Noise Amplification Breaks the Symmetry in Living Cells

Eitan Rotem[1,2], Adiel Loinger[1], Irine Ronin[1,2,3], Irit Levin-Reisman[1,2], Chana Gabay[1], Noam Shoresh[4], Ofer Biham[1] and Nathalie Q. Balaban[1,2,3]

Symmetry breaking in a population of living cells occurs even under uniform conditions, leading to heterogeneity in the population. An example for such a process can be seen in the response of bacterial populations to antibiotics, termed 'persistence'. The mechanism for persistence is unknown. We present a detailed study of the module implicated in antibiotic persistence. We find that noise, which is inherent in biological systems, results in the co-existence of dormant and growing cells. Stochastic simulations of protein-protein interactions offer predictions for the molecular mechanism behind high persistence, which have been confirmed by our measurements

I. Background

BIOLOGICAL systems are inherently noisy [1]. Analysis of the way cells either combat or utilize noise has led to new insights into the design and evolution of genetic networks [2-3]. In particular, it has been shown that the stochastic differentiation of a population of genetically identical cells into two distinct phenotypes can provide a strong advantage in an unpredictable and fluctuating environment [4]. Bacterial persistence, which plays a major role in the failure of various antibiotic treatments against pathogens, is a striking example of the advantage of variability. In contrast to resistance, which is genetically acquired, persistence is a transient phenotypic recalcitrance to antibiotics observed in only a small fraction of the bacterial population [5]. Persistence was shown to be due to an inherent bi-modality of growth rates in bacterial populations [6].

Moyed *et al*. isolated a strain of *E.coli* with a thousand-fold increase in persistence [7] and the mutation, named *hipA7*, was mapped to a gene encoding the HipA toxin [8] of the *hipBA* toxin-antitoxin module. Toxin-antitoxin modules (TA) consist of pairs of genes, where one gene acts as a toxin and the other cancels out its effect. They were first identified on plasmids and the discovery that TA modules are chromosomal triggered a search for their functions and this unresolved issue has prompted much debate and research [9-10].

Acknowledgements: This work was supported by the Human Frontier Science Foundation and the Israel Science Foundation.

[1] Racah Institute of Physics; eitanr@phys.huji.ac.il
[2] Center for Nanoscience and Nanotechnology;
[3] Sudarsky Center for Computational Biology;
The Hebrew University, Jerusalem 91904, Israel
[4] Broad Institute of Harvard and MIT, Cambridge 02142, MA, USA

II. Objectives

Our goal was to characterize the mode of action of toxin-antitoxin network motifs in general, and the *hipBA* module in particular, and understand how they can generate phenotypic variability.

III. Results

1) We used single cell time lapse measurements of HipA induction fused to mCherry fluorescent protein. We saw the correlation between the level of mCherry expression and the duration of growth arrest leading to persistent bacteria.
2) Detailed analysis of the module showed that HipA effect is seen only above a threshold of expression, and that the level of the threshold depends on HipB expression. This provides a mechanism for the appearance of two populations.
3) Stochastic (Monte Carlo) simulations of the hipBA module investigate the effect of weaker complex-DNA bonding vs. weaker complex formation. The results show that only the latter case affects the persisters 'phase space' and increases the probability of a cell to become persistent. We confirmed this prediction by Colocalization and FRET experiments.

References

[1] Elowitz, M. B., et al. (2002) Stochastic gene expression in a single cell *Science* **297,** 1183-1186.
[2] Losick, R. & Desplan, C. (2008) Stochasticity and cell fate *Science* **320,** 65-68.
[3] Kaern, M., et al. (2005) Stochasticity in gene expression: from theories to phenotypes *Nature reviews* **6,** 451-464.
[4] Lachmann, M. & Jablonka, E. (1996) The inheritance of phenotypes: an adaptation to fluctuating environments *J Theor Biol* **181,** 1-9.
[5] Bigger, J. W. (1944) Treatment of staphylococcal infections with penicillin by intermittent sterilisation *The Lancet***,** 497-500.
[6] Balaban, N. Q., et al. (2004) Bacterial persistence as a phenotypic switch *Science* **305,** 1622-1625.
[7] Moyed, H. S. & Bertrand, K. P. (1983) hipA, a newly recognized gene of Escherichia coli K-12 that affects frequency of persistence after inhibition of murein synthesis *J. Bacteriol.* **155,** 768-775.
[8] Korch, S. B., Henderson, T. A., & Hill, T. M. (2003) Characterization of the hipA7 allele of Escherichia coli and evidence that high persistence is governed by (p)ppGpp synthesis *Molecular Microbiology* **50,** 1199-1213.
[9] Gerdes, K., Christensen, S. K., & Lobner-Olesen, A. (2005) Prokaryotic toxin-antitoxin stress response loci *Nat Rev Microbiol* **3,** 371-382.
[10] Van Melderen, L. & Saavedra De Bast, M. (2009) Bacterial toxin-antitoxin systems: more than selfish entities? *PLoS Genet* **5,** e1000437.