Engineering Models to Scale

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Developing organisms can regulate and scale patterns to achieve consistent proportions despite immense changes in size. In this issue, Cao et al. build a model system, using engineered *E. coli,* to understand how regulatory dynamics can produce pattern scaling without the need for a morphogen gradient.

The physicist Richard Feynman famously wrote, "What I cannot create, I do not understand," at the top of his final blackboard. This philosophy has inspired many in the emerging field of synthetic biology, which harnesses the power of biology to rationally engineer biomolecular systems for a variety of purposes, such as whole-cell biosensing and in vivo diagnostics (Slomovic et al., 2015). The "build-to-understand" approach (Elowitz and Lim, 2010) is complementary to top-down systems biology approaches and borrows concepts and techniques from engineering and computer science. By creating biological systems with desired architectures and functions, it aims to test design principles in relative isolation by exploring how biology's building blocks, such as DNA-encoded genes, can be rearranged and altered to produce different phenotypes. In this issue, Cao et al. use this approach to tackle the question of how self-organizing systems maintain a constant ratio of physical pattern features with changing size, a property known as scale invariance (Cao et al., 2016).

Living cells possess a remarkable capacity to reliably scale and self-pattern over time and space. The complexity of these natural systems has hindered our ability to isolate and study the fundamental biological mechanisms that can give rise to emergent pattern scaling. In many cases, multiple models can explain patterning and pattern scaling, but the large number of unmeasured parameters and interlinked systems can hinder connecting such models to experiments. Since the early 1980s, there have been efforts to establish a quantitative framework for understanding the biology of scaling (Calder, 1984), and a rich body of molecular biology has since been established in model systems, including *Drosophila* development (Gregor et al., 2005). However, we have traditionally only been able to study such effects by probing and perturbing endogenous cellular networks.

To create a tractable model for studying scaling properties, Cao et al. engineered *E. coli* using a synthetic gene circuit that self-patterns rings of gene expression in bacterial colonies (Payne et al., 2013). The system incorporated fast positive feedback and slow negative feedback delayed by accumulation of a morphogen, AHL, to produce self-organized spatial patterns that were read out by reporter gene expression (Figure 1A). While previously engineered patterns (Basu et al., 2005) required a morphogen gradient, in this system, the bacteria instead used morphogen concentration to sense the size of the physical space inhabited by the colony and then created the appropriately scaled pattern based on that information. AHL accumulation depended on the size of the agar domain environment in which the bacterial colony was growing, and gene expression capacity depended on the cells' position within the colony (highest at colony edge and decreasing toward the center). Only in conditions of high AHL and high gene expression capacity could the reporter gene be fully expressed; thus, a ring of high expression formed around the colony edge. The resulting rings showed scale invariance, as the widths were pro-

portional to both the colony and agar domain radii.

Since this was an engineered microbial model, all of the genetic parts providing this scale invariant behavior were known and could be manipulated to understand the key underlying mechanisms. Specifically, the engineered bacterial cells were designed to utilize multiple components to create patterns, namely, T7 RNA polymerase (T7-RNAP) to create positive feedback, fluorescent CFP and mCherry to act as reporters, luxI to produce the morphogen AHL, and luxR to sense AHL. The fully integrated synthetic system functioned as follows: T7-RNAP transcribed luxR, luxI, CFP, and more T7-RNAP. The luxI enzyme produced AHL. As the small molecule AHL diffused out of the cell, it sensed domain size by activating luxR when it had accumulated in the environment. Then, activated luxR promoted transcription of fluorescent mCherry and T7-lysozyme, which inhibited T7-RNAP transcription to create negative feedback. As the colony grew, it depleted nutrients in the agar, limiting colony expansion. Together, the combination of AHL accumulation and nutrient consumption set the boundaries for ring initiation as well as the edge of the colony.

The synthetic system was modeled mathematically to gain insight into the mechanism of pattern scaling. The model captured several levels of dynamics: gene circuit dynamics, cellular growth, and gene expression dependence on spatial position within the colony. This multi-level approach allowed the authors to find the essential requirements for scale invariance and efficiently explore
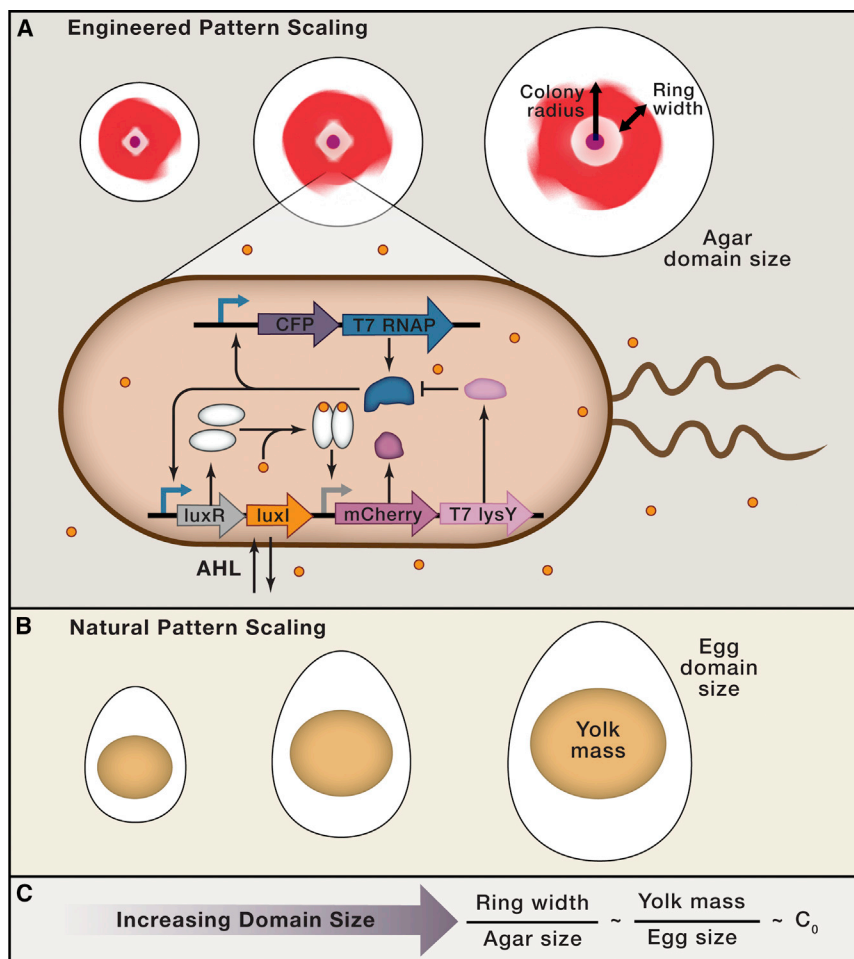
Figure 1. Engineered Microbial Model Allows Mechanistic Study of Pattern Scaling Properties
(A) An engineered model system of *E. coli* forms ring patterns with scale invariance. The ring is formed by differential gene expression throughout the colony, with high CFP and mCherry in the center and a ring of only mCherry expression along the colony circumference. An *E. coli* cell with a genetic circuit schematic is depicted below the ring pattern diagrams. T7-RNAP transcribes CFP, luxR, luxI, and T7-RNAP. As AHL builds up from luxI production, luxR forms active dimers that promoter transcription of mCherry and T7 lysozyme (lysY) that inhibits transcription by T7-RNAP.
(B) A natural model system such as an avian egg exhibits scale invariance.
(C) As domain size increases for the natural and engineered system, both maintain a constant ratio (C_0) of a physical feature (yolk mass or ring width) to domain size (egg or agar domain size).

consistent with the model's predictions. To test the effect of T7-RNAP feedback strength, the authors varied a chemical inducer that controlled the transcriptional positive feedback. Weaker positive feedback followed the mathematical predictions as the range of scale invariance and fluorescence intensity decreased.

The work by Cao et al. nicely shows how a build-to-understand synthetic biology approach can be used to derive, test, and validate hypotheses relating a particular phenotype, such as pattern scaling, to potential underlying regulatory mechanisms. While it remains to be seen whether similar biological regulation controls pattern scaling in nature, there are geometrically analogous systems that could be studied—for instance, that of certain avian eggs (Figure 1B) in which eggs of all sizes have the same relative amount of yolk and albumen (Dzialowski and Sotherland, 2004). Looking forward, it will be exciting to see synthetic biologists team up with molecular biologists and adopt a build-to-understand approach to elucidate principles that govern complex phenotypes.

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the system's large parameter space. The model predicted several critical conditions for creating robust scale invariance, namely, minimal metabolic burden of T7-RNAP and lysozyme expression and strong positive feedback of T7-RNAP.

The predicted effects of increased metabolic burden were then tested experimentally by expressing an additional gene with T7-lysozyme. This extra metabolic burden reduced the ring width and size range that had scale invariance,