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Spatial Patterning from an Integrated Wnt/ β -catenin and Notch/Delta Gene Circuit

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Abstract

Classically, the Wnt/ β -catenin and Notch/Delta signaling pathways were thought to operate through separate mechanisms, performing distinct roles in tissue patterning. However, it has been shown that β -catenin activates transcription of Hes1, a signaling intermediate in the Notch/Delta pathway that controls its lateral inhibition mechanism. To investigate this non-canonical crosstalk mechanism, a new gene circuit, integrating the two pathways, is proposed and simulated in two-cell and multi-cell environments. This model also captures both Paneth cell-mediated and mesenchymal Wnt production. The simulations verify that the gene circuit is temporally bistable and capable of forming a pattern on a multi-cell grid. Last, the model exhibits a bifurcation based on the steady state concentration of Wnt and the relative amount of control β -catenin has over the Hes1 promoter, providing a possible mechanism to explain why a homogeneous population of transit amplifying cells is observed directly above the more diverse stem niche.

I. Introduction

Intestinal crypts are small invaginations at the base of each intestinal villus, composed of clonal populations [2] of approximately 2000 cells [3], and are the primary site of stem cell proliferation in the intestinal epithelium [4]–[6]. At the base of the cylindrical crypts, stem cells rapidly divide to replace damaged cells while lysozyme-secreting Paneth cells defend

the stem cells from bacterial attack and provide signaling cues to maintain the crypt's "stemness." When stem cells are pushed out of the crypt base, they permanently differentiate into goblet cells and absorptive enterocytes [4]. Excluding Paneth cells, almost all cells in the crypt are replaced every 2 – 6 days [7]. While the intestinal stem cells are some of the fastest dividing cells in the body, aberrant growth and tumorigenesis are relatively rare in the intestine due to the robust regulatory networks that control crypt growth and spatial organization (Fig 1) [8].

The spatial organization of the crypt is primarily controlled by the evolutionarily-conserved Notch/Delta [9] and Wnt/ β -catenin signaling [10] pathways. At the base of the intestinal crypts, Notch/Delta juxtacrine signaling creates a characteristic checkerboard pattern of stem cells and Paneth cells that minimizes the stem-stem and Paneth-Paneth contact through a positive feedback, lateral inhibition mechanism (Figures 1, 2a). [6]. qPCR and RNA-seq experiments on the stem cell and Paneth cell populations have demonstrated that stem cells express Notch Receptors 1 and 2 along with Frizzled Receptor 7 and that Paneth cells express Delta-like Ligand 1, Delta-like Ligand 4, Jagged 1, and Atoh 1 [1], [6]. (Notch Receptors 1 and 2 will be referred to as Notch/N, and DLL1, DLL4, JAG1, and ATOH1 will be referred to as Delta/D).

When a Notch receptor is activated by the Delta of a neighboring cell, its intracellular domain (NICD/F) acts as a transcription factor (TF) that autocatalyzes the production of Notch receptors while also upregulating Hes1 (H), which inhibits production of its Delta-like ligands that it presents to its neighboring cells leading to pattern formation. Wnts (W) on the other hand tend to have a stabilizing effect on cell populations. When a stem cell receives Wnt 2b from the mesenchyme or Wnt 3 and Wnt 11 from its neighboring Paneth cells, the destruction of the TF β -catenin (B) by the core destruction complex is inhibited leading to increased stemness and growth rate [6]. While the Wnt/ β -catenin pathway has been studied extensively, numerous questions exist about why Paneth cells produce Wnt since the stem cells can survive Paneth cell ablation using mesenchymal Wnt [5], [6], [11], why Paneth cells secrete Wnt in a pseudo-juxtacrine manner to only their neighboring stem cells [12], and why β -catenin forms a complex (I) with NICD to upregulate the expression of Hes1 and other Notch signaling pathway molecules [9], [13]–[17].

We construct three distinct models of Wnt production and secretion (mesenchymal/constant, paracrine/diffusing, and juxtacrine) and implement them in two-cell and multi-cell simulations to investigate the gene circuit's robustness and pattern forming capabilities. Specifically, we attempt to elucidate the design principles underlying the redundancy of mesenchymal and Paneth cells as Wnt sources and the distributed control of Hes1 production by the β -catenin/NICD complex and β -catenin alone.

II. Methods

A. Model Formulation

Extensive modelling has been performed for both the Wnt/ β -catenin and Notch/Delta pathways in isolation. Most significantly, Lee et al created the first Wnt modelling framework [18], Mirams et al reduced Lee's model to a single ODE approximating the

asymptotic dynamics of β -catenin [19], Chen et al first described the combined Positive Feedback and Lateral Inhibition mechanisms of Notch signaling [1], and Kay et al compiled the first model depicting Notch/Wnt crosstalk controlling Hes1 production along with the most complete list of parameter estimates for both pathways [13]. The following proposed model combines elements of these previous model while also reducing the dimensionality of the system and allowing us to investigate the effects of different Wnt sources for the first time.

$$N'_i = \theta_1 \left(r_N + (1 - r_N) \frac{(I_i^*)^3}{K_1^3 + (I_i^*)^3} \right) - (\mu_N + \alpha_F \langle D_j \rangle) N_i \quad (1)$$

$$H'_i = \frac{\sigma_{DSH} \sigma_H^3 \zeta_H}{(\sigma_{DSH} + \langle W \rangle)(\sigma_H^3 + H_i^3)} \left(\theta_4 \frac{(I_i^*)^3}{K_4^3 + (I_i^*)^3} + \theta_5 \frac{B_i^3}{K_5 + B_i^3} \right) - \mu_H H_i \quad (2)$$

$$D'_i = \theta_7 \left(r_D + (1 - r_D) \frac{\theta_6^3 K_6^6}{\theta_6^3 K_6^6 + K_7^3 (K_6 + H_i)^3} \right) - (\mu_D + \alpha_F \langle N_j \rangle) D_i \quad (3)$$

$$B'_i = \alpha_B - \left(\frac{\theta_9 (\sigma_B + \langle W \rangle)}{(K_{10} + \alpha_w \langle W \rangle)(K_9 + B_i)} + \theta_{10} \right) B_i - \alpha_I B_i F_i^* \quad (4)$$

$$F'_i = \frac{\theta_2 N_i \langle D_j \rangle}{K_2 + B_i} \quad (5)$$

$$I_i^* = \frac{\theta_3 B_i N_i \langle D_j \rangle}{K_2 + B_i} \quad (6)$$

$$\theta_4 + \theta_5 = 1 \quad (7)$$

All variables refer to the same chemical species as in the introduction, asterisks denote the use of the pseudo-steady state assumption, angle brackets denote an average over adjacent cells, and r_w, r_N, r_D, θ_4 are defined on $[0, 1]$. The r variables indicate the relative amount of gene expression due to noise or an actual feedback. In contrast, θ_4 and θ_5 control the relative amount of control β -catenin and the complex I have over the production of Hes1, allowing us to determine the effects of the Wnt and Notch submodules on stem cell differentiation without affecting the overall rate of Hes1 production. Last, unlike previous models, we consider the transient production of Wnt by Paneth cells. Since the epigenetic factors that drive Paneth cell Wnt production are poorly understood, it is assumed that complex I is a transcriptional repressor for Wnt production. The models for constant, paracrine, and juxtacrine Wnt secretion are presented below.

$$\text{Constant: } W' = 0 \quad (8)$$

$$\text{Paracrine: } W' = \theta_8 \left(r_W + (1 - r_W) \sum_{i=1}^N \frac{K_8^3}{K_8^3 + (I_i^*)^3} \right) - \mu_W W \quad (9)$$

$$\text{Juxtacrine: } W'_i = \theta_8 \left(r_W + (1 - r_W) \frac{K_8^3}{K_8^3 + (I_i^*)^3} \right) - \mu_W W \quad (10)$$

For the constant and paracrine models, all the cells experience the same Wnt concentration W . In contrast, the cells in the juxtacrine model experience the average of adjacent cells Wnt concentrations W_i . Default parameter values are given in Table 1 in the Appendix.

B. Two-Cell Simulation

The equations above were implemented in Matlab using the ode45 function. Parameters r_W , r_N , θ_4 , θ_5 , θ_8 and the initial condition for Wnt (W_0) were varied while all others were left at their default values. The initial conditions were selected so that the stem cell would have 2% higher concentrations of Notch and Hes1 while the Paneth cell would have 2% higher concentration of Delta. (Without the perturbation, the system fails to exhibit bistability.) Due to the high dimensionality of the system, a summary variable called Polarization (P) was introduced.

$$P = \frac{N_1 H_1 D_2}{N_2 H_2 D_1} \quad (11)$$

Polarization will be close to one if there is no bifurcation of cell fates. For contour maps, the polarizations are inverted to bound the function from [0, 1] rather than [1, ∞).

C. Multi-Cell Simulation

To further explore the pattern forming capabilities of the circuit, a circular grid (typically with 30 cells) approximating the crypt base was constructed using a Voronoi tessellation [20], [21] and used to create an adjacency matrix. Iterating over the nodes of the graph at each time step, the average concentrations of Wnt, Delta, and Notch at adjacent nodes were calculated and used to calculate the derivatives. The equations were integrated with ode15s (a variable time step, stiff integrator) with two nodes selected at random to have the Paneth cell initial condition while all others were given the stem condition. As with the two-cell simulations, we introduce a summary variable called stemness.

$$S_i = \ln \left(\frac{N_i H_i}{D_i} \right) - \ln \left(\left\langle \frac{N H}{D} \right\rangle \right)_{All} \quad (12)$$

III. Results

A. Dynamics of the Two-Cell System

For the given parameter choices, all three models indicate that a cell fate decision occurs as shown in Figure 2b. While not shown, if the cells' initial conditions are reversed, the cells switch fates but achieve the same steady states. In short, the system is bistable, and if implemented stochastically, the model may demonstrate switching behavior. Parameters were chosen such that all three models gave the same final Wnt concentration of 1, but it is interesting to note that the constant and paracrine models converged to steady state in 7 hours versus 9 hours for the juxtacrine model. Additionally, the polarizations of the constant and paracrine models were almost 5 times higher than the polarization of the juxtacrine model. This suggests that juxtacrine secretion could allow for greater plasticity after tissue damage. While not shown, the juxtacrine and paracrine models are extremely sensitive to Wnt's initial condition, both taking an additional 6 hours to converge to steady state if the initial concentration is reduced from 0.5 to 0. Last, by including the autoinhibiting Hes1 module to inhibit Delta instead of assuming direct inhibition by NICD, short term oscillations (~4 hrs) are observed.

B. Two-Cell Bifurcation Analysis

The bifurcation analysis for the ratio of Notch, Delta, and Hes1 between the cells along with the polarization for the constant model is presented in Figure 3. At Wnt levels from 0 – 3 with $\theta_4 > 60\%$ (upper left corners of Figure 3a–d), the cells always achieve distinct fates. Oddly, the model predicts an inversion of the Notch levels of the cells for Wnt levels between 3 – 4 that would be equivalent to a high Notch/high Delta state that is not experimentally observed. However, at Wnt levels above 4 or $\theta_4 < 50\%$, the cells fail to make a cell fate decision. Likewise, the other models exhibited similar bifurcation behavior.

From a biological standpoint, it is thought that a Wnt gradient exists with a maximum at the base of the crypt ($W \sim 2$) and a minimum at the top of the crypt ($W \sim 0$). While the crypt base has an almost 1:1 ratio of stem to Paneth cells, epithelial cells significantly outnumber Goblet and other secretory cells in the crypt walls where the Wnt levels are lower. This is consistent with the system having a θ_4 in the range of [0.6, 0.7] since it would transition from bistability to monostability as Wnt decreased. Additionally, colorectal tumors require elevated levels of Wnt 3 to grow which is consistent with the bifurcation diagram indicating a transition to monostability as Wnt increases above homeostatic levels [22].

C. Dynamics of the Multi-Cell System

Last, the model was implemented on a spatial grid as shown in Figure 4a and generated the time courses in Figure 4b. As predicted, the model generated the characteristic checkerboard pattern of the crypt base. Additionally, the same pattern was obtained for $n = 20, 30, 50$, and 100 cells suggesting that the pattern is scale invariant.

Most notably, the constant and paracrine models converge to almost identical solutions within 20 hours. In the juxtacrine model, the total process takes close to 80 hours because the polarization process travels in a wave like manner across the grid. However, the time for

a cell to commit to the Paneth lineage is still approximately 10 hrs, and these events appear to occur consecutively. In a more realistic scenario, only a subpopulation of the cells would be differentiating so this difference in time to steady state would be more abrogated as in the two-cell model. Last, the results of the juxtacrine model show a reduced range of stemness but appear to have more tightly defined stem and Paneth states (reduced variance in the stemness of the Stem and Paneth populations).

IV. Discussion

In conclusion, the distributed control of Hes1 production and redundant sources of Wnt play critical roles in intestinal crypt organization. If Hes1 production was entirely controlled by Notch/Delta signaling, the models predict that the lateral inhibition effect could potentially be too strong leading to secretory cell hyperplasia further up the crypt. In contrast, all three models, regardless of mode of Wnt secretion, predict a bifurcation behavior that when coupled with the experimentally observed Wnt gradient in the crypt would naturally give rise to differentiation at the crypt base and more homogeneous cell fates along the crypt walls. Assuming a constant level of Wnt production by the mesenchyme, the short range diffusion of the Paneth cells may both provide the extra boost required to reach the bifurcation region while also providing tight control over the size of the stem cell niche.

Future work should focus on two features of the model. Crypt recovery after Paneth cell ablation and the crypt's ability to control and plasticity in response to noise.

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V. Appendix

Table 1

Default parameter values for the proposed model.

Var	Value	Var	Value	Var	Value	Parameter	Value
μ_N	0.017 nM/s ¹	K_5	10 nM	θ_4	Variable	r_D	0
μ_H	0.065 nM/s	K_6	1.21 nM	θ_5	1- θ_4	α_I	6.8 (nM · s) ⁻¹
μ_D	0.0098 nM/s ^{1\ \}	K_7	14.7 nM	θ_6	25.714 nM/s	α_F	0.0272 (nM · s) ⁻¹
μ_F	0.00385 nM/s	K_8	1 nM	θ_7	0.1 nM/s	α_B	2.5 nM/s ¹
μ_I	0.04 nM/s	K_9	1200 nM	θ_8	Variable	α_w	1.075
μ_W	1 nM/s	K_{10}	0.0165 nM	θ_9	33.6 nM/s	σ_{dsh}	1.0 nM
K_1	0.4 nM	θ_1	0.1 nM/s	θ_{10}	2.57E-4 1/s	σ_H	3.5 nM
K_2	5.66E-4 nM	θ_2	0.040 1/s	r_N	Variable	σ_B	0.1 nM
K_4	0.9 nM	θ_3	0.680 (nM · s) ⁻¹	r_W	Variable	ζ_H	0.5

REFERENCES

- [1]. Chen K-Y et al., "A Notch positive feedback in the intestinal stem cell niche is essential for stem cell self-renewal," *Mol. Syst. Biol.*, vol. 13, no. 4, p. 927, 4 2017. [PubMed: 28455349]
- [2]. Snippert HJ et al., "Intestinal Crypt Homeostasis Results from Neutral Competition between Symmetrically Dividing Lgr5 Stem Cells," *Cell*, vol. 143, no. 1, pp. 134–144, 10 2010. [PubMed: 20887898]
- [3]. Nicolas P, Kim K-M, Shibata D, and Tavaré S, "The Stem Cell Population of the Human Colon Crypt: Analysis via Methylation Patterns," *PLOS Comput. Biol.*, vol. 3, no. 3, p. e28, 3 2007. [PubMed: 17335343]
- [4]. Barker N, "Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration," *Nat. Rev. Mol. Cell Biol.*, vol. 15, no. 1, pp. 19–33, 1 2014. [PubMed: 24326621]
- [5]. Sato T et al., "Single Lgr5 stem cells build crypt–villus structures in vitro without a mesenchymal niche," *Nature*, vol. 459, no. 7244, pp. 262–265, 5 2009. [PubMed: 19329995]
- [6]. Sato T et al., "Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts," *Nature*, vol. 469, no. 7330, pp. 415–418, 1 2011. [PubMed: 21113151]
- [7]. Williams JM, Duckworth CA, Burkitt MD, Watson AJM, Campbell BJ, and Pritchard DM, "Epithelial Cell Shedding and Barrier Function: A Matter of Life and Death at the Small Intestinal Villus Tip," *Vet. Pathol.*, vol. 52, no. 3, pp. 445–455, 5 2015. [PubMed: 25428410]
- [8]. Cho K-H, Baek S, and Sung M-H, "Wnt pathway mutations selected by optimal β -catenin signaling for tumorigenesis," *FEBS Lett.*, vol. 580, no. 15, pp. 3665–3670, 6 2006. [PubMed: 16764864]
- [9]. Katoh M and Katoh M, "Notch ligand, JAG1, is evolutionarily conserved target of canonical WNT signaling pathway in progenitor cells," *Int. J. Mol. Med.*, vol. 17, no. 4, pp. 681–685, 4 2006. [PubMed: 16525728]
- [10]. Valenta T et al., "Wnt Ligands Secreted by Subepithelial Mesenchymal Cells Are Essential for the Survival of Intestinal Stem Cells and Gut Homeostasis," *Cell Rep.*, vol. 15, no. 5, pp. 911–918, 5 2016. [PubMed: 27117411]
- [11]. Durand A et al., "Functional intestinal stem cells after Paneth cell ablation induced by the loss of transcription factor Math1 (Atoh1)," *Proc. Natl. Acad. Sci.*, vol. 109, no. 23, pp. 8965–8970, 6 2012. [PubMed: 22586121]
- [12]. Farin HF et al., "Visualization of a short-range Wnt gradient in the intestinal stem-cell niche," *Nature*, vol. 530, no. 7590, pp. 340–343, 2 2016. [PubMed: 26863187]
- [13]. Kay SK et al., "The role of the Hes1 crosstalk hub in Notch-Wnt interactions of the intestinal crypt," *PLOS Comput. Biol.*, vol. 13, no. 2, p. e1005400, 2 2017. [PubMed: 28245235]

- [14]. Hayward P et al., "Notch modulates Wnt signalling by associating with Armadillo/ β -catenin and regulating its transcriptional activity," *Dev. Camb. Engl*, vol. 132, no. 8, pp. 1819–1830, 4 2005.
- [15]. Li B et al., "Interaction of Wnt/ β -catenin and notch signaling in the early stage of cardiac differentiation of P19CL6 cells," *J. Cell. Biochem*, vol. 113, no. 2, pp. 629–639, 2 2012. [PubMed: 21956839]
- [16]. Borggrefe T, Lauth M, Zwijsen A, Huylebroeck D, Oswald F, and Giaimo BD, "The Notch intracellular domain integrates signals from Wnt, Hedgehog, TGF β /BMP and hypoxia pathways," *Biochim. Biophys. Acta*, vol. 1863, no. 2, pp. 303–313, 2 2016. [PubMed: 26592459]
- [17]. Muñoz-Descalzo S, de Navascues J, and Arias AM, "Wnt-Notch signalling: An integrated mechanism regulating transitions between cell states," *BioEssays*, vol. 34, no. 2, pp. 110–118, 2 2012. [PubMed: 22215536]
- [18]. Lee E, Salic A, Krüger R, Heinrich R, and Kirschner MW, "The Roles of APC and Axin Derived from Experimental and Theoretical Analysis of the Wnt Pathway," *PLOS Biol*, vol. 1, no. 1, p. e10, 10 2003. [PubMed: 14551908]
- [19]. Mirams GR, Byrne HM, and King JR, "A multiple timescale analysis of a mathematical model of the Wnt/ β -catenin signalling pathway," *J. Math. Biol*, vol. 60, no. 1, pp. 131–160, 1 2010. [PubMed: 19288106]
- [20]. "CVT_CIRCLE_UNIFORM - Uniform Centroidal Voronoi Tessellation on Circle" [Online]. Available: https://people.sc.fsu.edu/~jburkardt/m_src/cvt_circle_uniform/cvt_circle_uniform.html. [Accessed: 10-Dec-2017].
- [21]. Du Q, Faber V, and Gunzburger M, "Centroidal Voronoi Tessellations: Applications and Algorithms," *SIAM Rev*, vol. 41, no. 4, pp. 637–676, 1 1999.
- [22]. Voloshanenko O et al., "Wnt secretion is required to maintain high levels of Wnt activity in colon cancer cells," *Nat. Commun*, vol. 4, 10 2013.

Figure 1.

Stem cells and Paneth cells form checkerboard patterns in the intestinal crypts. A) Transverse cross-sections of intestinal crypts. stem cells are green (LGR5-EGFP), and Paneth cells are red (RFP-anti-Lysozyme). B) Intestinal crypt cross-section schematic. C) A longitudinal cross-section of a crypt-villus structure (villus not to scale) depicting the various cell types.

Figure 2.

a) Gene circuit diagrams for three modes of Wnt secretion. Arrows denote activation, and dots denote inhibition. Red and blue represent distinct cells. i) Cells experience constant Wnt (C). ii) Both cells experience total Wnt (P). iii) Cell experience neighbor's Wnt (J) b) Concentration profiles of all species (nM) as a function of time (hrs, log) along with polarization. Bifurcation of cell fates is observed for all species except β -catenin for $r_W = r_N = 0.1$, $r_D = 0$, $\theta_4 = 0.75$, $W = 1$ (C), $\theta_8 = 0.7$ nM/min (P), $\theta_8 = 1.2$ nM/min (J)

Figure 3.

Bifurcation analysis of the constant/mesenchymal Wnt model where the Wnt concentration (W) and the relative control of Hes1 (H) production by NICD/ β -catenin (I) compared to β -catenin alone (B). Ideally, all the colormaps should be bounded from [0, 1] with numbers under 0.4 indicating bifurcation since the ratios are inverted.

Figure 4.

a) Spatial simulation of the model (nodes colored by stemness) where green indicates stem cells and red indicates Paneth cells. All three models generate a checkerboard, but juxtacrine produces fewer Paneth cells. b) Time courses for the same simulation. $r_w = r_N = 0.1$, $\theta_4 = 0.75$, $W = 2$ (C), $W_0 = 0$ (P/J), $\theta_8 = 3$ (J), and $\theta_8 = 0.1$. Parameters tuned to give final average Wnt concentration of 2.