

Classifying kinetic models of ppGpp in stringent response and growth regulation

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Introduction

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. Similar changes occur when bacteria undergo diauxic shift. In both cases the growth rate is reduced dramatically. Under some circumstances growth is eventually resumed at a lower rate. A clear, quantitative understanding of the precise mechanism is of immediate utility given its possible 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.tuberculosis*, 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.

Genomic data (Chang, 2002) indicates that the reprogramming of transcription associated with (p)ppGpp during stringent response is very similar to that during transient growth arrest occurring during diauxie. Measurements during **exponential growth** (Bremer, 1996) also exhibit a correlation between increased (p)ppGpp concentration, downregulation of rRNA transcription, as well as reduced growth rates.

While it is accepted that (p)ppGpp plays a role in the reprogramming of transcription, there is some disagreement in the literature regarding the **specific mechanism** that achieves the differential regulation.

Objectives

We constructed a generic **mathematical model** for transcriptional control by (p)ppGpp, which encompasses the **complete causal cycle** of the stringent response: differential regulation driven by (p)ppGpp; change in translational activity as a result of differential regulation; production and destruction of (p)ppGpp controlled by the level and status of translation.

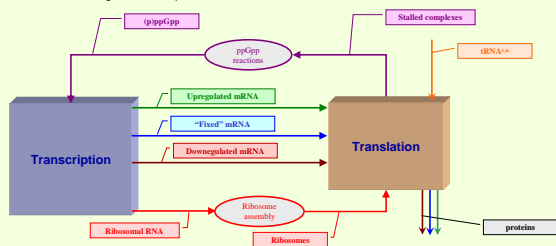
With a relatively large number of model parameters that are not directly measured *in vivo*, our model is in a sense **underdetermined**. Many possible parameter sets are compatible with the experimental information we implemented so far. Our approach is to use the model as a vehicle to **integrate and compare diverse experimental information** by mapping out those sets of model parameters that are consistent with the respective data.

Similarly, theoretical statements (models) regarding a specific mechanism can be formulated as mathematical constraints on model parameters or model predictions (the latter are ultimately mapped back onto parameters). The validity or feasibility of these **theoretical constraints** can be assessed by comparing their constraints on model parameters with those resulting from experimental data.

Our working assumption is that the mechanism outlined above is responsible both for the fast and dramatic changes in transcription that occur during growth arrest and the relatively small adjustments that accompany long term variations in during exponential growth. This assumption may be disproved if no parameter sets are found that are compatible both with exponential growth data and time series for stringent response.

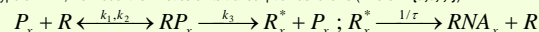
Model definition

Below is a 'block diagram' of the processes described in the model.

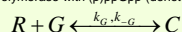


Transcription

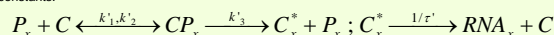
We follow four types of RNA, one representative each for the mRNA species that are upregulated (*u*), downregulated (*d*), respectively unchanged (*f*) in the presence of (p)ppGpp, and ribosomal RNA (*r*). For each type of RNA, we model the kinetics of its transcription as follows (where $x=(u,d,f,r)$):



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 modeled by defining a separate species of RNA polymerase, C, which is a complex formed by RNA polymerase with (p)ppGpp (denoted by G below).



This complex RNAP participates in the same reactions as R, but is characterized by a different set of kinetic constants:



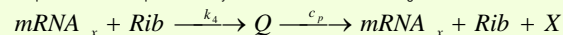
At some finite (p)ppGpp concentration [G], a fraction of all RNAP is in the modified form. The ratio of partition is an increasing function of [G].

$$[R_{total}^0] = [R_{total}] + [C_{total}] \quad ; \quad [C_{total}]/[R_{total}] = f([G])$$

Translation and (p)ppGpp

Amino-acid availability is modeled by a variable parameter, the ratio of uncharged to charged tRNA, $r = \frac{[tRNA^-]}{[tRNA^+]}$.

Translation proceeds via a complex formed by a ribosome and the mRNA being translated.



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 and the elongation rate decreases accordingly:

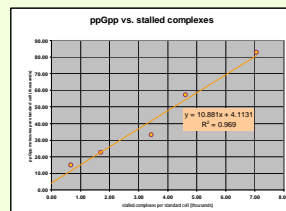
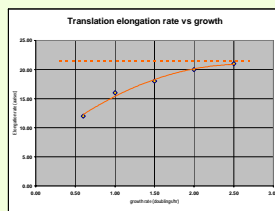
$$\frac{c_p^{stall}}{c_p} = 1 + r \quad . \quad \text{At equilibrium, the number of stalled complexes is: } Q^* = \frac{r}{1+r} Q$$

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

The additional (p)ppGpp reduces the transcription of stable RNA, eventually reducing the number of ribosomes and transcribing complexes, and the system settles at a lower transcription-translation rate appropriate for the reduced amino-acid availability.

Parameter assessment

The definition of the four promoter groups is based on the genomic study by (Chang, 2002). The majority of the kinetic data in our model is from the compilation of (Bremer, 1996). Many parameter values are directly found there. Some are estimated as illustrated for the maximum translation rate and the dependence of (p)ppGpp on stalled complexes.



The case of the transcription kinetic parameters for the four promoter groups is more challenging. They are not directly measured, and their values in the absence and presence of ppGpp are subject to theoretical debate. We developed a procedure to search the space of these parameters for points (sets) which provide a reasonable fit to the total mRNA and stable RNA transcription rates given in (Bremer, 1996).

We first convert our three constants into a promoter strength (same as our k_j) and a Michaelis-Menten saturation constant (where the promoter is the 'enzyme' and free RNAP is the 'substrate': $K_m = (k_2 + k_3) / k_1$). One parameter set specifies the four (K_m, k_3) values (only six are different) in the absence of (p)ppGpp, as well as the x and y factors that relate the 'base' values to those at saturation with (p)ppGpp. The parameter x increases the K_m of stable RNA and the 'downregulated' mRNA group, and y increases the transcription time for all promoter groups. For each parameter set we calculate (if possible) the normal to complex RNAP ratios at which the model prediction for stable RNA transcription matches exactly the values given in (Bremer, 1996) for the five different growth rates. We also calculate the predicted total mRNA transcription rates for these ratios. We score the parameter set based on (i) the linear fit to the correlation between the measured (p)ppGpp concentrations and the calculated RNAP ratios and (ii) fit between predicted and measured total mRNA transcription rates.

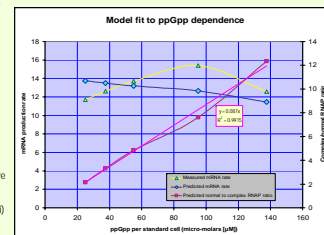
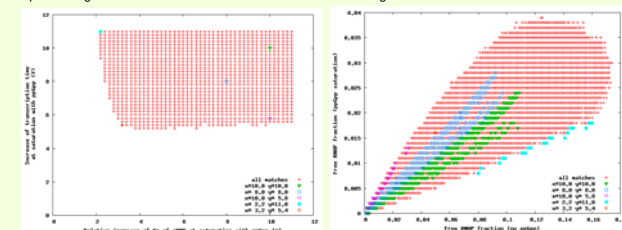


Illustration of the fit for one of the many parameters sets we found compatible with the experimental transcription data

Results and outlook

We used our framework to compare the predictions of different sets of parameters consistent with two 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. We allow for both an increase in the K_m for stable RNA (parameter x) as well as an increase in transcription time (y). We found parameter sets compatible with the (Bremer, 1996) exponential growth data for various combinations of the two strengths.



Dynamical simulations with the parameter sets calibrated with exponential growth data exhibit a surge of (p)ppGpp following nutritional downshift. This is an early indication that possibly a single kinetic model may be appropriate for both stringent response and exponential growth. We are currently comparing time course data from the literature with simulations using the above parameter sets.

Our parameter sweep algorithm will benefit from implementing a Monte Carlo search method. We are also considering using a reachability algorithm to efficiently predict the measurements of the (p)ppGpp surge. The model may be extended to predict the growth rate which is now an input.

We will include more available experimental datasets on *E.coli* and will apply this approach to *M.tuberculosis*, for which significantly less data is available. The acceptable parameter sets are currently quite large. Further data will provide tighter constraints. We plan to make these sets publicly available in the form of a database.

Acknowledgements

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Bremer, H and P. P. Dennis 1996. *Modulation of chemical composition and other parameters of the cell by growth rate*. p1553-1569 In: *E.coli and S.typhimurium*: cellular and molecular biology, American Society for Microbiology, Washington, D. C.
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