

Model for Comprehensive Growth Rate Control Mediated by (p)ppGpp

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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.

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.

The stringent response is mediated by guanosine 5'-diphosphate 3'-diphosphate and 5'-triphosphate 3'-diphosphate, or (p)ppGpp. It has been long established (Lazzarini and Cashel, 1975) that (p)ppGpp concentrations increase during the stringent response. 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.

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 a diauxic shift.

While it is accepted that (p)ppGpp plays a role in the reprogramming of transcription, there is some disagreement in the literature (see for example, Dennis *et al*, 2004 and Paul *et al*, 2004) regarding the **specific mechanism** that achieves the differential regulation.

A clear, quantitative understanding of the precise mechanism of transcriptional regulation as well as of the wider role of (p)ppGpp in log phase growth rate adjustment and entry into stationary phase is of immediate utility given its possible role in the dormancy of *M. tuberculosis*, still one of the most important infectious diseases worldwide. Our model also proposes a general method for predicting the growth rate based on transcription and translation dynamics and a limiting condition on the aggregate density of cells.

Model

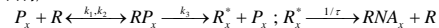
We constructed a generic **mathematical model** for transcriptional control by (p)ppGpp, which encompasses the **complete causal cycle** of the stringent response: differential regulation driven by (p)ppGpp; change in translational activity as a result of differential regulation; production and destruction 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. Currently we use data from (Bremer, 1996) on growth rate dependence of transcription rates and (Chang, 2004) for the size of the four promoter groups..

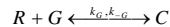
Our working assumption is that the mechanism outlined above is responsible both for the fast and dramatic changes in transcription that occur during growth arrest and the relatively small adjustments that accompany long term variations in during exponential growth. This assumption may be disproved if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

Transcription

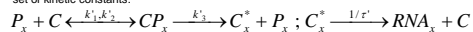
We follow four types of RNA, one representative each for the mRNA species that are upregulated (*u*), downregulated (*d*), respectively unchanged (*f*) in the presence of (p)ppGpp, and ribosomal RNA (*r*). For each type of RNA, we model the kinetics of its transcription as follows (where $\mathbf{x}=(u,d,f,r)$):



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:



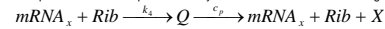
At some finite (p)ppGpp concentration [G], a fraction of all RNAP is in the modified form. The ratio of partition is an increasing function of [G].

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



Translation and (p)ppGpp

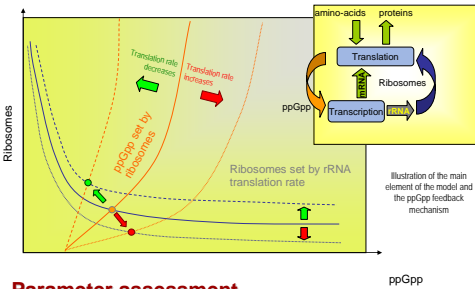
A measure of [un]favorability of external conditions is amino-acid availability, represented by an independently variable parameter, the ratio *r* of uncharged to charged tRNA. This parameter modifies the translation elongation rate and determines the (p)ppGpp concentration, as follows. Translation proceeds 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: $\frac{c_p}{c_p} = 1 + r$. At equilibrium, the number of stalled complexes is: $Q^* = \frac{c_p}{1+r} Q$

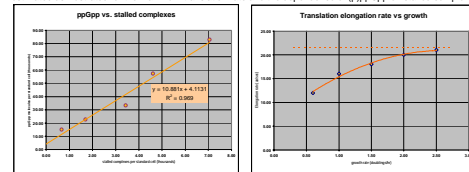
The reactions that produce and those that destroy (p)ppGpp are influenced by the presence of stalled translational complexes. The production reactions are enhanced, so the presence of these complexes leads to an increase in (p)ppGpp.

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_4) and a Michaelis-Menten saturation constant (where the promoter is the 'enzyme' and free RNAP is the 'substrate': $K_m = (k_1 + k_2)/k_3$). One parameter set specifies the four (K_m, k_{sat}) 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.

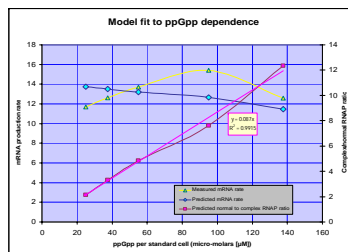


Illustration of the fit for one of the many parameter sets we found compatible with the experimental transcription data

Closure condition necessary to determine the growth rate

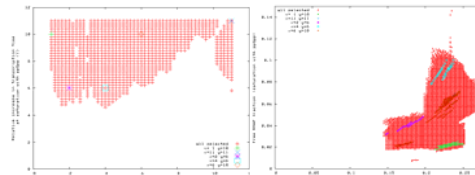
The knowledge of net production (and degradation) rates as a function of concentrations for all species can be used to predict the equilibrium (steady state) concentrations, for a given growth rate. However, this information in itself is not enough to predict the growth rate.

An additional mathematical condition is necessary. This condition should quantify the fact that newly produced material (proteins, RNA, DNA) must lead to the expansion of the physical volume occupied by a cell colony, otherwise the concentrations would increase indefinitely.

To a first approximation, if the mass density of cells can be assumed constant, then the sum of concentrations of all species, weighted with their respective atomic mass, must add up to a constant. Based on this argument, we sought a linear combination of concentrations of RNA, DNA and protein that is close to constant for all growth rates according to the experimental data from (Bremer, 1996). We use this condition to calculate the growth rate.

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 ; mechanism put forward by Gourse *et al*) 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.



Post-stringent decrease of (p)ppGpp under-estimated

Dynamical simulations with the parameter sets calibrated with exponential growth data exhibit a surge of (p)ppGpp following nutritional downshift, consistent with experimental results (Lazzarini and Cashel, 1975). This is an indication that a single mechanism may account for both stringent response and exponential growth.

However, in the model described here, the decay rate of (p)ppGpp following the surge is essentially given by the growth rate. This is due to the fact that (p)ppGpp is 'pegged' to the concentration of stalled complexes, which in turn is a fixed fraction (as given by the tRNA ratio) of the active ribosomes. The only mechanism that reduces the concentration of ribosomes is dilution due to growth. The experimental data indicates a half life on the order of 20 minutes for the excess (p)ppGpp, even in situations of growth arrest.

Clearly, an additional mechanism is needed to explain the post-stringent decrease of (p)ppGpp. One candidate is the ribosome modulation factor or *rmf* (Aiso, 2005), which converts ribosomes into inactive 100s form. A more likely possibility is the *relBE* mechanism (Christensen, 2004) which probably leads to the prompt destruction of excess ribosomes following the onset of starvation.

Acknowledgements

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