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Review

The cell giveth and the cell taketh away: An overview of Notch pathway activation by endocytic trafficking of ligands and receptors

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ABSTRACT

Notch signaling is firmly established as a form of cell-to-cell communication that is critical throughout development. Dysregulation of Notch has been linked to cancer and developmental disorders, making it an important target for therapeutic intervention. One aspect of this pathway that sets it apart from others is its apparent reliance on endocytosis by signal-sending *and* signal-receiving cells. The subtle details of endocytosis-mediated molecular processing within both ligand- and receptor-presenting cells that are required for the Notch signal to maintain fidelity remain unclear. The endosomal system has long been known to play an important role in terminating signal transduction by directing lysosomal trafficking and degradation of cell surface receptors. More recently, endocytic trafficking has also been shown to be critical for activation of signaling. This review highlights four models of endocytic processing in the context of the Notch pathway. In ligand-presenting cells, endocytosis may be required for pre-processing of ligands to make them competent for interaction with Notch receptors and/or for exerting a pulling force on the ligand/Notch complex. In receptor-presenting cells, endocytosis may be a prerequisite for Notch cleavage and thus activation and/or it could be a means of limiting ligand-independent Notch activation. Recent advances in our understanding of how and why endocytosis of Notch receptors and ligands is required for activation and termination of signaling during normal development and in disease states are discussed.

Contents

Abbrevations: EGF, epidermal growth factor; NRR, negative regulatory region (structural domain of Notch that shields S2 cleavage site in absence of ligand binding and activation); LNR, Lin12/Notch repeat; HD, heterodimerization domain; NECD, Notch extracellular domain; NICD, Notch intracellular domain (the portion of Notch that traffics and signals to the nucleus); CSL, CBF/Su(H)/Lag-1; DSL, Delta/Serrate/Lag-2 (family of ligands that activate Notch); Lgd, lethal giant disks; Hrs, hepatocyte growth factor-regulated tyrosine kinase substrate; ESCRT, endosomal sorting complexes required for transport; S1 cleavage, (furin-mediated cleavage resulting in heterodimerization of Notch); S2 cleavage, (ADAM/TACE cleavage resulting in release of NECD fragment); S3 and S4 cleavage, (γ-secretase cleavages resulting in release of NICD fragment).

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Introduction

In the past decade, endocytic trafficking has been shown to be a critical component of many signaling pathways – including the well-studied Notch signaling pathway, which is essential for a wide range of developmental processes. Many features of the Notch signal transduction cascade have been elucidated, but a key question that remains to be fully answered is why endocytic trafficking is required in both signal sending and receiving cells for the pathway to function. This brief review will cover what is known about the role of endocytosis in Notch signaling and will highlight questions remaining in the field.

Several recent reviews provide an in depth description of the core features of Notch signaling (Kopan and Ilagan, 2009; Tien et al., 2009). Briefly, Notch proteins are single pass transmembrane receptors that transduce signals via a unique mechanism involving receptor proteolysis, resulting in the release of an active intracellular Notch fragment. The extracellular domain of the prototypical Notch receptor contains tandem arrays of Epidermal Growth Factor (EGF)-like repeats and a conserved negative regulatory region (NRR) that consists of three Lin12/Notch repeats (LNRs) and a heterodimerization (HD) domain (Fig. 1A). The NRR functions to prevent ligand-independent activation of Notch, as illustrated by the fact that mutations within this domain generate a constitutively active receptor, leading to developmental defects and cancers (Greenwald and Seydoux, 1990; Weng et al., 2004). During intracellular receptor maturation, mammalian Notch is cleaved at the S1 site within the HD domain by furin, or a related member of the proprotein convertase family. This generates extracellular and transmembrane subunits that are held together by the HD domain (Fig. 1A). Furin cleavage is not required for mammalian Notch to reach the cell surface (Bush et al., 2001), but it is required for activation of Notch signaling. Curiously, *Drosophila* Notch lacks a consensus furin cleavage site and only the uncleaved form is detected on the cell surface (Kidd and Lieber, 2002), suggesting that pathway activation differs between vertebrates and invertebrates.

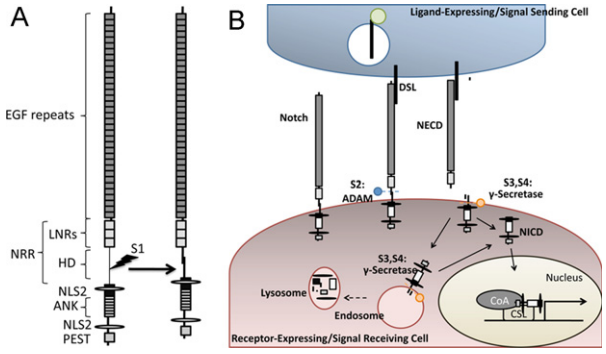

Fig. 1. Schematic illustration of Notch structure and pathway activation. (A) Notch receptors have an extracellular domain composed of reiterated epidermal growth factor (EGF)-like repeats and a conserved negative regulatory region (NRR) consisting of Lin12/Notch repeats (LNRs) and a heterodimerization (HD) domain. The intracellular portion of Notch contains repeated ankyrin (ANK) repeats, nuclear localization signals (NLS) and a PEST domain that controls receptor half life. Vertebrate Notch undergoes S1 cleavage within the secretory pathway to generate the heterodimeric receptor that is found on the cell surface. (B) Notch is activated by binding to ligands of the Delta/Serrate/Lag-2 (DSL) family. The ligands are ubiquitinated (green circle) and internalized into signal sending cells before and/or after receptor activation. Activated Notch undergoes sequential cleavage, initially at the S2 site by members of the ADAM family of metalloproteases (blue ball), and then at the S3 and S4 sites by γ-secretase (orange circle). S2 cleavage occurs at the cell surface and releases the Notch extracellular domain (NECD) from the heterodimer. γ-secretase mediated cleavages take place on the plasma membrane and/or in endosomes. These cleavages release the Notch intracellular domain (NICD), which translocates to the nucleus where it interacts with members of the CBF1/Su(H)/Lag-1 (CSL) family of transcription factors, and recruits co-activators (CoA) to activate transcription of Notch target genes. NICD signaling is terminated by lysosomal degradation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Ligands of the Delta/Serrate/Lag-2 (DSL) family activate Notch. These ligands are membrane-anchored proteins and thus receptor activation requires cell–cell contact in most circumstances. Ligand binding triggers a sequential cascade of cleavages within Notch, named S2, S3 and S4 (Fig. 1B). The S2 site, which resides within the carboxyl (C)-terminal portion of the HD domain, is cleaved by members of the ADAM/TACE metalloprotease family (Mumm et al., 2000). This cleavage releases the Notch extracellular domain (NECD) from the heterodimer (Fig. 1B) (Kopan et al., 1996; Struhl and Adachi, 1998). A recent structural analysis showed that the S2 cleavage site is normally masked by extensive interdomain interactions within the NRR (Gordon et al., 2007). Thus, ligand induced conformational changes in the Notch receptor are presumably required to expose the S2 site for ADAM-mediated cleavage. S2 cleavage is a prerequisite for subsequent intramembranous cleavage of Notch at the S3 and S4 sites by the γ-secretase complex. These cleavages release the Notch intracellular domain (NICD) (Struhl and Adachi, 2000; Fiuza and Arias, 2007). The NICD then translocates to the nucleus, where it interacts with members of the CBF1/Su(H)/Lag-1 (CSL) family of transcription factors, displacing co-repressors and recruiting co-activators to activate transcription of Notch target genes (Fortini and Artavanis-Tsakonas, 1994; Fiuza and Arias, 2007).

Studies in *Drosophila* have shown that dynamin-dependent endocytosis is essential in both the ligand- and receptor-presenting cells for successful transduction of Notch signals (Seugnet et al., 1997), and several models have since been proposed to explain this requirement (illustrated in Fig. 2). First, endocytosis has been proposed to direct DSL ligands to an intracellular compartment where they undergo essential post-translational modifications prior to recycling to the cell surface for receptor activation (Fig. 2A) (Wang and Struhl, 2004; Wang and Struhl, 2005). Alternatively, or perhaps in addition, endocytosis of DSL ligand bound to the Notch receptor may be necessary to provide a pulling force that dissociates the Notch heterodimer and/or induces a conformational change, thereby exposing the S2 ADAM cleavage site (Fig. 2B) (Parks et al., 2000; Nichols et al., 2007). In the signal-receiving cell, Notch endocytosis may be required for γ-secretase cleavage of Notch, perhaps because the enzyme complex is primarily active in an intracellular compartment (Fig. 2C) (Gupta-Rossi et al., 2004). Finally, Notch receptor endocytosis and lysosomal targeting may be required to prevent "accidental" ligand-independent activation of Notch (Fig. 2D) (Childress et al., 2006; Gallagher and Knoblich, 2006; Jaekel and Klein, 2006). In the following sections, we present evidence for and against each of these models, which are not mutually exclusive.

Ligand endocytosis: required for ligand activation?

As summarized above, the requirement for endocytosis in signal sending cells might reflect a need to internalize the ligand prior to receptor interaction in order to generate an active ligand and/or a need to internalize the ligand–receptor complex in order to activate signaling. There is good evidence supporting both hypotheses and, indeed, endocytosis in the signal-presenting cell may serve multiple functions. The observation that DSL ligand activation requires a specialized endocytic pathway, rather than simple bulk endocytosis, supports the first model. However, the