Emergent Oscillation Induced by Nutrient-Modulating Growth Feedback

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Background

Rational design and forward engineering of synthetic gene circuits based on general principles have demonstrated its power in successfully constructing, testing, and debugging circuits for many remarkable applications in the past [1-3]. However, the complex interplay between the gene circuit and the host cell physiology at various scales often makes the circuit functions fragile to the environmental fluctuations [4-6]. The transcription and translation of heterologous gene circuits steal a considerable amount of the host cell's resources that are originally allocated for host activities, thus inevitably placing a significant metabolic burden on the host cell [7-9]. The altered physiology of host cells, in turn, affects the circuits' intended functions [10-12]. Furthermore, the host environmental fluctuations make the circuit-host interplay more complicated and circuit behavior hard to predict. Thus, it is essential to understand how these circuit-host interactions affect the functionality and design of synthetic gene circuits.

Methods

E. coli DH10B (Invitrogen, USA) was used for all the cloning construction experiments. Measurement of the self-activated circuit was performed in E. coli K-12 MG1655ΔlacIΔaraCBAD. Cell density (optical density, OD) and Green Fluorescent Protein (GFP) were measured at 30-minute intervals.

For the mathematical modeling we followed our previous work [13] to model the dynamics of the circuit gene expression and cell density. For this study, the dependence of the production rate of the gene circuit on growth rate of the host cell was considered in one model. In order to understand the underlying molecular mechanism for the oscillatory dynamics, we also developed another model by explicitly considering the ribosome allocation for cell maintenance, growth feedback, and circuit gene given that the ribosome is regarded as a primary determinant of growth rate [14].
Figure 1: Emergent Oscillation Induced by Nutrient-Modulating Growth Feedback. (A) Dynamics of growth (Optical Density, OD) and GFP/OD after 1:100 dilution of cells into fresh medium with 20% LB in the culture medium (dotted lines with error bars). Fitting of the model to the dynamics of the host cell growth and circuit gene expression (solid lines). (B) The diagram of the model by considering the ribosome regulation and allocation. (C) Model prediction about the dynamics of circuit gene expression level with various carrying capacity (OD\textsubscript{max}). (D) Experimental verification of the model prediction. The nutriment level was controlled by the fraction of LB in the culture medium. Data indicate mean±s.d.(n=3).

Results

We found an unexpected oscillatory behavior of a self-activation gene circuit induced by nutrient modulating growth feedback. Specifically, after dilution of the E. coli strain carrying activated self-activation switch into the fresh medium with moderate nutrient, its gene expression first decreased as the cells grew and then showed a significant overshoot before reaching the steady states, leading to unexpected oscillation dynamics (Fig. 1A, dotted lines).

The underlying molecular mechanism of the oscillation was demonstrated by a mathematical model, which includes the ribosome allocation towards gene production, cell growth, and cell maintenance (Fig. 1B). Upon data fitting, this model successfully captured the oscillation-like dynamics of the circuit gene expression (Fig. 1A, solid lines).

Interestingly, the model predicted a counterintuitive dependence of oscillation...
amplitude on the nutrient level, where the highest peak was found in the medium with a moderate nutrient level but not the richest (Fig 1C). We experimentally verified this prediction by tuning the nutrient level in the culture medium (Fig. 1D).

**Conclusion**

Our results demonstrated a new nonlinear emergent behavior mediated by growth feedback, which depends on the dynamic ribosome allocation between gene circuit expression and host cell growth.

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**References**