Notch proteins are transmembrane receptors that participate in a highly conserved signaling pathway that regulates morphogenesis in metazoans. Newly synthesized Notch receptors are proteolytically cleaved during transit to the cell surface, creating heterodimeric mature receptors comprising noncovalently associated extracellular (N\(^\text{EC}\)) and transmembrane (N\(^\text{TM}\)) subunits. Ligand binding activates Notch by inducing two successive proteolytic cleavages, catalyzed by metalloproteases and gamma-secretase, respectively, that permit the intracellular portion of N\(^\text{TM}\) to translocate to the nucleus and activate transcription of target genes. Prior work has shown that the presence of N\(^\text{EC}\) prevents ligand-independent cleavage of N\(^\text{TM}\), but the mechanisms involved are poorly understood. Here, we define the roles of two regions at the C-terminal end of N\(^\text{EC}\) that participate in maintaining the integrity of resting Notch receptors through distinct mechanisms. The first region, a hydrophobic, previously uncharacterized portion of N\(^\text{EC}\), is sufficient to form stable complexes with the extracellular portion of N\(^\text{TM}\). The second region, consisting of the three Lin12/Notch repeats, is not needed for heterodimerization but acts to protect N\(^\text{TM}\) from ligand-independent cleavage by metalloproteases. Together, these two contiguous regions of N\(^\text{EC}\) impose crucial restraints that prevent premature Notch receptor activation.

Notch proteins define a unique class of highly conserved transmembrane receptors regulating cell growth, differentiation, and death in different tissues of multicellular organisms (reviewed in reference 1). Aberrations in Notch signaling have been linked to several diseases in mammals. The first human homologue of Notch, NOTCH1, was identified through its involvement in chromosomal translocations found in human T-cell acute lymphoblastic leukemias/lymphomas (T-ALL) (12). In nonhuman mammals, retroviral activation of each of the other three mammalian Notch homologues (NOTCH2, NOTCH3, and NOTCH4) has also been linked to several inherited development disorders of humans, CADASIL syndrome (19) and Allagile syndrome (29), respectively.

Human NOTCH1 (hN1) has a crucial influence upon several stages of lymphocyte development (reviewed in reference 31). Expression of constitutively active hN1 in hematopoietic stem cells induces the development of CD4\(^+\) CD8\(^+\) double-positive (DP) immature T cells in the bone marrow, inhibits normal marrow B-cell development, and eventually leads to the appearance of fatal T-cell leukemias (32). Conversely, inducible notch1 knockout mice fail to develop mature T cells due to a requirement for notch1 during early stages of intra-thymic T-cell development (33). Thus, although Notch receptors are essential for the development of multicellular organisms, their activation must be precisely regulated.

Notch receptors are first synthesized as 300- to 350-kDa type I single-pass transmembrane glycoproteins. During maturation, Notch precursor polypeptides are proteolytically processed by a furin-like convertase at a site called S1 (Fig. 1A), yielding two noncovalently associated subunits (26). The resulting two associated subunits, here termed extracellular Notch (N\(^\text{EC}\)) and transmembrane Notch (N\(^\text{TM}\)), constitute the mature heterodimeric form of the protein present at the cell surface (2, 7).

The N\(^\text{EC}\) subunit of hN1 (Fig. 1A) contains 36 N-terminal epidermal growth factor (EGF)-like repeats that include the region responsible for ligand binding (23, 36). The EGF-like repeats are followed by a negative regulatory region (NRR), which participates in restraining premature activation of Notch receptors (15, 34). In hN1, the NRR includes the three LNR modules and an additional C-terminal tail of 103 residues (N\(^\text{EC}\)C). The N\(^\text{TM}\) subunit (Fig. 1A) includes a short extracellular region (N\(^\text{TM}\)X) containing a pair of conserved cysteines (25, 27), followed by a transmembrane segment, and an intracellular region (ICN). ICN consists of a RAM domain, seven ankyrin (ANK) repeats flanked by two nuclear localization signals, a transactivation domain (TAD), and a PEST region thought to regulate protein degradation (37).

Notch ligands are transmembrane proteins that are mem-
bers of the DSL (Delta, Serrate, and LAG-2) family. These proteins contain a cysteine-rich DSL domain essential for binding to the EGF-like domain in the NEC subunit of Notch receptors (16). In the current model for Notch activation, ligand binding to repeats within the EGF-like domain induces two sequential proteolytic events termed S2 and S3 cleavages. S2 cleavage is catalyzed by disintegrin-metalloproteases like tumor necrosis factor alpha (TNF-α)-converting enzyme (TACE) at a site lying 13 residues external to the transmembrane segment of the NTM subunit (8, 27). Following cleavage at S2, γ-secretase cleavage within the transmembrane domain at site S3 releases active ICN (39, 46). Once released from the plasma membrane, ICN translocates to the nucleus, where it interacts with members of the CSL (for CBF-1/RBPJκ, Suppressor of Hairless, and Lag-1) family of transcription factors, resulting in transcriptional activation of target genes (4, 11, 18, 20, 24, 39, 40, 43, 47, 48). The RAM and ANK domains of ICN participate in CSL binding, while the ANK domain is also essential for recruitment of coactivators and transactivation (21, 28, 38).

Enforced expression of forms of Notch lacking the NEC subunit results in phenotypes characteristic of constitutively active Notch signaling in a variety of model organisms (10, 13, 25, 30, 35, 42). Additionally, disruption of the mature heterodimeric form of the receptor leads to nuclear localization of a polypeptide of the size of ICN and increased transcriptional activation of a CSL-specific reporter gene (34). In contrast, forms of Notch lacking the EGF-like repeats, but retaining the NRR, are restrained in an inactive state and are unable to respond to ligand (20, 25, 35, 42). Furthermore, certain deletions and point mutations within the NRR lead to gain-of-function phenotypes in Drosophila (25, 42) and Caenorhabditis elegans (6, 15). Taken together, these studies suggest that a major role of the NRR region of the NEC subunit is to restrain activation of Notch receptors in the absence of ligands, yet permit activation in response to ligand binding.

Although it is clear that the NRR participates in the regulation of Notch activity, there is limited information about the mechanism by which this region restrains signaling. Here, we establish that the NEC region within the NRR is sufficient to

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**FIG. 1.** (A) Schematic representation of hN1. NEC, Notch extracellular subunit; NTM, Notch transmembrane subunit; NRR, negative regulatory region; LNR, domain comprising the three LIN12/Notch repeats; NEC-C, C-terminal segment of NEC; NTMX, NTM extracellular region; TM, transmembrane segment; ICN, intracellular Notch; TAD, transactivation domain; S1, furin cleavage site. (B) Truncated hN1 polypeptides used in these studies.
form a heterodimer with the extracellular region of N

"TMX" and that the LNR domain is necessary for preventing metalloprotease cleavage at the S2 site in the absence of ligand, even though it is not required for heterodimerization. In addition, Notch polypeptides lacking both the ligand-binding and the LNR domains activate CSL reporter genes in cultured cells and drive the appearance of CD4+CD8+ DP T cells in the peripheral blood when expressed in hematopoietic stem cells of mice, confirming that the removal of the LNR domain results in a ligand-independent gain-of-function phenotype. Together, these findings identify two distinguishable activities residing within the NRR: heterodimerization, mediated through the N9C-C region; and prevention of ligand-independent proteolysis, which also requires the LNR domain.

MATERIALS AND METHODS

Expression plasmids. The various forms of mammalian Notch used in this study are summarized in Fig. 1. Constructs for expression of N1, N2, and N3 polypeptides were designed for the purposes of expression in E. coli and mammalian cells by PCR amplification of human NOTCH1 (42), human NOTCH2 (41), and mouse NOTCH3 (22) cDNAs as templates. Eukaryotic hN1, hN2, and mouse N3 (mN3) constructs used for the subunit association studies were engineered in the plasmid vector pCDNA3 (Invitrogen). cDNAs encoding hN1 NRR-NTMX (residues Glu-1447 to Gln-1734), hN1 NRR-NCC-NTMX (residues His-1565 to Gln-1734), hN2 N2C-NTMX (residues Asp-1537 to Gln-1677), and mN3 N3C-NTMX (residues Glu-1504 to Ser-1614) were inserted in frame into an engineered BamHI site at the 3' end of the sequence encoding the hN1 signal peptide (bp +15 to +69). FLAG and hemagglutinin (HA) epitope tags were then introduced at the N and C termini, respectively. For transcriptional activation studies, cDNAs encoding hN1 N9C-NTM (residues Met-1 to Lys-2556), hN1 NRR-NTMX (residues Glu-1447 to Lys-2556), N9C-NTMX (residues Ala-1563 to Lys-2556), and ΔΔ (residues Glu-1674 to Lys-2556) were engineered in frame at the 3' end of the hN1 signal peptide in the pCDNA3 plasmid vector. For metalloprotease sensitivity experiments, three myc epitope tags were added in series at the C-terminal end of the N9C subunit of each construct. The bacterial hN2 N9C-NTM-XHA expression construct (residues Asp-1537 to Gin-2556) was engineered in the plasmid vector pET-21a (+) (Novagen). The construct was designed such that only a single additional methionine was added at the N terminus of the encoded hN2 polypeptide and a hexahistidine tag was added to the C terminus. To permit stable expression in bone marrow cells, cDNAs encoding hN1 NRR-NTMX and N9C-NTMX were cloned into the backbone of the retroviral shuttle vector MigRI (32).

Expression of Notch proteins in cultured cells. U2OS and 293T cells (American Type Culture Collection) were transiently transfected with Lipofectamine added to the C terminus. To permit stable expression in bone marrow cells, the N terminus of the encoded hN2 polypeptides and a hexahistidine tag was added 44 to 48 h after transfection with the Promega dual luciferase kit. Normalized firefly luciferase activities were measured in whole-cell extracts prepared 44 to 48 h after transfection with the Promega dual luciferase kit.

Metalloprotease sensitivity assay. U2OS cells transiently transfected with myc-tagged forms of hN1 (NRR-N9C-ntm and N9C-ntm) were treated with the presenilin inhibitor compound E (CalBiochem) at a concentration of 1 μM or with carrier alone (0.01% dimethyl sulfoxide) as described previously (45). Cells were washed once with ice-cold Hanks buffered saline and lysed in buffer F (50 mM Tris [pH 7.5], 10 mM methylsulfonyl fluoride, 10-μg/ml aprotinin, 10-μg/ml leupeptin, 1% NP-40) for 15 min on ice. Lysates were cleared by centrifugation at 16,000 × g for 15 min at 4°C. hN1 polypeptides were immunoprecipitated from whole-cell lysates (2 x 106 cells) by incubation for 2 h at 4°C with anti-myc antibody (9E10) cross-linked to protein G-Sepharose beads (Sigma). After incubation, beads were washed twice with buffer F and once with a mixture of 50 mM Tris, pH 7.5, and 100 mM NaCl. Anti-myc immunoprecipitates were treated with lambda protein phosphatase (New England BioLabs) as recommended by the manufacturer prior to Western blot analysis.

Murine bone marrow transplantation assays. All experiments were performed as described previously (4) and were conducted in accordance with National Institutes of Health guidelines for the care and use of animals and with an approved animal protocol from the University of Pennsylvania Animal Care and Use Committee. Briefly, cDNAs cloned into the MigRI vector were packaged into retroviruses by transient transfection of Bone23 cells. After titration of viruses on NIH 3T3 cells, green fluorescent protein (GFP)-normalized retroviral supernatants were used to “spinoculate” bone marrow cells from lethally irradiated (900 rads) C57BL/6 mice (Taconic Farms). The retrovirally transduced bone marrow cells were then injected into lethally irradiated (900 rads) 4- to 8-week-old female syngeneic recipients. Three weeks posttransplantation, peripheral blood sampled from retro-orbital sinuses was assessed for the presence of GFP+ immature T cells by flow cytometric analysis (Becton Dickinson; FACSCalibur). Cells were incubated with biotinylated CD8 (Becton Dickinson; FACSCalibur). Cells were incubated with biotinylated CD8 and phycoerythrin-conjugated CD4 (RM4-5) (Pharmingen). Biotinylated anti-CD8 and phycoerythrin-conjugated CD4 were collected, biphosphorylated, resuspended in water, and analyzed by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry.

RESULTS

The NRR-NTMX precursor from hN1 is properly processed into noncovalently associated subunits. Several lines of evi-
idence suggested that association between the two subunits of hN1 after S1 cleavage results from contacts between the NRR of the NEC subunit and the extracellular stub of the N TM subunit (NTMX). Therefore, we first designed a construct for expression of the NRR-NTMX precursor, predicting that this polypeptide would be processed at the S1 site during maturation and secreted into the culture medium when expressed in mammalian cells.

To determine whether the precursor is processed at the S1 site during maturation and whether the NRR and N TMX fragments remain associated with each other after cleavage at the S1 site during maturation, we expressed the NRR-NTMX precursor polypeptide of hN1 with FLAG and HA epitopes at the N and C termini, respectively, to facilitate detection of the individual subunits of the complex in immunoprecipitation assays (Fig. 1B). Following transient transfection into 293T cells, the single-chain precursor (NRR-NTMX) was detected in whole-cell extracts (Fig. 2A), whereas immunoprecipitates prepared from conditioned media using either FLAG or HA-coupled beads contained both epitope-tagged subunits, NRR and N TMX (Fig. 2B), but little or no uncleaved precursor. Thus, the two subunits remain stably associated as an NRR-NTMX heterodimer after furin cleavage and secretion into the conditioned media.

Recombinant furin-cleaved N ECC-NTMX forms a stable heterodimer. To prove unequivocally that other factors are not required for the formation of stable N ECC-NTMX heterodimers, we purified a histidine-tagged, recombinant form of the N ECC-NTMX precursor from hN2 (Fig. 4A) and tested whether the two subunits remained associated after furin cleavage. Before furin cleavage, a sample of the purified N ECC-NTMX-H6 precursor elutes as a single peak when analyzed by denaturing HPLC on a C18 column (Fig. 4B, top). After furin cleavage, however, two peaks are present in the HPLC chromatogram (Fig. 4B, bottom), even though the two subunits copurify as a single peak on gel filtration with the same volume of elution as the precursor polypeptide. The first peak (retention time, 46 min) results from N TMX-H6 and the second from N ECC (retention time, 68 min), based on masses determined by MALDI-TOF mass spectrometry (N ECC ex-

**FIG. 2.** Recovery of hN1 heterodimeric complexes secreted into conditioned media. Notch1-derived polypeptides with FLAG and HA epitope tags were resolved by SDS-PAGE under reducing conditions and detected on Western blots. Antibodies used to detect NRR and N TMX were anti-FLAG and anti-HA, respectively. (A) The NRR-NTMX single-chain precursor (36 kDa), detected in whole-cell extracts by Western blot with anti-FLAG. (B) Noncovalently associated NRR (27 kDa) and N TMX (8.4 kDa) subunits, coimmunoprecipitated (IP) from conditioned media with anti-FLAG or anti-HA as indicated, and detected by Western blot using anti-FLAG (top panel), or anti-HA (lower panel). (C) Dissociation of the heterodimer upon treatment with SDS. Coimmunoprecipitated NRR and N TMX subunits were treated with 1% NP-40, or 0.025% SDS prior to Western blotting with anti-FLAG (top panel) or anti-HA (lower panel).
The N\textsuperscript{NEC}C region is sufficient for formation of stable heterodimers with N\textsuperscript{TMX}. (A, B) N\textsuperscript{NEC}-N\textsuperscript{TMX} heterodimers were recovered from conditioned media by immunoprecipitation (IP) with either anti-FLAG or anti-HA and were subjected to SDS-PAGE under reducing conditions. Each subunit was then detected by Western blotting for its respective epitope tag. (Top panel) Detection of N\textsuperscript{NEC} with anti-FLAG; (bottom panel) detection of N\textsuperscript{TMX} with anti-HA. (A) hN1 heterodimers. (B) hN2 and mN3 heterodimers. The asterisk denotes a polypeptide of slightly smaller molecular weight than hN2 N\textsuperscript{NEC} that is not competent for association with N\textsuperscript{TMX}.

C

<table>
<thead>
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<th>Protein</th>
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<td>cN 1593 ENKTCQSEPFLAGLMSVMVPNVEA REILACPLIGRHSILTVVRKHDAH 1645</td>
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Expected molecular mass = 8,419 kDa, actual = 8,420 kDa; N\textsuperscript{TMX}-H\textsubscript{6} expected molecular mass = 8,494 kDa, actual = 8,489 kDa). These results demonstrate that the N\textsuperscript{NEC}C region of hN2 is sufficient to form a stable heterodimer with N\textsuperscript{TMX}.

LNR modules prevent ligand-independent Notch activation in cells. Prior studies suggesting that the LNR domain is needed to restrain Notch signaling were conducted with mutations or deletions involving the LNR domain within recep-
tors that retained the EGF repeat ligand-binding region. Thus, increases in Notch function stemming from these mutations could have resulted from either ligand-independent signaling or hyperresponsiveness to ligand. To distinguish between these possibilities, we compared the activities of two hN1 constructs: NRR-N\textsuperscript{TM}, which lacks the ligand-binding domain, and N\textsuperscript{EC}-N\textsuperscript{TM}, which lacks both the ligand binding and LNR domains. We first compared the abilities of these two constructs to activate transcription from a CSL-dependent luciferase reporter gene in cultured cells (Fig. 5). As anticipated, NRR-N\textsuperscript{TM} had no intrinsic signaling activity in this assay. In contrast, NECC-N\textsuperscript{TM} demonstrated substantial ability to activate CSL, confirming that the LNR domain is needed to prevent ligand-independent receptor activation. The extent of receptor activation at equivalent doses of input was more modest than that observed with a construct that lacks the entire NEC subunit (Fig. 5; designated H9004). In Western blots, we consistently observe that NECC-N\textsuperscript{TM} expresses at lower levels than does H9004 (as in Fig. 6B) (data not shown), possibly explaining the observed difference in activity.

The LNR domain prevents ligand-independent proteolysis of Notch receptors. Since the LNR domain is not necessary to maintain stable association of the two Notch subunits, we hypothesized that it protects N\textsuperscript{TM}X from ligand-independent proteolysis. To test this idea, we compared the extents to which NRR-N\textsuperscript{TM} and N\textsuperscript{EC}-C-N\textsuperscript{TM} forms of hN1 are cleaved at the S2 site (Fig. 6A).

Normally the product of S2 cleavage (N\textsuperscript{TM*}, Fig. 6A) is rapidly cleaved at the S3 site by gamma-secretase to release ICN1 (i.e., the active form of Notch), a species that has a very short half-life. However, in the presence of gamma-secretase inhibitors, N\textsuperscript{TM*} is stabilized, allowing it to accumulate and be detected (45). Cells transiently expressing N\textsuperscript{EC}-C-N\textsuperscript{TM} accumulate readily detectable amounts of N\textsuperscript{TM*} in the presence of a gamma-secretase inhibitor, whereas cells expressing NRR-N\textsuperscript{TM}, a molecule that still has the LNR modules but is quiescent in...
the reporter assay, do not (Fig. 6B). Together, these results show that although the LNR domain is not essential for association between the two Notch subunits, it does play a critical role in the regulation of receptor activity by preventing premature proteolytic activation in the absence of ligand.

Loss of the LNR modules causes Notch gain of function in a mouse model. To assess the biological activity of N Ecc-NTM in vivo, we scored its activity in a murine bone marrow transplantation assay. When used to reconstitute irradiated mice, bone marrow stem cells transduced with gain-of-function forms of Notch1 rapidly give rise to an abnormal CD4+ CD8+ DP T-cell population in the bone marrow that spills into the peripheral blood by 3 weeks posttransplantation (4, 31). In these experiments, we used the retroviral vector MigRI, which also drives the expression of GFP from a bicistronic mRNA with an internal ribosomal entry sequence, in order to gate on transduced cells. The GFP+ cells in the peripheral blood of a mouse reconstituted with hematopoetic stem cells transduced with MigRI virus (4, 32). In contrast, 70% of the GFP+ cells in the peripheral blood of a mouse reconstituted with N Ecc-C-N TM transfused marrow cells are CD4+ CD8+ immature DP T cells (Fig. 7). Similar results were obtained with nine mice in each group (not shown). Thus, the LNR region is necessary to prevent ligand-independent receptor activation in vivo.

DISCUSSION

Because Notch signaling controls so many developmental events, its activation must be precisely controlled. Here, we define the roles of two regions at the C-terminal end of the N Ecc subunit that participate in maintaining the integrity of resting Notch receptors through distinct mechanisms. The domain mapping experiments define a previously uncharacterized region, termed N Ecc-C, that associates directly with N TM to create the stable Notch heterodimer (Fig. 2 to 4). The LNR modules, which are not required for heterodimerization, are needed to prevent premature proteolytic cleavage at the S2 site of NTM. This NECC region ranges in size from 70 to 103 residues among the mammalian Notch receptors and includes a number of highly conserved residues.

FIG. 6. The LNR domain of hN1 confers resistance to proteolysis at the S2 site. (A) Schematic representation of the hN1 polypeptides used in the proteolysis experiments, noting the position of the myc epitope tag, the site of S2 cleavage, and the difference in polypeptide length between native N TM and the product after S2 cleavage, denoted N TM*. (B) The S2 proteolysis product N TM* is generated when the LNR domain is deleted from hN1. U2OS cells transfected with the indicated hN1 variants were incubated in the presence of a presenilin inhibitor (DFP-AA). N TM and N TM* polypeptides were then recovered by immunoprecipitation with an anti-myc antibody, resolved by SDS-PAGE under reducing conditions, and detected by Western blotting using anti-myc.

FIG. 7. A form of Notch1 lacking both ligand-binding EGF repeats and the LNR domain, N Ecc-C-N TM, causes abnormal T-cell development. Peripheral blood was obtained from mice (n = 9 for each group) on day 54 after reconstitution with hematopoetic stem cells transduced with MigRI retroviruses encoding either NRR-N TM, which lacks only the ligand-binding domain, or N Ecc-C-N TM. Representative flow cytometric results are shown.

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conserved hydrophobic residues that might participate in the association with N\textsuperscript{TMX} (Fig. 3C). Most striking among the conserved sequences is the cluster of aliphatic amino acids that lies near the N\textsuperscript{EC}C N-terminal end (Fig. 3C).

N\textsuperscript{EC}C is not as well conserved in \textit{Drosophila} Notch, consistent with the possibility that fly Notch may not require S1 cleavage for activation. However, significant sequence similarity does exist in this region, both within the hydrophobic stretch near the N-terminal end and at a series of invariant residues scattered throughout the region. Efforts to determine a high-resolution structure of a mammalian Notch heterodimer, which will reveal the nature of the interface in detail, are now under way.

Mutations in N\textsuperscript{EC}C and in its heterodimerization partner, the extracellular stub of N\textsuperscript{T}M (N\textsuperscript{T}MX), have been previously associated with gain-of-function phenotypes in both flies and worms. In worms, the S642N mutation in N\textsuperscript{EC}C of the glp-1 notch homolog causes a gain-of-function phenotype (6). In flies, simultaneous replacement of the two conserved cysteines in N\textsuperscript{T}MX with serine residues leads to gain of function, as assessed by the development of an antineurogenic phenotype (25), whereas point mutations elsewhere within N\textsuperscript{T}MX are associated with gain of function in worms (15). The biochemical studies reported here provide a rationalization for the observed gain of function in the genetic studies; various mutations in either N\textsuperscript{EC}C or N\textsuperscript{T}MX can destabilize the heterodimer, causing premature subunit dissociation after S1 cleavage and subsequent Notch activation by the normal proteolytic cascade of S2 and S3 cleavages.

LNR domains are a unique feature of Notch receptors, existing in all members of the family as three tandemly repeated modules. Although the N\textsuperscript{EC}C region is sufficient to form a stable heterodimer with N\textsuperscript{T}MX, it is not sufficient to prevent proteolytic activation in the absence of the adjacent LNR modules (Fig. 5 and 6). The implication of these studies is that LNR mutations cause gain-of-function phenotypes because they allow proteolysis at the S2 site even in the absence of ligand, although without promoting premature dissociation of the subunits. The effects we observed previously showing that EDTA dissociates and activates Notch heterodimers may be explained by loss of this “anti-protease” activity, as these experiments were performed (whole-cell lysates or intact cells at 25 to 37°C) under conditions in which proteases may have been active.

More generally, our studies show that stable heterodimer association and prevention of ligand-independent activating cleavages are separable functions that can be attributed to two different regions of the extracellular domain of Notch and raise further mechanistic questions. Most notably, does the LNR domain prevent ligand-independent proteolysis merely by sterically preventing access of metalloproteases to the S2 site, or do more complex mechanisms come into play? It will also be important to resolve the puzzle of how physiologic ligand binding overcomes the intrinsic restraint on signaling imposed by the LNR domain.

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ADDITIONAL IN PROOF

A report coauthored by several of us now shows that activating mutations of human Notch1 are frequently found in human T-cell acute lymphocytic leukemia. The majority of these gain-of-function mutations are located within the heterodimerization domain defined by these studies (Science, in press).

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