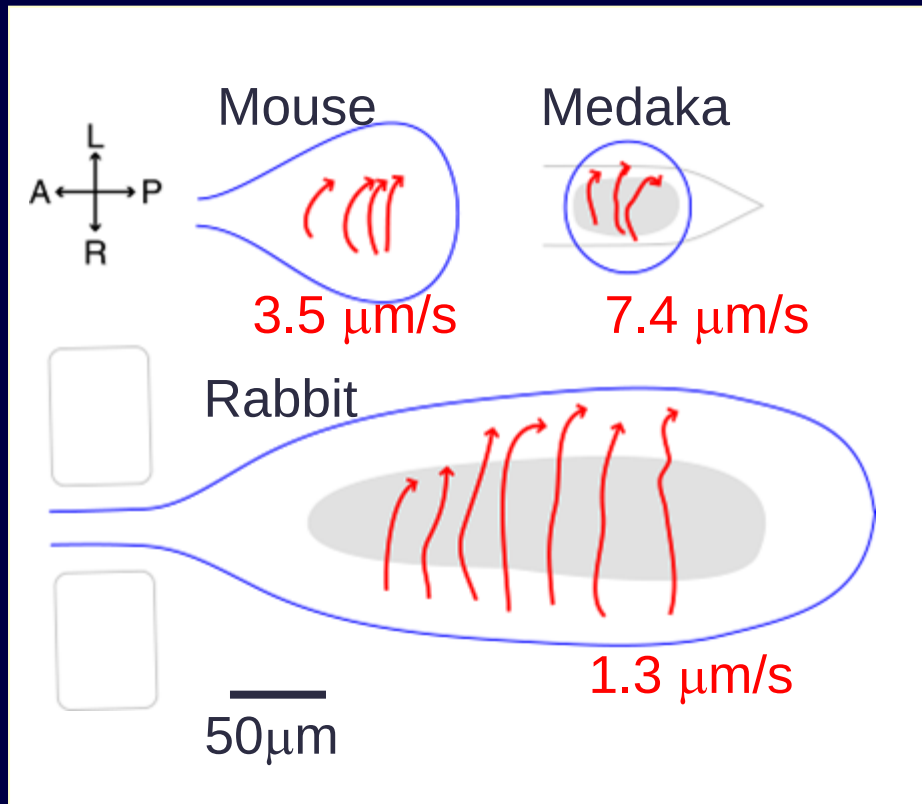
Medaka embryo:
courtesy of Dr.
Shima
(Univ.Tokyo)

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

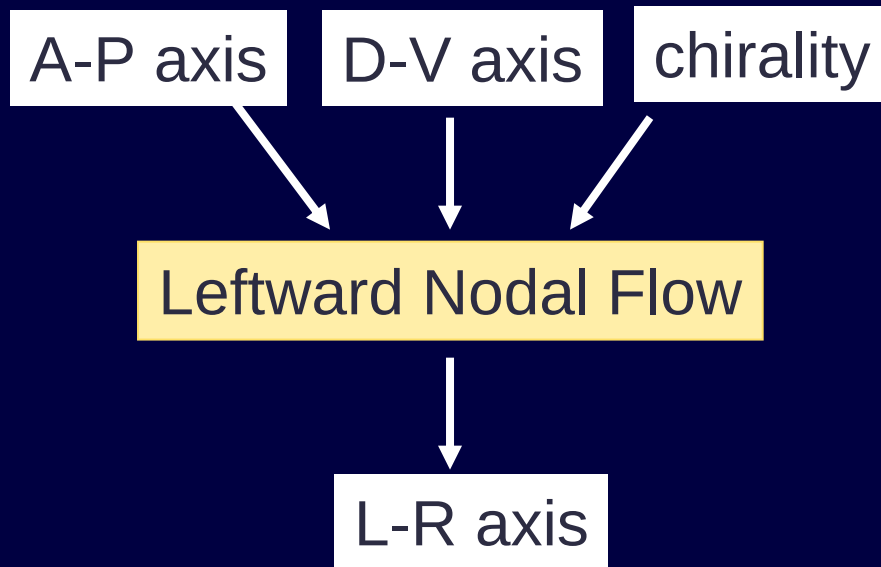
1. Leftward Nodal Flow
2. Left-specific expression of master genes
3. Left/right asymmetric morphogenesis

Tautology!

Doesn't answer why left is left.

Central Question:

What directs the flow to the left?

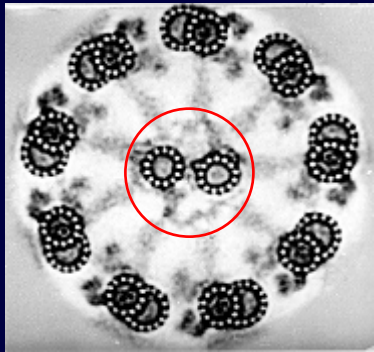


- How are the information of the A-P axis, D-V axis and the chirality integrated to determine the directionality of the nodal flow?
-

What produces the leftward flow?

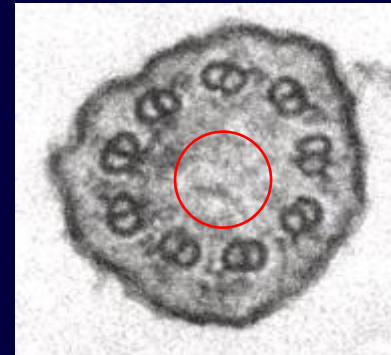
- Rotation of the primary cilia in the node.

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.

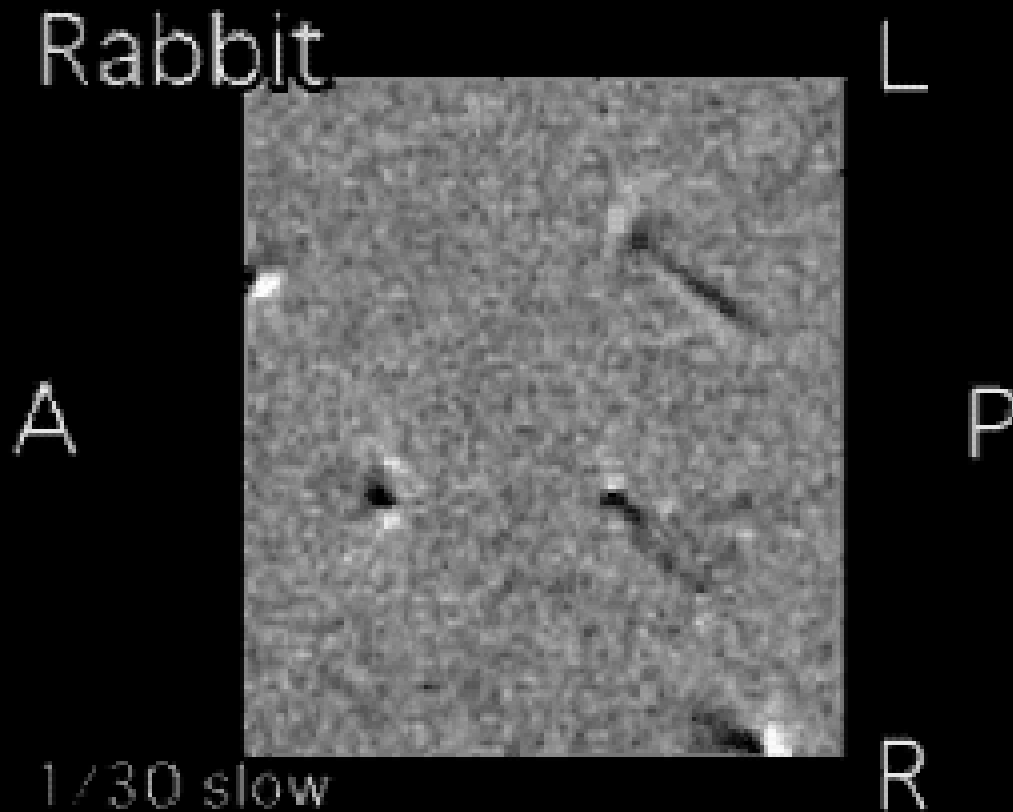
Some mechanism(s) exist for the conversion into the laminar leftward flow.

Rotation of Monocilia



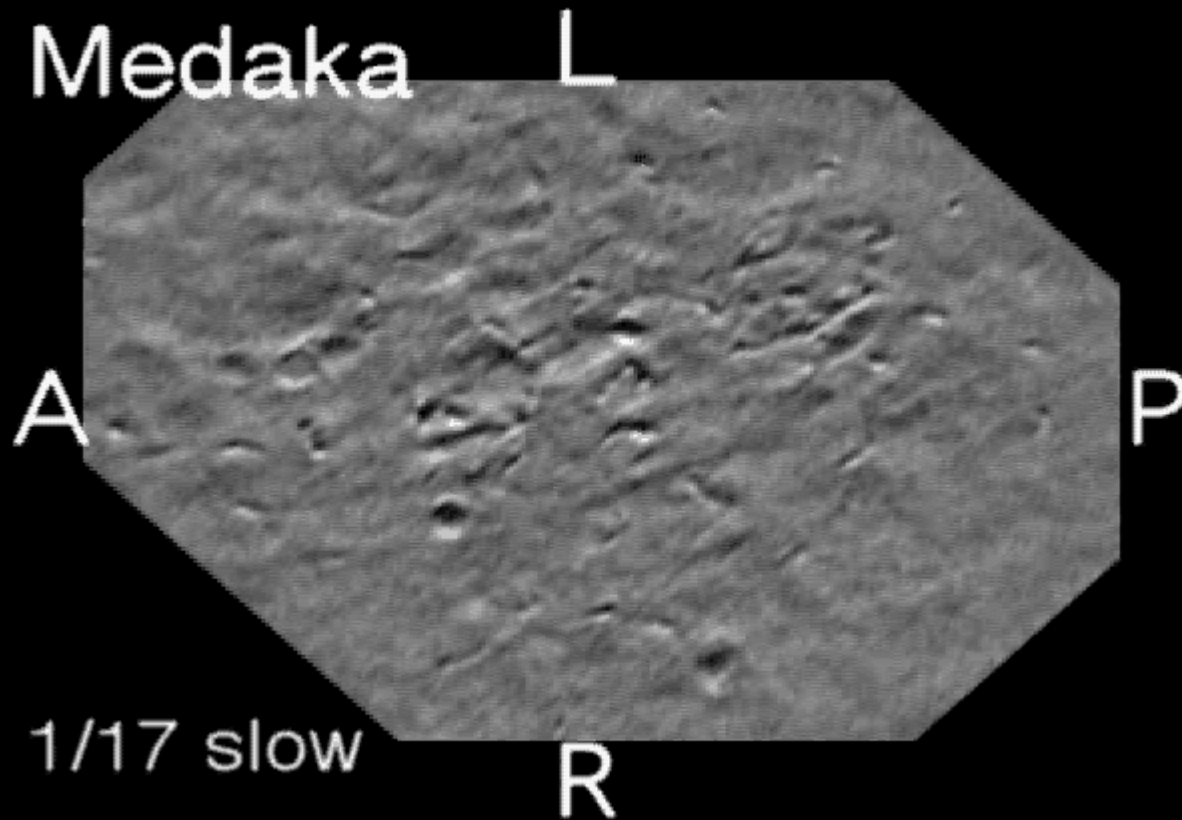
Recording frame rate:
500 frames / sec

Posteriorly Tilted Rotation of Monocilia



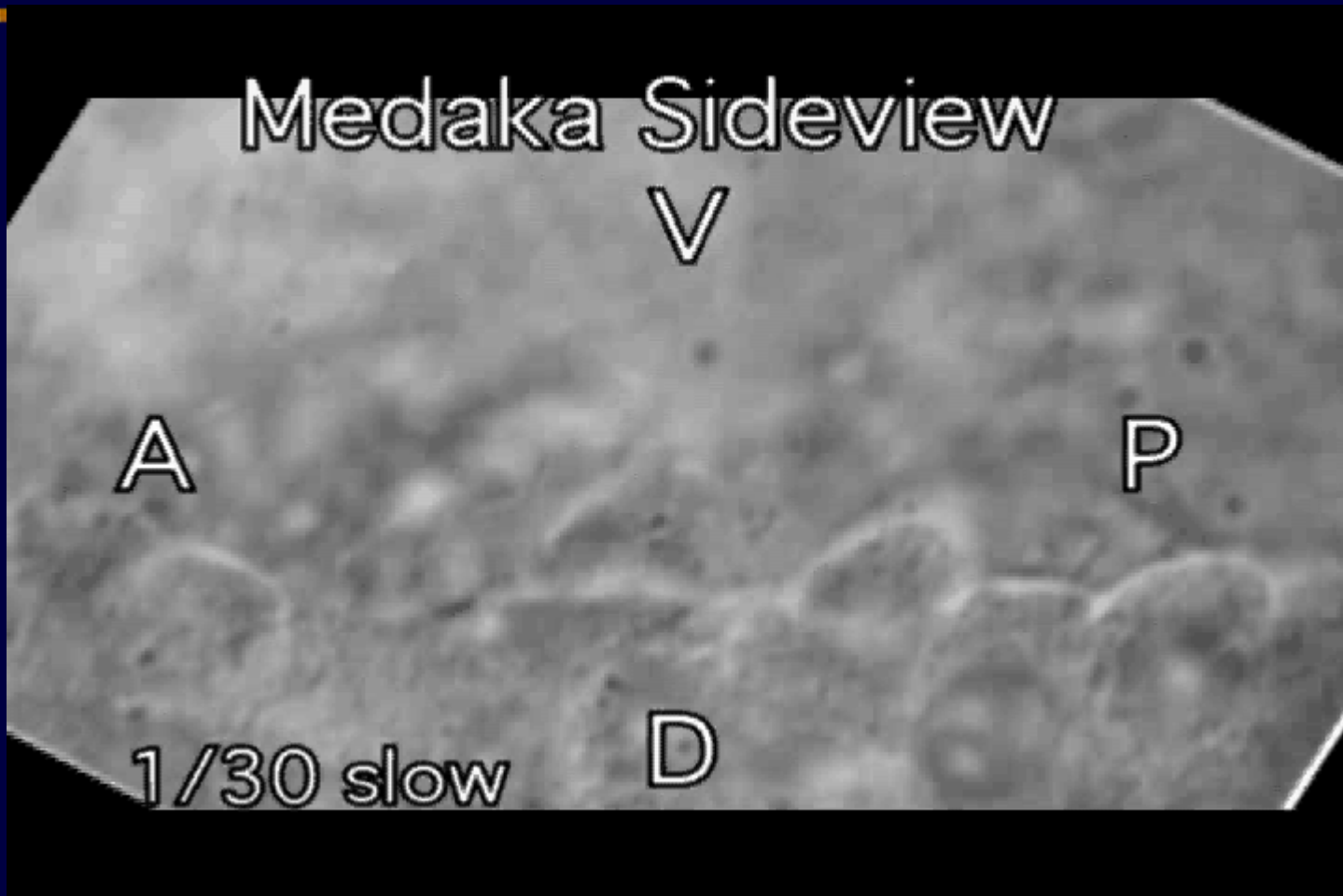
Recording frame rate:
500 frames / sec

Posteriorly Tilted Rotation of Monocilia



1000 frames / sec

Posteriorly Tilted Rotation of Monocilia

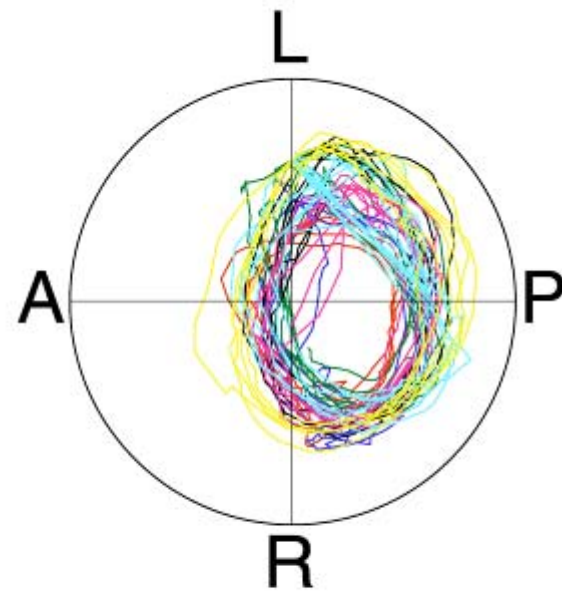
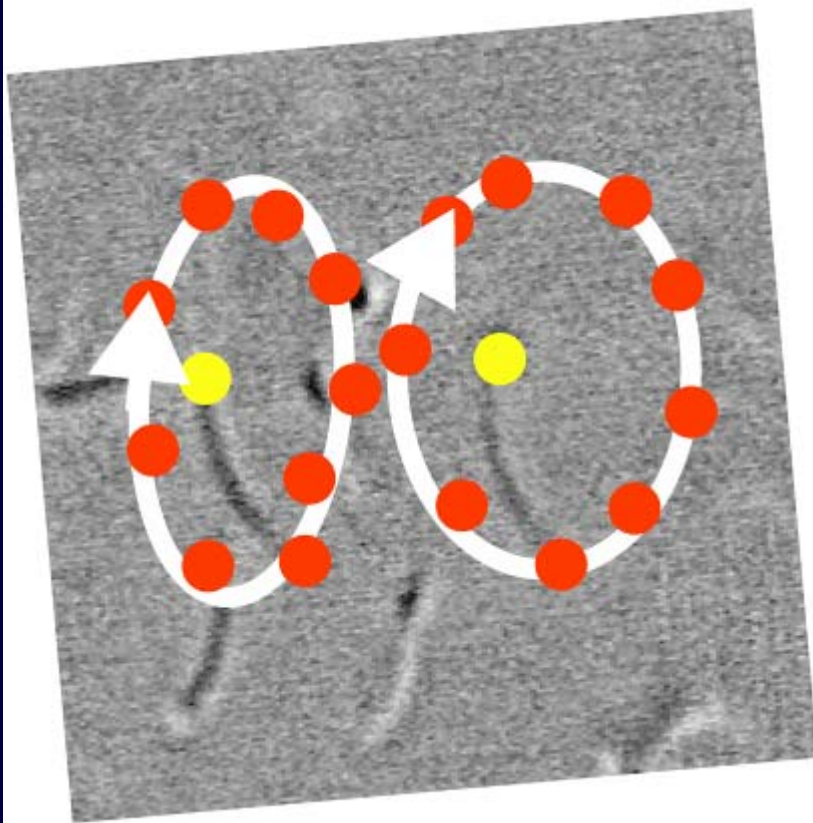


Side view



1000 frames / sec

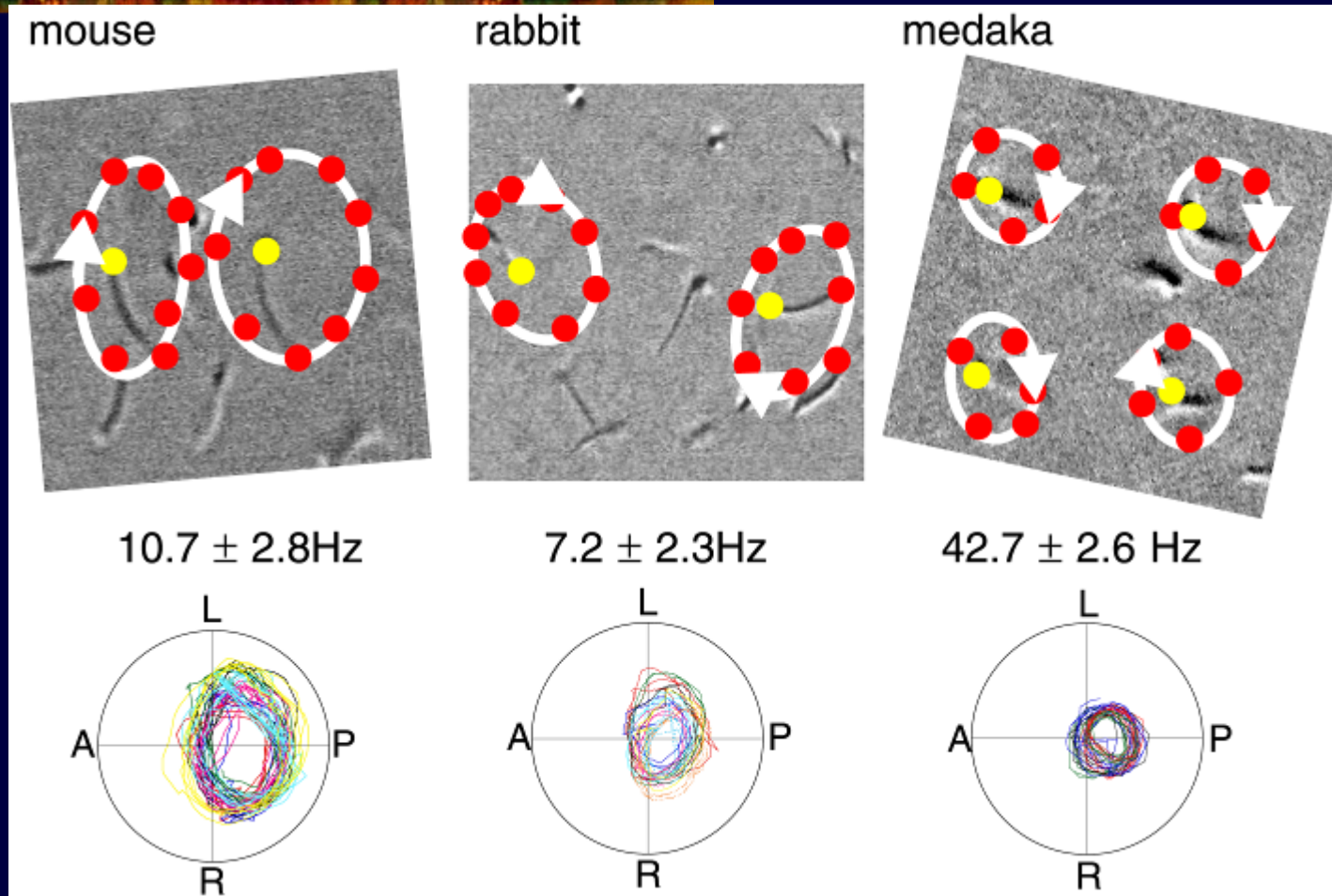
Posteriorly tilted rotation of cilia



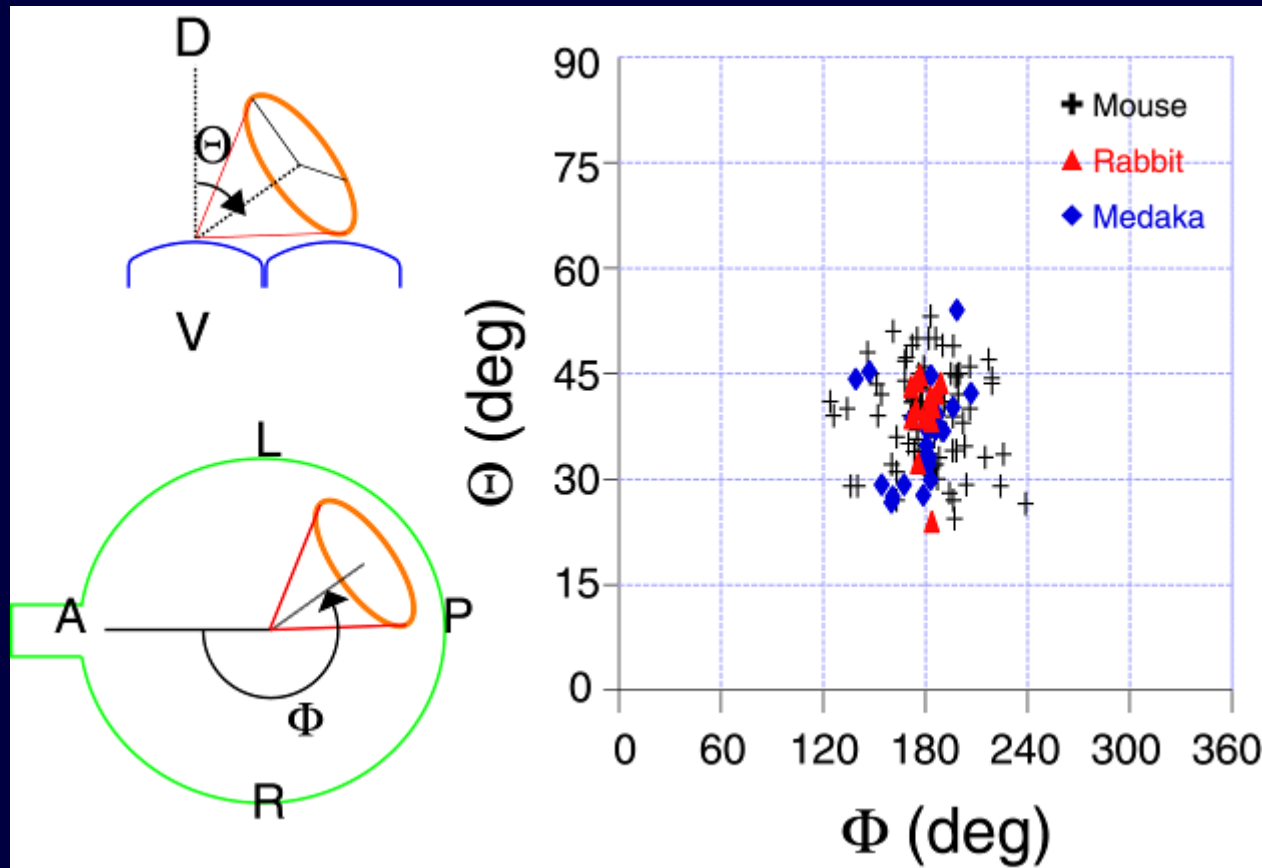
$10.7 \pm 2.8\text{Hz}$



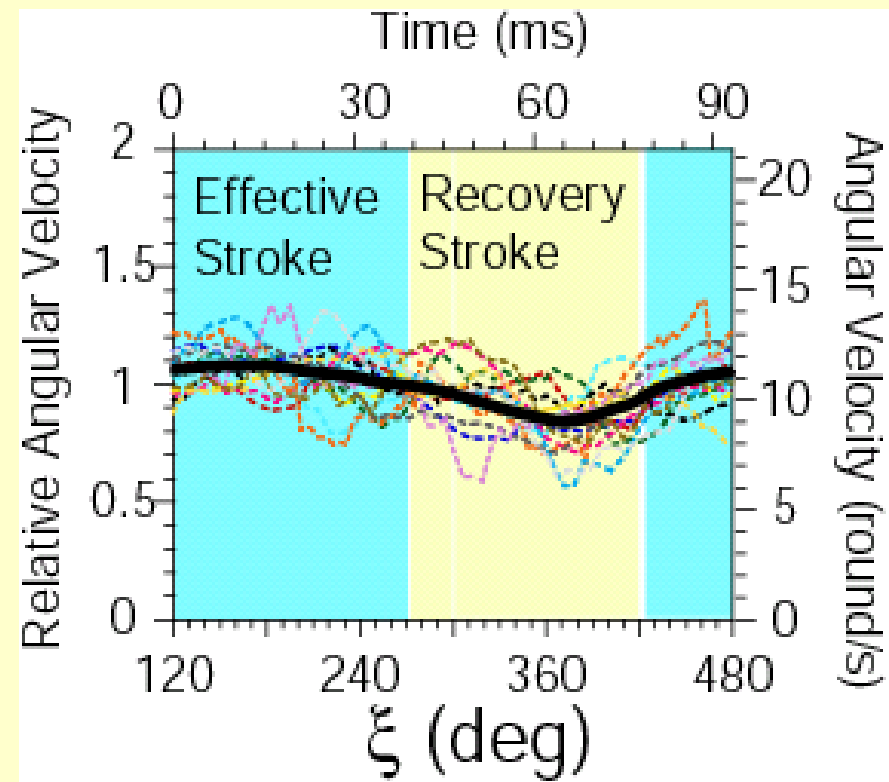
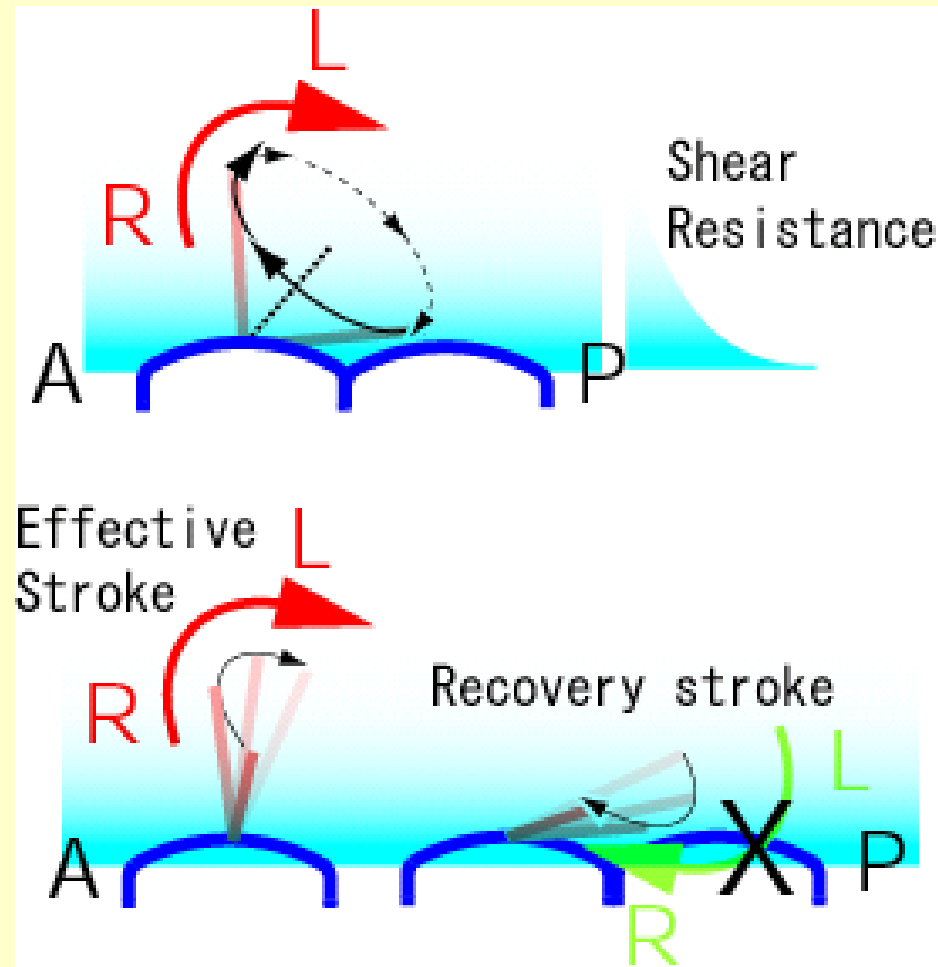
Posteriorly tilted rotation of cilia



Axis of Rotation is Tilted $\sim 40^\circ$ to the Posterior.



Hydrodynamic Mechanism of the Generation of the Leftward Flow



Cilia integrate the information of the axes and the chirality

