Supporting Information

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SI Text

Model Functions and Fitting Data to Obtain the Parameters. Fig. S1*A* shows the functions we use for f_r, f_p, and f_a. We used the following functional forms for them in Eqs. **5–7**:

$$f_r(A) = \begin{cases} 0, & \text{if } A < D_r \\ f_r^{\infty} \cdot \left(1 - \frac{D_r}{A}\right), & \text{if } A \geq D_r \end{cases}, \quad [S1]$$

$$f_p(A) = f_p^{\infty}, \quad [S2]$$

$$k_a(G) = k_a^{\infty} \cdot f_g(G) \cdot f_a(A), \quad [S3]$$

$$f_g(G) = \frac{G^{1.5}}{G^{1.5} + D_g^{1.5}}, \quad [S4]$$

$$f_a(A) = \frac{D_a}{D_a + A}. \quad [S5]$$

The functions $f_r(A)/f_r^{\infty}$ and $f_p(A)/f_p^{\infty}$ give the normalized dependences of the respective rates on ATP concentration. $(f_p/f_p^{\infty}) \approx 1$ is a good approximation under fast growth; it reflects the near independence of protein synthesis rate on ATP (1). The empirical form $f_r(A)/f_r^{\infty}$ approximates complex regulations underlying ribosome biogenesis and deployment (2) and derives from a Michaelis–Menten (MM) expression

$$\frac{f_r(A)}{f_r^{\infty}} = \frac{(A - D_r)}{D_{ra} + (A - D_r)}, \quad [S6]$$

where D_r is an activation energy to synthesize ribosome and D_{ra} is an affinity parameter. Comparing with experimental data, $A(\lambda)$ provides similar activation, and the affinity parameters $D_{ra} \sim D_r$ and substituting in the MM form gives Eq. **S1**. Its validity is observed in Fig. 2*B*. Because $f_r(A)$ is intended to capture the behavior of increase and saturation with A, our results would be similar if we choose other functional forms. Functions $f_g(G)$ and $f_a(A)$ capture, respectively, catabolism of glucose and its feedback inhibition (3). Although the choices of $f_r(A)$, $f_g(G)$, and $f_a(A)$ affect the growth law $\lambda(G, \mathbf{c_m})$ and predictions in Fig. 4 *C–H*, they do not affect the correlations $\phi - \lambda$ (Eqs. **16** and **17**), $\epsilon - \lambda$ (Eq. **S16**), the fitness optimum (Eq. **14**), and the flux matching (Eq. **15**). Our modeling here does not treat external molecules other than glucose.

We estimate the parameters piecewise by fitting our model expressions with data as described below. (*i*) We get $f_p^{\infty} \sim 0.7$ and $\gamma \sim 0.1\ \text{h}^{-1}$ as the best fit of the data of the RP fraction vs. growth rate using Eq. **16** with the known constant k_p'=9.7 h^{-1} or 20 aa/s per ribosome (Fig. 2*A*); (*ii*) we get $D_r \sim 0.18$ mM and $\lambda^{\infty} \sim 1\ \text{h}^{-1}$ as the best fit of the data of ATP concentration vs. growth rate using Eq. **S11** (Fig. 2*B*); (*iii*) we get $D_a \sim 4$ mM, $D_g \sim 0.07$ mM, and $k_a^{\infty} \sim 120\ \text{h}^{-1}$ by fitting one of the three analytical roots of Eq. **12** against data of growth rate vs. glucose concentration (Fig. 2*C*); (*iv*) we choose $k_r = \lambda_p \sim 5\ \text{h}^{-1}$ based on theory; and (*v*) $f_r(A) + f_p(A) < 1$ indicates the cell is subsaturated with energy as far as ribosome function is concerned. For our numerical ODE model $f_r^{\infty} + f_p^{\infty} = 0.9$ (Table 2) implies 90% of ribosomes are active (4).

ATP as Cellular Energy Status. Energy status is upshifted with growth rates in *E. coli* and is reflected in the concentration upshift of metabolites such as pyruvate and phosphoenolpyruvate (5) and cofactors such as ATP, GTP, and NADH (6) with specific growth rates. Therefore, there are different choices that we could have made for the internal energy cache, but ATP concentration is a reasonable surrogate for any of them, because it correlates, and ATP concentration does not violate stringent response, the guanosine tetraphosphate (ppGpp)-mediated inhibition of ribosome synthesis during amino acid starvation, because ppGpp itself is derived from ATP (7).

The Fitness Expressions. Here we show the steps that we use for deriving Eq. **12**. Substituting the definitions of the rates J_a, J_r, and J_p (Eqs. **5–7**) into Eq. **10** gives

$$\left(m_r k_r f_r + m_p k_p f_p\right)\left(\frac{R}{P}\right) = m_a k_a. \quad [S7]$$

Further substituting Eq. **8** for R/P and using Eq. **9** converts Eq. **S7** to

$$(\lambda + \gamma)\left[\lambda + \left(\frac{m_p k_p}{m_r}\right) f_p\right] = \left(\frac{m_a k_a}{m_p}\right)\left(\frac{m_p k_p}{m_r}\right) f_p. \quad [S8]$$

Defining the terms $\lambda_p \equiv (m_p k_p / m_r)$ and $\lambda_a \equiv (m_a k_a(G)/m_p)^*$ and then rearranging Eq. **S8** gives

$$\frac{\lambda^2}{\lambda_p f_p} + \lambda\left(1 + \frac{\gamma}{\lambda_p f_p}\right) + (\gamma - \lambda_a) = 0. \quad [S9]$$

Based on observations (Fig. S2*B*), $f_p[A(G)]$ reaches constant values with growth rates, so we make the approximation of $f_p[A(G)] \to f_p^{\infty}$ over the entire growth range. Next, to obtain the cubic polynomial in λ (Eq. **12**) and its coefficients, we expand Eq. **S9** completely in terms of λ by writing λ_a explicitly in terms of λ

$$\lambda_a[G, \lambda(G)] = \left(\frac{m_a k_a^{\infty}}{m_p}\right) \cdot \frac{G^{1.5}}{D_g^{1.5} + G^{1.5}} \cdot \frac{D_a}{D_a + \frac{D_r}{1 - (\lambda/\lambda^{\infty})}}, \quad [S10]$$

using the correlation of ATP concentration and λ from Eqs. **9**, **11**, and **S1**

$$A = \frac{D_r}{1 - (\lambda/\lambda^{\infty})}. \quad [S11]$$

We get

$$a_3\lambda^3 + a_2\lambda^2 + a_1\lambda + a_0 = 0, \quad [S12]$$

where the coefficients are

$$a_0 \equiv \lambda^{\infty}\lambda_p f_p^{\infty}\left[\delta\gamma - \lambda_a^{\infty}\left(\frac{G^{1.5}}{D_g^{1.5} + G^{1.5}}\right)\right], \quad [S13]$$

$$a_1 \equiv \lambda_p f_p^{\infty}\left[\lambda_a^{\infty}\left(\frac{G^{1.5}}{D_g^{1.5} + G^{1.5}}\right) + \delta\lambda^{\infty} - \gamma\right] + \delta\gamma\lambda^{\infty},$$

$$a_2 \equiv \delta\lambda^{\infty} - \lambda_p f_p^{\infty} - \gamma,$$

$$a_3 \equiv -1,$$

*λ_a is the ratio of a cell's total ATP generation flux to the ATP cost of making 1 NRP molecule; it has units of hours^{-1} and represents a driver of biomass growth. It has an impression of efficiency of the metabolic proteins for energy production and could also be measured from experiments.

and where we defined $\lambda_a^\infty = (m_a k_a^\infty)/m_p$ and $\delta = 1 + (D_r/D_a)$. The solution of Eq. **S12** yields three real roots, of which only one determines the observed glucose dependence of the specific growth rate $\lambda(G)$, the Monod law. It is readily found that $\lambda \equiv \lambda(G)$ in Eq. **12** gives a Monod-like growth function, the shape of which depends on constants λ_a^∞, D_g, δ, λ_p, f_p^∞, λ^∞, and γ.

Ribosomal Efficiency: The Net Peptide Elongation Rate. Here, we derive the relationship between the peptide elongation rate k_{per} and ribosomal fraction $\phi(\lambda)$. We begin by defining k_{per} in terms of fluxes J_r and J_p defined in Eqs. **5** and **6**

$$k_{per} = \left(N_r J_r + N_p J_p \cdot \chi\right)/R = N_r\left(\lambda + k_p' f_p \cdot \frac{\lambda}{\lambda+\gamma}\right), \qquad \textbf{[S14a]}$$

$$= N_r \frac{\lambda}{\phi(\lambda)}. \qquad \textbf{[S14b]}$$

Here, $N_r = 7336$ and $N_p = 325$ are the respective number of amino acid residues per ribosome and NRP molecule, and the latter's likelihood for turnover is $\chi = \left(\frac{\lambda}{\lambda+\gamma}\right)$. In Fig. 5, we plot Eq. **S14a** using $f_p = f_p^\infty$.

The Energy Efficiency ε: Definition and Properties. We define the energy efficiency of growth as the mass rate of proteins produced per mole rate of ATP spent

$$\varepsilon = \frac{\text{mass flux of all proteins produced}}{\text{molar flux of ATP synthesized}} = \frac{\rho\lambda}{m_a J_a}, \qquad \textbf{[S15]}$$

and, it is expressed in units of gram-weight of cells per mole of ATP consumed. Because energy is also spent in the synthesis of nonprotein material, including lipids, carbohydrates, and fatty acids, we make an estimate using the theoretical ATP requirements from Neijssel et al. (ref. 8, table 4) for *E. coli* growing in glucose that our model is neglecting about 15% of the total energy produced. Therefore, our estimate of ε is overestimated by this percentage. The protein density is denoted by ρ.

To derive Eq. **13**, we start from Eq. **S15** and substitute Eq. **10** for $m_a J_a$

$$\varepsilon(\lambda, f_p) = \frac{\rho\lambda}{m_a J_a} = \frac{\rho\lambda}{\rho\phi\left[\left(\frac{\lambda}{\varepsilon_r}\right) + \left(\frac{k_p' f_p}{\varepsilon_p}\right)\right]} = \frac{\lambda}{\left[\frac{\lambda+\gamma}{\lambda+\gamma+k_p' f_p}\right]\left[\left(\frac{\lambda}{\varepsilon_r}\right) + \frac{k_p' f_p}{\varepsilon_p}\right]}. \qquad \textbf{[S16]}$$

In the second line, we replaced ϕ from Eq. **16**. The term in the square bracket of the denominator of the first equation is the total cost of synthesizing all proteins (RP + NRP) per unit time per ribosome. $(1/\varepsilon_r)$ and $(1/\varepsilon_p)$ are the respective costs of making RPs and NRPs in units of moles of ATP per gram of ribosome or NRP. Experimental $\phi(\lambda)$ data can be transformed to $\epsilon(\lambda)$ from Eq. **S16** by eliminating $k_p' f_p$ via Eq. **16**

$$\epsilon(\lambda; \gamma, \epsilon_r, \epsilon_p) = \frac{\frac{\lambda}{\lambda+\gamma}}{\frac{\lambda}{\lambda+\gamma}\cdot\frac{\phi}{\epsilon_r} + \frac{(1-\phi)}{\epsilon_p}}. \qquad \textbf{[S17]}$$

To derive Eq. **14**, we substitute the constraint $f_r^\infty + f_p^\infty = 1$ into Eq. **S16** to get

$$\varepsilon\left(f_p^\infty\right) \equiv \varepsilon\left[k_r\left(1 - f_p^\infty\right), f_p^\infty\right]. \qquad \textbf{[S18]}$$

Then, to find the value $f_p^\infty = f_p^{\infty,*}$ that maximizes $\varepsilon(f_p^\infty)$, we set the derivative to zero, i.e., $d\varepsilon(f_p^\infty)/df_p^\infty = 0$, and solve for f_p^∞. This gives the result shown in Eq. **14**, where $\varepsilon_{rp} = (\varepsilon_p - \varepsilon_r)/\varepsilon_r$, and we removed small terms involving γ^2 and γ^3. It also gives the growth rate at the point of optimal energy efficiency

$$\lambda^{\infty,*} = k_r\left(1 - f_p^{\infty,*}\right) \approx \frac{\gamma}{\varepsilon_{rp}}\left(1 - \frac{k_p'}{k_r}\right) + \sqrt{\frac{\gamma}{\varepsilon_{rp}} k_p'}, \qquad \textbf{[S19]}$$

where $\varepsilon_{rp} = (\varepsilon_p - \varepsilon_r)/\varepsilon_r$. Eqs. **14** and **S19** do not depend on the form of $f_r(A)$ and $f_p(A)$.

To split efficiency contributions from R and P components in Fig. 4*A*, Eq. **S16** can be exactly written as

$$\epsilon = \epsilon_r' + \epsilon_p' = \epsilon_r j_r + \epsilon_p\left(\frac{\lambda}{\lambda+\gamma}\right) j_p, \qquad \textbf{[S20]}$$

where $j_r = j_r(\lambda) = \lambda/(\lambda + \lambda_p f_p)$ and $j_p(\lambda) = 1 - j_r(\lambda)$ are the fractional ATP fluxes along the respective R and P paths, and the factor $\left(\frac{\lambda}{\lambda+\gamma}\right)$ corrects ϵ_p due to protein turnover with rate γ.

Flux Matching at High Speeds. Here, we derive Eq. **15**: $\lambda^\infty = k_r f_r^\infty = \lambda_a f_p^\infty$. First, we note that under fast-growth conditions, all ribosomes are busy either making ribosomes or making NRPs; hence, under those conditions, we have the constraint

$$f_r^\infty + f_p^\infty = 1. \qquad \textbf{[S21]}$$

Under slower-growth conditions, some ribosomes are typically unused. Under fast growth, we also have $\gamma = 0$. Now, $\lambda \equiv \lambda(\lambda_a, k_r, \lambda_p)$ is a function of three rates. By combining Eqs. **S9**, **11**, and **S21**, we get

$$\left(\frac{1}{\lambda_p} - \frac{1}{k_r}\right)\lambda^2 + \lambda - \lambda_a f_p^\infty = 0 \Rightarrow \left(\frac{1}{\lambda_p} - \frac{1}{k_r}\right)\lambda^2 + \left(1 + \frac{\lambda_a}{k_r}\right)\lambda - \lambda_a = 0, \qquad \textbf{[S22]}$$

The positive solution of Eq. **S22** can be written as

$$\lambda^\infty = \frac{\lambda_a}{1 + \lambda_a/k_r}\left[1 - \beta + 2\beta^2 - \cdots\right], \qquad \textbf{[S23]}$$

where we have simplified this expression by defining

$$\beta \equiv \lambda_a \frac{1/\lambda_p - 1/k_r}{(1 + \lambda_a/k_r)^2}, \qquad \textbf{[S24]}$$

for $0 \le |\beta| \ll 1/4$. β is expected to be small in general. By setting $\beta \approx 0$, a line of steepest ascent in the fitness landscape, we get a limiting expression for growth rate (9)

$$\lambda^\infty \sim \lambda_p \frac{\lambda_a(G)}{\lambda_p + \lambda_a(G)}. \qquad \textbf{[S25]}$$

Comparing against Eq. **11**, we find that $f_r^\infty = \lambda_a/(k_r + \lambda_a)$. Because $f_p^\infty = 1 - f_r^\infty$, we have $f_p^\infty = k_r/(k_r + \lambda_a)$, giving the result in Eq. **15**.

An interesting result is an estimate of the relative rank ordering of rate coefficients. By using $f_p^\infty = 0.8$ and $f_r^\infty = 0.2$, we get

$$\lambda_a < k_r \le k_p'. \qquad \textbf{[S26]}$$

This indicates that the slowest rate coefficient (under fast growth) is λ_a, which characterizes metabolism and not the production of protein.

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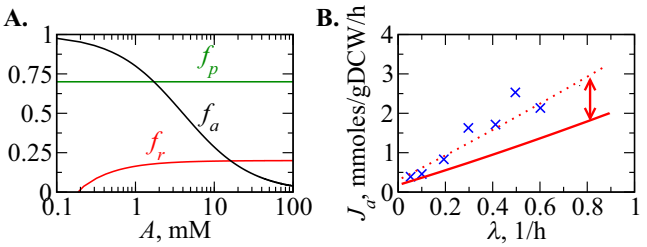

Fig. S1. (*A*) The rate functions $f_p(A)$ and $f_r(A)$ for protein and ribosome synthesis, respectively, and $f_a(A)$ for ATP as functions of ATP concentration. (*B*) Flux of glucose, J_a, conversion to ATP, from experimental oxygen uptake rate data, J_O, compared against model. We use the theoretical conversion factor $J_a = J_O/n$ where $n = 6$ is the theoretical maximum number of moles of oxygen per mole of glucose for respiration. The converted data, × [Vemuri et al. (1)], exceeds the model prediction by a factor of ~1.7 (. . .), due to nonrespirational oxygen, not treated here.

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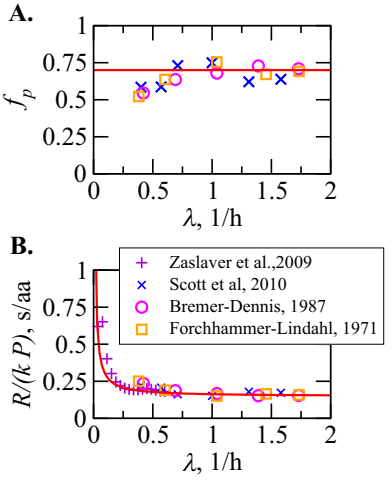

Fig. S2. (*A*) Fraction of ribosomes translating NRPs, $f_p(\lambda)$, is constant between moderate to fast growth rates, λ. Hence, we assume $f_p(\lambda) = f_p^{\infty}$ (solid red line). $f_p(\lambda)$ is computed from data, $\phi(\lambda)$, via Eq. **16**, where $\gamma = 0.1\ \mathrm{h}^{-1}$. (*B*) Average time in seconds to extend an average NRP by one peptide bond, $R/(\lambda \cdot P)$ vs. λ. Symbols are for experimental data of $\phi/[\lambda(1-\phi)]$, from the following: ×, Scott et al. (1); +, Zaslaver et al. (2); ○, Bremer and Dennis (3); □, Forchhammer et al. (4). Red line is the model prediction.

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