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Role of Cell Morphology in Classical Delta-Notch Pattern Formation

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***Abstract*—Notch signaling is responsible for creating contrasting states of differentiation among neighboring cells during organism's early development. Various factors can affect this highly conserved intercellular signaling pathway, for the formation of fine-grained pattern in cell tissues. As cells undergo dramatic structural changes during development, one of the factors that can influence cell-cell communication is cell morphology. In this study, we elucidate the role of cell morphology on mosaic pattern formation in a realistic epithelial layer cell model. We discovered that cell signaling strength is inversely related to the cell area, such that smaller cells have higher probability/tendency of becoming signal producing cells as compared to larger cells during early embryonic days. In a nutshell, our work highlights the role of cell morphology on the stochastic cell fate decision process in the epithelial layer of multicellular organisms.**

***Index Terms*—Cell Morphology, Lateral Inhibition, Direct cell-cell signaling, 2D vertex model, Cell growth simulation.**

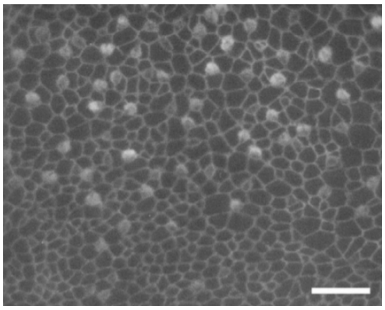

Fig. 1. Classical checkerboard pattern observed during embryonic stage E6 in auditory organ of chick, reproduced from Goodyear *et al.* [24]. The bright cells represent the hair producing/Delta cells whereas black cells represent supporting/Notch cells. Scale bar, 10 mm.

I. Introduction

During the early embryonic development of metazoans, cells undergo dramatic structural changes due to cell birth, growth, migration, and apoptosis in a cell tissue [1]. During the same period, cells cross-talk with each other to coordinate the cell fate decision process through feedback mechanisms [1]–[3]. The epithelial-mesenchymal transition [2] and tip-stalk cell selection during angiogenesis [3] are a few examples of morphogenesis occurring concurrently with Notch signaling. We hypothesized that the cell geometry can regulate robust tissue patterning during development [4]–[8].

Tissue patterning during early developmental stages is controlled by lateral inhibition phenomenon, mediated by Notch signaling. Canonical Notch signaling is an evolutionarily conserved signaling mechanism [9], that falls under the category of juxtracrine signaling i.e. contact-dependent signaling. It triggers when transmembrane proteins i.e. Delta and Notch of adjacent cells interact with each other. This interaction results in cleavage of the Notch receptor and release of Notch Intracellular Domain (NICD) [10]. NICD then travels to the nucleus and control gene regulatory processes. Therefore, Notch signaling is considered responsible for many cellular processes including stem cell proliferation [11], apoptosis [12], development [13], and survival [14].

Several models have been proposed to understand the complex Delta-Notch signaling process, that drives checkerboard pattern formation due to lateral inhibition in an organism, as depicted in Fig 1. These mathematical models highlight the role of different factors/attributes that are responsible for assigning distinct fate to cells from a sheet of identical cells in a tissue [4]. One of the factors that can affect Notch signaling significantly is cell geometry, as both Delta-Notch proteins are colocalized on the cell perimeter [5]. Earlier studies suggest that signaling strength can be affected by cell geometry during angiogenesis [6], bristle patterning in *Drosophila* [4], and asymmetric cell divisions in zebrafish [7]. Khait *et al.* further explore this factor i.e. cell-cell contact geometry, by considering cell membrane dynamics contribution in classical Delta-Notch pattern generation [5]. Shaya *et al.* studied Notch signaling on a computational cell model and proposed that the cell morphology alone can generate the classical Notch-mediated lateral inhibition pattern in a disordered hexagonal cell lattice [8]. Recent studies have also considered cell geometry role in addition to other factors that can affect the Notch signaling mechanism. Saleh *et al.* takes into account the effect of morphogenesis while studying Notch heterogeneity role on cell fate decision process [15]. Although these models explained the pattern formation in their respective cell types, they did not propose a generalized mathematical and computational model for Delta-Notch signaling in adjacent cells of the realistic epithelial layer.

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To model complex biological processes like cell dynamics, tissue patterning and protein-protein interactions various computational cell models have been proposed. Cell models fundamentally complement experimental studies, where it is difficult to identify the effects of different attributes of individual cells on cellular pattern formation. The models available for simulating intercellular signaling mechanism in the epithelial layer are the Potts model [16], center-based model [17], viscoelastic cell model [18], and the vertex model [19], [20]. We used a two-dimensional mechanical vertex model that is capable of modeling cell birth, cell shrinkage, cell growth, cell division, cell death, cell rearrangement, cell collisions, and tissue fusion [20]. All these features are required to generate a realistic cell mosaic and are not available together in any of the other existing cell models.

In our work, we explore the influence of cell morphology on the cell fates decision process on a realistic epithelial layer. By modeling protein-protein interactions on a computational cell model, we gain quantitative insights of the dynamics of the relationship between cell morphology and cell-cell signaling. Results indicate that cell area plays a critical role in the formation of highly ordered mosaic pattern and cell fate determination process.

II. Materials and Methods

A. Cell Model

We use a two dimensional dynamic cellular model to describe the lateral inhibition process in the epithelial layer [20]–[23]. The features/properties that make it stand out from other cellular models are the accurate representation of the geometric properties of a single cell as well as the topological properties of cells in monolayered tissues, without the requirement of periodic boundary conditions and an initial population of cells.

To control the cell fate decision process through the Notch signaling pathway, the two proteins, Delta ligand and Notch receptor require direct physical contact. To model it, we consider ligands and receptors concentration of only adjacent/direct neighboring cells to measure each cell signaling strength. Cis-interactions are not taken into account during the study of contact-dependent Notch signaling, as previous studies [8] indicate that its inclusion doesn't change the results. As the cell model used is independent of periodic boundary conditions, therefore boundary edges have no neighboring cells. Hence, for boundary edges the concentration of cell proteins i.e. Delta and Notch receptor is set to zero, as both are proteins are membrane-bound [9]. For simplicity, we assume the morphogenetic development process of tissue to be slower as compared to fate determination time [8].

B. Cell Morphology Role in Cell-Cell Signaling

Our general strategy to simulate the role of cell morphology in cell fate determination on an irregular cell lattice is depicted in Fig 2. The numerical equations of the dimensionless model are solved in Visual Studio using Boost C++ libraries. It is noteworthy that the initial conditions are randomly generated.

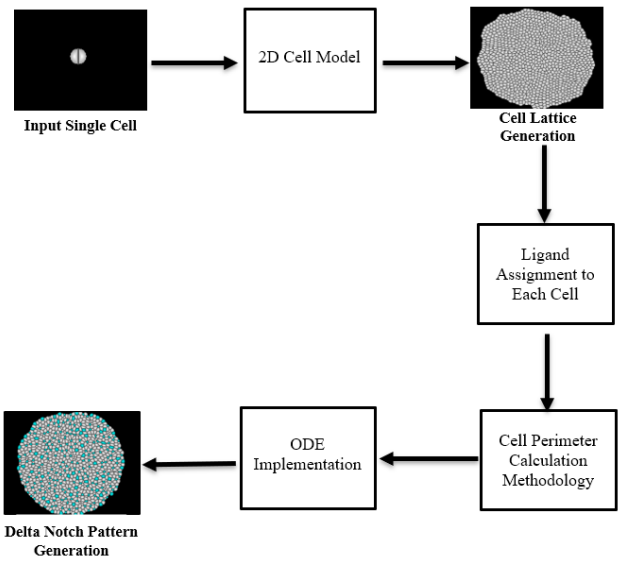

Fig. 2. Schematic of lateral inhibition model while taking into account cell geometry on Delta-Notch pattern formation in an epithelial layer.

To model cell morphology role in Notch signaling, cell lattice is assumed to remain fixed/static during intercellular signaling mechanism. Proteins i.e. Delta and Notch, are assumed to be homogeneously distributed on each boundary of a cell. Initially, using uniform distribution all edges of the cells were assigned Notch value close to one, whereas Delta ligand concentration was set close to zero. Following Shaya *et al.* [8], we proposed a set of ordinary differential equations that take into account cell perimeter during the lateral inhibition process.

$$\frac{dN_{i,j}}{dt} = \frac{\beta_N}{L_i} - N_{i,j} - kN_{ij}D_{ji} \tag{1}$$

$$\frac{dD_{i,j}}{dt} = \frac{\beta_D}{L_i(1+\frac{q(\sum N_{ij}D_{ji}l_{ij})^n}{1+(\sum N_{ij}D_{ji}l_{ij})^n})} - D_{ij} - kN_{ji}D_{ij} \tag{2}$$

In this dimensionless model, (1) and (2) describe the rate of change of Notch and Delta in cell i, on the boundary of cell j respectively. k, β_D and β_N are constants defined as the association constant of Delta-Notch protein, the production rate of Delta ligand and Notch receptor respectively. l_{ij} represents edge length of cell i that shares the boundary with cell j and L_i is the perimeter of cell i. The constants n and q in Delta's equation, are defined as the Hill equation coefficient and binding rate of Delta-Notch protein respectively. In the above equations, D_{ij} and N_{ij} represent the cumulative concentrations of Delta and Notch in cell i on the boundary with cell j. These two coupled differential equations represent the phenomena of classical Delta-Notch pattern formation while taking into account the cell geometry and direct protein-protein interactions of Delta ligands and Notch receptors of neighboring cells respectively. The

TABLE I
SENSITIVITY ANALYSIS PERFORMED ON VARIOUS PARAMETERS OF CELL MORPHOLOGY MODEL

Parameter	Definition	Optimum Range [a.u.]	Value Used [a.u]
n	Hill equation coefficient	$1< n <7$	3
β_N	Production rate of Notch	$1< \beta_N <100$	3.8
β_D	Production rate of Delta	$1< \beta_D <500$	3.8
k	Association constant of Delta-Notch binding	$0.001< k <100$	0.1
q	Binding rate of Delta Notch protein	$150< q < 200$	194

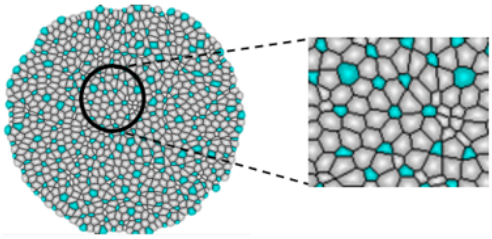

Fig. 3. Simulation of cell morphology model on 2D stochastic cell lattice. The magnified view (10x) of cell lattice signifies that smaller cells have a higher proability of becoming Delta cells (cyan in color) as compared to larger cells. Supporting cells are represented by white color.

changing Delta concentration depends upon the summation of the effects brought by the degradation of Delta, activation of inactive Delta ligands caused by neighboring cell Notch receptors and production of new Delta ligand in the cell (Eq (2)). The changing Notch receptor concentration in each cell with time is described in (1). The rate of change of Notch N is a combination of effects arising from Notch decay, production of Notch on cell perimeter as well as activation of inactive Notch receptor, caused by neighboring cell's Delta. Sensitivity analysis is performed on cell morphology model to assign k, β_N, β_D, q and n constants the values of 0.1, 3.8, 3.8, 194 and 3 respectively (Table I). This mathematical model is implemented on a 2D dynamic vertex model to check the dependency of the Notch signaling process on cell perimeter, as illustrated in Fig 2.

III. RESULTS

We use a stochastic cell model to grow the cell lattice, comprising ∼1,000 cells from a single cell [20], [21]. The distinctive features of this model include incorporating properties of individual cells, the dynamic growth process and accounting for all topological changes. In addition, the cell mosaic generated by this 2D model can closely resemble the natural epithelial layer commonly observed in metazoans (as shown in Fig 1). Naveed *et al.* [21] showed that the stochastic vertex cell model can generate the natural epithelial layer of *Drosophila* and other metazoans with an error rate of ∼5%. Therefore, we use the cell mosaic generated by this model to study the effect of morphogenesis on the Notch signaling mechanism in tissue at early embryonic days. As the cell lattice is stochastically generated, 20 simulations are performed to obtain robust results. We performed quantitative analysis to evaluate the role of cell area in classical Notch pattern formation. It is worth mentioning that all results are evaluated at the steady-state.

To study the role of morphogenesis on cell fate determination, we model the Notch signaling mechanism on a realistic two dimensional cell model. Equations (1) to (2) are implemented on monolayered cell lattice, to model the effect of cell perimeter on protein-protein interactions during Notch signaling pathway. To quantitatively assess/evaluate cell morphology role in Notch pattern formation on the epithelial layer, we calculated Pearson's correlation coefficient of cell perimeter and Delta ligand in cell tissues. Our results indicate the existence of a relationship between the Delta ligand level and cell area. The average result of 20 simulations shows a negative dependency of Notch patterning on cell size, as the correlation coefficient between Delta level and cell area came out to be -0.44. To further explore the influence of cell geometry contribution on the Notch signaling pathway, we compared the cell size of Delta cells. Our analysis shows that ∼70% of signal-producing cells/Delta cells are smaller in size, as shown in Table II. Hence, cells with a smaller area have a higher probability of becoming Delta cells as compared to larger cells in a realistic epithelial layer cell model. This is consistent with the previous study of Shaya *et al.* [8], who performed the analysis on a disordered hexagonal cell lattice.

Experimentally, our analysis is supported by Goodyear *et al.* [24], who had studied the Notch signaling process in the basilar papilla of chick. The basilar papilla is the auditory organ of the chick, in which hair cells are arranged in a highly organized mosaic pattern such that each hair cell is separated from the neighboring cell through supporting cells. It is observed that during early embryonic development of chick from E6-E8, cells smaller in the perimeter are more likely to become hair cells as compared to cells with larger perimeter as depicted in Fig. 1. Although in later stages, hair cells can grow larger as compared to supporting cells. But the initial selection of hair cells relies on cell area.

IV. DISCUSSION

For many years, scientists were puzzled about the science of interacting processes, that are responsible for the development of diverse structural features of an organism during its life cycle. Recent advancement in technology has permitted

TABLE II
QUANTITATIVE ANALYSIS OF CELL SIZE WITH DELTA CELL SELECTION

Cell Size	Cell's Perimeter Range (Normalized)	Average Number of Delta cells
Small	0.70 - 0.90	177 ± 4.03
Medium	0.91 - 1.16	39 ± 16
Large	1.17 - 1.33	33 ± 19

researchers to study the developmental mechanisms at the microscopic level and discovered the hidden attributes that play a critical role in an organism's growth and development. All the developmental processes in metazoans occurring at the genetic, cellular, molecular, and evolutionary levels, fall under the umbrella of developmental biology. The three fundamental processes of developmental biology that caters the embryonic development are tissue patterning, tissue growth, and tissue morphogenesis.

In our work, we elucidate the role of the interdependency of two major factors of developmental biology i.e. tissue patterning and morphogenesis. Both of these factors play a critical role during the early embryonic development of metazoans. Tissue patterning during embryogenesis is controlled by a ubiquitous mechanism called Notch signaling [1]. Notch signaling is a direct cell-cell signaling mechanism that is considered responsible for the formation of the well-ordered and well-spaced pattern of cells in metazoans and plays an important role during embryogenesis [25] and central nervous system development [26]. One of the possible factors that can affect this highly conserved signaling pathway is morphogenesis, as this phenomenon occurs concurrently with Notch signaling. Morphogenesis is defined as the biological process that is responsible for developing cells, organs, and tissues shape.

To understand the role of morphogenesis on tissue patterning, we proposed a mathematical model that takes into account cell shape in addition to protein-protein interactions. Previously, researchers have studied the effect of cell geometry on tissue pattern formation [4]–[8]. Although these studies explained the pattern formation in their respective cell types, yet they did not propose a generalized model for Delta-Notch signaling in the epithelial layer. In our work, we simulated the proposed mathematical model on a realistic 2D vertex cell model. The simulation results provide insights into the complex signaling process occurring at the cellular level, which is difficult to observe in experimental life science.

Our model predicts the dependency of cell geometry with the Notch signaling mechanism (as shown in Fig. 3). Quantitative analysis shows that during early embryonic days, cell selection is negatively correlated with the dimensions of the contact area. This suggests that in a disordered and realistic cell lattice the phenomena of cell fate selection is biased by the cell geometry. These results obtained are inline with the experimental results of Goodyear *et al.* [24], which show that during the early development of basilar papilla smaller cells have a higher chance of becoming hair cells as compared to larger cells.

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