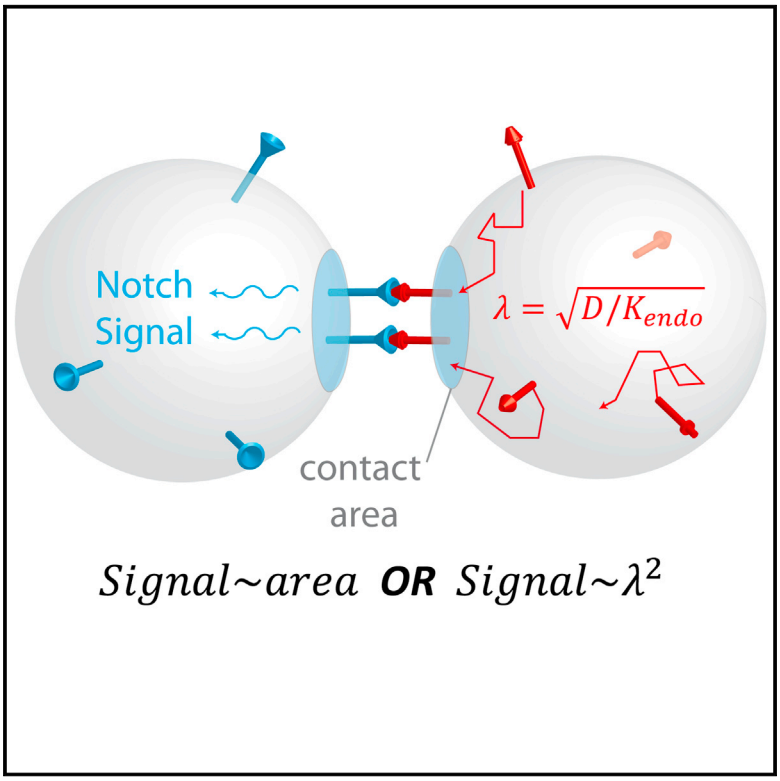
Report

Cell Reports

Quantitative Analysis of Delta-like 1 Membrane Dynamics Elucidates the Role of Contact Geometry on Notch Signaling

Graphical Abstract



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In Brief

Khait et al. show large cell-to-cell variability in the diffusion coefficients of the Notch ligand Delta-like 1. A combination of quantitative FRAP-TIRF imaging and mathematical modeling is used to examine the implications of this result on Notch signaling and its dependence on cell-cell contact geometry.

Highlights

- Diffusion coefficients of Dll1 exhibit large cell-to-cell variability
- A model shows how membrane dynamics and contact area affect Notch signaling
- For small contact areas, signal depends on diffusion-length scale of Dll1
- Observed variability can lead to different behaviors in different cellular contexts

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Quantitative Analysis of Delta-like 1 Membrane Dynamics Elucidates the Role of Contact Geometry on Notch Signaling

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SUMMARY

Notch signaling is ubiquitously used to coordinate differentiation between adjacent cells across metazoans. Whereas Notch pathway components have been studied extensively, the effect of membrane distribution and dynamics of Notch receptors and ligands remains poorly understood. It is also unclear how cellular morphology affects these distributions and, ultimately, the signaling between cells. Here, we combine live-cell imaging and mathematical modeling to address these questions. We use a FRAP-TIRF assay to measure the diffusion and endocytosis rates of Delta-like 1 (Dll1) in mammalian cells. We find large cell-to-cell variability in the diffusion coefficients of Dll1 measured in single cells within the same population. Using a simple reaction-diffusion model, we show how membrane dynamics and cell morphology affect cell-cell signaling. We find that differences in the diffusion coefficients, as observed experimentally, can dramatically affect signaling between cells. Together, these results elucidate how membrane dynamics and cellular geometry can affect cell-cell signaling.

INTRODUCTION

Notch signaling is a highly conserved juxtacrine-signaling pathway, which is repeatedly used for the coordination of differentiation between neighboring cells in metazoans (Artavanis-Tsakonas and Muskavitch, 2010; Artavanis-Tsakonas et al., 1999). Interaction between Notch receptors in one cell, and Notch ligands on a neighboring cell, results in two consecutive proteolytic cleavage events of the Notch receptor (Bray, 2006; Gordon et al., 2008) and the release of its intracellular domain, which then translocates to the nucleus and serves as a co-transcription factor (Nam et al., 2006).

Notch ligand endocytosis is known to be essential for Notch signaling, although its exact role is still controversial (Heuss et al., 2008; Koo et al., 2005, 2007; Le Borgne, 2006; Weinmaster and Fischer, 2011). It has been suggested that endocytosis and recycling processes are involved in priming of the Notch ligands (Le Borgne, 2006) as well as in exerting a pulling force on the Notch receptors in adjacent cells, required for their activation (Meloty-Kapella et al., 2012; Nichols et al., 2007).

In addition to recycling processes, the membrane distribution of Notch receptors and ligands can be affected by lateral diffusion. Although the role of membrane diffusion has been studied extensively in other signaling pathways (Chung et al., 2010; Jaskolski and Henley, 2009; Niv et al., 1999; Wang et al., 2008), its role in Notch signaling is still largely unknown (Narui and Salaita, 2013).

The geometry of the contact between cells can also be an important factor affecting Notch signaling. Interestingly, Notch signaling is found to operate in different tissues with very different cell-cell contact morphologies. For example, Notch signaling can be found in adherens junctions where the boundary between cells can extend over several microns (Couturier et al., 2012). In contrast, it has been suggested recently that Notch signaling can be transduced through very thin filopodial contacts between non-adjacent cells (on the order of 0.1–0.2 μm in diameter; Cohen et al., 2010). Such signaling events were shown to be important for proper patterning in bristle spacing in *Drosophila* (Cohen et al., 2010) and in pigment patterning in the zebrafish (Hamada et al., 2014).

How do the lateral diffusion and recycling processes affect the distribution and dynamics of Notch receptors and ligands on the contact between cells? How does the geometry of the contact affect these distributions and ultimately the signaling between cells? Here, we address these questions using a combination of quantitative experiments and mathematical modeling. We performed fluorescence recovery after photobleaching (FRAP) coupled to total internal reflection fluorescence (TIRF) microscopy to measure the diffusion and endocytosis rates of the Notch ligand Delta-like 1 (Dll1) in a cell culture assay. We developed a mathematical modeling approach that, together with our experimental results, provides a framework for understanding how the interplay among lateral diffusion, membrane recycling, and geometry of cell contact affects Notch signaling.