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Review

Notch signaling: Distinct ligands induce specific signals during lymphocyte development and maturation

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Abstract

Notch signaling is a highly conserved pathway involved in cell fate choice during development with Delta and Jagged constituting the two evolutionary conserved families of Notch ligands. These ligands are transmembrane proteins with conserved biochemical structure that share their receptors and signal through a common mechanism. Upon ligand binding Notch receptors are proteoliticaly cleaved, the intracellular domain of Notch (NICD) is released and translocated to the nucleus, where it activates target genes. In mammals, four receptors and five ligands have been described. Delta-1, Delta-3 and Delta-4 are homologues to *Drosophila* Delta and Jagged-1 and Jagged-2 to *Drosophila* Serrate. Despite strong domain homology, there is growing evidence that signals transmitted through Delta or Jagged ligands can differentially affect the target cell. At least during embryonic development, Notch receptors and Notch ligands functions cannot be compensated by other members. Knock-out mice for Notch-1, Notch-2, Delta-1 and Jagged-1 are embryonic lethal [Swiatek PJ, Lindsell CE, del Amo FF, Weinmaster G, Gridley T. Notch1 is essential for post-implantation development in mice. Genes Dev 1994;8:707–19; Shimizu K, Chiba S, Kumano K, Hosoya N, Takahashi T, Kanda Y, Hamada Y, Yazaki Y, Hirai H. Mouse jagged1 physically interacts with notch2 and other notch receptors. Assessment by quantitative methods. J Biol Chem 1999;274:32961–9; Hrabe de Angelis M, McIntyre 2nd J, Gossler A. Maintenance of somite borders in mice requires the Delta homologue DII1. Nature 1997;386:717–21; Xue Y, Gao X, Lindsell CE, Norton CR, Chang B, Hicks C, Gendron-Maguire M, Rand EB, Weinmaster G, Gridley T. Embryonic lethality and vascular defects in mice lacking the Notch ligand Jagged1. Hum Mol Genet 1999;8:723–30]. Similarly, mice heterozygous for Delta-4 inactivation also die before birth [Gale NW, Dominguez MG, Noguera I, Pan L, Hughes V, Valenzuela DM, Murphy AJ, Adams NC, Lin HC, Holash J, Thurston, G, Yancopoulos, GD. Haploinsufficiency of delta-like 4 ligand results in embryonic lethality due to major defects in arterial and vascular development. Proc Natl Acad Sci USA 2004;101:15949–54]. Invalidation of Jagged-2 results in defaults in thymus morphology and γδ development [Jiang R, Lan Y, Chapman HD, Shawber C, Norton CR, Serreze DV, Weinmaster G and Gridley T. Defects in limb, craniofacial and thymic development in Jagged2 mutant mice. Genes Dev 1998;12:1046–57]. Altogether, these data suggest that each Notch member can exert unique specific effects.

In this review, we will thus focus on recent data about differential effects of Notch ligands on T cell development and differentiation. In light of recent biochemical and molecular advances on Notch-signaling pathway, we will examine how specific effects can be mediated by a given ligand.

Keywords: Notch; T cell development; Notch ligands; T cell homeostasis

Contents

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1. Specific effects exerted by Notch ligands

Notch receptors and ligands are expressed in developing and mature lymphocytes and in lymphoid tissues (see § 3). This pattern of expression suggests a role in both lymphocytes development and peripheral maturation. The role of Notch signaling in early B and T lymphocyte development has been extensively studied [7,8], while its influence on mature T cell function only recently emerged (review in Ref. [9]). It was recently shown that each ligand of Delta and Jagged family could exert specific effects on T cell development and maturation. Indeed, Delta-1 ligand has been involved in T cell development, whereas Jagged-1 has been mostly described for its influence on peripheral T cell differentiation.

1.1. Notch ligands and T cell development

To better evaluate the contribution of each ligand in lymphocyte development, a series of experiments were performed comparing directly the Delta and Jagged effects. Using the stroma cell line S17 over expressing human Delta-1 or human Jagged-1 on human $CD34^+$ hematopoietic stem cells, Jaleco et al. have shown that Delta-1 allows the emergence of cells with characteristics of T/NK precursors, while Jagged-1 did not apparently interfere with lymphoid development from hematopoietic progenitors [10]. Later on, using the M-CSF-deficient BM stroma-derived cell line OP-9, it was shown that over expressing of the Notch ligand Delta-1 can induce full T cell differentiation in vitro [11]. The ectopic expression of Delta-1 inhibits B cell maturation but sustain αβ and γδ T cell differentiation [11]. In this study, only $CD8^+$ T cells can be obtained, probably, because of the absence of MHC class-II molecules expression by OP-9 cells. We have shown using nude mice that in absence of thymus, both Delta-1 or Delta-4 over expression is sufficient to induce T cells development in vivo [12]. Thus, Delta ligands provide a signal sufficient for the induction of T cell lineage commitment even in absence of thymus [11,12]. In apparent contrast, it was shown that the absence of Delta-1 do not affect T cell development in mice [13], however, in absence of Delta-1, another ligand expressed in the thymus, i.e. Delta-4, could substitute Delta-1 and support T cell development. Indeed, Delta-4 over expression was tested both in vitro in OP-9 cell line [13] and in vivo in absence of thymus [12] and was shown to induce T cell commitment.

Using the OP-9 cell line Lehar et al. compared Delta-1 and Jagged-1 effects on murine BM-derived hematopoietic stem cells and thymus precursors at the DN1 stage [14]. They showed that the majority of BM-derived stem cells do not respond to Jagged-1 signal, similarly to human cells [10], whereas only the Delta-1-expressing stroma cells promote the proliferation and maturation of T cells progenitors [11]. They also showed that T cell progenitors at the DN1 and DN3 stages respond to Jagged-1 by differentiating along the NK and γδ cell lineages [14]. A role of Jagged ligands in γδ development has been already suggested since Jagged-2 deficient mice exhibit a decrease in γδ T cell lineage differentiation [6]. Concerning NK cells development, it was recently shown that OP-9 cell line over expressing Jagged-2 stimulates the development of NK cells from BM-derived HSC [15]. In this concern, we have reconstituted the immune system of normal mice with fetal liver cells over expressing Jagged-1 or Jagged-2, but we failed to observe any differences with control mice (de La Coste, unpublished data).

1.2. Delta ligands and lymphocytes maturation

Others and we have more recently shown that Delta ligands are involved in peripheral T cell maturation and that their functions are not redundant. In absence of a thymus, we showed that mature $CD4^+$ T cells developed in the presence of Delta-1 or Delta-4 show unique patterns of cytokine production after in vitro stimulation [12]. We showed that $CD4^+$ T cells developed in presence of Delta-1 only produced IFNγ, whereas $CD4^+$ T cells developed in presence of Delta-4 produce IFN-γ, IL-4 and IL-5. These observations suggest that Delta-1 is associated with Th1 polarization while Delta-4 induced a mixed Th1–Th2 phenotype. A similar Th1 polarization has been obtained in vitro using a soluble form of Delta-1 on TCR-activated T cells [16]. However, Amsen et al. found that Delta ligands promote Th1 differentiation using co-culture of naïve transgenic $CD4^+$ T cells with APCs

expressing Notch ligands, while Jagged-1 promotes Th2 differentiation [17]. These apparent discrepancies may be due to the different experimental system used. Nevertheless, in the Amsen study, Delta-4 expression was correlated with the ability of LPS to promote Th1 response and only the Delta-1 effects have been directly tested by over expression approach. It is, therefore, possible that the enforced expression of Delta-4 leads to different pattern of cytokines expression when compared with Delta-1 [17].

By over expressing Delta-1 and Delta-4 during lymphoid development, we also observed that Delta-4- or Delta-1-induced T cell development is associated with a significant increase in the number of ectopic developing DP T cells [12]. However, in mice over expressing Delta-4, we detected a marked increase in the number of peripheral DP cells (especially, in LNs), which was never observed in Delta-1 mice. Other groups [18] previously described the development of a lymphoproliferative disease associated with Delta-4 over expression [19]. In one study, the DP ectopic cells were not transplantable into secondary recipients suggesting that the observed phenotype was more likely due to lymphocyte proliferation rather than a neoplastic transformation-lymphoma [18]. In the second study, DP T cells were injected into recipient mice and lead to the development of acute lymphoma [19]. The proliferative disease described by Dorsh and co-workers was often lethal, resembling a pre-oncogenic situation, which we never observed after Delta-1 over expression [12]. These findings suggest that Delta-1 and Delta-4 ligands either bind the same receptor(s) with different affinities or bind distinct Notch receptors. Development of T lymphomas was also described in transgenic mice over expressing an activated form of Notch-3 [20], whereas retroviral over expression of Notch-1 was not associated with lymphoproliferative disorder [21,22]. Thus, these results suggest that T cell development induced by Delta-1 or Delta-4 in vivo is not equivalent.

Concerning peripheral $CD8^+$ T cells, we found that both Delta-1 and Delta-4 promote IFNγ production [12], therefore, confirming previous data showing that blocking of Notch inhibits IFNγ production by $CD8^+$ T cells [23]. Different results have been obtained in a model of organ transplantation [24]. In this study, the authors showed that pretreatment of spleen $CD8^+$ T cells with cells expressing Delta-1 ligand in presence of allo-antigen is able to inhibit the response to subsequent exposure to the same antigen [24]. This mechanism, which can result in graft survival, was shown to be $CD8^+$ T cells-dependant. Such $CD8^+$ responder T cells exhibit an altered cytokine production resulting in a decreased IFNγ production and an enhanced IL-10 expression [24].

1.3. Jagged ligands

The role of Jagged ligands on peripheral T cell function has been first suggested by in vitro data from Hoyne et al. [25]. Hoyne et al. have shown that murine-antigen-presenting cells over expressing human Jagged-1 in presence of antigen induce naïve $CD4^+$ T cells to become regulatory T cells and these T cells can induce tolerance in naïve mice [25]. It has also been shown that allo-antigen-presenting cells over expressing Jagged-1 when co-cultured with naïve T cells induce a decrease in IFNγ production, IL-2 and IL-5, consistent with induction of a regulatory phenotype [26]. This mechanism of antigen-specific tolerance induction mediated through activation of T cells by Jagged over expressing APCs is also occurring in human T cells. Epstein-Barr virus positive APCs over expressing Jagged-1 co-culture with autologous human T cells can induce antigen-specific regulatory T cells and modify immune response to viral antigens [27]. Transgenic mice over expressing an activated form of Notch-3 in thymocytes and T cells [20] contain a significantly increased population of $CD4^+$ $CD25^+$ T cells in thymus and spleen [28]. These cells were tested for regulatory functions and the authors showed that these transgenic mice are protected against induced-autoimmune diabetes [28]. These results support the idea of a central role for Notch pathway, possibly via Notch-3 and Jagged-1 interaction in sustaining regulatory T cells differentiation and function. In contrast to its peripheral effects, the role of Jagged ligands on hematopoietic stem cell self-renewal and differentiation is still controversial [29–33].

From all these data, it appears that Delta ligands are involved in T and B lymphocytes commitment and on T lymphocyte peripheral maturation, while Jagged ligands are more often involved in γδ and NK lymphocytes development and peripheral T cell differentiation. Overall, these data raised an intriguing question, how can the combination of Notch receptors with distinct ligands underlay so diverse biological outcomes of Notch-signaling pathway?

Notch ligands share a conserved extra-cellular domain containing multiple EGF repeats and a Delta–Serrate–Lag2 (DSL) motif that is required for receptor binding. Jagged proteins possess a distinct cystein-rich region proximal to the transmembrane domain and which it is not present in Delta ligands (for review, see Ref. [34]). This structural difference can constitute one explanation for Delta and Jagged distinct biological functions. However, this is not sufficient to explain how different ligand–receptor combinations can trigger distinct downstream events. The intracellular side of Notch ligands is variable among the homologs both in sequences and length [34]. Interestingly, it was observed that the conserved DSL motif, shared by all Notch ligands and involved in Notch–Notch ligand interactions is not functionally interchangeable [35]. Thus, the DSL motif alone is not capable to transmit a specific signal and the whole ligand sequence is necessary to generate a specific signal by a given ligand. Different features now emerge that might help to understand how can diverse outcomes be induced by the same set of receptors and ligands molecules. Notch signaling can possibly be regulated at three different levels, the Notch receptor itself, the Notch ligand or Notch–Notch ligand interaction. Regulation and modulation can occur at different steps of Notch signaling. We will now discuss about various – but not all – aspects

of Notch-signaling regulation and modulation that can help to understand how specific outputs can be induce by distinct ligands.

2. Expression of Notch receptors and ligands

In mammals, the presence of four Notch receptor and five ligands increases the possibility of different combinations of receptor–ligand binding. However, the four Notch receptors and five ligands are differentially expressed and tightly regulated during lymphoid development [36–39]. Therefore, the restrictive and temporal expression of all Notch genes can constitute by itself a mechanism for Notch-signaling regulation and specificity. By immunohistochemical analysis, it has been shown that Notch-1 is differentially expressed during T cell development with high level of expression at the most immature stages of development, i.e. double negative stage (DN), a decreased intensity at the double positive stage (DP) and an intermediate level of expression at the single positive (SP) stage [37]. These data show that Notch-1 expression is dynamic during thymus differentiation. In accordance with this pattern of expression during lymphocyte development, absence of Notch1 in hematopoietic stem cells blocks T cell development at the DN-1 stage [7], while conditional invalidation of Notch-1 at the DN-3 mice stage has no effect on T cell development, suggesting that Notch-1 activity is temporally restricted to early T cell development [40]. Notch-3 is also expressed in thymocytes while Notch-2 expression is weaker [36]. Notch-2 expression is associated with B cell development has been observed at the pro-B stage [41]. Consistently, conditional inactivation of Notch-2 does not affect T cell development [42]. Over expression of an activated form of Notch-2 permits early pro-B cell development but blocks B cell maturation at the pre-B stage [43]. Thus, pro-B cells may need to down-regulate Notch-2 expression to continue through B cell development. These data highlight the need of a dynamic pattern of Notch expression to ensure efficient signaling [43].

In the thymus, Delta-1 and Delta-4 expression is mostly found on stromal cells while Delta-3 expression is not detectable [39,13]. Expression of Delta ligands is higher in the cortex when compared with the medulla, reinforcing the idea that Delta-induced Notch activation is important for early T cell development. Jagged-1 expression is restricted to the thymus epithelium, whereas Jagged-2 is expressed in both lymphoid and stromal cell compartments of the thymus suggesting that Jagged ligands may play distinct role in Notch mediated T cell development [36]. It has been proposed that Jagged-2 is involved in reciprocal thymocytes interactions, whereas Jagged-1 action may be restricted to the cross talk between thymocytes and stromal cells [36].

Concerning peripheral hematopoietic populations, it is generally observed that APCs expressed Notch ligands [25,44] while lymphocytes sub-population expressed Notch receptors [16,17]. Nevertheless, the picture is not so simple and contradictory patterns of Notch receptors expression by peripheral naive T cell sub-populations have been reported using quantitative RT-PCR [16,17] indicating that potent antibodies, especially, for immunohistochemical analysis will be necessary to precisely determine Notch–Notch ligand expression profile for each hematopoietic sub-population.

3. Modulators of Notch signaling: the role of Fringe

One possibility to explain the differential effects of Notch ligands is a variation of Notch-signaling intensity depending on the ligand. The regulation of Notch signal intensity could possibly be due to the activity of Fringe modulator. Glycosylation of different EGF repeats are involved in interaction between Notch receptors and Notch ligands (review in Ref. [45]).

Fringe does not regulate Delta and Jagged ligands in an equivalent manner [46]. It has been shown that Lunatic Fringe suppresses Jagged-1 signaling while enhancing Delta-1 signaling, underlying a certain degree of diversity between modulators of Notch that belong to the same family [47]. One interesting point is that Lunatic Fringe can suppress Jagged-1-induced signaling even though Jagged-1 can still bind to Notch-1, suggesting that suppression occurs after ligand binding [47]. By using mutant proteins, the authors clearly demonstrated that Fringe modulation of ligand-induced Notch-1 signaling is strictly dependent on Fringe glycosyltransferase activity, but how changes in glycosylation can affect ligand-induced Notch signaling remains unknown. Losses in glycosylation could alter the structural properties of Notch [48]. However, it was next demonstrated that Fringe glycosylation of Notch-1 affects differentially Notch-1 proteolysis induced by ligand binding [46]. In this study, the authors showed that Notch-1 signaling induced by Delta-1 is enhanced by all Fringe proteins supporting the idea that Fringe proteins potentiate Delta-1-induced Notch-1 signaling through increased ligand binding. In response to Jagged-1, Lunatic and Manic but not Radical Fringe suppresses signaling without affecting ligand binding. This suggests that ligand–receptor interactions do not promote the proteolysis required for activation of downstream signaling events [46]. Binding assays on cultured cells and functional analysis of Notch mutations indicated that the EGF domain #12 repeat is important for Serrate–Notch interaction while others EGF repeats are involved in Delta–Notch interactions ([49], for discussion, see Ref. [45]).

Altogether, these findings suggest that different combinations of Notch ligands, receptors and Fringe proteins can deliver different levels of signaling and lead to different biological outcomes. How could glycosylation affect Notch–Notch ligand interactions to either positively or negatively regulated Notch signaling? One proposed hypothesis is that glycosylation of Notch potentiate signaling by enhancing

ligand binding, but this is not the case for Jagged-1. Biochemical analysis of modulation by Fringe proteins of Delta-1 and Jagged-1 binding on Notch-2 lead to the observation that each Fringe protein acts on a different site of the extra cellular region of Notch2 [50]. The authors proposed that the difference in modulator function of multiple Fringe proteins may result from the distinct amino acid sequence specificity targeted by these glycosyltransferases [50].

4. Proteolytic cleavage of Notch ligands

Biochemical studies have shown that Delta ligand is cleaved resulting in a soluble form constituted by the extra cellular domain (Delta–ECD), which function is unclear. The DSL ligands are proteolytically processed, but in contrast with Notch receptor, such cleavage seems to be constitutive [51–53]. The question now is to understand the function of such secreted ligand, does it act as a Notch activator or does it antagonize normal Delta-induced activation?

A secreted form of Notch ligand LAG-2 has been identified in Elegans [54]. This soluble ligand acts as a signaling molecule ([54,55], whereas in *Drosophila*, secreted forms of Delta and Jagged were shown to have antagonistic effects during eye and wing development [56]. In mammals, soluble forms of Delta and Jagged have been studied, however, depending on the cellular context and experiments they act as antagonist or agonist of Notch ligand or even as neutral molecules. It is thus difficult to have a clear scheme for the function of soluble Notch ligands in mammals [53,57–59]. Nevertheless, various hypothesis on soluble ligands function have been proposed: (1) the soluble forms of Notch ligands would regulate Notch-signaling pathway by competing with their transmembrane counterpart or (2) Notch ligands are cleaved to clear the cell surface of signal-delivering cell and thus stop activation of Notch pathway [60], a situation that does not agree with a constitutive cleavage. However, in both situations, Notch ligand cleavage permits the regulation of Notch signaling and thus, constitutes an important step of regulation between various ligands.

As far as we know this process of extra cellular shedding has been described only for Delta-1 in mammals and for both ligands in *Drosophila*. Additional data about the cleavage of other Delta ligands, especially, Delta-4 and Jagged ligands will be necessary.

5. Control of Notch–Notch ligands processing and endocytosis

Endocytosis was recently described as a major mechanism in Notch-signaling pathway and its role in Notch regulation was highlighted by recent findings. The first evidence for the role of endocytosis in Notch signaling came from the analysis of the shibire protein mutant in *Drosophila*. Shibire is a dynamic homolog necessary for the formation of endocytic vesicles and was shown to be necessary both in signal-generating cell (Delta-expressing cell) and signal-receiving cell (receptor-expressing cell) for normal Notch signaling [61]. Endocytosis concerns both Notch ligand and Notch receptor and thus, signal-generating cell and signal-receiving cell. A mechanism of *trans*-endocytosis was also described [62].

5.1. Delta endocytosis

The study of various mutants in *Drosophila* lead to the observation that Delta is transported to the cell surface and is then internalized by endocytosis [63,64]. Recent data suggest that this Delta ligands endocytosis is required for Notch activation [65]. Ubiquitine residues serve as a signal for endocytosis and two E3 Ubiquitine ligases are responsible for Delta ubiquitination; Mindbomb (Mib) was described in *Zebrafish* [66] and Neuralized (Neur) in *Drosophila* [67]. Mib physically interacts with Delta and promotes its ubiquitination and internalization [66], which have been shown to up-regulate Notch activity. In *Zebrafish mib* mutants, a reduced lateral inhibition mediated by Notch is observed due to a reduction in Notch-signaling activity [66]. Two models have been proposed to explain Delta endocytosis function. In the first one, Mib acts in the signal-delivering cell by clearing Delta from Notch at the cell surface and thus preventing *cis*-inhibition of Notch via Delta (see Ref. [68]). The second hypothesis is that Mib acts directly as an activator of Delta activity, allowing to conformational changes that unmask the S2 Notch cleavage site necessary for subsequent Notch signaling. This second hypothesis is supported by a number of accumulating data. Delta proteins, deficient for endocytosis, exhibit reduced signaling capacity in vivo during imaginal development [65] and fail to support *trans*-endocytosis of the extra-cellular domain of Notch (NECD) [65]. Delta was shown to be co-localized with NECD, suggesting that NECD forms a complex with Delta. This step is critical for Notch activation and is required to achieve processing and dissociation of Notch protein [65]. Thus, subsequent Notch signaling in signal-receiving cell is dependent on Delta endocytosis and NECD *trans*-endocytosis. Delta mutants lacking their intracellular domain inhibit Notch activation and act as dominant negative proteins [69,70]. One hypothesis is that by the absence of the intra-cellular domain, Delta is not endocytosed and thus failed to activate Notch signaling.

Neur and Mib exert similar roles in Delta endocytosis. However, the role of Neur was described in the developing eye of *Drosophila* [67] and its role in vertebrates remain unclear since loss of function experiments in mouse exhibit only a slight phenotype [71,72]. Recently, the function of the *Drosophila* ortholog of Mib (D-Mib) was tested and shown to be required for multiple Neur-independent/Notch-dependent developmental processes [73]. D-Mib is able to rescue various aspects of Neur mutant phenotype, suggesting that, at least in *Drosophila*, Neur and Mib exert overlapping

functions [73]. An interesting observation is that Neur preferentially regulates Delta, whereas D-Mib preferentially acts on Serrate [73], while they are both able to interact and regulate the two DSL ligands. These results suggest a certain degree of specificity between the ubiquitine ligase and the target ligand. Thus, it could be that these two ubiquitine ligases act synergistically in vertebrates, but more probably, that they are specifically co-expressed in different tissues with the appropriate Notch ligand, an issue that needs to be investigated.

5.2. Serrate–Jagged endocytosis

Until recently, only one study on *Drosophila* cultured cells, expressing Serrate, described a mechanism of *trans*-endocytosis of Notch receptor [74]. Endocytosis of Jagged ligands was never described in vivo. However, some new findings emerge suggesting that endocytosis of Notch ligands is a general mechanism of Notch-signaling activation and thus Notch regulation. It was demonstrated that D-Mib interacts with both Serrate and Delta ligands [73] even if D-Mib/Jagged association appears to be weaker than D-Mib/Delta one. Moreover, Mib targets both ligands for endocytosis, promoting their signaling activities in *Drosophila* [73]. However, the phenotypes observed in the *Drosophila* Neur mutant or in the *Zebrafish* and *Xenopus* Mib mutants strongly support the notion that ubiquitination of DSL ligands is required both in invertebrates and vertebrates. If Delta endocytosis and NECD *trans*-endocytosis are required and necessary for Notch-signaling activation in signal-receiving cell, it is then difficult to imagine a totally distinct mechanism for Jagged-induced Notch activation. Thus, it is more likely that all the different steps and partners necessary for Notch–Notch ligand endocytosis (Shi/dynamine, Mib, Neuralized) are differentially regulated and expressed depending on which Notch ligand is activating the target cell.

6. Transcriptional regulation

Upon ligand binding, Notch receptors are cleaved and the NICD is translocated to the nucleus, where it acts as a transcriptional regulator by displacing co-repressor proteins. The NICD contains domains mediating signal transduction. When in the nucleus, NICD associates with a transcription factor called CSL (CBF-1/RBP–Jk, suppressor of hairless (Su(H)), Lag-1) and recruits a co-activator protein Mastermind. Other transcriptional co-activators (p300) are thereafter recruited to form a multiproteic complex capable of transcriptional activation of target genes. In absence of NICD, the multiproteic complex is inhibited by the co-repressor Groucho and transcription is blocked (for review, see Ref. [75]). Thus, NICD is a potent regulator of gene expression as a nuclear co-activator.

Given the highly conserved domains of the intracellular region of the Notch receptors [34], the question of how distinct functions of Notch receptors can be induced is still open. The observation that over expression of constitutive active forms of Notch-1 and Notch-3 under the control of the lck promoter does not lead to the same results further supports that different Notch receptors may have distinct functions [21,36,76]. In addition, the inability of other Notch receptors, i.e. Notch-3, to compensate for the loss of Notch-1 during T cell development indicates that these proteins are not interchangeable [7]. Furthermore, in vitro studies have shown that the ICD of Notch-3, by contrast with Notch-1, is a poor transcriptional activator of HES [77]. In fact, they show that Notch-3 ICD acts as a Notch-1 repressor by competing with Notch-1 ICD for access to RBP–Jk and also that Notch-1 and Notch-3 compete for a common co-activator present in limiting amounts [77].

Notch-1 and Notch-2 have also been studied for their capacity to inhibit granulocyte differentiation [78]. While Notch-1 ICD inhibits granulocytic differentiation induced by G-CSF but not GM-CSF, Notch-2 ICD inhibits GM-CSF-induced granulocyte differentiation but not G-CSF [78]. The authors define a Notch cytokine response (NCR) region that is associated with differences in post-translational modifications and sub-cellular localization [78]. The phosphorylation of a critical serine residue in Notch-2 NCR is responsible for such specificity [79].

Thus, there are biochemical evidences that NICD contain specific regions and amino acid sequences that give a certain degree of specificity. Moreover, nuclear regulators of Notch may also regulate Notch signaling. For instance, Mint inhibits the transcriptional activity mediated by RBP–Jk. Double hybrid experiments showed that Mint competes with Notch1–ICD to interact with RBP–Jk [80]. Another negative regulator of Notch signaling, the Notch-regulated ankyrine-repeat protein (Nrarp) has been shown to block CBF-1-dependent transcriptional activation of Notch-responsive genes [81].

Notch-induced transcriptional activity can also be regulated by the quantity of Notch–ICD present in the nucleus. Two proteins have been identified as regulators of Notch and were identified as responsible for Notch ubiquitination and subsequent degradation. Sel10 is a negative regulator of Notch signaling [82], which interacts with the nuclear form of Notch [83] and is responsible for its ubiquitination and degradation by the proteasome [84]. Sel-10/NICD interaction takes place in the nucleus and permits to control the activation of target genes. The second regulator Deltex was first identified as a positive regulator of Notch pathway in *Drosophila* and encodes for a putative ubiquitine ligase. Deltex can act as a positive or negative regulator of Notch depending on the species [85]. Deltex directly interacts with NICD and possibly inhibits Su(H)–NotchIC interaction in the nucleus [86,87]. Further evidences demonstrated that Deltex is also associated with Notch-dependent transcriptional events [87]. It was shown in vitro on cultured cells that an important fraction of Deltex-1, one of the four mammalian homolog of *Drosophila* Deltex, is localized in the nucleus and physically interacts with p300 a co-activator molecule recruited into the

active complex by NICD in order to activate target genes expression [88]. However, the nuclear localization of Deltex remains controversial and it is generally admitted that Deltex is one of the cytoplasmic Notch regulators. Deltex and Sel-10 appear to be potent Notch-signaling regulators as they allow NICD degradation and thus repress the activation of specific targets genes.

A new type of regulation has been recently described. Numb an adaptator protein, which recruits the ubiquitination machinery, promotes Notch-1 ubiquitination at the cell membrane. When ubiquitinilated at the membrane, NICD is directly degraded, therefore, circumventing nuclear translocation and downstream activation of Notch-1 target genes [89]. A transgenic mouse model over expressing murine Numb under the control of the lck promoter has been created [90]. These mice do not exhibit any defects in T cell development again suggesting that Notch-1 activation is necessary at very early developmental stage and that a restricted window is open for Notch-1 to exert its activity and activate target genes.

7. Conclusions

Over the last 10 years, accumulating data came from analysis of Notch-signaling activation in mammals. One important observation is that post-translational modifications play a critical role in controlling Notch-signaling activity. Positive or negative regulators can regulate each step of Notch signaling. These regulators can be ubiquitous protein shared by various signaling pathways or specifics to Notch signaling. For instance, it has been shown that spacially restricted factors cooperate with Notch in the regulation of HES genes, the main Notch-signaling target genes [91] The diversity of proteins involved in Notch-signaling pathway strongly supports the notion that in a given cell, at a given time point, the induced output will be unique and specific because it will depend on the proteins available in the signal-receiving cell.

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