Questions of shape, growth and patterning in organogenesis

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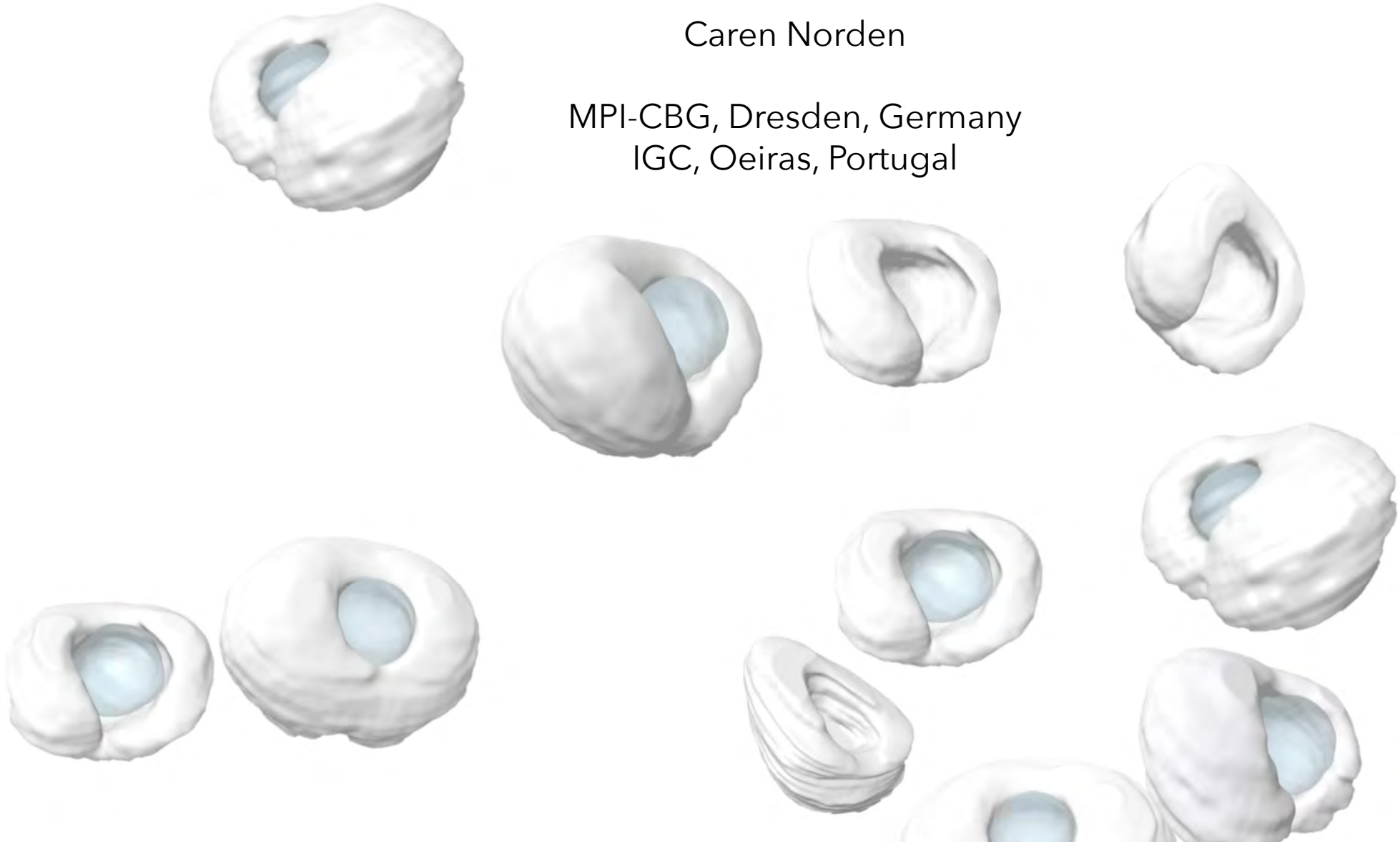


NORDEN LAB

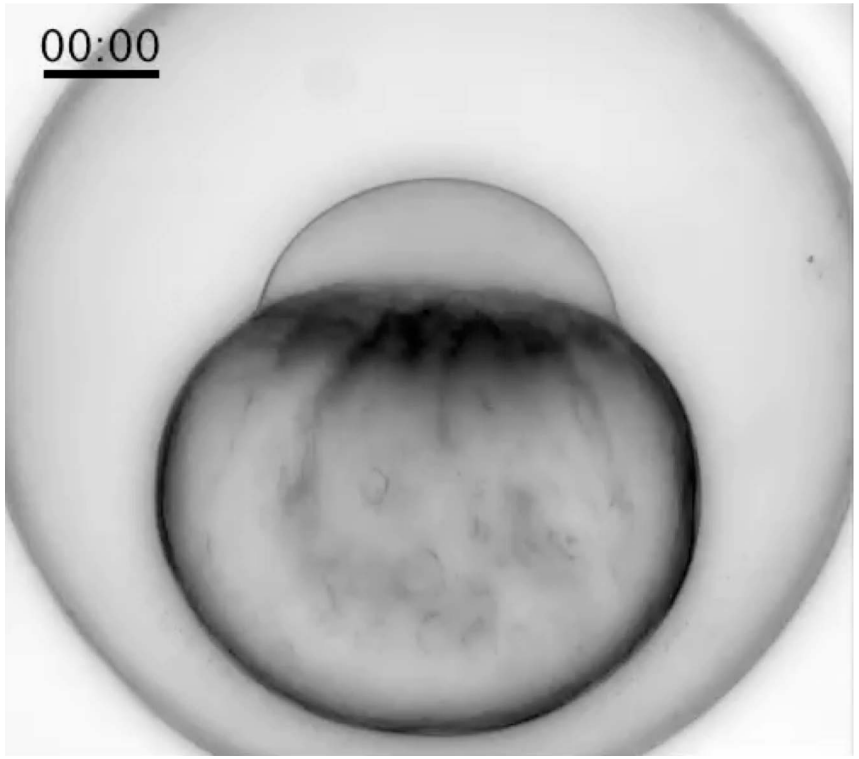
How do cells form tissues, organs and organisms?

?

How do single pieces come together?

Understanding development means understanding multicellularity

Kaufmann et al. 2012



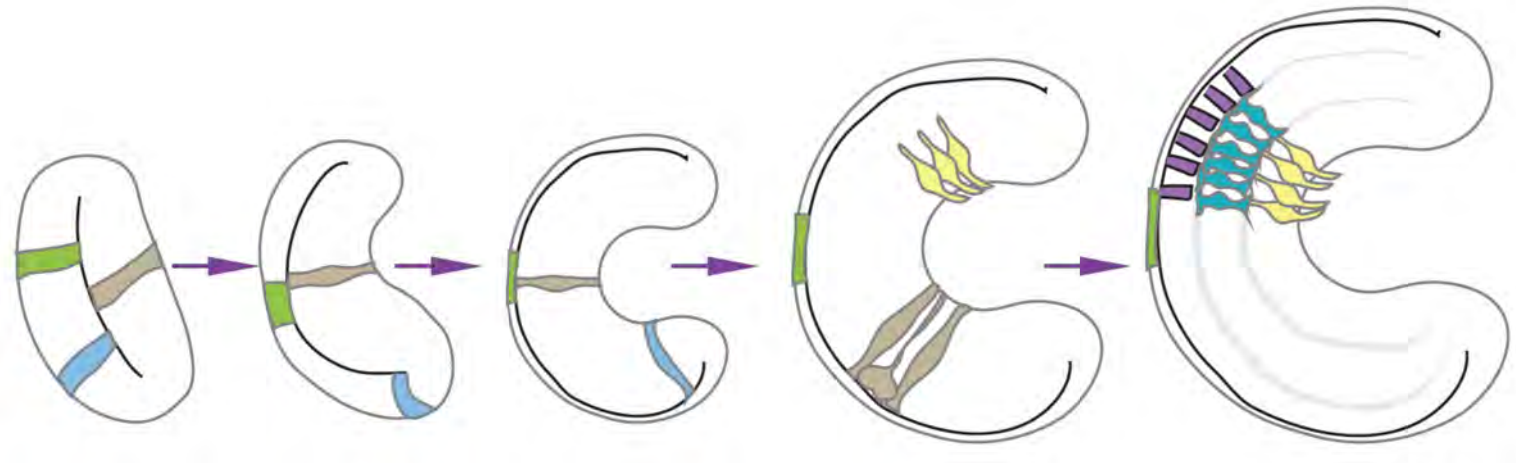
During organogenesis

- the right cells
- need to proliferate or differentiate
- at the right time
- and move to the
- right place

How is this orchestrated?

We need to understand processes from ‘cells to tissue’ and their mechanics

Retinal organogenesis

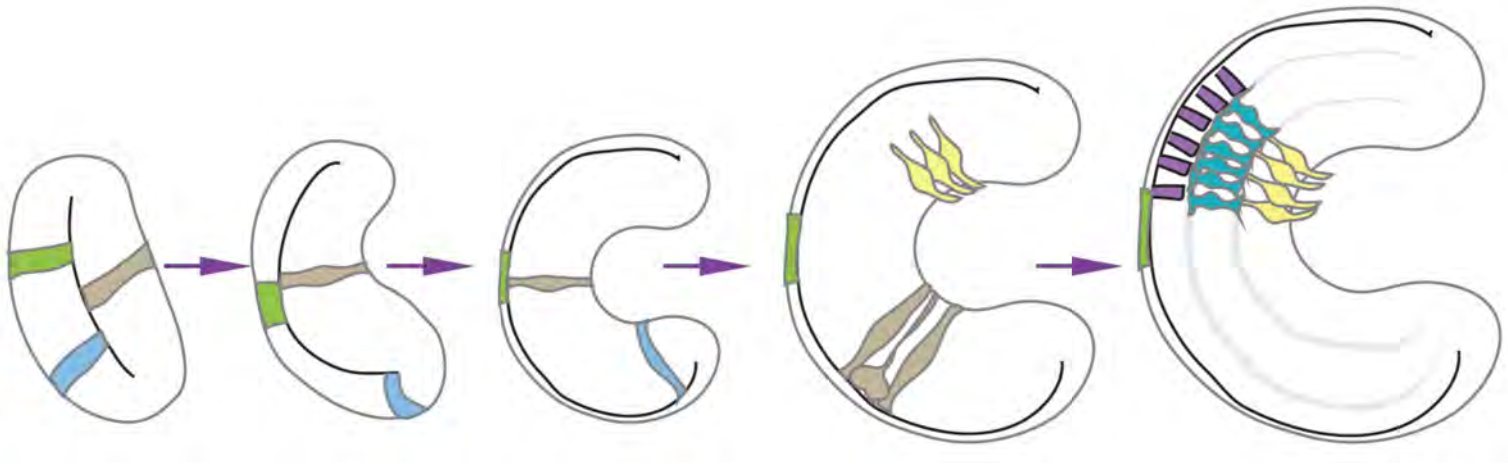


- How does shape arise from epithelial rearrangements?

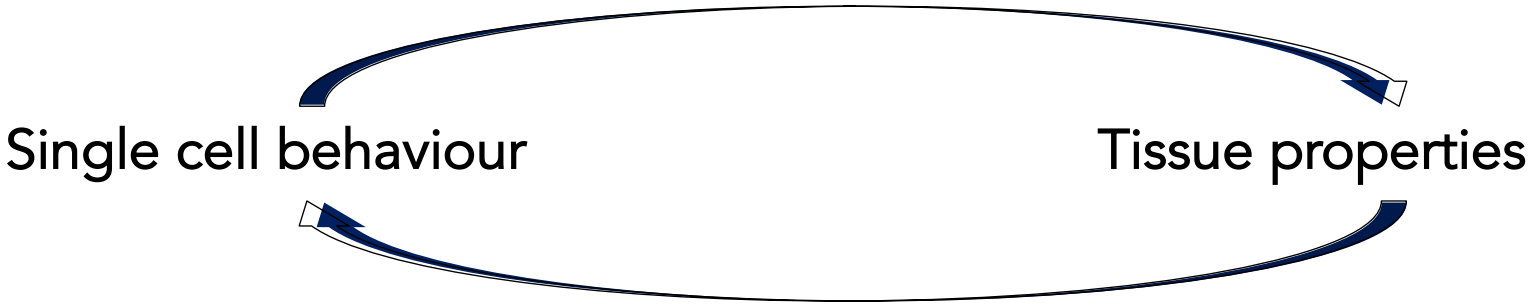
- How is proliferation and differentiation regulated in neuroepithelia?

- How do neuronal migration and lamination pattern the retina?

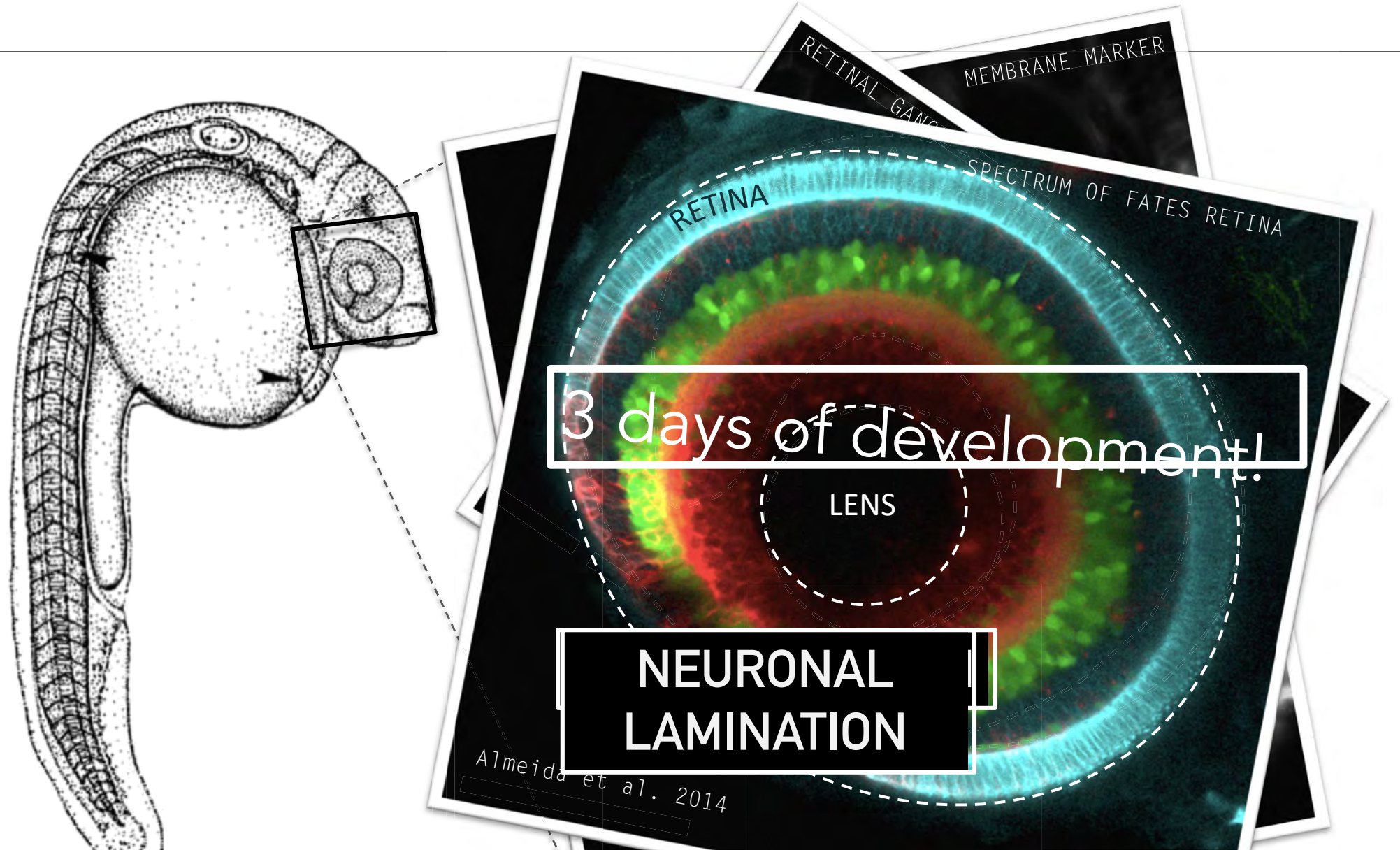
Retinal organogenesis



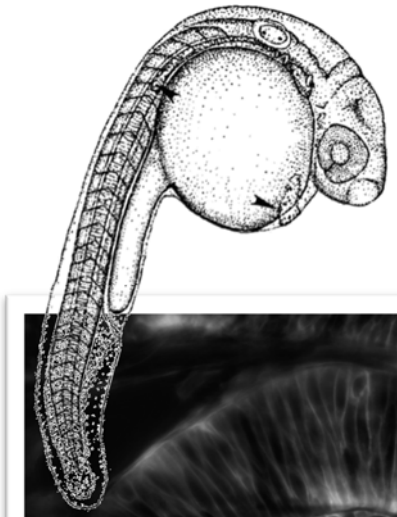
Shape Growth Patterning

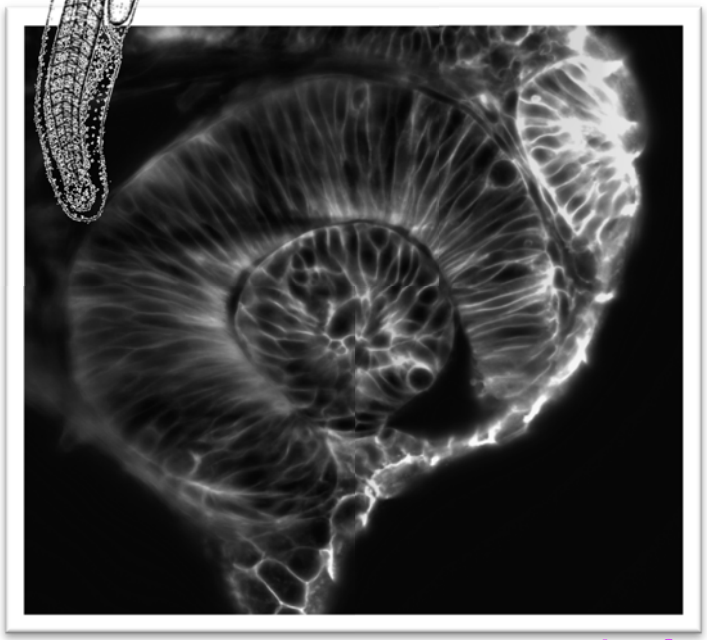


Zebrafish retina as an accessible model

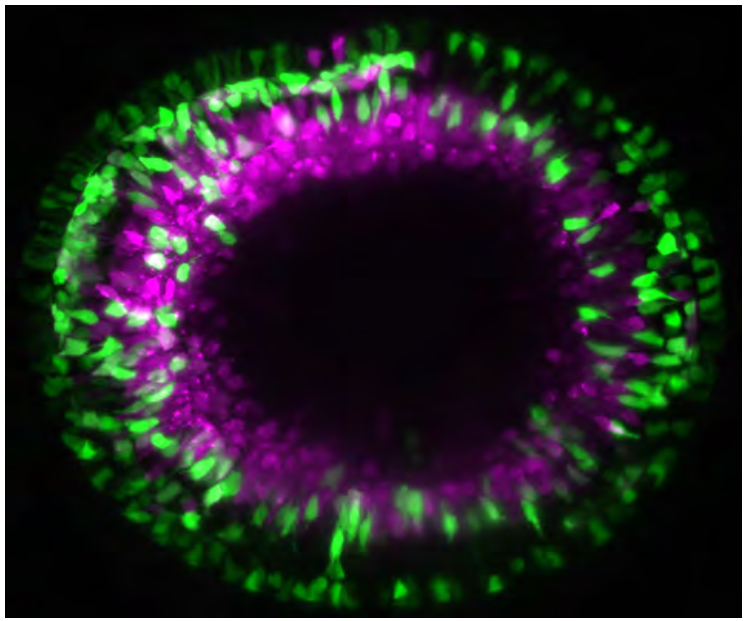


Order-Chaos-Order

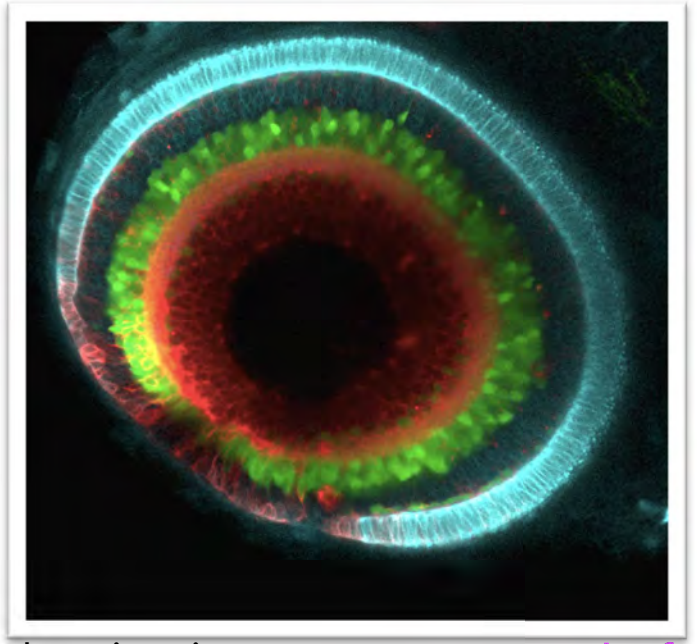




Neuroepithelium 30 hpf

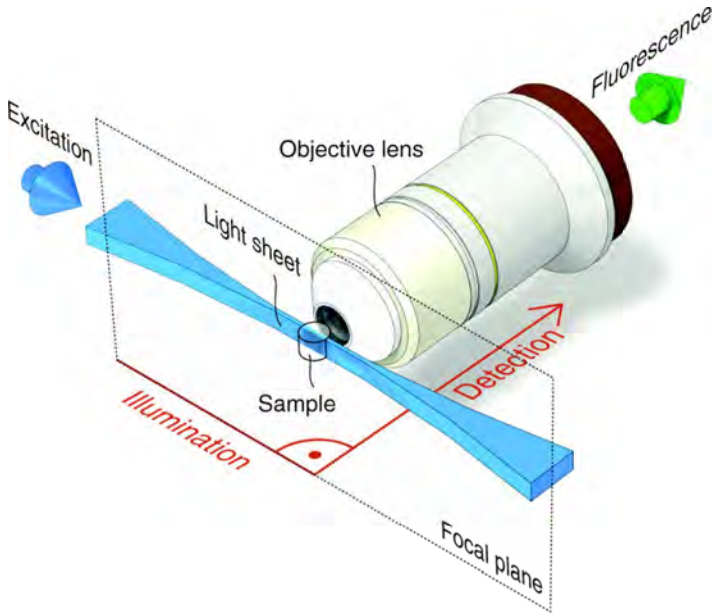


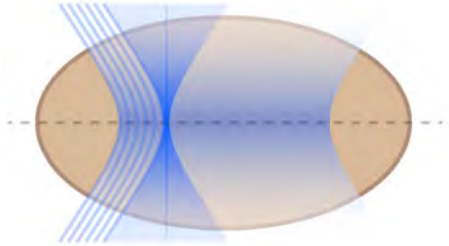
Migration
and Lamination 56 hpf



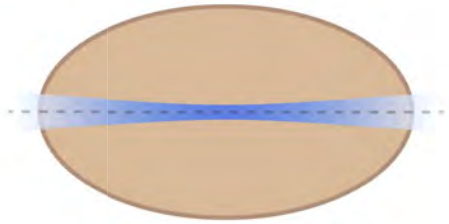
Lamination 80 hpf

Light sheet microscopy for quantitative experiments

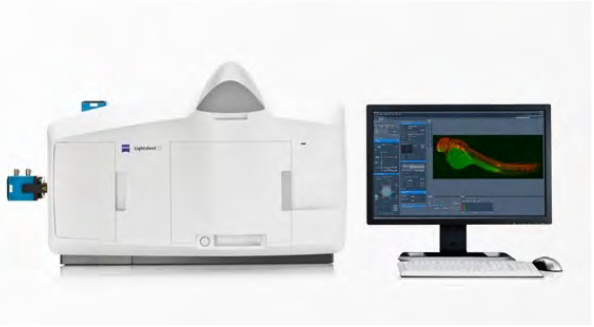




Confocal

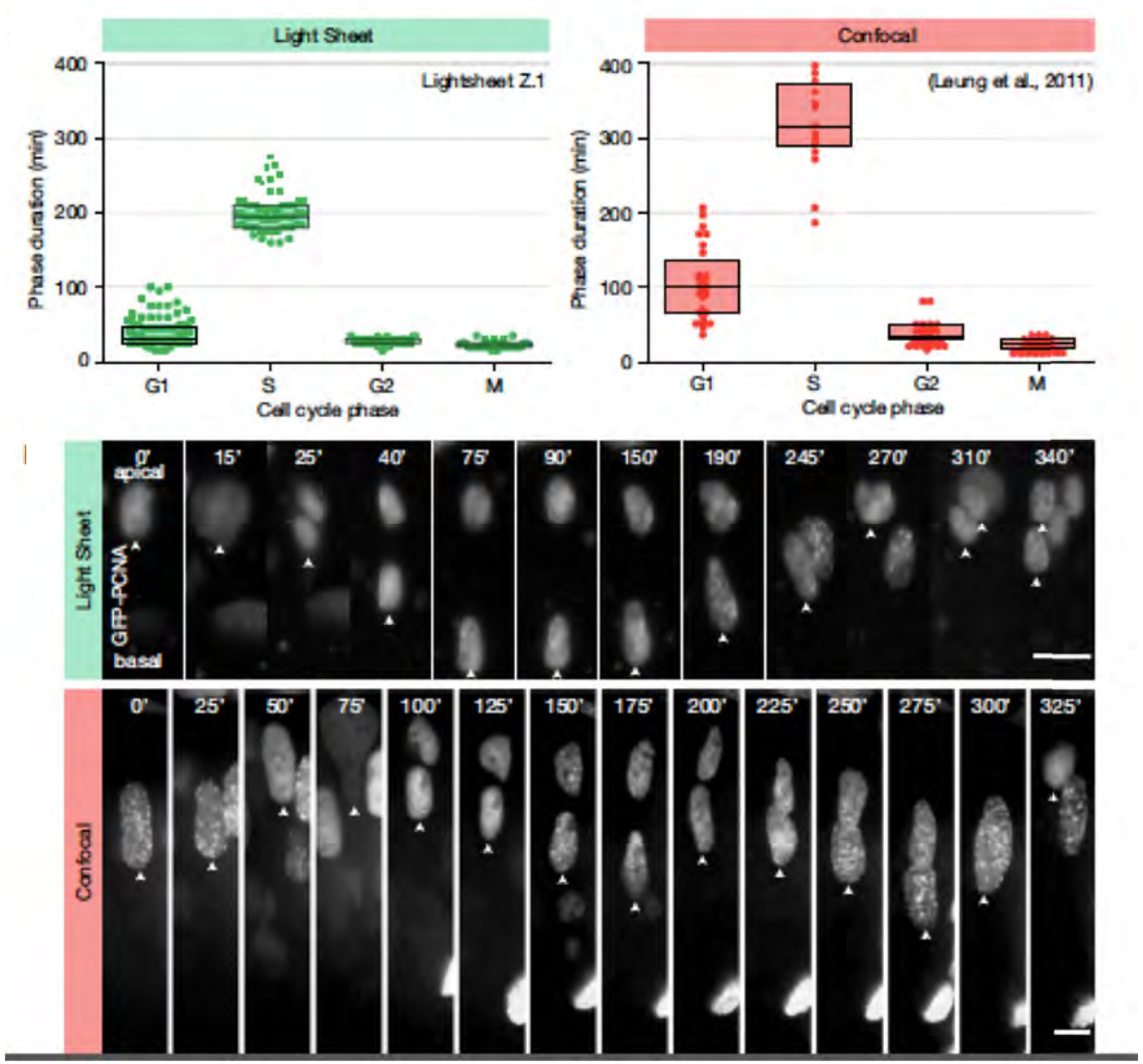


Light sheet



Lightsheet Z.1 (Zeiss)

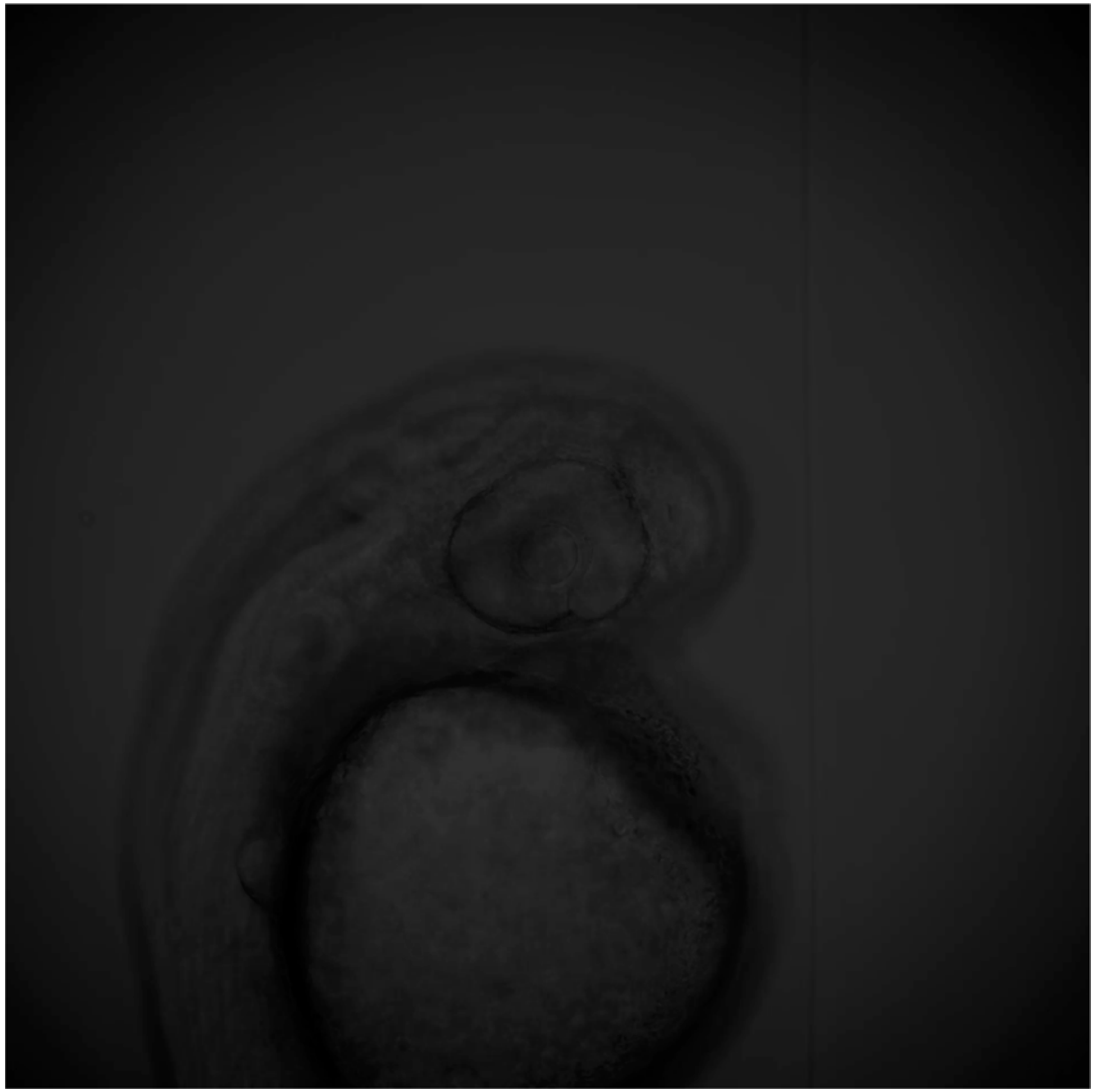
Reduction of phototoxicity



Icha, Weber et al., BioEssays 2018

Experiments over dozens of embryos

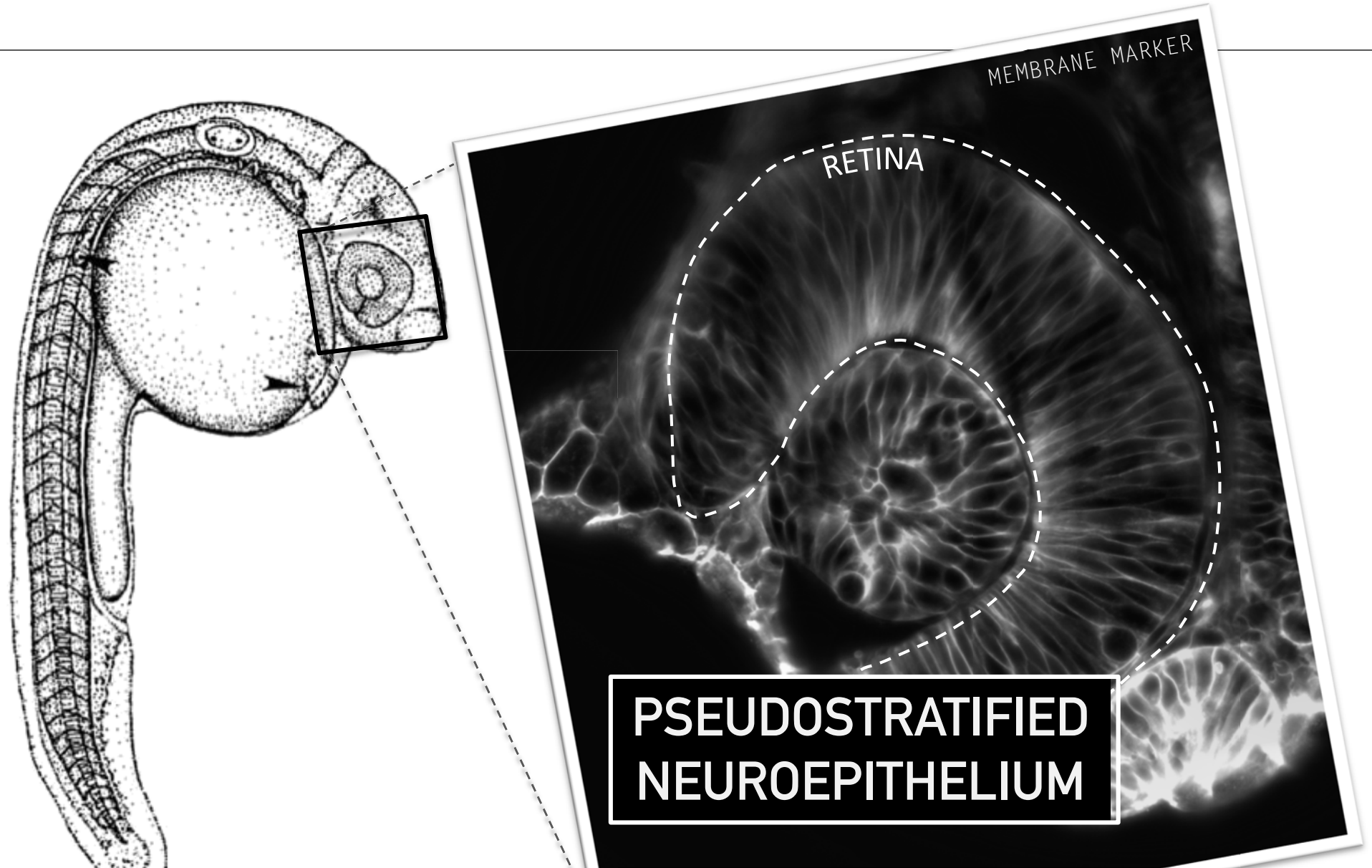
Ath5 Gap-GFP



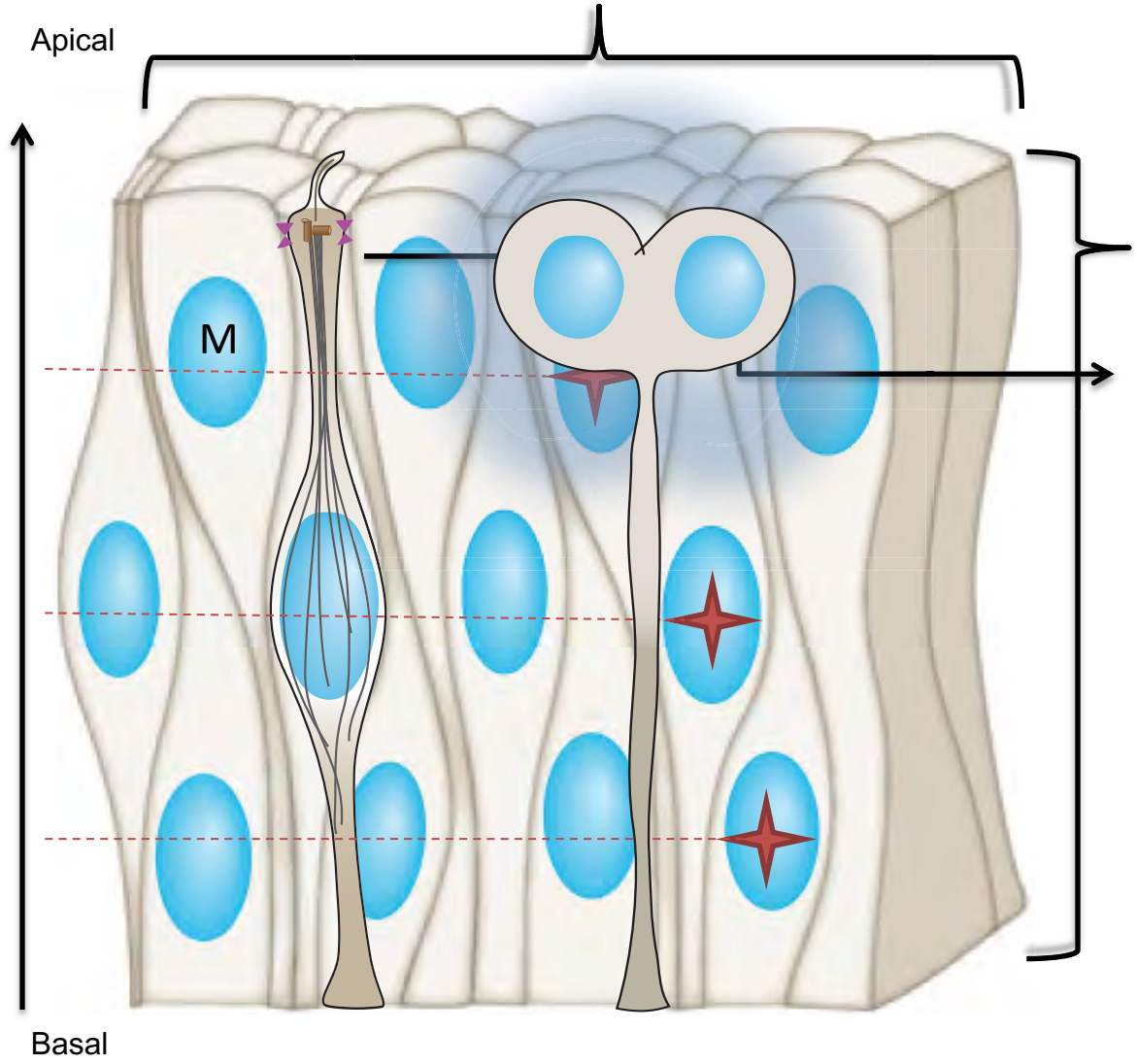
Quantitative analysis of

- Stereotypicity
- Plasticity
- Robustness
  of cell and tissue behaviour

The pseudostratified retinal neuroepithelia

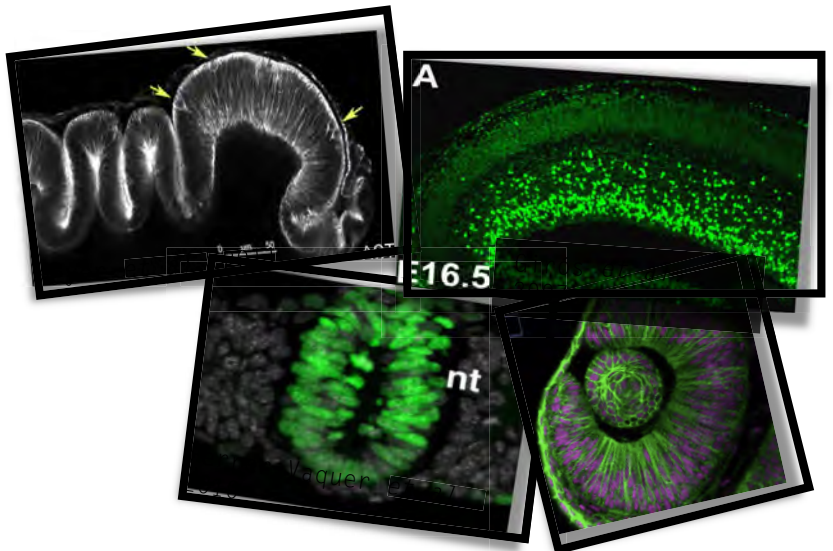


What are pseudostratified epithelia?

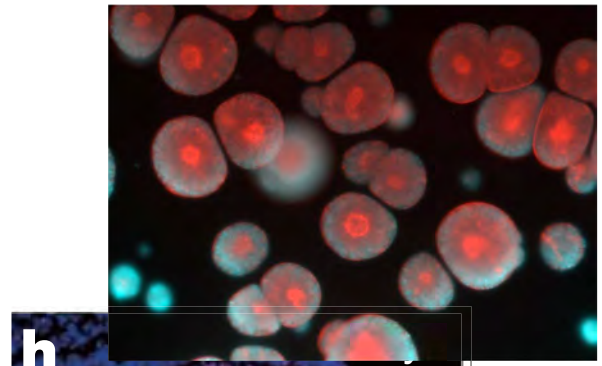


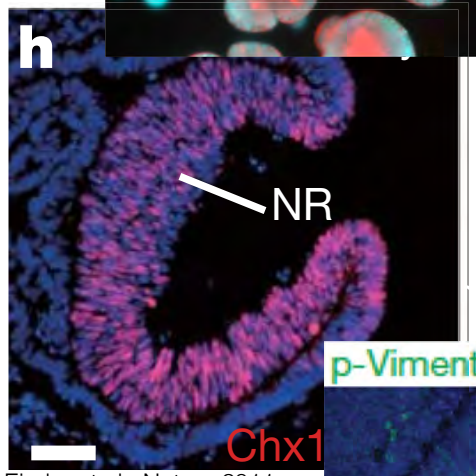
- Densely packed
- Single layer of elongated cells
- Cells are polarized
- Nuclei can be localized at any position along the apico-basal axis in interphase
- Mitosis always occurs apically

Why care about pseudostratified epithelia?

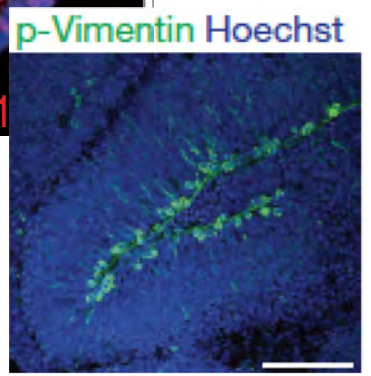


Keisuke Ishihara



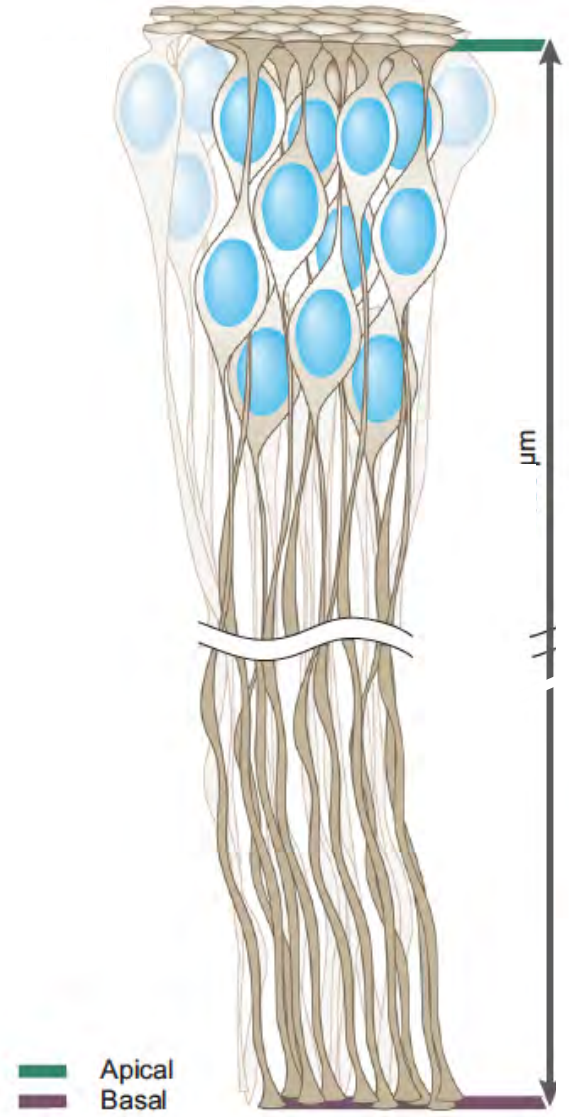


Eiraku et al., Nature 2011

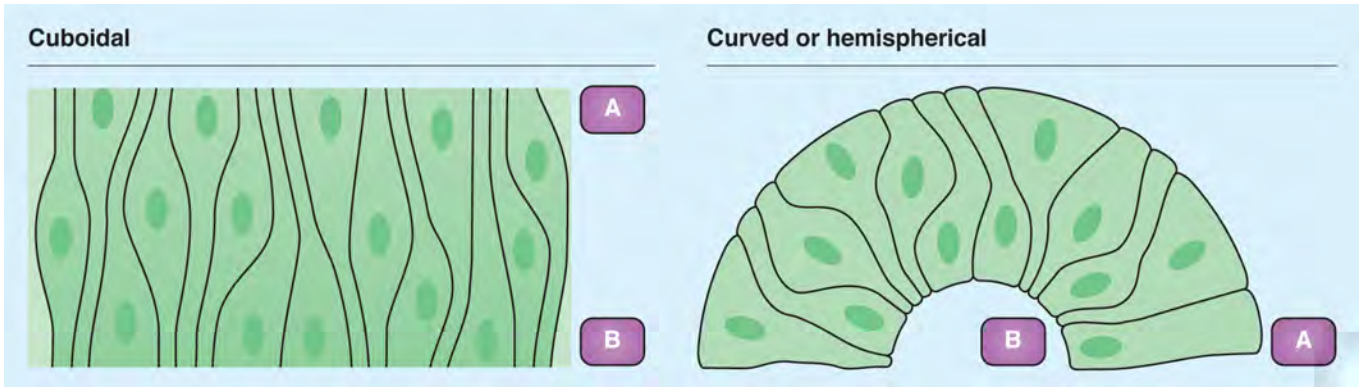


Lancaster et al., Nature 2013

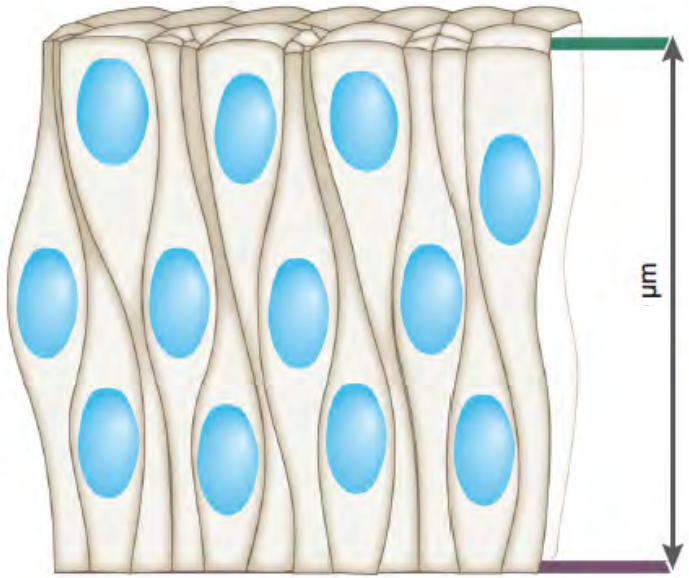
Pseudostratified epithelia are diverse in length and shape



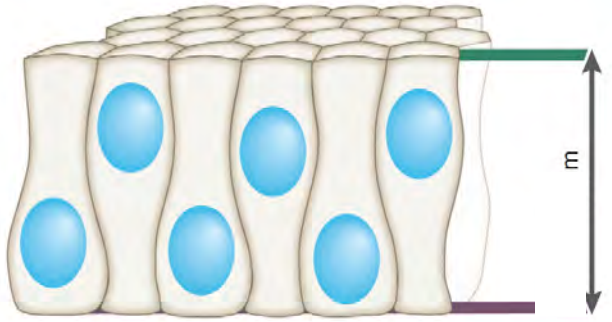
Long PSE



Norden, Journal of Cell Science, 2017



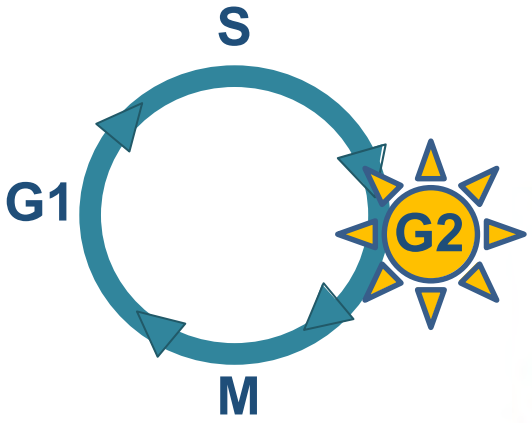
Intermediate PSE

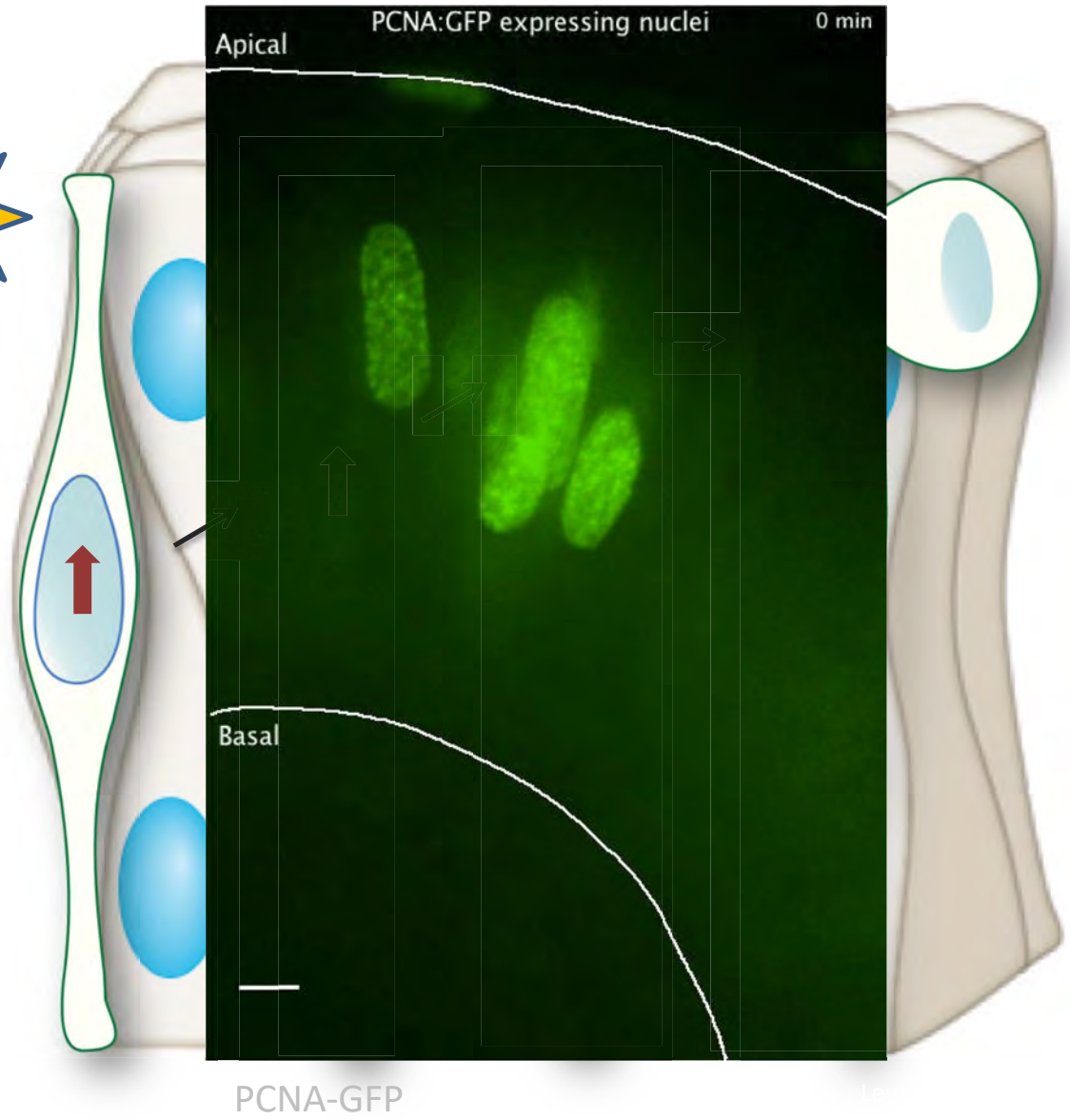


Short PSE

Strzyz, Matejcic, Norden International Review of Cell and Molecular Biology, 2016

One phenomenon they all have in common

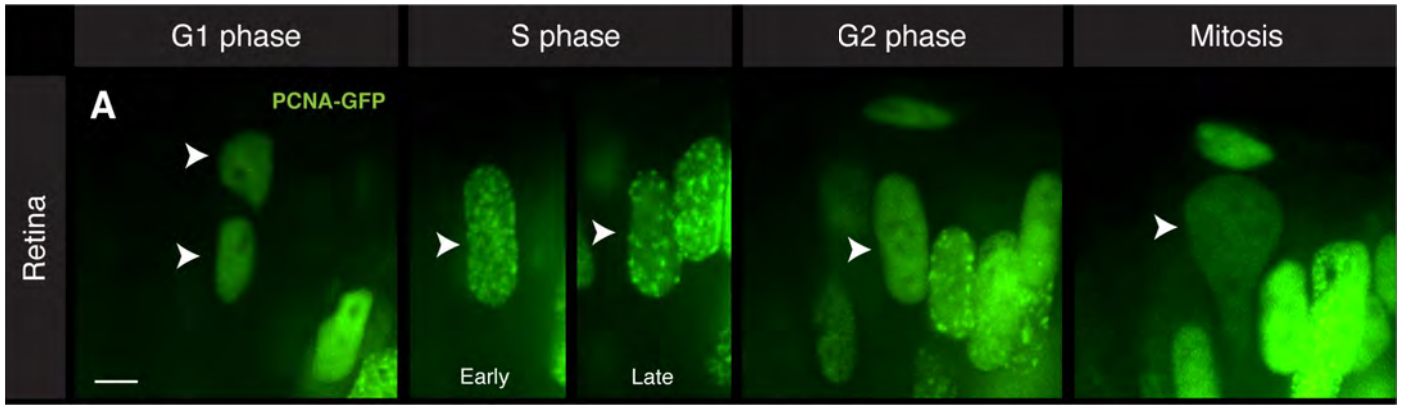


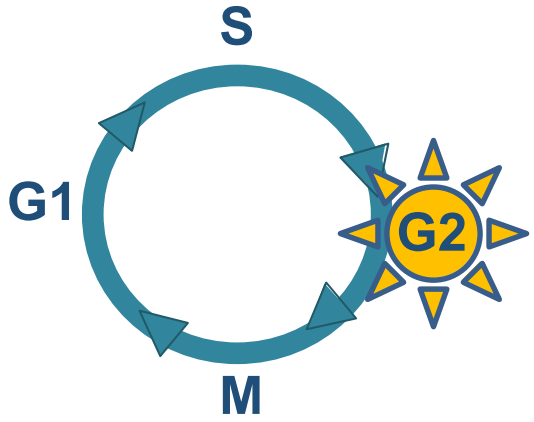


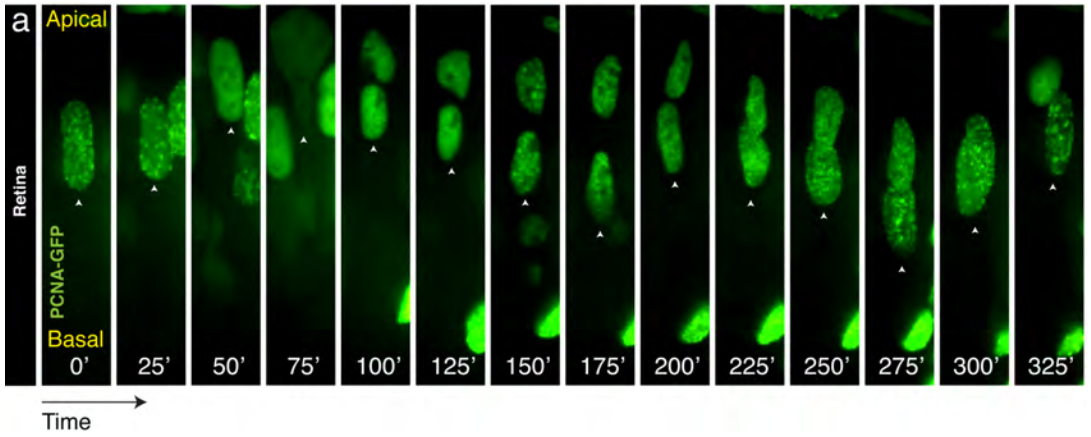
PCNA-GFP

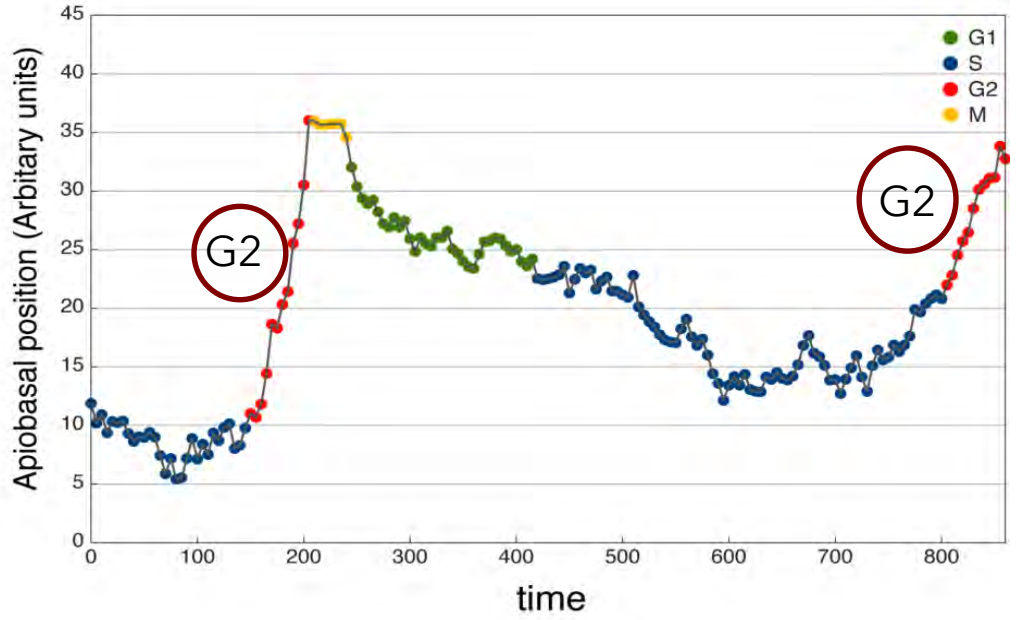
INTERKINETIC NUCLEAR MIGRATION ≙ APICAL MOVEMENT OF NUCLEI BEFORE MITOSIS

Apical nuclear migration occurs in G2 only



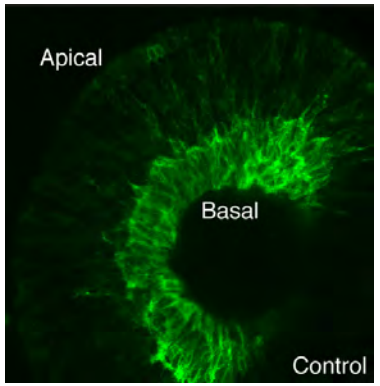


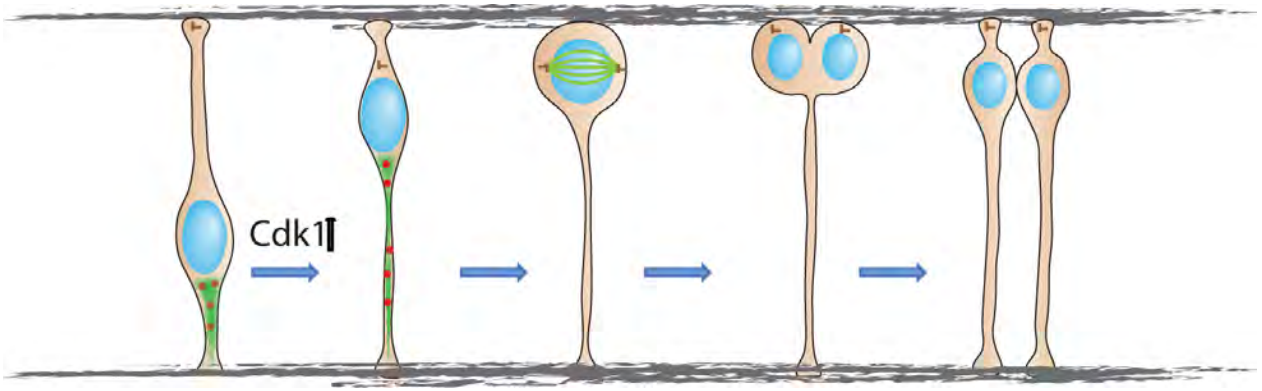


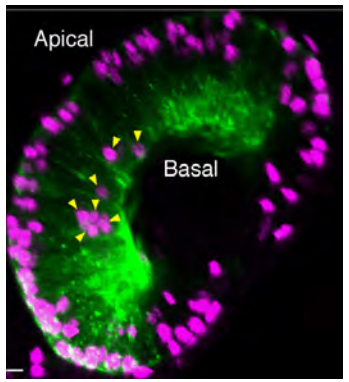


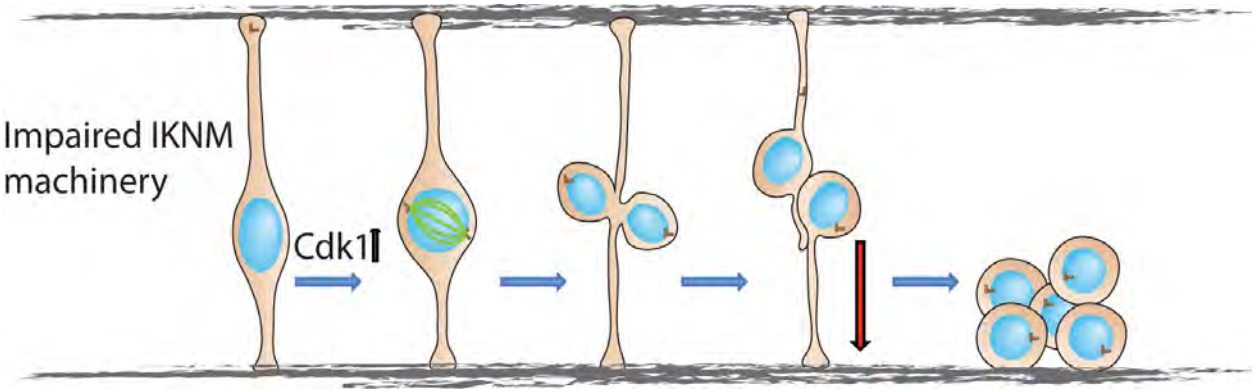
Leung…Norden 2011 Development

Apical divisions are crucial to maintain tissue integrity







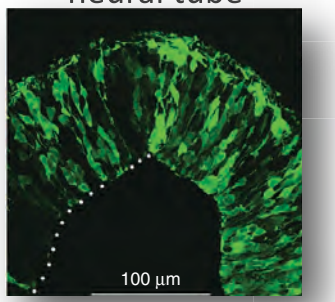


How is the force generated to move nuclei apically?

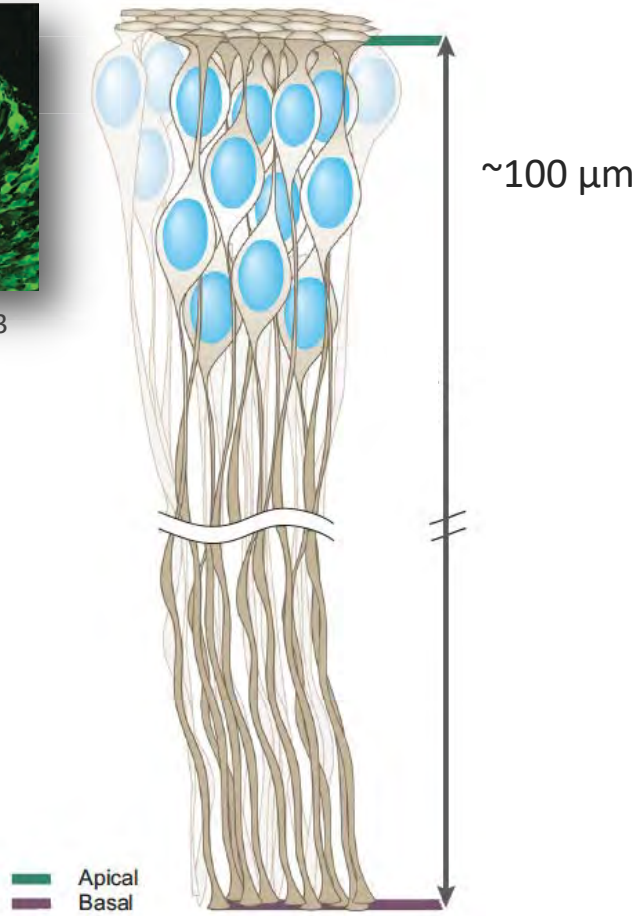
Strzyz …Norden Developmental Cell, 2015

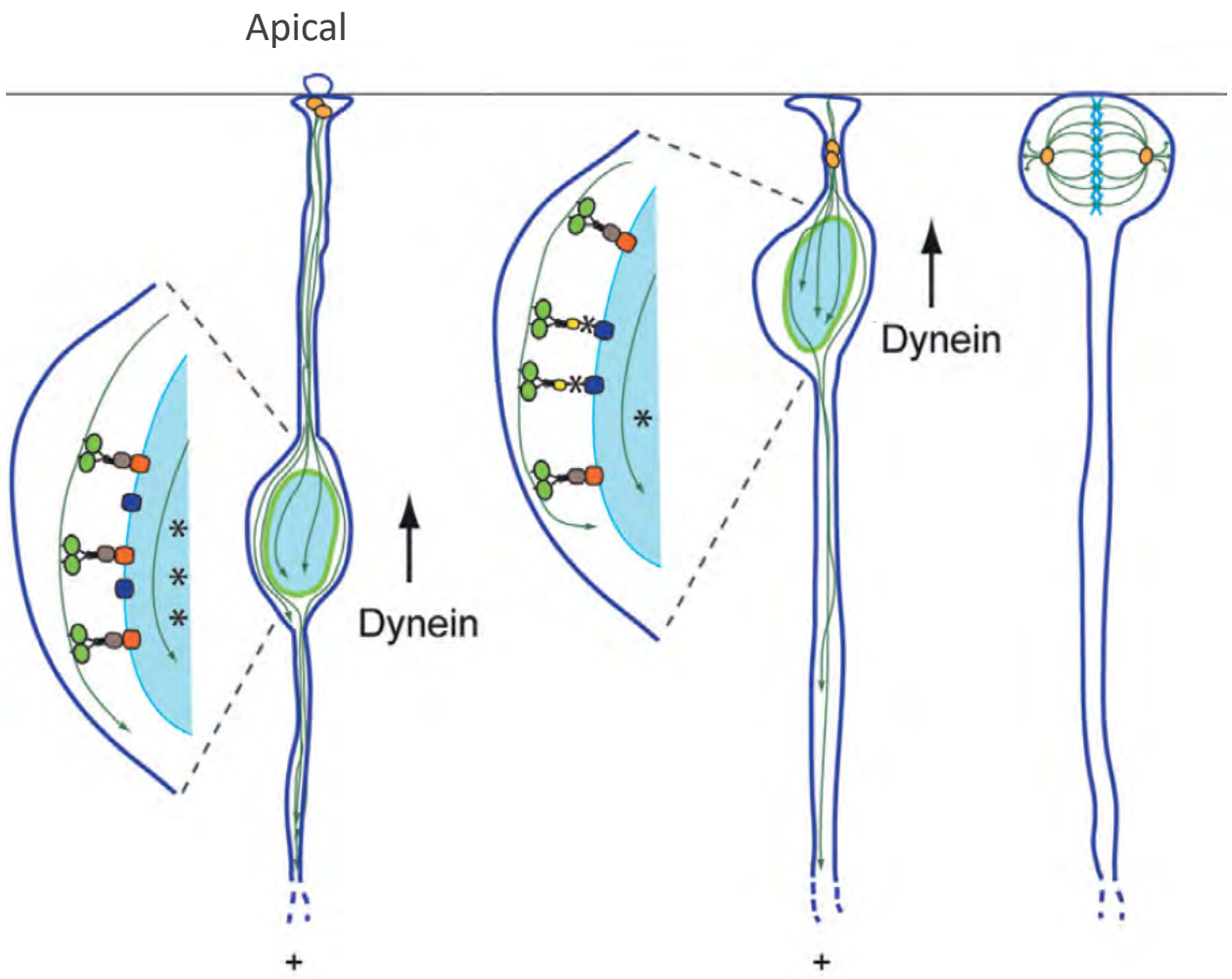
In elongated neocortex epithelia microtubules move nuclei

Mammalian neural tube



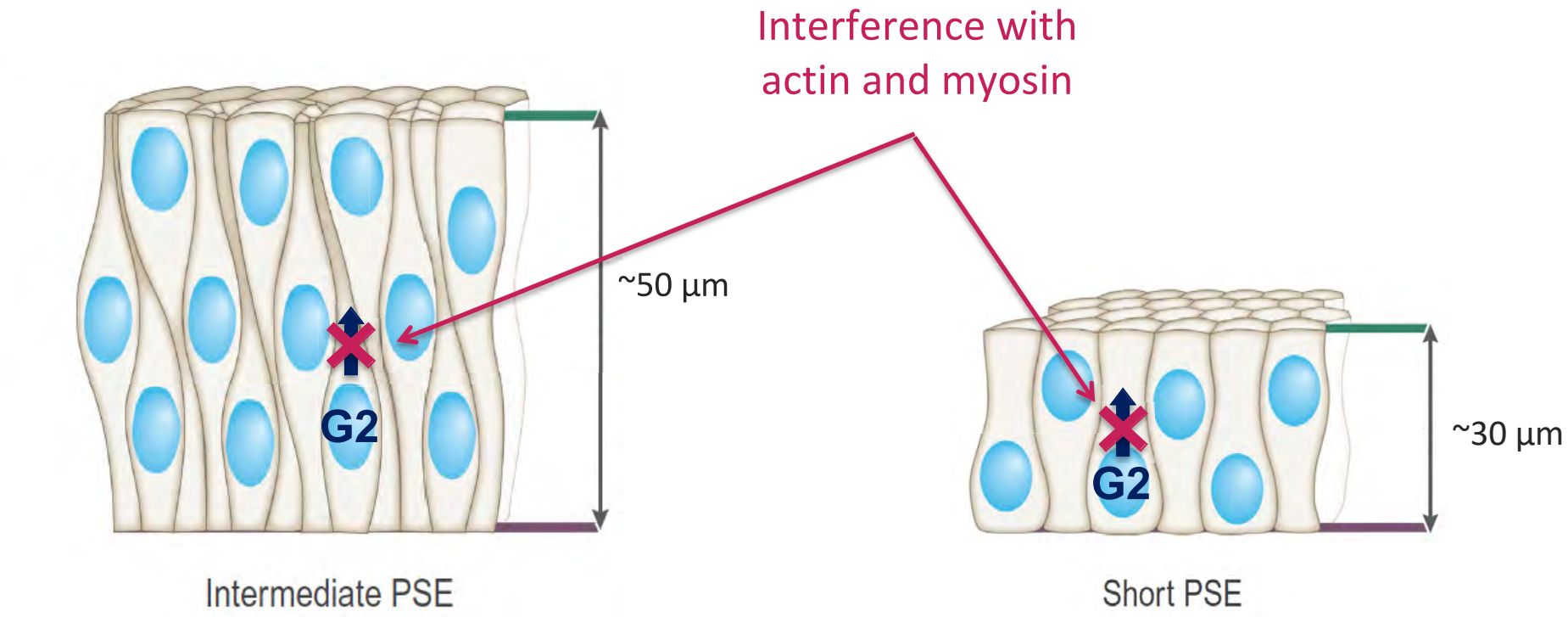
Okamoto et al 2013





Bertipaglia et al., 2017, with changes

In shorter neuroepithelia actin is involved

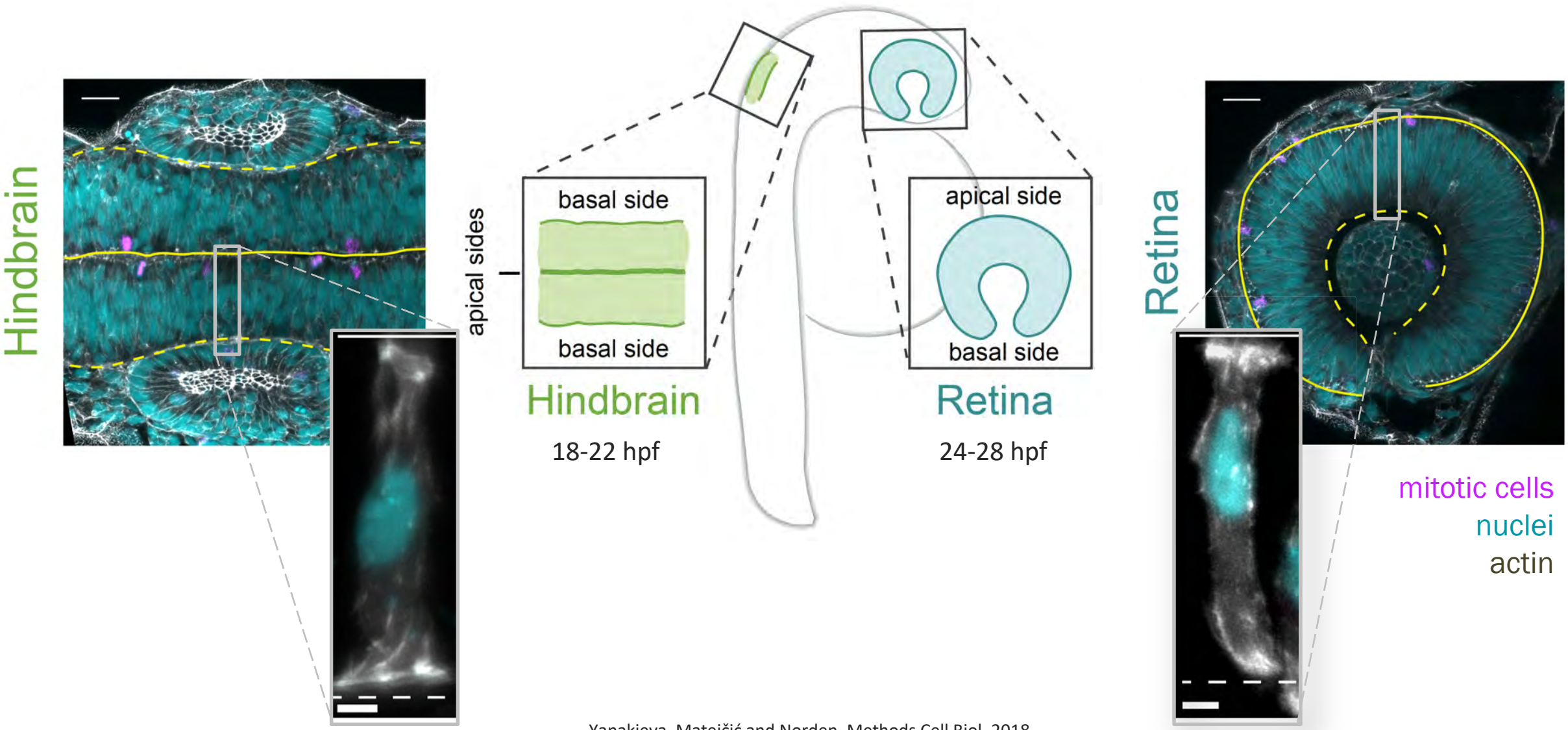


How does actin move nuclei?

And is it all the same?

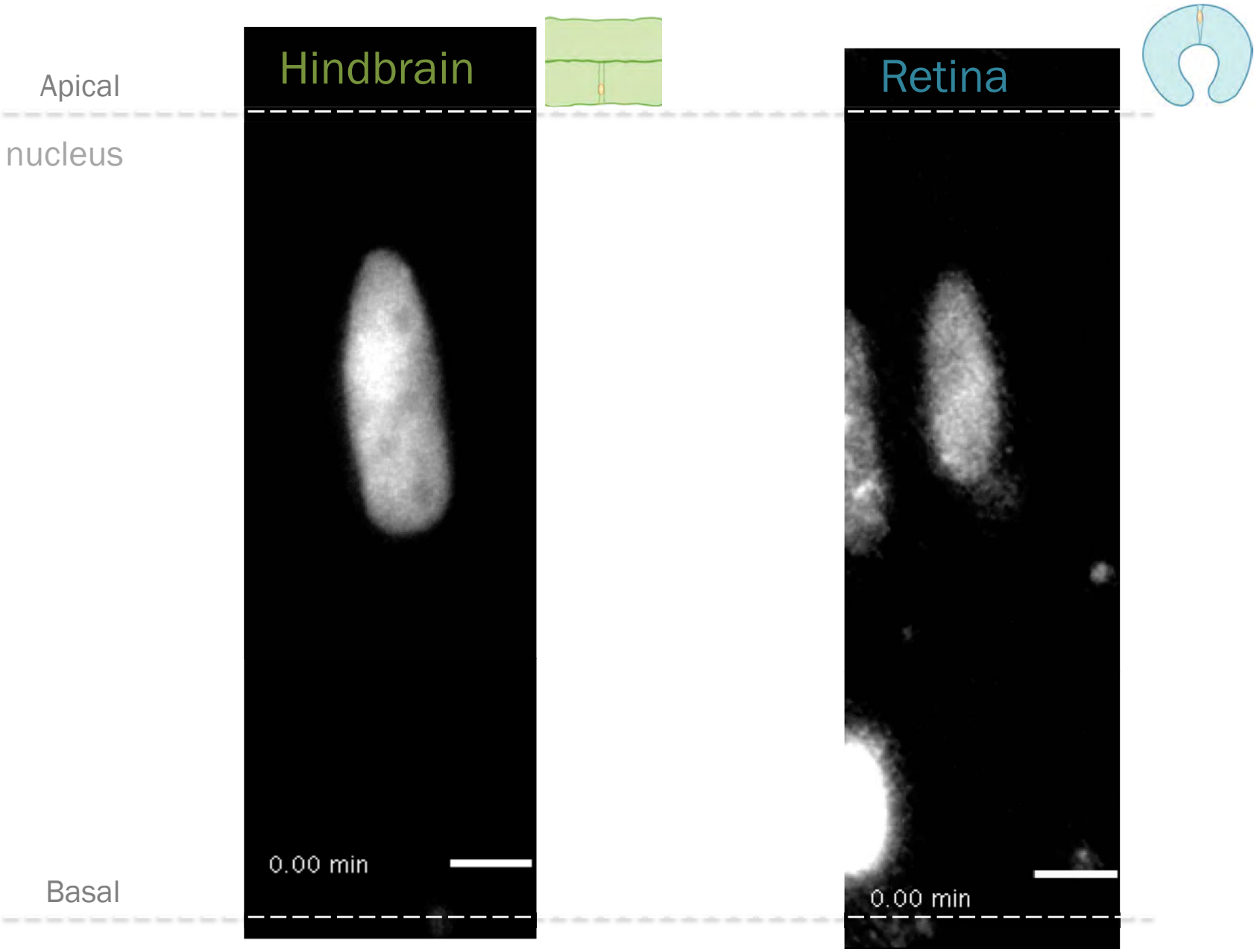
Norden et al., 2009
Leung and Klopper et al., 2011
Meyer et al., 2011
Rujano et al., 2013
Strzyz et al., 2016

Studying apical nuclear positioning *in situ* in zebrafish embryos

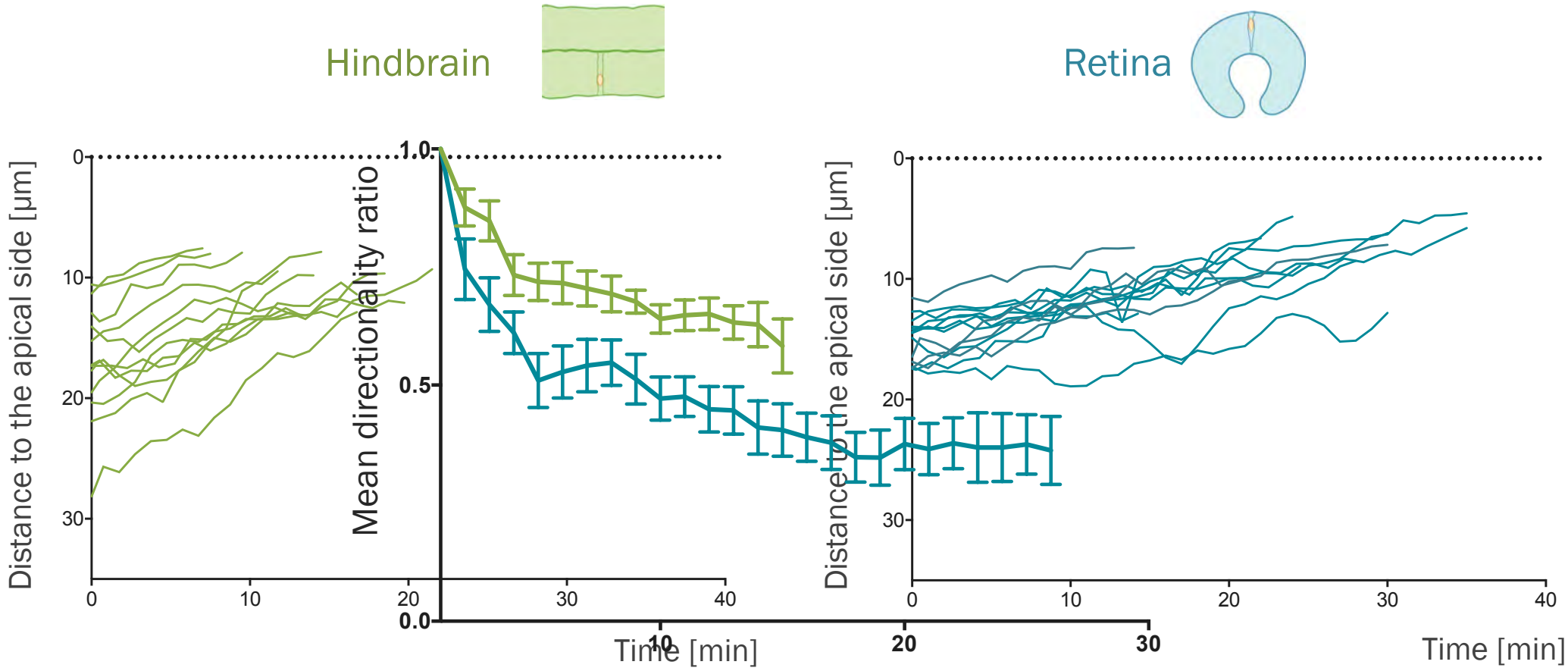


Yanakieva, Matejčić and Norden, Methods Cell Biol, 2018

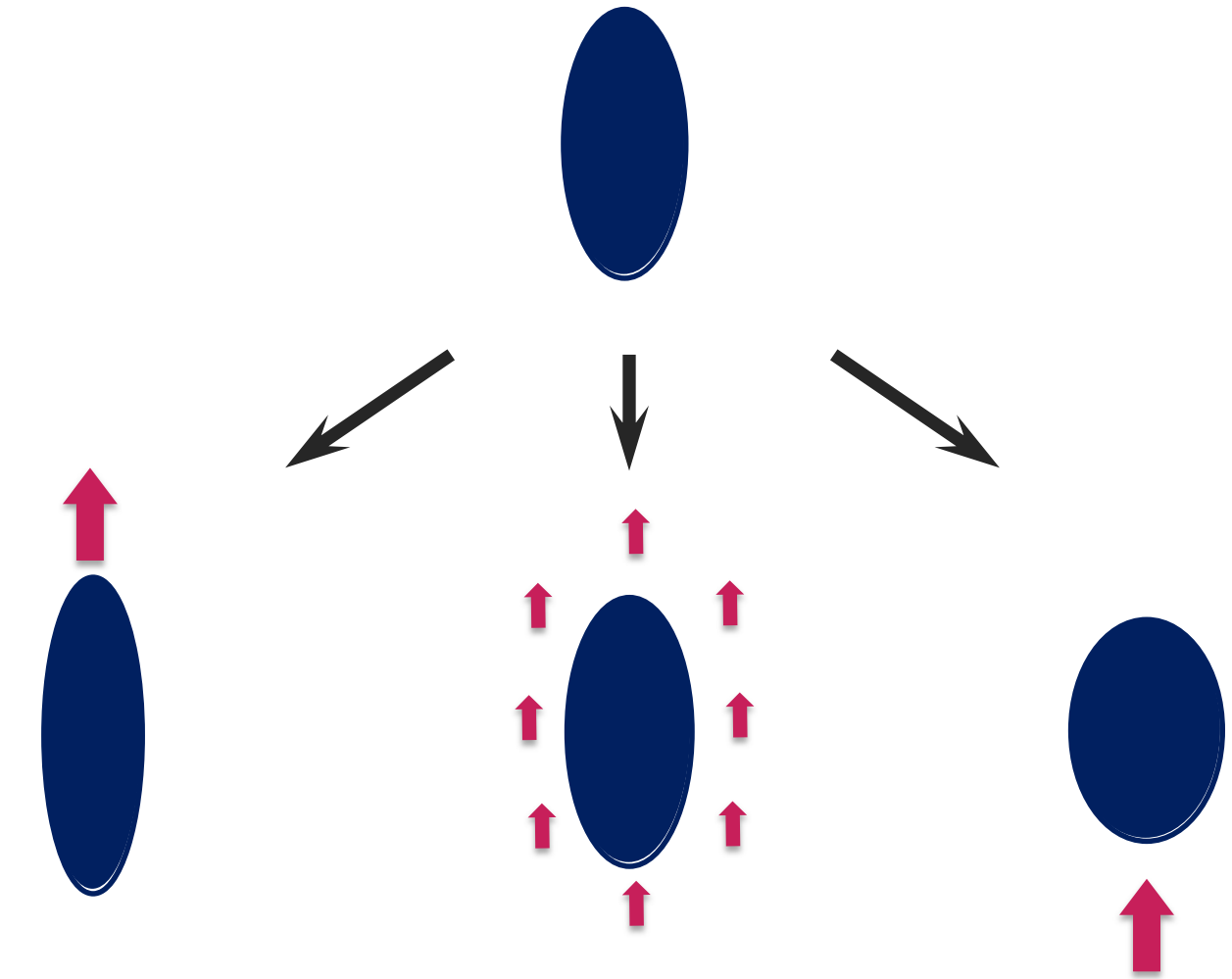
On first sight movements look similar



Hindbrain nuclei move faster and more directed than retinal nuclei



Are nuclei exposed to different forces?



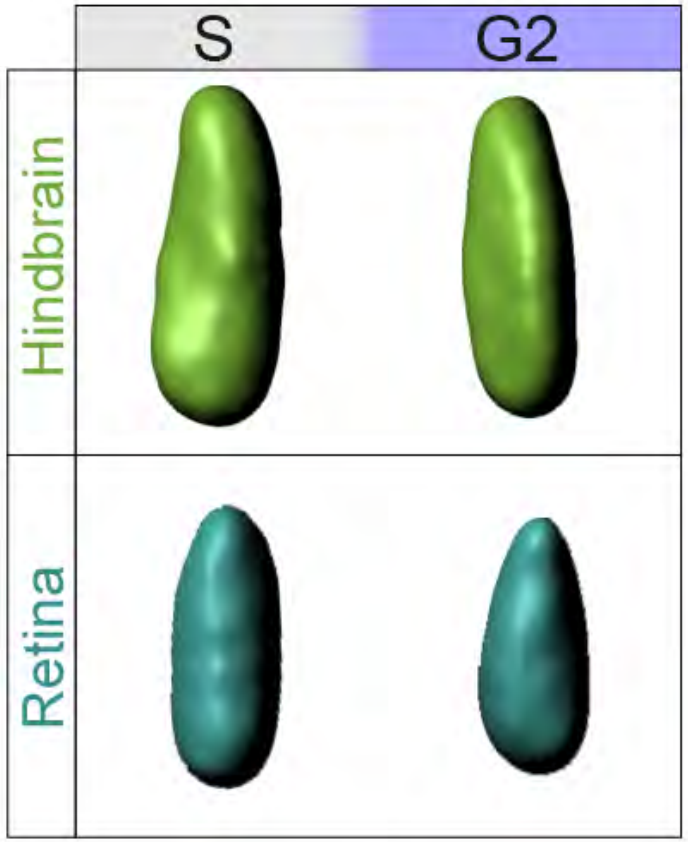
Pulling?

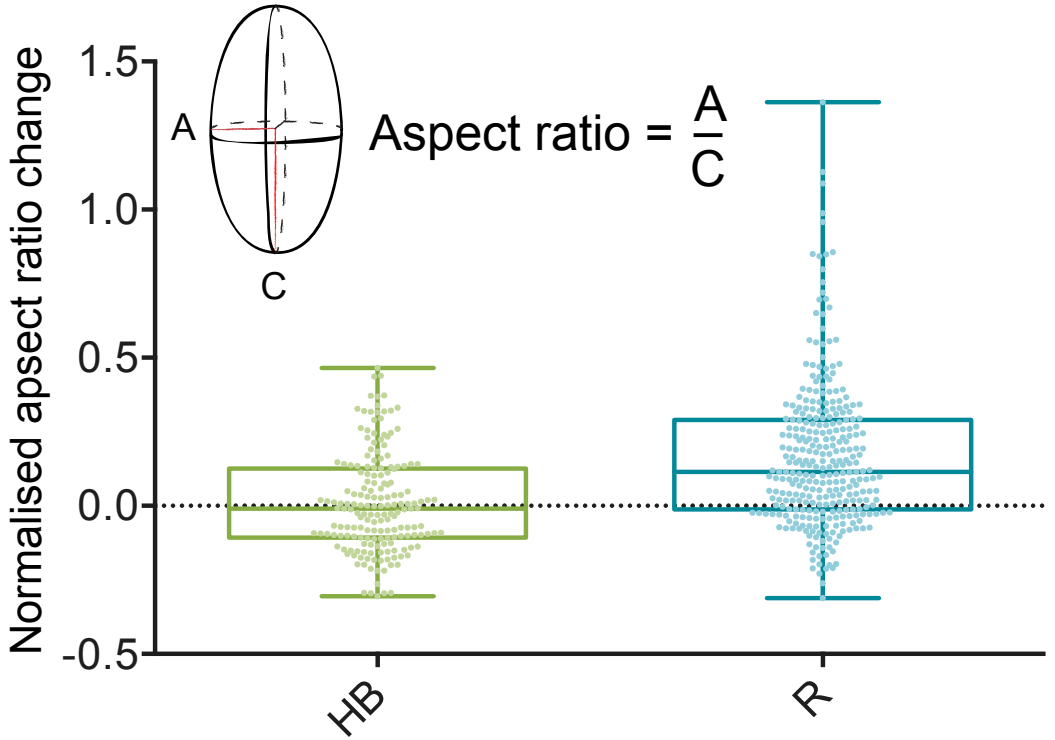
Evenly distributed?

Pushing?

nuclei
forces

Only retinal nuclei deform basally during apical migration

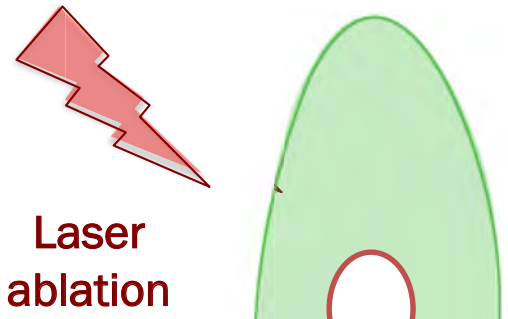




Laser ablation to distinguish between pushing and pulling forces

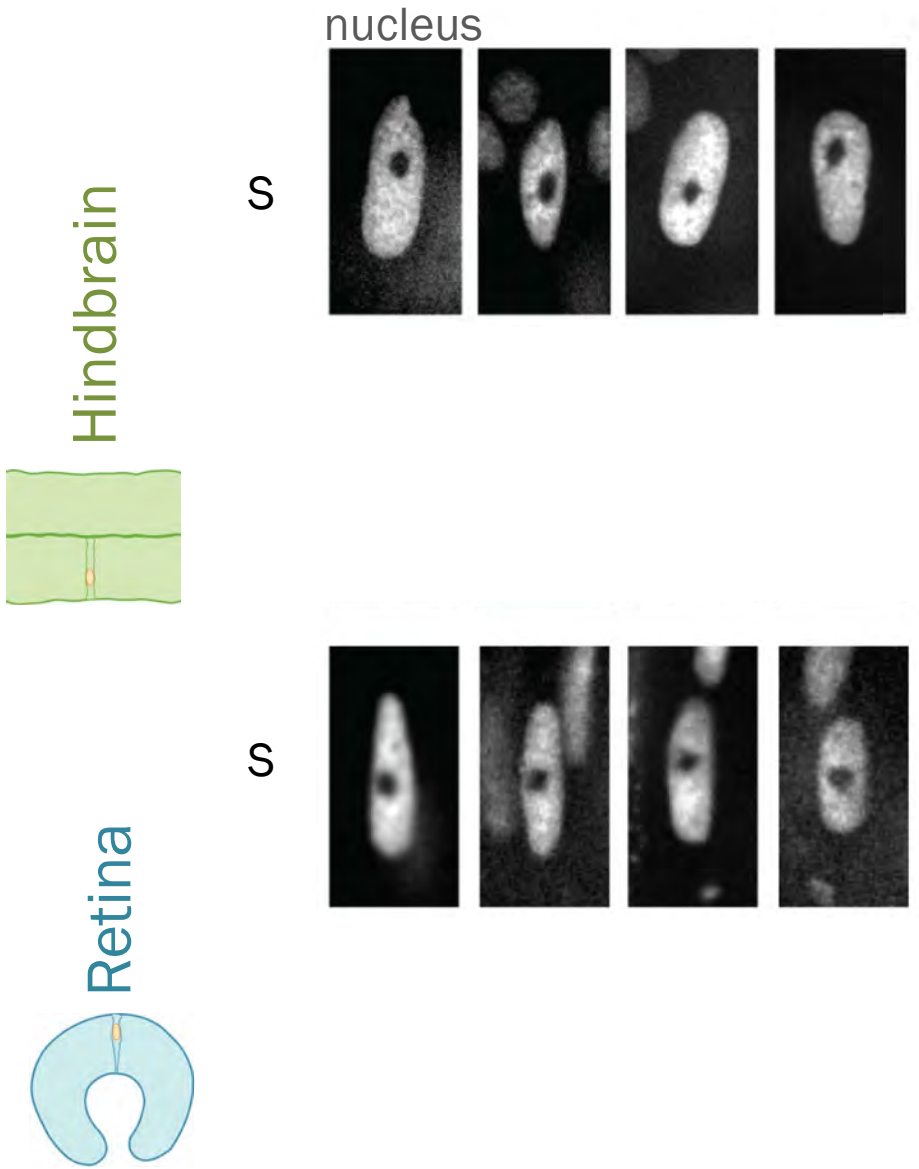
Apical

S

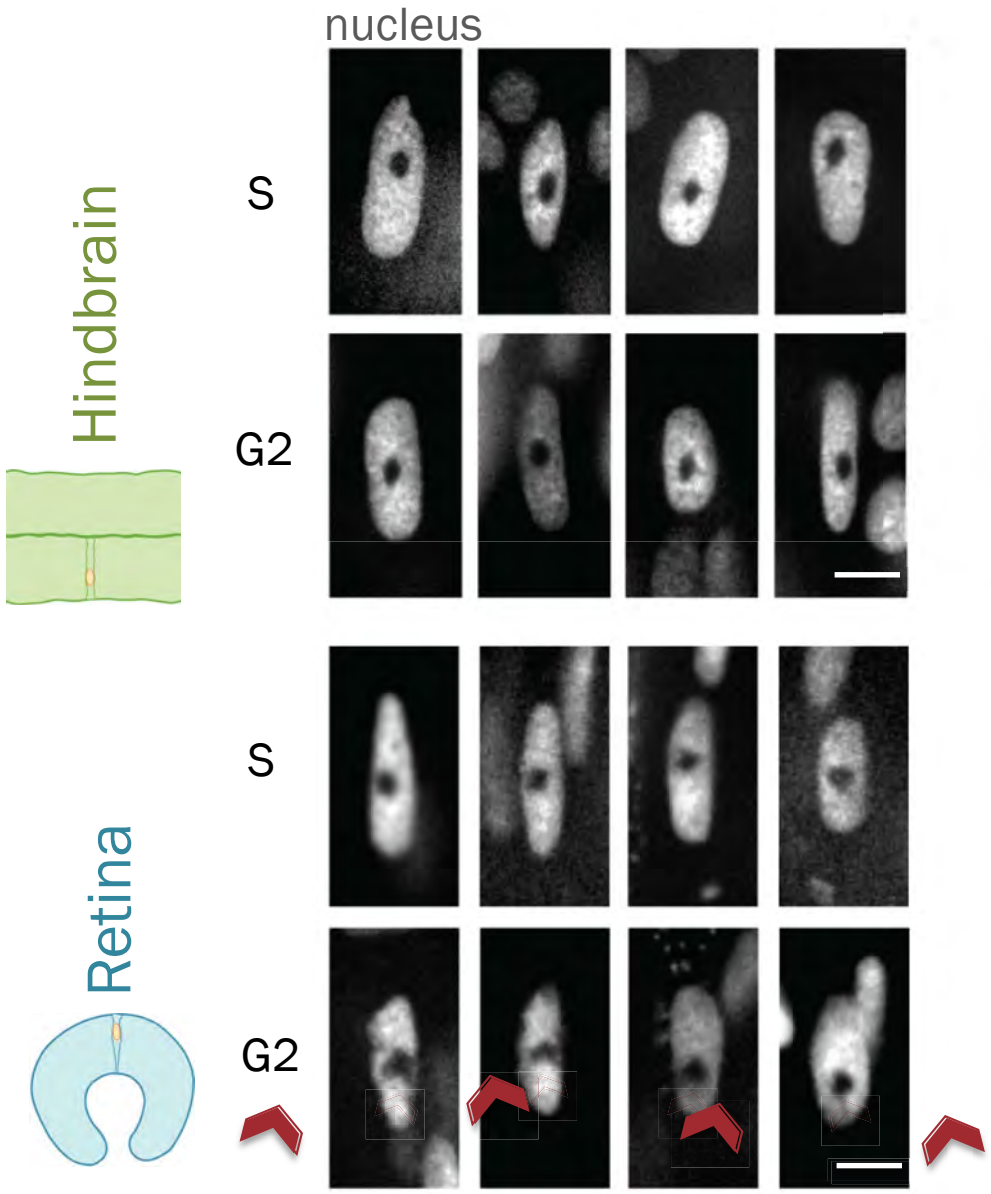


Basal

Retinal nuclei are pushed



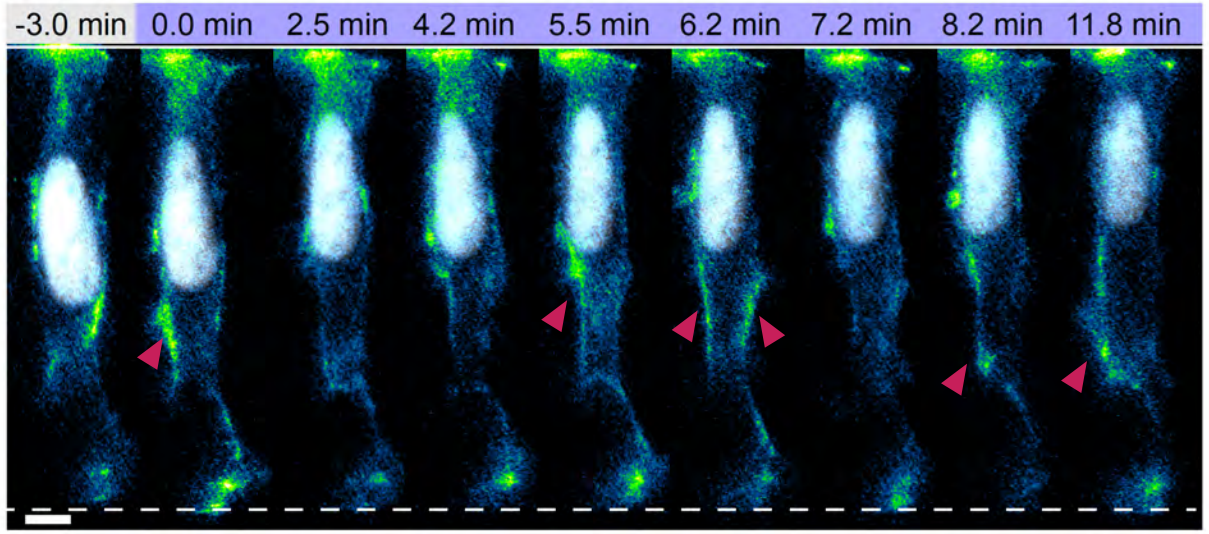
Retinal nuclei are pushed

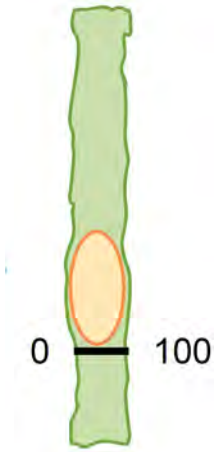


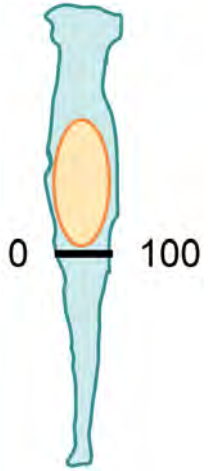
	# concave deformations
HB (S)	0 out of 12
HB (G2)	0 out of 10
R (S)	0 out of 10
R (G2)	7 out of 14

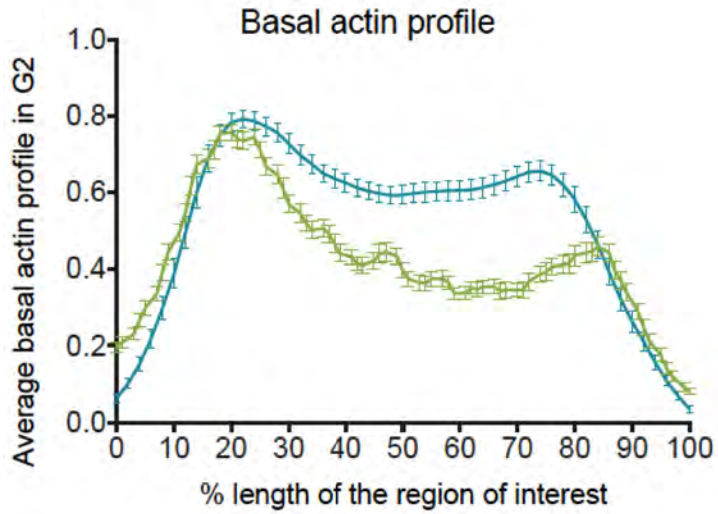
Differences of actin activity in retinal and hindbrain cells?

Actin is differently localized during apical nuclear migration









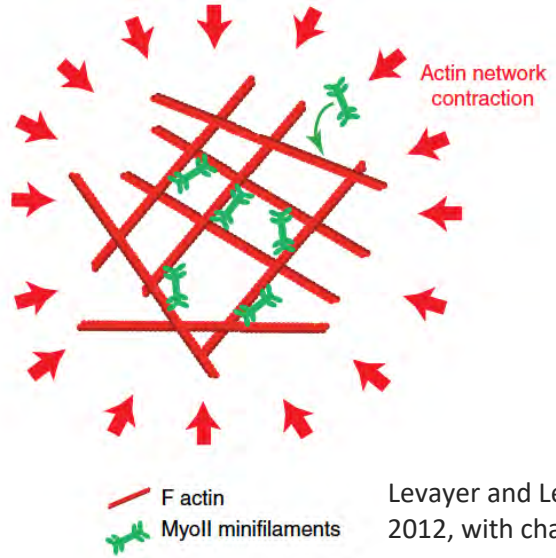
nuclei, actin

min max

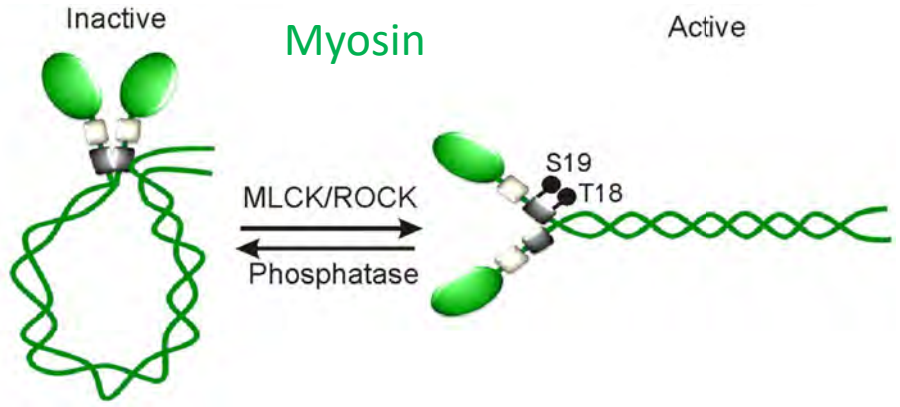
Different molecular mechanisms?

Force generation by actin

- Network contraction

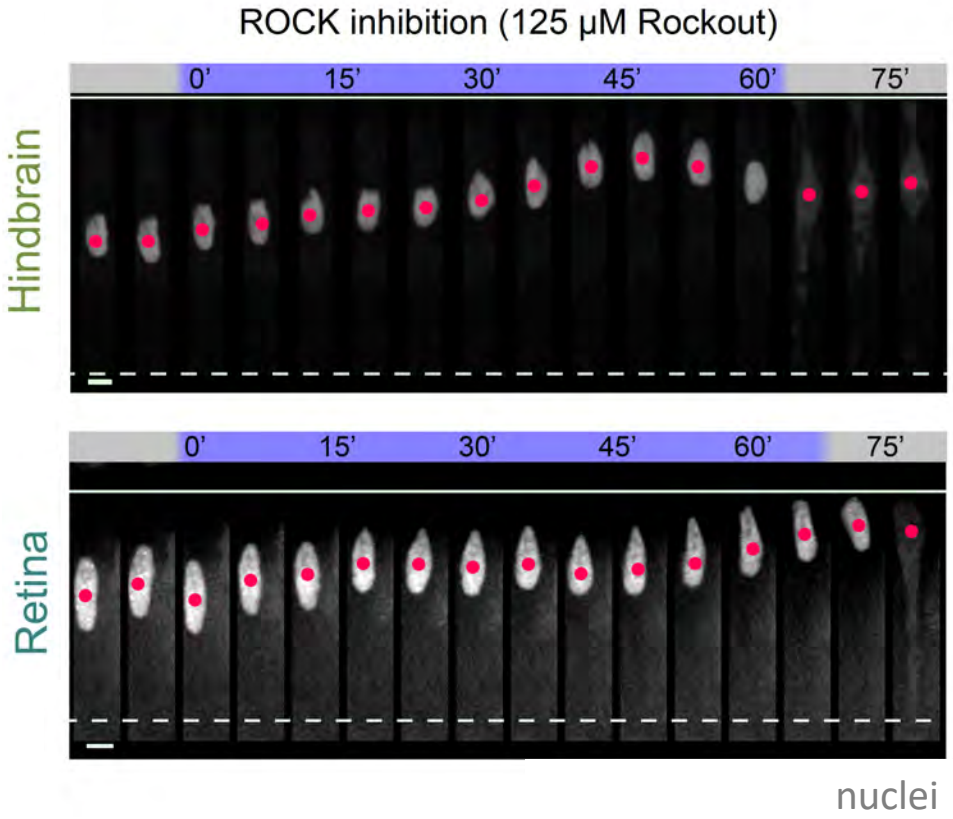


Levayer and Lecuit, 2012, with changes

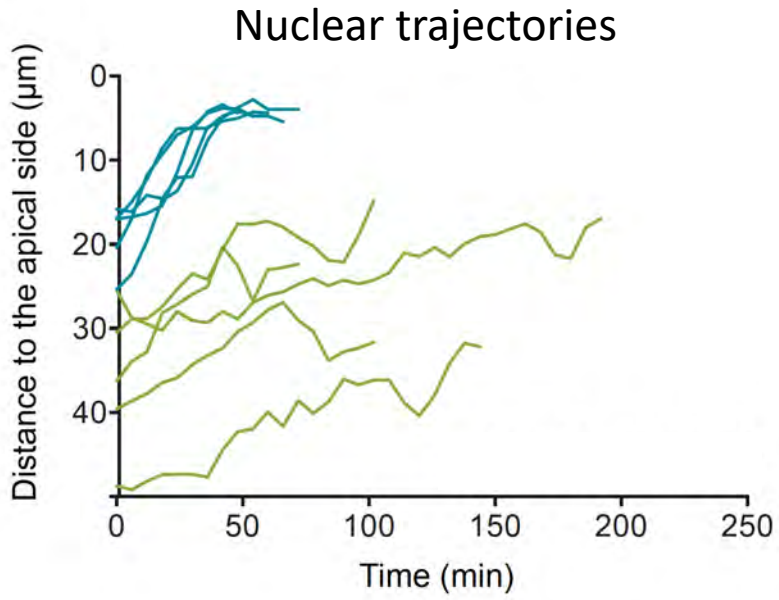


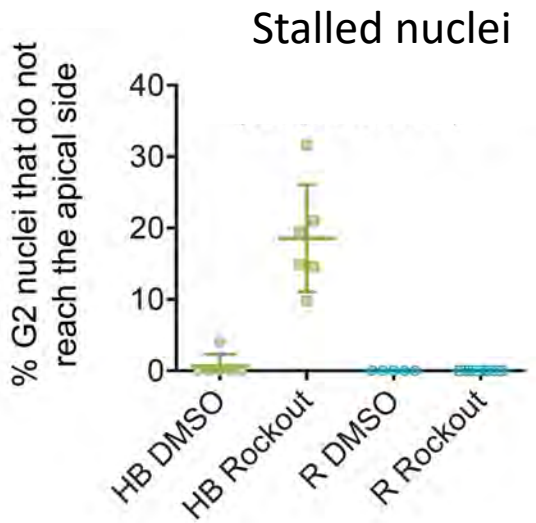
Betapudi, 2014, with changes

Inhibition of ROCK activity perturbs nuclear positioning only in hindbrain



nuclei

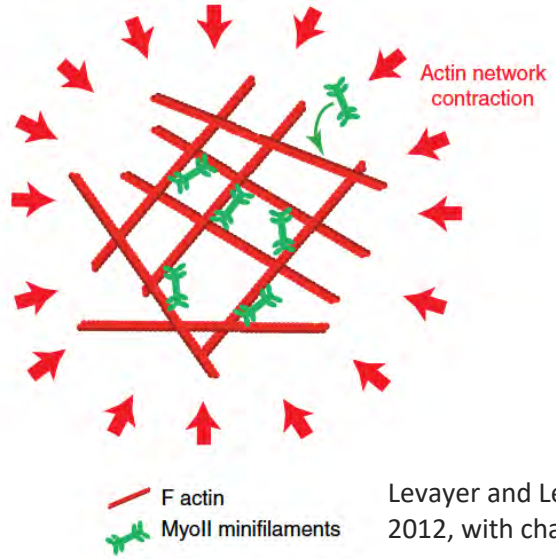




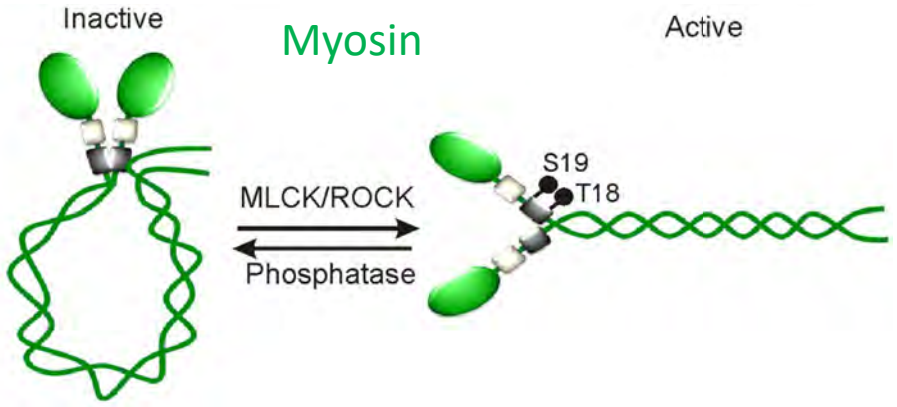
Mean ± SD

Force generation by actin

- Network contraction



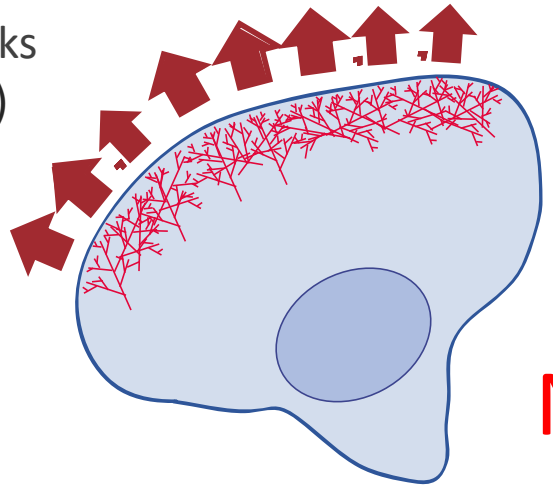
Levayer and Lecuit, 2012, with changes



Betapudi, 2014, with changes

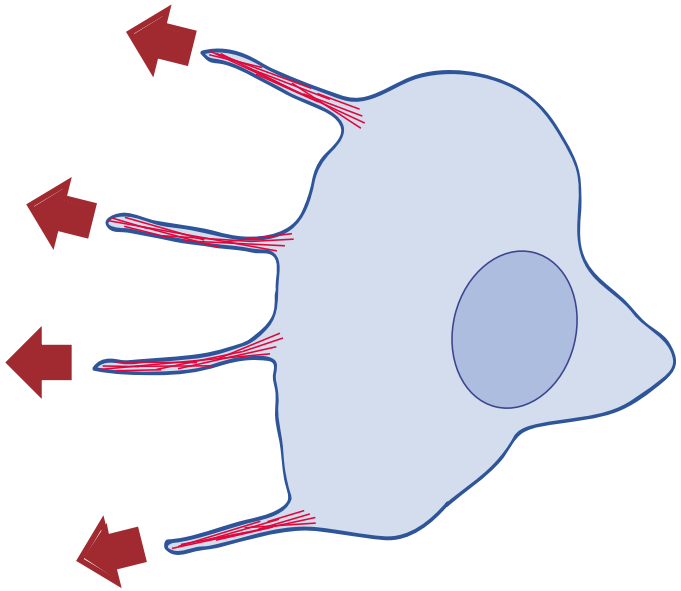
- Network expansion

Meshworks (Arp2/3)

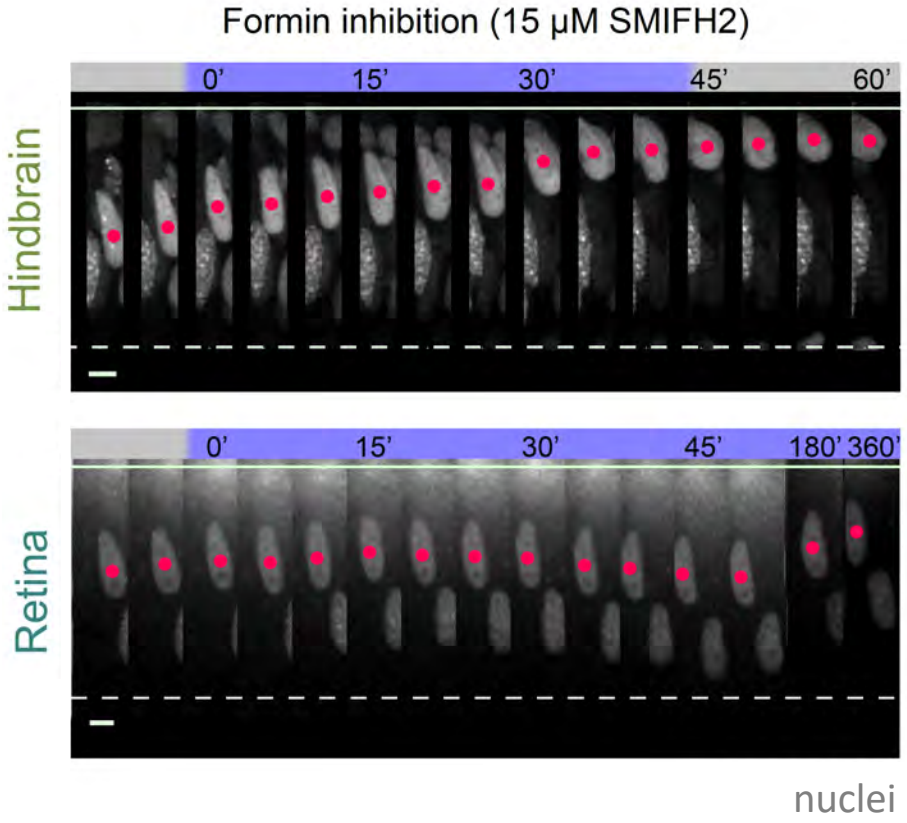


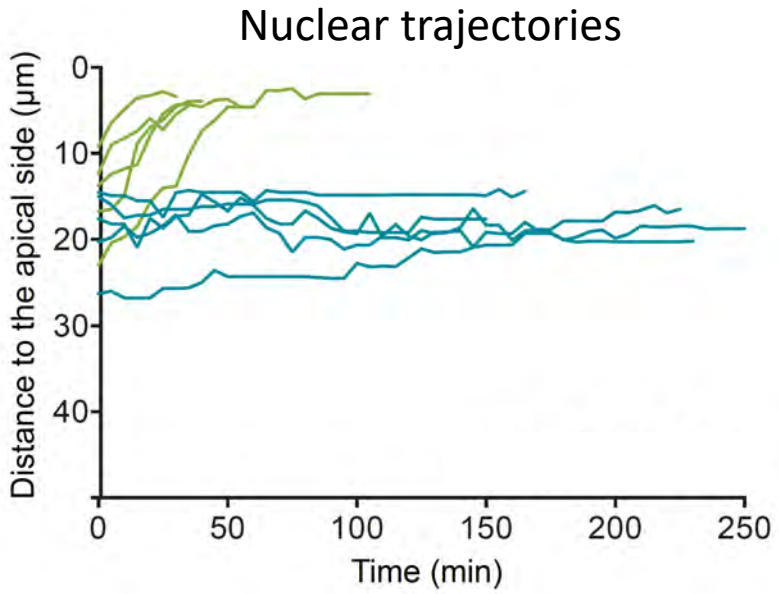
NO!

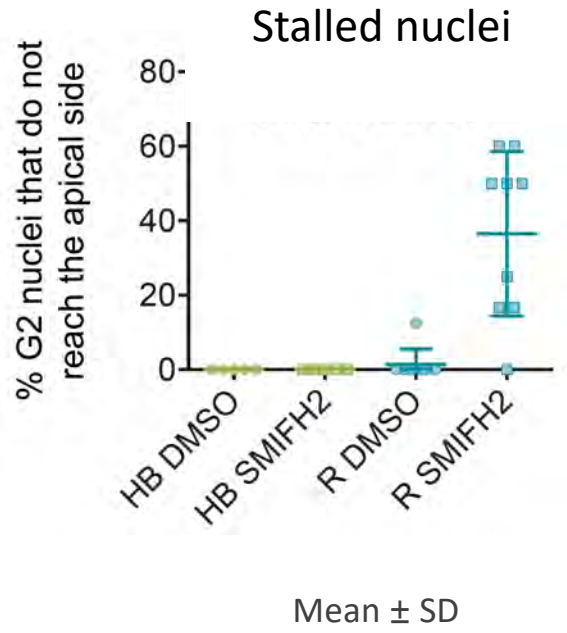
Parallel bundles (formins)



Inhibition of formin activity perturbs nuclear positioning only in retina

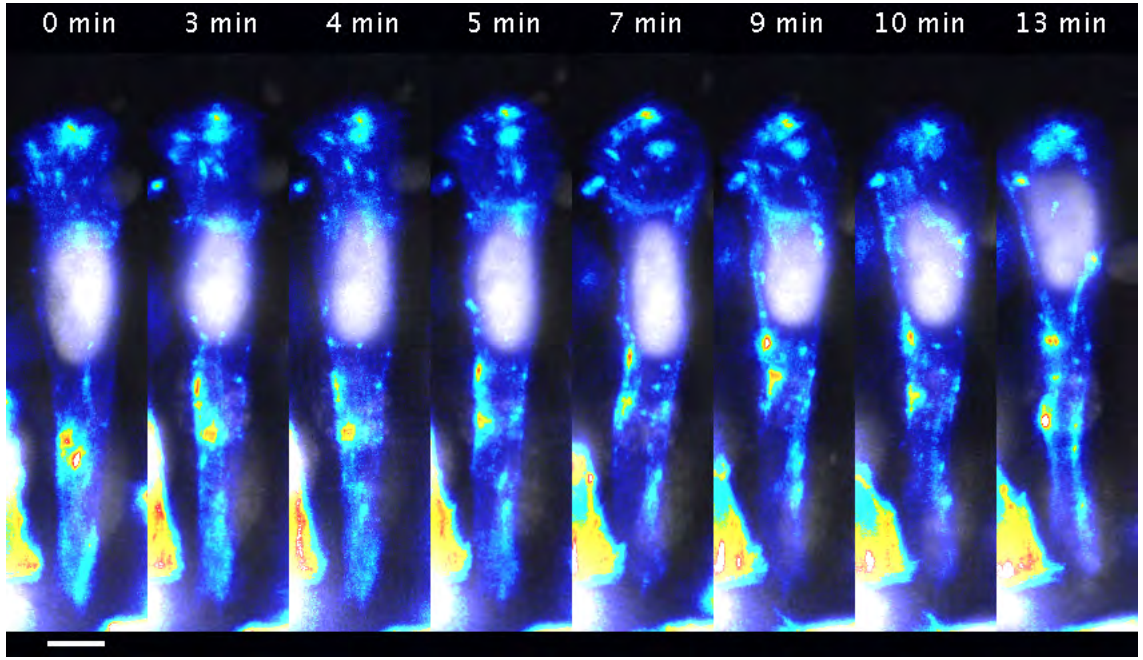




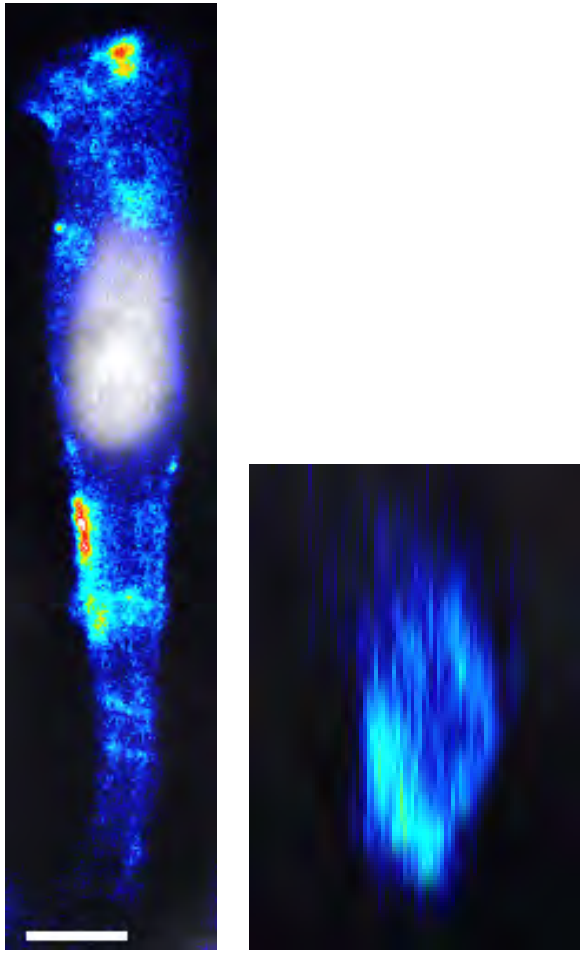


Mean ± SD

Formin3 localizes basal to the nucleus during apical migration



Formin3
Nucleus



x/y axis

x/z axis

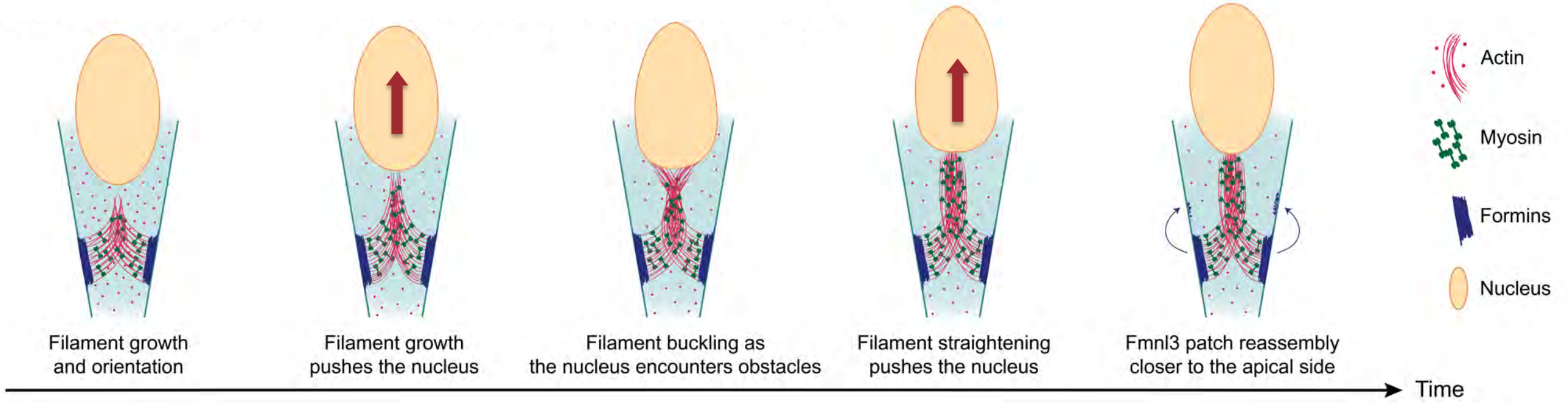
Formin3 DN overexpression (Fmnl3ΔC) leads to nuclear stalling in the retina

Overview of drug conditions

	Small molecule inhibitor	Inhibited process	Apical migration perturbed	
			Hindbrain	Retina
Positive controls	Blebbistatin	Myosin II activity	✓	✓
Positive controls	Latrunculin A + Jasplakinolide	Actin turnover	✓	✓
Contraction	Rockout	Rho-kinase-dependent myosin activation	✓	X
Contraction	ML-7	MLCK-dependent myosin activation	X	✓
Expansion	ck-666	Arp2/3-dependent actin nucleation	X	X
Expansion	SMIFH2	Formin-dependent actin nucleation	X	✓

All findings were backed up with genetic conditions

Proof-of-principle toy model of formin dependent positioning



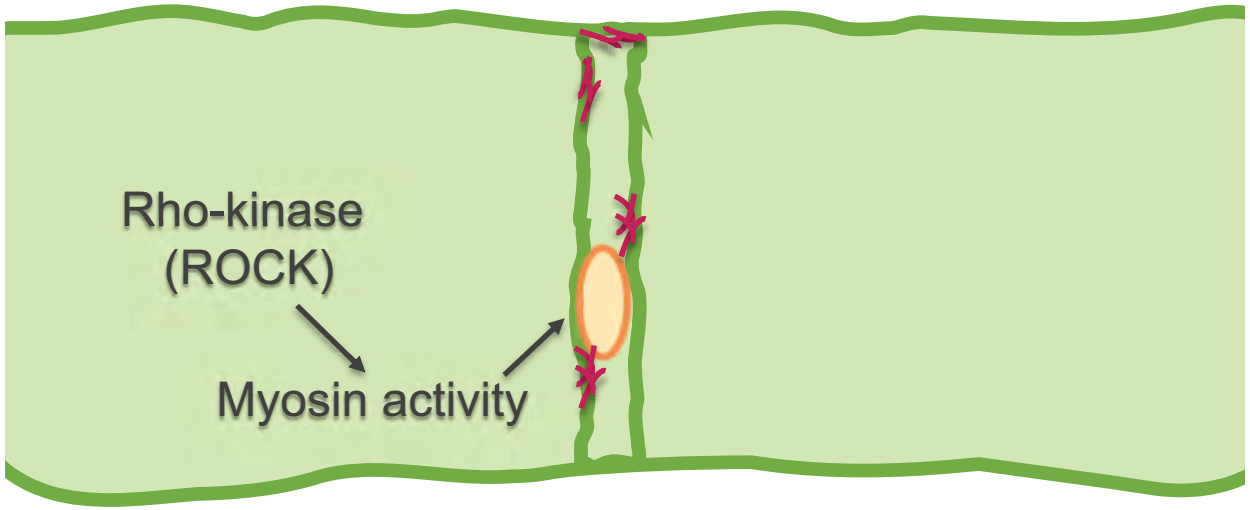
with Carl Modes

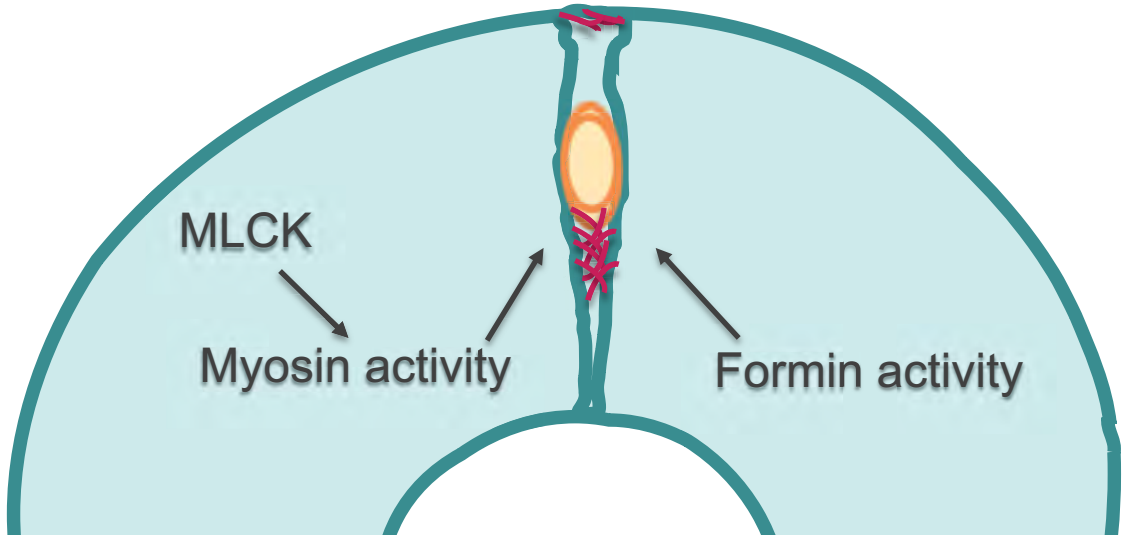
$\kappa_+ = 0.3\ \mu m/min$ * Hotulainen and Lappalainen, 2006

$v = 0.3\ \mu m/min$

Contractility based model also a possibility

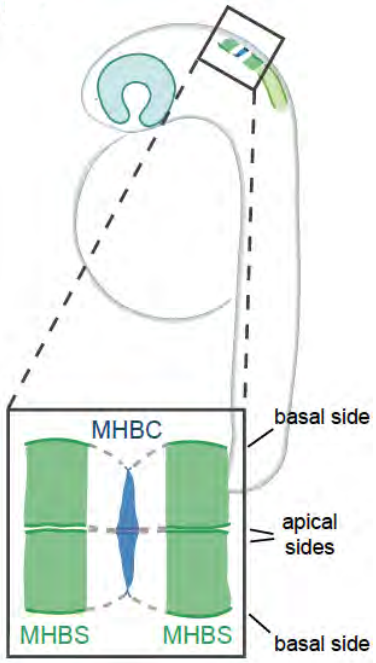
Nuclear positioning mechanisms differ in close neuroepithelia





Is this related to differences in tissue shape?

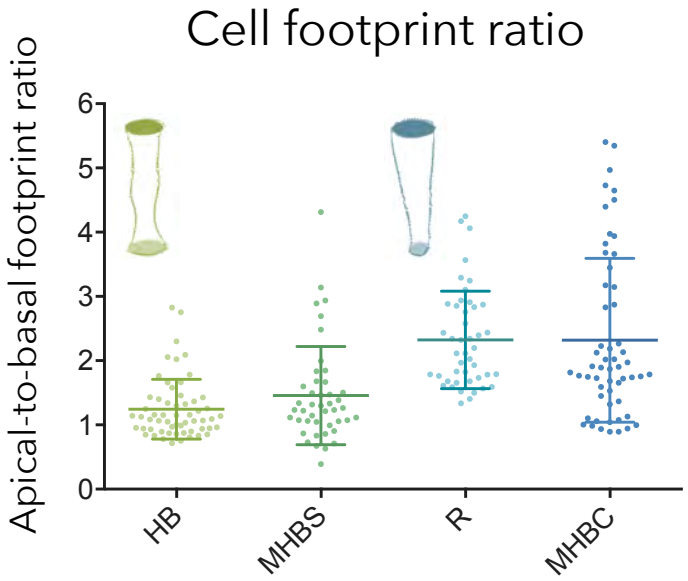
Basally constricted and straight tissues have different features



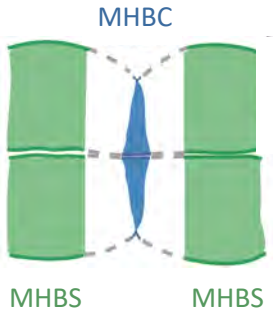
Midbrain-hindbrain boundary (MHB)

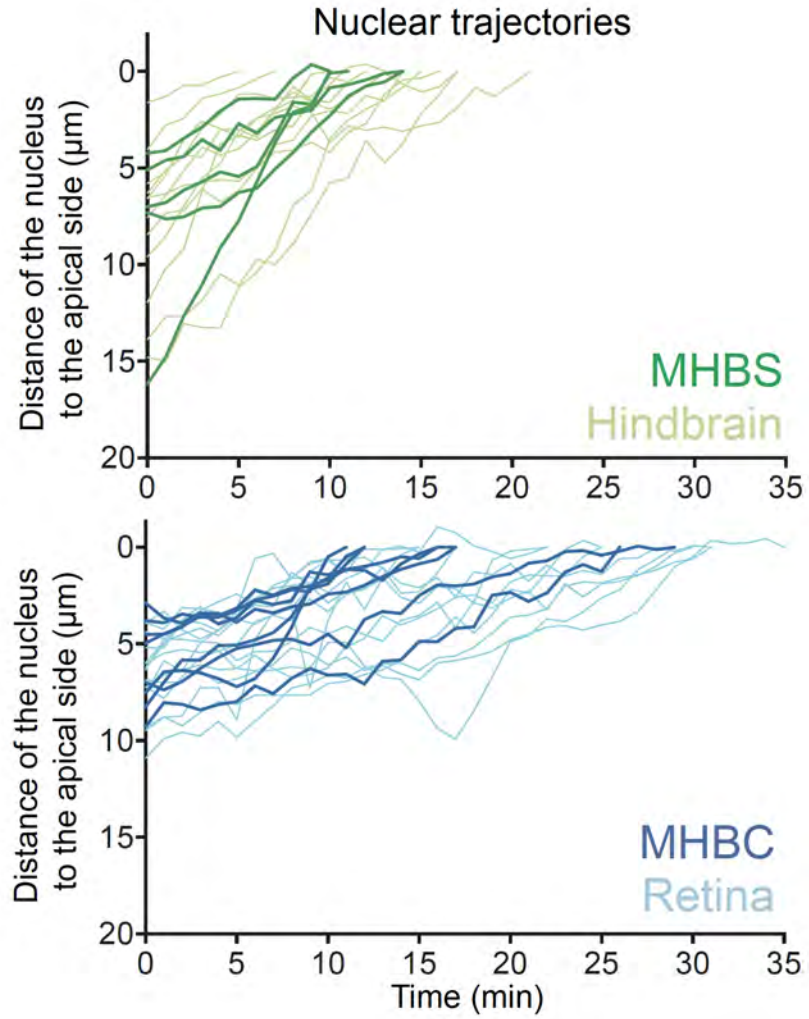
MHBS → straight

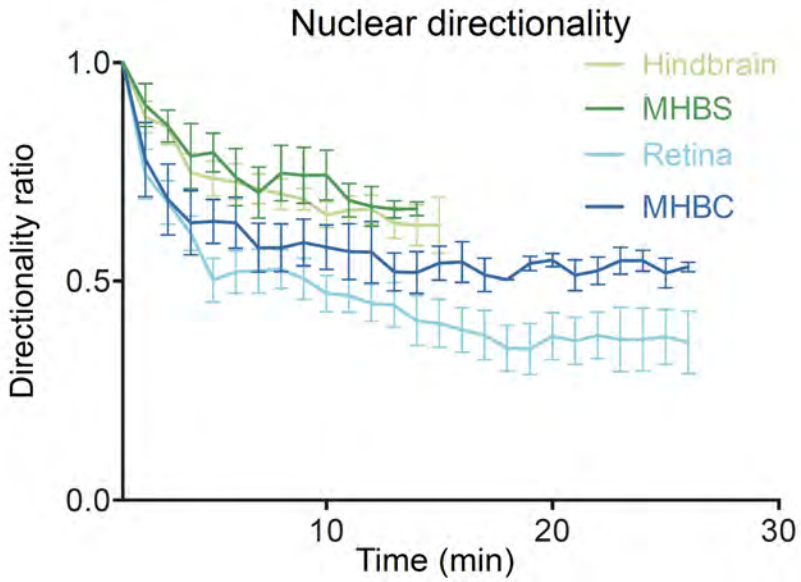
MHBC → constriction



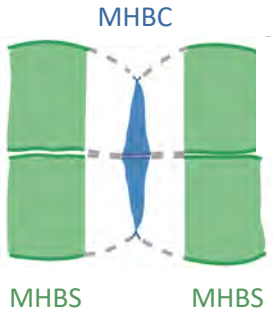
Nuclear positioning kinetics are tissue shape dependent

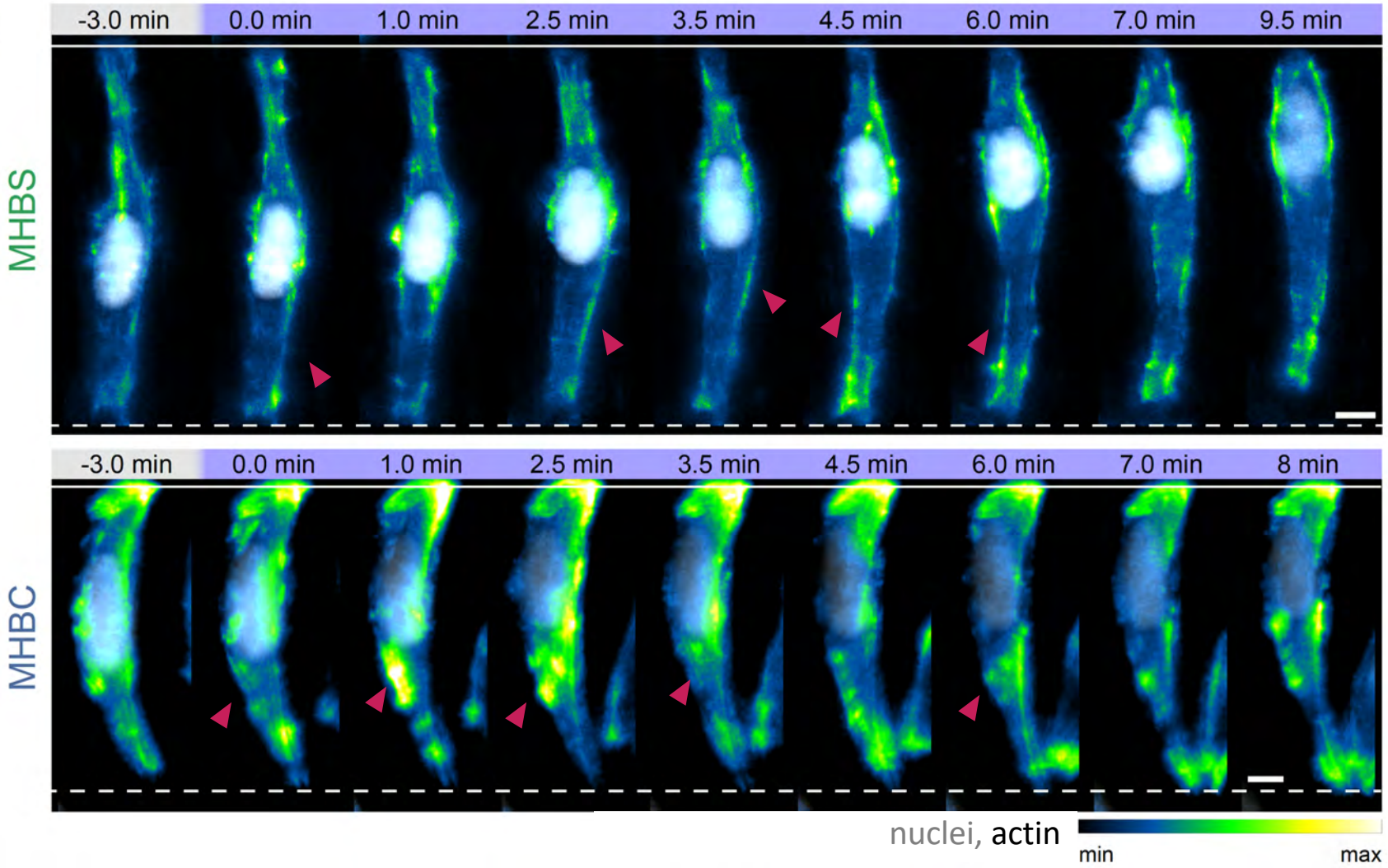




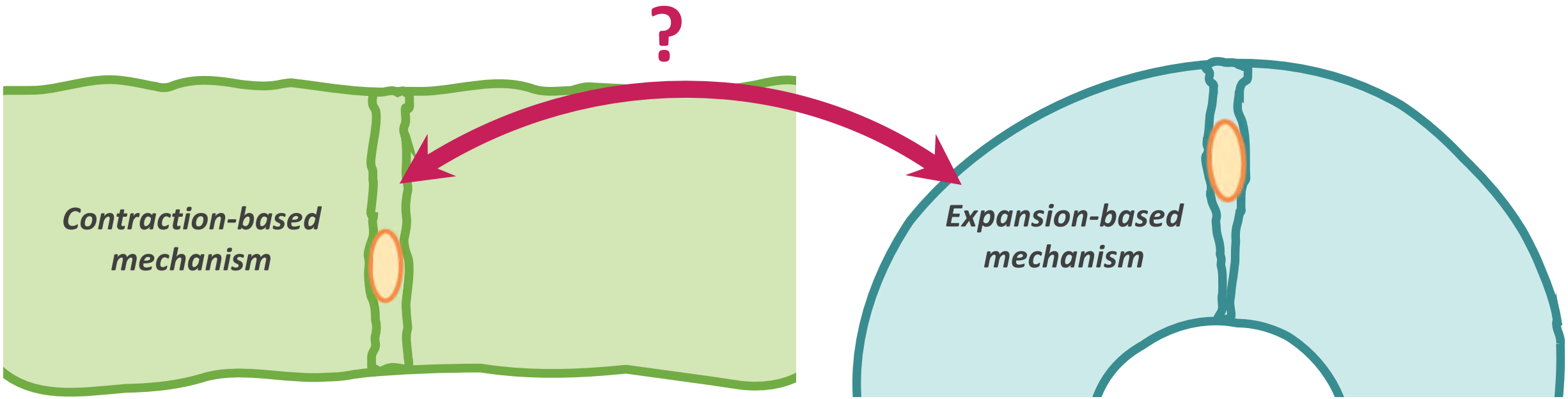


Actin distribution during nuclear positioning is tissue shape dependent

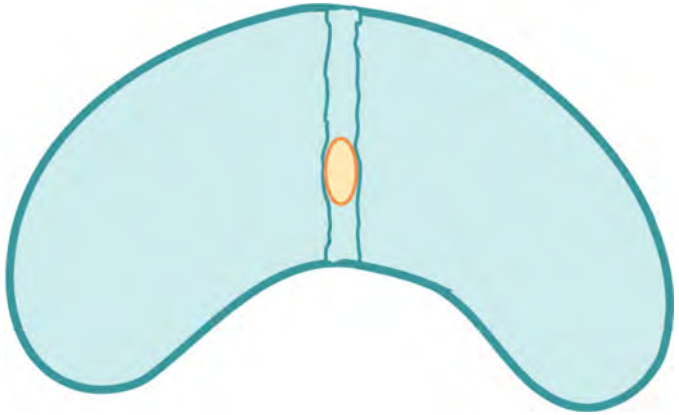


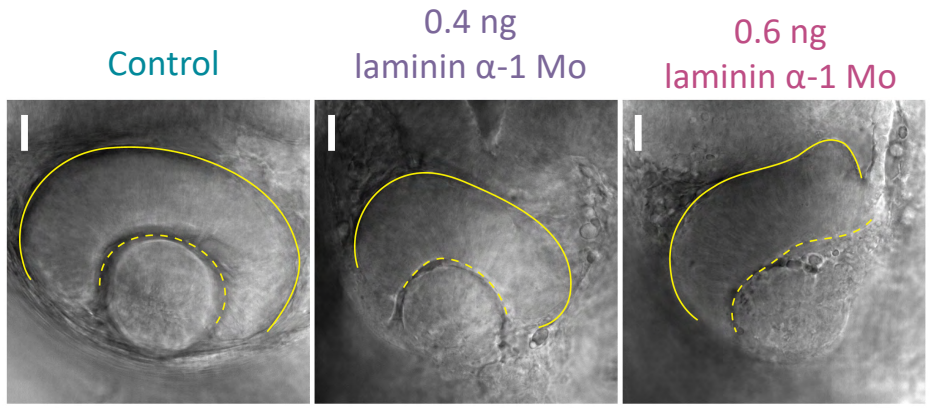


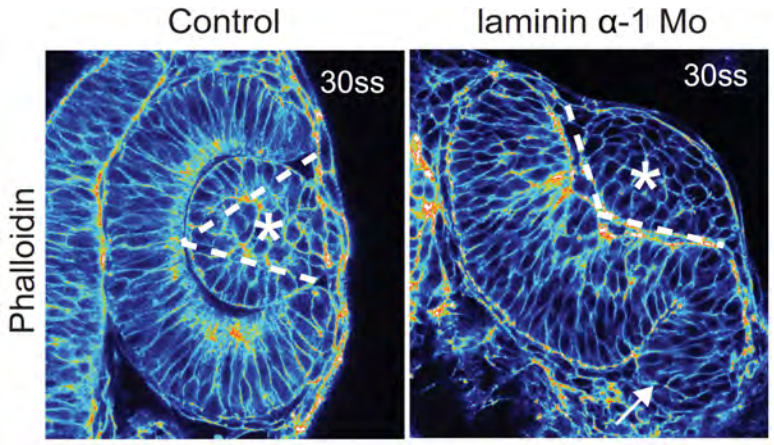
Do tissue shape changes result nuclear positioning mechanism changes ?



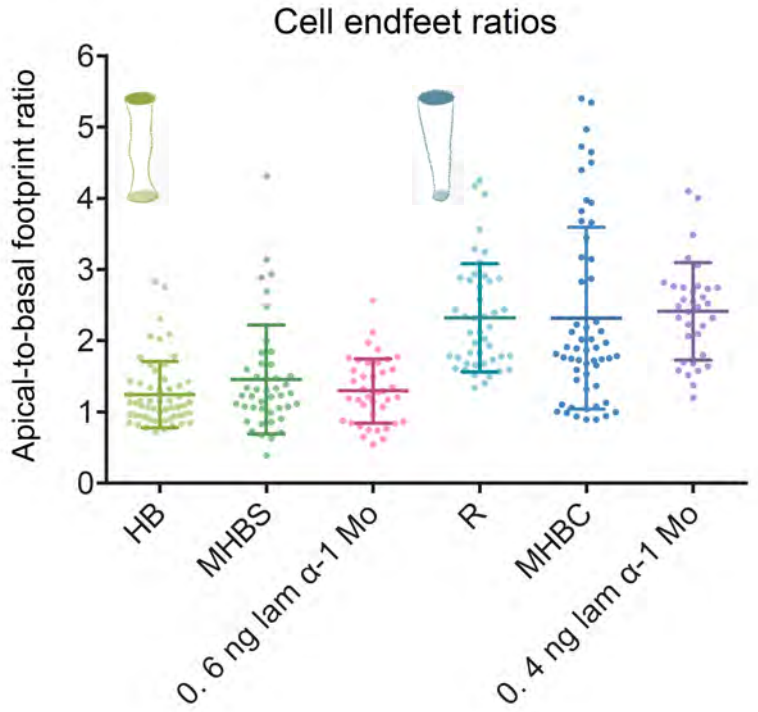
Flattening the retina leads to cell shape changes



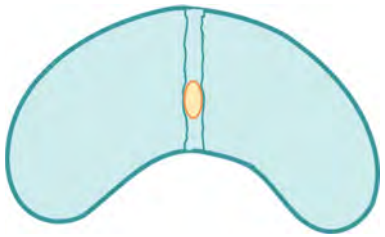


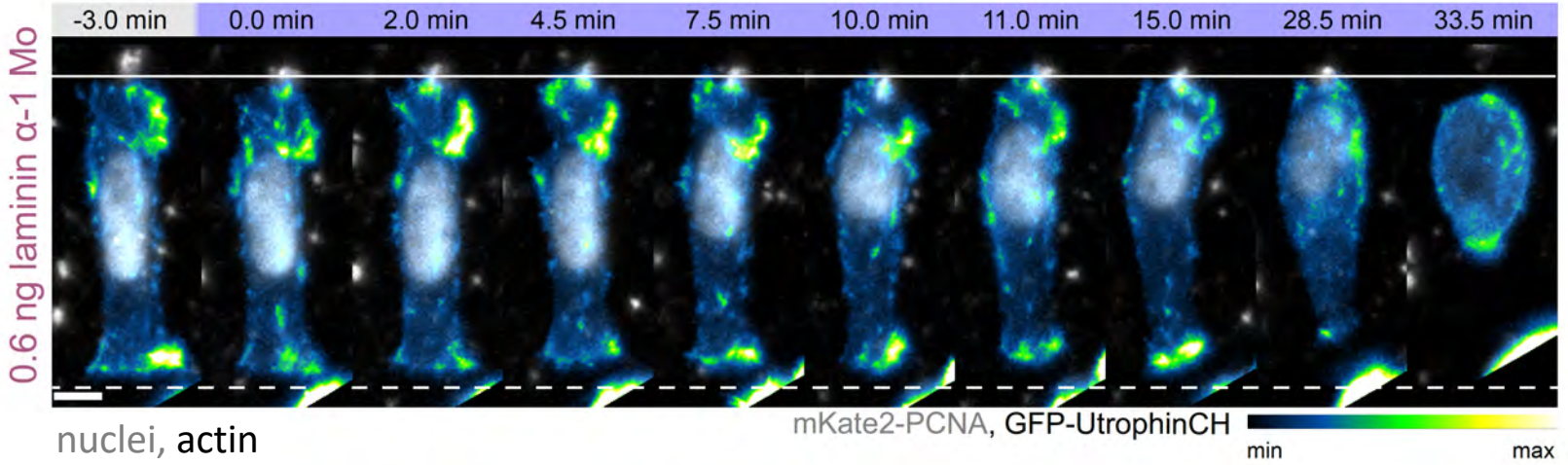


Sidhaye and Norden, 2017



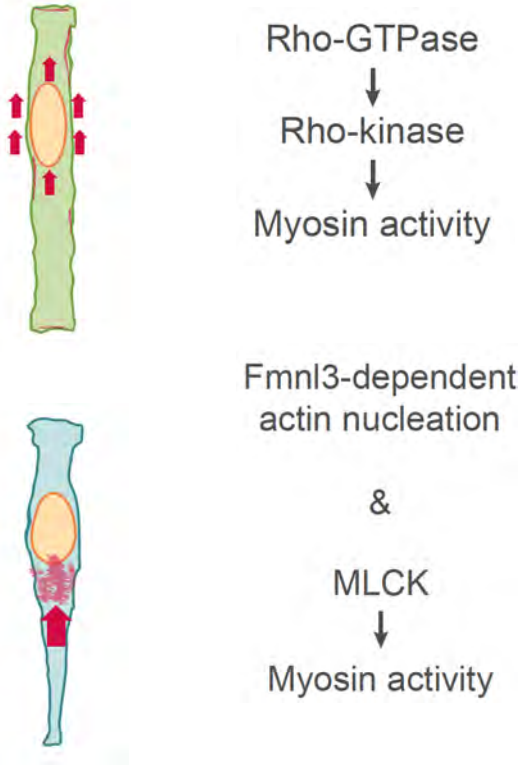
Cell shape changes lead to changes in apical nuclear positioning



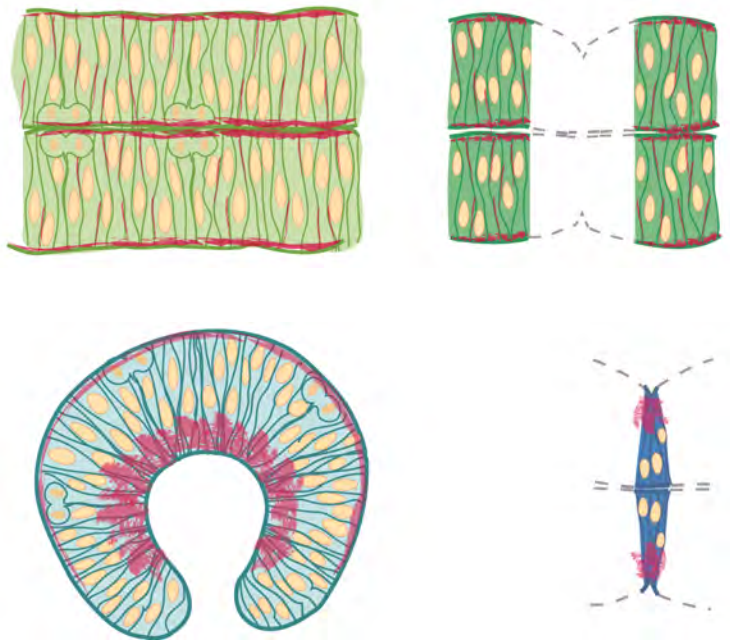


Summary

Distinct actin-dependent mechanisms move nuclei in different tissues



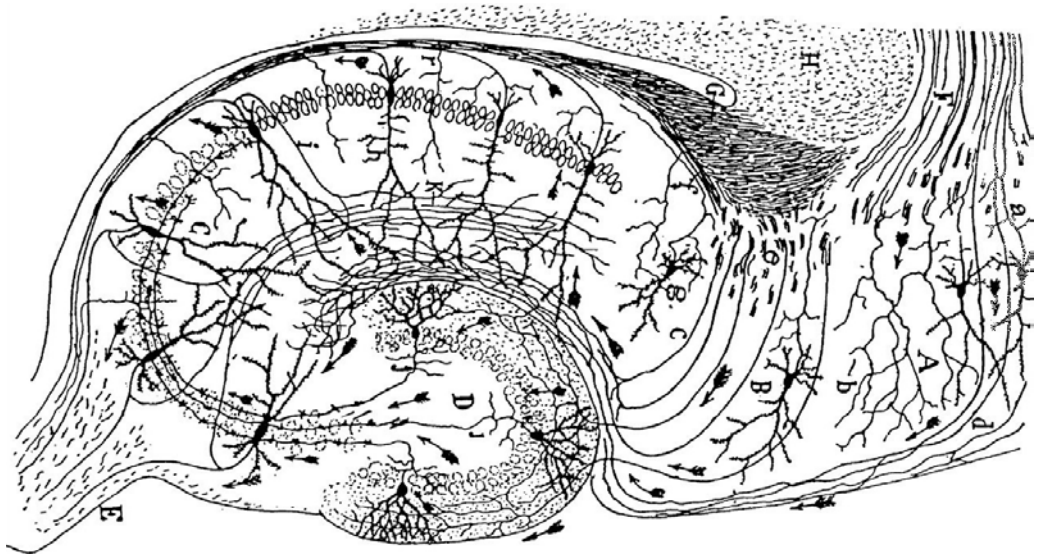
Tissue shape influences the mechanism of apical migration



Yanakieva, Erzberger, Matejčić, Modes and Norden (JCB, accepted and on BioRxiv)

In tissue morphogenesis the end result can justify the many means!

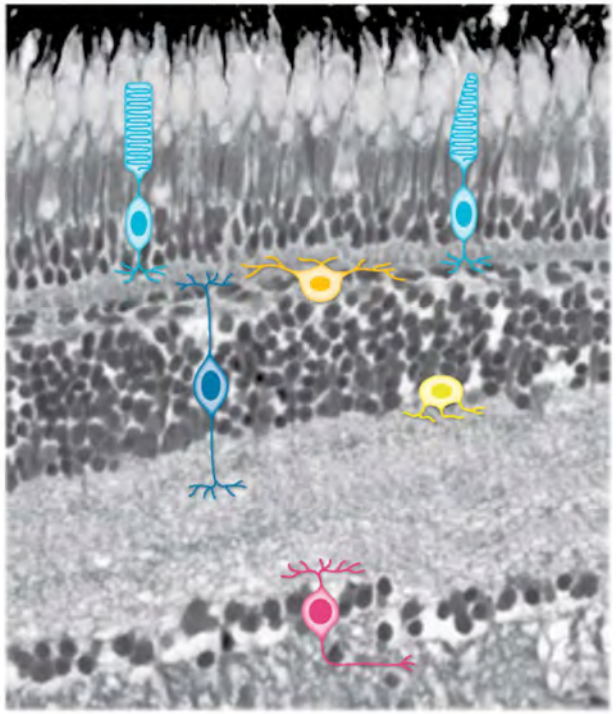
How do neurons navigate to correct positions and build functional interconnected layers?



Santiago Ramon y Cajal

Correct retinal lamination is a prerequisite for function

Zebrafish Retina

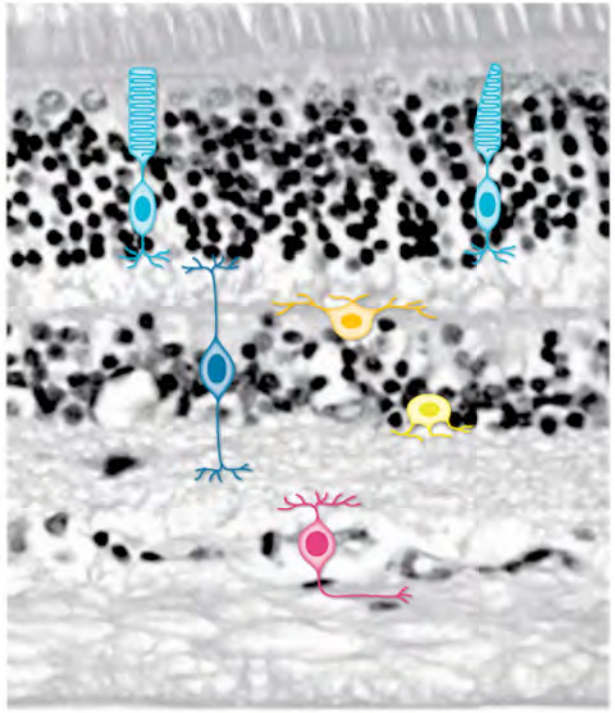


ONL

INL

GCL

Human Retina



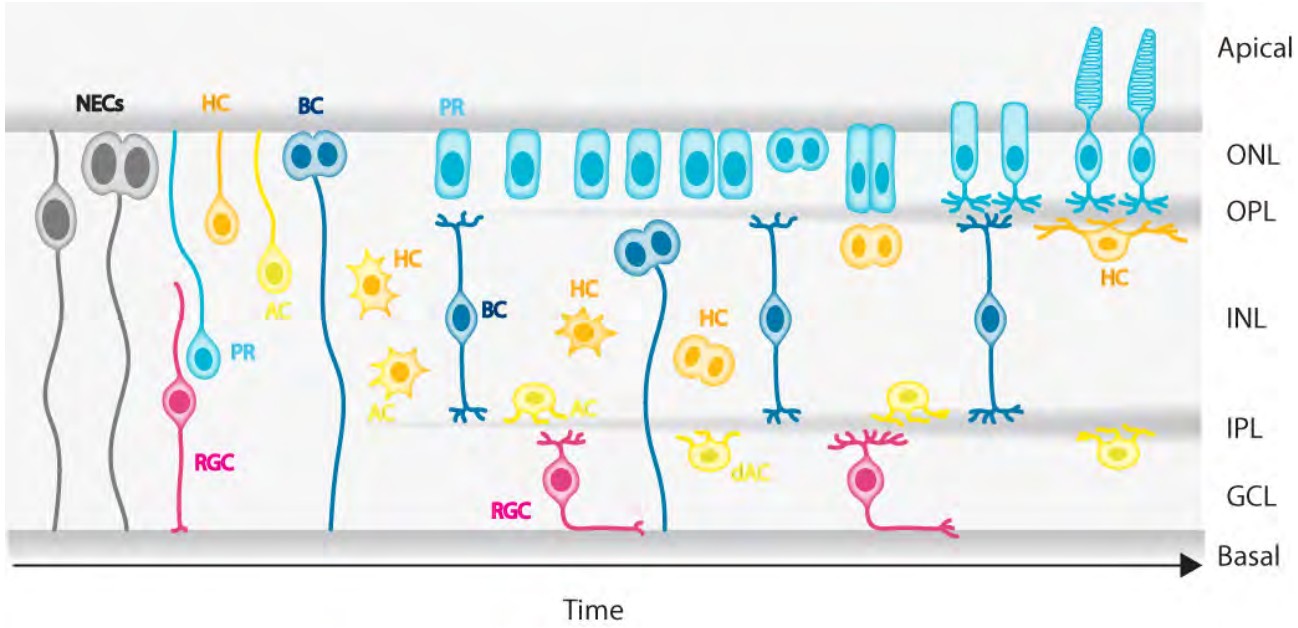
Outer nuclear layer

Inner nuclear layer

Ganglion cell layer

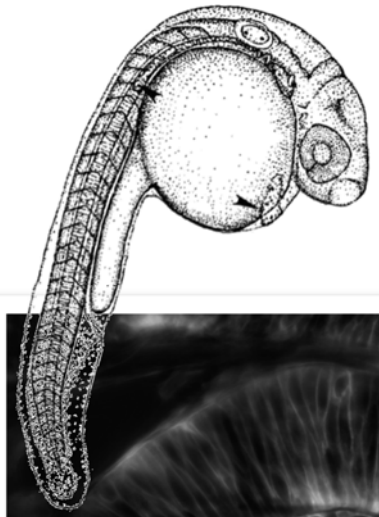
Retinal neuronal migration and lamination is complex

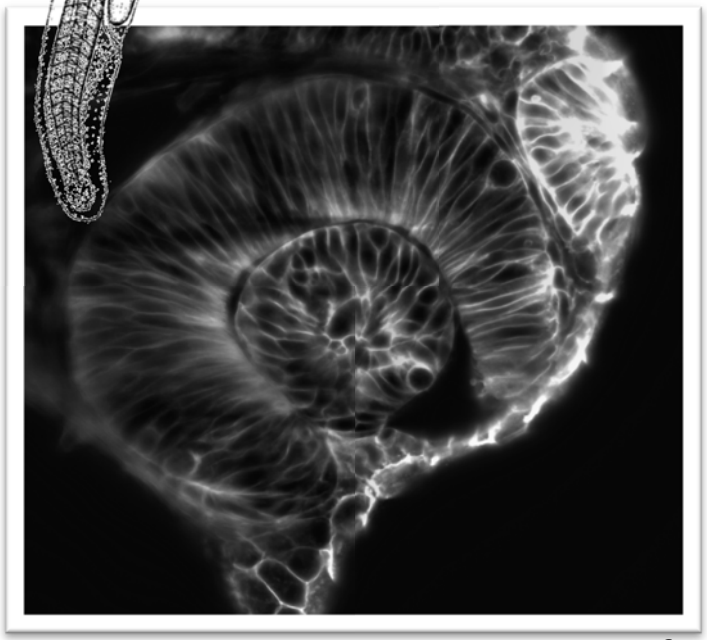
Zebrafish Retina



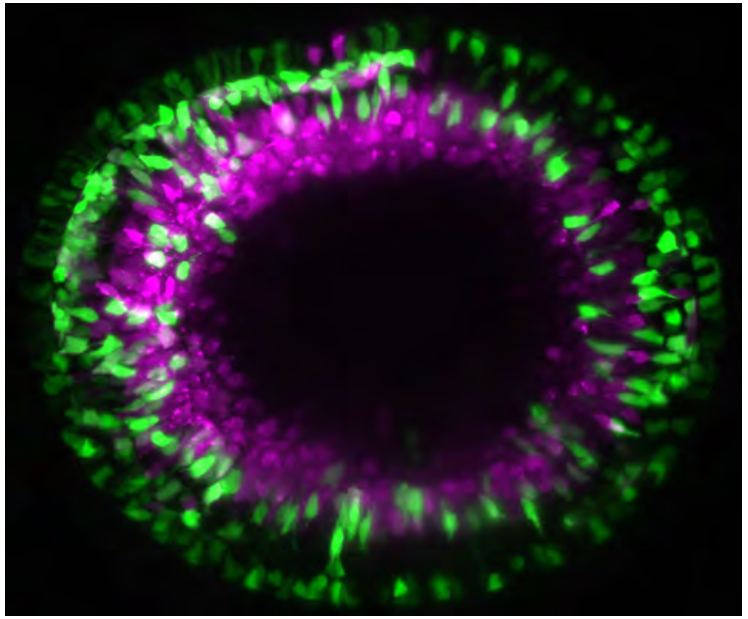
Weber...Norden, Cell Reports 2014, Chow…Harris and Norden, Development 2015, Icha…Norden, JCB 2016, Amini, Rocha, Norden, Frontiers, 2018

Order-Chaos-Order

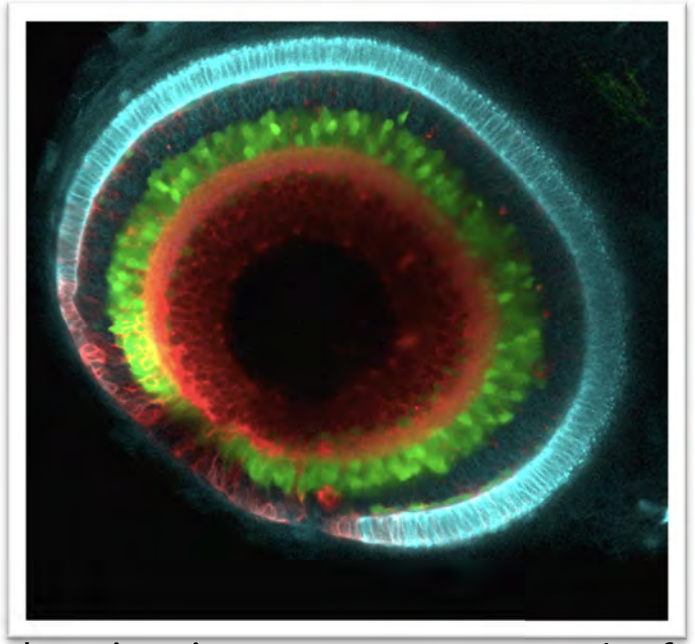




Neuroepithelium 30 hpf



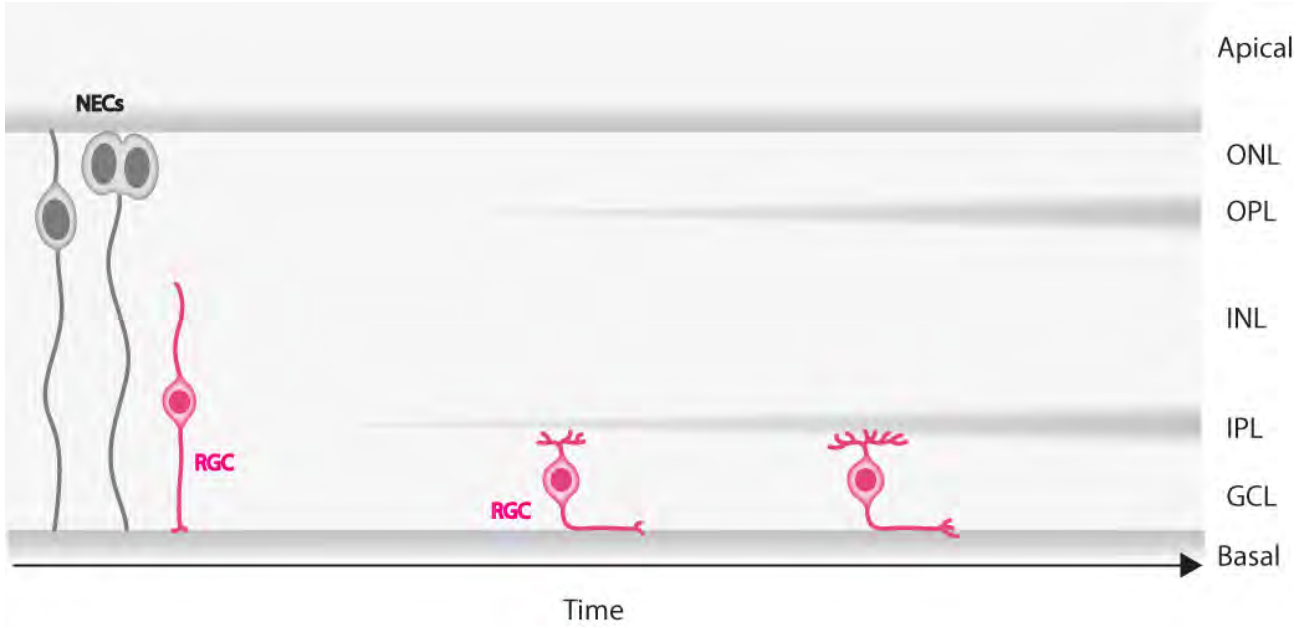
Migration and Lamination 56 hpf



Lamination 80 hpf

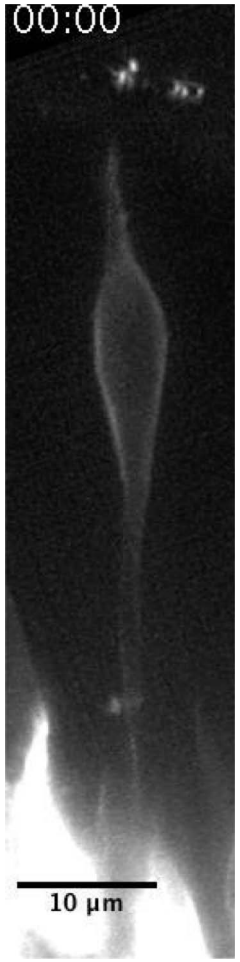
Starting at the beginning: Retinal ganglion cell migration

Zebrafish Retina

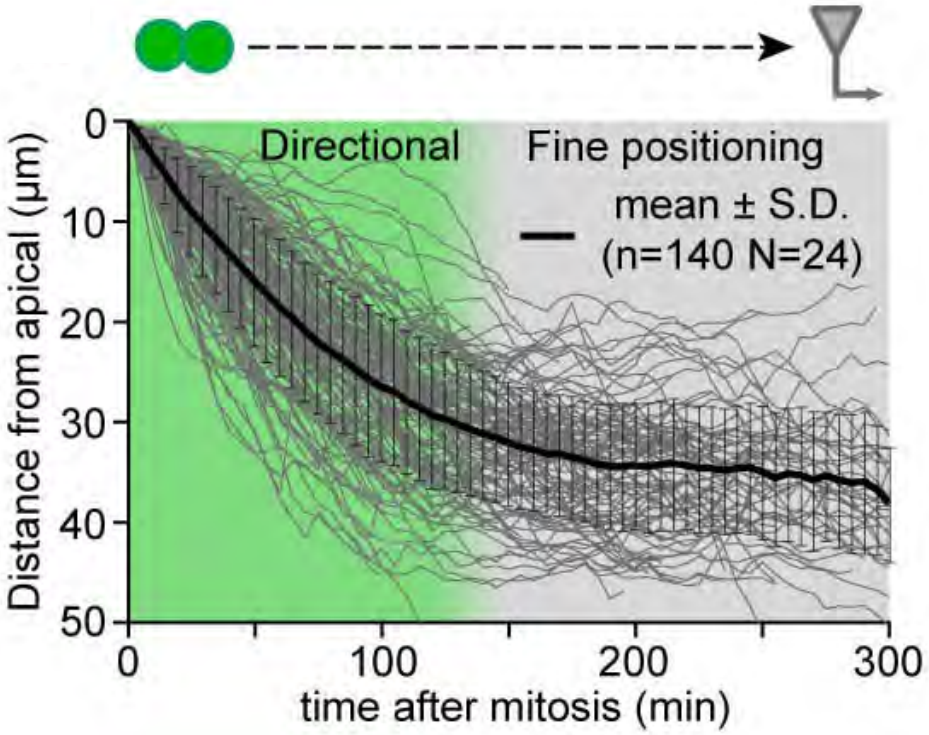


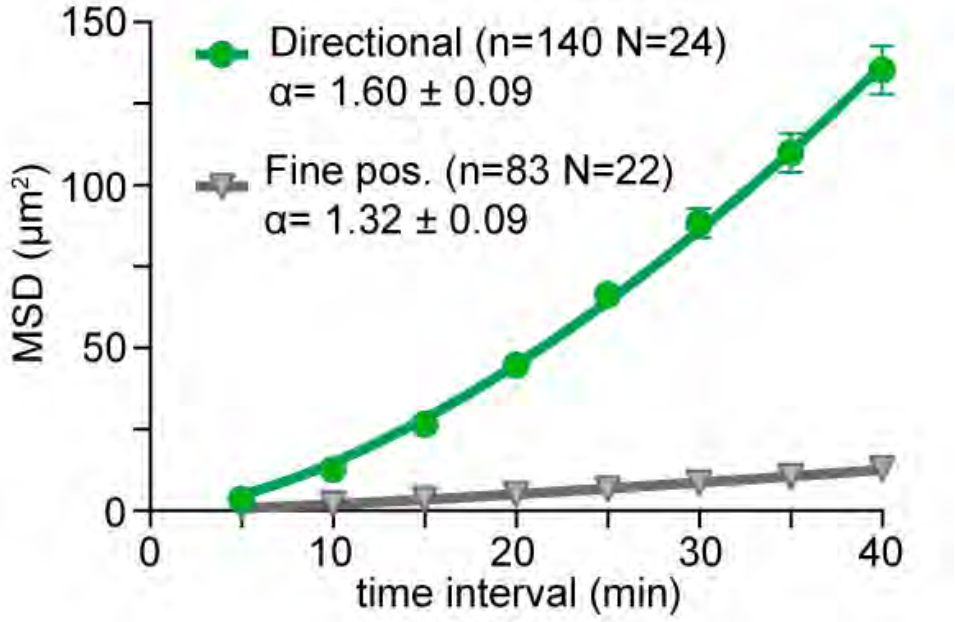
Weber...Norden, Cell Reports 2014, Chow…Harris and Norden, Development 2015, Icha…Norden, JCB 2016, Amini, Rocha, Norden, Frontiers, 2018

Quantitative analysis of retinal ganglion cell movements



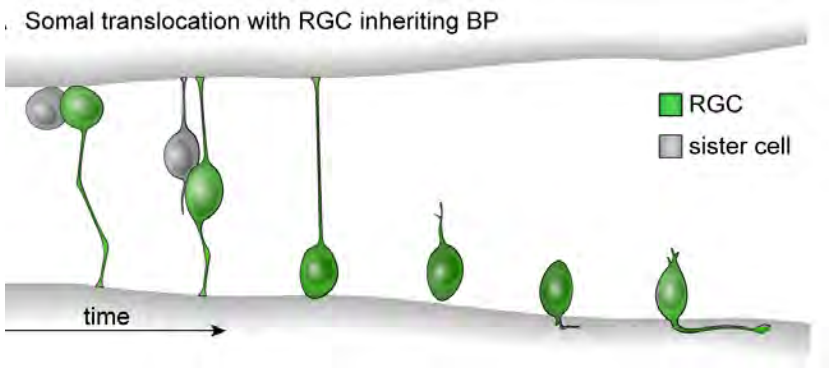
control





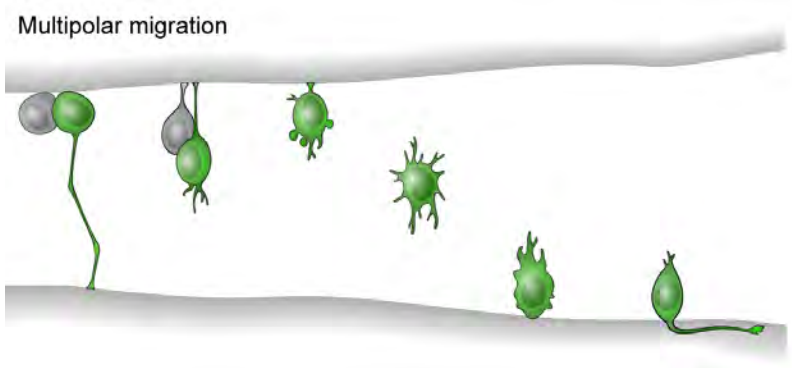
Two types of retinal ganglion cell translocation

Somal translocation



- Directed with fast kinetics
- Basal process reattachement
- Stabilized apical microtubules

Multipolar translocation

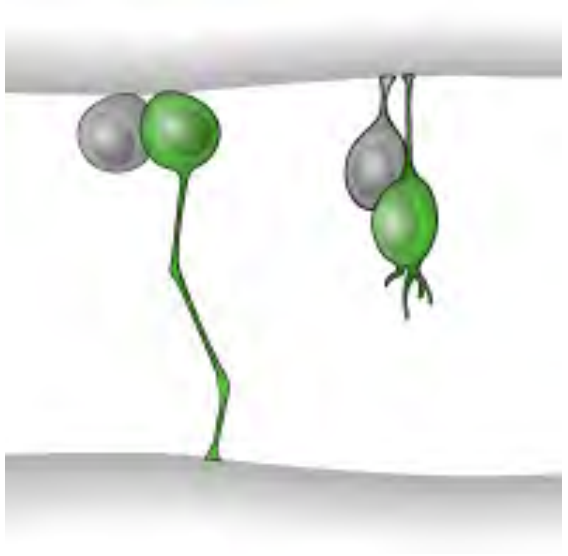


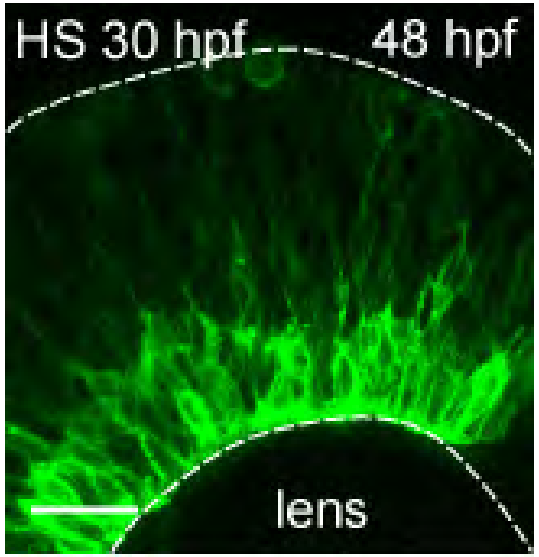
- Directed with slower kinetics
- Loss of apical process
- Actin dependent

End result is similar

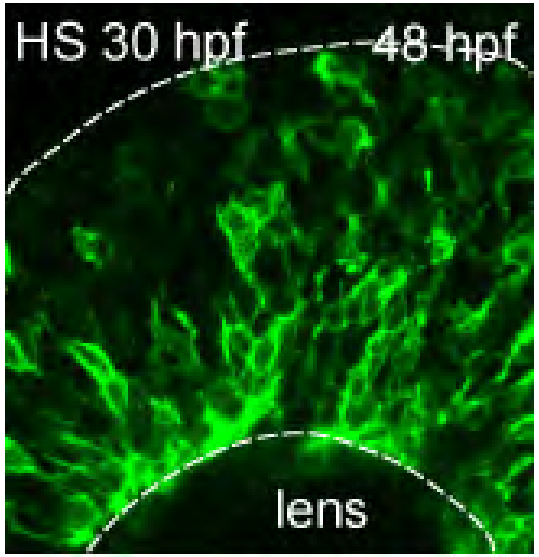
Icha et al., J. Cell Biol. 2016

Both modes impaired: translocation fails



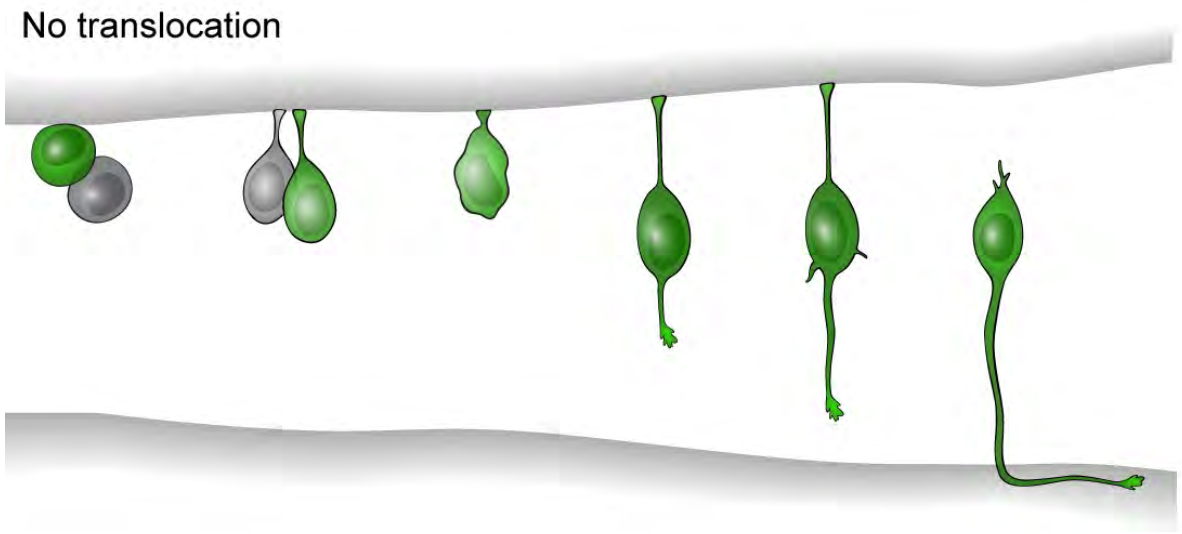


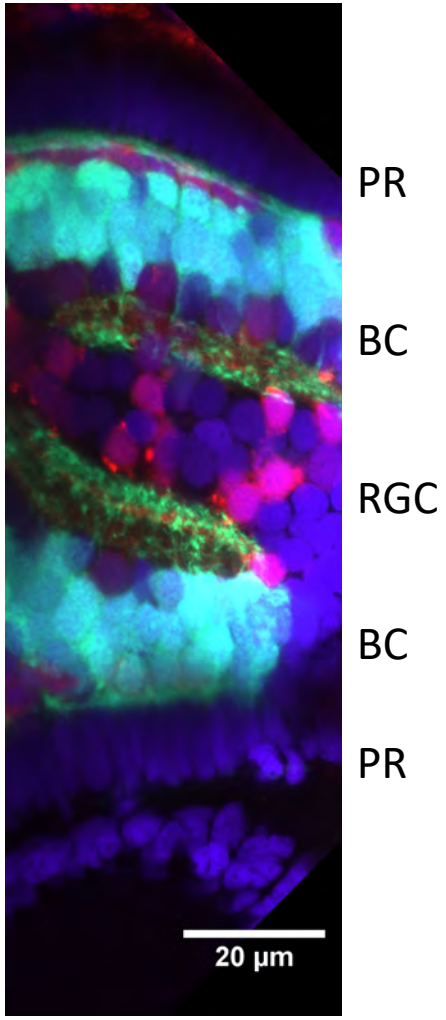
Ath5-GFP 48hpf control



Ath5-GFP 48hpf basal process block apical process stays

Impairment of both modes: Ectopic lamination





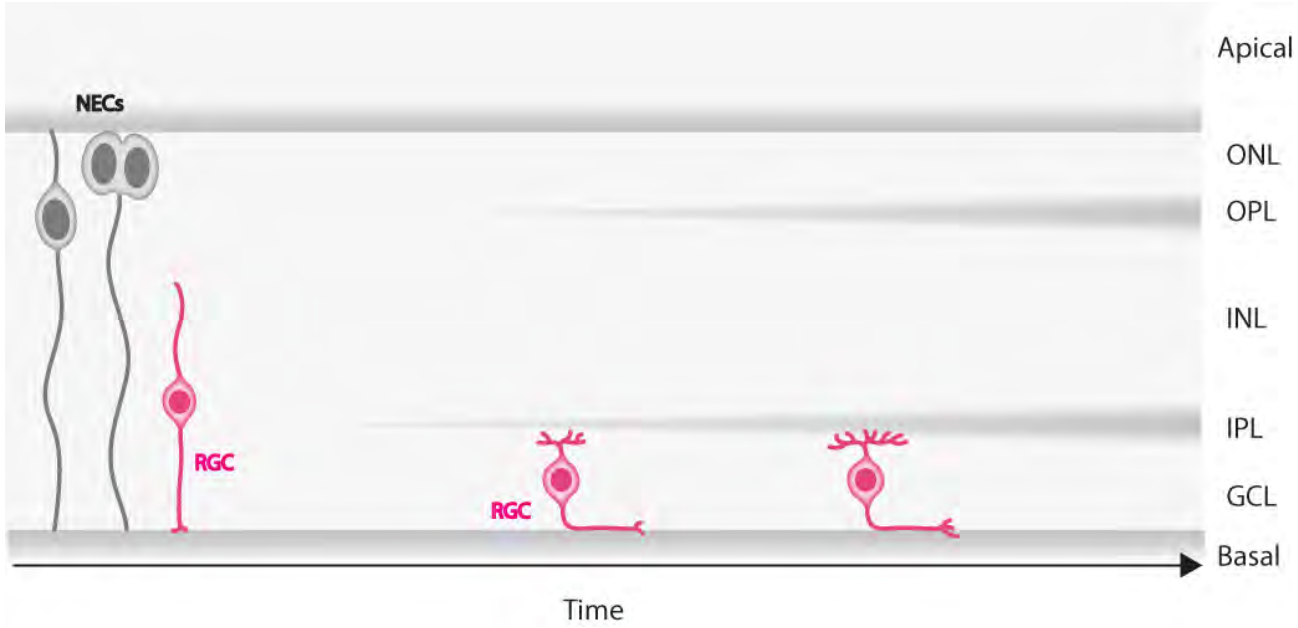
Serious problems with retinal lamination

Retinal ganglion cell translocation sets the stage for all further retinal lamination

Icha et al., J. Cell Biol. 2016

Retinal ganglion cell migration

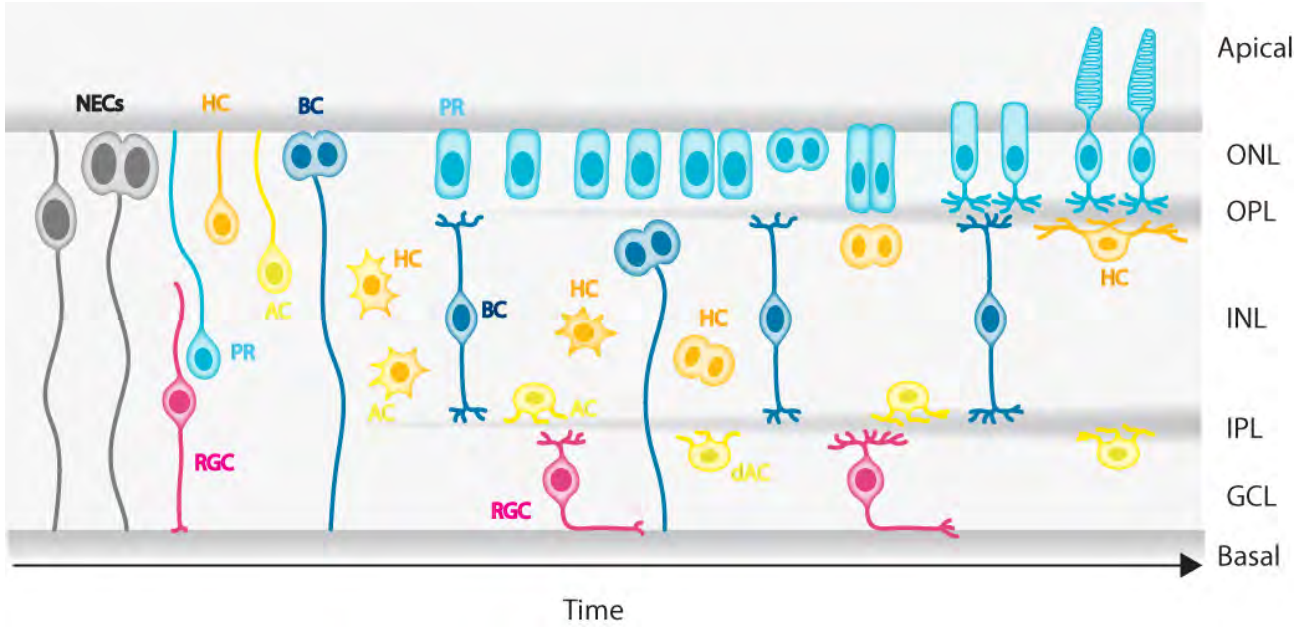
Zebrafish Retina



Icha…Norden, JCB 2016

Retinal neuronal migration and lamination is complex

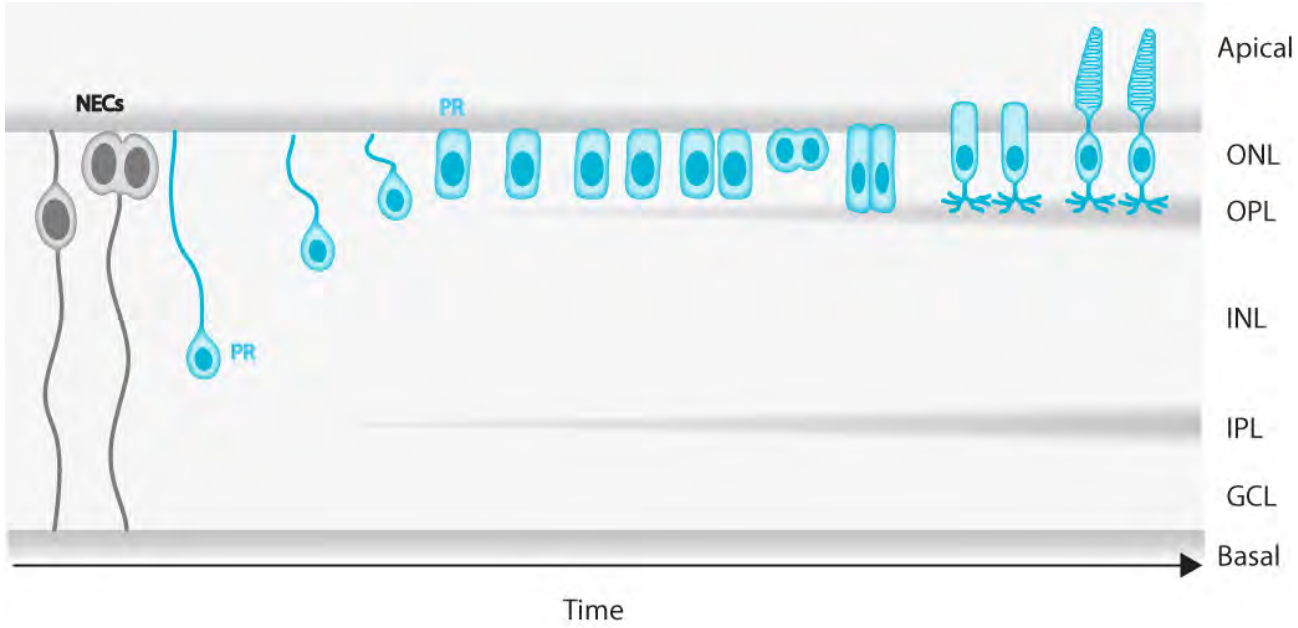
Zebrafish Retina



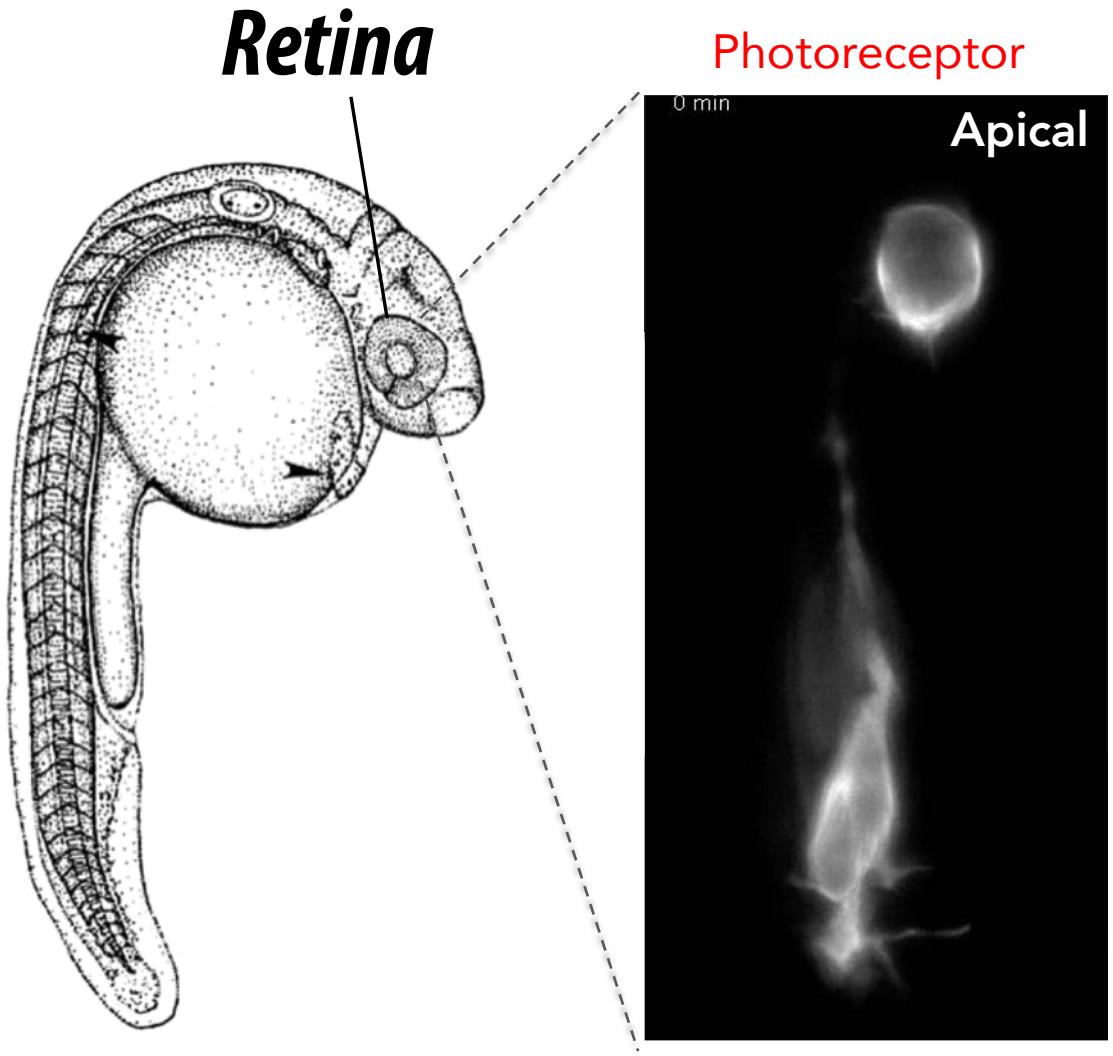
Weber...Norden, Cell Reports 2014, Chow…Harris and Norden, Development 2015, Icha…Norden, JCB 2016, Amini, Rocha, Norden, Frontiers, 2018

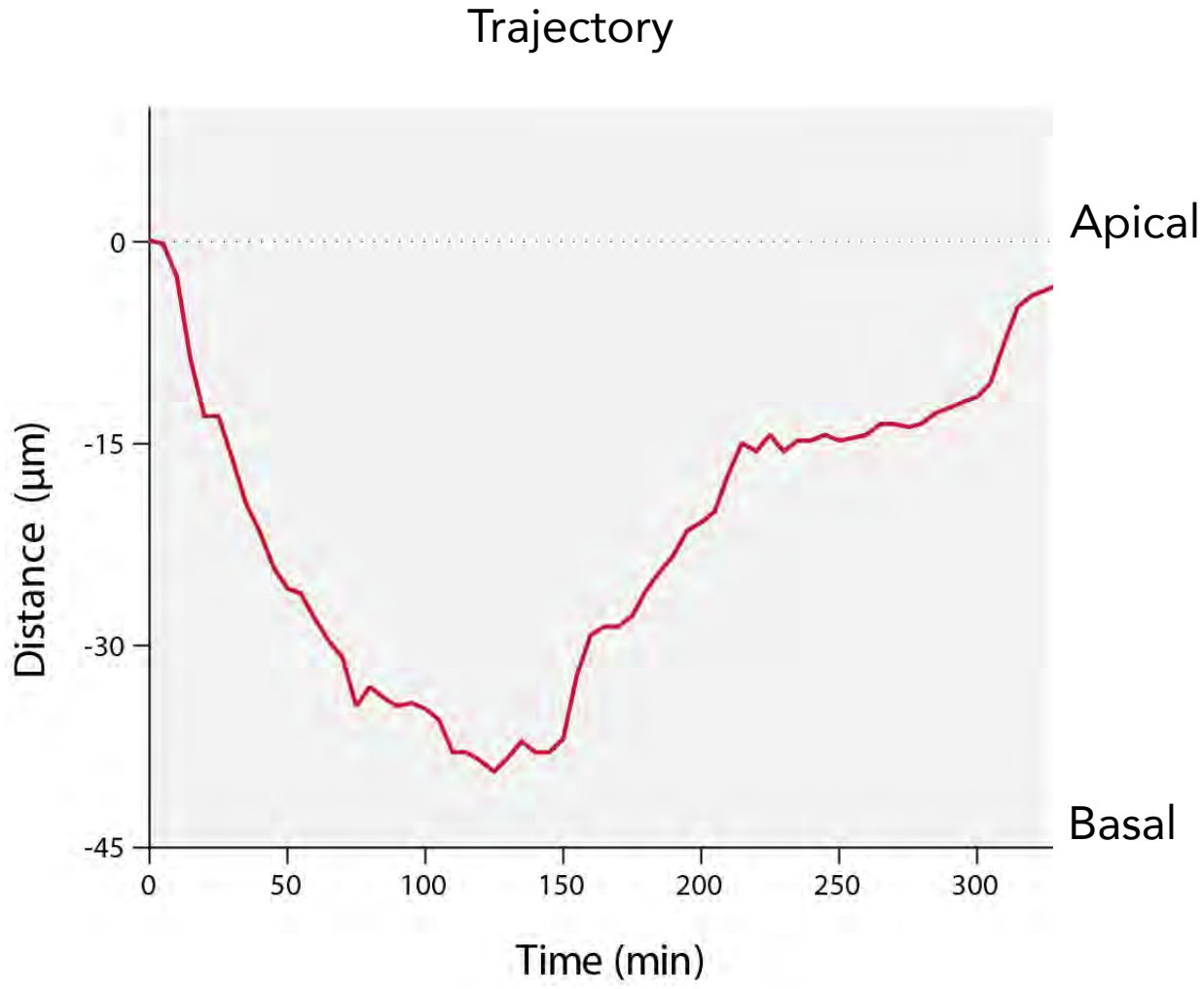
Moving on: Photoreceptors

Zebrafish Retina

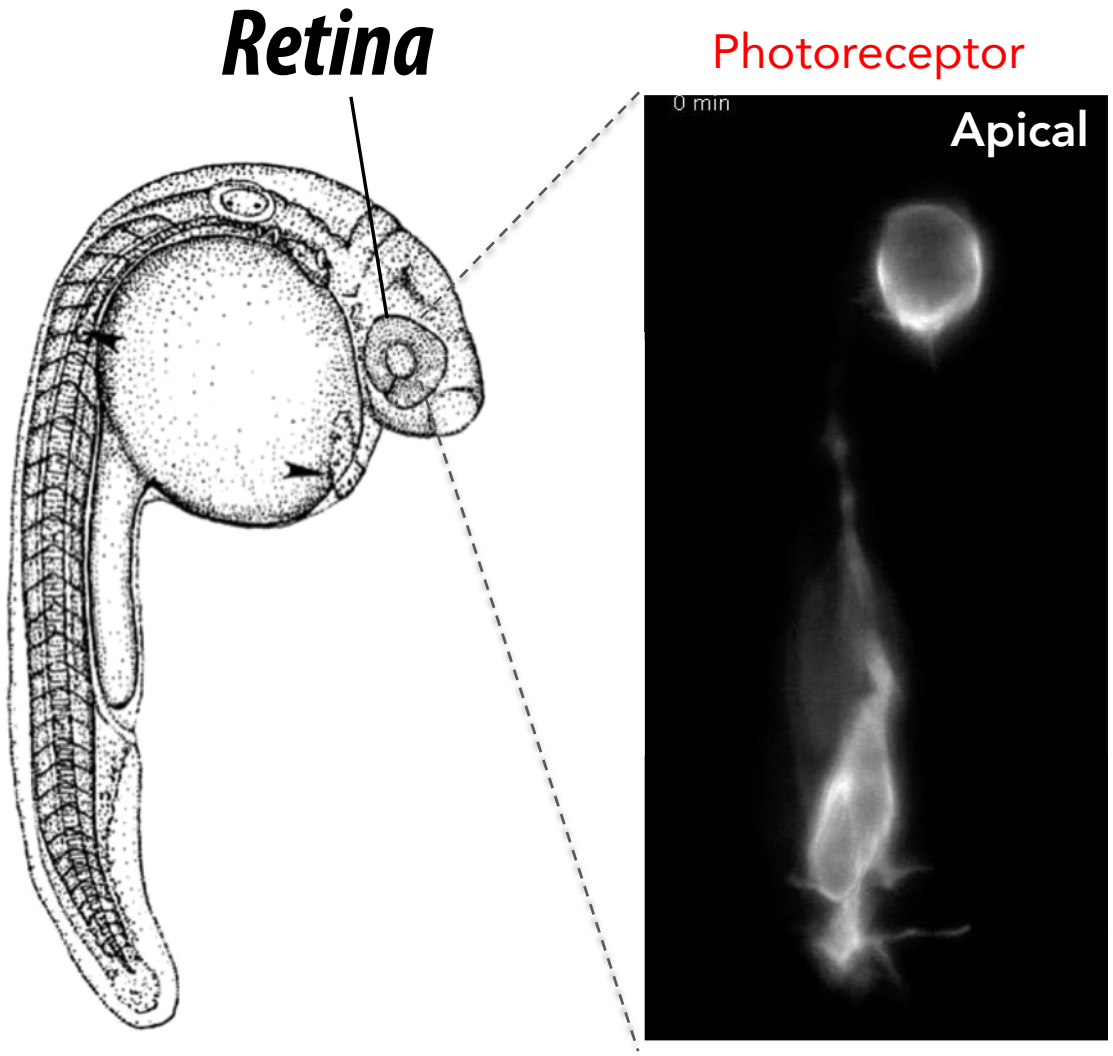


Photoreceptors move bidirectionally before lamination



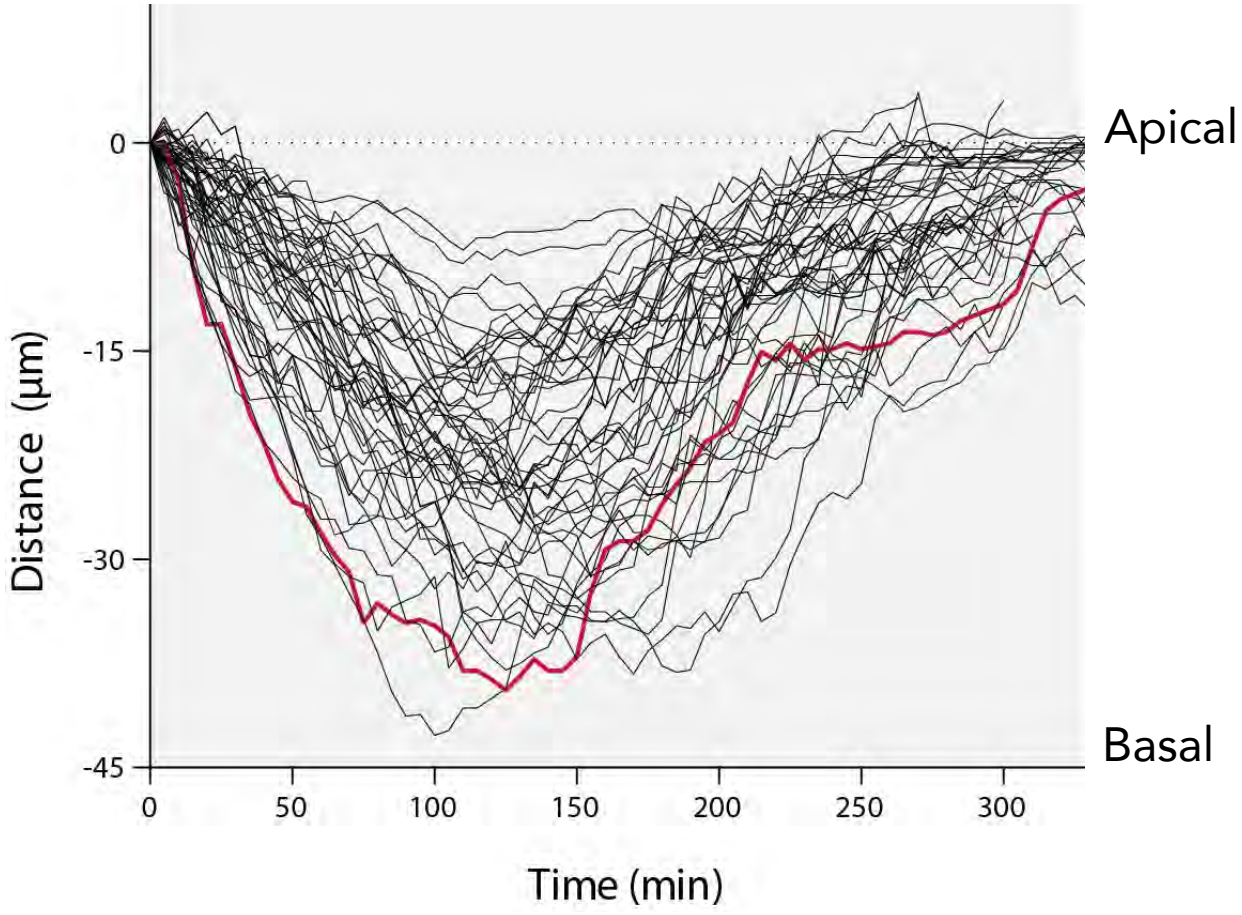


Photoreceptors move bidirectionally before lamination



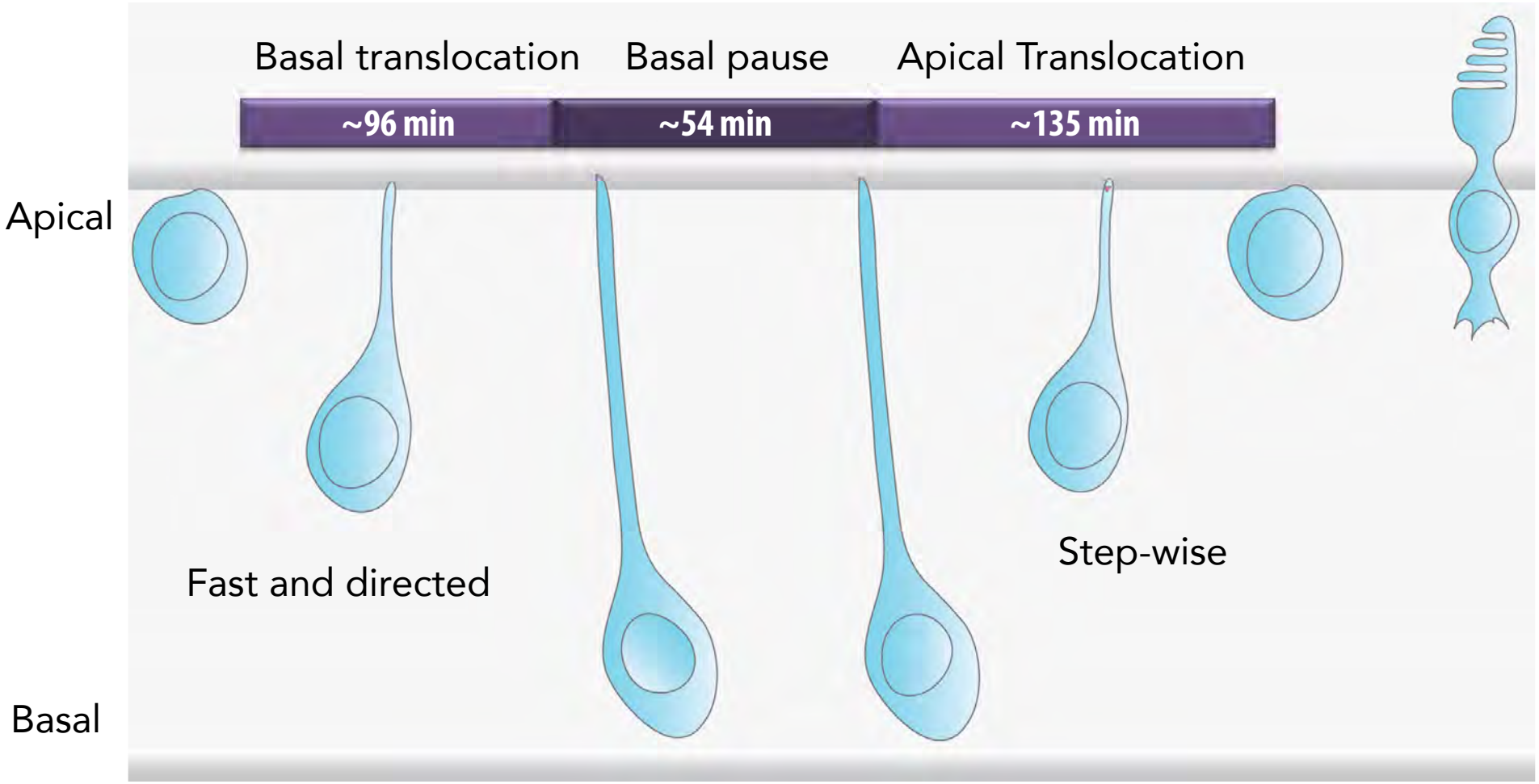
LZ1 light-sheet

Trajectories



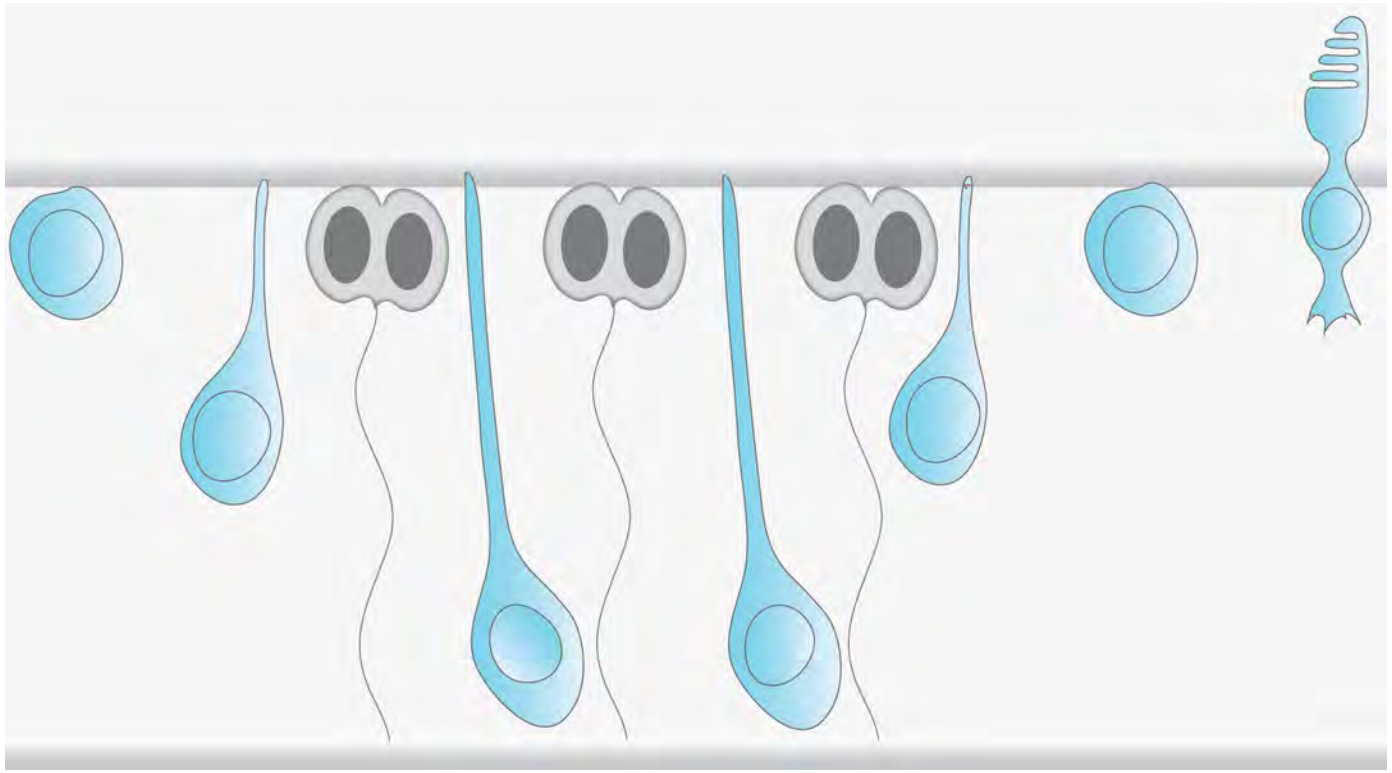
n=41 cells from 14 embryos

Basal and apical movement show different kinetics



n=41 cells from 14 embryos

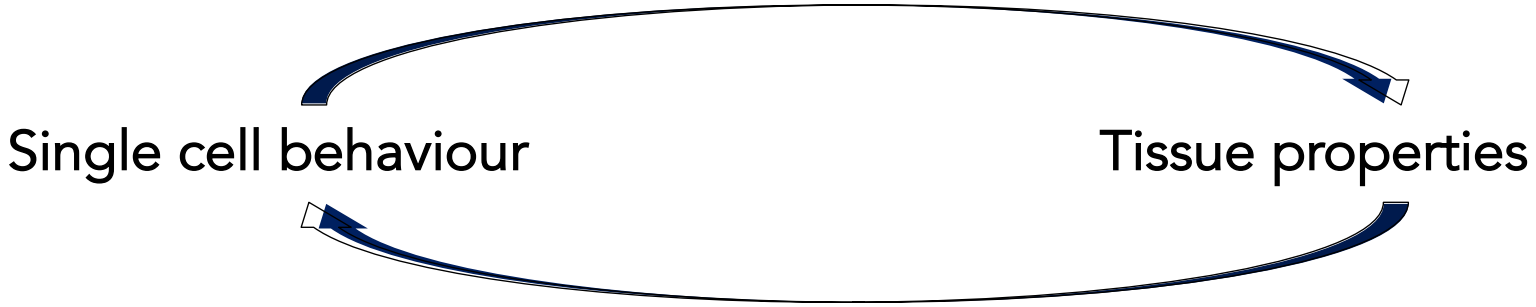
Understanding cells in tissue needs understanding of the tissue



1- PRs undergo bidirectional migration

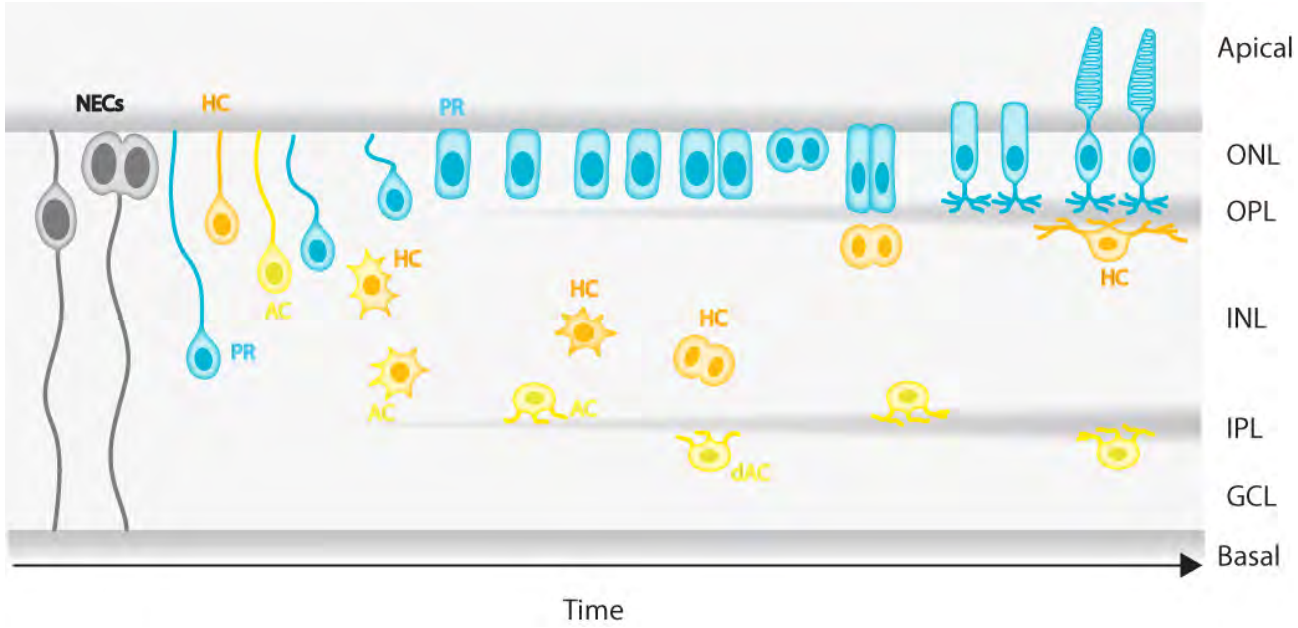
2- Different movements have different kinetics and mechanisms

3- PRs move to make space for progenitor cells



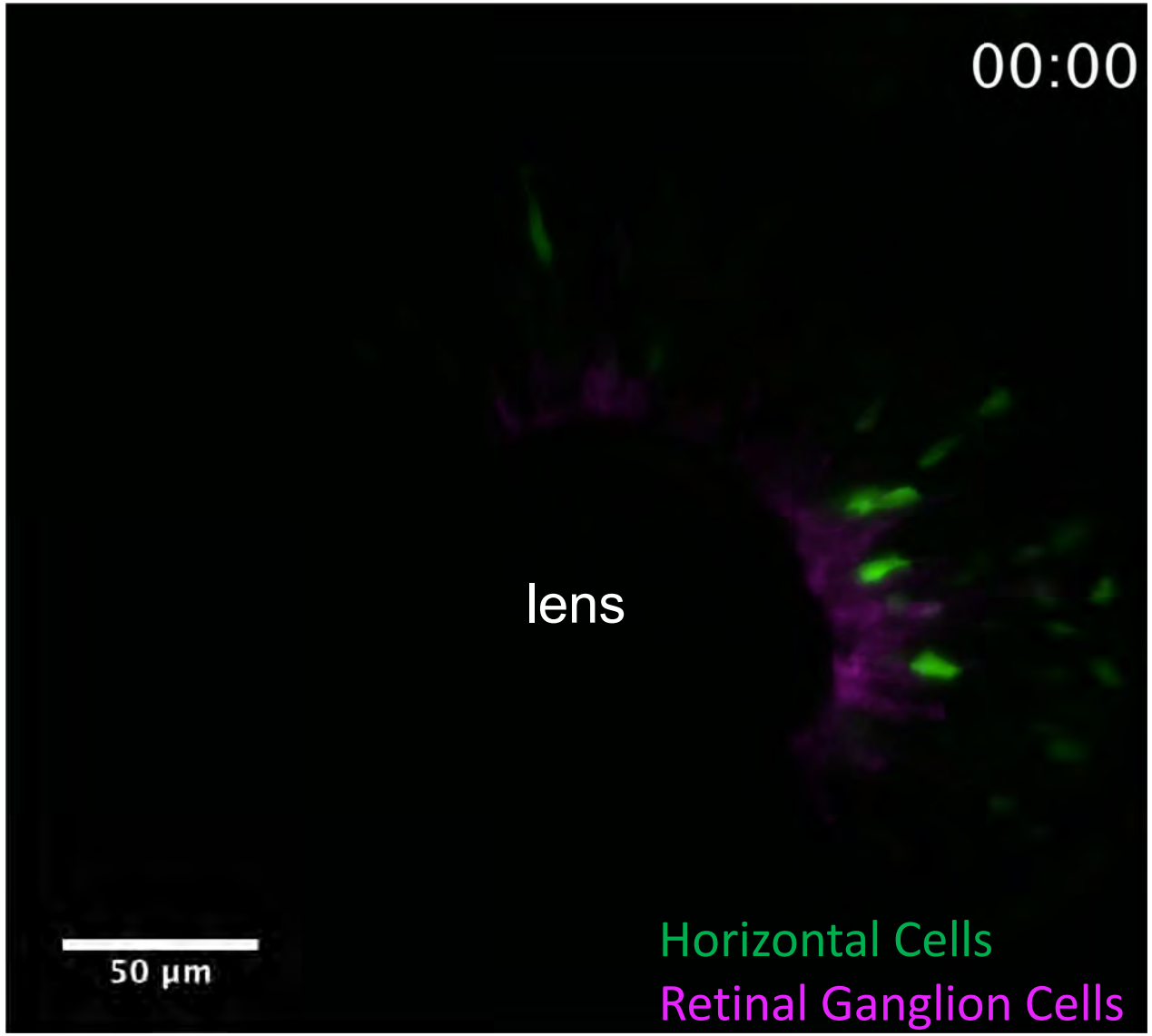
Retinal neuronal migration and lamination is complex

Zebrafish Retina



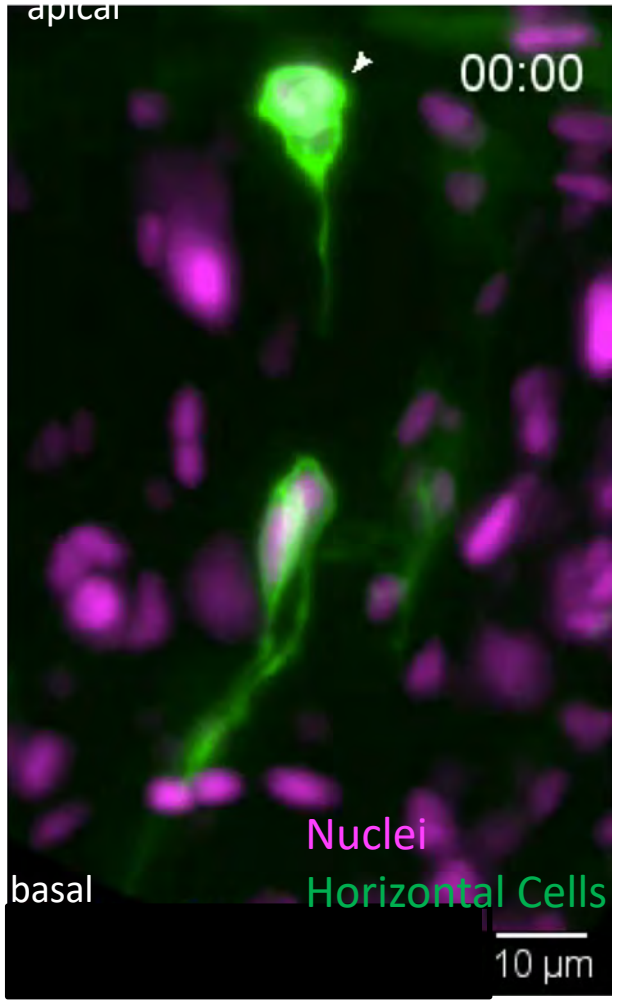
Weber...Norden, Cell Reports 2014, Chow…Harris and Norden, Development 2015, Icha…Norden, JCB 2016, Amini, Rocha, Norden, Frontiers, 2018

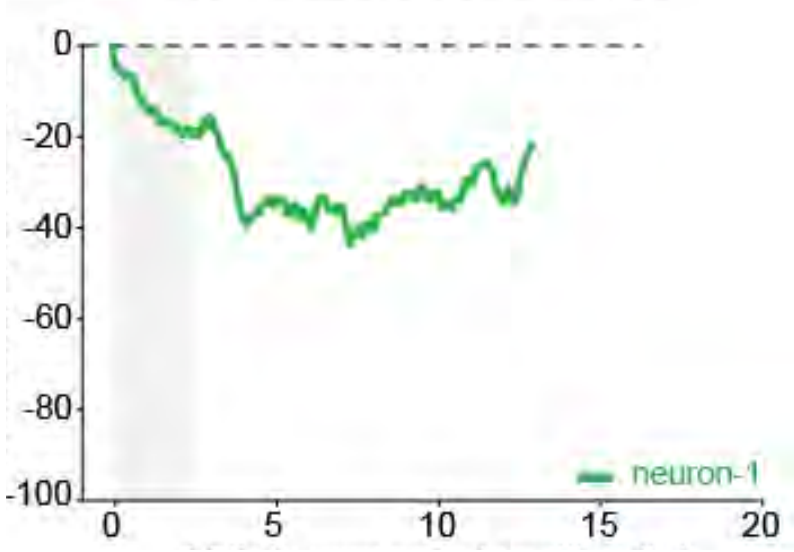
You ain’t seen nothing yet: Horizontal cell migration…



Horizontal cell migration behavior is non-predictable

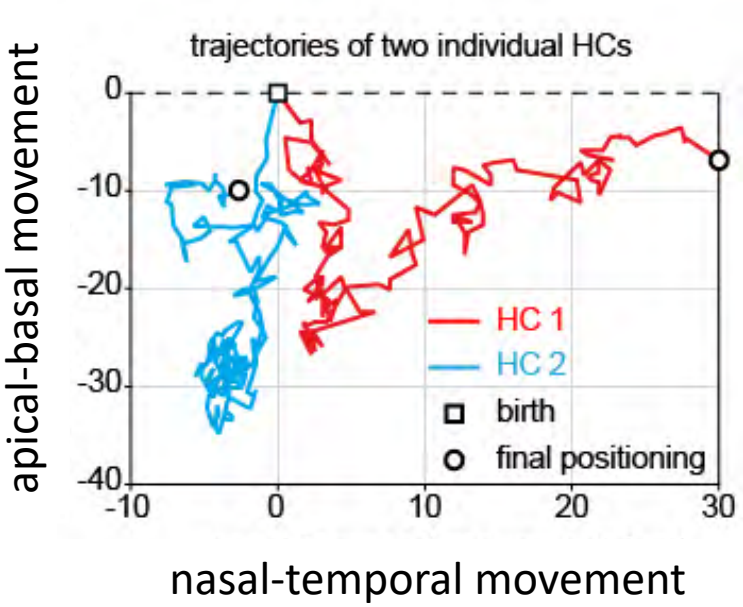
neuron-1



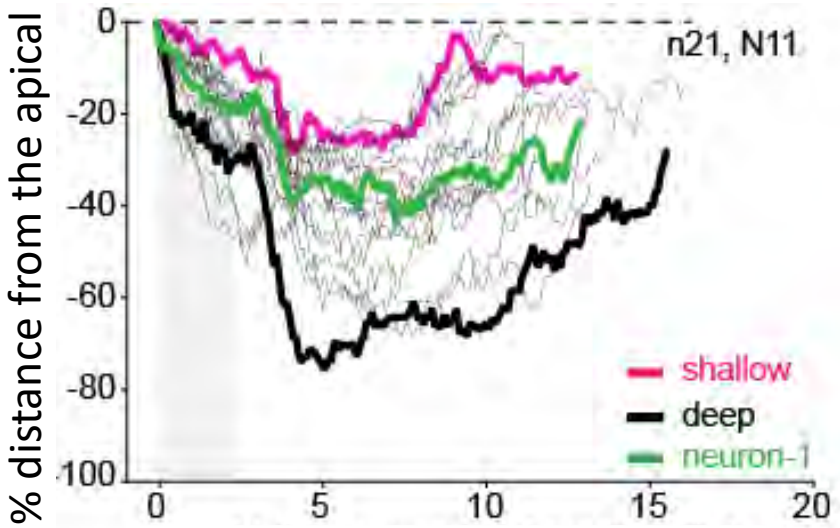


Horizontal cell migration behavior is non-predictable

migration path

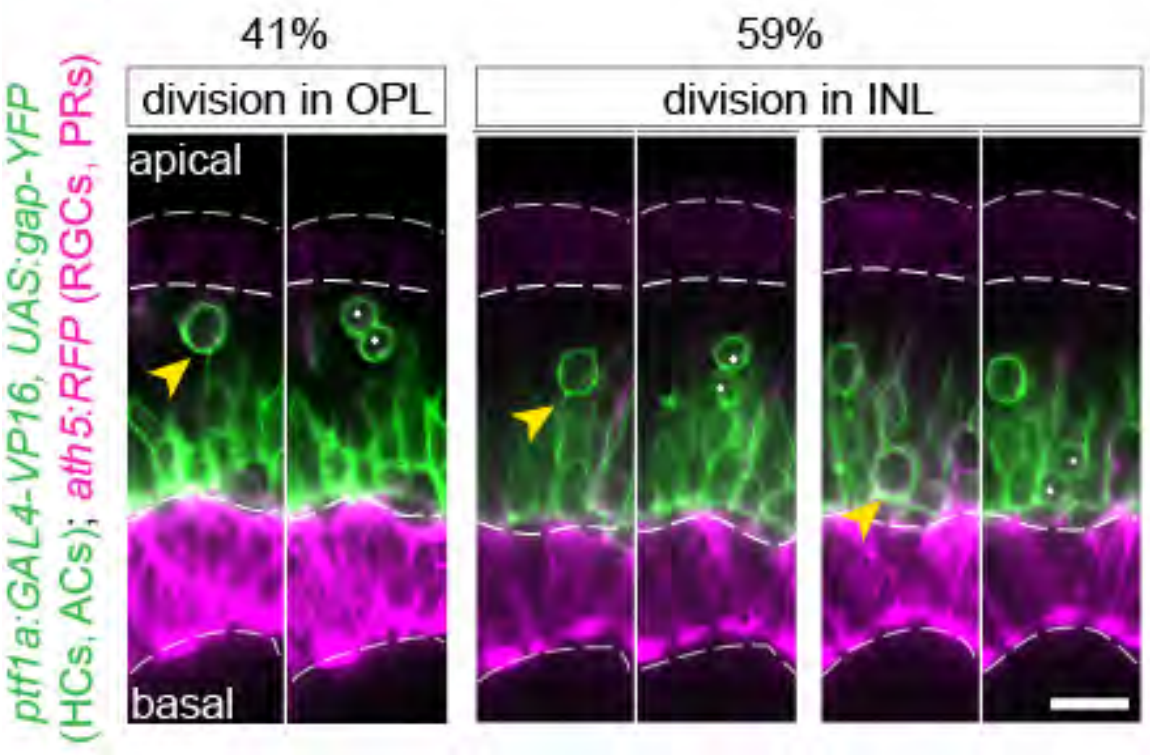


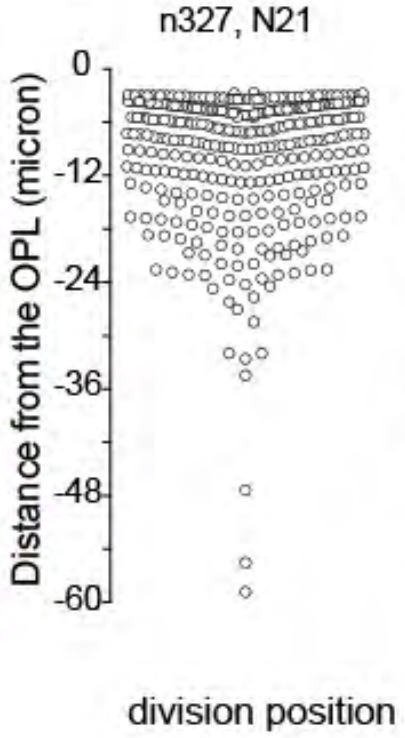
migration depth

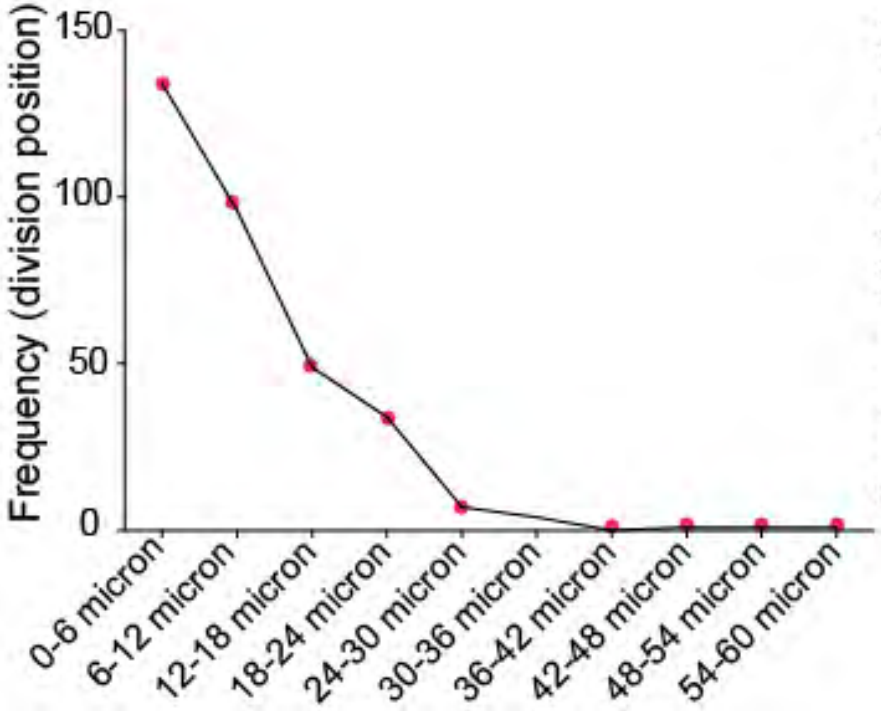


Amini et al., 2019 Development

Horizontal cells divisions occur all along apico-basal axis

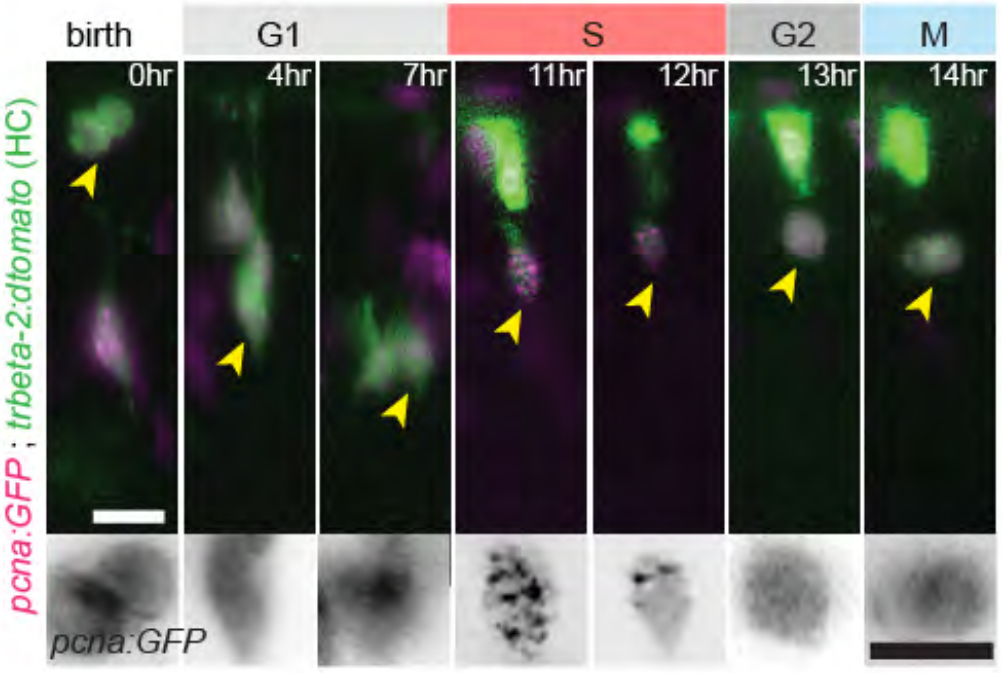


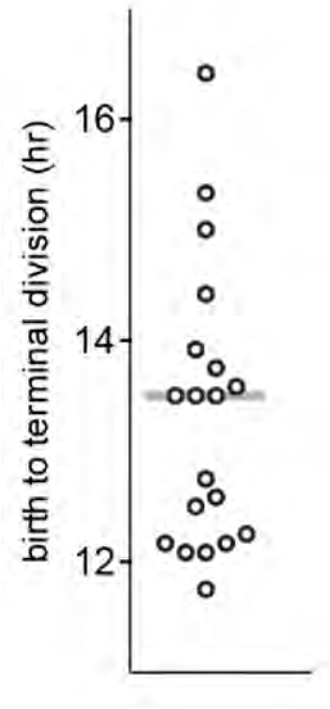


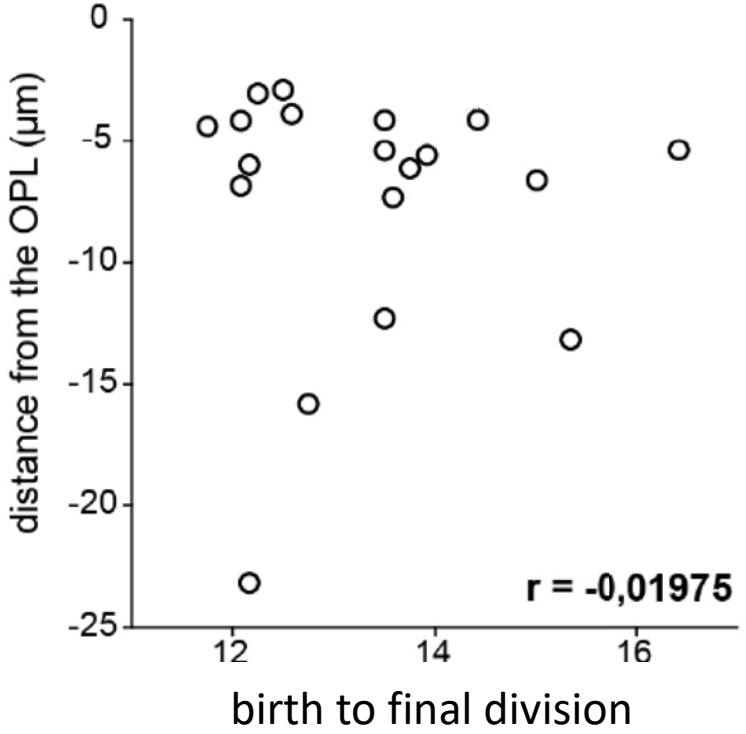


Does a cell cycle timer lead to varying migration and division behavior?

Horizontal cells do not follow a cells cycle timer

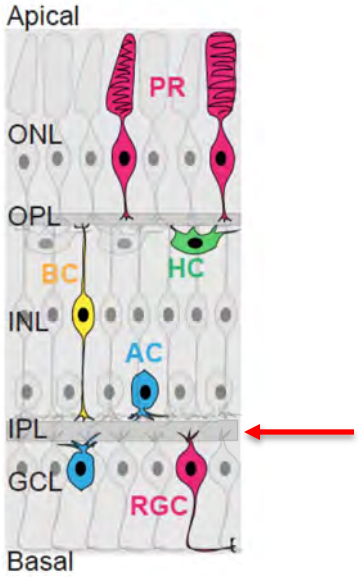


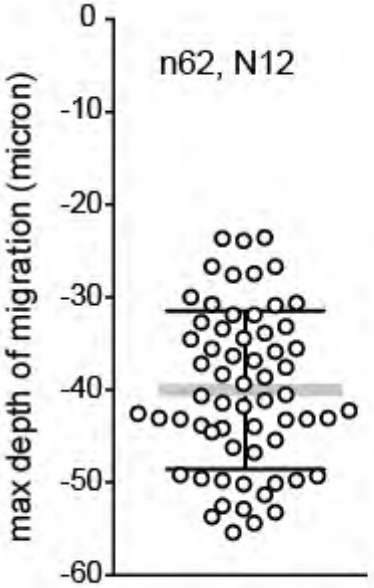


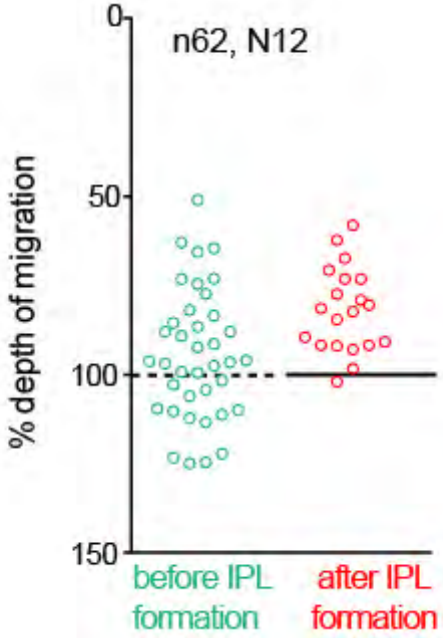


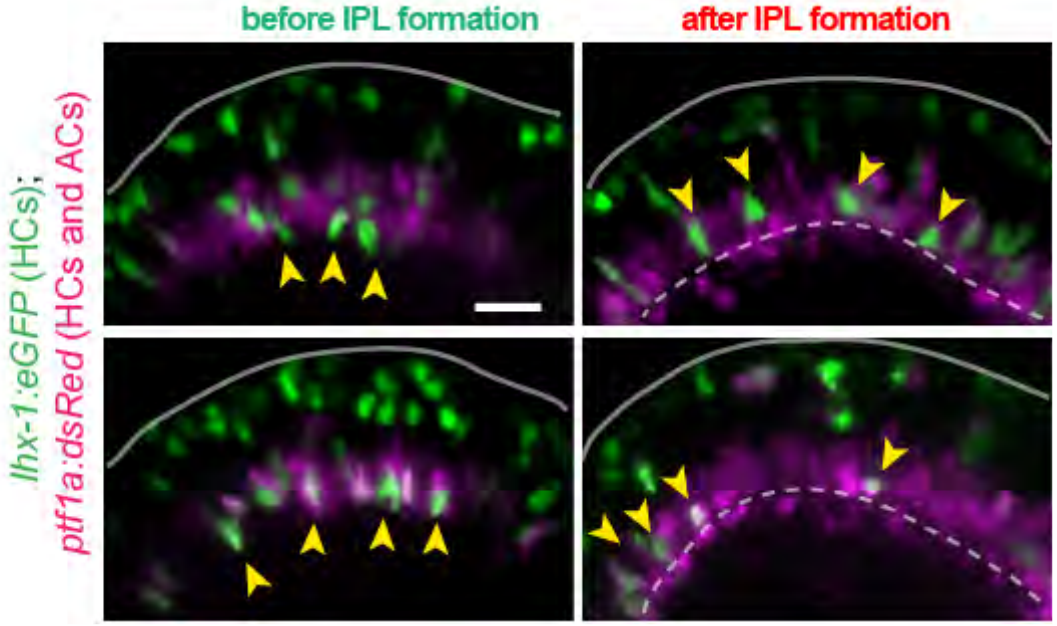
Is there any reproducible hall mark of horizontal cell behavior?

Inner plexiform layer formation defines depth of migration

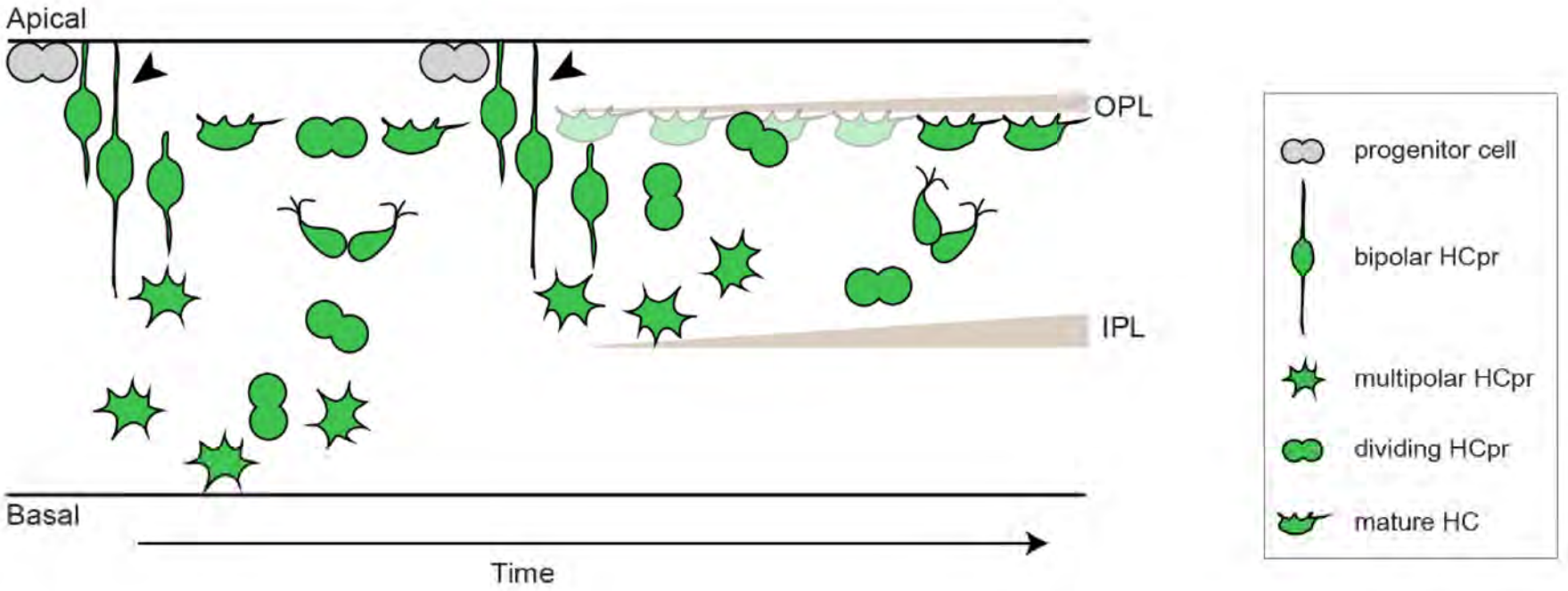








Horizontal cell behavior findings and open questions

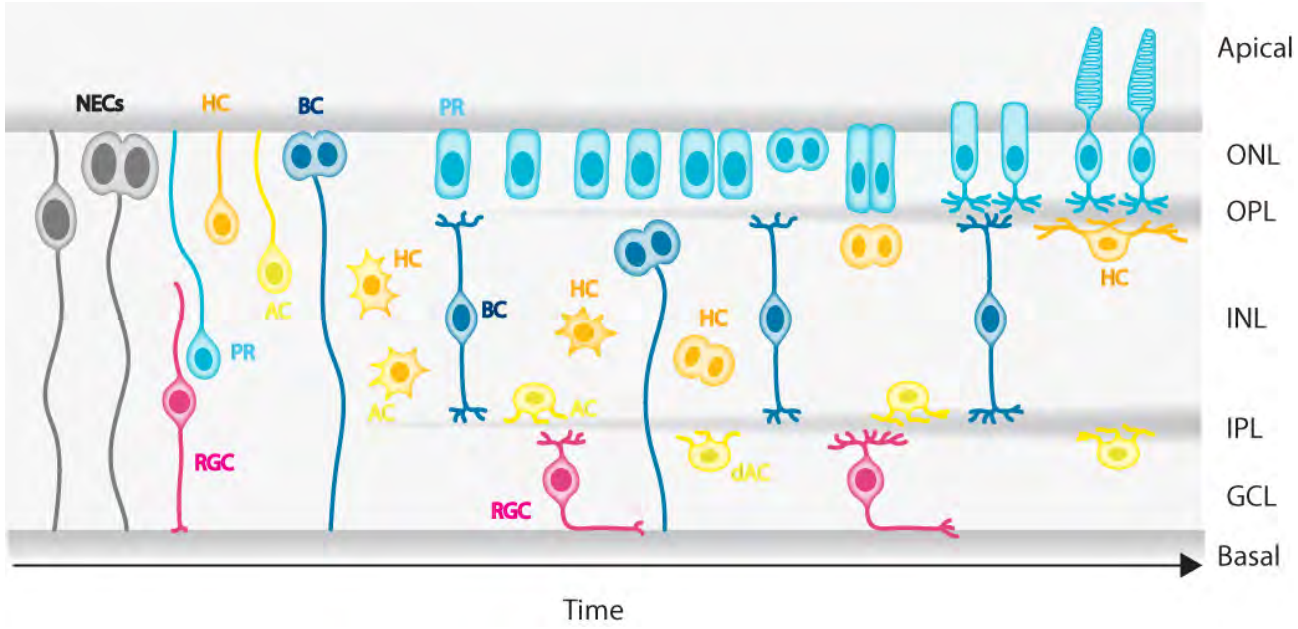


Amini et al., Development 2019

- Finding 1: Quantitative analysis reveals that none of the followed parameters are stereotypic
- Finding 2: All horizontal cells reach the amacrine cell layer before apical turning

- Question 1: What is the role of cell and tissue mechanical properties?
- Questions 2: What sets the turning point? What is sensed?

The future of retinal lamination research is bright!



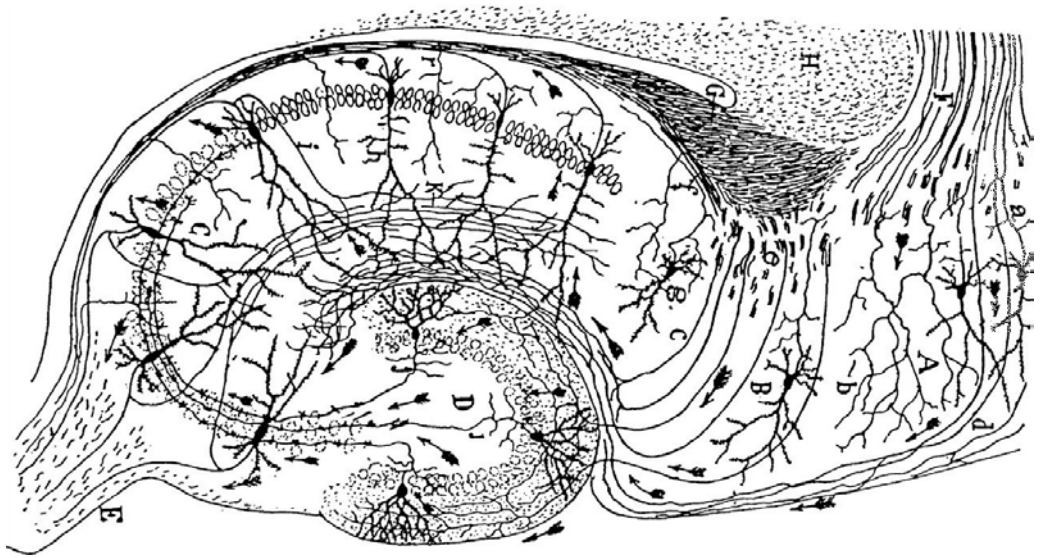
Weber...Norden, Cell Reports 2014, Chow…Harris and Norden, Development 2015, Icha…Norden, JCB 2016, Amini, Rocha, Norden, Frontiers, 2018

Single cell behaviour

Tissue properties

And neural lamination does not only occur in retina

Hippocampus

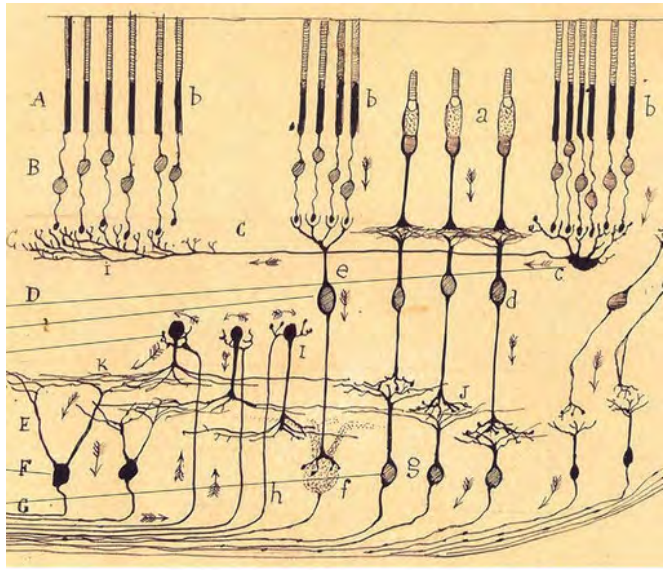


Santiago Ramon y Cajal

Neocortex

Santiago Ramon y Cajal

Retina



Santiago Ramon y Cajal

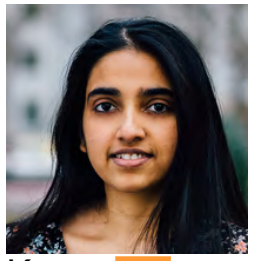
Crucial for efficient neuronal circuit formation

Key Step of brain development

THANKS!


Patricia


Caren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DFG-SFB 655

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Light microscopy facility
Fish facility

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