Current topics in theoretical biophysics

Erik van Nimwegen	@NimwegenLab
Richard Neher	@richardneher
Mihaela Zavolan	@ZavolanLab
Knut Drescher	@knutdrescher

BIOZENTRUM
Universität Basel
The Center for Molecular Life Sciences

SIB
Swiss Institute of Bioinformatics

Format of the course

- Each week we introduce a particular topic and one or two associated seminal papers in the field (1pm-2pm).
- You are given the papers plus a set of questions about the papers on adam.unibas.ch.
- You have to read the papers and prepare your answers to these questions.
- The next week, we discuss the papers together (10.15am-noon).
- You are supposed to attend at least 80% of the discussions and are expected to actively contribute to the discussion. **You may be called out to give your answer to specific questions.**
- At the end of the course, you pick one paper that we discussed and write a short essay/report on this paper.

The lecturers

Richard Neher
Microbial evolution and adaptation

Knut Drescher
Development and functions of bacterial communities

Erik van Nimwegen
Function and evolution of genome-wide regulatory networks

Mihaela Zavolan
Key regulators of gene expression and cell identity

BIOZENTRUM
Universität Basel
The Center for Molecular Life Scienc

SIB
Swiss Institute of Bioinformatics

Overview of the topics

Date	Topic	Teacher
Sept 26/Oct 3	Bacterial growth laws	Erik van Nimwegen
Oct 3/10	Gene expression constraint on evolutionary rate	Mihaela Zavolan
Oct 10/17	Translational control	Mihaela Zavolan
Oct 17/24	Error correction by kinetic proofreading	Richard Neher
Oct 24/31	Stochastic gene expression	Mihaela Zavolan
Oct 31/Nov 7	Scaling laws in genomics	Erik van Nimwegen
Nov 7/14	Temperature compensation in the circadian clock	Richard Neher
Nov 14/21	Liquid-liquid phase transitions	Richard Neher
Nov 21/28	Frequency-modulated gene regulation	Erik van Nimwegen
Nov 28 /Dec 5	Physical constraints for the evolution of multicellularity	Knut Drescher
Dec 5/12	Growth and form in the gut	Richard Neher
Dec 12/19	Stochastic phenotype switching in adaptation	Erik van Nimwegen

What is systems biology?

- Field of study: the study of interactions between components of a biological system, aiming to determine how the behavior of the whole system emerges.

- Approach to the study of biological systems: involving large-scale measurements, development of quantitative models, validation of the models through further experiments.

- Paradigm (thought pattern): the function of biological systems can be understood through analysis of the system in its entirety as opposed of individual components (reductionist approach).

Origins of Systems Analysis

• In contrast to the objects studied in physics, chemistry, etcetera, biological objects are *systems* that have been *designed* by evolution to perform particular functions.

• In this sense, biological systems are analogous to machines designed by humans.

• The general theory for analyzing `systems' finds its origin in the analysis of the first complex machines that were designed by humans.

Typical questions/topics:

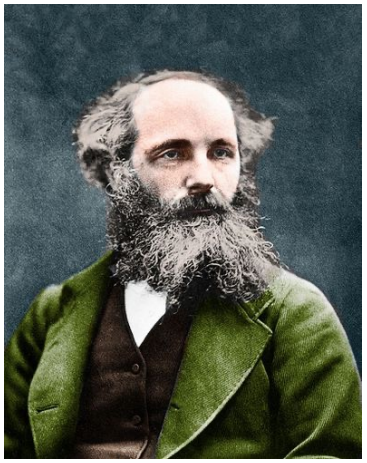
• Are there general design principles for achieving particular functionalities?

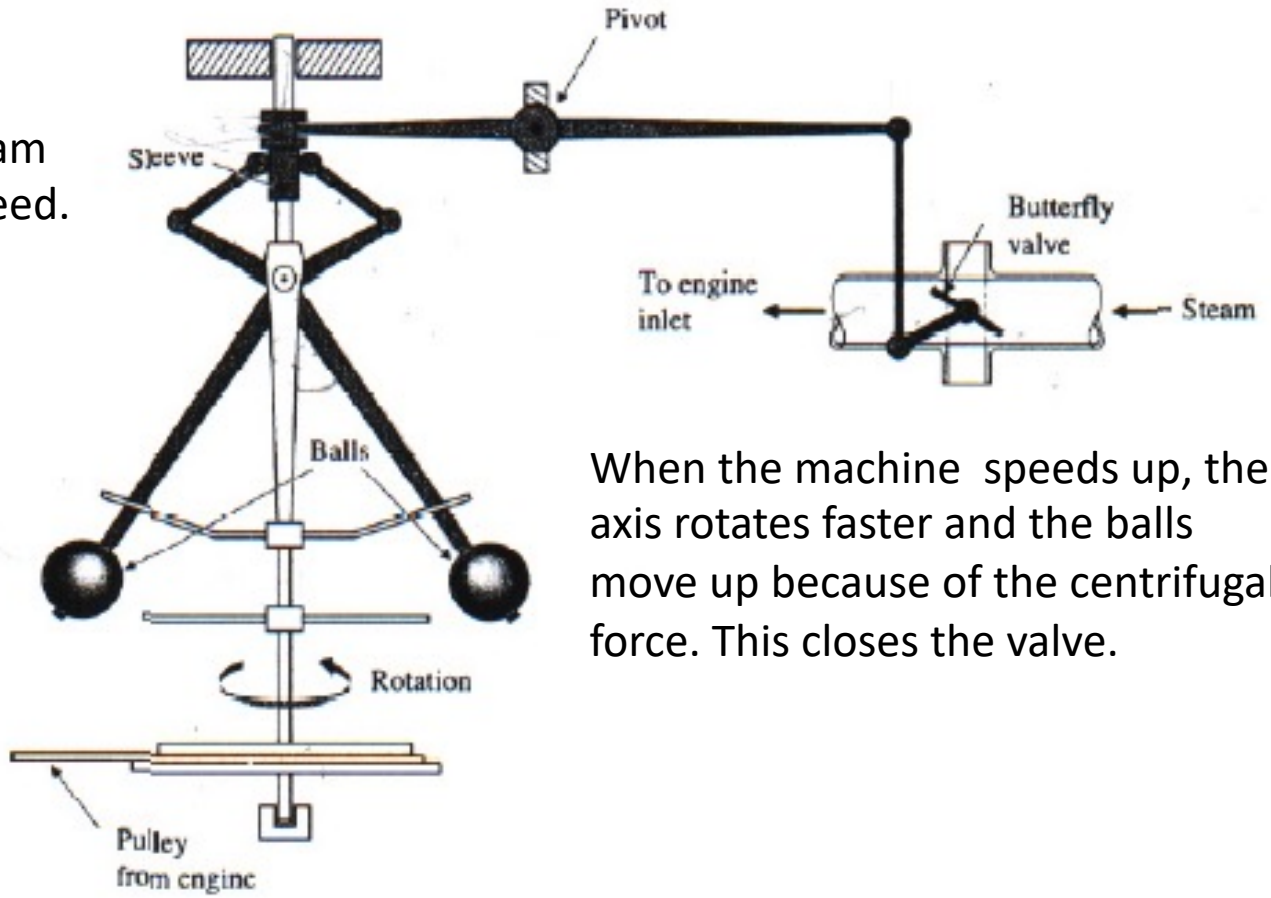
• Principles for constructing complex functionalities from simpler functional 'modules' (switches, amplifiers, filters, etc).

• How to make the system stable to outside disturbances and failures of components?

The governor of James Watt's steam engine

A device for keeping the steam engine running at a fixed speed. This device allows the steam engine to *regulate itself*.





When the machine speeds up, the axis rotates faster and the balls move up because of the centrifugal force. This closes the valve.

In 1868, James Clerk Maxwell (of electromagnetic equations' fame) analyzed the governor's dynamical stability using differential equations. This is probably the first example of system's control theory.

How does quantitative science actually work

Richard Feynman (Lectures on Physics):

This, then, is one of the important steps in the development of physical law: first we observe an effect, then we measure it and list it in a table; then we try to find the rule by which one thing can be connected with another.....
Now in the further development of science, we want more than just a formula. First we have an observation, then we have numbers that we measure, then we have a law which summarizes all the numbers. But the real glory of science is that we can find a way of thinking such that the law is evident.

My paraphrase:

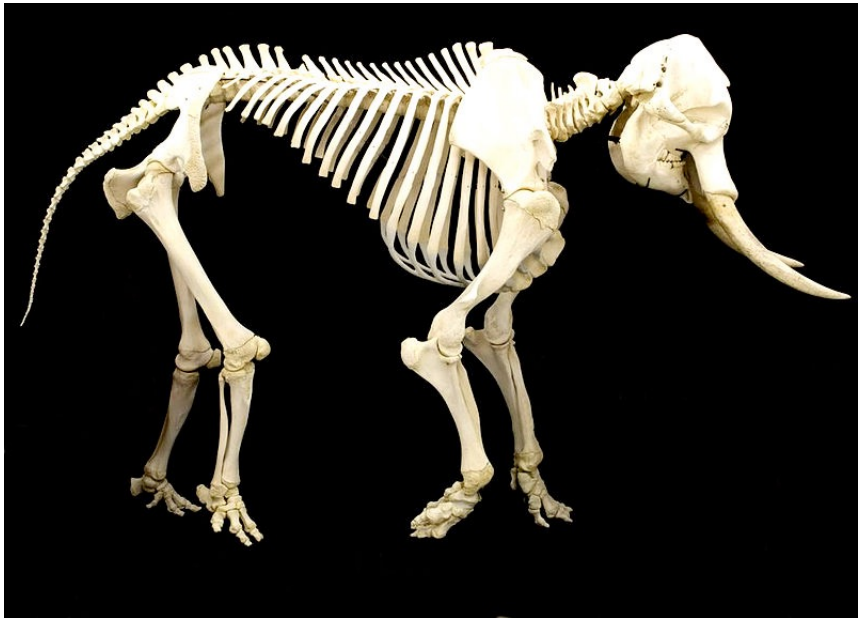
1. We observe a pattern that interests us.
2. We formalize the pattern by rigorously defining measurable quantities that obey particular quantitative relationships, i.e. `formulas' that connect measurable quantities.
3. We develop a theory that, in one fell swoop, explains many of the formulas and makes them self-evident.

Examples:

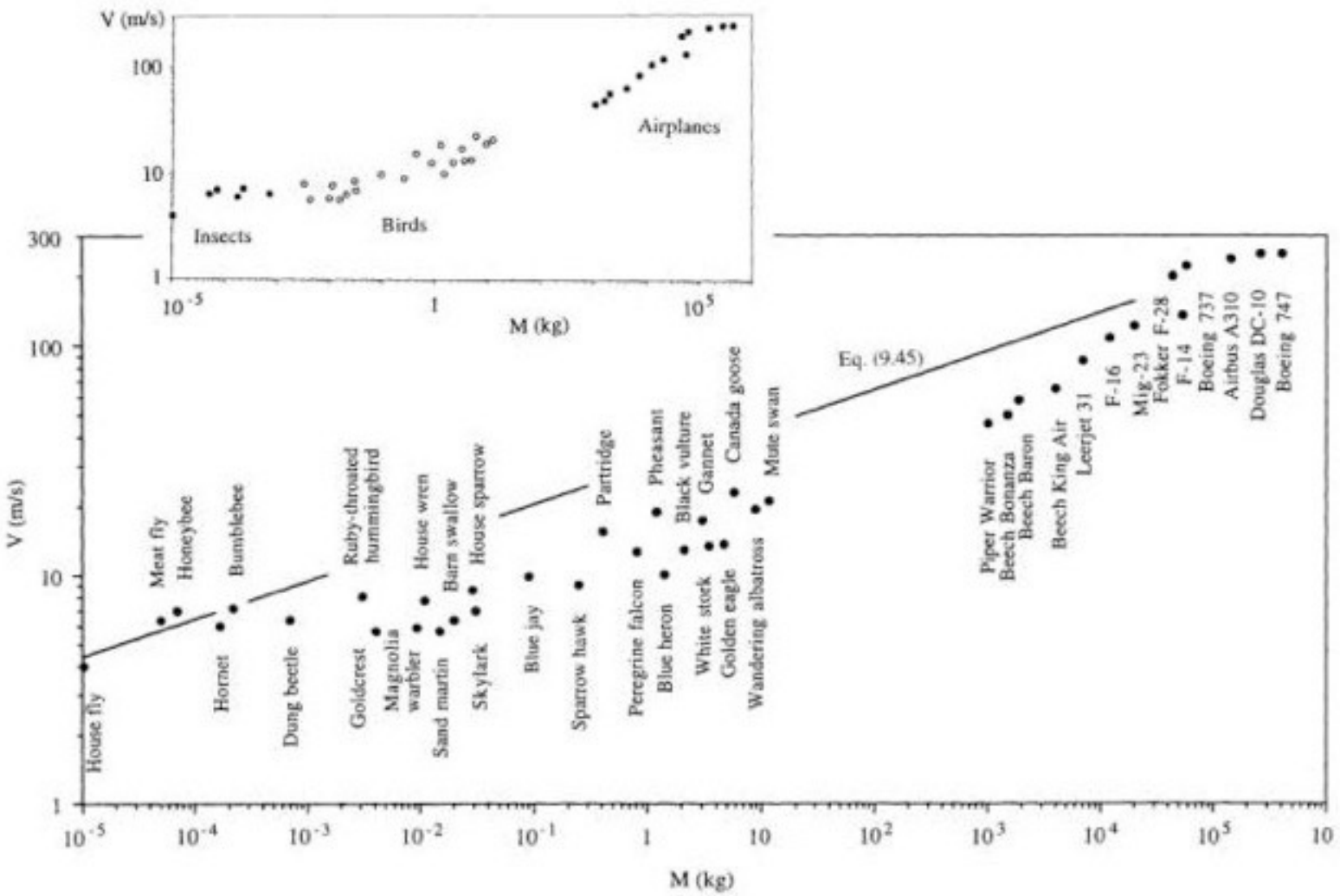
- Mechanics and gravity: First Galileo's and Kepler's laws, then Newtonian mechanics.
- Thermodynamics: First thermodynamic laws, later microscopic models.
- Electrodynamics: First Ohm's law, Faraday's laws, later Maxwell's equations.

Allometry

- Study of the relationship of body size to shape, anatomy, physiology and behavior.
- Origin in the work of Otto Snell (1892), D'Arcy Thompson (1917) and Julian Huxley (1932)
- Isometric scaling: changes in size do not lead to changes in proportion, Allometric scaling: changes that differ from isometry.



Example of allometry: Flying speed as a function of weight



A Bejan, S Lorente. Philos Trans R Soc Lond B Biol Sci. 2010 May 12;365(1545):1335-47.

Kleiber (1932) – Basal metabolic rate vs body mass (3/4 power)

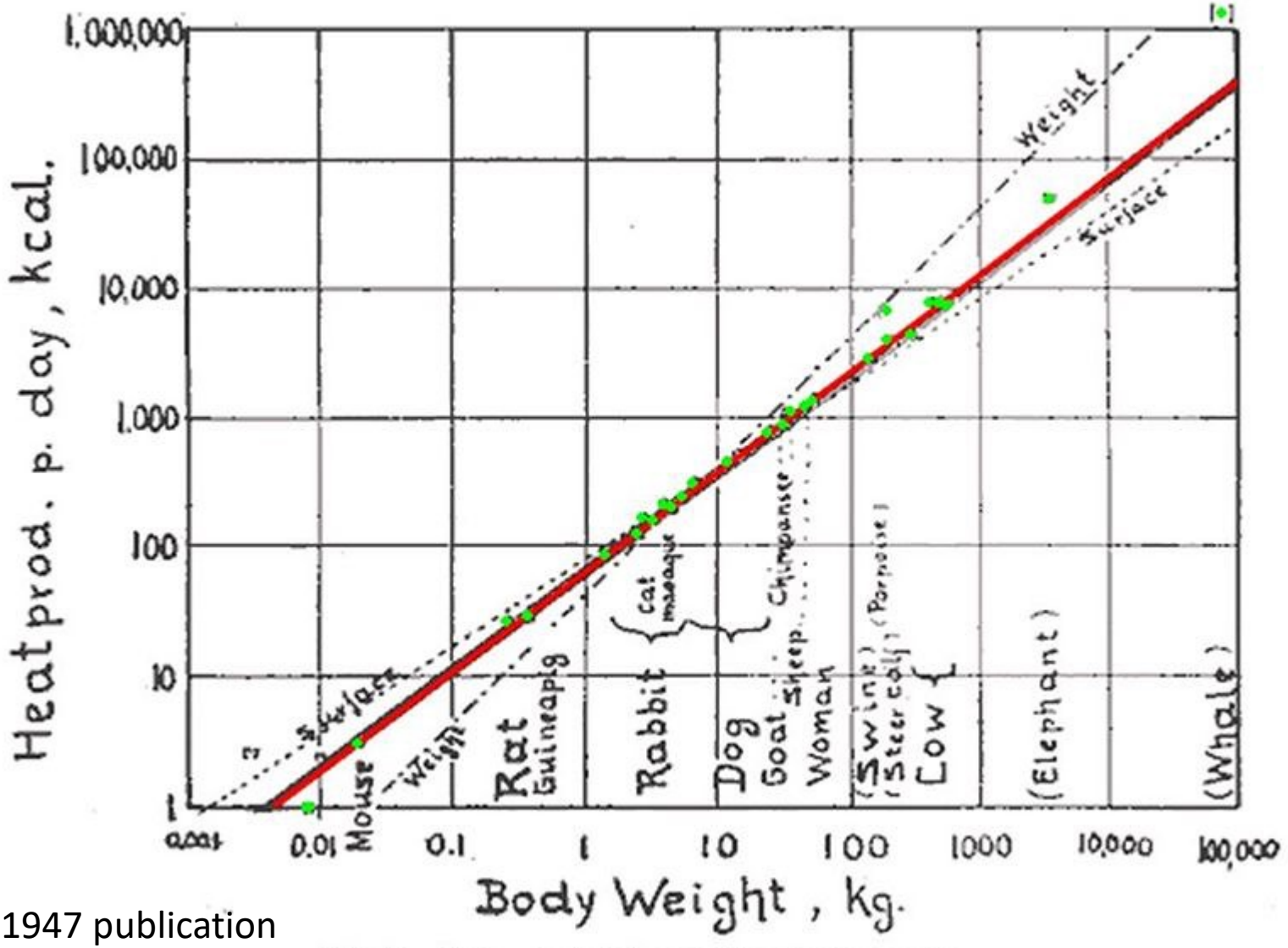


Fig. 1. Log. metabol. rate/log body weight

From a 1947 publication

Allometry in one equation

$$Y = Y_0 M^b \Leftrightarrow \log(Y) = \log(Y_0) + b \log(M)$$

- Equations of this type are called scaling laws.
- They correspond to straight lines in a log-log plot.
- Some of the oldest and intriguing general quantitative observations in biology correspond to such scaling laws.
- This week we will look at scaling laws in bacterial growth.
- Later we will also look at scaling laws in genomic quantities.

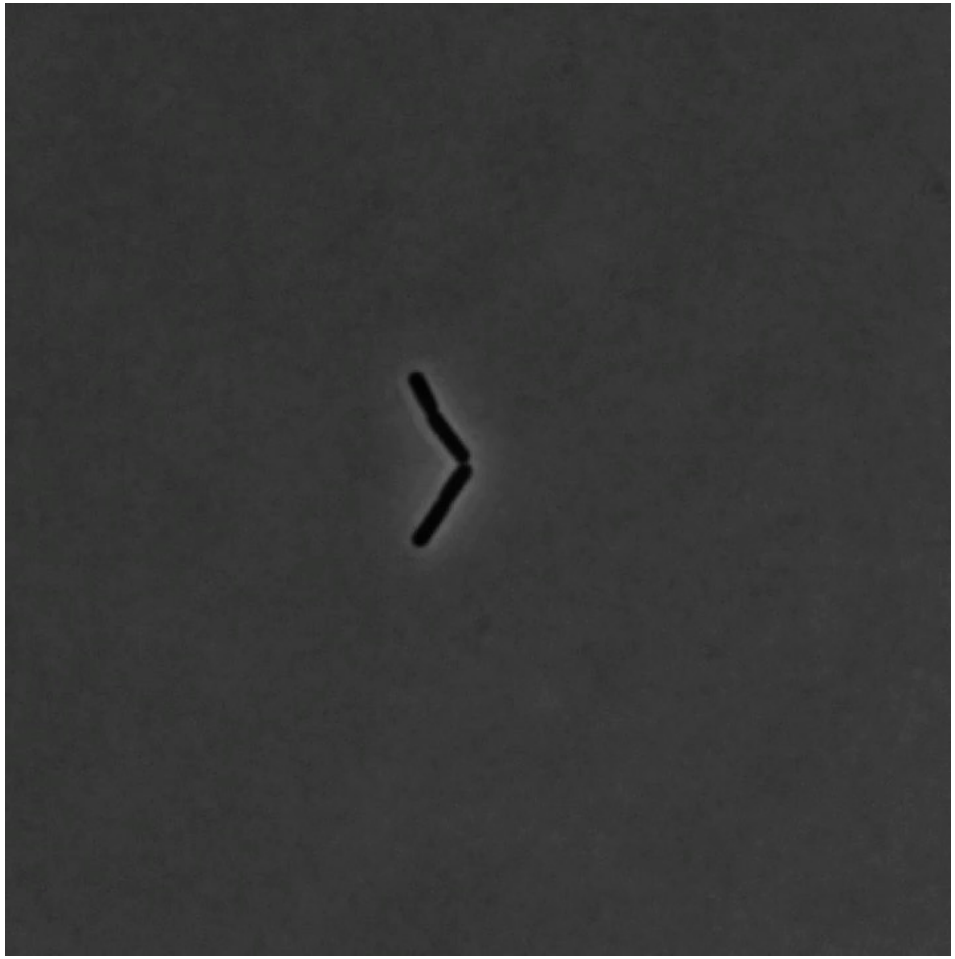
Bacterial growth laws

• This week's paper looks at universal quantitative relationships that relate the physiology of bacterial cells to the rate at which the cells are growing.

• The key observable from which all this work starts is the exponential growth of populations of genetically identical bacteria in some environment.

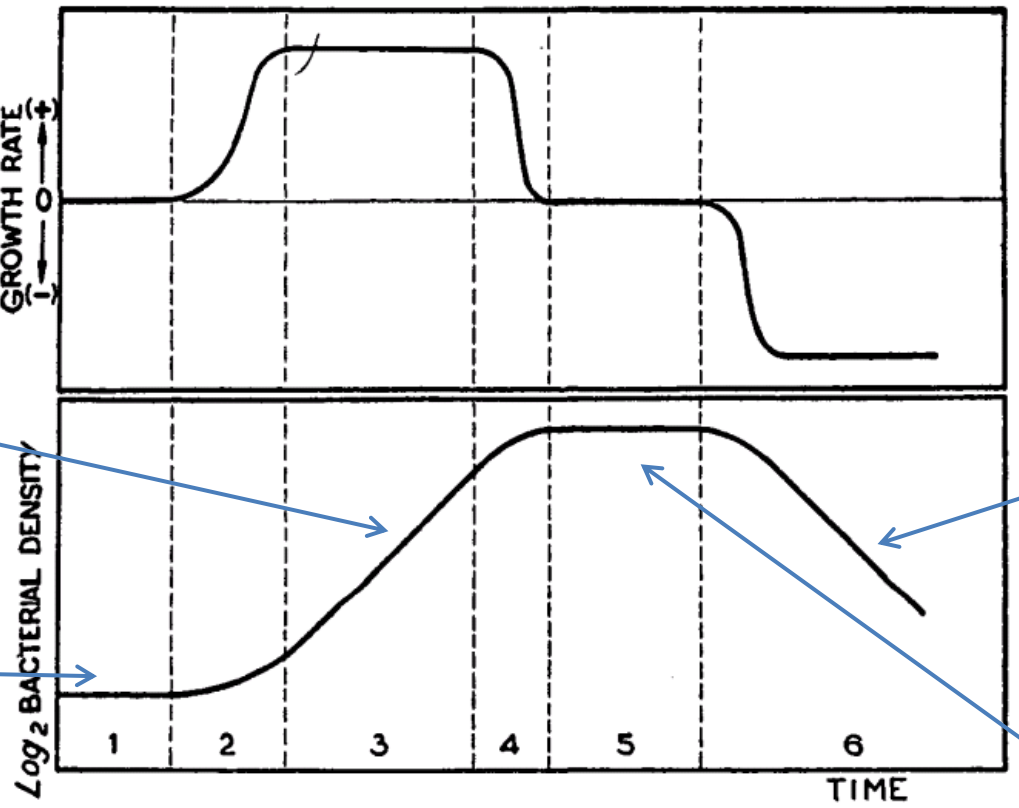
• We imagine that a small set of bacteria is put in a medium that contains nutrients that allows the cells to replicate and we follow the cells as they grow.

E. coli growing and expressing GFP



Video courtesy of Michael Elowitz
These E. coli have been engineered to express GFP.

Growth phases of exponentially growing bacteria



Exponential growth phase

Lag phase

Death phase

Stationary phase

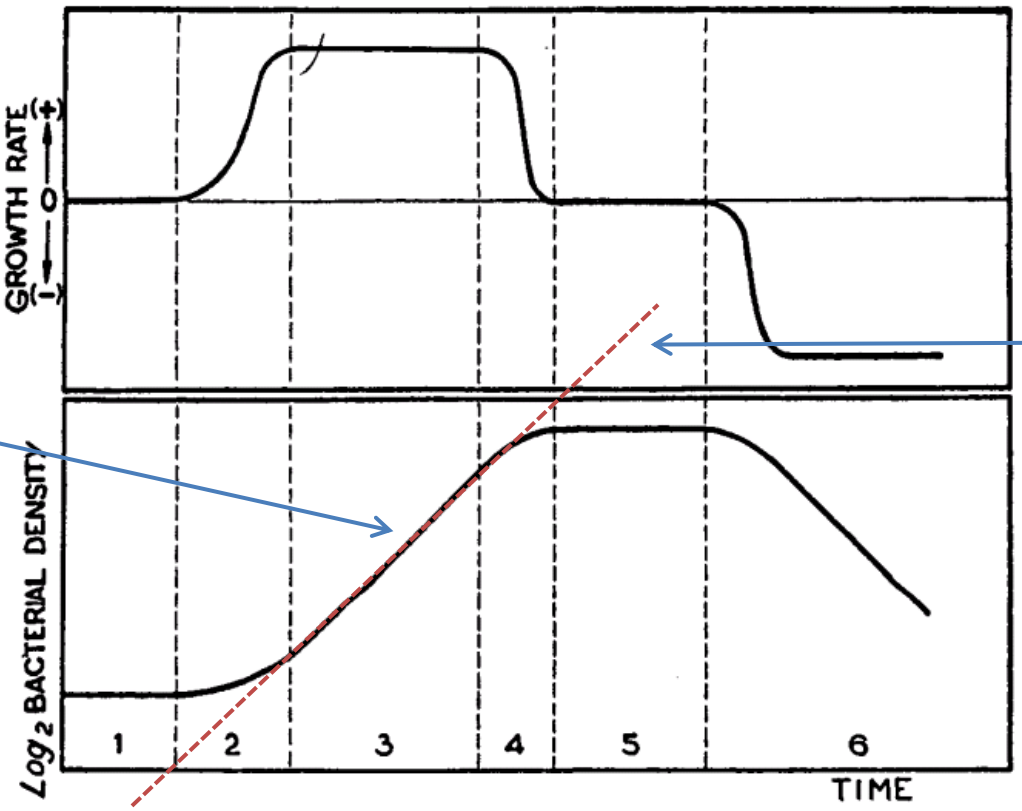
FIG. 1.—Phases of growth. Lower curve: log bacterial density. Upper curve: variations of growth rate. Vertical dotted lines mark the limits of phases. Figures refer to phases as defined in text (see p. 373).

From: Jacques Monod. The growth of bacterial cultures
Annu. Rev. Microbiol. 1949

Growth phases

Exponential growth phase

$$n(t) \propto e^{\lambda t}$$



Growth-rate:

λ

FIG. 1.—Phases of growth. Lower curve: log bacterial density. Upper curve: variations of growth rate. Vertical dotted lines mark the limits of phases. Figures refer to phases as defined in text (see p. 373).

From: Jacques Monod. The growth of bacterial cultures
Annu. Rev. Microbiol. 1949

Growth rate depends on growth condition

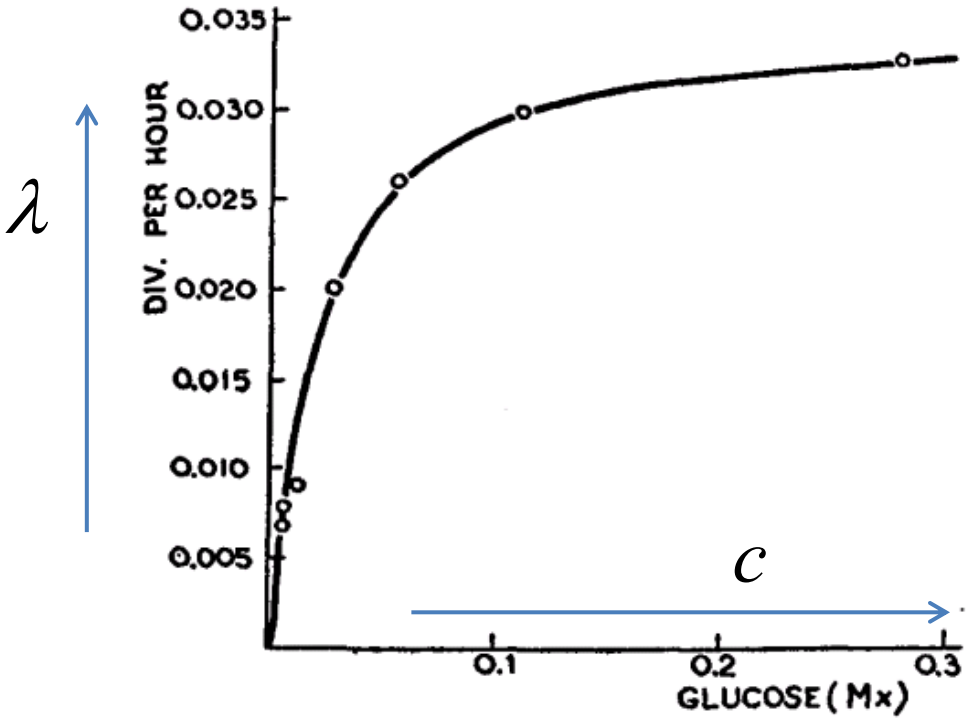


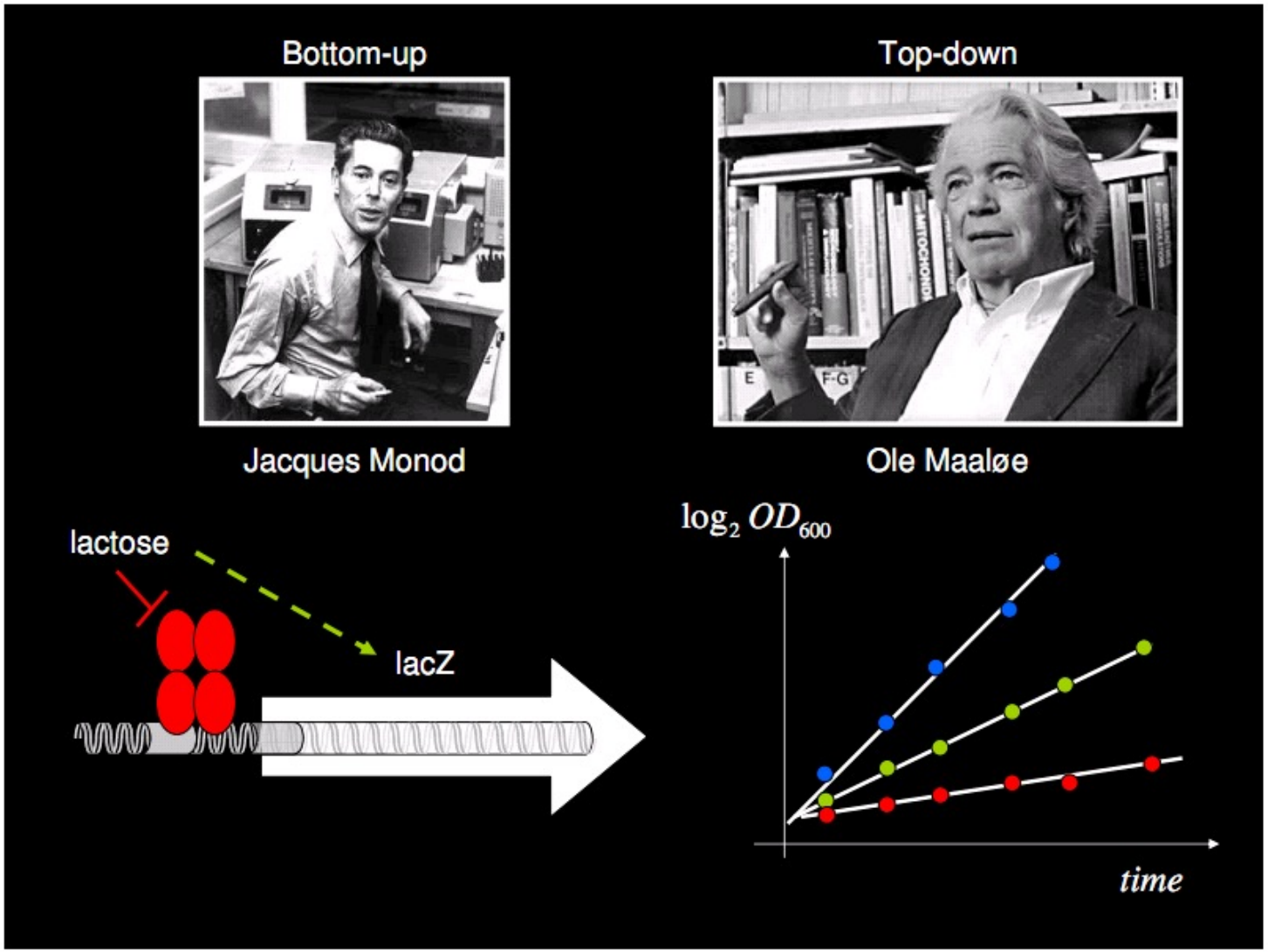
FIG. 5.—Growth rate of *M. tuberculosis* in Dubos' medium, as a function of glucose concentration. Solid line drawn to equation (2) with $R_K = 0.037$ and $C_1 = M/45$ (20).

For example, varying the concentration of a growth-limiting nutrient, growth-rate follows *Monod's equation*:

$$\lambda(c) = \lambda_{\max} \frac{c}{c + c_0}$$

Two ways of approaching the question:

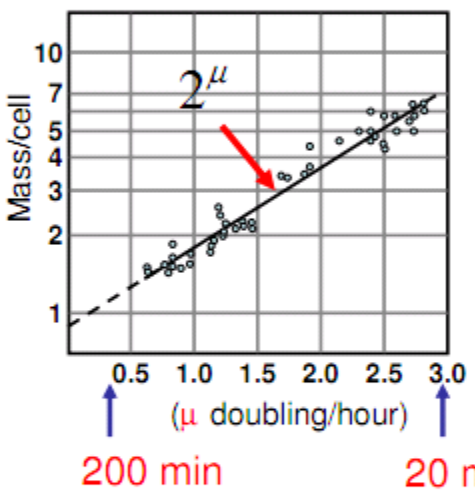
Study what happens at the molecular level or look for universal laws.

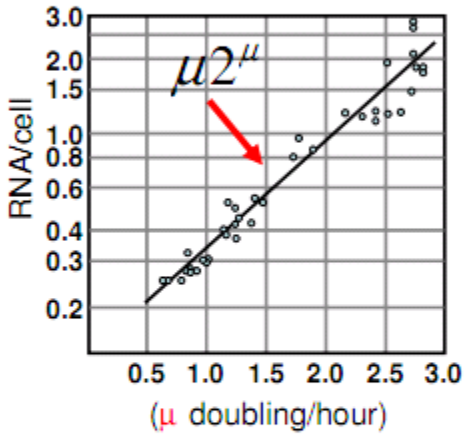


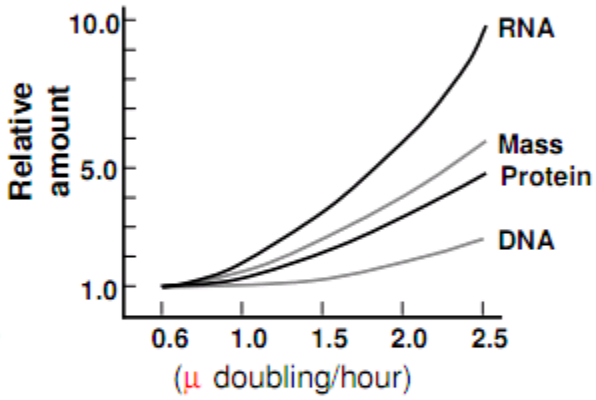
Slide courtesy of Matthew Scott.

The bacterial growth laws – Schaechter, Maaløe and Kjeldgaard (1958)

- Using more than 20 distinct growth media, observe in Salmonella macromolecular composition is a function of ***growth rate alone***.







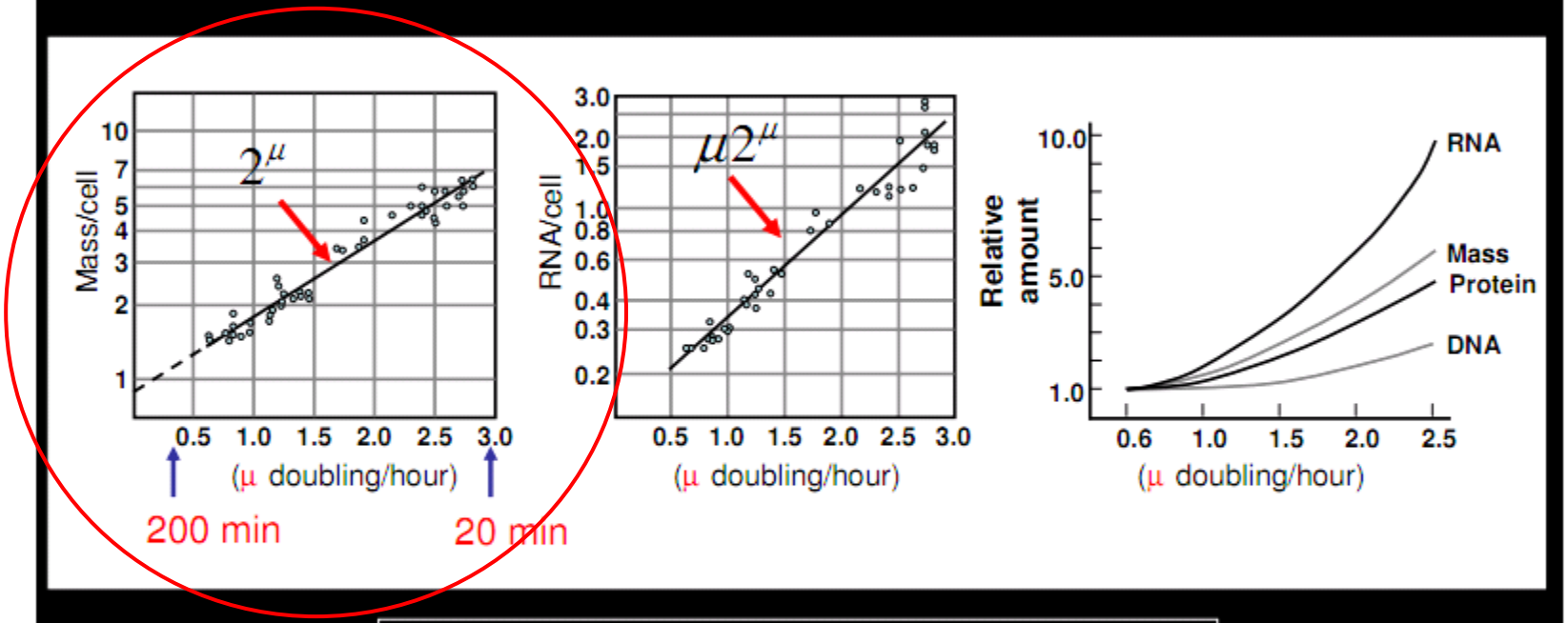
RNA/Protein is a linear function of growth rate μ

Schaechter, Maaløe and Kjeldgaard (1958) *Journal of General Microbiology* **19**: 592.

Slide courtesy of Matthew Scott.

The bacterial growth laws – Schaechter, Maaløe and Kjeldgaard (1958)

- Using more than 20 distinct growth media, observe in Salmonella macromolecular composition is a function of ***growth rate alone***.



RNA/Protein is a linear function of growth rate μ

Schaechter, Maaløe and Kjeldgaard (1958) *Journal of General Microbiology* **19**: 592.

Slide courtesy of Matthew Scott.

Why does cell volume grow exponentially with growth rate?

Nature. 1968 Sep 7;219(5158):1077-9.

Relationship between cell size and time of initiation of DNA replication.

Donachie WD.

PMID: 4876941 [PubMed - indexed for MEDLINE]

Several observations (supported by data):

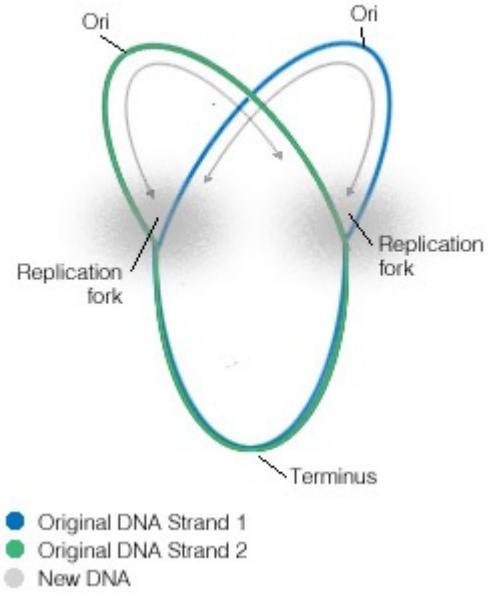
• During exponential growth at rate λ the mass inside a single cell is also growing exponentially at the same rate: $M(t) = M_0 e^{\lambda t}$

• The density of the cell stays the same, that is the volume of the cell also grows at the same rate: $V(t) = V_0 e^{\lambda t}$

• The key assumptions of Donachie's model are:

1. Independent of condition, DNA replication forks move at the *same speed*.
2. The number of replication forks per unit volume is held constant.
3. The cell divides whenever one replication fork is done copying the entire DNA.

Bacterial replication



• In contrast to eukaryotes bacteria (often) have circular chromosomes with a *single* origin of replication.
• If we assume that replication forks move at a fixed speed, then it takes a fixed amount of time to replicate the entire DNA. Let us call this time *T*.
• However, it has been found that bacteria can often have growth rates that correspond to division time *less than T.*

How is this possible?
Answer: Bacteria let multiple replication forks run *in parallel*. By the time it divides there are already other replication forks partially done as well.

• Donachie's model assumes that there is a critical cell volume V_c at which the first replication fork is started. The second fork starts when the volume is $2V_c$ etcetera.
• The bacterial divides when the first fork is done replicating the entire chromosome.

Cell volume versus growth

- The cell volume is growing exponentially: $V(t) = V_0 e^{\lambda t}$
- The number of forks is also growing exponentially, i.e. $n_f(t) = \left\lfloor \frac{V(t)}{V_c} \right\rfloor = \left\lfloor \frac{V_0 e^{\lambda t}}{V_c} \right\rfloor$

- The *age* of the nth fork depends logarithmically on *n*: $t_n = \frac{\log(n)}{\lambda}$

- Assume that, at growth-rate λ, there are in steady-state *n* forks per cell, and the cell divides when this *n*-th fork finishes.
- The age of this fork *must* precisely equal the replication time *T, i.e.*

$$t_n = T \Rightarrow T = \frac{\log(n)}{\lambda} \Rightarrow n = e^{\lambda T}$$

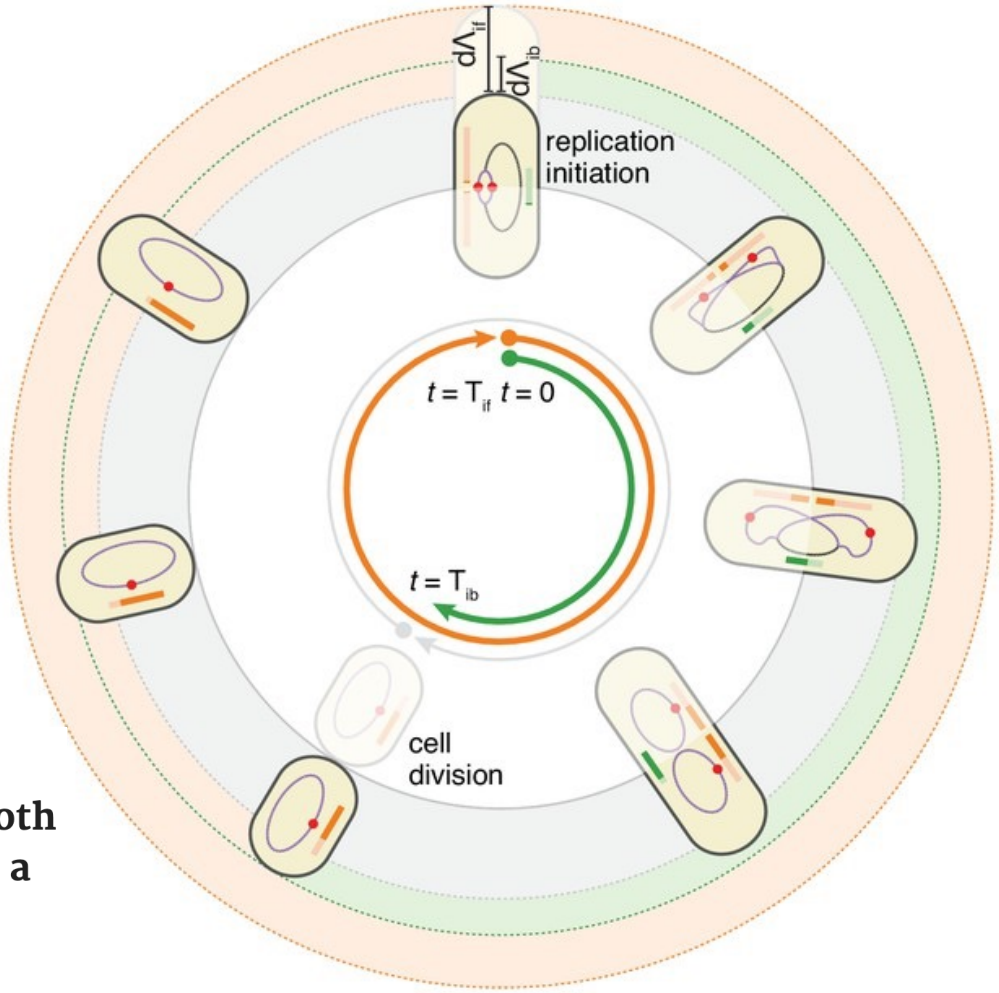
- But because there is a fixed number of forks per volume we have:

$$V = V_c n = V_c e^{\lambda T}$$

That is, the volume grows exponentially with growth-rate
This theory *predicts* the slope of the relationship to be *T*.

Unfortunately Donachie's model is incorrect

- Recent experimental methods have made it possible to track growth and division *at the single cell level.*
- These observations have shown that cells do not initiate replication at a fixed size.
- Instead, of using a 'sizer' cells use an *adder* mechanism: they add a fixed amount of volume between consecutive replication initiation experiments.
- The amount of added volume increases with growth-rate.
- This can be explained if we assume that a fixed amount of some key protein needs to be produced *and* that the production rate of this protein is *independent* of growth-rate.



› Elife. 2019 Nov 11;8:e48063. doi: 10.7554/eLife.48063.

Initiation of chromosome replication controls both division and replication cycles in *E. coli* through a double-adder mechanism

Guillaume Witz [1 2], Erik van Nimwegen [1 2], Thomas Julou [1 2]

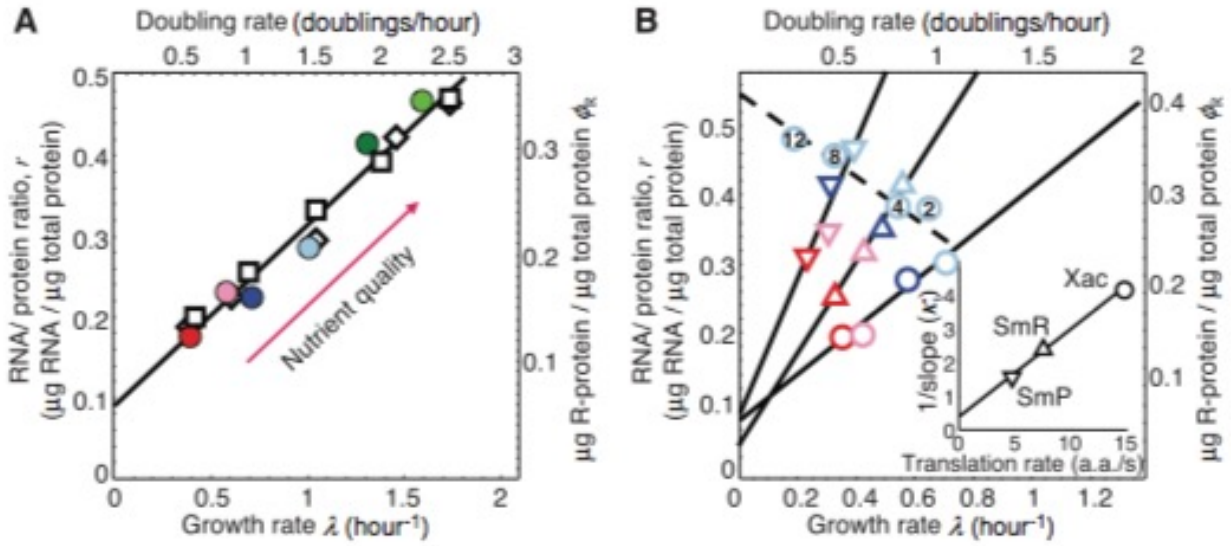
This week's paper

Interdependence of Cell Growth and Gene Expression: Origins and Consequences

Matthew Scott,[1]*† Carl W. Gunderson,[2]* Eduard M. Mateescu,[1] Zhongge Zhang,[2] Terence Hwa[1,2]‡

In bacteria, the rate of cell proliferation and the level of gene expression are intimately intertwined. Elucidating these relations is important both for understanding the physiological functions of endogenous genetic circuits and for designing robust synthetic systems. We describe a phenomenological study that reveals intrinsic constraints governing the allocation of resources toward protein synthesis and other aspects of cell growth. A theory incorporating these constraints can accurately predict how cell proliferation and gene expression affect one another, quantitatively accounting for the effect of translation-inhibiting antibiotics on gene expression and the effect of gratuitous protein expression on cell growth. The use of such empirical relations, analogous to phenomenological laws, may facilitate our understanding and manipulation of complex biological systems before underlying regulatory circuits are elucidated.

Growth laws



Strain / Medium	EQ2	Xac	SmR	SmP	Xac in cAA+glc Cm conc. (μM)	
M63+glyc	●	○	△	▽	2	(2)
M63+gluc	●	○	△	▽	4	(4)
cAA+glyc	●	○	△	▽	8	(8)
cAA+gluc	●	○	△	▽	12	(12)
RDM+glyc	●	Historical data:	□ Strain B/r; Ref. (10)			
RDM+gluc	●		◇ Strain 15τ-bar; Ref. (12)			

Fig. 1. Correlation of the RNA/protein ratio *r* with growth rate λ for various strains of *E. coli*. (**A**) Comparison among *E. coli* strains grown in minimal medium: Strain B/r [(*10*), squares], 15τ-bar [(*12*), diamonds], and EQ2 (this work, solid circles). The growth rate is modulated by changing the quality of nutrients as indicated in the key at lower left. The fraction of total protein devoted to ribosome-affiliated proteins (ϕ_R) is given by the RNA/protein ratio as $\phi_R = \rho \cdot r$ (table S1). (**B**) The RNA/protein ratio for a family of translational mutants SmR (triangles) and SmP (inverted triangles) and their parent strain Xac (circles) (*27*), grown with various nutrients (see key at lower left) (table S2). Translational inhibition of the parent Xac strain via exposure to sublethal doses of chloramphenicol (circled numbers; see legend table) gave RNA/protein ratios similar to those of the mutant strains grown in medium with the same nutrient but without chloramphenicol (light blue symbols). Dashed line is a fit to Eq. 2. Inset: Linear correlation of κ_t values obtained for the Xac, SmR, and SmP strains (table S2) with the measured translation rate of the respective strains (*14*) ($r^2 = 0.99$).

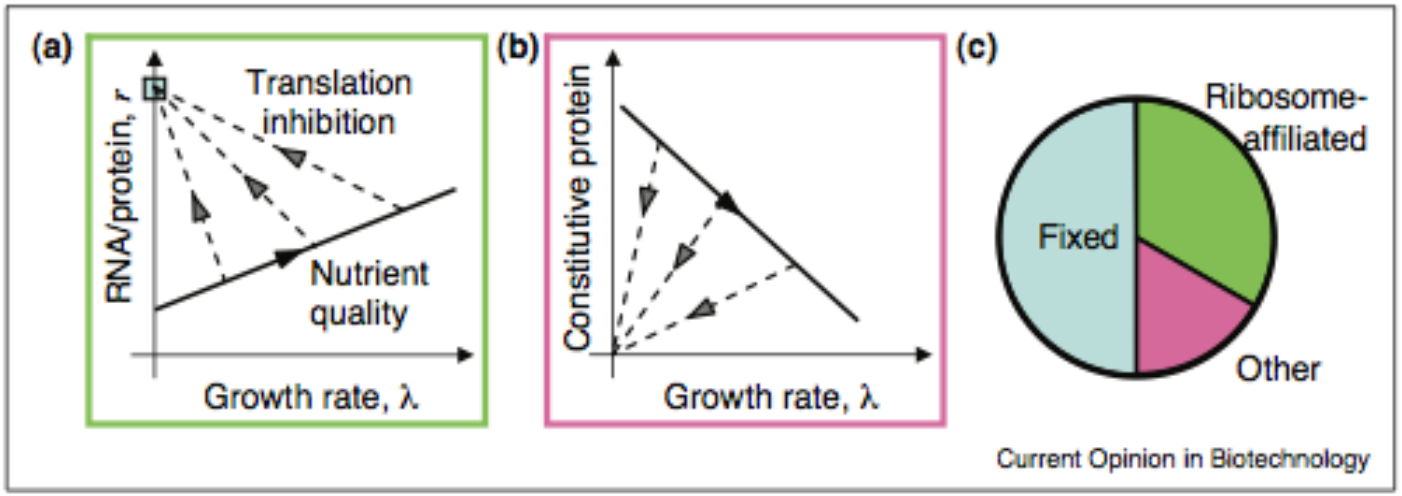
$$r = r_0 + \frac{\lambda}{\kappa_t} \qquad r = r_{\max} - \frac{\lambda}{\kappa_n}$$

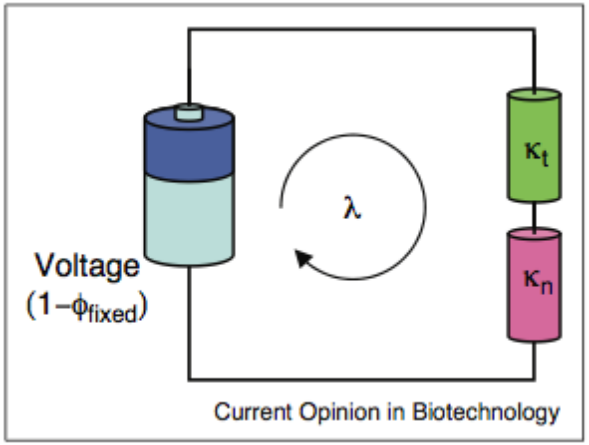
Review paper

Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Biotechnology
ELSEVIER

Bacterial growth laws and their applications

Matthew Scott[1] and Terence Hwa[2,3]





BIOZENTRUM
Universität Basel
The Center for Molecular Life Sciences
SIB
Swiss Institute of Bioinformatics

Qualitative origin of the scaling laws

Matching fluxes

- Catabolism: Nutrients are taken from the environment and transformed into basic constituents that cells are made of (nucleotides, amino acids, lipids).
- Anabolism: Basic constituents are put together by molecular machines (e.g. polymerases and ribosomes) into DNA, proteins, and cell walls.
- Catabolic and anabolic fluxes must match: rate of producing basic constituents must match rate at which they are put together into new biomass.

Universal machines

- All new DNA is made by DNA polymerases (replication forks) stringing together nucleotides.
- All new proteins are made by ribosomes stringing together amino acids.

Conservation laws

- DNA polymerases run at the same rate independent of growth condition.
- Ribosomes run at the same rate independent of growth conditions.
- The total concentration of protein in the cell is constant independent of growth condition.

Combining these leads to the observed growth laws.