RICE UNIVERSITY

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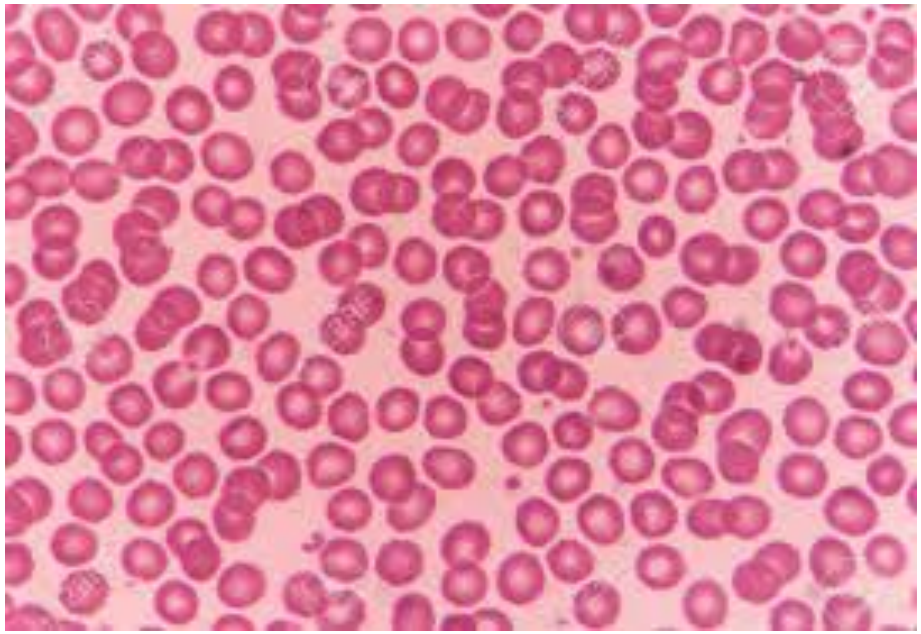
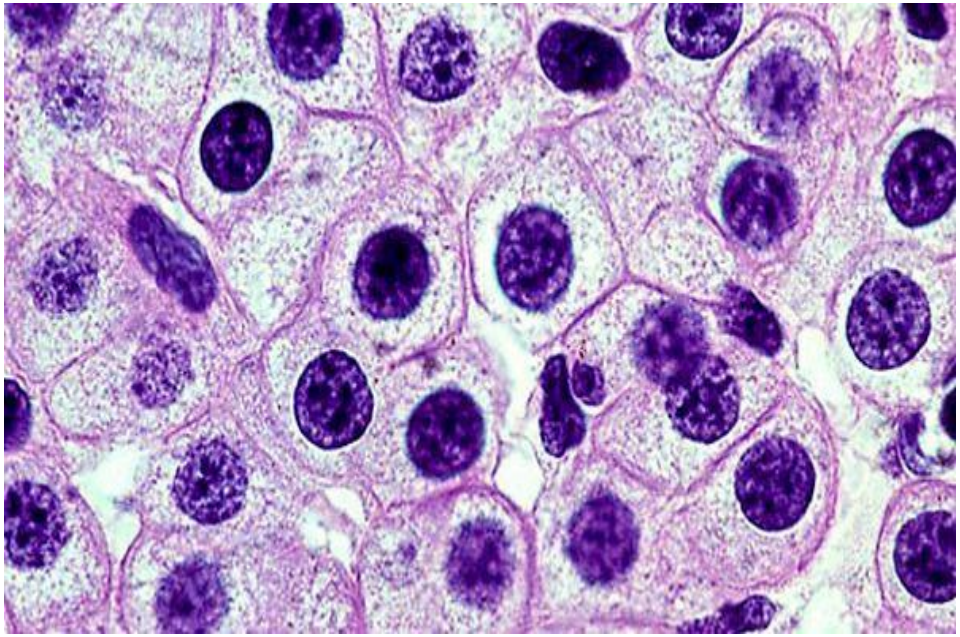
Stochastic Mechanisms of Cell-Size Regulation in Bacteria

Sizes and Shapes of Biological Cells



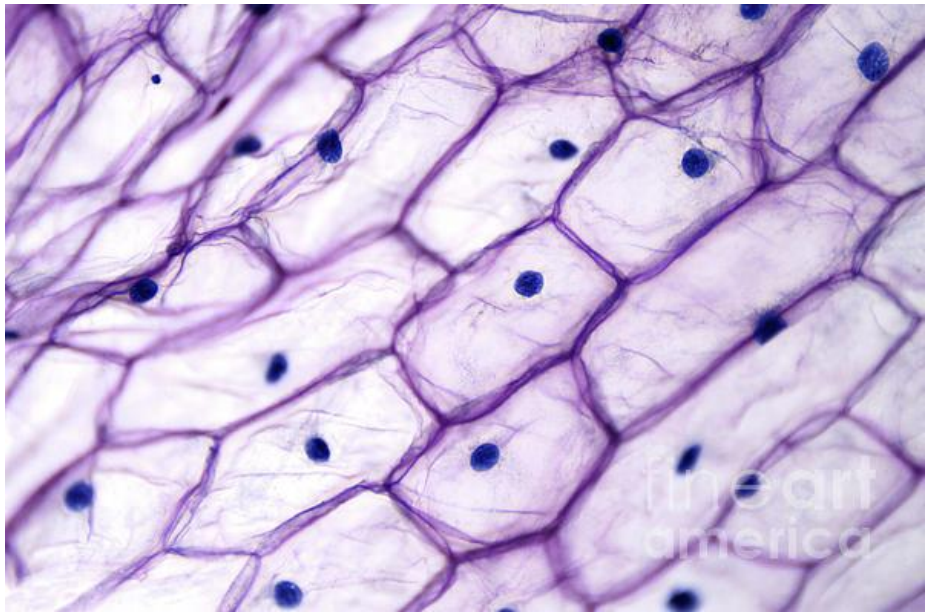
Normal blood cells under microscope



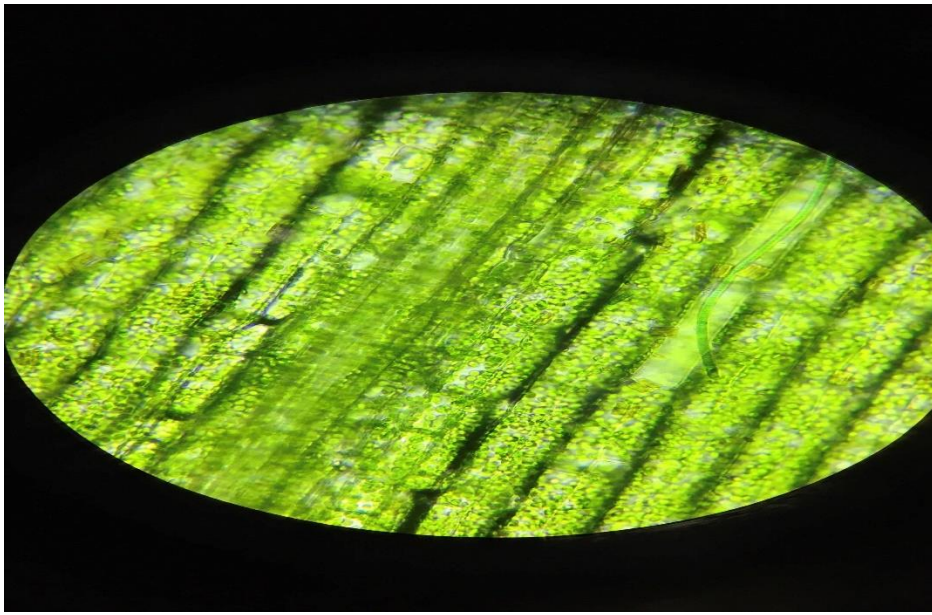
Human skin cells under microscope

Cells – building blocks of all biological matter

Sizes and Shapes of Biological Cells

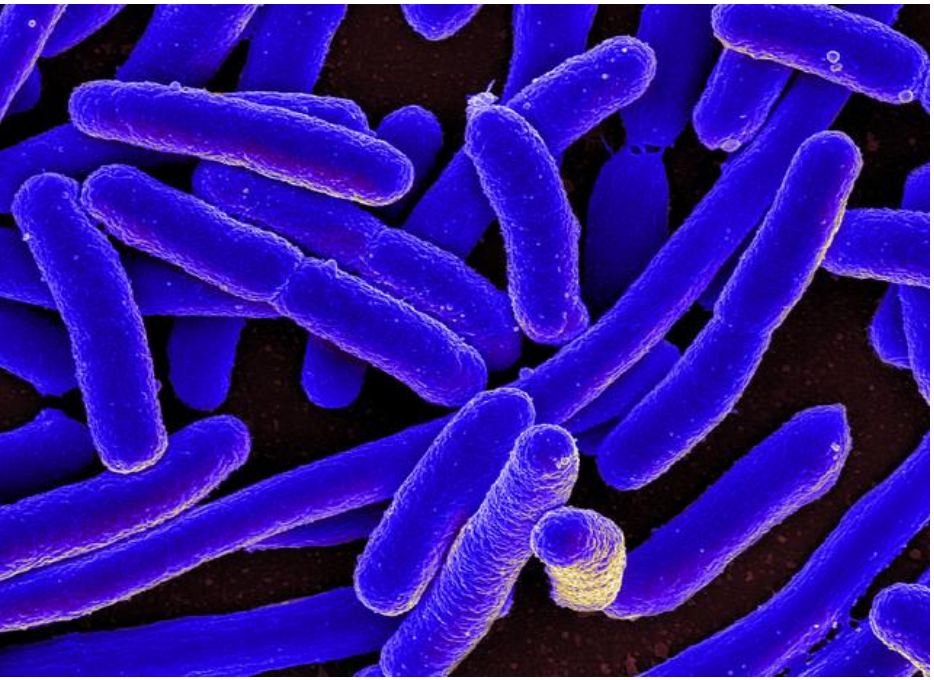


Onion epidermis cells under microscope

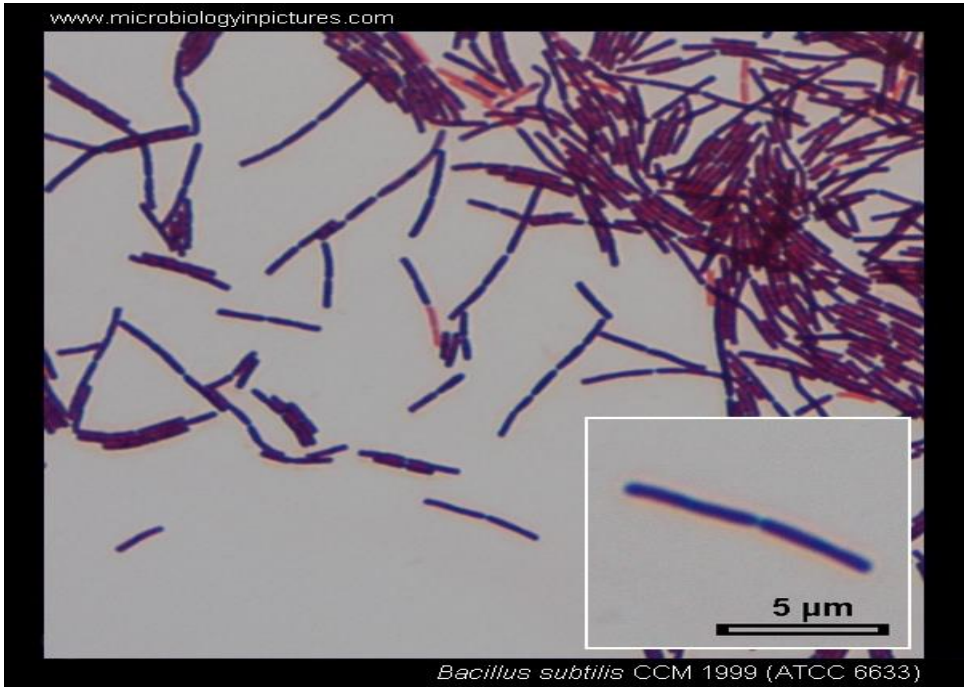


plant cells under microscope

Sizes and Shapes of Biological Cells



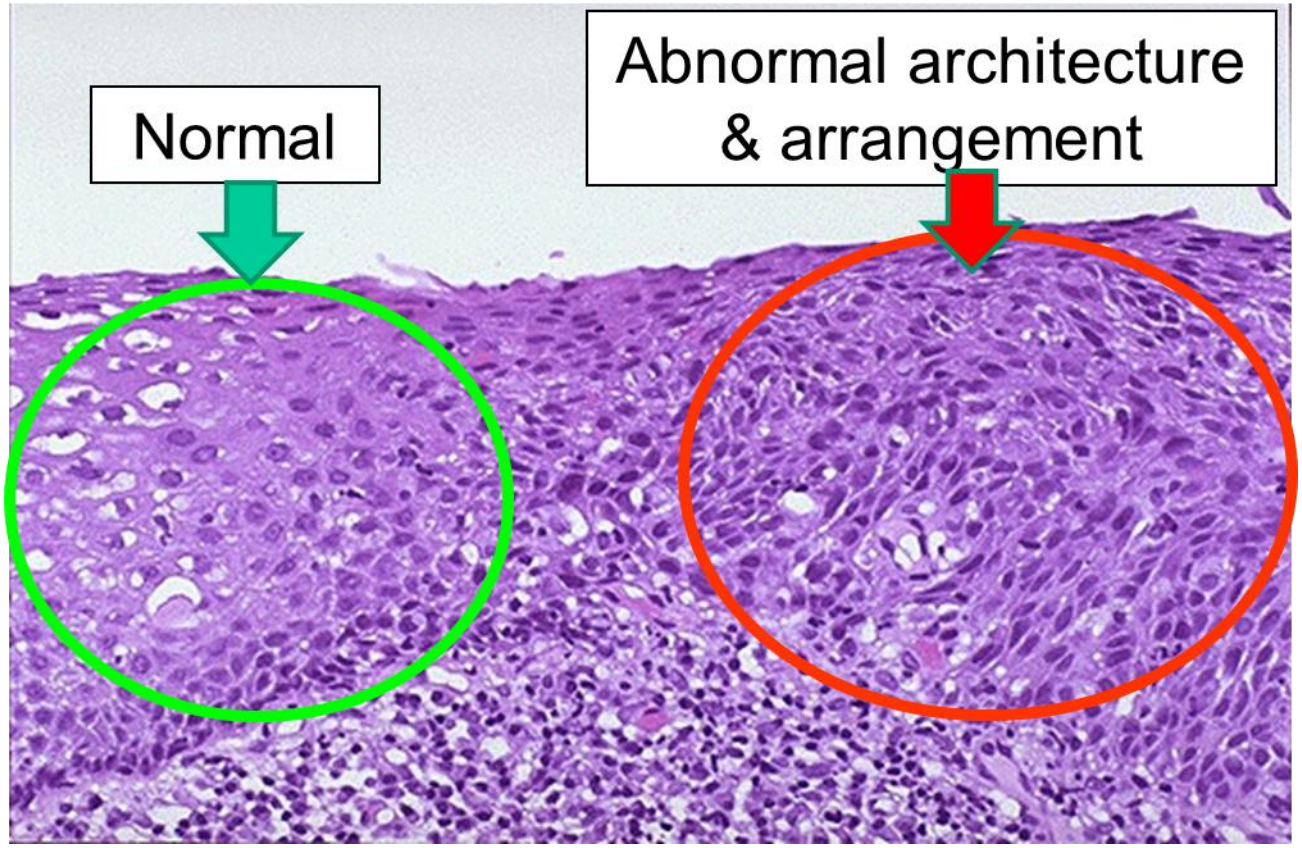
E.Coli bacteria



Bacillus subtilis bacteria

Striking observations: while for different organisms cell sizes and cell shapes might deviate strongly, for a given organism and tissue they are remarkably similar

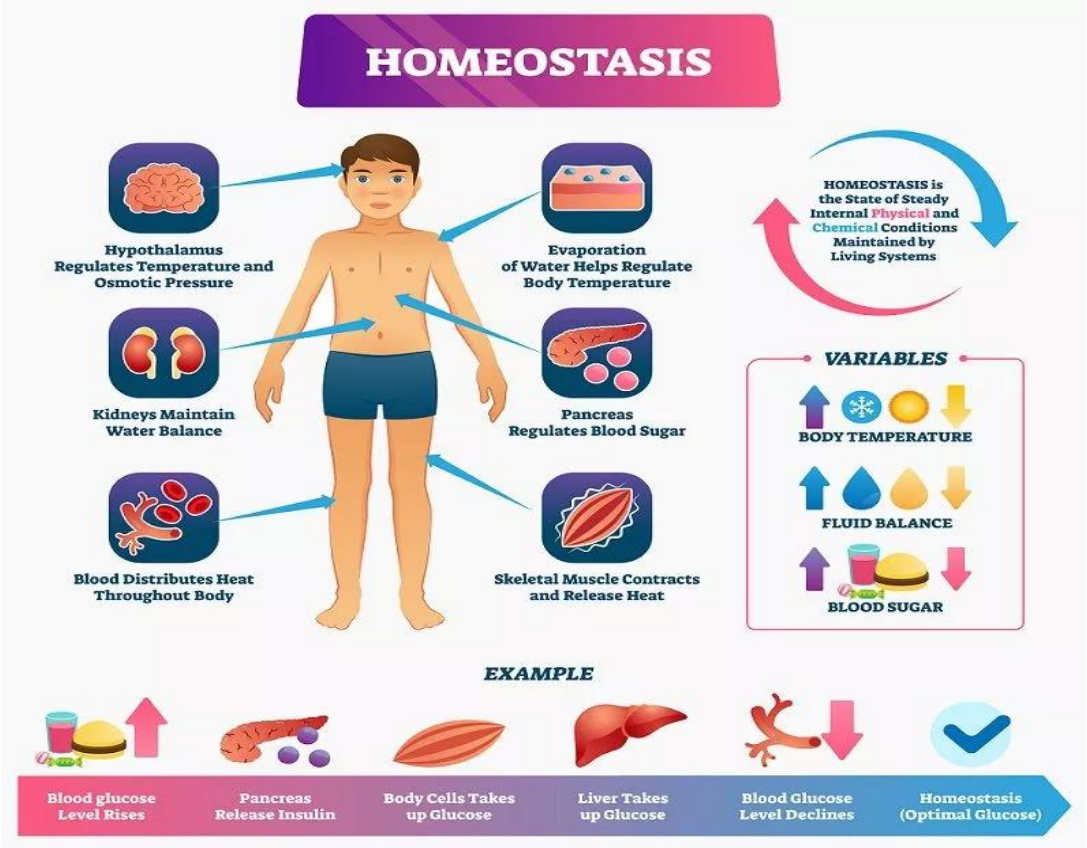
Loss of Cell Size Uniformity is a Sign of Deceases



Deviations from the cell size uniformity is still one of the best markers for cancer!

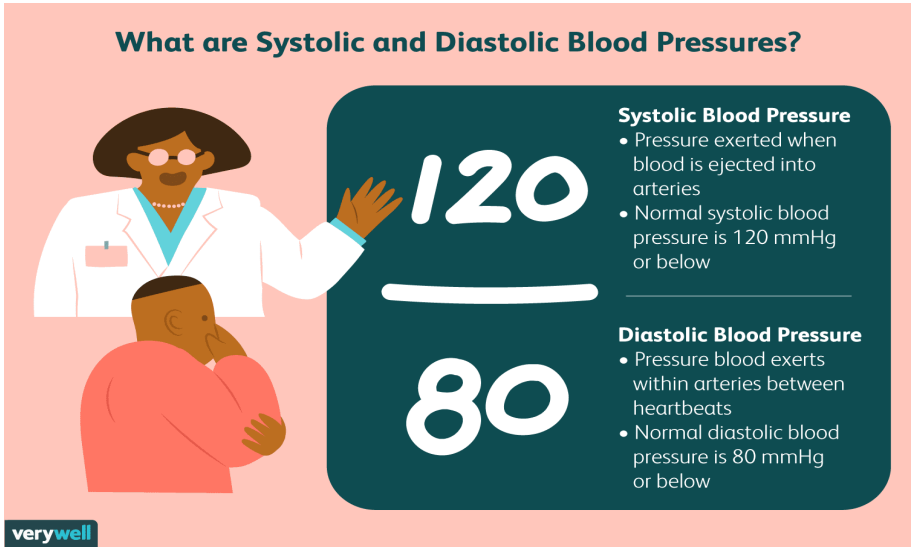
Cell-Size Uniformity is a Consequence of Homeostasis

Tendency to maintain constant physiological properties (blood pressure, sugar level, body temperature, etc.).



It is believed that cell size uniformity leads to the most efficient functioning of organism.

Homeostasis



Healthy Blood Sugar Levels

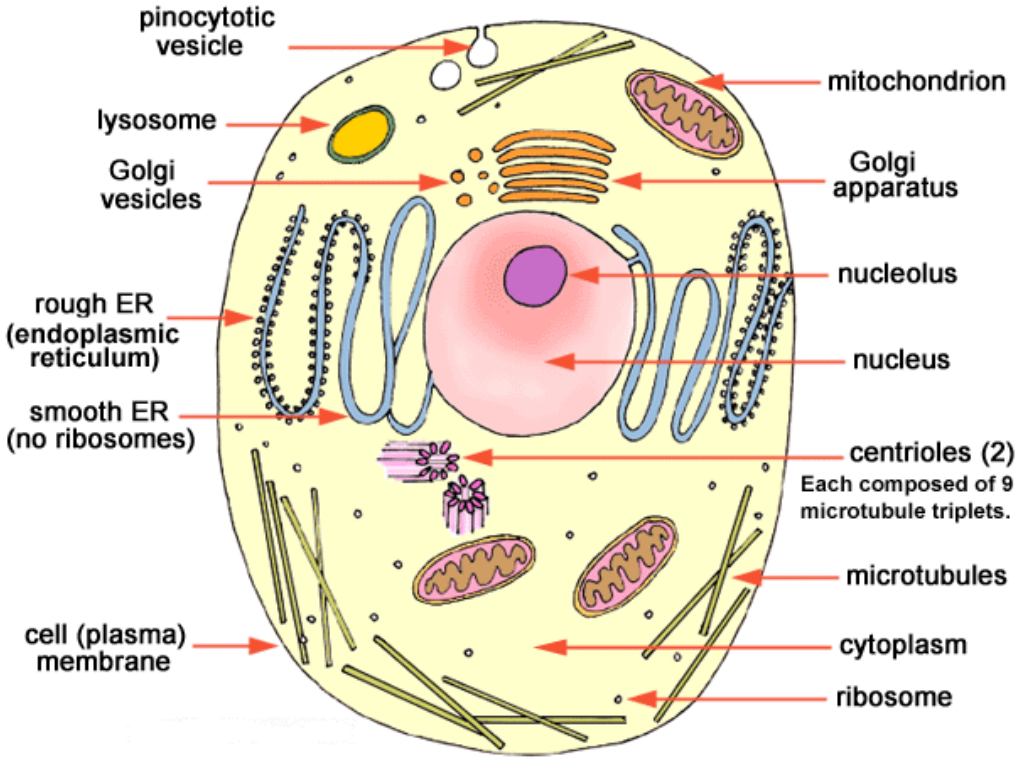
TIME	WITHOUT DIABETES	WITH DIABETES
Fasting	70–99 mg/dL	80–130 mg/dL
1–2 hours after meals	< 140 mg/dL	< 180 mg/dL
A1C test	< 5.7%	< 7%

Source: American Diabetes Association

INSIDER

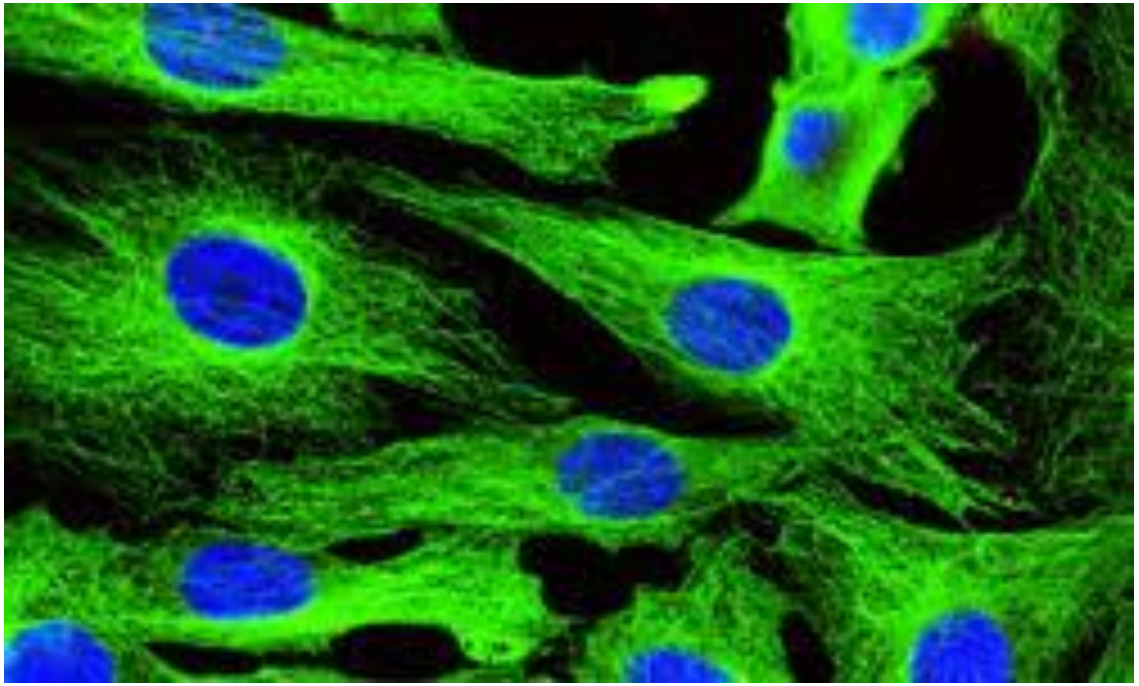
Homeostasis is a dynamic state of "equilibrium" (actually, steady-state) in biological systems that describes the most optimal functioning of the organism.

Regulation of Cell Size



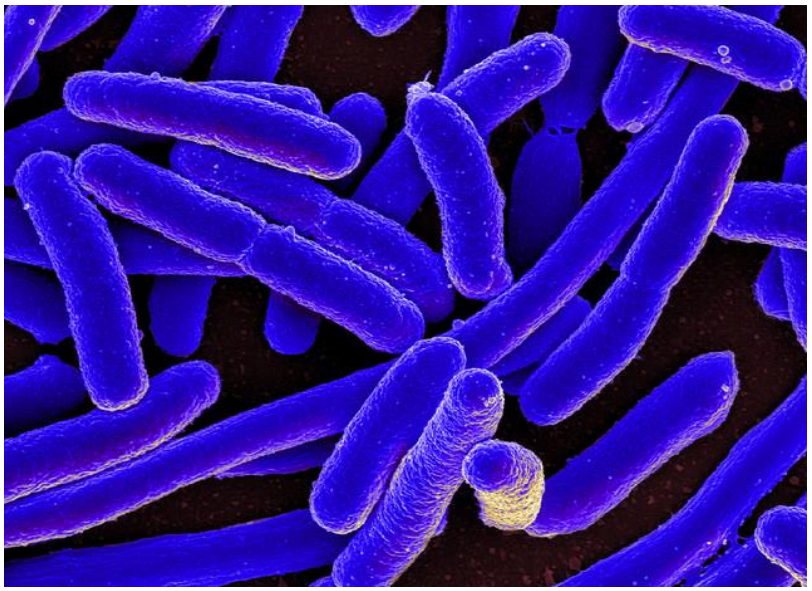
Specific cell size is the result of the balance between cell growth (mass accumulation) and proliferation (cell division). But both of these processes are independent. What are the mechanisms of the cell-size regulation?

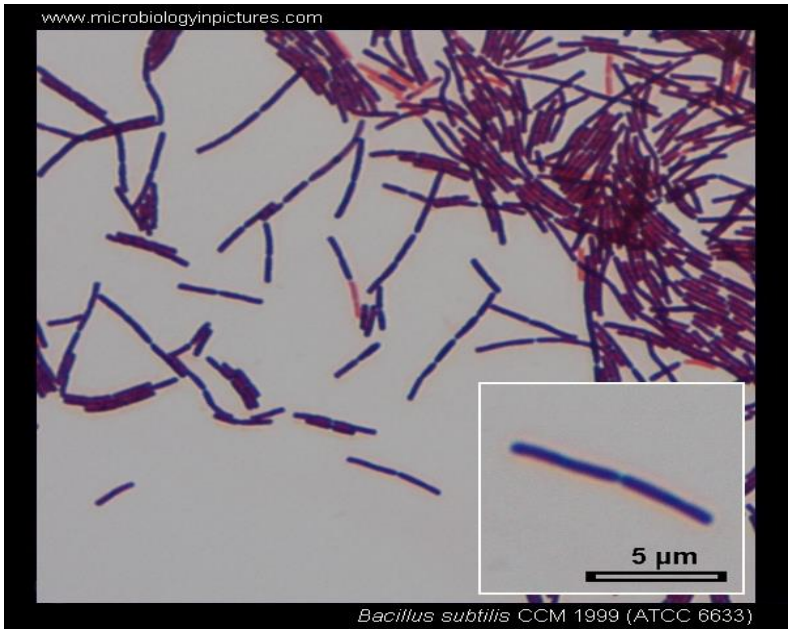
Regulation of Cell Size



A concept of "cell size checkpoint" have been proposed for mammalian cells. The idea is that there are specific biochemical pathways that regulate the cell size.
Some supporting information has been received – but still very limited.

Bacterial Cells



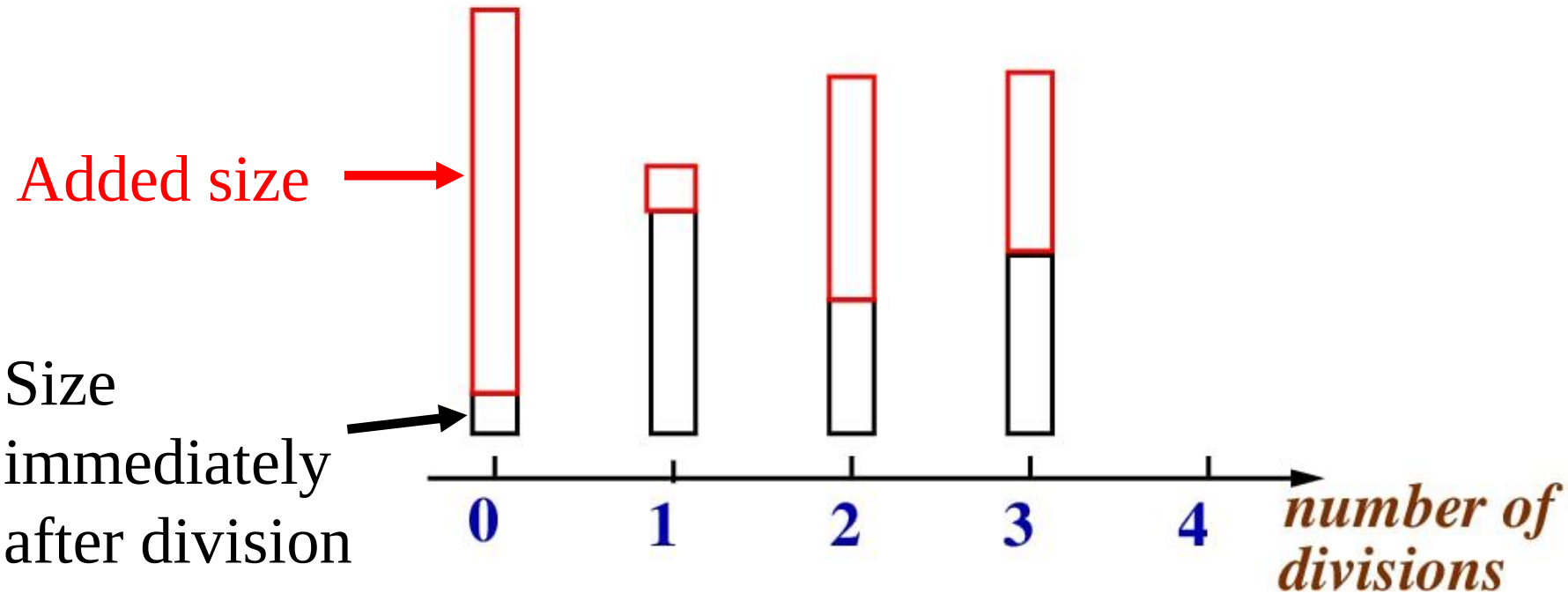


Unlike eukaryotes, bacteria do not have cell-size checkpoints.

But what are the mechanisms of cell-size control in bacteria?

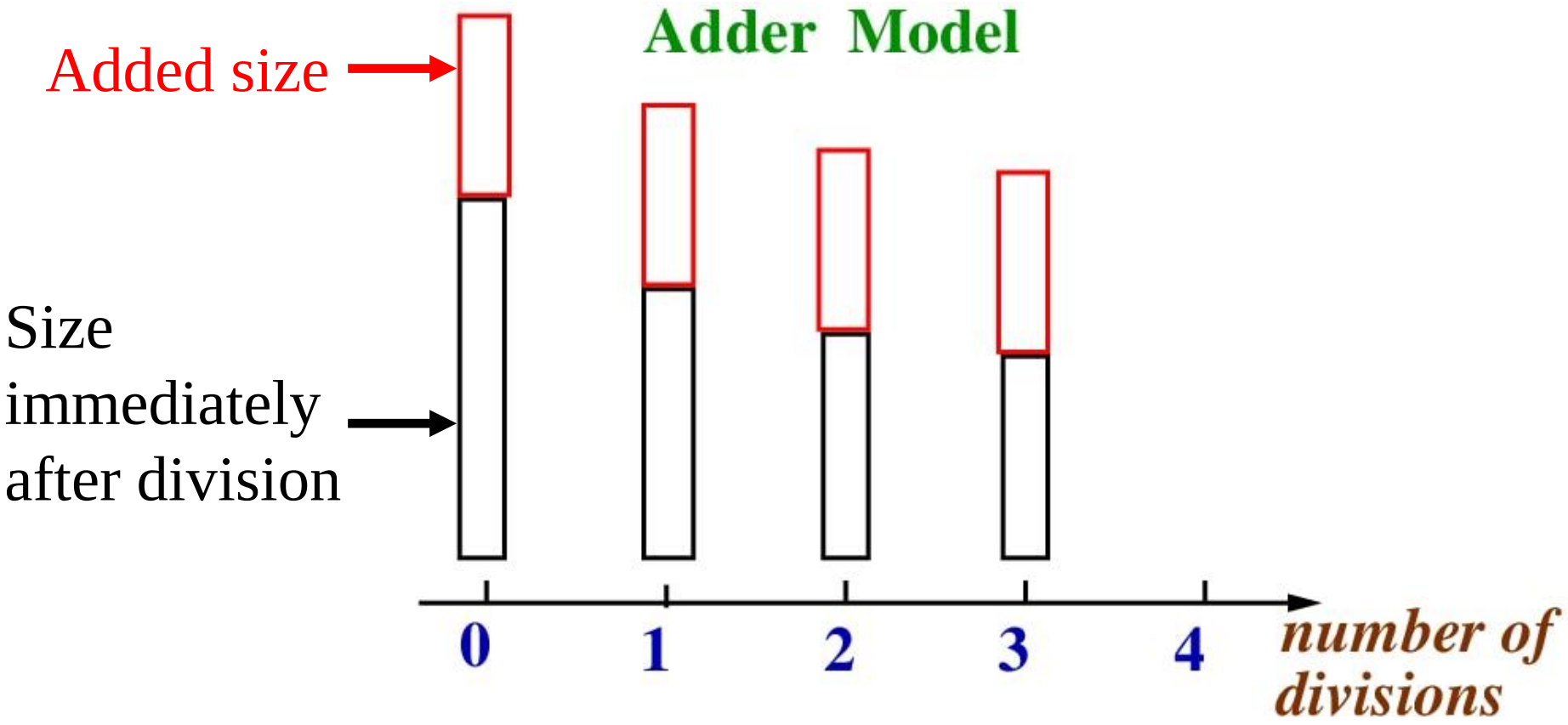
Theoretical Ideas

Sizer Model



The sizer model assumes that the constant cell size is a result of a regulation mechanism that selectively restricts the growth of large cells and promotes the growth of small cells.

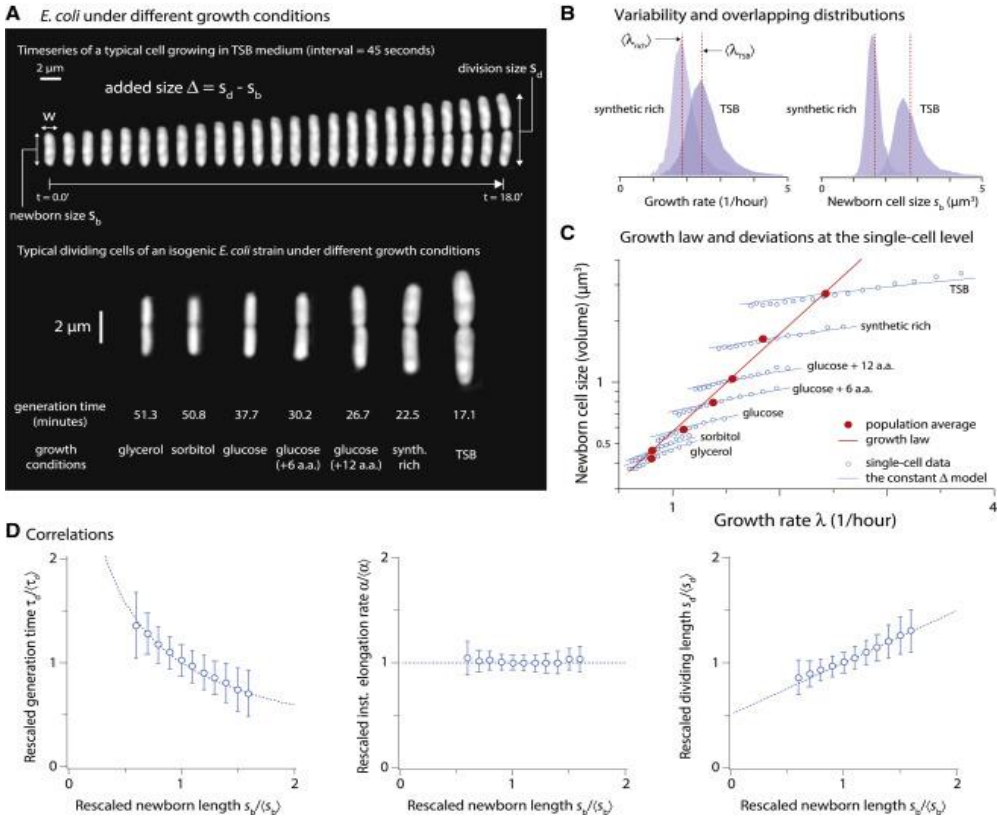
Theoretical Ideas



The adder model assumes that all types of cells, small or large, between divisions accumulate the same amount of mass.

Experimental Studies

Population data for mammalian and yeast cells are difficult to interpret. But the use of single-cell microfluidic devices (“mother machine”) for bacteria provided a lot of quantitative information on mechanisms of cell-size regulation.

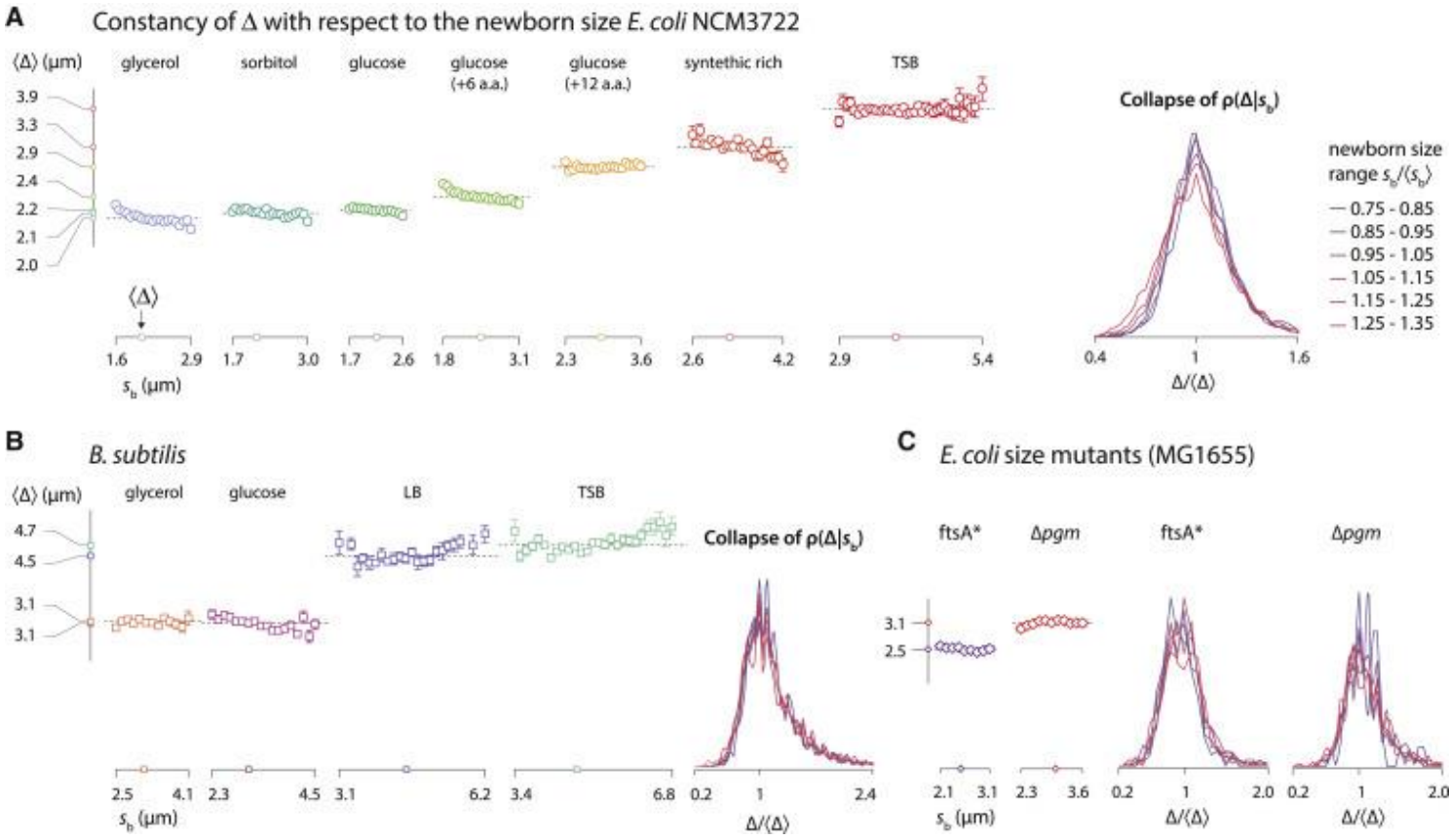


Cell-Size Control and Homeostasis in Bacteria, S. Taheri-Araghi et. al., *Current Biology*, **25**, 385-391, 2015

Experimental Studies

E.coli

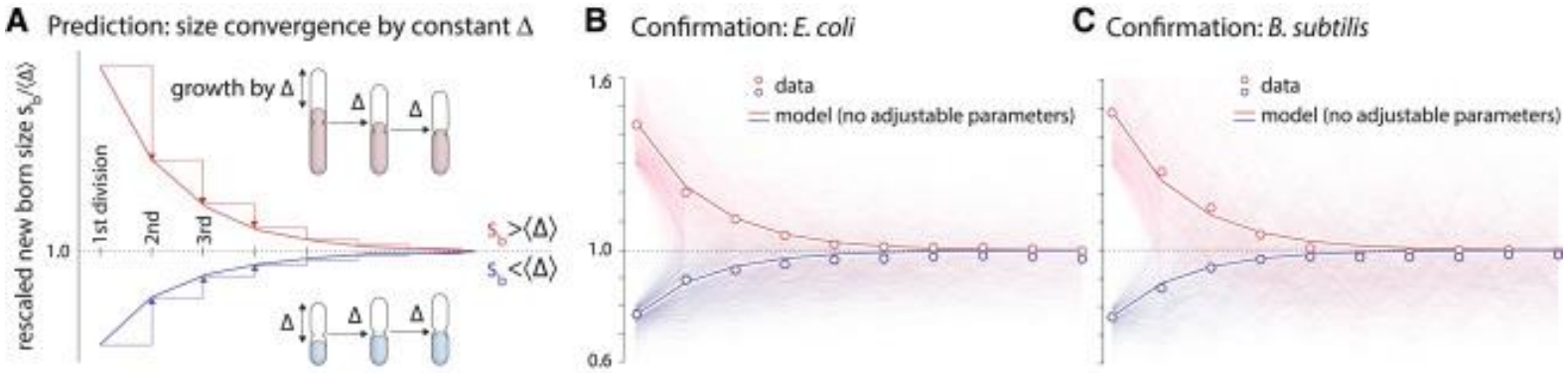
B. subtilis



Experimental data clearly support the Adder Principle

Cell-Size Control and Homeostasis in Bacteria, S. Taheri-Araghi et. al., *Current Biology*, **25**, 385-391, 2015.

Current Theoretical Views

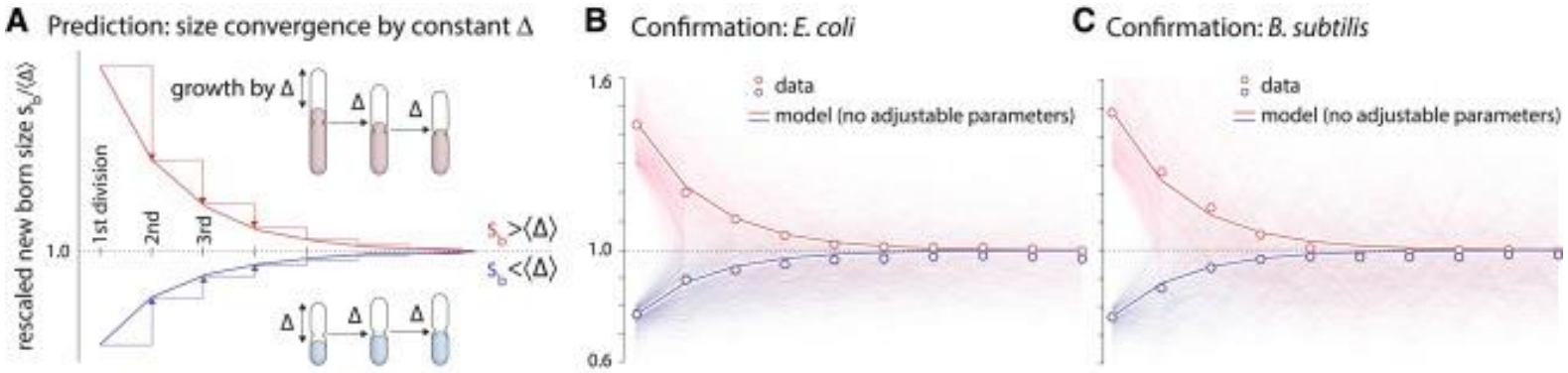


Cell-size regulation is a consequence of two general processes:

1) Balanced biosynthesis – in other words, the number of proteins responsible for cell growth and cell division is always proportional to the cell volume and it is established much faster than growth or proliferation time scales.

This is very reasonable.

Current Theoretical Views

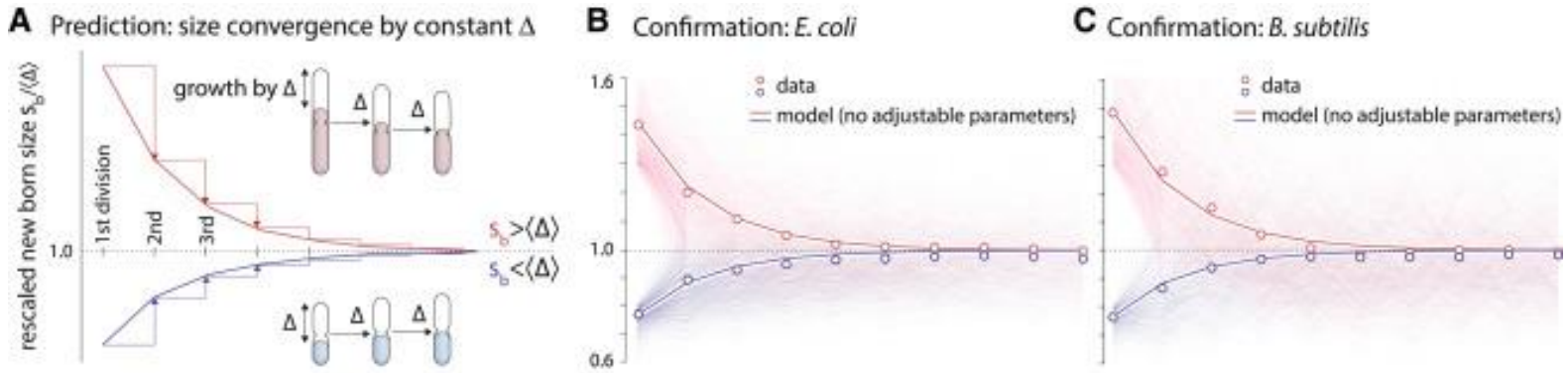


Cell-size regulation is a consequence of two general processes:

2) A threshold accumulation of division initiators and precursors to a fixed number.

Very problematic!!! A specific set of biochemical and biophysical process must be responsible for the appearance of such thresholds, but none have been identified in experiments.

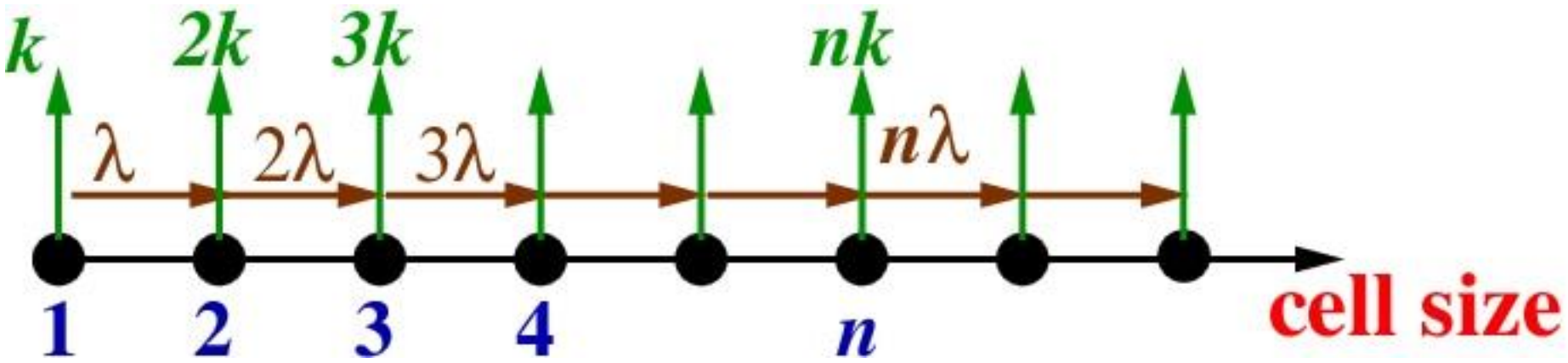
Our Hypothesis



Cell-size regulation is governed by, at least, two stochastic processes (cell division and cell growth) that are coupled together.

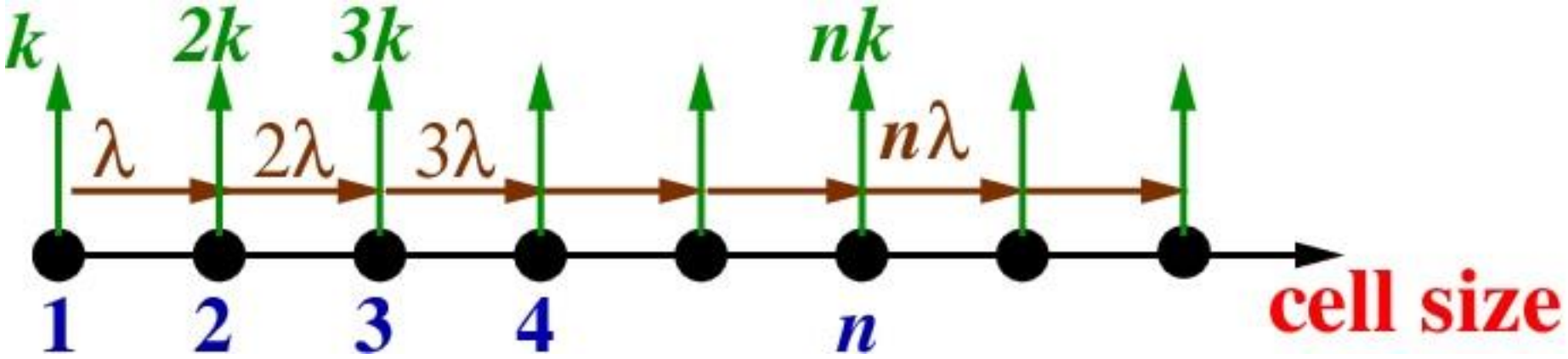
The assumption of stochasticity eliminates the need of using the thresholds, which is the weakest point of current theoretical approaches.

Discrete-State Stochastic Model of Cell-Size Regulation



1) 1D description of the cell growth. Because the majority of the bacteria are rod-like and mostly change their length.
2) The size of the bacteria is associated with a discrete variable ***n***, which corresponds to the number of proteins responsible for the growth or division. It can be a number of FtsZ proteins, for example.

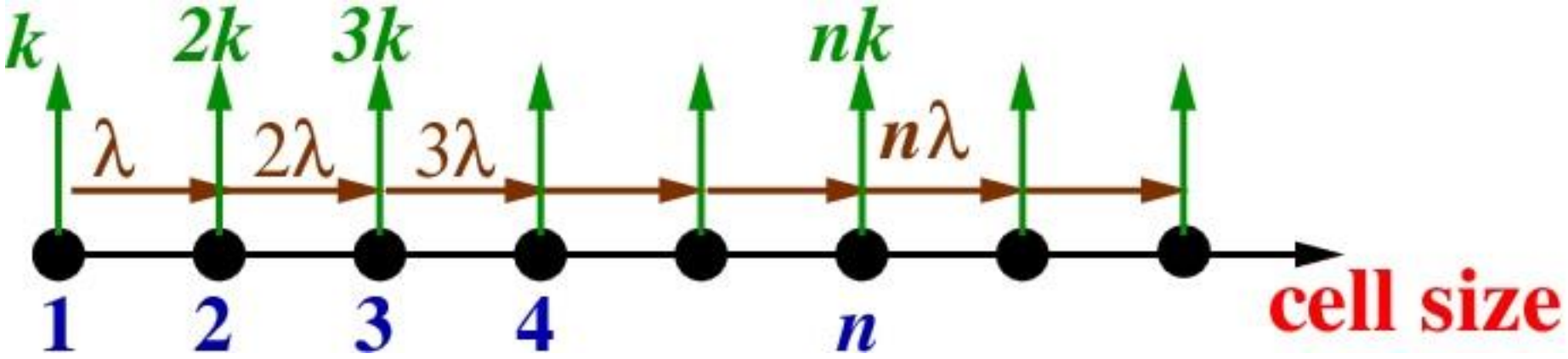
Discrete-State Stochastic Model of Cell-Size Regulation



3) It is assumed that proteins responsible for cell growth and division are formed much faster that the growth and division rates. Reasonable for typical conditions when nutrients are sufficiently available.

4) The number of proteins responsible for growth and division is always proportional to ***n***. Consistent with experimental findings of the balanced biosynthesis.

Discrete-State Stochastic Model of Cell-Size Regulation

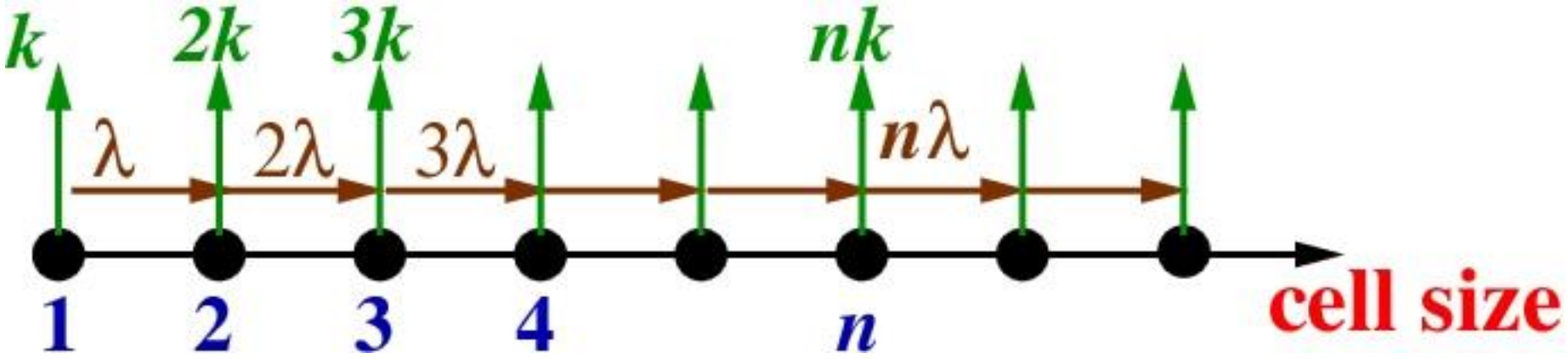


A minimal theoretical model.

Only 2 processes can happen: growth with a rate λn and division with a rate kn.

The growth and division processes are viewed as "chemical reactions." This allows to naturally introduce the stochasticity in the system.

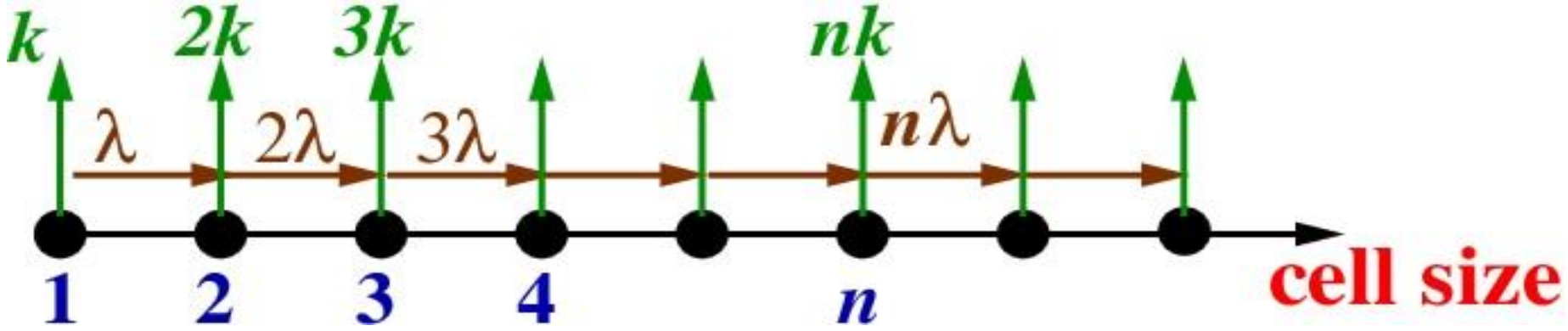
Discrete-State Stochastic Model of Cell-Size Regulation



IMPORTANT: the division can take place for the cells of ANY size.

But the rate of division is not the same and due to coupling to the growth, the division happens preferable at a relatively narrow range of cell sizes. This eliminates the need of using thresholds.

Theoretical Analysis



If the number of proteins responsible for growth and division is very high ($n >> 1$), a simple mean-field approach can be used to describe the dynamics of cell size.

Temporal evolution of average length:

$$\frac{d < n >}{dt} = \lambda < n > -2k < n > \frac{< n >}{2}$$

growth

Shortening: every division removes <n>/2, creates 2 cells

Theoretical Analysis

Temporal evolution of average length:

$$<n(t)> = \frac{c\lambda e^{\lambda t}}{1 + kce^{\lambda t}}$$

$$c = \frac{n_0}{\lambda - kn_0}$$

n_0 – initial length

$$n_{st} = \frac{\lambda}{k}$$

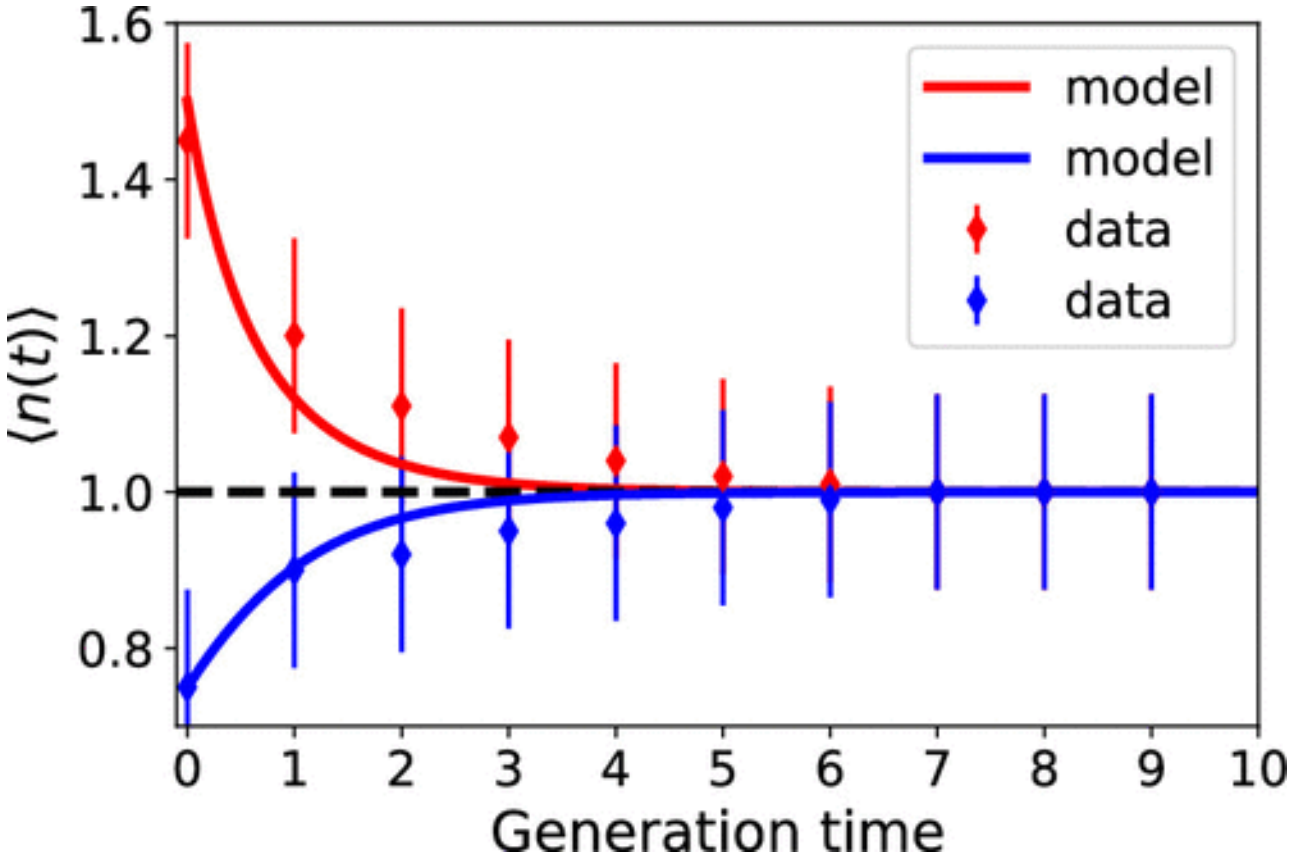
Stationary average length size (very large times)

$$\frac{< n(t) >}{n_{st}} = \frac{e^{\lambda t}}{\frac{n_{st}}{n_0} - 1 + e^{\lambda t}}$$

Temporal evolution of the average length in the dimensionless form

Theoretical Analysis

Normalized cell length as a function of the number of divisions:

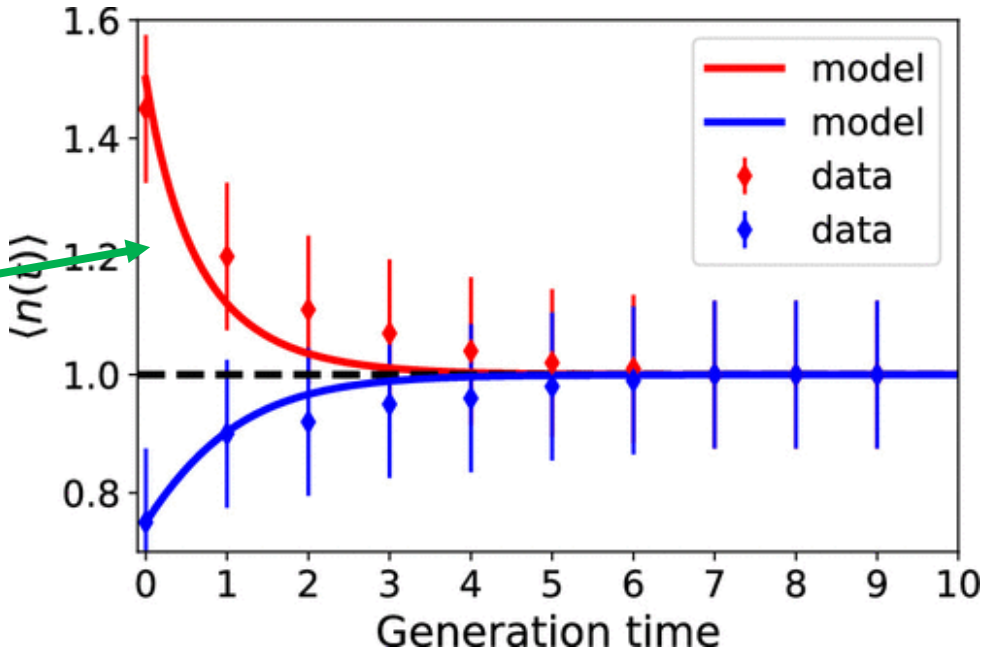


$$\frac{< n(t) >}{n_{st}} = \frac{e^{\lambda t}}{\frac{n_{st}}{n_0} - 1 + e^{\lambda t}}$$

Theoretical Analysis

Several observations:

1) Excellent agreement with experimental data on E.coli and B.subtilis bacteria (without fitting parameters!);
2) Bacteria can reach the homeostasis quickly (after 4-5 divisions) even when the original lengths deviate more than 50%



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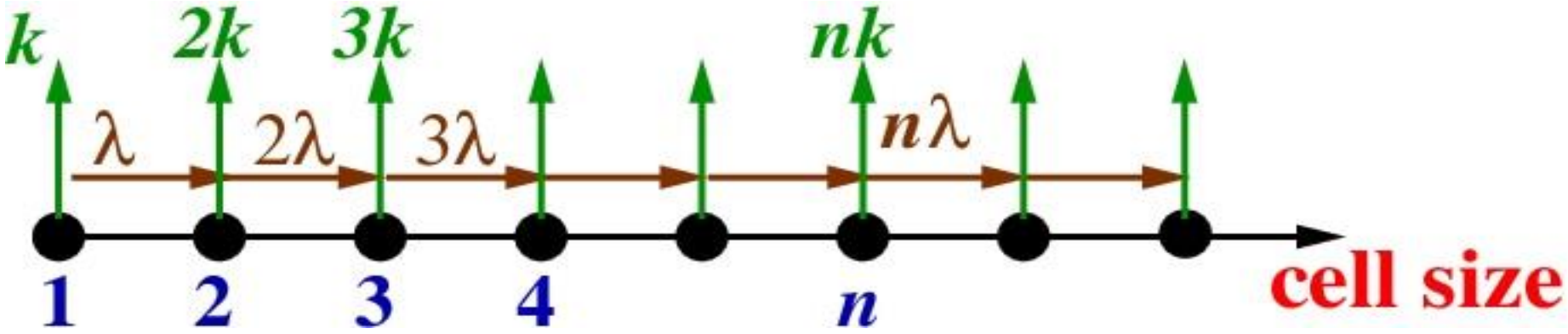
Theoretical Analysis



At steady-state, more precise analysis (without mean-field assumptions) can be made.

The probability to divide at the cell length m:

$$p_m = p = \frac{km}{km + \lambda m} = \frac{1}{1 + \lambda/k}$$

In our stochastic model, the probability of division is always constant and independent of the cell length!

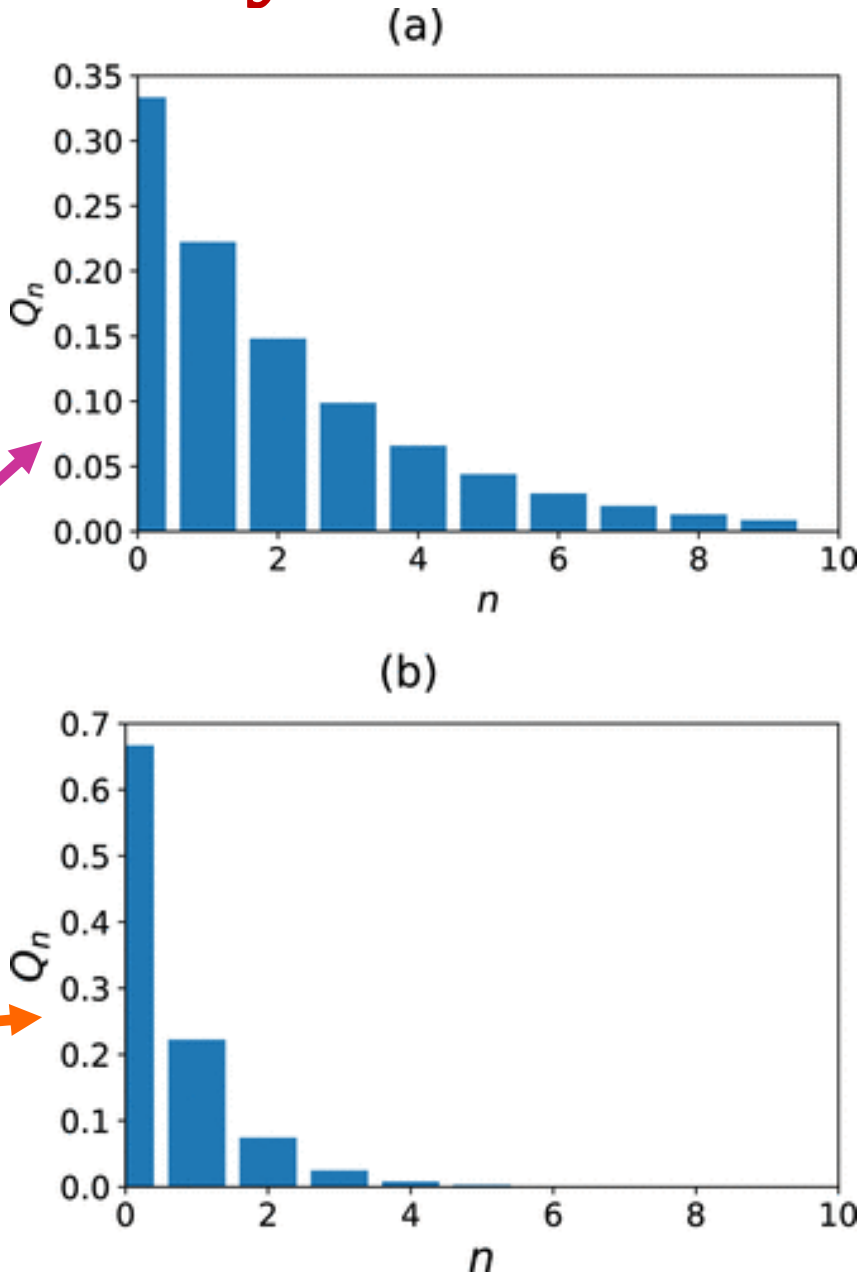
Theoretical Analysis

The probability to divide after adding exactly $\boldsymbol{n}$ units to the cell with the original length $\boldsymbol{n_0}$:

$$\boldsymbol{Q_n = p(1-p)^n}$$

Growth is faster

Division is faster



Theoretical Analysis

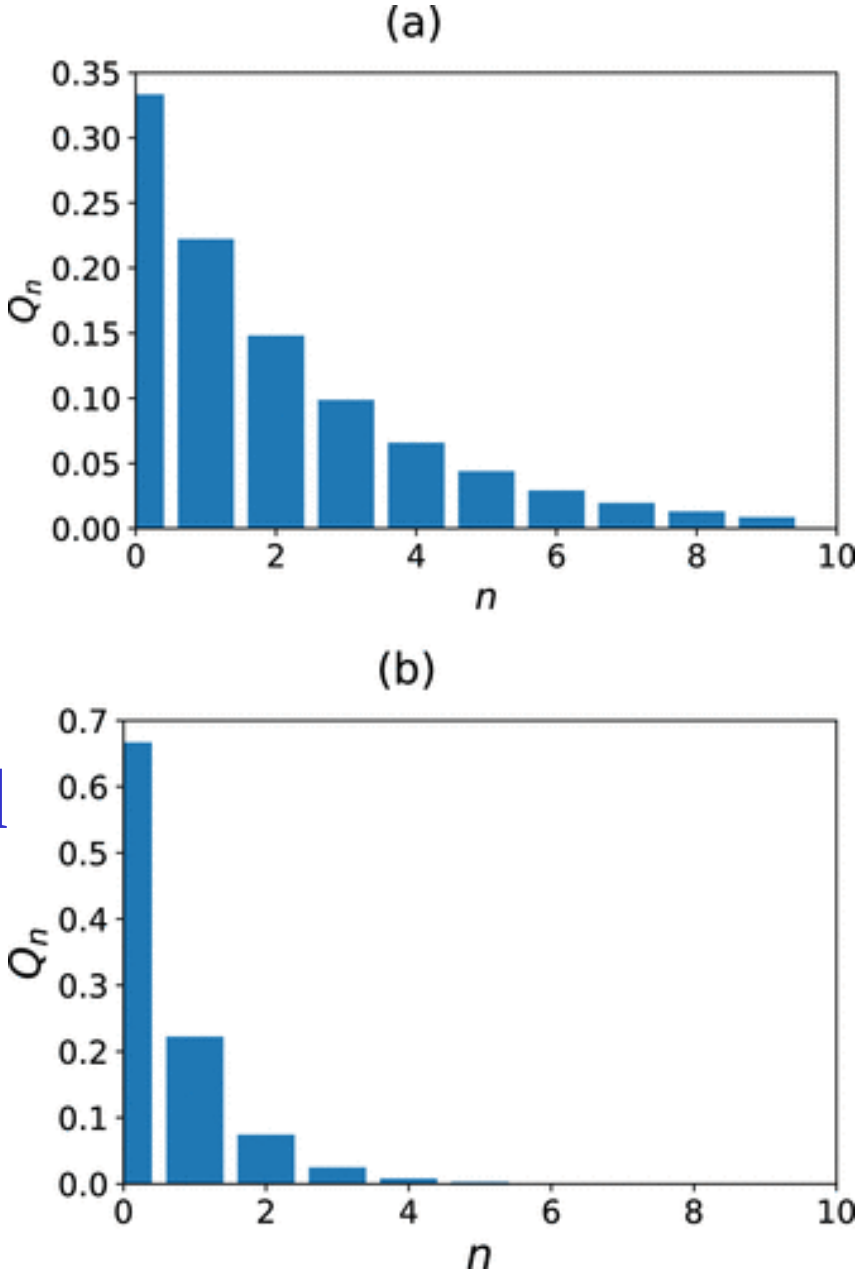
Average added length can be explicitly calculated:

$$< l > \equiv l = \sum_{n=0}^{\infty} n\, Q_n = \lambda / k$$

Important observations:

1) Independently of the initial size, the same length is added between divisions – agrees with experimentally supported Adder Principle!

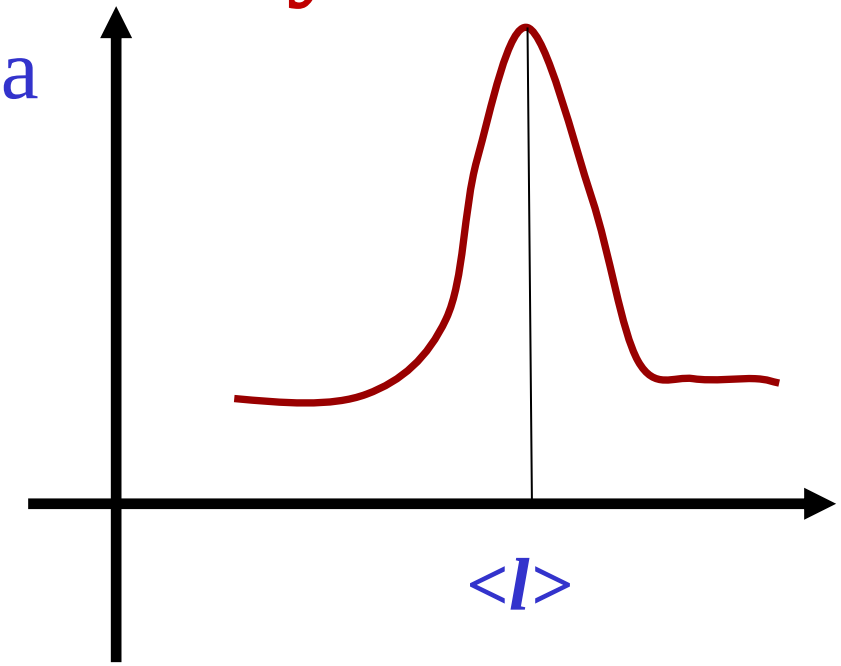
2) Average added length is equal to the average cell length.

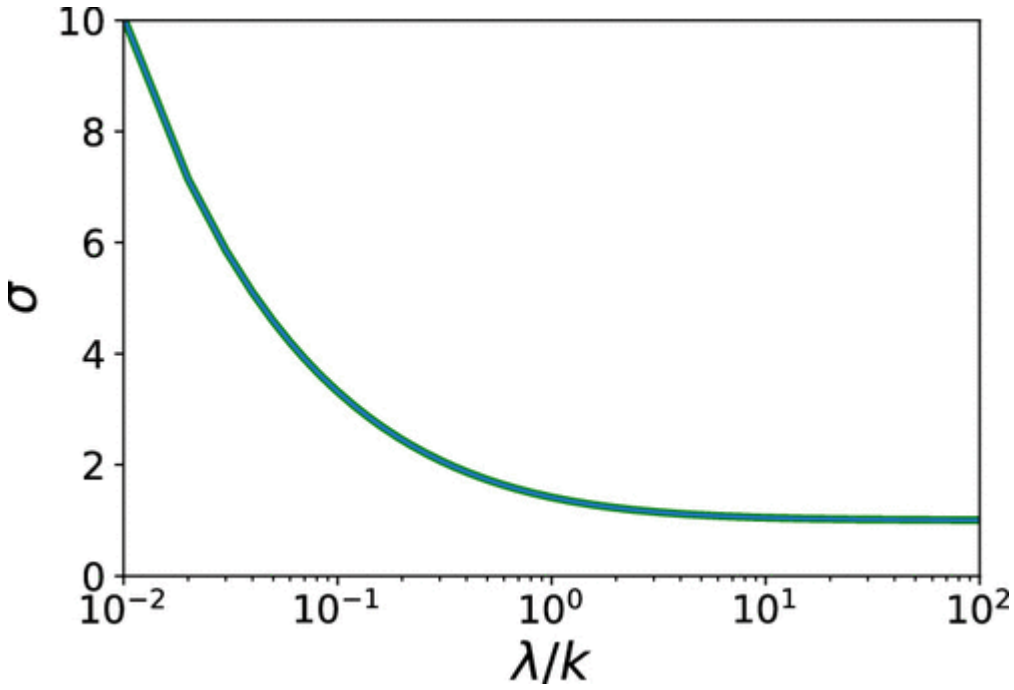


Theoretical Analysis

Due to stochasticity, there is a distribution of added lengths

$$\sigma = \sqrt{\frac{1 + \lambda/k}{\lambda/k}}$$

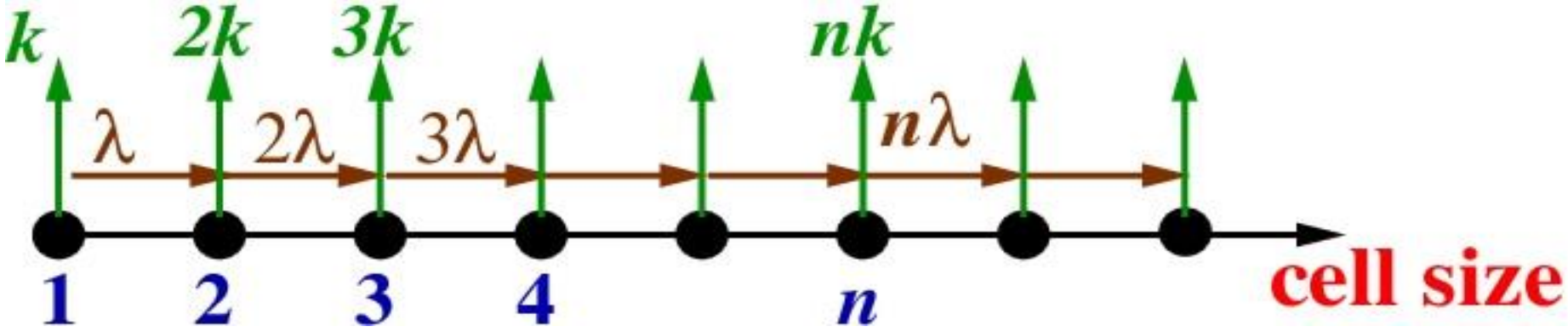




Normalized variance (CV) of the added cell length.

The faster the growth, the narrower the distribution.

Theoretical Analysis

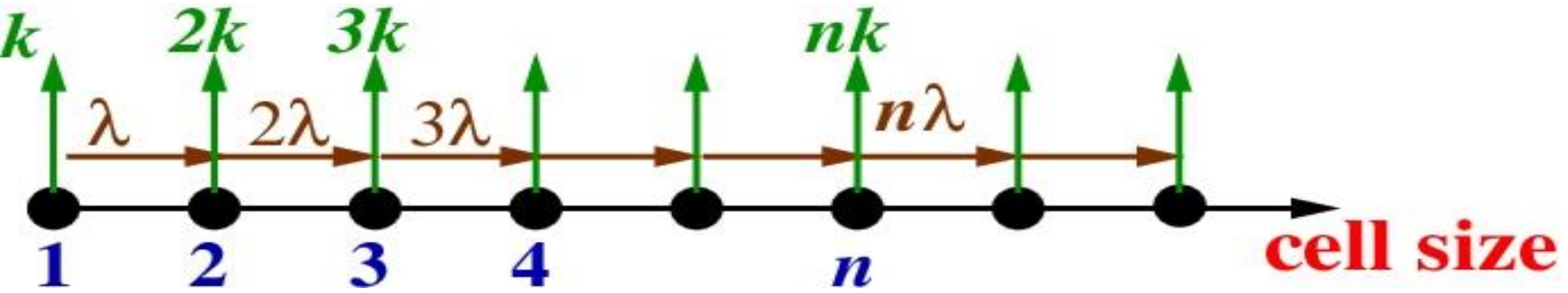


Our theoretical approach can also evaluate the dynamics of cell-size regulation. It can be done using the method of first-passage probabilities.

We can explicitly evaluate the distribution of division times and its moments – probability of division, mean inter-division times, variances, etc.

J. Phys. Chem. Lett. 2020, **11**, 8777-8782

Theoretical Analysis



Consider that the system starts from the size n_0 and the division happens exactly after adding the size l. One defines then a function $F_m(t)$ as a probability density function of dissociating exactly at the length n_0+l for the first time at time t if at $t=0$ the system started at the length m. Temporal evolution of these functions are governed by backward master equations.

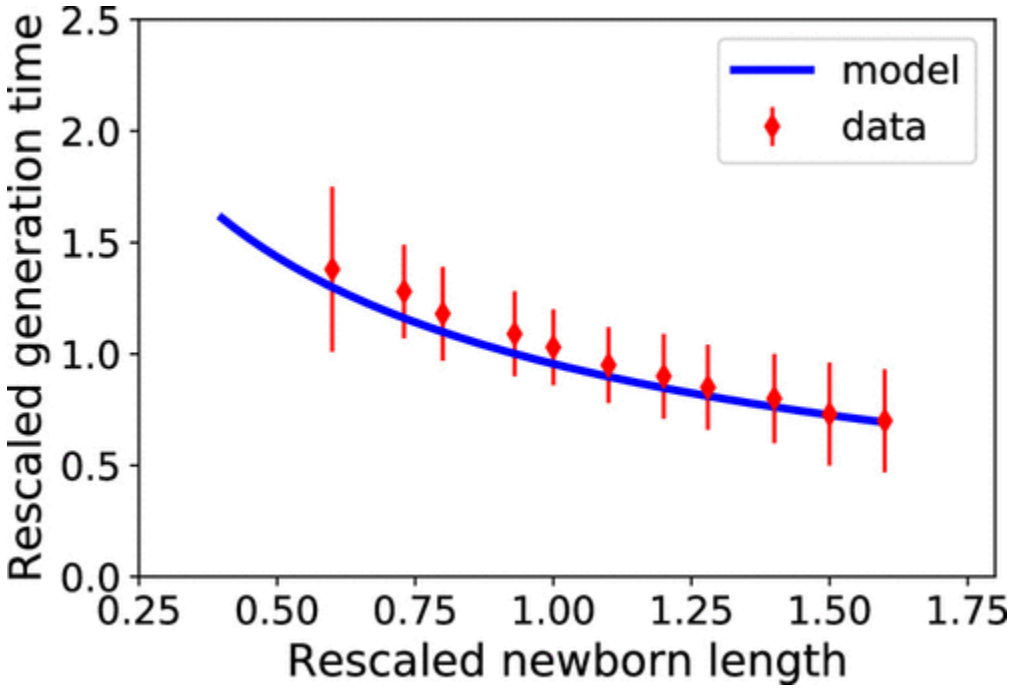
Theoretical Analysis

Probability of adding exactly the length l

$$\Pi_{n_0} = p(1-p)^l$$

Mean inter-division times after adding exactly the length l

$$T_{n_0} = \frac{1}{k+\lambda} \sum_{j=0}^{l} \frac{1}{n_0 + j}$$

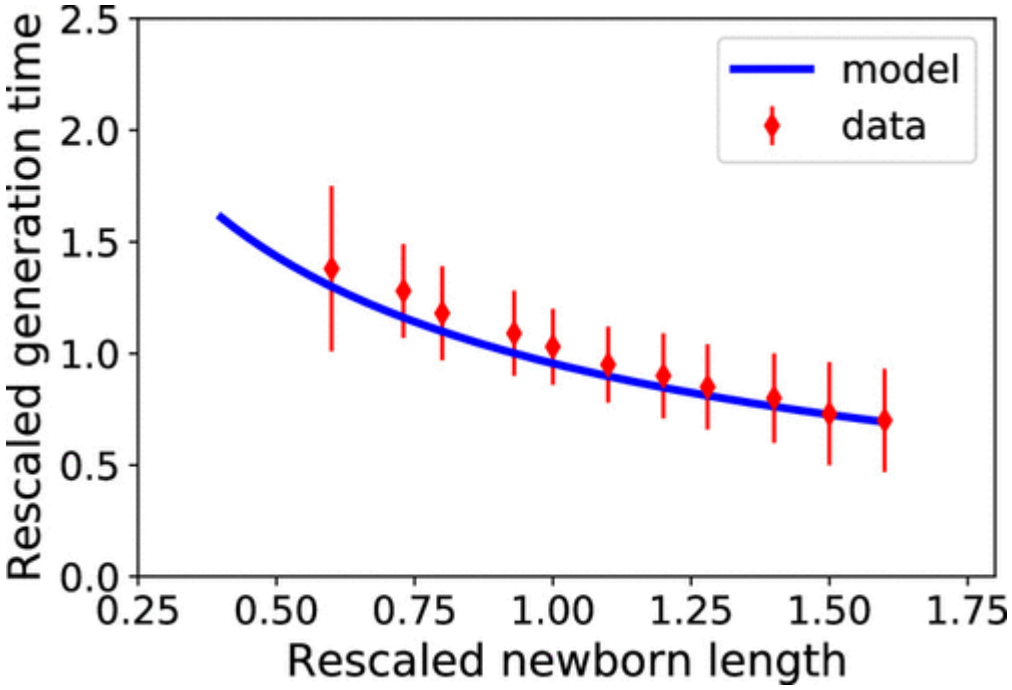


Comparison with experimental observations on E.coli bacteria

Theoretical Analysis

We predict that the mean inter-division times decrease with the newborn length. This is because for larger cell sizes the length ***l*** can be added faster.

Fully agrees with experimental observations!!!

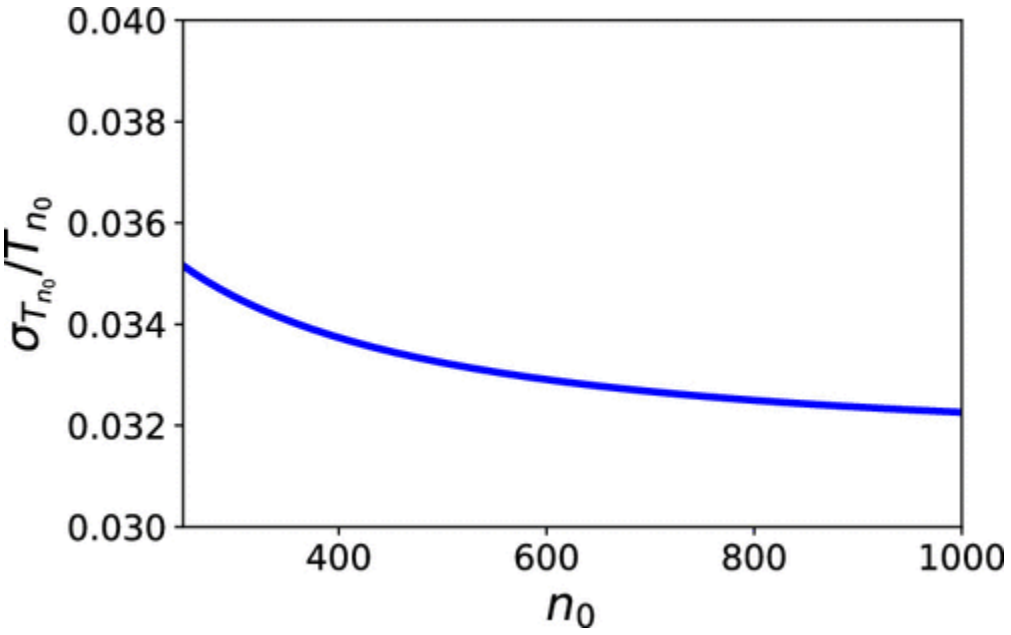


$$T_{n_0} = \frac{1}{k + \lambda} \sum_{j=0}^{l} \frac{1}{n_0 + j}$$

Theoretical Analysis

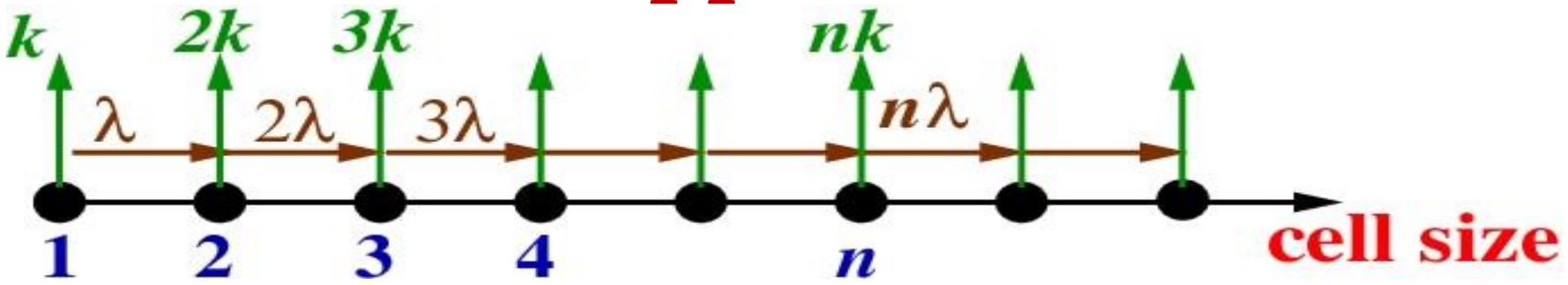
We predict that the stochastic noise in the system (viewed as a normalized variance of division times) is almost independent of the newborn length.

The trend is consistent with experimental observations!!!



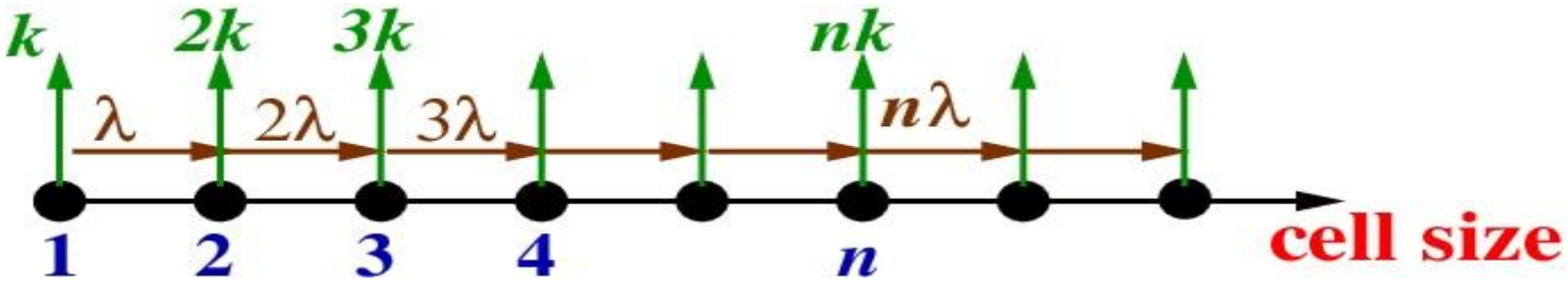
$$\sigma = \frac{1}{k+\lambda}\sqrt{\sum_{j=0}^{l}\frac{1}{(n_0+j)^2}}$$

Advantages of Our Theoretical Approach



1) Presents a physically simple stochastic picture of cell-size regulation in bacteria;
2) Simple model that can be solved analytically for both size distributions and for dynamics of cell regulation;
3) The reliance on stochastic mechanisms allows us to eliminate the need to use the concept of thresholds.
4) A very good agreement with single-cell experimental data on various bacteria is obtained.

Our View of Cell-Size Regulation



Almost deterministic cell-size regulation is achieved via a proper coupling of several stochastic processes (cell growth and cell division).

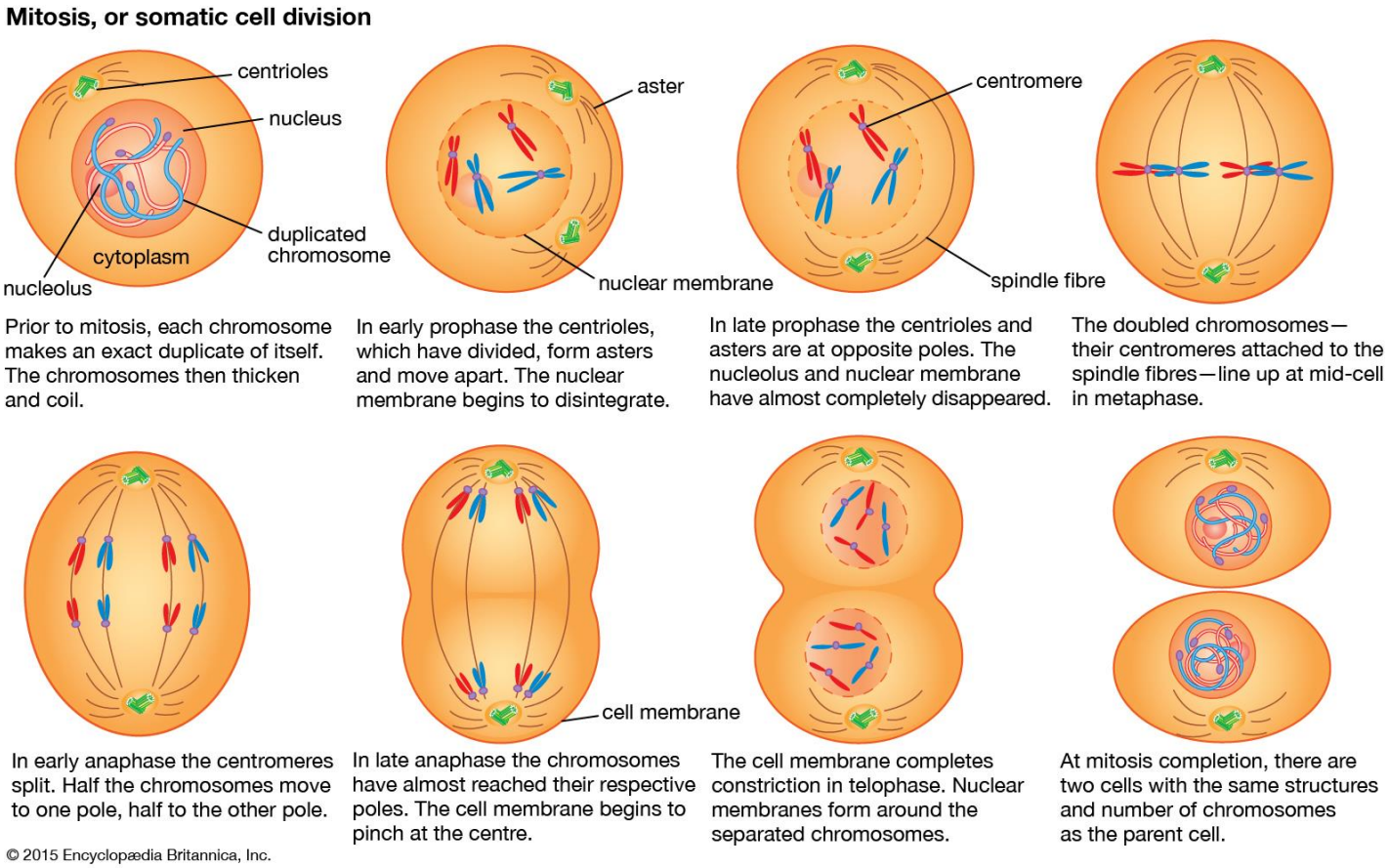
For short lengths, division rates are too small and they grow forward.

For long lengths, the division rate are high and such lengths cannot exist.

The average length is determined by the balance by growth and division.

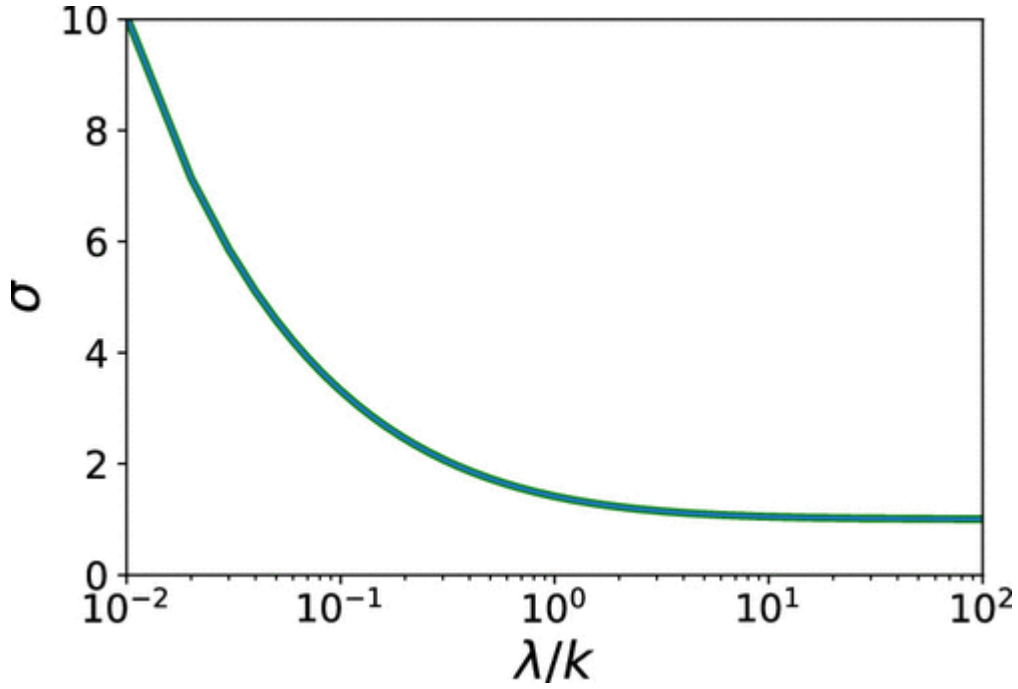
Critical Comments

1) A complex set of multiple biochemical and biophysical transitions are lumped into two stochastic processes (cell growth and cell division).



Critical Comments

2) Some experimental data are not fully captured.



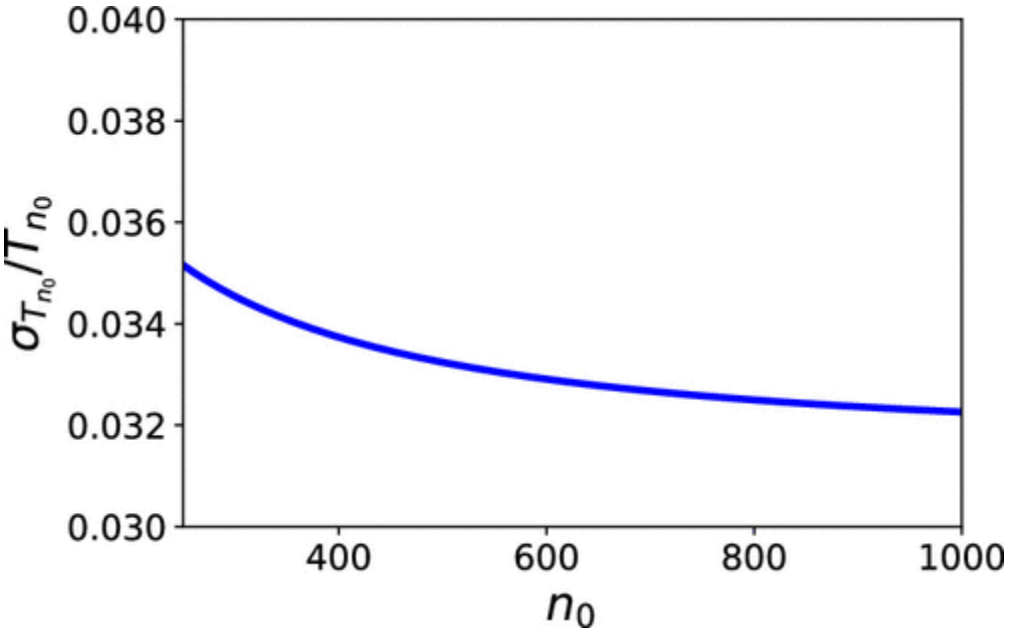
Normalized variance of the added cell length.

We predict that for real cellular conditions CV~1, while experiments suggest CV~0.2-0.3.

Can be corrected by taking into account more intermediate chemical states during the growth and division?

Critical Comments

2) Some experimental data are not fully captured.



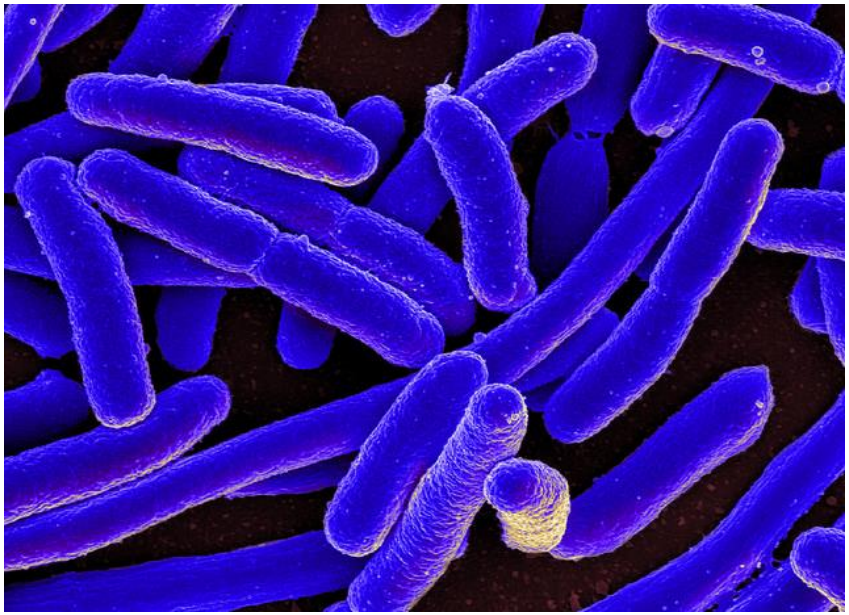
Normalized variance of inter-division times.

We predict for interdivision times CV~0.03-0.04, while experiments suggest a larger noise, CV~0.1.

Because we assumed the division after adding exactly the length ***l***. But in reality smaller or larger values are also possible.

Critical Comments

3) One-dimensional model for complex 3D systems.



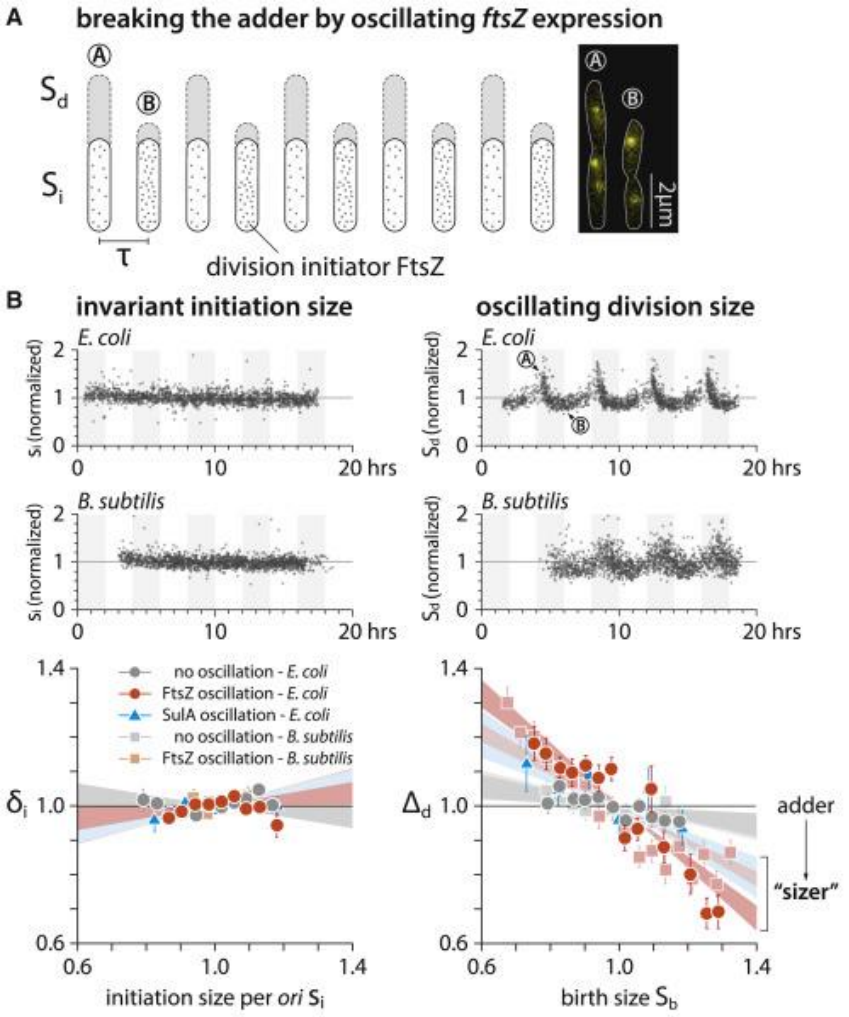
Bacteria grow in volume, and more advanced description is needed.

Critical Comments

4) Good nutrient conditions are considered only. The approach has to be extended to poor nutrient conditions and to dynamic fluctuations in the protein precursors.

Mechanistic Origin of Cell-Size Regulation

Recent experiments test the mechanistic origin of the cell-size regulation by imposing the fluctuations of proteins responsible for growth and division



Si et al., 2019, *Current Biology* **29**, 1760-1770

Conclusions:

- A new theoretical framework to explain the cell-size regulation in bacteria is presented
- It is based on the hypothesis that the mechanisms are purely stochastic via properly coupling of cell growth and cell division
- Our theory eliminates the need of using the concept of thresholds that are experimentally not supported
- Exact analytical solutions provide a clear physical picture of underlying processes
- A very good agreement with available experimental data on bacteria is found

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