Emergence of Travelling Waves from a Synthetic Oscillatory Gene Network

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Abstract

Multicellularity and collective cell behavior exemplify the emergence of complex patterns and structures across scales in living systems. When cells interact they can generate higher order patterns of gene expression or patterns of mechanical stresses and strains (Chan et al., 2017; Vining and Mooney, 2017). There are a wide range of phenomena in which a key element to a developmental process is the appearance of a traveling wave of chemical concentration, mechanical deformation, electrical or other type of signal. Thus, studying traveling waves is relevant to our understanding of fundamental mechanisms underlying pattern formation. We have developed a study of the coupling between a synthetic genetic oscillator and constraints on cell growth in colonies via protein dilution (Yáñez-Feliú and Vidal, 2020). Our theory predicts that these mechanical constraints generate characteristic patterns of growth rate inhomogeneity in growing cell colonies, inducing the emergence of traveling waves of gene expression. Supporting our predictions, we have preliminary experimental evidence that colonies of bacterial cells carrying the synthetic oscillatory circuit do indeed form traveling waves.

Background

The emergence of complex patterns takes place in natural phenomena such as embryonic development, tumor formation, wound healing, among others (Santos-Moreno and Schaerli, 2019). Understanding how these patterns are generated and maintained will enable applications in tissue engineering and regenerative medicine. Synthetic biology applies design principles to generate combinations of genetic parts that perform a given function, for example oscillation, which at the same time helps us to understand the complexity inherent to natural systems. A variety of genetic circuits have been designed, analyzed, simulated, and then implemented in this way. These synthetic circuits simplify biological systems reproducing a specific function such as oscillators (Elowitz and Leibler, Potvin-Trottier et al., 2016), among others.

While these circuits are often studied as dynamical systems in single cells or well mixed populations, the function of genetic circuits has also been studied in cell colonies (Luo et al., 2019; Santos-Moreno and Schaerli, 2019). We focus here on the

repressilator (Elowitz and Leibler, 2000), a gene network that encodes a ring oscillator topology consisting of three repressors, where repressor 1 inhibits repressor 2, repressor 2 inhibits repressor 3, and repressor 3 inhibits repressor 1 (Figure 1). Recently, the circuit was revisited by Potvin-Trottier et al. (2016) with microfluidics systems that allowed them to observe single cells oscillating synchronously in chemostatic conditions for long periods of time. In this work sources of noise were reduced in several ways.

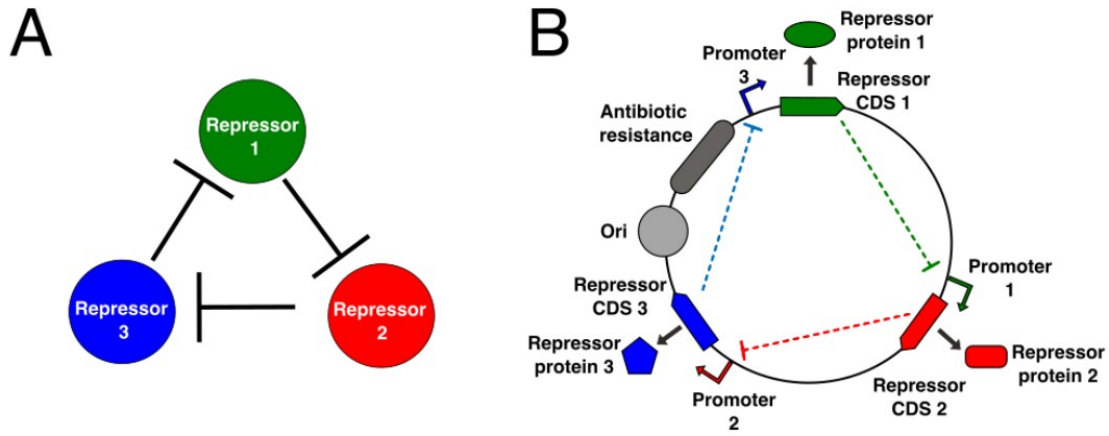

Figure 1. Repressilator genetic oscillator circuit. (A) Network representation of the repressilator, in the schematic 1 represses 2, 2 represses 3, and 3 represses 1. (B) Genetic circuit diagram of a plasmid encoding the repressilator.

Methods

We have used two approaches for predicting traveling waves. First, we developed an analytical one dimensional model (in colony radius) that explicitly considers dilution by growth. And second, to test if these predictions hold in constrained growing microcolonies of cells we used our individual based biophysical model of bacterial cell growth and division (Rudge et al., 2012).

First, we show in silico how mechanical constraints generate characteristic patterns of growth rate heterogeneities in growing cell colonies. Based on simple bacterial colony growth (Rudge et al., 2012), we derive an expression for the pattern of growth-rate across the colony, which describes the growth rate as exponentially decaying from center to border:

$$\bar{\mu}(t) = e^{-r(t)/r_0} \tag{1}$$

Next, we develop a simple mathematical model coupling the repressilator genetic circuit to growth rate variation via simple dilution of proteins. A simple two-step model of the balanced repressilator genetic circuit, a type of genetic ring oscillator,

can be formulated as follows,

$$\frac{dm_i}{dt} = \frac{a + b(p_j/K_j)^n}{1 + (p_j/K_j)^n} - \delta m_i \tag{2}$$

$$\frac{dp_i}{dt} = cm_i - \gamma p_i - \mu(t)p_i \tag{3}$$

where i is one of the three genes, j is its corresponding repressor gene, mi, pi are the mRNA and protein concentrations respectively, a is the constitutive transcription rate, b is the leaky or repressed transcription rate, μ is the instantaneous growth rate of the cell or population of cells, γ is the protein degradation rate, and δ is the mRNA degradation rate. Since mRNAs are typically short lived, we may assume quasi-steady state concentrations. Rescaling protein concentration and time we obtain,

$$\frac{dp_i}{dt} = \frac{\alpha}{1 + p_j^n} - \bar{\gamma} p_i - \bar{\mu}(t)p_i \tag{4}$$

Finally, we have developed microscopy protocols that allow imaging of fluorescence and phase contrast simultaneously over 12-24 hour periods of growth of cell colonies on agarose hydrogel substrates at single cell resolution. Using these techniques we have measured and analyzed the repressilator circuit developed by Potvin-Trottier et al. growing constrained to a quasi-two-dimensional monolayer, and subject to viscous drag from the hydrogel substrate.

Results

This simple one-dimensional model predicts that coupling the repressilator to this pattern of growth rate via protein dilution, generates traveling waves of gene expression (Figure 2C). A kymograph represents the spatial dynamics of a one-dimensional system evolving over time. Each point in the kymograph represents the state of the system at a given time (in the x-axis) and its position with a color (in the y-axis). By taking radial averages the kymograph fully characterizes radially symmetric spatio-temporal patterns with the vertical axis representing distance from the center of the colony. The kymograph represents the symmetric structure of the pattern, whereby the growth of the colony can be seen as two linearly expanding borders forming a triangular shape. The color represents the radially averaged repressor protein concentrations (red, green, and blue) at each position at each time point, then normalized to their maximum values. Hence we confirm our theoretical

prediction of traveling wave patterns emerging from the repressilator circuit (Figure 2C).

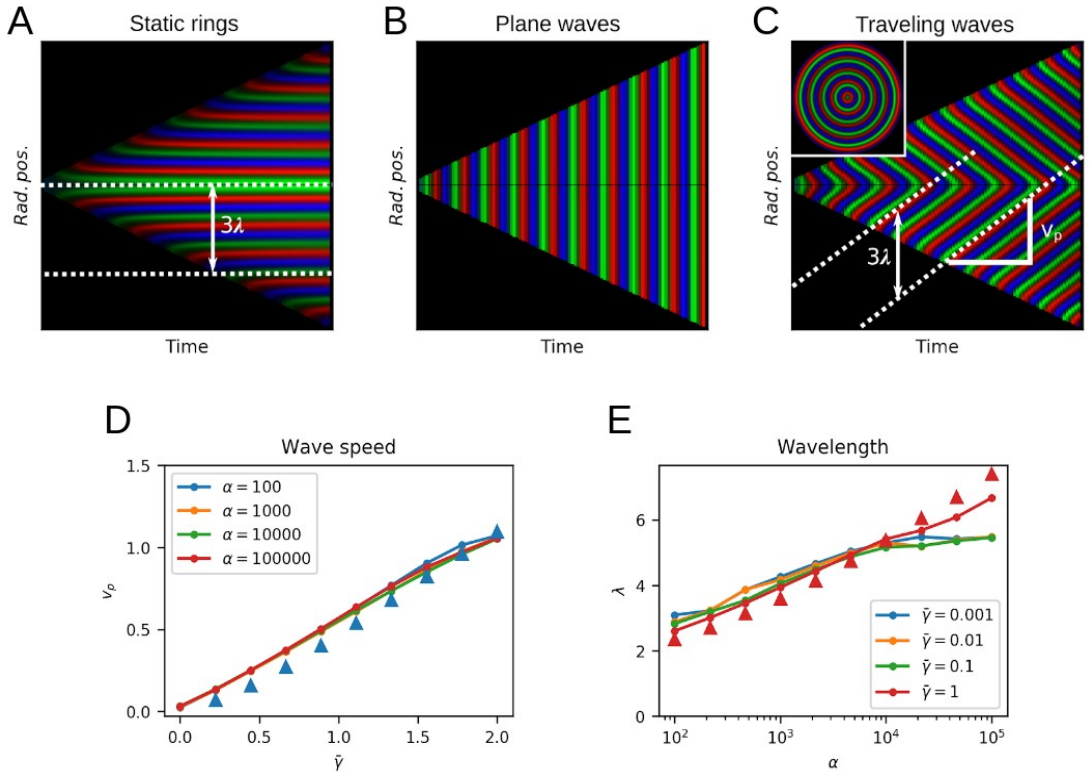

Figure 2. Results for 1D model. (**A–C**) Kymographs of growing colonies show protein concentrations as a function of radial position over time, with no protein degradation (**A**, $\bar{\gamma}$=0, α = 10^4) we obtain static rings, with no growth rate dilution (**B**,$\bar{\gamma}$=1.5, α = 10^4) we see plane waves, and with growth rate dilution and protein degradation (**C**,$\bar{\gamma}$=1.5, α = 10^4) we have traveling waves. White dashed lines show wave trajectories. The distance between two trajectories is the wavelength λ, and the slope is the wave speed *vp*. Inset in © is the whole colony at the end of the experiment. (**D**). Effect of $\bar{\gamma}$ on wave speed *vp* at different α. (**E**). Effect of α on wavelength λ at different $\bar{\gamma}$. Triangles in (**D,F**) show analytical estimates for α = 100 and $\bar{\gamma}$=1, respectively.

We show that the dynamics of these spatio-temporal patterns are determined by two parameters; the protein degradation and maximum expression rates of the repressors. We derive simple relations between these parameters and the key characteristics of the traveling wave patterns: firstly, wave speed is determined by protein degradation and secondly, wavelength is determined by maximum gene expression rate (Figure 2D,E).

Next, using our individual based biophysical model of bacterial cell growth and division (Rudge et al., 2012), we grew colonies from a single cell up to 60,000 cells, tracking each cell's motion and protein expression levels according to the equation for protein production without the growth rate term (since dilution was computed by the biophysical model). The results show, as predicted, the formation of symmetrical traveling rings relative to the center of the colony (Figure 3).

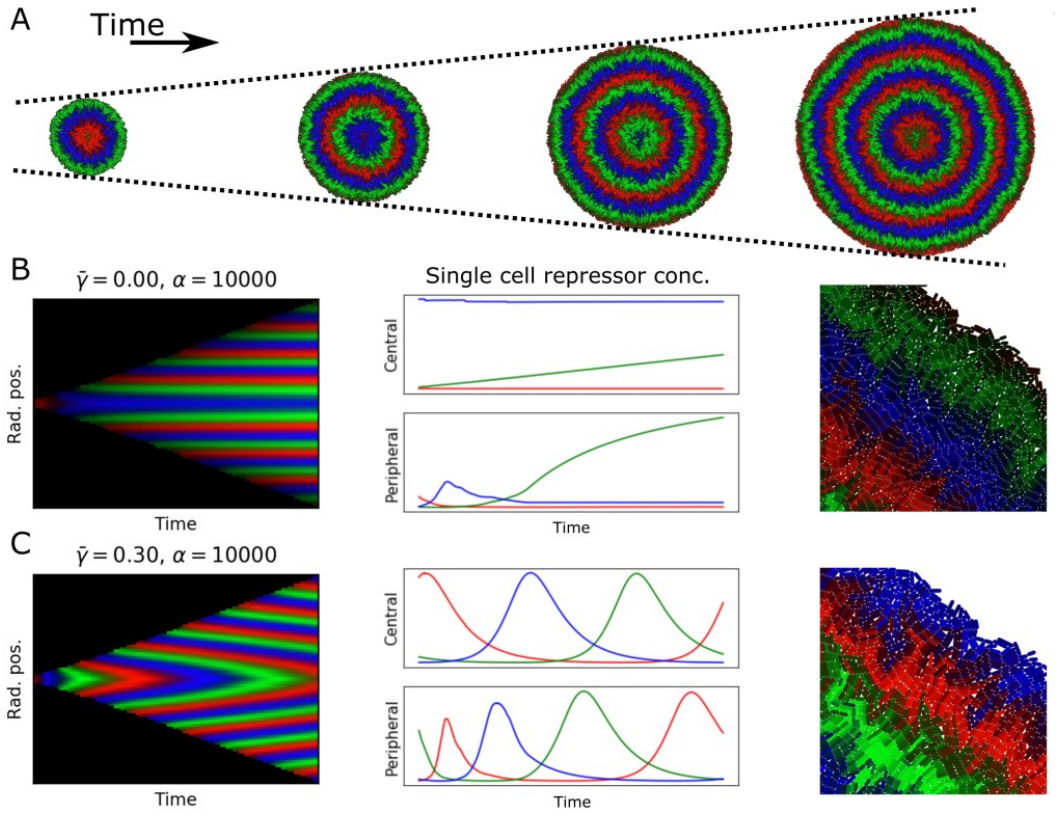

Figure 3. Results for Individual Based Model (IBM). (**A**) Colonies with 5,000, 20,000, 35,000, and 50,000 cells, equally spaced 9.3 doubling times apart, with $\bar{\gamma}$=0.3, α=10.000. (**B–C**) Each panel shows: kymograph of repressor concentrations (51 doublings, approximately 60,000 cells); time dynamics for central cell and peripheral cell in colony; close up of edge of colony at end of experiment. Parameters: (B) $\bar{\gamma}$=0, α = 10, 000. (C) $\bar{\gamma}$=0.3, α = 10, 000.

Finally, microscopy and image analysis reveal the emergence of traveling waves in growing cell colonies (Figure 4). We observed distinct rings of gene expression (Figure 4A), which when analyzed dynamically via the kymograph (Figure 4B) reveal traveling waves originating from the edge of the colony and moving toward the center as predicted. As shown above for simulations, the kymograph represents the radial average fluorescence at each time point, and diagonal stripes indicate traveling waves.

Here we have marked the position of the YFP and RFP wave fronts at each time. These waves are due to phase differences in the peaks of gene expression, as observed in the time traces of the center of the colony (Figure 4C). We note the low frequency of these waves (we only observed one period during a 12 hour experiment), which our theory predicts may be due to high maximal gene expression rate. Hence, our preliminary results suggest that changing the RBS of the repressor genes may increase the number of observed wave fronts.

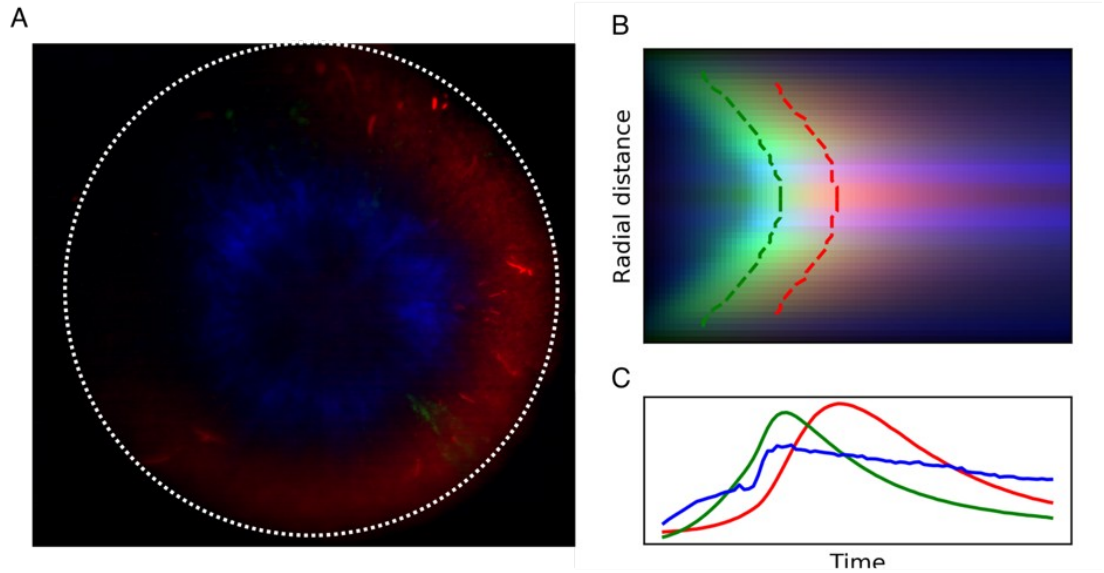

Figure 4: Time-lapse fluorescence microscopy of repressilator colonies show ring patterns (**A**) that form traveling waves, seen in kymograph (**B**) due to phase offsets (**C**).

Conclusion

This work demonstrates that mechanical constraints give rise to higher order gene expression patterns in cell colonies, and provide a simple system for their design and analysis. Experimental results support our theory and future work will be focused on changing genetic parts in order to tune the wavelength and wave speed of the traveling waves. The understanding of complex multicellular behaviors at multiple scales in order to control how these patterns are generated and maintained will enable applications in natural phenomena such as embryonic development, tumor formation, wound healing and tissue engineering.

Funding

GV was supported by a scholarship from the Institute for Biological and Medical Engineering, Pontificia Universidad Católica de Chile. GY was supported by Beca

Ayudante Doctorando scholarship from the Department of Chemical and Bioprocess Engineering, Pontificia Universidad Católica de Chile. TR, GV, and GY acknowledge financial support from the National Agency for Research and Development (ANID)/PIA/ACT192015.

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