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Review

Ligand-Independent Mechanisms of Notch Activity

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Interaction between the Notch receptor and Delta–Serrate–Lag2 (DSL) ligands is generally deemed to be the starting point of the Notch signaling cascade, after which, Notch is cleaved and the intracellular domain acts as a transcriptional coactivator. By contrast, Notch protein can become activated independent of ligand stimulus through recently identified endosomal trafficking routes as well as through aberrant regulation of Notch components during Notch trafficking, ubiquitination, and degradation. In this review, we summarize genes implicated in ligand-independent Notch activity and remark on the mechanisms by which this process could occur.

Trends

Notch, a transmembrane receptor and transcriptional coactivator, can be activated independent of ligand through a trafficking-dependent route.

Multiple endosomal routes exist that can lead to ligand-independent Notch activation, which are dependent on temperature and competing E3 ligases.

Notch ligands expressed in the same cell as Notch help to buffer against ligand-independent Notch activity.

Crystal cells use ligand-independent Notch activity endogenously for survival.

Defects in trafficking and ubiquitination of Notch cause Notch accumulation and activation, possibly through different mechanisms.

The Many Ways to Activate Notch Signaling

The Notch pathway has emerged as one of the major signaling cascades activated throughout development and its misregulation has been associated with many diseases. Canonical Notch signaling begins upon DSL ligand [Delta (Dl) or Serrate (Ser) in *Drosophila*; Delta or Jagged orthologs in mammals] binding to the extracellular domain of Notch (NECD), allowing subsequent proteolytic cleavage of the Notch receptor (reviewed in [1]). This cleavage releases the intracellular domain of the Notch receptor (NICD), which can translocate to the nucleus and form a complex with a CSL transcription factor (CBF-1 in mammals, Suppressor of Hairless in *Drosophila*, and Lag-2 in *Caenorhabditis*), resulting in expression of downstream target loci [2–5]. However, recent work has shown that the Notch pathway can be used noncanonically, including signaling independent of CSL transcription factors through the Wnt pathway (reviewed in [6]) or in a DSL ligand-independent manner. This ligand-independent activation of the Notch receptor is primarily caused by the disruption of genes that control endosomal sorting and ubiquitination, resulting in accidental and often detrimental pathway activity (reviewed in [7,8]). Conversely, mechanisms are emerging by which ligand-independent Notch activity is controlled endogenously.

When Notch is not required to signal in a cell, the receptor must be tightly regulated to make certain no aberrant signal is produced. Furthermore, there is a constant turnover of Notch in the cell, as pulse–chase experiments show the disappearance of labeled Notch within hours (e.g., [9]). Notch is translated, processed into its heterodimer form, and transported to the membrane, where it awaits ligand presentation from a neighboring cell. If ligand is not presented, Notch will be marked for degradation. This occurs by the addition of a monoubiquitin signal to the Notch intracellular domain by the E3 ubiquitin ligase, Deltex (Dx), spurring its internalization [10]. Another E3 ubiquitin ligase, Kurtz (Krz) has been shown to complex with Dx and Notch, promoting polyubiquitination of the receptor [11,12]. The Endosomal Complex Required for Transport (ESCRT) is able to recognize polyubiquitinated proteins, and has been implicated in the regulation of many membrane-bound receptors [13,14]. There are four distinct ESCRT complexes, which work sequentially to sort Notch into intraluminal vesicles (ILVs) of the multivesicular body (MVB). Eventually, the cargo in the lumen of the MVB is transferred to the lumen of the lysosome for degradation. If malfunctions in this process leave Notch ensnared on the outer endosome membrane, then NICD can be accidentally removed from NECD, mimicking

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ligand-dependent Notch activation. This model is primarily supported by genetic loss- and gain-of-function studies in *Drosophila*, although some genes have been characterized in zebrafish as well. Among the genes identified are E3 ligases, endosomal sorting proteins, metalloproteases, basal body proteins (in zebrafish) [15], small GTPases [16,17], *hif1-alpha*, and Notch's own ligands (Table 1). Note that some of these genes are also involved in ligand-dependent Notch signaling. Most of these factors have been implicated in endosomal regulation [18–22] and appear to act in the same overarching process. For example, the ESCRT-III component, Shrub, serves as a link between the ligand-independent Notch activation observed in ESCRT complex mutants and that witnessed in both *lethal giant discs* (*lgd*) mutants and *dx*-expressing cells [7,11,17].

While the endogenous regulation of Notch requires more investigation, a clearer picture is emerging of the mechanism of ligand-independent activation that occurs in mutant tissue. In this review, we address the role and mechanisms of endogenous ligand-independent Notch

Table 1. Genes Implicated in Positive or Negative Regulation of Ligand-Independent Notch Activity

Gene(s)	Function	Regulation	Mechanism	Refs
lethal(2) giant discs (lgd)	Phospholipid binding	Negative	Trafficking defect in *lgd* mutant cells where ubiquitinated Notch accumulates on lysosomal limiting membrane	[41–43,81,82]
deltex (Dx)	E3 ligase	Positive	Promotes monoubiquitination, endocytosis, and stabilization of Notch on lysosomal limiting membrane	[23–28,35]
Su(Dx)* and *dNedd4	E3 ligase	Both	Promotes internalization of Notch, resulting in either inhibition (via polyubiquitination) or promotion (via Dx-mediated route) of ligand-independent Notch activity depending on temperature	[30–35]
ESCRT I–III complexes	Sorting of ubiquitinated proteins	Negative	MVB biogenesis and sorting of ubiquitinated proteins is disrupted in ESCRT mutants (e.g., *Vps25*, *tsg101*, *shrub*) leading to high levels of Notch accumulating in malformed endosomes	[7,9–11,44–49,52]
kuz* and *TACE	ADAM proteases	Positive	Removes Notch ectodomain, leaving as a viable substrate for presenilin-mediated S3 cleavage	[64,71]
Ras-like Protein A	Small GTPase	Negative	Mechanism unclear, although has been implicated in exocysts and endocytosis	[16–19]
BBS1/BBS4	BBSome components	Negative	Shifts subcellular location of Notch from membrane, recycling endosome, and lysosome towards the MVB and late endosome	[15]
similar (sima)	Hypoxic stress response	Positive	Stabilizes Notch in *hrs* positive endosomes	[22]
***cis*-DSL ligands**	Notch ligands	Negative	Ligands in same cell as receptor buffer against accidental activation	[37]