Computational Model for the Bacterial Stringent Response

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Introduction and Objectives

The **stringent response** is the set of metabolic and regulatory changes that take place in a bacterium as a consequence of a downshift in the availability of nutritional substances, especially amino-acids. A clear, quantitative understanding of the its precise mechanism is of immediate potential utility given its role in the dormancy of *M. tuberculosis*, still one of the most important infectious disease worldwide.

Typically, the stringent response is accompanied by a **down-regulation** of the transcription of **stable RNA** and certain species of mRNA. Other species of mRNA are up-regulated. As a result of this **differential regulation**, the translation machinery is greatly slowed down and significant resources are shifted to the production of amino-acids. Both in *E.coli* and *M.tb,* the stringent response is believed to be mediated by guanosine 5'-diphosphate 3'-diphosphate and 5'-triphosphate 3'-diphosphate, or **(p)ppGpp**. Experimentally it has been established that (p)ppGpp concentrations increase during the stringent response. Some observations suggest the presence of a **surge** of (p)ppGpp that lasts a relatively short time followed by the establishment of a lower equilibrium level.

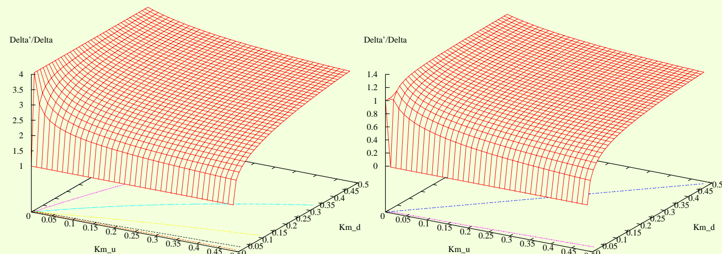
While the main features of the stringent response mechanism outlined above are generally accepted, there is disagreement regarding the specific mechanism that achieves the differential regulation. We wish to define a comprehensive *in silico* model for the stringent response which encompasses the complete causal cycle of the stringent response: the differential regulation driven by (p)ppGpp, the change in translational activity as a result of differential regulation, and the production/destruction of (p)ppGpp controlled by the level and status of transcription.

Results

We use our framework to compare the predictions of different sets of parameters consistent with mechanisms proposed in the literature. One point of disagreement is in the details of how differential regulation takes place in the presence of (p)ppGpp. Our kinetic constants for transcription initiation relate to the Michaelis-Menten constant as follows: $K_m = (k_2 + k_3)/k_1$

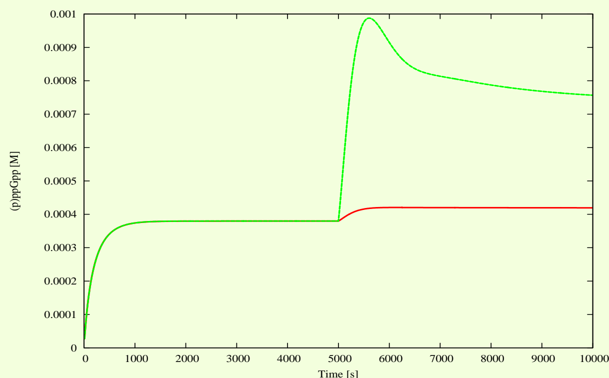
In the view of Zhang et al., stable RNA promoters are unsaturated and amino-acid promoters are not, and the main effect of (p)ppGpp is to increase the transcription time, leading to a decrease in the concentration of free RNAP, hence a lowered transcription rate for the amino-acid promoters. In our model this comes down to an increased τ parameter for all RNA species.

In work by Barker et al. it is proposed that it is the amino-acid promoters which are unsaturated, and the effect of (p)ppGpp is a decrease of the association constant (equivalent to our k_1) for stable RNA promoters as well as an overall increase of the equivalent of k_2, both leading to an increase of K_m, but only for stable RNA promoters. This leads to the release of RNAP by the previously saturated stable promoters, resulting in increased transcription of amino-acids.



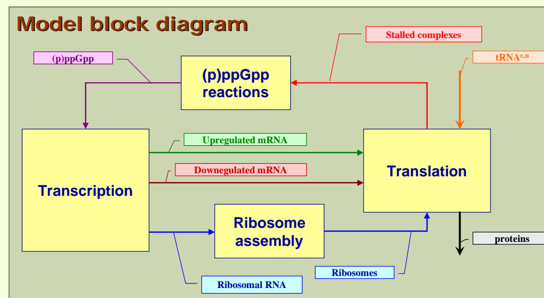
We calculated the predicted change in transcription rates for the two types of RNA ('downregulated" mRNA and stable and "upregulated' mRNA) in both scenarios, for a variety of K_m values (above). This illustrates that there are many ways to achieve a given differential regulation factor, compatible with either of the two models, so differential regulation does not clearly favor either of them.

The effect of an increase in transcription time is evident in dynamical simulations as shown below, where we plot the time evolution of the (p)ppGpp concentration during a nutritional downshift, for two different magnitudes of this increase, both quadratic in the (p)ppGpp concentration. In this case the qualitative features of the time evolution are strongly influenced by the details of the model.



Model

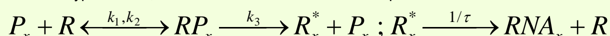
The model is structured into a number of functional units, whose inputs and outputs are the concentrations or production rates of different substances.



Transcription

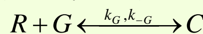
We follow three types of RNA, one representative each for the mRNA species that are upregulated, respectively downregulated in the presence of (p)ppGpp, and ribosomal RNA.

For each type of RNA, we model the kinetics of its transcription as follows:



$$P_x + R \xleftrightarrow{k_1,k_2} RP_x \xrightarrow{k_3} R_x^* + P_x \; ; \; R_x^* \xrightarrow{1/\tau} RNA_x + R$$

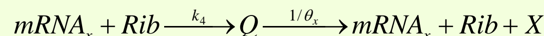
where R is RNA polymerase, P_x is the promoter, R_x^* and is the elongating complex. The kinetic constants change in the presence of (p)ppGpp. This is done by defining a separate species of RNA polymerase, C, which is a complex formed by RNA polymerase with (p)ppGpp (denoted by G below).



$$R + G \xleftrightarrow{k_G,k_{-G}} C$$

Translation and (p)ppGpp

Amino-acid availability is described via a variable parameter, the ratio of uncharged to charged tRNA, $r = tRNA^u / tRNA^c$. Translation proceeds via a complex formed by a ribosome and the mRNA being transcribed. The transcription time θ acts as the lifetime of the complex Q:



$$mRNA_x + Rib \xrightarrow{k_4} Q \xrightarrow{1/\theta_x} mRNA_x + Rib + X$$

We take the total amount of tRNA fixed. When during an elongation step, an uncharged tRNA is engaged instead of a charged one, the process *stalls.* Assuming the uncharged tRNA is bound for the same time as it would take to complete the normal elongation step, the elongation time increases in the presence of uncharged tRNA: $\theta_x' = \theta_x(1+r)$.

At equilibrium, the number of stalled complexes is: $Q^* = \frac{r}{1+r} Q$

The reactions that produce and those that destroy (p)ppGpp are influenced by the presence of stalled translational complexes. The production reactions are greatly enhanced, so the presence of these complexes leads to an increase in (p)ppGpp.

Conclusions and Outlook

The stringent response mechanism is well studied and can be reasonably isolated from the rest of the biochemical machinery of the cell. Yet, even in this case the difficulty of direct measurement of in vivo parameters results in significant room for ambiguity and guesswork.

This unfulfilled need of reliable parameter values is a general feature of the current status of system biological modeling. Our intention is to turn the relationship around and use modeling to integrate a variety of experimental observations. The requirement of matching a given experimental result places an indirect constraint on the model parameters, leading to a maximal range of those parameter values that are compatible with all the available observations.

As a first step in this endeavor, we present a draft of a complete model of the stringent response, and illustrate how qualitatively different observations/predictions are related to the same model parameters. While both the presence or absence of a slowdown in transcription in the presence of (p)ppGpp appears compatible with reasonable levels of differential regulation, the two possibilities lead to observable differences in the time evolution of the model during the onset of the stringent response.

We are planning to review more of the existing experimental observations relevant to the stringent response and identify the constraints posed on the model parameters.

Acknowledgements

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