# A gradient modulated intercellular feedback controlling pattern formation inside intestinal crypts

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***Short Abstract* — Intestinal crypts regulate homeostasis by creating a stem cell niche at the base, where stem cells and niche cells form a soccer-ball-like pattern. Divided cells migrate out of the niche and differentiate into a different pattern at the top. However the mechanisms behind crypt pattern formation remain unclear. Here we show that a paracrine gradient can modulate a Notch-dependent intercellular feedback loop to create diverse patterns at different crypt locations. Furthermore, mutual inactivation between Notch ligands and receptors creates an ultra-sensitive juxtacrine switch for robust and speedy pattern formation during the rapid regeneration of the crypt epithelium.**

***Keywords* — pattern formation, intestinal crypt, stem cells, Notch-dependent lateral inhibition.**

## I. Purpose

The small intestine and colon are lined with a single layer of epithelium cells. The epithelium is full of crypts, which are invaginations into the underlying connective tissue. The intestinal epithelium is replaced every 3-5 days, making it the fastest regenerative tissue in the body. To maintain homeostasis, stem cells are tightly controlled by a niche at the bottom of the crypt. In side the niche, 12~14 Lgr5+ stem cells form soccer-ball-like pattern with CD24+ Paneth (niche) cells [1]. Divided cells leave the niche and migrate up while differentiating into absorptive (enterocyte) and secretory (Goblet) lineages, eventually forming more random cell fate patterns at the top. However, it remains unclear how local cell interaction mechanisms give rise to the diverse patterns at different crypt locations.

## II. Materials and Methods

Steady-state and dynamic analyses were performed on an ODE model to analyze the robustness and speed of pattern generation. The stability of patterns is evaluated by the Lyapunov exponent. Intestinal cells were cultured in 3D organoid culture seeded with Lgr5+ stem cells. mRNA levels were measured by RT-qPCR and protein levels were measured by western blots. Immunofluorescence was performed on cryosectioned crypts harvested from mice.

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## III. Results

Notch signaling depends on ligands on a cell activating receptors on a neighboring cell. Stem cells and enterocytes express high levels of Notch receptors while Paneth and Goblet cells express high levels of Notch ligands, suggesting that Notch plays a role in pattern formation [2].

We first built an intercellular Notch signaling model based on transcriptional feedback. Even though the model can generate individual patterns, it is not capable of replicating the spatially varying patterns inside the crypt.

We then investigated an alternative mechanism, in which activated Notch receptors form a positive feedback by upregulating their own expression while receptors and ligands mutually inactivate each other in the same cell [3]. Coupled with a paracrine Wnt gradient, this model faithfully replicated the spatially varying crypt patterns.

We experimentally tested whether such a mechanism exists in intestinal cells. RT-pPCR and wester blots confirmed the expected upregulation of Notch receptors and downregulation of Notch ligands after addition of recombinant Notch ligands to 3D intestinal organoid culture. Immunohistochemistry on cryosectioned mouse intestinal crypts further confirmed the model predictions.

Last, we asked the question why evolution has favored this alternative mechanism over transcriptional feedback. Computational analysis revealed that this alternative mechanism generates more robust patterns in a more speedy fashion, which is important for maintaining homeostasis during the rapid turnover of the intestinal epithelium.

## IV. Conclusion

Our mathematical model demonstrates that, coupled with a paracrine signaling gradient, an intercellular circuit involving positive feedback and mutual inactivation between Notch ligands and receptors can generate the different patterns in a robust fashion. The computational predictions are supported by *in vitro* and *ex vivo* experimental evidence.

## References

[1] Snippert HJ et al. (2010) Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* **143**, 134-44.

[2] Crosnier C et . (2006) Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. *Nat Rev Genet* **7**, 349-59

[3] Sprinzak D, et al. (2011) Mutual inactivation of Notch receptors and ligands facilitates developmental patterning. *PLoS Comput Biol* **7**