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Cell fate determination is influenced by Notch heterogeneity

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***Abstract*—Notch signaling (NS) determines the fate of adjacent cells during metazoans development. This intercellular signaling mechanism regulates diverse development processes like cell differentiation, proliferation, survival and is considered responsible for maintaining cellular homeostasis. In this study, we elucidate the role of Notch heterogeneity (NH) in cell fate determination. We studied the role of NH at intercellular, intracellular and the coexistence of Notch variation simultaneously at the intracellular and intercellular level in direct cell-cell signaling on an irregular cell mosaic. In addition, the effect of intracellular Notch receptor diffusion on an irregular cell lattice is also taken into account during Delta-Notch lateral inhibition (LI) process. Through mathematical and computational models, we discovered that the classical checkerboard pattern formation can be reproduced with an accuracy of 70-81% by accounting for NH in a realistic epithelial layer of multicellular organisms.**

***Index Terms*—Notch Signaling, Notch Heterogeneity, Direct cell-cell signaling, 2D vertex model, Cell growth simulation.**

I. Introduction

During the development of an organism, cells communicate with each other, in order to control gene regulatory processes. There are a number of signaling mechanisms through which cells can communicate, but one of the signaling pathway that stands out because of its critical role is the Notch signaling (NS) pathway. This pathway is considered responsible for many cellular processes including stem cell development [1] and survival [2].

NS is triggered when Delta, a trans-membrane ligand, interacts with neighboring cell's Notch receptor. Ligand binding leads to cleavage and release of Notch Intracellular Domain (NICD) [3]. Under certain conditions, NICD travels to the nucleus and downregulates Delta expression. This intercellular signaling mechanism is responsible for assigning distinct cell fate and the formation of fine-grained pattern in cell tissues. Mutation or overproduction of intercellular signaling pathway can have drastic effects on human health. At the cellular level, malfunctioning of NS will result in the increased number of connected Delta cells [4]. These interlinked Delta cells are the root cause of shutting down the various systems of metazoans. Malfunctioning of NS can cause Alzheimer's disease [5], congenital heart disease [6], and genetic disorders like Alagille syndrome [4]. Several models are proposed to study the factors that affect complex NS process. Notch heterogeneity (NH) and tension dependant rate of Delta-Notch binding is studied by Koon *et al.* [7] during sprouting angiogenesis process. Shaya *et al.* [8] proposed cell morphology as the symmetry breaking mechanism in the classical Notch-mediated lateral process of the basilar papilla. The role of membrane dynamics on NS is studied extensively [9], as both proteins are co-localized on the plasma membrane of the cell. In addition to trans interactions, cis interactions [10] and filopodia dynamics [11] are also taken into account during pattern refinement process due to NS. Although these studies explained the pattern formation in their respective cell types, yet they did not propose a generalized model for NS in the epithelial layer.

In our work, we proposed a generalized model for the epithelial layer to investigate the role of NH in the highly conserved NS pathway. To understand the role played by NH during pattern formation, we for the first-time studied variation in the Notch receptor concentration while taking into account cell morphology at four different levels, i.e., intracellular NH, intercellular NH, the coexistence of intracellular and intercellular NH and the effect of intracellular Notch receptor diffusion in tissue. To emphasis the role played by cell morphology on the Delta-Notch LI model, we implemented the proposed NH mathematical model on an ideal yet unrealistic cell mosaic, i.e., square cell lattice. Results show that these under explored mechanisms can contribute towards generating of typical Delta-Notch pattern, that is observed during the LI process in the epithelial layer.

II. Materials and Methods

A. Cell Model

We use a previously developed dynamic cellular model to describe the LI process in the epithelial layer [12]–[15]. This 2D cellular model can accurately represent 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. The cell mosaic generated by this model, closely resembles natural epithelial layer of *Drosophila* and other metazoans with an error rate of $\sim$5% [12].

To model NS on 2D cell model, we take into account ligands and receptors concentration of only adjacent neighboring cells, as NS requires direct physical contact between cells. Since

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both cell proteins, i.e., Delta and Notch are membrane-bound proteins [3], therefore the concentration of these proteins on the boundary edges of neighboring cells is set to zero. For simplicity, we assume the morphogenetic development process of tissue to be slower than the fate determination time [8].

B. Role of Notch Heterogeneity (NH) in Cell-Cell Signaling

Role of variation in Notch receptor concentration on lateral inhibition (LI) model is studied at four levels. In all four NH models, initial conditions are randomly generated and results are evaluated at the steady-state. We use Boost C++ libraries in Visual Studio to solve numerical equation of these dimensionless models.

Intracellular Notch Heterogeneity: To model intracellular NH, we assume homogeneous distribution of Delta ligand, close to zero, across the perimeter of each cell in the tissue. To study NH role at the intracellular level, we hypothetically divide each cell into four compartments i.e. left, right, up and down. Due to realistic shape of cells in our 2D cell model, each cell has different number of edges in each compartment. Initially, using a uniform distribution a random value of Notch is assigned to each section of the cell by ensuring the total level of Notch at the intercellular level remains the same. The mean and variance of the Notch receptor in the left compartment is 0.2 and 0.003 while all other compartments have 0.45 and 0.037 respectively. The Notch concentration is then evenly distributed among all edges, that share the same compartment.

Following the 1D model of Koon *et al.* [7], we proposed a 2D model that takes into account intracellular NH in the epithelial layer.

$$\frac{dN_{x,j}}{dt} = -k_d N_{x,j} + k_f(\sum_{n=0}^{X} D_n)(1 - N_{x,j}) \tag{1}$$

$$\frac{dD_j}{dt} = -D_j + \frac{b_o}{1 + \frac{[\frac{N_{l,j}+N_{r,j}+N_{u,j}+N_{d,j}}{E}]^m}{K}} \tag{2}$$

In the proposed mathematical model, D_j stands for cumulative Delta ligand concentration, whereas $N_{x,j}$ in (1), represent a generic expression of Notch receptor concentration in each compartment, i.e., left, right, up and down, of cell j respectively. The summation term over D_n in (1) represents cumulative effect of the neighboring cell's Delta of compartment x, on activation of inactive Notch receptor. The two rate constant terms, i.e., k_d and k_f in (1) represents degradation rate of Notch and the binding reaction rate between Delta and inactive Notch receptor . The constant term E, m, b_o and K in (2) represents the number of edges associated with each cell, hill coefficient, the maximum expression rate of Delta and Delta's inhibitory coefficient for each cell.

Intercellular Notch Heterogeneity: To study the role of intercellular NH on pattern formation, we assigned all cells a random concentration of Notch receptor, having mean 0.35 and variance 0.04 respectively. A uniform distribution is used to assign Delta ligand concentration close to zero for each cell. We proposed the following set of differential equations, to model the role of intercellular NH on adjacent cell's signaling mechanism in cell tissue.

$$\frac{dN}{dt} = -k_d N + k_f(\sum_{n=0}^{neigh} D_n)(1 - N) \tag{3}$$

$$\frac{dD}{dt} = -D + \frac{b_o}{1 + \frac{N^m}{K}} \tag{4}$$

Equation (4) describes that the changing Delta concentration depends upon the summation of the effects brought by the degradation of Delta and the inhibition of activated Notch. The equation (3) represents that rate of change of Notch N is a combination of effects arising from Notch decay as well as activation of inactive Notch receptor, caused by neighboring cell's Delta.

Coexistence of Intracellular and Intercellular Notch Heterogeneity: To investigate the role of NH existence at both intracellular and intercellular level, we extend our previously proposed model of intracellular NH. To cater NH at both levels, we initially assigned a random value of Notch to each edge of the cell using uniform distribution having mean 0.35 and variance 0.04 respectively. This random Notch receptor assignment to each edge of the cell compartment, is responsible for introducing intercellular NH in a tissue besides intracellular NH. Equations (1) and (2) are implemented to understand the effect of coexistence of intracellular and intercellular NH on classical Delta-Notch pattern formation.

Role of Diffusion in Intracellular Notch Heterogeneity: Variation in concentration of Notch receptors at the intracellular level, ultimately leads towards diffusion process [17]. To study diffusion affect on NS pattern formation, we extended the previously proposed model of intracellular NH by adding a diffusion term W, similar to the one proposed in [18]. As Delta ligand concentration is set close to zero in all models therefore its diffusion is not taken into account.

$$\frac{dN_{x,j}}{dt} = -k_d N_{x,j} + k_f(\sum_{n=0}^{X} D_n)(1 - N_{x,j}) + W((N_{v1,j} + N_{v2,j}) - 2N_{x,j}) \tag{5}$$

$$\frac{dD_j}{dt} = -D_j + \frac{b_o}{1 + \frac{[\frac{N_{l,j}+N_{r,j}+N_{u,j}+N_{d,j}}{E}]^m}{K}} \tag{6}$$

The diffusion term W will affect the rate of activated Notch on each side of the cell which will ultimately affect Delta ligand concentration of cell. The Notch receptor concentration on the adjacent sides of compartment x are represented by $N_{v1,j}$ and $N_{v2,j}$ in (5).

After performing the sensitivity analysis, we find that the optimum range for the constant terms k_d, k_f, b_o, K and m

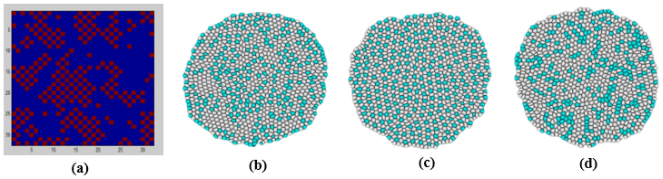

Fig. 1. (a) Sample run of the intracellular NH model implemented on square 32×32 cell lattice. Delta cells are marked red whereas Notch cells are in blue color. (b) Intracellular NH model, (c) Ideal Delta-Notch pattern and (d) Random Delta cell selection model, implemented on irregular cell lattice having ∼1000 cells. Delta cells are marked cyan whereas Notch cells are in white color.

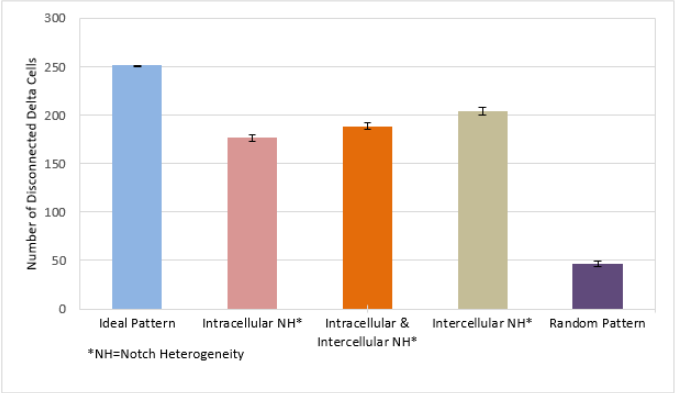

Fig. 2. Comparison of various LI model of NH with the ideal and random Delta-Notch pattern in the epithelial layer.

lies between 0.05 to 7, 0.6 to 17, 0.3 to 100, 0.001 to 3 and 1 to 500 respectively. The values we choose, for all four NH models, are 1, 0.3, 0.8, 0.01 and 2 respectively.

III. Results

A stochastic cell model is used to grow the cell lattice, comprising ∼1,000 cells, from a single cell [12], [15]. To obtain robust results, 10 simulations are performed for each factor that affects NS.

A. Lateral inhibition model with Notch Heterogeneity

Most LI models of NS, make the inherent assumption of the homogeneous distribution of Delta and Notch proteins in each cell [4]. Although, Delta ligands are uniformly distributed among cells undergoing LI [7], however Notch receptors concentration can vary widely among cells during early embryonic days [7]. In our work, we studied NH role at four levels in the epithelial layer.

To evaluate our results, we generate an ideal Delta-Notch pattern on an irregular cell lattice, as shown in Fig. 1(c). In an ideal pattern, no two adjacent cells become Delta cells and only 25% of total cells in the tissue can be selected as Delta cells. This is similar to 1:3.9 of hair cells to supporting cells ratio observed in basilar papilla of *Drosophilia* [19]. Moreover, we simulated a cell tissue in which 25% of total cells are randomly selected as Delta cells, shown in Fig. 1(d). In order to observe the effect of NH model on an ideal cell mosaic, we implemented our NH model on a square 32×32 cell lattice, as depicted in Fig. 1(a). These three metrics provide us a benchmark to evaluate the performance of proposed models of NH on an irregular cell mosaic.

Effect of Intracellular NH on Notch Pattern Formation: To study intracellular NH role explicitly with cell geometry on NS, we compare the results of intracellular NH model with the ideal and random Delta-Notch pattern. Quantitative analysis show that on an average 176 ± 17 isolated Delta cells are found using this model (Fig. 1(b)). The graph of Fig. 2 show that the results obtained using this model are far better than random Delta cell selection model (46±3 isolated Delta cells in a tissue). However, ideal Delta-Notch pattern is still not achievable by considering only Notch receptor variation at the intracellular level. To fully assess the role of cell morphology on NH model, we implemented our intracellular NH model on an ideal square lattice. Simulation results indicate that ideal pattern is achievable on an ideal square lattice, as shown in Fig 1(a). Hence, one of the factors contributing to the less number of isolated Delta cells in the realistic epithelial layer is the irregular shape of the constituent cells.

Effect of Intercellular NH on Notch Pattern Formation: The results obtained after implementing intercellular NH model on an irregular cell mosaic resembles closely with ideal Delta Notch pattern, as depicted in Fig. 2. Quantitatively, the number of isolated Delta cells in each simulation are 203 ± 18 (as compared to the 251 ± 2 Delta cells in the ideal model). The high ratio of isolated Delta cells signifies the role of intercellular NH in direct cell-cell signaling mechanism.

Effect of Coexistence of Intracellular and Intercellular NH on Notch Pattern Formation: To fully assess NH role in classical Delta-Notch pattern formation in an epithelial layer, we studied variation in Notch receptor concentration at both levels, i.e., intracellular and intercellular, using equation (1) and (2). Our results in Fig. 2, show that the number of isolated Delta cells achieved considering NH at both levels is 188±20, which is far better than the random Delta cell selection, i.e., 46±3 in a tissue. However, the deviation of results from ideal pattern signifies that there are other factors too that play a role in pattern formation.

Role of Notch Receptor Diffusion on Notch Pattern Formation: The phenomena of NH cannot be modeled accurately without taking into account the role of Notch receptor diffusion in the cell. We studied the effect of varying diffusion coefficient term W for both models, i.e., intracellular NH model and coexistence of intracellular and intercellular NH model. The result in Fig. 3 shows that as long as W is small, i.e., $W<7$, it is possible to obtain classical Delta-Notch pattern with an average accuracy of ∼73%.

By closely observing the cell lattice at high diffusion

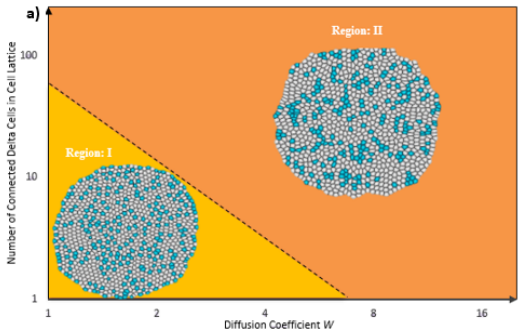

Fig. 3. General trend of Delta-Notch pattern achieved by varying diffusion coefficient (W) in the intracellular NH model and the coexistence of intracellular and intercellular NH model. As long as W <7, i.e., in region I, the pattern achieved in cell lattice resembles classical salt pepper pattern of NS. But as the diffusion coefficient W is increased, i.e., region II, the pattern deviates from ideal Delta-Notch pattern.

coefficient value, i.e., W>7, we observe existence of Delta cells in filaments form. To quantitatively assess the presence of filaments, we measure the filament length using digital image processing technique with MATLAB and compare it with the filaments found in the randomly selected Delta cell model. The average filament length for both intracellular NS model came out to be 81±4 pixels. This value is much higher than the average filament length found in randomly selected Delta cell, i.e., 68±4 pixels. We also implemented this model on ideal square shaped cell lattice, and observed filament formation at high W value. It will be interesting to investigate the reason behind this phenomena, as our study indicate that Notch receptor polarization is not responsible for cluster formation.

The result obtained fundamentally complement the experimental studies, which show that the rate of diffusion of Notch is typically slow as compared to the rate of reaction. Mean diffusion time of Notch receptor is 500s [20] in a cell of radii 10 μm whereas Delta-Notch binding time is 1ms [21]. Hence, the slow diffusion rate might be essential to generate a mosaic pattern in cell tissue.

IV. Discussion

In developmental biology, an enduring challenge for many years was to find the mechanism that is responsible for assigning different character to adjacent cells in metazoans tissues. In the 20th century, the NS pathway was discovered in *Drosophila* [22]. Later, it was found that NS is responsible for the formation of the well-ordered and well-spaced pattern of cells in other metazoans as well [3], [4].

In this study, we explored the contribution of NH in the cell fate determination process on a realistic epithelial layer cell model. We show that Delta-Notch pattern can be achieved with the help of (a) intracellular NH, (b) intercellular NH, (c) coexistence of intracellular and intercellular NH and (d) Notch receptor diffusion at the intracellular level to varying degrees, i.e., ∼70% to ∼81%. The best model among these four, that can replicate results close to the ideal pattern is intercellular NH model. However, the deviation of all NH model from classical Delta-Notch pattern indicates that other factors like cell adhesion, cell tension might also be required. To test NH model under ideal conditions, we implemented it on an ideal 2D cell lattice. Our simulation results show that ideal checkerboard pattern can be achieved on an ideal cell lattice, as shown in Fig. 1(a). However, due to the non-existence of ideal cells in the real world, we cannot attain classical Delta-Notch pattern only by NH in metazoans tissues.

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