Entrainment of bacterial synthetic clocks

Octavio Mondragon-Palomino[1], Tal Danino[1], Jangir Selimkhanov [1], Lev Tsimring [1, 2] and Jeff Hasty [1, 2, 3]

***Abstract* — We force a self-sustained synthetic gene oscillator in growing colonies of *E. coli* cells, exploring a wide range of forcing amplitudes and periods. From single-cell data, we are able to determine the entrainment region in the amplitude-period plane. For parameters in this region, the phase and period of oscillations are locked to the external signal. We use a detailed mathematical model to account for our observations.**

***Keywords* — Synthetic Oscillator, Entrainment**

I. Background

Quite often biological oscillators become synchronized to environmental cues. A paradigmatic case is the circadian clocks in animals, plants and some bacteria, that become entrained to the periodic 24 hour cycle of light and darkness [3].

In an effort to understand the fundamental mechanisms of biological rhythms, our lab has adopted a synthetic biology approach, developing a self-sustained, tunable and robust genetic oscillator in single *E. coli* cells [1]. Oscillations are persistent, but as cells divide they run out of phase within a few generations. In analogy with the environmental driving of circadian clocks we force the bacterial oscillator to study its dynamics. In this case, the driving signal is a harmonically modulated concentration of Arabinose. This saccharide molecule acts on the genetic construct as an inducer of transcription. We find that the double feedback oscillator can become phase locked to the external input, allowing us to define the main entrainment region (or Arnold tongue [4]) in the amplitude-period space.

II. Experiments and results

To perform the forcing experiments we developed a novel microfluidic device that permits the observation of multiple colonies of bacterial cells for many generations while being subjected to an external stimulus. In time lapse fluorescence microscopy experiments, we scanned multiple frequencies and amplitudes of forcing. Images were analyzed with custom made software for single-cell tracking. In this way we obtained reliable statistical distributions for the period of single-cell oscillations and for the relative phase between cell oscillations and the external signal.

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[1]Department of Bioengineering, University of California, San Diego. E-mail: omondrag@ucsd.edu

[2] BioCircuits Institute, University of California, San Diego.

[3]Biological Sciences Division, University of California, San Diego.

Entrainment to the external stimulus can be appreciated in experimental time-lapse movies. Qualitatively, we found single-peaked distributions of the relative phase and the adjustment of oscillator's rhythm to the periodic signal. From the statistical distributions, we quantified the intensity of locking, enabling us to contrast our experimental results to the predictions of a detailed mathematical model [1].

III. Conclusion

Through the combination of fluorescence microscopy, microfluidics and automated image analysis, we study quantitatively the dynamics of a forced self-sustained synthetic gene oscillator. Our work provides the first experimental demonstration that synthetic oscillators can become entrained to external cues.

References

[1] Stricker J. *et al.* (2008) A fast, robust and tunable synthetic gene oscillator. *NATURE* 456 (7721), 516-519.

[2] Mather W. *et al.* (2009) Delay-Induced Degrade-and-Fire Oscillations in Small Genetic Circuits. *PRL* 102 (6):068105(4).

[3] Bell-Pedersen *et al.* (2005) Circadian rhythms from multiple oscillators: lessons from diverse organisms. *Nature Reviews Genetics,* 544-556.

[4] Pikovsky A, Rosenblum M and Kurths J (2001) Synchronization. A universal concept in nonlinear sciences. Cambridge University Press, Cambridge,UK