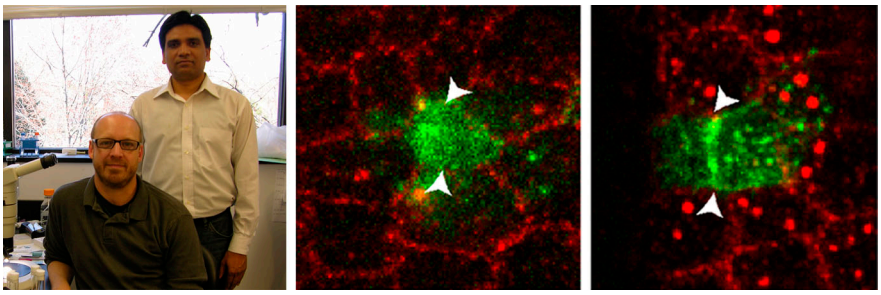
Text by Ben Short
bshort@rockefeller.edu

Sanpodo seals precursors' fate

Study reveals how a membrane protein boosts Notch signaling in some daughter cells while suppressing it in others.

Focal Point



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Fabrice Roegiers (left), Alok Upadhyay (right), and colleagues (not pictured) investigate how a protein called Sanpodo regulates Notch signaling to direct the developmental fate of fly sensory organ precursor cells. The precursors divide asymmetrically to generate a pIIa cell, in which Notch signaling is activated, and a pIIb cell, in which the pathway is inhibited. Upadhyay et al. find that, in pIIa cells, Sanpodo promotes Notch signaling by binding to the γ-secretase complex that cleaves and activates the Notch receptor. In pIIb cells, however, Sanpodo restricts Notch signaling by removing the receptor from the plasma membrane. Compared with SOP cells expressing wild-type Sanpodo (green, center), cells expressing a mutant unable to be endocytosed (green, right) accumulate Notch (red) at the apical cell surface, leading to inappropriate Notch activation.

During *Drosophila* development, sensory organ precursor (SOP) cells undergo a series of asymmetric divisions to generate mechanosensory bristles along the thorax of adult flies. Notch signaling controls the fate of the SOP cells' progeny, ensuring that each bristle contains a single neuron surrounded by hair, socket, and sheath cells. Upadhyay et al. describe how a protein called Sanpodo regulates this process by activating Notch in some cells but inhibiting it in others (1).

SOP cells initially divide asymmetrically to generate an anterior pIIb cell that contains an inhibitor of Notch signaling called Numb and a posterior pIIa cell that lacks Numb and is therefore able to activate the Notch pathway (2). But Numb isn't the only protein that regulates Notch signaling. "My lab is interested in how you flip this Notch switch after mitosis," says Fabrice Roegiers from the Fox Chase Cancer Center in Philadelphia. "We thought that Sanpodo might be the key to this."

Sanpodo is a four-pass transmembrane protein that interacts with both Numb and the Notch receptor (3). Flies lacking Sanpodo fail to activate Notch in pIIa cells, leading to an excess of sensory neurons and a loss of hair and socket cells. On the other hand, Sanpodo might be involved in suppressing Notch in pIIb cells because it is removed from the plasma membrane of these cells in a Numb-dependent manner (4). "We wanted to understand how Sanpodo promotes Notch signaling during asymmetric cell division and what the nature of its interaction with Numb is," Roegiers explains.

> **"These two trafficking motifs function together to limit Notch signaling."**

Roegiers and colleagues, led by postdoc Alok Upadhyay, first looked at Sanpodo's function in promoting Notch activity in pIIa cells (1). After the Notch receptor binds its ligand, it undergoes a series of proteolytic cleavages that allows the Notch intracellular domain (NICD) to translocate to the nucleus and regulate gene transcription. Overexpressing NICD rescues Sanpodo-mutant flies, suggesting that Sanpodo acts at the stage of NICD production. Upadhyay et al. found that Sanpodo binds to Presenilin, the catalytic subunit of the γ-secretase complex that cleaves NICD from the rest of the Notch receptor. A Sanpodo mutant lacking its Presenilin-binding domain was unable to rescue bristle formation in Sanpodo-deficient flies. "That suggests that the interaction with Presenilin is how Sanpodo promotes Notch activation," says Roegiers, who thinks that, because Notch is only present in small amounts at the pIIa cell membrane, Sanpodo might act as an adaptor that helps the γ-secretase complex cleave its substrate.

The researchers then turned their attention to Sanpodo's function in pIIb cells. A Sanpodo mutant lacking the conserved endocytic motif NPAF no longer binds Numb and accumulates at the plasma membrane of pIIb cells. But this mutant has no effect on SOP differentiation (5). Upadhyay et al. noticed, however, that a second endocytic signal, a dileucine motif, was present in Sanpodo's N-terminal cytoplasmic domain. A version of Sanpodo lacking both endocytic signals restored Notch signaling in the SOP cells of Sanpodo-null flies, preventing the formation of excess sensory neurons. "But in a subset of sensory organs we saw no neurons and extra socket cells, which is a hallmark of inappropriate Notch activation," Roegiers explains. "That suggested that these two trafficking motifs function together to limit Notch signaling in the pIIb cell."

By expressing Sanpodo in cells that don't normally produce the protein, Upadhyay et al. found that Sanpodo helps remove Notch from the plasma membrane, as long as one or both of its endocytic motifs are intact. Notch accumulated at the apical membrane of pIIb cells expressing the double Sanpodo mutant, explaining the pathway's inappropriate activation.

Sanpodo therefore inhibits Notch signaling in pIIb cells by removing the receptor from the cell surface, while simultaneously activating the pathway in pIIa cells by linking the receptor to γ-secretase. Roegiers and colleagues now want to investigate how Numb and Sanpodo cooperate to regulate Notch receptor trafficking.

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4. Roegiers, F., et al. 2005. *Mol. Biol. Cell*. 16:3480–3487.
5. Tong, X., et al. 2010. *Mol. Biol. Cell*. 21:802–810.