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Notch signaling: Where is the action?

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Summary

It has been a long-standing question whether the activation of Notch by its ligands occurs in a specific region of the plasma membrane. A study now shows that this is indeed the case in the *Drosophila* sensory organ precursor cell lineage.

Main text

Cell-to-cell signaling is a fundamental process in multicellular organisms frequently linked to cell fate decision-making. Many aspects of signaling have been studied in great detail, but what remains unclear in most cases is where in cells signal transduction takes place. Notch signaling is one of the main pathways to control cell fate decisions during animal development. It occurs locally and is triggered by binding of a transmembrane ligand of the Delta/Serrate/Jagged (DSL) family to the transmembrane receptor Notch on the surface of

an adjacent cell. Ligand binding followed by endocytosis of the ligand is thought to exert mechanical stress on the Notch receptor, leading to a conformational change that exposes an extracellular cleavage site for the protease ADAM10/Kuzbanian. The proteolytic fragment of Notch produced after cleavage consists of the transmembrane domain and the intracellular domain and is subject to a further cleavage by a protease of the γ-secretase family, leading to the release of the Notch intracellular domain (NICD) and its translocation to the nucleus. In the nucleus, NICD binds to CBF1/Suppressor of Hairless/LAG1 (CSL) transcription factors and stimulates transcription of target genes [1, 2]. Although the Notch signaling pathway is rather short and appears simple at first glance, many questions arise when it comes to the issue of how specificity of signaling is achieved. In many tissues, both Notch and its ligands are broadly expressed in numerous cells, many of which are in direct contact with each other, yet Notch activation occurs only in a small subset of cells. In several contexts Notch directly regulates different cell fates of the two daughter cells upon division. How is Notch signaling restricted to the sibling cells? A study by Trylinski and colleagues published in this issue of Current Biology [3] uses the sensory organ precursor (SOP) cell lineage to address this question and now demonstrates that the major source of nuclear NICD in the pIIa cell is a pool of Notch localized at the newly formed cell contact basal to the midbody at cytokinesis (Figure 1).

The body surface of adult *Drosophila* flies is covered with mechanosensory bristles that are generated at the pupal stage in a series of asymmetric cell divisions. The first division of the SOP generates two different daughter cells: the anterior pIIb cell giving rise to the neuron and the sheath cell of the sensory organ, and the posterior pIIa cell giving rise to the shaft and socket cells of the bristle. The different cell fates of pIIb and pIIa are controlled by Notch signaling [4]. Notch is activated in the pIIa cell where NICD enters the nucleus [5] and is

suppressed in pIIb by the asymmetric inheritance of Numb, a negative regulator of Notch signaling [6]. Trylinski and colleagues asked the simple question whether they could trace back the origin of the pool of NICD appearing in the pIIa nucleus to where in the cell it was produced.

To that aim they generated several transgenic fly lines expressing fluorescently tagged versions of Notch, its transmembrane ligand Delta and Neuralized, a ubiquitin ligase required for endocytosis of Delta [7]. All fluorescent fusion proteins were expressed under control of the respective endogenous promoters, ensuring their expression at physiological levels. The analysis of the subcellular localization of the proteins in living and fixed samples at the pIIb-pIIa interface revealed that at cytokinesis Notch, Delta and Neuralized accumulated both at the newly formed contact site basal to the midbody and in a second region apical to the midbody that harbors the adherens junctions. Intriguingly, only the levels of the basal pools of Notch and Delta were affected in animals mutant for *Numb* and *presenilin* (γ-secretase) or upon inhibition of Neuralized, pointing to the predominant relevance of Notch-Delta interaction at the basal interface between pIIb and pIIa for Notch signaling mediated cell fate decision [3, 5].

To test this hypothesis, the authors performed a series of elegant state-of-the-art life imaging experiments. They first used a version of Notch fused to both GFP and Cherry in tandem to check whether nuclear NICD is produced from a pool of Notch that is rapidly turned over at the plasma membrane or from a more slowly turned over pool of Notch passing through late endosomes. The rationale of this experiment is based on the fact that it takes longer for Cherry to mature to a fluorescent state than for GFP and that GFP fluorescence is quenched in late endosomes due to their low pH. Indeed, only GFP-tagged NICD was detectable in the pIIa nucleus, confirming that nuclear NICD is derived from a pool

of Notch that turns over rapidly, most likely at the plasma membrane. In the next approach, Trylinski et al. selectively photobleached GFP-tagged Notch at either the basal or the apical interface between pIIb and pIIa and measured nuclear NICD levels in pIIa after bleaching. Whereas bleaching of the apical pool of Notch-GFP had little effect, bleaching of the basal pool of Notch-GFP led to a strong reduction of nuclear NICD, strongly indicating that the pool of Notch at the membrane abutting its sibling cell basal to the midbody is the source for most of pIIa's nuclear NICD. To finally nail down this hypothesis, a transgenic fly line expressing a photoconvertible version of Notch fused to the mMaple3 fluorescent protein was used. mMaple 3 switches its color from green to red upon illumination with UV light. mMaple3 photoconversion at the apical pIIb-pIIa interface did not lead to an increase of red fluorescent NICD above background values in the pIIa nucleus, whereas photoconversion at the basal pIIb-pIIa interface led to a weak but statistically significant increase of photoconverted nuclear NICD in pIIa. Altogether, these data show for the first time that a sub-pool of Notch located at the plasma membrane between two recently born sibling cells undergoing a cell fate decision is actively engaged in signaling.

The Schweisguth lab previously showed that symmetry of Notch between the pIIb and pIIa cells is broken at cytokinesis when Numb triggers the removal of Notch from pIIb membranes by increased endocytosis [5]. Trylinski et al. now demonstrate that Notch and its ligand Delta localize to the newly formed abutting membranes that separate the two sister cells upon cytokinesis. How this is achieved remains to be determined, but it provides an elegant explanation for how Notch signaling is confined to sister cells.

A different mechanism for Notch signaling between pIIb and pIIa that is also linked to cytokinesis has recently been proposed by Coumailleau et al. [8]. These authors have shown that Notch and Delta travel together in specific endosomes marked by the presence of the

protein Sara [9] that are asymmetrically segregated into pIIa upon cytokinesis. They further propose that Notch is activated in the limiting membrane of Sara endosomes rather than at the plasma membrane. Sara endosomes bind to the central spindle via the plus-end directed kinesin Klp98A, which confers bidirectional motility on the spindle. Key to the asymmetric segregation of Sara endosomes into pIIa is the fact that the central spindle itself is made asymmetric by the activities of the microtubule depolymerizing kinesin Klp10A and its antagonist Patronin [10].

Together these studies reveal that the process of cytokinesis is intimately linked to Notch mediated cell fate decisions in the fly sensory organ lineage. It further becomes clear that Notch activation is highly compartmentalized, whether it occurs at a specific region of the plasma membrane, at the limiting membranes of endosomes or both. The ultimate answer to precisely determine where in cells Notch activation takes place will require the development of tools that allow to measure tension induced conformational changes and/or cleavage of Notch by live imaging.

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Figure 1. Notch signaling is linked to cytokinesis of the SOP cell. The image shows where

Notch signaling occurs at the newly formed cell contact between pIIb and pIIa.