

Bound for destruction: Ubiquitination protects against improper Notch signaling

December 29, 2004 – The Notch pathway is an important molecular signaling mechanism whose existence has been known, or at least hinted at, for nearly a century since the identification of a mutant strain of *Drosophila* fruit flies with “notched” wings in Thomas Hunt Morgan’s lab in 1910. Later studies revealed that the Notch gene encodes a receptor protein that extends through both sides of the cell membrane and which is capable of interacting with a ligand partner, such as the protein Delta, presented on the surface of a neighboring cell. This “juxtacrine” interaction causes the cleavage of an intracellular region of the Notch protein, loosing it into the cytoplasm and triggering the activation of transcription factors within the cell’s nucleus. In addition to its effects on wing structure in flies, Notch signaling is known to be important in a number of neural cell fate determination and developmental processes, and is conserved in species from human to roundworm. In all processes in which it participates, Notch signaling shows the ability to sense a small change in cell fate and amplify it, acting as a sort of contrast enhancement mechanism in cell fate determination.

Notch is activated by a protease that is present ubiquitously in the cell membrane. What has long remained a mystery, however, is the question of how Notch receptors that have not been activated by a ligand are protected from digestion by that protease. Now, in a report published in the December 29 issue of *Current Biology*, Shigeo Hayashi (Group Director, Laboratory for Morphogenetic Signaling) and colleagues at the RIKEN Center for Developmental Biology (Kobe, Japan) have identified the means by which unstimulated cells protect the Notch receptor from activation.



Adult *Drosophila* wing in which Nedd4 was overexpressed. Nedd4 inhibited wing margin formation and promoted expansion of the wing vein. The defects resemble the typical Notch mutant phenotypes.

Recent studies by other labs had shown that a number of stages in the Notch cascade were subject to ubiquitination, in which proteins are tagged by a complex of ubiquitin proteins. This system is best known for its function in marking proteins for degradation by a waste disposal unit known as a proteasome. Hayashi et al. sought to study the possibility that ubiquitination might play a part in rendering the

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unbound Notch receptor inert. Their attention was drawn to Nedd4 (a member of the ubiquitin ligase family of molecules that directly bind to proteins marked for degradation), as it had previously been shown that Nedd4 plays a role in the processing of other types of transmembrane proteins. Proteins in the cell membrane must first be internalized through a process known as endocytosis before they can be digested by the proteasome, and indeed other types of ubiquitin ligase have been shown to operate in the endocytosis of ligand-activated Notch.

The group first showed that an increase in *nedd4* activity resulted in the nicked wing phenotype characteristic of Notch loss of function and in reductions of the total amount of the Notch intracellular domain in the cytoplasm of treated cells. Taken together, these results suggested that Nedd4 works as an antagonist of Notch signaling at an early stage, prior to the proteolytic cleavage of the receptor's intracellular domain. Further investigation revealed the specific domain by which Nedd4 interacts with Notch and pinpointed the site of origin for this interaction at the cell membrane, a finding congruent with the idea of Nedd4 as an agent of endocytosis. Nedd4's role as a suppressor of Notch was illustrated even more plainly when the lab showed that inhibition of Nedd4 results in the upregulation of ligand-independent activation of the Notch pathway.

Nedd4's place in the greater scheme of Notch signaling became clearer when the group next turned to examine the interaction between Nedd4 and Deltex (Dx), a putative ubiquitin ligase known to bind and activate the Notch receptor. Hayashi's group found evidence that Nedd4 and Dx vie with each other to regulate Notch activity during endocytosis, and that Nedd4 actually destabilizes Dx in the presence of Notch. This competition between two ubiquitin ligases to permit or suppress activation of a signaling pathway represents a neat solution to the problem confronting cells of how to prevent molecular loose cannons from fouling their precisely ordered workplans.