Notch: a membrane-bound transcription factor

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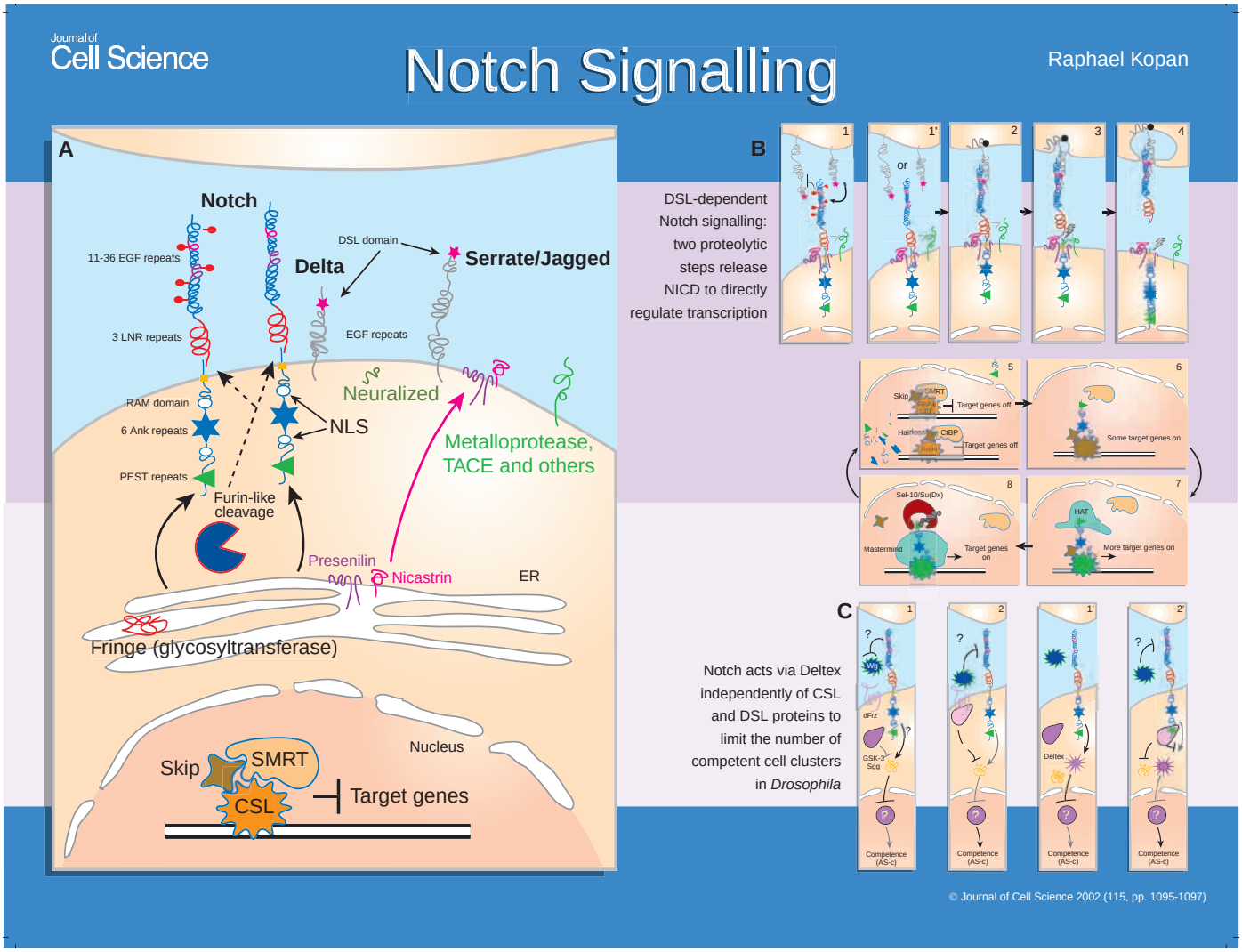
Notch signaling is both simple and complex. It is simple because no secondary messengers are required as Notch can directly report to the nucleus after contact with ligand at the plasma membrane. Notch acts as a membrane-bound transcription factor that is released in response to ligand binding by two proteases acting sequentially. The freed intracellular domain enters the nucleus, where it interacts with a DNA-bound protein to activate transcription of selected targets. Notch signaling is complex because it regulates an astonishingly large number of cellular decisions during development (Artavanis-Tsakonas et al., 1999) and in the adult (Milner and Bigas, 1999). In addition, the regulation of Notch proteolysis is complex, involving an array of accessory proteins whose functions have been elucidated only recently (Kramer, 2001; Mumm and Kopan, 2000). Finally, genetic experiments indicate that Notch may also have a separate, proteolysis-independent function at the membrane, where it acts together with Wnt to affect developmental competence (Brennan et al., 1999a; Ramain et al., 2001).

In the poster, panel A summarizes the proteins in the Notch signal transduction pathway for which biochemical functions have been proposed. Panel B outlines the events that occur in response to ligand binding and during the regulation of transcription and return to the basal state. In panel C a speculative pathway, independent of core components, is presented.

Panel A

The core elements of the Notch signaling system include the Notch receptor, DSL ligands (Delta and Serrate/Jagged in *Drosophila* and vertebrates, Lag-2 in *C. elegans*) and CSL DNA-binding proteins (CBF1/RBPjk in vertebrates, Suppressor of hairless in *Drosophila*, Lag-1 in *C. elegans*). Only some target genes are known and the first identified are



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members of the HES (Hairy/Enhancer of Split) family of basic helix-loop-helix transcriptional regulators (not illustrated). Notch proteins (and ligands) contain extracellular EGF (epidermal growth factor)-like repeats. The repeats 11-12 of Notch (pink) interact with the DSL domain of ligands (pink). The EGF repeats in Delta-like ligands are continuous whereas in the Serrate-like ligands they are interspersed with short linker sequences. Notch can be modified in the Golgi by a glycosyltransferase called Fringe (Blair, 2000) on specific fucose-modified EGF repeats. Fringe is expressed only in a subset of cells; Notch is hyperactivated when Fringe-expressing and -nonexpressing territories abut, forming a boundary (Irvine and Rauskolb, 2001; Wu and Rao, 1999). Notch is secreted to the cell surface in a Furin-convertase-dependent step (Logeat et al., 1998). The LNR (Lin/Notch Repeat; red) domain maintains the association between the polypeptides resulting from the Furin cleavage (at site 1 or S1). The intracellular domain contains the RAM23 domain (RAM), which enhances interaction with CSL protein. The Notch intracellular domain (NICD) contains nuclear localization signals (NLS), a CDC10/ankyrin repeat domain (ANK), which mediates interactions with CSL and other proteins, and a domain rich in proline, glutamate, serine and threonine residues (PEST). Also shown are neuralized (an E3 ubiquitin ligase), presenilin (an intramembrane cleaving protease; I-CliP) (Huppert and Kopan, 2001) and Nicastrin, a protein that interacts with presenilin and is required for plasma membrane localization and stabilization of presenilin (Kopan and Goate, 2002). Presenilin and Nicastrin are part of γ-secretase, an activity contributing to Alzheimer's disease (Kopan and Goate, 2000). A metalloprotease at the cell surface is also required for Notch signaling (Mumm and Kopan, 2000). In the nucleus, CSL binds SMRT (silencing mediator of retinoid and thyroid hormone receptor) and SKIP (ski-related protein). These proteins facilitate nuclear localization of CSL (Zhou and Hayward, 2001) and, together with histone deacetylase (HDAC), repress transcription from target genes (Mumm and Kopan, 2000). In *Drosophila*, CSL interacts with Hairless and C-terminal-binding protein (CtBP) to mediate repression (see panel 5) (Morel et al., 2001). This is the basal state of cells in which Notch is inactive or absent.

Panel B

Notch signaling starts with ligand binding (1-1′). Although all DSL proteins bind Notch that has not been modified by Fringe (1′), in cells expressing Fringe (1) ligands show distinct preferences. Delta prefers Fringe-modified Notch, whereas Serrate would much rather bind unmodified Notch. These preferences are the basis for Notch hyperactivation at boundaries between Fringe-expressing and -nonexpressing territories.

Despite being associated with the presenilin complex, Notch cannot be cleaved since its extracellular domain somehow blocks presenilin activity. After ligand binding, neuralized adds a ubiquitin to the intracellular domain of delta and triggers its endocytosis (2) (Kramer, 2001). Ligand endocytosis triggers a conformational change in Notch that permits metalloproteases to cleave near the membrane at a second site (S2), then the extracellular domain is trans-endocytosed to the ligand-expressing cell (3) (Parks et al., 2000). This event permits presenilin to cleave Notch at a third site (S3) located within its transmembrane domain (4) (Mumm and Kopan, 2000). NICD is now free to translocate to the nucleus. In cells expressing both Notch and ligand, ligand interferes with this process by an unknown mechanism; this interference may be relieved by the neuralized protein, which targets Delta for degradation (Kramer, 2001).

Once in the nucleus, NICD converts CSL from a transcriptional repressor to a transcriptional activator (5-8). Our current understanding proposes that this conversion occurs by direct protein-protein interactions between the Notch intracellular domain, SKIP and CSL, which leads to SMRT/HDAC dissociation (reviewed by Mumm and Kopan, 2000). This alone is sufficient to express some target genes. Notch/CSL can recruit histone acetylases (HATs) to assist in chromatin remodeling, and Mastermind/Lag-3 to activate additional targets. The metabolism of NICD in the nucleus is controlled by phosphorylation and ubiquitination by the E3 ubiquitin ligase Sel-10 (Gupta-Rossi et al., 2001; Oberg et al., 2001; Wu et al., 2001) and Su(Dx) (Cornell et al., 1999). NICD degradation resets the cell and prepares it for the next round of Notch signaling.

Panel C

Another activity of Notch, independent of DSL ligands and CSL proteins, was observed during *Drosophila* myogenesis and neurogenesis (Brennan et al., 1999a; Ramain et al., 2001; Rusconi and Corbin, 1999). The evidence for the existence of such a signaling axis in *Drosophila* is compelling, although still at an earlier phase of discovery compared with the Notch/CSL axis (A,B**)**. Therefore, many of the arrows in this panel describe genetic interactions rather than biochemical mechanism.

Two specific regions of Notch are required for this activity: the 'Abruptex' region of Notch (EGF repeats 17-29, dark blue) (Brennan et al., 1997; Brennan et al., 1999b; Ramain et al., 2001) and the region C-terminal to the ANK repeats that contains the PEST domain (Brennan et al., 1997; Ramain et al., 2001). This region was previously shown to bind Dishevelled (Dsh). Dsh is a mediator of Wnt signals downstream of the Frizzled (Frz) receptors (Huelsken and Birchmeier, 2001). During *Drosophila* neurogenesis, CSL-dependent Notch signals restrict neural competence to a small number of cells within an equivalence group (Kopan and Turner, 1996). In this newly recognized role, Notch acts to prevent cells from acquiring neural or myogenic competence earlier in development. Although it is unclear how Notch mediates this effect, or how many additional proteins are involved, this activity requires Deltex, a cytoplasmic ring finger protein (Ramain et al., 2001) and the kinase GSK3β (Sgg) (Brennan et al., 1999b; Ramain et al., 2001). Deltex and CSL appear to be antagonistic, possibly because both compete for the ANK domain of Notch. This cytoplasmic Notch activity may not require proteolysis (1,1′), however, it has not been determined yet whether

presenilin or other proteases are required for the Deltex-dependent Notch activity. Wnt acts to block Notch either directly, via the Abruptex domain (Wesley and Saez, 2000) (1-2) or, more likely, indirectly by stimulating Dsh to block GSK3 or to circumvent Deltex/Notch interaction (or both; 1′,2′). Interference with Notch/Deltex activity by Dsh requires the Dsh-binding region of Notch; deletions of this region and some Abruptex mutations render Notch a constitutive repressor of neural competence (2′) (Ramain et al., 2001). Removal of both Notch and wingless restores competence, suggesting that the only role of Wnt in acquisition of neural or myogenic competence is to antagonize Notch.

It is clear that not all the proteins facilitating inhibition of competence have been described. There is at this time no biochemical mechanism proposed for this process. Similar Wnt/Notch interactions in other organisms are yet to be discovered, casting doubt on the generality of these observations. The importance of the Abruptex mutations was recognized early – the name was given even before it was realized that locus is identical to Notch – but a biochemical explanation for the complex genetic behavior of Abruptex mutations is still lacking. These recent observations may begin to provide an answer.

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