Modeling and Analysis of Cell Differentiation using Hybrid Systems

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Abstract—The capacity of cells to differentiate into specialized types is fundamental to the development of organisms. However, the underlying cell differentiation process is not yet well understood. In this work, we consider cell differentiation resulting from interactions of the Delta and Notch proteins. We model the protein concentration dynamics using switched affine hybrid automata. Applying tools from monotone dynamical system theory, we prove steady-state properties of the model. We also find, by exploiting symmetries in the system, conditions on the model parameters to ensure that the steady-state reached is consistent with biological observations. We conclude that the piecewise affine hybrid model is a good framework for modeling the cell differentiation process because the consistency of the model with biological observations can be analyzed theoretically for cellular networks of arbitrary size.

I. INTRODUCTION

Cell differentiation is the process by which specialized cell types emerge starting from a homogeneous distribution. In developing tissues, a network of cells forms various spatial patterns of differentiated and undifferentiated types [15]. Although the exact mechanism for pattern formation is not well understood, it has been generally accepted that patterns form due to cell signaling [11]. One such signaling mechanism, known as lateral inhibition, is such that a cell that reaches a certain state of differentiation inhibits its neighbors from reaching that particular state of differentiation.

Notch and Delta are trans-membrane proteins involved in the cell signaling that leads to differentiation of cells. Notch is a cell membrane protein that functions as a receptor. Notch production in a given cell is promoted as Delta protein from the neighboring cells bind with the Notch receptor in the cell. As the Notch protein concentration increases in a cell, Delta production in the same cell decreases. These intra-cellular and inter-cellular interactions gives rise to a feedback loop that amplifies differences between adjacent cells [6].

Previous work has addressed developing mathematical models based on biological understanding of the cell differentiation process [6], [21], [16]. In most of the proposed models, the system under study is defined as a network of cells on a 2-dimensional surface. The state of the system is defined to be Delta and Notch protein concentrations for each cell in the network. The dynamics of the Delta and Notch concentrations are modeled by nonlinear differential equations that capture the inhibition and promotion aspects of the cellular interactions. However, due to complex interactions arising in a large network, pattern forming properties and temporal performance of the models cannot be theoretically analyzed. Researchers have previously fully analyzed the proposed models for small networks, such as two interacting cells, and have then used insight and simulations to generalize the results to larger numbers of cells [6], [9]. Stochastic models have also been proposed in [16], [5]. The authors in [16] explain why stochasticity in cell differentiation is advantageous and sometimes necessary. They validate their model with simulations.

We adopt a hybrid system model as proposed in [10] in which the protein concentration dynamics is modeled as a switched affine system. The switching behavior is motivated by the observation that as protein concentrations reach certain threshold levels, the production of other proteins becomes activated or deactivated [9], [12], [17]. Although piecewise affine systems have simple dynamics in each mode, they can exhibit complex behaviors such as closed orbits and Zeno executions [19], [3], [7], [12] and analyzing their steady-state properties remains a challenge. In this work, we provide theoretical analysis of the hybrid model and prove that the model parameters can be chosen to provide biologically feasible results.

Our main contributions are as follows: First, we show that for any model parameters, and for almost all initial conditions, the switched affine automata does not exhibit closed orbits or Zeno executions. Second, we provide necessary and sufficient conditions on the parameters of the model to guarantee that the patterns formed in steady-state are consistent with biological observations. Unlike previous work, our results are independent of the number of cells in the network, the initial concentration levels of Delta and Notch, and the spatial location of the cells in the network. In addition, our results are robust with respect to parameter variations in the sense that we provide a range of values under which each observed pattern is formed.

This article is organized as follows: In Section II we describe the biological observations of the Delta-Notch protein network and formulate the problem under study based on these observations. In Section III we describe the hybrid model for Delta-Notch interactions for a network of cells. In Section IV we analyze steady-state properties of the model and find conditions on model parameters to ensure the model produces biologically feasible results. In Section V we show consistency of simulations with our theoretical analysis. Finally, in Section VI we review why this model is a good framework for the problem under study.

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II. PROBLEM DESCRIPTION

Many researchers have focused on understanding interactions of the Delta and Notch proteins resulting in cell differentiation [11], [16], [15], [6], [10], [9]. Experimental studies have shown that:

- Delta production in a given cell is inhibited by high Notch concentrations in the same cell
- Notch production is promoted by high Delta concentrations in neighboring cells
- Concentrations of Delta and Notch converge exponentially to their steady-state values

It has been observed that in steady-state, the Notch or Delta concentration for any given cell converges to either a high or a low level. A cell that exhibits high Delta and low Notch levels in steady-state is referred to as a differentiated cell, and a cell that exhibits low Delta and high Notch levels in steady-state is referred to as an undifferentiated cell. The arrangement of differentiated and undifferentiated cells in a network of cells forms patterns. We summarize the properties of these patterns from experimental and biological observations [15], [11].

Biologically observed patterns in steady-state:

1) Each cell exhibits either high Delta or high Notch levels but not both, i.e., each cell converges to a differentiated or an undifferentiated type
2) Every undifferentiated cell has at least one differentiated neighbor
3) No two differentiated cells are neighbors

The problem we address in the remainder of the paper is as follows:

**Problem statement:** Develop a mathematical model for the Delta-Notch interaction based on the biological observations of the process. Prove that the proposed model produces results that are consistent with the observed patterns as described above.

We start by adopting the piecewise affine model introduced in [10]. In [10] the authors theoretically analyzed the steady-state properties of the model for a network of four cells. To address the problem for larger networks, a reachability algorithm was developed [9]. Given a steady-state pattern, this algorithm determines the set of initial conditions of all proteins that result in that steady-state pattern. However, the algorithm is not computationally feasible for large networks. In this work, we extend their approach by proving that if the parameters of the switched affine model are chosen properly, the model results in biologically observed patterns for a network of arbitrary size cells.

III. MODEL

Let \( x_D \) and \( x_N \) denote the concentrations of Delta and Notch respectively. We model the dynamics of Notch and Delta concentrations in cell \( i \) as [9]:

\[
\begin{align*}
\dot{x}_D^i &= -\lambda_D x_D^i + u_D^i \\
\dot{x}_N^i &= -\lambda_N x_N^i + u_N^i
\end{align*}
\]  

(1)

Here, we use superscript \( i \) to denote variables for cell \( i \), \( \lambda_N \) and \( \lambda_D \) to denote the decay rates for Notch and Delta respectively. Since there is finite amount of protein in a cell, each variable lies inside some bounded interval of non-negative values \([0, s_{\text{max}}]\). The input to Delta takes on two values: \( u_D^i \in \{\mu_D, \nu_D\} \) where \( \mu_D < \nu_D \). We refer to the case in which \( u_D^i = \mu_D \) as the *Off* mode or the deactivated mode for Delta and the case in which \( u_D^i = \nu_D \) as the *On* mode or activated mode. The input \( u_D^i \) switches to its high value if and only if \( x_D^i \leq h_D \) where \( h_D \) is the Delta activation threshold that needs to be determined. Similarly, \( u_N^i \in \{\mu_N, \nu_N\} \) where \( \mu_N < \nu_N \). The input, \( u_N^i \) switches to its high value if and only if \( \sum_{j \in N_i} x_D^j > h_N \), where \( N_i \) denotes neighbors of cell \( i \) and \( h_N \) is a threshold value to be determined.

Spatially, cells are arranged in hexagonal close-packed 2-dimensional lattices as shown in Fig. 1. For a lattice of finitely many cells, each cell, except for those on the boundary of the lattice, has six neighbors. If one generalizes the dynamic model in (1) to any cell in the network, then, one needs to take into account the spatial location of the cells in the network when analyzing the steady-state properties. This is because the boundary cells have a different number of neighbors from the interior cells.

![Fig. 1: Lattice of the cellular network.](image)

In order to overcome the inconsistency in the neighborhood properties of the cells, we identify the opposite edges of the lattice as shown in Fig. 2. In Fig. 2a, the sides of the square with the same arrows, i.e., opposite sides are identified and the resulting space is a Torus as shown in Fig. 2b. The reader can verify that it is always possible to do this identification while ensuring that each cell has six distinctive neighbors if each of the rows and columns of the lattice contains at least four cells. The identification results in a homogeneous network of cells in the sense that every cell is now an interior cell with six neighbors. We can study any given cell in the network and generalize its steady-state properties to all other cells in the network.

![Fig. 2: Opposite edges of the rectangle (sides with the same arrows) are identified. The resulting manifold is a Torus.](image)
We develop a hybrid automata model for cell $i$ based on the model (1) as follows:

$$\mathcal{H}^i = \{Q^i, X^i, Init^i, f^i, Dom^i, R^i\}$$  

- $Q^i = \{q_1, q_2, q_3, q_4\}$, is the set of discrete modes
- $X^i = (x_N, x_D)^T \in \mathbb{R}^2$, is the set of continuous states
- $Init^i = Q^i \times \{x_N^i : 0 \leq x_N^i, x_D^i \leq x_{\text{max}}\}$, is the set of initial conditions
- $f^i = Ax + b^i_q$, are vector fields describing the continuous evolution of $x \in X^i$ for each mode $q \in Q^i$. $A = \text{diag}(-\lambda_D, -\lambda_N)$ and $b^1_q = (\mu_D, \mu_N)$, $b^2_q = (\nu_D, \nu_N)$, $b^3_q = (\mu_D, \nu_N)$, $b^4_q = (\nu_D, \nu_N)$;
- $Dom^i : Q^i \rightarrow X^i$ defines the domain for each mode:
  - $\text{Dom}(q_1) = \{x_N^i > h_D, \sum_{j \in N} x_D^i \leq h_N\}$
  - $\text{Dom}(q_2) = \{x_N^i \leq h_D, \sum_{j \in N} x_D^i \leq h_N\}$
  - $\text{Dom}(q_3) = \{x_N^i > h_D, \sum_{j \in N} x_D^i > h_N\}$
  - $\text{Dom}(q_4) = \{x_N^i \leq h_D, \sum_{j \in N} x_D^i > h_N\}$
- $R^i : Q^i \times X^i \rightarrow Q^i$, is a reset relation that describes the discrete transitions of the hybrid system. Here, discrete transitions from a given mode occur as the state reaches any boundary of the domain of that mode

The hybrid model of a network with $N_c$ number of cells is the composition of the hybrid model for each cell. It is a switched affine dynamical system with $2N_c$ states (one Delta and one Notch protein variable for each cell) and $4^{N_c}$ modes. The domain of each mode is a $2N_c$-dimensional polytopes. The continuous dynamics in each discrete mode is given by a diagonal state transition matrix and a constant input vector. Mode switches occur as the continuous state crosses a $(2N_c - 1)$-dimensional switching hyperplane defined by the threshold values $h_D$ and $h_N$. The discrete transitions are not accompanied by a continuous state reset and hence trajectories of the system are continuous.

Notice that as any protein concentration crosses a threshold value, causing a switch in the mode of the hybrid automata, the dynamics of that particular protein concentration does not change. Crossing any one switching hyperplane only changes the dynamics of the neighboring proteins. Consequently, the flow of the vector field in the direction of the normal vector to any $(2N_c - 1)$-dimensional switching hyperplanes does not change sign and along any one switching hyperplane the vector field can be defined uniquely.

IV. ANALYSIS

Many tools for stability analysis and reachability have been developed to study steady-state behavior of piecewise affine systems [22], [4], [9], [12]. The main difficulty in applying these tools is that the number of modes in the hybrid model of the cellular network is exponential in the number of cells in the network. In order to study behavior of the model for an arbitrary number of cells we take advantage of the symmetry in the network and we also use tools from monotone system theory. We briefly review monotone systems. The interested readers could refer to [20] for an overview on this topic.

A. Monotone Systems

Monotone systems are a class of dynamical systems in which interactions of different components of the state result in robust and predictable behavior [20]. Robustness is particularly desirable in modeling biological systems due to the fact that in many cases there are uncertainties in model parameters. As a result, the interest in using monotone system theory tools in analysis of complex biological networks has increased.

Consider $\dot{x} = f(x)$ with domain $D \subset \mathbb{R}^n$. Let $\phi_t(x)$ denote the solution of this differential equation starting at point $x \in \mathbb{R}^n$ at time 0. $\phi_t(x)$ is referred to as the flow generated by the vector field $f$. Fix $m = (m_1, \ldots, m_n)$, with $m_i \in \{0, 1\}$ for $i \in \{1, \ldots, n\}$ and define $K_m = \{x \in \mathbb{R}^n : (-1)^{m_i}x_i \geq 0, \forall i \in \{1, \ldots, n\}\}$. Here, $K_m$ is a cone that defines a partial ordering:

$$x \leq K_m y \iff y - x \in K_m.$$  

**Definition 1:** (Hirsch and Smith [13]) The dynamic system is called type-$K_m$ monotone if $\forall x, y \in D$:

$$x \leq K_m y \rightarrow \phi_t(x) \leq \phi_t(y), \quad \forall t$$

Most of the previous works on monotone systems provides sufficient conditions for a dynamic system with continuous vector field to be monotone. These results are not directly applicable to the problem at hand because the vector fields are piecewise continuous. However, the notion of monotonicity is extended for piecewise affine systems in [2] and this notion will be described below.

First, we define a piecewise affine system on a compact domain $D \subset \mathbb{R}^n$ as a set of affine differential equations:

$$\dot{x} = A^t x + b^t, \quad \forall x \in C^t$$  

where $C^t \subset \mathbb{R}^n$ are bounded $n$-dimensional polytopes. The union of the closure of these polytopes covers the domain $D$ and the intersection of any two polytopes is empty. Since the vector field defined by (4) may be discontinuous at the boundary of the polytopes, the flow $\phi_t(x)$ is defined as a solution of the vector field in the sense of Filippov [8]. Let $\mu$ denote the Lebesgue measure in $\mathbb{R}^n$.

**Definition 2:** (Aswani et al. [2], Definition 2) A piecewise affine system is a type-$K_m$ almost everywhere (a.e.) monotone system if there exists $Z \subset D$ with $\mu(Z) = 0$ such that for all $x, y \in D \setminus Z$, $x \leq K_m y$ implies that $\phi_t(x) \leq \phi_t(y)$, for all time $t \geq 0$ for which the flow remains in $D$.

The difference between the classical definition of monotone systems and the above definition of a.e. monotone systems is that in the latter, the ordering property stated in (3) may not be satisfied for a set of initial conditions. However, this set must have measure zero. We note that the authors in [2] consider the domains $C^t$ to be rectangular. Generalizing rectangular domains to polytope domains is an easy extension of their work.

It may not be easy to prove monotonicity from studying condition (3) on the flow. There is a simple graph theoretic
test for determining if a system is monotone. This test was originally developed for monotone systems and has been extended for piecewise monotone systems in [2]. Consider a differential equation with \( n \) states, where the dynamics of state \( i \) is given by:

\[
\frac{dx_i}{dt} = f^i(x_1, x_2, \ldots, x_n)
\]

We represent the interactions between the states \( x_1, \ldots, x_n \) using a signed graph \((V, E, S)\), in which the vertices \( V \) represent the states, \( E \) and \( S \) represent the signed edges which are constructed as follows. Let \( \zeta \in \mathbb{R}^n \), \( h \in \mathbb{R} \), and \( x(\zeta; j, h) = (\zeta_j + h)e_j + \sum_{i \neq j} \zeta e_i \), where \( e_i \) are unit vectors in \( \mathbb{R}^n \). Define

\[
f^i(x(\zeta; j, h)) := f^i((\zeta_j + h)e_j + \sum_{i \neq j} \zeta e_i) \tag{6}
\]

If \( f^i(x(\zeta; j, h)) \) is non-decreasing for all increasing \( h \) then we draw an edge from vertex \( j \) to vertex \( i \) with a positive sign. If \( f^i(x(\zeta; j, h)) \) is non-increasing for all increasing \( h \) we draw an edge from vertex \( j \) to vertex \( i \) with a negative sign. If \( f^i(x(\zeta; j, h)) \) is constant for all \( h \) then no edge is placed between vertex \( i \) and vertex \( j \). Next, define a loop, \( L := \{ u_1, \ldots, u_m, u_{m+1} | u_{m+1} = u_1, (u_i, u_{i+1}) \in E \} \). The sign of the loop \( L \) is defined as the product of the sign of the edges in \( L \). The sufficient conditions for monotonicity are based on two assumptions:

**Assumption 1:** For every \( \zeta = (\zeta_1, \ldots, \zeta_n) \in \mathbb{R}^n \), \( f^i(x(\zeta; j, h)) \) does not both increase and decrease for increasing \( h \).

**Assumption 2:** For every \( (n-1) \)-dimensional switching hyperplane, the flow of the vector field in the direction of the normal vector to the hyperplane is nonzero and does not change sign.

**Theorem 1:** (Aswani et al. [2], Theorem 2) If the signed, directed graph associated with the piecewise affine system has only positive loops and the Assumptions 1 and 2 hold then the system is almost everywhere (a.e.) monotone.

As a result of existence of only positive feedback loops, every state in a monotone system responds consistently with respect to perturbations of other states. One of the important consequences of monotonicity is that there are no stable periodic orbits in monotone systems [18], [13]. It follows from the definition of a.e. monotone systems that for almost all initial conditions, there are no stable periodic orbits. Next, we apply the above results to the problem at hand.

**B. Steady-state Analysis of the Delta-Notch network**

Here, we determine the range of parameters such that the Delta-Notch model presented in Section III results in biologically feasible results. For the remainder of this section, we make the following assumption on thresholds \( h_D \) and \( h_N \):

**Assumption 3:** \( h_D \notin \{ \frac{h_N}{x_N}, \frac{v_N}{x_N} \} \) and \( h_N \notin \{ \frac{c_D x_D}{k_D}, \frac{s_D x_D}{k_D} + \frac{y_D}{k_D}, \ldots, \frac{v_N x_N}{k_N} \} \).

The above assumption ensures that the flow of the vector field in the direction of the normal vector to any one of the switching hyperplanes is nonzero and that Assumption 2 is satisfied. We now study the steady-state properties of the model using tools from monotone system theory.

**Theorem 2:** The piecewise affine model of the Delta-Notch interaction network is an almost everywhere (a.e.) monotone system.

**Proof:** We represent the interaction between proteins using a signed graph as discussed in the previous section. For a network with \( N \) number of cells, there are \( 2N \) vertices representing the set of all Notch and Delta proteins in all cells. Based on the sign assignment for a piecewise affine system described in the previous paragraphs, one can verify that there is an edge with a negative sign connecting Notch protein to Delta protein in the same cell. Intuitively, this is true because high Notch concentration in a cell inhibits Delta production in the same cell. There is a positive edge from Delta protein of a given cell to Notch protein of all its neighboring cells. Fig. 3 shows the edges assigned to a cell and its neighbors. One can verify that any loop in the graph has to go through an even number of edges connecting Notch to Delta proteins. Hence, there are even number of negative edges in any loop and the sign of any loop is positive. The system is almost everywhere monotone by Theorem 1.

![Fig. 3: The signed edge assignment for a cell and its six neighbors. The positive edges from Delta protein of the center cell to all the neighboring Notch vertices are not shown for legibility.](image)

The result that the system is a.e. monotone does not depend on the model parameters and is solely based on the inhibition and promotion properties of the interactions. Hence, the result is robust with respect to variations in model parameters. One consequence of monotonicity is the following:

**Corollary 1:** In the Delta-Notch network, for almost all initial conditions, the steady-state does not exhibit a stable periodic orbit.

Notice that this does not exclude unstable periodic orbits. However, existence of noise in the biological system results in perturbation of the trajectories from the periodic orbits. Due to instability of these orbits, the trajectories will not converge back to them.

Piecewise affine systems may exhibit infinite mode switches in finite time interval and this phenomena is referred to as Zeno behavior [1], [14]. In [10] it was shown that the piecewise affine model applied to network of two cells has a Zeno trajectory corresponding to both cells having identical initial conditions. For a network of arbitrary size we have:
**Proposition 1:** The set of initial conditions that lead to Zeno trajectories has measure zero.

**Proof:** Assume that there exists a Zeno trajectory. Then, at least one protein concentration, for example $x_{D,i}$, the Delta protein in cell $i$, must increase and decrease infinitely often. This is possible only if Notch concentration in the same cell, $x_{N,i}$, crosses the threshold $h_{D}$ infinitely often. Applying this argument again, we conclude the Delta concentration for at least one of the neighbors of cell $i$, say $x_{D,i}$, must increase and decrease infinitely often and hence its associated Notch protein $x_{N}$ must cross the threshold $h_{D}$ infinitely often. In addition, since infinite switching happens in finite time, both $x_{N}$ and $x_{D}$ must reach the switching hyperplane at the same time. However, the set of initial conditions that simultaneously reach intersection of two or more switching hyperplanes has measure zero as shown in Lemma 5 in [2]. Hence, we conclude that the set of initial conditions that lead to Zeno executions has measure zero. ■

From the first-order dynamics of the protein concentrations in (1), it is clear that for each cell, there are up to four possible equilibria $(x_{D}, x_{N}) = (\frac{\mu}{h_{N}}, \frac{\nu}{h_{N}})$ where $h_{D} \in \{h_{D}, v_{D}\}$ and $x_{N} \in \{v_{N}, v_{V}\}$. This will result in up to $4^{N_{c}}$ possible equilibria for network of $N_{c}$ cells. We need to determine the range of parameters of the model such that the equilibria reached are biologically feasible, i.e. they satisfy conditions (1-3) described in Section II. Let us recall that a cell that has low Notch level and high Delta level in steady-state is referred to as differentiated type and a cell that has high Notch level and low Delta level is referred to as undifferentiated type.

**Proposition 2:** The following are necessary and sufficient conditions on parameters to ensure the model results in each of the following biologically feasible results:

1) Each cell converges to a differentiated or an undifferentiated type: (c1) $\frac{\mu}{h_{N}} < h_{D} < \frac{\nu}{h_{N}}$  
2) Every undifferentiated cell has at least one differentiated neighbor: (c1) $\frac{\mu}{h_{N}} < h_{D} < \frac{\nu}{h_{N}}$, and (c2) $6\frac{\mu}{h_{N}} < h_{N}$  
3) No two differentiated cells are neighbors: (c1) $\frac{\mu}{h_{N}} < h_{D} < \frac{\nu}{h_{N}}$ and (c3) $h_{N} < 5\frac{\nu}{h_{D}}$  

**Proof:**

(a) Suppose $x_{N}$ reaches the high steady-state value of $\frac{\nu}{h_{N}}$. Then, condition $h_{D} < \frac{\nu}{h_{N}}$ ensures that Delta is deactivated and hence reaches its low steady-state value. Similarly, if $x_{N}$ reaches the low steady-state level, then, $h_{D} > \frac{\nu}{h_{N}}$ would ensure that Delta does not become deactivated and Delta reaches its high level.

(b) Suppose conditions (c1) and (c2) hold but there exists an undifferentiated cell, call it cell $i$, with no differentiated neighbor. From (c1) we conclude that cell $i$ and all its neighbors have high Notch level and low Delta level. Then, in steady-state, the sum of Delta concentrations in the six neighbors is $6\frac{\mu}{h_{D}}$. This is not a sufficient concentration to activate the Notch in cell $i$ because by condition (c2) the threshold is set to $h_{N} > 6\frac{\mu}{h_{N}}$. This contradicts that cell $i$ is undifferentiated. Hence, an undifferentiated cell has at least one differentiated neighbor.

(c) Now, suppose conditions (c1) and (c3) and that cell $i_{1}$ is differentiated and has a differentiated neighbor $i_{2}$. Then the Delta concentration of this differentiated neighbor has reached the high level $\frac{\nu}{h_{N}}$. Hence, $\sum_{j \in N_{i_{1}}} x_{D,j} \geq 5\frac{\mu}{h_{D}} + \frac{\nu}{h_{N}}$, and by condition (c3) this implies that the input to the Notch protein of cell $i_{1}$ has to be at the high value. Hence, the Notch concentrations in cell $i_{1}$ has reached the high level.

The analysis above does not depend on the initial conditions, or the network size. Also the results are robust in the sense that we have given a range for $h_{N}$ and $h_{D}$ that guarantee desired equilibrium properties. As can be seen from above proposition, not only can we predict steady-state properties for parameters satisfying conditions above, but also we can predict how the steady-state properties change if any of the above conditions are violated. For example, if condition (c3) of Proposition 2 is not satisfied, it is possible that the model will result in two adjacent cells both being differentiated.

**V. SIMULATIONS**

Simulation was performed for lattices of cells with various number of rows and columns. Figures 4 and 5 show two such simulations for a lattice of $20 \times 20$ cells. The boundaries of the lattice are identified as described previously such that every cell has six distinct neighbors. The parameters are set to: $\lambda_{N} = -1$, $\lambda_{D} = -2$, $\mu_{N} = \mu_{D} = 1$, $v_{N} = v_{D} = 4$. The thresholds $h_{N}$ and $h_{D}$ are chosen randomly from the allowable ranges derived in the previous section. In our case the ranges are given by: $h_{N} \in [6, 7]$, $h_{D} \in [1, 4]$. The initial conditions of Notch and Delta concentrations in each cell are taken from a uniform distribution between zero and their maximum values.

In steady-state each cell converged to either a differentiated cell or an undifferentiated cell. The simulations were consistent with the experimental observations on the two properties of the desired equilibrium: every undifferentiated cell had a differentiated neighbor, and no two differentiated cells were neighbors. Although the details of the pattern depended on the initial conditions, a large number of simulations indicated that the ratio of differentiated to undifferentiated cells was approximately 1 to 3.

Our simulations for large numbers of initial conditions and parameters indicated that on average there would be two mode switches before steady-state is reached. Fig. 6 shows the trajectories of Notch and Delta for a randomly chosen cell in the network. We can see that the inputs for both Notch and Delta dynamics switch between high and low values up to three times until steady-state is reached.

**VI. CONCLUSIONS**

We presented a switched affine system to model the Delta and Notch protein interaction network responsible for cell differentiation. We showed that the dynamic system is almost everywhere (a.e.) monotone. Hence, for almost all initial conditions, there are no stable periodic orbits or Zeno trajectory. We found necessary and sufficient conditions
on the parameters of the model to ensure that it produces biologically feasible results. The analysis was independent of the initial concentration of the proteins in the network and the number of cells in the network. Our simulations validated the theoretical analysis. We conclude that the piecewise affine model captures the observed dynamical and steady-state properties of the system.

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