What determines the posterior tilting of the nodal cilia?

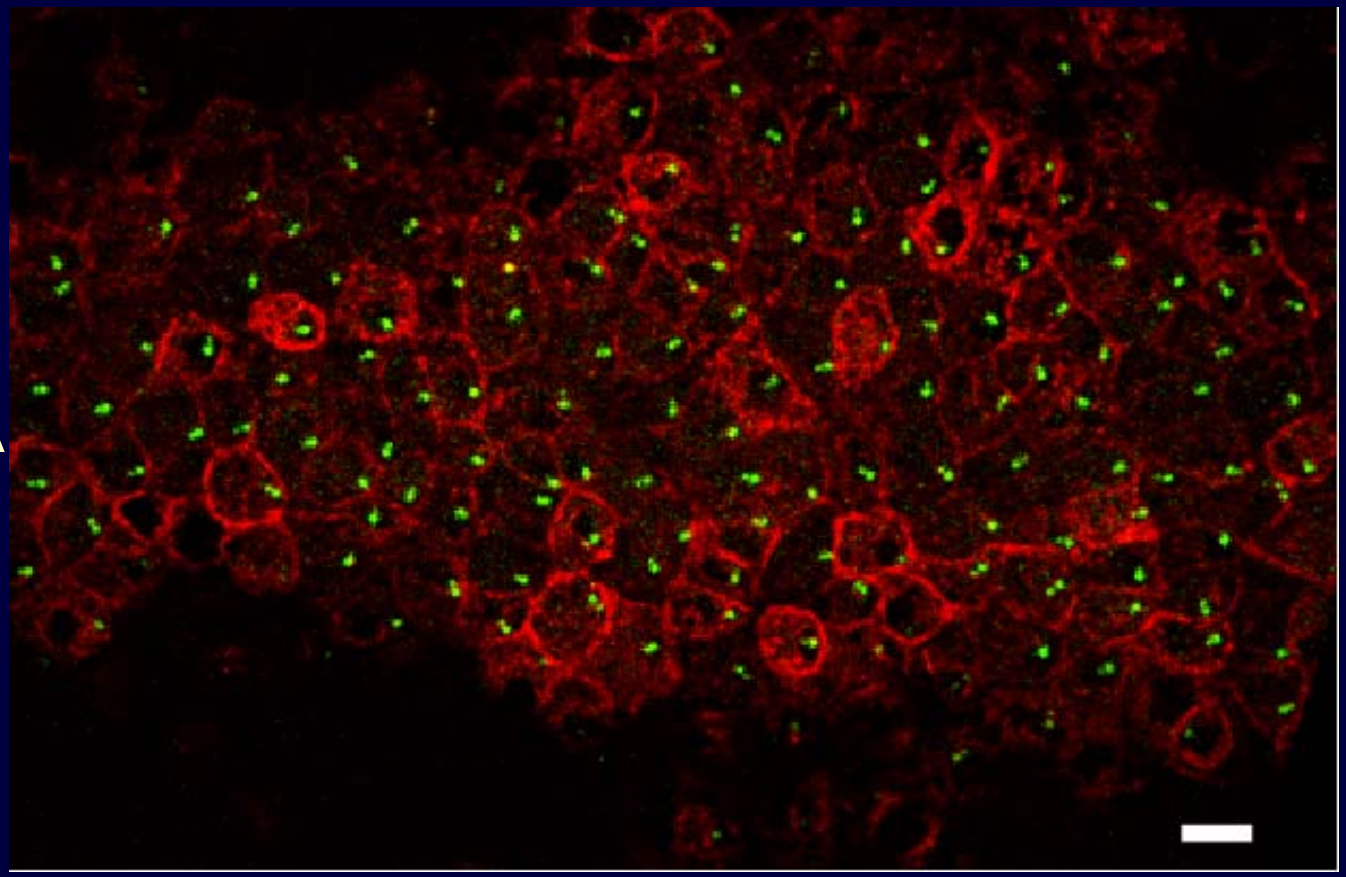
Posterior projection of nodal cilia

Mouse Rabbit Medaka

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

Posterior Positioning of Basal Body

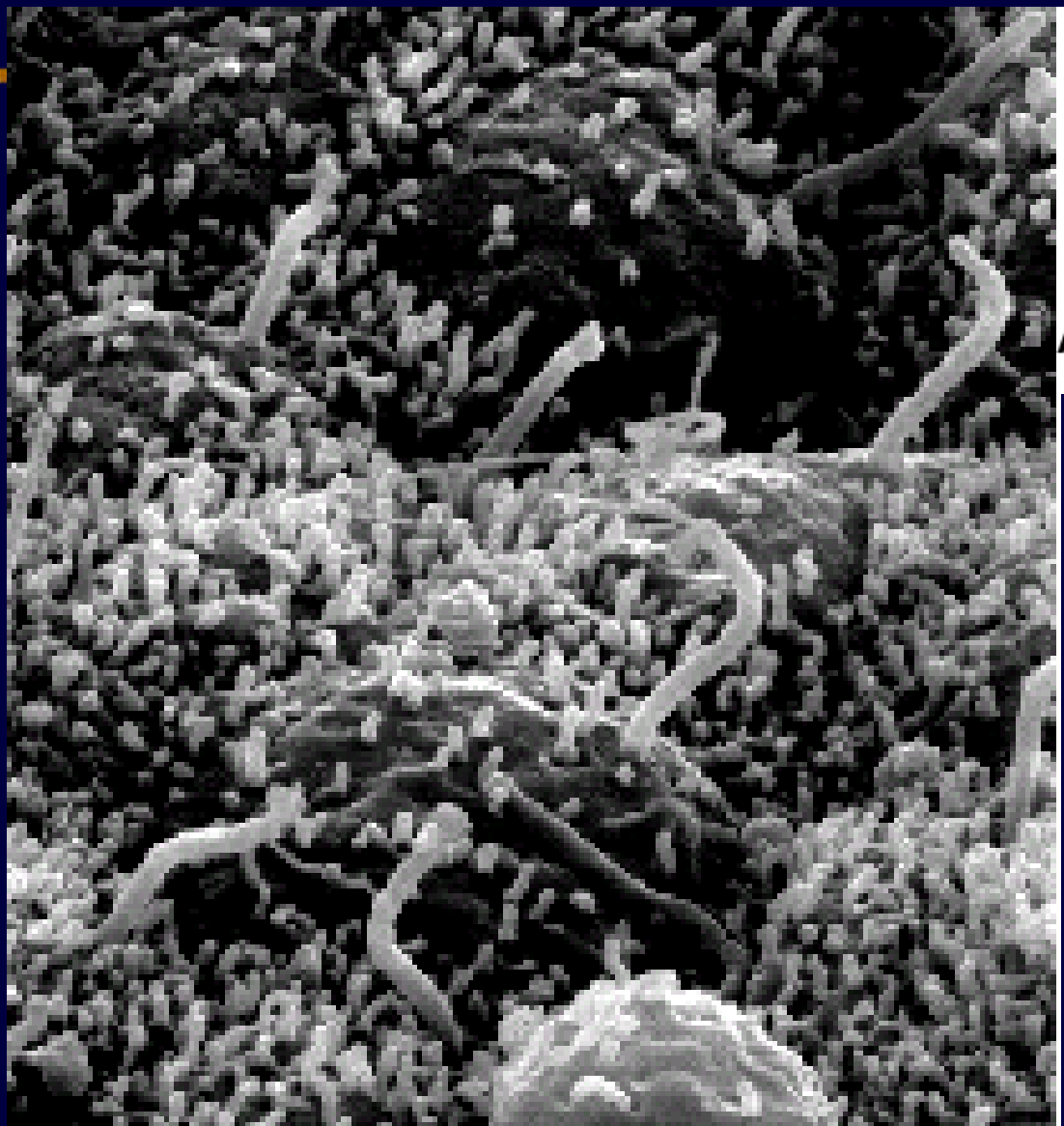
A

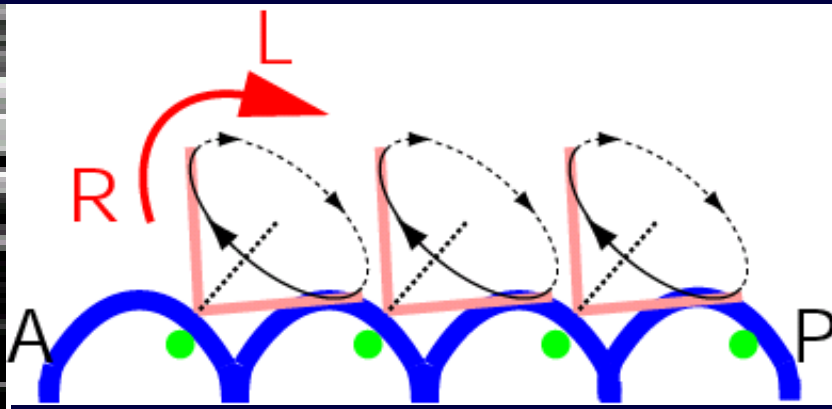


P

Green: γ–tubulin, Red: apical cell surface

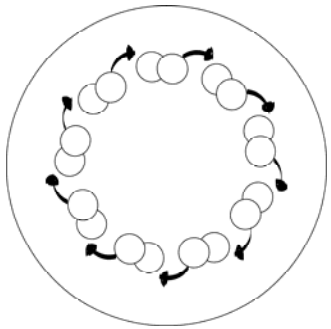
Potential link to planar cell polarity





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

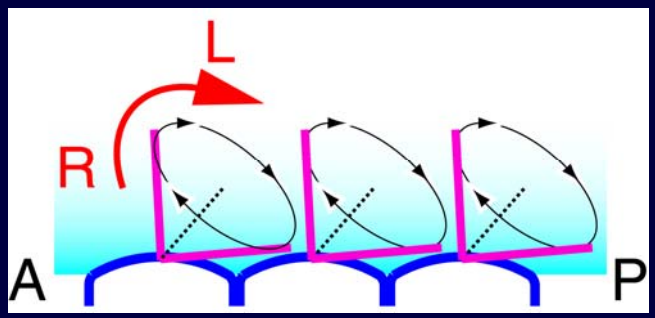
AP axis

PCP?

Ventral projection

Posterior tilting

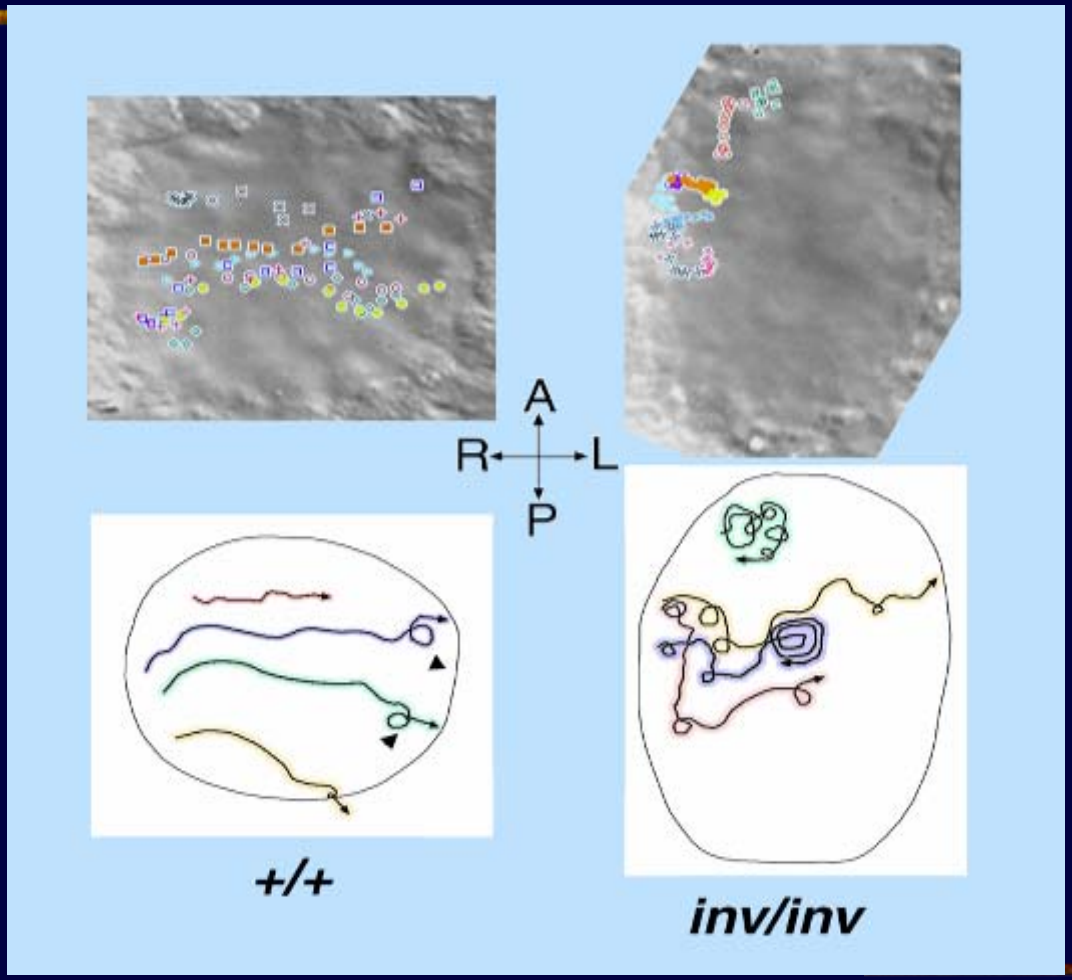
Clockwise rotation



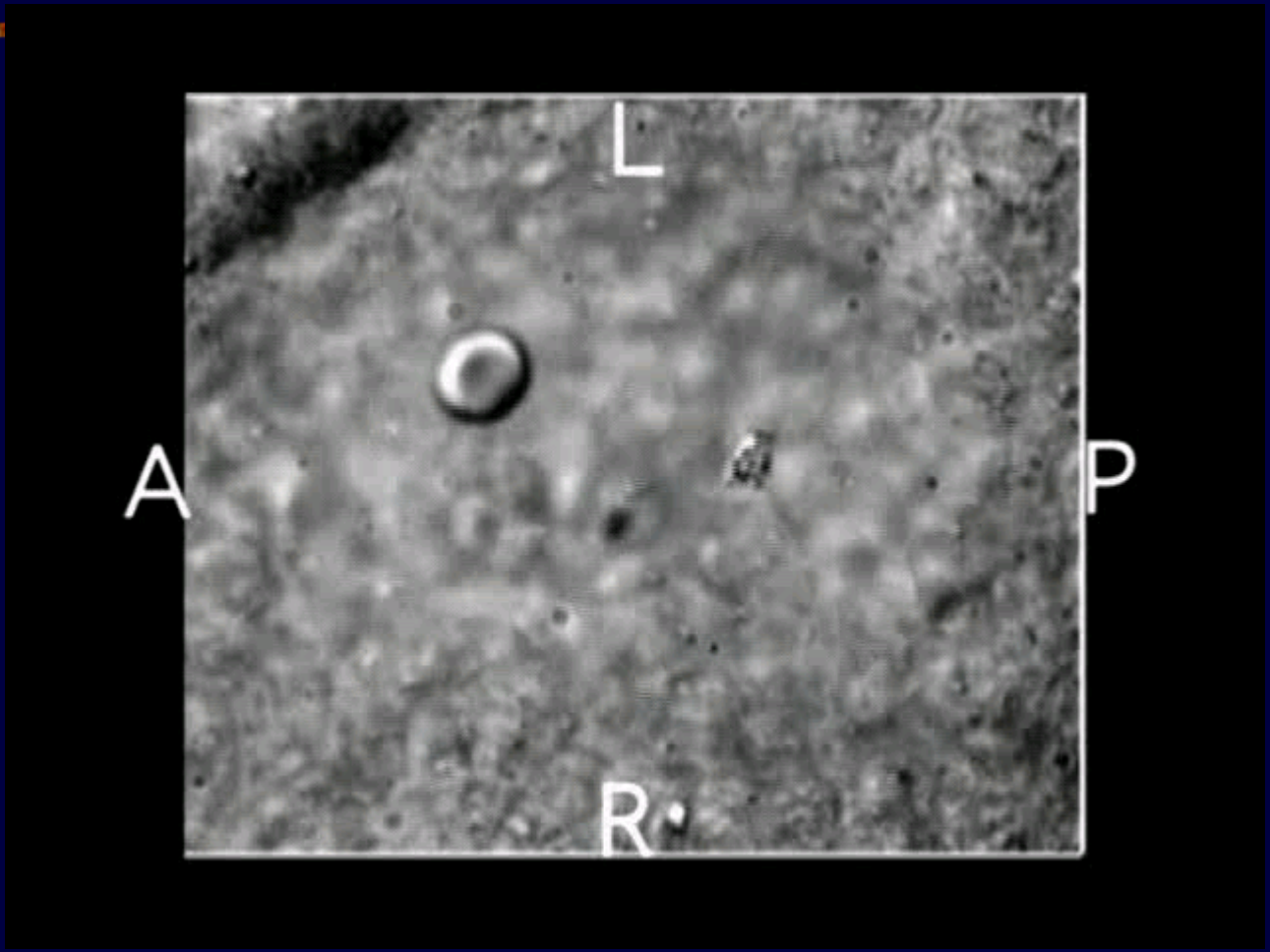
Hydrodynamic Mechanism

Leftward Flow

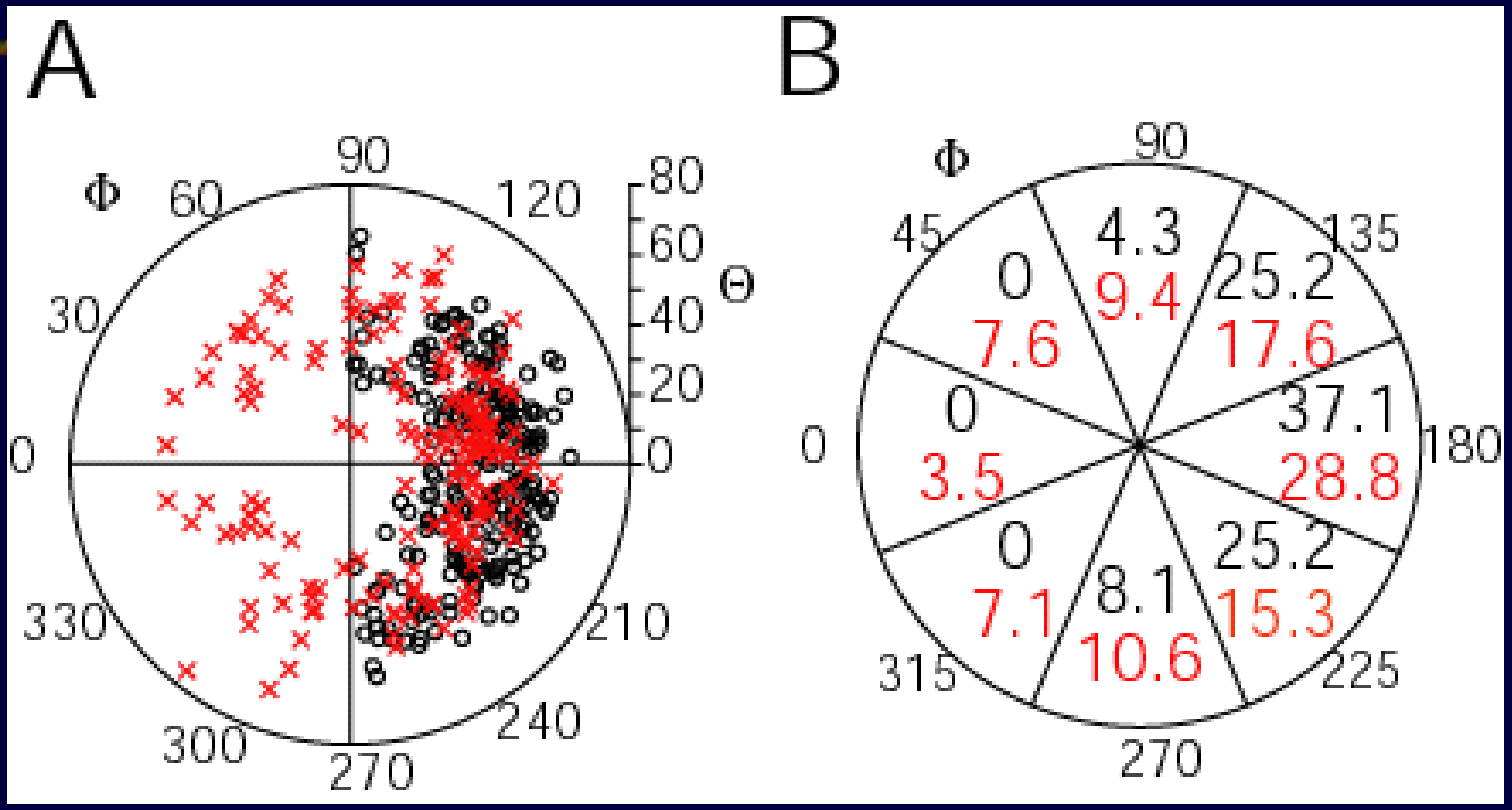
Slow leftward flow with meandering streamline in *inv* mutant mice.



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



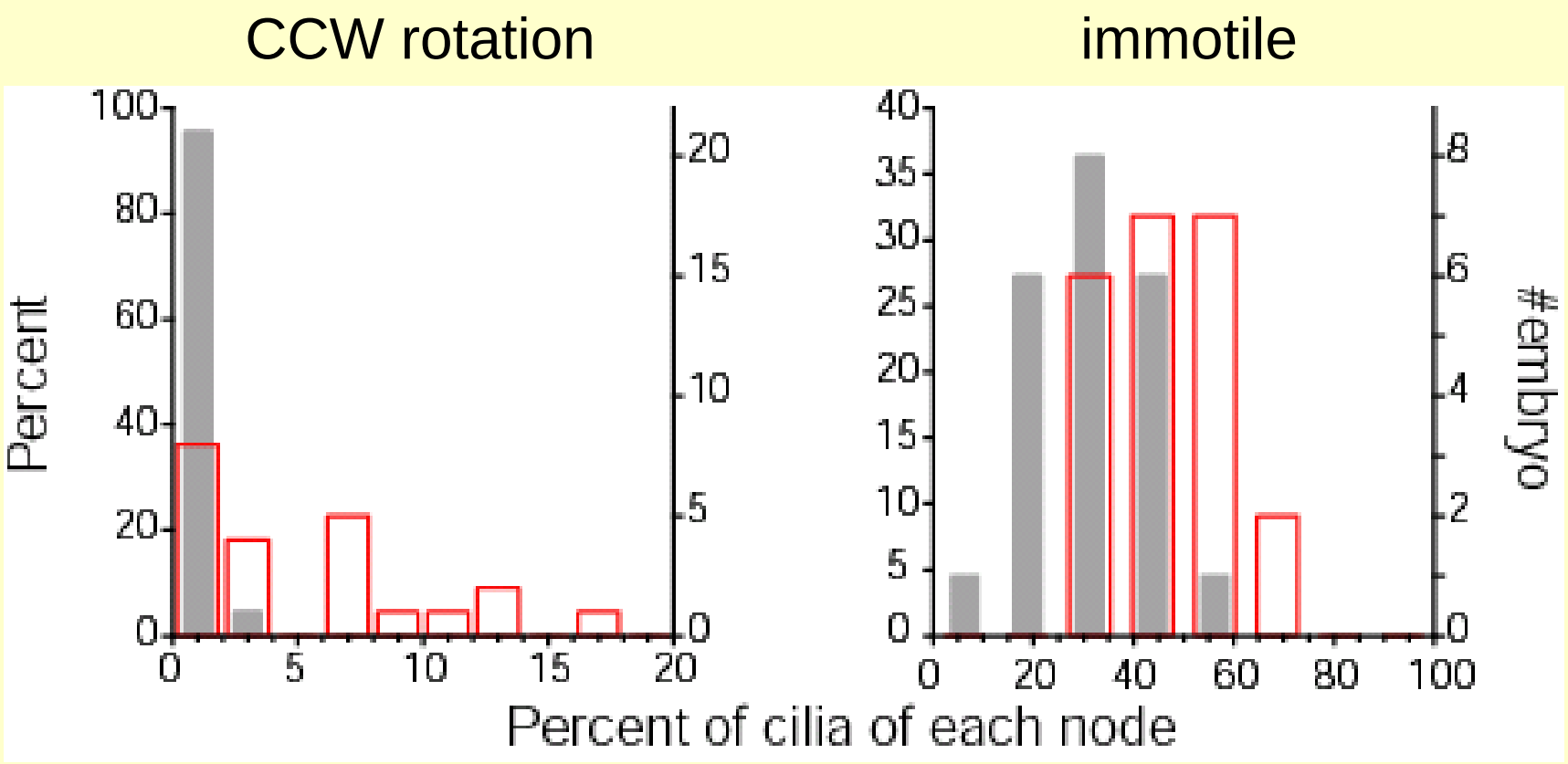
Wider distribution of the direction of the rotation axis in *inv* mutant.



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

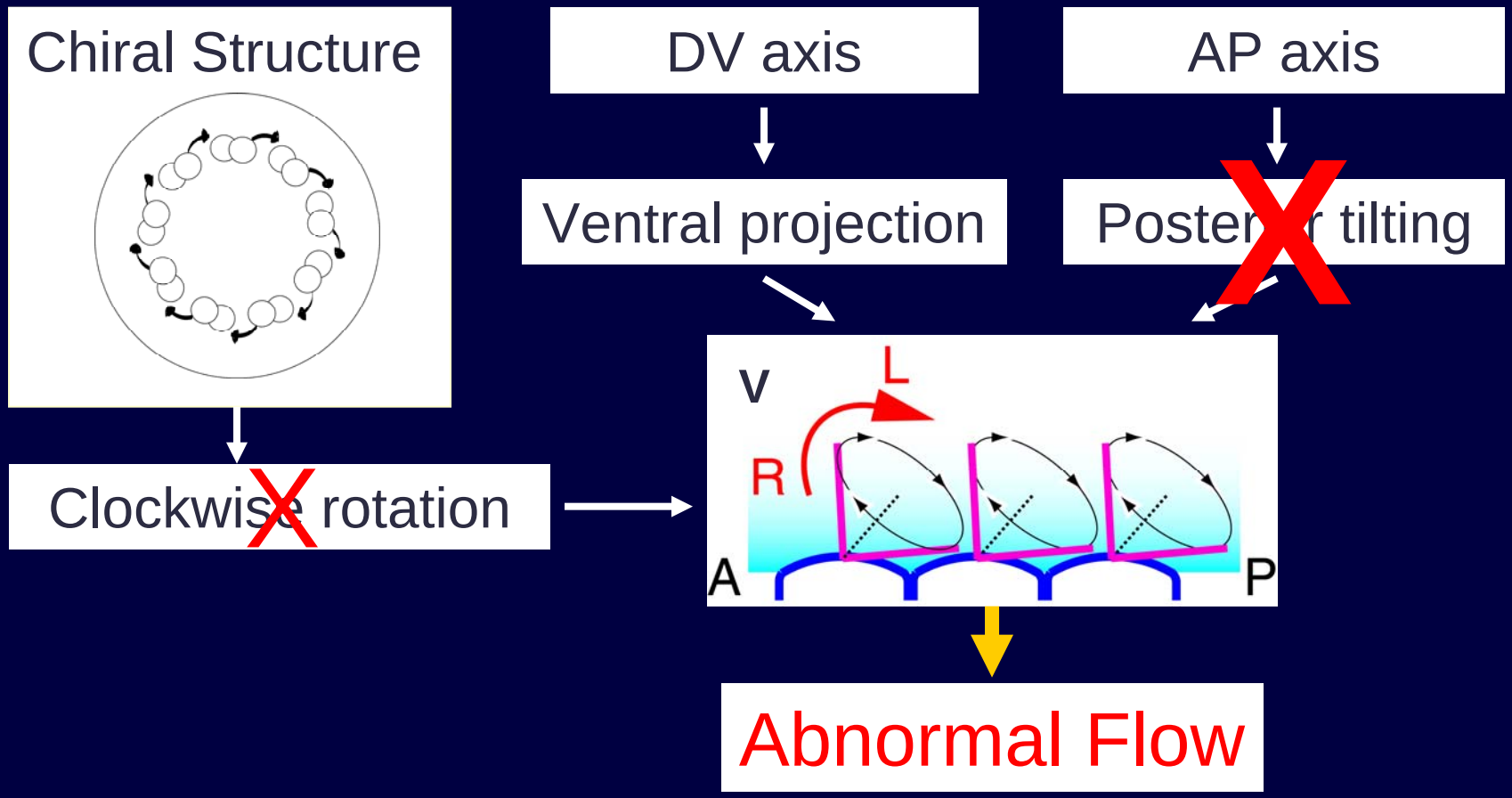
~20% of cilia were anteriorly tilted in *inv/inv* mice.

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



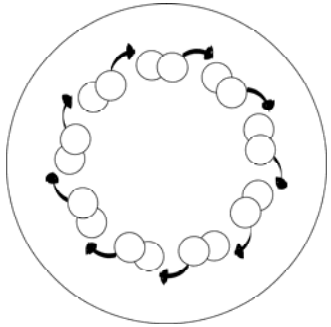
Red: *inv/inv*, Black: *inv/+*

Mechanism of abnormal flow in *inv* mutant mice.



Cilia integrates the information of the axes and the chirality

Chiral Structure



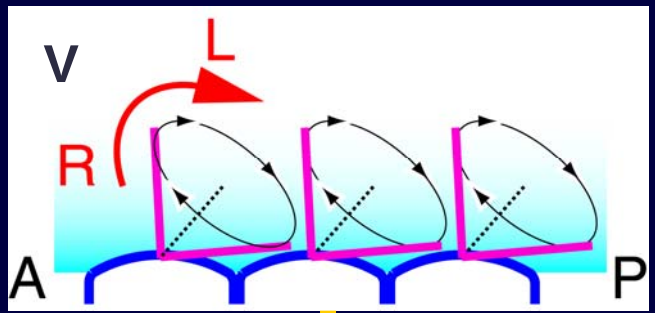
Clockwise rotation

DV axis

Ventral projection

AP axis

Posterior tilting



Leftward Flow

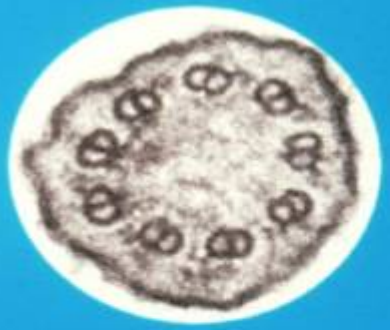
Kartagener's Syndrome

Immotile Cilia syndrome

Immotile Cilia
(Male Infertility + Respiratory Failure etc.)

?

Situs Inversus



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

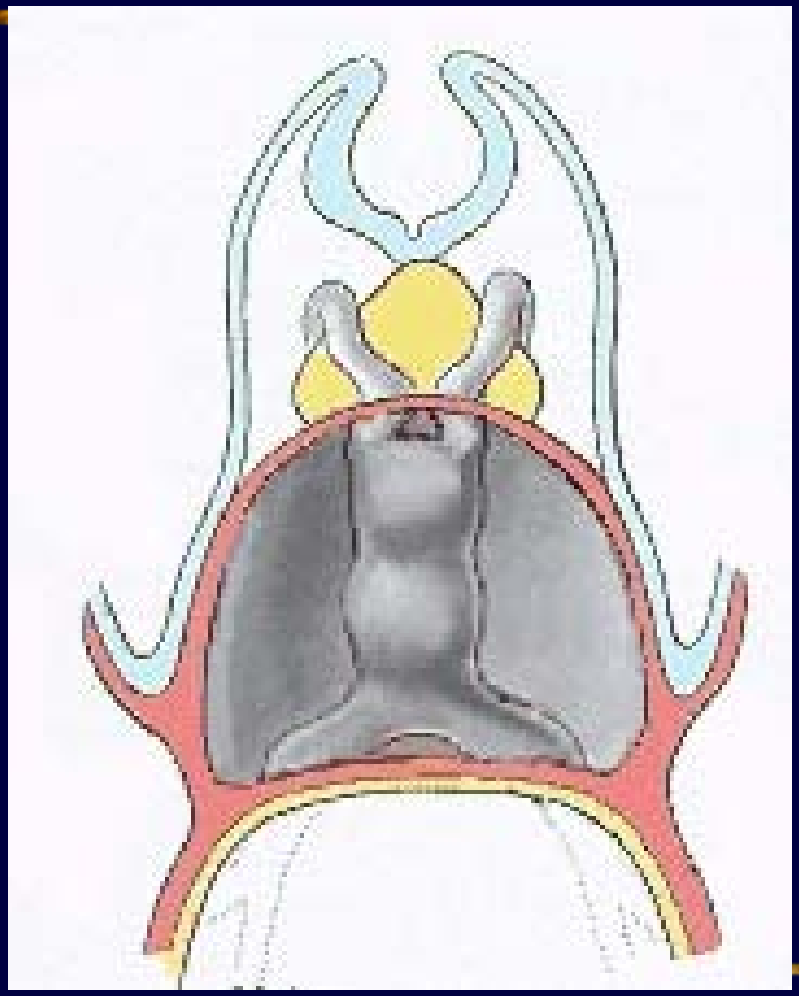
FGF-induced vesicular release of Sonic hedgehog and retinoic acid in leftward nodal flow is critical for left right determination

Nature 435:172-177, 2005

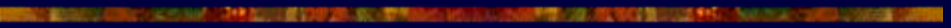
Yosuke Tanaka, Yasushi Okada & Nobutaka Hirokawa

Dept Cell Biol & Anat, Grad Sch Med, Univ Tokyo

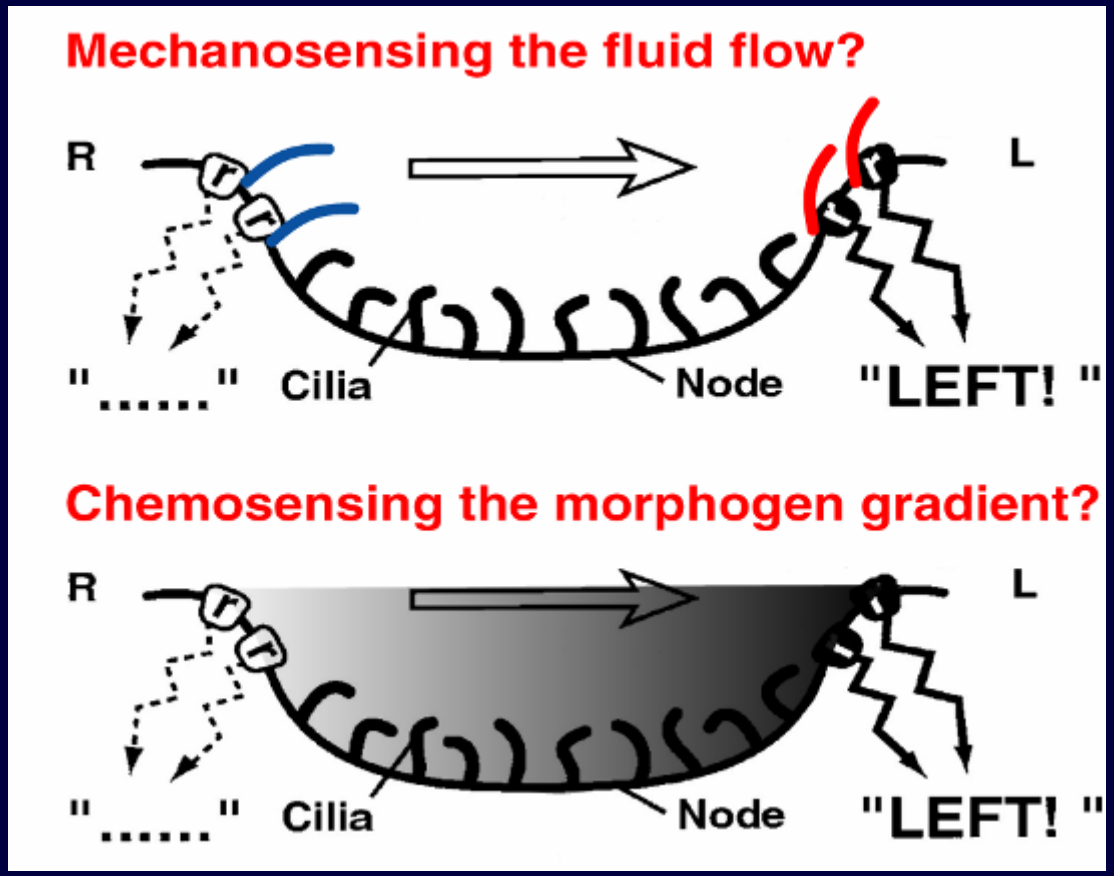
Symmetry breaking is essential for developing your internal organs



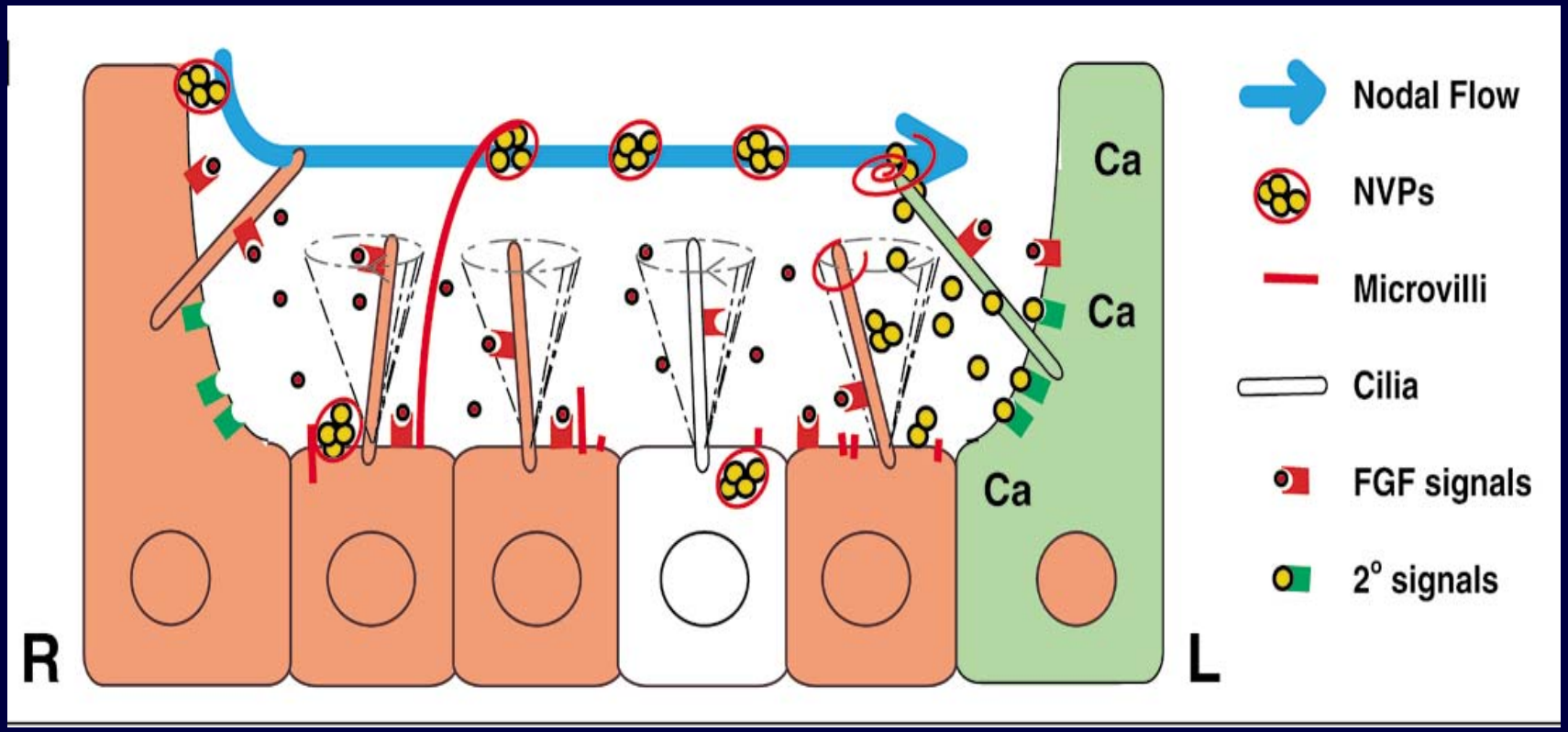
(William Larsen's Human Embryology website)



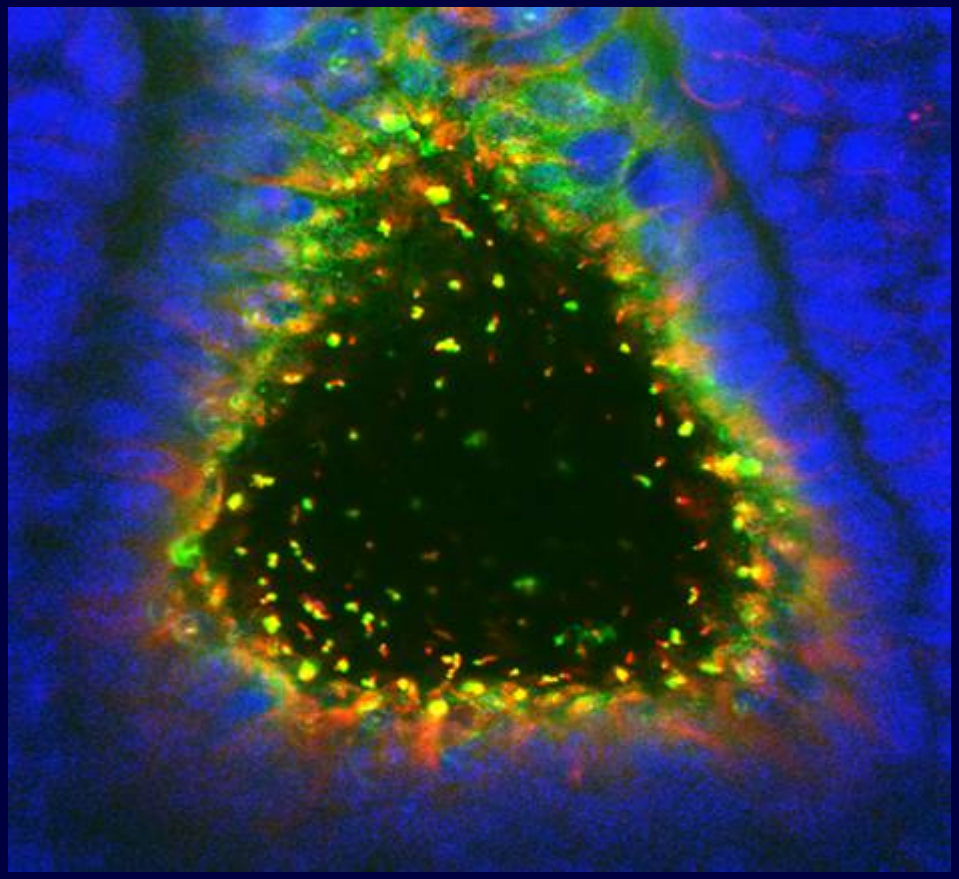
The sensing mechanism of nodal flow is very much controversial

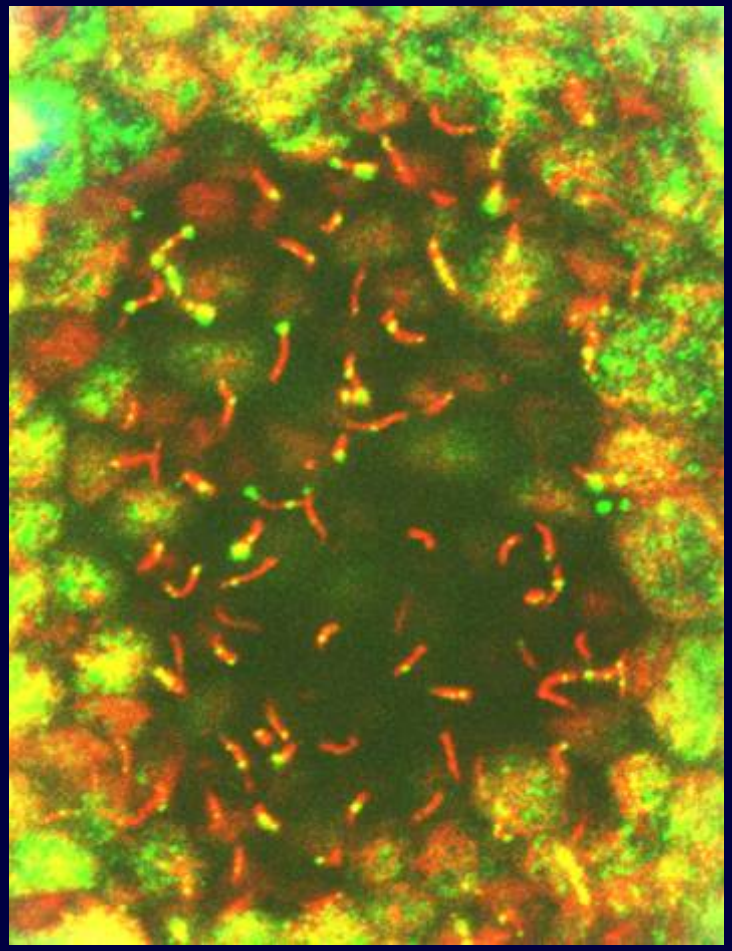


We identified morphogen-carrying vesicular parcels flowing to the left

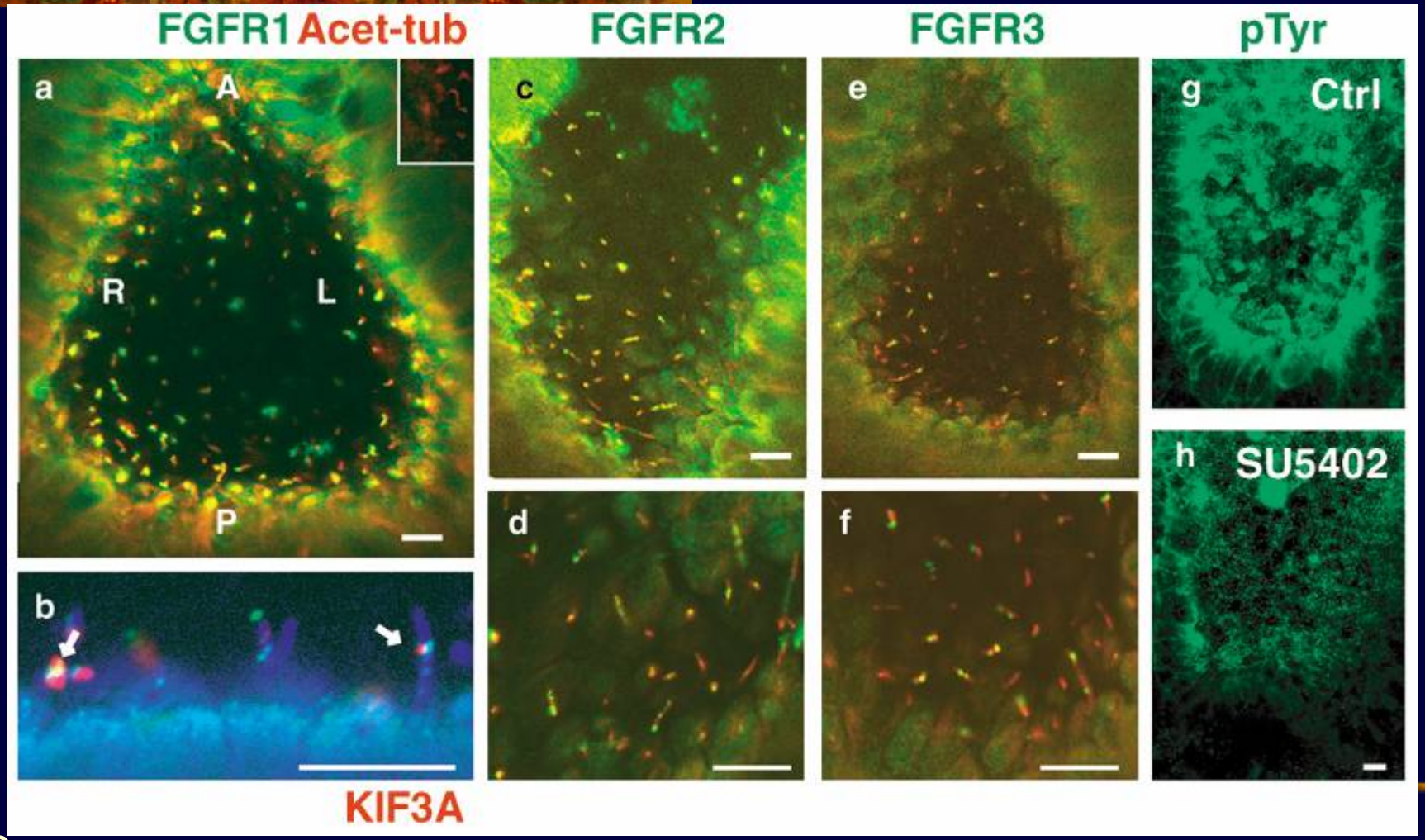


FGF receptors in ventral node

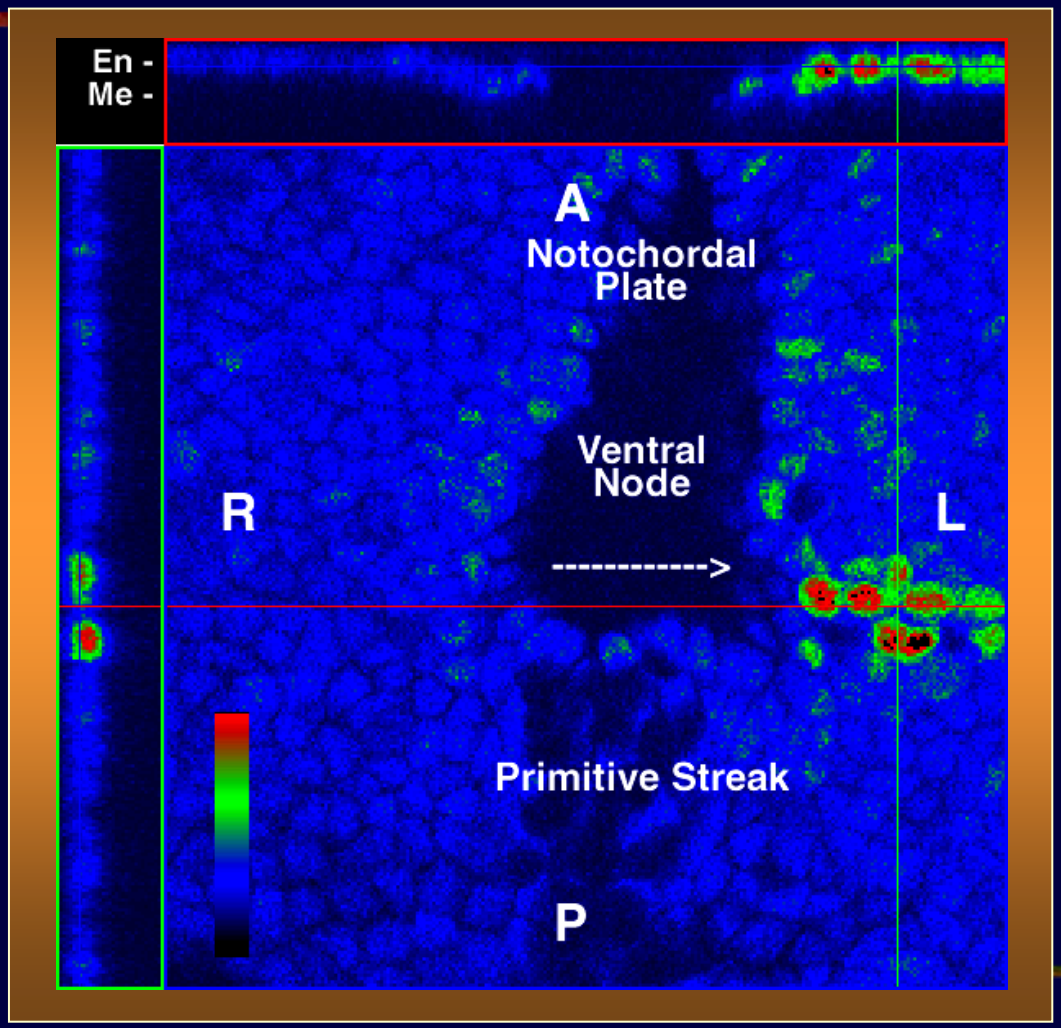




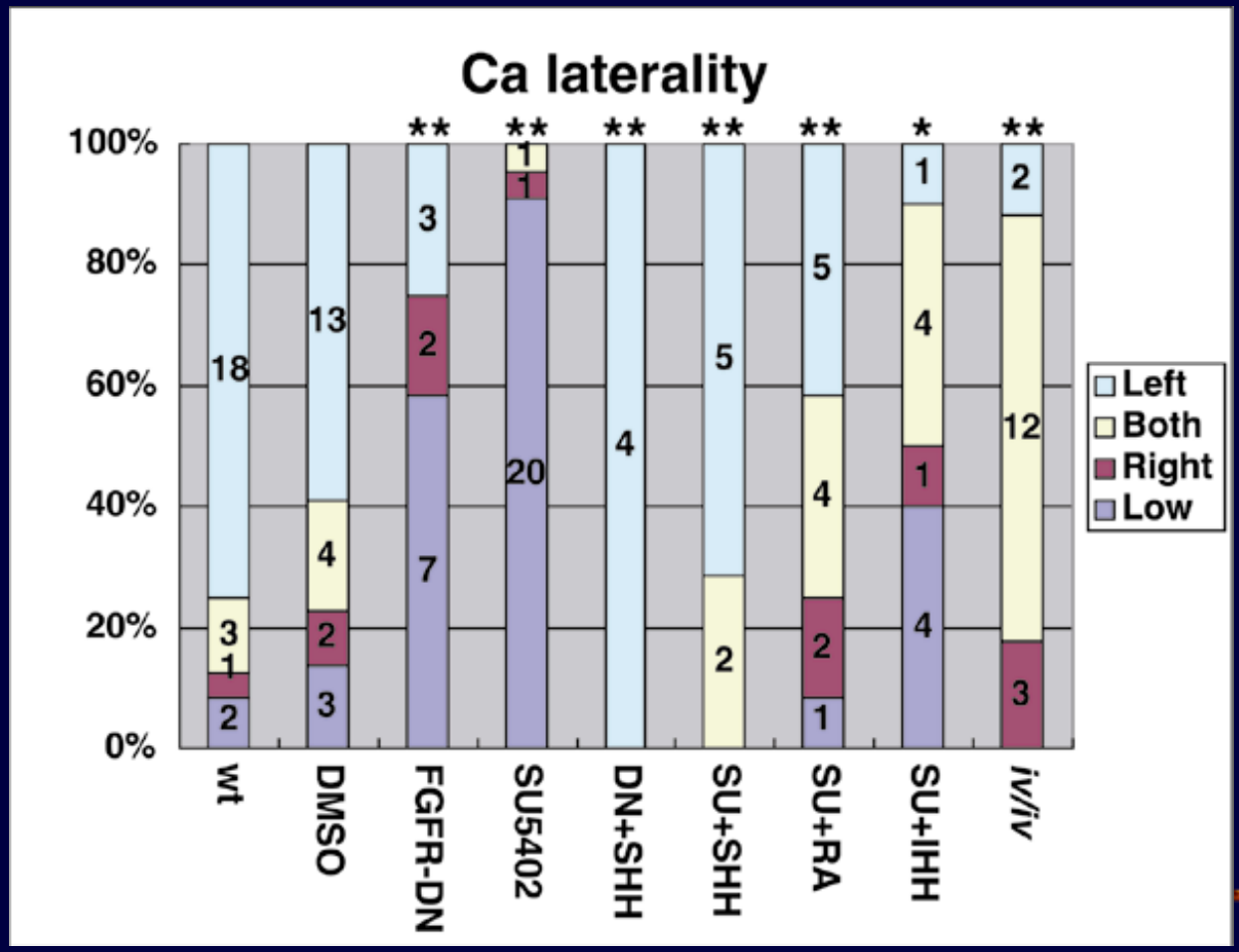
Active FGF signals in ventral nodal region



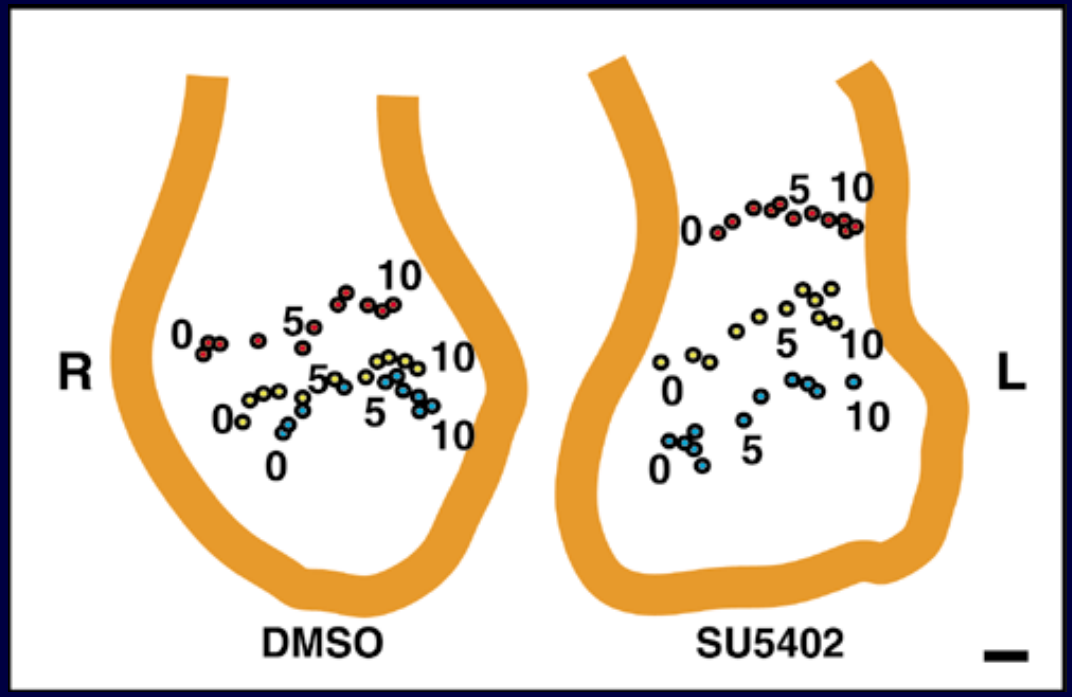
Ca elevation in left definitive endoderm

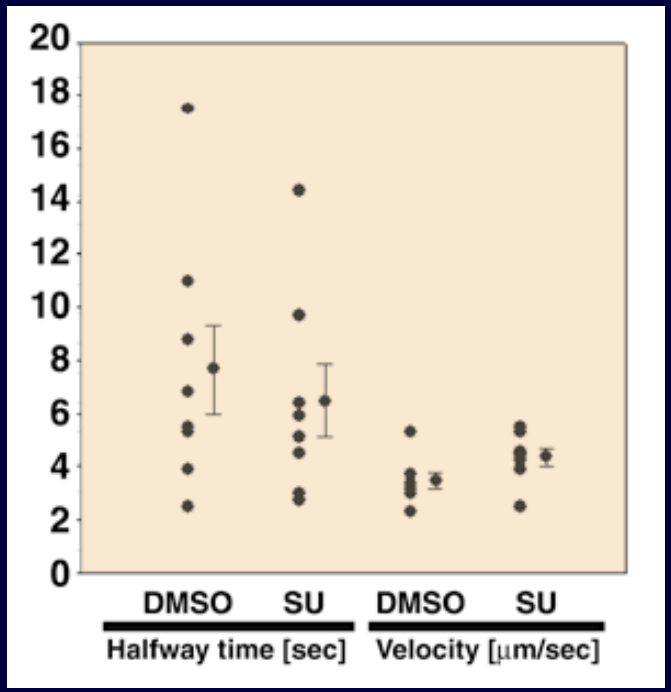


RA and SHH elevated Ca on the left side in the presence of SU5402

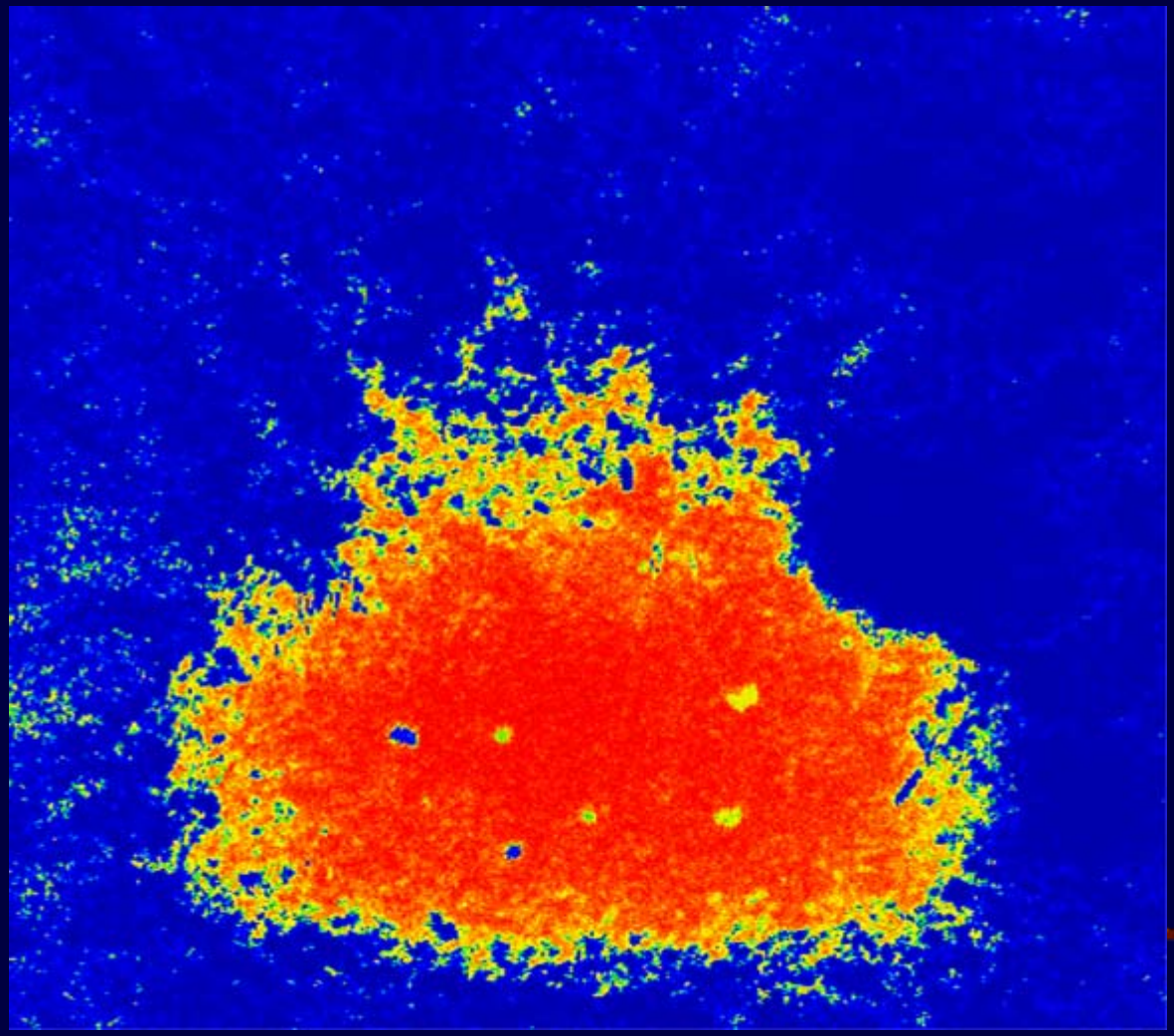


SU5402 did not apparently impair the fluid flow on 1-3 somite stage

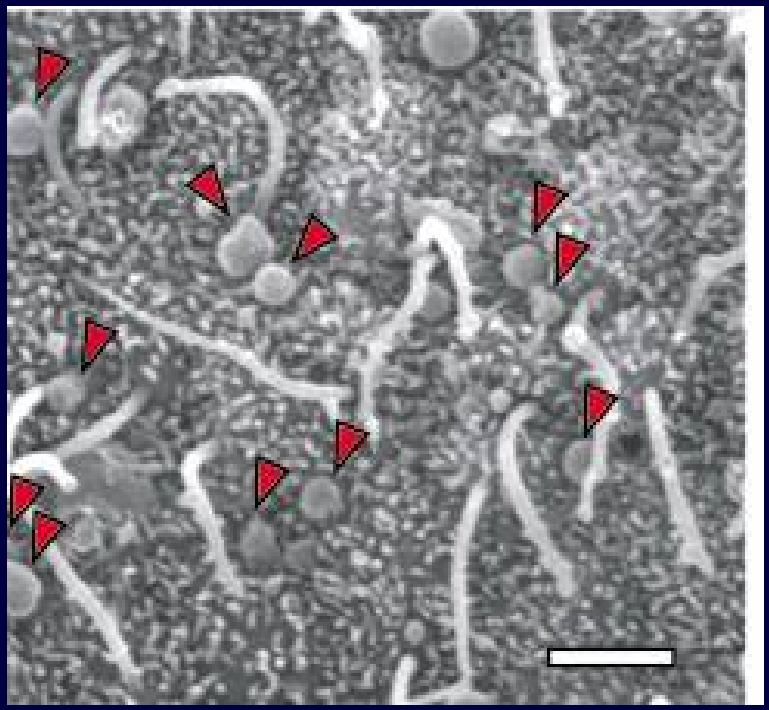


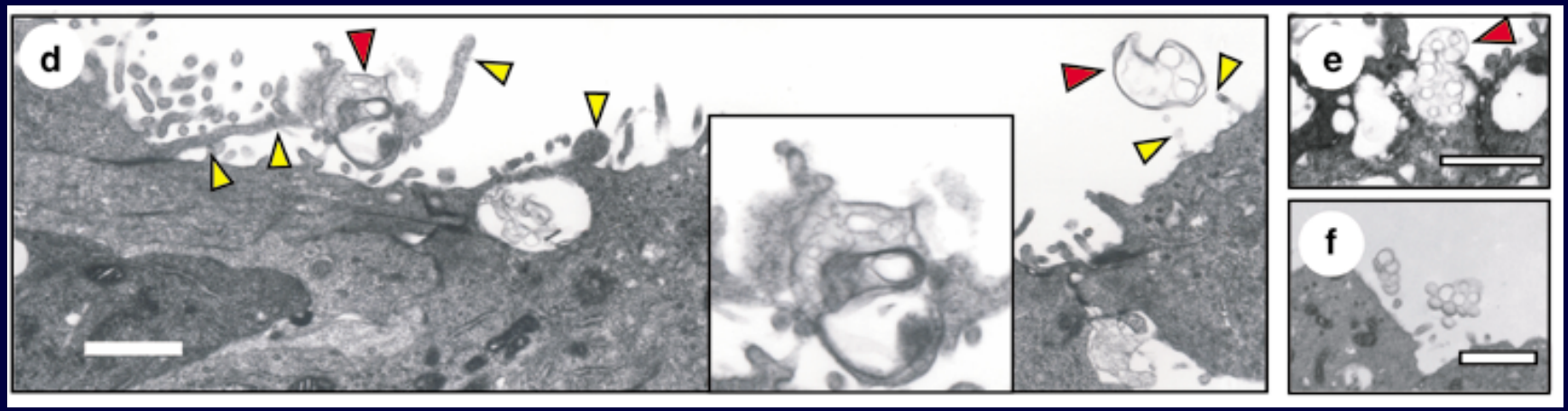


Nodal Vesicular Parcels (NVPs) flows from the right to the left



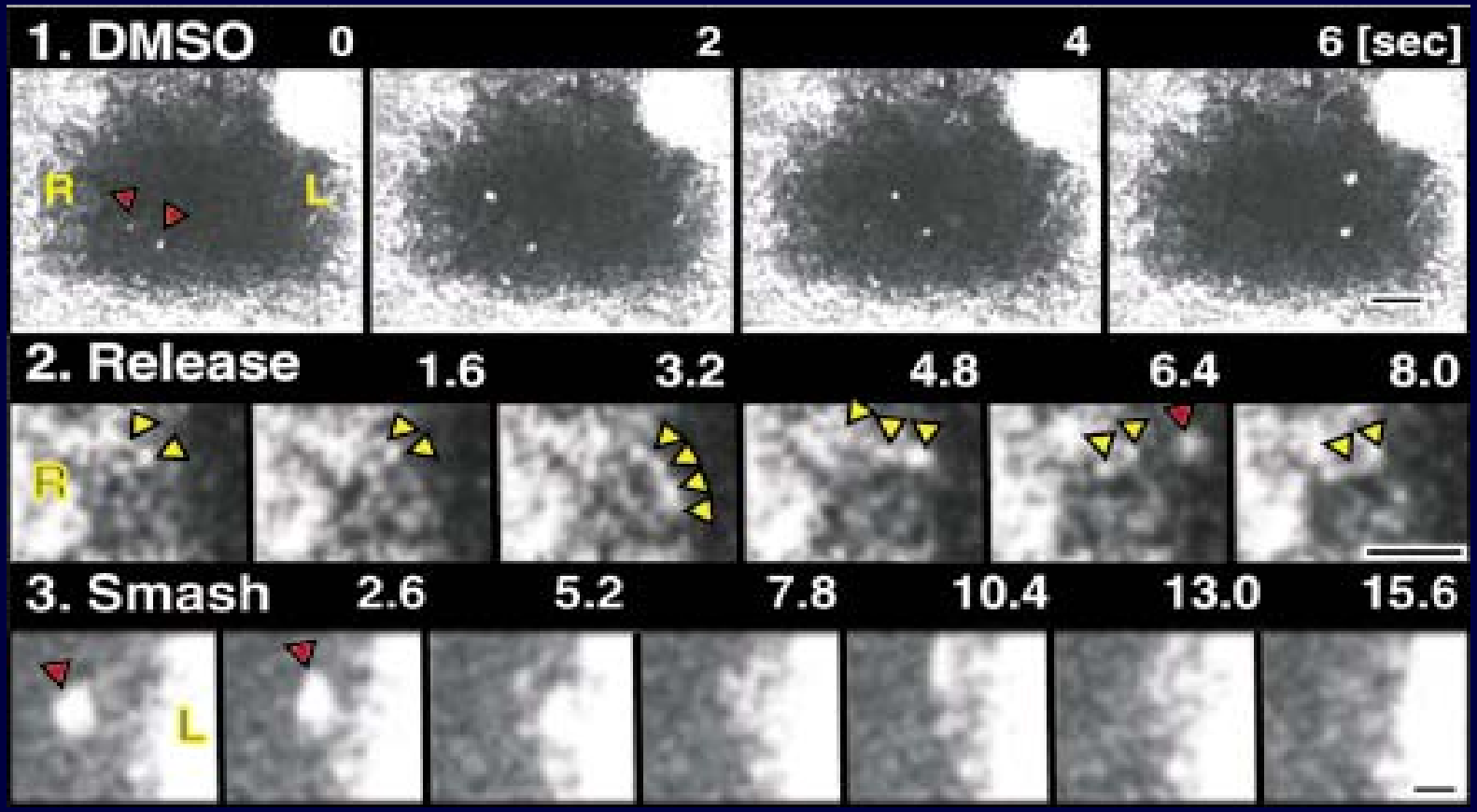
NVPs are associated with microvilli on its release



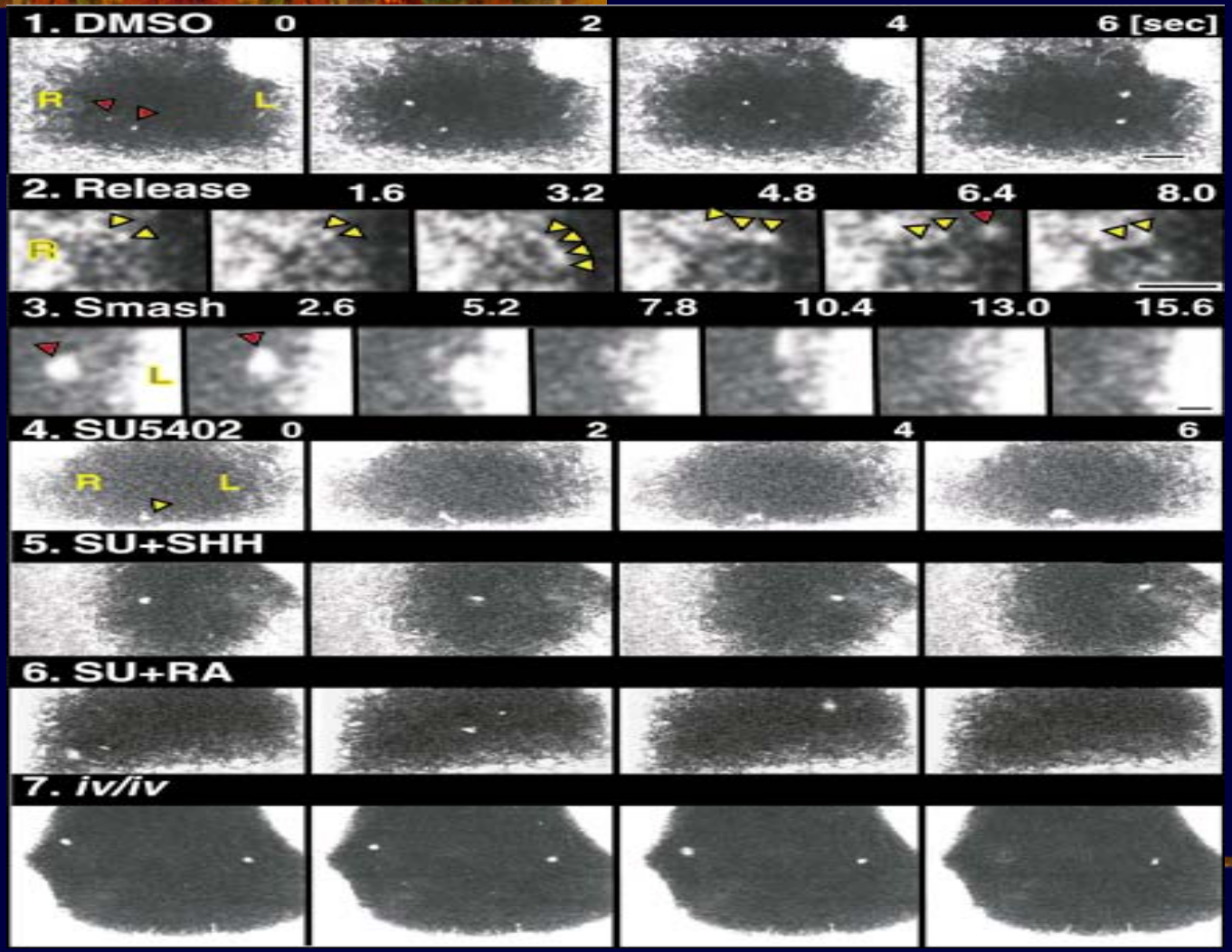


e & f, SU5402-treated

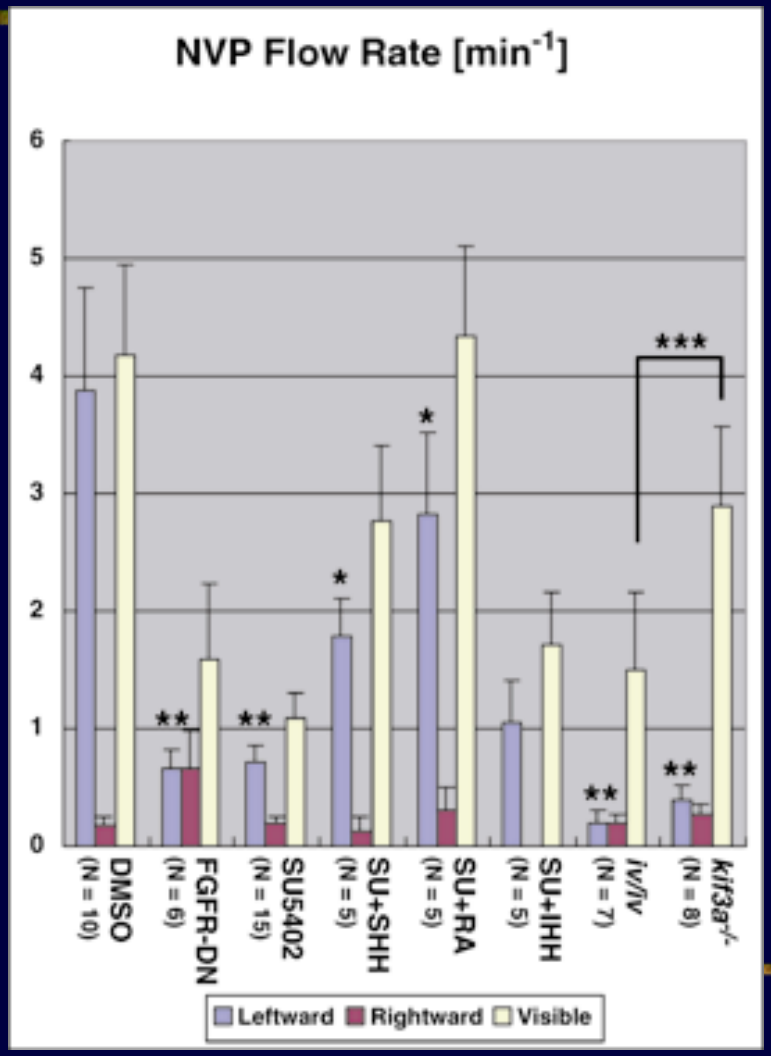
Unidirectionality of NVP flow is ensured by its fragmentation



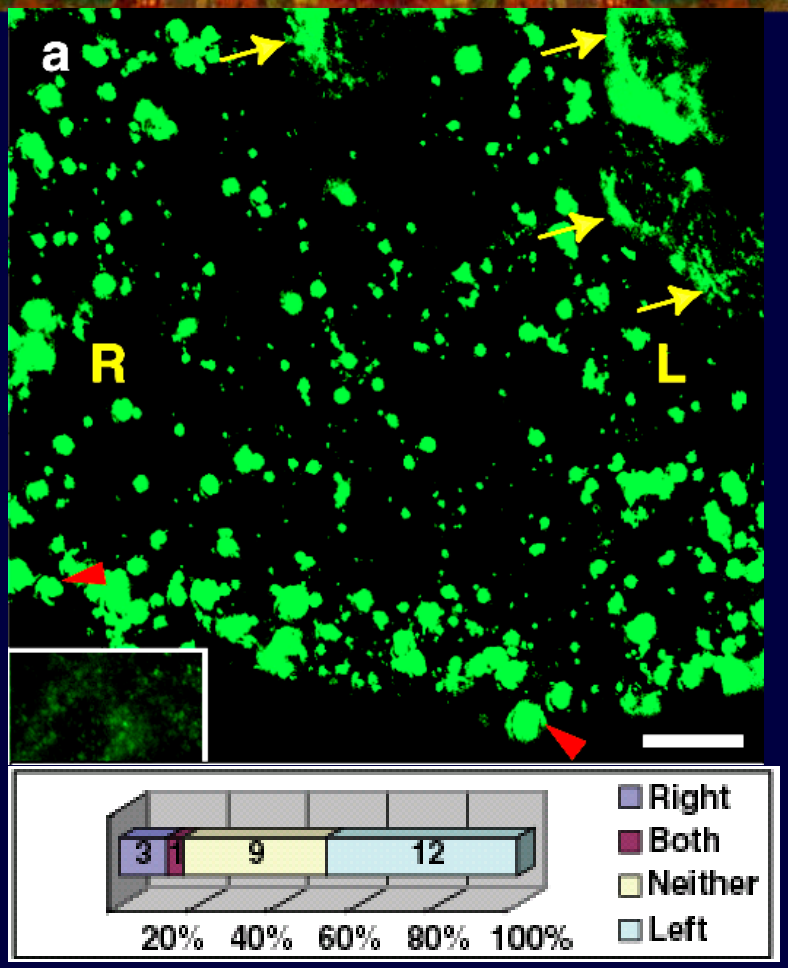
NVP flow can be modulated by pharmacological perturbations



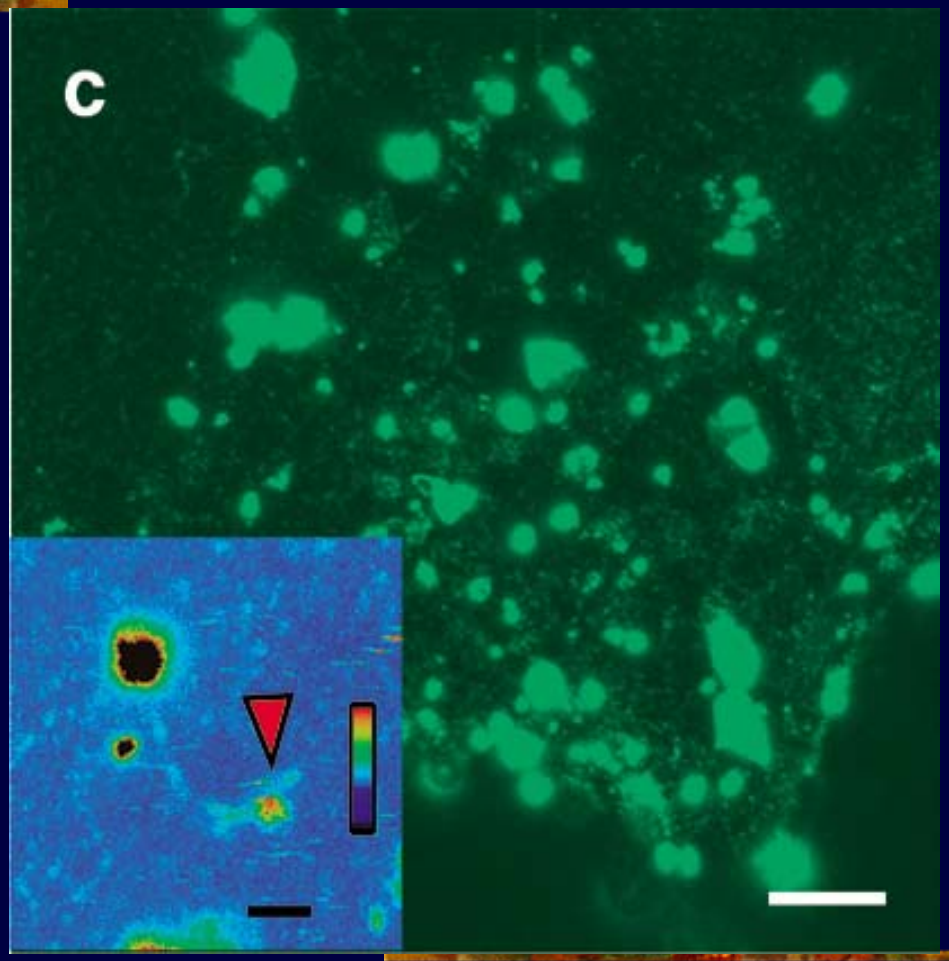
A non-ciliated node has more NVPs than a node with immotile cilia



SHH and RA localize on NVPs

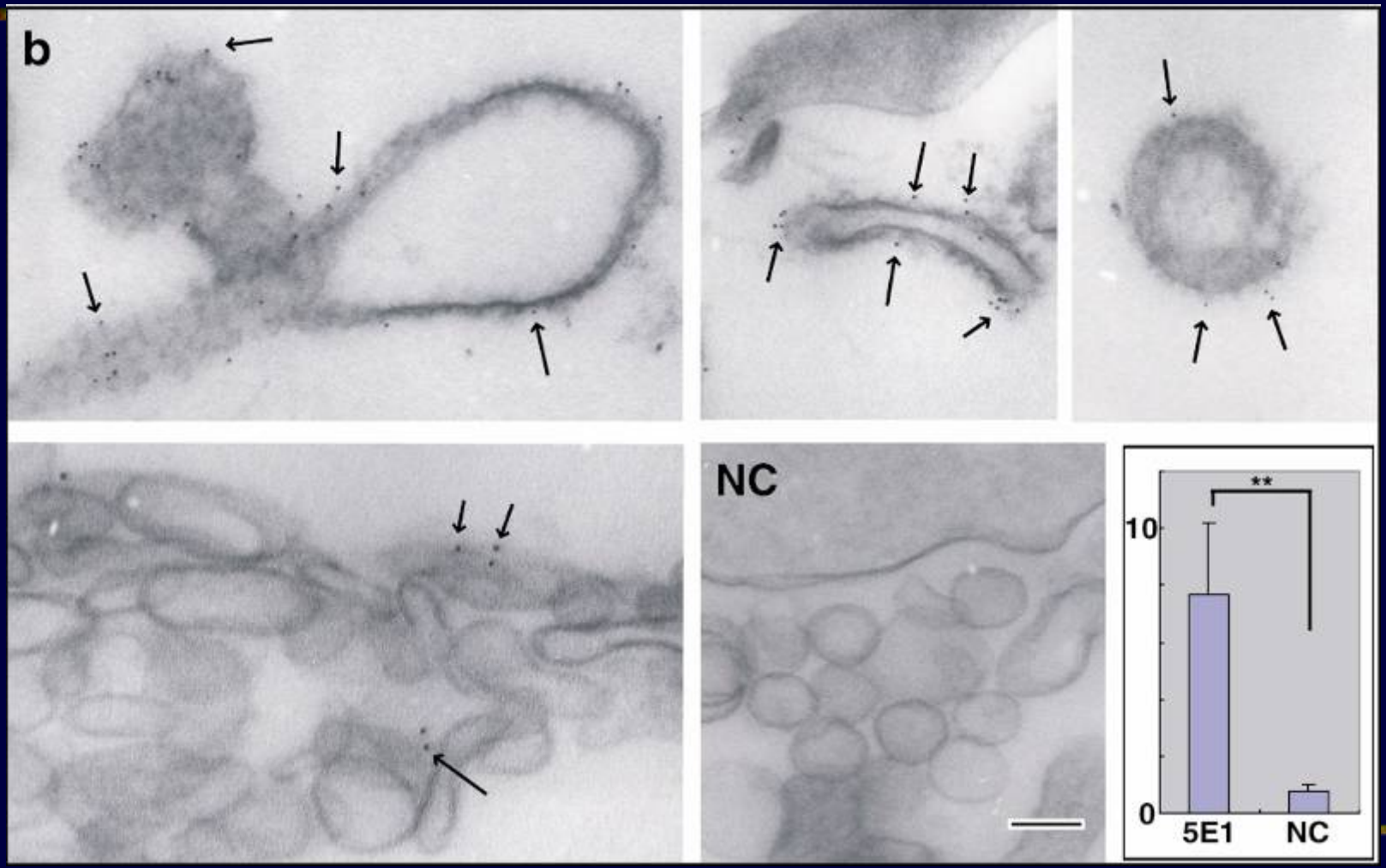


SHH

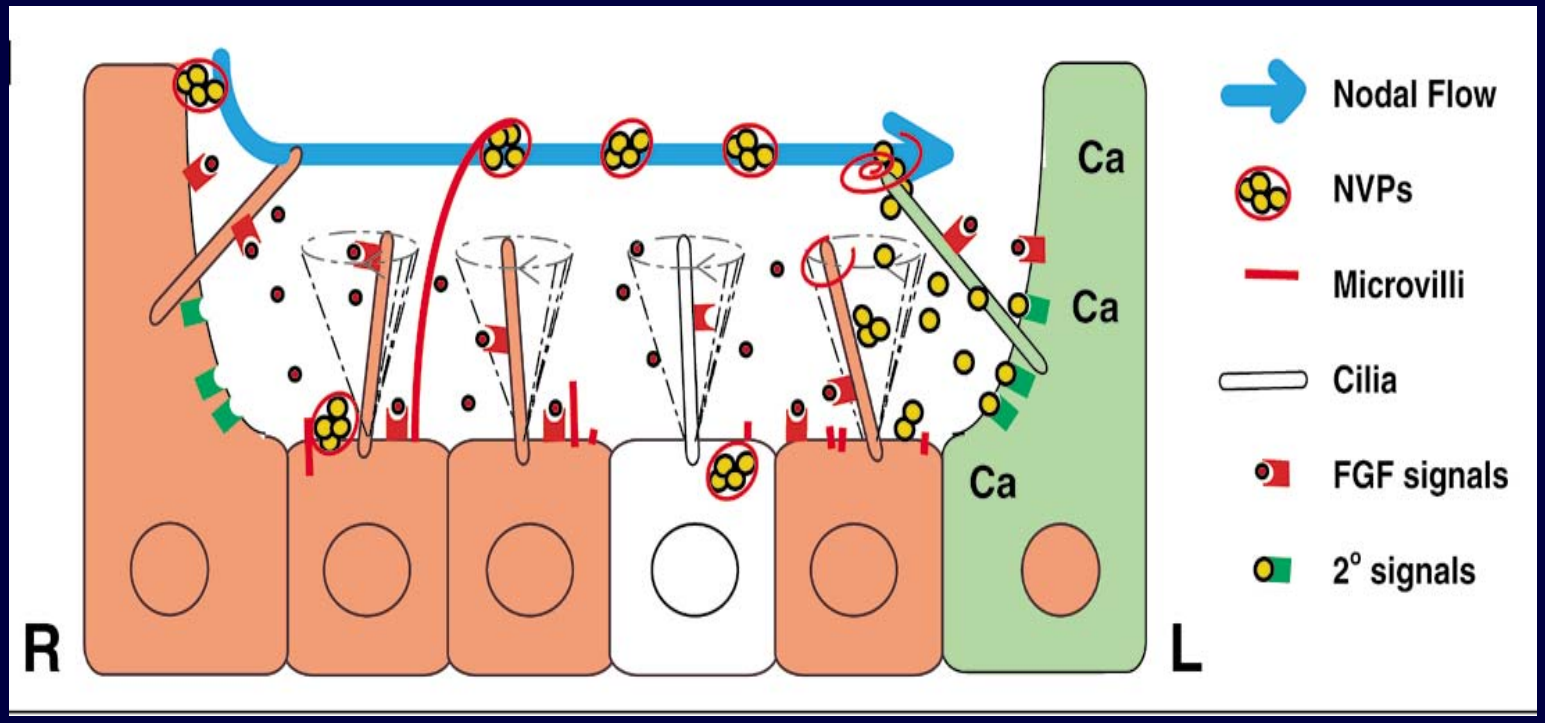


RA

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.