2

E. coli as a model system

2.1 The *E. coli* cell

2.3.1 *Assume an E.coli cell with a volume of* $1\,\mu\mathrm{m}^3$*, containing 25% proteins by weight. An amino acid weighs on average 100 Daltons (*$1\ \mathrm{Da} = 1.7\cdot 10^{-27}\,\mathrm{kg}$*), and amino acids have the same density as water. How many amino acids are incorporated in the proteins in the cell?*

Answer Bacterial volume $V_{\mathrm{bact}} = 10^{-18}\,\mathrm{m}^3$. Assuming the same density as water the mass of a bacterium is $m_{\mathrm{bact}} = V_{\mathrm{bact}} \cdot 1000\,\mathrm{kg\ m}^3 = 10^{-15}\,\mathrm{kg}$. 25% of this weight is $m_{\mathrm{amino}-B} = 2.5\cdot 10^{-16}$ kg. This gives a number of amino acids $n_{\mathrm{amino_B}} = m_{\mathrm{amino_B}}/(170\cdot 10^{-27}) = 2.5\cdot 10^{-16}/1.7\cdot 10{-}25 \sim 10^9$. Thus one bacteria contain about 1 billion amino acids, a number that is comparable to the 1.5 billion glucose molecules that are needed to make the bacterium (a glucose molecule $C_6H_{12}O_6$ has a mass of 180 dalton, and is thus slightly heavier than an amino acid).

2.3.2 *Assume that an average protein consists of 360 amino acids. How many proteins are there in the above E. coli cell?*

Answer There are $10^9/360 \sim 3\ 000\ 000$ proteins in the *E. coli* cell.

2.3.3 *Assume that one bacterial generation takes 30 minutes, and that it contains 40 000 active ribosomes. What is the average rate of translation in the cell (codons per second per ribosome).*

Answer 40000 ribosomes have to translate 1 000 000 000 amino acids in 1800 s. This makes a translation rate of $10^9/(40\,000\cdot 1800) = 100/7.2 = 14$

amino acids per second (dependent on growth conditions, translation rates between 10 and 20 amino acids have been measured).

2.3.4 *Assume that at any time there are 1000 RNA polymerases (RNAP) in an E. coli cell that transcribe mRNA. Assume further that transcription and translation occur at the same rate. Calculate the average number of proteins produced per mRNA.*

Answer The activity of 40 000 ribosomes should be balanced by the transcription of 1000 RNAP. This means that one RNAP serves the activity of 40 Ribosomes, or that the number of translations per message is 40.

2.3.5 *What time does it takes to clear a promoter site before a new RNAP can bind (RNAP occupies 75 bp on the promoter)? What is the expected theoretical maximum activity for a promoter?*

Answer With a co-occurring translation/transcription of 12.5 codons per second, the RNAP should move an average speed of $v = 37.5$ base pairs per second. Thus a new RNAP should be able to enter when the previous RNAP has cleared a distance of $x = 75$ base pairs, taking a time of $x/v = 2$. The expected maximum promotor firing rate should be 0.5 s^{-1} which in fact is much smaller than the measured maximum firing rate of ribosomal promotors.

2.4.1 *Show that the form of Eq. (2.9) is maintained when one considers that both γ and μ can be represented by Michaelis–Menten growth curves:*

$$\nu = \nu_{\max} \cdot \frac{[\text{nutrient}]}{[\text{nutrient}] + K} \quad \text{and} \quad \gamma = \gamma_{\max} \cdot \frac{\Lambda}{\Lambda + \Lambda_{\mathrm{g}}} \tag{2.1}$$

Here $\gamma_{\max}$ corresponds to a max translation speed of $20\,\mathrm{aa\ s}^{-1}$ *and* $\Lambda > \Lambda_{\mathrm{g}} = 0.5\,\mathrm{doublings\ h}^{-1}$ *(from [75]).*

Answer Consider again:

$$T \;=\; Q + R + M$$

where

$$\lambda \cdot T = \gamma \cdot (R - R^{*})$$
$$\gamma = \gamma_{max} \cdot \frac{\lambda}{\lambda + \lambda_{\mathrm{g}}}$$

meaning that $1/\gamma = (1/\gamma_{max}) \cdot (1 + \lambda_{\mathrm{g}}/\lambda)$. Metabolic proteins M, on the other hand, are proportional to the rate ν at which they can convert nutrients to amino acids:

$$\lambda \cdot T = \nu \cdot M \quad \text{with} \;\; \nu = \nu_{\max} \cdot \frac{[\text{nutrient}]}{K + [\text{nutrient}]}$$

meaning that $1/nu = (1/\nu_{\max}) \cdot (1 + K/[\text{nutrient}])$. Putting the equations together:

$$1 = \frac{Q}{T} + \frac{R}{T} + \frac{M}{T} \Rightarrow$$
$$1 = \frac{Q}{T} + \frac{\lambda}{\gamma} + \frac{R^*}{T} + \frac{\lambda}{\nu} \Rightarrow$$
$$1 = \frac{Q}{T} + \frac{\lambda}{\gamma_{\max}}(1 + \frac{\lambda_g}{\lambda}) + \frac{R^*}{T} + \frac{\lambda}{\nu_{\max}}(1 + \frac{K}{[\text{nutrient}]}) \Rightarrow$$
$$1 = \frac{\lambda_g}{\gamma_{\max}} + \frac{Q}{T} + \frac{R^*}{T} + \frac{\lambda}{\gamma_{\max}} + \frac{\lambda}{\nu_{\max}} + \frac{\lambda}{\nu_{\max}} \cdot \frac{K}{[\text{nutrient}]} \Rightarrow$$
$$1 \quad - \frac{\lambda_g}{\gamma_{\max}} - \frac{Q}{T} - \frac{R^*}{T} = \lambda \left(\frac{1}{\gamma_{\max}} + \frac{1}{\nu_{\max}} + \frac{1}{\nu_{\max}} \cdot \frac{K}{[\text{nutrient}]} \right) \Rightarrow$$
$$\lambda = \left(1 - \frac{\lambda_g}{\gamma_{\max}} - \frac{Q}{T} - \frac{R^*}{T}\right) \cdot \frac{[nutrient]}{\frac{K}{\nu_{\max}} + (\frac{1}{\gamma_{\max}} + \frac{1}{\nu_{\max}})[\text{nutrient}]}$$

which is indeed the Monod growth equation, just supplemented with some more complicated prefactors.

2.4.2 *From Eq. (2.9), and inserting Eq. (2.7)*

$$\frac{R}{T} = 1 - \frac{Q}{T} - \frac{M}{T} = 1 - \frac{Q}{T} - \frac{\Lambda}{\nu} \tag{2.2}$$

one may investigate RNA per protein by changing γ through reduction of translation rates by certain antibiotics [102]. Sketch the corresponding RNA per protein versus Λ dependencies in Fig. 2.13 and identify ν and overheads Q from the curves. Interpret the ribosome expression response to reduced translation ability of individual ribosomes.

Answer Fig. 2.1 shows that the ribosome fraction increases when one makes translation slower.

2.4.3 *Growth may be limited by more than nutrients, for phytoplankton in the ocean by light and photosynthesis, suggesting an extension:*

$$T = Q + R + M + L \tag{2.3}$$

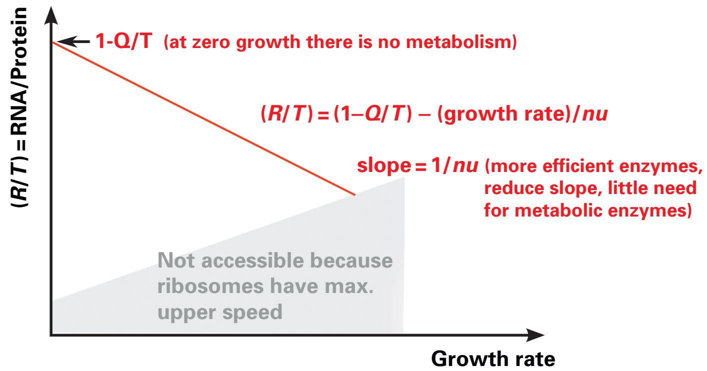

Figure 2.1 Ribosomal fraction increases when growth is slowed down by reducing the ability of ribosomes to translate (moving from right to left in the figure).

where L is the part of the protein mass allocated to photosynthesis. Assume in addition to Eqs. (2.5), (2.6), (2.7) that $\Lambda T = \alpha L$ and express $1/\Lambda$ as function of $1/\alpha$.[13] *With subdivided metabolism, the final growth can be limited with respect to any of these nutrients.*

Answer

$$1 = \frac{Q}{T} + \frac{R}{T} + \frac{M}{T} + \frac{L}{T} \Rightarrow$$

$$1 = \frac{\lambda}{\nu} + \frac{\lambda}{\gamma} + \frac{\lambda}{\nu} + \frac{\lambda}{\alpha} \Rightarrow$$

$$\frac{1}{\lambda} = \frac{1}{\nu} + \frac{1}{\gamma} + \frac{1}{\alpha} + \frac{1}{\nu} \Rightarrow$$

$$\lambda = \frac{1}{\frac{1}{\nu} + \frac{1}{\gamma} + \frac{1}{\alpha} + \frac{1}{\nu}}$$

an equation that expresses a Michaelis–Menten relation with respect to available nutrients (through ν) and a Michaelis–Menten reaction with respect to available photosynthetic energy (through α).