Week 1: Bacterial growth laws

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Growth laws

At the foundation of molecular biology lie studies in microbiology of the behavior of bacteria across different conditions. In the early days it was of course impossible to easily monitor the molecular components of bacterial cells and so, naturally, researchers were focusing on variables that they could easily measure, such as the total growth that was observed in different conditions.

Typically, when a small batch of genetically identical bacteria are put in fresh media that contain nutrients the number of bacteria as a function of time typically goes through the following stages

1. A lag phase where the number of bacteria stays constant.
2. An exponential growth phase where the number of bacteria $B(t)$ grows exponentially with time $B(t) \propto e^{\lambda t}$.
3. A stationary phase where the number of bacteria stays again fixed.
4. A death phase where the number of bacteria drops roughly exponentially with time $B(t) = \exp(-\delta t)$.

Thus, bacterial growth curves can be characterized by the lengths of these phases and by the *growth-rate* λ during the exponential phase, and the *death-rate* δ during the death phase. In the paper that we are discussing the growth-rate λ is going to be a key quantity of interest, i.e. the paper investigates how other general characteristics of cellular state depend on λ.

It was found very early on that, apart from varying between different strains of bacteria, the growth-rate for a given strain depends on many variables such as the temperature, the acidity and salt concentration of the medium, and the kinds of nutrients that are available in the media. One of the founders of molecular biology, Jacques Monod, was one of the first people to formulate a

quantitative law that related growth-rate λ to nutrient concentration c, i.e. the well-known *Monod equation*

$$\lambda = \lambda_{\max} \frac{c}{c + k_c}, \tag{1}$$

where k_c is a constant. This is a hyperbolic relationship between the concentration of the nutrient and the growth-rate. At low concentration the growth-rate increases linearly with slope $1/k_c$, at a concentration $c = k_c$ the growth-rate has reached half of its maximum, and at very high concentration the growth-rate saturates at $\lambda_{\max}$. By the way, if you have 15 minutes to spare, I can highly recommend this video interview with Jacques Monod from 1966.

In the paper we are reading this week it is investigated how certain quantities, like the ratio between total amount of RNA and protein in the cell, depends on the growth-rate λ as this growth-rate is varied either by changing nutrients or by adding antibiotics to the growth-media.

Questions about: Interdependence of Cell Growth and Gene Expression: Origins and Consequences

The paper we will discuss is entitled "Interdependence of Cell Growth and Gene Expression: Origins and Consequences" written by Matthew Scott, Carl W. Gunderson, Eduard M. Mateescu, Zhongge Zhang, and Terence Hwa. It was published in Science in 2010.

As with all papers written in high-profile journals such as Nature and Science, this paper is very condensed in its presentation. It is therefore very helpful to also read the supplementary material that comes with the paper. You will probably find especially the sections on the 'Theoretical Analysis' necessary reading to understand the key points of the paper. As an additional resource, I have also uploaded a review paper by Matthew Scott and Terry Hwa that, in my opinion, presents some of the key theoretical ideas in a slightly more intuitive way.

Question 1

The first main 'growth-law' that is discussed in the paper is shown in Figure 1A and written in equation (1). It shows a linear relationship between two quantities. Which are these quantities on the horizontal and vertical axis? What does each symbol/dot in the plot correspond to? What is being varied across these symbols/dots? There are both colored and non-colored symbols/dots. What do they correspond to? What do the different colors correspond to? The vertical axis has a scale both on the left and the right. What are these quantities? Apparently, these quantities are directly proportional to each other. Does this make sense? What does it imply that these are directly proportional? In particular it seems to imply something about the ratio between ribosomal RNA and total RNA in different growth conditions. What does it imply?

Question 2

A key thing that we want to understand is the relationship (1), i.e. as cells grow in different environments at different rates, the fraction of all proteins that are ribosomes increases linearly with the growth rate itself. In the theoretical analysis part of the supplementary materials one derivation is given for this observation.

The key ingredients are that:

1. When the cells are undergoing exponential growth at rate λ, the total amount of new protein that is created per unit time is λP, where P is the current total amount of protein.

2. All new proteins are made by ribosomes. Thus, the total amount of protein made per unit time is also given by the total number of ribosomes (or more precisely the total number of ribosomes that is actively translating) times the average translation rate.

3. Another key assumption is that the average translation rate is *the same* in all different conditions, i.e. independent of the growth-rate (Extra: Do you find *a priori* plausible? Is there a reason why evolution might have selected cells to always translate at the same rate, independent of growth-condition?).

Use these assumptions to derive equation (1). Why is the inverse of the slope $1/\kappa_t$ proportional to the translation rate in this explanation? What does the off-set r_0 correspond to?

Question 3

In figure 1B the authors now look at a set of 'translational mutants', i.e. in these mutants the ribosome moves at a different speed over the mRNAs. What is plotted in figure 1B? What is being varied for the points that roughly fall onto the solid straight lines? What is shown in the inset? What do the three points in this inset correspond to? How does this fit with the 'theory' that we derived in question 2? Finally, what is being varied along the dotted line?

Question 4

A 'new' linear growth law is identified from figure 1B and given in equation (2). Precisely what in Figure 1B does this equation correspond to? What is being varied that leads to different growth rates in this case, and what is being kept the same? More extensive data supporting this law is shown in Figure 2. Clearly the lines fitted to each set of points with a given color have different slopes $1/\kappa_n$. Thus, κ_n is a quantity that is associated with the color of the dot. What is this? Note that in supplementary figure S3A a relationship is shown

between κ_n and the growth-rate λ_0 when there are no anti-biotics, i.e. without translation-rate inhibition, which may help interpret κ_n.

Question 5

Figure S3B shows the fitted intercepts $r_{\max}$ for the solid lines shown in figure 2A, i.e. the $r_{\max}$ of equation (2). The findings suggest that $r_{\max}$ depends very little on the nutrients in which the cell is growing. How does $r_{\max}$ relate to the fraction of all proteins in the cell that are associated with ribosomes? The authors now suggest that, because there is an $r_{\max}$ corresponding to much less than 100% ribosomes, the set of proteins that are not ribosomes can be subdivided into 2 classes: a "class-Q" of proteins that always correspond to a fixed fraction of all proteins in the cell, and a "P-class" whose concentration goes down when the fraction of ribosomes ("R-class") goes up. Is there any evidence that the authors provide that there is a class of proteins that are always present at a fixed fraction (ϕ_Q) of the total proteome? Hint: Look at supplementary figure S4.

Question 6

The three classes of proteins together have to correspond to the entire proteome, i.e. $\phi_R + \phi_Q + \phi_P = 1$. How is this used to derive equation (3). This is then combined with equation (2) to derive equation (4). Again, it is important to check what is being varied in equation (4) and what is being kept the same. Can you give an intuitive explanation about what is happening?

Question 7

Figure 2C is a test of the prediction of equation (4). What is used to test this prediction? What is meant with a constitutive promoter? What is shown in figure S5B? What part of the equation (4) does this test?

Question 8

Figure 3A and the discussion of it in the text eventually reach up to the equation (5), which derives a general Michaelis-Menten relationship between growth rate and nutrient levels, i.e. exactly Monod's equation. We want to derive this relationship here ourselves. For this we need to combine the three 'laws' that have been derived in the paper.

We start with the first growth-law

$$r = r_0 + \frac{\lambda}{\kappa_t} \tag{2}$$

We can use the fact that RNA/protein ratio is directly proportional to the fraction ϕ_R of the proteome that is ribosomes, i.e.

$$\phi_R = \rho r. \tag{3}$$

Using this we get the first growth-law in terms of ribosome fraction of the proteome

$$\phi_R - \phi_0 = \frac{\rho\lambda}{\kappa_t}. \tag{4}$$

The second constraint we are going to use is that the fraction of ribosomes ϕ_R and the fraction in the P-class ϕ_P much sum up to a constant $\phi_R^{\max} = 1 - \phi_Q$, i.e.

$$\phi_P + \phi_R = \phi_R^{\max}. \tag{5}$$

Finally, we use equation (4) of the paper

$$\phi_P = \frac{\rho\lambda}{\kappa_n}. \tag{6}$$

Combine these three equations to derive equation (5) of the paper.

The way figure 3 presents this derivation is that $\kappa_n\phi_P$ is like a flux (material per unit time) of nutrients into basic 'building blocks' (e.g. amino acicds). The idea is that, the more proteins are expressed in class P, the bigger this flux, i.e. these are proteins that help turning nutrients into the basic building blocks for the cell. The parameter κ_n says something about the quality of the nutrients in the environment. That is, when κ_n is high, only a small number of proteins ϕ_P is needed to get a big flux.

Besides the influx from nutrients to basic building blocks there is also an outflux from the basic building blocks into proteins. This outflux is proportional to how many ribosomes there are, times translation rate, i.e. $\kappa_t\phi_R$.

The idea is that the two fluxes need to be balanced, i.e. $\kappa_n\phi_P = \kappa_t\phi_R$. Combining this with the constraint that $\phi_P + \phi_R = 1 - \phi_Q$ (i.e. there is a fixed fraction ϕ_Q needed for 'cell maintenance') this then allows one to solve for both ϕ_P and ϕ_R in terms of κ_t and κ_n.

Question 9

Finally, figure 4 investigates the effect on the growth-rate of uselessly expressing a gene (β-galactosidase). The authors claim that their theory (cumulating into equation (5) of the paper) predicts the effect on growth-rate without any free additional parameters. What is the key idea to this prediction, i.e. what is the assumed only effect that the extra expression has? Which value in the derivations of the previous question has now been changed? Derive equation (6) of the paper from equation (5).