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Notch catches a Jagged edge

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Abstract

Notch signaling is an essential cell-cell communication pathway that influences numerous cell fate decisions during development. Structural and biochemical studies of a Notch-Jagged complex dramatically advance current understanding of ligand recognition, and reveal evidence of catch-bond behavior in the complex.

Notch proteins are large, modular single-pass transmembrane receptors in a fundamental developmental signaling pathway that influences numerous cell fate decisions in all metazoan organisms. Signal transduction relies on the ability of canonical Delta or Jagged/Serrate family ligands to induce proteolysis of the Notch receptor and liberate its intracellular domain. Prior to ligand stimulation, receptor quiescence is established by a negative regulatory region, which masks a critical ligand-dependent processing site immediately external to the plasma membrane¹. Ligand stimulation unmasks this site, enabling cleavage by an ADAM metalloprotease, and the ADAM-truncated receptor then becomes a substrate for gamma secretase, which releases the Notch intracellular region (NICD) from the membrane, allowing it to enter the nucleus and form a transcriptional activation complex that regulates transcription of Notch target genes (Fig. 1a). O-linked sugar modifications of the Notch EGF repeats play an important role as modulators of Notch signal transduction by influencing the responsiveness of Notch receptors to their various Delta and Jagged ligands². Protein O-fucosyltransferase-1 (*POFUT1*) transfers O-fucose residues to specific serine and threonine receiver sites on Notch receptors, and these fucosylated residues can be further elaborated with N-acetylglucosamine by fringe glycosyltransferases and with additional sugars thereafter. How ligand recognition occurs, how recognition is modulated by receptor glycosylation, and how engagement of ligands at a binding site distant from the membrane induces sensitivity to proteolytic cleavage at a juxtamembrane site are questions that have been the focus of researchers in the field for more than two decades. In a recent *Science* article³, Luca and colleagues reported the X-ray structure of a complex between interacting fragments of the Notch1 receptor and a high-affinity evolved Jagged1 ligand, which, together with biochemical studies investigating the force dependence of the affinity of this interaction, provide new insight into how the Notch signal transduction machinery responds to Jagged and Serrate family ligands.

The complex visualized in the X-ray structure includes EGF-like domains 8–12 (of 36 total) of the Notch1 receptor, and a high-affinity engineered fragment of Jagged1 that extends from its N-terminal MNLL and DSL domains through its third EGF-like repeat (Figure 1A).

Both receptor and ligand adopt elongated conformations, with an antiparallel contact interface that extends over a remarkable distance of 120 Å (Figure 1B). This interface can be divided into two discrete sites of engagement, with the first encompassing EGF repeats 11–12 of Notch1 and the MNNL and DSL domains of Jag1, and the second derived from EGF repeats 8–10 of Notch1 and EGF repeats 1–3 of Jag1. Both contact zones contribute to the binding affinity of the interaction, distinguishing the Jag1 complex from the related DLL4-Notch1 complex⁴, which appears to derive its binding energy exclusively from an interface with EGF repeats 11–12 of Notch1. The importance of the second interface zone in Jag1 recognition was foreshadowed by signaling assays showing that stimulation by both Delta and Jagged ligands requires EGF8⁵, and by the discovery of a *Drosophila* Notch V324M point mutation in EGF8, called “jigsaw,” which selectively interferes with signaling by Serrate as opposed to Delta⁶.

Another striking feature of the Notch1-Jag1 complex is the direct engagement of Notch1 O-linked fucose residues on Thr466 of EGF12 and Thr311 of EGF8 with residues from the MNNL and EGF3 domains of Jag1 at the binding interface. The O-linked fucose on Thr466 also contacts DLL4 directly in the Notch1-DLL4 complex, but O-fucosylation of Thr311 exhibits greater importance in functional assays probing Jag1-induced Notch1 signaling, suggesting that discrimination among ligands can be encoded by different combinations of sugar modifications of the receptor⁷. Previous studies had suggested that Jagged1 contains a calcium-binding site in its MNNL domain that facilitates phospholipid binding⁸, but there is no evidence of bound calcium in the MNNL domain of Jag1 in the structure of the Notch1-Jag1 complex, raising a question about the functional significance of the prior observations.

The leading model for unmasking of the metalloprotease cleavage site in response to ligand binding is that bound ligand exerts a mechanical force to expose the metalloprotease processing site. Recent studies have provided direct support for such a mechanotransduction model, with the negative regulatory region acting as a mechanosensor⁹. One paradoxical aspect of the model, however, has been that the monovalent affinity of ligands for the receptor lies in the relatively weak micromolar range^{5,10}, raising the question of how the ligand-receptor interaction withstands the force required to induce proteolytic cleavage. Luca and colleagues used a biomembrane force probe setup to measure the lifetime of Notch-ligand complexes as a function of applied force, and discovered that the lifetimes of the Notch1-Jag1 and Notch1-DLL4 complexes *increase* with increasing force, reaching maxima at roughly 10 pN of force. The decrease in dissociation rate constant in response to increasing force is characteristic of “catch bonds” (also known as flex bonds), and explains how ligand-receptor complexes remain stable enough to allow the ligands on signal-sending cells tug on the receptor and induce a signal. At the molecular level, the catch bond results from an interdomain rotation of the Jag1 fragment at the joint following the MNNL domain upon receptor engagement (Figure 1C), allowing it to “hook” the receptor when under tension, rather than slip.

The new findings also raise a number of unanswered questions. Do the differences that distinguish the Jag1-Notch1 complex from the DLL4-Notch1 complex indeed reflect intrinsic differences in the mode of receptor engagement by Jagged and Delta-like ligands? Is the handshake between the two proteins fully described in the complex between these two

receptor fragments, or are there additional contacts between the N-terminal EGF repeats of Notch with the more distal EGF domains of the ligands that might enhance catch-bond behavior? If the repeats C-terminal to EGF12 on Notch have no role in ligand engagement, do they facilitate ligand capture by extending the ligand-binding domain away from the cell surface? Do they create a lever arm for the delivery of mechanical force? Do they promote clustering of ligand-receptor complexes? The structure and biochemistry of the Jag1-Notch1 complex will serve as a key point of departure driving future work that will answer these and other questions about the Notch signal transduction mechanism.

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Figure 1. Notch-Jagged Interaction Schematic and Structural Features

A. Cartoon diagram of full-length Notch1 and Jagged1 proteins. Schematic illustrating the domain organization of the Notch1 receptor and the Jagged1 ligand. For Notch1, the EGF repeats are colored purple, the negative regulatory region is in pink and blue, and the Notch intracellular region is shaded gray. EGF repeats 8–12 are more darkly shaded to represent the region seen in the structure, and the fucosylation sites are shown as asterisks. For Jagged1, the MNNL domain is yellow, the DSL domain is brown, the EGF repeats are orange, and the von Willebrand factor type-C domain is shown as a gray oval. The sending and receiving cell membranes are shown as curved black lines. B. Structural model of the Jagged1-Notch1 complex (PDB ID code 5UK5), shown in surface representation. Center panel shows the complex as a transparent molecular surface, over cartoon representations of

the backbone for each protein. Top: Jagged1 surface representation, colored dark brown at the contact interface and light brown elsewhere. Bottom: Notch1 surface representation, colored dark purple at the contact interface, and light purple elsewhere. C. Structure of the isolated Jagged1 fragment (blue), superimposed on the structure of Jagged1 in the complex with Notch1 (brown). The superposition of the DSL domains was used to make the comparison; note the upward rotation of the MNBL domain of Jagged1 upon complexation.