Model for DNA-less cell fate.

Guillaume Garnier
Team GO Paris–Saclay 2019

Université Paris–Saclay

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Introduction

Intoduction

Last year, Team Go Paris Saclay decided to work on free DNA cell.

- What are the consequences of destroying DNA within a cell?
- Could a DNA-less cell be still useful?

Introduction

An attempt was made to answer these questions with the help of a mathematical model

- We developed a model of DNA-less cell fate
- We started from an deterministic whole cell model [WODS15]. We modeled the absence of DNA as a stop to all transcription, and adapted the initial hypotheses of Weiße and al. (PNAS, 2015)

Part I – The whole–cell dynamic

The nutrient quantity

The variation of the nutrient quantity is depending on :

$$\boxed{\frac{ds_i}{dt} = v_{imp}(e_t, s) - v_{cat}(e_m, s_i) - \lambda s_i,}$$

where

- v_{imp} is the import rate of nutrient

$$v_{imp}(e_t, s) = e_t \frac{v_t s}{K_t + s}.$$

- $v_{cat}(e_m, s_i)$ is the rate of transformation of nutrient in energy.

$$v_{cat}(e_m, s_i) = e_m \frac{v_m s_i}{K_m + s_i},$$

- λ is the dilution rate.

The energy quantity

The variation of the energy quantity is depending on :

$$\frac{da}{dt} = v_{cat}(e_m, s_i) - \sum_x n_x v_x - \lambda a,$$

where

- $v_{cat}(e_m, s_i)$ is the rate of transformation of nutrient in energy.

Hypothesis

Here we made the approximation that energy use is only due to the translation process.

The RNA and ribosome quantities

In this model, we have 2 types of ribosomes

- free ribosomes (not involved in a translation process) : r
- ribosomes bound to mRNAs (actively translating mRNA): c_x.

From this quantity depends the translation rate of a protein:

$$\boxed{v_x = \frac{\gamma(a)}{n_x} c_x,}$$

and where $\gamma(a)$ is the effective rate of protein elongation:

$$\boxed{\gamma(a) = \frac{\gamma_{max} a}{K_y + a}.}$$

The RNA and ribosome quantities

It gives this equation for the pool of 'bound ribosomes':

$$\boxed{\frac{dc_x}{dt} = k_b m_x r - k_u c_x - v_x - \lambda c_x,}$$

where

- k_b and k_u denote the rates of binding and unbinding of a ribosome to an mRNA
- $m(x)$ is the mRNA quantity encoding a protein x

We also have an equation for the 'free-ribosomes' pool:

$$\boxed{\frac{dr}{dt} = v_r - \lambda r + \sum_x [v_x - k_b m_x r + k_u c_x].}$$

The RNA and ribosome quantities

We can deduce from that a differential equation for the mRNA quantity

$$\boxed{\frac{dm_x}{dt} = \omega_x(a) - k_b m_x r + k_u c_x + v_x - d_m m_x - \lambda m_x,}$$

where

- ω_x is the rate of transcription and $x \in \{e_t, e_m, r\}$:

$$\omega_x = \frac{w_x a}{\theta_x + a},$$

- w_x is the maximal transcription rate and θ_x is the threshold amount of energy at which transcription is half-maximal.

The protein quantity

In this model we are considering 4 proteins quantities:

- ribosomes r
- transporter enzymes e_t which import nutrient inside the bacteria
- metabolic enzymes e_m which transform the nutrient in energy
- the house keeping protein quantity q, that doesn't matter for the proper functioning of the model

It gives these equations where $x \in \{e_t, e_m, q\}$:

$$\boxed{\frac{dx}{dt} = v_x - (\lambda + d_x)x.}$$

To simplify we are considering $d_{e_t} = d_{e_m} = d_q$ where d_x is a degradation rate.

The dilution term

The growth rate λ connect the cellular processes with growth.

Hypothesis

The total mass of the cell is defined as (Proteins + Ribosomes)

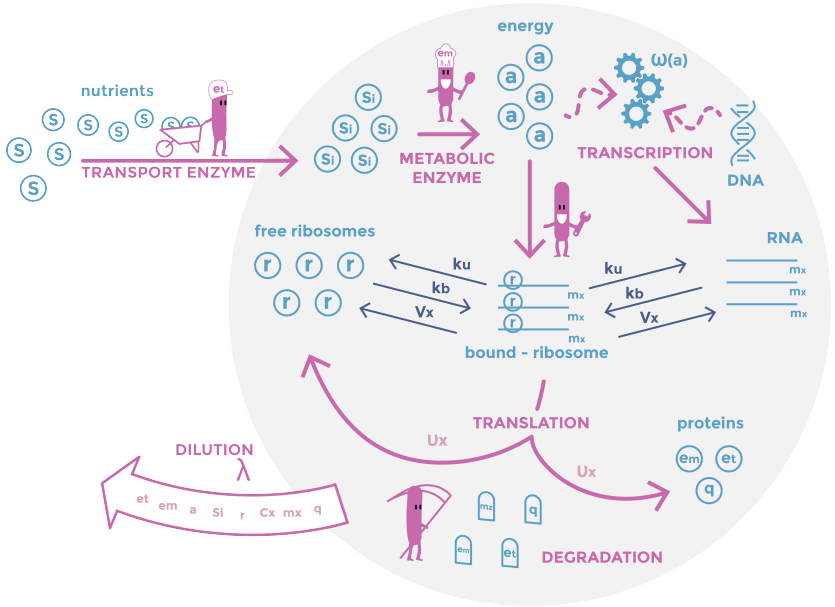
$$M = \sum_x n_x x + n_r \sum_x c_x.$$

It follows that $\frac{dM}{dt} = \gamma(a)\sum_x n_x - \lambda M - \sum_x n_x x d_x$.

The mass of a bacteria is assume constant during the first part of the model $\frac{dM}{dt} = 0$.

$$\boxed{\lambda = \frac{\gamma(a)\sum_x n_x - \sum_x n_x x d_x}{M}.}$$

Summary



Nutrients inside the bacteria	$\frac{ds_i}{dt} = v_{imp}(e_t, s) - v_{cat}(e_m, s_i) - \lambda s_i$
Energy	$\frac{da}{dt} = v_{cat}(e_m, s_i) - \sum n_x v_x - \lambda a$
Bound ribosomes	$\frac{dc_x}{dt} = k_b m_x r - k_u c_x - v_x - \lambda c_x$
Free ribosomes	$\frac{dr}{dt} = v_r - \lambda r + \sum [v_x - k_b m_x r + k_u c_x]$
mRNA	$\frac{dm_x}{dt} = \omega_x(a) - k_b m_x r + k_u c_x + v_x - d_m m_x - \lambda m_x$
Proteins	$\frac{dx}{dt} = v_x - (\lambda + d_x)x$

Figure: Scheme of the first part of the model

Part II – The effect of nucleases

Modelling the effect of nucleases

Nucleases are enzymes degrading DNA.

$$\text{No DNA} \quad \Longrightarrow \quad \text{No Transcription}$$

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But the effect of the nucleases is not instantaneous. It's progressive. Therefore, instead of arbitrarily setting the transcription rates to 0 at the beginning of the simulation, we preferred to reduce them as:

$$\omega'_x(a,t) = \omega_x(a) * e^{-\alpha t},$$

where α is the effect rate of the nuclease.

Modelling the effect of nucleases

We compute α with our experiments, which permit to reduce of 99% the transcription rate after 15 min.

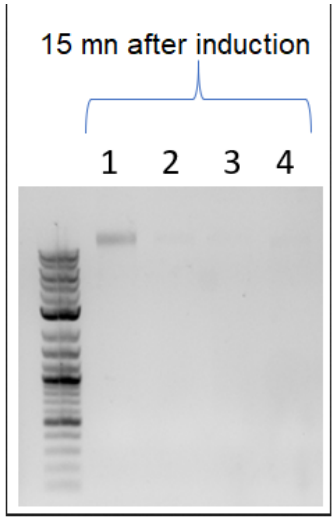


Figure: A result of gDNA gel electrophoresis obtained by our team. We can observe that there is completely removed after 15 min

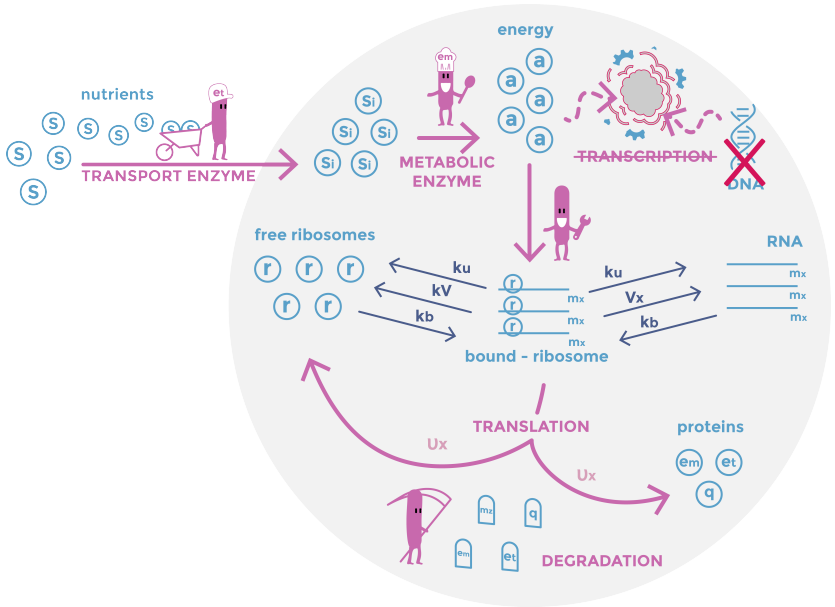
What purpose for the model

The principal purpose of the model is to evaluate the fate of DNA-less bacteria: how long are they still metabolically active?

- As they ca not divide, we decided to set

$$\lambda = 0.$$

What purpose for the model



Nutrients inside the bacteria	$\frac{ds_i}{dt} = v_{imp}(e_t, s) - v_{cat}(e_m, s_i)$
Energy	$\frac{da}{dt} = v_{cat}(e_m, s_i) - \sum n_x v_x$
Bound ribosomes	$\frac{dc_x}{dt} = k_b m_x r - k_u c_x - v_x$
Free ribosomes	$\frac{dr}{dt} = v_r + \sum [v_x - k_b m_x r + k_u c_x]$
mRNA	$\frac{dm_x}{dt} = \omega'_x(a,t) - k_b m_x r + k_u c_x + v_x - d_m m_x$
Proteins	$\frac{dx}{dt} = v_x - d_x x$

Figure: Scheme of the second part of the model

Some results

The total amount of ribosomes stop increasing

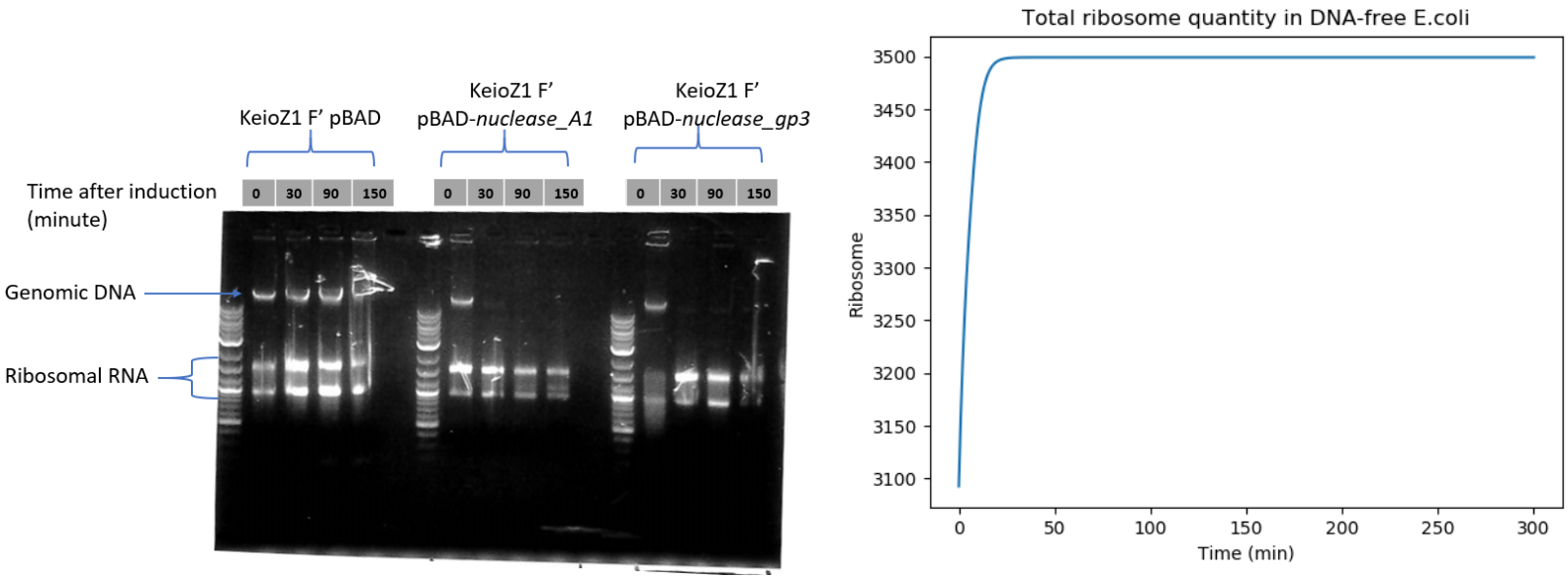


Figure: The quantity of ribosomal RNA decreases

Some results

Until 15mn, the DNA-less cell continues to produce proteins at similar level than the healthy DNA-proficient cell.

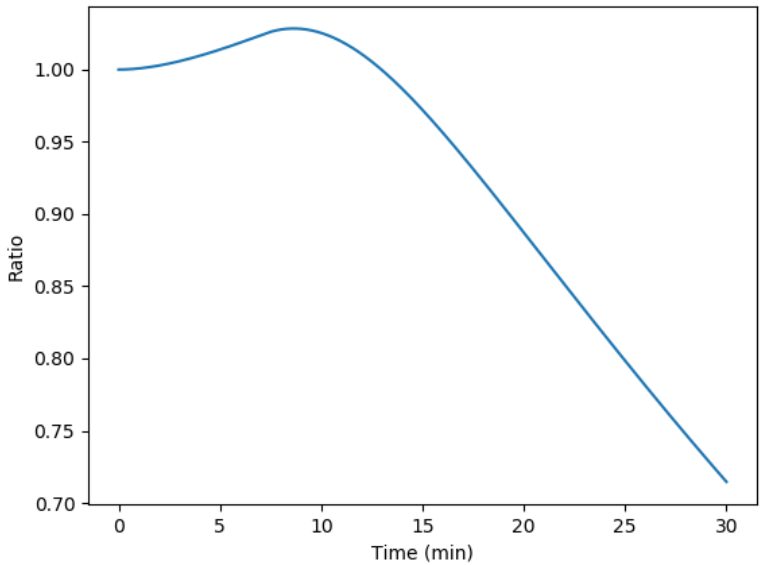


Figure: The housekeeping proteins ratio between the bacteria without and with DNA.

Some results

In theory, if cell components are remaining, maybe DNA-less cells can still divide.

These aspects were difficult to evalute with our model since bacteria are not considered individually but rather as a whole (biomass).

Part III – The phage infection

The phage infection

- One of the main experimental result of our team was the replication of an RNA information within DNA-less bacteria.
- This was obtained by infecting DNA-free bacteria with the MS2 RNA phage.
- An intriguing result was the drastic decrease of the number of phages produced by DNA-less bacteria compared to a normal bacteria.

For this reason we decided to model phage infection in DNA-less and DNA-proficient bacteria.

The phage infection

- Phage RNA (vRNA, for 'viral RNA') are replicated by the viral RDRP (RNA-dependent-RNA-polymerase) protein
- The RDRP protein is produced by translation of the vRNA by host ribosomes.
- The RDRP replicates vRNA into a minus strand, which serves as template to produce new positive strand vRNA genomes.

The phage infection

vRNA encodes 4 proteins:

- the maturation protein A,
- CP (capsid/coat protein),
- L (lysis protein)
- the RDRP polymerase

CP proteins bind to a vRNA (positive strand), so that new viral particles are assembled within the cytoplasm. This vRNA packaging into the capsids prevents the ribosomes from binding to vRNA.

The phage infection

Hypothesis

- Duplication is exponential and depends on RDRP enzyme quantity.
- We assume that all RDRP proteins find a free vRNA.

This gives this duplication rate for vRNA:

$$w_f = \frac{rdrp \times G}{N_f},$$

where

- $rdrp$ is the RDRP quantity already produced,
- G is the RDRP speed of polymerisation (duplication of RNA),
- N_f the nucleotide size of the vRNA.

The phage infection

In order to represent the capsid effect we modify the binding rate of vRNA to the ribosomes as followed:

$$kb' = kb \times \frac{K_{caps}}{K_{caps} + capside},$$

where K_{caps} is find due to the litterature.

The phage infection

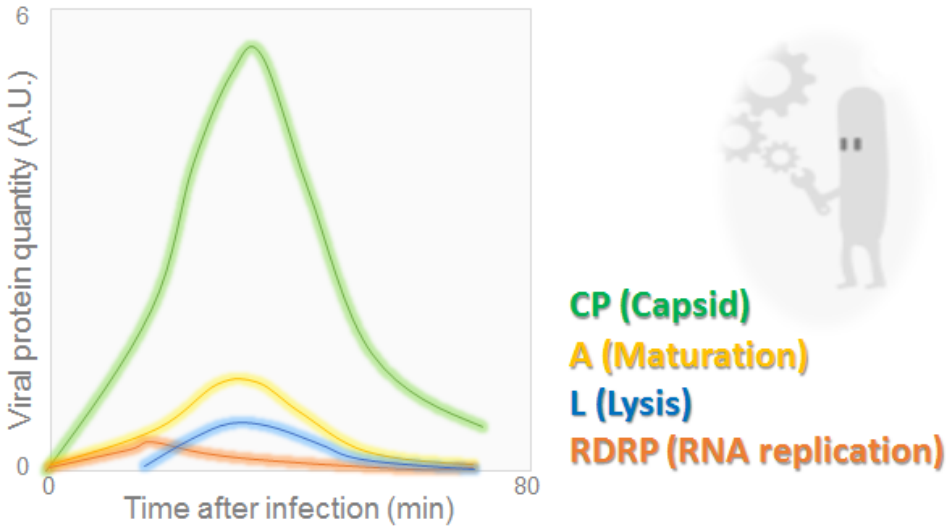
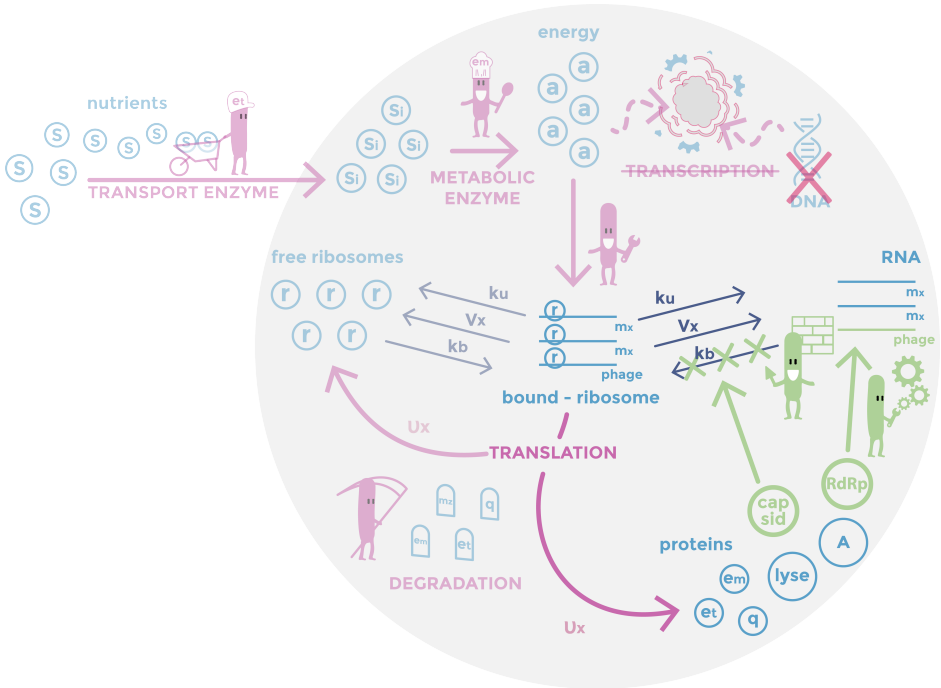


Figure: The graph, adapted from [BB79], illustrates the evolution of lysis, maturation, RDRP and capsid proteins over the time. It may be seen that all quantities have reached a peak at 30 min. Then they dropped off

The phage infection



Bound ribosomes to vRNA	$\frac{dc_f}{dt} = k'_b m_f r - k_u c_f - v_f$
vRNA	$\frac{dm_f}{dt} = w_f - k'_b m_f r + k_u c_f + v_f$
Phage proteins	$\frac{dx}{dt} = v_x$

Figure: Scheme of the phage part of the model. RDRP are replicating vRNAs and capsid which prevent the ribosomes from binding to vRNAs.

Back to the problem

Why there are less virions produced in DNA-less cell ?

To answer, we compared the production rate of phage proteins in DNA-less and DNA-proficient bacteria.

The production rate of proteins directly depends on the quantity of ribosomes bound to vRNA. Therefore we compared the quantities of bound ribosomes in DNA-less and DNA-proficient bacteria.

Back to the problem

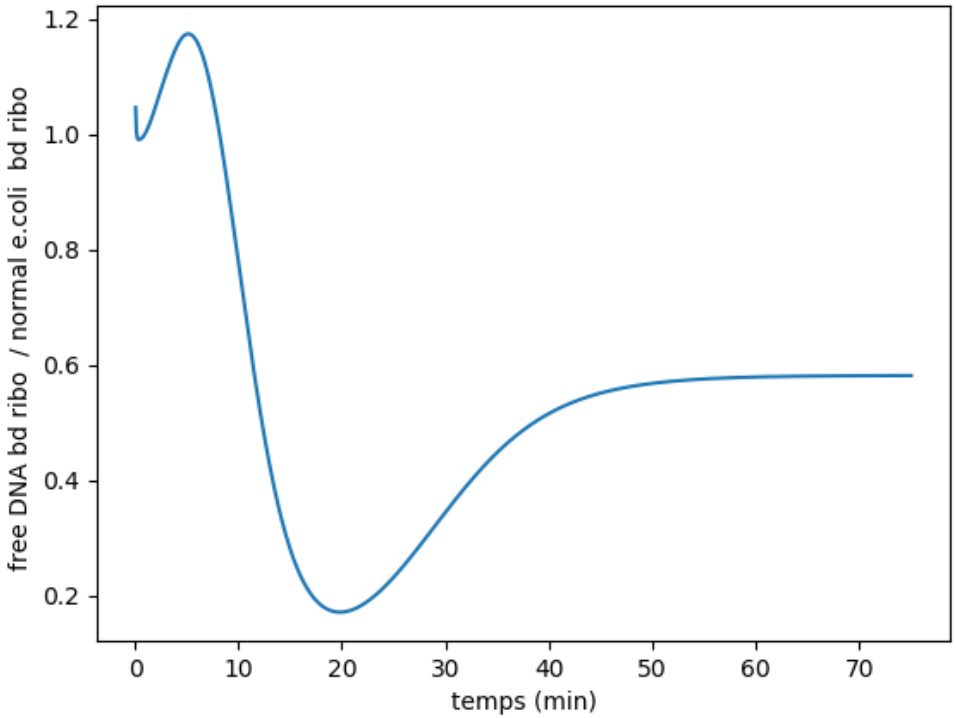


Figure: Ratio plot of bound ribosomes quantity in DNA-less and DNA-proficient infected bacteria.

Back to the problem

- After DNA-less bacteria completely stop producing ribosomes so less ribosomes are available to bind to vRNAs
- After 20mn, the ratio increases again to reach 0.6. Maybe the ribosomes that were translating the host mRNAs detached from them and started translating vRNAs.

Bound ribosomes ratio is quasi-equivalent to proteins production rate ratio. Therefore we can conclude that average production rate for phage proteins is higher on the bacteria with than without gDNA.

Conjecture

the lower phage production in DNA-less bacteria is due to a lower quantity of host ribosomes

Thanks for you attention !

References I

Marian N Beremand and Thomas Blumenthal, *Overlapping genes in rna phage: a new protein implicated in lysis*, Cell **18** (1979), no. 2, 257–266.

Andrea Y Weiße, Diego A Oyarzún, Vincent Danos, and Peter S Swain, *Mechanistic links between cellular trade-offs, gene expression, and growth*, Proceedings of the National Academy of Sciences **112** (2015), no. 9, E1038–E1047.