Supplemental Information

Collective space sensing coordinates pattern scaling in engineered bacteria

Yangxiaolu Cao[1], Marc D. Ryser[2], Stephen Payne[1], Bochong Li[1], Christopher V. Rao[3], Lingchong You[1,4,*]

[1]Department of Biomedical Engineering, Duke University, Durham, NC, USA,

[2]Department of Mathematics, Duke University, Durham, NC, USA,

[3]Department of Chemical and Biomolecular Engineering, University of Illinois, Urbana Champaign, IL, USA

[4]Duke Center for System Biology, Duke University, Durham, NC, USA

[*]Corresponding author. Department of Biomedical Engineering, Duke University, CIEMAS 2355, 101 Science Drive, Box 3382, Durham, NC 27708, USA

Tel.: +1 (919)660-8408; Fax: +1 (919)668-0795; E-mail: you@duke.edu

Supplemental Experimental Procedures

Supplemental tables

Supplemental References

Supplemental Experimental Procedures

I. Strains, media, and device

Bacterial strains

Unless noted otherwise, MG1655 cells carrying the circuit or the control plasmids were used for the printing experiments.

Circuit and plasmids

- The full circuit consists of two plasmids: pET15bLCFPT7 and pTuLys2CMR2, as described previously(Payne et al., 2013)
- ptetmCherry served as our control for constitutive expression of mCherry. The pattern_LuxIKO circuit is the pattern-formation circuit with *luxI* knocked out (Payne et al., 2013). The pattern_curli and pattern_desulf circuits each carries an additional effector gene. The *CsgA* gene (Chen et al., 2014) was inserted downstream of the T7 lysozyme gene to form the pattern_curli circuit. The cysteine desulfhydrase gene (Wang et al., 2001) was inserted downstream of T7 lysozyme gene to form the pattern_desulf circuit.

Growth media

- **The LB medium**: 25gLB Broth Powder, pH7 (MO BIO Laboratories, Inc) was added into 1L deionized H_2O. After autoclaving for 45mins, the LB medium was stored at room temperature. LB was used to prepare pre-culture for inkjet printing experiments. The medium was supplemented with appropriate antibiotics (75 μg/mL carbenicillin and 50 μg/mL chloramphenicol, or both) when applicable.
- **The 2×YT medium**(Sambrook and Russell, 2001): 16g tryptone (Difco Laboratories), 5g NaCl (Sigma), 10g yeast extract (Difco Laboratories), and 20.92g MOPS (Sigma) were added into 1L deionized H_2O. The 2xYT medium was adjusted with 1.0M KOH (Sigma) solution to PH = 6.5 by VWR Symphony SB70P PH Meter.

Overnight liquid culture

MG1655 cells carrying the full circuit (pET15bLCFPT7 and pTuLys2CMR2) were streaked on an agar plate supplemented with carbenicillin and chloramphenicol, and incubated at 37℃ for 16 h. Then, a single colony was picked and inoculated in 3mL LB medium supplemented with 75 μg/mL carbenicillin and 50 μg/mL chloramphenicol for 16 h.

Culture well (Figure S1B)

The culture well used in this system is Culture Well™ multiwell chambered coverslip (Grace Bio-Labs; Bend, OR, USA; Item #103310). Each chip has two 1mm deep wells. The radius of each well is 7500 µm. Before culturing the cells, the rest of the silicon gasket flap was excised using a razor blade.

II. Methods

Measurement of cell density in liquid culture

Cell densities of liquid cultures were quantified using optical density (OD) measured at 600 nm absorbance using a Perkin-Elmer VICTOR3 plate reader.

Fluorescence microscopy

A Leica DM16000B fluorescence microscope with a mercury excitation lamp at 5X was used to image samples. Capture resolution was set as 8 bits for depth and 2 for binning. To measure CFP, the excitation filter was set to 436/20, and the emission filter was set to 480/40. The offset of the filter was set to 33/255, gain 100/255. To measure mCherry, the excitation filter was set to 575/25, and the emission filter was set to 632/60. The offset of the filter was set to 0/255; gain 150/255. All these parameters were kept the same between experiments.

Inkjet printing (Figure S1A)

We used the Epson Stylus Photo R280 Ultra Hi-Definition Photo Printer (C11C691201) for printing experiments for three reasons(Cohen et al., 2009). First, this printer contains a CD tray, which provides the capability of printing on a solid flat surface. After the culture well was loaded on the CD tray, the printing template could be designed to match the corresponding position of the culture well. Second, the inkjet is piezo-activated, which will not affect the cell viability. Third, the printer has a high resolution: 5760 × 1440 pixels at the maximal dots per inch (dpi), which enables precise control of initial seeding positions of bacteria.

To facilitate manipulation and sterilization, the outer shell of the printer was disassembled and removed. We then used PrintPayLess six packs Empty Refillable Ink Cartridges instead of the original ink cartridges.

Print heads were cleaned thoroughly before and after each experiment. First, the printer head box was repositioned to the middle of the printer trail and absorbent paper towels were placed under the printer head to collect the liquid flushing through the printer heads. Second, the printer heads were flushed with 75% ethanol once, followed with washing with deionized water three times gently using a syringe. The absorbent paper towels were removed and the printer head box was then placed back in its original spot.

To prepare 0.3% agar for printing, we mixed 0.15g of agar (214530 Difco™ Agar, Granulated) in 50ml of 2×YT medium, and microwaved the mixture until it was homogenous with no aggregates. We then cooled the agar below 50℃ at room temperature, and supplemented it with 75 μg/mL carbenicillin, 50 μg/mL chloramphenicol, and 1000 µM β-D-1-thiogalactopyranoside (IPTG). We next pipetted 170 μL of the agar into each culture well, and let it solidify at room temperature.

An overnight culture of MG1655 cells carrying the full circuit was diluted to 0.2 absorbance (measured by Victor 3 plate reader) and then diluted another 50 fold into fresh LB broth. The diluted culture was transferred into a tone empty ink cartridge using a sterile syringe. The other five cartridges were filled with deionized water with a 0.2 µm filter (VWR® Syringe Filters, # 28145-477).

Printing templates were designed in software GIMP using 1-pixle diameter spot. Each template was exported to an Epson CD printer program to direct printing of bacteria onto the agar surface. After printing, a 24 mm×50 mm glass coverslip was placed on the top of the culture well. There are two reasons for using the coverslip: First, the coverslip allows us to control the agar volume. In our typical experiments, the thickness was confined to ~20µm, which allows the cells to grow into a uniform and thin layer. Second, the coverslip seals with the silicon gasket, which minimizes evaporation of water from the soft agar.

All of the inkjet-printed samples were incubated at 30˚C for 16-43 h.

Precise control of seeding configuration by inkjet printing (Figure S1C)

We tested the inkjet-printing platform by printing MC4100 cells constitutively expressing the mCherry protein. A 3ml LB culture derived from a single colony from MC4100 containing the ptetmCherry plasmid was grown for 16 h at 37℃. The culture was diluted to OD of 0.2 and then diluted another 10 fold before being loaded into an ink cartridge. We placed a 25 mm × 25 mm glass cover in the bottom of the 100 mm petri dish, and then added 7.85 ml molten LB agar supplemented with 50 μg/ml chloramphenicol to generate a 1mm-depth agar layer. After the agar solidified, the embedded glass cover and the above agar were taken out from the petri dish by using a razor blade to cut off the excess agar.

We then printed bacteria onto the agar layer with 1 mm spacing distance between printed spots. The inkjet-printing indeed achieved precise control of the colonies positions. The actual distances between colonies were 969.19 ± 24.28 μm when we intended to print them 1mm apart. The actual distances for the indented 500 μm distances were 506.96 ± 35.57 μm.

Domain size calculation (Figure S1D)

We made array templates with different spacing distance to control the effective domain area per colony, which is determined as $\frac{agar\ surface\ area}{number\ of\ visible\ colonies}$. The domain radius for each colony is that of a circle with the same area as the effective domain area of the colony. To minimize boundary effects of initial cells printed close to the edge of a culture well, the array was designed to be symmetric and evenly distributed.

Control experiments (Figures S1G and S2A)

We used MG1655 cells carrying ptetmCherry plasmid and the same experimental system described in Figure S1G, by varying the domain radii from 1500 to 2500 μm. In the experiment shown in Figure S2A, after incubation at 30˚C for 24 h, no core-ring patterns were observed in the absence of circuit induction by IPTG. Pattern-forming dynamics were evident upon addition of 1mM IPTG.

Colony radius measurement (Figure S1H)

In our experiments, the glass coverslip on top of the culture well confines the height of the cell colony. Confocal microscopy images indicate the gap between the agar surface and the top glass cover was ~20μm, consistent with an estimated value of 15μm. Towards the center of the colony, the height is confined by the air gap; the colony height decreases toward to edge because of the motility of the cells.

Movie S2 was split into frames with a gray color map. After locating the center of the colony, distances with greatest gray scale gradient away from the center were recorded. The average value of these distances represents the colony radius (green line in Figure S1H left panel). The ring width is defined as the distance between the inner and outer edge of the ring (described in Figure S1E).

In simulation, the colony radius (green line in Figure S1H right panel) is defined as the distance from the colony center to the position where the cell density is half of the maximum cell density. The definition of the inner and outer edge of the ring follows the same rules as in Figure S1E.

III. Model development

We previously modeled colony growth and gene circuit dynamics using an agent-based approach(Payne et al., 2013). The PDE model used in the current study corresponds to the hydrodynamic limit of the stochastic agent-based model from (Payne et al., 2013). The PDE formulation has two advantages. First, it is computationally less expensive to solve the PDE model numerically than the stochastic agent-based model. This increased computational efficiency makes intensive parameter estimation studies feasible. Second, the PDE formation better facilitates development of mechanistic insights into the patterning dynamics. Because the air pocket between glass plate and dense agar is only 20μm high (Figure S1H), we model the system in two spatial dimensions and neglect vertical variations in gene expression profiles.

The circuit dynamics can be described by the following PDEs:

$$\begin{cases} \dfrac{\partial C}{\partial t} = \kappa_C \Delta C + \alpha_C \dfrac{1}{1+\alpha T+\beta L}\cdot\dfrac{N}{K_N+N} C\left(1-\dfrac{C}{\bar{C}}\right), \\ \dfrac{dN}{dt} = -\dfrac{\alpha_N}{|\Omega|}\displaystyle\int_\Omega C\left(1-\frac{C}{\bar{C}}\right)\frac{N}{K_N+N}\, d\sigma, \\ \dfrac{dA}{dt} = \dfrac{\alpha_A}{|\Omega|}\displaystyle\int_\Omega C\,\frac{T}{K_T+T}\frac{K_P}{K_P+P}\varphi(x,C)d\sigma - d_A A\,, \\ \dfrac{\partial T}{\partial t} = \kappa_C \dfrac{\nabla \mathrm{T}\cdot\nabla \mathrm{C}}{C} - \alpha_C T\dfrac{N}{K_N+N}\left(1-\dfrac{C}{\bar{C}}\right) - d_T T + \alpha_T \theta(C)\dfrac{T}{K_T+T}\dfrac{K_P}{K_P+P}\varphi(x,C) - k_1 TL \\ \qquad\qquad + k_2 P, \\ \dfrac{\partial L}{\partial t} = \kappa_C \dfrac{\nabla \mathrm{L}\cdot\nabla \mathrm{C}}{C} - \alpha_C L\dfrac{N}{K_N+N}\left(1-\dfrac{C}{\bar{C}}\right) + \alpha_L \theta(C)\dfrac{T}{K_T+T}\dfrac{A^m}{K_A^m+A^m}\varphi(x,C) - d_L L - k_1 TL \\ \qquad\qquad + k_2 P, \\ \dfrac{\partial P}{\partial t} = \kappa_C \dfrac{\nabla \mathrm{P}\cdot\nabla \mathrm{C}}{C} - \alpha_C P\dfrac{N}{K_N+N}\left(1-\dfrac{C}{\bar{C}}\right) + k_1 TL - k_2 P, \end{cases} \tag{1}$$

where

- $C(t,x)$ is the cell density
- $N(t)$ is the nutrient concentration
- $A(t)$ is the AHL concentration
- $T(t,x),\ L(t,x),\ P(t,x)$ are cellular T7RNAP, lysozyme and the T7-lysozyme complex density respectively

In deriving these equations, we make the following assumptions:

1. Cells are assumed to perform an unbiased random walk; their movement is modeled as diffusion(Kenkre, 2004; Maini, 2004; Murray et al., 1998). We considered "diffusion" as an approximation of the observed colony expansion, so that cell movement can be described by a single lumped parameter. Intracellular components are modeled with passive-tracer equations (see derivation below).

2. Cell growth is modeled with a logistic term, along with a Monod function. The Monod function is to account for contribution of nutrient to overall colony growth. The nutrient here refers to one or more limiting factors that constrain growth. The logistic term accounts for the limit of cell growth in a particular location. This carrying capacity is unlikely limited by nutrient availability. Instead, it is limited by the spatial confinement imposed by our device, which is the colony height is confined to be ~20 μm between the coverslip and the agar surface.

3. Fast diffusion of AHL and nutrient.

4. Gene expression capacity:

$$\varphi(x,C)=\begin{cases}\dfrac{K_\varphi^n}{K_\varphi^n+\left(R_\varphi-x\right)^n}, & x\le R_\varphi\\ 1, & x>R_\varphi\end{cases} \tag{2}$$

where R_φ is defined as the distance between colony center and the location where cell density is 95% of the carrying capacity (coefficients are all described in Table S1).

Derivation of passive tracer equations

T7RNAP, lysozyme and the T7-lysozyme complex cannot diffuse across the membrane of their host cell, and consequently follow the movement of the host cell.

To derive the passive tracer PDE, consider a generic cell-bound substance without sources and sinks, and assume that the cells (C) evolve according to a generic reaction-diffusion equation

$$\frac{\partial C}{\partial t}=\kappa\Delta C+f(C), \tag{3}$$

where κ is the diffusion constant and f is a smooth function. We denote by $Y(t,x)$ (with units $[mol/cell]$) the per cell concentration at location x of the passive tracer, and by $y(t,x)$ (with units $[mol/mm2]$) its surface concentration. Here we invoke again the above assumption on uniform vertical distribution of cell content. In other words, we assume that the per-cell concentration of the substance is uniform in vertical direction and only depends on the location of the cell, but not its height in the stack of cells. Integrating over a control volume V, using the conservation of mass, and applying the divergence theorem, we find the following conservation law in global form

$$\frac{\partial}{\partial t}\int_V Y(x,t)C(x,t)dx=\int_{\partial V}Y(x,t)\,\kappa(x)\nabla C(x,t)\cdot d\sigma=\int_V \nabla\cdot\left(\kappa(x)Y(t,x)\nabla C(t,x)\right)dx. \tag{4}$$

In the first equality, we used the fact that diffusion is driven along the gradient: the flow is proportional to $\kappa(x)\nabla C$. Together with equation (3), the above conservation equation (4) becomes in local form

$$\frac{\partial Y}{\partial t}=\kappa\frac{\nabla Y\cdot\nabla C}{C}-f(C)Y.$$

Non-dimensionalization of the model

First, we rescale the time and space variables as

$$\hat{t}=\alpha_C t,\quad \hat{x}=\frac{x}{\mathcal{L}}$$

where $\mathcal{L}$ is a length scale to be chosen later.

We next rescale the state variables,

$$\hat{C}=\frac{C}{\bar{C}},\quad \hat{N}=\frac{N}{N_0},\quad \hat{A}=\frac{A}{K_A},\quad \hat{L}=\frac{d_L}{\alpha_L}L,\quad \hat{T}=\frac{d_T}{\alpha_T}T,\quad \hat{P}=\frac{P}{K_P}$$

With these dimensionless variables, and by defining $\hat{\varphi}\left(\hat{x},\hat{C}\right)=\varphi(x,C)$, we can rewrite the model equations in a dimensionless form. Introducing the parameter groups $G_i,(i=1,\dots,12)$ (see Table S2), the nondimensional equations become (omitting the hats for notational simplicity):

$$\begin{cases} \dfrac{\partial C}{\partial t} = G_1 \Delta C + \dfrac{1}{1+\alpha T+\beta L} C(1-C)\dfrac{N}{G_2+N}, \\ \dfrac{dN}{dt} = -G_3 \displaystyle\int_{\Omega} C(1-C)\dfrac{N}{G_2+N} d\sigma, \\ \dfrac{dA}{dt} = G_4 \displaystyle\int_{\Omega} C \dfrac{T}{1+T}\dfrac{1}{1+P}\varphi(x,C) d\sigma - G_5 A, \\ \dfrac{\partial L}{\partial t} = G_1 \dfrac{\nabla \mathrm{L}\cdot\nabla \mathrm{C}}{C} - L\dfrac{N}{G_2+N}(1-C) - G_6 L + G_7\theta(C)\dfrac{T}{1+T}\dfrac{A^m}{1+A^m}\varphi(x,C), \\ \dfrac{\partial T}{\partial t} = G_1 \dfrac{\nabla \mathrm{T}\cdot\nabla \mathrm{C}}{C} - T\dfrac{N}{G_2+N}(1-C) - G_8 T + G_9\theta(C)\dfrac{T}{1+T}\dfrac{1}{1+P}\varphi(x,C), \\ \dfrac{\partial P}{\partial t} = G_1 \dfrac{\nabla \mathrm{P}\cdot\nabla \mathrm{C}}{C} - P\dfrac{N}{G_2+N}(1-C), \end{cases} \tag{5}$$

Due to the reversible first order kinetics of T7RNAP bind with T7 lysozyme to form T7-lysozyme complex is fast(Kumar and Patel, 1997), we add the additional constraint that L, T and P are at equilibrium:

$$P = \frac{G_{10}}{G_{11}G_{12}} TL$$

mCherry and CFP equations

Experimentally, the circuit dynamics are reported by mCherry and CFP , which are co-expressed with lysozyme and T7RNAP, respectively. To allow for a direct comparison between model and experiment, we also model the dynamics of mCherry (ψ_R) and CFP (ψ_C):

$$\frac{\partial \psi_R}{\partial t} = G_1 \frac{\nabla\psi_R\cdot\nabla C}{C} - \psi_R \frac{N}{G_2+N}(1-C) + G_7\theta(C)\frac{T}{1+T}\frac{A^m}{1+A^m}\varphi(x,C), \tag{6}$$

$$\frac{\partial \psi_C}{\partial t} = G_1 \frac{\nabla\psi_C\cdot\nabla C}{C} - \psi_C \frac{N}{G_2+N}(1-C) + G_7\theta(C)\frac{T}{1+T}\frac{A^m}{1+A^m}\varphi(x,C), \tag{7}$$

Domain shape, initial conditions, and boundary conditions

The physical domain (see Figure S1D) is much larger than the emerging patterns, and its total size enters the model equations only through G_3 and G_4, which both scale as $1/|\Omega|$ (see equation (1) and Table S2). Since the computational domain only provides the computational range for running the simulation, consequently, we choose a computational domain large enough to avoid pattern interference with the boundary and to enforce no-flux boundary conditions.

The initial conditions are chosen to reflect the experimental configuration. Since seeding cells are printed as small droplets on top of the agar, we choose a highly localized, radially symmetric initial field of cells $C(x,t)|_{t=0}$, placed at the center of the domain. The initial nutrient concentration is given by N_0, and the A, L and P concentrations are initially 0. We introduce a small amount of T into the initial cell colony at time 0. Finally, homogenous von Neuman, or no flux conditions are used for all PDEs in system(Murray et al., 1998).

Numerical solver for the PDE model

To solve the model numerically in MATLAB, we exploit the radial symmetry of the system and reduce it to a PDE in polar coordinates, only depending on one spatial variable, namely the radius $r \in [0, R]$. We then use a fractional multistep method(Ryser et al., 2012; Tyson et al., 2000). The goal of the method is to split each equation into the respective advection, diffusion and reaction contributions, and to update each part separately over the basic time step Δt. In addition, we introduce a fourth step, which

equilibrates L, T and P after each 3-step update. Next, we briefly describe the numerical methods used in each step.

Step 1: Advection. To update the advection contributions, we combine the MATLAB built-in Runge-Kutta solver ode45 with a second order centered finite difference scheme for discretization of the gradients.

Step 2: Diffusion. Since the diffusion dynamics are stiff, we combine the MATLAB built-in ODE solver ode15s with a second order centered finite difference scheme for discretization of the Laplacian.

Step 3: Reactions. We again use the MATLAB built-in Runge-Kutta solver ode45 to solve the intracellular reaction kinetics.

Step 4: L-T-P equilibration. After completion of steps 1-3, the L-T-P system is updated by projecting it onto the manifold defined by $P = \frac{G_{10}}{G_{11}G_{12}}TL$. With this constraint, the concentrations after steps 1-3 (L_0, T_0, P_0) are updated during step 4 to (L_1, T_1, P_1) as follows:

$$L_1 = \frac{1}{2}\left(L_0 - G_{10}T_0 - G_{11} + \sqrt{(L_0 - G_{10}T_0 - G_{11})^2 + 4G_{11}(L_0 + G_{12}P_0)}\right),$$

$$P_1 = P_0 + \frac{1}{G_{12}}(L_0 - L_1),$$

$$T_1 = T_0 + \frac{1}{G_{10}}(L_0 - L_1).$$

Parameters screening and execution of PDE model

The dimensionless model has 22 parameters (Table S2). Each dimensionless parameter is a combination of several parameters with units. Rather than estimating dimensionless parameters directly, we searched values of dimensional parameters in a realistic range, and then determine the corresponding dimensionless parameters. Ten parameters were estimated or measured in other studies; three parameters were estimated through new experiments (Table S1).

We examined the contributions from the other nine parameters using a simple search algorithm. We first searched 18,231 parameter sets (Figure S2C, light blue lines) to identify those able to generate the core-ring pattern. During this step, we applied three criteria: 1) ability to generate mCherry core-ring pattern similar to the experimental observations; 2) transient CFP dynamics; 3) maintenance of mCherry ring within at least three-fold change in the domain size. Our search yielded 409 parameter sets (Figure S2C, dark blue lines). However, many of these sets did not generate scale invariance (e.g., see Figure S2D, step 1, blue curve).

We next searched the vicinity of each of these sets to determine an optimal set able to generate scale invariance. In each round of search, we introduced a small normally distributed perturbation to each parameter value (Figure S2D, step 2). With the perturbed parameter set, we simulated the patterning dynamics for different domain radii and calculated a score for the parameter set according to its performance in generating scale invariance. This score accounts for two aspects:

(1) The linear correlation between the ring width and the domain radius, as well as that between the colony radius and the domain radius. Each correlation is quantified on the basis of the coefficient of determination (R-squared) to value the linear fit. Higher R-squared value results in a higher score;
(2) The standard deviation (SD) of the ratio of the ring width to the colony radius. Smaller SD value results in a higher score.

We repeated this perturbation 10 times and identified the parameter set with the highest score. We then used this new set as a starting point for the next round of perturbation analysis. The red curve in Figure S2D step 3 is the parameter set that has the highest score after a total of 100 perturbations (10 rounds, each with 10 perturbations to the same parameter set). This parameter set generates a high degree of scale invariance (bottom panel in step 3).

Comparison with previous patterning dynamics

Experimentally, the key differences in the circuit parameters include:

(1) Greater cell motility of MG1655 cells (than MC4100Z1 cells), such that it can still form a sufficiently large colony even when growing on agar with higher density (0.3% in the current study vs. 0.07% in the previous study). This difference is only critical for making our inkjet-printing protocol feasible – it is more challenging to print on very soft agar. In other words, the greater motility of MG1655 compensates for the higher agar density in the current study.

(2) A shallower gradient of the gene expression capacity. This notion is consistent with our observation of the gene expression pattern of constitutive mCherry from MG1655 cells (Figure S1G).

(3) Less metabolic burden of circuit activation on the host cell. To illustrate this point, we measured growth in MC4100Z1 (the cell strain used in the previous study) and MG1655 (current study) cells carrying the pattern-formation circuit and its different variants. These variants include the positive-feedback module, the pattern-formation circuit with the *luxI* gene knocked out, and the pattern-formation circuit with an effector gene co-expressed with the T7 lysozyme. As Figure S2F shows, compared with MG1655 cells, MC4100Z1 cells experienced a higher metabolic burden when different circuits were induced. Furthermore, the pattern-formation circuits carrying an effector gene (pattern_desulf) caused a higher metabolic burden than did the pattern-formation circuit by itself.

Computationally, the model is able to generate two types of patterns, depending on parameter values. In the presence of a strong metabolic burden by circuit activation (α=4) and fast decaying gene expression capacity (n=4 and $K_\varphi = 2$), the patterns do not exhibit scale invariance (indicated as a green dashed line in Figure S2C). These correspond to our previous experimental data (Payne et al., 2013). In this case, mCherry formed a ring during the development, and the ring width did not change with domain radius (Figure S2G, green box). The steep gene expression capacity profile in this configuration results in very high expression of mCherry and T7 lysozyme on the edge relative to the interior. Also, a very narrow ring will occur towards the edge of the colony and since this results in a very high metabolic burden, a pause in colony growth occurs, causing less dilution of circuit components and therefore reinforcement of a very bright, narrow ring.

The other set of parameters have a moderate metabolic burden (α=0.4) and a slowly decaying gene expression capacity profile (n=1) (indicated as a red dashed line in Figure S2C). mCherry here formed a long lasting pattern consisting of a core and a ring, and CFP formed a transient ring before the mCherry ring initiated. Also, both the width of the mCherry ring and the colony radius scaled linearly with respect to the domain radius (Figure S2G, red box). This is consistent with the experimental results generated in the current study. In the current experimental setup, the cells grew on top of the agar, which provided better access to nutrients for all cells in the colony. Compared with the previous experiment, the gene expression profile in this case exhibited a flatter profile and the metabolic burden caused by circuit activation is weaker.

Emergence of scale invariance

The scale invariance requires a balance between colony growth and timing of pattern formation. In particular, the simulations indicate three conditions that are critical for the generation of scale

invariance. First, the metabolic burden by the circuit is small. Second, the T7RNAP feedback is strong. Third, the gene expression capacity declines slowly toward the colony center. Here we illustrate the mathematical basis on how these conditions can lead to scale invariance.

Variables:

- D: domain radius; δ_n: initial nutrient concentration; h: the height of the culture well
- t_1: ring initiation time; t_2: when the nutrient concentration reaches $0.001N_0$(complete exhaustion); $\Delta T = t_2 - t_1$
- R_C: colony radius when the system reaches steady state; σ is the proportion coefficient; $R_C = \sigma \cdot D$
- R_φ(t): the distance from colony center to the position where cell density is 95% of the carrying capacity
- F_W: distance between R_φ and R_C, also defined as half-width of the wavefront
- x_{inner}: distance between the colony center and the inner edge of the ring
- x_{outer}: distance between the colony center and the outer edge of the ring
- W_R: width of the mCherry ring; $W_R = x_{outer} - x_{inner}$.

Proportionality between R_c and D

With a small metabolic burden, the growth dynamics can be decoupled from the circuit dynamics. As a result, the equation governing cell dynamics (1) admits traveling wave solutions:

$$\dot{r} \approx v\frac{N}{G_2 + N} \tag{8}$$

where v is the maximum traveling wave speed.

Next, we notice that nutrient consumption is restricted to the moving colony edge of width $2F_W$. Thus the nutrient consumption is approximated by:

$$\dot{N} = -G_3 2\pi \cdot 2F_W r(t)\frac{N}{G_2 + N} \tag{9}$$

Combining equations (8) and (9) yields:

$$\dot{r} = v\frac{\dot{N}}{-G_3^1 \cdot r(t)}$$
$$\dot{r^2} = -\frac{2v}{G_3^1}\dot{N}, \tag{10}$$

where $G_3^1 = 4\pi F_W G_3$.

Integrating both sides of equation (10) yields:

$$r(t) = \sqrt{\frac{2v}{G_3^1}(N(0) - N(t))}$$

Eventually, all nutrient will be consumed, and hence:

$$R_c = \lim_{t\to\infty} r(t) = \sqrt{\frac{2v}{G_3^1}N(0)} = \sqrt{\frac{2v}{G_3^1}\pi D^2 h\delta_n} = \sqrt{\frac{2v}{G_3^1}\pi h\delta_n} \cdot D.$$

Or:

$$R_c = \sigma D,$$

where $\sigma = \sqrt{\frac{2v}{G_3^1}\pi h\delta_n}$, a constant.

Proportionality between t_1 and D

To simplify the estimation, we rewrite equation (5) as follows:

$$\begin{cases} \frac{da}{dt} = \alpha_A \frac{T}{K_T + T} \frac{K_P}{K_P + P}, \\ \frac{\partial T}{\partial t} = \alpha_T \frac{T}{K_T + T} \frac{K_P}{K_P + P} - \frac{\partial P}{\partial t}, \\ \frac{\partial L}{\partial t} = \alpha_L \frac{T}{K_T + T} \frac{A^m}{K_A^m + A^m} - \frac{\partial P}{\partial t}, \\ \frac{\partial P}{\partial t} = k_1 TL - k_2 P, \\ A = \frac{1}{|\Omega|} \sum a \end{cases}$$

Here we make several assumptions:

(1) a is AHL production per cell. This is a function of time and location.

(2) Gene expression only takes place over a certain width along the edge of the colony. This assumption is made with reference the gene expression capacity formula, in which half width is applied as a constant.

(3) Because the metabolic burden is low, we separate colony growth from the gene expression equations.

It can be shown that, when $A \gg 1, P(x,t) \approx a(x,t)$. The relation between t_1 and D is determined by how quickly AHL accumulates and the amount of this accumulation.

AHL accumulation rate

The rate of AHL accumulation over time is given by:

$$\frac{dA}{dt} = G_4 \int_{\Omega} C \frac{T}{1+T} \frac{1}{1+P} \varphi(x, C) d\sigma - G_5 A ,$$

At the beginning of cell growth, $C \frac{T}{1+T} \frac{1}{1+P}$ is the same for different domain sizes. Near time 0, A is negligible; thus, the degradation term can be eliminated. Therefore,

$$\frac{dA}{dt} \propto G_4 \propto \frac{1}{|\Omega|} \propto \frac{1}{D^2}$$

AHL accumulation amount

The total amount of AHL accumulation is also determined by the same rate equation but can be approximated by considering the time window when AHL approaches its maximum (or when time approaches t_1)

$$\frac{dA}{dt} = G_4 \int_{\Omega} C \frac{T}{1+T} \frac{1}{1+P} \varphi(x, C) d\sigma - G_5 A = G_4 \frac{T}{1+T} \frac{1}{1+P} * 2\pi R_C * K_\varphi - G_5 A$$

As AHL approaches its maximum, we have $A \gg 1$, $T(x,t) \ll 1$. Also, $T(x,t)$ does not change significantly over time due to the lysozyme-mediated negative feedback; thus, $\frac{T}{1+T} \approx small\ constant$. Under this condition, we also have $P \approx a$. Hence:

$$\frac{dA}{dt} \propto \frac{R}{D^2}\frac{1}{1+P} - G_5 A = \frac{R}{D^2}\frac{1}{1+a} - G_5 A = \frac{R}{D^2}\frac{1}{1+\frac{A*D^2}{R_C}} - G_5 A$$

When AHL reaches its maximum (A_{max}), we have $\frac{dA}{dt}|_{A=A_{max}} = 0$, or:

$$\frac{R_C}{D^2}\frac{1}{1+\frac{A_{max}*D^2}{R}} - G_5 A_{max} = 0 \rightarrow {A_{max}}^2 D^2 + R_C A_{max} = \frac{{R_C}^2}{D^2 \cdot G_5}$$

Because $R_C \propto D, {A_{max}}^2 D^2 + R_C A_{max} \propto 1 \rightarrow A_{max}|_{t=t_1} \propto \frac{1}{D}$. Therefore,

$$t_1 = \frac{A_{max}|_{t=t_1}}{AHL\ acucmulation\ rate} \propto \frac{\frac{1}{D}}{\frac{1}{D^2}} \propto D$$

<u>Proportionality of the mCherry ring width to D</u>

After t_1, colony radius expansion is negligible. According to (6), advection of mCherry and T7RNAP within R_c is negligible as $\nabla C \approx 0$. The profile of mCherry $\psi_R(x,t)$ at radius x and time t can be expressed as

$$\psi_R(x,t) = \psi_R(x,t_1) + G_7 \int_{t_1}^{t} \varphi(x,t) \cdot \frac{T(x,t)}{1+T(x,t)} \cdot \frac{A(t)^m}{A(t)^m+1} dt$$

Therefore, when $t = t_2$, mCherry profile is

$$\psi_R(x,t_2) = \psi_R(x,t_1) + G_7 \int_{t_1}^{t_2} \varphi(x,t) \cdot \frac{T(x,t)}{1+T(x,t)} \cdot \frac{A(t)^m}{A(t)^m+1} dt \quad (11)$$

Conditions required for scale invariance (as revealed by numerical simulations):

- **A strong T7 positive feedback**, coupled with negative feedback from lysozyme, leads to a flat T7RNAP profile across space after t_1. Also because of the negative feedback, $T(x,t) \ll 1$ and does not change significantly over time (Figure S3D). That is,

$$\frac{T(x,\ t)}{1+T(x,t)}|_{t_1 \le t \le t_2} \approx T(x,t) \approx \tau \quad (12)$$

- Between t_1 and t_2, AHL remains $\gg 1$ (Figure S3E). Since the AHL concentration is not space dependent,

$$\frac{A(t)^m}{A(t)^m+1}|_{t_1 \le t \le t_2, m=2} \approx 1 \quad (13)$$

- Given equations (12) and (13), the accumulation of mCherry is mainly determined by the gene expression capacity (equation (2)), a space and time dependent function. Simulations indicate that scale invariance is favored when $n \approx 1$; that is, the **gene expression capacity profile is shallow**. After the ring initiates at t_1, colony expansion is negligible compared to the final cell radius, R_C. Therefore, $r(t)|_{t_1 \le t \le t_2} \approx R_C \approx \sigma \cdot D$.

Since $R_\varphi(t)$ is defined as the position where cell density is 95% of the carrying capacity, $R_\varphi(t)$ is a function of R_C. Based on the search results: $K_\varphi = F_W(t_1) \Rightarrow K_\varphi + R_\varphi(t)|_{t_1 \le t \le t_2} = R_C = \sigma \cdot D$ (Figure S3F). Hence:

$$\varphi(x,t)|_{t_1 \le t \le t_2} = \frac{K_\varphi}{K_\varphi + R_\varphi(t) - x} = \frac{K_\varphi}{\sigma \cdot D - x}$$

Therefore,

$$\psi_R(x,t_2) = \psi_R(x,t_1) + G_7\tau \int_{t_1}^{t_2} \frac{K_\varphi}{\sigma \cdot D - x} dt$$

$$= \psi_R(x,t_1) + \frac{G_7\tau\Delta T K_\varphi}{\sigma \cdot D - x}$$

However, the match between K_φ and the half-width of the colony wavefront is not an absolute constraint. The system can still generate approximate scale invariance even when these two variables are slightly different.

Calculating the mCherry ring width (Figure 3C, S3G):

The ring width refers to the distance between the valley of mCherry profile to its horizontal intersection at t_2. To compute the ring width, we examine the mCherry profile in two steps: mCherry profile at t_1 and the accumulation of mCherry from t_1 to t_2 (the maturation phase).

Based on simulations, the mCherry profile at ring initiation time, $\psi_R(x,t_1)$, scales with the domain radius D. This scaling results from the initial phase of the circuit dynamics coupled with colony growth and expansion. A strong T7RNAP positive feedback loop induces fast lysozyme synthesis. Meanwhile, a small metabolic burden decouples cell growth from the circuit dynamics. The fast dilution from the cell growth gives the cell in the relative same location of the colony same amount of time to synthesizes mCherry. Given this scaling, we use a normalized distance, $\hat{x} = \frac{x}{D}$ to continue the calculation.

$$\psi_R(\hat{x},t_2) = \psi_R(\hat{x},t_1) + \frac{G_7\tau\Delta T K_\varphi}{\sigma \cdot D - \hat{x} \cdot D} \quad (14)$$

Due to the scaling of mCherry profile at t_1, $\psi_R(\hat{x},t_1)$ is approximately the same for varying domain sizes on normalized axis (Figure S3G, left).

The time for nutrient exhaustion, t_2, is proportional to the initial nutrient concentration, which is proportional to D. The time for ring initiation, t_1, is the time when AHL reaches a maximum. This time is also proportional to D according to our simulation. Thus, $\Delta T = t_2 - t_1 = \delta \cdot D$ (δ is a positive constant).

$$\frac{G_7\tau\Delta T K_\varphi}{\sigma \cdot D - \hat{x} \cdot D} = \frac{G_7\tau\delta K_\varphi}{\sigma - \hat{x}}$$

Therefore, the accumulation of mCherry during the maturation process $\frac{G_7\tau\delta K_\varphi}{\sigma-\hat{x}}$ is independent of D (Figure S3G, middle), when using the normalized x-axis.

Taken together, the mCherry profile on a normalized axis at t_2 is:

$$\psi_R(\hat{x},t_2) = \psi_R(\hat{x},t_1) + \frac{G_7\tau\delta K_\varphi}{\sigma - \hat{x}}$$

Again, both $\psi_R(\hat{x},t_1)$ and $\frac{G_7\tau\delta K_\varphi}{\sigma-\hat{x}}$ are independent of D. If there is an inner edge $\hat{x}_{inner}$, it will be the **same value** for varying domain radius (Figure S3G, right). That is, $x_{inner} = D \cdot \hat{x}_{inner} \propto D$; the position of the inner edge always scales with D. x_{outer} is approximately equal to $R_C \approx \sigma \cdot D \propto D$. Therefore, ring width $W_r = x_{outer} - x_{inner} \propto D$.

Effects of adding exogenous AHL (Figure S4A-E)

Both simulation using the full model and experiments demonstrate that addition of exogenous AHL has two major effects (**Figure 4B**):

(1) Formation of smaller rings.
(2) Disruption of the proportionality between the ring width and domain radius, while maintaining their linear correlation for a smaller range of domain radii.

These effects can also be intuitively interpreted based on the same mathematical framework outlined above. Compared with the base case, equations (12) and (13) still hold in the presence of exogenous AHL. However, the extra AHL induces more lysozyme expression, which induces higher metabolic burden on cell growth (Figure S4C). Compared to cell growth, diffusion will be the dominant factor to determine local cell density, which leaves the half-width of wavefront wider than in the base case.

$$K_\varphi + \varepsilon = F_W(t_1), \qquad (\varepsilon > 0)$$

$$\Rightarrow K_\varphi + R_\varphi(t)|_{t_1 \le t \le t_2} = R_C - \varepsilon = \sigma \cdot D - \varepsilon$$

Equation (11) becomes:

$$\psi_R(\hat{x}, t_2) = \psi_R(\hat{x}, t_1) + \frac{G_7 \tau \Delta T K_\varphi}{\sigma \cdot D - \varepsilon - \hat{x} \cdot D} \tag{15}$$

Based on simulation, the maturation time is still proportional to D: $\Delta T = t_2 - t_1 = \delta_{ahl} \cdot D$. However, $\delta_{ahl} > \delta$ as the ring initiates earlier (t_1 is smaller than the base case).

$$\psi_R(\hat{x}, t_2) = \psi_R(\hat{x}, t_1) + \frac{G_7 \tau \delta_{ahl} K_\varphi}{\sigma - \frac{\varepsilon}{D} - \hat{x}}$$

To understand why the relationship of ring width to domain radius has a positive intercept on y-axis, the position of the ring's inner edge needs to be calculated. In Figure S4D (left), from unit 2 to unit 7 on x-axis, the mCherry profile is approximately linear: $y = -k\hat{x} + a_0$, (k, a_0 are positive constants).

The inner edge position, x_{inner}^{+ahl}, satisfies $\frac{\partial \psi_R(\hat{x}, t_2)}{\partial \hat{x}}|_{\hat{x} = \widehat{x_{inner}^{+ahl}}} = 0$. Therefore we have:

$$\frac{\partial \psi_R(\hat{x}, t_1)}{\partial x} + \frac{G_7 \tau \delta_{ahl} K_\varphi}{\left(\sigma - \frac{\varepsilon}{D} - \hat{x}\right)^2} = 0$$

$$\widehat{x_{inner}^{+ahl}} = \sigma - \frac{\varepsilon}{D} - \sqrt{\frac{G_7 \tau \delta_{ahl} K_\varphi}{k}}$$

Eventually,

$$W_R = \left(\widehat{x_{outer}^{+ahl}} - \widehat{x_{inner}^{+ahl}}\right) D$$

$$= \sigma D - \left(\sigma - \frac{\varepsilon}{D} - \sqrt{\frac{G_7 \tau \delta_{ahl} K_\varphi}{k}}\right) D$$

$$= \varepsilon + \sqrt{\frac{G_7 \tau \delta_{ahl} K_\varphi}{k}} D$$

That is, the ring width is a linear function of the domain radius but with a positive

intercept.

Effect of higher metabolic burden (Figure S4F)

According to our simulation using the full model and experiment, having significant additional metabolic burden (e.g. by expressing another gene) would lead to the loss of scale invariance (**Figure 4C**).

Conditions:

- Compared with the base case, the AHL concentration accumulates slowly to pass the half activation threshold (=1). After t_1, AHL decreases but remains $\gg 1$. As with the base case, the AHL concentration is not space-dependent due to its fast diffusion.
- With a higher metabolic burden, the colony growth slows down substantially. The T7RNAP profile is no longer flat across space after ring initiation at t_1. Instead, it will have a higher distribution around colony center.
- Different from the base case, a higher metabolic burden has a significant effect on cell growth. Cell diffusion is the dominant factor to determine local cell density, which leaves the half-width of the wavefront wider than in the base case. Under this condition, the relationship of K_φ and $R_\varphi(t)$ is similar to the case of adding exogenous AHL. Hence,

$$\psi_R(x,t_2) = \psi_R(x,t_1) + G_7\tau\int_{t_1}^{t_2}\frac{T7(x,\ t)}{1+T7(x,t)}\cdot\frac{K_\varphi}{\sigma\cdot D-\varepsilon_2-x}dt$$

Here, the inner edge cannot be expressed as a simple mathematical function. Due to a smaller synthesis rate of AHL, ΔT is shorter. **The smaller ΔT, together with the perturbed T7RNAP distribution, will lead to a ring width with a smaller width.** Because of the additional metabolic burden, colony radius does not rely solely on nutrient availability (**Figure 4C**).

Effects of reducing the positive feedback strength (Figure S5)

According to our simulation using the full model and experiment, a significant reduction in the strength of the T7RNAP positive feedback would lead to loss of scale invariance (**Figure 4D**). However, a linear dependence between the two is maintained within a smaller range of domain radius, in comparison with the base case.

Conditions:

- Compared with the base case, the AHL concentration accumulates slowly to pass the half activation threshold (=1). After t_1, AHL decreases but remains $\gg 1$. As with the base case, AHL concentration is not space dependent.
- With a weak positive feedback, the T7RNAP profile is no longer flat across space after ring initiation, t_1 (Figure S5A). Instead, it is dictated by the gene expression capacity and the T7RNAP level is greater near the colony edge.
- Similar to the base case, circuit activation does not cause a significant metabolic burden; thus $K_\varphi + R_\varphi(t)|_{t_1\le t\le t_2} = R_C = \sigma\cdot D$. (Figure S5C). Hence

$$\psi_R(x,t_2) = \psi_R(x,t_1) + G_7\tau\int_{t_1}^{t_2}\frac{T7(x,\ t)}{1+T7(x,t)}\cdot\frac{K_\varphi}{\sigma\cdot D-x}dt$$

Here, the mCherry profile at t_1 no longer scales with the domain radius as mCherry accumulation is affected by the T7RNAP spatial distribution. The inner edge cannot be expressed in a simple

mathematical function. However, qualitative analysis of the process can give us the insights of most features.

Due to a smaller synthesis rate of AHL with a weak T7RNAP positive feedback loop, ΔT is shorter. The shorter ΔT leads to a shorter maturation time for the ring, thus a smaller **ring width**. In the base case, ΔT increases proportionally with the domain size. However, when positive feedback is too weak, ΔT decreases with the domain size (Figure S5B). When the domain size is too large, there is not enough time to initiate the ring. **The linear regime of ring width vs. domain radius shrinks and shifts to small domain sizes.**

Comparison with representative mechanisms for pattern generation and scaling

Researchers have sought to define mechanisms underlying pattern formation and scaling as early as the 1950s. These mechanisms either focus on generation of patterns by self-organization or by interpreting pre-defined gradients of chemicals (morphogens).

Turing was the first to propose a reaction-diffusion (RD) model consisting of interlocking positive and negative feedback loops involving two diffusible morphogens, to explain self-organized pattern formation (Turing, 1952). The original Turing model cannot generate scale invariance. However, variants of the Turing model have been proposed to account for scale invariance. These include models that assume concentration-dependent diffusion coefficients or size-dependent reactions.

If the diffusion coefficients of morphogens depend on the concentration of another diffusible molecule produced by all cells at a constant rate, the Turing model can support generation of patterns that scale with size (Aegerter-Wilmsen et al., 2005; Hunding and Sørensen, 1988; Pate and Othmer, 1984). This mechanism has been proposed to explain pattern formation and scaling during the embryonic development, such as scaling during the slug stage of *Dictyostelium discoideum*. However, it requires some stringent dynamic constraints that lack direct experimental evidence. These include: 1) the morphogen diffusivities must be proportional to the square of the length scale, and 2) matching between the production rate and leakage rate of the morphogen into the surroundings.

Another extension of the Turing model is the **size-dependent reaction model** (Boissonade, 2009; Ishihara and Kaneko, 2006; Ricard, 2010; Rosen, 1978). In addition to the two morphogens assumed in the Turing model, this model introduces another chemical whose concentration depends on the size of the reaction environment. The model further assumes that chemical is generated in a localized region of the system and it diffuses fast. As a result, its concentration is proportional to the inverse of the square of the environmental size. In this theoretic model, each morphogen has two states – active or inactive. Transition between the two states is catalyzed by this chemical. Only morphogens in the active state can participate in reactions. Satisfying these conditions, scale invariance can arise from the modified Turing model. A limitation of this model is the lack of direct experimental evidence for the key model assumptions. However, the AHL in our system can be considered as fulfilling some of the roles proposed for the additional chemical in the size-dependent reaction model. AHL concentration also approximately scales with the inverse of the square of the domain size; it also plays a critical role in modulating some reactions in our system.

In contrast to models mentioned above, the other class of models focuses on interpretation of pre-defined chemical gradients by downstream processes. A classical model is the positional information model (or the French Flag model), proposed by L. Wolpert (Wolpert, 1969). The essence of this mechanism is a pre-defined morphogen gradient that is interpreted by downstream genes, where different genes are activated at different ranges of the morphogen concentration. In this framework, the scaling property of the final patterns is determined by the scaling property of morphogen gradients. Several models have been proposed to generate scaling morphogen.

A perfect sink model assumes that the morphogen is generated at a source and degrades at a distant edge. As a result, it forms a linear gradient from source to sink. This mechanism could support

scale invariance (Aegerter-Wilmsen et al., 2005; Crick, 1970). This theoretic model requires strict conditions to ensure a perfect sink to ensure scale invariance in the morphogen gradients.

Another model assumes **integration of two opposing gradients**, where two morphogens are produced at opposite ends of the developing field. There is an effective annihilation reaction between the two morphogens. Cells control the size of the pattern by the ratio of two gradients (McHale et al., 2006).

A shuttling-based mechanism requires two pre-defined morphogens: an activator and an inhibitor. Efficient shuttling requires binding of the ligand to the inhibitor to facilitate its diffusion and subsequent release of the ligand by cleavage of the complex. The morphogen profile is reshaped by the physical translocation of the activator to the midline, mediated by its binding to the inhibitor. This mechanism is supported by data in *Drosophila* and *Xenopus* embryos (Ben-Zvi et al., 2008). This mechanism is further generalized to **an expansion-repression (ExR) model**. In the ExR model, a single morphogen is secreted from a local source and diffuses in the field of cells to form a distribution profile that has a peak at the source. The diffusion of this morphogen is facilitated by a diffusible molecule, the "expander". However, the production of the expander is repressed by the concentration of morphogen. With appropriate parameters, the ExR model can generate a scale-invariant morphogen gradient (Barkai and Shilo, 2013; Ben-Zvi and Barkai, 2010; Ben-Zvi et al., 2011a; Ben-Zvi et al., 2011b).

The mechanisms above have focused on generation or interpretation of morphogen gradients by feedback control only. Recent studies have suggested an important role of tissue growth and expansion in establishing the morphogen gradients, by contributing to the transport and accumulation of the morphogen molecules (Averbukh et al., 2014; Fried and Iber, 2014). The resulting morphogen gradients in turn can influence the tissue growth and expansion.

Aspects of our mechanism are related to the mechanisms mentioned above:

1. Our model is analogous to the Turing model in two aspects: (1) our model also relies on self-organized pattern formation; (2) our circuit logic is identical to the Turing model. The critical difference is that the activator is transported by advection in our system but by diffusion in the Turing model.
2. AHL serves a similar role of the catalyzing chemical in the size-dependent reaction model. Its concentration is reflects the dimension of the domain, and it regulates rates of reactions involving other molecules.
3. Our circuit logic also resembles that of the expansion-repression model, where T7RNAP serves as the expander, T7 lysozyme serves as the repressor, and morphogen AHL serves as the regulator between them. However, a critical difference is that AHL in our system primarily serves as a timing cue in modulating the rates of other reactions (as in point 2).
4. In our system, colony growth and expansion play a major role in establishing the initial profile of mCherry during the first stage of pattern formation, by contributing to the transport and accumulation of intracellular proteins. This aspect is reminiscent of the computational analysis by (Averbukh et al., 2014; Fried and Iber, 2014), which emphasizes the role of tissue growth and expansion in establishing morphogen gradients in a natural system.
5. The integration of the mCherry profile at the end of the first stage and the mCherry increment during ring maturation (**Figures 3, 5**) is analogous to the two opposing gradients model.

Supplemental Tables

Table S1. Related to Figure 2. Definition and the value of parameters used in the PDE model

Parameter	Description	Value	Base Unit	Search range or Reference
κ_C	Cellular diffusion coefficient on 0.3% 2xYT agar	2.5×10^{-3}	$cm^2 \cdot h^{-1}$	(Song et al., 2009)
α_c	Cell growth rate on 2xYT agar	1	h^{-1}	Fit with experiments
α_n	Nutrient depletion rate	155	$molecule \cdot h^{-1} \cdot cell^{-1}$	Fit with experiments
K_n	Half-saturation for nutrient uptake	20	nM	Fit with experiments
K_A	Concentration threshold of AHL to half-maximum of the pLuxI promoter	20	nM	(Collins et al., 2006)
α_A	AHL synthesis rate	9600	$molecule \cdot h^{-1} \cdot cell^{-1}$	(You et al., 2004)
d_A	AHL degradation rate	0.3	h^{-1}	(You et al., 2004)
α_L	Synthesis rate of T7 lysozyme	4500	$molecule \cdot h^{-1} \cdot cell^{-1}$	$0 - 9 \times 10^3$
d_L	Degradation rate of T7 lysozyme	0.0144	h^{-1}	(Payne et al., 2013)
K_T	Half activation constant of T7RNAP	1200	$molecule \cdot cell^{-1}$	$0 - 5 \times 10^3$
α_T	Synthesis rate of T7RNAP	6000	$molecule \cdot h^{-1} \cdot cell^{-1}$	$0 - 8 \times 10^3$
d_T	Degradation rate of T7RNAP	0.3	h^{-1}	(Payne et al., 2013)
k_1	Combination rate of T-Lys complex	400	$molecule^{-1} h^{-1} \cdot cell$	(Kumar and Patel, 1997)
k_2	Dissociation rate of T-Lys complex	10800	h^{-1}	(Kumar and Patel, 1997)
k_D	Equilibrium association constant of T7-lysozyme complex	0.037	$molecule^{-1} \cdot cell$	(Kumar and Patel, 1997)
K_P	Half inhibition of T-Lys complex	400	$molecule \cdot cell^{-1}$	$0 - 5 \times 10^3$
α	Inhibition factor of T7RNAP on Growth	1		0 - 5
β	Inhibition factor of T7 lysozyme on Growth	100		$0 - 2 \times 10^3$
m	Hill coefficient of AHL mediated gene expression	2		(Payne et al., 2013)
n	Hill coefficient for distance-dependent gene expression capacity	1		0 - 5
K_φ	Half activation distance for gene expression	2	cm	0 - 10
$\bar{C}$	Cell carrying capacity	3×10^5	$cells \cdot ml^{-1}$	

Table S2. Related to Figure 2. Expression and value of coefficient in nondimensional model

Nondimensional parameter	Expression	Value
G_1	$\frac{k_c}{\alpha_c \mathcal{L}^2}$	0.07*
G_2	$\frac{K_n}{n_0}$	0.3*
G_3	$\frac{\alpha_n \bar{c}}{\alpha_c n_0} \frac{\mathcal{L}^3}{\lvert\Omega\rvert} \frac{1}{10^{-4} cm}$	0.0046*
G_4	$\frac{\alpha_a \bar{c}}{\alpha_c K_a} \frac{\mathcal{L}^3}{\lvert\Omega\rvert} \frac{1}{10^{-4} cm}$	0.955
G_5	$\frac{d_a}{\alpha_c}$	0.3
G_6	$\frac{d_L}{\alpha_c}$	0.0144
G_7	$\frac{\alpha_L d_L}{\alpha_c \alpha_L}$	0.0144
G_8	$\frac{d_T}{\alpha_c}$	0.3
G_9	$\frac{\alpha_T}{\alpha_c K_T}$	5
G_{10}	$\frac{K_T d_L}{\alpha_L}$	0.0038
G_{11}	$\frac{d_L}{\alpha_L k_D}$	8.64×10^{-5}
G_{12}	$\frac{K_P d_L}{\alpha_L}$	0.0013
$G_{mCherry}$	$\frac{1}{\alpha_c}$	1
G_{CFP}	$\frac{1}{\alpha_c}$	1

* G1, G2, G3 are estimated by comparing the experiment colony expansion with Fisher-KPP's traveling wave solution with wave speed (Aronson and Weinberger, 1978). After fitting G_1 with colony expansion curve, $\mathcal{L} = \sqrt{\frac{k_c}{\alpha_c G_1}}$ =0.18898 cm=1889.8 μm;

Supplemental References

Aegerter-Wilmsen, T., Aegerter, C.M., and Bisseling, T. (2005). Model for the robust establishment of precise proportions in the early Drosophila embryo. Journal of theoretical biology *234*, 13-19.

Aronson, D., and Weinberger, H. (1978). Multidimensional nonlinear diffusion arising in population genetics. Advances in Mathematics *30.1*, 33-76.

Averbukh, I., Ben-Zvi, D., Mishra, S., and Barkai, N. (2014). Scaling morphogen gradients during tissue growth by a cell division rule. Development *141*, 2150-2156.

Barkai, N., and Shilo, B. (2013). Developmental biology: Segmentation within scale. Nature.

Ben-Zvi, D., and Barkai, N. (2010). Scaling of morphogen gradients by an expansion-repression integral feedback control. Proceedings of the National Academy of Sciences *107*, 6924-6929.

Ben-Zvi, D., Pyrowolakis, G., Barkai, N., and Shilo, B. (2011a). Expansion-Repression Mechanism for Scaling the Dpp Activation Gradient in Drosophila Wing Imaginal Discs. Curr Biol *21*, 1391-1396.

Ben-Zvi, D., Shilo, B., and Barkai, N. (2011b). Scaling of morphogen gradients. Current Opinion in Genetics & Development.

Ben-Zvi, D., Shilo, B.Z., Fainsod, A., and Barkai, N. (2008). Scaling of the BMP activation gradient in Xenopus embryos. Nature *453*, 1205-1211.

Boissonade, J. (2009). Self-organization phenomena in non-equilibrium chemical systems. Origins of Life: Self-Organization and/or Biological Evolution?, 23-36.

Chen, A.Y., Deng, Z., Billings, A.N., Seker, U.O., Lu, M.Y., Citorik, R.J., Zakeri, B., and Lu, T.K. (2014). Synthesis and patterning of tunable multiscale materials with engineered cells. Nat Mater *13*, 515-523.

Cohen, D.J., Morfino, R.C., and Maharbiz, M.M. (2009). A Modified Consumer Inkjet for Spatiotemporal Control of Gene Expression. Plos One *4*.

Collins, C.H., Leadbetter, J.R., and Arnold, F.H. (2006). Dual selection enhances the signaling specificity of a variant of the quorum-sensing transcriptional activator LuxR. Nature biotechnology *24*, 708-712.

Crick, F. (1970). Diffusion in embryogenesis. Nature *225*, 420-422.

Fried, P., and Iber, D. (2014). Dynamic scaling of morphogen gradients on growing domains. Nat Commun *5*, 5077.

Hunding, A., and Sørensen, P. (1988). Size adaptation of Turing prepatterns. J Math Biol.

Ishihara, S., and Kaneko, K. (2006). Turing pattern with proportion preservation. Journal of theoretical biology *238*, 683-693.

Kenkre, V.M. (2004). Results from variants of the Fisher equation in the study of epidemics and bacteria. Physica A *342*, 242-248.

Kumar, A., and Patel, S.S. (1997). Inhibition of T7 RNA polymerase: Transcription initiation and transition from initiation to elongation are inhibited by T7 lysozyme via a ternary complex with RNA polymerase and promoter DNA. Biochemistry-Us *36*, 13954-13962.

Maini, P.K. (2004). Using mathematical models to help understand biological pattern formation. Comptes rendus biologies *327*, 225-234.

McHale, P., Rappel, W.J., and Levine, H. (2006). Embryonic pattern scaling achieved by oppositely directed morphogen gradients. Phys Biol *3*, 107-120.

Murray, J.D., Cook, J., Tyson, R., and Lubkin, S.R. (1998). Spatial pattern formation in biology: I. Dermal wound healing. II. Bacterial patterns. J Franklin I *335B*, 303-332.

Pate, E., and Othmer, H.G. (1984). Applications of a model for scale-invariant pattern formation in developing systems. Differentiation *28*, 1-8.

Payne, S., Li, B., Cao, Y., Schaeffer, D., Ryser, M.D., and You, L. (2013). Temporal control of self-organized pattern formation without morphogen gradients in bacteria. Molecular systems biology *9*, 697.

Ricard, J. (2010). Systems biology and the origins of life? part II. Are biochemical networks possible ancestors of living systems? networks of catalysed chemical reactions: non-equilibrium, self-organization and evolution. Comptes rendus biologies *333*, 769-778.

Rosen, R. (1978). Self-Organization in Non-Equilibrium Systems - Nicolis,G, Prigogine,I. Int J Gen Syst *4*, 266-269.

Ryser, M.D., Qu, Y.D., and Komarova, S.V. (2012). Osteoprotegerin in Bone Metastases: Mathematical Solution to the Puzzle. PLoS computational biology *8*.

Sambrook, J., and Russell, D.W. (2001). Molecular cloning : a laboratory manual, 3rd edn (Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press).

Song, H., Payne, S., Gray, M., and You, L. (2009). Spatiotemporal modulation of biodiversity in a synthetic chemical-mediated ecosystem. Nature chemical biology *5*, 929-935.

Turing, A. (1952). The chemical basis of morphogenesis. Transactions of the Royal Society of.

Tyson, R., Stern, L.G., and LeVeque, R.J. (2000). Fractional step methods applied to a chemotaxis model. J Math Biol *41*, 455-475.

Wang, C.L., Clark, D.S., and Keasling, J.D. (2001). Analysis of an engineered sulfate reduction pathway and cadmium precipitation on the cell surface. Biotechnol Bioeng *75*, 285-291.

Wolpert, L. (1969). Positional information and the spatial pattern of cellular differentiation. Journal of theoretical biology *25*, 1-47.

You, L., Cox, R.S., 3rd, Weiss, R., and Arnold, F.H. (2004). Programmed population control by cell-cell communication and regulated killing. Nature *428*, 868-871.