Stringent Response and Persistence in *E.coli*

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***Short Abstract* — We present an example of a very simple bacterial signaling system, the one underlying the stringent response, and its role in the seemingly unrelated phenomenon of persistence. Our main contention is that noise-induced fluctuations along the (p)ppGpp-dependent pathway are responsible for rare, transient shutdowns of the translation machinery, which in turn result in intermittent periods of growth arrest. The few individual cells from a fast-growing bacterial population which are going through such shutdowns may present a phenotype consistent with that of persisters, non-growing cells which are more resilient to antibiotic exposure than the rest of the population.**

***Keywords* — Bacterial persistence, stringent response, (p)ppGpp.**

I. Background

THE stringent response mechanism [1,2] is responsible for quick adaptation of bacteria to adverse changes in the environment, typically pertaining to nutrient availability. The stringent response is mediated by the alarmone (p)ppGpp, whose best studied effect is a re-programming of transcription. In the presence of (p)ppGpp, genes related to the growth machinery are down-regulated while others, such as those related to amino-acid synthesis, are up-regulated. Another branch of the stringent response mechanism ensures the quick shutdown of translation, through toxin-antitoxin pairs, such as RelB and RelE. When, starvation-induced (p)ppGpp spikes activate toxin-antitoxin modules by reducing antitoxin lifetime [3], the released toxins reversibly disable the translating ribosomes.

Persisters are a small fraction of a genetically homogeneous bacterial population that is significantly more likely to survive antibiotic exposure. In *E. coli*, persisters have a much slower growth rate *prior to* antibiotic exposure [4,5]. Some of these persisters are spontaneously generated at a low frequency, from normally growing cells. The molecular mechanism behind this process is not known.

II. Results

Through mechanistic modeling of the two branches of the stringent response mechanism, we show that antitoxin fluctuations in the presence of a low (p)ppGpp concentration can initiate translation shutdowns even in the absence of starvation. Growth resumes following the slow deterministic recovery of the translational machinery.

We argue that spontaneously occurring persisters in *E. coli* are the result of such transient growth arrests caused by spontaneous, stochastically occurring activation of the stringent response system containing (p)ppGpp linked toxin-antitoxin modules. Our model generates kill curves that qualitatively match multimodal curves obtained experimentally. Our model predicts that the absence of (p)ppGpp in a RelA/SpoT knockout will further decrease the extremely low likelihood of shutdown events. We confirmed this experimentally demonstrating a dramatic reduction of persister frequency in the knockout strain.

III. Conclusion

A consistent quantitative model of the stringent response mechanism and its effect on growth rate control has led us to a plausible explanation for a seemingly unrelated phenomenon, bacterial persistence. If further experimental evidence ascertains our hypothesis, we have identified a simple example of the emergence of a secondary function of a primitive signaling system.

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