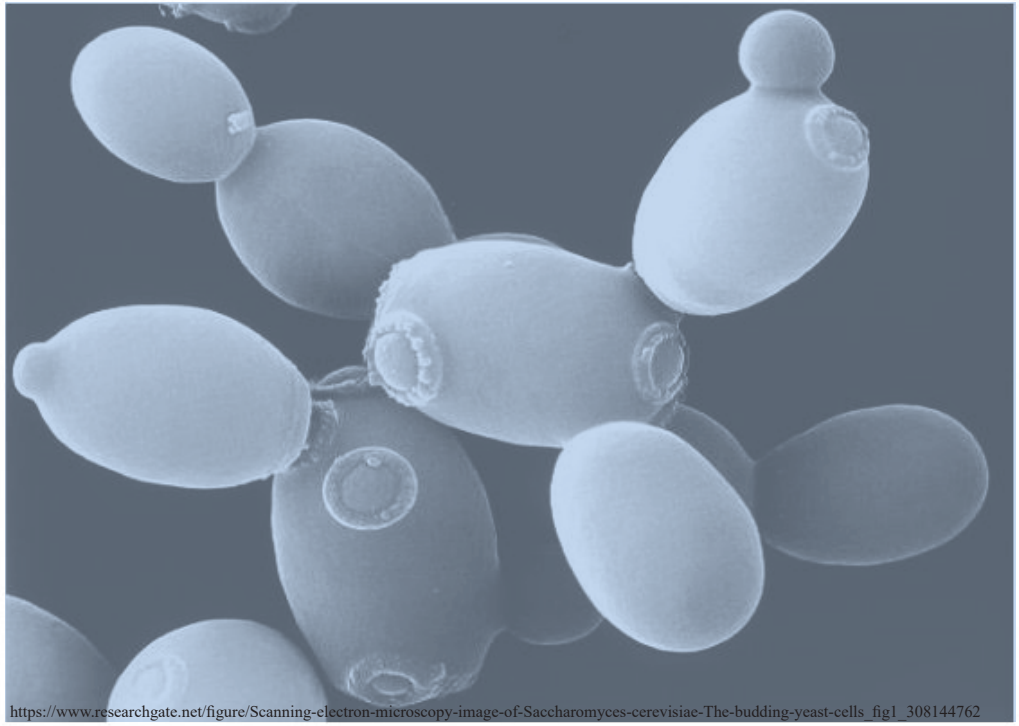
Cellular Growth and Form


https://www.researchgate.net/figure/Scanning-electron-microscopy-image-of-Saccharomyces-cerevisiae-The-budding-yeast-cells_fig1_308144762

Course 3: Cell Growth and Division

Thomas Lecuit
chaire: Dynamiques du vivant

COLLÈGE DE FRANCE
1530

Cell size – volume

Statement of the problem:

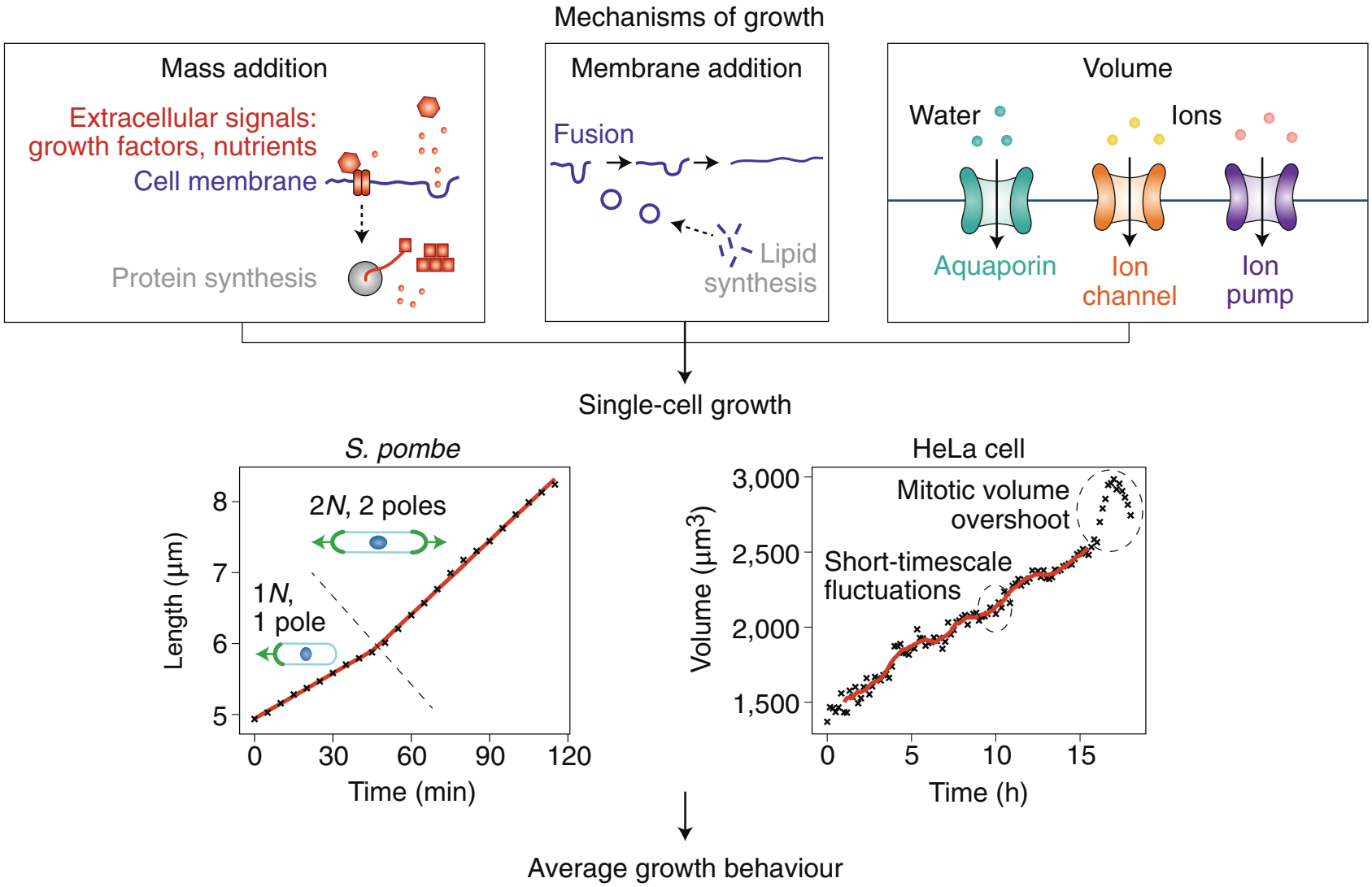
- Cell size varies between cell types in Animals but within a given cell type there is very little size variation (ex. epithelial cell). So cells control their volume tightly.
  —Why is cell size regulated? How is cell size regulated?

- Cell size is tightly coupled to cell function, for example, neuron, oocyte, red blood cells, Ciliates etc.

- Cell size is physically constrained, e.g.:
  —diffusion of metabolites limits cell size.
  —surface to volume ratio for exchange with environment
  —diffusion of signalling molecules limits communication within cells (unless other transport mechanisms operate such as motor driven or by trigger waves)
  —energetically: synthesis of ribosomes and translational capacity limits cell growth. Given maximal rate of rRNA transcription, there is a limit to cell growth, unless polyploidy or multinucleation (ex. muscle cells, ciliates etc).

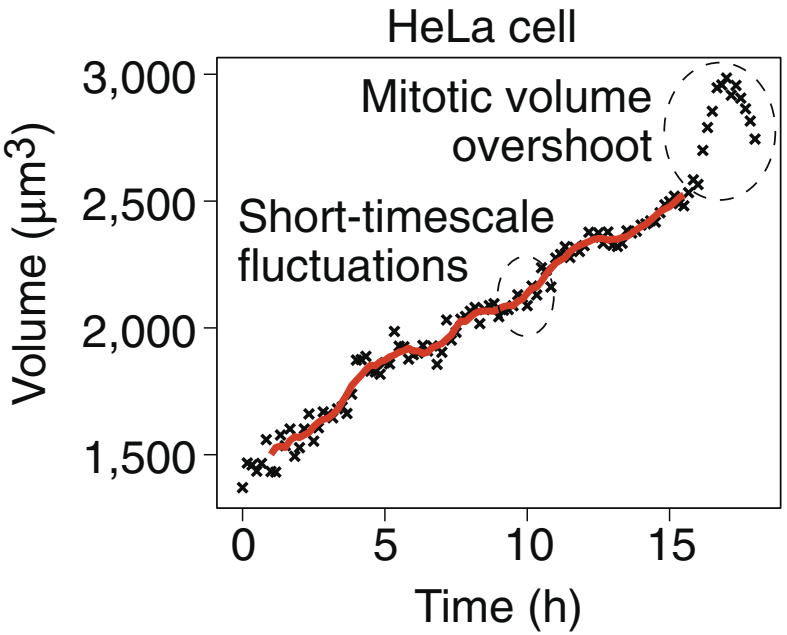
- Cell size is governed by protein synthesis, osmotic flow and cell cycle which operate at different time scales: how is this integrated?

Mechanisms of cell growth

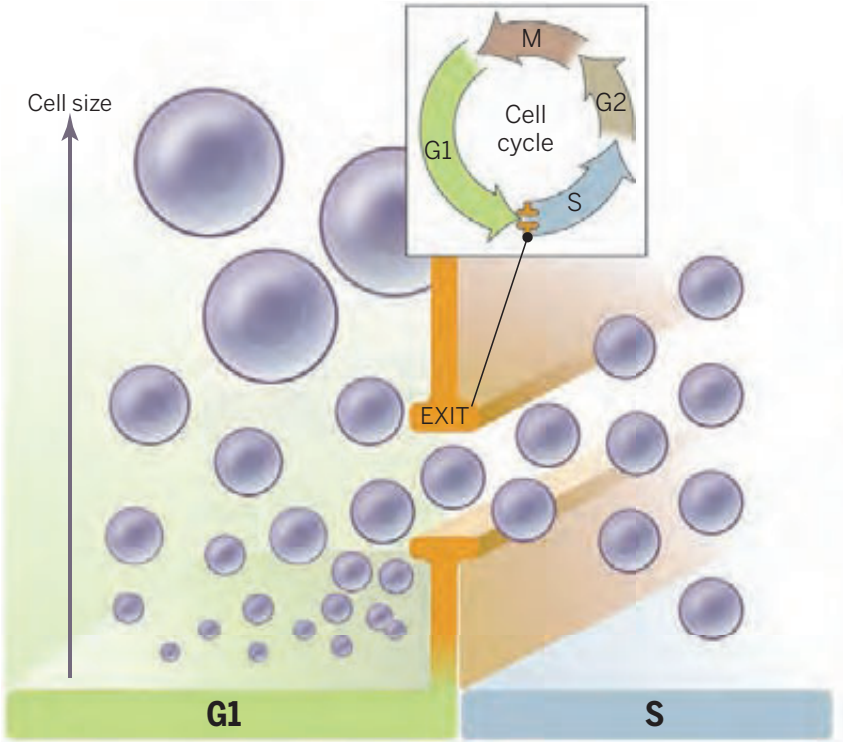


C.Cadart L. Venkova, P. Recho, M. C. Lagomarsino and M. Piel
Nature Physics 15: 993–1004 (2019)

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

Cell size — Questions

- Control of the cell size mean by extracellular signals (eg. IGF, EGF) that act on cell cycle length and cell growth rate.

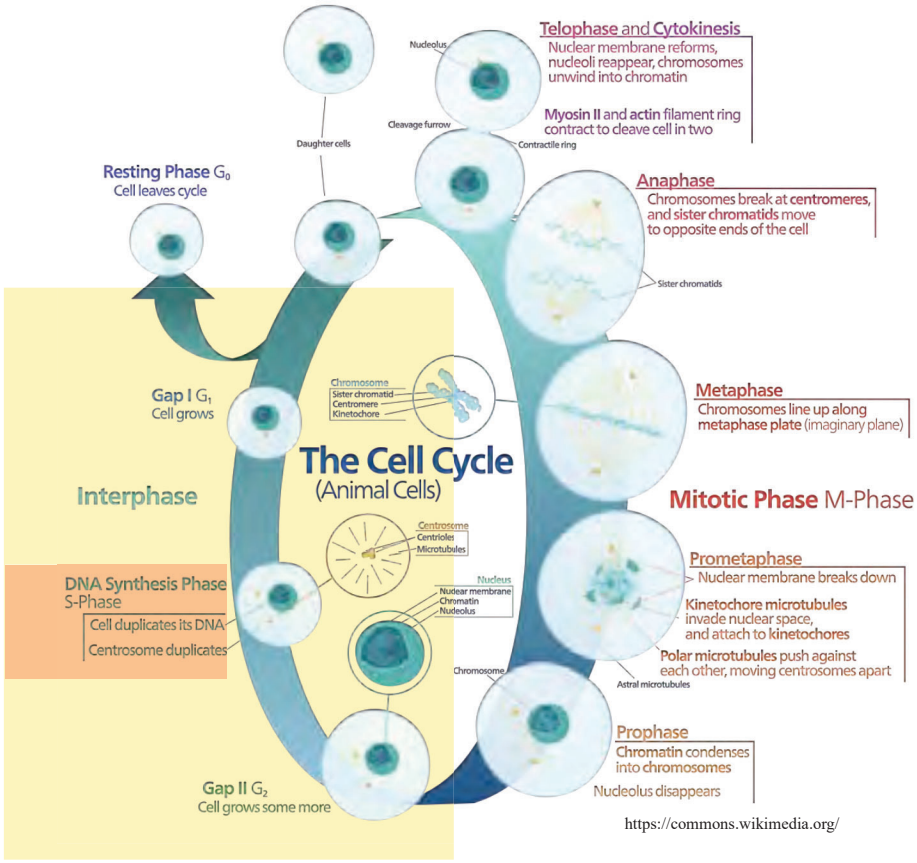
See Lectures 2019: (26 Nov & 17 Dec 2019)

- Control of cell size heterogeneity: mechanisms to reduce variation.

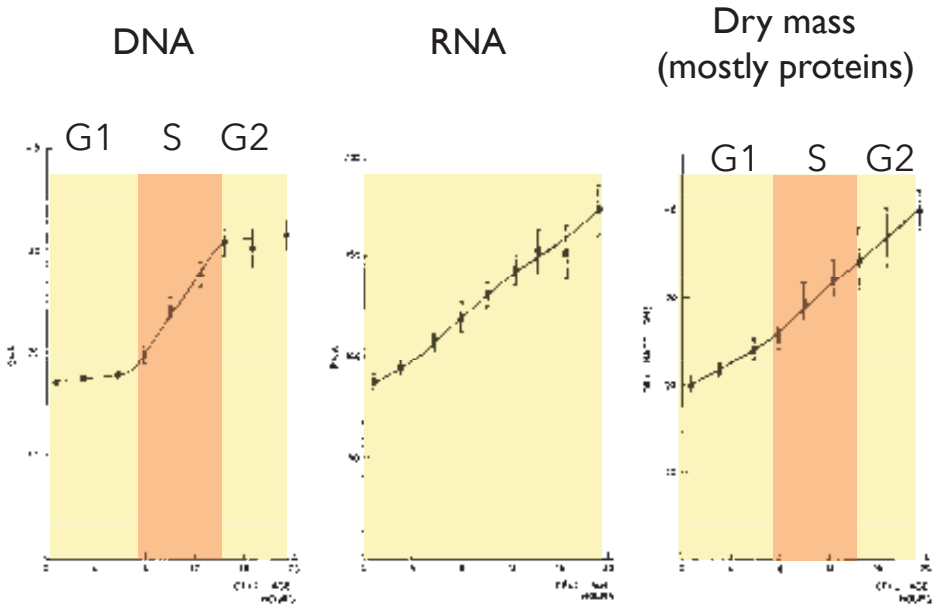
Size sensor and mechanism that adjusts cell cycle length as a function of cell size (feedback)

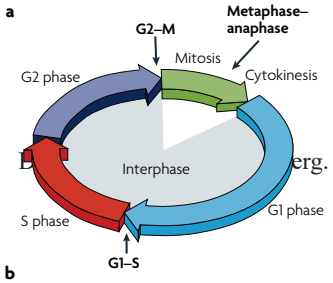
Cell growth and cell division

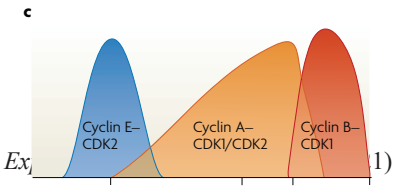
- DNA, RNA and protein content doubles during interphase (growth phase)

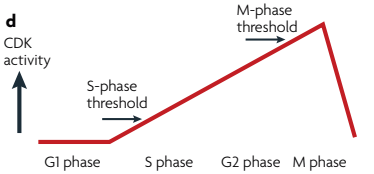


https://commons.wikimedia.org/



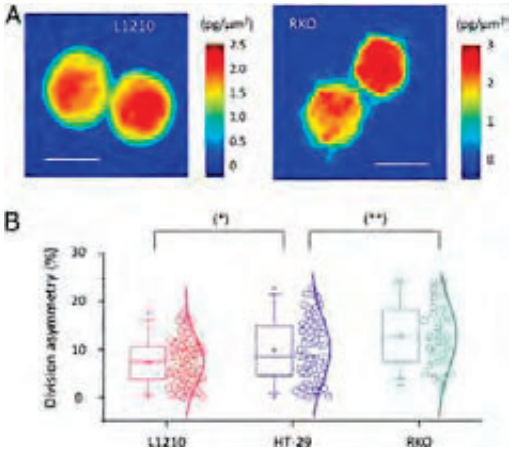






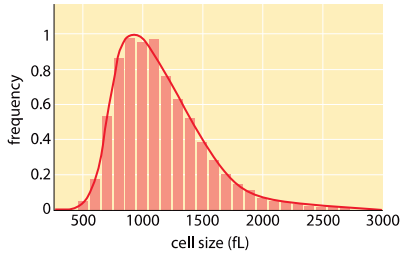
Why cell size must be regulated during the cell cycle

- There is a division asymmetry in cell mass/volume in the order of 10%



Y. Sung et al and M. Kirschner. *PNAS* 2013.
www.pnas.org/cgi/doi/10.1073/pnas.1315290110

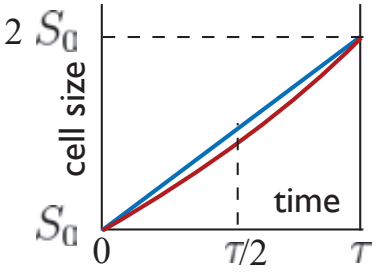
- If cell growth rate in a population is constant (linear growth) cell size heterogeneity will be modest in the population (but grow slowly)
- But if cell growth rate is exponential, namely proportional to cell size, cell size heterogeneity will increase acutely within a few generations.
- Following cell divisions, size difference between daughter cells will then be amplified in the next cell cycle.
- Over several cell generations this would result in strong cell size heterogeneity in a population of a given cell type in a given environment
- However, this is not the case. Cell size is highly constrained.
- There must exist mechanisms to regulate cell size during the cell cycle so as to limit cell size variation in a population



L1210, a mouse lymphoblast cell line

Why cell size must be regulated during the cell cycle

- The relative cell size difference between a linear and an exponential growth model is at most 5.63% during cell cycle
- Detecting this in single cell data requires high precision methods (see later)



Linear Growth $S_l(t) = S_0 + \beta t$

Exponential Growth $S_e(t) = S_0 e^{kt}$

t - time

τ - length of cell cycle (time)

S_0 - size of cell at birth

$2 \times S_0$ - size of cell at division

A. Tzur, R. Kafri, V. S. LeBleu, G. Lahav, M. W. Kirschner, *Science* 325, 167–171 (2009)

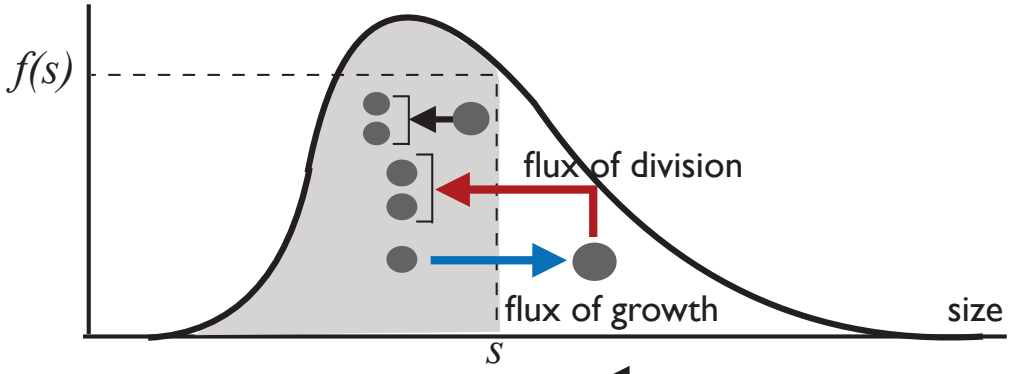
- There is a large body of evidence supporting exponential cell growth in Bacteria, Yeast and mammalian cells.

Why cell size must be regulated during the cell cycle

- Evidence that cell growth rate is size dependent (ie. growth is exponential)
- Statistical argument based on mass conservation law (Collins & Richmond method, 1962)

J. F. Collins, M. H. Richmond, *J. Gen. Microbiol.* 28, 15–33 (1962).

At steady-state the flux of newborn cells by cell division, born with a size smaller then s is balanced by the rate at which cells grow past the size threshold (s)

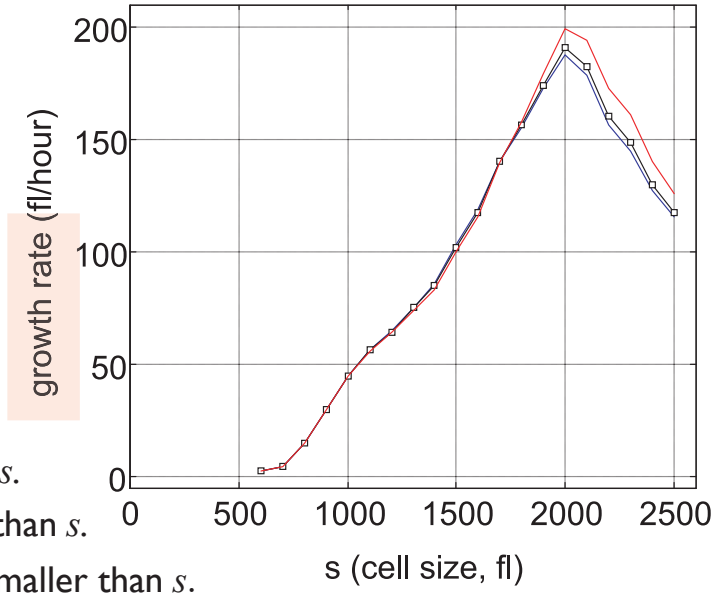


$$\underbrace{N_t \alpha F_a(s)}_{\text{total population increase}} = \underbrace{2N_t \alpha F_0(s) - N_t \alpha F_m}_{\text{cell divisions}} \underbrace{- v(s) N_t f_a(s)}_{\text{cell growth}}$$

Given knowledge of:
—cell size distribution in asynchronous population at steady state
—cell size distribution of newborn cells
—distribution of cell size difference between sister cells δ

$$F_m(s) = (F_0 * \delta)(s)$$

Infer growth rate:



N_t total number of cells in the population at time t

α frequency of cell divisions in the asynchronous population.

v growth rate

$F_0(s)$ cumulative size probability distribution of newborn cells smaller than s.

$F_a(s)$ cumulative size probability distribution of asynchronous cells smaller than s.

F_m cumulative size probability distribution of the mitotic subpopulation smaller than s.

A. Tzur, R. Kafri, V. S. LeBleu, G. Lahav, M. W. Kirschner, *Science* 325, 167–171 (2009)

What do cells measure to control division?

Sizer: cells divide at *target* size

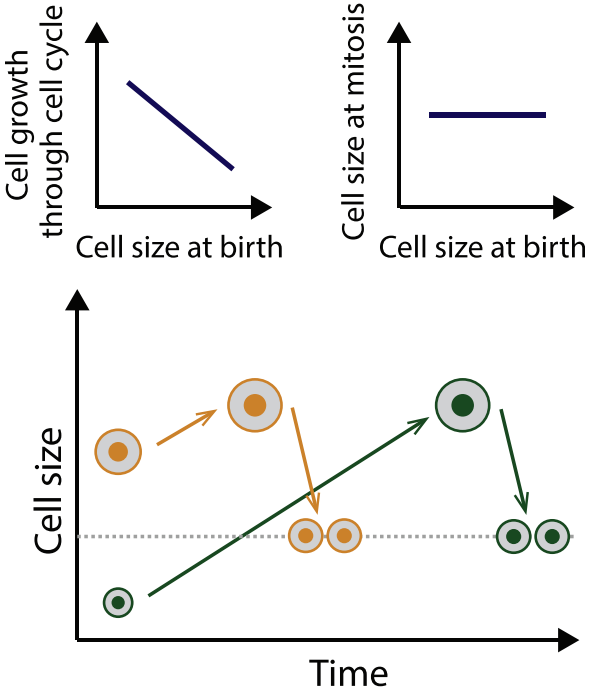
Different cells in the same environment (in situ) have different target size.
So there must be an absolute measurement of size, not simply relative.
Otherwise a smaller newborn cell would not reach the normal target size

Timer: cells divide after fixed duration

Adder: cells divide after fixed size increase

Intrinsic mechanism to control cell size

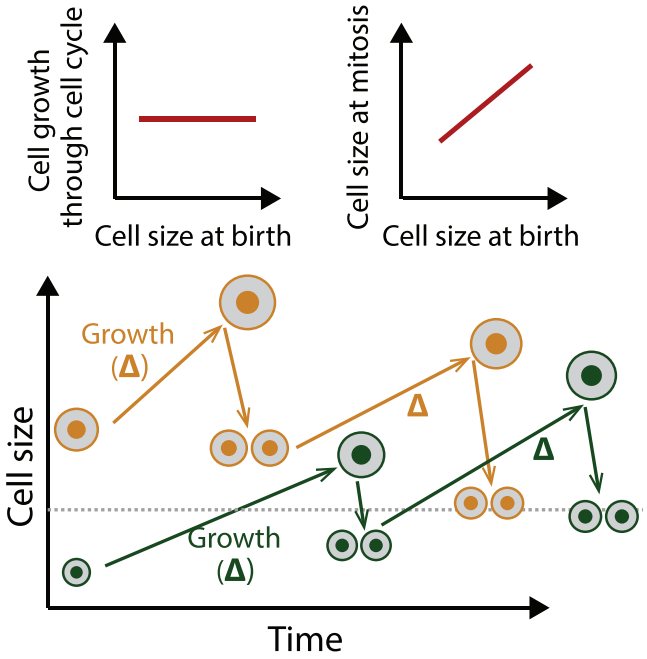
Sizer mechanism



Immediate change to target cell size

Adder/Timer mechanism

Cells add a fixed size.
Cells may do so by timing cell growth (given a set growth rate)



Progressive change to target cell size

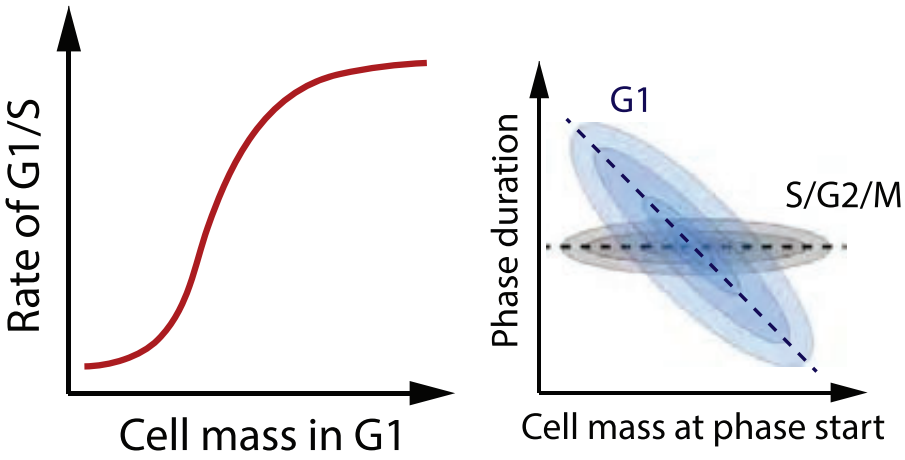
E. Zatulovskiy and J.M. Skotheim. *Trends in Genetics*, 36: 360 -372 (2020)

Intrinsic mechanism to control cell size

Deterministic vs Stochastic sizer

- Cells grow to a *fixed* target size
- Cells add a *fixed* added size

- Rate of G1/S transition is the *probability* of transition

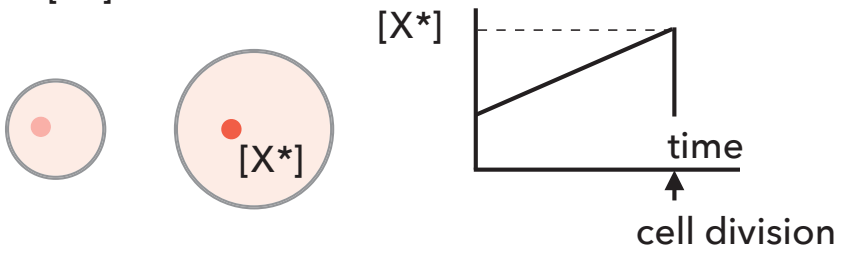


E. Zatulovskiy and J.M. Skotheim. *Trends in Genetics*, 36: 360 -372 (2020)

Is cell size sensed and how?

Parameters that can be used as a proxy for cell size:

- **Cell volume (or cell mass or protein content):**
  – amount of protein X (whose concentration is fixed because protein synthesis scales with volume).
  [X] could be measured at a *subcellular location, by titration mechanism* to a fixed-number of target sites (eg. in the genome)
  Threshold value [X*] defines cell size

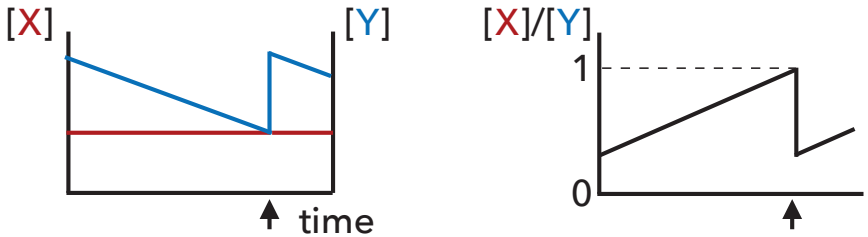


ex: Cln3 in *S. cerevisiae*

Wang, H., Carey, L. B., Cai, Y., Wijnen, H. & Futcher, B. Recruitment of Cln3 cyclin to promoters controls cell cycle entry via histone deacetylase and other targets. *PLoS Biol.* 7, e1000189 (2009).

Ratio of [X]/[Y] where Y is produced in fixed amount early in cell cycle and is diluted as volume increases.

ex: Whi5 and Cln3 in *S. cerevisiae*
RB in Mammals

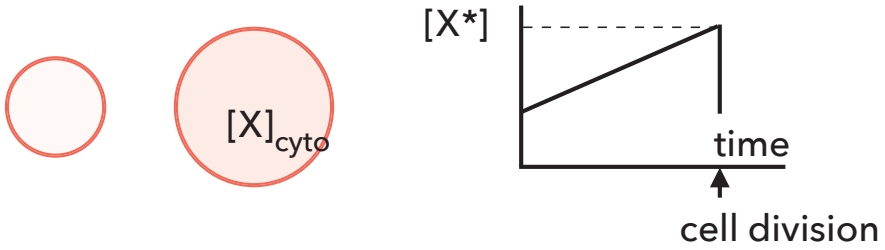


Is cell size sensed and how?

Parameters that can be used as a proxy for cell size

- **Surface to volume ratio:** S/V scales as 1/R so it decreases with cell size.

– An inducer of cell division synthesized in cytoplasm and targeted at the membrane would be predominantly at the membrane when the cell is small but increase in the cytoplasm beyond a certain cell size.



- Cell length:

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

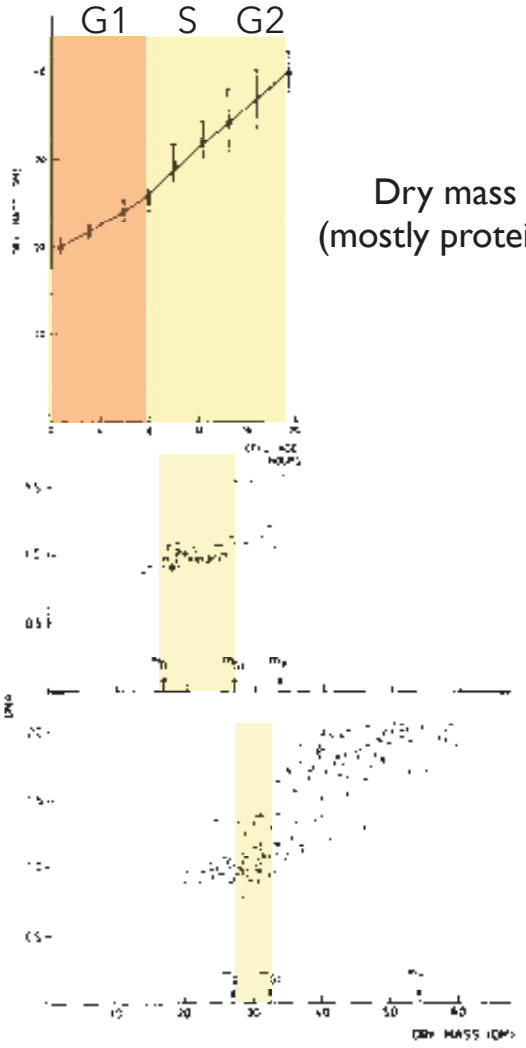
ex: MinCD in Bacteria
Pom1 in *S. pombe*

Plan

1. Evidence of size sensing mechanism
2. Cell sizer: control of G1 length
3. Cell sizer: control of cell division process
4. Cell adder mechanism

1. Evidence of size-sensing mechanism in animal cells

- Cell-size threshold gating G1 exit?
- A critical cell mass is required to enter S phase



Dry mass
(mostly proteins)

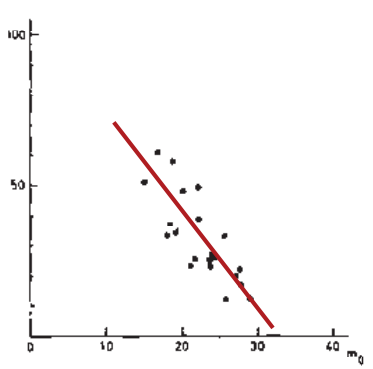
SUMMARY

The mass of cells entering interphase after cell division, initial mass (m_b), and the cell mass at the stage of initiation of DNA synthesis (m_{i1}) were determined in 21 populations of mouse fibroblasts *in vitro* (L-cells) which exhibited different m_i values. Cells in populations with low initial masses had to synthesize more mass before the onset of DNA synthesis than cells in populations with high initial masses. In addition, these smaller cells required more time for this mass synthesis than the bigger cells. The generation times were found to be almost identical for the different L cell populations. Cells in populations with low initial masses therefore spent a relatively long time in the G1 and a relatively short time in the S + G2 phases of the cell cycle. The opposite applied when the cell populations had high initial masses.

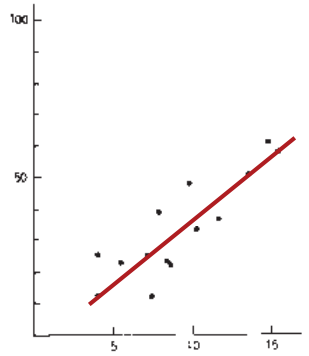
These findings support the hypothesis that initiation of DNA synthesis is dependent on the cell mass.

%mass increase
in G1 until onset of S phase

$$\left(\frac{m_{G1} - m_b}{m_b} \times 100\right)$$



mass at birth



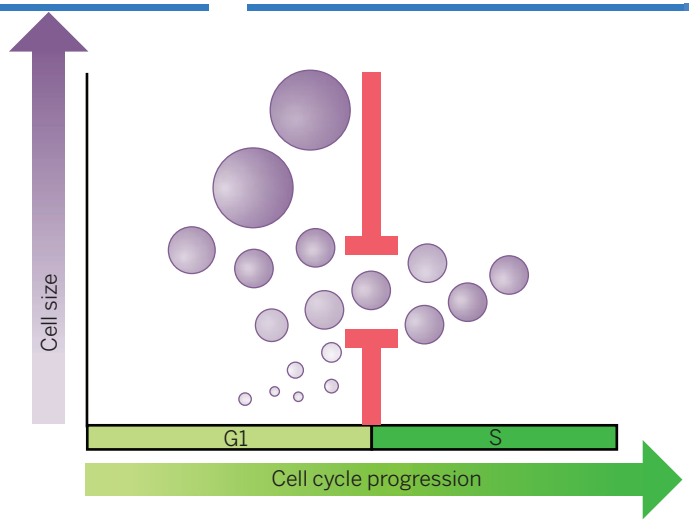
Length of G1 (hours)

D. Killander and A. Zetterberg. *Experimental Cell Research* 38, 272–284 (1965). doi: 10.1016/0014-4827(65)90403-9

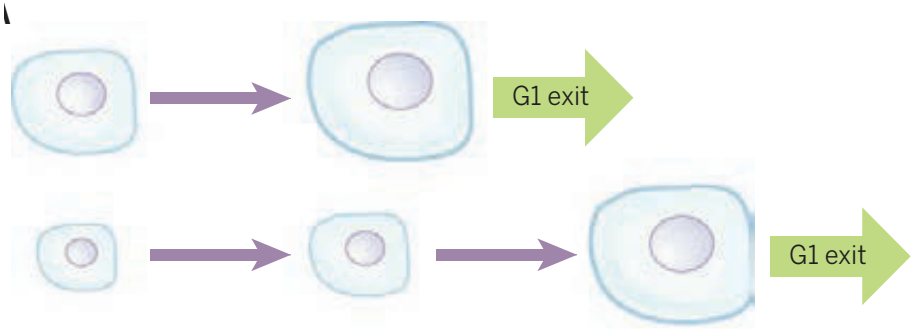
D. Killander and A. Zetterberg. *Experimental Cell Research* 40, 12-21) (1965)

1. Evidence of size-sensing mechanism in animal cells

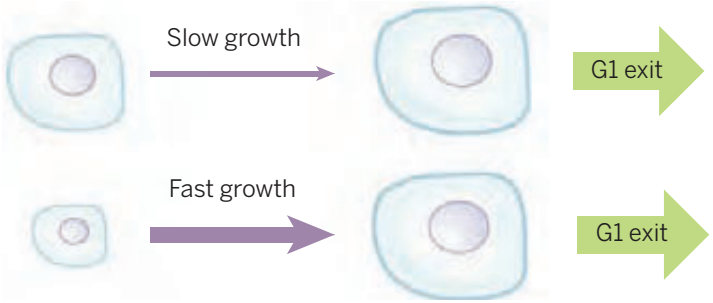
- Cell-size threshold gating G1 exit



- Duration of G1 phase



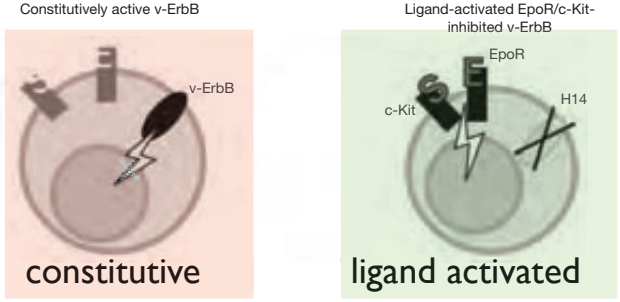
- Growth rate in G1 phase

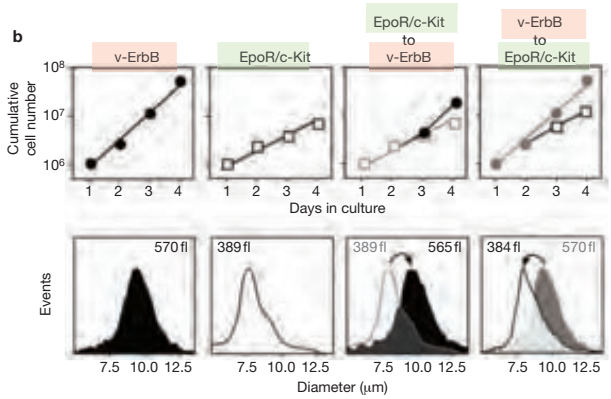


Miriam B. Ginzberg et al. and M Kirschner. *Science* 348, (2015);
DOI: 10.1126/science.1245075

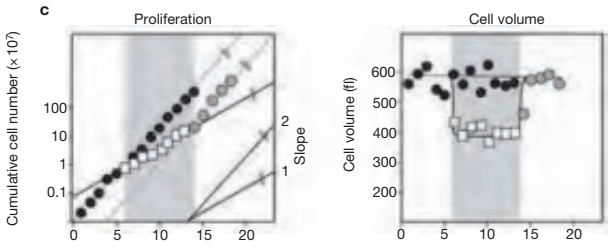
1. Evidence of size-sensing mechanism in animal cells

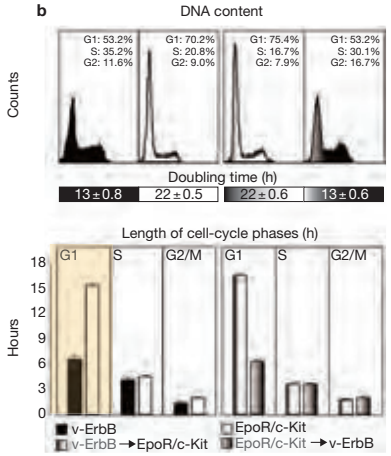
- Two modes of cell growth in haematopoietic cell lines differ in growth rate
- Cells can reversibly switch between modes
- Cells differ in rate of protein synthesis associated with cell growth

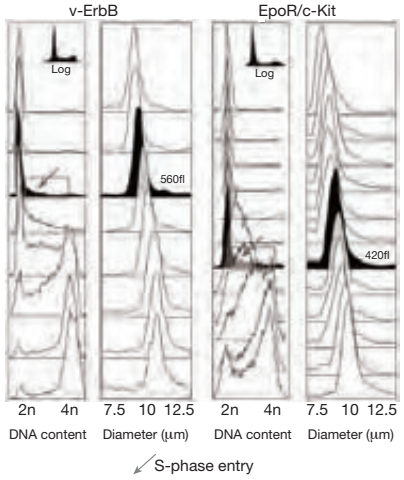




- Cells will reduced growth rate stayed longer in G1 compared to faster growing cells
- Duration in S and G2/M phases had similar length
- Cells that switched growth condition adjusted G1 phase length
- As a result cells had different mean size in S phase





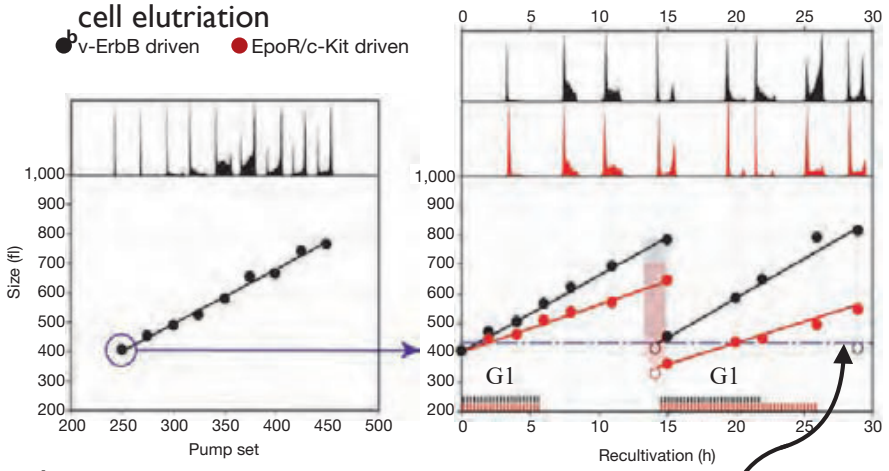


H. Dolznig, F. Grebien, T. Sauer, H. Beug, E. W. Müllner,
Nat. Cell Biol. 6, 899–905 (2004).

1. Evidence of size-sensing mechanism in animal cells

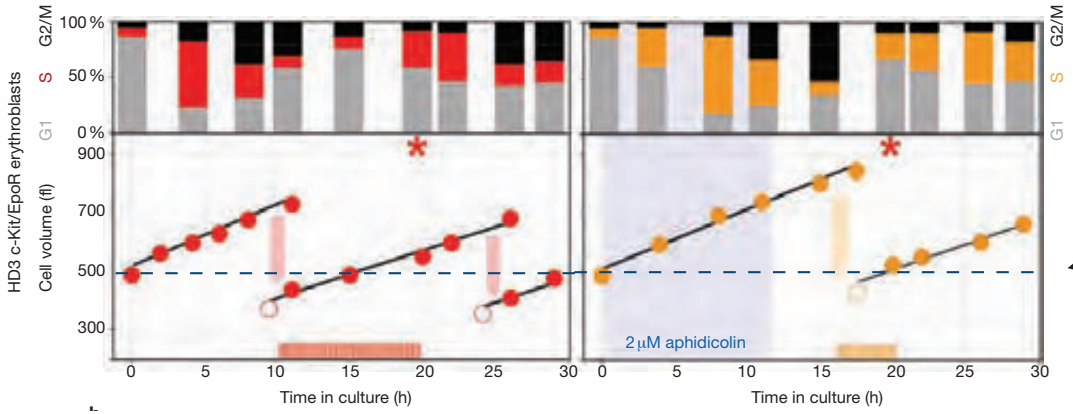
- **A 'size information state' is passed on through M phase to daughter cells,** and determines G1 duration of the following cycle

- v-ErbB driven cells in G1 switched to a slower growth mode immediately reduce their growth rate but keep a short G1 characteristic of v-ErbB cells because cells already have a size sufficient to enter S phase
- However the cells are smaller when they divide and newborn cells are smaller
- The next G1 phase is longer so cells reach a hypothetical thresold value of size.



- Prolonged S phase (with low dose aphidicolin) prolongs growth in slow growing cells resulting in larger cells
  In the next cycle, newborn cells are larger and cells have a shorter G1

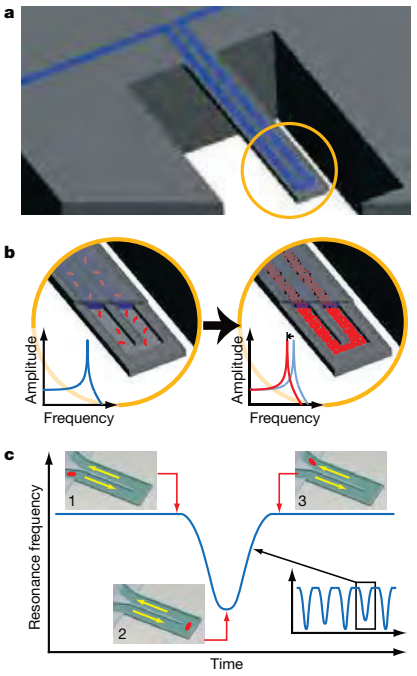
Hypothetical size threshold



H. Dolznig, F. Grebien, T. Sauer, H. Beug, E. W. Müllner, *Nat. Cell Biol.* 6, 899–905 (2004).

Measuring cell mass and cell volume

Suspended microchannel resonator (SMR)



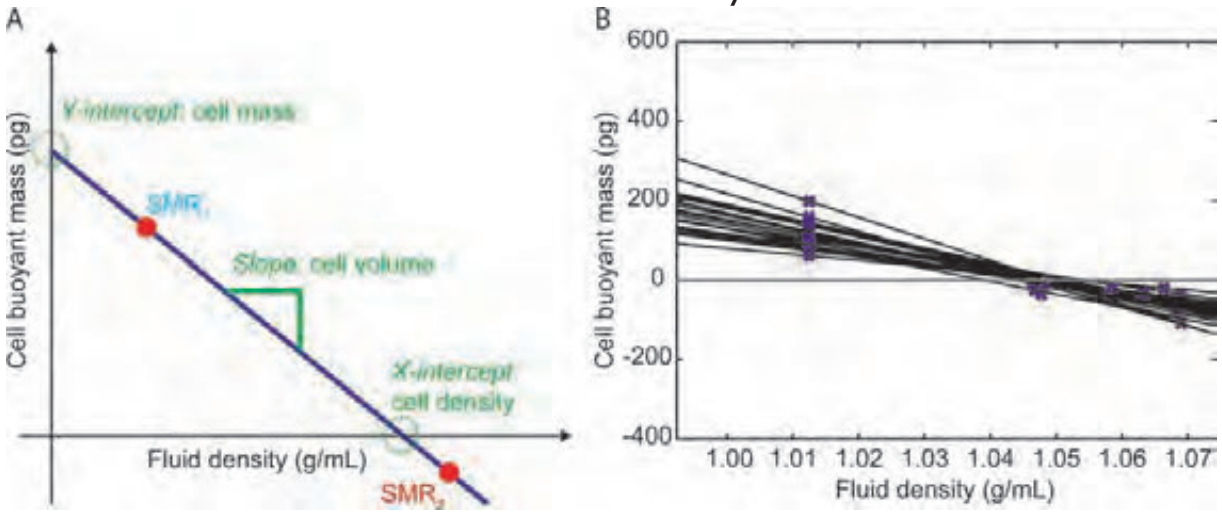
- Cantilever resonates at a frequency dependent on mass of cantilever
- When a cell enters the channel embedded in cantilever, the frequency changes as a function of buoyant mass of cell.

$$m_{\mathrm{B},1} = V_{\mathrm{cell}} \times \left(\rho_{\mathrm{cell}} - \rho_{\mathrm{Fluid},1}\right)$$

T. P. Burg et al., S. Manalis *Nature* 446, 1066–1069 (2007)

- Using two different fluid densities one can extract cell volume cell density and cell mass

Precision of 0.01% !!

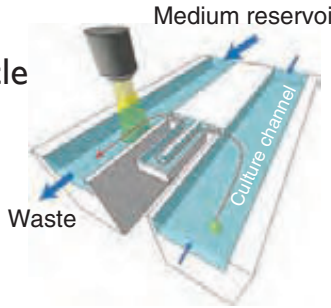


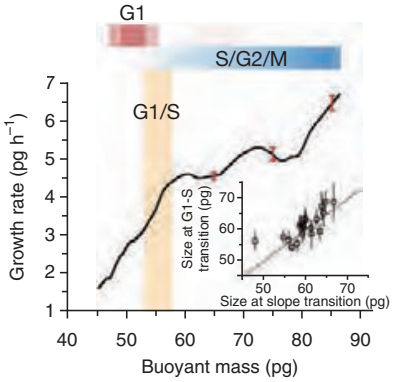
A. Bryan et al. S. Manalis *LabChip (*2014) ,14,569–576

1. Evidence of size-sensing mechanism in animal cells

- **G1/S transition is not gated by cell size but by cell growth rate**

A system to track individual growth rate and mass through the cell cycle

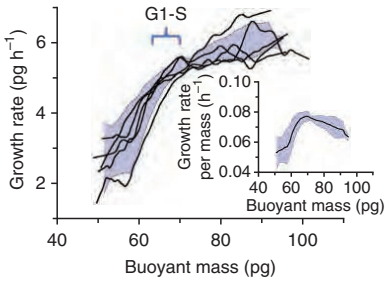


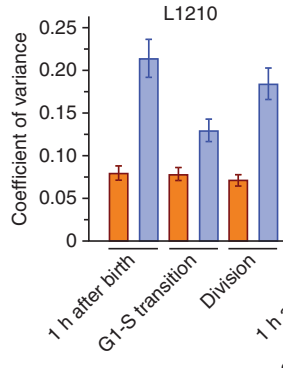


- Individual growth rate is neither constant (linear growth) nor simply proportional to mass (exponential).

- Growth rate is globally biphasic: growth rate is reduced at G1/S transition

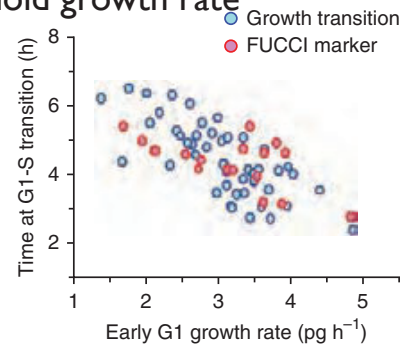
- Variance of growth rate is minimal at G1/S transition
- All cells entered S phase with similar growth rate, suggesting a threshold growth rate.

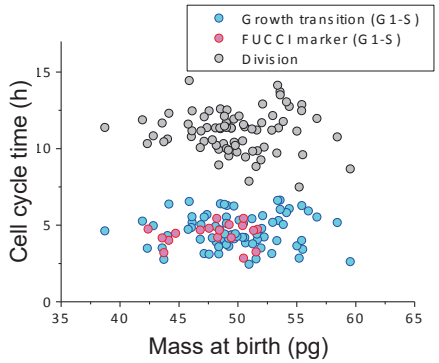




- **Consistent with a growth rate threshold:**

— the time until G1/S transition negatively correlates with growth rate at G1 entry: cells need more time to reach threshold growth rate





- In contrast, time until G1/S of division does not correlate with mass at birth and cell size.

S. Son et al., M. Kirschner and S. Manalis. *Nat. Methods* 9, 910–912 (2012).

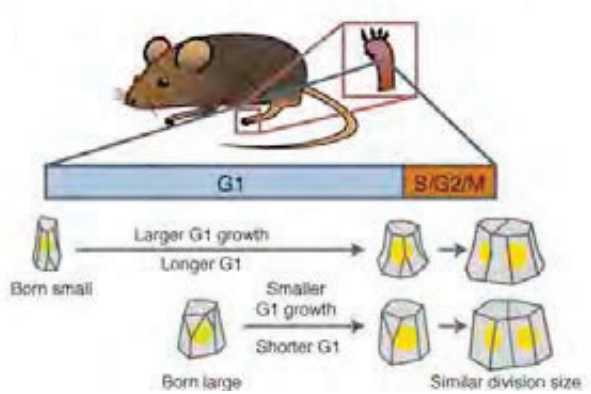
Plan

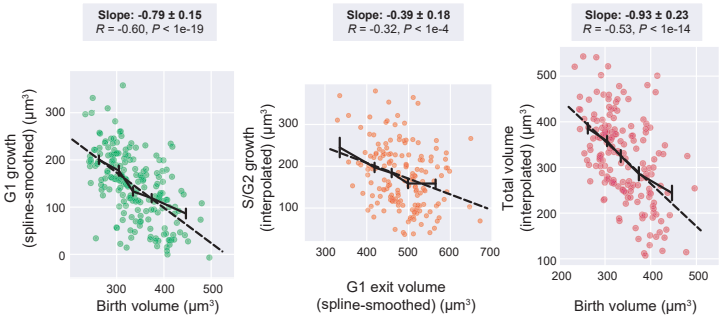
1. Evidence of size sensing mechanism
2. Cell sizer: control of G1 length
3. Cell sizer: control of cell division process
4. Cell adder mechanism

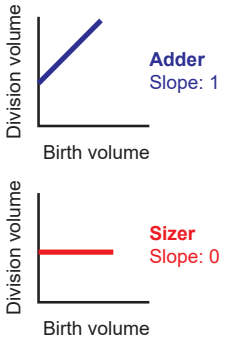
2. Cell sizer: control of G1 length

- Size dependent regulation of G1 length *in vivo:* Sizer mechanism

- Quantification of single-cell growth from longitudinal imaging of mouse epidermal stem cells over 1 week.
- Epidermal stem cells grow faster than linearly *in vivo*
- Cells couple cell growth and cell-cycle progression consistent with the existence a G1 sizer

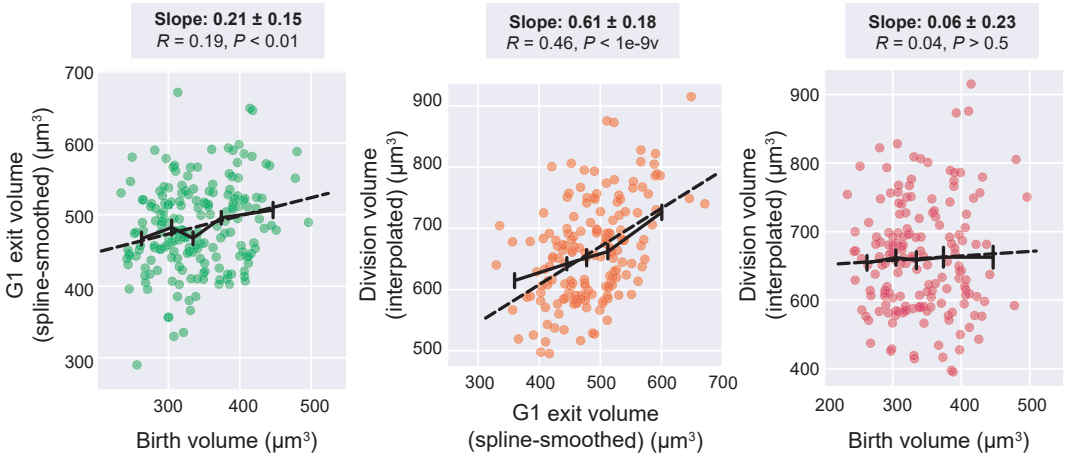






near sizer

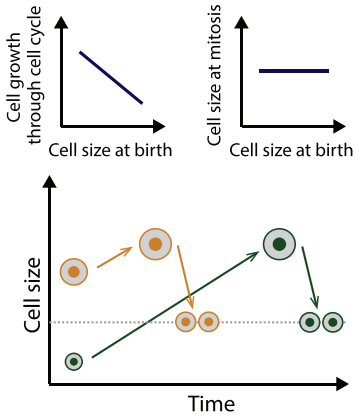
near perfect sizer

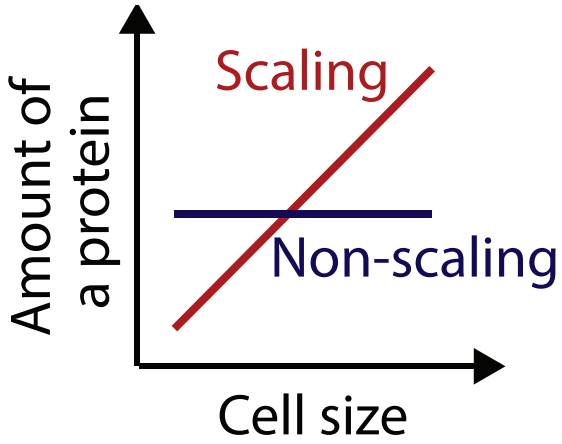


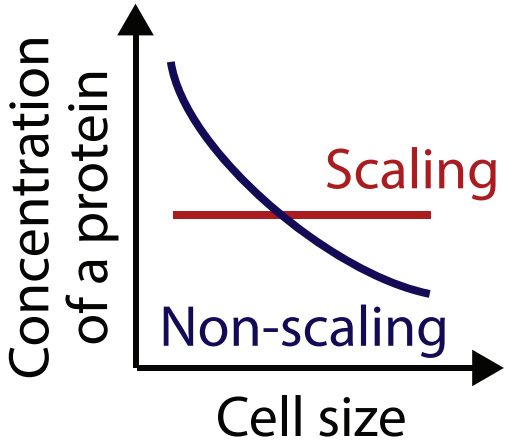
Xie & J.M. Skotheim, 2020, *Current Biology* 30, 916–924

2. Cell sizer: control of G1 length

- Size dependent regulation of G1 length
- Mechanism 1: dilution of mitotic inhibitor







E. Zatulovskiy and J.M. Skotheim. *Trends in Genetics*, 36: 360 -372 (2020)

2. Cell sizer: control of G1 length

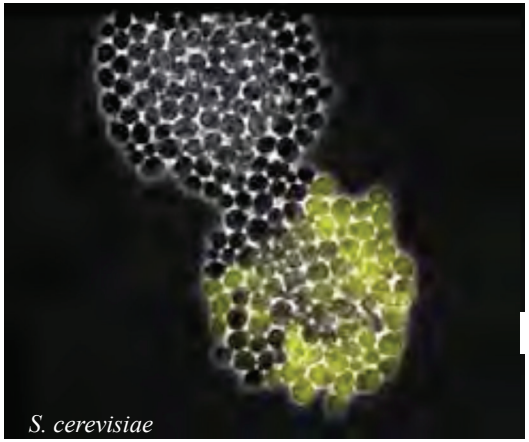
When Wee Meets Whi

A Whi dram. The isolation of the *S. pombe wee* mutants by Paul Nurse and Peter Fantes spurred Bruce Carter and me to screen for small-size mutants in *S. cerevisiae*. I bet Bruce a bottle of the best Irish Whiskey that we would not be able to isolate such mutants. Happily, I lost the bet, and we identified *S. cerevisiae* small-size mutants, which we called *Whi* in honor of the bet. Bruce can be seen here enjoying the spoils of victory, a bottle of Black Bushmills.

Peter Sudbery

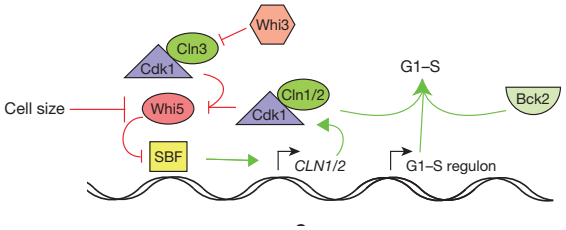
- Size dependent regulation of G1 length
- Mechanism 1: dilution of mitotic inhibitor

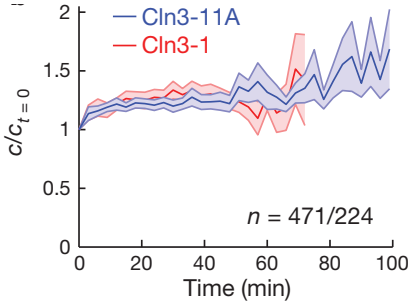
—Budding Yeast: Differential scaling of cell cycle regulators with cell size

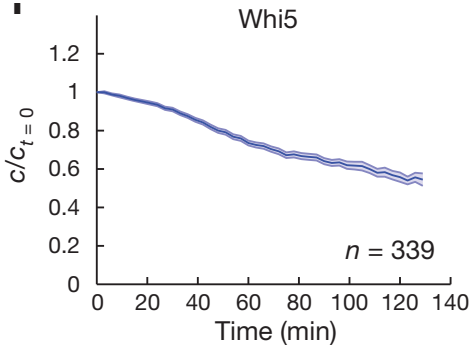


www.waterfordwhisky.com/element/yeast

- Whi5 inhibits G1 progression and cell division
- Cln3 (and CdK1) block Whi5 and promote G1-S and cell cycle

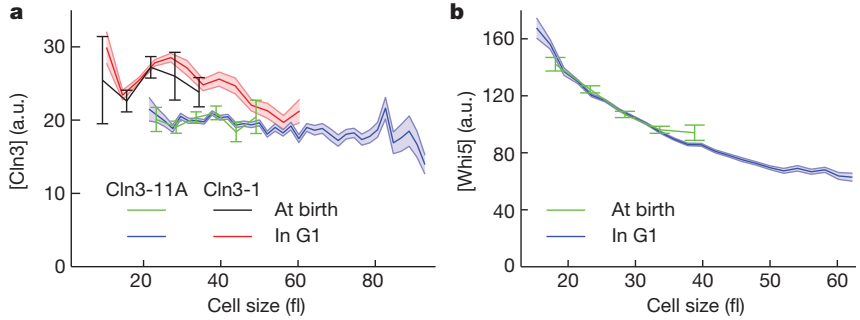






The cell cycle inhibitor Whi5 is diluted by growth in G1.

- How could smaller cells grow more to attain target size?
- This would require that smaller cells have a higher concentration of Whi5, as observed.
- Differential size dependence of Cln3 and Whi5.



Schmoller KM, Turner JJ, Kõivomägi M, Skotheim JM. *Nature*. 2015 Oct 8;526(7572):268-72. doi: 10.1038/nature14908.

2. Cell sizer: control of G1 length

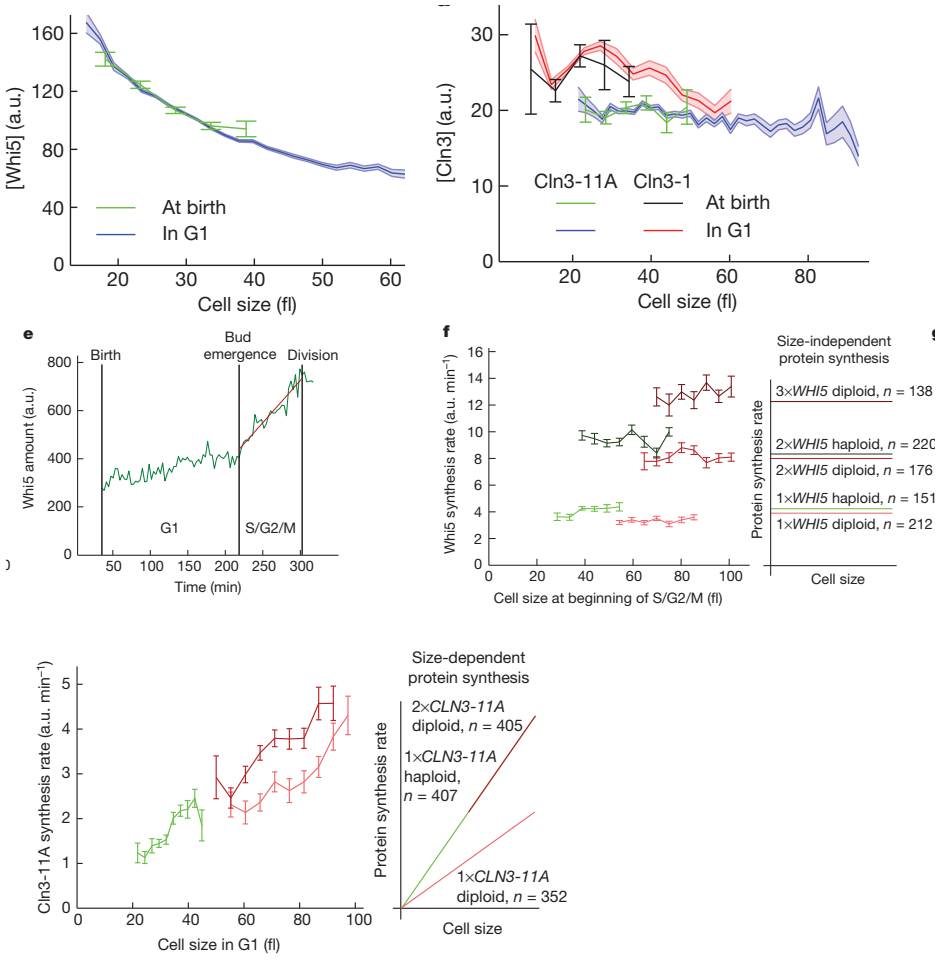
- Size dependent regulation of G1 length
- Mechanism 1: dilution of mitotic inhibitor

—Budding Yeast: Differential scaling of cell cycle regulators with cell size

- Differential size dependence of Cln3 and Whi5:

Smaller cells have a higher concentration of Whi5

- Whi5 is a stable protein.
- Whi5 is produced in S/G2 in a size independent manner and large and small cells produce a similar amount of Whi5. Since bigger mother cells produce bigger daughters, larger newborn cells have a lower concentration of Whi5. This is essential for the inhibitor dilution size-dependent model.
- In contrast, the rate of Cln3 synthesis is proportional to cell size consistent with the fact that RNA and Protein synthesis rate scale with cell size for most proteins. Thus, Cln3 has a constant concentration in G1 like most proteins (see Lin & Amir. *Nature Com* | (2018) 9:4496))
- Whi5 synthesis rate is determined by copy number but not by ploidy (nor cell size), in contrast to Cln3



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2. Cell sizer: control of G1 length

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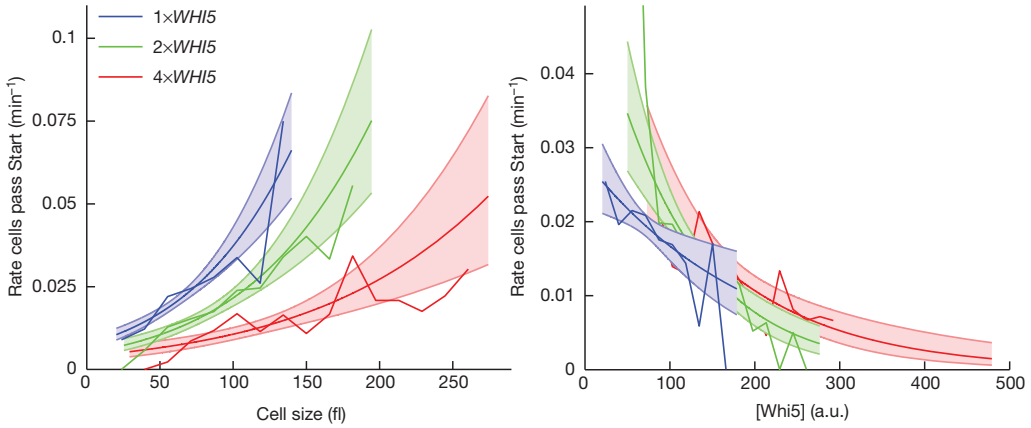
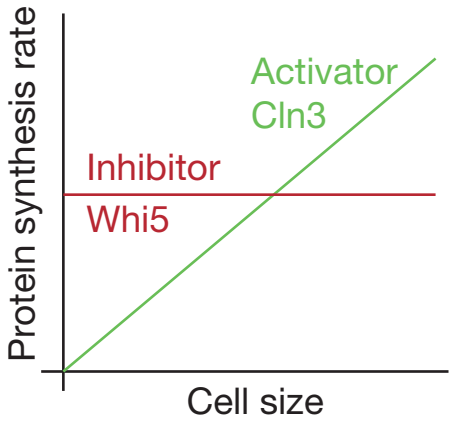
—Budding Yeast: Differential scaling of cell cycle regulators with cell size

According to the inhibitor-dilution model, the rate at which cells pass Start is determined by the concentrations of Whi5 and Cln3. If Cln3 concentration is constant in pre-Start cells, the Whi5 concentration alone should predict the rate at which cells progress through Start.

- Whi5 concentration determines the rate at which cells progress through Start
- Increasing the concentration of Whi5 reduces the rate at which cells go to Start





Schmoller KM, Turner JJ, Kõivomägi M, Skotheim JM. *Nature*. 2015 Oct 8;526(7572):268-72. doi: 10.1038/nature14908.

2. Cell sizer: control of G1 length

- Size dependent regulation of G1 length
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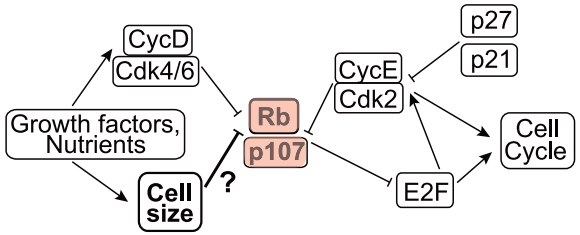
—Mammalian cells RB: Differential scaling of cell cycle regulators with cell size

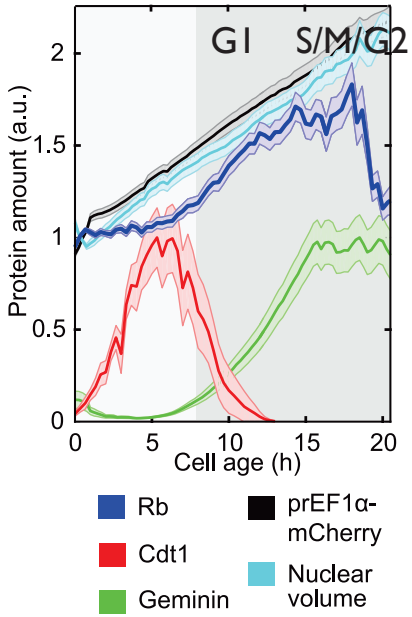
Retinoblastoma proteins Rb and p107 are the functional orthologs of Whi5

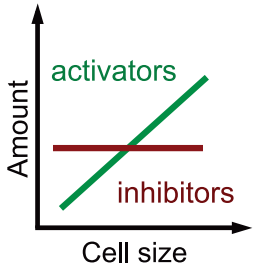
Deletion of Rb, like Whi5 in budding Yeast reduces cell size

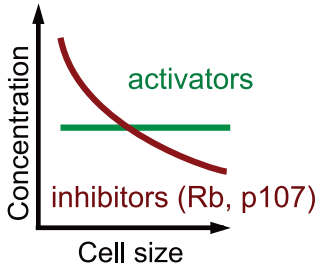
Rb is constant in G1 while nuclear volume increases

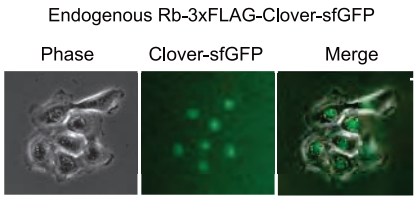
Other proteins increase through the cell cycle











E. Zatulovskiy et al., and J.M. Skotheim *Science* 369, 466–471 (2020)

2. Cell sizer: control of G1 length

- Size dependent regulation of G1 length
- Mechanism 1: dilution of mitotic inhibitor

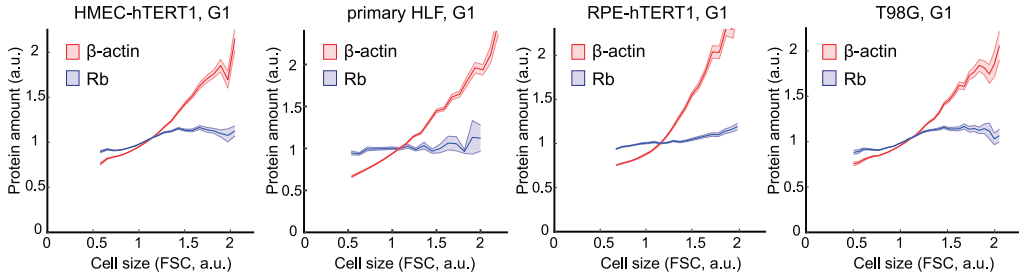
—Mammalian cells RB: Differential scaling of cell cycle regulators with cell size

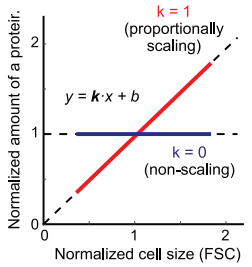
The amount of Rb is independent of cell size, contrary to other proteins

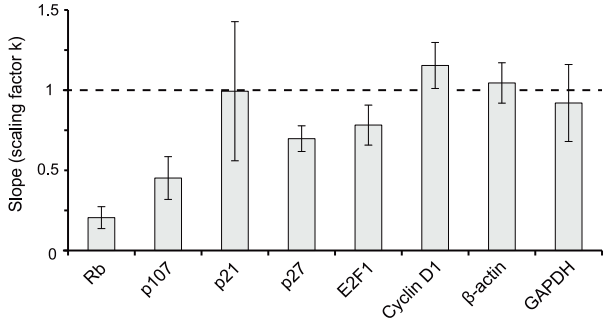
The absence of scaling is consistent with an Rb dilution model and titration of E2F activation

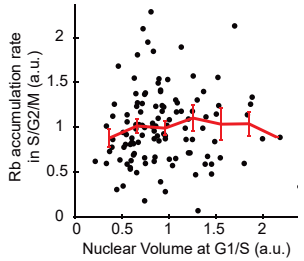
Rb is very stable (29h vs 16h for cell cycle) and its amount is determined by synthesis in mother cell

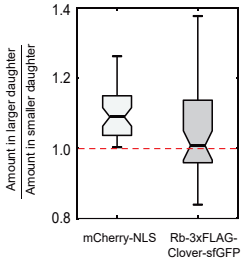
Rb is produced at the same rate in S-G2 independent of cell size and partitioned equally in daughter cells (more than control proteins, may be due to association with chromatin?)

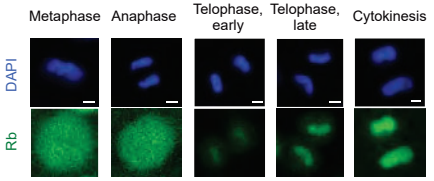












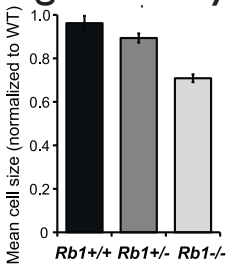
E. Zatulovskiy et al., and J.M.. Skotheim *Science* 369, 466–471 (2020)

2. Cell sizer: control of G1 length

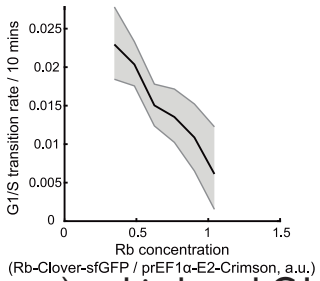
- Size dependent regulation of G1 length
- Mechanism 1: dilution of mitotic inhibitor

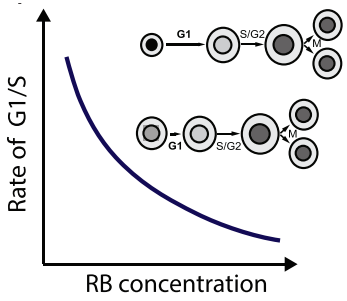
—Mammalian cells RB: Differential scaling of cell cycle regulators with cell size

A key prediction of the Rb dilution model is that Rb dosage should affect cell size in vivo

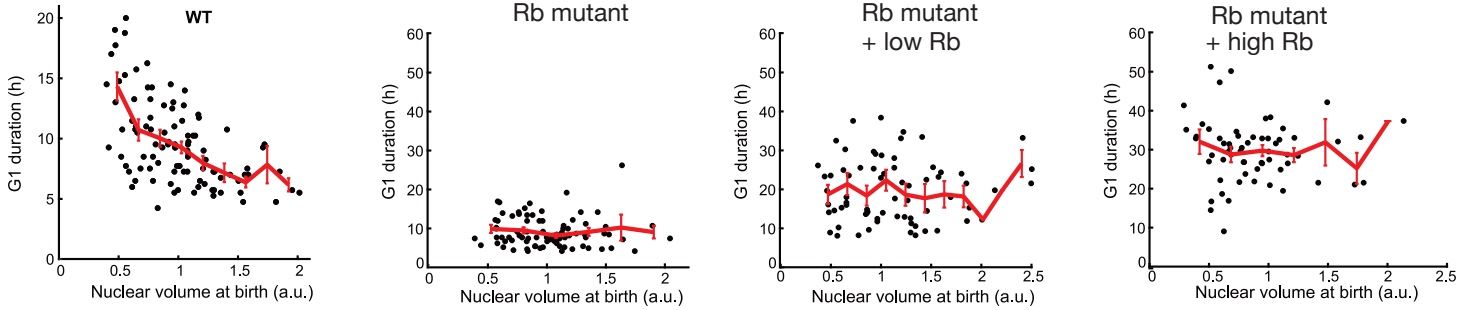


Reducing by half the dose of Rb in cell culture triples the rate of G1/S transition consistent with Rb dilution model





Inverse correlation between cell size (nuclear volume) at birth and G1 duration is another prediction of the model. Such correlation is lost when Rb is mutant. And G1 duration depends on the concentration of Rb. Hence, it is the dilution of Rb that matters, not its simple presence.



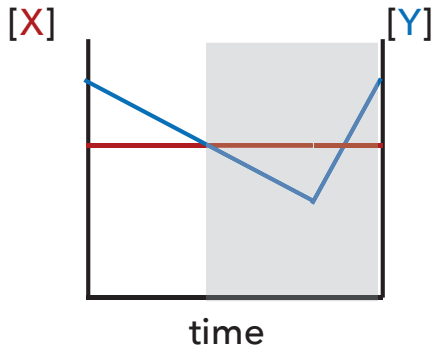
E. Zatulovskiy et al., and J.M.. Skotheim *Science* 369, 466–471 (2020)

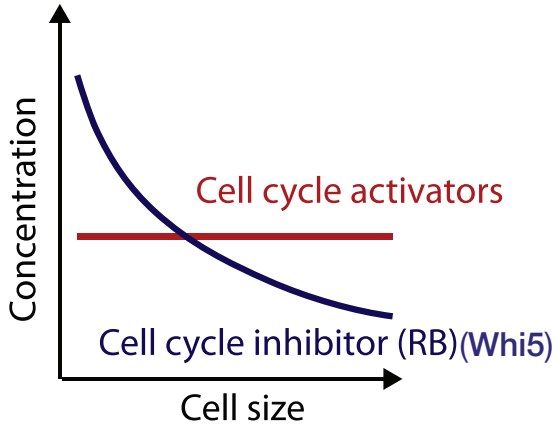
Deep conservation of Cell Sizer mechanism

- Size dependent regulation of G1 length by dilution of cell cycle inhibitor

Ratio of [X]/[Y] where Y is produced in fixed amount early in cell cycle and is diluted as volume increases.

ex: Whi5 and Cln3 in *S. cerevisiae*
Rb in Mammals



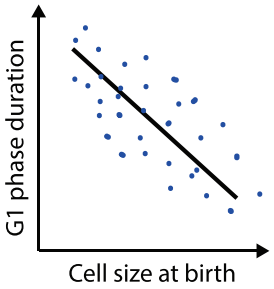


E. Zatulovskiy et al., and J.M.. Skotheim *Science* 369, 466–471 (2020)

Schmoller KM, Turner JJ, Kõivomägi M, Skotheim JM. *Nature*. 526:268-72 (2015)

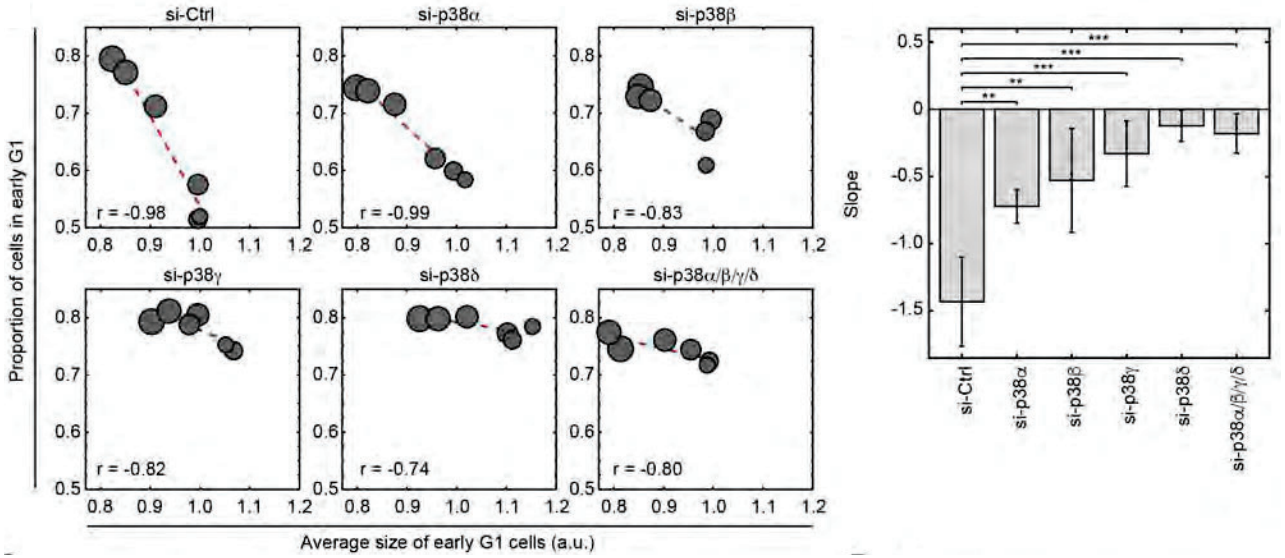
2. Cell sizer: control of G1 length

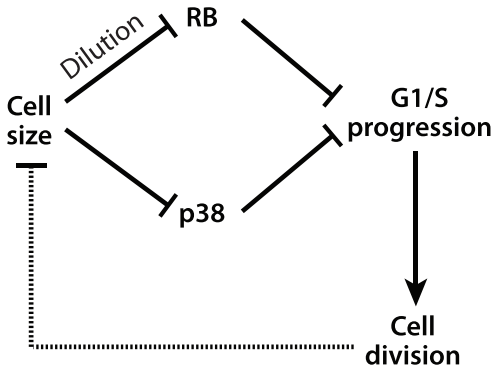
- Size dependent regulation of G1 length
- Mechanism 2: p38 kinase



The proportion of cells in early G1 reflects the duration of G1

when p38 is knocked down small cells and larger cells have similar G1 length, such that cell size is no longer coupled with cell division properly





Liu et al. and M. Kirschner and R. Kafri. *eLife* 2018;7:e26947. DOI: https://doi.org/10.7554/eLife.26947

Plan

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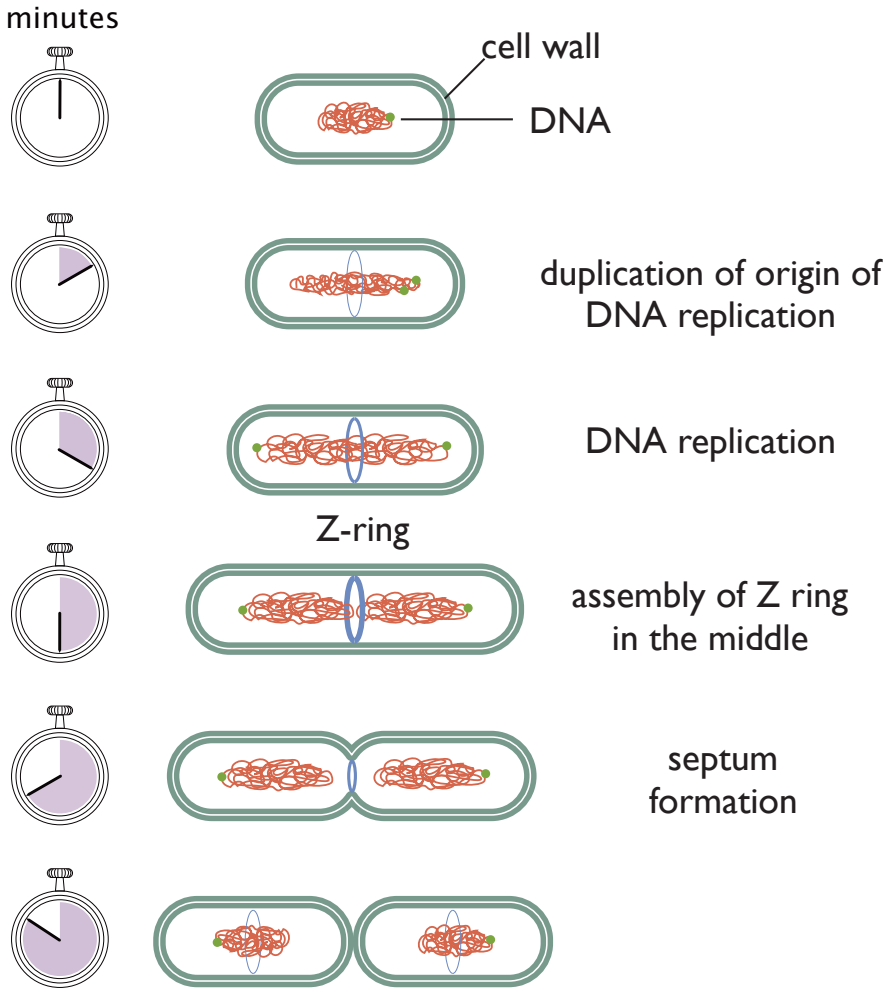
3. Cell sizer: control of cell division process

- Spatial and temporal control of cell division in Bacteria

- Bacteria have a cell wall and divide by binary fission
- The cell cycle initiates with the duplication of the origin and DNA replication then proceeds in an orderly fashion around the circular chromosome.
- At the same time, a group of cell division proteins beginning with the tubulin analog FtsZ form a ring (the Z ring) at the center of the cell that will dictate the future site of septum formation.
- Proper cell size requires that cell division happens:
  - — at the cell center
  - — at the right time



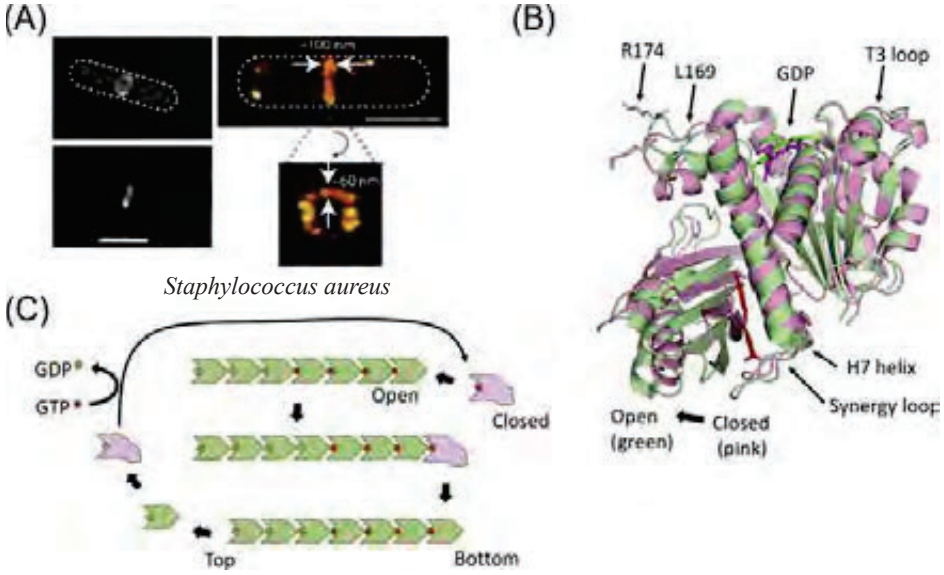
Rob Philipps, Jane Kondev, Julie Theriot, Hernan G. Garcia.
illustration: Nigel Orme
Physical Biological of the Cell (Garland Science)

3. Cell sizer: control of cell division process

- The *cell division machinery* in Bacteria

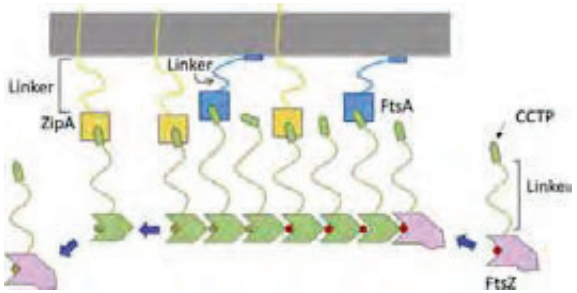
  - The Z ring is a universal structure involved in cell division by septation in all Bacteria
  - The Z ring consists of patches of FtsZ filaments tethered to the membrane (via FtsA in most Bacteria and also via ZipA in *E. coli* and other Gammaproteobacteria)
  - FtsZ is a tubulin homolog which binds GTP and has GTPase activity
  - FtsZ polymerises in dynamic filaments. GTPase activity promotes filament treadmilling
  - Dynamic scaffolding: FtsZ filaments treadmilling distributes components of cell wall synthesis machinery (peptidoglycan) during septum assembly

Cooperative Assembly of FtsZ



Staphylococcus aureus

Membrane association of Z-ring

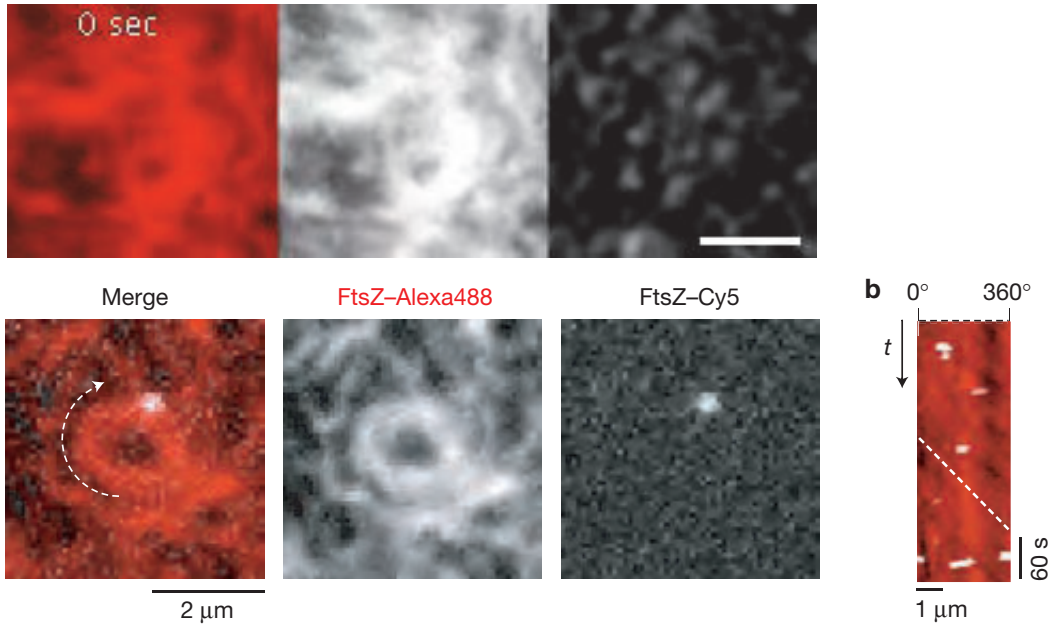


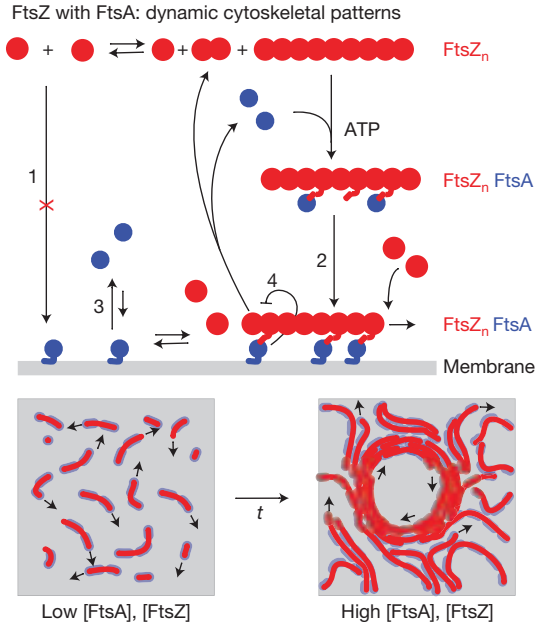
S. Du and J. Lutkenhaus. *Trends in Microbiology* 27: 781-791 (2019)

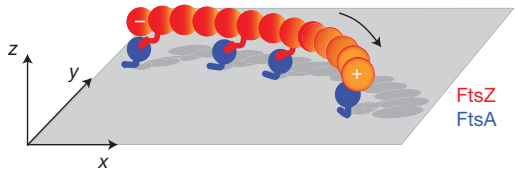
3. Cell sizer: control of cell division process

- The *cell division machinery* in Bacteria

The bacterial cell division proteins FtsA and FtsZ self-organize into dynamic treadmilling cytoskeletal patterns







Loose M, Mitchison TJ. *Nat Cell Biol.* 16(1):38-46. doi: 10.1038/ncb2885 (2014)

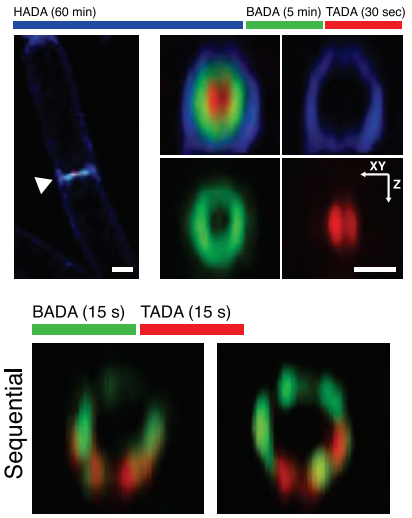
3. Cell sizer: control of cell division process

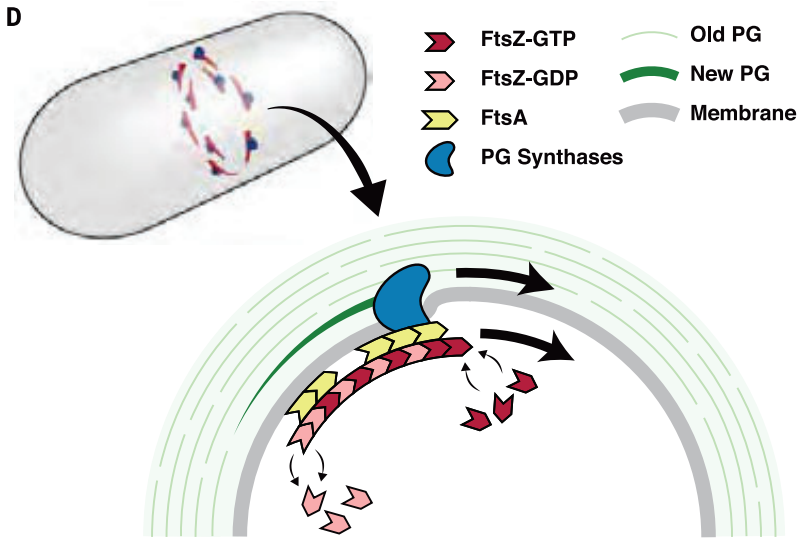
- The *cell division machinery* in Bacteria

Dynamic scaffolding of peptidoglycan (PG) synthesis by FtsZ

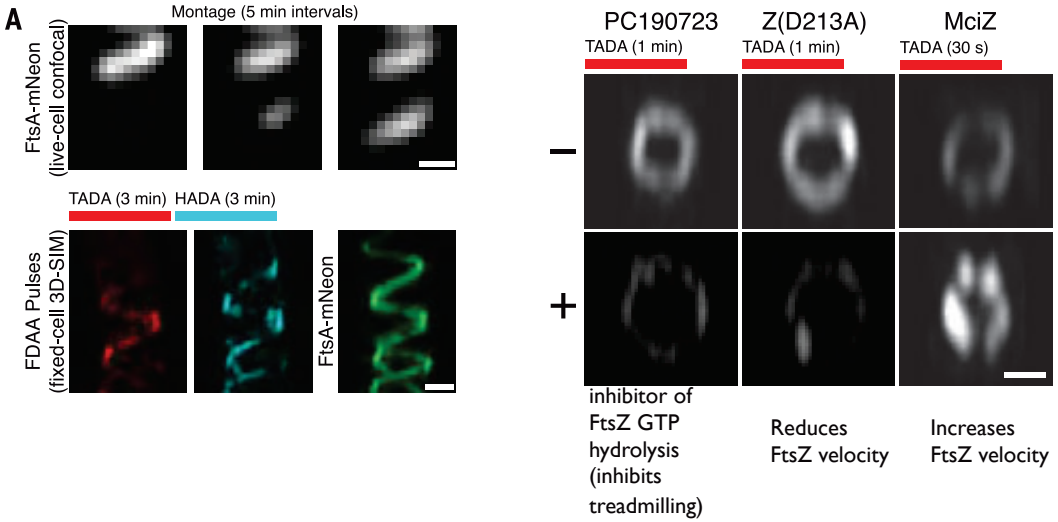
Septal PG synthesis occurs sequentially from an outer ring inward

PG synthesis occurs at discrete, mobile sites





Cytokinesis is controlled by directional motion of FtsAZ filaments



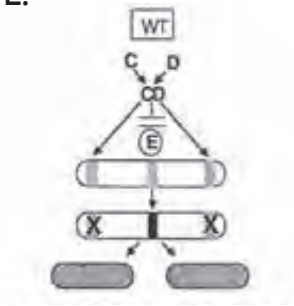
Bisson-Filho et al., *Science* 355, 739–743 (2017)

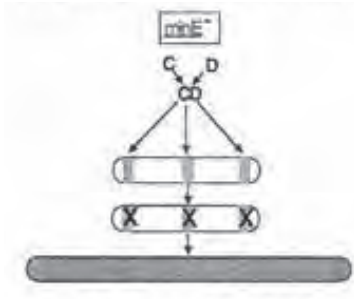
3. Cell sizer: control of cell division process

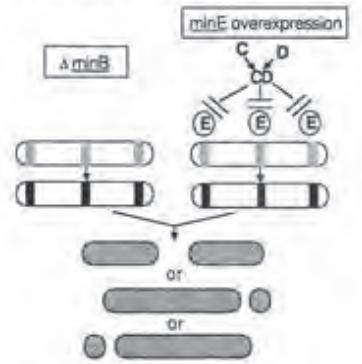
- *Spatial control* of cell division in Bacteria

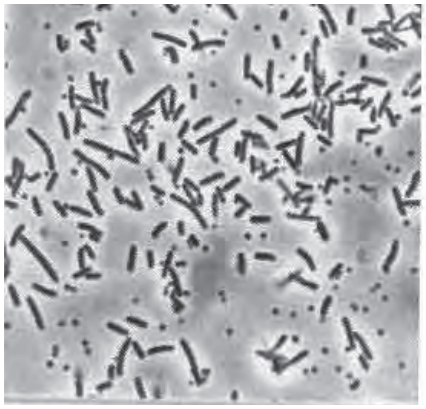
- The minB locus controls cell size in *E* coli by regulation of septation
  In the mutant, septation can happen in the middle or at the tips of cells
- *minB* codes for 3 proteins: MinC, D and E.

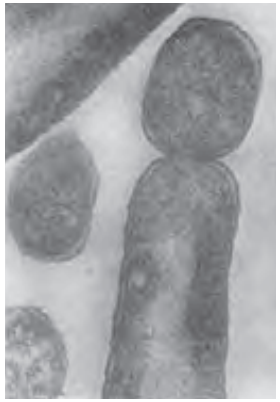
- MinC and MinD repress septation
- MinE promotes septation
  by antagonising MinC and D







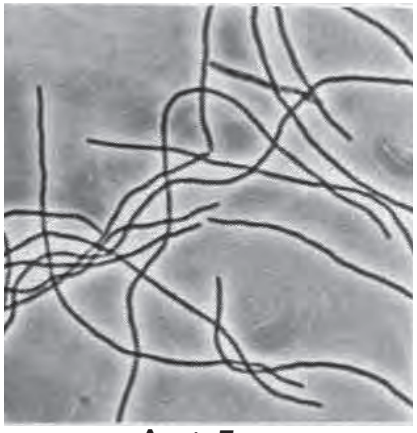




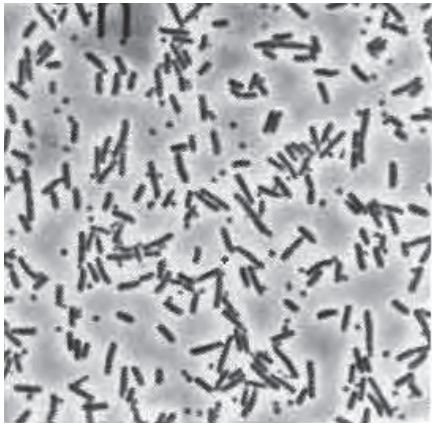
ΔminB locus — mutant produces minicells in *E coli*

(originally from F. Jacob at Institut Pasteur)

H. I. Adler, W. D. Fisher, A. Cohen, and Alice A. Hardigree
PNAS 57 (2) 321-326 (1967)



ΔminE or
minCD overexpression



ΔminB locus or
minE overexpression

De Boer PA, Crossley RE, Rothfield LI *Cell.* 56 (4): 641–649. (1989)

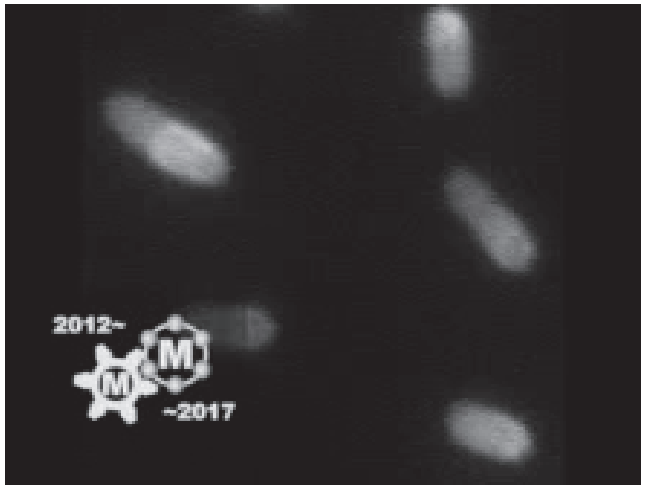
3. Cell sizer: control of cell division process

- *Spatial control* of cell division in Bacteria

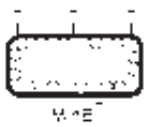
—The MinCDE system defines the cell center through polar oscillations of a Z-ring inhibitor

- Oscillations of MinD
- MinC recruited by MinD
  >mean MinC concentration is lowest in middle

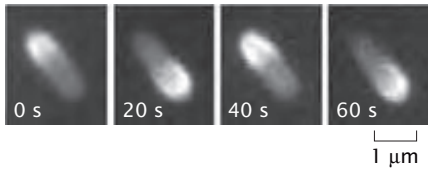
- MinC inhibits FtsZ
- Therefore FtZ assembles in the cell center



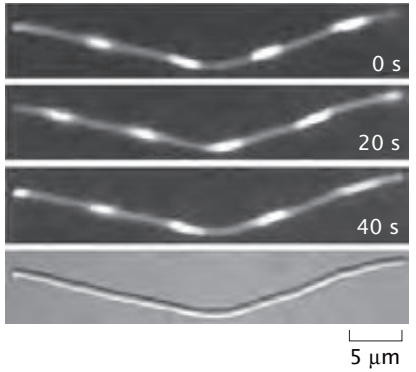
Gfp-MinD localization in minE-

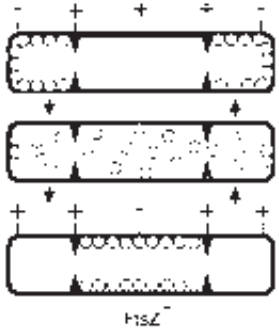


Gfp-MinD localization in WT



Gfp-MinD localization in FtsZ- filaments



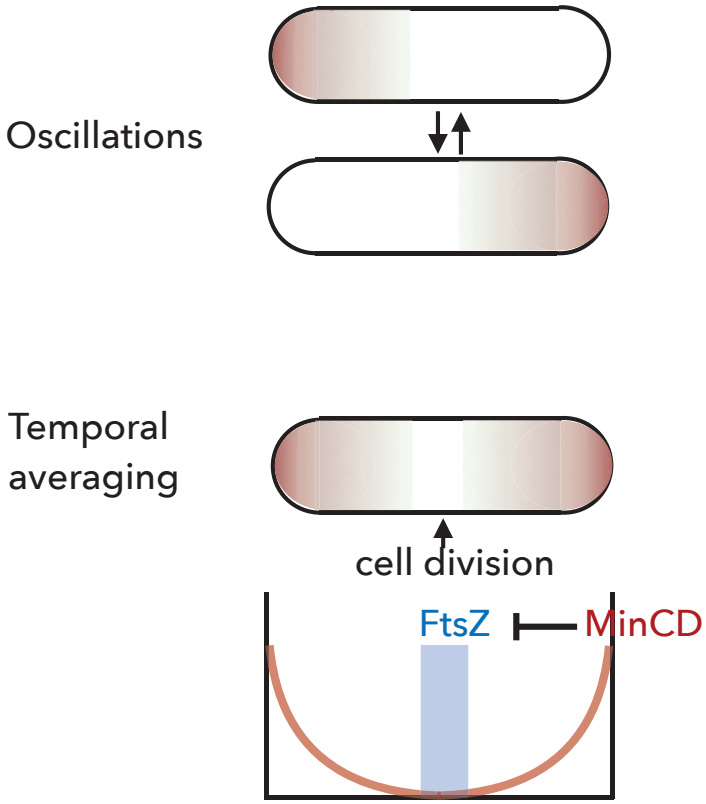


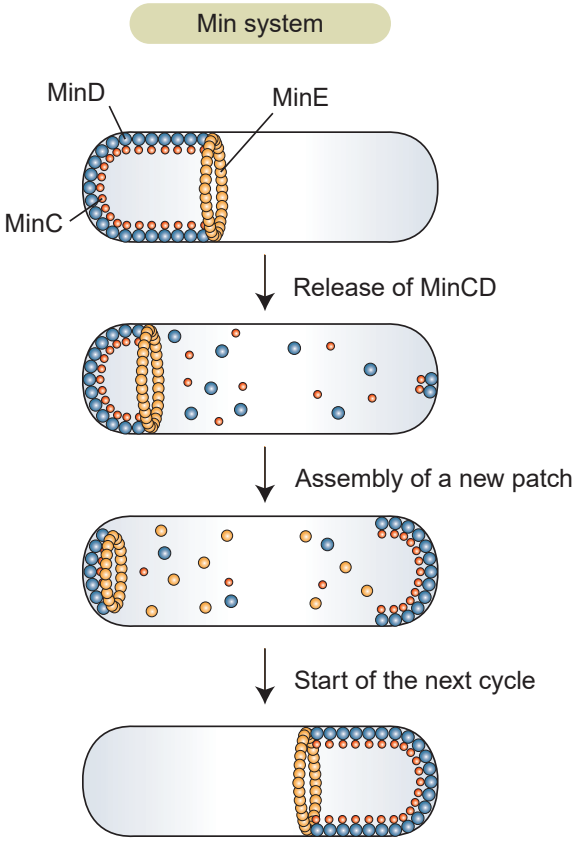
D. Raskin and P. de Boer. *Proc. Natl. Acad. Sci. USA 96:4971–4976 (1999)*

3. Cell sizer: control of cell division process

- *Spatial control* of cell division in Bacteria

—The MinCDE system defines the cell center through polar oscillations of a Z-ring inhibitor



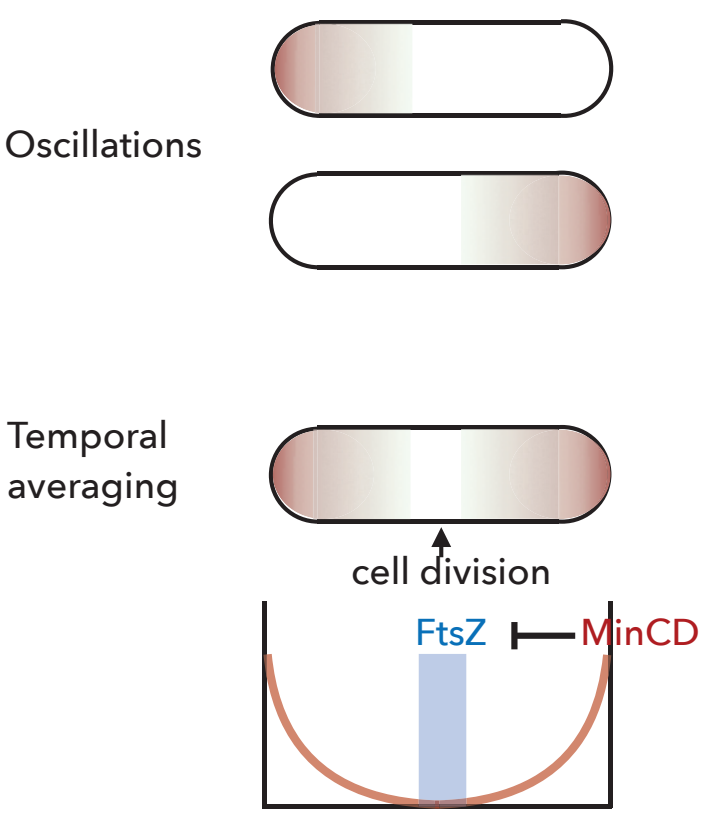


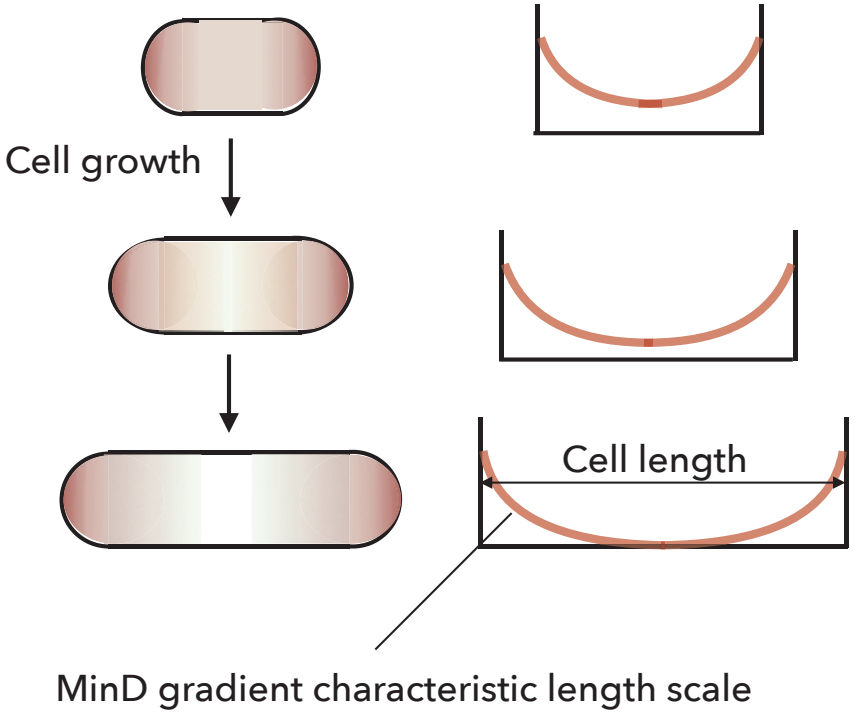
M. Thanbichler *Cold Spring Harb Perspect Biol* 2010;2:a000331 (2010)

3. Cell sizer: control of cell division process

- *Temporal control* of cell division sets cell size in Bacteria

—Min system as a Cell Ruler:
Cell length emerges from length scale of MinCD spatial temporal pattern





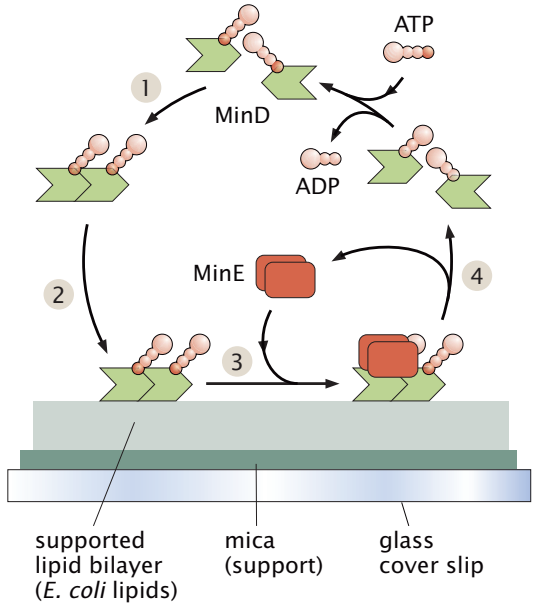
3. Cell sizer: control of cell division process

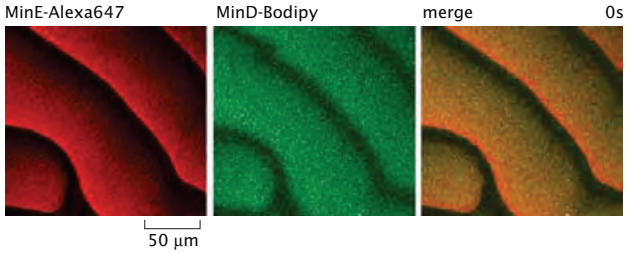
- *Spatial and temporal* control of cell division in Bacteria

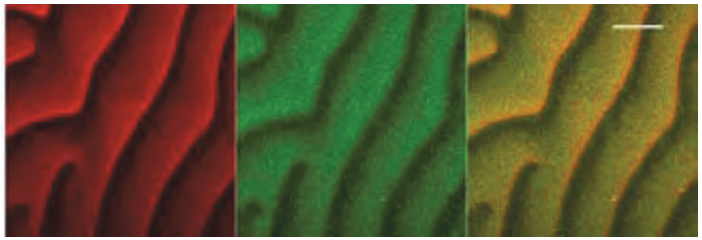
—Cell size is set by characteristic length scale of a reaction/diffusion system

Turing instabilities: travelling waves in 2D

MinD is an autocatalytic activator
MinD recruits its own inhibitor MinE
MinE induces its dissociation via MinD dissociation







see Course 27 Nov 2018: Mechano-chemical instabilities

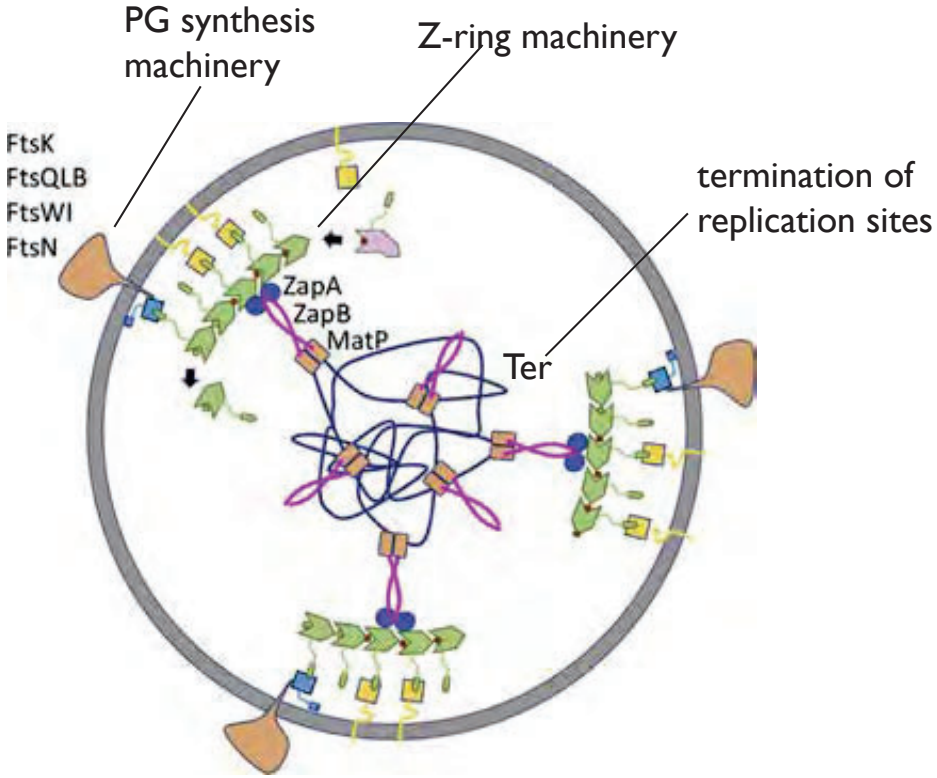
Loose M, Fischer-Friedrich E., Ries J. Kruse Karsten and Schwille Petra. *Science*, 320:789-792 (2008)

Rob Philipps, Jane Kondev, Julie Theriot, Hernan G. Garcia.
illustration: Nigel Orme
Physical Biological of the Cell (Garland Science)

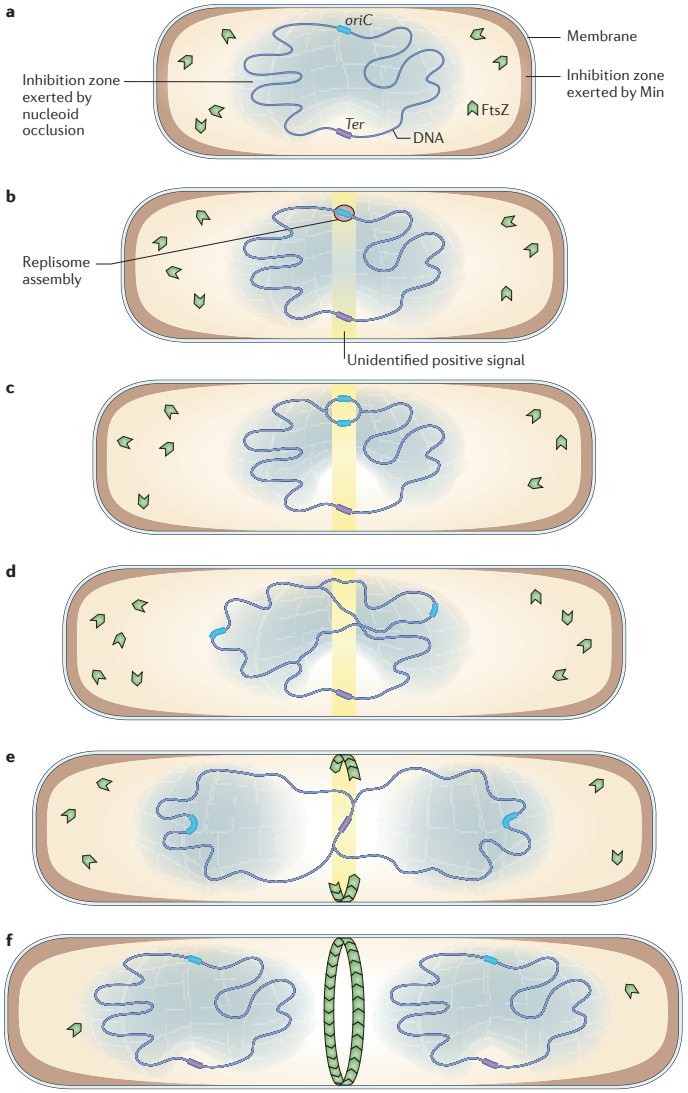
3. Cell sizer: control of cell division process

- *Spatial and temporal* control of cell division in Bacteria

- Temporal and spatial regulation of cell division by nucleoid occlusion and the Min system in rod-shaped bacteria
- Termination of replication sites inhibits FtsZ ring assembly
- The duration of DNA replication is a timer of cell division



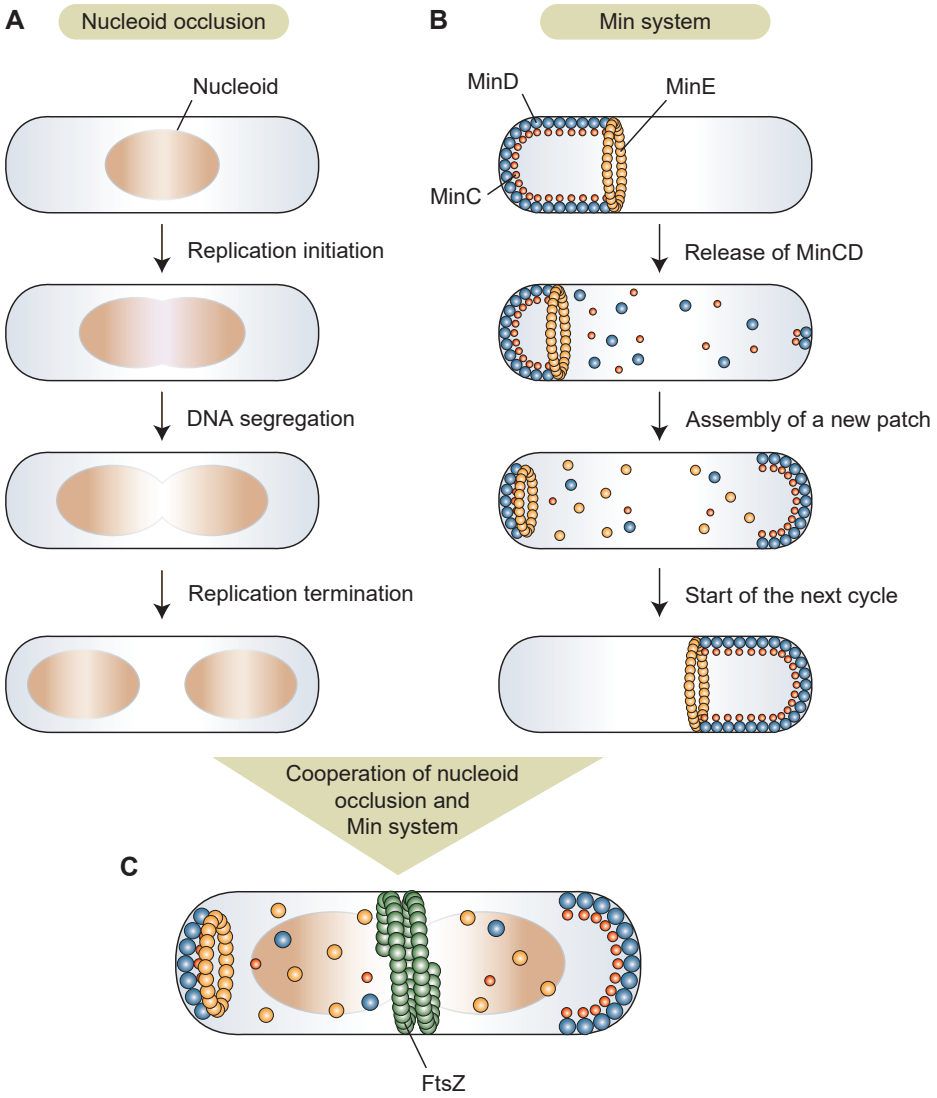
S. Du and J. Lutkenhaus. *Trends in Microbiology* 27: 781-791 (2019)



LJ Wu and J. Errington *Nature Reviews Microbiology*, 10:8-12 (2011)

3. Cell sizer: control of cell division process

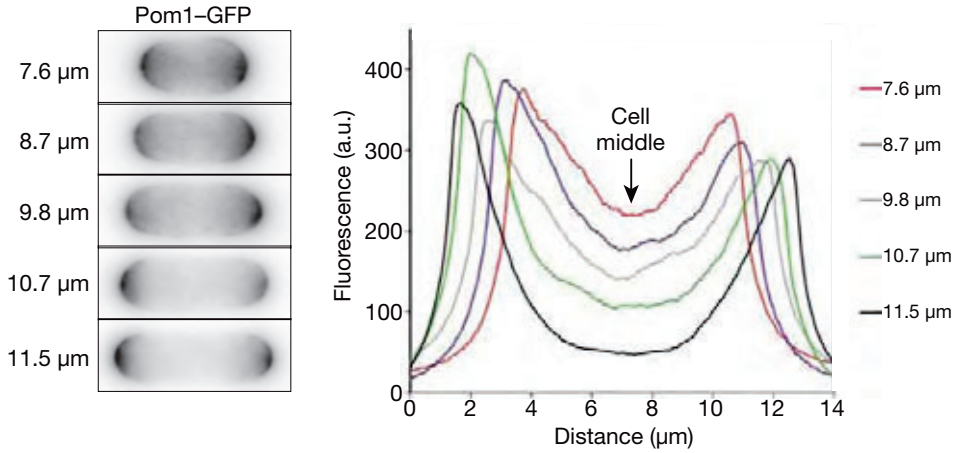
- *Spatial and temporal* control of cell division in Bacteria

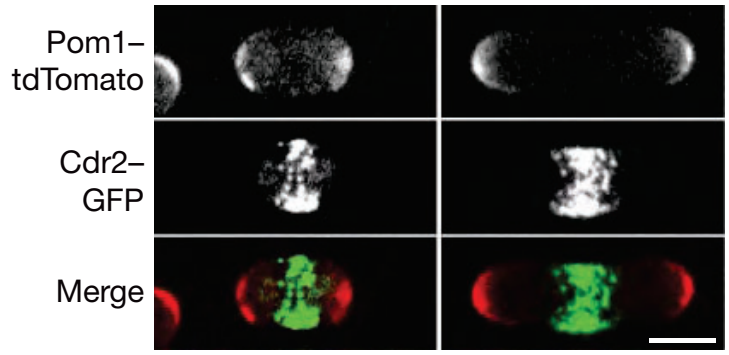


M. Thanbichler *Cold Spring Harb Perspect Biol* 2010;2:a000331 (2010)

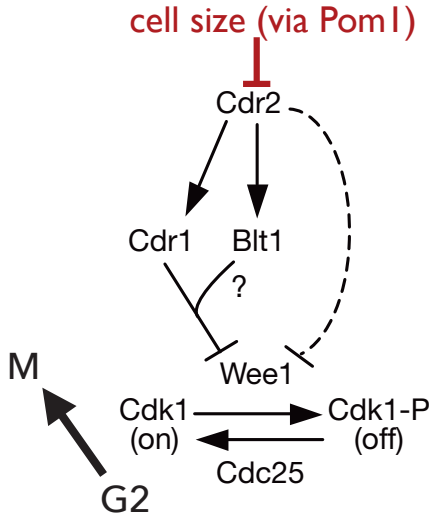
3. Cell sizer: control of cell division process

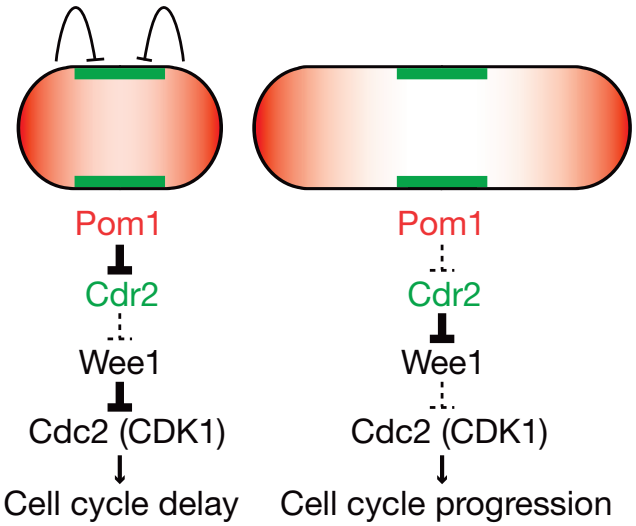
- *Spatial and temporal* control of cell division in Fission Yeast





Strain	Length at division (µm)
WT	14.3 ± 0.9
pom1Δ	11.7 ± 2.0
cdc25^{ts}	21.0 ± 2.4
wee1^{ts}	6.8 ± 0.9
P3nmt1-pom1^{+}	17.7 ± 1.4
P3nmt1-pom1^{+} cdc25^{ts}	33.9 ± 5.4

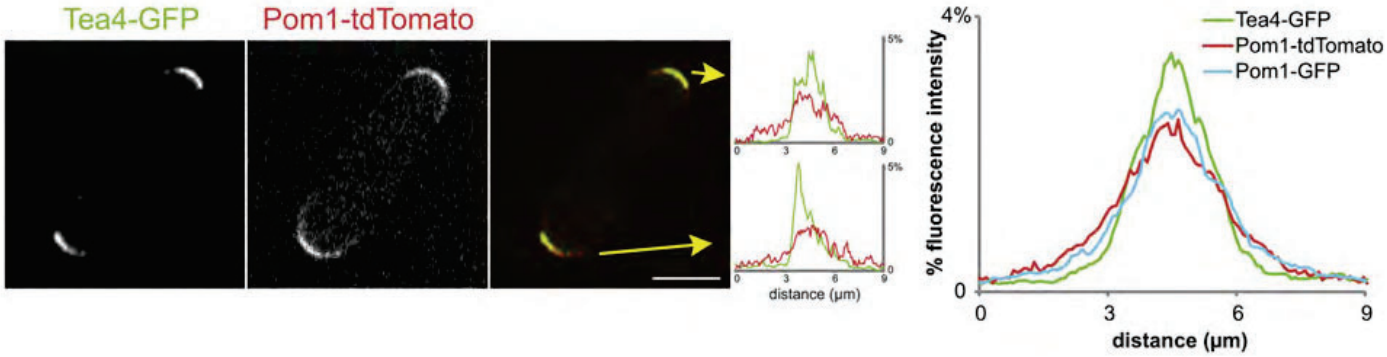


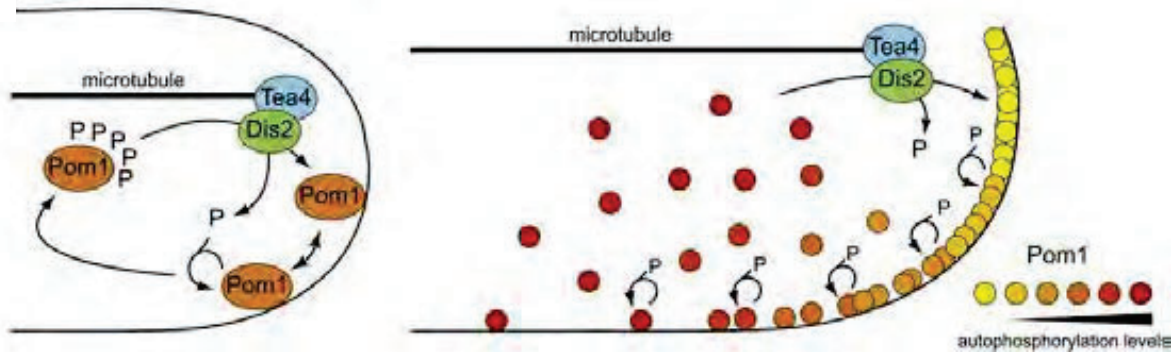


Moseley JB, Mayeux A, Paoletti A, Nurse P: *Nature* 459:857-860 (2009)
48. Martin SG, Berthelot-Grosjean M: *Nature* 459:852-856 (2009)

3. Cell sizer: control of cell division process

- *Spatial and temporal* control of cell division in Fission Yeast
  - Gradient of Pom1 induced by membrane association at cell poles, Pom1 lateral diffusion and dissociation through autophosphorylation
  - Microtubules enrich at cell poles Tea4 which recruits the phosphatase Dis2 and promotes Pom1 membrane association
  - Dephosphorylation gradient of Pom1 induced by microtubules





Hachet O, Berthelot-Grosjean M, Kokkoris K, Vincenzetti V, Moosbrugger J, Martin SG *Cell* 145:1116-1128. (2011)

Plan

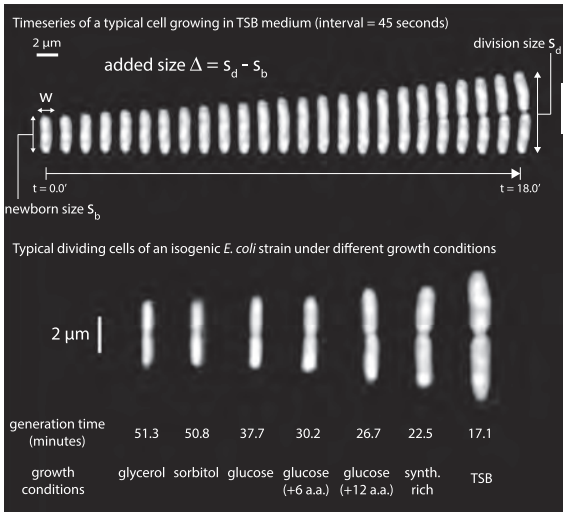
1. Evidence of size sensing mechanism
2. Cell sizer: control of G1 length
3. Cell sizer: control of cell division process
4. Cell adder mechanism

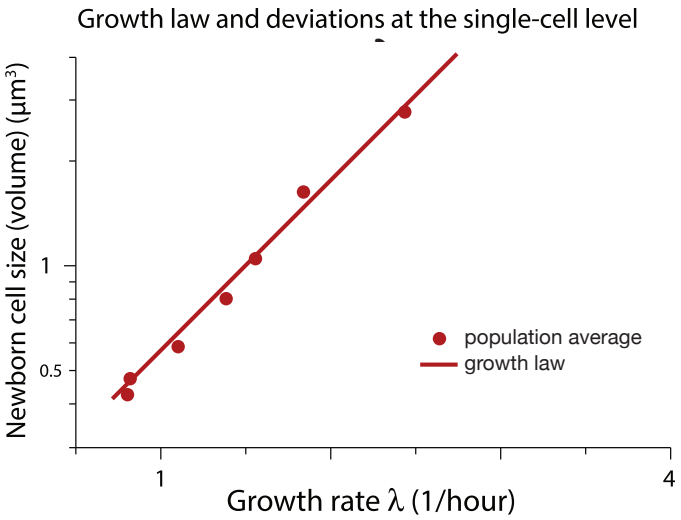
4. Cell « adder » mechanism

- Bacteria

- Population data are consistent with the « growth law »:

- growth rate λ increases with nutrients
- newborn cell size (V_b) increases with nutrients and exponentially with growth rate (—● —● —)
  $\langle V_b \rangle = A \exp(B\lambda)$
- time until division decreases with increasing growth rate





Taheri-Araghi et al., and M. Vergassola and S. Jun *Current Biology* 25, 385–391 (2015)

4. Cell « adder » mechanism

- Bacteria

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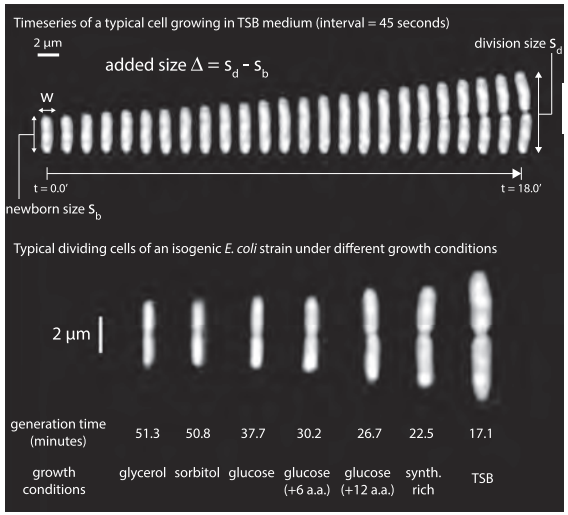
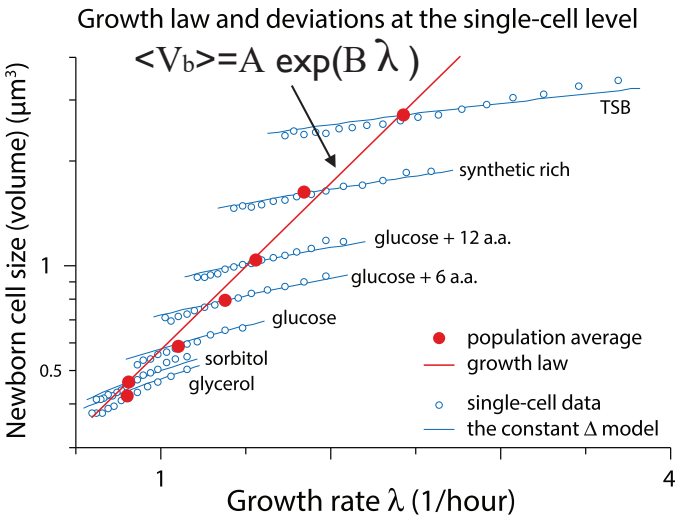
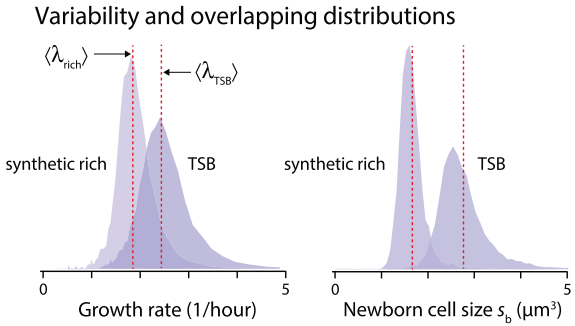


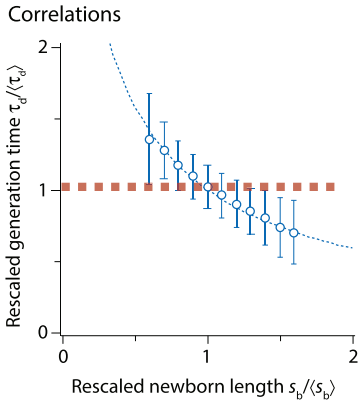
- But single cell level analysis shows cell size follows a different law



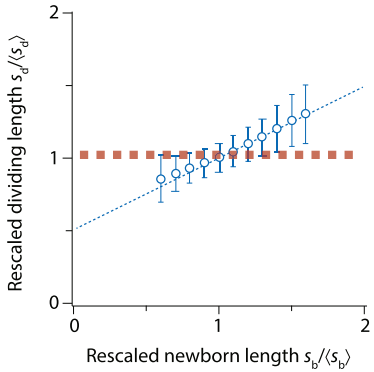


- Cells with the same growth rate under different conditions should have the same volume, following $\langle V_b \rangle = A \exp(B\lambda)$
- However this is not the case

Inconsistency with « timer » model



Inconsistency with « sizer » model

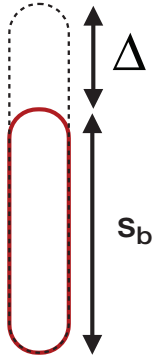


Taheri-Araghi et al., and M. Vergassola and S. Jun *Current Biology* 25, 385–391 (2015)

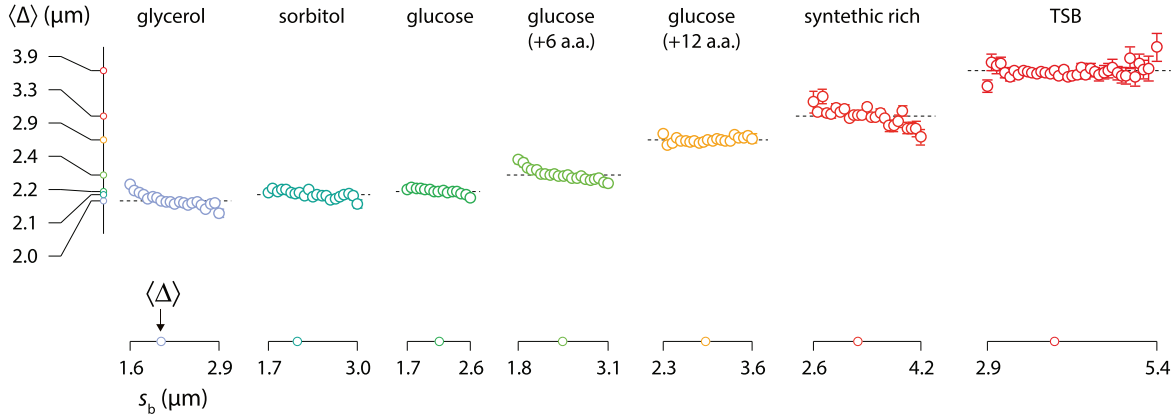
4. Cell « adder » mechanism

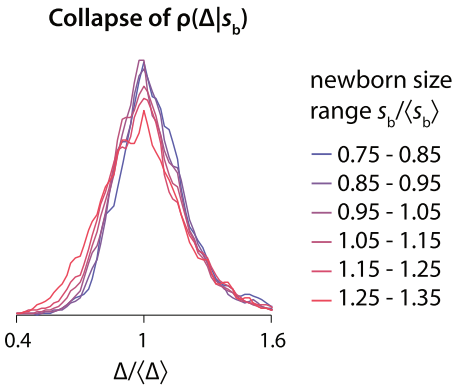
- **Convergence towards homeostatic cell size by constant « adder » mechanism**

  - The constant « adder » model proposes that a constant size Δ is added during cell cycle independent of the size at birth s_b
  - Data: Δ depends on growth conditions (eg. nutrients) and fluctuates between cells in a given environment. However it is not correlating with cell size at birth
  - The distribution of Δ conditioned on cell size at birth rescaled to their mean is invariant



Constancy of Δ with respect to the newborn size *E. coli* NCM3722

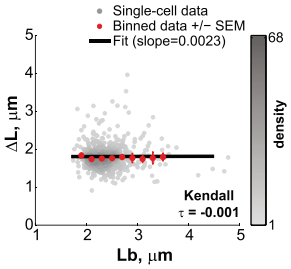




Taheri-Araghi et al., and M. Vergassola and S. Jun *Current Biology* 25, 385–391 (2015)

Similar findings in *Caulobacter crescentus*:

M. Campos et al. and C. Jacobs-Wagner *Cell* 159, 1433–1446 (2014)

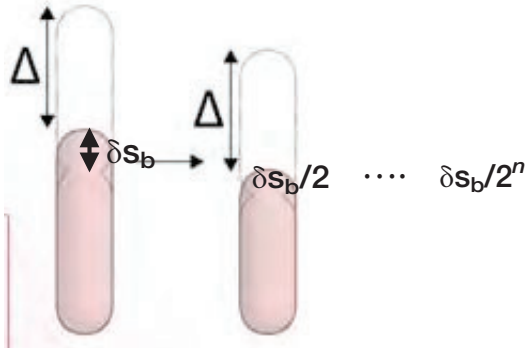


4. Cell « adder » mechanism

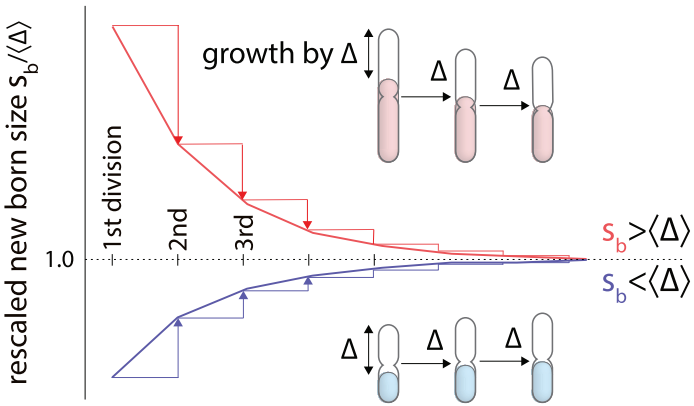
• Convergence towards homeostatic cell size by constant « adder » mechanism

- The constant « adder » model explains the convergence of cell size towards a population homeostatic value:

$$s_b = \langle s_b \rangle + \delta s_b$$
$$\langle s_b \rangle + \delta s_b/2$$
$$\ldots.$$
$$s_b = \langle s_b \rangle = \Delta$$

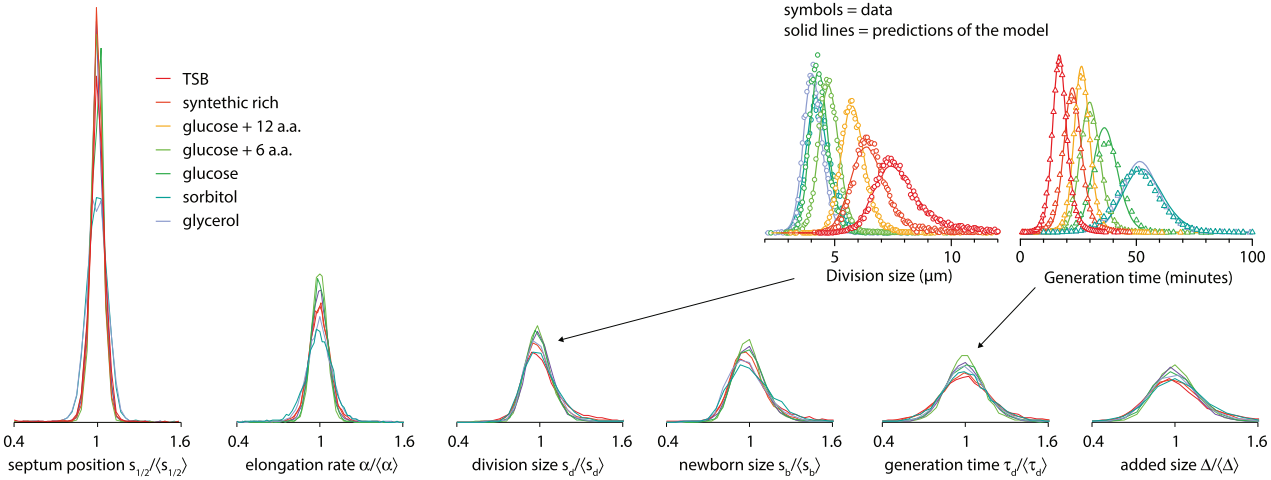


Prediction: size convergence by constant Δ

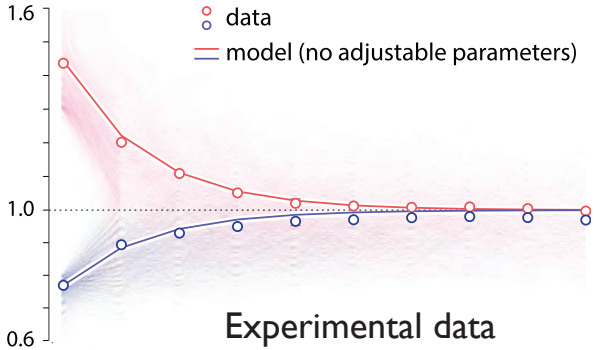


- Scale invariance of parameter distributions results from scale invariance of ρ(Δ)

Distributions & their collapse



Predictions of the Δ model and agreement with data

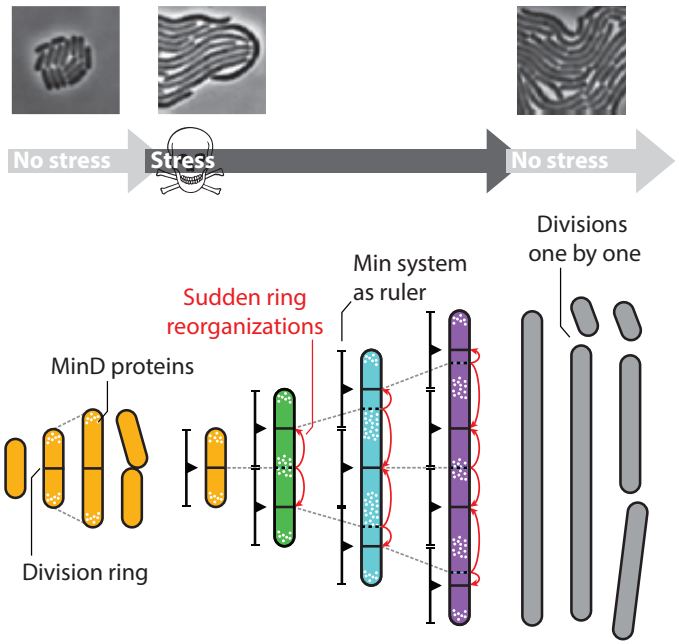


Experimental data
E coli (and *B. subtilis*, not shown)

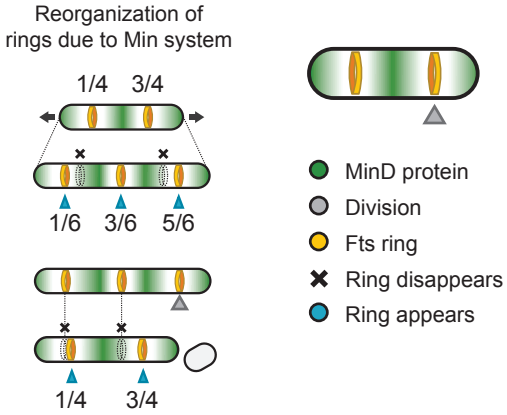
Taheri-Araghi et al., and M. Vergassola and S. Jun *Current Biology* 25, 385–391 (2015)
see also: M. Campos et al. and C. Jacobs-Wagner *Cell* 159, 1433–1446 (2014)

Cell « sizer » and cell « adder »

Filamentous bacteria grow under stress:
the release of stress induces cell division in single locations, one by one



Location of cell division is determined by the MinCD system



Cell division timing is determined by the time it takes to add a constant size

The « adder » mechanism serves as a timer

>>Mechanisms underlying constant « adder »: protein synthesis?

Wehrens et al., 2018, *Current Biology* 28, 972–979

4. Cell « adder » mechanism

Article

Current Biology

The Adder Phenomenon Emerges from Independent Control of Pre- and Post-*Start* Phases of the Budding Yeast Cell Cycle

Highlights

- Haploid daughter cells grow a size-independent amount in their cell cycle
- Single-cell imaging identifies predictive parameters for cell-cycle transitions
- The adder arises from independent regulation of the pre- and post-*Start* cell cycles
- Mutations of G1/S regulators disrupt the adder

Authors

Devon Chandler-Brown, Kurt M. Schmoller, Yonatan Winetraub, Jan M. Skotheim

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In Brief

Budding yeast daughter, but not mother, cells grow a size-independent amount in their cell cycle, known as the adder. Chandler-Brown, Schmoller, et al. show that the adder arises from independent regulation of the pre- and post-*Start* cell cycle rather than from a molecular adder mechanism. Accordingly, mutations of G1/S regulators disrupt the adder.

Chandler-Brown et al., 2017, *Current Biology* 27, 2774–2783

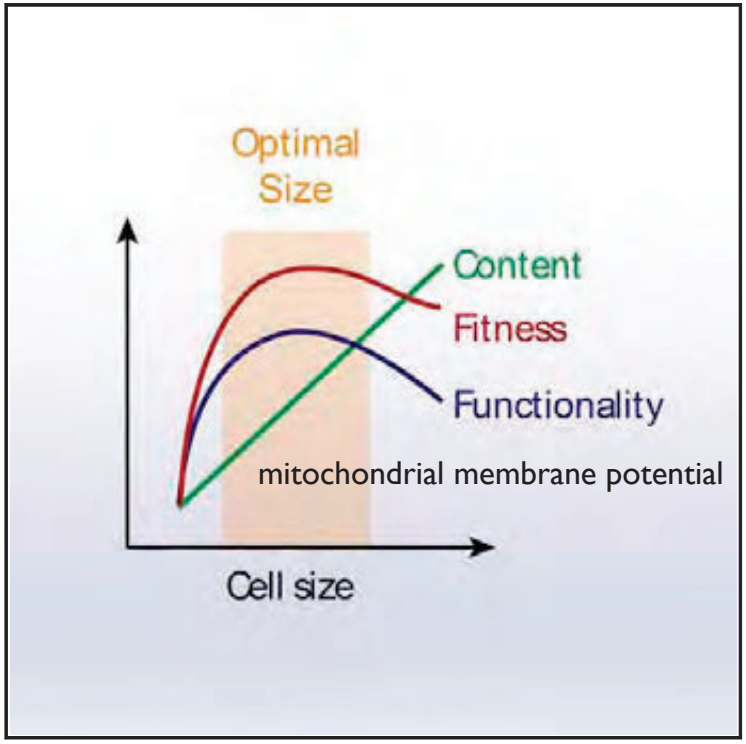
Is there an optimal cell size?

Article

Developmental Cell

Cellular Allometry of Mitochondrial Functionality Establishes the Optimal Cell Size

Graphical Abstract



Authors

Teemu P. Miettinen, Mikael Björklund

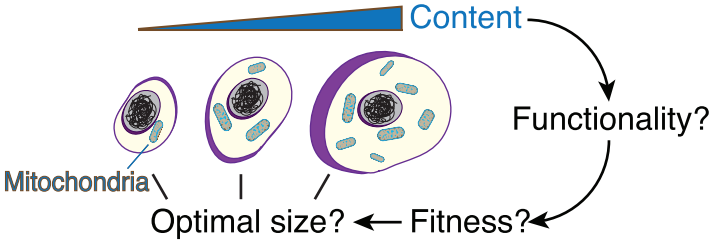
Correspondence

mikael.bjorklund.lab@gmail.com

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 mitochondia



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

COLLÈGE DE FRANCE
1530

CHAIRE DYNAMIQUES DU VIVANT
Année académique 2020-2021

Thomas LECUIT

Taille, croissance et organisation cellulaires

Cours les mardis de 10h à 11h30
Amphithéâtre Guillaume Budé

Cours :

17 novembre 2020	Du tissu à la cellule : taille et complexité
24 novembre 2020	Volume cellulaire : déterminants physico-chimiques et régulation
01er décembre 2020	Croissance et division cellulaires : la cellule mesure-t-elle ses dimensions ?
08 décembre 2020	Lois de proportions cellulaires

Colloque :

Contraintes et plasticité au cours du développement et de l'évolution
(avec Denis Duboule, chaire Évolution des génomes et développement)

Les 03 & 04 juin 2021
Amphithéâtre Maurice Halbwachs