bining rare earth elements with other elements makes other colors. For example, titanium and cerium make a yellow color.

The largest growth area for rare earth elements is magnetic applications. Samarium-cobalt and iron-neodymium-boron alloys give extremely stable magnets with high remanence and coercive field strengths (*11*). These magnets form an integral part of hard disk drives, electric motors, and compact headphones.

It is likely that the use of rare earth elements will increase, but many applications are held back by the cost of these elements. The report by Uda *et al.* may well lead to simpler and less complicated routes for the separation of these elements, reducing cost and opening up further opportunities for applications of these unique elements.

References and Notes

1. T. Uda, K. T. Jacob, M. Hirasawa, *Science* **289**, 2326 (2000).
2. T. Moeller, *Comprehensive Inorganic Chemistry*, I. C. Bailar *et al.*, Eds. (Pergamon, Oxford, 1971), vol. 4, pp. 1–101.
3. C. G. Brown and L. G. Sherrington, *J. Chem. Technol. Biotechnol.* **29**, 193 (1979).
4. J. Alstad *et al.*, in *Comparative Study of the Rare Earth Elements in Extraction by HDEHP/Shell Sol T from Nitric and Sulphuric Acid Solutions, Proceedings of the International Solvent Extraction Conference*, G. V. Jeffreys, Ed. (Society of Chemical Industry, Lyon, France, 1974), pp. 1083–1102.
5. F. Habashi, Ed., *Handbook of Extractive Metallurgy*, vol. 3 (Wiley-VCH, Weinheim, Germany, 1997), pp. 1695–1760.
6. The separation factor is the ratio of the concentrations of the rare earth element in the organic and aqueous phases.
7. Uda *et al.* used both experimental and estimated data to devise their separation process. A difference in volatility between divalent chlorides and tetravalent chlorides is already known for tin(II) and tin(IV) compounds, and the separation of volatile chlorides is used in the purification of titanium tetrachloride.
8. A. Raman, *Z. Metallkd.* **68**, 163 (1977).
9. K. H. J. Buschow and A. R. Miedema, in *Hydrogen Absorption in Rare Earth Intermetallic Compounds, Hydrides Energy Storage Proceedings International Symposium*, A. F. Andresen and A. J. Maeland, Eds. (1977), pp. 235–249.
10. V. K. Pecharsky and K. A. Gschneidner, *J. Magn. Magn. Mater.* **200**, 44 (1999).
11. K. A. Gschneidner and L. Eyring, Eds., *Handbook on the Physics and Chemistry of Rare Earths*, vol. 12 (North-Holland, Amsterdam, 1989), pp. 133–212.

PERSPECTIVES: NEUROSCIENCE

An Accomplice for γ-Secretase Brought into Focus

Sangram S. Sisodia

Alzheimer's disease (AD) is thought by many to be intimately, if not causatively, associated with the deposition of short β-amyloid (Aβ) peptides in the cerebral cortex and hippocampus of affected individuals (*1*). These Aβ peptides are liberated from β-amyloid precursor proteins (APPs) after cleavage of APPs in the membrane by β- and γ-secretase enzymes. Identified last year, β-secretase (BACE 1) is a membrane-tethered aspartyl protease that generates an Aβ fragment from the APP amino terminus (see the figure) (*2*). In contrast, the identity of γ-secretase—which cleaves APP within its transmembrane segment to generate the carboxyl-terminal Aβ fragment—has remained a mystery. Various researchers have all but declared that the serpentine presenilin proteins (PS1 and PS2) are the γ-secretases responsible for intramembranous processing of APP (and other proteins such as Notch). These declarations are likely to be tempered by the recent report in *Nature* from St George-Hyslop and his colleagues (*3*). These investigators describe a new protein, dubbed nicastrin, that associates with PS1 and PS2 and, most provocatively, affects γ-secretase processing of APP. This hints that, even if PS1 and PS2 do have γ-secretase activity, they may require help from additional proteins to cleave APP efficiently.

Some AD patients with early-onset disease carry mutations in either the *PS1* or *PS2* gene. These mutations induce a shift in the preferred γ-secretase cleavage site in APP by two amino acids. The ill-fated consequence of this shift is the generation of a slightly longer Aβ fragment (42 rather than 40 amino acids in length) called $A\beta_{42}$, which forms fibrillar clumps that are toxic to neurons (*4*). Genetic analysis of the PS homolog, *sel-12*, in the worm and phenotypic examination of mice lacking either *PS1* or *PS2* or both genes strongly suggest that the PSs facilitate signaling through the *lin12/glp1/Notch* pathway during animal development. The ability of Notch, a membrane receptor, to move to the nucleus and activate gene expression depends on its proteolytic processing within the membrane (*5*). It is intriguing that the PSs facilitate two seemingly distinct processes, namely $A\beta_{42}$ production and Notch activity. Interest in this observation escalated even further with the discovery that secretion of Aβ peptides is compromised in *PS1*-deficient neurons, resulting in the intracellular accumulation of membrane-tethered, β-secretase–derived "β stubs" (the substrates for γ-secretase). Indeed, γ-secretase activity is completely abrogated in cells derived from mouse blastocysts lacking both *PS1* and *PS2* (*6*). Concurrently, Wolfe and colleagues (*7*) demonstrated that expression of PS1, harboring substitutions of the two aspartate residues in transmembrane domains six and seven, also compromised Aβ secretion. Together these findings led to the proposal that PSs are unusual diaspartyl proteases that hydrolyze peptide bonds in membrane-associated proteins. Consistent with this observation, intramembranous processing of Notch 1 and its nuclear signaling activity are also attenuated in *PS1*-deficient cells or in cells expressing mutant PS1 (*6*). Finally, the recent demonstration that PS1 and PS2 are selectively cross-linked to potent, transition-state γ-secretase inhibitors has led many to indict the PSs as the culprit γ-secretases (*8*).

Although the notion that PS1 and PS2 are γ-secretases is appealing, the evidence is not as air-tight as it might appear. First, PSs are predominantly found in the early compartments of the endoplasmic reticulum (ER), yet intramembranous processing of APP and Notch occurs in late compartments of the secretory pathway (the Golgi and beyond) (*6*). Moreover, the rate at which APP moves through the ER is decreased in PS1-deficient neurons, suggesting that PS1 could be a chaperone (or scaffold protein), recruiting APP (or its β stubs) to those secretory compartments in which γ-secretase activity resides (*6*). In a similar fashion, it has been proposed that SREBP (a membrane-anchored protein that regulates cholesterol synthesis) is delivered by SCAP (a cholesterol-sensing ER membrane protein) from the ER to the Golgi, where it is cleaved by the Site-1 protease (*9*). Second, PS1-dependent γ-secretase processing of APP is promiscuous, generating heterogeneous carboxyl-terminal Aβ fragments, which indicates that this protease has relaxed sequence specificity. In contrast, γ-secretase processing of Notch 1 is precise, occurring at only one position in the transmembrane segment of the protein (*5*). Finally, it is now abundantly clear that accumulation of the PSs is tightly regulated through their association with limiting cel-

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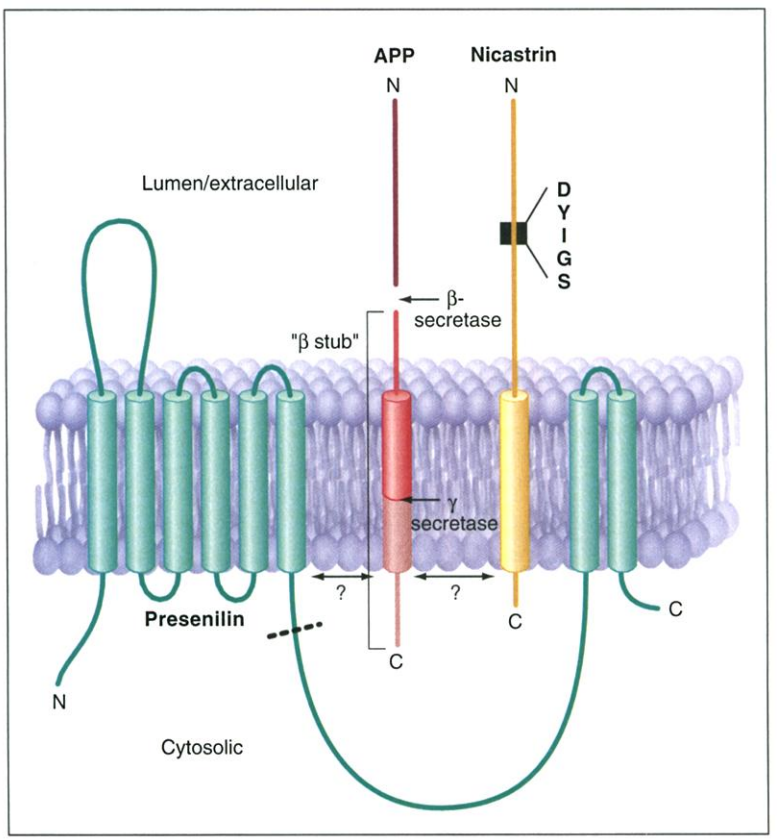
lular components (*4*). The ~43- to 50-kD PS polypeptides exist in larger heteromeric complexes with estimated molecular sizes ranging from ~250 kD to more than 10^6 daltons; the largest of these detergent-solubilized complexes perfectly cofractionates with γ-secretase activity (*10*). Mutations in the two PS aspartates reduce the size of the heteromeric PS complex (*3*), suggesting that the aspartates are necessary for assembling the γ-secretase complex but do not have enzymatic activity per se.

In the new work, St George-Hyslop and colleagues extend the notion that PS1 and PS2 are components of a multiprotein γ-secretase complex. They isolated PS1 along with associated proteins from detergent-solubilized extracts of mammalian membranes using immobilized antibodies against PS1 (*3*). A prominent ~80-kD polypeptide, immunopurified in stoichiometric amounts with PS1, was cleaved with trypsin and analyzed by mass spectrometry. Comparison of the full-length sequence of this polypeptide, called nicastrin, with cDNA database sequences suggested that it might adopt the topology of a type I integral membrane protein (see the figure). Orthologs of nicastrin are expressed in evolutionarily divergent organisms, including the worm, the fruit fly, and the plant *Arabidopsis*. But nicastrin sequences from these organisms have no homology to, or motifs in common with, any known proteins. Coincidentally, similarities between the embryonic worm phenotypes associated with reduced activity of the nicastrin homolog, *aph-2*, and of the PS homologs, *sel-12* and *hop-1*, indicate that nicastrin may also facilitate *glp-1/Notch* signaling during development.

This functional parallel aside, St George-Hyslop and co-workers provide intriguing (yet, admittedly baffling) information about nicastrin's involvement in cleavage of APP and production of Aβ peptides. First, antibodies to nicastrin equivalently coprecipitate wild-type PS1 or PS1 carrying FAD-linked or aspartate mutations. Surprisingly, both full-length APP and its β stubs are also coprecipitated, despite the authors' assertion that APP is not a component of the complex from which nicastrin was isolated. Second, the effect of nicastrin on Aβ production yields paradoxical findings. Overexpression of nicastrin has no apparent effect on the production of Aβ; however, nicastrin harboring substitutions of alanine for aspartate and tyrosine in the conserved sequence aspartate-tyrosine-isoleucine-glycine-serine (DYIGS) not only remains fully capable of associating with PS1, but also enhances secretion of Aβ peptides, most prominently of $Aβ_{42}$. In contrast, expression of nicastrin with a deletion of the DYIGS motif and neighboring sequences leads to a reduction in secreted Aβ and a substantially reduced association with PS1. Currently, there is neither a satisfying explanation for the incongruous effects of nicastrin on Aβ production, nor is it immediately obvious how the nicastrin domain that projects into the ER lumen interacts with PS1 (see the figure). While we eagerly await resolution of these outstanding issues, the new findings have led to speculation that nicastrin function is intimately coupled to γ-secretase activity. Clearly, this notion must now be validated by analysis of Aβ peptide production and Notch processing in cells from mice lacking nicastrin.



The complexities of amyloid processing. The transmembrane proteins nicastrin and presenilin are associated together in a complex that has β-secretase activity. The amyloid precursor protein (APP) is first cleaved by β-secretase to yield a β stub, which is then cleaved by γ-secretase to yield a β-amyloid fragment that is secreted. Nicastrin associates in some way with presenilin through the DYIGS motif in its extracellular domain. The β stub is associated (probably transiently) with the presenilin-nicastrin complex.

It is clear that portraying γ-secretase as a single entity (a PS) is an oversimplification. The way forward is to reconstitute full γ-secretase activity with purified components, a rather challenging proposition given that there could be a large number of different proteins in the multimeric PS complex. Even with the best characterized example of intramembranous proteolysis—the processing of SREBP-2 by the Site-2 protease—this reaction has not been reproduced in vitro with isolated components (*9*). Nevertheless, identification of the entire repertoire of polypeptides that associate with PS in the γ-secretase complex is the next step. This should yield new therapeutic targets for blocking Aβ production and for ameliorating the symptoms of AD. In addition, it will be instructive to see whether some familial and sporadic AD patients carry mutations in nicastrin or in other components of the γ-secretase complex. These efforts should provide mechanistic insights into how the transmembrane domains of proteins are enzymatically cleaved in the membrane, a process that regulates a diverse list of cellular functions (*9*).

Perhaps Jean-Luc Godard, the French film director, was thinking about the contribution of science to medicine when he quipped "counter vague ideas with sharp images." For a sharper image of γ-secretase—stay tuned.

References

1. D. J. Selkoe, *Trends Cell Biol.* **8**, 447 (1998).
2. R. Vassar *et al.*, *Science* **286**, 735 (1999).
3. G. Yu *et al.*, *Nature* **407**, 48 (2000).
4. D. L. Price and S. S. Sisodia, *Annu. Rev. Neurosci.* **21**, 479 (1998).
5. E. H. Schroeter *et al.*, *Nature* **393**, 382 (1998).
6. C. Haass and B. De Strooper, *Science* **286**, 916 (1999).
7. M. S. Wolfe *et al.*, *Nature* **398**, 513 (1999).
8. B. DeStrooper, *Nature* **405**, 627 (2000).
9. M. S. Brown, J. Ye, R. B. Rawson, J. L. Goldstein, Cell **100**, 391 (2000).
10. Y. Li *et al.*, *Proc. Natl. Acad Sci. U.S.A.* **97**, 6138 (2000).