Intramembrane proteolysis at a glance: from signalling to protein degradation

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ABSTRACT

Over the last two decades, a group of unusual proteases, so-called intramembrane proteases, have become increasingly recognized for their unique ability to cleave peptide bonds within cellular membranes. They are found in all kingdoms of life and fulfill versatile functions ranging from protein maturation, to activation of signalling molecules, to protein degradation. In this Cell Science at a Glance article and the accompanying poster, we focus on intramembrane proteases in mammalian cells. By comparing intramembrane proteases in different cellular organelles, we set out to review their functions within the context of the roles of individual cellular compartments. Additionally, we exemplify their mode of action in relation to known substrates by distinguishing cleavage events that promote degradation of substrate from those that release active domains from the membrane bilayer.

KEY WORDS: Regulated intramembrane proteolysis, Rhomboid, Presenilin, γ-secretase, Signal peptide peptidase, SPP-like protease, Site-2 protease, RCE1, ZMSTPE24, Limited proteolysis, Protein homeostasis

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Introduction
Eukaryotic cells are highly structured and contain numerous organelles. To guarantee proper functionality of these distinct cellular entities, cells have to tightly control distribution, abundance and activity of each and every compartment-specific protein. Furthermore, damaged protein species need to be efficiently recognized and removed. Although cells have developed several safeguarding mechanisms that control protein homeostasis (proteostasis), including the ubiquitin proteasome system (Ciechanover and Kwon, 2015) and lysosomes (Winckler et al., 2018), intramembrane proteases are additional key regulators of the organelle proteome (Avci and Lemberg, 2015). In a biological context, proteolysis is an irreversible reaction; hence, an intramembrane cut introduces directional changes to membrane-associated processes. From a structural point of view, all intramembrane proteases are polytopic membrane proteins that form aqueous active sites within the lipid bilayer and harbour catalytic residues or a prosthetic zinc ion either adjacent to or inside the plane of the membrane (Sun et al., 2016). These unusual proteases are divided into four families according to their catalytic mechanism: metalloproteases, aspartyl proteases, serine (rhomboid) proteases and one unique glutamyl protease (see Box 1). With the exception of peroxisomes, at least one intramembrane protease is found in every membrane-enclosed cellular compartment (see poster).

Since cellular organelles fulfil a wide range of functions, different intramembrane proteases contribute to a large variety of different pathways. From what is known about intramembrane proteolysis, we differentiate here between two biological consequences, namely protein activation and degradation. On the one hand, limited proteolysis that generates a stable protein fragment with a specific function is a key step in protein activation. On the other hand, intramembrane proteolysis can also generate metastable fragments that are subsequently turned over by cellular degradation systems such as the proteasome. In this Cell Science at a Glance, we will focus on known functions of intramembrane proteases in mammalian cells. We will compare intramembrane proteases present within particular cellular compartments instead of reviewing individual examples of their respective mechanistic class. With this, we aim to describe how organelle-specific protease functions mirror the compartment’s requirements. Initially, intramembrane proteolysis referred to cleavage of transmembrane proteins within the hydrophobic core of the lipid bilayer (Brown et al., 2000), we extend our definition to proteases that position their active sites in the broader context of the membrane. Therefore, we include ZMPSTE24 (zinc metalloprotease Ste24 homologue), which is a zinc metalloprotease with a large hollow barrel-shaped membrane-integral chamber that encloses the active site in the membrane head group region (see Box 2 for further details). Focusing on mammalian cells, we will describe key functions of intramembrane proteases within all major cellular organelles, starting with mitochondria, followed by the inner nuclear membrane, and the secretory and the endocytic pathways.

Mitochondria
Mitochondria fulfill a plethora of essential functions including respiration, biosynthesis of lipids and other metabolites, calcium signalling, and control of apoptosis. To ensure adequate function, a number of different mechanisms constantly survey the mitochondrial membrane proteome (Haynes and Ron, 2010). The only mitochondrial intramembrane protease, the rhomboid protease PARL (presenilin-associated rhomboid-like – named by an artefact observed in a yeast two hybrid interaction screen) is localized at the inner mitochondrial membrane (Jeyaraju et al., 2006). PARL provides an important safeguarding system to target damaged protein activation and degradation. On the one hand, limited proteolysis that generates a stable protein fragment with a specific function is a key step in protein activation. On the other hand, intramembrane proteolysis can also generate metastable fragments that are subsequently turned over by cellular degradation systems such as the proteasome. In this Cell Science at a Glance, we will focus on known functions of intramembrane proteases in mammalian cells. We will compare intramembrane proteases present within particular cellular compartments instead of reviewing individual examples of their respective mechanistic class. With this, we aim to describe how organelle-specific protease functions mirror the compartment’s requirements. Initially, intramembrane proteolysis referred to cleavage of transmembrane proteins within the hydrophobic core of the lipid bilayer (Brown et al., 2000), we extend our definition to proteases that position their active sites in the broader context of the membrane. Therefore, we include ZMPSTE24 (zinc metalloprotease Ste24 homologue), which is a zinc metalloprotease with a large hollow barrel-shaped membrane-integral chamber that encloses the active site in the membrane head group region (see Box 2 for further details). Focusing on mammalian cells, we will describe key functions of intramembrane proteases within all major cellular organelles, starting with mitochondria, followed by the inner nuclear membrane, and the secretory and the endocytic pathways.

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Box 1. Architecture of intramembrane proteases
Intramembrane proteases have the unusual property of cleaving their substrates in the plane of cellular membranes. Based on their structure and catalytic mechanism they can be grouped into different families.

Aspartyl intramembrane proteases
Members of the GxGD aspartyl proteases have been intensively studied as their inaugural member, presenilin, is linked to familial Alzheimer’s disease (Langosch and Steiner, 2017; Wolfe, 2019). Presenilin associates with the non-catalytic subunits nicastrin, presenilin enhancer 2 (PEN2) and anterior pharynx-defective 1 (APH1) to form a 250 kDa protease complex known as γ-secretase (Langosch and Steiner, 2017; Wolfe, 2019). Mammals have two presenilin paralogues and several APH1 isoforms (three in human and four in rodents) that form functionally distinct complexes (Sannerud and Annaert, 2009). A related group of enzymes, with the signal peptide peptidase (SPP) as the first identified example and four SPP-like (SPPL) proteases, also carries a presenilin fold but has an inverted active-site topology (Weihofen et al., 2002). The structures of the archaeal SPP/presenilin homologue MCMJR1 (Li et al., 2013) and the human γ-secretase complex (Yang et al., 2019) revealed the architecture of the conserved membrane-embedded active site domain and the first mechanistic insights of how substrates are recognized.

Rhomboids
Rhomboids are serine proteases that were first discovered in D. melanogaster where they act as key activators of epidermal growth factor secretion by cleaving membrane-tethered precursors (Lee et al., 2001). Crystal structures of the E. coli rhomboid protease GlpG showed that rhomboids consist of a conserved six transmembrane domain core with an active site cavity that opens to the periplasmic (luminal) side of the membrane (Wang et al., 2006).

Site-2 protease
Site-2 protease (S2P) is a zinc metalloprotease that is conserved in all kingdoms of life, except the budding yeast S. cerevisiae. The structure of an archaeal S2P showed that catalytic zinc is coordinated in the centre of a six transmembrane helix bundle with a channel-like opening connecting the active site to the cytoplasmic side (Feng et al., 2007).

Ras-converting enzyme 1
Ras-converting enzyme 1 (RCE1) belongs to a group of so-called CAAX proteases that process prenylated peptides at the cytoplasmic interface of the ER. Although, so far, no bona fide membrane-protein substrates for CAAX proteases have been identified, the RCE1 crystal structure revealed that the active site catalytic glutamate residues are positioned in the plane of the membrane (Manolaridis et al., 2013).

Box 2. ZMPSTE24 has a membrane-integral substrate-binding chamber
The second CAAX protease, ZMPSTE24 (known as Ste24 in yeast), plays a crucial role in the maturation of farnesylated proteins (Michaelis and Barrowman, 2012). Although these canonical ZMPSTE24/Ste24 substrates are peripheral membrane proteins, crystal structures of ZMPSTE24 and Ste24 revealed a membrane-spanning hollow barrel-shaped active site comprising seven antiparallel transmembrane helices (Pryor et al., 2013; Quigley et al., 2013). Whereas three α-helices close this barrel from the ER luminal site to the cytoplasmic and nucleoplasmic side, a zinc-metalloprotease domain with two openings allows substrates to enter the membrane-integral active-site chamber. Since substrates are supposed to access the catalytic zinc ion from within the membrane, we suggest the inclusion of ZMPSTE24/Ste24 in the class of intramembrane proteases. Consistent with this, ZMPSTE24/Ste24 cleaves proteins that clog the Sec61 translocon channel by an unknown mechanism (Ast et al., 2016; Kayatekin et al., 2018).
mitochondria to the macroautophagy (mitophagy) pathway, which, when defective, is associated with familial Parkinson’s disease (Pickrell and Youle, 2015; Spinazzi and De Strooper, 2016). In this context, PARL cleaves the serine/threonine kinase PTEN-induced kinase 1 (PINK1) while it is imported into mitochondria (Jin et al., 2010; Meissner et al., 2015). This processing releases the C-terminal cleavage fragment of PINK1 into the cytoplasm, where it is subsequently degraded by the ubiquitin proteasome system (see poster). However, when the mitochondrial membrane potential is disrupted by the ionophore CCCP, PINK1 does not reach the inner mitochondrial membrane (Narendra et al., 2010) and is not cleaved by PARL. Instead, PINK1 is targeted to the outer mitochondrial membrane and becomes fully activated (Pickrell and Youle, 2015). Among other events, this leads to the recruitment of the E3 ubiquitin ligase Parkin and removal of damaged mitochondria by mitophagy (for more details see Pickrell and Youle, 2015). Interestingly, upon CCCP treatment, the serine/threonine phosphatase PGAMS5, another factor linked to mitochondrial homeostasis, is cleaved by PARL, leading to its mature dodecameric assembly, which results in the formation of large fibres that eventually get released from mitochondria by an unknown mechanism (Ruiz et al., 2019; Sekine et al., 2012). Consistent with this inverse regulation of PARL activity upon CCCP-induced chemical stress, a regulatory complex consisting of the matrix protein SLP2 (also known as STOML2) and the inner membrane AAA-protease YME1L1 controls substrate access (Wai et al., 2016). Moreover, a recent proteomic survey has identified several additional PARL substrates (Saia et al., 2017). One of those is the apoptosis factor SMAC (also known as DIABLO), which is cleaved in response to inner membrane depolarization, resulting in its release into the cytosol where it sets the stage for apoptosis. Overall, PARL occupies an important role in modulating the mitochondrial membrane proteome by performing cleavages that lead to protein activation, as well as triggering protein degradation, in concert with the cytoplasmic proteasome and other mitochondrial proteases.

**Endoplasmic reticulum and inner nuclear membrane**

The endoplasmic reticulum (ER) is the largest cellular organelle and forms a continuous compartment with the nuclear envelope. For the secretory pathway, the ER is the main entry point of newly synthesized proteins and, therefore, serves as the first protein quality control checkpoint. Since all translocated proteins begin to fold and mature here, the ER faces a huge burden of misfolded proteins, which, if they are not removed, lead to stress (Christianson and Ye, 2014; Ellgaard et al., 2016). The ER membrane accommodates several proteases including the rhomboid serine protease rhomboid-like protein 4 (RHBDL4, also known by its gene name Rhbd41) (Fleig et al., 2012), the aspartyl protease signal peptide peptidase (SPP, encoded by HM13) (Weihofen et al., 2002), the glutamyl protease Ras-converting enzyme 1 (RCE1) (Manolaridis et al., 2013) and the metalloprotease ZMPSTE24 (Quigley et al., 2013). Although most ER-membrane proteins are excluded from the inner nuclear membrane, RCE1 and ZMPSTE24 localize to peripheral ER, as well as to the inner nuclear membrane (Barrowman et al., 2008; Burrows et al., 2009).

The unique proteome of the inner nuclear membrane is ensured by nuclear pore complexes that restrict diffusion between the peripheral ER and the nucleoplasm (Schirmer et al., 2003). The inner nuclear membrane proteins, including lamins, give the nucleus its unique shape and are crucial for chromosome organization, and DNA replication and repair (Guerrero and Kind, 2019). Interestingly, RCE1 and ZMPSTE24 are important for the removal of the last three amino acids from the so-called CAAX proteins (where ‘C’ is a prenylated cysteine that is important for membrane attachment, ‘A’ is an aliphatic amino acid and ‘X’ is any amino acid) (see poster). The CAAX motif is found in a number of cytoplasmic and/or nuclearoplasmic peripheral membrane proteins, including the Ras GTase and lamins, and its processing is a crucial step in maturation of these essential factors (Michaelis and Barrowman, 2012). In addition, ZMPSTE24 is known to catalyse another cut in pre-lamin A at a site distinct from the CAAX motif (see poster) (Barrowman et al., 2008). This cleavage removes the prenylated C-terminal domain that anchors the protein at the membrane and is essential to generate mature nuclear lamin A (Pendas et al., 2002). Homozygous loss-of-function mutations in ZMPSTE24 or mutation of the pre-protein cleavage site both lead to the accumulation of unprocessed pre-lamin A, which is linked to a severe laminopathy known as Hutchinson–Gilford progeria syndrome (Michaelis and Barrowman, 2012). Yet, the molecular function of lamin A maturation and how cleavage affects its interplay with the nuclear lamina are important open questions.

In the peripheral ER, ZMPSTE24 serves as a crucial proteostasis factor by removing proteins that get stuck in the Sec61 translocon channel, one of the main protein transport pathways into the ER, as observed for oligomers of the human islet amyloid polypeptide (IAPP) (see poster) (Ast et al., 2016; Kayatekin et al., 2018). This shows that ZMPSTE24 has a dual role as a protease involved in maturation and/or activation, and protein degradation. The same holds true for SPP, which has been initially described to remove signal peptides from the ER membrane after they have been released from nascent chains by the signal peptidase complex (SPase) (see poster) (Weihofen et al., 2002). For most ER-targeting signal peptides this is predicted to lead to turnover. However, for some, such as the signal peptide of the MHC class I molecule HLA-A (SP-HLA-A; see poster), a regulatory peptide is generated, which, after its transport into the ER lumen and loading on HLA-E receptors, is monitored at the cell surface by natural killer cells, thereby reporting integrity of the antigen-presenting machinery (Lemberg et al., 2001). Another variation of this two-edged function is the role of SPP as a non-canonical factor of the ER-associated degradation (ERAD) pathway (Christianson and Ye, 2014). Although most ERAD substrates are displaced from the ER to the cytoplasm by a dislocon complex, which consists of an ERAD E3 ubiquitin ligase and several auxiliary factors (Wu and Rapoport, 2018), SPP-mediated proteolysis directly triggers the release and degradation of several homeostatic ER-membrane proteins, including the unspliced form of the unfolded protein response regulator XBPI (Chen et al., 2014), heme oxygenase 1 (Boname et al., 2014) and the SNARE protein syntaxin-18 (Avci et al., 2019). For the purpose of this regulated abundance control, SPP is part of a 500 kDa ERAD complex that consists of the E3 ligase translocation in renal carcinoma on chromosome 8 (TRC8, also known as RNF139) and the rhomboid pseudoprotease Derlin1 (Boname et al., 2014; Chen et al., 2014; Stagg et al., 2009). Although the molecular mechanism remains to be determined, the emerging picture is that the ERAD factors prime SPP-released fragments for turnover by the proteasome. Very recently, a testis-specific SPP homologue SPPL2C (SPP-like 2C) has been shown to promote male germ cell development by cleaving phospholamban and ER-resident SNARE proteins (Niemeyer et al., 2019; Papadopoulos et al., 2019). Last but not least, the rhomboid serine protease RHBDL4 funnels certain unstable membrane proteins such as the orphan subunit of the pre-T cell antigen receptor α (pTα, also known as PTCRA) into a non-canonical branch of the ERAD pathway (see poster) (Fleig et al., 2012). In addition, RHBDL4 may also have regulatory functions in...
controlling protein secretion (Paschkowsky et al., 2016; Wan et al., 2012; Wunderle et al., 2016). In conclusion, there is a fine line between limited proteolysis that tunes the specific properties of a substrate and proteolytic processing that destabilizes the protein, leading to its subsequent turnover by other degradation systems.

**Golgi apparatus**

With its central role in protein maturation and trafficking, the Golgi complex hosts three intramembrane proteases, namely site-2 protease (S2P, also known as MBTPS2) (Rawson et al., 1998), the aspartyl protease SPP-like 3 (SPPL3) (Nyborg et al., 2006), and the rhomboid serine protease rhomboid-like protein 1 (RHBDL1) of unknown function (Lemberg and Freeman, 2007). The metalloprotease S2P was the first intramembrane protease identified. It acts as a key rheostat in the secretory pathway by regulating the proteolytic release of membrane-tethered transcription factors, such as the sterol regulatory element-binding proteins (SREBP)-Rs (Rawson et al., 1998), cyclic AMP-dependent transcription factor (ATF6) (Ye et al., 2000) and cyclic AMP-responsive element-binding protein, hepatocyte-specific (CREBH, also known as CREB3L3) (Zhang et al., 2006). This regulation by limited proteolysis is achieved through a combination of controlled trafficking and by the site-1 protease (SIP, also known as MBTPS1)-catalysed shedding of the luminal portion – either a loop connecting two transmembrane domains of SREBP proteins or the ectodomain of ATF6 and CREBH – that prepares the membrane-tethered transcription factors for cleavage by S2P (see poster) (Sakai et al., 1998). In contrast, SPPL3 influences the maturation of other proteins by triggering the proteolytic release of Golgi glycosyltransferases such as N-acetylgalactosaminyltransferase V (GnT-V, also known as MGAT5), which is thought to lead to their degradation within lysosomes or by extracellular proteases (see poster) (Voss et al., 2014). Once again, some of the outlined Golgi intramembrane proteases play a role in the activation of regulatory molecules whereas others contribute to regulated degradation control of membrane-anchored enzymes.

**Plasma membrane and endosomes**

The plasma membrane and endosomal system together contain six intramembrane proteases: the two catalytic subunits of γ-secretase, presenilin-1 (PSEN1) and presenilin-2 (PSEN2) (Mentrup et al., 2017; Wolfe, 2019), two additional aspartyl proteases, namely SPP-like 2A and 2B (SPPL2A and SPPL2B), and two rhomboid serine proteases, rhomboid-like proteins 2 and 3 (RHBDL2 and RHBDL3) (Lohi et al., 2004). The emerging picture is that one version of each protease type localizes to the plasma membrane, while a second parologue is found in endosomal vesicles (see poster) (Friedmann et al., 2006; Lohi et al., 2004; Sannerud and Annaert, 2009). At the interface to the extracellular space, intramembrane proteases of the late secretory pathway and the endosomal system join a large number of sheddases that remodel the cell surface and trigger secretion of bioactive molecules (Lichtenthaler et al., 2018). Presenilin proteins form the catalytic active site subunit of the γ-secretase complex, which, similarly to PARL and SPP, joins non-proteolytic subunits and auxiliary factors (see Box 1) (Shah et al., 2005) in order to select its substrates. Currently, around 100 membrane proteins have been identified as substrates (Haapasalo and Kovacs, 2011; Hemming et al., 2008; Lichtenthaler et al., 2018). A unifying feature is that all known γ-secretase substrates face the extracellular or luminal environment with their N-terminus, a topology commonly referred to as type I membrane protein orientation. However, the physiological consequence of intramembrane cleavage has been revealed for only a small fraction of these γ-secretase substrates, such as its role in Alzheimer’s disease as reviewed elsewhere (Langosch and Steiner, 2017; Wolfe, 2019). Here, we will solely exemplify its activating and degrading function on cell-surface receptors. Upon ligand binding, receptors such as neurogenic locus notch homologue protein 1 (Notch1) undergo shedding by a member of the a metalloprotease of the disintegrin and metalloproteinase (ADAM) family (Lichtenthaler et al., 2018), which prepares the remaining membrane-integral portion for a subsequent cut by the γ-secretase complex (Struhl and Adachi, 2000). The released intracellular domain of Notch1 functions as a transcription activator in signalling (De Strooper et al., 1999). However, for the vast majority of other cell-surface proteins, cleavage by γ-secretase is believed to be crucial to maintain plasma membrane proteostasis, thereby preventing the accumulation of remaining transmembrane anchors (see poster) (Kopan and Ilagan, 2004). However, given the redundancy with the endolysosomal system, which removes the vast majority of plasma membrane proteins (Winckler et al., 2018), the physiological relevance of γ-secretase-mediated protein degradation remains to be determined.

The second group of aspartyl intramembrane proteases in the endosomal system are SPPL2A and SPPL2B, which cleave single-pass membrane proteins with the N-terminus facing the cytoplasm, referred to as type II membrane proteins (Friedmann et al., 2006; Mentrup et al., 2017). Under overexpression conditions, SPPL2A and SPPL2B have overlapping substrate specificity; however, work in mice suggests distinct physiological functions (Schröder and Saftig, 2016). SPPL2A-catalysed cleavage of CD74, which is the invariant chain of MHC class II molecules, has been shown to control B-cell development (Beisner et al., 2013; Bergmann et al., 2013; Schneppenheim et al., 2013). During transport of MHC class II molecules to late endosomes, CD74 is proteolytically processed by the soluble cysteine protease cathepsin S (CatS, also known as CTSS), generating a membrane-anchored stub that is further cleaved by SPPL2A (see poster). While the released intracellular domain of CD74 impacts activation of nuclear factor κB (NF-κB), defects in B-cell maturation in SPPL2A-knockout mice have been primarily attributed to accumulation of the uncleaved CD74 transmembrane stubs that disturb membrane trafficking by an unknown mechanism (Schröder and Saftig, 2016). Likewise, in the absence of SPPL2A and/or SPPL2B, the lectin-like oxidized LDL receptor 1 (LOX1, also known as OLR1) accumulates in late endosomes, leading to its prolonged activation and increased atherosclerosis (Mentrup et al., 2019). Whether SPPL2A and SPPL2B also play a direct role in regulated protein degradation remains to be determined.

Rhomboid proteases were initially identified in *Drosophila* screen as regulators of growth factor secretion (Freeman, 2014); however, in mammalian cells, this function is primarily performed by ADAM proteases (Lichtenthaler et al., 2018). As a recent proteomics screen identified a heterogenous set of substrates to be cleaved by the plasma membrane rhomboid RHBDL2 (Johnson et al., 2017), it is attractive to speculate about a more general role in the proteostasis surveillance. Although the physiological function for the majority of identified substrates remains to be determined, RHBDL2-catalysed cleavage of thrombomodulin has been shown to control remodelling of cell–cell contact points during wound healing (Cheng et al., 2011; Lohi et al., 2004), whereas cleavage of the C-type lectin domain family 14 member A (CLEC14A) has been implicated in regulation of angiogenesis (Noy et al., 2016). For RHBDL3, which localizes mainly to endosomes (Lohi et al., 2004), so far no substrates are known. The emerging picture is that in both
the late secretory pathway and the endosomal system, intramembrane proteases act as bivalent proteostasis factors, with wide physiological functions ranging from regulated intramembrane proteolysis to bulk protein degradation.

Conclusions and outlook

Along our way through the secretory and endocytic pathways, we encounter homologous intramembrane proteases in different cellular compartments. While the γ-secretase complex and rhomboids cleave primarily type I membrane proteins, the SPP family shows the opposite substrate orientation and cleaves type II membrane proteins. Some of these intramembrane cleavage events require a preceding shedding of their substrate for recognition, whereas others can recognize full-length proteins (Lichtenthaler et al., 2018). Moreover, cleavage of polytopic membrane proteins has been described (Fleig et al., 2012). Together with a large set of protein sheddases, intramembrane proteases are able to target all membrane topologies. With the two CAAX motif-processing proteases RCE1 and ZMPSTE24 joining the intramembrane proteases (see Boxes 1 and 2), the substrate spectrum broadens to peripheral membrane proteins. Furthermore, because bacterial RCE1 homologues such as Bacillus subtilis BrsW trigger degradation of membrane-anchored anti-sigma factors (Heinrich et al., 2009), we may speculate that mammalian CAAX proteases can also cleave transmembrane substrates. For most intramembrane proteases, it is sufficient to overexpress the protease together with its substrate to observe robust cleavage. However, for RHBDL1 and RHBDL3, no substrates are yet known. Hence, under physiological conditions, additional factors may be required. In agreement with this, certain intramembrane proteases have been described to form membrane protein complexes that are potentially involved in substrate selection and regulation (Chen et al., 2014; Lichtenthaler et al., 2018; Sekine et al., 2012; Wolfe, 2019). Other possible means of controlling intramembrane proteolysis are regulated substrate trafficking, or the prerequisite for tailoring the substrate by shedding the substrate’s ectodomain, which prevents interaction with some intramembrane proteases (Lichtenthaler et al., 2018). However, some proteases, such as rhomboids, can cleave membrane proteins without prior shedding, pointing to a more diverse substrate-selection mechanism. In some cases, this is linked to recognition of metastable transmembrane domains, but site-specific recognition of consensus sequences has also been observed (Langosch et al., 2015). This bivalent substrate recognition mechanism, including sampling transmembrane helix stability as well recognizing putative cleavage-site motifs, mirrors the wide range of different functions. For certain intramembrane proteases such as the SPP and presenilin families, a function in activation and degradation is already known. Owing to the growing understanding of the protease–substrate relationship as outlined in this review, it emerges that in contrast to most other proteases, a given intramembrane protease can do both—activate and degrade their membrane protein substrates. It will be important to reveal whether the cleavage decision-making process is entirely determined by the substrates, or whether differences on the protease side such as varying adaptor proteins govern this biological distinction. A detailed understanding of the intramembrane protease mechanism as well as defining their physiological substrate spectrum will reveal whether such a dual role in activation cleavage and protein degradation is a common principle.

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Competing interests

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