Precise Programming of Bacterial Pattern Formation by a Synthetic Circuit and Inkjet Printing

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***Short Abstract* — To probe mechanisms underlying self-organized pattern formation in biological systems, we have established an experimental framework to examine pattern formation in engineered bacteria. The pattern formation is programmed by a gene circuit, whereas its initial conditions are precisely controlled by inkjet printing. Guided by modeling, we used this technique to quantitatively examine how various features of bacterial patterns could be modulated by environmental factors. Our results suggest that our system provides a mechanism to realize environmental sensing, whereby the size increases with environmental domain size. Our technique represents a novel strategy to examine spatiotemporal dynamics in natural or engineered bacteria.**

I. Purpose

In developmental biology, self-organized pattern formation is ubiquitous and essential for a large variety of processes, including feather branching [1] and tissue stratification [2]. Its importance has driven an intense investigation of what mechanisms can give rise to complex patterns from an initial group of undifferentiated cells. In our system, the pattern formation process is fundamentally driven by the interplay between intracellular gene expression, cell growth, and cell-cell communication, programmed by a synthetic gene circuit. In contrast to the vast majority of proposed mechanisms to explain pattern formation that requires spatial morphogen, our study investigates pattern formation in the absence of a spatial morphogen gradient.

II. Results and Discussions

Our synthetic gene circuit consists of a T7 RNA polymerase that activates its own expression and the expression of LuxR and LuxI. Upon activation by T7 RNAP, LuxI can mediate synthesis of acyl-homoserine lactone (AHL), which can induce expression of T7 lysozyme upon binding LuxR. T7 lysozyme then inhibits T7 RNAP. Fig 1a shows the inkjet-printed microcolony of MG1655 ptetmCherry (motile cells constitutively expressing mCherry) with a spacing distance of 1mm on a 0.3% rigid agar surface without a glass coverslip after 24 h growth. The microcolony placement pattern can maintain the relative distance (deviation of measured spacing distance to 1mm is ±24.28μm) between different cell colonies during the growth. By combining the inkjet printing technology with pattern formation capability driven by the synthetic gene circuit, we can achieve microcolonies with self-organized pattern (Fig 1b-e). At early stages of the patterning process, T7 lysozyme inhibits cell growth significantly as AHL concentration accumulates above a critical threshold throughout the entire spatial domain, giving rise to a mCherry ring coinciding with a pause in cell expansion (Fig 1c-d). This phenomenon correlates with temporal circuit dynamics, where the global morphogen concentration serves as a timing mechanism to trigger formation and maintenance of the ring patterns in two dimensions (the height of each cell colony layer is ~10-30μm). By varying the spacing distance between each cell colony, we observed that the ring size increases with the effective domain size (the surface area per cell colony), consistent with the model prediction.

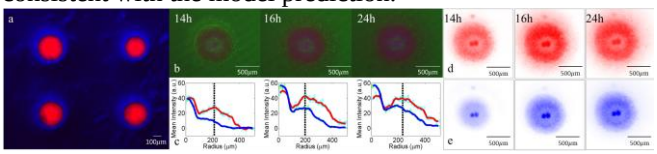

Figure 1 a) Inkjet-printed microcoloy of MG1655 ptetmCherry; b) raw composite fluorescent image of inkjet-printed microcolony of MG1655 pET15bLCFPT7 pTulys2CMR2; c) CFP (blue line) and mCherry(red line) at varying radial distance from center; d-e) mCherry (d) & CFP (e) images.

III. Conclusion

We have achieved robust, predictable, regular, and reproducible microcolonies at precisely predefined locations using inkjet printing. When integrated with the pattern-forming gene circuit, we have reproducibly achieved precise, self-organized ring patterns in a growing microcolony of cells. Our experimental data and computational results show that the pattern formation process is mediated by the temporal dynamics of morphogen concentration. The dependence of ring size on the effective domain size may provide insights regarding a plausible mechanism to explain scale invariance in natural developmental systems.

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