Central Questions Regarding Cell Sizes and Growth Rates

- How do cell division rates scale with resource availability?
- How do cells decide when to divide, i.e., coordination of growth rate and size at maturity?
- How does the investment in cellular composition alter when a cell grows?
- Does the evolutionary relationship between cell size and growth rate reflect the physiological response within species?

Simple Growth-Response Models

Exponential growth in numbers, given a value of r: $N_t = N_0 e^{rt}$

Doubling time: $t_D = \ln(2)/r$

The Monod equation for the growth-rate response to external nutrient concentration, S:

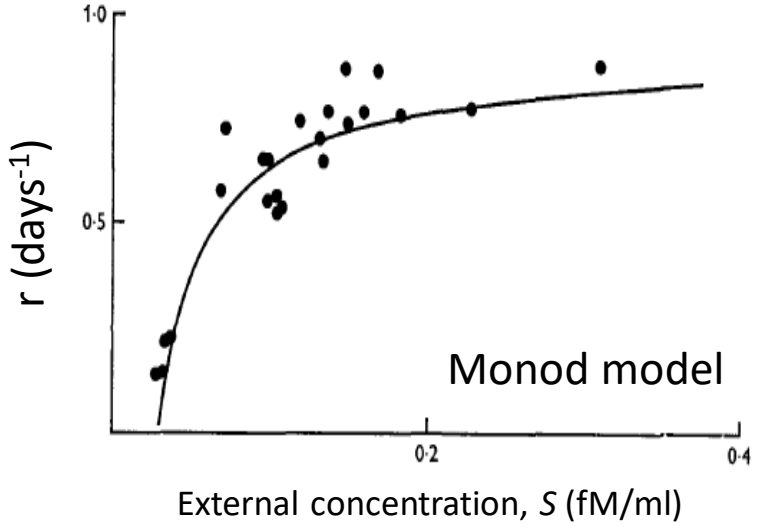
$$r = r_{\max}\left(\frac{S}{K_r + S}\right)$$

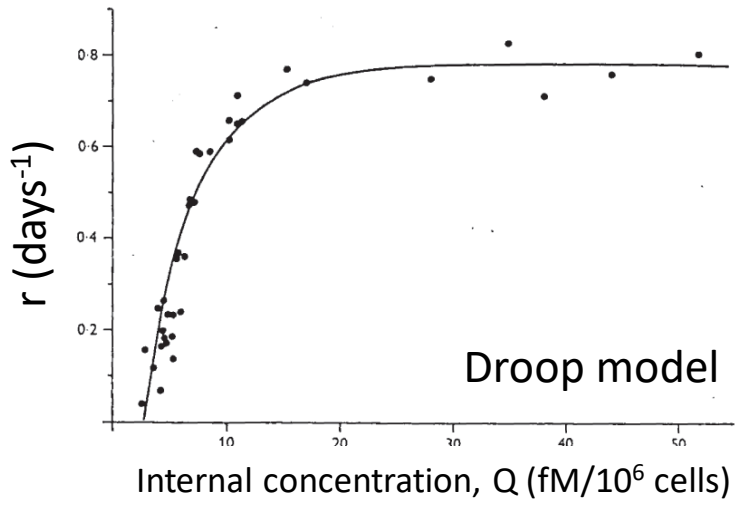
- A hyperbolic relationship similar to the Michaelis-Menten form for enzyme kinetics.

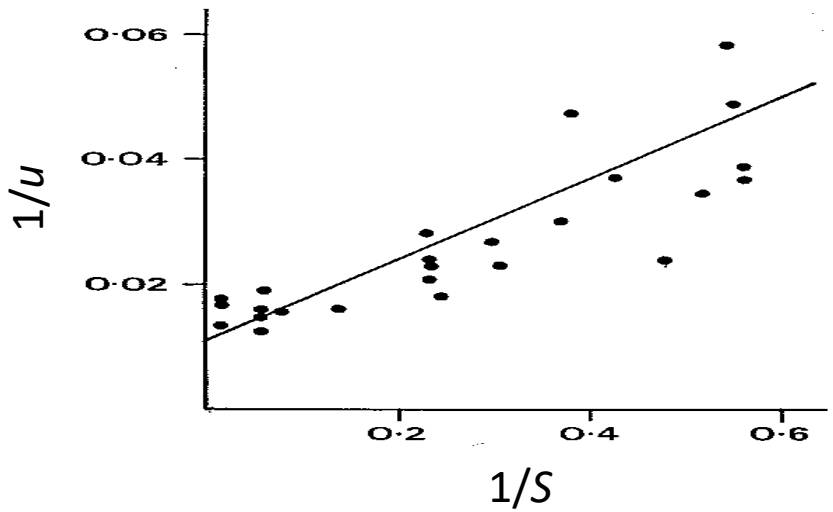
The Droop equation for the growth-rate response to internal nutrient concentration, Q:

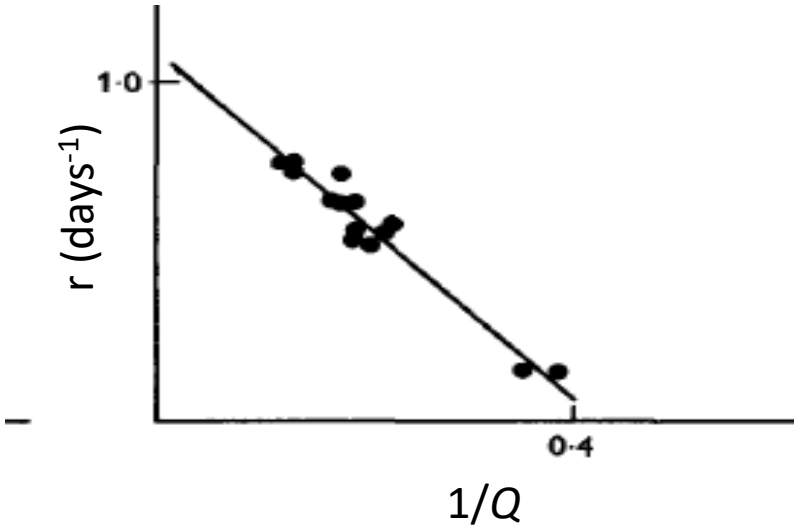
$$r = r'_{\max}\left(1 - \frac{\phi}{Q}\right)$$

Response of the Chrysophyte *Monochrysis* to Vitamin B_{12} Concentration

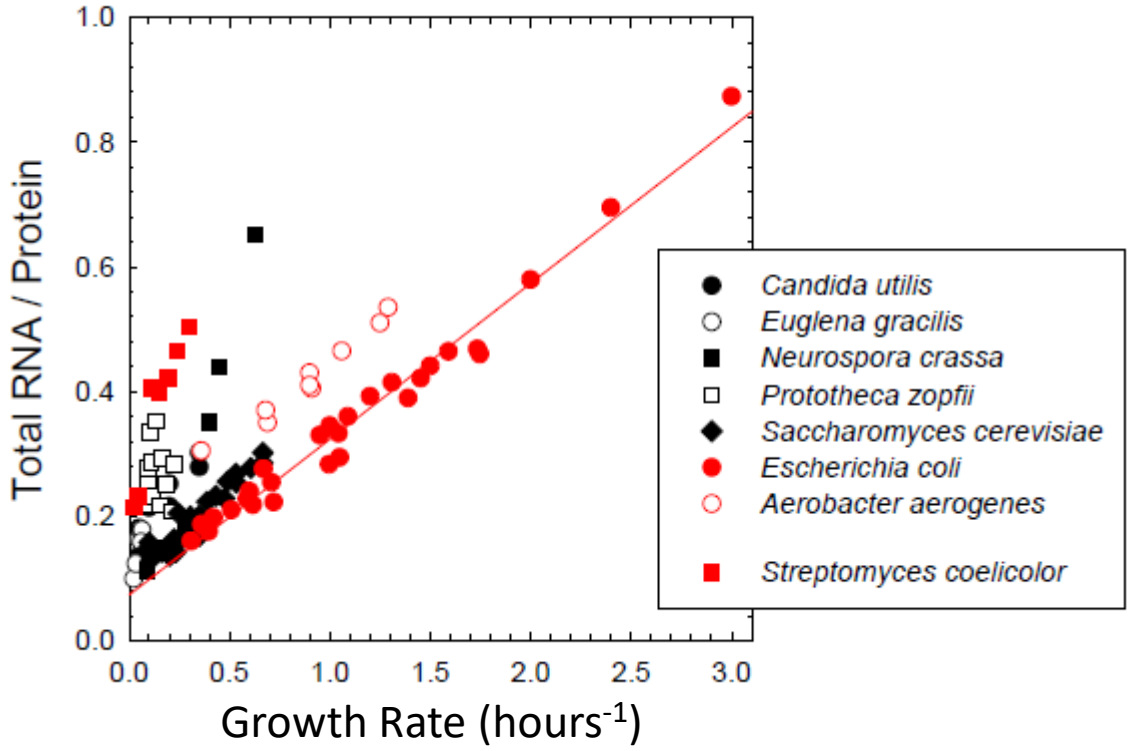








Cellular Remodeling in Response to Nutrient Availability: Scaling of Ribosome Use with Growth Rate



- An example of a ``growth law'' by identified by microbial physiologists.

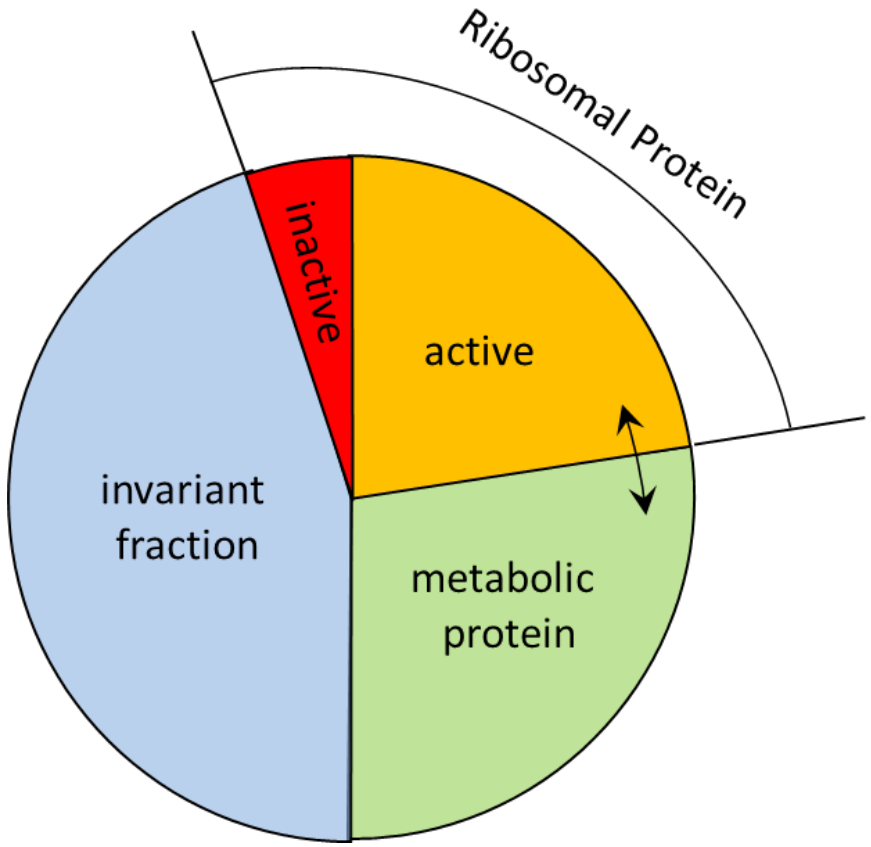
- The universal increase in the relative investment in ribosomes with increasing cell-division rate presumably reflects the conflict of the high energetic cost of ribosomes and their necessity for building cellular material.

- Typically, 60 to 70% of the RNA in cells is associated with ribosomes.

- In *E. coli*, ~5 to 50% of cellular protein is associated with ribosomes and translation-associated proteins.

A Tradeoff Between Investment in Translation and Resource Acquisition?

- If there is a fixed fraction of inactive ribosomes, and active ribosomes translate at a rate independent of the cell's physiology, cell growth rates are a function of the relative allocation of resources to nutrient harvesting vs. biomass production.



Rate of increase of total protein mass (M):

$$\frac{dM}{dt} = m_{AA} \cdot k_T \cdot N_R$$

- N_R: number of ribosomes
- k_T: translation rate / ribosome
- m_{AA}: mass of an amino acid

$$\frac{dM}{dt} = rM$$

What is the optimal allocation of mass to different protein sectors in environments with different nutritional capacity?

The Upper Limit to Cellular Growth Rates?

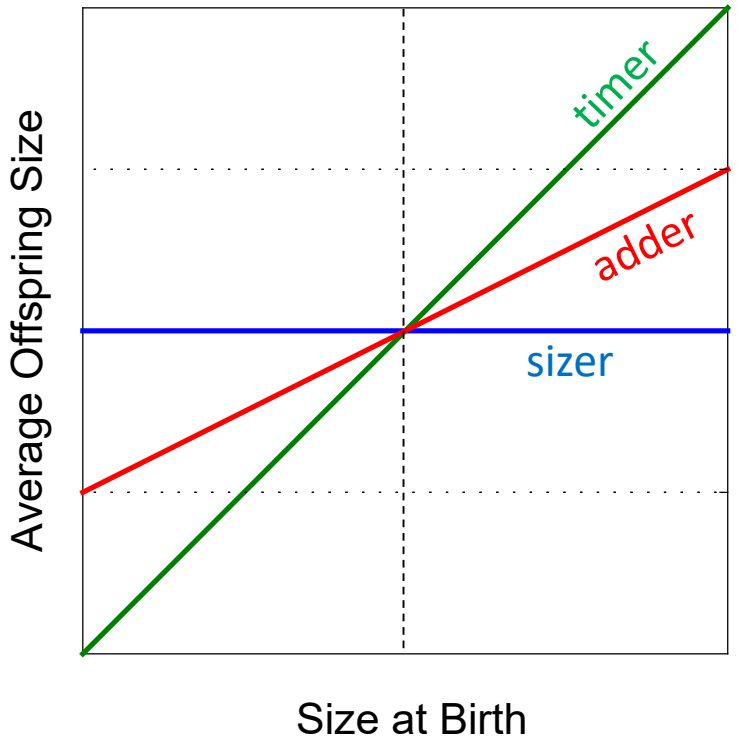
- Upper limit to the translation rate per ribosome ≈ 20 amino acids / second.
- A bacterial ribosome contains about 7500 amino acids, and accessory proteins contain about the same amount.
- 15,000 AAs / (20 AAs incorporated / second) = 750 seconds = 12.5 minute minimum doubling time.

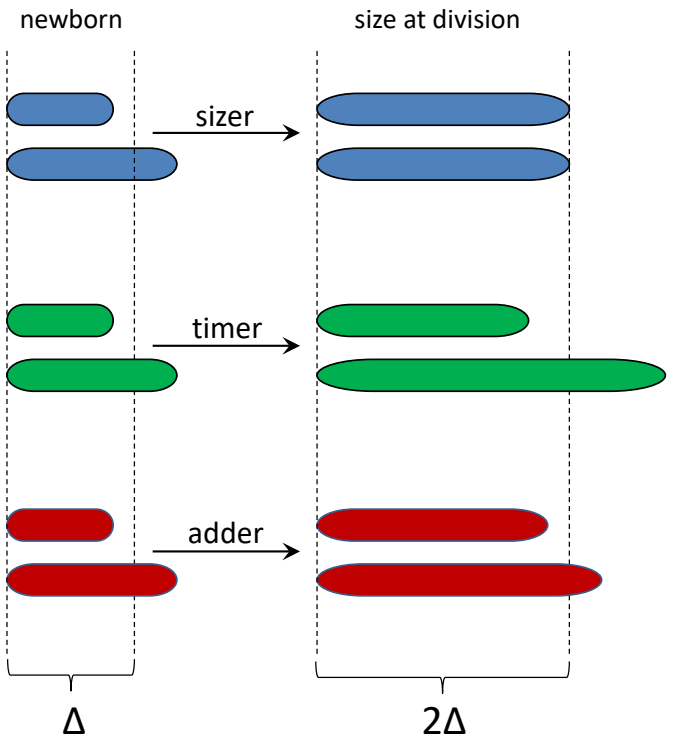
How Do Cells Know When to Divide?

- Exponential growth implies that the metabolic features of cells remain constant independent of size.
- Sizer model – division once a critical cell volume is reached.
- Timer model – division after a fixed period of time.
- Adder model – division after adding a specific increment.

How Do Cells Know When to Divide?

- Sizer model – division once a critical cell volume is reached.
- Timer model – division after a fixed period of time.
- Adder model – division after adding a specific increment.



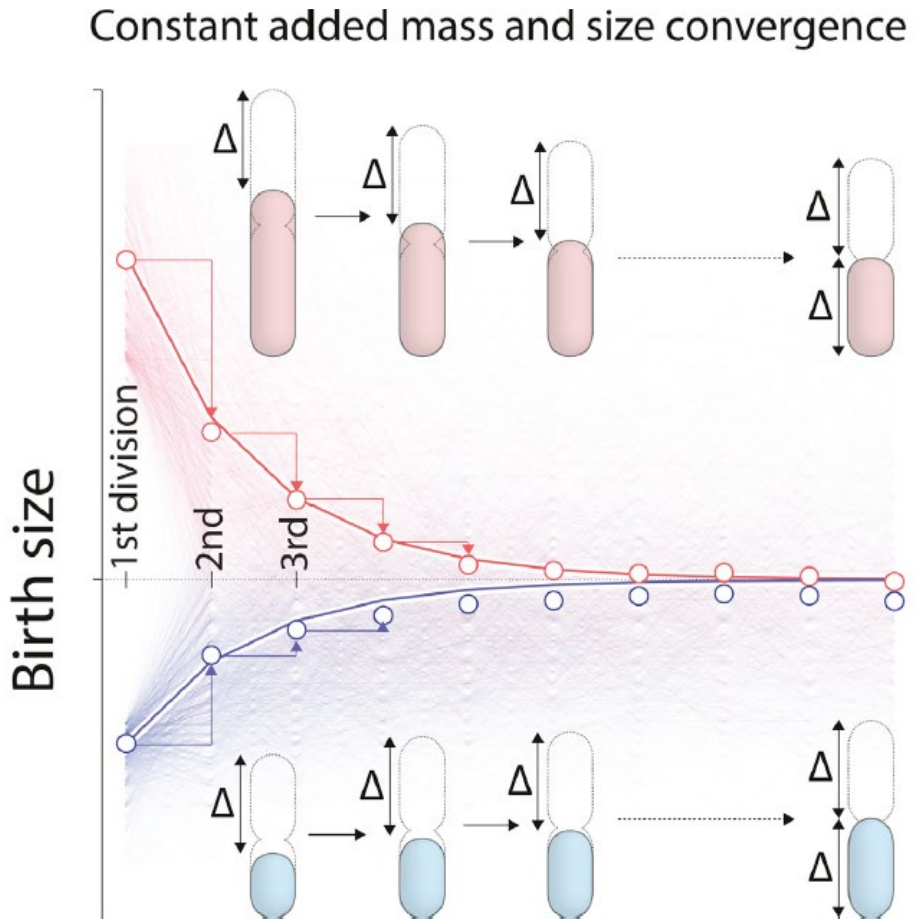


Cell-Size Homeostasis Under the Adder Model

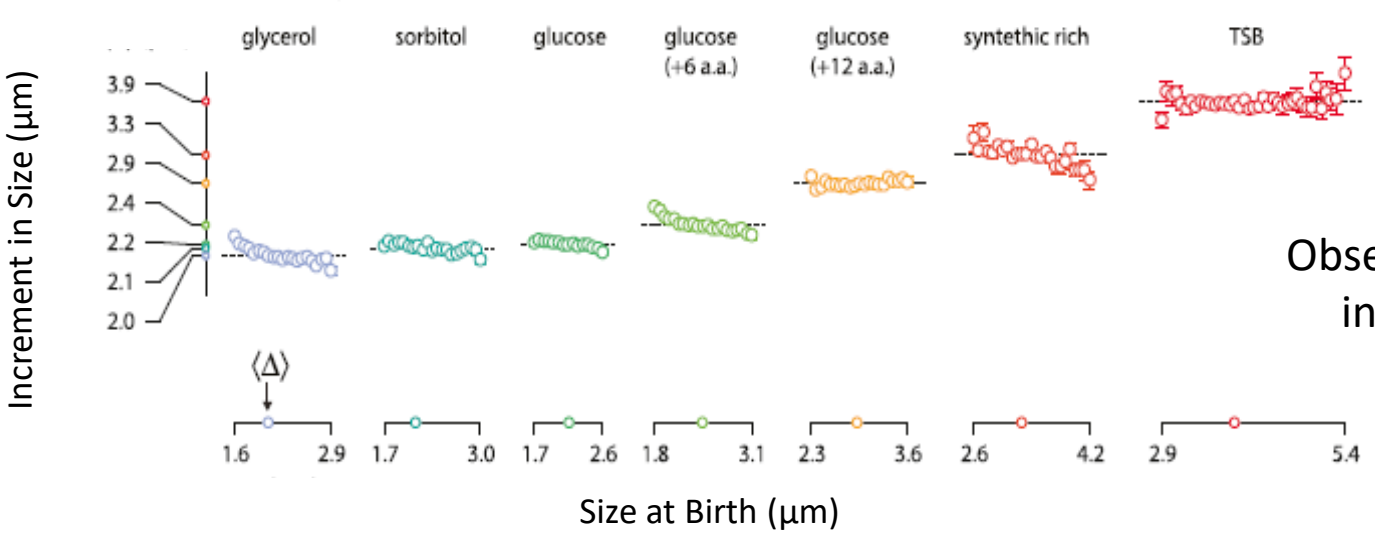
Let Δ be the increment per cell division.

If a cell is v larger than Δ at birth, having size (v+Δ), the expected size at division is (v+Δ) + Δ.

After cell division, the average size is (v/2)+Δ, so the deviation has been reduced by 50%.



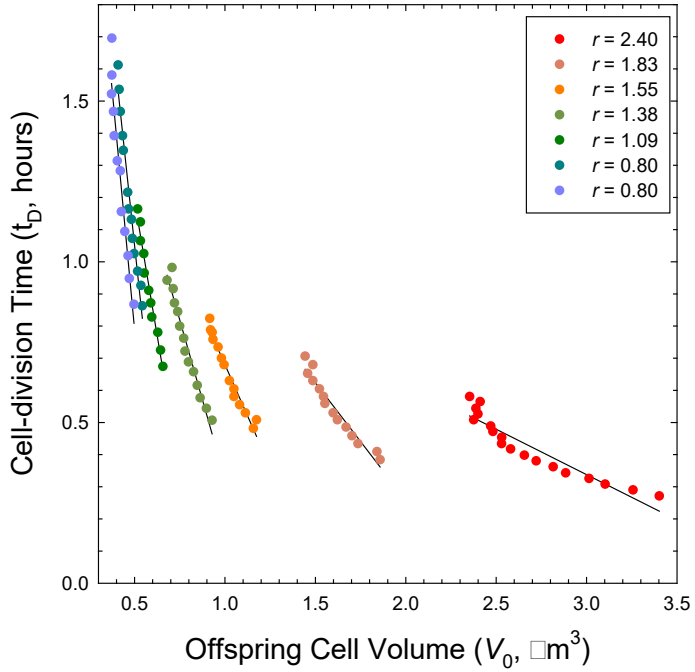
In *E. coli,* the Growth Increment in Size is Nearly Independent of the Size at Birth



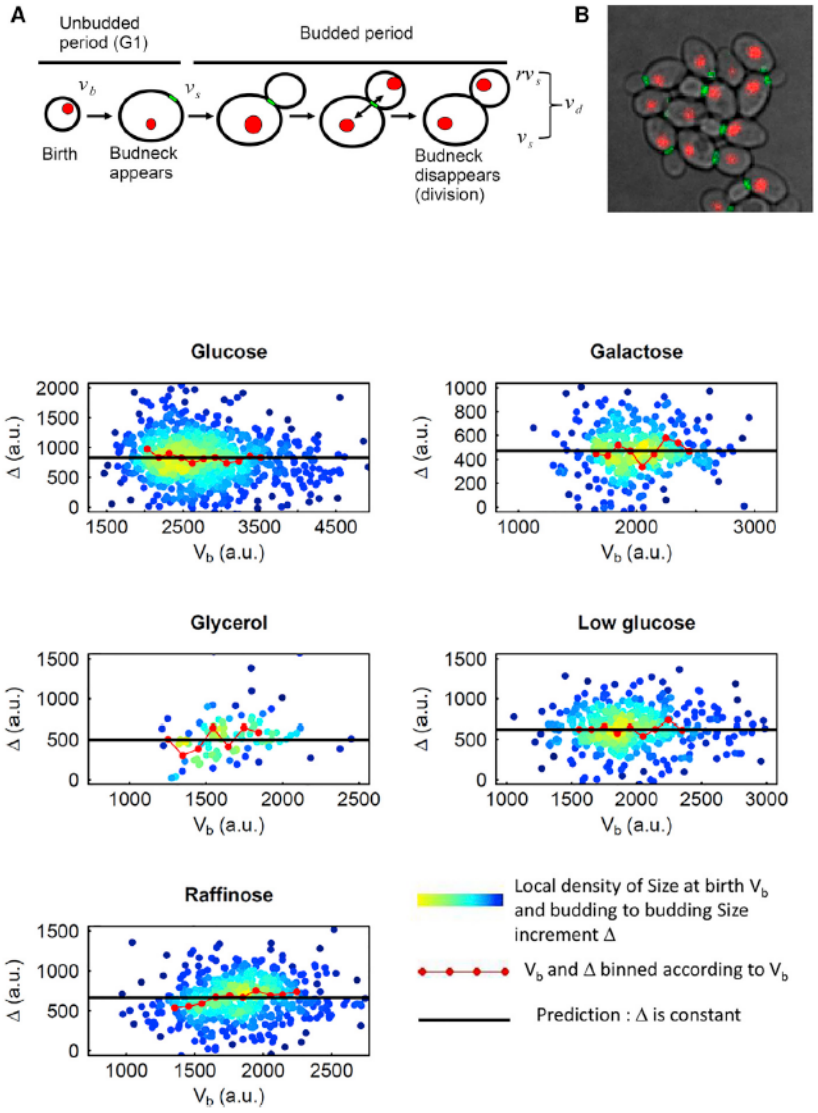
Observations from different growth media with increasing nutritional value. Taheri-Araghi et al. (2015)

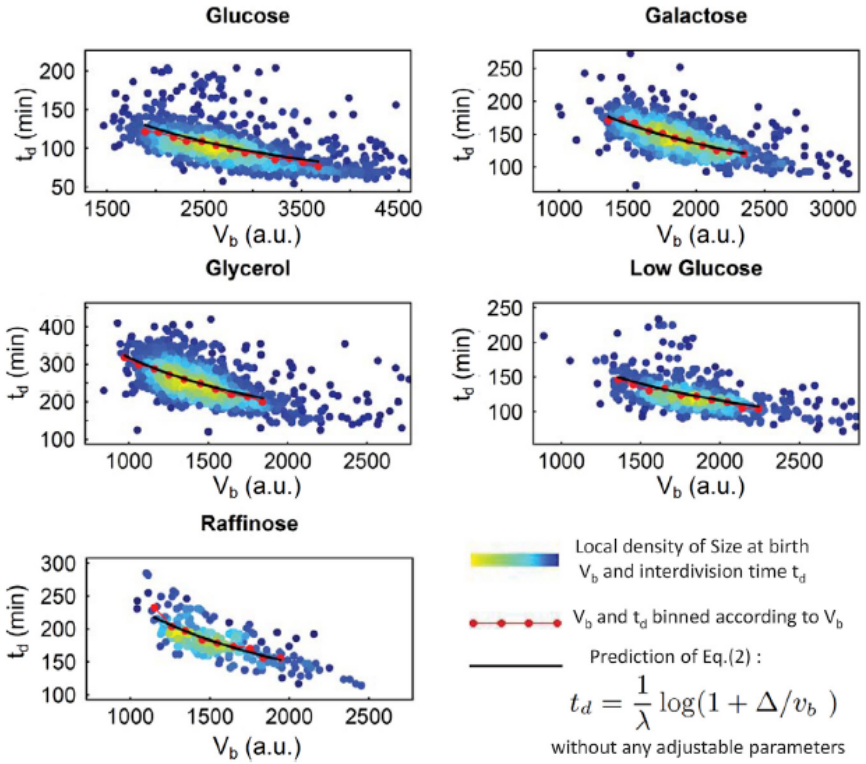
Because growth is exponential, cells that are smaller at birth take longer to achieve a given increment in size.

Inverse relationship between cell-division time and size at birth is stronger with media with low nutritional content.



Support for the Adder Model for Growth in Budding Yeast





Soifer et al. (2016)

Moving From Phenomenological Growth Laws to Mechanisms of Cell-Size Determination: a Continuously Diluted Inhibitor in *S. cerevisiae*

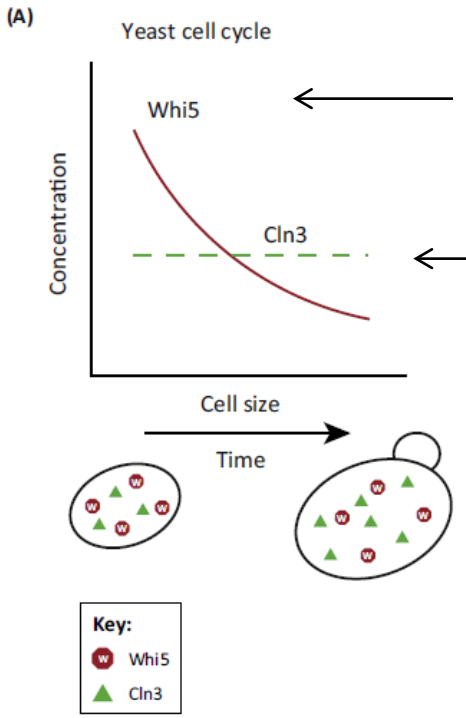


Figure 2. To Link Cell Size with Division, Cells Employ Regulatory Molecules with Cell Size-Dependent Concentrations. (A) In budding yeast, cell size control is based on the differential synthesis of the cell cycle activator Cln3 and the inhibitor Whi5. While Cln3 is produced in proportion to cell size so that its concentration is constant, Whi5 is produced at a rate independent of cell size so that its concentration is smaller in larger cells. This promotes cell cycle entry in larger cells. (B) In early frog embryos, a similar mechanism senses cell size to control the timing of the mid-blastula transition (MBT) at the 12th division cycle. Histones inhibit the MBT and are at constant concentration while genomic DNA promotes the MBT. DNA concentration doubles at each cell division because in the early frog embryo cells divide without growing. The decreasing histone-to-DNA ratio can then measure cell size to control the timing of the MBT.

← Short burst of mitotic inhibitor at cell division.

← Inhibitor of the inhibitor is maintained at constant concentration, and only becomes effective when Whi5 is sufficiently dilute.

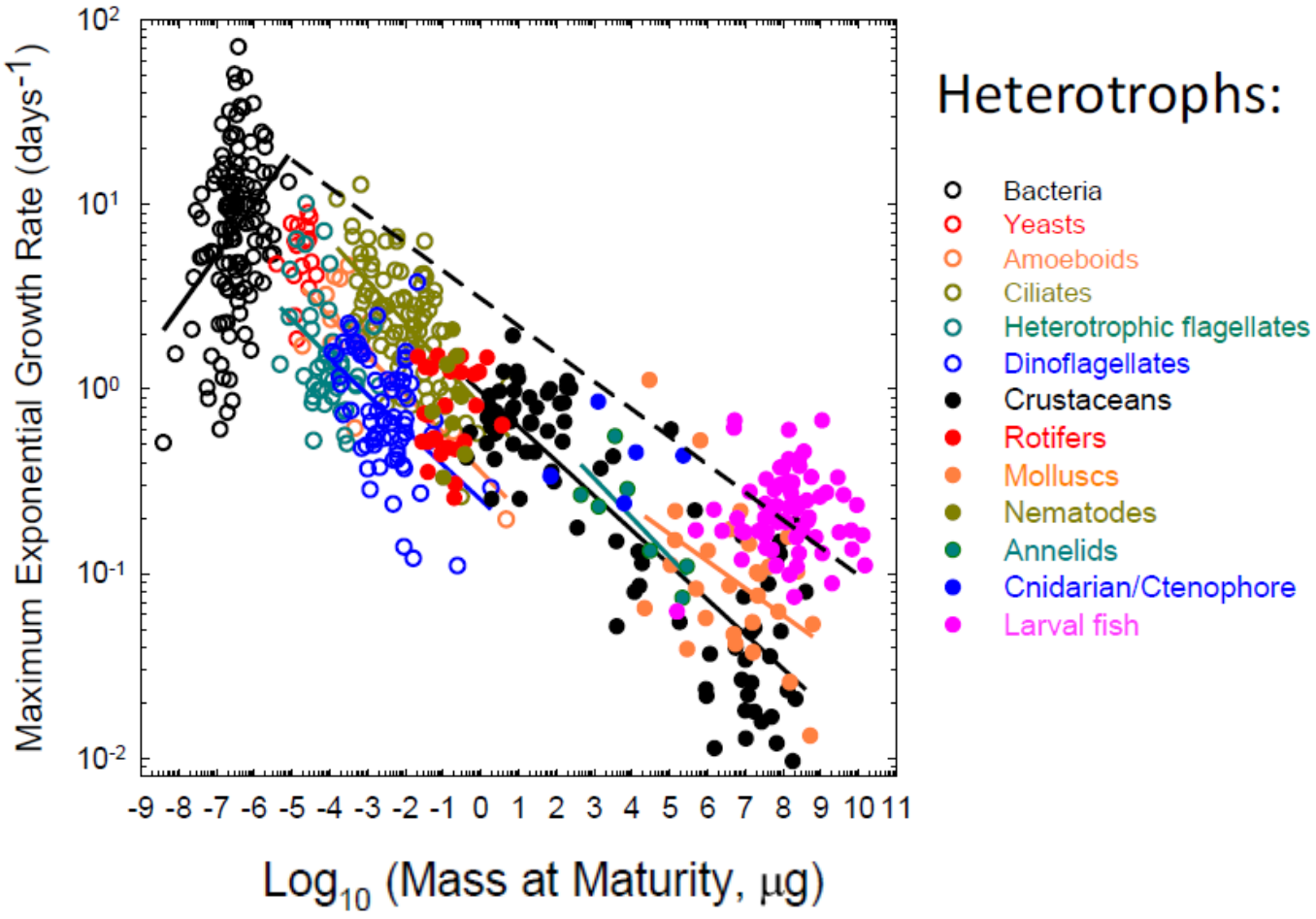
In *S. pombe*, an inhibitor mechanism is also used, but here an activator of mitosis is centrally located.

An inhibitor of the activator has a gradient from the cell poles, and the concentration declines as the cell grows.

Bacteria use still different mechanisms that vary among species.

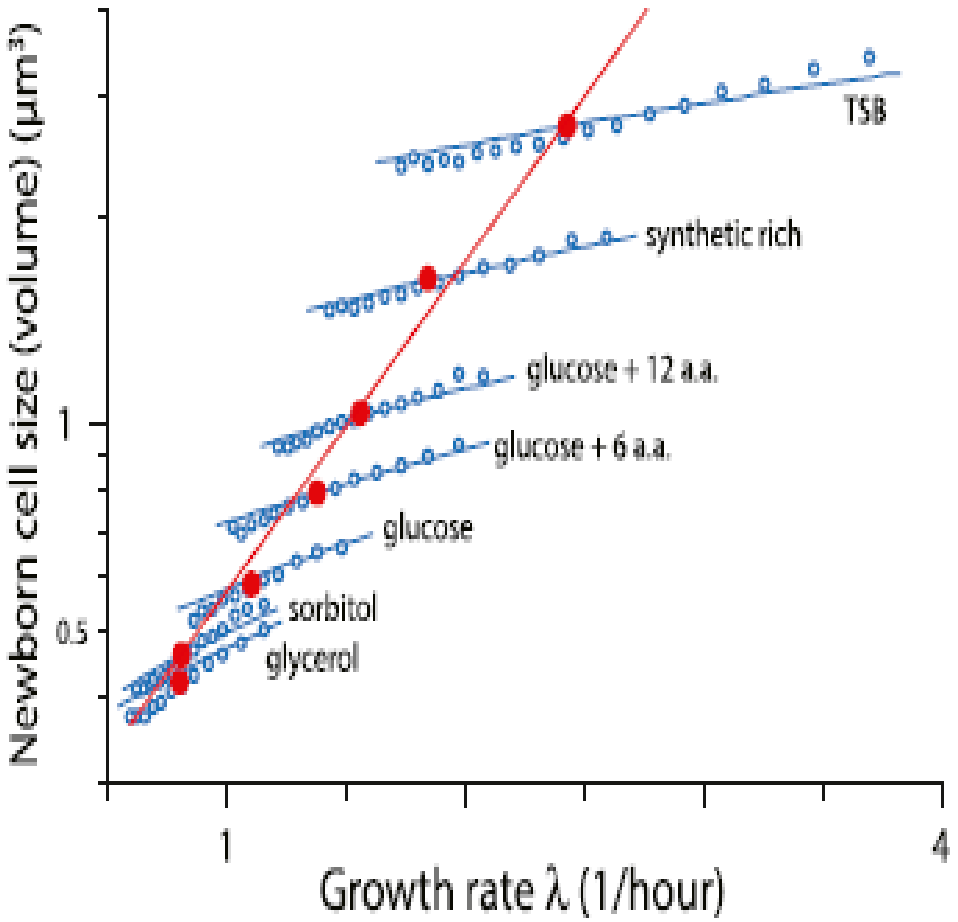
Schmoller and Skotheim (2015)

Scaling of Maximum Growth-Rate with Organism Size Across the Tree of Life



- Bacteria and unicellular eukaryotes scale in opposite directions.

Cell Size and Growth Rate Increase with Nutrient Availability: a phenotypic response, not a genetic change.

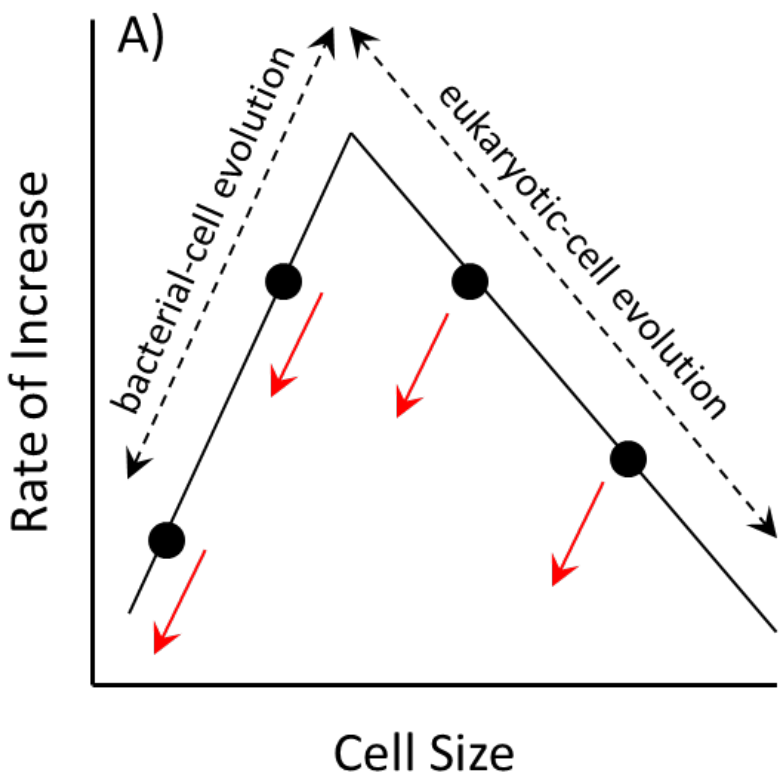


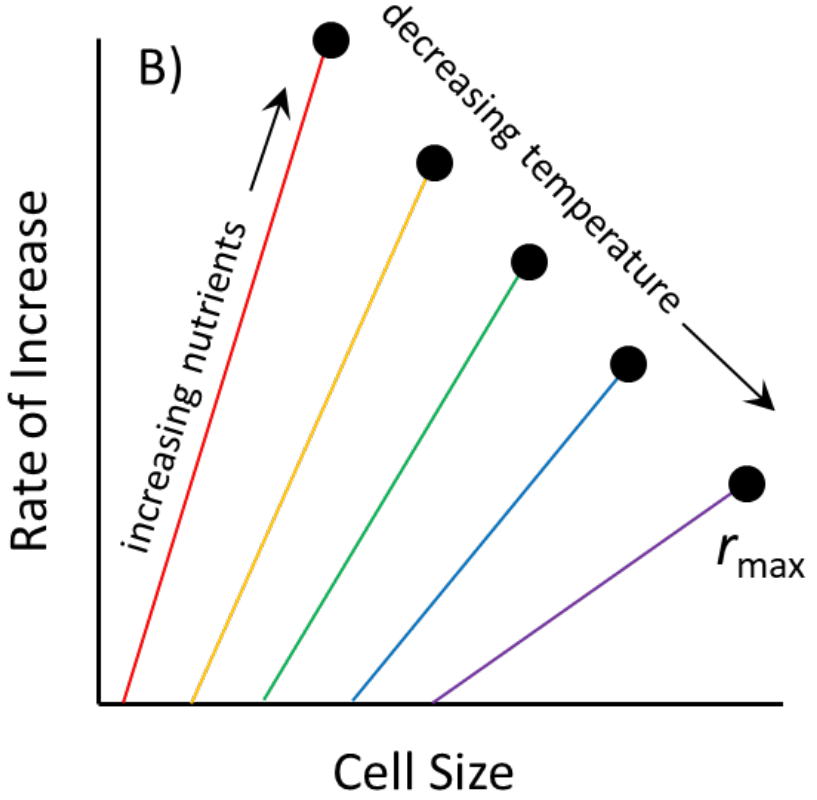
- Is this type of reaction an intrinsic physiological response, i.e., a by-product of the underlying molecular mechanisms by which cells commit to division?
- Are such shifts are adaptive in any way?
- Are such changes transient responses to selection, with larger cells making larger cells, and doing so more rapidly?

Taheri-Araghi et al. (2015)

The Phylogenetic Growth Rate / Cell Size Allometry in Prokaryotes Resembles the Phenotypic Response to Nutrients.

The Eukaryotic Pattern Resembles the Response to Temperature.





How Do Cells “Regulate / Count” the Numbers / Mass of Their Individual Parts as They Grow?

The green alga *Chlamydomonas* can sense both the absolute and relative sizes of its flagella.

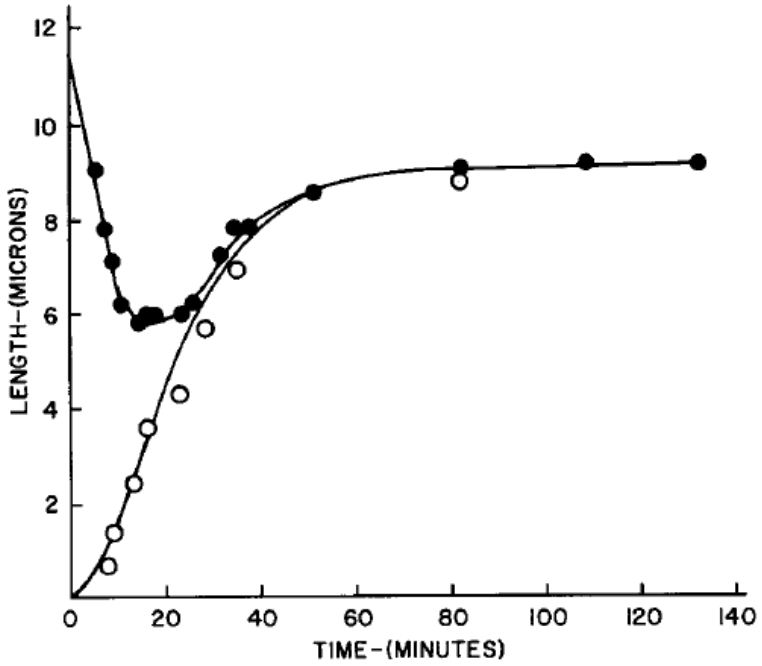
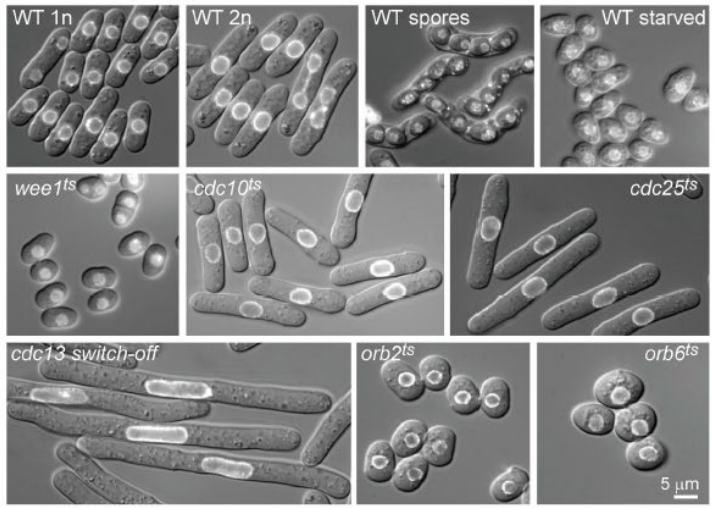
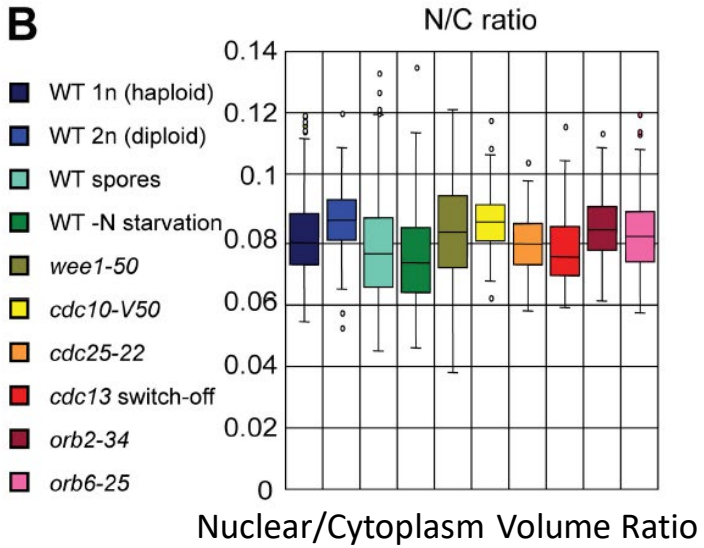


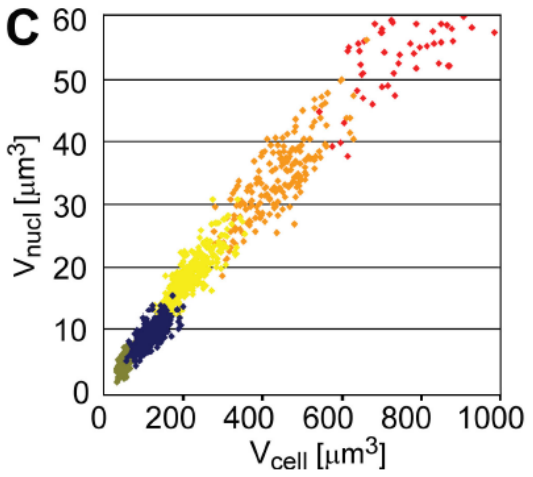
FIGURE 4 Amputation of one of the two flagella of a single *Chlamydomonas* (pf 16) showing partial shortening of the intact flagellum. Open circles, amputated flagellum; closed circles, intact flagellum.

Rosenbaum et al., 1969, J. Cell Biology

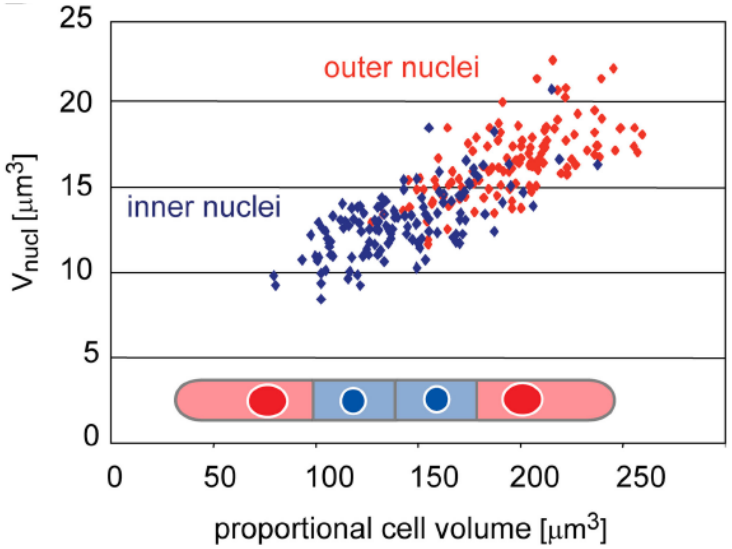
In Both Fission and Budding Yeast, Cell Volume Determines Nuclear Volume







- A constant "karyoplasmic" ratio (≈ 0.08) in haploid and diploid cells.
- The same ratio is maintained throughout cell growth.
- Cell-division mutants demonstrate that the local cytoplasmic environment determines nuclear volume, not the other way around.



Neumann and Nurse, 2007, J. Cell Biol.; Jorgensen et al., 2007, Mol. Biol. Cell

Cytoplasmic Factors Regulate the Size of the Nucleus in the African Frog *Xenopus*

- Cytoplasmic extract from the species with a large genome forms larger nuclei regardless of the source of DNA.

- The species with a larger genome has higher levels of the import factor importin-α in its cytoplasm, and addition of this to the extract from the small- genome species leads to larger nuclei.

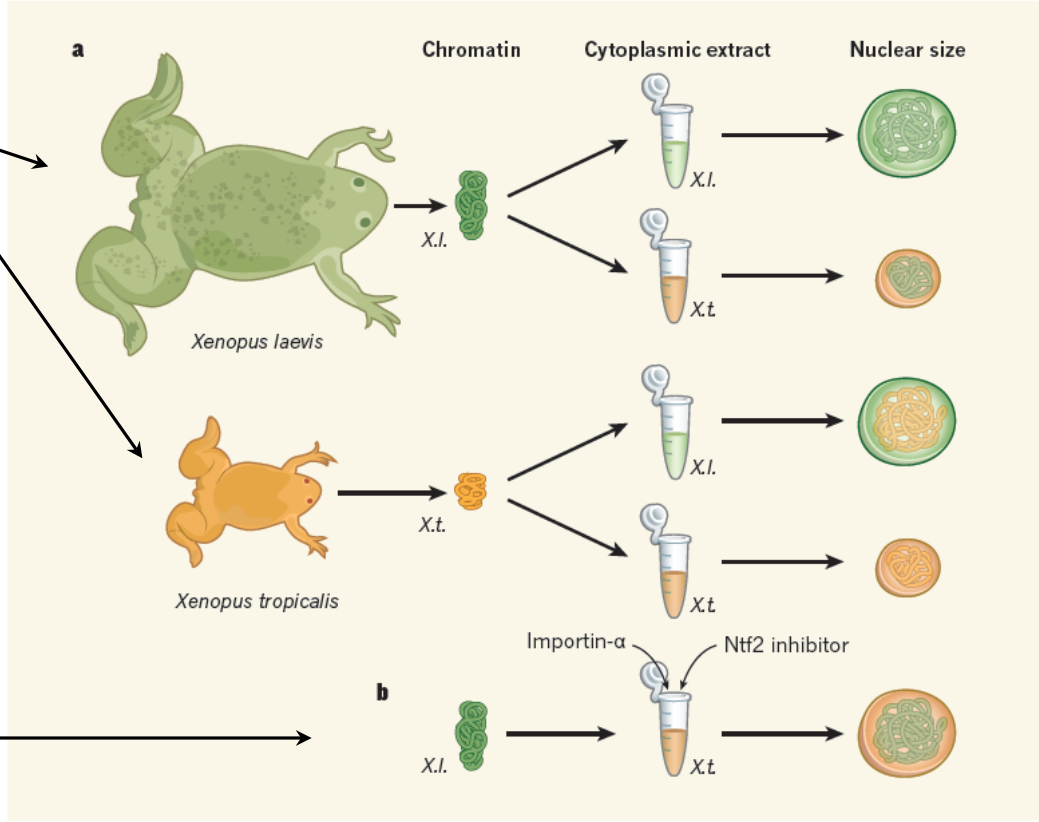


Figure 1 | The cytoplasm regulates nuclear size. **a**, To examine factors affecting nuclear size, Levy and Heald[2] mixed sperm chromatin from either *Xenopus laevis* (*X.l.*) or *X. tropicalis* (*X.t.*) with cytoplasmic extracts from the eggs of either *X. laevis* or *X. tropicalis*. They find that, regardless of the chromatin used, *X. laevis* extracts promote formation of large nuclei, whereas *X. tropicalis* extracts promote formation of smaller nuclei. (The drawings of *X. laevis* and *X. tropicalis* are to scale). **b**, Two proteins, importin-α and Ntf2, account for the differences between the *X. laevis* and *X. tropicalis* extracts: addition of importin-α and inhibition of Ntf2 activity in *X. tropicalis* extracts lead to formation of larger nuclei.

Levy and Heald (2010, Cell); Cohen-Fix (2010, Nature)

The Ontogenetic Response of Cell Composition to Cell Volume is Generally Isometric During Cell Growth:

the relative proportions of cell contents remain constant during cell growth.

Saccharomyces	mitochondrial volume	1%
	vacuole volume	6%
Candida	mitochondrial volume	10%
Cryptococcus	mitochondrial volume	9%
HeLa cells	mitochondrial volume	1%
Euglena	mitochondrial volume	6%
	plastid volume	16%
Chlorella	mitochondrial volume	3%
	plastid volume	40%
	vacuole volume	10%

Two Models for Intracellular Growth Regulation

1) Independent exponential growth rates of volumes of the total cell and its parts:

$$V_t = V_0 e^{rt} \qquad z_t = z_0 e^{\beta t}$$

$$\ln(z_t) = \left(\frac{\beta}{r}\right) \ln(V_t) + c$$

Slope of a log-log plot yields the ratio of growth rates.

Isometric growth = slope 1.0.

---

2) Growth rate of parts directly dependent on cell volume: $\frac{dz}{dt} = \beta \cdot V_t = \beta \cdot V_0 e^{rt},$

- Same key parameter.
- Linearity is expected on an arithmetic scale.
- If growth is isometric, the two models will be difficult to discriminate.

$$z_t = \left(\frac{\beta}{r}\right) V_t + c,$$

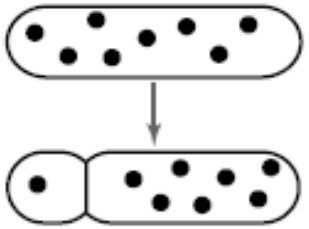
Stochastic Inheritance of Molecular Features and Distributions of Growth Features

- What are the non-genetic sources of cell-to-cell variation?
- What is the level of phenotypic variation among genetically uniform cells, and how does this compare with levels of variation in multicellular species?
- What are the evolutionary consequences of phenotypic variation?
  - Evolutionarily hardwired as an enhancer of survivability, or an impediment to evolutionary progress?
- Transient response to selection.

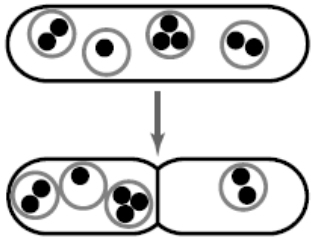
Nongenetic Sources of Variation

- Variation associated with micro-environmental differences.
- Inaccuracies in the growth-increment target, Δ.
- Variation in transcription and translation rates, and in rates of molecular decay.
- Simple binomial partitioning of parental cell contents to the two daughters.
- Asymmetrical partitioning of parental cells.
- In eukaryotes, stochastic assortment of organelles, including mitochondria, which can cause further variation-generating feedback.

Asymmetrical Partitioning



Disordered Clustering Among Organelles

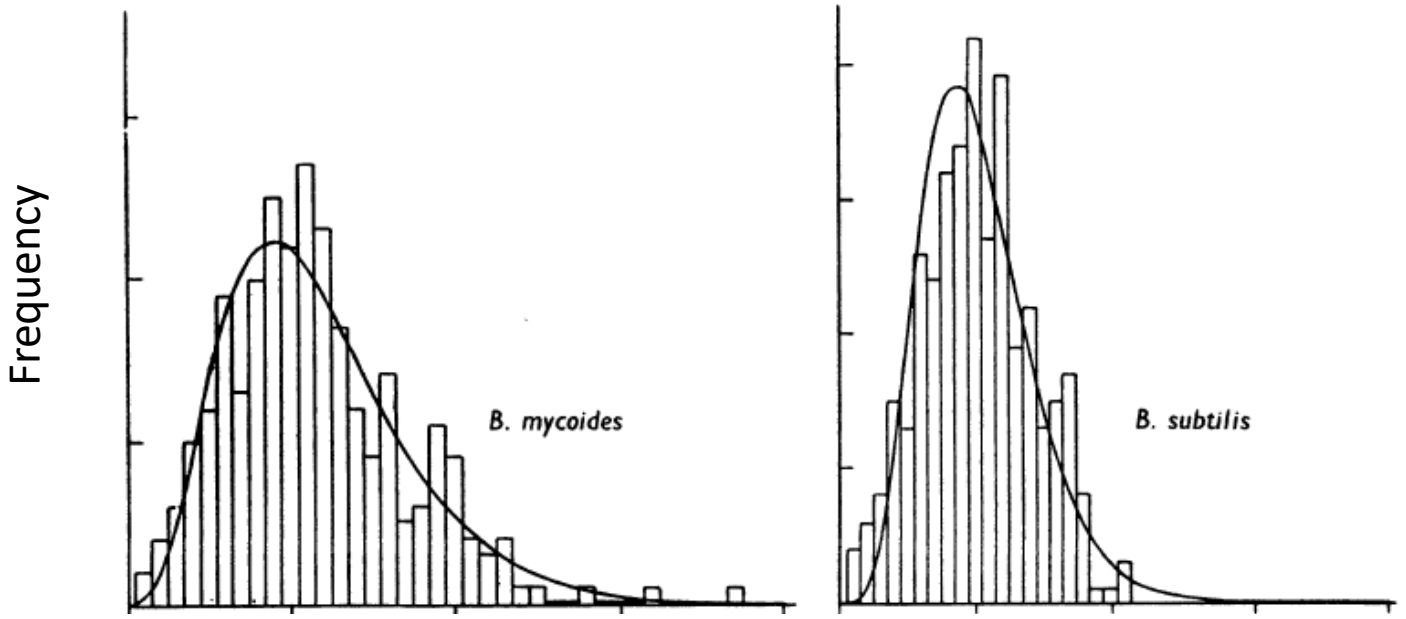


Potential Statistical Models for Distributions

Yule – all parts have to be duplicated independently with fixed probability, with division occurring at the time of duplication of final part.

Pearson Type III – cell division occurs after a series of consecutive steps has been completed.

Fitted Pearson Type III Distributions for Two *Bacillus* Species



Variation in Cell Biological Features is Typically Much Greater Than That For Morphometric Traits in Multicellular Species, Which Have CVs ≈ 0.05 to 0.10.

Table 4.1. Coefficients of variation (CV, standard deviation divided by the mean) for growth-related features of cells.

Species	Trait	CV	Reference
Bacteria:			
Aerobacter cloacae	Generation time	0.180	Powell 1958
Azotobacter agilis	Elongation rate	0.100	Harvey et al. 1967
	Generation time	0.224	Harvey et al. 1967
Bacillus mycoides	Generation time	0.478	Powell 1956
Bacillus subtilis	Generation time	0.538	Powell 1956
Bacterium aerogenes	Generation time	0.298	Powell 1956
Escherichia coli	Elongation rate	0.076	Taheri-Araghi et al. 2015
	Division length	0.140	Taheri-Araghi et al. 2015
		0.120	Harvey et al. 1967
	Birth length	0.162	Taheri-Araghi et al. 2015
	Generation time	0.209	Taheri-Araghi et al. 2015
		0.280	Harvey et al. 1967
	Added length	0.240	Taheri-Araghi et al. 2015
Proteus vulgaris	Generation time	0.319	Powell 1956
Pseudomonas aeruginosa	Generation time	0.138	Powell 1958
Serratia marcescens	Generation time	0.167	Powell 1958
	Generation time	0.138	Tyson 1989
Streptococcus faecalis	Generation time	0.273	Powell 1956
Eukaryotes:			
Saccharomyces cerevisiae	Length of G1 phase	0.458	Di Talia et al. 2007
Schizosaccharomyces pombe	Division length	0.068	Tyson 1989
Tetrahymena pyriformis	Generation time	0.125	Scherbaum and Rasch 1957
	Division size	0.125	Scherbaum and Rasch 1957

How does natural selection promote permanent change?

- A permanent response to natural selection requires resemblance between relatives.

- Resemblance between relatives is a function of the fraction of phenotypic variation that has a genetic basis.

- The efficiency of natural selection declines with increasing environmental variation for the trait.

The Concept of Heritability

- The phenotypic value of an individual (P) is defined as the sum of an expectation based on the underlying genotype (G) and a random environmental deviation (E):

$$P = G + E$$

- The phenotypic variance in the population is equal to the sum of that at the genotypic and environmental levels:

$$\sigma_P^2 = \sigma_G^2 + \sigma_E^2$$

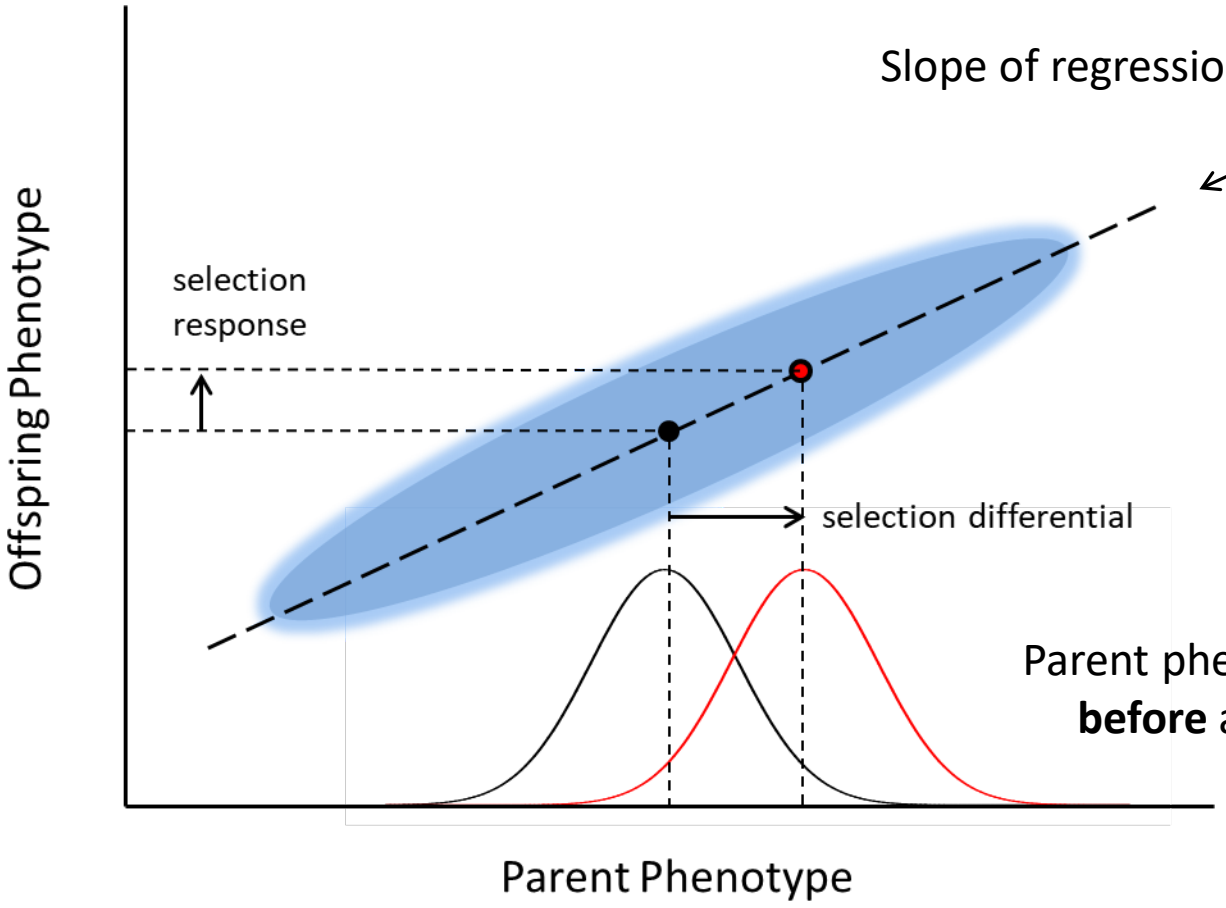
- The phenotypic covariance between relatives is equal to the genetic variance (for asexually reproducing individuals):

$$\begin{aligned}\sigma(P_o, P_p) &= \sigma[(G_o + E_o), (G_p + E_p)] \\ &= \sigma(G_o, G_p) \\ &= \sigma_G^2\end{aligned}$$

- The heritability of a trait is equal to the fraction of total variation with a genetic basis; can be thought of as the efficiency of the response to selection:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_E^2}$$

The response to directional selection is equal to the product of the change in mean phenotype due to selection (the selection differential) and the slope of the parent-offspring regression (the heritability):



Parent phenotype distributions **before** and **after** selection.

Genetic variation increases the response to selection; environmental variation reduces it.

Two Final Issues

- Because binary fission results in substantial sharing of the contents of parent and offspring cells, unicellular species are subject to significant inheritance of nongenetic effects, which can lead to transient shifts in phenotypic values in the absence of genetic change.

- Although there has been considerable speculation that such high levels of phenotypic variation represent adaptations molded by natural selection to cope with variable environments, there is little empirical or theoretical support for this contention.

Transient Evolution Without Genetic Variance Through Partial Propagation of Environmental Effects

- Persistent selection leads to a steady-state amount of change – the new progress each generation is balanced by the loss of previous progress by the dilution of inherited environmental effects:

Size of an adult cell at the time of reproduction: $V_a = V_0 + \Delta + e_\Delta$

where V_0 is the size at birth, Δ is the expected growth in size, and e_Δ is the deviation of the actual growth increment from Δ owing to background variation

In absence of selection, the mean phenotype remains constant: $\overline{V}_0 = \overline{V}_a/2 = \Delta$

After one generation of selection: $\overline{V}_0(1) = \Delta + (\overline{e}_\Delta/2)$

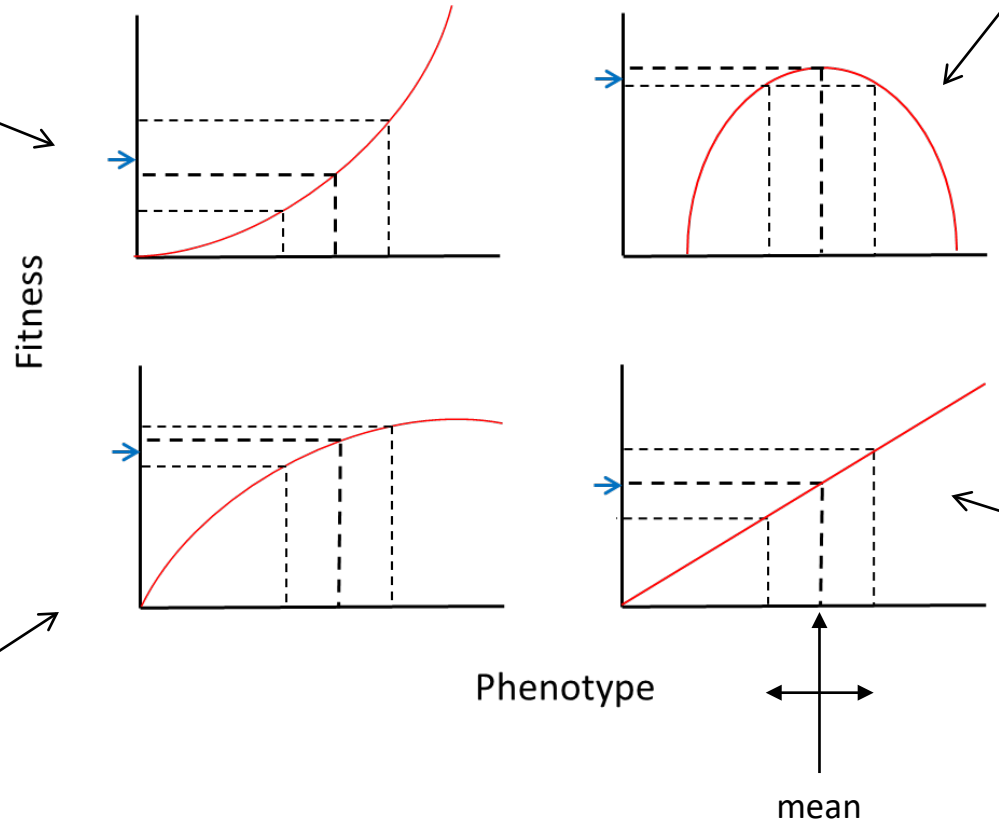
After two generations of selection: $\overline{V}_0(2) = \Delta + (\overline{e}_\Delta/2) + (\overline{e}_\Delta/4)$

After several generations: $\longrightarrow \quad \Delta + \overline{e}_\Delta$ as t increases.

The Influence of Variation on the Response to Selection Depends on the Form of the Fitness Function

Concave fitness function:
variation increases the mean.

Stabilizing fitness function:
variation reduces the mean.



Convex fitness function:
variation reduces the mean.

Linear fitness function:
variation has no effect.