Notch/LIN-12 signaling: transduction by regulated protein slicing

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Intercellular signaling through the Notch/LIN-12 transmembrane receptors regulates growth and differentiation during animal development. Moreover, defects in the conserved Notch/LIN-12 pathway are linked to human diseases. Here, we review models for two key steps in Notch/LIN-12 signaling: ligand-mediated activation of the receptor and receptor-mediated activation of transcription. Ligand binding appears to permit proteolysis of the receptor; as a result, the receptor’s intracellular domain can enter the nucleus and function as a transcriptional co-activator.

Notch/LIN-12 SIGNALING regulates an enormous array of cell interactions during animal development, thereby controlling growth, differentiation and patterning in many tissues. In nematodes, for example, the specification of blastomere fates during early embryogenesis, proliferation of germline tissue and vulval development, all rely on this pathway. In Drosophila melanogaster and vertebrates, the pathway controls the cell-fate decision between epidermis and neuron, as well as a multitude of other fate decisions in nearly all tissues. Consistent with its key role in development, dysfunction of the Notch/LIN-12 pathway has been implicated in numerous human diseases, including leukemia, cervical and colon carcinomas, Alzheimer’s, stroke (CADASIL) and Alagille syndrome. A systematic structure–function analysis (Table I) can be explained, at least in part, by the need to impose precise spatial and temporal regulation upon their expression.

In this review, we focus our discussion upon the three-component, stripped-down version of the Notch/LIN-12 signaling pathway. Although these three components might not be essential for all Notch/LIN-12 signaling, they appear to be critical for the vast majority of signaling by these receptors. In addition to these core components, other factors and target genes clearly play key roles. Of particular note are Drosophila kuzbanian and its C. elegans homolog sup-17 (which encode ADAM proteases that affect signaling)3, C. elegans SEL-12 and its human homologs, presenilins PS1 or PS2 (which can regulate LIN-12/δEF-1 signaling), and fringe (which encodes a secreted regulator that differentially affects Delta and Serrate ligands)6. A detailed consideration of these and other regulators and target genes is beyond the scope of this review.

Table I. The Notch/LIN-12 signaling pathway

<table>
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<tr>
<th>DSL ligand</th>
<th>Receptor activation</th>
<th>Notch/LIN-12 receptor</th>
<th>Transcription-factor activation</th>
<th>CSL effector</th>
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<tr>
<td>Caenorhabditis elegans</td>
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<td>Notch/LIN-12</td>
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<tr>
<td>LAG-2, APX-1</td>
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<td>Drosophila melanogaster</td>
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<tr>
<td>Notch/LIN-12 receptor</td>
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<tr>
<td>Serrate, Delta</td>
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<td>Delta 1 and 2, Jagged 1 and 2</td>
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Negative regulators, positive regulators, downstream target genes and regulatory loops have been removed from the pathway for simplicity. CBF1 is also known as RIP-1a and RIP-2.
LAG-2, which include the signal sequence, and the N-terminal and DSL regions, are sufficient for unregulated signaling but not for mutant rescue. This small fragment can induce excess germine proliferation in an otherwise-wild-type background, but it cannot rescue a null mutant. To achieve mutant rescue, membrane association is required. This suggests that the N-terminal and DSL regions are localized or concentrated at the membrane association is required. It LAG-2 carries a deletion of either the N-terminal region or the DSL domain, it cannot signal; suggesting that both regions are critical for signaling 

Unlike the N-terminal and DSL regions of the ligand, neither the EGF-like repeats nor the IC region is essential. However, mutations in the EGF-like repeats can influence ligand activity and, because these repeats are present in all DSL proteins, it seems unlikely that they have no function. They play an ancillary role, such as stabilization of the ligand–receptor complex. Alternatively, they might play a negative regulatory role, such as competing with the receptor for binding to the DSL domain, in which case their removal would not be expected to reduce signaling. The role of the IC region is similarly enigmatic: in C. elegans, its removal increases LAG-2 activity, while in Drosophila and vertebrates, a similar deletion has a dominant negative effect. These two contrasting results suggest that the IC region of the ligand plays a regulatory role that has diverged during evolution.

The specificity of DSL-receptor interactions has been examined by constructing chimeric transgenes that place the coding region for one ligand under the control of regulatory sequences of another. LAG-2 and APX-1 were found to be interchangeable in C. elegans, similarly, either Delta or Serrate could support the decision between neural and epidermal fates in the Drosophila embryo. However, Delta and Serrate are not always equivalent, and their differences appear to map to their N-terminal and DSL domains.

The ligand-binding site within the EC fragment has been explored in tissue culture cells by assessing the aggregation of Delta- and Notch-expressing cells. These studies suggest that two Ca²⁺-binding repeats (EGFL11, EGFL12) in Drosophila Notch are critical for a Ca²⁺-dependent association with Delta. Missense mutations in EGFL7 of C. elegans GLP-1 (Ref. 24) and EGLL2 of Drosophila Notch (unpublished) can abolish receptor activity; however, the nematode EGL7 repeat does not bear a Ca²⁺-binding repeat signature, and the significance of these mutations for ligand binding has not been tested. Therefore, binding studies will be essential to show definitively which EGF-like repeats are critical for mediating the ligand–receptor interaction. The LAG repeats, as well as the region between those repeats and the transmembrane domain, are not only required for receptor function but also have been implicated in receptor regulation. Thus, certain missense mutations in either the LAG repeats or in the LNG–TM interval can activate the receptor and render it ligand-independent.
A reasonable interpretation is that these mutations affect amino acid residues that are critical for the proposed EC inhibition and, therefore, release the receptor from that inhibition.

Figure 3 presents two models for activation of the receptor by the ligand. In the first model, binding of the ligand to the receptor’s EGF repeats induces receptor dimerization, thereby activating the receptor (Fig. 3a). In support of this idea, the ligand appears to bind the EGF repeats (see above), and receptor dimerization has been suggested by genetic studies. In the second model, the ligand interacts with the LNG repeats and disrupts an inhibitory interaction between the EC and TM–IC fragments of the receptor (Fig. 3b). Dimerization of either ligand or receptor can be included in this second model but, for simplicity, is not illustrated. This second model is suggested by the influence of the LNG repeats and the LNG–TM interval on ligand-regulated repression of the receptor (see above). Clearly, both models are speculative, and neither is probably correct in detail. Nonetheless, the mechanism probably involves the EGF and the LNG repeats, because both are critical for receptor activity. Perhaps the actual mechanism combines features of the two models in Fig. 3. For example, the ligand could dock on the EGF repeats, which, in turn, might lead to an interaction with, or conformational change within, the LNG repeats that results in release from EC inhibition. One approach to understanding this step in the signaling mechanism is to compare, at a biochemical level, the wild-type receptor, which is repressed unless bound by ligand, with mutant ligand-independent receptors, which are derepressed. Such a comparison could shed light on how the transition from the inactive to the active state of the receptor occurs.

Receptor activation of transcription

How does signaling activate the CSL transcription factor? Unlike many receptors, the Notch/LIN-12 receptor appears to possess no enzymatic activity of its own. Nonetheless, functions can be ascribed to three distinct regions within the intracellular (IC) region of the receptor (Fig. 2). Of most importance is the ANK-repeat region. Missense mutations in the ANK repeats eliminate the activity of both normal and constitutively activated receptors. Moreover, overexpression of a full-length Notch receptor containing the ANK repeats (plus approximately 30 amino acid residues at either end) activates the pathway in a fashion that is dependent on the ANK repeats (Refs. 33, 35; V. Kodoyianni and J. Kimble, unpublished). Furthermore, the ANK-repeat region can bind to the CSL transcription factor, although nonspecifically, and can also dramatically enhance transcription by the CSL transcription factor in tissue-culture cells. Together, these data support the idea that the ANK-repeat region can act in the nucleus as a transcriptional co-activator. The RAM domain, which lies just inside the transmembrane domain, binds to the CSL transcription factor in vitro and in vivo. However, overexpression of the RAM domain neither activates the pathway nor interferes with endogenous signaling. Finally, the C-terminal region negatively regulates receptor activity by poorly understood mechanisms that appear to be species specific.

Two models to explain CSL activation by the receptor have been suggested. The first proposes regulated transport of the CSL transcription factor from the cytoplasm to the nucleus (Fig. 4a); the second proposes regulated proteolytic cleavage of the receptor, followed by entry of the receptor IC into the nucleus (Fig. 4b). In this second model, the receptor IC cleavage product is predicted to act as a transcriptional co-activator when bound to the CSL protein. Evidence for each model has been obtained using overexpressed and, in some cases, mutant proteins. However, both models predict a change in the subcellular distribution of either the receptor IC domain or the CSL transcription factor, but no such change has been observed in vivo by careful examination of the endogenous proteins (Refs. 21, 44, 45; V. Kodoyianni and J. Kimble, unpublished).
Recently, a second proteolytic-cleavage site has been identified in the Notch1 receptor of vertebrates; in this case, the receptor IC domain is released by proteolysis. This cleavage site, which is distinct from the maturation cleavage in the LNG–TM interval, is in the receptor's transmembrane domain at its C-terminal border. Of particular significance is that ligand binding induces this cleavage, and mutations of the cleavage site dramatically reduce receptor activity and signaling. The fragment generated by this cleavage is called the Notch1 intracellular domain (NICD). Cleavage might release either the NICD alone, or an IC–CSL complex for entry into the nucleus. Intriguingly, signaling by the NICD in tissue-culture cells occurred at concentrations that were not detectable by immunocytochemistry, which might explain why the NICD has not been observed in the nucleus during development.

A similar mechanism (i.e. one in which proteolytic cleavage releases a transcription factor) has been demonstrated for activation of the sterol regulatory element binding protein (SREBP). In this case, ligand-induced proteolysis releases the SREBP transcription factor from a membrane-bound form in the endoplasmic reticulum. One minor difference is that SREBP is a DNA-binding protein, whereas the NICD appears to be a transcriptional co-activator that does not itself bind to DNA. However, in both cases, the linkage between ligand binding and transcriptional regulation is remarkably simple and direct.

Conclusions and future prospects

The Notch/LIN-12 signaling pathway is a ubiquitous pathway that controls a variety of processes during development. The signal-transduction mechanism that is emerging involves at least two steps: (1) ligand-induced activation of the receptor, perhaps by release from EC inhibition; and (2) receptor-induced activation of the CSL DNA-binding protein, perhaps by generation of an IC proteolytic fragment that acts in the nucleus with the CSL protein to promote transcription.

Although these broad strokes paint a reasonable picture, many questions remain. How does the receptor EC domain inhibit receptor activity, and how does ligand binding relieve such inhibition? Does ligand binding induce dimerization and thereby generate a substrate for the protease, or does ligand binding permit the transmembrane domain to readjust its position and expose a site for proteolytic cleavage? What role do the presenilins play in signaling? As mentioned at the beginning of this review, the presenilins can regulate LIN-12/GLP-1 signaling in nematodes. Recent work supports a role for presenilins in the facilitated cleavage of APP. Do the presenilins also facilitate cleavage of Notch/LIN-12 receptors? What is the role of the ADAM proteases in signaling? Do they mediate the newly identified ligand-induced cleavage? Does the NICD join the CSL protein in the nucleus, or is a complex formed with the CSL at the membrane, so that the two are released together for entry into the nucleus? Finally, can the receptor act through mechanisms other than those outlined?
here? Although our understanding of the signaling mechanism used by Notch/LIN-12 receptors remains limited, major inroads have clearly been made. Challenges for the future include the biochemical detail and the generation of reagents to intervene in the pathway for therapeutic uses.

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