Supplemental Data S3

A model for the altered expression of the arginine utilization (*roc*) operons

Among the most strongly upregulated genes in the genome-reduced strains are the genes of the σ^L-dependent *roc* operons (*rocABC*, *rocG*, *rocDEF*) that are controlled by the activator RocR. σ^L is the sigma factor that exhibits the most pronounced change in the genome-reduced strain (an approximately 7-fold increase in PG10, Fig.), but no strong upregulation was seen for other σ^L-dependent, but not RocR-dependent operons, indicating a role of rocR. Indeed RocR is also strongly upregulated in the genome-reduced strains. To test whether the observed increase in σ^L and RocR are sufficient to explain the changes in roc gene transcription, we analyzed a mathematical model for the regulation of the roc operons.

The model is based on the thermodynamic (or quasi-equilibrium) models for gene regulation (Bintu et al., 2005), which are extended here by a non-equilibrium step to account for the ATP-dependent activation of σ^L-dependent transcription. Transcription is described by Michaelis-Menten-like kinetics, with a reversible binding step of the RNA polymerase $E\sigma^L$ to the promoter, characterized by a Michaelis constant *K* that can be approximated by the equilibrium dissociation constant, followed by an irreversible and ATP-dependent initiation step. This description results in the transcription rate

$$J = k(a)\frac{[E\sigma^L]}{K + [E\sigma^L]}.$$

The rate *k*(*a*) of the irreversible initiation step is taken to depend on the concentration of the activator RocR, *a*, in the following way:

$$k(a) \approx k_{max}\frac{a^{*n}}{K_a^n + a^{*n}},$$

where K_a is the characteristic concentration scale for half-maximal activation and n is a Hill coefficient describing the cooperativity of activation. Since activation by the related activator NtrC in E. coli depends on the interaction of two dimers (Mettke et al. 1995) and the *roc* operons each have two binding sites for RocR, the Hill coefficient is taken to be $n \approx 2$. a* in this expression denotes the concentration of activator in its active conformation, which it assumes upon binding of its inducer, ornithine (Gardan et al, 1997). Activation by ornithine is described by equilibrium binding, which leads to the following expression for the concentration of active RocR:

$$a^* = a\frac{o}{K_{o+o}}.$$

Here *o* is the concentration of ornithine and K_o is the equilibrium dissociation constant for binding of ornithine to RocR.
Thus, in summary we arrive at the following expression for the transcription rate:

$$J = k\frac{[E\sigma^L]}{K + [E\sigma^L]}\frac{1}{1 + \left(\frac{K_a(K_o + o)}{ao}\right)^n} \approx k\frac{[E\sigma^L]}{K}\left(\frac{ao}{K_aK_o}\right)^n.$$

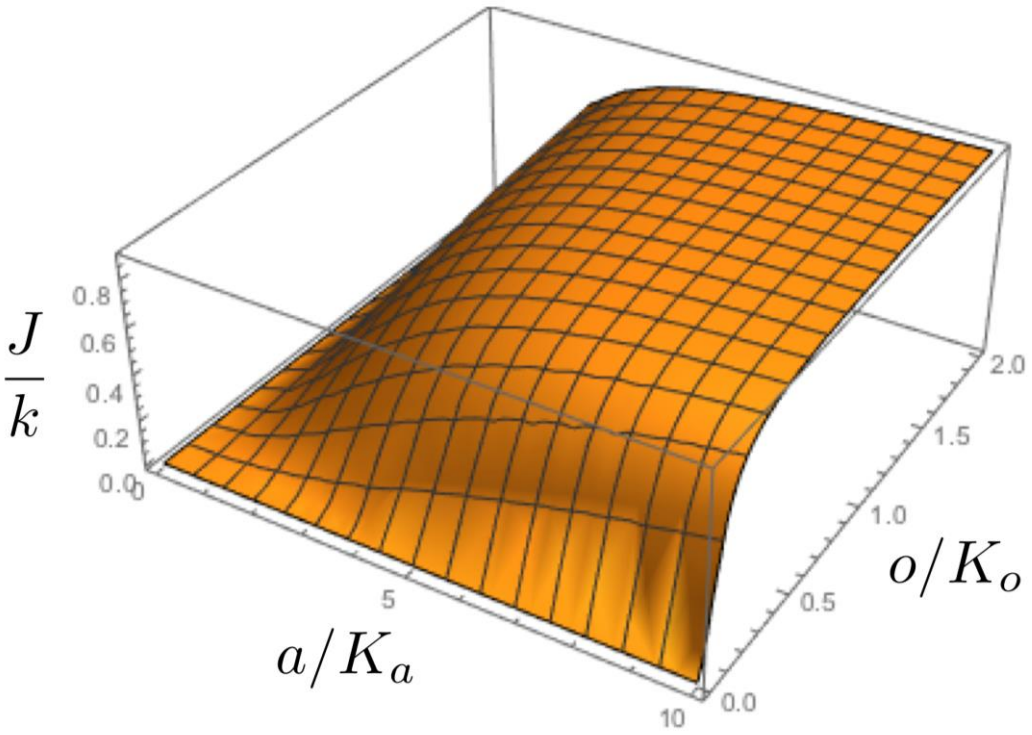

Figure 1: Normalized transcription rate as a function of the concentrations of the activator RocR (a) and the inducer ornithine (o), both expressed relative to the relevant dissociation constants.

The last line is an approximation assuming that none of the 3 binding reactions (ornithine-RocR, RocR–operator site, RNAP-promoter) is saturated. Under these conditions the strongest response to changes in the concentrations occurs. If any of the 3 reactions is closer to being saturated, the response will be less.

The calculated transcription rate is plotted in Fig. 1 as a function of the concentrations of RocR (*a*) and ornithine (*o*).

The concentrations of σ^L, RocR, and ornithine are increased in the genome reduced strains compared to the parental strain, 7-fold, 3.9-fold and 16-fold in PG10 and 5-fold, 1.6-fold and 2-fold for PS38. Thus the maximal potential increase in the transcription rate in PG10 is then 7-fold due to σ^L, 3.9^n-fold due to RocR, and 16^n due to ornithine, and with $n \approx 2$, overall an up to ≈27000-fold increase in the transcription rate is possible. If we assume that the promoter is saturated with RNAP, which for σ^N-type sigma factors is often a good approximation ("poised RNA polymerases"), the maximal increase is still ≈3900-fold, which may be an underestimate if the promoter is only saturated in the reduced strain, but not in the parental strain. On the other hand, the concentration of the RNA polymerase holoenzyme with σ^L may increase more strongly than the concentration of σ^L itself, if sigma factor competition is changed from one strain to the other. Thus, the strong observed upregulation of *roc* genes in

the genome-reduced strain (1400-fold for RocA in PG10) can be explained by the observed increase in the concentration of the relevant regulators σ^L, RocR, and ornithine. For pS38, the maximal increase expected from this calculation is 50-fold compared to the parental strain, less than what is observed (a 250-fold increase), indicating either an additional contribution (5-fold) to the regulation, for example due to changed sigma factor competition, or a requirement for larger cooperativity ($n \approx 3.5$).

References

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