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Review

Sending the right signal: Notch and stem cells ☆

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ABSTRACT

Background: Notch signaling plays a critical role in multiple developmental programs and not surprisingly, the Notch pathway has also been implicated in the regulation of many adult stem cells, such as those in the intestine, skin, lungs, hematopoietic system, and muscle.
Scope of review: In this review, we will first describe molecular mechanisms of Notch component modulation including recent advances in this field and introduce the fundamental principles of Notch signaling controlling cell fate decisions. We will then illustrate its important and varied functions in major stem cell model systems including: *Drosophila* and mammalian intestinal stem cells and mammalian skin, lung, hematopoietic and muscle stem cells.
Major conclusions: The Notch receptor and its ligands are controlled by endocytic processes that regulate activation, turnover, and recycling. Glycosylation of the Notch extracellular domain has important modulatory functions on interactions with ligands and on proper receptor activity. Notch can mediate cell fate decisions including proliferation, lineage commitment, and terminal differentiation in many adult stem cell types. Certain cell fate decisions can have precise requirements for levels of Notch signaling controlled through modulatory regulation.
General significance: We describe the current state of knowledge of how the Notch receptor is controlled through its interaction with ligands and how this is regulated by associated factors. The functional consequences of Notch receptor activation on cell fate decisions are discussed. We illustrate the importance of Notch's role in cell fate decisions in adult stem cells using examples from the intestine, skin, lung, blood, and muscle. This article is part of a Special Issue entitled Biochemistry of Stem Cells.

1. The molecular basis of Notch signaling

Mutations in the *Notch* genetic locus were originally described almost 100 years ago as sex-linked lethal mutations in *Drosophila*, where heterozygous females had notches in their wings [1]. Later, *Notch* was characterized as a "neurogenic locus", as homozygous mutations in *Notch* were found to result in cell fate changes during neurogenesis: null embryos contained many more neural cells at the expense of the epidermal cells [2,3]. Almost 30 years ago, the *Notch* locus was sequenced and shown to encode a transmembrane protein [4–6]. Later, work showed that Delta and Serrate acted in the Notch pathway [7–9], and elegant mosaic studies provided evidence of their ligand function [10]. Most components of Notch signaling have been first characterized in *Drosophila* and have been later found to be present in mammals. The essential components of Notch signaling have been found to be highly conserved, functioning in a similar manner in all Metazoans.

1.1. Notch signaling basics: receptors and ligands

Notch receptor activation by its ligands is thought to be short range, occurring between juxtaposed cells. The Notch receptor (Notch in *Drosophila*, GLP-1 and Lin-12 in *Caenorhabditis elegans*, Notch1–4 in mammals) is a transmembrane protein that is activated by its transmembrane ligands of the DSL family (Delta and Serrate in *Drosophila*, Lag-2 in *C. elegans* and Delta-like and Jagged in vertebrates) expressed in neighboring cells. Upon activation of Notch by its ligands, the Notch protein undergoes two proteolytic cleavages that will release an intracellular Notch domain (NICD) allowing its consequent translocation to the nucleus. In the nucleus, NICD interacts with the DNA binding protein CSL/RBP-Jk (human CBF1 and RBP-Jk, fly Suppressor of Hairless and nematode Lag-1), and the co-activator Mastermind, promoting the transcription of the Notch target genes (Fig. 1) (reviewed in [11–14]). A number of other proteins control the activity of Notch ligands and receptors and will be further detailed below (Section 3). In addition to the activation of the Notch receptor by ligands expressed in neighboring

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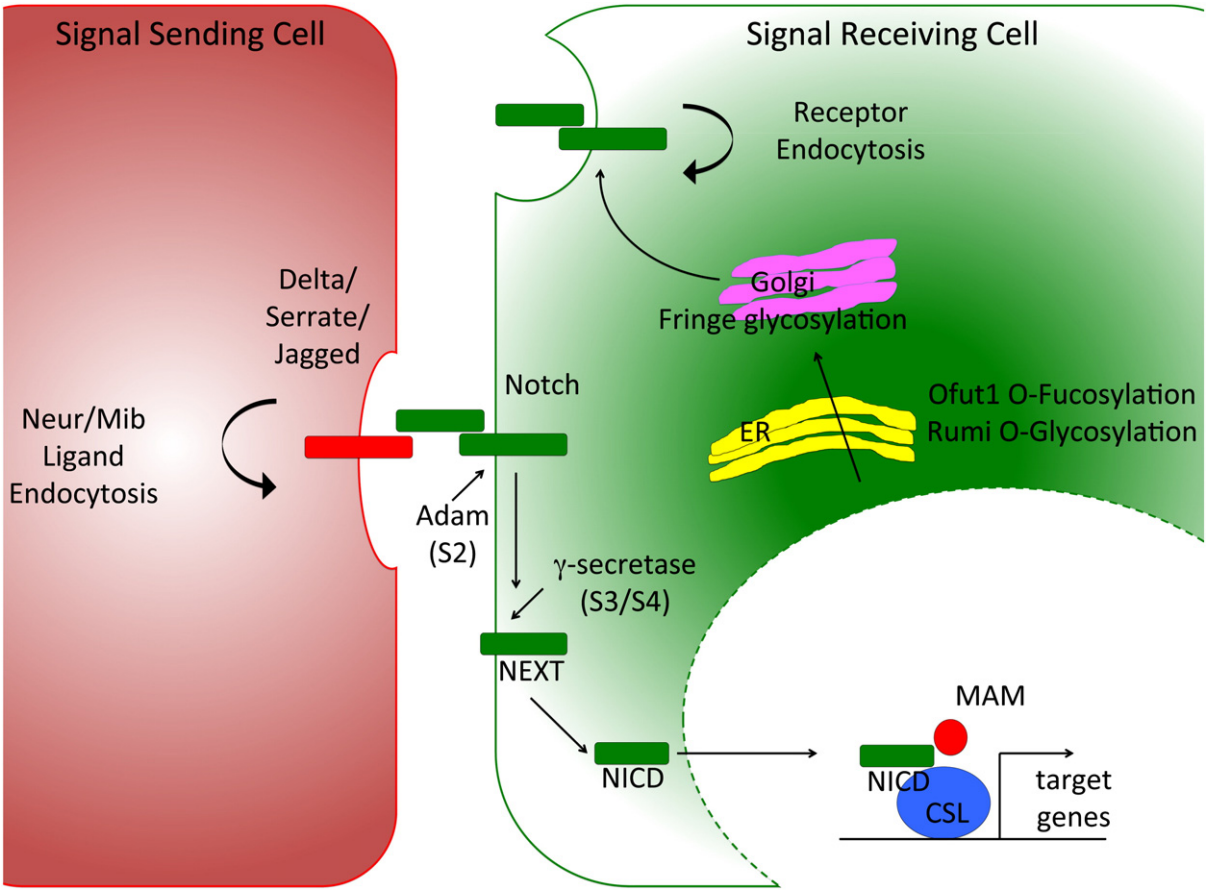

Fig. 1. Notch signaling in cells. Notch receptor and ligands are trans-membrane proteins. Ligands expressed in adjacent cells activate the receptor, which undergoes successive proteolytic cleavages. The Notch intracellular domain (NICD) translocates to the nucleus where it will associate with CSL/RBP-Jk and MAM to form a transcriptional complex and activate the expression of the Notch target genes. The Notch receptor is glycosylated in the ER and the Golgi complex. Both ligand and receptor are regulated by endocytosis.

cells, there can also be *cis*-interaction between the receptor and the ligands expressed in the same cell that results in inhibition of Notch signaling.

1.2. Domain organization of the Notch receptor and its ligands

Both the Notch receptor and its ligands are type I transmembrane proteins. Notch is found at the plasma membrane as a heterodimer composed of a large Notch Extra Cellular Domain (NECD) and a membrane tethered intracellular domain produced by furin-mediated Notch proteolytic cleavage (site 1 or S1 cleavage) [15,16]. Fig. 2A illustrates the structure of the four mammalian Notch receptors (mNotch1–4) and the *Drosophila* receptor (Notch) The extracellular domain of Notch contains tandem Epidermal Growth Factor-like (EGF) repeats, which vary in number in the different Notch proteins. Specific EGF repeats mediate interaction with the ligands and contain consensus motifs for sugar modifications (described in more detail bellow). The EGF repeats are followed by the negative regulatory region (NRR) of Notch, which contains three Lin-12-Notch repeats (LNR) and a hetero-dimerization domain (HD). The NECD and the membrane tethered intracellular domain interact non-covalently in a calcium dependent manner.

The Notch transmembrane domain (TMD) is followed by the RBPJk association module (RAM), nuclear localization sequences (NLS), a seven ankyrin repeats (ANK) domain and a transactivation domain (TAD) that contains a proline/glutamic acid/serine/threonine-rich motifs (PEST), which target NICD for degradation (reviewed in [14]).

The structure of the ligands of the DSL family of ligands is represented in Fig. 2B. The better-characterized DSL ligands, *Drosophila* Delta and Serrate as well as mouse mJagged1–2 and mDll1, contain at their N-terminus, a DSL motif followed by a "Delta and OSM-11-like proteins" (DOS) motif and EGF repeats. *Drosophila* Serrate as well as mammalian mJagged1–2 proteins have an additional cysteine-rich domain before their transmembrane domain [14], whereas other DSL ligands, like mDll3 and mDll4 only contain the DSL motif and EGF repeats in their extracellular domain [17].

1.3. Activation of the Notch receptor by its ligands

How exactly the interaction of the ligands leads to activation of the receptor is not entirely understood. The NRR appears to play a critical role in preventing the proteolytic cleavage of the receptor in the absence of ligand binding, as the cleavage site is normally protected by a hydrophobic interface between the LNR motifs and the HD domain (Fig. 3B). It has been proposed that ligand binding induces conformational changes in the NRR, leading to relaxation of the interaction of the LNR motifs with the HD and exposing the cleavage site [18,19]. This model is consistent with evidence indicating that the activation of the Notch receptor by its ligands first leads to cleavage of Notch by the ADAM/TACE/Kuzbanian family metalloproteases at site 2 (S2), which releases the ectodomain of Notch and creates an activated, membrane-tethered intermediate called the Notch Extracellular Truncation (NEXT) [20,21]. γ-Secretase activity of the Presenilin–Nicastrin–Aph1–Pen2 protein complex cleaves NEXT at two endomembrane sites (S3) releasing the NICD [22–26].

NICD is the transcriptionally active form of the protein and will ultimately translocate to the nucleus [27–29]. Once in the nucleus,

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