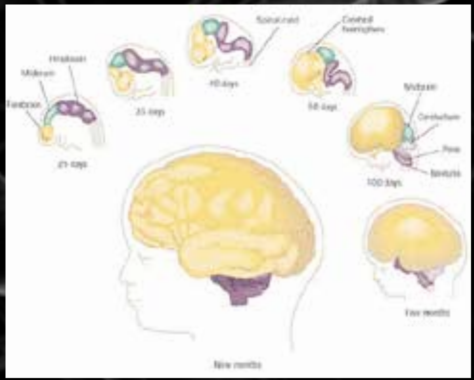
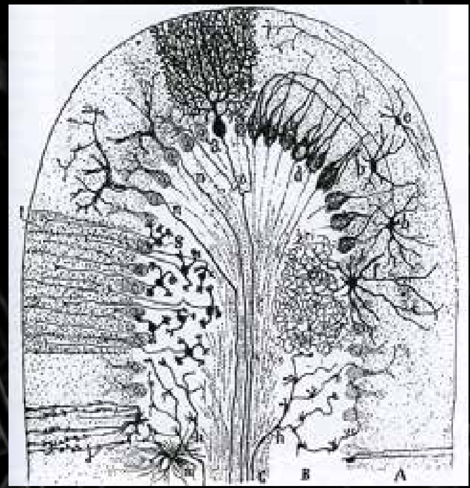
Dynamics and mechanisms of nuclear migration in neurons

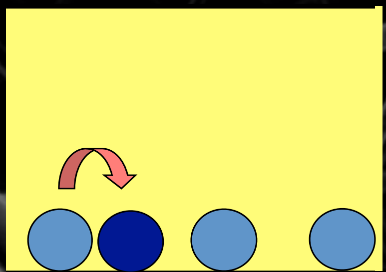
KUIAS-iCeMS

Mineko Kengaku

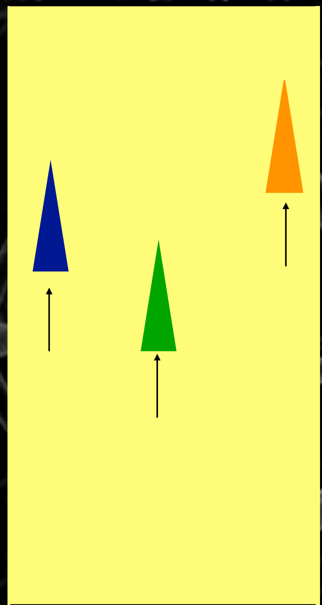
Dynamic cell motility in developing brains



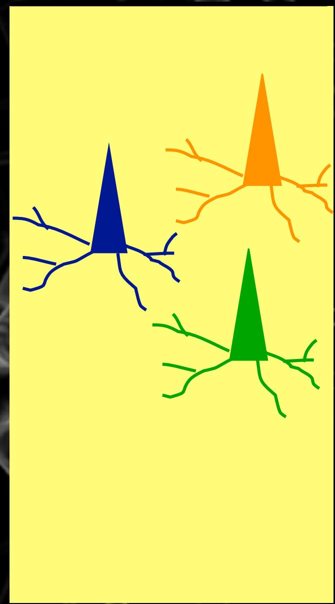




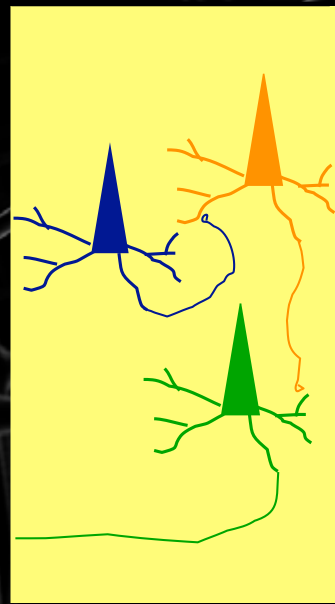
cell differentiation
細胞分化



Cell migration
細胞移動



Process arborization
突起形成

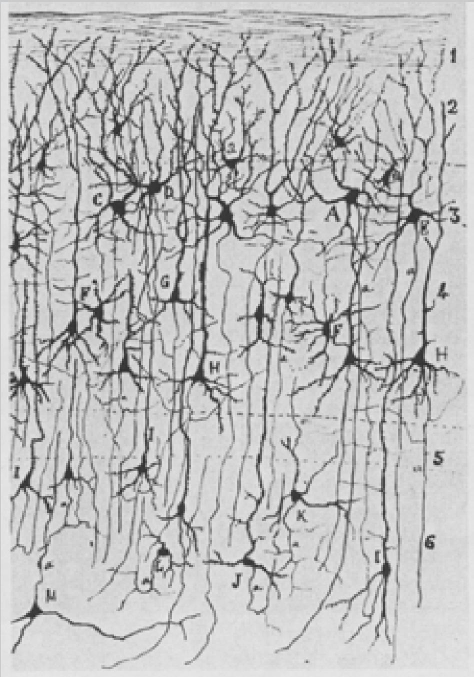


neural network formation

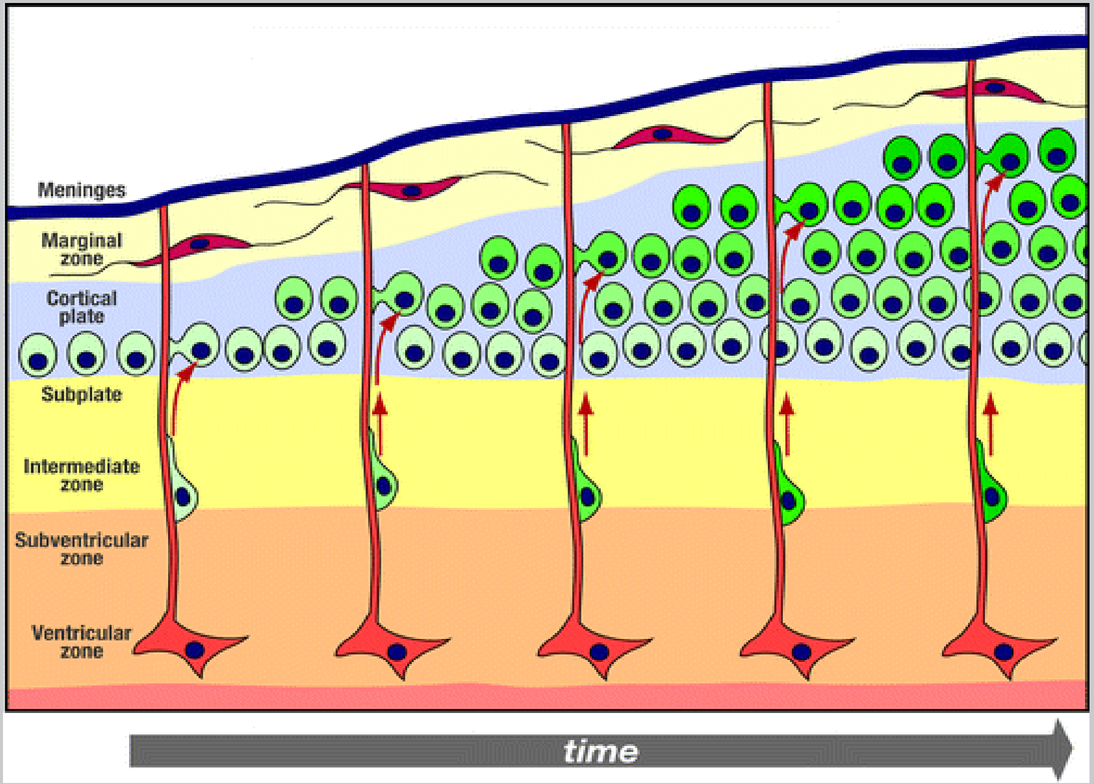
Neurons reach appropriate positions

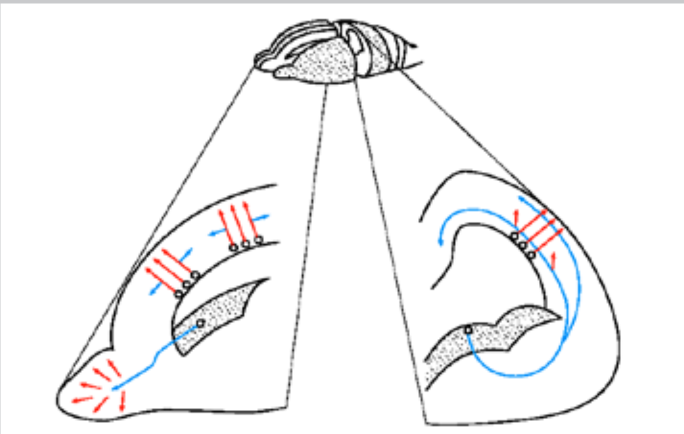
Process formation and coupling

Migration of neurons in the central nervous system

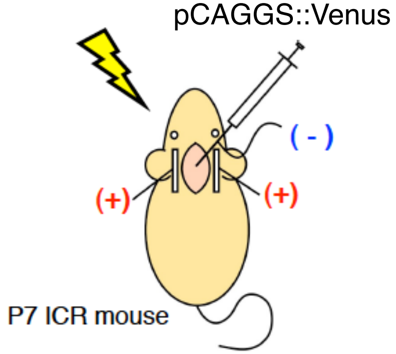


Summary diagram of Golgi staining of cerebral cortex. Ramón y Cajal (1911)

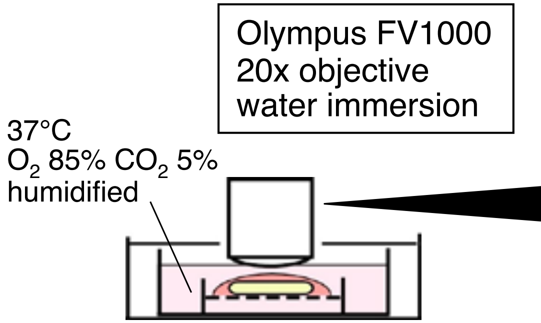




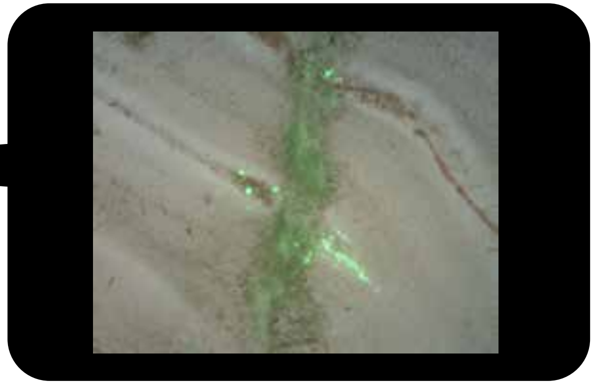
Live-cell observation of granule cell migration in slice culture



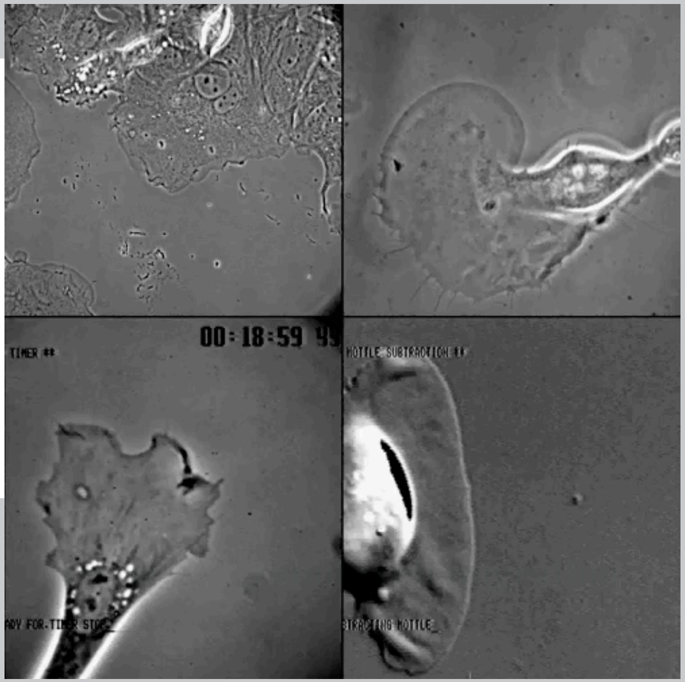
***in vivo* electroporation**



time-lapse recording of the cerebellar tissue culture



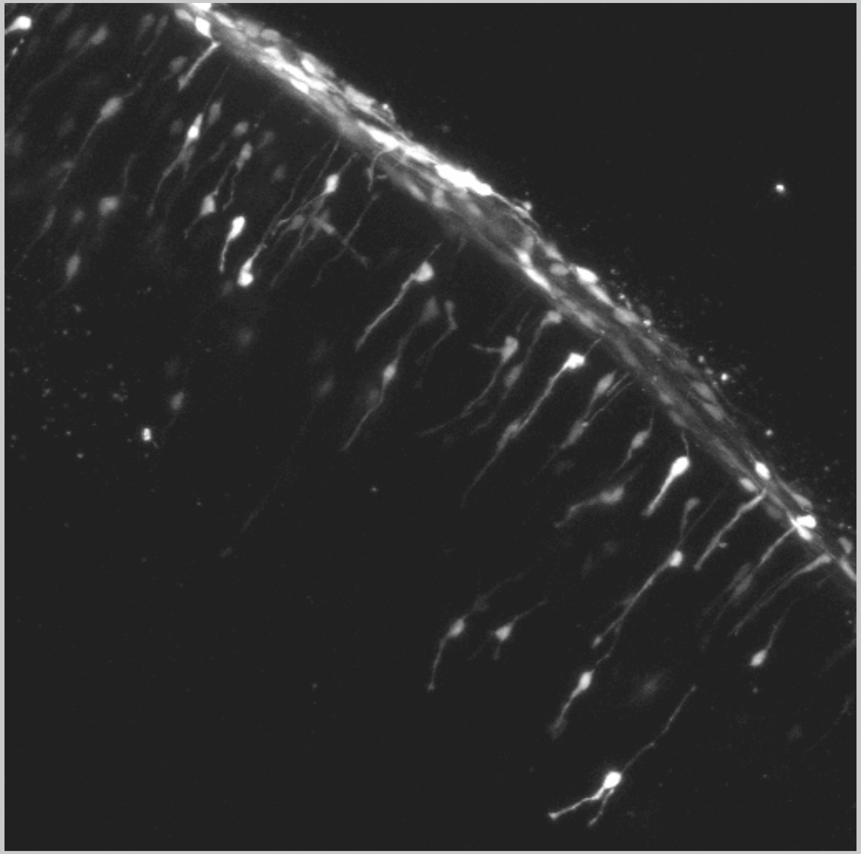
Umeshima et al., PNAS 2007
Umeshima et al., Mol Cell Neurosci. 2012



The Cytoskeleton and Cell Migration: Vic Small lab Website

pia ↑

cortex ↓



20-min intervals
8 frames/sec

Nuclear movement in fibroblasts and neurons

Fibroblast migration

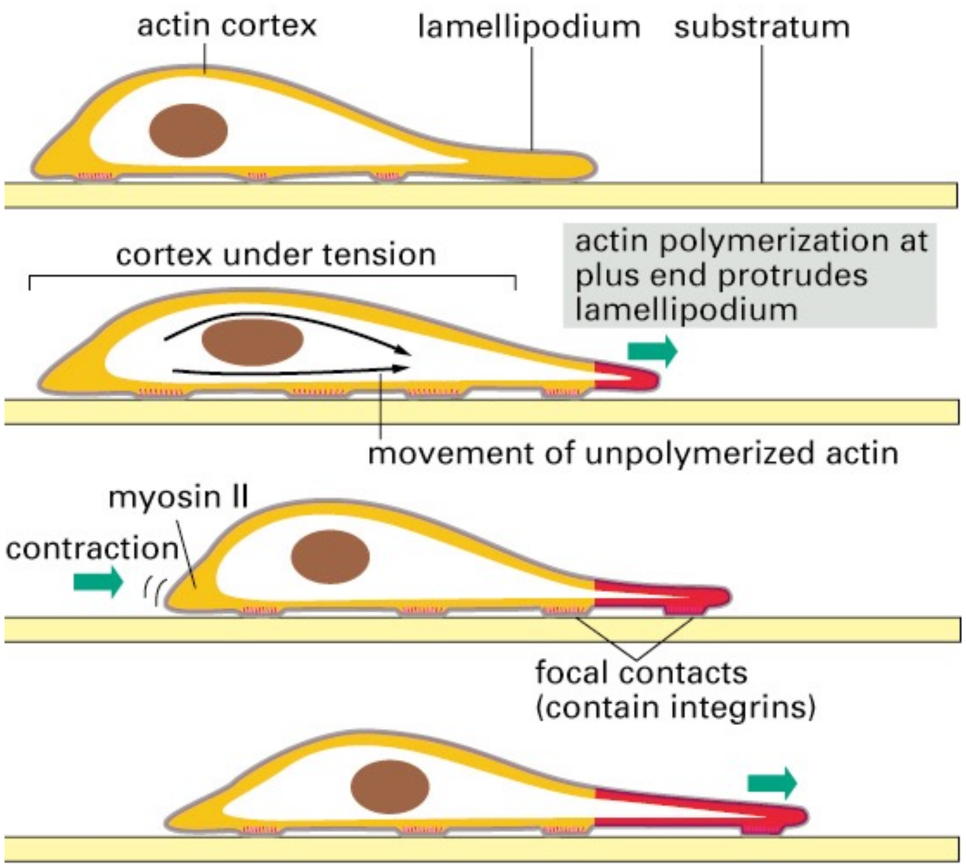
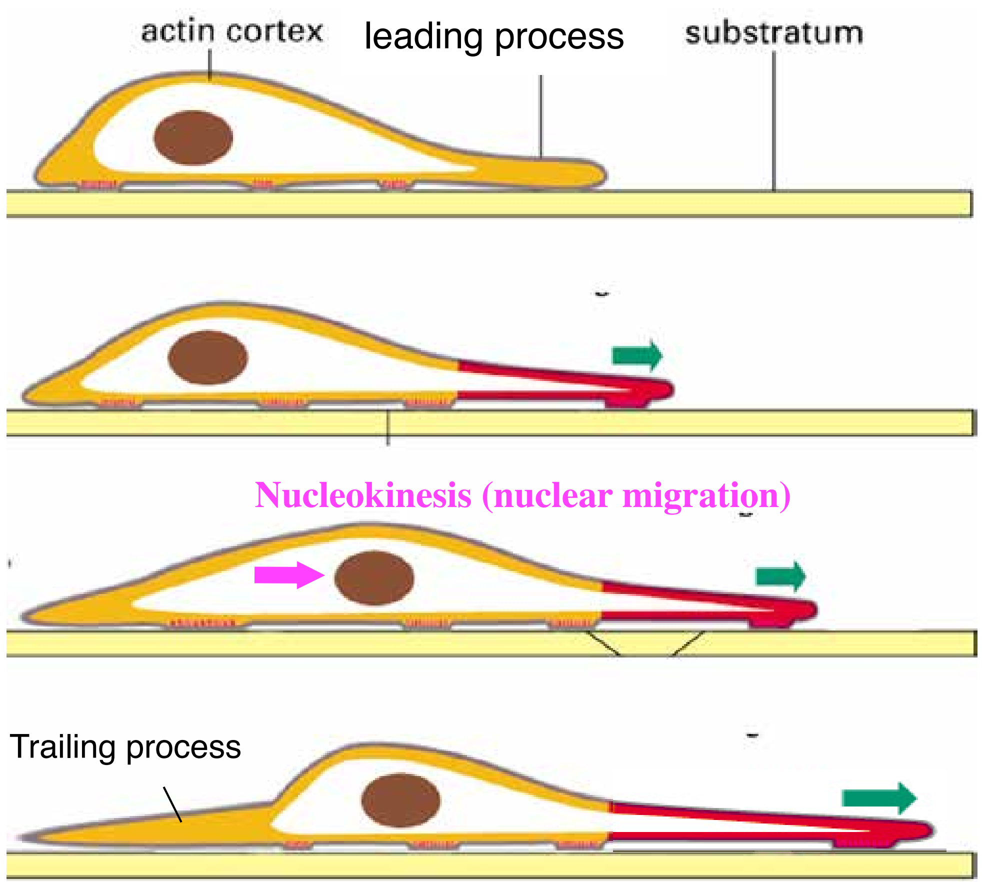


Figure 16–85. Molecular Biology of the Cell, 4th Edition.

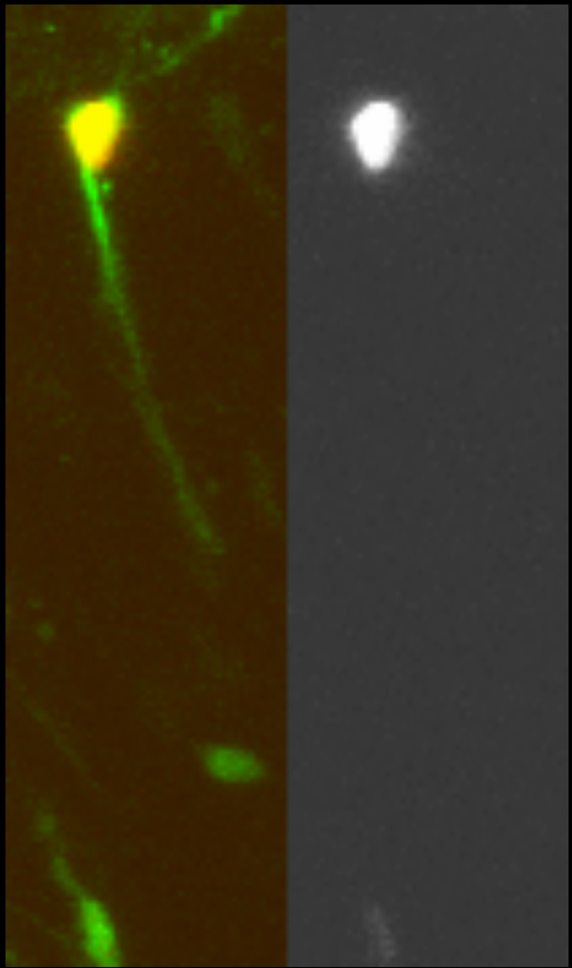
Neuronal migration



actin-dependent

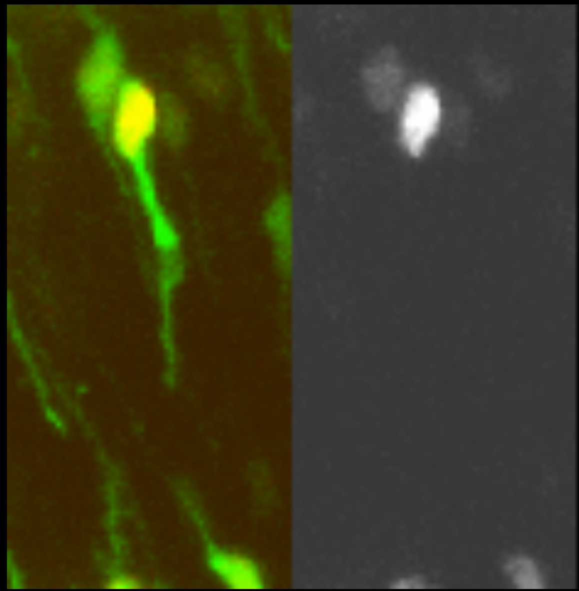
actin+microtubule-dependent

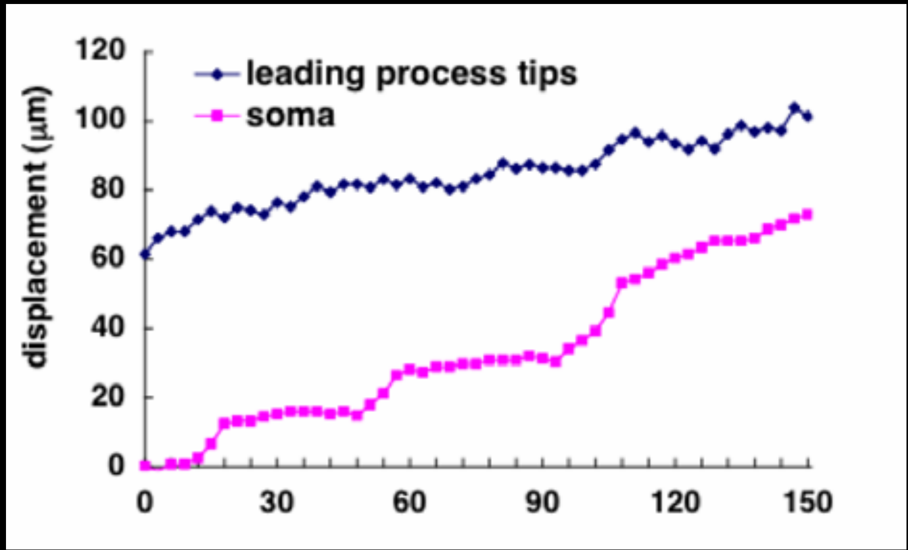
Nucleus migrates independently of leading process elongation



GFP
DsRed-Nuc

DsRed-Nuc

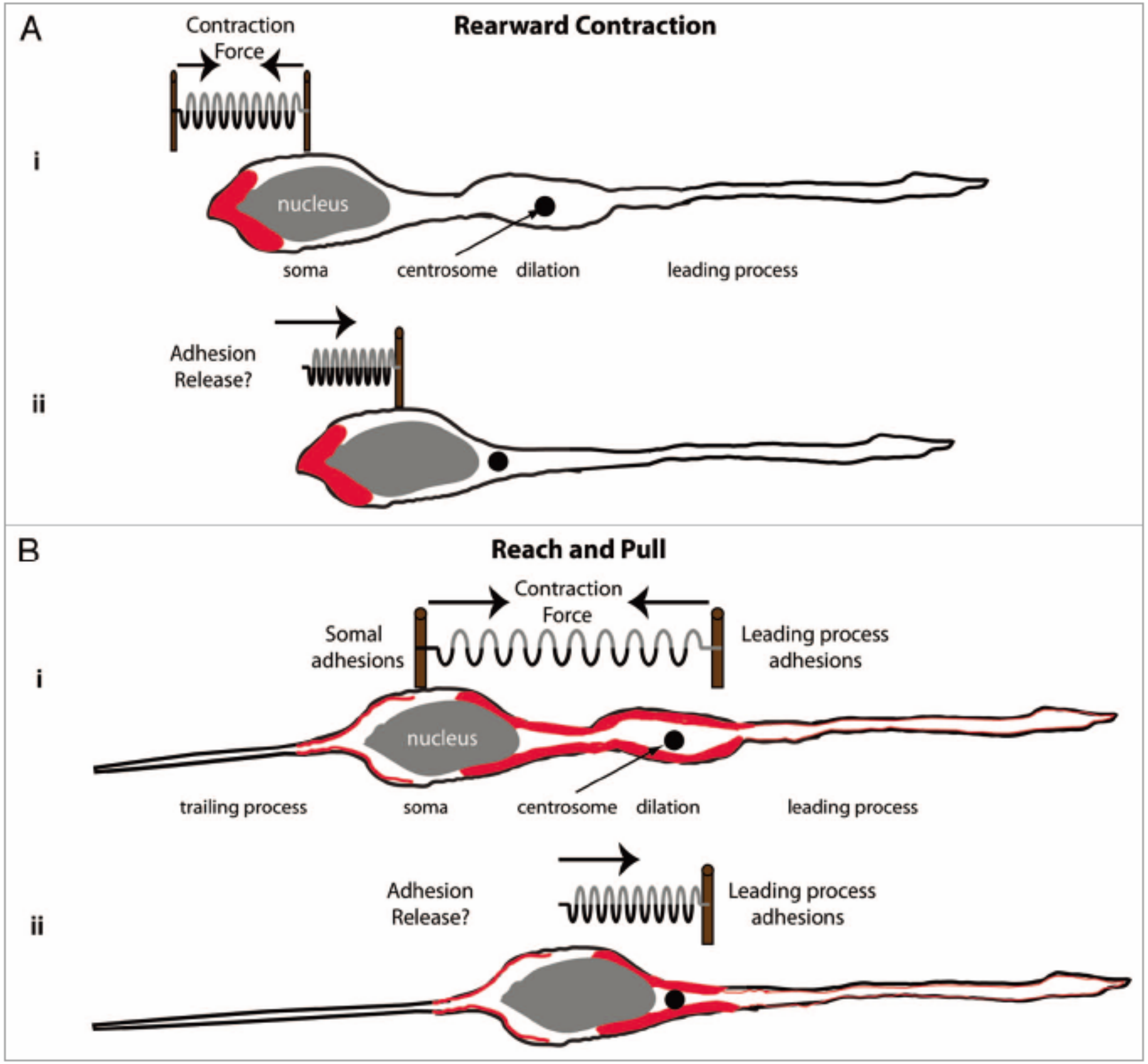




3-min intervals
8 frames/sec

Push or pull?

What do cytoskeletons do for nuclear migration?



Invasive strategies (ablation, disruption) may indirectly affect cell behaviors

Trivedi and Solecki 2011

High-speed, high-resolution live-cell imaging by the CSU confocal microscopy

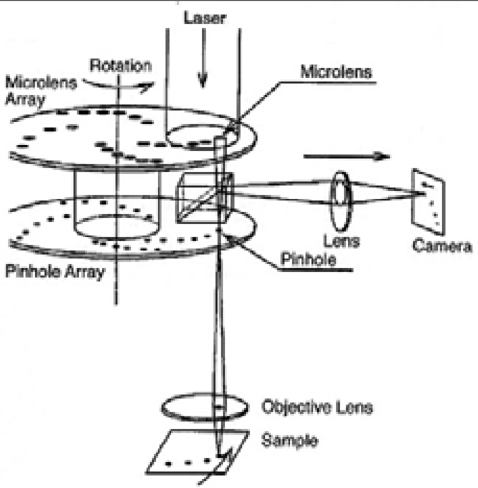


FIGURE 2-71. Yokogawa high-speed confocal system. Microlenses on a second Nipkow disk increase disk transmission to 40–60% instead of a fraction of a percent as in conventional, single-Nipkow-disk systems. The microlens and pinhole arrays are patterned to give a homogeneous field with no sign of scan lines. (From Ichihara *et al.*, 1996.)

"Video Microscopy", Inoue and Spring, 1997

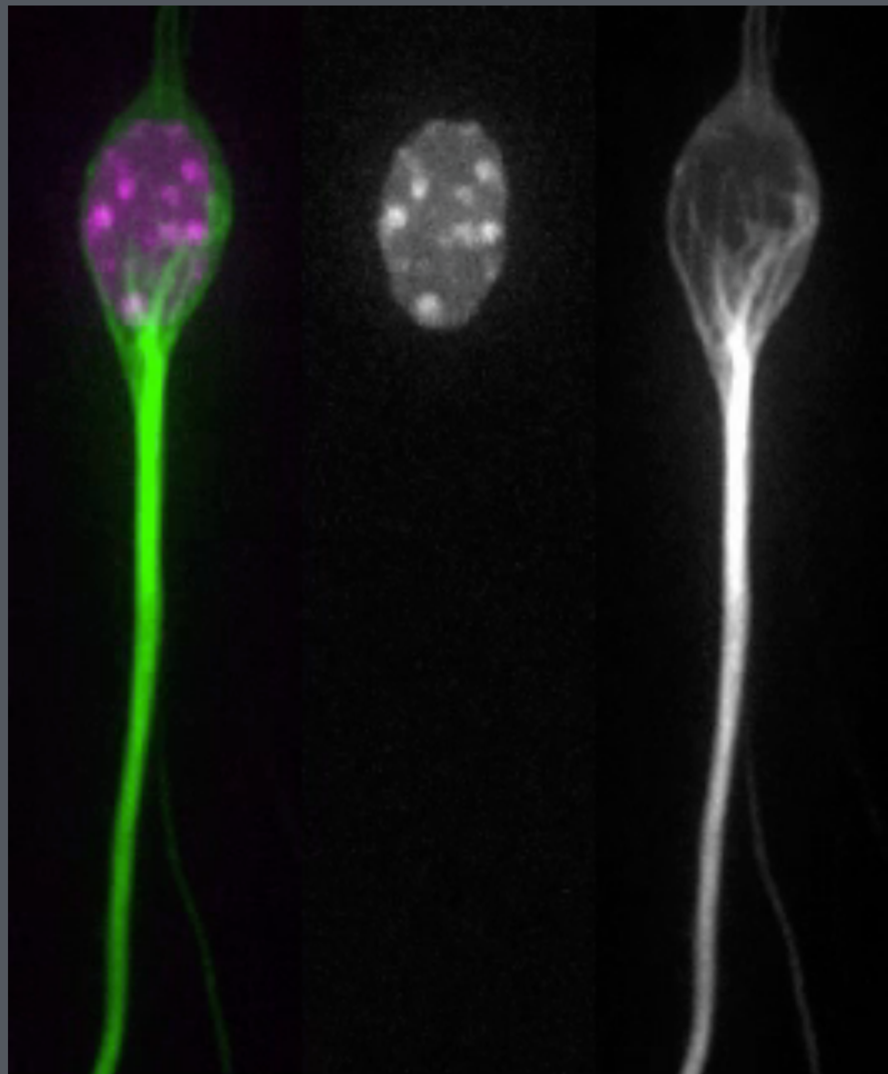
Nipkow disc confocal microscopy

100x objective

Z: 10~12 planes
3 channels (GFP, RFP, DIC)
⇒total ~36 planes (less than 8 sec)

time-lapse imaging:
every 15 sec for 1~2 hours

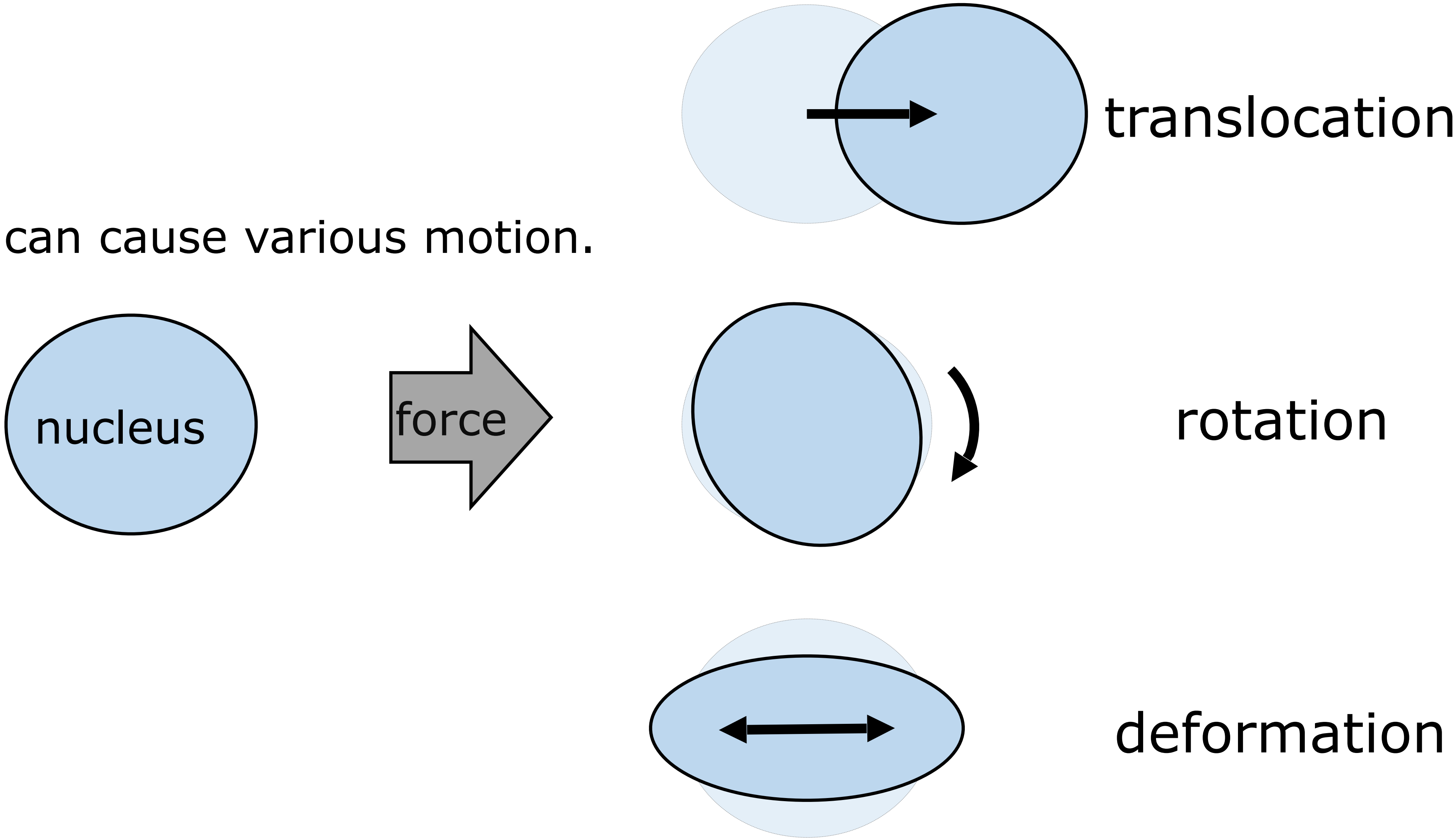
cell nucleus **microtubules**



15-sec interval
20 frames/sec

Estimate the force applied to the nucleus from its motion

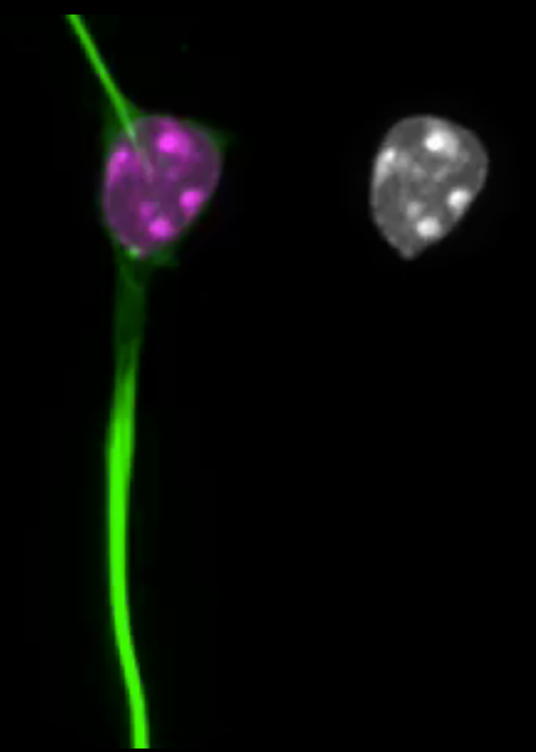
Force can cause various motion.



Nucleus rotates in *migrating* neurons

young neuron (2 days in culture)

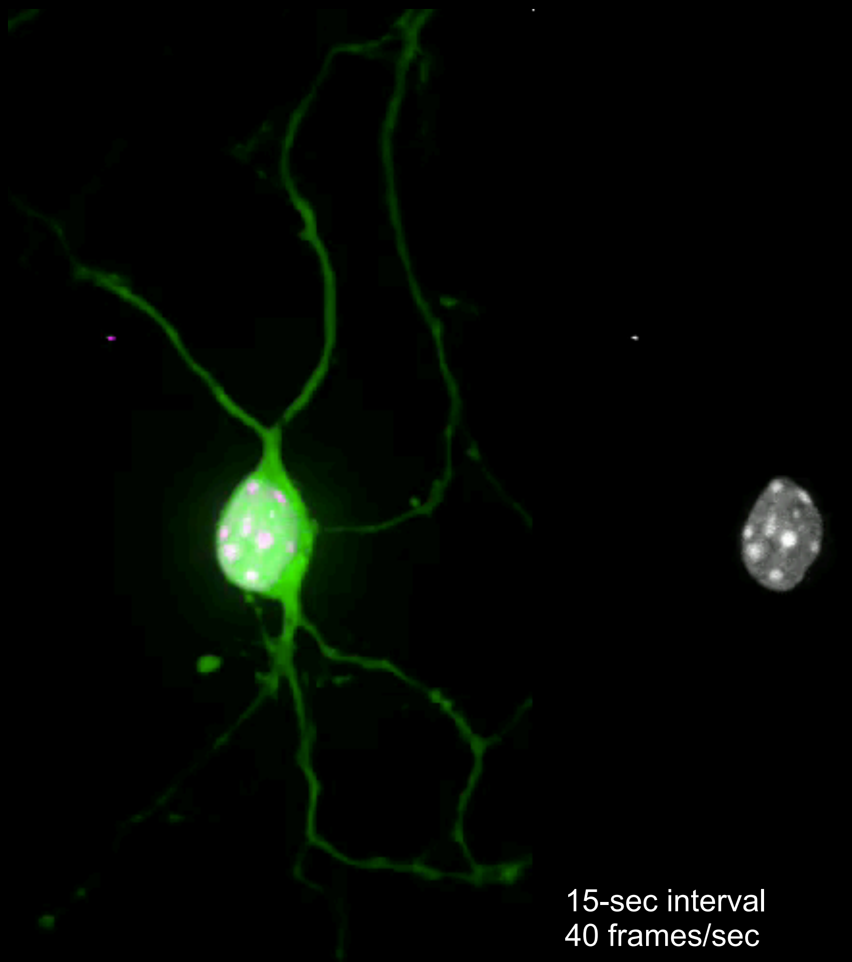
Merge **Nucleus**



15-sec interval
20 frames/sec

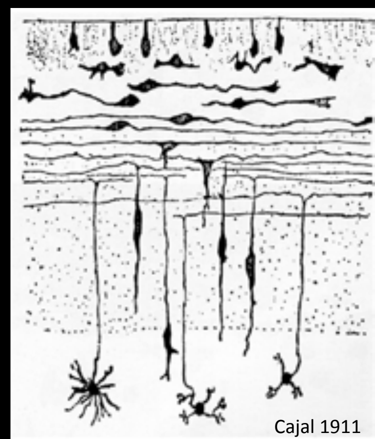
mature neuron (6 days in culture)

Merge **Nucleus**



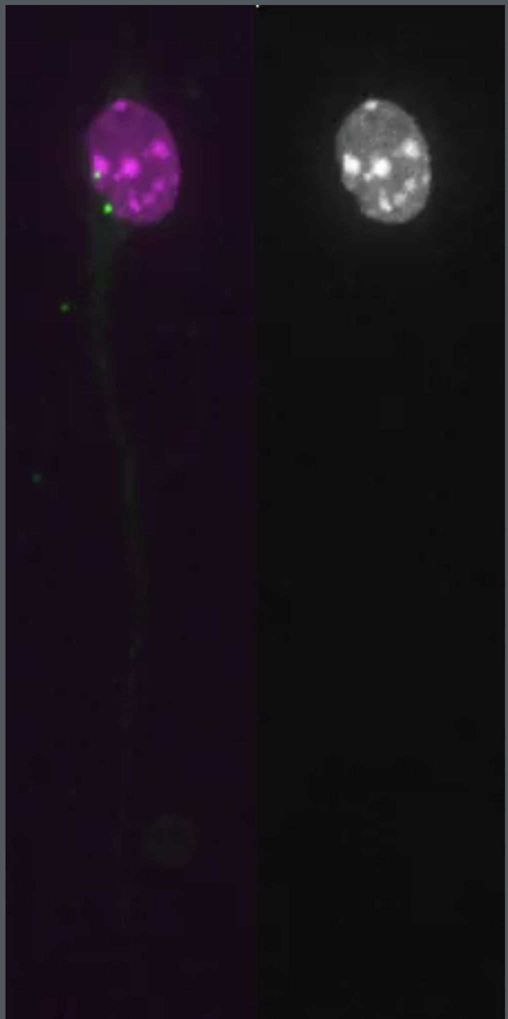
15-sec interval
40 frames/sec

Microtubules: EGFP-DCX
Nucleus: DsRed2-Nuc

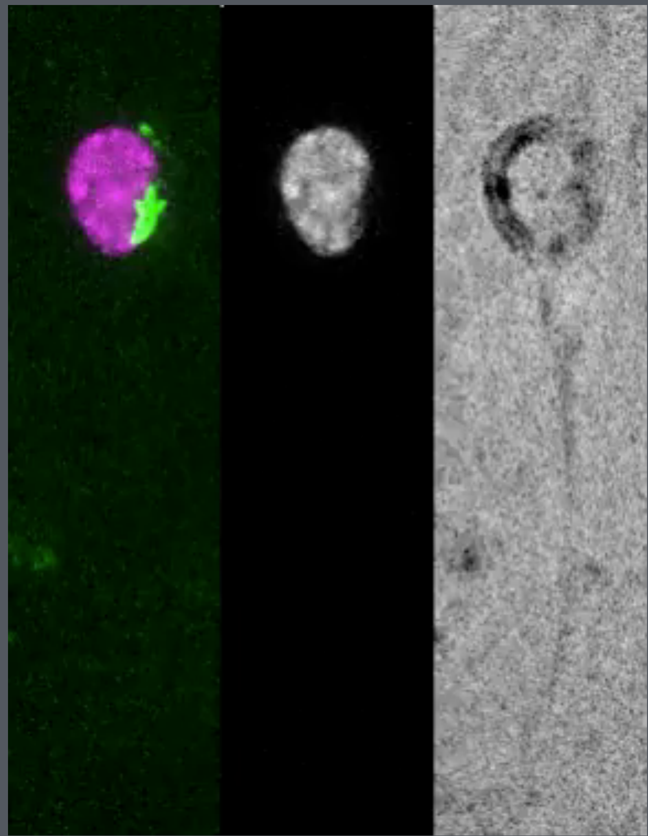


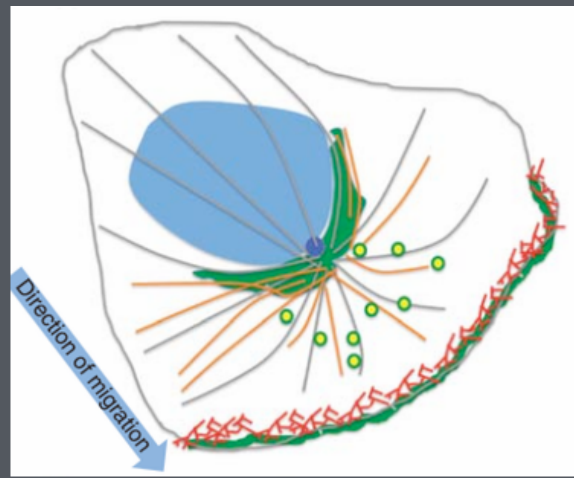
Centrosome and Golgi do not rotate with the nucleus

Cetn2-GFP
HP1β-mCherry



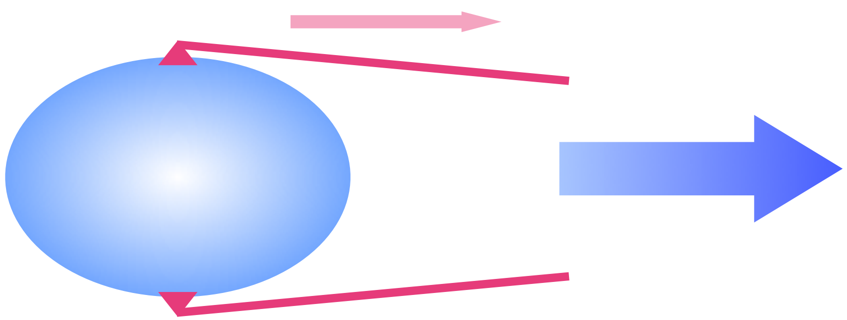
Golgi1-GFP
HP1β-mCherry
DIC



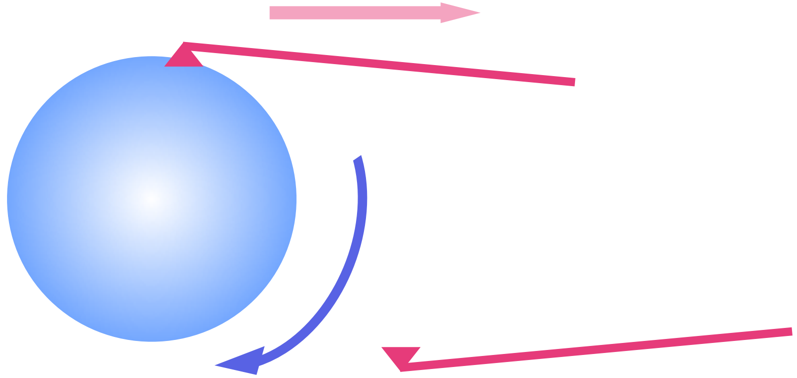


Hypothetical Mechanism of the Nuclear Rotation

Uniform (bilateral) force drives migration

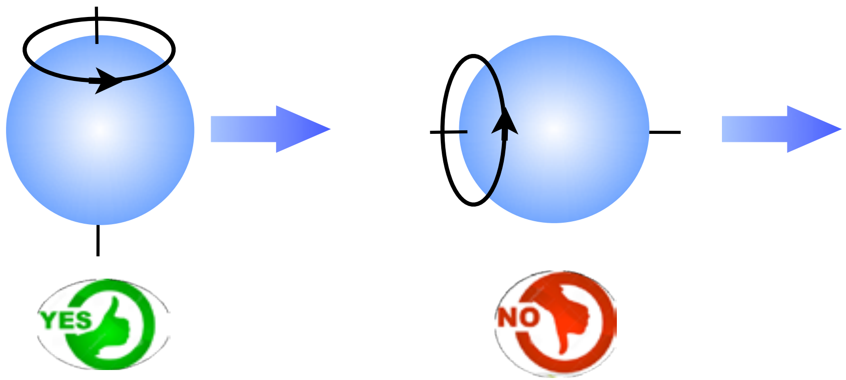


Unbalanced (unilateral) force drives rotation



If so...

1. Rotation axis should be orthogonal to the migration trajectory



2. Rotation should stop when the nucleus is disconnected with cytoskeletons

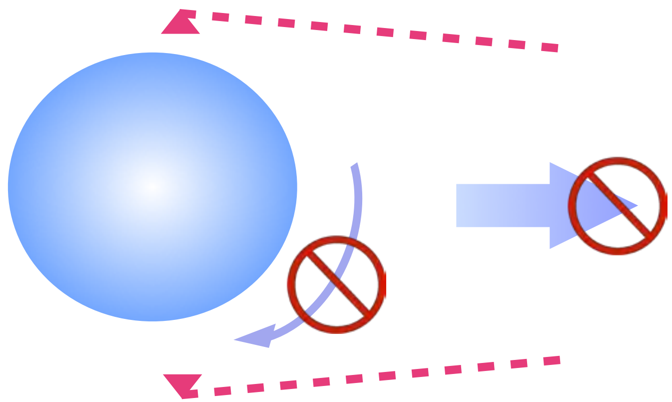
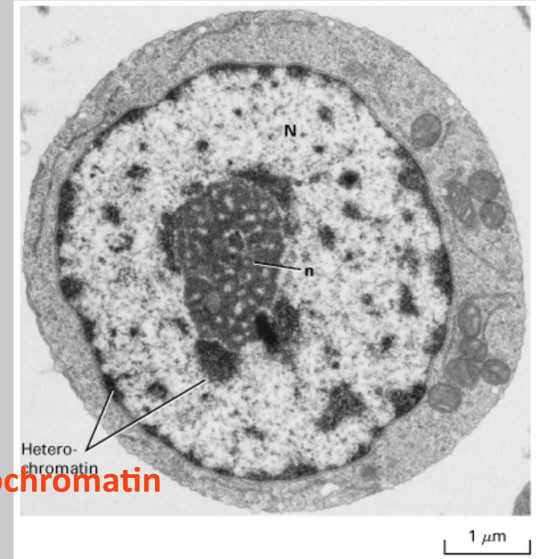
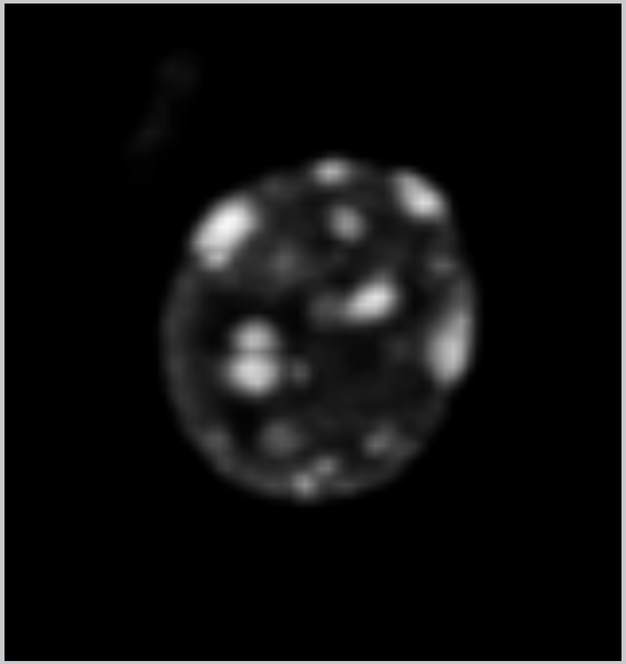
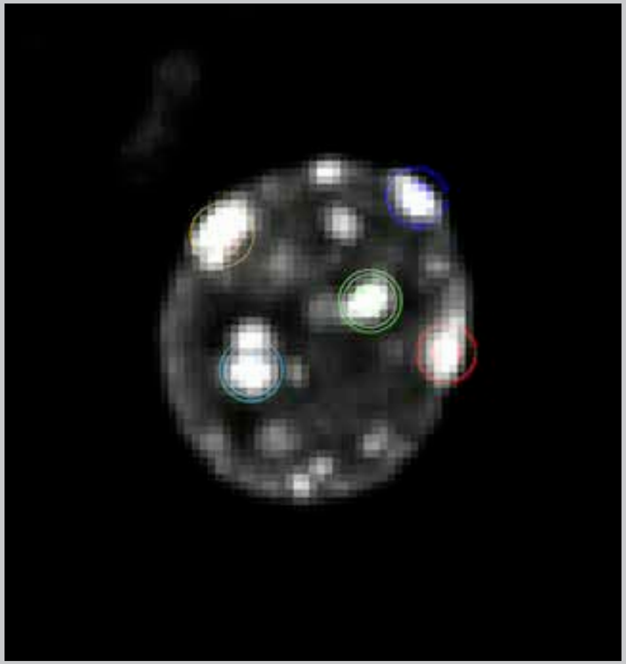


Image analysis of nuclear rotation

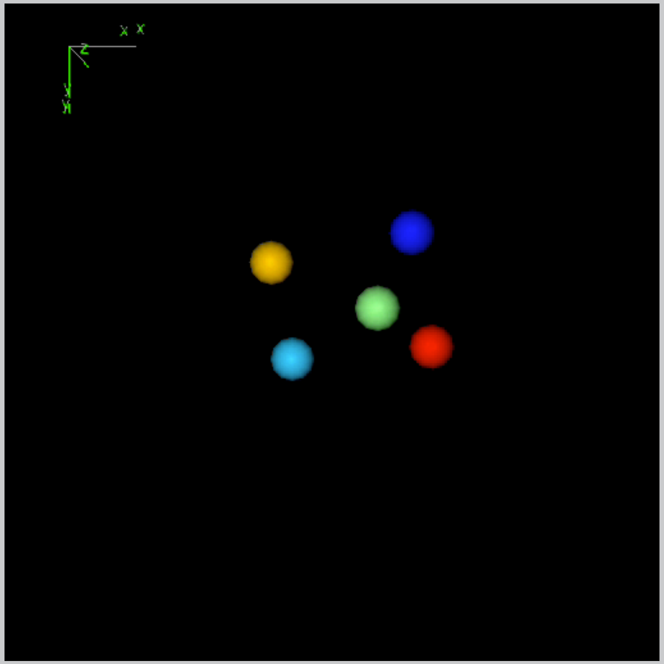




HP 1β-mCherry
MIP images



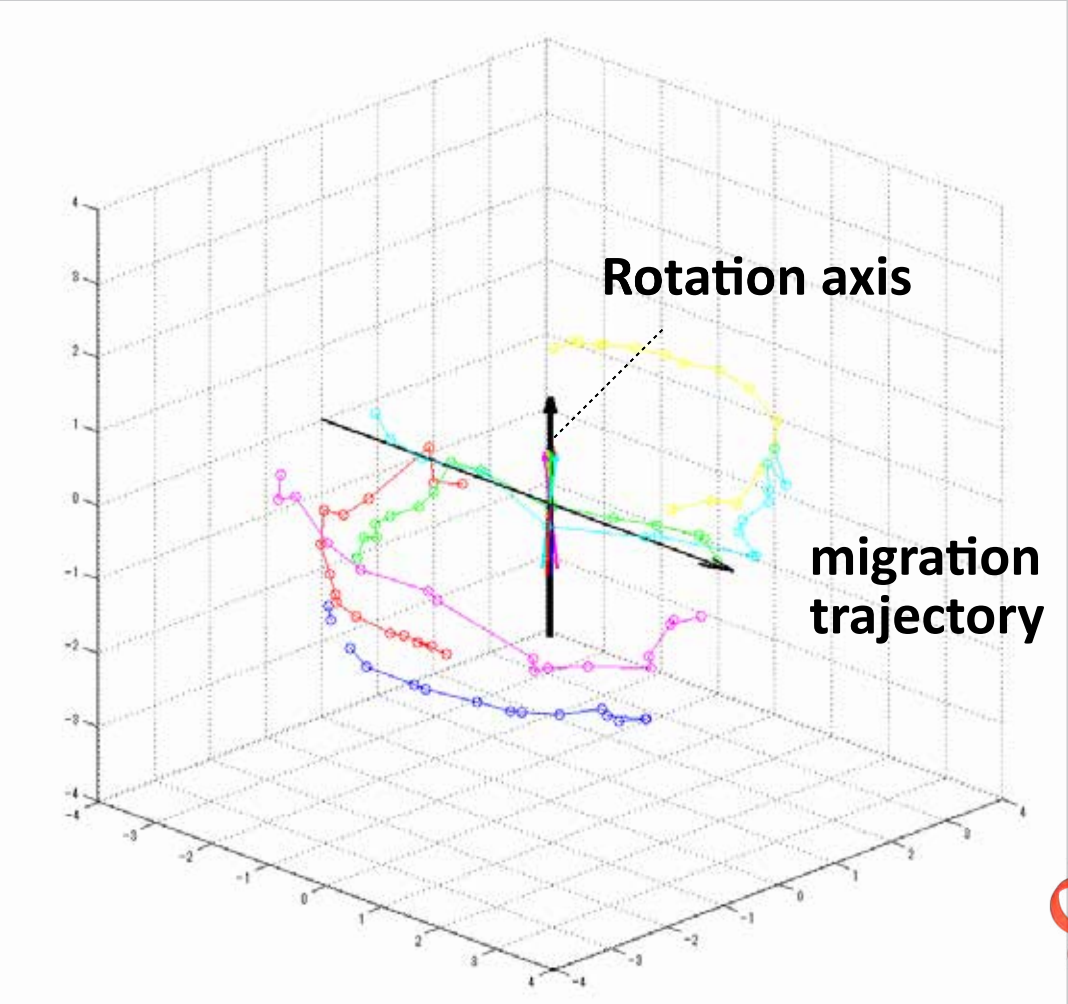
Spot tracking

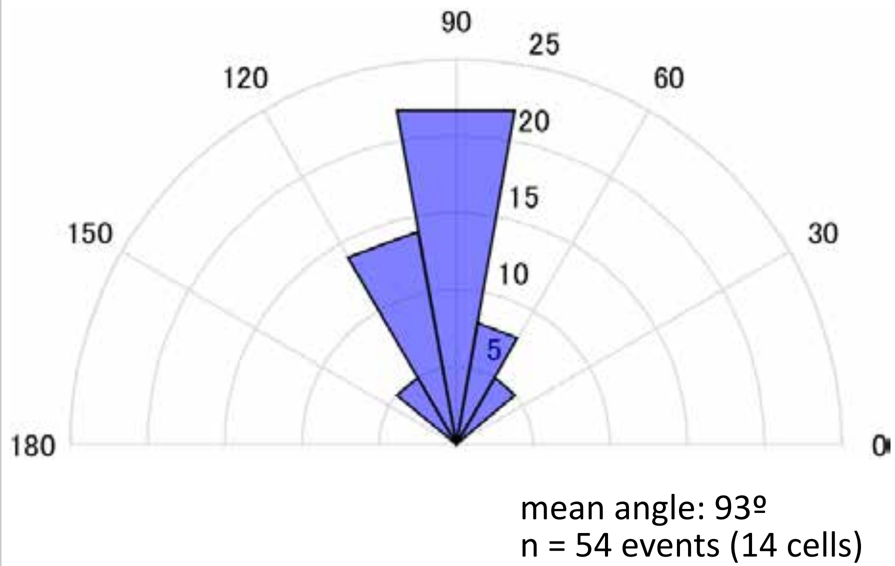


3D movie

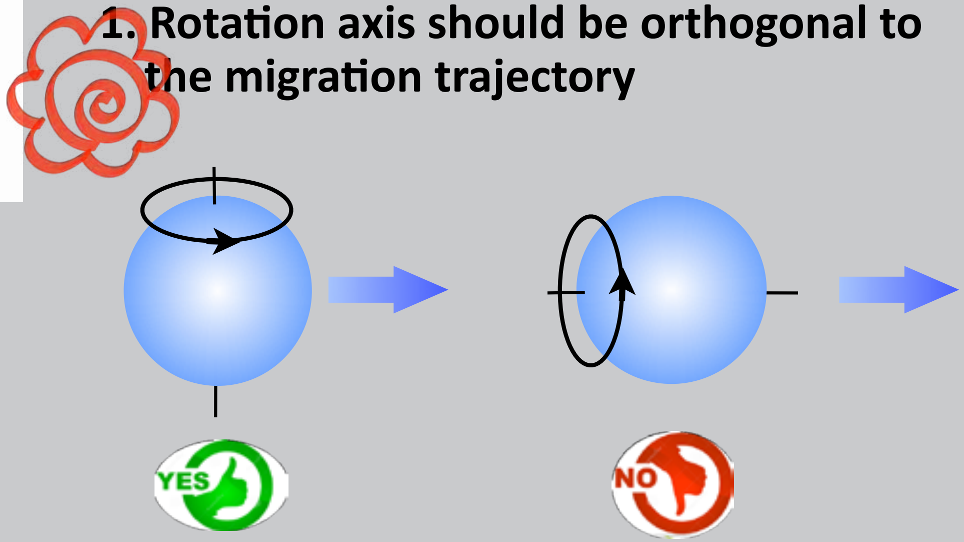
1 hr (interval: 15 sec)

Rotation axis is relevant to migration direction

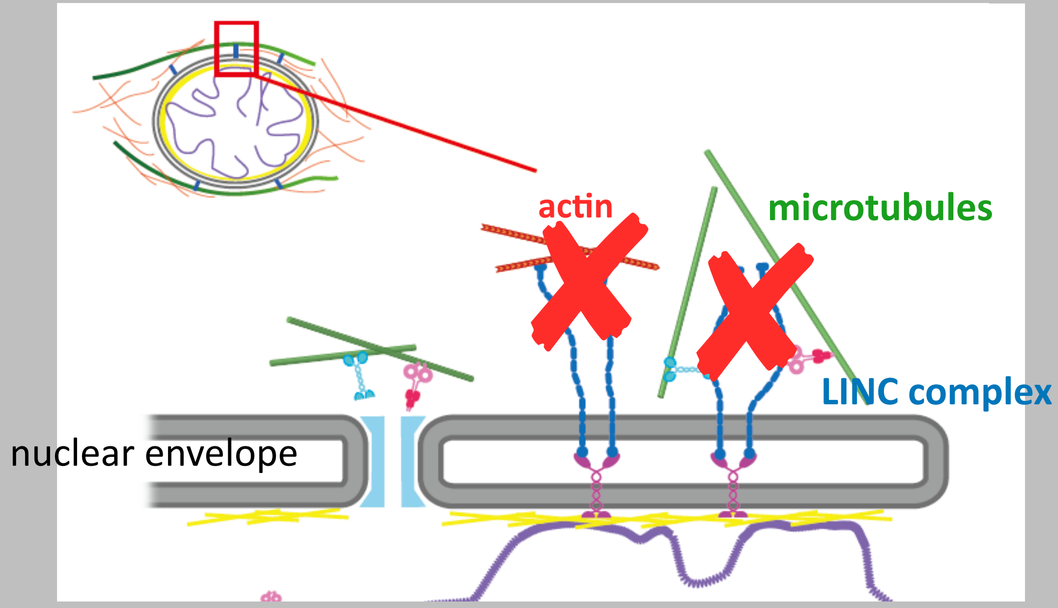


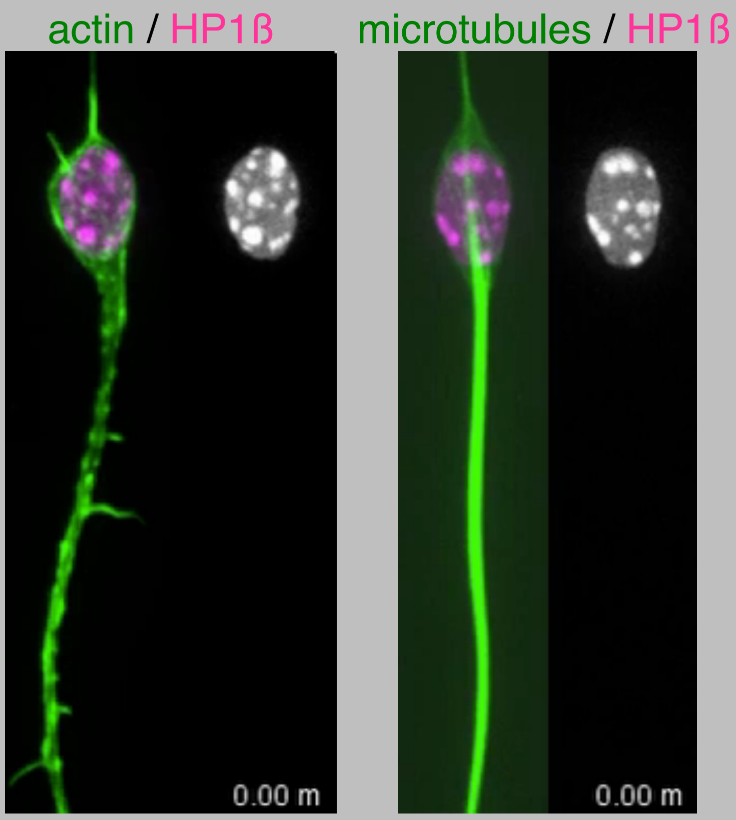


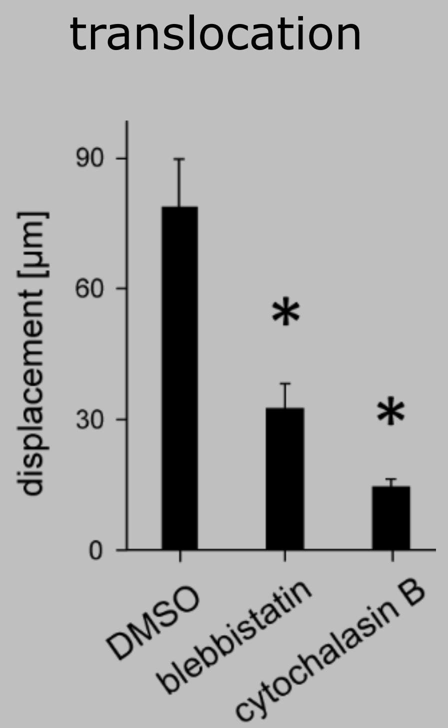
1. Rotation axis should be orthogonal to the migration trajectory

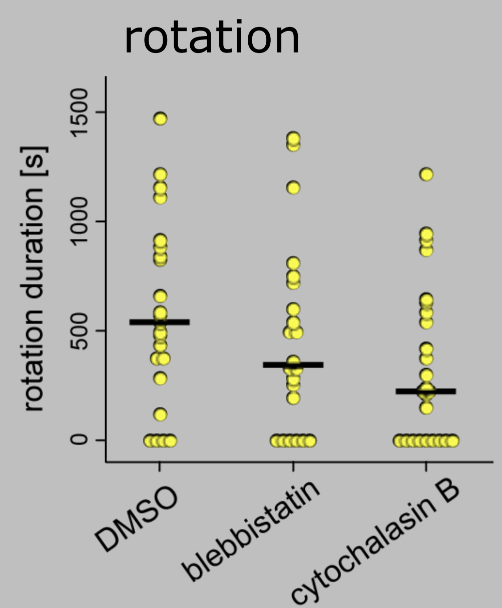


Microtubules, but not actin, are involved in nuclear rotation



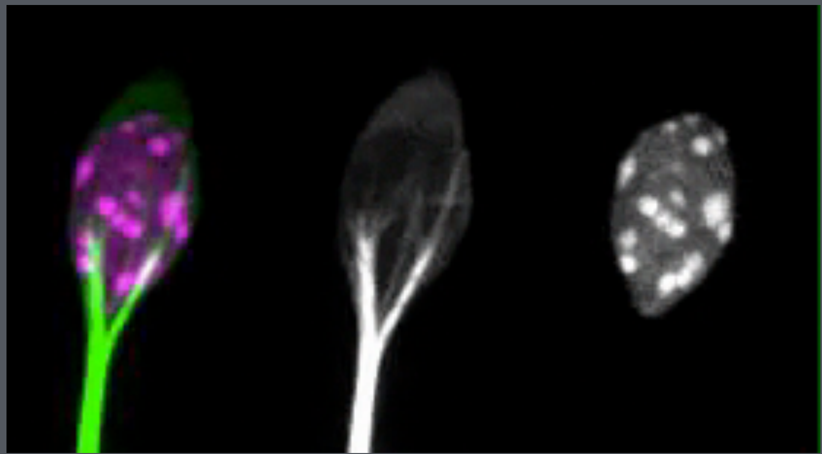




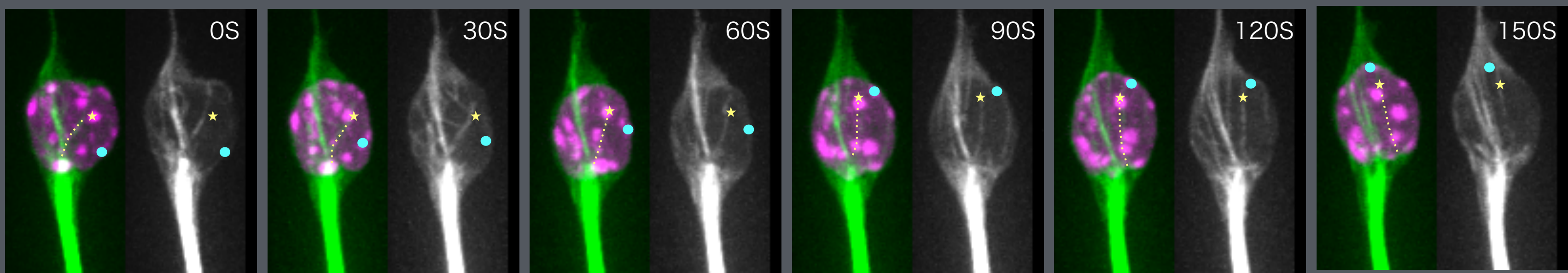


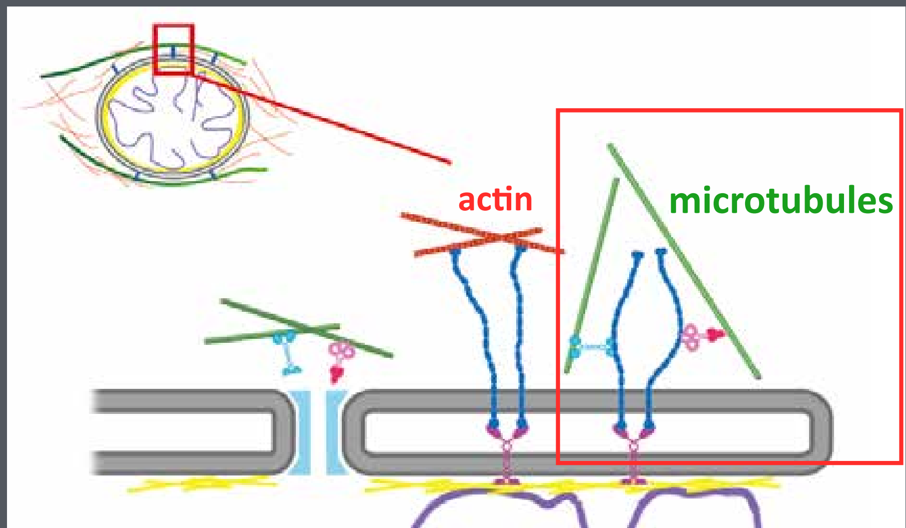
Synchronous movement of microtubule network and nucleus

HP 1β-mCherry
microtubules



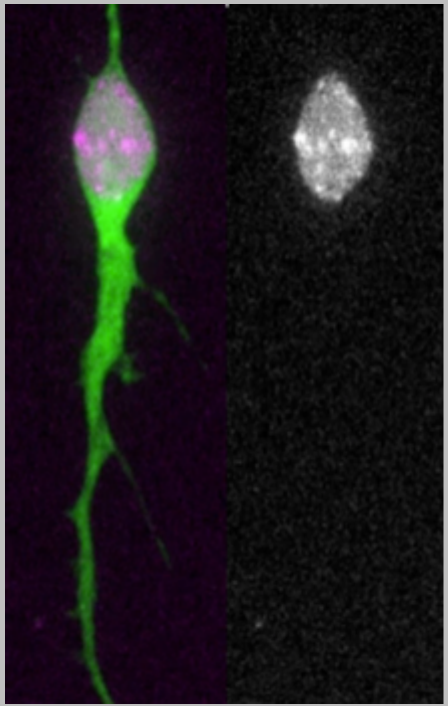
15-sec interval
7 frames/sec





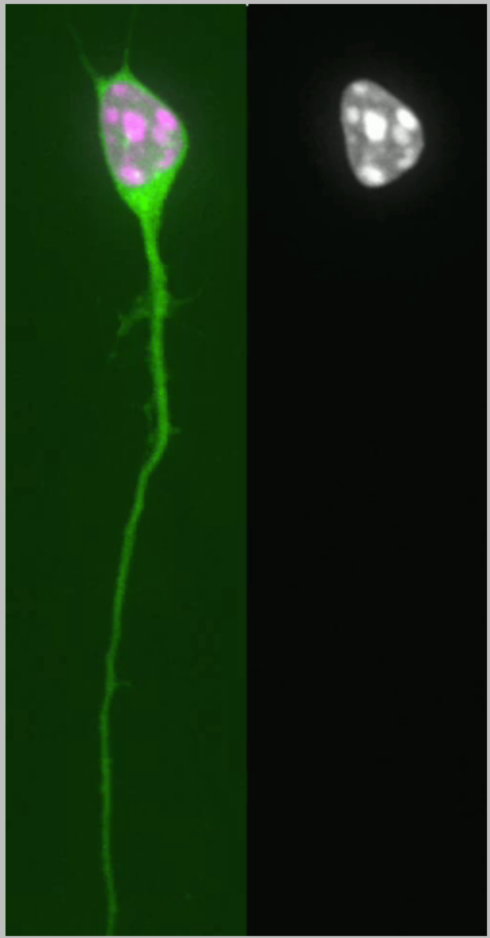
Dynein and kinesin are involved in nuclear rotation

Lis1N
(dynein inhibition)

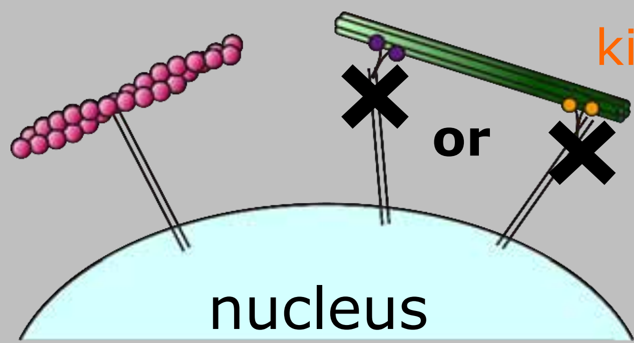


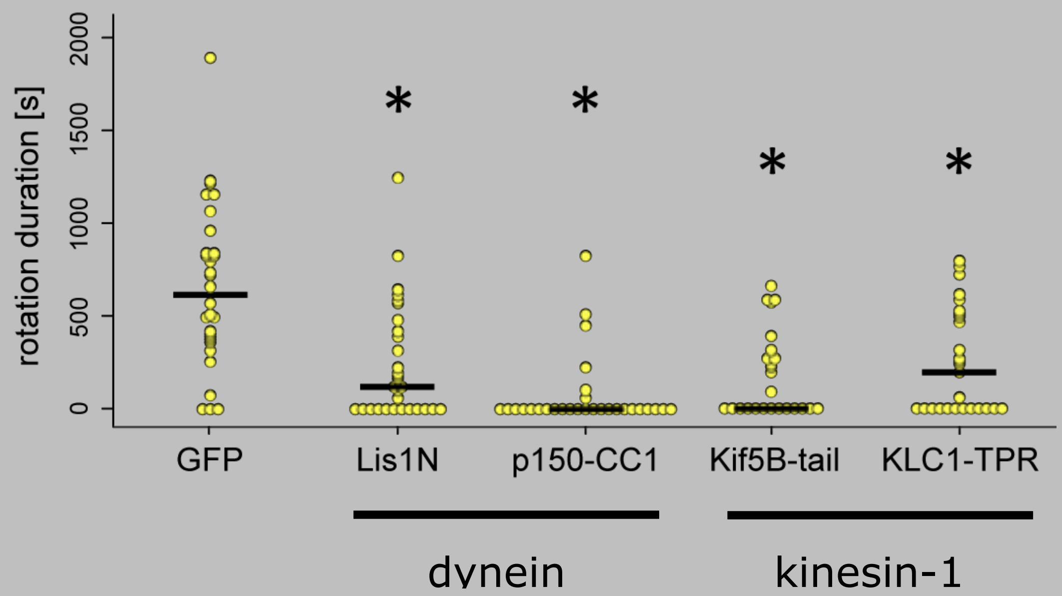
HP 1β-mCherry
Lis1N

KLC1-TRP
(kinesin inhibition)



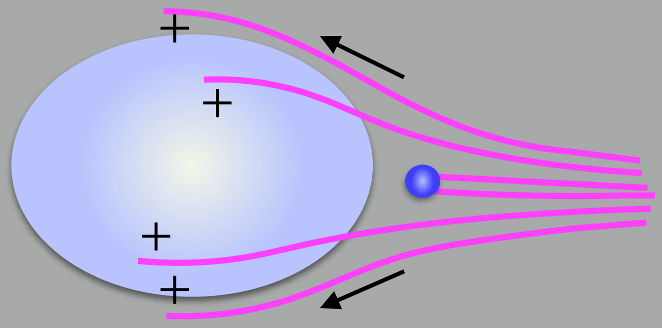
HP 1β-mCherry
KLC1-TRP

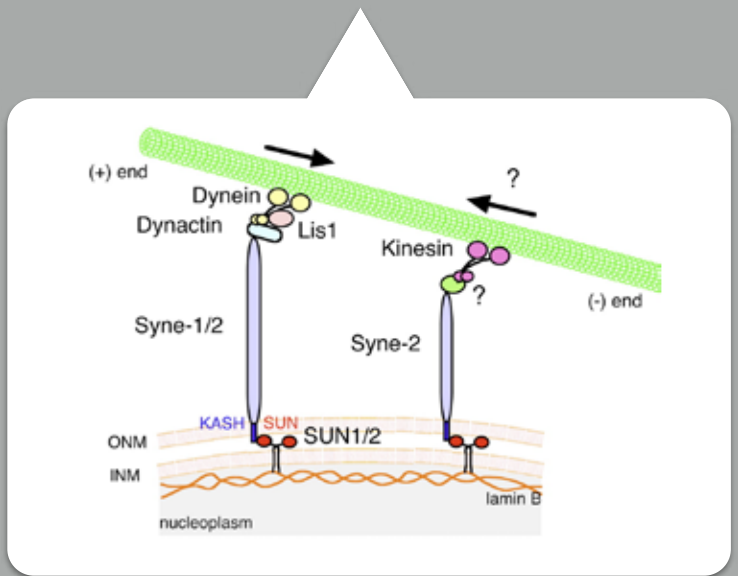




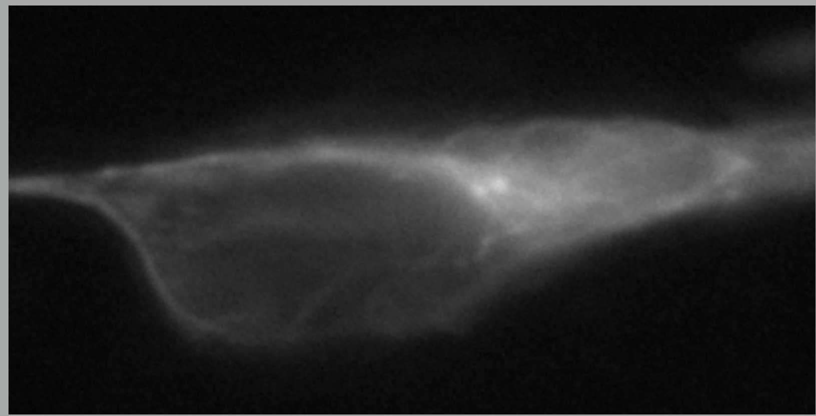
Perinuclear microtubules are bidirectional in migrating neurons

previous view:
MTs are uniformly oriented
with (+)-toward the nucleus



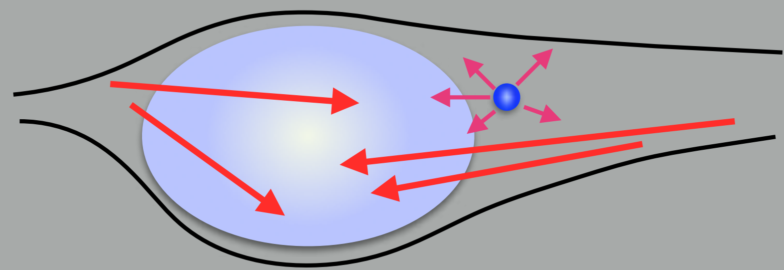


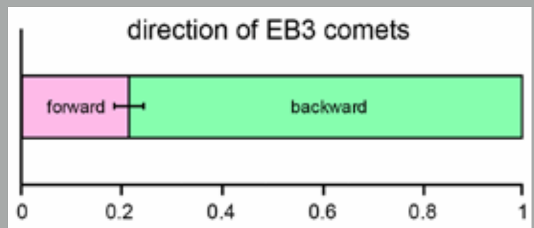
How can kinesin function?



EB3-GFP
centrin2-GFP

5-sec intervals
6 frames/sec

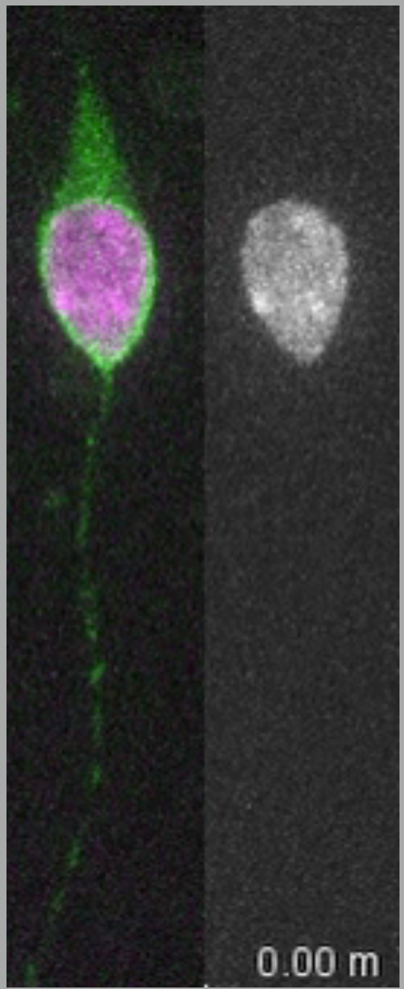




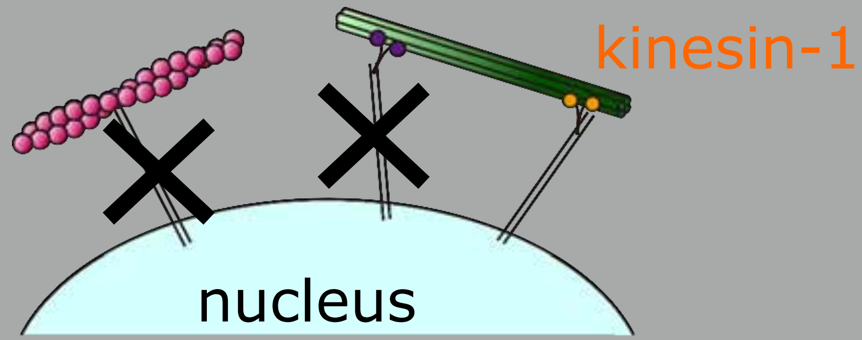
Both kinesin and dynein can contribute to nuclear transport

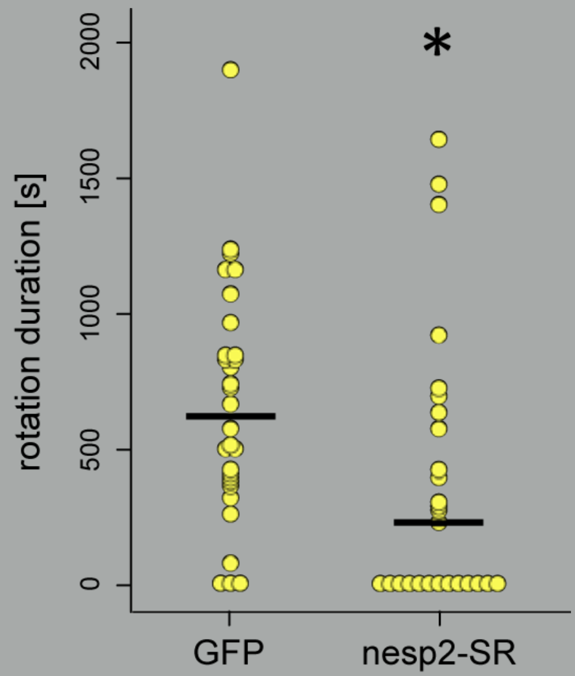
Kinesin-1 is not sufficient to drive nuclear rotation

nesp2-SR / Nuc



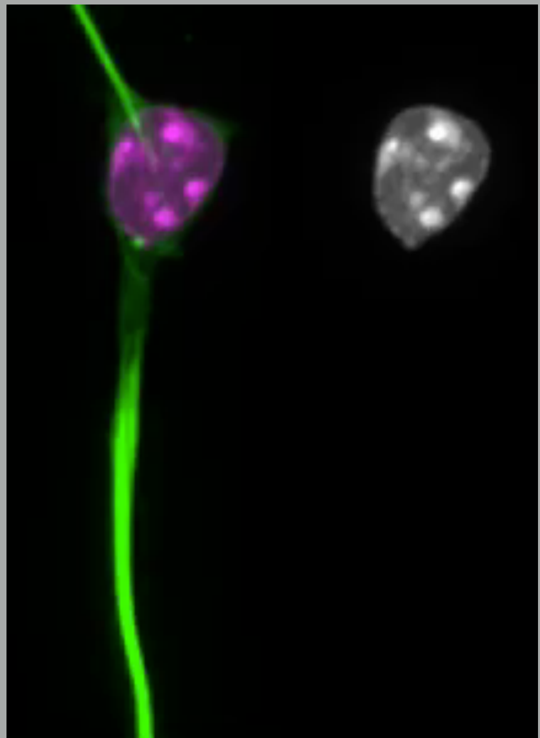
The nucleus retains sharp deformation in the front



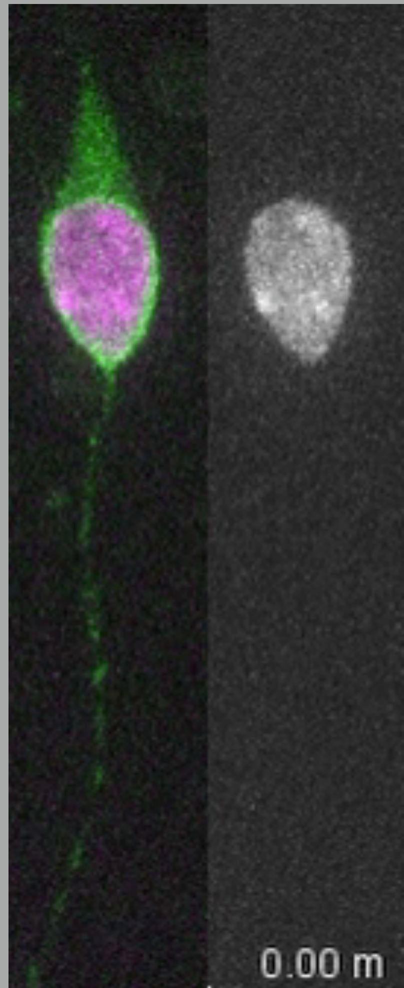


Kinesin/MTs generate point force on the nuclear surface

GFP / Nuc

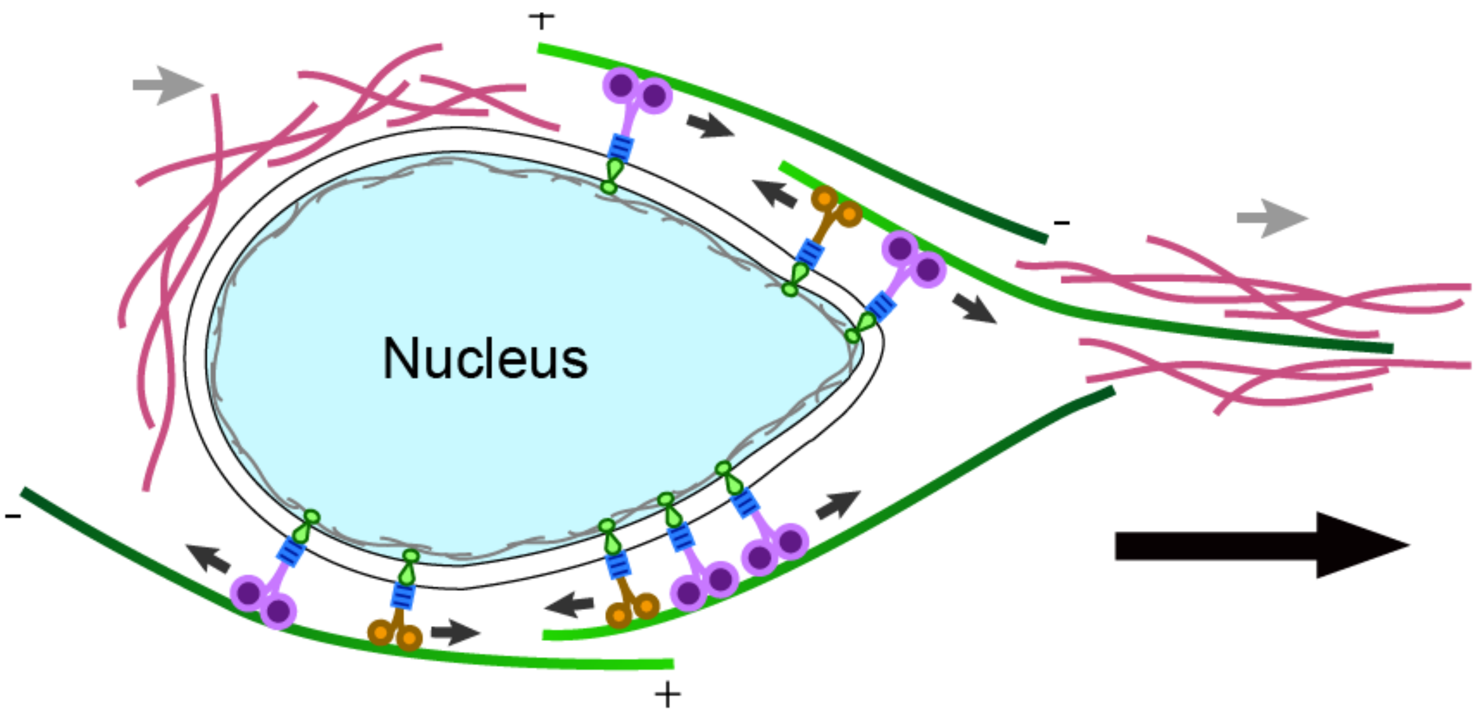


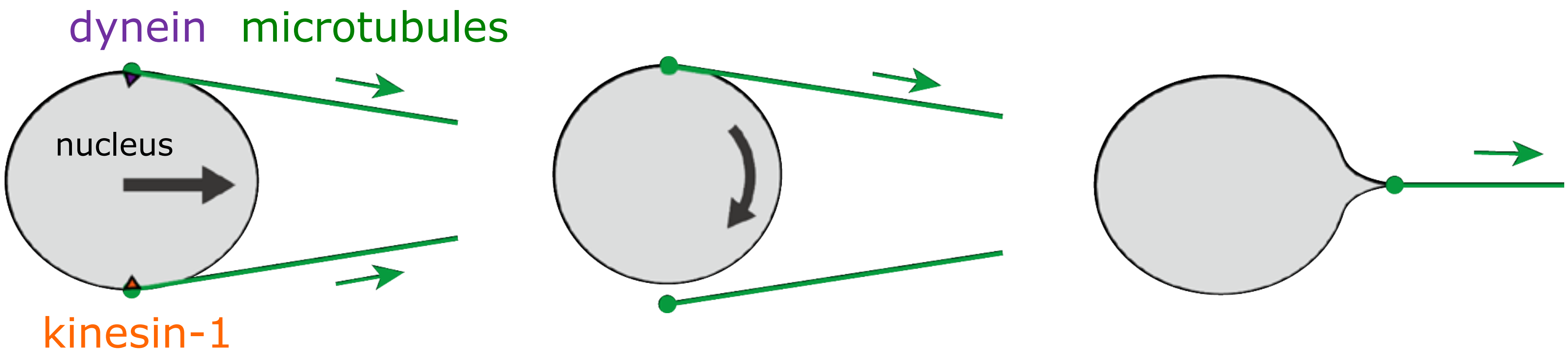
nesp2-SR / Nuc



Dynamic interaction of MT motors is critical for driving nuclear rotation

Summary and conclusion





Wu et al., Development 2018

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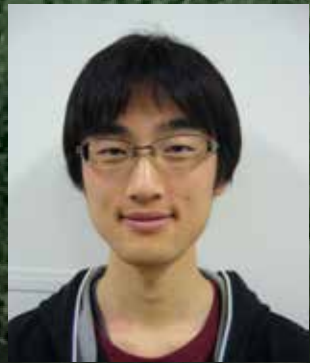
You Kure Wu


Hiroki Umishima


Naotaka Nakazawa


Chuying Zhou


William Theoputra

Interplay of developmental clock and extracellular environment in brain formation

wpi World Premier International Research Center Initiative

BIO Assembler

JSPS