Sheddases and intramembrane-cleaving proteases: RIPpers of the membrane

Symposium on Regulated Intramembrane Proteolysis

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The Ringberg Symposium on Regulated Intramembrane Proteolysis took place between 27 and 30 November 2006, at the Ringberg Castle, Rottach-Egern, Germany, and was organized by C. Haass.

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disorder. The meeting was the first of its kind in this research field and, as all participants agreed, was long overdue. RIP occurs in all organisms studied so far and is a regulated proteolytic cleavage mechanism typically required for either signal transduction or the degradation of membrane proteins (Brown *et al.*, 2000). Although much has been learned about RIP, the meeting made it clear that many questions remain.

In many cases, RIP starts with an initial cleavage of a single-span membrane protein substrate with type I or II orientation. This cleavage, which is referred to as ectodomain shedding, occurs within the ectodomain at a peptide bond close to the trans-membrane domain (TMD), either constitutively or in response to a ligand. Shedding results in the release of the ectodomain into the extracellular milieu and the generation of a membrane-bound stub, which then undergoes a second cleavage within its TMD, called intramembrane proteolysis. Members of the disintegrin and metalloprotease (ADAM) family, matrix metalloproteases and the aspartyl proteases β -site APP-cleaving enzymes 1 and 2 (BACE1 and BACE2) carry out the ectodomain-shedding step in most cases that have been studied. The intramembrane cleavage is mediated by intramembrane-cleaving proteases (I-CLiPs). These are multi-span integral membrane proteins, the active sites of which are located in their hydrophobic regions, typically in the predicted TMDs. Metallo- and aspartyl protease I-CLiPs depend on a previous cleavage event in order to conduct TMD hydrolysis, which releases peptides into the extracellular space and intracellular domains (ICDs) into the cytosol. ICD release can, in some cases, have a signalling function (Wolfe & Kopan, 2004). By contrast, I-CLiPs of the serine protease-type—the rhomboids—cleave their substrates without the requirement of a preceding ectodomain cleavage step (Wolfe & Kopan, 2004). The mechanistic basis for these differences is unclear. Interestingly, I-CLiPs of the cysteine protease-type have not yet been discovered.

For type I membrane proteins, intramembrane proteolysis is catalysed by the γ -secretase complex, which consists of the aspartyl protease presenilin 1 or 2 (PS1 and PS2, respectively) as the catalytic subunit and three other proteins essential for activity—nicastrin, presenilin enhancer 2 (PEN2) and anterior pharynx-defective phenotype 1 (APH1; Wolfe & Kopan, 2004). By contrast, type II membrane

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Introduction

At the end of November 2006, 60 scientists met at Ringberg Castle in the beautiful Bavarian countryside of Lake Tegernsee to present and discuss recent findings in the field of regulated intramembrane proteolysis (RIP). The meeting was funded by the Collaborative Research Centre 'Molecular Mechanisms of Neurodegeneration' (SFB 596) of the German Research Foundation (DFG) and was organized by the co-ordinator of the SFB, Christian Haass. RIP is an important area of research of the SFB 596 because it has a crucial role in Alzheimer disease—the most common neurodegenerative

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proteins that have undergone ectodomain shedding can subsequently become substrates for either the metalloprotease site 2 protease (S2P) or an aspartyl protease I-CLiP of the signal peptide peptidase (SPP)-like (SPPL) family (Wolfe & Kopan, 2004). The prototype enzyme of the SPPL family, SPP, removes membrane-anchored signal peptides from the endoplasmic reticulum membrane after an initial cleavage by signal peptidase, which can be considered to be analogous to shedding. SPP also cleaves the hepatitis C virus (HCV) polyprotein and might have a crucial role in the life cycle of this virus.

Function of sheddases

With regard to sheddases of the ADAM and BACE protease family, significant progress was reported at the meeting in elucidating their function and substrate spectrum, as well as in understanding different ways of controlling their activity. Many single-span cell-surface membrane proteins undergo shedding by a metalloprotease, but the identity of the protease is unknown in many cases. At the meeting, it became clear that this situation is changing as a result of the availability of more specific metalloprotease inhibitors and knockout mice deficient in one or several of the ADAM proteases. The use of these reagents has been particularly fruitful for ADAM10, for which various new functions and substrates were described. C. Blobel (New York, NY, USA) identified ADAM10 as the main enzyme that releases the low-affinity immunoglobulin E receptor CD23 (Weskamp *et al*, 2006). This reveals a role for ADAM10 in the allergic response, in which shedding of CD23 is considered a crucial event. P. Saftig (Kiel, Germany) and A. Capell (Munich, Germany) reported a role for ADAM10 in cell adhesion. Saftig showed that ADAM10 is involved in the shedding of different members of the cadherin protein family, including E-cadherin. This fits with his observation of increased ADAM10 expression and less E-cadherin cell-surface staining around blisters in eczematous skin disease. To cleave E-cadherin, ADAM10 needs to be transported to adherence junctions, which requires a putative SH3-domain-binding motif in its cytoplasmic domain (Capell).

As well as ADAM10, BACE1 has attracted a lot of attention. Initially, BACE1 was identified as a sheddase of the amyloid precursor protein (APP) and is considered to be a crucial drug target for Alzheimer disease because it catalyses the first cleavage step in the generation of the disease-causing amyloid β -peptide (A β) from APP. The physiological functions of BACE1 were initially unclear because *Bace1*-knockout mice only present subtle phenotypic changes compared with wild-type mice. However, data presented by M. Willem (Munich, Germany) and R. Yan (Cleveland, OH, USA) identified a crucial developmental role for BACE1. *Bace1*-knockout mice show a striking reduction of myelination in the peripheral (Hu *et al*, 2006; Willem *et al*, 2006) and central nervous systems (Hu *et al*, 2006). Both speakers presented evidence that this hypomyelination phenotype results from a reduction in shedding of type III neuregulin 1, which is required for myelin sheath formation in the peripheral nervous system. BACE1 function has also been studied in the zebrafish as reported by B. Schmid (Munich, Germany), who showed that knockdown of BACE1 expression affects the kinetics of the startle response, resulting in altered and shortened swim paths. The same phenotype was observed after treatment with a small molecule BACE1 inhibitor, suggesting that the fish could be used for *in vivo* BACE1 inhibitor screens.

Apart from APP and neuregulin 1, only a few other BACE1 substrates have been identified. Interestingly, some of the BACE1

substrates, such as APP, P-selectin glycoprotein ligand 1, neuregulin 1 or β -subunits of voltage-gated sodium channels are not only shed by BACE1 but also by ADAM proteases. BACE1 cleavage might be an additional option for other ADAM-protease substrates, as suggested by S. Lichtenthaler (Munich, Germany), who reported the results of a survey of different ADAM-protease substrates that were tested for cleavage by BACE1. He suggested that redundancy between BACE1 and ADAMs in the shedding process might explain the mild phenotype observed in *Bace1*-knockout mice, in which the ADAM proteases still provide sheddase activity for BACE1 substrates.

Control of sheddase activity

Although BACE1 is a promising drug target for Alzheimer disease, the development of specific BACE1 inhibitors for therapeutic purposes has been slow. This has generated a lot of interest in finding alternative molecular mechanisms to control the amount of APP shedding by BACE1. B. De Strooper (Leuven, Belgium) reported on the identification of microRNAs (miRNAs) that inhibit BACE1 translation. He suggested that changes in the expression level of these miRNAs might provide an explanation for the increased BACE1 protein levels observed in Alzheimer disease brains. In addition, he proposed that changes in miRNA levels might account for the astonishing change in BACE1 expression early in life: in mice, BACE1 expression declines in the second postnatal week and remains low in adult animals (Willem *et al*, 2006). Mechanisms of APP and BACE1 endocytosis are being studied by J. Tang (Oklahoma City, OK, USA), who pointed out that wild-type APP has to reach endosomes to be cleaved efficiently by BACE1. He reported that binding of apolipoprotein E (ApoE) to its receptor ApoER2 triggers co-endocytosis of APP and BACE1, resulting in increased BACE1-mediated cleavage of APP. Interestingly, the ApoE4 isoform is a risk factor for Alzheimer disease, prompting Tang to speculate that it efficiently promotes the association of APP and BACE1. A different approach towards reducing APP cleavage by BACE1 could involve enhancing the activity of ADAM10 to increase α -cleavage of APP. Both proteases compete for APP as a substrate, but have opposite effects on A β generation. In fact, F. Fahrenholz (Mainz, Germany) reported that the pituitary adenylate cyclase-activating polypeptide (PACAP) stimulated ADAM10-mediated cleavage of APP and reduced A β generation. This effect could also be seen in mouse models of Alzheimer disease after nasal administration of PACAP. However, enhancing ADAM10 activity will also have adverse consequences by enhancing the allergic response, as pointed out by Blobel.

For some diseases, an inhibition and not an activation of ADAM proteases might prove useful. In this regard, tissue inhibitors of metalloproteases (TIMPs) provide a relatively safe way to control ADAM protease activity. R. Black (Seattle, WA, USA) reported that TIMP3 reduced cartilage degeneration and bone lesions in a rat osteoarthritis model. He also pointed out that TIMPs might be superior to small molecule protease inhibitors because their distribution in the body will be markedly different as they are bound to matrix proteins rather than being freely diffusible. Thus, they might act in a localized manner without inhibiting the shedding of other substrates in different parts of the body.

Progress on the function of I-CLiPs

New functions for I-CLiPs continue to emerge and several were reported at the meeting. M. Freeman (Cambridge, UK) discussed the unexpected finding that the rhomboid protease AarA mediates a

quorum-sensing signal in the bacterium *Providencia stuartii* by cleaving the twin-arginine translocase protein TatA (Stevenson *et al*, 2007). Rhomboids were further implicated in protein processing in the secretory pathway (M. Lemberg, Cambridge, UK) and in host invasion by the *Toxoplasma gondii* and *Plasmodium falciparum* (malaria) parasites (S. Urban, Baltimore, MD, USA). In addition, T. Rudka (Leuven, Belgium) showed that cleavage of the dynamin-related Optic atrophy 1 (OPA1) by the mitochondrial presenilin-associated rhomboid-like (PARL) protein in mice controls apoptosis through the opening of the cristae. In *Drosophila* and yeast, however, mitochondrial rhomboid-mediated cleavage of OPA1 controls mitochondrial fusion, as Freeman pointed out.

B. Martoglio (Basel, Switzerland) and R. Flührer (Munich, Germany) attributed a functional role to SPPL2a and SPPL2b, which were shown to process tumour necrosis factor α (TNF α ; Flührer *et al*, 2006; Friedmann *et al*, 2006). Cleavage of this substrate releases the TNF α ICD, which in turn triggers the production and the release of interleukin 12; this indicates a crucial role for SPPL2a and SPPL2b in the regulation of adapted and innate immunity. It will be interesting to see whether and how the distinct knockdown phenotype of the zebrafish SPPL2b homologue reported by Schmid—that is, erythrocyte accumulation in an enlarged caudal vein—relates to TNF α signalling.

The implication of SPP as a drug target for certain infectious diseases received further substantiation at the meeting. J. McLauchlan (Glasgow, UK) showed that SPP also processes the core protein from GB virus B, a close relative of HCV that is used as a surrogate to study HCV infection. This validates SPP as a possible target for anti-viral therapy to treat hepatitis C.

Owing to its cleavage of the Notch cell-surface receptor to release the transcriptional regulator Notch ICD, γ -secretase controls a key signalling pathway in cell differentiation during embryonic development as well as later in life. However, R. Kopan (St Louis, MO, USA) pointed out that presenilin also has developmental functions through the Akt and Wnt signalling pathways, independent of its γ -secretase activity. Reinforcing this theme, Kopan presented data showing that conserved γ -secretase-independent presenilin activity exists in a moss, *Physcomitrella patens* (a primitive plant), which lacks the Notch and Wnt signalling pathways. Interestingly, a function of γ -secretase in fertilization and cell fusion in the unicellular green alga *Chlamydomonas* was reported by G. Yu (Dallas, TX, USA).

Towards the structure of I-CLiPs

As well as new data on the function of intramembrane proteases, structural and mechanistic information on these proteases was reported at the meeting. By definition, the active-site residues of intramembrane proteases must be located in close proximity inside the membrane and be accessible to water in order to catalyse the hydrolysis of a peptide bond. How do intramembrane proteases accomplish the task of hydrolysing proteins in a lipid bilayer? Structural biologists have investigated this intriguing process as Y. Ha (New Haven, CT, USA) nicely pointed out at the meeting with his remark that “the nature of the problem demands crystallographic proof.” The presentation of the first crystal structure of an intramembrane protease—the *Escherichia coli* rhomboid GlpG—by Ha therefore represented the main highlight of the meeting (Wang *et al*, 2006). The crystal structure of a rhomboid shows that the active-site residues serine and histidine, which are remote in the primary sequence, are indeed close enough to form hydrogen bonds and

form the earlier proposed catalytic dyad (Lemberg *et al*, 2005) rather than a classical serine protease catalytic triad. An important feature revealed by the crystal structure is that the active-site residues are located in a water-containing cavity approximately 10 Å below the membrane surface. Therefore, as proposed for an intramembrane protease, the active site of the rhomboid is within the plane of the membrane. Urban presented the crystal structure of the same enzyme packed in a different symmetrical unit, which he resolved in collaboration with Shi and colleagues (Wu *et al*, 2006) by using the published GlpG coordinates (Wang *et al*, 2006). Both structures agree on the principal finding that the active site is provided by the serine–histidine dyad located in a membrane-embedded cavity. However, probably because of differences in the crystallization procedure, the two structures differ in the location of TMD5, which Ha proposes to be part of the enzyme transmembrane bundle (Wang *et al*, 2006), whereas Urban suggests that its top tilts outwards (Wu *et al*, 2006). Accordingly, the two structures allow different interpretations of how the substrate gains access to the active site (Fig 1). Ha suggested that substrate entrance is controlled by the highly flexible loop 5, the so-called ‘cap’ (Wang & Ha, 2007) and not by loop 1 as originally proposed (Wang *et al*, 2006), but Urban argued that gating of the protease requires substrate-mediated movement of TMD5 (Wu *et al*, 2006; Baker *et al*, 2007). The heated debate at the meeting about which of the two crystals reflects the real structure will set the direction for future structural studies, which must include a bound substrate or inhibitor to define further open and closed states of the protease.

As γ -secretase comprises at least four integral membrane proteins, high-resolution crystal structure information for this complex is still very remote. Nevertheless, the recently published low-resolution electron microscopy structure of the complex showed interesting features (Lazarov *et al*, 2006). As pointed out by M. Wolfe (Boston, MA, USA), it reveals a particle with a pore-like central cavity connected to the outside by two holes that might represent exit sites for the γ -secretase cleavage products. Similar structural information might be attainable for the presenilin-related SPP. As Wolfe further reported, amino-terminally truncated SPP can be purified from *E. coli* in an enzymatically active form and might therefore be amenable to structural determination by using nuclear magnetic resonance. Wolfe and H. Steiner (Munich, Germany) reported on their efforts to obtain information on subunit arrangement and subunit stoichiometry of γ -secretase. There is now broad agreement in the field that the γ -secretase complex has a molecular weight of approximately 500 kDa on native polyacrylamide gels, suggesting either that the complex contains additional components or that one or the other of the four core components is present in multiple copies (Wolfe). Both speakers showed that chemical cross-linking strategies are useful to reveal inter- and intramolecular subunit interactions of γ -secretase complexes as a tool to obtain basic structural information. T. Iwatsubo (Tokyo, Japan) and A. Tolia (Leuven, Belgium) presented data on the water accessibility of the catalytic site of γ -secretase, which was probed by systematic scanning of cysteine residues artificially introduced into TMD6 and TMD7 of presenilin using cysteine-modifying reagents (Sato *et al*, 2006; Tolia *et al*, 2006). Residues of the highly conserved GxGD region that includes the active-site aspartate of TMD7 were found to be water-accessible, suggesting that the active-site region is in a hydrophilic environment. The functional importance of this region was emphasized by Steiner, who showed

Fig 1 | Putative mechanism of intramembrane cleavage catalysed by the rhomboid intramembrane cleaving proteases family. (A) Loop 5 cap movement-gating mechanism (Wang & Ha, 2007). Opening of loop 5 (dark blue) causes increased access of water molecules (black dots) to the cavity (yellow) and exposes the active site (histidine (H) and serine (S) residues). This allows access and cleavage of the substrate (green). The α -helical transmembrane domain (TMD) of the substrate becomes partly unfolded in order to be cleaved. (B) TMD5 movement-gating mechanism (Wu *et al*, 2006; Baker *et al*, 2007). The open conformation of the protease is achieved by a structural switch of TMD5 (dark blue), which makes the active site accessible to the substrate. I-CLiP, intramembrane-cleaving proteases.

that the GxGD motif has a role beyond the catalytic function of γ -secretase in substrate identification (Yamasaki *et al*, 2006). Tolia reported a further observation, namely that the two aspartates in TMD6 and TMD7, when substituted by cysteines, could be cross-linked, suggesting that they face each other.

Substrate cleavage by I-CLiPs

Several speakers reported on the substrate sequence requirements of intramembrane proteases. Rhomboids have a sequence requirement for helix-breaking residues in their substrates and, as reported by Urban, cleave their substrates in a similar manner to other I-CLiPs at two sites that are four and six residues into the TMD (Baker *et al*, 2007). Interestingly, however, Y. Akiyama (Kyoto, Japan) reported that *E. coli* GlpG, for which no physiological substrate is known, cleaves a model rhomboid substrate apparently outside the membrane. Similarly, as Freeman reported, AarA cleaves its substrate TatA at its TMD close to the predicted extracellular space–membrane edge (Stevenson *et al*, 2007). These observations are not easy to understand considering the relatively deeply membrane-embedded active site of rhomboids that was revealed by the crystal structure. Helix-breaking residues are also important structural features of substrates of SPP, which typically cleaves signal peptides at one main site. However, as shown by McLauchlan, there are other contributory factors for efficient cleavage of viral core proteins by SPP. Fluhrer showed that

SPPL2b cleaves its substrate TNF α at two distinct major sites, similar to γ -secretase, which cleaves its well-studied substrates APP and Notch at two major sites in the TMD, one of which is close to the respective membrane border for releasing its cleavage products (Fluhrer *et al*, 2006). However, it is known that γ -secretase cleaves its substrates in a non-specific manner without strict substrate sequence requirement, whereas the substrate sequence requirements of SPPL2b have not yet been investigated.

The cleavage precision of γ -secretase at the γ -site of APP can be modulated with a subset of non-steroidal anti-inflammatory drugs (NSAIDs), such that less of the pathogenic A β 42 is produced. This finding is of considerable interest for γ -secretase as a drug target for Alzheimer disease therapy. T. Kukar (Jacksonville, USA) presented data obtained with an NSAID-related affinity reagent, fenofibrate-biotin, which indicate that NSAIDs might bind to the substrate rather than the protease itself. Kukar obtained similar results for an SPP substrate. Interestingly, Wolfe reported that cleavage of SPP could also be modulated by NSAIDs, which is inconsistent with NSAID binding to substrates.

An unresolved question is why presenilin needs partner proteins to function as γ -secretase, whereas its related proteases SPP and the SPPL family do not. Yu showed that nicastrin functions as the γ -secretase substrate receptor, and that it probably ensures that potential substrates are of the appropriate length. It was shown earlier that γ -secretase substrates need to be short enough to be cleaved efficiently, with the extracellular stub left after ectodomain shedding being typically around 20–30 amino acids. Fluhrer reported the observation that both full-length and ectodomain-shed TNF α could be co-isolated with an inactive aspartate mutant of SPPL2b. This suggests that the intramembrane cleavage of type II proteins, which is executed by SPPLs, does not require initial ectodomain shedding of the substrate; therefore, additional partner proteins with receptor function might not be needed for SPPLs.

L. Kroos (East Lansing, MI, USA) reported a new mode of protease activation for the S2P family protease SpoIVFB, which is required for bacterial endospore formation. During this process, a bound inhibitory protein is degraded to activate this intramembrane protease (Zhou & Kroos, 2005). Therefore, partner protein removal seems to have an important role for certain proteases of the S2P family to cleave their substrates.

Conclusions

The Ringberg Symposium on Regulated Intramembrane Proteolysis was the first meeting that brought together the leaders of this research field. The highlight of this historic meeting and a milestone for the RIP field was certainly the crystal structure of a rhomboid protease showing a membrane-localized active site only five years after its discovery. Thus, it is now proven that intramembrane proteases exist and that the cleavage of a peptide bond in the hydrophobic environment of a membrane is no longer a mystery.

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REFERENCES

- Baker RP, Young K, Feng L, Shi Y, Urban S (2007) Enzymatic analysis of a rhomboid intramembrane protease implicates transmembrane helix 5 as the lateral substrate gate. *Proc Natl Acad Sci USA* (in press)
- Brown MS, Ye J, Rawson RB, Goldstein JL (2000) Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. *Cell* **100**: 391–398
- Fluhrer R *et al* (2006) A γ -secretase-like intramembrane cleavage of TNF α by the GxGD aspartyl protease SPPL2b. *Nat Cell Biol* **8**: 894–896
- Friedmann E *et al* (2006) SPPL2a and SPPL2b promote intramembrane proteolysis of TNF α in activated dendritic cells to trigger IL-12 production. *Nat Cell Biol* **8**: 843–848
- Hu X, Hicks CW, He W, Wong P, Macklin WB, Trapp BD, Yan R (2006) Bace1 modulates myelination in the central and peripheral nervous system. *Nat Neurosci* **9**: 1520–1525
- Lazarov VK, Fraering PC, Ye W, Wolfe MS, Selkoe DJ, Li H (2006) Electron microscopic structure of purified, active γ -secretase reveals an aqueous intramembrane chamber and two pores. *Proc Natl Acad Sci USA* **103**: 6889–6894
- Lemberg MK, Menendez J, Misik A, Garcia M, Koth CM, Freeman M (2005) Mechanism of intramembrane proteolysis investigated with purified rhomboid proteases. *EMBO J* **24**: 464–472
- Sato C, Morohashi Y, Tomita T, Iwatsubo T (2006) Structure of the catalytic pore of γ -secretase probed by the accessibility of substituted cysteines. *J Neurosci* **26**: 12081–12088
- Stevenson LG, Strisovsky K, Clemmer KM, Bhatt S, Freeman M, Rather PN (2007) Rhomboid protease AarA mediates quorum-sensing in *Providencia stuartii* by activating TatA of the twin-arginine translocase. *Proc Natl Acad Sci USA* **104**: 1003–1008
- Tolia A, Chavez-Gutierrez L, De Strooper B (2006) Contribution of presenilin transmembrane domains 6 and 7 to a water-containing cavity in the γ -secretase complex. *J Biol Chem* **281**: 27633–27642
- Wang Y, Ha Y (2007) Open-cap conformation of intramembrane protease GlpG. *Proc Natl Acad Sci USA* **104**: 2098–2102
- Wang Y, Zhang Y, Ha Y (2006) Crystal structure of a rhomboid family intramembrane protease. *Nature* **444**: 179–183
- Weskamp G *et al* (2006) ADAM10 is a principal 'shedase' of the low-affinity immunoglobulin E receptor CD23. *Nat Immunol* **7**: 1293–1298
- Willem M *et al* (2006) Control of peripheral nerve myelination by the β -secretase BACE1. *Science* **314**: 664–666
- Wolfe MS, Kopan R (2004) Intramembrane proteolysis: theme and variations. *Science* **305**: 1119–1123
- Wu Z *et al* (2006) Structural analysis of a rhomboid family intramembrane protease reveals a gating mechanism for substrate entry. *Nat Struct Mol Biol* **13**: 1049–1051
- Yamasaki A, Eimer S, Okochi M, Smialowska A, Kaether C, Baumeister R, Haass C, Steiner H (2006) The GxGD motif of presenilin contributes to catalytic function and substrate identification of γ -secretase. *J Neurosci* **26**: 3821–3828
- Zhou R, Kroos L (2005) Serine proteases from two cell types target different components of a complex that governs regulated intramembrane proteolysis of pro-sigmaK during *Bacillus subtilis* development. *Mol Microbiol* **58**: 835–846

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