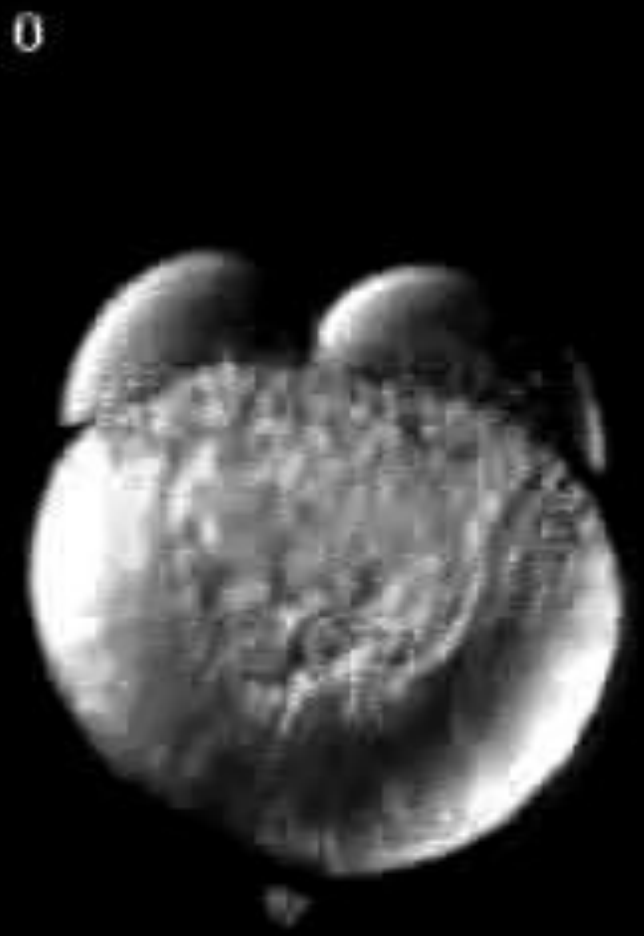
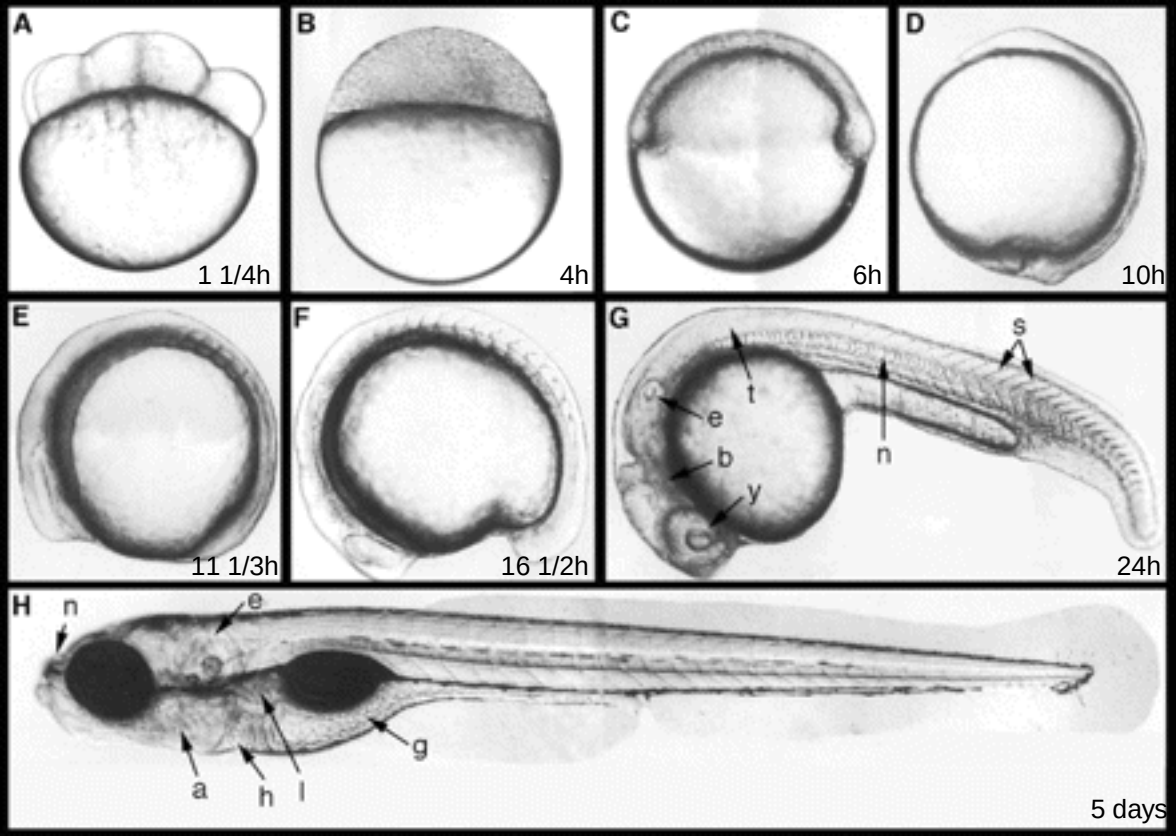
The zebrafish as a model to explore cell biology of morphogenesis

Caren Norden
MPI-CBG Dresden

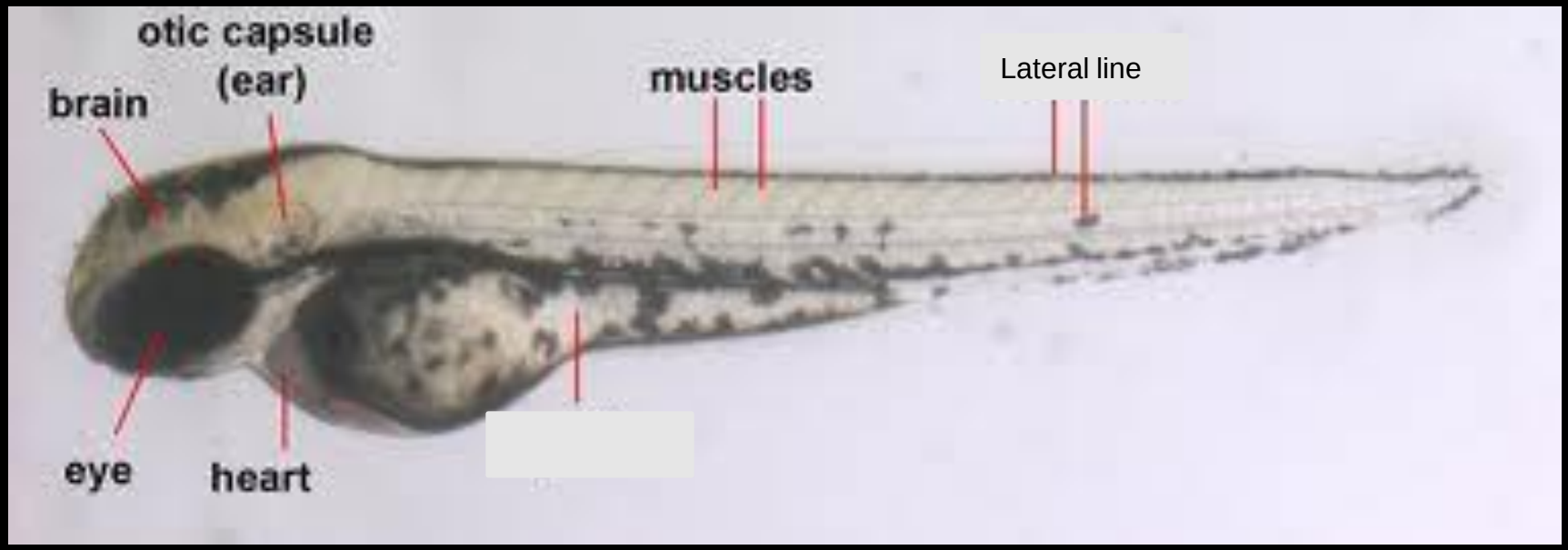
Advantage No1of zebrafish as a vertebrate model organism: Embryos develop externally and fast!



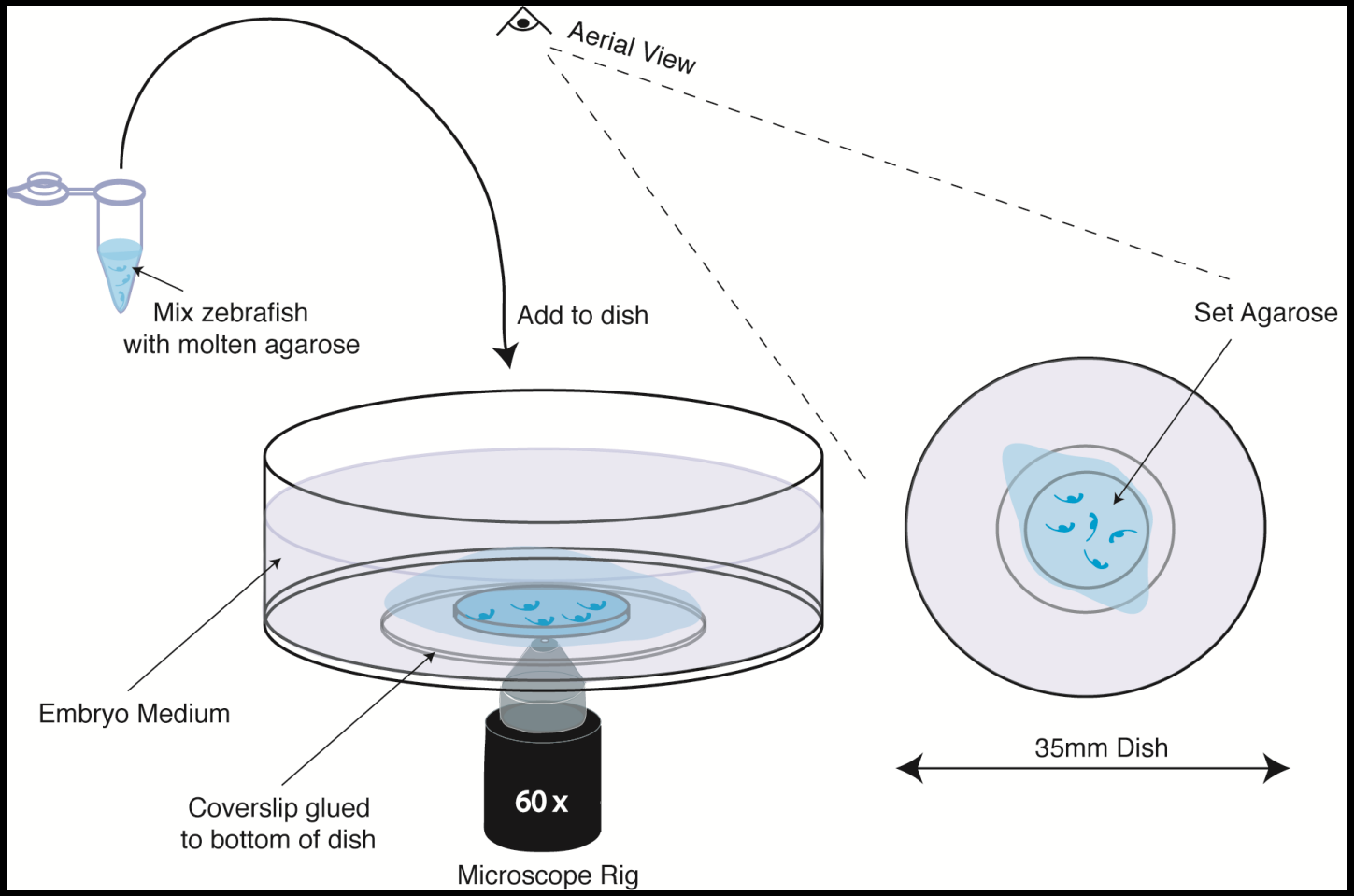
Advantage No2 of zebrafish as a vertebrate model organism: Embryos develop rapidly and are transparent for 72h+!



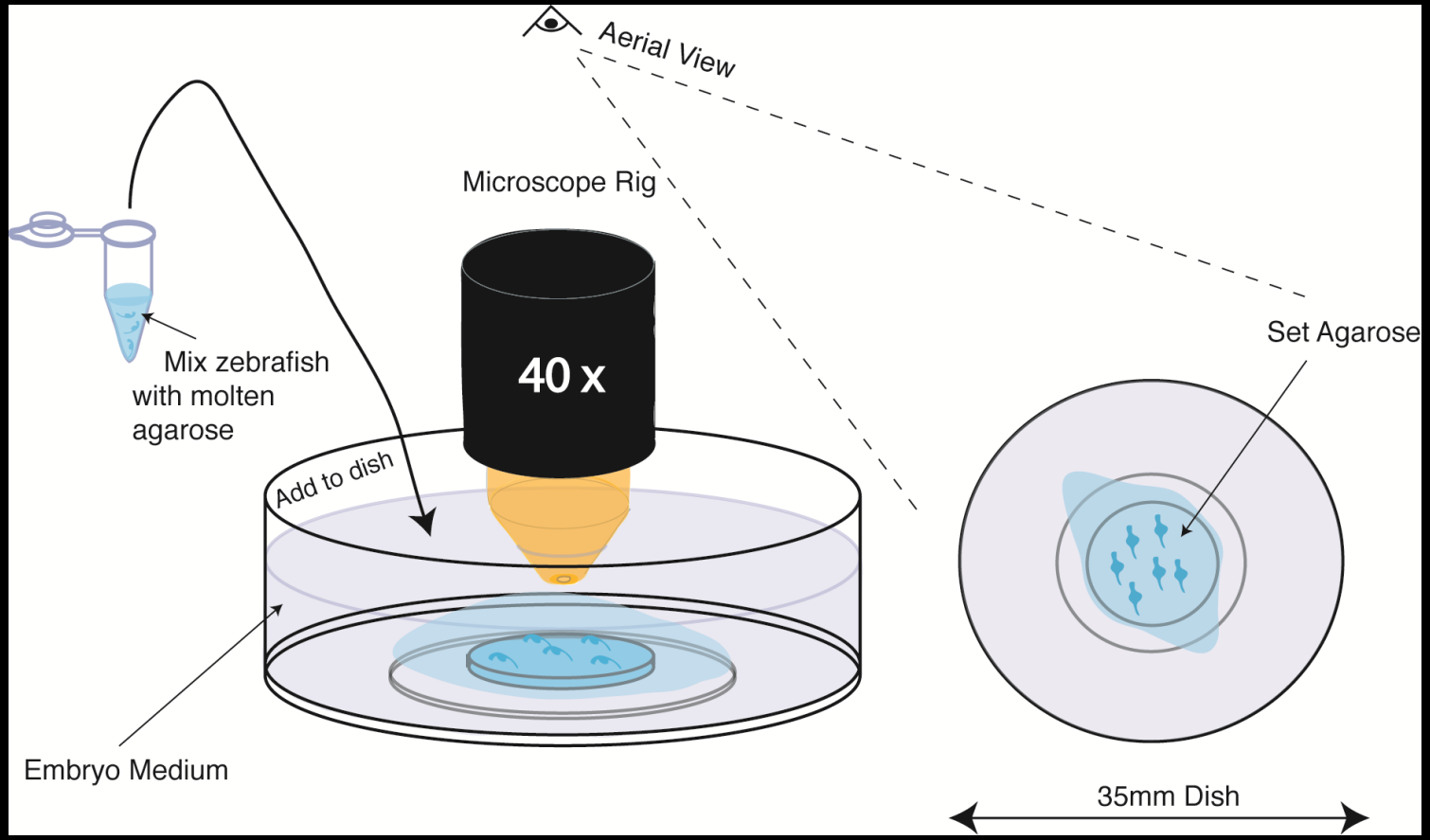
Advantage No2 of zebrafish as a vertebrate model organism:
Embryos develop rapidly and are transparent for 72h+!
Perfect to study organ development!



Advantage No3 of zebrafish as a vertebrate model organism: Preparing embryos for imaging is very easy!



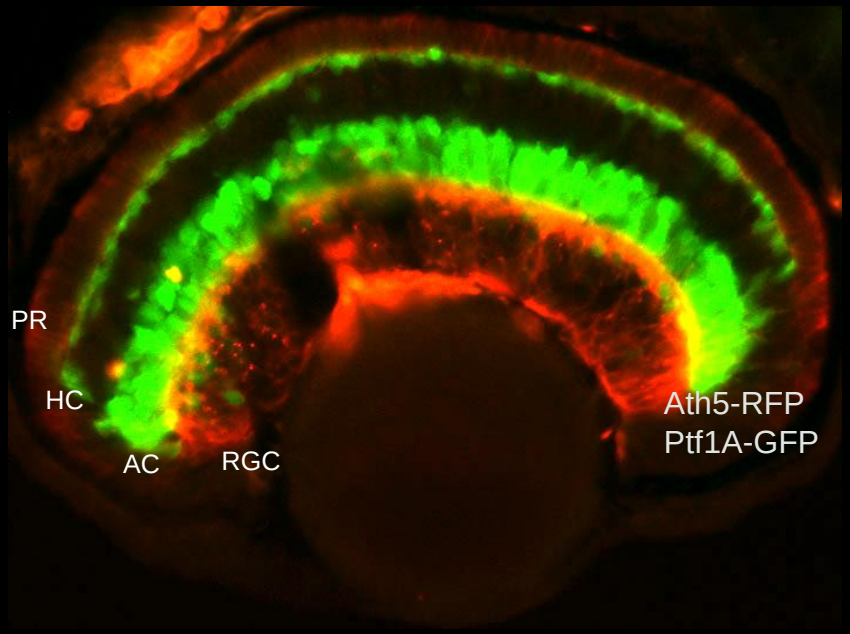
Advantage No3 of zebrafish as a vertebrate model organism: Preparing embryos for imaging is very easy!

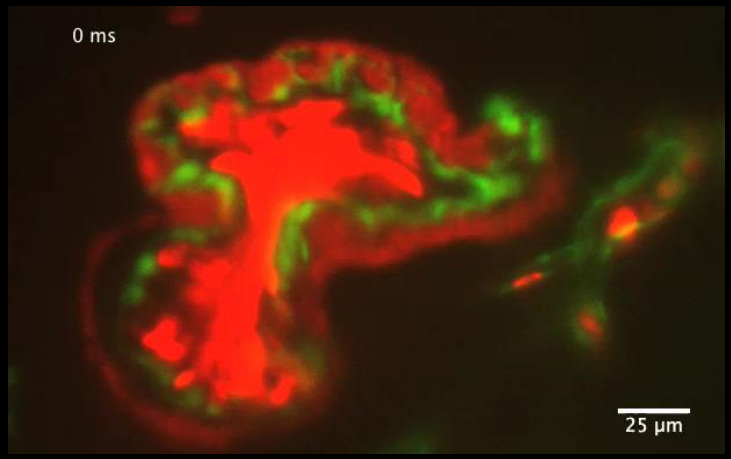


Virtually every microscope (within reason) can be used for imaging: Widefield, point scanning confocal, spinning disk, SPIM, …etc…

Tools to do follow subsets of cells during organdevelopment

1) Plethora of transgenic lines to label specific neuronal subsets (usually transcrition factor promotors but also others)

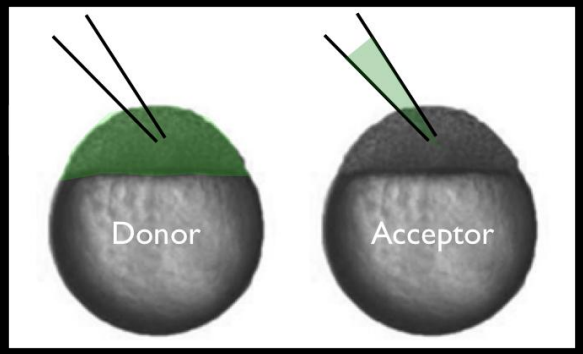


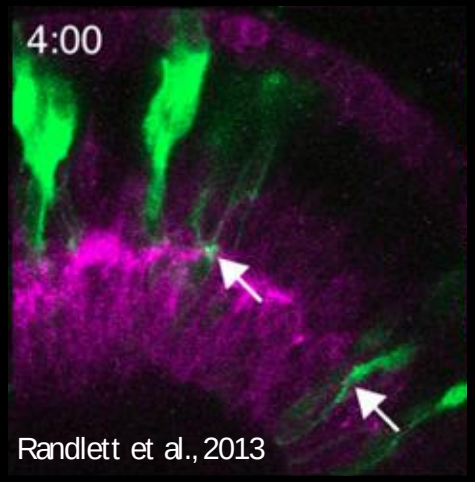


Endocardium Blood cells/Myocardium (Jan Huisken)

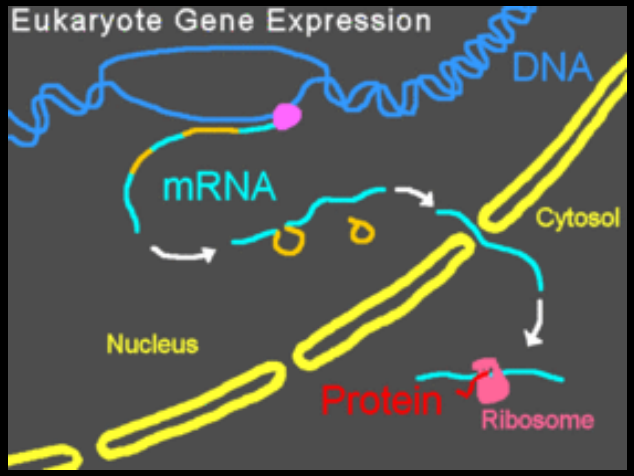
Tools to do follow subsets of cells during organ development

2) Transplantation techniques





Tools to do knock down proteins in zebrafish: morpholinos (Steric block mechanism)

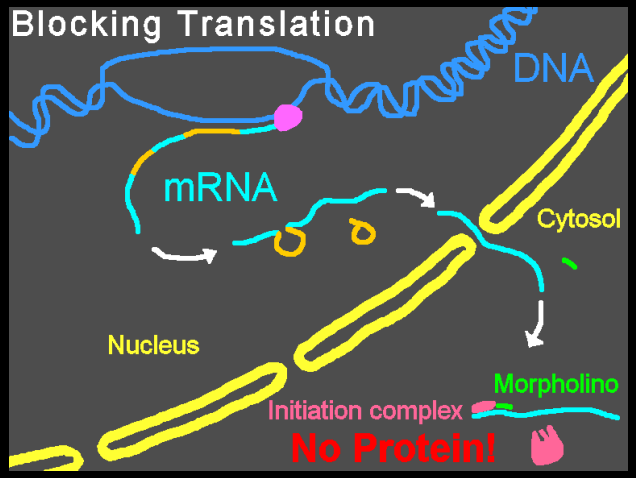


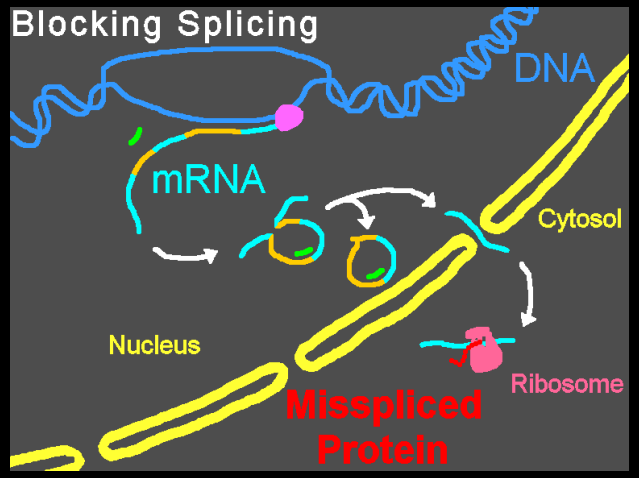
Advantages:

- *global loss of proteins.*
- *easy to use.*
- *Titration possible to understand additive effects.*

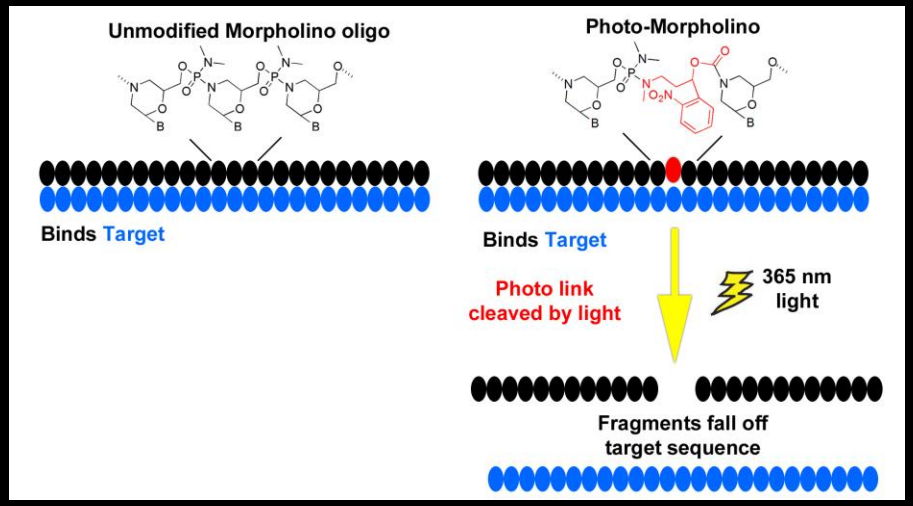
Disadvantages:

- *global loss of proteins.*
- *Dilution of morpholino during development.*
- *Very general effects in case of ubiquitous targets.*
- *Pricey.*





Some tools to do knock down proteins in zebrafish: photomorpholinos

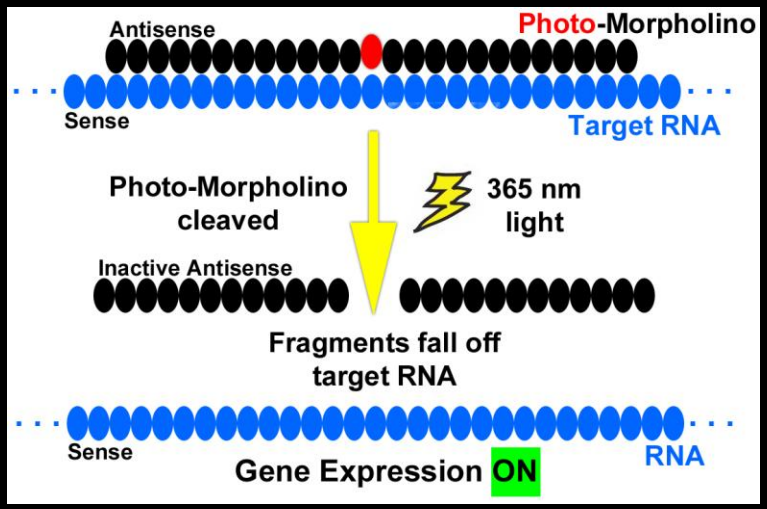


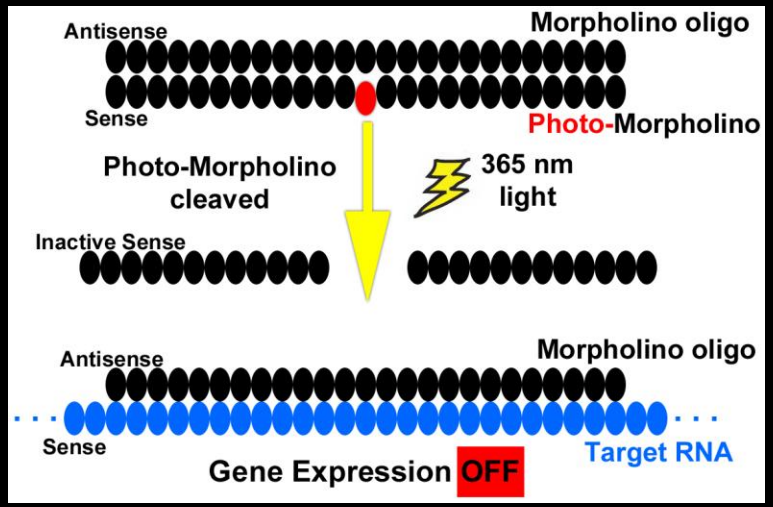
Advantages:

- *Spatial and temporal control of protein loss.*
- *In principle genes can be turned on and off.*

Disadvantages:

- *Needs additional equipment.*
- *Not so easy to use, needs a lot of calibration.*
- *Still in proof of principle stage.*
- *Very pricey.*

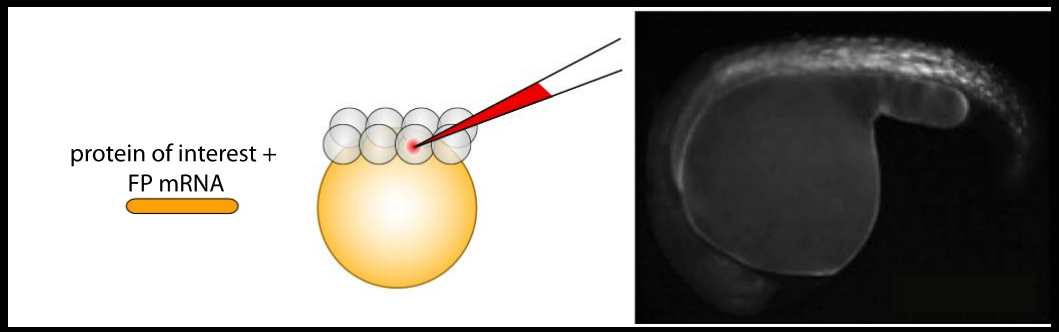




Some tools we use to investigate cell biological problems in developing zebrafish

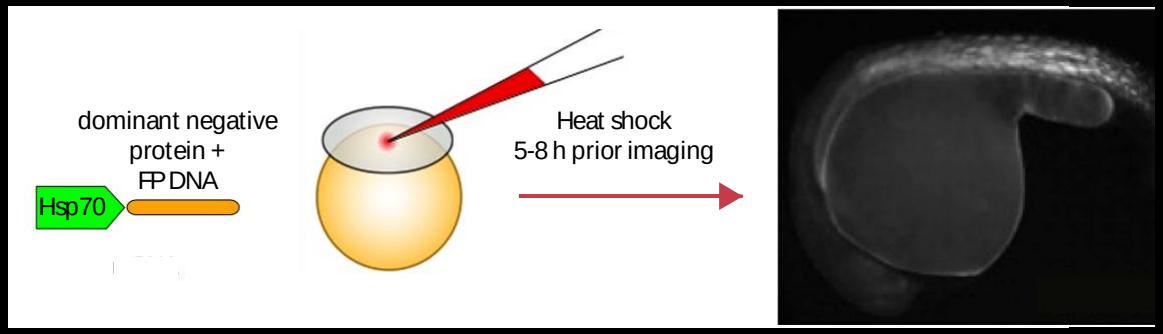
- Injection into zebrafish embryos

1. Labeling intracellular structures: mRNAs encoding fluorescently tagged proteins



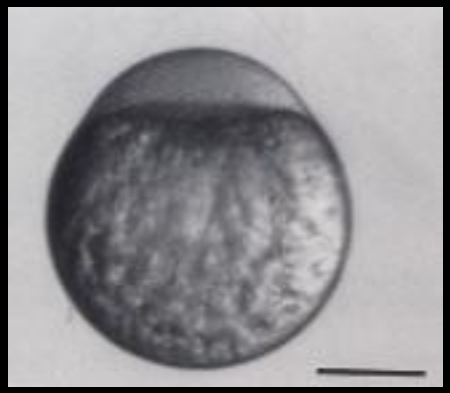
2. Modifying protein function: Dominant negative constructs

- Competitive inhibitors of proteins
- Inactive
- ‘Distract’ endogenous proteins from their function

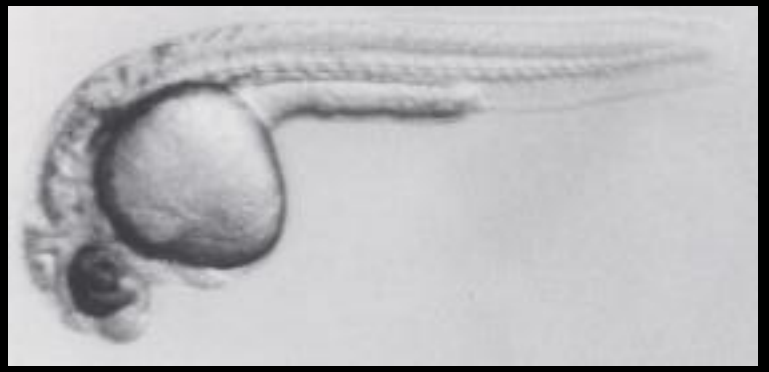


Small molecule (Drug) approaches: (cytoskeletal inhibitors, cell cycle inhibitors…)

Morphogenesis from the one cell embryo to the organism - the zebrafish as a trackable example.



24 h



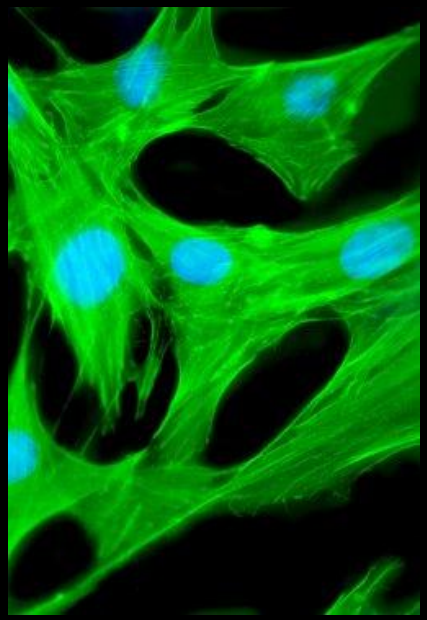
Changes on the single cell level

→ changes of groups of cells like epithelia

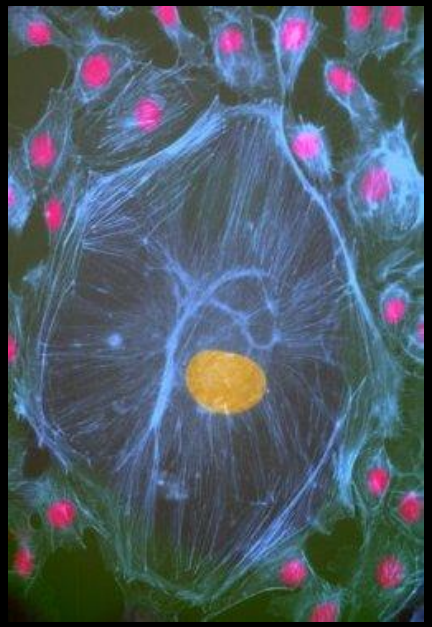
→ changes on the tissue level

→ organ formation.

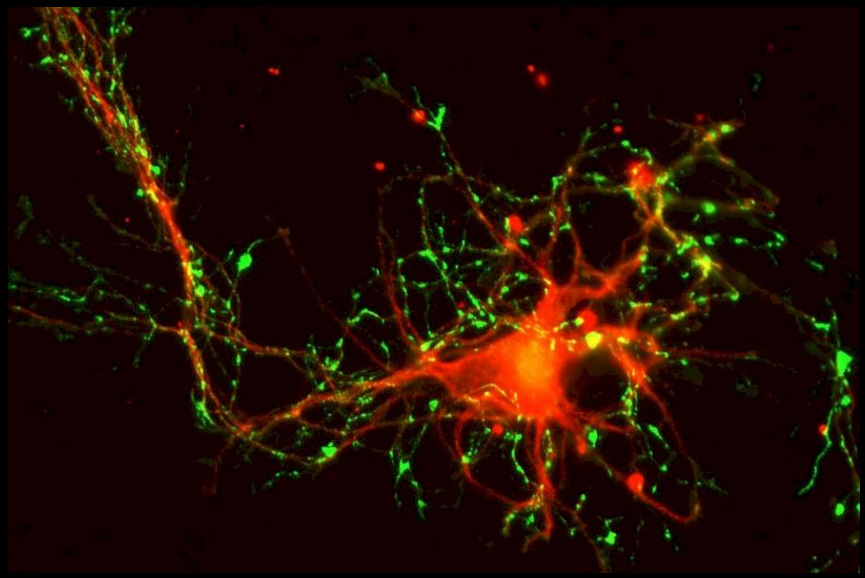
Single cell morphology ultimately dictates organismal morphology.



Fibroblast



Hepatocyte



Neuron

…however, single cell dynamics in culture are most likely not sufficient to holistically understand the morphogenesis of a developing organ

→ In vivo and in embryo approaches are needed.

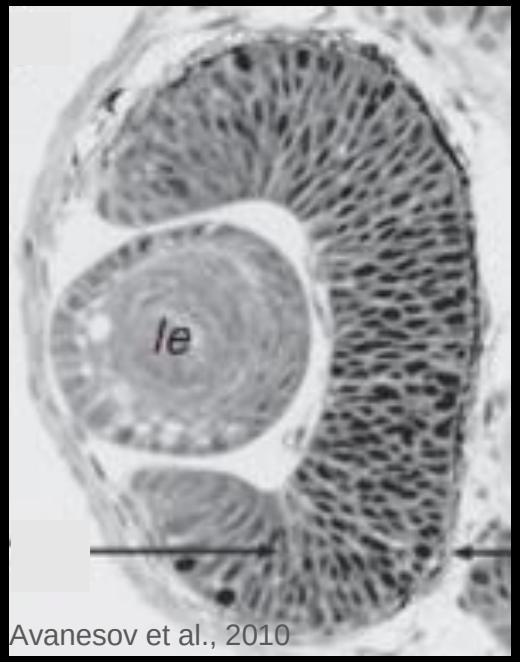
The tissue we use to understand aspects of morphogenesis: the zebrafish retina.

- rapid development

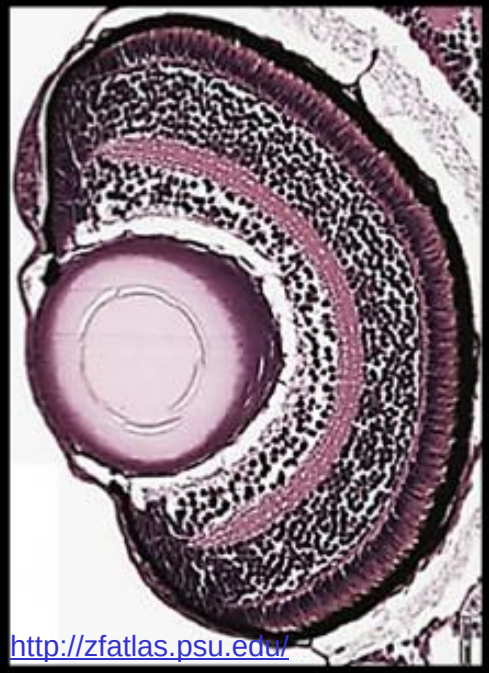
- easy accessibility

- composition similar to human retina

36 hpf



Avanesov et al., 2010

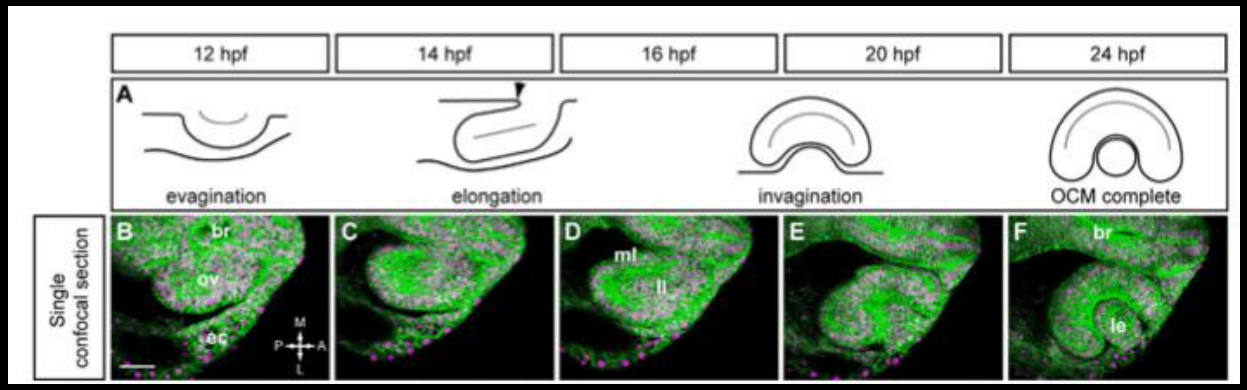


http://zfatlas.psu.edu/

72 hpf

Developmental stages in zebrafish retinal development.

1) Optic cup morphogenesis (Kwan et al. 2011)



Cell number increases:

1.140 12hpf

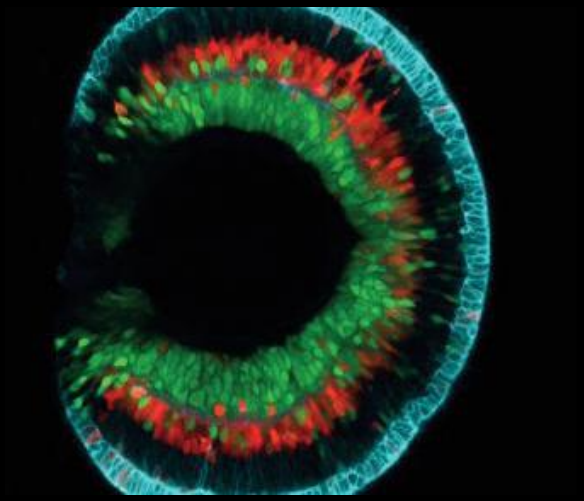
2.470 24hpf

11.000 48hpf

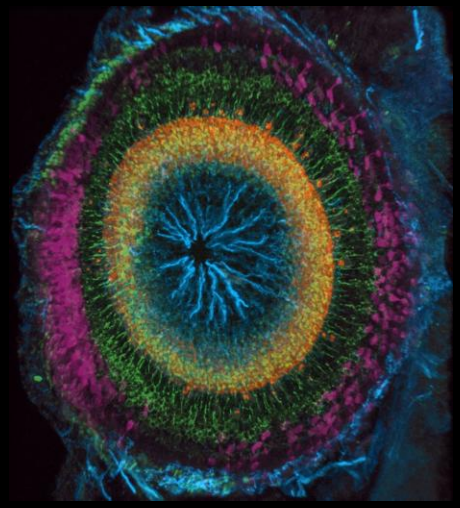
21.000 72hpf

2) Pseudostratification

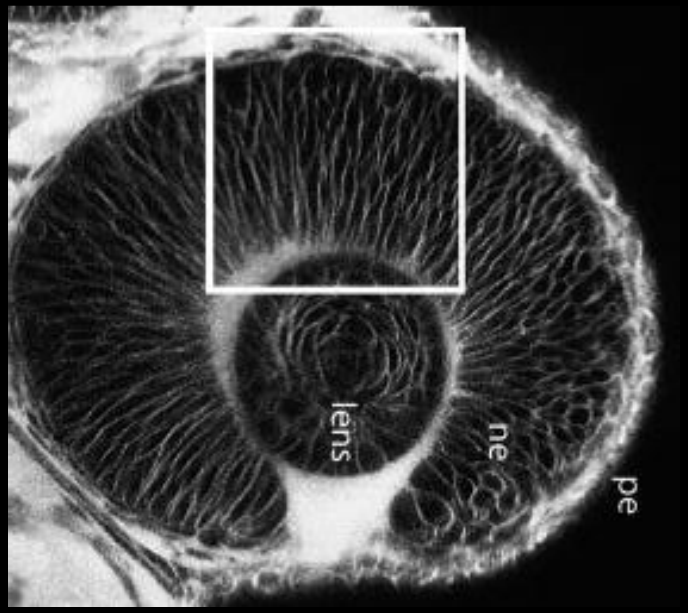
3) Neurogenesis (Harris lab page)



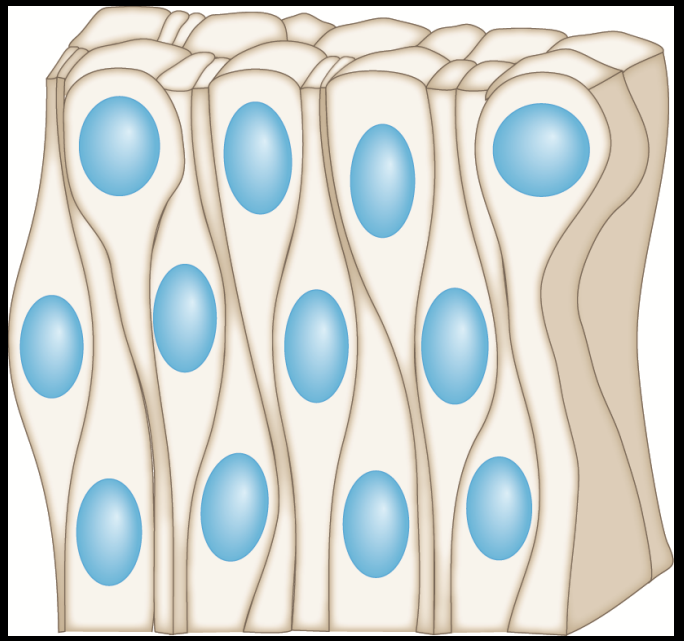
4) Connectivity (Wong lab page)



Concentrating on Step 2) pseudostratified neuroepithelium



Das *et al* 2003



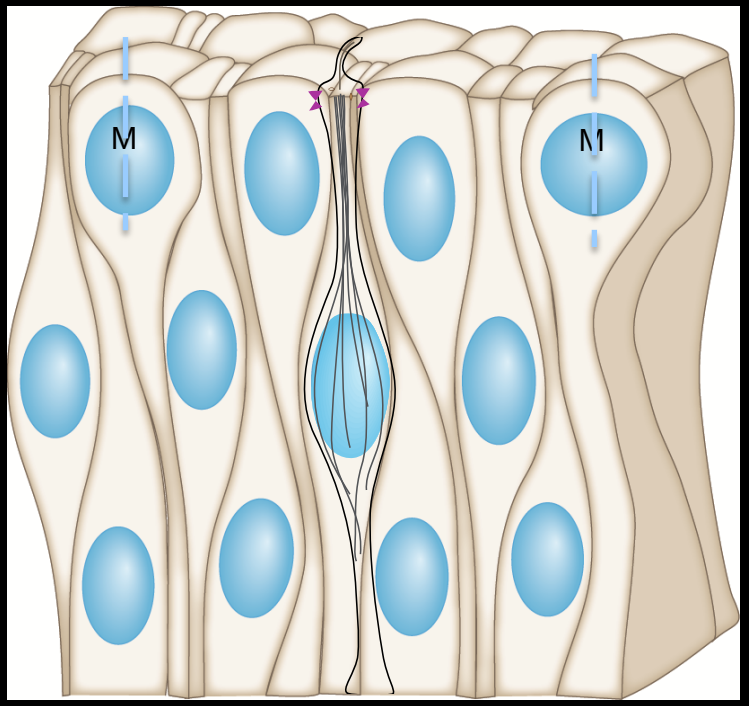
apical

basal

Pseudostratified epithelia including neuroepithelia occur during development of many multicellular organisms

apical

basal

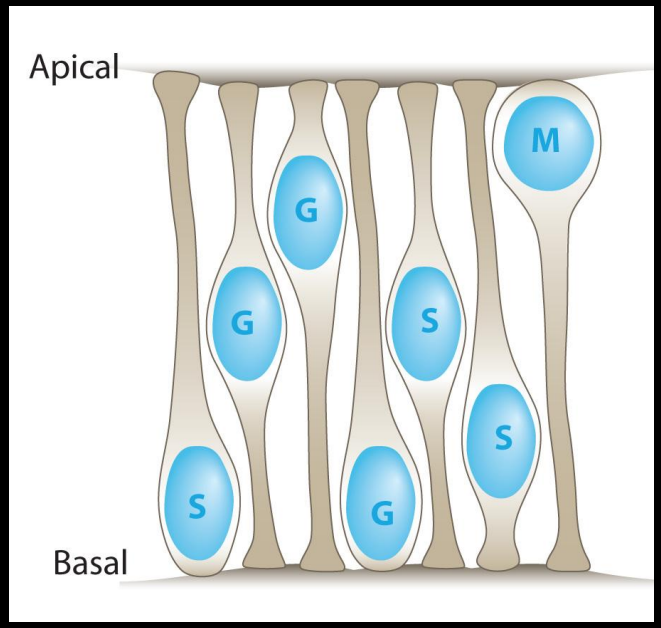


digestive

airway

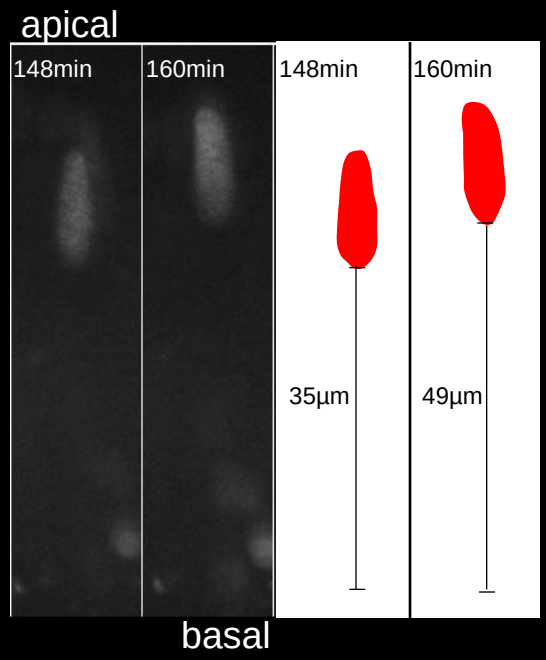
neuroepithelia

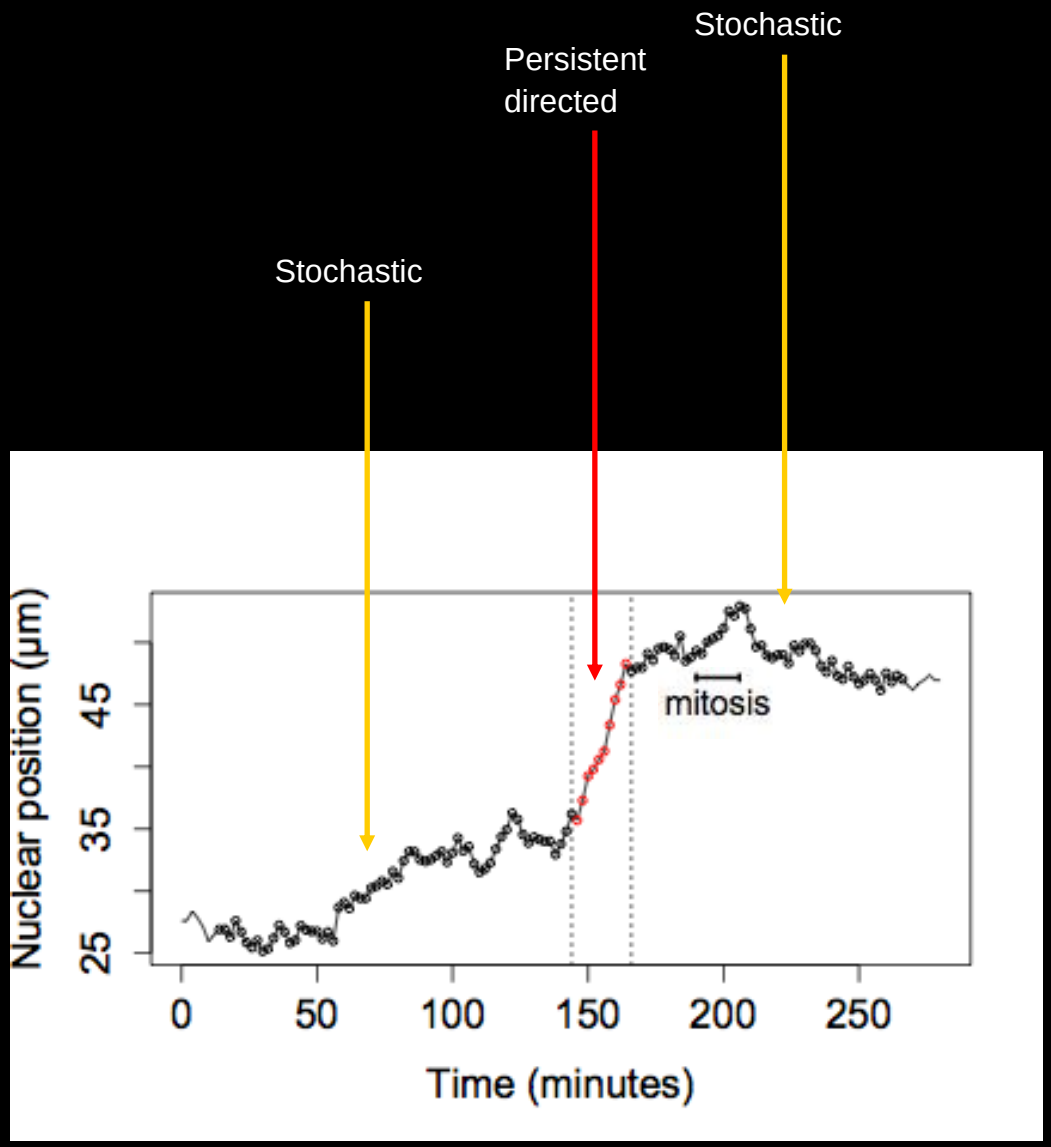
Pseudostratified epithelia feature interkinetic nuclear migration (IKNM).



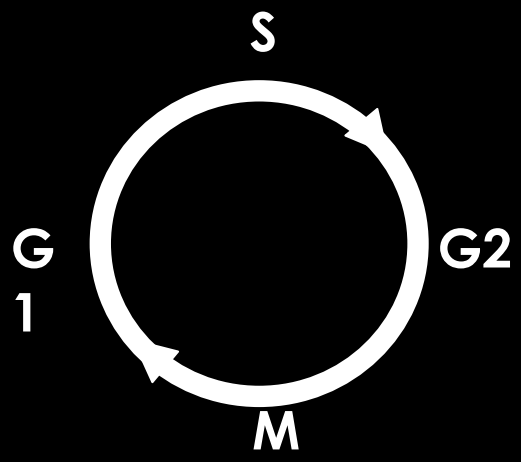
H2B-RFP

IKNM represents stochastic motion of nuclei intermitted by rapid apical migration.

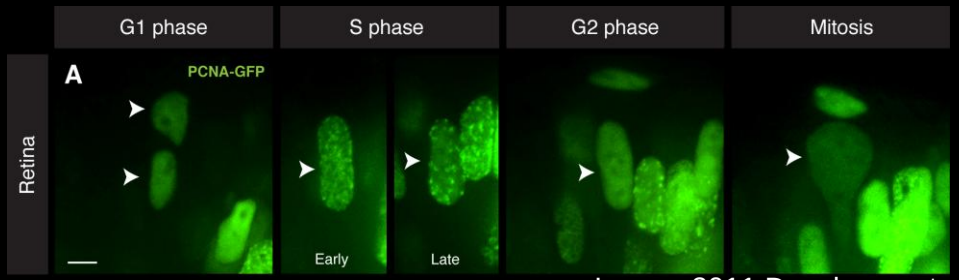




PCNA-GFP as a tool to analyse how nuclear movements correlate to cell cycle phases in retina.



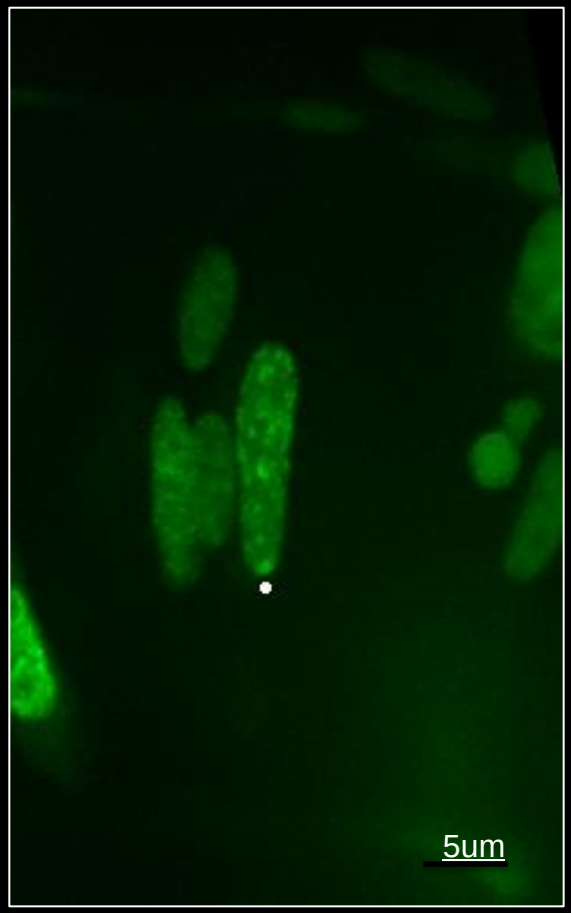
PCNA-GFP



Leung, 2011 Development

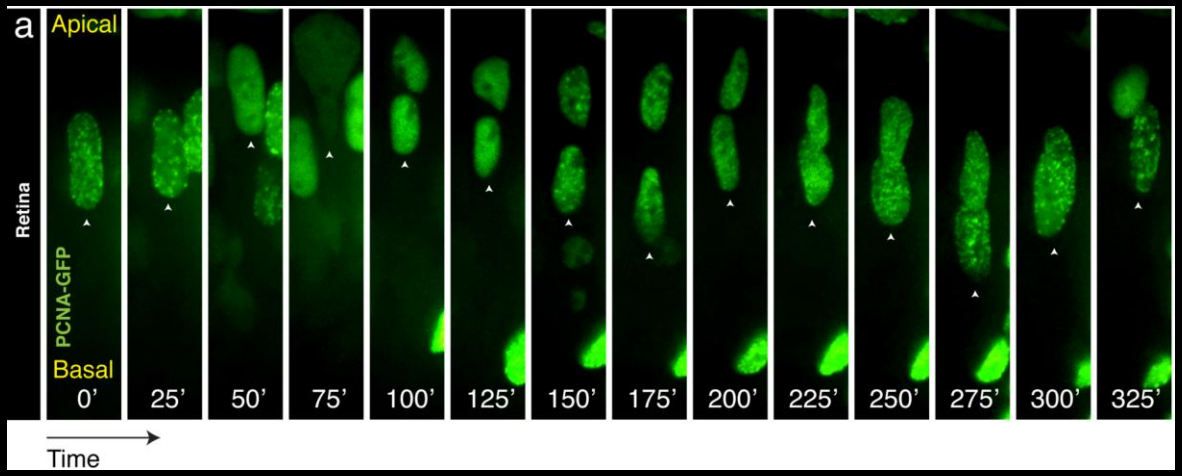
PCNA-GFP

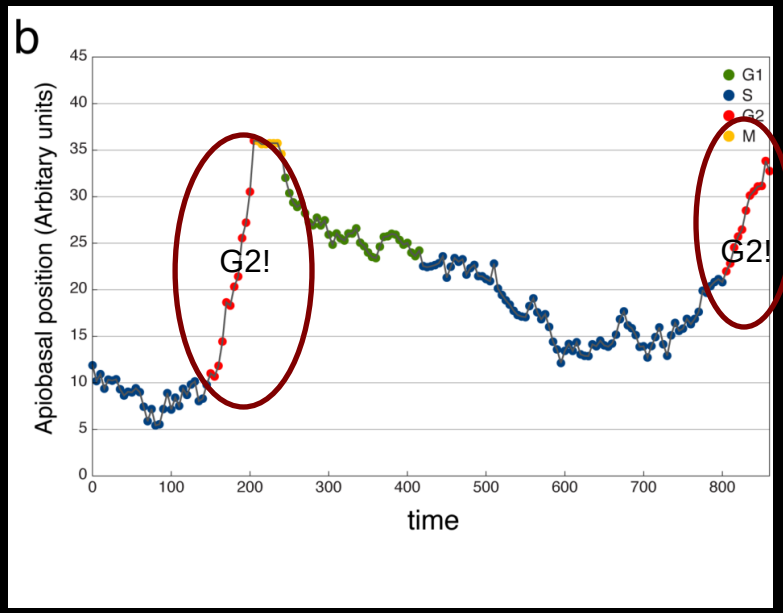
apical



basal

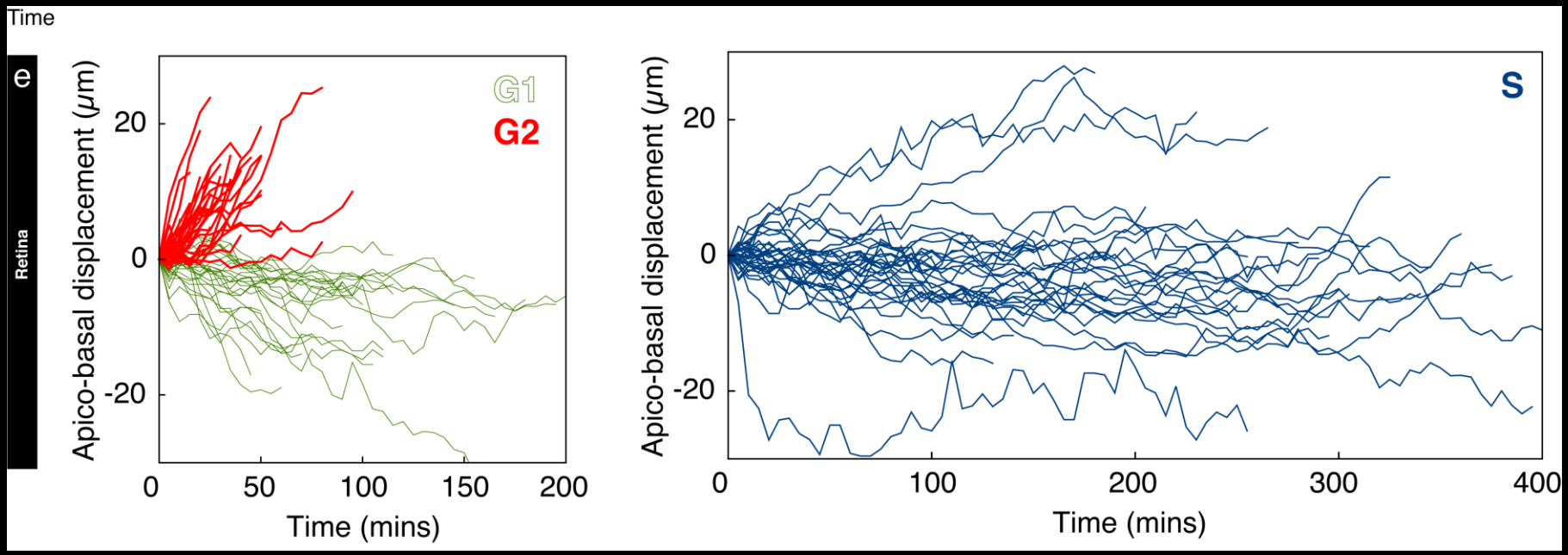
PCNA-GFP reveals that rapid apical migration occurs in G2 and G2 only.





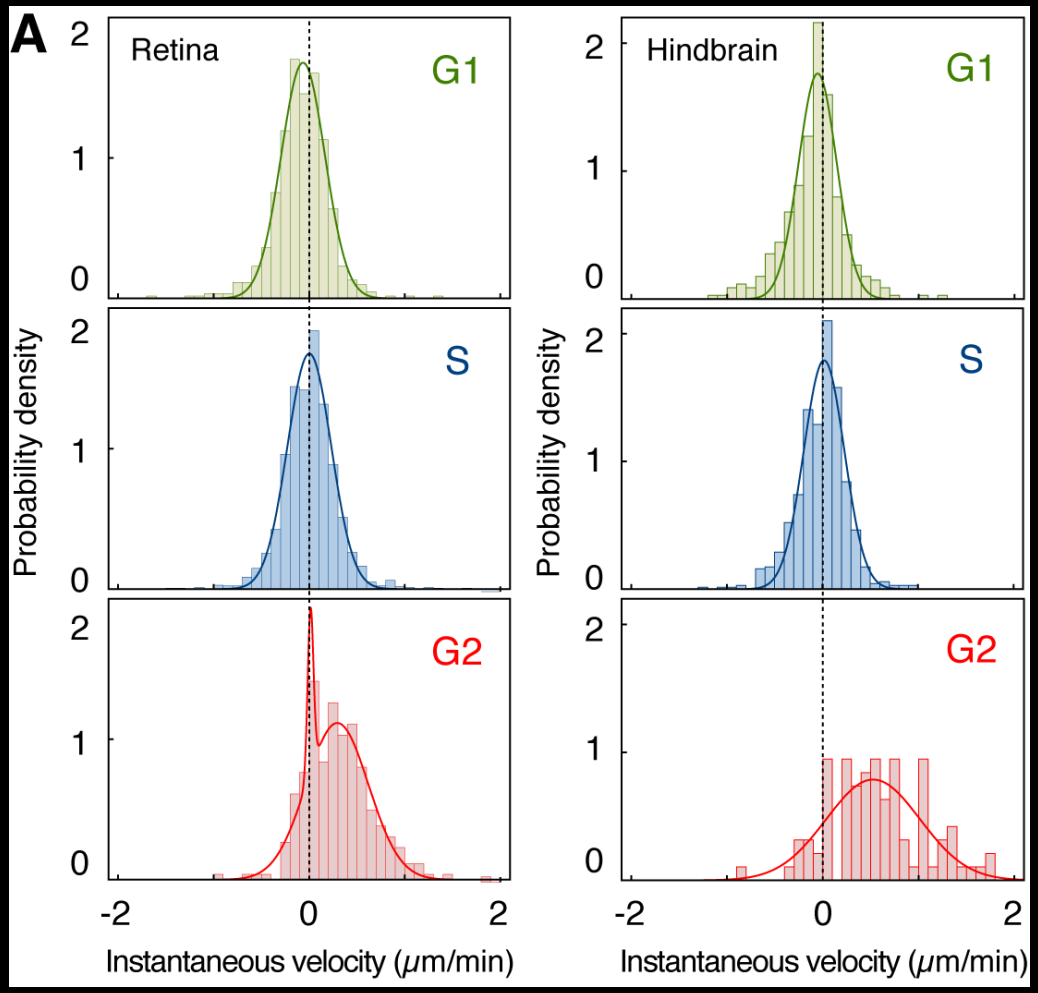
Leung, 2011 Development

G2 is the only cell cycle phase in which nuclei exhibit unidirectional movement.



Leung et al., Development 2011

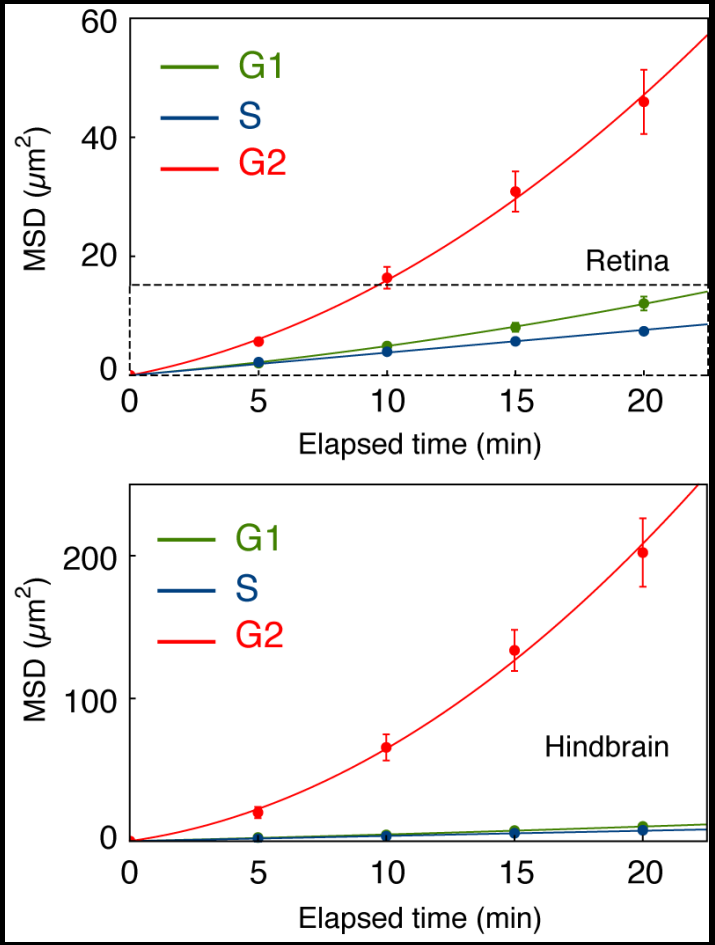
Velocity distribution analysis



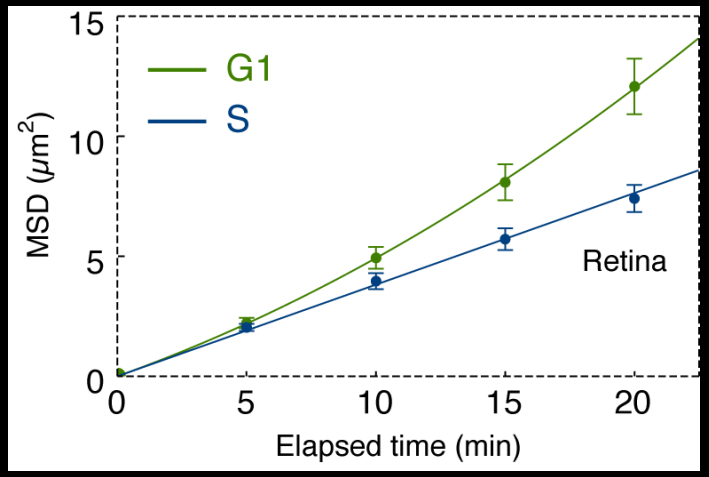
G2 velocity distributions show an apical drift.
Distributions in G1 and S phase are centered around zero

Leung et al., 2011

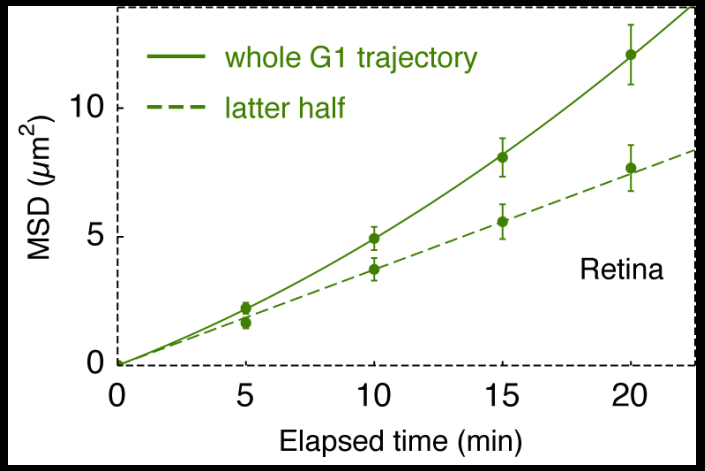
Mean Square displacements analysis



MSD for G2 shows a positive curvature with time. This means movement is directed. Nuclei move to a higher extend than in G1 and S.



G1 MSD shows a slight positive curvature with time. Is basal movement directed after all? Did we miss it?

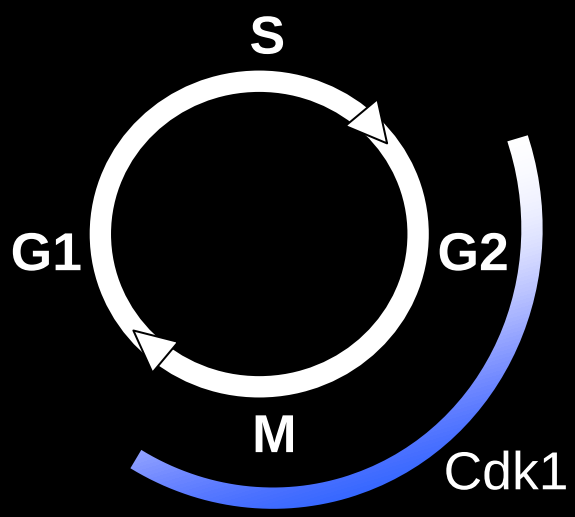


We think basal drift is mainly caused by the apical membrane that acts as an apical barrier

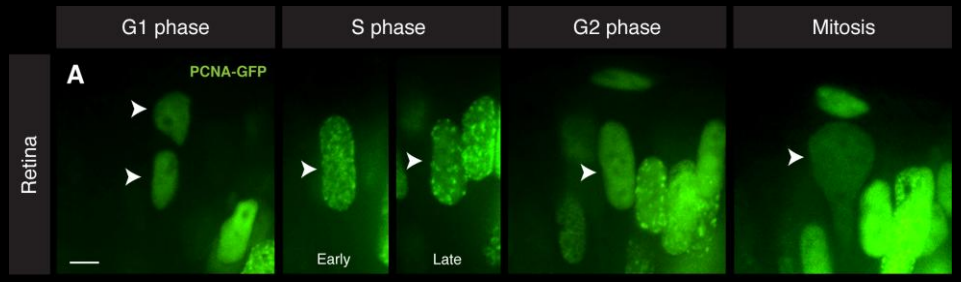
Leung et al., 2011

Movements in G2 are actomyosin dependent.
How are they linked to the cell cycle?

Cdk1/cyclinB plays a role in G2-M transition.

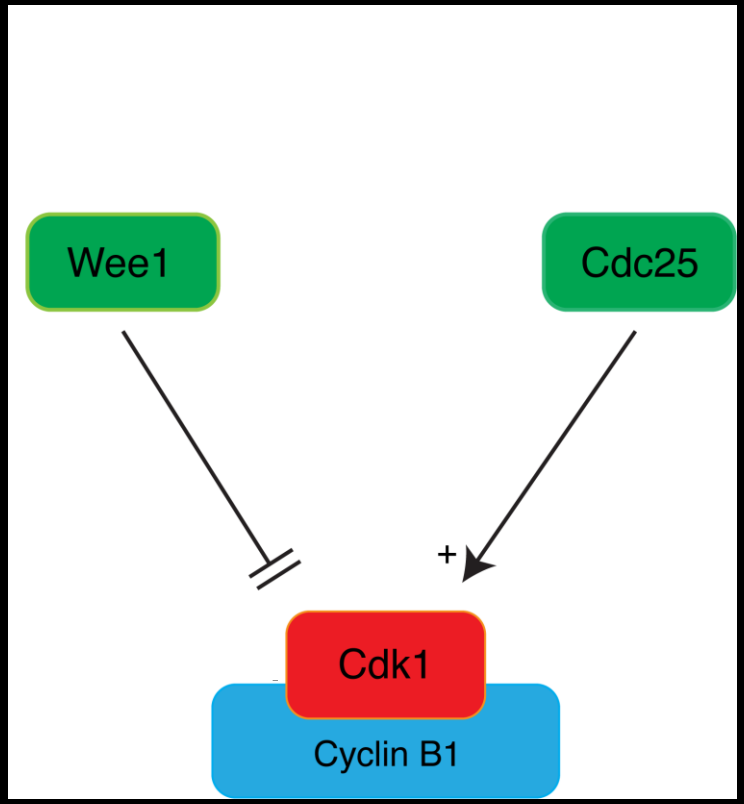


→ Is there a link between Cdk1 and nuclear movements in G2?

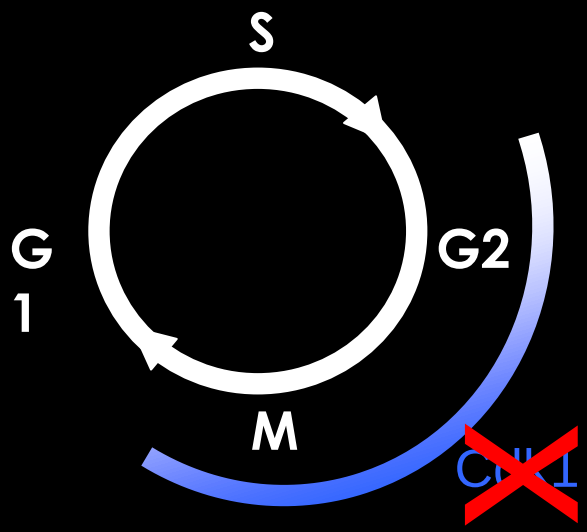


Leung, 2011 Development

How does Cdk1 activity influence IKNM onset in G2?

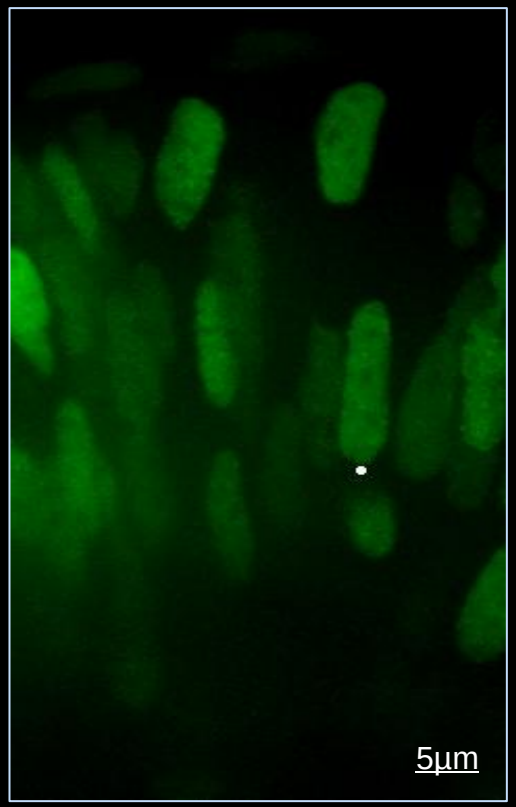


Is there a link between Cdk1 activity and nuclear movements in G2? Cdk1 inhibition:



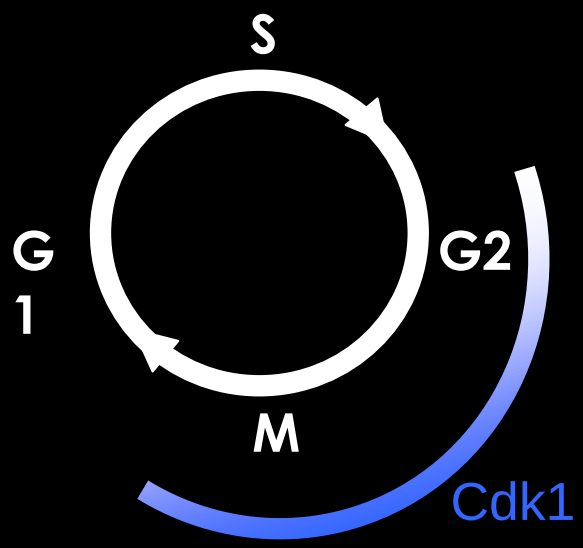
PCNA-GFP

Cdk1 inhibition



Cdk1 activity is necessary for rapid apical migration.

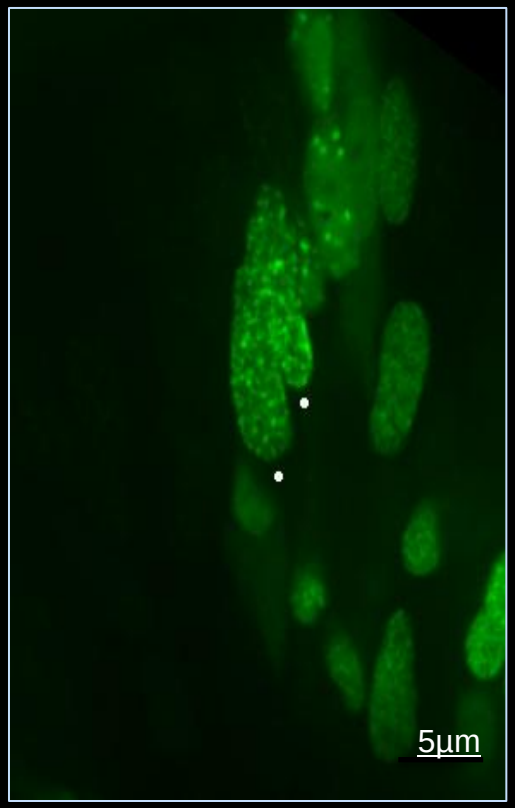
Is there a link between Cdk1 activity and nuclear movements in G2? Premature Cdk1 spike.



PCNA-GFP

Premature Cdk1 activity (Wee1 inhibitor)

apical

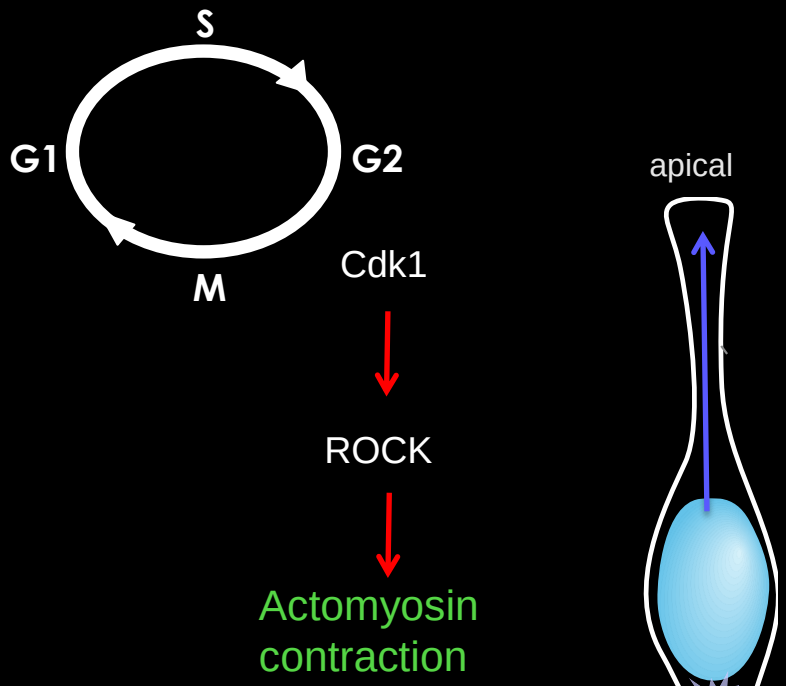


basal

Cdk1 activity is necessary and sufficient for rapid apical migration.

How is force generated to move nuclei in G2?
Molecular cascade responsible for actomyosin activity!

The cascade we uncovered so far:

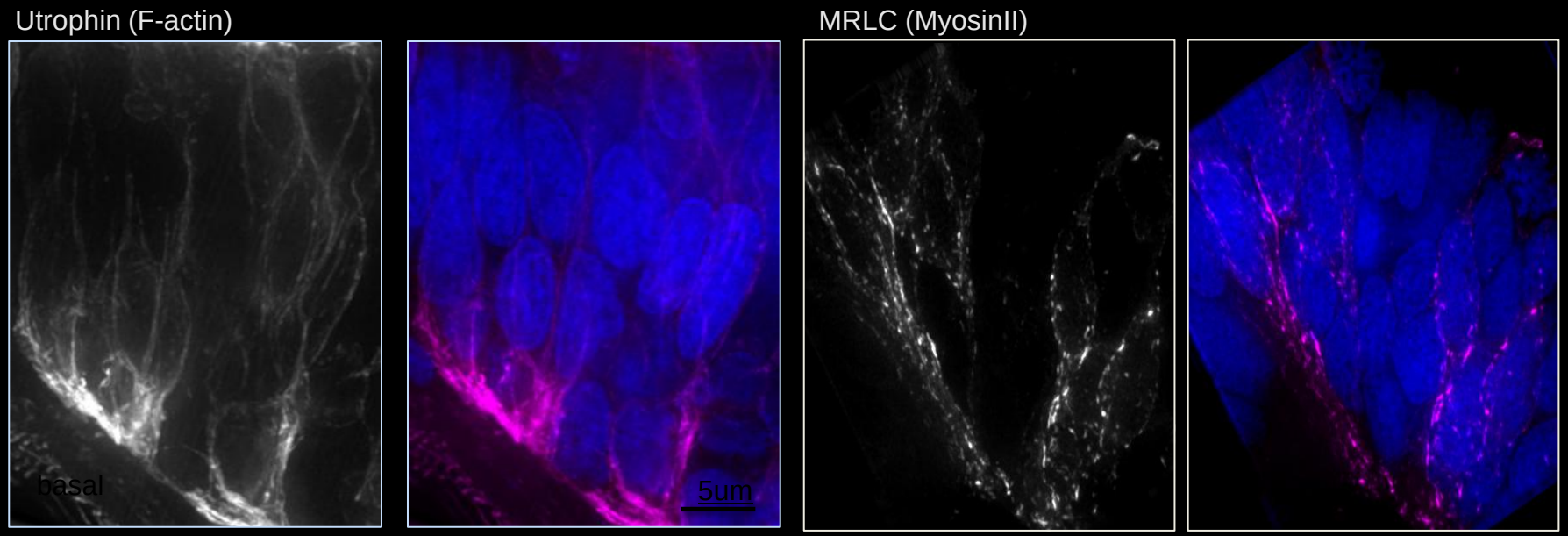


How does Cdk1 activity lead to directed actomyosin contraction and thereby directed nuclear movement in G2?

Cdk1 Bias?

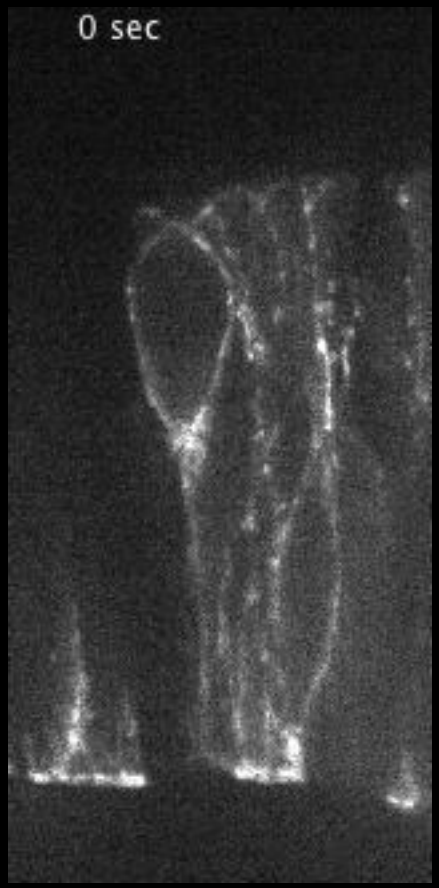
Actomyosin Bias?

Pseudostratified epithelia show a basal bias of actomyosin distribution. This bias is independent of cell cycle stage.

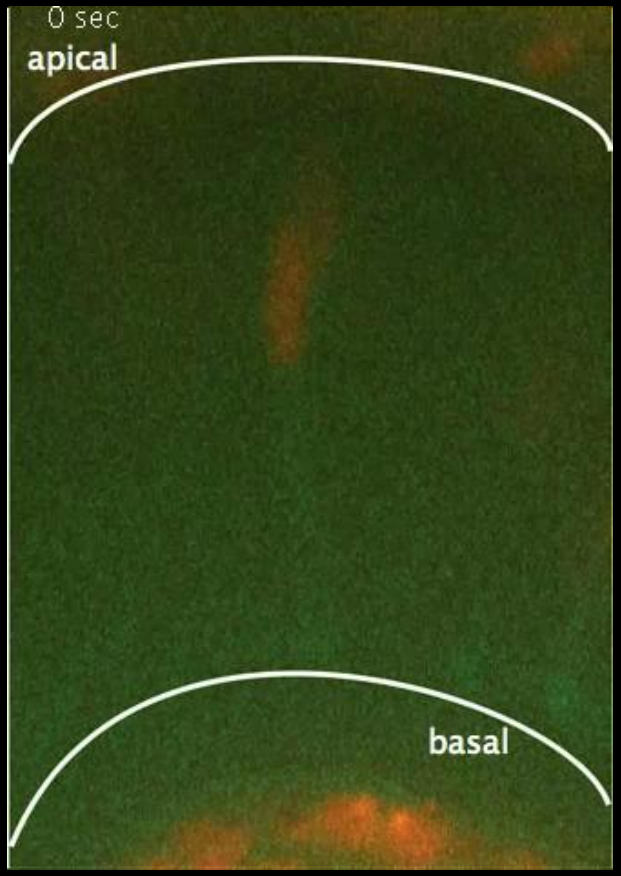


Pseudostratified epithelia show a basal bias of actomyosin distribution which is due to a constant apicobasal actin flow.

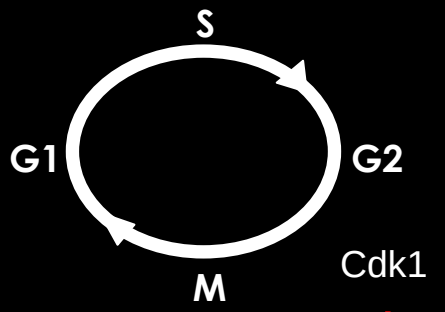
Utrophin (F-actin)



PA-Utrophin (F-actin)



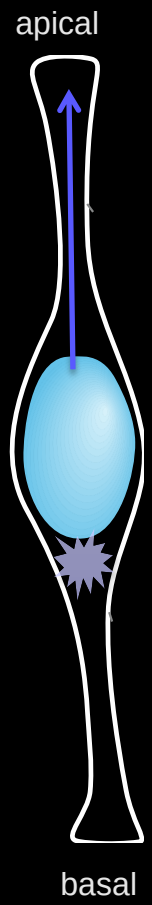
The cascade we uncovered so far:



Cdk1

ROCK

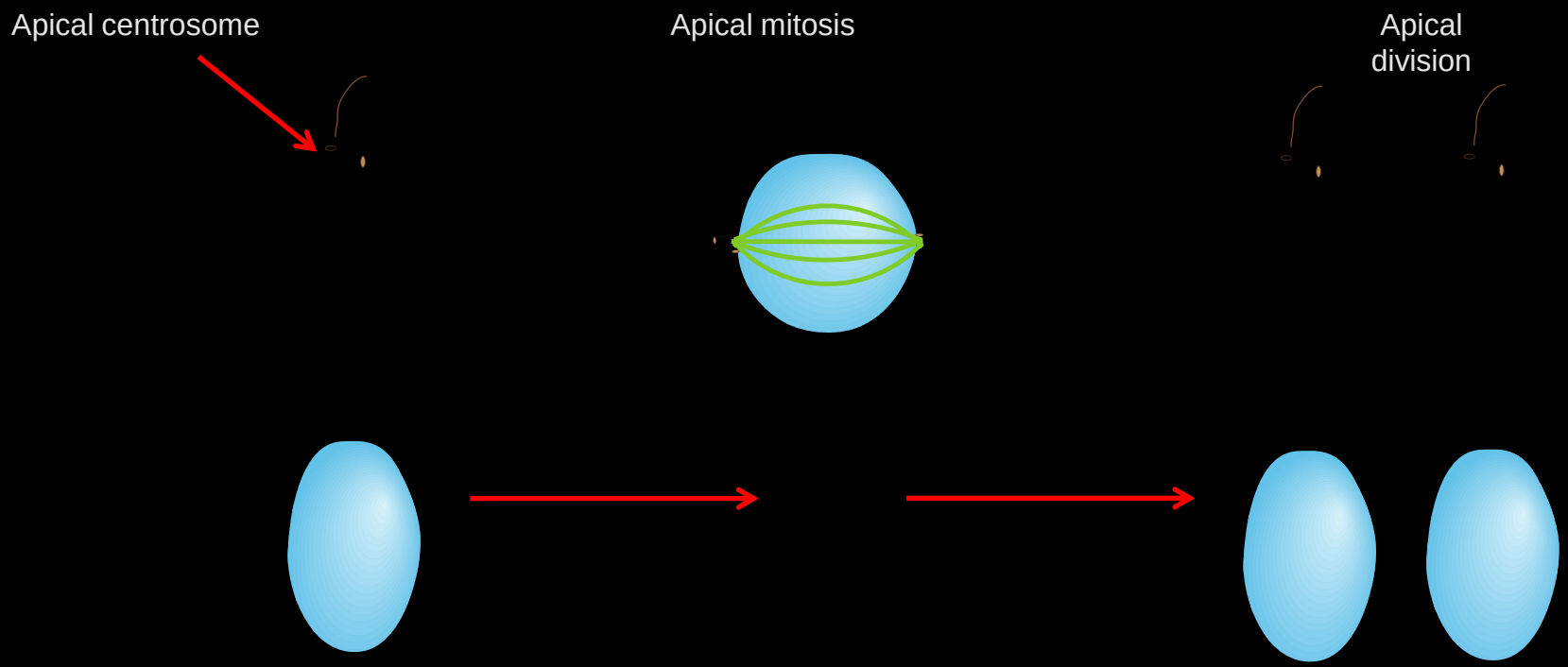
Actomyosin contraction



A constant apico-basal actin flow leads to basal bias of actomyosin contractility upon Cdk1 activation.

Ok, now we know more about the machinery but what is the reason for all mitosis to occur apically anyhow…?

Textbook hypothesis:

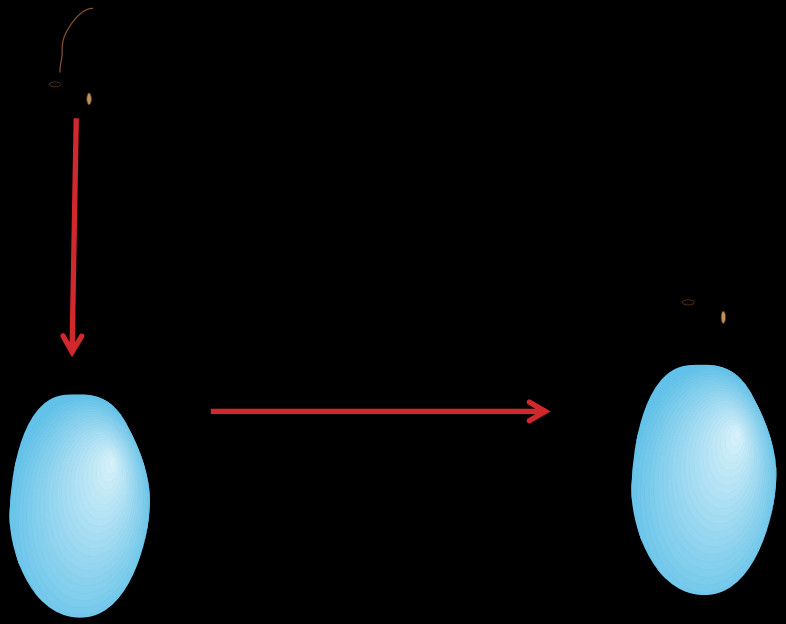


Is the apically located centrosome responsible for rapid apical migration of nuclei…?

It is the mere vicinity to a centrosome that allows entry into mitosis?

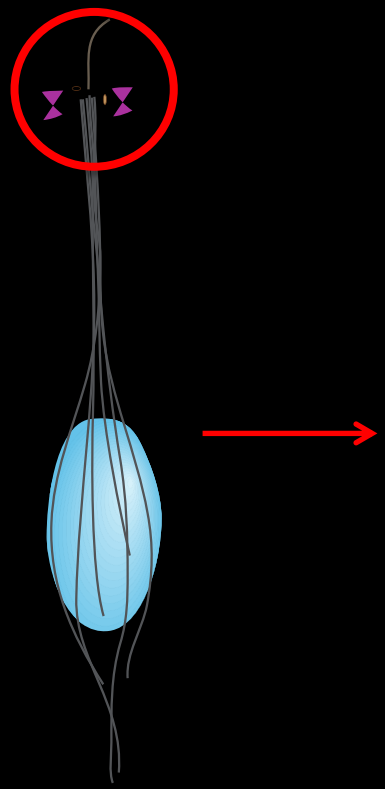
What happens when we supply basal centrosomes?

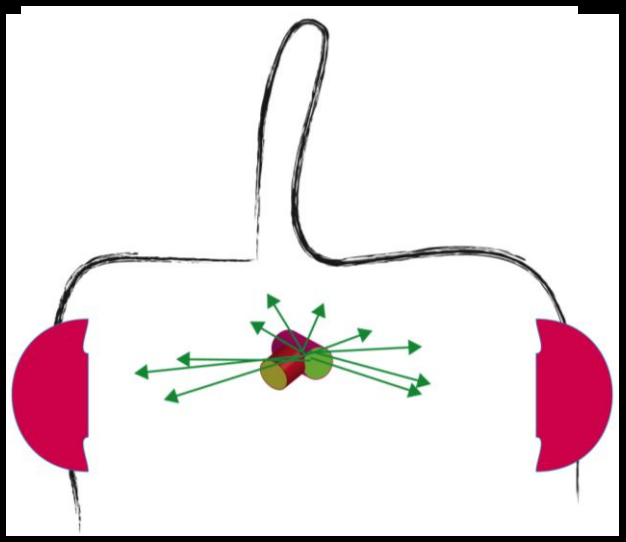
First approach: Basal mispositioning of centrosome.



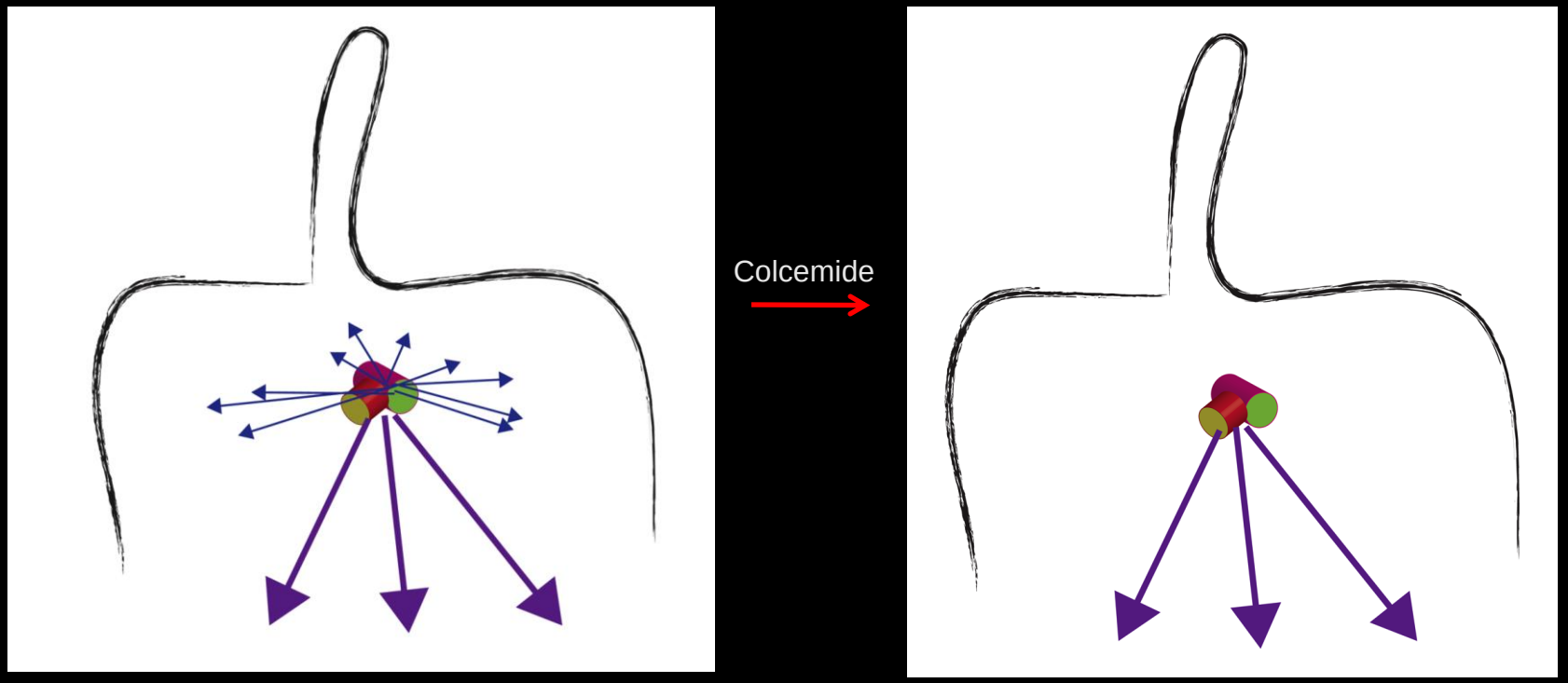
Structures responsible for tethering the centrosome apically.

A) Primary cilium
B) Microtubules
C) Adherens junctions

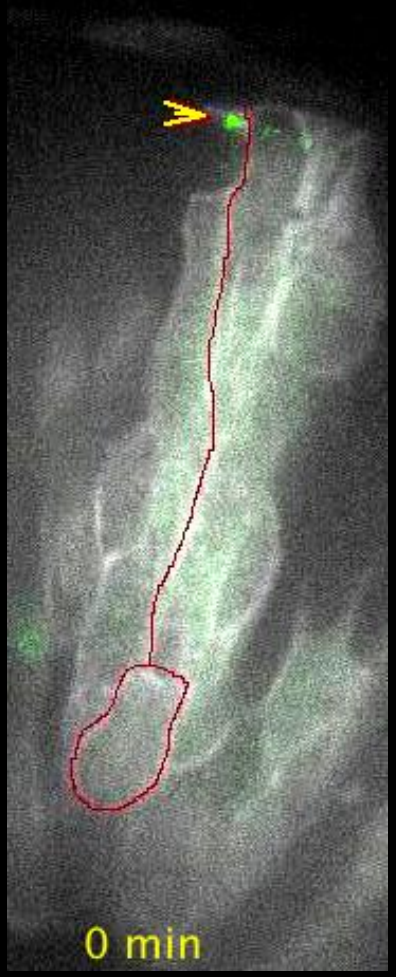




Interference with dynamic astral microtubules using microtubule destabilizing drug colcemide.



Interference with dynamic astral microtubules using microtubule destabilizing drug colcemide: IKNM still occurs even when NEB happens basally!



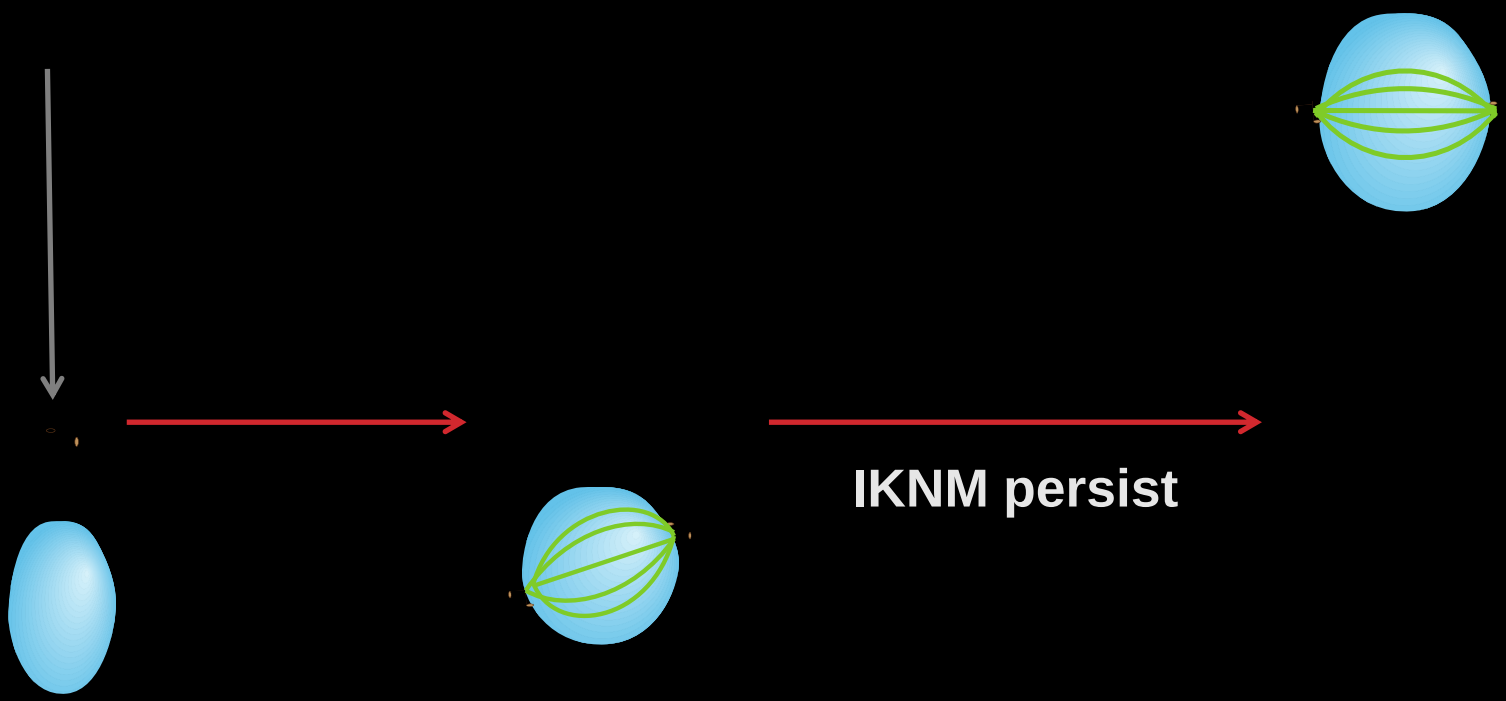
Colcemide => mild MTs depolymerizing drug

Centrin-GFP

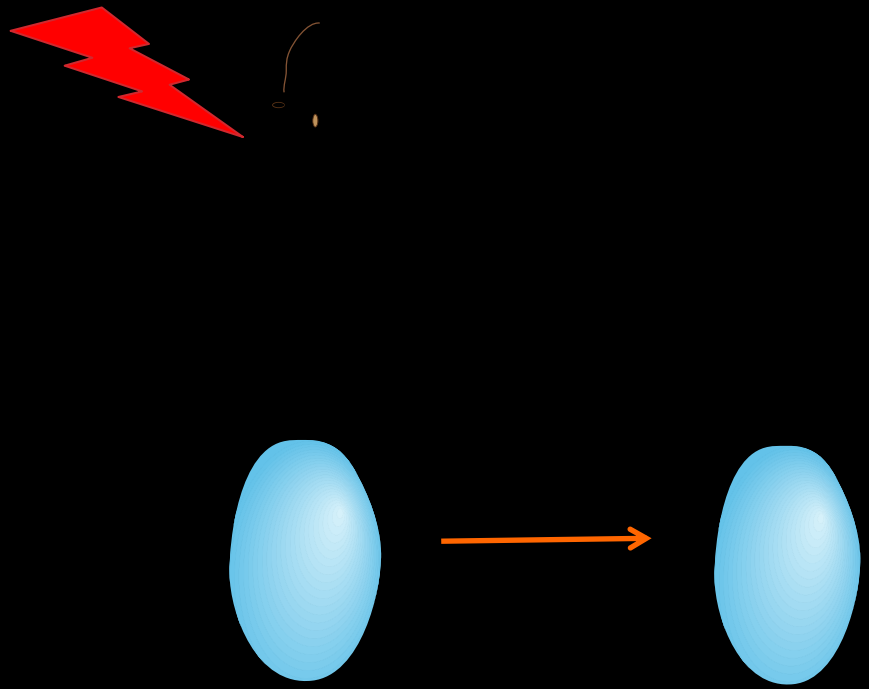
H2B-RFP

Mem-mKate2

Summary: centrosomes can be mispositioned towards basal location by interference with dynamic astral microtubules or adherens junctions. HOWEVER IKNM persists!



Second approach: Full centrosome ablation.

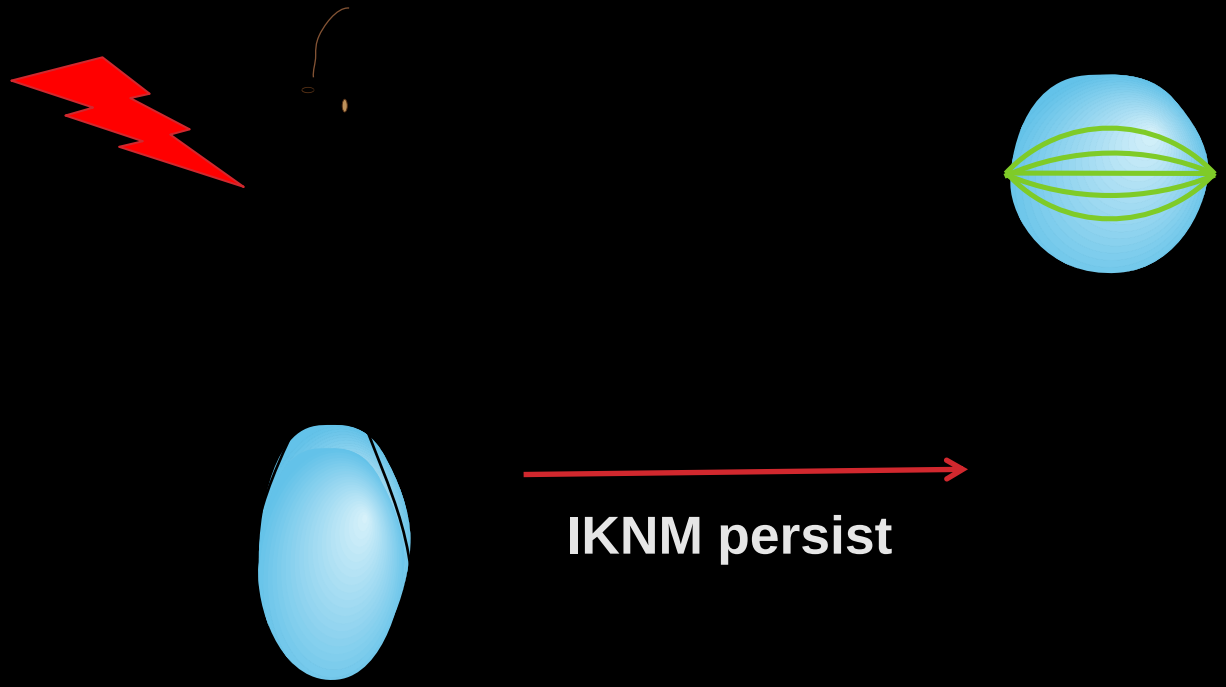


Second approach: Full centrosome ablation.

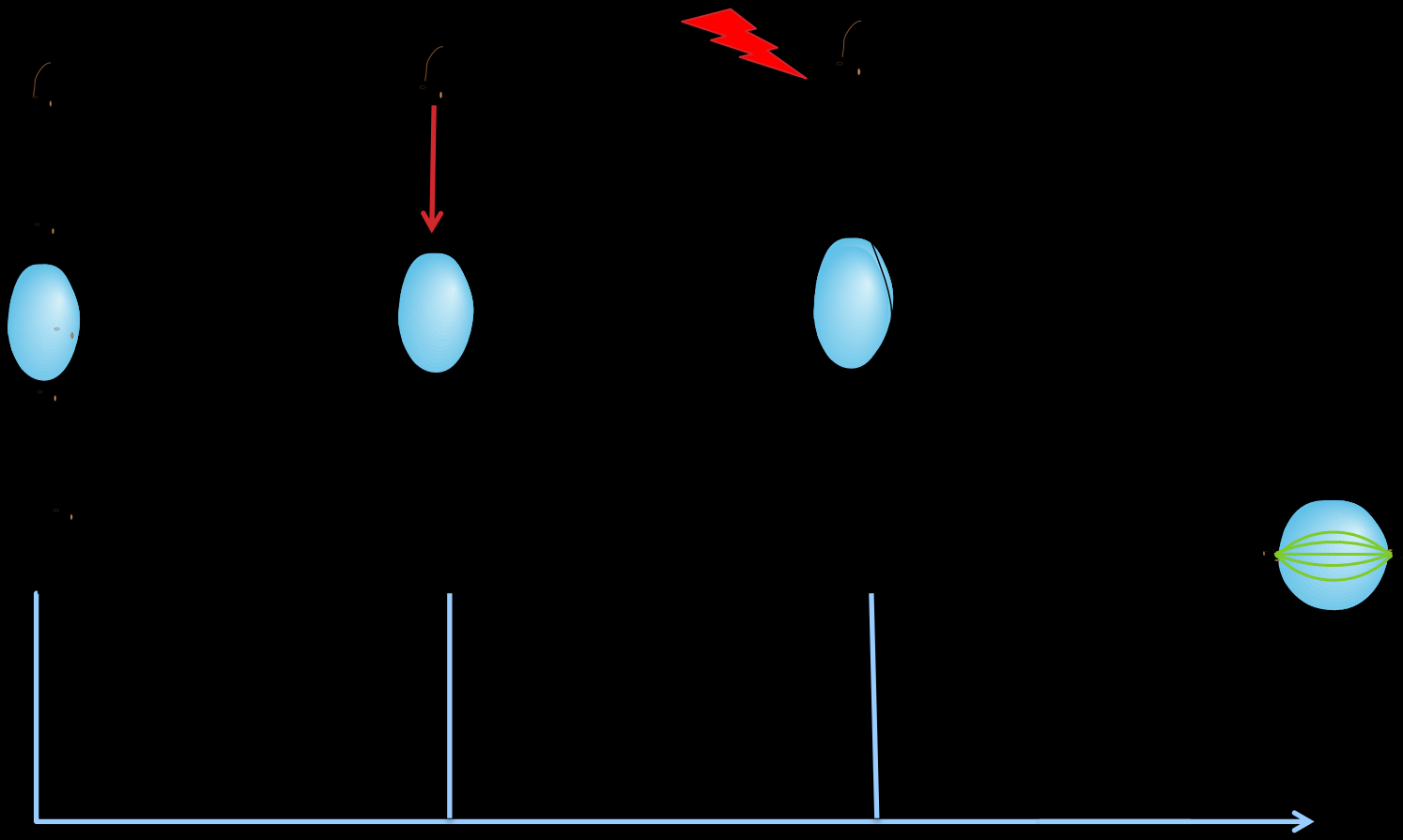
Second approach: Full centrosome ablation. Statistics:

	No evident centrin signal	Weaker centrin signal	Weak ablation/ non-targeted ablation	Cell death/ extrusion
Total	5	9	3	9
Divides normally	2	4	1	
Divides obliquely/not in plane	1	2	1	
Stuck in mitosis apically following NEB	2	x	x	
Shows apical migration but does not perform NEB	x	2	1	
Stalls non-apically	x	1	x	

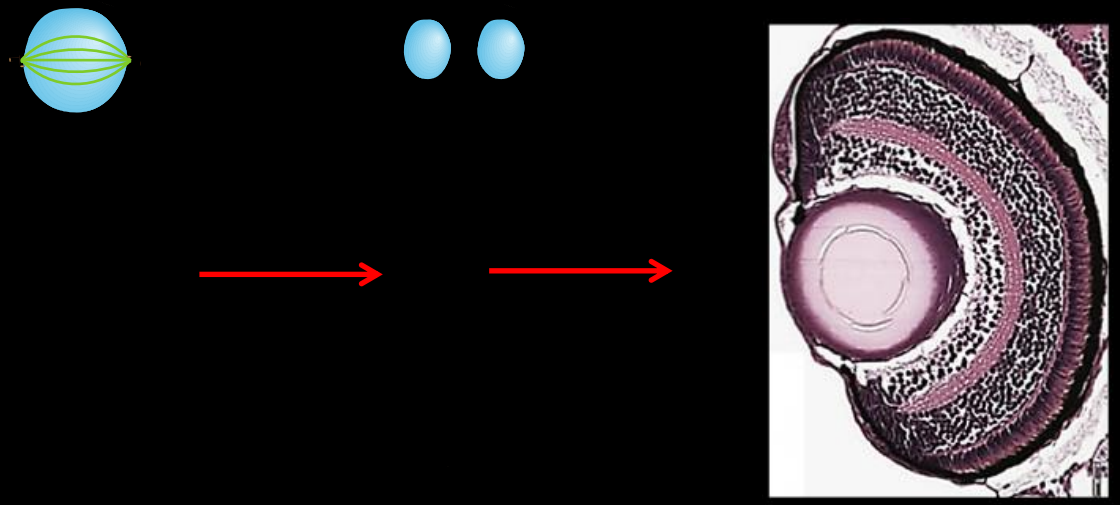
Centrosome removal does not inhibit IKNM.

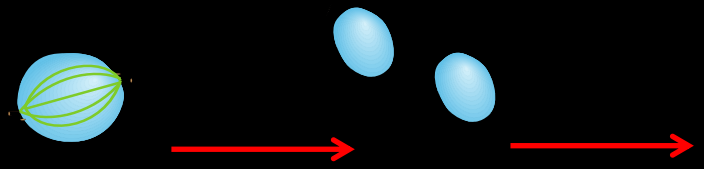


Summary centrosome modifications:



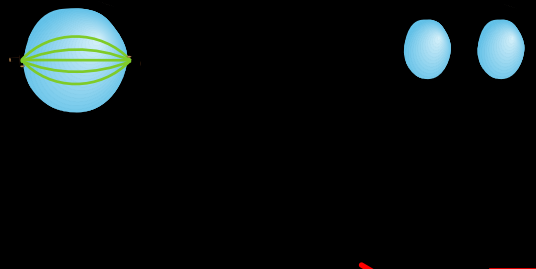
WHY does the cell ensure apical mitosis under all circumstances?
MAYBE because apical mitosis is the only way to reproducible ensure perpendicular division angles….





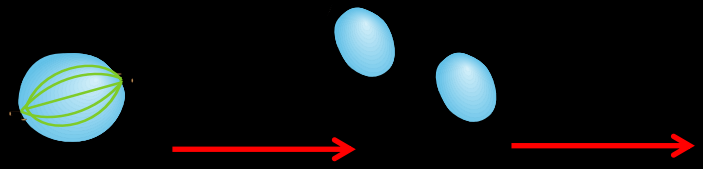
???????

Apical mitosis is most likely so robust to ensure proper tissue formation and equilibrium between proliferation and differentiation!



Equal distribution of cell fate determinants/api co-basal components

Proper tissue architecture and differentiation



Unequal distribution of cell fate determinants /apico-basal components

Perturbed tissue architecture and differentiation