Coming to Grips with Notch

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Notch in Invertebrate Development. Notch proteins are highly conserved transmembrane receptors that are involved in cell fate regulation in invertebrates (1). While Notch receptors are initially synthesized as single polypeptide chains proteolytic processing results in the formation of a heterodimeric receptor in which the extracellular domain is noncovalently attached to the transmembrane and intracellular (IC) part. The Notch–IC is responsible for signaling and contains a series of ankyrin repeats similar to those found in nuclear factor (NF)-κB. The ligand-dependent release of Notch–IC requires processing by the membrane-associated presenilin which in a mutant form is responsible for familial Alzheimer's disease. The biochemical pathway of Notch–IC dissociation from the receptor is not completely understood but requires at some stage proteolytic cleavage (Fig. 1).

The Notch receptor ligands include Delta, a transmembrane protein that affects adjacent cells expressing Notch receptors (2), but may also interact with Notch receptors expressed by the very same cell. Also, soluble Delta ligands have been reported the action of which, however, appears restricted to immediately neighboring cells.

The detailed biochemical events that lead to the dissociation of Notch–IC from the heterodimeric Notch receptor after ligation are still unknown. There is also relatively little known of how Notch–IC regulates transcriptional activity: Notch–IC contains conserved nuclear localization sequences. However, Notch–IC may not directly enter the nucleus but may form a complex with the suppressor of hairless (Su(H)) protein which may function as a transcriptional regulator. Alternatively this complex may allow further posttranslational modifications. What appears to be clear is that only tiny amounts of Notch–IC reach the nucleus.

One of the more often discussed modes of Notch activity consists of so-called lateral signaling whereby apparently stochastic small differences of Notch receptors and Delta ligands on apparently otherwise equivalent neighboring cells are exaggerated by feedback loops resulting in cells that either express high levels of Notch receptors or high levels of Delta ligands. Again the biochemistry of these feedback loops is poorly understood. The important point is that the cells with high levels of Notch or high levels of Delta assume different developmental fates (2). However, there are clearly also interactions between Notch receptors and ligands on nonequivalent cells i.e., cells in which the developmental potential differs before the receptor–ligand interaction.

Notch in the Mammalian System. In mammals four different Notch receptors (Notch 1–4) and four different ligands (Jagged–1 and –2 as well as Delta-like 1 and 3) have been identified. Expression of Notch receptors and ligands is found in lymphoid tissue including bone marrow and thymus (3). The intracellular signaling pathways involve the association of Notch–IC with the mammalian equivalent of Su(H), CBF-1. CBF-1 is present in most mammalian cells and in the absence of the Notch functions as a transcriptional repressor. The association with Notch–IC may convert this function into a transcriptional activator, again by largely unknown biochemical events. Notch–IC binds also to the intracellular zinc finger protein Deltex which does not translocate to the nucleus. The formation of the CBF-1 Notch–IC and Deltex Notch–IC complexes may have different consequences, the former resulting in the activation of NF-κB and a family of basic helix loop helix (bHLH) proteins named Hairy Enhancer of Split (HES-I) whereas the latter may result in the repression of another bHLH protein E47. Both HES-I and E47 have important functions in the immune system (1; Fig. 1).

Notch in the Immune System. Initial studies on a putative role of Notch–1 in determining T cell fate in the mammalian immune system involved transgenic overexpression of Notch–IC that was associated with an increased ratio of CD8 over CD4 single-positive (SP) thymocytes (4), the commitment of which is controlled by the specificity of the α,βTCR for class I and class II MHC molecules, respectively (5). Such an effect was not apparent with a different transgenic Notch–IC construct that increased survival of immature thymocytes (6). The physiological relevance of these observations is not clear for several reasons: first, the increased number of SP CD8$^+$ thymocytes included abnormal cells that did not leave the thymus (4). Second, conditional inactivation of a floxed Notch–1 gene by the Cre-recombinase under control of a CD4 promoter failed to reveal any essential role of Notch–1 in the survival and ratio of SP CD8 and CD4 cells (7). These apparently contradictory results are consistent either with Notch re-

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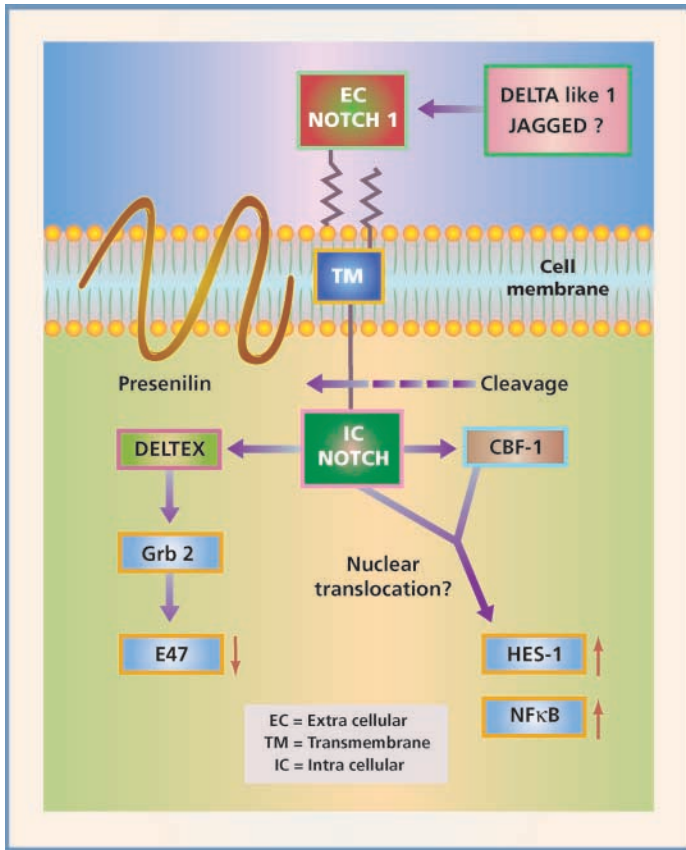
Figure 1. Regulation of transcription by Notch-1. Following binding of the Delta-like ligand, the Notch-IC part of the Notch-1 receptor is released, interacts with CBF-1, and regulates transcription. Binding of Notch-IC to the Deltex-ligand regulates transcription by a different pathway.

dundancy or transgenic artifacts of unphysiological overexpression. Third, the Notch-1 transgenic mice developed regularly tumors (4, 6) implying an unphysiological function of the transgene.

Analysis of loss and gain of Notch activity was, however, quite complementary at an earlier time in lymphoid development, namely the commitment of precursors to either the B or T cell lineage. An early report on the loss of function of the floxed Notch-1 gene by the Cre-recombinase under the control of the regulatable MX-promoter revealed a complete block in T cell development starting at the earliest double negative (DN1) activity precursors in the thymus (8). Subsequent reports on loss or gain of Notch-1 function, which are discussed in the following, significantly contribute to our understanding of T and B cell commitment in early precursors in the bone marrow or thymus as well as to the understanding of the mechanisms involved in the generation of acute T cell leukemias (9–12).

Instruction of T Cell Commitment by Notch. Recent studies by Wilson et al. (9) on the ablation of Notch-1 in adult mice are best compatible with the view that normally Notch-1 instructs T cell lineage commitment and that in its absence B cell development takes over: after inactivation of Notch-1 in lymphoid precursors the DN 1, 2, and 3 thymocyte subsets were rapidly diminished and in the thymus a concomitant increase in B cell precursors was observed. As B cell precursors were not detected in
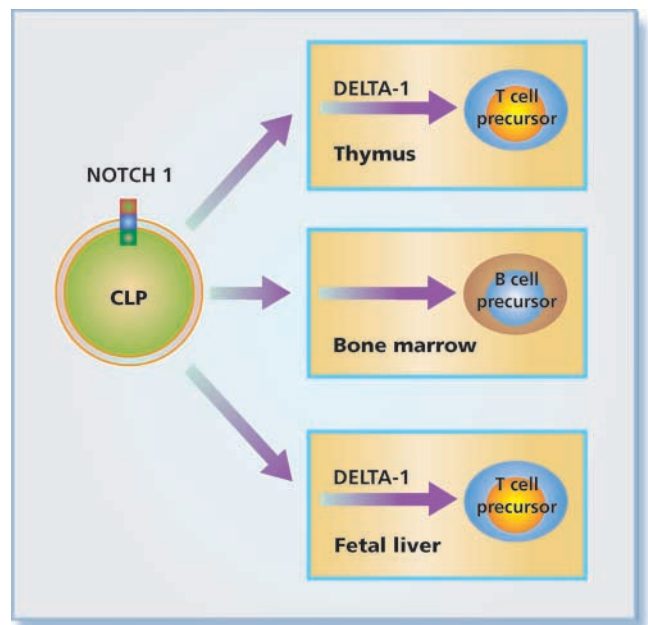

Figure 2. Hypothetical scheme of T versus B lineage commitment. When common lymphoid precursors (CLP) encounter the Notch-1 ligand Delta-like-1 in the thymus or fetal liver they become T committed precursors at the expense of B committed precursors. In the absence of Delta-like-1 ligands B cell development predominates.

the blood and B cells were generated after intrathymic injection of Notch-1$^{-/-}$ bone marrow, the authors concluded that B cell precursors did not migrate into the thymus but that instead of T cells B cells were much more efficiently produced from precursors in the thymus. Similarly, the ectopic expression of lunatic fringe, a modifier of Notch-1 signaling, in thymocytes caused in a non-cell-autonomous fashion the intrathymic development of B cells (10).

These analyses of loss of Notch-1 activity in the thymus are supported by studies on gain of Notch-1 activity in the bone marrow (11): a Notch-IC transgene blocked B cell differentiation in bone marrow while permitting the accumulation of CD4$^+$CD8$^+$ lymphoid cells in bone marrow and spleen. In pre-TCR–deficient mice (i.e., RAG$^{-/-}$, TCRβ$^{-/-}$, or SLP 76$^{-/-}$ mice) the Notch-IC transgene resulted only in the accumulation of cells with the phenotype of DN2 and DN3 thymocytes arguing that Notch-IC could not replace pre-TCR function (11, 13) but increased the number of T cell precursors before the onset of TCR rearrangement. These cells expressed in addition to the CD44/25 surface markers CD3ε and pTα at the RNA level. The authors interpreted these results to indicate that a Notch-IC transgene instructs T cell development in the bone marrow at the expense of B cell precursors, perhaps by inducing commitment to the T lineage in a common lymphoid precursor (11, 14). This possibility is consistent with a third study on Notch-1 activity (12) which analyzes the impact of expression by either Jagged-1 or Delta-like-1 ligands by murine stromal cells on the differentiation of human CD34-positive cells in an in vitro culture system: B cell development was blocked by Delta-like-1 but not Jagged-1 ligands in spite of the fact that both ligands were expressed in a functionally meaningful fashion. In addition, the authors observed that Delta-like-1 ligands on stromal cells permitted the accumulation of cells with characteristics of early T cells.

While none of the three studies directly addresses the question whether Notch-IC or Notch-ligands affect a common lymphoid precursor, the quantitative aspects as reported by Wilson et al. (9) and Allman et al. (11) are consistent with this idea while Jaleco et al. are careful in pointing out (12) that Notch may not necessarily instruct commitment in a common precursor but may arrest B cell committed precursors while allowing differentiation of T cell committed precursors. Thus there is still opportunity for more definitive experiments.

Notch-induced T lineage commitment may be achieved by the Notch-IC-Deltex complex dependent repression of the E box binding E47 protein, E47 being required for B lymphopoiesis (15). However, as pointed out (11) E2A activity appears to be required also at early stages in T cell development. Additionally, Notch-1 may activate through binding to CBF-1 another member of the bHLH proteins, the HESI that plays a critical role in the expansion of early T cell precursors (16). More detailed studies are required to define the mechanisms by which Notch-1 activity favors the T cell lineage.

At present a scenario can be envisaged where the absence of inductive signaling by Notch-1 in the thymus through Delta-like-1 ligation leads to the adoption of B cell fate by common lymphoid precursors whereas Delta-like-1–dependent Notch-1 activation diverts these precursors to the T cell lineage Delta-like-1 ligands may normally be only poorly expressed in the bone marrow (12) such that no T cell committed precursors could be formed outside the thymus in the adult organism. In fetal liver, however, T cell committed precursors have been described (16) and perhaps Delta-like 1 ligands are expressed there (Fig. 2).

Cooperation of Notch and the Pre-TCR in Acute Leukemia. Notch may induce T cell fate through HES-1 activation. It has also been reported that overexpressed Notch-IC enhances pre-TCR expression (6). It is clear, however, from the data reported in (10, 12) that overexpressed Notch-1 cannot replace pre-TCR function even though it may activate NF-κB to some extent (1).

It has perhaps not been sufficiently stressed that to date all mice harboring Notch-IC transgenes develop regularly tumors that exhibit markers of immature thymocytes. Such acute leukemia-like tumors can be generated through Notch-1 (4, 6, 17, 18) and Notch 3 transgenes (19) and require in all cases a functional pre-TCR (reference 10, and Screpanti, personal communication). It has been established that constitutive pre-TCR signaling, independent of any putative ligands on thymic stroma results in NF-κB activation (20) that may at least in part be responsible for anti-apoptotic as well as proliferation signals at the pre-TCR controlled checkpoint. Overexpressed Notch-IC may influence transformation in several ways: it may continually upregulate the pre-TCR and thus be responsible for continuous pre-TCR signals. It may also synergize with pre-TCR signals through direct activation of antiapoptotic pathways as well as NF-κB (1). Finally, pre-TCR signals may set the stage for Notch-induced transformation. It is not clear, at present, whether some translocation event results in Notch activation in these tumors. The fact, however, that Notch and the pre-TCR cooperate both in developmental progression and tumorigenesis (references 11 and 19, and Screpanti, personal communication) makes Notch and pTα possible targets for tumor therapy.

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