A Bio-Inspired Spatial Patterning Circuit

Kai-Yuan Chen, Danial J. Joe, James B. Shealy, Bruce R. Land, and Xiling Shen

***Abstract*— Lateral Inhibition (LI) is a widely conserved patterning mechanism in biological systems across species. Distinct from better-known Turing patterns, LI depend on cell-cell contact rather than diffusion. We built an *in silico* genetic circuit model to analyze the dynamic properties of LI. The model revealed that LI amplifies differences between neighboring cells to push them into opposite states, hence forming stable 2-D patterns. Inspired by this insight, we designed and implemented an electronic circuit that recapitulates LI patterning dynamics. This biomimetic system serve as a physical model to elucidate the design principle of generating robust patterning through spatial feedback, regardless of the underlying devices being biological or electrical.**

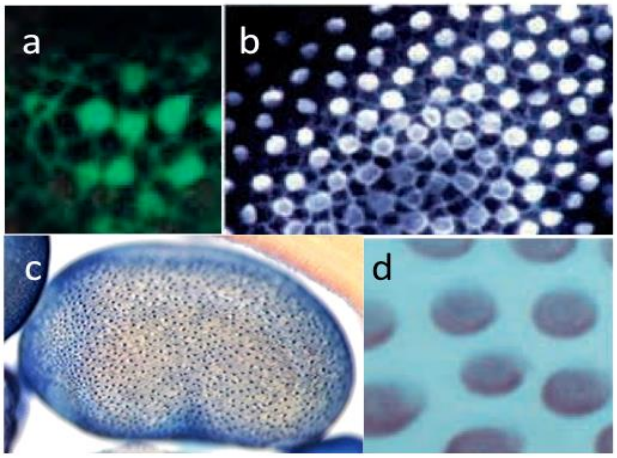

Figure 1. Pattern Formation in Biology. a) Sensory cell pattern formation in the zebrafish ear (green cells: hair cells, dark cells: supporting cells.)[1] b) Pattern formation in basilar papilla of the chick ear (bright cells: hair cells, dark cells: supporting cells.)[2] c) Pattern formation of cillilated cell precursors in Xenopus embryo (black dots are the cilliated cell precursors labeled by α-tubulin.)[3] d) Notch 1 expression during feather differentiation in chick embryo[4].

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I. Introduction

Developmental pattern formation in biological systems has attracted much interest. It is often associated with Turing patterns, which rely on a reaction-diffusions mechanism involving long range sensing of concentration gradient (morphogenetic gradient) and short distance diffusion of cell-cell communication molecules (paracrine signaling) [5, 6]. However, Turing pattern is not the only mechanism capable of generating patterns. During development and tissue homeostasis, cells also employ lateral inhibition (LI) to form boundaries and "pepper-salt"-like 2-D patterns [7] (Figure 1). Highly conserved in many species [1, 2, 4, 8, 9], LI depends on Notch signaling and direct cell-cell contact, where Notch receptors and ligands on the surfaces of neighboring cells interact with each other to determine a highly organized global pattern. This pattern provides a great demonstration that local cell-cell interactions can lead to coordinated group behaviors, though how LI manages to achieve it robustly remain somewhat unclear.

Thanks to emerging fields like systems biology and synthetic biology, there has been increasing appreciation that biological regulatory circuits and man-made control systems share similar "design principles", because they both have to meet certain requirements such as speed, robustness, stability and noise rejection. Classic electrical circuits such as latch and oscillator have been implemented as genetic circuits in living cells [10-12]. Conversely, genetic circuits have also been implemented as electrical circuits to elucidate the design principles[13, 14].

Inspired by such work, we designed an electronic circuit that mimics intercellular LI interactions. The circuit was first simulated in LTSpice and the simulation was compared with a mathematical model of LI. We then physically implemented the electronic LI circuit and measured its patterning dynamics, which recapitulates both predictions from theoretical simulations and observations in biological systems.

II. Material and Method

A. Lateral Inhibition of Notch Signaling

Notch signaling is activated when a Notch receptor is bound by a Notch ligand from an adjacent cell. The binding causes the cleavage of the Notch intercellular domain (NICD), which translocates into the nucleus and activates a group of transcriptional factors (TFs). These TFs subsequently regulate the downstream targets such as members of the HES family, which play important roles in regulating cell fate. For LI to function, these TFs need to suppress the expression of ligands in the same cells (Figure 2a). LI essentially forms an intercellular double negative feedback loop between neighboring cells.

B. Mathematical Model

A mathematical model was built to describe the LI regulation mechanism described above [15].

$$\frac{dN_i}{dt} = \beta_N[\frac{< D_j >_i^n}{(K_N + < D_j >_i^n)}] - \alpha_N N_i \quad (1)$$

$$\frac{dD_i}{dt} = \beta_D[\frac{K_D}{(K_D + N_i^m)}] - \alpha_D D_i \quad (2)$$

N_i and D_i refer to the expression level of Notch signaling (NICD) and Notch ligands in cell i respectively. $<D_j>_i$ represents the average ligand level of adjacent cells surrounding cell i. $\beta_{(N,D)}$ and $\alpha_{(N,D)}$ are production rate and degradation rate of Notch signaling (N) and Notch ligand (D) respectively. Transcriptional regulation is approximated by the Hill function [15-17]. The Hill function in Equation 1 represents activation of Notch signaling, while the Hill function in Equation 2 describes lateral inhibition. In the Hill functions, m and n are Hill coefficients and $K_{(N,D)}$ are saturation constants.

C. Numerical Analysis and Simulation

Based on the above equations, a simple two-cell model was analyzed to investigate the stability of LI. The Symbolic Math Toolbox of MATLAB was used to analyze the two-cell model. For spatial patterning simulation, cell populations with various connectivity configurations (line, ring, square matrix) were tested with random initial conditions were assigned. The ODE solver based on Runge-Kutta method, ode45, in MATLAB was utilized for the spatial simulation.

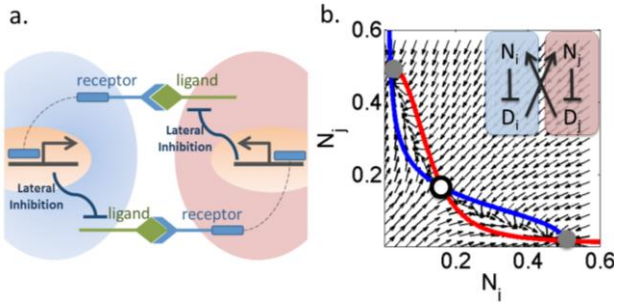

Figure 2. a) Illustration of Lateral Inhibition in Notch signaling. Notch signaling is activated when the Notch ligand from adjacent cell binds to the Notch receptor on a cell, which causes cleavage of the NICD. Subsequently, the cleaved NICD then translocate to nucleus and forms transcriptional complex with other factors, which suppress the expression of the Notch ligand on the same cell. This process is called lateral inhibition (LI). The LI regulated ligand expression then feedback to the adjacent cell, which forms an intercellular double-negative feedback loop. b) Dynamics analysis of lateral inhibition in a two-cell system. The upper-right diagram shows the Notch LI circuit in a 2-cell system. N_i and N_j refer to the Notch signaling level in cell i and cell j respectively. The red line in the figure is the nullcline of N_i, and the blue line is the nullcline of N_j. The circles mark the steady states: filled circles are stable points, and unfilled circle in the middle is an unstable point. (β_N=1, β_D=1,α_N=1,α_D=1,K_N=1,K_D=0.001,n=1,m=3)

D. SPICE simulation

The electronic LI patterning circuit was designed and simulated in LTspice (Figure 3a). The opamps were used to impose a piece-wise linear approximation of the hill functions using its saturation and gain characteristics (Figure 3b). In the circuit (Figure 3a), U4 implements the Notch activation and computes the average input value from several source cells, U3 forms the differential equation integrator of *dN/dt* (Equation 1), U2 implements the LI, and U1 forms the *dD/dt* differential equation integrator. (Equation 2)

E. Implementation of the Physical Circuit

The electronic LI patterning circuit was implemented on a breadboard with LM358 opamps, resistors and capacitors of designed values.

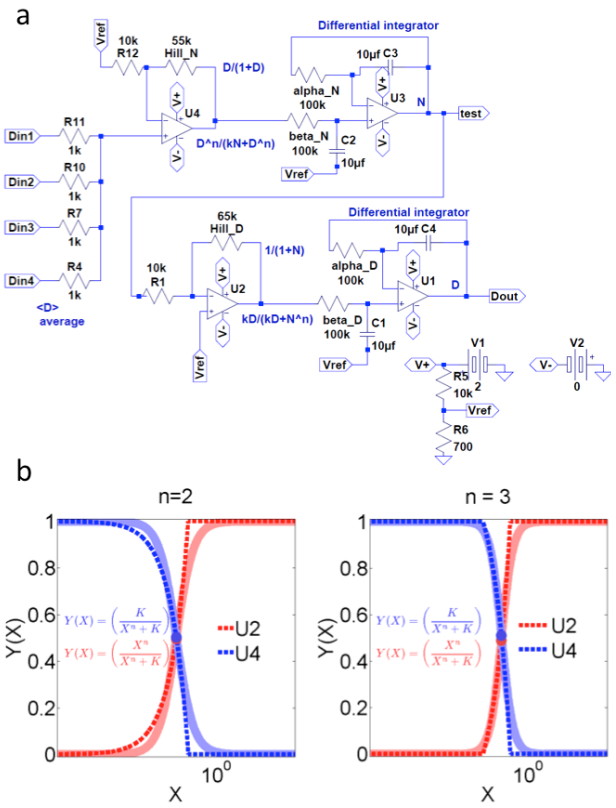

Figure 3. a) Electronic circuit of LI in a single cell. Opamp U4 implements Notch activation, while opamp U2 implements LI. Opamp U1 and U3 are the differential equation integrators of *dN/dt* and *dD/dt* respectively. b) The linear piece-wise approximation of hill functions. The solid lines refer to the mathematical hill functions, while the dashed lines are linear piece-wise approximations of electronic circuits. Red color represents the increasing hill function, and the blue color represents the decreasing hill function. The dots in the figures refer to the maximum absolute sensitivity used for linear approximation of the gains in circuits. n is the hill coefficient in the hill functions. (K = 0.01 in the analyses)

III. Result

A. Systems analysis of the LI circuit

Analysis of a simple two-cell system reveals that LI generates bistability through the intercellular double negative feedback loop. For analytical purpose, the dimension of the two-cell system was reduced from 4 variables to 2 variables, so that the nullclines and equilibrium points could be presented on a 2-D phase plane (Figure 2b). Based on the phase plane, there are two stable equilibrium points located at opposite steady states: high Notch signaling in one cell, while low Notch signaling in another one. Besides the two stable

steady states (solid dots in Figure 2b), there is an unstable steady state residing in the middle (unfilled dot). The analysis suggests that any difference between two cells in contact will be amplified to achieve opposite Notch signaling states, which will lead to distinct cell fate outcomes.

B. Pattern Formation of LI Circuit

The 2-cell analysis suggests that LI generates bistability between neighboring cells (Figure 4a). We then investigated how local cell fate bifurcation gives rise to global pattern formation in different spatial configurations, we performed multicellular simulation in the arrangements of a line, a ring, and a square matrix respectively (Figure 4b-d). Bi-stable patterns are robustly observed in these various configurations. Dynamical tracking of Notch signaling levels (Figure 4e) in the cell matrix (Figure 4d) shows that cells bifurcate and reach opposite steady states from the initially random initial conditions to form the observed pattern. This multicellular simulation suggests that the LI circuit is a robust patterning mechanism that is insensitive to initial conditions and spatial configurations.

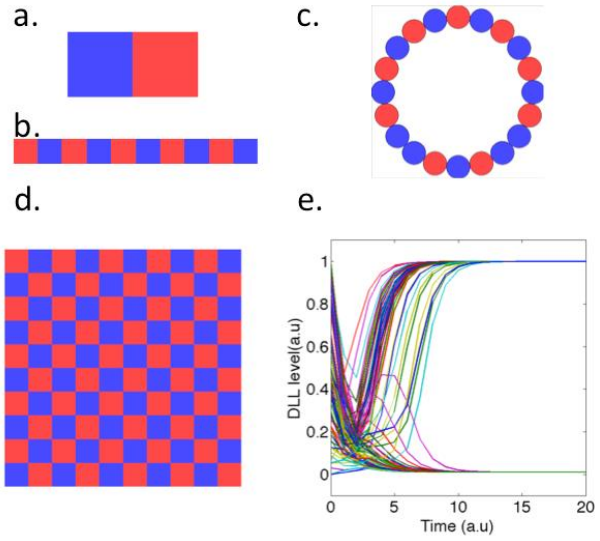

Figure 4. Mullticellular Simulation of LI circuit. a) LI pattern formation in a 2-cell system, b) cells in a line, c) cells in ring structure, d) in 10x 10 cell matrix. The red color refers to high DLL level, and the blue color represents low DLL level. e) LI patterning dynamics in a 10 x 10 cell matrix. (β_N=1, β_D=1,α_N=1,α_D=1,K_N=0.01,K_D=0.01,n=m=3)

C. SPICE simulation of Electronic LI Circuit

To show the LI patterning dynamics in a cell population, 9 cells with identical LI circuit were connected in a ring structure (Figure 5a). LTSpice simulations (Figure 5c) shows that Notch signaling levels bifurcate and reach opposite steady states similar to the numerical simulation (Figure 5b). Slight differences in initial transient responses could be observed between the mathematical model and the electronic circuit. This was caused by the linear piece-wise approximation of non-linear Hill functions by the electrical circuit implementation (Figure 3b). However, this approximation has no bearing on the final patterning outcomes, suggesting that LI is a robust patterning mechanism that is insensitive to the exact characteristics of the underlying mechanism (biological or electrical). This may reflect an evolutionary design principle of biological system, which has to tolerate uncertainties and fluctuations of biochemical reaction rates.

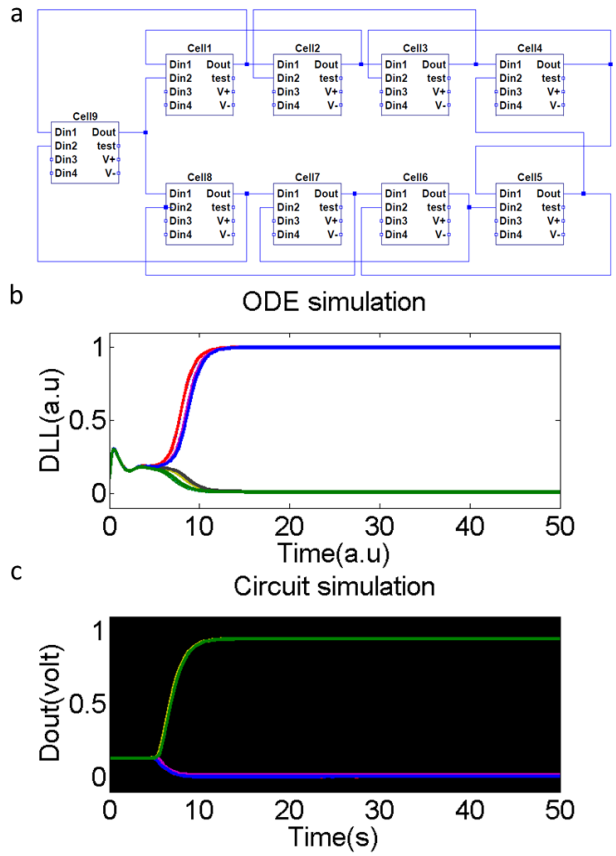

Figure 5. Simulation of LI circuit in 9 cells. a) Connectivity of the electronic circuits (Figure 3a) in 9 cells. b) Patterning dynamics of the mathematical model (Equation 1,2) from numerical simulation (same connectivity in Figure 5a). c) Patterning dynamics of electronic LI circuits from SPICE simulation (same connectivity in Figure 5a)

(β_N=1, β_D=1,α_N=1,α_D=1,K_N=0.01,K_D=0.01,n=m=2)

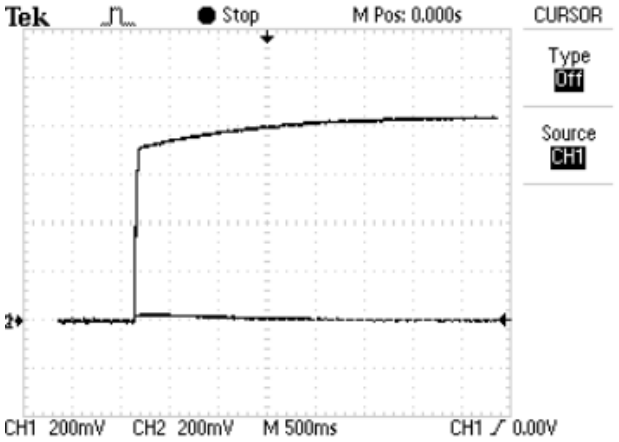

Figure 6. Measurement of the physical LI circuit. Each line refers to the dynamics of a cell.

D. Implementation of a Physical LI Circuit

An electronic LI circuit was implemented based on the simulated design (Figure 3a). We measured the output voltages that represent ligand levels in neighboring cells (Figure 6). The ligand levels bifurcate transiently and eventually reach distinct stable levels, consistent with the simulations (Figure 5 b,c). The slight transient variation before approaching steady states is due to the nonlinearity of LM358 opamps at low voltage levels. Overall, this physical LI circuit gives rise to a patterning behavior that is consistent with observations from biological systems.

We are currently scaling up the number of interconnected LI circuit elements to understand how local and global complexity will affect LI pattern formation. Extensive measurements are being performed to understand how variables such as the number of connected LI modules (representing neighboring cells), the opamp gain and nonlinearity (representing cooperativity of biochemical reactions), and changes of connectivity (representing cell migration, division and death) affect the stability and robustness of pattern formation.

IV. Discussion

A lot of efforts have been put into understanding the underlying mechanisms of biological pattern formation. However, issues critical to the performance of an engineered system, e.g., robustness, dynamics, and stability, have been largely ignored.

Here, using *in silico* models and biomimetic electrical circuits, we demonstrate that LI forms robust cell fate patterns by acting as an intercellular bistable switch that amplify differences between neighboring cells. This switch is robust against initial conditions and 2D cellular configurations. The fact that the electronic LI circuit faithfully recapitulates the bifurcation dynamics of biological systems demonstrates a design principle – a robust patterning mechanism does not depend on the exact characteristics of its underlying devices, regardless of whether they are electrical or biological. Hence LI is rather insensitive to noise, fluctuations, and variations, explaining its prevalence among biological systems.

This represents a deviation from the common practice of electrical circuit design, which relies on precisely characterized devices that are fast and reliable. With modern day applications of low power sensing and computing (e.g., in sensor networks, implanted devices and neural networks), where reliability and robustness often trump accuracy due to unpredictable environments, biomimetic circuits such as LI may provide an alternative design approach. Physical implementations of bio-inspired circuits may also serve as "test beds" for validating insights from theoretical modeling of biological systems before moving into the wet lab, where experiments are often time-consuming and severely limited in their capabilities.

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