

Quantitative Model of Stringent Response in *E.coli* Explains Spontaneous Persister Generation

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Abstract—Persisters are a small fraction of a genetically homogeneous bacterial population that is significantly more likely to survive antibiotic exposure. A sizeable fraction of persisters are generated during normal growth. The molecular mechanism behind this process is not known. We argue that spontaneous persistence in *E. coli* is the result of transient growth arrests caused by random activation of an extended stringent response system containing (p)ppGpp linked toxin-antitoxin (TA) modules. Through mechanistic modeling, we show that antitoxin fluctuations in the presence of a low (p)ppGpp concentration can initiate translation shutdowns. Our model generates kill curves that qualitatively match multimodal curves obtained experimentally.

I. BACKGROUND

Bacterial persistence has been described soon after the introduction of antibiotics (Bigger, 1944). Persisters are responsible for the typical bimodal or multi-modal kill curves obtained when plotting the concentration of colony forming units (CFUs) against exposure time, whose slope decreases with exposure time.

During killing experiments in a microfluidic device (Balaban *et al.*, 2004) persisters behaved differently from the other cells *before antibiotic exposure*, exhibiting very slow growth or not growing at all. Some persisters are generated by spontaneous transitions of normally growing cells to the persister phenotype. The simultaneous existence of several phenotypes is a form of adaptation to changing environments (Kussell *et al.*, 2005). The molecular mechanism of persistence is not known. When microorganisms encounter an environment with limited nutrients, they enter a dramatically slowed

growth state characterized by a decrease in rRNA, tRNA and protein synthesis. This is known as the *stringent response* and is mediated by the rapid accumulation of (p)ppGpp (Cashel *et al.*, 1996).

Results of (Korch *et al.*, 2003) and our own experiments show that the persister phenotype is eliminated or severely reduced in *E.coli* knockouts incapable of synthesizing (p)ppGpp. Gene expression in persister cells (Keren *et al.*, 2004; Shah *et al.*, 2006) indicates the over-expression of toxins that are part of toxin-antitoxin (TA) modules. TA modules such as RelBE are also important in the stringent response (Gerdes *et al.*, 2005; Kuroda, 2006).

II. RESULTS

We constructed a minimal, biologically correct mechanistic model of the stringent response, with parameters taken from the literature or obtained through global fitting to published observations of *E.coli*. The model includes: the transcriptional effect of (p)ppGpp; its RelA-dependent synthesis; an estimation of the growth rate; the dynamics of the TA module RelBE, including its Lon-mediated control by (p)ppGpp; the inhibitory effect of toxin release on translating ribosomes; reversal of ribosome inhibition by tmRNA.

We add noise to this model, by describing the enzyme RelA and the elements of the TA module RelBE stochastically. Simulations exhibit rare shutdown events caused by fluctuations of the toxin level in excess to that of the antitoxin, often leading to translation shutdown. Such events are less frequent in a version of the model where the effect of (p)ppGpp on the TA module was turned off, corresponding to a (p)ppgpp knockout.

To compare with the phenomenology of persistence, we extracted growth rate distributions for a population of cells by sampling one long noisy

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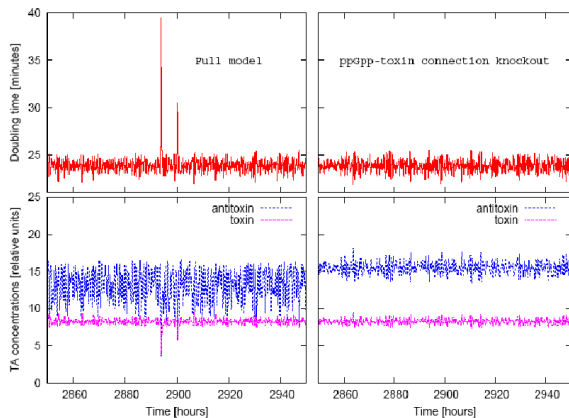


Fig. 1. RelBE (top) and growth rate (bottom) fluctuations in a single cell, in the full model (left) and a knockout where the (p)ppGpp-Lon-antitoxin degradation effect is disabled, mimicking the (p)ppGpp knockout strain. The presence of a basal level of (p)ppGpp in the full model makes excess toxin fluctuations more likely.

simulation, for the complete model (corresponding to WT) and the (p)ppGpp-toxin knockout version. We constructed kill curves assuming that the killing rate is proportional to the growth rate of a cell at the time of initial exposure. Both curves exhibit multimodal behavior similar to experiments, with a much reduced persister fraction for the (p)ppGpp-toxin knockout.

III. DISCUSSION

This is to our knowledge a first dynamical, mechanistic model that brings together the elements of the extended stringent response, including the (p)ppGpp-lon-toxin pathway, and the translation inhibition effect of toxins. While the notion of toxin fluctuations being at the origin of spontaneous switching of cells from normal growth to persistence has been postulated previously, (Keren *et al.*, 2004) we have shown that this is made possible entirely by elements of the extended stringent response mechanism. We also explain the role of (p)ppGpp in the phenomenon of bacterial persistence.

Our results indicate that the stringent response and persistence result from same molecular mechanism, engaged deterministically by starvation in the first case, and stochastically, by molecular fluctuations, in the second. We speculate that it is likely that the stringent response has evolved primarily under the evolutionary pressure of frequent episodes of starvation, and persistence has appeared as a

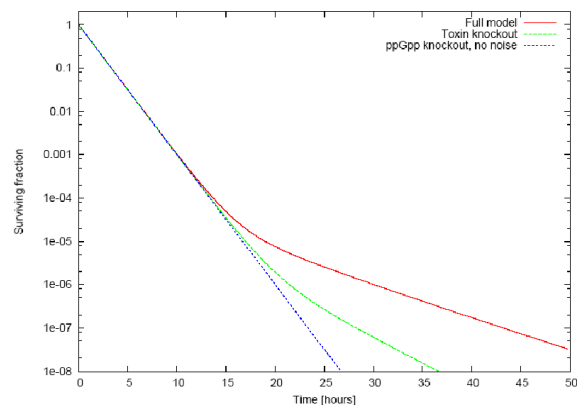
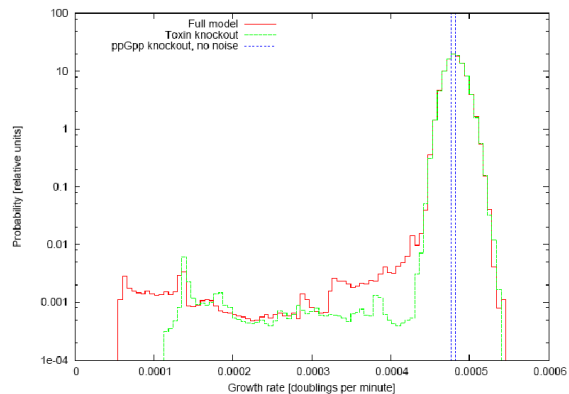


Fig. 2. (Top:) Growth rate histograms derived from the long term simulations partially shown in Figure 1, by sampling the same cell at different times. Assuming the sampling times are well separated, this is equivalent to a snapshot of an ensemble (colony) of statistically independent cells. The (p)ppGpp-toxin knockout has a smaller number of very slowly growing cells. Knocking out (p)ppGpp altogether also turns off all sources of noise, since toxin excess fluctuations become practically impossible. (Bottom:) Simulated kill curves in the presence of an antibiotic for a colony of cells, derived from the histograms shown above. We assume that the killing rate (probability of death per unit time) for each cell is proportional to the growth rate of the cell at the time of initial exposure. The complete (p)ppGpp knockout has no persisters whatsoever, while the (p)ppGpp-toxin connection knockout better approximates the observed (p)ppGpp knockout strain.

secondary function, relevant under less frequent episodes of exposure to antibiotics.

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