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Notch Cleavage: Nicastrin Helps Presenilin Make the Final Cut

Dispatch

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Presenilin is thought to be the proteolytic component of γ-secretase, responsible for the intramembranous cleavage of substrates that include the activated Notch receptor. Recent studies have identified the novel protein Nicastrin as another essential component of the Presenilin/γ-secretase complex.

Notch is a transmembrane receptor that is the focal member of a widely utilized, eponymous signal transduction cascade; it mediates cell–cell interactions and pattern formation in all metazoan organisms examined to date. Nearly a decade ago, several groups independently noticed that a constitutively active Notch fragment consisting of its intracellular domain (N^{IC}) is nuclearly localized, a provocative observation that suggested a function for this transmembrane protein in the nucleus (reviewed in [1]). Although the evidence for this hypothesis took some years to accumulate, it is now generally accepted that activation of Notch at the cell surface by ligand triggers proteolytic cleavage and release of N^{IC}, which then translocates to the nucleus and functions as a transcriptional coactivator. Now a new player has been identified: the novel protein Nicastrin, which recent studies [2–4] show plays an important part in the Presenilin-mediated cleavage and release of N^{IC} from the plasma membrane.

Notch Cleavage: It's As Easy As S1, S2, S3

Notch processing is unexpectedly complex and includes multiple proteolytic events (Figure 1) referred to as the S1, S2 and S3 cleavages (reviewed in [5]). S1 cleavage in the extracellular domain of Notch occurs constitutively in the trans-Golgi network and is mediated by a furin-like convertase; reassembly of the fragments creates a heterodimeric Notch receptor at the cell surface. This event has been most closely characterized with respect to mammalian Notch, but there is evidence that fly Notch is similarly processed. S2 cleavage by a disintegrin/metalloprotease occurs in response to ligand binding and releases the majority of the extracellular domain. This cleavage is believed to be mediated by TACE in vertebrates and may be mediated by the related but distinct protein Kuzbanian in *Drosophila*, although the precise role of the latter protein is controversial at present.

The resulting membrane-anchored fragment, referred to as Notch extracellular truncation (NEXT), is subject to intramembranous S3 cleavage by γ-secretase, which finally releases N^{IC}. The mechanism of this particular cleavage has attracted considerable interest for two reasons. First, it is the key event that regulates nuclear translocation of N^{IC} and its second life as a transcriptional coactivator. Second, a processed carboxy-terminal fragment of the β-amyloid precursor protein (β-APP) is subject to a highly similar intramembranous cleavage, an event that underlies the formation of cytotoxic plaques found in patients afflicted with Alzheimer's disease. Understanding the mechanism of γ-secretase-mediated cleavage is thus potentially of great medical relevance as well.

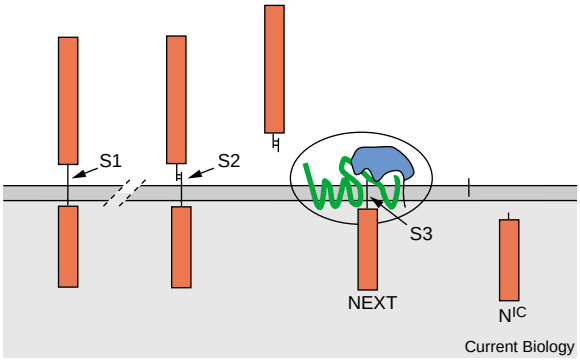

Figure 1. Summary of Notch cleavage events.

S1 cleavage in the extracellular domain of Notch (red), catalysed by a furin-like convertase, occurs constitutively in the Golgi. The two halves are reassembled as an intramolecular heterodimer present at the plasma membrane. Interaction with ligand induces S2 cleavage by a disintegrin/metalloprotease (TACE, and possibly Kuzbanian), generating the Notch extracellular truncation (NEXT). NEXT is processed by the γ-secretase complex (oval), which includes Nicastrin (blue) and Presenilin (green). Nicastrin is a single pass transmembrane protein that might aid in the assembly or trafficking of the γ-secretase complex to the plasma membrane and/or help to recruit substrates. Presenilin is an eight-transmembrane domain, putative aspartyl protease thought to be the catalytic component of γ-secretase. The order and timing of γ-secretase/substrate assembly is not well defined, and they may exist as a complex as Notch is trafficked to the cell surface (i.e., prior to S2 cleavage). However, only the NEXT fragment, and not heterodimeric Notch, is efficiently cleaved by γ-secretase. S3 cleavage in the transmembrane domain releases N^{IC}, which subsequently translocates to the nucleus.

Presenilin and Nicastrin in S3 Cleavage

A wealth of studies using invertebrate and vertebrate model systems demonstrated that Presenilin is a key component of γ-secretase which is biochemically required for Notch S3 cleavage and genetically essential for Notch signal transduction (reviewed in [5]). Presenilin is an eight-transmembane domain, putative aspartyl protease which is widely thought to be the enzyme that cleaves γ-secretase substrates. Presenilin co-purifies with the γ-secretase complex and is labeled by transition-state analogue inhibitors of γ-secretase. However, direct demonstration of Presenilin-mediated substrate cleavage *in vitro* has

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not yet been possible, probably because of the requirement for additional factors in the large ~2000 kD γ-secretase complex.

In a satisfying convergence of genetic and biochemical approaches, orthologs of a novel single-pass transmembrane protein involved in Notch signal transduction and γ-secretase activity were recently isolated independently from worms and humans. This protein, first known as APH-2, was identified from a screen for maternal-effect mutants of the nematode *Caenorhabditis elegans* that have phenotypes resembling those resulting from mutations in GLP-1, one of two nematode Notch receptors [6]. APH-2 localizes to cell-surface membranes and is required for GLP-1-mediated cell–cell interactions in the early embryo. It is subsequently required for signaling by LIN-12, the other worm Notch receptor, during development of the somatic gonad [7].

Complementary biochemical studies [8] identified the human homolog of APH-2, Nicastrin, as a protein that could be stoichiometrically immunopurified with Presenilin. Yu *et al.* [8] named the protein after the Italian village Nicastro, home to members of an extended family that were key to studies that eventually linked mutations in the two human presenilin genes to the most frequent forms of familial Alzheimer's disease. Nicastrin forms a ternary complex with Presenilin and the γ-secretase substrates NEXT or carboxy-terminal β-APP [8,9]. Furthermore, misexpression of various forms of Nicastrin was found to modulate processing of β-APP, indicating a functional link between Nicastrin and γ-secretase. Finally, Nicastrin function has been conserved, as human Nicastrin can partially complement the *aph-2* mutant phenotype [7].

In spite of these compelling links between Nicastrin and Notch signaling, some important connections remained to be made. For example, misexpression of various Nicastrin mutants that strongly affect β-APP processing only mildly affect Notch processing [9]. Also, *aph-2* mutations affect some, but not all settings of Notch activity [7]. These negative data could be reasonably explained, however, if Notch processing was efficient enough to withstand the presence of putative dominant-negative Nicastrin isoforms, while maternal contributions might obscure the apparent genetic requirements for *aph-2*. The Struhl, St. Johnston and Fortini groups [2–4] have simultaneously characterized *Drosophila* Nicastrin and now reported powerful genetic evidence for its general requirement during Notch S3 cleavage *in vivo*.

Ovary and post-embryonic clones of *nicastrin* mutant cells in *Drosophila*, as well as embryos deficient for maternal and zygotic Nicastrin, display a panoply of *Notch*-like defects which encompass many different settings of Notch activity [2–4]. These include a failure of ovarian follicle cells to cease cell division and begin differentiation, hyperplasia of the embryonic and adult nervous system, thickening of wing veins and failure to specify the wing margin. Although *ex vivo* blastomere recombination experiments seemed to indicate that APH-2 might function in either the signal-sending or signal-receiving cell [6], mosaic clonal analysis demonstrated that Nicastrin functions strictly cell autonomously *in vivo*, and that cells do not require Nicastrin to send the Notch signal [2,3]. Thus, Nicastrin is generally required for Notch signaling and functions in signal-receiving cells in *Drosophila*.

To determine the site of Nicastrin function in signal reception, these researchers took advantage of two types of constitutively active Notch deletion variant previously used to characterize Notch and Presenilin function. Although these variants signal independently of ligand in wild-type tissue, they differ in their requirement for Presenilin. The first, ΔECN, resembles NEXT in that it lacks most of the extracellular domain but remains membrane anchored; it requires Presenilin for S3 cleavage and access to the nucleus. The second, N^{IC}, is soluble, translocates freely to the nucleus, and signals independently of Presenilin. These Notch derivatives display identical requirements for Presenilin and Nicastrin for access to the nucleus and signal transducing activity, indicating that Nicastrin also functions in S3 cleavage of Notch (Figure 1) [2–4]. These genetic data were substantiated with biochemical data demonstrating an accumulation of NEXT in the absence of either Nicastrin or Presenilin. Although the cellular location of S3 cleavage has been the subject of debate, *nicastrin* and *presenilin* follicle cell clones accumulate NEXT at the apical membrane, suggesting that the plasma membrane is indeed the location of S3 cleavage [3].

Possible Functions of Nicastrin

Nicastrin exhibits independent interactions with Presenilin and substrates, which led to the proposition that it might help to recruit substrates to the γ-secretase complex for processing by Presenilin [8,9]. Surprisingly, Presenilin-dependent intramembranous cleavage does not absolutely require any specific sequence motif. Instead, a primary determinant appears to be that the size of the substrate extracellular domain be small [10]. As some 95% of Nicastrin is located extracellularly, it was speculated that Nicastrin might help to measure the size of the extracellular domain of potential γ-secretase substrates [2,4].

Nicastrin also appears to regulate Presenilin stability, as Presenilin levels are strongly reduced following prolonged reduction of Nicastrin in cell culture or in large clones *in vivo* [2–4]. Nicastrin, perhaps complexed to substrate, might positively influence Presenilin endoproteolytic maturation or incorporation into the complex, either of which could stabilize Presenilin. Alternatively, Nicastrin may be required for transport of the Presenilin/γ-secretase complex to the plasma membrane. This possibility is suggested by the observation that the normally high level of *Drosophila* Presenilin at the apical membrane is nearly absent in *nicastrin* imaginal disc clones, although subapical Presenilin persists [2].

Curiously, APH-2 displays the reciprocal requirement for nematode Presenilins SEL-12 and HOP-1 for its normal localization to the cell surface [11], further suggesting that the γ-secretase complex may need to be substantially assembled prior to trafficking to the cell surface. In fact, it is conceivable that Notch normally arrives at the plasma membrane already

associated with components of γ-secretase, as Notch–Nicastrin and Presenilin–Notch interactions can be detected in the secretory pathway [9,12]. A more detailed understanding of how the γ-secretase complex is assembled, both in terms of order of addition as well as subcellular location of interactions, should help to resolve some of these possibilities. Finally, it should be noted that Nicastrin has some limited homology to aminopeptidases, but directed tests of this enzymatic activity have not given positive results [13].

More to RIP than Notch

The lifestyle of Notch might seem highly unusual, but at least two other transmembrane proteins, β-APP and the tyrosine kinase ErbB-4, have now been found to lead highly similar lives. Both are subject to Presenilin-mediated intramembranous cleavage and nuclear translocation of their intracellular domains, which can function as nuclear transcriptional modulators [14,15]. The existence of additional Presenilin/Nicastrin targets can be inferred from the finding that *nicastrin* and *presenilin* clones display phenotypes more severe than those of *Notch* clones, including specific defects in the spectrin cytoskeleton and cadherin adhesion complexes [3]. The relevant targets in these settings remain to be identified. A variety of other artificial substrates that share the properties of being single-pass transmembrane proteins with small extracellular domains are also cleaved in a Presenilin- and/or Nicastrin-dependent manner in *Drosophila* [2,10], which might reflect a more general role for γ-secretase in the scavenging and removal of degraded or truncated membrane proteins. The relatively specific phenotypes of *presenilin* and *nicastrin* worm and fly mutants would seem to indicate, however, that their essential functions encompass the processing of a fairly limited number of substrates.

Nicastrin/Presenilin-mediated cleavage undoubtedly involves additional factors. One good candidate is the newly discovered protein APH-1. Like Presenilins SEL-12 and HOP-1, APH-1 is a multipass transmembrane protein that is required for Notch signaling and localization of APH-2 in nematodes [11]. APH-1 has been conserved in flies and vertebrates, and we await further functional characterization of this novel protein. We also anticipate that proteomic approaches may eventually allow all components of the purified γ-secretase complex to be identified, which should greatly aid efforts to understand the mechanism of proteolysis by γ-secretase.

Protein cleavage within a transmembrane domain has recently been dubbed ‘regulated intramembranous proteolysis’ (RIP), and a large number of other RIP substrates that are processed by other types of membrane-embedded protease — collectively termed intramembrane-cleaving proteases, or I-CliPs — have recently been characterized (reviewed in [16,17]). An exploding field unto itself, RIP happens in the ER, the Golgi, and at the plasma membrane, and occurs in organisms ranging from bacteria to humans. Thus, it is an ancient strategy for regulating the function of select transmembrane proteins that live a second life elsewhere — extracellularly, cytoplasmically, or in an intracellular compartment. The coming years will surely see the identification of new RIP substrates and the elucidation of the many different mechanisms for catalysing RIP.

References

1. Greenwald, I. (1998). LIN-12/Notch signaling: lessons from worms and flies. Genes Dev. *12*, 1751–1762.
2. Chung, H.-M. and Struhl, G. (2001). Nicastrin is required for Presenilin-mediated transmembane cleavage in *Drosophila*. Nat. Cell Biol. *3*, 1129–1132.
3. Lopez-Schier, H. and St. Johnston, D. (2002). *Drosophila* Nicastrin is essential for the intramembranous cleavage of Notch. Dev. Cell *2*, 79–89.
4. Hu, Y., Ye, Y. and Fortini, M. (2002). Nicastrin is required for γ-secretase cleavage of the *Drosophila* Notch receptor. Dev. Cell *2*, 69–78.
5. Kopan, R. and Goate, A. (2000). A common enzyme connects Notch signaling and Alzheimer’s disease. Genes Dev. *14*, 2799–2806.
6. Goutte, C., Hepler, W., Mickey, K. and Priess, J.R. (2000). *aph-2* encodes a novel extracellular protein required for GLP-1-mediated signaling. Development *127*, 2481–2492.
7. Levitan, D., Yu, G., George-Hyslop, P. and Goutte, C. (2001). APH-2/Nicastrin functions in LIN-12/Notch signaling in the *Caenorhabditis elegans* somatic gonad. Dev. Biol. *240*, 654–661.
8. Yu, G., Nishimura, M., Arawaka, S., Levitan, D., Zhang, L., Tandon, A., Song, Y.Q., Rogaeva, E., Chen, F., Kawarai, T. (2000). Nicastrin modulates presenilin-mediated Notch/GLP-1 signal transduction and β–APP processing. Nature *407*, 48–54.
9. Chen, F., Yu, G., Arawaka, S., Nishimura, M., Kawarai, T., Yu, H., Tandon, A., Supala, A., Song, Y.Q., Rogaeva, E. (2001). Nicastrin binds to membrane-tethered Notch. Nat. Cell Biol. *3*, 751–754.
10. Struhl, G. and Adachi, A. (2000). Requirements for presenilin-dependent cleavage of Notch and other transmembrane proteins. Mol. Cell *6*, 625–636.
11. Goutte, C., Tsunozaki, M., Hale, V. and Priess, J. (2002). APH-1 is a multipass membrane protein essential for the Notch signaling pathway in *Caenorhabditis elegans* embryos. Proc. Natl. Acad. Sci. U.S.A. *99*, 775–779.
12. Ray, W.J., Yao, M., Nowotny, P., Mumm, J., Zhang, W., Wu, J.Y., Kopan, R. and Goate, A.M. (1999). Evidence for a physical interaction between presenilin and Notch. Proc. Natl. Acad. Sci. U.S.A. *96*, 3263–3268.
13. Fergani, A., Yu, G., George-Hyslop, P. and Checler, F. (2001). Wild-type and mutated nicastrins do not display aminopeptidase M- and B-like activities. Biochem. Biophys. Res. Comm. *289*, 678–680.
14. Cao, X. and Sudhof, T. (2001). A transcriptionally active complex of APP with Fe65 and histone acetyltransferase Tip60. Science *293*, 115–120.
15. Ni, C.-Y., Murphy, M., Golde, T. and Carpenter, G. (2001). γ-secretase cleavage and nuclear localization of ErbB-4 receptor tyrosine kinase. Science *294*, 2179–2181.
16. Huppert, S. and Kopan, R. (2001). Regulated intramembrane proteolysis takes another twist. Dev. Cell *1*, 590–592.
17. Brown, M., Ye, J. and Goldstein, J. (2000). Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. Cell *100*, 391–398.