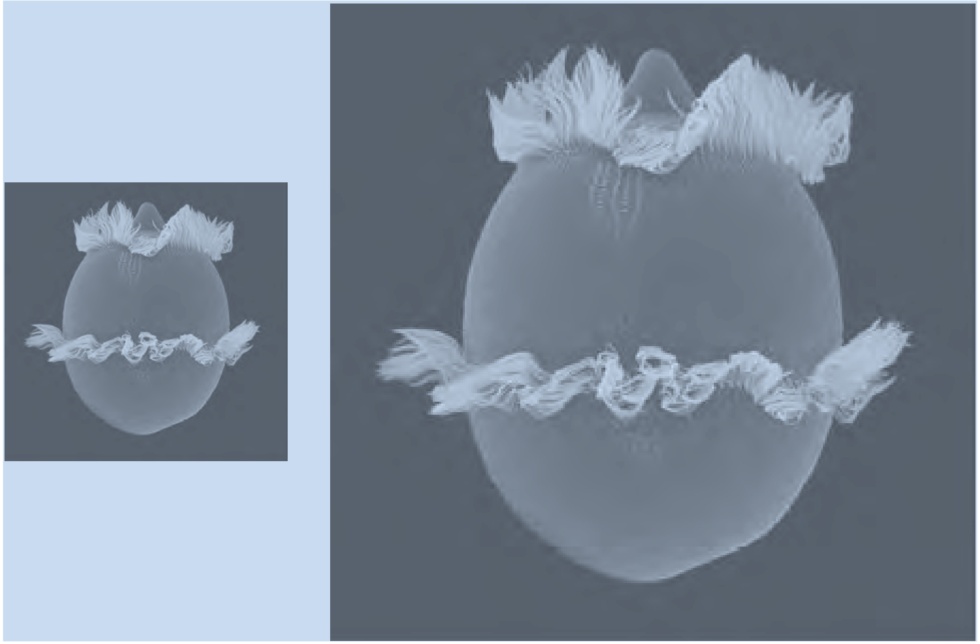
Cellular Growth and Form



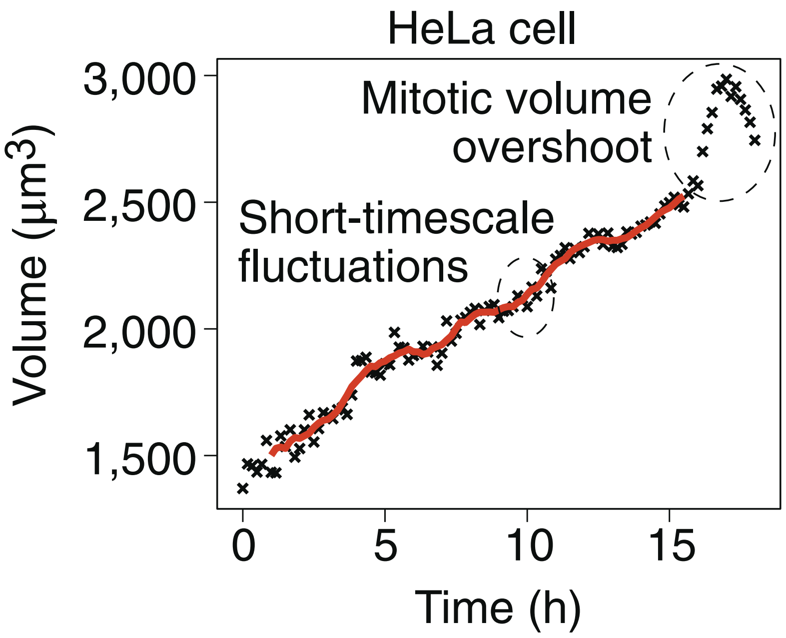
Course 4: Cellular scaling

Thomas Lecuit

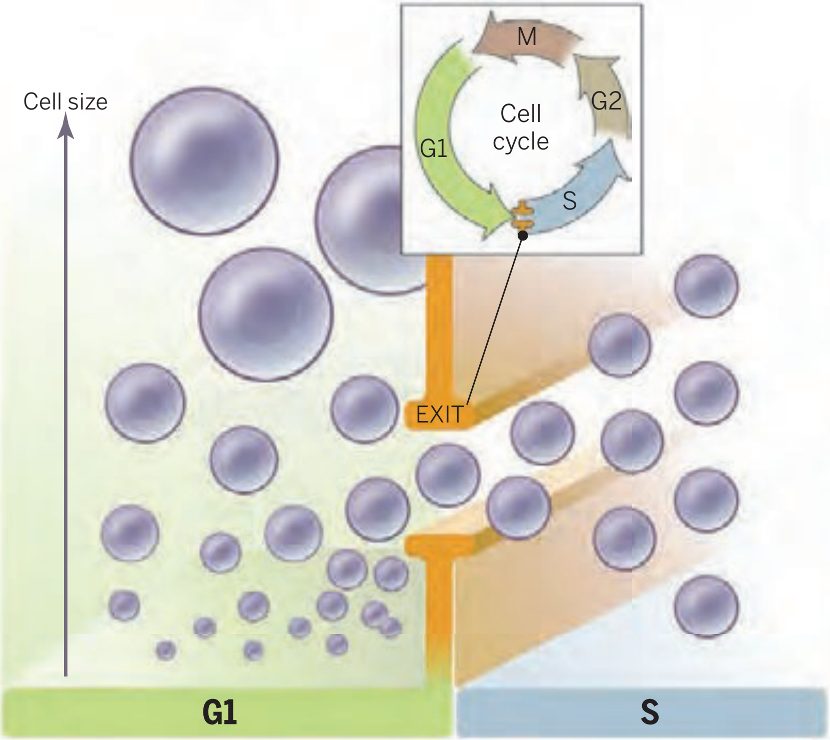
chaire: Dynamiques du vivant

COLLÈGE
DE FRANCE
1530

Cell size is set by co-regulation of cell division and cell growth



C.Cadart L. Venkova, P. Recho, M. C. Lagomarsino and M. Piel *Nature Physics* 15: 993–1004 (2019)

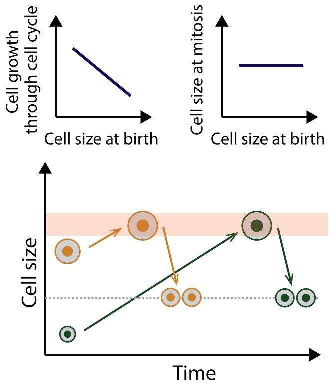


Miriam B. Ginzberg et al. and M Kirschner. *Science* 348, (2015); DOI: 10.1126/science.1245075

What do cells measure to control division?

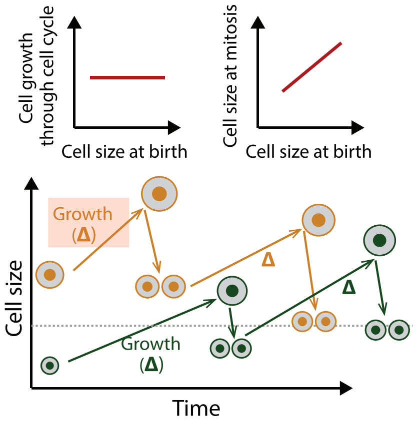
- **Sizer:** cells divide at *target* size

Deterministic vs Stochastic sizer



- **Timer:** cells divide after fixed duration
- **Adder:** cells divide after fixed size increase

Cell level vs population scale control

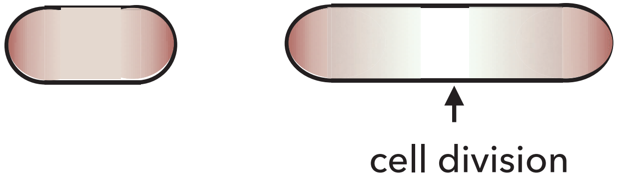


Scaling: Do cells measure their size?

Mechanisms that procure information about size

- Ratio of two length scales: cell geometrical length and biochemical length scales

  – An inhibitor of cell division is inhibited from the poles. As cell size increases assembly of cytokinetic ring is possible beyond critical cell size.

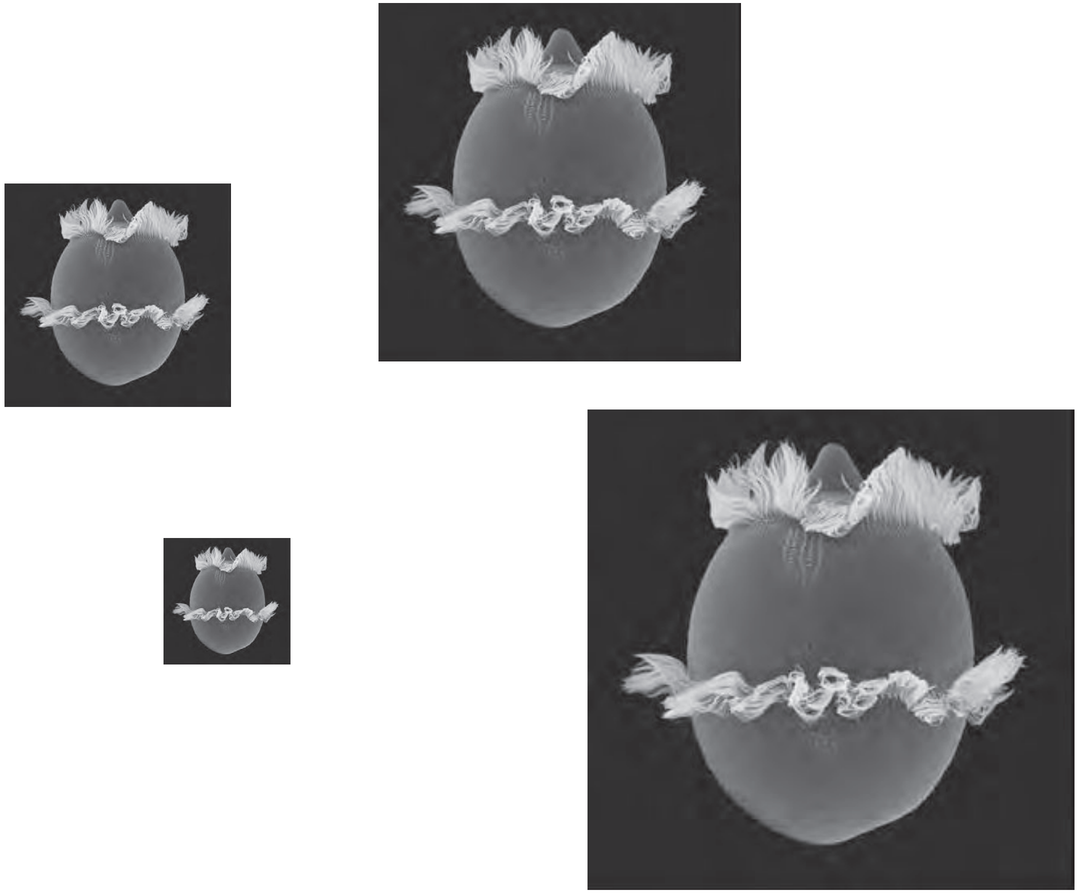


ex: MinCD in Bacteria
Pom1 in *S. pombe*

- Indirect measure of cell volume:

  Production of a size-invariant negative regulator of cell cycle progression provides an indirect feedback information about size.

Scaling: How do cells adjust their proportions?



Scaling in living organisms

Scaling of body parts, internal (organs, skeleton) and external (limbs) in animals

- Size range spans 6 orders of magnitude in land vertebrates

Julian S. Huxley

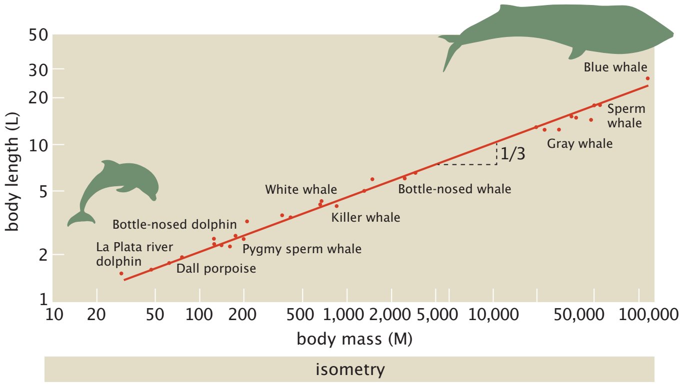
Georges Teissier

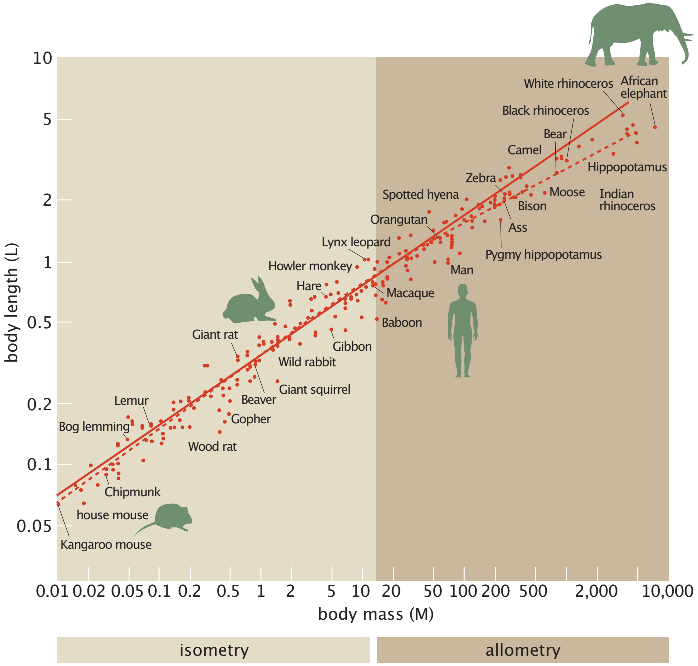
scaling law: $f(x) = b \cdot x^{\alpha}$

scaling invariance: $f(cx) \propto f(x)$

allometry: $\alpha > 1$ or < 1

isometry: $\alpha = 1$





(M ≤ 20 kg): $L = 0.329M^{0.336}$

(M < 20 kg): $L = 0.441M^{0.266}$

COLLÈGE DE FRANCE 1530

Thomas LECUIT 2020-2021

Cellular scaling between species

Cell internal organisation: number of organelles

<1 µm

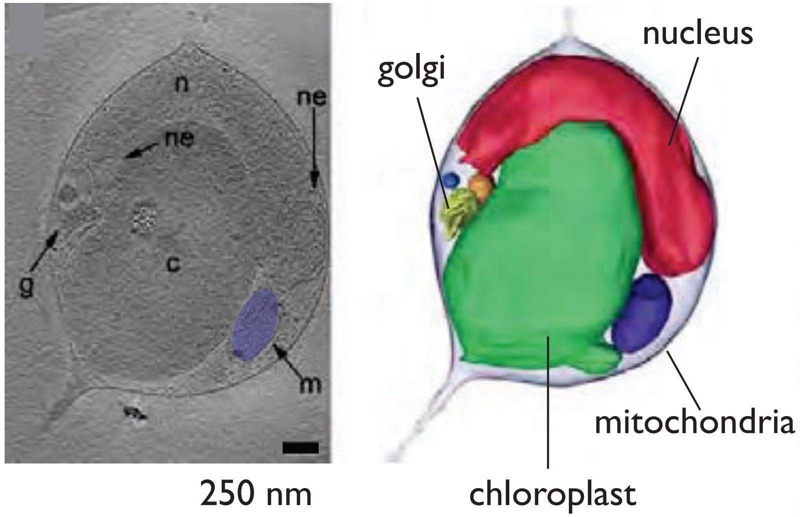
1 mitochondria

5-10 µm

10 mitochondria

200 µm

few 1000s mitochondria



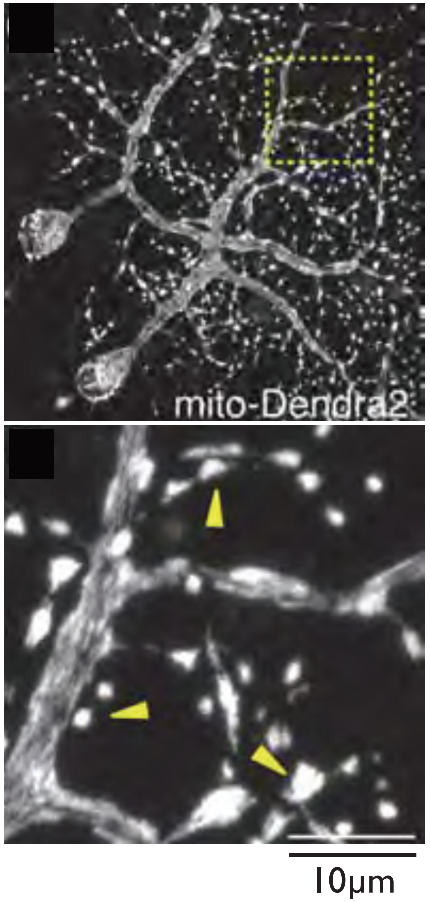
Ostreococcus tauri

picoplankton

Henderson GP, Gan L, Jensen GJ (2007)
PLoS ONE 2(8): e749.
doi:10.1371/journal.pone.0000749

Saccharomyces cerevisiae

T. Klecker and B. Westermann *Biological Chemistry* 401 (2020)
doi.org/10.1515/hsz-2019-0439

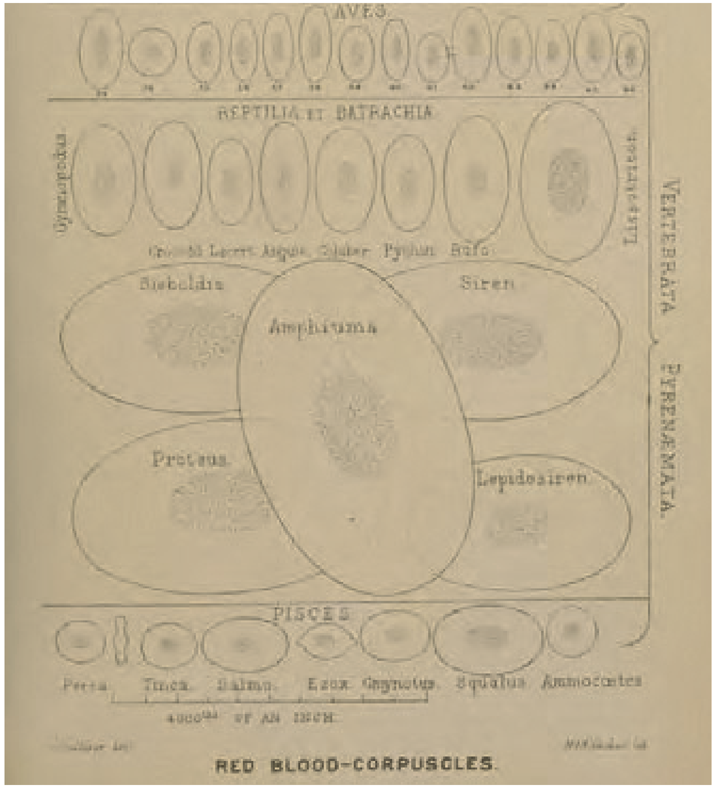


Purkinje neuron
mouse

Cellular scaling between species…

Cell internal organisation: size of organelles

1875: G. Gulliver's observation of red blood cells in different vertebrate species revealed approximate scaling of nuclei to cell size



Gulliver, G. (1875). On the size and shape of red corpuscles of the blood of vertebrates. *Proc. Zool. Soc. Lond.* 474–495.

…and within species

This was later further documented within a given species:

1900':

- Richard Hertwig (1850-1937): sea urchins and protists.
  Kern-plasma relation: ratio between nuclear and plasma (cytoplasm) volumes

  (1903) Uber Korrelation von Zell- und Kerngrosse und ihre Bedeutung für die geschlechtliche Differenzierung und die Teilung der Zelle. *Biol. Centralb.*, Bd. 22.

- Theodor Boveri (1862-1915): sea urchins

  (1905) Zellenstudien V. Uber die Abhangigkeit der Kerngrosse u. Zellenzahl der Ausgangszellen. Jena.

- Edwin Conklin (1863-1952): *Crepidula plana*

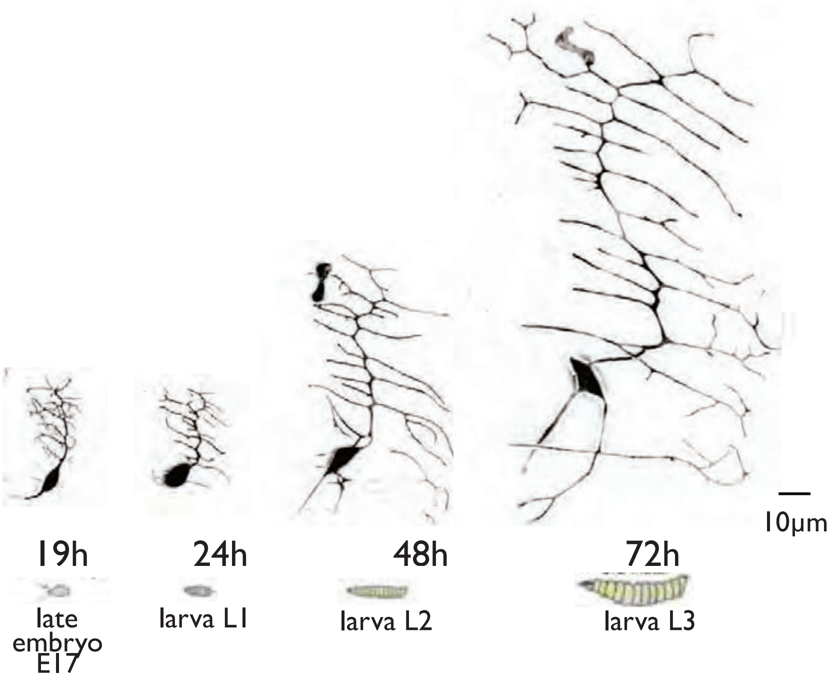
  (1912) Conklin, E. *J. Exp. Embryol.* 12, 1–98.

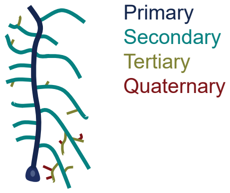
R. Hertwig

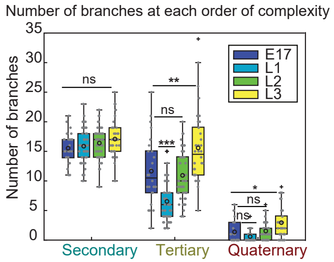
T. Boveri

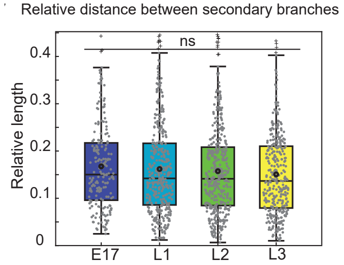
Cellular scaling within species

Cell internal organisation — Cell shape and external organisation

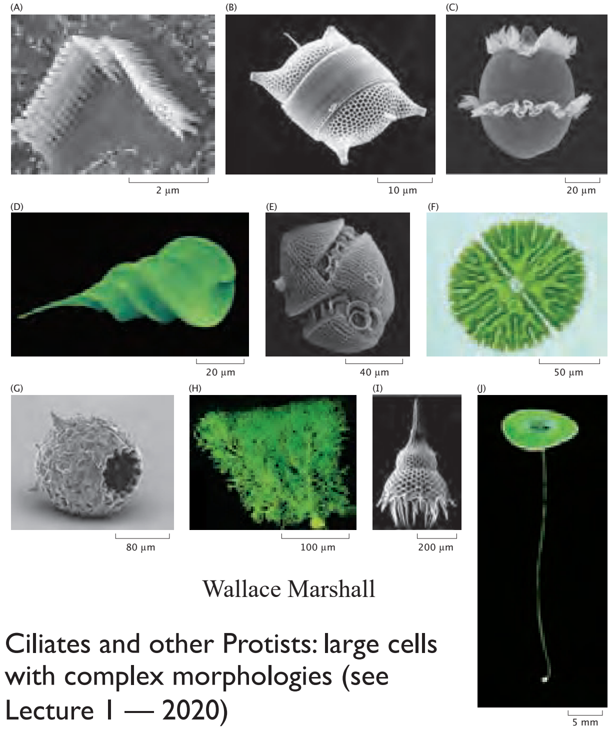






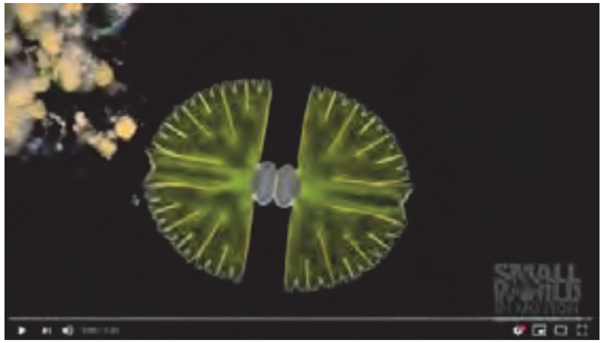


A. Palavalli, N. Tizon-Escamilla, J-F. Rupprecht, T. Lecuit, *Current Biology* (2020), https://doi.org/10.1016/j.cub.2020.10.054



Wallace Marshall

Ciliates and other Protists: large cells with complex morphologies (see Lecture 1 — 2020)

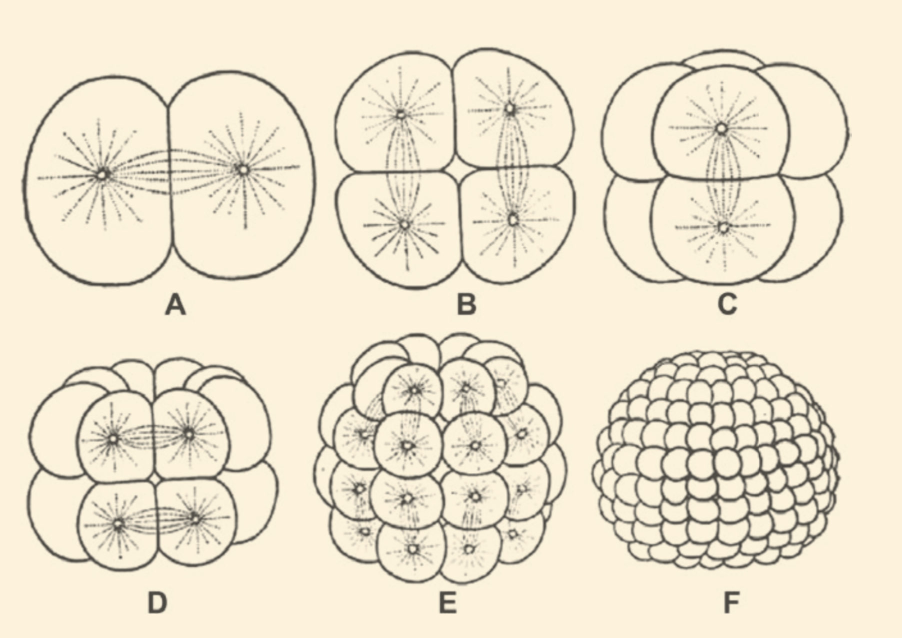


Green alga — *Micrasterias rotata*

Cell size change during embryonic cleavage

Cells decrease exponentially their size (volume) during early steps of animal development

First 10 hours of amphibian embryogenesis, cell diameter decreases around 100-fold, from a 1.2 mm egg to 12 µm diameter blastomeres



The cell in development and inheritance
EB Wilson (1897)

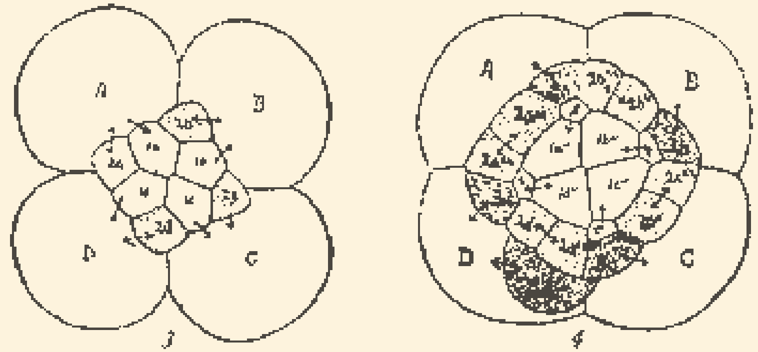


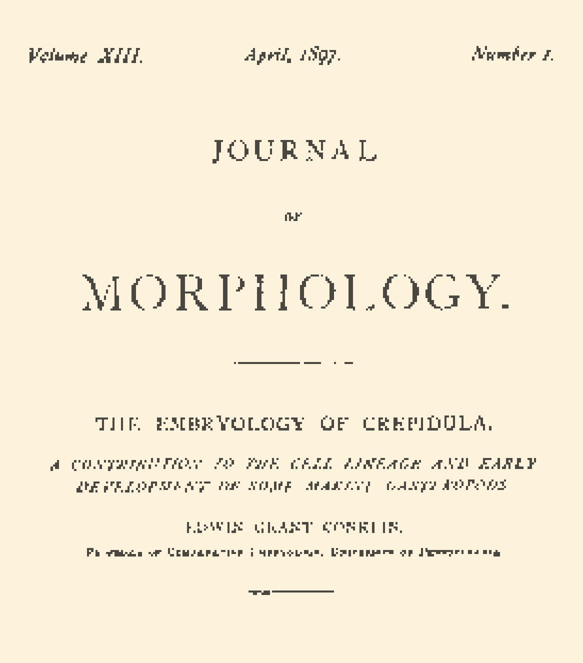
DIAGRAM 3.— Crepidula, twelve cell stage.

DIAGRAM 4.— Crepidula, twenty-five cells; *t*, turret cells (trochoblasts). In these and some of the following diagrams the macromeres and first quartette are unshaded; the second quartette is stippled; the third quartette is shaded with lines; and the fourth quartette (4d) with dots and circles. The direction of the various cleavages is shown by means of arrows.

The Embryology of *Crepidula* (Conklin 1897)

Cell size change during embryonic cleavage

Cells decrease exponentially their size (volume) during early steps of animal development



Volume XIII. *April, 1897.* *Number 1.*

JOURNAL

OF

MORPHOLOGY.

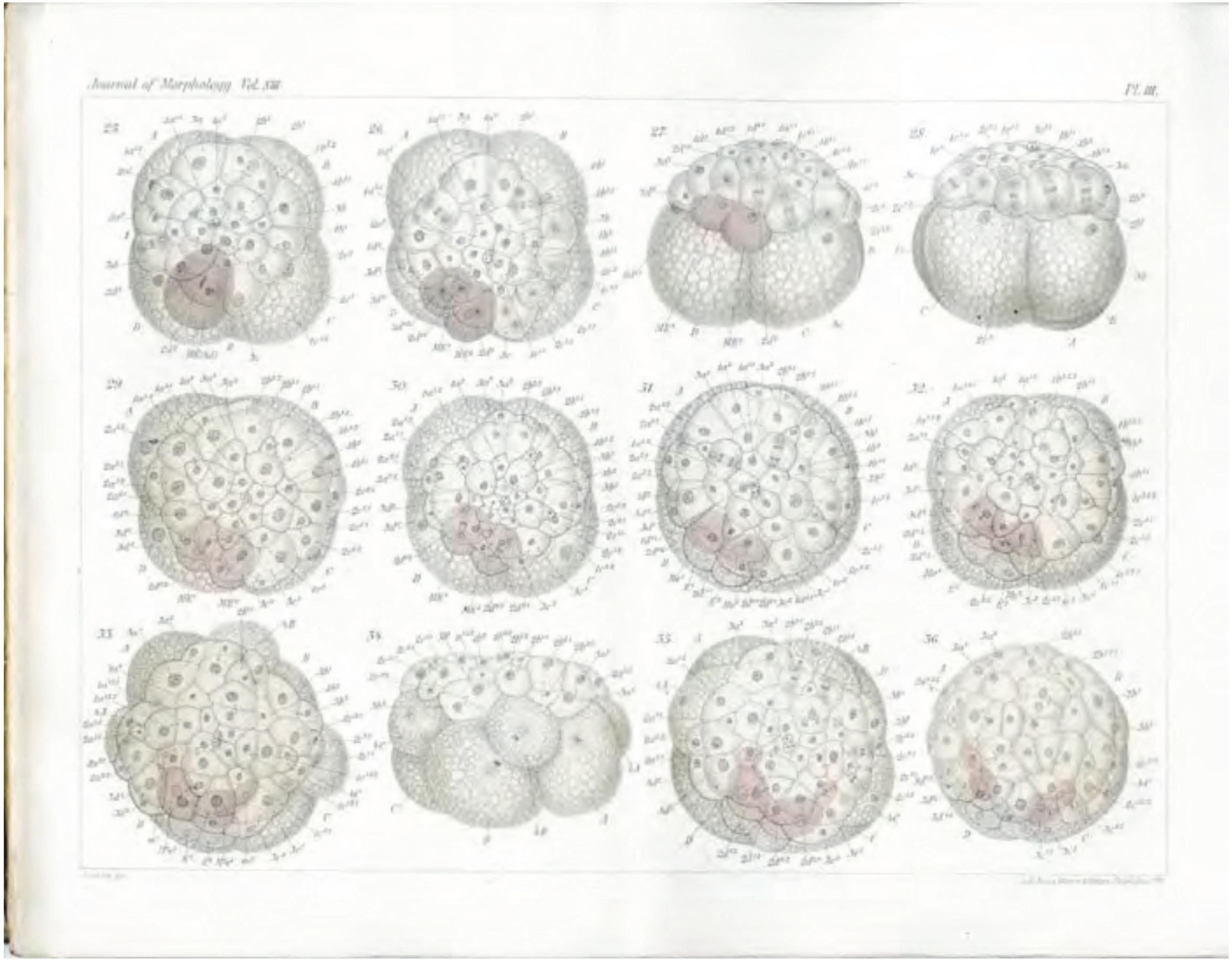
THE EMBRYOLOGY OF CREPIDULA.

A CONTRIBUTION TO THE CELL LINEAGE AND EARLY DEVELOPMENT OF SOME MARINE GASTEROPODS.

EDWIN GRANT CONKLIN,

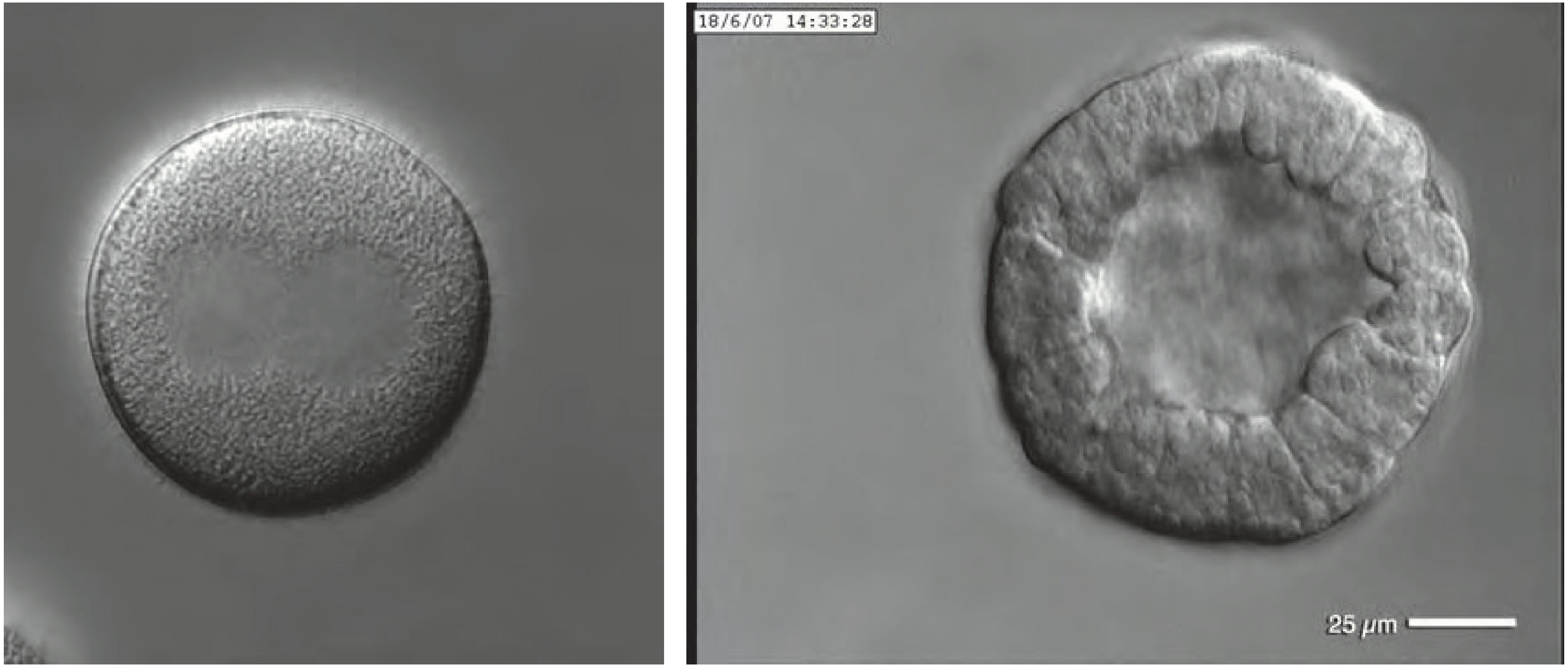
Professor of Comparative Embryology, University of Pennsylvania.

Marine Mollusk Gasteropod



Cell size change during embryonic cleavage

Cells decrease exponentially their size (volume) during early steps of animal development



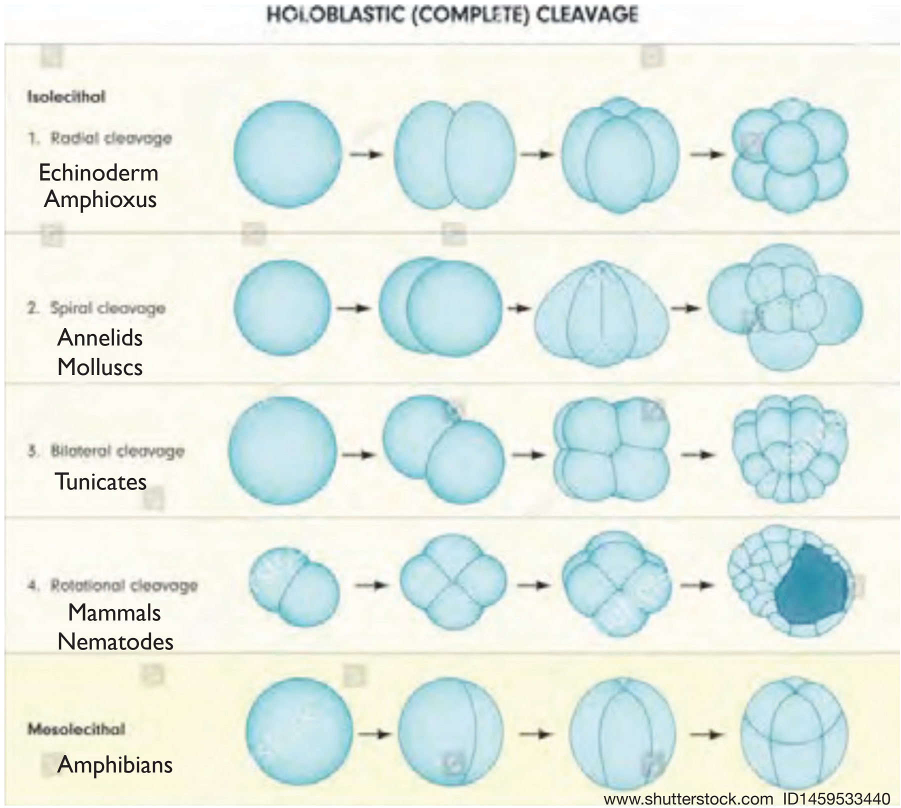
Strongylocentrotus droebachiensis —Green urchin

Sarsia sp. — Jellyfish

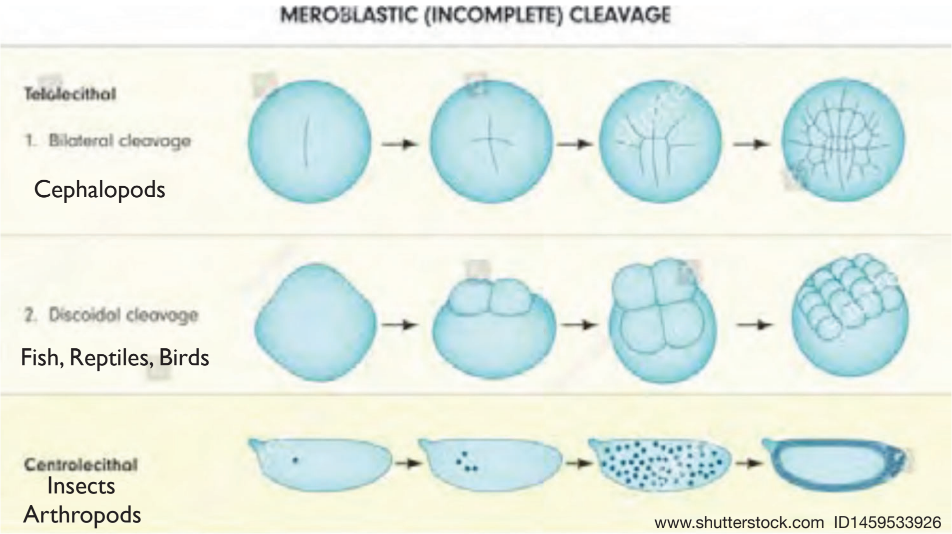
Georges von Dassow
http://www.gvondassow.com/

Cell size change during embryonic cleavage

Cells decrease exponentially their size (volume) during early steps of animal development



www.shutterstock.com ID1459533440



www.shutterstock.com ID1459533926

Cell size – scaling

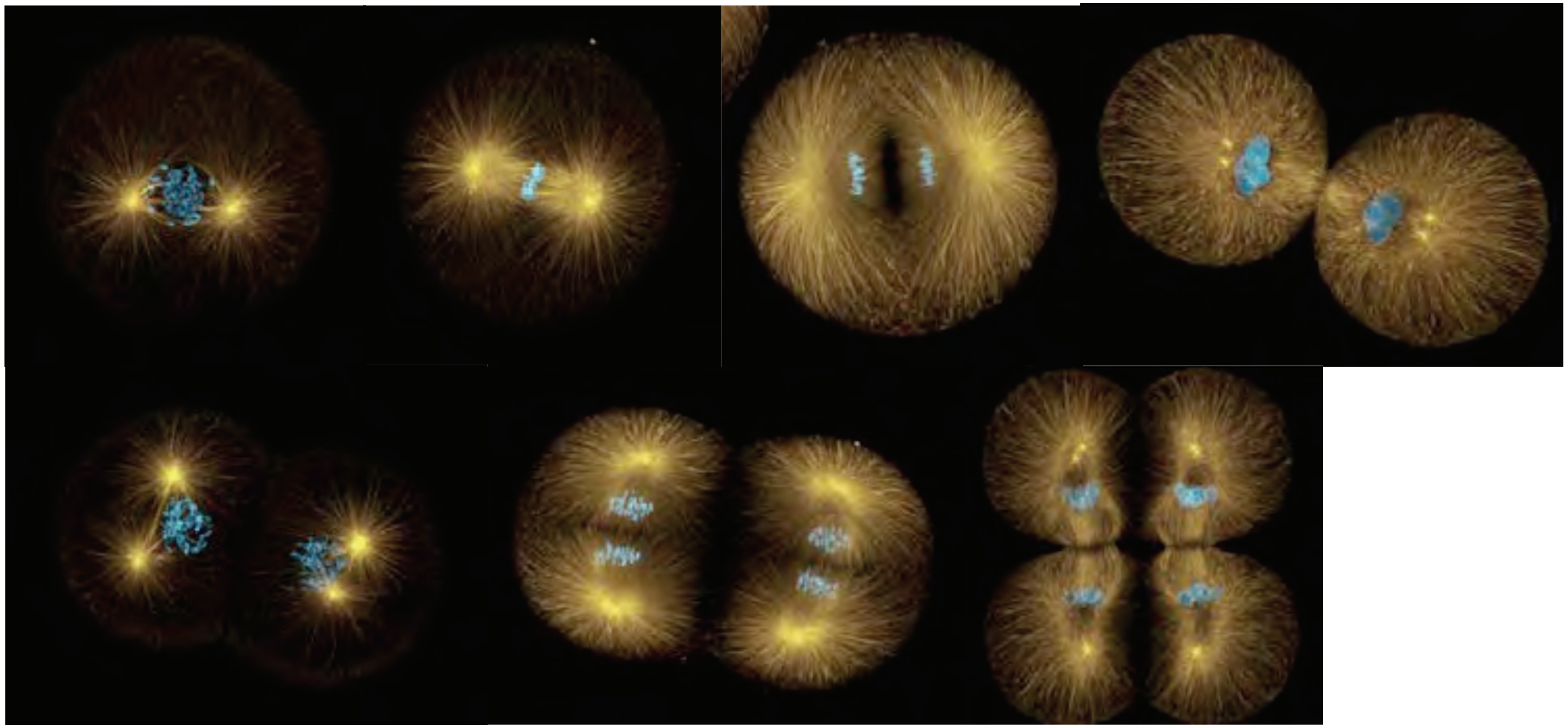
Statement of the problem:

- Cell size is tightly regulated but varies strongly during embryonic cleavage (by a factor of 2^N for N divisions)
- Cells grow 2-fold before they divide or may grow while they increase their ploidy
- As cells change their volume do internal organelles scale? What are the mechanisms of scaling?
- When cells display symmetric structures (eg. flagella or cilia) how is the size of these structures controlled

Q: Do internal cell structures and organelles scale with cell size?

Cell size change during embryonic cleavage

Q: Do internal cell structures and organelles scale with cell size?

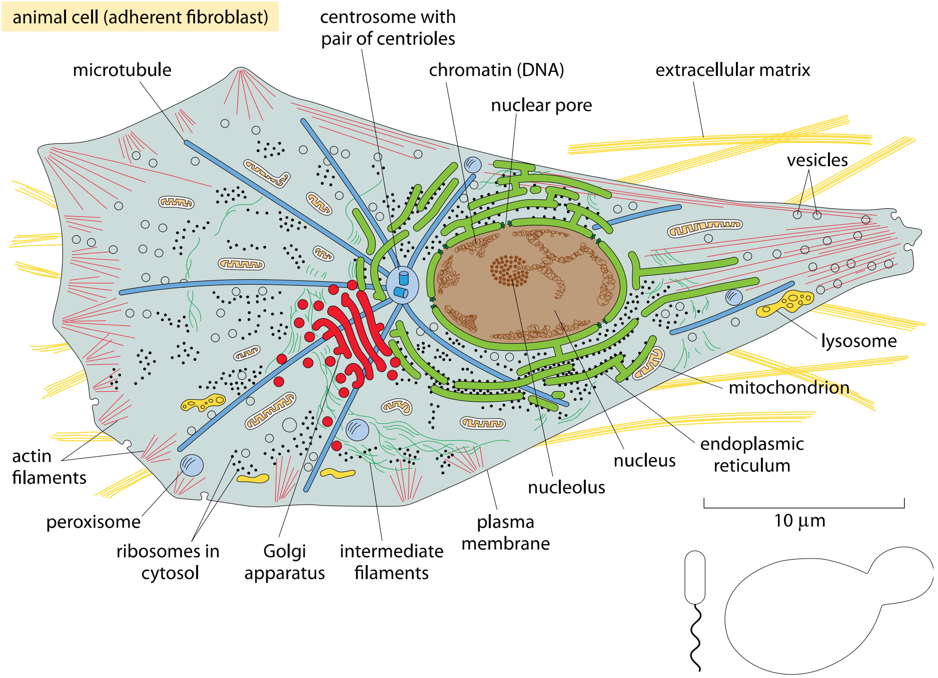


Cerebratulus marginatus

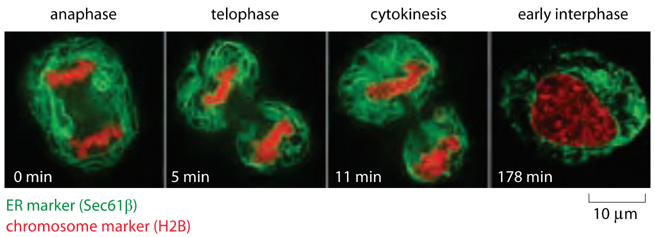
Georges von Dassow
http://www.gvondassow.com/

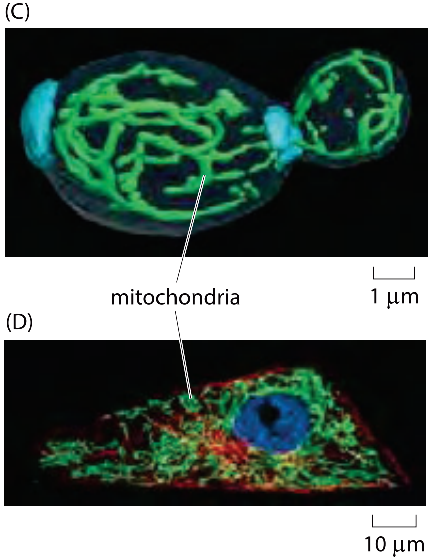
Internal cell organisation

Organelles
Cytoskeletal structures



Cell Biology by the numbers. Ron Milo, Rob Phillips, *illustrated* by Nigel Orme.
Garland Science 2012

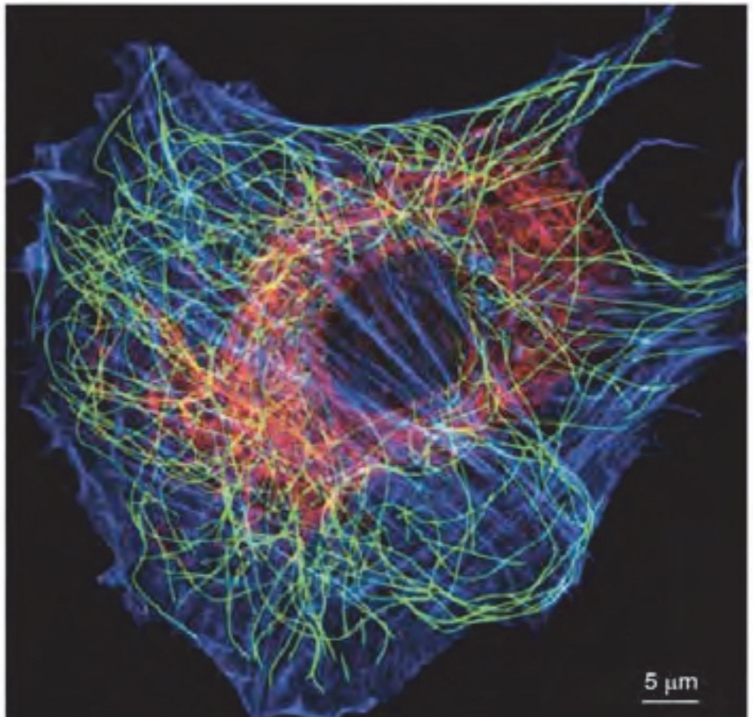




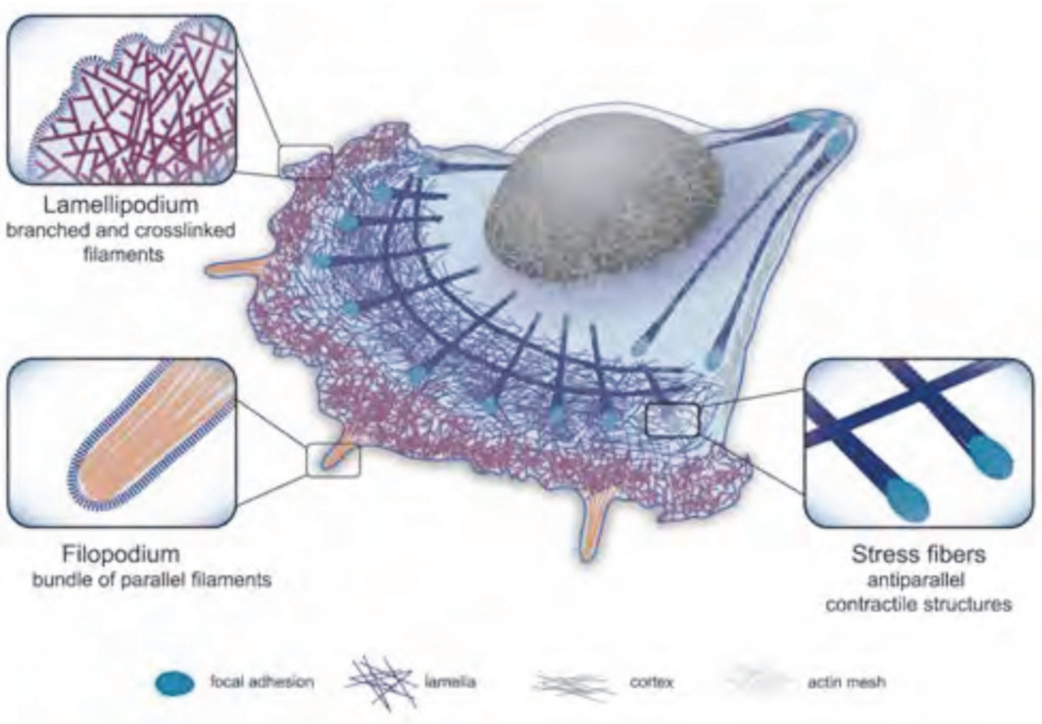
membrane type	percentage of total cell membrane	
	liver hepatocyte	pancreatic exocrine cell
plasma	2	5
rough ER	35	60
smooth ER	16	<1
Golgi apparatus	7	10
mitochondria outer	7	4
mitochondria inner	32	17
nucleus inner	0.2	0.7
secretory vesicle	-	3
lysosome	0.4	-
peroxisome	0.4	-
endosome	0.4	-

Internal cell organisation

Cytoskeletal structures

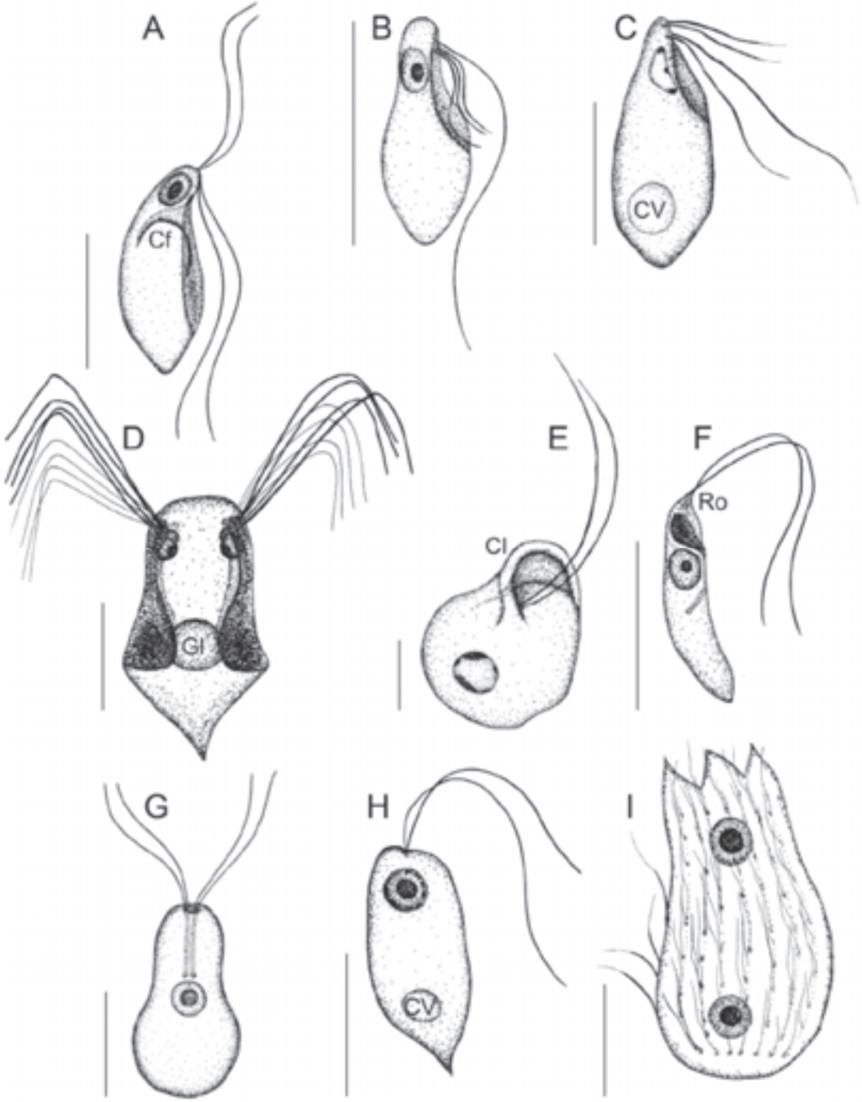


Microtubules (green)
Actin filaments (blue)
Intermediate filaments (red)



Letort G, Ennomani H, Gressin L *et al.*
https://doi.org/10.12688/f1000research.6374.1

External cell organisation



Appendages — eg. Flagella

- Protists: unicellular organisms, autotrophic or heterotrophic (neither animals nor plants)
- Flagellates

A , *Pharyngomonas kirbyi* ; **B** , *Percolomonas cosmopolitus* ; **C** , *Percolomonas descissus* ; **D** , *Psalteriomonas lanterna* ; **E** , *Heteramoeba clara* ; **F** , *Pleurostomum flabellatum* ; **G** , *Trimastigamoeba philippinensis* ; **H** , *Naegleria gruberi* **I** ,. *Stephanopogon minuta* .

Cf – cytopharynx; Cl – collar; CV – contractile vacuole; Gl – globule of hydrogenosomes; Ro – rostrum.

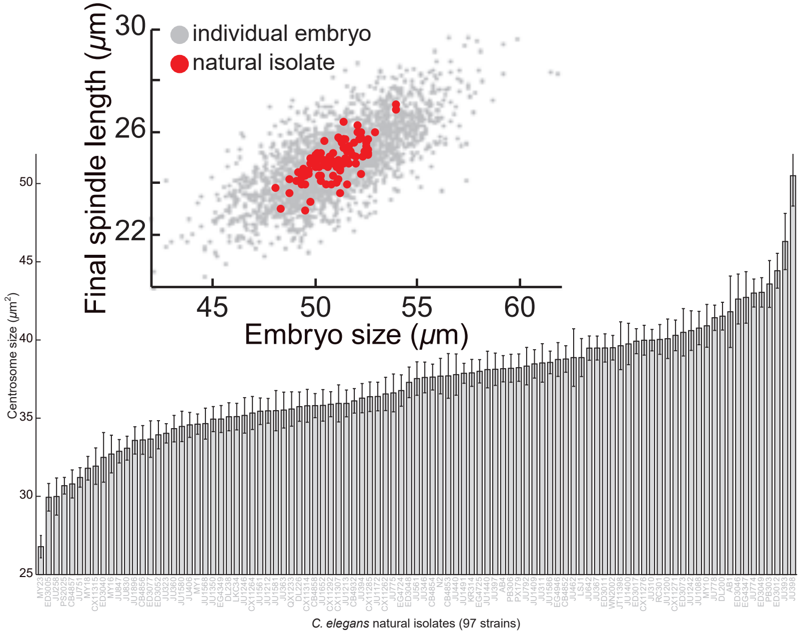
Scale bars = 10 µm.

After Broers et al., 1990; Bovee, 1959; Brugerolle & Simpson, 2004; Droop, 1962; Fenchel & Patterson, 1986; Page, 1967, 1988; Park et al., 2007; Park & Simpson, 2011; Yubuki & Leander, 2008.

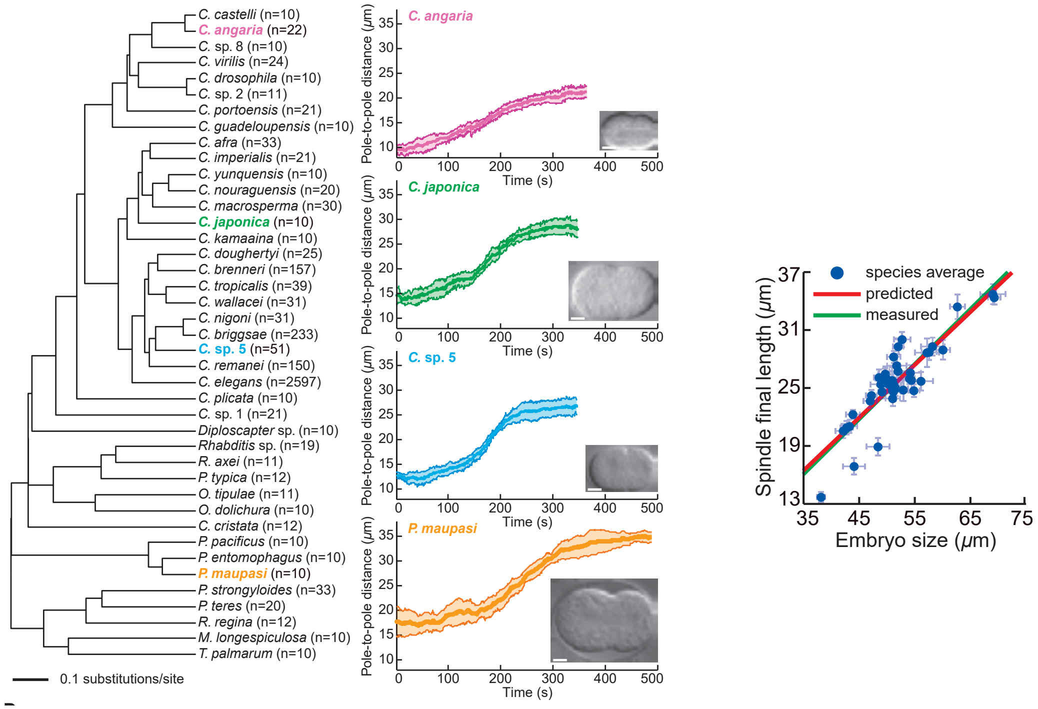
Within- and between-species scaling

Example: mitotic spindle

- Spindle size (and shape) vary among different species and cell types to ensure chromosome segregation fidelity, and proper spindle positioning
- In nematodes, embryo size, and thus cell size, is subject to stabilising selection
- Stabilising selection on embryo size quantitatively predicts within-species and between-species variation of spindle traits (eg. length)



Within-species analysis



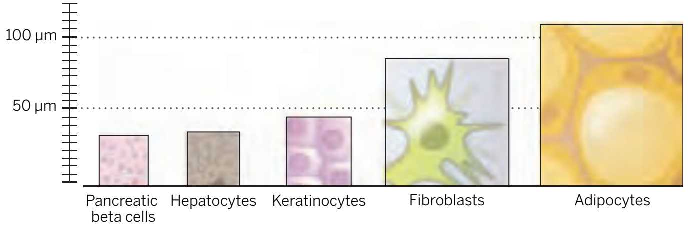
Between-species analysis

Farhadifar et al., and Marie Delattre and Dan. J Needleman. *Current Biology* 25, 732–740 (2015)

Cellular scaling

Between species

Between cell types within species



Within cell type within species

- Organelles must scale with cell volume: exponential?
- Yet the genome is not scaling with cell size

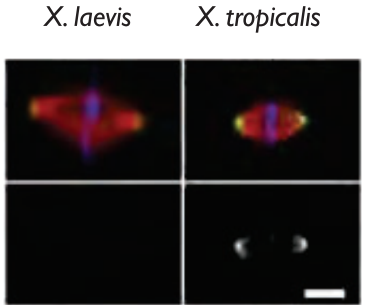
Cellular scaling

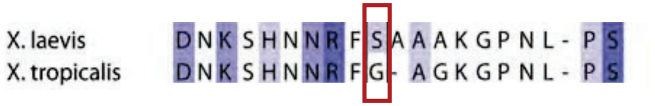
- Biochemical composition of cell:

Potential role of genes and gene regulation between species and cell types


Science 328, 633-636

Xenopus tropicalis *Xenopus laevis*

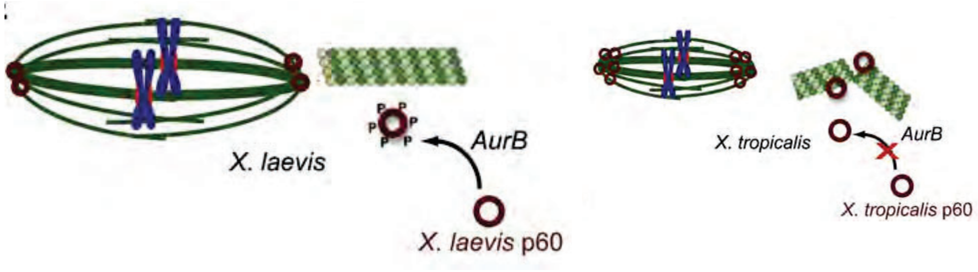




Katanin: Microtubule severing protein reduces spindle size

In *X. laevis*, Katanin p60 is phosphorylated by Aurora kinase B and inactivated

A single amino acid change renders *X. tropicalis* refractory to AuroraB inactivation

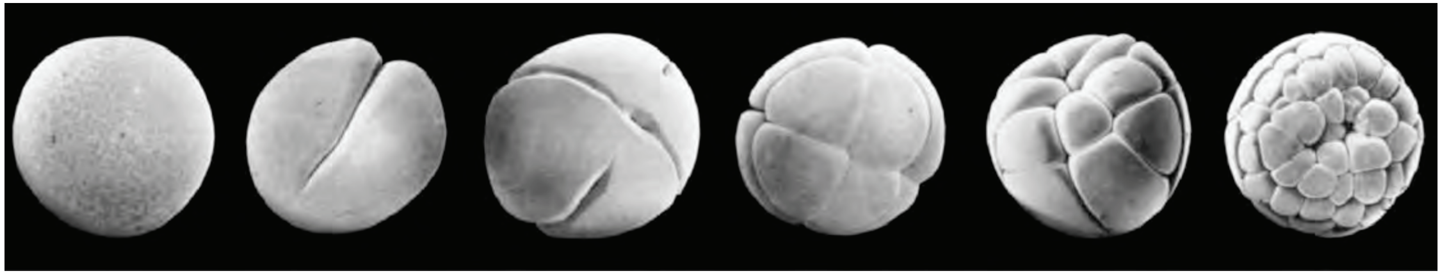


R. Loughlin et al. F. Nédélec and R. Heald. *Cell* 147, 1397–1407 (2011)

Cellular scaling

- Non genetic/biochemical adaptation to size:

— geometric cues, size of pool of constituents?
— how does biochemistry respond to geometry?



First 12 cleavage cell divisions. The cell radius decreases 16-fold with little change in biochemistry

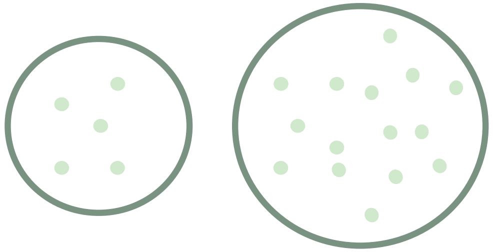
T. Mitchison et al. *Cold Spring Harb Perspect Biol* 2015;7:a019182 (2015)

Cellular scaling

- Number of organelles/subcellular structures

— mitochondria, Golgi, endosomes etc.

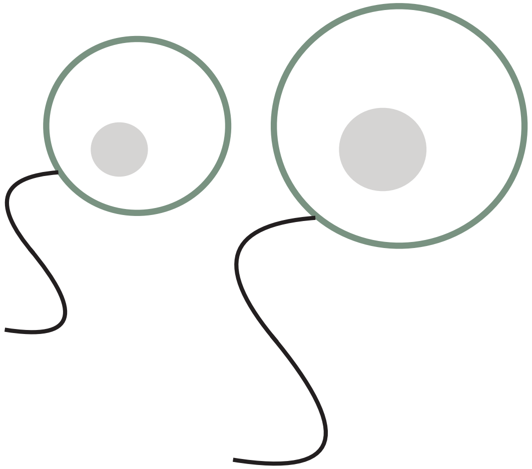
— fixed size



- Size of organelles/subcellular structures

— nucleus, endoplasmic reticulum, centrosomes, spindles, cilia etc.

— adaptation of size



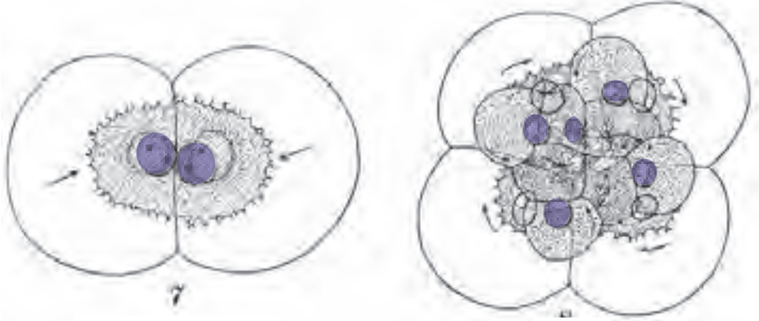
Scaling to cytoplasmic volume not cell dimension

CELL SIZE AND NUCLEAR SIZE

EDWIN G. CONKLIN

From the Department of Biology, Princeton University

- Observation of *Crepidula* cleavage divisions

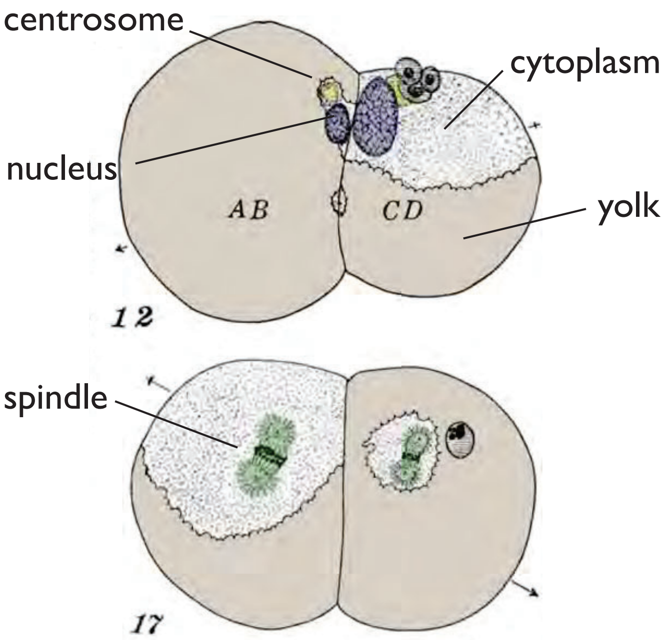


nucleus: 24µm

14µm

- Due to centrifugation along the indicated axis (arrows) during cleavage, cell size and the amount of cytoplasm (dotted) and yolk (brown) are not proportional in the two blastomeres.
- The size of organelles (nucleus, spindle and centrosome) scales with cytoplasm volume but NOT cell size *stricto sensu.*

- Centrifugation experiments:

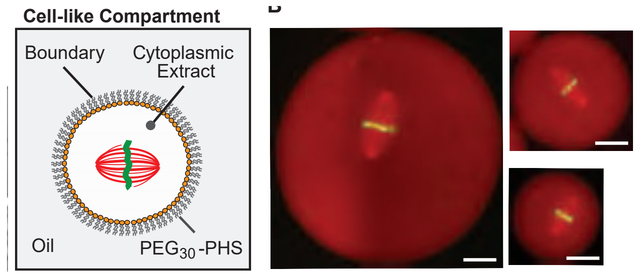


Conklin, E. (1912). *J. Exp. Embryol.* 12, 1–98.

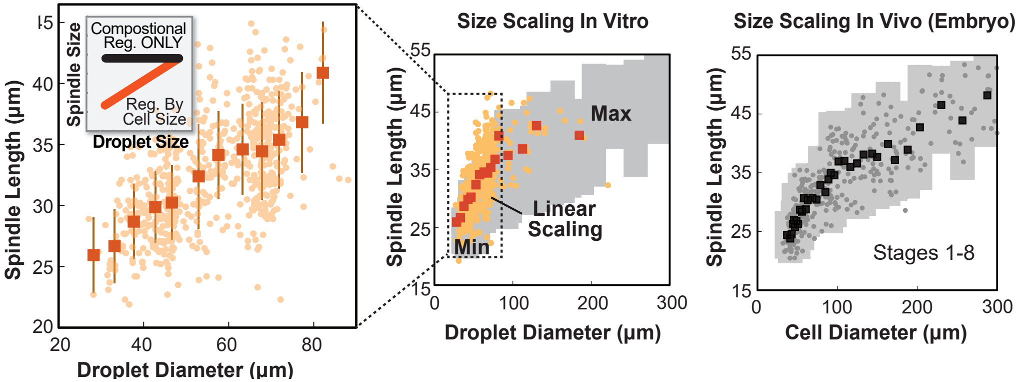
N. Goehring and A. Hyman *Current Biology* 22, R330–R339 (2012)

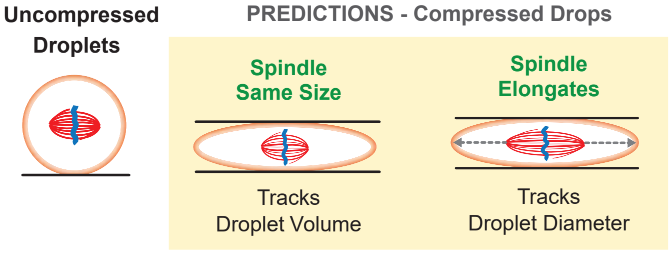
Scaling to cytoplasmic volume not cell dimension

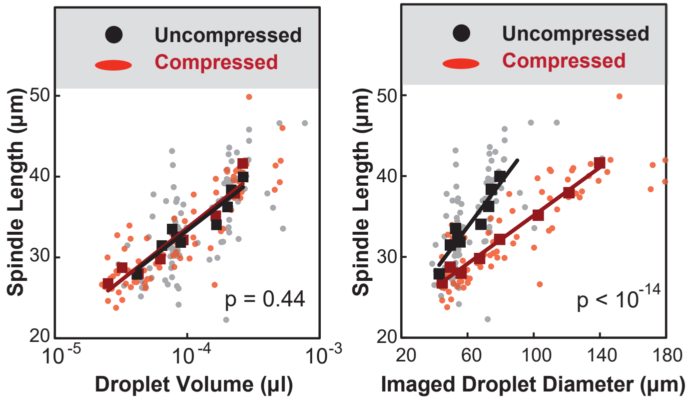
- Encapsulation of *Xenopus laevis* extracts into droplets reveals 2 phases: linear size scaling of spindles and « saturation » where the maximum size is set by extract composition (eg. developmental stage, or species)



- Disentangling the role of physical constraints (spatial extent of droplet) from the role of cytoplasmic volume.
- Compression alters the droplet geometry but not its volume.
- Spindle length scales with droplet volume, not geometry





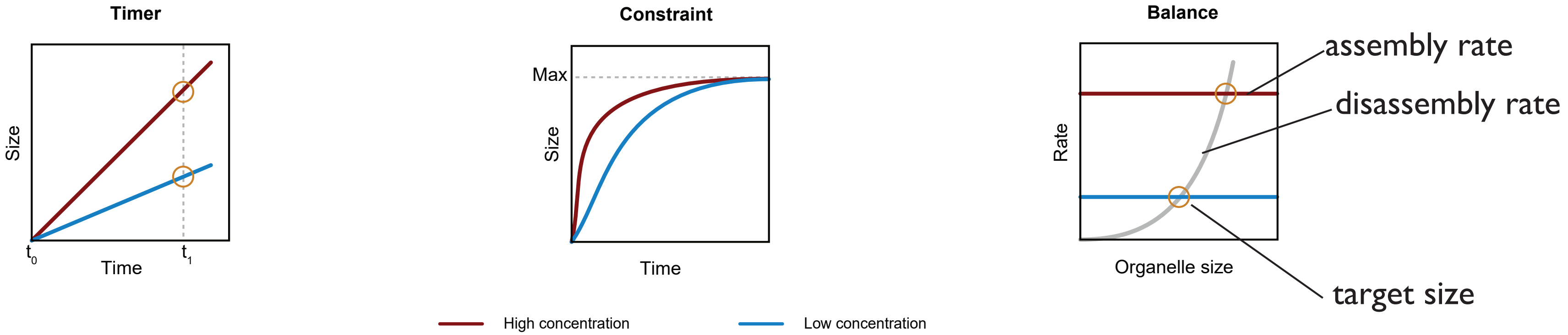


Matthew C. Good, et al and Rebecca Heald. *Science* 342, 856-860. (2013)
DOI: 10.1126/science.1243147

see also: J. Hazel et al. *Science* 342, 853-856. (2013)
DOI: 10.1126/science.1243110

Size control mechanism of organelles

- The rate of biochemical reaction is typically a function of substrate concentration
- The assembly kinetics of organelles is proportional to the cytoplasmic concentration of constituents.



- Size could be set by the **duration of assembly**.
- Different concentrations would yield different size of internal structures

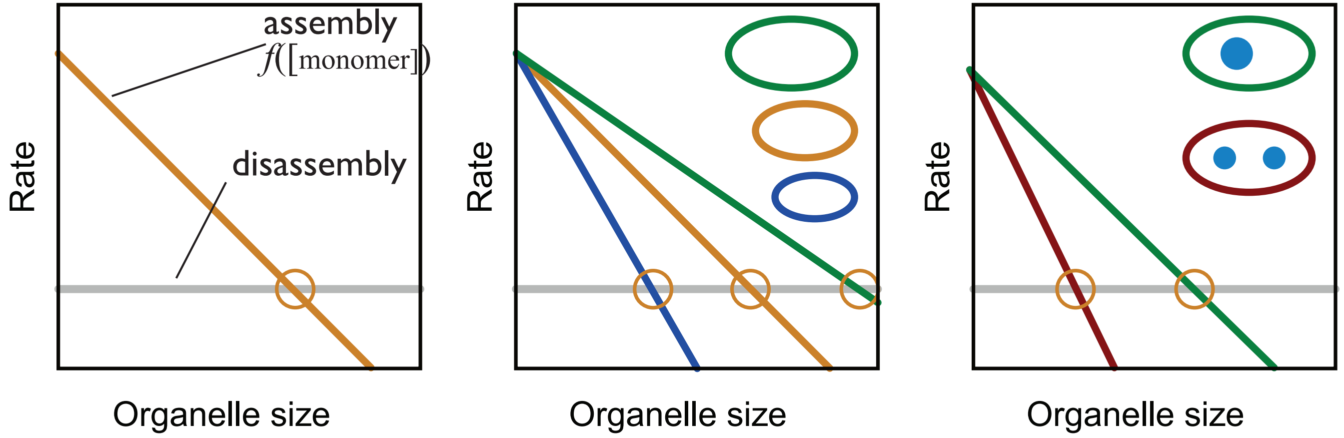
- Size could be limited by a **physical constraint** such that despite different assembly kinetics the maximum size is set

- Size could be limited by the **balance between assembly and disassembly.**
- Negative feedback between size and assembly rate:

N. Goehring and A. Hyman *Current Biology* 22, R330–R339 (2012)

Limiting pool mechanism

Scaling without need to measure the size of the cell



- If the monomers form a finite, limiting pool, then assembly of the organelle consumes monomers and monomer concentration decreases
- As a result, assembly rate decreases as the organelle size increases up to a steady state

$$N_{tot} = N_{monomer} + N_{organelle}$$

- If the monomer concentration is size-independent (ie. synthesis of monomer is proportional to cell size), then a smaller cell decreases the concentration of monomer faster than a larger cell because the amount of monomer is lower.

- As a result, assembly rate decreases faster as organelle size increases and the target size is smaller in smaller cell, hence scaling

- Competition for monomer in a common pool, and rapid diffusion has two consequences:
- increasing the number N of organelles decreases their size as $1/N$
- The N different organelles have the same size unless additional mechanisms are considered (in fact not really, see later)

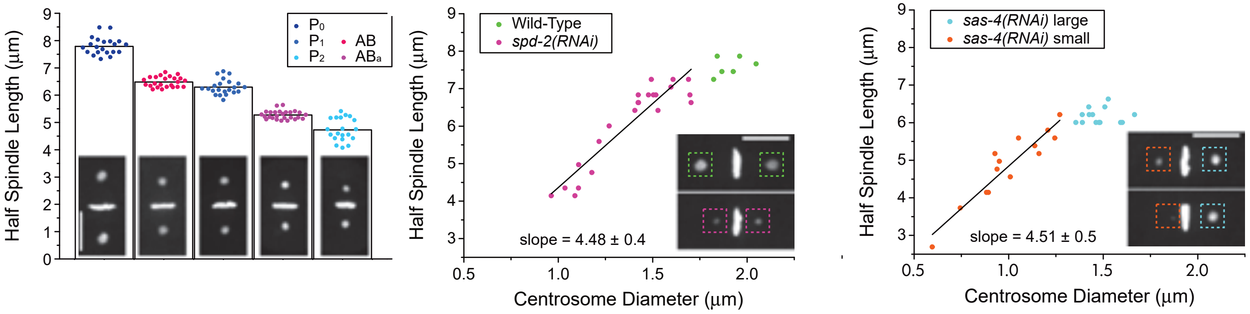
N. Goehring and A. Hyman *Current Biology* 22, R330–R339 (2012)

Limiting pool mechanism

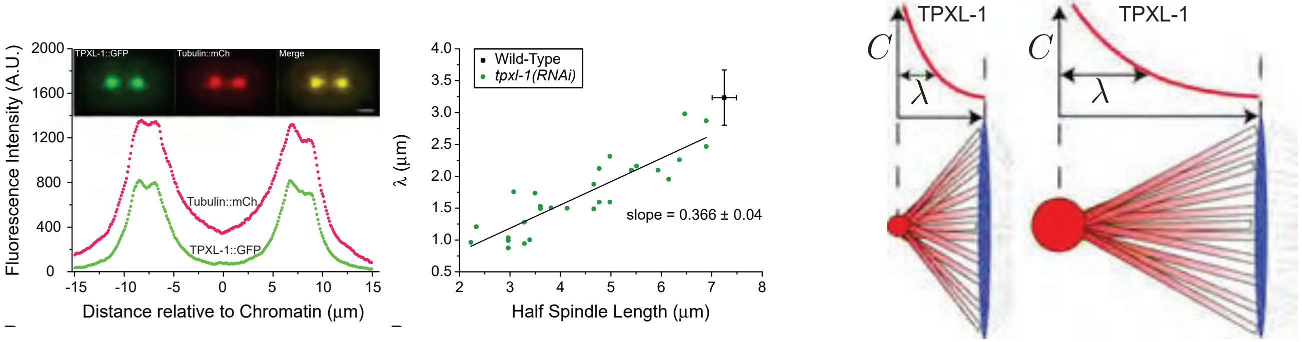
Centrosome scaling - Spindle scaling

- The centrosome nucleate microtubules
- The size of centrosome determines the size of the mitotic spindle

- The size of centrosomes and mitotic spindles are correlated between cells and within cells (asymmetric spindles)



- TPXL-1 targets Aurora kinase to MTs and controls spindle length.
- TPXL1 concentration at centrosomes correlates with spindle size
- Centrosomes control spindle size by regulating the length scale of a gradient of TPXL-1 on microtubules



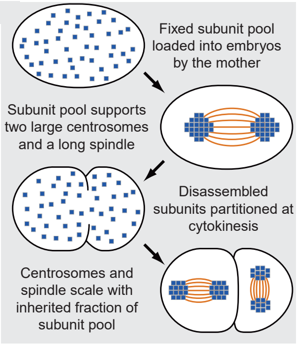
Greenan, G., Brangwynne, C.P., Jaensch, S., Gharakhani, J., Jülicher, F., and Hyman, A.A. (2010). *Curr. Biol.* 20, 353–358.

TPX2, Aurora Kinase and spindle length: Bird, A.W., and Hyman, A.A. (2008). *J. Cell Biol.* 182, 289–300.

Limiting pool mechanism

Centrosome scaling

- The centrosome nucleate microtubules
- Centrosome size is governed by a limiting pool mechanism of centrosomal components that scale with cell size



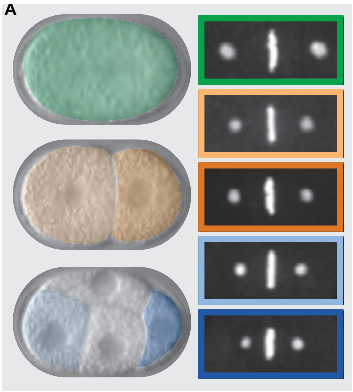
Fixed subunit pool loaded into embryos by the mother

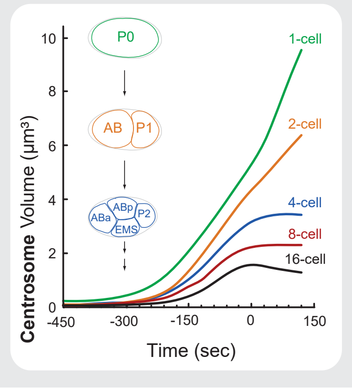
Subunit pool supports two large centrosomes and a long spindle

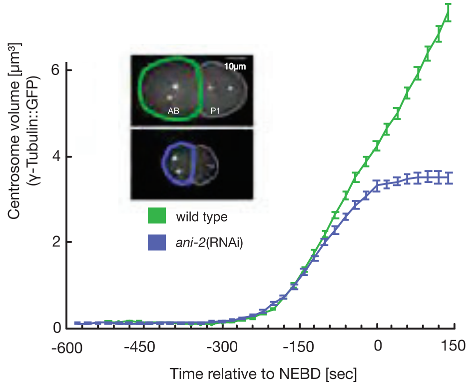
Disassembled subunits partitioned at cytokinesis

Centrosomes and spindle scale with inherited fraction of subunit pool

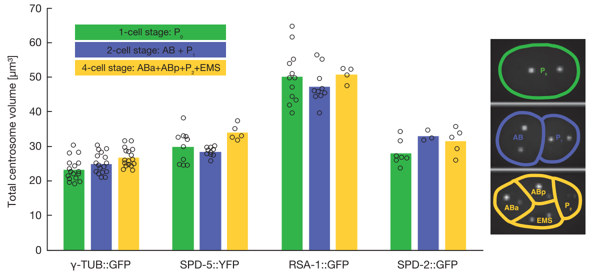
- Centrosome volume scales with cell size, and is independent of cell fate.

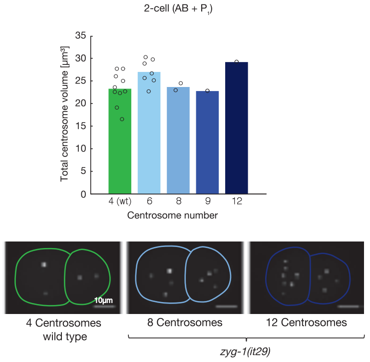






- The total volume of centrosome remains constant over time as cells divide
- Increasing the number of centrosomes reduces the size of individual centrosomes but the total volume of centrosomes remains invariant
- The size of centrosomes is sensitive to total SPD2 in a cell.
- This is consistent with a limiting pool of maternally inherited SPD-2 governing centrosome size and scaling

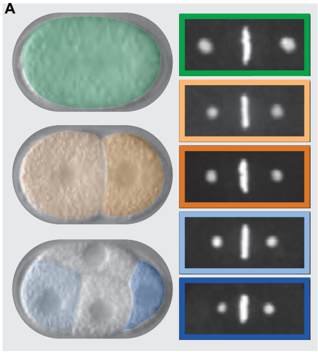


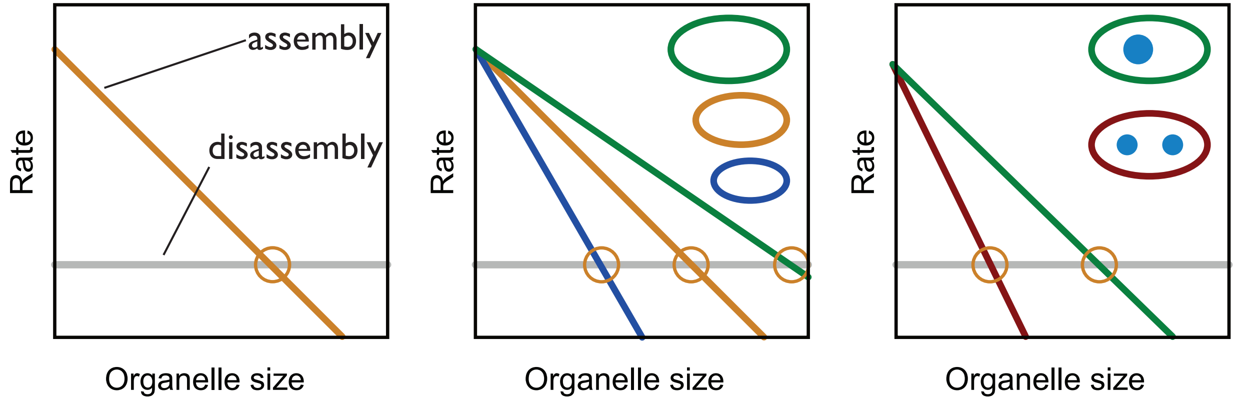


Decker, M., et al., and Hyman, A.A. (2011). *Curr. Biol.* 21, 1259–1267.

Limiting pool mechanism

- Scaling of centrosome size by limiting pool of centrosomal components
- Scaling of mitotic spindles with centrosomes



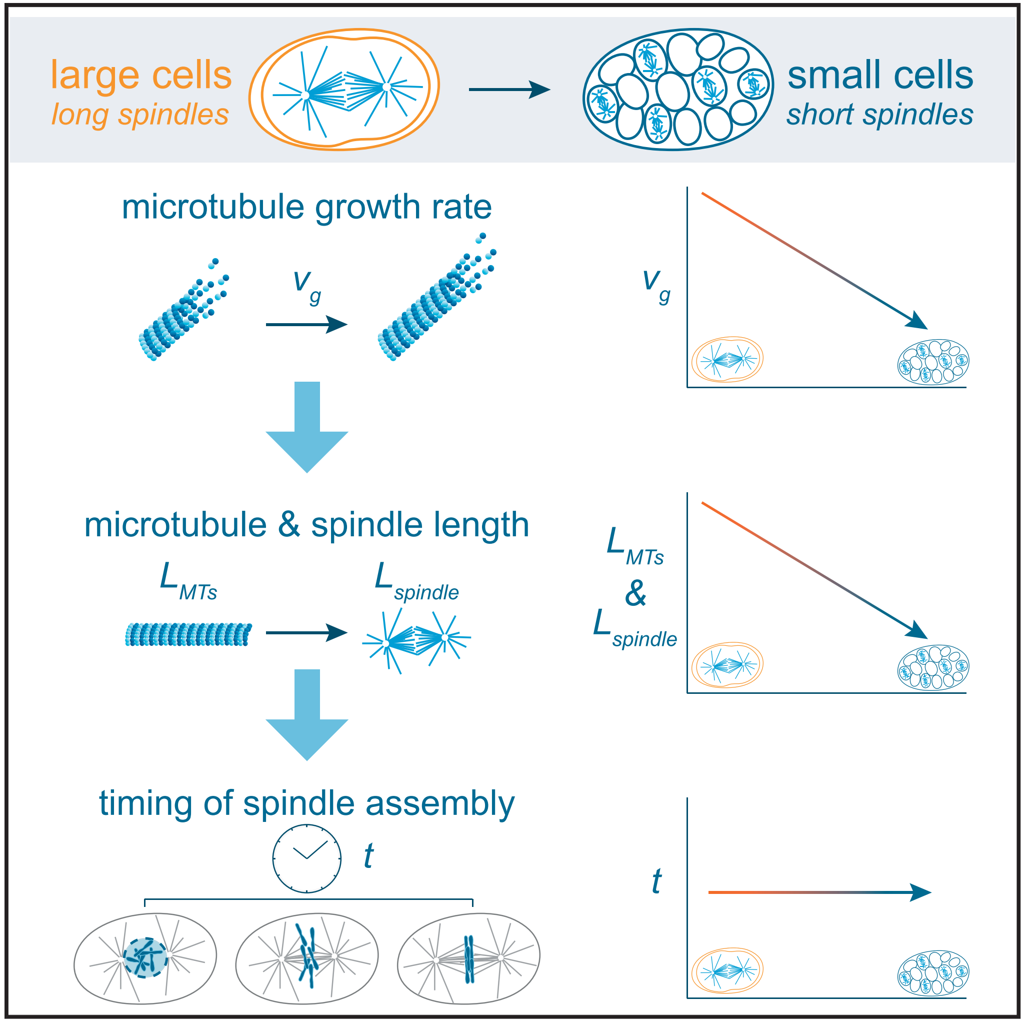


N. Goehring and A. Hyman *Current Biology* 22, R330–R339 (2012)

Limiting pool mechanism

Microtubule Dynamics Scale with Cell Size to Set Spindle Length

Scaling of MT dynamics with cell volume
can be explained by existence of a limiting pool of a regulator of MT dynamics

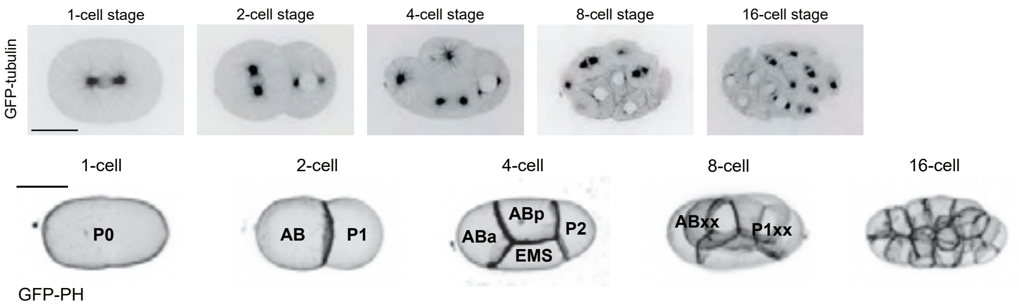


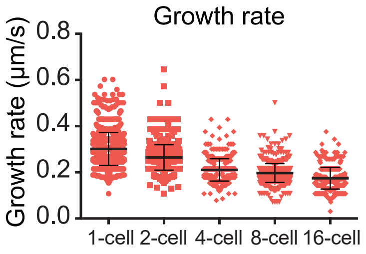
Lacroix et al., F. Nedelec and J. Dumont. 2018, *Developmental Cell* 45, 496–511

Limiting pool mechanism

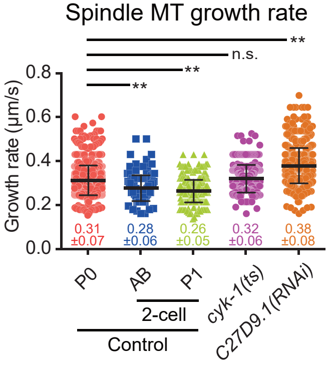
Microtubule Dynamics Scale with Cell Size to Set Spindle Length

Caenorhabditis elegans
Nematode

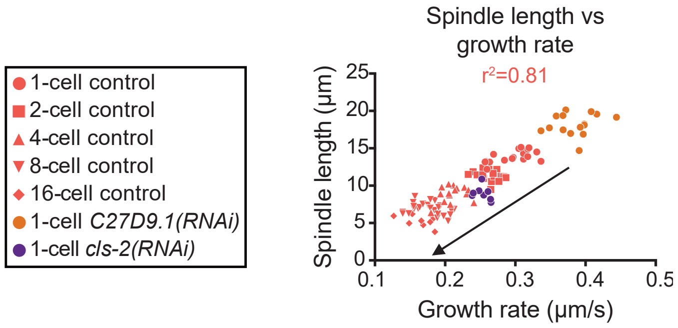


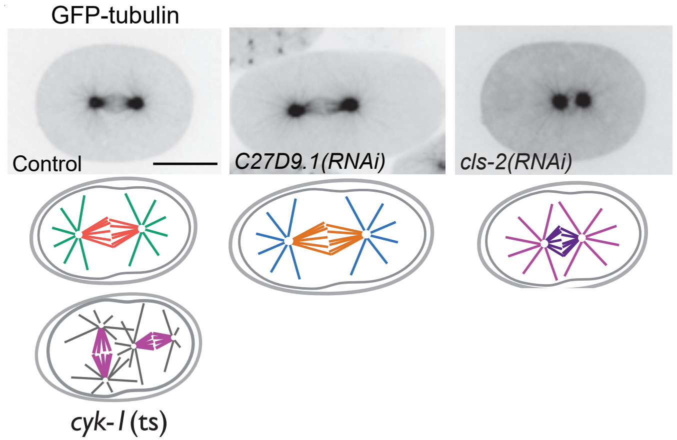


- Microtubule average length depends on 2 kinetic parameters, growth and shrinkage rates, and 2 switch probabilities (catastrophe and rescue)
- Growth rate is reduced over time while other parameters are unchanged
- The reduction in growth rate is due to a reduction in cell volume but not to change in cell composition (fate)



- Spindle length scales with and is tuned by spindle microtubule growth rate.

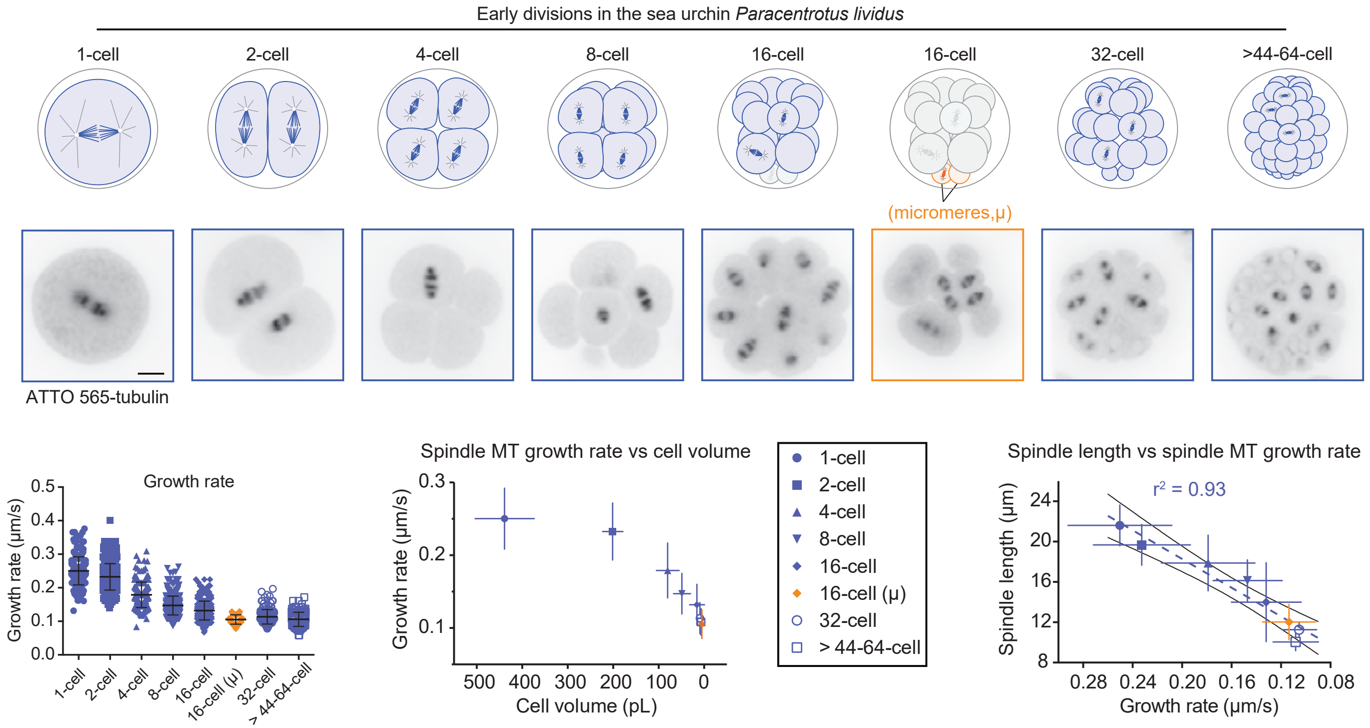




Lacroix et al., F. Nedelec and J. Dumont. 2018, *Developmental Cell* 45, 496–511

Limiting pool mechanism

Microtubule Dynamics Scale with Cell Size to Set Spindle Length



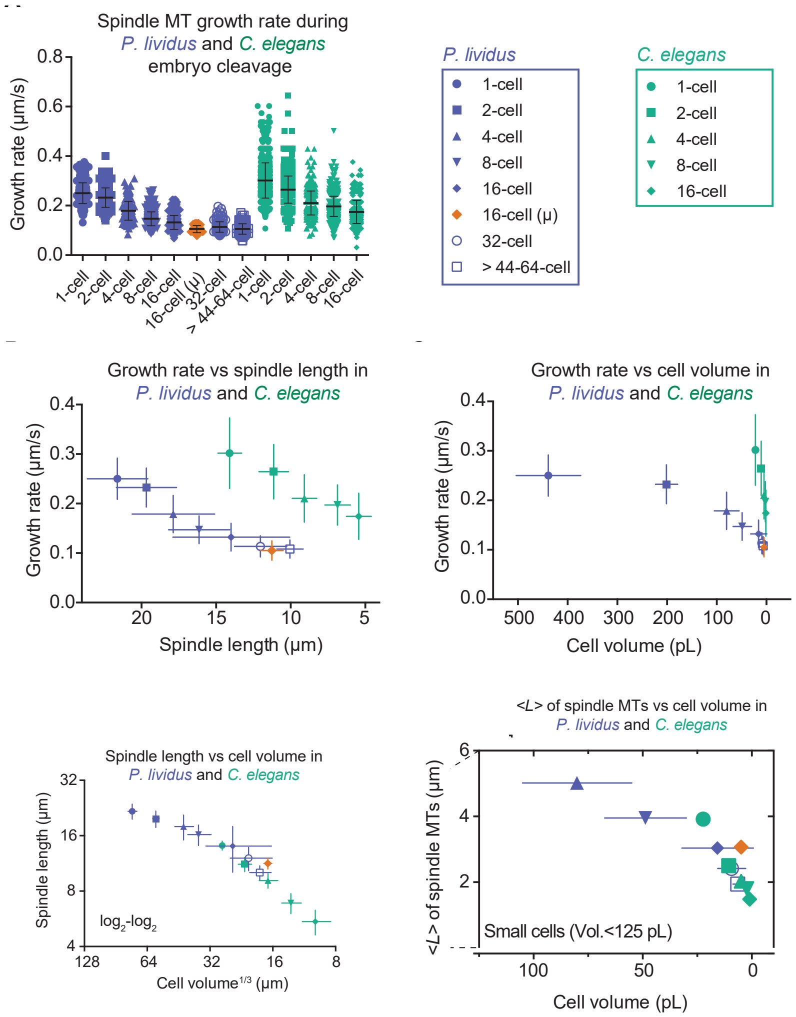
Paracentrotus lividus
Echinoderm

Lacroix et al., F. Nedelec and J. Dumont. 2018, *Developmental Cell* 45, 496–511

Limiting pool mechanism

Microtubule Dynamics Scale with Cell Size to Set Spindle Length

- *C.elegans* embryos are about 20 times smaller than *P. lividus* embryos.
- Though spindle microtubule growth rate determines spindle size and scales with cell volume in each species
- MT growth rate is not an absolute predictor of spindle size: growth rate is different in cells of different volume across species.



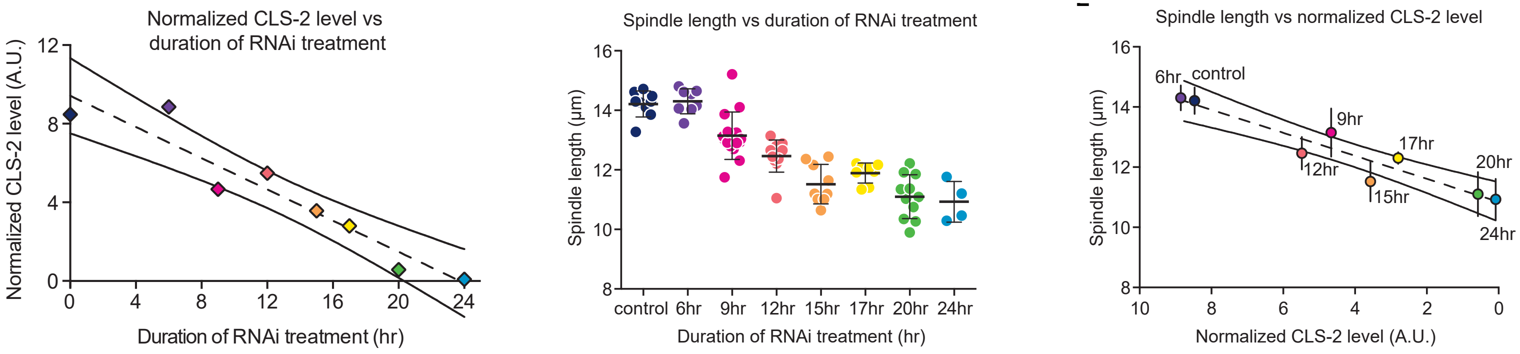
- However, cells of similar size across species have similar average microtubule length and spindle length
- So the dynamic parameters of MT polymerisation scale with cell volume

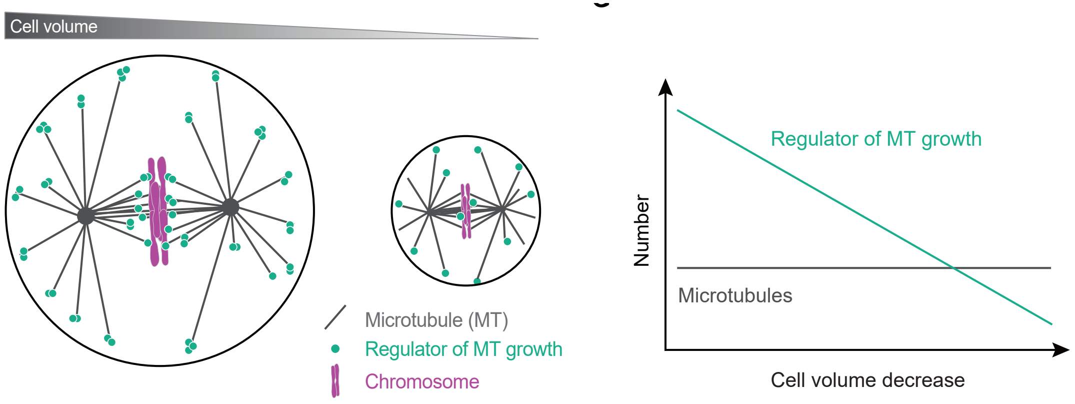
Lacroix et al., F. Nedelec and J. Dumont. 2018, *Developmental Cell* 45, 496–511

Limiting pool mechanism

Microtubule Dynamics Scale with Cell Size to Set Spindle Length

- The dynamic parameters of MT polymerisation scale with cell volume
- This can be explained by Limiting pool model
- Tubulin is unlikely to be limiting and evidence indicate that it is not in many instances
- However, MT associated proteins, MAPs, could be limiting
- MAPs affect MT dynamics, in particular growth rate: CLS-2 (CLASP)
- CLS-2 titration reduces spindle length





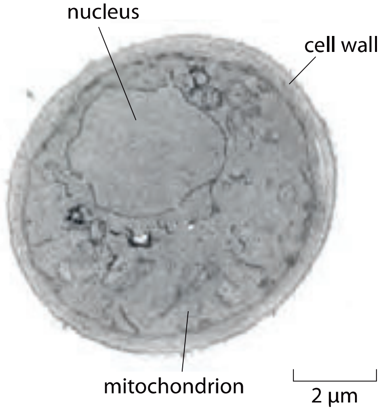
Lacroix et al., F. Nedelec and J. Dumont. 2018, *Developmental Cell* 45, 496–511

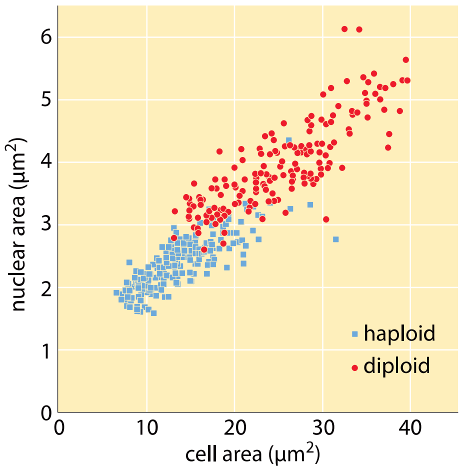
Limiting pool mechanism

—Nuclear scaling

Nuclei scale to cell size: *Kern-plasma relation* from Hertwig

- Yeast

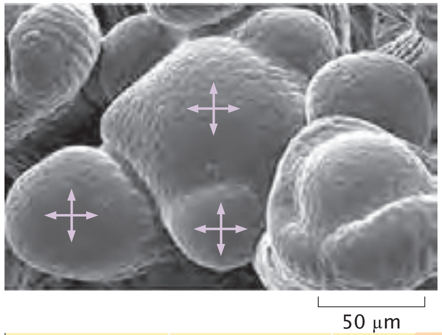


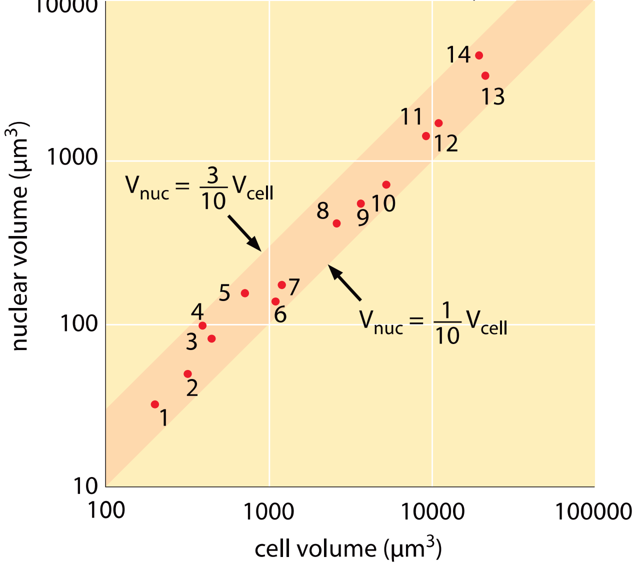


P. Jorgensen et al., *Molecular Biology of the Cell,* 18:3523, 2007

Cell Biology by the numbers. Ron Milo, Rob Phillips, *illustrated* by Nigel Orme. *Garland Science* 2012

- Shoot apical meristem





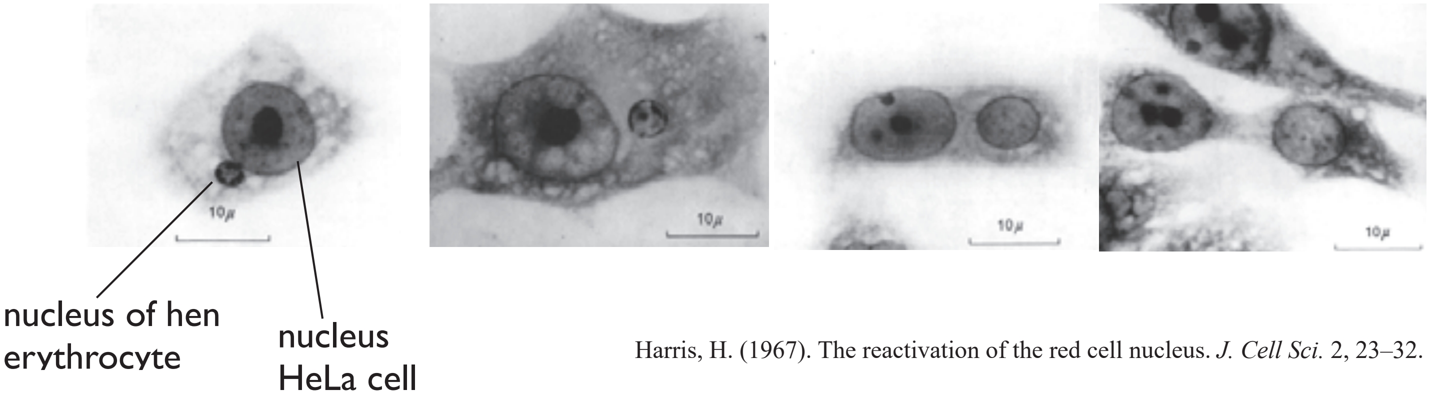
1 *Arabidopsis thaliana*, 2 *Lobularia maritime* (Sweet Alison), 3 *Hypericum virginicum* (Marsh St. John's wort), 4 *Cicer arietinum* (chickpea), 5 *Nelumbo lutea*, 6 *Spinacia oleracea* (spinach), 7 *Cyanotis pilosa*, 8 *Anemone pulsatilla* (Meadow Anemone**)**, 9 *Tradescania navicularis* (day flower), 10 *Convallaria majalis* (Lily of the valley), 11 *Fritillaria laneeolata* (chocolate lily), 12 *Fritillaria camtschatcensis*, 13 *Lilium longiflorum* (Easter lily)(4 x), 14 *Sprekelia formosissima* (Aztec lily). (Adapted from H. J. Price *et al.*, Experientia, 29:1028, 1973.)

Limiting pool mechanism

—Nuclear scaling

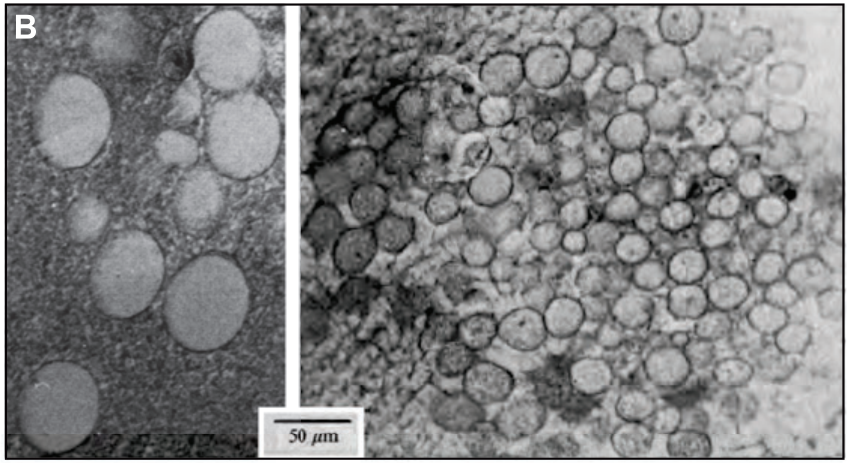
Nuclei scale to cell size: *nuclei grow to scale with cell size*

- Injection of small nuclei into larger cells (Hela cells) cause nuclear growth



Harris, H. (1967). The reactivation of the red cell nucleus. *J. Cell Sci.* 2, 23–32.

- Injection of Hela cells nuclei into large *Xenopus* oocytes causes nucleus enlargement. Enlargement is reduced as the number of injected nuclei increases.
- This is consistent with nuclei competing for a limited component released by the germinal vesicle. Competition between nuclei reduces nuclear growth



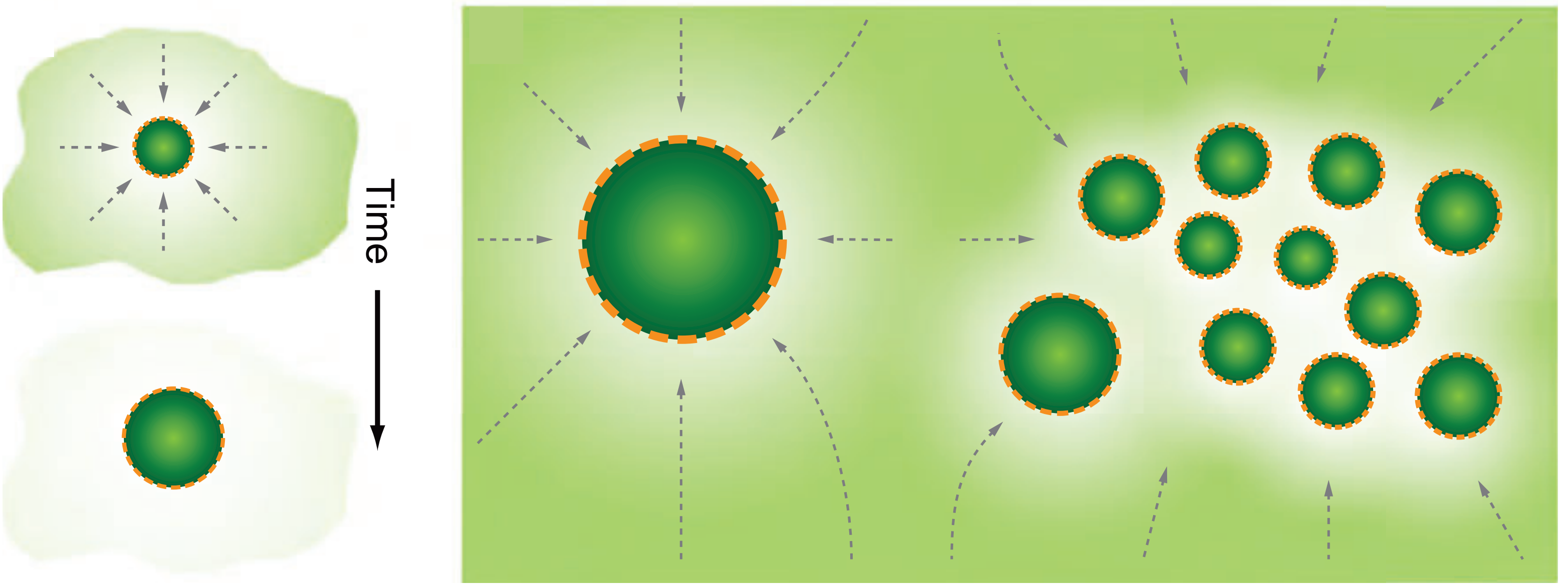
Gurdon, J.B. (1976). Injected nuclei in frog oocytes: fate, enlargement, and chromatin dispersal. *J. Embryol. Exp. Morphol.* 36, 523–540.

Limiting pool mechanism

—Nuclear scaling

Nuclei scale to cell size: *nuclei grow to scale with cell size*

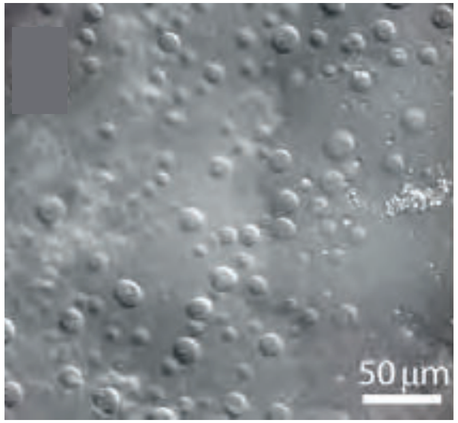
A model of limiting pool of component of nuclear envelope growth is consistent with experimental observations



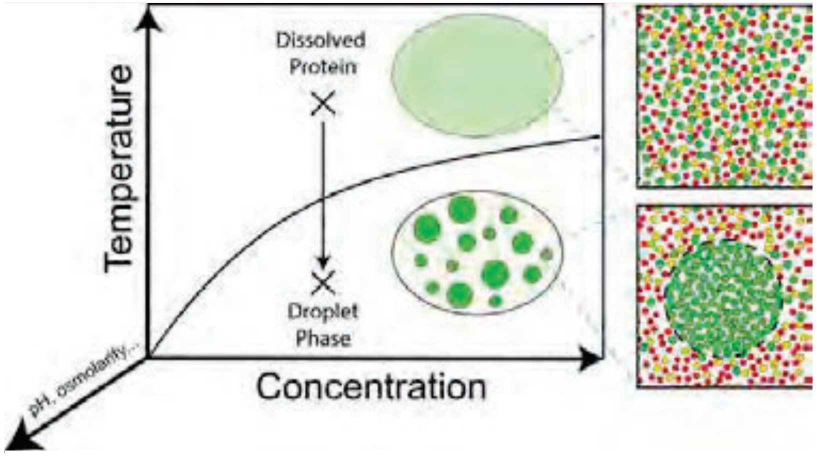
N. Goehring and A. Hyman *Current Biology* 22, R330–R339 (2012)

A variant: Phase transition based scaling

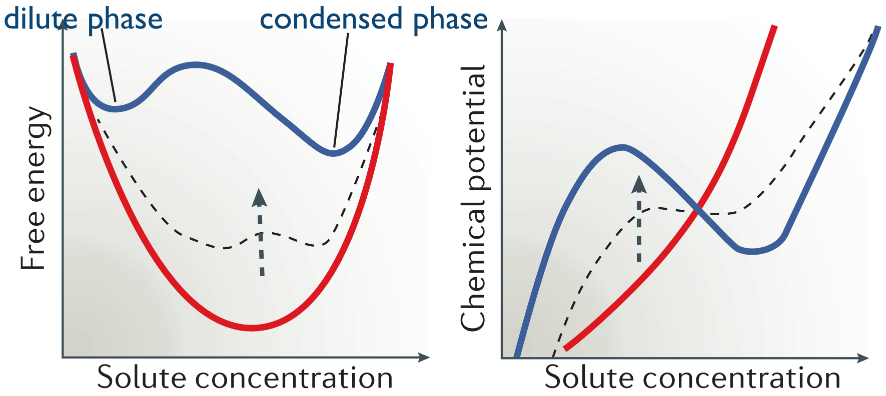
- Proteins and RNAs in cells form liquid mixtures and so-called membrane-less organelles: centrosomes, centrioles, P granules etc
- Proteins and RNA mixtures phase separate above a critical concentration.



Nucleoli (and other RNP droplets) in nucleus of *X. laevis* oocyte



Brangwynne. *J. Cell Biol*. 203:875–881 (2013)



🟥 A solution of proteins/RNAs forms a homogeneous mixture when solutes don't interact. Solutes distribute evenly on average to maximise the entropy of the system.

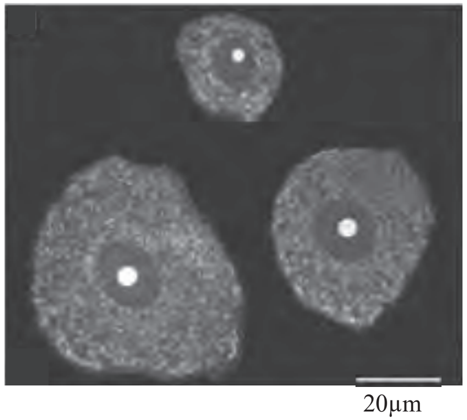
🟦 When proteins/RNAs interact, the free energy is multimodal and the system can exist in 2 configurations where the concentration is different but the chemical potential is the same. At equilibrium there is no net flux between the two phases despite rapid exchanges. Phase separation occurs at the concentration of proteins/RNAs where the solute-solute interactions overcome the entropic tendency of the system to remain homogenous.

Banani, Lee, Hyman and Rosen. *Nat Rev Mol Cell Biol* 18(5):285-298. (2017)

Hyman, Weber, Jülicher. *Annu. Rev. Cell Dev. Biol.* 30:39–58 (2014)

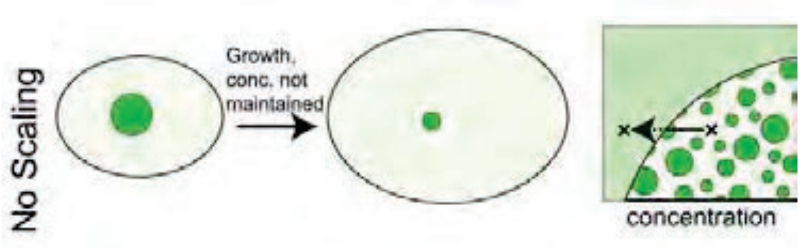
A variant: Phase transition based scaling

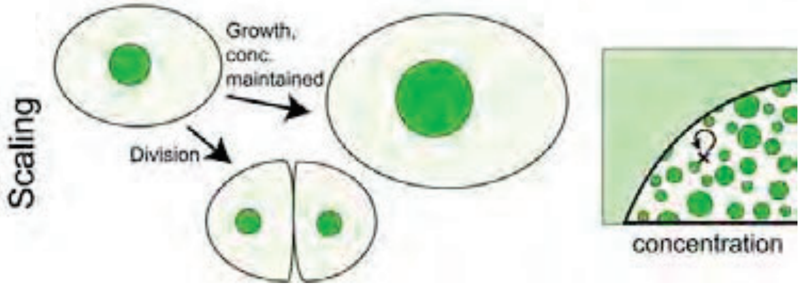
- Scaling of liquid-liquid de-mixing phases
- Proteins and RNA mixtures phase separate above a critical concentration.



Scaling of nucleoli, nuclei and cells in dorsal root ganglia neurons

Berciano et al.
J. Struct. Biol. 158:410–420. (2007)





- As a cell grows, if the protein/RNA synthesis does not scale with cell size, the concentration of solutes decreases, and liquid condensates dissolve when the concentration goes below the critical concentration
- This results in the absence of scaling with cell size

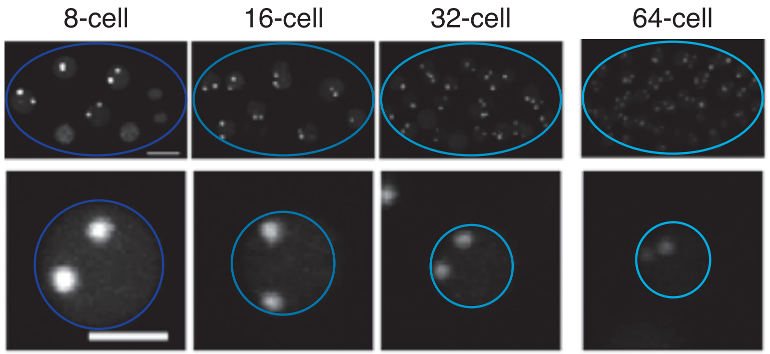
- If concentration is kept constant (scaling of synthesis to volume as commonly observed, see Lecture 2, « What sets cell volume? »), then scaling occurs
- the size of the condensate is set by the size of the pool of solutes if it is limiting. When concentration will drop below the critical concentration, growth of the condensates ceases

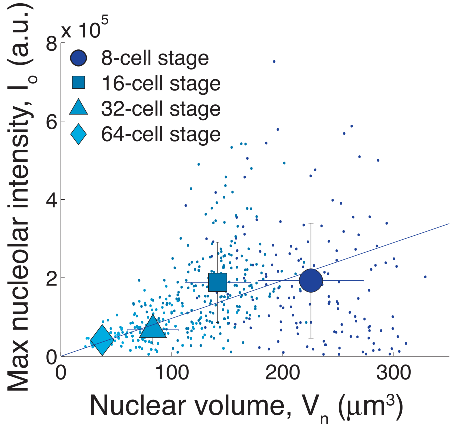
Brangwynne. *J. Cell Biol*. 203:875–881 (2013)

A variant: Phase transition based scaling

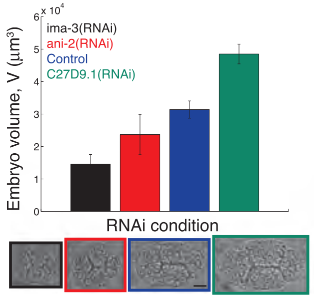
—Nucleolus scaling is highly relevant to cell growth

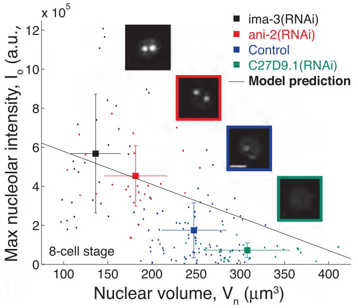
- Nucleoli are the site of ribosome biogenesis
- Ribosome synthesis and assembly is limiting for cell growth due to limits on the synthesis of rRNAs (See Lecture 2: « What sets cell volume? »)
- Nucleolar size scales directly with cell size during development (in *C. elegans*) and in dorsal root ganglia neurons

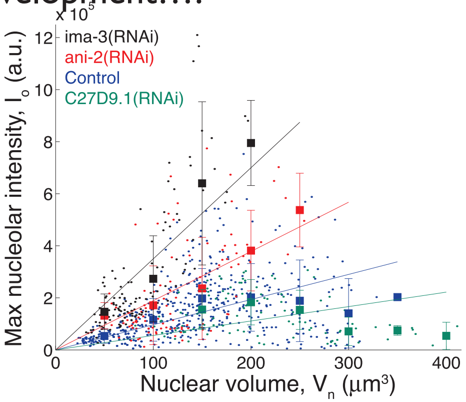




- However, comparison of 8-cell stage cells of different size (using RNAi conditions), shows that smaller cells have brighter nucleoli, indicating inverse scaling.
- In each condition however, nucleoli scale directly with cell size during development…!







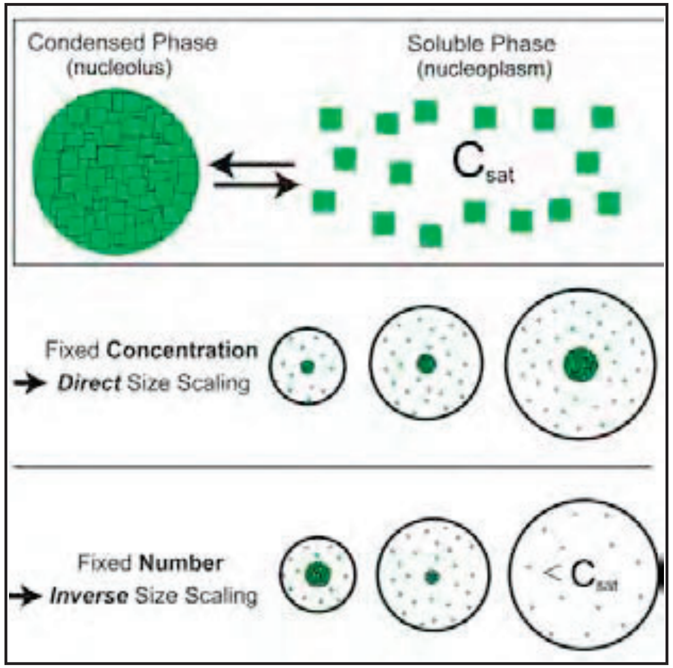
Weber & Brangwynne, 2015, *Current Biology* 25, 641–646

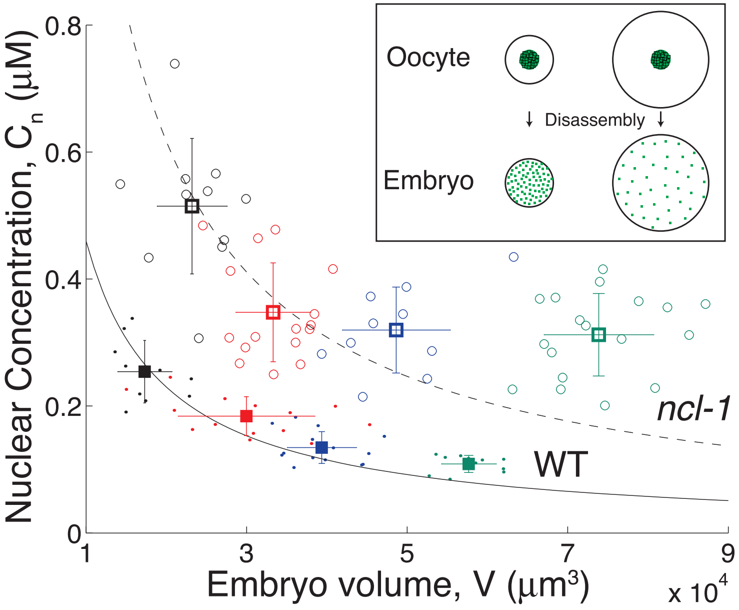
A variant: Phase transition based scaling

—Nucleolus scaling is highly relevant to cell growth

- Nucleoli assembly depends on the concentration of components
- This concentration is higher in smaller embryos (and smaller cells)
- Components are loaded maternally in oocytes independent of the size of oocytes
- As a result, **smaller embryos inherit a higher concentration of nucleolar components**

- A phase separation based model explains quantitatively both:
  - —Direct size scaling during development
  - —Inverse scaling across embryo size





Weber & Brangwynne, 2015, *Current Biology* 25, 641–646

Thomas LECUIT 2020-2021

Scaling by surface to volume ratio sensor

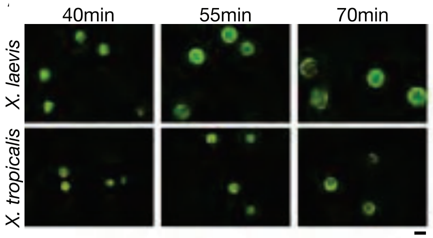
Nuclear size Is regulated by Importin α and Ntf2 in *Xenopus*

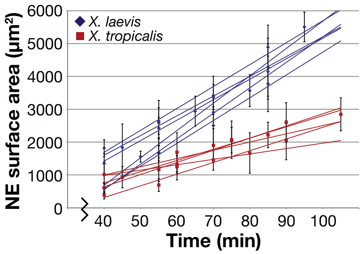

Science 328, 633-636

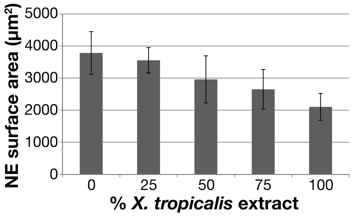
- *Xenopus laevis* and *tropicalis* have different size.
- *X. laevis* is tetraploid while *X. tropicalis* is diploid

- Difference in nuclear size in 2 species Different kinetics of growth of the nuclear envelope (NE) are observed in extracts.

- Mixed extracts indicate that titratable cytoplasmic factors are responsible for determining nuclear size

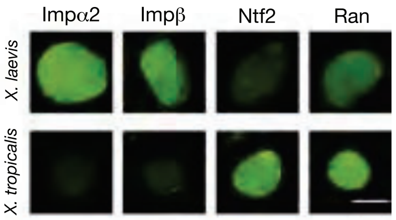


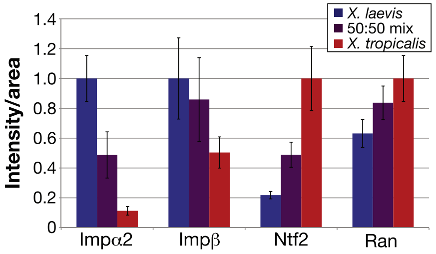




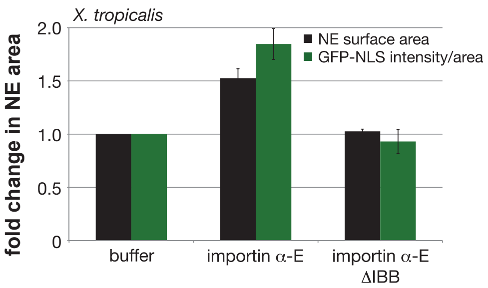
- <u>Compositional differences in extracts:</u>

Importinα2 and Ntf2 levels are different in 2 species (Importinα2 promotes nuclear import of NLS-containing proteins such as Lamins which are required for nuclear growth)





- <u>Functional tests:</u>
- Adding Importin α2 to *X. tropicalis* extracts that formed nuclei increases nuclear size



COLLÈGE DE FRANCE 1530

Thomas LECUIT 2020-2021

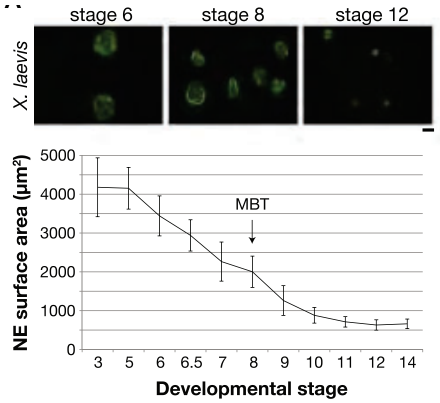
D. Levy and R. Heald. *Cell* 143, 288–298 (2010)

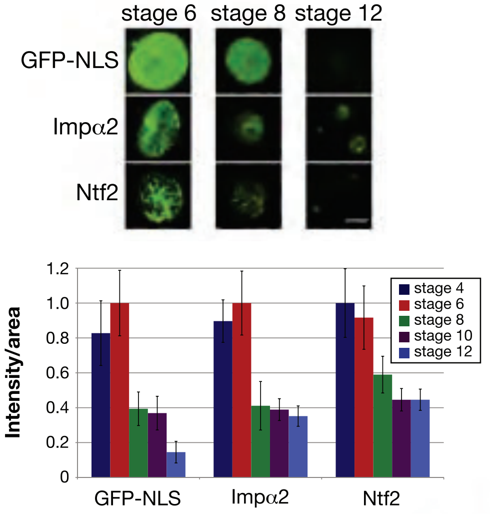
Scaling by surface to volume ratio sensor

Nuclear scaling is regulated by Importinα and Ntf2 in *Xenopus*

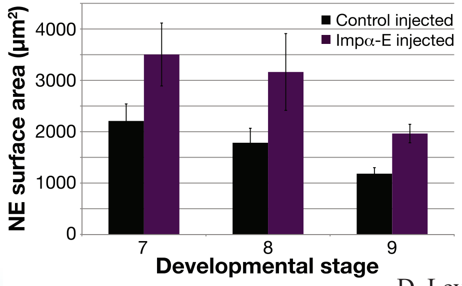
Xenopus embryonic cleavage

- Nuclear scaling:
- The nuclei reduce their size during progressive embryonic cleavage, together with cell size
- This correlates with a reduction in the concentration of Importin α2 and Nft2 in nuclei





- Injection of Importing α2 increases nuclear size during developmental cleavage and scaling



D. Levy and R. Heald. *Cell* 143, 288–298 (2010)

Scaling by surface to volume ratio sensor

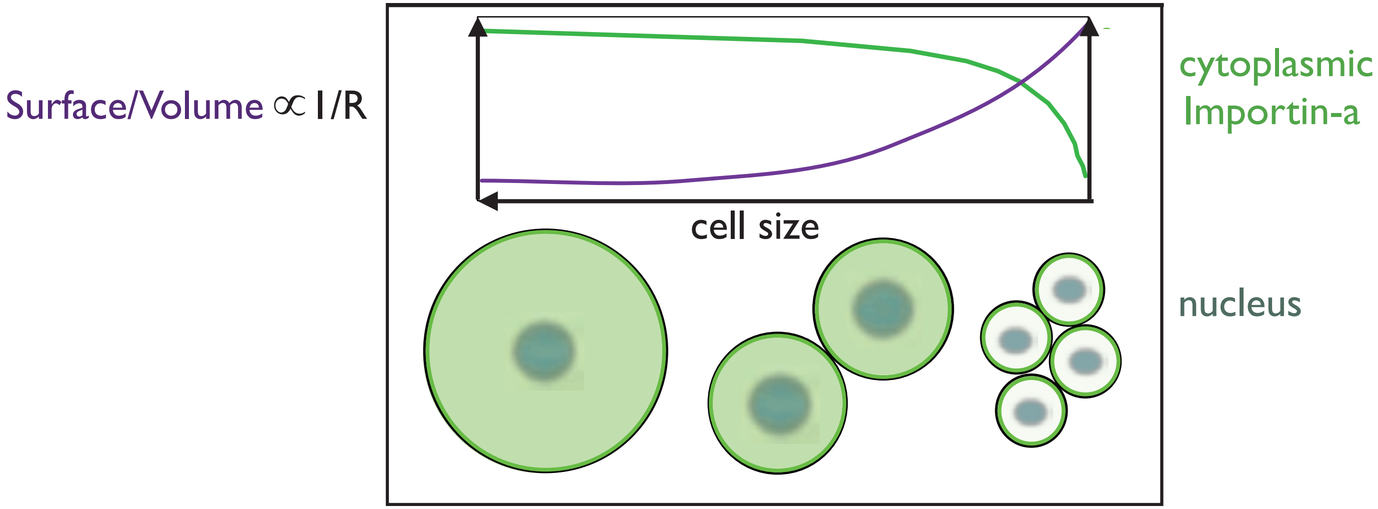
—Nuclear scaling

Importin α partitioning to the plasma membrane regulates intracellular scaling

Question: how to couple change in biochemical composition and change in size?

- Change in surface to volume ratio couples cell size to cytoplasmic Importin α
- Smaller cells titrate cytoplasmic Importin α to the plasma membrane, thereby reducing nuclear size
- Importin α is a surface area-to-volume sensor that scales intracellular structures to cell size.

(NB: this model cannot apply to cells with very different geometry than sphere)



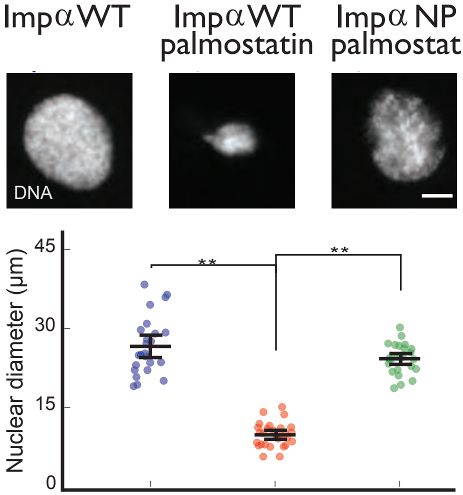
C. Brownlee and R. Heald. *Cell* 176, 805–815 (2019)

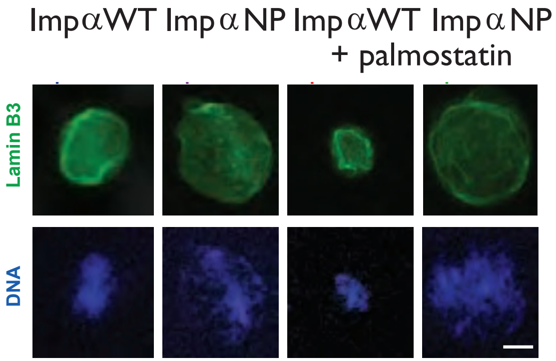
Scaling by surface to volume ratio sensor

—Nuclear scaling

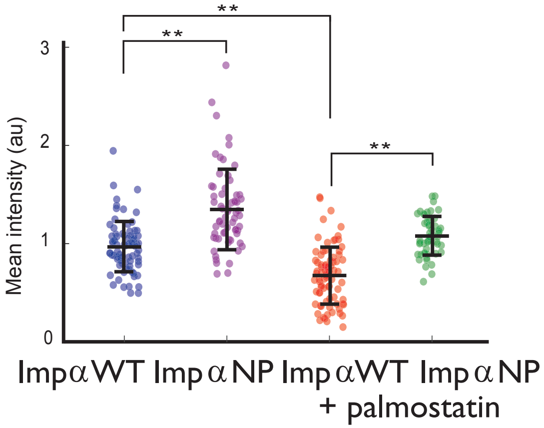
Importinα partitioning to the plasma membrane regulates intracellular scaling

- Importin a is palmitoylated and partitions to the plasma membrane
- Palmitoylation of Importin a reduces nuclear size
- And reduces nuclear recruitment of LaminB3 (an NLS containing protein)





Lamin B3 intensity

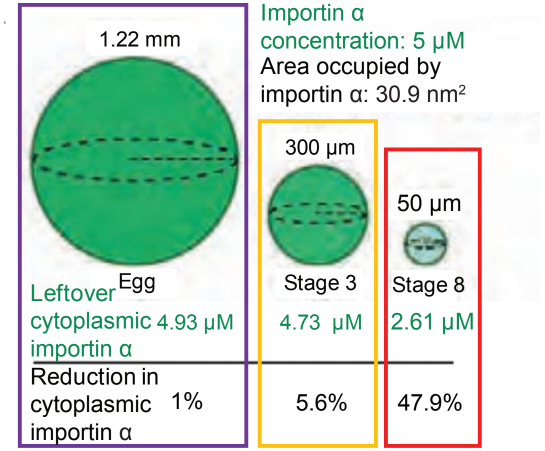


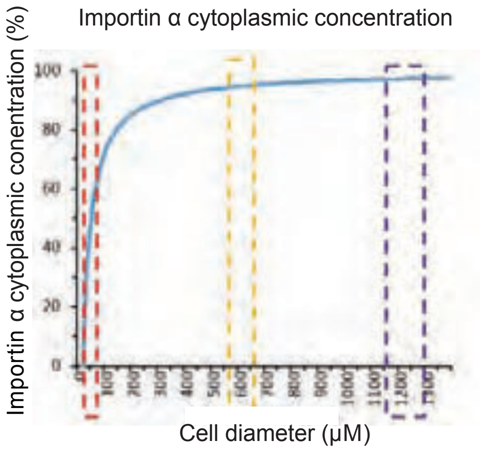
C. Brownlee and R. Heald. *Cell* 176, 805–815 (2019)

Scaling by surface to volume ratio sensor

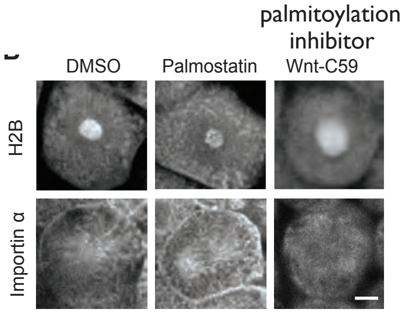
Importin a partitioning to the plasma membrane regulates intracellular scaling

- Model of membrane versus cytoplasmic partitioning of Importin a as a function of cell size.
- Non linear decrease of Importin a.

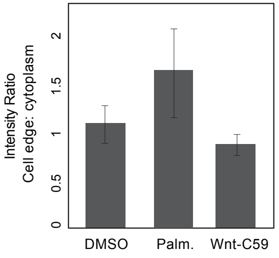


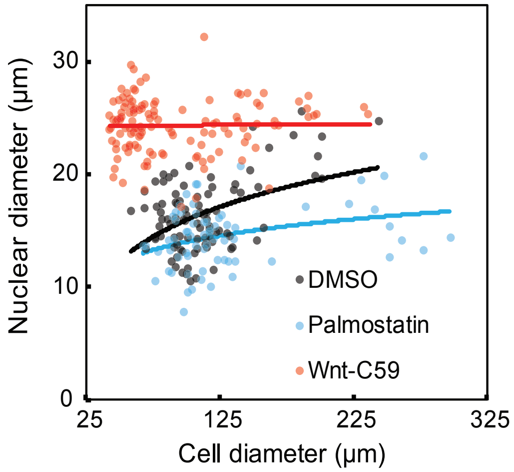


- Scaling of nuclear size to cell size is lost when palmitoylation is inhibited in embryos.



stage 7 embryo



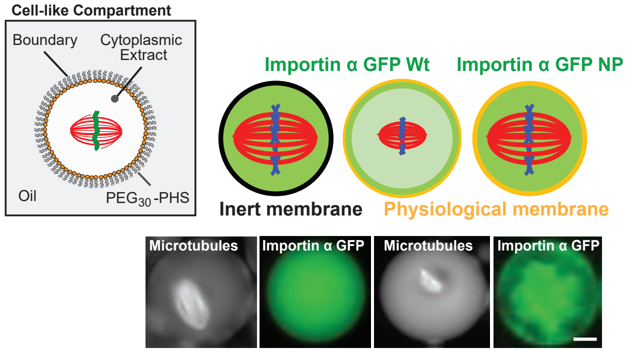


C. Brownlee and R. Heald. *Cell* 176, 805–815 (2019)

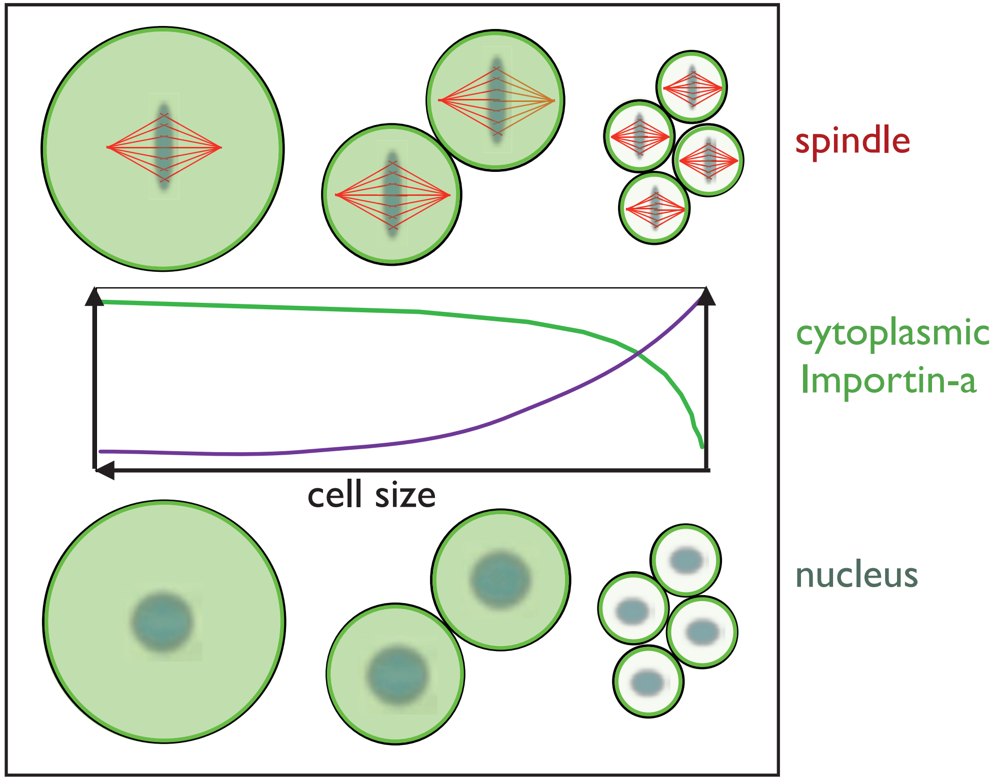
Scaling by surface to volume ratio sensor

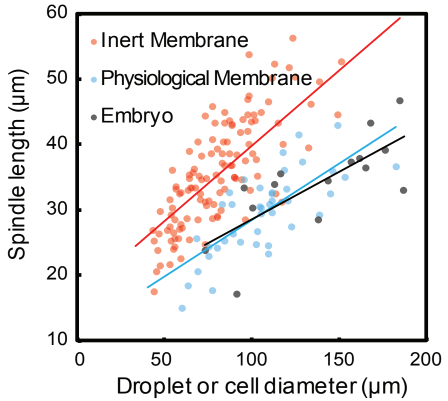
Importin a partitioning to the plasma membrane regulates spindle size

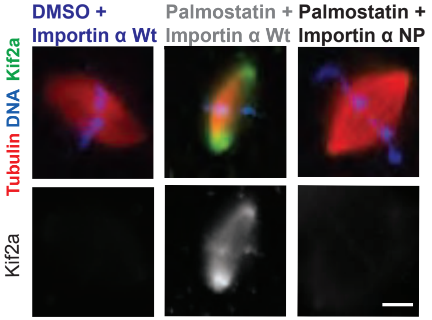
- Importin-a binds the kinesin Kif2 which reduces spindle size.
- Membrane recruitment of Importin-a increases Kif2 binding to spindles.
- Hence in smaller cells, where cytoplasmic Importin a is lowest, Kif2 reduces spindle size



Surface/Volume ∝ 1/R





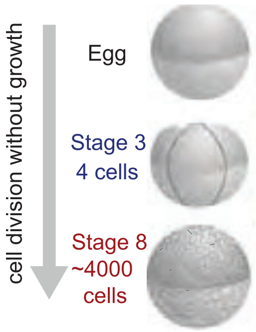


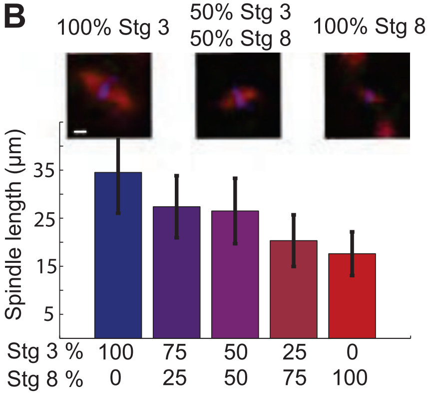
C. Brownlee and R. Heald. *Cell* 176, 805–815 (2019)

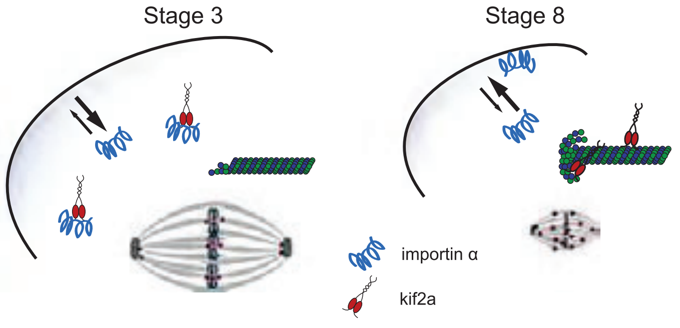
Scaling by surface to volume ratio sensor

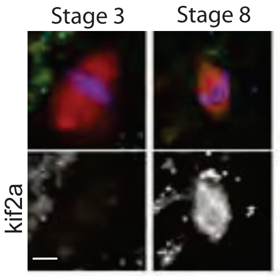
Importin a partitioning to the plasma membrane regulates spindle size

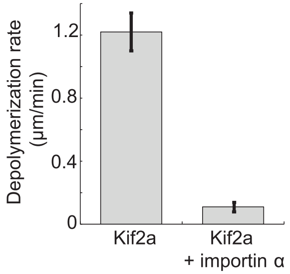
- The kinesin Kif2 is an NLS-containing protein which binds mitotic spindles in stage 8 but not in stage 3 embryos
- Importin-a binds and inhibits Kif2 microtubule depolymerising activity
- Importin a antagonises Kif2 dependent reduction of spindle size
- Hence in smaller cells, where cytoplasmic Importin a is lowest, Kif2 reduces spindle size

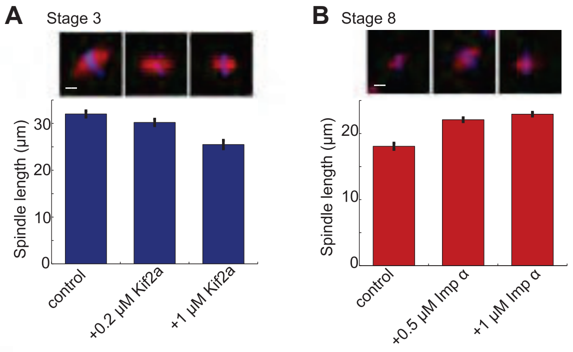












Wilbur and Heald. *eLife*;2:e00290. DOI: 10.7554/eLife.00290 (2013)

Scaling mechanisms – Summary

- Limiting pool mechanism

- Sensor of surface to volume ratio:

  tunes the effective cytoplasmic concentration of regulator of organelle size

These mechanisms rely on *non local* sensing: geometry of environment

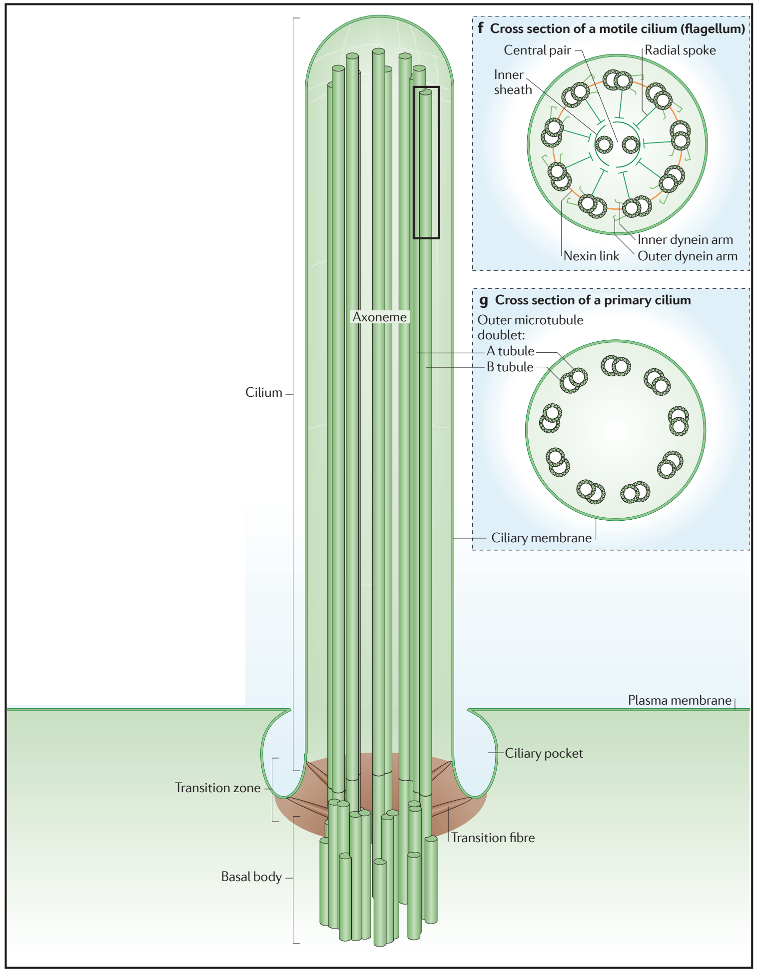
- Let's consider *local* size sensing mechanisms

Limits of « Limiting pool mechanism »

Controlling the size of multiple organelles - Cilia

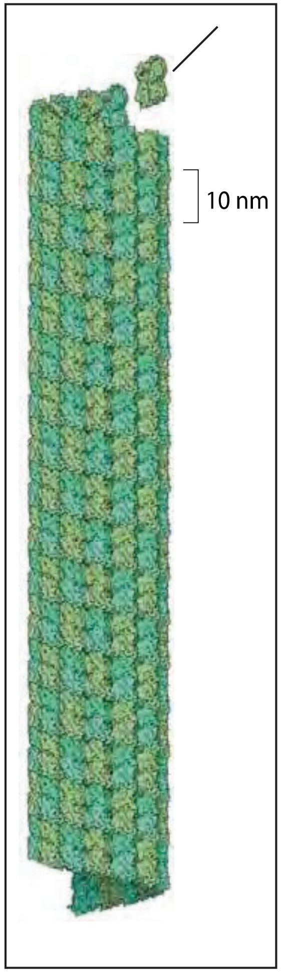
https://www.sciencesource.com/archive/Chlamydomonas--SEM--SS2526322.html

Chlamydomonas reinhardtii
Green algae



cilium

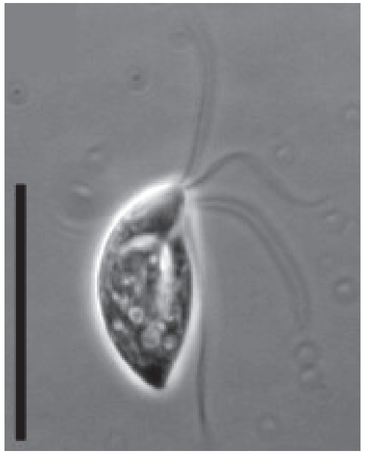
H Ishikawa and WF Marshall *Nature Reviews Mol Cell Biol* 12: 222-234 (2011)



microtubule

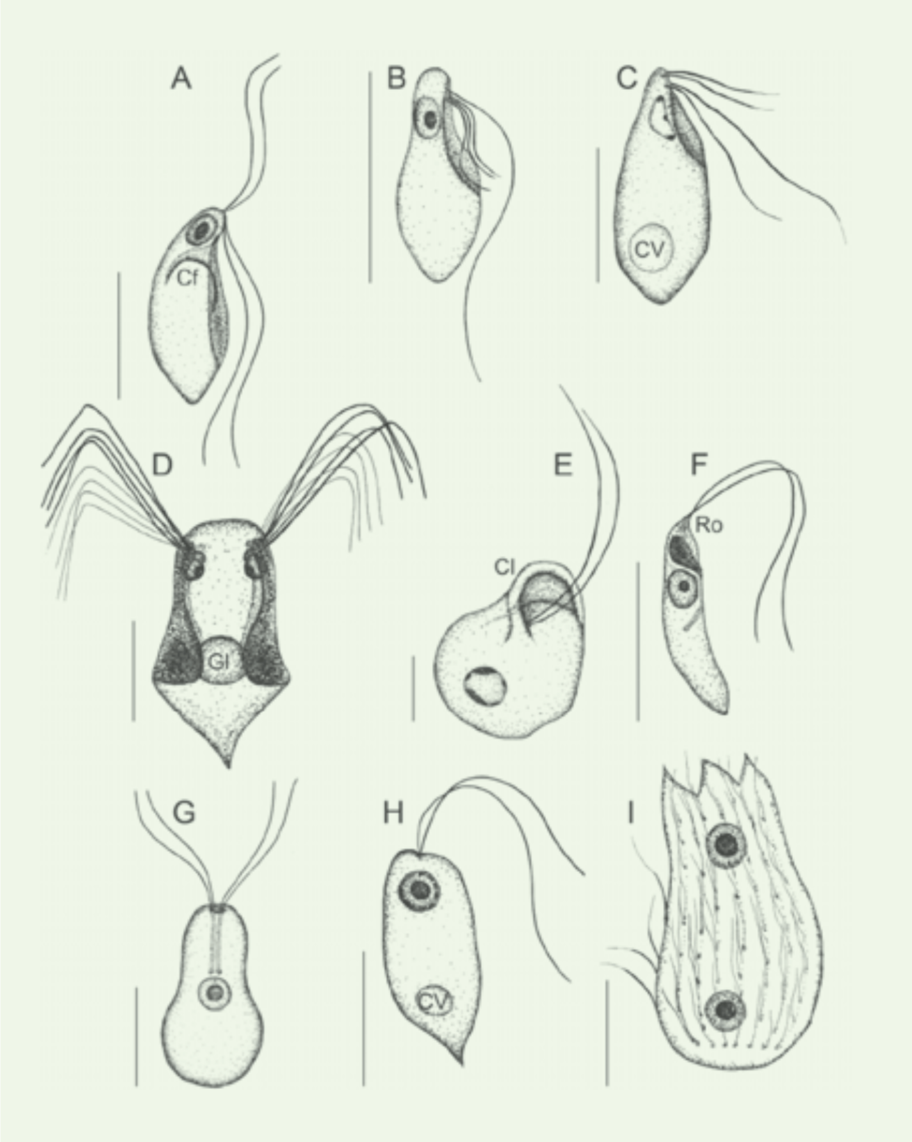
Limits of « Limiting pool mechanism »

Controlling the size of multiple organelles - Cilia



Pharyngomonas kirbyi

Protist, 162, 691–709 (2011)



A , *Pharyngomonas kirbyi* ; **B** , *Percolomonas cosmopolitus* ; **C** , *Percolomonas descissus* ; **D** , *Psalteriomonas lanterna* ; **E** , *Heteramoeba clara* ; **F** , *Pleurostomum flabellatum* ; **G** , *Trimastigamoeba philippinensis* ; **H** , *Naegleria gruberi* **I** ,. *Stephanopogon minuta* .

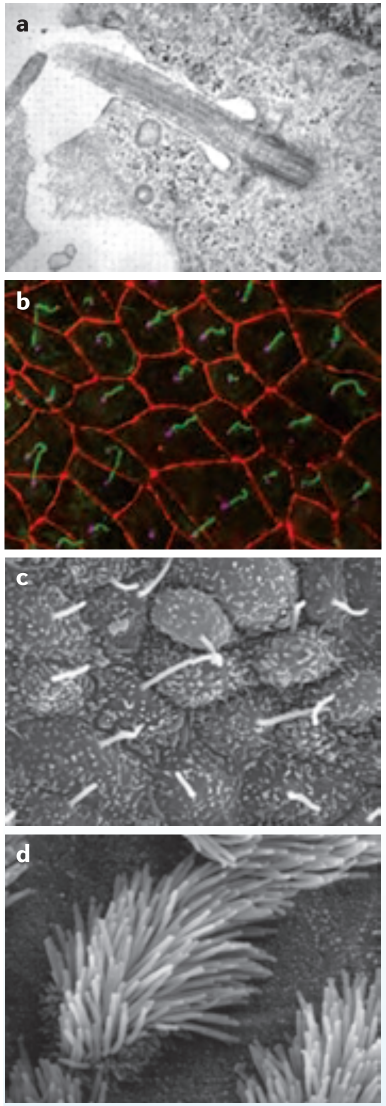
Cf – cytopharynx; Cl – collar; CV – contractile vacuole; Gl – globule of hydrogenosomes; Ro – rostrum.

Scale bars = 10 µm.

After Broers et al., 1990; Bovee, 1959; Brugerolle & Simpson, 2004; Droop, 1962; Fenchel & Patterson, 1986; Page, 1967, 1988; Park et al., 2007; Park & Simpson, 2011; Yubuki & Leander, 2008.

Limits of « Limiting pool mechanism »

Controlling the size of multiple organelles - Cilia

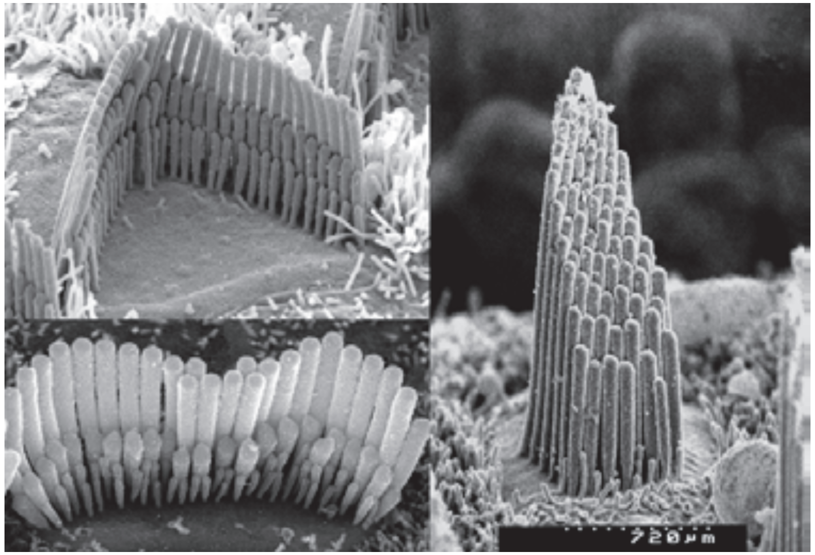


retinal pigment epithelial (RPE1) cells

inner medullary collecting duct (IMCD 3) cells.

mouse nodal cilia

mouse tracheal motile cilia



hair cells inner ear

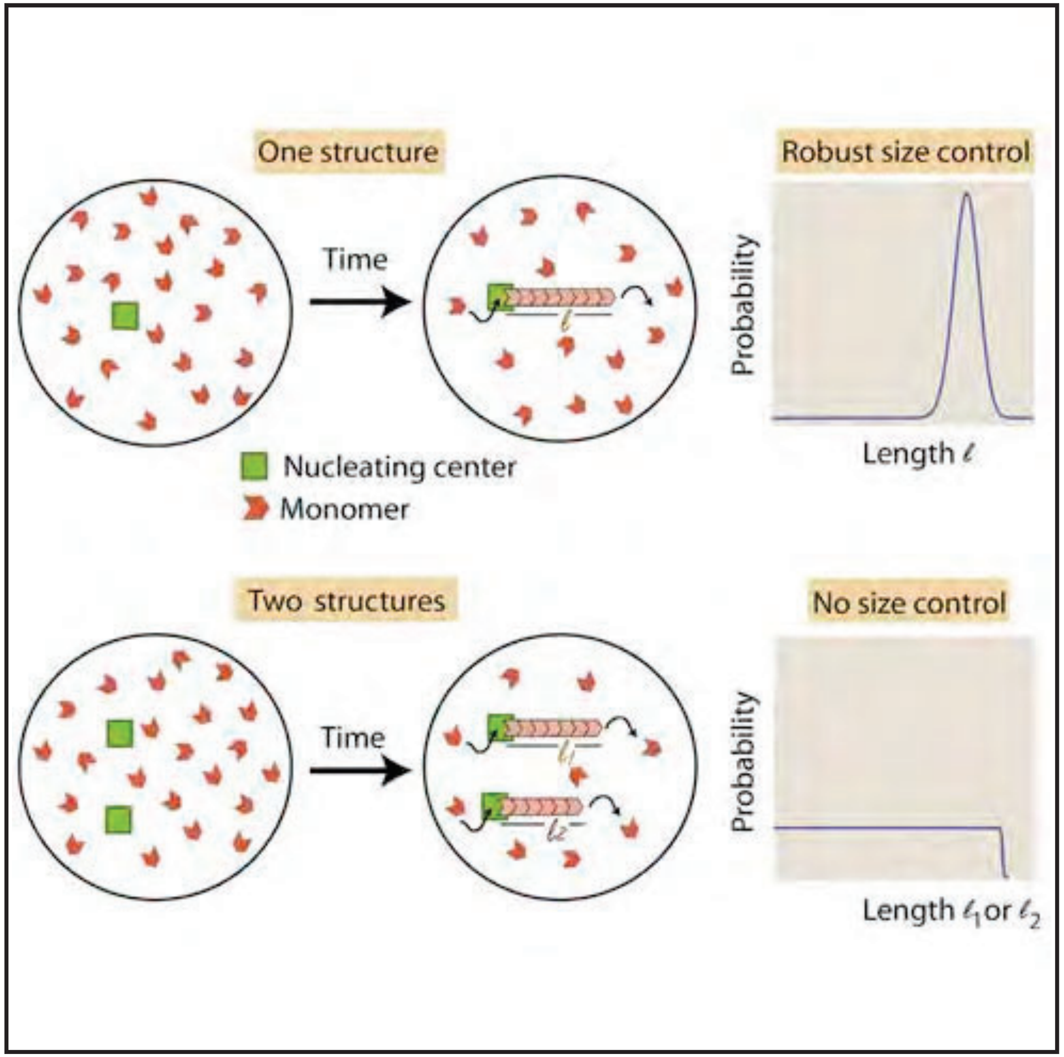
David Furness

H Ishikawa and WF Marshall *Nature Reviews Mol Cell Biol* 12: 222-234 (2011)

Limits of « Limiting pool mechanism »

Controlling the growth of multiple organelles

- The limiting-pool mechanism of size control successfully assembles one structure of a well defined size
- Multiple structures assembled from a common limiting pool exhibit large size fluctuations
- Assembly of multiple structures exhibits three characteristic timescales



Mohapatra et al. and Jane Kondev, *Cell Systems* 4, 559–567 (2017)

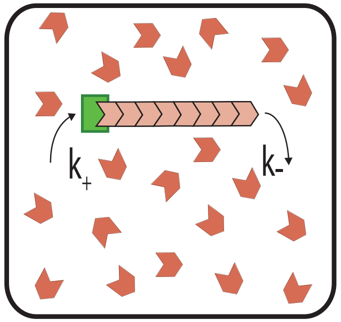
Limits of « Limiting pool mechanism »

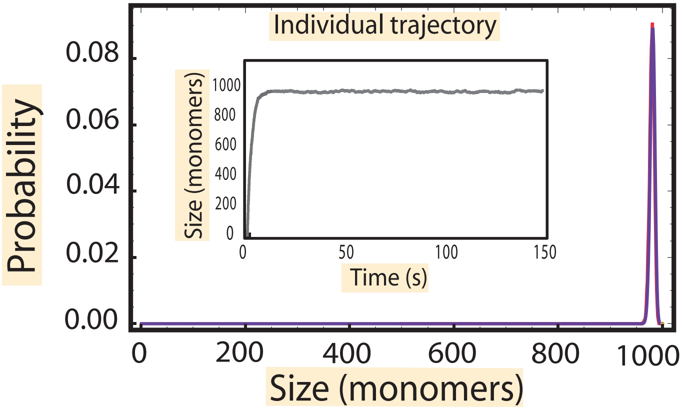
Controlling the growth of multiple organelles

- Single nucleating center

$$\frac{d\,p(l,t)}{dt} = k'_+(N-l+1)p(l-1,t) + k_- p(l+1,t) - k'_+(N-l)p(l,t) - k_- p(l,t). \qquad \text{(Equation 1)}$$

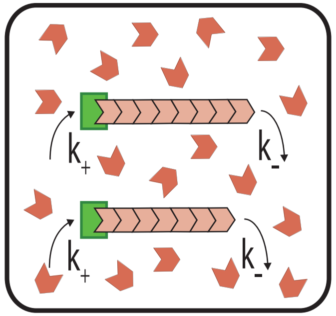
—determine *p(l,t)* at steady state given detailed balance: $p(l)k'_+(N-l) = p(l+1)k_-$

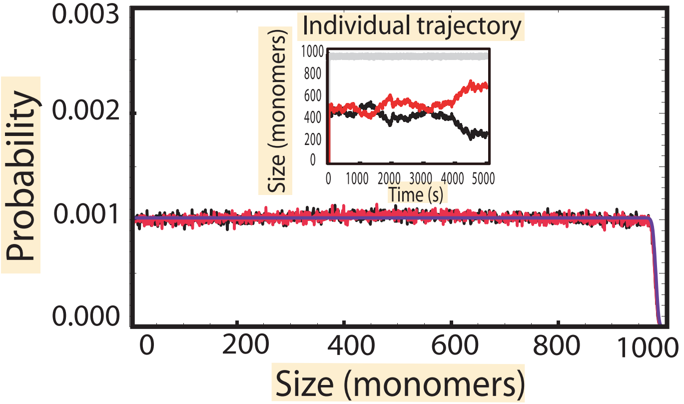




- Two nucleating center

$$\frac{d\,p(l_1,l_2,t)}{dt} = k'_+(N-l_1-l_2+1)(p(l_1-1,l_2,t) + p(l_1,l_2-1,t)) + k_- p(l_1+1,l_2,t) + k_- p(l_1,l_2+1,t) - 2\left(k'_+(N-l_1-l_2)+k_-\right)p(l_1,l_2,t). \qquad \text{(Equation 2)}$$





- So the growth of multiple cilia of same length is evidence that other mechanisms than limiting pool mechanism are involved

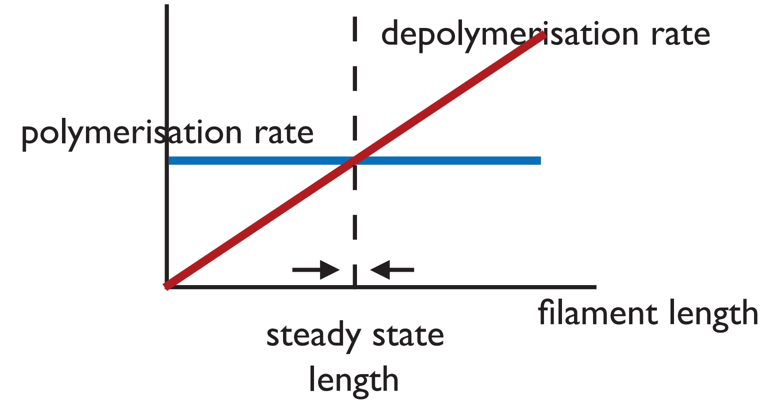
Mohapatra et al. and Jane Kondev, *Cell Systems* 4, 559–567 (2017)

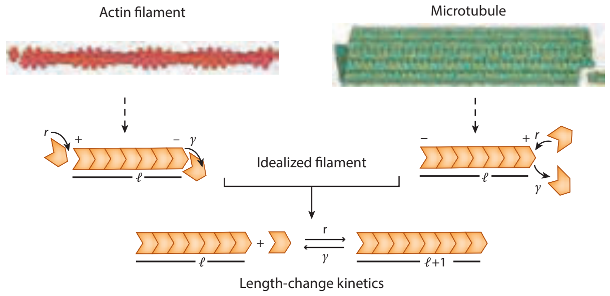
« Antenna » model of length control

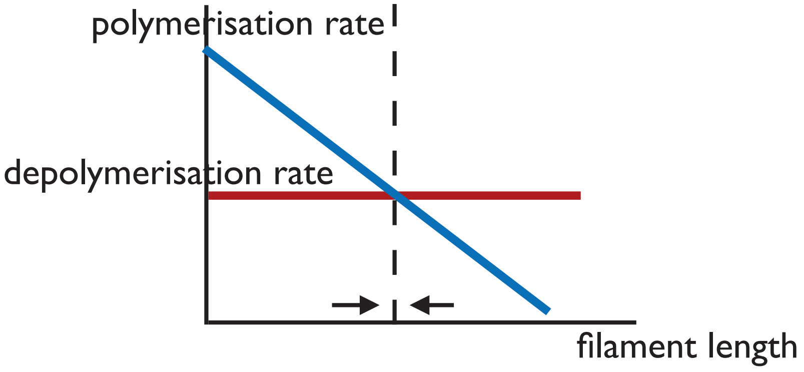
1D length control: how filaments control their length

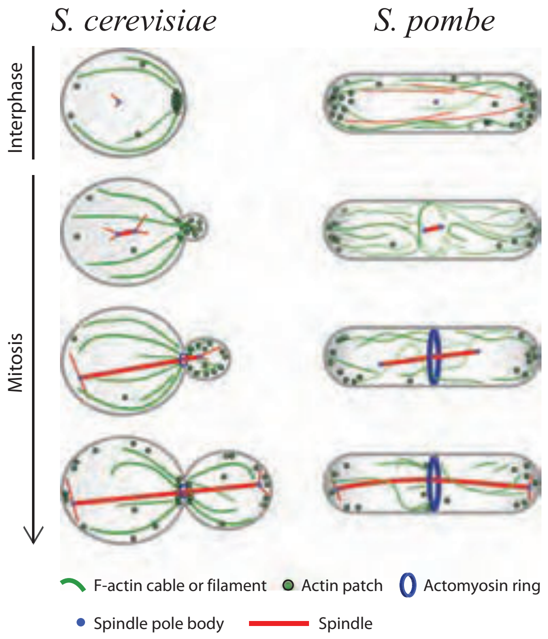
Feedback of length on local dynamics

- Short microtubules grow fast
- Long microtubules grow slowly
- Above critical length growth is arrested





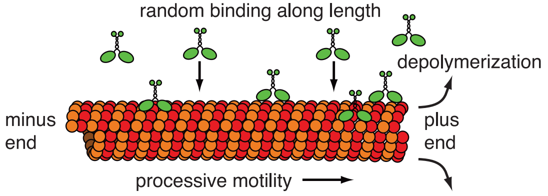


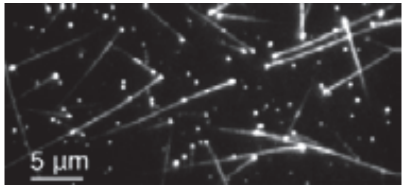


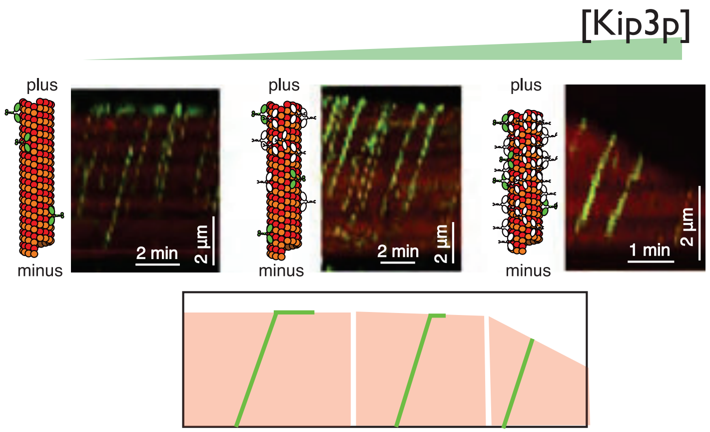
Mishra et al *FEMS Microbiol Rev* 38 (2014) 213–227

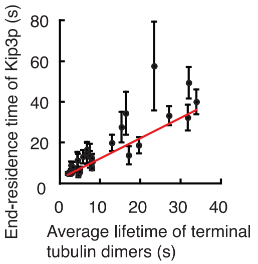
« Antenna » model of length control

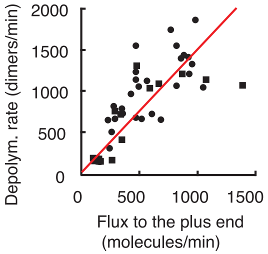
Feedback of length on dynamics



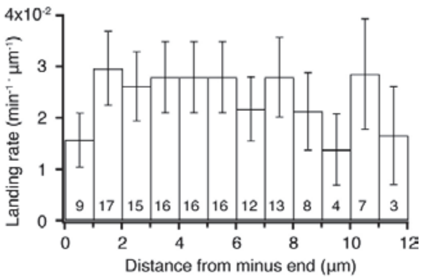






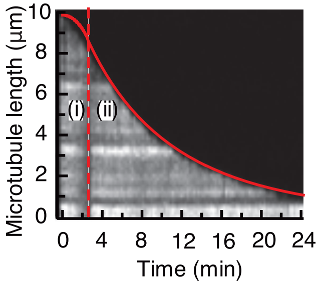


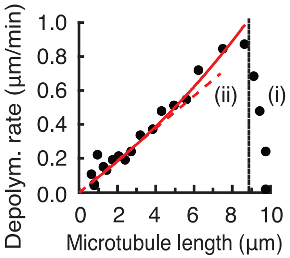
(1) the total rate at which Kip3p molecules land on a microtubule is proportional to the microtubule's length
(2) Kip3p is a highly processive motor over a wide range of Kip3p densities on the microtubule lattice
(3) new Kip3p arriving at the + end of MT induces dissociation of stalled Kip3p
(4) each dissociation of Kip3 removes 1-2 tubulin dimers
(5) the rate of depolymerization is proportional to the flux of Kip3p molecules to the microtubule end



- The antenna model predicts well the length dependent depolymerisation rate
- Negative feedback of length on MT depolymerisation.

- Energetic cost: few 1000 ATPs for dissociation of 1-2 dimers!!
energy used to collectively « compute » MT length and control depolymerisation rate



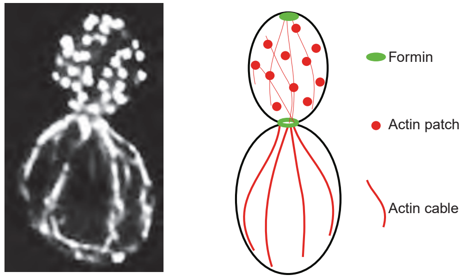


Varga, V., Leduc, C., Bormuth, V., Diez, S., and Howard, J. (2009). *Cell* 138, 1174–1183.

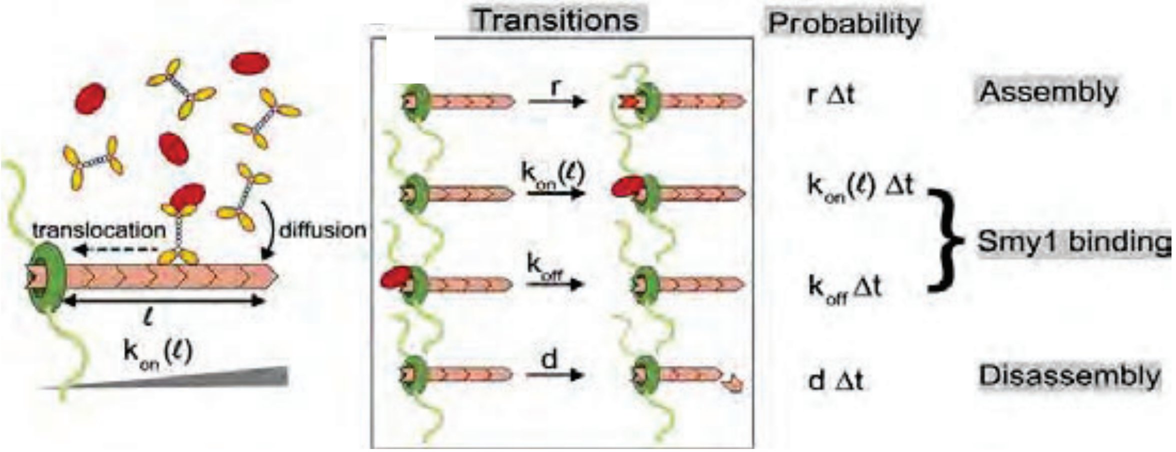
« Antenna » model of length control

Antenna Mechanism of Length Control of Actin Cables.

- « Antenna mechanism" involves three key proteins:
  —Formins, polymerize actin,
  —Smy1 proteins, bind formins and inhibit actin polymerization,
  —Myosin motors, deliver Smy1 to formins,
- This leads to a length-dependent actin polymerization rate.



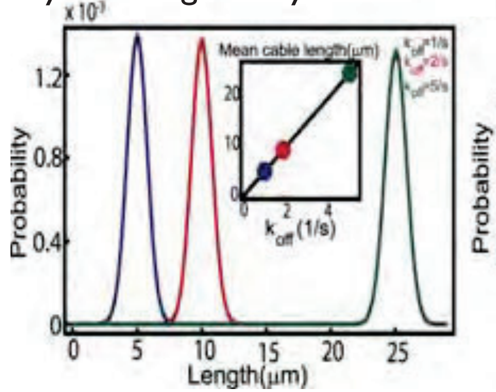
Lee. *Nature Cell Biology* 4: E29–E30(2002)

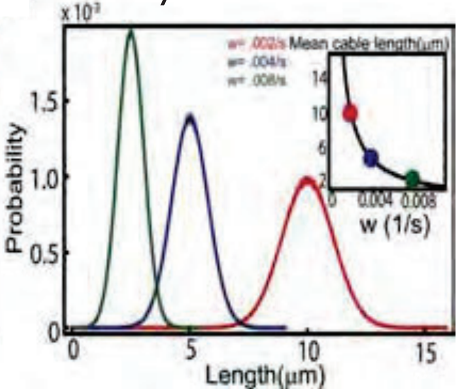


- Steady state cable length distributions depend on Smy1 concentration and binding affinity of Smy1 to formins

$$k_{on}(l) = wl$$

w :: Smy1 concentration

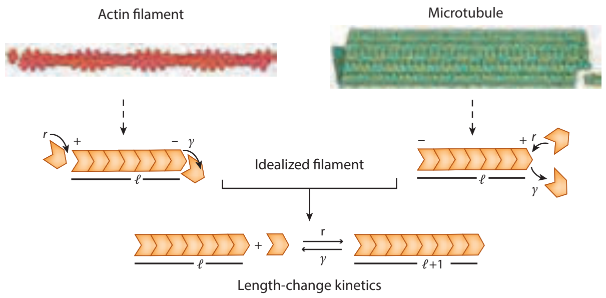

Smy1 binding affinity to formins


Smy1 concentration

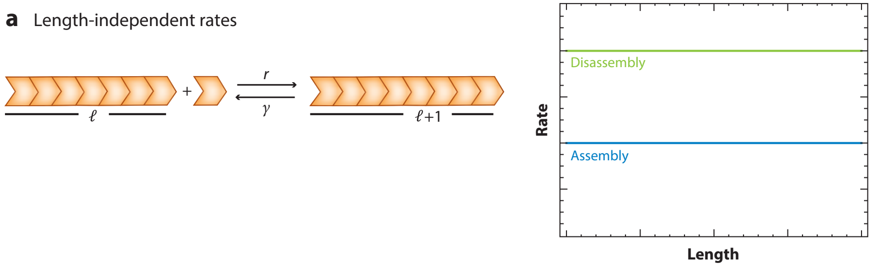
Mohapatra L, Goode BL, Kondev J (2015)
PLoS Comput Biol 11(6): e1004160. doi:10.1371/journal.pcbi.1004160

Design principles of length control of cytoskeletal structures

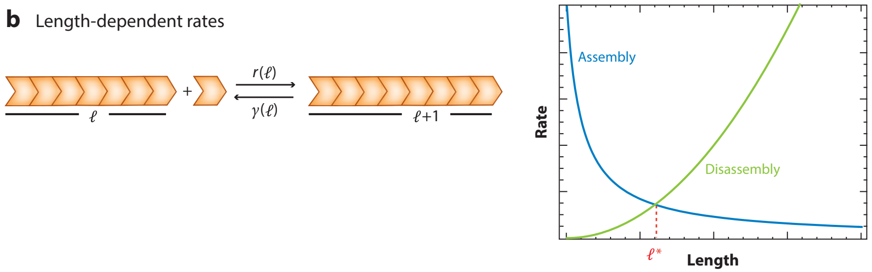
- stochastic dynamics of filaments
- What are the principles of length control?



- Size invariant assembly and disassembly: no characteristic length scale



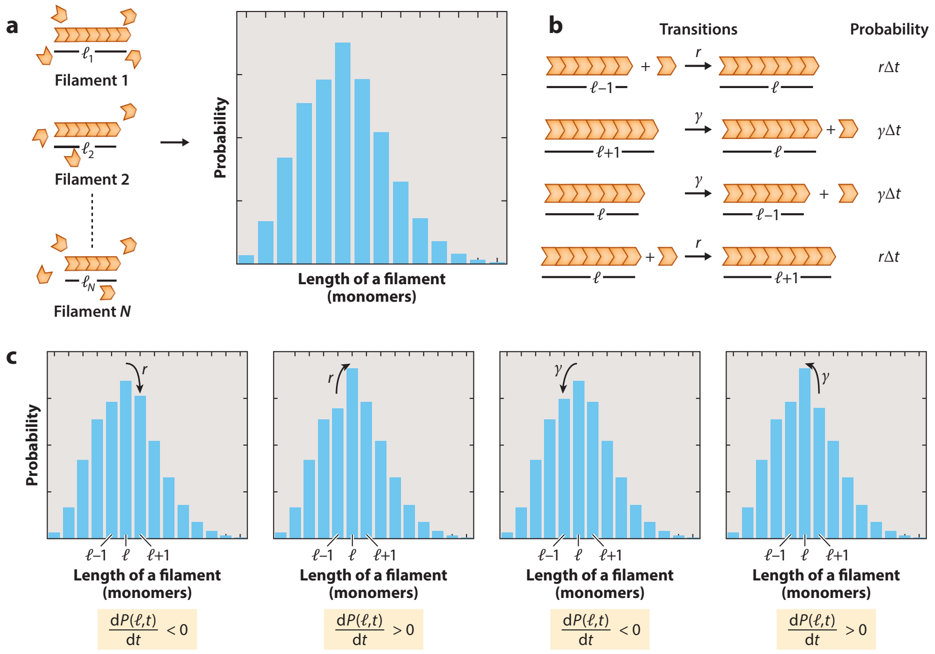
- Size dependent assembly and disassembly: characteristic length at steady state
- Negative feedback: increased polymerisation in shorter filaments and/or increased disassembly in longer filaments



L. Mohapatra, B. Goode, P. Jelenkovic, R. Phillips and Jane Kondev. *Annu. Rev. Biophys*. 45:85–116 (2016)
doi: 10.1146/annurev-biophys-070915-094206

Design principles of length control of cytoskeletal structures

$P(l, t)$: probability that filament is of length l at time t



$$\boxed{\frac{\mathrm{d}P(l,t)}{\mathrm{d}t} = r\,P(l-1,t) - r\,P(l,t) + \gamma\,P(l+1,t) - \gamma\,P(l,t).}$$

Calculate steady state distribution with detailed balance: $r(l)P(l) = \gamma(l+1)P(l+1)$

- The rates of growth r and shrinkage γ need not be constant
- In fact, their dependency on length l will give rise to interesting properties

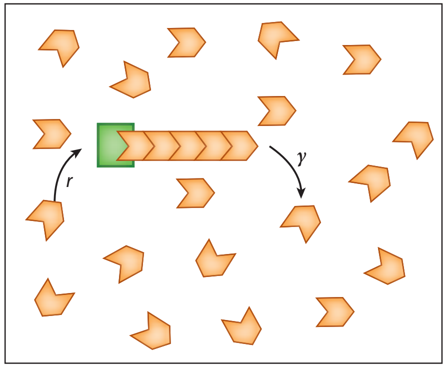
$$P(l) = f(r(l), r(l-1), \ldots r(0), \gamma(l), \gamma(l-1), \ldots \gamma(0))P(0).$$

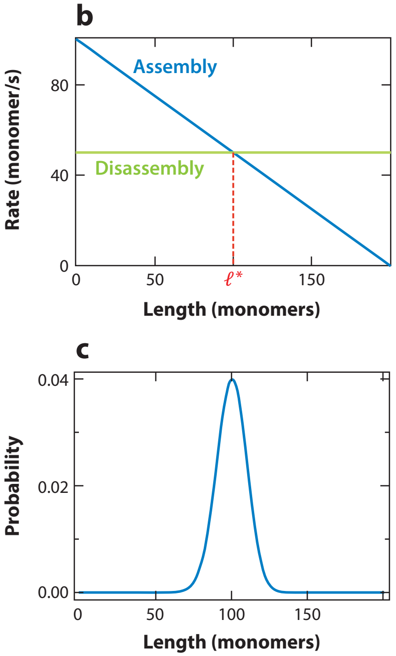
L. Mohapatra, B. Goode, P. Jelenkovic, R. Phillips and Jane Kondev. *Annu. Rev. Biophys*. 45:85–116 (2016)
doi: 10.1146/annurev-biophys-070915-094206

Design principles of length control of cytoskeletal structures

Length control by assembly

- **Finite/limiting pool model**





Probability distribution

$$P(l) = \left(\frac{r'}{\gamma}\right)^{l} \frac{N_{\mathrm{t}}!}{(N_{\mathrm{t}} - l)!} P(0)$$

$$r = r' N_{\mathrm{t}}$$

Mean length: $\langle l \rangle = N_{\mathrm{t}} - \frac{\gamma}{r'}$

Limited by N_t : length increases with size of pool (N_t)
Higher dissociation reduces size

Variance $\frac{\gamma}{r'}$

L. Mohapatra, B. Goode, P. Jelenkovic, R. Phillips and Jane Kondev. *Annu. Rev. Biophys*. 45:85–116 (2016)
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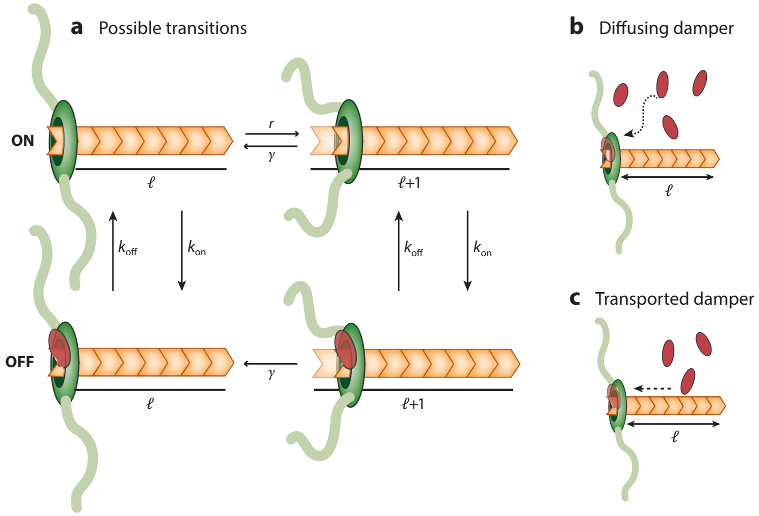
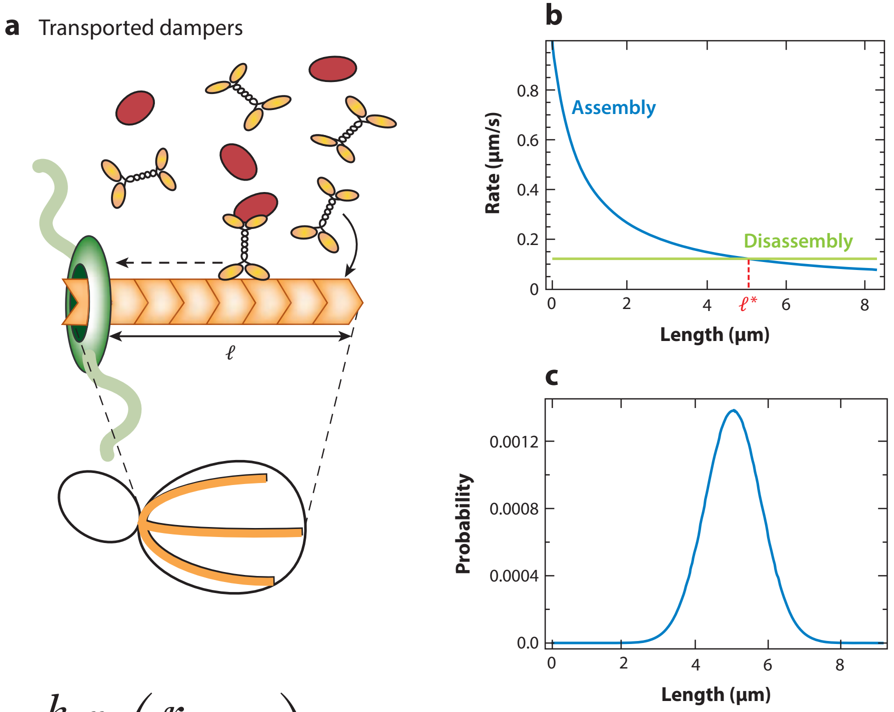
Design principles of length control of cytoskeletal structures

Length control by assembly

- **Antenna model:** negative feedback by length dependent recruitment of assembly inhibitor





Mean length: $$\langle l \rangle = \frac{k_{\mathrm{off}}}{w}\left(\frac{r}{\gamma}-1\right)$$

Increases as affinity of damper decreases (increased dissociation k_{off})
Increases as concentration of damper decreases

L. Mohapatra, B. Goode, P. Jelenkovic, R. Phillips and Jane Kondev. *Annu. Rev. Biophys*. 45:85–116 (2016)
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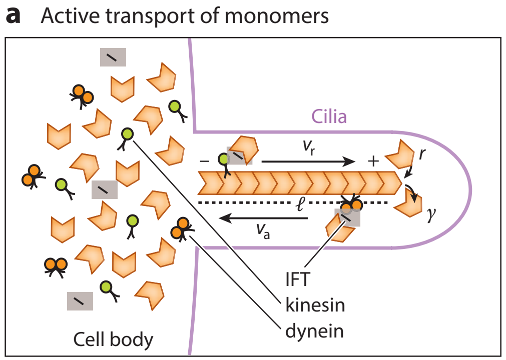
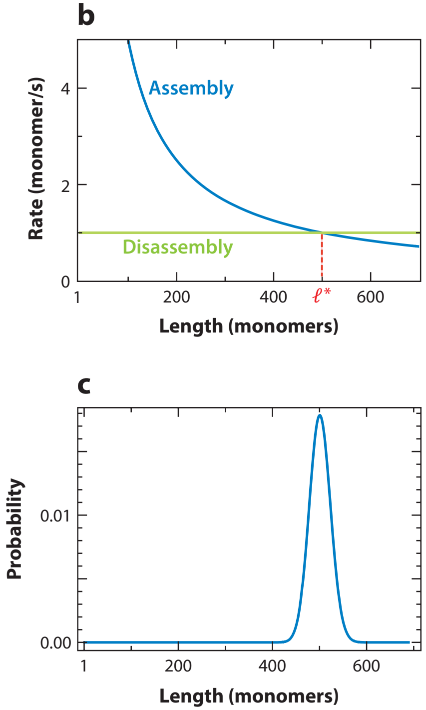
Design principles of length control of cytoskeletal structures

Length control by assembly

- **Antenna model:** negative feedback by active transport of monomer

- microtubules grow at the tip of cilia
- diffusion of monomers is slow (10s to reach tip of 10µm long cilila)
- active transport of monomers to the tip controls the flow of monomers: 10s of monomers every second
- The number of IFT molecules that link monomer to motor is fixed and independent of cilia length.
- time of transport of monomers increases with length of filament
- the assembly rate decreases with filament length
- longer filaments grow slowly and shorter grow faster





Chlamydomonas reinhardtii

- mean length $\langle l \rangle = \dfrac{r'}{\gamma} + 1$

$$r' = \alpha N v$$

Increases with the number of transporters (IFT)

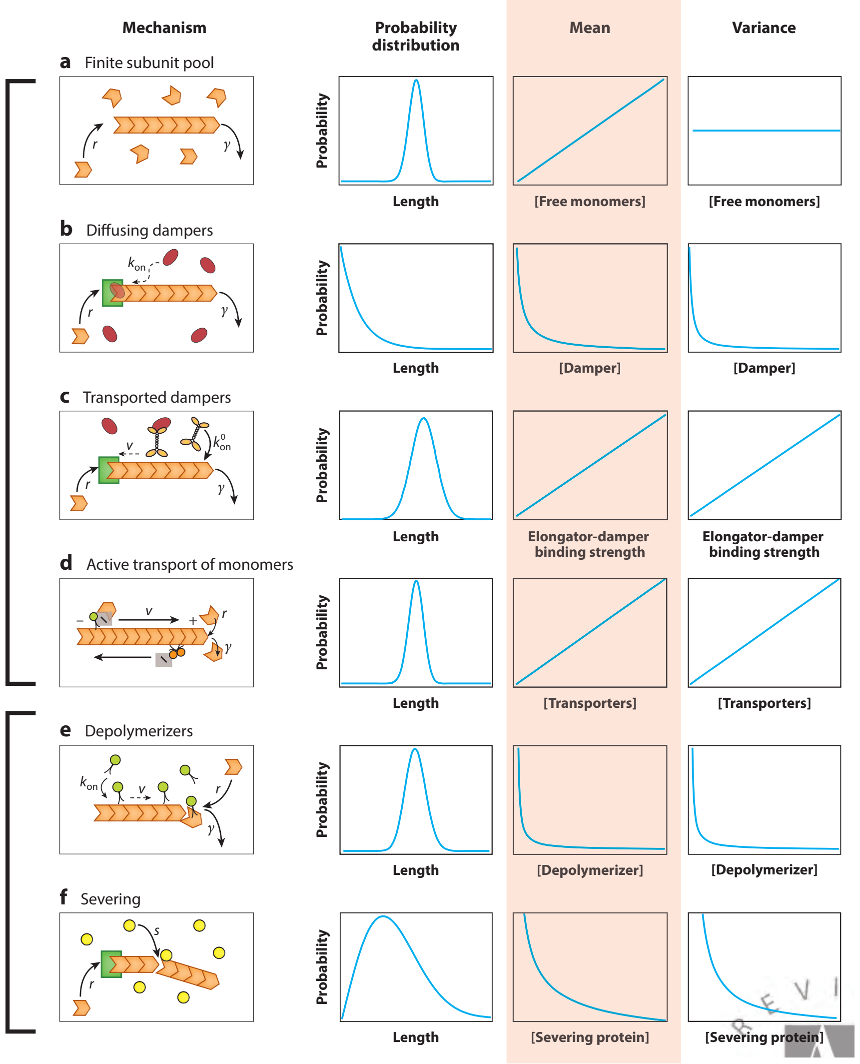
L. Mohapatra, B. Goode, P. Jelenkovic, R. Phillips and Jane Kondev. *Annu. Rev. Biophys*. 45:85–116 (2016)
doi: 10.1146/annurev-biophys-070915-094206

Design principles of length control of cytoskeletal structures

- Statistical features of different models

- Length control by assembly

- Length control by disassembly



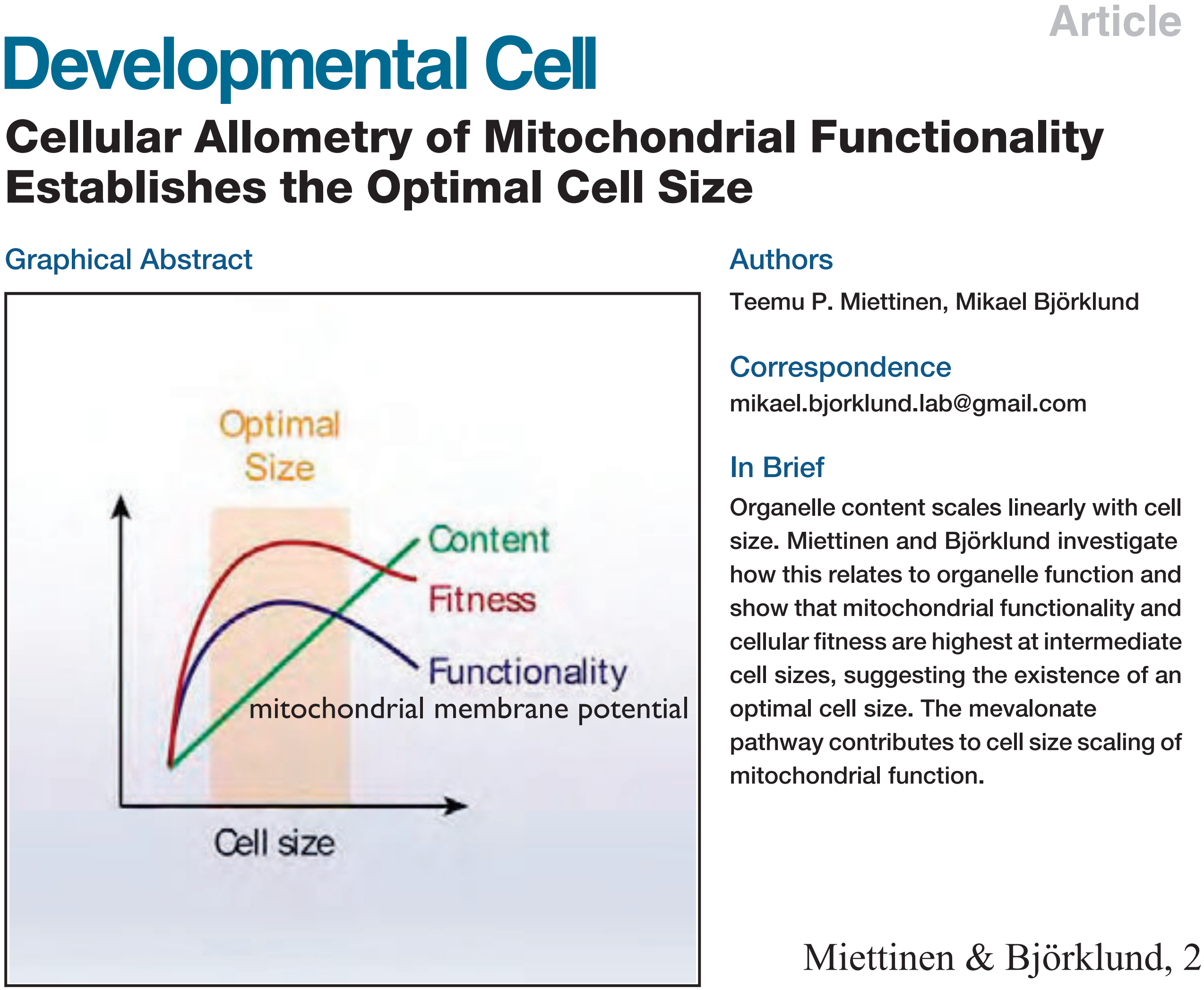
- Condition for scaling with cell size

monomer pool scales with cell size

transporter number scales with cell size

L. Mohapatra, B. Goode, P. Jelenkovic, R. Phillips and Jane Kondev. *Annu. Rev. Biophys*. 45:85–116 (2016)
doi: 10.1146/annurev-biophys-070915-094206

Cellular scaling and cell size control



Article

Developmental Cell

Cellular Allometry of Mitochondrial Functionality Establishes the Optimal Cell Size

Graphical Abstract

Authors

Teemu P. Miettinen, Mikael Björklund

Correspondence

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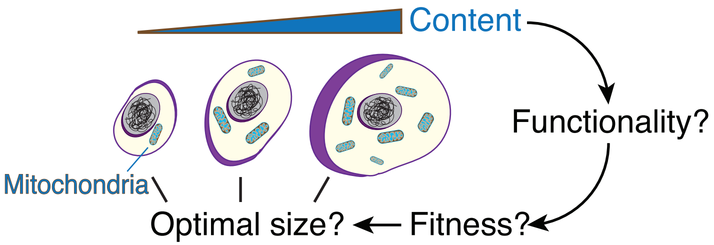
In Brief

Organelle content scales linearly with cell size. Miettinen and Björklund investigate how this relates to organelle function and show that mitochondrial functionality and cellular fitness are highest at intermediate cell sizes, suggesting the existence of an optimal cell size. The mevalonate pathway contributes to cell size scaling of mitochondrial function.

Cell size scaling of mitochondria



Highlights

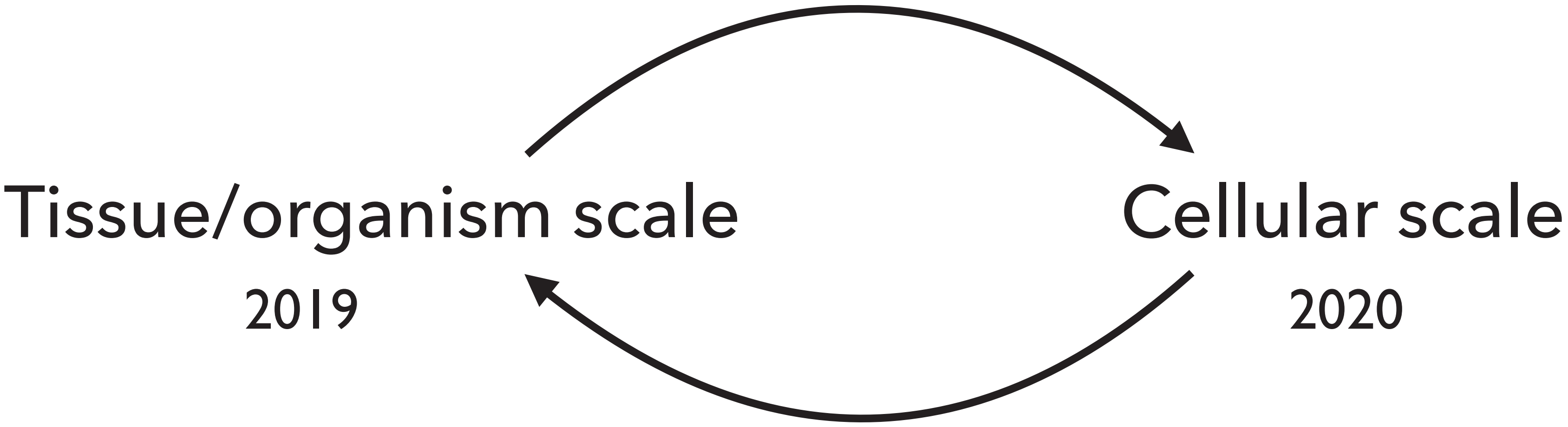
- Mitochondrial functionality is highest in intermediate-sized cells in a population
- Mitochondrial membrane potential changes with cell size, not cell cycle
- Evidence for an optimal cell size, whereby functionality and fitness are maximized
- Mitochondrial dynamics and mevalonate pathway required for the optimal cell size

Miettinen & Björklund, 2016, *Developmental Cell* 39, 370–382

Conclusion

How is biological size encoded?

Coupling scales: development arrest of growth
tissue homeostasis



Tissue/organism scale
2019

Cellular scale
2020

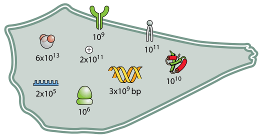
- Motor, Constraints and Regulation of Growth

Energy supply

carbohydrates
amino-acids
ions etc

→

Metabolism



Synthesis: ATP, mRNAs, proteins, ribosomes
lipids, carbohydrates

↓↑ Feedback

Osmotic flow: ions, H_2O

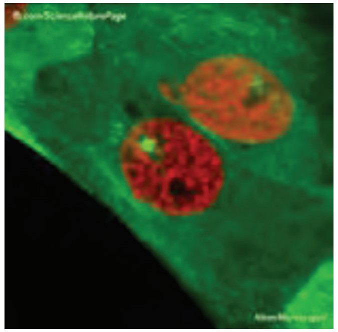
→

Cell growth

Feedback ↓↑

Cell division

Sizer
Adder



kidney cell

Energy uptake
↓
Energy delivery

→ Energy → Metabolism → Cell growth ↓↑ Cell division

→ Kidney, liver
→ Organ growth
→ Gut, muscle, bones, …

Feedback #3 coordination ↓↑

Sizer

Feedbacks: #1 Patterning, #2 Mechanics

Colloque :

Constraints and plasticity in Development and Evolution

3-4 Juin 2021 – 9h-18h

Amphithéâtre Maurice Halbwachs

Organisateurs:
Denis Duboule (chaire: Evolution des génomes et développement)
Thomas Lecuit (chaire: Dynamiques du vivant)

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1530

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Hopi Hoekstra (Harvard)
Laurent Keller (Univ. Lausanne)
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Shigeru Kuratani (Kobe)
L. Mahadevan (Harvard)
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