# A Notch positive feedback controlling intestinal stem cell niche patterning

Kai-Yuan Chen[1], Tara Srinivasan[2], Kuei-Ling Tung[1], Pengcheng Bu[1], and Xiling Shen[1]

***Short Abstract* — The intestinal epithelium is the fastest regenerative tissue in the body, fueled by rapid-cycling stem cells. How the underlying regulatory scheme manages this dynamic stem cell niche is not entirely clear. We discovered that intestinal stem cells employ a positive feedback mechanism via direct Notch binding to the 2nd intron of the Notch1 gene. Inactivation of the positive feedback by CRISPR/Cas9 mutation alters the mosaic stem cell niche pattern and hinders regeneration in organoids. Dynamical system analysis and agent-based multiscale stochastic modeling suggest that the positive feedback enhances the robustness of niche patterning. This study highlights the feedback mechanisms in spatiotemporal control of the dynamic stem cell niche**.

***Keywords* — Intestinal Stem Cell, Gene Editing, Notch Signaling, Positive Feedback, Systems Biology, Organoid.**

## I. Introduction

STEM cell niche provides a spatial environment that regulates stem cell self-renewal and differentiation[1]. One example is at the base of the intestinal crypt, where self-renewing LGR5+ intestine stem cells (ISC) and lysozyme-secreting Paneth cells interdigitate to form a mosaic pattern [2, 3].

Regulation of the niche is a concerted effort involving various signaling pathways. Among the pathways, Notch signaling pathway is often linked to developmental patterning [4, 5]. Notch signaling is mediated through direct cell-to-cell contact of membrane-bound Notch ligands on one cell and trans-membrane Notch receptors on adjacent cells, which causes cleavage and release of Notch Intercellular Domain (NICD) that further activate downstream Notch signaling.

In this study, we characterized the response of Notch signaling components in LGR5+ ISCs from intestinal organoids and identified a direct Notch positive feedback (PF) loop. Perturbation to the positive feedback by CRISPR/Cas9 mutation of the binding sequence significantly reduced the number of ISCs in the stem cell niche. Computational modeling suggests that the positive feedback may contribute to robustness of the system when proliferation rates are high.

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[1]Department of Biomedical Engineering, Duke University, USA.
E-mail: kai.yuan.chen@duke.edu, xiling.shen@duke.edu, kt173@duke.edu, bupc@ibp.ac.cn
[2]Department of Biomedical Engineering, Cornell University, USA
E-mail: ts594@cornell.edu

## II. Results

### A. NICD directly activates Notch1 Transcription

Notch signaling activation by recombinant JAG1 ligands and EDTA in intestinal organoid cells showed Notch1 upregulation, while Notch signaling inhibition by DAPT showed Notch1 downregulation. ChIP-Seq data (H3K4me1 and H3K27ac) and motif analysis of mouse small intestinal cells identified a novel NICD binding site on a highly active enhancer region on the 2nd intron of Notch1, showing a direct Notch feedback loop that has not been reported in other systems.

### B. PF enhances robustness of niche dynamics in silico

In the single-, pair-, and multi-cell mathematical models, dynamical system analysis shows that Notch1 positive feedback enhance robust bi-stability of Notch signaling, and therefore helps patterning in stem cell niche. A multi-scale, agent-based stochastic crypt model was constructed, and the simulation further supports that Notch1 positive feedback can maintain stable mosaic ISC niche pattern in fast-regenerative intestine crypts.

### C. PF is essential to niche patterning in organoids

CRISPR/Cas9 editing system was applied to mutate Notch1 binding site in intestine organoid cells. The mutated binding site significantly altered the stem cell niche pattern. The forming efficiency and growth in CIRSPR/Cas9 mutated mouse and human organoids were significantly hindered consequently, impairing intestinal capability for regeneration.

## III. Conclusion

The novel Notch1 positive feedback is essential to the intestinal stem cell niche patterning by enhancing the stability of spatiotemporal signaling pattern and cell-cell communication.

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