be used in future experiments, together with an appropriate outcoupler to release atoms continuously from the trap, to obtain a truly continuous atom laser beam. The new approach is technically challenging but conceptually simple. It shows how BECs can be routinely manipulated, transported, and merged. As another milestone in atom optics is passed, what will be next?

It might take longer than just 1 day, but experiments to demonstrate first applications for continuous atoms lasers are under way. Many open questions remain. What are the spectral properties of continuous atom lasers? What is the effect of the merging process on the phase of the BEC? How narrow can the line of an atom laser get? Can it be a precise spectroscopic tool? Will it be possible to produce easy-to-use, compact sources for everyday atom optics applications? Can matter waves also be amplified continuously (*11*, *12*)?

With the experimental results of Chikkatur *et al.*, answers to many of these questions seem to be within reach. Their technically sophisticated but conceptually simple solution to the continuous atom laser problem has been successful where sophisticated concepts have failed. The next challenge will be to extend these concepts and develop "high power" atom lasers.

References

1. M. H. Anderson *et al.*, *Science* **269**, 198 (1995).
2. M. R. Andrews *et al.*, *Science* **275**, 637 (1997).
3. M.-O. Mewes, *Phys. Rev. Lett.* **78**, 582 (1997).
4. B. P. Anderson, M. A. Kasevich, *Science* **282**, 1686 (1998).
5. I. Bloch *et al.*, *Phys. Rev. Lett.* **82**, 3008 (1999).
6. R. J. C. Spreeuw *et al.*, *Europhys. Lett.* **32**, 469 (1995).
7. M. Holland *et al.*, *Phys. Rev. A* **54**, R1757 (1996).
8. L. Santos *et al.*, *Phys. Rev. A*, **63**, 063408 (2001).
9. E. Mandonnet *et al.*, *Eur. Phys. J. D* **10**, 9 (2000).
10. A. P. Chikkatur *et al.*, *Science* **296**, 2193 (2002).
11. S. Inouye *et al.*, *Nature* **402**, 641 (1999).
12. M. Kozuma *et al.*, *Science* **286**, 2309 (1999).

PERSPECTIVES: BIOCHEMISTRY

Intramembrane Proteases—Mixing Oil and Water

Michael S. Wolfe and Dennis J. Selkoe

Proteases and peptidases, enzymes that hydrolyze other proteins and peptides, are critical to many normal and pathological events and are often important targets for therapeutic interventions. The last place in the cell to expect enzymes to hydrolyze their substrates is within the hydrophobic environment of membrane lipid bilayers. Nonetheless, several families of hydrolytic enzymes now appear to carry out this seemingly paradoxical process. The latest installment in this continuing saga is reported on page 2215 of this issue by Weihofen and colleagues (*1*). They characterize a hydrolase called signal peptide peptidase (SPP), pinpoint its active site to amino acids within a membrane, and reveal its similarity to presenilin, a hydrolase implicated in Alzheimer's disease.

There are four classes of proteases and peptidases classified according to which amino acid residues in the enzyme catalyze the breaking of an amide bond in the substrate. These classes are the serine/threonine proteases exemplified by the enzymes of the proteasome (the cell's protein degradation factory), the cysteine proteases such as the caspases involved in apoptosis, the metalloproteases exemplified by angiotensin converting enzyme, and aspartyl proteases such as the HIV protease. All four categories contain many examples of fully water-soluble proteases as well as membrane proteases that span the membrane once and have their active sites in the aqueous compartments of the cell.

The authors are at the Center for Neurologic Diseases, Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115, USA. E-mail: mwolfe@rics.bwh.harvard.edu; dselkoe@rics.bwh.harvard.edu

Remarkably, the same mechanistic principles seem to apply to the recently recognized multipass intramembrane proteases that traverse the membrane many times and have their active sites buried within the lipid bilayer (see the table). This suggests that there are only a few biochemical solutions to the general problem of how to cut an amide bond. For instance, the S2P family of proteases cleaves membrane-anchored transcription factors involved in cholesterol biosynthesis (*2*), and contains a conserved and essential HEXXH motif that is characteristic of many soluble metalloproteases. Another example is the newly described rhomboid protease family responsible for releasing transforming growth factor–α (TGF-α) from a membrane-bound precursor (*3*). Conserved amino acid residues (asparagine, histidine, and serine) required for activity of rhomboid are reminiscent of the catalytic triad typically found in serine proteases. Indeed, inhibitors of soluble serine proteases also block rhomboid-mediated proteolysis. Both S2P and rhomboid cleave amide bounds within the transmembrane regions of their substrates, and the residues responsible for this hydrolysis are located either within the membrane or at the membrane-cytosol interface. Thus, these two families are members of the rapidly expanding group of intramembrane-cleaving proteases (I-CliPs) (*4*).

Presenilin is the founding member of the aspartic I-CliPs. Presenilin contains eight transmembrane domains and is required for the intramembranous proteolysis of the amyloid-β precursor protein (APP), the Notch and Erb-B4 receptors, E-cadherin, and probably numerous other single-transmembrane substrates (*5*–*8*). This protease activity, called γ-secretase, releases the cytosolic tails of APP and Notch that exemplify part of a new signaling mechanism (*9*, *10*). Processing of APP by γ-secretase also produces the amyloid-β peptide, which plays a central part in the pathogenesis of Alzheimer's disease. Thus, γ-secretase is considered a major therapeutic target. The notion that presenilin contains the active site of γ-secretase arose from the observation that two conserved intramembrane aspartates are critical for both γ-secretase activity and the "presenilinase" activity that cleaves full-length presenilin into its biologically active heterodimeric form (*11*). These data suggest that presenilins are unique intramembranous aspartic proteases that are activated by autoproteolysis.

Aspartyl protease transition-state analog inhibitors of γ-secretase bind directly to both presenilin fragments (*12*, *13*). This finding and the fact that each fragment contributes one of the two key aspartates strongly imply that the active site of γ-secretase resides at the interface of the heterodimeric fragments. However, presenilin heterodimers alone do not constitute γ-secretase. The formation and stabilization of the heterodimers is tightly regulated by other cellular factors (*14*), and these are thought to be integral membrane proteins that combine with the heterodimers to form a large active protease complex. Nicastrin and aph-1 are postulated to be members of this complex (*15*, *16*), but whether these proteins together with presenilin can reconstitute γ-secretase activity remains to be seen.

Although the cumulative evidence strongly suggests that the catalytic component of γ-secretase resides in presenilin, this idea has not been without its skeptics (*17*, *18*). Four apparent inconsistencies have been aired. First is the so-called "spatial paradox": Presenilin is located mainly in the endoplasmic reticulum and Golgi apparatus, whereas γ-secretase cleavage of Notch and APP is thought to take place at or near the cell surface. However, presenilin localization studies used antibodies that do not distinguish between full-length and heterodimeric presenilin or between

those heterodimers that have entered high molecular weight bioactive complexes and those that have not. Moreover, presenilin heterodimers have actually been detected in cell-surface labeling experiments (*19*). Second, Notch and APP are not processed in the same way: APP is cleaved at several sites, whereas Notch seems to be cut at a single site. However, only one of the two Notch cleavage products, the cytosolic tail, has been analyzed. Isolation and characterization of the Notch counterpart of amyloid-β peptide may reveal heterogeneous cleavage as well. Third, mutating one of the two presenilin aspartates affects APP and Notch processing differently. But this mutation does inhibit γ-secretase cleavage of both substrates, and the apparent differences are difficult to interpret with endogenous wild-type presenilin in the background. Fourth, a class of γ-secretase inhibitors blocks the γ-secretase cleavage of APP but not of Notch in cultured cells. These compounds, however, do not interact with γ-secretase directly in cell-free assays (*20*), and their mechanism of action may be upstream of the enzyme.

POLYTOPIC MEMBRANE PROTEASES

Mechanistic class	Name	Substrates	I-CliP
Metallo	S2P family	SREBP, ATF6	Yes
	Ste24p, Rce1p	CAAX prenylated proteins	No
Serine	Rhomboid family	TGF-α	Yes
Aspartic	Presenilins	APP, Notch, Erb-B4, E-cadherin	Yes
	SPP family	Signal peptide remnants	Yes
	TFPP family	Leader peptides of type 4 prepilins	No
Cysteine	?	?	?

The new work by Weihofen *et al.* on the presenilin-like SPP provides compelling evidence that presenilins are proteases and should quell objections of the critics. Many integral membrane proteins require a short hydrophobic stretch of amino acids, the signal sequence, for proper insertion into the membrane. The signal sequence is promptly clipped off by a signal peptidase. The resulting signal peptide remaining in the membrane is then processed by SPP. In this way, SPP produces epitopes from the major histocompatability complex (MHC) class I molecules that signal MHC biosynthesis to cells of the immune system. In search of SPP, Weihofen and colleagues applied the same affinity-labeling strategy used to identify the active site of γ-secretase (*13*, *14*). They converted a peptidomimetic SPP inhibitor into an analog containing a photoactivated group (for covalent binding to the target) and biotin (for detection of the tagged target). Two glycosylated forms of the same seven-transmembrane protein were identified. Database mining for homologs and orthologs revealed four families of highly conserved proteins found in many species. These same proteins were recently identified by Golde and colleagues in a database search for presenilin homologs (*21*).

All SPPs contain two conserved transmembrane aspartates, and these aspartates are part of two signature motifs found in presenilin and in a bacterial family of multipass (polytopic) aspartyl proteases called type 4 prepilin peptidases (*22*, *23*). Weihofen *et al.* show that expression of SPP in the yeast *Saccharomyces cerevisiae*, which contains no endogenous SPP gene, results in SPP proteolytic activity in solubilized membrane fragments. Mutation of one of the two conserved aspartates abrogated protease activity. The formal possibility remains that the SPP protein activates a closely related protease in the yeast host. However, this is highly unlikely, and the reconstitution of activity in yeast by the investigators suggests that this presenilin homolog is indeed the signal peptide peptidase.

Presenilin and SPP differ from each other in important respects. Unlike SPP, presenilin does not undergo glycosylation. SPP appears to act alone, whereas presenilin requires association with other membrane proteins for activity. Moreover, presenilin undergoes highly regulated processing into heterodimers, whereas SPP is apparently active as a full-length protein. Interestingly, the orientation of SPP in the membrane is opposite to that of presenilin, and accordingly, their respective substrates are inserted in the membrane in opposite directions. Despite these differences, the finding that expression of the SPP protein yields proteolytic activity eliminates the major objection to presenilin being the catalytic component of γ-secretase, namely, that presenilin bears no resemblance to known proteases. Presenilin has been postulated by some to be an essential cofactor or chaperone for γ-secretase but not the catalytic component itself (*17*, *18*). With the discovery of SPP, it is now clear that among the suspected members of the γ-secretase complex, only the presenilins resemble a known protease.

The discovery of I-CliPs represents a major change in our conception of proteases and peptidases and raises many fascinating questions for future study. No I-CliP has so far been purified to homogeneity and shown to have proteolytic activity. This stems from the inherent difficulties of working with multipass membrane proteins. However, one polytopic metalloprotease, Ste24p, retains activity after purification (*24*). This enzyme cleaves COOH-terminal CAAX motifs after cysteine prenylation. Although the catalytic residues of Ste24p apparently do not lie within the membrane (it is not an I-CliP), this protease is nevertheless a multipass membrane protein and does not resemble soluble or membrane-tethered metalloproteases in its primary amino acid sequence.

Purification of I-CliPs will be critical for discerning the details of their mechanism and structure. Because water is required for catalysis, the active site must be sequestered from the hydrophobic environment of the lipid bilayer. This raises the question of how membrane-embedded substrates, which can only move in the two-dimensional environment of the lipid bilayer, access the internal active site. These proteases are likely to have an initial docking site for substrate that is distinct from the active site. After initial binding, conformational changes would allow the substrate to enter the inner sanctum of the active site. The I-CliPs so far discovered probably represent just the tip of the proverbial iceberg. Many genes encode membrane proteins of unknown function, and new polytopic membrane proteases, including I-CliPs, are likely to be among them. The rest of the decade will be spent writing this new chapter in protease biochemistry.

References

1. A. Weihofen, K. Binns, M. K. Lemberg, K. Ashman, B. Martoglio, *Science* **296**, 2215 (2002).
2. R. B. Rawson *et al.*, *Mol. Cell* **1**, 47 (1997).
3. S. Urban, J. R. Lee, M. Freeman, *Cell* **107**, 173 (2001).
4. M. S. Wolfe, J. De Los Angeles, D. D. Miller, W. Xia, D. J. Selkoe, *Biochemistry* **38**, 11223 (1999).
→ B. De Strooper *et al.*, *Nature* **398**, 518 (1999).
→ G. Struhl, I. Greenwald, *Nature* **398**, 522 (1999).
7. C. Y. Ni, M. P. Murphy, T. E. Golde, G. Carpenter, *Science* **294**, 2179 (2001).
8. P. Marambaud *et al.*, *EMBO J.* **21**, 1948 (2002).
→ E. H. Schroeter, J. A. Kisslinger, R. Kopan, *Nature* **393**, 382 (1998).
10. X. Cao, T. C. Sudhof, *Science* **293**, 115 (2001).
→ M. S. Wolfe *et al.*, *Nature* **398**, 513 (1999).
12. Y. M. Li *et al.*, *Nature* **405**, 689 (2000).
13. W. P. Esler *et al.*, *Nature Cell Biol.* **2**, 428 (2000).
14. G. Thinakaran *et al.*, *J. Biol. Chem.* **272**, 28415 (1997).
15. G. Yu *et al.*, *Nature* **407**, 48 (2000).
16. C. Goutte, M. Tsunozaki, V. A. Hale, J. R. Priess, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 775 (2002).
17. S. S. Sisodia, P. H. St George-Hyslop, *Nature Rev. Neurosci.* **3**, 281 (2002).
18. S. S. Sisodia, W. Annaert, S. H. Kim, B. De Strooper, *Trends Neurosci.* **24**, S2 (2001).
19. W. J. Ray *et al.*, *J. Biol. Chem.* **274**, 36801 (1999).
20. W. P. Esler *et al.*, *Nature Cell Biol.* **4**, E110 (2002).
21. C. P. Ponting *et al.*, *Hum. Mol. Genet.* **11**, 1037 (2002).
22. C. F. LaPointe, R. K. Taylor, *J. Biol. Chem.* **275**, 1502 (2000).
23. H. Steiner *et al.*, *Nature Cell Biol.* **2**, 848 (2000).
24. A. Tam, W. K. Schmidt, S. Michaelis, *J. Biol. Chem.* **276**, 46798 (2001).