Text by Ben Short
bshort@rockefeller.edu

Glycosphingolipids keep signaling in top-Notch condition

Membrane lipids promote the endocytosis and signaling capacity of Notch receptor ligands.

Cell surface proteins are absolutely essential to activating the Notch signaling pathway, but the lipids that surround them in the plasma membrane have a significant influence too, say Hamel et al. (1).

Notch receptor ligands (Delta and Serrate in flies) are transmembrane proteins that bind receptors on the surface of neighboring cells. Mysteriously, the ligands must be endocytosed before they can activate their receptors—whether this happens before or after their interaction is unclear. One possibility is that the ligand is internalized after binding, pulling the receptor with it to induce a conformational change that triggers downstream signaling. Alternatively, the ligand may by endocytosed before receptor binding, only to be recycled back to the cell surface in a modified form capable of stimulating the Notch receptor (2).

Either way, Delta and Serrate endocytosis is essential, and is promoted by E3 ubiquitin ligases called Mindbomb and Neuralized, both of which add ubiquitin to the ligands' intracellular tails. Mutations in either of these two genes block Notch signaling (3).

To understand more about Notch ligand endocytosis, Sophie Hamel and François Schweisguth, from the Pasteur Institute in Paris, France, screened for new regulators of the process. They looked for genes that, when overexpressed, restored normal Notch signaling to flies carrying a dominant-negative version of Mindbomb (1). Using this approach, the two researchers found that increased amounts of an enzyme called α1,4-*N*-acetylgalactosaminyltransferase-1 (α4GT1) rescued the block in Delta and Serrate endocytosis caused by the Mindbomb mutant. The enzyme also counteracted defects in the Neuralized ubiquitin ligase.

α4GT1 is a Golgi-localized protein involved in the synthesis of glycosphingolipids (GSLs) that are transported to the cell surface where they may promote the formation of lipid raft membrane domains (4). Overexpressing α4GT1 increased the amounts of a particular GSL called N5 on the surface of *Drosophila* cells. But how would this activity promote Delta and Serrate endocytosis? Hamel and Schweisguth turned to a collaborator—Jacques Fantini from the University of Aix-Marseille, France—who determined that GSLs like N5 bind to a specific domain in the extracellular portions of the Notch ligands. "You might imagine that the ligands interact with membrane patches rich in glycosphingolipids," says Schweisguth, which would cluster the proteins into raft-like domains, facilitating their endocytosis and subsequent signaling activity.

> "The role of glycosphingolipids is only revealed if you sensitize the system."

It remains to be seen whether this is how increased N5 production by α4GT1 promotes Notch ligand internalization. But unlike Mindbomb and Neuralized, α4GT1 isn't an essential part of the process. Although the absence of the enzyme exacerbates the developmental defects of *Drosophila* with a partial Notch receptor deficiency, flies lacking the enzyme alone appear completely normal. So how important is α4GT1 to Notch signaling?

"There are so many layers of regulation that the system can compensate for the loss of one specific component like α4GT1," explains Schweisguth. "The role of glycosphingolipids is only revealed if you sensitize the system by, for example, inhibiting Mindbomb or reducing Notch receptor levels."

According to Schweisguth, the significance of GSLs to Notch signaling is better indicated by the fact they have a similar function in *C. elegans*: hyperactive Notch signaling is suppressed by the removal of enzymes that synthesize GSLs (5). "This evolutionary conservation is a strong argument that glycosphingolipids have an important function in this pathway," says Schweisguth. Adding to this proposal is the fact that mutations in *Jagged1*, the human homologue of Serrate, map to the region predicted to bind GSLs and cause the multi-system disease Alagille syndrome.

Schweisguth and colleagues are now making similar mutations in fly Notch ligands to investigate how the interaction with GSLs promotes their ability to activate Notch receptors. "We also want to know how this is controlled in time and space," Schweisguth adds. "The concentration of glycosphingolipids probably doesn't change during development, but their organization into membrane nanodomains might be regulated genetically."

Focal Point

Sophie Hamel (right) and François Schweisguth (left), together with Jacques Fantini, demonstrate that glycosphingolipids (GSLs) promote Notch signaling by boosting endocytosis of the ligands Delta and Serrate. Ligand endocytosis—a prerequisite for receptor activation—is inhibited by a mutant version of the E3 ubiquitin ligase Mindbomb, resulting in decreased Notch signaling and an abnormal wing shape (left). Endocytosis—and Notch activity—is restored by overexpressing an enzyme that synthesizes the GSL N5 (right). Delta and Serrate bind GSLs directly, suggesting that their endocytosis may be stimulated by clustering into lipid raft–like membrane domains.

1. Hamel, S., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200907116.
2. D'Souza, B., et al. 2008. *Oncogene.* 27:5148–5167.
3. Le Borgne, R., et al. 2005. *Development.* 132:1751–1762.
4. Sillence, D.J. 2007. *Int. Rev. Cytol.* 262:151–189.
5. Katic, I., et al. 2005. *Genetics.* 171:1605–1615.