SUPPLEMENTAL MATERIAL

A Repression dynamics

As mentioned in the main text, free operons (O_F) can be bound by either mRNA polymerase molecules (P) or active repressor molecules (R_A). After binding, the mRNAP-DNA complex has to undergo a series of isomerizations before it can assemble the first mRNA nucleotide. This whole process is assumed to take place with a rate $k_p P M_F(t)$. On the other hand, the repression process is taken as a reversible first order reaction, with forward and backward constant rates k_r and k_{-r}, respectively. All the parameters involved in these processes are estimated in Section B and shown in Table 3. They reveal that repression is about two orders of magnitude faster than the binding of mRNAP to free operons. This fact justifies a quasi-steady state assumption for repression, which implies the following chemical equilibrium equation,

$$k_r O_F(t) R_A(t) = k_{-r} O_R(t), \tag{A.1}$$

where $O_R(t)$ represents the concentration of repressed operons. Let O denote the total operon concentration and O_P the concentration of operons with a mRNAP in the controlling section. Assume that DNA replication produces enough new *trp* operons to balance dilution so that O keeps constant. Thus, $O = O_F(t) + O_R(t) + O_P(t)$. By solving for $O_F(t)$ from this and Eq. A.1 we obtain

$$\begin{aligned} O_F(t) &= O - O_P(t) - O_R(t) \\ &= \frac{K_r}{K_r + R_A(t)}\big(O - O_P(t)\big), \end{aligned} \tag{A.2}$$

with $K_r = k_{-r}/k_r$.

After an mRNAP binds a free operon and assembles the first mRNA nucleotide, it moves along the operon continuing with transcription. It takes a time τ_p (estimated in Section B) for a polymerase to move far enough to free the operon controlling section. From these facts, the binding rate of mRNAP to free operons is $k_p P O_F(t)$, while the rate of operons freed by polymerases who have moved far enough equals the rate of mRNAP that bound free operons a time τ_p ago, times a dilution factor due to the assumption that the bacterial culture is exponentially growing at a rate μ, *i.e.:* $k_p P O_F(t-\tau_p) e^{-\mu\tau_p}$. The equation governing the dynamics of O_P resulting from all these considerations is

$$\frac{dO_P}{dt} = k_p P\big[O_F(t) - OF(t-\tau_p)e^{-\mu\tau_p}\big] - \mu O_P(t). \tag{A.3}$$

The equation for the O_F dynamics can be derived from Eq. (A.2) and (A.3) as

$$\begin{aligned} \frac{dO_F}{dt} &= \frac{K_r}{K_r + R_A(t)}\Big\{\mu O - k_p P\big[O_F(t) - \\ &\qquad O_F(t-\tau_p)e^{-\mu\tau_p}\big]\Big\} - \mu O_F(t). \end{aligned} \tag{A.4}$$

The term $K_r/\big[K_r + R_A(t)\big]$ in the above equation is the fraction of non-repressed operons, while the term μO is the operon production rate, which is such that it balances dilution to keep O constant.

B Parameter estimation

Bremer and Dennis [B1] reported growth rates of *E. coli* cultures under different temperature and nutrient conditions. These rates vary from $0.6\,\mathrm{h}^{-1}$ to $2.5\,\mathrm{h}^{-1}$. Bliss [B2] reports experimental results about the kinetics of tryptophan production in *E. coli* cultures. In those experiments, the growth rate is $2.0\times10^{-4}\,\mathrm{s}^{-1}$. Here, we employ the smallest growth rate reported by Bremer and Dennis [B1],

$$\mu \simeq 0.6\,h^{-1} = 1.0\times10^{-2}\,\mathrm{min}^{-1},$$

because it is the most likely to correspond to bacteria growing in minimal media (the experiments with which we compare the model in Section 3.

Bremer and Dennis [B1] further reported average steady-state concentrations of free mRNA polymerase molecules (P) and free ribosomes (ρ) at different growth rates. From these data, $P \simeq 1250$ molecules/cell and $\rho \simeq 1400$ molecules/cell for the growth rate considered here. *E. coli* are rod-like bacteria $3-5\,\mu$m long and $0.5\,\mu$m in diameter, so they have a volume in the range from 6.0×10^{-16} liters

to 9.8×10^{-16} liters. Taking a mean volume of 8.0×10^{-16} liters, the average molar concentrations of free mRNA polymerase molecules and ribosomes are calculated to be:

$$P \simeq 2.6\,\mu\text{M},$$

and

$$\rho \simeq 2.9\,\mu\text{M}.$$

From Gunsalus *et al.* [B3], the normal concentration of aporepressor (R_I) in a tryptophan free culture medium is:

$$\overline{R_I} \simeq 375\,\text{molecules/cell} \simeq 0.75\,\mu\text{M}.$$

In normal *E. coli*, there is only one tryptophan operon O per genome. *E. coli* cells can be genetically manipulated by introducing plasmids of tryptophan operon so the number of them can be increased up to a few hundred. In the present work, we consider normal cells. When a given cell is undergoing DNA replication, it generally has a complete and a second partially assembled genome. In very rapid growing cultures, it is possible to find two partially assembled genomes in cells undergoing DNA replication. This explains why the average number of genome equivalents per cell is larger than one, and according to Bremer and Dennis [B1] it is around 1.6. Therefore

$$O \simeq 3.32 \times 10^{-3}\mu\text{M}.$$

Schmitt *et al.* [B4] studied the activation of the *trp* repressor by tryptophan. In this process, two molecules of tryptophan bind the *trp* repressor in two independent sites with identical affinities and no cooperativity [12-15] (N.B. References in this supplemental material without a preceeding letter are to be found in the main text). The corresponding forward and backward reaction rate constants were respectively estimated by Schmitt *et al.* [B4] at physiological temperature as

$$k_t \simeq 3.48 \times 10^2\,\mu\text{M}^{-1}\text{min}^{-1},$$

and

$$k_{-t} \simeq 2.1 \times 10^4\,\text{min}^{-1}.$$

The dissociation constant of the tryptophan repressor-operator complex $K_r = k_{-r}/k_r$, was measured by Klig *et al.* [B5], who report the value $K_r = 2.6 \times 10^{-3}\,\mu$M. Klig *et al.* also measured the tryptophan repressor-operon complex degradation rate as

$$k_{-r} \simeq 1.2 \times 10^{-2}\,\text{min}^{-1},$$

so k_r is

$$k_r \simeq 4.6\,\mu\text{M}^{-1}\text{min}^{-1}.$$

Anthranilate synthase is feedback inhibited when two molecules of tryptophan bind each one of the *TrpE* subunits. Caligiuri and Bauerle [11] studied this process and concluded that *Trp* binding is cooperative in the wild type enzyme, with a Hill coefficient of

$$n_H \simeq 1.2.$$

They also estimated the forward (k_i) and backward (k_{-i}) reaction rates as

$$k_i \simeq 1.76 \times 10^{-2}\,\mu\text{M}^{-1}\text{min}^{-1},$$

and

$$k_{-i} \simeq 7.20 \times 10^{-2}\,\text{min}^{-1}.$$

Trp feedback inhibition of *TrpE* activity results in approximately 50% inhibition of *TrpE* activity in cultures growing in the minimal medium, which in turn results in the *trp* operon being expressed at twice the level it would be if there were no feedback inhibition (C. Yanofsky, personal communication). In our model, the activity of anthranilate synthase is proportional to the non-inhibited enzymatic concentration and its production rate is assumed to be one half that of *TrpE*. Thus, the above mentioned experimental fact may be interpreted as that one half of the anthranilate synthase pool is feedback inhibited by tryptophan in cultures growing in the minimal medium, as was done by Bliss *et al.* [4]. From this interpretation and Eq. 5 of Table 2 which defines the relation between the concentration of active enzymes (E_A) and the total enzymatic pool (E), the *Trp* concentration for bacteria growing in the minimal medium can be calculated as

$$\overline{T} = \frac{k_{-i}}{k_i} \simeq 4.1\,\mu\text{M}.$$

In this model, we take the normal steady-state conditions to be those of the bacterial culture growing in the minimal medium. Based on this, $\overline{T}$ is the normal steady state tryptophan concentration.

From the data reported by Draper [B6], the translation initiation rate k_ρ is estimated to be in the range from $k_\rho \simeq 60\,\mu\mathrm{M}^{-1}\mathrm{min}^{-1}$ to $k_\rho \simeq 600\,\mu\mathrm{M}^{-1}\mathrm{min}^{-1}$. Further, efficient mRNA's have been observed to initiate translation every $3\,\mathrm{s} = 5.0\times10^{-2}\,\mathrm{min}$ [B6]. This translation time implies that

$$\tau_\rho \simeq 5.0\times10^{-2}\,\mathrm{min}.$$

Given that initiation is the rate-limiting step of translation [B6], we must have $k_\rho\overline{\rho} \simeq \tau_\rho^{-1}$. From this relation, we estimate the value of k_ρ to be

$$k_\rho = \frac{1}{\tau_\rho\rho} \simeq 6.9\,\mu\mathrm{M}^{-1}\mathrm{min}^{-1}.$$

Mulligan *et al.* [B7] measured transcription initiation rates for several operons. The values they report range from $0.43\,\mu\mathrm{M}^{-1}$ to $3,420.0\,\mu\mathrm{M}^{-1}$. To estimate a proper value for k_p, we use the fact that tryptophan operon allows transcription initiation every $6\,\mathrm{s} = 0.1\,\mathrm{min}$ [B8]. From this, the time delay τ_p is

$$\tau_p \simeq 0.1\,\mathrm{min}.$$

Initiation is also the rate limiting process for transcription. i.e. $k_pP = \tau_p^{-1}$. Thus, k_p can be alternately estimated as

$$k_p = \frac{1}{\tau_pP} \simeq 3.9\mu\mathrm{M}^{-1}\mathrm{min}^{-1}.$$

The *TrpE* polypeptide is 520 amino acids long. This means that the length of the *trpE* gene is 1560 nucleotides long. On the other hand, Bremer and Dennis [B1] report a mRNA chain elongation rate of about 39 Nucleotides/second at the growth rate considered here. From this, τ_e can be estimated as

$$\tau_e \simeq 0.66\,\mathrm{min}.$$

Functional half-lives for different kinds of mRNA have been reported [9]. They range from 40 s to 20 min. These half-lives imply degradation rates in the range from $3.5\times10^{-2}\,\mathrm{min}^{-1}$ to $1.03\,\mathrm{min}^{-1}$. Bliss *et al.* [4] consider a degradation rate of $0.96\,\mathrm{min}^{-1}$. Here, we estimate the mRNA degradation rate by making use of the experimental fact that under normal conditions, around 30 ribosomes are bound to a single mRNA [B8]. The binding rate for ribosomes should then be about 30-fold bigger than that for D-enzymes. The ribosome binding rate can be estimated as ρk_ρ. Therefore

$$k_dD \simeq \frac{\rho k_\rho}{30} \simeq 0.6\,\mathrm{min}^{-1}.$$

The assumption of constant repressor concentration can be read as $R = R_I + R_A$. This assumption and Eq. 6 of Table 2, which determines the concentration of active repressor molecules, permit the estimation of the total repressor concentration as

$$\begin{aligned} R &= \frac{K_t+\overline{T}}{K_t}\overline{R_I} \\ &\simeq 0.8\,\mu\mathrm{M}, \end{aligned}$$

as well as the active repressor steady-state concentration as

$$\begin{aligned} \overline{R_A} &= \frac{\overline{T}}{\overline{T}+K_t}R \\ &\simeq 5.09\times10^{-2}\,\mu\mathrm{M}, \end{aligned}$$

where $K_t = k_{-t}/k_t$.

Landick *et al.* [B9] note that under excess tryptophan conditions, 85% of the mRNAP's initiating transcription halt due to transcriptional attenuation. There is also evidence that transcriptional attenuation is only released under severe tryptophan starvation. The function

$$A\,(T) = b\Big(1 - e^{-T(t)/c}\Big),$$

with

$$b \simeq 0.85$$

and

$$c \simeq 4.0\times10^{-2}\,\mu\mathrm{M},$$

satisfies those experimental observations.

Following Bliss [4] and Sinha [5], we take the enzyme degradation rate γ to be approximately zero,

since according to their estimates, it is much smaller than other terms like the growth rate:

$$\gamma \simeq 0\,\mathrm{min}^{-1}.$$

From the equations that govern the evolution of O_F [Eq. 1 of Table 2] the steady-state concentration of free operon controlling sections is

$$\begin{aligned}\overline{O}_F &= \frac{\mu}{k_p P\left(1-e^{-\mu\tau_p}\right)+\mu\dfrac{K_r+R_A(\overline{T})}{K_r}}O \\ &\simeq 1.54\times10^{-4}\,\mu\mathrm{M}.\end{aligned}$$

The normal concentration of free *TrpE*-related ribosome binding sites can be estimated from the steady state solution of the equation for dM_F/dt [Eq. 3 of Table 2] as

$$\begin{aligned}\overline{M}_F &= \frac{k_p P e^{-\mu\tau_m}\left[1-A(T)\right]}{k_\rho\rho\left(1-e^{-\mu\tau_\rho}\right)+k_d D+\mu}\overline{O}_F \\ &\simeq 3.78\times10^{-4}\,\mu\mathrm{M}.\end{aligned}$$

The steady-state concentration of anthranilate synthase enzyme can be calculated from the equation for $dE/dt = 0$ [see Eq. 4 of Table 2]:

$$\overline{E} = \frac{k_\rho\rho e^{-\mu\tau_e}}{2(\gamma+\mu)}\overline{M}_F \simeq 0.378\mu\mathrm{M}.$$

According to Bliss *et al.* [4], the internal consumption rate of tryptophan can be modeled using a Michaelis-Menten type relation:

$$g\frac{T}{T+K_g}.$$

They also assert that K_g is equal to or less than one tenth of the normal *Trp* concentration. From this value we have

$$K_g \simeq 0.2\mu\mathrm{M}.$$

The constant g stands for the maximum tryptophan consumption rate. The rate of tryptophan consumption under normal conditions can be estimated by noting that tryptophan is primarily consumed in the assembly of proteins. Bremer and Dennis [B1] report that the average cell dry weight at the growth rate considered in this work is $148\times10^{-9}\,\mu$g. By multiplying by the growth rate ($\omega \simeq 1\times10^{-2}\,\mathrm{min}^{-1}$) and dividing by the average cell volume ($8\times10^{-16}\,$liters) we get the protein production rate estimated as $1.85\,\mathrm{g\,liter}^{-1}\mathrm{min}^{-1}$. Since 20 to 25% of the cell dry weight corresponds to protein mass, and noting that tryptophan accounts for around 1% of this dry weight [4] the tryptophan consumption rate under normal conditions is

$$\begin{aligned}g\frac{\overline{T}}{\overline{T}+K_g} &\simeq 4.63\times10^{-3}\,\mathrm{g\,liter}^{-1}\mathrm{min}^{-1} \\ &\simeq 22.7\,\mu\mathrm{M\,min}^{-1}.\end{aligned}$$

This permits us to estimate g by solving for it in terms of $\overline{T}$, and K_G:

$$g \simeq 25.0\,\mathrm{min}^{-1}.$$

E. coli are capable of efficiently transporting tryptophan from its environment. To achieve this transport, *E. coli* synthesize three tryptophan permeases. Two of them are tryptophan specific, whereas the other also transports phenylananine and tyrosine [16]. The expression of the operons that encode these enzymes is dependent, in general, on the tryptophan and tryptophan repressor concentrations. Furthermore the tryptophan permeases may also play the role of maintaining a high intracellular tryptophan concentration when there is no source of extracellular tryptophan. According to Drozdov-Tikhomirov and Skurida [B10], the tryptophan uptake rate can be modeled by the following equation in terms of the internal and external (T_{ext}) tryptophan concentrations.

$$F(T,T_{\mathrm{ext}}) = d\frac{T_{\mathrm{ext}}}{e+T_{\mathrm{ext}}\left[1+T(t)/f\right]}.$$

The value of parameters e and f was also estimated by Drozdov-Tikhomirov and Skurida as

$$e \simeq 0.9\,\mu\mathrm{M},$$

and

$$f \simeq 380\,\mu\mathrm{M}.$$

Parameter d can be estimated from the experimental fact that for bacteria growing in a media with a high

tryptophan concentration (where the *Trp* uptake rate is approximately d), the enzyme activity is about one tenth of the activity corresponding to the minimal medium [17]. The value of d compatible with this experimental result is

$$d \simeq 23.5\,\mu\mathrm{M\,min}^{-1}.$$

The tryptophan production rate constant K can be estimated from the steady state equation for $dT/dt = 0$ [see Eq. 9 of Table 2 and notice that $F(T,0) = 0$] as

$$\begin{aligned} K &= \frac{G(\overline{T}) + \mu\overline{T}}{E_A(\overline{E},\overline{T})} \\ &\simeq 126.4\,\mu M^{-1}\mathrm{min}^{-1}. \end{aligned}$$

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