A complete biophysical genotype phenotype fitness map based on antibiotic resistance.

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1 Abstract

2 Introduction

- History of people working on predicting evolution
- Recent efforts with emphasis on data based approaches
- Connect statistical mechanics models of gene expression to antibiotic resistance and growth rate
- summary of the results

3 Methods

3.1 Promoter sequence import

3.2 Reporter construct design

3.3 Barcode Mapping

3.4 Genome Integration

4 Results

5 Discussion

- discuss how to scale to 1000 genes

6 To do list

- Write Introduction
- Collect references from reg-seq paper and new references
- write paragraphs about genes chosen
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Supplemental Information for: Whatever the title will be

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S1   Plate reader Growth Rate Measurements

Strains are streaked on LB-Agar plates from frozen stocks to obtain single colonies. Single colonies are picked and grown in 3ml of LB (TR: name brand) overnight in an incubator without shaking. In the morning, cultures are diluted 1:100 into 3ml of minimal media and grown in a shaker until they reach an OD600 of 0.4, which is indicative of exponential phase. Doing the pre-growth in an incubator instead of shaker has shown to have cultures that are grown simultaneously reach exponential phase more consistently (TR: cite paper and possibly show a figure of data?). When cultures reach exponential phase, they are diluted 1:40 into 300µl of growth media in a well plate. For each well plate, growth media and antibiotic is mixed right before the experiment. To reduce the preparation time, for each antibiotic concentration used in the experiments, a stock of tetracycline (Zymo Research, A1004-25) is prepared in 100% ethanol such that 50µl of stock can be mixed with 6.5ml of growth media to obtain the target concentration. This ensures less variation in antibiotic concentration across replicates. We used Nunc edge 96 well plates (ThermoFisher Scientific catalog number 167425), which have a moat around the wells which can be filled with water to reduce evaporation during growth. Plates are grown in a Biotek Cytation (TR: Check if either 5 or 7) plate reader at 37C, with measurements every 7 min and linear shaking between measurements (check what speed). Each plate is grown for about 24h, or less if growth has stalled.

S2   Empirical Bacterial Growth Laws

In recent years, there has been a tremendous advancement in our understanding bacterial physiology from a theoretical perspective. The group of Terrence Hwa has proposed and tested a series of empirical "growth laws" based on experimental observations of cellular behavior [1, 2]. A major observation is the law that describes a linear relationship between the fraction of the proteome dedicated to ribosomes and growth rate of the cell,

$$\lambda = (r_u - r_{\min})\kappa_t, \tag{S1}$$

where λ is the growth rate, r_u is the proteomic fraction of active ribosomes, $r_{\min}$ is the minimal fraction of ribosomes needed for growth and κ_t is an empirically determined proportionality factor called translational capacity. Another result of their study was that the observed cells responded to translation inhibiting drugs by increasing the ribosome fraction of the proteome. Surprisingly, this relationship was also found to be linear,

$$\lambda = (r_{\max} - r_{\text{tot}})b \tag{S2}$$

In the absence of drug, these two equations are equal to each other, and $r_{\text{tot}} = r_u$. We can use this to determine the proportionality factor b,

$$b = \frac{1}{\lambda_0}(r_{\max} - r_u)$$

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$$= \frac{1}{\lambda_0}\left(r_{\max} - \frac{\lambda_0}{\kappa_t} - r_{\min}\right)$$

$$= \frac{\Delta r}{\lambda_0} - \frac{1}{\kappa_t}, \tag{S3}$$

where we inserted equation S1 for r_u. Now we can solve for the total ribosomes r_{tot},

$$r_{\text{tot}} = r_{\max} - b\lambda$$

$$= r_{\max} - \lambda\left(\frac{\Delta r}{\lambda_0} - \frac{1}{\kappa_t}\right)$$

$$= r_{\max} - \Delta r\lambda\left(\frac{1}{\lambda_0} - \frac{1}{\kappa_t \Delta r}\right). \tag{S4}$$

S3 Derivation of Drug Inhibition

Tetracycline binds the small subunit of the ribosome and stalls translation by inhibiting tRNA from binding [3] and enter the cell by passive diffusion through the outer membrane and subsequently energy dependent transport through the cytoplasmic membrane [3]. We denote the transport through the membrane by an constant term J_{in} (TR: Another step could be to include the energy dependent transport),

$$J_{\text{in}} = P_{\text{in}}a_{\text{ex}} - P_{\text{out}}a, \tag{S5}$$

where a_{ex} is the extracellular concentration of drug, a is the intracellular drug concentration and j is a proportionality constant. In our model, cells gain resistance by expressing drug specific secondary efflux pumps (antiporter), which draw energy from a chemical gradient. In the case of the tetracycline antiporter, the drug is exported against a protein gradient. We assume that the proton gradient is constant for the duration of the experiment (something we could think about more in detail later, apparently the import also depends on pH, so those effects might cancel). In this case, the drug kinetics can be described by a Michaelis-Menten equation describing a flux J_{ex}

$$J_{\text{ex}} = V_{\max}\frac{a}{a + K_M}, \tag{S6}$$

where K_M is the Michaelis constant of the efflux pump and $V_{\max}$ is the maximum efflux rate which depends on the expression of the resistance gene. In this model we include growth mediated feedback to gene expression [2]. That is, if growth rate is reduced, so is the expression of genes in the cell [4],

$$V_{\max} = \frac{\lambda}{\lambda_0}V_0, \tag{S7}$$

where V_0 and λ_0 are the catalytic rate and growth rate in the absence of drug. A detailed dissection of V_0 and how it depends on regulation of the resistance gene is given in section ??.

Tetracycline binds reversibly to the ribosome, therefore we can describe the binding kinetics with

$$f(r_u, r_b, a) = -k_{\text{on}}a\left(r_u - r_{\min}\right) + k_{\text{off}}r_b, \tag{S8}$$

where k_{on} is the binding rate of drug to free ribosome, k_{off} is the dissociation rate of the drug-ribosome complex, and r_b is the concentration of bound ribosomes. In the binding term, we consider

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the difference of free ribosome to minimum ribosome concentration needed for growth, the model is based on the empirical growth laws, which are not defined for $r_u < r_{\min}$. Using these terms, we can write down the ODE's describing the concentration of drug and ribosomes,

$$\frac{da}{dt} = -\lambda a + f(r_u, r_b, a) + J(a_{\mathrm{ex}}, a) - V_0 \frac{\lambda}{\lambda_0} \frac{a}{a + K_M}, \tag{S9}$$

$$\frac{dr_u}{dt} = -\lambda r_u + f(r_u, r_b, a) + s(\lambda), \tag{S10}$$

$$\frac{dr_b}{dt} = -\lambda r_b - f(r_u, r_b, a), \tag{S11}$$

where $s(\lambda)$ is the rate at which new ribosomes are synthesized. Here we are looking for steady state solutions to find a relationship between growth rate and extracellular drug concentration. To start, we solve the first empirical growth law S1 for the concentration of free ribosomes, $r_u = \lambda/\kappa_t + r_{\min}$ and use it to find the concentration of bound ribosomes by solving equation S11

$$0 = -\lambda r_b + k_{\mathrm{on}} a \left(r_u - r_{\min} \right) - k_{\mathrm{off}} r_b$$

$$= -\lambda r_b + k_{\mathrm{on}} a \frac{\lambda}{\kappa_t} - k_{\mathrm{off}} r_b$$

$$\Rightarrow r_b = k_{\mathrm{on}} a \frac{\lambda/\kappa_t}{\lambda + k_{\mathrm{off}}} \approx \frac{k_{\mathrm{on}} a \lambda}{k_{\mathrm{off}} \kappa_t} = \frac{a\lambda}{K_d \kappa_t}, \tag{S12}$$

where we simplified $k_{\mathrm{off}} + \lambda \approx k_{\mathrm{off}}$ ($k_{\mathrm{off}} \sim 10^{-2} s^{-1}$ [5] and $\lambda < 5 \times 10^{-4} s-1$ (20 min doubling time)) and introduced the dissociation constant of drug-ribosome binding, $K_d = k_{\mathrm{off}}/k_{\mathrm{on}}$. Next, we use these two results to solve for the intracellular drug concentration in S9,

$$0 = -\lambda a - \frac{k_{\mathrm{on}} a \lambda}{\kappa_t} + k_{\mathrm{off}} \frac{a\lambda}{K_d \kappa_t} + P_{\mathrm{in}} a_{\mathrm{ex}} - P_{\mathrm{out}} a - V_0 \frac{\lambda}{\lambda_0} \frac{a}{a + K_M}$$

$$= -\lambda a(a + K_M) + P_{\mathrm{in}} a_{\mathrm{ex}}(a + K_M) - P_{\mathrm{out}} a(a + K_M) - V_0 \frac{\lambda}{\lambda_0}$$

$$= a^2 \left(-\lambda - P_{\mathrm{out}} \right) + a \left(-\lambda K_M - V_0 \frac{\lambda}{\lambda_0} - P_{\mathrm{out}} K_M + P_{\mathrm{in}} a_{\mathrm{ex}} \right) + P_{\mathrm{in}} a_{\mathrm{ex}} K_M, \tag{S13}$$

Above we found expressions for both unbound ribosomes r_u and bound ribosomes r_b, which we can compare to equation S4 by noting that $r_{\mathrm{tot}} = r_u + r_b$,

$$r_{\max} - \Delta r \lambda \left(\frac{1}{\lambda_0} - \frac{1}{\kappa_t \Delta r} \right) = \lambda/\kappa_t + r_{\min} + \frac{a\lambda}{\kappa_t K_d}$$

$$r_{\max} - \frac{\Delta r \lambda}{\lambda_0} + \frac{\lambda}{\kappa_t} = \lambda/\kappa_t + r_{\min} + \frac{a\lambda}{\kappa_t K_d}$$

which we solve for the intracellular drug concentration

$$a = \frac{\Delta r \kappa_t K_d}{\lambda} \left(1 - \frac{\lambda}{\lambda_0} \right) \tag{S14}$$

By inserting equation S14 into equation S13 we obtain a polynomial of degree 3. The roots of this polynomial need to be found numerically. We obtain at least one real root for all non-negative a_{ex}. At increasing resistance, there is a non-continuity in the response (Figure **??**).

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S4 Statistical Mechanics of the Simple Repression Motif

Supplemental References

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