# Cell-cycle synchronized oscillations of a negatively self-regulated gene in E. coli

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***Short Abstract* — Robust, tailored behaviours such as genetic oscillations have been successfully implemented in prokaryotic and eukaryotic organisms. However, periodic processes such as gene doubling and cell division are rarely accounted for. Accordingly, we studied a chromosome-integrated, negative-feedback circuit based upon the bacteriophage λ transcriptional repressor Cro and observed strong, feedback-dependent oscillations in single-cell time traces [1]. This finding was surprising due to a lack of cooperativity, long delays or fast protein degradation [2]. Moreover, we found feedback-dependent oscillations to be synchronized to the cell cycle by gene duplication, with phase shifts predictably correlating with estimated gene doubling times.**

***Keywords* — Oscillations, self-regulation, gene-doubling, timelapse imaging, λ lysis/lysogeny, synthetic biology, stochastic modelling.**

## I. Background

ADVANCED applications in synthetic biology require the design of genetic networks that are both *predictable* and *robust*. However, many critical aspects of cell and molecular biology are still poorly characterized and, as a consequence, engineering even simple genetic circuits remains challenging. This is further accentuated by the requirement that synthetic circuits must reliably operate within noisy and heterogeneous environments. In fact, identical challenges apply to developing predictive models of natural biological systems. Thus, among many uses, simple circuits can be studied to refine complex gene-regulation models, providing powerful insights into natural organisms.

There are surprisingly few studies of negative feedback networks with the single-cell, time-lapse resolution required to study gene expression dynamics. Some recent studies show how repressor-binding strength impacts protein expression, and others how negative feedback increases the bandwidth of expression noise [4]. Notably, oscillations in Lac repressor expression have been attributed to reaction delays combined with fast repressor degradation [5]. However, all known experiments utilized genetic constructs with multiple high-affinity binding sites and, in some cases, additional degrees of cooperativity in autoregulation.

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## II. Methods

To facilitate comparison with recent theoretical and stochastic simulation results, we sought to create a simpler circuit in which the bacteriophage λ transcriptional repressor Cro represses its own expression. We then tracked and analysed single-cell expression dynamics over several hours for tens of individual microcolonies.

Timelapse imaging revealed oscillations in Cro expression that were in phase with the cell cycle, while strong oscillations did not occur in the absence of negative feedback. The circuit was then integrated at different genomic loci to shift the gene doubling time; this produced phase shifts with Cro expression peaks tending to lag estimated doubling times by ~20 minutes. Consistent with our theoretical predictions and simulations [2-3], as well as previous experiments, negative feedback increased the coefficient of variation and reduced the Fano factor. Aside, the size and resolution of our single-cell data sets made it possible to calculate the power spectral density of Cro expression time series, which showed that negative feedback increased the noise bandwidth of Cro expression.

## III. Conclusion

Our results suggest that accurate models of bacteriophage λ lysis/lysogeny must necessarily include cell-cycle dependence and genome-location effects, and suggest a molecular and evolutionary basis for biases in chromosome integration sites of lamboid prophages. Moreover, these factors should also be accounted for in the rational design of chromosome-integrated synthetic genetic networks, especially in light of the recent development of simplified recombineering techniques.

## References

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